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

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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.

15

A CLASSIFICATION OF NEUROSECRETORY CELLS OF CRUSTACEA*

15.1. Introduction

In Crustacea, the distribution of NSCs is limited to the brain, thoracic ganglia, circum-oesophageal connectives and eyestalk. Enami (1951) first described different types of NSCs and mapped them in the brachyuran crab, *Sesarma dehaani*. Following this, there were numerous reports on the classification of NSCs in a variety of crustaceans belonging especially to the superior orders (Durand, 1956 ; Matsumoto, 1958 ; Adiyodi and Adiyodi, 1970). One of the compelling inference from these studies is that the morphology of the NSCs in different forms is highly variable and species-specific. In the present experiment a classification of NSCs of an anomuran crab is attempted employing various criteria such as size, shape, location, tinctorial properties and cytological details.

15.2. Material

Brain and thoracic ganglion of the anomuran crab, *Albunea symnista*.

15.3. Procedure

1. Observe the slides prepared in the Expt. No. 14.
2. Note the size, shape, location and other cytological details of the NSCs.
3. Classify the NSCs into different types based on the characteristics mentioned in the step 2.
4. Draw the camera lucida drawings of NSCs (Fig. 1).

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

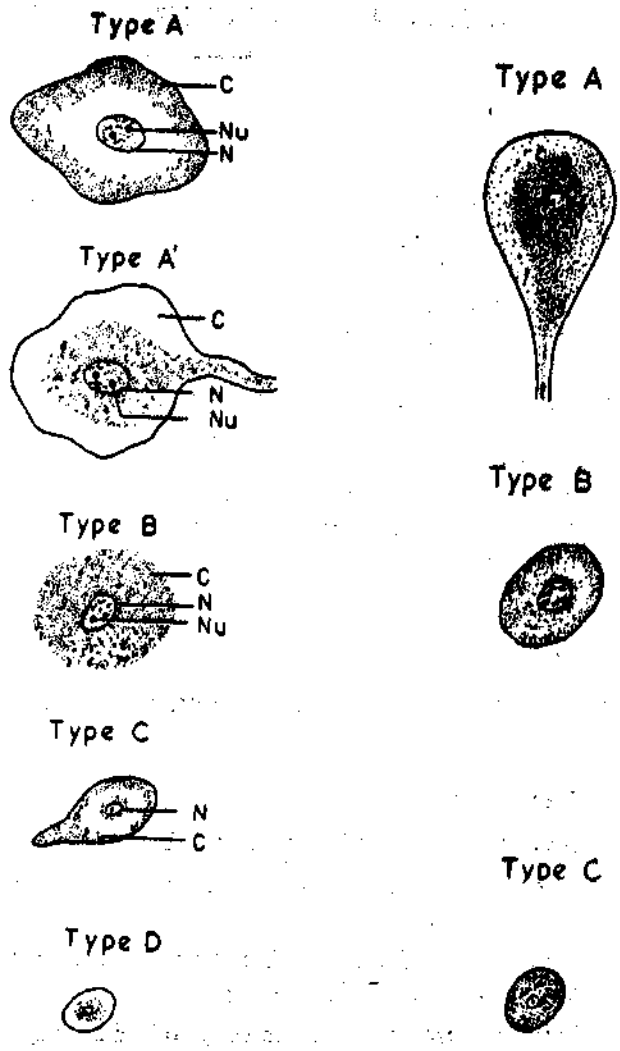

Fig 1.

Types of NSCs in the brain (*a*) and thoracic ganglion (*b*) of *A. symmista*.

5. Map the distribution of NSCs in different regions of the brain and thoracic ganglion. For this, first draw the outline of intact neuroendocrine centres. By using various symbols, indicate the location of individual NSCs as shown in Figs. 2 and 3.

15.4. Observations

15.4.1. NSC types of the brain (Fig. 1a)

A type NSC: These NSCs are 45 μm-50 μm in diameter. The nucleus is oval in outline and possesses two nucleoli. The cytoplasm is flaky due to the accumulation of secretory granules. These cells are distributed in the dorsal and ventral median regions of the protocerebrum. The axons are indistinguishable.

A' type NSC: It is similar to the A type NSC, except for the presence of an axon. Cells of this type are mainly located along with the A type in the dorsolateral and ventromedian regions of the protocerebrum.

B type NSC: These cells are oval and 35 μm-40 μm in diameter. The nucleus is prominent, but without a distinct nucleolus. These cells are known to be distributed in the anterolateral region of the protocerebrum with a few cells scattered in the ventrolateral region. As in the A type, axons are wanting.

C type NSC: These are conical or club shaped, and are few in number, measuring 25 μm - 30 μm in diameter. They are distributed in the dorsal and ventral regions of the deutocerebrum. A few cells are also seen near the A type.

D type NSC: This type is spherical without an axon, and measures 8 μm -10 μm in diameter. The cytoplam is scanty, but possesses dark blue granule. Cells of this type are confined exclusively to the dorsal region of the protocerebrum.

15.4.2. NSC type of the thoracic ganglion (Fig. 1b)

A type NSC: It is monopolar, uninucleolated and 25 μm-30 μm in diameter. The shape of the nucleus varies from flat to crescent. These cells are located in the dorsal and ventral regions of the neuropile.

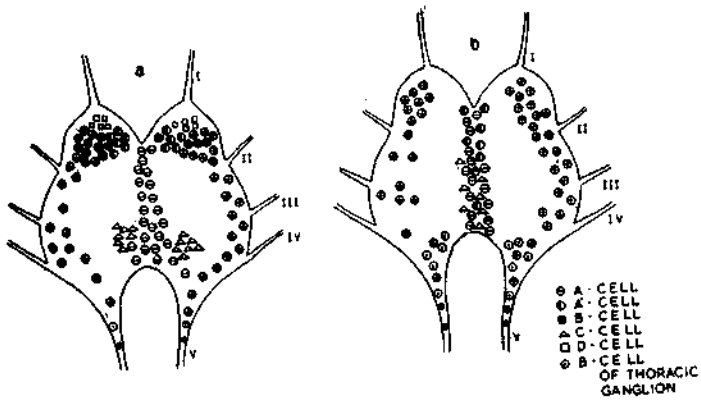

Fig. 2.

Distribution of NSCs in the brain of *A. symnista*. (a) dorsal side, (b) ventral side. I—optic nerve, II—Antennulary nerve, III—Antennary nerve, IV—Maxillary nerve, V—Circum-oesophageal connective.

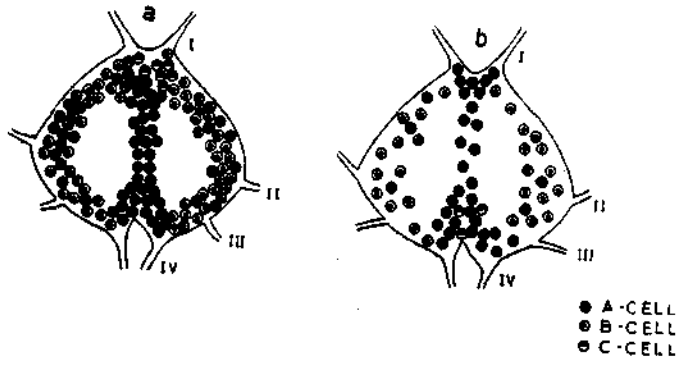

Fig. 3.

Distribution of NSCs in the thoracic ganglion of *A. symnista*. (a) dorsal side, (b) ventral side. I and IV—Thoracic nerves, III and IV—Nerves to thoracic appendages.

B type NSC : These cells are oval shaped, more abundant and measure 15 μm-20 μm in diameter. Cells of this type are binucleolated and found distributed in the dorsal and ventral regions of the thoracic ganglion. A few cells are also noticeable among the A type NSC.

C type NSC : This type NSC is round with a diameter of 10 μm-15 μm and distributed in the dorsal side of the anterior region.

Tabulate the observations in the table given below :

NSC types	size	shape	location	tinctorial affinities	cytological details

15.5. References

Adiyodi, K. G. and R. G. Adiyodi, 1970. Endocrine control of reproduction in decapod Crustacea. *Biol. Rev.*, **45** : 121-165.

Durand, J. B. 1956. Neurosecretory cell types and their secretory activity in the crayfish. *Biol. Bull.* (Woods Hole), **111** : 62-76.

Enami, M. 1951. The source and activities of two chromatophorotrophic hormones in the crab of the genus *Sesarma*. II—Histology of incrementary elements. *Ibid.*, **101** : 241-258.

Matsumoto, K. 1958. Morphological studies on the neurosecretion in crabs. *Biol. J. Okayama Univ.*, **4** : 103-186.