



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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MARINE FISHERIES RESEARCH INSTITUTE HELD AT THE UNIVERSITY
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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 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.

**EYESTALK LIGATION EXPERIMENTS ON THE
FAIRY SHRIMP *STREPTOCEPHALUS DICHOTOMUS****

18.1. INTRODUCTION

The endocrine system of lower crustaceans such as anostracan also shows a similarity with that of decapod crustaceans, especially in the possession of a stalked eye in which is present the X-organ sinus gland complex (Lake, 1969). In the fairy shrimp *Streptocephalus dichotomus*, the sinus gland is located inbetween the lamina ganglionaris and the optic medulla. The cells in this gland show variation in their secretory contents during reproductively quiescent and reproductively active periods (Munuswamy, 1982). The effect of eyestalk ligation on vitellogenesis depends on the phase of the annual reproductive cycle (Weitzman, 1964). The present experiment is designed to study the effect of eyestalk ligation on ovaries and clutch formation, during different phases of the female reproductive cycle of the fairy shrimp *S. dichotomus*.

18.2. MATERIALS

Specimens of *S. dichotomus* (males and females).

18.3. PROCEDURE

1. Select 3 sets of fairy shrimps of different maturity.
 - Group I* : immature stage (10-12mm)
 - Group II* : mature stage (15-25 mm)
 - Group III* : spent stage (15-25 mm)
2. Prechill the animals for a few seconds in ice.

Prepared and verified by N. Munuswamy and T. Subramoniam, Unit of Invertebrate Reproduction, Department of Zoology, University of Madras, Madras-600 005.

3. Ligate the eyestalks of the above three groups of fairy shrimps using human hairs ; loope around the base of the eyestalk.
4. Maintain experimental as well as control females (without ligation), in separate finger bowls with pond water.
5. Perform all the experiments under normal day light condition.
6. Do not feed the fairy shrimps during the experiments.

18.4. OBSERVATION

Observe the nature of ovary, shell glands, ovisac and frequency of clutch formation in the three groups of fairy shrimps. Compare the results with that of control groups.

18.5. INFERENCE

As the reproductive cycle of the fairy shrimp *S. dichotomus* is short (48 hours), the efficacy of the ligation could be evaluated within 6 to 15 hours after ligation. Direct observation on the changes occurring in the appearance of eggs in the lateral part of the oviduct, eggs in the ovisac and the secretory nature of the shell gland could be made through the transparent body wall.

18.6. REFERENCES

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