Energy flow through microbial food webs

Bacterial growth and consumption of organic matter

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Bacterial growth and consumption of organic matter

1. Relevance of bacterial production
2. Basic understanding of the methods of choice
3. Ranges of BP and large-scale patterns (depth, season, ecosystem)
4. But, wait... how did we get to these numbers? The Leucine-to-Carbon CF
5. Why are so low?
   - leucine recycling
   - leucine respiration
   - leucine/TdR ratios
6. Energy and the bacterial cell- A bit of BGE ecology
7. Linkages between bacterial C processing, leucine respiration, LCF and BGE
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• ~1975: things smaller than 1 μm do most organic C respiration/mineralization in the ocean (e.g. Azam & Hodson 1977)

• ~1977-1980: Bacteria are shown to be very abundant (10^5-10^6 ml⁻¹)

• “...it is now recognized by most marine bacteriologists, and also by some oceanographers, that aerobic, heterotrophic bacteria make up a very large and dynamic component of the biomass in the euphotic layers of the coastal and open ocean”

  *Ducklow, H. W. 1983. Bioscience 33: 494-499*

• “... the (microbial loop) ideas did not get widespread acceptance until high abundance of bacteria and large bacterial production were shown to be general”

Different concepts

- **Activity**: exoenzymes, end-product evolution, MAR-FISH...
- **Cell Division**: cell number enumeration, DVC, FDC,...
- **Growth**: $^3$H-Thymidine, BrdU, cell number+size...
- **Production**: POC increase, $^3$H-Leucine...
- **Cell(biomass) turnover**: growth(production)/standing stock
- **C consumption**: POC/DOC disappearance, respiration +production,...

These processes result in ecosystem-level biogeochemical processes, gas exchanges between ocean and atmosphere, etc.

To determine the importance of microbes to ocean food webs, to nutrient and carbon cycling, we need to quantify at least some of these parameters.
Just to remember...

Figure 3 | Adaptive strategies of bacteria in the ocean. The adaptations that are shown are relevant to the structuring of marine ecosystems by bacteria at the nanometre-millimetre scale, but they also affect ocean-basin-scale processes, including the cycling of carbon, nitrogen and phosphorus and the biogeochemical behaviour of organic matter (for example, sinking). The strategies that are depicted here include motility, environmental sensing, permeases and cell-surface hydrolases. They enable coupling between bacteria and organic matter. For example, phytoplankton or cell debris, depicted as particulate organic matter (POM) and bacteria. Polymers also require hydrolysis to direct substrates before trans-membrane transport. Note the ability of bacteria to take up, as well as release, \( \text{NH}_4 \) and \( \text{PO}_4^- \).

More definitions...

- **BACTERIAL GROWTH EFFICIENCY (BGE)**
- **BACTERIAL CARBON CONSUMPTION (BCD)**

### FOOD WEB
- **PHYTOPLANKTON**
- **DOC**
- **POC**
- **BACTERIOPLANKTON**

### BIOMASS PRODUCTION (BP)
Carbon recycled to the MFW

### RESPIRATION (BR)
Carbon lost to the atmosphere

### Formulas:

- \[ BCD = BP + BR \]
- \[ BGE = \frac{BP}{BP + BR} \]

**When scaled to abundance:** Cell-specific resp./prod./C consum.

**When scaled to biomass:** C-specific r/p/c

\[ C\text{-sp } R = \mu \]
But also...

What is "bacterial production?"

The graph shows the growth phases of bacteria: Lag phase, Exponential phase, Stationary phase, and Death phase. The growth rate, \( \mu \), is defined as the change in number of bacteria per time, normalized by the number of bacteria, i.e., \( \frac{dN}{dt}/N \). This is approximately equal to [time\(^{-1}\)].

The maximum biomass per unit time, \( BP = \mu B \), has units of biomass per unit of time.


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Estimating bacterial production...

1.- Specific for bacteria ?
2.- Conversion factor, a function of SGR ?
3.- Manipulation, can it affect SGR ?
4.- Sensitive enough to allow short incubations ?

<table>
<thead>
<tr>
<th>Changes in #s</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP increase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FDC</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$^{35}$S-sulfate assim.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$^3$H-Ade inc. in RNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$^3$H-TdR inc. in DNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$^3$H-Leu inc. in protein</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Methods for measuring bacterial activity

- Bulk community activity (community growth)
  - change in cell numbers (w/o predators)
  - increase in ATP, lipopolysaccharide, muramic acid...
  - FDC (Hagström et al. 1979)
  - dark $^{14}C$ uptake (Sorokin 1961)
  - $^{35}S$-sulfate incorporation (Monheimer 1972/1974)
  - $^{3}H$-adenine (Karl 1979)
  - $^{3}H$-thymidine (Fuhrman & Azam 1980/1982)
  - $^{3}H$/$^{14}C$-leucine (Kirchman et al 1985)
  - BrdU immunocapture (Steward & Azam 1999)

13/27 years w/o new methods added to our toolbox

Does that mean we should be happy ??
Estimating bacterial production...

a) Growth without predators

Ivanov 1955
Sieburth et al. 1977

< 1 µm / inhibitors
unfiltered

NO₃, PO₄...

b) Incorporation of radioactive tracers

Fuhrman & Azam 1980
Karl et al. 1981
Kirchman et al. 1985

FC: carbon or cells per mol of TdR or Leu
Radioactive tracing...

Thymidine: DNA precursor
- difficult to measure intracellular pools
- sometimes catabolized
- usually $^3$H

Leucine: aminoacid, protein building block
- difficult to measure isotope dilution
- some algae uses it
- can be $^3$H or $^{14}$C

• TdR 10% less sensitive

• TdR measures growth, Leu measures production
Procedure scheme

- Take sample
- Add rad. substrate at saturating level.
- Incubate at in situ temperature. In the dark**
- Process.
  - filter on 0.2 µm filter
  - centrifugate in eppendorf
  - (rinse with ethanol)
  - precipitate with TCA
- Count radioactivity by liqui scintillation (dpms)
- Convert dpms into pmols Leu
- Convert pmols Leu into µgC
What happens to the added label?

- Diluted with external pool
- Diluted with internal pool
- Incorporated nonspecifically (to e.g. lipids, proteins)
- Recycled
- Respired / degraded / metabolized

Simon & Rosenstock 1992, L&O.
What happens to the added label?

- Is leucine diluted with outside leucine?
  free leucine is commonly <5 nM
  leu is ~5% (often < 1%) of all DFAA, which are 40-150 nM
  leucine is “expensive” to be synthesized de novo


- Is leucine recycled during experiment?

ship. We checked effects of ethanol rinse and BSA addition in our protocol, because in most published studies BSA is not added and ethanol rinse is often used to remove unspecific ³H labelling (Wicks and Robarts, 1998; Ducklow et al., 2002; Kirchman et al., 2005) although sometimes ethanol rinse did not change the results (Van Wambekke et al., 2002; Granéli et al., 2004). There was no significant difference among the different treatments (+ or − ethanol, + or − BSA added, data not shown). As we also man-
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Vertical patterns of bacterial “production”
Vertical patterns of oceanic bacterial “production”
Longitudinal patterns of oceanic bacterial “production”
## Ecosystem patterns of oceanic BP

<table>
<thead>
<tr>
<th></th>
<th>BB</th>
<th>PB</th>
<th>B/P bm</th>
<th>BP</th>
<th>PP</th>
<th>B/P prod</th>
<th>B µ</th>
<th>P µ</th>
<th>B/P µ</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Atlantic</td>
<td>1000</td>
<td>4500</td>
<td>0.11</td>
<td>275</td>
<td>1083</td>
<td>0.25</td>
<td>0.30</td>
<td>0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>Eq. Pacific</td>
<td>1300</td>
<td>1800</td>
<td>0.72</td>
<td>230</td>
<td>1200</td>
<td>0.19</td>
<td>0.12</td>
<td>0.70</td>
<td>0.2</td>
</tr>
<tr>
<td>Subarctic Pac.</td>
<td>1140</td>
<td>1270</td>
<td>0.90</td>
<td>56</td>
<td>629</td>
<td>0.09</td>
<td>0.05</td>
<td>0.50</td>
<td>0.1</td>
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<tr>
<td>Arabian Sea</td>
<td>1440</td>
<td>1240</td>
<td>1.20</td>
<td>257</td>
<td>1165</td>
<td>0.22</td>
<td>0.18</td>
<td>0.93</td>
<td>0.2</td>
</tr>
<tr>
<td>Hawaii</td>
<td>1500</td>
<td>447</td>
<td>3.60</td>
<td>106</td>
<td>486</td>
<td>0.22</td>
<td>0.14</td>
<td>1.10</td>
<td>0.1</td>
</tr>
<tr>
<td>Bermuda</td>
<td>1317</td>
<td>573</td>
<td>2.70</td>
<td>70</td>
<td>465</td>
<td>0.15</td>
<td>0.05</td>
<td>0.81</td>
<td>0.1</td>
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<tr>
<td>Ross Sea</td>
<td>217</td>
<td>11450</td>
<td>0.02</td>
<td>55</td>
<td>1248</td>
<td>0.04</td>
<td>0.25</td>
<td>0.11</td>
<td>2.3</td>
</tr>
<tr>
<td>NW Med Sea</td>
<td>420</td>
<td>585</td>
<td>0.75</td>
<td>48</td>
<td>330</td>
<td>0.25</td>
<td>0.12</td>
<td>0.55</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>median=</strong></td>
<td></td>
<td></td>
<td></td>
<td>83%</td>
<td>21%</td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
</tbody>
</table>

Ducklow 1999, FEMS ME & Pedrós-Alió et al. 1999, DSRI
Small temporal and spatial variability in bacterial “production”
Seasonal variability in bacterial "production"
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Converting incorporation into production

Leucine incorporation into bacterial protein: fast, easy, direct, sensitive, universal...

Theoretical (Semiempirical) Conversion Factor
Empirical

pmol Leu [time]⁻¹ [vol]⁻¹       \(\rightarrow\)       \(\mu g C [time]⁻¹ [vol]⁻¹\)

“The problem of picking the correct conversion factor is the difficult part of using either leucine or thymidine incorporation as a measure of bacterial production” (Kirchman 2001)

**Theoretical LCF:** Based on cell leu and protein average content and isotope dilution
3.10 kgC mol leu⁻¹  2x dilution
1.55 kgC mol leu⁻¹  no dilution

**Empirical LCF Principle:** Bacteria are allowed to grow in a water sample on naturally occurring substrates and biomass and leucine incorporation simultaneously registered and compared.
Converting incorporation into production

- dpms to Leucine incorporation rates
- Leucine incorporation rates to Carbon production

\[
\text{Prod (} \mu gC \text{ l}^{-1} \text{ h}^{-1}) = \text{Leu (} \mu \text{mol} \text{ l}^{-1} \text{ h}^{-1}) \times C/F 2.2 \times (\%\text{Leu})^{-1} \times (C/\text{protein}) \times \text{ID}
\]

3.1 kgC mol Leucine\(^{-1}\)

Simon & Azam, 1989 MEPS best estimates
**The Empirical Leucine-to-Carbon CF**

**Principle:** Bacteria are allowed to grow in a water sample on naturally occurring substrates (or in combinations with nutrient enrichments), and biomass and leucine incorporation simultaneously registered and later compared.

We need growth to occur. Thus, we commonly

- dilute the sample with < 0.2 µm water,
- filter through 0.6 / 0.8 µm to reduce predator relevance

**Advantages (Kirchman 1993)**

- they are calculated with natural bacterial assemblages
- the factors are calculated for the system under study
- the factors “correct” errors in the method

**Disadvantages**

- physiological information on macromolecular synthesis is ignored
- growth rates not affected by the dilution ?
- natural bacterial assemblages ?
- filtration artifacts ?
- time consuming
- can’t be measured at a rate similar to the rate of LIR measurement
The Empirical Leucine-to-Carbon CF

- Fuhrman paper ca. 1000, Kirchman paper(s) ca. 700 citations
- Literature review 1986 - 2010
- 348 papers with BP data of natural marine ecosystems
- 267 Leu (77%), 81 TdR
- 290 (83%) made inferences about C processing by bacteria

- 56 did measure ECF (19%)
- 32 another study ECF (11%)
- 259 did not measure ECF (89%)
1.55 kgC mol leucine\(^{-1}\)

3.1 kgC mol leucine\(^{-1}\)

119 literature estimates
135 own estimates
### The Empirical Leucine-to-Carbon CF

**Patterns in offshore - inshore gradients**

<table>
<thead>
<tr>
<th>Site</th>
<th>Position</th>
<th>CF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE Pacific</td>
<td>midshelf</td>
<td>2.40</td>
<td>Sherr et al. 2001-AME</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>offshore</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>NE Pacific</td>
<td>midshelf</td>
<td>2.11</td>
<td>del Giorgio et al. 2012-L&amp;O</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>offshore</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Mediterranean Sea</td>
<td>midshelf</td>
<td>2.00</td>
<td>Pedrós-Alió et al. 1999-DSR2</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>offshore</td>
<td>0.36</td>
<td></td>
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<tr>
<td>NE Atlantic</td>
<td>estuary</td>
<td>3.55</td>
<td>Morán et al. 2002-AME</td>
</tr>
<tr>
<td></td>
<td>midshelf</td>
<td>1.45</td>
<td></td>
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<td></td>
<td>slope</td>
<td>1.03</td>
<td></td>
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<tr>
<td></td>
<td>offshore</td>
<td>0.65</td>
<td></td>
</tr>
</tbody>
</table>
The Empirical Leucine-to-Carbon CF

Patterns with site depth

[Graph showing data points on a scatter plot comparing Carbon-to-leucine Conversion Factor (kgC mol Leu⁻¹) against Maximal depth (m) with a title "All data"]
The Empirical Leucine-to-Carbon CF

Patterns with temperature

Carbon-to-leucine Conversion Factor (kgC mol Leu⁻¹) vs Temperature

All data

Gasol & Pedrós-Alió, unpubs.

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The Empirical Leucine-to-Carbon CF

Patterns with system richness

Experiment Mesomed'98

Nutrient supply (µM Nitrate) vs. Carbon-to-leucine Conversion Factor (kgC mol leucine⁻¹)

Chlorophyll a (mg m⁻³) vs. Carbon-to-leucine Conversion Factor (kgC mol leucine⁻¹)

\[ y = 1.19 + 0.48 \log(x) \quad R^2 = 0.99 \]

\[ y = 1.10 + 0.48 \log(x) \quad R^2 = 0.92 \]
Should we expect a relationship between the LCF and the Leu:TdR ratio?
The Empirical Leucine-to-Carbon CF

- In general oceanic LCFs are <<<< theoretical CF
- Patterns with water depth, temperature, nutrients,...
- Lower LeuCFs are more dissimilar to TdRCFs than high LeuCFs
- Mesopelagic LeuCFs are not very different from surface CFs (Baltar et al. 2010-AME, Gasol et al. 2009-PiO)
- So, what happens with Leucine incorporation, then?
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Why such small Leu-to-C CFs?

- literature patterns in the LCF
- why are the LCF so low?
  - protein recycling
  - leucine respiration
- why do bacteria respires the leucine?

Average oceanic LCF < 1

if bacteria are turning over their newly synthesized protein at the time scale of the analysis, we would detect a decrease in the label accumulated: the label would be diluted (i.e. less label than it should)
Is leucine recycled?

Approach: cold chase

Measured in 10 stns: non significant turnover (ave. 2% per h)
Note viruses ¿?
Why such small Leu-to-C CFs?

- literature patterns in the LCF
- why are the LCF so low?
  - protein recycling
  - leucine respiration
- why do bacteria respire the leucine?

Average oceanic LCF < 1
Not!

if bacteria are sometimes respiring the added leucine, there would be a decoupling between uptake (what we really measure) and incorporation (assimilation into cell proteins). A variable part of the uptake would be respired, and not assimilated.
Measuring leucine respiration

1. Sample
2. $^{14}$C-leucine
3. Acidify ($H_2SO_4$)
4. Add PEA (2-phenylethylamine)

$^{14}$C-leucine to $^{14}$CO$_2$

Filter paper

Hobbie & Crawford 1969, L&O

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Wecoma cruise 2002 NE Pacific

Coca cruise 2003 NE Atlantic

Blanes Bay Microbial Observatory 2003-2004 NW Mediterranean

Alonso-Sáez et al. 2007, L&O
del Giorgio et al. 2011, L&O
Measuring leucine respiration

Is leucine respiration (at the time-scale of the analyses) significant?

LeuResp = 5.01*LeuInc^{0.44}, r^2 = 0.63
Measuring leucine respiration

less active communities respire most of the added leucine!
TdR vs. Leucine incorporation

Gasol & del Giorgio 2000, SciMar

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TdR vs. Leucine incorporation

- Wecoma cruise
- Coca cruise
- Blanes Bay
Bacteria respire the leucine because they are “stressed” (energy-stressed), the media is “hostile” and need energy. So, no matter how nice is to keep the added Leu for growth, bacteria must burn it.
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BGE captures the energetic status of a community

Catabolic and anabolic pathways that influence growth efficiency in aquatic bacteria:

a. Rate of ATP production from the oxidation of organic compounds
b. Energetic cost of active transport of substrates
c. ATP utilization for biosynthesis and growth
d. ATP utilization for maintenance
e. ATP production from endogenous metabolism
Uncoupling between BP and BR in incubations

del Giorgio et al. in prep
...but large scale covariation

\[ BR = 2.61 \times BP^{0.60} \]

\[ r^2 = 0.50 \quad n = 831 \]

*del Giorgio et al. in prep*
BGE increases with system productivity

del Giorgio & Cole 2000 in Kirchman (ed)
BGE varies according to ecosystem type

del Giorgio et al. in prep
The nature and source of DOC influences BGE

The nature and source of DOC influences BGE


Wednesday, May 30, 2012
Evidence for energy limitation of bacteria

\[ \mu (h^{-1}) \]

Spec substrate consumption

\[ m_e (\mu = 0.0 \ h^{-1}) \]

\[ m (\mu) \]

\[ e (\mu) \]

Death

Dormancy

Growth rate \( \mu \) (h\(^{-1}\))
BGE and hostility of the environment

Carlson et al. 2007, Oceanography
A few concepts on BGE

- Bacterial carbon consumption (BCC) can be estimated from the sum of BP and BR or from ΔPOC and ΔDOC in bacteria-only SW cultures.
- For every BR measurement, there to 100 to 500 measurements of BP...
- Most estimates of BCC are based on BP measurements and on assumptions concerning BGE... not good!
- BGE tend to be low (< 15%) in unproductive, open ocean areas, and increase with system productivity
- Marine bacteria quickly react to changes in the resource environment by shifting the catabolic/anabolic pathways. BGE is thus very variable, and this variability is a reflection of the metabolic versatility of marine bacteria
- Low BGE imply high respiration rates for any given level of BP
- Total bacterial carbon consumption (BP+BR) accounts for 70% to over 150% of primary production in oligotrophic areas with low BGE
- This may be evidence of net ecosystem heterotrophy
- BGE can be used to estimate the “hostility” of the environment, and reflects the energetic level of the communities
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Observation: LCF in oceanic waters are low, very low
Observation: This is not due to protein recycling, but to leucine respiration

Why is the added leucine resired?  because bacteria are energy-limited

Thus, the LCF (and the respiration of the leucine) should be related to other indicators of physiological status

- bulk BGE
- growth rates
- the ratio of leucine uptake to thymidine uptake (Leu:TdR)

A test in 2 oceanic cruises (NE Pacific, Wecoma, NE Atlantic, Coca) + one coastal station (Blanes Bay Microbial Observatory)
Patterns of C metabolism (BR)

A slight tendency for more respiration in the coastal stations
Patterns of C metabolism (BP)

No clear trend for BP (using standard CF)
Patterns of C metabolism (BGR)

BGE quite variable, slight tendency to decrease towards offshore NOT predictable from Temp
More Leu Resp in offshore waters (at least in surface)

Patterns of C metabolism (Leu resp)

Gasol et al., submitted Wednesday, May 30, 2012
Distance to coast: the best predictor of LCF (not CHL, nor Temp)
Patterns of C metabolism (BP) w/o and w EmpCF

LCF: 1.55 kgC mol Leu$^{-1}$

Distance from the coast (km)

Bacterial Production ($\mu$gC l$^{-1}$ d$^{-1}$)

Gasol et al., submitted

Wednesday, May 30, 2012
Patterns of C metabolism
(ratio Leu:TdR)
Leucine-to-Carbon conversion Factor (KgC mol Leu$^{-1}$)

Log $L_T = 1.24 - 0.332 \text{LCF}$, $R^2 = 0.57$

The LCF has to do with the physiology of the bacterial community

Higher LCF $\approx$ lower Leu:TdR

The LCF has to do with the physiology of the bacterial community
is the LCF related to other estimates of the way in which bacteria process C?
in particular, are LCF a reflection of BGE?
are an indication of the amount of leucine respired and not incorporated?

\[
\log \text{LCF} = 0.79 + 1.28 \log \text{BGE}
\]
N= 25, P <0.001, \( r^2 = 0.48 \)

Matrix of pairwise correlations

<table>
<thead>
<tr>
<th></th>
<th>CHL</th>
<th>Emp CF</th>
<th>L:TdR</th>
<th>GR (d-1)</th>
<th>Total Leu inc-</th>
<th>Leu Resp</th>
<th>% Leu Resp</th>
<th>BGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHL</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td>0.82</td>
<td>0.77</td>
<td>-0.29</td>
<td></td>
</tr>
<tr>
<td>Emp CF</td>
<td>0.42</td>
<td>-0.38</td>
<td>0.49</td>
<td></td>
<td>0.55</td>
<td>-0.46</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>L:TdR</td>
<td></td>
<td>-0.38</td>
<td>-0.70</td>
<td>-0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR (d-1)</td>
<td></td>
<td></td>
<td>-0.70</td>
<td></td>
<td>-0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Leu inc-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.82</td>
<td>-0.39</td>
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<td></td>
<td></td>
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</tbody>
</table>
Bacterial growth and consumption of organic matter

1.- Relevance of bacterial production
2.- Basic understanding of the methods of choice
3.- Ranges of BP and large-scale patterns (depth, season, ecosystem)
4.- But, wait... how did we get to these numbers? The Leucine-to-Carbon CF
5.- Why are so low?
   - leucine recycling
   - leucine respiration
   - leucine/TdR ratios
7.- Energy and the bacterial cell- A bit of BGE ecology
8.- Linkages between bacterial C processing, leucine respiration, LCF and BGE
9.- Conclusion: The varying scales of microbial C consumption and their linkages

(10.- The ecological vs. filogenetic determination of CFs)
### Different processes operating at different scales

<table>
<thead>
<tr>
<th>Instantaneous</th>
<th>min-to-hr</th>
<th>hr-to-days</th>
<th>&gt;days</th>
</tr>
</thead>
</table>

- **Uptake**
  - Outside
  - Inside

- **Respired**
  - Incorporation
  - Assimilated

- **Respired catabolism**

- **Growth**

**Note:**

- ATP content
- Macron...
Conclusions

- Evidence that BGE is rather variable (more than predicted by some models)
- Open ocean LCFs are $\ll 1 \text{ kgC mol}^{-1}$
- LCF varies with distance to coast and availability of limiting nutrients
- The LCF is well related to the Leu:TdR ratio and to BGE
- The LCF, BGE, %LeuResp all reflects the in situ physiological state of the assemblage, and the basic physiological responses of bacteria to energy limitation
  - A large part of the variability in LCF is ecological.
- The coast-to-land pattern might have to do with the phylogenetic composition of the bacterial assemblage)

... there is physiologically-relevant information in the LCFs that can be exploited
... there is potential for finding surrogates of BGE and LCFs that might allow us to measure these variables at a rate similar to the rate of measurement of LIR.
Bacterial growth and consumption of organic matter

1. Relevance of bacterial production
2. Basic understanding of the methods of choice
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7. Linkages between bacterial C processing, leucine respiration, LCF and BGE
8. Conclusion: The varying scales of microbial C consumption and their linkages
(10.- The ecological vs. filogenetic determination of CFs)
A CF experiment in the Pacific
Biases in LeuCF experiments

Bacteria are allowed to use the existing DOC pool (DOC production and consumption processes are uncoupled), which might not be representative of the molecules that support in situ production on short time scales, and might bias growth estimates.

“In dilution experiments, uptake doesn’t necessarily mean growth !!! And even biosynthesis might not mean growth: at the start of starvation protein synthesis is detected at the same time than cells become smaller and smaller” (Güde 1990)

Have the factors obtained in those experiments any ecological sense ?

do they represent phylotype-specific properties or truly ecologically properties ?
Example 1 - Antarctica

Bacterial diversity changes during incubations

Massana et al. 2001, L&O.
Incubations during cruise
DCHARMA

Massana et al. 2001, L&O.

Wednesday, May 30, 2012
Conversion factors from the Dharma cruise experiments

The same bacterial assemblage is selected in each experiment, but the factor does vary systematically with temperature and enrichment.
### Example 2 - Blanes Bay

<table>
<thead>
<tr>
<th>eCF</th>
<th>Unamm</th>
<th>Amm</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Aug</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Sep</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Dec</td>
<td>3.6</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Gamma-*

*Bacteroidetes*

*Alfa-*

*Alonso-Sáez et al. 2010, ENM.*
With thanks to:

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XAG Morán

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I Lekunberri
E Vázquez-Domínguez
R Massana
R Simó

• C-More course organizers
• You (for listening)
• and all the poor little bugs for helpful cooperation…