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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.

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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.

MARINE INVERTEBRATE REPRODUCTION: AN EXPERIMENTAL APPROACH*

Reproductive biology is central to biological science and an understanding of it is vital to proper animal management. Since marine invertebrates have representations in all phyla, some of them exclusively, their reproductive biology has been studied with the aim of extending the basic ideas over a wider phyletic and environmental range. Experimental studies on invertebrate reproduction in the past were limited to insects alone inasmuch as they formed the major agricultural pests. A proper control of them, however, entails a thorough knowledge of the sexual, reproductive and developmental biology.

With the advent of intensive aquaculture of useful marine invertebrates such as prawns, crabs and molluscs, not only a basic knowledge of the reproductive process of these invertebrates but an experimental approach to problems such as extrinsic and intrinsic factors controlling reproduction is very much in need. Basic information relating to reproductive periodicity, fecundity and mode of fertilization in the unstudied candidate species of marine invertebrates is, however, helpful in identifying the potential species for the purposes of aquaculture.

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

The main area in which the experimental studies have been concentrated is the endocrine manipulation of reproductive activities such as gonad maturation and spawning. Again, gametogenesis, the central event in the reproductive cycle, is shown to be controlled by endocrine processes that differ markedly among the various invertebrate groups (Highnam, 1978). Perhaps, crustaceans have received the maximum attention in this regard in view of their aquaculture importance as well as the easy way in which the endocrinological manipulation can be achieved. For example, the localization of gonad inhibitory hormone in the eyestalk of decapod Crustacea is helpful in the easy extirpation of this source by simply ablating the eyestalk without much injury to the organism (Panouse, 1943). Similarly, implantation of neurosecretory organs such as brain and thoracic ganglia as well as grafting of ovary and androgenic glands have also been successful. In fact, most of our information on the endocrine regulation of reproduction in Crustacea has been obtained by such experimental studies (Subramoniam, 1981). A recent trend in the study of invertebrate endocrinology is organ culture, allowing the direct action of hormones on target tissues without interference from other systems (Gomot **et al.**, *1980).*

Among the marine invertebrates, the reproductive adaptations are in accordance with the life style of the organisms concerned. It is therefore difficult to generalize on the research methodologies for different groups of marine invertebrates. Bearing this in mind, the research methods given in this manual

have been so designed as to place the emphasis on the accurate assessment of morphological, structural and biochemical parameters used in the study of reproductive biology of typical marine invertebrate forms. The possibility of extending these methods to other invertebrates, showing variations in the reproductive anatomy and physiology is also explored.

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