CMFRI SPECIAL PUBLICATION
Number 7

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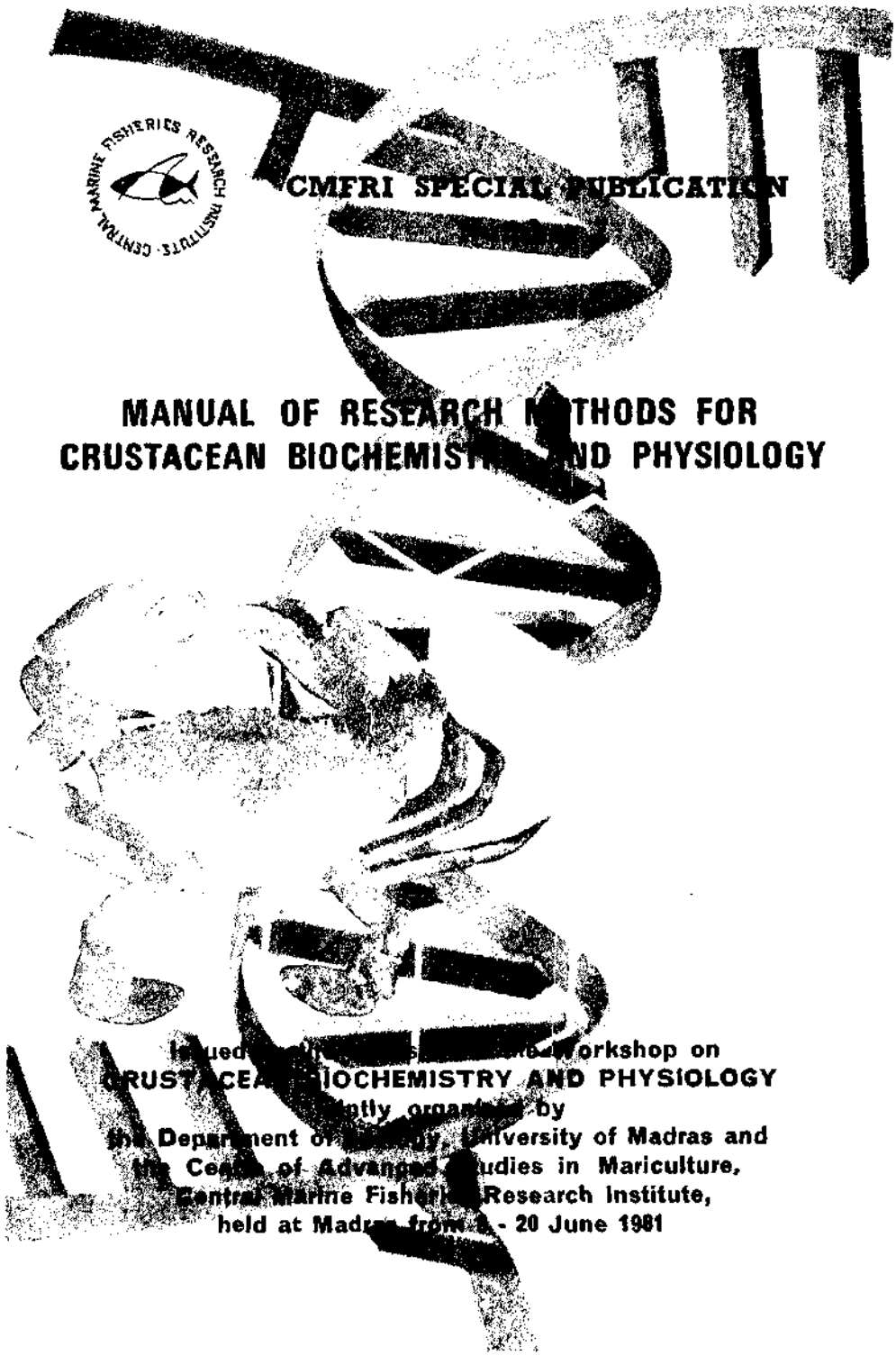
Issued on the occasion of the Workshop on
CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Madras from 8 - 20 June 1981



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Manual of Research Methods for Crustacean Biochemistry and Physiology

EDITED BY

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3 BLOOD VOLUME *

3.1. PRINCIPLE

The principle underlying the methodology of blood volume determination involves the determination of degree of dilution of an injected dye by comparing the concentration of the injected dye and the concentration of the dye in the blood after a particular period of injection. The degree of dilution reflects blood volume (Lee, 1961).

3.2. REAGENTS

1. 0.9% *sodium chloride solution*: Dissolve 900 mg of sodium chloride in 100 ml of distilled water.
2. 0.1% *Congo red in* 0.9% *sodium chloride solution* (*stock solution*): Dissolve 100 mg of Congo red in 100 ml of 0.9% sodium chloride solution.
3. 0.001% *Congo red* (*working solution*): Take 1 ml of the stock solution and make it upto 100 ml with 0.9% sodium chloride solution.

3.3. PROCEDURE

3.3.1. Preparation of standard graph]:

1. Take 0.5,[1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml of the working solution and make it upto 5 ml with 0.9% sodium chloride solution using a 5 ml standard flask. The solutions contain 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 μg/ml respectively.

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

2. Measure the optical density of these solutions at 480 nm in a spectrophotometer against the blank (5 ml of 0.9% sodium chloride solution).

3. Plot the O.D. in Y axis against the concentration in X axis and draw a slope.

3.3.2. Estimation of blood volume :

1. Withdraw 0.1 ml of blood by cutting the dactylus of the walking leg and make it up to 5 ml with 0.9% sodium chloride solution to serve as a blank.

2. Inject 0.1 ml of 0.1% Congo red through the arthrodial membrane at the base of the uropod.

3. Place the crab in a tank containing 50% sea water.

4. After 40-45 minutes, collect 0.1 ml of blood and make it upto 5 ml with 0.9% sodium chloride solution.

5. Find out the optical density of Congo red in the sample at 480 nm in spectrophotometer against the blank.

6. The optical density is referred to the standard graph to find out the concentration.

3.4. Calculation

The blood volume (V) can be calculated following the equation given by Lee (1961) :

$$V=(dg1/g2)-a$$

where $g1$ is the concentration of dye injected, $g2$ is the concentration of dye in the sample, d is the volume of the sample and a is the volume of saline injected with the dye.

3.5. Interpretation

One of the necessary prerequisites in this method is to empirically assess the time taken for the complete dilution of the injected dye. It should also be noted, as the dye is getting diluted there is a possibility of accumulation and excretion of the dye. Therefore, the dye concentration in the blood has

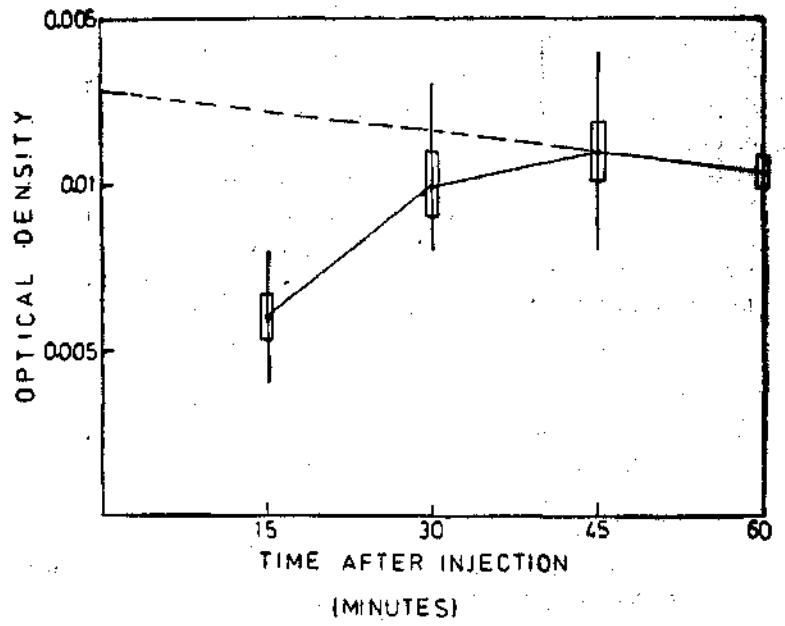

Fig. 1 : Concentration of the dye Congo red in the blood after different hours of injection in *Scylla serrata*. The extrapolation of the declining point is indicated by the dotted line.

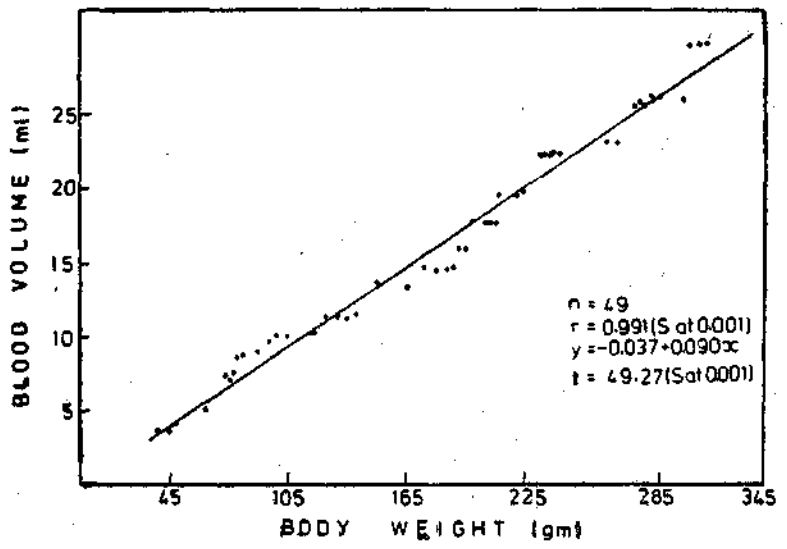

Fig. 2 : Variation in the blood volume in relation to body weight in *Scylla serrata*.

to be determined at different intervals after the injection of the dye (Mullainadhan, 1979). Figure 1 indicates the rate of dilution as well as the rate of elimination of the dye from the blood. Therefore, the accurate blood volume may be assessed by extrapolating the curve at the declining point. For crustaceans, the weight of the animal should be taken into note of (Fig. 2). During blood collection, sufficient care should be exercised to prevent loss of blood. Cut end of the dactylus may be sealed with molten wax.

3.6 References

Lee, R. M. 1961. The variation in the blood volume with age in the desert locust (*Schistocerca gregaria*). *J. Insect Physiol.*, **6** : 36-51.

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