



CMFRI SPECIAL PUBLICATION

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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CENTRE OF ADVANCED STUDIES IN MARICULTURE, CENTRAL
MARINE FISHERIES RESEARCH INSTITUTE HELD AT THE UNIVERSITY
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(LIMITED CIRCULATION)

Published by: **E. G. SILAS**
Director
Central Marine Fisheries
Research Institute
Post Box No. 1912
Cochin 682 018

PRINTED IN INDIA
AT THE DIOCESAN PRESS, MADRAS-7—1982 C3626

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.

PERMEABILITY STUDIES AND DEHISCENCE OF SPERMATOPHORES*

11.1. INTRODUCTION

The mechanism of release of sperm from crustacean spermatophores has long been debated. Many factors such as external physical pressure, imbibition of water by substances within and an oviducal secretion have been suggested to be responsible for the opening up of spermatophores (Mouchet, 1931; Bloch, 1935; Subramoniam, 1977). In other forms, such as lobsters where the spermatophore is in the form of a complex gelatinous ribbon, the powerful chelae of the fifth leg is used for breaking the spermatophore and then gouges it open, thus releasing the spermatozoa (Fielder, 1964). In *Scylla serrata*, the free spermatozoa are seen in the spermathecal smear only at the spent stage of female (Ezhilarasi & Subramoniam, 1982). This suggests that the entry of spermathecal or ovarian fluid into the spermatophores may bring about the dissolution of spermatophore layers and spermatophoric substances. In the light of the above observations, permeability experiments using acids, alkalies and vital dyes have been made on the spermatophores of *Scylla serrata*.

11.2. PERMEABILITY STUDIES ON SPERMATOPHORES

11.2.1. Reagents

1. *Glacial acetic acid*
2. *Concentrated hydrochloric acid*
3. *Concentrated sulphuric acid*
4. *4% Sodium chloride*: 4 gm of sodium chloride in 100 ml of distilled water.

* Prepared and verified by K. Uma and T. Subramoniam, Unit of Invertebrate Reproduction, Department of Zoology, University of Madras, Madras-600 005.

5. 2% Potassium hydroxide : 2 gm of potassium hydroxide in 100 ml of distilled water.

6. 4% Sodium hydroxide : 4 gms of sodium hydroxide in 100 ml of distilled water.

11.2.2. Procedure

1. Collect the freshly separated spermatophores in a cavity slide.
2. Treat the spermatophores with weak acids, strong acids, sodium chloride solution and alkaline solutions.
3. Tabulate the results

Chemicals	Changes occurring in the spermatophores
Acetic acid	
Conc. hydrochloric acid	
Conc. nitric acid	
4% Sodium chloride	
2% Potassium hydroxide	
4% Sodium hydroxide	

11.3. DEHISCENCE OF SPERMATOPHORES

11.3.1. Reagents

1. 0.1% Basic fuchsin
2. 0.1% Congo red
3. 0.1% Toluidine blue
4. 0.1% Methylene blue

(For preparation of the above solutions *vide* section 8.2.1)

11.3.2. Procedure

1. Add 0.1% solutions of basic fuchsin, toluidine blue, methylene blue and congo red to the spermatophores.
2. Observe the spermatophores using compound microscope after 5 minutes, 10 minutes and 15 minutes and note the colour intensity of layers and sperm mass.

3. Record the staining reactions of the spermatophore layers in the Table given below.

Dyes	5 minutes		10 minutes		15 minutes	
	SL	SM	SL	SM	SL	SM
0.1% Basic fuchsin						
0.1% Congo red						
0.1% Toluidine blue						
0.1% Methylene blue						

if there is no colour (—) ; if there is light colour (+) ; if the colour is intense (++) ; SL—Spermatophore layer ; SM—Sperm mass.

11.4. REFERENCES

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