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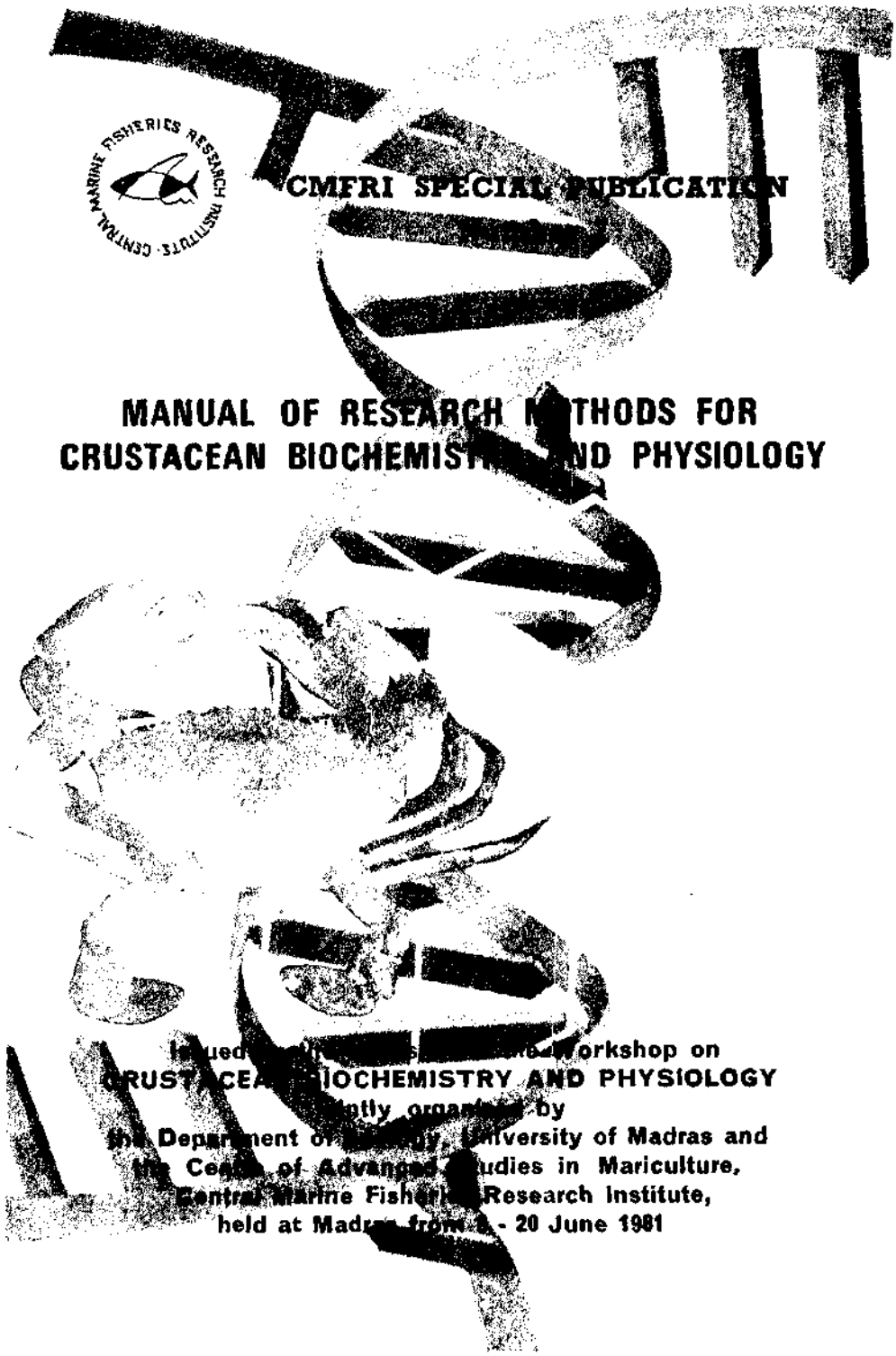
Issued on the occasion of the Workshop on
CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Madras from 8 - 20 June 1981



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Manual of Research Methods for Crustacean Biochemistry and Physiology

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ISSUED ON THE OCCASION OF THE WORKSHOP ON CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY 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 MADRAS FROM 8-30 JUNE, 1981

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EFFECTS OF THE SURGICAL EXCISION OF THE SINUS GLAND AND EYESTALK ABLATION ON OSMOTIC REGULATION *

20

20.1. Introduction

Sinus gland is a neurohaemal organ wherein hormones from different neurosecretory centres are stored. The sinus gland of *Scylla serrata* is well developed and macroscopically visible owing to its well known characteristic opacity and slightly bluish-hue. It is located at the dorsal aspect of the junction between the medulla interna and the medulla terminalis in the eyestalk. Since it is a compact structure it is possible to remove the sinus gland from the eyestalk. There are two surgical procedures for the removal of the sinus gland. The first procedure involves the removal of the retinal portion of the eye cap and the other involves surgical excision without disturbing the eye cap and thereby the vision of the crab (Kleinholz, 1947).

20.2. Removal of the Retinal Portion of the Eye Cap

20.2.1. Procedure I :

1. Prechill the crab and make it immobile ;
2. Make a sharp cut at the retinal portion of the eye cap and expose the medulla interna.
3. The gland will be visible as an opaque organ of bluish-hue dorsally at the corner of the medulla interna.
4. Using a fine pair of forceps and a needle, the sinus gland can be lifted and transferred to physiological saline (0.9% NaCl).
5. The cut end of the retinal portion may be sealed with cold paraffin wax to avoid loss of blood.

* Prepared and verified by G. Dayanithi & M. H. Ravindranath, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

20.2.2. Procedure II :

1. Animal is anesthetized as in procedure I.
2. Make a rectangular cut (length 5 mm × width 3 mm) at the posteriorad of the long axis of the eyestalk approximately in the centre of the non-retinal portion.
3. Remove the rectangular piece of the exoskeleton and the underlying hypodermis using a fine pair of forceps.
4. Place the removed exoskeleton and hypodermis in physiological saline (0.9% NaCl).
5. Remove carefully, the apparently visible bluish-hue glandular part (sinus gland) by lifting the gland from its base using a fine pair of forceps.
6. Replace the hypodermis and the exoskeleton to their respective positions.
7. Seal the groove between the margin of the opening and the rim of the replaced exoskeleton with cold paraffin wax.
8. Place the crab in a tank containing 50% sea water.

20.3. Eyestalk Ablation

Bliss *et al.* (1954) have observed that the effects of sinus gland removal is different from that of bilateral eyestalk removal. Bilateral eyestalk removal involves the removal of X-organ, sinus-gland and excision injury to the optic peduncle. Eyestalk can be removed very easily at the base and the loss of blood can be arrested by applying cold paraffin wax. The operation can be simplified if the crabs are wrapped with cloth leaving the eye exposed so that they cannot struggle and placed them on crushed ice to reduce their activity.

20.4. Influence of Eyestalk Hormones of *Scylla serrata* on Osmotic Regulation

Scylla serrata is exposed to fluctuating salinities (Joel, 1973). It is able to control to some extent its body fluid concentration. It is known that the salt and water balance is controlled at least in part, by hormone secreted by X-organ in eyestalk. The

hormone is also stored in the sinus gland. By extirpating the source of the hormone as well as the storage site (sinus gland), the osmoregulatory ability of the animal can be changed.

20.4.1. Procedure

1. Select 6 sets of intermoult, male and unautotomized crabs for this experiment.
   (*a*) *Animal* 1 : Normal, uninjured.
   (*b*) *Animal* 2 : Sinus gland removed after cutting the retinal portion of the eye cap.
   (*c*) *Animal* 3 : Sinus gland removed by simple excision without cutting the retinal portion of the eye cap.
   (*d*) *Animal* 4 : Bilateral eyestalk ablation.
   (*e*) *Animal* 5 : Eye cap covered with black paint to blind the animal.
   (*f*) *Animal* 6 : Dactylus injury as suitable control for the experiment.
2. Keep these animals immersed in separate tanks filled with water of particular salinity for two hours.
3. Determine the ammonia and chloride concentrations in the blood as well as in the media before keeping the crab in the media following the methods mentioned in 8.0 & 14.0.
4. After two hours, determine ammonia and chloride concentrations in the medium as mentioned earlier.
5. Then collect the blood from the crabs and determine ammonia and chloride concentrations.
6. Repeat the same experiment with different sets of animals with different salinities.

20.5 References

Bliss, D. E., J. B. Durand & J. H. Welsch, 1954. Neurosecretory systems in decapod Crustacea. *Z. Zellforsch.*, 39 : 520-536.

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For your own notes

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