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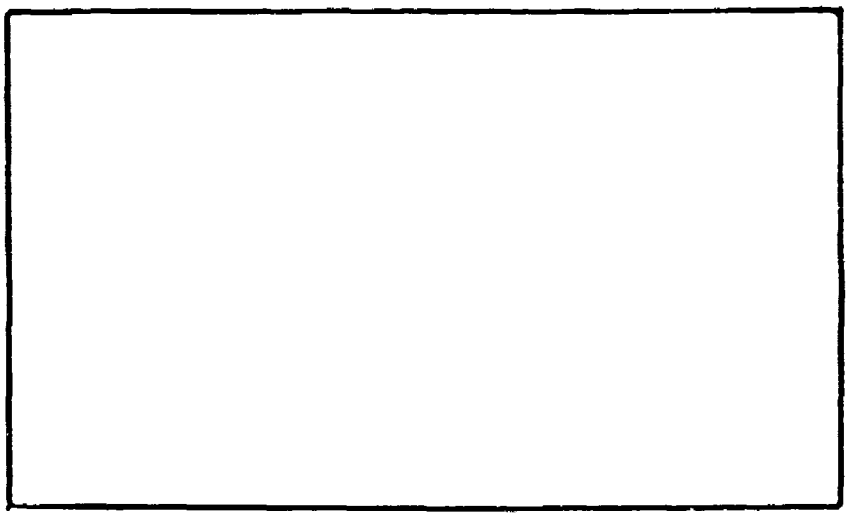

TECHNICAL MEMORANDUM

U.S. NAVAL APPLIED SCIENCE LABORATORY
NAVAL BASE
BROOKLYN I, NEW YORK

A SUMMARY OF THE DEVELOPMENT OF PROCEDURES FOR LABORATORY MARINE-FOULING STUDIES - PART 2

LAB. PROJECT 9300-22, TECHNICAL MEMORANDUM NO. 3

SR 007-08-05, TASK 1201

3 JUL 1964

MATERIAL SCIENCES DIVISION

APPROVED: D. H. Kallas

D. H. KALLAS
Associate Technical Director

U.S. NAVAL APPLIED SCIENCE LABORATORY
NAVAL BASE
BROOKLYN, NEW YORK 11251

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Ref: (a) BUSHIPS ltr R 007-08-05, Ser: 633P-1441 of 26 Jul 1962
(b) MATLAB NAVSHIPYDNYK Project S999-4, Progress Report 3 of 7 Dec 1962
(c) NAVAPLSCIENLAB Project 9300-22, Technical Memorandum No. 1 of 25 May 1964
(d) NAVAPLSCIENLAB Project 9300-22, Technical Memorandum No. 2 of 17 Jun 1964

INTRODUCTION

1. The development of procedures for studying marine fouling in the laboratory, authorized by reference (a), is continuing at the U.S. Naval Applied Science Laboratory. The current work on this program concerns, mainly, the development of techniques for the mass rearing of juvenile barnacles, from embryo to the planktonic cyprid stage, under controlled laboratory conditions. The cyprid barnacles will then be used in connection with this Laboratory's methodology for studying fouling attachment and evaluating marine antifoulants.

2. This memorandum is Part 2 of a brief summary of the development, since submittal of reference (b), of procedures for the rearing of barnacles in the laboratory, and for the study of the settlement of these barnacles upon test surfaces under completely controlled conditions. The work which is currently underway may be arranged into five main categories as follows:

a. Acquisition, delivery, identification and study of several species of adult barnacles.

b. Laboratory facilities for providing environment for living marine organisms.

c. Development of procedures for maintaining and feeding juvenile barnacles in the laboratory.

d. Design of special equipment and techniques which are required for laboratory fouling methodology.

e. Rearing of juvenile barnacles from embryo through successive naupliar and cyprid stages.

3. Part 1 of this summary, reference (c), covered categories a and b above. Part 2 covers categories c, d and e. The material presented in this summary is intended to provide a general description of the problems which are being encountered and the methods and techniques which are being employed for their solution, and to present an overall picture of the approach to the fouling

problem which is being pursued by the Laboratory. More complete and technically detailed reports on the progress of individual phases of the work will follow.

BACKGROUND

4. Although attempts to combat marine fouling are as old as ships themselves, it is only within fairly recent times that antifouling has been subject to systematic scientific inquiry. At present, many new toxic materials and new coating systems to contain them are being developed which may provide a degree of antifouling effectiveness. In order to adequately study large numbers of such materials and to determine their exact mode of action upon the fouling community, facilities which afford far greater control than are provided by harbor test stations, test rafts and ship-bottom tests are required.

5. It is generally agreed that the field of antifouling studies may be divided into two main areas:

To develop improved antifouling coatings with effective service life of from three to five years.

To develop better and quicker methods for studying marine fouling and for evaluating antifouling coatings.

Studies, currently underway at this Laboratory, of antifoulants and of antifouling coating formulation, fall within the first area, while the development of this Laboratory's methodology for studying marine fouling and evaluating antifouling coatings falls within the second area.

6. When operative, the Naval Applied Science Laboratory methodology for studying the attachment of barnacles to coatings under laboratory conditions will constitute an important first step toward systematic, controlled study and evaluation of marine antifouling coatings. With continued development, techniques for laboratory mass rearing of other fouling organisms will be devised which may eventually permit laboratory simulation of complex fouling environments.

ADVANTAGES OF METHODOLOGY

7. Procedures, currently in widespread use, for studying the fouling resistance of coatings generally involve exposure of coatings to a natural fouling environment at harbor test stations or on a test raft. The advantages of using a laboratory methodology for marine-fouling studies were set forth and described in reference (c), and are listed here as follows:

a. Elimination of natural variations.

b. Usable without regard to season.

c. Independent of location.

d. Permit close observation of fouling attachment.

e. Separation of contributing factors which control fouling.

f. Permit screening of new coating formulations.

g. Indication of optimum performance of a series of similar formulations.

h. Provide a production control.

i. Reduction of evaluation time.

j. Provide research tool for marine-fouling research.

OUTLINE OF METHODOLOGY

8. Briefly, the Naval Applied Science Laboratory methodology for studying and evaluating marine antifoulants consists of the following steps:

a. Collection, from natural sources, of adult barnacles.

b. Removal of embryo barnacles from the adult.

c. Rearing the juvenile barnacles, through the successive naupliar stages, to the presettled cyprid stage.

d. Exposure of antifouling test panels to a fixed number of cyprid barnacles under controlled conditions.

e. Count of the number of barnacle settlers.

f. Interpretation of the barnacle settlement data as an indication of service antifouling effectiveness.

SUMMARY OF DEVELOPMENT OF LABORATORY PROCEDURES

9. Development of procedures for maintaining and feeding juvenile barnacles in the laboratory.

a. Culturing algae - The chief source of food for juvenile barnacles, in their natural habitat, and therefore in the laboratory, are algae of the types

known as diatoms and nanoplankton. Diatoms are microscopic, unicellular, chlorophyll-bearing organisms of the class Bacillariophyceae, typically characterized by a perforated silaceous cell wall. The many species of diatoms may be divided into two subclasses, namely, Pennales which are oblong, feather, or boat shaped, and Centrales which are cylindrical or disc-shaped. Planktonic, marine species of both subclasses of diatoms are currently being cultured in the Laboratory. The term nanoplankton is generally used to describe micro-algae less than 10 microns in size. These organisms, which are usually green in color, consist of a nucleated mass of protoplasm contained within a cellulose cell wall. Many species of algae of this type possess flagella and are capable of locomotion. The culturing of algae in the laboratory is, in itself, a complex problem requiring considerable knowledge, experience and facilities. A continuous, adequate supply of the proper species of algae is prerequisite for maintaining the quantities of juvenile barnacles required by the laboratory fouling methodology. The following is a brief outline of the more important considerations which bear upon the culturing of algae as a food source for the juvenile barnacles being reared in the laboratory.

(1) Algae inoculums - Inoculums of algae have been supplied by the Haskins Laboratory and by the U.S. Bureau of Commercial Fisheries Biological Laboratory. In general, these have been non-sterile transfers of algae stock culture. Upon receipt, they are successfully stored in a viable condition, for long periods of time, in the Laboratory's incubator-shaker.

(2) Species - The six species listed in the following table have been selected for culturing on the basis of published reports and private communications on the feeding habits of barnacle larvae.

MARINE ALGAE BEING CULTURED IN THE LABORATORY

Species	Subclass	Approximate size (microns)	Color of Mass Culture
Cyclotella nana	Diatom (Centrales)	2 to 4	brown
Dunaliella euchlora	(Nanoplankton) Chlorophyta	6 to 8	green
Phaeodactylum tricornutum	Diatom (Pennales)	25 to 30	brown
Nitzschia closterium	Diatom (Pennales)	50 to 70	brown
Cryptomonas sp.	(Nanoplankton) Cryptophyta	--	brown
Monochrysis lutheri	(Nanoplankton) Chrysophyta	6	brown

(3) Size of culture - Algae culturing is currently being carried out in several sizes of vessels which include small-scale "starter" cultures comprising an inoculum of 1 deciliter in 9 deciliters of enrichment medium, and larger-scale cultures comprising the growth of the "starter" culture in 9 liters of enrichment medium. The quantities of algae resulting from these procedures have been sufficient for the needs of the barnacle-rearing program.

(4) Enrichment media - Two enrichment media have been successfully employed for the culturing of algae, as follows: (a) Erdschreiber culture solution - a seawater infusion of soil with additional salts, prepared in the Laboratory under carefully controlled conditions, and (b) Universal enriching medium - a seawater solution of nutrients and vitamins prepared in the Laboratory in accordance with a fixed formula.

(5) Environmental - Attention is given to controlling the environment for algae culturing as follows: (a) Temperature. The temperature in the incubator-shaker for inoculums and "starter" cultures is 60°F; in the laboratory for large-scale cultures, 70°F. (b) Aeration and agitation. "Starter" cultures thrive without forced aeration and require only occasional shaking to discourage agglomeration of the individual cells; large-scale cultures are aerated and agitated by forced dispersal of filtered-air bubbles. (c) Light. Three light sources are provided for the growth of algae: tungsten-filament lamps, cold fluorescent tubes, and Grow-Lux tubes. (d) Carbon-dioxide. CO_2, a basic ingredient for the growth of algae, is currently supplied by the ambient air in the incubator shaker and by the forced aeration in the large-scale cultures. The addition of pure CO_2 to these cultures will be studied, should it become desirable to increase the yield of algae.

(6) Selection of species for feeding: The six species of algae, listed in (2) above, have been fed to juvenile barnacles in the laboratory with a variety of results. It has been observed that certain species are more readily ingested by the barnacle larvae, and that even within a single species, variations in its usability exist. The size of the algae and the nature of its cell wall probably play an important role in this connection. Also, since it is difficult, within the present capabilities of the Marine Biology Facility, to maintain cultures on a long-term self-regenerating basis with adequate control of contamination, the age of certain cultures seems to be an important factor in their value as food. It was observed that the introduction of certain cultures of the diatom Cyclotella nana into thriving populations of nauplii, resulted in complete mortality within a short time. However, techniques have been developed which provide reasonable assurance that only healthy "non-toxic" cultures are used for nauplius feeding. Current preference, among the species being cultured, for

feeding to laboratory-reared juvenile barnacles, is as follows:

Excellent	Cyclotella nana
Fair	Dunaliella euchlora Phaeodactylum tricornutum
Poor	Nitzschia closterium Cryptomonas sp. Monochrysis lutheri

(7) Additional species of algae There are many other species of algae which, by virtue of their size and nutritional content, could be considered as potentially useful as a food source for juvenile barnacles. As these become availabe, they will be cultured and studied in the Laboratory. An inoculum of chlamydomonas sp. has been received recently, and is undergoing examination.

b. Quantitative feeding considerations.

Experience gained in laboratory-rearing of barnacle larvae indicates that the quantity and concentration of algae in the rearing vessels should be kept within well defined limits. In the case of the diatom Cyclotella nana, which is currently the chief food source, the following quantities have been found to yield satisfactory results, and have resulted in rapid development from the ova, through the six naupliar stages, to the presettled cyprid stage for three species of barnacle - Balanus eburneus, B. improvisus and B. amphitrite.

Seawater	750 ml
Nauplii	100 to 150
Diatom culture	75 ml
Culture density	1.0 - 1.5 X 10^6 cells per ml

These quantities result in a diatom density (initially) in the rearing vessel of approximately 1.0 - 1.5 X 10^5 cells per ml. In order to determine the cell density of a culture of algae, a hemacytometer is used. This instrument (generally used for determining blood-cell counts) provides a means for counting, under the microscope, the number of cells in a given volume of culture-solution. From this count, the overall density can be calculated. Culture solutions are also centrifuged, and an indication of the relative volumes of algae and solution are obtained. As more data on hemocytometer-counts and centrifuged-volumes become availabe, a correlation will be established which will provide the basis for a quick, standard method for cell-density determination of algae cultures using the centrifuge-volume data alone. The hemacytometer can also be used to count the number of cells, in a given culture, which are in the process of cell division. These data may be interpreted as an indication of the general health and vigor of the culture.

c. Food sources other than algae.

The need for supplementing the algae-diet for laboratory-reared barnacle larvae has not been established. Some investigators, who have had limited success in maintaining juveniles in the laboratory, report than an additional protein-rich food source is required, while others make no mention of feeding problems or methods. Although this Laboratory has successfully reared three species of barnacle larvae through the complete cycle of metamorphosis from fertilized ova, through the six successive naupliar stages, to the presettled cyprid stage on a diet of diatoms alone, the possibility that additional nutrients may be needed cannot be overlooked. Among the food sources and diet supplements, which have been studied in connection with the laboratory-rearing of barnacle larvae, or which are being considered for use, are the following:

desiccated beef liver powder
fresh fish liver
nutrient agar
nutrient gelatin
nutrient beef-extract and bacto-peptone broth
yeast extract
amino acids
egg albumin
vitamins

d. Control of infection and poisoning.

In any simulated environments, such as have been designed by the Laboratory for rearing juvenile barnacles and for culturing algae, precautions against infection and baterial growth are necessary. In the absence of all of the "balancing factors" present in nature, bacteria, if unchecked, often thrive in abnormally large numbers; and on occassion, ideal conditions for bacterial growth and spread of infection may exist in a laboratory environment. Bacteria enter the laboratory environments from three sources: (1) from the raw seawater, (2) from the air, and (3) from the adult barnacles which supply embryos for laboratory rearing. Some of the precautions which have been placed in effect in connection with the Laboratory's rearing techniques are described below:

(1) Filtration and irradiation - Installation of filtration and irradiation equipment is underway. When installation is completed, incoming raw seawater will be filtered with specially designed, orlon wound, pressure-tube filters which will remove all particulate matter larger than 15 microns. The seawater will then be passed through an ultraviolet water-treatment system which has been designed to eliminate the greater part of fungi and bacteria in the water. (See reference (c), Part I of this memorandum, paragraph 10.). In this manner, seawater used in the laboratory for culturing algae and rearing barnacle larvae will be relatively free of ciliates, fungi and bacteria which would otherwise infect the aquaria.

(2) Algae cultures - Additional precautions are necessary to prevent or minimize contamination in algae culture systems. While seawater is a delicately balanced, complex material which does not respond well to extreme temperatures, it has been found that most algae can be successfully cultured in seawater which has been pasturized by heating to 160°F. In addition to pasturization of seawater for algae culturing, all equipment used for storing, transfering and culturing algae is sterilized, and culture tanks are covered, in the prescribed manner, with cotton plugs.

(3) Hatching nauplii from embryos - Bateria and fungi are undoubtedly present in great variety on the barnacle accretion panels upon which the majority of adult, ova-bearing barnacles are brought to the laboratory, and bacteria and fungi are therefore associated with the lamellae (egg masses) which are removed from the adults for laboratory rearing. This source of infection is kept under control by the introduction of 300 units of penicillin per cc of seawater in the hatching finger bowls. First and second stage nauplii remain in this antibiotic environment for anywhere from a few minutes to several hours before they are removed to the seawater-algae environment of the rearing vessel.

(4) Contact with metals - All systems at the Laboratory's Marine Fouling Facility which come in contact with any phase of algae culturing and barnacle-larvae rearing have been constructed of non-metallic materials. In addition to the necessity of eliminating obviously toxic metals such as copper and copper alloys, it was found that sources of possible contamination and toxics could be introduced by aluminum and ferric metals as well, and these were also eliminated. Experiments are currently underway to determine the harmful effects, if any, of using stainless steel sieves, in place of cloth-mesh sieves, for the process of refreshing the environment in the nauplius rearing vessels. The U.S. Bureau of Commercial Fisheries Marine Laboratory reports that although a ten-minute exposure to stainless steel will kill mollusk larvae, an exposure of only a few seconds, such as for sieving, is harmless.

(5) Glass vessels - Although not enough data are as yet available, there is some indication that the composition of the glass of the barnacle-larvae rearing vessels may affect the naupliar survival rates. On several occasions it was observed that the mortality rates for juvenile barnacles being reared in vessels of so-called soft glass, probably containing lead oxide, was higher than for juveniles being reared in borosilicate glass (Pyrex) vessels. For this reason, borosilicate glass has been specified for all glass systems.

(6) The types of infection and poisoning, and the methods used to combat them, are summarized in the following table:

CONTROL OF INFECTION AND POISONING

Types of Infection or Poison	Source	Methods Used
Particulate matter	Raw seawater	Filtration
Fungi and bacteria	Raw seawater	Ultraviolet irradiation
		Pasturization
	Adult barnacles	Antibiotics
	Algae culture equipment	Sterilization
Metal and soft glass poisoning		Contact with metals eliminated. Borosilicate glass used.

10. Design of special equipment and techniques which are required for laboratory fouling methodology. In order to pursue a program of rearing barnacles in the laboratory and of developing a methodology for studying barnacle-attachment and evaluating the effectiveness of antifouling coatings, much new equipment and many special techniques had to be devised. The more important of these are described below:

a. Seawater treatment system - The equipment for filtration, irradiation and aeration of incoming raw seawater is described in reference (c), Part I of this memorandum, paragraph 10. In addition, a tank for heating seawat was designed and installed; the tank is constructed of marine plywood, li with poly(vinyl chloride) sheet, and is equipped with a battery of 14 quar immersion heating elements. This equipment can deliver seawater from ambient to about 120°F.

b. Rearing vessels for juvenile barnacles - During the course of the development of procedures for laboratory mass-rearing of juvenile barnacles, several types of barnacle-rearing vessels have been used. Basically, the rearing procedures can be divided into two general types: (1) continuous

circulation procedures, and (2) 'batch environment-change" procedures. A "batch environment change' procedure is described in paragraph c below. Continuous circulation provides continuous addition of fresh seawater and algae culture solution, discharge of waste material, and retention of the microscopic barnacle larvae. For this purpose, a quantity of 3-liter capacity, cylindrically shaped, borosilicate glass vessels were designed and fabricated. A glass, funnel-shaped drain is provided in the center of the bottom of each vessel; and a tube leading from the drain, up the outside of the vessel to the desired height, maintains the water-level in the vessel. On the inside, the drain-funnel is covered with a close-fitting, perforated ceramic disc. A layer of gravel is placed in the bottom of the rearing vessel, and this is covered with a layer of sterilized fine sand.

c. Use of sieves for renewing rearing environment - A "batch environment-change" procedure has been found to be satisfactory for rearing juvenile barnacles. This procedure, modeled after techniques employed at the U.S. Bureau of Commercial Fisheries Marine Laboratory, Milford, Connecticut, involves the periodic passing of the contents of the rearing vessel through a sieve which collects the barnacle larvae and discharges the seawater, unused algae, and other waste and unwanted products. The larvae are then bathed with a gentle stream of seawater and subsequently washed into a clean rearing vessel containing fresh seawater and algae culture. Currently in use for this procedure, are expendable sieves, fabricated at the Laboratory, consisting of finely woven plankton cloth in stiff paper frames. The use of stainless steel sieves, see paragraph 9.d (4) above, is being considered.

d. Settlement vessels for studying barnacle attachment - Equipment has been designed and constructed which will permit the simultaneous evaluation of 24 different antifouling test panels; each evaluation taking place in a separate glass vessel called a settlement vessel. A gravity-feed seawater system has been devised which supplies an identical rate of flow to each of the 24 settlement vessels. The equipment consists of three seawater distributors each surrounded by eight settlement vessels. A constant head is maintained by the height differential between the overflow holes in the settlement vessels and the overflow tube in the distributor. The seawater flows down through a length of capillary tubing and empties into the bottom of the settlement vessel. The water then flows upward through the settlement vessel at a controlled speed of about 1 knot. The entire system of distributors and settlement vessels is constructed of glass and may be easily disassembled for cleaning. A layer of sterilized sand is placed in the bottom of each settlement vessel. A finely-woven nylon cloth apron is suspended around the inside of each vessel to prevent both the loss of cyprids and their attachment to the glass sides of the vessel. A four-inch square test panel, coated with antifouling material will be suspended in the settlement vessel, and a fixed number of planktonic cyprids (probably between 50 and 100) will be introduced. The number of cyprids which attach to the test panel and begin to function as shell-

building barnacles will be interpreted as an indication of the coating's antifouling effectiveness.

e. Leaching of antifoulant toxics from coating formulations - There is considerable evidence that the effectiveness of antifouling paints is related to the rate of release of toxic material from the paint into the seawater. This rate of release or leaching rate, is of primary importance to the evaluation of antifouling paints. The leaching of antifouling coatings will be accomplished in the laboratory, with reasonable control, in a tank designed for this purpose. This tank, measuring about 4 feet by 2 feet by 1-1/2 feet deep, provides for the movement of coated test panels through seawater. The panels are mounted on speed-controlled rotating racks which are submerged in a bath of running seawater. A controlled program of leaching and exposure to cyprid settlement will provide information about the rate of decrease in antifouling effectiveness with time as well as an indication of the ultimate effective life of a coating. In addition, as the needs of the antifouling program and the methodology require, studies of absolute leaching rates by periodic sampling of the leach wash-water, and correlations of laboratory-tank leaching with leaching rate in the sea, will be conducted.

f. Other special apparatus - The Marine Fouling Laboratory has been well equipped with a variety of apparatus requisite to the tasks of identifying and rearing juvenile barnacles, providing the methodology for studying barnacle attachment, and evaluating the antifouling effectiveness of coatings. Of interest are the following:

(1) Stereo Zoom Microscope, Model BVB-73. Bausch and Lomb.

(2) Polarizing Microscope, Model LM-4. Bausch and Lomb. Magnifications of 20X to 2000X. Equipped with Baush and Lomb Eyepiece Camera, Model M.

(3) Microtome, Rotary, Model 820. A.O. Spencer.

(4) Hot-water bath. (for preparing wax-mounted microtome-section slides)

(5) pH Meter, Model 72. Beckman. (accurate to ± 0.05 unit)

(6) Incubator - Rotary Shaker, Model G27. New Brunswick Scientific.
Temperature Control: 0°C to 80°C ± 0.5°.
Shaking cycles: 50 to 400 per second
Cold illumination, atmosphere control.

(7) Fresh water Heater.

(8) Incubator, 60°C maximum. Precision Scientific.

(9) Autoclave, 258°F maximum at 20 psi. Burpee.

(10) Refrigerator.

(11) Centrifuge, Clinical, 3000 rpm. International.

(12) Hemacytometer. C. A. Hanssen.

(13) Stone Tank Aquaria. Seawater or freshwater.
Sizes: 4 ft. x 1-1/2 ft. x 1 ft. deep
2 ft. x 1-1/2 ft. x 1 ft. deep
2 ft. x 1-1/2 ft. x 1 ft. deep

11. Rearing of juvenile barnacles from embryo through successive naupliar and cyprid stages. The laboratory rearing of juvenile barnacles, from embryos, through the successive naupliar stages, to the presettled cyprid stage is a key step in the Laboratory's antifouling methodology. (See paragraph 8 above). The Laboratory has recently succeeded in mass-rearing three species of barnacle; Balanus eburneus, B. improvisus and B. amphitrite; of the five species currently being studied, to the cyprid stage with high survival rates. It is anticipated that in the near future, all five species will have been reared to the cyprid stage. This success constitutes an important breakthrough in the field of marine biology; for the first time, several species of barnacle have been laboratory-reared in mass quantities, on a repeatable and routine basis. Reference (d) reported on the initial Laboratory success in rearing cyprids from embryos, and provided a general discussion of the background information on this subject. A complete description of standardized methods for the laboratory mass-rearing of juvenile barnacles will be the subject of a future report. For the purpose of this memorandum summarizing the development of laboratory procedures, the following outline of steps in the rearing process is given:

a. Adult barnacles, usually measuring about 10 to 20 mm at the base, are removed from the plywood accretion panel with the use of a sharp-edged spatula. When properly done, the basal plate of the barnacle's calcareous shell remains attached to the panel and the animal is thus completely exposed. Should the basal plate part from the surface of the panel and remain with the barnacle, the plate is carefully pried away with a forceps. Balanus balanoides does not have a basal plate and its body is always accessible upon removal from its natural site.

b. Embryos, when present, are contained in two lamellae, each consisting of from 300 to 800 embryos, located laterally within the body sac of the barnacle. The body sac is cut and drawn aside to expose the lamellae which are removed intact with a dissecting needle. The Laboratory has classified lamellae according to the development of their embryos into three groups, as follows:

(1) Group 1 - These are the least developed (youngest) embryos. The lamella is white or light tan; the embryos, closely packed, do not have the nauplian eye spot. Microscopic examination (200X) does not reveal any motion by individual embryos. Hatching to first stage nauplii will commence in about 3 days to 2 weeks.

(2) Group 2 - The color of the lamella varies from light tan or pink to dark brown or red, depending upon the species and the age of the embryos. The embryos, still contained within an intact lamella, each have a characteristic nauplian eye spot. Microscopic examination reveals rhythmic motion of individual embryos at the periphery of the lamella. Hatching to first stage nauplii will commence in about 6 hours to 3 days.

(3) Group 3 - These embryos have begun to hatch. The lamella has ruptured, is no longer intact and cannot be removed as an entity. The embryos are drawn off with the use of a pipette. Hatching to 1st stage nauplii is either completed or will take place within 6 hours.

Upon removal from the parent barnacle, the lamellae are placed in glass finger-bowls containing a solution of 200 units of penicillin per milliliter of sterilized sea-water, and allowed to hatch.

c. Soon after hatching takes place in the penicillin solution, the first stage nauplii are transferred to a rearing vessel. The transfer is facilitated by the fact that 1st stage nauplii are phototropic and will travel towards a light source. By placing a small spotlight at the side of the finger-bowl so that a beam of light traverses the solution horizontally, the nauplii can be made to concentrate at the side of the bowl where they are drawn off with a pipette and discharged into the rearing vessel. In this manner, about 100 to 150 nauplii are placed in each rearing vessel which also contains 750 ml of treated seawater and 75 ml of algae culture of proper density.

d. Once every 24 hours, the contents of the rearing vessel are passed through a plankton cloth sieve which collects the barnacle larvae and discharges the seawater, unused algae, and other waste products. After bathing in a stream of seawater, the larvae are washed into a clean rearing vessel containing fresh seawater and algae culture. This daily "sieving" of the contents of the rearing vessels continues until the cyprid stage is reached. (for Balanus eburneus: 5 days, for B. improvisus: 6 days, and for B. Amphitrite: 6 days).

e. It is the barnacle cyprid, a swimming and crawling organism measuring about 0.5 mm in length, which settles upon and attaches itself to submerged surfaces, builds a calcareous shell and becomes "fouling". It is the observation of this settling and attaching of barnacle cyprids, carried out under controlled laboratory conditions in connection with potential antifouling coating systems, that constitutes the basis of this Laboratory's antifouling methodology.

12. FUTURE WORK AND REPORTS

a. Work on all phases of the development of the laboratory methodology for studying marine fouling are underway. These include:

(1) Improvement and standardization of techniques for mass-rearing juvenile barnacles with mortality kept to minimum levels.

(2) Studies of leaching rates of antifouling coatings and of laboratory leaching equipment and techniques.

(3) Development and standardization of laboratory techniques for exposing test surfaces to barnacle cyprid attachment, and evaluation of frequency of attachment.

(4) Development of correlations between laboratory cyprid-attachment and service antifouling effectiveness.

b. A technical memorandum, describing in detail the Laboratory's marine fouling facility, is being prepared and will be forwarded in August 1964.

c. A progress report on standardized laboratory procedures for rearing juvenile barnacles will be prepared and will be forwarded in December 1964.