

OFFICE OF NAVAL RESEARCH
BOSTON BRANCH OFFICE

DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH SCIENTIFIC DEPARTMENT
207 WEST 24TH STREET
NEW YORK, NEW YORK 10011

IN REPLY REFER TO:

RSS: fgr
4330/N00014-1468-033400

20 Nov 1968

Dr. Pat Wennekens
Oceanographer
Office of Naval Research
San Francisco Area Office
1076 Mission Street
San Francisco, California 94103

Dear Pat:

By way of this letter I wish to introduce Dr. Dryce Lacombe of the Brazilian Naval Research Institute. Dr. Lacombe has been conducting research on the morphology, histochemistry, and histology of various species of barnacles at the Osborne Laboratories of Marine Sciences since May 1968. This research was funded by ONR under Contract N00014-1468-033400.

During Dr. Lacombe's stay in New York, I introduced her to several scientists working in similar areas. She will be visiting scientists at the California Academy of Sciences, San Francisco, from 25 November to 6 December 1968. Should she have time and your schedule permits it, you may wish to introduce her to people in your area doing research on barnacles.

With best wishes to you and yours, I remain,

Sincerely yours,



RICHARD S. STEVENS
Scientific Department

Copy to:
ONR (480D - F.D. Jennings)
Boston - Sci Dept

relatoris 7-2.

EDUCATION · RESEARCH · CONSERVATION

NEW YORK ZOOLOGICAL SOCIETY

NEW YORK AQUARIUM and

OSBORN LABORATORIES OF MARINE SCIENCES

Telephone: 266-8500

Cable Address: AQUAPARK NEW YORK

Seaside Park, Coney Island
Brooklyn, New York 11224

November 13, 1968

Dr. Ross F. Nigrelli
 Director, New York Aquarium and
 Osborn Laboratories of Marine Sciences
 Seaside Park, Coney Island
 Brooklyn, New York 11224

Dear Dr. Nigrelli:

The following report summarizes my research efforts in the Department of Microbiology, Osborn Laboratories of Marine Sciences, from May 1, 1968 to date.

At the invitation of Dr. Ross F. Nigrelli, Director of the Osborn Laboratories of Marine Sciences, I was asked to collaborate on the histological, histochemical and biochemical research of barnacles; the cement apparatus of these organisms and the isolation, purification and identification of the cement. This plan of studies, already in progress at the Osborn Laboratories of Marine Sciences was in effect, a continuation of my own work at the Brazilian Naval Research Institute, Rio de Janeiro.

Admiral Carlos Mesiano, Director of the Brazilian Naval Research Institute, gave permission for a leave of absence from my own research so that I might accept Dr. Nigrelli's invitation.

My affiliation with the Osborn Laboratories began on May 1, 1968. The research work, accomplished in the Microbiology Laboratory, consisted of the following three phases:

- A. The acquisition and preparation of equipment and materials for the histological and histochemical methods.
- B. The development of the research.
- C. Analysis of the results and their preparation for publication.

A. Preparatory phase:

The first several weeks after my arrival were occupied with obtaining and setting up the required equipment and fixatives needed in the histological procedures; the collecting of suitable specimens for study; fixation of the tissues; dehydration and paraffin inclusion in preparation for tissue sectioning.

In these studies I prepared and used the following fixatives:

- I. 1. Bouin according to Duboscq-Brazil
2. Bouin with sea water
3. Susa according to Heidenhain
4. Susa with sea water
- 5.. Carnoy's fluid
6. Gilson
7. Flemming
8. Lantanio
9. Ciaccio
10. Cajal
11. Formol-acetate
12. 95% alcohol
13. Acetone

Following fixation, the material was dehydrated in the Alcohol-Benzene series, imbedded in paraffin and sectioned serially at 5, 7, 10 and 12 microns, according to the specimen. The sections were stained with one or several of the following stains which I prepared.

- II. 1. Delafield hematoxylin
2. Heidenhain iron hematoxylin
3. Ehrlich hematoxylin
4. Azan method
5. Harris hematoxylin
6. Nuclear fast red
7. Chromotrope 2R
8. Naphthol green
9. Eosin
10. Congo red
11. Orange GG
12. Trypan blue
13. Alcian blue
14. Gallocianin
15. Janus green
16. Sudan III
17. Toluidine blue
18. Light green

III. Application of histochemical methods in enzyme studies:

1. Cytochromoxidase
2. Glycerophosphatase, alkaline
3. Alkaline naphtholphosphatase
4. Gomori ATP/ase
5. Padilha and Herman, ATP/ase
6. Wachstein and Maisel, ATP/ase
7. Kahffman and Hill, SDH/ase
8. Peroxydase

IV. Species of barnacles used in the study:

1. Balanus nubilis
2. " psittacus
3. " eburneus
4. " balanoides
5. " amphitrite
6. " tintinnabulum var.

IV. Species of barnacles used in the study, cont'd.

7. *Conchoderma aurita*
8. *Mitella polimera*
9. *Lepas anatifera*

V. Stations sampled for barnacles used in the study:

1. Sea Gate, Brooklyn
2. Rock jetty on the seashore in front of Aquarium
3. Sheepshead Bay, Brooklyn, N. Y.
4. Woods Hole Oceanographic Institute, Cape Cod, Massachusetts, Cape Cod Canal
5. Nahaut Bay and neighboring islands of the Marine Biological Station, Falmouth, Mass.
6. Boston Harbor

B. Development of the research:

The barnacle studies were developed in the following way:

1. The tissues were either fixed "in situ" or they were excised and fixed in the fixatives listed above in A 1.
2. After dehydration and paraffin infiltration, the tissues were cut in serial section on the microtome. Fixed on slides.
3. The tissues were then stained according to the staining techniques listed in A 2.
4. Examination of the tissues using microscopic techniques followed the staining in order to study the cytology of the cement glands and canals.
5. Drawings of the cement glands and canals were made using the Camera Lucida.
6. Microphotographs were made using black and white and color film to show the cement glands, their position, the canals and the secretion in the glands and canals.
7. Copies of the photographic material have been made to include in work to be published.
8. The data was analyzed and prepared for publication.

C. Results of the research:

The results of the research have been reported in several papers now in preparation for publication or presently in press.

1. Preliminaire observations sur les "cement glands".
2. Studies on the biology of barnacles: Alkaline phosphatase histochemically detectable in the cement glands of the Balanidae.
3. Comparative studies of the cement apparatus in Lepas anatifera and Balanus tintinnabulum.

4.

4. Comparative histology of the cement glands of the Balanidae and the mechanism of cement secretion.
5. Histoenzymological studies of the cement glands in the Balanidae: Cytochrome oxydase, Succinic dehydrogenase, and Peroxydase in *B. eburneus*, *B. balanoides* and *B. nubilis*.

III. Research in progress:

1. Histochemistry of the cement glands in different species of barnacles.
 2. Isolation and chemical analysis of the cement in *Balanus eburneus*.
 3. Ultrastructure of the cement glands of the Cirripedia.
- IV. Scientific Institutes visited under the auspices of the U. S. Navy, Office of Naval Research.
1. Lamont Geological Observatory, Department of Marine Biology
 2. Marine Biological Laboratory, Woods Hole, Mass.
 3. Harvard University, Marine Biological Station, Nahaut Bay.

During my stay at the Osborn Laboratories of Marine Sciences, I prepared:

1800 histological slides;
 700 histenzymological slides;
 650 paraffin blocks;
 400 color microphotographs;
 200 black and white microphotographs and many line drawings in India ink.

V. Acknowledgements:

I wish to acknowledge the support provided by Dr. Ross F. Nigrelli, Director, The New York Aquarium and the Osborn Laboratories of Marine Sciences, during the course of my research at the laboratories. I would also like to thank him for giving me the opportunity to continue my research on the barnacles native to the United States.

I also wish to thank Dr. Vincent R. Liguori, Head, Department of Microbiology, for his cooperation and assistance in the barnacle studies and for placing his laboratory at my disposal. In addition, I would like to express my gratitude to Mr. John Blair of the New York Aquarium who constantly made me feel welcome in his home with his family and who did all he could to make my stay in the United States pleasant and successful.



Ponta da Jararaca

ILHA DOS PORCOS

6 Enseada do Forno

Ponta do Forte

Ponta d'Água

Enseada dos Aryós

Morro da Atalaia

Ponta do Veado

Arrebentações com vento de NE

Praia Brava

5 Ponta Maramutá

ILHA DO CABO FRIO

Antigo Farol (390)

Boqueirão

Praia do Farol

Ponta do Oratório

Enseada da Parede