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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.

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EFFECT OF ALTERED PHOTOPERIODISM (ALTERED INTENSITY AND DAY LENGTH) ON NEUROENDOCRINE FUNCTION AND OVARIAN MATURATION IN AN OCYPOD CRAB, *OCYPODA MACROCERA**

19.1. Introduction

In marine invertebrates, light forms a significant external signal (' Zeitgeber ', Aschoff, 1960) to control various reproductive activities (Segal, 1970). The initiation and synchronization of spawning, gonad maturation and sex determination are light-dependent reproductive activities and appear to be mediated through photo-neuroendocrine pathways (Scharrer, 1964). In *Porcellio dilatatus*, the extension of photoperiod lengthens the period of reproduction and delays the molt (Mocquard *et al.*, 1976 a, b). In decapod crustaceans such as *Pachygrapsus marmoratus* (Pradeille-Rouquette, 1976) and *Palaemonetes pugio* (Little, 1968) the administration of longer photoperiod accelerates reproduction. The above mentioned examples clearly suggest the role of day length on the initiation of reproductive processes in lower as well as higher crustaceans.

However, the specific role of wavelength and light intensity on reproductive processes has received only meagre attention. In sea pen, *Cavernularia obesa*, the intensity of illumination determines the time course for gamete release (Mori, 1960). More secretory activity is noticed in MTGX1 (Medulla terminalis ganglionaris X-organ) and MTGX2 of eyestalk in *Palaemon serratus* when subjected to light-dark experiments (Van Herp *et al.*, 1977). Likewise, in crayfish *Orconectes clypeatus*, Finger mann and Oguro (1962) found the stimulation of neurosecretory

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cells in medulla externa of eyestalk when administered to continuous light. So it is evident that the photoperiod regulates the molting and reproduction through neuroendocrine pathways.

19.2. Principle

Artificial illumination is effected through the neuroendocrine pathways and regulates the process of molting and reproduction by altering the neuroendocrine secretion in endocrine centres. The light regime suppresses the MIH factor in the eyestalk and stimulates the MH when light is administered at the time of molt. During the intermolt period the inhibition of MIH factor stimulates the production of GSH in the brain and TG of females and androgenic gland hormone in males.

19.3. Photoperiodism Chamber

The square photoperiodism chamber is made with wood at the size of 193L × 140 W × 148 H (in cms) and is covered by black cloth. At the corners of upper side of the chamber, inverted 'U' shaped tubes are fixed for free aeration. Three 4300 K fluorescent white tube lights are fixed inside the chamber. The intensity of light is altered by changing the height of the light and the same is measured with EEL Lux meter.

19.4. Altered Intensity and Constant Daylength

19.4.1. Procedure

1. Prior to experiment, assess the ovarian stages of all crabs by window method (*vide* Expt. No. 17).
2. Select the following four sets of crabs at early stage of vitellogenesis (vitellogenesis-I).

Experimental

I. With intact eyestalk.

II. With painted (black paint) eyestalk.

Control

I. With intact eyestalk.

II. With painted eyestalk.

3. Keep the experimental sets inside the photoperiodism chamber (4 crabs/tank) and the control sets outside the chamber.
4. Maintain the same field salinity and simulate the environmental conditions in all the tanks.
5. Use different light intensities such as 140, 300, 450, 640, 800 Lux by altering the height of the fluorescent tube light.
6. At a time, take the single light intensity along with fixed daylength (12L : 12D) to study the immediate effect on the ovarian maturation. Likewise, alter the intensity five times (five intensities) with constant daylength (12L : 12D).

19.4.2. Observation

1. Compare the ovarian stages of control and experimental crabs.
2. With a 5 day interval observe the cytological and cytophysiological changes of ovary and the neuroendocrine centres.

19.5. FIXED INTENSITY AND ALTERED DAYLENGTH

19.5.1. Procedure

1. Repeat the steps 1-4 of the previous experiment (19.4).
2. Use the suitable intensity which is standardized in the first experiment (19.4).
3. Alter the daylength as OL : 24D ; 4L : 20D ; 8L : 16D ; 12L : 12D ; 16L : 8D ; 20L : 4D and 24L : 0D.
4. At the time of experiment, use the fixed intensity and select a single daylength among the seven daylengths mentioned above. Likewise, conduct seven experiments with seven daylengths with fixed intensity and find out the suitable daylength, favourable for the ovarian maturation.

19.5.2. Observation

(i) Make a comparison between the ovarian stages of control and experimental crabs.

(ii) Observe the cytological and cytophysiological changes of ovarian maturation and the neuroendocrine centres with the interval of five days.

19.6. References

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