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IMPROVED CULTURE OF *AURELIA AURITA* SCYPHISTOMAE FOR BIOASSAY AND RESEARCH[1]

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Abstract. A number of significant pollutants are being found in aquatic environments. Generally, there are no standardized methods or commonly used animal species for bioassay of toxicants. Our study describes a method by which genetically cloned scyphistoma larvae can be grown in chemically defined media and under standardized conditions. We report a 10-step procedure to culture *Aurelia aurita* scyphistomae successfully on frosted glass microscope slides. Attached scyphozoa larvae are particularly appropriate for both bioassay and physiological research. We reared continuous generations of *Aurelia aurita* in our laboratory for the past 15 years on a diet consisting of nauplii of *Artemia salina*. We have found that all available strains of *Artemia* cysts provide an adequate diet for the *Aurelia* polyp.

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Current emphasis on cnidarian research has been stimulated by the development of controlled laboratory culturing methods for *Hydra* spp. by Loomis (1953, 1954). Subsequently, other hydroid forms have been cultured by Hauenschild and Kanellis (1953), Fulton (1960), Braverman (1962a, 1962b), and Toth (1965). Investigations with cultured hydroids have added considerable knowledge in development, regeneration, cellular reassociation, aging, cell ultrastructure, tissue culture, physiology, and nutrition and feeding. Conspicuous by its absence is research in bioassay with marine coelenterates. Stewart and Schurr (1980) have shown a startling mortality of *Artemia salina* to asbestos fibers at levels commonly found in several marine and fresh water environments. It is obvious that a bioassay using marine pollutants is needed. Bioassay with marine invertebrates has not kept pace with the highly developed studies reported for the insects, bacteria, plants and laboratory rodents.

Our laboratory is participating in the "Intercalibration exercise for bioassay with *Artemia salina*" coordinated by the Artemia Reference Center, State University of Ghent, Belgium, for example One species, however, will not serve for the entire marine environment. Furthermore, there is no genetically homogeneous variety of *Artemia* similar to inbred strains of bacteria, insects and mice. Our method of *Aurelia* culture may be ideal for both bioassay and for physiological research with coelenterate polyps.

A review of the studies of the growth of the moon-jelly fish *A. aurita* indicates that culture conditions have varied almost as much as the range of experimentation, although there currently exist 3 general types of culture. Crowder (1926), Southward (1955) and Custance (1964, 1966) maintained *A. aurita* scyphistomae in laboratory aquaria with circulating natural sea water (NSW) and achieved growth and development on various substrates under varying physical conditions. This technique, although inexpensive and easily maintained, is limited to research in coastline facilities. Unfortunately, we are finding many toxins, carcinogens and pollutants in "natural" sea water. This method may not serve for research where a high degree of accuracy is needed.

Another technique, developed by Spangenberg (1965, 1970) for her strobilation studies, was successful in maintaining various life stages of a Texas strain of *A. aurita*. This technique em-

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ployed 2 defined media—Artificial Sea Water-Spangenberg Formula (ASW_S) and Artificial Sea Water-Instant Ocean Formula (ASW_{10})—and the periodic swirling of the culture medium and scyphistomae to discourage normal basal attachment of the polyps to the sides and bottom of the culturing vessels. These polyps reproduced asexually both by budding and generation of ephyrae and buds; scyphistomae, ephyrae and wastes were then separated by centripetal force.

Groat and Toth (1965) developed and tested a new procedure in an inland facility involving hydroids such as *Hydractinia, Podocoryne*, and *Bougainvillia*, which were cultivated on glass microscope slides in Artificial Sea Water-Instant Ocean Formula (ASW_{10}). This technique allowed normal basal attachment and morphological development to occur and provided the possibility of completely definable culturing conditions as well as ease in care and viewing. This technique possessed unique possibilities, such as statistical analysis of the rate of growth of clonal populations under experimental conditions and adaptability to a wide range of experimental designs. Because of these possibilities, we conducted a further study and modification of the basic method to refine this technique in the cultivation of *A. aurita* scyphistomae.

MATERIALS AND METHODS

The apparatus and procedure for developing this culturing technique were devised with readily available laboratory equipment. It is a flexible, time-saving technique applicable to a wide range of experimentation. Figure 1 shows the apparatus necessary to initiate the culture procedure. All items were autoclaved and rinsed initially in glass-distilled water and rinsed again in Artificial Sea Water—Instant Ocean Formula (ASW_{10}), which we obtained from Aquarium Systems, Inc., Eastlake, OH (prepared using glass-distilled water instead of tap water). The salinity of this preparation at 20 °C±2 °C was 32‰±1 (pH 7.9 to 8.2).

The initial stock of *A. aurita* scyphistomae was obtained in 1964 from the Supply Department of the Marine Biological Laboratory, Woods Hole, MA and was maintained in a constant temperature incubator at 20±2 °C in ASW_{10}. *A. aurita* scyphistomae of average uniform shape and size of 2 to 4 mm in length and 1 to 3 mm in diameter were selected. Buds, branches, polytentacular conditions, and other morphological variables did not significantly hinder the rate of attachment. Maximum size was achieved by increasing feedings from twice weekly to once every 2 days. *Aurelia aurita* scyphistomae were pre-fed and the medium changed to eliminate *Artemia salina* eggs, nauplii, and debris from the culture trays. Eggs of *A. salina* were obtained from various commercial producers. These were cultured in wide-mouth gallon jars (3.79 liters) filled with ASW_{10} and aerated. The solution was kept at approximately 30 °C with a 30 watt bulb and eggs hatched after 48 hours. In order to separate the newly hatched nauplii from the eggs, aeration was stopped and the positively phototaxic nauplii swam toward the light where they were easily removed. They were concentrated in fresh ASW_{10} where density estimates could be determined by dilution and microscopic examination. The *A. aurita* scyphistomae were fed 25±5 nauplii per polyp twice weekly over a period of 12 hours. The medium was changed completely after each feeding, and the polyps were carefully rinsed to remove remnants of *A. salina*. The ASW_{10} culture medium used throughout was allowed to age 24 hours before use.

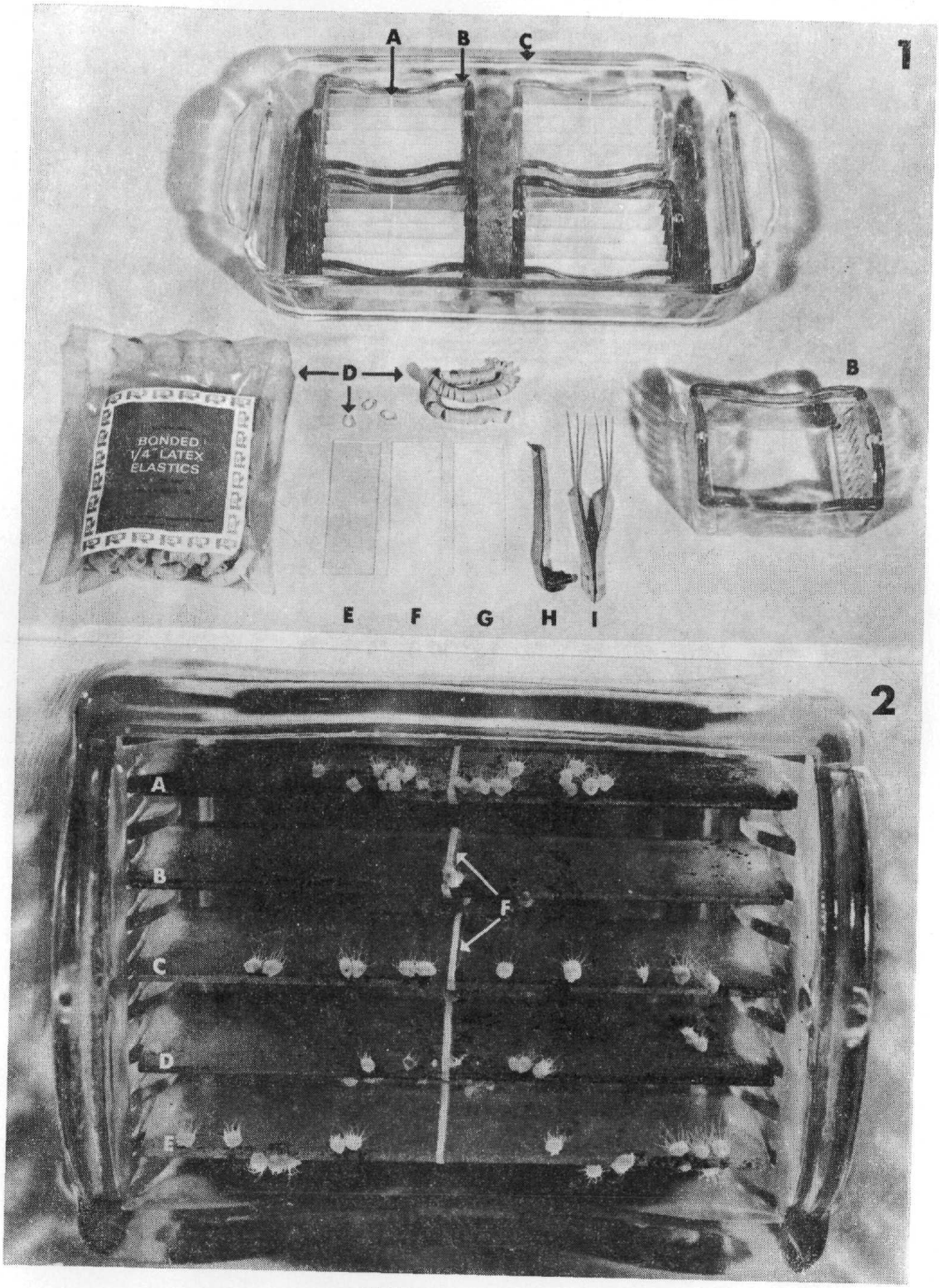
Culturing Process

1. Glass microscope slides were frosted by rubbing one surface on a glass plate with distilled water and carborundum dust. This procedure resulted in a uniformly roughened surface that provided a better substrate for basal attachment of the polyp. Slides were rinsed thoroughly with glass-distilled water and soaked in fresh ASW_{10} for 12 hours to remove the minute glass and carborundum particles.

EXPLANATION OF FIGURES 1 AND 2

FIGURE 1. Apparatus used in the culturing procedure: A) frosted microscope slide with attached latex dental band; B) glass microscope slide carriage holder; C) culturing tray; D) latex dental bands; E–G) progression in slide preparation—plain slide, frosted slide, frosted slide with dental band; H) aluminum microscope clip used to dislodge scyphistoma from the original substrate; and I) aluminum forceps used to handle the scyphistomae.

FIGURE 2. A glass slide carriage holder with five slides, each covered with a colony of *Aurelia aurita* scyphistomae in different stages of development. Slides A, C, and E are representative of 5 to 6 weeks of growth after the initial culturing procedure began; slide B is representative of colonies 1 to 2 weeks after the beginning of culturing; slide D is representative of a colony approximately 4 weeks old; F is the location of original points of attachment, the latex dental band.



FIGURES 1 AND 2

2. Twenty frosted slides were placed 5 to a carriage in each culture tray (fig. 1). The tray was then filled to the top of the slides with fresh ASW_{10}.

3. A clean latex dental band was stretched with forceps and placed on each frosted slide. The slides were then returned to the culture tray and allowed to soak for 20 min.

4. An aluminum microscope clip (see figure 1) was used to gently dislodge a minimum of twenty *A. aurita* scyphistomae from the original substrate (which consisted of frosted slides to which the stock scyphistomae had previously been attached). These polyps were carefully transferred to a clean beaker of ASW_{10} and allowed to stand at room temperature (20 °C) for 20 min. In the fresh medium, the scyphistomae relaxed and the extended polyps were carefully inspected for damage to the basal region. Injured individuals were discarded at this point, and any debris inadvertantly transferred from the original culture tray was removed.

5. The first banded slide was removed from the glass tray, and an aluminum microscope clip placed between the dental band and the frosted surface and several drops of ASW_{10} placed beneath it.

6. One *A. aurita* scyphistoma was removed from the beaker and placed under the elevated dental band. This polyp was carefully positioned so that it was on a vertical axis perpendicular to the band, and approximately equidistant from the basal disk region and the proximal region of the hypostome.

7. To avoid rupturing the polyp, the dental band was slowly lowered over the *A. aurita* scyphistoma. Occasionally the pressure of the lowered band caused some polyps to disgorge the enteric cavity contents but without observable prolonged damage to the specimens.

8. The slide with the bound polyp was carefully rinsed in ASW_{10} and placed in the carriage holder in the culturing tray. Steps 5 through 8 were repeated until all slides contained at least one specimen (figure 2).

9. The tray containing the bound polyps was placed in a 20 °C incubator or (experimental temperature). The top of the tray was covered with plastic wrap to avoid contamination and evaporation. An air line with an air stone was placed in each culturing tray for moderate aeration and maintainance of an adequate oxygen concentration (approximately 9.0 ppm).

10. *A. aurita* individuals that became dislodged from their position under the dental band were replaced. The ASW_{10} was changed 3 times weekly, and salinity, temperature, and pH were adjusted. Within 2 weeks, the polyps firmly attached to the slides and the dental band was removed by lifting it with a probe and severing the rubber.

Optimal Conditions For Culture

We completed more than 19 tests of growth rate over the 15 years we have been culturing *Aurelia*. Optimal growth conditions were measured by determining the best rate of budding of new individuals from all budding types (Gilchrist 1937). In our laboratory, the best conditions were salinity between 25‰ and 32‰, temperature of 18 to 20 °C, feeding of about 25 *Artemia salina* nauplii per *Aurelia* scyphistoma per day, constant but moderate aeration, and an inverted position for the scyphistoma to aid in regurgitation of undigested material from the gut. Average growth rates under best conditions were 36.95 *Aurelia* scyphistoma larvae cloned from a single scyphistoma per slide in 7 weeks.

The 10-step culturing procedure described is valuable because it increases the potential for accurate experimentation with the scyphistoma stage of *Aurelia aurita*. Other marine and freshwater hydroid forms may be cultivated similarly (Groat and Toth 1965, Toth 1965). Because the scyphistomae are attached to a clean and translucent substrate (the frosted glass slide), precise observation and record of the rates of clonal increase are easily done. Individual polyps can be tagged and studies on changes in form, position, motility, and migration from the place of attachment can be easily observed. Because the scyphistomae are attached to a substrate elevated above the bottom of the culture vessel, food and waste remnants do not interfere with the growth and development of the polyps. Clearly, this method is economical for the production of a large number of experimental specimens. Bioassay with *Aurelia aurita* scyphistoma is possible using this culture method, which is particularly advantageous because genetically similar test animals can be cloned from a single polyp in a relatively short time.

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