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Number 9

MANUAL OF RESEARCH METHODS FOR MARINE INVERTEBRATE REPRODUCTION



Issued on the occasion of the Workshop on
MARINE INVERTEBRATE REPRODUCTION
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute, Cochin
held at the University of Madras
from 25th October to 10th November 1982

The Centre of Advanced Studies in Mariculture was started in 1979 at the Central Marine Fisheries Research Institute, Cochin. This is one of the Sub-projects of the ICAR/UNDP project on 'Post-graduate agricultural education and research'. The main objective of the CAS in Mariculture is to catalyse research and education in mariculture which forms a definite means and prospective sector to augment fish production of the country. The main functions of the Centre are to :

- provide adequate facilities to carry out research of excellence in mariculture/coastal aquaculture ;
- improve the quality of post-graduate education in mariculture ;
- make available the modern facilities, equipments and the literature ;
- enhance the competence of professional staff ;
- develop linkages between the Centre and other Institutions in the country and overseas ;
- undertake collaboration programmes ; and
- organise seminars and workshops.

Under the programmes of the Centre, post-graduate courses leading to M.Sc. (Mariculture) and Ph.D. are offered in collaboration with the University of Cochin since 1980.

Front cover : SEM picture showing surface topography of *Streptocephalus dichotomus* egg.

Manual of Research Methods for Marine Invertebrate Reproduction

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MARINE FISHERIES RESEARCH INSTITUTE HELD AT THE UNIVERSITY
OF MADRAS FROM 25TH OCTOBER TO 10TH NOVEMBER, 1982.

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PREFACE

The technologies of controlled reproduction, induction of spawning, sex reversal, artificial fertilisation, sterilisation and preservation of gametes are increasingly applied in aquaculture to obtain quality seed, quality fish stock and better yield. In this context, researches on different aspects of reproduction, developmental biology and physiology have assumed considerable importance besides their values in understanding of the ontogeny of the organisms. Extensive researches carried out in recent years from several laboratories in the world have not only accumulated a body of information, but also brought forth several new concepts to our understanding of the development and reproductive behaviour of finfishes and shellfishes.

In India, directed research on reproductive physiology and biology is taken up only recently and the field is still in an infant stage. In view of its emerging importance, it is identified as an area for priority research and for expertise development in the programmes of the Centre of Advanced Studies in Mariculture at the Central Marine Fisheries Research Institute, and several programmes of research are being taken up in this field with particular reference to the reproductive behaviour of the culturable finfishes and shellfishes.

Advances made on the frontiers of invertebrate reproduction in recent years have been significant enough to organise a national workshop and to prepare a manual on research methodologies for the study of the subject. Several histological, histochemical and biochemical methods and sophisticated instruments have been introduced in these studies making it essential that the scholars who desire to work and specialise in the field are given adequate basic information on the research methods so as to enable them to appreciate and advance research to understand the problems confronted in the field.

The present manual, the third in the series, is prepared and compiled by Dr. T. Subramoniam, Leader of the 'Unit of

Invertebrate Reproduction' of the Zoology Department of the University of Madras, Tamil Nadu. During the past decade, a team of research scholars are working on different aspects of marine invertebrate reproduction including the cultivable crustaceans such as *Scylla serrata*, *Panulirus homarus* and *Macrobrachium* spp. under his leadership. Contributing to our knowledge on the subject, the research results achieved so far in these aspects by the Unit have unfolded several new concepts in oogenesis, spermatogenesis, sperm transfer strategy, fertilization and endocrine control of reproduction and gamete formation.

I wish to express my great appreciation to Dr. T. Subramoniam and his team of Scholars, who by their dedication and interest evolved a series of tested research methods and set a theme of investigation through insight and skill on marine invertebrate reproduction. I am sure that this manual will be of immense use to the research scholars and scientists who would like to specialise in the subject and cognate fields.

This is the second workshop we are organising in close collaboration with the University of Madras. I wish to express my gratitude to Dr. M. Santappa, Vice-Chancellor, University of Madras for the keen interest evinced in such collaborative programmes and for the advice. I am also indebted to Dr. K. Ramalingam, Professor and Head of the Department of Zoology, University of Madras for productive discussions, continuous support and suggestions. I wish to thank Shri P. T. Meenakshisundaram and Shri K. Rengarajan, Scientists of the Central Marine Fisheries Research Institute for their help in the preparation of this manual.

E. G. SILAS,
Director, C.M.F.R.I.

VI. REPRODUCTIVE ECOLOGY

**DETERMINATION OF REPRODUCTIVE
PERIODICITY IN THE INTERTIDAL MOLE
CRAB *EMERITA ASIATICA****

20.1. INTRODUCTION

Food supply may be regarded as the primary factor controlling growth rate and egg production in the natural populations of Crustacea (Wenner *et al.*, 1974). The faster growth rate can also lead to an altered age/size at sexual maturity. Thus environmental factors not only influence the percentage of berried female in a population, but also the size of sexual maturity. Therefore, in determining the reproductive cycle of any population of Crustacea, the minimum size at sexual maturity must be considered first. Among the various methods employed to determine the reproductive activity of marine invertebrates (Giese, 1959), by far the gonad index is the most common. This is calculated as the ratio of the gonad wet weight (or volume) to the wet weight (or volume) of the whole animal, expressed as a percentage (Giese and Pearse, 1974). Since most of the decapod crustaceans carry the eggs in the pleopods till hatching, the percentage occurrence of ovigerous females in a population can also be used as an index of reproductive activity. Again, the rate of egg development also varies according to the sea water temperature and hence an assessment of the mean egg developmental rate is also necessary for correctly assessing the reproductive activity. In this experiment various methods as applied to estimating the female reproductive cycle of an anomuran crab *Emerita asiatica* are described.

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20.2. METHODS

20.2.1. Determination of stage at sexual maturity

Collect female crabs of different sizes and measure the carapace length (CL).

Find out the lowest size class of the ovigerous females in a population. This should be determined for all the samples collected throughout the year.

Determine arbitrarily the size range at which the females begin to lay eggs (In *Emerita* 19 to 22 mm CL). For gonad index and other methods, consider only females above this size range.

20.2.2. Incidence of ovigerous female

Find out the percentage of ovigerous females in the samples collected in different months of the year. Plot the percentage of ovigerous females against time.

20.2.3. Egg mass index

Wash the crab to remove the adhering sand particles and weigh in a physical balance.

Remove the pleopods along with the attached eggs and weigh after blotting dry.

Strip off the eggs from the pleopodal hairs and determine the weight of the pleopod. The weight of the eggs can be calculated by subtracting the weight of the pleopods from the initial weight of the pleopods with attached eggs.

Calculate the egg mass index using the formula :

$$\text{Egg mass index} = \frac{\text{Weight of the egg}}{\text{Weight of the body}} \times 100$$

20.2.4. Mean egg developmental stages (MEDS)

To find out the mean egg developmental stages, determine the different stages in the egg development occurring in the pleopod using the key given in Table 1.

Classify the eggs of the collection sample into one of the ten developmental stages (Table 1). Suppose we have a sample

TABLE 1. Classification of Egg Development in *Emerita asiatica*

Stage	Description
I	Yellow yolk granules seen ; egg mass bright orange in colour.
II	Cleavage has taken place and blastomeres are seen ; egg mass bright orange in colour.
III	A yolk-free white streak makes its appearance in the animal pole ;
IV	One quarter of the yolk cleared ; the white band encircles the yolk material which is now in the centre ; at the animal pole a periodic twitching is recognized ; red pigment spots are seen at the edge of the yolk ; colour of the egg mass is dull orange.
V	One third of the yolk cleared ; two eye spots appear ; red spots prominent and seen at the end of the animal pole ; colour of the egg mass dull orange.
VI	Egg mass brownish orange in colour ; eyes well developed ; yolk is found in the vegetal pole ; two thirds of the yolk cleared ; red pigments seen all over the white space.
VII	Egg mass greyish orange in colour ; yellow yolk is found as two clusters in the centre ; appendages of the embryo are developed ; heart beat seen ; eye spots very well developed.
VIII	Egg mass pale grey in colour ; colourless yolk in the form of oil globules seen just below the eyes as two pockets ; heart beat more prominent ; embryo almost completely developed.
IX	Embryo fully formed ; egg mass white in colour ; no yolk globules seen ; about to be released.
X	Released zoea larvae.

Adapted from Subramoniam (1979).

collection of ten animals in January, 3 of them falling into 3rd stage, 2 of them falling into 5th stage and 5 of them falling into 8th stage, then the frequency distribution for the 10 animals will be represented as follows :

Month	No. of animals	Developmental stages										MEDS± Sd
		1	2	3	4	5	6	7	8	9	10	
Jan.	10	-	-	3	-	2	-	-	5	-	-	

Compute the MEDS using the formula

$$\text{MEDS} = (\sum Si \times fi) / \sum fi$$

where Si = stage 1, 2, 3,, 10 and

fi = the number of animals falling in the i^{th} developmental stages.

\sum = stands for the summation for all 10 stages.

From the above example

$$\begin{aligned} \text{MED} &= \sum (Si \times fi) / (\sum fi) \\ &= 59/10 \\ &= 5.9 \end{aligned}$$

Standard deviation (Sd) can be computed using the formula

$$\text{Sd} = \frac{(\sum Si^2 \times fi) - (\sum Si \times fi)^2 / (\sum fi)}{(\sum fi - 1)}$$

Find out the MEDS for all the 12 months in a year.

20.2.5. Gonad and hepatic indices

Dissect the crab and drain away the blood completely.

Carefully remove the ovary and hepatopancreas and transfer them separately to previously weighed cellophane paper without blotting. Blotting the tissue with the filter paper will result in the mature ovary containing fully ripe oocytes sticking to the paper.

Weigh the ovary and hepatopancreas in a monopan balance to the nearest 0.1 mg.

Calculate the gonad as well as hepatopancreatic indices by expressing their respective weights as a percentage of total

weight of the animal. For the ovigerous female the weight of the animal excludes the weight of the pleopodal eggs.

20.3. OBSERVATION

Plot the values of the egg mass index, MEDS, gonad and hepatic indices in the same graph and delineate the breeding intensities for atleast three consecutive years.

20.4. REFERENCES

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