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 competance 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

EDITED BY

**T. SUBRAMONIAM**

*Unit of Invertebrate Reproduction, Department of Zoology, University of Madras, Madras-600 005*


**CMFRI SPECIAL PUBLICATION**

**Number 9**

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

(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 broughtforth 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 cultivable 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.

# III. SPERM MORPHOLOGY AND SPERMATOPHORES OF CRUSTACEA

# 8

# *IN VITRO* OBSERVATION ON SPERM MORPHOLOGY IN A FEW DECAPOD CRUSTACEANS*

## 8.1. INTRODUCTION

Based on the classification of Afzelius (1971), the spermatozoa of marine invertebrates can be categorized into 1) primitive 2) simplified 3) modified and 4) atypical type. He has also correlated the morphological features of the spermatozoa with the type of fertilization. Decapod crustaceans are peculiar in possessing non-flagellate, vesiculiform spermatozoa (Nath, 1956). Among the different groups of decapods, the morphology of the spermatozoa is highly variable and is species-specific. The organisation of these vesiculiform spermatozoa also differs from that of the typical spermatozoa in that it is not possible to discern the typical structures such as head, mid piece and tail. In general, the acrosomal vesicle, under light microscopic observation, is found to be present in the centre of the sperm cells, when viewed from above. The mitochondria are completely fragmented and are found mixed with chromatin granules, forming the outermost layer, namely nuclear-mitochondrial nebenkern. In between the central acrosomal vesicle and the nuclear mitochondrial nebenkern are found two vesicles (primary and secondary). Though immotile, decapod spermatozoa possess variable numbers of rays or spikes. These structures are not to be confused with the flagellum of the typical spermatozoan, as they lack the 9 + 2 filamental pattern.

## 8.2. MATERIALS

Live specimens of *Emerita asiatica, Albunea symnista, Clibanarius longitarsus, Penaeus indicus* and *Scylla serrata.*

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

**8.2.1. Reagents**

1. 1%*Neutral red* : Dissolve 1 gm of neutral red in 100 ml of distilled water.
2. 1%*Congo red* : Dissolve 1 gm of Congo red in 100 ml of distilled water.
3. 0.1%*Acetocarmine* : Dissolve 100 mg of acetocarmine in 100 ml of distilled water.
4. 0.1%*Methylene blue* : Dissolve 100 mg of methylene blue in 100 ml of distilled water.
5. 0.1%*Fast green* : Dissolve 100 mg fast green in 100 ml of distilled water.
6. 0.1% *Basic fuchsin* : Dissolve 100 mg of basic fuchsin in 100 ml of distilled water.
7. 0.1% *Janus green* : Dissolve 100 mg of Janus green in 100 ml of distilled water.
8. 0.1% *Bromophenol blue* : Dissolve 100 mg of bromophenol blue in 100 ml of distilled water.
9. 1% *Periodic acid* : *Vide* section 2.5.1.
10. *Schiff's reagent* : *Vide* section 2.4.3.

8.3. METHOD

**8.3.1. Staining with vital dyes**

1. Dissect the specimen and separate the male reproductive system.
2. Cut a bit of the distal portion of the vas deferens to separate the spermatophoric components.
3. Transfer to a clean slide and apply a coverslip on it and press gently.
4. Add a few drops of prepared vital stain (*e.g.* 0.1% methylene blue) at the edges of coverslip and allow them to stain the material for a few minutes.
5. Wipe off the excess stain before observation.

The same procedure is used for different materials with different stains.

### 8.3.2. Periodic acid-Schiff method (Pearse, 1968)

1. Treat the prepared fresh smear in 1% periodic acid for 10 minutes.
2. Wash in running water for 5 minutes.
3. Immerse in Schiff's reagent for 10 minutes.
4. Wash in running water for 5 minutes.
5. Observe the intensity of magenta colour.

## 8.4. Observation

After staining with the vital dyes, describe the morphology of the spermatozoa of the crustaceans given above. Find out the differences in the number as well as the shape of the spikes/rays. Also, distinguish and characterize the various organelles from specific staining reaction. PAS positivity is obtainined in the acrosomal region. Nuclear mitochondrial nebenkern will specifically stain with Janus green and the nuclear stains. The vesicles will remain unstained.

## 8.5. References


Afzelius A. B. 1971. Sperm morphology and fertilization biology. *Proc. Int. Symp. The Genetics of the spermatozoon. Edinburgh.*, pp. 16-20.

Nath, C. 1956. Cytology of spermiogenesis. *Int. Rev. Cytol.*, *V*. 395-453.

Pearse, A. G. E. 1968. *Histochemistry : Theoritical and applied J. & A. Churchill Ltd.*, London, 3rd edition, Vol. I, pp. 758.