# STUDIES ON MEROPLANKTON

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By

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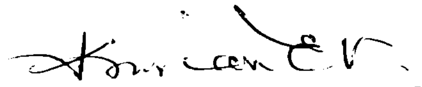
This is to certify that this thesis is an authentic record of the work carried out by the candidate, Mr. T. Balachandran, M.Sc., under my supervision and guidance in the Indian Ocean Biological Centre and Regional Centre of National Institute of Oceanography, Cochin and that no part thereof has been presented before for any other degree in any University.



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# P R E F A C E

Victor Hensen in 1887 coined the word plankton meaning something that floats passively hither and thither. Plankton, as it formed the basic food supply of marine life and indicator of water masses, has been the subject of detailed study among marine and fishery scientists. This great community is drawn from a very wide variety of animal phyla. Moreover, while a large number of zooplankton animals often referred as holoplankton remain planktonic throughout their existence (e.g. copepods, thaliaceans, chaetognaths), the others occur in the plankton only during a part of their lives. The term meroplankton generally refers to this portion of the zooplankton which is transient in nature. This would include the larvae of many of the invertebrate phyla, as well as fish eggs and larvae and certain species which occur on a seasonal basis. Meroplankton usually spending the early part, of their lives in the plankton remain for the rest of their lives in the nektonic or benthic environment. However, some of the hydromedusan groups spend the early part of their lives fixed to the substratum as hydroids and float about in the plankton later as sexual medusae. Similarly mature stages of certain benthic nereids and syllids (Polychaeta) occur in plankton during their spawning period. Also included in the mero-

plankton are young cephalopods which when adult become part of the nekton. Compared to the far higher proportion of meroplanktonic forms in neritic zooplankton, oceanic zooplankton, though it may include a few larvae such as phyllosoma and leptocephalus which may be long living, in general has mostly holoplanktonic members. In the case of benthic organisms the larvae do not, as a rule, have time to drift far from their place of origin, since sooner they have to develop to a stage when they must sink to the sea bed for settlement. In cold and less saline areas, and in high latitudes the benthic animals tend to produce young ones that develop miniatures of their parents and so miss out meroplanktonic stage.

Substantial information has been accumulated dealing chiefly with the systematics, distribution, biochemical composition and preservation aspects of holoplankton from most of the oceans. However, work on meroplankton especially from the tropical Indian Ocean is scarce and the few publications available refer to the decapod and fish larvae only. Considering the role of meroplankton in the economy of the sea, this group has become an attractive subject of intensive research. The present study besides providing information regarding the fixation and preservation techniques and biochemistry of tropical meroplankton, also consolidates information regarding their zoogeography in the

Indian Ocean region, with a view to amplifying the limited information available from this area. The distribution studies are based on the collections made during the International Indian Ocean Expedition (1960-'65) and kept at the Indian Ocean Biological Centre, Cochin, whereas the material for the fixation and preservation and biochemical studies was collected by the author during 1968-'78 from the west and east coasts of India.

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# STUDIES ON MEROPLANKTON

BY

T. BALACHANDRAN.

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# 1. FIXATION AND PRESERVATION

## 1.1. Introduction.

### 1.1.1. Historical resume:

The fixation and preservation study found its origin in 1967 when SCOR-WG 23 (Scientific Committee on Oceanic Research-Working Group 23) was evolved from the studies conducted by SCOR-WG 13, on zooplankton sampling methods (SCOR, 1966 and UNESCO, 1968), which had begun its work in 1964. During the discussions in 1968, conflicting reports were given relating to the various methods of fixation and preservation. Techniques used by some workers with success did not appear to find favour with others. Specialists gave observations which were both extensive and accurate, but reasons for success or failure with most of the methods used were matters of opinion or conjecture. Also preservation methods may vary according to the type of samples and organisms. In zooplankton studies minute forms such as protozoa, jelly forms such as medusae, impenetrable forms such as large crustacea and osmotically sensitive forms such as sipunculoid larvae, present a variety of problems peculiar to this branch of biological science. The 1927 zooplankton samples collected during the IIOE (International Indian Ocean Expedition) period 1960-1965 by different ships from different areas in the Indian Ocean showed varying state of preservation. It was

clear that insufficient data was available in most of the cases to permit accuracy of comparison. When badly preserved specimens were present, it was not known as to whether this was due to shipboard procedure - such as delay in fixation or the quality of the formaldehyde used, its original strength when purchased or to an excessive fall in pH, because the latter had not been measured with sustained accuracy over a period of time. The net result of these deficiencies, and many others was the institution of experiments which would provide evidence of a factual nature in the future handling of zooplankton. Because of the diversity of marine zooplankton in many natural communities and the specific requirements necessary to achieve optimal results with many taxa, a variety of associated methods had to be evolved for dealing with a wide range of marine plankton. The amount of fixative required for fixation varied according to the nature and amount of animal protein, and of lipid present. Accordingly, SCOR-WG 23 designed the lay out of experiments to be conducted. The overall and desired was the production of a series of techniques - practicable, reliable, and reproducible - which will include old and new methods with their relationships to different types of plankters. This part presents the result of such an investigation, carried out by the author at the Indian Ocean Biological Centre in Cochin on the lines suggested by SCOR-WG 23, into the best techniques of fixation and preservation of marine zooplankton for taxonomic and morphological study. The SCOR-WG 23 programme was international in

character and scope, with the terms of reference "to suggest methods for preserving zooplankton samples for taxonomic study and for biomass determination". Surprisingly the monograph published (UNESCO, 1976), but for scattered references did not deal with any aspect of meroplankton fixation and preservation. Also the results were pertaining to studies in a temperate country (temperature >20°C) and the effect of tropical climate on preservation was completely ignored. As such research was continued in the National Institute of Oceanography with reference to meroplankton and the results obtained so far are presented here.

In addition, this work also includes a discussion on the treatment of zooplankton samples containing meroplankton, on board the vessel and in the laboratory from the time the net is lowered into the water. Meroplankton shrinkage, volume and weight loss with time of preservation and preservation of pigments and colour were studied side by side. An understanding of the nature and causes of deterioration noted in the different taxa by the specialists concerned is a prerequisite for an insight into the problems of fixation and preservation. So a brief review of the damages observed in the IIOE collections of meroplankton held at the Indian Ocean Biological Centre have been dealt with under respective taxa. Chemicals, leading to relaxed death of meroplankton organisms preventing contraction and distortion at fixation, were tested as narcotic, relaxant and anaesthetic agents. Among other

invertebrates these agents produced inconsistent results. In the selection of a particular narcotic, considerable difficulty was experienced due to the immense variation existed between different animals and their acceptance of narcotics. Response to narcotics varied with age and from species to species. Many narcotics in use required a period of 24 hours or more for full narcotic effect. Hence need was felt to seek quicker-acting narcotics by the use of new chemicals. Thus 32 narcotics were screened with reference to each group to select the most appropriate one.

This study extending over a period of 10 years from July 1968 to June 1978 is expected to yield additional information on the long term effect of climatic variations (temperature and light) on tropical zooplankton fixatives and preservatives.

While no attention was given to the underlying physiological mechanisms involved, interest was evinced only in the behavioural response of zooplankton.

### 1.1.2. Survey of literature:

General techniques for fixation:- The most important publications on the subject are: the preservation of natural history specimens Vol. I (1955) and Vol. II (1968) published by Wagstaffe, Reginald and Fidler; osmotic pressure of fixing solutions by Young (1935); the rate of penetration of fixatives by Underhill (1932); use of glacial acetic acid for killing parasitic nematodes by Borland (1961); carbondioxide fixation

in marine invertebrates by Hamon, Card and Susan (1962); polyvinyl alcohol-fixative-adhesive for small helminths and protozoa by Hoffmann (1954); and noted on methods for the narcotization, killing, fixation and preservation of marine organisms by Russel (1963).

Baker, Dow and Fishman (1958) used a chloral hydrate formaldehyde fixative solution in enzyme histochemistry. Baker (1966) dealing with cytological techniques advocated use of aldehydes as fixatives and preservatives for zooplankton in view of their non-coagulative nature of animal proteins. Pearse (1968) found that it is the hydrated monomer methylene glycol produced in weak solutions of formaldehyde which is the reactive part with the biological material.

Marr (1963) recommended fixation in formaldehyde solution and then transfer to 75 percent ethanol aqueous for benthic fauna. McGee-Russell (1958) used alcohol and 40 percent formaldehyde in equal proportions for fixing calcium. Steedman (1976) used propylene glycol as an additive to fixing and preserving fluids for zooplankton to bring about softening effect, inhibit mould growth, and to assist penetration of formaldehyde fixative.

<u>Preservation by freeze-dry technique</u>:- Since the publications of Altmann technique for fixation by drying while freezing, developed by Gersh (1932), a number of modifications were introduced to freeze-drying to suit the taste of researchers and nature of specimens. Rowe (1960 and 1971)

dealt with the theory and practice and machinery and methods of freeze drying. Harris (1968) designed a new apparatus for freeze-drying whole biological specimens. Others in the series were Hower (1967), Pearse (1963) and Simatos and Rey (1965).

<u>Miscellaneous preservation techniques</u> have been dealth with in about two hundred and fifty publications. Bassett (1947) used ethyl methacrylate as a preserving medium for gross anatomical serial sections. Berry (1944) dealt with antibacterial values of ethylene glycol monophenyl ether. Bruno and Sahyun (1944) made comparative studies of preservative agents. Fraser (1961) used polyethylene glycol in preservation. Held (1915) tested the preserving action of benzoic acid. Jamieson and Powell (1931) and Powell and Jamieson (1938) used merthiolate as a preservative for biological products. Chloral hydrate as a preservative of anatomical objects was used by Keen (1875). Levi (1946) preserved biological specimens with clarite X. Noble and Jaeckle (1929) preserved animals by infiltration with paraffin. Walmsley (1869) preserved objects in glycerine jelly. Wheatley (1941) preserved biological specimens with isobutyl methacrylate polymer. Wright (1870) published a note on the preservation of minute animals in acetic acid. Williamson and Russell (1965) used ethylene glycol as a preservative for marine organisms. Owen (1955) and Owen and Steedman (1956 and 1958) used phenoxetol and propylene phenoxetol as a preservative of animal tissues in view of their bactericidal.

fungicidal and antioxidant properties.

Schutner (1900), Gilles (1900) and Russell (1895) used formalin as a preservative. Smith (1944) used a neutral solution of formaldehyde for biological purposes. Miller (1932) used treated formalin as a permanent preservative. Walker (1953) has published a voluminous book on formaldehyde. Nasarenko (1960) studied the effect of formaldehyde on aquatic organisms. Taube (1962, 1965) and Mahoney (1963) used para-formaldehyde as a field preservative. Armstrong and Wickstead (1962) have published a note on the preservation of plankton samples with formalin employing neutralisers.

Atkins (1922) prepared permanently non-acid formalin for preserving calcareous specimens. Burke (1933) knowing the importance of pH of formalin as a fixative and preservative made adjustments and stabilisation of the hydrogen ion concentrations of formalin solutions with neutralisers as 5 percent pyridine which gave a pH around 7.8. Smith (1944) Neutralised 40 percent formaldehyde solution for biological purposes using hexamine at 20 percent strength. According to Romeiss (1948) calcium carbonate produced a pH of 6.4. Carriker (1950 and 1963) developed a specific preservative fluid for bivalve larvae. Hill (1966) used propylene phenoxetol as a preservative for living holothurians (*Thyone mosbii*). Gregg (1968) preserved invertebrates in alcohol. Levi (1966) dealt with the care of alcoholic collections of small invertebrates. Novak *et al.* (1966) used liquid nitrogen for shell fish preservation. Pettinger (1951) studied effect

of ascorbic acid on keeping quality of frozen oysters. Tarjan (1967) developed an abbreviated lactophenol-glycerine procedure for preserving nematodes. Tahir (1960) developed new technique for preserving prawn larvae. Van Bruggen (1965) studied preservation of molluscs. Subrahmanyam *et al.* (1963) experimented possible use of ammonia for fish preservation. Tarr and Bailey (1940) tried effectiveness of benzoic acid for fish preservation.

Beers and Stewart (1970) preserved the spicular skeleton of acantharians adding strontium chloride 80 mg per litre sea water. Beers (1966) while studying chemical composition of major zooplankton groups in the Sargasso Sea, noted interference with neutralising agents. Fudge (1968) during biochemical analysis of preserved zooplankton, Hopkins (1968) during the estimation of carbon and nitrogen content of fresh and preserved euphausiid crustacean, when hexamine was used as a buffer; Lovegrove (1966) during the determination of dry weight of plankton; Raymont *et al.* (1967) estimating the biochemical composition of certain oceanic zooplankton decapods and Sutcliffe (1957) trying on an improved method for the determination of preserved plankton, noted the effects of various neutralising and buffering agents on the plankton specimens. The above studies showed loss of both weight and volume after preservation in formaldehyde solution. Miller (1962) recorded the slight solvent action of sea water upon calcareous plankters. Petersen (1976) has published a note on fixation and preservation of planktonic coelenterates. Rose (1933), Newell and

Newell (1963), Fantin (1964) and Wickstead (1965) have dealt with certain aspects of crustacean fixation and preservation during their routine studies.

Preservation of fish has been studied by Gross (1962), Hora (1939), Mc Allister (1965) and Smith (1968). Ohm and Fellers (1926) preserved fish with hypochlorites. Clark (1888) preserved fish using acetic, boracic, salicylic and other acids. Gruen and Wirtz (1964) studied fish preservation with or without hexamethylenetetramine. Haraguchi *et al.* (1969) studied preserving effect of ozone to fish.

*On relaxing agents*:- Methods of relaxation, narcotisation and anaesthetisation have been dealt with in over one hundred publications. The most important work among them is a study of general anaesthesia in teleosts with a discussion of its implications to the transportation of fishes which formed the Ph.D. dissertation of McFarland (1959). Quinaldine was tried as an anaesthetic for fish by Muench (1958). Shark was immobilised by means of ethanol by Sadak (1966). Bagenal (1963) found propylene phenoxetol as a good anaesthetic for *Pleuronectes* sp. Bell (1964) has summarised the properties, characteristics and uses of some general anaesthetics for fish. Burrows (1952) used chloretone to anaesthetise salmon fingerlings. While Fish (1943) used high carbondioxide concentrations to anaesthetise fish, Beckmeyer (1953), Griffith *et al.* (1941) and Allison (1954) tried the effect of ether anaesthesia. Sehdev *et al.* (1963) anaesthetised sockeye salmon with 2-phenoxy ethanol. Trott (1965) used nicotine sulphate in fish narcotisation. Thorson (1959) anaesthetised *Petromyzon marinus*

with tricaine methanesulfonate. This narcotic was first used by Rothlin (1932) and Randall (1962) used it as a teleost fish anaesthetic. Ball and Cowan (1959) used urethane as an anaesthetic for fishes, and Johnson (1954) used it in brook trouts. Kisch (1947) immobilised fish with procaine. M-amino ethylbenzoate was tried as anaesthetic for amphibian embryos by McGovern and Rugh (1964) whereas ethyl p-aminobenzoate was tested as an anaesthetic for cold blooded vertebrates by McErlean (1967). The above works mainly pertain to fishes and other vertebrates.

General techniques for relaxation have been dealt with by few workers. Marr (1963) made studies on the preservation of marine animals especially dealing with narcotisation and anaesthesia. Griffin (1910) used magnesium sulphate for the anaesthetisation of marine animals. Osterhout and Hill (1933) produced anaesthesia by distilled water. Parker (1939) tried cooling as general anaesthesia.

Techniques and methods developed for relaxation of invertebrates are varied and the leading works are given below. Gohar (1937) used formaldehyde for the preservation of contractile marine animals in an expanded condition. Lemche (1963) also used formaldehyde for narcotising gastropoda. Runham _et al._ (1965) used a modification of Gohar's method by the addition of menthol and propylene phenoxetol. Abdel-Malek (1951) relaxed helminths before fixation with menthol. Also Gray (1953) used menthol with chloral hydrate as a narcotic for coelenterates. Owen (1955) and Owen and Steedman (1958) used one percent propylene phenoxetol as a relaxing and

preserving agent for molluscs. Rosewater (1963) though found propylene phenoxetol as an anaesthetic for giant clams and other molluscs, reported temporary ill-effects. He also found a one percent solution too strong for small molluscs. Mckay and Hartzband (1970) used propylene phenoxetol narcotic agent for unsorted benthic invertebrates. Bowman (1968) used succinylcholenies and other drugs for narcotising gastropod molluscs. Foley *et al.* (1966) anaesthetised the lobster, *Homarus americanus*, with isobutyl alcohol and methyl pentynol. Hargis (1953) used chloretone as a trematode relaxer. Hewatt (1943) developed a method for narcotising holothurians. Joosse and Lever (1959) narcotised a pulmonate gastropod, *Limnaea stagnalis* with nembutal and M.S. 222 one after another. Lalli (1972) recommended M.S. 222 or Urethane in sea water for gymnosomatous pteropod. Ahmad (1969) found anaesthetic effects of tricaine methanesulphonate (M.S. 222) on an amphipod, *Gammarus pulex* (L). The narcotic agents nembutal and M.S. 222 were used separately but produced faster narcotisation when used together after initial treatment with nembutal (Joosse and Lever, 1959; Lever *et al.*. 1964; McGowan and Bough, 1964; Randall, 1962; Rothlin, 1932). Lever *et al.* recommended nembutal to be used at 0.1 percent solution at 20°C and 0.08 percent at 27°C. Runham *et al.* (1965) found the above combination producing quickest result on slugs in 1 to 10 minutes. Methods on narcotisation have been developed in connection with the microscopical studies made by Pantin (1948, 1960) using 7.5 percent magnesium chloride or 20 percent magnesium

sulphate. Gannon and Gannon (1975) found carbonated water the best narcotising agent, when added in the ratio of 1:20 to a sample containing cladocera and copepoda.

Clement and Cather (1957) relaxed veliger larvae by placing them in a mixture of one percent saturated aqueous solution of chloretone and 2 parts sea water in 3 to 4 minutes. Leibowitz (1976) narcotised veligers using propylene phenoxetol/ magnesium chloride method. Hannerz (1956) found cocaine to be the best anaesthetic for larval spionids. The bibliography is not exhaustive. Still the above survey clearly shows lack of information on meroplankton narcotisation and relaxation. The details clearly show that the effectiveness of the various chemicals, the amount used, and the time required for relaxation varied with the different taxa and conditions. This necessitated extensive experimental studies with meroplankton.

<u>Colour preservation</u>:- In early years glycerine was used for the preservation of the natural colours of marine organisms. Methods for preservation of colour in biological specimens and marine organisms have been developed by Elmhirst (1929), Rohdenburg (1930), Sosby (1906) and White and Peters (1969). Waller and Eschmeyer (1965) found copepods preserved in a 1:9 dilution of 2.2 ml butylated hydroxytoluene (BHT) concentrate/ litre of strong 40 percent formaldehyde in good condition and retaining colour for variable periods. Colour preservation and fading of museum specimens have been studied by McCormick (1961), Ritchie (1924), Harmer (1922) and Shein (1951). Studies for preservation of colour in fishes were made by many -

Elias (1872) and Collingworth (1939) used alcoholic preservation; Borodin (1930), Crawford and Breder (1922), Haly (1891), Marie and Marie (1962), Samokhvalova (1955) and Sawinski (1953) used other methods. Garrick (1968) used antioxidants. Yoshida (1962) preserved fish specimens with their original body colour, injecting sodium ascorbate solution. Yamaga and Morioka (1962) preserved body colour of red fishes chemically. Toyama and Miyoshi (1963) retained red colour in preserved fish and crustacean specimens using an antioxidant Butylated Hydroxyanisole (BHA). Ueyanagi (1966) and Matsumoto *et al.* (1972) succeeded in maintaining red pigmentation of larval tuna fish.

Verrill (1865) preserved star fishes with natural colour. Jusmosyk (1952) preserved skin colours of amphibia and Windsord (1971) used ammonium sulphate to maintain frog colour. A method of colour preservation in opisthobranch mollusc was studied by Robilliard (1969). The nature of animal colours has been extensively dealt with by Fox and Vevers (1960).

*Shrinkage, volume and weight loss:* Methods for determination of plankton volume have been developed by Haley (1954), Frolander (1957), Sutcliffe (1957), Yentsch and Hebard (1957) and Tranter (1959). The effects of formaldehyde fixation and preservation on marine organisms have been studied by Sutcliffe (1957), Wimpenny (1949), Raymont *et al.* (1967), Lovegrove (1966), Omori (1968), Beers (1966) and Fudge (1968). Wilson (1953) observed differences between measurements of living and preserved aquatic nymphs caused by injury and preservation.

Jones (1936) made comparative measurement of living and fixed protozoa. Orton (1937) noted shrinkage of oyster tissues in Bouin's fluid. Leckey (1938) noted changes in river planktonic organisms due to formalin preservation. Parker (1963) studied effects of formalin on length and weight of fishes. Farris (1963) has similarly observed shrinkage of sardine (*Sardinops caerulea*) larvae upon preservation in buffered formalin. Devi (1972) also noted shrinkage of fish larvae according to the strength of formaldehyde used and developmental stages of fish larvae. The magnitude of the volume loss of as much as 13 to 85 percent of the live volumes was realised by Ahlstrom and Thrailkill (1962) by the systematic quantitative remeasuring of plankton volume change with time of preservation. They found greater percentage decrease in samples predominant with non-crustacean plankton and thus related plankton volume loss to constituent composition.

1.1.3. Work done at the Indian Ocean Biological Centre:

In India, the experimental project on the preservation of plankton was supported by the National Institute of Oceanography and carried out by the author at the Indian Ocean Biological Centre, Cochin. The series of experiments designed for the SCOR Working Group 23 were carried out with suitable modifications to suit the tropical conditions. According to the availability of zooplankton material, experiments were replicated. The summary of observations and experiments carried out between 1968 and 1972 was presented

before the Symposium on the Fixation and preservation of marine zooplankton held at Bath, University of Bath, U.K., 13 - 20 July, 1972, under the auspices of UNESCO and SCOR and published in the UNESCO Monograph (1976). The results in detail were published by the author in 1973 and 1974 through a series of papers as follows: A review on fixatives and preservatives (1973c) deals with existing techniques in this field. Another paper (1973b) reviews the nature and causes of deterioration observed in zooplankton samples. Methods of collection, handling and storage of zooplankton in tropics have been dealt with in another paper (1974b). A paper (1974d) on Dowicill '100' deals with its use as a fixative-cum-preservative for zooplankton. Similarly a chemical, phenoxetol was tried as a sorting medium-cum-preservative for zooplankton in the tropics (1974e). About fixation and preservation of calcareous and gelatinous plankters separate publications were made (1974a and 1974c). Also the results of a thorough investigation on formaldehyde as a fixative and preservative for zooplankton have been published (1973a).

However, the above series of publications mainly deal with the holoplanktonic marine zooplankton only. Hence these studies were continued with reference to marine meroplankton. The results obtained during the period 1968 - 1978 are presented here.

Fig. 1. ZOOPLANKTON.

## 1.2. Material, design of experiments and methods.

### 1.2.1. Material:

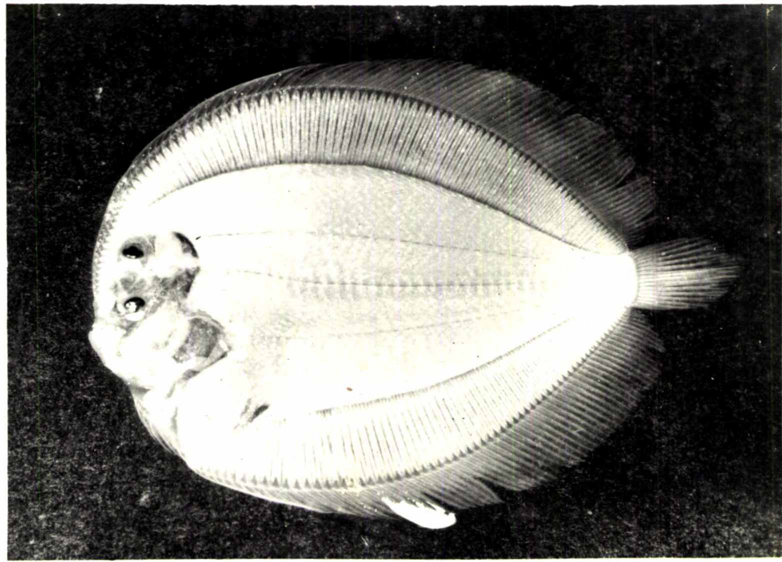
Materials for the study was specially collected from Bay of Bengal and off Kerala coast during 1968-1978 using low vessel speeds of 0.5 to 1 knot, and taking care to minimise the damages caused to meroplankton samples during collection and on handling as outlined by Balachandran (1973b and 1974b). Tornaria larvae rise to the surface at midnight and begin to leave the surface before dawn. Hence for collections of tornaria their negative response to light has been taken into consideration. In general meroplankton were collected by sampling a water column of 100 m to surface during the three hour period after sun rise and after sun set. Freshly collected meroplankton (Fig. 1) was preserved in bulk with the sole aim of retension of morphological characters. Also meroplankton taxa were separated from the total catch of mixed plankton, narcotised, fixed and preserved with a view to select the most appropriate reagents. The various meroplankton taxa sorted out thus were grouped under the following 13 categories (Figs. 2, 3 & 4):

1. Medusae of the hydromedusan type as Aglantha and anthozoan larvae belonging to Zoanthidea and Ceriantharia.
2. Muller's larvae of polyclad turbellarians.
3. Pilidium larvae of Nemertini.
4. Meroplanktonic polychaetes consisting of trochophore larvae and mature stages of benthic nereids and syllids.

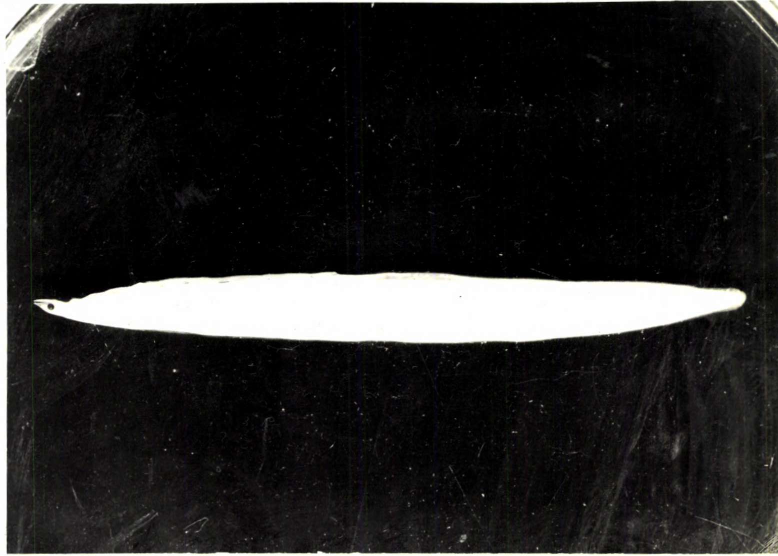
Fig. 2a. LARVA OF *[illegible]* Sp.

Fig. 2b. LEPTOCEPHALUS LARVA OF EEL.

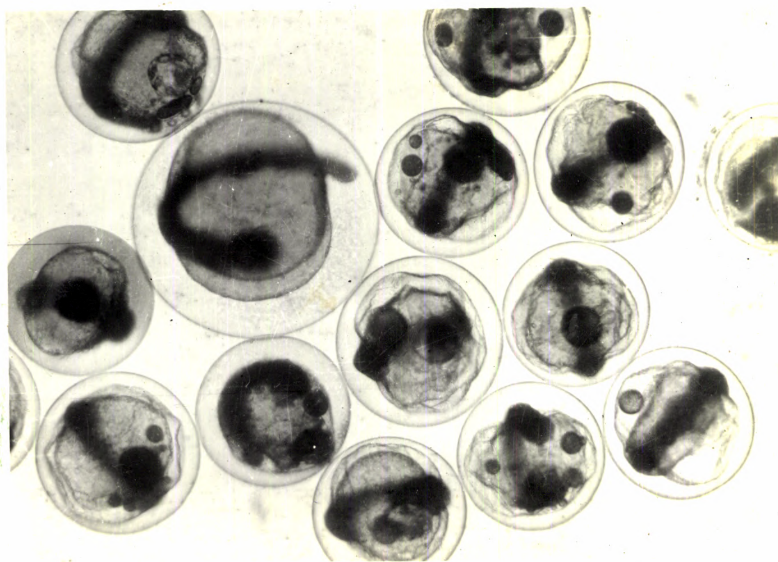
Fig. 2c. FISH EGGS.



2a



2b

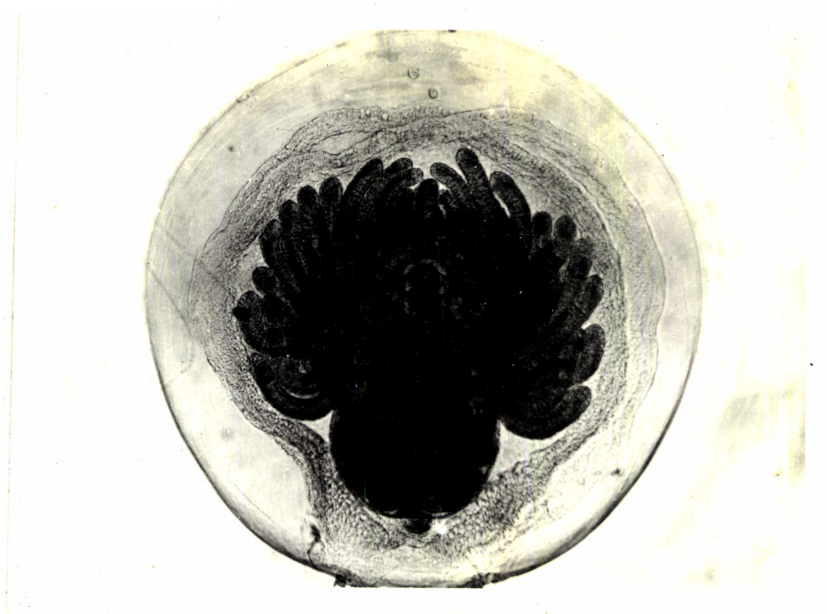


2c

Fig. 3a. LINGULA LARVA.

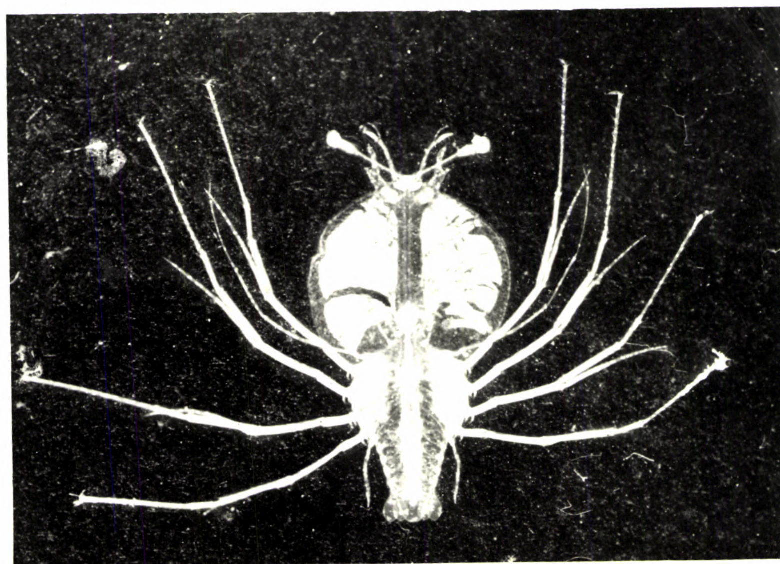
Fig. 3b. CEPHALOPOD JUVENILES.

Fig. 3c. PHYLLOSOMA LARVA.



3 a

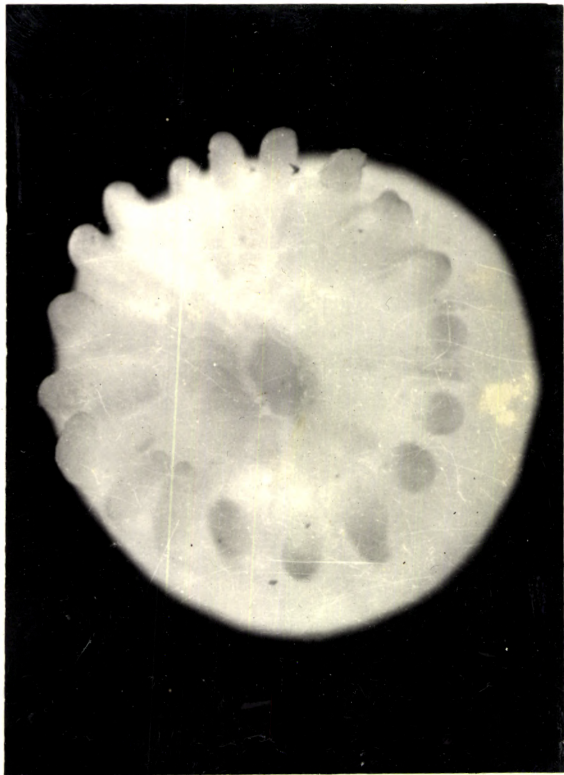
3 b



3c

Fig. 4a. CERIANTHARIA LARVA. Fig. 4b. BRACHIOLARIA LARVA.

Fig. 4c. ACTINOTROCHA LARVA. Fig. 4d. MEGALOPA LARVA.



4a

4b

4d

4c

5. Sipunculoid larvae and postlarvae.

6. Meroplanktonic crustacea such as (a) nauplius, metanauplius, protozoea, zoea and mysis stages of benthic and nektonic crustacea, (b) cirripede larva, (c) alima larva, (d) zoea of anomuran crabs, (e) zoea and megalopa of brachyuran crabs, (f) phyllosoma larva of scyllarid decapod and (g) larval stages of prawns and shrimps.

7. Meroplanktonic molluscs such as Lamellibranchiata postlarvae, gastropod veligers and juvenile cephalopods.

8. Cyphonautes larvae of Bryozoa.

9. Actinotrocha larvae of Phoronidea.

10. Lingula larvae of Brachiopoda.

11. Echinoderm larvae such as bipinnaria and brachiolaria of Asteroidea, ophiopluteus of Ophiuroidea, echinopluteus of Echinoidea, auricularia and doliolaria of Holothuroidea and crinoid larva of Antedon.

12. Tornaria larvae of Enteropneusta and

13. Fish eggs and larvae.

1.2.2. Design of experiments:

Histological studies made on meroplankton fixed in coloured fixatives using Nile Blue sulphate 5 ppm demonstrated the effect of varying concentrations of fixatives on the specimens, depending on the variations in their cuticular coverings. These studies also showed the importance of various amounts of fixative required depending on the nature of protein and amount of lipid present in the specimens. The amount of fixative required by the specimens was measured by the fall in the concentration of the chemical in the fixative. The fixation time was calculated from the assay of fixative

and found to be less than ten days. Once fixation is completed the specimens are transferred to a storage or preservative fluid in order to remove the nutrient for bacterial growth, interfere with their metabolism by adjusting the environment beyond the tolerance of bacterial cell by dehydration and pH, or stop microbial invasion by chemical attack. At times the fixed protein, lipid and calcium become subject to dissolution resulting in volume and weight loss in specimens. These deteriorations have to be controlled by the use of additives available for different purposes.

Certain criteria have been observed in the selection of new reagents, and toxicity, irritability, evaporation rate, and price, as well as practicability and convenience, have been held to be of major importance. Similarly the following criteria for selection of preservatives were also adopted as far as possible - must be colourless, odourless, non-volatile, non-transparent, non-poisonous, able to preserve the natural colour of specimens, bactericidal and fungicidal, low in price, able to maintain good condition of specimens prolonging the duration of their shelf-life inhibiting biotically induced deterioration of tissues and able to maintain weight and volume unaltered.

Keeping the above points in view, a series of experiments were designed principally to investigate the value of old and new fixing and preserving methods for mesoplankton. The existing techniques of fixation and preservation (Balachandran, 1973e) were under examination in detail, with a view to

eliminating as far as possible the present lack of data regarding precise reasons for success or failure of the various mixtures of reagents which are used at present.

In most of the experiments, reagent grade formaldehyde (Walker, 1944) produced by the oxidation of methanol and sold in glass containers at a storage temperature around 30°C and 35 to 40 percent in strength, with a pH around 3.5, was used as a standard fixative (Balachandran, 1973a). The qualities which made the formaldehyde unfavourable as a preservative - activity, changeability, quick penetration of living tissues - are strongly in its favour as a fixative, and its ready miscibility with sea water and distilled water makes its application to plankton fixation a matter of relative ease. The fact that as a fixative it requires no lengthy washing-out process afterwards, is greatly in its favour when soft delicate organisms are concerned. The details on the use of formaldehyde and other solutions as fixatives in the present experimental series are given on page 32 . The fixation of zooplankton, used in the series of experiments on preservation, was accomplished in 2 percent formaldehyde on site sea water solution. In this way it was possible to follow the effects of various preservatives without greatly multiplying the total number of experiments, as would be the case had a number of different fixatives were used. In all, there are 11 series of experiments on preservatives, most of the series containing 7 to 17 separate formulae. Each series, under a separate descriptive heading, contained details of preserving

fluids, some of which may also be used as fixatives. Many of these when indicated the right lines to follow were subjected to further refinement. The descriptive titles of the series are as follows and the details are given in Series 1 to 11.

Series 1 - Basic solution: Formaldehyde-Sea water I. (1 percent formaldehyde in on site sea water plus additives).

Series 2 - Basic solution: Formaldehyde-Sea water II. (2 percent formaldehyde in on site sea water plus additives).

Series 3 - Basic solution: Formaldehyde-distilled water I. (1 percent formaldehyde in distilled water plus additives).

Series 4 — Basic solution: Formaldehyde-distilled water II. (2 percent formaldehyde in distilled water plus additives).

Series 5 - Basic solution: Dowicill 100 - Sea water 10, 20 or 30 percent. (10, 20 or 30 percent Dowicill 100 in on site sea water plus additives).

Series 6 - Basic solution: Dowicill 100 - Distilled water 10, 20 or 30 percent. (10, 20 or 30 percent Dowicill 100 in distilled water plus additives).

Series 7 - Hydroxyl bearing chemicals.

Series 8 - Water soluble esters.

Series 9 - Miscellaneous fixing and preserving fluids.

Series 10 - Methods for jelly plankters.

Series 11 - Methods for calcareous plankters.

The above series of experiments consisted of four parts as follows:

Specification sheet 1 - Details of preserving fluid formula.

" " 2 - pH record.

Specification sheet 3 - Formaldehyde assay.
" " 4 - Specimen condition record - by eye and low power binocular microscope.

Specification sheets 2, 3 and 4 are common for Series 1 - 11. The details of observations (data collected) were noted in the specification sheets and these number over 2400. These original data sheets are not presented here owing to their large volume. But the consolidated observations are given under results and discussion. Only sample forms of these sheets are given in the Appendices I, II and III. Chemical abbreviations are listed on page 33 .

The reasons underlying the experimental series on preservation are:

Series 1-4:

Formaldehyde is being examined as a preservative at 1 and 2 percent strength, made up in different diluents and with additives to modify the pH (3.5 to 10), the rate of evaporation, the rate of penetration, the hardening effects, and to resist the attack by bacteria, moulds and yeasts.

These experiments are expected to provide answers for the following questions:-

1. Does it make any difference in the long run which strength of formaldehyde is used?
2. Is it advantageous to make up the formaldehyde solution in distilled or sea water?
3. Does the pH of formaldehyde solution reach a steady low level and remain there?

4. Does buffering really operate over long periods of time with formaldehyde plus macroplankton?

5. Which are the best buffers for macroplankton?

6. Which is the best way of keeping the pH of formaldehyde solutions plus macroplankton near to pH 7.0.

7. Do reagents with hydroxyl groups, such as glycols, help to keep macroplankton flexible in formaldehyde solution?

8. Is it better to use a reagent such as magnesium chloride to assist muscle flexibility in preserved macroplankton?

<u>Series 5-6</u>:

In these experiments one formaldehyde donor-Dowicil 100 is being examined. These series may provide answers to the following questions:-

1. Do Dowicil 100 liberate sufficient active formaldehyde to keep formalin fixed macroplankton in good condition?

2. Is it an advantage to use these reagents in distilled water or are they better in sea water?

3. Is it necessary to employ with these reagents bactericidal or fungicidal additives?

4. Do the donor break down in time and cease to function?

5. After the donor has parted with its formalin, does the reagent which remains has a deleterious effect on fixed tissues?

<u>Series 7-8</u>:

These series deal with the effects of glycols and esters on formaldehyde fixed macroplankton. The answers expected from these experiments are:-

1. Do glycols, by virtue of their hydroxyl groups keep formalin fixed macroplankton soft without actually softening them too much over a long period of time?

2. Do water soluble esters preserve fixed protein?

3. Or do they need additives such as bactericides and fungicides?

4. Do partly esterified reagents such as glycol monoacetate occupy an intermediate position in the preservation of plankton between the glycols and the esters?

5. Do these reagents dissolve macroplankton? If so, which particular groups?

6. Do these maintain such a steady pH at 7.0 that they would be suitable for the preservation of calcareous plankters?

__Series 9:__

Deals with a small number of reagents which do not fall into the previous categories, and with certain formulations put forward by various researchers for fixation and preservation.

__Series 10:__

The preservative fluids are tested for the efficiency in the preservation of gelatinous plankters. Whether the additive used can reduce volume and weight loss? Can they help in reducing shrinkage caused to the specimens?

__Series 11:__

The formulae developed are designed to keep the pH in the region of 7.0 or slightly over. To avoid precipitation of calcium oxalate, sea water is omitted from the formulae where potassium oxalate is used. In the formulae which follow.

several are similar but for a difference in the amount of formaldehyde.

1.2.3. Methods:

Zooplankton was collected as detailed in 1.3.1. Sorted meroplankton taxa were allowed to swim about in suitable containers. They were anaesthetised using specific agents mentioned on pages 30 & 31 before fixation was done. Then the fixatives mentioned on page 32 were added slowly, keeping the ratio of fixatives to meroplankton at 9 : 1. At times taxa were slowly transferred to the fixatives in the same manner as above. A fixation time of 10 days was allowed, during which fixative qualities were examined and recorded.

Meroplankton used for preservation series was fixed in 2 percent formaldehyde in sea water. Then they were slowly transferred to the preservatives mentioned in series 1 to 11 and vice-versa. Observations were made at frequent intervals of 1, 2, 3, 5, 7, 15, 30, 60, 180, and 365 days followed by yearly observations. Respective taxonomic criteria may be evaluated differently by various workers. Specimens must be stiff and transparent. All features mentioned should be fixed and preserved satisfactorily because specific criteria in recent taxonomic research have become more exact and detailed. State of preservation was judged by the general appearance, colouration, condition of appendages and internal body structures. In making up the formulae comparable,

measurements used were normally grams and millilitres. With fluids volumetric measurements were used as it was quicker and easier.

The above series of experiments were replicated as and when macroplankton was available. The first series of experiments were carried out in the ambient room temperature, 28° to 32°C. A second series was carried out in a temperature controlled room having 20°C in order to study the effect of high tropical temperatures. As mentioned above in order to study the effect of photochemical reactions on specimens taking place in the direct and indirect sun light, one series was kept in the normal room light and another set was kept in a dark room. Monitoring of specimen condition, formaldehyde assay, pH etc. as given in the specification sheets (Appendices I, II & III) were done regularly. Sensitive indicator papers or pH meter was used to measure the pH of fixatives and preservative solutions. A comparison with results obtained either way showed values close enough in accuracy. In the case of formaldehyde donor as Dowicil 100 the amount of free formaldehyde present in the solution was measured.

Formaldehyde Assay-Sodium Sulphite Method:

The simple and accurate method of sodium sulphite was used throughout the experimental series in the assay of the formaldehyde content in the fixative and preservative solutions.

<u>Principle</u>:- The basis of this method is the quantitative liberation of sodium hydroxide when formaldehye reacts with sodium sulphite and water according to the following formula:

$$CH_2O(Aq) + Na_2SO_3 + H_2O = NaOH + CH_2(NaSO_3)OH$$

The change in pH may be followed as the reaction proceeds by using thymolphthalein as an indicator, and the amount of sodium hydroxide liberated is estimated by titration using the above formula. The amount of formaldehyde present in the fluid examined may be calculated as follows:

<u>Method</u>

1. Pour about 50 ml of a 30 percent solution of sodium sulphite in distilled water into a clean conical flask.
2. Add 4-5 drops of thymolphthalein indicator solution.
3. Add a few drops of normal sodium hydroxide solution until the solution gives a faint blue colour.
4. Add normal sulphuric acid solution in drops until the blue colour just disappears.
5. Weigh out 3 g of the formaldehyde sample. Add it to the sodium sulphite solution. The solution will turn blue.
6. Shake the solution and titrate with normal sulphuric acid or with normal hydrochloric acid until the blue colour just disappears.
7. The percentage of formaldehyde may be calculated from the following formula:

$$\text{Percentage of formaldehyde} = \frac{\text{Acid titre} \times \text{Normality of acid} \times 3.}{\text{weight of sample}}$$

<u>Note</u>: A small glass or polythene thimble will be found convenient for weighing the sample. Prepare fresh solutions of sodium sulphite daily.

Method of narcotisation:-

The meroplankton was brought on the deck of the vessel and placed in an aerated container filled with on site sea water. Preliminary tests were conducted to determine the relative effects of different concentrations of each narcotising agent on the larvae. The concentrations that appeared to have the most favourable narcotising effect on the organisms were then utilised in the following experiments. About 10 larvae were transferred to a finger bowl containing 90 ml of on site sea water. Then the predetermined concentration of a narcotising agent was added and any changes in behaviour of the organisms were recorded. Identical experiments were conducted with other narcotics using fresh larvae. To facilitate observation of the meroplankters under a stereo-microscope, larvae were also kept in petri dishes. The narcotising agents were chosen on the basis of their availability, relative low cost, and possible adaptability to meroplankton. A particular narcotising agent was deemed successful if it acted as a relaxer, gently decreasing locomotor activity of the organisms and immobilising them in a relatively short time without indication of frenzied movements. While no attention was given to the underlying physiological mechanisms involved, interest was evinced only in the behavioural response of meroplankton. Also other methods were used to establish the condition of the narcotised meroplankton - such as prodding with a needle or gently poking it with a glass rod. For the oxygen starvation method sea

water was boiled for 5-10 minutes, cooled to the temperature of sea water in which the plankton lived and replaced the natural sea water with the boiled. Carbon dioxide excess was made by the addition of dry ice or by bubbling pure carbon dioxide from a cylinder of gas. Details of all narcotics used are given on pages 30 and 31.

Plankton ratio:

Experiments on the ratio of the volume of fixative/preservative to that of plankton revealed 9 : 1 to be reasonable when 2 and 1 percent formaldehyde was used. A proportion lesser than 9 : 1 leads to decomposition of specimens due to insufficient fixative/preservative fluids.

Shrinkage, volume and weight loss.

Material and methods.

The test samples were collected from the west coast of Kerala. The meroplankton taxa viz. larval forms of coelenterates, polychaetes, sipunculoids, crustaceans, echinoderms, enteropneusts and fishes, when present in large numbers, were separated quickly and preserved in 2 percent formaldehyde solution in on site sea water. Their volume was measured immediately, soon after preservation and subsequently at the end of 1, 2, 5, 10 and 25 days as well as 1, 3, 6, 12 and 24 months. The volumetric measurement is a determination of the space occupied by the plankton. Of the 2 basic direct volumetric techniques, the method of measuring by displacement was

adopted and kept uniform throughout the experiments. In this method the space occupied by the plankton is measured in terms of the equivalent volume of liquid that they displace. The water and interstitial fluids can be quantitatively removed from a sample of known total volume, measured, and the difference from the total taken as a measure of the plankton volume. The size of the graduate cylinder used will depend upon the sample size but should generally allow for atleast a 10 percent difference in the readings with and without the plankton sample present. In this procedure the use of suction or air pressure to remove interstitial water must be done with care in order to avoid damage to plankton. Removal of interstitial fluid from the plankton sample is an important step in making accurate and precise determination of displacement volume. Several types of apparatus have been developed which provide for both the removal of these fluids and the measurement of volume. The following method was used. Frolander (1957) described an apparatus, constructed of common laboratory glassware, in which the liquid and interstitial fluids from a plankton sample held in a chamber filled to a known total volume were removed by aspiration through a sintered glass filter and measured in a graduated cylinder. The difference between the chamber volume and the volume of fluids removed by aspiration is taken as a measure of the plankton volume.

For shrinkage studies random samples of larvae were measured individually with an ocular micrometer. The individual larvae were placed in numbered vials and killed with 2 percent

borax buffered formaldehyde. The larvae were measured again at the end of 1, 2, 3, 10, 20 and 30 days and recorded as L1, 2, 3, 4, 5 and 6. Leptocephalus larvae were grouped in 3 lengt groups, and the others were averaged and the mean preserved lengths recorded.

Anaesthetics, Narcotics and Relaxants experimented.

| Serial Number | Name | Range of dosage/ Amount required |
|---|---|---|
| 1 | Oxygen starvation | |
| 2 | Carbondioxide excess | |
| 3 | Gentle heating to 40°C | |
| 4 | Carbonated water | 1:10 to 1:20 ratio |
| 5 | Benzamine hydrochloride | 0.1 percent |
| 6 | Butyn 1 percent | 10 percent |
| 7 | Cocaine | 0.1 percent |
| 8 | Chloroform | 15 drops/100 ml |
| 9 | Chloretone 1 percent | 8 percent; 1:2500 - 1:3000 |
| 10 | Chloral hydrate crystals | 16/100 ml |
| | " " liquid 2% | 10 percent |
| 11 | Ether | 1.5 to 2 percent |
| 12 | Ethanol | 10 percent |
| 13 | Formaldehyde 40 percent | 1:20 |

Anaesthetics, Narcotics and Relaxants experimented. (contd.)

| Serial Number | Name | Range of dosage/ Amount required |
|---|---|---|
| 14 | Hydrogen peroxide 3 percent | 10 percent |
| 15 | Hydroxylamine 10 percent | 10 percent |
| 16 | Methyl alcohol | 10 percent |
| 17 | Methyl pentynol | 15 drops/100 ml |
| 18 | Menthol crystals | 1g/100 ml |
| 19 | Menthol:Chloral hydrate 12:13 | 0.1 percent |
| 20 | Magnesium Chloride (isotonic with sea water) | 3.5 percent; 40-160 m |
| 21 | Magnesium sulphate 3 percent | 10 to 20 percent |
| 22 | Nembutal | 0.08 to 12 percent |
| 23 | Propylene phenoxetol | 0.002 to 1 percent |
| 24 | Procaine | 0.1 percent |
| 25 | Physostigmine Salicylate | 8 percent |
| 26 | Quinaldine:Acetone:Water (1:16:32) | 4 percent |
| 27 | Sodium amytal | 1.2 g/litre |
| 28 | Tertiaryamyl alcohol | 8 percent |
| 29 | Tubocuraine | 12 percent |
| 30 | Tribromoethanol | 5 - 30 ppm |
| 31 | Tricaine methane sulphonate | 0.3 to 3 percent; 1:15,000 |
| 32 | Urethane 10 percent | 12 percent |

## Fixatives used.

| Serial Number | Chemical | Strength | Diluent |
|---|---|---|---|
| 1 | Formaldehyde | 4 percent | Sea water |
| 2 | " | " | Tap water |
| 3 | " | " | Distilled water |
| 4 | " | 2 percent | Sea water |
| 5 | " | " | Tap water |
| 6 | " | " | Distilled water |
| 7 | " | 1 percent | Sea water |
| 8 | " | " | Tap water |
| 9 | " | " | Distilled water |
| 10 | Bowicil 100 | 20 percent | Sea water |
| 11 | " | " | Distilled water |
| 12 | " | 10 percent | Sea water |
| 13 | " | " | Distilled water |
| 14 | Ethanol | 80 percent | Distilled water |
| 15 | " | 90 percent | Distilled water |
| 16 | Acetaldehyde | 2 percent | Sea water |
| 17 | Acrolein | 2 percent | Sea water |
| 18 | Paraformaldehyde | 2 percent | Sea water |
| 19 | Glutaraldehyde | 2 percent | Sea water |
| 20 | Bouin's fluid | 75:25:5 | Picric acid: Formaldehyde: Acetic acid |
| 21 | Carnoy's fluid | 60:30:10 | Ethanol: Chloroform: Acetic acid |
| 22 | Chromic acid | 1 percent | Sea water |
| 23 | Osmic acid | 0.3 percent | Sea water |
| 24 | Tri-chloroacetic acid | 1 percent | Sea water |
| 25 | P-toluene sulphonic acid | 1 percent | Sea water |
| 26 | Potassium hydroxide: Glycerine | 1:2.5 percent | Distilled water |

## Chemical abbreviations used

| | | | |
|---|---|---|---|
| 1 | HEX | - | Hexamethylene tetramine |
| 2 | BOR | - | Sodium tetraborate |
| 3 | $NaHCO_3$ | - | Sodium bicarbonate |
| 4 | SGP | - | Sodium glycerophosphate |
| 5 | Na Ace | - | Sodium acetate |
| 6 | $CaCO_3$ | - | Calcium carbonate |
| 7 | Cal Sil | - | Calcium silicate |
| 8 | Mg Sil | - | Magnesium silicate |
| 9 | Al Sil | - | Aluminium silicate |
| 10 | EGDA | - | Ethylene glycol diacetate |
| 11 | EGMA | - | Ethylene glycol monoacetate |
| 12 | GMA | - | Glycerol monoacetate |
| 13 | GDA | - | Glycerol diacetate |
| 14 | GF | - | Glycol formal |
| 15 | PG | - | Propylene glycol |
| 16 | PGDA | - | Propylene glycol diacetate |
| 17 | PGMA | - | Propylene glycol monoacetate |
| 18 | PEG 1500 | - | Polyethylene glycol 1500 |
| 19 | PHEN | - | Phenoxetol |
| 20 | PROP PHEN | - | Propylene phenoxetol |
| 21 | DOW A | - | Dowicide A |
| 22 | PROGP | - | Progallin P |
| 23 | Sod Benz | - | Sodium benzoate |
| 24 | BSTN CD | - | Basyntan CD |
| 25 | TRIS | - | 2-amino-2-(hydroxymethyl)-1,3-propanediol. |

## Preserving fluid formulae

Series 1: Basic solution (referred to as FSW-1)

2.5 ml 40% formaldehyde; 97.5 ml on site sea water.

Specification Sheet No. 1

| Expt. No. | FSW-1 | HEX | BOR | $NaHCO_3$ | SSP | NA Ace | $CaCO_3$ | Cal Sil | Mg Sil | Al Sil | BDMA | GMA | GP | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 98 | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 96 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 98 | - | 2 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 99 | - | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 8 | 98 | - | - | - | 2 | - | - | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 16 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 17 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

**Preserving fluid formulae**

Series 2: Basic solution (referred to as FSW-2)

5 ml 40% formaldehyde; 95 ml on site sea water.

Specification Sheet No.1

| Expt. No. | FSW-2 | HEX | BOR | $NaHCO_3$ | SBP | NA AGS | $CaCO_3$ | Cal Sil | Mg Sil | Al Sil | EGMA | GMA | OP | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 98 | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 96 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 98 | - | 2 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 99 | - | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 8 | 98 | - | - | - | 2 | - | - | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 16 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 17 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

# Preserving fluid formulae

Series 3: Basic solution (referred to as FDW-1)

2.5 ml 40% formaldehyde; 97.5 ml distilled water

Specification Sheet No.1

| Expt. No. | FDW-1 | HEX | BOR | $NaHCO_3$ | SHP | Na AOS | $CaCO_3$ | Cal Sil | Mg Sil | Al Sil | EDMA | GMA | GP | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 98 | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 96 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 98 | - | 2 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 99 | - | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 8 | 98 | - | - | - | 2 | - | - | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 16 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 17 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

# Preserving fluid formulae

**Series 4: Basic solution (referred to as FDW-2)**

**5 ml 40% formaldehyde; 95 ml distilled water**

**Specification Sheet No. 1**

| Expt. No. | FDW-2 | HEX | BOR | $NaHCO_3$ | SGP | NA AGS | $CaCO_3$ | Cal Sil | Mg Sil | Al Sil | HEMA | GMA | GP | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 98 | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 96 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 98 | - | 2 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 99 | - | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 8 | 98 | - | - | - | 2 | - | - | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 16 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 17 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

## Preservation fluid formulae

Series 5: Basic solution (referred to as DSW 10 or 20%)

10 ml Dowicil 100, 90 ml on site sea water.
20 ml Dowicil 100, 80 ml on site sea water.

Specification Sheet No. 1

| Expt. No. | DSW 10% | DSW 20% | HEX | DOR | $NaHCO_3$ | SOP | NA AGS | $CaCO_3$ | Cal Sil | Mg Sil | Al Sil | EGMA | GMA | GP | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | - | 99 | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | - | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 8 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

## Preserving fluid formulae

Series 6: Basic solution (referred to as DDW 10 or 20%)

10 ml Dowicil 100, 90 ml distilled water.
20 ml Dowicil 100, 80 ml distilled water.

Specification Sheet No.1

| Expt. No. | DDW 10% | DDW 20% | MEK | DOR | $NaNO_3$ | SOP | Na.AOS | $CaCo_3$ | Cal Sil | Mg Sil | Al Sil | MDMA | GMA | GF | TRIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | - | 99 | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 99 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | - | 99 | - | 1 | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 95 | - | - | - | 5 | - | - | - | - | - | - | - | - | - | - |
| 6 | 99.5 | - | - | - | - | 0.5 | - | - | - | - | - | - | - | - | - |
| 7 | 95 | - | - | - | - | - | 5 | - | - | - | - | - | - | - | - |
| 8 | 95 | - | - | - | - | - | - | 5 | - | - | - | - | - | - | - |
| 9 | 95 | - | - | - | - | - | - | - | 5 | - | - | - | - | - | - |
| 10 | 95 | - | - | - | - | - | - | - | - | 5 | - | - | - | - | - |
| 11 | 95 | - | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| 12 | 95 | - | - | - | - | - | - | - | - | - | - | 5 | - | - | - |
| 13 | 95 | - | - | - | - | - | - | - | - | - | - | - | 5 | - | - |
| 14 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | 5 | - |
| 15 | 95 | - | - | - | - | - | - | - | - | - | - | - | - | - | 5 |

## Preserving fluid formulae

### Series 7: Hydroxyl-bearing chemicals.

Specification Sheet No.1

| Expt. No. | PG | PEG 1500 | PHEN | PROP PHEN | SEA WATER | DIST. WATER | DOW A | PROG P | BHEN OD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | - | - | - | 70 | - | - | - | - |
| 2 | 30 | - | - | - | - | 70 | - | - | - |
| 3 | - | 30 | - | - | 70 | - | - | - | - |
| 4 | - | 30 | - | - | - | 70 | - | - | - |
| 5 | 10 | - | 2 | - | 88 | - | - | - | - |
| 6 | 10 | - | 2 | - | - | 88 | - | - | - |
| 7 | 10 | - | - | 1.5 | 88.5 | - | - | - | - |
| 8 | 10 | - | - | 1.5 | - | 88.5 | - | — | - |
| 9 | 10 | - | - | - | 78 | - | 2 | - | 10 |
| 10 | 10 | - | - | - | - | 78 | 2 | - | 10 |
| 11 | 20 | - | 3 | - | 77 | - | - | - | - |
| 12 | 10 | - | 2 | - | - | 86 | - | 2 | - |
| 13 | 10 | - | 2 | - | 86 | - | - | 2 | - |
| 14 | 20 | - | - | 1 | - | 76 | 1 | 2 | - |
| 15 | 10 | - | - | 1 | 82 | - | 2 | 5 | - |
| 16 | 10 | - | 2 | - | 82 | - | 1 | 5 | - |
| 17 | 30 | - | - | - | - | 67 | 1 | 2 | - |

## Preserving fluid formulae

**Series 8: Water soluble esters**

**Specification Sheet No.1**

| Expt. No. | GMA | GDA | EGMA | PGMA | EGDA | PGDA | DIST. WATER | SEA WATER | DOW A | PROG P | DOW 100 | Sod. Benz. | BSTN CD | PHEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | - | - | - | - | - | 77 | - | 1 | 2 | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | 77 | 1 | 2 | - | - | - | - |
| 3 | - | 20 | - | - | - | - | 77 | - | 1 | 2 | - | - | - | - |
| 4 | - | - | 10 | - | - | - | - | 87 | 1 | 2 | - | - | - | - |
| 5 | - | - | 10 | - | - | - | 83 | - | - | 2 | - | - | 5 | - |
| 6 | - | - | 10 | - | - | - | 79 | - | - | 1 | - | 10 | - | - |
| 7 | - | - | - | 10 | - | - | - | 83 | 1 | 1 | - | 5 | - | - |
| 8 | - | - | - | 20 | - | - | 70 | - | - | - | 10 | - | - | - |
| 9 | - | - | - | 20 | - | - | - | 70 | - | - | 10 | - | - | - |
| 10 | - | - | - | - | 10 | - | 78 | - | - | 2 | - | - | 10 | - |
| 11 | - | - | - | - | 10 | - | - | 78 | - | 2 | - | - | 10 | - |
| 12 | - | - | - | - | - | 10 | 88 | - | - | 2 | - | - | - | - |
| 13 | - | - | - | - | - | 20 | 77 | - | - | 1 | - | - | - | 2 |
| 14 | - | - | - | - | - | 20 | - | 77 | - | 1 | - | - | - | 2 |
| 15 | - | - | 20 | - | - | - | 77 | - | - | 1 | - | - | - | 2 |
| 16 | - | - | - | - | 20 | - | 77 | - | - | 1 | 2 | - | - | - |

## Preserving fluid formulae

**Series 9: Miscellaneous fixing and preserving fluids.**

Specification Sheet No.1

| Expt. No. | Formula | |
|---|---|---|
| 1 | Phenoxetol | 2.0 ml |
| | Propylene glycol | 10.0 ml |
| | Progallin P | 0.1 g |
| | Nipastat | 3.0 g |
| 2 | Propylene phenoxetol | 1.5 ml |
| | Propylene glycol | 10.0 ml |
| | Progallin P | 0.1 g |
| | Phenonip | 0.5 ml |
| | Water | 87.9 ml |
| 3 | Propylene phenoxetol | 1.5 ml |
| | Propylene glycol | 10.0 ml |
| | Progallin P | 0.1 g |
| | Water | 88.4 ml |
| 4 | Rasyntan GD | 10.0 g |
| | Propylene glycol | 20.0 ml |
| | Propylene phenoxetol | 1.0 ml |
| | Water | 69.0 ml |
| 5 | Dowicil 100 | 10.0 g |
| | Propylene glycol | 2.0 ml |
| | Methylcellulose | 0.1 g |
| | Water | 87.9 ml |
| 6 | Tannic acid | 5.0 g |
| | Propylene glycol | 15.0 ml |
| | Dowicide A | 1.0 g |
| | Sodium nitrate | 3.0 g |
| | Water | 76.0 ml |
| 7 | Sodium benzoate | 1.5 g |
| | Nipastat | 1.0 g |
| | Propylene glycol | 2.0 ml |
| | Water | 95.5 ml |

## Preserving fluid formulas

**Series 10: Miscellaneous fluids for jelly plankters.**

| Specification Sheet No. 1 | | |
|---|---|---|
| Expt. No. | Formula | |
| 1 | 40% formaldehyde | 2.5 ml |
| | Sea water | 97.5 ml |
| 2 | 40% formaldehyde | 2.5 ml |
| | Distilled water | 97.5 ml |
| 3 | 40% formaldehyde | 2.5 ml |
| | Sodium acetate | 5.0 g |
| | Sea water | 92.5 ml |
| 4 | 40% formaldehyde | 2.5 ml |
| | Sodium benzoate | 5.0 g |
| | Sea water | 92.5 ml |
| 5 | Glycerol | 10.0 ml |
| | Phenoxetol | 1.5 ml |
| | Distilled water | 88.5 ml |
| 6 | 50% isopropanol | 95.0 ml |
| | Sodium benzoate | 5.0 ml |
| 7 | 80% ethanol | 95.0 ml |
| | Sodium acetate | 5.0 ml |
| 8 | Dowicil 100 | 10.0 ml |
| | Sea water | 90.0 ml |
| 9 | Dowicil 100 | 10.0 ml |
| | Distilled water | 90.0 ml |
| 10 | Borax | 0.5 g |
| | 40% formaldehyde | 2.5 ml |
| | Sea water | 97.0 ml |

## Preserving fluid formulas

**Series 11: Methods for calcareous meroplankters.**

F = fixative; P = preservative; FP = both

Specification Sheet No.1

| Expt. No. | F, P FP | Formula | |
|---|---|---|---|
| 1 | FP | Pot. oxalate | 3.0 g |
| | | 40% formaldehyde | 10.0 ml |
| | | Distilled water | 87.0 ml |
| 2 | FP | Pot. oxalate | 3.0 g |
| | | 40% formaldehyde | 5.0 ml |
| | | Distilled water | 92.0 ml |
| 3 | FP | Pot. oxalate | 3.0 g |
| | | 40% formaldehyde | 2.5 ml |
| | | Distilled water | 94.5 ml |
| 4 | FP | Pot. oxalate | 5.0 g |
| | | 40% formaldehyde | 5.0 ml |
| | | Distilled water | 90.0 ml |
| 5 | FP | Pot. oxalate | 3.0 g |
| | | 40% formaldehyde | 5.0 ml |
| | | Sodium benzoate | 5.0 g |
| | | Distilled water | 87.0 ml |
| 6 | FP | 40% formaldehyde | 5.0 ml |
| | | Distilled water | 95.0 ml |
| 7 | FP | 40% formaldehyde | 2.5 ml |
| | | Distilled water | 97.5 ml |
| 8 | FP | 40% formaldehyde | 2.5 ml |
| | | Hexamine | 0.5 g |
| | | Sea water | 97.0 ml |
| 9 | P | Dowicil 100 | 5.0 g |
| | | Sod. acetate | 5.0 g |
| | | Sea water | 90.0 ml |
| 10 | FP | Cal. carbonate | 5.0 g |
| | | 40% formaldehyde | 2.5 ml |
| | | Sea water | 92.5 ml |

Preserving fluid formulae

Series 11: Methods for calcareous meroplankters (Contd.)

F = fixative; P = preservative; FP = both

| Specification Sheet No. 1 | | | |
|---|---|---|---|
| Expt. No. | F, P, FP | Formula | |
| 11 | FP | Borax<br>Pot. oxalate<br>Distilled water<br>40% formaldehyde | 2.0 g<br>5.0 g<br>91.5 ml<br>2.5 ml |
| 12 | P | 80% ethanol, aqueous | |
| 13 | P | 50% isopropanol, aqueous | |
| 14 | P | 90% ethanol aqueous | |
| 15 | P | Pot. oxalate<br>80% ethanol aqueous | 3.0 g<br>97.0 ml |
| 16 | P | Prop. phen.<br>Sea water | 1.0ml<br>99.0 ml |
| 17 | P | Prop. phen.<br>Glycerine<br>Tap water | 1.0 ml<br>5.0 ml<br>94.0 ml |
| 18 | P | Phenoxetol<br>Sea water | 1.0 ml<br>99.0 ml |
| 19 | FP | Pot. oxalate<br>Sodium tetraborate<br>40% formaldehyde<br>Distilled water | 3.0 g<br>2.0 g<br>2.5 ml<br>92.5 ml |
| 20 | FP | Sod. glycerophosphate<br>40% formaldehyde<br>Sea water | 0.5 g<br>2.5 ml<br>97.0 ml |
| 21 | P | 40% formaldehyde<br>Sod. bicarbonate<br>Sea water<br>Sugar | 10.0 ml<br>5.0 g<br>1000.0 cc<br>100.0 g |
| 22 | FP | 40% formaldehyde<br>Sod. glycerophosphate<br>Distilled water | 2.5 ml<br>0.5 g<br>97.0 ml |

1.3. Results and discussion.

1.3.1. Shipboard and curating techniques.

Though majority of methods on shipboard and curating techniques deal with collection of plankton samples as a whole, only a few methods have been developed for the collection of specific taxa. Barnes (1950) dealt with a note on the barnacle larvae of the Clyde Sea area as sampled by the Hardy continuous plankton recorder. Costello et al. (1957) has given methods for obtaining and handling marine eggs and embryos. Southward (1970) developed improved methods of sampling postlarval young fish and macroplankton. Tamura (1948) described the method of collecting the plankton while the boat is in motion. Cushing investigated the mesh selection factor for plaice larvae. Avoidance of plankton samples was another important problem in the collection of larvae. Criteria in recent taxonomic research have become more detailed and specific. So it is desirable that all features must be fixed and preserved satisfactorily.

The deterioration and the poor quality noted in the zooplankton collected from the tropical seas can be on various accounts and warrants several precautions to be taken. The points raised here are the outcome of observations made by the author during his participation in the regular oceanographic cruises during 1968-'78.

A number of specimens have been found dead and many others had appendages (especially those adapted for planktonic life) broken, before the haul is brought up. The death ratio and

breakage can be reduced with nets having a rigid cod end, towing for a minimum length of time, and by gentle wash down and removal from net. The net may be raised at a hauling speed of 45 metres/minute, thus giving a towing duration of about 2½ minutes for the 100 m water column over the plankton rich continental shelves. A towing speed of 0.5 to 1 knot brought better specimens than one collected in towing at 2 knots. Small hauls of short duration collected better quality specimens than big hauls of longer duration. The interaction between net and water which constituted filtration performance influenced the condition of the catch. The organisms filtered from the water accumulated upon the filtering surface. Damage to the organisms caught appeared to be caused by the pressure drop across the meshes (filtration pressure) which varied as the square of the approach velocity. Hence the condition of the catch can probably be improved by lowering the speed of tow, by reducing the mouth area of the net or by increasing the area of gauze. Frictional damages are caused to zooplankton during netting by clogging due to the smaller mesh size of gear. Specimens caught early during a tow will be damaged more than those caught late by abration and pressure exerted, by increased flow of water on using gear of larger mesh size. When collection of zooplankton in live conditions are desired, the bucket of the net must have a reasonably large volume of water - 500 ml or more - below the windows made of a metal gauze of the same mesh as the net. Buckets made of polyvinyl chloride or brass of light construction are preferred.

Depending on the size of meroplankton required nets of different meshes 150 - 500 micron must be used. Plankton nets made of nylon gauze Nytal 7P caused less damage than polyester estal mono P.E. nets, perlon monodur nets, silk nets and metal gauzes, all nets being made of monofilament, basket weave netting. Escapement behaviour exhibited by meroplankton during collection caused damages to spiny, squarish, or irregularly shaped meroplankton, particularly those covered with bristles or provided with long appendices or those with a coarse, rough or sticky surface. The streamlined, smooth or slimy organisms are not damaged in the nets. Meroplankton can be obtained in large numbers by sampling during the three-hour periods after sun rise and after sun set. Hosing down the sides of the net with a strong jet of water caused rupture of body and breakage of appendages.

During the transfer of plankton from the bucket to the fixative, exposure to air must be avoided to prevent desiccation and formation of artefacts. The high temperature of the atmosphere compared to the water from which samples were collected, initiates histolysis and promotes bacterial growth. Hence soon after collection is over, material was transferred to the temperature controlled room (20°C). Lecithotrophic larvae especially fish larvae with yolk sac deteriorated immediately after their death. As long as meroplankton could be kept alive fixation can be delayed. Soon after arrival on deck, the meroplankton taxa was sorted out at 20°C. Trapping of veliger larvae on coarse filters turned out to be injurious.

Hence they have to be handled carefully by means of a pipette.

On isolation of each meroplankton taxon, they required narcotisation to avoid the reaction to formaldehyde. Formaldehyde is unlikely to be replaced by other chemicals in the near future as a fixative to meroplankton in general. One part of concentrated formaldehyde should be added to nineteen parts of the sea water containing the meroplankton sample. It is essential that the stock formaldehyde be neutralised before use by addition of 2 percent sodium tetraborate or marble powder. With very small larvae lugol or osmic acid can be used. A gentle shaking to prevent formation of layers in the beginning ensured a good fixation. The formaldehyde assay experiments indicated that on an average a maximum of ten days, was required for complete fixation of meroplankton when formaldehyde was used. The final penetration of formaldehyde by plankton depended on the strength of formaldehyde used rather than on the time given for fixation. The reactive group in fixation being the protonated derivative + $CH_2OH$ which is an electrophile reacting at areas of high electron density, provided best fixation at a pH 6.0 to 7.0. So the acidity caused by cannizzaro reaction, sorensen reaction, oxidation to formic acid and its reaction with animal protein has to be removed using buffers and neutralisers. In specimens with high lipid content, use of a lipid solubiliser increased penetration rate. It is advisable to fill the containers completely to avoid sloshing while the ship rolls and pitches, and thus to avoid damage caused to specimens. Studies on

effect of variations in the volume of zooplankton to fixative/preservative showed a ratio of 1:9 to be fully satisfactory in tropics. A specimen condition more than 10 percent lead to deterioration. Faulty storage containers have to be avoided as it leads to evaporation of volatile constituents as water and formaldehyde, and concentration of non-volatile constituents as buffers and neutralising agents. Polyethylene containers with their walls permit gases such as oxygen to diffuse through the material and to oxidise the contained products. Thus, the use of thin polyethylene bottles as containers for plankton in formaldehyde has to be discouraged, because of the possibility that oxidation may be taking place with a consequent production of formic acid and lowering of pH. It is better to use previously numbered thick walled plastic bottles or translucent, strong relatively unbreakable and if possible impermeable styrene jars having phenolic cap with plastic coated liners with suitable labels. Of late since formaldehyde was found dissolving paraffins and softening agents from the polyethylene, use of amber coloured glass jars have been recommended. It is advisable to use vials that have screw-on lids made of hard plastic and provided with a liner. Soon after fixation for 10 days are over, the specimens can be transferred to specific preservatives found good for each zooplankton taxa. In the storage of specimens if formaldehyde is used as a preservative, at high temperatures cannizzaro reaction is increased and at low temperatures polymers are produced. Hence it is better to store them in a temperature controlled room around 20°C.

Alcohol-based preservatives often required additional protectio[n] from drying up. Storing the specimens in dark room helped to reduce photochemical reactions increasing colour preservatio[n]. Also formaldehyde preservatives when used required formaldehyde assay at intervals to maintain a one percent strength. Sample preservatives required replacements with fresh stock solutions rather than by the addition of additives, until the pH stabilis[ed]. In storage bottles containing meroplankton occasional gentle inversion of the jar is necessary to make sure that dispersal of preserving fluid takes place to avoid layering of preservative fluid.

### 1.3.2. Shrinkage, volume and weight loss.

Volumetric measurements made on 10 meroplankton samples before and 15 minutes after preservation, and at intervals of 1, 2, 5, 10, 15 and 25 days and 1, 3, 6, 12 and 24 months are given in Table I. On preservation all the 10 samples underwent immediate shrinkage ranging from 8 to 55 percent due to loss of water. The amount of volume shrinkahe undergone by the different groups are, crustacean larvae 8 - 17 percent, Coelenterate larvae 35 percent, sipunculoid larvae 40 percent, polychaete trochophores 33 percent and fish larvae and eggs 16 percent. But for crustacean larvae, other groups continued their volume loss in the first day. Most marked volume decrease on the first day was noticed for tornaria, echinoderm and sipunculoidea amounting to 70, 60 and 60 percent respectiv[ely]. In general loss on first day was significantly more than that o[f]

Table I

Measurements of volumes of 10 meroplankton samples to determine change in volume with time (in ml).

| Taxa | Before preser-vation | After preser-vation | Days after preservation | | | | | | Months after preservation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 5 | 10 | 15 | 25 | 1 | 3 | 6 | 12 | 24 |
| Larval Coelenterates | 8.4 | 6.3 | 3.8 | 3.7 | 3.6 | 3.5 | 3.4 | 3.3 | 3.3 | 3.2 | 3.2 | 3.2 | 3.2 |
| " | 5.7 | 4.7 | 3.0 | 2.7 | 2.5 | 2.4 | 2.4 | 2.3 | 2.3 | 2.3 | 2.2 | 2.0 | 2.0 |
| Trochophore larvae | 1.2 | 0.8 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Sipunculoidea " | 4.2 | 2.6 | 1.6 | 1.5 | 1.5 | 1.4 | 1.3 | 1.2 | 1.2 | 1.1 | 1.1 | 1.0 | 1.0 |
| Crustacean larvae | 4.6 | 3.8 | 3.8 | 3.7 | 3.7 | 3.7 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| " " | 6.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| " " | 6.5 | 6.0 | 6.0 | 5.7 | 5.6 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| Echinoderm " | 2.3 | 1.5 | 0.9 | 0.7 | 0.6 | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 | 0.3 | 0.3 |
| Tornaria " | 13.2 | 6.0 | 4.1 | 4.0 | 3.1 | 2.6 | 2.6 | 2.6 | 2.6 | 2.4 | 2.4 | 2.3 | 2.3 |
| Fish larvae and eggs | 5.0 | 4.2 | 4.0 | 3.9 | 3.7 | 3.6 | 3.5 | 3.5 | 3.4 | 3.3 | 3.2 | 3.2 | 3.2 |

subsequent days after preservation. While the volume of trochophore larvae got stabilised in one day, that of crustacean larvae took 15 days to attain stability. For the rest of meroplanktonic larval groups volume loss continued at a slow pace up to 1 2 months when they attained stability. The percentage loss in volume of meroplankton from the starting time up to 2 years is given in Table II at 5 time intervals. The table shows a maximum loss of 87 percent for echinoderm larvae whereas one set of crustacean larvae lost only 15 percent.

Table II

Percentage loss in plankton volume from the volume before preservation

| Taxa | Time interval | | | | |
|---|---|---|---|---|---|
| | 1 day | 10 days | 30 days | 1 year | 2 years |
| Larval Coelenterates | 55 | 60 | 63 | 62 | 62 |
| " | 50 | 60 | 60 | 65 | 65 |
| Trochophore larvae | 50 | 50 | 50 | 50 | 50 |
| Sipunculoidea " | 60 | 70 | 71 | 76 | 76 |
| Crustacean larvae | 17 | 20 | 24 | 24 | 24 |
| " " | 17 | 17 | 17 | 17 | 17 |
| " " | 8 | 15 | 15 | 15 | 15 |
| Echinoderm " | 60 | 80 | 82 | 87 | 87 |
| Tornaria " | 70 | 80 | 80 | 85 | 85 |
| Fish larvae and eggs | 20 | 28 | 32 | 36 | 36 |

From the above 2 tables it is clear that crustacean meroplankton attained equilibrium in 15 days' time and thus weight or volume change was at the minimum. The trochophore larvae too was not affected by the preservative in the long-run but for the initial loss. In all other samples the change after an year was considerably negligible. Meroplanktonic crustacea and fish larvae formed important constituents of the general zooplankton catch, as they were present in almost all samples. Fish eggs shrank only slightly. Fish larvae shrank on preservation, but equilibrium was attained in 6 months' time.

Tables III, IV and V show the amount of shrinkage undergone by three groups of larvae on preservation.

Table III

Shrinkage of leptocephalus larva in 2 percent formaldehyde sea water.

| Original length | Percentage reduction in standard length after | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | 2 days | 5 days | 10 days | 20 days | 30 days |
| 4 - 5 mm | 8.6 | 9.5 | 11.1 | 12.3 | 16.4 | 20.2 |
| 5 - 8 mm | 9.2 | 10.1 | 11.7 | 12.9 | 18.9 | 22.3 |
| 8 -10 mm | 9.8 | 11.1 | 12.3 | 14.0 | 18.2 | 21.1 |

In each length group the values obtained on 5 leptocephalus larvae was averaged and given in the above table. In 30 days time the larvae swell about 2-5 times their original volume like a white ball, which on slightly pricking got reduced to a thin transparent ribbon.

Table IV

Shrinkage of phyllosoma larvae in 2 percent formaldehyde sea water.

| Original length | Percentage reduction in standard length after | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | 2 days | 5 days | 10 days | 20 days | 30 days |
| 1 to 2 mm (average of 7 larvae 1.4mm) | 1.2 | 2.12 | 2.68 | 3.1 | 3.3 | 3.3 |

In the case of phyllosoma larva provided with an exo-skeleton shrinkage was far less (Table IV) compared to lepto-cephali. After 10 days more or less stable condition was attained.

Table V

Shrinkage of Arachnactis larva in 2 percent formaldehyde sea water.

| Original length | Percentage reduction in standard length after | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | 2 days | 5 days | 10 days | 20 days | 30 days |
| 5 to 8 mm (average length 6.2mm) | 16.5 | 17.8 | 18.5 | 22.6 | 25.8 | 30.2 |

The arachnactis larvae of cerianthid anthozoans underwent violent contraction by the shortening of longitudinal muscles. On the first day 16 percent contraction was noted which on the 30th day got increased to 30 percent (Table V). Thereafter almost a steady state was attained.

Meroplanktonic larvae have been found undergoing varying degrees of shrinkage in the fixing and preserving fluids, during the present study. According to the water content of meroplankton in their tissues, coelomic fluid and mesogloea, fixation brings about contraction of organisms, the high water content larvae loosing maximum body fluid. Ahlstrom and Thrailkill (1962) also observed a decrease in volume of plankton amounting to 13 to 85 percent of the live volume as a result of fixation and preservation in formaldehyde. The ten test samples attained stability within an year of collection. So for all practical purposes, preserved meroplankton volumes can be considered to become stabilised in one year's duration. The decrease in volume that occurred in the 10 test samples (Tables II and III) ranged from 15 to 87 percent of their live volumes. The amount of decrease in volume is related to the nature of specimens. It was least in crustacean and fish larvae and maximum in tornaria, sipunculoidea and echinoderm larvae. Ahlstrom and Thrailkill noted 13 percent shrinkage in crustacea and 80 percent shrinkage in jelly plankton after 22 months. So crustacean samples reached equilibrium soon. The study shows the importance of making reproducible, volume measurements of meroplankton only after one year. Wet volumes of meroplankton were found over-estimated due to included interstitial liquid, the average value of which varied in the present study from 25 to 45 percent of wet plankton volume. Physical and chemical analysis of fresh and fixed plankton by Rudge (1968), Lovegrove (1966) and Raymont et al. (1967) showed most plankters suffering

loss of both weight and volume after formaldehyde preservation. Major change in volume or weight is due to water loss which has the effect of concentrating organic constituents. But inorganic salts are lost lowering ash content. Glucose and glycogen also may be lost by plankton. Oil or fat globules are less fully retained and the extracted oil is seen as free-floating droplets. The above water loss may be due to osmotic imbalance between the original body fluids and the preserving fluids. Osmotic reactions have been studied by Maunsbach (1966) and Bone and Denton (1971). The body easily shrink when exposed to osmotic variation because there are spacious body cavities as in sipunculoids. The body of sipunculoids may be regarded as a muscular bag filled with fluid. As such a succession of workers (Quintin, 1900; Bekmaysm, 1921; Harms and Dragendorff, 1933; Adolph, 1936; Hallen, 1939 and Gross, 1954) have investigated osmotic changes in adult sipunculoids; and found that sipunculoids operate by hydrostatic pressure and they lack osmotic control. Since the coelomic fluid has about the same osmotic pressure as sea water, when the larva is placed in diluted sea water, takes in water and increases its weight, and looses water and decreases its weight in concentrated sea water. These changes continue until the coelomic fluid has become isotonic with the surrounding medium. If returned to the original medium, the original weight is regained. Such water exchanges are possible in a number of meroplanktonic larvae. Thus due to loss or gain of fluids, body wall ruptures leading to coming out of body contents. Hopkins (1968) found hexamine increasing weight of copepods, chaetognaths and

euphausiids by 10 to 25 percent of dry weight. This may be the factor responsible for rupture of their body wall. At a pH higher than 8.0 in alkaline condition protein dissolves and it comes out of the body leading to volume and weight loss. Solubility of different proteins depends on their isoelectric points. When ethylene glycol monoacetate, glycol monoacetate and magnesium chloride are used as an additive to formaldehyde solutions lateral shrinkage took place in most specimens in 2 years. Sodium acetate produced general shrinkage. Fish eggs developed signs of shrinkage in solutions containing glycol monoacetate and Dowicide A.

During the experiments it was possible to transfer specimens from a low to a higher osmotic pressure in fluids like ethylene glycol and formaldehyde without cracking, splitting or bursting, the reverse transfer proving destructive. Devi (1972) studying the shrinkage - percentage reduction in standard length of the material - in plankton samples, found shrinkage related to strength of formaldehyde used and the stage of maturity. The shrinkage was 5 times greater in early fish larvae, than in advanced forms. The length measurement during the present study also revealed mixed reactions of macroplankton to the 2 percent sea water formaldehyde solution. The forms with exoskeleton reacted entirely different from those with internal skeleton and without skeleton. The length increment noted by Farris (1963) in _Sardinops caerulea_ was not observed in the present series. This increment may be due to insufficient fixation. Devi (1972) also noted increments after preservation and attributed this be due to a change in body shape

caused by continuous dehydration, that reduced volume and at the same time caused stretching or elongation. Such lengthening was not observed during the present study. The percentage shrinkage of tuna fish larvae of standard length varied from 3 to 15 percent in 3 months time compared to 3.9 to 8.2 percent observed by Devi. It was evident from the present studies that volume determination of preserved material will be less than live volumes by a variable amount depending on constituent composition and the time interval intervening since preservation. The greatest amount of shrinkage took place in the first 30 minutes. Thus contraction was the first and almost invariable reaction of protein to fixation, its intensity depending on the water content of meroplankton and their age. Contraction of internal and external protein fibres of the meroplankton, while still under the influence of the living animal, provides a squeezing effect, and hyperosmotic pressures may exert a draining effect.

### 1.3.3. Colour preservation.

The vast and widely scattered literature referred revealed attempts to preserve the various organisms with their natural colours. The survey also revealed lack of data regarding colour preservation in plankton especially meroplankton. Many of these publications suffered from insufficient detail or lack of information regarding environmental data. The meroplankton samples held at the Indian Ocean Biological Centre was found completely bleached of their natural colour and pigmentation.

This bleaching effect was attributed to the various additives added, the preservatives used, and to the environmental factors. Hence an attempt was made during the present studies to preserve pigmentation of various kinds occurring in the meroplankton by using specific additives and maintaining constant environmental factors - pH, dissolved oxygen, acidity, light, temperature - of preservatives used.

<u>On pigmentation and their taxonomic importance.</u>

Majority of the meroplankton mainly in the upper layers are more transparent as it has a selective survival value among plankton - the more transparent they are, the less chance will there be of their being seen, seized and eaten. Depending on the filtration of light rays, the preponderance of blue in the upper illuminated zone and of red or dark colours in the shaded zone has survival value for this distribution. The newly hatched fish is discerned by spots of pigment on its body and the black pigment of its eyes. The melanistic pigments and their patches are intense in many fish larvae and these were found fading if allowed to remain in the light for a period of time. Ueyanagi (1966) and Matsumoto *et al.* (1972) showed the importance of red pigments in the identification of tuna larvae. Auxis larvae are distinguished by the presence of 2 or more rows of chromatophores (pigment spots) and choroid pigment of the eyes (jet black or very dark brown) are characteristic of leptocephali. Pigmentation has an important role in the identification of anchovy larvae. Silas (1974) indicated

presence of a post vent row of chromatophores along the ventral margin of the body, reaching up to the urostyle as a specific character of Rastrelliger larvae.

Meroplanktonic crustacea from higher latitudes and from the bathypelagic zone often contained large quantity of colourless, yellow or orange lipids. In the scyllarid lobsters, during development, the colour of eggs changes from orange to brown and shortly before hatching, the eggs are almost colourles In Veliger larvae the early intermediate stages were readily distinguishable by black pigment, patch between tentacles. The later swimming creeping stage has dark-red pigment patches in the distal portion of each lobe. Pigmented border in velum may be conspicuous. Later stages have darkly pigmented hind gut. Certain veligers have dark red pigmented border throughout larva life. Spionidae trochophore has black chromatophore (melanophor Trochophores of ampharetidae are red due to yolk, protodrillidae has orange pigmented spot, phyllodocidae has green pigment. Polychaete trochophore pigmentation is variable with a general tendency to increasing pigmentation with increasing age. Glandular pigments are lost completely in preservative. Among echinoderm larvae, Ophiopluteus [illegible] has arm tips with orange or red pigment, while Laganum species has yellow pigment.

Tornaria larvae have coloured body along with eye pigmentation. The pigmentiferous amoebocytes in the body of Actinotroc larvae are coloured as the orange, yellow and brown pigments of Actinotrocha branchiata, yellowish white colour of Actinotr pallida and the light brown pigment of Actinotrocha hippocrepi

The free swimming trochophore larvae of sipunculoid has a pair of red ocelli in the apical nervous thickening. Muller's larvae are brilliantly coloured. Very often after preservation these colours are either completely lost or faded and the state of preservation in the preservative fluids was judged from their body colours.

<u>Nature and cause of disappearance of pigment.</u>

Loosanoff and Davis (1963) found colours of the algal food greatly affecting the colour of bivalve larvae. By feeding larvae with differently coloured food organisms, it was possible, especially in the early straight-hinge stages, to change the colour of the soft body within hours. Temperature along with light affect the colouration of zooplankton. The beautifully coloured lipid present in crustacean larvae dissolved in the preservative fluid. Pigment spots (especially eye pigments) readily faded in fluids with hexamine, and borax which cannot be considered as ideal additives, for specimens in which pigment spots may be used in identification. The intense melanistic pigments of fishes were found fading, when kept in the light for a period of time. Hydrogen ion concentration, dissolved oxygen and formic acid in the solution were the internal factors causing discolouration. Oil oxidation in fishes also adds to deterioration of colour.

Fish larvae and other zooplankton preserved in formaldehyde lost colour in 3 to 30 days. But formaldehyde preservative containing the phenolic antioxidant butylated

hydroxyanisole (BHA) preserved the colour of macroplankton including fish larvae up to 1 year, whereas preservative fluid containing butylated hydroxytoluene (BHT) and Na-Erythorbate could maintain colour only up to 4 months and 1 month respectivel. The above antioxidants produced better colouration at 0.1 percent strength than 0.05 percent. Increase in strength above 0.1 did not improve the colouration. Macroplanktonic crustacea preserved in a 1:9 dilution of 2.2 ml BHT concentrate per litre of strong formaldehyde produced a supple and cleared condition. But Tayama and Miyoshi (1963) found fish larvae treated with BHT or Na-Erythorbate loosing colour in 30 days of preservation whereas BHT displayed original colour of prawns; while Na-Erythorbate did not show any effect. Fish larvae kept in 1 percent formaldehyde sea water of pH about 6.5 having 0.6 percent sodium ascorbate as an additive maintained natural colours for 6 months thereafter undergoing slight fading. Boiled sea water, due to exhaustion of oxygen when used as a diluent helped in prolonging colour preservation. Thus distilled water as a diluent also produced better results. Specimens held in polythene material bleached more than those in glass containers due to entry of oxygen through the porous polythene container. Other salts, reducing agents and antioxidants used for colour preservation proved harmful. Examination of specimens in the pH range 5.5, 6.0, 6.5, 7.5, 8.0 and 8.5 showed disappearance of eye pigments from specimens in the higher pH range of 8.0 and 8.5. Similarly a low pH also increased colour fading. When borax is used in sufficient quantity to produce a pH of

over 8.0 in preserving fluids, the majority of pigments in marine zooplankton become bleached or dissolved within 1 - 2 years. The final result is a white or bleached specimen. For colour preservation, therefore, borax is unsuitable. It was found that the eyes of meroplankton, which are normally black, lost their colour and changed over the course of 1 or 2 years to brown or pink. A definite bleaching of meroplanktonic crustacean eyes occurred with borax and hexamine. It was possible that the softening effects of a high pH lead to a partial break down of the proteins associated with eye pigments. The mero-plankton stored in 20°C had better colouration than those kept in room temperature of 28° to 32°C. Similarly meroplankton samples held in the dark room had their brilliant colours maintained over one year than those kept exposed to direct and indirect sunlight. Those kept in indirect light had better pigmentation than those in direct light.

1.3.4. <u>Taxa-wise studies.</u>

<u>Meroplanktonic Coelenterates</u>

<u>Taxonomic characters of preservative value:</u> The meroplanktonic coelenterates include mainly anthozoan larvae and the medusae of the hydromedusan type as Aglantha. Actiniarian larvae swim about in the plankton as tiny oval ciliated bodies, with or without an aboral tuft and often grooved along the septal attachments. They have a mouth, stomodaeum, siphonoglyphs, and 8 to 24 septa, but tentacles are absent. Mesenteries are

visible in transparent forms. The pelagic larvae of cerianthids known as Semper's larvae taken in the plankton frequently very large, with 12 septa, mouth and pharynx are of two types - the cerianthina larva, oval with a latitudinal girdle of particularly long cilia near the oral pole with up to 30 tentacles, and the cerianthella larva, elongated with a ventral longitudinal band of very long cilia without tentacles. A considerable number of genera of cerianthids have been known mainly from the pelagic meroplankton larvae as Arachnactis, Ovanthina, Anactinia, Apiactis, Cerianthula and Calpanthula. These are provided with 2 crowns of tentacles, with or without an anterior odd member, separated by peribuccal disc.

<u>Nature of deterioration noted in the IIOE collection:</u> The condition of the above mentioned gelatinous meroplankton, with high water content, sorted out from the mixed zooplankton was not found satisfactory due to the mechanical damage caused to these specimens by the sharp spicules of the crustacea and the spines of pteropod. By increasing, the proportion of the supernatant solution to sample and gentleness in handling, the damages can be reduced considerably. A proportion of the sample/supernatant in the ratio of 1:9 was essential in the container.

Often a region or the whole of their bodies did not represent any more the normal form and a portion of their internal organs were found to be projecting outside the actinopharynx. The respective dimensions of their sarcosepta

varied very much, not only from one larva to the other, but also from the left side as compared to the right, although on the whole, the two sides gave a fairly good idea of the relationship of height. In a large number of these larvae, the ectoderm had come off from the major portion of the surface of their bodies and their tentacles, either on rubbing on the wall of the collecting nets or by maceration, in the preservative fluid. A good number of acanthideans were shrunken and adpressed out of shape. The ciliary band was missing.

In the case of meroplanktonic medusae, the musculature and mesogloea decomposed leaving only a thin, collapsed, structureless epidermal envelope, occasionally the velum remained, the manubrium and tentacles were rarely seen.

<u>Narcotisation</u>: When good specimens are expected they are to be collected in low vessel speeds of 0.5 to 1 knot, sorted out from the bulk specimens and fixed separately. During fixation, 90 percent of ceriantharian larvae underwent a violent contraction at the moment of putting them in the fixing liquid. Hence the need was felt to narcotise these larvae for excellent preservation. Of the 32 narcotics tried (see pages 30 and 31) 0.3 percent tricaine methanesulfonate and 0.1 percent benzamine hydrochloride acted quickly compared to those already in use namely 3.5 percent magnesium chloride, in 20 percent magnesium sulphate and 0.002 percent propylene phenoxetol in sea water. Narcotising effects were easily felt with formaldehyde too in

about 6 hours by adding 40 percent formaldehyde into large jars containing the larvae, to make a final strength of 2 percent in sea water. The narcotics could help to avoid distortion of umbrella and manubrium, and contraction of tentacles, especially when minute structural details were essential. However Peterson (1976) could not gain any advantage by narcotising other coelenterates as medusae or siphonophore.

Fixation and preservation: Twenty six fixatives (see page 32) and preservatives (Series 1-10) were tried. Alcohol fixation of coelenterates lead to heavy contraction of specimens. Fixatives such as Bouin's fluid, and Carnoy's fluid also were not able to provide good fixation. The sea water collected off Cochin and the 40 percent formaldehyde purchased, having a pH of 8.0 and 3.5 respectively, when used to prepare a 2 percent formaldehyde fixing-cum-preservative fluid of pH 6.7, produced good fixation and preservation. But soon the pH fell considerably (pH 6.0), on addition of specimens. Hence various neutralisers and buffers had to be added for prolonged maintenance of pH around 7.0 with specimens. Depending on the pH variation from 6.0 to 8.5, a change in translucency of coelenterate zooplankton from opaque to clear appearance, was noticed. Thus as pH increased, gelatinisation increased finally leading to the break down of specimens. The various neutralising agents used with specimens were found to damage the tissues propotionate to their concentration. Russell (1948) found excess calcium carbonate being able to produce

a pH of 6.4. Calcium carbonate powder being insoluble has to be added only with stock solution (either 40 percent formaldehyde or diluted with sea water) since if added with specimens it obscured taxonomic details. Thus as it was absent in the specimen jars, lowering of pH could not be stopped. Hence occasional change of preservative was required. This insoluble neutralising agent at 5 percent strength produced a pH of 7.9 with 1 percent formaldehyde without specimens and with specimens a pH around 7.0 was maintained.

Silicates of calcium, magnesium and aluminium also acted similar to calcium carbonate. These being insoluble also covered specimens as fine deposits. Aluminium silicate at 5 percent strength when added to 2 percent formaldehyde sea water showed an initial pH of 7.8 which was reduced to 6.0 after 2 years. But formaldehyde at 1 percent strength with 5 percent silicate had an initial pH of 7.9 which was reduced to 6.2 after 2 years. Magnesium silicate at 5 percent strength when added to one percent formaldehyde sea water maintained a pH of 7.4 after 2 years from the initial pH of 7.9. Two percent formaldehyde in sea water, when neutralised with magnesium silicate had an initial pH of 8.0 which was reduced to 7.5 after 2 years. The initial pH of 8.9 produced by the addition of 5 percent calcium silicate to 1 percent formaldehyde sea water was reduced to 7.2 after 2 years, while that of 2 percent formaldehyde was reduced to 7.1 from the initial pH of 8.8. After 2 years all the above preservatives required replacement by decantation from the stock neutralised fluid.

Dissolved neutralising and buffering chemicals when used in large amounts were good as they prevented a fall in pH. Sodium acetate when used as neutralising agent at 5 percent strength with preservatives maintained a pH of 6.5 even after two years, compared to the initial pH of 7.5. Sodium bicarbonate as a neutralising agent had the disadvantage of carbon-dioxide production. Also the sodium hydrogen carbonate got converted as sodium carbonate which raised the initial pH of 7.5 to 9.0 leading to dissolution of protein. Even with slight evaporation, the pH tended to rise, when sodium bicarbonate, hexamine or borax was used as an additive in excess of 1 percent strength. Due to the cementing and bleaching action of borax present in the bottom of jar and disintegrating action of the mesogloea by hexamine, these nonvolatile reagents have to be avoided as buffers in the case of coelanterates. Borax considered to be the most suitable reagent for keeping formaldehyde solutions in sea water at pH 7.0 to 8.6 (with hexamine the second highest for general zooplankton) was found completely detrimental to macroplanktonic coelenterates. Borax dissolved in distilled water up to 6 percent, whereas it was only 2 percent in sea water. Soon this was precipitated and the excess amount settled on the macroplankton specimens as white granules. In strong formaldehyde borax dissolved up to 7 percent initially which got precipitated gradually, maintaining a pH between 8.0 and 8.4. Walker (1944) has cited borax as being suitable for preventing 40 percent formaldehyde from becoming acidic. Borax at 2 percent, in 2 percent formaldehyde sea water has a buffering range between 7.0 and 8.6. At 0.5

percent strength with specimens, buffering effect was very little. Excess borax means 1 to 2 percent of dissolved borax in the strong formaldehyde which when diluted becomes 0.1 to 0.2 percent. Borax acted as a macerating agent, in weak formaldehyde solution. Pigments were found getting bleached at a pH above 8.0 after one or two years.

Smith (1944) suggested use of hexamethylene tetramine as a biological buffer at 20 percent strength with 40 percent formaldehyde. Hexamine operated in three ways: as a mild base, as an anti-oxidant and by removal of acid in solution. Hydrolysis produced a pH of about 8.4, which is maintained in the presence of formaldehyde. The buffering property ranged from 6.2 to 6.9 depending on the amount of material used. At 2 percent it was between 6.4 and 5.5. With 2 percent hexamine in 4 percent formaldehyde distilled water the buffering range varied from 6.4 to 6.9. The 4 percent hexamine preserved specimens in general, become transparent, soft and swollen leading to disintegration. At 2 percent strength hexamine could not produce a pH above 7.0. Thus hexamine was found unfit as a buffer for coelenterate meroplankton.

Tris an organic buffer as it developed a high pH around 10.5 with a buffering action in the range of 7.8 to 9.5 was found unfit for meroplankton.

Sodium glycerophosphate was introduced as a buffer by Steedman (1976). This at 2, 1 and 0.5 percent strength maintained pH of 40 percent formaldehyde at 7.1, 6.7 and 6.3

respectively. On dilution with sea water however the buffering range was reduced to 5.5 and 6.8. The 1 percent formaldehyde sea water with meroplankton maintained a pH between 6.0 and 7.0 over an year when buffered with 0.5 percent sodium glycerophosphate. Also in the preservative fluids no precipitate was formed as was the case with other phosphates. The rest of additives used were found to be of no value in improving quality of preservation. The experimental series 5 and 6 proved Dowicil 100 at 10 percent strength dissolved in sea water to be a good substitute for 1 percent formaldehyde sea water. But considering its cost and availability formaldehyde was found better over Dowicil 100. The experimental series using hydroxy bearing chemicals, water soluble esters, miscellaneous fluids and fluids for jelly plankters could not provide a better fixation and preservation for the meroplanktonic coelenterates. Preserving formulae containing 1 percent propylene phenoxetol and those with additives as propylene glycol and phenoxetol showed better preservation, after formaldehyde fixation. The additives acted as bactericide, fungicide and antioxidant.

To sum up, of the various fixatives tried (see page 32), meroplanktonic coelenterates were best fixed in 2 percent formaldehyde in sea water, in 10 days. As a preservative, use of 1 percent formaldehyde in sea water either neutralised with excess calcium carbonate or 5 percent sodium acetate or buffered with 0.5 percent sodium glycerophosphate to maintain a pH around 7.0 was found good, provided a specimen to preservative ratio of 1:9 was kept. While 20 percent Dowicil

in distilled water or sea water proved as a good fixative, a 10 percent solution was enough as a preservative. One percent propylene phenoxetol in distilled water also preserved well the jelly forms. 4.5 percent propylene glycol and 0.5 percent phenoxetol if added to above preservatives maintained the specimen condition better. The specimens stored in a temperature controlled room at 20°C gave better results compared to room temperature (28°-32°C). Also specimens stored in a dark room either at 20°C or room temperature, compared to those exposed to light produced good results.

## Muller's larvae

Taxonomic features of preservative value: Polyclad turbellarians, lacking direct development, have a larval stage known as Muller's larva. This larva has a finely ciliated ovoid body and eight finger shaped ectodermal lappets, which are also ciliated. This larva about 0.4 mm in size possesses an apical tuft of cilia, eyes, frontal gland and a mid ventral mouth opening which leads into a muscular pharynx and the larval digestive cavity. In *Stylochus* sp. larvae are liberated with only 4 lobes, the 2 absent pairs being developed later. Such 4 lobed larvae are known as Goette's larvae.

Nature of deterioration: In view of their small size, Muller larvae were not sorted out from the plankton samples collect during the International Indian Ocean Expedition. However larvae collected subsequently and kept in 4 percent formald solution deteriorated. The brilliant colouration of these

larvae were entirely lost. The preoral strongly ciliated band was broken. The parenchyme which filled the ectodermal lobes oozed out in few and the rest became shrunken.

Narcotization: Of the 32 chemicals used, a few helped to prevent their contraction and distortion at fixation so that later they may be more readily identified in preserved condition. The narcotics were used only for a short while. Only quicker-acting narcotics were selected in view of the lipid content of larvae. Simple methods, of oxygen starvation, by replacing the natural sea water with the boiled and of carbondioxide excess by bubbling carbon dioxide, relaxed Muller' larvae in 10 minutes. Addition of ethanol and methyl alcohol drop by drop produced extension and death. Gohar's formaldehyde method also killed larvae in a relaxed manner. Most of the analgesics were found either ineffective or they produced harmful effects such as dissolution of lipid content.

On fixatives, preservatives and additives: The 26 fixatives mentioned on page 32 were tested. Fixation in acetaldehyde, acrolein, paraformaldehyde or glutaraldehyde did not produce better results than formaldehyde, and in view of their adverse effects, they are not recommended. Osmic acid, chromic acid, Bouin's fluid, and Carnoy's fluid though fixed the material well, in view of the contraction caused and the colour imparted to the larvae, their use as fixatives were ruled out. Larval fixation was excellent in 2 percent formaldehyde and 20 percent Dowicil 100, all diluted either with sea water or distilled water

Fixation was complete in 10 days. The above fixative in 4 percent strength was too strong as it caused contraction and 1 percent seemed rather insufficient. Fixative strength was found proportional to the cuticular coverings of the larvae. Lipid material present acted as a penetrant resistant. This could be rectified with the addition of a lipid solvent as propylene phenoxetol at 0.5 percent strength. In the preservative formulae, use of either sea water or distilled water, produced equally satisfactory results. Distilled water was disastrous only when the specimens were osmotically sensitive. One percent formaldehyde in sea water with an initial pH of 7.2 preserved the larvae in good condition. Neutralising agents were necessitated to prevent subsequent fall in pH. Addition of 5 percent sodium acetate maintained a pH between 6.8 and 7.4, for 2 years and was not harmful to Muller's larvae. Sodium acetate was frequently used in histology and pathology to keep formaldehyde solutions at a pH of 6.9 to 7.3. Without specimens sodium acetate maintained a pH of 7.6 for 6 months and with specimens pH never fell below 6.5 or seldom rose above 7.5. In 2 and 4 percent hexamine buffered and 1 and 2 percent borax buffered solutions, preservative qualities maintained were poor. Use of 5 percent calcium carbonate, calcium, magnesium and aluminium silicates with stock formaldehyde could not maintain pH indefinitely. The excess reagents settled on the minute larvae causing obstruction to taxonomic observation. Similarly use of ethylene glycol monoacetate, glycol monoacetate glycol formal and tris did not improve preservative qualities.

Sodium glycerophosphate when used in 0.5 percent strength produced good preservation, maintaining a pH between 6.0 and 7.0. Addition of 4.5 percent propylene glycol to the preservative formulae helped to reduce shrinkage. One percent propylene phenoxetol in sea/distilled water got the pH reduced to 5.5 from 6.5 in one year. With the addition of 0.5 percent sodium glycerophosphate, or 5 percent sodium acetate the pH was maintained above 6.5 with specimens. Specimen condition was good. Most of the preservatives mentioned in series 1-10 could not provide a better preservation. Not a single additive used could maintain the pH of preservative solutions at 7.0 or slightly over at the same time being a tissue preservative. Muller's larvae not exposed to light and varying room temperatures (28° - 32°C) exhibited better preserved conditions. Hence it is advisable to keep the preserved specimens in temperature controlled (20°C) dark rooms.

## Pilidium larvae

Taxonomic features of preservative value: Heteronemertines have characteristic pilidium or Desor's larvae of size 0.3 to 0.6 mm. Larvae are gelatinous, transparent and helmet shaped due to a down growth of 2 oral lateral lobes carrying with it a part of the ciliated band. The periphery of pilidium are covered by long cilia. They possess a thin ciliated band round the wide larval mouth, a stomach and an apical sense organ. Desor's larva is an oval ciliated postgastrula lacking oral lobes and ciliated oral band.

<u>Failure of determination</u>: In view of their small size these larvae were not sorted from the plankton samples collected during the International Indian Ocean Expedition. Pilidium larvae collected during the local cruises when preserved in hexamine buffered formaldehyde solution either became opaque or gelatinised. The ciliary bands were broken or separated. These gelatinous larvae suffered considerable volume and weight change in the fixatives and preservatives leading to oozing out of body contents. Certain larvae became so shrunken as to make their taxonomic features invisible.

<u>Narcotization</u>: Of the 32 narcotics mentioned on pages 30 and 31 majority were found unfit as they produced unfavourable effects. Use of ethanol 10 percent, methyl alcohol 10 percent, or tertiary amyl alcohol 8 percent as narcotic caused considerable shrinkage. Oxygen starvation, carbon dioxide excess, gentle heating and addition of carbonated water did not find success. Addition of 0.002 percent propylene phenoxetol and/or nembutal 0.08 percent produced good relaxation of larvae in 1-10 minutes. Tricaine methanesulfonate produced a cloudy suspension hindering observation of pilidium.

<u>On fixatives, preservatives and additives</u>: Of the 26 fixatives (see page 32) tried, ethanol 80 and 90 percent caused considerable shrinkage. Osmic and chromic acid imparted colouration. Trichloroacetic acid and P-toluenesulphonic acid 1 percent strength produced good fixation. Also 2 percent formaldehyde in sea water as a fixative precipitated protein constituents of

larvae in a way that retained the cell, its contents and the entire organisms in an essentially natural state. In view of the high water content of larvae sea water proved to be a better diluent than distilled water. Of the various preservative formulae (Series 1-10) a few provided best preservation. One percent propylene phenoxetol in distilled water with a pH 5.5 to 6.5 could preserve pilidium larvae without shrinkage, provided 5 percent propylene glycol was added. Hydroxyl bearing chemicals, water soluble esters and miscellaneous fluids were not favourable as preservatives. The universal preservative formaldehyde at 1 percent strength diluted with sea water having 4.5 percent propylene glycol and 0.5 percent phenoxetol as an additive produced excellent preservation. Bowicil 100 at 10 percent strength was in no way better than formaldehyde. In addition it imparted an yellowish colour to the specimens. At a pH higher than 7.5 the body protein got swelled, became more translucent and were in a process of gelatinisation and final break down. Volume loss was more due to the most watery nature. Comparative studies on pilidium larvae stored in dark room and those kept exposed to normal light showed better preservation in the former. Larvae exposed to ambient temperatures of room (28° - 32°C) revealed more deterioration than those kept at 20°C in a temperature controlled room.

## Meroplanktonic Polychaetes

This group includes larval stages of benthic polychaete species, the majority of which has a planktonic phase lasting for a few hours or days, but occasionally as long as several months as in *Poecilochaetus* sp. and swarming mature stages of benthic forms, such as many of the Nereidae and Syllidae. Usually they occur in shallow waters of neritic zone.

Characters of taxonomic value: The characters used for the identification of late larval and mature stages are division of body, form of prostomium, peristomium, tentacles, tentacular cirri, gills, proboscis with its jaws and denticles. Parapodia with their bristles and larval setae and ciliation are of high specific value. The larvae are elongated with a large number of segments and very variable external shape. The larval ciliation arranged in girdles called trochia consists of a proto, telo, gastro, noto and neurotroch ending in a ciliary pit. Body pigmentation is very important. Pigmentation is variable with a general tendency to increasing pigmentation with increasing age. The poecilochaetus larva has two pelagic stages - one, the metatrochophora attaining about 34-40 segments and two, the nectosoma stage growing over 10 mm.

Nature of deterioration noted in the IIOE collections: These larvae with low water content became soft, sticky and transparent due to deterioration of internal tissues, but due to a tough cuticle, the general shape and consistency was retained. The

body form and colouration were often changed. Very often the glandular pigmentation completely disappeared. The abundance of yolk present in lecithotrophic larvae was completely deteriorated. Very often the body segments were separated. The ciliation over the larval body was more or less lost. The larval muscular cells were deformed. In the mitraria larva (Wilson, 1932) the two tufts of long usually ear-shaped larval setae on either side of body degenerated.

Narcotization: Very often the preserved polychaetes were found fragmented. Mature syllids, nereids and larval spionids were found breaking into pieces during fixation. Hence a series of narcotics (see pages 30 and 31) were tried to induce relaxation so that "formalin frenzy" did not affect these polychaetes. The narcotic agents were chosen on the basis of their availability relative low cost and possible adaptation to meroplankton polychaetes. Sea water diluted with an equal amount of isotonic magnesium salt solution (7.5 percent magnesium chloride or 20 percent magnesium sulphate) induced complete relaxation of polychaetes on their immersion in it. Hannerz (1956) found cocaine to be the best anaesthetic for larval spionids. During this study procaine was tested for all meroplanktonic polychaetes and got excellent anaesthetic effect. Carbonated water when added in the ratio 1:10 to sea water containing specimens, relaxed them in few minutes. Nembutal 12 percent, tubo curaine 12 percent and ter-amyl alcohol 8 percent also were found as good narcotics, whereas chloretone, chloral hydrate, urethane etc. could not induce relaxation in the safe doses.

Relaxing effect was felt by the drop by drop addition of alcohol to the sea water containing specimens. Contraction of the specimens could be prevented by keeping them initially in 30 percent alcohol and latter immersing in 70-90 percent alcohol. Wilson (1932) avoided distortion of delicate larval stages by pipetting Bouin's fluid of pH 1.3 heated up to 60°C into a small volume of sea water containing the swimming larvae. After killing, the larvae were transferred to cold Bouin's fluid for 3 hours, thereafter transferring to alcohol after a wash.

On fixation, preservation and additives: For histological purposes Bouin, Brasil and Zenker's fluids were generally used. Permanent preparations were made by the addition of a drop of melted gelatin-glycerine and warming slightly after placing a cover slip. To prevent larvae from becoming transparent and to enable the fine structures discernible, those mounted in Canada balsam required colouration. Of the 26 fixatives tried (see page 32) formaldehyde was found quite detrimental to good preservation, as the specimens rapidly became soft, sticky and nearly useless. However, on initial fixation in 90 percent aqueous alcohol, the specimens were hardened completely and then could be removed to formaldehyde for preservation. Trochophore larvae can be best fixed in a mixture of one volume of sea water with an equal volume of osmium tetroxide.

Of the various preservatives shown in series 1-10, 70-80 percent alcohol provided good preservation. With alcohol, change of preservative was necessitated at times to ensure the

alcoholic content. The sticky nature of polychaete was avoided by maintaining a pH between 6.0 and 7.0. At this pH protein did not become soft and gelatinous. The material hardened during alcohol fixation was preserved in a better condition in 1 percent formaldehyde. Dilution of formaldehyde for preservation with sea water or distilled water did not make any difference. When 1 percent formaldehyde was used as a preservative, the buffer borax at 1 and 2 percent strength proved bad as it raised the pH above 7.0 (alkaline nature) making the protein soft. Similar deteriorating condition in the specimen was noted with 2 and 4 percent hexamine. The preservative fluids, made of stock formaldehyde neutralised with 5 percent insoluble aluminium, or calcium silicate or calcium carbonate maintained a pH between 6.0 and 7.5, with polychaete meroplankton. In these preservatives quality of preservation was better. One percent formaldehyde in sea water neutralised with 0.5 percent sodium glycerophosphate having a pH between 6.0 and 7.0 maintained the condition of the specimen better, than other neutralising and buffering chemicals tested in this series.

To conclude 90 percent aqueous ethanol provided good fixation of polychaetes after narcotisation. This also proved as the best preservative. Alternately one percent sea water formaldehyde buffered with 0.5 percent sodium glycerophosphate or neutralised with 5 percent calcium silicate could be opted for preservation. Effect of light and temperature on polychaete larvae were studied by maintaining identical collections

in dark and temperature controlled rooms. Specimens kept in dark rooms and those kept in temperature controlled rooms at 20°C maintained higher quality in preservation.

## Meroplanktonic Crustacean larvae

The meroplanktonic crustacea includes generally nauplius, metanauplius, protozoea, zoea and mysis stages of benthic and nektonic adult crustaceans. Among them are bivalved cypris and post-cypris stages of Cirripedia, alima larva of Stomatopoda zoea (larva) and postlarva of anomuran mole crabs, zoea (larva) and megalopa (postlarva) of Brachyuran crabs, leaf-like transparent phyllosoma (zoea/larva) and postlarva of scyllarides macrurans and larval stages of prawns and shrimps whose adults are not planktonic. Berried eggs hatch either into a nauplius as in penaeinae or into a zoea as in other groups.

<u>Taxonomic features of preservative value</u>: All decapods have a resilient, chemically resistant, non-calcified, chitinous exoskeleton. The systematic characters are drawn almost entirely from the features of this exoskeleton. Hence the value of such material from a taxonomic point of view should therefore be unaffected by the fixative and preservative used. The integument is usually thin and articulating surfaces especially in the limbs of preserved specimens are often fragile. Thus risk of breakage and subsequent loss of diagnostic structure is high. Specialised adaptations for planktonic life, such as eye, prolongation of limbs, setae and body out growths are

particularly vulnerable to damage during handling. Antennae or legs of zoeas of some families were found breaking by abrasion in the net during collection. The laterally compressed cypris larva has a bivalve shell, the two valves being fused along the mid-dorsal line.

<u>State of preservation of IIOE material</u>: Seventy five percent of plankton samples contained decapod larvae. Twenty percent of sorted specimens were well preserved and undamaged. Thirty five percent showed poorly preserved protoplasm, but their systematic value was not appreciably reduced. Twenty eight percent had systematic value reduced, usually by loss of appendages, but other specimens of same species being intact. Fifteen percent did not have any intact specimens of species, and had systematic value reduced, usually by loss of appendages. Severely damaged specimens of little value were only 2 percent. In all, half of the decapod larvae showed some degree of decay of the soft protoplasm, a few specimens having only the exoskeleton. The cypris larvae had the empty shells sometimes separated. The pleopods of megalopa and pereiopods of phyllosoma were damaged to various degrees.

<u>Narcotization</u>: During fixation crustacean zooplankton in general were found undergoing a violent locomotor activity prior to death often causing rejection of gut contents, spilling of eggs from brood pouches, or dropping of egg sacs. Elbourn (1966) suggested that a high percentage of empty guts in

*[illegible] [illegible]* was due to rejection of gut contents when preserved with formalin. Similar reaction termed as "formalin frenzy" was found to occur among the meroplanktonic crustacean too. Megalopas of some families of the Anomura (Galatheidae) and Brachyura (Portunidae) tended to autotomise their large chelae during the addition of formalin to sea water containing them. This necessitated use of an anaesthetising agent to circumvent unfavourable effects of formalin frenzy. Various techniques have been developed empirically for specific cases utilising oxygen starvation, carbon dioxide excess, gentle heating to 40°C or narcotising (see pages 30 and 31) agents. Though [illegible] (1963) had made comments on the ill-effects of propylene phenoxetol being used as a narcotic, with crustacean meroplankton this worked well. 0.002 percent of propylene phenoxetol was found sufficient in the present study. Carbonated water added to a sample in the ratio of 1:20 containing specimens was sufficient to narcotise larvae in minutes. Chloroform 15 drops/100 ml and methyl alcohol 10 percent also were found suitable to crustacean meroplankton. The above acted in 5 minutes reducing locomotor activity. The chemicals nembutal 12 percent, tubocuraine 12 percent, hydroxylamine 10 percent, tert-amyl alcohol 8 percent, chloral hydrate 16 crystal/100 ml and 1 percent butyn 10 percent though could relax the meroplankton, acted too slow with safe doses. Three percent hydrogen peroxide 10 percent, 1 percent physostigmine salicylate 8 percent, quinaldine in acetone and water 4 percent, methyl pentynol 15 drops/100 ml and tricaine methanesulfonate (M.S.222)

4 percent caused irregular locomotor activity and so were not good for crustacean larvae. Two percent chloral hydrate 10 percent, 1 percent chloretone 8 percent, 10 percent urethane 12 percent, 3 percent magnesium sulphate 10 percent and menthol crystals 16/100 ml were ineffective because locomotor activity were not slowed down in normal dosages. Thus of the varying narcotics used 0.002 percent phenoxetol and 5 percent carbonated water though found effective in general, variations were noticed with individual species.

Fixatives and preservatives with additives: Of the several fixatives (see page 32) and preservatives used (series 1-10) 2 percent sea water formaldehyde solution as a fixative and 1 percent sea water formaldehyde solution as a preservative was found fully satisfactory from the point of view of meroplanktonic crustacea, although this can cause considerable discomfort to the taxonomists. Many crustaceans retained flexibility of muscle which prevented limbs breaking off too easily when fixed in formaldehyde as it is non-coagulative. When chromic acid used as a fixative proteins were coagulated loosing flexibility of muscles. Other aldehydes as acrolin and acetaldehyde with their toxic properties, unpleasant odour, narcotic effect and high price were not found better than formaldehyde. A fixative solution containing 1 percent potassium hydroxide, 2.5 percent glycerine and 96.5 percent distilled water, when added in drops to a dish containing the larvae, killed them in 5 minutes in a well extended condition avoiding flexure. These larvae required

2 or 3 changes in the preservative fluid for removing traces of potassium hydroxide. As the pH was lowered, the protein contracted more leading to the brittle tendency of limbs ending in breakdown. pH was controlled by the amount of organic matter, and the mineral constituents as calcium phosphate and calcium carbonate in their integument. Thus pH values below 7.0 were noted with meroplanktonic crustacea in formaldehyde storage solutions, in which hexamine was present. At a higher pH a degree of softness and flexibility was incorporated and tissues got damaged at a pH above 8.0. Specimens preserved in fluids containing 4 percent hexamine became clear in 2 years and appendages started to break away. At 2 percent level hexamine failed to keep pH above 7.0. Borax buffered one percent formaldehyde with specimens maintained a pH of 7.0 to 8.6. In preservative fluids containing borax, it precipitated as white granules in or on crustacea, creating difficulties in identification. The tendency for 0.5 percent borax, particularly if used with formaldehyde distilled water is to produce an internal swelling of crustacea which makes them turgid. Hexamine and borax also bleached or whitened pigments, colour of black eyes changing to brown or pink. When 2 percent sodium glycerophosphate was added as a buffer to 1 percent formaldehyde sea water in the resulting pH of 7.0 to 7.1, specimens developed embrittlement of limbs due to retension of calcium within muscles or integument. But at 0.5 percent strength of sodium glycerophosphate having a pH of 6.0 to 7.0, the above brittleness was reduced considerably, especially with

the addition of 2 to 5 percent propylene glycol. Ethanol 60 percent in distilled water also proved to be a good preservative. These meroplanktonic larvae often having a soft flaccid integument, after fixing in a formaldehyde-sea water solution, on transfer to 70 percent ethanol provided good condition. This reduced handling difficulties by hardening tissues and also by increasing their rigidity. A few drops of glycol added to the ethanol helped preservation. Ethanol glycol 50 percent in strength was found a satisfactory preservative for majority of these larvae. Moreover, this was more pleasant and convenient in handling. In certain caridian larvae flexure of the abdomen and distortion of natural form resulted when preserved in alcohol or formaldehyde. This could be avoided by the addition of 10 percent glycerine to the 1 percent formaldehyde solution in distilled or sea water.

The large quantities of unsaturated fats present in the meroplankton very often leached out discolouring the preservative, as well reducing the pH. This could be highly reduced by increasing the volumetric ratio of meoplankton to preservative as 1:9. As long as the preservative fluid with the specimens showed pH instability, it was found better to change the fluid occasionally.

In short one percent formaldehyde in sea water buffered with 0.5 percent sodium glycerophosphate with additives like 0.5 percent propylene phenoxetol and 4.5 percent propylene glycol was found to be a good preservative for meroplanktonic crustacea. Maintenance of these larvae in dark room and at

a lower temperature of 20°C showed improvement in the condition of crustacean larvae.

## Sipunculoid larvae

Sipunculoids are exclusively marine, worm like animals, having a spiral type development, resulting in a trochophore type larva that metamorphoses into a young worm. The larva of Sipunculus sp. about 1 mm in length, swims around in the plankton for about a month. The larva being transparent, the well developed digestive tract can be seen from outside. A well developed metatroch and fat globules are present. Pelagosphaera larva obtained was a transparent spherical one about 6 mm in diameter with typical sipunculoid features.

Nature of deterioration noted in the larvae collected during IIOE: The sipunculoid features namely mouth at the anterior pole, recurved digestive tract with dorsal anus, ventral nerve cord, retractor muscles, and pair of nephridia had undergone partial to complete deterioration. Preservation had destroyed the cilia and metatroch. As these larvae lack osmotic control and they operate by hydrostatic pressure, in the fixative and preservative fluids, considerable volume loss or gain take place resulting in the rupture of body wall.

Narcotisation: The sipunculoid larvae after narcotisation could produce better specimen conditions. These larvae on transfer to a new media were found undergoing considerable volume regulation. As the coelomic fluid has about the same osmotic

pressure as sea water, the intact animal when placed in diluted sea water took in water and increased its weight, and lost water and decreased its weight in concentrated sea water. These changes continued until the coelomic fluid had become isotonic with the surrounding medium. Also a differential permeability of the body wall to water was noticed. Hence it was essential to allow sometime for the larvae to stabilise the water exchange. Use of a relaxant helped to complete this process undisturbed. Of the 32 chemicals (see pages 30 and 31) tried as narcotics and relaxants, 0.02 percent propylene phenoxetol in sea water proved to be the best anaesthetic, though it also took about one hour to relax. Best anaesthetic effect was produced as follows: Sipunculoid larvae were allowed in on site sea water to expand. Prepare a formaldehyde phenoxetol mixture in on site sea water in the ratio 2.5 : 1: 96.5 ml. Add 1 ml of above mixture to the container. Add 6 ml more at the rate of 1 ml per 30 minutes. After 10 hours the sipunculids become anaesthetised. There after remove them to 1 percent formal-dehyde diluted with sea water for fixation.

On fixation, preservation and additives: Any fixative solution having an osmotic pressure equal to that of its coelomic fluid could produce an excellent fixation. The coelomic fluid of larval body constitutes about 50 to 60 percent body volume and the water content varies from 85 to 89 percent of the wet weight. As such 2 percent formaldehyde in sea water proved to be the best fixative of the 26 solutions (see page 32) tested.

Bouicil 100 in sea water at 20 percent strength also fixed well. Of the various preservative formulae (Series 1-10), only few produced excellent preservation of sipunculoid characters. One percent formaldehyde and 10 percent Bouicil 100 in sea water with additives provided good preservation. Borax buffered stock 40 percent formaldehyde solution of pH 8.0 to 8.2, when diluted to 1 percent could maintain a pH of 7.0 to 8.6 suitable for sipunculid larvae. The pH of about 8.0 was maintained by the occasional addition of 0.2 g borax to 100 ml preservative. The pH of larval coelomic fluid being alkaline, having a pH of 7.6, only those additives which can maintain this level and a slightly higher osmotic pressure than sea water could be preferred. The ionic content of this larvae having more potassium than in sea water, necessitated addition of potassium ions in the preservative fluid. The addition of 5 percent sodium potassium tartrate served this purpose. Addition of potassium oxalate was deferred as it produced precipitate with sea water. Instead, if distilled water was used, then the ionic concentration of coelomic fluid could not be maintained The buffer tris though maintained a high pH was found detrimental to sipunculoids. In order to maintain the ionic concentration of preserving fluids equal to that of coelomic fluid, dilution of formaldehyde with distilled water or tap water was avoided. The condition of sipunculid larvae stored in dark room as well as in a temperature controlled room (20°C) was found to be superior to those kept in the normal conditions.

## Meroplanktonic molluscs

This group comprises shell-bearing forms such as lamellibranchiate postlarvae, gastropod veliger larvae and juvenile cephalopods. Typically, marine bivalves have two free-swimming larval stages, the trochophore and the veliger (similar to gastropod veliger, but symmetrical) having a larval period of up to 21 days. Most marine gastropods pass through the trochophore stage within the egg, and the free swimming larva is the veliger.

Nature of deterioration: Among the veligers, the velum with 2 to 6 lobes, the well developed foot with the operculum, the asymmetric larval shell and the pair of larval tentacles were found undergoing deterioration. In the juvenile cephalopod the yolk sac, the size of which varied in the different species was not fixed and preserved properly. The larval body endowed with shell thickening, large, sometimes pedunculated eyes, the tentacles and fins underwent several kinds of damages as loss of eyes, fins and tentacles separating, dissolution of shell and torn mantle. Pigmentation when present is lost completely. Balachandran (1973) has dealt with the nature of deterioration found in calcareous plankters. The sequence of dissolution of shells observed in these experiments were as follows: Glassy and transparent shells turned opaque, became chalky, flakey and fell apart, the flakes dissolving completely at a pH below 7.0. The sculptural details noted in the live shells were not distinct in the deteriorating samples. Deterioration was noted mainly in the first six months only.

Problems in fixation and preservation: In the shelled forms one has to consider the fixation and preservation of the soft parts as well as that of calcareous shell. Identification of bivalve postlarvae is dependent on the characters of the shell - their shape, hinge structure and dimensions (Rees, 1950; Loosanoff, Davis and Chanley, 1966) and consequently it is essential that they be preserved in good condition. The shell is composed of aragonite which is the most soluble form of calcium carbonate occurring in the molluscan shells. Break down or dissolution or structural modifications of these shells very often took place due to a low pH of the aqueous solutions of formaldehyde and other chemicals used for preservation. It was found equally true that when the pH rose above 8.5, calcareous plankters disintegrated because the protein which binds the calcium salts softened and swelled permitting the calcium salts to separate into small pieces and fall apart. The transparent veliger larvae have been identified by the pattern of locomotion, feeding behaviour and careful documentation of appearance using cine-microphotography. Here the problem of preservation is centred around the ciliated velum, shell and the visceral mass. Among cephalopod juveniles the yolk sac fixation and preservation required special methods.

On narcotisation: During the present study on fixation veliger larvae were found undergoing violent contraction and distortion. Hence invariably veligers have to be narcotised to fix in a fully relaxed condition. Thirty two chemicals (see pages 30 and 31) were tried during the study. The method reported by Leibowitz

(1976) was tried as follows and found successful. The gastropod veligers were exposed to solutions of increasing magnesium chloride concentration until a strength of 2.3 percent was reached, where they were mildly narcotised but had not begun to counteract. Then the concentration of propylene phenoxetol was increased in steps from zero to 0.25 percent by volume. The propylene phenoxetol acted as the final narcotic, while the magnesium chloride served to desensitise the veligers to the propylene phenoxetol. Depending on the developmental stages, veliger larvae were also narcotised using varying dilutions of magnesium chloride (40 - 180 mgm) alone and found successful without propylene phenoxetol. Similarly following Owen (1955) and Owen and Steedman (1956, 1958) propylene phenoxetol in concentrations of 0.01 to 0.025 was tried to narcotise veligers and bivalve larvae and obtained good results. In general use of the above two relaxants one after another produced better results than when used separately. Rosewater (1963) used 1 percent propylene phenoxetol as an anaesthetic for giant clams. Carriker (1963) has published notes on method for narcotisation, killing, fixation and preservation of marine organisms. A combination of nembutal (0.08 percent solution) and Tricane methanesulphonate (M.S. 222) (3 percent) tried during this study, also narcotised veligers and bivalve larvae in 3 to 5 minutes. Addition of a mixture of menthol and chloral hydrate in equal proportions (0.1 percent strong) when added to the dish containing larvae, produced complete narcotisation in 30 minutes. Clement and Cather (1957) used a mixture of one

part saturated aqueous solution of chloretone and two parts of sea water to narcotise veligers in 3 to 4 minutes. In the present study 1 percent chloretone in 8 percent strength was found to be an effective narcotic to veliger and bivalve larvae, requiring only 2 to 3 minutes. Thus a good number of narcotic agents listed on pages 30 and 31 were found effective with meroplanktonic molluscan larvae. However, narcotisation had no improvement in the preservative qualities of cephalopod juveniles.

<u>On fixatives, preservatives, and additives</u>: Of the 26 fixatives mentioned (see page 32) ethanol aqueous 80 percent, 2 percent formaldehyde in sea water or distilled water, and 20 percent Dowicil 100 in sea or distilled water produced excellent fixation criterion. Formaldehyde stock solution was neutralised with excess calcium carbonate. Three percent potassium oxalate was added when distilled water was used as a diluent. Rest of the fixatives was found ineffective on various grounds. Formaldehyde when used as a fixative or a preservative tended to oxidise to formic acid which dissolved calcareous structures within six months as a result of fall in pH of 1 percent formaldehyde sea water from 6.8 to 6.0. The only means of avoiding reaction with the shell carbonates was by adding a buffer which is dynamically more reactive than a carbonate, so that a pH of about 8.0 which is the level of normal sea water was maintained. So frequently 3 percent potassium oxalate was used since calcium oxalate produced then is more resistant to acid solution than calcium carbonate. Therefore in the presence of potassium

oxalate, the calcium carbonate found in calcareous meroplankton was not only less liable to dissolve but converted to calcium oxalate in the long run. Sea water was omitted from the formula where potassium oxalate was used due to the instant reaction of chloride, precipitating calcium oxalate in the fluid and on the specimens. This precipitation could be avoided by washing the specimens in distilled water. The results showed that one percent formaldehyde in distilled water with 3 percent potassium oxalate was the best preservative for bivalve larvae. However the stock formaldehyde has to be neutralised with 2 percent borax so as to keep the pH around 8.2. The pH of preservative fluids showed irregular fluctuations. The borax used maintained the pH around 8.0 by hydrolysis in the preservative fluid. But borax when added in excess, softening action of borax exceeded the preserving action of the preservative. Hence addition of borax at the rate of 0.1 percent must be done at intervals often checking the pH. Excess sodium tetraborate often settled on the specimens as white crystals. Also borax reacted with calcium producing less soluble calcium borate. One percent formaldehyde in sea water or distilled water with calcium carbonate added to saturation proved to be another less satisfactory preservative, as the additive settled on the shells obscuring taxonomic details and failed to maintain pH around 8.0

Carriker's solution (1950) - 10 cc 40 percent formaldehyde buffered with sodium bicarbonate to pH 10.0, 1000 cc sea water + 100 g sugar - was tried as a preservative (Series 11). Due to the considerable fall of pH to 6.5 in few weeks and as a

result of formation of precipitates, it was not found satisfactory.

A good molluscan preservative must be able to retain the sculpture, colour, and texture of shells and in veligers, flexibility of hinge ligament, prevent shell corrosion and formation of precipitates and preserve soft parts. Thus well preserved meroplanktonic shells had shiny, translucent shells retaining colour and hardness.

Hexamethylene tetramine used since 1944 was found unsatisfactory in the warm storage conditions, of the present study, as it lead to disintegration of calcified material at 0.5 percent strength in one percent formaldehyde sea water. The pH of such a preservative with specimens was found always less than 7.0. Hexamine at high concentrations disintegrated calcified material. Similarly sodium acetate, sodium bicarbonate and sodium benzoate all at 5 percent strength were found unsatisfactory. Due to the rising tendency for pH up to 9.0, solutions having sodium bicarbonate on its conversion to sodium carbonate, disintegrated the protein of shells. Sodium glycerophosphate 0.5 percent in one percent formaldehyde sea water or formaldehyde distilled wat[illegible] produced a pH of 7.0 to 7.2 on the addition of calcareous plankters. This preservative though it showed a pH above 7.0, calcareous plankters showed little sign of breakdown on long-term preservation.

The pH of storage fluid between 6.0 and 7.5 regarded as suitable for most taxonomic purposes, dissolved calcareous plankters. Thus use of 5 percent potassium oxalate with

1 percent formaldehyde distilled water or borax buffered 40 percent formaldehyde on dilution with distilled water to make 1 percent, gave better preservation than rest of the preservative formulae given in Series 1-11. Due to the high solubility of calcium carbonate in sea water, it was found better to use distilled water as a diluent. Occasional replacement of preservative during the first 6 months was found necessary to compensate sorensen's fall in pH.

A simple way of preservation of shell parts of meroplankton was tried by keeping them as dry specimens in tubes lined with cotton. They maintained excellent condition during the last 10 years.

Fixation in buffered 2 percent formaldehyde and subsequent transfer to 80 percent ethanol aqueous for preservation (Series 11) gave good results, inspite of the evaporation rates, price and poor solvent properties of sea water salts. As sea water produced flocculation and tap water haziness, distilled water should be used for dilution. In undiluted ethanol, specimens were found brittle. One of the advantages of alcohol is that it will shrink protein and hold calcareous components tightly. A specimen to preservative ratio of 1:9 was essential to reduce acid formation and to provide good preservation. Specimens kept in a temperature controlled room (20°C) and in a dark room had increased preservative qualities compared to those kept in ordinary room temperature and light. However shells were not affected by the ambient light and temperature of rooms.

## Cyphonautes larvae

**Taxonomic features:** The larvae of ctenostomata and cheilostomata of ectoprocta are known as cyphonautes. They lead a somewhat extended free life, up to 2 months. The larva has a triangular outline, strongly compressed laterally by the two movable chitinoid valves united by a strong adductor muscle. At the aboral pole there is a knob-like apical nervous organ provided with long bristles. The oral surface possess a ciliary girdle. Within the bivalved shell is a U-shaped digestive tract of pharynx, stomach and intestine and the pyriform organ. The planktotrophic larvae are characterised by the variations in the shape of shell (Ryland, 1964).

**Nature of deterioration in the larvae collected during the IIOE:** An examination of the larvae, revealed very often the presence of only the bivalved shell in the preservative fluid. The shells very often were in a state of decay. The 2 valves got separated as the adductor muscles binding them got dissolved. The internal organs when present were in a state of deteriorati

**Narcotisation:** The empty shells were the result of an initial unfavourable reaction to the formaldehyde during fixation and preservation. This reaction known as formalin "frenzy" caused violent activity of these larvae ending rejection of shell contents. Also due to sudden closure of the valves the fixati was not able to fix the internal organs, which in due course decayed completely. So in order to avoid the above unfavoural

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reaction, cyphonautes larvae were successfully anaesthetised before fixation. Of the 32 narcotics (see pages 30 and 31) utilised, one was deemed successful if it acted as a relaxer, gently decreasing movements of the organisms and relaxing them in short duration without any violent reaction. 0.005 percent phenoxetol added to sea water containing the larvae anaesthetised them in one hour. In this condition the larvae kept open their bivalved shell. Similarly a 0.1 percent mixture of chloral hydrate and menthol narcotised these larvae in 30 minutes Magnesium chloride 40 mg dissolved in 100 ml sea water left the cyphonautes larvae in a highly relaxed condition. Tertiary-amyl alcohol 8 percent, ethanol in drops or 1 percent butyn 10 percent on addition to sea water containing larvae caused closure of the valves. 0.08 percent nembutal, tubocuraine 12 percent and hydroxylamine 10 percent can be accepted with reservations, as they acted too slowly. In other words these could be effective only on the addition of too much narcotising agents.

<u>On fixatives, preservatives, and additives:</u> The cyphonautes larvae narcotised as above were fixed in the various fixatives given on page 32. Fixation in 2 percent formaldehyde in sea water for about 10 days gave good results. The stock formaldehyde was buffered with 2 percent borax to produce a pH around 8.1. On dilution it gave a pH between 7.0 and 8.4 depending on sea water and formaldehyde used. After fixation these larvae were transferred to various test preservatives mentioned in series 1 to 11. One percent solution of propylene phenoxetol in distilled water proved an efficient preservative of larvae.

The 2 valves appeared in satisfactory condition. The main advantage in the use of phenoxetol will obviously be the absence of irritating and unpleasant vapours, associated with formaldehyde. A further advantage noted in the present study was prevention of the formation of borax crystals which occurred in the formaldehyde preservatives. The shells were not affected by light and temperature fluctuations.

## *Actinotrocha larvae*

*Features of taxonomic interest:* These larvae leading a pelagic life of several weeks belonged to the exclusively marine lophophorates consisting of 15 species and were collected from the upper littoral zonal waters. The fully developed L-shaped larva had a characteristic appearance. The elongated enormous larvae varied in length from 1 to 5 mm with species. This had a large preoral lobe bent forward above the mouth like a hood. The tentacular girdle had 6 to 24 pairs of tentacles varying with species. A strongly ciliated telotroch was present and the larvae were completely ciliated. The digestive tract was tubular with a large stomach. Body was transparent with pigmentiferous amoebocytes.

*Nature of deterioration noted in the IIOE collection:* The orange, yellow and brown pigments of *Actinotrocha branchiata*, yellowish white colour of *Actinotrocha pallida* and the light brown and dark brown pigments of *Actinotrocha hippocrepia* have been completely bleached in the preservatives. The body

ciliation was more or less completely lost. The ciliated telotroch, tentacles, preoral lobe etc. were in a process of complete degeneration. In about 50 percent of larvae the characteristic L-shape was almost lost due to shrinkage and distortion.

__Narcotisation:__ The larvae were anaesthetised before fixation in order to have all morphological features well expanded and reduce the ill-effects of formalin frenzy. The 32 narcotic agents mentioned on pages 30 and 31 were tried to promote a relaxed death in the fixative. Immense variation existed between different species and size range in their acceptance of narcotics. Simple methods of oxygen starvation, carbondioxide excess, gentle heating met with failure. Addition of ethanol drop by drop did not produce extension and death. Gohar's (1937) method of formaldehyde addition, found successful with nudibranchs was modified and tried with actinotrochs. In this method larvae were allowed to expand in sea water to which a mixture of 1 percent phenoxetol in 1 percent formaldehyde was slowly added at the rate of 1 ml per 30 minutes. The larvae were anaesthetised in 1 to 2 hours. During this study addition of 1 drop of propylene phenoxetol alone to 100 ml sea water containing the larvae narcotised them in 3 to 6 hours. Rest of the chemicals could not be recommended either due to their slow action or ineffectiveness as a relaxant. Whenever a rapid relaxation in 10 minutes was desired a 4 percent tricaine methane-sulphonate solution could produce the result.

On fixatives, preservatives and additives: Actinotrocha larvae well expanded in the narcotic reagent were fixed in the differen fixatives mentioned on page 32 and their fixative qualities were observed for 10 days. The best fixation was provided by 2 percent formaldehyde in on site sea water. A vigorous shaking of the fixative fluid plus the specimens caused disintegration while a gentle movement improved distribution of fixative. Fixation in 20 percent Dowicil 100 in distilled or sea water and 2 percent formaldehyde in distilled water did not improve the fixative qualities than the above fixative. During fixation a specimen to fixative ratio of 1:9 was important. The pH of fixative around 6.0 did not affect the condition of larvae durin the period of fixation. Protein fixation with 80 percent ethano in distilled water caused shrinkage and volume loss of larvae. Trichloroacetic acid and p-tolune sulphonic acid 1 percent in sea water fixed actinotroch proteins, but had no advantage over that of formaldehyde fixative. Similarly chromic acid, 2 percent glytaraldehyde, 2 percent paraformaldehyde, Acrolein 2 percent, Acetaldehyde 2 percent, Bouin's fluid, and Carnoy's fluid failed to produce quality fixation of larvae.

Good quality specimens fixed in 2 percent formaldehyde in sea water, after 10 days, was transferred to the various test preservatives mentioned in series 1 to 10. Preservative quality was decided evaluating the various taxonomic criteria expected during identification. All the taxonomic features mentioned above was preserved satisfactorily in a number of

preservatives. The two percent borax buffered stock formaldehyde when diluted to 1 percent formaldehyde either with distilled or sea water imparted good preservation to larval actinotrocha. This maintained a pH between 7.0 and 8.6. Additive such as 5 percent ethylene glycol gave good results though at the end of 2 years the pH fell to 7.0 making the specimens more transparent. In hexamine buffered preservatives, larvae became softened and were more transparent. The pigmentation faded in all the preservatives. On neutralising the 1 percent formaldehyde preservative with insoluble excess calcium carbonate, calcium, magnesium and aluminium silicate, it preserved the larvae in good condition up to 2 years, though they showed a tendency to stick to fine cilia. But care has to be taken to use only the decanted fluid. A pH varying from 6.2 to 7.2 was maintained up to one year, thereafter requiring a replacement of preservative fluid. Ethylene glycol monoethylether acetate and glyceryl monoacetate as an additive softened the larvae in 6 months of preservation along with shrinkage. Addition of 5 percent magnesium chloride produced lateral shrinkage and transparency. Sodium acetate as an additive was of no use. Fifty percent ethylene glycol and 1 percent distilled water phenoxetol with a pH 6.0 to 6.5 preserve the larvae in good condition. Dark room conditions and lowered room temperature (20°C) increased specimen qualities.

## Brachiopod larvae

Taxonomic features: The brachiopod embryo, at the stage of three pairs of tentacles, becomes a free-swimming larva for about 2 months, with the main parts of the adult already defined. The relatively small body containing the digestive tract and bearing distally the lophophore is enclosed between the mantle lobes that are covered externally by the larval shell or protegulum. The characteristic inarticulate larvae with a bivalve shell enclosing a body bearing a bilateral lophophore with a limited number of ciliated tentacles are well known objects in the plankton. The planktotrophic larvae are characterised by the variations in the shape of the shell, discinid larvae possessing circular shaped valves and lingulids with oval or oblong shells.

Nature of deterioration noticed in the IIOE collections: To comment on the quality of preservation in general it can be said that the brachiopod larvae seems to have undergone moderate to extensive degeneration of soft internal tissues. Very often only the triangular compressed bivalved shell was present in the preservative fluid. Only about 25 percent had intact larval structures. In the inarticulates the two valves were separated due to swelling and dissolution of muscles holding the shells. The prismatic layer, periostracum (organic layer) and the calcareous layer were subject to varying degrees of dissolution in the acidic and alkaline fluids. The larval character namely principal setae were lost in nearly 50 percent of specimens.

<u>Narcotisation</u>: Brachioped larvae on the addition of fixatives suddenly closed their valves preventing fixation of internal body parts. Twenty five percent of larvae reacted violently to the fixative leading to ejection of internal organs. Hence 32 chemicals listed on pages 30 and 31 were tested as anaesthetic relaxant and narcotic agents. Selection of a particular chemic was done based on the behavioural response of brachioped larvae to these agents. Propylene phenoxetol 0.005 percent dropped in sea water containing the larvae was the most acceptable relaxing agent. This acted very slowly as it took time to diffuse throughout the total volume. Thus it required up to 12 to 24 hours for complete relaxation. This time could be reduced to 2 hours using stronger solutions (0.01 percent). Menthol and chloral hydrate mixture (0.1 percent) put forward by Gray (1953) for coelenterates and supported by Van Eeden (1958) for limnic gastropods acted well with brachiopoda. Complete narcotisation followed in 6 to 12 hours. Nembutal and M.S. 222 reagents used by Joose and Lever (1959), Lever et al. (1964), McGowan and Baugh (1964) and Randall (1962) produced faster narcotisation when used together. Addition of 0.08 percent nembutal in the ratio 1:8 followed by 0.3 percent M.S. 222 in the ratio 1:15000 produced narcotisation in 10 to 20 minutes. Rest of the chemicals were found unsatisfactory as few had no narcotic effect on these larvae and others were required in large amounts based on observation of locomotor activities.

<u>On fixatives, preservatives and additives:</u> The inarticulate families Lingulacea and Discinacea except Craniacea have shells with calcium phosphate as their mineral constituent, whereas in the articulate larvae the shell has calcium carbonate in the form of calcite as mineral constituent. Also the phosphatic inarticulate shells have a large amount of (25-40 percent) organic matter in the form of chitin, while that of articulate larval shells have only a low (1-5 percent) organic matter in the form of chitin. Hence in choosing a good fixative and preservative fluid for the inarticulate larvae, care was taken to avoid chemicals dissolving phosphate and chitin. Of the various fixatives listed on page 32, Bouin's fluid, Carnoy's fluid and chromic acid hardened the tissues excessively though rapid fixation took place. In addition due to their pungent odour and corrosive action they were found unfit for fixation. Two percent formaldehyde solution in sea water prepared by dilution of stock formaldehyde buffered with 2 percent borax, having a pH 7.0 to 8.6 proved to be a good fixative. Though alcohol fixation is unsatisfactory histologically, 80 percent aqueous ethanol produced good fixation morphologically. The formaldehyde donor Dowicil 100, 20 percent in sea water, buffered with borax and having a pH of 7.0 to 8.4 also worked as a good fixative.

Rest of the fixatives as acrolein and glutaraldehyde in view of varying drawbacks are not recommended here.

In practice it was found better to use a separate fluid as a preservative, though this had the objection of being more expensive. In this connection several preservative formulae dealt with in series 1 to 11 were tested. But for the disadvantages such as cost and high evaporation rate, 80 percent aqueous ethanol preserved well the larval specimens. Based on the advantages Marr (1963) recommended initial fixation in formaldehyde and then transfer to ethanol, for a better looking specimen. Sea water was unsatisfactory as a diluent for strong alcohol because its dissolved salts were thrown out of solution and got deposited on the larvae in such quantity as to obscure some of the minutia required for taxonomic purposes. For preservation of calcareous material a high pH above 7.5 was necessary. This high pH of 7.5 to 8.0 was produced using potassium metaborate 0.1 percent or potassium tetraborate 0.3 percent with 80 percent ethanol aqueous. Potassium salts were preferred as they had more solubility than sodium salts in water. In the case of potassium metaborate decanted solution was used as the excess settled at the bottom. In alcohol preservative without alkalising agents, shell became translucent to opaque and also more brittle. Two percent borax buffered 1 percent phenoxetol in distilled water gave excellent preservation to brachiopod larvae as it maintained a pH above 7.0. Also 1 percent phenoxetol in sea water buffered with 2 percent borax produced a pH above 7.5 and preserved well brachiopod shells. Preservative formulae in series 11 containing 4.5 percent propylene glycol as additive, kept the shelled larvae with

improved flexibility and less brittleness. Storing of larval shells either in the dark room or in the low temperature room of 20°C could not add their preservative qualities.

## Echinoderm larvae

Exclusively marine and largely pentaradiate bottom dwellers, the echinodermata comprises about 5400 species. Most of these release their ripe gametes into the surrounding water, where fertilisation occurs and where the bilaterally organised planktonic larvae develop until they become more radially organised to metamorphose within a month. These larvae collected with the general plankton are the following: Bipinnaria and brachiolaria of Asteroidea; bipinnaria, pluteus and ophiopluteus of Ophiuroidea; Bipinnaria, pluteus and echinopluteus of Echinoidea; auricularia and doliolaria of Holothuroidea and doliolaria larva of Crinoidea and crinoid larva of Antedon. These larvae are very delicate in construction, body being covered and protected by a thick girdle of cilia broken up into a number of arms supported by calcareous rods.

Nature of deterioration noted in the IIOE collections: In order to identify these larval forms, a high degree of quality preservation is necessary. These larvae with bristled arms sorted out from the zooplankton samples collected during IIOE and held at the Indian Ocean Biological Centre were in a highly deteriorating stage, unable to make any taxonomic studies. The ciliated

arms drawn out into long processes, girdles and arms were broken or disintegrated, the calcareous rods supporting the arms dissolved. As regards auricularia only indistinct masses and ribbon like fragments were seen. In about 30 percent of samples no identifiable material was present. The sharp spicules of crustacea and spines of pteropods produced cuts on these larvae eventually leading to disintegration of specimens. The transparent larvae after death changed very rapidly becoming opalescent and then opaque.

<u>Narcotisation</u>: These delicate larvae when fixed in 2 percent formaldehyde solution underwent violent contractions on account of an initial reaction due to this universal fixative. In order to circumvent unfavourable effects of formalin frenzy it would be desirable to find a substance that would effectively narcotise echinoderm larvae. Of a number of chemicals utilised as relaxants (see pages 30 and 31) a few were found suitable. Cocaine and procaine anaesthetised 80 percent larvae. 0.002 percent propylene phenoxetol narcotised them in 30 minutes. Carbonated water in the ratio 1:20, chloroform at the rate of 15 drops/100 ml, nembutal 12 percent and chloral hydrate 16 crystals/100 ml made the larvae immovable within 5 minutes. Chloretone 8 percent, urethane 12 percent and tubocuraine 12 percent were ineffective narcotics as swimming behaviour continued. Giant sized (15 mm) auriculatia larva however required a greater concentration of the relaxant. To summarise the amou of narcotic agents used depended on the size of larvae and numb of larvae experimented. In general a volume of 1 ml per larva was required.

On fixatives, preservatives and additives: A comparison of the state of fixation of these transparent creatures of pelagic habitat fixed in different fixatives (see page 32) showed that most satisfactory fixation was with 2 percent formaldehyde in sea water. Giant sized auricularia larvae up to 15 mm showed better fixation with 1 percent trichloroacetic acid or 1 percent p-toluene sulphonic acid. Within 30 minutes their transparent appearance slightly changed to translucency. However they remained whole without showing any sign of shredding. In these cases use of a narcotic was effective as death occurred instantly. These auricularia larvae have to be transferred soon to a preservative solution. Bouin's fluid, osmic acid and chromic acid fixation were avoided due to the yellow to dark-brown colouration imparted to the specimens. A major problem with these larvae was the steady and slow loss of volume. The pH of fixatives and preservatives would appear to play a minor role in the preservation of soft bodied echinoderm larvae, in case extremes were avoided. Of the various preservatives with proportional variations investigated (Series 1-10) a few were proved to be most satisfactory. The solutions which preserved larvae in good condition contained calcium carbonate, calcium silicate and aluminium silicate as additives. These insoluble chemicals were added with stock strong formaldehyde and only decanted solutions were used for dilution purposes. Also borax buffered formaldehyde when diluted to 1 percent with sea water preserved specimens well. A limited amount of borax should therefore be accepted as a very effective additive when

echinoderm larvae were preserved which could maintain a pH around 7.6 to 8.5 up to 2 years. At the same time, hexamine at 1 percent level caused disintegration of larvae. A well preserved auricularia larva of size 0.5 to 1 mm had the continuous flagellated band arranged in good condition. Also the curved digestive tract with its sacciform stomach and 3 coelomic sacs were well preserved. In the case of giant auricularia the excessively frilly flagellated band was intact. In asteroidea larvae the ciliary band, the projections, the digestive tract and the coelomic compartments were in good shape. The tiny early echinopluteus larva possessed four arms supported by skeletal rods, as is the case with the 3 brachiolar arms of the brachiolaria larva. Here preservation of skeletal rods was of great importance. Ophiopluteus larvae retained the ciliated bands accompanying outgrowths and edges. Preservation at 20°C in a dark room improved specimen conditions.

## Tornaria larvae

Features of taxonomic importance: The characteristic swimming larva of Enteropneusta known as tornaria, having no resemblance to the adult is uniformly ciliated initially which later developed to sinuous ciliary bands. In the genus *Ptychodera* the larva developed a row of small tentacles and hence known as tentaculate type. The larvae belonging to 70 species are planktonic for about 3 to 4 weeks and ranged in size from 1 to 9 in height. The translucent earlier larva becomes opaque at later stages. *Planktosphaera pelagica* a transparent spherical

larva about 10 mm in diameter is of an unknown type of hemi-chordate. The interior is filled with a glassy jelly. Viscera occupied about one-fourth of the interior. Over the surface is a very sinuous and highly branched ciliary band.

<u>Nature of deterioration noticed in the 1101 samples:</u> Seventy five percent of the helmet shaped or ovoid larvae kept preserved in hexamine buffered 4 percent formaldehyde sea water solution were found broken into pieces. The eye pigmentation was completely bleached or changed its original colour. The coelomic pouches and the complete digestive tract with curved intestine (present in live larvae) were disintegrated in 50 percent of larvae. To a certain extent damage was caused by the crustacean spicules and pteropod spines piercing their body while they were in the mixed plankton catch. These cuts hastened in oozing out of body protein. Planktosphaera larva was found swollen like a ball which on agitation disintegrated.

<u>Narcotization:</u> The tornaria larvae reacted adversely to formaldehyde when added as fixative. The violent locomotor action exhibited, caused damages to the sinuous ciliary bands. Hence the 32 narcotics listed on pages 30 and 31 were tried as narcotising agents, to alleviate the above problems. Majority caused unfavourable erratic movements before the commencement of narcotic effect. A few narcotics were ineffective on the larvae tested. One percent propylene phenoxetol added to sea water containing the tornaria larvae relaxed them within 2 hours. A few drops of 95 percent alcohol added to the container hastened

diffusion of phenoxetol. Anaesthetisation, by cocaine or procaine was effective with tentaculate larvae in keeping their tentacles extended. Three percent hydrogen peroxide on addition to sea water containing larvae in the ratio 1:8 required only 2 to 4 minutes to anaesthetise. Quinaldine and methyl pentynol were not acceptable as they caused initial erratic swimming movements.

<u>On fixatives, preservatives and additives</u>: From the nature of deterioration noted it appeared that the tornarian protein was not truly fixed in the usual formaldehyde fixative. So it imbibed fluid from the fixative and preservative solution due to the high internal osmotic pressure in such a way that the swolle larval body got burst. Tornaria had a low specific gravity of 1.035 (higher than sea water) due to a large empty blastocoel. For taxonomic purposes a medium stiff with irreversible conditio of the specimen proteins had to be produced. To serve this purpose a special fixative consisting of 2 percent sea water formaldehyde and 0.5 percent aluminium potassium sulphate was used. Alum on dissolution in the fixative produced a pH of 4.0. This fixative theoretically seemed suitable for tornaria fixatio as it could produce excellent specimen condition. Also a 10 to 15 percent mixture of ethylene glycol with formalin acetic alcoh was fully satisfactory fixative for tornaria. Due to the highe percent of water present in tornaria, fixation and preservation in formaldehyde solutions lead to contraction which could be observed within few minutes after fixation and this continued at a steady rate for hours and days until the final volume contracti

reached a stable state. All the water soluble materials unaffected by formaldehyde might dissolve into the surrounding fluid. Dowicil 100 at 20 percent strength when replaced by 2 percent formaldehyde, also gave similar results. Fixation in other fixatives such as 1 percent trichloroacetic acid, p-toluenesulphonic acid 1 percent, chromic acid, Bouin's fluid, Carnoy's fluid etc. were found effective to a certain degree only. Ethanol produced too much brittleness as well as shrinkage due to dehydration for the larvae. In fixatives using solutions of osmic acid and chromic acid, specimens developed an yellow to dark brown discolouration. The above fixatives caused disintegration of specimens as a result of steady slow loss of water sooner or later. During fixation addition of larvae to the fixative gave better results than the reverse.

The larvae fixed as above in good condition were transferred to the various test preservatives given in series 1 to 10. Tornaria having body fluid of pH 6.5 was best preserved in that pH. One percent formaldehyde in distilled water with a pH of 6.4 and 1 percent formaldehyde sea water solution with a pH of 6.8 maintained a pH of 6.0 on addition of larvae and found suitable for storage purposes. The formaldehyde donor Dowicil 100, 10 percent in sea water or distilled water preserved the tornaria well. Polymers of formaldehyde failed as preservatives. As a low pH less than 7.0 was satisfactory, preservative fluids with additives were of little use. Anyhow addition of 5 percent ethylene glycol prevented larvae from becoming brittle in formaldehyde.

Tornaria preserved in 80 percent ethanol aqueous underwent about 50 percent shrinkage. So ethanol preservation could not be recommended. Ethylene glycol having the advantage of less violent osmotic reactions was used for preserving tornaria larvae. Even after 3-5 years the larvae were present without any change in gross morphology. Also tornaria showed very little wrinkling and hardening which usually might accompany their storage in alcoholic media. The volume loss due to evaporation was prevented with pure ethylene glycol. Propylene phenoxetol one percent in distilled water as a preservative for tornaria could keep well fixed material in good condition for the last 10 years having a pH in the range of 6.0 to 6.5. The above fluid with propylene glycol improved preservative qualities. Most of the preservative formulae mentioned in series 1 - 10 failed to preserve tornaria. These preserved larvae kept in a dark room and in a temperature controlled room (20°C) appeared superior to those kept in ambient light and temperature conditions.

## Fish larvae

**Features of taxonomic interest:** Primary requirements for identification of larvae are meristic (numerable) characters, such as number of vertebrae corresponding to number of myomeres, position and number of fin rays and spines (all fins), position of fins particularly of dorsal and pelvics, and number of gill rakers. The sequence of fin development is often important. Some kinds of larvae possess distinctive larval characters such as stalked eyes, trailing guts, elongated fin rays, elaborate

head spines, and opercular spines. However majority do not possess distinctive specialised structures. Fish larvae with embryonal fin folds (without fin-rays) and finned larvae (having fin--rays) were studied together. Delay in the develop ment of adult form is a common feature of teleosts. Some larvae carry a small to a large yolk sac. The tuna larvae are characteristic by their red pigments.

Nature of deterioration noticed in the IIOE fish larvae: Ichthyo plankton material collected during the IIOE and kept at the Indian Ocean Biological Centre, belonging to about 50 families revealed different stages of preservation ranging in condition from good to very poor. Some samples ranging from passable to very poor condition contained only remnants of larvae, their melanin pigments either lost or faded to brown or pale yellow and having white spots sedimented on the surface of their bodies. Damages to specimens were of several kinds. On some (thin elongate larvae of clupeids, engraulids etc.) eyes were lost and intestine ripped. Some specimens were badly deteriorating myomeres separating and swollen, flesh being separated from skeletal parts, occasionally only skeletal parts remaining. The extent of damage was proportional to the different stages of development of fish larvae, the larvae with yolk sac being more fragile than others which were stubby and compact. Considerable shrinkage of fish larvae was noted in the preservative fluids due to dehydration and protein solubilisation.

Narcotisation: A detailed discussion on the use of general anaesthetics for fish has been summarised by Bell (1964). There in, he had dealt with the characteristics and uses of eleven general anaesthetics, so as to permit ready selection of the most appropriate anaesthetic for laboratory or field work with reference to large fishes. Hence in the present series of experiments dealing with larval fishes the dosage - response data was reanalysed. These anaesthetic chemicals were found useful during transportation of fish, mass killing, for general handling during tagging, photography, operation, bleeding etc. As the primary requirement for identification of fish larvae is meristic, anaesthetisation before fixation helped to fix the meristic characters as fin rays stretched. Of the 32 chemicals listed on pages 30 and 31, a number of them were found suitable for larval fishes. Bagenal (1963) used propylene phenoxetol solution weaker than 0.025 percent to anaesthetise plaice in the size range 16 - 33 cm, up to 1 hour. In the present studies 0.005 percent phenoxetol made the plaice larvae limp in 15 minutes. Tertiary amyl alcohol of 8 percent concentration immobilised fish larvae in 5 minutes. Chloral hydrate crystals when added at the rate of 16 crystals per 100 ml sea water containing larvae immobilised them in 3 minutes. Similarly 10 percent urethane at 12 percent strength, 1 percent chloretone in the ratio 1:2500, 1 percent ether, 0.3 percent tricaine methanesulphonate, 4 percent quinaldine acetone water mixture in the ratio 1:16:32 and tribromoethanol 5 ppm immobilised fish larvae in less than 5 minutes. The above narcotics

are found successful based on the results obtained by the empirical investigation made during the present study. The problem faced in using the narcotic reagents with fish larvae was due to their lipid solvent property and presence of yolk sac in many fish larvae. Hence it was important to keep the fish larvae in the fluids for the minimum time required.

<u>On fixatives, preservatives and additives</u>: Of the various fixatives tested (see page 32) 2 percent formaldehyde was found sufficient to fix the larvae. Fixatives stronger than 2 percen formaldehyde cause damages to larvae. Twenty percent Dowicil 100 also proved to be good fixative. The fixatives required 10 days duration for complete fixation.

External factors such as temperature and light were found responsible for the discolouration of fish larvae. Hydrogen ion concentration, dissolved oxygen and formic acid in the solution were the internal factors causing discolouration. Yet, use of neutralised 1 to 2 percent formaldehyde or 80 percent ethanol aqueous and storing in low temperature dark room, could not solve the problem. Dehydration in sodium chloride solution and subsequent immersion in glycerine had preserved the colour to a certain degree. The method of Yoshida (1962) has been tried during these studies and found good. Accordingly a four percent formaldehyde solution in 30 percent sea water containing 0.6 g sodium ascorbate was used for immersing the larvae and kept air tight. Immersion of fish larvae in one percent formal dehyde sea water solution containing 1 percent sodium ascorbate

also proved to be good. Ueyanagi (1966) and Matsumoto *et al.* (1972) had shown the importance of red pigments in the identification of tuna larvae. Use of butylated hydroxytoluene has been of some value in preserving these red pigments during the present study.

Shrinkage of fish larvae in the fixative and preservative fluids as noted by Farris (1963) and Devi (1972) has been observed in the present studies too. This has been attributed to the dehydration caused by the fixatives and preservatives and the protein solubilisation at pH higher than 7.0.

Other fixatives listed on page 32 and tested during the study such as Bouin's fluid, Carnoy's fluid, acrolein, acetaldehyde, chromic acid and ethanol could not provide a better fixation criteria than that of 2 percent formaldehyde in sea wat

Properly fixed fish larvae were preserved in the various preservative fluids mentioned in series 1-10 . Of these one percent formaldehyde in sea water made by the dilution of 2 percent borax buffered 40 percent formaldehyde with on site sea water having a pH 7.0 to 8.4 was found sufficient to keep qualit fish larvae over an extended period of ten years, but for the shrinkage noted. The Sorensen's fall in pH was made up by replacement with new preservative fluid or by the addition of 0.1 g borax/litre as and when pH fell. Fish larvae kept in hexamine buffered formaldehyde suffered muscular disintegration. The modified aldehyde Dowicil 100 is a formaldehyde donor. Its solid nature and instant solubility in on the spot sea water

makes it a convenient field fixative. Though this imparted a slight yellowish brown colour to fish larvae, a 10 percent solution in sea water liberated sufficient formaldehyde, to preserve the fish larvae. However this required a buffer to raise the initial pH of 3.5 to one around 7.0. 0.005 percent sodium glycerophosphate served this purpose. Thus Dowicil 100 did not have any superiority or gain over formaldehyde in preserving fish larvae. Calcium carbonate 5 percent when used as a means of neutralising formaldehyde should be used only with stock solution, either 40 percent formaldehyde or 1 percent formaldehyde. In view of its absence in the specimen jars occasional change of preservative was necessitated. This maintained a pH of 7.0. Similarly insoluble neutralisers such as calcium, magnesium and aluminium silicate at 5 percent strong could maintain the preservative fluids containing fish larvae in good condition. Soluble neutralising agent like sodium acetate also maintained pH of 1 percent formaldehyde around 6.5 to 7.5 at 5 percent strength.

The shrinkage noted in fish larvae was due to contraction of protein in the fixative and preservative solution used and all attempts in avoiding this shrinkage met with failure. For preservation of fish larvae the gentle, inert, long lasting properties of non-formaldehyde preservatives were tested. In 80 percent ethanol aqueous, due to dissolution of fat and oil from the fish larvae, an yellow colour was imparted. In view of its cost, high evaporation rates, shrinkage and the brittleness it imparted to fish larvae it is better to avoid ethanol.

Ethyleneglycol first used by Williamson and Russel (1965) as a preservative was tried in 50 percent strength and found fish larvae were well preserved. Propylene glycol being an inhibitor of mould growth, penetrant aid, bringing softening effects to fixed protein, when added to preservative fluids, added the preserving qualities of fish larvae. Propylene phenoxetol showing strong bactericidal and fungicidal properties and being anti-oxidant was used for the preservation of fish larvae. This chemical at 1 percent strength in distilled water maintained a pH 6.5 to 7.5 when buffered with 0.5 percent borax. Fish larvae preserved in this preservative maintained the taxonomic features. The discolouration of fish larvae noted in specimens kept preserved in the varying temperature and light conditions of the room was found considerably reduced when they were stored in dark and temperature controlled (20°C) rooms.

## Leptocephali larvae

Eel larvae known as leptocephali has been identified by their body shapes, length, pigmentation, nature of delicate medium fin and the number and disposition of larval teeth. Criteria observed for satisfactory fixation and preservation of eel larva are - (a) undistorted, relatively flexible body, (b) black or very dark brown pigment spots and eye pigment, (c) translucent lateral muscle segments and (d) complete larval teeth and undamaged fins.

The very attenuate larvae of snipe eels and notacanthids are readily damaged in nets and subsequently by poor handling and preservation. The larvae collected during the IIOE and preserved in 4 percent formaldehyde buffered with 2 percent hexamine, underwent distortion and swelling of body in 50 percent, followed by a split body in the rest. Melanophores and choroid pigments got faded completely, larval teeth lost, fins damaged and myomeres especially in the caudal region disintegrated. In short 90 percent of leptocephali had muscle tissue entirely disappeared and their body reduced to a thin, diaphanous, transparent ribbon. Growth studies using the preserved specimens could not be done in the present studies due to 7 percent shrinkage noted in the body length. Increase in length on preservation of larvae observed by others was not found in the present studies.

The above observations lead to the conclusion that protein of eel larvae was not fixed properly in the 4 percent formaldehyde fixative used. As a result the improperly fixed body protein takes in more fluid, having a higher osmotic pressure than the external medium, leading to swelling of body and rupture. Leptocephalus protein was found best fixed at a pH around 4.0. This pH 4.0 was maintained by the dissolution of 0.5 percent aluminium, potassium sulphate in the 2 percent formaldehyde in sea water. Once fixed this can be best preserved in 1 percent formaldehyde in sea water or distilled wate and 1 percent phenoxetol irrespective of its pH. Preservation

in 80 percent ethanol aqueous reduced body length up to 7 percent and hence could not be used for preservation.

## Fish eggs

General appearance and transparency was used as the criteria with fish eggs for their identification. Oil globules were present in fish eggs serving as reserve food in times of need or as floatation adjunct. Anchovy eggs on fixation in formaldehyde became slightly smaller but more rigid. Damages to specimens during netting ruptured their tissues which lead to escape of oil globules later. These oil globules inside the eggs are in liquid form and so their preservation is accompanied by chemical, thermal and mechanical problems.

Formaldehyde which preserved protein well is not a good fixative/preservative for fish eggs, lecithotrophic larvae, and other plankters having oil/yolk. Lipid breakdown is a continuous process in formaldehyde solutions. The higher the pH, the greater was the likelihood of oil droplet size reduction and hence the greater the dispersion and possibility of oil saponification leading to breakdown or disappearance in oily plankters. Fish eggs showed signs of shrinkage in solutions containing glycerol monoacetate and Dowicide A and they became opaque in solutions containing Dowicide A, ethylene glycol monoacetate, glycerol diacetate, trioxane and borax. A satisfactory state of preservation of fish eggs throughout the first year of observation was found following the use of

5 percent calcium carbonate in one or two percent formaldehyde solutions in sea water; 5 percent calcium chloride in 2 percent formaldehyde in sea water; and 2 percent formaldehyde solution in sea water made up with borax buffered 40 percent formaldehyd At a pH higher than 8.0 due to saponification and breakdown of lipid, oil globules were kept in good condition for prolonged periods by fixation in neutral 2 percent formaldehyde and subsequent transfer to an anti-oxidant as 0.5 percent propylene phenoxetol solution. Sea water was found better than distille water as a diluent for formaldehyde fixatives and preservatives for use with oily plankters. Also oil globules were preserved in a semi solid state by placing the specimens in a refrigerato Oil globules got stained by the addition of terasil blue G.R. liquid (2 drops in 100 ml sea water containing eggs) can be used for observing oil disintegration. Alcohol, a good fixati of glycogen and protein dissolved oil/fat readily producing an yellow colour to alcohol. The slimy film developed on specime transferred from alcohol to formaldehyde was due to the non-solubility of oil in formaldehyde solution which has been force out of alcoholic solution. At dilutions below 75 percent, oil previously dissolved is forced out as emulsion. Oxidation of oil also bleached the colour of the specimens. Progallin-P as a lipid anti-oxidant was not satisfactory as it discoloured preservative fluids.

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# 2. BIOCHEMICAL INVESTIGATIONS.

## 2.1. Introduction.

### 2.1.1. General.

Methods of quantitative evaluation were introduced to the study of marine plankton by Hensen (1887). Zooplankton biomass measurements can be considered as indices to the amount of living matter present in the form of one or more of the various kinds of organisms comprising a plankton population. One of the classical measures of biomass is chemical, which includes the analysis of plankton for their content of specific elements such as carbon, nitrogen, and phosphorus and for biochemical components like protein, lipid, and carbohydrate or for more specific forms of these general classes of organic materials. The various biochemical substances which have been used to provide estimates of biomass have a variable relationship to the total organic content depending upon the type of organism examined, the stage of development, the season of capture and other environmental factors.

Much interest has developed recently in the studies on biochemical composition of zooplankton particularly in relation to seasonal changes as studied by Mauchline and Fisher (1969), Kryuchkova and Makarov (1969), Raymont *et al.* (1971a) and Ferguson and Raymont (1974).

A review of the earlier literature on the food requirements of zooplankton shows that the assumption has usually been made that either carbohydrate or lipid, or a mixture of these substrates, is used for respiration purposes. One would presume that a considerable knowledge of the biochemical composition of selected zooplankton species is available if we are to assess the likely substrates used for respiratory purposes.

Therefore it is surprising to find that the information available on the biochemical composition of marine zooplankton is quite insufficient. Much of the earlier work involved the use of mixed zooplankton hauls or mixture of species of copepods or other groups of zooplankton organisms. But since diet, respiratory uptake and other physiological characteristics vary between one species and another, analysis of mixed hauls is unlikely to yield useful information. Moreover, even when selected species were chosen, frequently only lipid or protein was analysed, often not on the same haul of zooplankton. Frequently there is a large range of values in the biochemical fraction which has been investigated and it is uncertain how far such variations were due to changes in the area and time of sampling and stocks used.

The precise values for protein may also be open to question, since in most of the earlier works nitrogen was analysed by Kjeldahl determinations; the protein content has then been calculated on the assumption that protein contains 16 percent

nitrogen. But recently it has been pointed out (Raymont et al., 1966) that protein may have a nitrogen content varying from 12 to 20 percent. Though criticism of both Biuret and Folin methods for determining protein are to some degree justified, a direct estimate of protein is probably preferable to Kjeldahl determinations.

A further difficulty in the earlier work is that carbohydrate was very rarely analysed. An exception is the work of Krey (1950) and also the analysis of Millar and Scott (1967) but in many other cases where carbohydrate content has been quoted it has been calculated by substracting the total lipid, protein, ash and chitin from a theoretical 100 percent. It seemed therefore important in investigating the biochemical composition of selected species of plankton.

A detailed investigation, therefore, of the components of all the three major biochemical fractions in one and the same species might help us to a better understanding of the substrates utilised and even perhaps of some of the metabolic pathways involved. The general significance of biochemical studies on marine zooplankton has been reviewed by Corner and Cowey (1968).

Interest in the chemical composition of plankton, especially with regard to their nutritional value in fisheries investigations can be traced back to Hensen (1887) and Brandt (1898). Since then our knowledge of plankton chemistry has increased during the past 2 decades. Where the organic

fraction has been analysed, work has been restricted to a few species belonging to major groups of zooplankton (See e.g. Raymont *et al.*, 1964, 1966, 1967 and 1968; Raymont and Linford, 1966; Reeve *et al.*, 1970; Ikeda, 1971; Ferguson and Raymont, 1974; Raymont *et al.*, 1969a and 1969b, 1971a and 1971b and Mayzaud and Martin, 1975).

The above study can reveal the role of planktonic organisms in the transfer of organic constituents through the aquatic food web. The need for biochemical studies on marine zooplankton is now widely accepted in view of the relatively few studies, which have been made and of their importance in any understanding of the metabolism of zooplankton. Also the importance of investigating single species of zooplankton and the significance of proximate analysis in the general understanding of the biochemistry of zooplankton have been emphasised. More or less simultaneous complete analysis permits observation of any reciprocal relationship between the constituents. Knowledge of the proximate biochemical composition of a variety of zooplankton species is a pre-requisite for detailed investigations of the constituent lipids, amino acids and other nitrogenous compounds, carbohydrates, etc. In turn, the knowledge of the variations in these fractions may lead to some understanding of the biochemical pathways in zooplankton. Jeffries (1970) discussing the effect of seasonal changes in the environment on the fatty acid composition of zooplankton suggested that such variations in chemical patterns could be

useful in studying biological organization at the community levels, since fatty acid composition varied with seasonal succession and productivity. Also Jeffries (1969) studying the free amino acid pool in a temperate zooplankton community found free amino acid pattern representing a picture of an organism's metabolic activities and suggested the use of these changes to monitor subtle environmental changes. Free amino acid levels appear to be an index of physiological condition and productive capacity. Sriraman and Reddy (1977) by comparing the electrophoregrams of adults and juveniles of *Penaeus indicus* and *P. monodon* observed ontogenetic variations in the protein fractions, attributable to biochemical changes during different phases of growth and suggested their usefulness in confirming the identity of planktonic juveniles. Such ontogenetic differences have been reported in fishes also (Herzberg and Pasteur, 1975). Tsuyuki *et al.* (1965) have pointed out their usefulness in the biochemical taxonomy of fishes.

### 2.1.2. Survey of literature.

*On proteins:* The determination of protein in zooplankton has been relatively less studied than lipids, since protein is assumed not to have an energy storage function though subsequent work by Marshall and Orr (1955); Raymont *et al.* (1966); Reeve *et al.* (1970) through their studies disproved this assumption.

Ferguson and Raymont (1974) and references cited therein have analysed a large number of zooplankton species (decapods, mysids and euphausiids) for their biochemical constituents and concluded that in all their analysis protein is the major organic component (53 - 64 percent dry weight) in terms of dry weight regardless of the species except when zooplankton is storing large amounts of lipids and carbohydrates.

Orr (1934a and b) is one of the few earlier workers who analysed separate species of copepods. Orr got protein values ranging from 31 to 44 and 30 to 77 percent, for Euchaeta norvegica and Calanus finmarchicus respectively. His figures are not so high as those of Krey (1950). Krey restricts his data to analysis of "copepods" - presumably a mixture of a few species and got protein values of 71 - 77 percent, calculated from total nitrogen values. Nakai (1955) has analysed protein in six copepod species and found varying from 34.8 percent (Calanus plumchrus) to 82.6 percent (Acartia clausi) and suggested that the amounts of protein and lipid are complementary. Cowey and Corner (1963a) suggested a protein content of 50 percent for Calanus. Mayzaud and Martin (1975) got 44.75 percent for Calanus finmarchicus similar to Orr's value (1934b). Vinogradova (1964) gave 40 - 60 percent protein for general plankton, with 43 percent for Calanus helgolandicus in contrast to 75.2 percent recorded by Nakai (1955).

Euphausia superba was analysed for its protein content by Burkholder et al. (1967), Vinogradova (1960), Sidhu et al. (1970),

Raymont *et al.* (1971b) and Ferguson and Raymont (1974). While Sidhu *et al.* (1970) and Burkholder *et al.* (1967) got protein content of 49 (N×6.25) and 63.8 percent respectively based on nitrogen values, others estimated protein directly and got a mean range from 50 to 61. But revised estimations of protein percentage based upon determination of amino acid nitrogen gave a value of 37.35 only. Though Nakai (1955) found a very high protein content of 79.3 percent for *Euphausia pacifica*, Jawed (1969) got only 58 percent. Suyama *et al.* (1965) analysed the protein and non-protein nitrogen constituents of *Euphausia* and found the amount of crude protein around 17 percent in fresh sample. Raymont *et al.* (1964, 1966) estimated 71 percent protein content with a monthly mean, varying from 60 to 73 percent for *Neomysis integer*. Similar values of 72 and 70 percent were recorded by Seguin (1968) and Raymont *et a* (1966) for *Praunus flexuosus* and *Leptomysis lingvura* respectiv But *Neomysis rayii* had only 57 percent (Jawed, 1969). The bathypelagic mysids *Eucopia* and *Gnathophausia* (Raymont *et al.*, 1969b) gave a protein value of 58 and 65 percent of the total organic matter respectively excluding chitin.

Among the chaetognaths, only three species were studied. While Maynaud and Martin (1975) got 54.2 percent for *Sagitta elegans*, Reeve *et al.* (1970) observed a mean of 53 percent fo *Sagitta hispida*. These values were more or less identical with that of euphausiids. A deep sea chaetognath *Eukrohnia fowleri* however had a very high protein content (77 percent total organic matter excluding chitin) similar to neritic my

Raymont *et al.* (1967) investigating biochemical composition of oceanic decapods (*Acanthephyra*, *Gennadas* and *Sergestes*) found relatively high values of 60, 62 and 58 percent respectivel though the level is not as great as in mysids. The North Atlantic decapods *Systellaspis*, *Pasiphaea*, *Oplophorus* and *Acanthephyra* gave protein values of approximately 53, 64, 45 and 57 percent respectively (Raymont *et al.*, 1969b). Burkholder *et al.* (1967) based on Kjeldahl nitrogen estimated a protein content of 14.12 percent and based on amino nitrogen 13.19 percent in *Munida gregaria*.

Thus some aerial and vertical differences were encountered in protein fraction fluctuations. Neritic mysids had very high protein (70 - 72 percent). Euphausiids, decapods and chaetognaths had a medium range of 50 to 60 percent. But copepods showed low and high protein contents. To a certain extent the variations noted are due to analytical errors.

Differences in the techniques of analyses used, contribute to the variability. The precise values for protein might also be open to question, since protein was estimated by early workers, on the basis of the total nitrogen content (analysed by Kjeldahl determination) using the classical conversion factor of 6.25. Also it was assumed that almost all the nitrogen was protein nitrogen. But Fenton and Simmonds (1958) and Raymont *et al.* (1966) pointed out that protein may have a nitrogen content varying from 12 to 30 percent, instead of the assumed 16 percent, thus preferring a direct estimation of protein.

Cowey and Corner (1963a) have reported that 90 percent of the total nitrogen was amino acid nitrogen (14 percent as free amino acid nitrogen and 76 percent as protein nitrogen) in *Calanus finmarchicus*. They (1963b) also found that 80 percent of the total nitrogen in *Calanus helgolandicus* is present as amino acid nitrogen. Contrary to their earlier results of 14 percent, Corner and Cowey (1968) found that the non-protein nitrogen forms only a small part of the total body nitrogen. Raymont *et al.* (1966, 1968) confirmed non-protein nitrogen amounting to 10 to 20 percent of total nitrogen in *Neomysis integer* (Austin, 1965), and this varied with the environmental salinity. The protein of *Neomysis integer* contained 13.3 percent nitrogen. Raymont *et al.* (1967) also found a non-protein fraction of 20 to 25 percent of the total nitrogen in the three decapod species - *Acanthephyra*, *Gennadas* and *Sergestes*. Raymont *et al.* (1971a) observed in *Meganyctiphanes norvegica*, non-protein nitrogen forming approximately 22 percent of the total nitrogen. Mayzaud and Martin (1975) found large amounts of non-protein nitrogen - 19 percent of total nitrogen in *Calanus finmarchicus* stage V and 42 percent of total nitrogen in *Sagitta elegans* similar to a mean of 37 percent of total nitrogen reported in *Sagitta hispida*, by Reeve *et al.* (1970). The non-protein nitrogen fraction of the total nitrogen fluctuated (30-46 percent) and averaged over a third of the total. Sidhu *et al.* (1970) analysed *Euphausia superba* and obtained a non-protein nitrogen of 3.1 percent similar to the 3.81 percent of the total nitrogen reported for *Euphausia pacifica*. These

large and variable fraction of non-protein nitrogen may account for the fact that the sum of the organic fractions does not equal 100 percent and the great variability in protein content.

Size effect: An analysis (Raymont et al., 1969a and 1971a) of protein values according to size groups, in contrast to lipid, shows that there is no obvious relationship between protein and body size in northern krill. However he gave some indication that very small Meganyctiphanes taken during winter had somewhat higher protein levels. In an earlier study on Euphausia superba (Vinogradova, 1960) the protein content was relatively constant, ranging from 55 to 61 percent both seasonally and in young and adults. Ferguson and Raymont (1974) analysing Euphausia superba ranging in body size from 10 to 400 mg dry weight, illustrated that the proportion of protein declines fairly steadily with increasing body size. The protein values range from 38 to 67 percent (mean 50 percent) and are similar to the data obtained by Raymont et al. (1971b) when a range of 40 - 61 percent (mean 52 percent) was obtained. For specimens with a mean size of 200 mg, the protein content was 27 - 42 percent (mean 37 percent).

Seasonal variation: Many workers have attributed differences noted in protein content in part be due to seasonal changes in composition for a single species. Raymont et al. (1964) suggested that the fluctuations previously reported for zooplankton are due partly to the analysis of the different fractions being carried out on material captured at different

times. It seems essential for any critical appraisal of the biochemical fractions in a species of zooplankton that the different analyses are carried out practically simultaneously on the selected species taken in the same plankton haul and maintained under standard conditions.

Orr (1934 a, b) studied the variation of protein levels in *Euchaeta norvegica* and *Calanus finmarchicus* and found it varying from 31 to 44 and 30 to 77 respectively. But there is little information on seasonal changes of protein in zooplankton other than that of Raymont *et al.* (1966) for *Neomysis integer*, in which protein showed almost no seasonal variation and Raymont *et al.* (1969a) for *Meganyctiphanes* from Norwegian waters with little variation (50 in February to 62 percent in April, mean 59 percent). The overall mean for the whole *Meganyctiphanes* gave 57 percent protein content. Raymont *et al.* (1971a) however suggested for *Meganyctiphanes norvegica* from Scottish waters, a range of monthly mean protein from 51 percent (October) to 64 percent (March). The variation between individual determinations of protein for any month usually ranged from a minimum of about 50 percent to a maximum of some 65.68 percent. The overall mean for the whole material gave an average protein content of 57 percent dry weight similar to earlier studies. Reeve *et al.* (1970) analysing the proximate biochemical composition of *Sagitta hispida* of warm waters observed protein fraction averaging 53 percent fluctuating widely throughout the year (39 to 70 percent), with highest

values in December and January, intermediate values in summer, and lowest values in spring and autumn. Since experimentally starved animals showed no such protein variability, the fluctuations were attributed to environmental parameters other than food.

*Protein as a metabolic substrate*: The very variable protein content suggests that this material may be utilised as a metabolic substrate (for oxidative purposes), atleast under conditions of stress. Raymont (1972) suggested the possible use of some part of the protein for metabolism. Support to this idea comes from the work of Conover and Corner (1968) in which they suggest that, though lipid may first be utilised as a food reserve by the copepod, *Calanus hyperboreus*, protein may be called on later as a metabolic substrate. Javed's (1969) studies on *Neomysis rayii* and *Euphausia pacifica*, also suggest that those animals metabolise protein atleast during starvation as indicated by a low O/N ratio $< 8$. The variable protein content in *Sagitta hispida* with an O/N ratio 7:1 indicate protein metabolism (Reeve *et al.*, 1970). Starvation experiments on *Neomysis integer* (Ferguson, 1973) showed that protein was the most important metabolic reserve accounting for 90 percent of the total weight lost. *Euphausia superba* (Ferguson and Raymont, 1974) and *Meganyctiphanes norvegica* (Raymont *et al.*, 1971a) rely to some extent on protein metabolism, *Meganyctiphanes* due to limited lipid content may require early use of protein.

**Relationship with other fractions:** Raymont *et al.* (1969a, b) showed that for *Meganyctiphanes* the seasonal relationship between protein and ash content was the reciprocal of the lipid content. Raymont *et al.* (1971a) indicated that a reciprocal relationship between lipid and protein is observable in the 3 size categories of *Meganyctiphanes norvegica* studied. The fluctuations in protein are almost a mirror image of the changes in lipid. These values strongly support the reciprocal complementary relationship between the lipid and protein, fractions advocated first by Nakai (1955). This reciprocal relationship indicates a portion of the lipid and the protein being utilisable as substrates.

**On carbohydrates:** Brandt (1898) made the first estimation of carbohydrate and got a value of 20 percent for copepods, which is ten times more than the recent values. Krey (1950) gave a range of 0 - 4.4 percent and 13.9 percent for carbohydrate in copepods and *Sagitta* spp. respectively. His values indicated fairly constant levels for carbohydrate. His observation on *Sagitta* is significant as it suggests a higher proportion of carbohydrate for *Sagitta* than for copepods and this value is certainly much above that for other zooplankton studied. On the other hand, it appears that *Sagitta* has a lower lipid fraction (1.51 to 6 percent). Though this high content was attributed to the carnivorous nature of *Sagitta*, Raymont and Conover (1961) found very little carbohydrate for the essentially carnivorous copepod *Euchaeta*.

Results of earlier biochemical studies by Raymont and Krishnaswamy (1960), Raymont and Conover (1961) and Marshall and Orr (1962) have indicated that the amount of carbohydrate in several marine planktonic species is less than one percent of dry body weight. A few determinations by Raymont and Conover (1961) gave fairly low values for eleven oceanic zooplankton species from American waters such as *Meganyctiphanes norvegica*, *Thysanoessa* sp. (0.05 to 0.08 percent wet weight), *Nematoscelis megalops* (0.08 to 0.17 percent wet weight), *Para-euchaeta norvegica* (0.06 to 0.11 percent wet weight), *Pleuromamma* sp. (0.1 percent wet weight), *Hyperia galba* (0.15 percent wet weight), *Conchoecia* (0.31 percent wet weight), *Calanus finmarchicus* (0.11 to 0.4 percent wet weight) *Neomysis americana* (0.19 to 0.25 percent wet weight) and *Calanus hyperboreus* (0.14 to 0.29 percent wet weight). From these studies, it is conclud that the animals in question do not contain sufficient carbo-hydrate reserves to meet energy requirements, though Raymont and Krishnaswamy (1960) showed that carbohydrate levels in zooplankt tended to lower during starvation. Also this study shows a rather variable carbohydrate content for coastal species and constant content for deep and open ocean forms due to conversion of fat to carbohydrates.

Vinogradova (1960) suggested varying carbohydrate contents ranging from 6 to 28 percent for *Euphausia superba*. Similarly Vinogradova (1964) got high carbohydrate values ranging from 16 to 34 percent for general plankton from Black Sea and rather

low carbohydrate value for Calanus helgolandicus (9 percent). In these studies, carbohydrate was obtained by substraction of the sum of the percent ash, protein and lipid from a theoretical 100 percent. Hence the above high values require re-examination.

Raymont and Krishnaswamy (1960) found Neomysis integer having a carbohydrate content of mean value of 0.20 and 0.21 percent wet weight. But, Raymont et al. (1964) on using a modified method for carbohydrate estimation in Neomysis integer got a total carbohydrate content of 2.4 percent of dry body weight, the low level suggesting that glycogen cannot be a signi ficant storage material. Raymont et al. (1964 & 1966) found in Neomysis integer mean level of 2.3 and 2.4 percent dry weight declining to lower levels in November and January. Seguin (196 got 3 percent carbohydrate content in Praunus flexuosus.

Raymont and Linford (1966) analysed carbohydrate content in the Mediterranean open sea zooplankton. The littoral subtropical mysid Leptomysis lingvura gave a carbohydrate value of 2.6 percent of dry weight. Since dry weight was not measured, the results were recalculated on a dry weight basis using dry weight values for similar taxa. Thus, the carbohydrate content expressed as a percentage of the dry weight would be 3.15 percent for Temora stylifera; 2.4 percent for Sagitta inflata and S. serratodentata, and 7 percent for Doliolum sp. Doliolum being a pure herbivore is expected to store greater amounts of carbohydrate giving high value.

Raymont *et al.* (1967) analysed biochemical composition of 3 species of oceanic decapod zooplankton from Gulf of Aden and suggested low 2-3 percent carbohydrate content in *Acanthephyra purpurea*, *Gennadas clavicarpus* and *Sergestes* sp. Burkholder *et al.* (1967) estimated "carbohydrate etc." by difference in galatheid decapods, *Munida gregaria* and *Euphausia superba* to be 45 and 48 percent respectively of the dry matter.

Raymont *et al.* (1969b) estimated carbohydrate constituent in deep sea zooplankton collected from the North Atlantic. As percentage wet weight, the decapods *Acanthephyra*, *Systellaspis*, *Funchalia* and *Oplophorus* had very low carbohydrate content of 0.4 to 0.5 and in terms of percentage expressed as total organic content, excluding chitin, it was 2 percent. But the bathypelagic chaetognath *Eukrohnia* had a higher fraction of 0.7 percent wet weight or low value of 3 percent of total organic content. This is far below than Krey's value for *Sagitta* spp. The bathypelagic mysids *Eucopia* and *Gnathophausia* had a carbohydrate content of 0.5 to 0.6 percent wet weight and when expressed as percentage of total organic content, values were 2 and 5 respectively. The high value for *Gnathophausia* needs rechecking. Thus the neritic, oceanic and offshore mysids have similar carbohydrate values.

Raymont *et al.* (1969a) observed exceedingly low values averaging 2 percent throughout the period in *Meganyctiphanes norvegica*. The differences between monthly means are relatively small, with a maximum of 2.3 percent in May and a

minimum of 1.8 percent in April. Reckoned as percentage of the total organic matter, it varies only between 2.4 and 2.8 percent. This low contribution by carbohydrate is almost identical with what have already been reported for *Neomysis* (Raymont *et al.*, 1964, 1966) and for *Leptomysis* (Raymont and Linford, 1966). Analysis of *Thysanoessa inermis* gave the same carbohydrate value as in *Meganyctiphanes*. Raymont *et al.* (1971a) estimated carbohydrate content of *Meganyctiphanes* from Scotland and observed a rather greater change in the total carbohydrate than for Norwegian material. The extreme range was from 1.1 to 3.7 percent and in August it was 1.7 to 3.7 percent. The low value suggests that glycogen does not form a substantial part of the body reserves as is the case with many marine animals. Marginally higher carbohydrate value was given (2.8 percent) by fresh specimens than the deep frozen material. The values from Norwegian and Scottish specimens reveal that little regional differences are observable with *Meganyctiphanes*.

The biochemical composition of *Euphausia superba* with a wide range of body size, about 10 to 400 mg dry weight, was studied by Raymont *et al.* (1971b) and Ferguson and Raymont (1974). Carbohydrate was the lowest of the three biochemical fractions. The smaller krills (about 24 mg) showed higher values (mean 3.1 and 4.1) and the larger krills (100 - 135 mg) showed lower values (mean 2.1). Still larger ones (about 200 mg) also showed a mean value of 2.1. Raymont *et al.* (1971b) gave

slightly greater carbohydrate values - a mean of 4.7 percent in December, declining to 3.1 percent in January. Despite the low values, a decline in carbohydrate levels with body size and advancing season was noted. Thus smaller krills found in early December showed double the carbohydrate value of the larger krills found in January-February. In earlier studies by Nakai (1955) and Javed (1969), *Euphausia pacifica* gave a carbohydrate value of 2.7 percent.

Reeve *et al.* (1970) analysing the biochemical fractions of a tropical warm water chaetognath, *Sagitta hispida* observed carbohydrate as usual, remaining the smallest fraction, ranging between 2 and 6 percent (mean 3.5 percent). Maynard and Martin (1975) obtained 1.5 percent only for temperate species of chaetognaths - *Sagitta elegans*, whereas on analysing temperate copepods got a carbohydrate content of 1.69 percent.

It thus appears that zooplankton from a variety of habitats (estuarine, inshore, oceanic) and from several different geographical localities (Britain, Gulf of Aden, western North Atlantic, Norway, Mediterranean) all show the same low insignificant contribution of carbohydrate content.

As far as known, the only attempts to fractionate zooplankton sugars is that of Raymont *et al.* (1968) on *Neomysis integer*. Their results indicate that almost 50 percent of the total carbohydrate is probably ribose from nucleic acids, glycogen 25 percent, and free pentose about 5 percent. They failed to detect any glucose or galactose and so the fate of the

remaining 20 percent could not be solved. Studies on *Calanus finmarchicus* and Sagitta *elegans* by Raymond and Martin (1975) showed that major part of carbohydrate (80 - 92 percent) is insoluble in TCA indicating nucleic acid pentose and only trace amounts of free D-glucose (4-8 percent) can be detected.

*On lipids*: Lipids, the major metabolic fuel being the most efficient storage form of energy having a caloric value of more than twice that of protein and carbohydrate is found having very high variability in its distribution, among zooplankton species. The biochemical composition of surface-living zooplankton has been investigated by a number of workers (Ackman and Eaton, 1967; Conner and Cowey, 1968; Culkin and Morris, 1969, 1970; Fischer, 1962; Jeffrey *et al*., 1966; Linford, 1965; Littlepage, 1964; Raymont *et al*., 1964, 1966, 1969; Raymont and Conover, 1961; Reeve *et al*., 1970; Sipos and Ackman, 1968; Vinogradova, 1960; Yamada, 1964). Studies on meso and bathypelagic forms are few (Culkin and Morris, 1969; Lee *et al*., 1970a, 1971a, 1971b, Lee and Hirota, 1973; Raymont *et al*., 1966, 1969b). Results for the copepods have been tabulated by Ikeda (1971) and the data for the other species have been reviewed by Raymont *et al*. (1969). Nakai (1955) indicated values ranging between 2.7 percent of the dry weight for *Euphausia pacifica* and 53.9 percent for *Calanus plumchrus*. Lee *et al*. (1971a) attempting to relate the amount and composition of lipid in copepods to their vertical and latitudinal distributions reported values ranging from 3 percent for *Candacia curta*

to 61 percent for Heterorhabdus sp. Conover (1962, 1964) found that Calanus hyperboreus may contain at times up to 60 percent fat. Maynard and Martin (1975) got values ranging between 44.4 percent for Calanus finmarchicus and 7.8 percent for Sagitta elegans. Lee (1974) analysing lipid content in 17 zooplankton species from an inlet in British Columbia got values ranging from 9 percent for a ctenophore to 47 percent in copepods.

Others have got high lipid values as high as 40 percent for Calanus finmarchicus (Klem, 1932), 47 percent for Calanus finmarchicus (Orr, 1934b), 53.9 percent for Calanus plumchrus (Nakai, 1955) and 60-70 percent for various euphausids (Fischer, 1962). Littlepage (1964) has quoted maximum lipid values of 46 and 36 percent respectively for two Antarctic species, Euchaeta antarctica and Euphausia crystallorophias.

Both seasonal and life history changes have been found causing lipid content variations in Calanus finmarchicus (Orr, 1934b; Rudanova, 1940; Conover, 1962), Calanus hyperboreus (Conover, 1962) and two Antarctic crustaceans (Littlepage, 1964). A seasonal study by Conover (1962) showed that lipid ranged from 15 to 50 percent with the highest values in summer as it stored fat when blooms of phytoplankton occur. Lee (1974) observed a build-up of reserve lipid in Calanus hyperboreus collected from Arctic ice-land changing with season (blooms) - highest lipid value (74 percent) in August slowly decreasing to the very low value (29 percent) in June - and with depth

(deep-water fauna differing in food habits and life history from upper water community). Conover and Corner (1968) found in *C. hyperboreus* from the Gulf of Maine lipid varying from 15 percent in April to 55 percent in summer. Seasonal lipid studies of the polar copepods *Metridia longa* from the Arctic with 33 percent (Lee *et al.*, 1972a) and *Euchaeta antarctica* with 46 percent (Littlepage, 1964) demonstrated that these carnivorous copepods also had a large increase in lipid during the summer.

Lee *et al.* (1970a, 1971 a & b, 1973) have shown an increase of lipid (due to increase in wax esters) in copepods from deep water or high latitudes relative to species from near surface waters or low latitudes. Lee and Hirota (1973) analysed lipid content in 32 tropical species of copepods from different depth intervals. They found near-surface copepods from the upper 250 m having a mean of 14 and range of 3-37 percent lipid. The migrating copepods had a mean of 14.8 with a range 7-28 percent lipid whereas the deep-living copepods had a mean of 29 and a range 18-68 percent. Their lipid data for copepods collected in the upper 500 m at tropical, sub-tropical, temperate and polar latitudes revealed the progressive increase in the mean percentage of lipid from the tropical to polar latitudes. Also they showed that over wide geographic areas of the world's oceans the distribution of lipids in copepods follows definite patterns.

Summarising environmental data of Sheard (1953), Littlepage (1964) and Lee et al. (1971a) two factors - temperature or temporal distribution and rate of food supply were found responsible for the lipid content variation. Littlepage (1964) got 36 percent lipid in winter beginning in *Euphausia crystallorophias* dropping to 9 percent before the bloom started, the lipid appearing as a metabolic reserve. Kryuchkova and Makarov (1969) found the mean lipid content of a herbivorous krill increasing as summer advances from 3 to 30 percent. The seasonal lipid content of *Meganyctiphanes norvegica* varied from 10 to 29 percent (Raymont, 1969a and 1971a) with a mean of 17 to 18 percent, the fluctuation suggesting a reciprocal relationship between protein and lipid contents. Due to the limited lipid reserve of this boreal euphausid, it metabolises only protein which is reflected in the omnivorous diet. The high lipid values of greater than 40 percent got for *Euphausia superba* may be a reflection of the higher latitude habitat of antarctic krill. Ferguson and Raymont (1974) found in *Euphausia superba* the lipid content increasing with the advance of season. *Thysanoessa* sp. also exhibited a similar lipid change. There is strong evidence for the accumulation of lipid towards the end of the summer to a maximum of 30 percent with intensive grazing (Moiseev, 1970; Kryuchkova and Makarov, 1969). Raymont and Martin (1975) concluded that cold water plankton with low food supply have larger lipid storage. Small plankton like neritic copepods in view of their higher level of turn-over of their biomass compared to small body size as an alternative

to high storage (Maynard, 1963) do not contain more lipid materials.

Ferguson and Raymont (1974) found a relative increase in the proportion of lipid with increase in body weight; whereas in smaller krill lipid is the smallest fraction, in large specimens of *Euphausia superba* lipid and protein are virtually identical in amount. In *Euphausia superba* from 10 mg to 500 mg (dry weight) lipid content changed more and more, up to 30 mg having 16 percent and 200 to 300 mg having 35 percent lipid. A similar direct relationship between lipid and body size was suggested by Mauchline and Fisher (1969) for *Meganyctiphanes* and was confirmed by Raymont *et al.* (1969a, 1971a), though the evidence was less striking than for *Euphausia superba*. Further *Meganyctiphanes* did not attain the extremely high lipid values ( $>$ 40 percent) found in *Euphausia superba*, possibly this is a reflection of the higher latitude habitat of Antarctic krill.

Littlepage (1964) noted a large increase in lipid in *Euchaeta antarctica* during the summer when egg production occurs Lee *et al.* (1974) indicated mature females of *Euchaeta japonica* in the process of accumulating lipid stores for later transfer to eggs. Thus maximum egg production takes place during summer The eggs of *Euchaeta marina* and *Euchaeta media* were also rich in lipids with wax esters accounting for 72 and 58 percent of their lipids respectively. Corkett and McLaren (1969) suggests that *Pseudocalanus* lengthens its period between egg production

during times of low food. Mauchline and Fisher (1969) also noted seasonal fat change correlated to egg production.

Raymont *et al.* (1969b) in all their deep sea species of zooplankton analysis found lipid content varying approximately inversely from 33 to 13 percent when expressed as percentage of total organic matter, excluding chitin, showing aerial and vertical differences, the semi-planktonic tidal pool *Neomysis integer* (Raymont *et al.*, 1966), neritic *Leptomysis lingvura* (Raymont and Linford, 1966) and inshore *Praunus flexuosus* (Seguin, 1968) having a mean low lipid content of 13, 11 and 13 percent respectively, with year round variations of 7-14 percent, while deep sea and offshore species *Eucopia* and *Gnathophausia* had a higher lipid content of greater than 30 per-cent of total organic content excluding chitin. The above studies show very little food reserve as fat in mysids, their omnivorous diet meeting the immediate requirements. But according to Lee and Hirota (1973) the mesopelagic tropical mysid *Gnathophausia* sp. had a lipid content of 11 to 24 per-cent with appreciable amounts of stored wax esters (32 percent of the lipid). Lee *et al.* (1971a) found 60 percent wax esters of total lipids in a subtropical species of *Gnatho-phausia* with 42 percent lipid.

Raymont *et al.* (1967) noted the variation between the biochemical composition of three Gulf of Aden oceanic decapod species. The lipid values of 12 and 15 percent respectively for *Acanthephyra purpurea* and *Gennadas clavicarpus*, found

similar to those obtained for *Leptomysis* and *Neomysis* (11 and 13 percent), are within the 3-15 percent variation (Fischer, 1962) obtained for the 2 species of oceanic decapod - *Acanthephyra*. Galkin and Morris (1969) noted absence of wax esters in a mesopelagic decapod *Gennadas valens*. Lee *et al*. (1971a) found 21 percent lipid with only 10 percent of lipid as wax esters in a subtropical species of *Gennadas*. Lee and Hirota (1973) observed in a tropical species of *Gennadas* only 10 to 14 percent of lipid with very low wax ester. The tropical penaeid decapod *Sergestes robustus* and *Sergestes challengeri* with 10 and 15 percent lipid (Lee and Hirota, 1973) having 22-23 percent of lipid as triglyceride differed much from the high (29 percent) lipid content noted by Raymont *et al*. (1967) in *Sergestes* sp. from Gulf of Aden. Raymont *et al*. (1969b) analysing biochemical fractions of deep sea decapods observed a lipid content of 10, 12 and 14 percent for the three species of *Funchalia*, *Acanthephyra* and *Systellaspis* respectively. The Oplophoridae caridean decapod *Systellaspis* sp. from tropical seas (Lee and Hirota, 1973) showed a variation in lipid content from 7 to 11 percent with a triglyceride value of up to 40 percent of lipid content. The decapod *Pasiphaea pacifica* with 21 percent lipid had a large store of neutral lipid (69 percent of total lipid).

Lee *et al*. (1971a) analysed lipid content of zooplankton from subtropical seas to relate the amount and composition of lipid in organisms to their vertical and latitudinal distribution. The polychaete *Alciopidae*, amphipod *Gammaridae* and

Hyperiidae and chaetognaths Eukrohnia sp. showed appreciable amounts of wax esters with a lipid content of 55, 57, 7 and 40 percent respectively. Lee and Hirota (1973) made an interesting observation with a tropical hyperiid amphipod species having 18 percent lipid, that it has no reserve lipid. Also they found tropical chaetognaths having 27 percent lipid content, a lower value than the subtropical chaetognaths (38-40 percent, Lee et al., 1971a). The tropical Diacria and Clio specimens had only 2 to 3 percent lipid, compared to the subtropical pteropod species with 5 percent lipid. Lee (1974) analysed lipid contents of 17 zooplankton species collected from Inlet, British Columbia. The polychaete Tomopteris septentrionalis, chaetognath Sagitta elegans, ctenophore Pleurobrachia pileus with a lipid content of 22, 14 and 9 percent respectively were characterized by little storage lipid (7-10 percent of lipid). It is assumed that these species transfer most new organic matter into new tissue rather than storage lipid. The ostracod Conchoecia elegans, pteropod Clione limacina, euphausiid Euphausia pacifica, amphipods Eurimno chrysalis and Hyperia galba, decapod Pasiphaea pacifica and ctenophore Beroe cucumis had a lipid content of 17, 31, 19-26, 26, 19, 21 and 13 percent respectively. These specimens except Beroe had primarily triglycerides. But the copepod taxa with high lipid content (28-47 percent) had very high level of storage wax ester (53 to 90 percent of the lipid). Oncaeidae sp. collected from subtropical ocean gave a lipid fraction of

25 percent (Lee *et al.*, 1971a). The only tropical, exclusively carnivorous species studied in detail is the surface living warm water neritic species, *Sagitta hispida* (Reeve *et al.*, 1970). In this, lipid though distinctly variable (9-27 percent) does not make a very substantial fraction of the body weight.

Most pertinent references on the analytical data of lipid studies on crustacean zooplankton are listed and discussed by Ackman and Eaton (1966a, 1967); Klem (1932); Saiki and Mori (1956); Saiki *et al.* (1959); Lasker and Theilacker (1962); Lovern (1964); Tsuyuki *et al.* (1964a, b); Williams (1965); Jeffrey *et al.* (1966); Bottino *et al.* (1967 a, b); Lewis (1967); Arnold, (1968); Raymont *et al.* (1968); Culkin and Morris (1969); Pierce *et al.* (1969) and Lee *et al.* (1970a,b; 1971a&b; 1972a,b; 1973 and 1974). The significant differences noted in fatty acids of two North Atlantic Krills - *Meganyctiphanes norvegica* and *Thysanoessa inermis* by Fischer (1962) were elaborated by Ackman *et al.* (1970). Lee *et al.* (1971b) found that the lipid content and spectrum of fatty acids and alcohols were largely dependent on the copepod diet. Thus they noted a linear correlation between the amount of food fed and total lipid content of the zooplankton; also the composition of wax esters changed with increased diet. Lee *et al.* (1970a, b and 1971a, b) found wax esters as an important reserve lipid in copepods. Lee *et al.* (1974) analysing the lipid changes during life cycle of a North Pacific marine copepod *Euchaeta japonica*

found all stages from egg to adult (6 naupliar and 6 copepodid stages) containing wax esters in their lipid stores, while triglycerides were important only in the eggs, early naupliar stages and adults.

2.1.3. Tropical Indian Ocean Zooplankton.

A survey of literature pertinent to the biochemical analysis of zooplankton, clearly shows the dearth of data with reference to Indian Ocean zooplankton especially in the tropical area of 20°N to 20°S. Nair et al. (1975) made a preliminary analysis of the biochemical constituents in few zooplankton collected from the estuarine waters of Cochin. The calculated average values expressed as percentage of dry weight of copepods, mysids, Acetes spp., decapod larvae, fish larvae and Sagitta bedoti, for the different constituents were 52 - 64 percent protein, 12 - 21 percent lipid, 3 - 5 percent carbohydrate, 9 - 15 percent ash and 1.4 - 4 percent chitin respectively. Also the water content ranged from 10 to 92 percent wet weight. Gopalakrishnan et al. (1977) analysed major biochemical constituents of two zooplankton species collected during periods of abundance in Cochin backwaters. The two species Acetes cochinensis and Acartia species studied had an average protein, carbohydrate, chitin, ash and water content of 51.2, 26.5, 4.7, 2.7, 8.6 and 77 percent respectively. Rosamma et al. (1979) studied biochemical composition of copepods, mysids

and euphausiids from the Laccadive Sea and found that the protein, carbohydrate and lipid content fluctuated between 5.3 and 62, 2.5 and 5 and 22 and 32 percent of dry weight respectively. The moisture content varied from 82 to 90 percent wet weight. These results indicate that the variations between neritic and oceanic forms are not appreciable. Madhupratap *et al.* (1979) made biochemical studies on some tropical estuarine species. They noted variations in the protein, carbohydrate and lipid contents of a ctenophore, three hydromedusae, one chaetognath, three copepods and two sergestids varying between 30 and 77, 0.4 and 2, 6 and 14 respectively. Austin (1972) analysed the biochemical composition of the tropical intertidal mysid *Gastrosaccus* sp. and got a protein, carbohydrate, lipid, chitin and ash content of 60, 4, 10, 12 and 12 percent of dry weight respectively. Also he found 80 percent of wet weight as water content.

## 2.1.4. Tropical meroplankton.

Nair *et al.* (1975) estimated biochemical composition of decapod larvae and fish larvae collected from estuarine areas of Cochin. On comparing the average values for their major biochemical constituents, fish larvae are found having a higher protein and lipid content, with a lower carbohydrate content than decapod larvae. Also the fish larvae had only very small water content (10.1 percent) compared to the high water content (85.2 percent) in decapod larvae.

## 2.2. Material and Methods.

### 2.2.1. Preparation of material.

Meroplankton larvae were collected during local cruises and on board R.V. Gaveshani from Arabian Sea and Bay of Bengal during 1976 to 1978. But for the fish and decapod larvae, all other larval forms which were small in size were collected as and when available, sorted under a binocular dissecting microscope, quickly rinsed with distilled water or 3 percent ammonium formate solution and kept frozen at -20°C. When sufficient quantity was accumulated, the material was dried in an oven at 70°C to constant weight. The material was homogenised and redried for 5 hours at 70°C and then stored in a desiccator in polythene bags until taken for analysis.

For wet weight determination the freezed specimens were allowed to thaw before analysis in the room temperature. Excess water on specimens was drained gently on a glass filter paper to avoid artificially increased values. During this process loss of body fluid was assumed to be minimal. These were transferred to previously ashed and weighed aluminium foil cups and dried in an oven at 70°C to constant weight, cooled and weighed again. The difference gave the amount of water content in these larvae as percent wet weight.

Raymont *et al.* (1971a) found deep frozen material suitable for biochemical analysis. One of the disadvantages noted was the accurate determination of dry/wet weight ratio. During

thawing of specimens, especially if drainage is not entirely complete, high water contents may be recorded especially in small specimens. Generally smaller specimens have a greater water content and this is accentuated by the difficulty of draining smaller animals effectively. Another difficulty is that, with the lapse of time before analysis a certain amount of drying may occur.

The ash contents were determined on animals which had first been used for dry weight determinations. They were ashed (carbon burnt) to constant weight in a muffle furnace at 450°C to 500°C in a 24 hour period without causing any loss of the relatively volatile ions i.e. sodium, potassium and bicarbonate. Ashed samples were reweighed after cooling in a desiccator. Since during ashing aluminium foil due to oxidation gains weight a gain of 0.4 percent of the original weight - it is necessary to ash the aluminium foil before experiments.

Chitin was estimated using the method of Strickland and Parson (1968) and Raymont et al. (1964) and found both methods giving identical values. A known weight of larvae was treated with 50 percent sodium hydroxide overnight, subsequently washed with distilled water followed by methanol-chloroform, and then dried to constant weight. This was ashed and the loss in weight was taken to be that of chitin.

## 2.2.2. Biochemical Techniques.

Carbohydrate: Several methods are employed for measuring the total carbohydrate in animal tissue. Most frequently used in studies on marine invertebrates are the anthrone method (Seifter *et al.*, 1960), the method of Mendel *et al.* (1954) and the phenol-sulphuric method of Dubois *et al.* (1956). A later modification by Marshall and Orr (1962) to the method of Dubois *et al.* is considered to be more accurate (cf. Raymont *et al.*, 1964) than the previous methods. This method also found suitable for dried material was therefore adopted in the present study for its accuracy and simplicity.

The dried macroplanktonic material was weighed and placed in a boiling tube. In experiments where a limited amount of dried material was available, 1 ml of the homogenate prepared for the protein estimation was further diluted to a known volume and 1 ml aliquots placed in the previously dried boiling tubes. One ml of 5 percent phenol solution was added followed by 5 ml of concentrated analar sulphuric acid, the latter delivered from a sman off 5 ml pipette. The optimal density was read after 30 minutes at 490 μ in a Beckman Spectrophotometer against blanks of 1 ml distilled water, 1 ml phenol and 5 ml concentrated sulphuric acid, and compared with those of a standard curve for glucose. This was prepared from standard solutions of glucose in the range of 10 - 100 μg/l. This curve is linear over the range used.

Lipid: Because of the fairly low lipid content in macroplankton, many of the extraction techniques used are inapplicable to this study. Linford (1965) reviewed several methods available for the determination of lipids in small invertebrates. She found that the continuous reflux method, using the soxhlet apparatus and petroleum ether as the only solvent, did not extract the total lipid present and that lipo-protein complexes, largely remained in the extracted tissues. The colorimeteric micromethod for the determination of total lipids in serum (Swahn, 1952), was also investigated but no reproducibility of results could be obtained. Similar problems occurred when using the method of Brandt et al. (1952). The gravimetric technique of Folch et al. (1956) was found to give the highest lipid extraction. This method of estimation was adopted in the present study.

About 100 mg of wet tissue was homogenised in a solution of 2:1 chloroform/methanol, (v/v analytical grade), to a final dilution of 20-fold the volume of the tissue sample, (assuming the relationship 1 g of tissue = 1 ml). The homogenate was filtered through a sintered glass filter under vacuum into a graduated centrifuge tube and rinsed through with small quantities of the chloroform/methanol solution. The filtrate was washed thoroughly with 0.2 of its volume of a 0.05 N KCl solution to remove non-lipid contaminants. After centrifuging to give two distinct phases, the upper phase containing the water and contaminants was removed as completely as possible

by pipetting. The lower phase was then washed with the KCl supernatant from a previously prepared chloroform/methanol/ 0.5 N KCl solution in the proportions of 8:4:3 V/V respectively. After centrifuging, the lower phase was transferred to a weighed container for evaporation of the solvent in the dark for 24 hours. The container was then reweighed to give the weight of the total lipid extracted.

Protein: The total protein content of mesoplankton was measured colorimetrically by a quantitative adaptation of the Biuret reaction (Krey *et al.*, 1957; Raymont *et al.*, 1964). Biuret gives a purple colour with substances including protein, containing two peptide groups attached either to each other, to the same nitrogen atom, or the same carbon atom. The intensity of colour produced is dependent on the peptide bond concentration and not on the amino acid composition. A wide variety of proteins give a quantitative colorimetric reaction and as there are almost no substances other than protein occurring in biological material which give this reaction to any significant extent, the method is useful for measuring small quantities of protein.

The Biuret reagent was prepared as follows using analar grade reagents: 1.5 g $CuSO_4$. 5 $H_2O$ and 6 g NaK tartrate. 4 $H_2O$ were dissolved in 500 ml of distilled water in a 1 litre volumetric flask. 300 ml of 10 percent sodium hydroxide was added with constant swirling and the volume made up to 1 litre with distilled water. The reagent was stored in a polythene bottle

The dried material was weighed and homogenised with 1 ml of distilled water and 4 ml Biuret reagent in a Potter-Elvehjem homogeniser until no purple stained particles remained. When a limited number of animals were available 3-6 were homogenised in a known volume of distilled water and one ml aliquots treated with the Biuret reagent. The solution prepared was left to stand for 30 minutes to allow the colour to develop and then filtered through a glass filter paper to remove chitinous debris. The optical density of the clear filtrate was read at 540 μ in a Beckman Spectrophotometer. Glass fibre filters were used in the filtration as paper containing cellulose absorbs cupric ions from the reagent. The optical density obtained was compared with those of a standard curve for bovine plasma albumen over the range 1-10 mg. In all optical density readings blanks of 1 ml distilled water and 4 ml Biuret were used.

The protein content of small samples, was measured by the colorimetric method of Lowry et al. (1951), which is 100 times more sensitive than the Biuret method. The proteins in the sample react with Folin-ciocalteu phenol-determining reagent, containing phosphotungstic and phosphomolybdic acids and the intensity of colour developed depends upon the quantity of tyrosine and tryptophan residues present. The method therefore assumes a similarity in level of these two amino acids in the protein of the sample and the standard. Free amino acids do not affect the colour significantly. The method has the disadvantage that the amount of colour varies with different protein

and is not strictly proportional to concentration. In the present study the quantities of samples to be analysed were kept very small in order that the protein levels did not rise above 30 µg/ml. This minimised any error due to a nonlinear relationship, between concentration and optical density.

The reagents were prepared as described by Lowry *et al.* (1951) with the exception of the alkaline copper solution, (reagent C). This was made up according to Erice (1965) to avoid exceeding the solubility product of copper tartrate in any stock solution. Solutions were prepared as follows:

| | | |
|---|---|---|
| Reagent A | - | 2 percent W/V sodium carbonate in 0.1 N sodium hydroxide. |
| Reagent B-1 | - | 5 percent W/V copper sulphate. |
| Reagent B-2 | - | 10 percent W/V potassium sodium tartrate. |
| Reagent B | - | 1 volume each of B-1 and B-2 with 8 volumes distilled water, mixed (discarded after 1 day). |
| Reagent C | - | 50 volumes of A and 1 volume of B mixed (discarded after one day). |
| Reagent D | - | Folin-ciocalteu phenol reagent. A sample was titrated with 1 N NaoH, using phenolphthalein as indicator, to give the normality of the stock solution. The appropriate amount of distilled water was added to make the final concentration 1 N. |

To determine the protein content, dried samples were weighed and homogenised in 1 ml distilled water and 5 ml reagent C. This solution was filtered through GF/C filters and after 10 minutes 0.5 ml reagent D was added and mixed thoroughly within

3 seconds. After 30 minutes the optical density was read at 750 μ in a Beckman Spectrophotometer against reagent blanks. A standard curve was prepared from solutions of bovine plasma albumen (10 - 30 μg/ml) which was linear over this range.

Reporting data on chemical content in terms of ash-free dry weight provides the most direct relationship for comparative purposes. However, other bases such as dry weight are commonly used since many methods of chemical analysis call for the use of dried samples. These are most acceptable when a single species and age/size class, is studied. In samples of mixed populations the amount of inorganic materials and water may be highly variable.

## 2.3. Results.

Data on major biochemical constituents of meroplankton studied are given in the Table VI, a to i on pages 162 to 166. The tables indicate the values for protein, lipid, carbohydrate, chitin, ash and water content for different larval meroplankton taxa - Coelenterata, Polychaeta, Sipunculoidea, Cirripedia, Decapoda, Lamellibranchiata, Echinodermata, Tornaria and Fish larvae. Except otherwise mentioned, the values are expressed as percentage dry weights together with the range of variation. Water content is expressed as percentage wet weight. In view of their small size a number of meroplankton larvae are pooled together for analyses and mean values are recorded.

Table VI (a - i)

Protein, lipid, carbohydrate, chitin and ash content in various macroplankton taxa expressed as percentage dry weight and water content given as percentage wet weight.

| Taxa (a) Macroplanktonic coelenterates | Protein | Lipid | Carbo-hydrate | Chitin | ash | Water content |
|---|---|---|---|---|---|---|
| Semper's larva | 38.1 | 32.4 | 1.6 | 10.2 | 7.2 | 80.2 |
| Semper's larva | 18.2 | 61.1 | 1.2 | 9.7 | 6.4 | 40.6 |
| Semper's larva | 22.1 | 50.9 | 1.6 | 12.1 | 5.7 | 41.3 |
| Ceriantharian larva (planktotrophic) | 54.6 | 7.7 | 1.9 | 11.7 | 7.1 | 95.1 |
| " | 46.4 | 8.1 | 1.8 | 10.8 | 6.9 | 92.4 |
| " | 42.8 | 7.9 | 1.8 | 11.2 | 7.9 | 94.6 |
| " | 64.2 | 8.9 | 2.1 | 10.7 | 8.4 | 95.8 |
| (lecithotrophic) | 9.1 | 70.3 | 1.1 | 6.3 | 6.3 | 20.4 |
| " | 45.3 | 24.6 | 1.6 | 8.4 | 7.1 | 59.4 |
| " | 39.3 | 28.4 | 1.8 | 7.6 | 7.4 | 54.3 |

| Taxa (b) Polychaete larvae | Protein | Lipid | Carbo-hydrate | Chitin | ash | Water content |
|---|---|---|---|---|---|---|
| Lecithotrophic | 21.7 | 62.4 | 3.2 | 6.4 | 4.8 | 40.9 |
| " | 23.4 | 55.3 | 2.9 | 6.8 | 5.1 | 38.7 |
| " | 22.8 | 50.1 | 3.2 | 6.9 | 5.4 | 36.4 |
| " | 24.9 | 45.8 | 3.1 | 7.1 | 5.2 | 35.4 |
| " | 28.1 | 30.2 | 2.7 | 8.4 | 6.8 | 49.4 |
| " | 23.7 | 47.3 | 3.1 | 6.6 | 5.8 | 41.6 |
| Planktotrophic | 57.6 | 11.4 | 6.2 | 9.2 | 5.2 | 61.5 |
| " | 57.1 | 9.9 | 4.9 | 10.4 | 5.4 | 62.9 |
| " | 58.1 | 10.2 | 5.1 | 9.8 | 5.6 | 68.1 |
| " | 62.7 | 12.1 | 5.7 | 11.4 | 6.3 | 67.3 |
| " | 58.7 | 12.2 | 6.2 | 8.2 | 5.2 | 63.2 |
| " | 60.3 | 10.6 | 5.8 | 10.5 | 5.8 | 71.4 |
| Taxa (c) Sipunculoidea larvae | 7.9 | 22.1 | 1.2 | - | 68.6 | 86.2 |
| " | 13.4 | 21.7 | 1.0 | - | 64.1 | 88.2 |
| " | 15.3 | 22.6 | 0.9 | - | 62.3 | 84.6 |
| " | 18.1 | 18.7 | 0.8 | - | 62.9 | 86.7 |
| " | 12.3 | 7.4 | 0.7 | - | 76.8 | 88.5 |
| " | 13.1 | 8.2 | 0.6 | - | 77.1 | 88.3 |

| Taxa (d) Cirripedia larvae | Protein | Lipid | Carbo-hydrate | Chitin | ash | Water content |
|---|---|---|---|---|---|---|
| Nauplius I-IV + cypris stage | 43.2 | 23.2 | 8.2 | 3.2 | 8.7 | 63.9 |
| " | 38.4 | 16.2 | 10.2 | 3.5 | 17.6 | 70.4 |
| " | 39.9 | 21.7 | 9.5 | 3.3 | 12.7 | 68.3 |
| Post cypris | 54.1 | 22.3 | 11.7 | 3.5 | 8.0 | 70.1 |
| " | 50.2 | 5.5 | 3.5 | 3.4 | 19.2 | 90.2 |
| " | 58.2 | 14.1 | 3.8 | 3.2 | 12.4 | 85.6 |
| **Taxa (e) Decapod larvae** | | | | | | |
| Crab zoea | 22.6 | 25.2 | 4.8 | 8.1 | 16.2 | 78.4 |
| " " | 27.3 | 42.7 | 4.6 | 8.2 | 6.8 | 72.3 |
| Megalopa | 33.0 | 42.1 | 4.8 | 7.3 | 7.1 | 75.8 |
| Phyllosoma | 38.1 | 36.6 | 5.1 | 7.2 | 12.3 | 75.9 |
| Decapod larvae | 57.8 | 12.2 | 5.3 | 7.6 | 15.1 | 80.5 |
| " " | 60.1 | 11.8 | 2.7 | 5.6 | 17.1 | 83.6 |
| " " | 60.0 | 15.1 | 3.1 | 6.2 | 15.6 | 81.2 |
| " " | 52.3 | 25.1 | 2.6 | 5.1 | 14.2 | 72.3 |
| " " | 47.8 | 18.5 | 4.6 | 6.8 | 9.4 | 81.1 |
| P. _indicus_ < 15 mm | 13.2-16.7 | 6.1-8.2 | 0.8-0.11 | 5.2 | 16-17 | 80.1 |
| " 15-20 mm | 20.6-24.3 | 7.5-9.1 | 0.11-0.15 | 4.9 | 18-19 | 86.4 |
| " > 20 mm | 21.1-27.2 | 7.4-8.8 | 0.22-0.24 | 6.3 | 20-29 | 82.3 |
| P. _monodon_ < 15 mm | 15.4-21.6 | 8.1-9.5 | 0.06-0.10 | 5.1 | 18-20 | 82.1 |
| " 15-20 mm | 16.7-20.5 | 9.2-13.1 | 0.09-0.10 | 5.6 | 24-20 | 81.5 |
| " > 20 mm | 21.3-28.3 | 10.4-12.1 | 0.38-0.39 | 6.1 | 29-30 | 86.4 |

| Taxa (f) Bivalve larvae | Protein | Lipid | Carbo-hydrate | Chitin | Ash | Water content |
|---|---|---|---|---|---|---|
| Bivalve larvae | 14.1 | 3.1 | 10.1 | - | 73.2 | 17.6 |
| " " | 8.2 | 1.3 | 6.8 | - | 80.9 | 11.2 |
| " " | 10.1 | 1.6 | 3.6 | - | 80.2 | 11.3 |
| " " | 9.1 | 1.4 | 6.9 | - | 79.2 | 11.6 |
| " " | 10.5 | 1.8 | 4.2 | - | 78.3 | 12.7 |
| " " | 13.6 | 3.4 | 6.7 | - | 72.4 | 18.1 |
| " " | 24.1 | 5.8 | 9.2 | - | 60.4 | 30.3 |
| " " | 25.1 | 3.5 | 8.7 | - | 60.5 | 29.6 |
| " " | 22.2 | 4.2 | 8.6 | - | 62.2 | 28.3 |

| Taxa (g) Echinoderm larvae | | | | | | |
|---|---|---|---|---|---|---|
| Lecithotrophic larva | 15.3 | 68.2 | 2.8 | - | 13.4 | 40.7 |
| " | 17.7 | 59.6 | 3.1 | - | 13.5 | 44.3 |
| " | 20.4 | 54.7 | 2.4 | - | 14.2 | 48.6 |
| " | 25.1 | 34.3 | 2.2 | - | 19.2 | 59.2 |
| " | 25.2 | 23.7 | 2.4 | - | 20.3 | 67.3 |
| Planktotrophic larva | 20.9 | 18.3 | 2.3 | - | 29.4 | 79.4 |
| " | 18.8 | 16.9 | 2.7 | - | 28.3 | 85.2 |
| " | 19.7 | 15.3 | 1.9 | - | 27.1 | 84.1 |

| Taxa (h) Tornaria larvae | Protein | Lipid | Carbo-hydrate | Chitin | Ash | Water content |
|---|---|---|---|---|---|---|
| Tornaria larvae | 29.1 | 6.1 | 0.6 | - | 64.2 | 93.2 |
| " " | 27.8 | 5.8 | 0.7 | - | 65.6 | 90.6 |
| " " | 23.3 | 4.2 | 0.8 | - | 70.8 | 96.1 |
| " " | 21.2 | 4.6 | 0.9 | - | 72.2 | 92.6 |
| " " (aged) | 34.3 | 5.3 | 0.8 | - | 58.4 | 83.8 |
| " " (aged) | 33.8 | 4.9 | 0.6 | - | 59.1 | 84.6 |

| Taxa (i) Fish eggs & larvae | Protein | Lipid | Carbo-hydrate | Chitin | Ash | Water content |
|---|---|---|---|---|---|---|
| Fish eggs | 14.8 | 68.6 | 2.5 | - | 1.2 | 85.0 |
| Carangidae with yolk | 19.2 | 68.4 | 2.4 | - | 6.9 | 11.4 |
| " without yolk | 74.9 | 4.1 | 2.1 | - | 2.2 | 78.4 |
| Engraulidae with yolk | 22.3 | 64.8 | 2.9 | - | 6.2 | 13.8 |
| " without yolk | 66.3 | 18.4 | 2.7 | - | 2.4 | 72.4 |
| Serranidae " | 64.2 | 23.6 | 2.1 | - | 4.6 | 75.2 |
| Sciaenidae " | 60.8 | 24.1 | 2.9 | - | 4.3 | 68.4 |
| Myctophidae " | 59.4 | 23.1 | 1.9 | - | 3.9 | 70.7 |
| Gonostomidae " | 70.6 | 18.6 | 2.0 | - | 4.2 | 69.4 |

Larval coelenterates are grouped as lecithotrophic, planktotrophic and Semper's larvae. The individual variations among the 10 analyses (Table VI a) are considerable according to the age class, size group, feeding mechanism, time of collection etc. In the lecithotrophic larvae protein contents increased from 9.1 to 45.3 percent as the yolk content depleted from 70.3 to 24.6 percent. With the lowering in lipid content, an increase in water content from 20.4 to 59.4 percent of wet weight occurred. The chitin and ash content range, more or less remained same i.e. 6.3 to 8.4 percent and 6.3 to 7.4 percent respectively. Similar increase in protein content from 18.2 to 38.1 percent with simultaneous lowering in lipid content from 61.1 to 32.4 percent was observed in the Semper's larva. Also the water content increased from 40.6 to 60.2 percent of wet weight. The chitin content ranging from 9.7 to 12.1 percent was higher than other lecithotrophic larvae, but the ash content remained with slight variation, i.e. from 5.7 to 7.2 percent. In the ceriantharian larvae the protein content ranged from 42.8 to 64.2 percent, probably in relation to their stages of growth. The lipid content was uniformly low with the range of 7.7 to 8.9 percent. The water content was very high reaching a value of 92.4 to 95.1 percent wet weight. The ash content varied from 6.9 to 8.4 percent and chitin gave a higher content of 10.7 to 11.7 percent. The data for carbohydrate in coelenterate larvae showed rather low values with smaller range from 1.1 to 2.1 percent in all the 10 analyses

made. The generally low values suggest that glycogen, the usual storage carbohydrate in many marine animals, does not form a substantial part of the body reserves in coelenterate larvae. The slightly higher values in the ceriantharia larvae may be the result of their feeding on phytoplankton. In general the protein, lipid and water content show considerably large variations in their amount depending on their developmental stages. But for the higher chitin contents in the ceriantharian larvae, the ash and chitin contents did not show any evidence of seasonal variation.

The polychaete larvae were grouped into lecithotrophic and planktotrophic. Depending on the area and period of collection, in all twelve analyses (Table VI b) were carried out. Each series consisted of larvae collected from one particular area in a particular season in order to get enough material for all analyses. The protein content in the lecithotrophic larvae varied from 21.7 to 28.1 percent, whereas the planktotrophic larvae had a protein content of more than double the amount ranging from 57.1 to 62.7. The predominance of protein and its slight variation in the planktotrophic larvae indicate their vigorous feeding nature and identical developmental stages. The lipid content in the lecithotrophic trochophore larvae varied considerably from 30.2 to 62.4 percent. This indicates either their differential rate of yolk metabolism or different stages of yolk absorption. Those with a higher lipid content may be the early larvae and others with a low lipid content may be the

advanced stages. The planktotrophic larvae showed only a low lipid content. The variations from 9.9 to 12.2 percent may be the result of differential conversion efficiency.

Compared to the low carbohydrate content of coelenterate larvae, the polychaete trochophore showed a very high percentage. Carbohydrate in lecithotrophic larvae varied from 2.7 to 3.2 percent and in planktotrophic larvae varied from 4.9 to 6.2 percent. The planktotrophic larvae as they feed on phytoplankton accumulate double the amount of those found in lecithotrophic larvae, carried over through the egg. The ash content in trochophores varied from 4.8 to 6.8 percent. The higher ash content of 6.8 may be the result of a low lipid and protein content. The very low ash content of 4.8 percent can result from the high lipid content. Chitin amount varied from 6.4 to 8.4 percent in lecithotrophic larvae, generally the planktotrophic larvae having a high chitin content of 8.2 to 11.4 percent. The water content was less varying i.e. from 35.4 to 49.4 percent in the lecithotrophic larvae and higher values of 61.5 to 71.4 percent occurred in planktotrophic larvae owing to the depletion of lipid reserve.

The larvae of Sipunculoidea were collected during 6 cruises from the neritic waters of Kerala coast, kept frozen and analysed in 6 groups (Table VI e). The lipid content varied from 7.4 to 22.6 percent indicating presence of few fat globules, in the free swimming larvae. The water content was very high ranging from 84.6 to 88.5 percent. This high water content possessing

a high proportion of inorganic salts lead to a very high percent of ash content (62.3 to 77.1 percent). Carbohydrate content as in other zooplankton was far less having a range of 0.6 to 1.2 percent. Protein content also indicated very low values (7.9 to 18.1 percent), lower than that of the lipid. Of the 6 analyses, the first four were having more lipid and carbohydrate representing the early larvae and the last two analyses gave low lipid contents (7.4 to 8.2 percent) and carbohydrate content (0.6 to 0.7 percent) representing the advanced larvae ready to metamorphose, having depleted the lipid contents.

The cirriped larvae (Table VI d) collected belonged to various stages. They were grouped into naupliar and post-cypris stages. The nauplier and the cypris stages were obtained from 3 areas only in large numbers. Similarly post-cypris stages were collected on 3 occasions. The nauplier stages which were nonfeeding types had a protein content varying from 38.4 to 43.2 percent and a lipid content varying from 16.2 to 23.2 percent. The carbohydrate varied from 8.2 to 10.2 percent. These higher carbohydrate values may be the outcome of their contribution to the metabolic substrates. The chitin content varied from 3.2 to 3.5 percent only. But the ash content showed considerable variation from 8.7 to 17.6 percent. The higher ash content (percent) may be a reflection of the reduced fat content. The water content fluctuated from 65.9 to 70.4 percent. Generally water content was found to increase with fall in lipid content.

The post-cypris larvae had a higher protein content varying from 50.2 to 58.2 percent. The analyses suggest that lipid is the most variable with a range of 5.5 to 22.3 percent. The low value of 5.5 percent may reflect the starved or insufficiently fed ones, in which lipid is used to the maximum possible. The high lipid content of 22.3 percent may represent the well fed ones where in the lipid utilisation is minimum. The carbohydrate content, compared to the very low percent noticed in general zooplankton, gave higher values of 3.5 to 11.7 percent. A tendency for reduction in the carbohydrate content was noticed as the larvae were nearing metamorphosis. The chitin content more or less remained constant with only slight, variation from 3.2 to 3.5 percent. However the ash content showed substantial variation, fluctuating between 8.0 and 19.2 percent. The high ash content of 19.2 was associated with a very low lipid content. Similarly the low ash content of 8.0 percent denoted a high lipid content of 22.3 percent. The water content varied from 70.1 to 90.2 percent. The low and high water contents were related to the high and low lipid contents.

In general the variations noted in the biochemical constituents of cirriped larvae are considerable, indicating different stages in the development. The fluctuations may be a function of changes before and after metamorphosis.

The crustacean meroplankton collected was sorted out into 6 categories. Each analysis represented a number of larvae homogenised together, in view of their small size and requirement of large amount for all the analyses. Crab zoea and megalopa

were analysed (Table VI e) separately. Phyllosoma of lobsters also was separately considered. *Penaeus indicus* and *Penaeus monodon* larvae were obtained from culture tanks. Rest of the crustacean meroplanktonic larvae was grouped as decapod larvae. The comparatively low lipid levels of the zoea stages of crabs, prawns, lobsters and other decapods may reflect considerable utilization of lipid reserves during embryonic development prior to hatching. Crab zoea, megalopa and phyllosoma were having a higher lipid content (25.2 to 42.7 percent) compared to the other decapods including penaeid larvae (6.1 to 25.1 percent). In the two analyses, crab zoea gave lipid contents of 25.2 and 42.7 percent. The megalopa had a higher lipid content of 42.1 percent whereas phyllosoma had 36.6 percent. The data also showed lipid depletion during development having a wide range. The decapod larvae showed lipid fraction varying from 11.8 to 25.1 percent, the low amount indicating considerable lipid usage in the egg itself. The penaeid larvae contained very low amounts of lipid, *P. indicus* having 6.1 to 9.1 percent and *P. monodon* from 8.1 to 13.1 percent, thus exhibiting species differences.

Protein content in these meroplanktonic larvae varied considerably from 13.8 to 60.1 percent. Even in the same species, protein varied as observed in *Penaeus indicus* from 21.3 to 28.3. *Penaeus indicus* and *Penaeus monodon* had the lowest protein content compared to other larvae. The range of protein in *Penaeus indicus* (13.2 to 27.2 percent) was almost same as observed in

*Penaeus monodon* (15.4 to 28.3 percent). The range of variation in the same species emphasises the building up of body material as the larvae grew. Crab zoea had lesser protein content (22.6 percent) than megalopa (33.0 percent). The phyllosoma larvae had 38.1 percent protein. Most of the decapod larvae had high protein content - 47.8 to 60.1 percent. Compared to a low lipid content these larvae had very high protein content.

Carbohydrate content in crustacean larvae in general varied from 2.6 to 5.3 percent. But the very low carbohydrate content in *Penaeus* spp. indicates considerable variation - 0.06 to 0.39 percent. Carbohydrate content increased as the larvae grew older. The low value suggests absence of carbohydrate in the newly released larvae and later additions in small amounts are the results of larval growth. The low values with little variations in other analyses reveal its lack of any important role in the larval development.

Ash content averaged 22 percent. The variation might be the result of seasonal patterns, and associated with inorganic salts in the body water. As the prawn grows ash content is on the increase. The higher ash content may also be associated with the low protein and lipid content. In *Penaeus indicus* and *Penaeus monodon* (larvae and postlarvae) ash content increased from 16 to 29 and 18 to 30 percent respectively as they grew. While crab larvae had an ash content of 6.8 to 16.2 percent, the lobster larvae gave 12.3 percent ash content. In the other decapod larvae ash content varied between 9.4 and 17.1 percent.

The chitin content fluctuated between 4.9 and 8.2 percent. This was associated with the stage of larvae used for analysis. Chitin content was highest immediately before or after moulting. The crustacean larvae during and immediately after moulting absorbs water. Tissue production and accumulation of organic reserve occurs after size increase. Thus the water content in crustacean larvae fluctuates considerably and depending on the addition of organic content, percent of organic fractions vary. Thus water content in *Penaeus* spp. ranged from 80.1 to 86.4 percent, whereas in crab larvae the range was from 72.3 to 78.4 percent. In other decapod larvae the water content fluctuated between 72.3 and 83.6 percent.

Bivalve larvae were analysed (Table VI f) as such. In view of their small size calcareous shells having more than 95 percent inorganic matter were also included in the analyses. The larvae were collected from 10 areas on different seasons. The protein content varied from 8.2 to 25.1 percent. The variation may be due to the seasonal difference or the age group analysed. Lipid content varied from 1.3 to 5.8 percent. Generally bivalve larvae in the early stages and during food scarcity in later stages do not feed, instead utilise the carbohydrate, protein and lipid content of the body. Hence very low concentrations of organic fractions are of common observations. Thus the amount of carbohydrate showed variations between 3.6 and 10.1 percent. The higher values obtained for carbohydrate than lipid show its highly restricted use. As

the larvae grow in size the percentage of carbohydrate content also increases. The ash content showed very high values due to the inclusion of shells containing a large percent of inorgani matter. These values ranged from 60.4 to 80.9 percent. The water content varied from 11.2 to 30.3 percent. In these analyses also water content gave low values due to inclusion of shell material.

Echinoderm larvae (Table VI g) were separated into two groups namely lecithotrophic and planktotrophic larvae. Lecithotrophic larvae were obtained from 5 areas and planktotrophic larvae from 3 areas. The lecithotrophic larvae had a very high lipid content depleting from 68.2 to 23.7 percent as they utilized lipid reserves for metabolic purposes. During this period a small percent was converted as protein which thus increased in its content from 15.3 to 25.2 percent. Carbohydrate content was low and remained without much variation in the range 2.2 to 3.1 percent. The echinoderm skeleton is mainly calcite and accumulation of this calcite in its body for future use may contribute for the high ash content (13.4 to 20.3 percent). Also the amount of water in proportion to the lipid depletion increased from 40.7 to 67.3 percent.

Compared to the above observations planktotrophic larvae had a low lipid content of 15.5 to 18.3 percent. Amount of protein varied from 18.8 to 20.9 percent and that of carbohydrate from 1.9 to 2.7 percent. The ash content was high ranging from 27.1 to 29.4 percent. This may be due to low organic content and high water content. Amount of water varied from 79.4 to 84.1 percent.

Sesarmia larvae were collected from six different areas during this study and analysed (Table VI h) separately. The data presented are average values for a number of larvae collected together during a particular period from a particular area. Two collections contained aged larvae. The water content was highest ranging from 90.6 to 96.1 percent. But in the aged larvae the water content fell to 83.8 to 84.6 percent. These aged larvae had a higher protein content perhaps indicating its preparation for metamorphosis. Thus while the aged larvae had a protein content of 33.8 to 34.3 percent the young larvae had a lesser protein content of 21.2 to 29.1 percent. The lipid content in all larvae was low ranging from 4.2 to 6.1 percent. Carbohydrate content varied from 0.6 to 0.9 percent. These low organic matter content may be a reflection of the higher metabolic requirement of these larvae. In view of the very high water content, the inorganic salts present in the body water lead to a very high ash content ranging from 58.4 to 72.2 percent, low values being noticed in aged larvae due to reduced water content.

Fish larvae collected from different areas were sorted out into 6 families irrespective of their length and age class. In each family about 30 larvae in groups of ten each were analy for the different fractions and the results (Table VI i) given are the average values obtained. Only in the Carangidae and Engraulidae families larvae with and without yolk-sac could be sorted out. In the rest of the families yolk-sac stage larvae

could not be collected. Also larvae of Gonostomidae and Myctophidae fishes had very small yolk-sac only. The larvae with yolk-sac had a very high lipid content ranging from 64.8 to 68.4 percent. In the Engraulidae even when the yolk-sac was depleted still a high lipid content of 18.4 percent occurred compared to the 4.1 percent in the Carangidae. Rest of the larvae had a lipid content ranging from 18.6 to 24.1 percent. On the other hand these larvae had a very high protein content ranging from 59.4 to 70.6 percent. The Engraulidae and Carangid larvae with yolk had a low protein content of 19.2 to 22.3 per- cent, while those without yolk-sac had a very high protein conten of 66.3 to 74.9 percent. The carbohydrate content present in fish larvae (1.9 to 2.9 percent) was like the normal contents occurring in general zooplankton and adult fishes (0.5 to 3.0 percent). Amount of ash varied from 6.2 to 6.9 percent in larva with yolk-sac and 2.2 to 2.4 percent in larvae without yolk sac. Water content in larvae with and without yolk sac fluctuated from 11.4 to 13.8 and 72.4 to 78.4 percent respectively. Fish eggs in general had 85 percent water, 68.6 percent lipid, 14.8 percent protein, 2.5 percent carbohydrate and 1.2 percent ash contents.

## 2.4. Discussion.

### Meroplanktonic coelenterate larvae

In the meroplanktonic stage, larval anthozoans are seen with or without tentacles. The larvae without tentacles are either large or small. The relatively large thenarian larvae

are yolky, lecithotrophic, and unwieldy with a long pelagic life up to 25 days. These larvae take no feed from the plankton. A few of these large lecithotrophic larvae as like *Alcyonium* sp. has only a short pelagic life lasting for a few days to a week. The relatively small thenarian larvae are planktonic, feeding on phytoplankton during the very short pelagic life (1 week). The athenarian tentaculate larvae are rather small, lecithotrophic having a long pelagic life up to one month. The tentaculate larvae belonging to ceriantharia are rather large (up to 2300 microns long) planktotrophic larvae feeding on microplankton and growing rapidly during the plankton stage, which is very long as in *Cerianthus* sp. The older larvae feed on zooplankton including fish larvae (Lebour, 1922). The Semper's larvae of zoanthideans are fully lecithotrophic.

So far, no attempt has been made to estimate the biochemical fractions of these larvae. But few studies on the adult coelenterates indicate presence of large amounts of chitin in their bodies (Young, 1971 and Wainwright, 1963). The chitin content in the ceriantharian larvae and Semper's larvae varied from 9.7 to 12.1 percent and this amount was greater than ash content.

The ceriantharian larvae possessed a very high water content up to 95 percent whereas the Semper's larvae and large thenarian larvae which are lecithotrophic had low water content varying from 40 to 60 percent in relation to the amount of lipid used up. Water was absorbed as the larvae metabolised more and more lipid reserve.

The carbohydrate content was low ranging from 1.6 to 2.1 percent in both lecithotrophic and planktotrophic larvae. Nadimpratap *et al.* (1979) got 0.64 percent carbohydrate content for *Pleurobrachia globosa* from Cochin estuarine waters and Krishnaswamy (1960) got 1.4 percent for *Pleurobrachia pileus* from temperate waters. Also Nadimpratap *et al.* (1979) got low carbohydrate values ranging between 0.39 and 1.96 percent for three species of hydrozoan medusae. These figures indicate that carbohydrate is the least important metabolite for coelenterate larvae.

The protein content varied from 22 to 38 percent in Semper's larvae, 9 to 45 percent in lecithotrophic larvae and 43 to 64 percent in ceriantharian planktotrophic larvae. Nadimpratab *et al.* (1979) found a protein content varying from 30 to 39 percent in the 3 species of hydrozoans and *Pleurobrach globosa*.

Lipid content formed an important metabolite of lecithotrophic larvae having a content of up to 70 percent, decreasing to 24 percent as the larvae utilised this. In cerianthariam larvae lipid content formed a low percentage (about 8 percent) as observed in three hydrozoan species (Nadimpratap *et al.*, 1979). The ctenophore specimens from temperate seas contained about 9 to 13 percent lipid.

<u>Polychaete larvae</u>

The meroplanktonic trochophores can be grouped under three types according to the degree in which they depend on the plankton as a source of food. Few polychaetes such as *Autolytus* sp., *Arenicola* sp. and *Spirorbis* sp. have plankto-trophic larvae with a short pelagic life of only few hours to very few days, feeding on plankton with almost negligible growth. Majority of polychaete larvae are planktotrophic having a pelagic life lasting up to 3 months. These trochophores on the third day of development complete first 3 worm segments. These early segmented larvae and other trochophores are rather hardy and require no food during the first 5 or 7 days after which they either settle or lead a pelagic life feeding on plankton. These are small larvae as they originate from very small eggs poor in yolk and so are totally dependent on the food conditions in the plankton. As soon as they develop setigerous appendages, jaws begin to grow and consume microplankton and diatoms. The older larvae of the polychaetes show external features and an organisation very similar to those of the parent animals (with cirri, bristles, eyes etc.). These larvae differ from the adult in having organs which enable them to swim and float in the water, e.g. bands or tufts of cilia and the provisional bristles. Also these larvae have the mouth and the intestine which will also function in the adults. Nutritionall these larvae are characterised by an impressive diversity.

Some of the trochophore larvae belonging to *Syllis*, *Eunice*, and *Nereis* originate from rather large yolked eggs which develop into pelagic larvae of clumsy shape rather unfit for locomotion and are often carried passively by the currents in the water masses. They take no food from the plankton, but feed exclusively on the content of yolk within the egg cell from which they originate. Thus they are able to lead a long pelagic life usually longer than 3 months in tropics.

Thus, the wide variations noted in the percent composition of biochemical fractions of polychaete larvae can be explained from the type of larvae analysed. The lecithotrophic larvae have very high lipid content (up to 62.4 percent) which is inversely proportional to the duration of pelagic life. The lowest lipid content noted was 30.2 percent. These larvae generally had a protein content lower than 28.1 percent and the carbohydrate content more or less constant around 2.7 to 3.2 percent. The reduced carbohydrate content may be the result of high water content. The chitin and ash content more or less gave similar values with an average water content of 40 percent. But the planktotrophic larvae had almost similar proportions of the biochemical constituents like the adult polychaetes. Gopalakrishnan *et al.* (1977) obtained a protein, carbohydrate and lipid content of 57.8, 8.3 and 12.5 percent of dry weight respectively for a benthic polychaete, *Diopatra neapolitana* from Cochin backwaters. Lee *et al.* (1971) in Alciopidae and Lee (1974) in *Tomopteris septentrionalis* found 55 and 22 percent of lipid

content respectively. Thus, while the lipid content of planktoni polychaetes was comparatively higher than the benthic forms, the carbohydrate content showed a decrease. The higher values for ash content of planktonic polychaetes may be attributed to the large amount of biologically inert ash derived from the salt content. The planktotrophic larvae had a protein content varying from 57.1 to 62.7 percent, which amounts to more than double the protein content in lecithotrophic larvae with 21.7 to 28.1 percent. Similarly the lipid content of planktotrophic larvae (9.9 to 12.2 percent) showed a 3 fold reduction from that of lecithotrophic larvae. The carbohydrate content showed a higher proportion of 4.9 to 6.2 percent compared to 2.7 to 3.2 percent in lecithotrophic larvae. Also the ash and chitin content of planktotrophic larvae showed high values. A large variation in the protein, lipid and carbohydrate content among the planktotrophic larvae were not observed since a major portion of food consumed was mainly utilised for their metabolic activities and thus addition of tissues was the minimum. Beers (1966) indicated the occurrence of only very little carbohydrate fraction (0.33 to 1.43 percent of dry weight) in holoplanktonic polychaetes. Also the wet to dry weight ratio varied from 3.7 to 17.5 percent.

Though Raymont and Krishnaswamy (1960) and Raymont and Conover (1961) confirmed the low levels of carbohydrate in zooplankton, a higher level was noticed in tropical plankton (Nair et al., 1973; Gopalakrishnan et al., 1977; and Rosamma et al., 1979), the latter again confirmed by the present results on polychaete larvae.

Lee *et al.* (1974) studying lipid changes during the life history of a marine copepod, *Euchaeta japonica* found reduction of lipid content from 64.4 percent of the eggs to about 14.2 percent in copepodite stage one which gradually rose to 52.2 percent in mature females. The data presented here demonstrate the important role of lipid in the provision of energy for the young larval stages especially during the non-feeding period.

From the very early stages of lecithotrophic larva lipid fraction makes up a decreasing proportion of the dry weight until it begins to feed. Once it starts feeding the lipid values rise to attain a peak as it attains maturity. Thus Gopalakrishnan *et al.* (1977) recorded a lipid fraction of 12.5 percent in benthic polychaete, *Diopatra neapolitana*. This high value noted in January got decreased drastically until June as a result of lipid portion being used for the production of eggs. It is this lipid present in eggs that contributed to the yolky larvae.

Littlepage (1964) has noted a large increase in lipid content in *Euchaeta antarctica* when egg production occurred. Maclaren (1965) suggested that *Pseudocalanus* sp. lengthens its period between egg production during periods of low food availability. The high lipid content in polychaete larvae observed in the present study can be explained from the above observations.

## Sipunculoidea larvae

The dry to wet weight ratio of these larvae (13.4 to 11.3 percent) were in the range 10 - 20 percent as noted for penaeid larvae. The ash content of 62.3 to 77.1 percent agreed with that of bivalve larvae (60.4 to 80.9 percent), and tornaria larvae (58.4 to 72.2 percent). Similar high ash content occurred in gelatinous holoplanktonic forms also. The carbohydrate content of 0.6 to 1.2 percent was slightly higher than that of penaeid larvae and tornaria larvae and lesser than coelenterate larvae (1.1 to 2.1 percent). The variable lipid content from 7.4 to 22.6 percent agreed with that observed in Cirripedia larvae (14.1 to 23.2 percent). The other meroplanktonic larvae also had a highly variable lipid content. While general zooplankton analyses showed a high protein content (53 to 64 percent) sipunculid larvae gave a low protein content of only 7.9 to 18.1 percent similar to penaeid larvae (13.2 to 28.3 percent). Most of the meroplankton also had a higher protein content. Peebles and Fox (1933) and Gross (1954) found the water content of adult _Dendrostomum zostericolum_ varying from 80 to 85 percent of the wet weight. Also they found slightly lower water content in larger, hence older specimens. Adolph (1936) reported 88 percent water content in _Golfingia gouldi_. Thus the water content in larval forms might have been very high but for the fat globules present. Adolph (1936) also reported _Golfingia gouldi_ adult containing 3.9 percent wet

weight (32.5 percent dry weight) of lipid content. Wilber (1947) observed after a month without food the lipid content falling to 0.8 percent, being lost mainly from the coelomic fluid, not from the body wall. The sipunculoid larvae too after spending some time in plankton just before metamorphosis might undergo a sort of starvation, thus resulting in very low lipid, protein and carbohydrate contents as observed in the last two analyses. This has lead to the conclusion that body wall is not made of lipids. As the cuticle did not dissolve in hot potassium hydroxide, it cannot be chitinous. So the major protein fraction may be derived from the body wall and musculature.

## Cirripedia larvae

Cirripede larvae generally completing their development up to the settling stage (cypris) in 7 to 9 days, sometimes leads a planktonic life up to 2 months. Many of these cirriped nauplii are non-feeding type up to post-cypris stage. These feeding larvae use flagellates, diatoms etc as food. The main food-catching organ of cirripede larvae is the antenna (Moyse and Knight-Jones, 1967). It bears delicate plumose setae and setules, which form a close-meshed filtering area in the basal region of the endopodites. The fine food particles collected are scraped by the setae of the mandible endopodites and pushed into the mouth by the gnathobases and neighbouring structures. In the later naupliar stages, the

very long processes trailing posteriorly increase the volume of water filtered retaining particles as small as 5 microns. The above mentioned feeding mechanisms suggest use of different feed items, in increasing concentrations as the larvae grow. So in the estimation of different biochemical fractions of these larvae, difference occurs in their concentrations. Thus the lipid fraction declines from the very high level in the first naupliar stage to stage IV nauplius and cypris in the non-feeding larvae. But in the feeding larvae, lipid content increases from the cypris stage onwards. The type of organisms fed also controls the amount of biochemical fractions as lipid, protein and carbohydrate.

Barnes (1965) studied the biochemistry of cirripede eggs. During development he noted a net loss of all fractions, and small increases of glucose and soluble nitrogen, at certain stages. The protein content of eggs, varying from 56 to 61 percent, was definitely more or less equal to 53-62 percent protein present in adults. The larval protein also showed more or less same amount in the present study. But the lower limit reached 38.4 percent is probably in less fed ones. The lipid fraction of egg showed slightly higher values than the adult. But the larvae showed very low values indicating liberal use of lipid in the non-feeding early stages. The lipid content got reduced by 30-45 percent. Carbohydrate fraction of the larvae showed more or less same content as in the egg and adult of cirripeds. Claus et al.(1979) estimated biochemical composition of the larvae of Artemia salina starved

for 48 hours and fed on two different algal foods. During a 48 hours starvation period the percent of carbohydrate and lipid of freshly hatched larvae decreased from 11.24 to 10.37 and 25.53 to 21.95 percent respectively, whereas the ash content increased by 40 to 100 percent, from 8.17 to 19.97 percent. When fed, the protein level of the larvae increased significantly from 8.17 to 10.15 percent and the relative increase in ash content from 8.17 to 10.15 percent, was lower than in the case of starvation. On feeding, the increase in organic content surpasses the increase in inorganic content, which result in low percent of ash content. Similarly cirripede larva also showed major reduction in lipid content, and slight lowering in carbohydrate content. Protein content was highest compared to other factors, an increase similar to that observed in fed ones occurred in the post-cypris stage.

Ash content varied considerably from 8.0 to 19.2 percent. The higher ash content is an index of low lipid value.

Meroplanktonic decapod larvae

These larvae remain in plankton up to 80 days or more before reaching the juvenile stage, depending on the availability of suitable food. Many early larvae are predominantly herbivorous, later becoming carnivorous. The decapod larvae, atleast in their older stages, seem to live exclusively on animal food (Lebour, 1933). As larval development proceeds, structural modifications occur in the feeding appendages, these modi-

fications parallel changes in feeding habit. Several natantians have an abbreviated development or possess larvae which have a large yolk supply and require no, or only little, feeding prior to metamorphosis. Generally the naupliar stages do not feed. The specimens analysed for biochemical fractions consisted of naupliar, protozoea, mysis and postlarval stages, numbering about 13 or more. Certain penaeids with larger eggs hatch directly into predatory zoea. Few larvae attain up to 6 cm.

The crab and lobster larvae belonging to Decapoda Reptantia generally must take up external food. The phyllosoma larvae of lobsters survive up to 114 days. After several months the puerulus stage has attained an intermediate phase between pelagic and bottom life having 1.5 cm in size. Phyllosoma is generally cannibalistic. The average length of time required for larvae of crab to pass through planktonic stage is 28 days. They feed on phyto and zooplankters. Frank *et al.* (1975) raised *Rhithropanopeus harrisii* larvae with *Artemia salina* nauplii and reported on biochemical changes (Protein, total lipid, alkaline phosphates and glutamic oxaloacetic transaminase) during larval development.

| Larval stages | zoea 1 | zoea 2 | zoea 2 | zoea 4 | megalopa |
|---|---|---|---|---|---|
| Protein (Percent wet wt.) | 3.4 | 3.1 | 4.1 | 6.3 | 6.6 |
| Lipid (Percent wet wt.) | 3.8 | 2.9 | 6.4 | 6.8 | 8.4 |

The above authors noted protein and lipid content of larvae increasing during the 2nd, 3rd and 4th zoea stages. Increase in 3rd stage indicates nutritional requirements prior to metamorphosis. Biochemical changes may be a more relevant indicator of growth during larval development than either size increase or moult frequency, when assessing the value of various diets in the culture of crabs.

In the present analysis protein and lipid content as percentage dry weight varied considerably among crab larvae but the carbohydrate remained constant. The lipid content variation was considerably high and might be a picture of premoulting stage requiring high lipid content for moulting. Protein content increased in megalopa stage, indicating growth by intensive feeding. A reduction in ash content of megalopa observed was in relation to an increase in the protein content. The lipid depletion during the development of larvae from egg ranged from 11.5 to 60 percent of initial reserves (Frank et al., 1975). In crustacea, lipids are the only principal organic reserve and source of metabolic energy. The increase in lipid content in zoea during development from 25.2 to 42.1 percent may reflect metabolic preparation for the critical metamorphic period. According to Passano (1960) the size increase associated with crustacean moulting can be attributed to water uptake during and immediately after ecdyses. The dry weight during this period is considerably reduced due to very high water content. Tissue production as indicated by an increase in protein content

and accumulation of organic reserves occur after size increase. This cyclic phenomenon characteristic of adult crustaceans occur during larval development of all crabs as evidenced by the changes in protein. The amount of carbohydrate found expressed as percentage of dry weight did not vary much. In view of such small differences no correlation could be observed between the carbohydrate content and the size of larvae.

## Meroplanktonic molluscs

Majority of lamellibranch and entire prosobranch larvae are planktotrophic. All these larvae show external features and an organisation very similar to those of the parent animals. Thus the lamellibranchiate larvae within 48 hours of hatching are protected by fully developed bivalve shells and the prosobranchiate larvae by the twined shells as found in the adults. The only difference of these larvae from the adults is the provision in larval life of a floating and swimming device, the velum. Typically, marine bivalves have two free swimming larval stages. the trochophore and the veliger (similar to gastropod veliger but asymmetrical). The intertidal oyster and other bivalve larvae have a planktonic life of up to 21 days. Larval growth to a large extent depended upon the food available. Majority are suspension feeders on a variety of unicellular algae. Most molluscan larvae are herbivorous, some latter becoming omnivorous or more rarely carnivorous. It has been stated that in _Ostrea virginica_ (Prytherch, 1924) and

*Ostrea gigas* (Fujita, 1934) which feed on the plankton, feeding does not begin until a certain degree of larval development has been reached. These larvae have accordingly at first a lecithotrophic stage, and the length of their planktotrophic larval life is shorter than their lecithotrophic life. Bivalve larva starts metamorphosing on reaching a length of 200 to 250 microns. The meroplanktonic bivalves usually reach a size varying from several microns to 2 millimetres.

The highly calcified larval shells contribute to a major portion of inorganic carbon. Hence the organic matter present is less and vary from 20 to 40 percent of dry weight. (It has to be remembered that in view of the small size, the shells are not separated before analyses). The ability of bivalve larvae to withstand starvation and resume normal growth when food becomes available is well known (Bayne, 1965; Millar and Scott, 1967). Thus during food scarcity energy is supplied by the protein, lipid and carbohydrate as shown in the table. In six analyses the protein content varied from 8.2 to 14.1 percent followed by carbohydrate content ranging from 3.6 to 10.1 percent and fat content varying from 1.4 to 3.4 percent. These low values may represent larvae with less food consumed. But in the rest 3 analyses the protein content gave high values up to 25.1 percent. The lipid and carbohydrate respectively showed high values of 5.8 and 9.2 percent. The carbohydrate content was 2 to 5 times higher than lipid values. These 3 collections may be the well fed ones.

Beer (1966) noted low carbohydrate contents in planktonic pteropods, averaging less than 1.12 percent of dry weight. Compared to the very low carbohydrate occurring in temperate zooplankton, bivalve larvae had a higher content (10 percent) higher than other tropical zooplankton. Millar and Scot (1967) found loss of carbohydrate, in relation to the initial amount present in oyster larvae during the initial stages of starvation. Also they found that of the three types of substrates, quantitatively, carbohydrates are the least important. Collyer (1957) found 13.4 - 15.4 percent of the dry weight of organic matter in oyster larvae to be glycogen and showed that the viability of larvae, when fed under normal cultural conditions, was not related to varying amounts of glycogen. The carbohydrate in benthic adults constitutes an important reserve material. Russel (1923) and Le Gall (1947) got a carbohydrate content of 27 to 34.9 percent in benthic adults. Gopalakrishnan *et al.* (1977) got 14.1 percent carbohydrate in benthic bivalve, *Meretrix casta*.

Millar and Scot (1967) observed in oyster larvae loss of protein independent of the amount initially present, at times of food scarcity and starvation. The variations in protein content from 8.2 to 23.1 percent during the present study may reflect such loss of protein. The protein content in the benthic adult bivalves (Russell, 1923; Le Gall, 1947; Ansell *et al.*, 1973; Gopalakrishnan *et al.*, 1977) gave lower values compared to the larvae.

Millar and Scott (1967) observed oyster larvae with 15.2 to 44.6 percent of organic matter as triglyceride, using lipid for energy than protein and carbohydrate. But in the adult oysters with only an average lipid content of 9 percent, it is found less important, though in the female phase the adult oysters contained more lipid than male phase. In the present studies the lecithotrophic larvae released from eggs with high lipid content, possessed large amounts of lipid, carrying from eggs. Lee and Hirota (1973) obtained low lipid values (2-3 percent) for holoplanktonic *Sagitta* sp. and *Clio* sp. and very high lipid values (40 percent) for *Gaussia* sp. and (31 percent) for *Clione limacina* (Lee, 1974).

Wafar (1974) estimated biochemical composition of two Eulamellibranch bivalves, *Meretrix casta* and *Sanguinolaria diphos*. The water content of *Meretrix casta* (81.96 percent) and *Sanguinolaria diphos* (80.32 percent) were similar to those mentioned by Vinogradov (1953) for *Ostrea edulis* (78.70 - 87.36 percent), for *Mya arenaria* (83.46 percent), *Pecten irradians* (80.32 percent) and *Venus mercenaria* (84.56 percent). Srinivasan and Krishnaswamy (1964) reported water content of 77 percent in *Martesia fragilis*. Similarly high values were reported by Ansell *et al.* (1973) for *Donax* sp. Venkataraman and Chari (1951) found water content of *Meretrix casta* of Ennore backwaters varying from 73.18 to 84.02 percent whereas Gopalakrishnan *et al.* (1977) reported an average water content of 66.3 percent only. Thus for the same species 3 authors got three different values.

Similar variation in the water content was observed in the bivalve larvae in the present studies too. The low percent of water content observed in the present studies was due to inclusion of shells in the analysis.

## Echinoderm larvae

The bilaterally organised echinoderm larvae develop in plankton until they become more and more radially organised, metamorphose and settle down to a bottom life. These larvae have a larger gap between the larval stages and the adult stage. Their pelagic life lasts from 1 week to 3 months. The lecithotrophic pelagic larvae hatching out from large sized eggs will take no nourishment from the plankton, but will develop until metamorphosis exclusively on their own content of yolk. Their planktonic stage is only of value for the distribution of the species, and is of no importance as a feeding stage. However, the decapod larvae are known to feed regularly on larval echinoderms. These lecithotrophic larvae have a very high (68.2 percent) lipid content initially which go on declining as the larvae grow (up to 23.7 percent). While a gradual decrease in fat content occurs as the larvae grows the protein content remains more or less same. Thus a reduction in lipid content in proportion to an increase in protein content as a mirror image, commonly noticed in holoplankton, does not take place in larval echinoderms. The carbohydrate content more or less

remained constant, varying from 2.2 to 3.1 percent. A very high growth rate could not be observed in this study since majority of yolk is used for maintenance rather than production of body substance. The early pluteus stage reached in 2 to 6 days after fertilization, metamorphoses in 20 to 30 days. The late pluteus larvae required food in order to continue their development. Similar to this, many echinoderm larvae have a pelagic, planktotrophic larvae with a long pelagic life up to 3 months. These larvae are generally small in size as they develop from very small eggs poor in yolk. They totally depend on the food conditions in the plankton. In view of their small size two third of what they consume is used up for metabolic energy, and thus very little is deposited as body protein until they metamorphose. The analytical data showing very low levels of protein (18.8 to 20.9 percent) and lipid content (13.5 to 18.3 percent) may represent these planktotrophic pelagic larvae. The last three sets of data pertained to small sized larvae are assumed to be planktotrophic. The carbohydrate content was comparatively low. The ash content was low in the larvae with large amount of yolk. But the planktotrophic larvae had a higher ash content. The larvae with their naked surface exposed to the surrounding water, imparts them a very high water content (up to 96 percent). On drying, the water escapes and carries along with it a large proportion of inorganic salts, dissolved in it. Thus during the analysis though an ash content of below 27 percent was

obtained actually the larvae possess a higher ash content. Also the high concentration of organic constituents is due to concentration of organic portion while the water content escapes during drying. These planktotrophic larvae with a dry weight ratio of 1-4 percent, due to high water content included in its dry weight a relatively large amount of biologically inert ash derived from the salt content. The lecithotrophic larvae with a dry weight of 4 to 16 percent have only a low ash content.

## Tornaria larvae

These are found constantly increasing in size and the very high water content imparts it a clear glassy appearance. The large blastocoel contains scarcely any organ except the digestive tract, conferring on them a low specific gravity, which necessitates constant swimming to prevent sinking. Thus the tornaria as it requires large amounts of metabolic energy, the organic matter present as reserve is very low. Also they are able to feed only on minute planktonic organisms as such deriving the minimum energy only. As the tornaria ages, they get reduced in size with the onset of metamorphosis. These features are well reflected in their biochemical compositions. Thus high water content and ash content as encountered among tunicates and ctenophores (70 percent) are noted in the sipunculoid larvae also. The high ash content in bivalve larvae was due to the shell material whereas in tornaria it was

due to high inorganic salt content in body water. Compared to other zooplankton, lipid content is very low. Similar low lipid content was observed in bivalve larvae. In the aged tornaria due to reduction in size accompanied by reduction in water content, protein content increased, with a fall in ash content. The dry weight ratio is lower than the range 10-20 percent noted for copepods, euphausids and similar planktonic organisms.

Fish larvae

Compared to the low water (75 percent) content, high ash content (5 percent) and high fat content indicated by a relative higher calorific content (6500 cal/g dry organic substance) per unit egg weight in demersal fish eggs, pelagic fish eggs are characterised by a high percentage of water content (91 percent) low ash content (1 percent) and a low fat content indicated by low calorific content (5000 cal/g dry organic substance) (Lasker 1962; Mengi, 1965; Paffenhofer and Rosenthal, 1968; Fluchter and Pandian, 1968). According to Sverdrup et al. (1942) high water content and small size may be considered as a special adaptation to pelagic life. In the present study fish eggs in general collected from estuarine and coastal waters were analys and got a water content of 85 percent with an ash content of 1.2 percent. The tropical eggs on an average have a lipid content of 68.6 percent. Immediately after hatching, the larvae of most marine fishes exist on yolk reserves, they can

feed only after their eyes, mouth parts and intestine have become functional, i.e. usually shortly before the yolk is completely absorbed. Due to minute size of tropical fish larvae yolk sac was smaller compared to those in temperate waters. In the Engraulidae larvae (Hunter, 1972) yolk absorption, come to an end in the four day old larvae and complete metamorphosis (35 mm) in 74 days. *Solea solea* larvae begin to feed 3 or 4 days after hatching (Fluchter, 1965; Rosenthal, 1966). The truely pelagic phase of the larvae is limited to a few days after the end of yolk sac stage. Larvae of *Belone belone* begin to feed when less than half of their yolk is absorbed (Rosenthal and Fonds, 1973). Fish larvae are characterised often by their specific nutritional requirements and very limited capacity for bridging even short period of starvation. Mortality of fish larvae due to starvation at the end of the yolk sac stage seems indeed to play a major role. Once the larvae had started feeding well, mortality is decreased. Younger larvae require higher feed concentrations (Hunter, 1972) than older ones, which can command larger potential food resources. Rosenthal (1969b) estimated an increase in the number of copepod nauplii from 30 to 120 required by the clupea larva as it grew in length from 10 to 14 mm. Prey catching success in fish larvae increases with age. In *Clupea* sp. the prey catching success increases from an initial one percent to nearly 60 percent within 30 to 35 days (Rosenthal, 1969a). Within one and the same species, the degree of selectivity tends to increase

with age, size and/or experience. The planktonic fish larvae feed on micro-organisms, protozoans, planktonic algae, planktonic animals and non-living food items. In the absence of sufficient food, fish larvae reach a "point of no return" at which only 50 percent of the larvae are still able to feed if sufficient food becomes available (Blaxter and Ehrlich, 1974). Also during advanced stage of starvation a tendency to head-heaviness increase due to an increase in hypo-osmotic body water as well as a decrease in body protein. Finally osmotic regulation fails and the larvae dehydrate. The above observations reveal the presence of fish larvae in plankton under the influence of a number of variables which control the biochemical fractions of fish larvae.

Nair *et al.* (1975) got average values of 63.9, 21.1 and 2.8 percent for the protein, lipid and carbohydrate portion respectively for fish larvae collected from estuarine waters. Fish is supposed to be one of the most valuable sources of high grade protein available to man and this protein in fish flesh constitute 20 to 85 percent. Das (1978) got protein content in *Mugil cephalus* varying from 15.8 to 18.64 percent. In the fish larvae in which major portion of yolk is present the protein content is 19.2 percent which goes on increasing up to 74.9 percent until the larvae leave the meroplanktonic life. The protein content is considerably low in the lecithotrophic larvae (19.2 to 22.3 percent).

The fat content varied much more widely than the water, protein or mineral content. While the ratio of the highest to the lowest value of protein or water content is not more than three to one, the ratio between highest and lowest fat values is more than 300 to one in adult fishes (0.1 to 31 percent wet weight). While the lipid content of lecithotrophic larvae is 68.4 percent, the lipid content go on reducing as the larvae exhaust its yolk sac (4.1 percent). Juvenile <u>Gonostomatidae</u> sp. gave a lipid content of 13 to 22 percent (Lee, <u>et al.</u>, 1971) and 9-16 percent (Lee & Hirota, 1973). Also they found lipid content in various species of juvenile fish ranging from 6 to 41 percent. Fish larvae also showed a very high fluctuation in their lipid content (68.4 to 4.1 percent). Fatty fishes like sardines and mackerels are found having a seasonal variation in fat content varying from 2.0 to 50 percent Fat content in <u>Mugil cephalus</u> (Das, 1978) from Goan waters varie between 2.11 and 6 percent. As the fat content rises, the water content falls, and vice versa; the sum of water and fat in fatty fish is fairly constant at about 80 percent of wet weight. Similar changes occur in the larval fishes also. As the larvae use lipid, the weight lost is balanced by the water absorbed.

The amount of carbohydrate is generally too small to be of any significance. Beer (1966) analysing fish and fish larvae from Sargasso Sea showed a variation in carbohydrate content from 0.36 to 1.38 percent. Das (1978) found the carbohydrate content in both the sexes of <u>Mugil cephalus</u> fluctuating between

0.80 and 2.80 (average 1.73) percent. Nair *et al.* (1975) obtained a carbohydrate content of 2.8 percent for fish larvae from Cochin backwaters. The fish larvae analysed during the present studies too provided such low carbohydrate values (1.9 to 2.9percent) indicating the insignificant role of the carbohydrate metabolism during larval development.

While the ash content of fishes in general varied from 1 to [illegible] percent, in the present study ash content fluctuated between 1.2 and 6.9 percent and it seems that this did not have any relationship with other factors.

Nair *et al.* (1975) got only 10.1 percent of wet weight as moisture content for the fish larvae analysed from Cochin backwaters. Das (1978) found moisture content in adult *Mugil cephalus* varying from 71.95 to 76.11 percent of wet weight similar to the observations made by various authors in other adult fishes (Chari, 1948; Arevalo, 1949; Parulekar and Bal, 1969 and Thomson, 1966). Generally the fish flesh had a water content between the extremes of 30 and 90 percent wet weight. In cod fish water content is usually found increasing with a decrease in protein during spawning time and thus the condition of fish is estimated by measuring water content.

In the present studies, the low water content of 11.4 percent of wet weight occurred in the newly hatched fish larvae with full yolk and as the yolk is used up the water content increased to 78.4 percent, and the larvae become planktotrophic.

There after the water content fluctuated between 68 and 78 percent of wet weight. It is not clear, whether the entire increase in water content is due to the absorption of water and/or due to the retention of metabolic water. Oxidation of different nutrient matter releases varying quantities of metabolic water. Thus the water content rises as the fat content falls.

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## 3. ZOOGEOGRAPHY.

### 3.1. Introduction.

#### 3.1.1. Scope and purpose of study of IIOE Zooplankton.

The survey of the early concepts of oceanographic phenomena of the Indian Ocean indicates that in the course of centuries empirical knowledge pertaining to certain oceanographic features of the Indian Ocean had reached a sufficient level to be of practical use even during the time of early civilizations. Thus they knew about the winds, the waves, the tides, the currents, monsoon phenomena, and the reversal of winds as proved by the early maritime contacts between India and Mediterranean countries.

Indian Ocean including the Red Sea and Persian Gulf with its 75 million square kilometres covering 14.0 percent of the earth's surface is the least explored of the world oceans. A number of expeditions have collected oceanographic data in the Indian Ocean and incorporated them in their reports. The more important of these expeditions are Novara (1857-'59), Challenger (1873-'74), Gazelle (1874-'76), Elisabeth (1887), Investigator (1887, 1892, 1893, 1925-'38), Penguin (1891), Waterwitch (1895), Stork (1897), Valdivia (1898-'99), Gauss (1902-'03), Sealark (1905-'09), Siboga (1906), Planet (1906-'07), Mowe (1912-13), Merlin (1920),

Amiraglio Magnaghi (1924), Ommando (1927), W. Snellius (1929), Dana (1928-'30), Mabahiss (1933-'34), Discovery II (1930-'31), Albatross (1947-'48), Charcot (1948-'49), William Scoresby (1935, 1936, 1950), Galathea (1950-'52), Ob (1955-'57), Laperouse (1956), Norsel (1955, 1956, 1957), Umitaka Maru (1956), Oven (1957, 1958), Atlantis (1959), Vityaz (1960) and Gaillard (1961).

Of these Sealark and Mabahiss are the only expeditions which spent considerable time in a detailed investigation of some parts of the Indian Ocean. The reports of these expeditions and the papers published by Sewell (1913) in the Journal of the Royal Asiatic Society of Bengal constituted the early important source of oceanographic data for the Indian seas.

Inspite of the above works involving 34 expeditions and about 1300 stations vast areas in the Indian Ocean remained unexplored. So the International Council for the Exploration of Sea recommended a large scale comprehensive study that involved methodically collected samples from a wider area. Based on this, Scientific Committee on Oceanic Research of the ICSU, in 1958-'59 initiated a programme which materialised in the International Indian Ocean Expedition (IIOE), held during 1960-'65 involving 75 million rupees and traversing 291,000 kilometres of water. UNESCO through the Intergovernmental Oceanographic Commission joined the endeavour by co-sponsoring the expedition and establishing the Indian Ocean Biological

Centre (IOBC) at Cochin in 1962 for processing the zooplankton collections made during the IIOE.

A study of the marine pelagic food web involving the five trophic levels - phytoplankton, zooplankton (herbivores), zooplankton (carnivores), fish (planktivores), fish (piscivores), illustrates the important role of marine plankton community especially zooplankton consisting of mero and holoplankton. But as mentioned above our previous knowledge on the distribution of marine plankton, in the Indian Ocean is mainly based on single voyages across the ocean. Therefore the earlier biogeographers found it difficult to state precisely the faunal provinces and relative abundance in each.

One of the main objectives of the IIOE was the study of the qualitative and quantitative distribution (zoogeography) and speciation (diversity) of planktonic organisms. The distributional studies of this sort will also be helpful in (1) evaluating the adaptation of plankton to physical, chemical and ecological properties of the environment and to know more about their community structure, ecology and behaviour and species diversity, (2) proper determination of the species (identification keys) of various plankton groups that occur in this area of investigation and point out their ecophenotypic variations in the different water masses if any, (3) to deduce taxonomic features characteristic of larvae of particular species, so as to trace phases of phylogenetic significance and to draw evidence from larval ontogeny in confirming classification,

(4) to estimate their frequency of occurrence and abundance to some extent in relation to the diurnal, seasonal and annual variations, (5) to elucidate the longitudinal and latitudinal zonation (variation) of zooplankton, (6) to explain the pattern of distribution as tracing the faunal provinces (zoogeographical regions) in relation to hydrological parameters as temperature, salinity, oxygen concentration, nitrate and phosphate concentrations of water masses and (7) to correlate their distribution pattern with the physical factors of the environment as upwelling, eddies, thermocline, light, depth, turbidity, hydrodynamics, seasonal reversal of monsoon circulation and large gyral oceanic streams.

The meroplanktonic distributive stages of marine animals are affected by a combination of several environmental factors as temperature and next in importance salinity resulting in direct and indirect effects. Survival of individual stages leading to population maintenance, extension of species range occupying new niches and inhibition of metamorphosis beyond the time when optimal environmental factors are maximal for survival of juveniles, contribute to direct effects. Excessive mortality and extended planktonic life caused by unfavourable environmental factors leading to food shortage amounts to indirect effect. Also their zoogeography is controlled by the prevailing currents and the seasonal changes related to monsoons and upwelling. But no attempt has so far been made to study in detail the fluctuations in intensity, distribution

and composition of these larvae in relation to the environ-mental factors.

However, in the present study the scope has been restricted to a general treatment of the distribution and fluctuation of larvae of anthozoa, cirripedia, sipunculoidea and gastropoda in the entire Indian Ocean and of all meroplanktonic larvae along the pelagic ecosystems of 78°E and 110°E. Also their distributional aspects in relation to the thermocline during day and night in the western Indian Ocean has been studied.

3.1.2. <u>Survey of literature</u>.

<u>Studies on meroplankton from the coastal waters around India</u>: A bibliography of plankton of the Indian Ocean prepared by Prasad (1964) cites 881 publications. It would appear from the above papers mostly dealing with the systematics of the different holoplanktonic groups with frequent reference to hydrography that we have only a very meagre account of the meroplankton species from coastal and oceanic waters around India. The early studies on meroplankton concerning chiefly with the taxonomic and developmental aspects are available only as scattered descriptions along with their general plankton studies as follows: The earliest study dates back to Menon (1931) who gave a preliminary account of the Madras plankton followed by Aiyar *et al.* (1936) who described the plankton records for the year 1929 and 1930 off Madras. Also Panikkar and Aiyar

(1939) and Paul (1942) made observations on breeding in brackish water animals of Madras. Menon (1945) studied the seasonal distribution of the plankton of the Travancore coast. Prasad *et al.* (1952) and Prasad (1954, 1956) have given accounts of the plankton in the nearby Gulf of Mannar. Ganapati and Rao (1958) made a quantitative study of plankton off Lawson's Bay. George (1958) made observations on the plankton off the Cochin backwaters, while George (1953) described marine plankton off the coastal waters of Calicut. Mukundan (1967) reported on the plankton of Calicut inshore waters. Chacko (1950, 1956 and 1959) has given an account of the plankton in and around Krusadi Islands. Bal and Pradhan (1945 and 1952) made a preliminary note on the plankton of Bombay Harbour.

While the above studies gave brief remarks about the seasonal distribution of larval forms off Madras, Lawson's Bay, Krusadi, Gulf of Mannar, Trivandrum, Cochin, Calicut and Bombay, specific meroplanktonic taxa have been dealt with by only few researchers. Prasad (1954) made observations on the distribution and fluctuation of planktonic larvae off Mandapam. Menon (1914) made a preliminary note on the metamorphosis of zoanthella. Panikkar (1936, 1937, 1938 and 1947) and Kumari (1954) gave general accounts of the anthozoan larvae. Nair (1944 and 1950) dealt with larval Ceriantharia. Ganapati and Lakshmana Rao (1958, 1962) dealt with Scyphistoma larvae. Devanesan and Varadarajan (1940) have reported on the occurrence of Tornaria larvae at Krusadi. Sudarsan (1958) studied the

Brachiopod larvae from the west coast of India. Gravely (1927a and b) described Pilidium and Actinotrocha larvae from Palk Bay and Gulf of Mannar respectively. Menon (1931, 1933, 1937, 1940, 1949 and 1951) and Prasad and Tampi (1953) gave accounts of decapod larvae from the Madras plankton. Of these, the work of Menon (1951) describing all the larval stages of *Metapenaeus dobsoni* Miers from the plankton is significant as it formed a pioneer work. Recent studies on penaeid larvae were made by Subrahmanyam (1965), Mohamed *et al.* (1968), Paulinose (1974) and Gopalakrishnan (1976). Raje and Ranade (1972) described developmental stages of *Metapenaeus monoceros* and *Penaeus merguiensis* from laboratory culture. Rao (1973) described larval stages of *Parapenaeopsis stylifera*. George and Goswami (1977) made observations on the larvae of penaeid prawns of commercial importance in the coastal waters of Goa. Pillai (1955) reported on decapod larvae of Travancore. Chhapgar (1956) described larval stages of some crabs of Bombay. Alikunhi (1944a, b, 1949, 1950, 1951 and 1967) and Alikunhi and Aiyar (1942 and 1943) gave accounts of the metamorphosis of phyllosoma and stomatopod larvae from the Madras plankton. Prasad and Tampi (1957, 1959) dealt with phyllosomas of Mandapam and Laccadive Seas respectively. And Mohamed *et al.* (1971) described the first phyllosoma stage of the Indian deep-sea spiny lobster. Aiyar (1933 and 1935) dealt with polychaete larvae of Madras coast while Ganapati and Radhakrishna (1958) gave an account of the polychaete larvae in the plankton off the

Waltair coast. Gopinath (1942, 1946) studied the concentration of post-larval fishes along the Trivandrum coast during November-March period. Menon (1945) found fish eggs and larvae most common off Trivandrum coast from September to November and May to July. George (1953) noted scarcity of fish eggs and larvae during the monsoon months off Calicut. Mukundan (1967) observed abundance of fish eggs and larvae off Calicut from August to December. George (1979) made studies on the distribution and abundance of fish eggs and larvae off the south-west coast of India with special reference to scombroids, collected during 1971-'75. Venkataramanujam and Ramamurthi (1974, 1977) dealt with seasonal variation in fish eggs and larvae of Porto Novo coastal waters. Nair (1946, 1947 & 1952) studied the fish eggs and larvae of the Madras plankton. Bal and Pradhan (1945, 1946, 1947 and 1951) studied fish eggs and larvae collected in zooplankton during 1944-'47 from Bombay waters. Balakrishnan (1959, 1961, 1963, 1969 and 1971), Balakrishnan and Devi (1974) Bilpey (1977), Premalatha (1977) and Sreekumari (1977) gave accounts of certain groups of fish larvae from the Kerala coast. Ganapati and Raju (1961a, b; 1963) and Raju and Ganapati (1969) made studies on fish eggs and larvae from Waltair coast and Bay of Bengal respectively. Jones and Kumaran (1964 b) reported on eggs and larvae of scombroid fishes.

<u>Studies on zooplankton of the Indian Ocean:</u> The results of the International Indian Ocean Expedition have been published by UNESCO in the form of IIOE collected reprints, Volumes 1 to

during 1965-'72. In addition, Indian Ocean Biological Centre has published Atlases, Volumes I to V (1968-'73) showing zones of occurrence and abundance of zooplankton. Indian Ocean Biological Centre has also issued a series of Handbooks, Volume I to V (1969-'73) based on the International Zooplankton Collections.

Recent papers include those read at the following four Symposia;

1. NISI/INCOR Symposium on "Indian Ocean" held in New Delhi (1967).
2. The International Symposium on "Indian Ocean and adjacent seas, their origin, sciences and resources", conducted at Cochin (1971).
3. The Biology of the Indian Ocean held in Kiel (1971), a
4. On Warm Water Zooplankton held in Goa (1976).

Studies on meroplankton from the Indian Ocean are mainly on larval forms of economic importance collected during the IIOE.

One of the notable early works on meroplankton is that of Delsman (1921-'38) on fish eggs and larvae of the Java Sea. Later Jones and Kumaran (1963, 1964a) based on "Dana" material (1928-'30) from the Indian Ocean gave an account of the distribution of tuna and billfish larvae in the area.

Peter (1969a) recorded the very early larval stages of mackerel - Rastrelliger kanagurta from the Indian Ocean. Balasubramanyan et al. (1969) from the larval stages studied

indicated that flying fishes spawn around the coasts of Ceylon. Devi (1977) studied the distribution of the larvae of flat fish (Heterosomata) in the Indian Ocean. Ueyanagi (1969) and Peter (1977) made observations on the distribution of tuna larvae in the Indian Ocean and Arabian Sea respectively. Peter (1969b) reported on the density of fish eggs and larvae of the Indian Ocean and (1974) studied seasonal variation of ichthyo-plankton in the Arabian Sea in relation to monsoon. Nellen (1973) dealt with the fish larvae in the Arabian Sea and the Persian Gulf based on "Meteor" collections, 1964-'65. Also Ali Khan (1972) studied distribution of fish larvae in the Gulf of Aden and off the coast of West Pakistan based on materi collected during 1964-'67. Silas (1974) based on larval stag of mackerel collected by "Varuna" in 1964 prepared distribution maps in the south-west coast of India and Laccadive Sea.

Saraswathy (1972) described the distribution of Indian Ocean bivalve larvae. Aravindakshan and Sakthivel (1973) located cephalopod nurseries in the Indian Ocean.

Ranjamma and Gopalakrishnan (1977) described the distribution of tornaria and echinoderm larvae in the Indian Ocean, whereas Nair (1974) observed discontinuity in the distribution of phoronid and brachiopod larvae in the Indian Ocean.

Most important papers published on crustaceans are: Menon et al. (1969) made a preliminary note on decapod larvae of Arabian Sea based on expedition material. Menon and

Williamson (1971) studied larval stages of *Thalassocaris* spp., while Menon (1972) studied larval development of *Heterocarpus* sp. George and Paulinose (1973) traced larval history of pasiphaeid prawn *Leptochela robusta*. Paulinose (1973) studied the developmental stages of *Penaeopsis rectacuta* and (1979) made a detailed study on taxonomy, distribution and developmental stages of 22 species of penaeid larvae from the Indian Ocean.

Studies on phyllosomas from Laccadive Seas, Arabian Sea and Indian Ocean are limited to Prasad and Tampi (1959, 1960 and 1966). Tampi (1971) and Tampi and George (1975) dealt with the systematics of the 84 specimens of phyllosoma larvae, belonging to most of the commercially important spiny lobsters of the Indian Ocean region, sorted out from the International Indian Ocean Expedition collections. Studying their distribution, the authors noted paucity of larvae from near the coastal areas of India and Ceylon.

## 3.2. Material and Methods.

### 3.2.1. Material.

The material dealt with during the present study was collected during the IIOE (1960-'65). The collection was made mainly from the upper 200 m area occupying only 5 percent of the total volume of Indian Ocean estimated to be 29195 x $10^4 m^3$ (Pollak, 1958). Only 18 research vessels belonging to 9 nations participated in the biological (zooplankton collection) programme of the International Indian Ocean

Expedition during 1960-'65. The major part of the collections (90 percent) was made during 1962 (386 samples), 1963 (743 samples) and 1964 (586 samples). The number of collections made by each ship during this period is given in Table VII. The number of zooplankton samples collected during 72 cruises and received at the Indian Ocean Biological Centre amounts to 1927 excluding 218 samples from Agulhas Bank. The station list and their environmental data are published by IOBC (1969-'71).

Geographical coverage: Among the 1927 plankton samples only 1906 are from the Indian Ocean area. The limit of Indian Ocean is assumed to be from 30°N to 40°S and 20°E to 120°E excluding South China Sea; 21 samples are outside this limit.

An attempt made to illustrate the density of stations of the IIOE collections in terms of spatial, diurnal, hourly and seasonal coverage revealed their heterogeneous distribution, as a result of lack of co-ordination for simultaneous observations. The Table VIII shows the density of observations in the 7 zones of the Indian Ocean based on day and night collecti and seasonal collections - April to September and October to M

Bay of Bengal had the maximum density of collections in terms of area and number - Bay of Bengal covering 6.26 percent area only having 352 collections compared to the Arabian Sea covering 12 percent area with only 420 collections. Numerical of the 1927 collections, 22 percent were collected from the Arabian Sea, 18.5 percent from the Bay of Bengal, 19.6 percen

TABLE VII : The participant nations and the research vessels in the IIOE Programme, the general area explored and the number of samples collected during 1960-'65.

| Country | Ship | 1960 | 1961 | 1962 | 1963 | 1964 | 1965 | Night samples | Day samples | NE Monsoon samples | SW Monsoon samples | Total | Area explored |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Australia | Diamantina | - | - | 36 | 107 | 36 | 19 | 97 | 101 | 34 | 164 | 198 | South-east Indian Ocean. |
| " | Gascoyne | - | - | 15 | 32 | - | - | 21 | 26 | 32 | 15 | 47 | Along the meridian 110°E. |
| " | Patanela | - | - | - | - | 21 | 5 | 25 | 1 | 26 | - | 26 | Bet. 60°-115°E, 33°-51°S. |
| Germany | Meteor | - | - | - | - | 41 | 81 | 51 | 71 | 122 | - | 122 | Gulf of Aden, Somalia, Kenya & west coast of India. |
| India | Conch | - | - | - | 6 | - | - | 1 | 5 | 6 | - | 6 | Off Cochin. |
| " | Kistna | - | - | 39 | 153 | 77 | 83 | 176 | 176 | 204 | 148 | 352 | Bay of Bengal, Arabian Sea and central equatorial region. |
| " | Varuna | - | - | - | 71 | - | - | 28 | 43 | 20 | 51 | 71 | West coast of India. |
| Japan | Kagoshima Maru | - | - | - | 24 | 8 | - | 31 | 1 | 32 | - | 32 | Meridian 78°E & 86°E, bet. 8°N & 25°S. |

TABLE VII (Contd.)

| Country | Ship | 1960 | 1961 | 1962 | 1963 | 1964 | 1965 | Night samples | Day samples | NE Monsoon samples | SW Monsoon samples | Total | Area explored |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Japan | Koyo Maru | - | - | 23 | 18 | 6 | - | 46 | 1 | 47 | - | 47 | Meridian 94°E & 100°E, bet. 8°N-20°S. |
| " | Oshoro Maru | - | - | 16 | 45 | 21 | 2 | 33 | 51 | 84 | - | 84 | Off Sumatra & off Java. |
| " | Umitaka Maru | - | - | 13 | 20 | 15 | - | 17 | 31 | 48 | - | 48 | Meridian 78°E, 100°-120°E, 5°-25°S. |
| Pakistan | Zulfiquar | - | - | - | - | 22 | - | 13 | 9 | 22 | - | 22 | Off Pakistan. |
| S. Africa | Natal | - | - | 100 | 35 | - | - | 54 | 81 | 63 | 72 | 135 | Southeast African coast. |
| Soviet Union | Vitiaz | 14 | 3 | 30 | - | 7 | 5 | 34 | 45 | 37 | 42 | 79 | 70°-95°E and 10°N-35°S. |
| U.K. | Discovery | - | - | - | 41 | 162 | - | 111 | 92 | 33 | 170 | 203 | 40°-70°E and 20°N-20°S. |
| U.S.A. | Anton Bruun | - | - | - | 191 | 118 | - | 89 | 220 | 139 | 170 | 309 | Bay of Bengal, Arabian Sea & western half of S. Indian Ocean. |
| " | Argo | - | - | 94 | - | 12 | - | 53 | 53 | - | 106 | 196 | Somali coast, bet. 45°E-100°E, 6°N-6°S. |
| " | Pioneer | - | - | - | - | 40 | - | 40 | - | 4 | 36 | 40 | 80°-105°E, 21°N-6°S. |
| Toal | 18 | | | | | | | 920 | 1007 | 953 | 974 | 1927 | |

TABLE VIII : Density of IIOE Zooplankton observations on selected zones: Comparison between Day and Night and two seasons.

| Area | Limit from | to | No. of 1° sq. | % | Total No. of collections | % | Day No. of collections | % | Night No. of collections | % | April-Sept. No. of collections | % | Oct-March No. of collections | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Arabian Sea | 30°N 32°E | 5°N 78°E | 501 | 11.934 | 420 | 22.00 | 253 | 25.46 | 187 | 20.48 | 168 | 16.96 | 252 | 27.51 |
| 2. Bay of Bengal | 25°N 78°E | 5°N 101°E | 263 | 6.265 | 352 | 18.46 | 165 | 16.61 | 187 | 20.48 | 218 | 22.02 | 134 | 14.62 |
| 3. Equatorial | 5°N 39°E | 5°S 104°E | 589 | 13.934 | 374 | 19.62 | 177 | 17.82 | 197 | 21.57 | 196 | 19.79 | 178 | 19.43 |
| 4. S. West | 5°S 20°E | 40°S 55°E | 716 | 17.055 | 200 | 10.49 | 126 | 12.68 | 74 | 8.10 | 108 | 10.90 | 92 | 10.04 |
| 5. S. East | 5°S 100°E | 40°S 120°E | 574 | 13.673 | 322 | 16.89 | 178 | 17.92 | 144 | 15.77 | 173 | 17.47 | 149 | 16.26 |
| 6. South Central (North) | 5°S 55°E | 20°S 100°E | 673 | 16.079 | 164 | 8.60 | 74 | 7.45 | 90 | 9.85 | 90 | 9.09 | 74 | 8.07 |
| 7. South Central (South) | 20°S 55°E | 40°S 100°E | 900 | 21.438 | 74 | 3.88 | 40 | 4.02 | 34 | 3.72 | 37 | 3.73 | 37 | 4.03 |
| | Total | | 4198 | 100.000 | 1906 | 100.00 | 993 | 100.00 | 913 | 100.00 | 990 | 100.00 | 916 | 100.00 |

from the equatorial zone, 10.5 percent from the south-west, 17 percent from the south-east and 12.5 percent from the south-central Indian Ocean though it occupied 38 percent of the Indian Ocean. But the northern Indian Ocean extending up to 5°N, and having 20 percent of total area had 38 percent of total collections. Latitudinally maximum number of samples (495) were collected from an area between 20°N and 10°N i.e. more than 25 percent of collections are from 10 percent area. Most of the observations are clustered along the coastal areas of India and South Arabia. This discrepancy in the sampling density will affect the outcome and reliability of biogeographical studies.

Day and Night coverage: Of the 1906 collections, 993 are made during day time and 913 during night. On an hourly basis early morning hours (0100-0500) and afternoon hours (1600 and 1800 hrs) have very few collections (45 to 56 and 55 and 57 respectively) and maximum collections (135, 121, 187 and 124 respectively) were made at 1000, 1100, 2000 and 2100 hours. Thus half of the Indian Ocean water has coverage only for one hour. Cassie (1963) showed that no two samples are comparable unless they are taken at the same time of day and under the same lighting conditions. Bogorov (1958) found zooplankton undergoing diurnal vertical migration to different depths and the main day level varying from species to species. This can introduce a disparity in the estimation of abundance since the organisms of

diurnal vertical migration have been incompletely sampled. The apparent absence of a species in such an area might also be due to lack of sampling at depth.

Monsoon coverage: An analysis of sample distribution in relation to monsoon changes though showed 655 collections during April to September and 615 collections during October-March in the area north of 10°S, the area sampled during one season (April to September) was not properly repeated in the other season (October to March) leading to considerable disparity. Studies on monthwise distribution of samples revealed that December, January, April, July and August had 185-228 collections while November and June had only 111-117 collections.

Special cruises: Collections made during 8 cruises - Discovery 3/64, Umitaka Maru cruise 23/62, Kagoshima Maru cruise 3/63-64, Diamantina cruise 1/63, 2/63, 3/63 and Gascoyne 4/62 and 1/63 were used for zooplankton distribution studies in relation to the thermocline, the annual variation along 78°E and the seasonal variation along 110°E (Fig. 5). Though Discovery made collections from 62 stations (35 night + 27 day) to study the effect of thermocline, the 200 - 0 m haul and the thermocline to 0 m were not made simultaneously which might have resulted in sampling different areas.

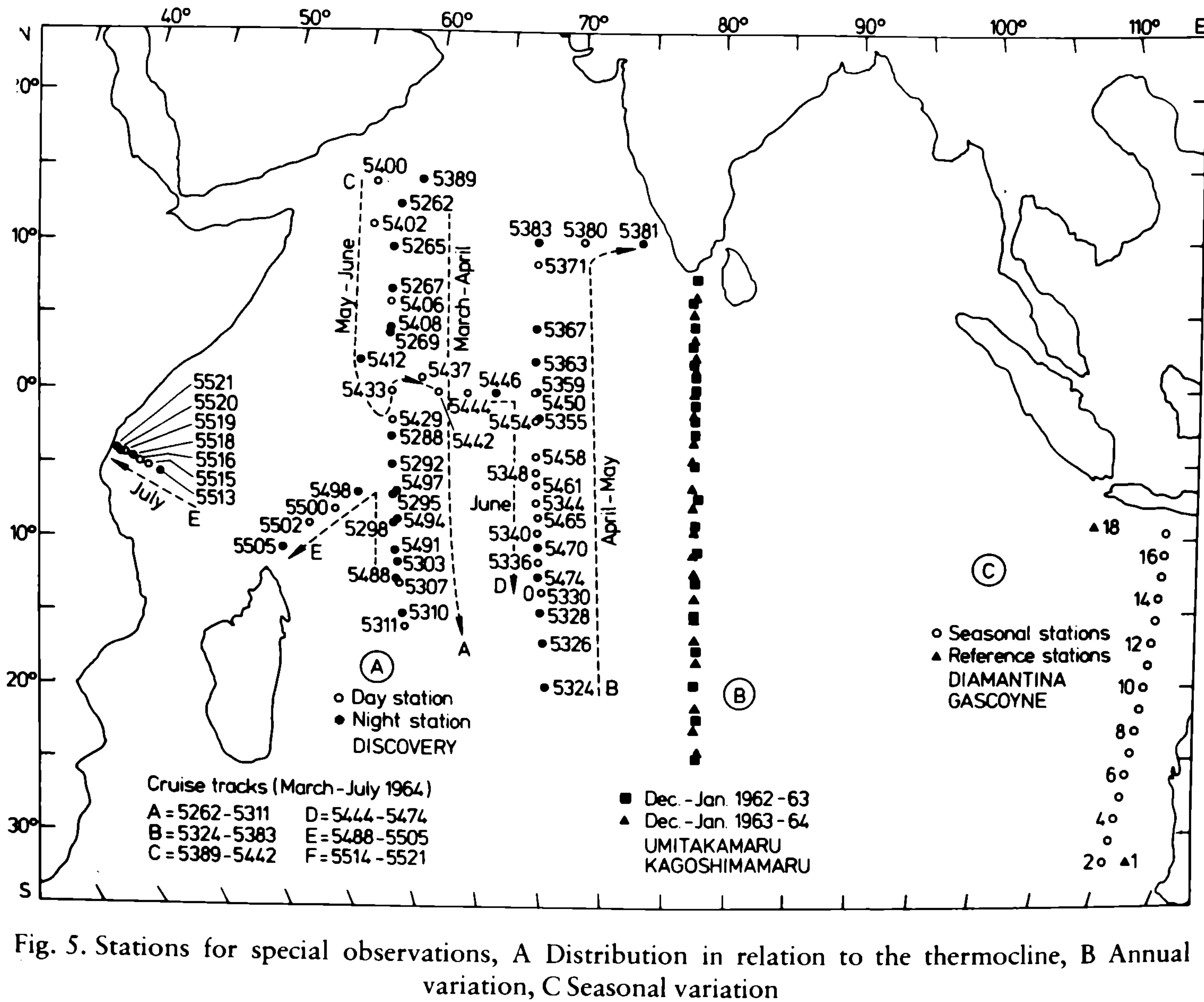



Fig. 5. Stations for special observations, A Distribution in relation to the thermocline, B Annual variation, C Seasonal variation

3.2.2. <u>Methods</u>.

Sakthivel (1972) and Sakthivel and Rao (1973) have made a critical study of the methods employed during the IIOE zooplankton collections. Based on proposals, made by a team of zooplankton experts who met at Cochin in 1961, that a standard sampling device is necessary for uniformity of collections for quantitative studies, Currie (1963) designed the Indian Ocean Standard net. Prior to this, in the absence of a standard gear, different types of nets - organdie net, Juday net, N 70 V net and 75 m net were used by different ships. Table IX gives the number of collections made by different types of gear from different depths of haul illustrating zooplankton sampling variability.

The Indian Ocean Standard net is a low-speed net ($<3$ knots) made of medium gauge (nylon gauze having a mesh width of 330 microns and porosity of 0.46). This ring net having a mouth area of 1 square metre and an open area ratio of 4.3 has a cylinder-cone form with a total length of 5 metres. <u>Motoda et al.</u> (1963) measured the filtration efficiency at 70-90 percent (0.96). Therefore a vertical haul from 200 m would filter approximately 192 $m^3$ of water. According to Tranter and Smith (1968) the net is not likely to clog when hauled vertically, in central water masses, but may do so when the wire angle is high or the water rich in plankton. The net was hauled from stationary or drifting ships vertically without a flow-meter from a depth of 200 m to the surface at an average speed of 1 m

TABLE IX : IIOE Zooplankton sampling variability.

| Gear | Mouth Diameter (cm) | Mesh width (micron) | Number of collections made<br>Wire out (m)<br>10-50 | 51-100 | 101-199 | 200 | 201-300 | 301-400 | 401-680 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| IOSN | 113 | 330 | 78 | 104 | 35 | 1047 | 433 | 29 | 2 | 1728 |
| Organdie | 50 | 60 | 1 | 1 | - | 104 | 4 | 2 | - | 112 |
| N-70 | 70 | 540<br>240 | 4 | 5 | 4 | 49 | 2 | - | - | 64 |
| Juday | 113-80 | 170 | - | - | 15 | 1 | 4 | 1 | 1 | 22 |
| 75 N | 75 | 60 | - | - | - | 1 | - | - | - | 1 |
| Total samples | | | 83 | 110 | 54 | 1202 | 443 | 32 | 3 | 1927 |

per second using 4 mm wire cable in deep waters and in coastal waters where the sounding is less than 200 m, a vertical haul from bottom to surface was preferred. While few ships made collections from 200 m to the surface, others paid out more wire to compensate for the wire angle measured. 247 coastal collections include 83 at a depth lesser than 54 m, 110 in the range 50 to 100 m and 54 in the range of 100 to 200 m depth. Discovery made collections from the thermocline to surface and these depths varied from 30 to 120 m. 478 collections had wire paid out more than 200 m, 443 collections at the range between 200 to 300 m, 32 collections between 300 and 400 m and 3 collections in the range 400 to 880 m depth. 1202 collections were taken with only 200 m wire paid out. In view of the considerable sampling variabilities noted, at IOBC, samples were classified as standard and nonstandard. Thus 1548 samples were classified as standard and 379 as nonstandard. A standard sample can be defined as the plankton in the water column under 1 $m^2$, the stratum samples being the upper 200 m in deep water and the entire water column where the sounding is less than 200 m. A non-standard sample is one which was taken in deep water from strata considerably shallower or deeper than 200 m ($\pm$ 30%), one taken at considerable wire angle ( $>45°$) and also one taken with a non-standard net. Juday net with a mouth area of 1 $m^2$ had the shape of a reduction cone-cone form. Having a mesh width of 0.17 mm, it had a porosity of 0.32 and open area ratio of 4.2 (Bogorov, 1959). This fine gauze net was used on board Vitiaz during its 31st and 33rd cruises and

collected 22 samples. N 70 net used during cruises Natal I and IV collected 64 samples. It has a mouth area of 0.37 $m^2$, with a cone form. According to Foxton (1956) this net with a mesh width of 170 microns had a porosity of 0.32 and an open area ratio of 2.4. Organdie net was used during the cruises of Kistna II, III and part of IV, V, VIA and VIB cruises and collected 112 samples. This ring net having 0.5 m diameter had a mesh width of 0.2 mm. The 75 m net with mouth diameter of 75 cm and mesh width of 60 microns was used to collect only one sample during Anton Bruun cruise I.

The zooplankton samples generally fixed and preserved in 4 percent formaldehyde buffered with hexamine were sent to IOBC, Cochin. Although there were certain standard procedures adopted for fixation and preservation of samples, a good percentage of samples had undergone deterioration resulting in the pigment disappearance, dissolution of skeletal matter and disintegration of soft parts as already dealt with in the first part.

At the table, displacement volume was measured after which the larger organisms, fish eggs and larvae were removed. Rest of the sample was subsampled either with a Lea's plankton fractionator (Wiborg, 1951) or by a Folsom plankton splitter (McEwen *et al.*, 1954 and Gopalakrishnan, 1973). Only aliquots (3 to 5 ml or 90 percent of small samples) were sorted out into various taxa retaining the rest as archives. The macroplankton which emerged from the sorting excluding those studied by others formed the material for the present study.

Reliability of samples: The drawbacks noted in the collection methods are (1) IOSN may tend to clog when the wire angle is high (Tranter and Smith, 1968) or the water is rich in plankton. (2) Flow meter was not used during collections. (3) Limitation of the depth of haul to 200 m made the day hauls incomplete for most of the diurnal migratory species. So Banse (1964) has suggested desirability of a hydrologically meaningful depth. (4) The uneven distribution of stations and unexplored areas in between affect abundance estimation. (5) Fluctuating monsoon can lead to wrong interpretation. (6) Fractionation and sub-sampling procedures can introduce large scale errors. (7) According to Cushing (1962) the patchiness can introduce large errors in the estimation of abundance. (8) According to Banse (1964) the population density obtained from a haul with a net of 1 square metre may hardly occur along the path of net. (9) According to Clutter and Anraku (1968) avoidance of samplers by plankton and Vannucci (1968) loss of organisms through the meshes can affect proper sampling of a particular water column. (10) Variability in kinds, size, mobility, abundance and distribution of marine zooplankton precludes use of the same sampler and methodology for all purposes. But during the IIOE the decision to use only IOSN restricts the precision of data dealing with abundance and occurrence.

Presentation of data: The Indian Ocean is a seasonal ocean (Ramage, 1969; Wyrtki, 1973). Hence for a meaningful presentation of data, the entire collections were split on a seasonal basis to study the seasonal variation in the occurrence and abundance. In view of the limited collections the year was split into 2 seasons only i.e. the SW monsoon dominated period from April 16 to October 15 and the NE monsoon dominated period from October 16 to April 15. South of 10°S these periods correspond to winter and summer. Prasad (1968) found this division more meaningful in the biomass studies of zooplankton. A south-north section was chosen for the seasonal study along the 110°E meridian. Non-standard samples were included in the study to have wide geographical coverage of stations in terms of occurrence rather than abundance. As the species undergo diurnal migrations, night and day collections were separately analysed. For distribution and abundance studies, it was recommended that samples be compared on the basis of catch per unit standard haul (IOBC, 1969) and not on the number in volume of water filtered, since flow meter was not used and wire angle was recorded only in few cases. So, the total number of meroplankton taxa were estimated per unit standard haul from each station and plotted on a map. When stations were clustered or repeated average value was taken.

Numerical abundance was used to indicate the main centre of distribution. For contouring, four ranges (1-9, 10-99, 100-999 and >1000 per unit standard haul) were chosen to indicate

the different grades of density and this helped to nullify the varying depth of hauls. While open circles indicated absence of organisms, in these stations, closed circles indicated presence of organisms. As the number of stations sampled during an expedition can hardly be considered a representative neither in space nor in time, the data can give only incidental information. Thus Sakthivel (1972 and 1977) found 6 cruises sampling the 110°E repeatedly, having 20 species of euthecosomes whereas other areas which are sampled only once had a lesser number of species. But based on the encouraging results of Sakthivel (1972 and 1977) on pteropods this study was extended to meroplankton taxa also.

The meridional zone 78°E controlled by 5 water masses (Uda, 1966) was sampled (23 collections) by Umitaka Maru in December-January 1962-'63 and by Kagoshima Maru (20 collections) in December-January 1963-'64 in order to study the distributional pattern of plankton in different water masses and their annual variation. Certain meroplankton taxa sorted out from the 43 samples collected from this area were used for this purpose.

Between 32°S and 9°S along the meridian 110°E six biological cruises were conducted (Rochford, 1969) at a monthly interval of 2 months during August 1962 to August 1963. Sixteen stations, each at a distance of 145 km apart along the track were sampled twice per cruise - on the way north and on the way south once in the evening and another in the morning hours. Part of the samples collected during Ga 4/62 and all samples of

Dm 4/62 by Clark Bumpus Samplers (Tranter, 1966) were not sorted at IOBC. The rest of the 133 samples were used for studying seasonal, diurnal and latitudinal variations of meroplankton (except a few taxa) along 110°E. The details of sampling with field data are given in the cruise reports (CSIRO, 1965a, b, c, d and 1966a, b). Tranter and Kerr (1969) and Tranter (1973) dealt with this ecosystem along 110°E meridian in detail.

During March-August 1964 as part of the IIOE, R.R.S. Discovery sampled 62 stations in the western half of the Indian Ocean (area under the monsoonal influence) in order to study the distribution of meoplankton in relation to the thermocline (Fig. 5). This is the first study providing information on vertical distribution of certain meroplanktonic groups in relation to the thermocline. According to the period and area of collection the 62 stations are grouped under 6 cruise tracks - A, B, C, D, E and F. Of the total of 62 stations 35 are in night and 27 in the day. Two samples, one from 200 m to the surface and the other from the thermocline (30 m to 120 m deep) to the surface, with 6 to 12 minutes interval between hauls, are collected from each station. One of the serious drawbacks in these collections is that both the collections were made in separate hauls at different times, hence the possibility of sampling different area.

Some of the groups such as crustacean larvae, especially of decapod and trochophore larvae were not dealt with in the present studies.

## 3.3. General distribution of Anthozoa, Sipunculoidea, Cirripedia and Gastropoda.

For general distribution studies only the above four taxa were dealt with since rest of meroplankton taxa in the IIOE collections were studied by others. However, for specific studies as done under 3.4, 3.5 and 3.6, taxa whose general distribution have been studied by others also were included as they have not covered these aspects.

### 3.3.1. Larvae of Anthozoa.

Anthozoan larvae constitute an important collection by virtue of their number and their scientific interest. These larvae whose adults are not identified are classified into 3 orders namely Actiniaria, Zoanthidea and Ceriantharia, of which Ceriantharia consists of 31 genera and 75 species. Within the limits of the vast ocean zone explored by IIOE, the large majority of the planktonic larvae are obtained from neritic area of the Indian Ocean. These neritic larvae strictly adapted to pelagic life, floating passively, almost motionless at the surface of water, can be diverted to far off places. The transportation by the currents explain the widespread horizontal distribution of some larvae even if their representatives are generally few per station. Many of these mixed epiplanktonic stages with or without vitelline remains may pass their entire existence in the meroplankton or they may constitute normaly sedentary tubicle forms which have secondarily given up the sub-soil life.

The occurrence of anthozoan larvae according to nature of hauls is given in Table X.

TABLE X : Occurrence of anthozoan larvae according to category of hauls.

| Nature of hauls | No. of hauls | % of total No. of hauls | No. of hauls in which larvae are present. | % of hauls in which larvae are present. |
|---|---|---|---|---|
| Total | 1927 | 100.0 | 1065 | 55.0 |
| Standard | 1548 | 81.0 | 943 | 61.0 |
| Non-standard | 379 | 19.0 | 122 | 33.0 |
| Day | 1028 | 53.5 | 621 | 61.0 |
| Night | 892 | 46.5 | 444 | 50.0 |
| SW monsoon | 987 | 51.0 | 563 | 57.0 |
| NE monsoon | 939 | 49.0 | 502 | 54.5 |

Out of a possible total of 220 five degree squares in the Indian Ocean, collections are available from 170; of these, 39 squares were without larvae; 79 squares contained only 1 to 3 larvae; 29 squares contained 4 to 6 larvae; 16 squares contained 7 to 9 larvae; 4 squares contained 10 to 12 larvae; one square contained 13 to 18 larvae and only 2 squares had more than 18 larvae.

Numerical abundance, indicating population density in a statistical sense, ranged from 0 to 190 per haul. The maximum average representation in a five degree square is 20 and that

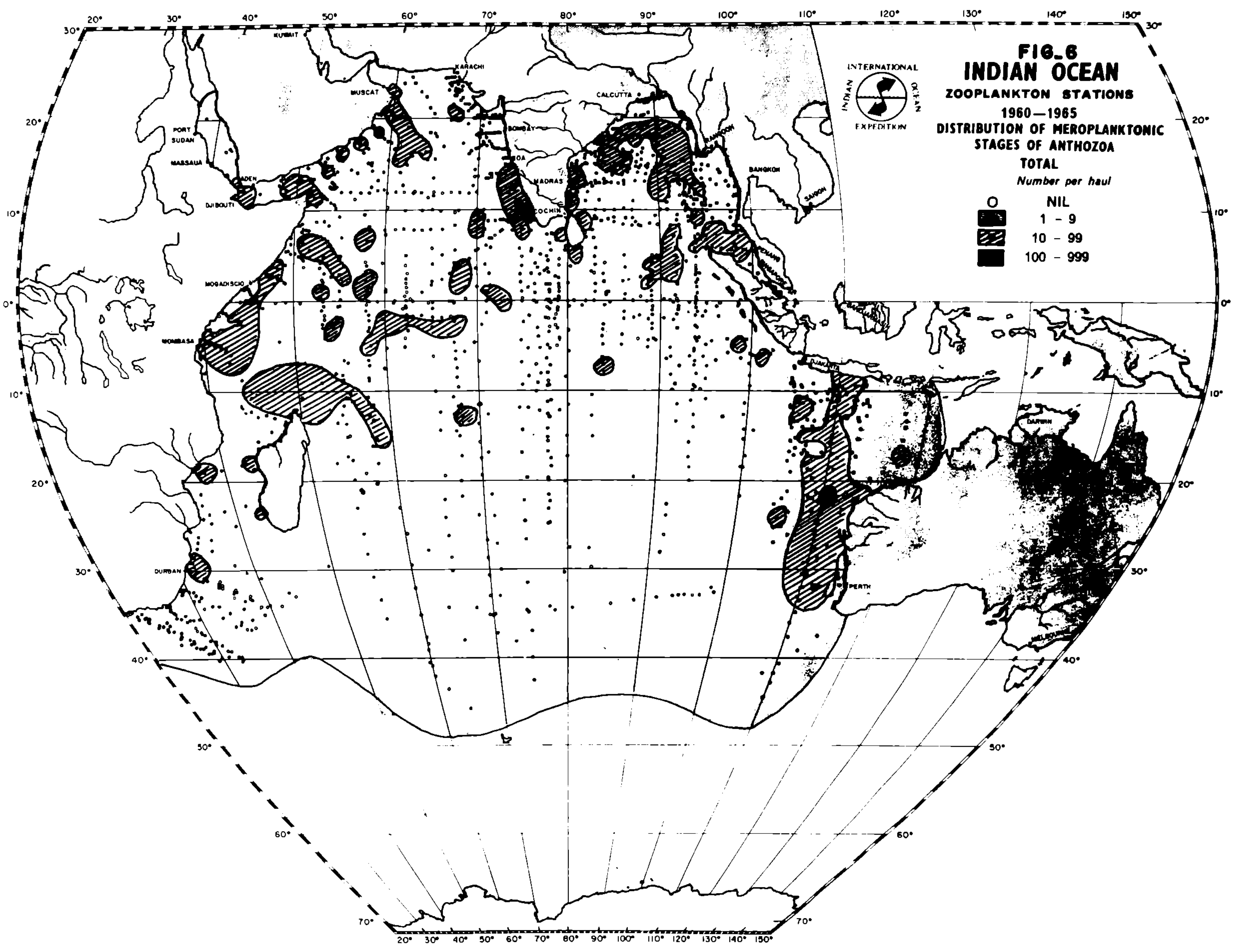


FIG_6
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
STAGES OF ANTHOZOA
TOTAL
Number per haul
NIL
1 - 9
10 - 99
100 - 999
INTERNATIONAL INDIAN OCEAN EXPEDITION
KUWAIT
KARACHI
MUSCAT
CALCUTTA
BOMBAY
GOA
MADRAS
COCHIN
BANGKOK
SAIGON
PORT SUDAN
MASSAUA
ADEN
DJIBOUTI
MOGADISCIO
MOMBASA
DJAKARTA
DARWIN
DURBAN
PERTH

of a 10° zone of latitude 16. Of the 1065 hauls which contained anthozoan larvae, only 71 hauls had more than 20. The 71 hauls had 2858 larvae, i.e. 40 per unit haul while the rest 994 hauls had 4881 larvae, hence an average of 5 per haul.

Areas of greater larval abundance are as shown in Figs. 6, 7 and 8 described below: (a) Along the west Australian coast mainly along 110°E longitude from 8°S to 34°S; (b) in the Gulf of Aden and off muscat; (c) off the coast of Somalia and Kenya, 5°S to 7°N; (d) off Durban; (e) North-east of Madagascar; (f) west coast of India; (g) around Ceylon; (h) in the central and western Bay of Bengal; (i) Andaman Sea and (j) isolated patches along the equatorial region of western Indian Ocean.

The Central Southern Indian Ocean is almost devoid of larvae. A gradual reduction is noticed from north to south in the oceanic region. The trend of distribution in general shows comparable high values towards the western and eastern sides of the Indian Ocean and along the Indian coasts. Thus larvae are more abundant in the neritic areas far from land. There is a relation between numbers of larvae and proximity to land. The larvae were completely absent in an area between 33°S and 46°S and 39°E and 114°E from where the "Patanela" collections were obtained.

Anthozoan larvae are present in 61 percent of night hauls and in only 50 percent of day hauls. The catch per night haul is 0 - 9 and per day haul 1 - 4, depending on area of catch and season and probably other factors that interfere with

vertical migration, e.g. stratification of water. Hauls made during "Diamantina" cruises 1/64 and 1/65 and "Kagoshima Maru" cruise 3, "Anton Bruun" cruises 2 and 5 indicate that the hour of collection is less important than the area of collection.

Of the hauls having more than 20 larvae, 43 night hauls (1725 larvae) averaged 40 per haul, and 28 day hauls (1133 larvae) averaged 40 per haul. This probably shows lack of diurnal effect. Though 30 percent of hauls are made at 2000 to 2100 hours, hauls made at 0100 to 0600 hours, 0800 to 1200 hours, 1500 to 1800 hours and 2200 to 2300 hours had fairly good catches, probably indicating an absence of hourly effect on larval catch. While one haul at 2100 hours had 190 larvae, one haul at 0900 hours had 112, indicating patchiness. Vanhoffen (1895) and Panikkar (1947) have noted the occurrence of _Arachnactis albida_ in swarms at the surface in day and night time respectively.

Water movements caused by wind and hurricane waves may carry the actiniarian larvae to distant places from its normal centre of distribution, thus contributing for a wider distribution in the neritic as well as oceanic region. This transportation of larvae to areas far away from where they are spawned creates considerable difficulty in locating their breeding centres. The larval presence noted thus in oceanic areas is known as expatriation areas.

Table XI shows total number of larvae obtained during the IIOE distributed monthwise. From the table it is clear that

peak catch is from August declining to a minimum in November and then increase with peaks in April and May. Cruise-wise analysis indicates that the area sampled rather than the month determine the presence of larvae. Thus while Anton Bruun cruise I collected an average of 10 larvae per haul in April 1963 from an area 13°N to 20°N and 87°E to 94°E, Anton Bruun cruise V collected only an average of 1 larva per haul in April 1964 from another area 6°N to 40°S and 74°E to 75°E.

TABLE XI : Total number of Anthozoan larvae obtained during the IIOE distributed monthwise.

| Month | No. of Marsden squares sampled | Total No. of hauls | Total No. larvae collected | Average catch/haul |
|---|---|---|---|---|
| January | 29 | 228 | 473 | 2 |
| February | 24 | 140 | 505 | 4 |
| March | 15 | 127 | 585 | 5 |
| April | 30 | 201 | 1,299 | 7 |
| May | 22 | 169 | 813 | 5 |
| June | 21 | 117 | 307 | 3 |
| July | 37 | 184 | 709 | 4 |
| August | 26 | 198 | 1,460 | 7 |
| September | 24 | 136 | 550 | 4 |
| October | 25 | 108 | 380 | 4 |
| November | 16 | 111 | 192 | 2 |
| December | 32 | 203 | 476 | 2 |
| Grand Total: | 301 | 1,922 | 7,749 | 4 |

FIG_7
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
STAGES OF ANTHOZOA.
DURING APRIL 16th – OCTOBER 15th

*Number per haul*

| Symbol | Number per haul |
|---|---|
| O | NIL |
| dot in box | 1 - 9 |
| hatched | 10 - 99 |
| solid | 100 - 999 |

In the Bay of Bengal there are 2 peaks of occurrence, one in April-May and the other one in July-August. In the western Arabian Sea the peaks are noticed in February and November and in the eastern Arabian Sea in July. In the equatorial area irregular peaks are noticed during the different months of the year, while November and December recorded poor catch. In the eastern Indian Ocean north of 30°S larvae are collected round the year in large numbers and the peak catch is noticed in August. In the western Indian Ocean the maximum abundance is found in February and August. In the south central Indian Ocean, larvae occurred in very few numbers. In general, the scarcity of anthozoan larvae are noticed in November and December while the peak is in August.

Gallagher (1966) based on the seasonal regime of currents, classified four periods in the Arabian Sea. The present observation on anthozoan larvae in the Arabian Sea during the above periods revealed the highest availability in spring transition and lowest in fall transition.

The larval distribution during the SW and NE monsoon periods is shown in Figs. 7 and 8. From Table X it can be noted that, in the SW monsoon period 51 percent of hauls and in NE monsoon period 54.5 percent hauls recorded anthozoan larvae probably indicating absence of monsoon effect. The distribution pattern during SW monsoon and NE monsoon is almost similar to the total distribution except for the wider area covered during SW monsoon. However, areas of abundance along 110°E

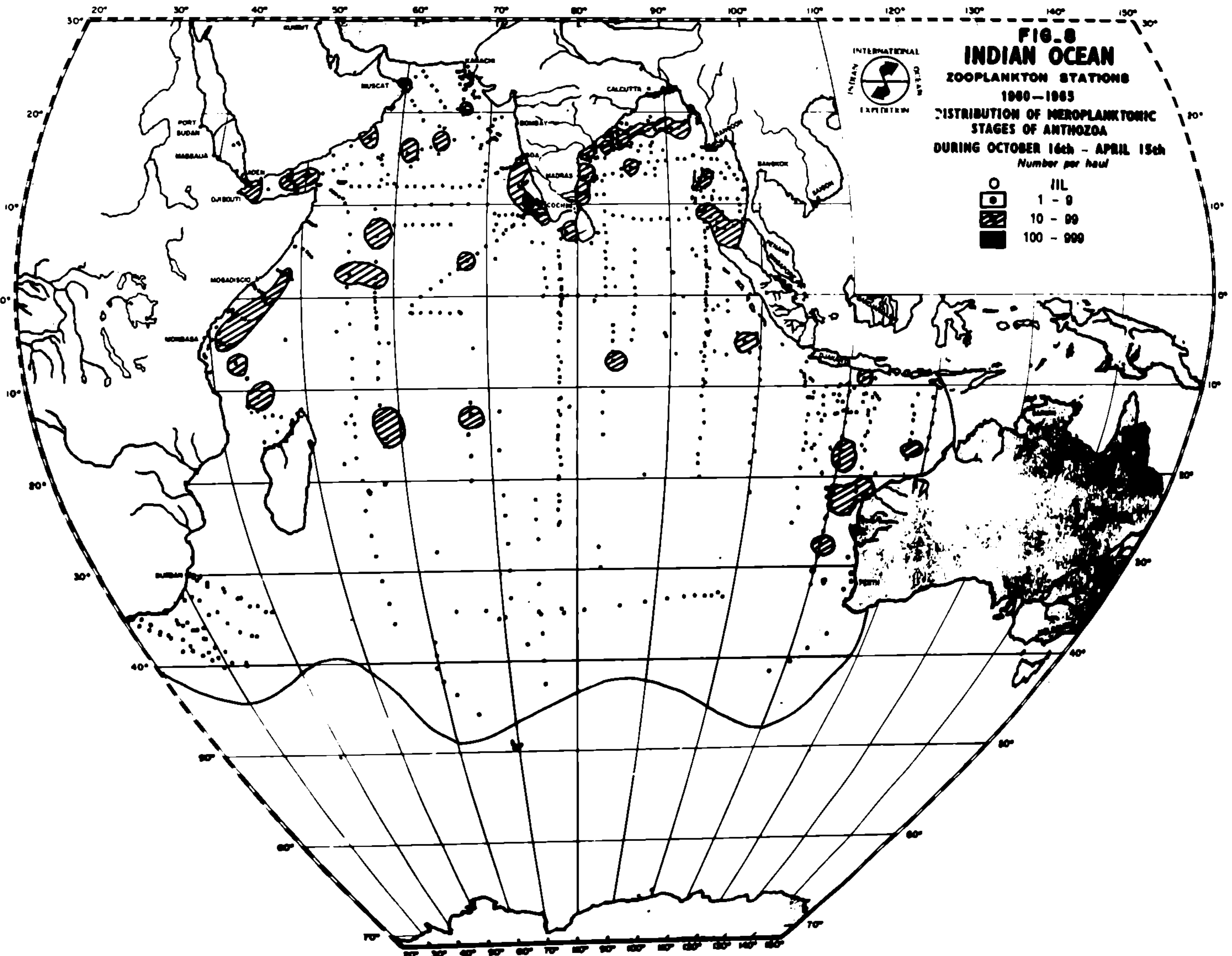


FIG. 8
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
STAGES OF ANTHOZOA
DURING OCTOBER 16th - APRIL 15th
Number per haul
NIL
1 - 9
10 - 99
100 - 999
INTERNATIONAL INDIAN OCEAN EXPEDITION
MUSCAT
KARACHI
BOMBAY
CALCUTTA
MADRAS
COCHIN
BANGKOK
PORT SUDAN
MASSAUA
ADEN
DJIBOUTI
MOGADISCIO
MOMBASA
DURBAN
PERTH

and north-east Madagascar is considerably reduced during NE monsoon.

Based on distribution of anthozoan larvae, Indian Ocean can be divided into 4 plankton regions: They are: (1) Pelagic ecosystem along 110°E; (2) Bay of Bengal waters; (3) Tropical zone (25°N to 10°S) and (4) Subtropical and transitional zones (10°S to 45°S).

3.3.2. Larvae of Sipunculoidea.

Sipunculoids assigned to 250 species under 13 genera (Fisher, 1952 and Edmonds, 1955, 1956) are exclusively marine benthos and are found in all seas except in brackish areas, at all latitudes, mostly in the littoral zone, but also extent into abyssal waters, to a depth of 5000 m. They have a wide distribution as they are adaptable to a range of temperature and depth. Wanting in polar waters, they are centred in warmer waters. The sipunculoids of Indo-West Pacific waters have been treated in detail in a series of articles. In view of such a distribution pattern for the adults, their larvae occurring in plankton collection also can be expected to have a wide distribution. Surprisingly larval sipunculoids are present only in 308 out of 1927 collections, making a small percent of 16 only. Larval distribution is shown in Fig. 9. Majority of larvae are collected from neritic waters on par with adult habitat. On a day/night basis they are present in 178 day and 130 night samples. Associated with the low frequency of occurrence their numerical

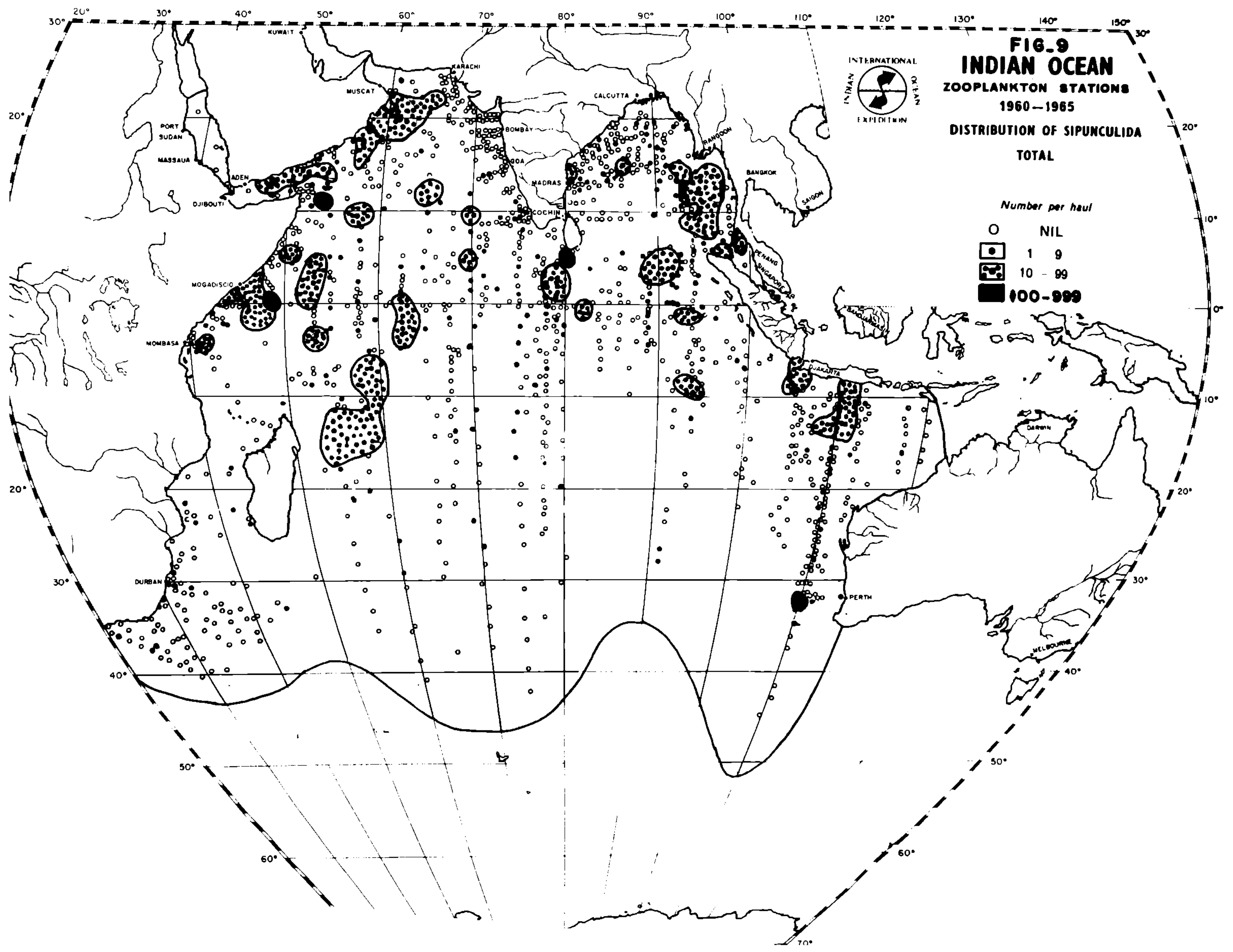


FIG.9
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF SIPUNCULIDA
TOTAL
INTERNATIONAL INDIAN OCEAN EXPEDITION
Number per haul
NIL
1 - 9
10 - 99
100-999
KUWAIT
MUSCAT
KARACHI
BOMBAY
GOA
MADRAS
COCHIN
CALCUTTA
RANGOON
BANGKOK
SAIGON
PENANG
SINGAPORE
DJAKARTA
DARWIN
PERTH
MELBOURNE
PORT SUDAN
MASSAUA
ADEN
DJIBOUTI
MOGADISCIO
MOMBASA
DURBAN

abundance also is low. 215 collections representing 70 percent of total had sipunculoidea larvae numbering 1 to 9 per haul, whereas 58 collections (19 percent) had larvae numbering 11 to 20 per haul and 35 samples making up 11 percent had larvae ranging from 21 to 660 per haul. The reason for this can be their restricted larval periods. Generally sipunculoids spawn in summer. The larvae generally slightly less than 1 mm in length, recorded first by Muller (1850) and described by Krohn (1851) swim around in the plankton for about a month. So only if the water column is sampled during this period the larvae can be collected in large numbers. Thus Fischer (1947) recorded a considerable number of Pelagosphaera larvae taken in plankton tows in the West Indian region during the spawning period. During the IIOE period the maximum number of 660 larvae per haul is collected by 'Diamantina' in a night collection in September from the south-west coast of Australia. But for this collection the pelagic ecosystem along meridian 110°E is poor in Sipunculoidea larvae. Larval concentrations of 14 to 37 numbers per haul occurred north of tropical boundary along 110° off Java coast. In the highly productive Bay of Bengal waters, enriched at regular intervals through land drainage in the monsoonal cycle, frequency of occurrence was more in Andaman Sea. Compared to the northern and southern Bay of Bengal the central Bay of Bengal had isolated patches along east coast of India from Madras to Visakhapatnam. But for the patchy occurrence off Cochin the eastern Arabian Sea waters had very few

sipunculoid larvae. The upwelling waters off Arabian and Muscat coasts and Gulf of Aden waters had a large number of larvae. An isolated patch of 180 larvae per haul is collected from a day collection in July off Mogadishu. But for this, along the African coasts from 10°N to 40°S, sipunculoid larvae occurred in few numbers. Along Somali coast only one day collection in December off Ras Hafun had 153 larvae per haul. Rest of the Somali coast between equator and 12°N had only poor representation. However upwelling waters off Somali coast had a different percentage of composition from that of Arabian Sea waters.

In the Southern Indian Ocean gyral waters sipunculoids are present rarely. Along north of equator and south of peninsular tip of India frequency of sipunculoid larval occurren is more. On a monthly basis 45 samples in August and 45 sample in September contained these larvae, while October, November and December months had larvae in 13, 11 and 19 samples respectively, indicating settlement of majority of larvae prior to October. Also of the total 3800 larvae collected 1160 are obtained during September.

Based on sipunculoid larval distribution 10 areas can be recognised in the Indian Ocean, even though the boundaries between these regions do not form inseparable barriers. These areas are: (1) Red Sea and Persian Gulf, (2) Arabian and Oman coasts in the West Arabian Sea up to 10°N as southern limit, (3) West coast of India and east Arabian Sea up to 10°N,

(4) Eastern Bay of Bengal waters, (5) Andaman Sea, (6) Equatorial Current region 10°N to 10°S, (7) Southern subtropical gyre and transitional zone 10°S to 45°S, (8) Agulhas Current area, (9) Off Sumatra and (10) West coast of Australia.

3.3.3. Larvae of Cirripedia.

Adult cirripeds except some parasites are the only sessile crustaceans specialised for suspension feeding mostly inhabiting marine and estuarine environments. They possess planktonic nauplius larvae with 1 to 4 stages developing into cypris to post-cypris stages. The food habitat of tropical, arcticboreal and oceanic species vary much. Depending on the variation in the environmental requirements the cirripeds produce one to several broods per breeding season and their larval development lasts from 1 to 2 weeks. The above factors decide the abundance and frequency of larval occurrence in plankton catches.

The low frequency of occurrence and low abundance in the catches is perhaps due to their minute size. Either they were inefficiently filtered with IOSN having 330 microns mesh size or they were not completely sorted out from the IIOE zooplankton samples. In general, warm water holds more larvae than cold water forms.

Of the 1927 samples, 45 percent had cirripede larvae. Fig. 10 shows distribution of cirripedia larvae. Numerically over 100 larvae are obtained only from about 20 stations, of which only 5 had more than 1000 larvae. A maximum of 1972 larvae

FIG_10
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF CIRRIPEDIA

INTERNATIONAL INDIAN OCEAN EXPEDITION

*Number per haul*

| Symbol | Number per haul |
| --- | --- |
| ○ | NIL |
| ⊡ | 1 - 9 |
| ▨ | 10 - 99 |
| ■ | 100 - 999 |
| ■ | Over 1000 |

KUWAIT
MUSCAT
KARACHI
BOMBAY
GOA
MADRAS
COCHIN
CALCUTTA
BANGKOK
SAIGON
PORT SUDAN
MASSAUA
ADEN
DJIBOUTI
MOGADISCIO
MOMBASA
DURBAN
DARWIN
PERTH
MELBOURNE

is obtained from Cape Agulhas in a night haul in October 1962. Also high larval concentrations are noted in 40 percent of 135 samples collected by 'Natal' during July, October and November 1962 and January 1963, from the South African coast. Thus the South African coast (Agulhas Bank) under the influence of Agulhas Current appears to be a very rich area for cirripede population. One night haul near northern tip of Somali coast in August 1964 made by 'Discovery' fetched 1450 larvae. Similarly one day haul in November 1964 made by 'Zulfiquar' off Karachi collected 1618 larvae. Oceanic waters compared to neritic waters are very poor as they had only 1 to 9 larvae per haul. Yet 'Vityaz' during its 36th cruise collected in a night haul in July 1963, 1017 larvae from a station from the South-Central Indian Ocean (34°S and 78°E).

Larval population in the range 100 - 999 per haul as isolated patches is found in Agulhas Bank area, near Aden in Red Sea, off Karachi, in the Andaman Sea, in the northern Bay of Bengal and near Kerguelen Island.

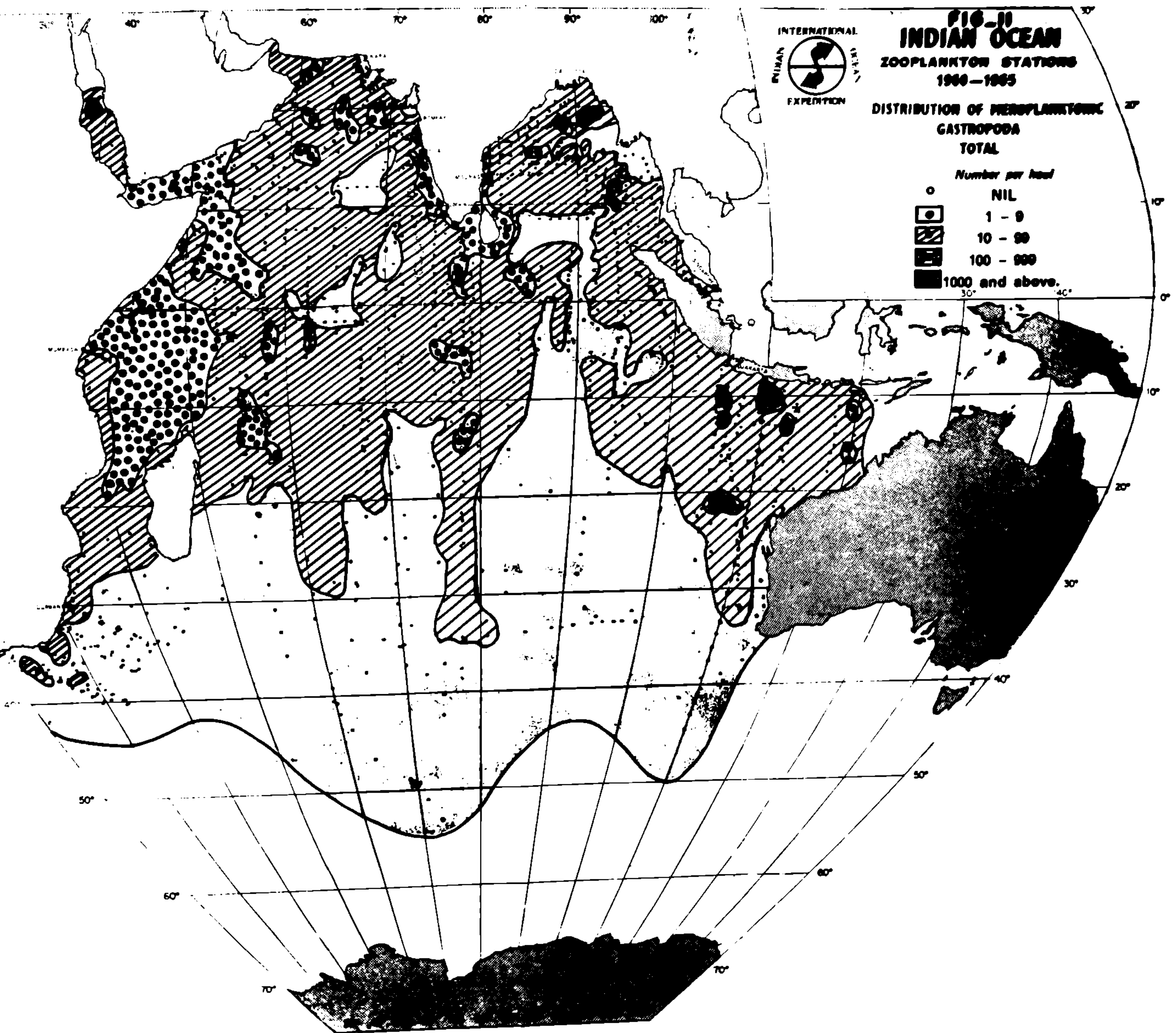
The larvae ranging in numbers from 10 - 99 per haul occurred in vast stretches along the coasts. The areas include Agulhas Bank, off Somali and Kenya coast, Red Sea and Gulf of Aden, off Muscat and Karachi, Maharashtra coast, off Cochin, off Madras, north-west Bay of Bengal, Andaman Sea, off Penang and south of Java. Rest of the Indian Ocean area had very poor representation and turned to be an impoverished region. Compar to the Arabian Sea, hydrological conditions in the Bay of Benga

seem to favour proliferation of adult cirripedia, leading to abundance of their meroplanktonic larvae. In general, the percentage of cirriped larvae to the total zooplankton is high in the upwelling waters and eutrophicated areas. Numerical abundance seems associated closely with the low saline waters of Bay of Bengal and high saline waters of Somali and Agulhas Current waters. Also the high production along Somali coast may be the result of prevailing geotrophic upwelling. Their scarcity along the west coast of India excluding off Cochin area may be due to the upslope of oxygen minimum layer entering inshore waters which is detrimental to the living organisms (Carruthers *et al.*, 1959).

Based on cirripede larval distribution, 6 areas are recognised in the Indian Ocean. They are: (1) Agulhas Current region, (2) Somali Current region extending to Gulf of Aden and Red Sea, (3) Bay of Bengal waters up to 15°N as southern limit, (4) Andaman Sea, (5) Java waters and (6) rest of the Indian Ocean including the subtropical and transitional zones.

### 3.3.4. Larvae of Gastropoda.

With over 36,000 species, the largest molluscan class, Gastropoda is not so commercially important as bivalvia and cephalopoda and so received less attention from zoogeographers. Our knowledge on distribution pattern of gastropods is very poor as they inhabit a great variety of biotopes in accordance with more variations in the environmental and nutritional requirements, changing in the course of life cycle. The meroplanktonic free swimming veliger larvae generally employing a



INTERNATIONAL
INDIAN
OCEAN
EXPEDITION
FIG-11
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
GASTROPODA
TOTAL
Number per haul
NIL
1 - 9
10 - 99
100 - 999
1000 and above.

ciliary feeding mechanism, being suspension feeders, use phytoplankters as main food source. Hence these larvae are almost absent in the ecosystems where phytoplankton is scarce. These larvae generally feeding in the surface waters also feed well below the compensation depth.

The present study is based on numerical data on macroplanktonic gastropods as a whole sorted from 1927 collections. The total distribution of larvae is shown in Fig. 11 whereas Figs. 12 and 13 represent their distribution in the SW monsoon and NE monsoon periods respectively. These larvae have fairly wide distribution up to 30° south, throughout the year. The occurrence of a dense patch in the Red Sea (about 3360 per haul) and another one (over 1000 per haul) south of Java is noteworthy. Also dense patches having numbers between 100 and 999 per haul occurred adjacent to the shore, along the west and east coasts of India up to 16°N, around Ceylon, in the Gulf of Aden along the South Arabian coast extending far off Somali coast, up to 20°S in the Mozambique channel. The South Arabian coast and the Somali coast are well known for upwelling and high production (Kabanova, 1968; Prasad, 1968 and Cushing, 1971). Interestingly the above vast patches off Tanzania and Mozambique occur at a great distance of 100 to 250 km from the shore. Such isolated distant patches also occur in the northern part of Bay of Bengal, around Andamans, off Sunda Islands, in the tropical zone, south-west of Ceylon along 78°E meridian, off Kutch, northern and central parts of Arabian Sea and north-east of Madagascar.

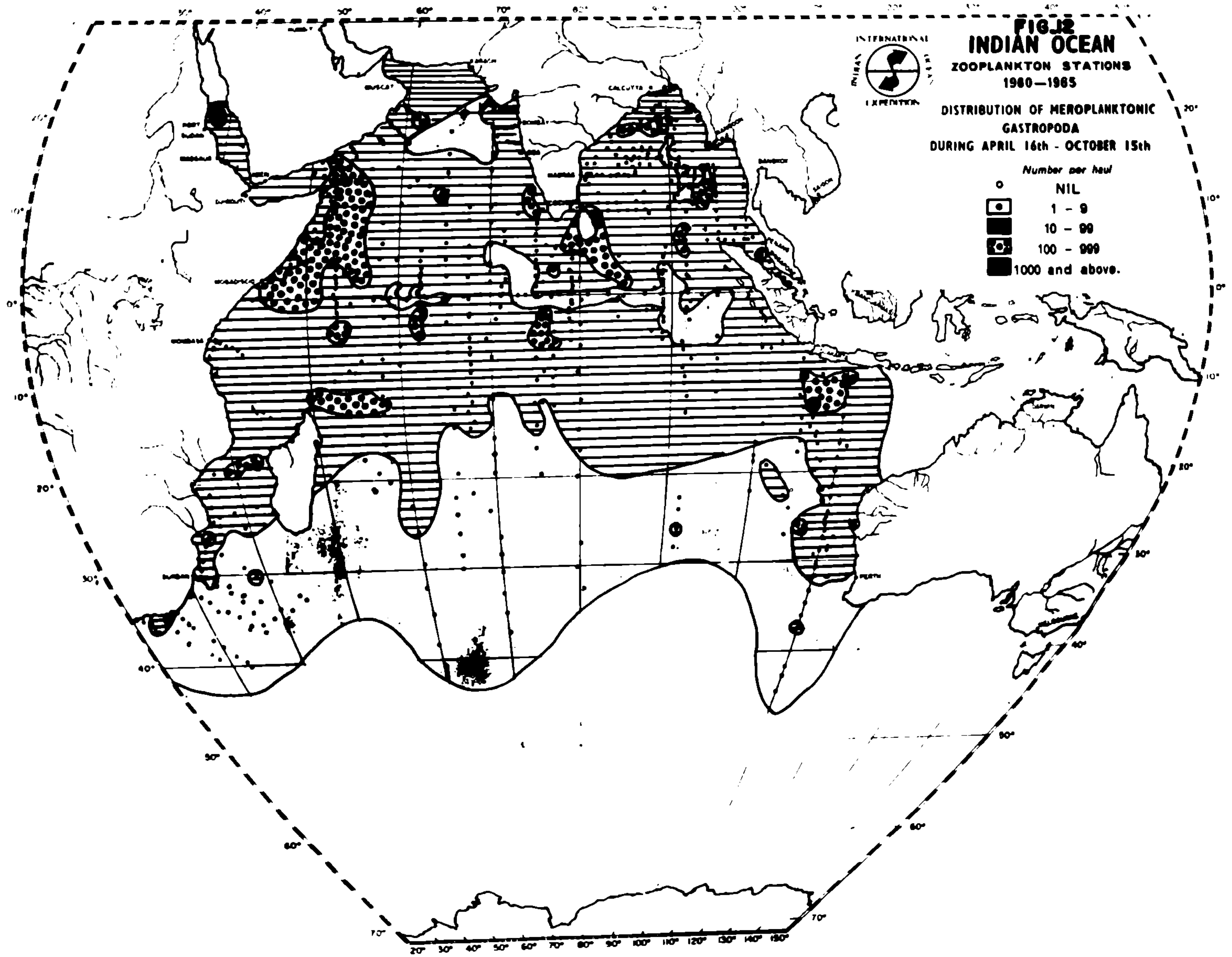


FIG.12
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
GASTROPODA
DURING APRIL 16th - OCTOBER 15th
Number per haul
NIL
1 - 9
10 - 99
100 - 999
1000 and above.
INTERNATIONAL INDIAN OCEAN EXPEDITION
CALCUTTA
MUSCAT
MADRAS
BOMBAY
PERTH
DURBAN

Their abundance along the west coast of India than the east coast may be on account of wide continental shelves off the west coast coupled with the upwelling phenomenon. In general, they are more abundant in the western Indian Ocean, gradually increasing in an east-west axis. Also the numbers decrease southwards. But for the head of the Bay of Bengal, the pattern of distribution coincides with the zooplankton abundance along the south Arabian and Somali coasts. As noted by Ortmann (1893) and Gurney (1924), the relationship between the number of larvae and proximity to land of the station at which they are collected imply that larvae of littoral species usually remain in neighbourhood of land masses and do not move out into the open sea. This fact is well confirmed in the present analysis.

Average numbers of 10 to 99 per haul spreads out in a wide area over Indian Ocean up to 20°S. The area of high density off Somali coast spreads out from the area of upwelling in the direction of the prevailing currents. The Indian Ocean south of 15°S in general has poor collections with 1 to 9 larvae in each haul. The distribution of meroplanktonic gastropods exhibit a pattern independent of other molluscan groups such as Thecosomata, Heteropoda, Cephalopoda, Gymnosomata, Nudibranchiata and lamellibranchiata. While Bay of Bengal generally act as a nursery for larval forms, surprisingly meroplanktonic gastropods are sparsely represented. The area of divergence along the equatorial zone shows moderate abundance. The larvae are scarce in the anticyclonic gyre lying in between the zonally oriented equatorial waters and the subantarctic cyclones probably due to the convergence and sinking of surface waters.

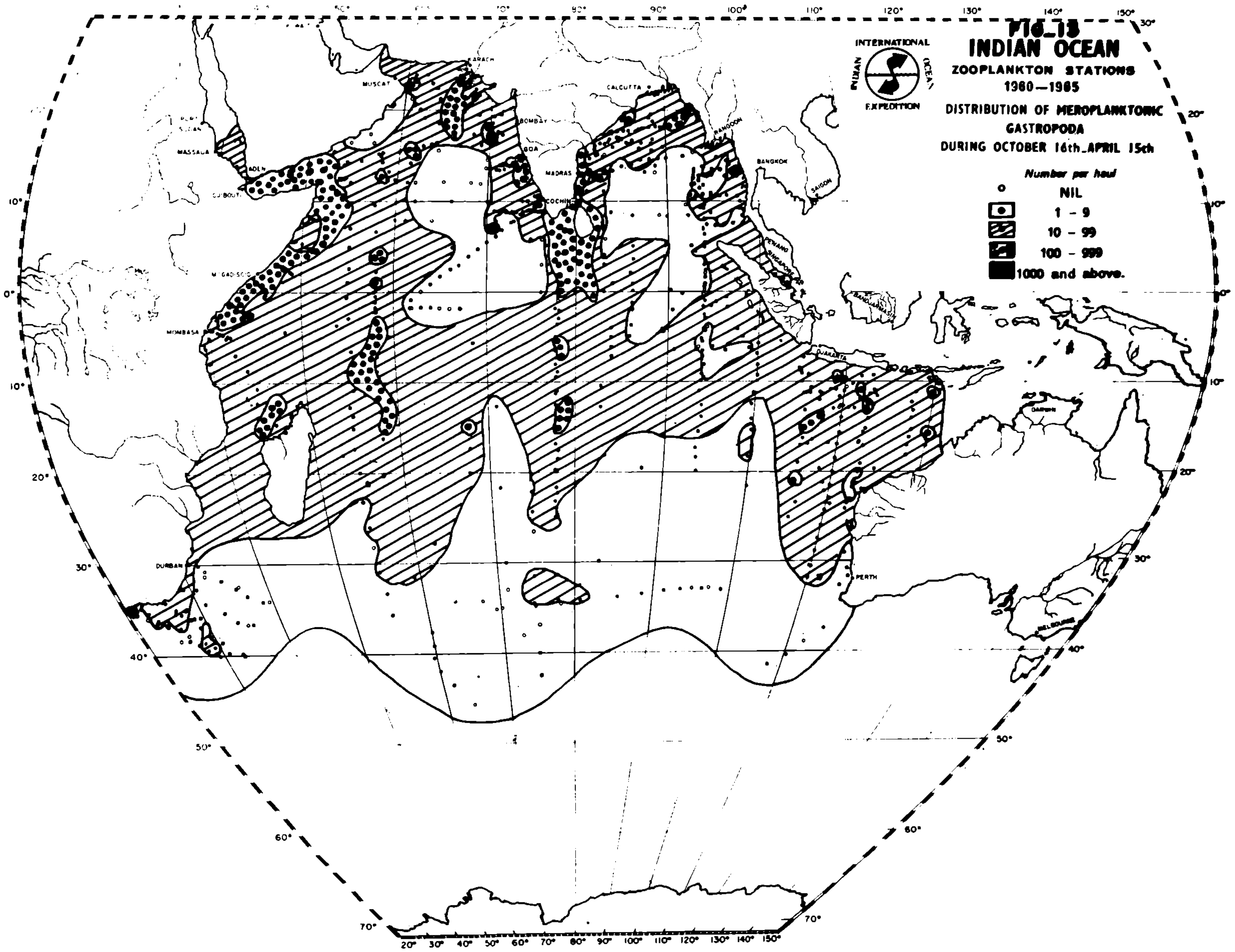


FIG_13
INDIAN OCEAN
ZOOPLANKTON STATIONS
1960—1965
DISTRIBUTION OF MEROPLANKTONIC
GASTROPODA
DURING OCTOBER 16th_APRIL 15th
INTERNATIONAL INDIAN OCEAN EXPEDITION
Number per haul
NIL
1 - 9
10 - 99
100 - 999
1000 and above.
MUSCAT
KARACHI
BOMBAY
GOA
MADRAS
COCHIN
CALCUTTA
RANGOON
BANGKOK
SAIGON
PENANG
ADEN
DJIBOUTI
MASSAUA
MOGADISCIO
MOMBASA
DURBAN
DJAKARTA
PERTH
MELBOURNE
DARWIN

Season-wise distribution of these gastropods show much more extensive distribution in the SW monsoon than NE monsoon. Numerical abundance also shows considerable fall during NE monsoon developing vast stretches of low density areas ( 1 to 9 per haul) in the southern waters of Arabian Sea and Bay of Bengal. The rich areas of Indian coasts during NE monsoon show a reduction during SW monsoon. Around Ceylon and off Somali coast abundance was noted irrespective of season. The area of abundance noted around Ceylon coincides with the region of the main tuna fishery (Cushing, 1971). These larvae are important as they form the food of tuna. The seasonal variation is not much pronounced in the equatorial zone. West Australian Sea also does not show any appreciable seasonal cycles. A month-wise study reveals periodic steady rise and fall. During peak periods gastropod larvae form an important constituent of the larval population of the plankton catches. Based on the distribution pattern of gastropods 4 zoogeographical regions are recognised in the Indian Ocean. They are: (a) East coast of Africa up to 20°S including Persian Gulf, (b) coastal upwelling areas of India and the seas around Ceylon, (c) rest of the Indian Ocean up to 25°S and (d) the area between 25°S and 45°S.

### 3.4. Latitudinal, seasonal and diurnal variations along the meridian 110°E longitude.

The study is based principally on data available at the Indian Ocean Biological Centre from 133 collections made during the 5 seasonal biological cruises in a south-north section along this meridian (Fig. 5). These cruises are of great interest in view of the seasonal nature of Indian Ocean. The anti-cyclonic Indian Ocean gyre penetrates this section in the west. In the north it crosses the South Equatorial Current and in the south enters the northern fringe of the west wind drift. The boundary between the tropical and subtropical zones lie near 18°S.

The primary productivity is higher in the tropical zone particularly during NE monsoon season (August-November). Also primary productivity, chlorophyll 'a', and seston (particulate carbon) each reached their seasonal minimum during the period of the late summer thermocline (January-February). Surface nutrients are generally low, especially in the subtropical zone. Chlorophyll 'a' concentrated at about 75 m. The depth of photosynthetic layer ranged from 60 m (January-February) to 130 m (October). This ecosystem provided evidence for the concept of trophic succession among phytoplankton, zooplankton and micronekton as there are high correlation between the phyto-plankton of one cruise and the zooplankton of the next, and between the zooplankton of one cruise and the micronekton of the next.

Seasonal variation is usually expressed in terms of the annual range. This is satisfactory for properties that vary in a regular way, but less satisfactory for those that have large, non-seasonal components of variability. To compare one biological property with another and one part of an ocean with another, a better measure of seasonal amplitude as the standard deviation of monthly mean is required. For the purpose of comparison, the value is expressed as a percentage of the annual mean (coefficient of variation). Using this index of mean seasonal amplitude, various meroplankton taxa are compared. Earlier studies showed a general trend towards increasing seasonal amplitude, from nutrients and biomass to plankton numbers with greater variation at the species level than at the taxa level. In the present study, within the various meroplankton taxa the magnitude of seasonal amplitude varied from 100 to 400 percent. Fish larvae being present in all the collections showed the lowest seasonal amplitude (100 percent) followed by fish eggs (140 percent). Stomatopoda larvae showed 312 percent, cirripedia 364 percent and lamellibranchiate 375 percent. Most of the larval groups - actinotrocha, tornaria, sipunculoidea, phyllosoma and brachiopoda showed a mean seasonal amplitude of greater than 400 percent. However meoplankton as such showed only 20 percent variation. The reason for these high values may be due to their low frequency of occurrence, the variation increasing with lowering in the frequency of occurrence.

Table XII gives mean levels for different meroplankton taxa, cruise by cruise. Separate values are given for tropical and subtropical zones and for night and day collections. In general, mean values for zooplankton biomass increased from January cruise to August cruise. Accordingly fish larvae also showed an increase from January to August both in the tropical and subtropical zones. Similar increases though not so distinct as for fish larvae, could be seen among all meroplankton taxa. On a zonal basis in tune with the high zooplankton biomass in the tropical zone, fish larvae and bivalve larvae also are more in abundance compared to other taxa. Comparison of day and night catches showed a higher value at night than day especially with reference to fish larvae and bivalve larvae. On a zonal basis fish larvae had greater difference in the tropical zone. Anthozoan larvae and fish eggs showed only a narrow marginal difference. Elsewhere differences were slight.

Table XIII shows seasonal amplitudes separately for tropical and subtropical zones, by day and night. In general, there was a greater day-night difference in seasonal amplitude for the tropics, than for the subtropics, particularly in the case of fish eggs, fish larvae and bivalve larvae. The large seasonal amplitude for particular strata (200 - 0 m) are caused by vertical migrations from one stratum to another. The purpose of this study is to establish the magnitude of seasonal variation in this part of the ocean in order to make a comparison in this respect with other better known regions of the world ocean. This comparison revealed the seasonal amplitudes along 110°E (eastern Indian Ocean) to be low, but none the less significant.

TABLE XII : Mean levels for various meroplankton taxa in the tropical and subtropical zones along 110°E during 5 cruises in 1962-'63 on a day-night basis. (Day value/night value)

| Meroplankton taxa | Cruises | | | | |
|---|---|---|---|---|---|
| | Jan-Feb. 1963 | March-April 1963 | May-June 1963 | July-Aug. 1963 | August 1962 |
| **Subtropical zone (south of 15°S)** | | | | | |
| 1. Zooplankton biomass ($mgm^{-3}$) | 41/51 | 44/53 | 21/35 | 28/58 | 46/72 |
| 2. Fish eggs | 6/3 | 4/3 | 5/4 | 11/11 | 5/6 |
| 3. Fish larvae | 15/34 | 14/44 | 6/25 | 8/67 | 11/73 |
| 4. Bivalve larvae | 2/1 | 2/2 | 1/1 | 1/2 | 2/2 |
| 5. Tornaria " | 2/3 | 3/1 | 1/1 | 1/1 | 6/6 |
| 6. Anthozoa " | 4/2 | 5/8 | 16/21 | 16/20 | 29/61 |
| 7. Stomatopoda " | 1/1 | 1/1 | 1/1 | 1/2 | 3/1 |
| 8. Cirripedia " | 1/0 | 1/0 | 1/1 | 1/1 | 1/0 |
| 9. Actinotrocha " | - | - | - | - | 1/1 |
| 10. Sipunculida " | - | 1/0 | - | 1/1 | - |
| 11. Phyllosoma " | 0/1 | 0/1 | - | - | - |
| 12. Brachiopoda " | - | - | - | - | - |

TABLE XII (Contd.)

| Meroplankton taxa | Cruise | | | | |
|---|---|---|---|---|---|
| | Jan-Feb. 1963 | March-April 1963 | May-June 1963 | July-Aug. 1963 | August 1962 |
| **Tropical zone (north of 10° S)** | | | | | |
| 1. Zooplankton biomass ($mgm^{-3}$) | 31/87 | 47/71 | 42/63 | 63/109 | 84/113 |
| 2. Fish eggs | 2/13 | 1/3 | 8/4 | 11/5 | 4/6 |
| 3. Fish larvae | 4/48 | 9/64 | 7/53 | 17/62 | 24/81 |
| 4. Bivalve larvae | 8/6 | 4/6 | 7/49 | 24/6 | 2/2 |
| 5. Tornaria " | - | 2/1 | 2/1 | - | 1/0 |
| 6. Anthozoa " | 1/1 | 7/3 | 5/13 | 3/5 | 18/8 |
| 7. Stomatopoda " | 1/0 | 1/1 | 1/1 | 1/1 | 1/1 |
| 8. Cirripedia " | 1/1 | 1/0 | 1/1 | 4/1 | 1/1 |
| 9. Actinotrocha " | - | - | - | 1/0 | - |
| 10. Sipunculida " | - | 1/1 | 0/2 | 1/0 | - |
| 11. Phyllosoma " | 0/1 | 0/1 | - | - | - |
| 12. Brachiopoda " | 1/0 | - | 0/1 | - | - |

TABLE XIII : Seasonal variation in 3 meroplankton taxa during 1962-'63 in the tropical and subtropical zones.

| Taxa | Seasonal variation % (Coefficient of variation) | | | |
|---|---|---|---|---|
| | Tropical | | Subtropical | |
| | Day | Night | Day | Night |
| Fish larvae | 95 | 53 | 73 | 65 |
| Fish eggs | 182 | 155 | 113 | 121 |
| Bivalve larvae | 125 | 292 | 119 | 112 |
| Zooplankton biomass | 35 | 32 | 27 | 26 |

3.5. Annual variation along the meridian 78°E longitude.

As part of the International Indian Ocean Expedition, 'Umitaka Maru' cruise 23 sampled the water column along the meridian 78°E between 8°N and 25°S during December 1962 and January 1963 and collected 20 zooplankton samples between 1900 and 2400 hours. Similarly 'Kagoshima Maru' cruise 3 also sampled the same area during December 1963 and January 1964 and collected 23 samples between 1700 and 2100 hours. Thus most of the collections were made during 2000 and 2100 hours, from a water column 200 m to surface using IOSN. In the hauls where there was wire angle, more wire was paid out to reach a depth of 200 m, and in such cases a correction factor based on wire out was applied. The station list and sampling details are given in the IOBC Handbook (1969) and the station positions are shown in Fig. 5. Chlorophyll-a data is taken from the phytoplankton production atlas of the IIOE (Krey and Babenerd, 1976). Night collections were made in order to avoid the effect of vertical migration and avoidance of surface waters during the day, if any, by the larvae. Based on the vertical temperature gradients and the occurrence and abundance of the majority of the taxa, 3 faunal zones were selected for latitudinal comparison and the mean number of organisms for each zone indicating their annual variation in abundance is given in Table XV. The vertical temperature gradient in the upper 100 m was very narrow at the equator during both years. The stations up to the equator was taken as zone A, stations between

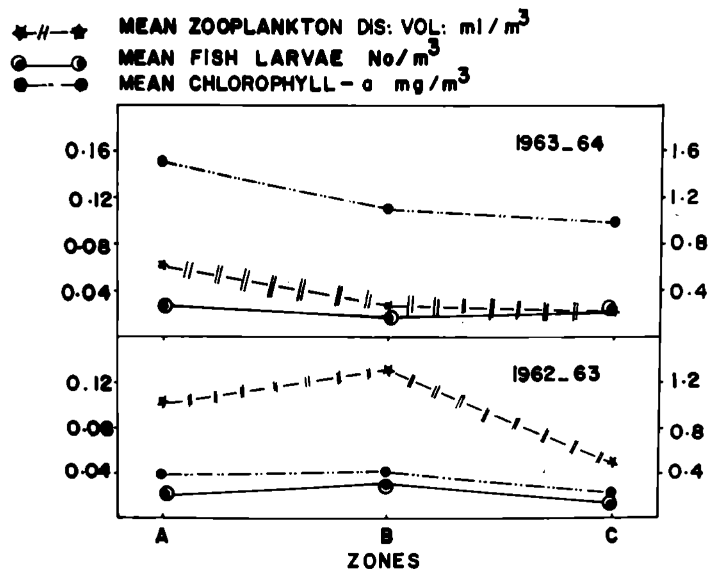



FIG.14a MEAN ANNUAL VARIATION IN ABUNDANCE OF FISH LARVAE IN RELATION TO CHLOROPHYLL-a AND ZOOPLANKTON DISPLACEMENT VOLUME IN THREE SELECTED ZONES OF THE MERIDIAN 78° E.

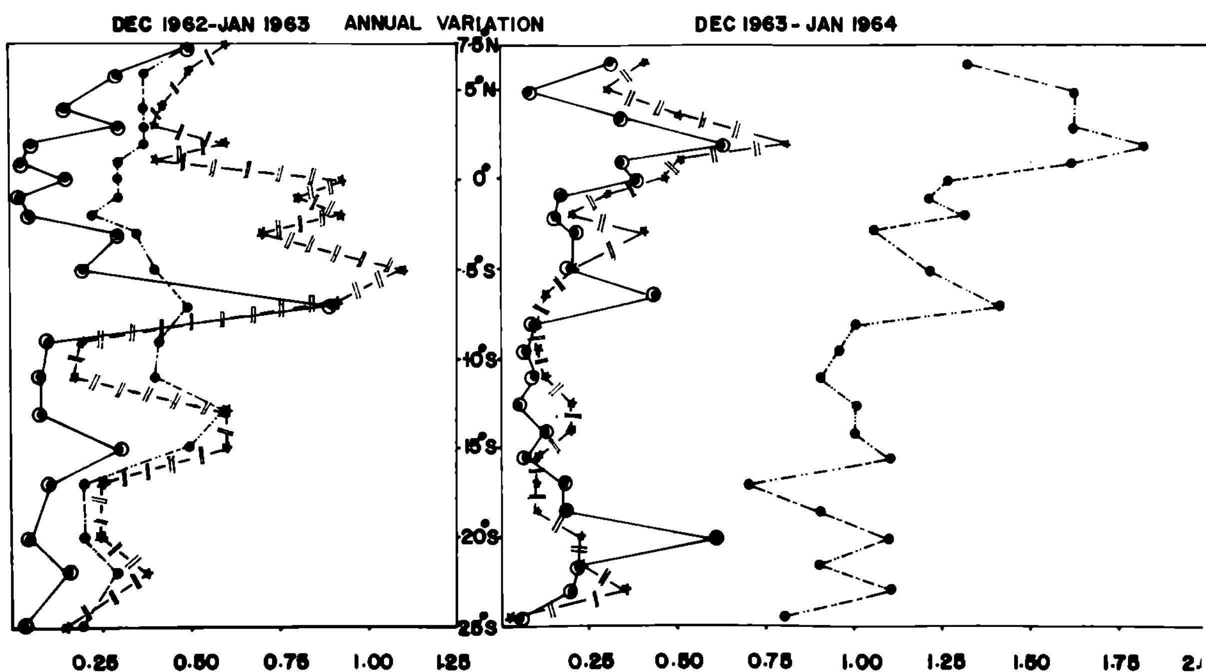



FIG. 14b THE ANNUAL VARIATION IN ABUNDANCE OF FISH LARVAE IN RELATION TO CHLOROPHYLL—a AND ZOOPLANKTON DISPLACEMENT VOLUME

the equator and 15°S as zone B, and the stations between 15°S and 25°S as zone C. The data collected from each zone in the form of mean values for chlorophyll 'a', zooplankton displacement volume and fish larvae are given in Fig. 14a and the data collected from the 43 stations for chlorophyll 'a' (mean value in the upper 200 m), zooplankton displacement volume and numbers of fish larvae are plotted in Fig. 14 b.

The mean annual variation in fish eggs and larvae in relation to chlorophyll 'a' and zooplankton displacement volume is given in Table XIV.

Table XIV : Mean annual variation in abundance of fish eggs and larvae in relation to chlorophyll 'a' and zooplankton displacement volume along 78°E.

| Year | Mean Chlorophyll 'a' ($mg/m^3$) | Mean zooplankton displacement vol. ($ml/m^3$) | Mean number of Fish eggs ($No./m^3$) | Mean number of Fish larvae ($No./m^3$) |
|---|---|---|---|---|
| December 1962-January 1963. | 0.034 | 0.053 | 0.02 | 0.20 |
| December 1963-January 1964. | 0.118 | 0.025 | 0.007 | 0.23 |

The table indicates high concentration of chlorophyll 'a' in the second year, compared to the low concentration of zooplankton, caused perhaps by a shift in the production of phytoplankton bloom which zooplankton depends. From the chlorophyll value and biomass of zooplankton, it appears that the phyto-

plankton bloom has started earlier and grazing by zooplankton had advanced to the extent that the secondary producers have established their population well in the environment. But in the second year, the grazing has just started and the zooplankton might have hardly reached the ground for grazing. The periodical changes in the set up of the irregular NE monsoon might have introduced this late or earlier bloom of the phytoplankton in succession of the changes in the properties of the environment. Sugawara and Saijo (1966) observed higher primary production in the first year than in the second year. Similarly an year to year distinct variation in abundance (Table XV) occurred with respect to all meroplankton groups, probably related to the movement of eddies and areas of divergence. The differences noted in the meroplankton population south of 15°S may be attributed to the boundary observed by Miyake and Sugiura (1966) at about 15°S on both sides of which the property of the ocean considerably differed.

TABLE XV : Mean annual variation in abundance of certain meroplankton taxa along 78°E.

| Larval Taxa | Average number of specimens/haul | | | | | |
|---|---|---|---|---|---|---|
| | Zone A (8°N - 5°S) | | Zone B (5°S - 15°S) | | Zone C (15°S - 25°S) | |
| | I Year | II Year | I Year | II Year | I Year | II Year |
| Fish eggs | 4 | 2 | 5 | <1 | 2 | 1 |
| Fish larvae | 38 | 55 | 57 | 29 | 26 | 43 |
| Bivalve | 4 | 3 | 4 | <1 | 1 | <1 |
| Anthozoa | 2 | 2 | 3 | 3 | 1 | 1 |
| Cephalopoda | <1 | 2 | 2 | 1 | 1 | 1 |
| Sipunculoidea | <1 | 2 | 1 | 0 | 0 | 0 |

Fish larvae numbering from 5 to 173 per haul were present in all the 43 stations sampled. In the first year average larval catch was 38 which rose to 45 in the second year. Compared to the considerable increase in the chlorophyll 'a' value during the second year, mean number of fish larvae showed only slight increase (Table XIV). However, as fish larvae form one of the major components among secondary producers, they follow the general trend of zooplankton abundance (Fig. 14 a). While the northern and southern sectors showed an increase in the average number of fish larvae in the second year, middle sector recorded a lowering in the abundance of fish larvae (Fig. 14 b). This figure also indicates the pattern of annual variations in the abundance of fish larvae more or less dependent of chlorophyll 'a' values and similar to values of zooplankton displacement volume. Fish larvae while in the first year recorded one high peak and 4 to 5 small peaks, second year had 3 to 4 small peaks only. Since fish eggs are known to hatch out into larvae in one to two days, one can expect larval distribution pattern overlapping that of eggs especially in density gradients. But the prevailing current pattern may carry the larvae to far off places away from the spawning areas and thus inspite of the discontinuous distribution of fish eggs recorded distribution of fish larvae is wide spread along the meridian with increased densities in certain water masses and varying with spawning activity of fishes. While Peter (1969b) observed no clear or uniform volumetric relationship between plankton and fish larvae in the IIOE samples from the Indian Ocean,

George (1979) recorded a general positive relationship between volume of plankton and the number of eggs and larvae collected from coastal waters of south-west coast of India. George (1979) also noted preceding peaks of eggs and larval numbers to those of plankton volumes. However in the Gulf of Aden, Ali Khan (1972) recorded an inverse relationship between number of larvae and zooplankton volumes.

Fish eggs were present in 21 out of 43 collections along 78° meridian. While 78 eggs were obtained from 10 stations in the first year, only 33 eggs were obtained from 11 stations in the second year. Thus considerable variation in the occurren of fish eggs was noticed annually. The year 1962-'63 showed two and half times more eggs than 1963-'64. Distributional studies along the north-south axis showed total absence of fish eggs north of 3°N in the first year and north of 5°N in the second year. Maximum eggs were obtained from the Arabian Sea water mas of high salinity, high temperature and low oxygen. West flowin south Equatorial Current and the central water mass had very few eggs in both years. From the results obtained it is clear that in the second year fish eggs were considerably lower than in the first year. George (1979) also noticed variations from the generalised patterns in the distribution of fish eggs in the different years, 1971-'75 along the south-west coast of Indi in tune with the spawning activity of fishes.

Brachiopod larvae having high tolerance to changes in salinity as indicated by their abundance in Bay of Bengal, Gulf of Aden, off south-west coast of India, were absent along the

78°E meridian, although they were present in 52 (2.7 percent) out of 1927 zooplankton samples collected from the Indian Ocean. Their absence in the Arabian Sea water mass extending to the central Indian Ocean between 7°N and 10°S may be the result of their absence in the northern Arabian Sea. Muir-Wood (1959) has recorded a discontinuous distribution in the three genera of adult brachiopods of the Indian Ocean and observed the limited motility of larvae, produced by the adults generally living in congregation, for dispersal even with the aid of currents and winds. Thus the discontinuity noted in the distribution of adults and larval motility may account for the discontinuous distribution of larvae also, leading to their total absence from many areas in the Indian Ocean especially along 78°E meridian.

Tornaria larvae were not found along 78°E meridian. Generally enteropneusts tend to live in aggregations of the same or different species favouring warm and temperate waters, but this may be the mere consequence of the settling of larval stages in favourable sites and their avoidance of other places. This may explain their total absence from 78°E meridian. Also as no significant difference was noticed in the day and night collections, their absence cannot be attributed to the time of collection. While SW monsoon contributed significantly higher numbers than in NE monsoon, in the Indian Ocean, the absence of tornaria larvae along the meridian 78°E may be due to restriction of collection during the NE monsoon period only.

Phoronida in northern hemisphere breed in spring or summer months from March to December and in southern hemisphere from November to May. These benthonic forms generally exist in the upper littoral zone (above 50 m) of tropical and temperate zones. So these larvae generally preferring low salinity waters were present in 80 (4.9 percent) out of the 1927 zooplankton samples collected from the Indian Ocean. They are present very rarely in the Arabian Sea, central Indian Ocean and eastern part of the Indian Ocean between equator and latitude 30°S and longitude 68°E to 120°E. This may explain their absence along 78°E meridian However, 3 specimens were obtained in December 1962 from 5°N and this may be a chance of collection by drifting.

Cirripedia larvae were obtained from 3 zooplankton collections out of 43 made along 78°E meridian. Of this one station at 5°30'N contained 43 larvae. The collections belonged to the Arabian Sea water mass of high salinity, high temperature and low oxygen. These larvae were absent south of equator along 78°E meridian. Generally these larvae were found increasing in number with increasing temperature and increased availability of food. While 49 specimens were collected during December 1963, none was obtained during December 1962.

The pelagic life of phyllosoma larvae continues until 3 moults or puerulus stage is attained-an intermediary phase between pelagic and bottom life. The larva preferring high temperature (27°-30°C) high salinity (31‰) and strong sunlight was absent in the 43 collections made along the 78°E meridian, except for one specimen obtained from 15°S from the East Indies

water mass of low salinity and high temperature. Their absence may be related to their spawning period in early spring or summer.

Of the 43 samples analysed from the 78°E meridian, only 3 samples had stomatopod larvae. The stations located at 2°S, 3°30'N and 6°30'N belonged to the Arabian Sea water mass influenced by the Equatorial Current and Equatorial Counter Current. These larvae were collected in December 1963 only. They are absent beyond 2°S.

Saraswathy (1972) studying the distribution of bivalve larvae in the Indian Ocean, found 50 percent of samples having bivalve larvae. She also noticed their absence in the open ocean especially within an area between 60° and 100°E and 15° and 45°S. In the present study analysis of bivalve larvae from the 43 samples collected along 78°E meridian also showed their absence south of 20°S. But larvae were present in large numbers in the Arabian Sea water mass of high temperature, high salinity and low oxygen along this meridian between 7°N and 10°S. On a north-south axis larval abundance decreased from 34 numbers (6°N) to 4 numbers (20°S). This reduction in the number of larvae far out in the open sea can be a reflection of shallow water habitat of the adult. These larvae collected far away from the coasts can possibly be those from the nearshore areas drifted along with the currents. While 63 larvae were collected from 6 stations in 1962-'63, only 30 larvae were collected from 6 stations in 1963-'64, exhibiting marked annual variation. In both years more larvae were present in the west flowing north equatorial current.

Of the 1927 collections from the Indian Ocean, cephaloped juveniles were present in 63 percent of total collections, the Bay of Bengal accommodating the largest nursery in the Indian Ocean (Aravindakshan and Sakthivel, 1973). Cephalopods being a demersal spawner, during late winter and early spring, in vast areas of ocean bottom, the larvae released there have wide range in distribution having wide tolerance to salinity and temperature. Of the 43 collections along 78°E meridian, 17 had cephaloped juveniles. Of the 46 juveniles collected 12 (from 7 stations) were obtained during 1962-'63 and 34 (from 10 stations) during 1963-'64. Thus the larval abundance showed a three fold annual variation from first year to second year. While in the first year only one specimen was collected from an area north of equator, second year gave 22 specimens (from 6 stations). While in both years larvae were spread out south of 7°S, they were more abundant in the second year than in the first year.

Sipunculoids adaptable to a range of depth and temperature are strictly benthic in habitat and inhabit mainly the littoral zone and extend to abyssal waters to a depth of 5000 m. Though cosmopolitan, they centre in warmer waters and have a number of endemic species. The Indo-West Pacific region being the home of numerous species, during the IIOE, the 1927 zooplankton samples collected had sipunculoid larvae in abundance. Spawning generally occurs in summer. Yet the larvae were present in only 8 out of the 43 stations sampled along 78°E meridian. But for one specimen collected from the Bay of Bengal water mass of low salinity and high temperature all the remaining 28 specimens were

taken from the Arabian Sea water mass. Not a single specimen was obtained from south of 7°S. While they were not present in the east flowing equatorial current during December 1962, 19 larvae occurred in this current in December 1963. Also in December 1962, ten larvae were collected from the west flowing north equatorial current and the eddy formed in between 5°S and 10°S.

### 3.6. Distribution in relation to the thermocline in the western Indian Ocean during day and night.

The numerical data of certain meroplankton taxa collected from each station in relation to the thermocline is presented in the form of graphs in Figs. 15 to 18. In presenting the data the unit standard haul system is found more meaningful than the number per $m^3$ since the initial assumption that the number of organisms is greater than the volume of water filtered is greater is not applicable for long vertical hauls, especially in relation to the non-random distribution of the organisms. But in fact the number of organisms are really more in the long haul (200 - 0 m) than in the short haul (thermocline to surface). Also the regression analysis showed the relationship between the two hauls, 200 - 0 m and the thermocline to 0 m (No./$m^3$) to be highly significant (99% level).

The distribution of fish larvae in relation to the thermocline is shown in Fig. 15. Fish larvae showed nocturnal abundance having 25 larvae per haul above the thermocline and

EXPLANATIONS FOR THE ABBREVIATIONS USED IN FIGS. 13-18.

NEC 3 - North Equatorial Current during March.

ECC 3 - Equatorial Counter Current during March.

SEC 3-4 - South Equatorial Current during March-April.

ECC 4 - Equatorial Counter Current during April.

MC 5-6 - Monsoon Current during May-June.

SEC 6-7 - South Equatorial Current during June-July.

SC 7 - Somali Current during July.

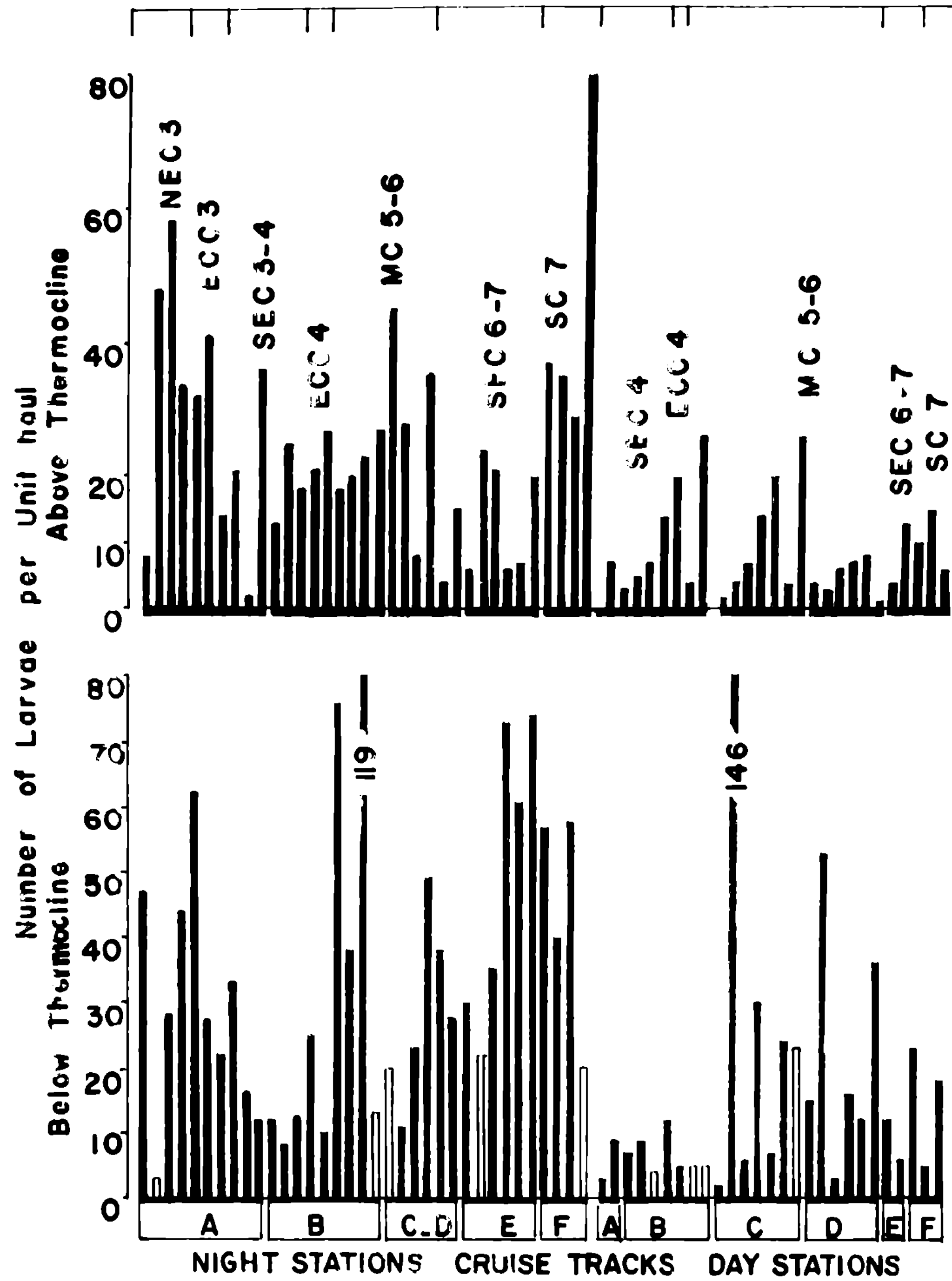



FIG. 15 THE DISTRIBUTION OF FISH LARVAE (IN TERMS OF UNIT HAUL
BLANK COLUMNS REPRESENT NEGATIVE VALUES

33 larvae per haul below the thermocline, whereas they are sparsely populated above (7/haul) and below (17/haul) the thermocline in the day hauls, in the 200 m water column. Fish larval abundance below the thermocline in the day time indicates the vertical migration undergone by fish larvae to avoid surface light. The mean day level may vary according to the penetration and intensity of light in different stations.

The area explored being under the influence of monsoon and having thermocline in shallow depths due to mixing of bottom and surface waters, light penetration is low. And so fish larvae occur in most day stations but in lesser numbers. The occurrence of fish larvae above and below the thermocline indicates that the thermocline is not a barrier for the distribution of fish larvae. However, George (1979) found fish larvae of Rastrelliger, sardine and tuna being abundant at specific temperature ranges.

Ahlstrom (1959b) found no consistent difference in the day and night catches of larvae of Trachurus symmetricus off California. While Richards et al. (1971) found no difference in catches of Auxis larvae between day and night, Klawe (1963) had noted lesser catches of Auxis larvae at night. Ali Khan (1972), Silas (1974) and Peter (1977) recorded increased catches of fish larvae at night in the Indian Ocean, while Boonprakob and Dahtaranon (1974) found positive phototaxis reaction in the larvae of Rastrelliger neglectus, large number of the larvae congregating at 5 to 10 m during day time and sinking to greater

depths from dusk through night. George (1979) studying day and night larval catches showed in most cases a marginal increas in number of larvae caught at night than in the day. The larvae of *Vinciguerria*, showed 100 percent increase in the night catch, while *Sardinella* spp. and *Euthynnus affinis* showed a 50 percent increase and all the other tuna larvae and larvae of *Rastrellige* *kanagurta* showed only a marginal increase in night collections.

Thus the diurnal variation seen in the above studies indic that the major factor in controlling the vertical migration of fish larvae in the region under study is light rather than temperature. Of the 6 cruise tracks, Tracks A, E and F show relatively high abundance of fish larvae (50, 49 and 59 per haul respectively) which perhaps might be due to the influence of th SW monsoon during June and July. In March-April (Track A) fis larvae are comparatively less in the South Equatorial Current (SEC) than the North Equatorial Current (NEC) and Equatorial Counter Current (ECC). In April-June (Tracks B, C and D) stations of monsoon current show less abundance than SEC and ECC. Track E falling in the area of divergence in July near north-east Madagascar and the Track F in July under the influen of Somali Current show significant increase in the number of fish larvae. Nine stations showed negative values below thermocline, giving the impression that no larvae were present. This can be due to the non-random distribution of the organism or due to an artefact of the method used.

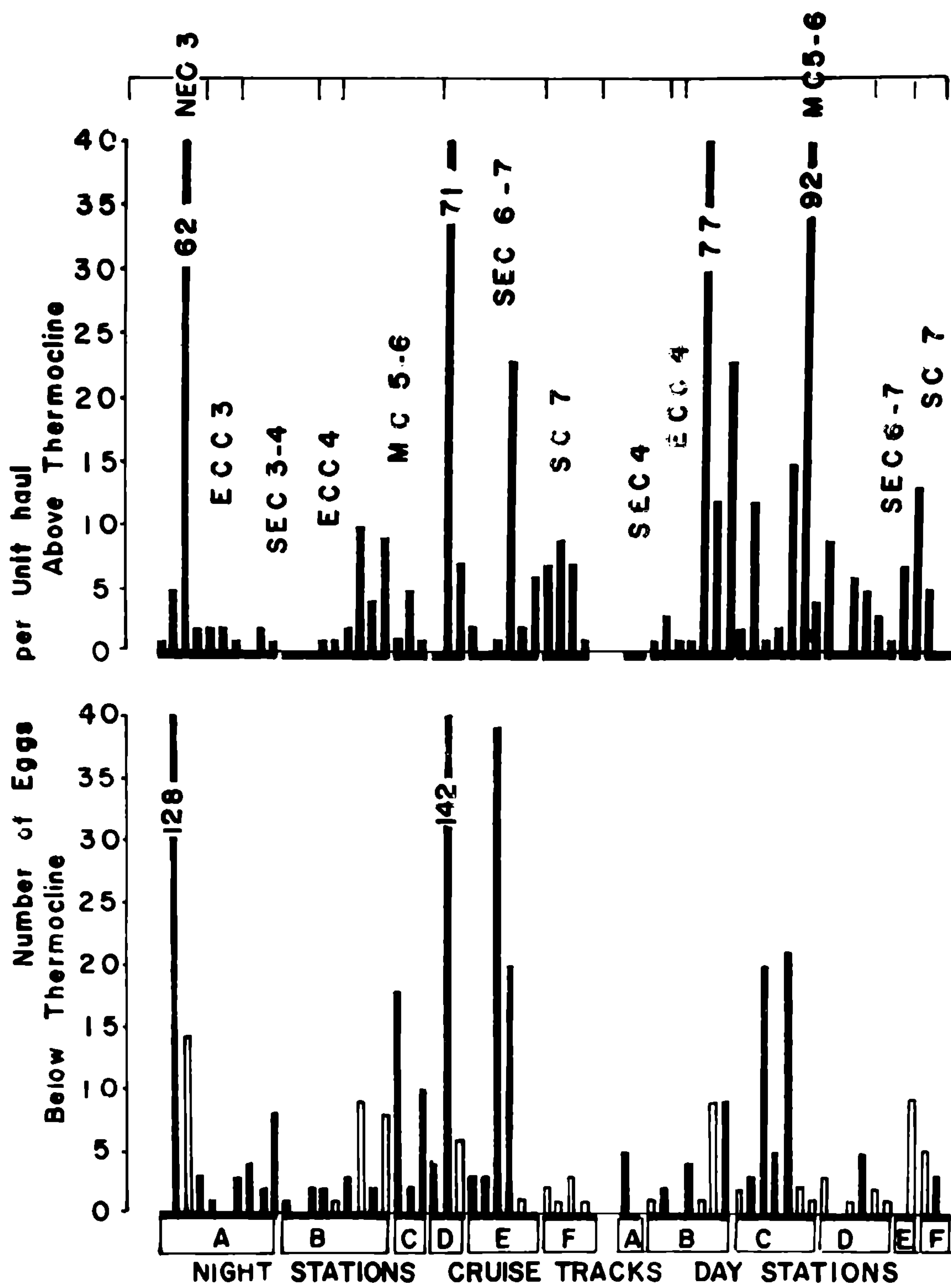



FIG. 16 THE DISTRIBUTION OF FISH EGGS (IN TERMS OF UNIT H/
BLANK COLUMNS REPRESENT NEGATIVE VALUES

The distribution of fish eggs (Fig. 16) indicates only narrow nocturnal abundance (18 per haul) compared to the day hauls (13 per haul). Of the 35 night hauls, while on an average 7 eggs were obtained from hauls above thermocline, hauls below thermocline had 11 eggs per haul. Similarly of the 27 day hauls made, collections above thermocline contained 11 eggs per haul while the collections below thermocline contained only 3 per haul. On a track-wise, Tracks C and D sampled during April-June from the area of monsoon current showed more abundance revealing highest spawning activity. The presence of large number of eggs in certain areas can be taken as proof of congregation of spawners and of spawning ground there. Above results indicate absence of light or temperature effect on the distribution of fish eggs.

Bivalve larvae (Fig. 17) were present in 32 out of 35 night stations, on an average of 3.2 larvae per haul and in only 18 out of 27 day stations with an average of 1.6 larvae per haul. This indicates nocturnal abundance of bivalve larvae in all the collections. While the night hauls made above thermocline had 2.4 larvae per haul, those collections below thermocline had 3.3 larvae per haul. In the day stations collections above the thermocline had an average of 1.3 larvae per haul and collections below the thermocline had an average of 2.4 larvae per haul. Half of the collections made below the thermocline showed negative values.

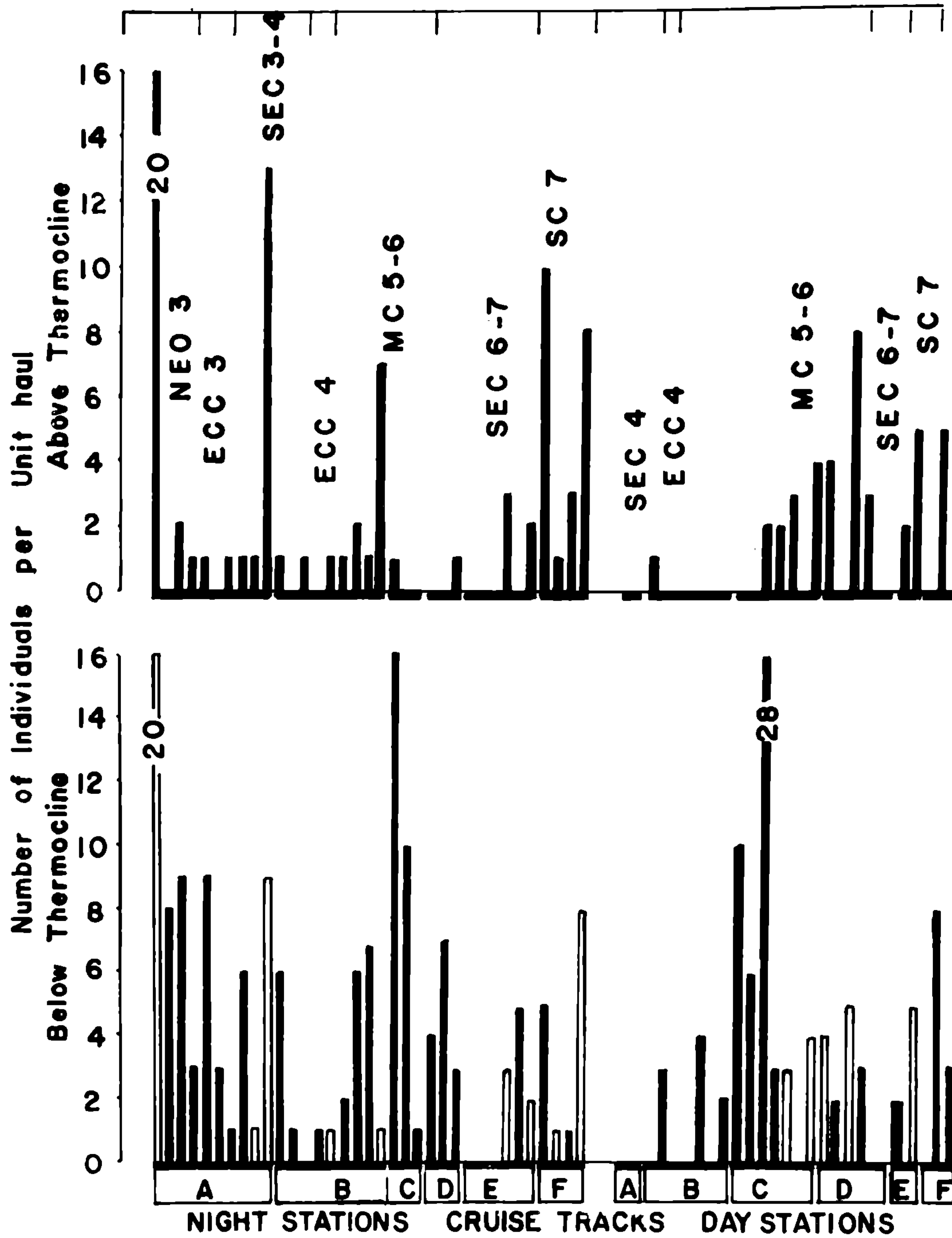



FIG. 17 THE DISTRIBUTION OF BIVALVE LARVAE (IN TERMS OF UNIT HA
BLANK COLUMNS REPRESENT NEGATIVE VALUES

Similar to fish larvae, bivalve larvae were also influenced more by light than the temperature during vertical migration. Of the 6 tracks sampled, the order of abundance was C, A, F, D, B and E. Track C with maximum abundance was under monsoon currents.

Anthozoan larvae (Fig. 18) are present in 24 out of 35 night stations and 22 out of 27 day stations. Frequency of occurrence are same (20 out of 35) in samples above and below thermocline in the night collections and less (12 out of 27) above thermocline than below thermocline (17 out of 27) in the day collections. Larvae are more abundant (about 4 per haul) below thermocline than above thermocline (about 2 per haul) in both the day and night stations. However 5 night stations and 4 day stations bel thermocline showed negative values. The above results indicate that more than the temperature, the light factor acts as a contro in the vertical distribution of anthozoan larvae as noted for fish larvae and bivalve larvae. Of the six tracks sampled, the stations of track F which was covered in July falling under the area of the Somali Current had the maximum abundance of 12 larvae per haul. Next to it was the Track A stations covered in March and April under the influence of major Equatorial Currents. The stations in Tracks C and D under the influence of the Monsoon Current and the South Equatorial Current had very poor larval occurrence (2 per haul).

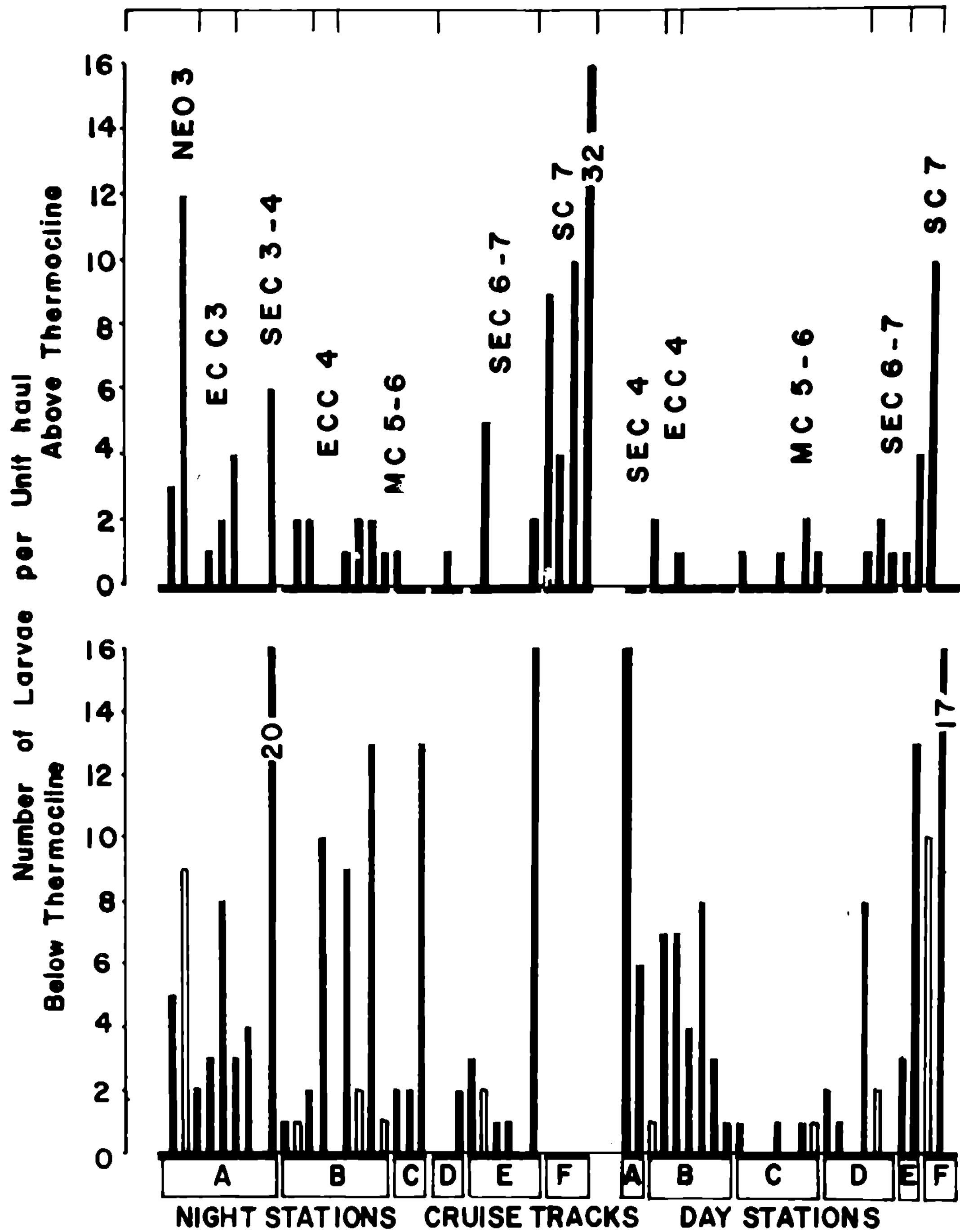



FIG. 18 THE DISTRIBUTION OF ANTHOZOAN LARVAE (IN TERMS OF UNIT HAUL)
BLANK COLUMNS REPRESENT NEGATIVE VALUES

Sipunculoidea larvae are present in 13 out of 62 stations sampled. They are mainly collected from stations in Tracks A and E during March and April period. They are mainly present in the night samples especially below thermocline.

Actinotrocha larvae are present in only 2 out of 62 stations. One day station in Track B under the influence of South Equatorial Current had 37 larvae below thermocline and only one larva above the thermocline. Another day station also in Track B influenced by the Monsoon Current had 4 larvae below the thermocline.

Tornaria larvae are present in 25 out of 62 stations. Sixteen night stations had an average number of 7 larvae per haul, whereas 9 day stations had an average number of 6 larvae per haul. This indicates nocturnal abundance. Tornaria are found abundant above the thermocline in contrast to other larval forms. Tornaria are absent below the thermocline in the day stations. Maximum larvae are collected during July from the Somali Current area.

Cirripedia larvae are present in 17 out of 62 stations. Of these 10 night stations had 9 larvae in each and 7 day stations had 4 larvae in each. This indicates nocturnal abundance. While the collections above thermocline had an average of one larva per haul, that of below the thermocline had an average of 6 larvae per haul.

Of the 62 stations only one day station above the thermocline in the Track C had one phyllosoma larva. Similarly four brachiopoda larvae are present in a night station above the thermocline in Track E.

Stomatopoda larvae are present in 20 out of 62 collections made. But for the 54 larvae obtained from a night station above the thermocline they are present in very few numbers in the day and night hauls and above and below the thermocline.

Cephalopod juveniles are present in 24 stations out of 62 stations sampled, 13 night stations had an average of 8 larvae per haul whereas 11 day stations had an average of 3 larvae per haul. On an average more cephalopod juveniles are present below the thermocline. Of the 6 tracks sampled tracks E and F covered during June and July had maximum number of juveniles.

Pages

4. SUMMARY .. .. 265-277

## 4. SUMMARY.

1. The studies on the meroplankton are based on a series of collections of tropical zooplankton from the west and east coasts of India and Indian Ocean during 1960-'78. The experiments carried out on the fixation and preservation techniques, biochemistry and zoogeographic distribution of meroplankton in the Indian Ocean are dealt with.

2. The shipboard and curating techniques deal with careful collection and appropriate preserving procedures beginning with the lowering of nets into the water and culminating in well preserved plankton samples which can be stored and retrieved for future study.

3. For fixation of sample immediately after collection on board the vessel, 2 percent buffered formaldehyde is recommended and this is done by adding borax-buffered 40 percent formaldehyde (2 gm borax to 98 ml formaldehyde) to sea water containing plankton. For proper fixation there should be 9 parts of the fixative to 1 part of plankton. The meroplankton requires a fixation time of up to 10 days before they are transferred to the preservatives.

4. During fixation, meroplankton underwent shrinkage due to loss of 15 to 67 percent water. Whereas trochophore larvae which lost 30 percent of volume in one day maintained it afterwards, other meroplanktonic larvae which showed a

continuing but decelerating decrease with time, attained their equilibrium in about twelve months. Thus repro-ducible volume measurements of meroplankton in general could be done only after a time lapse of twelve months.

5. Natural pigmentation being of great value in the taxonomy of meroplankton, colour fading or bleaching which occurs within 3 to 30 days in the preservative caused by temperature, light, pH above 7 and below 4, dissolved oxygen, formic acid and the additives added, can be reduced by storing in dark room in low temperature (20°C), avoiding hexamine and borax and by the addition of anti-oxidants such as butylated hydroxyanisole and butylated hydroxytoluene.

6. Freshly collected meroplankton during fixation in formal-dehyde undergoes initial unfavourable reaction expressed in violent movements, leading to damages. This problem can be alleviated by narcotisation with specific agents.

7. Meroplanktonic coelenterate larvae generally subjected to mechanical damage and violent distortion/contraction could be fixed in good condition on prior narcotisation with 0.3 percent tricaine methanesulphonate or 0.1 percent benzamine hydrochloride. Two percent formaldehyde in sea water as a fixative and 1 percent formaldehyde in sea water as a preservative with a pH around 7 maintained by the addition of 0.5 percent sodium glycerophosphate, 5 percent sodium acetate or excess calcium carbonate proved

good. Addition of 0.5 percent phenoxetol and 4.5 percent propylene glycol to the above preservative improved the condition of the specimens. Also 1 percent propylene phenoxetol in distilled water preserved these larvae satisfactorily.

8. The contraction and distortion noted in Muller's larvae can be avoided by anaesthetisation with ethanol or by oxygen starvation. Two percent formaldehyde in sea water or 20 percent Dowicil 100 in sea or distilled water produced excellent fixation in these larvae provided a lipid solvent such as 0.5 percent propylene phenoxetol is added. One percent formaldehyde in sea water neutralised with 5 percent sodium acetate or 0.5 percent sodium glycerophosphate preserved the specimens. Also 1 percent propylene phenoxetol in sea/distilled water maintained the good condition of the specimens.

9. Propylene phenoxetol and/or nembutal were found good relaxing agents for pilidium larvae. Two percent formaldehyde in sea water or one percent P-toluene sulphonic acid formed suitable fixing solutions. One percent propylene phenoxetol in distilled water or 1 percent formaldehyde in sea water preserved the larvae satisfactorily and 4.5 percent propylene glycol as an additive increased preserving qualities.

10. Effect of formalin frenzy on polychaete trochophores can be avoided by inducing complete relaxation using sea water

diluted with an equal amount of isotonic magnesium salt solution or cocaine. Best fixation of polychaete larvae was obtained by a mixture of one volume of sea water with an equal volume of osmium tetroxide or 90 percent aqueous alcohol. Good preservation was maintained by 70 to 80 percent alcohol.

11. Unfavourable effects of formalin frenzy noted among larval crustaceans may be eliminated using an anaesthetizing agent like 0.002 percent phenoxetol or five percent carbonated water. Two percent formaldehyde solution in sea water as a fixative and one percent formaldehyde solution in sea water as a preservative maintained fully satisfactory results for macroplanktonic crustacea, provided 0.5 percent sodium glycerophosphate and 4.5 percent propylene glycol are added as additives. Eighty percent ethanol in distilled water containing few drops of glycol also preserved well these larval stages.

12. Propylene glycol 0.02 percent relaxed the Sipunculoidea larvae, thus avoiding the disturbances during the period of initial volume regulation. Two percent formaldehyde solution in sea water or 20 percent solution of Dowicil 100 in sea water, both having an osmotic pressure equal to that of sipunculoid larval coelomic fluid provided excellent fixation. Either one percent formaldehyde solution in sea water or ten percent solution of Dowicil 100 in sea water

preserved well these larvae. Addition of five percent sodium potassium tartrate or borax to maintain a pH around 7.6 increased the preservative qualities.

13. Meroplanktonic stages of molluscs require fixation and preservation of soft parts as well as calcareous shells. Distortion and contraction occurring in gastropod veligers can be reduced by narcotising with a mixture of 2.3 percent magnesium chloride and 0.25 percent phenoxetol. Larvae can be well fixed in 80 percent ethanol aqueous, two percent formaldehyde solution in sea water or 20 percent Dowicil 100 solution in sea water. One percent formaldehyde solution in distilled water neutralised with borax having three percent potassium oxalate as an additive or 80 percent ethanol aqueous preserved well the larvae. For molluscan larvae distilled water as diluent acted better.

14. Closure of shell valves and rejection of shell contents by cyphonautes larvae can be averted by narcotisation with 0.005 percent phenoxetol, magnesium chloride or chloral hydrate-menthol mixture. Good fixation occurred in two percent formaldehyde solution in sea water buffered with borax. One percent propylene phenoxetol in distilled water preserved the fixed larvae in good condition.

15. Actinotrocha larvae were narcotised using propylene phenoxetol or a mixture of propylene phenoxetol and formaldehyde or tricaine methane sulphonate to avoid the effect

of formalin frenzy. Larvae were well fixed in two percent formaldehyde solution in sea water. One percent formaldehyde solution in sea or distilled water buffered with borax imparted good preservation.

16. Anaesthetization of brachiopod larvae with 0.005 percent phenoxetol helped prevention of violent reaction leading to closure of shell valves in these larvae. While two percent formaldehyde solution in sea water buffered with borax to maintain a pH around 7 to 8.6 proved to be a good fixative, 80 percent ethanol aqueous containing potassium metaborate preserved the larvae in good condition. Equally good was preservation in one percent phenoxetol solution either in distilled water or sea water buffered with two percent borax and having 4.5 percent propylene glycol as an additive.

17. Cocaine, procaine, 5 percent carbonated water, chloroform, nembutal and chloral hydrate-menthol mixture, quietened echinoderm larvae and avoided contraction. Larvae were most satisfactorily fixed by two percent formaldehyde solution in sea water or one percent trichloroacetic acid or one percent P-toluene sulphonic acid. One percent formaldehyde solution in sea water buffered with borax or neutralised with calcium carbonate perfectly preserved them.

18. One percent propylene phenoxetol added to sea water along with a few drops of ethanol helped to relax the tornaria larvae exhibiting violent activities during fixation. Tornaria as it imbibed water and burst being swollen, necessitates a special fixative consisting of two percent formaldehyde sea water solution and 0.5 percent aluminium potassium sulphate with a pH around 4. On fixation these larvae having a body fluid of pH 6.5 are best preserved in one percent formaldehyde solution in sea water. Use of ethylene glycol as an additive reduced brittleness.

19. Damages to fish larvae can be easily avoided by immobilisation using narcotics such as 0.005 percent phenoxetol, tertiary amyl alcohol, chloral hydrate crystals or chloretone. These larvae can be well fixed with two percent formaldehyde or 20 percent Dowicil 100 solution in sea water. Quality of preserved fish larvae can be maintained using one percent formaldehyde solution in sea water buffered with borax. Equally good was one percent propylene phenoxetol buffered with borax. Leptocephali can be best fixed at a pH around 4 using 2 percent formaldehyde solution having 0.5 percent aluminium potassium sulphate or 1 percent phenoxetol in sea water.

20. Fish eggs were kept in good condition for prolonged periods by fixation in two percent buffered formaldehyde solution and by subsequent transfer to an antioxidant such as 0.5 percent phenoxetol for quality preservation.

21. Studies on the biochemical composition of dried samples of various meroplankton taxa have shown individual variations according to their age class, size group, metamorphosing stage, feeding mechanism, type of organism fed and time of collection.

22. In proportion to a depletion in the yolk (lipid) content, lecithotrophic larvae and Semper's larvae of coelenterates showed an increase in protein and water content. In planktotrophic ceriantharian larvae with low lipid and high water content, protein content varied in relation to their growth stages. The storage carbohydrate (glycogen) content in coelenterate larvae was very low. Seasonal variation was not evident in the ash and chitin contents.

23. The planktotrophic polychaete trochophore larvae possessed a protein content double than the lecithotrophic larvae. Compared to the low yolk content in the planktotrophic larvae the lecithotrophic trochophore larvae had varying lipid contents indicating the difference in yolk metabolism. The carbohydrate percent was very high. Planktotrophic larvae had higher chitin and water content than the lecithotrophic.

24. Sipunculoidea larvae are characterised by a high ash content, variable low lipid content and low protein content.

25. Cirripedia larvae, mainly nonfeeding type up to postcypris stage, leads a planktonic life from 7 to 60 days. Lipid fraction goes on declining from first naupliar to cypris stage, as they are nonfeeding and increases in the feeding stages from cypris onwards. Associated with the low lipid value, high ash and water content were noted. In cirriped larvae carbohydrate content was high making substantial contributions to metabolic substrates. Considerable variations occurred in cirriped biochemical constituents perhaps indicating development stages. Meroplanktonic decapod larvae remaining in plankton as long as 80 days and undergoing a change from herbivore to carnivore exhibited considerable changes in their biochemical composition depending on their growth and moult stages. In penaeids, lipid and carbohydrate content was very low and the ash content increased with age.

26. Seasonal changes and the age group modified the biochemical composition of bivalve larvae. In general these larvae had a very low concentration of organic fraction, though carbohydrate content increased with size.

27. Lecithotrophic larvae of echinoderm had a very high to low percent of lipid depending on their stages of metamorphosis. Carbohydrate values remained more or less low. The calcite accumulation increased the ash content.

28. Tornaria larvae had very high water as well as ash content. Protein content increased with the ageing of larvae. Lipid and carbohydrate contents were low.

29. The biochemical composition of fish larvae varied from one spawning ground to another and from season to season, depending on size and age group. But the basic causes of differences are usually due to variation in the feeding nature and the draw made on larval reserves of fat and protein.

30. General distribution studies of four meroplankton taxa collected from the Indian Ocean during the IIOE showed abundance of larvae in the coastal areas especially during the SW monsoon period. Based on the larval distribution different zoogeographical areas in the Indian Ocean are differentiated.

31. Anthozoan larvae, ranging in numerical abundance from 0 to 190 per haul had comparatively high values (neritic abundance) towards the western and eastern Indian Ocean and along the Indian coasts, oceanic region showing a gradual reduction from north to south. Larval swarms

and expatriation areas were also observed. Cruise-wise analysis indicated that the area sampled rather than the season determined the abundance of larvae. Similarly hour of collection was found less significant than the area of collection. Though no monsoon effect was noted, spring transition period had more larvae than fall.

32. Cirripedia larvae, generally more abundant in coastal waters than in the open ocean, have high population in Bay of Bengal associated with its low saline waters and peak abundance was noticed in patches at selected areas like the South African coast.

33. Associated with the low frequency of occurrence perhaps due to a restricted larval period and spawning period in August-September (summer spawner) the numerical abundance of sipunculoid larvae also is low. Majority of larvae are collected from the neritic waters especially from the upwelling areas.

34. Meroplanktonic gastropods having wide distribution up to 45°S throughout the year gradually increasing in an east-west axis and reducing southwards had dense patches in restricted areas especially in the neighbourhood of land masses as is the case with larvae of other littoral species. Bay of Bengal collections showed lesser number of gastropod larvae. SW monsoon period showed more larvae than during NE monsoon.

35. Seasonal, latitudinal and diurnal variations of mero-plankton taxa in a pelagic ecosystem between 9°S and 32°S along the meridian 110°E longitude has been studied based on 133 collections made during 5 biological cruises in 1962-'63. Within the various meroplankton taxa, the magnitude of seasonal amplitude varied from 100 to 400 percent, fish larvae showing the lowest seasonal amplitude of 100 percent. The meroplankton taxa showed an increase in value from January to August. Study of the day and night catches showed a higher value at night than during day. Comparison with the magnitude of seasonal variation in this part of the ocean with other regions of the Indian Ocean revealed seasonal amplitude along 110°E to be low, but none the less significant.

36. Studies on annual variation along the meridian 78°E longitude based on 43 samples collected in 1962-'63 revealed annual distinct fluctuations in abundance of all groups.

37. Based on 35 night and 27 day collections made by 'Discovery' from a depth of 200 to 0 m and the thermocline to 0 m, distribution in relation to thermocline was studied in the western Indian Ocean. The abundance of fish larvae both above and below the thermocline indicated that the thermo-cline is not a barrier for the distribution of fish larvae. However their less abundance above thermocline during day

time indicated vertical migration to avoid light. Thus the results showed that light controlled vertical migration more than the temperature. The six cruise tracks sampled showed variations in abundance along with narrow nocturnal abundance noted among fish eggs, bivalve larvae, sipunculoid larvae, tornaria larvae and cirriped larvae.

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Abdel-Malek, E.T., 1951. Menthol relaxation of helminths before fixation. *J. Parasit.*, *37*: 321.

Ackman, R.G. and C.A. Eaton, 1966a. Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters III. *Can. J. Biochem.*, *44*: 1561-1566.

Ackman, R.G. and C.A. Eaton, 1967. Fatty acid composition of the decapod shrimp, *Pandalus borealis*, in relation to that of the euphausiid, *Meganycti-phanes norvegica*. *J. Fish. Res. Bd. Canada*, *24*: 467-471.

Adolph, E., 1936. Differential permeability to water and osmotic changes in Phascolosoma. *Jour. Cell. Comp. Physiol.*, *9*.

Ahlstrom, E.H., 1959b. Vertical distribution of pelagic fish eggs and larvae off California and Baja California. *U.S. Fish. Bull.*, 161, *60*: 107-143.

Ahlstrom, E.H. and J.R. Thrailkill, 1962. Plankton volume loss with time of preservation. *Rapp. P.V. Reun. Cons. perm. int. Explor. Mer.*, *153*: 78.

Ahmad, M.F., 1969. Anaesthetic effects of tricaine methane sulphonate (MS 222 Sandoz) on *Gammarus pulex* (L) (Amphipoda). *Crustaceana*, *16*: 197-201.

Aiyar, R.G., 1933. Preliminary observations on the life history of *Acentrogobius neilli*. *Curr. Sci.*, *2*: 216-217.

Aiyar, R.G., 1935. Observations on the development of *Acentrogobius neilli*. *Zool. Anz.*, *3*: 83-92.

Aiyar, R.G., 1936. Early development and metamorphosis of the tropical echinoid *Salmacis bicolor* Agassiz. *Proc. Indian Acad. Sci.*, *3B*: 714-728.

Ali Khan, J., 1972. Distribution and abundance of fish larvae in the Gulf of Aden and in the waters off the coast of W. Pakistan in relation to the environment. *Diss. Kiel*: 191.

Alikunhi, K.H., 1944a. Growth stages of Lysio-squilla tigrina Nobili. Curr. Sci., 13: 18-19.

Alikunhi, K.H., 1944b. Final pelagic larva of Squilla hieroglyphica Kemp. Curr. Sci., 13: 237-239.

Alikunhi, K.H., 1949. Note on the metamorphosis of Phyllosoma larvae from Madras. Proc. Indian Sci. Congr. (35th Sess.), Pt.3: 1931.

Alikunhi, K.H., 1950. Observations on some larval and post-larval stomatopoda. J. Bombay nat. Hist. Soc., 49: 101-107.

Alikunhi, K.H., 1951. An account of the stomatopod larvae of the Madras plankton. Rec. Indian Mus., 49: 239-319.

Alikunhi, K.H., 1967. An account of the postlarval development, moulting and growth of the common stomatopods of the Madras coas Proc. Symp. Crustacea, Mar. biol. Ass India, II: 824-945.

Alikunhi, K.H. and R.G. Aiyar, 1942. On some squilla larvae from the Madra plankton. Curr. Sci., 11: 56-58.

Alikunhi, K.H. and R.G. Aiyar, 1943. Growth in some stomatopoda. Curr. Sci., 12: 80-82.

Allison, L.N., 1954. Brown trout anesthetized with ether for spawn taking. Progve. Fish. Cul 16 (4): 171.

Ansell, A.D., P. Sivadas and B. Narayanan, 1973. The ecology of two sandy beaches in South-west India. IV. The biochemica composition of four common invertebrates. J. mar. biol. Ass. India Spl. Publ: 333-348.

Aravindakshan, P.N. and M. Sakthivel, 1973. The location of cephalopod nurseries in the Indian Ocean. IOBC Handbook, V: 70-75.

Arevalo, A., 1949. Variations in the chemical compositio of the Sorel (Trachurus trachurus). Chemical Abstracts, 142: 3118.

Armstrong, F.A.J. and J.H. Wickstead, 1962. A note on the preservation of plankt samples with formalin. J. Cons. int. Explor. Mer., 27(2): 129

Arnold, C.R., MS. 1968. The lipids of selected organisms of the *Sargassum* community from the Gulf of Mexico. *Ph.D. Thesis*, Texas A and M University, College Station, Texas, 113 pp.

Atkins, W.R.G., 1922. The preparation of permanently non-acid formalin for preserving calcareous specimens. *J. mar. biol. Ass. U.K.*, *12*: 792-94.

Austin, J., 1965. Aspects of nitrogen metabolism in *Neomysis integer* (Mysidacea). *Ph.D. Thesis*, University of Southampt

Austin, J., 1972. The biochemical composition of the tropical intertidal mysid *Gastrosaccus* sp. *J. mar. biol. Ass. India*, *14*: 219-220.

Bagenal, T.B., 1963. Propylene phenoxetol as a fish anaesthetic. *Nature, Lond.*, *197*: 1222-1223.

Baker, J.R., 1966. The fine structure resulting from fixation by formaldehyde: the effects of concentration, duration and temperature. *J. R. Micr. Soc.*, *85*: 391-399.

Baker, J.R. II, K. Hew and W.H. Fishman, 1958. The use of a chloral hydrate formaldehyde fixative solution in enzyme histochemistry. *J. Histochem. Cytochem.*, *6*: 244.

Ball, J.N. and P.N. Cowen, 1959. Urethane as a carcinogen and as an anaesthetic for fishes. *Nature Lond.* *184*: 370.

Bal, D.V. and L.B. Pradhan, 1945. A preliminary note on the plankton of the Bombay harbour. *Curr. Sci.*, *14* (8): 211-212.

Bal, D.V. and L.B. Pradhan, 1945, 1946, and 1947. First, second and third Progress report on "Investigation of fish eggs and fish larvae from Bombay waters". 1944-1947, Govt. Press, Bombay.

Bal, D.V. and L.B. Pradhan, 1951. Occurrence of fish larvae and post-larvae in Bombay waters during 1944-1947. *J. Univ. Bombay (New Ser. B)*, *20*: 1-15.

Bal, D.V. and L.B. Pradhan, 1952. Records of zooplankton in Bombay waters during 1944-47. *J. Univ. Bombay*, B. **20**: 75-80.

Balachandran, T., 1973a. Investigations on formaldehyde as a fixative and preservative for zooplankton. *Curr. Sci.*, **42** (9): 322-323.

Balachandran, T., 1973b. A review of the nature and causes of deterioration in zooplankton samples. *Mahasagar*, **6** (1): 25-30.

Balachandran, T., 1973c. A review of fixatives and preservativ[es]. *Mahasagar*, **6** (3 & 4): 182-187.

Balachandran, T., 1974a. A note on fixation and preservation of calcareous zooplankton. *Curr. Sci.*, **43** (5): 153-154.

Balachandran, T., 1974b. On methods of collection, handling and storage of zooplankton in tropics. *Curr. Sci.*, **43** (5): 154-155.

Balachandran, T., 1974c. Phenoxetol as a good sorting medium-cum-preservative for zooplankton in the tropics. *Curr. Sci.*, **43** (12): 380-381.

Balachandran, T., 1974d. Dowicil '100' - A fixative-cum-preser[vative] for zooplankton. *Mahasagar, Bull. natn. Inst. Oceanogr.*, (1 & 2): 107-109.

Balachandran, T., 1974e. On fixation and preservation of gelatinous plankters. *Mahasagar, Bull. natn. Inst. Oceanogr.*, (1 & 2): 111-117.

Balakrishnan, K.P., 1959. Fish eggs and larvae of Kerala coast. Abst. *Symposium on First All India Congress of Zoology, Jabalpur, Section A.*

Balakrishnan, K.P., 1961. Fish eggs and larvae collected by Research Vessel 'Conch' during 1958-[59]. 1. Larvae of *Cynoglossus semifasciat[us]* Day. *Bull. Res. Inst. Univ. Kerala, Ser. C.*, **7**: 131-140.

Balakrishnan, K.P., 1963. Fish eggs and larvae collected by the Research Vessel 'Conch'. *Bull. Dept. Mar. biol. and Oceanogr. Univ. Kerala*, **1**: 81-96.

Balakrishnan, K.P., 1969. Eggs and early larvae of *Thrissocles* species (Engraulidae; Pisces). *Marine Biology*, 2 (3): 224-227.

Balakrishnan, K.P., 1971. Occurrence of larvae of *Bregmaceros* s along the west coast of India. *Bull. Dept. Mar. Biol. Oceanogr., Univ. Cochin*, 5: 17-24.

Balakrishnan, K.P. and C.B.L. Devi, 1974. Larvae of some flat fishes from a tropical estuary. *The early life history of fish*. Ed. J.H.S. Blaxter, Springer-Verlag, Berlin: 263-264.

Balasubrahmanyan, K., K.S.P.B. Rao and R.S. Subbaraju, 1969. Larval and juvenile stages of the flying fish *Exocoetus volitans* L. and *Hirundichthys coromandelensis* (Hornell) from the Bay of Bengal. *Proc. Symp. Indian Ocean, New Delhi*, 1967. *Bull. natn. Inst. Sci. India*, 38, Part II: 805-811.

Banse, K., 1964. On the vertical distribution of zooplankton in the sea. *Progress in Oceanography*, 2: 53-125, Pergamon Press, Oxford.

Barnes, H., 1965. Studies in the biochemistry of cirripede eggs. 1. Changes in the general biochemical compounds during development of *Balanus balanoides* and *Balanus balanus*. *J. Mar. Biol. Ass. U.K.*, 45: 321-339.

Bassett, D.L., 1947. Ethyl methacrylate as a preserving medium for gross anatomical serial sections. *Anat. Rec.*, 99 (2): 145-50.

Bayne, B.L., 1965. Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L). *Ophelia*, 2 (1): 1-47.

Beeman, R.D., 1968. The use of succinylcholine and other drugs for anaesthetizing or narcotizing gastropod molluscs. *Pubbl. Sta. Zool. Napoli*, No. 36: 267-70.

Beers, J.R., 1966. Studies on the chemical composition of the major zooplankton groups in the Sargasso Sea off Bermuda. *Limnol. Oceanogr.*, 11: 520-528.

Beers, J.R. and G.L. Stewart, 1970. The preservation of acantharians in fixed plankton samples. Limnol. Oceanogr., 15: 825-827.

Bell, G.R., 1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Bull. Fish. Res. Bd. Canad., 148: 4.

Berland, B., 1961. Use of glacial acetic acid for killing parasitic nematodes for collection purposes. Nature, Lond. 191: 1320-1321.

Berry, H., 1944. Antibacterial values for ethylene glycol monophenyl ether. Lancet, 247: 175-178.

Blaxter, J.H.S. and K.F. Ehrlich, 1974. Changes in behaviour during starvation of Herring and Plaice larvae In: J.H.S. Blaxter (Ed.), The early life history of fish. Springer-Verlag, Berlin: 575-588.

Bliss, R. Jr., 1872. Appearance of colour in fish kept in alcohol. Amer. Pop. Sci. Rev., 11: 335-336.

Bogorov, B.G., 1958. Biogeographical regions of the plankton of the North-western Pacif Ocean and their influence on the deep sea. Deep-Sea Res., 5: 149-161

Bogorov, B.G., 1959. On the standardisation of marine plankton investigations. Int. Rev. ges. Hydrobiol. Hydrogr., 44 (4): 621-642.

Bone, Q. and E.J. Denton, 1971. The osmotic effects of electron microscope fixative. J. Cell. Biol 49: 571-581.

Boonprakob, V. and Y. Dhuteranon, 1974. Diurnal vertical distribution of t larvae of the Indo-Pacific mackerel Rastrelliger neglectus (Van Kampen Proc. IPFC 15th Sess., 1972: 131-1

Borodin, N.A., 1930. Effective methods of preserving fishes in natural colour. Mus. News Wash., 8 (6): 11-12.

Bottino, N.R., L.M. Jeffrey and R. Reiser, 1967a. Metabolism of long-chain poly unsaturated fatty acids in Antarctic euphausia. *Fed. Proc.*, 26: 793.

Bottino, N.R., L.M. Jeffrey and R. Reiser, 1967b. The lipids of Antarctic fish. *Antarctic J. U.S.*, 2: 194-195.

Brandt, K., 1898. Beitrage zur Kenntnis der chemischen Zusammensetzung des Planktons. *Wiss. Meeresuntersuch. Abt. Kiel, N.F.*, 3: 43-90.

Brandt, T., P.P. Weinstein, B. Mehlman and E.C. Weinback, 1952. Observations on the metabolism of bacteria free larvae of *Trichinella spiralis*. *Exp. Parasitol.*, 1: 245-255.

Bruno, M.M. and Melville Sahyun, 1944. Comparative studies of preservative agents. *J. Bact.*, 48 (2): 255.

Burke, 1933. Pyridine as a neutralizing agent for formaldehyde. As referred by H.F. Steedman in *Zooplankton fixation and preservation*. The Unesco Press, Paris, 1976, page. 129.

Burkholder, P.R., R.M. Mandelli and P. Centeno, 1967. Some chemical properties of *Munida gregaria* and *Euphausia superba*. *J. Agric. Fd. Chem.*, 15: 718-720.

Burrows, R.E., 1952. Chloretone used to anaesthetise salmon fingerlings. *Progve. Fish. Cult.*, 14 (2): 78.

Carriker, M.R., 1950. Killing and preservation of bivalve larvae in fluids. *Nautilus*, 64: 14-17.

Carriker, M.R., 1963. Some recent methods for narcotization killing, fixation, and preservation of marine organisms. Appendix I. In: H.B. Russell (Comp.), *Notes on methods for the narcotization, killing, fixation and preservation of marine organisms*. Woods Hole, Mass., Systematics-Ecology Programme, Marine Biological Laboratory: 70 pp.

Carruthers, J.N., S.S. Gogate, J.R. Naidu and T. Laevastu, 1959. Shoreward upslope of the layer of minimum oxygen off Bombay, its influence on marine biology, especially fisheries. *Nature, Lond.*, 183: 1084-1087.

Cassie, R.M., 1963. Microdistribution of plankton. Oceanogr. Mar. Biol. A. Rev., 1: 223-55

Chacko, P.I., 1950. Marine plankton from waters around the Krusadai Island. Proc. Indian Acad. Sci. 31 (3) B: 162-174.

Chacko, P.I., 1956. Annual report of the Marine Biological Station, Tuticorin, April 1954-March 1955 (Section on Plankton). Fish. Stn. Reports: 37-56.

Chacko, P.I., 1959. Annual report of the Marine Biological Station, Krusadai Island, Gulf of Mannar for 1955-56 (Section on Plankton Fish. Stn. Reports: 7-10.

Chhapgar, B.F., 1956. On the breeding habits and larval stages of some crabs of Bombay. Rec. Indian Mus., 54: 33-52.

Chari, S.T., 1948. Nutritive value of some of the west coast marine food fishes of Madras Province. Indian Jour. Med. Res., 36 (3): 253.

Chen, T.P., and L.E. Fellers, 1926. Fish preservation by hypochlorites. Univ. Wash. Publs. Fish., 1: 205-227.

Clark, A.H., 1888. Fish preservation by the use of acetic boracic, salicylic and other acids and compounds. Trans. Am. Fish. Soc., 1887: 28-34.

Claus, C., P. Benijts and G. Vande Putte, 1979. The biochemical composition of the larvae of two strains of Artemia salina (L.) reared on 2 different algal foods. J. exp. Mar. Biol. Ecol., 36: 171-183.

Clement, A.C. and J.N. Cather, 1957. A technique for preparing whole mounts of veliger larvae. Biol. Bull., 113: 340.

Clutter, R.I. and M. Anraku, 1968. Avoidance of samplers in Monographs on Oceanographic Methodology. 2, Zooplankton sampling, UNESCO: 57-76.

Collyer, D.M., 1957. Viability and glycogen reserves in the newly liberated larvae of Ostrea edulis L. J. mar. Biol. Ass. U.K., 36: 335-37.

Conover, R.J., 1962. Metabolism and growth in *Calanus hyperboreus* in relation to its life cycle. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer.*, 153: 190-196.

Conover, R.J., 1964. Food relations and nutrition of zooplankton. *Occas. Publs. Proc. Symp. exptl. mar. Ecol.*, No. 2: 81-91, University of Rhode Island.

Conover, R.J. and E.D.S. Corner, 1968. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. *J. mar. biol. Ass. U.K.*, 48: 49-75.

Corkett, C.J. and I.A. McLaren, 1969. Egg production and oil storage by the copepod *Pseudocalanus* in the laboratory. *J. exp. Mar. Biol. Ecol.*, 3: 90-105.

Corner, E.D.S. and C.B. Cowey, 1968. Biochemical studies on the production of marine zooplankton. *Biol. Rev.*, 43: 393-426.

Costello, D.P., M.E. Davidson, A. Eggers, M.H. Fox and C. Henley, 1957. Methods for obtaining and handling marine eggs and embryos. *Woods Hole, Mass.*, *Marine Biological Laboratory*.

Cowey, C.B. and E.D.S. Corner, 1963a. Amino acids and some other nitrogenous compounds in *Calanus finmarchicus*. *J. mar. biol. Ass. U.K.*, 43: 485-493.

Cowey, C.B. and E.D.S. Corner, 1963b. On the nutrition and metabolism of zooplankton. II. The relationship between the marine copepod *Calanus helgolandicus* and particulate material in the Plymouth Sea Water, in terms of amino acid composition. *J. mar. biol. Ass. U.K.*, 43: 495-511.

Crawford, D.R. and C.M. Breder, Jr., 1922. Notes on the preservation of the colours of fishes. *Copeia*, 105: 25-30.

Cross, F.B., 1962. Collecting and preserving fishes. *Misc. Publs. Mus. nat. Hist., Univ. Kans.* No. 30: 41-44.

CSIRO Australia, 1965a. Oceanographical observations in the Indian Ocean in 1963, H.M.A.S. Gascoyne, Cruise G1/63. *CSIRO Aust. Oceanogr. Cruise Rep.*, 21.

CSIRO Australia, 1965b. Oceanographical observations in the Indian Ocean in 1963. H.M.A.S. Diamantina, Cruise Dm 1/63. CSIRO Aust. Oceanogr. Cruise Rep., 23.

CSIRO Australia, 1965c. Oceanographical observations in the Indian Ocean in 1963. H.M.A.S. Diamantina Cruise Dm 2/63. CSIRO Aust. Oceanogr. Cruise Rep., 24.

CSIRO Australia, 1965d. Oceanographical observations in the Indian Ocean in 1963. H.M.A.S. Diamantina Cruise Dm 3/63. CSIRO Aust. Oceanogr. Cruise Rep., 25.

CSIRO Australia, 1966a. Oceanographical observations in the Indian Ocean in 1962. H.M.A.S. Gascoyne Cruise G4/62. CSIRO Aust. Oceanogr. Cruise Rep., 17.

CSIRO Australia, 1966b. Oceanographical observations in the Indian Ocean in 1962. H.M.A.S. Diamantina Cruise Dm 4/62. CSIRO Aust. Oceanogr. Cruise Rep., 20.

Culkin, F. and R.J. Morris, 1969. The fatty acids of some marine crustaceans. Deep-Sea Res., 16: 109-116.

Culkin, F. and R.J. Morris, 1970. The fatty acid composition of two marine filter feeders in relation to a phytoplankton diet. Deep-Sea Res., 17: 861-65.

Cullingworth, A.W., 1939. Methods employed in sealing glass display jars containing specimens in spirit and of displaying fishes with their natural colours in spirit. Bull. S. Afr. Mus. Assoc., 1: 261-264.

Currie, R.I., 1963. The Indian Ocean Standard net. Deep-Sea Res., 10: 27-32.

Cushing, D.H., 1962. Patchiness. Rapp. Proces-Verbaux Reunions Conseil Perm. Intern. Explor. Mer., 15 (26): 152-163.

Cushing, D.H., 1971. Upwelling and fish production. Adv. Mar. Biol., 9: 255-334.

Das, H.P., 1978. Seasonal variations in the chemical composition and calorific content of Mugil cephalus Linnaeus from the Goa waters. Mahasagar, Bull. natn. Inst. Oceanogr., 11 (3 & 4): 177-184.

Dekhuyzen, M., 1921. Les parois de certains animaux marins halisotoniques sont biologiquement semipermeables. Arch. Neerland Physio 5.

Delsman, H.C., 1921-1938. Fish eggs and larvae of the Java Sea. Treubia, 1 to 16.

Devanesan, D.W. and S. Varadarajan, 1940. The occurrence of Tornaria larva at Krusadai. Curr. Sci., 9: 375-377.

Devi, C.B.L., 1972. Fixation and preservation of plankton samples. In: Handbook to the International Zooplankton Collections. III: Proc. Workshop on Plankton Methods: 24-3

Devi, C.B.L., 1977. Distribution of larvae of flat fishes (Heterosomata) in the Indian Ocean. Proc. Symp. Warm Water Zoopl. Spl. Pub. UNESCO/NIO: 18-20.

Dileep, M.P., 1977. The larval development and distribution of Saurida tumbil (Bloch) of south-west coast of India. Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO: 460-473.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Analyt. Chem., 28: 350-356.

Ealey, E.H.M., 1954. A new method of net plankton determination. J. Cons. CIEM., 19: 368.

Edmonds, S.J., 1955, 1956. Australian Sipunculoidea I, II. Austral. Jour. Marine Freshw. Res., 6, 7.

Elbourn, C.A., 1966. Some observations on the food Orelena [illegible] [illegible] (Fischer). Ann. Mag. nat. Hist., 9 (13): 227-231.

Elmhirst, R., 1929. Preservation of colour in marine organisms. Museums J., 29 (1): 6-8.

Eschmeyer, R.H., 1953. The effect of ether anaesthesia on fin-clipping rate. Progve. Fish Cult., 15 (2): 80-82.

Farris, D.A., 1963. Shrinkage of sardine (Sardinops caerulea) larvae upon preservation in buffered formalin. Copeia, 1: 185-186.

Ferguson, C.F., 1973. Aspects of nutrition and metabolism of Neomysis integer. Ph.D. Thesis, University of Southampton.

Ferguson, C.F., and J.E.G. Raymont, 1974. Biochemical studies on marine zooplankton. XII. Further investigation on Euphausia superba Dana. J. mar. biol. Ass. U.K., 54: 719-725.

Fish, F.F., 1943. The anaesthesia of fish by high carbondioxide concentrations. Trans. Amer. Fish. Soc., 72: 25-29.

Fischer, W.K., 1947. New genera and species of echiuroid and sipunculoid worms. Proc. U.S. Nat. Mus., 97.

Fischer, W.K., 1952. The sipunculoid worms of California and Baja California. Proc. U.S. Nat. Mus., 102.

Fischer, L.R., 1962. The total lipid material in some species of marine zooplankton. Rapp. P.V. Réun. Cons. perm. int. Explor. Mer., 153: 129-136.

Flüchter, J., 1965. Versuche zur Brutaufzucht der Seezunge Solea solea in kleinen Aquarien. Helgoländer wiss. Meeresunters., 12: 395-403.

Flüchter, J. and T.J. Pandian, 1968. Rate and efficiency of yolk utilisation in developing eggs of the Sole Solea solea. Helgoländer wiss. Meeresunters., 18: 53-60.

Folch, J., M. Lees and G.H. Sloane Stanley, 1956. A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem., 226: 497-509.

Foley, D.M., J.E. Stewart and R.A. Holley, 1966. Isobutyl alcohol and methyl pentynol as general anaesthetics for the lobster, Homarus americanus, Milne Edwards. Canad. J. Zool., 44: 141-143.

Fox, H.M. and G. Vevers, 1960. The nature of animal colours. Sidgwick and Jackson, London.

Foxton, P., 1956. The distribution of the standing crop of zooplankton in the southern ocean. 'Discovery Rep.', 28: 191-236.

Frank, J.R., S.D. Sulkin and R.P. Morgan, II., 1975. Biochemical changes during larval development of the Xanthid crab *Rhithropanopeus harrisii*. I. Protein, total lipid, alkaline phosphatase and glutamic oxaloacetic transaminase. *Mar. Biol.*, 32: 105-111.

Fraser, J.H., 1961. Use of polyethylene glycol in biology. *Nature, Lond.*, 189 (4760): 241-242.

Frolander, H.F., 1957. A plankton volume indicator. *J. Cons. CIEM*, 22: 278-283.

Fruton, J.S. and S. Simmonds, 1958. *General Biochemistry.* John Wiley and Sons., Inc., New York, 1077 pp.

Fudge, H., 1968. Biochemical analysis of preserved zooplankton. *Nature, Lond.*, 219: 380-81

Fujita, T., 1934. Note on the Japanese oyster larvae. *Proc. 5th Pacif. Sci. Congr.*, 4111-4117.

Gallagher, C., 1966. The variability of water masses in the Indian Ocean. *Nat. Oceanogr. Data Centre, General Series, Publ. G* 11.

Ganapati, P.N. and M.V. Lakshmana Rao, 1958. The occurrence of Scyphistoma larva in Visakhapatnam Harbour. *Curr. Sci.*, 27: 396-397.

Ganapati, P.N. and M.V. Lakshmana Rao, 1962. Observations on the development of Scyphistoma larvae from Visakhapatnam Harbour. *Proc. All India Congr. Zool., Jabalpur*, 1: Part 2: 299-304 (1959), also Abstract: 25 (1959).

Ganapati, P.N. and Y. Radhakrishna, 1958. Studies on the polychaete larvae in the plankton off Waltair coast. *Andhra Univ. Mem. Oceanogr.*, 2: 210-237.

Ganapati, P.N. and N.S. Raju, 1961a. On the occurrence of pelagic fish spawn. *Curr. Sci.*, 30: 61.

Ganapati, P.N. and N.S. Raju, 1961b. On the eggs and early development of eels off Waltair coast. *J. Zool. Soc. India*, 1960, 12 (2): 229-238.

Ganapati, P.N. and N.S. Raju, 1963. Fish eggs off Waltair coast. *Curr. Sci.*, 32: 361-362.

Ganapati, P.N. and D.V.S. Rao, 1958. Quantitative study of plankton off Lawson's Bay, Waltair. *Proc. Ind. Acad. Sci.*, (B), 48: 189-209.

Gannon, J.E. and S.A. Gannon, 1975. Observations on the narcotization of crustacean zooplankton. Crustaceana, 28 (2): 220-224.

George, K.C., 1979. Studies on the distribution and abundance of fish eggs and larvae off the south-west coast of India with special reference to Scombroidea. Ph.D. Thesis, University of Cochin, 197 pp.

George, M.J., 1958. Observations on the plankton of the Cochin backwaters. Indian J. Fish., 5 (2): 375-401.

George, M.J. and V.T. Paulinose, 1973. Leptochela robusta Stimpson (Decapoda, Caridea, Pasiphaeidae) from the south-west coast of India and its larval development. IOBC Handbook, 5.

George, M.J. and S.G. Goswamy, 1977. Observations on the larvae of Penaeid prawns of commercial importance in the coastal waters of Goa. Proc. Symp. Warm Water Zooplankton Spl. Publ. NIO/UNESCO: 148-154.

George, P.C., 1953. The marine plankton of the coastal waters of Calicut with observations on the hydrological conditions. J. Zool. Soc. India, 5 (1): 76-107.

Gersh, I., 1932. Altmann technique for fixation by drying while freezing. Anat. Rec., 53: 309-337.

Gerrick, D.J., 1968. A comparative study of antioxidants in colour preservation of fish. Ohio J. Sci., 68 (4): 239-240.

Gilles, 1900. Formalin as a preservative. Entomologist, 33: 90.

Gohar, H.A.F., 1937. The preservation of contractile marine animals in an expanded condition. J. mar. biol. Ass. U.K., 22: 295-299.

Gopalakrishnan, K., 1976. Larval rearing of red shrimp Penaeus marginatus (Crustacea). Aquaculture, 9 (2): 145-154.

Gopalakrishnan, T.C., 1973. Folsom Splitter accuracy. Proceedings of the Workshop on Plankton Methods. In: Handbook to the International Zooplankton Collections, 5: 35.

Gopalakrishnan, T.C., K.K.C. Nair, M. George Peter, V.N. Sankaranarayanan and T.S.S. Rao, 1977. Major biochemical constituents of some faunal components of the Cochin backwaters. Indian J. mar. Sci., 7: 151-152.

Gopinath, K., 1942. Distribution and feeding of the post-larvae of fishes of the Trivandrum coast. Curr. Sci., 11: 337-338.

Gopinath, K., 1946. Notes on the larval and postlarval stages of fishes found along the Trivandrum coast. Proc. natl. Inst. Sci. India, 12: 7-21.

Gravely, F.H., 1927a. Nemertinea. Bull. Madras Govt. Mus. N.S., 1: 53-54.

Gravely, F.H., 1927b. The littoral fauna of Krusadai Island in the Gulf of Mannar. Polyzoa. Bull. Madras Govt. Mus., New Ser., 1 (1): 89-94.

Gray, P., 1953. Handbook of basic microtechnique. Constable, London.

Gregg, R.R., 1968. The preservation of invertebrates in alcohol. Ent. News., 79: 1-12.

Griffin, L.E., 1910. A method of using magnesium sulphate for the anaesthetization of marine animals. Philipp. J. Sci., Sec. D., 5: 86.

Griffiths, F.P., G. Webb and P.W. Schneider, 1941. Ether anesthesia of steelhead trout. Trans. Amer. Fish. Soc., 70: 272-273.

Green, W., 1954. Osmotic responses in Dendrostomum. Jour. Exptl. Biol., 31.

Gruen, L. and M. Wirtz, 1964. Fish preserves with or without hexamethylene-tetramine. Dt. Lebensmittelrdsch. 60 (1): 14-18.

Gurney, R., 1924. Crustacea, 9. Decapod larvae. Nat. Hist. Rep. Br. Antarc. Terra Nova Exped. Zoology, Crustacea, 8: 37-202.

Haly, A., 1891. A medium for preserving the colours of fish and other animals. Nature, Lond., 43: 212.

Hammen, C.S. and Susan C. Lum, 1962. Carbondioxide fixation in marine invertebrates. J. Bio. Chem., 237 (8): 2419-2429.

Hannerz, L., 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae n. fam. in the Gullmar Fjord (Sweden). Zool. Bidr. Upps., 31: 1-204.

Harmer, S.F., 1922. Experiments on the fading of museum specimens. Museums J., 21: 205-222.

Haraguchi, T., Y. Simidu and K. Aiso, 1969. Preserving effect of ozone to fish. Bull. Jap. Soc. Scient. Fish., 35: 915-919.

Hargis, W.J. Jr., 1953. Chloretone as a trematode relaxer and its use in mass collecting techniques. J. Parasit., 39 (2): 224-225.

Harms, W. and O. Dragendorff, 1933. Osmotische Untersuchungen an Physcosoma lurco. Zschr. Wissensch. Zool., 143.

Harris, R.H., 1968. A new apparatus for freezedrying whole biological specimens. Med. Biol. Illust., 18: 180-182.

Held, D., 1915. The preserving action of benzoic acid. Arch. Hyg., 84: 289.

Hensen, V., 1887. Uber die Bestimmung des Planktons oder des im Meere treibended materials des Pflanzen and Tieren. Bericht d. Kom. z. Wiss. Unters. d. deutschen Meere.

Herzberg, A. and R. Pasteur, 1975. The identification of grey mullet species by disc electrophoresis. Aquaculture, 5: 99-106.

Hewatt, W.G., 1943. A method for narcotising Holothurians. Science, 97: 588.

Hill, R.B., 1966. Propylene phenoxetol as a preservative for living holothurians (Stichopus moebii). Nature, Lond., 211 (5046): 304-305.

Hoffmann, G.L., 1954. Polyvinyl alcohol-fixative-adhesive for small helminths and protozoa. Trans. Amer. Microsc. Soc., 73: 328-329.

Hopkins, T.L., 1968. Carbon and nitrogen content of fresh and preserved *Nematoscelis difficilis*. *Journal du Conseil*, 31: 300-304.

Hora, S.L., 1939. A note on the collection, preservation, labelling and transport of fish. *J. Darjeeling nat. Hist. Soc.*, 13: 123-31

Hornell, J., 1893. The use of formalin as a preservative medium for marine animals. *Natur. Sci.*, 5 (7): 416.

Hower, R.O., 1967. The freeze-dry preservation of biological specimens. *Proc. U.S. nat. Mus.*, 119 (3549): 24 pp.

Huheey, J.E., 1963. Concerning the use of paraformaldehyde as a field preservative. *Copeia*, No. 1: 192-193.

Hunter, J.R., 1972. Swimming and feeding behaviour of larval anchovy *Engraulis mordax*. *Fish. Bull. Calif.*, 70: 821-838.

Ikeda, T., 1971. Changes in respiration rate and in composition of organic matter in *Calanus cristatus* under starvation. *Bull. Fac. Fish. Hokkaido Univ.*, 21: 280-298.

IOBC, 1969. 1969. International Zooplankton Collections. *IOBC Handbook*, 1. Station List.

IOBC, 1968-1973. International Indian Ocean Expedition *Plankton Atlases*, I-V, NIO, CSIR, New Delhi.

IOBC, 1969-1973. *Handbooks to the International Zooplankton Collections*, I-V, NIO, CSIR, New Delhi.

Jamieson, W.A. and H.M. Powell, 1931. Merthiolate as a preservative for biological products. *Amer. J. Hyg.*, 14 (1): 218-224.

Jawed, M., 1969. Body nitrogen and nitrogenous excretion in *Neomysis rayii* Murdoch and *Euphausia pacifica* Hansen. *Limnol. Oceanogr.*, 14: 748-754.

Jeffries, H.P., 1969. Seasonal composition of temperate plankton communities: free amino acids. *Limnol. Oceanogr.*, 14: 41-52.

| | | |
|---|---|---|
| Jeffries, H.P., | 1970. | Seasonal composition of temperate plankton communities: fatty acids. Limnol. Oceanogr., 15: 419-426. |
| Jeffrey, L.M., N.R. Bottino and R. Reiser, | 1966. | The distribution of fatty acid classe in lipids of Antarctic euphausiids. Antarctic. J., 1: 209. |
| Johnson, L.D., | 1954. | Use of Urethane anesthesia in spawnin eastern brook trout. Progve. Fish. Cult., 16 (4): 182-183. |
| Jones, E.P., | 1936. | Comparative measurements of living and fixed protozoa. Trans. Amer. Mic Soc., 55: 316-318. |
| Jones, S. and M. Kumaran, | 1963. | Distribution of larval tuna collected by the Carlsberg Foundation's Dana Expedition (1928-30) from the Indian Ocean. FAO Fish. Rep., 3 (6): 1753-7 |
| Jones, S. and M. Kumaran, | 1964a. | Distribution of larval bill fishes in the Indo-Pacific with special referen to the collections made by the Danish Dana Expedition. Proc. Symp. Scombroid. Fishes. Mandapam Camp. Pt. 1: 483-498. |
| Jones, S. and M. Kumaran, | 1964b. | Eggs and larvae of Scombroid fishes. Proc. Symp. Scombroid Fishes. Mar. Bi Ass. India, Part 1: 343-378. |
| Joosse, J. and J. Lever, | 1959. | Techniques of narcotization and operation for experiments with Limnae stagnalis (Gastropoda, Pulmonata). Proc. K. ned. Akad. Wet., Ser. C, 62: 145-149. |
| Juszczyk, W., | 1952. | The preservation of natural colours in skin preparations of certain amphi Copeia, No. 1: 35-38. |
| Kabanova, J.G., | 1968. | Primary production of the northern pa of the Indian Ocean. Oceanology, 8: 214-225. |
| Keen, W.W., | 1875. | Chloral as a preservative of anatomic objects. Nature, Lond., 12: 484. |
| Kisch, B., | 1947. | A method to immobilize fish for cardi and other procedures with procain. Biol. Bull. (Woods Hole), 93: 208. |

Klawe, W.L., 1963. Observations on the spawning of four species of tuna in the eastern Pacific Ocean based on the distribution of their larvae and juveniles. Bull. Inter-Am. Trop. Tuna Comm., 6(9):449-51

Klem, A., 1932. Contributions to the study of the oils of marine crustacea. I. The oils of Meganyctiphanes norvegica M. Sars and Calanus finmarchicus Gunn. Hvalrådets Skr., 6: 24.

Kollen, G., 1939. Über die Nephridien von Phascolosoma japonicum. Verhandlg. Dtsch. Zool. Gesellsch., 41: In: Zool. Anz. Suppl. Bd. 12.

Krey, J., 1950. Eine neue Methode zur quantitativen Bestimmung des Planktons. Kieler Meeresforsch., 7: 58-75.

Krey, J., K. Banse and Hagmeier, 1957. Über die Bestimmung von Eiweiss im Plankton mittels der Biuret reaktion. Kiel. Meer., 13: 35-40.

Krey, J. and B. Babenerd, 1976. Phytoplankton production Atlas of the International Indian Ocean Expedition. Kiel, p.70.

Krishnaswamy, S., 1959. Metabolic studies on marine parasitic copepods and on certain zooplankton species. Ph.D. Thesis, University of Southampton.

Krohn, A., 1851. Über die Larve des Sipunculus nudus. Müller's Arch. Anat. Physiol.

Kryuchkova, M.I. and G.P. Makarov, 1960. Technochemical characteristics of Krill (Translation by National Lending Librar

Kumari, B.K., 1954. Plankton studies. The occurrence and distribution of the siphonophores, anthozoan larvae and pelagic tunicates, off Visakhapatnam coast. M.Sc. Thesis, Andhra University, Waltair.

Kunjamma, P.P.M. and T.C. Gopalakrishnan, 1977. Distribution of Echinoderm larvae and Tornaria larvae in the Indian Ocean. Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO, Goa: 128-131.

Lackey, J.B., 1938. The manipulation and counting of river plankton and changes in some organisms due to formalin preservation. U.S. Public Health Rep., No. 53: 2080-209

Lalli, C.M., 1970. Morphology of Crucibranchaea macrochira (Meisenheimer) a Gymnosomatous pteropod. Proc. Malacological Soc. Lond., 39: 1-14.

Lasker, R., 1962. Efficiency of yolk utilization by developing embryos and larvae of the Pacific Sardine *Sardinops caerulea* (Girard). J. Fish. Res. Bd. Can., 19: 867-875.

Lasker, R. and G.H. Theilacker, 1962. The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. J. Lipid. Res., 3: 60-64.

Lebour, M.V., 1922. The food of plankton organisms. J. Mar. Biol. Ass. U.K., 12: 644-677.

Lebour, M.V., 1933. Rearing marine animals in a plunger jar. Science Progress, No. 107: 494-502.

Lee, R.F., J.C. Nevenzel and G.A. Paffenhofer, 1970a. Wax esters in marine copepods. Science, 167: 1510-1511.

Lee, R.F., J.C. Nevenzel and G.A. Paffenhofer, 1970b. The metabolism of wax esters and other lipids by the marine copepods, *Calanus helgolandicus*. J. Lipid Res., 11: 237-240

Lee, R.F., J. Hirota and A.M. Barnett, 1971a. Distribution and importance of wax esters in marine copepods and other zooplankton. Deep-Sea Res., 18: 1165-67.

Lee, R.F., J.C. Nevenzel and G.A. Paffenhofer, 1971b. Importance of wax esters and other lipids in the marine food chain: Phytoplankton and copepods. Mar. Biol., 9: 99-108.

Lee, R.F., J.C. Nevenzel and R. Sauerheber, A.A. Benson and A.R. Lewis, 1972a. Lipids in the marine environment. Calif. Mar. Res. Comm. CALCOFI Rep., 16: 95-102.

Lee, R.F., J.C. Nevenzel and G.A. Paffenhofer, 1972b. The presence of wax esters in marine planktonic copepods. Naturwissenschaften, 59: 406-411.

Lee, R.F. and J. Hirota, 1973. Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. Limnol. Oceanogr., 18: 227-239.

Lee, R.F., 1974. Lipids of zooplankton from Bute Inlet, British Columbia. J. Fish. Res. Bd. Can., 31: 1577-1582.

Lee, R.F., 1974. Lipid composition of the copepod *Calanus hyperboreus* from the Arctic Ocean changes with depth and season. *Mar. Biol.*, 26: 313-318.

Lee, R.F., J.C. Nevenzel and A.G. Lewis, 1974. Lipid changes during life cycle of marine copepod, *Euchaeta japonica* Marukawa. *Lipids*, 9 (11): 891-898.

Legall, J.V., 1947. Valeur nutritive et valeur therapeutique del l' huitre. *Notes Mem. Off. Scient. tech. Pech. marit.*, No.? : 1-72.

Leibovitz, D.H., 1976. Propylene phenoxetol/magnesium chloride method for narcotising gastropod veligers. In: H.F. Steedman (Ed.) *Mono. Oceanogr. Methodology*, 4. Zooplankton fixation and preservation: 301-304.

Lemche, H., 1963. Personal communication cited by N.W. Runham, K. Isarankura and B.J. Smit 1965. Methods for narcotising and anaesthetising gastropods. *Malacologia* 2 (2): 231-238.

Lever, J., J.C. Jager and A. Westerveld, 1964. A new anaesthetisation technique for freshwater snails tested on *Lymnaea stagnalis*. *Malacologia*, 1: 331-38

Levi, H.W., 1946. Preservation of biological specimens with clarite X. *Science*, 103 (2667): 17

Levi, H.W., 1966. The care of alcoholic collections of small invertebrates. *Syst. Zool.*, 15 (3): 183.

Lewis, R.W., 1967. Fatty acid composition of some marine animals, from various depths. *J. Fish. Res. Bd. Can.*, 24: 1101-1115.

Linford, E., 1965. Biochemical studies on marine zooplankt II. Variations in the lipid contents of some mysidacea. *J. Cons. perm. int. Explor. Mer.*, 30: 16-27.

Littlepage, J.L., 1964. Seasonal variation in lipid content of two antarctic marine crustacea. In: *Biologie antarctique*: 463-470. Ed. Carrick *et al.*, Paris: Hermann, *Actual. Sci. Ind.*, 1312: 463.

Loosanoff, V.L. and H.C. Davis, 1963. Rearing of bivalve mollusks. In: F.S. Russell (Ed.), *Advances in Marine Biology*, 1, Academic Press, New York: 1-136.

Loosanoff, V.L., H.C. Davis and P.E. Chanley, 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. *Malacologia*, 4 (2): 351-435.

Lovegrove, T., 1966. The determination of the dry weight of plankton and the effect of various factors on the values obtained. In: H. Barnes (Ed.), *Some contemporary studies in marine science*. London, Allen & Unwin Ltd., 429-467.

Lovern, J.A., 1964. The lipids of marine organisms. *Oceanogr. Mar. Biol. Ann. Rev.*, 2: 169-191.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Madhupratap, M., P. Venugopal and P. Haridas, 1979. Biochemical studies on some tropical estuarine species. *Indian J. Mar. Sci.* 8: 155-158.

Marie, M. and T. Marie, 1962. A solution for the preservation of colour in fishes. *Turtox News*, 40 (4): 122-123.

Marr, J., 1963. Some notes on the preservationof marine animals. *J. Cons. int. Explor. Mer.*, 28 (1): 121-125.

Marshall, S.M. and A.P. Orr, 1955. *The Biology of a marine copepod*. Oliver and Boyd, Edinburgh, 188 pp.

Marshall, S.M. and A.P. Orr, 1962. Carbohydrate as a measure of phytoplankton. *J. mar. biol. Ass. U.K.*, 42: 511-519.

Matsumoto, W.M., E.H. Ahlstrom, S. Jones, W.L. Klawe, W.J. Richards and S. Ueyanagi, 1972. On the clarification of larval tuna identification particularly in the genus *Thunnus*. *Fish. Bull.*, 70 (1): 1-12.

Maunsbach, A.B., 1966. The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. II. Effect of the varying osmolality, ionic strength buffer system and fixative concentration of glutaraldehyde solutions. J. Ultrastruct. Res., 15: 283.

Mauchline, J. and L.R. Fisher, 1969. The biology of euphausiids. Adv. mar. Biol., 7: 1-454.

Mayzaud, P. and J.L.M. Martin, 1975. Some aspects of the biochemical and mineral composition of marine plankton. J. exp. mar. Biol. Ecol., 17: 297-310.

McAllister, D.E., 1965. The collecting and preserving of fishes. Bull. natn. Mus. Canada, No. 69: 152-170.

McCormick, J.B., 1961. Colour preservation of gross museum specimens. Arch. path., 72 (1): 96-99.

McErlean, A.J., 1967. Ethyl p-aminobenzoate: An anaesthetic for cold-blooded vertebrates. Copeia, No. 1: 239-240.

McEwen, G.F., M.W. Johnson and T.R. Folsom, 1954. A statistical analysis of the performance of the Folsom Plankton Sample Splitter, based upon test observations. Arch. Met. Geophys. Bioklimatol. Ser. A., 7: 302-327.

McFarland, W.N., 1959. A study of general anesthesia in teleosts with a discussion of its implications to the transportation of fishes. Ph.D. Dissertation, Univ. Calif., Los Angles, 204 pp.

McGee-Russell, S.M., 1958. Histochemical methods for calcium. J. Histochem. Cytochem., 6: 22-42.

McGovern, B.H. and R. Rugh, 1944. Efficacy of M-amino ethyl benzoate as an anaesthetic for amphibian embryos. Proc. Soc. Exp. Biol. Med., 57: 127-130.

McKay, C.R. and D.S. Hartzband, 1970. Propylene phenoxetol narcotic agent for unsorted benthic invertebrates. Trans. Amer. microsc. Soc., 89(1): 53-54.

McLaren, I.A., 1965. Some relationship between temperature and size development rate and fecundity of the copepod Pseudocalanus. Limnol. Oceanogr., 10: 528-538.

Mendel, B., A. Kemp and D.K. Myers, 1954. A colorimetric method for the determination of glucose. J. Biochem., 56: 639-646.

Mengi, T., 1965. Veränderungen in der chemischen Zusammensetzung des reifenden Ovariums des Ostseedorsches. Kieler Meeresforsch., 21: 107-121.

Menon, K.R., 1914. Note on the genus Acaetinia. Rec. Ind. Mus., 10: 211-212.

Menon, K.S., 1931. A preliminary account of the Madras plankton. Rec. Ind. Mus., 33: 489-516.

Menon, M.A.S., 1945. Observations on the distribution of the plankton of Trivandrum coast. Proc. Indian Acad. Sci. B., 22: 31-62.

Menon, M.K., 1933. The life-histories of decapod crustacea from Madras. Bull. Madras Govt. Mus. (New Ser. Nat. Hist.), 3 (3): 1-45.

Menon, M.K., 1937. Decapod larvae from the Madras Plankton. Bull. Madras Govt. Mus. (New Ser. Nat. Hist.), 3 (5): 1-56.

Menon, M.K., 1940. Decapod larvae from the Madras Plankton. II. Bull. Madras Govt. Mus. (New Ser. Nat. Hist.), 3 (6): 1-47.

Menon, M.K., 1949. The larval stages of Periclimenes indicus Kemp. Proc. Indian Acad. Sci. B., 30: 121-135.

Menon, M.K., 1951. The life history and bionomics of an Indian Penaeid prawn Metapenaeus dobsoni Miers. Proc. Indo-Pacific Fish. Council, 11: 80-93.

Menon, M.K., P.G. Menon and V.T. Paulinose. 1969. Preliminary notes on the decapod larvae of the Arabian Sea. Proc. Symp. Indian Ocean, Bull. Nat. Inst. Sci. India, 38 (2): 753-757.

Menon, P.G., 1972. Decapod crustacea from the International Indian Ocean Expedition. The larval development of [illegible] (Caridea). J. Zool. London, 167 (3): 371-397.

Menon, P.G. and D.I. Williamson, 1971. Decapod crustacea from the International Indian Ocean Expedition. The species of Thalassocaris (Caridea) and their larvae. J. Zool., London, 165: 27-51.

Miller, R.R., 1952. Treated formalin as a permanent preservative. Turtox News, 30 (10): 178-179.

Miller, R.H., 1962. As quoted by H.F. Steedman in Zooplankton fixation and preservation. The Unesco Press, Paris, 1976, p.146.

Millar, R.H. and J.M. Scott, 1967. The larva of the oyster Ostrea edulis during starvation. J. mar. biol. Ass. U.K., 47: 475-484.

Miyake, Y. and Y. Sugiura, 1966. General report of the participation of Japan in the International Indian Ocean Expedition. V-5. Chemical Oceanography Rec. Oceanogr. Work in Japan, 8 (2):72-

Mohamed, K.H., P. Vedavyasa Rao and M.J. George, 1968. Postlarvae of the penaeid prawns of the southwest coast of India, with a key to their identification. FAO Fish. Rep., (57), 2: 487-503.

Mohamed, K.H., P. Vedavyasa Rao and C. Suseelan, 1971. The first phyllosoma stage of the Indi deep-sea lobster, Puerulus sewelli Ramadan. Proc. Indian Acad. Sci., 74 (4): 208-215.

Moiseev, P.A., 1970. Some aspects of the commercial use of Krill resources of the Antarctic Seas. Antarctic Ecology, 1 : 213-216, (Ed. M.W. Holdgate), London Academic P

Motoda, S., K. Kouno, A. Kawamura and K. Osawa, 1963. Proposed method of estimation of quantity of water filtered by vertical net haul, and its application on illustrating distribution of zooplankt biomass in the eastern Indian Ocean. Inf. Bull. Planktol. Japan, No. 10: 22-2

Moyse, J.J. and R.W. Knight-Jones, 1967. Biology of Cirripede larvae. In: Symposium on Crustacea, Part II, Mar. Biol. Ass., Mandapam, India: 595-

Muench, B., 1958. Quinaldine, a new anesthetic for fish. Progve. Fish. Cult., 20 (1): 42-44.

Mukundan, C., 1967. Plankton of Calicut inshore waters and its relationship with coastal pelagic fishery. Indian J. Fish., 14: 271-292.

Muir-Wood, H.M., 1959. The John Murray Expedition 1933-34. Scientific Reports, 10: 283.

Müller, M., 1850. Über eine den sipunculiden Würmer ähnliche Wurmlarve. Müller's Arch. Anat. Physiol.

Nakai, Z., 1955. The chemical composition, volume, weight and size of the important marine plankton. Tokai Reg. Fish. Res. Lab. Spl. Publ. No. 1: 12-24.

Nair, R.V., 1944. On the larvae cerianthoria from Madras plankton. Curr. Sci., 13: 132-133.

Nair, R.V., 1947. On the metamorphosis of two leptocephali from the Madras Plankton. Proc. Indian Acad. Sci., B.25: 1-14.

Nair, R.V., 1946. Fish eggs and larvae of the Madras Plankton. Abstract. Proc. Indian Sci. Congr., 33rd Sess., Pt. 3: 127.

Nair, R.V., 1950. On the new Cerianthorian larvae from Madras Plankton. Rec. Indian Mus., 47: 239-291.

Nair, R.V., 1952. Studies on fish eggs and larvae of the Madras Plankton. Proc. Indian Acad. Sci B. 35: 181-208.

Nair, V.R., 1974. Discontinuity in the larval distribution of Phoronida and Brachiopoda in the Indian Ocean. Curr. Sci., 43 (21):676-

Nair, V.R., M. Saraswathy, K. Sarala Devi and T.C. Gopalakrishnan, 1975. Biochemical composition of Sagitta bedoti and other zooplankton organisms from an estuarine area. Mahasagar - Bull. natl. Inst. Oceanogr., 8: 109-111.

Nasarenko, I.U., 1960. The effect of formaldehyde on aquatic organisms. Trudy. Vses. Gidrobiol. Obshch., 10: 170-174.

Nellen, W., 1973. Kinds and abundance of fish larvae in t Arabian Sea and the Persian Gulf. In: 'The Biology of the Indian Ocean', edited by B. Zeitzschel, Springer-Verlag Berlin: 415-430.

Newell, G.E. and R.C. Newell, 1963. Marine Plankton, London, Hutchinson: 17-

Noble, G.K. and M.E. Jaeckle, 1929. Preservation of animals by infiltration with paraffin. Mus. Jour., 29: 8-10.

Novak, A.F., M.R. Ramachandra Rao and G.R. Bright, 1966. Use of liquid nitrogen for shell fish preservation. *Chem. Engng. Prog. Symp. Ser.*, 62 (69): 75-79.

Orr, A.P., 1934a. On the biology of *Calanus finmarchicus*. Part IV. Seasonal changes in the weight and chemical composition in Loch Fyne. *J. mar. biol. Ass. U.K.*, 19: 613-632.

Orr, A.P., 1934b. The weight and chemical composition of *Euphausia norvegica* Leach. *Proc. R. Soc. Edinb.*, B 54: 51-55.

Ortmann, A., 1893. Plankton-Expedition der Humbolt-Stiftun Decapoden und Schizopoden. *Kiel und Leipzig. Bd.*, 11: 120, 10 pls.

Orton, J.H., 1937. Note on shrinkage of oyster tissues in Bouin's fixative. *J. R. microsc. Soc.*, 57 (4): 255.

Osterhout, W.J. and S.E. Hill, 1933. Anesthesia produced by distilled water. *J. gen. Physiol.*, 17 (1): 87-98.

Owen, G., 1955. Use of propylene phenoxetol as a relaxing agent. *Nature, Lond.*, 1753: 43

Owen, G. and H.F. Steedman, 1956. Preservation of animal tissues with a note on staining solutions.

Owen, G. and H.F. Steedman, 1958. Preservation of molluscs. *Proc. Malac. Soc. Lond.*, 33 (3): 101-103.

Paffenhofer, G.A. and H. Rosenthal, 1968. Trockengewicht und kaloriengehalt sich entwickelnder Heringseier. *Helgolander Wiss. Meeresunters.* 18:45-5

Panikkar, N.K., 1936. On *Anisactis bengalensis* species nova, a new pelagic larvae of ceriantharian from the Madras Plankton. *Zool. Anz.*, 115: 250-260.

Panikkar, N.K., 1937. Observations on Arachnactis of the Madra plankton together with a general accour of the Anthozoan larvae of the Madras coast. Abstract. *Proc. Indian Sci. Congr. 24th Sess*: 292.

Panikkar, N.K., 1938. On the occurrence of Isarachnactis in the Arabian Sea. *Curr. Sci.*, 7: 282-28

Panikkar, N.K., 1947. Observations on the structure and devel mental stages of a new species of Arachnactis from the Madras plankton. *Annls. Sci. Nat., Zool.*, 9: 227-251.

Panikkar, N.K. and R.G. Aiyar, 1939. Observations on breeding in brackish-water animals of Madras. *Proc. Indian Acad. Sci.*, B, 9: 343-364.

Pantin, C.F.A., 1948. *Notes on microscopical technique for zoologists.* London, Cambridge Universit Press, 76 pp.

Pantin, C.F.A., 1964. *Notes on microscopical techniques for zoologists.* Cambridge University Press, London, 77 pp.

Parker, G.H., 1939. General anaesthesia by cooling. *Proc. Soc. exp. Biol. N.Y.*, 42: 186-1

Parker, R.R., 1963. Effects of formalin on length and weight of fishes. *J. Fish. Res. Bd. Can.*, 20: 1441-1456.

Parulekar, A.H. and D.V. Bal, 1969. Observations on the seasonal changes i the chemical composition of *Bregmaceros mcclellandi* (Thomson). *Jour. Univ. Bombay*, 38 (64): 82-92.

Passano, L.M., 1960. Moulting and its control. In: *The Physiology of Crustacea.* Vol. I, (Ed. Waterman, T.H.), Academic Press, London, 670 pp.

Paul, M.D., 1942. Studies on the growth and breeding of certain sedentary organisms in the Madras Harbour. *Proc. Indian Acad. S* B. 15: 1.

Paulinose, V.T., 1973. Developmental stages of *Penaeopsis rectacuta* Bate (Decapoda: Penaeidae) from the Indian Ocean. *IOBC Handbook* 5: 97-110.

Paulinose, V.T., 1974. Decapod crustacea from the Internati Indian Ocean Expedition: The species of *Funchalia* (Penaeidae) and their postlarvae. *J. nat. Hist.*, 8: 433-4

Paulinose, V.T., 1979. Studies on decapoda larvae (Crustac Decapoda: Penaeidae) of the Indian *Ph.D Thesis*, University of Kerala.

Pearse, A.G.E., 1963. Rapid freeze-drying of biological tissues with a thermoelectric unit. *J. Scient. Instrum.*, 40: 176.

Pearse, A.G.E., 1968. *Histochemistry, Theoretical and App* Vol. I, London, J. & A. Churchill.

Peebles, F. and D. Fox, 1933. The structure, functions and general reactions of Dendrostoma zostericola. Bull. Scripps Inst. Oceanogr. Tech. Ser., 3.

Peter, K.J., 1969a. Larvae of Rastrelliger (Mackerel) from the Indian Ocean. Bull. natn. Inst. Sci. India, 38: 771-777.

Peter, K.J., 1969b. Preliminary report on the density of fish eggs and larvae of the Indian Ocean. Bull. natn. Inst. Sci. India, 38: 854-863.

Peter, K.J., 1974. An appraisal of the larval fishes of The Arabian Sea. Seafood Export Journal, 6 (6): 47-49.

Peter, K.J., 1977. Distribution of tuna larvae in the Arabian Sea. Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO, 1977: 36-4

Peterson, K.W., 1976. Fixation and preservation of planktonic coelenterates. In: Zooplankton fixation and preservation: 260-269.

Pierce, R.W., J. Van Der Veen and H.S. Olcott, 1969. Proximate and lipid analyses of Krill (Euphausia sp.) and red crab (Pleuroncodes planipes). Agr. Food. Chem., 17: 367-369.

Pillai, N.K., 1955. Pelagic crustacea of Travancore. 1. Decapod larvae. Bull. Cent. Res. Inst. Univ. Travancore, C., 4: 47-101.

Pollack, M.J., 1958. Frequency distribution of potential temperatures and salinities in the Indian Ocean. Deep-Sea Res., 5: 128-13

Pottinger, S.R., 1951. Effect of ascorbic acid on keeping quality of frozen oysters. Comml. Fish. Rev., 13 (8): 5-7.

Powell, H.M. and W.A. Jamieson, 1958. On the efficiency of merthiolate as a biological preservative after 10 years' use. Proc. Indian Acad. Sci., 47: 65-70.

Prasad, R.R., 1954. The characteristics of marine plankton at an inshore station in the Gulf of Mannar near Mandapam. Indian J. Fish., 1: 1-36.

Prasad, R.R., 1954. Observations on the distribution and fluctuation of planktonic larvae off Mandapam. Tech. Pap. Proc. Indo-Pacif. Fish. Coun., 5th Meeting, Bangkok, 1954, 2: 21-24.

Prasad, R.R., 1956. Further studies on the plankton of the inshore waters off Mandapam. Indian J. Fish., 3: 170-185.

Prasad, R.R., 1964. A bibliography of plankton in the Indian Ocean. Indian Nat. Com. Ocean Res., 3: 1-86.

Prasad, R.R., 1968. Maps on total zooplankton biomass in the Indian Ocean. IIOE Plankton Atlas, 1, Fasc.2, IOBC, NIO, (CSIR), Cochin, India.

Prasad, R.R., S.V. Bapat and P.R.S. Tampi, 1952. Observations on the distribution of the plankton of the six inshore stations in the Gulf of Mannar. J. Zool. Soc. India, 4: 141-151.

Prasad, R.R. and P.R.S. Tampi, 1953. A contribution to the biology of the blue swimming crab, Neptunus pelagicus (Linnaeus), with a note on the zoea of Thalamita crenata. J. Bombay nat. Hist. Soc., 51: 674-689.

Prasad, R.R. and P.R.S. Tampi, 1957. On the phyllosoma of Mandapam. Proc. nat. Inst. Sci. India, 23 (1-2): 48-67.

Prasad, R.R. and P.R.S. Tampi, 1959. On a collection of Palinurid Phyllosomas from the Laccadive Seas. J. mar. biol. Ass. India, 1 (2): 143-164.

Prasad, R.R. and P.R.S. Tampi, 1960. Phyllosomas of Scyllarid lobsters from the Arabian Sea. J. mar. biol. Ass. India, 2 (2): 241-249.

Prasad, R.R. and P.R.S. Tampi, 1966. A preliminary report on the phyllosomas of the Indian Ocean collected by the Dana Expedition 1928-1930. J. mar. biol. Ass. India, 7 (2): 277-283.

Premalatha, P., 1977. A study of the development and distribution of the larvae of leatherskin along the southwest coast of India. Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO, Goa: 430-439.

Price, G.A., 1965. A membrane method for determination of total protein in dilute algal suspensions. Analytical Biochem., 12: 213-218.

Prytherch, H.F., 1924. Experiments in the artificial propagation of oysters. Appendix XV. Rep. U.S. Comm. Fish. Bur. Fish. Doc. No.961: 1-14, Washington.

Quintin, R., 1900. Communication osmotique chez l' invertebre marin. *C.R. Acad. Sci., Paris*, **131**.

Raje, P.C. and M.R. Ranade, 1972. Larval development of Indian penaeid shrimps. I. *Penaeus merguiensis* de Man. *J. Ind. Fish. Ass.*, **2** (1 & 2): 1-16. Larval development of Indian Penaeid shrimps. II. *Metapenaeus monoceros* (Fabricius). *J. Ind. Fish. Ass.*, **2** (1 & 2).

Raju, N.S. and P.N. Ganapati, 1969. Distribution of fish eggs and larvae in the Bay of Bengal. *Proc. Symp. Indian Ocean*, New Delhi, 1967, *Bull. natn. Inst. Sci. India*, No. **38**, Pt. 2: 797-804.

Ramage, C.S., 1969. Indian Ocean surface meteorology. *Oceanogr. Mar. Biol. ann. Rev.*, **7**: 11-30

Randall, D.J., 1962. Effect of an anaesthetic on the heart and respiration of teleost fish. *Nature, Lond.*, **195**: 506.

Rao, P.V., 1973. Studies on the larval development of the commercially important penaeid prawns of India. *J. mar. biol. Ass. India*, **15** (1): 95-124.

Raymont, J.E.G., 1972. Some investigations on the biochemical composition of marine zooplankton. *Essays in Hydrobiology* (Univ. of Exeter) 65-91.

Raymont, J.E.G. and S. Krishnaswamy, 1960. Carbohydrate in some marine planktonic animals. *J. mar. biol. Ass. U.K.*, **39**: 239-248.

Raymont, J.E.G., J. Austin and E. Linford, 1964. Biochemical studies on marine zooplankt 1. The biochemical composition of *Neomysis integer*. *J. Cons. perm. int. Explor. Mer.*, **28**: 354-363.

Raymont, J.E.G., J. Austin and E. Linford, 1966. Biochemical studies on marine zooplankt III. Seasonal variation in the bio-chemical composition of *Neomysis integer*. In: *Some Contemporary studies in Marine Science* (H. B. Barnes): 597-605.

Raymont, J.E.G., J. Austin and E. Linford, 1967. The biochemical composition of certain oceanic zooplanktonic decapoda. *Deep-Sea Res.*, **14**: 113-115.

Raymont, J.E.G., J. Austin and E. Linford, 1968. Biochemical studies on marine zooplankton. V. The composition of the major biochemical fractions in *Neomysis integer*. *J. mar. biol. Ass. U.K.*, 48: 735-760.

Raymont, J.E.G., and R.J. Conover, 1961. Further investigations on the carbohydrate content of marine zooplankton. *Limnol. Oceanogr.*, 6: 154-164.

Raymont, J.E.G. and E. Linford, 1966. A note on the biochemical composition of some Mediterranean zooplankton. *Int. Revue ges. Hydrobiol.*, 51: 485-488

Raymont, J.E.G., R.T. Srinivasagam and J.K.B. Raymont, 1969a. Biochemical studies on marine zooplankt VI. Investigation on *Megancytiphanes norvegica* (M. Sars). *Deep-Sea Res.*, 16: 141-156.

Raymont, J.E.G., R.T. Srinivasagam and J.K.B. Raymont, 1969b. Biochemical studies on marine zooplankt VII. Observations on certain deep-sea zooplankton. *Intern. Revue. ges. Hydrobiol.*, 54: 357-365.

Raymont, J.E.G., R.T. Srinivasagam and J.K.B. Raymont, 1971a. Biochemical studies on marine zooplankt VIII. Further investigations on *Meganyctiphanes norvegica*. (M. Sars). *Deep-Sea Res.*, 18: 1167-1178.

Raymont, J.E.G., R.T. Srinivasagam and J.K.B. Raymont, 1971b. Biochemical studies on marine zooplank IX. The biochemical composition of *Euphausia superba* Dana. *J. mar. biol. Ass. U.K.*, 51: 581-588.

Rees, C.B., 1950. The identification and classification of lamellibranch larvae. *Hull. Bull. mar. Ecol.*, 3: 73-104.

Reeve, M.R., J.E.G. Raymont and J.K.B. Raymont, 1970. Biochemical studies on marine zooplank VI. Investigations on *Meganyctiphanes norvegica*. *Deep-Sea Res.*, 16: 141-15

Richards, W.J. and David C. Simmons, 1971. Distribution of tuna larvae (Pisces, Scombroidae) in the northwestern Gulf of Guinea and off Sierra Leone. *U.S. Fish. Bull.*, 69 (3): 555-568.

Ritchie, J., 1924. Preservation of zoological specimens in fluid to preserve their natural colour. *Museums J.*, 23: 191-193.

Robilliard, G.A., 1969. A method of colour preservation in opisthobranch molluscs. *Veliger*, 11 (3): 289.

Rochford, D.J., 1969. Seasonal variations in the Indian Ocean along 110°E. I. Hydrological structure of the upper 500 m. *Aust. J. Mar. Freshw. Res.*, **20** (1): 1-50.

Rohdenburg, G.L., 1930. The preservation of specimens in colour. *Science*, **71** (1831): 134-135.

[illegible], 1948. As referred by H.F. Steedman in *Zooplankton fixation and preservation*. The Unesco Press, Paris. 1976: 127.

Rosamma Stephen, S.U. Panampunnayil, T.C. Gopalakrishnan and V.N. Sankaranarayanan, 1979. Biochemical studies of zooplankton from the Laccadive Sea. *Indian J. Mar. Sci.*, **8** (4): 257-2[illegible].

Rose, M., 1933. Copepodes pelagiques. P. 1-16, Paris, P. Lechevalier. (*Faune de France*, **26**).

Rosenthal, H., 1966. Beobachtungen uber das verhalten der seezungenbrut. *Helgolander Wiss. Meeresunters.*, **13**: 213-228.

Rosenthal, H., 1969a. Untersuchungen uber das Beutefangverhalten bei larven des Herings clupea harengus. *Mar. Biol.*, **3**: 208-221.

Rosenthal, H., 1969b. Verdauungsgeschwindigkeit, Nahrungswahl und Nahrungsbedarf beiden larven des Herings clupea harengus L. *Ber. dt. Wiss. Komm. Meeresforsch.* **20**: 60-96.

Rosenthal, H. and M. Fonds, 1973. Biological observations during rearing experiments with the garfish *Belone belone*. *Mar. Biol.*, **21**: 203-218.

Rosewater, J., 1963. An effective anaesthetic for giant clams and other molluscs. *Turtox News*, **41** (12): 300-302.

Rothlin, E., 1932. M.S. 222 (Losliches Anaesthesin), ein Narkoticum fur Kaltbluter. *Schweiz. Med. Woch. Schr.*, **62**: 1042.

Rowe, T., 1960. The theory and practice of freeze drying. *Ann. N.Y. Acad. Sci.*, No. **85**: 679-681.

Rowe, T.W., 1971. Machinery and methods in freeze drying. *Cryobiology*, **8**: 153-172.

Runham, N.W., K. Isarankura and B.J. Smith, 1965. Methods for narcotising and anaesthetising gastropods. Malacologia, 2 (2): 231-238.

Russell, E.S., 1923. Report on seasonal variation in the chemical composition of oysters. Fishery Invest., Lond. Ser. 2, 6 (1): 1-24.

Russell, H.D., 1963. Notes on methods for the narcotization, killing, fixation and preservation of marine organisms. Systematics-Ecology Program Marine Biological Lab., Woods Hole, Mass., 70 pp.

Ryland, J.S., 1964. The identity of some cyphonautes larvae. J. mar. biol. Ass. U.K., 44: 645-654.

Saiki, M., S. Fang and T. Mori, 1959. Studies on the lipid of plankton. II. Fatty acid composition of lipids from Euphausiacea collected in the Antarctic and Northern Pacific Oceans. Bull. Jap. Soc. Sci. Fish., 24: 837-39.

Saiki, M. and T. Mori, 1956. Studies on the whale oil. XII. On the lipid of Calanus cristatus (Part I). Bull. Jap. Soc. Sci. Fish., 21: 1041-44.

Sakthivel, M., 1972. Studies on Euthecosomata of the Indian Ocean. Ph.D. Thesis, Univ. of Cochin.

Sakthivel, M., 1977. Further studies of Plankton Ecosystems in the eastern Indian Ocean. VIII. Seasonal, diurnal and latitudinal variations in abundance of Euthecosomata along the 110°E meridian. Aust. J. Mar. Freshw. Res., 28: 663-671.

Sakthivel, M. and T.S.S. Rao, 1973. A few comments on the zooplankton investigations of the International Indian Ocean Expedition. Handbook to the IIOE Collections, IV: 151-165, IOBC, Cochin.

Samokhvaleva, G.V., 1955. Collection of small fish and preservation of their colours (In Russian). Zool. Zh., 34: 1178-1179.

Saraswathy, M., 1972. Distribution of bivalve larvae in the Indian Ocean. Proc. Workshop Plankton Methods. In: Handbook to the International Zooplankton Collections, IOBC, Cochin, 3: 43-53.

Savinski, P.L., 1953. Methods of preserving form and other natural colours of insect larvae, fish and other animals. *Zool. Zh., Moscow*, 32 (6): 1285-1287.

SCOR, 1966. Reports on zooplankton sampling methods: Report of Working Party I (Micro-zooplankton). *SCOR Proceedings*, 2: 37 and 53, La Jolla, Calif, Working Group [illegible].

Seguin, G., 1968. Contribution a l'etude biochimique de Pranunus flexuosus (Muller). *Deep-Sea Res.*, 15: 491-492.

Sewell, R.B.S., 1913. Notes on the biological work of the R.I.M.S.S. Investigator during the survey seasons, 1910-1911. *J. Proc. Asiat. Soc. Beng. (New Ser.)*, 9: 329-390.

Sehdev, H.S., J.R. McBride and V. Fagerlund, 1963. 2-Phenoxy-ethanol as a general anaesthetic for sockeye salmon. *J. Fish. Res. Bd. Can.*, 20 (6): 1435-1440.

Seifter, S., S. Dayton, B. Novic and E. Muntwyler, 1950. The estimation of glycogen with the anthrone reagent. *Arch. Biochem.*, 25: 191-200.

Sheard, K., 1953. Taxonomy, distribution and development of the Euphausiacea (Crustacea). *Rep. B.A.N.Z. antarct. Res. Exped. (Ser. B)*, 8: 1-72.

Shein, M., 1951. Colour restoration and preservation of gross museum specimens. *Bull. int. med. Mus.*, 32 (1951): 117-23

Sidhu, G.S., W.A. Montgomery, G.L. Holloway, A.R. Johnson and D.M. Walker, 1970. Biochemical composition and nutritive value of krill (*Euphausia superba* Dana). *J. Sci. Fd. Agric.*, 21: 293-296.

Silas, E.G., 1974. Larvae of the Indian mackerel, *Rastrelliger kanagurta* from the west coast of India. *Indian J. Fish.*, 21 (1): 233-253.

Simatos, D. and L. Rey, 1965. Freeze-drying of biologic materials. [illegible], 24, Suppl. 15: 213-216.

Sipos, J.C. and R.G. Ackman, 1968. Jelly fish (*Cyanea capillata*) lipids: Fatty acid composition. *J. Fish. Res. Bd. Can.*, 25: 1561-1569.

Smith, J.L.B., 1944. A neutral solution of formaldehyde for biological purposes. *Trans. Roy. Soc. S. Afr.*, September: 279-282.

Smith, J.L.B., 1968. The collecting and preservation of fishes. *Bull. S. Afr. Mus. Ass.*, 9 (6): 202-[illegible].

Sorby, H.C., 1908. On the preservation of marine animals with their natural colour. *Museums J.*, 7: 223-227.

Sreekumari, A., 1977. Development and distribution of the larvae of the white-bait *Stolephorus zollingeri* along the southwest coast of India. *Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO, Goa*: 440-449.

Srinivasan, V.V. and S. Krishnaswamy, 1964. The chemical composition of *Martesia fragilis*, a wood boring pholad of Madras. *Zool. Jb. Physiol.*, 70: 539-46.

Sriraman, K. and P.S. Reddy, 1977. Biochemical studies in planktonic juveniles and adults of *Penaeus indicus* (Milne Edwards) and *Penaeus monodon* (Fabricius). *Proc. Symp. Warm Water Zoopl. Spl. Publ. UNESCO/NIO, Goa*: [illegible].

Steedman, H.F., 1976. Zooplankton fixation and preservation. *Monographs on Oceanographic Methodology* 4: UNESCO Press, Paris.

Strickland, J.D.H. and T.R. Parsons, 1968. A manual of sea water analysis. *Bull. Fish. Res. Bd. Can.*, No. 167: 311pp

Subrahmanyam, M., 1965. Studies on the biology of some crustacea. *M.Sc. Thesis*, Andhra University, Waltair.

Subrahmanyan, V., N.L. Lahiry, M.N. Moorjani, R. Balakrishnan and M.A. Krishnaswami, 1963. Ammonia, possible use for preserving fish. *Science*, 142 (3589): 233-234.

Sudarsan, D., 1968. Brachiopod larvae from the west coast of India. Proc. Indian Acad. Sci., B. 68 (2): 59-68.

Sudak, F.N., 1966. Immobilization of large sharks by means of ethanol. Copeia, No.3: 611-12

Sugawara, K. and Y. Saijo, 1966. General report of the participation of Japan in the International Indian Ocean Expedition. Primary Production V-6. Rec. Oceanogr. Wks. (In Japanese), 8 (2): 85-91.

Sutcliffe, W.H.Jr., 1957. An improved method for the determination of preserved plankton volumes. Limnol. Oceanogr., 2: 295-296.

Suyama, M., K. Nakajima and J. Nonaka, 1965. Studies on the protein and non-protein nitrogenous constituents of Euphausia. Bull. Jap. Soc. Scient. Fish., 31: 302-306.

Sverdrup, H.U., M.W. Johnson and R.H. Fleming, 1942. The Oceans. Their physics, chemistry and general biology. Prentice Hall, Englewood Cliffs, N.J., 1087 pp.

Swahn, B., 1952. A micromethod for the determination of the total lipid in serum. Scand. J. Clin. Lab. Invest., 4: 247-249.

Tampi, P.R.S., 1973. The Phyllosoma larvae of the Indian Ocean. J. mar. biol. Ass. India, 15 (2): 560-565.

Tampi, P.R.S. and M.J. George, 1975. Phyllosoma larvae in the IIOE (1960-'65) collections - Systematics. Bull. natn. Inst. Oceanogr. 8 (1 & 2): 15-44.

Tamura, T., 1948. The method of collecting the plankton while the boat is in motion. J. Fish. Hakodate, 53: 19-22.

Tarjan, A.C., 1967. An abbreviated lactophenol-glycerine procedure for preserving nematodes. Nematologica, 13: 153-154.

Tarr, H.L.A. and B.E. Bailey, 1940. Effectiveness of benzoic acid for fish preservation. J. Fish. Res. Bd. Can., 4 (5): 327-336.

Tambe, A.M., 1962. The use of paraformaldehyde as a field preservative. *Copeia*, 1: 209-210.

Tambe, C.M., 1965. The use of paraformaldehyde as a biological preservative. *The Progressive Fish Culturist*: 165-168.

Thomson, J.M., 1966. Synopsis of biological data on the grey mullet *Mugil cephalus* Linnaeus 1758. *CSIRO Fish. Oceanogr. Fishery Synopsis* No. 1.

Thorson, T.B., 1959. Tricaine methanesulfonate (M.S. 222) as an anaesthetic for the sea lamprey, *Petromyzon marinus*. *Copeia*, 2: 163-165.

Toyama, K. and G. Miyoshi, 1963. Prevention from fading of aquatic animals under preservation. 1. Test on preservatives to retain red colour in fish and crustacean specimens. *J. Tokyo Univ. Fish.*, 22 (1): 43-49.

Tranter, D.J., 1959. A method for determining zooplankton volumes. *J. Cons. CIEM* 25: 272-278.

Tranter, D.J., 1966. The Australian Clarke-Bumpus Sampler and calibration tank. *CSIRO Aust. Div. Fish. Oceanogr. Tech. Pap.* No. 19.

Tranter, D.J., 1973. Seasonal studies of a Pelagic Ecosystem (Meridian 110°E) In: *The Biology of the Indian Ocean*. Edited by B. Zeitzschel, 487-520, Springer-Verlag, Berlin.

Tranter, D.J. and J.D. Kerr, 1969. Seasonal variations in the Indian Ocean along 110°E. V. Zooplankton biomass. *Aust. J. mar. Freshw. Res.*, 20: 77-84.

Tranter, D.J. and P.E. Smith, 1968. Filtration performance. *Monographs on Oceanographic Methodology*, 2, Zooplankton Sampling: 27-56, Unesco.

Trott, L.B., 1965. The use of nicotine sulphate in fish narcotisation. *Micronesica*, 2: 71-72.

Tsuyuki, H., U. Naruse, A. Mochizuki and S. Ito. 1964a. Studies on the lipids of Euphausiacea, *Euphausia superba* L. I. Acetone soluble lipid. *[illegible]*, 13: 203-206.

Tsuyuki, H., U. Naruse, A. Mochizuki and S. Ito, 1964b. Studies on the lipids of Euphausiacea Euphausia superba L. II. Properties of phospholipids. Eikagaku, 12: 477-480.

Tsuyuki, H., E. Roberts and W.E. Vanstone, 1965. Comparative zone electropherogram water of muscle myogens and blood haemoglobins of marine and fresh water vertebrates and their application to biochemical systematics. J. Fish. Res. Board Can., 22: 203-13.

Uda, M., 1966. Upwelling of subpolar intermediate water in the tropical zone of world oceans and its relation with the location of regions of high biological productivity including regions favourable for tuna fishing. II. Inter. Oceanogr. Congr. Moscow, Abst. : 415.

Ueyanagi, S., 1966. On the red pigmentation of larval tuna and its usefulness in species identification. Rep. Nankai Reg. Fish. Lab., 24: 41-4

Ueyanagi, S., 1969. Observation on the distribution of tuna larvae in the Indo-Pacific Ocean with emphasis on the delineation of spawning areas of albacore, Thunnus alalunga. Bull. Far. Seas. Fish. Res. Lab., 2: 177-256.

Underhill, D.M.L., 1932. The rate of penetration of fixatives. J. R. Microsc. Soc., June: 113-120.

UNESCO, 1968. Zooplankton sampling. Monographs on Oceanographic Methodology, 2. Paris, Unesco, 2nd impr., 1974.

UNESCO, 1976. Zooplankton fixation and preservation. Monographs on Oceanographic Methodology, 4. Paris, Unesco, 350 pp.

Van Bruggen, A.C., 1965. Preservation of molluscs and storage and maintenance of malacological study collections. Bull. S. Afr. Mus. Ass., 8: 209-213

Van Eeden, J.A., 1958. Two useful techniques in fresh water malacology. Proc. Malac. Soc., 33 (2).

Vanhoffen, E., 1895. Untersuchungen uber die Anatomie und Entwicklung von Arachnactis albida M. Sars. Bibliotheca zoologica, Bd. [illegible], Lf.1.

Vannucci, M., 1968. Loss of organisms through the meshes. In: Monographs on Oceanographic Methodology 2. Zooplankton Sampling, Unesco: 77-86.

Venkataraman, R. and S.T. Chari, 1951. Studies on Oysters and Clams; Biochemical variations. Ind. Jour. Med. Res., 39 (4): 533-541.

Venkataramanujam, K. and K. Ramamoorthy, 1974. Seasonal variation in fish eggs and larvae of Porto Novo coastal waters. Indian J. Fish., 21: 254-262.

Venkataramanujam, K. and K. Ramamoorthy, 1977. Seasonal variation in fish eggs and larvae of Porto Novo coastal waters. Proc. Symp. Warm Water Zoopl. Spl. Publ. NIO/UNESCO, Goa: 474-485.

Verrill, A.E., 1865. Preservation of star fishes with natural colours. Am. J. Sci., 2nd Ser., [illegible]: 228.

Verrill, A.E., 1870. Glycerine for preserving natural colours of marine animals. Am. Nat., 3: 156.

Vinogradova, Z.A., 1960. Study of the biochemical composition of Antarctic Krill (Euphausia superba Dana). Dokl. Akad. Nauk. S.S.S.R., 133: 680-682 (N.L.L. Translation).

Vinogradova, Z.A., 1964. Some biochemical aspects of a comparative study of plankton from the Black Sea, the sea of Azov and the Caspian Sea. Okeanologiya, 4 (2): 232-242 (N.L.L. Translation).

Wafar, M.V.M., 1974. Biochemical composition of the Lamellibranchs Meretrix casta (Chemnitz) and Sanguinolaria diphos (Gmelin). Indian J. Fish., 21 (1): 289-292.

Wagstaffe, Reginald and J. Havelock Fidler, 1955. The Preservation of natural history specimens. Vol. I. H.F. Witherby Ltd., London, 205 pp.

Wagstaffe, Reginald and J. Havelock Fidler, 1968. The Preservation of natural history specimens. Vol. II. H.F. Witherby Ltd., London, 404 pp.

Wainwright, S.A., 1963. Skeletal organization in the coral Pocillopora damicornis. Q. Jour. Microsc. Sci., 104: 169-183.

Walker, J.F., 1944. Formaldehyde. 1st Ed., 397 pp. Reinhold Publishing Corporation, N.Y.

Walker, J.F., 1953. Formaldehyde. Reinhold Pub. Co., New York, 575 pp.

Waller, R.A. and W.N. Eschmeyer, 1965. A method for preserving colour in biological specimens. Bioscience, 15 (5): 361.

Walmsley, W.H., 1869. Preservation of objects in glycerine jelly. Nature, Lond., 1: 172.

Wheatley, M.D., 1941. Preservation of biological specimens with isobutyl methacrylate polymer. Science, 94: 49.

White, R.A. and E.J. Peters, 1969. A method of preserving colour in aquatic vertebrates and invertebrates. Turtox News, 47 (9): 296-297.

Wiborg, K.F., 1951. The whirling vessel, an apparatus for the fractioning of the plankton samples. Fiskeridir. Skr. Havunders. 11 (3): 1-16.

Wickstead, J.H., 1965. An introduction to the study of tropical plankton. Hutchinson, London, 160 pp.

Wilber, C., 1947. The effect of prolonged starvation on the lipids in Phascolosoma gouldi. Jour. Cell. Comp. Physiol., 29.

Williams, P.M., 1965. Fatty acids derived from lipids of marine origin. J. Fish. Res. Bd. Can. 22: 1107-1122.

Williamson, D.I. and G. Russell, 1965. Ethylene Glycol as a preservative for marine organisms. Nature, Lond., 206 (4991): 1370-1371.

Wilson, D.P., 1932. On the Mitraria larva of *Owenia fusiformis* Delle Chiaje. *Phil. Trans. R. Soc., Ser. B.*, 221: 231-234.

Wilson, D.P., 1953. On the settlement of larvae. *J. mar. biol. Ass. U.K.*, 31: 413-438.

Wimpenny, R.W., 1949. The dry weight and fat content of plankton. *Ann. biol., Copenh.* 4: 89-90.

Windsor, D.A., 1971. Ammonium sulfate as a preservative which does not remove colour from frogs. *Copeia*, No. 2: 336-337.

Wright, T.S., 1870. Note on the preservation of minute animals in acetic acid. *J. Anat. Physiol. Lond.*, 4: 239.

Wyrtki, K., 1973. Physical oceanography of the Indian Ocean. In: B. Zeitzschel and S.A. Gerlach (Eds.), *The Biology of the Indian Ocean*. Springer-Verlag, Berlin, Heidelberg: 18-36.

Yamada, M., 1964. Studies on the lipid of plankton. I. The lipid of *Neomysis nakazawai*. *J. Japan Oil Chem. Soc.*, 13: 236-239.

Yamaga, M. and K. Marioka, 1962. Chemical preservation of the body colour of red fishes. *Bull. Jap. Soc. Sci. Fish.*, 28: 1104-1108.

Yoshida, Y., 1962. A way of making fish specimens with their original body colours kept. *Bull. Misaki Mar. Inst.*, No. 3: 67-78.

Young, J.Z., 1935. Osmotic pressure of fixing solutions. *Nature, Lond.*, 135 (3420): 823.

Young, S.D., 1971. Organic matrices associated with $CaCO_3$ skeletons of several species of hermatypic corals. In: H.M. Lenhoff, L. Muscatine and L.V. Davis (Eds.), *Experimental coelenterate biology*. Univ. of Hawaii, Honolulu: 260-284.

Zhdanova, O., 1940. Chemical composition of *Calanus finmarchicus* in the Barents Sea. *Dokl. Akad. Nauk S.S.S.R.*, 29: 218-224.

Zehntner, 1900. Formalin as a preservative. *Illte. Z. Ent.*, 5: 216.

APPENDICES - I - III.

APPENDIX - I (Sample form used)

Series 1-11. Specification Sheet No.2. pH Sheet

| Experiment Nos. | pH when made up | Changes in pH of preservative fluids with specimens after | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 day | 2nd day | 3rd day | 4th day | 5th day | 2 weeks | 4 weeks | 2 months | 6 months | 1 year | 2 years |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |

## APPENDIX - II (Sample form used)

Series 1-11 Specification Sheet No.3. <u>Formaldehyde Assay.</u>

| Experiment Nos. | Formaldehyde content in the preservative fluid with specimens | | | | | | |
|---|---|---|---|---|---|---|---|
| | When made up | On adding specimens | End of 1st week | End of 1st month | End of 6 months | End of 1 year | End of 2nd year |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| 9 | | | | | | | |
| 10 | | | | | | | |

## APPENDIX - III (Sample form used)

Series 1-11 | Specification Sheet No.4. | Specimen condition.

| Experiment Nos. | Specimen condition in the preservative fluids as observed by naked eye and low power binocular | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Condition at the end of | | | | | | | | | | |
| | 1 month | 3 months | 6 months | 9 months | 1 year | 2 years | 3 years | 4 years | 5 years | 7 years | 10 years |
| 1 | | | | | | | | | | | |
| 2 | | | | | | | | | | | |
| 3 | | | | | | | | | | | |
| 4 | | | | | | | | | | | |
| 5 | | | | | | | | | | | |
| 6 | | | | | | | | | | | |
| 7 | | | | | | | | | | | |
| 8 | | | | | | | | | | | |
| 9 | | | | | | | | | | | |
| 10 | | | | | | | | | | | |