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Fuhrman J A & Azam F. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl. Environ. Microbiol.* 39:1085-95, 1980. [Scripps Institution of Oceanography, Univ. California, San Diego, La Jolla, CA]

Heterotrophic production rates of native marine planktonic bacteria were estimated by increases in direct cell counts in 3 µm-filtered seawater and also via incorporation of tritiated thymidine into DNA. Results indicated that about 25 percent of primary production is consumed by bacteria, suggesting they are a major component of marine food webs. [The *SCI*® indicates that this paper has been cited in more than 330 publications.]

Bacterial Growth in the Sea

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Oceanographers and ecologists are very interested in knowing the pathways and rates of energy and material flow in aquatic and marine food webs. The most difficult organisms to study in this regard are the microorganisms. Epifluorescence microscopy in the mid-1970s showed that, although they are very small, bacteria contribute significantly to the biomass of the marine systems.[1] But biomass alone does not demonstrate that the organisms are actively participating in ecological processes, because they may be dormant or growing very slowly. What was needed was a means of measuring growth rates of mixed populations of bacteria in natural habitats.

On entering graduate school, and on the advice of my undergraduate research advisor, Penny Chisholm at MIT, I began working with Farooq Azam at Scripps Institution of Oceanography in La Jolla, California. He had already been experimenting with estimating RNA and DNA synthesis rates via incorporation of thymidine, uridine, and adenine into these macromolecules, and I had done similar work with pure cultures as an undergraduate. My graduate research project centered on obtaining quantitative growth rate estimates from DNA synthesis as measured by incorporation of tritiated thymidine in field samples.

My first summer (1978), everyone from the lab traveled to Saanich Inlet, British Columbia, to work on an interdisciplinary project that involved several principal investigators from different institutions studying plankton in large enclosures. It was an enjoyable and intellectually stimulating time. The following winter, I traveled to Antarctica with other Scripps scientists, continuing these experiments in McMurdo Sound. All the sampling was through the ice, and we traveled to different locations by helicopter and sled (human-powered), which was fun. Instead of making holes through the ice ourselves, we decided to use breathing holes conveniently maintained by Weddell seals. Sampling was never interrupted by a surprised seal coming up to breathe, but curious penguins often hopped or tobogganed over to investigate. Additional experiments were performed in La Jolla that spring, and by summer a pattern of results had emerged; the heterotrophic production estimates suggested that bacteria were consuming about 20-25 percent of the total primary production, indicating that their utilization of dissolved organic matter is a major route in marine carbon cycling. While we were writing this up for submission, A. Hagstrom *et al.*[2] published very similar conclusions, based on a completely different approach.

An excellent review by P.J. le B. Williams[3] cited these data as well as others in a major rethinking of the roles of bacteria in the sea. Our own subsequent work included detailed tests of various assumptions we had made, and the results decreased the uncertainties in our prior conclusions.[4] In the last decade, many investigators around the world have used the thymidine incorporation approach, largely because of its relative ease and simplicity. There has been little change in the overall conclusions from a decade ago; however, there has been considerable discussion and debate about methodologies and how best to calculate production rates from thymidine incorporation data.[5]

Extensive interest in the ecological roles of planktonic bacteria and the popularity of the thymidine method probably explain why this paper has been cited so often. Recently, additional methods for estimating bacterial growth and production, particularly leucine incorporation into protein,[6] have become widely accepted. Together, these various tools should help us to understand how microorganisms fit into our picture of the world.

1. **Ferguson R L & Rublee P.** Contribution of bacteria to standing crop of coastal plankton. *Limnol. Oceanogr.* 21:141-5, 1976. (Cited 190 times.)
2. **Hagstrom A, Larsson U, Horstedt P & Normark S.** Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. *Appl. Environ. Microbiol.* 37:805-12, 1979. (Cited 185 times.)
3. **Williams P J le B.** Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. *Kieler Meeresforsch. Sonderh.* 5:1-28, 1981. (Cited 165 times.)
4. **Fuhrman J A & Azam F.** Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.* 66:109-20, 1982. (Cited 335 times.)
5. **Ducklow H W & Carlson C A.** Oceanic bacterial production. (Marshall K C, ed.) *Advances in microbial ecology.* New York: Plenum, 1992. Vol. 12. (In press.)
6. **Simon M & Azam F.** Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol.—Prog. Ser.* 51:201-13, 1989.

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