CMFRI SPECIAL PUBLICATION
Number 7

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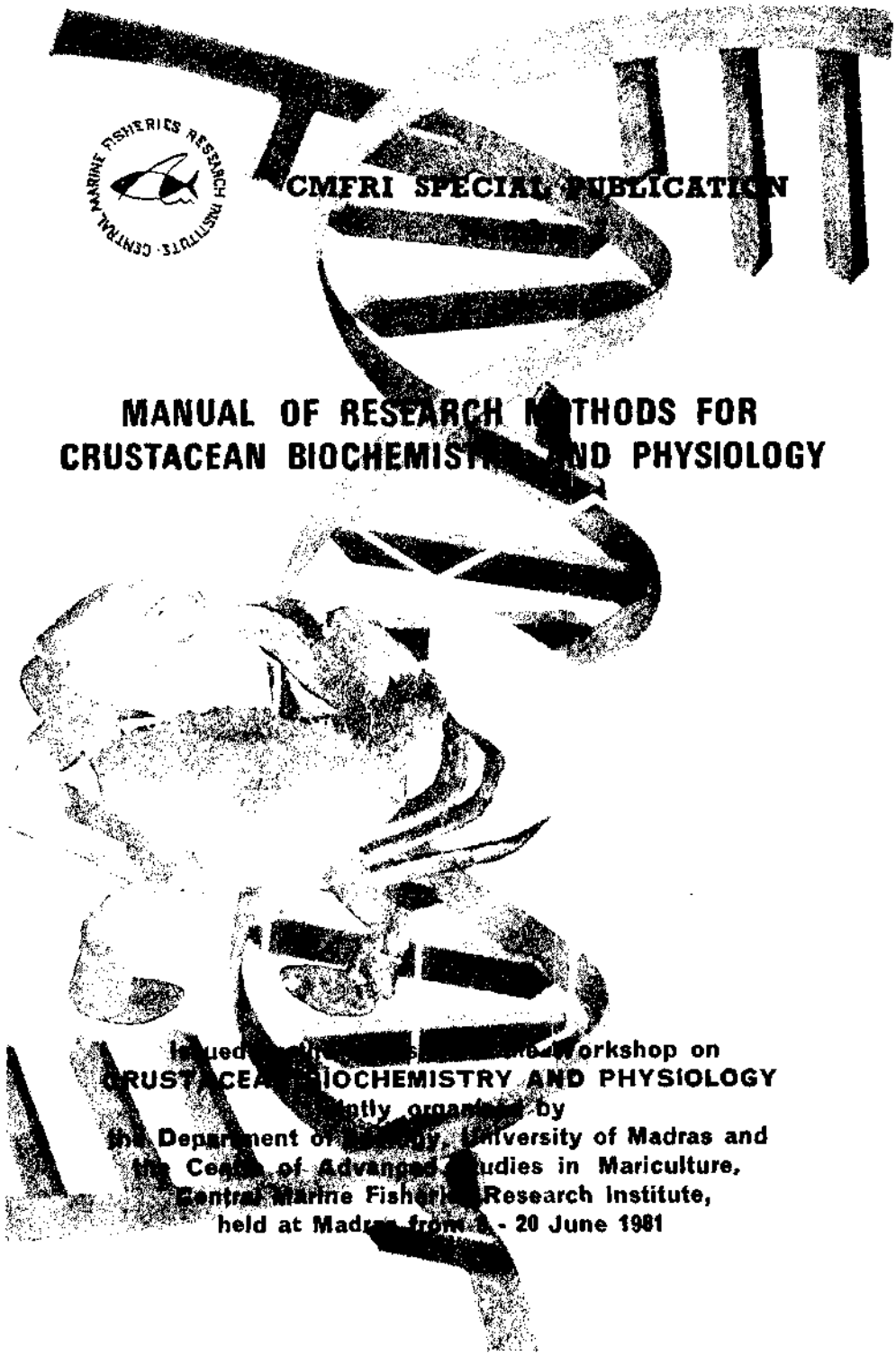
Issued on the occasion of the Workshop on
CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Madras from 8 - 20 June 1981



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Manual of Research Methods for Crustacean Biochemistry and Physiology

EDITED BY

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AMMONIA * 8

8.1. PRINCIPLE

Sodium hypochlorite ($NaOCl^-$) combines with ammonia present in the sample to produce NH_2Cl and OH^- ions. Sodium nitroprusside catalyses the reaction. The NH_2Cl in the presence of 3 $0H^-$ combines with phenol and forms a quinonoid complex. This quinonoid complex combines with another phenol molecule to form indophenol, a coloured compound. The colour thus formed is directly proportional to the ammonia present in the sample (Boltz & Howel, 1978).

8.2. REAGENTS

1. *Reagent A*: Dissolve 10 gm of phenol with 50 mg of sodium nitroprusside in 500 ml of water (This solution is stable for one month if kept in stoppered amber bottle in refrigerator).
2. *Reagent B* : Dissolve 5 gm of sodium hydroxide in 10 ml of sodium hypochlorite and dilute to 500 ml of water.
3. *Ammonia standard* :
   (*a*) *Stock solution* : Dissolve 0.3819 gm of anhydrous ammonium chloride in 1 litre of water.
   (*b*) *Working solution* : Dilute 1 ml of stock solution to 1000 ml with water (1 ml=0.122 μg of NH_2=0.1 μg of N_2).
4. *Deproteinizing agent* :
   80% *ethanol* : As mentioned in 4.2.2.

8.3. PROCEDURE

1. Add 0.2 ml of blood in 2 ml of deproteinizing agent.

* Prepared and verified by M. H. Subhashini, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

2. Centrifuge it at 5000 rpm for 5 minutes and collect the supernatant.
3. Add 2.5 ml each of reagent A to 1 ml of the supernatant (sample), 1 ml of 80% ethanol (Blank) and 1 ml of standard ammonia solution (standard).
4. After five minutes, to each tube add 2.5 ml of reagent B.
5. After five minutes incubate all tubes at 37°C for 20 minutes.
6. Read the optical density after 30 minutes at 625 nm.

8.4. Calculation

$$\text{Amount of } NH_3 \text{ present in the sample} = \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{0.00122}{0.1} \times 100 \text{ mg\%}$$

where 0.00122 refers to the amount of NH_3 present in mg in the standard ; 0.1 refers to the amount of blood (in ml) present in the sample.

8.5 Reference

Boltz D. F. & J. A. Howel, 1978. *Colorimetric determination on non-metals* Vol. 8 Second edn., pp. 210-213. Wiley-Interscience Publication, New York.