

Provasoli L, McLaughlin J J A & Droop M R. The development of artificial media for marine algae. *Arch. Mikrobiol.* 25:392-428, 1957.
[Haskins Labs., New York and Marine Station, Millport, Scotland]

The principles involved in formulating chemically defined artificial media for a variety of bacteria-free marine algae are discussed. The strategies used in the US and UK to avoid precipitates in high pH, salinity, and calcium and magnesium media are described. [The SC[®] indicates that this paper has been cited in over 415 publications; it is the most-cited paper published in this journal.]

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Soon after immigrating to the US during the summer of 1948, I became very interested in work being done by Bostwick Ketchum at the Woods Hole Oceanographic Institution. Ketchum was studying the correlations between yearly fluctuations in the sea of nitrogen (N) and phosphorus (P) and algal growth and successions. But these studies were based on experiments with the only available bacteria-free marine alga, *Nitzschia closterium*. By contrast, the Pringsheim culture collection at Cambridge had over 100 bacteria-free cultures of freshwater algae and a variety of chemically defined media were available for studying their nutrition. No such media were available for marine algae; the bacterized cultures at Plymouth (England) were grown on seawater enriched with N, P, and soil extract. The frequency of citation to my paper reflects the great need to fill this vacuum, notably the need to avoid precipitation of P, calcium (Ca), and magnesium (Mg) during autoclaving in high salinity and at the pH of solutions mimicking seawater.

The paper did not result from a collaboration of authors working alongside each other; instead, it pooled independent research by Droop and by McLaughlin and me. Droop joined us for several months when he became aware of our work. My mo-

tive for studying the nutrition and physiology of marine algae was shared by McLaughlin (my graduate student), who was an active collaborator in the research at Haskins Laboratories. Droop probably was interested in doing for the study of marine algae what his mentor, E.G. Pringsheim, had done for freshwater algae. The common goal, though actually reached independently, had often followed parallel lines of research and led to similar media. Obviously, the first areas to be investigated were the needs and tolerances of selected marine algae toward the salinities and concentrations of Ca, Mg, and P conducive to precipitate formation on autoclaving. Most littoral species grow well in dilute seawater, but oceanic species need seawater concentrations. Precipitation during autoclaving was largely eliminated by addition of the chelator ethylenediaminetetraacetic acid (EDTA) and the seldom-toxic alkaline buffer TRIS. Hutner *et al.*,¹ at Haskins Laboratories, had found that EDTA-trace-metal complexes, including those with iron (Fe), remain soluble at high pH and are suitable metal-ion sources for microorganisms and algae. Discoveries that thiamine² and vitamin B₁₂^{3,4} were indispensable for several freshwater and marine algae permitted substitution of soil extract as a source of vitamins as well as trace metals; reproducible chemically defined media thus became available. Media essentially similar to ours now serve for many marine unicellular algae representing all the algal groups and seaweeds.

The trace-metal mixes were empirical; the "active," i.e., available, concentration of each trace metal to the algae was unknown. Morel *et al.*⁵ employed a computer program to define the speciation and molarity of each component of the media, thereby formulating "Aquil," a high-purity medium successfully used for trace-metal studies of marine diatoms and dinoflagellates. Brand *et al.*⁶ using EDTA as a chelator, have found that selected littoral and oceanic species have different optima and toxicity to zinc, manganese, and Fe.

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6. Brand L E, Sunda W G & Guillard R R L. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28:1182-98, 1983.