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THE MICROBIAL LOOP

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Assigned Readings

REQUIRED

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. *10*: 257-263. *[Note the alphabetical authorship. Fenchel experimentally demonstrated the importance of flagellate grazing in controlling bacterial standing stocks]*

Ducklow, H. 2000. Bacterial production and biomass in the oceans. Pp. 85-120 in D. L. Kirchman, ed., Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

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SUPPLEMENTAL

Fenchel, T. 1988. Marine plankton food chains. Ann. Rev. Ecol. Syst. *19*: 19-38.

Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology *66*: 109-120.

Giovannoni, S and M. Rappé. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes. Pp. 47-84 in D. L. Kirchman, D. L., ed.. Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

Hoppe, H.-G., K. Gocke, R. Koppe, and C. Begler. 2002. Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. Nature *416*: 168-171. *[Meridional tritiated leucine & thymidine assays. The equatorial region (8° N to 20° S) is a net heterotrophic region.]*

Jumars, P. A., D. L. Penry, J. A. Baross, M. J. Perry and B. W. Frost. 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. Deep-Sea Res. *36*: 483-495. *[The source of DOM, fueling the microbial loop may be from inefficient (but optimal) grazer guts]*

Jumars, P. A. 1993. Concepts in Biological Oceanography: An interdisciplinary primer. Oxford University Press, New York. 348 pp. *[Read Chapter 10, pp. 179-197 is on 'microbial loops']*

Jürgens, K. and R. Massana. 2008. Protistan grazing on marine bacterioplankton. Pp. 383-441 *in* D. L. Kirchman, ed, Microbial ecology of the oceans, 2nd edition. Wiley-Blackwell, New York. 593 pp.

Nagata. T. 2000. Production mechanisms of dissolved organic matter. Pp. 121-152 *in* D. L. Kirchman, ed, Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

Nagata, T. 2008. Organic matter - bacteria interactions in seawater. Pp. 207-241 *in* D. L. Kirchman, ed, Microbial ecology of the oceans, 2nd edition. Wiley-Blackwell, New York. 593 pp.

Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovonanni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature *418*: 630-633. *[PCR revealed this clade makes up about 1/4 of marine bacteria, but it was unculturable. Using very low-nutrient medium and dilution, 18 isolates were obtained.]*

Riemann, B. and R. T. Bell. 1990. Advances in estimating bacterial biomass and growth in aquatic systems. Arch. Hydrobiol. *118*: 385-402.

Thingstad, T. F. 2000. Control of bacterial growth in idealized food webs. Pp. 229-260 *in* D. L. Kirchman, ed., Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

General Comments

We will only have one class on the enumeration and production of marine heterotrophic bacteria. Dr. Shiaris offers a semester-long graduate course on Microbial Ecology. I have tried to make this handout sufficiently detailed so that those who want additional information on this important field can find it.

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MICROBIAL STANDING STOCKS AND PRODUCTION

Brock (1987) stressed that bacterial ecology should be studied in the field. If you transfer bacteria back to the laboratory, you may destroy the micro-environments that control bacterial growth rates. Techniques to study *in situ* growth and standing stock have emerged within only since the early 1970's to study *in situ* activities and standing stocks.

The most widely accepted method to enumerate natural bacterial abundances in the field are epifluorescent microscopic techniques using either acridine orange (AO) or DAPI DNA stains. Staining with AO was introduced by **Daley & Hobbie (1975)** & **Hobbie et al. (1977)**, and staining with DAPI was introduced for pelagic bacteria by **Porter & Feig (1981)** and for benthic bacteria by **DeFlaun & Mayer (1983)**. Both AO and DAPI are fluorescent dyes which react with DNA. DAPI stains adjacent thymine-thymine nucleotides (dimers) in DNA. DAPI exhibits less background staining than AO and is usually preferred. All DNA stains with AO and DAPI, so neither of these stains is specific for heterotrophic bacteria. The similarly sized autotrophic picoplankton will stain too.

Epifluorescent enumeration of bacteria with AO and DAPI revolutionized the field of microbial ecology. Before these techniques were introduced, microbial ecologists had to rely on laboratory culture techniques to estimate field populations. In the most probable number (mpn) technique, a field sample is diluted until the highest dilution factor that produces growth is found. From this dilution factor, the most probable number of bacteria in the original sample can be estimated. The most probable number method is still used routinely for enumerating pathogenic bacteria and viruses in the marine environment. Unfortunately, there is no universal medium and set of culture conditions for growing marine bacteria. Only a small percentage of the marine bacteria found in a given patch of mud or ml of water will grow in cultures in the laboratory. When the AO technique was introduced, microbial ecologists realized that they'd been underestimating the abundance of marine bacteria by at least 1000 times. Now, the next important question to be answered was, "What percentage of the bacteria were active, and how fast are they growing?"

The most widely used technique for estimating bacterial production is and Azam's (**1980**, **1982**) tritiated thymidine technique. **Moriarty (1986)**, in his review of the tritiated thymidine method, noted that the technique had been used for environmental samples since the early 1970's. Fuhrman and Azam were the first to work out the specificity, incubation times, and conversion factors sufficiently to apply it to large-scale marine surveys.

There is still considerable controversy about the tritiated thymidine method. **Riemann & Bell (1990)**, one of the supplemental readings, discusses the assumptions behind the method. Thymidine is a precursor to thymine, a nucleotide use only in DNA. The method relies on the assumption that when bacteria encounter thymidine in nature, they curtail their own synthesis of thymidine (*de novo* synthesis) and incorporate the labelled thymidine *via* a scavenge pathway. This external pool of thymidine has a known specific activity (radioactivity/concentration), which can be used to calculate the rate of DNA synthesis. If the bacteria continued to synthesis thymidine *de novo*, then an unknown fraction of the thymidine incorporated would have the radioactive label and the true rate of DNA synthesis would by underestimated.

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Box 1. Steps in estimating bacterial production using the *in situ* tritiated thymidine (TdR) method.

1. Determine the specific activity [dpm/mole thymidine] of the TdR
2. Add TdR to samples to achieve desired activity:
   a. The concentration should be low enough that only bacteria, which have high surface:volume ratios, take up significant amounts
      i. 5 nM for water column
      ii. 20 nM or more for sediments
   b. For sediments, the TdR should be injected into porewater to achieve uniform labeling
3. Perform incubation under "natural conditions"
4. Extract the DNA from samples using cold TCA.
5. Measure the specific activity of DNA (*i.e.*, the radioactivity per gram of DNA) using liquid scintillation counting
6. Using conversion factors, calculate microbial production. The calculation usually assumes no isotope dilution and that the thymidine incorporated into DNA has the same specific activity as the TdR added. Conversion factors are essential to estimate production or microbial specific growth rate.
   a. Conversion factors:
      i. Thymidine-to-biovolume conversion factor: 15.2×10^{17} μm^3 mol TdR^{-1}
      ii. 121-580 fg C μm^{-3} have been used in the literature (**Riemann & Bell 1990**), with 350-580 fg C μm^{-3} being used in recent studies.
   b. Calculate microbial carbon production using: using:

$$\frac{dC}{dt} = \frac{I^*}{A^* T} F_{CT}$$

where, A^* = *Spec. act. of HdT* $\left[\frac{dpm}{mol\ thymidine}\right]$.

C = *microbial carbon.*

F_{CT} = *Conversion factor* $\left[\frac{g\ microbial\ Carbon}{mol\ thymidine}\right]$.

I^* = *Spec. act. of DNA in sample* $\left[\frac{dpm}{sample}\right]$.

T = *Incubation time* $[h]$.

Gilmour *et al.* (1990) discovered a major limitation of the thymidine method: sulfate-reducing bacteria, a major and diverse group of heterotrophic bacteria, lack the scavenge pathway to utilize external thymidine. The tritiated thymidine technique could not estimate their production at all.

A second major assumption is that both the external and internal pools of thymidine are small relative to the amount of added thymidine. If these pools were high, the labeled thymidine would be significantly diluted and the production would be underestimated. All organisms are capable of utilizing thymidine. The specificity of the thymidine technique for microbial production relies on the assumption that bacteria, with their high surface: volume rations are responsible for the vast majority of short-term thymidine uptake in a sample. Thymidine is added at low concentrations to increase the specificity for microbes. Rivkin (**Rivkin & Seliger 1981**, **1986a**, **1986b**, **Rivkin & Voytek 1986**) uses thymidine addition to estimate diatom specific growth rates, but he added the thymidine at much higher concentrations (mM not nM). A final major set of assumptions involves the factors needed to convert thymidine synthesis per sample volume to microbial carbon production per sample volume. Box 1 provides a simplified procedure for estimating microbial

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production using the thymidine method.

THE MICROBIAL LOOP HYPOTHESIS

Bacterial abundances in the ocean are probably largely controlled by heterotrophic nanoflagellate grazing, as described by **Azam *et al.* (1983)**, and reviewed by **Fenchel (1988)**. However, substrate limitation and bacteriophage mortality can not be ruled out.

The microbial loop hypothesis links primary production and trophic transfer through microbes to higher trophic levels. The term 'loop' is because the microbial community may be regarded as a scavenge pathway for transferring organic matter from primary producers to the macrozooplankton. **Ducklow (2000)** defines the microbial loop as "the bacterial recovery through uptake and metabolism of dissolved organic matter (DOM) otherwise "lost" from the trophic system via excretion, exudation, and diffusion." p 88 **Pomeroy (1974)** was one of the first to stress the importance of the microbial loop.

Azam *et al.* (1983) described the carbon flow from DOM release by phytoplankton through bacteria to heterotrophic nanoflagellates and then perhaps to ciliates and macrozooplankton and framed the **microbial loop hypothesis**:

1. Bacteria utilize dissolved organic matter (DOM) as an energy source *[The source of DOM is from phytoplankton, perhaps mediated by zooplanktonic grazing or viral lysis]*
2. Bacterial numbers are controlled by heterotrophic flagellates which can reach densities of $3x10^3$ cells/ml.
3. Flagellates are preyed on by the microzooplankton in the 10 to 80 μm size range (*e.g.*, ciliates).
4. DOM is only inefficiently returned to the main food chain.

The source of organic matter for the microbial loop is controversial. It could be dissolved organic matter release by phytoplankton or release of DOM by grazers (**Jumars *et al.* 1989**). **Bjørnsen (1988)** argued that small cells must leak low-molecular-weight organic compounds. Fogg (**1966**, **1977**, **1983**) argued that the leakage of glycolic acid was the result of photorespiration. **Sharp (1977)** sharply criticized the methods used in many early studies of DOM excretion by phytoplankton. Many of these earlier studies had filtered the phytoplankton with filtration pressures that could have ruptured phytoplankton cells producing high apparent DOM release rates. Recent work has shown that viral lysis of phytoplankton can be a major source of dissolved organic matter.

Is the microbial loop an efficient scavenge pathway for the return of photoautotrophic production to higher trophic levels (*e.g.*, macrozooplankton)? This sink-link question was clearly framed by **Banse (1984)** in a book review on marine microbial processes:

> *"In any case, the issue of whether the small phagotrophs of the open sea are producing much organic matter accessible to copepods or are principally mineralizing organic matter in a long,*

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> *"inefficient" food chain (the link versus the sink in regard to the traditional food web) is not adequately addressed. I note, however about ten values of gross growth efficiency (growth over ingestion) scattered throughout the book, which seem to all be below 50% (or much below). These low efficiencies would mean large respiratory (and fecal) losses on each transfer between the small phagotrophs and hence little food for copepods. So- are we pico-, nano- and microzooplanktologists all fascinated by a huge sink?"*

Ducklow *et al.* (1986) brought the sink-link debate to a boil with a controversial mesocosm study, rebutted by **Sherr *et al.* (1987)**. **Ducklow *et al.* (1986)** argued that the microbial loop was a sink and did not return significant amounts of photoautotrophic carbon to macrozooplanktonic trophic levels. They found only about 2% of their radioactively tagged carbon, released as glucose, ended up in macrozooplankton after 50 days. Strangely, **Ducklow et al. (1989)** recanted this view, stating the Loch Ewe bag experiment was done during the spring and that later in the year, more DOM would have been transferred to macrozooplankton. They used network modeling to show that nearly 70% of the energy of the macrozooplankton might pass through heterotrophic bacteria, not directly from phytoplankton:

> *"However when recycling is considered, it can be seen from Table 8.9 that up to 69% of the macrozooplankton input was mediated by the bacteria. It has been observed in mesocosm experiments that only a few per cent of labeled bacteria pass into large zooplankton (**Ducklow et al. 1986**). Since the dependency of macrozooplankton appears to rise as recycling does, it is tempting to speculate that the mesocosm results were due to low recycling. This was probably the case for the early spring experiment in a Scottish sea loch described by **Ducklow et al. (1986)**."*

Fenchel (1988) argues that with gross growth efficiencies of about 30%, less than 10% of organic matter released by phytoplankton is returned to zooplankton larger than ≈100 µm.

OUTLINE OF MARINE MICROBIOLOGICAL PATTERNS, PROCESSES, AND TECHNIQUES

1. **Some BIG questions about the microbial loop:**
   - a. **Water column:**
     - i. What is the rate of gross primary production in the world's oceans and what fraction of this gross primary production is released as dissolved organic matter (DOM) to fuel the microbial loop?
     - ii. What processes lead to the release of DOM:
       - Phytoplankton leakage (*e.g.*, photorespiration)
       - Sloppy zooplankton grazing (*e.g.*, **Jumars *et al.* 1989**)?
       - Viruses
     - iii. What **are** the concentrations of labile dissolved organic matter in the ocean?

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iv. What processes control the abundance of bacteria in the water column?
- Substrate limitation
  - (a) Organic carbon
  - (b) Nitrogen
- Grazing
- Viruses

v. What group of organisms control nutrient regeneration?
- Bacteria
  - (a) **Goldman *et al.* (1987)** note that for bacteria to remineralize N, the C:N of bacterial biomass > (C:N ratio of substrate*(Gross bacterial growth efficiency))]
  - (b) Do bacteria compete with phytoplankton for nutrients (*i.e.*, nitrogen and phosphorus)?
- Microzooplankton
- Macrozooplankton

vi. What is the C:N ratio of the DOM released by phytoplankton and zooplankton?

vii. What are the gross growth efficiencies (GGE=ingestion/growth) for members of the microbial loop (literature values range from ≈50% {Banse} to over 85% {Paine and Wiebe 1978}).

viii. Is there a functional relationship between GGE and organism size (Banse thinks not, Caron thinks so)?

b. **Benthos:**

i. What are the major food resources for benthic macrofaunal deposit feeders?
- bacteria
- Microphytobenthos
- detritus & organic coatings on mineral grains

ii. What fraction of organic matter utilization (benthic metabolism) is due to the macrofauna, meiofauna and microfauna?

iii. Is dissolved organic matter a significant source of reduced organic carbon for infaunal organisms?

iv. Are large deposit feeders better competitors for scarce food supplies?

2. **Methods for enumerating microbes in sediments and in the water column**

a. Direct-count procedures

i. A list of techniques:
- Electron microscopy.
  - (a) SEM & TEM
  - (b) Can be combined with vital stains to separate living from dead particles
- Coulter™ or EPICS™ counters
  - can't separate living from dead
- Fluorescence microscopy or fluorescent activated cell sorting (FACS)
  - (a) DNA stains
    - (i) Acridine orange (AO)
    - (ii) DAPI
      -stains thymine-thymine dimers.
  - (b) Immunofluorescence
    - (i) Can be combined with autoradiography to enumerate active cells
    - (ii) Can be used with Fluorescence activated cell sorter.
  - (c) INT, respiring bacteria deposit INT intracellularly as dark red spots (separates actively respiring bacteria)
  - (d) autofluorescence of Chl *a*, appropriate for cyanobacteria and other photoautotrophs

ii. Advantages of direct counting procedures.
- Don't require separation of microbes from surrounding particles
- Two orders of magnitude higher numbers than with culture techniques.

iii. Drawbacks
- Do not measure biomass (David White's criticism)
- Methods usually don't distinguish active from inactive.

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b. Indirect or viable count
   i. List:
      – Plate counts for bacteria
      – bacteriophage plaque assays
      – Selective enrichment media
         (a) can separate different components
         (b) alternatives exist for many groups
      – Most probable number
         (a) Some advantages:
            (i) permits liquid culture
            (ii) can be used for enteric bacteria and viruses
         (b) Some disadvantages:
            (i) Requires selective enrichment media
            (ii) Destroys the micro-environments utilized by both water-column and benthic bacteria.
   ii. Drawbacks to plate counts:
      – The micro-environment of marine bacteria can't be duplicated on plates.
      – total viable count is a misnomer
      – agar can contain contaminants
      – bacteria can't utilize agar

c. Biochemical methods
   i. List
      – ATP assays
         (a) Advantages:
            (i) All bacteria have ATP
            (ii) [ATP] is relatively easy to assay
            (iii) [ATP] is related to biomass not cell numbers
         (b) Disadvantages
            (i) all organisms have ATP
            (ii) ATP conversion factor of 250-286 is not constant (ATP to cellular carbon, 120 for soil samples).
      – Total adenylate pool
      – chlorophyll (for photoautotrophic bacteria)
         (a) Types
            (i) chl *a*
            (ii) bacteriochlorophyll
         (b) Advantages
            (i) all photoautotrophic bacteria have chlorophyll
            (ii) Assay is straightforward
         (c) Disadvantages
            (i) Conversion factors not constant
            (ii) Only photoautotrophic bacteria assayed.
      – Lipopolysaccharide (LPS):
         (a) advantages: specificity: a major fraction of gram negative cell wall is composed of LPS. *Limulus* amoebocyte lysate reacts specifically with the LPS to form a turbid solution
         (b) disadvantage: doesn't work for gram positive forms.
      – Muramic acid assay (murein = peptidoglycan=mucopeptides)
         (a) advantages:
            (i) all bacteria have muramic acid
            (ii) not found in other organisms
            (iii) directly related to surface area and hence to biomass
         (b) disadvantages of muramic acid assay:
            (i) analytically difficult; usually requiring gas chromatography to assay
            (ii) Gram-positive bacteria have a much thicker murein layer. Must assume a ratio of gram positive to gram negative bacteria in the field

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(iii) **MA:C conversion factor** still required
1) Gram positive:44 µg MA/mg C
2) Gram negative:12 µg MA/mg C

– specific lipid components (White)
(a) Advantage
(i) can separate different microbial components
(ii) can be used with ^{13}C to estimate production
(b) Disadvantage
(i) must convert to biomass
(ii) technically difficult, usually requiring gas chromatography
– protein: too nonspecific
– gene probes (DNA or RNA):
(a) List:
(i) 16S rRNA or 16s-like RNA probes (**Giovannoni *et al.* 1990**)
(ii) specific DNA probes (*e.g.*, X-Chung Wang's TDH probe for *Vibrio parahaemolyticus* [Biology M.Sc. dissertation UMASS/Boston 1990])
(b) Advantages
(i) Highly specific for individual groups. Probes now exist to separate eubacteria from archaebacterial and eucaryotic DNA (**Giovannoni et al. 1990**)
(ii) RNA probes have been produced to estimate the abundance and activity of marine nitrifying bacteria
(iii) Can be quantitative
(iv) Efficiency not dependent on separation of bacteria from particles
(v) Can be combined with the Polymerase Chain Reaction (PCR) to attain very high sensitivity
(c) Disadvantages
(i) Probe must be available for DNA flanking sequences
(ii) Gene copy number must be known for quantitative enumeration (a drawback for many groups)

3. **Methods of estimating bacterial growth rates or activity**
a. List
i. Measure average biomass and assume a production: biomass ratio (P:B) (often with an assumed temperature factor, Q_{10})
ii. O_2 flux
– Advantages:
(a) relatively simple analytically
(b) directly related to production
– Disadvantage:
(a) other terminal electron acceptors can and are used in the sediments (much of the respiration can be anaerobic using SO_4^{-2} as the terminal electron acceptor)
(b) bacterial inhibitors, used to separate bacterial and eucaryotic respiration are often not specific and may affect meiofauna more than bacteria (Montagna)
– Examples of oxygen-flux studies:
(a) Smith `82, Castle Island
(i) Bacteria 42-47%
(ii) meiofauna 1.6-1.7%
(iii) macrofauna 1.9-2.6%
(iv) other microfauna: 51-55%
(b) Fenchel (1969): estimated the metabolic contributions of microfauna, meiofauna, and macrofauna
iii. Monitoring changes in bacterial numbers or particle size classes (& the dilution method):
– advantages:
(a) simple and reliable.

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(b) can be used with Landry's dilution technique to estimate production with reduced grazing (*e.g.*, **Ducklow & Hill 1985**)

– disadvantages:
  (a) bacterial production may be in steady state with heterotrophic nanoflagellate grazers.
  (b) bottle effects.
  (c) can't separate active from inactive, or live particles from dead

– empirical relationships can be used to estimate activity from size spectra and assumed size-specific growth rate relationships (*e.g.*, Sheldon's linear biomass hypothesis)

iv. Monitoring changes in antibiotic resistant mutants (*e.g.*, **Plante *et al.* 1989**)
Advantages: specific to selected microbial groups

v. Molecular tracers: change in lipid biosynthesis (David White) Can be used with ^{32}P to estimate production based on phospholipid synthesis.

vi. **Radiolabeling procedures**

– Overall advantages:
  (a) Autoradiography can be used with any of the radiotracers to estimate activity per cell
  (b) high sensitivity
  (c) analytically straight forward

– disadvantages
  (a) "isotope dilution effect": The `natural' concentration of the substrate must be known in order to calculate growth rates using techniques like the tritiated thymidine procedure [see Pollard and Moriarity 1984, King and Berman 1985]
  (b) enrichment effects: addition or a Radiolabeled, rich carbon or nitrogen source may produced unrealistically high growth rates.

– A list of radiolabeling techniques.
  (a) ^{14}C-bicarbonate incorporation for chemoautotrophs
    (i) generally too non-specific for estimating only chemoautotrophic growth rates.
    (ii) dark bottle sometimes used.
  (b) ^{14}C-labeled carbohydrates (*e.g.*, glucose)
  disadvantages:
  bacteria may not grow well on organic substrates having low N:C ratios if they are nitrogen limited.
  (c) ^{14}C-labeled or tritiated amino acids (*e.g.*, radio-labeled leucine)
    (i) incorporation into proteins measured
    (ii) advantage: C:N ratio is often low (≈ 2)
    (iii) disadvantage: may produce unrealistically high growth rates if the population is nitrogen limited
  (d) **Fuhrman & Azam's (1982)** tritiated thymidine incorporation in DNA
    (i) advantages:
      1) specific for bacteria (eucaryotes cannot take up the low concentrations added)
      2) Can be quantified
      3) Can be combined with autoradiography
      4) Can be used with frequency of dividing cells equations to estimate specific growth rates
    (ii) Drawbacks.
      1) Many methodological problems (from LaRock and Moriarity 1990 AGU presentations)
        a) -added radiolabel may be incorporated into other cellular products
      -de novo synthesis of thymidine
        b) -isotopic equilibration
      2) doesn't work well in anaerobic sediments

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3) C:DNA ratio must be assumed to estimate specific growth or production
4) **C:DNA conversion factors** not constant (40 to 200 range at least)

(e) **Karl's (1984)** adenine incorporation method and **Bossard & Karl's (1986)** adenine nucleotide pool turnover: designed to measure the growth of the total microbial community
Problems:
(i) adenine response not uniform
(ii) bacteria dominate adenine uptake

vii. Frequency of dividing cells
- Developed by many, including:
  (a) **Hagstrom et al. 1979**
  (b) Eppley & Weiler
  (c) Penny Chisholm
- **McDuff & Chisholm (1982)** provide the correct equations for estimating μ:
  μ (mean over the interval t, $+t_d$)= $1/t_d \ln[1 + f(t)]$
  where, μ = specific growth rate
  t_d = time spent dividing
  f(t) =the frequency of dividing cells in the population
- Ways of estimating frequency dividing
  (a) visual
    (i) paired nuclei
    (ii) INT zones
    (iii) DNA content with fluorescence activation
  (b) radioisotopically
    (i) Rivkin's ≥ (for diatoms only)
    (ii) Rivkin's ^{14}C (**Rivkin & Seliger 1981**)
    (iii) Rivkin's (**1986a**, **1986b**, **Rivkin & Voytek 1986**) tritiated thymidine

viii. Adenylate energy charge: higher in active bacteria, but conversion factors widely varying

ix. Electron transport system (ETS) activities (Packard, Christenson and others)
- advantage: works no matter what the terminal electron acceptor
  (a) O_2
  (b) NO_3^{--}
  (c) NO_2^{-}
  (d) SO_4^{--}
- Methods
  (a) macerate cells
  (b) incubate with $NADPH_2$, succinate
  (c) add tetrazolium chloride, INT
- Problems
  (a) estimates potential not actual activity
  (b) not easily converted to production estimates

x. Heat flux measurements (Pamatmat `82 Science *215*; 395)
- advantage: all organisms, whether using aerobic or anaerobic respiration (or fermentation) produce heat
- disadvantages:
  (a) placing sediment in a calorimeter severely disrupts sediment structure.
  (b) difficult analytically
    (i) heat measurements fluctuate wildly initially.
    (ii) stabilization
  (c) estimates potential respiratory activity, not actual

xi. specific enzyme activities
- dehydrogenase
- phosphatase
- cellulase
- nitrogenase

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4. **Estimates of heterotrophic protist standing stocks.**
   a. Visual microscopy with vital staining.
   b. Epifluorescence microscopy with vital staining.
      Using Caron's technique, the heterotrophic nanoflagellates can be separated from the autotrophic nanoflagellates by the absence of autofluorescence in the former group.
   c. Scanning electron microscopy
   d. Specific lipid components:White has found lipid components which distinguish prokaryotes and eukaryotes.
      i. advantage: specificity & estimates biomass
      ii. disadvantage: difficult analytically.
      e. Serology.
      i. advantages:
         – specificity
         – sensitivity
         – simply analytically (*e.g.*, dot blots)
      ii. disadvantage:
         – protist diversity.
            A `good' antiserum will react specifically with cell surface antigens on only one or a few species. If the heterotrophic protist assemblage is diverse, a battery of antisera would be required to enumerate the abundance of heterotrophic protists.
         – background staining and cross-reactions
         – Estimates abundance, not biomass.

5. **Estimates of bacterivory:**
   a. Field studies:
      i. Monitoring predator and prey populations (*e.g.*, with direct counts or Coulter™ counter & epifluorescence microscopy)
      ii. Experimental removal of grazer population
         – filtering
            (a) difficult because of size overlap
            (b) Damages phytoplankton cells
         – eucaryotic chemical inhibitors (**Fuhrman & McManus 1984**)
            (a) cycloheximide
            (b) colchicine
      iii. Dilution methods (with filtration of predator populations)
         – Since predation rate is heavily density dependent, the dilution of a bacterial sample with natural medium will drastically reduce rates of bacterivory while. supposedly leaving bacterial growth rate unaffected.
         – Landry
         – **Ducklow and Hill (1985)**
      iv. Fluorescently labeled particles
         – **Caron et al. (1999)** apply the method
      v. Radioisotopic labeling
         – add radiolabel specific for bacteria (*e.g.*, tritiated thymidine, glucose)
         – monitor change in size fractionated activity with time (*e.g.*, **Ducklow *et al.* 1986**, **Fuhrman & McManus 1984**).
         – monitor closely through time
      vi. Stable isotope analysis (predators resemble the isotopic composition of their prey)
         – In some environments (*e.g.*, hydrothermal vents) bacteria have characteristic $\delta^{13}C$ and $\delta^{15}N$-signals.
   – Laboratory studies:
      – Incubation of predators and known densities of prey.
      – Dilution techniques
      – Chemical inhibition of bacterial growth rate (*e.g.*, penicillin, **Fuhrman & McManus 1984**). Grazing rate can be estimated from the pulsed decrease in bacterial numbers.

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- Chemical inhibition of eukaryotes: cycloheximide, colchicine (Newell *et al.* 1983, Fuhrman & McManus 1984)
- Addition of labeled bacteria
  - Radiolabeled bacteria
  - fluorescently labeled bacteria.
- Addition of bacterial sized particles
  - Types of particles
    - Radiolabeled particles
    - fluorescently labeled bacteria.
    - latex spheres
  - Examples of studies
    - **Caron et al. (1999)**
    - **McManus & Fuhrman (1988)**
    - **Nygaard *et al.* (1988)**
    - **Pace & Barliff . (1987)**
    - **Sherr *et al.* (1987)**
  - Problems
    - bacterivores may use chemical clues to detect prey
- Model approaches
  - Estimate biomass of size classes and assume a P:B ratio
  - Construct ecosystem simulation models (*e.g.*, **Frost 1988**)
  - Estimate rates of bacterial lysis by phage through estimates of bacterial and phage abundance.

- **Microbial loop hypothesis:**
  - Bacteria utilize DOM, excreted by phytoplankton, as an energy source
  - Bacterial numbers kept (<5 x 10^6 cells/ml) in check by heterotrophic flagellates which can reach densities of 3x10^3 cells ml.
  - Flagellates are preyed on by the microzooplankton in the 10 to 80 µm range.
  - The microzooplankton are, in turn, preyed on by the macrozooplankton
  - DOM ineffectively returned to the main food chain.

Terms and Concepts

Gram reaction (**Stanier *et al.* 1970**): See Appendix 1

Gross growth efficiency — See Appendix 1

isotope dilution effect — Principle of isotope dilution: *"A series of samples are incubated with a constant amount of radioactive thymidine to which increasing amounts of unlabeled thymidine are added. The DNA is extracted and the reciprocals of the amounts of radioactivity in DNA are plotted against the amounts of thymidine present (Fig. 5). If there is no dilution of the isotope incorporated into DNA by any sources other than the unlabeled thymidine that was added, the plot will pass through zero (e.g., Fig. 5A). A negative intercept on the ordinate is an estimate of the amount of dilution by isotope by other sources of thymine in DNA (e.g., Fig. 5B). It is not strictly a pool of thymidine, but represents the sum of all pools that dilute the tritiated thymidine prior to incorporation into DNA"* **Moriarty (1986, p. 262)**

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Size groups (from **Fenchel 1988**, **Sieburth 1978**)
- picoplankton 0.2 -2 µm
- nanoplankton 2-20 µm
- microplankton 20-200 µm
- mesoplankton 200 µm - 20 mm

Outline of Papers

ASSIGNED

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. *10*: 257-263. [6, 26, 27]

- Abstract:
  - Bacterial growth coupled to that of phytoplankton
  - Bacteria utilize 10 to 50% of primary production
  - Bacterial numbers controlled by nanoplanktonic heterotrophic grazers
  - Nanoplanktonic bacteriovores preyed upon by microzooplankton
  - microbial loop returned to the food chain.
- Introduction
  - Biochemical methods for enumerating bacterial standing stocks:
    - ATP
    - Muramic acid assay
    - LPS assay
  - Problems with these biochemical methods: conversion factors
  - direct counts: AO, DAPI, TEM & SEM
  - Bacterial production rates
    - frequency of dividing cells
    - tritiated thymidine incorporation
- Old view: bacteria as remineralizers.
  - Is this true?
  - If so, how does it occur?
- Bacterial biomass and production:

Azam et al. (1983) Table 1. Numbers and biomass of heterotrophic bacteria in the marine environment.

Environment	Numbers (10^8/l)	Biomass (µg C/l)
Estuaries	50	?
Coastal waters	10-50	5-200
Offshore waters	0.5-10	1-5
Deep waters	0.1	?

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- Relatively constant bacterial number 10^4 to $5x10^6$ cells/ml
  - Does a homeostatic control exist?
  - 10-20% attached to particles
- Bacterial production
  - 2 to 250 µg C $(l\ day)^{-1}$
  - 5 to 30% of primary production
- Conversion efficiencies vary from 40 to 80%
- Proportion of carbon respired ranges from 40% to 90%

- Conditions favoring bacterial growth
  - good correlation with Chl *a*
  - seasonal patterns of abundance
- Factors limiting bacteria in the sea
  - carbon, nitrogen or other nutrients
  - Fenchel showed that heterotrophic microflagellates in the size range 3 to 10 µm are effective bacteriovores in the sea.
    - choanoflagellates
    - colourless chrysomonads
    - these reach densities of 10^3 cells/ml

Fig. 2. predator-prey oscillations with a 4-day lag

  - flagellates have 24-h generation times.
- **Microbial loop hypothesis**
  - bacteria utilize DOM as an energy source
  - bacteria kept in check by heterotrophic flagellates which can reach densities of $3x10^3$ cells ml^{-1}.
  - Flagellates are preyed on by the microzooplankton in the 10 to 80 µm range.
  - DOM inefficiently returned to the main food chain.
- **Discussion**

heterotrophic flagellates and microzooplankton are the remineralizers of nutrients

Ducklow, H. 2000. Bacterial production and biomass in the oceans. Pp. 85-120 in D. L. Kirchman, ed., Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp. [6]

1. Introduction
   a. What is bacterial production?
      i. Specific growth rate
   b. Is bacterial production Net or gross?
      i. ^{14}C & O_2 methods for primary production
      ii. net bacterial production usually measured.
   c. Why measure bacterial production?
      i. Importance of the microbial loop
         - defined as "the bacterial recovery through uptake and metabolism of dissolved organic matter (DOM) otherwise "lost" from the trophic system via excretion, exudation, and diffusion." p 88
         - Bacterial production is the key process controlling flux through the loop
      ii. Quantifying biogeochemical fluxes of carbon and other elements.
         - bacteria dominate DIM incorporation (Azam & Hodson 1977)
      iii. Estimating growth rates
2. Methods: a survey and update
   a. Bacterial biomass
   b. Epifluorescent microscopy
      i. AODC (Hobbie et al. 1977)
      ii. DAPI (**Porter & Feig 1980**; should be 1981)
      iii. SYBR Green (**Noble & Fuhrman 1998**)
   c. Flow cytometry
   d. Cell volume and Mass.

Table 1. Phytoplankton and bacterial biomass in the ocean.
Table 2. Carbon content and carbon density of bacterial cells

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Ducklow Table 2. Carbon content and carbon density of bacterial cells

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

3. **BACTERIAL PRODUCTION**
   - a. Introduction: tritiated thymidine
   - b. Earlier approaches
   - c. Thymidine and leucine incorporation
     - i. Introduced by Fuhrman & Azam **1980**, **1982**, **Fuhrman et al. 1980**)
       - ushered in a new era
     - ii. New developments
       - 1-2 ml samples in microcentrifuge (Smith & Azam 1992)
       - bromodeoxyuridine as a non-radioactive tracer (Steward & Azam 1992)
   - d. [^{3}H]-leucine (Leu) incorporation into bacterial protein
     - i. introduced by **Kirchman et al. 1985**
       - required conversion factor
     - ii. variability in Leu:TdR methods
4. **GROWTH RATES AND VARIABILITY**
   - a. Cell kinetics: bacterial abundance and biomass.

Figure 2
Figure 3

   - b. Application to determination of conversion factors
5. **THE ECOLOGY OF GROWING AND NONGROWING CELLS**
6. **BACTERIOPLANKTON STANDING STOCKS AND PRODUCTION RATES**

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Ducklow Table 5. Bacterioplankton and phytoplankton production in the open sea.

Property		N. Atlantic	Eq. Pac-Spr	Eq. Pac-Spr	Sub N Pac	Arabian	Hawaii	Bermuda	Ross Sea
Euphotic zone m		50	120	120	80	74	175	140	45
Biomass mg C m^{-}	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	**.02**
Production	Bacteria	275	285	176	56	257	nd	70	5.5
	Phytoplankton	1083	1083	1548	629	1165	486	465	1248
	B:P	0.25	**0.26**	0.11	0.09	0.22	nd	0.18	**0.04**
Growth	Bacteria	0.3	0.13	0.12	0.05	0.18	nd	0.05	0.25
	Phytoplankton	0.3	0.64	0.8	0.5	0.93	1.1	0.81	0.11
	B:P	1	0.2	0.15	0.1	0.19	nd	**0.06**	2.3

Figure 4.

7. Summary
   a. Bacterial standing stocks in the euphotic zone average about 0.5-2 g C m^{-2} across a range of environments
      i. The ratio of bacterial to phytoplankton standing stocks from 0.1 to 2.0 in the gyres.
   b. Bacterial production maintained at a remarkably constant ratio to primary production (0.15-0.2)
   c. Bacterial stocks seem to be limited principally by resource limitation in lower productivity systems, but removal processes more intesne in coastal and estuarine systems, suppressing standing stocks to below oceanic levels
   d. Estimating bacterial biomass using C, or N is still techically difficult and uncertain
   e. Better recognigition, detection and understanding of inactive cells are needed to specify rates and mechanisms of bacterial growth.

SUPPLEMENTAL

Ducklow, H. W., D. A. Purdie, P. J. LeB. Williams and J. M. Davies. 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science *232*: 865-867. [7, 19, 28, 48, 49] [See Critique by **Sherr et al. (1987)** with rejoinder by Ducklow]

I. **Abstract.**
   A. Estimates of high production rates implicate free-living marine bacterioplankton as a link in the microbial loop.
   B. enclosed water column of 300 cubic meters used to test the microbial loop hypothesis
   C. followed the fate of ^{14}C-labeled glucose for 50 days.
      1. only 2% ended up on larger organisms.
      2. 20% in particulate fraction
      3. most respired by heterotrophic bacterioplankton.
   D. **secondary production by organisms smaller than 1 µm may not be an important food source in marine food chains.**

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E. bacterioplankton may be a sink for carbon in planktonic food webs and may serve principally as agents of nutrient regeneration than as food.

II. Introduction
- A. bacteria can comprise 20% of carbon biomass
- B. growth efficiencies greater than 50%
- C. growth rates of 2 per day.
- D. do bacterioplankton supplement the diets of microzooplankton
- E. Bacterioplankton form the microbial loop.
- F. Are bacteria a salvage pathway supplementing primary production for herbivores? (The sink vs Link question)

III. The experiment
- A. representative coastal marine ecosystems: Loch Ewe Scotland
- B. 15-m deep water column, 5-m diameter enclosed bag
- C. added 6 m Ci of [^{14}C]glucose & assumed only bacteria would take of glucose.
- D. Pools followed: DOC, DIC, POC (divided into 6 size fractions)

IV. Results
- A. Two stages of tracer fate
  1. 4-6 h first phase: 90% of labeled glucose removed
     - a. 90 percent metabolized by bacterioplankton
     - b. 4% in greater than 1 µm POC size fraction
  2. 55-d second phase
     - a. redistribution among the dissolved and to a lesser extent particulate pools.
     - b. less than 5 percent passed to fractions greater than 1 µm.
     - c. recovered 80% of the label as POC, Doc or DIC.
       -missing label due to respiration with the atmosphere.
- B. only a small fraction of the tracer was detected in classes larger than 1 µm (Fig. 2 and Table 1)
- C. no evidence for the transfer of carbon through to 10- to 30- to 30 - 100 µm fractions to the >100 µm fractions.

V. Discussion
- A. evidence that the bacterioplankton removed by protozoan grazers.
- B. Results do not support the idea of a microbial loop.
- C. in some areas up to 80% of PP is by cyanobacteria (*Li et al.*)
- D. 10 to 60% of primary production passes directly through the bacterioplankton
- E. results suggest that carbon assimilated by plankton smaller than 1 µm may not pass into conventional food chains leading to metazoan secondary production. Thus significant fractions of the total carbon fixed by organisms smaller than 1 µm may be lost from the trophic system.
- F. Heterotrophic flagellates may be important for the regeneration of nutrients.

Postscript: See explanation in **Ducklow *et al.* 1989.** *"However when recycling is considered, it can be seen from Table 8.9 that up to 69% of the macrozooplankton input was mediated by the bacteria. It has been observed in mesocosm experiments that only a few per cent of labeled bacteria pass into large zooplankton (**Ducklow et al. 1986**). Since the dependency of macrozooplankton appears to rise as recycling does, it is tempting to speculate that the mesocosm results were due to low recycling. This was probably the case for the early spring experiment in a Scottish sea loch described by **Ducklow et al. (1986)**."*

Fenchel, T. 1988. Marine plankton food chains. Ann. Rev. Ecol. Syst. *19*: 19-38.[6, 7, 15]

I. Introduction: the classical view of plankton food chains.
- A. change in paradigm **Williams (1981)**
- B. linear food chains out
- C. early history of biological oceanography

II. A new picture of plankton communities
- A. The composition of plankton communities
  picoplankton, nanoplankton, microplankton, mesoplankton
  1. picoplankton: coccoid cyanobacteria
  2. nanoplankton: photosynthetic and phagotrophic forms.
     - a. phagotrophs: choanoflagellates, cryptomonads, chrysomonads, bicoecids, and helioflagellates.

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b. autotrophs: cryptomonads, chrysophytes, haptophytes, prasinophytes, some euglenoids and dinoflagellates, chlorophytes, some tiny diatoms

3. microplankton
   a. autotrophs: dinoflagellates and diatoms
   b. phagotrophs: ciliates (aloricate and loricate "tintinnid" oligotrichs dominate

B. Plankton food chains and the microbial loop
1. nano- and picoplankton dominate production, but are inefficiently grazed by macrozooplankton
2. 10-50% of primary production may be leaked.
3. estimates of high bacterial production
4. stable bacterial numbers, but large zooplankton can't graze them
5. heterotrophic nanoflagellates graze cyanobacteria and the smallest eucaryotic primary production
6. ciliates and heterotrophic dinoflagellates graze heterotrophic nanoflagellates
7. "microbial loop" Fig. 1

III. Properties of plankton organisms and food chains
A. Steady state phagotrophic food chains
1. prey: predator sizes are constrained
2. types of feeding
   a. raptorial
   b. filter feeding
   c. diffusion feeding
3. typical prey: predator ratios are 1:10 with 1:1 to 1:100 ratios possible
4. planktonic tunicates can retain bacteria King *et al.* 1980
5. allometry
6. Sheldon size spectrum
7. Lotka-Volterra type predator-prey cycles.

B. Uptake and excretion of dissolved materials
Bjornsen: being small means you leak

C. Remineralization and mineral cycling
D. Microscale patchiness
E. Symbiosis and mutualism
F. The "sink or link" problem
1. non-sensical question
2. only 3-9% of primary production to larger animals

G. Sedimentation and the supply of food for benthic organisms
1. spring sinking of diatoms
2. late summer sinking of dinoflagellates

IV. Current and future problems
Gross growth efficiency of 30% (10% too low)

Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology *66*: 109-120 [4, 17, 39]

I. Abstract
A. Technique uses tritiated-thymidine
B. autoradiography used to test accuracy
specific for active bacteria
C. Measurement of:
1. isotope dilution effect
2. DNA content of natural bacteria

D. conversion factor documented

II. Introduction
A. Method introduced by **Fuhrman & Azam 1980**
B. Questions:
1. Do organisms other than bacteria incorporate tritiated thymidine from nM concentrations in seawater? *[some benthic diatoms may, but at low rates **Rivkin 1986a**]*

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2. What proportion of natural bacteria are "active" and what portion incorporate tritiated thymidine in seawater?
3. How is the specific activity affected by isotope dilution, both inside and outside cells?
4. How specifically does tritiated thymidine label DNA compared to other macromolecule?
5. What is the DNA content of natural marine bacteria?

C. Microbial groups overlap in size, making the separation of heterotrophic bacterial processes difficult.

III. Materials and Methods

A. tritiated thymidine from New England Nuclear
1. methyl ^{3}H-thymidine
2. >50 Ci /mmole

B. Incubation and processing
1. Seawater samples collected by 5 l Niskin bottles
2. kept in dark
3. *in situ* incubations with **5 nM** tritiated thymidine
4. Samples processed after 1 and 3 h
5. 10 % TCA used to extract soluble pools from the cells
6. cold-TCA insoluble material collected by filtration using 0.45 µm Millipore™ filters
7. Filters rinsed 5 times with 1 ml ice cold 5% TCA
8. Placed in scintillation vial
9. 1 ml ethyl acetate used to dissolve the filter.
10. Radioactivity assayed by liquid scintillation spectrometry
11. Efficiency determined with internal ^{3}H toluene standard

Moles of thymidine incorporated = dpm * $(SA)^{-1}$ 4.5 x$10^{-13.}$

dpm = disintegrations per minute
SA = specific activity of the thymidine
4.5 x 10^{-13} is the number of curies per dpm.
Blank values from formalin or mercury killed controls subtracted.

C. Autoradiography.
1. Simultaneous measurement with AO epifluorescence
2. developed silver grains
3. Methods for
   a. gelatin covered filters
   b. in situ incubations
   c. AO incubation
   d. Autoradiogram using nuclear track emulsion
   e. slides placed in the dark

D. Extraction of specific molecules by hydrolysis.
1. Acid-base hydrolysis used to extract specific macromolecule
2. RNA
3. mitomycin C, a DNA inhibitor, added to stop DNA synthesis

E. Incorporation of radioactive phosphate and thymidine into DNA
1. Water samples divided into 4 subsamples
2. radioactive ^{32}P or ^{33}P added
3. after 1-5 h incubation, the water was filtered through Nucleopore filters.

F. Bacterial DNA content
1. microfluorometric method
2. 80% acetone extraction

G. Field measurements: sample locations in Southern California Bight

IV. Results and Discussion

A. Specificity of tritiated thymidine for bacteria
1. silver grains almost never associated with larger organisms
2. 1% of silver grains associated with some pennate diatoms but might be due to leakage of DOM by bacteria and heterotrophic uptake by larger organisms

B. Generality of tritiated thymidine incorporation for all non-photosynthetic bacterioplankton.
1. Compared various measures of activities (*e.g.*, 15 tritiated amino acids, glucose)
2. different sensitivities a problem

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3. No significant differences between the percentages of bacteria labeled with amino acids, thymidine and a combination of both even though there is a slight trend that the percentage of cells utilizing thymidine may be 3% smaller than the percentage using the amino acids.

Latent image fading: p. 114 fading with time of the images caused by the earliest disintegrations

C. Macromolecular specificity of thymidine labeling
1. TCA-insoluble easier to measure than labeling in total pure DNA
2. 80-90% of TCA-insoluble fraction is DNA, but can be as low as 65%
3. Assumed 65% to 80% of TCA-insoluble fraction is DNA.

D. Labeling of precursor pools by exogenously added thymidine
i.e., What is effect of isotope dilution?

Isotope dilution p. 115. Without correction for isotope dilution, the synthesis rate will be underestimated by an unknown amount. For example if the extracellular thymidine pool is large, most of the thymidine fixed will not be labeled.
1. Extracellular concentration < 1 nM
2. K_m is a few nM so uptake rate is nearly constant
3. the addition of 5 nM results in little isotope dilution.
4. Intracellular specific activity
   a. dTTP is the precursor of DNA
   b. dTTP contaminated by algal dTTP
5. Moriarty and Pollard suggest that the total extracellular and intracellular pool sizes of thymidine and its derivatives can be measured by an isotope dilution approach. They assume that the rate-controlling step in DNA synthesis occurs after the synthesis of dTMP and therefore all the extracellular and intracellular precursor pools up to dTMP are in isotopic equilibrium.
6. F & A do not directly test for isotopic equilibrium. They estimate thymidine incorporation and use supposedly independent methods and compare results.
7. Compared methods using ^{32}P and tritiated thymidine
   - assumed a 4:1 molar ratio
8. Thymidine incorporation underestimates DNA synthesis by factors of 2.7 to 7.1
   a. multiply by 3 to 6 for nearshore
   b. multiply rates by 6 to 7 for offshore

E. Bacterioplankton DNA content
"The amount of DNA in the bacteria must be known if one is to convert the quantity of DNA synthesized into the number of bacteria produced"
1. 2.4×10^{-15} g DNA per bacterium
   a. 10% of bacterial dry weight
   b. earlier estimates by Holm-Hansen were low

F. Application to production rate estimates
Conversion factors:
Thymidine per cell:
Moles thymidine incorporated x 1.7×10^{18} = cells produced (nearshore)
Moles thymidine incorporated x 2.4×10^{18} = cells produced (offshore)
These estimates do not necessarily apply to different environments or with different methods.

G. Test of the method: Figure 6 thymidine *vs*. change in cell numbers
Moles thymidine incorporated x 1.6^{18} = cells produced (nearshore) r^2=0.69

H. Application to field data
1. **bacterial growth doubling every 1 to 4 days**
2. offshore, bacterial doubling greater than 1 week
3. Bacterial secondary production is from 5% to 25% of primary production
4. Bacteria consuming from 10% to 50% of primary production, assuming a growth yield of 50%
5. In general, the bacterioplankton consume from a third to half of the primary production.
6. Growth is density dependent.

I. Role of bacteria in food chains
1. Are bacteria consumed by grazers?
2. Links between DOM and bacterioplankton and (2) between bacterioplankton and bacteriovores significant.

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Giovannoni, S and M. Rappé. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes. Pp. 47-84 in D. L. Kirchman, D. L., ed.. Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

I. Introduction
II. Ribosomes: deciphering the evolution of life on earth
Figure 1. Consensus phylogenetic tree illustrating the major lineages of the domain bacteria
Figure 2. Composite phylogenetic tree displaying relationships among the most widespread SSU rRNA gene clusters from marine prokaryotic plankton
III. Molecular sleuths: solving the riddle of marine bacterioplankton diversity
IV. Why culturable and nonculturable?
V. The major bacterioplankton groups
    A. Systematics and the culturable heterotrophic bacterioplankton
        1. Culturable gamma proteobacteria
Figure 3. Phylogentic dendrogram of the gamma subclass of the Proteobacteria
        2. Culturable alpha proteobacteria: the Roseobacter and Sphingomonas clades
Figure 4.
    B. Marine Methyltrophs
    C. The cytophaga-Flavobacterium-Bacteroides group
    D. Planctomycetales
    E. Oxygenic phototrophs: the cyanobacteria
        1. *Synechococcus*, Prochlorococcus
    F. The dominant uncultured bacterioplankton groups
        1. The upbiquitous SAR11 Cluster
        2. The SAR116 cluster
        3. the uncultivated gamma proteobacteria: SAR86
        4. Gram-positive bacterioplankton: the marine actinobacteria clade
        5. SAR202 and the mesopelagic non-sulfur species
        6. The marine Group A clade
        7. The Marine Group B/SAR324 clade
        8. The marine Archaea
VI. Gene clusters and bacterioplankton population genetics
VII. Coastal versus open-ocean bacterioplankton species
VIII. Bacterioplaknton population dynamics
    A. The stratification of bacterioplankton populations
IX. Link between community structure and biogeochemical cycles
X. Research Horizons
XI. Summary
    A. The most abundant bacterioplankton have never been cultured
    B. The major marine prokaryotic groups appear to have cosmopolitan distributions
    C. A relatively small number of uncultured marine bacterioplankton clades (9)_ accoutn for 80% of marine Bacteria 16S rRNA gene clones recovered from seawater
    D. Marine Archaea are abundant and almost invariably fall within two phylogentic groups
    E. High genetic diversity; unknown ecological specialization but some groups distributed differently with depth
    F. Particle-associated and freely suspended bacteria are different
    G. Stratification of bacterioplankton typical of ocean surface.

Hoppe, H.-G., K. Gocke, R. Koppe, and C. Begler. 2002. Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. Nature *416*: 168-171. *[Meridional tritiated leucine & thymidine assays. The equatorial region (8° N to 20° S) is a net heterotrophic region.]*

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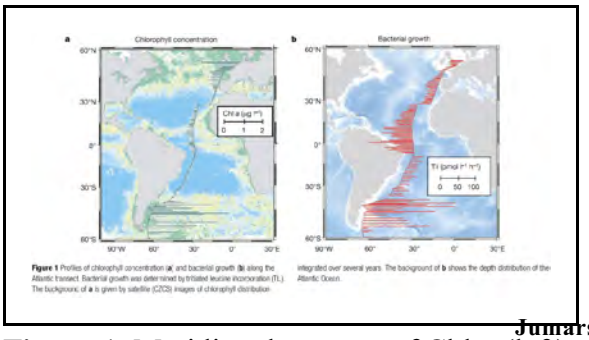

Figure 1. Meridional transect of Chl *a* (left) and bacterial production (leucine incroporation

Jumars, P. A. 19

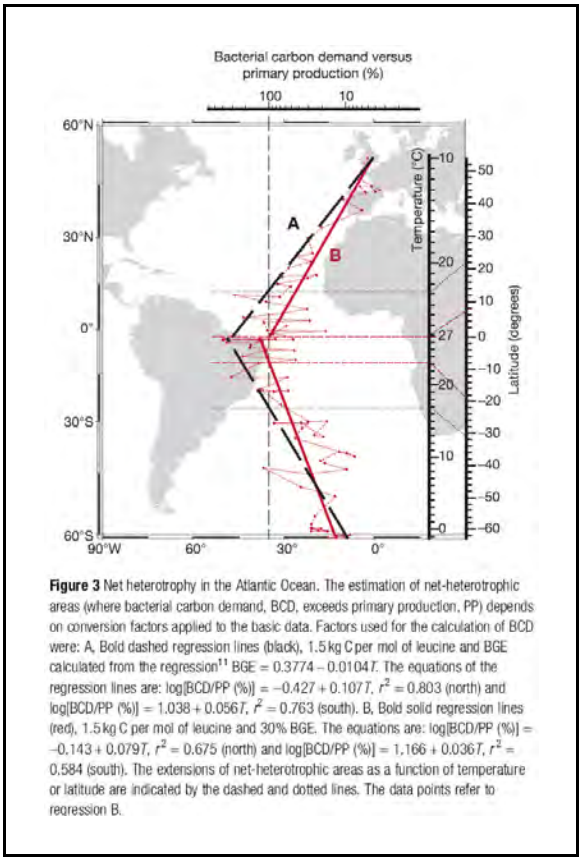

Figure 3 Net heterotrophy in the Atlantic Ocean. The estimation of net-heterotrophic areas (where bacterial carbon demand, BCD, exceeds primary production, PP) depends on conversion factors applied to the basic data. Factors used for the calculation of BCD were: A, Bold dashed regression lines (black), 1.5 kg C per mol of leucine and BGE calculated from the regression[11] BGE = 0.3774 – 0.0104*T*. The equations of the regression lines are: log[BCD/PP (%)] = –0.427 + 0.107*T*, r^2 = 0.803 (north) and log[BCD/PP (%)] = 1.038 + 0.056*T*, r^2 = 0.763 (south). B, Bold solid regression lines (red), 1.5 kg C per mol of leucine and 30% BGE. The equations are: log[BCD/PP (%)] = –0.143 + 0.079*T*, r^2 = 0.675 (north) and log[BCD/PP (%)] = 1.166 + 0.036*T*, r^2 = 0.584 (south). The extensions of net-heterotrophic areas as a function of temperature or latitude are indicated by the dashed and dotted lines. The data points refer to regression B.

Figure 1.Bacterial production vs. Primary production. The equator is a zone of net heterotrophy.

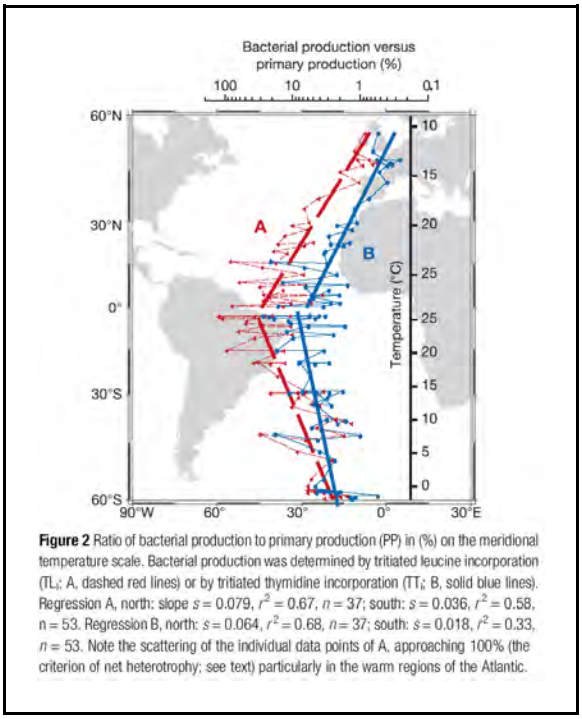

Figure 2 Ratio of bacterial production to primary production (PP) in (%) on the meridional temperature scale. Bacterial production was determined by tritiated leucine incorporation (TL_i; A, dashed red lines) or by tritiated thymidine incorporation (TT_i; B, solid blue lines). Regression A, north: slope s = 0.079, r^2 = 0.67, n = 37; south: s = 0.036, r^2 = 0.58, n = 53. Regression B, north: s = 0.064, r^2 = 0.68, n = 37; south: s = 0.018, r^2 = 0.33, n = 53. Note the scattering of the individual data points of A, approaching 100% (the criterion of net heterotrophy; see text) particularly in the warm regions of the Atlantic.

Figure 1. Bacterial & primary production using leucine (red) and thymidine (blue)

93. Concepts in Biological Oceanography: An interdisciplinary primer. Oxford University Press, New York. 348 pp. ***[Read Chapter 10, pp. 179-197 is on 'microbial loops']*** [?]

X. Many microbial loops

A. *The loop hypothesis*

1. key questions poised by **Pomeroy (1974)**

Fig. 10.1 Azam et al. like-Loop diagram

2. restatement of linear biomass hypothesis (see his chapter 7)

3. **Pomeroy's (1974)** questions remained unanswered until Fenchel (1984)

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4. Fenchel's 1984 review developed the mechanistic arguments of chapter 4: *"From them he concluded that neither flagellates nor larger organisms could make net energetic profit by ingesting picoplankton of cell densities below $10^5 ml^{-1}$*
   a. due to mechanical constraints
   b. small ciliates can be bacterivores too (Sherr and Sherr (1987)
5. Bacterivores can't reduce picoplankton cell densities which fall below 10^5 ml^{-1} due to the physical constraints of Brownian motion
6. **Proctor and Fuhrman (1990)** on marine viruses.

B. *Sources of labile, dissolved organic matter.*
"Measurements of bacterial production with new radionuclide techniques often reveal bacterial production .. in excess of 10% of primary production and as high as 40% or even more. thus, it seems unlikely from mass balance that the source of the organic fuel for this secondary production is very far in the food web from the phytoplanktonic source of the organic fuel."
1. **Fogg (1983)**: glycolate excretion [But see Colman 1989]
2. Only 0.4% of the fluid volume experiences exudate concentrations>10% background (see Chapter 4 and Azam and Ammerman (1984)
   a. Clever use of the Poisson distribution to calculate the distances between bacteria and phytoplankton cells.
   b. **Fig. 10.2**: Probability of a bacterium being within 90 µm of a phytoplankton cell is ≈0.003
3. **Jackson (1989)** analyzes large phytoplankton cells with high leakage rates.
4. Calculation of encounter rates per bacterial cell.

Equation 10.2. 220 µm phycosphere from Azam and Ammerman: "One encounter of a [phytoplankton] diffusional shell per bacterium every 10 min.
Phycosphere distorted by shear
5. Sloppy grazing
   a. Raised by **Pomeroy (1974)**
   b. **Jumars *et al.* (1989)**

C. Biological sources and sinks of inorganic nutrients
1. Lehman and Scavia
2. Scavia *et al.* (1984) phytoplankton cells entrained in the same fluid stream containing zooplankton exudation, providing a mechanism for encounters.
3. Lehman (1987) developed a theory of close encounters of a nutrient kinds
4. Porter (1976) described an extreme example in which freshwater blue-green algae benefit in growth rate, presumably from nutrient acquisition by being ingested and passing thought the guts of zooplankton

D. **One functional group of bacteria.**
1. Ward's nitrifying bacteria
2. Currin's assay for nitrogen fixers

E. Benthic microbial loops
1. Most sediment bacteria attached to mineral grains.

Jumars, P. A., D. L. Penry, J. A. Baross, M. J. Perry, and B. W. Frost. 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. Deep-Sea Res. *36*: 483-495. [6, 7, 25, 26, 27]

Abstract
A. extension of digestion theory.
B. DOM in seawater is a byproduct of animal ingestion and relatively low assimilation efficiencies.

I. Introduction:
A. Heterotrophic bacteria require 20-40% of mean carbon fixation rate (Azam and Fuhrman, Hagstrom, Lancelet and Billen)
B. existing models, Fasham and Peterson require 10% of phytoplankton loss from the phytoplankton cell.
C. Lancelet and Billen indicate low loss rates.

II. Theory
A. Reactions for incomplete digestion: an optimal foraging grazer should not completely assimilate labile DOM for prey.
B. Fates of solutes ejected in fecal pellets.

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III. Discussion

Nagata. T. 2000. Production mechanisms of dissolved organic matter. Pp. 121-152 *in* D. L. Kirchman, ed, Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

I. Introduction

II. Release of DOM by phytoplankton

Figure 1. Percent extracellular release (PER) of DOC relative to primary production

- A. Phytoplankton release a variety of compounds
  - 1. Lab
    - a. 2-10% of production in exponential growth (Figure 1)
    - b. Higher when nutrients depleted or growth conditions suboptimal
    - c. Or cells in stationary or senescent phase growth
    - d. abrupt changes in light intensity
  - 2. Field
    - a. ^{14}C incubations

Table 1. Percent extracellular release (PER) of DOC in marine environments

    - b. Up to 80% percent extracellular release (PER) during declining phase of blooms (Larsson & Hägstrom 1982, **7**, **26**, **27**, **28**, **Lancelot 1983**)
    - c. viral infection can increase DOM release (**Gobler et al. 1997**)
    - d. Higher near surface with higher irradiance (cell damage?)
    - e. **Baines & Pace (1991)** is about 13% of primary production across a wide array of environments.
      - (1) premature to conclude this is a global average
      - (2) bacteria consume 40% to 50% of primary production across systems, so 2/3 of bacterial organic matter demand must be met by other sources. **(Cole et al. 1988**, **Ducklow & Carlson 1992**).

III. MECHANICAL MODELS OF DOM RELEASE

- A. Overflow model
- B. leakage model

IV. Production of DOM by grazers

- A. Release of DOM by protozoa
  - 1. release about 10-30% of ingested pray organic matter as DOM
  - 2. may be dominant source of DOM release
  - 3. exceeds release by phytoplankton
  - 4. release Fe
  - 5. release DOM during egestion
    - a. see Fig. 2

Figure 2. Feeding sequence for protozoan grazers

    - b. Assimilation efficiency ((G+R)/I x 100) of 60-70% (**Fenchel 1982**, **1987**) implies egestion of 30-40% of ingested material
    - c. picopellets=colloids
    - d. **Jumars et al. 1989** modeled grazing "optimum digestion": release of DOM relative to ingestion increases with prey abundance.
      - (1) consistent with Nagata & Kirchman (1991)
      - (2) other studies find retention time of prey in vacuoles varies little with food abundance
    - e. Flagellates excrete Urea and purines (Caron & Goldman 1990)
- B. Release of DOM by zooplankton
  - 1. zooplankton consume a large fraction of primary production dominated by large phytoplankton (White & Roman 1992, Dagg 1993).
  - 2. The release of DOC by zooplankton can represent 10-20% of ingestion (**Copping & Lorenzen 1980**, **Strom et al. 1997**)

Table 4. Release of DOM by crustacean zoolankton.

  - 3. 4 modes of release of DOM by zooplankton
    - a. sloppy feeding
      - (1) Lampert (1978): 4-17% of DOM lost during feeding by Daphnia
    - b. excretion
    - c. egestion

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d. release from fecal pellets
(1) Following **Jumars et al. 1989**, "We may hypothesize that a dominant control of DOM release by zooplankton is egestion and rapid dissolution of solutes from pellets"

V. RELEASE OF DOM BY VIRAL INFECTION
A. Gobler et al. (1997): viral infection important for DOM release during the declining phase of phytoplankton blooms
B. need to reevaluate the conventional explanation that the high flux of DOC release during declining phase of blooms is due solely to algal extracellular release

VI. Which mechanisms are important for bacteria?
A. Multiple trophic pathways provide DOM, a view different from **Azam et al. (1983)** who argued that the major process was algal extracellular release
B. Heterotrophs play a major role in DOM release
C. Oligotrophic environments

Figure 4

1. grazers the dominant source of DOC, 65% of total DOC production
a. protozoa are the most important grazers (can consume 80% of primary production **Landry et al. 1997**, **Liu et al. 1995**)
b. Consistent with **Jumars et al. (1989)**
c. viruses, 3% of primary production and 50% of bacterial production
2. Total heterotrophic production far exceeds primary production (Table 7)
a. counterintuitive but arises because organic carbon is recycled primarily by DOC-microbial food chains (**Scavia 1988**, **Strayer 1988**)

VII. New perspectives on production pathways of refractory DOM
A. >90% of DOM released to seawater is consumed and respired on a time scale of days or less.
B. refractory carbon has an average > 1000 years (Williams & Druffel 1987)
C. bacterial membrane proteins, peptidoglycan may form refractory DOM

VIII. Conclusions
A. In cultures, exponentially growing phytoplankton relae about 5% of total primary production as DOC
B. Protozoan grazers can release 20-30% of ingested prey organic carbon as DOC
1. metazoan zooplankton: 10-20%
2. egestion of unassimilated material may be the major mechanism
C. Viral infection of host cells may result in substantial release of DOM, potentially large but poorly understood source of DOM
D. Oligotrophic ocean model indicates protozoa are major contributors to DOM
E. A substantial portion of refractory DOM is derived from bacteria.

Riemann, B. and R. T. Bell. 1990. Advances in estimating bacterial biomass and growth in aquatic systems. Arch. Hydrobiol. *118*: 385-402. [4, 5]

I. Abstract
A. ^{3}H-thymidine and ^{3}H-adenine incorporation into DNA and ^{3}H-leucine incorporation into protein are currently in use in many laboratories.
B. leucine and thymidine methods together give reasonable estimates of bacterial growth.

II. Introduction
A. Revolution due to **Azam *et al.* (1983)** , **Sherr & Sherr 1988**
B. new fluorometric techniques for enumerating bacteria and assessing growth
C. DOC release is readily used by bacteria.
D. Wright (1988) concern over methods was detracting from conceptual advances.

III. Microscopic sizing of bacteria
A. AO and DAPI
B. mean cell volume from photograms, image analysis,

IV. Conversion of cell volume to cell carbon
A. 121 fg C μm^{-3} has been applied (Ferguson & Rublee 1976, Watson *et al.* 1977)
B. Bratbak and Dundas 220 fg C μm^{-3}
C. 350-580 in recent studies.

V. **Bacterial growth rates**
-see **Moriarty (1986)** and earlier reviews.

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VI. **^{3}H-thymidine incorporation into DNA**
- A. specific for bacteria at nM concentrations
- B. two pathways
  1. *de novo* route and salvage pathway
  2. *de novo* synthesis must be eliminated or it contribution assessed
- C. introduced by **Fuhrman & Azam (1980)**
- D. ice cold TCA to remove non-bound ^{3}H-thymidine
- E. ^{3}H remaining assumed to be bound to DNA
- F. Problems
  1. variation in ^{3}H-thymidine in DNA vs other molecules
  2. 10-fold range in conversion factors.

Fig. 1. Pathways of thymine and thymidine metabolism from **Moriarty 1986**

VII. **Specificity of macromolecular labeling**
- A. N-starved cells may use thymidine nitrogen Cho & Azam (1988)]
- B. Is purification of DNA essential?

VIII. **Conversion factor for calculating cell production or carbon from rates of thymidine incorporation**

Fig. 2. Empirical conversion factors.
use higher concentration of ^{3}H-thymidine

Fig. 3. The effects of increasing concentrations of ^{3}H-thymidine on the rate of ^{3}H-thymidine incorporation. *[Note asymptote at ≈5nM]*

IX. **^{3}H-thymidine incorporation in anaerobic environments.**
- A. sulfate reducers and chemolithotrophic bacteria don't incorporate thymidine (**Gilmour *et al.* 1990**)
- B. chemolithotrophic bacteria may not take it up

X. **^{3}H-adenine incorporation into DNA and RNA.**
incorporated by both bacteria and algae into RNA and DNA: measures total microbial production

XI. **^{3}H-leucine incorporation into protein**
- A. protein constitutes over ½ dry weight of bacterial cells
- B. applied by Cuhel (1982) to seawater
- C. Kirchman *et al.* (1985): most leucine incorporated into protein
- D. when bacteria are not in balanced growth, the ratio of leucine to ^{3}H-thymidine incorporation varies greatly (Fig. 5)
- E. leucine incorporation more sensitive than ^{3}H-thymidine

XII. Frequency of dividing cells
- A. proposed by **Hagstrom *et al.* (1979)**
- B. FDC can be 2 orders of magnitude higher than ^{3}H-thymidine (Riemann *et al.* 1984)

XIII. Conclusions. Four methods available
- A. ^{3}H-thymidine incorporation
- B. ^{3}H-leucine incorporation into protein
- C. ^{3}H-adenine incorporation into RNA and DNA
- D. frequency of dividing cells

Sherr, E. B., B. F. Sherr, and L. J. Albright. 1987. (with response by H. W. Ducklow, D. A. Purdee, P. J. LeB. Williams and J. M. Davies. Bacteria: Link or Sink? Science *235*: 88-89.[7, 18, 45]

1. Criticism of **Ducklow *et al.* 1986.**
2. one can't generalize from a single link or sink experiment.
3. Criticism.
   - a. **Ducklow *et al.* (1986)** don't provide information on the components of the planktonic assemblage and without this information the experiment can't be interpreted
   - b. Parsons *et al.* experiment provided direct evidence that bacteria can be a link in marine food webs. Glucose addition stimulated production
   - c. The idea that cyanobacteria may not be utilized is not supported.
     i. grazed by ciliates
     ii. ciliates grazed by metazooplankton.
   - d. ^{14}C bicarbonate spike showed that at the end of 10 days, herbivorous zooplankton had incorporated only 1.2% of ^{14}Cactivity.

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Rejoinder by Ducklow:

1. In earlier study, there was appreciable net primary production on the 3 sunniest days. This experiment had rapid uptake of bacterial pool.`
2. Tritiated thymidine experiment showed that only 0.5% of label found in >100 µm particles.
3. Friday Harbor 12 -20% of leucine label transferred to zooplankton larger than 53 µm.
4. Parsons *et al.* yield was 0.1% with glucose enhancement experiment.
5. 10 copepods per liter present.
6. Protozoan bacterivores removing 50% to 100% of bacterial production each day.

"We do not share the view of Sherr et al.. that if the results of an experiment fail to fit the hypothesis, then one rejects the experiment or its site"

Thingstad, T. F. 2000. Control of bacterial growth in idealized food webs. Pp. 229-260 *in* D. L. Kirchman, ed., Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp.

1. Introduction

Figure 1

Figure 2. Idealized scheme of carbon flow through the pelagic food web.

   a. Idealized food web (Fig. 2)
      i. complications: mixotrophic protiess, parasites, appendicularians (baleen whales of the microbial world)
2. Top-down or bottom-up control
   a. Correlating bacterial biomass to bacterial production
   b. "Bottom-up" refers to the causal chain in which resource limitation influences the consumer and the consumer's predators, and so on, up the food chain. "Top-down" refers to the cascading effects of predators controlling their prey, which again may control their prey and so on down the food chain (e.g., **McQueen et al. 1989**)
   c. Bacterial carbon demand
   d. **Billen et al. (1990)** plotting method
      i. smaller variation in growth rate than biomass
      ii. suggestion of less bottom-up and more top-down control in oceanic environments
      iii. Ducklow (1992)
   e. Control of bacterial biomass
      i. heterotrophic nanoflagellate potential generation time 5 hours **(Fenchel 1982)**
      ii. predator control taken over as the standard explanation for control of bacterial biomass
      iii. simple Lotka-Volterra model predicts steady state bacterial population under flagellate predation control of 8 x 10^5 cells mL^{-1}, close to levels observed
      iv. freshwater: higher bacerial abundance
      v. sediment porewater bacterial abundance: 10^9 cells mL^{-1} (**Schmidt et al. 1998**)
         – reduced flagellate predation rate in sediments
   f. ciliate predator added to model
      i. Hutchinson's paradox
3. Substrate control of bacterial growth rate

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a. The case of P-limited bacterail growth rate
b. C-limited bacterial growth rate
c. Effects of eutrophication
d. The quantitative importance of bacteria in food webs

4. Summary
   a. Conditions controlling bacterial growth require a model to analyze
   b. Lotka-Volterra model
   c.
   d. top-down and bottom-up can't be readily separated at steady state.
   e.
   f.
   g. silica supply can have a largeeffect on bacterial production

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(AO and DAPI)

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MICROBIAL DIVERSITY

Bano, N. and J. T. Hollibaugh. 2000. Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the β subdivision of the class Proteobacteria in the Arctic Ocean. Appl. Env. Microbiol. *66*: 1960-1969. *[PCR amplification of 16sRNA DNA unique to ammonium oxidizers followed by denaturing gradient gel electrophoresis. Low diversity, dominated by Nitrospira-like organisms]*

Breitbart, M., P. Salomon, B. Anderson, J. M. Mahaffy, A. M. Segall, D. Mead, F. Azam, and F. Rohwer. 2002. Genomic analysis of uncultured marine viral communities. Proc. Nat. Acad. Sci. 99: 14250-14255.

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Cottrell, M. T. and D. L. Kirchman. 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Env. Microbiol. *66*: 5116-5122.

Crump, B. C., C. S. Hopkinson, M. L. Sogin, and J. E. Hobbie. 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. Appl. Env. Microbiol. 70: 1494-1505. *[Denaturing gel gradient electrophoresis of 16S RNA DNA used to document seasonal and spatial patterns [^{14}C]leucine incorporation used to estimate growth]*

Curtis, T. P., W. T. Sloan & J. W. Scannell. 2002. Estimating prokaryotic diversity and its limits. Proc. Nat. Acad. Sci. *99*: 10494-10495. *[Lognormal distribution used to predict total bacterial species in the sea unlikely to exceed a few million. Take with a grain of salt.]* {?}

Curtis, T. P. and W. T. Sloan. 2004. Microbial diversity and its limits: microbial community structure in nature and implications for microbial ecology. Current Opinion in Microbiology *7*: 221-226. *[They extend their earlier models of alpha diversity, assuming a lognormal distribution, to beta diversity (diversity among habitats) and global metacommunity diversity]* {?}

DeLong, E. 2003. Oceans of Archaea. ASM News *69*: 503-511. *[The archaea were raised to the 3rd monophyletic domain, along with the Bacteria and Eucarya) in the 1970's by Woese. They are cosmopolitan but species poor consisting largely of extreme thermop?hiles, extreme halophiles and methanogens]{?}*

DeLong, E. F. and D. M. Karl. 2005. Genomic perspectives in microbial oceanography. Nature *437*: 336-342. *[A review of 30 years of rapid advance, including the discovery of prochlorophytes and astounding microbial diversity (using PCR, QPCR, and whole genome shotgun sequencing)]{?}*

Dolan, J. R. and C. L. Gallegos. 2001. Estuarine diversity of tintinnids (planktonic ciliates). J. Plankton Res. *23*: 1009-1027. *[Classic methods with Shannon's H' used to analyze the estuarine gradient]*

Dufresne *et al.* 2003. Genome sequence of the cyanobacterium Prochlorococcus marinus SS120, a nearly minimal oxyphototrophic genome PNAS *100*: 10020-10025. *[Whole genome sequenced and key enzymes of TCA and Calvin cycles identified]*

Finlay, B. J. 2002. Global dispersal of free-living eukaryote species. Science 296: 1061 *[Small eukaryotes are likely to be ubiquitous in the world's ocean; so too are prokaryotic species]* {?}

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonium oxidizing archaea in water columns and sediments of the ocean. Proc.Natl. Acad. Sci. USA *102*: 14683-14688 *[Ammonium oxidizers AMO were once thought to be exclusively members of the proteobacteria, but archeal AMO primers reveal widespread and very diverse archaeal AMO.]*

Fuhrman, J. A. and L. Campbell. 1998. Microbial microdiversity. Nature *393*: 410-411. *[Introduces Moore et al.'s study documented different Prochlorococcus genetic strains adapted to different light regimes]*

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Gast, R. J., M. R. Dennett, and D. A. Caron. 2004. Characterization of protistan assemblages in the Ross Sea, Antarctica, by denaturing gradient gel electrophoresis. Appl. Env. Microbiol. *70*: 2028-2037. *[Significant genetic diversity of diatoms, dinoflagellates, ciliates, flagellates, and unidentified eukaryotes]*

Hughes, J. B., J. A. Hellman, T. H. Ricketts, and B. J. M. Bohannan. 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. Applied Env. Microbiology 67: 4399-4406. *[Reviews diversity appraoches, especially those described in **Colwell & Coddington (1994)** and applies them to microbial diversity] {**53**}*

Jenkins, B. D., G. F. Steward, S. M. Short, B. B. Ward, and J. P. Zehr. 2004. Fingerprinting diazotroph communities in the Chesapeake Bay by using a DNA macroarray. Appl. Env. Microbiol. 70: Appl. Env. Microbiol. 70: 1767-1776. *[Clone libraries created using the nifH DNA sequence. DNA macroarrays using 360 bp of the nifH precursor for nitrogenase mapped in Chesapeake Bay]*

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science *276*: 734-740. *[Reviews emerging molecular tools for idenifying patterns of microbial diversity]*

Palenik, B. et al. 2003. The genome of a motile marine *Synechococcus*. Nature *424*: 1037-1042.

Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovonanni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature *418*: 630-633. *[PCR revealed this clade makes up about 1/4 of marine bacteria, but it was unculturable. Using very low-nutrient medium and dilution, 18 isolates were obtained.]* {?}

Rocap, G. et al. 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature *424*: 1042-1047. *[A high- and low-light genotype differ markedly in number of genes and genome size 1350 of 1716 or 2725 genes shared, but unique genes confer relative fitness on ecotypes in different light regimes]*

Schloss, P. D. and J. Handelsman. 2004. bial mensus. Microbiology and Molecular Biology Reviews *68*: 1092-2172. *[Rarefaction curves indicate little leveling off, but Chao1 estimators indicate that previous estimates of 10^7 to 10^9 bacterial species may be too high]*

Steward, G. F., B. D. Jenkins, B. B. Ward, and J. P. Zehr. 2004. Development and testing of a DNA microarray to assess nitrogenase (*nifH*) gene diversity. Appl. Env. Microbiol. *70*: 1455-1465.{}

Taroncher-Oldenburg, G., E. M. Griner, C. A. Francis, and B. B. Ward. 2003. Oligonucleotide microarray for the study of functional gene diversity in the nitrogen cycle in the environment. Appl. Env. Microbiol. *69*: 1159-1171.

Thompson, J. R., S. Pacocha, C. Pharino,V. Klepac-Ceraj, D. E. Hunt, J. Benoit, R. Sarma-Rupavtarm, D. L. Distel, and M. F. Polz. 2005. Genotypic Diversity Within a Natural Coastal Bacterioplankton Population. Science 307: 1311-1313. *[Incredible genetic diversity (>1000 distinct genotypes) within one 16sRNA group Vibrio splendidus. "Overall, the genomes show extensive allelic diversity and size variation. Individual genotypes rarely recurred in samples, and allelic distribution did not show spatial or temporal substructure. Ecological considerations suggest that much genotypic and possibly phenotypic variation within natural populations should be considered neutral."]*

Tringe, S. G., C. von Mering, A. Kobayashi, A. A. Salamov, K. Chen, H. W. Chang, M. Podar, J. M. Short, E. J. Mathur, J. C. Detter, P. Bork, P. Hugenholtz, and E. M. Rubin. 2005. Comparative metagenomics of microbial communities. Science *308*: 554-557. *[Incredibly high gentotypic diversity within one bacterial population, sampled seasonally at Plum Island MA.]*

Venter, J., K. Remmington, J. Heidelberg, A. Halpern, D. Ruscch, J. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science *304*: 66-74. *[Whole-genome shotgun sequencing used to generate 1.045e9 base pairs from 1800 microbial species, including 148 new*

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species and 1.2e6 new genes. Abstract: "We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity."]

Ward, B. B. 2002. How many species of bacteria are there? Proc. Nat. Acad. Sci. *99*: 10234-10236 *[An introduction to and review of* ***Curtis et al. (2002)****]*

Zehr, J. P., M. T. Mellon, and S. Zani. 1998. New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (nifH) genes. Appl. Env. Microbiol. *64*: 3444-3450. *[nifH sequences amplified from gyres revealing greater diversity of species and habitat types than previously realized]*

AEROBIC PHOTOHETEROTROPHIC BACTERIA

Kolber, Z. S., F. G. Plumley, A. S. Lang, J. T. Beatty, R. E. Blankenship, C. L. VanDover, C. Vetriani, M. Koblizak, C. Rathgeber, and P. G. Falkowski. 2001. Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science *292*: 2492- *[Aerobic anoxygenic photoheterotrophs AAPs characterized with infared fast-repitition rate fluorometer. When organic matter is high, they are heterotrophs; but can become photoautotrophs when organic matter is low. These AAP's constituted 10% of the microbial community in the euphotic zone in the NE Pacific 48° N, 128° W, and the % could be higher in oligotrophic areas.]*

Schwalbach, M. S. and J. A. Fuhrman 2005. Wide-ranging abundances of aerobic anoxygenic phototrphic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. Limnol. Oceanogr. *50*: 620-628. *[Reports from* ***Kolber et al. (2001)*** *on high abundances of AanPB bacteria inspired this study, which found low abundances in coastal waters (1.17 ± 1.56%)]{?}*

THE MICROBIAL LOOP HYPOTHESIS

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. *10*: 257-263. *[The key early conceptual paper, note the alphabetical order of authors]*

Banse, K. 1992. Grazing, temporal changes of phytoplankton concentrations, and the microbial loop in the open sea. Pp. 409-440 *in* P. G. Falkowski and A. D. Woodhead, *eds*., Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York.

Davidson, K. 1996. Modelling microbial food webs. Mar. Ecol. Prog. Ser. *145*: 279-296. *[A review of trophic interactions and C & N flow from phytoplankton, bacteria, to grazers]*

Ducklow, H, M. J. R. Fasham and A. Vezina. 1989. Derivation and analysis of flow networks for open ocean plankton systems. pp. 159-205 *in* F. Wulff, J. G. Field, and K. H. Mann, *eds*.. Network analysis in marine ecology. Lecture notes on coastal and estuarine studies, Vol. XX. Springer Verlag, New York. 284 pp.

Fenchel, T. 1988. Marine plankton food chains. Ann. Rev. Ecol. Syst. *19*: 19-38. *[An excellent concise review]* [**6**, **7**, **15**]

Fuhrman, J. A. 1992. Bacterioplankton roles in cycling of organic matter: the microbial food web. pp. 361-383 *in* P. G. Falkowski and A. D. Woodhead, eds, Primary productivity and biogeochemical cycles in the sea. Plenum.

Jumars, P. A. 1993. Concepts in Biological Oceanography: An interdisciplinary primer. Oxford University Press, New York. 348 pp. *[Chapter 10, pp. 179-197 is on 'microbial loops']* [?]

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Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. BioScience *24*: 499-504. *[Describes the microbial loop - a paper before its time]*[6, 24, 25]

Tobiesen, A. 1991. The succession of microheterotrophs and phytoplankton within the microbial loop in Oslofjorden, May-October, 1984. J. Plankton Res. *13*: 197-216.

Extracellular release of DOM by phytoplankton

Azam, F. and J. W. Ammerman. 1984. Cycling of organic matter by bacterioplankton in pelagic marine ecosystems. Pp. 345-360 *in* M. J. R. Fasham, *ed.*, Flows of Energy and Materials in Marine Ecosystems. Plenum Press, New York. *[Includes analyses of the diffusional constraints on the phycosphere, the zone of high DOM concentrations around phytoplankton cells. See also Jackson]*

Baines, S. B. and M. L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol. Oceanogr. 36: 1078-1090. [Extracellular release a constant 13% of production, see **Nagata 2000**] [26]

Bjørnsen, P. K. 1988. Phytoplankton release of organic matter: why do healthy cells do it? Limnol. Oceanogr. *33*: 151-154. [6]

Bowen, J. D., K. D. Stolzenbach, and S. W. Chisholm. 1989. Simulating bacterial clustering around phytoplankton cells in a turbulent ocean. Limnol. Oceanogr.

Colman, B. 1989. Photosynthetic carbon assimilation and the suppression of photorespiration in the cyanobacteria. Aquat. Bot. 34: 211-231. *[Photorespiration not important in normal cyanobacteria; little glycolate is formed under natural cyanobacterial photosynthesis: "It is doubtful, therefore, whether glycolate excretion or release has any major ecological significance" (p. 219)]*

Eppley, R. W., S. G. Horrigan, J. A. Fuhrman, E. R. Brooks, C. C. Price and K. Sellner. 1981. Origins of dissolved organic matter in Southern California coastal waters, experiments on the role of zooplankton. Mar. Ecol. Prog. Ser. *6*: 149-159.

Fogg, G. E. 1966. The extracellular products of algae. Oceanogr. Mar. Biol. *4*: 195-212.[6]

Fogg, G. E. 1977. Excretion of organic matter by phytoplankton. Limnol. Oceanogr. *22*: 576-577.[6]

Fogg, G. E. 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. Botanica marina *26*: 3-14. *[Glycolic acid, a major source of labile DOM, is released as a result of photorespiration.]*[6]

Jackson, G. A. 1987. Simulating chemosensory responses of marine microorganisms. Limnol. Oceanogr. *32*: 1253-1266. *[Bacteria and copepods can only sense the diffusive plumes from phytoplankton greater than about 5 µm]* [?]

Jackson, G. A. 1989. Simulation of bacterial attraction and adhesion to falling particles in an aquatic environment. Limnol. Oceanogr. *34*: 514-530. *[Analysis of plumes emanating from large phytoplankton cells]* [25]

Jensen, L. M. 1985. Characterization of native bacteria and their utilization of algal extracellular products by mixed-substrate kinetic model. Oikos *45*: 311-322.

Lancelot, C. 1979. Gross excretion rates of natural marine phytoplankton and heterotrophic uptake of excreted products in the southern North Sea as determined by short-term kinetics. Mar. Ecol. Prog. Ser. *1*: 179-186.

Lancelot, C. 1983. Factors affecting phytoplankton extracellular release in the Southern bight of the North Sea. Mar. Ecol. Prog. Ser. *1*: 115-121. *[Reviewed by **Nagata 2000**]* [26, 43]

Larsson, U. and A. Hägstrom. 1982. Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Marine Biology *67*: 57-70. [Reviewed by **Nagata 2000**, see also **Lancelot 1983**] [26]

Nagata. T. 2000. Production mechanisms of dissolved organic matter. Pp. 121-152 *in* D. L. Kirchman, ed, Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp. [35, 36, 39, 42, 43, 45, 46, 50]

Sharp, J. H. 1977. Excretion of organic matter: do healthy cells do it? Limnol. Oceanogr. *22*: 381-

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399. *[Earlier studies of high DOM losses may have been technical artefacts]*[6]

Thingstad, T. F., Å. Hagstrom, and F. Rassoulzadegan. 1997. Accumulation of degradable DOC in surface waters: is it caused by a malfunctioning microbial loop? Limnol. Oceanogr. *42*: 398-404. *[DOC can accumulate if bacterial growth kept low by competition with phytoplankton for nutrients and numbers kept low by predators. A steady-state model produced].*

Tolbert, N. E., H. D. Husic, J. V. Moroney, and B. J. Wilson. 1985. Relationship of glycolate excretion to the DIC pool in microalgae. Pp. 211-227 *in* W. J. Lucas and J. A. Berry, eds., Inorganic carbon uptake by photosynthetic organisms. Amer. Soc. Plant Physiol., Rockville MD. *[Discusses the conditions leading to glycolate excretion by microalgae]*

Williams, P. J. LeB., C. S. Yentsch 1976. An examination of photosynthetic production, excretion of photosynthetic products, and heterotrophic utilization of dissolved organic compounds with reference to results from a coastal subtropical sea. Marine Biology *35*: 31-40.

Heterotrophic Protist standing stocks & growth

Bloem, M-J., B. Bär-Gilissen, and T. E. Cappenberg. 1986. Fixation, counting and manipulation of heterotrophic nanoflagellates. Appl. Env. Micro. *57*: 1266-1272.

Caron, D. A. 1983. Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures. Applied and Environmental Microbiology 46: 491-498. *[Nanoplankton are stained and examined for Chl a. Stained bacteria, not containing Chl a are presumed to be heterotrophs]*

Geider, R. J. 1987. An improved method for the observation and enumeration of heterotrophic and photoautotrophic microplankton. J. exp. mar. Biol. Ecol. *110*: 19-25.

Ohman, M. D. and R. A. Snyder. 1991. Growth kinetics of the omnivorous oligotrich ciliate Strombidium sp. Limnol. Oceanogr. *36*: 922-935. *[Grows poorly on bacteria]*{?}

Sherr, E. B., B. F. Sherr, R. D. Fallon, and S. Y. Newell. 1986. Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol. Oceanogr. *31*: 177-183.

Silver, M. W., M. W. Gowing, D. C. Brownlee, and J. O. Corliss. 1984. Ciliated protozoa associated with oceanic sinking detritus. Nature *309*: 246-248.

Stoecker, D. K., A. E. Michaels, and L. H. Davis. 1987. Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature *326*: 790-792.

Grazing on bacteria (especially by heterotrophic nanoflagellates):

Albright, L. J., E. B. Sherr, and R. D. Fallon. 1987. Grazing of ciliated protozoa on free and particle-attached bacteria. Mar. Ecol. Prog. Ser. 38: 125-129.

Alonso, M. C., V. Rodriguez, J. Rodriguez, and J. J. Borrego. 2000. Role of ciliates, flagellates, and bacteriophages on the mortality of marine bacteria and on dissolved-DNA concentration in laboratory experimental systems. J. Exp. Mar. Biol. Ecol. *244*: 239-252. *[Phage and flagellates more important sources of bacterial mortality than the ciliate Uronema]*

Andersen, P. and T. Fenchel. 1985. Bacterivory by microheterotrophic flagellates in seawater samples. Limnol. Oceanogr. *30*: 198-202.

Anderson, A., U. Larsson and A. Hagstrom. 1986. Size-selective grazing by a microflagellate on pelagic bacteria. Mar. Ecol. Prog. Ser. *33*: 51-57.

Anderson, O. K., J. C. Goldman, D. A. Caron, and M. R. Dennett. 1986. Nutrient cycling in a microflagellate food chain: III. Phosphorous dynamics. Marine Ecology Progress Series *31*: 47-55. *[Phagotrophic microflagellates, while grazing, are responsible for most of the P regeneration in this lab system.]*

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. *10*: 257-263.

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Azam, F. and J. W. Ammerman. 1984. Cycling of organic matter by bacterioplankton in pelagic marine ecosystems: microenvironmental considerations. Pp. 345-360 in M. J. R. Fasham, ed., Flows of energy and Materials in Marine Ecosystems. Plenum Publishing.

Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. Limnol. Oceanogr. *27*: 1059-1071. *[Ciliates have a lower weight-specific growth than simple allometry indicates (the y-intercept differs. Their importance may have been overemphasized]*

Banse, K. 1984. Review of P. Bougis, ed., Marine Pelagic Protozoa. Limnol. Oceanogr. *29*: 445-446. *[Banse frames the sink versus link question in his concluding sentence.]*[6]

Billen, G., P. Servais, and S. Becquevort. 1990. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia 207: 37-42. [29]

Bird, D. F. and J. Kalff. 1986. Bacterial grazing by planktonic lake algae. Science *231*: 493-495. *[Lake algae ingest bacteria.]*

Bjønsen, P. K., B. Riemann, S. J. Horsted, T. Gissel Nielsen, and J. Pock Sten. 1988. Trophic interactions between heterotrophic nanoflagellates and bacterioplankton in manipulated seawater enclosures. Limnol. Oceanogr. *33*: 409-420.

Bloem, J. and M.-J. Bår Gilissen. 1989. Bacterial activity and protozoan grazing potential in a stratified lake. Limnol. Oceanogr. *34*: 297-309.

Brendenberger, H. 1991. Filter mesh sizes of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. *36*: 884-894. *[Retention from 33-154%. Although cladocerans don't sieve, mesh size associated with retention efficiency]*

Caron, D. A., P. G. Davis, L. P. Madin, and J. M. Sieburth. 1982. Heterotrophic bacteriovorus protozoa in oceanic microaggregates. Science *218*: 795-797.

Caron, D. A., P. G. Davis, L. P. Madin, and J. McN. Sieburth. 1986. Enrichment of microbial populations in microaggregates (marine snow) from surface waters of the North Atlantic. J. Mar. Res. *44*: 543-565. *[Most probable number statistics used to count bacterivores. Bacterivores dependent on detrital aggregates.]*

Caron, D. A., J. C. Goldman, and M. R. Dennett. 1988. Experimental demonstration of the role of bacteria and bactiverous protozoa in plankton nutrient cycles. Hydrobiologia *159*: 27-40.

Caron, D. A., E. R. Peele, E. L. Lim & M. R. Dennett. 1999. Picoplankton and nanoplankton and their trophic coupling in the surface waters of the Sargasso Sea South of Bermuda. Limnol. Oceanogr. 44: 259-274. *[Tritiated thymidine for bacterial production and bacterial grazing by fluorescently labeled particles. Small nanoplanktonic protists <5µm responsible for most of the bacterial grazing. 0.9 to 18-d doubling times for the heterotrophic nanoplankton]* {13, 14}

Davis, P. G. J. McN. Sieburth. 1984. Estuarine and oceanic microflagellate predation of actively growing bacteria: estimation by frequency of dividing bacteria. Mar. Ecol. Prog. Ser. *19*: 237-246.

Dolan, J. R. and K. Šimek. 1997. Processing of ingested matter in *Strombidium sulcatum*, a marine ciliate (Oligotrichida). Limnol. Oceanogr. *42*: 393-397. *[constant digestion rate in cells feeding or deprived of prey, proecessing latex spheres or bacterial cells]*

Ducklow, H. W. and S. M. Hill. 1985. The growth of heterotrophic bacteria in the surface waters of warm core rings. Limnol. Oceanogr. 30: 239-259. *[Uses the dilution technique to estimate bacterial growth and bacterivory. Bacteria double 1-2 times daily and consume 40% of primary production daily. Only 3-7% of primary production converted to protozoan biomass.]*

Ducklow, H. W., D. A. Purdie, P. J. LeB. Williams and J. M. Davies. 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science *232*: 865-867. *[See the rebuttal E. B. Sherr et al. (1987) below, followed by a rejoinder by Ducklow et al.]* {7, 19, 28, 48, 49}

Epstein, S. S. and M. P. Shiaris. 1992. Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. Appl. Env. Microbiol. *58*: 2426-2431.

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Estep, K. W., P. G. Davis, M. D. Keller and J. McN. Sieburth. 1986. How important are oceanic algal nanoflagellates in bacterivory? Limnol. Oceanogr. *31*: 646-649.

Fenchel, T. 1980. Suspension feeding in ciliated protozoa: feeding rates and their ecological significance. Microbial Ecology *6*: 13-25.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Marine Ecology Progress Series *8*: 225-231. [Assimilation efficiency of 60%, 60-70%, cited by **Nagata 2000**] [**26**, **29**]

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. III. Adaptations to heterogeneous environments. Marine Ecology Progress Series *9*: 25-33.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Marine Ecology Progress Series *9*: 35-42.

Fenchel, T. 1984. Suspended marine bacteria as a food source. Pp. 301-315 *in* M. J. R. Fasham, ed., Flows of energy and materials in the marine environment. Plenum Press, New York. *[Macrozooplankton and ciliates are not effective bacterivores. The important bacterivores are less than 10 μm in size.]*

Fenchel, T. 1987. Ecology of protozoa: the biology of free-living phagotrophic protists. Springer-Verlag, Berlin [Cited by **Nagata 2000**: assimilation efficiency of 60%] [**26**]

Fenchel, T. 1988. Marine plankton food chains. Ann. Rev. Ecol. Syst. *19*: 19-38. *[An excellent concise review]*[**6**, **7**, **15**]

Fuhrman, J. A. and G. B. McManus. 1984. Do bacteria-sized marine eukaryotes consume significant bacterial production? Science *224*: 1257-1260. *[Chemical inhibitors of eucaryotic growth used to show that small eukaryotes consume more than 50% of the bacterial production.]*[**13**, **46**]

Fuhrman, J. A., R. W. Eppley, A. Hagström, and F. Azam. 1985. Diel variations in bacterio-plankton, phytoplankton, and related parameters in the Southern California Bight. Marine Ecology Progress Series *27*: 9-20. *[They followed submerged drogues, measuring diel changes in chl, ^{14}C uptake, bacterial uptake, ^{3}H-thymidine uptake, bacterial abundance, non-pigmented flagellates, POC, N, C/N, and O_2.]*

Geider, R. J. 1989. Use of radiolabelled tracers in dilution grazing experiments to estimate bacterial growth and loss rates. Microb. Ecol. *17*: 77-87.

del Giorgio, P. A. and J. J. Cole. 2000. Bacterial energetics and growth efficiency. Pp. 289-325 *in* D. L. Kirchman, ed., Microbial ecology of the oceans. Wiley-Liss, New York. 542 pp. *[Bacterial Growth Efficiency low (usually < 30%)] [?]*

Goldman, J. C., D. A. Caron, O. K. Anderson, and M. R. Dennett. 1985. Nutrient cycling in a microflagellate food chain: I. Nitrogen dynamics. Marine Ecology Progress Series 24: 231-242. *[This phagocytic microflagellate preys on both bacteria and diatoms, regenerating ammonium.]*

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DO BACTERIA AND PHYTOPLANKTON COMPETE FOR NUTRIENTS AND ARE BACTERIA REALLY REMINERALIZERS OF NITROGEN?

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OCEANS: NET AUTOTROPHIC OR HETEROTROPHIC?

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Duarte, C. M., S. Agusti, del Giorgio and J. J. Cole. 1998. Regional carbon balances in the oceans [a response to **Williams & Bowers 1998**]. Science *284*: 1735.

Duarte, C. M. and S. Agusti, J. Arestegui, N. González, and R. Anadón. 2001. Evidence for a heterotrophic subtropical northeast Atlantic. Limnol. Oceanogr. 46: 425-428. *[Off NW Africa from 1991-2000, the P/R ratio was less than 1 for most of the stations with photosynthesis < 3 g O_2 $m^{-2}d^{-1}$]*

Geider, R. J. 1997. Photosynthesis or planktonic respiration. Nature *388*: 132-133. *[Geider criticizes the allometric models of **del Giorgio et al. (1997)** who'd proposed that low productivity systems are heterotrophic. The low productivity systems accumulate O_2 and export POC, unlikely for net heterotrophic systems. Cyanobacteria may have been counted as heterotrophs by del Giorgio et al. , producing a 200% overestimate of heterotrophic respiration. A PQ of 1.25 raises the overestimate to 250%. Del Giorgio & Cole respond, agreeing with Geider somewhat but arguing that with BGE of 20%, bacterial respiration exceed gross photosynthesis]*

González, N., R. Anadón, B. Mouriño, E. Fernández, B. Sinha, J. Escánez, and D. de Armas. 2001. The metabolic balance of the planktonic community in the North Atlantic subtropical gyre: the role of mesoscale instabilities. Limnol. Oceanogr. 946-952. *[Communities were net heterotrophic in August but in balance in the spring. Mesoscale features reduced heterotrophy relative to photosynthesis.]*

Hoppe, H.-G., K. Gocke, R. Koppe, and C. Begler. 2002. Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. Nature *416*: 168-171. *[Net heterotrophy, in which phototrophic carbon fixation is less than bacterial carbon demand, Meridional tritiated leucine & thymidine assays. The equatorial region (8° N {27 °C}to 20° S{17°C}) is a net heterotrophic region.]*

Karl, D. M., E. A. Laws, P. Morris, P. J. lB. Williams and S. Emerson. 2003. Metabolic balance of the open sea. Nature *426*: 32. *[A hypothesis proposed to account for the presence of net heterotrophic gyre ecosystems.]*

Kirchman, D. L. 1997. Microbial breathing lessons. Nature *385*: 121-122. *[Kirchman introduces and summarizes the arguments in **del Giorgio et al. 1997**]{**52**}*

Rivkin, R. B. and L. Legendre 2001. Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science *291*: 2398-2400. *[100 data points from the literaure used to find a regression of bacterial growth efficiency vs. temperature. Near 0°C, BGE ≈ 50%, but BGE<10% at 30 °C]*

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Williams, P. J. leB. 1998. The balance of plankton respiration and photosynthesis in the open oceans. Nature *394*: 55-57. *[Data from 5 open-ocean regions analyzed, showing net autotrophy and organic carbon export.* ***Del Giorgio et al. (1997)*** *using individual photosynthesis and respiration data may not be as valid as using depth-integrated photosynthesis and respiration]*

Williams, P. J. leB and D. G. Bowers. 1998. Regional carbon imbalances in the oceans. Science *284*: 1735. *[A critique of* ***del Giorgi et al. 1997*** *and* ***Duarte & Agusti 1998****. They argue that one allometric equation might have been fit to a relationship requiring 2 or more allometric equation, producing a statistical artifact indicating that respiration exceeded production.* ***Duarte et al. 1998*** *responded]*

MISCELLANEOUS

Colwell, R. K, and J. A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. Phil Trans. Roy. Soc. B *345*: 101-118. *[Cited,* ***Hughes et al. (2001)****]{?}*

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Landry, M. R., R. T. Barber, R. R. Bidigare, F. Chai, K. H. Coale, H. GT. Dam, M. R. Lewis, S. T. Lindley, J. J. McCarthey, M. R. Roman, D. K. Stoecker, P. G. Verity, and J. R. White. 1997. Iron and grazing constraints on primary production in the central equatorial Pacific: An EqPac synthesis. Limnol. Oceanogr. *42*: 405-418. *[EqPac is HNLC, Fe enters via upwelling but is low in concentration. Most phytoplankton cells Fe-limited and controlled by microzooplankton. f-ratio low (0.07-0.12)]* [27]

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McDuff, R. E. and S. W. Chisholm. 1982. The calculation of *in situ* growth rates of phyto-plankton populations from fractions of cells undergoing mitosis: a clarification. Limnol. Oceanogr. *27*: 783-788.[12]

McQueen, D. J., M. R. S. Johanes, J. R. Post, T. J. Stewart, and D. R. S. Lean. 1989. Bottom-up and top-down inputs on freshwater pelagic community structure. Ecol. Monogr. *59*: 289-309. *[In the Carpenter cascade, a decrease in fish leads to an increase in planktivores, a decrease in herbivores and an increase in chl a. In the bottom up/top down concept (which McQueen introduced and supports), chl a concentration is independent of fish abundance.]* [29]

Web Resources

Table 1. Web resources on marine microbial processes

URL	Description	PI/Webmaster
http://snow.tamu.edu/	Biocomplexity project: analyzing the biodiversity of the nitrogen cycle with microarrays	George Jackson
http://www.biotech.umb.edu/	UMASS/Boston biotechnology center	Roderick Jensen

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Table 1. Web resources on marine microbial processes		
http://www.gene-chips.com/	Gene chips/DNA microarrays	
http://www.bio.usyd.edu.au/Protsvil/leeandpaddy.htm	Diversity and Geographic Distribution of Free-Living Heterotrophic flagellates - Analysis by PRIMER	W.J. Lee and D.J. Patterson
http://megasun.bch.umontreal.ca/protists/protists.html	Protist image data	Molecular Evolution and Organelle Genomics program at the University of Montreal
http://tolweb.org/tree/phylogeny.html	Tree of life project	
http://www.awi-bremerhaven.de/Biomeer/molecular-genetics-top08-e.html	Micro-Arrays for the Detection of the Abundance and Distribution of Pathogenic Protozoa, Flagellated Algae and Diatoms (EU project MICROPAD)	K. Metfies, S. Rabold, L. Medlin
http://www.sb-roscoff.fr/Phyto/PICODIV/PICODIV_publications.html	PICODIVa European FP5 program Monitoring the diversity of photosynthetic picoplankton in marine waters	

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