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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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DETECTION AND CHARACTERIZATION OF ESTERASE ISOZYME BY DISC GEL ELECTROPHORESIS USING INHIBITORS*

27.1. Introduction

The non-specific esterases include different types of esterases, acetyl, aryl, carboxyl and cholin esterases. These forms are identified on the basis of their differential activity towards various inorganic inhibitors (Holmes and Master, 1968 ; Dickinson and Johnson, 1978). Similarly isozymes of a type of esterase are detected by the differences in their molecular weight, as shown by their relative mobilities in disc gel electrophoresis (Ruddle and Harrington, 1976). Knowledge on the occurrence of different types of esterases and isozymes of esterases helps to pinpoint their functional role in the lipid metabolism in different tissues.

27.2. Principle

Non-specific esterases are fractionated on polyacrylamide gel and stained by the method of Ruddle and Harrington (1976). The coupling reaction products of napthyl substrate with non-specific esterases are stained with a diazoniam salt Fast blue RR. As the dye is more stable at the neutral pH of the phosphate buffer (Kapin and Ahamad, 1980 ; Ezhilarasi, 1982), staining of esterases are carried out only in pH 7.0 of phosphate buffer.

27.3. Material

Eggs of *Emerita asiatica.*

27.4. Inhibitors

To inhibit the activity of different esterases, the following inorganic inhibitors are used in the specified concentration.

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

1. *p-chloromercuric benzoic acid* (p-CMB) : 10^{-1}M (0.1 M)*
2. *Ethylene-diaminetetra acetic acid* (EDTA) : 10^{-1}M (0.1 M)
3. *Malathion (Organophosphate)* : 10^{-2}M (0.01 M)
4. *Silver nitrate* ($AgNO^3$) : 10^{-2}M (0.01 M)
5. *Eserine sulphate* : 10^{-2}M (0.01 M)

TABLE 1. Key for identification of different species of esterases based on inhibition reaction

Chemicals		acetyl	aryl	carboxyl	cholin
P-CMB	..				
EDTA	..		— —		
Malathion	..			— —	— —
$AgNO_3$	..	—	— —	— —	
Eserine sulphate	..				— —

— Partial inhibition; — — Complete inhibition (Holmes and Master, 1968 ; Dickinson and Johnson, 1978).

27.5. PROCEDURE

27.5.1. Fractionation of Non-specific Esterases

Follow the same procedure as given in Expt. No. 3.

27.5.2. Staining of Esterases

Incubate the gels in 5 ml of staining mixture (Dissolve 40 mg of α-napthyl acetate in 1 ml acetone. Add 99 ml of M/15 phosphate buffer (pH 7.0). Dissolve 70 mg of Fast blue RR in the above solution at 37°C and store in 5 : 5 : 1 ratio of methanol, water and acetic acid).

* Molar solutions are prepared by dissolving the chemical (Gram molecular weight of the chemical X desired molar) in 1 litre double distilled water.

27.5.3. Characterization of Esterases

1. Before staining for esterases incubate the gels in the inhibitor solutions for 30 minutes separately.
2. Wash in double distilled water.
3. Stain for esterases.
4. For each experiment maintain a control without treating in the inhibitor solution.
5. Visually compare the effect of inhibition by the inhibitors and tabulate the results based on Table 1. (Oxford, 1973).

27.6. Observation

Detect the different types of esterases and isozyme of esterase in the given sample.

27.7. References

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1. Manual of Research Methods for Crustacean Biochemistry and Physiology—CMFRI Special Publication No. 7, 1981, 172 pp.

2. Manual of Research Methods for Fish and Shellfish Nutrition—CMFRI Special Publication No. 8, 1981, 125 pp.

3. Manual of Research Methods for Marine Invertebrate Reproduction—CMFRI Special Publication No. 9, 1982, 214 pp.

Back cover : SEM picture showing surface topography of *Branchinella kugunumaensis* egg.