Growth and loss in the ocean: Planktonic bacteria as links or sinks in marine food webs

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“As on land and everywhere else the animal life depends for its very existence on the assimilation of the plants, but while on land animals of every size up to elephants can partake directly of the food provided by the plants, there must be, and is, in the water of the open ocean a special fauna mainly composed of microscopic and just barely visible animals which alone are able to utilize the small phytoplankton organisms. These in turn are eaten by somewhat larger animals, and so on right up to the largest fishes. This is of course the only possibility, but it is a wasteful way of conducting life’s business and means a considerable reduction in the total mass of animal which can be supported on a definite production of phytoplankton food.” - August Krogh, 1934
What is it we want to know?

- **Carbon flow** (bacterial production, respiration, DOC utilization rates)

- **Bacterial growth** (cell physiology, nutritional status)

- **Biomass** (biogenic carbon, trophic linkages)
Motivation
Bacterial growth largely regulates fluxes of carbon and nutrients, and dictates energy flow through marine ecosystems.

DOM (C, N, P, Fe, O, S, Zn, etc., etc.)

Bacterial Biomass

CO$_2$, NH$_4^+$, PO$_4^{3-}$

Higher trophic levels

Link

Sink
To determine the importance of microbes to ocean food webs/carbon/nutrient fluxes, we need to:

1. **Quantify bacterial population size and mass**
   - Biomass, abundance, cell sizes

2. **Quantify growth rates and production**
   - Biomass production, respiration, cell division, and turnover

3. **Understand growth limiting factors**
   - Metabolic flexibility, physiology
1950 - The Prevailing Concepts

• Low bacterial biomass-based largely on plate counts
• Slow growing
• Predominately using resistant/refractory substrates
• Low (c. 3%) growth yields (Waksman)
• Minor contribution to community biomass (Harvey)
• Low contribution to overall metabolism (<1% to total respiration)
• Growth was mainly on the surface of particles
Cycling of matter in the sea circa 1960

Bacteria are relegated to the benthos

Steele 1974
Limnologists had already recognized a role for planktonic bacteria in both mineralization of nutrients and as an energy source to predators.
1950-1974 – the Seeds of Change and the Growth of the New Paradigm

• Realization of the dynamic nature of DOM

• Recognition the plate count technique seriously underestimated bacterial abundances

• Inability to account for recycling of nitrogen (Dugdale & Goering, 1966)
1950-1974 – the Seeds of Change and the Growth of the New Paradigm

• One example of the dynamic nature of DOM (Harvey 1955)
Plate counts tend to provide estimates of total bacterial abundances that are 0.01-0.1% of cell abundances estimated by epifluorescence microscopy. Why?

- Many bacteria in seawater are difficult culture?
- Wrong substrates in media?
- Non-viable or dead bacteria?
A Revolution Begins

The Ocean’s Food Web, A Changing Paradigm

Lawrence R. Pomeroy

Fig. 1. The classical paradigm of the ocean’s food web in simplified form is enclosed within the circle. More recently conceived pathways are outside the circle. The possible relative magnitude of the pathways is discussed in the text.

Pomeroy 1974
The microbial loop hypothesis: Photosynthetically produced DOM is consumed by heterotrophic bacteria; these bacteria are consumed by heterotrophic flagellates, which are in turn are preyed on by microzooplankton. Thus, energy and matter lost from the food web as DOM is returned through bacterial growth.
Microbial Loop

• Heterotrophic bacterial growth results in recovery of non-living pool of dissolved organic matter back into living pool of biomass.
• Transfer of bacterial biomass to higher trophic levels
• Remineralization of nutrients through the various stages of growth and predation.
Quantifying the importance of the microbial loop to ocean ecosystems

• Determine rate of bacterial biomass production and bacterial growth

• Determine flux of DOM into bacteria

• Determine the efficiency of organic matter and energy flow through bacteria

• Determine rate of removal and efficiency of predators
Bacterial Production

• Bacterial production (BP) is the rate that bacterial biomass is produced. It is the net movement of organic matter from a nonliving pool (DOM) to a living pool (bacterial biomass).

• Typically when marine ecologists speak of bacterial production, they refer only to heterotrophic production (secondary production).

• Mathematically

\[
BP = \mu B
\]

\[\mu = \text{specific growth rate (time}^{-1}\text{)}\]

\[B = \text{bacterial biomass (mg C L}^{-1}\text{)}\]

• **Note that** \[\mu = \frac{BP}{B}\]

• Thus, BP has units of mg C L\(^{-1}\) d\(^{-1}\)
Measuring bacterial growth in seawater

• Most direct method is to measure changes in cell abundance + volume (biomass) over time in the absence of predation.
The phases of bacterial growth in a closed system

Closed system; variable growth rate – cells are inoculated into media and grow until resources are depleted (logistic growth model).
Bacterial growth in a chemostat

Open system: constant supply of limiting nutrients; growth rate held constant by rate of substrate addition (or removal). Typically use an exponential growth model.
Ideally we could estimate $B$ and $\mu$ to get $BP$ ...

Unfortunately, $\mu$ of marine bacteria is very difficult to measure

From an exponentially growing population the specific growth rate ($\mu$) can be derived from:

$$\frac{dN}{dt} = \mu N$$

$$N_t = N_0 e^{\mu t}$$

or alternatively:

$$\mu = \frac{\ln N_t - \ln N_0}{t}$$

$\mu$ has units of time$^{-1}$

Doubling time ($d$) is the time required for the population to increase by 100%; it is related to $\mu$ by:

$$N_t = N_0 e^{\mu d}$$

$$\frac{N_t}{N_0} = e^{\mu d} = 2$$

$$d = \ln 2 / \mu$$

$d$ has units of days or hours.
Factors that regulate bacterial growth in nature

- **Resources (bottom up)** - inorganic and organic substrates
- **Temperature**
- **Sunlight** – newly discovered bacterial metabolisms
- **Predation (top down)** – does not strictly control growth rate, but controls biomass accumulation, thereby influencing growth rate determinations
Measuring bacterial growth in seawater cultures – provides information on predation control and resource control of growth

Carlson et al. (1996)

Fig. 3. Time course response of bacterial carbon (■) and DOC concentration (○) in unamended seawater cultures. (A) Cultures that demonstrated measurable growth utilized a significant portion of the bulk DOC pool. (B) Cultures that showed minimal growth did not significantly remove DOC.
Pitfalls in determining growth rates of natural marine bacteria

- Mixed assemblage of microbes with variable growth rates.
- Losses via predation and/or viral lysis tend to balance growth (i.e. no net change in population standing stock over time).
- Uncertainties in measurements of production and biomass.
Measuring bacterial growth rates \textit{in situ} can be difficult, but estimates of cell production can be estimated and related to growth.

Commonly used methods of estimating bacterial production

\[ \text{Production} \]
\[ (\Delta \text{biomass/time}) \]
\[ (\text{mg C L}^{-1} \text{ d}^{-1}) \]

- $^3$H-thymidine
- $^3$H/$^{14}$C-leucine
- $^3$H-adenine
Bacterial Production – no direct measures... advantages and disadvantages of several methods

- **Adenine** - purine base; RNA and DNA precursor (see Karl 1979). Can be used to measure nucleic acid production rate.
  - **Pros**: ability to determine intracellular isotope dilution by measuring ATP.
  - **Cons**: labels DNA, RNA, and ATP pools, non-specific - potentially incorporated by all microbes

- **Thymidine** - nucleoside of thymine; DNA precursor (see Fuhrman and Azam 1980). Used to estimate rates of DNA production.
  - **Pros**: specific to heterotrophic bacteria
  - **Cons**: difficult to measure intracellular dilution; catabolism under some conditions

- **Leucine** - amino acid; incorporated into protein (see Kirchman et al. 1992). Measures Protein production rates.
  - **Pros**: more sensitive than thymidine (intracellular protein >> DNA)
  - **Cons**: some cyanobacteria can utilize; difficult to measure isotope dilution.
The basics of measuring bacterial production

Concentrate plankton on filter (0.2 µm)

Whole SW

SW + isotope

$^{3}\text{H}$-thymidine
$^{3}\text{H} / ^{14}\text{C}$-leucine

Incubate at \textit{in situ} temperature, typically in the dark (heterotrophic production)

Extract RNA, DNA, protein from filters. Count radioactivity by liquid scintillation and convert to rate of precursor incorporation into nucleic acid or protein (nmol leu L$^{-1}$ hr$^{-1}$)

SW + isotope
The pathways of intracellular precursor incorporation, degradation, and dilution

- **Salvage pathway**
  - DNA (thymidine)
  - Protein (leucine)

- **Degradation pathway**
  - Possible metabolism and non specific labeling of proteins, RNA, DNA

- **De novo synthesis**
  - of leucine, thymidine from internal pools of C, N, P

- **3H-Thymidine/Leucine**
More conversion factors required to get to an ecologically meaningful unit from cellular incorporation rates

Conversion factors can vary more than an order of magnitude, directly influencing estimate of production. Also, factors are variable in time and space – should be determined each time production is measured.

<table>
<thead>
<tr>
<th>Region</th>
<th>Thymidine conversion (10^{18} cells mole^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic Mediterranean</td>
<td>1.7</td>
</tr>
<tr>
<td>Ross Sea, Antarctica</td>
<td>0.2-1.3</td>
</tr>
<tr>
<td>Subarctic North Pacific</td>
<td>1.74</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>0.2-5.6</td>
</tr>
<tr>
<td>Oligotrophic North Pacific</td>
<td>1.46</td>
</tr>
</tbody>
</table>

Fig. 6. (A) Thymidine and (B) leucine conversion factor plots (cumulative change in cell abundance vs integrated incorporation). (---) Linear regressions through all points shown (slope is conversion factor in cells per mol). See Tables 1 to 3 for details of experiments and designations. (O) Carboy 94-4 experiment, (●) combined data from all other experiments.

Fig. 7. (A) Thymidine and (B) leucine conversion factors determined from slopes of individual plots of cumulative cell production versus cumulative incorporation rates. Bars are 95% confidence interval. Values do not differ significantly between experiments sharing letters above bars (t-test, p > 0.05). Experiment designations as in Table 1.
Vertical Profiles of Bacterial Production

Bacterial Production (ng C L\(^{-1}\) d\(^{-1}\))

Depth (m)

Depth (m)

0 500 1000 1500 2000 2500

0 200 400 600 800 1000 1200

Polar Front
Eq-Pac
HOT
Arabian Sea

Bacterial Production (ng C L\(^{-1}\) d\(^{-1}\))

Polar Front

Eq-Pac

HOT

Arabian Sea
## Bacterial production and growth in the upper ocean of various ocean ecosystems

<table>
<thead>
<tr>
<th>Location</th>
<th>Bacteria Biomass (mg C m⁻²)</th>
<th>Phyto. Biomass (mg C m⁻²)</th>
<th>BB:PB</th>
<th>BP (mg C m⁻² d⁻¹)</th>
<th>PP (mg C m⁻² d⁻¹)</th>
<th>BP:PP</th>
<th>BP/BB (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargasso Sea</td>
<td>659</td>
<td>573</td>
<td>1.2</td>
<td>70</td>
<td>465</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>North Atlantic</td>
<td>500</td>
<td>4500</td>
<td>0.11</td>
<td>275</td>
<td>1083</td>
<td>0.25</td>
<td>0.55</td>
</tr>
<tr>
<td>Subarctic North Pacific</td>
<td>571</td>
<td>447</td>
<td>1.2</td>
<td>56</td>
<td>629</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Station ALOHA</td>
<td>750</td>
<td>447</td>
<td>1.7</td>
<td>106</td>
<td>486</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>Arabian Sea</td>
<td>724</td>
<td>1248</td>
<td>0.58</td>
<td>257</td>
<td>1165</td>
<td>0.22</td>
<td>0.35</td>
</tr>
<tr>
<td>Average Stand dev.</td>
<td>641</td>
<td>1443</td>
<td>0.96</td>
<td>153</td>
<td>765</td>
<td>0.18</td>
<td>0.25</td>
</tr>
<tr>
<td>CV (%)</td>
<td>105</td>
<td>1740</td>
<td>0.62</td>
<td>105</td>
<td>334</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>16%</td>
<td>120%</td>
<td>64%</td>
<td>69%</td>
<td>44%</td>
<td>35%</td>
<td>79%</td>
</tr>
</tbody>
</table>
The importance of the microbial loop in different marine ecosystems depends on at least four factors:

1. The rate that DOC is produced

2. The rate that bacteria convert DOC into biomass (this is bacterial production)

3. The rate that DOC is respired during growth

4. The rate of bacterial removal and subsequent passage of material to higher trophic levels
Main points

- Bacterial biomass is a large component of plankton biomass in marine ecosystems (~30->100% of phytoplankton).

- Bacterial production tends to range from 10-30% of photosynthetic production of particulate organic matter.

- Bacterial growth rates typically range 0.1-1.0 d⁻¹ (equivalent to doubling times of ~0.7 to 7 days).
Great plate count anomaly

- Plate counts tend to provide estimates of total bacterial abundances that are 0.01-0.1% of cell abundances estimated by epifluorescence microscopy
- Why?
  - Many bacteria in seawater are difficult culture?
  - Wrong substrates in media?
  - Non-viable or dead bacteria?
Non-living and inactive bacteria?

- Various staining, destaining, and microautoradiography methods have suggested ~30-60% of the “DAPI” stainable cells are inactive or dormant.

Zweifel and Hagstrom (1995)

Choi et al. (1996)
Addition of organic nutrients to seawater culture increased the abundance of nucleoid-visible cells and the proportion of actively respiring cells. These data suggest inactive cells may have low cellular DNA concentrations.
Merging molecular biology and marine bacterial ecology

Cottrell and Kirchman 2003
Efforts to determine the activity and cell sizes of various phylotypes by combining microautoradigraphy and FISH.
Motivation

Bacterial growth regulates fluxes of carbon and nutrients, and dictates energy flow in marine ecosystems.

DOM (C, N, P, Fe, O, S, Zn, etc., etc.)

Bacterial Biomass

CO$_2$, NH$_4^+$, PO$_4^{3-}$

10-20% of PP into BP

Higher trophic levels

Link

DOM uptake to support $\mu$

Sink ??
Energetic costs of growth

a. Oxidation of organic matter to form ATP, b. energy expense of active transport, c. anabolic reactions utilize energy, d. maintenance energy expenditures, e. degradation of biomass via endogenous metabolism.

del Giorgio and Cole (2001)
Major energy consuming processes for growth

- Solute transport
- Maintenance
- Growth and reproduction
- Regulatory
We can estimate production, biomass, and growth…but what about the total carbon flux supporting bacterial growth?

• Total amount of carbon that supports bacterial growth includes carbon used for biomass synthesis and carbon metabolized.

• Total flux of carbon supporting bacterial growth or Bacterial Carbon Demand (BCD) equals:
  \[ \text{BCD} = \text{BP} + \text{Respiration} \]

• The bacterial growth efficiency (BGE) is the growth yield or the amount of biomass synthesized relative to total carbon required for growth.
  \[ = \text{BGE} = \frac{\text{BP}}{\text{BP} + \text{Respiration}} \]

If we can constrain BGE, then \( \frac{\text{BP}}{\text{BGE}} = \text{BCD} \)