CC/NUMBER 38
SEPTEMBER 20. 1993

This Week's Citation Classic®

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. [Inst. Marine Resources. Scripps Inst. Oceanography, Univ. California. San Diego, La Jolla, CA]

A method was developed and tested for measurement of heterotrophic bacterial growth in marine plankton by incorporation of tritiated thymidine. Results indicated bacteria consume 10-50 percent of the total primary production of fixed carbon, showing they are important in major element cycling in the sea. [The *SCI*® indicates that this paper has been cited in more than 450 publications, making it the most-cited paper published in this journal.]

Measuring Marine Bacterial Growth

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Until the mid-1970s, heterotrophic microorganisms were generally ignored as agents of material and energy transfer in the sea. Most common methods missed the vast majority of them. In 1974, a visionary article by LR. Pomeroy[1] showed that bacteria and other microbes could be major components of marine food webs. Then, new epifluorescence microscopy techniques, greatly facilitating direct counts, showed that bacterial biomass was substantial in relation to other plankton.[2]

At this point (1977), I was entering graduate school at Scripps Institution of Oceanography. At the excellent advice of Penny Chisolm (at MIT, where i was an undergraduate), I was linked up with Farooq Azam as an advisor, and I can't imagine having made a better choice. He suggested measuring bacterial growth rates to see if this large bacterial biomass turns over rapidly and thus consumes a large proportion of the primary production of fixed carbon. We decided to measure bacterial DNA synthesis by incorporation of tritiated thymidine. This was an approach that other labs had used as an index of relative growth, but not absolute growth rates of mixed species in the field.

Experiments started in summer, 1978, in British Columbia. The whole lab was there, including Tim Hollibaugh, a postdoc who taught me a lot about lab work and the fine art of harvesting crab claws for dinner. (Start by wading through eelgrass until a crab grabs your foot...) We taught ourselves epifluorescence microscopy, originally with slightly fluorescent immersion oil that made it a real challenge! We also found that in field samples, thymidine goes primarily into bacterial DNA.[3] The following winter, I went to Antarctica, where I traveled by helicopter and sampled through seal "breathing holes" in the ice. This was great fun. Even in -2°C water, the method gave interpretable results. Back in California, further measurements revealed a consistent pattern: The bacterial growth rates suggested that bacteria consume perhaps 25 percent or more of the total primary production. As we were writing up the results, A. Hagstrom et al.[4] published the same conclusion based upon a completely independent approach.

Our original published growth estimates[5] used some untested assumptions. The work for this paper was to check each assumption with ocean samples. A new autoradiography technique showed that thymidine is taken up almost exclusively by bacteria and that essentially all active bacteria take it up. Bacterial DNA content was measured from field samples. The percent of incorporated tritium specifically in DNA was shown to fall in a predictable range. Intracellular isotope dilution of the thymidine was estimated with labeled PO_4. These results were combined to generate a "conversion factor" to estimate bacterial production from thymidine incorporation. An independent conversion factor was determined empirically from experiments with native marine bacteria grown in filtered seawater, and this agreed well with the other one. It all pointed to bacterial consumption of roughly 10-50 percent of the primary production.

Since then, our method has been used extensively around the world,[8] probably because it is easy, it apparently works, and the measured parameter is necessary for many studies. Discussion continues about the ideal methodologies and conversion factors to "fine tune" the data,[6] but the basic conclusions from a decade ago have not changed. Additional approaches have been developed to measure bacterial growth,[7] and together these tools are helping us to understand the roles of microbes in the sea.

1. **Pomeroy L R.** The ocean's food web, a changing paradigm. *Bioscience* 24:499-504. 1974. (Cited 205 times.)
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3. **Hollibaugh J T, Fuhrman J A & Azam** F. Radioactively labeling of natural assemblages of bacterioplankton for use in trophic studies. *LimrtoL Oceanogr.* 25:172-81, 1980.
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6. **Ducklow H W & Carlson C A**. Oceanic bacterial production. *Advan. Microb. Ecol.* 12:113-81. 1992.
7. **Simon M** *&* **Azam F.** Protein content and protein synthesis rates of planktonic marine bactena. ***Mar. EcoL—Progr. Ser.* 51**:201-13. 1989**.**

Received July 26, 1993