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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.

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Y-ORGANECTOMY IN THE CRAB, *SCYLLA SERRATA**

16.1. Introduction

Gabe (1953, 1956) first described the Y-organ in 58 species of Malacostraca. The Y-organ is a glandular structure of epidermal origin. It is located in the antennary/maxillary segments. The Y-organ varies in gross morphology and location in different species of crustaceans (Spindler *et al.*, 1980). It is conical in the Brachyura, tenticular in the Natantia and foliaceous in Isopoda. In position, appearance and function, the Y-organ is similar to the prothoracic gland of insects. In Crustacea, there are two types of Y-organ. They are (1) Y-organs isolated from the hypodermis (brachyurans) and (2) Y-organs connected to or even integrated into the hypodermis (macrurans).

Bilateral removal of the Y-organs prevents molting in crustaceans and reimplantation leads to the resumption of the normal molting process (Echalier, 1954, 1955, 1959 ; Passano and Jyssum, 1963 ; Burghause, 1975). The molt inhibiting hormone (MIH) from the X-organ/sinus gland complex controls the synthesis and secretion of molting hormone (ecdysone) from the Y-organ (Kleinholz and Keller, 1979 ; Spindler *et al.*, 1980).

In recent years, the importance of the ecdysial glands of Crustacea namely the Y-organ has been realised in the reproductive process especially the multiplication of oogonial cells and the stimulation of vitellogenin synthesis in the extra-ovarian sites (Arvy *et al.*, 1956 ; Demeusy, 1962).

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

In this experiment the method of Y-organ ablation closely follows the technique of Echalier (1959).

16.2. Material

Alive crab, *Scylla serrata*.

16.3. Reagents

Methylene blue solution : Dissolve 0.2 gm of methylene blue in 100 ml of distilled water. Add 10 ml of the above solution to 90 ml of 0.9% physiological saline (0.9 gm sodium chloride in 100 ml distilled water).

16.4. Procedure

1. Hold the specimen immobile on a dissection board with the face uppermost under a dissection microscope.
2. Place the dissection board in a tray containing 0.9% physiological saline.
3. Cut a 2.5 mm square piece of the pterygostomian region of the exoskeleton, bordering on the ventral edge of the sub-orbital region and directly below the cornea of the stalked eye by means of a dental drill.
4. Incise the epidermis on three edges of the hole and lift back carefully, revealing the Y-organ which is attached to the epidermis (The Y-organ has a yellowish appearance against the bluish white colour of the surrounding muscle and connective tissue.
5. Remove the Y-organ with sharpened watch maker tweezers.
6. Replace the epidermis in the original position and seal with molten paraffin wax.
7. Keep the organ in a cavity block with the physiological saline containing one drop of methylene blue solution.
8. Observe the gland under low magnification.

16.5. Results

The cells of the Y-organ with secretory products stain deep blue.

16.6. References

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