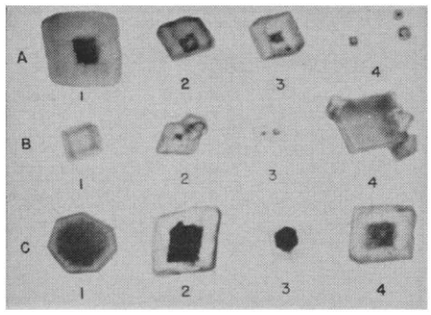

Fig. 1. Photomicrographs of typical dolomite crystals that occur in modern unconsolidated marine carbonate muds along the southern coast of Florida. Crystals range in size from about 16 to 62 μ, although the degree of enlargement varies from photograph to photograph. *A-1*, *A-2*, and *A-3*: euhedral crystals with dark organic material inside. *A-4*: three crystals contrasting lack of etching (lower two) with high degree of etching and loss of crystal faces (upper one). *B-1*: euhedral crystals lacking an inclusion. *B-2*, *B-3*, and *B-4*: interpenetrating euhedral crystals. *C-1*: seven-sided crystal that appears to be in initial growth stage. *C-2*, *C-3*, and *C-4*: euhedral crystals with rhombohedral-shaped dark inclusions.

tion of Na_2CO_3. The suspensions were decanted to effect a sizing based on settling velocity and particle size. Dolomite was suspected when rhombohedrons were found with an electron microscope in the size fraction of less than 1 μ. Many preparations, ranging in size between 62 and 16 μ, were acidified in very dilute (1:30) HCl to remove the more soluble (in dilute acid) aragonite, calcite, and magnesian calcite that constitute more than 95 percent of the total carbonate. The insoluble residue was studied by x-ray diffraction on a Norelco high-angle Geiger-Mueller counter goniometer. As shown by Fig. 2, the insoluble residue consists of dolomite and quartz.

The origin of the dolomite is incompletely known. The presence of complex clusters of these crystals suggests that it has not been transported. The apparent concentration of dolomite near the top of the sediment cores suggests that dolomite is forming at the sediment-water interface rather than after burial in some diagenetic process. If exposure of the mud bank coincides with a time of high evaporation, concentration of the dissolved solids in the surface film of water will rise markedly. The presence of organic material and a high concentration of dissolved salts may possibly be the conditions necessary to initiate growth of the original dolomite crystal on which later crystals may grow.

Fairbridge (*1*) has reviewed the problem of the origin of dolomite. In brief, dolomite is an important constituent in ancient calcareous rocks, but is not known to be forming in modern carbonate sediments whose depositional environment is considered to be typical of ancient deposits. As shown by the present study, however, dolomite is forming along the western margin of Florida Bay. Further geochemical study of this occurrence should help to clarify the origin of some ancient dolomites (*2*).

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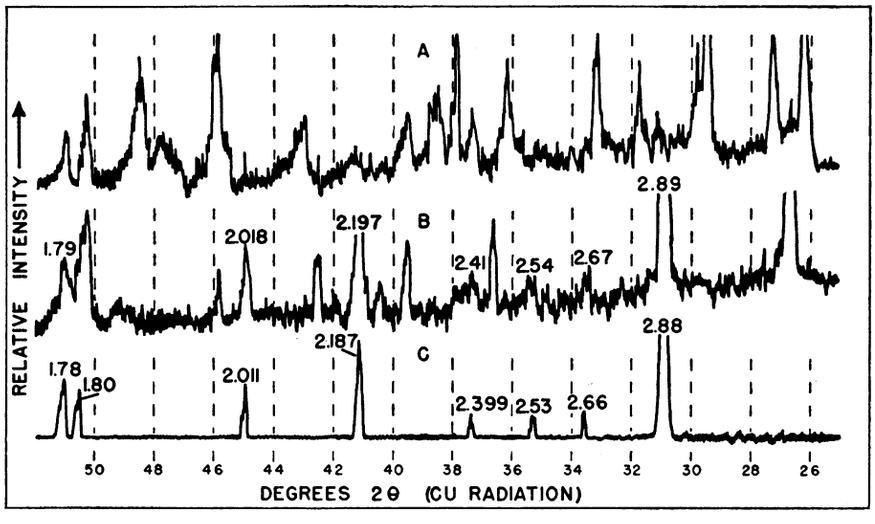

Fig. 2. Comparision of three x-ray diffraction patterns that confirm presence of dolomite in Florida carbonate muds. (*A*) Diffraction pattern of untreated carbonate mud. Note that no dolomite peak appears at 31°. (*B*) Diffraction pattern of residue of sample *A* after calcite, aragonite, and magnesian calcite have been leached away with dilute HCl. Note presence of strong dolomite peak at 31°. Also note that d_{hkl} values for dolomite peaks are listed; other peaks are quartz which also occurs in the residue. (*C*) Diffraction pattern of U.S. Bureau of Standards No. 88 standard dolomite shows dolomite peaks comparable to those in *B*.

References and Notes

1. R. W. Fairbridge, *Soc. Econ. Paleontol. Mineral. Spec. Publ. No. 5*, 126 (1957).
2. This investigation is part of a long-range carbonate sediment study initiated by John W. Harbaugh at Stanford University. Dr. and Mrs. Ramon Somavia, the American Association of Petroleum Geologists, Curt Dietz, the Shell Fund for Fundamental Research at Stanford, and N.S.F. contract No. G6597 provided financial assistance which is gratefully acknowledged.

15 May 1961

Mass Culture of Phytoplankton as Foods for Metazoans

Abstract. An apparatus for mass culture of photosynthetic microorganisms has been developed to grow algae for use as foods for larval and juvenile mollusks in studies of their physiological requirements. The apparatus consists of a series of 5-gal growth chambers, and the system can be enlarged to yield any desired volume of algae by replication of basic units. Approximately 50 lit. of algal suspension, averaging about 0.5 ml of packed wet cells per liter, are produced daily.

In developing a standard technique for rearing lamellibranch larvae, one of the requirements was to provide an adequate quantity of food. To be ingested by early-swimming larvae of the American oyster, *Crassostrea virginica*, food particles must be in suspension and must not be larger than about 7 μ in size.

Photosynthetic nannoplankton appeared to be the most promising natural food. It was found, however, that various species of microorganisms not only differ in food value, but that some are not utilized at all, while others are actually toxic (*1*, *2*). Some produce toxins that appear to be intracellular but, more commonly, toxins occur as external metabolites. Certain bacteria also produce toxins (*3*). In low concentrations these toxins only retard the growth of bivalve larvae, but sometimes concentrations are sufficient to prevent development of eggs, or kill larvae (*4*).

To provide food for the daily feeding of the larval and juvenile mollusks at this laboratory, from 20 to 60 lit./day of a relatively dense culture of food organisms in unialgal and,

preferably, bacteria-free culture are needed. For a pilot or commercial hatchery for clams or oysters this requirement could easily be from 100 to 1000 gal daily. The objective, therefore, was to develop a culture apparatus that is inexpensive, simple to operate, and capable of producing any desired volume of the food culture. A number of comparatively small basic units is preferable to a single larger unit, since several species of algae can then be produced simultaneously. This is important because a mixture of several species of microorganisms often causes better growth of larvae than any single species (*2*), and because larvae of different bivalves require different foods. Moreover, the best foods for larvae are not necessarily the best foods for juveniles of the same species.

Use of algae as food is not, of course, confined to bivalves and their larvae. There are filter-feeding representatives of almost every phylum of aquatic invertebrates that subsist on algae either during early stages of their development or throughout their lives. Development of a dependable method for growth and maintenance of mass cultures of algae is, therefore, a requirement for keeping large colonies of these aquatic animals healthy.

Our culture apparatus accommodates 16 5-gal Pyrex carboys or 20 9-lit. Pyrex serum bottles as growth chambers. Vigorous agitation, by bubbling a mixture of air and CO_2 through the cultures, keeps the contents of the chamber thoroughly mixed. This prevents stratification and helps to expose all cells to periods of intense illumination. The growth chambers are immersed to a depth of 3 or 4 inches in a water bath kept at 19° ± 1°C by a force-flow constant temperature unit. Lighting is provided by four 40-watt, cool-white fluorescent tubes situated behind the back row of growth chambers and three 40-watt tubes suspended above the front row (Fig. 1).

About 1.5 lit. of culture are withdrawn daily from each serum bottle growth chamber, or 3 lit./day from each carboy. Although the various species of algae differ in their rate of growth and final density maintained, some of our cultures have been maintained at this rate of productivity for more than a year, yielding about 0.75 ml of packed wet cells daily from each 9-lit. serum bottle, or 1.5 ml/day from each 5-gal carboy. A volume of sterile sea water and nutrient salts,

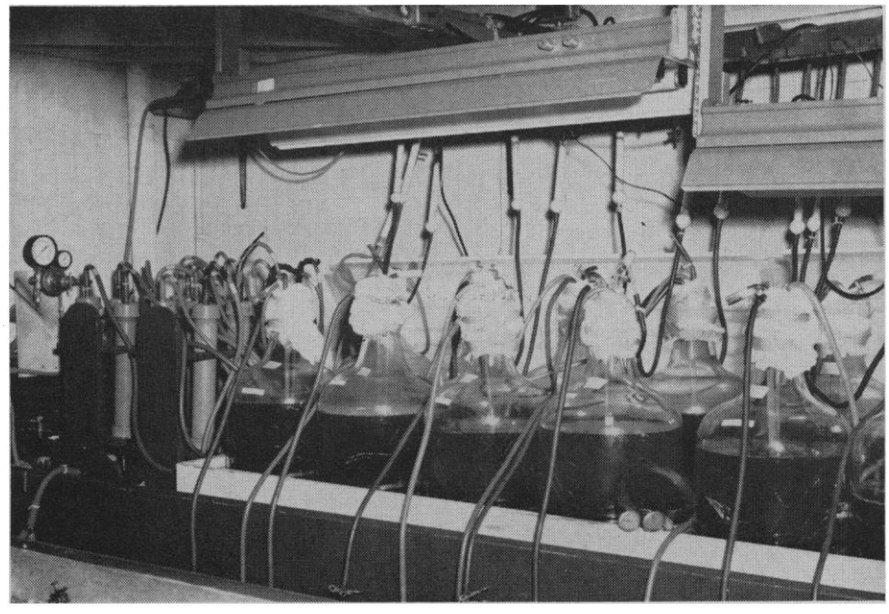

Fig. 1. Culture apparatus, showing carboy growth chambers in water bath and arrangement of lights.

equal to the volume of culture drawn off, is aseptically added to the growth chamber each day.

Mass cultures of chrysomonads, cryptomonads, chlorophytes, and diatoms have been grown successfully in our apparatus. They include *Isochrysis galbana, Monochrysis lutheri, Dicrateria inornata, Dicrateria* sp. (BII), *Chromulina pleiades, Phaeodactylum tricornutum, Cryptomonas* sp., *Dunaliella euchlora, Dunaliella primolecta, Chlorella* sp. (580), and *Skeletonema costatum.*

Much of the labor involved in older methods that use heat sterilization has been eliminated by using a "cold" method of sterilization of the media added to the growth chambers. A number of "cold" methods of sterilization were tested but, until lately, none had proved satisfactory. Recently, by placing a ceramic filter (Selas No. FP-128-03) in the intake line for each growth chamber (Fig. 2), a satisfactory system has been developed. The sea water is first passed through "Ful-flo" Orlon filters to remove larger particles, nutrient salts are added, and this solution is forced through the ceramic filter (maximum pore size 0.6 μ) to replace the culture drawn off.

The growth chamber and Selas filter, together with the tubing connecting

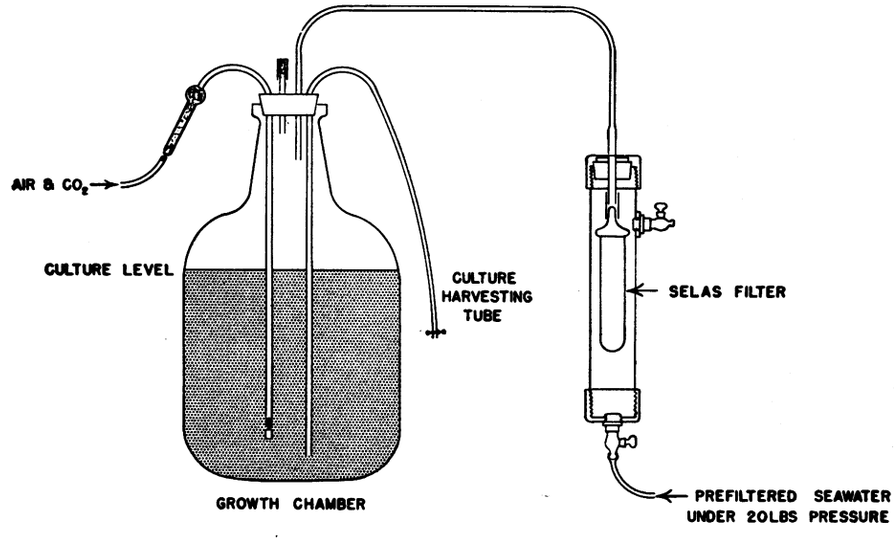

Fig. 2. Diagram of culture apparatus, which uses Selas filter for sterilizing sea water.

them, can be autoclaved already assembled. The casing, into which the Selas filter fits, is of polyvinyl chloride, and the stopcocks are of hard rubber and are not autoclaved. The cap at the upper end of the case is slotted so that it can be fitted over the tubing and stopper after the sterile Selas filter is inserted. This permits periodic disassembly and washing of the outside of the filter in hot water without disturbing the sterile system.

Occasionally growth of algae in enriched sea water is poor, either because of the absence of some essential nutrient normally present in sea water or because of the presence of some toxin. Some artificial sea waters, such as that used by Provasoli *et al.* (*5*), are satisfactory and give uniform results, but their use would be expensive and their preparation time-consuming if used for large-volume cultures. When the nutrient requirements of each of the species of algae under cultivation are known, it should be possible to add a complete nutrient mixture to sea water so that algal cultures will grow well at all times, except during those probably rare periods when toxic elements or metabolites are present in high enough concentrations to retard growth.

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14 March 1961

Blood Flow Measured by Doppler Frequency Shift of Back-Scattered Ultrasound

Abstract. The Doppler shift of ultrasound, scattered from moving elements within a stream of blood, is related to the velocity of blood flow. A flowmeter based on this principle has been constructed and was used to record blood flow through intact vessels in dogs.

If two piezoelectric crystals (barium titanate) are positioned in a plastic cylinder so that they are directed toward a point in the center of the channel (Fig. 1), a sound beam of ultrasonic frequency (5 Mcy/sec) generated from one crystal passes through pure water with so little scattering that no detectable sound energy reaches the second (receiver) crystal. Foreign particles, such as small bubbles or particles, provide interfaces so that some of the sound energy in the beam is scattered and a small fraction reaches the crystal on the opposite side. Normal blood produces sufficient scattering of a 2-watt/cm², 5-Mcy/sec sound beam to provide a detectable level of sound energy at the receiver crystal. If the blood in the cylinder is stationary, the frequency of sound at the receiver crystal is precisely the same as the transmitted frequency. When blood flows along the cylinder, the frequency of the back-scattered sound is altered by the Doppler shift. Since the various particles move at different velocities across the stream, the frequency spectrum of the sound returning from the various interfaces is broadened. However, the frequency of the reflected signal, determined by the frequency meter, was found to be linearly related to the instantaneous flow velocity of the blood during both steady and sinusoidal flow. Thus, the mean Doppler shift in frequency can be employed as a measure of the instantaneous flow velocity of blood.

The plastic transducer was clamped about a length of thin-walled rubber tubing, and the spaces between the crystals and tubing wall were filled with water to couple into the tubing. The received signal was mixed with the transmitted signal to develop a beat signal corresponding in frequency to the Doppler shift. The received frequency differed from the transmitted frequency by 0 to 3500 cy/sec as flow velocity varied from 0 to 100 cm/sec. All frequencies above 15 kcy/sec were rejected by a filter, and the signal was coupled to a frequency meter of the type which develops an analogue voltage proportional to the number of voltage zero crossings per unit time. The voltage from this frequency meter was found to be a linear indication of flow velocity within 5 percent of full scale deflection (100 cm/sec). Furthermore, zero flow levels could be quickly and reliably established by merely removing the voltage input to the frequency meter.

The plastic cylinder was clamped about an unopened artery (descending aorta) of an anesthetized dog, and the recorded signal produced a fairly typical pattern of arterial flow for this site. However, since this simple developmental device does not sense direction of flow, a transient retrograde surge of flow would not be detected. There is some danger that the sonic intensity required to produce detectable reflected sound energy at the receiver crystal may produce hemolysis. However, the

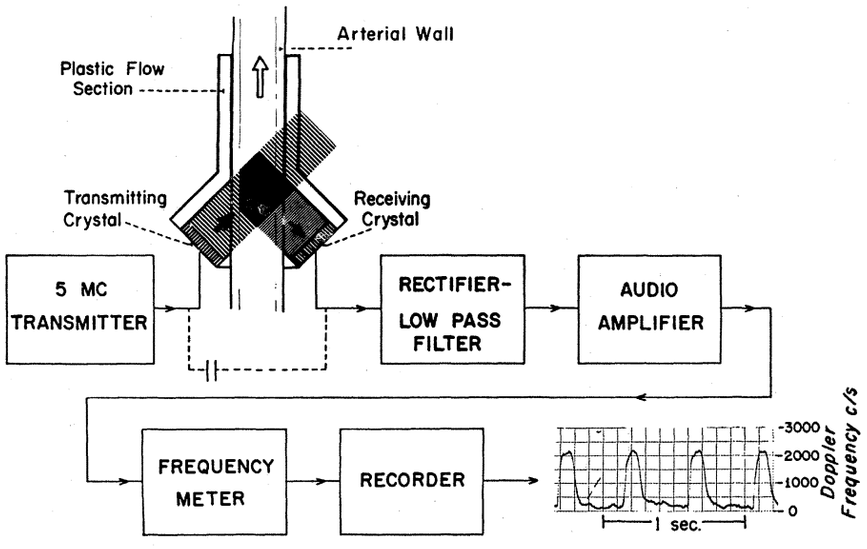

Fig. 1. A method of recording blood flow by measuring the Doppler shift in frequency of ultrasound scattered in the moving blood. High frequency (5 Mcy/sec) sound is beamed diagonally into the blood stream where a part of the incident sound is scattered by the particulate components of blood to the receiving crystal. A beat note with a frequency equal to the Doppler frequency shift is developed from the transmitted and received signals. A frequency meter develops a d-c voltage proportional to the frequency of the beat note. This output voltage is recorded and calibrated in terms of flow. A wave form of flow through the intact descending thoracic aorta of a dog is illustrated.