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BACTERIAL PRODUCTION AND BIOMASS IN THE OCEANS

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The study of bacterial growth dynamics in the sea is a relatively new field of investigation. The subject of bacterial growth is not treated by ZoBell in his classic treatise *Marine Microbiology* (ZoBell 1946), even though Henrici (1938) provided a surprisingly familiar look at bacterial dynamics in freshwater lakes. Brock (1971) reviewed other early attempts to investigate bacterial growth processes in nature, but few of his references are to the marine realm. Yet following the introduction of new methods for assessing bacterial abundance and production rates, bacterial production studies became commonplace on oceanographic cruises and in the literature. Nearly all approaches until quite recently have been limited to addressing bacterioplankton as a homogeneous assemblage, which may explain the success of the field; the new measurements were directly amenable to compartmental modeling just when that activity began its own renaissance, aided by the rapid evolution of the personal computer. In this chapter, I survey recent developments in technique and provide a synthesis of current understanding of bacterioplankton productivity and biomass levels in the sea.

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WHAT IS BACTERIAL PRODUCTION?

Bacterial production is *secondary production*: the synthesis of bacterial biomass, primarily from organic precursors with some inorganic nutrients. The net effect is to move organic matter from one pool to another. Bacterial production can be expressed as the rate of synthesis of cells (N) or cell mass B*:

$$P = \mu B \tag{1}$$

where μ is the specific growth rate of the population expressed in units of inverse time t^{-1},

$$\mu = \frac{1}{B}\frac{dB}{dt} \tag{2}$$

and B (or N) is the mass (number) of cells, expressed per unit of volume. As we'll see later on, the definition, while exact mathematically, contains in practice an element of ambiguity, or circularity. This is because we do not always derive estimates of P through *a priori* measurements of μ and B. In fact, one message of this chapter is that it is easiest in a practical sense to measure P, but the key to understanding the meaning and regulation of bacterial production is still precise and unambiguous determination of in situ values of μ (see, e.g., Ducklow et al., 1992, 1999). Kemp et al. (1993) provide a practical guide to measurements of the three terms in Equation (1). There are many treatments of bacterial growth in the laboratory, but few in nature. Cooper (1991) is the standard text on the physiology and biochemistry of bacterial growth at the cellular level. Appendix 6 in Cohen (1995) provides a lucid, insightful, and entertaining discussion of the mathematics of *human* population growth, which can be applied to bacteria with few if any modifications. I will try to provide a guide for understanding the biological processes and practical pitfalls associated with measuring and understanding bacterial production in the sea. I will consider in turn measurement of μ and P. I discuss N and B in less detail, mostly insofar as they pertain to understanding and measuring μ and P. Finally I review the magnitude of bacterial production in various marine systems.

Is Bacterial Production Net or Gross?

The development of practical and reliable (but see below) approaches for measuring bacterial production allowed a meaningful dialog between marine bacteriologists and biological oceanographers for the first time. Only after bacterial processes could be expressed in the same units used by other oceanographers could bacteria be fitted into current paradigms of marine

*In this chapter, I address production mostly in terms of the biomass as carbon produced, but cell numbers are also discussed. Most readers can consider mass and numbers to be more or less interchangeable for general understanding.

trophodynamics (Williams 1981, 1984; Azam et al. 1983). However there is still some misunderstanding concerning the meaning of bacterial production, especially when bacteriologists talk to phytoplankton ecologists. Scientists working on phytoplankton have the luxury of being able to specify primary production directly in terms of measured fluxes of mass or energy. They measure carbon fixation rates using $^{14}CO_2$ (Steeman-Nielsen 1952) or ^{18}O-labeled water (Bender and Grande 1987), determine changes in total CO_2 or O_2 dissolved in seawater (Williams 1993, Emerson et al. 1993), or quantify light absorption with optical sensors (Marra et al. 1999). From such measurements two quantities, *gross* and *net* primary production, can be estimated. Gross primary production is the total fixation of carbon during photosynthesis in the light, whereas net primary production (NPP) is the gross production in the light less the amount of carbon respired by phytoplankton over a 24-hour period (Falkowski and Raven 1997). Unambiguous determination of gross and net primary production rates is complicated by the presence in most water samples of microheterotrophs, which carry out respiration in addition to that accomplished by the phytoplankton (Williams 1998). But the point here is that phytoplankton ecologists are alert to the distinctions between net and gross production, and they expect bacteriologists to be also. What is it that bacteriologists measure?

The short answer is that they measure net bacterial production (BP_{net}) but usually just call it bacterial production, as I will do in the remainder of this chapter (similarly, when I say "primary production," I am referring to NPP). *All* approaches for determining bacterial production provide estimates in some fashion or other of the *net* rate of biomass synthesis, without including bacterial respiration in the estimates (Jahnke and Craven 1995). Bacterial biomass synthesis plus respiration (R) can be termed gross production (BP_g) in loose analogy to phytoplankton production. Further misunderstanding arises from the use of the term *bacterial carbon demand* (BCD) in place of gross production. In physiological terms, BCD is determined from the gross growth efficiency Y (Lancelot and Billen 1986), or GGE (Goldman et al. 1987):

$$BCD = BP_g = \frac{BP_{net}}{Y} = BP_{net} + R \tag{3}$$

$$GGE = Y = \frac{BP_{net}}{BP_g} \tag{4}$$

where Y is a traditional term from bacterial physiology for *fractional growth yield*, defined as the biomass synthesized per unit total limiting nutrient utilized (Stanier et al. 1976). Bacterial growth yields are addressed in detail in Chapter 10 and in del Giorgio and Cole (1998). Here I simply clarify the relationships among bacterial growth efficiency, carbon demand, and net and gross bacterial production. I should also note that while the GGE can be defined physiologically, it is harder to specify its meaning, or value ecologically, especially over longer time scales (Jahnke and Craven 1995).

One further comment about bacterial production is in order. It is common to try to assess the significance of particular bacterial production estimates by comparing the bacterial production to a simultaneous determination of primary production, usually using carbon-14. Since many routine primary production measurements do not include estimates of the production of DOC (and many estimates do include it: see Cole et al. 1988), one should be careful to avoid using these ratios to claim "bacterial production is X% of primary production," as I occasionally say in this chapter. This form of statement implies that bacteria are directly using some share of the contemporaneous primary production, when in fact what we really wish to convey is that they are using an amount of carbon equivalent to X% of particulate primary production. Only if a total estimate of primary production (particulate plus dissolved) is available can one properly claim that bacterial production was some fraction of the primary production, in that time and place.

WHY MEASURE BACTERIAL PRODUCTION?

The advantages of measuring bacterial production should be obvious to bacterial ecologists, but the rationale for focusing on this measurement may still not be immediately apparent to other biological oceanographers and biogeochemists. In fact, there are clear advantages for bacteriologists and biogeochemists, as implied by the relationships shown in Eqations (1)–(4). This rationale for bacterial production measurements is articulated nicely in Cole and Pace (1995).

Importance of the Microbial Loop

Establishing the existence, functioning, and magnitude of the microbial loop and microbial food webs in the sea has been a major theme of biological oceanography over the past two decades, since the introduction of more easily used methods for determining bacterial production (Fuhrman and Azam 1980; Ducklow 1983; Azam 1998). The term "microbial loop" per se refers to the bacterial recovery through uptake and metabolism of dissolved organic matter (DOM) otherwise "lost" from the trophic system via excretion, exudation, and diffusion (Azam et al. 1983; Jumars et al. 1989). Bacterial production is the key process originating the flux of DOM through the loop, and so estimates of bacterial production establish the importance of the microbial loop and of microbial food webs initiated by bacterivory, in marine ecosystems.

Quantifying Biogeochemical Fluxes of Carbon and Other Elements

Marine bacterioplankton are usually free-living and are sustained by the flux of low molecular weight DOM (LMW-DOM) into the cell. Furthermore for all practical purposes, they dominate DOM incorporation (Azam and Hodson 1977). Only molecules below 500–1000 Da are recognized and transported

through cell membranes by bacterial permeases. Some variable and perhaps large fraction of the LMW-DOM is derived from the breakdown of high molecular weight DOM (HMW-DOM) by extracellular enzymes (Somville 1983; Hoppe 1983; D. C. Smith et al. 1992). Both the LMW and HMW DOM pools consist potentially of hundreds or thousands of individual compounds. These pools cannot yet be fully characterized chemically, and we cannot measure the aggregate fluxes directly (see Chapters 5–7 for discussion of DOM composition and dynamics). In other words it is not yet possible to measure directly the total flux of DOM into bacteria, BP_g. The most practical approach is to determine bacterial production and the GGE and then use equations (3) and (4) to derive the BCD, even though because of interbacterial and viral carbon cycling, the BCD is not a unique function of bacterial production and GGE, (Jahnke and Craven 1995). Even without direct measurements of GGE, we can make first-order estimates showing that DOM fluxes are large terms in the budgets of organic carbon in marine ecosystems, just by knowing that bacterial production is an appreciable fraction of primary production (see below).

Estimating Growth Rates

It is fiendishly difficult to measure bacterial growth rates in nature (Brock 1971). Although in principle it is straightforward to determine the right-hand terms in Equation (2), in practice it is seldom possible to obtain unambiguous estimates of dB/dt. Rates of change of cell populations in nature are usually underestimates of the actual growth rate because there is simultaneous removal of prey cells by predators (Landry and Hassett 1982; Ducklow and Hill 1985a; Chapter 12) and viruses (Chapter 11). Strategies that have been employed for minimizing or independently accounting for the removal terms include dilution, size fractionation, and specific metabolic inhibitors; these are reviewed elsewhere in this book (Chapters 12). Growth rates are of course intrinsically interesting to know, and they are required to parameterize models. Further, if we could measure growth rates unambiguously, and relate them to other, more easily measured variables (e.g., chlorophyll, temperature), then the derived growth rates could be used to estimate bacterial production from equation (1) for large-scale system comparison. But in practice, it is easier to measure bacterial production and biomass, and calculate μ from equation (1), instead. Thus, measuring bacterial production remains our best approach to obtaining large data sets on growth rates. The approach is flawed, however, unless we can specify the fraction of bacterial production (or N) that is actually growing (Zweifel and Hagström 1995; Sherr et al. 1998).

METHODS: A SURVEY AND UPDATE

Marine bacteriology has always been challenged by methodological difficulty, imposed in large part by the exceptionally small size and dilute concentrations

of cells in a complex mix of contaminating organisms and dead particles (Kemp et al. 1993). Any treatment of bacterial biomass dynamics has to address methods to place critical understanding of the data in proper context. It is important to recognize that very few direct determinations of bacterial biomass or production have ever been made in unmanipulated or minimally manipulated samples. There is no carbon-14 assay for bacterial production. Instead, both biomass and production are derived from measurements of related quantities through application of conversion factors. Both the choice of property analyzed and the conversion factor values influence the conclusion of the measurements. The following review is meant to guide the reader toward both deeper and more comprehensive treatments of each subject (e.g., Karl 1986).

Bacterial Biomass

The overwhelming majority of published studies are based on microscopic determinations of bacterial abundance. There are other methods for estimating bacterial numbers or biomass, notably detection of gram-negative cell walls using *Limulus* amebocyte lysate (LAL; Watson et al. 1977). I limit this review to a discussion of direct detection of cells by microscopy or flow cytometry, but it is worthwhile noting that new, automated, and sensitive colorimetric assays of LAL may make this technique more attractive. I am not aware of any published observations using this modernized approach in field study.

Epifluorescence Microscopy Direct microscopy had long been understood to yield substantially higher numbers of bacterial cells in lake waters (Henrici 1933, 1938) and seawater (Jannasch and Jones 1959) than plate count and other cultural techniques. But the difficulty in resolving cells by light or phase contrast optics limited the application of the method, and so cultural estimates remained in favor, despite the lower estimates. As ZoBell (1946, p. 52) concluded, "At best direct counts give data which only supplement and aid in the interpretation of results obtained by cultural procedures."

In spite of the shortcomings of direct microscopy, the approach was followed by Soviet oceanographers, who obtained bacterial biomass estimates of the same order of magnitude as other plankton groups. They formed a modern dynamic viewpoint about oceanic bacterioplankton considerably in advance of Western bacteriologists (e.g., Sorokin 1964). Direct microscopy was finally adopted widely following the introduction of a practical method for concentrating bacteria on optically flat polycarbonate filters for direct counting with acridine orange epifluorescent microscopy (AODC) (Hobbie et al. 1977; Watson et al. 1977). Other, brighter and/or more specific DNA fluorochromes have been introduced (e.g., DAPI; Porter and Feig 1980; SYBR Green: Noble and Fuhrman 1998), but the original AODC protocol remains largely unmodified, irrespective of the dye employed. Direct microscopy remains the most

widely used approach to measuring bacterioplankton abundance and is irreplaceable as a ground truth baseline on which microbiological interpretation can be based. Experienced microscopists are remarkable image processors and data reducers, but it is hard to document properly the information obtained visually during direct counting.

Flow Cytometry With the development of sensitive optics, practical laser systems, and higher fluorescence yield fluorochrome dyes, the flow cytometric detection and enumeration of marine heterotrophic bacteria is becoming an attractive alternative to microscopy. The method is preferable because many more cells are counted in each sample than is possible by epifluorescence microscopy and because the heterotrophic bacteria can be distinguished from prochlorophytes and coccoid cyanobacteria of similar size and fluorescence characteristics (Campbell et al. 1994); these phototrophs are usually counted as heterotrophic bacteria with microscopy. Sample preparation is much less labor intensive, requiring only an adequate supply of liquid nitrogen at sea. Because of the ease of sample throughput and the greater yield of information per unit effort, flow cytometry should replace epifluorescence microscopy for most routine applications over the next few years. The two approaches appear to detect essentially the same population of cells (Figure 1), although region- or perhaps cruise-specific variability remains to be resolved (Ducklow et al. in press).

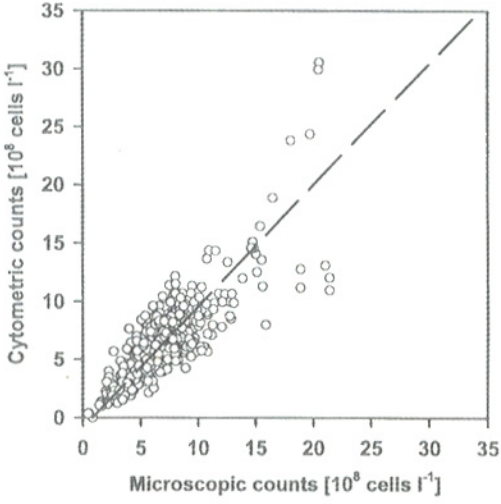

Figure 1. Comparison of epifluorescence microscope versus flow cytometric counts of heterotrophic bacteria in the upper 200 m of northwestern Arabian Sea in January, March, and December 1995. The model II regression line is $Y = 1.04X - 0.87$; $r^2 = 0.69$, $n = 421$. (Data from Ducklow et al. in press; Campbell et al. 1999.)

Cell Volume and Mass Bacterial biomass cannot be measured directly. Rather, biomass estimates are derived from abundance or biovolume measurements multiplied some factor for carbon per cell. Several papers (Cho and Azam 1990; Ducklow and Carlson 1992; Fukuda et al. 1998) show graphs of bacterial biomass versus chlorophyll *a*, indicating that bacterial biomass tends to equal or exceed phytoplankton mass at low chlorophyll concentrations. Table 1 indicates why this might be true, using typical carbon conversion factors (CCF) for deriving biomass. The critical dependence of relative biomass level on assumptions about conversion factor values is obvious.

Estimation of cell volume remains technically difficult by microscopy and cytometry. Cell volume is now routinely measured with epifluorescence microscopy using digital image analysis of video images (Bjørnsen 1986; Ducklow et al. 1995; Carlson et al. 1996; Pomroy and Joint 1999). Especially when large numbers of samples must be analyzed for oceanographic surveys, the analysis is limited for practical considerations to about 300–400 cells per sample, usually without replication. A serious problem with epifluorescent determina-

Table 1. Phytoplankton and bacterial biomass in the ocean, estimated using different C:Chl ratios for calculating phytoplankton carbon (Phyto-C) from chlorophyll (Chl) and carbon conversion factors (CCF) for estimating bacterial carbon (Bact-C) from cell counts

Regime	Chl ($\mu g\,L^{-1}$)	Number of Bacteria (10^9 cells L^{-1})	C:Chl ($\mu g\,\mu g^{-1}$)	CCF ($fg\,C\,cell^{-1}$)	Phyto-C ($\mu g\,C\,L^{-1}$)	Bact-C ($\mu g\,C\,L^{-1}$)
Open sea	0.1	0.5	50	10	5	5
Coastal	1	1	50	10	50	10
Estuary	10	5	50	10	500	50
Open sea	0.1	0.5	100	10	10	5
Coastal	1	1	100	10	100	10
Estuary	10	5	100	10	1000	50
Open sea	0.1	0.5	50	20	5	10
Coastal	1	1	50	20	50	20
Estuary	10	5	50	20	500	100
Open sea	0.1	0.5	100	20	10	10
Coastal	1	1	100	20	100	20
Estuary	10	5	100	20	1000	100
Open sea	0.1	0.5	50	30	5	15
Coastal	1	1	50	30	50	30
Estuary	10	5	50	30	500	150
Open sea	0.1	0.5	100	30	10	15
Coastal	1	1	100	30	100	30
Estuary	10	5	100	30	1000	150

tion of cell volume is lack of authentic standards. Fluorescent microspheres are commonly used to calibrate measuring algorithms, but their emission wavelengths and fluorescence yields differ from those of native bacterioplankton, (i.e., they are different colored and brighter) and this complicates edge detection by image processing. Thus it is not possible to compare cell volumes objectively. For example, Wiebinga et al. (1997) reported relatively large mean cell volumes of 0.11 μm^3 $cell^{-1}$ for the northwestern Indian Ocean during the Southwest Monsoon in 1992, while Pomroy and Joint found cells averaging 0.03 μm^3 $cell^{-1}$ slightly further northeast in 1994. The latter value is more characteristic of oceanic regimes, yet while regional and interannual differences cannot be discounted, the standardization problem renders the debate somewhat futile. Newer, brighter fluorochromes might alleviate this problem.

Deciding which objects seen or detected under the microscope should be counted and measured as bacterial cells presents another difficulty. Most image analysts rely on experience and subjective criteria such as shape, size, and brightness to edit noncellular objects prior to analysis. This approach is generally reliable for open-ocean samples, which contain smaller numbers of detrital particles and other contaminants of bacterial image fields; but even experienced microscopists report difficulty when analyzing inshore and estuarine waters with high and diverse populations of noncellular objects. Recently, Blackburn et al. (1998) reported an image discrimination technique using neural network–based algorithms whereby operators can "teach" image analyzers to reject certain classes of particles. With new stains and more sophisticated numerical approaches, microscope-based determination of cell number and size should remain a benchmark for some time to come.

Flow cytometry appears to offer some hope of improvement. Mie theory suggests that light-scattering characteristics should be a function of cell volume or mass, making it possible to derive mass from cytometric determination of mean forward angle light scatter (FALS) per sample. Robertson et al. (1998) report a good relationship between FALS and dry mass for cultured cells and a natural population. A current drawback is that marine bacterioplankton still lie right near the lower limit of resolution for most flow cytometers, rendering extrapolation of the relationship between FALS and cell volume down to the sizes characteristic of native populations uncertain (P. del Giorgio, personal communication). Another cytometric approach is to relate the mean fluorescence per sample to cell volume, determined on parallel samples with image analysis. This approach is intuitively reasonable, inasmuch as larger cells containing more DNA should absorb more stain and fluoresce more brightly (Sherr et al. 1999). However calibration relies on microscopy, with the difficulties already noted. Further, the presence of inactive, nondividing cells with low DNA content, and of small, rapidly growing cells with multiple genomes (Wiebinga et al. 1999) would also confound straightforward interpretation of cell-specific fluorescence information. Nonetheless the large sampling rates and multiparameter data collecting capability of flow cytometers make approaches toward cytometric cell sizing highly attractive.

Reliable translation to cell mass is required to convert bacteriological measurements of abundance and cell volume into biogeochemically useful mass units. Initially, Fuhrman and Azam (1980), followed by others, used a value expressed in femtograms of carbon per micrometer cubed, namely, 120 fg C μm^{-3} cited in Watson et al. (1977) to derive relevant biomass estimates. That value was based on measurements of cultured *E. coli* which are 100-fold larger than native bacterioplankton. Bratbak and Dundas (1984) and Bratbak (1985) triggered a small revolution in bacterial appreciation with new estimates ranging 160–930 fg C μm^{-3}, which overnight increased bacterial standing stocks in the sea by a factor of 3 or more. These estimates were based on pure cultures of marine bacteria grown on lab media and natural samples grown in enriched seawater. The first estimates of carbon per cell for native bacterioplankton grown on naturally occurring substrates were given by Lee and Fuhrman (1987), who grew natural assemblages from small (<0.8 μm) filtrates in particle-free seawater and related microscopic volume estimates to C and N masses measured with a CHN analyzer. Interestingly, they determined that carbon per unit volume was itself inversely proportional to cell volume, such that carbon per cell was relatively constant at 20 fg over the observed size range of 0.036–0.077 μm^3. Twenty femtograms of carbon per cell has assumed something of a canonical status in marine bacteriology (Cho and Azam 1990; Ducklow and Carlson 1992). Using similar cellular mass estimates, many investigators have concluded that bacterial biomass must equal or even exceed phytoplankton biomass in many oceanic regions (Fuhrman et al. 1989; Cho and Azam 1990; Li et al. 1992).

The 20 fg C $cell^{-1}$ value represents a high cellular carbon density, especially for the small (<0.05 μm^3) cells characteristic of many oceanic regimes (≥ 400 fg C μm^{-1}). This is toward the upper end of the higher estimates reported by Bratbak and Dundas and others. Independent estimates of bacterial biomass indicate that bacterial carbon content might be somewhat lower. Several investigators with access to synoptic data on biomass of a comprehensive range of plankton groups ranging from phytoplankton and bacteria through protozoans and zooplankton attempted to constrain the bacterial carbon content using plankton biomass and total particulate carbon and/or living carbon. Christian and Karl (1994) and Caron et al. (1995) obtained estimates of 10–15 fg C $cell^{-1}$ for these "constrained" conversion factors. In another novel approach Carlson et al. (1999) used high precision analyses of DOC and TCO_2 to recover the bacterial carbon by difference in a mass balance approach, which also yielded GGE estimates. Their bacterial carbon content ranged from 7 to 13 fg C $cell^{-1}$ for cells of 0.06–0.09 μm^3.

The cellular carbon conversion factors reported by Lee and Fuhrman (1987) and others address cultured material derived from natural bacterial assemblages, but they are not direct estimates of actual in situ bacterial populations. As pointed out by Fukuda et al. (1998), these estimates might be biased by species succession during culture and by growth on substrates supplied as artifacts of the filtrations used to prepare the seawater culture media.

Table 2. Carbon content and carbon density of bacterial cells

Region	Density (fg C μm^{-3})	Content (fg C $cell^{-1}$)	Method	References
Pure cultures	160–930		CHN analysis	Bratbak (1985)
Estuarine: coastal shelf				
Norwegian fjord		7–12	X-ray diffraction	Fagerbakke et al. (1996)
Long Island Sound	210–600	15–24	CHN analysis	Lee and Fuhrman (1987)
Otsuchi Bay, Japan		17–53	CHN analysis	Kogure and Koike (1987)
Ross Sea Antartica	77–165	7–13	C mass balance	Carlson et al. (1999)
Oceanic				
Hawaii		10	Biomass constraints	Christian and Karl (1994)
Bermuda		15	Biomass constraints	Caron et al. (1995)
Southern Ocean		12	Direct measurement	Fukuda et al. (1998)

Fukuda et al (1998) reported the first direct measurements of the carbon and nitrogen content of marine bacterial assemblages. After preparing filtrates of surface waters from a wide range of coastal and oceanic sites, with minimal phytoplankton contamination, they analyzed carbon using the high temperature catalytic oxidation (HTCO) methodology. The analytical approach avoided the need to concentrate samples on GF/F filters for CHN analysis, with the attendant loss of small (possibly carbon-dense) cells through the filters. Fukuda et al. (1998) report mean carbon per cell of 12.4 ± 6.3 and 30.2 ± 12.3 fg C $cell^{-1}$ for oceanic and coastal locations, respectively, noting that if their estimates are representative of most marine areas, use of a uniform factor like 20 fg C $cell^{-1}$ would overestimate bacterial biomass in oceanic habitats and underestimate it in coastal regions. Bacterial carbon content is summarized in Table 2, and some estimates of bacterial standing stocks are given in the sections that follow.

BACTERIAL PRODUCTION

Bacterial production, defined in equation (1), is commonly measured indirectly using radioisotope-labeled precursors of DNA and/or protein synthesis to yield

synthesis rates, which must be converted to production rates using empirical factors. The two most common approaches use [^{3}H]-thymidine (Fuhrman and Azam 1980) and [^{3}H]-leucine (Kirchman et al. 1985), and these approaches (especially thymidine) have been reviewed and debated extensively (Moriarty 1985; Robarts and Zohary 1993; Karl 1986; Karl and Winn 1984; Ducklow and Carlson 1992; Kemp et al. 1993). The reader is directed to these reviews for more detailed discussion of bacterial production methodology.

Earlier Approaches

Karl (1979) proposed the first modern method for estimating bacterial production in the sea. He initially measured incorporation of [^{3}H]-adenine into RNA in upper- and midwater samples from the Caribbean Sea, and later extended studies to pure cultures of phytoplankton and bacteria as well as various oceanic and other aquatic environments (Karl et al. 1981; Karl and Winn 1984). In many (but possibly not all: see Fuhrman et al. 1986) marine environments, [^{3}H]-adenine is incorporated by bacteria, phytoplankton, and perhaps other microorganisms, which prompted Karl to apply the adenine technique to estimating total microbial production rates. The ambiguity or nonspecificity of this approach is the main reason for the decision of many investigators not to adopt it in studies calling for specific information on heterotrophic bacterial production. Nonetheless the adenine approach had several advantages that have not yet been approached by other methods. With adenine it is possible to measure the intracellular specific activity of the labeled tracer precisely by extracting [^{3}H]- and/or [^{32}P]-labeled ATP, which quickly achieves isotopic equilibrium with the other macromolecular constituents of the cell (Karl et al. 1981). If one knows the turnover rates of macromolecular pools, it is possible to estimate the specific turnover rates (= growth rates) of the population directly from isotopic data. Finally, [^{3}H]-adenine is taken up very rapidly by microbial assemblages, providing a high sensitivity method. Christian et al. (1982), Hanson and Lowry (1983), and Ducklow et al. (1985) applied this approach to various marine environments.

At about the same time the adenine method was introduced, Hagström et al. (1979) proposed that the frequency of dividing cells (FDC) in bacterial assemblages could be used to derive growth rates, and thus, production rates. This approach is based on both theoretical and empirically established relationships between the frequency of dividing cells (cells that have formed invaginations in the cell wall and a division plate but have not separated) in a population and the population division rate. A major attraction of this approach is that once it is calibrated, no incubations are required to obtain growth rate information—a simple collection of preserved samples can be examined following cruises or experiments to recover the rate data. However as Hagström et al. (1979) showed, the relationship between FDC and growth rates is temperature dependent and nonlinear, so calibration requires incubations, much as in the thymidine method and other approaches (see below).

Christian et al. (1982) showed that in experimentally manipulated samples, FDC was related to adenine and changes in cell numbers. I am not aware of a systematic comparison of FDC with more widely used bacterial production methods. More significantly, the difficulty of resolving dividing cells precisely, especially small oceanic cells, renders the method impractical for many marine environments, even given the no-incubation advantage. The FDC method probably should be reevaluated, given the recent wider application of flow cytometry and image analysis techniques for investigating cell morphology and DNA content.

Thymidine and Leucine Incorporation

The introduction of [^{3}H]-thymidine (TdR) incorporation as a measurement of heterotrophic bacterial production (Fuhrman and Azam 1980, 1982; Fuhrman et al. 1980) really did usher in a new era in the study of bacterial dynamics in marine and freshwater. The TdR approach offered a relatively simple protocol that was specific for estimating the production rate of actively growing bacterial cells. The pros and cons of TdR have been debated at great length (Karl and Winn 1984; Moriarty 1985), and there are several drawbacks (Hollibaugh 1988; Robarts 1993), but it remains the most widely used of all bacterial production methods (Kemp et al. 1993).

Two recent developments extend the utility of the TdR method. An adaptation allowing processing of 1–2 mL samples in a microcentrifuge has vastly reduced the volumes of radioisotope and reagents required, decreasing cost and waste production (D. C. Smith and Azam 1992). Steward and Azam (1999) propose the application of bromodeoxyuridine as an alternative, non-radioactive precursor of DNA synthesis for estimating bacterial production. These two approaches will help investigators who are limited by money or by radioactive materials prohibitions on vessels. Both approaches may even turn out to be more sensitive than the original approach.

The measurement of [^{3}H]-leucine incorporation (Leu) into bacterial protein was proposed as an alternative to TdR by Kirchman et al. (1985). As Kirchman (1992) and several others have shown, TdR and Leu incorporation rates do covary over a variety of time and space scales, suggesting that both methods address bacterial production–related processes. Comparison of bacterial production estimates from the two methods provides some idea of the uncertainty in determining bacterial production. As originally described by Kirchman, the Leu approach required an empirical conversion factor (CF) for estimating bacterial production, in a manner analogous to TdR. Kirchman (1992) used empirically determined conversion factors to estimate bacterial production by the two methods in the subarctic Pacific Ocean. He obtained slopes of 0.92 ± 0.09 and 0.76 ± 0.08 for (model I) regressions of Leu-BP on TdR-BP in the 0–40 and 40–80 m layers, respectively. This data set points to the complications involved in comparing the two methods, which measure separate though related physiological processes.

Agreement between Leu- and TdR-based bacterial production estimates will be exact if unbiased conversion factors can be obtained and if the ratio of the conversion factors is equal to the inverse ratio of the incorporation rates. Kirchman (1992) found that the mean ratio of conversion factors for TdR and Leu (Leu:TdR) was 16.1 ($n = 17$ and 14 for TdR and Leu), and also reported that the mean ratio of Leu:TdR incorporation rates for the entire water column data set was 16.8 ($n = 481$). This is very good agreement, accounting for the good match between the bacterial production estimates. But since the ratio of the conversion factors was slightly less than the incorporation ratio, the TdR-based estimates of bacterial production were slightly greater than the Leu-based estimates. If Leu:TdR incorporation rates vary substantially through a water column, reliance on a single set of conversion factors (usually derived from just one or two depths), will probably yield differing estimates of bacterial production.

Variations in Leu:TdR incorporation ratios can be substantial (Table 3), but there have been just a few investigations of the meaning of this variability. Chin-Leo and Kirchman (1990) showed how Leu:TdR can change with changes in growth rates and/or physiological state of bacterial assemblages. Departures from balanced growth should change the incorporation ratio, since cellular composition changes when cells shift up or down to a new growth rate (Cooper 1991). The meaning of balanced versus unbalanced growth is not straightforward in mixed natural populations probably growing at a range of rates (see below). Tibbles (1996) showed that Leu:TdR is temperature dependent, with ratios increasing with temperature. Since Leu and TdR incorporation appear to differ in their temperature dependence, conversion factors should not be constant with respect to temperature. Shiah and Ducklow (1997), following

Table 3. Ratio of leucine to thymidine incorporation rates (pmol:pmol) in the upper 200 m at selected oceanic sites

	Region		
	North Atlantic (47°N, 20°W)	Equatorial Pacific (0°N, 140°W)	
	May 18–31, 1989	March 23–April 1992	October 2–21, 1992
Mean Leu:TdR ratio	23.0	19.7	12.8
Standard deviation	18.6 (81%)	6.1 (31%)	2.9 (22%)
Range	2.6–116.3	9.1–52.5	4.6–23.0
N	156	206	223

Source: Ducklow et al. (1993, 1995).

Brunschede et al. (1977), suggested that "unfavorable" conditions (e.g., excessively low or high temperatures in temperate estuaries) led bacterial populations to invest more cellular resources in biomass synthesis (measured by Leu) than cell division (TdR). These studies all address relative changes in Leu:TdR ratios, but no one to date has been able to explain quantitatively the significance of the value of the ratio in any given sample, or regional differences in the ratio (Table 3). Further insight into relationships will probably require improved models of bacterioplankton physiology, explicitly addressing protein, RNA, and DNA synthesis, and cell division. Numerical simulation models of bacterial biosynthetic pathways exist (Stephanopoulos and Vallino 1991; Vallino et al. 1996) but have not been applied to bacterial production measurement scenarios.

Simon and Azam (1989) introduced another approach to estimating bacterial production employing Leu. They showed that the ratio of protein to carbon was highly invariable in bacterioplankton cells. From this observation it can be deduced that carbon-based bacterial production can be derived from Leu incorporation without recourse to a cell-based conversion factor or knowledge of carbon per cell (see above). If the intracellular isotope dilution is known or can be measured, a single conversion factor can be established for converting Leu to bacterial production. Simon and Azam (1989) claimed intracellular dilution of [^{3}H]-leucine varied by a factor of about 2 and suggested that this conversion factor would have a range of just 1.5–3 kg C per mol of Leu incorporated. The proliferation of converstion factors for TdR and Leu complicates cross-system comparison of bacterial production (Ducklow and Carlson 1992). Simon and Azam's (1989) approach provides a somewhat universal factor for bacterial production estimation.

Moreover the ratio of biomass production from Leu to cellular production from TdR:

$$\frac{\mu g\, C^{-1} l^{-1} h^{-1}}{\text{cells}\ L^{-1} h^{-1}} \tag{5}$$

yields the biomass of the average newly produced cell (μg C cell^{-1}). For example, Ducklow et al. (1993) derived a TdR CF of 2.65×10^{18} cells mol^{-1} to estimate bacterial production in the North Atlantic in 1989. The mean Leu:TdR ratio given in Table 3 and Simon and Azam's (1989) Leu CF indicate new cells had 13–26 fg C cell^{-1} [i.e., $(23 \times 1500$ to $3000)/(2.65 \times 10^{18})$], a value within the range of cell masses reviewed above.

If ambient cell mass were known reliably, it could be compared to the estimated mass of daughter cells to make inferences about removal rates and size-selective grazing. If, for example, newly produced cells are small, but the mean observed cell sizes were significantly greater, one might assume that bacterivory was not intense, or at least that grazers did not seem to be selecting larger cells to ingest. Unfortunately, as suggested earlier, we cannot easily or routinely specify cell mass reliably.

GROWTH RATES AND VIABILITY

Cell Kinetics; Bacterial Abundance and Biomass

In most natural water samples, simultaneous removal of bacterial cells by bacteriovores and viruses can reduce or balance the specific growth rate of the prey bacterial population, giving the appearance that the bacteria are not growing, as shown for phytoplankton preyed upon by microzooplankton (Landry and Hassett 1982). This is a very common case for natural bacterial assemblages in the ocean. In experiments, however, grazing and viral lysis can be minimized if not eliminated completely by dilution with filtered water; grazers can be eliminated by filtration. In these experiments, bacterial abundance increases over time, which provides an estimate of bacterial production and specific growth rates.

Observation of changes in cell numbers or mass over time is the most direct but perhaps not the easiest way to measure bacteria growth. Nonetheless the approach is worth the effort: it affords us the most fundamental access to cell growth per se, since it is based on direct visualization of the cell assemblage. Further, by providing an estimate of production independent of precursor incorporation assays, this approach is commonly used to derive empirical values for conversion factors (Kirchman et al. 1982). The slope of a plot of the natural log of cell numbers versus time yields the specific growth rate μ (equation (1), Figure 2A). Figure 2A illustrates a uniform population of cells (e.g., a pure culture or a seawater culture of a natural assemblage dominated by a single population) growing exponentially with $\mu = 1.0\ d^{-1}$. This population is incorporating thymidine at a constant cell-specific rate of 2.8×10^{-20} mol cell^{-1} h^{-1}. As Kirchman et al. (1982) pointed out, the slope of the plot of the incorporation rate versus time is identical to the abundance plot. When cells are in balanced, exponential growth, specific growth rates can be determined from the increase in incorporation rate. This direct approach utilizing either the cell or incorporation plots can be used to estimate growth rates and conversion factors (Christian et al. 1982; Cuhel et al. 1982; Li, 1984; Ducklow and Hill 1985a, 1985b; LaRock et al. 1988; Ducklow et al. 1992; Chrzanowski et al. 1993). Kirchman et al. (1982) suggested that it was easier to measure incorporation rates than to count cells, a view I suggest is a matter of individual choice.

One problem is the presence of nongrowing cells in the culture assemblage, or more generally, heterogeneity of population growth rates. This problem has not been systematically treated even though new techniques are beginning to show how it may be solved. Kirchman et al. (1982), Cuhel et al. (1982), and Li (1984) all suggested that plots of incorporation rates would reveal the mean growth rate of the cell population or fraction thereof actually incorporating the isotopes employed. If TdR were used, this by definition would address the dividing cells. If only part of the total cell population were growing or actively incorporating isotope, the incorporation rates would increase faster than the

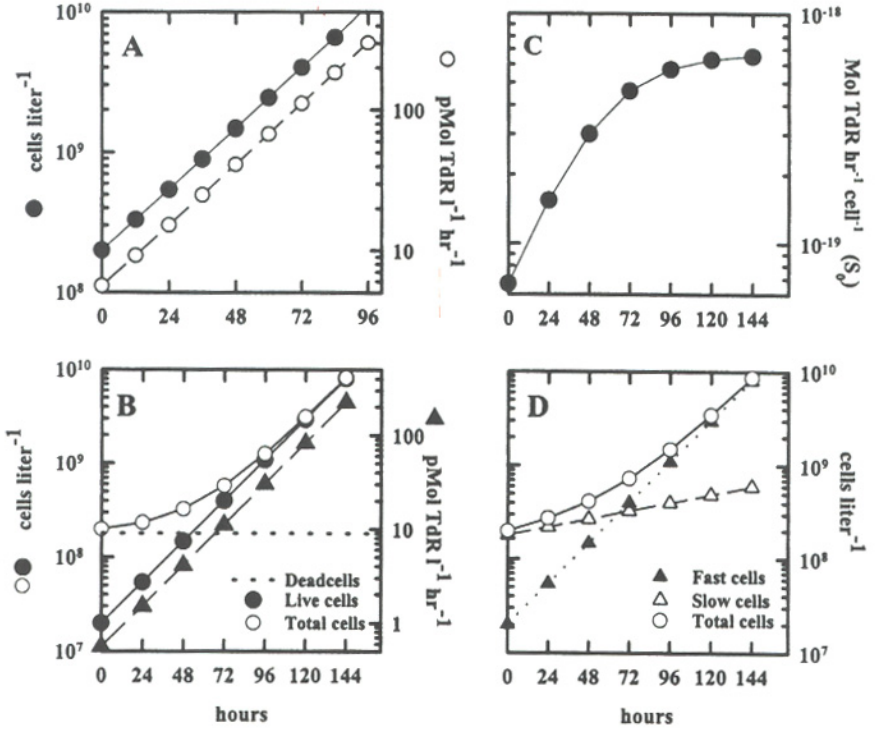

Figure 2. Exponential growth in idealized bacterial assemblages. (A) Solid circles, 100% active cells, growing at 1 d^{-1}; open circles, thymidine incorporation by the growing cells. (B) 90% nongrowing "dead" cells and 10% "live" cells growing at 1 d^{-1}; triangles, thymidine incorporation by growing cells. (C) Apparent cell-specific rate of Tdr incorporation by the total (live + dead) cell population in (B). (D) A small "fast" and a 10-times-larger, "slow" population growing at 1 and 0.2 d^{-1}, respectively. Note the nonexponential growth of the total population.

total cell count because the cells are "diluted" by the inactive or nongrowing fraction. Figure 2B shows a bacterial assemblage with initially 10% of the cells growing at 1 d^{-1} as in Figure 2A, and the remainder of the assemblage nongrowing and constant. TdR incorporation by the growing fraction again parallels the growing cell plot. Note though, that the total population appears to have a lag period, caused by the gradual overgrowth of the nongrowing cells by the active fraction (Zweifel and Hagström, 1995). Extrapolation from the later stages of growth in the total counts back toward time zero provides an estimate of the original size of growing fraction, provided population structure has not changed. Torreton and Dufour (1996) used a similar "nongrowing fraction" model to estimate that just 0.1–5% of the total cells were active in the coral atoll lagoons of the Tuamoto Archipelago.

This example shows how the problem of nongrowing cells can be addressed simply by counting the total cell population long enough to permit extrapola-

tion back to time zero. The scenario requires that nongrowing cells remain nongrowing in culture. In most cases in which bacterial growth in seawater cultures has been studied, this scenario has not been addressed directly—for example, by means of autoradiography or vital stains like CTC to monitor the growth of the active fraction. Choi et al. (1996: their Figure 3A) show results from a seawater culture incubation with total (DAPI) and "active" (CTC-stained) cell counts. The total counts have an apparent lag period similar to the one shown in Figure 2B, while the CTC counts grew without lag and converged with the total counts, consistent with the "inactive subpopulation" model.

Figure 2D shows a more general case with two growing populations. It is easy to see from this plot that increasing contrasts in growth rate and/or size of the two populations will enhance the curvature of the "lag" period. Even with greatly contrasting populations, the lag period may not be detected if

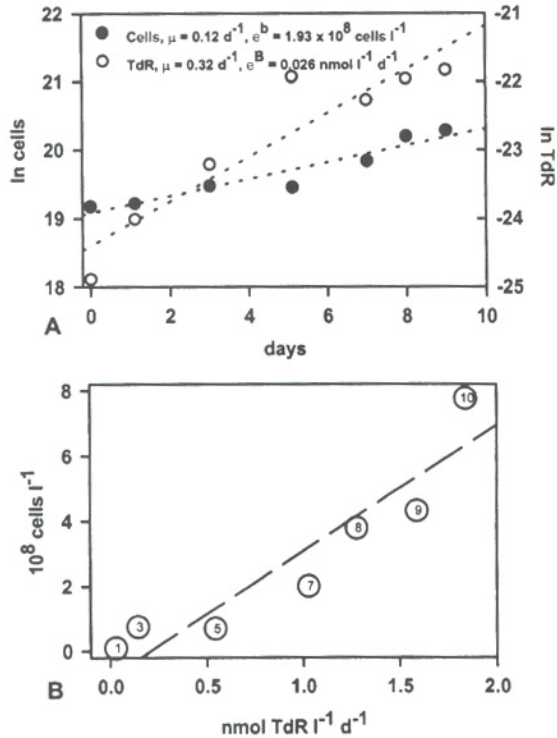

Figure 3. Seawater culture growth experiment from the Ross Sea, Antarctica. (A) Growth of cells and increase in TdR incorporation rates during a 10-day incubation. (B) Cumulative cells produced plotted against integral TeR incorporation. The slope of this plot is the thymidine conversion factor (Bjørnsen and Kuparinen 1991). Days sampled are given by numbers in circles.

sampling is infrequent, as the example shows: even daily sampling over 4–6 days would not yield a significant departure from linearity to confirm existence of the telltale "lag" phase. Using flow cytometry, Wiebinga et al. (1999) detected growing (at 2.4 d^{-1}) and nongrowing bacterial populations in seawater cultures from the Arabian Sea, showing a plot almost precisely like Figure 2B. But with samples taken at approximately 3, 6, 12, and 18 hours, curvature in the plot of total cells cannot be demonstrated, and the experiment did not last long enough for the plots to converge. However because the investigators detected both subpopulations directly (using cytofluorimetrically detected DNA content), the essential structure of the assemblage was apparent.

When a total cell count is made up of growing and nongrowing, or fast and slow populations, the incorporation rates will increase faster than cells, as noted earlier. This effect was observed by Ducklow and Hill (1985b), Ducklow et al. (1992), and Pomeroy et al. (1994). Figure 2C shows how incorporation rates which increase faster than cells result in increasing cell-specific incorporation rates. The leveling off of the curve is a reflection of the convergence of the active and total populations (cf. Figure 2B). From the simple models shown here, and indirectly suggested by Choi et al. (1996) and Wiebinga et al. (1999), it now appears that observations of differential specific growth rates determined from cell kinetics and incorporation rates can be explained by postulating two or more populations with different growth rates. I am not aware of a direct test of this hypothesis using autoradiography or cytometric sorting of labeled cells to follow the active subpopulation responsible for the growth of the incorporation plot.

Application to Determination of Conversion Factors

Fuhrman and Azam (1980, 1982) originally proposed a "theoretical" value for the TdR conversion factor of $0.2–2.4 \times 10^{18}$ cells produced per mole of TdR incorporated and showed that the TdR-derived bacterial production matched that independently estimated by counting cells in filtrates smaller than 3 μm. With an independent estimate of the specific growth rate or production rate, the conversion factor (CF) required to derive the bacterial production from the TdR incorporation, T, is just

$$CF_{\text{der}} = \tau \frac{N_0}{T_0} \tag{6a}$$

where τ is the slope of ln T versus time, and with all three parameters derived from the plot shown in Figure 3A. This approach was termed the "derivative approach" (Kirchman et al. 1982) because it was calculated from the slope of the regressions shown in Figure 3A. A difficulty arises when there is heterogeneity among the active and total populations. To address this problem, Ducklow et al. (1992) and Kirchman and Ducklow (1993) proposed a modified form of

equation (6a):

$$CF_{mod} = \mu \frac{e^B}{e^b} \tag{6b}$$

and used regressions of cells and TdR incorporation versus time to estimate the parameters B and b, the y-intercepts of the regressions. This is just the same as equation (6a), except that observed N_0 and T_0 are replaced by the derived time zero estimates, e^B and e^b. It is easy to see that if there is significant curvilinearity in either cell or incorporation plots as shown in Figure 2, equation (6a) will probably result in high CF values as a result of dividing a high N_0 by a low T_0 value. Equation (6B) is an attempt moderate this effect, but most comparisons still indicate that this approach tends to yield high CF values.

The so-called cumulative approach (Bjørnsen and Kuparinen (1991) is an alternative empirical approach, that also employs time-course data on cell growth and incorporation rates. In this approach the cumulative cells produced to each time point are regressed on the integrated thymidine incorporation, and the calculated slope is the CF. This method tends to give lower values than those of Kirchman et al. (1982): either the "derivative" method [equation (6a)] or its modified form [(equation (6b)]. This is because more rapidly increasing TdR incorporation relative to cumulative cell production lowers the slope of the plot (Figure 3B).

Figure 3 shows results of an experiment conducted in the Ross Sea in November 1994 (Ducklow et al. 1999: temperature $\approx -1°C$) to illustrate cell dynamics and calculation of conversion factors. The upper plot shows time courses of ln cells and ln TdR incorporation. The slope of the TdR data is clearly greater than the slope of the cells plot, suggesting that not all cells were incorporating TdR. Although it is difficult to discern clearly, there was an apparent lag period in the cells plot, also indicative of slower growing or nongrowing cells, consistent with the larger TdR slope. Extrapolation of the cell counts between days 5 and 9 yields an estimate of 5×10^8 cells L^{-1} for the growing cell population, which is 20% of the observed initial cell abundance. Conversion factor values calculated from these data using equations (6a) and (6b) give values of 4.4 and 1.6×10^{18} cells mol^{-1}, respectively. Cumulative cell production is plotted against integral TdR incorporation for the same data set in the lower graph. The model II regression slope of the plot yields a conservative CF estimate of 3.9×10^{17} cells mol^{-1}.

Thus three methods of deriving conversion factors from the same data set result in estimates that range over an order of magnitude. Which value is correct? The original derivative approach is correct if there are indeed nongrowing or slowly growing cells in the assemblage. The high value of this estimate makes sense. A smaller population incorporating TdR will probably have low incorporation rates requiring a large CF to obtain the correct bacterial production. Without independent observations to verify that some

Table 4. Bacterial production and conversion factors in the Ross Sea[a]

	Bacterial Production ($\mu g\ C\ L^{-1}\ d^{-1}$)		
Cell mass ($fg\ C\ cell^{-1}$)	Derivative	Modified Derivative	Cumulative
10	1.14	0.43	0.10
20	2.27	0.85	0.20
30	3.41	1.28	0.30

[a]Rates based on TdR incorporation, using conversion factors calculated by the indicated procedures [equation (5) and the different C per cell factors. Rates based on Leu incorporation were 0.12–0.42 $\mu g\ C\ L^{-1} d^{-1}$.

percentage of the cells was nongrowing, there is no a priori way to decide whether this is the valid formula to use. The cumulative approach makes the fewest assumptions about cell growth and composition of the populations and their growth, but it clearly yields an underestimate if the growing population is small.

At present there is no very satisfying alternative to decide on which approach to take if only total cell counts are available. One approach would be to compare the bacterial production estimates to those obtained from leucine incorporation using the conversion factor suggested by Simon and Azam (1989). A difficulty is that these two estimates cannot be compared directly without also assuming a carbon-per-cell factor to convert the TdR-based estimates of bacterial production into carbon production. For the experiment shown in Figure 3, L_0, the initial rate of leucine incorporation, was 0.14 $nM\ d^{-1}$, which using Simon and Azam's (1989) conversion factor of 1.5–3 $kg\ C\ mol^{-1}$, gives a bacterial production of 0.21–0.42 $\mu g\ C\ L^{-1}\ d^{-1}$. The T_0 of 0.03 $nM\ d^{-1}$ gives bacterial production of 0.1–3 $\mu g\ C\ L^{-1}\ d^{-1}$, using the three conversion factor estimates and three choices of C per cell (Table 4). The derivative approach seems to yield high values of bacterial production, whereas the other conversion factors give values closer to the leucine value, depending on the cell mass.

THE ECOLOGY OF GROWING AND NONGROWING CELLS

All the models discussed thus far are based on the assumption that there can be substantial populations of inactive or nongrowing cells in natural assemblages. There is no question that at least some bacterial species are exquisitely well adapted for long-term survival (days to centuries) in media with no energy sources. There is an enormous literature on the physiology and biochemistry

of bacterial starvation–survival in cultures, and in various natural media and aquatic environments (Poindexter 1981; Kjelleberg et al. 1993; Morita 1997). Morita (1997) put forward the view that most of the biosphere is highly oligotrophic with respect to bacterial nutrition, and most bacteria in most habitats are in the starvation–survival state. There is substantial evidence that sometimes sizable fractions (from < 10 to $> 75\%$) of marine bacterial assemblages are not active, as indicated by autoradiography (Hoppe 1976; Douglas et al. 1987) or vital respiratory stains (e.g., CTC: del Giorgio et al. 1997; Sherr et al. 1998). Because of the lack of good operational definitions for these terms, and methods to address them, it is difficult and perhaps impractical to establish whether all the inactive cells are truly dormant, inactive, or nongrowing.

It seems unlikely that, as posited in some of the foregoing models, a static cell population could be maintained at a fixed size in nature for an extended period of time. Such cells would be cropped from the population by grazers unless they were nutritionally inferior to growing cells, and grazers strongly preferred growing cells. Nonetheless this view was reenergized by Zweifel and Hagström (1995), who used a modified DAPI staining/destaining technique to suggest that many marine bacterial cells lacked nucleoids, hence by definition were nongrowing "ghosts." Later Choi et al. (1996) showed that cells initially observed to be ghosts grew actively in seawater culture and later had nucleoids. Morita (1997) showed that starving cells undergo a loss of DNA as part of the starvation–survival adaptation. Thus Zweifel and Hagström's "ghosts" are probably similar to cells showing up as inactive in autoradiographic assays or CTC stained samples: they contain nucleoids too small to show up on the microscope, but they are viable cells.

The importance of these observations is as Zweifel and Hagström (1995), del Giorgio and Cole (1998), and others have suggested. Bacterial assemblages in nature appear to be dominated by small, highly active subpopulations coexisting with larger groups of less active, or perhaps temporarily inactive cells. The ubiquity of removal processes (e.g., bacterivory, viral lysis, adsorption, sinking) demands exchange between the active and inactive fractions of the bacterial assemblage. Perhaps some fraction of the growing population is intermittently or continuously "turned off" while parts of the inactive populations are reactivated, as observed by Choi et al. (1996). Blackburn et al. (1996) attempted to account for the presence of ghost cells in a numerical simulation model by including processes by which cells could be inactivated, but they did not include reactivation and recruitment of cells back into the growing fraction.

It is important to point out that production rates and fluxes measured by current methods are not affected by these considerations, but specific rates of growth and activity must be higher if fewer cells are actively engaged in carrying out measured activities. This argument opens up the possibility that bacteria in nature might be growing at rates substantially greater than estimated from bulk considerations, to maintain measured production rates. Mean growth rates are commonly reported to lie in the range $0.1–1\ d^{-1}$ for habitats reaching from the equator (Kirchman et al. 1995) to the poles

(Ducklow et al. 1999; Rich et al. 1998). If the actively growing fractions of these assemblages are as small as 10% of the total assemblage, growth rates must be scaled up accordingly. But if the active and inactive fractions exchange substantially on the time scales of growth, then growth rates integrated over the exchange timescale may lie somewhere in between these extremes. Clearly, better insight into the biology and ecology of bacterioplankton requires reliable ways to penetrate and resolve the demographic structure of natural assemblages.

BACTERIOPLANKTON STANDING STOCKS AND PRODUCTION RATES

Bacterioplankton biomass and production estimates are uncertain at least by a factor of 2, owing to unexplained variability and imprecisely specified conversion factors, discussed earlier. Further, variability in conversion factors and independent behavior of TdR and Leu incorporation (Table 4) complicate comparisons of stock and production estimates in the literature. Ducklow and Carlson (1992) approached this problem by using mean values for cell and thymidine conversion factors to back-calculate a consistent set of estimates. One possible objection to this approach is that it ignores empirical conversion factors calibrated for a particular study (Rivkin et al. 1996). Another complication is the basis of comparison. Many estimates use the euphotic zone (depth of 1% surface irradiance) as a common depth for integration. This is a logical basis for comparison to phytoplankton stocks and photosynthetic rates, but it leaves open the question of what processes support bacterial stocks below the illuminated layer.

Wiebinga et al. (1997) and others have pointed out that carbon produced in the euphotic zone must ultimately support bacterial carbon demand throughout the water column. Choice of some greater depth is arbitrary unless one could specify the time and space scales over which the products of local photosynthesis are dispersed prior to bacterial utilization. This exercise could be accomplished using three-dimensional numerical models, but only if we also knew how to parameterize the carbon flux relevant to bacterial metabolism! Mixed layer comparisons have the added complication that mixed layers vary over diel to interannual time scales (Gardner et al. 1995; Michaels and Knap 1996). There is no satisfying answer to this minor dilemma, except perhaps to provide estimates integrated over several characteristic scales. Here I compare euphotic zone integrals, simply because I wish to scale the resulting estimates to phytoplankton properties.

Cole et al. (1988) synthesized data extending over seasonal to annual scales to conclude that bacterial production was equivalent to about 20–30% of the local primary production. They reported the data as originally presented, using the conversions applied in each respective study. Their exercise should be repeated. In the ensuing decade a great many additional studies have been

published, including many for the open sea, which was not well represented in the original summary. It is not clear that the ratio of Cole et al. (1988) holds for the open sea. Bacterial production was 5–15% of ^{14}C primary production in the subarctic Pacific in 1987–1988 (Kirchman et al. 1993). The researchers used an empirical conversion factor of 1.7×10^{18} cells mol^{-1} TdR and 20 fg C $cell^{-1}$ to derive their estimates. Ducklow et al. (1993) concluded that bacterial production was 15–80% of the ^{14}C primary production during the spring phytoplankton bloom in the subarctic NE Atlantic, using a mean TCF of 2.7×10^{18} cells mol^{-1}, and 20 fg C $cell^{-1}$. Li et al. (1993) used TCF of $1–2.3 \times 10^{18}$ cells mol^{-1} and 20 fg C $cell^{-1}$ to conclude that bacterial production was 8–18% of the ^{14}C primary production in the northwestern Atlantic at the same time. Later, Kirchman et al. (1995) and Ducklow et al. (1995) again used 20 fg C $cell^{-1}$ to estimate bacterial production in the central equatorial Pacific during the 1992 El Niño. They did not determine empirical conversion factor but used a mean value from the literature of 2.2×10^{18} cells mol^{-1} to convert TdR to bacterial production, obtaining bacterial production to primary production ratios of 12–20%. In the Sargasso Sea off Bermuda, bacterial production was 15% of primary production, using 1.6×10^{18} cells mol^{-1} TdR and a volumetric conversion factor of 120 fg C μm^{-3}. Wiebinga et al. (1997) and Pomroy and Joint (1999) estimated bacterial production in the Arabian Sea using a variety of TCF and CCF, obtaining a larger range of 3–50% of primary production (average 10–18%). In general, bacterial production averages about 15% of primary production, excluding a few nonequilibrium situations like decaying blooms (Ducklow et al. 1993). These estimates all used conversion factors within a factor of about 2 of each other, lending at least ease of comparability, if not absolute reliability to the estimates.

Recent observations of heterotrophic bacterial stocks and production rates are summarized in Table 5 and compared to corresponding, synoptic phytoplankton data. I tried to minimize some of the concerns cited above by reporting observations for which the raw data were accessible, and by using a consistent set of conversion factors to derive the carbon-based estimates. These data are discussed in more detail in Ducklow (1999). This summary provides data on bacteria in open-ocean regimes including the subarctic North Atlantic and Pacific, oligotrophic gyres in both basins, the Arabian Sea region influenced by monsoonal upwelling, the Antarctic shelf seas, and the central equatorial Pacific. The subarctic and equatorial Pacific are "high-nutrient, low-chlorophyll" (HNLC) regimes (Longhurst 1998). As a simple generalization, bacterial biomass in the euphotic zone averages about 1–2 g C m^{-2}, except in the Antarctic, where it is usually much lower. Production rates, and thus mean turnover rates, vary more widely, as do relationships with phytoplankton properties. However it is notable that with a few exceptions, bacterial production is usually about 10–20% of the corresponding primary production.

It is generally accepted that bacterial populations decline in size from estuaries and inshore areas of greater organic and inorganic enrichment toward the more oligotrophic open sea (Sieburth 1979). Volumetric estimates of

Table 5. Bacterioplankton and phytoplankton properties in the open sea[a]

Property	N Atlantic[b]	Eq Pac–Spr[c]	Eq Pac–Fall[d]	Sub N Pac[e]	Arabian[f]	Hawaii[g]	Bermuda[h]	Ross Sea[i]
Euphotic zone m	50	120	120	80	74	175	140	45
Biomass, mg C m^{-2}								
Bacteria	1000	1200	1467	1142	1448	1500	1317	217
Phytoplankton	4500	1700	1940	1274	1248	447	573	11450
B:P	0.2	0.7	0.75	0.9	1.2	3.6	2.7	0.02
Production, mg C m^{-2} d^{-1}								
Bacteria	275	285	176	56	257	nd	70	5.5
Phytoplankton	1083	1083	1548	629	1165	486[j]	465	1248
B:P	0.25	0.26	0.11	0.09	0.22	nd	0.18	0.04
Growth Rates, d^{-1}								
Bacteria	0.3	0.13	0.12	0.05	0.18	nd	0.05	0.25
Phytoplankton	0.3	0.64	0.8	0.50	0.93	1.1	0.81	0.11
B:P	1	0.2	0.15	0.1	0.19	nd	0.06	2.3

[a] All stock estimates based on 20 fg C $cell^{-1}$. Data may overestimate actual heterotrophic eubacterial biomass as a consequence of lower C contents and/or interference by *Prochlorococcus* and *Archaea*. Production estimated from 3000 g C mol^{-1} leucine incorporation.

[b] Eastern North Atlantic spring phytoplankton bloom, 47°N, 20°W; May, 1989, $n = 13$ (Ducklow et al. 1993).

[c] Equatorial Pacific, 0°N, 140°W; March–April 1992, $n = 18$ (Ducklow et al. 1995).

[d] Equatorial Pacific, 0°N, 140°W; September–October 1992, $n = 19$ (Ducklow et al. 1995).

[e] Subarctic North Pacific, 45°N, Kirchman et al. (1993).

[f] Northwest Arabian Sea, 10–20°N, 165°E, January–December 1995, $n = 21$ (Ducklow et al. in press).

[g] Hawaiian Ocean Time Series (HOT); 1995–1997; $n = 21$; (http://hahana.soest.hawaii.edu/hot/methods/pprod.html).

[h] Bermuda Atlantic Time Series (BATS); 1991–1998, $n = 106$ paired comparisons; for BP and phytoplankton biomass calculations, see Carlson et al. (1996). The ratios are means of the ratios, not ratios of the means. BP calculated from TdR(1.6×10^{18} cells mol^{-1}).

[i] Ross Sea, Antarctica; 76°S, 180°W; 1994–1997; Carlson et al. (1998); Ducklow, unpublished data.

[j] 1989–1996; $n = 64$. Data source as for note *g*.

bacterial abundance (cells per liter) range from about $1–5 \times 10^8$ cells L^{-1} in the most oligotrophic regions studied (Cho and Azam 1990), to over 2×10^{10} cells L^{-1} in rich estuaries (Ducklow and Shiah 1993). Therefore it is surprising to compare integrated bacterial stocks in euphotic zones from different oceanic regimes (Figure 4A). Estimates from four areas of the North Atlantic region [Chesapeake Bay, the Sargasso Sea and nearby Gulf Stream, and the northeastern subarctic (*sensu* Longhurst 1998)] range from 0.5 to 120×10^{12} cells m^{-2}. There is a significant but not strong positive relationship with euphotic zone depth ($Y = 0.22X + 31.74$, $r^2 = 0.26$, $n = 320$). Chesapeake Bay is rich in nutrients, chlorophyll *a*, organic matter and light-absorbing material, with correspondingly shallow euphotic zones (mean depth, 5 m: Malone et al. 1988). The shallow euphotic depth obviously counteracts the effect of enrichment on bacterial accumulation in the estuarine water column (Figure 4B). In spite of high abundance, the standing stock integrated over the euphotic zone is about 50% lower than in the northwestern Sargasso Sea off Bermuda (Figure 4A and cf. Carlson et al. 1996). There are exceptions to the general trend. The euphotic zone in the northeastern Atlantic subarctic became highly enriched with bacteria following the peak of the 1989 spring bloom (Ducklow et al. 1993). In coastal Antarctic waters of the Ross Sea (Figure 4A) and other polar regions

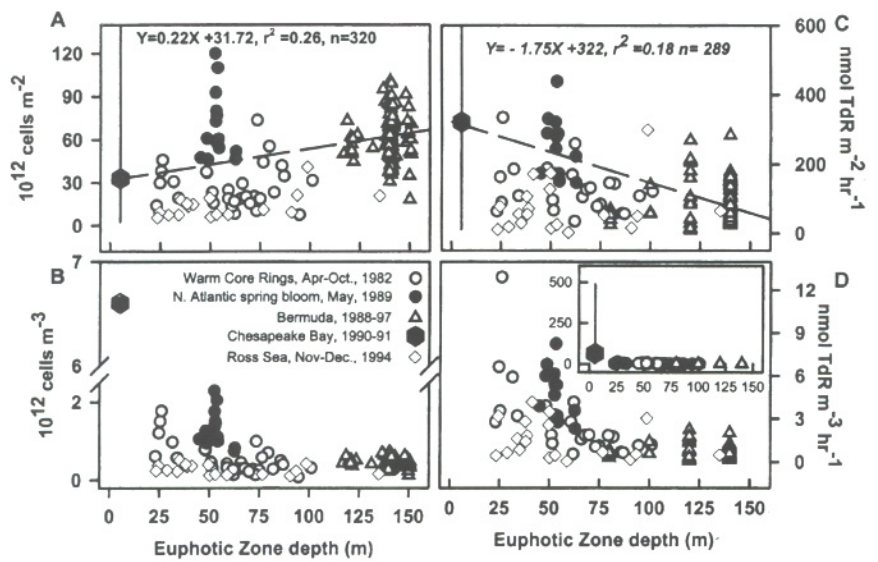

Figure 4. Bacterial standing stock and production in the euphotic zone. (A) Euphotic zone (EZ) standing stocks. (B) EZ mean abundance (stock/depth). (C) EZ integrated TdR incorporation. (D) EZ mean TdR incorporation rates (integrated rate/depth). *Inset:* Same plot including Chesapeake Bay data, presented as mean and range (heavy line) of 162 monthly measurements made at 10 stations up and down the bay in 1990–1991 (Shiah and Ducklow 1994). Other data sources cited in text.

(Karl 1993), bacterial accumulation seems to be strongly suppressed by temperature or factors related to plankton community structure (Carlson et al. 1998). Generally, however, even though the mean primary production per unit volume is clearly higher in inshore habitats, including the Ross Sea (W. O. Smith and Gordon 1997), oligotrophic regimes with deep euphotic zones have greater integrated bacterial biomass than estuaries and coastal oceans.

This pattern becomes more surprising when we analyze bacterial production (as thymidine incorporation). There is a significant *negative* relationship between euphotic zone thymidine incorporation, in nanomoles per square meter per hour, and depth of the photosynthetic layer (Figure 4C; $Y = -1.75X + 322$; $r^2 = 0.18$, $n = 289$). In this case bacterial activity is six times greater in the estuary (Figure 4D, inset), but there is no difference among integrated production rate in the other sites. Bacterial utilization of organic matter results in greater euphotic zone production levels in estuaries, but also seems to result in greater integrated biomass accumulation in the open sea. It follows from this that turnover rates (specific growth rates) are about tenfold greater in the estuaries.

We do not have a satisfactory theory of the regulation of bacterial stocks and production in the sea. There is indirect evidence for bottom-up control by resource availability (Billen et al. 1990; Ducklow 1992). Bacterial abundance and production are significantly correlated with phytoplankton biomass (chlorophyll *a*) and primary production, respectively (Cole et al. 1988). The latter relationships are reflections of the strong and ultimate dependence of bacterial metabolism on local primary production. This dependence may be obscured or nonexistent in estuaries where bacterial productivity can exceed phytoplankton production (Ducklow and Shiah 1993) and is supported by exogenous inputs of terrestrial organic matter. Dominant bottom-up effects suggest that bacterial stocks should increase with increasing organic matter supply. Removal processes (top-down controls) counterbalance the bottom-up effect. Sanders et al. (1992) compared the relationships between bacteria and heterotrophic nanoplankton bacteriovores in high and low productivity regimes of both marine and freshwater habitats. They concluded that bottom-up effects were predominant in oligotrophic systems, while top-down control was stronger in eutrophic systems. The data presented here indicate that top-down effects exert a strong effect across the trophic spectrum. Removal processes maintain relatively uniform bacterial abundance throughout the ocean outside the land–sea margin (Figure 4B). Suppression of estuarine water column stocks seems especially intense (Figure 4A), in spite of high local inputs of organic matter and high bacterial production (Figure 4D).

Thus it appears that in the open sea over a wide range of geographic and trophic habitats, integrated bacterial stocks are high (about twice as high as in estuaries) but possibly not as productive (averaged over the active and inactive assemblage) as once believed. This is apparently because bacterial GGE is low, also averaging about 20% (Chapter 10). If these mean figures are truly accurate and representative, bacterial carbon demand is about the same magnitude as

the local particulate net primary production estimated from ^{14}C measurements. Better estimates of total and bacterial respiration rates would provide a rigorous constraint on this conclusion (Jahnke and Craven 1995). Bacterioplankton are forced to scavenge DOM from diverse sources (Pomeroy 1974; Williams 1981; Azam 1998; Chapter 6). The effective flux of DOM into bacterial cells must approach the magnitude of the daily primary production. Top-down controls (including viral lysis) appear to be weaker in the open sea than in estuaries. Bacteria may constitute a stronger link to higher trophic levels inshore than offshore, where large amounts of carbon are sequestered in the bacterial biomass. But methods for specifying DOM flux, removal rates, bacterial production, and respiration must be improved by at least an order of magnitude in precision before these conclusions can be verified.

There is one important caveat to the foregoing speculation. Bacterial abundance is not biomass and thymidine is not carbon production. The patterns shown in Figure 4 will not reflect actual stocks and production in nature if, for example, cell volumes and carbon content are larger in estuaries than in the open sea (Fukuda et al. 1998), or if TdR conversion factors vary significantly and systematically (e.g., Rivkin et al. 1996). Patterns of carbon flux are valuable for biogeochemical studies and modeling, but actual patterns of abundance and biovolume are also important: bacteriovores select and ingest cells, not carbon units. Both approaches are needed for complete understanding.

SUMMARY

1. Bacterial standing stocks in the euphotic zone average about 0.5–2 $g\,C\,m^{-2}$ across a range of oceanic systems. The ratio of bacterial to phytoplankton stocks varies widely, from less than 0.1 in polar coastal seas to over 2.0 in the oligotrophic gyres.
2. Bacterial production is maintained in a remarkably constant ratio to primary production, averaging about 0.15–0.2 across oligotrophic and oceanic HNLC and upwelling and blooming systems. Bacterial production is generally much lower during polar coastal blooms, but it can be high following the peak phase of blooms in temperate and subpolar regimes.
3. Bacterial stocks seem to be limited principally by resource limitation in lower productivity systems; but removal processes are more intense in coastal and estuarine systems, suppressing integrated standing stocks to below oceanic levels.
4. Estimating bacterial biomass and production in geochemical mass units (C- or N-based estimates) is still technically difficult and uncertain. Order-of-magnitude increases in precision and perhaps accuracy are needed to gain deeper understanding of bacterial ecology in the sea.

5. Better recognition, detection, and understanding of inactive cells are needed to specify rates and mechanisms of bacterial growth.

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