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 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

EDITED BY

T. SUBRAMONIAM

Unit of Invertebrate Reproduction, Department of Zoology,
University of Madras, Madras-600 005

CMFRI SPECIAL PUBLICATION

Number 9

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.

(LIMITED CIRCULATION)

Published by: **E. G. SILAS**
Director
Central Marine Fisheries
Research Institute
Post Box No. 1912
Cochin 682 018

PRINTED IN INDIA
AT THE DIOCESAN PRESS, MADRAS-7—1982 C3626

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.

26

QUANTITATIVE ASSAY OF NON-SPECIFIC ESTERASES IN THE DEVELOPING EGG OF *EMERITA ASIATICA**

26.1. Introduction

It is a well known fact that crustacean yolk contains a considerable quantity of storage lipids to be utilized during embryogenesis. Esterases are the main hydrolytic enzymes responsible for converting complex storage lipids into easily utilizable glycerides and free fatty acids. Esterases exist as isozymes which could be characterized histochemically after separating them on polyacrylamide gel. Different species of the non-specific esterases could be further characterized using specific inhibitors. In addition, it is also possible to assay quantitatively different levels of esterase activity both in the developing ovary and developing eggs in the pleopod. This will specifically indicate the storage of esterases during vitellogenesis and their subsequent utilization during embryogenesis.

26.2. Principle

α-napthol liberated from α-napthyl acetate by the enzymatic activity of the sample combines with Fast blue RR to produce a coloured compound and this is measured at 590 nm to quantify the esterase activity.

26.3. Materials

Different stages of developing eggs in the pleopods of *E. asiatica*. For the classification of eggs refer Expt. No. 20.

---

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

26.4. REAGENTS

1. *Substrate :* Prepare 0.003 M α-napthyl acetate by dissolving the substrate in 1 ml of acetone. Make up this 1 ml to 100 ml with double distilled water.

2. *Arresting reagent :* Mix two parts of 1% (W/V) Fast blue RR with five parts of 1% (W/V) sodium laryl sulphate.

26.5. PROCEDURE

26.5.1. Enzyme source

Prepare dilute aqueous egg homogenates in chilled double distilled water. Centrifuge at 2,000 g for 10 minutes. Collect the supernatant without the contamination of lipid cap.

26.5.2. Reaction mixture

Each reaction mixture consisted of 2.5 ml of substrate, 2.5 ml of (M/15) phosphate buffer from pH 6 to 9 at an interval of 0.5 and 1.0 ml of tissue homogenate. Incubate the reaction mixtures at 37°C for 30 minutes. Arrest the enzyme reaction by adding freshly prepared arresting reagent. Read all the samples and controls against the blank at 590 nm in a spectrophotometer.

26.5.3. Control I

Mix 2.5 ml of substrate with 2.5 ml of (M/15) phosphate buffer. To this mixture add 1 ml of tissue homogenate and read immediately to note initial 'zero' reading.

26.5.4. Control II (Kapin and Ahamad, 1980)

Non enzymatic hydrolysis (self hydrolysis) of acetate substrates are commonly reported. Hence to avoid the effect of non-enzymatic hydrolysis, buffered substrate without enzyme at every pH is incubated for 30 minutes and then arrested as per the other experimental tubes.

26.5.5. **Estimation of Protein** (Lowry *et al.*, 1951)

Protein content of the samples were determined side by side using bovine serum albumin as standard. (For procedure refer Expt. No. 12).

Express the enzyme activity as mg α-napthol liberated/mg protein/30 minutes.

26.6. Observation

Quantify the non-specific esterase activity in different stages of egg development. Find out the optimum pH for esterase activity by plotting the values obtained in a graph.

26.7. References

Kapin, M. A. and S. Ahamad, 1980. Esterases in larval tissues of gypsy moth, *Lymantria dispar* (L.): Optimum assay conditions, quantification and characterizations, *Insect Biochem.*, **10** : 331-337.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193** : 265-275.