NOTES AND NEWS


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CULTURE OF THE BARNACLE BALANUS EBURNEUS GOULD IN ARTIFICIAL SEAWATERS 1)

BY

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INTRODUCTION

The barnacle Balanus eburneus Gould has been used extensively for studies on settlement and toxicology. It was first successfully reared in the laboratory over two decades ago (Costlow & Bookhout, 1957). Nevertheless, the rearing methods employed to date remain relatively crude. We reared B. eburneus in natural and artificial seawaters free of most extraneous microorganisms. The larvae were fed Dunaliella viridis grown axenically in two different media. The nutritional value of the algae was shown to be dependant on the nature of the enrichments carried by the algal and perhaps the barnacle rearing medium.

METHODS AND MATERIALS

During the summer months of 1974 and 1975, gravid Balanus eburneus were collected from Accabonac Harbor in Springs, New York. During the cold months adults were brought into the laboratory and conditioned to spawn by placing them in 20° C seawater and feeding them freshly hatched Artemia salina (L.) nauplii

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and/or a suspension of *Dunaliella viridis*. The embryo sacs from the gravid barnacles were removed from the mantle cavity and washed three times in autoclaved, charcoal treated seawater (CHSW) whose salinity had been adjusted to 28 ppt with distilled water. The lamellae were allowed to hatch in covered glass dishes containing 6 embryo sacs per 100 ml of CHSW. All experiments were kept at 21.5°±0.5° C and a (22:2) (L:D) regimen. Bacterial contamination was minimized by including in the washing and rearing media a mixture of antibiotics consisting of Dihydrostreptomycin sulfate, Nutritional Biochemicals Corporation (NBC), 25 ug/ml; Penicillin “G”, NBC, 100 ug/ml; Polymixin B sulfate, NBC, 25 ug/ml; and Declomycin, Lederle Laboratory, 5 ug/ml. The antibiotic mixture (SPPD) was particularly effective against the bacterial flora associated with *B. eburneus* collected in Accabonac Harbor (Landau & D'Agostino, 1977).

The rearing media tested were CHSW and three artificial seawaters known as: Trace Marine Biological Laboratory (TMBL) (Cavanaugh, 1964), the Zarogian et al. (1969) formulation of artificial seawater for rearing molluscan larvae (ZPM), and Moore's Calcium-free seawater (cited in Cavanaugh, 1964). The salinities were adjusted to 28 ppt.

Newly hatched nauplii were transferred by pipette through three successive washes of sterile CHSW plus SPPD. The larvae were allowed to swim for 10 minutes in each fresh aliquot of wash media. After washing, five animals were distributed into each of the 1 ml depressions of a nine-hole Pyrex spot plate. Each depression contained 0.9 ml of the test medium plus SPPD. The depression slides were housed in Petri dishes. Moistened filter pads were included in order to prevent dehydration. The nauplii were fed axenic cultures of *Dunaliella viridis* grown in either STP, an enriched seawater medium (Provasoli & Shiraishi, 1959) or DC, a chemically defined medium (Provasoli et al., 1957). The nauplii usually developed into cyprids between the sixth and tenth day; the cyprids settled and metamorphosed into spat by the fifteenth day. The experiments were terminated after 20 days.

**RESULTS**

The results for the 20 day test period are summarized in table I. Statistical analysis was done by an approximate Z-test on ratio estimates for cluster sampling, where the clusters are the five nauplii per depression (Cochran, 1963). The medium ZPM + DC grown algae produced significantly fewer (95% confidence limit) cyprids than any of the other conditions. ZPM + STP grown algae gave significantly greater numbers of cyprids than CHSW + STP grown algae, CHSW + DC grown algae, and TMBL + DC grown algae. However, metamorphosis from the cyprid to the spat stage occurred significantly more frequently in CHSW + DC grown algae, CHSW + STP grown algae, and TMBL + STP grown algae than in TMBL + DC grown algae, ZPM + DC grown algae, or ZPM + STP grown algae. Larvae kept in Moore's calcium-free seawater became moribund in a few minutes.