

**Proceedings of the Summer Institute in Recent Advances
on the Study of Marine Fish Eggs and Larvae**

14 JUNE to 3 JULY, 1989



CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

Dr. SALIM ALI ROAD

COCHIN - 682 031.

PROCEEDINGS OF THE SUMMER INSTITUTE
IN
RECENT ADVANCES ON THE STUDY OF MARINE
FISH EGGS AND LARVAE

The Indian Council of Agricultural Research, having recognised the importance of the study of marine fish eggs and larvae, has sanctioned a Summer Institute to be held at the Central Marine Fisheries Research Institute's Regional Centre at Mandapam Camp, from 14th June to 3rd July, 1989.

There were seventeen candidates who have undergone the Summer Institute as its participants, sponsored by the Heads of various research, educational and developmental organisations dealing with fish and fisheries in the country.

The Summer Institute comprised of lectures, practicals, training, field visits and group discussions,

covering the latest development and recent advances in the field of marine fish eggs and larvae.

The Summer Institute was inaugurated by Dr. M.Lakshmanan, Vice Chancellor, Madurai Kamaraj University, on the forenoon of 14th June, 1989. Dr. A.G.Sathyanesan, Emeritus Scientist, Council of Scientific and Industrial Research has presided over the function; and later he has delivered a lecture on "Neuroendocrine Control of gonadotropin secretion with special reference to gonadal growth and spawning", for the benefit of the participants.

On 3rd July, 1989, the valedictory function was organised, when Dr. K.Subbaramaiah, Senior Scientist, Central Salt and Marine Chemicals Research Institute, Marine Algal Station, Mandapam, has delivered the valedictory address and also distributed Certificates to all the 17 participants who have successfully completed the Summer Institute.

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FOREWORD

For the rational exploitation, conservation and management of marine fisheries, it is essential to survey the spawning grounds of different species determine the spawning seasons, study their recruitment, growth and mortalities. This would be possible only if proper identities of the eggs, larvae, postlarvae and other developmental stages of the target species are established. Apart from this studies on marine fish eggs and larvae are essential for identification of new fish stocks of commercial importance as well as to correlate the distribution and abundance of early developmental stages of target species in relation to the prevailing environmental parameters and their influence on the stocks. In coastal aquaculture operations, it is imperative that the characteristic features of the early life history stages of the target species are known accurately.

In view of the above, it has become imperative to identify the early developmental stages of marine fishes precisely and document them in different geographical areas. Many contributions from different parts of the world have emanated over the years on account of these reasons; and two international symposia as well as a training course have been conducted in recent years.

In India, among about 1,300 species of marine bony fishes present, the early developmental stages of only about 100 species have been identified and documented so far, forming only about 8% of the number of the species. But, for a rational exploitation, conservation and management of our marine finfish resources, there is urgent need to fill up the lacunae existing in our knowledge at present. Hence, in order to focus attention on this aspect as well as to train some scientists and teachers in the country, the Indian Council of Agricultural Research has sanctioned a Summer Institute in "Recent Advances on the study of Marine Fish Eggs and Larvae" at the Mandapam Regional Centre of the Central Marine Fisheries Research Institute, from 14th June to 3rd July, 1989. The present volume contains the technical papers prepared and presented at the above Summer Institute by the Faculty.

The Director and Faculty Members express their gratitude to the ICAR for the financial assistance provided. They are also thankful to the Heads of various organisations for sponsoring the candidates for the Summer Institute.



P.S.B.R. James
Director, Summer Institute and
Director, CMFRI

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IMPORTANCE OF THE STUDY OF EGGS AND LARVAE IN
FISHERIES DEVELOPMENT

By

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Director

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Research in Fisheries biology of commercially important species have assumed importance only during the 19th and 20th centuries in different parts of the world. With progress of the above work over the years, it was realised that an absolute knowledge on the early developmental stages of marine fishes is required to assess their distribution and abundance in space and time (Ahlstrom, 1954; 1966). Such a study is also an essential prerequisite in undertaking the spawning surveys of target species, monitoring the changes in exploitable stocks and yields and forecasting the trends of their production (Ahlstrom and Moser, 1976). For instance, in the European Plaice, a correlation has been found to exist between the abundance of the early developmental stages in the plankton and the subsequent recruitment of the year - classes to the fishery. For the herring, a similar relation has been found between the spawning stock and egg production (Russell, 1976). The rate of survival from newly spawned eggs to the end of planktonic phase of life in the Pacific sardine was found to be about one in one thousand (Ahlstrom, 1954). From the above facts it is obvious that only if and when proper identities of eggs, larvae, postlarvae of the target species are established, will it be possible to determine the above events as well as to manage the respective fisheries in space and time. Hence, as drawn attention

to by Smith (1974) among many others, the above aspects constitute the most important objectives in the study of fish eggs and larvae.

The general practice has been to collect fish eggs and larvae from the natural environment and identify them. This method is often beset with a number of problems and uncertainty to a great extent. The alternate and sure method of identifying the eggs and larvae of fishes is to artificially fertilize the eggs of known species through induced breeding or stripping (parents are precisely known) and follow the development to describe later stages. There is great need for such work to solve many other problems related to study of fish eggs and larvae.

Apart from these, studies on marine fish eggs and larvae are important in the identification of new fish stocks of commercial potentialities, as explained by Ahlstrom (1968) and in the evaluation of fish resources, as drawn attention to by Ahlstrom and Moser (1976). Also, in order to correlate the distribution and abundance of early developmental stages of target species in relation to prevailing environmental parameters (physical, chemical and biological), the studies are important (Ahlstrom, 1954, 1966).

In coastal aquaculture operations, one of the basic requirements is to collect young stages, popularly called "Seeds", of fishes from natural seed-resources centres for stocking in grow-out ponds, cages etc.,. For this purpose, it is imperative that the characteristic features of the early life history stages of the target species are known accurately. Only if and when the above condition is fulfilled, will there be certainty with regard to the identity of the species stocked in culture and released in ranching. Character variability of young stages such as pigmentation, morphometric features and meristic

characters occurring in different areas should be understood properly through an indepth study of characters. This helps to avoid any confusion in the separation of the young stages of the target species from those of undesirable species. Also, the distinguishing characters of different developmental stages should be studied and properly documented, in order to segregate the most desirable stage for bestowing adequate attention in nursery practices. This is also essential to determine the effect of certain ecophysiological factors on a particular developmental stage, in order to explore the possibilities of undertaking remedial measures. Besides, character differences between developmental stages occurring in natural state and those obtained by artificial means (induced breeding) should be known, in order to assess the role of such difference in subsequent development.

Ahlstrom and Moser (1976, 1981) have elucidated that accurate identification and documentation of fish eggs, larvae and other developmental stages form a basis in fish taxonomy such as for clarification of taxonomic characters based on ontogenetic differences. The study is important for delimiting, spawning grounds, breeding seasons, migrations etc.,

From the study of eggs and larvae and their distribution and abundance, it is also possible (if fecundity of the species is known) to estimate the females in a population and thereby also the males (if sex ratio is known) and hence the strength of total spawning population which is essential for studies on recruitments mortality and fish stocks, that are vital in the rational exploitation, management and conservation of the resources. In order to make an assessment of the quality and quantity of Ichthyoplankton as an important component of the planktonic biomass and to gather information to determine

the whole spectrum of ichthyofauna in an area, a study of the early life history stages of fishes is important.

As a biological indicator brought by certain water masses, eggs and larvae have to be identified and documented. For use as a general study material, as an experimental organism in bioassays, in aspects of fish toxicology, embryology and other areas, a study of the early developmental stages of fishes is important.

In view of these compelling reasons, it has become imperative to study the early developmental stages of marine fishes with accuracy as well as to document them in different geographical areas. Publications such as those of Uchida, Imai et al. (1958) and Mit (1966) from Japan, Russell (1976) from Britain, Jones, Martin and Hardy Jr (1978), Hardy Jr (1978 a, b), Johnson (1978), Fritzsche (1978) and Martin and Drewry (1978) all from the Mid-Atlantic Bight and Ozawa (ed., 1986) from Western North Pacific are contributions in this direction and need emulation in other parts of the world as well. Realising these facts, an international training course on fish eggs and larval studies was conducted during 1973 (FAO, 1974) as well as two international symposia were held, one in 1973 (Blexter, Ed. 1974) and another in 1979 (Lasker and Sherman, 1981).

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RELEVANCE OF THE STUDY OF MARINE FISH EGGS AND LARVAE
IN INDIA

By

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India is a tropical, peninsular country, situated between about Lat. 3° and 38° N and between about Long. 68° and 80° E. There is an extensive coastline of more than 6,500 Km, dotted with many estuaries, creeks, backwaters bays, lagoons, etc., frequented by quite a few species of fishes. According to Talwar and Kacker (1984), there are about 1,400 marine and estuarine fish species in India. Of these, about 100 species belong to the group of sharks, rays and skates (Esasmobranchii), which are mostly viviparous, giving birth to their youngones and hence do not pose any problems with regard to their identity in their young stages. But, the rest of the number of species, about 1,300, belong to the group of bony fishes (Osteichthyes) and most of them are found along both the east and west coast of the peninsula, in Bay of Bengal and Arabian Sea respectively.

Among the many species of bony fishes, it has been observed that unlike as in temperate regions of the world where only one or two species contribute to fisheries, in the seas of a tropical country such as India, a number of species are present in the same genus and are allied genera, contributing to multispecies fisheries. A well known example of this kind is the Order Clupeiformes, represented by genera such as Sardinella, Dussumieria, Escualosa, Hilsa, Ilisha, Opisthopterus, Raconda, Stolephorus

Thryssina, Thryssa, Setipinna and Coilia. In many genera, each is represented by quite a few species. For instance, the genus Sardinella is represented by 13 species including the subgenus Amblygaster in India, vide Fircher and Bianchi (Ed., 1984), viz., S. albella, brachysoma, davi, fimbriata, gibbosa, jussieui, longiceps, melanura, neglecta, sindensis, S. (Amblygaster) clupeoides, amblygaster, sirm; and the genera Thryssa, Stolephorus and Ilisha have 11, 8 and 6 species respectively. Another such group is the family Mugilidae which, as may be seen from Tircher and Bianchi (1984) has 6 closely allied genera and among which the genus Liza has 13 species and the genus Valamugil has 6 species. Such a multiplicity of species is said to be the result of a more rapid rate of speciation in tropical waters than in temperate regions; and, in many localities, groups of congeneric species as well as species of several genera contribute to a fishery, ranging from 3 - 5 to 30 - 32 numbers.

Apart from the presence of closely allied species and/or genera in the same locality, most of the species are observed to spawn in the same area and at the same time. Bensam (1981) has reported the spawning of quite a few species of Clupeids and Engraulids at Cannanore, Tuticorin and Porto Novo, during the same spawning season. In many cases, the spawning seasons appeared to be throughout the year, as evidenced by the occurrence of eggs, larvae and juveniles, vide, Bal and Pradhan (1945, 1946, 1951), Gopinath (1946). According to Qasim (1973), spawning of most marine teleosts fishes in India is protracted, usually beginning at the onset of monsoon rains along both the coasts. In the west coast of India, the south-west monsoon rains commence from June-July and end up in September-October and in the east coast the north-east monsoon rains start from September-October and end up in November-December.

Perhaps the most baffling problem encountered in the study of fish eggs and larvae in Indian Waters is the overlapping sets of characters of the developmental stages of various species and/or genera. Although in a very few cases the characteristic features are distinct enough (Delsman, 1926 b, 1929 b, 1930, 1932 a, 1932 b, 1933, Jones and Menon, 1950, 52, Bensam, 1968, 1971), in the vast majority of cases the overlapping sets of characters have forced most workers to rely very much on circumstantial evidence, such as neritic-pelagic distribution of adult fish and occurrence of eggs and larvae, coincident occurrences of eggs and larvae in the locality of capture of prespawners, spawners and postspawners. Most workers have generally followed such a method, although in some cases cogent reasons of diagnostic in value have also been advanced by them. Some groups of species with overlapping sets of characteristic features are these of Sardinella, Thryssa, Liza, Leiognathus, etc., These overlapping sets of characters will be dealt with in detail in some of the future lectures. For the present it is enough to indicate that these are: egg diameters, diameter of oilglobule, number and disposition of muscle segments called myomeres and the like.

The interest of mankind towards Ichthyoplankton studies commenced only towards the end of the last century (Russell, 1976) when, in the year 1865, the eminent Norwegian planktologist has discovered that the eggs of the European cod, haddock and gurnard are planktonic. However, in India the first attempt to study marine fish eggs and larvae was made after another about fifty years when Bhattacharya (1916) described the early stages of three estuarine fishes. Thereafter, there was a progressive increase of research in this subject. An appraisal of work carried out till todate in the country recently

made by the present writer (Bensam, in press) shows that the number of species whose one or the other developmental stage has been identified is about 290 only, forming only about 23% of the marine bony fishes reported to occur in India (Talwar and Kacker, 1984). But, a more in-depth analysis shows that only in the cases of 100 species that almost all the vital developmental stages, viz., eggs, larvae, postlarvae and juveniles are known adequately, forming only 8% of the number of species in India. This fact warrents that for rational exploitation, management and conservation of our marine fisheries there is urgent need to fill up the lacunae in our knowledge in the case of the vast majority of the species as well as to document these of species not yet covered.

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NEED FOR A SOUND KNOWLEDGE ON THE ICHTHYOFAUNA OF THE
LOCALITY, WHERE WORK IS TAKEN UP-SPAWNING SEASON AND
SPAWNING GROUNDS

By

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For a study of the early developmental stages of marine fishes occurring in the locality where work is taken up, it is essential to acquire a sound knowledge on the various species occurring in that area. The Ramanathapuram District wherein Mandapam and the adjoining areas are located, has a coast-line of about 260 km covering the sea front of Gulf of Mannar and Palk Bay. These seas have lucrative fishing grounds with good concentration of finfishes, shellfishes, seaweeds and other organisms of economic importance. These are mainly exploited by indigenous crafts and gears in the nearshore waters extending to a depth of 10-15 m. With the technological advancement and establishment of infrastructure facilities, the annual marine fish production of the District increased from 30,000 tonnes to over 60,000 tonnes during the past one and half decades.

A large variety of fishes constitute to the catches. The most important of these are: Silver bellies, elasmobranchs, croakers, clupeids, goat fishes, perches, cat fishes, lizard fishes and carangids. The annual fish production by mechanised boats at Mandapam fluctuated between 2533 tonnes in 1980 and 721 tonnes in 1984, the average catch being 5557 tonnes. Silver bellies (2692 t) accounted for 48% of the catch followed by penaeid prawns (12%). Croakers and elasmobranchs contributed to the tune of 270 and 240 t respectively, the percentage composition being 5 and 4% of the total yield. Goat

fishes, carangids, cat fishes, flat fishes, clupeids and other miscellaneous fishes together accounted for about 1700 t (30%). The names of some of the oviparous bony fishes, the gear in which they are caught, size at maturity, spawning season, etc. are as follows:-

I. CLUPEIDAE

1. Sardinella gibbosa

Gear	: Gill net
Peak period of occurrence	: May-June
Depth of occurrence	: Upto 15 m
Length range in commercial fishery	: 100 - 180 mm
Size at first maturity	: 120 mm
Spawning season	: Jan-April

2. Sardinella albella

Gear	: Gill net
Peak period of occurrence	: May-June
Depth of occurrence	: Upto 15 m
Length range in commercial fishery	: 100-165 mm
Size at first maturity	: 115 mm
Spawning season	: Jan-April

II. LEIOGNATHIDAE

(Silver bellies)

1. Leiognathus jonesi

Gear	: Trawl net/shore seine
Peak period of occurrence	: Throughout the year
Depth of occurrence	: 5-20 m
Length range in commercial fishery	: 35-95 mm
Size at first maturity	: Males-70 mm Females - 65 mm

- Spawning season : Throughout the year
2. Leiognathus brevirostris
- Gear : Trawl net/Shore seine
Peak period of occurrence : Throughout the year
Depth of occurrence : 12-15 m
Length range in commercial fishery : 45-95 mm
Size at first maturity : Male : 68 mm
Female : 63 mm
Spawning season : May-Jun and Oct-Nov.
3. Leiognathus dussumieri
- Gear : Trawl net/Shore seine/
Gillnet.
Peak period of occurrence : Throughout the year
Depth of occurrence : 20-40 mm
Length range in commercial fishery : 40-90 mm
Size at first maturity : Males : 78 mm
Females : 83 mm
Spawning season : Apr-May and Nov-Dec.
4. Leiognathus berbis
- Gear : Trawl net/Shore seine
Peak period of occurrence : Throughout the year
Depth of occurrence : 3-8 m
Length range in commercial fishery : 60-90 mm
Size at first maturity : --
Spawning season : --
5. Leiognathus equulus
- Gear : Trawl net/gillnet
Peak period of occurrence : Throughout the year
Depth of occurrence : 10-25 m
Length range in commercial fishery : 125-200 mm

- Size at first maturity : --
Spawning season : Jan-Mar and May
6. Gazza minuta
- Gear : Trawl net/Shore seine
Peak period of occurrence : Throughout the year
Depth of occurrence : 7-20 m
Length range in commercial fishery : 45-115 mm
Size at first maturity : --
Spawning season : Jan-Apr and Aug-Dec.
7. Secutor ruconius
- Gear : Trawl net/Shore seine
Peak period of occurrence : Throughout the year
Depth of occurrence : 5-20 m
Length range in commercial fishery : 40-50 mm
Size at first maturity : 45 mm
Spawning season : Oct-Dec.
8. Secutor insidiator
- Gear : Trawl net/Gill net
Peak period of occurrence : Throughout the year
Depth of occurrence : 7-20 m
Length range in commercial fishery : 15-85 mm
Size at first maturity : 70 mm
Spawning season : Oct-Feb.

III. SCOMBRIDAE

1. Rastrelliger kanaqurta (Indian mackerel)
- Gear : Drift gill net/
Shore seine
Peak period of occurrence : Sep-Mar.
Depth of occurrence : 5-18 m

Length range in commercial fishery : 220-240 mm
Size at maturity : --
Spawning season : Jun-Jul and Jan-Mar.

IV. SERRANIDAE, SIGANIDAE, SCARIDAE AND LETHERINDAE

(Perches)

1. Epinephelus tauvina

Gear : Trap/Hooks & line
Peak period of occurrence : Oct-Mar
Depth of occurrence : 10-30 m
Length range in commercial fishery : 180-790 mm
Size at first maturity : --
Spawning season : --

2. Siganus canaliculatus

Gear : Trap/Hooks & line
Peak period of occurrence : Oct-May
Depth of occurrence : 2-5 m
Length range in commercial fishery : 150-300 mm
Size at first maturity : --
Spawning season : --

3. Lethrinus nebulosus

Gear : Traps/Gillnet/Hooks & line/Trawl net
Peak period of occurrence : Oct-Mar.
Depth of occurrence : 10-30 m
Length range in commercial fishery : 70-320 mm
Size at first maturity : --
Spawning season : --

4. Callyodon ghobban

Gear	: Traps/Hooks & line
Peak period of occurrence	: Nov-May
Depth of occurrence	: 2-5 m
Length range in commercial fishery	: 150-300 mm
Size at first maturity	: --
Spawning season	: --

A list of the more dominant bony fishes of Mandapam area is given below:-

Group names/Scientific names

Common name

SHADS, SPRATS AND SARDINES

<u>Dussumieria acuta</u>	: Rainbow sardine
Hilsa ilisha	: Indian shad
<u>Pellona ditchella</u>	: Indian pellona
<u>Sardinella gibbosa</u>	: Gold striped sardine
<u>S. albella</u>	: Short body sardine

ANCHOVIES

<u>Stolephorus bataviensis</u>	: Batavian anchovy
<u>S. devisi</u>	: Devisi anchovy
<u>S. commersonii</u>	
<u>S. indicus</u>	: Indian anchovy
<u>Thryssa mystax</u>	
<u>T. dussumieri</u>	

WOLF HERRING

<u>Chirocentrus dorab</u>	: Dorab wolf herring
<u>C. nudus</u>	: White fin wolf herring

MILK FISH

<u>Chanos chanos</u>	: Milk fish
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LIZARD FISHES

Saurida tumbil

: Greater lizard fish

S. undosquamis

: Brush tooth lizard fish

CAT FISHES

Tachysurus thalassinus

I. dussumieri

I. serratus

HALF BEAKS

Hemirhamphus spp.

FLYING FISHES

Cypselurus sp.

FLUTEMOUTHS

Fistularia villosa

SQUIRREL FISH

Holocentrus rubrum

Red squirrel fish

H. sammara

Blood spot squirrel fish

MULLETS

Mugil cephalus

Flat headed grey mullet

Valamugil seheli

Blue spot grey mullet

Liza parasia

Gold spot mullet

L. macrolepis

Borneo mullet

THREADFINS

Polynemus indicus

Indian thread fin

P. sexfilis

Golden six-thread
threadfin

P. heptadactylus

Seven-finger threadfin

P. sextarius

Black-spot threadfin

SEA PERCHES

Ambassis commersoni

A. gymnocephalus

Lates calcarifer

Epinephelus tauvina

E. diacanthus

E. fasciatus

E. malabaricus

Giant sea perch

Greasy reef cod

Six-barred reef cod

Banded reef cod

Malabar reef cod

WHITINGS

Sillago sihama

Silver whiting

WHITEFISH

Lactarius lactarius

White fish

CARANGIDS

Carangoides malabaricus

Atol mate

Selaroides leptolepis

Caranx sexfasciatus

C. ignobilis

Malabar trevally

One finlet scad

Dusky trevally

Yellowfin trevally

BLACK POMFRETS

Formio niger

Black pomfret

SNAPPERS

Lutianus argentimaculatus

L. fulviflamma

L. johni

L. kasmira

L. malabaricus

L. russelli

Pristipomoides typus

SILVER BELLIES

Gazza achlamys
Leiognathus berbis
L. bindus
L. brevirostris
L. daura
L. dussumieri
L. equulus
L. fasciatus
L. ionesi
L. leuciscus
L. lineolatus
L. splendens
•Secutor insidiator
S. ruconius

MOJARRAS

Gerres filamentosus
G. setifer

CROAKERS

Johnieops sina
Johnius carutta
J. dussumieri
J. maculatus
Kathala axillaris
Otolithes argentius
Otolithoides microdon

Sin croaker
Karutta croaker
Bearded croaker

EMPEROR BREAMS

Lethrinus nebulosus
L. ornatus

Starry emperorr bream
Ornate emperor bream

GOAT FISHES

Parupeneus indicus
Upeneus traquila
U. sulphureus
U. vittatus

Indian goat fish
Yellow goatfish
Yellow-striped
goatfish

CORAL FISHES AND ANGEL FISHES

Chaetodon spp.

Pomacanthodes spp.

PARROT FISHES

Callyodon ghobban

C. scaber

Flame parrot fish

Five band parrot fish

SPINE FOOTS

Siganus canaliculatus

S. javis

S. eramin

S. stellatus

Estuarine spinefoot

Streaked spinefoot

White-spotted spinefoot

Blotched spinefoot

MOORISH IDOL

Zanclus cornutus

RIBBON FISHES

Lepturacanthus savala

Trichiurus lepturus

MACKEREL AND SEERFISHES

Rastrelliger kanagurta

Scomberomorus commerson

S. guttatus

S. lineolatus

Indian mackerel

Narrow barred seerfish

Indo-Pacific seerfish

Streaked seerfish

SAIL FISHES

Istiophorus platypterus

Makaira indica

Sailfish

Black marlin

SWORD FISHES

Xiphias gladius

Swordfish

POMFRETS

Pampus argenteus .

Silver pomfret

P. chinensis

Chinese pomfret

FLAT FISHES

Psettodes erumei

Indian halibut

Cynoglossus bilineatus

C. dubius

C. macrostomus

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MORPHOMETRIC AND MERISTIC CHARACTERS OF FISHES

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Introduction

Both from the taxonomic as well as the management point of view, a correct identification of marine fishes is important. The 'Folk taxonomies' that developed in earlier times contained 250 to 800 kinds of animals. The invention of printing in the fifteenth century and world explorations have made expansion of taxonomy both possible and inevitable. A number of attempts to classify animals were made, but were limited in scope until Linnaeus introduced the binomial nomenclature in the eighteenth century. He has recognised species as the basic unit in nature. This meant that it is necessary to describe only one individual to know of an entire species. But later Linnaeus and his successors have encountered natural variation within each species and they were forced to recognise 'varieties'. The scientific names based on binomial nomenclature provide names that are recognised all over the world. Each name has two parts: the genus name which is always capitalised and the trivial name or the species epithet which is not capitalised. The two names together constitute a species name.

Continuing the effort to catalog all kinds of animals, taxonomists are concerned not only with the description of new forms but also with the placing of each form within a taxonomic system that shows its relationship to other forms.

Individuals are grouped into populations (sub species - geographically isolated and morphologically different from other similar populations), populations into species, species into genera, genera into families, order, phylum etc., and upto the Animal Kingdom.

TAXONOMIC METHODS

A modern taxonomist may be able to draw information from such varied fields as biochemistry, genetics, behaviour, physiology, geographical distribution, palaeontology and cytology to supplement and strengthen the more conventional laboratory data on morphology and anatomy. Foremost among these tools is the computer which permits complex multivariate analysis of large amount of data. Sufficient sampling and adequate coverage of the characteristics are important. The data are gathered using both old and new techniques. These include: 1. Morphometric measurements and ratios, 2. Meristic counts, 3. Anatomical characteristics, 4. Colour patterns, 5. Karyotype, 6. Electrophoresis and 7. Test for reproductive isolation.

Morphometric and Meristic Characters

Morphometric Measurements:

Measuring the linear dimensions of the whole or part of a fish is probably the most widely used technique in fisheries biology studies. Morphometric measurements are any standard measurements that can be taken on a fish such as Standard Length, Snout Length, length of largest fin ray of the dorsal fin, depth of the caudal peduncle and so on. Since these measurements change as the fish grows, these are usually expressed as ratios to Standard Length. Such ratios are only useful if comparisons are made between samples of fish of approximately the same size and sex, since the growth of a fish is not always proportional in all directions and sexual dimorphism is also noticed among fishes. Thus morphometric measurements while vital for

describing fish species may be of limited usefulness.

Three overall length measurements in common use are 1. Standard length, 2. Fork length and 3. Total length. The latter two measurements are more commonly used in fishery biology. Overall length measurements are made between perpendiculars along the median longitudinal axis from snout (U, the position of the maxillary symphysis) or from the tip of the lower jaw (L, the mandibular symphysis), vide Fig. 3.2.1. Measurements from L are taken with the mouth closed. If the lower jaw is projecting, measurements from the symphysis may necessitate provision of a special stepped nose piece on the measuring board. Generally measurements are made on the left side of the fish, with the right side of the fish resting on the measuring board. For definitions of positions, reference may be made to the next section.

1. Standard Length: Taken from U to the tip of the hypural bone (urostyle). This varies from species to species.
2. Fork Length: Measured from U or L to the cartilaginous tip of shortest or median caudal ray.
3. Total Length: Measured from U or L to the longest caudal fin ray, upper or lower, or an average of them both.

Longitudinal measurements other than overall length are also made between perpendiculars using measuring board with, for example a sliding cursor. When these are made radially from point U, calipers are recommended. Point-to-point measurements are sometimes made on big fishes such as tunas by tapes. These would be indicated by the word 'Surface' as these are not generally recommended. All measurements from LX to LM and also their 'upper' equivalents are grouped under the general name 'total length' LT. LM has been called 'bilobular length' and total 'auxiliary length'. The word 'Extreme' is used in LX,

LX' instead of 'maximum length to avoid confusion with the asymptotic length. LF and LF' are also called 'median' length or 'midcaudal' length. The term 'depth' is used instead of 'height'. Again the term 'width' is not recommended as an alternative to 'breadth' but 'thickness' would be an ideal term. Pectoral and ventral fins are to be measured in the folded position opposed to the body side (to keep the rays straight) from foremost visible point of insertion to the distal tip of the membranous edge.

Definitions of position

- U Maxillary symphysis.
- L Mandibular symphysis.
- OO Anterior edge of orbit.
- O' Posterior edge of orbit.
- J Posterior edge of mandible (buccal commissure).
- Y Gill-cover notch.
- G Posterior bony edge of operculum.
- G' Posterior membranous edge of gill cover.
- P Anterior point of insertion of the first pectoral fin ray.
- D1 Insertion of anterior dorsal (intersection of anterior margin of first dorsal spine, fin held erect with the contour of the back).
- D1' Position of last ray of anterior dorsal.
- D2 Insertion of first ray of posterior dorsal.
- D2' Position of last ray of posterior dorsal.
- Z Anterior edge of cloaca.
- A Insertion of first anal fin ray.
- A' Position of last anal fin ray.
- B Insertion of dorsal lobe of caudal fin.
- S Posterior tip of urostyle (forward protuberance of hypural blade).
- S' Posterior edge of fleshy peduncle or of pigmented zone.
- S'' Point of upper caudal keel.
- S''' Posterior limit of silvering (either last scale of the lateral line or the posterior zone limit of

- the scale covered by the peduncle).
- F Cartilaginous tip of shortest (median) caudal ray.
F' Membranous edge of caudal fin at fork.
N Distal tip of the longest caudal fin ray with lobe normally extended.
N' Distal tip of the longest ventral fin ray with lobe normally extended.
M Point where line NN' intersects median longitudinal axis.
M' Mid point of line NN'.
X Distal tip of longest dorsal caudal fin ray, with the lobe brought to the median longitudinal axis.
X' Distal tip of the longest ventral caudal fin ray, with the lobe brought to the median longitudinal axis.

Overall length measurements:

- LT and UT total length (any extreme or normal length).
LX Dorsal extreme length.
LX' Ventral extreme length.
LX'' Greater extreme length (LX or LX', whichever is greater).
LN Dorsal normal length.
LN' Ventral normal length.
LN'' Greater normal length (LN or LN', whichever is greater).
LM Median normal length.
LM' Mean normal length.
LF Midcaudal length.
LF' Fork length.
LS Standard length to urostyle (or to some external feature corresponding with it).
LS' Standard length to peduncle (or to the pigment under scales).
LS'' Standard length to keel.

LS''' Standard length to silvering.
LB (Dorsal) Body length.

Other longitudinal measurements

UJ Maxillary sheath length.
LJ' Mandibular length.
UO Snout length.
UY Upper head length.
LG Opercular head length.
Lg Greatest head length.
OO' Orbital diameter.
Id Longitudinal iris diameter (cf, Ih and Ig).
Ed Longitudinal pupil diameter (cf, Eh and Eg).
O'Y Postorbital distance.
UD1 Preanterior dorsal distance.
UP Prepectoral distance.
UV Preventral distance.
UD2 Preposterior dorsal distance.
D1D1' Anterior dorsal fin base length.
D2D2' Posterior dorsal fin base length.
UA Preanal distance.
AA' Anal fin base length.

Vertical measurements (Perpendicular unless otherwise stated)

Oh Orbital depth (from orbital crest to lower edge of maxillary, passing over middle of pupil).
Ih Perpendicular iris diameter.
Eh Perpendicular pupil diameter.
YJ' Head length.
D1P Back depth (oblique).
D1V Anterior dorsal depth (or dorsoventral depth).
h Greatest depth.
D2Z Posterior dorsal depth.
E2A Dorsoanal depth (slightly oblique).
h' Perpendicular anal depth.
q (Least) peduncle depth.

Lateral measurements

- PP Pectoral breadth.
b Greatest breadth.
OO Interorbital distance (at level of pupil centre)

Other measurements

- D1h Anterior dorsal height distance from insertion to tip of longest spine).
D2h Posterior dorsal height (distance from insertion to tip of longest spine).
Ph Pectoral fin length.
Vh Ventral fin length.
Ah Anal fin height.
Ch Dorsal caudal fin length.
Ch' Ventral caudal fin length.
Ch'' Greater caudal fin length.
Ig Greatest iris diameter.
Eg Greatest pupil diameter.
g Greatest girth.
VV Length of interventral flap.
NN' Spread caudal distance.

Skeletal dimensions

- Ax Axial length (anterior face of vertebra 1 to tip of urostyle).
Sk Skull length (maxillary symphysis to posterior occipital boundary).
An Anatomical length (= Ax + Sk).

Meristic counts

These counts are generally considered to be the most reliable taxonomic characteristics because most are easy to make and reliable. It includes anything on a fish that can be counted, such as the number of vertebrae, fin rays, spines, scale rows, pyloric caecae, lateral line

scales, pores, finlets and gillrakers. Since there is often considerable variation in these characteristics within species, it is important to make the counts on adequate number of individuals so that their mean, range and standard error can be determined, if the fishes involved are to be compared with other populations.

Number and disposition of the vertebrae

The backbone of a fish is a piece of machinery performing a variety of functions. At the anterior end it is designed to make connection with the head and the trunk. At the posterior end it is modified to act as the basal support and frame work for the caudal fin. It gives support to the median fins, provides attachment for the muscles, houses the central nervous system and the haemal artery, gives suspension and protection of the viscera and so on. It is a vital part in the turning and propulsion mechanism. Hence the form of the vertebral elements changes in its length to suit various functions. Every structural feature of a vertebral segment, even to the smallest zygapophyses forms one unit of a discrete gradation series and the natural compounding of these series gives a pattern which is distinctive to species. Furthermore, after comparing species with species, it is impossible to escape the impression that phylogenetic relationship is made manifest by agreement both in the character of individual gradation series and in their compounded pattern. The suggestion is all gadoid backbone conform to a gadoid pattern, all clupeoids to a clupeoid pattern and so on.

General pattern of division of a vertebral column

The total number of vertebrae is quite variable in fishes and within genera and species. They may be divided conveniently into precaudal (abdominal) and caudal portions. The first caudal vertebra is that which possesses an elongated haemal spine. Depending upon various other features,

the vertebrae may be grouped, but their number varies from fishes to fishes. The total number, number of vertebrae showing common features, their range and mean are important.

Some general features are as follows (Fig.3.2.2):

1. The post cranial vertebrae bear stout neural arches and spines.
2. The mesabdominals follow the post cranials, bear ribs, but do not possess haemal arches.
3. The postero-abdominals have closed haemal arches and bear ribs.
4. The antero-caudals greatly resemble the postero-abdominals except that they have lost the ribs and have developed haemal spines.
5. In the tail segment the vertebrae have their neural and haemal spines entering into the support of the caudal fin.
6. The hypural complex is almost symmetrical and fan like, receiving the rays of the caudal fin. The rays of the caudal fin are supported by altered vertebral elements (penultimate hypurals, epurals, urostyle).

The number and characters in each of these divisions in the vertebral column may be compared to arrive at meaningful conclusions.

Anatomical characteristics

These include features such as shape, completeness and position of the lateral line, position and size of the internal organs, special anatomical features (such as air bladder, air breathing apparatus, electric organs, otoliths, arrangement of the musculature etc.), secondary sexual characters (breeding tubercles in males, enlarged fin rays, etc.), shape size and interrelationship of bones and muscles.

Most of these are "yes" or "no" characters, either a fish has them or it does not have. These can be definitive characteristics for separating species as well as higher taxa.

Colour patterns

Colour patterns are quite variable with age, time or environment. These are part of the species description and are species-specific. The main problem in using colour pattern as a taxonomic tool is that it tends to fade in preservatives and descriptions of living fish tend to be highly subjective.

Karyotype

These are descriptions of the number and morphology of chromosomes. The number and position of chromosomes are conservative characters and so may be used as an indicator of the closeness of species interrelationship within families.

Electrophoresis

This technique of evaluating the protein similarities in fishes could be used as a taxonomic tool. The protein can be identified and genetic similarity of individuals and species can be compared.

Taxonomic tools in racial investigations

A combination of all or some of these taxonomic methods have been used for racial investigations from time to time with interesting and at times with negative results. These studies are important in fishery biology for evolving suitable management policies for judicious exploitation of the resources, among which the identification of the eggs and larvae to the species to which they belong is one important aspect.

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DEVELOPMENT OF COCYTES TO MATURITY AND SPAWNING

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Spawning in fishes is closely associated with the development of intra-ovarian eggs. Measurements of diameters of intra-ovarian eggs have been found to be an useful tool in studying the development of oocytes to maturity and spawning. An attempt to study the maturity by the measurements of ova was first made by Clark (1934) on the California sardine (Sardina caerulea). Her pioneering work was followed by those of Hickling and Rutenberg (1936) and De Jong (1939).

The method proposed by the above workers is essentially as follows: Ovaries are fixed in 5% formaldehyde and a small portion of the ovary is teased out on a slide in the same medium and measurements of all the ova in the field of the microscope are made, until about 500 ova are measured. In case the eggs appear asymmetrical due to preservation, the micrometer may be placed in horizontal position and the diameters are measured parallel to the graduations on the micrometer. Ovaries after fixing in Bouin's solution, may be cut in rotary microtome and the diameter of the oocytes in the sections could also be measured. Prabhu (1956) suggests that measurements of at least 1000 eggs from each ovary are necessary to mitigate the probable error in the representation of various groups of eggs in different stages of maturity and represented by various modes in the graphs. Normally the first batch of immature eggs are avoided in the measurements.

The intra-ovarian eggs vary not only in their size but also in their inclusions in ovaries which are fully

ripe or in the penultimate stage of ripeness. There are several batches of oocytes which take their origin from the germ cells of the ovigerous lamellae and, as the spawning process continues every season, these batches pass on from one stage to the other. An examination of the ovary in the penultimate stage of development shows chiefly the following four types of ova: Fig. 3.3.1.

- (1) Immature ova: ~~minute transparent ova~~ minute transparent ova possessing a nucleus and a protoplasmic layer.
- (2) Maturing ova: small, opaque ova in which yolk formation has just commenced, but not completed.
- (3) Mature ova: Opaque ova, fully yolked, but still contained within the follicle.
- (4) Ripe ova: Large fully or partially transparent ova which have burst out from the follicles.

Ovarian maturity stages are determined based on the predominance of the above mentioned types of development.

Histologically, oocyte development could be broadly classified into a primary growth phase, a secondary growth phase and a final maturation to be followed by ovulation and spawning.

- (1) Primary growth phase: The immature oocytes, known as the oogonia, are seen multiplying by mitosis in the stroma of the ovigerous folds. The oogonia are transformed to the primary oocytes by arresting the chromosomes at the prophase of the first meiotic division; this process is known as oogenesis. In the oogonia, the nucleo-cytoplasmic ratio is high, but as the growth progresses, this ratio decreases. Highly spiralized lamp-brush chromosomes are usually seen in the nucleus of the primary oocyte. Nucleoli multiply and arrange along the periphery of the nucleus, called peri-nucleolar stage. The follicular

wall is thin and less prominent in the oocytes during primary growth phase (Fig. 3.3.2).

(2) Secondary growth phase: Appearance of different types of yolk bodies in sequence is the characteristic feature of the secondary growth phase. Normally carbohydrate-rich cortical alveoli make their appearance along the periphery of the oocyte. Oil droplets are present in most of the marine fish oocytes. The protein laden yolk appear in the form of yolk granules and these along with the lipid yolk fill the entire ooplasm during the advanced stages of secondary growth phase. Size of the oocyte increases considerably and follicular wall becomes prominent (Fig. 3.3.3).

(3) Final maturation: Primarily, oocyte maturation involves the resumption of meiosis. It is commonly regarded that the chromosomal activity proceeds to the metaphase of the second meiotic division. Resumption of meiosis is heralded by a peripheral migration of the germinal vesicle (nucleus) and by the dissolution of the germinal vesicle (Germinal Vesicle Breakdown or GVBD). GVBD is commonly used as an indicator of oocyte maturation. In some species, co^oalescence of yolk granules resulting in the oocyte becoming increasingly transparent. Concomitant with maturation in many teleosts, especially marine forms with pelagic eggs, oocyte enlargement is due to hydration. Hydration in some species may result in a 300-400% increase in oocyte volume.

The present contention is that oocyte maturation and ovulation are dependent on pituitary gonadotropin. Concomitant with maturation or after maturation is complete, oocytes are ovulated into the ovarian lumen.

Various authors have classified the different maturity stages into 4 to 7 stages, calling them as "Stage I", "Stage II", etc., and taking into account the dominant size of the ova as well as the gross volume of the ovary in relation to the body cavity (Clark, 1934; Hickling and ^{Ruttenberg} ~~Euterberg~~).

1936; Prabhu 1956, Nair, 1959; James and Badrudeen, 1981)

Based on ova diameter measurements, four different types of spawning periodicities have been recognized in teleostean fishes:

Type A: Spawning taking place only once a year during a definite, short period. In this case, the eggs which are destined to be spawned are withdrawn from the immature stock in a single group, sharply distinguishable at least in the later stages of maturation from the stock of small eggs from which it was derived (Fig. 3.3.5). The oocyte development in this case is said to be synchronous, as in Therapon jarbua, Macrones vittatus and Chirocentrus dorab.

Type B: Spawning taking place only once a year, but with a longer duration. In species exhibiting this type of spawning, the range in size of the mature ova, irrespective of the number of modes representing them, have been found to be nearly half of the total range in size of the entire intra-ovarian eggs in the whole ovary (Fig. 3.3.4), as in Pelates quadrilieatus and Cypsilurus oligolepis.

Type C: Spawning twice in an year. In the ovaries of fishes exhibiting this type of spawning, in addition to the batch of eggs in ripe condition, another batch of eggs in which yolk formation has already commenced could be seen (Fig. 3.3.4), as in Psammoperca waigensis, Therapon puta and Selaroides leptolepis.

In the B and C types of spawning, the oocyte development is described as group synchronous.

Type D: Spawning throughout the year, but intermittently. Withdrawal of eggs from the immature stock is a continuous process; and there will be no sharp separation between the general egg stock and the maturing eggs (Fig.3.3.5). The pattern of oocyte development in this case is said to

be 'asynchronous', as observed in Stolephorus indicus.

It may be mentioned here that a protracted spawning season cannot always be equated with multiple spawns for each female. Protracted spawning season may simply reflect lack of population synchrony in terms of gonadal development. The term multiple spawner is generally applied to a species in which a female spawns more than once in a spawning season. The term fractional spawner has been used to refer to a species which spawns a part of the ovulated clutch or which mature ovulate, and spawn a part of a post-vitellogenic clutch at intervals over a relatively short period.

Clark (1934) points out that if only one batch of eggs is spawned, the ratio between the number of eggs in the maturing group and the number of eggs in the mature group should remain constant and on the other hand, if more than one batch is spawned, the ratio gradually decreases. Based on this principle she proves that individual California Sardines spawns an average of three batches. Inferred multiple spawning, she provides four lines of evidence, viz., multiplicity of modes in the ova diameter frequency curves, a high degree of correlation between the growth of successive groups of eggs, occasional presence in the ovary of a few ripe, unspawned eggs and decrease in the ratio of the number of eggs in the maturing and mature groups as the breeding season advances.

Fertilisation

When the egg becomes ripe, a small opening known as the microyle appears on it, through which the sperm enters at the time of fertilisation. It is assumed that soon after fertilisation, water enters through the pores of the egg membrane, lifting it from the yolk to form the perivitelline space. It is not known whether before fertilisation the micropliar canal is closed by a substance requiring an enzyme from the sperm to break it down.

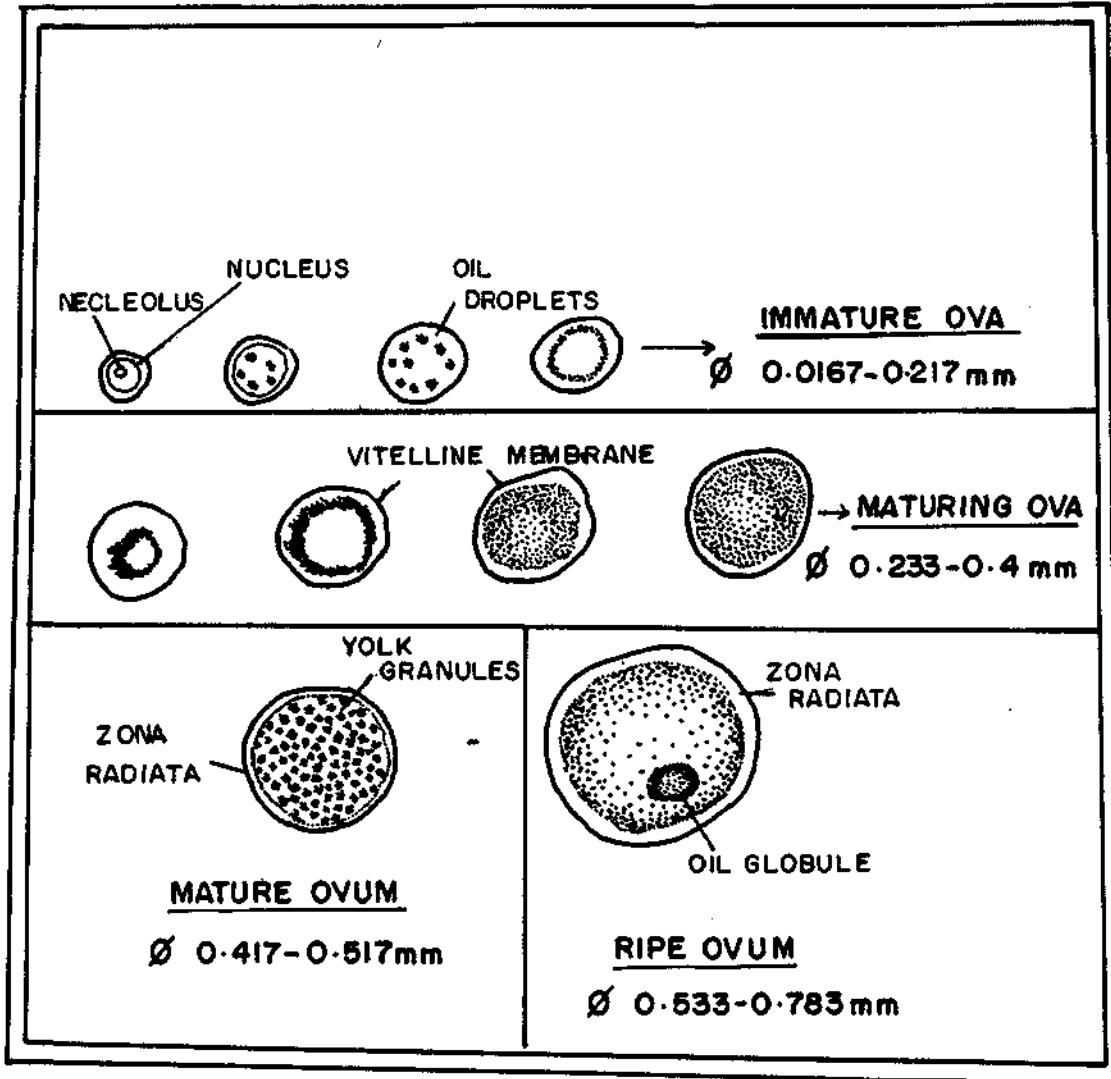
When the egg is laid free in the water, the outer covering (chorion) at once becomes hardened. The hardened chorion becomes thinner as development advances and the egg increases in size. This process called 'water hardening' is advantageous in that it offers protection to the eggs from predators. It is assumed that a part of the substance of the egg membranes is withdrawn and absorbed by the embryo and this seems to occur to a greater extent in the demersal eggs, where the embryo is more advanced on hatching, than in the pelagic eggs.

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Fig.3.3.1



PRIMARY GROWTH PHASE

Fig.3.3.2

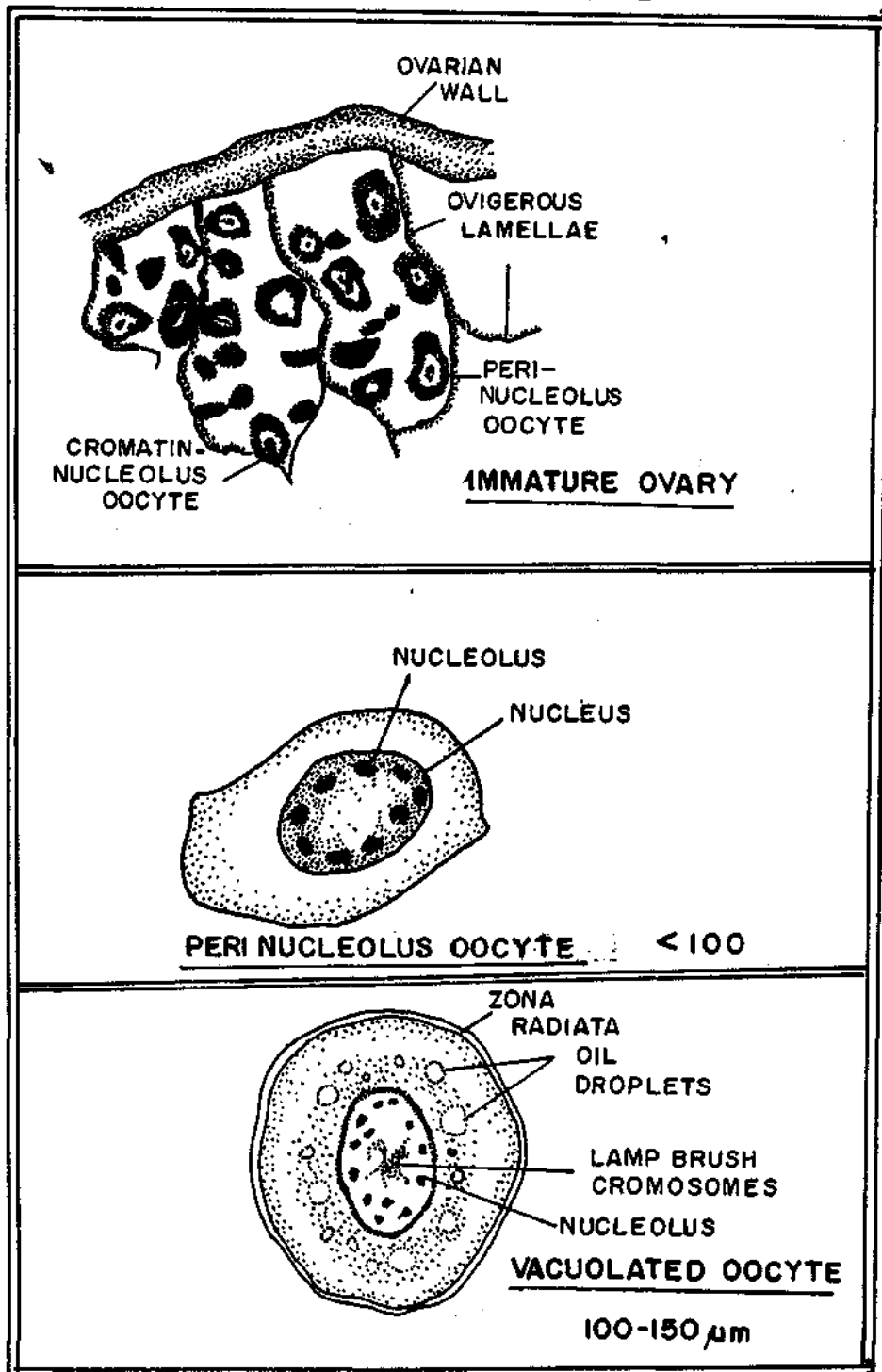


Fig. 3-3-3

SECONDARY GROWTH PHASE AND FINAL MATURATION

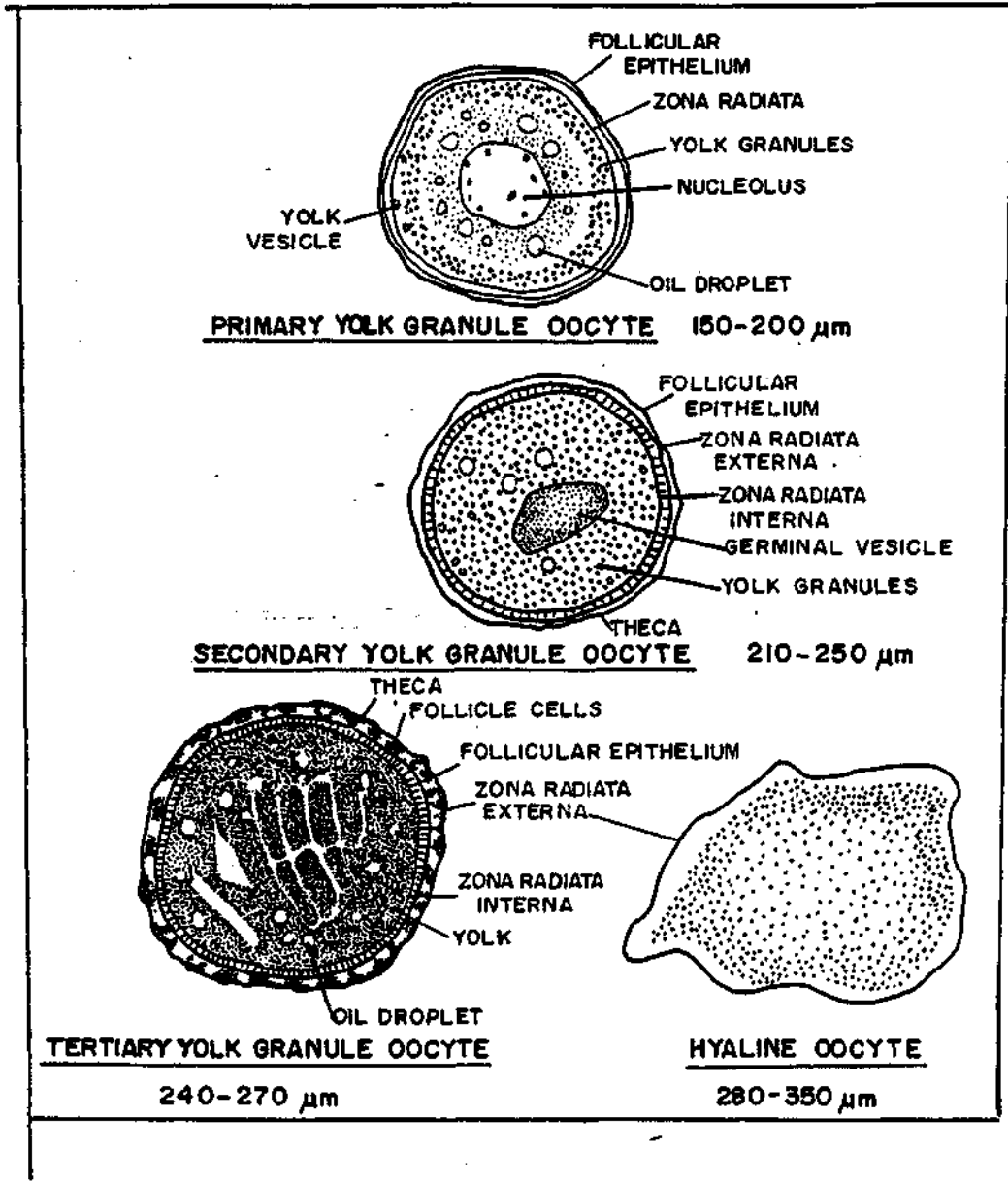
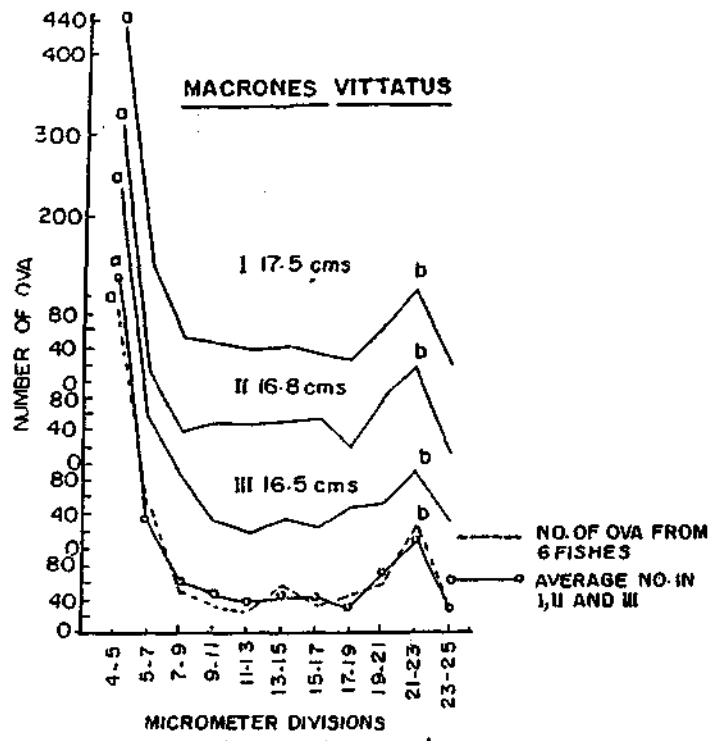
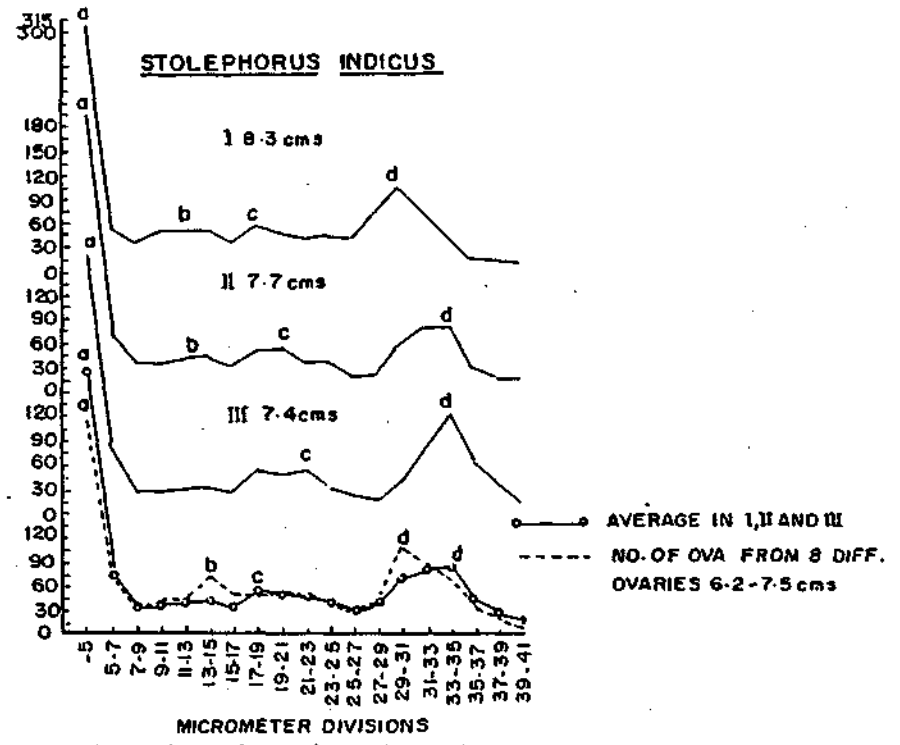


Fig.3.3.5



OVA DIAMETER POLYGON OF
MACRONES VITTATUS

(AFTER PRABHU 1956)



OVA DIAMETER POLYGON OF
STOLEPHORUS INDICUS

(AFTER PRABHU 1956)

DEVELOPMENT OF EGGS AND LARVAE OF MARINE FISHES

By

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Introduction

For an individual fish, the beginning of development is fertilisation of the egg by spermatozoum. Most marine teleosts have pelagic eggs which drift with the plankton in all water layers between the surface and the bottom. However, fishes like the British herring (Clupea harengus) and blennies and gobies have demersal eggs, which attach to substrata like stones, shells and weeds. Usually, pelagic eggs are transparent and spherical, with the exception of the eggs of certain engraulids (Stolephorus) which are oblong. When the embryo is fully developed, it hatches out as larva and undergoes further development.

Fertilisation and Embryonic development (Fig. 4.1):

There is an outer egg membrane called chorion. It is slightly thickened at one spot to form what is called the micropyle. Micropyle has a small outer funnel-shaped depression, leading to a canal, connecting with an inner conical elevation. The external surface of the chorion is usually smooth, but in the eggs of certain fishes like Callionymus there is an external raised hexagonal sculpturing and in the eggs of fishes like Belone there are fine tendrils. Within the chorion is the spherical yolk mass mixed up with protoplasm and surrounded by a thin delicate layer of protoplasm. The latter contains within it the nucleus in a lens-shaped thickening at the position of the future blastoderm. The sperm enters the egg through

the micropyle and at fertilisation it is thought that sea water enters through the pores of the chorion, lifting it from the yolk and forming the perivitelline space, which is usually narrow but in sardines it becomes very large.

Segmentation appears at first vertically to form the blastodisc. The upper cells are fully made of protoplasm while the lowest cells are continuous with the thin protoplasmic acellular layer of the yolk. Nuclei migrate into this acellular layer and increase in number to form a periblastic tissue without distinguishable cell walls, called "syncytium". The blastoderm gradually forms a coating over the yolk, process called Epiboly, leaving an area of yolk not covered by it, called "blastopore", which becomes smaller and smaller until the whole yolk area is covered. Accompanied by the above coverage, the blastodermal layer becomes a cylindrical rod like structure pressing into the surface of the yolk. The outline of the eyes and auditory sacs soon appear, together with formation of the muscle segments called myomeres. About 2 or 3 days after the closure of the blastopore, when the embryo is about half-way round the yolk, pigmentation usually appears as fine dots and the heart becomes functional.

The primordial larval fin membrane arises as a median fold enclosing a space filled with a jelly-like lymph. With further development, the caudal end of the embryo becomes raised up from the yolk. By the time these events happen externally, apart from the notochord (which is formed by movement of cells over the dorsal lip of the blastopore), five organ-forming tubes of tissue appear internally. These are the body covering epidermal tissue, neural tissue giving rise to the nervous system, endodermal marking the alimentary system and two

lateral mesodermal tubes of tissue marking the muscular tissue and body cavity. The mesodermal tubes of the trunk region segregate into dorsal, intermediate and lateral divisions, of which the dorsal division becomes divided vertically into block-like somites. Each somite becomes further subdivided into three regions (Fig.4.2, 7 a and b) called (a) dermatome which is the outer part of the somite and which is responsible for the development of the skin and its derivatives (b) sclerotomes which is the ventral part of the inner somite giving rise to the axial skeleton and (c) myotomes or myomeres which are the dorsal parts of the inner somite resulting in the formation of trunk musculature. In fishes and other lower vertebrates the myomere is the largest part of the somite, the sclerotome being inconspicuous. Their number correspond to the total number of vertebrae in the adults.

By the time the embryo has two eyes, auditory vesicles heart myomeres and larval finfold more or less covers the yolk and shows movements of the body, it is ready for hatching. In certain species with prolonged embryonic period, the eyes get pigmented before hatching and in certain other cases pigments also develop before the event.

Incubation and factors influencing pace of development

Although the period of incubation, i.e., the time taken from the time of fertilisation to the time of hatching is under genetic control, within each species, there are certain external factors which influence the pace of development and alter the period of incubation. Size of the egg and the quantity of yolk present therein are some initial factors. Temperature is the principal direct and indirect factor influencing these aspects. In temperate countries, the incubation period is longer during winter than in summer Blaxter (1969). Winkler (1986) deals with the relationship between temperature and incubation, the higher the temperature

within lethal limits the quicker the rate of development. Blaxter (1969) has shown that the Atlantic herring took about 25 days to hatch at a temperature of 6°C but only about 9 days at a temperature of about 14°C. Temperature is also found to reduce the size and weight at hatching, increase the efficiency of yolk absorption as well as to play an indirect role through oxygen capacity of water, phytoplankton production, etc. Similarly, salinity may accelerate or retard the time of hatching, while lack of oxygen has a retarding effect on the pace of development.

Hatching:

Hatching is controlled specifically as well as environmentally, the latter factors being temperature and oxygen. A softening of the chorion resulting from an enzyme secreted usually from ectodermal glands on the anterior surface of the body takes place, assisted by the activity of the embryo inside result in breaking of the chorion and hatching. It is believed that a part of the nutrient material in the chorion may be utilised by the embryo through the perivitelline fluid, thus resulting in its softening, vide, Blaxter (1969).

Larva (Fig. 4.2, 1-3):

The embryo which comes out of the chorion is called the larva (the newly hatched larva). It is usually transparent with pigments in some cases, the function of which are not understood. Notochord and myomeres are prominent with little development of cartilage or bone. Only the embryonic vertical finfold is present, mouth and jaws not yet developed and the gut is usually a straight tube. Blood is colourless and the circulatory as well as respiratory systems are poorly developed. Yolk is enormous, and it presumably has a hydrostatic function. Eye pigmentation is usually absent in the newly hatched larva, but develops only at

the end of the larval phase. The kidney is usually pronephric; and very little is known on the presence of gonads and other organs of the body cavity.

Most species hatch with <- shaped myomeres acting against the notochord as a skeleton. Additional numbers may be added posterior only and the final number is attained during the larval period. The myomeres become more complex as development progresses.

Postlarva (Fig. 4.2, 4-6)

As the yolk is resorbed, the mouth is formed, the eyes are pigmented, branchial arches are developed, the pectoral fins appear and the larva becomes fitted for a change to sources of external food from the yolk available till then. These mark the beginning of the postlarval phase of development. Branchial respiration replaces cutaneous one and the gill arches and filaments appear. The air bladder may or may not be present. One of the systems to develop early is the one for locomotion. The pectoral fins are the first to make their appearance as flap or fan like structures. These remain as such until a very late stage in postlarval development and get ossified much later than all the other fins. The caudal fin is the next to develop through invasion of the caudal end of the larval finfold by skeletogenous tissue which produces fin rays, thus transforming the caudal part of the larval finfold into the adult caudal fin. A similar development takes place for the dorsal fin and the anal fin which develop almost simultaneously appearing first as thickness of the finfold and transforming parts of the larval finfold. The thickenings are the interspace areas which will further split into many such areas. Between each pair of such areas a true fin ray develops. Parts of the larval finfold anterior to the dorsal and anal fins and in between each other

disappear gradually in the course of development. In fishes with an anterior and posterior part of the dorsal fin, usually the latter is the one to make its appearance, followed by the former. The pelvic or ventral fin is the last one to make its appearance. Usually the fin rays are the first to appear, followed by spines and their ossification.

Metamorphosis and Juvenile:

Metamorphosis or a change of body form from the larval or postlarval condition to the juvenile stage when the fish resembles the adult in all characters excepting sexual maturity, is rather slow in most of the marine teleosts. This is recently drawn attention to by Russell (1976) who points out that unlike the larval phase of development, the postlarval phase is very much longer, without any sharply demarcated termination. Different adult characters such as the numbers of fin rays and of vertebrae are often already developed, before the developing fish has lost certain postlarval characters such as pigmentation pattern. Although in the flatfishes there is a rotation of the optic region and a change in the normal orientation of the body so that both the eyes come to lie on one side accompanying metamorphosis, many postlarval characters are retained even after the above event. Thus, Russell (1976) points out that in most marine teleosts, it is impossible to determine a decisive point when a postlarva becomes a juvenile. Thus the term "postlarva" may be more appropriately defined as the period after the termination of the larval stage, during which there is a sequence of development leading to the juvenile stage.

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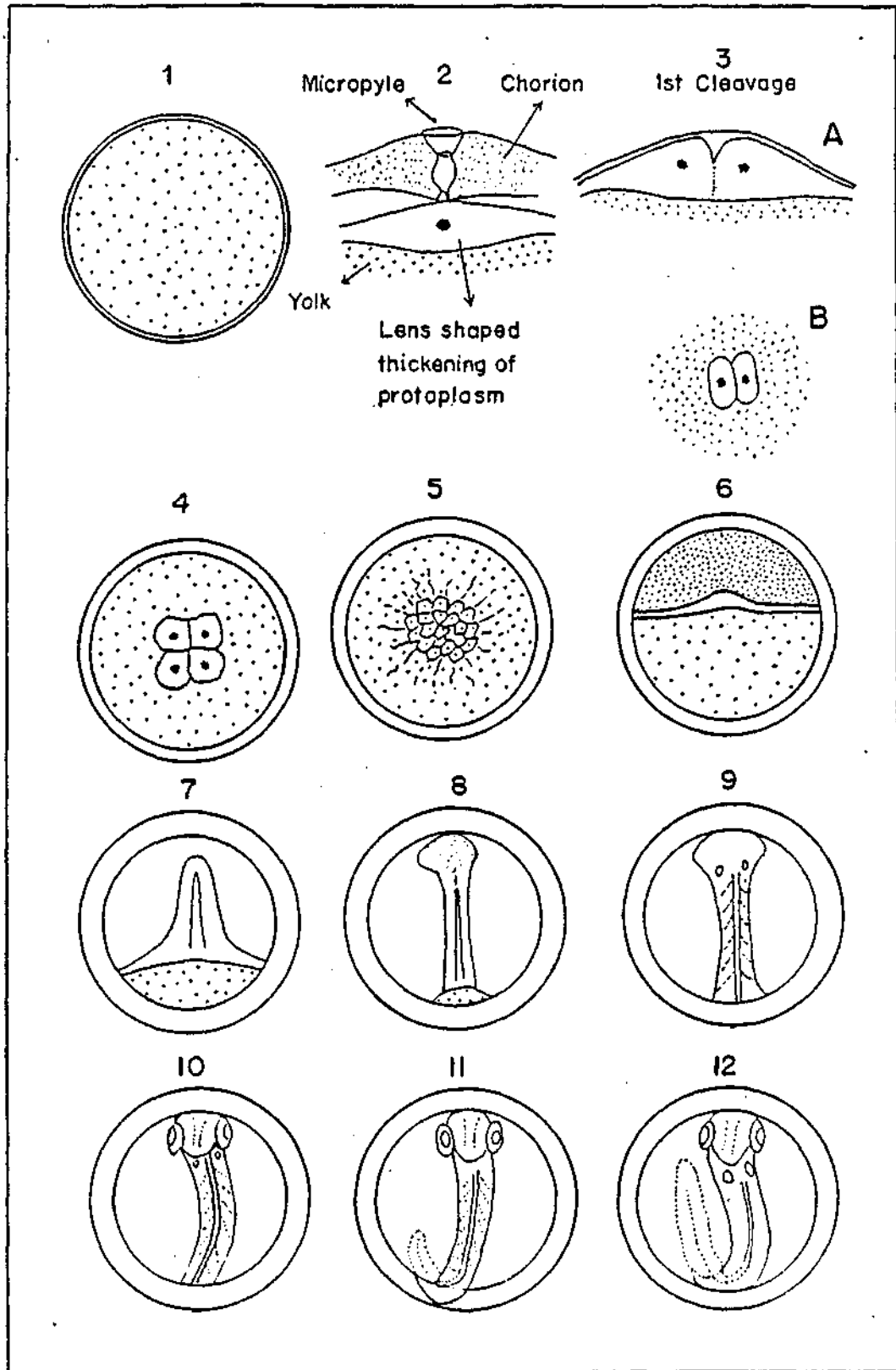


Fig. 4-1

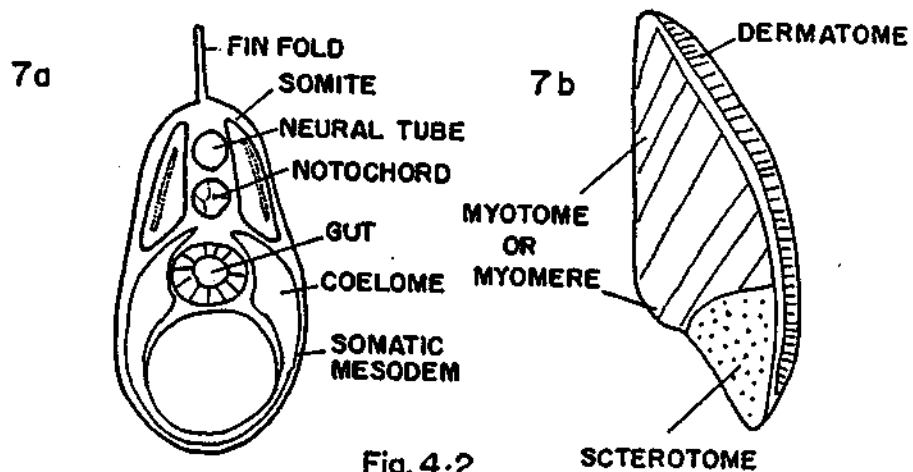
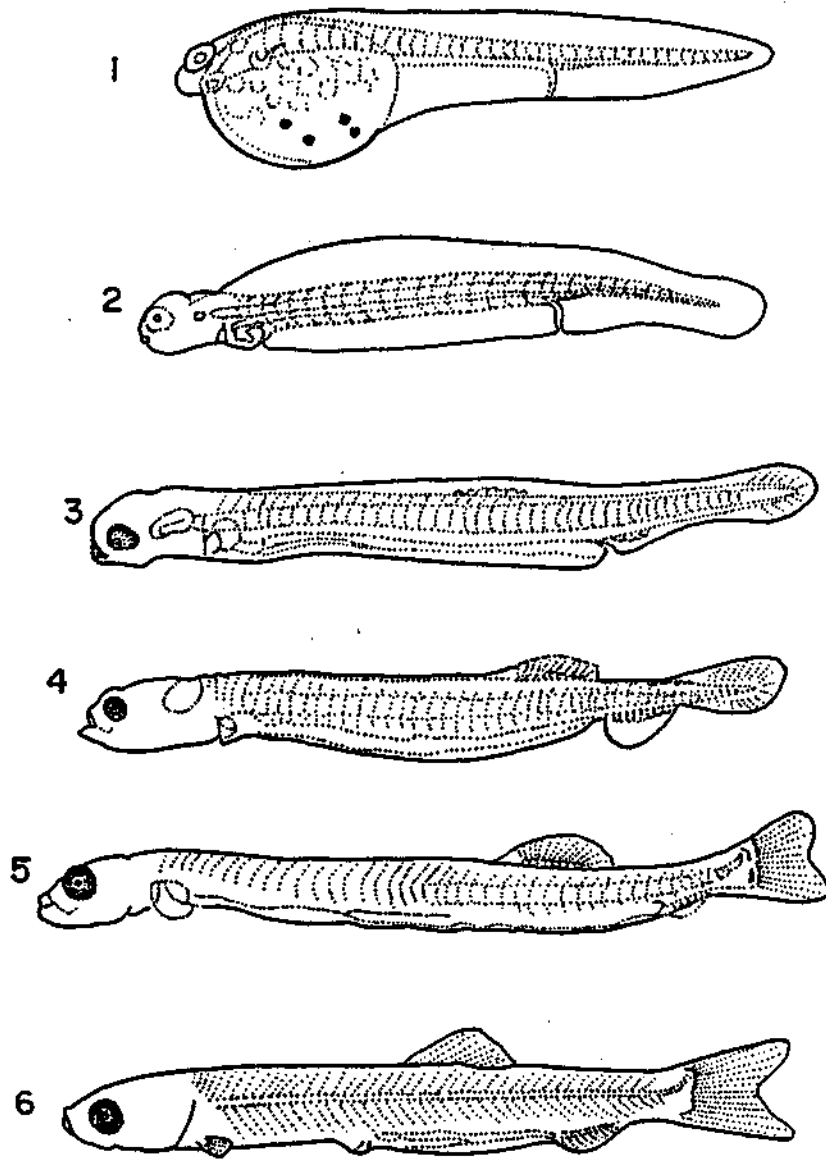


Fig. 4-2

CMFRI/SI/1989/Th.V

IDENTIFICATION OF EGGS AND LARVAE

By

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History of identification of fish eggs and larvae:

Towards the end of the 19th century Marine Biologists, Holt and Scott (1898) M'Intosh and Masterman (1897), Cunningham (1897) and Ehrenbaum (1905-1909) succeeded in describing the eggs and larvae of a large number of marine teleostean fishes in European waters. The Danish scientists, Schmidt (1904-1918) and Petersen (1892-1919) described several postlarval stages of demersal fishes. These efforts were supplemented by Clark (1920), Ford (1920-1931) and Lebour (1919-1927) at Plymouth. The prolific contributions of Japanese workers as well as those from USSR and Germany have not been freely accessible to Indian workers either due to language problems or for other reasons. However the contributions of Mito (1960) Ueyanagi (1959-63), Matsumoto (1958, 1959) Nakamura (1951, 1956) etc. are now well known to specialists working in the field. Likewise, the works of the Russian author Gorbunova (1963-1967) are also fairly well documented in English language.

The results of earlier workers showed that all marine food fishes except the herring and the capelin have pelagic eggs. The sand eels (Ammodytes) too are found to have demersal eggs.

Eggs and larval studies of marine fishes in Indian waters:

Studies on the natural history of marine fishes in India were pioneered by the erstwhile Madras Presidency

Fisheries Department (Hornell, 1910, 1922, Nayudu, 1922, Hornell and Nayudu, 1924). Several contributions to the knowledge of eggs and larvae of different commercial fishes were also made by the above Department in later years (John, 1939, Devanesan and John, 1940, 1941, Devanesan and Varadarajan 1942, Devanesan, 1943, Devanesan and Chacko, 1944, Chidambaram, 1943, Chidambaram and Venkataraman, 1946, Jacob, 1949, Chacko, 1950, 1954, Chacko and Mathew, 1955, Chacko and Gnanamekhalai, 1963).

Maritime Universities like Madras, Bombay and Travancore (erstwhile) also contributed to the studies on fish eggs and larval taxonomy (Aiyar 1935, Jones, 1937, Panikkar and Aiyar 1939, Panikkar and Nair, 1945, Nair, 1952, John, 1951, Vijayaraghavan, 1957, 1959, Kuthalingam, 1957, 1958, 1959, 1960, 1961, Bal and Pradhan, 1945, 1946, 1947, 1951, Menon, 1945 and Gopinath, 1942, 1946, 1950).

Recent studies at the Department of Marine Science, University of Kerala, Cochin have resulted in a number of contributions on the fish eggs and larvae of the southwest coast of India (Balakrishnan, 1959, 1961, 1963, 1969, 1971, Balakrishnan and Devi, 1974, Dileep, 1977, Premalatha, 1977, Sreekumari, 1977). Likewise, Andhra and Annamalai Universities also took up similar studies on the east coast (Ganapathi and Raju, 1961, 1963, Ganapathi and Rao, 1962, Dutt 1966, Rao, 1963, Raju and Ganapathi, 1949, Balasubramanyan, 1973, Balasubramanyan et al. 1969, Venkataramanujam and Ramamurthi 1974, 1977).

With the establishment of the Central Marine Fisheries and the Central Inland Fisheries Research Institute in 1947, studies on eggs and larvae were taken up as a regular programmes resulting in a number of publications (Jones and Menon 1950, 1951, 1952, 1953, Pantalu and Jones, 1951, Jones and Pantalu, 1958, Jones, 1958-1967, Jones and Kumaran, 1963, 1964, Sarojini and Malhotra, 1952, Karamchandani and Motwani, 1952, Balakrishnan,

1957, Kuthalingam, 1960. Bapat and Prasad, 1952, Bapat, 1955, Nair, 1948, 1952, 1959, 1961, Nair and Mohamed 1961, Rao, 1964, Subrahmanyam 1964, 1968, Chandra, 1964, James, 1967, Kotwal, 1967, Balakrishnan and Rao, 1971, Bensam, 1968, 1969, 1971, 1973, Gupta, 1972, Achari and Vincent, 1972, Vijayaraghavan, 1973, Girijavallabhan and Gnanamuthu, 1974, Silas and George 1971, Silas, 1974). Eggs, larvae and juveniles of several species like Sardinella longiceps, S. fimbriata, S. gibbosa, Kowala coval, Rastrelliger kanagurta, Scomberomorus spp., Auxis sp., Katsuwonus pelamis, Thunnus albacares, Euthynnus affinis, Xiphias gladius, Caranx kalla, Gempylus serpens, Stolephorus spp., Myripristis murdjan, Holocentrus sp., Dactyloptena orientalis and the eels have been the subject of studies by the above authors. While these efforts have been localised and generally taxonomy oriented they have resulted in a number of contributions on the life-history and spawning of several commercial species.

Delsman (1922-'38) in his series of publications on the fish eggs and larvae from the Java sea, contributed a wealth of information on the taxonomy of eggs and larvae of a number of species relevant also to the Indian region. A concerted effort to collect marine ichthyoplankton from a wider area, particularly off the west coast of India using ocean going research vessels was made since late fifties by the Central Marine Fisheries Research Institute, in collaboration with the erstwhile Indo-Norwegian Project using the research vessels 'Kalava' and 'Varuna' (Jones 1967). This has led to the collection of several eggs and larval samples from the shelf waters of the SW coast

For full references cited in page 2 & 3 also refer "An annotated bibliography on the breeding habits and development of fishes of Indian region" Bull.No.3 CMFRI (Jones & Bensam, 1968).

and the Laccadives archipelago. These materials have been used by and large for qualitative studies and also to indicate the spawning grounds, mainly of tunas, (Jones, 1958-1967). Jones and Kumaran (1963, 1964 a) based on Dana Expedition (1928-30) material from the Indian ocean gave an account of the distribution of tuna and billfish larvae in the area. Available information on eggs, larvae and juveniles of Indian scombroid fishes have also been compiled by these authors (Jones and Kumaran, 1964).

Identification of fish eggs:

Identification of fish eggs or larvae will be easier if the parents of the spawn products are already known. However in most cases this is not the case and we have to deal with planktonic material. In such a situation the only way is to apply certain salient sets of characters to a series of different stages of growth and connect them to juvenile stage. Comparison and linking wherever possible with past records of stages of the material will also help in arriving at the identities. M'Intosh and Masterman (1897) Hock and Ehrenbaum (1911), Simpson (1956) gave identifying clues for several fish eggs. Hiemstra (1962) developed a correlation table for identifying pelagic eggs.

A review on the early life histories of Clupeiformes from Indian waters with provisional keys for identifying the eggs and early larvae has been made by Bensam (1971). The important characters generally used in identifying fish eggs are:

1. The shape of the egg.
2. Size (diameter)
3. Nature of egg membrane - smooth, sculptured etc.
4. Extent of perivitelline space.
5. Presence or absence of oil globules.
6. Size of oil globule.
7. Homogenous or segmented yolk.

In later stages of development of the embryo the following characters are useful.

1. Presence or absence of pigmentation on yolk sac or oil globule.
2. Pigmentation pattern of the embryo.
3. Degree of pigmentation of the eyes.

Types of fish eggs and certain examples

Most fish eggs are spherical in shape.

Oval or pear shaped eggs	: <u>Stolephorus</u> , Gobies, Blennies some Pomaenitrids, <u>Ammodytes</u>
Demersal eggs	: Herring (<u>Clupea harengus</u>) <u>Jenkinsia</u> , Capelin (<u>Mallotus villosus</u>)
Ornamented/spiny/egg membrane	: Lizard fishes (<u>Saurida</u> , <u>Saurus</u>) <u>Chirocentrus</u> , Macruridae, <u>Apogon</u>
Double egg membrane (outer gelatinous coat)	: <u>Pellona</u> , <u>Ilisha</u> , <u>Hilsa</u> , <u>Sardinella albella</u> , <u>Fistularia</u> , <u>Exocoetus</u> , <u>Vinciguerria lucetia</u>
Filamented egg membrane	: Atherinidae, <u>Hemiramphus</u> , <u>Cypselurus</u> ,
Eggs in cluster	: <u>Triacanthus</u>
Spawn mass	: <u>Lophius</u> , cottidae
Wide perivitelline space	: <u>Sardinella</u> spp.
Segmented yolk	: Clupeids, carangids coarse segmented apodes (eels), <u>Vinciguerria lucetia</u> (irregularly segmented).
Stalked nature of yolk in embryo	: Ophichthid eels

- No oil globule : Sardinella sirm., Stolephorus zollingeri; Opisthopterus tardoore, Chanos chanos, Muraenid eels - Most Pleuronectid flat fishes
- Many oil globules : Setipinna, kowala, Anodontostoma, Cynoglossus, Pellona, Chirocentrus, Atherinidae, Siganus, Triglidae
- Oil globule of considerable size : Trichirus (0.65 mm)
- Oil globule in yolk at anterior part : Caranx, Mullidae
- Pigmented embryo : Gadidae, Barracuda, Mulletts
- Pigment on oil globule : Caranx, Trichiurus (not conspicuous).

Size of eggs:

Size of eggs are stated as diameter or as length of the longest axis when nonspherical. Most marine fish eggs are 0.5 mm above in diameter.

Range of diameter of egg

Some examples

0.5 - 1.0 mm

- : Caranx
Cynoglossus
Kowala
Anodontostoma
Vinciguerrria
Opisthopterus
Platycephalus
Dorosoma
Mackerel

1.0 - 1.5 mm	<u>Saurida</u>
	<u>Sardinella longiceps</u>
	<u>S. fimbriata</u>
	<u>Coilia</u>
	<u>Auxis</u>
	<u>Thrissocles</u>
1.0 - 1.5 mm	<u>Setipinna</u>
	<u>Chanos chanos</u>
	<u>Scomberomorus</u>
1.5 - 2mm	<u>Chirocentrus</u>
	<u>Fistularia</u>
	<u>Sardinella leiogaster</u>
2 mm	Eel, <u>Alosa</u> , <u>Trichiurus</u>

The hatching method for identification of fish larvae

The identity of free planktonic eggs at group, family or generic level may be possible in some cases from published information. Further, development of the fertilised eggs after hatching will throw more light on the egg as well as the larvae to the closer semblance of the material to the actual adult.

These observations are possible by the hatching and rearing method for the eggs in the laboratory. For successful accomplishment of this process a closed circulating water system is the primary need. It is advisable to have some sort of automatic or semiautomatic circulating system where self filtering and waste eliminating and oxygenating systems are also incorporated.

The physical, chemical and biological parameters of the circulating water system are to be monitored regularly so that all these are kept within the tolerance limit of the organisms reared. The temperature, salinity, pH and live food population introduced if any, bacterial protozoon or fungal contamination etc. are to be monitored systematically in the rearing system.

Apart from hatching eggs, already fertilised in nature and collected from the plankton, it may be possible in some cases to artificially fertilise ripe eggs in the laboratory introducing milt from ripe male of the species. If successful fertilisation takes place all such fertilised eggs can be removed to the rearing system for further development.

It is well known that in the development of fish eggs, the yolk serves as a reservoir of food from which the yolk sac larva takes its nutrition. Once development goes beyond this stage and the larva develops the mouth and mobility they are to be fed with appropriate food items preferably unicellular planktonic organisms reared for the purpose or collected from nature. The supply and in take of food of suitable quality and particle size are critical for the survival and growth of the larvae.

All debris and dead organic matter are to be removed from the system as soon as they are found by siphoning them off carefully.

If successful progressive development happen it is required to collect and fix the larvae at suitable intervals of time 6,12,24,48,72 hours etc. and upto as many days till we are able to get juvenile stage or cross to it. Detailed examination of these series of material will enable linking the earliest stage to the latest and the adult.

The series method:

The series method of study of ichthyoplankton for identification is applied partly in the hatching method as well. However the method is more appropriately employed in case of material collected from plankton where their origin is not at all clear. In this case, as it usually happens a collection of ichthyoplankton from a station may contain eggs or larvae or both of a species in various

stages of development or such closely resembling material may be available from different collections. The observer has to sort out and assign the material to a series in sequences of size and development. Since the progressive developmental process is likely to leave a trace of the immediately previous stage and the latest stage may show close semblance to juvenile characteristics especially in meristic and partly morphological features of the adult, the built up series will enable confirmation of the identity of the material. In many cases, study of the series of larval stages may require staining for clarifying osteological features, fin counts etc.

Identification of larvae:

The larval stage includes that stage prior to the acquiring of juvenile characters; the transition stage when juvenile characters are acquired. The juvenile stage is defined as the stage in which all the fin elements are present. In some cases there is the specialised juvenile stage which is found only in a few groups of fishes. The standard larval terminologies used are as follows:

- Embryo - Developmental stages to the moment of hatching
Larva - Developmental stages well differentiated from the juvenile and intervening between the moments of hatching and transformation; commonly divided into Prolarva and post larva.

Prolarva Still bearing yolk.

Post larva Larva following the absorption of yolk applied only when the structure continue to be strikingly unlike that of juvenile.

Alevin Larva of species in which post larval stages are not recognised i.e. in which the yolk bearing larva transforms directly into the juvenile.

Juvenile

Young essentially similar to adult.

When larvae from a specific area is studied, basic information on the endemic and migrant adult species occurring in the area is to be known. It is also important to have clear knowledge of the meristic (countable) characters of the adult fishes.

At least four major characters are to be taken into account for identification of fish larvae. They are:

1. Morphometrics: Measurements of body parts over a size range of specimens from larva to early juvenile stage. Changes in body proportions such as in body depth, head size, gut length, shape of viscera; fin positions including size at end of yolk sac stage and size at transformation stages.
2. Meristics: Countable structures such as myotomes or vertebrae, number of fin rays etc.
3. Pigment patterns and their changes during early stages. Melanophores are somewhat variable on larvae of the same size; may be expanded or contracted at the time of preservation and can be destroyed by exposure to light or through improper preservation.
4. Specialised larval characters such as spines on opercular bones or head; shape of eyes (subcircular, stalked etc); elongated dorsal/ventral rays or spines, extended snout etc.

The very shape of the larvae itself broadly distinguishes the major groups from each other for eg. the elongate clupeids from the broad and laterally compressed scombroids, carangids and several perches. As examples it may be instructive to look at in detail the larvae of some of the important commercial fishes and their identification.

1. Family Clupeidae

The clupeids and engraulids of this family constitute a large fishery resource in our country as elsewhere in the world.

Two typical genera among them are the Sardinella and Stolephorus.

Both clupeids and engraulids have elongate, rod shaped larvae with long guts. The gut length of most clupeid, is greater than 80% of their notochord length (NL), while that of engraulids it is less than 75% NL. Engraulid larvae have slightly greater body depth than clupeids and they are less laterally compressed than clupeids. Median fin development begins at less than 6 mm NL in engraulids, but does not begin until 7 mm NL in the clupeids.

Engraulid larvae have pigment on the ventral midline posterior to the anus, at sizes less than 6 mm NL, but no clupeids develop pigment until much larger sizes. Engraulids have fewer melanophores in the foregut series at any given size, than do clupeid larvae of the same size. No other larvae are likely to be confused with the above two groups except perhaps certain gonostomatids like Vinciguerria sp. However the subcircular eyes, pigmentation pattern and development of photophores in later larvae enable distinguishing them from the clupeids and engraulids. The early larvae of clupeids generally show typical crossed muscle fibres on the body.

Identifying clupeid genera:

Meristics are the most useful characters in distinguishing the genera, especially the myotomes which closely tally with the vertebral counts of the adults. As examples we can cite Sardinella (45-47) Stolephorus (39-32), Mackerel (31), Tunas (39-42) etc.

Morphometrics are not very reliable to distinguish genera, except during the transforming stages, when measurement such as predorsal, prepelvic and preanal length may be useful in some cases.

Pigmentation associated with the caudal area in larvae less than about 8 mm NL may distinguish some genera if used along with myotome counts. Staining of smaller specimens before ossification is complete, may make it easier to count myotomes and the developing fin rays.

The Family scombridae:

The family includes the mackerel, tunas, frigate mackerel, bonitos, seer fishes bill fishes etc. About two dozen species of the family are recorded from Indian waters.

The salient features of the scombroid larvae is their short truncated shape, large head and presence of strong opercular spines (tunas) and pterotic spines (bill fishes; exception mackerel).

Mackerel larva:

The salient diagnostic characters of mackerel larvae can be summarised as follows:

- Characteristics short bodied larva with about 31 myotomes
- Anus placed well forward
- Larval fin-fold begins at the occiput
- Lack pre-opercular spines (unlike the larvae of tunas)
- A post vent row of melanophores along the ventral margin of the body, reaching upto the urostyle.

Tuna larvae:

Matsumoto (1958) has illustrated a typical tuna larva and has given its general features. Tuna larvae are characterised by a large head, with opercular spines,

a triangular visceral mass located well forward in the body, the pre-anal distance being less than half of total body length in specimens upto about 9 mm, myotome number between 38-42, pigmentation (melanophores/chromatophores) rather sparse, most of it concentrated over abdominal sac, over the brain and in the caudal region, larvae about 10 mm (S.L) lose most of the pigment characters.

The main characters relied upon for the identification of tuna larvae are some fairly consistent black pigmentation such as those over the forebrain, tip of jaws and posterior half of the trunk. Meristic characters such as numbers of the myotomes, vertical fin rays and morphometrics of the head and eye and sizes at which structures differentiate are also found useful.

A key for identification of larval tunas based on Chromatophores distribution is given below: **

- I. Chromatophores present on trunk
 1. Chromatophores present over fore-brain
 - 1.1 A distinct chromatophore mid-ventrally in the caudal region - no chromatophore at the symphysis of the pectoral girdle - Katsuwonus pelamis
 - 1.2 A series of chromatophores along ventral margin on the trunk, from base of anal fin to caudal region - chromatophore at symphysis of the petoral girdle. Series of chromatophores along mandible - Euthynnus affinis
 2. No chromatophores over fore-brain
 - 2.1 Three short series of chromatophores on the mid-dorsal, mid-lateral and mid-ventral lines of the caudal region - chromatophore at the symphysis of the pectoral girdle - Auxis sp.

** Adapted from Yabe, Yabuta, Ueyanagi, 1963; Matsumota, 1958, 1962 - referable to adults recorded from Indian waters.

2.2 1-3 chromatophores along the dorsal margin on the trunk, initial one being anterior to origin of second dorsal. 1-5 chromatophores along ventral margin of the trunk - Thunnus tonggol.

2.3 No chromatophore along the dorsal margin on the trunk. 1-5 chromatophores along ventral margin of the trunk - Thunnus obesus

II. No chromatophores on trunk

1. No chromatophores over forebrain, presence of chromatophores at tip of lower jaw - Thunnus albacares

Bill fish larvae:

Indian bill fishes include the sword fish (Xiphias gladius), the sail fish (Istiophorus gladius) the marlins (Tetrapturus audax and Makaira spp.) and the spear fish (Tetrapturus angustirostris). Young stages with prolonged beaks are found in the bill fishes and wahoo. In the wahoo larva there is neither a spiny supraorbital ridge as in sword fish nor the long pterotic and preopercular spines as in the sailfish.

Family carangidae:

The major genera involved in the commercial fishery in India are the Horse mackerel (Megalaspis spp.), scads (Decapterus spp.), and a variety of small and large species.

Among the carangid larvae two different types are distinguished on the basis of morphology. The elongate and the deep bodied.

Among the meristic characters the vertebral formula (consequently the myotomes in the larvae) is quite stable (10 + 14) in numbers except in few species like Nucrates ductor or Seriola sp.

Armature of the head is another important character distinguishing the larvae of some of the genera from each other -

Shape and number of spines, the biggest one being of particular interest when serrated or denticulated (Elegatis); suborbital crest - whether single and short (Decapterus, Caranx) double and long (Nucrates), long and serrated (Trachynotus). The sagittal crest - its presence or absence; shape low or high and the position of its denticulations appear to be quite useful for distinguishing some genera and species.

Other larvae looking like Carangid larvae are Leioognathidae, Lactarius, Theraponidae, Apogonidae, and Ambassidae. All have 24 myotomes, Leioognathids have both Occipital crest and opercular spines as in carangids. But in Leioognathids the supraoccipital and preopercular spines are serrated with a curved spine on supraoccipital. More over anal spines are long and strong and not separate from the rest of the fin as in carangids. Dorsal spines are more than VIII and the soft rays are less than 18, anal spines are III and rays 14-15 at the maximum. In the case of Lactarius, the proportion of the anal fin counts are higher than that of dorsal fin, whereas carangids in general have more dorsal fin rays than anal rays.

In some post larvae like those of Therapon the opercle is provided with about 6 spines, of which the dorsal one is the strongest and the longest. In carangids the long and strong spine on preopercle is at the corner i.e. between the horizontal and vertical edges. The early stages of theraponids also differ from that of carangids in their pigmentation pattern. Apogonids and Ambassids are easily identified by their transparent and less pigmented nature of the body.

The Leptocephali

The Leptocephali larvae are deeply compressed, with well developed larval teeth, eyes and nasal organs. The

myomeres are superficial. The interior of the larvae is filled with noncellular nucoïd substance. The common sizes met with are 50-100 mm TL, the largest known larvae is 1800 mm TL.

The larvae reportedly do not eat solid food but may utilise dissolved organic substances or bacteria. The function of the teeth is yet unknown.

Different types of Leptocephali:

1. Elopiformes:

has large forked caudal fin except in very small larvae. Myomeres less than 100, has ventral fin. Dorsal and anal fin have short bases.

2. Anguilliformes:

Small rounded caudal fin. Myomeres almost always more than 100. Ventral fins are absent. Dorsal and anal fin have long base and are confluent with caudal.

3. Notacanthiformes:

Caudal fin absent, instead they have a long single filament. Several hundreds of myomeres present has very small ventral fin. Dorsal fin short on anterior part of the body. Anal fin present.

Family Myctophidae and Gonostomatidae:

The larvae of lantern fishes and light fishes are relatively very abundant in the Indian seas, and those of the gonostomatids (eg. Vinciguerria) can be confused with clupeoid larvae by the inexperienced observers. The elongate larvae with subcircular or stalked eyes differentiate them clearly from the clupeids.

The formation of photophores, especially the second branchiostegal photophore and the sequence of formation can be used to distinguish some genera -

The pigments, size of pectorals, presence of preopercular spines and development of snout are other important larval characters of myctophids. Useful meristic characters include vertebral, branchiostegal, dorsal, anal and ventral fin count. The gonostomid like Vinciguerria the light organs are formed at the same time and the pattern of photophores also differ.

Salient diagnostic features applicable to different groups of fish larvae

<u>Short oval body</u>	Monacanthidae, Balistidae, Antennaridae
<u>Short depressed body</u>	Platycephalidae, Pagasidae, Dactylopteridae
<u>Crest on nape</u>	Holocentridae, Carangidae, Leiognathidae, Coryphaenidae, Scorpaenidae, Platycephalidae.
<u>Barbel on lower jaw</u>	Exocoetidae
<u>Elongated tentacle on operculum</u>	Champsodontidae.
<u>Bony ridge over eyes</u>	Carangidae, Stromateidae, Holocentridae, Histiophoridae, Scorpaenidae.
<u>Protruded snout</u>	Holocentridae, Histiophoridae, Pagasidae, Exocoetidae, Hemiramphidae
<u>Pelvic fins abdominal</u>	Isospondyli, Iniomi, Scomberosox. They are soft rayed fishes lacking spines in the dorsal, anal and pelvic fin.
<u>Single short dorsal fin</u>	Gonostomatidae, Clupeidae, Engraulidae, Dussumieridae.

<u>Single long dorsal fin</u>	: Bregmacerotidae, Serranidae, Carangidae, Coryphaenidae, Leiognathidae, Histiophoridae, Stromateidae, Bothidae, Pleuronectidae, Soleidae, Cynoglossidae.
<u>Two dorsal fins</u>	Mugilidae, Apogonidae, Mullidae Gobiidae,
<u>Pectorals enlarged</u>	Exocoetidae, Stromateidae, Callyonymidae, Platycephalidae, Champsodontidae.
<u>Ventral fins absent</u>	Anguilliformes, Syngnathidae, Tetradontidae.
<u>Elongated fin rays on dorsal</u>	Bothidae, Soleidae, Cynoglossidae, Bregmacerotidae.
<u>No spines on operculum</u>	Labridae, Gobiidae, Trachypteridae Spines on operculum - Scombridae, Carangidae.
<u>Elongated spines on the dorsal and ventrals</u>	Serranidae, Ballistidae, Acanthuridae
<u>Alimentary canal</u>	
<u>Long straight alimentary canal</u>	Many Gonostomatidae, Clupeidae, Synodontids. Bulged or sac like - Cynoglossi- dae, Soleidae.
<u>Short and coiled</u>	Majority of Perciformes.
<u>Anal opening</u>	At middle of body - Apogonidae, Carangidae, Thunnidae, Scombridae, Gobiidae, Scorpaenidae, Pleuronectidae, Bothidae.
<u>Behind middle of body</u>	Apodes, Hemirhamphidae, Exocoetidae, Fistularidae, Mugilidae, Sphyraenidae, Coryphaenidae.

Far backwards

Stomiatoidea, Clupeidae, Synodontidae.

Far forwards

Bregmacerotidae, Atherinidae,
Blennidae, Trypauchenidae.

Pigmentation

Dense

Exocoetidae, Hemirhamphidae,
Holocentridae, Mugilidae,
Coryphaenidae, Histiophoridae

Partial

Atherinidae, Bregmacerotidae,
Mullidae, Apogonidae, Stromateidae,
Theraponidae, Platycephalidae

Blotches, spots

Engraulidae, clupeidae, Synodontidae,
Carangidae, Apogonidae, Serranidae,
Leiognathidae, Thunnidae,
Scomberomoridae, Pleuronectidae,
Cynoglossidae.

Myctomes/Vertebrae

Less than 24

Callionomidae, Balistidae,
Monacanthidae, Diodontidae,
Tetradontidae, Molidae.

30-40

Gonostomidae, Engraulidae,
Myctophidae, Coryphaenidae, Labridae,
Scombridae, Thunnidae, Sillaginidae.

41-50

Clupeidae, Engraulidae, Chirocentridae,
Myctophidae, Scomberomoridae,
Bregmacerotidae

51-80

Elopidae, Albulidae, Megalopidae,
Chirocentridae, Beloniformes,
Syngnathidae.

100-200

Anguilliformes, Trichiuridae
Gempylidae.

Eye stalks

Asteronethidae, Bathylagidae,
Myctophidae, Idiacanthus

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6.1 COLLECTION OF MATERIAL

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6.1.1 History of plankton sampling:

Plankton sampling by nets started a little more than 150 years ago and it is therefore very much in its infancy compared to fishing operations. In 1828, a surgeon, Dr. J. Vaughan Thompson made a small net to sample crab and barnacle larvae. Darwin used a small net on the Beagle and in 1844 Muller used a small meshed conical net to catch a host of minute creatures. This simple tool was the foundation for our knowledge of plankton especially to the taxonomists.

Plankton was soon realised to be more than a systematist's concern, and because it had such significance in the productivity of the sea, the food chain and in the identification of water masses, planktologists wanted to know how to relate the number of organisms found in the volume of water filtered, their distribution in depth, space and time and their daily, seasonal and annual variations.

Ichthyoplankton research has played an important role in marine science and its application to fisheries since the end of the last century. It has contributed not only to the clarification of basic problems of fish taxonomy, ecology, zoogeography and life history but also to the exploitation of fishery resources, a better understanding of the fish behaviour and the study of fish population dynamics. The fishery oriented targets of ichthyoplankton surveys include exploration for potential

fishery resources, location of spawning concentrations of fish stocks, monitoring of long term changes in the composition and abundance of resources and in spawning times and areas (Hempel, 1973). The study can be much more effective in tracing of fluctuations in spawning stocks by estimating the abundance of their eggs and young larvae, forecasting year class strength on the basis of the abundance of older larvae, estimating abundance of a stock based on its spawning population, and discriminating between stocks of the same species. In short ichthyoplankton surveys can be an additional method of estimating fish stock abundance complimentary to other methods of resource evaluation.

The need for so much comprehensive quantitative knowledge brought with it a number of sampling problems such as the sampler, its mesh size and material, the volume of water filtered, closing devices, measurement of the depth of sampling, speed of tow, avoidance and escapement. This was more so when it became necessary to make taxa specific sampling as in the case of ichthyoplankton.

The early life history stages of fishes are restricted, by depth, usually to the upper mixed layers. The passive eggs and feebly swimming larvae are quite vulnerable to capture. Many marine fishes have pelagic eggs and most have pelagic larvae. Thus it is easy to quantitatively sample several species over broad areas with a simple plankton net.

6.1.2 Collection of ichthyoplankton

6.1.2.1 Collection with net

The most widely used apparatus for collecting plankton in general is a plankton net. Typically a plankton net consists of a cone of bolting silk or equivalent material mounted on a ring to which are

attached three thin bridles spliced on to a smaller ring by means of which the net can be shackled to a towing rope. Modern nets of all patterns differ from this simple plan by having the first part of the net, i.e., the part attached to the ring, of thin canvass, thus making the net stronger. Also the end of the cone is left open and is reinforced by strong material and a small container - the plankton bucket - is attached to this end. This bucket receives most of the plankton as the net is towed along. (Fig. 6.1.1).

6.1.2.2 Hensen egg and larval net

This net developed by Hensen (1895) is used for collecting mostly macroplankton particularly fish eggs and larvae. The frame of the net consists of two metal rings connected vertically by four metal bars. The outer ring has a diameter of 75 cm and the lower ring has a diameter of 100 cm. The filtering portion of the net is 130 cm long and it is attached to an upper canvass portion, 55 cm in length. The opening at the head is reduced to increase the ratio of filtering area of the net to its mouth area and at the same time by means of the canvass head piece the back wash is minimised while the net is towed. There is a collecting bucket attached to the lower end of the net through a canvass piece of 10 cm long. At the top there are three bridles attached to the upper ring which are spliced to form an eye to which is shackled the towing line. From the lower ring three thin ropes are attached to the bucket which protects the netting from strain while hauled up. To maintain the vertical position of the net while hauling a sinker is attached to the bucket. (Fig. 6.1.2.).

6.1.2.3 Juday net

Juday (1916) made a net in which the non-filtering cone was of the same size and tapered as the net itself. (Fig. 6.1.3).

6.1.2.4 Indian Ocean Standard Net

This is another vertically operated net designed by Currie (1963) for use during the International Indian Ocean Expedition. Since the basic plan of constructing the net is the same as that of a typical net, the material used and the various dimensions are given below (Fig.6.1.4).

Section	Diam (cm)	Length	Material	Mesh (mm)
A	113	70	Nylon netting	12.5
B	113	30	Terrylene sail cloth	-
C	113	100	Nylon	0.33
D	113-10 (Tapering)	300	Nylon	0.33

6.1.2.5. Closing net

Samples are taken vertically to give a measure of the plankton under one square metre surface. When it is required to sample a particular strata of the water column, the closing nets are used. Nansen has developed a simple type of net for this purpose. The net can be used for vertical hauls in which case it takes the form of step-wise catches from one depth to the next, in order to determine the vertical distribution of the plankton. The net can be closed at any desired depth, by means of a messenger which releases the bridles. (Fig. 6.1.5).

To sample the whole vertical column repeatedly by a single closing net is time consuming. This led to the development of multidepth sampling equipment (Be, 1962).

6.1.2.6 Bongo net

A high speed plankton sampler which is effectively used for catching fish eggs and larvae is the Bongo net.

(McGowan & Brown, 1966) It is a twin net fixed side by side on an axis or yoke to which the hauling line is attached.

Hanging from the axis in between the two nets is the depressor of prescribed weight. Two types of Bongo nets are usually used; one with 20 cm mouth diameter and the other with 60 cm mouth diameter. In the Bongo net, the turbulence in front of the net caused by the towing bridles is absent thus minimising the net avoidance by fast moving organisms like fish larvae. The Bongo net is used for making oblique hauls while the ship is in motion. The moving ship and the net hauling speed make the hauling faster and therefore the fast moving organisms like fish larvae are also caught in the net along with other zooplankton. (Fig. 6.1.6).

6.1.2.7 Continuous plankton recorder:

The plankton recorder developed by Hardy (1936, 1939) is a unique instrument. In this instrument the net gauze runs through the water in the same way as a film in a camera, filtering the plankton which streams through. The covering gauze then covers the collecting gauze and both together are rolled up into a container which is filled with formalin solution in order to preserve the plankton. The strip of gauze is moved by the action of a propeller, which is driven by purely mechanical means as the result of the movement of the vessel. The rate of transport of the gauze is regulated at the speed which is so small that the advance of 1 cm is equivalent to one knot of the travelling stretch. Despite the small mouth, measuring less than 2 cm² the collecting capacity - even for larger organisms - is very good. The instrument can sustain a travelling speed of 8 to 17 knots per hour. (Fig. 6.1.7).

6.1.3.1 Mesh size and material

Plankton varies considerably in size from microscopic

protozoans and minute larval forms to fast moving fish larvae and therefore a range is needed both in mesh size and in amount of water filtered. The gauze used in plankton nets influences not only the size of the organisms caught but also filtration efficiency, drag, clogging, velocity and condition of the catch.

For effective sampling plankton gauze should have the following properties: (1) the meshes should be square, (2) the mesh aperture should be uniform, (3) the material of the strands should be stiff enough to resist bending or stretching, but flexible enough to allow self cleaning action, (4) the nature of the weave should prevent strands from sliding out of place and should prevent the meshes from distorting diagonally, (5) the porosity should not change when the net is immersed in water, (6) the material of the strand should not abrade easily and (7) it should resist degradation by sunlight and by chemicals used in cleaning.

The plankton gauze are usually made of nylon or silk. General industrial screening gauges, made of stainless steel are used for rigid nets. Metal and most synthetic gauges have monofilament strands. Silk and some nylon gauges have multifilament strands. (Fig. 6.1.8). The mesh size used for collecting fish eggs and larvae is 0.505 mm. For the collection of general zooplankton the net fabric with 0.33 mm is used.

6.1.4.1 Types of haul:

6.1.4.2 Vertical haul:

In a vertical haul the entire water column is filtered through from the bottom to the surface, or only a top part is filtered. The net is lowered to the determined depth from the anchored ship and slowly hauled up. The hauling speed is determined principally by the mesh width of the net (1 m/sec. for a 300 μ net).

a weight is attached to the net bucket.

6.1.4.3 Horizontal haul: The horizontal haul is used to obtain plankton samples from a particular water layer. A weight holds the net while it is being towed in the depth. The depth position of the net can be determined from the wire angle and the length of the cable paid out. Horizontal haul can be done at any water depth from surface to bottom.

6.1.4.4 Oblique haul: It is a combination of the two other types of haul. The net is lowered to a particular depth from a stationary ship. Afterwards the vessel is slowly moved forward and slowly the net would come up to the surface filtering an oblique column of water. The advantage of this method is that the water column is more intensively filtered in this manner than in a vertical haul. In another type of oblique haul, the ship will be in motion while the net is being lowered as well as hauled up.

6.1.5 Volume of water filtered

One of the first essentials in quantitative plankton sampling is to know the volume of water filtered. The simple calculation is based on the length of tow and the area of mouth ($\pi r^2 h$). Clogging of the meshes introduces an error into this calculation. One method to overcome this was to increase the filtering area. An alternative was to reduce the area of the mouth by a nonfiltering cone. In spite of all these modifications, the unknown variable factor - clogging - limited the accuracy of calculation. Here the need of the flow meter becomes significant.

The simplest flow meters indicate the number of revolutions of the impeller blades on a series of dials or on a counter. The TSK flowmeter (Nakai, 1954) has proved to be a highly reliable and sturdy meter of the dial type.

The flowmeter used in recent times is the digital flow meter of the hydrobios type. It is a small device with a propeller at one end. There is a small window on one side where the revolutions of the propeller being transmitted through a series of toothed wheels are indicated in numbers.

The flowmeter is to be calibrated before using. The number of revolutions is to be transformed into the quantity of water filtered by the net in which it is used. For this, the flow meter fitted to the net has to be towed several times in an experimental tank for known distance and calculated for the volume of water filtered.

6.1.6. Some problems confronting plankton collection

The critical problem area in sampling as regards quality is that of net avoidance by larvae. To decrease this there has been a tendency to increase hauling speed. However, the value of the increased catch of larger larvae has to be weighed against two adverse effects of increased speed; increased loss of small eggs and larvae through mesh aperture of the net due to increased filtration pressure at higher speed (the extrusion problem) and poorer condition of the specimens retained. It has been found that for all gears fish eggs and larvae were in the best condition from hauls made at slow vessel speed and damage to specimens became increasingly greater with increase in speed of hauling.

6.1.7 Criteria for fixing and preserving eel larvae

Although many characters are used in identifying leptocephali, those of body shape and pigmentation are most likely to be affected by unsatisfactory preservation. Distortion of body can occur in formaldehyde fixed and preserved material through absorption of liquid into the tissues with resultant swelling and occasionally final splitting of the specimen. Considerable shrinkage in body length can occur in alcohol held material and it

may reach as much as 5%. Accurate body lengths are particularly important in growth studies.

Alcohol and cork combination should never be used because the muscle tissue of leptocephalus readily takes up the colouring matter which dissolves out of the cork. If this happens, important internal structures such as vertical blood vessels to and from the viscera are obscured as well as the deep pigment around the vertebral column and on the spinal cord if the latter is present.

Most preserved leptocephali can be held for microscopic observation between glass slips, but the more rigid bodied metamorphic forms should be preserved flat or extended. The number and disposition of larval teeth is often an important character as in the delicate median fin. Careful handling of leptocephali during preservation and storing is therefore essential. The specimen is satisfactorily preserved for identification if it shows the following characteristics: (a) body undistorted, relatively flexible, but still with a certain amount of rigidity, (b) melanophores and chorioid pigment of the eyes jet black or even very dark brown, (c) myomeres translucent white or colourless, not coloured brown, (d) larval teeth complete and (e) fins undamaged.

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SURVEY OF EGGS AND LARVAE IN SPACE AND TIME

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Ichthyoplankton surveys are carried out mainly to:

1. Obtain basic knowledge on taxonomy, distribution and ecology of this component in the plankton.
2. To use the quantitative data obtained for exploring new fishery resources and to determine the relative abundance and distribution of economically important species.
3. To complement knowledge of fish population dynamics and to use the results to estimate spawning biomass by estimating abundance of eggs and young larvae.
4. Forecasting year class strength on the basis of the abundance of older larvae.

Ichthyoplankton surveys are accepted as one of the useful methods to monitor the adult populations and estimate biomass.

The early life history stages of marine fishes are passive and are usually found in the upper mixed layers. Most eggs and almost all larvae are pelagic and it is easy to sample several species over a wide area with simple plankton nets. The index of larval abundance has been shown to provide fairly reliable estimates of biomass of their adults as in the case of pacific mackerel, sardine and anchovy.

However, there are problems associated with this like taxonomic, technical and statistical nature. Problems involved in taxonomy are mainly due to the limited number of specialists knowledgeable in the identification of

fish eggs and larvae. Technical problems relate to adequate vessel time and staff for collection and processing of samples which may be very large in numbers. Sampling process involving quantification need to be of standard nature and statistical problems are inherent in the sampling process as well as interpretation of data.

Of the early quantitative works in the nature of surveys on the eggs and larvae of marine fishes, the programmes on the Pacific sardine (Sardinops caerulea) initiated in the thirties deserve special mention (Scofield, 1934, Sette and Ahlstrom, 1941, Ahlstrom, 1948, 1952, 1954, 1959). Marak and Colton (1961), Marak et. al. (1962) and Ahlstrom (1971, 1972) reported on several eggs and larval surveys conducted annually, under the auspices of the U.S. Department of Fish and Wild life Service. Boonprakob (1962) published the results of fish eggs survey in the Gulf of Thailand. In Indian waters, Bal and Pradhan (1945, 1946, 1947) and Panikkar and Nair (1945) reported on the results of investigations on egg and larval collections in Bombay and Madras waters.

During the International Indian Ocean Expedition IIOE-1960-'65) collections of Ichthyoplankton from a wide area of the Indian ocean were made by participating ships with the Indian Ocean Standard Net and used for quantitative studies. These have resulted in the publication of some general accounts of fish eggs and larvae and their distribution in the Indian ocean, (Ahlstrom, 1968, Peter, 1969, 1974, 1977), Panikkar and Rao, 1973, Shomura, 1970) as well as Atlas on fish eggs and larvae distribution (Panikkar, 1973 ed).

The IIOE collections have been made on the whole from widely separated stations of the open ocean and shelf water coverage has been rather limited. However,

Nellen (1973) made a detailed study on the kinds and abundance of the fish larvae from the cruise of R.V. Meteor in the Arabian Sea and Persian Gulf during 1964-65, when some sections covering the shelf waters of the SW coast of India were also worked.

Another recent contribution with a quantitative approach, from areas adjacent to Indian coasts is of Alikhan (1972), who studied the distribution and abundance of fish larvae in the Gulf of Aden and off the coast of west Pakistan based on material collected during 1964-'67 period, in 9 different cruises.

Silas (1974) described in detail the different larval stages of Indian mackerel and mapped their distribution and abundance off the SW coast of India and the Laccadives sea, based on material from cruises of R.V. Varuna in 1964.

Sampling system for Ichthyoplankton surveys:

The field operations will have to be conducted from ships of appropriate size, with a hydrographic winch and meter block. An inclinometer to measure the wire angles is also required.

The 'Bongo 60', a twin ring (0.6 m dia) net towed at slow speed (2-3 knots) is recommended as a suitable gear for ichthyoplankton sampling. It is a bridle free net, the towing rope being connected to the yoke joining the two rings of the net. A hydrodynamic depressor (about 20 kg) is suspended beneath the frame.

The towing frame is fitted with two cylindrical conical nets made of monofilament synthetic netting. One net (0.505 mm mesh) can be used as a principal ichthyoplankton sampling net and the other (0.333 mm) may be used for plankton biomass studies or fish egg and larvae escapement and extrusion studies. The use of soft cod ends is recommended as a matter of handling ease and also spillage back into the net is also likely to be

less than with hard plankton bucket. The cod ends are made of the same type of material and mesh size as the net cones. A calibrated flow meter is fixed to the mouth of each cone if the cones are of different mesh sizes or only to one cone if they are of same mesh size.

Plankton tow:

The ship is stopped and the depth at station is recorded. The initial flow meter reading and winch meter reading are to be checked and zeroed. The net is lowered to surface of water and the ship set underway.

The nets are allowed to stream off at surface briefly and lowered without entangling. Enough wire length is released to reach the net to the desired depths. eg. to lower the net to a depth of 210 m with wire angle of 45°, requires that 300 m of wire be let out (wire angle is the deviation from the vertical).

$$\begin{aligned} \text{Length of wire out} \times \cosine 45^\circ &= \text{net depth} \\ 300 \text{ m} \times 0.707 &= 210 \text{ m} \end{aligned}$$

A depth of **tow** graph can be prepared to have instant check on the wire to be paid out for the desired depth. While the ship is underway, the wire is paid out and after reaching the desired depth, retrieved at the same speed. This is called a continuous oblique tow. After taking up the net on board and washing down, the flow meter final reading is recorded.

To be more accurate regarding the time of tow a stop watch can be used to record the sinking time (the time to reach the desired depth) and retrieval time.

Anomalies in flow meter reading can happen due to varying ship speed, clogging of the net etc. While a typical exercise in ichthyoplankton collection for quantitative studies has been detailed above, a survey to cover spawning of any species in a wide area and time requires efforts of several boats.

Efforts on these lines were made during the several surveys conducted by Ahlstrom (1941-1972) in the eastern Pacific for pilchard, and the sardine. In the Japanese waters during the post-war years (1949) co-operative Iwashi resources surveys incorporated eggs and larval surveys in the programme. (Nakai and Hattori, 1949-51). As the spawning grounds of the species were concentrated in the coastal areas, extension of the survey to offshore areas was not necessary as in the case of oil sardine, mackerel and other small pelagics in Indian waters.

Cost of egg and larval surveys:

The cost in terms of vessel time and human effort required for egg and larval surveys is considerable. However any other type of vessel survey like acoustic and fishing for objectives like fish stock size estimate would require vessel time cost, but the survey results can be made available more quickly than in the case of egg and larval surveys, where processing material and data will take relatively more time.

Processing of material and data:

The plankton sample is washed out to bottles of appropriate capacity and labels put with details on Date/ Station No./Ship/Cruise No./Time/Gear/Position/Depth of tow etc. Two labels one inside and one over the bottle are to be used. The sample is normally preserved in formaline of 4-5% strength taking care to add enough laboratory grade formaline required for the volume of the full sample bottle. The preserving liquid may be about 3 times the actual plankton material volume. To get the required strength of 5% solution of formaline in a half litre jar 50 ml of concentrated commercial formalin can be added. 10 ml of sodium borate solution in sea water is added for each litre of preserved sample to counteract acidity.

In tropical conditions airconditioned, temperature controlled storage of material is advised.

For standard ichthyoplankton survey physical oceanographic data at the stations are useful supplementary information. This includes, temperature salinity and dissolved O₂ of the column sampled.

Laboratory procedures:

Volume estimate

Net volume estimate by displacement method for each sample.

- (a) Total volume including all material
- (b) Volume excluding large items, whose individual volume exceeds 5 ml.

Sorting:

It is recommended that total samples be sorted for fish eggs and larvae whenever possible and fractioning may be limited to exceptionally large samples. The 'Folsom splitter' is a standard apparatus for dividing plankton samples into aliquot portions.

Sorting is done usually under a dissecting microscope (x 10 times).

Identification:

Preliminary identification to family level may be possible during sorting by skilled technicians, while final identification needs careful scrutiny by experts. However if a survey is for a particular species only such material need be isolated and studied.

Storing identified material:

It is usual to store identified eggs and larvae in vials and closed with cotton plug and finally stored in a mother bottle containing appropriate strength preservative.

Study of survey data and interpretation:

The basic data for estimation of abundance of eggs and larvae are obtained from systematic sampling at regular time intervals at a number of stations distributed rather evenly through space. Integration of data from different cruises in relation to space and time is needed.

Two assumptions underlie this method of treatment: (1) The distribution of egg concentrations through space and through time are continuous (2) the egg concentration gradients between points in space and time are linear.

The number of eggs/larvae taken from each haul is made comparable with the numbers from other samples by referring all collections to a common basis- the number of eggs under a standard unit area of the sea (say 10 square metres). The number of eggs taken under a standard area may represent an accumulation of eggs from spawning in one day or more as the case may be.

Reliable estimates of annual abundance of larvae are more difficult than for planktonic eggs because of net avoidance and variation in diurnal behaviour of larvae. However some of the difficulties have been overcome by the development of high speed nets and sampling day and night etc.

Methods of estimation of abundance of eggs and larvae:

1. Construction of lines of equal abundance (isometric lines) on charts of distributions and integrating the areas with the contour lines. (Buchanan - Wollaston, 1915, 1923, 1926).
2. Sette (1943) based his estimate of annual abundance of haddock eggs on the average catch of all the cruises.

The UNDP/FAO Pelagic fishery project, Cochin in its Ichthyoplankton survey adopted the first method and identified the areas of abundance of fish eggs and larvae.

However this work was not elaborated to estimate the stock size, but hither to little known information on the spawning time area and regionwise relative abundance of spawn products were gathered for the project area investigated. For calculation of standard indices of abundance, the number of eggs/larvae under one m² surface was computed. This was done by multiplying the original numbers for each station with sampling depth and dividing by the volume of water filtered. Volume of water filtered is computed from the calibrated flowmeter revolutions for each haul.

$$\frac{N \times D}{V}$$

These standardised numbers for each station are plotted for different periods or integrated for the year to get a synoptic picture of the relative densities of the spawn products. Now forecasting the fisheries on the basis of indices of abundance of eggs/larvae involves a series of further estimates giving allowance for mortalities in the progressive development of the spawn products. It is easier to base this on the older larvae, thus eliminating the factor of mortality. So a relative index of abundance of late larvae could possibly be an index of the magnitude of recruitment into the fishery. However, it is a pretty time consuming exercise to arrive at this index before the start of a seasons fishery, especially, if the fishery immediately follows as in the case of the oil sardine or mackerel. Nowadays O - group surveys for young recruits are adopted in temperate waters to forecast fisheries. It would appear that these two methods can supplement each other; the larval abundance index enabling to get an idea of the forthcoming trend of fishery, earlier than the O - group abundance index which can be built up only later. In this connection, it is to be borne in mind that egg and larval surveys applicable to some of the temperate region fisheries are less appropriate in the case of tropical fisheries.

This is due to the reason that in temperate waters the spawning of fishes is relatively restricted in space and time and the hatching and larval development are slow, which enable a full assessment of the abundance of the spawn products. In case of most tropical species eggs hatch out within 24 - 48 hours and the larval development is also much faster. Still as a standard method either by itself or in combination with methods like O - group abundance studies and acoustic surveys, egg and larval surveys are considered to be useful in the forecast of fisheries.

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TERMINOLOGY OF THE EARLY DEVELOPMENTAL STAGES OF
MARINE FISHES

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A perusal of literature shows that over the years various authors have used different terminologies for the various early developmental stages or phases of marine fishes. Some of these authors are: Hubbs (1943), Jones (1950), Ahlstrom and Counts (1955), Ahlstrom (1968 b) and Balon (1971, 1976). From all these papers, four principal phases are recognisable, which are: (a) Egg (b) Larva (c) Postlarva and (d) Juvenile.

(a) Egg: The term egg is applied to the stage from the time of fertilisation to the one at which the embryo hatches out of the chorion (egg capsule). The egg stage is characterised by an exclusively endogenous nutrition from the yolk of the ovum. Balon 1976) has divided the egg stage into three phases, viz., cleavage phase, embryonic phase and eleutheroembryonic phases. It may be noted in this connection that as per the definition given by Balon (1976), the eleutheroembryonic phase commences only with hatching and is not a phase undergone within the egg. Hence, the inclusion of this phase as part of the egg life is highly questionable. On the other hand Ahlstrom and Counts (1955) have divided the egg or embryonic period into three stages, i.e. (i) the early egg, from fertilisation to closure of blastophore, (ii) the middle egg, from the closure of blastophore to the

time when the tail begins to separate and curves laterally from the embryonic axis and (iii) the late egg, from the time the tail is curved away from the embryonic axis to the time of hatching. These terminologies for the egg period are quite canning and hence are adapted for the purpose of the present studies.

- (b) Larva: The term larva is generally used for the post-embryonic period from the time of hatching onwards until the yolk is absorbed, the mouth is formed and the eyes are pigmented, as was defined by early workers on marine fish eggs and larvae. Hubbs (1943), Jones (1950) and a few other later workers have subdivided the larval phase into "prolarva" or "yolk-sac larva" to denote the presence of yolk and the term "larva" to subsequent stages. But, Russell (1976) has pointed out that restricting the use of the term "larva" to the yolk-sac stage is convenient, at the end of which the mouth is formed and the eyes are pigmented. This usage by Russell (1976) is followed for the present purpose. Among the larval stages one distinct stage is the newly hatched larva; and if the larval development is followed every hour, convenient stages can be formulated such as 3 hours, 6 hours, 9 hours old larvae, etc.,

- (c) Postlarva: The stage extending from the absorption of yolk, formation of mouth and pigmentation of eyes is denoted as "postlarva". Over the years different terminologies have been proposed for the various stages during postlarval development of marine fishes, such as protopterygiolarvae, pterygiolarva, prejuvenile, etc. vide Balen (1976). But Russell (1976) draws attention to the fact ever since the termination of the larval period, in the vast majority of marine fishes, the postlarval sequence of development has no sharply demarkated termination. This is because different adult

characters such as the numbers of fin rays or vertebrae are already developed before the fish has lost other larval characteristics such as pigmentation pattern. Russell (1976) further emphasises the fact that in view of the above reason it is impossible to determine a point at which the fish definitely becomes a juvenile. In view of this reason, Russell (1976) states that the term "postlarva" can be applied to the stage from the termination of the larval stage during which there is a sequence of development to juvenile stage. The use of the term "prejuvenile" in a broad sense by certain authors has been criticised by Ahlstrom (1968 b) who points out that it can be used only in a narrower sense to certain strikingly modified or specialised pelagic life history stages possessed by only a few fishes such as Tholichthyes stages of Chaetodontids, Rhynchichthys stages of Holocentrids etc. and can not be applied to the early developmental stages of marine teleosts.

Within the postlarval phase of development, three principal stages are reckoned by Moser and Ahlstrom (1970), Ahlstrom et al (1976) and Moser et al (1977). These stages are associated with the development of the caudal fin and its supporting elements, before, during and after the upward flexing of the posterior tip of the notochord, which are termed as (i) Preflexion, (ii) Flexion and (iii) Post flexion stages in the postlarval development. Of these three stages the postflexion stage is of longest duration, leading to juvenile stage.

- (d) Juvenile: As per the considerations observed in the previous section, during postlarval development one or the other early developmental characters still persist while some characters resembling those of the adults have formed. In other words, during

juvenile phase of development also the specimens may differ from adults. For instance although in general body form and in meristic characters the developing stage may resemble the adults, in details of morphometric proportions and pigmentation (colouration) it may show a marked difference. Hence, juvenile phase of development in vast majority of marine teleosts also may not have a sharply marked termination at an early size or age. But, developmental processes of certain characters may be delayed till the specimen becomes older and reaches a fairly large size. In view of this reason, the term juvenile for most teleosts may be said to be defined as the stage at which the specimen has developed all the vital meristic characters and general morphometric and pigmentation pattern.

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CMFRI/SI/1989/Th.VIII

MEASUREMENTS AND DRAWING OF THE EGGS AND LARVAE OF
MARINE FISHES

By

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Measurements

In any description and identification of marine fish eggs and larvae, measurements and drawings are vitally important because if only improper measurements and drawings of eggs, larvae, etc. are made by any worker, it is likely to confuse the other workers and result in a doubt on the identity of the material reported and/or described. In view of this reason, exact and accurate measurements of the eggs, larvae, postlarvae etc., under study is an important and vital prerequisite in any study on fish eggs and larvae. The various measurements which are required for documentation are as follows:

- (a) Egg (Fig.8,1)
- (i) Total egg diameter in the case of spherical eggs or length and maximum width of the egg if the eggs are elliptical (eg. Stolephorus).
 - (ii) Diameter of the yolk in the case of spherical eggs or length and maximum width of the yolk if the eggs are elliptical (Stolephorus).
 - (iii) Width of the perivitelline space. If the perivitelline space is not of the same width all along, the measurement at the two poles of the egg as well as the middle region has to be recorded.

- (iv) Diameter(s) of the oilglobule if one is present and of all the oilglobules if more than one is present.

(b) Larva (Fig.8,2):

- (i) Total length, from tip of head to end of caudal finfold.
- (ii) Notochord length, from tip of head to end of the notochord.
- (iii) Head length, from tip of head to the hind margin of auditory vesicles.
- (iv) Preanal length, from tip of head to the anus (or vent).
- (v) Postanal length, from vent to the end of caudal finfold.
- (vi) Head width, dorso-ventral width at the widest part of head.
- (vii) Width of trunk, dorso-ventral width at the middle and widest axis of the yolksac.
- (viii) Width at vent.

(c) Postlarva (Fig.8,3):

- (i) Total length, from the tip of the head (or the snout) to the hind margin of caudal fin.
- (ii) Standard length, from the tip of the head (or the snout) to the hinder margin of the upper hypural plate of caudal region.
- (iii) Preanal length, from tip of snout to the vent.
- (iv) Postanal length, from the vent to the hind end of caudal fin.
- (v) Head length, from the tip of the snout to the opercular cleithrum.

- (vi) Width of head, at the level of the middle axis of the eye.
- (vii) Width of trunk, at the widest axis of the postcephalic part of preanal region.
- (viii) Width at vent.
- (ix) Postanal width, at its widest region.
- (x) Predorsal length, from the tip of the snout to the front margin of dorsal fin base if formed.
- (xi) Postdorsal length, from the hind end of the dorsal fin if formed to the hind end of caudal fin.
- (xii) Length of dorsal fin base.
- (xiii) Length of anal fin base.

(C) Juvenile: All the morphometric measurements required in ichthyotaxonomy, viz., total length, head length, prepectoral length, predorsal length, prepelvic length, width of caudal peduncle, etc., are necessary for documenting the juvenile stage in development.

While recording the above measurements, it is desirable to standardise the accuracy of each measurement, in order to facilitate easy comparison and comprehension of various stages. Workers on early developmental stages of fishes usually standardise the accuracy of measurement of the larger stages such as juveniles, postlarvae and larvae as well to the first decimal place. For smaller specimens such as eggs, the accuracy is usually standardised to the second decimal place. And, for still smaller parts of the developing stage, such as yolksac, oilglobule, etc., the accuracy is standardised to the third decimal place.

Drawings

Scientifically accurate drawings of early developmental stages are as much important as the measurements, while documenting them in literature. Larger specimens are usually drawn based on eye measurements of characters, practised in ichthyotaxonomy as well as by using a magnifying glass. But, smaller specimens the characters of which can be studied only under the microscope can be measured and drawn only by using a microscope. For recording measurements of eggs, larvae, postlarvae or certain parts of these stages, micrometers are used by inserting them in eye pieces of the microscopes and the calibrations of the micrometers are measured by tallying their calibrations with a stage micrometer. Microscopic drawings are usually made by using a camera lucida. After obtaining a scientifically accurate pencil drawing of the eggs, larvae, etc., as well as their characters, the drawings are finalised with Indian Ink.

Early workers on fish eggs and larvae have usually drawn the specimens, especially larvae and postlarvae such as these were, even if these were partly or fully curved upon preservation. But, over the years, this has been posing problems for subsequent workers in comprehending the accurate measurements for comparison and contrast. In view of this reason, it is desirable to avoid such partly or fully curved specimens in the case of such of the fishes, the early developmental stages of which are available in plenty. But, if the developmental stages are not available in adequate numbers and if it is absolutely essential to sketch partly or fully curved specimens of larvae, etc., drawing skills may be employed to present the sketches in an uncurved normal manner of the developing stage, without making any compromise on the various characters and measurements

thereof. Also, in order to make an easier comparison and contrast of the various sequences in the developmental process, it is desirable to present all the drawings of stages from larvae to the juveniles to an uniform final length for documentation. For instance, a postlarva of 5 mm when magnified to 10 times for the figure becomes 50 mm and a longer stage of 20 mm becomes 200 mm for its figure. Instead of presenting such figures, when the stages are magnified and drawn to the same final proportions for the figures, easier comprehension is possible. With the advent of magnifying and reducing the sizes of drawings this can be achieved quite easily.

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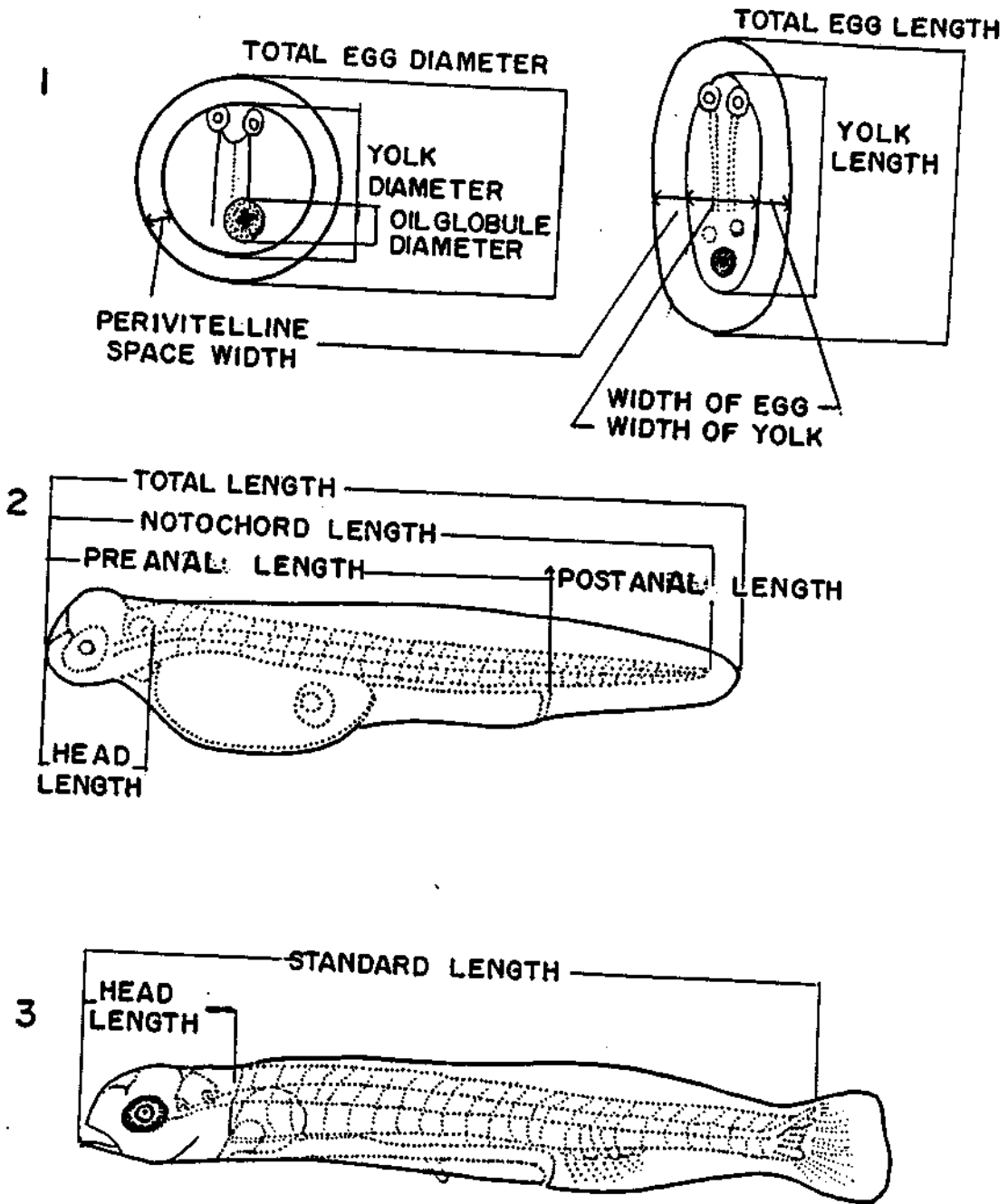


Fig-8

CMFRI/SI/1989/Th.IX

LABORATORY REARING OF THE EGGS AND LARVAE OF MARINE FISHES

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Quality of Water Medium:

The quality of water in which fish eggs and larvae are reared includes both physical and chemical factors that make aquatic environment suitable. Various chemical and physical parameters interact to produce a variety of distinct environmental complexities. Certain aspects which directly have some bearing on the quality of water are outlined below:-

Temperature:

For laboratory rearing purposes the most important physical aspect to be considered is temperature. Temperature of water has a definite influence on the successful rearing of the larvae. Vijayaraghavan (1955) collected hatchling of Anchoviella tri from the sea when the temperature was 28.6°C and maintained them at temperatures of 15°C, 20°C, 25°C and 30°C. After 8 hours he noticed less survival rate at 15°C and 30°C and maximum survival rates at 20°C and 25°C. With more experiments he ascertained that the best survival rate is between 20° and 25°C. He was successful in rearing the larvae of Decapterus russelli, Engraulis gravi, Anchoviella tri, Scomberomorus guttatum, Saurida tumbil and Cynoglossus sp. in temperatures ranging from 20°C to 25°C. In tropics the seasonal fluctuation in temperature is slight, and this may lead to a narrow range of tolerance. Based on the experiments on a total of 10 species of fish larvae, Kuthalingam (1959)

had shown that the range from lower to upper lethal limit in temperature was only to the tune of 3 to 4°C. It is possible that the tolerance may vary with the advancement of stage. The present author could collect the eggs of S. commerson and rear them till 72 hours at a temperature varying from 25 to 28°C.

Salinity:

Slight variation in salinities can affect the development at the early stages. Osmotic properties of water play a vital role in the life-history of fishes, especially at the marine and estuarine habitat. An increase or decrease in salinity brings about changes in the specific gravity of water and this has considerable impact on the fish at its critical period of development. Seer fish larvae were observed at salinity ranges of 31.80‰ - 35.12‰. Sphyraena borealis larvae were reared at salinity ranges of 33.0 - 34.6‰, and the present author has reared Scemberomorus commerson at 34.39 - 34.9‰.

Other factors:

The viscosity of sea water is high enough to protect the eggs and youngones from mechanical disturbances and help them to float on the surface of water. Dissolved substances in the water have both direct and indirect effect on fishes especially at their critical stage of development. The nutrients available in water can produce microscopic plants which in turn, act as food for many young and adults of fishes. Excess of dissolved salts create osmotic burden on young fishes.

Attack by ciliates.

The larvae reared are easily attacked by ciliates, as they flourish on dead eggs and then spread to healthy ones. It may be noted that the prolarvae are the most

vulnerable to ciliate attack. It is highly essential to keep the rearing system free from ciliates. Agitation of water has been widely considered useful by many workers. (Gross, 1939) By this the larvae are prevented from resting at the bottom where ciliates are abundant around decaying matter. Vijayaraghavan (1955) has made some studies on this aspect. It may be stated in this connection that the success with the rearing system depends to a large extent on absolute cleanliness while setting up the experiments.

Ambient Water:

Clean, unpolluted water with no suspended impurities is essential for successful rearing. Selection of the right quality of water from the right place and maintaining it in the right condition is an essential prerequisite. This can be achieved by ascertaining the natural spawning habits of fish, for utilising the water in which the fish spawns in the natural habitat. Water collected from any site where that particular fish breeds and undergo subsequent development is the most suitable one for rearing it through different stages. The ambient water thus collected, has to be filtered to remove all suspended particles and also the planktonic elements that are likely to develop, multiply or bloom subsequently, tampering with the experiments. Although considerable work has been done on the life history of fishes, little is known about the methods of laboratory rearing. Also, the approaches made in these study seem to differ from author to author, although the principles and objectives are one and same. Vijayaraghavan (1955) gave a brief account of the methods used by him to rear the eggs and larvae under tropical conditions. The methods adopted by the various workers are almost similar to these.

Methods of Rearing Eggs, Larval and Post Larvae

Methods used in rearing fish eggs and larvae vary from author to author. There is no standard procedure or equipment for the purpose. Bapat (1955) used troughs in the laboratory for hatching and for further studies as a routine measure. Seshappa and Bhimachar (1955) were able to keep alive Malabar sole larvae (Cynoglossus semifasciatus) in the laboratory through various stages of metamorphosis. Pelagic eggs of Sphyraena boreales were incubated and the larvae were reared in a 55 litre aquarium. Although, there are numerous instance of such rearing operations, the method adopted by Vijayaraghavan (1955) seem to carry more details. The water should be aerated and filtered frequently. According to needs, the size of the system may be changed. For few eggs or larvae even a finger bowl or breaker of one litre capacity may be enough. Glass troughs of 5 to 50 litre capacity may be used for larval rearing. The running water system can be better used for post larvae and Juvenile rearing. Use of light, constant aeration of water, proper maintenance of temperature and control of ciliates are the pertinent aspects which have to be taken care of during rearing.

Rearing Techniques used to rear eggs and larvae by the author

The present author succeeded in incubating the eggs and rearing the larvae of Scomberomorus commerson upto 72 hours hatching. The simple unpublished technique designed for rearing them in the laboratory and in situ is as follows:-

For the purpose of rearing the fertilised eggs, an improvised "Incubation unit" in which the eggs could pass through their critical stages, both before and just after hatching, and a 'Rearing Unit' in which one day old larvae could undergo further development, in situ,

were designed. The incubation unit (Fig. 9.1, a) consists essentially of a flat bottomed circular glass trough of 10 litre capacity and a glass beaker of 500 ml capacity. The beaker is filled to about 3/4th with fresh filtered seawater and the fertilised eggs were then transferred to the beaker. The mouth of the beaker is tightly closed with organdi cloth well soaked in filtered sea water. The beaker is then placed in the middle portion of the trough and the latter is filled gently with fresh, filtered sea water upto a level of about one centimeter below the mouth of the beaker. Besides providing ample circulation of water through the organdie cloth this also helps in preventing evaporation of water in the beaker and keeps the temperature of the water more or less as that of the surrounding sea water. The same unit may also be utilised for rearing the larvae for about a day after hatching. The rearing unit (Fig. 9.1, b) is helpful in rearing the larvae in situ from the second day onwards. It consists of a plastic bucket with slits cut all along its sides and a beaker of 1000 ml capacity. One day old larvae can be transferred to the beaker and the mouth is closed with organdie cloth of sufficiently large mesh size and it is then placed in the plastic bucket. The beaker is fastened tightly to the bucket and the entire unit is kept suspended by means of four bridles and kept at a depth of about 40 cm from the surface. Sufficient weight may be kept in the bucket to keep it upright in water.

Live Food of Postlarvae and Fry:

Little is known at present on the nutritional requirement and diet preference of the larvae and postlarvae of various species. Recent studies have indicated that the early postlarvae of fishes like the Seabass (Lates calcarifer) feed upon the rotifer

Brachionus plicatilis, nauplii of Artemia and the freshwater cladoceran Moina macrura. The diet requirement of postlarvae may be different from that of the fry. As growth advances mouth and jaws become more functional. Early in larval life, tuna were found to feed on small organisms measuring less than 100 microns; in the midlarval life, the feeding habits change abruptly to organisms of 500 microns or more. At the end of the mid larval life the fish takes larger organisms including other fish larvae. In the natural habitat the postlarva and juvenile fish feed on a wide variety of materials present in the water column, such as diatoms, phytoplankton and zooplankton. The food of young fish may vary from fish to fish. The intake of food items may also differ according to the feeding nature of the fish. A mullet fry at 20 mm stage feeds mainly on organic matter present in the water column, where as a seer, at the same size, prefer to feed mainly on larval fishes. The chemical compositions of some of the important live food items widely used in culture practices are incorporated in Table 9.1.

Table 9.1 Chemical composition of some important live food Organisms.

Organism	Percentage dry weight				Reference
	Protein	Carbohydrate	Fat	Ash	
<u>Tetraselmis maculata</u>	52	15.0	2.9	-	
<u>Dunaliella salina</u>	57	31.6	6.4	-	
<u>Chaetoceros</u> sp.	35	6.6	6.9	-	
<u>Skeletonema costatum</u>	37	20.8	4.7	-	Parsons et al. (1961)

<u>Phaeodactylum tricornatum</u>	33	24.0	6.6	-	Parsons et al. (1961)
<u>Exuviella</u> sp.	31	37.0	15.0	-	
<u>Chaetoceros</u> sp. (unialgal) Culture growing exponentially	48.6	9.2	9.5	-	Lewin and Guillard (1963)
<u>S. costatum</u> (unialgal culture growing exponentially)	60.6	34.71	7.7	-	
<u>S. costatum</u> (unialgal culture, grown 2-4 weeks)	43.52	34.55	21.93	-	
<u>P. tricornatum</u> (unialgal culture growing exponentially)	35.7	25.9	7.1	-	
<u>P. tricornatum</u> (fusiform cells from 16-d culture)	46.5	2.2	38.6	-	From Marshal and Orr (1960)
<u>P. tricornatum</u> (oval cells from 16-3 culture)	37.7	21.1	26.6	-	
Diatom (<u>Chaetoceros</u>)*	29.0	8.0	63.0	-	
Diatom (Mixed)*	24.5	14.2	61.3	-	Gallagher and Brown (1975)
Mixed Zooplankton*	46.0	6.0	23.0	25	
<u>Artemia</u> nauplii	55.60	-	15.20	15.25	Charles John Bhaskar (1982)
<u>Brachionus plicatilis</u>	59.07	8.44	24.05	8.44	
<u>Moina</u> sp.	56.69	13.47	23.73	6.11	Bardach et al (1972)
<u>Tubifex</u>	65.0	15.0	14.0	6.0	

* Grams per 100 gram Organic matter.

Adaptations of Mouth and Jaws

The type of food ingested and the feeding mechanism have correlation. Different types of mouth parts of the postlarval and juvenile fishes are given below:-

1. Centriscus sp. (22.5 mm TL)

Mouth, placed anteriorly at the tip of elongated tube like, snout. Jaws small and dorsally directed. At the younger stages it is pelagic and feeds on diatoms floating on the surface of water.

2. Mullet fry. (20 mm)

Protuded mouth, bearing no teeth, adapted to take mainly suspended organic matters.

3. Leptocephalus (120 mm)

Wide mouth, long and weak teeth, only to hold together the jaws when closed. Feeds on diatoms taken along with water. Mouth cavity is wide to suit it.

4. Caranx sp. (16.7 mm)

Mouth small no teeth on jaws characteristic of phytoplankton feeders.

5. Euthynnus affinis (5.62 mm TL)

Postlarvae, adapted to feed on plankton. Mouth wide, jaws prominent. Teeth pointed forward, to hold any live planktonic organism.

6. Saurida tumbil (33 mm TL)

Pelagic, fast feeder, feeding on decapods and copepodes. Teeth on jaws, bristle like pointed forward, closely arranged to capture large amount of pray and retain them in the mouth cavity. The teeth on tongue, recurved, help to take live zooplankters.

7. Seer fish (20 mm)

Mouth wide, jaws large, teeth strong mostly recurved. Few recurved teeth on palatine. Adopted for carnevorus type of feeding.

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- / postlarvae and juveniles of Polynemus indicus, Sardinella longiceps,

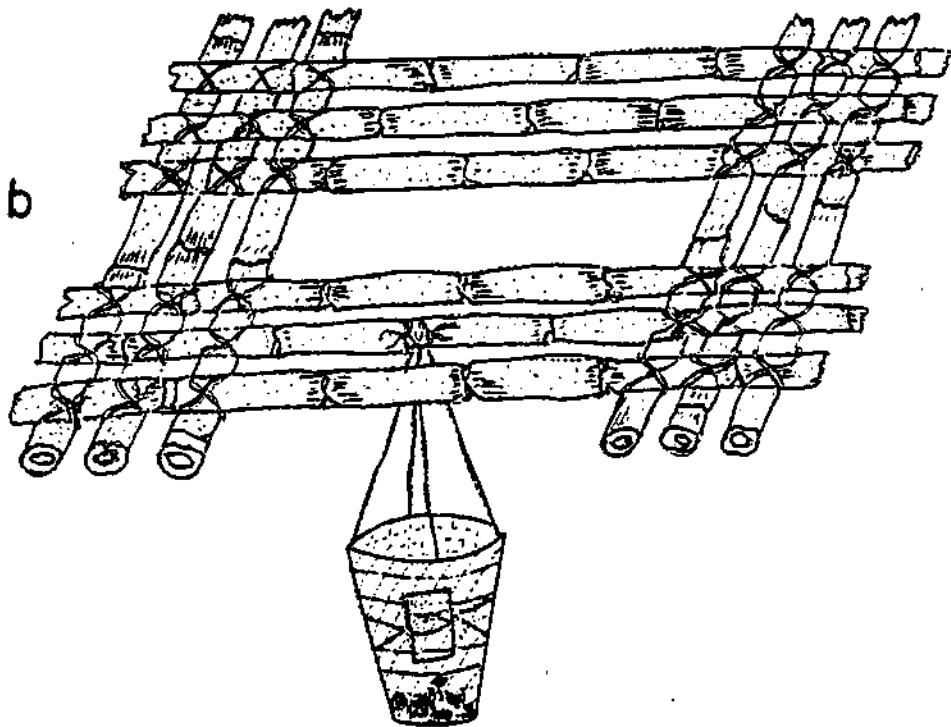
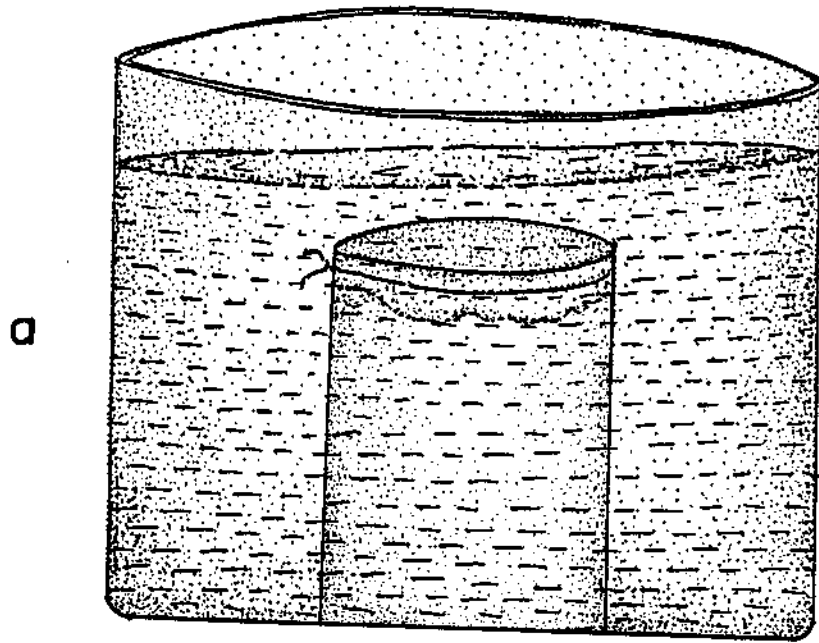


Fig. 9.1

GUIDELINES FOR THE IDENTIFICATION OF MARINE FISH EGGS

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Guidelines for identification may be considered under the following heads:

(1) Occurrences:

Occurrence of early developmental stages in the plankton and of spawners, spent, mature and/or recovering stages in fish catches can be used to identify the former as belonging to the latter only in a circumstantial manner. Delsman (1922-1938) has made use of such a method for assigning the early developmental stages; but, in addition to this he has also assigned cogent reasons of taxonomic nature in arriving at his conclusion. Thus, this circumstantial evidence of coincident occurrence of the eggs in the plankton and the spawning adults in the fishery can be only of limited value.

(2) Similarity with ripe ova:

Apart from the above guideline, another more valid guideline for identification of marine fish eggs is the similarities between the ripe ovarian ova of the parent and the planktonic eggs in the early stages of their development. In fishes such as Setipinna and Stolephorus the eggs, after extrusion in the water do not undergo increase in size due to hydration, as drawn attention to by Jones and Menon (1951 a). In such species, the size range of ripe ova and that of the planktonic eggs remain almost the same and hence the former is of great value to identify the latter. But, in other species like

Hilsa and Sardinella the eggs undergo hydration on coming into contact with water and as development progresses, thus altering the overall size of the planktonic egg from that of the ripe ovum (Jones and Menon, 1951 a). In the sardine Sardinops caerulea, Miller (1952) has found that the eggs diameter has increased from 1.15 mm at fertilisation to 1.64 mm after two hours and to a maximum of 1.83 mm at 10 hours after fertilisation. Although in such cases the overall diameter of the planktonic egg is different from that of the ripe ova, the diameter of the yolk remains the same as that of the ripe ova and is an useful guideline for identifying the eggs to the species to which these belong, as drawn attention to by Ahlstrom and Moser (1980).

(3) Egg Size:

According to Ahlstrom and Moser (1980), most of the marine fish eggs are in the size range of 0.6 to 1.6 mm in diameter. Bothid flat fishes are known to have small eggs of 0.5 to 0.8 mm; and pleuronectid flat fishes have large eggs of 4.0 to 4.5 mm and eels have still larger eggs of about 5 to 5.5 mm. In fishes like sardines and Anguilliformes, large sizes result due to hydration of the egg and progressive development of perivitelline space.

(4) Egg shape (Fig.10):

Planktonic eggs of vast majority of marine fishes are spherical, but there are some exceptions. For instance, the eggs of Stolephorus and certain Callionymus (Delsman, 1931) are ellipsoidal or globular (Fig. 10, A, B). Some eggs of Stolephorus, such as those of S. indicus and commersoni are provided with a knot at one pole. The eggs of certain gobioid fishes are attached to certain shells (Vijayaraghavan, 1973 b), somewhat ellipsoidal in shape, elongate and stumpy

and broader nearer the base (Fig.10,C). The attachment is by narrow, short hyaline fibres that are fused together forming a sucker shaped ring-like foot; these are also fused with similar structures of adjacent eggs.

(5) Chorion (Fig.10, D-H):

The outer egg capsule (also called egg shell, vitelline membrane, etc.,) in most marine fishes is smooth and unornamented. But, in certain groups of fishes it is ornamented such as Myctophiformes, Gadiformes, Pleuronectiformes, etc. The ornamentation may be in the form of a single swelling (or protuberance) as in some flat fishes (Fig.10, D) or in the form of an extensive honey comb-like polygonal network on the outer surface of egg membrane as in Chirocentrus (Delsman, 1923) (Fig.10, E), Saurida, flatfishes, etc. A less common type of ornamentation is spination (Fig.10, F), present on the eggs of Myctophiformes, certain exocoelids and allied fishes. In the demersal eggs of certain flying fishes adhesive filaments of 12-19 in number and of uniform size are present as in Cypselurus spilopterus (Vijayaraghavan, 1975, (Fig.10, G); and on the eggs of Hirundichthyes coramandelensis there are three distinct types of filaments (Vijayaraghavan, 1973a) (Fig.10,H).

(6) Inner egg membrane:

In most pelagic fish eggs, the chorion is the only egg capsule for the egg and an inner egg membrane is absent. But, in certain fish eggs, an inner egg membrane is present (Delsman, 1926). Ahlstrom and Counts (1958), the location of which may be either very close to the chorion or away from it (Fig.10,I).

(7) Yolk:

The yolk is the most dominant part of fish eggs, usually segmented or vacuolated in lower teleostean

groups such as clupeiformes but is homogeneous in most of the higher teleosts. The yolk is usually unpigmented; but in some groups it is pigmented as in certain Cynoglossus (Fig. 10, J).

(8) Oilglobule:

It may be absent in many species or groups as in Engraulidae, Cynodontidae, Gadidae, Pleuroneidae. If present, there may be only a single oilglobule as in many sardines; but the number may be many as in most Anguillaform eels, soleid and cynoglossid flatfishes, ranging from less than 10 to more than 50, the largest numbers being in most soleid flatfishes. Usually, the oilglobule is situated at the vegetal pole. It may be colourless usually, but in sardines it has a golden yellow colour. The single oilglobule may range in size from less than 0.1 to more than 1.0 mm in diameter. Usually, the oilglobule is unpigmented; but in the eggs of certain scombroids and Trichiurids, the oilglobule may be pigmented (Fig.10, K).

(9) Perivitelline space:

In most fish eggs which are "higher" in the group and which have a homogeneous yolk the perivitelline space is narrow, but in most fishes which are "lower" in the group and having vacuolated or segmented yolk, such as eels, clupeoid fishes, etc., a perivitelline space is present. Even in the latter category, a perivitelline space is absent to begin with (i.e. soon after spawned) but develops gradually, as observed by Miller (1952) in the Pacific sardine.

(10) Embryonic characters:

For the sake of convenience, Ahlstrom and Moser (1980) divide the embryonic development in the egg into three stages; (a) Early egg, from fertilisation to closure of blastopore (b) Middle Egg, from closure of

blastopore to the time when the tail begins to curve laterally away from the embryonic axis and (c) Late egg, from the time the tail is curved away from the embryonic axis to the time of hatching.

The pace of embryonic development differs from species to species group to group etc, because in some cases the embryo undergoes organogenesis even before the closure of blastopore (in early egg) while in others organogenesis takes place only in middle and late eggs. Pigmentation usually begins in middle eggs and reaches maximum in late eggs. Pigmentation may be present on the yolk and oilglobule also. Embryonic pigmentation is usually aligned along the dorsal margin of the body; and it is only after hatching the pigment migrate ventrally. But, in the case of the Japanese mackerel (Scomber japonicus), the ventral migration of the pigment takes place during the embryonic period itself. In the vast majority of marine fish eggs and larvae, the flexion of the caudal fin (notochord) and the formation of the fins take place only during larval/postlarval development. It is only in the eggs of flying fishes that flexion of the caudal fin and often the development of caudal anal and pelvic fin precedes hatching.

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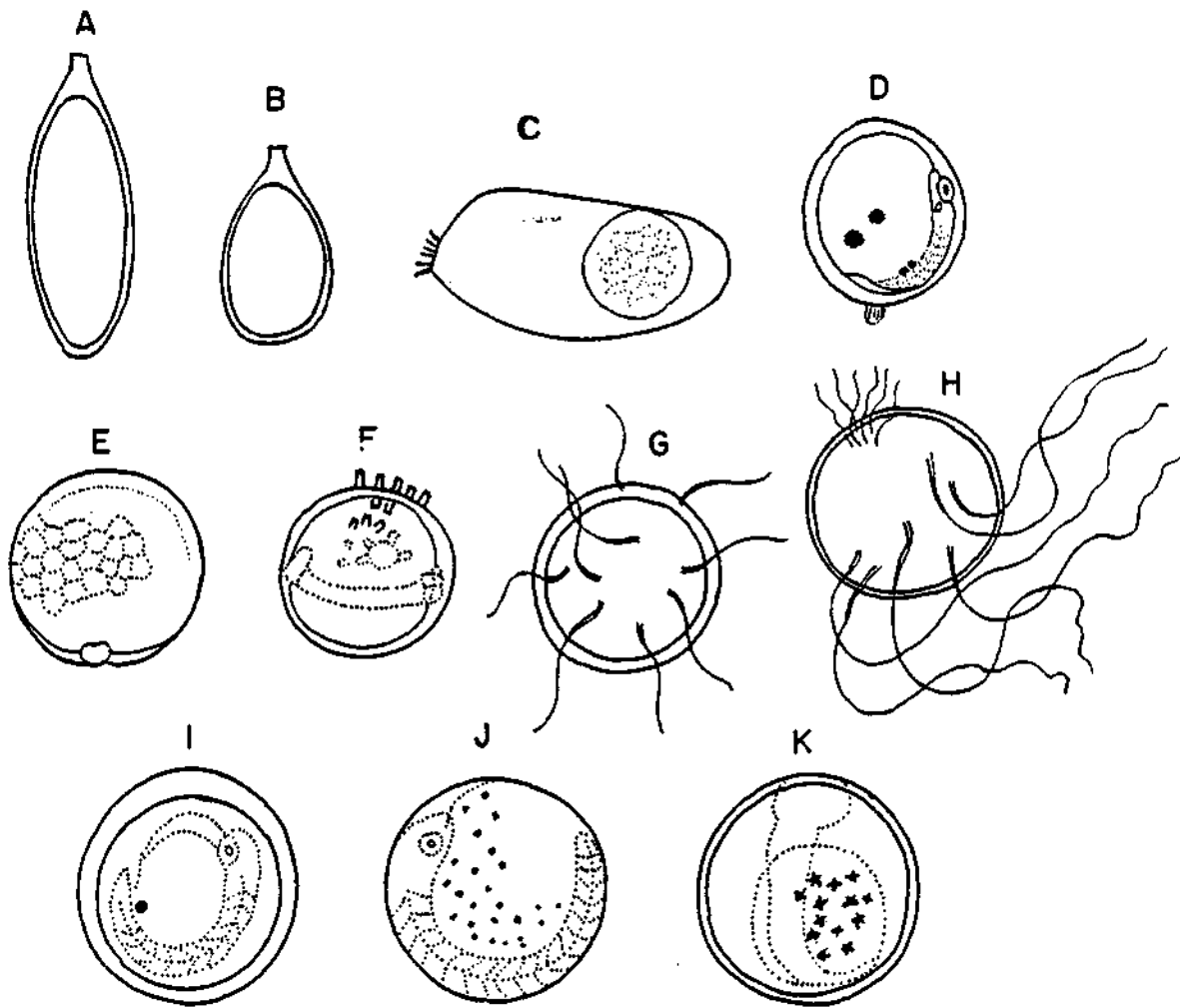


Fig.10

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GUIDELINES FOR THE IDENTIFICATION OF LARVAE

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Introduction

The different stages or phases in the life history of fishes markedly vary from the adult and the degrees of difference vary in different groups. However, there is no sudden metamorphosis in fishes and the change of form is slow. The differences between the larvae and adults of some fishes have led erroneously to the descriptions of the early stages of eel as Leptocephalus, Molidae as Molacanthus and Centaurus, Chaetodontidae as Tholichthys, Schindleria as Hemirhamphus and Lampreys as Ammocoetus. Later, these generic names were given the status of stages in the early life history of the fish concerned. It has been frequently generalised that there is higher development in smaller sizes in the tropical areas than in temperate areas. Larval fish development would conform with this generalisation. In the tropics, the postlarval period is short especially in marine teleosts where the yolk is completely absorbed by the second day. In elasmobranchs which are ovoviviparous or viviparous true embryo directly gives rise to the juvenile since the uterine embryo gets nourishment from the parent. In the viviparous poecilids like Gambusia and Lebistes young ones are only postlarvae.

Changes that take place from the early prolarval to the late postlarval stages vary in different groups or families and no generalisation can be made. There is considerable overlap in the size ranges at which the

various transitions take place and the occurrence of salient characters such as, the time of appearance of chromatophores, change in shape of eye, changes in body profile etc. show differences. These factors sometimes makes separation of growth stages difficult in many species.

Size and shape of the body:

The size of the newly hatched larva may vary from about a millimeter to a few centimetres (eel) in length. The newly hatched prolarva of Leiognathus ranges from 1.2 to 1.4 mm, Engraulis from 2.2 to 3.0 mm, Epinephelus from 1.4 to 1.6 mm, Sillago from 1.6 to 2.0 mm and Pleuronichthys from 3.6 to 3.7 mm. The prolarvae of most of the Clupeidae, Belonidae, Hemirhamphidae, Syngnathidae, Synodontidae, and Blenniidae are elongate. Slender bodied prolarvae are those of Sillaginidae, Sphyraenidae, Bregmacerotidae, Cepolidae, Gobiidae, Gerridae, Coryphaenidae and Cynoglossidae. The prolarvae of Muraenidae and Ophichthyidae have an elongated ribbon-like body. The prolarvae of Mugilidae, Pomadasyidae, Thunnidae, Scombridae, Scomberomoridae, Stromateidae, Scorpaenidae etc. have short fusiform body. The prolarvae of Ostracioidae and Tetraodontidae are globular in shape. The postlarvae of flatfishes have deeply compressed body and those of Platycephalidae, Pegasidae and Dactylopteridae are slightly depressed.

Nature of muscle fibres-Counting of number of myomeres:

Prolarvae of different groups could be distinguished by the number of myomeres which corresponds generally to the number of vertebrae in the adult and the general body proportions of the oldest metamorphosing stages available. The prolarvae of Balistidae, Aluteridae, Monacanthidae and Tetraodontidae have fewer than 24 myomeres. Mugilidae, Sphyraenidae, Carangidae, Mullidae,

cover

Istiphoridae, Theraponidae, Lelognathidae, Serranidae, Lutjanidae etc. have 23-24 myomeres. The myomere number in Clupeidae, Engraulidae, Thunnidae, Scomberomoridae, Sillaginidae, Coryphaenidae, Belonidae etc. vary between 35-50. Dussumieridae, Cepolidae, Bregmacerotidae, Belonidae, Cynoglossidae etc. have a little more than 50 myomeres. The muraenid Leptocephali could be broadly divided into two groups, a majority group of comparatively short forms having 120-137 myomeres and the numerically insignificant group of longer ones with 180-216 myomeres. Leptocephalus of Muraenesox talabon has 136 myomeres of which 58 are preanal.

Delsman (1933) has compared the adult vertebral number of some Clupeoids with their larval myomeres counts and has pointed out the differences in the myomeres numbers, total as well as preanal. Since the total myomere count in the early clupeoid larvae is generally higher than the adult vertebral number and gets stabilised only later in the course of development, identification of clupeoids based on myomere number alone may not be reliable, especially when two species having difference in only one or two myomere number is involved. Identification of prolarvae is reliable when the total myomere number corresponds to adult vertebral number if other related species having overlapping vertebral numbers are not involved. Kowala covai has 40 vertebrae and no other related species has the same number. All the other clupeoids have more than 42 vertebrae and therefore their larvae should have 42 or more myomeres. In the prolarvae of Kowala covai there are 32 preanal and 8 postanal myotomes. The larvae of Kowala covai and Stolephorus insularis are similar in appearance. But the larvae of S. insularis has 28 preanal myomeres whereas the preanal myomeres of Kowala covai becomes 28 by the forward shifting of the vent only in advanced postlarval

stage. The caudal fin shows the beginning of heterocercal condition only when the larva reaches about 8 mm (Bensam, 1969).

In the prolarval stage of carangids measuring about 2 mm there are 29 myomeres of which 13 are preanal and 16 postanal. But, in slightly advanced stage of 2.1 mm, though the total number of myomeres remains 29, the preanal myomeres is 11.

In the prolarva of Xiphias gladius measuring 3.2 mm, the vertebral column is straight and tapers posteriorly without any upturn (flexin). The caudal fin though homocercal at about 7.6 mm, the hypural bones have partly developed and the vertebral column is partly visible. The myomere formula of some of the larval tunas is 18 + 21 which tallies with the adult vertebral number 39 and this enables to distinguish them. The urostyle generally makes its appearance in the early postlarval phase (3.2 mm) with the evidence of hypurals ventral to it in Psenes cyanophrys. In this species the myomeres at the posterior region of the body are clear, forming a zig-zag pattern (Legapsi, 1956). In Bregmaceros the vertebral column remains straight even after the larva attains 4 mm and the elements of the urostyle begins to develop only at about 4.8 mm (Clancey, 1956).

Alimentary canal and position of vent:

The alimentary canal is visible through vertical muscle strands in most of the prolarvae of Clupeidae, Engraulidae, and Dussumieriidae as a straight tract. Sometimes the midgut region is slightly swollen in the late postlarval stage. In Cynoglossidae and Soleidae the anterior part of the alimentary tract bulges out like a sac. Changes in the alimentary canal take place gradually as the yolk is absorbed.

The vent is generally situated behind the midpoint

of the body in the prolarvae of Clupeidae, Dussumieriidae, Engraulidae and Synodontidae. The vent is far forward in the prolarvae of Bregmacerotidae, Atherinidae, Trypauchenidae and Blenniidae. The vent is almost below the middle of the body in the prolarvae of the families Holocentridae, Apogonidae, Gobiidae, Sillaginidae, Carangidae, Lutianidae, Thunnidae, Scorpaenidae, Cepolidae, Opisthognathidae, Scomberomoridae, Coryphaenidae, Sparidae, Champsodontidae etc. Vent is situated far behind the middle of the body in the prolarvae of Apodes, Hemirhamphidae, Exocoetidae, Fistulariidae, Sphyrinaeidae, Platycephalidae and Cephalacanthidae.

In the newly hatched prolarvae of Caranx sp measuring 1.73 mm there are 13 preanal and 16 postanal myomeres. The gut is short and opens immediately below the 13th myotome. In a slightly advanced 2.04 mm stage although the gut remains tubular, the vent has shifted anteriorly and opens below the 11th myomere (Kuthalingam, 1959). In leptocephali of eels the vent situated about the middle of the body or in the posterior half progressively shifts towards the anterior region as growth advances. In an advanced stage of leptocephali which is about 60 mm, the vent which has shifted still further anteriorly is situated opposite to the 88-90th myotome.

Origin and location of paired and unpaired fins:

Continuous finfold is present in the prolarvae of Bregmacerotidae, Serranidae, Theraponidae, Carangidae, Coryphaenidae, Lutianidae, Gerridae, Opisthognathidae, Istiophoridae, Trypauchenidae, Cynoglossidae, Soleidae, Pleuronectidae, Leiognathidae, Platycephalidae, Cephalacanthidae etc. In the prolarvae of Bregmaceros, the continuous finfold is seen in the region of the first dorsal whereas the second dorsal and caudal fins give very slight indications of ray formation and the pelvic fin is composed of two short rays.

Two separate dorsal finfolds are found in Mugilidae, Apogonidae, Mullidae, and Gobiidae in the prolarval stage. In Carangid prolarvae the dorsal and anal finfolds are broad and rudiments of pectoral are visible. In fishes with 2 dorsal fins and 2 anal fins (eg. Bregmaceros), the fin membrane appears to be continuous in the embryonic stage itself, but these would be later divided into two groups, i.e. dorsal fins and anal fins with undeveloped short rays between them. It is difficult to distinguish a break between the two anterior groups, especially in the early stages. Sometimes a tuft or group of tufts are seen at the base of the pelvic fin of more developed prolarvae of Bregmaceros.

In Psenes cyanophrys 2.5 mm prolarva the dorsal and anal fins though rudimentary are confluent with the rounded caudal. The pectoral fins are set on a fleshy base and are rounded, with evidence of developing finrays in the prolarva. Below the bases of the pectoral fins, rudiments of the pelvic fins are present. In a slightly advanced 3.2 mm stage, the first dorsal fin is seen in the finfold stage, whereas the second dorsal and caudal shows indications of rays.

In leptocephali prolarvae, pectorals are absent but a minute bud-like protuberance on each side immediately behind the gill opening represents them. In Muraenesox talabon, the dorsal originates opposite the 42nd myomere approximately between the snout and vent and the anal just behind the vent between the 97th and 98th myomere. Both the fins are continuous with the caudal and the tail is nearly 5.5 in total length. Dorsal origin in leptocephali shifts forward as growth advances in the prolarval stage. In the postlarvae of clupeoids the predorsal length is about double the postanal length. The pelvic fin in clupeoids originates in the form of a bulge in the prolarval stage. The caudal region is rhomboidal with a few striations on the dorsal and posterior aspects

and the caudal fin shows the beginning of heterocercal condition.

Changing pattern of pigmentation:

Based on the intensity of pigmentation, prolarvae falls under four broad categories. The prolarvae of Holocentridae, Belontiidae, Balistidae, Coryphaenidae, Blenniidae, Pegasidae, Istiophoridae and Cephalacanthidae are heavily pigmented. Only some parts of the body are pigmented in Exocoetidae, Atherinidae, Theraponidae, Mullidae, Stromateidae, Lobotidae and Platycephalidae. Only very few pigments are found in the prolarvae of Engraulidae, Apogonidae, Serranidae, Leiognathidae, Scomberomoridae, Thunnidae, Pleuronectidae and Cynoglossidae. The prolarvae of Gobiidae, Trypauchenidae, Bothidae and Soleidae are almost without pigments.

In the leptocephali of eels, chromatophores are generally present in the heart region and a row of 5 to 6 chromatophores on the sides of the posterior half of the lower jaw. An irregular row of minute chromatophores is present along the base of the anal fin and along the ventral margin of the gut. A dendritic black chromatophore is found on either side below the gill slit. In the prolarva of Muraenesox talabon (65 mm) four stellate chromatophores are present, one along the margin of the sides of the upper lip, one anterior to the posterior nares, one below the orbit and another slightly behind it. Chromatophores are present in the posterior region of the head. A row of similar chromatophores is evident along the entire length of the alimentary canal, and another row immediately below the spinal cord on all the myomeres except the first ten. Slightly less distinct chromatophores are present at the base of the anal and caudal fins.

Pigmentation pattern is an excellent diagnostic character in identifying larval tuna where the fins are not fully developed (Matsumoto, 1958). Pigmentation begins to appear in the early prolarval stage. In scombroids, the first dorsal fin is heavily pigmented. A few faint chromatophores are visible at the anterior basal portion of the second dorsal fin, which is otherwise colourless. A darkly pigmented area colours the top of the head and extends forward to the brain region. Scattered chromatophores in the peritoneum are visible through the body wall, particularly in the abdominal region.

In carangid prolarvae, brown and yellow pigment spots are scattered all over the embryo, yolk mass and faintly developed finfolds. Black pigments are present on the inner surface of the oil globule and on the margins of the embryo. In a slightly advanced stage of 2.02 mm, yellow chromatophores are present on the finfolds, on the dorsal and ventral margins of the myomeres, on the yolk sac and pectoral rudiment. Black pigment cells are found on the oil globule (Kuthalingam, 1959).

In the prolarvae of Psenes cyanophrys measuring 2.5 mm the chromatophores are quite evenly distributed on the head and nape, scattering through the cheeks and opercle. A few are also scattered at the base of the pectoral fins, on the side of the body cavity and on the ventral side of the body to the tip of the vertebral column. In a slightly advanced 3.2 mm stage, the chromatophores remain as such, but present on the dorsal side of the head in the form of a crown (Legapsi, 1956).

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GUIDELINES FOR IDENTIFICATION OF POSTLARVAE

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Introduction

From the morphological resemblances and the progress of changes in morphology accompanying growth, it is possible to trace the development of fishes. However, the easiest means in identifying fish larvae is to identify the largest stage and work down to the smallest.

Structure and shape of the head, trunk and tail:

Bony structures are found on the head in the post-larvae of Syngnathidae, Pegasidae, Ostraciontidae and Dactylopteridae. Spiny scales begin to form in the postlarvae of Balistidae, Aluteridae, Cephalacanthidae, Diodontidae and Tetradontidae. Postlarvae in some families like Lobotidae, Holocentridae, Cepolidae, Champsodontidae, Siganidae and Platycephalidae develop a bony crest of diverse types on the nape. Holocentridae, Istiophoridae, Cephalacanthidae and Dactylopteridae have a prominent spine on the supraoccipital part of the head. Preopercular spines are a common feature in the post-larvae of Holocentridae, Theraponidae, Carangidae, Coryphaenidae, Scomberomoridae, Istiophoridae, Thunnidae, Lutianidae, Cepolidae, Serranidae, Lobotidae, Pegasidae, Leiognathidae, Menidae, Sparidae, Platycephalidae, Champsodontidae and Scorpaenidae. Postlarval stages of Scombridae, Labridae, Gobiidae, Trypauchenidae, Clupeidae etc. have no preopercular spines. Scorpaenidae, Stromateidae, Cephalacanthidae, Cepolidae, Holocentridae,

Istiophoridae and Champsodontidae have a bony ridge above the eye in the postlarval stages. Postlarvae of Holocentridae, Istiophoridae, Xiphiidae and Pegasidae have protruding snout. A barbel is generally present on the chin in postlarval Exocoetids. Champsodontidae have an elongated tentacle on the operculum. The mouth in the postlarvae may be oblique (Hemirhamphidae, Exocoetidae, Bregmacerotidae, Apogonidae, Leiognathidae, Carangidae, Lutianidae, Serranidae and Opisthognathidae), inferior (Holocentridae, Cephalacanthidae), subterminal (Engraulidae) or tube-like (Fistulariidae, Syngnathidae).

Large size of the head relative to the rest of the body, the sharply pointed snout with well developed teeth on the jaws and prominent preopercular spines distinguish scombroid larvae from others. The preopercular spines gradually get reduced from above to below and in the postlarvae extend to beyond the operculum in Scomberomorus guttatus, whereas in the corresponding stages in S. commerson the second spine situated at the angle of the preopercle is conspicuously larger than the next and it extends beyond the operculum. The preopercular spines which are present in the larvae and early juveniles of most scombroids are absent in Rastrelliger kanagurta. Thus, the absence of preopercular spines is a useful criterion in identifying and isolating the postlarvae of Rastrelliger from those of other scombroids. In R. kanagurta at about 8.6 mm the shape of the head and the body, particularly the dorsal and ventral profiles are markedly adult-like and the transformation from the larvae to the juvenile is at about 9.5 mm. Silas and George (1971) state that during the larval and metamorphosing stages of Vinciguerrria nimbaria a progressive increase in the length of the head upto the postmetamorphic stage takes place. The head which has a more or less truncate snout in the larval stages, assumes the

general shape as seen in the juveniles and adult in the postmetamorphic stages. There is a conspicuous increase in the depth of the body on transition from larvae through metamorphosing stages.

Disposition of fins and their rays and spines:

As growth advances, the larval fin characters become pronounced while the adult characters make their appearance. Progressive shifting in the position of the dorsal and anal fins is a general feature in the development in the postlarval stages of many fishes. In the postlarvae of Rastrelliger kanagurta measuring 5.4 mm there is a general change in the body form and fins during the flexion process, (Silas, 1974). Indications of differentiation of the median fins are seen in the analages of the second dorsal and anal fins and incipient caudal rays are also seen. As growth progresses from the larval to the postmetamorphic stages, there is a relative decrease in the predorsal distance and a slight reduction in the preanal length.

In the eelver of eels, the vent and the dorsal have shifted still further anteriorly opposite the 58th and 12th myotome respectively and the caudal fin becomes pointed. In the postlarvae of Xiphias gladius measuring 6.9 mm, the dorsal and ventral fins are separated from the caudal fin and the fin rays appear. The caudal fin is still homocercal and the pelvic fins appear as small buds between the bases of pectorals. In Rastrelliger kanagurta the paired fins in the larvae upto 8 mm do not show any ossification of rays, while the median fins are fairly well developed. Among paired and median fins it is the caudal fin that shows more rapid development and the earliest indication of ossification of the rays (Silas, 1974). This is evident in larvae above 5 mm and by the time the larvae are about 9 mm, most of the

caudal rays are ossified.

Development of the fins in the postlarval stages is noticeably rapid in Psenes cyanophrys (Legapsi, 1956). The relatively small pelvic fins of P. cyanophrys helps to distinguish it from the postlarval stages of Carangidae to which it bears close resemblance. All the fins are present although rudimentary and not well differentiated. The pectoral fins in P. cyanophrys are set on a fleshy base and are rounded, with evidence of developing rays. Spines develop on the dorsal and anal fins which have started differentiating from the expanding caudal fin. The pelvic fins have grown but the ventral sheath is not yet visible. At about 4.7 mm the pectoral fins have several fully formed rays with indications of more in formation. In the pelvic fins a single spine develop and the fin ray elements are present. The dorsal and anal fins which have completely separated from the caudal have developing spines, but none of the fin rays is completely formed. The caudal is well defined, rather truncate preparatory to forking. Spines and rays are almost fully formed in the dorsal and anal when the postlarva reaches 6.9 mm. The pelvics are almost fully formed except for a complete adult fin ray count. Caudal fin with fully developed middle rays has a shallow cleft giving it a slightly emarginate posterior margin. When the larva reaches 9 mm, the pectorals assume a more definitely pointed shape and ray counts are those of the adult. Dorsal and anal have adult counts. The pelvic fins are fully developed and distinctly smaller than the pectorals.

In the postlarvae measuring 5.4 mm of Kowala coval, the pectoral fin is rounded with a few striations representing the future rays (Bensam, 1969). The predorsal length is almost double the postanal length. The pelvic

fin has not yet appeared. The anal fin is represented by a strip of thick tissue. Caudal region is rhomboidal. When the postlarva attains 8.9 mm, the position of ventral is indicated by a protuberance below the midgut region. By about 11 mm the dorsal has about 15 rays and its origin has moved still more anteriorly, thus making the precaudal and post dorsal length almost equal. The pelvic fin has developed and is supported by 8-9 rays.

It is possible to fix the identity of postlarvae of Bregmaceros by the pronounced absence of dorsal and anal rays after the continuous finfold stage as the pelvic rays (Clancy, 1956) are rather long. Pectoral fin is dorsolateral in position. The second dorsal and caudal fins give very slight evidence of ray formation. At about 4.9 mm, rays are visible in the dorsal and anal and there are 9 rays in the caudal. Three pelvic rays are recognisable. At about 7 mm the first dorsal cannot be divided into two portions, but it has developed 23 rays and the second anal has 19 rays. The somewhat round caudal fin has 19 rays now and at this stage, the first evidence of two rather distinct caudal lobes appear. Evidence of pectoral ray development is seen. The most anterior pelvic ray has a small tuft at its base. In late postlarvae, it is difficult to determine the break in the anal. The caudal fin has lost its roundness and is arrowhead shaped, composed of 23 rays. Pectoral fin is narrower at its base and signs of 2 developing rays are seen.

Pigmentation pattern:

In some groups identification of the postlarvae is possible by the difference in the nature and distribution of chromatophores in combination with the differences in body proportions, shape of the head and fin characters. Pigmentation patterns are often useful in identifying small tuna larvae. In the larva of Rastrelliger kanagurta

measuring 5.4 mm, a melanophore is present at the base of the urostyle and two at the distal margin of the hypural plate (Silas, 1974). On the ventral side of the abdomen there is a distinct melanophore and the primordium of the pelvic fin appears close to this. In a 6.6 mm stage, one or two chromatophores are present in addition at the base of the caudal fin just below the tip of the hypurals, besides the two at the lower caudal base. Only four melanophores are present at the posterior half of the base of the anal fin, while six are present at the anal finlets and the lower side of the caudal peduncle. In the late postlarva of mackerel, the basic pattern such as the post vent row of chromatophores, their numbers and disposition with size remain the same. The basic pattern of absence of branched chromatophores on the head until the Enlarges of the hypural plates are formed is seen in the larva. The post-vent row of melanophores vary from 11 to 14 and their number decrease with growth. The number and disposition of melanophores at the base of the caudal fin in the postlarvae show slight variations. The chromatophores on the dorsum of the viscera and intestine are sometimes diffuse (Silas, 1974). In Scomberomorus, chromatophores are present in patches at the tip of the snout, above and between orbitals, above the region of the hind brain and in the posterior region of the opercle (Jones and Kumaran, 1962). On the body they are present close to the base of the dorsal fin and along the mid-lateral line and a few at the base of the caudal and anal fins. The anterior region of the first dorsal is slightly pigmented.

In Psenes cyanophrys postlarvae measuring 4.9 mm, chromatophores are seen on the head and abdominal regions (Legapsi, 1956). In an advanced postlarva of 7 mm the pigmentation of the gut has intensified and numerous chromatophores cover the body. A row of melanophores

is seen along the anterior portion of the dorsal ridge and slight traces of the dorsal patches remain. In larger postlarvae, the body chromatophores seem to follow a definite pattern, those of the gut forming vertical stripes. Along the dorsal ridge two rows of chromatophores are seen separately widely anteriorly and fusing posteriorly. When the larva acquires adult characters at about 14 mm the intensity of pigmentation increases in these regions till the postlarvae attain a dark appearance.

A characteristic arrangement of pigmentation pattern is present in Bregmaceros spp. throughout the early postlarval stages (Clancey, 1956). In the early postlarval stage, chromatophores are seen in the head region, at the interorbital space behind the eye and below the mouth. On the body, chromatophores are found posterior to the head, along the gut region and posterior to the pectoral fin. Four distinct patches are found dorsal to anal finfold and two patches are ventral to the dorsal finfold. When the postlarva is 5.2 mm, pigmentation is more intense because chromatophores hinder to the eye have expanded forming a band extending across the head and showing patchiness behind the eye. Chromatophores appear along the upper and lower jaws. Pigmentation of the gut has become more intense and three definitive chromatophores are seen near the vent. Chromatophores are present behind the head and posterior to the pectoral fin. The four patches dorsal to the anal fin are larger and more intense. The patches below the dorsal fins now appear as four larger groups. In an advanced postlarva of 7 mm the pigmentation has intensified and numerous chromatophores cover the body. A row of chromatophores is seen along the anterior portion of the dorsal ridge and slight traces are still discernible. In larger postlarvae the number of chromatophores has increased and these are scattered throughout the body.

Myomeres and myosepta:

The number of myomeres in the postlarvae of Rastrelliger kanagurta are variable until the full complement of myomeres is formed by the time the larvae attain 4 mm (Silas, 1974). Myomeres grow obliquely with zigzagging in most of the segments by about 5.4 mm. The flexion of the notochord begins at about 5 mm and the simultaneous formation of the hypural plate also coincides with the zigzagging of the myomeres.

Migration of the eye in flat fishes:

According to Jones and Menon (1951), the duration of metamorphosis varies in different flat fishes. In Brachirus pan the pelagic life is short and transformation takes place early. The migration of eye is first indicated in the 3.4 mm stage and appears to be rather quick. At about 4.6 mm stage, the left eye reaches the dorsal edge and comes near to the right side when the fish becomes a postlarva. Among flat fishes Bothus pantherinus may be an example of late metamorphosis. The position of the eye in the postlarva of Cynoglossus lingua shows a condition different from that of Brachirus pan. They are so close that they appear to touch one another. During the first phase of migration the eyes become very close, but later these gradually move apart to give rise to the condition in the adult.

Development of scales:

In clupeoids, scales begin to appear when the larvae are about 15-16 mm in length. The scales are not formed uniformly all over the body. On the dorsal side anterior to the dorsal fin, these are formed subsequent to the other regions. The scales are very thin and fall off very easily. The scutes begin to develop in the juveniles of more than 20 mm and are formed in front of the pelvics and then gradually along the ventral edge of the abdomen. In mackerel, Rastrelliger kanagurta first

signs of formation of scales are observed in specimens of more than 20 mm (Balakrishnan and Rao, 1971). In Vinciguerria nimbaria Silas and George (1971) observed in a stage of 15.3 mm that the scales are deciduous and scale pockets are discernible on account of the fine rows of pigment spots.

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CHARACTERS AIDING IN THE IDENTIFICATION OF EGGS AND LARVAE
OF MAJOR GROUPS

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The various characters of the eggs, larvae, postlarvae of marine fishes as summarised by Ahlstrom and Moser (1976) may be considered group-wise, as follows. A perusal of the publications by Russell (1976), Jones, Martin and Hardy Jr. (1978), Fritsche (1978), Hardy Jr. (1978a,b), Johnson (1978) and Martin and Drewry. (1978) will also be of much use in this regard.

(1) Clupeiformes:

The Order Clupeiformes is composed of the Indian species of sardines (Sardinella), herrings (Ilisha, Pellona, Opisthopterus), shads (Hilsa), anchovies (Stolephorus, Thryssa, Coilia, Setipinna), wolf-herring (Chirocentrus) etc. The characters aiding in the identification of the Indian species have been summarised by Bensam (1971). The eggs of all the species have a vacuolated yolk eggs of anchovies elliphical; chorion unornamented; perivitelline space is quite wide in sardines but narrow in anchovies; single oilglobule present in most sardines, many in Escualosa, Nematalosa, Setipinna, Coilia, etc; embryonic pigmentation on the dorsal side present only in sardines. Larvae are pelagic, transparent, elongated, with anus situated far behind; Pigment spots present on the dorsal side of early larvae of only sardines; In newly hatched

larvae, the hinder end of yolk sac is globular and rounded off in sardines, but pyriform in anchovies; myomeres have an angular appearance and muscle fibres have a crossed arrangement. The position of vent changes with progressive development, thus altering the preanal and postanal number of myomeres. In postlarval development, the disposition of the dorsal fin in relation to the anal fin changes; in early postlarvae there is a slight increase in pigmentation and in late postlarvae the pigmentation increases; scales and scutes recognisable in the late postlarvae when the vent also occupies the adult position.

(2) Anquilliformes:

This Order comprises the eels, such as the Indian Anquilla, Echidna (Muraena) Urocongir, Muraenesea, Ophichthus, etc. The eggs are pelagic, spherical, with smooth chorion, vacuolated yolk, perivitelline specie wide and have one or more oilglobule. Larvae hatch out as leptocephali, with usually a straight gut and with a marked transformation to juvenile condition. Nair and Mohamed (1961) may be consulted for details in the development and metamorphosis.

(3) Gonorhynchiformes:

Represented only by a single species Chanos chanos found in India also, the eggs and larvae have many Clupeiform characters; but the muscle fibres have a parallel arrangement and not a crossed one.

(4) Myctophiformes:

In India this Order is represented by the Bombay Duck (Harpoden nehereus), lizard fishes (Saurida, Trachinocephalus), etc. The eggs are pelagic, spherical, with hexagonal pattern in some (Synodidae-Saurida, Trachinocephalus), yolk may be segmented or homogeneous, perivitteline space narrow and usually a single oilglobule is present.

The larvae are elongated, gut is straight or variously shaped, head spination may or may not be present and transformation may be either gradual or marked. Postlarvae with conspicuous ventrolateral spots of pigments.

(5) Atheriniformes:

In India this order is predominantly represented by the flying fishes (Exocoetus, Hirundichthys, Cypselurus), the half-beaks (Hemirhamphus, Tenarchopterus) and Garfishes (Belone or Tylosurus). Eggs mostly attached to other objects such as plants by means of well developed chorionic filaments. Usually most of the fins develop within the embryonic period itself; and the postlarvae are mostly pigmented.

(6) Perciformes:

This is perhaps the most diversified of all fish orders and the largest vertebrate order, encompassing the families of Indian salmon (Polynemidae), barracudas (Sphyraenidae), grey mullets (Mugilidae), mackerels, tunas, seerfishes (Scombridae), horse mackerels (Carangidae), perches (Epinephelidae), etc. The characteristic features of some of these families are summarised below:-

(a) Carangidae:

Eggs pelagic, spherical, with a single oilglobule, most species with segmented yolk and embryo, yolk and oilglobule pigmented. Alimentary canal short in the larvae, the body of which are pigmented. Pigmentation increases in postlarvae and juveniles.

(b) Sciaenidae:

Characters of eggs and larvae almost the same as those of Carangidae, but the yolk is homogeneous and unsegmented. Alimentary canal of larvae rather short with anterior anus. Postlarvae with distinctly oblique mouth and dense pigmentation.

(c) Scombridae:

Eggs, pelagic, spherical, with homogeneous yolk, with a single oilglobule and embryo as well as oilglobule with pigmentation. Alimentary canal of larvae short with anterior location of vent. Snout becomes prominent in later postlarval stages. Presence of preopercular spines is characteristic of the postlarvae of most genera but are absent in mackerels.

(d) Gobiidae:

Eggs mostly demersal, attached to submerged objects, shapes rather irregular, sometimes elliptical, characterised by enormous protoplasm and little yolk and with little pigmentation. Larvae elongated, with position of vent at about the middle of the specimens.

(e) Sphyraenidae:

Eggs pelagic, spherical, with an oilglobule, yolk segmented and the embryo as well as the oilglobule become pigmented as development progresses. Larval vent is placed towards the hinder end of the body which is pigmented. Postlarval development characterised by elongation of the snout.

(f) Mugilidae:

Eggs spherical, with a large oilglobule, which becomes pigmented along with the embryo and the yolk, as development progresses. Larval body and oilglobule usually heavily pigmented. Position of vent is almost at the middle region. Postlarval development characterised by dense pigmentation.

(7) Pleuronectiformes:

This order comprising flatfishes is made up in India of Indian halibut (Psettodes), left-eyed flounders (Pseudorhombus, Arnoglossus, Psettina, Bothus) right-eyed

soles (Solea, Synaptura) and left-eyed soles (Cynoglossus).

The eggs are predominantly pelagic and seldom demersal, spherical, usually with smooth chorion but with hexagonal pattern in some species, yolk homogeneous, perivitelline space is narrow and oilglobule is either absent or if present one or many in number (Embryo) and yolk sac may be pigmented. Larvae are with a coiled gut, with head spination and metamorphosis is marked with migration of one of the eyes to the other side in cases where both eyes are on one side.

(a) Bothidae:

Eggs pelagic, spherical having homogenous yolk, with a single oilglobule and pigmentation of embryo, yolk and oilglobule. Larvae with good pigmentation and position of vent at about the middle of body, which shifts forwards during postlarval development. Body becomes broader and the right eye shifts to the left side during metamorphosis. First dorsal fin ray usually long during postlarval phase.

(b) Pleuronectidae:

Eggs pelagic, spherical, having homogenous yolk usually without an oilglobule and the embryo with dense pigmentation. Larval pigmentation usually becomes localised as a few bands as development progresses. Postlarval development marked by a lateral broadening of the body and shifting of the left eye to the right side, involving metamorphosis.

(c) Soleidae:

Pelagic, spherical eggs, with a number of oil droplets, with homogeneous yolk and with stellate pigmentation for the embryo and the yolk. Larval finfold sometimes shows stellate pigments. During postlarval development there is a metamorphosis involving the shifting of the left eye to the right side.

(d) Cynoglossidae:

Eggs pelagic, spherical, yolk homogeneous, with many droplets of oil globule. In advanced stages the embryo is pigmented with stellate chromatophore on its body and larval finfold. A tentacle develops dorsal to the head in early postlarval stage which is actually the future first dorsal ray followed by the development of the second to a few more rays in further stages.

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QUANTITATIVE STUDIES AND PROJECTIONS OF MARINE
FISH EGGS AND LARVAE

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Regular sampling of fish eggs and larvae is essential for locating shoals of adult fishes and their spawning and nursery grounds. The implication of such studies in the estimation of spawning biomass is well stressed and documented by various authors (Ahlstrom, 1968; Tanaka, 1973; Smith and Richardson 1977; Regner et al., 1981). The following techniques are used for estimation of the number (quantity) of eggs and larvae in space and time as well as for their projections for fisheries research and developmental programmes.

Sampling for estimation:

Fish eggs and larval surveys have to be designed to overcome problems arising from spatial and temporal variations. Hence surveys have to be planned to cover all the spawning areas and seasons of the target species. With this in view, the Chief Scientist, the Captain and the technicians of the Ship plan the cruise and fix the stations. There are a lot of ways to distribute the stations over the survey area. From the statistical point of view, the random distribution of the stations will give best results, but this will consume a lot of ship's time. Colebrook (1973) has examined the different sampling patterns and he emphasizes that the evenly spaced grid in time and space has many advantages. It has been found by Switzer (1967) that a 2:1 rectangular grid is the most

convenient one for estimations and projections of the availability and abundance of fish eggs and larvae. After the plankton samples are taken ashore a measurement of the wet plankton volume, determined by displacement method is made. For this, the preserving liquid is removed from the plankton by pouring the sample through a calibrated filtering cone (Volume determiner) made of 0.33 mm Nitex. The plankton is retained in the cone until the drainage of liquid from the cone diminishes to an occasional drop. The volume of the drained plankton is then determined by using an automated burette. It is recommended to sort out total samples for fish eggs and larval studies. Only in the case of very large numbers of eggs and larvae, samples can be divided into subsamples and one or more of them sorted. In that case samples can be fractioned into aliquots using plankton splitters, such as Folsom splitter (Mc Ewen et. al., 1954). The Bogorov's counting tray is one of the best type of containers used for sorting and counting ichthyoplankton (Newell and Newell, 1963). A small quantity of sample to be sorted is poured into the counting tray and its contents are closely observed under the dissecting microscope. All the fish eggs and larvae are removed with a dropper and forceps, counted and placed in appropriately labelled dishes. After the eggs and larvae have been sorted out, its remaining contents are poured into a beaker labelled 'sorted'. This process is repeated until the entire sample or aliquot has been completely sorted. The total number of fish eggs and larvae removed from the plankton sample is recorded on the 'Ichthyoplankton Sorter's Work Sheet' which gives a list of all the genus/species likely to occur in the sample on one side of the page. For this, all that is needed is to tick in the appropriate column of the identified egg or larvae and then to finally count the

ticks when the sample has been worked over completely. Technicians who sort fish eggs and larvae must be trained to identify and separate eggs and larvae of the common families of fishes. It is advisable to establish a reference collection of identified fish eggs and larvae of important species in order to facilitate easy identification by comparison. Once the eggs and larvae of selected species are identified at the sorter's level, these can be enumerated and recorded separately. Before bottling, eggs or larvae of the selected species may be measured and the lengths recorded in data sheets. After the data are recorded the eggs and larvae are bottled for storage with appropriate labels. Identifications made at sorting level are rechecked by experts. After sorting all the samples from a cruise, a plankton sorter's sheet is prepared.

The purpose of the above studies is to estimate the number of eggs and larvae in each plankton haul to the number under a unit area of sea surface and for this the following formula is used.

$$C = 10 (a^{-1} b^{-1} c \cdot d)$$

where 'C' is the number of eggs or larvae beneath a unit surface area (10 square metres in this case); 'a' is the area of the mouth of the bongo net in square metres; 'b' is the length of the tow path in metres; 'c' is the number of eggs or larvae in the sample; and 'd' is the maximum depth of tow in metres.

The value 'a' is derived from the equation:

$$a = \pi r^2$$

The value 'b' is derived from the calibrated flowmeter:

$$b = fr$$

where 'f' is the calibration factor in metres per revolution (m/rev) for a given flowmeter at a given number of revolutions per second; and 'r' is the number of revolutions of the flowmeter during the tow. The value 'd' is determined from the tow data by the equation:

$$d = W \cos (\tan^{-1} \bar{T})$$

Where 'W' is the maximum length of wire out in metres (m); 'T' is the average tangent of the wire angle taken at 30 seconds intervals during the recovery phase of the plankton tow.

Thus, for an example where 'a' is 0.2827; 'b' is 773; 'd' is 199 and if 50 larvae (c = 50) were taken in the sample, the solution to the equation would be:

$$C = 10 \left(\frac{1}{0.2827} \times \frac{1}{773} \times 50 \times 199 \right) \\ = 455 \text{ larvae per } 10\text{m}^2 \text{ sea surface.}$$

The data so estimated may be compiled by area or station and time, with regard to the number of eggs and larvae per unit area (such as 1m² or 1m³ water filtered). Apart from the general information concerning the station such as longitude, latitude, depth, time, weather, etc., the hydrological parameters at different depths may also be noted.

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PRESENT STATUS OF WORK ON MARINE FISH EGGS AND LARVAE
IN INDIA AND OUTLOOK FOR THE FUTURE

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In India, interest on a study of marine fish eggs and larvae is found to have begun only in the first decade of the present century, when Bhattacharya (1916) has identified the larvae of a few estuarine fishes. Although there has been a steady increase in the output of research thereafter, most of the publications till the end of the thirties are on estuarine species only. It may also be seen that in the initial period many identifications are based on those made elsewhere, more so from Indonesia (formerly Java) by Delsman (1922-1938). An analysis of the quantum of publications made recently by Bensam (in press) shows that the main stay so far is from the fifties through the seventies, with the peak during the fifties (30%), followed by sixties (24%) and the seventies (22%). Species-wise also, the maximum coverage is during the fifties (30%), followed by sixties (20%) and seventies (19%). As already pointed out in one of the previous lectures, the total number of species whose one or more developmental stage has been identified so far is 29% of the known total number of species; and the number of species of which most of the vital stages are known at present is only 8% of the marine bony fishes present in Indian Waters. Hence, there is urgent need to intensify the studies on marine fish eggs and larvae in India.

In India, the "hatching method" of identification (vide lecture No.5) of marine fish eggs is possible only in the cases of a few estuarine and inshore fishes such as mullets. But, this method cannot be followed in the vast majority of inshore and offshore fishes because their oozing ovarian ova are difficult to collect. Thus, for a country like India, with the existing facilities, the hatching method of identification is not possible at present and hence the workers have to depend upon the "series method" of identification (vide lecture No.5). For this method to be effective, the whole series of stages should be available, in order to follow the vital changes in the developmental characters. But, a perusal of publications from India shows that only in some cases the whole series are available and that in most cases collections are not adequate to document all the important stages. It may be noted in this connection that the same body of water which contains one developmental stage need not necessarily contain one or more of the other developmental stages (Nellen and Hempel, 1970; Bailey, 1974; Russell, 1976). Hence it has become essential to make future collections of marine fish eggs and larvae much more extensively and intensively in space and time, so that as complete series of stages as is possible are collected for the series method of identification.

Apart from making extensive and intensive collections, as drawn attention to by Ahlstrom and Moser (1981), it is essential to enhance the quality of the specimens collected. Although it is desirable to study live eggs for their characters, in cases where it is not possible to do so, it is essential to improve the quality of the specimens. The value of the material becomes very much lesser if their condition does not facilitate accurate measurements and scrutiny of characters. One method to overcome poor preservation may be to narcotise the material before preserving in formalin. As drawn

attention to by Ahlstrom and Moser (1981) there is need for some basic research on ship-board handling and preservation techniques of marine fish eggs and larvae.

One factor that has been causing some difficulty for effective comparison and contrast of the developmental stages is the ambiguity prevailing in the definition and standardisation of the stages. In this connection, it is worthwhile to follow the division of developing eggs proposed by Ahlstrom and Counts (1955), such as (1) the early egg, (2) the middle egg and (3) the late egg. Also, it would be advantageous to standardise postlarval developmental sequences into three as followed by Moser and Ahlstrom (1970), Ahlstrom et al (1976) and Moser et al (1977), viz., (1) Preflexion, (2) Flexion and (3) Postflexion one is the longest period, involving a gradual development into the juvenile phase. These and other stages standardised are given in Table L

Besides, as seen in an earlier lecture, drawing skills have to be employed for documenting and presenting the figures in a manner, suitable for comparison and contrast (Fig. 1 and 2). Such a procedure has not been followed in most of the descriptions in India; and needs adoption in future work in this country.

A perusal of literature shows that in many instances, except for some prominent diagnostic features, certain subtle or elusive characters are not given due attention for tangible separation of the developmental stages of allied species. The fact that such subtle characters are valuable has been observed in recent studies (Bensam, 1984, 1986) on certain clupeids with overlapping number and disposition of myomeres. One such character is the difference in the pace or speed of development observed in the postlarvae of Sardinella clupeioides and S. slm. Between two almost comparable

sizes, the 10.2 mm postlarva of S. clupeioides and the 10.4 mm postlarva of S. sirm . the former shows markedly lesser developmental sequence in its narrow body, truncated caudal fin and lesser developed dorsal and anal fins when compared with the broader body, forked caudal fin and more advanced dorsal and anal fins in the latter species. Although the former is 0.2 mm shorter than the latter in total length, it is rather insignificant to account for all the above differences. In this connection it is suggested that for segregating comparable and/or similar sized developmental stages of closely allied species and/or genera, a tabulation of the characters of the developmental stages on the model proposed in Table 2 may be carried out. By devising such a mechanism, it may be possible to overcome some of the identification problems.

Similarly, much more intensive studies are required on the variability of such characters of developing stages as the location of the oilglobule and pigmentation between allied species. It is observed in recent studies (Bensam, 1984) among the larvae of the grey mullets Liza tade and L. subviridis which have the same number of myomeres that although the oilglobule in the larvae of both the species is situated in the front aspect of the yolk sac, the principal difference between the two is the presence of four narrow vertical streaks of pigments in L. tade but only a single prominent postanal band in L. subviridis.

In addition to such character differences, it is also essential to discover new characters for identification. Osteological and anatomical features of the early stages of one species may be different from those of an allied species. The advent of scanning electromicroscopy has opened up the possibilities for solving such intricate identification problems. By this method, Sumida et al. (1980) have found out differences between the chorion structure of the eggs of flatfishes. Similarly,

electrophoretic techniques may also be employed for discovering new distinguishing characters.

By adopting such techniques it will be possible to identify, distinguish and document the early developmental stages of such of the species and/or genera which are posing problems still.

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STUDY OF THE ICHTHYOFAUNA OF THE LOCALITY OF WORK

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- (A) Aim: To study the fish fauna at two centres, Mandapam and Pamban by making collections there; to study the characteristic features of various groups; and to identify the important species with the aid of literature (both in the field and in the laboratory)
- (B) Materials: Field note book, bucket with lid, ice box, polythene covers, scissors, scalpels, mounted needles, dividers, pins, measuring boards, scales, H.B.pencil, Indian ink, tags, labels, cotton, lab towels, hypodermic syringe, hand lens, microscope and chemicals such as methylated spirit, concentrated formalin (40%), Hexamine, Borax and Ammonia.
- (C) Methods:
- (I) COLLECTION
1. Visit the landing centre at the time of fish landings.
 2. Collect the different types of fishes from various gears and note the total length, fork length and standard length.
 3. Care should be taken to collect fresh specimens and to avoid mutilated and damaged specimens, unless otherwise interested.

4. Wash the fish thoroughly in sea water without damaging the fish.
5. Colour patterns are species-specific. Note this in the fresh condition itself as it may fade out after preservation.
6. Record details such as date, time, locality, depth, gear, nature of the fishing ground and additional information on the ecology if any, in the field note book. Keep the fish in polythene bags with proper labels or tags.
7. If the laboratory is far away, preserve the specimens collected in formalin in the field itself or keep them in ice box.
8. Bring the fish to the laboratory for detailed examination and preservation. Each specimen may be fixed. Fins, spines, rays etc., may be stretched and fixed by pins with least damage, on a soft wooden board. Do not allow the fish to dry. Add a few drops of formalin on the stretched out fins and rays.
9. Small fishes: If alive, drop them into a mixture of equal parts of methylated spirit and water for five minutes and transfer them to dilute formalin (1 part of concentrated formalin to 6 parts of water).
10. Medium fishes: When dead, in a state of as fresh as possible, place the better side down on a smooth board. By means of pins set all fins, spines and rays. Try to prevent the head from turning up. Make a small incision on the belly on the right side and preserve it in dilute formalin.
11. Large fishes: Apart from making incision on the belly, large fishes may be injected with formalin using a hypodermic syringe. Also some incisions may be made dorsally on both sides of the backbone.

12. If the specimens are to be left for several weeks in formalin, household Borax (1 teaspoon per each $\frac{1}{2}$ gallon of fixative) may be added to neutralise the acidic effect. Other methods are: a) To 1 pint (1 pint = 0.568 litre) of concentrated formalin add 2 oz (1 oz = 28 gm @) ordinary Hexamine and 6 pint water; b) To 1 pint concentrated formalin add 5 pints of water and 3 oz. Ammonia solution.
13. A good quality label is essential. Labels are best written with waterproof carbon ink or with a soft pencil.

(II) IDENTIFICATION

1. General features: Bony fishes are the largest class of living fishes coming under the Sub-Class Neopterygii which is divided into many orders. The characteristic features of the Sub-Class are that they have a firm skeleton of true bones, gillslits are covered with an operculum on both sides, caudal fin is more or less symmetrical and the fishes have vertical or unpaired fins and paired fins.
2. Body shape: Fusiform, oblong, elongated and cylindrical, usually laterally compressed, ventral side more convex than dorsal side; sometimes dorsoventrally flattened with eyes on one side, as in flatfishes.
3. Vertical or unpaired fins: The dorsal fin may consist of a single fin only or composed of a spiny dorsal and a soft dorsal. The spiny dorsal and soft dorsal may be continuous or separately placed. The numbers of spines and soft dorsal rays vary in different fishes. An adipose fin behind the dorsal fin is noticed in some fishes.

Apart from these, some swift and elongate fishes may have small detached finlets behind the dorsal. Note down these characters if any in the specimens collected. In scientific literature, the dorsal fin is abbreviated as D. Number of spines are to be indicated in Roman and soft rays in Arabic numerals, eg., D X 12 means the fish has two dorsals with ten spines in the first dorsal and 12 soft rays in the second dorsal. Follow the abbreviated methodology to identify the fishes.

4. Anal fin: Starts behind the vent. It has many rays and 1 to 3 spines. More than three spines are rarely found. Written as A III 10 means three spines and ten soft rays in the anal fin.
5. Caudal fin or tail fin: It may be pointed, rounded, truncate, emarginate, forked, lunate or wedge shaped.
6. Pectoral fin: The abbreviated form is P. P 16 means that the pectoral fin has 16 soft rays.
7. Ventral fin or Pelvic fin: Usually abbreviated as V. Note the position of the ventral fin in the fishes collected. If the ventral fin is located below the pectoral, they are said to be thoracic; further forward, jugular; and far back abdominal. Generally ventral fin may have one spine and 2 to 5 soft rays.
8. Mouth parts: Most of the fishes have some type of lips. The lower jaw is composed of mandibles and upper jaw is made up ^{of} premaxilla and maxilla. In some cases the maxillary bone may be elongated. The mouth may be at the tip of the snout, or sometimes the snout may overhang the mouth. Mouth may be oblique, with the lower jaw being the longer one; and sometimes it is protractile. Jaws are sometimes produced as beaks. Note down the number and disposition of pores on the chin as well as the number and disposition of barbels

if any. Examine the jaws for teeth pattern. Teeth may be villiform, conical, molariform, or in the form of canines or incisors. Sometimes teeth may be absent altogether. Vomerine and palatine teeth may be present in some fishes.

9. Branchial apparatus: The gill cover or the operculum is made up of four main bones - the opercle, preopercle, subopercle and interopercle. Observe for the presence or absence of scales as well as spines on them.
10. Gillrakers: These are important characters in fishes, seen as finger like or hair like cartilaginous projections of the gill arch, both on the upper and the lower limbs. In systematics these are denoted as GR (5-6) + (12-13), meaning 5-6 gill rakers on the upper limb and 12-13 gill rakers on the lower limb. Count is made on the 1st gill arch.
11. Scales: Note the scales in fishes. Some fishes possess the cycloid with smooth hind margin; and some the ctenoid, with comb like hind margin. See whether the scales are deciduous (easily shed) or adherent type. Count the number of scales in the lateral line (starting from the shoulder to caudal peduncle). Note and count the lateral transverse scales above and below the lateral line. It may be abbreviated as LI. 42, tr 3/9 meaning 42 scales in the lateral line and there are 3 rows above and 9 rows below the lateral line.
12. Lateral line: The position, curve and the branching if any of the lateral line may be noted. Some fishes may not have a lateral line at all.

(III) RECORDING OF DATA

1. After a careful study of the fish/fishes given or collected, note down the various characters in the abbreviated form, for example:-
D. X; I, 28-32. A. II, 8. L. lat. 50. Tr. (5-6) + (10-12). G.R (50-55) + (60-65).

2. Identify the fishes collected using standard identification manuals and texts (vide References)
3. Make an outline drawing of the fishes identified showing the various characters.
4. Briefly describe the important characters of each fish identified, such as body shape, fins, scale counts, lateral line, colouration etc. and also comment on the distribution.
5. Give the conventional hierarchy of nomenclature.
6. Preserve, if necessary, with proper labels.

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DETERMINATION OF THE MATURITY STAGES OF MARINE FISHES

By

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- (A) Aim: To determine the maturity stages of marine fishes based on eye examination.
- (B) Materials: (1) Unpreserved specimens of the species in various length ranges.
(2) Scissors, needles, scales, balance, note book and pencil.
- (C) Methods:
- (1) Cut open the abdominal cavity and spread out the skin sufficiently to permit a full view of the gonads.
 - (2) Note down the approximate proportion of the gonad in relation to the volume of the body cavity excluding the other visceral organs, in percentages such as 15%, 20%, 40%, 70% etc. or 1/3rd, 1/2, 3/4th etc.
 - (3) Record the unpreserved colour of the gonad.
 - (4) Determine whether based on the eye examination you can decide the fish is a male or a female; if so, record the sex.

- (5) If it is not possible to determine the sex, you may record the fact as 'indeterminate'.
- (6) Record the texture of the gonad such as "flat", "blade like", "leaf like", "tubular" etc.
- (7) If it is possible to determine the sex, then repeat the above procedures with specimens of different size groups and group them according to approximate 'stages' based on eye estimation and in consultation with literature (vide infra).
- (8) Usually, the maturity stages of female marine fishes are grouped into seven Stages:- (In total spawners - isochronal seven to eight stages and in partial spawners heterochronal, five stages).
 - (a) Stage I - Indeterminate, occupying about 5 to 10% of the body cavity.
 - (b) Stage II- Pinkish in colour, tubular and granular in appearance, occupying about 10 to 20% of the body cavity (early maturing).
 - (c) Stage III-Pale yellowish in colour, developing eggs fairly visible, the whole gonad occupying about 20 to 50% of the body cavity(maturing).
 - (d) Stage IV -Light pinkish in colour, the eggs increasingly much more visible and the gonad occupying about 40 to 70% of the body cavity, blood vessels prominent (late maturing).
 - (e) Stage V - Whitish in colour, eggs very well visible, not oozing out under gentle pressure and the gonad occupying 50 to 80% of the body cavity (mature).

- (f) Stage VI - Eggs transparent, resembling boiled sago, oozing out under gentle pressure and the gonad occupying 70 to 100% of the body cavity (ripe).
- (g) Stage VII- Ovary reduced in volume to about 20 to 30% of the body cavity, flacid, blood shot in appearance (spent).

(9) In the case of males, the testes are usually pale whitish in colour and thin in appearance through early maturing, maturing and late maturing stages (II through IV). In the mature (stage V) condition, the gonad occupies upto about 80% of the body cavity and is creamy white in colour, with milt not oozing out under pressure. In the ripe condition for males (stage VI), the milt oozes out under gentle pressure of the gonad which occupies about 70 to 100% of the body cavity. And under spent condition for males (stage VII) the gonad has become flacid and small in size about 20 to 30% of the body cavity, pale whitish in appearance and small amounts of milt still oozing out under gentle pressure (spent).

(10) Based on the above seven basic stages, the maturity stages of a few species may be determined and recorded. In some species the stages II to V may not be clearly recognisable and divisible. In such cases only the stages indeterminate, maturing, mature ripe and spent stages can be recognised on eye estimation, thus reducing the stages to only 5, instead of 7.

Note: After the above determination, the gonad may be gently removed from within the body cavity, wiped of the blood and body fluids with cotton and weighed before preservation. This is for the purpose of determining the ratio of the weight of the gonad in relation to the total weight of the fish and the maturing stages.

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DEVELOPMENT OF OOCYTES TO MATURITY

By

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- (A) Aim: To demonstrate the various stages of growth of the oocytes to maturity.
- (B) Materials: Sample of fishes (either fresh or preserved), monocular microscope, ocular and stage micrometers, scissors, needles, forceps, slides, cover slips, 5% formalin, plastic trays, pipettes with teats, towel, duster cloth.
- (C) Methods:
- (1) Cut open the fish from the vent towards the anterior region, examine the gonad and determine the sex.
 - (2) Select female fishes in different maturity stages from immature to spent, based on eye determination of size of gonad.
 - (3) Determine the maturity stage by macroscopic examination, such as colour, extent in the body cavity etc. of the gonads.
 - (4) Cut out anterior, middle and posterior parts of each pair of ovaries and examine the characters of the ova under microscope.
 - (5) Calibrate the ocular micrometer using a stage micrometer.
 - (6) Measure atleast 500 ova taken from each ovary and plot the diameter against the percentage occurrence.

- (7) Repeat the above for the various specimens of each species, group them into different maturity stages of Immature, Maturing, Early Mature, Late Mature, Ripe, Spawning, Spent, etc. in relation to the macroscopic studies you have done in the field and laboratory, vide CMFRI/SI/1989/Pr.II.
- (8) Study closely the characters of ripe ovum, such as overall diameter, diameter of yolk, diameter of oilglobule, etc.
- (9) Make Camera-lucida sketches of some mature and ripe ova, with appropriate scale (Ref.CMFRI/SI/1989/Pr.IX).
- (10) Preserve all the data for future reference.

EVALUATION OF THE MORPHOMETRIC AND MERISTIC
CHARACTERS OF FISHES

By

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- (A) Aim: To determine the body proportions, to prepare adult skeleton, to familiarise with staining technique for small specimens and to count vertebrae, spines and rays.
- (B) Materials: Monocular microscope, camera lucida, measuring boards, scales, dividers, scalpels, forceps, mounted needles, brushes, pipettes, heater, aluminium kettle, sodium hypochlorate, hand lens. For Alizarin staining, the following materials are required: Glacial acetic acid, Glycerine, Chloral hydrate, Alizarin, Petri dishes, embryo cups, cavity slides, cover slips etc.
- (C) Methods:
- (I) Morphometric measurements:
1. If the fish samples collected for the study are preserved, wash them in running water to remove formalin and its smell, for about 1 to 2 hours.
 2. It is better to examine the fish in fresh condition, but this may not be possible at all times. It is

desirable that measurements of freshly caught fish form the basis of comparison until such time as the effects of freezing or chemical preservation on the body proportions have been determined to have constant effects and these effects are finally established. The natural variability of most dimensions is relatively small so that the effect of preservation on differential shrinkage or expansion of different body parts might lead to spurious results if samples of preserved fish from one region are compared with samples of fresh fish from another or if samples of fish from two different regions had undergone different preservative treatment. But, this does not apply when countable (meristic) characters rather than measurements are concerned.

3. Overall length measurements are to be made between perpendiculars along the median longitudinal axis, keeping the mouth of the fish in closed condition.
4. The tail fin has to be extended to give normal (total) length or the tips of one or both the caudal lobes may be drawn to the longitudinal axis of extreme (total) length. Some times total length measurements are made with the caudal lobes partially drawn together so that their outer edges are parallel to each other and to the axis. Follow a uniform pattern throughout the study. If a normal length is the chosen dimension, it is necessary to standardise the procedure of laying the fish on the board. A common method is to place the head of the fish against the nose piece of the measuring board with the right hand, hold the fish in position with the left hand, and use the right hand to straighten the body of the fish and extend its tail with a single stroking movement.
5. Where a choice of sides is involved, all measurements and counts are made on the left side of the fish. When side-to-side comparisons are being made, or if necessary for other reasons, denote by prefixing "r" or "g" (right or

greater) to the notation (or term). This rule may be applicable to Ph and Vh, vide lecture notes III (2), Fig. 3.2.1.

6. Measurements by using calipers and dividers: The tip of the fixed arm of the calipers (or one point of the dividers) is applied to the point mentioned and the tip of the sliding arm of the calipers (or the other point of the divider) is applied to the second point mentioned.
7. Take morphometric measurements of the fishes (Refer lecture notes III (2), Fig. 3.2.1, definitions of position).

II. Meristic counts:

1. Examine and count the number of spines and soft rays in the median fins such as the spiny dorsal, soft dorsal, caudal and anal fins.
2. Count the number of spines (if any) and rays in the paired fins.
3. Count the number of scales in the lateral line and lateral transverse.
4. Count the number of gillrakers in the upper and lower limb of the first gill arch.
5. Count the number of chin pores and barbels.
6. Note the pattern of teeth in the jaws, vomer and palatine.
7. Note the number of pyloric caecae and colouration of the body cavity.
8. Note the shape and structure of the air bladder.
9. Note the number of myotomes and the pigment pattern.

III. Vertebral counts, number and disposition:

1. The vertebral column has to be prepared by boiling the fish in fresh water, just long enough to loosen the tissues from the bones. If the bones appear to be oily

it may be necessary to bleach them in a dilute solution of commercial "Clorax" (Sodium hypochlorite).

2. Note the total number of vertebrae (n) beginning with the 1st vertebra and counting one for each bony segment behind it, including the complex terminal or urostylar segment. (i.e. how many segments there are in the linear series of a vertebral column). This is given in systematic works on fishes, and is extensively employed in biometric investigations on fishable populations.
3. Note for any abnormality i.e., long and irregularly formed segments, which suggests local fusion of adjacent vertebrae. If these non-typical segments are counted as if they are single vertebrae, the total (n) for the back bone proves lower than normal. Complex segments at the posterior end of the back bone are widespread in some fishes.
4. Seeing that (n) is by definition an integer, it can be expressed as the sum of other integers. Group the vertebrae into Precaudal (A) and caudal (B). The precaudals may be again grouped into post cranial (a) and abdominal (b). Caudal vertebrae may be grouped into anterior caudal (c) and posterior caudal (d). This may be expressed as $n = (A + B) = (a + b + c + d)$. The summation of (n) as ($a + b + c + d$) is of practical utility in the study of variation from species to species.
5. Examine each segment of the vertebral column and the differences in the vertebral form which are structural rather than numerical or geometrical differences which may be figured and described rather than counted. These are no less important, although these cannot be expressed in concise mathematical terms.
6. Concerning the number of individuals, the greater the number of individuals examined, the greater the value of the results obtained.

7. Take out the vertebral columns of the fishes given and describe them.
8. Examine the vertebral variations in groups of fishes such as Leiognathids.

VI. Alizarin staining of fish larvae:

The alizarin preparation used to stain the hard parts of large fishes have to be slightly modified for staining the fish larvae and postlarvae, as these are delicate and require special care. Prepare the staining solution by the following formula:

Glacial Acetic Acid	0.5 ml.
Glycerine	3.0 ml.
Chloral hydrate	10.0 ml.
Alizarin stain	100.0 gms.

The fish larvae are to be hardened and kept in 5% formalin. After hardening, wash them, place them in 1 to 2% KOH. After these become transparent, these can be stained. The staining solution is added drop by drop to fresh KOH containing the specimen till it becomes violet - pink in colour. Once these are stained, the used up solution may be pipetted out, fresh KOH solution and increasing quantities of glycerine may be added at regular intervals and the stained material is preserved in pure glycerine.

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Practical guide lines

5. COLLECTION OF PLANKTON

WORK ON BOARD RESEARCH VESSEL

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5.1 Aim: To collect zooplankton from the sea using plankton nets for the study of ichthyoplankton.

5.2 Materials required:

1. Zooplankton net with collecting bucket and weight
2. Meter block
3. Flow meter
4. Inclinator
5. Rubber hose for washing the net
6. Wide mouthed polythene jars of 500 ml capacity
7. Concentrated formaldehyde solution
8. Measuring cylinder (50 ml capacity)
9. Polythene funnel of 15 cm mouth diameter
10. Labels
11. Log sheets
12. Field diary
13. Lead pencil
14. Permanent ink marker pen
15. Stop watch

5.3 Methodology

5.3.1 Procedure for conducting ichthyoplankton surveys

5.3.1.1 The planning stage

The planning stage is likely to be the most important part of the survey for this is where the objectives of the survey are compared with monetary and personnel resources.

5.3.1.2 Field operations

Field operations can be conducted from ships of 15-100 m in length which are equipped to make plankton tows and hydrographic observations.

5.3.1.3. Cruise plan

A cruise plan to satisfy the objectives of the survey may be made in advance. The station positions are to be determined beforehand.

5.3.1.4. Log sheets

The log sheets in which all particulars with regard to the plankton haul are to be entered have to be prepared (see model log sheet given below).

Name of vessel	Cruise No.	Station No.	Date
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Time		Position		Flow meter reading		
Net into water	Net out of water	Lat.	Long.	Initial	Final	Difference

Depth at station	Type of haul	Depth of haul	Warp released
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Net used	Mesh size	Wire angle	Ship speed
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5.3.1.5. Sampling system

The recommended sampling system requires that the ship be equipped with a hydrographic winch with more than 400 m of standard hydrographic wire of 4.8 mm, a meter block or a metering system and an angle indicator to measure the wire angle.

5.3.1.6. The sampler

The Bongo net is recommended as the best type of sampler for ichthyoplankton surveys. Nets of either 20 cm or 60 cm diameter are usually used. The mesh size selected is 0.505 mm.

A flowmeter is mounted in the mouth of one of the net frames to provide data on the volume of water filtered during each tow.

5.3.2.1. The towing procedure and data records

First of all the net may be examined for any cuts or holes and if present may be mended. Ensure that the plankton collecting buckets are securely attached to the net and have no gaps through which plankton may escape. Shackle the towing line to one of the loops at the centre of the yoke of the net assembly. Also shackle the depressor to the other loop on the yoke.

After the gear is assembled and the preshooting data are entered in the log sheet the tow is ready to begin. The tow is made off either side of the ship.

The ship is stopped at the station. After determining the bottom depth the tow depth is decided. To lower the net to 210 m with a wire angle of 45° requires that 300 m of wire be let out (wire angle is defined as deviation from vertical).

Length of wire out X cosine 45° = Net depth
ie. 300 X 0.707 = 210 m (net depth)

The initial reading on the flow meter is to be noted down in the log sheet before shooting the net.

The weight is lowered below the surface of the water. The winch meter is zeroed. The ship is set underway at two knots speed. Now the net is lowered into the water. If the net is set properly in the water release wire at the rate of 50 m/minute. The stop watch is started as soon the flow meter is seen to sink below the surface of water. The stop watch is used to record the sinking time and towing time in seconds. The duration of tow is used for calculation of mean velocity of towing. When the desired amount of wire has been paid out the stop watch is stopped. The sinking time is recorded in seconds. The stop watch is zeroed and restarted immediately. (Since the net is fishing on the way down, sinking time is as important as that of retrieval). When the stop watch is restarted, the nets are left at the desired depth for 30 seconds. At the end of 30 seconds the wire angle is recorded for that depth and retrieval is begun at the rate of 10 m per 30 seconds. Ship speed during sinking, during times at depth and during retrieval is maintained to keep the wire angle at $45^{\circ} \pm 3^{\circ}$ wire angle. Normally 2 knots per hour would maintain this wire angle. (Fig. 5.1.1. Flow meter).

The nets are brought directly out of the water at a steady rate. It is important not to allow the net to fish too long at the surface because of the bias that results from over sampling surface waters. When the flow meter breaks the water surface, the stop watch is stopped and its reading in seconds is recorded as the towing time.

The nets are washed down from the outside using a water jet. After all the plankton has been washed into the cod ends, the nets are brought aboard. The plankton collecting buckets at the cod ends are carefully removed without spilling and taken to the wet laboratory of the ship for preservation.

Before leaving the station, the flow meter is read and recorded as the final readings. The difference between the initial and final readings is calculated and recorded.

Total towing time is recorded. For a 300 m tow total time should be about 21 minutes and 30 seconds (6 minutes sinking time, 30 seconds settling time and 15 minutes retrieval time). Before every haul the net has to be examined for any cut or hole.

5.3.3. Handling the sample at sea

Fish and larvae are fragile and easily damaged. Proper care is needed in all stages of preservation and handling aboard the ship.

5.3.3.1 Preserving the sample

The plankton sample should be preserved immediately especially in tropical waters. The storage container in which the sample is preserved should be of sufficient size so that when filled the preserving fluid (5% formaldehyde solution) will occupy at least three times the volume of the plankton. Wide mouthed polythene jars of 500 ml capacity can be used as container.

The plankton collection is carefully poured from the collecting bucket into the container. The collecting bucket is then rinsed down to gather the last of the plankton. The jar containing the plankton is then filled three fourths full with sea water before adding the preservative. To obtain the recommended 5% solution of formalin in $\frac{1}{2}$ l jar, 25 ml of concentrated formaldehyde is to be added. The sample jar is then filled almost to the top with sea water, capped and shaken lightly to obtain immediate uniform preservation. While plankton fixing is done in 5% formalin, for prolonged storage 3% is enough.

5.3.3.2 Labeling

It is essential that samples are properly labelled. Information contained on the labels should be sufficient to identify the sample with certainty. One label is put inside the jar while a second one placed on the inner lid of the jar

both written with lead pencil and the jar is screw capped with the outer lid. Besides, the details are written outside the jar with a permanent ink marker pen. The labels are made with cartridge paper.

5.3.4. Laboratory procedures

5.3.4.1. Plankton volume determination

A measurement of wet plankton volume, determined by displacement is made for each plankton sample soon after the samples are taken ashore. The zooplankton volume measurement provides a rough measure of zooplankton biomass (Ahlstrom et al., 1969). Larger samples may have to be aliquoted for sorting and the size of the aliquot will often depend on sample size.

The process of determining wet plankton volume by displacement is rather simple. It is done using a specially designed volume determiner. It is a cylindrical apparatus of at least 75 ml capacity and 12 cm height made of perspex whose both ends are open. To one end is attached a piece of plankton netting of the mesh size of .505 mm. The plankton along with the fluid is poured into this apparatus. While the fluid is filtered out, the plankton will remain inside. When the fluid is completely drained off, the apparatus is tightly locked into a special frame so that the netted end becomes leak proof. The open top portion of the apparatus is then covered with a lid having a small hole on one side and a screw-adjusted needle hanging from the centre of the lid. The needle may be adjusted in such a way that its free tip will reach the 50 cc level of the apparatus. A 50 cc burette fixed on a stand is now filled with 5% formalin. The nozzle of the burette is inserted into the volume determiner through the hole on the lid and the fluid is poured along the inner side of the apparatus without bubbling. Continue pouring until the water level touches the tip of the needle. Now note the burette

reading. The water remaining in the burette will be equal to the volume of zooplankton in the volume determiner. (Fig.5.1.2)

5.3.4.2. Percentage of plankton to be sorted

It is recommended that total sample be sorted for fish eggs and larvae whenever possible and that fractioning of sample be limited to those containing exceptionally large numbers of eggs and larvae. The Folsom splitter (McEwen, Johnson and Folsom, 1954) is a standard apparatus for dividing plankton samples into aliquot portions. Normally a minimum of 5 cc sample has to be sorted. (Fig.5.1.3 Folsom splitter and Fig. 5.1.4 whirling splitter)

5.3.4.3. Sorting fish eggs and larvae

Before a sample is sorted, the preserving liquid should be drained off to avoid irritation to eyes and lungs. The sample can be sorted in a very weak formalin solution. If a sample has not been completely sorted out during the day it was started, the unsorted plankton should be put back into 5% formalin. Rough sorting can be done in a 15 cm diameter glass petridish but for finer sorting a counting chamber can be effectively used under a dissecting microscope. The sorted eggs and larvae are preserved in glass tubes. One label has to go into each tube giving the details of the plankton collection. The tubes are filled with preservative to the brim and plugged with cotton. Each tube will contain eggs and larvae collected from one station. All the tubes belonging to a particular cruise can be put together in a large container of either glass or polythene. Enough packing with cotton has to be given to ensure that the tubes do not rub each other and break. Enough cotton should ~~kw~~ be placed also at the bottom of the jar. Finally the remaining space in the jar can be filled with cotton so that the tubes will not displace even while transportation. The jars containing tubes also are to be filled with preservative ie. 3% formaldehyde solution. (Fig.5.1.5 Counting chamber).

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GUIDELINES FOR SORTING OUT EGGS AND LARVAE

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(A) Aim: To familiarise with the methods of sorting out fish eggs and larvae from live and preserved plankton samples.

(B) Materials:

- (i) Fresh live plankton.
- (ii) Clear, filtered sea water in a bucket/can.
- (iii) Binocular microscope.
- (iv) Monocular microscope.
- (v) Embryo cups, 3 - 5 Nos.
- (vi) Petridishes, 5 - 10 cm dia, 3 - 5 Nos.
- (vii) Ocular micrometer, 1 No.
- (viii) Stage micrometer, 1 No.
- (ix) Pipettes, long type with teat, 2 Nos.
- (x) Pipettes, small type with teat, 3 Nos.
- (xi) Formalin, 2% in sea water, 250 ml.
- (xii) Cavity slides, 3 - 5 Nos. and cover slips.
- (xiii) Laboratory towel.
- (xi) Dusting cloth.
- (x) Specimen tubes with bakelite screw cap.

(C) Methods:

- (i) Clean all the glassware first with freshwater and then with filtered sea water, to ensure them free from formalin.

- (ii) Examine a sample of the live plankton under the binocular microscope.
- (iii) observe the important macro characters such as
(a) the size and shape of the eggs, nature of the chorion, presence or absence of oilglobule, size of the oilglobule and perivitelline space if present, nature of yolk, pigmentation on the embryo yolk and oilglobule if present, and (b) shape of the larvae (linear or shorter), position of vent, pattern of pigmentation, presence of fins, arrangement of muscle fibres etc.,
- (iv) Based on the above observation of the macro characters, separate the eggs and larvae showing similarities and are of more or less the same developmental stages into embryo cups containing clear, filtered sea water.
- (v) Place a sample of 5 to 10 eggs/larvae, as the case may be, in a cavity slide in the live condition and their vital characters in various stages (vide lecture notes IV) may be recorded under a monocular microscope under one or more magnifications in the live condition with an adequate number (vide Practical No.7).
- (vi) If the embryo within an egg shows movements of its body (particularly seen in "late" eggs and those ready for hatching) which hampers the study of its characters add one drop of 2% formalin to kill the embryo. Record the characters and measurements rapidly before shrinkage.
- (vii) Since the larvae in live condition move actively, fix them as suggested above to record their characters and measurements.
- (viii) For preservation of eggs/larvae as a record and for future references, fix 5 to 10 eggs/larvae in formalin and keep them labelled in specimen tubes.

GUIDELINES FOR MICROSCOPIC STUDY OF CHARACTERS

By

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- (A) Aim: To familiarise with the microscopic study of the various characters of fish eggs and larvae.
- (B) Materials: As in Practical No.CMFRI/SI/1989/Pr.IV.
- (C) Methods:
- (i) Place the ocular micrometer inside the eye piece of the microscope.
 - (ii) Keep the stage micrometer on the stage of the microscope.
 - (iii) Make a note of the magnification of both the eye piece and the objective.
 - (iv) Synchronise the calibrations in the two micrometers and determine how many calibrations in the ocular micrometer (called Micro Meter Divisions or simply as MMD) are required to synchronise the calibrations of for example 0.5 mm/1 mm/1.5 mm/2 mm in the stage micrometer under the particular eye piece and objective.
 - (v) If X number of ocular micrometer divisions synchronise with, for example 1.5 mm in the stage micrometer, then 1 ocular micrometer division is equivalent to $\frac{1.5}{x}$ mm, under the particular consumption of eye piece and objective magnifications used.

- (v) After determining the specific measurement of one Micro-Meter Division (MMD), remove the stage micrometer, place the egg or larva as the case may be and record the various measurements, such as egg diameter, yolk diameter, oilglobule diameter etc. (for the eggs), total length of the postlarva, head length etc. (for larvae, post-larvae, early juveniles, etc. After determining the measurements in MMD, the value can be converted into millimetre (mm).
- (vii) Record the other characters such as pigmentation, colour of the yolk/oilglobule/etc. in the live condition, ornamentation on the chorion etc.
- (viii) Record the number of rays or spines in all the fins as well as the nature of muscle fibres.
- (xi) Record the number of myomeres before and after the position of vent (preanal and postanal myomeres).
- (x) Study the various characters as well as draw the specimen by using camera lucida (vide Practical No.CMFRI/SI/Pr.IX)

CMFRI/SI/1989/Pr.IX

DRAWING OF LIVE AND PRESERVED FISH EGGS AND LARVAE

By

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- (A) Aim: To familiarise with the microscopic drawing of fish eggs and larvae.
- (B) Materials: In addition to the materials listed in Practicals No.VII, the following:
- (i) Camera lucida and their attachments.
 - (ii) Good quality white paper.
 - (iii) A good pencil, eraser and pencil sharpener.
- (C) Methods:
- (i) Isolate the particular specimen for drawing with the aid of needles or brushes or pipettes.
 - (ii) While dealing with live eggs, ensure that the glassware used are free from formalin.
 - (iii) Mount the eggs/larva in the live/preserved condition in a cavity slide in the required Position.
 - (iv) Ensure that no air bubble(s) are locked up anywhere under the cover slip.
 - (v) Fix the camera lucida in position.

- (vi) Place the white paper alongside the microscope on the table, below the mirror, determine the extent of the figure on the paper and sketch the outline of the various characters including the overall profile of the specimen and pigmentation.
- (vii) Record the magnification of the specimen in the drawing sheet by checking up the magnification of each MMD under the particular combination of eye piece and objective used, vide Practicals No.VII, (B), (i) to (vi).
- (viii) After fully drawing the specimen take out an Indian Ink tracing of it at the time required.
- (ix) For drawing specimens which are very much longer (larger) than the viewing area of the microscope under the particular combination, use eye pieces or objectives of lesser magnifications.
- (x) If the size of the specimen is only slightly longer than the viewing area of the microscope, draw the figure in two parts and join them to get the whole figure.

CMFRI/SI/1989/Pr.X

EVALUATION OF THE CHARACTERS OF EGGS AND LARVAE

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Aim: To evaluate the characters of fish eggs and larvae.

The following characters may be evaluated in relation to the stage of development:-

(A) Eggs:

- (i) Total diameter.
- (ii) Yolk diameter.
- (iii) Extent of perivitelline space.
- (iv) Pattern of pigmentation on the embryo, yolk, oilglobule.
- (v) Ornamentation on the chorion.

(B) Larvae and Postlarvae

- (i) Number and disposition of myomeres.
- (ii) Stage of developmental features in relation to the length of the specimen.
- (iii) Pattern of pigmentation.
- (iv) Origin of various fins.
- (v) Bifurcation of caudal fin.
- (vi) Appearance of rays and spines in fins and the variations in their number.
- (vii) Relative position of dorsal, anal, pelvic and pectoral fins in relation to the length of the

specimen and the stage of development.

(C) Juveniles:

All the morphometric, meristic and colouration pattern of the specimen, as applied in ichthyotaxonomy (vide Practical, IV) are to be taken in to account.

CMFRI/SI/1989/Pr.XI

LIBRARY WORK ON A STUDY OF FISH EGGS AND LARVAE

By

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Aim: To assess whether the material worked out has already been reported in literature.

Procedure:

- (1) The literature recorded in the section on "References" in the Compendium may be consulted.
- (2) Also publications on Fish Eggs and Larvae to the extent available in the libraries which are accessible may be consulted.
- (3) Index cards may be prepared along with brief annotations.
- (4) Cross references may also be consulted.
- (5) Based on such a library work, it may be ascertained whether the material studied has not yet been reported in literature; and if so, gather the literature available on the eggs and larvae of species and genera allied to the material.

FINALISATION AND WRITING UP OF THE WORK ON
FISH EGGS AND LARVAE

By

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(A) Aim: To write up and finalise the work for publication.

(B) Materials:

- (i) The descriptive notes prepared on the material studied by you.
- (ii) Drawings of the eggs and larvae.
- (iii) Literature published on the eggs and larvae of species/genera allied to the ones studied by you.

(C) Procedure:

- (i) Make out an "Introduction", drawing out attention to the importance of the species and a brief review of the existing state of knowledge (literature) on the eggs and larvae of the species as well as of the genera related to it.
- (ii) Write up a section on material and methods giving out the way the work was carried out.
- (iii) Describe the various developmental stages under such subsections as "Eggs", "Larvae", etc. etc. drawing attention to the various salient features and referring to the figures (to be drawn with Indian Ink while sending for publication).

- (iv) In the section on "Discussion", compare and contrast your findings with the work on the eggs and larvae already published on those of allied species and/or genera.
- (v) List out the relevant references.