Requirements for the Culturing of Sea Animals

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Because of a renewed interest in keeping live animals in the school laboratory, the author presents his analysis of many of the difficulties associated with the maintenance of a salt water aquarium. He provides some practical suggestions for a solution to some of these problems.

The sea, because it is such a rich storehouse of animals and plants, has appealed to scientists ever since man first became interested in the things about him. Nevertheless, it has had to be almost ignored as a biotic source since keeping the animals alive in the laboratory has been so difficult.

Atz (1964) reviewed the literature concerning the reasons for 1) the instability of sea water when placed in aquaria or artificial circulatory systems, and 2) the inability of marine organisms to adjust to these water changes.

With the advent of satisfactory synthetic sea salts which maintain their chemical stability over long periods of time and with new culture systems which offer a chemically defined marine environment with methods for controlling this environment, it is now possible to keep marine organisms for indefinite periods and very often through their entire life cycles. In a well designed system, toxic nitrogen compounds decrease by oxidation through nitrite to nitrate, whereas in poorly designed systems ammonia, which is most toxic, usually remains at a very high level.

One of the essential differences between artificial and natural sea water is the quantity and perhaps the quality of organic content.

When one considers the organic content of natural sea water, he is generally thinking of the organic materials present in it as it exists in nature. These are presumably products of the activities of metabolism, growth, and reproduction of plants and animals in the sea. However, when one considers the organic content of artificial sea water, he is usually thinking of materials put into that water by the plants and animals living (or dying) in it or by the persons managing it (such as feeding the animals). Some organic matter could get into the artificial sea water from the fresh water used to prepare it or even from the salts used to constitute it, but it is generally assumed that this is insignificant in comparison to the material contributed as described above.

The differences in organic content may account for differences in animal behavior, if they exist, in aquaria or artificial sea water systems. Very little scientific work has been done which might show that behavioral differences exist except possibly that done by microbiologists who may have observed differences in the behavior of microorganisms in natural and in artificial sea waters.

As soon as natural or artificial sea water is put into a lighted tank some fresh water species of algae which adapt to a marine environment and bacteria start to grow but the exact difference in the rate of plant population increase between the two kinds of water has not yet been fully determined.

Indications are that both algae and bacteria increase at an equal or slower rate in the synthetic sea water. There is no reason to believe that it is not as life-supporting as the natural sea water. Microbiological growth, both qualitative and quantitative, in the dark, has not been completely determined. However, the relatively limited microbiological populations in newly made up synthetic sea water as compared with natural sea water, at least as far as bacteria are concerned, is one of the very desirable features of its use.

Other advantages are as follows: freedom from particulate contaminating materials which might be collected with natural sea water, relative stability of the water's chemical characteristics, avoidance of possible fluctuations in salinity, savings in travel time and inconvenience necessary by trips to coastal waters, the ease with which dry salts can be stored as contrasted with the storage of natural sea water, and the distinct advantage in knowing exactly what is in the prepared sea water.

The general requirements necessary in the designing of satisfactory sea water systems can be summarized as follows: 1) maintenance of a suitable alkaline reserve; 2) avoidance of contamination; 3) maintenance of adequate oxygen levels; 4) removal and/or detoxification of waste products; and 5) maintenance of suitable temperatures.

Control of pH is probably the most important of all chemical controls in a marine system or aquarium but it is not the only essential one. Without it, few of the others would be possible. Simply keeping up the alkaline reserve, however, will not insure a healthy marine aquarium or sea water system. For example, control of ammonia is not possible when the pH is low but too much ammonia can be present even if the pH is at the optimal level.

Biologically inert materials such as hard rubber (vulcanite or ebonite) or plastics (unplasticized polyvinyl chloride) should be used in the construction of life-support systems. Rapid circulation of the water by means of all plastic centrifugal pumps, properly screened, is required. For most laboratory organisms, twenty to twenty-four changes of water every twenty-four hours in a system would be ideal but some may do well with only ten changes. With such a system metabolic ammonia would be diluted and passed over to the filters for conversion to nitrate so rapidly that it would never reach a maximum of 0.05 ppm, and would average about 0.01 ppm.

Contact with air as the incoming water breaks the surface film will insure adequate free oxygen in solution as well as the carrying away of waste products through a biological filter at the rate as given previously.

The importance of bacteria in the biological breakdown of waste products has been recognized for well over fifty years by many biologists but there has been less agreement as to the nature of the bacterial flora. It is now known that the nitrifying activity of the filter bed is greatest in the upper layers of the bed at 30-35°C, and at a pH of 9.0.

The ratio of animals (one pound) carried in the tank to the volume of filtrant material (one cubic foot) of a known size (2-5 mm grains) and composition (calcareous gravel, limestone, coral, or crushed oyster shells) is another parameter in the design of an ideal circulatory system. The ratio of animals to tank size varies from 40 gallons/pound of animals as reported from the Steinhart Aquarium to 448 gallons/pound at the Plymouth Aquarium. One hundred gallons water to every pound of animals should be sufficient. It is recommended that two gallons of water be available for each one inch of fish, five gallons for a two-inch fish, and ten gallons for a three-inch fish.

The chilling of the water by a condensing unit (½ horsepower/150 gallons) or the heating of water by any good aquarium heater with a thermoregulator is essential. Thirteen degrees C is optimum for many common laboratory animals such as urchins, toadfish, and 27°C for tropical fish.

Open systems involve the continuous passage of suitable water (usually from the sea) at an optimal rate for maintaining the inhabitants. A closed system is one operating for three or more months without the addition of outside water. A semi-closed system requires the addition of new water periodically.

In closed systems, after the introduction of animals, the concentration of toxic ammonia will gradually increase as a result of animal metabolism, but if the system is well designed the ammonium concentration will be kept at a low level by the activity of nitrifying bacteria in the filter bed. In a warm fresh water system housing goldfish, the ammonium concentration has been known to rise from 0.055 ppm to 0.980 ppm within five days and then decrease to 0.010 ppm within the following ten days after the build-up of a bacterial flora in the filter bed.

Ammonia is oxidized to nitrite and then to nitrate so that there will be a gradual accumulation of the latter. Since the nitrate has a low toxicity for aquatic animals, it can be permitted to increase but a point will be reached when the health of the captive animals is endangered.

Octopuses appear to be comfortable in sea water with concentrations of 20 ppm of nitrate whereas *Pagurus* begin to show discomfort only after a concentration of 75-100 ppm is reached. Coral fishes will still maintain good health in water that has a content of 300 ppm of nitrate, twenty times higher than in the ocean. The permissible limits of a closed system are, therefore, variable although dependent upon the nitrate toxicity.

Laboratory checks, as described by Strickland and Parsons (1965), assist in the proper management of artificial sea waters. The ammonia test involves the linkage of two uncolored compounds through an ammonium nitrogen producing a colored com-

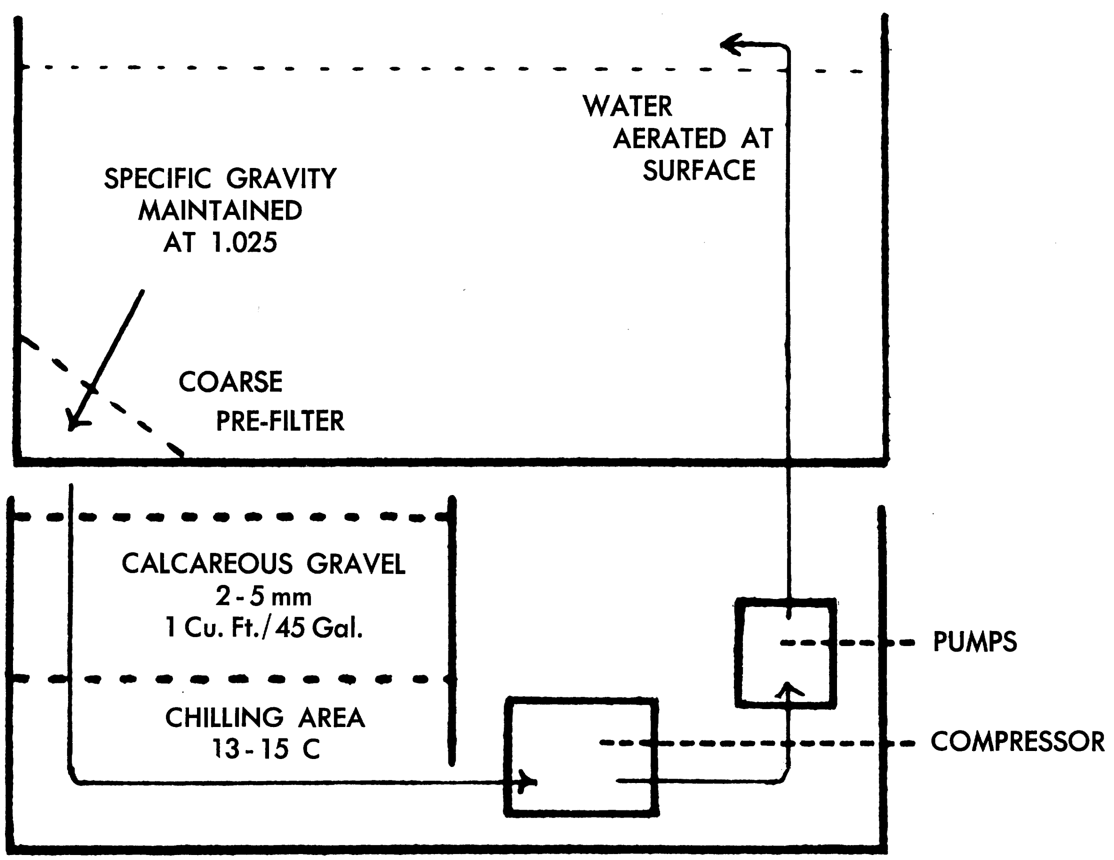

Diagram of Culture System Showing Water Circulating Counter Clockwise.

pound which is then concentrated by extraction in an organic solvent and measured photometrically. Salts interfere with the reaction, reducing the yield by a factor of 0.62 times that of distilled water so that standards must be run in deionized water and the results multiplied by a factor.

The nitrite test involves its linkage with sulfanilamide to form a diazo compound and then coupled to make a colored compound which can be measured as such. Nitrates are reduced with hydrazine sulfate to nitrites and measured photometrically.

High concentrations of ammonia in solution (more than a few ppm) varying according to species, must be avoided. Moderate and gradual loadings of marine organisms without over-feeding and a proper run-in period based upon the size of the initial inoculum of filter bacteria normally introduced with the higher organisms, should keep equilibrium volumes for ammonia as low as 0.055 ppm or less.

The length of the "run-in" period determined by other factors besides the size of the initial inoculum, include the effect of temperature on bacterial growth rates, the trace-element requirements of the bacteria, and the individual characteristics of the bacterial strain present.

Proper "breaking-in" of the aquarium will permit the culturing of even very delicate marine invertebrates without difficulty. Establishing the pH of the water at about 8.2, which is generally suitable for all marine animal organisms, will be maintained by aeration and by passage of the water through calcareous gravel in the filter bed.

To operate a closed or semi-closed system satisfactorily for an indefinite period, the water must be kept circulating at all times to allow for proper aeration and "purification." The specific gravity (1.025) of the water must be maintained. Temperatures must be maintained by a thermo-regulating device. Systems should never be overloaded with animals although maximum quantities as determined by laboratory checks of oxygen and ammonia content can be kept without difficulty. Only enough food should be added at any time to satisfy the hunger of the animals. Periodic laboratory checks of ammonia, nitrite, and nitrate should be carried out (Strickland and Parsons 1965). Approximately 25% of the water should be changed every four to six weeks as determined by laboratory nitrate checks to keep the nitrate level from getting too high. Not too much data are available as yet to allow the setting-up of a "permissible limits" scale for various animals but good management of the physico-chemical factors of closed systems should help to keep most marine animals alive for indefinite periods.

References

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Aquaria

Students in the elementary and secondary teacher education programs at Iowa State University, Ames, Iowa, have the opportunity now of learning about certain animal forms which geographical location has kept rather far away from the midwest. The Zoology and Entomology Department has had installed a series of self contained, temperature-controlled, constant-circulation salt water aquaria in which marine forms can be kept alive for long periods in conditions approaching those of the ocean. The manufacturer of the units refers to them as *Instant Oceans,* and sells the necessary natural salts for making up measured volumes of "ocean." The aquaria not only provide students who have never been to a marine laboratory a chance to see and study live salt-water forms of animals, but also make it possible for advanced students to work on special problems or do research on marine forms. The teaching in elementary zoology and invertebrate zoology has been particularly enhanced.