

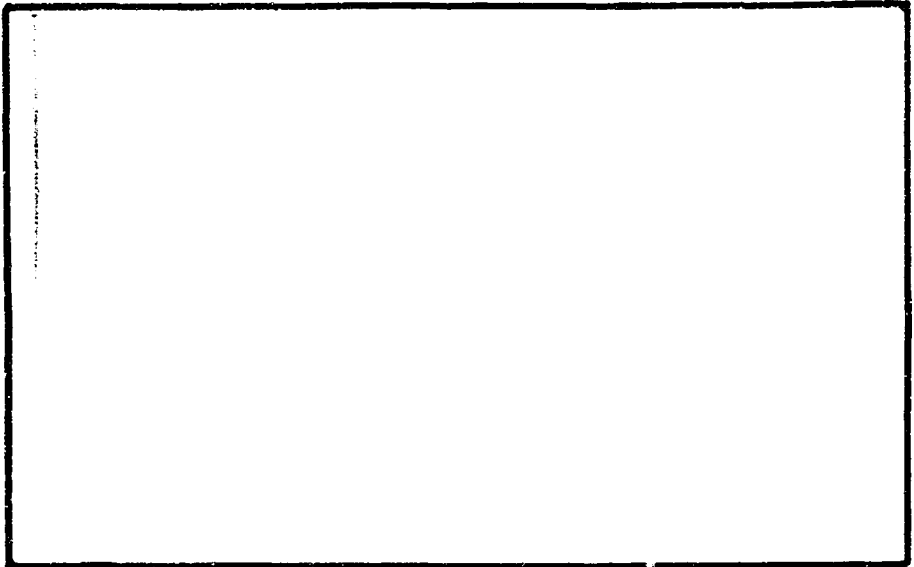
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# TECHNICAL MEMORANDUM

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

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A REPORT OF THE MASS-REARING  
OF SETTLED BARNACLE CYPRIDS  
IN THE LABORATORY

Lab. Project 9300-22, Technical Memorandum #2

SR 007-08-05, Task 1201

17 JUN 1964

MATERIAL SCIENCES DIVISION

Approved:



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Ref: (a) BUSHIPS ltr R007-08-05 Ser 633P-1441 of 26 Jul 1962  
(b) NAVAPLSCIENLAB Project 9300-22, Technical Memorandum #1 of  
25 May 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 under controlled laboratory conditions.
2. This memorandum is a preliminary report on the Laboratory's successful rearing of barnacle larvae through the complete juvenile cycle of metamorphoses from fertilized ova, through the six naupliar stages and the presettled cyprid stage, to settled young adult barnacles, under controlled laboratory conditions.

## BACKGROUND

3. The rearing of juvenile barnacles was undertaken at this Laboratory as part of the program to develop a laboratory methodology for studying marine fouling which, for the first time, will permit study and evaluation of the attachment of barnacles to surfaces under controlled laboratory conditions. A requisite for such a program is a continuous supply of presettled barnacle cyprids, reared in the laboratory and available as required by the needs of the program for evaluating the antifouling effectiveness of coatings or for studying other phases of fouling control.
4. After hatching from fertilized ova, juvenile barnacles undergo a series of metamorphoses and pass through several stages of development. The first six (in the case of the species being reared in the Laboratory) of these stages are called naupliar stages, the larvae being known as nauplii (singular: nauplius). The nauplius is a free-swimming micro-organism ranging in size from about 200 to 500 microns. The sixth-stage nauplius metamorphoses into a swimming-and-crawling larval stage called a cyprid. It is the barnacle cyprid which ultimately settles on a submerged marine surface, builds a calcareous shell, and becomes "fouling".
5. A brief review of the overall status of research on the laboratory-rearing of juvenile barnacles, including the latest Applied Science Laboratory data, is given in the following table which includes only those investigators who have had a degree of success.

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 Technical Memorandum #2

Research Organization (Investigator & Date)	Species Reared	Reported Survival Rates	Purpose of Research
Marine Biological Assn. Plymouth, England (R. Bassindale) 1936	Balanus balanoides	cyprids: none (5th stage nauplii)	Identification of larval stages and development of setation formulae
	Verruca stroemia	cyprids: 25% settlers: none	
Sheffield University Sheffield, England (R. Bassindale) 1936	Chthalamus stellatus	cyprids: none (6th stage nauplii: 10%)	Same as above.
Ministry of Agriculture and Fisheries Burnham, England (Knight-Jones & Waugh) 1949	Elminius modestus	cyprids: none (2nd stage nauplii)	Same as above.
Duke University Marine Laboratory Beaufort, N. C. (Costlow & Bookhaut) 1957-1958	Balanus oburneus	cyprids: yes settlers: 16%	Same as above.
	Balanus amphitrite	cyprids: yes settlers: 13%	
Marine Station Millport, Scotland (Barnes & Barnes) 1958	Balanus nubilis	cyprids: none (3rd stage nauplii)	Same as above.
Marine Biological Station Menai Bridge, Wales (B. Wisely) 1959	Elminius modestus	cyprids: yes settlers: 0.02-3.8%	Development of techniques for mass-rearing barnacle larvae in the labora- tory.
University College of Swansea Swansea, Wales (J. Moyse) 1960	Elminius modestus	cyprids: 90% settlers: 75%	Same as above.

Research Organization (Investigator & Date)	Species Reared	Reported Survival Rates	Purpose of Research
Marine Station Millport, Scotland (Barnes & Costlow) 1961	Balanus balanus	cyprids: none	Identification of larval stages and development of setation formulae.
University College of Swansea Swansea, Wales (J. Moyse) 1961	Acasta spongites	cyprids: yes	Same as above.
	Pyrgoma anglicum	cyprids: yes settlers: yes	
U. S. Naval Applied Science Laboratory Brooklyn, New York (Freiberger & Cologer) 1964	Balanus eburneus	cyprids: 80% settlers: 55%	Development of methodology for studying marine fouling, and antifouling methods under controlled laboratory condi- tions.
	Balanus improvisus	cyprids: 75% settlers: 50%	
	Balanus balanoides	cyprids: none (4th stage nauplii)	
	Balanus amphitrite	cyprids: none (5th stage nauplii)	
	Balanus trigonus	cyprids: none (5th stage nauplii)	

6. The most successful research of the laboratory-rearing of barnacle cyprids, before the work reported herein, was by J. D. Costlow and C. G. Bookhaut at Duke University Marine Laboratory and by J. Moyse at the University College of Swansea (Great Britain), about which the following additional descriptions are given.

a. Costlow and Bookhaut; cf. John D. Costlow, Jr. and C. G. Bookhaut, Biol. Bull. 112,313-24(1957) Ibid. 114,284-95(1958); successfully reared small quantities of barnacle larvae, each individual larva being reared in a separate "micro-container" with a capacity of 1.2cc. The primary purpose of this work was to observe each molt, examine and study the nauplius and its exuvium at each stage, and to develop and publish data and information on the identification of species and larval stages. These investigators reported that survival of juveniles to the settled stage was 16.3 and 12.7%.

b. Moyse; cf. John Moyse, Nature 185,120(1960); succeeded in mass-rearing juveniles of the species Elminius modestus, and briefly describes, with a minimum of detail and documentation, the rather simple procedures and techniques employed. This investigator reported that survival of juveniles to the settled stage was about 75%, and advances the opinion that the failure of other investigators to achieve like results is probably due to contamination of cultures with other organisms, especially ciliates, and to overcrowding, or stale or wrong food.

c. The procedures and techniques of Moyse were employed in earlier attempts by this Laboratory to rear barnacle larvae, and were found to be unsuccessful in connection with the species being reared and the conditions prevailing at the Laboratory's Marine Fouling Facility, and resulted in 100% mortality at the 3rd naupliar stage.

#### REARING CYPRIDS IN THE LABORATORY

7. This Laboratory has succeeded in mass-rearing barnacle larvae from the fertilized ova, or embryo, through the six naupliar stages, to the cyprid, or terminal juvenile, stage. About 800 embryos of two species, of the five species currently being reared for use with the fouling methodology, were hatched on 19 May 1964, passed through the six naupliar stages with very low mortality, and began to metamorphose to free-swimming cyprids after a rearing period of five days for species Balanus eburneus, and six days for B. improvisus. The estimated survival rates were: B. eburneus 80%, and B. improvisus 75%. About two-thirds of the cyprids settled on the glass walls of the rearing vessels, attached themselves thereto, underwent decortication and body rotation, and survived to the young barnacle stage. Two days later, another hatching of about 200 B. eburneus was conducted and reared to the attached young barnacle stage with similar results.

8. It is considered that the procedures and techniques which have been developed at the Laboratory for the mass-rearing of juvenile barnacles, will in the near future result in successful rearing of pre-settled cyprids of all five species of barnacle, common to the Eastern shores of North America, currently being studied.

9. A general description of the problems which have been encountered in this work, and a brief discussion of Laboratory procedures and techniques for culturing algae, rearing barnacle larvae, and developing the antifouling methodology are contained in Part 1 (reference (b)) and Part 2 (to be issued in July 1964) of a Naval Applied Science Laboratory Technical Memorandum entitled "A Summary of the Development of Procedures for Marine Fouling Studies". A detailed report on the

rearing of barnacle cyprids will be prepared and forwarded to the Bureau later this year.

FUTURE WORK

10. It is anticipated that, in the near future, Laboratory-reared barnacle cyprids of several species will be available in quantity and on a routine basis. This will permit the emphasis in laboratory work to progress to the next main phase in the methodology development; namely, the design of methods and equipment for the exposure of coated antifouling test panels to pre-settled barnacle cyprids under controlled laboratory conditions.

11. Work currently underway includes the following:

- a. Design and installation of water filtration and irradiation equipment.
- b. Development of techniques for culturing several species of algae for use as food for juvenile barnacles.
- c. Improvement of techniques for mass rearing juvenile barnacles to the cyprid stage with mortality kept to minimum levels.
- d. Studies of leaching rates of antifouling coatings and of laboratory leaching equipment and techniques.