A MARINE BIOLOGY LABORATORY FACILITY AND COURSE OF INSTRUCTION AT AN INLAND UNIVERSITY[1, 2]

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ABSTRACT

The development of an easily maintained marine biological facility, with artificial sea water and adequate filtration systems, has made possible a course of instruction in marine biology at an inland university. The laboratory facility and the instructional program are described in detail so that individuals at other institutions can adapt desirable elements to instructional and research use.

Instruction in marine biology at inland universities is not common. Biology students far from the ocean have little opportunity to study living marine animals and to appreciate the interesting varieties of marine inhabitants. However, with the development of various inert synthetic materials, it is now possible to maintain inland marine facilities of considerable size for both instructional and research use.

In the spring of 1963, the Department of Biology at Bowling Green State University, Bowling Green, Ohio, began to develop a pilot project with an adequate physical plant for inland marine study. Suitable sealants, a salt-water formulation, and methods of aeration, filtration, and circulation were selected with regard to utility and expense and were tested in a series of marine aquaria. Successful operation of these initial tests resulted in the development of a marine aquarium-laboratory and the establishment of a course in marine biology. The marine biology course includes a field trip to the ocean for collecting marine animals to be transported back to the university for study and research in the aquarium-laboratory. In addition, an inter-departmental course is being offered on oceanography.

THE LABORATORY FACILITIES

In initiating an inland marine laboratory, features which deserve careful consideration include the type of tanks, choice of sealants, selection of a salt water formulation, adequate filtration, and aeration systems, as well as design and construction of the laboratory room. The design and materials discussed below, currently in use at Bowling Green offer an expedient plan for the economical and successful operation of a marine aquarium constructed in a renovated class room. A more elaborate and permanent design can be achieved, at more expense, by constructing a special aquarium-laboratory with built-in tanks and other features.

Although molded fiberglass tanks are best for marine use, since they contain no toxic materials and require minimal maintenance, substitutes will serve adequately. A number of 10-gal, glass, bulb-edge aquaria and a variety of stainless steel frame tanks with capacities of 15 and 29 gal are presently being used in our laboratory. A 0.5-inch hole was drilled in the upper corner of each tank, approximately 2 inches from the rim, to allow for gravity circulation outside the tank (fig. 1). In order to prevent contamination of the salt water through corrosion of the stainless steel frame upon exposure to salt water, the rims and edges were coated with an epoxy-based plastic paint. Such paints are chemically inert in salt water and are purchased as a liquid synthetic resin and catalyst which, when mixed in proper proportions, form a durable and protective surface.

We found that an important factor in selecting marine tanks is the composition

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[2]A paper on this subject was presented at The Ohio Academy of Science meetings, 1964.

of the adhesives used in construction since, with constant exposure to salt water, many sealants deteriorate and contribute chemical impurities to the water. Questionable sealants were avoided by replacing or covering them with a sealant guaranteed to be non-toxic in salt water, such as "Jewel Aquarium Cement" (distributed by Ward's Natural Science Establishment, Rochester, New York). Stainless steel "Metaframe Aquaria," constructed with a sealant guaranteed inert in salt water, are also being used in our laboratory.

In order to retard evaporation and prevent the accumulation of dust and debris, cover lids were constructed so that the lid would fit down into the tank approximately three-eighths of an inch (fig. 1). The lids are held in place by two narrow

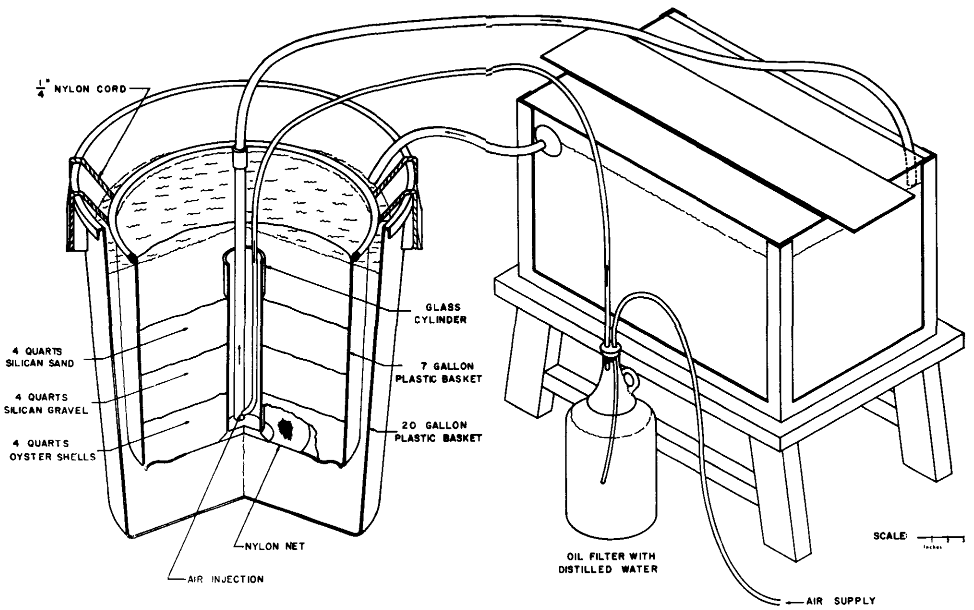

FIGURE 1. A diagram of an individual aquarium and external filter, showing the filtration, aeration and circulation systems.

glass strips, one the length of the lid and another slightly longer, bonded to the larger cover lid with a clear epoxy resin such as "Hysol 'Epoxi-Patch' No. 0151" (distributed by Servi-Supply, Inc., Meadville, Pennsylvania). The longer narrow strip extends 1.5 inches beyond each end of the tank in order to support the lid over the water and, in addition, to serve as handles.

We found that we could not use natural sea water because of shipping costs and the accumulation of decaying organic material during shipment. The use of a concentrate also involves expensive shipping charges, since about one-third of the total water volume remains, although it has the advantage of containing both the major and minor constituents of natural sea water. Our inland facility presently uses a synthetic sea-water formulation which contains all the major elements of natural sea water and a variety of trace elements in the form of packaged salts, but without the water volume.

We have found "Instant Ocean" completely satisfactory. It was developed by Aquarium Systems, Inc., 1450 East 289 Street, Wickliffe, Ohio (A new unitized aquarium system for marine use is also offered by this Company.). This formula contains both major chemical elements and a variety of trace elements, which are mixed with tap water and kept under constant aeration for several days to remove residual chlorine in the tap water. The composition of this formula is modified by the manufacturer for adaptation to the chemical composition of local tap waters. As presently used, the salts are measured for mixing with 100 gallons of tap water, although the formula is available in various quantities. Since the sea water must be mixed in a chemically inert container, a 145-gallon polyethylene drum is used. When mixing is completed, this solution has a specific gravity of 1.025 and a pH value of approximately 8.4 at 20 C. Correlated with the above values of density and temperature, the salinity of this solution approximates that of sea water (Harvey, 1957 and Sverdrup, Johnson and Fleming, 1942). Synthetic sea water is relatively sterile at the beginning of use, with a low initial plankton and bacterial population. This is disadvantageous for marine filter-feeding animals. Such organisms can be fed with *Artemia* nauplii as a substitute for the deficiency in natural nutrients. The presence of trace elements in a closed-system situation does not necessarily mean that such elements will be utilized directly by the marine organisms, since many inorganic materials are initially consumed by marine plants (Sverdrup et al., 1942).

A significant feature of a marine physical plant is the development of efficient filtration, aeration, and circulation systems. In many instances these systems are maintained separately, but in this laboratory these three systems are combined. The design and utilization of the outside filter was developed with cooperation from Mr. William E. Kelley, former Director, The Cleveland Aquarium, Cleveland, Ohio. This filter (fig. 1) is composed of two main parts, an outer filter housing and an inner filtrant chamber, both of which are non-toxic, polyethylene baskets. The inner basket is suspended from the handles of the outer basket by nylon cord, which then follows the circumference under the rim for support. The base of this basket is cut out and a circle of nylon netting is inserted, using nylon fishline as thread. Accumulation of dust and debris is prevented by a lid. The lid is raised slightly above the rim of the outer basket to allow for air circulation.

The filtrant chamber is composed of three equal layers of particulate materials which, together, fill the container to within 5 cm of the top. The lowest layer, next to the nylon netting, consists of $CaCO_3$ in the form of crushed oyster shells; the middle layer consists of silicon gravel (grain size of 2 to 5 mm); and the top layer consists of fine silicon sand (fig. 1). The buffering effect of the $CaCO_3$ layer maintains the pH of the salt water solution in an average range of 7.8 to 8.0. During a year of operation, the pH values have not gone below 7.4. This data corresponds to the pH range of about 7.5 to 8.4 in natural sea water (Sverdrup et al., 1942). The filtrant materials provide an increased surface area for the growth of bacteria important in the mineralization of organic nitrogen and the inter-transformation of ammonium, nitrite, and nitrate compounds (Harvey, 1957).

This filter, by containing, in most cases, a volume of water equal to or greater than the volume of the aquarium, and some 4 quarts of each particulate material, allows for an advantageous dilution of wastes and subsequently less fluctuation in the chemical factors of the water. We have found that an individual filter for each tank should be maintained in order to avoid contamination of a series of tanks served by a common filter. Since metabolic wastes of some marine organisms are highly toxic to other organisms, and since such poisons cannot be removed through normally available filtering methods, the individual filter and tank unit allows for separation of specimens and increases the safety factor. From one-third to one-half of the total water volume of each circulating unit should be replaced each month to reduce the amounts of accumulated nitrogeneous wastes concentrated as nitrites and nitrates (W. E. Kelley, private communication).

Current studies in this laboratory include a comparative analysis of the efficiency of several filtration systems. Circulation by means of an air-lift system has proved highly efficient and provides simultaneous aeration and water circulation between each tank and filter. The air-lift consists of an inflexible polyethylene tube, with the point of air injection at its base, connected to flexible Tygon polyethylene tubing at the upper end (fig. 1). Glass tubing may be substituted with a small portion of flexible Tygon tubing at the base for the air-line injection. This air-lift tube is placed inside a glass cylinder with a closed base, centrally located in the filtrant chamber.

In the circulation system, the air supply lifts the column of water in the air-lift tube through the flexible tubing and into the tank (fig. 1). Water is returned to the filter housing by gravity flow from the hole in the tank, and is forced up through the filtrant chamber materials to fill the glass cylinder for the air-lift. The advantage of this system is that particulate debris is deposited in the outer filter housing rather than in the filtrant chamber. This markedly reduces the need for frequent cleaning of the filtrant chamber and prevents reduction of the filtration rate from the matting of organic debris on the filtrant surface. This system has resulted in efficient filtration, accompanied with buffering action, and adequate aeration of the water. An average of about 8 ml/liter dissolved oxygen (determined by Winkler's method) is present in the water of the tanks. This is within the range of variation of natural sea water (Sverdrup et al., 1942). Recommended air pressure to operate the air-lift is approximately 7 lb/inch.[2] Higher pressures cause air bubbles to rise too rapidly to accomplish efficient water lift and may cause embolism in the organisms. A difference in height of 2 inches between the water level in the filter housing and the level of the overflow hole in the tank is used for the gravity flow return of the water into the outer filter housing. This arrangement has a circulation efficiency of approximately 10 gal an hour, or a turnover rate once every 90 min.

In addition to the use of a standard air filter on the air line the air should be bubbled through a container of distilled water, before entering the air-lift system, in order to prevent contamination of the salt water from oils and rust from the air lines. Such a filter can be made from a glass 1-gal bottle with the air lines placed in a two-holed stopper (fig. 1). These bottles will also add water vapor to the system, thus compensating for evaporation effects. Density variations of the salt water in this system have been relatively slight and have averaged 1.022 to 1.025 sp gr. In individual aquaria, the density is adjusted to correspond to natural conditions where the specimens were obtained.

This marine system at Bowling Green State University is an integral part of the newly constructed aquarium-laboratory, which contains facilities for salt and fresh water aquaria, adequate storage area, display and work space for preservation work, and individual study projects, refrigeration units, incubators and standard laboratory fixtures. The laboratory is maintained at a constant 20 C temperature. This corresponds to the average surface temperature (20.40 C) for the collecting areas where specimens have been obtained in the eastern Atlantic, between 30–40° North latitude (King, 1963). Figure 2 shows the top tier of aquaria, each with an individual filter supported by an extension of the table top. The lower level of tanks is at table level with filters placed on the floor. This figure also shows the various parts of the individual filter system in relation to the aquaria. The two levels of aquaria, together with the individual external filters, allow for approximately 650 gal capacity of salt water.

The brine shrimp *Artemia* is raised in the laboratory, using synthetic salt water, and the nauplii are used in feeding the filter-feeding organisms and the smaller animals, such as pipe fish, barnacles, coral, and sea horses. Bits of frozen shrimp serve as excellent food for the larger animals, such as the varieties of crustaceans and fish. Uneaten particles of food are removed soon after feeding to prevent decay in the water. Prior to feeding, the pH and density of the water in each aquarium are taken and the data recorded. Successful operation of this marine facility has been documented by several longevity records of over a year, with the maintenance of a number of pagurid crabs, lobsters, barnacles, a variety of fish

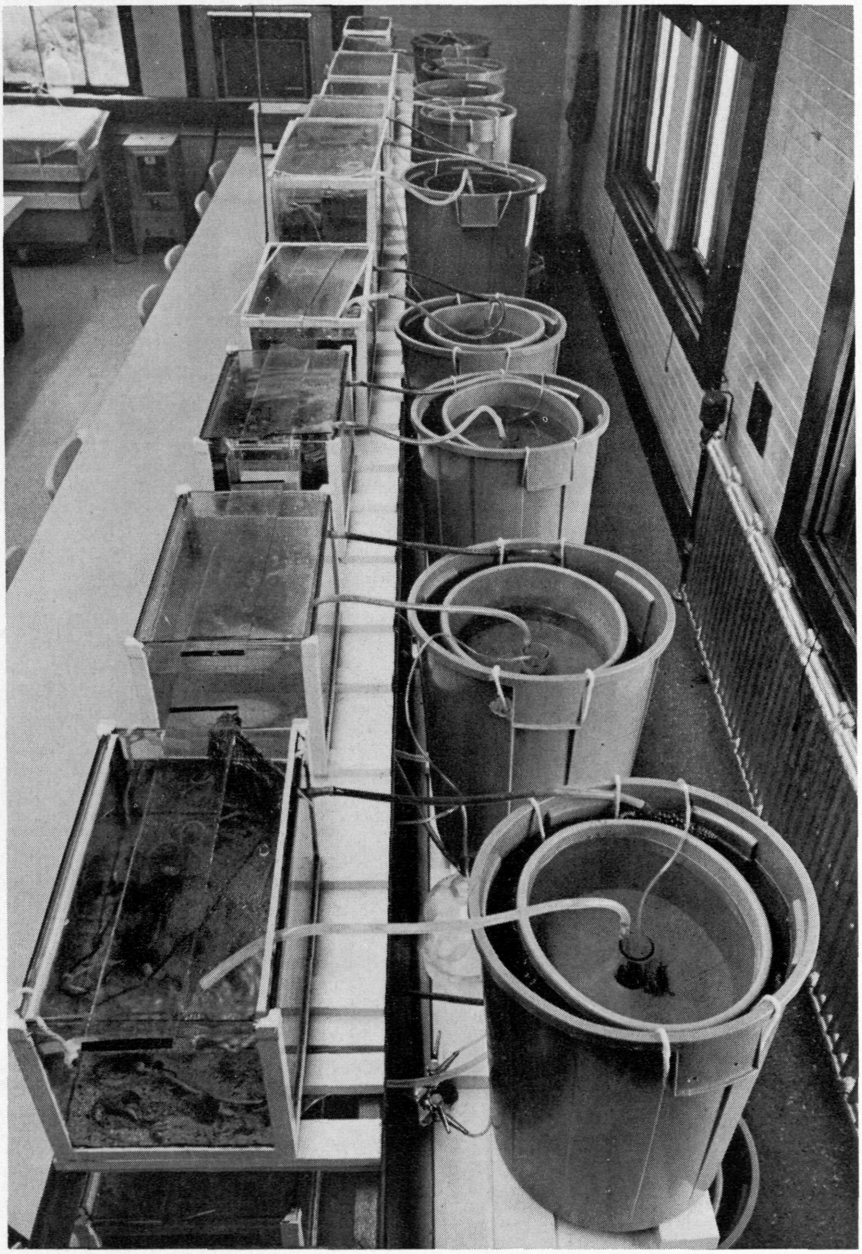

FIGURE 2. The aquarium-laboratory, showing the top tier of marine aquaria and individual external filters. A second row is housed under this shelf.

species, and the northern coral *Astrangia*. A total of 52 species from seven phyla have been successfully maintained.

THE INSTRUCTIONAL PROGRAM

Development of the marine laboratory described above has stimulated the establishment of a research course in marine biology for advanced undergraduate and graduate students. The program includes a collecting trip to the ocean in addition to research and study in the aquarium-laboratory, thus offering a unique educational opportunity for inland students interested in marine biology.

A major feature of instruction is the collecting field trip taken each semester. These trips are taken to the Atlantic coast before the commencement of classes in the fall and to the southern Atlantic or Gulf coast during spring vacation. There are many areas along each coast which offer excellent collecting and study opportunities; prior visiting arrangements are made with various marine stations. Field work includes collecting in a number of ecological environments such as mud flats, inter-tidal zones, tide pools, estuaries, rocky and sandy shorelines, as well as trawling at various depths when an ocean vessel is available. In each ecological zone, an analysis of the physical factors, such as substrate, pH, density, oxygen content, air and water temperatures, is made and recorded.

The collected specimens are kept alive during transportation from the coast to the inland marine aquarium, a distance of 800 to 1,100 miles depending upon the collecting location. Currently, we are using a permanent mobile transportation unit which includes a number of non-breakable containers with permanent air lines, all placed in a covered pick-up truck. A constant air supply is achieved by utilization of a 12-volt air pump driven by the truck battery, and an auxiliary air tank holding about 244 ft^3 of compressed air for emergencies.

During the semester, many aspects of the marine study program are conducted in the inland aquarium-laboratory. The students participate in the general maintenance of the aquaria. Each student is responsible for the upkeep and nutrition of several tanks, including analysis of the salt water, periodic changing of the water, cleaning of the tank and filters, and general physiological observations on the organisms in each tank. Precautions are taken, during any work with the aquaria, to avoid contact of toxic factors, such as tobacco, with the salt water (Simkatis, 1957; Straughan, 1959).

Weekly seminars on such topics as osmoregulation, nervous physiology, bioluminescence, crustacean molting, color changes in crustaceans and other animals, and circadian rhythms provide students with insight into various areas of research. In addition, a seminar discussion on marine ecology is held, correlated with the field trip data and studies. Various aspects of oceanography, meteorology, marine geology, and geophysics are discussed and supplemented by the use of available films. A textbook on marine biology with emphasis on physiological experimentation, such as Raymont (1963), Nicol (1960), or Prosser and Brown (1961), and a more general text such as Coker (1962), and a text on oceanography such as King (1963) have been useful.

The collecting experience and the seminars, in addition to independent library research, are designed to provide a background for the student in selecting a research project which will involve the use of the available living marine organisms in the laboratory. Demonstrations of various techniques of physiological and chemical studies contribute additional background for the students' projects. Research which lends itself to this program involves physiological studies with littoral organisms: Crustacean molting and chromatophore change; identification and culturing of plankton; tube construction in Polychaetes; physiological importance and sorption of trace elements; the efficiency of various filtration systems; the effects and tolerance levels of various physical factors on the organisms, such as temperature, oxygen, pH, and density; the effect of monochromatic light and polarized light on growth and development of organisms; adaptation to various salinity ranges; toxicity of metal ions; fungicidal effects in sea water; sustaining of life cycles (particularly coelenterates); and an analysis of bacterial growth curves correlated with physical conditions of the aquaria. We have found that chemically defined artificial sea water is more advantageous for certain physiological research than natural sea water, which is subject to seasonal fluctuations.

The presence of a living marine collection has generated student enthusiasm about the ocean and its inhabitants, and has provided a stimulating teaching aid for the other biology courses, including invertebrate zoology and general zoology. Interest in the marine aquarium has exceeded the limits of the university community, as evidenced by the numerous visits by individuals and groups from the surrounding area. Also, students who continue their studies at marine biological stations are better prepared to take advantage of the new opportunities.

SUMMARY

Interest in marine study and an initial pilot project has led to the development of a successfully operating inland marine facility, with synthetic salt-water formulation and a combined filtration-aeration-circulation system. A constant temperature aquarium-laboratory has been constructed for marine and freshwater study and research. The development of an inland marine laboratory has stimulated the establishment of a research course in marine biology for advanced students. This program offers a collecting field trip to the ocean in addition to research and study in the aquarium-laboratory.

The presence of a marine laboratory and its collection of living specimens not only provides a stimulating display and teaching aid in a biological curriculum, but also increases the scope of possible research at an inland university. An inter-departmental course in oceanography with instruction shared by chemistry, geography, geology, and biology complements the research in the marine laboratory and the field trips.

The artificial sea water is chemically defined and has been found to enhance the accuracy of certain physiological studies.

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