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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 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

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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.

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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 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.

13

ELECTROPHORETIC SEPARATION OF PROTEIN FRACTIONS OF SEMINAL SUBSTANCES OF *SCYLLA SERRATA*•

13.1. Introduction

Among crustaceans, the cirripede seminal plasma has been reported to contain a large quantity of proteins (Barnes and Blackstock, 1974). In *S. serrata* too, the seminal plasma il proteinaceous (Uma, 1982). Since a copious quantity of seminas plasma is transferred to the female reproductive tract and stored in the spermatheca for a long time, the seminal plasma is suggested to act as a nutrient medium for sperm maintenance. The present experiment is designed to verify this supposition by electrophoretically separating the proteins of seminal plasma stored in the vas deferens as well as in the spermatheca of the mated females.

13.2 Method of Collection of Seminal Plasma and Spermatophores

(*Vide* Expt. No. 12)

13.2.1. Sample preparation

Seminal plasma : Dilute 0.1 ml of seminal plasma with 1 ml of 40% sucrose and from this take 0.2 ml of sample per one gel tube.

Spermatophores : Homogenise 100 mg of spermatophores with 1 ml of 40 % sucrose, centrifuge and take 0.2 ml of supernatant.

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

13.3. Collection of Spermathecal Fluid

In *S. serrata*, the prepubertal females having a white thread like ovary is always unmated, which is evidenced from the absence of spermatophores in the spermathecal smear. However when the ovary matures to stage II, spermathecal smear is rich in spermatophores. Mated *S. serrata* is available from stage II to IV of ovarian maturation (Ezhilarasi, 1978 ; Ezhilarasi and Subramoniam, 1982).

13.3.1. Method of collection of spermathecal fluid from virgin crabs

Remove the entire spermatheca from the virgin female (stage II—ovary) ; wash the spermatheca with distilled water to remove the adhering haemolymph.

Transfer it into a clean watch glass ; puncture the spermatheca and collect the contents.

Dilute 0.2 ml of this with 1 ml of 40% sucrose. From this take 0.2 ml for one gel tube.

13.3.2. Method of collection of spermathecal fluid from the mated female crabs

Remove the spermatheca from the mated females (Stage II—ovary) ; wash the spermatheca with distilled water, transfer it into a clean watch glass. Puncture it ; collect the contents, contrifuge at 2000 rpm for 5 minutes to separate the fluid free from the spermatophores.

Dilute 0.2 ml of supernatant (spermathecal fluid) with 1 ml of 40% sucrose and take 1.2 ml for one gel tube.

Homogenise 150 mg of the spermatophores taken from the mated spermatheca with 2 ml of 40% sucrose, centrifuge, take 0.2 ml of the supernatant for one gel tube.

For electrophoretic method (Davis, 1964) *vide* Expt. No. 3.

13.4. References

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