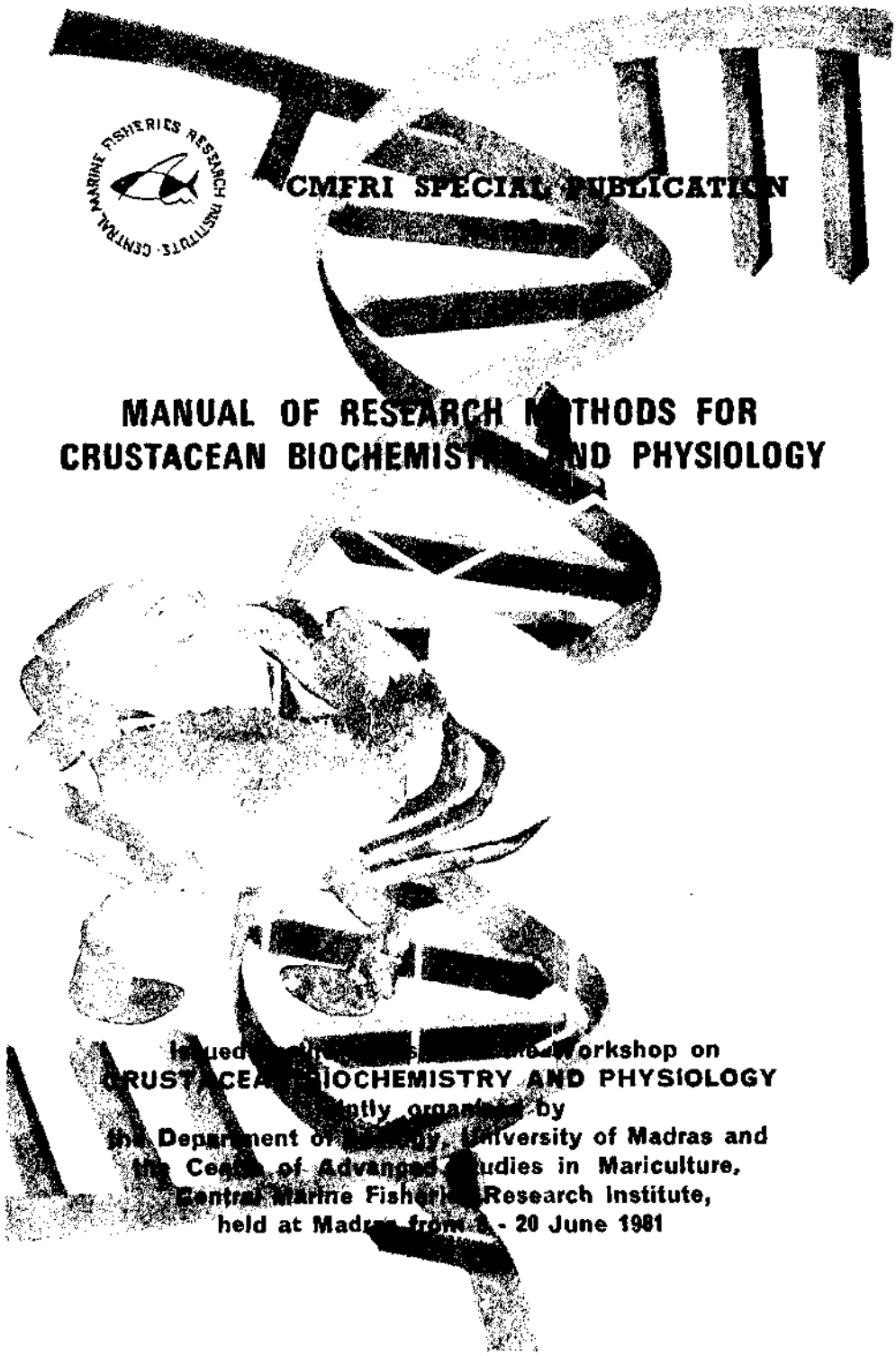
CMFRI SPECIAL PUBLICATION
Number 7

MANUAL OF RESEARCH METHODS FOR CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY

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

EDITED BY

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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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CHLORIDE * 14

14.1. PRINCIPLE

Chloride in the sample displaces thiocyanate from mercuric thiocyanate, which in turn combines with iron of ferric nitrate to become ferric thiocyanate, which is a coloured compound. The colour intensity is proportional to the iron complexed with thiocyanate which inturn depends on the amount of Cl^- ions which have displaced thiocyanate from mercuric thiocyanate (Schorenfeld & Lewellen, 1964).

14.2. REAGENTS

1. *Colour reagent*

   (*a*) *Saturated mercuric thiocyanate solution* : Dissolve 2 gm of mercuric thiocyanate ($Hg(SCN)_2$ in 1000 ml of water. Keep the solution at room temeprature for 48 hours or longer and shake frequently, filter it before use.

   (*b*) *Ferric nitrate solution* : Dissolve 13 gm of ferric nitrate in approximately 400 ml of distilled water and 1.5 ml of Conc. HNO_3. Then to the whole volume of solution b, add 500 ml of solution *a* and make upto 1000 ml. Then add 5to 6 ml of 6% mercuric nitrate until the absorbance of 80 m Eq/L Chloride standard is between 0.07 and 0.1.

2. *Standard Cl^- solution* : Dissolve 585 mg of dry pure NaCl in 100 ml of distilled water. This standard is equivalent to 100 m Eq Cl^-/L.

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* Prepared and verified by K. Kannan & M. Arumugam, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

14.3. PROCEDURE

1. 0.1. ml of blood, 0.1 ml of distilled water (blank) and 0.1 ml of standard solution are added to 1 ml of 80% of ethanol individually and centrifuge at 3500 rpm for 5 minutes.
2. Take 0.1 ml of supernatant from all the tubes and add 3 ml of colour reagent separately.
3. After 10 minutes, find the optical density at 480 nm in a spectrophotometer.

14.4. CALCULATION

$$\frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \text{Concentration of standard} \times \frac{1000}{\text{Vol. of sample}}$$

$$= \text{m Eq/L.}$$

14.5 REFERENCE

SCHOENFELD, R. S. & C. J. LEWELLEN, 1964. Colorimetric method for determination of serum chloride. *Clin. Chem.*, **10** : 533-539.

For your own notes

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