Energy flow through microbial food webs

Bacterial growth and consumption of organic matter

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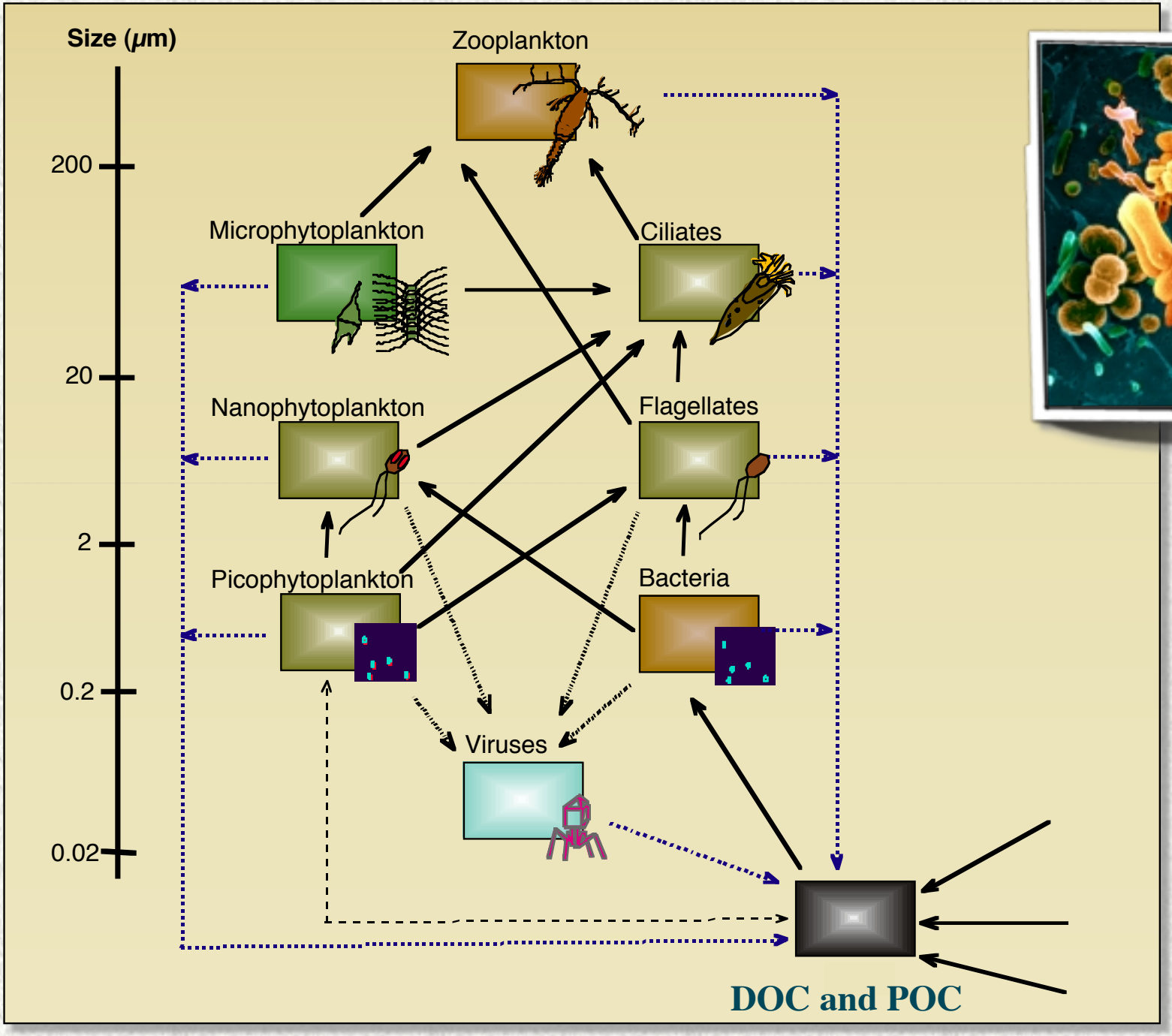
Size (μm)
200
20
2
0.2
0.02
Zooplankton
Microphytoplankton
Ciliates
Nanophytoplankton
Flagellates
Picophytoplankton
Bacteria
Viruses
DOC and POC

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
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Bacterial growth and consumption of organic matter

- ~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"

  Fuhrman, J.A. 1992. In: Falkowski, P.G. & A.D.Woodhead (eds). Primary productivity and biogeochemical cycles in the sea

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...

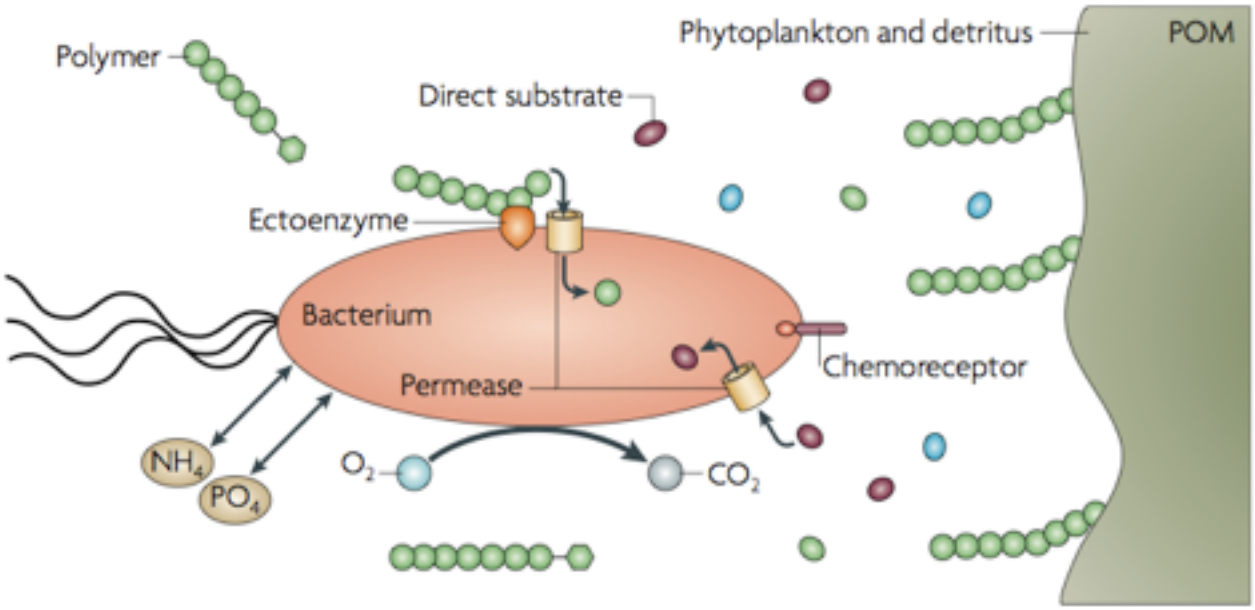
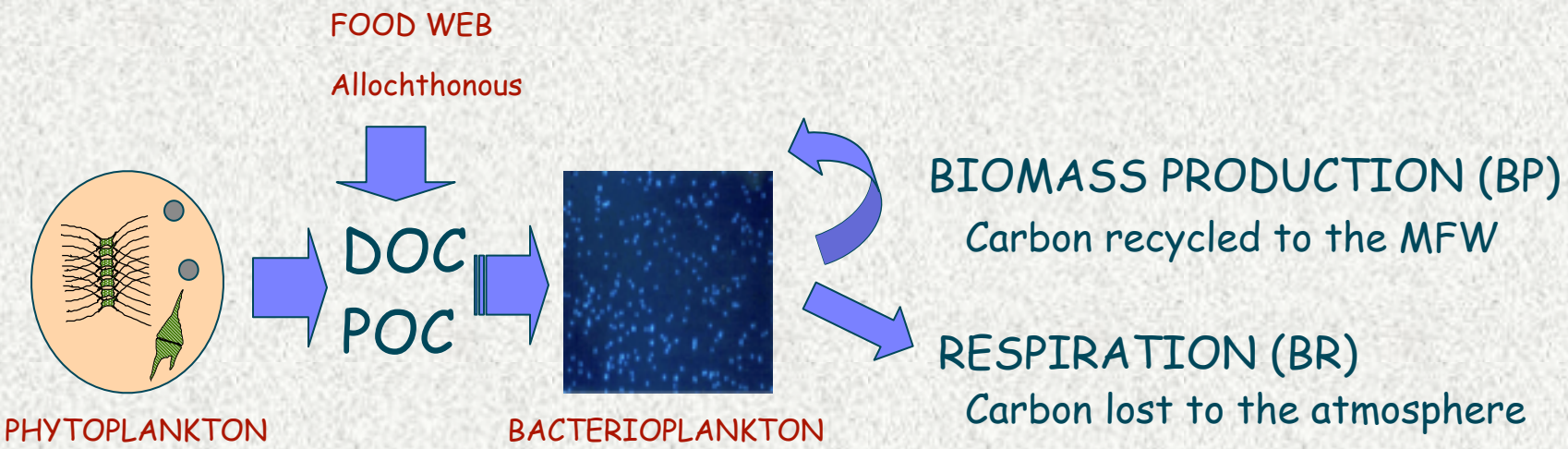


Figure 3 | **Adaptive strategies of bacteria in the ocean.** The adaptions that are shown are relevant to the structuring of marine ecosystems by bacteria at the nanometre–millimetre scale, but they also affect ocean-basin-scale processes, including the cycling of carbon, nitrogen and phosphorus and 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, NH_4 and PO_4.

Azam & Malafatti 2007, Nat. Rev. Microb.

More definitions...



$$BCD = BP + BR$$

BACTERIAL CARBON CONSUMPTION
Bacterial gross production

$$BGE = \frac{BP}{BP + BR}$$

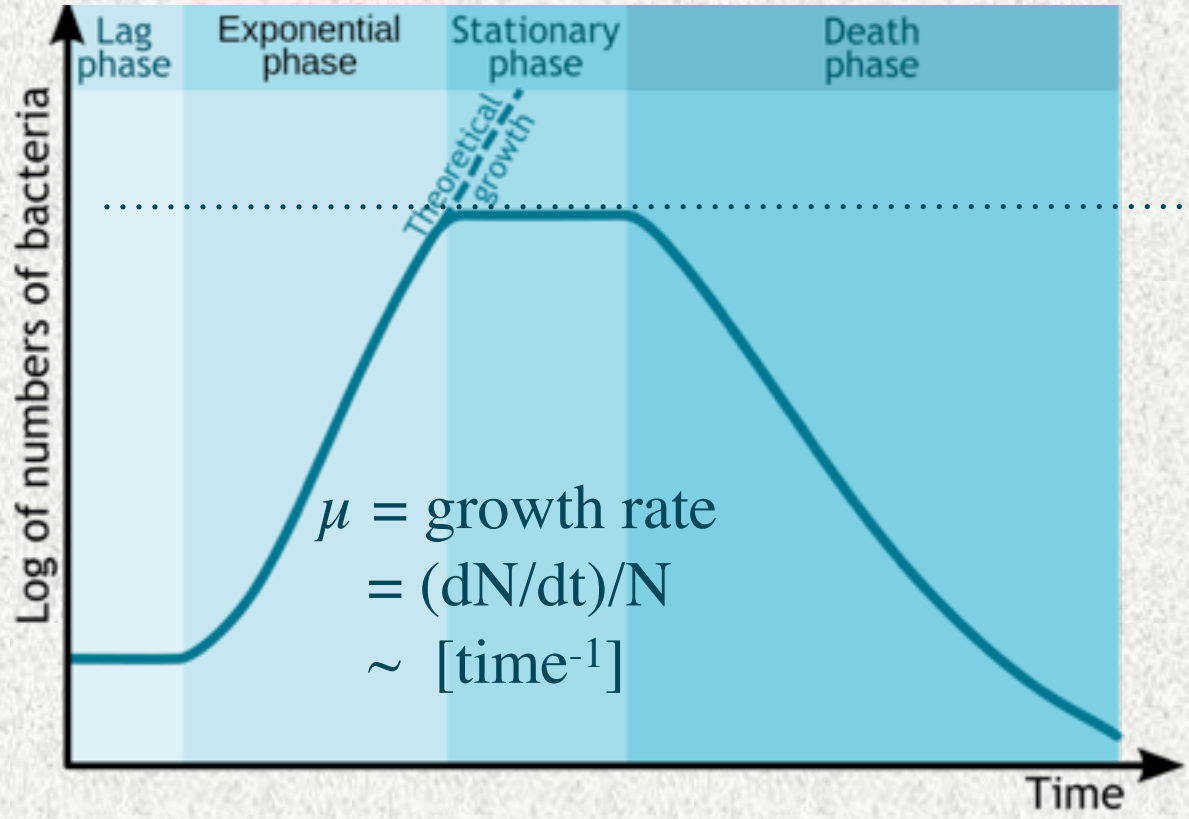
BACTERIAL GROWTH EFFICIENCY

When scaled to abundace — Cell-specific resp./prod./C consum.

When scaled to biomass — C-specific r/p/c — C-sp R = μ

But also...

What is "bacterial production?"



Max. allowed by resources

source Wikipedia

BP = μB 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 ?

	1	2	3	4
Changes in #s	++	++	-	-
ATP increase	-	+	-	+
FDC	++	-	++	++
^{35}S-sulfate assim.	--	+	+	+
^{3}H-Ade inc. in RNA	--	--	+	++
^{3}H-TdR inc. in DNA	++	-	+	++
^{3}H-Leu inc. in protein	+	-	+	++

Azam & Fuhrman 1984

Methods for measuring bacterial activity

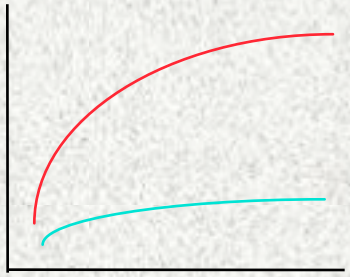
- Bulk community activity (community growth)
  - change in cell numbers (w/o predators)
  - increase in ATP, lipopolysacharide, muramic acid...
  - FDC (Hagström et al. 1979)
  - dark ^{14}C uptake (Sorokin 1961)
  - ^{35}S-sulfate incorporation (Monheimer 1972/1974)
  - ^{3}H-adenine (Karl 1979)
  - ^{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

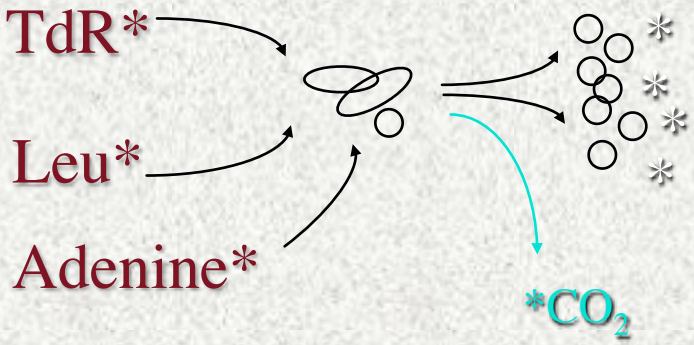
unfiltered

NO_3, PO_4...

FC: carbon per cell

b) Incorporation of radioactive tracers

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



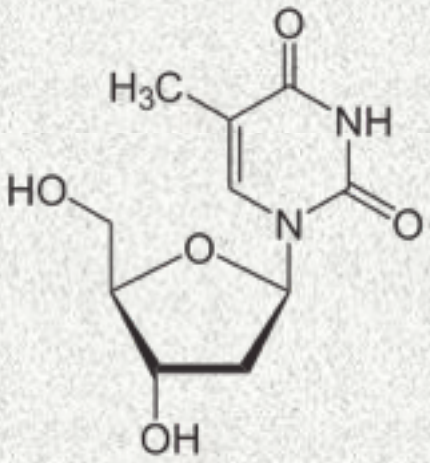
DNA*

protein*

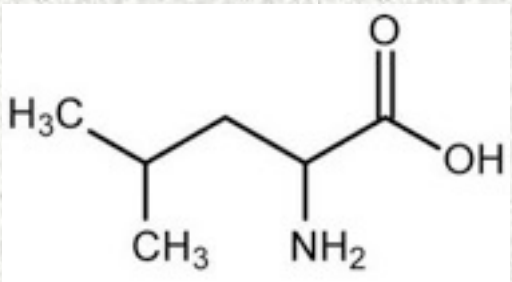
RNA*

FC: carbon or cells
per mol of TdR or Leu

Radioactive tracing...



Thymidine: DNA precursor
- difficult to measure intracell. pools
- sometimes catabolized
- usually ^{3}H



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

- TdR 10% less sensitive

- TdR measures growth, Leu meaures 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

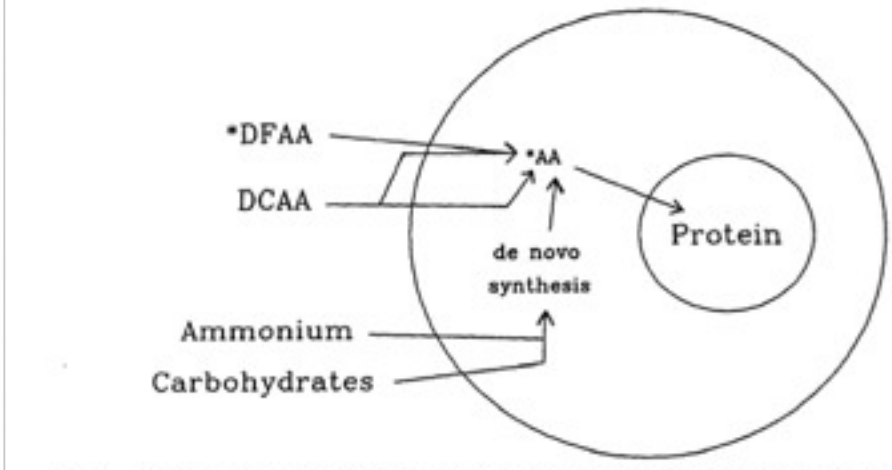


Fig. 1. Diagram for isotope dilution (ID) and the major precursors of amino acids in the intracellular pool. Outside the large circle is the extracellular environment of a bacterium and inside is its intracellular pool and the protein fraction. The specific activity (SA) of radiolabeled free amino acids in the intracellular pool (*AA), which are taken up as dissolved free amino acids (*DFAA), can be diluted by nonlabeled amino acids entering the pool via hydrolized dissolved combined amino acids (DCAA) or de novo synthesis from NH_4^+ and a carbon source like carbohydrates. ID = SA(*DFAA)/SA(*AA).

Simon & Rosenstock 1992, L&O.

What happens to the added label?

- Is leucine diluted with outside leucine?
  free leucine is commony <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 incorporated into lipids/nucleic acids? <10%, Servais 1995, Simon 1999)

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 ^{3}H labelling (Wicks and Robarts, 1998; Ducklow et al., 2002; Kirchman et al., 2005) although sometimes ethanol rinse did not change the results (Van Wambeke 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-

- Is leucine recycled during experiment?

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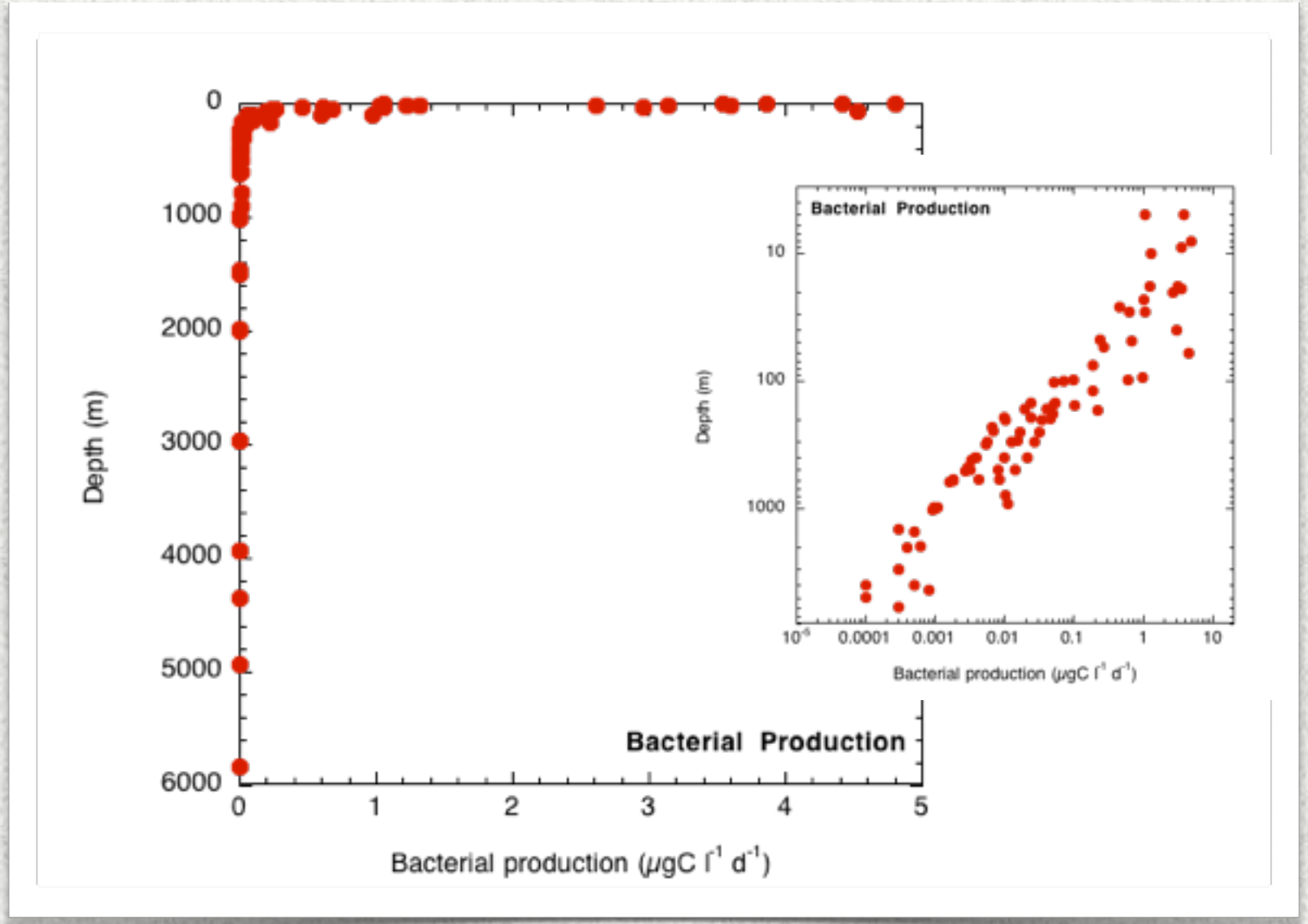
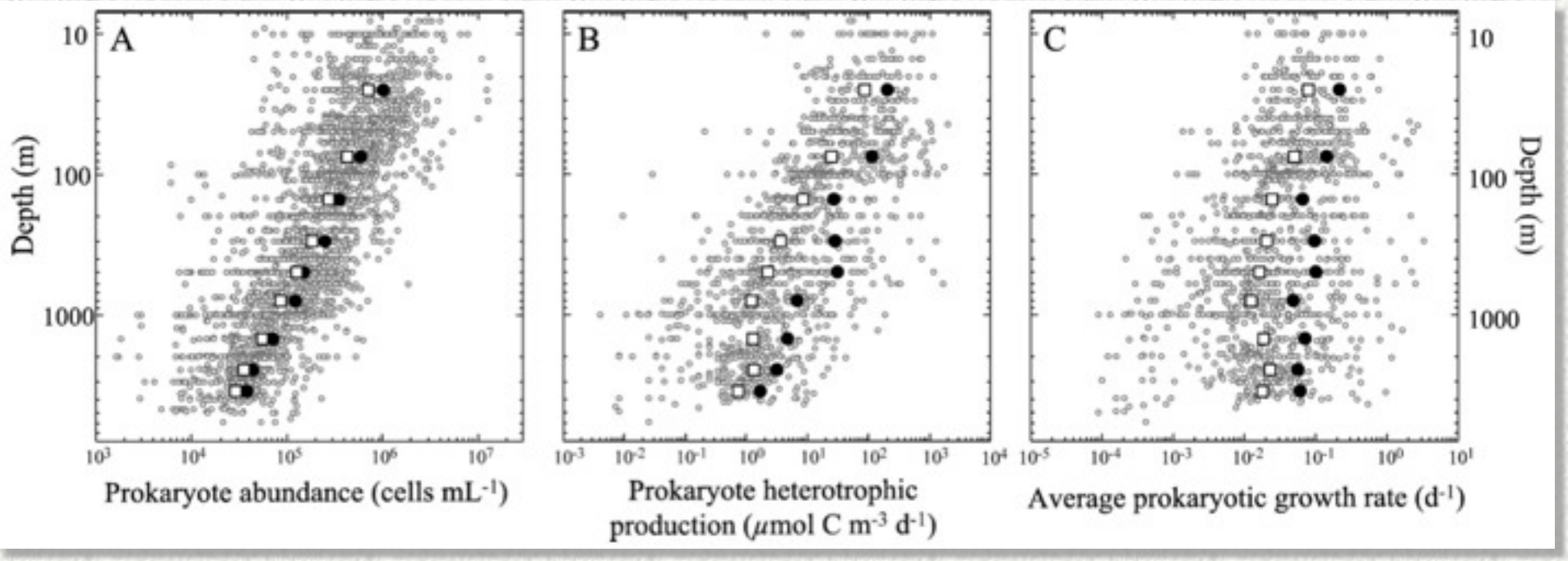
Vertical patterns of bacterial "production"

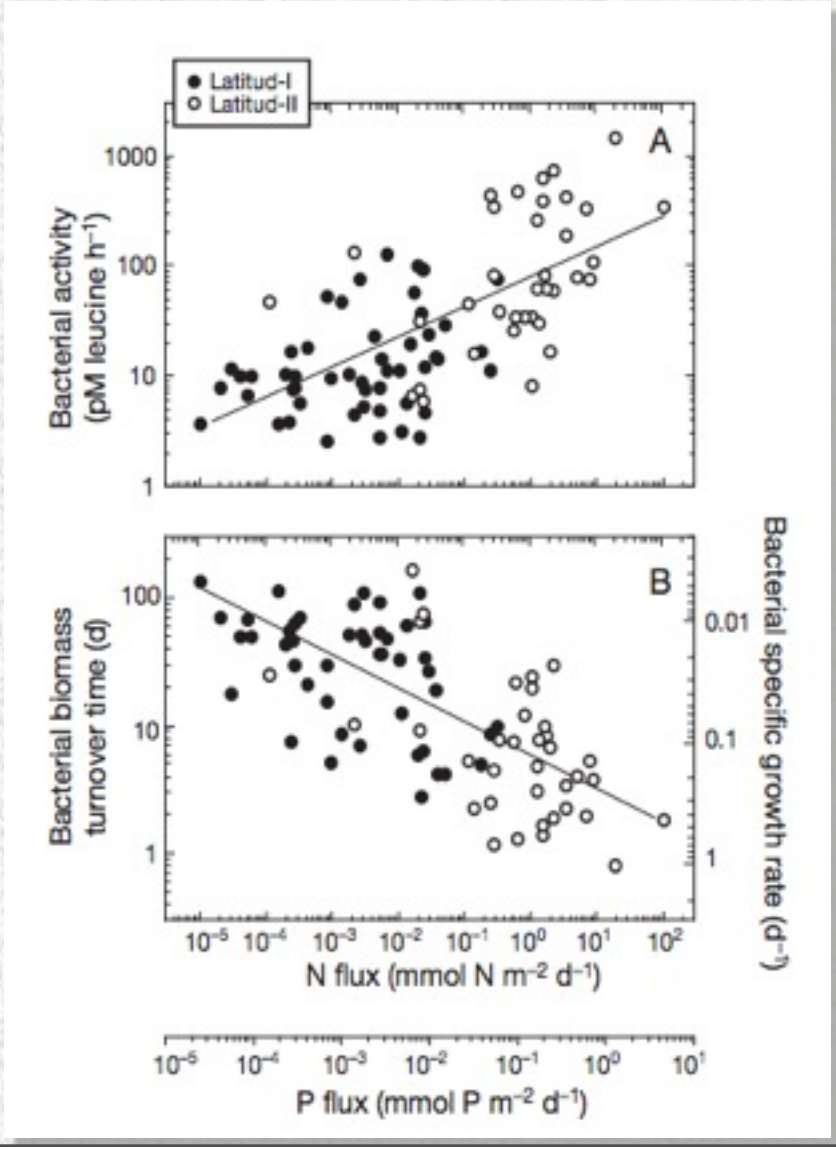


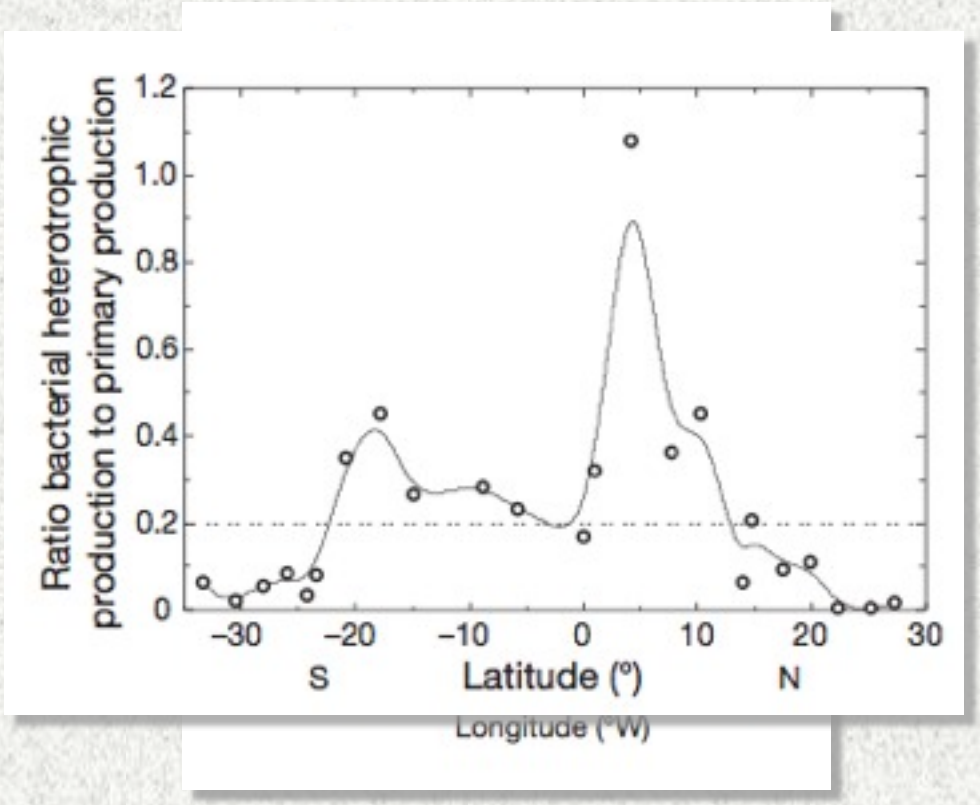
Vertical patterns of oceanic bacterial "production"



Arístegui et al. 2009, L&O.

Longitudinal patterns of oceanic bacterial "production"





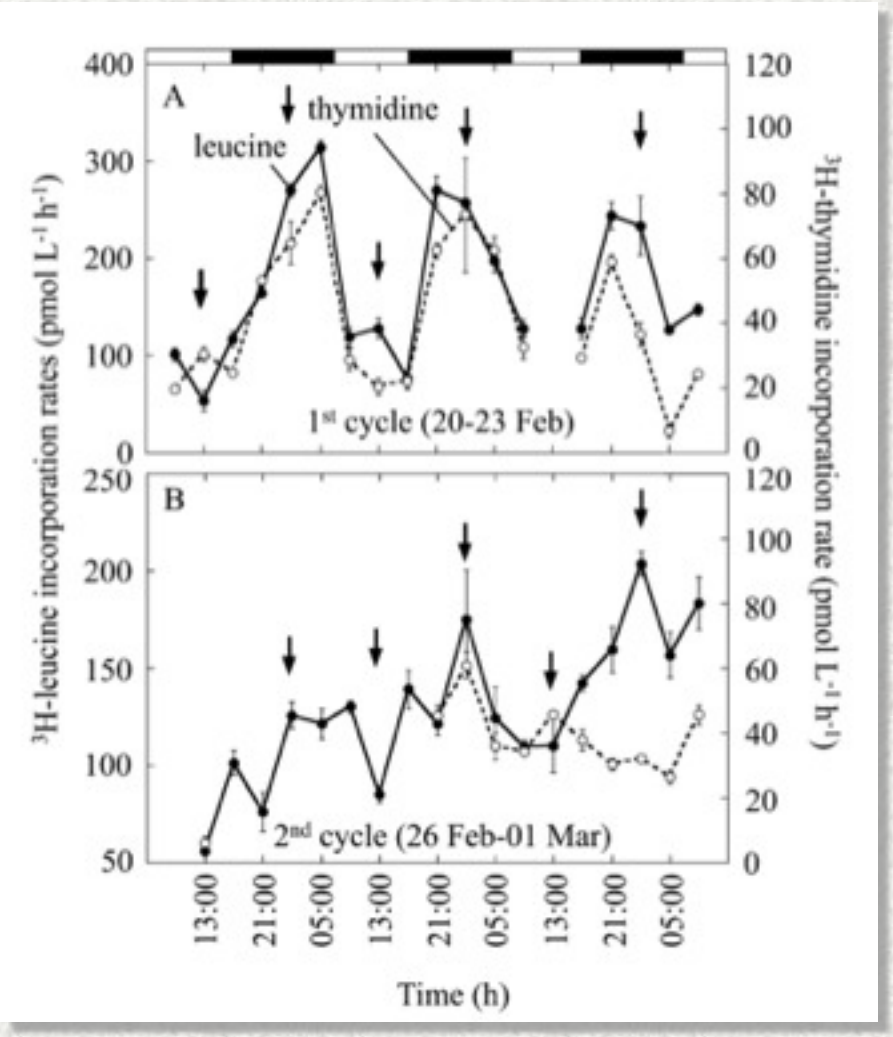
Gasol et al. 2009, AME

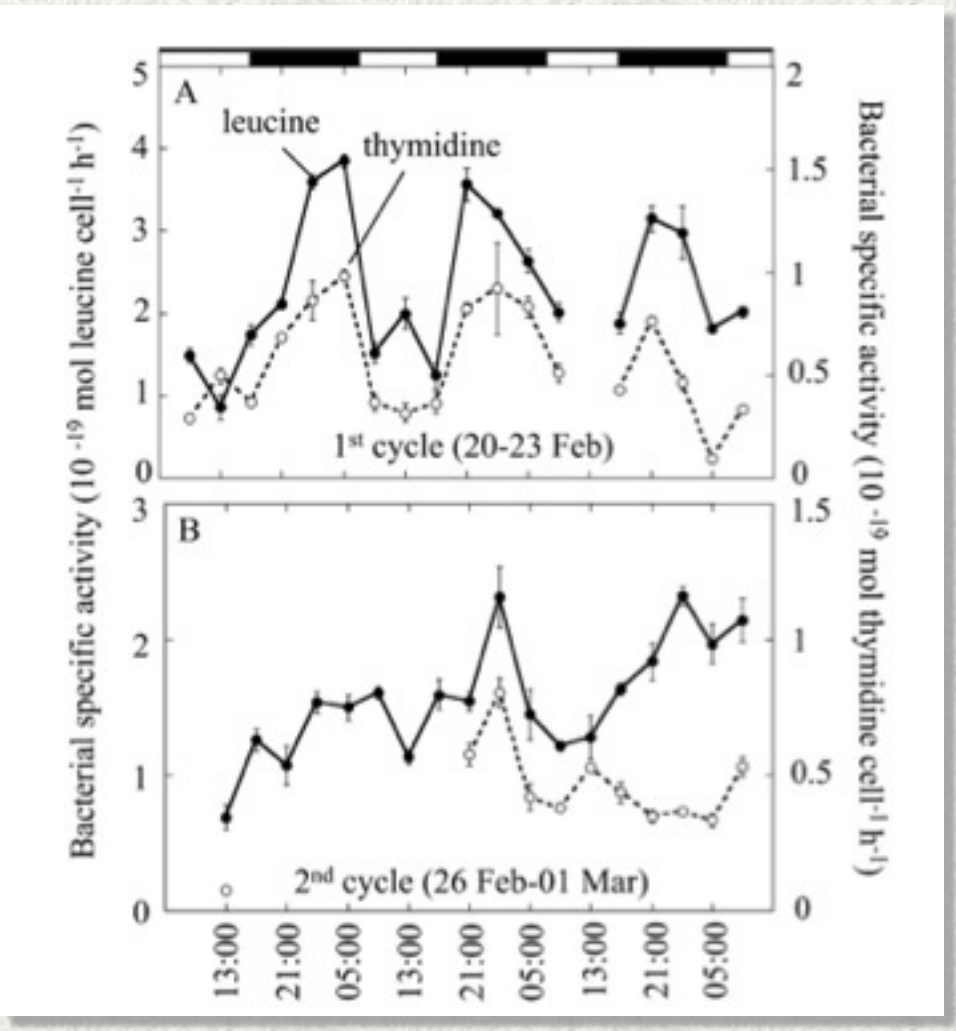
Ecosystem patterns of oceanic BP

	BB	PB	B/P bm	BP	PP	B/P prod	B μ	P μ	B/P μ
N. Atlantic	1000	4500	0.11	275	1083	0.25	0.30	0.30	1.0
Eq. Pacific	1300	1800	0.72	230	1200	0.19	0.12	0.70	0.2
Subarctic Pac.	1140	1270	0.90	56	629	0.09	0.05	0.50	0.1
Arabian Sea	1440	1240	1.20	257	1165	0.22	0.18	0.93	0.2
Hawaii	1500	447	3.60	106	486	0.22	0.14	1.10	0.1
Bermuda	1317	573	2.70	70	465	0.15	0.05	0.81	0.1
Ross Sea	217	11450	0.02	55	1248	0.04	0.25	0.11	2.3
NW Med Sea	420	585	0.75	48	330	0.25	0.12	0.55	0.2
median=			83%			21%			0.2
	mgC m^{-2}			mgC m^{-2} d^{-1}			d^{-1}		

Ducklow 1999, FEMS ME & Pedrós-Alió et al. 1999, DSRI

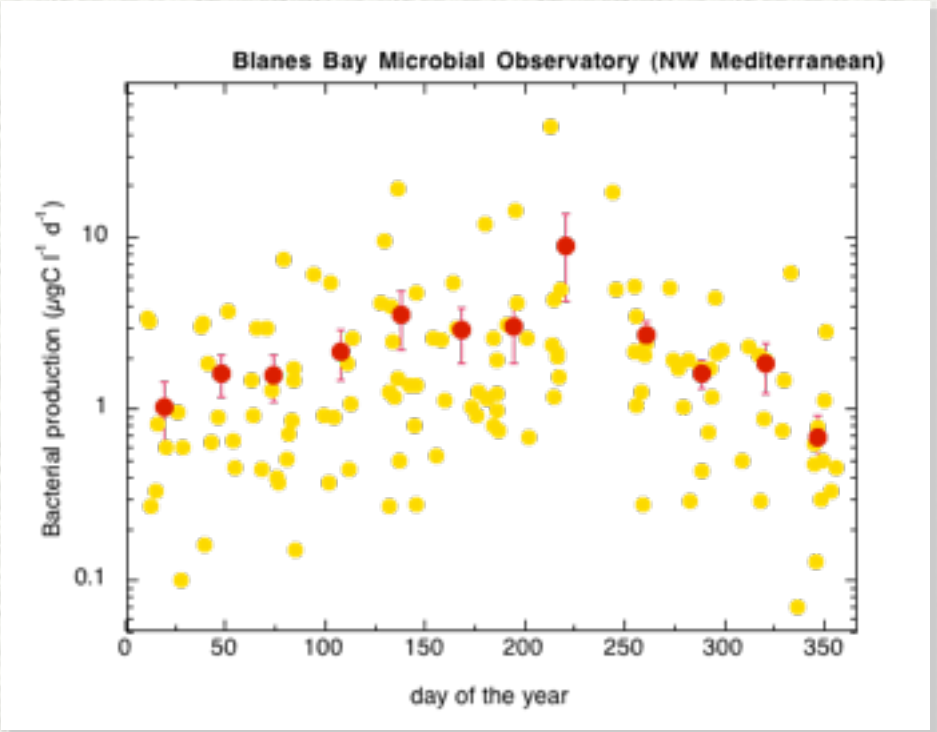
Small temporal and spatial variability in bacterial "production"

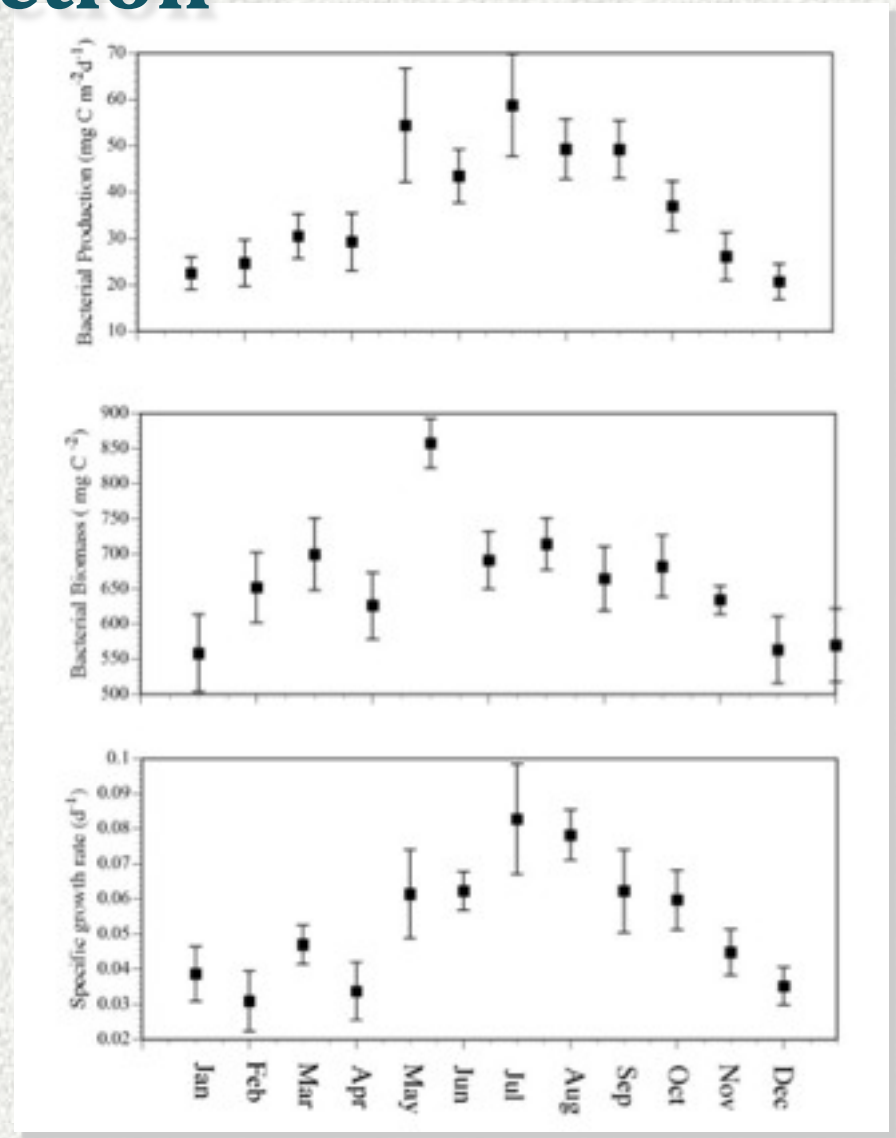




Ruiz-González et al. 2012, L&O

Seasonal variability in bacterial "production"





Steinberg et al. 2001, DSRI.

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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 $[time]^{-1}$ $[vol]^{-1}$ → μgC $[time]^{-1}$ $[vol]^{-1}$

" 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

$$\text{Prod } (\mu gC\ l^{-1}\ h^{-1}) = \text{Leu } (\mu mol\ l^{-1}\ h^{-1}) * CF2.2 * (\%Leu)^{-1} * ({}^{C}/_{protein}) * ID$$

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

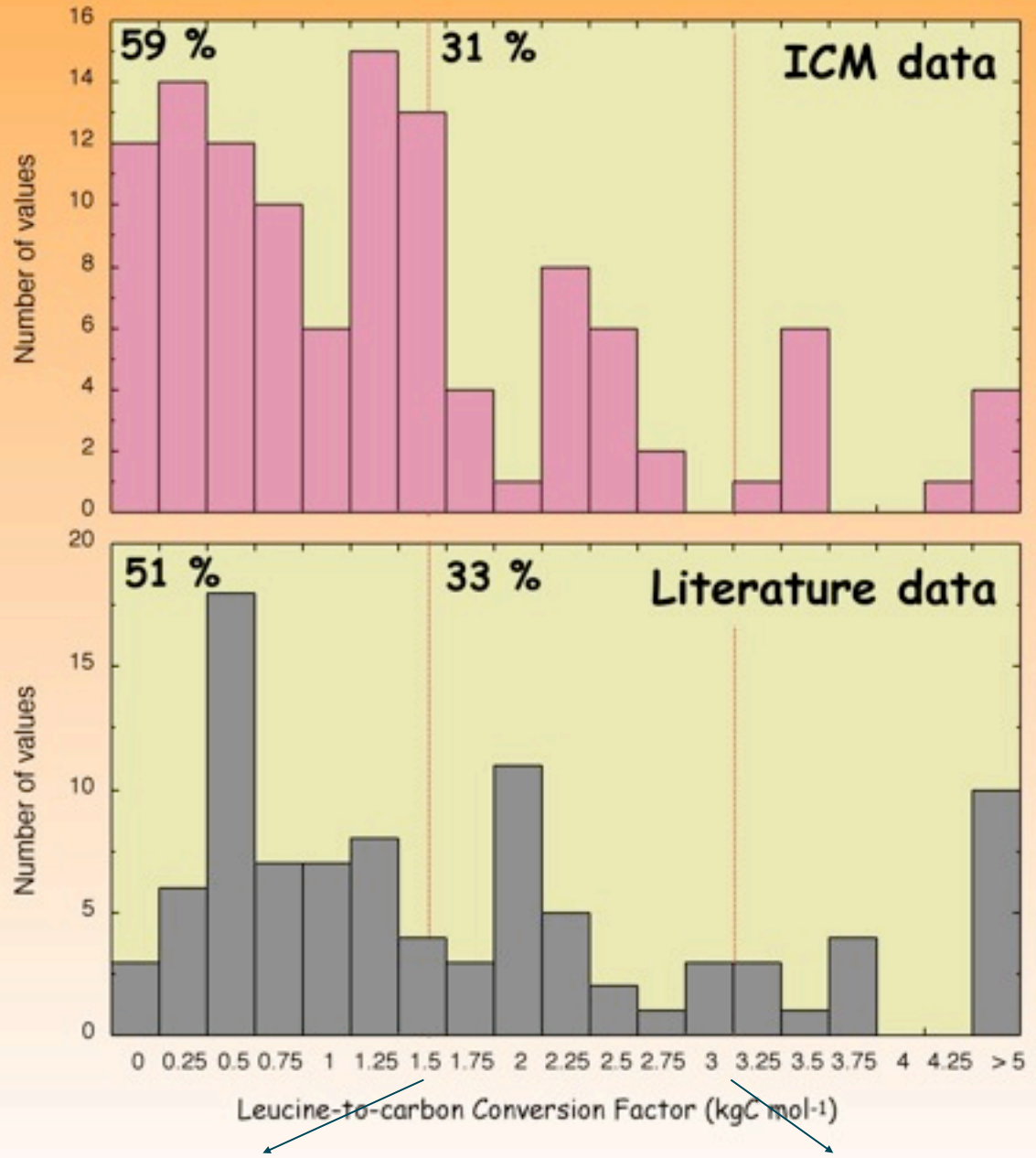
- physiological information on macromolecular synthesis is ignored
- growth rates not affected by the dilution ?
- natural bacterial assemblages ?
- filtration artifacts ?
- time consuming
- can't be measured at a rate similar to the rate of LIR measurement

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%)

Literature search

119 literature estimates
135 own estimates



1.55 kgC mol leucine^{-1}

3.1 kgC mol leucine^{-1}

Gasol & Pedrós-Alió, unpubls.

The Empirical Leucine-to-Carbon CF

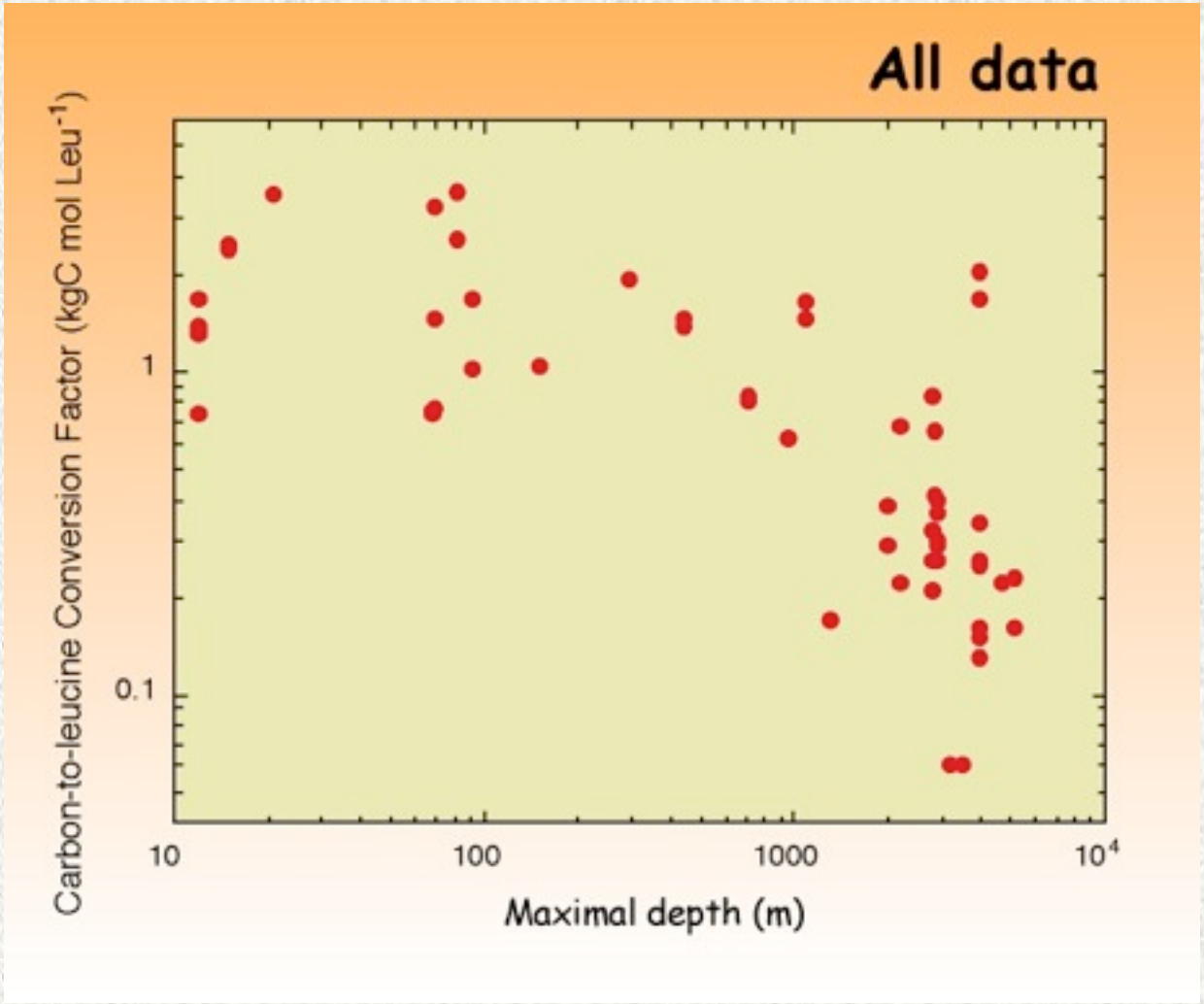
Patterns in offshore - inshore gradients

Site	Position	CF	Reference
NE Pacific	midshelf	2.40	Sherr et al. 2001-AME
	slope	1.80	
	offshore	0.40	
NE Pacific	midshelf	2.11	del Giorgio et al. 2012-L&O
	slope	0.75	
	offshore	0.31	
Mediterranean Sea	midshelf	2.00	Pedrós-Alió et al. 1999-DSR2
	slope	1.54	
	offshore	0.36	
NE Atlantic	estuary	3.55	Morán et al. 2002-AME
	midshelf	1.45	
	slope	1.03	
	offshore	0.65	

Gasol & Pedrós-Alió, unpubls.

The Empirical Leucine-to-Carbon CF

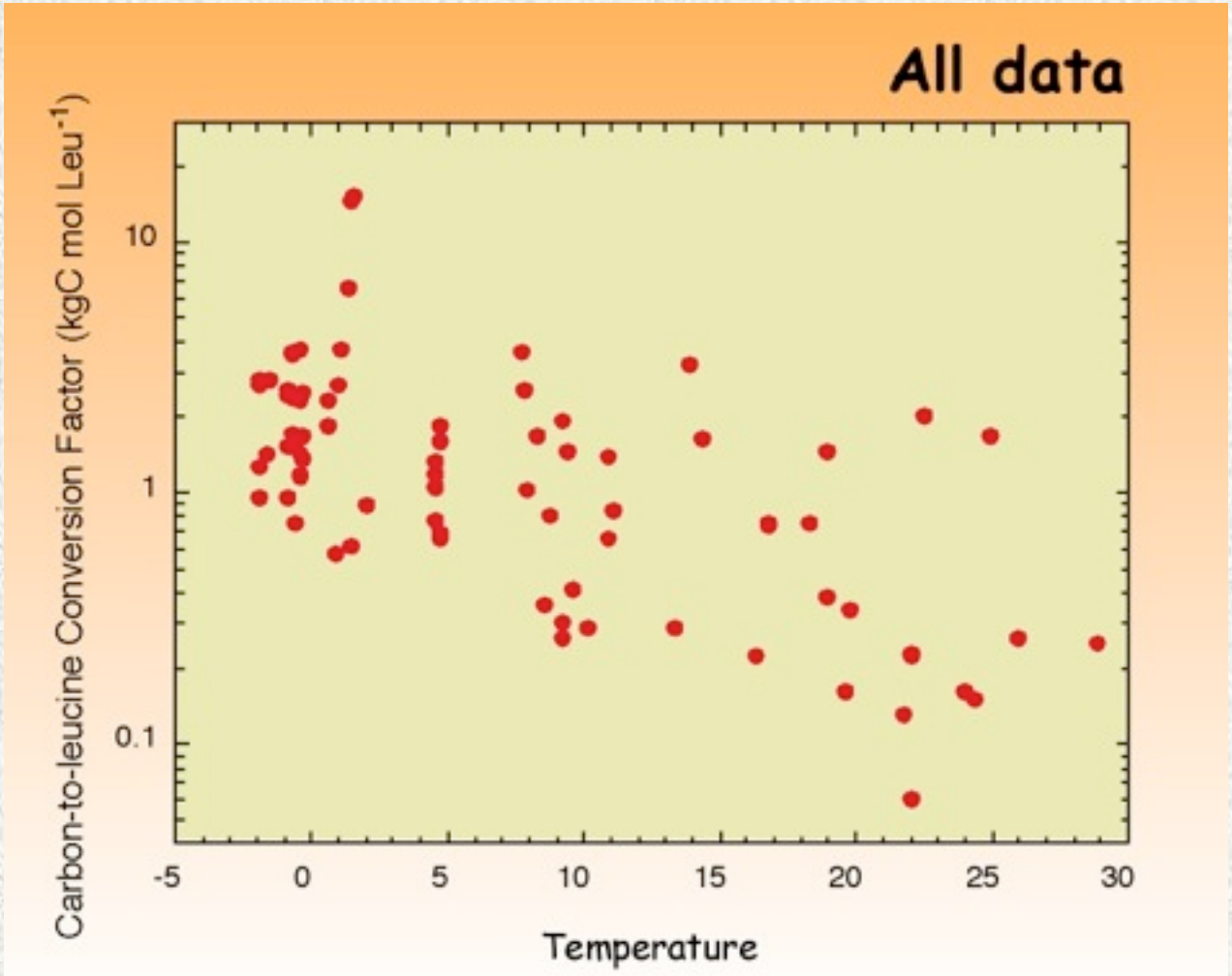
Patterns with site depth



Gasol & Pedrós-Alió, unpubls.

The Empirical Leucine-to-Carbon CF

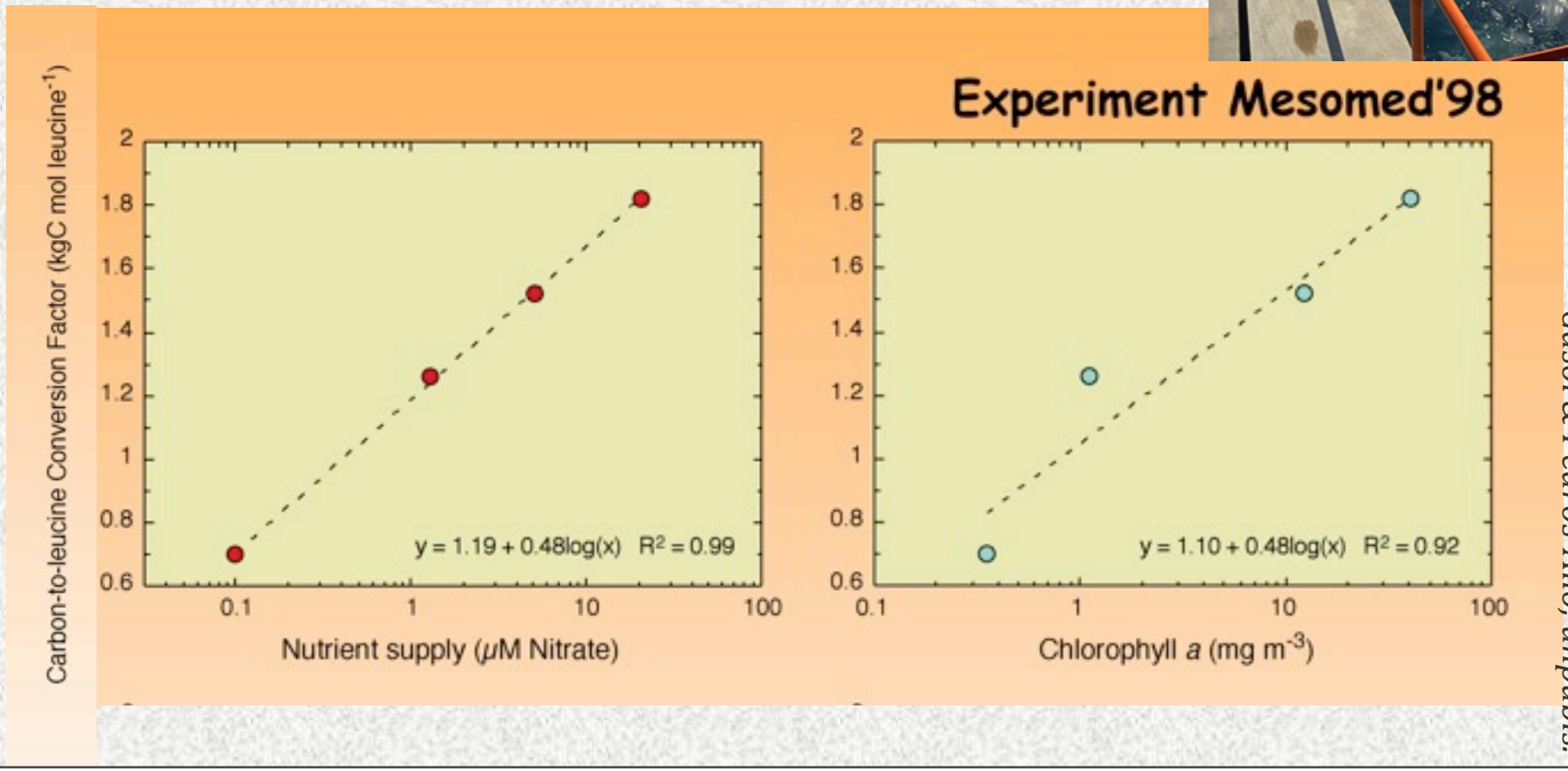
Patterns with temperature



Gasol & Pedrós-Alió, unpubls.

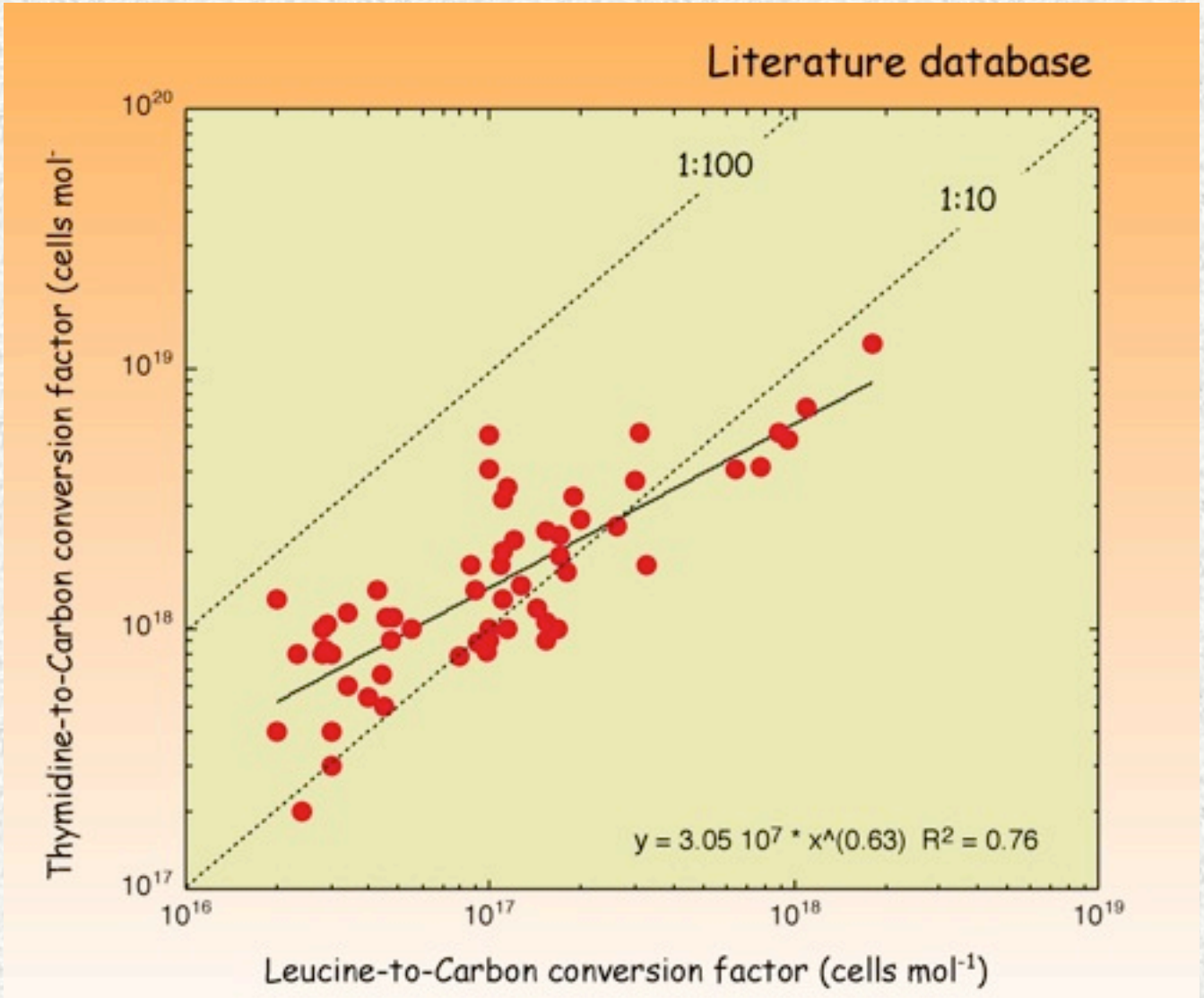
The Empirical Leucine-to-Carbon CF

Patterns with system richness



Gasol & Pedrós-Alió, unpubls.

The Empirical Leucine-to-Carbon CF



Should we expect a relationhsip between the LCF and the Leu:TdR ratio ?

Gasol & Pedrós-Alió, unpubls.

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?

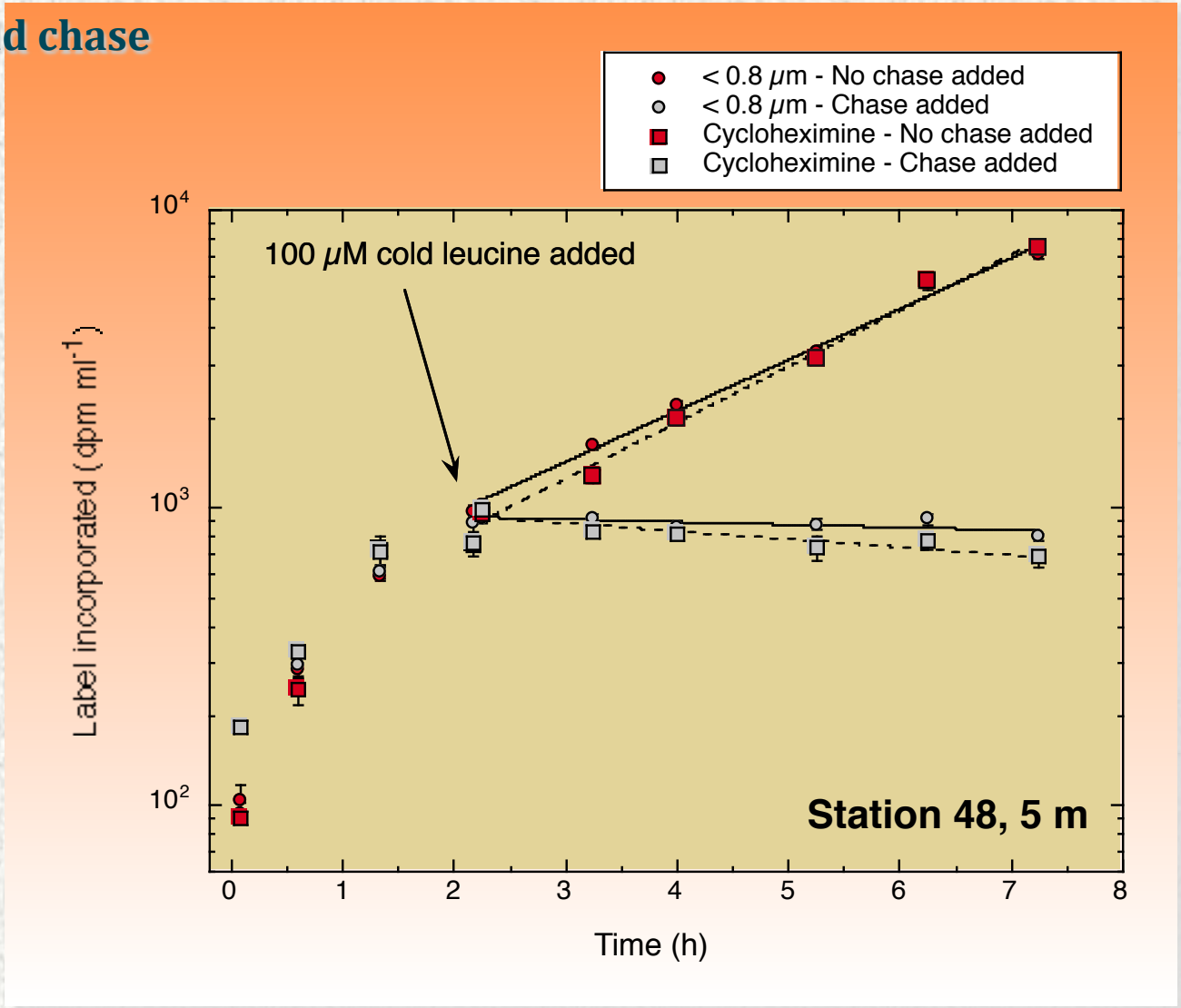
- literature patterns in the LCF
- why are the LCF so low ?
  - protein recycling
  - leucine respiration
- why do bacteria respire the leucine ?

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 ¿?

Alonso-Sáez et al. 2007, L&O

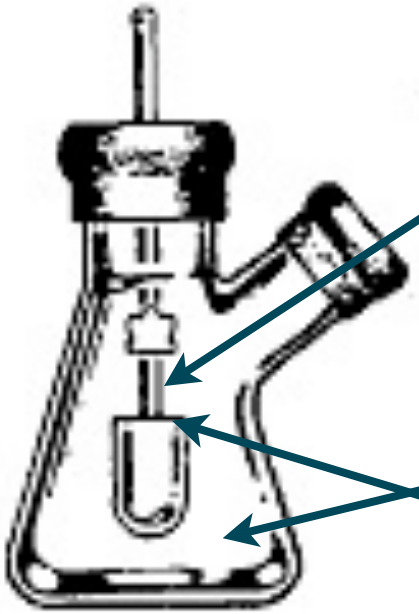
Why such small Leu-to-C CFs?

- literature patterns in the LCF — **Average oceanic LCF < 1**
- why are the LCF so low ?
  - protein recycling — **Not!**
  - leucine respiration
- why do bacteria respire the leucine ?

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

^{14}C-leucine to $^{14}CO_2$



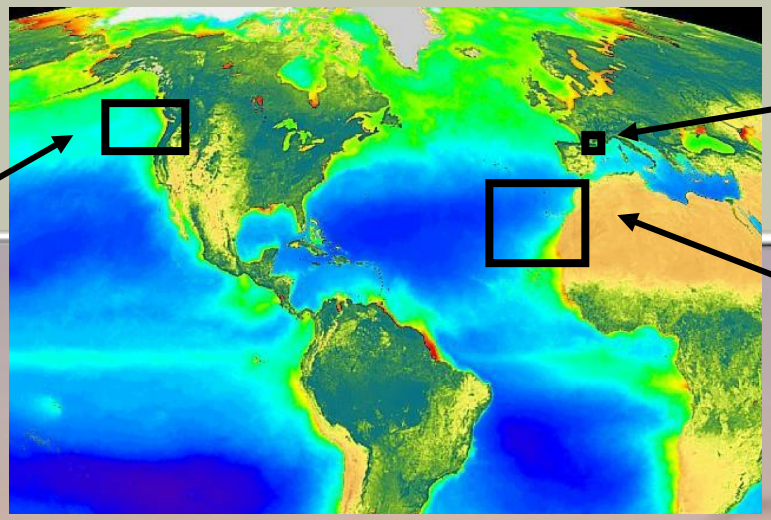
4.-Add PEA
(2-phenilethylamine)

3.- acidify (H_2SO_4)

1.-Sample
2.- ^{14}C-leucine

filter paper

Hobbie & Crawford 1969, L&O



Blanes Bay Microbial Observatory 2003-2004 NW Mediterranean

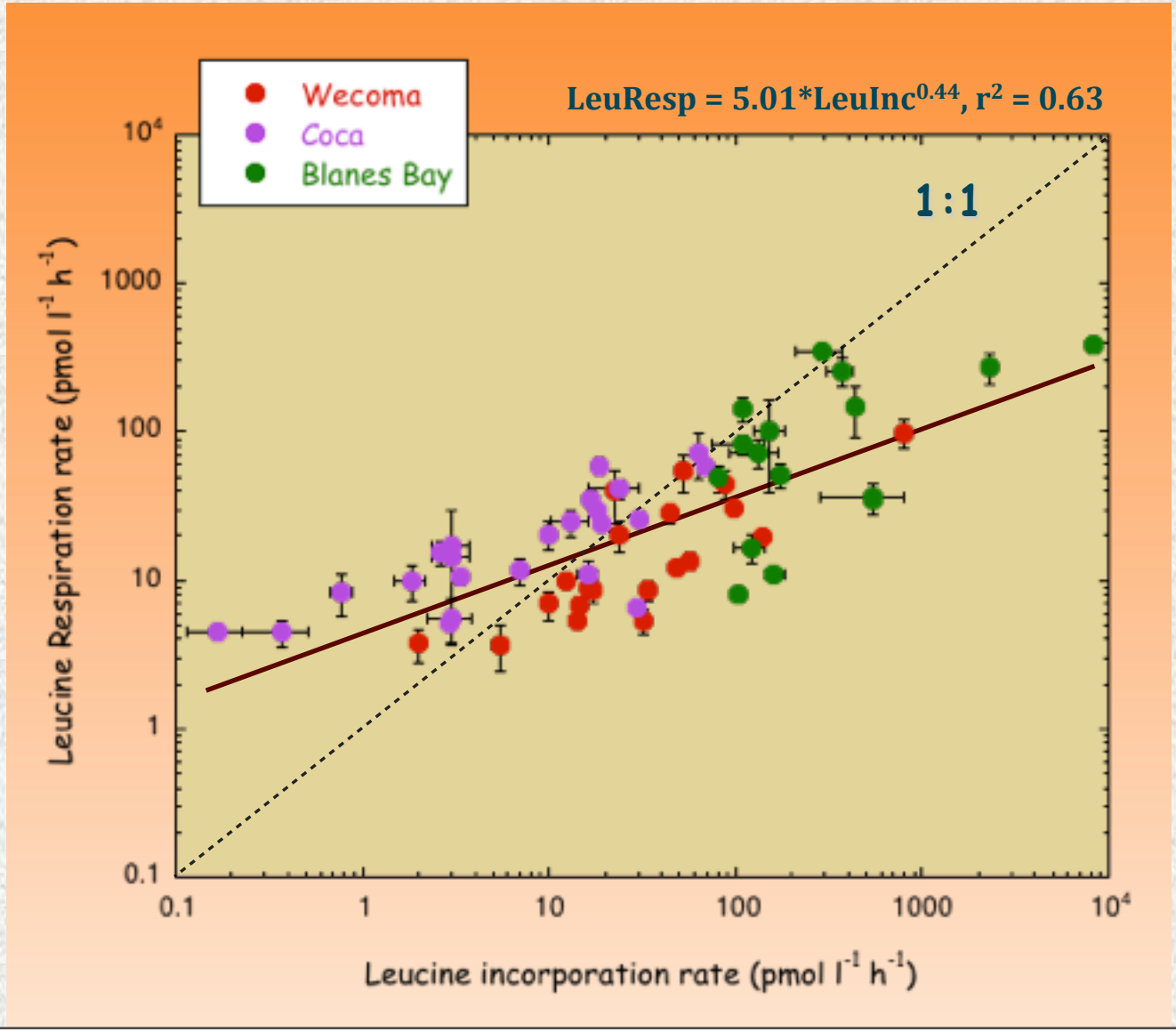
Wecoma cruise 2002 NE Pacific

Coca cruise 2003 NE Atlantic

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

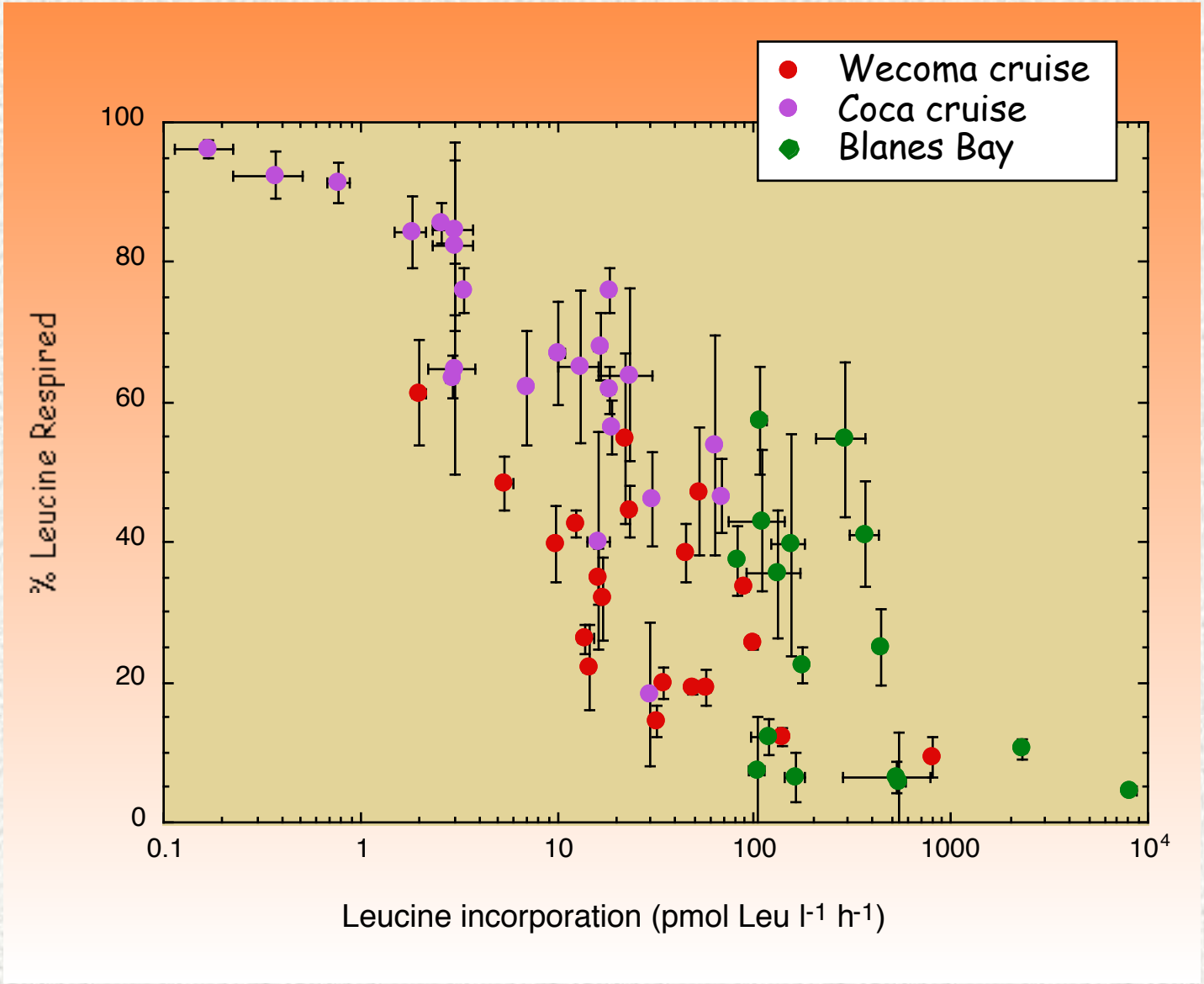
Measuring leucine respiration

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



Gasol et al, submitted

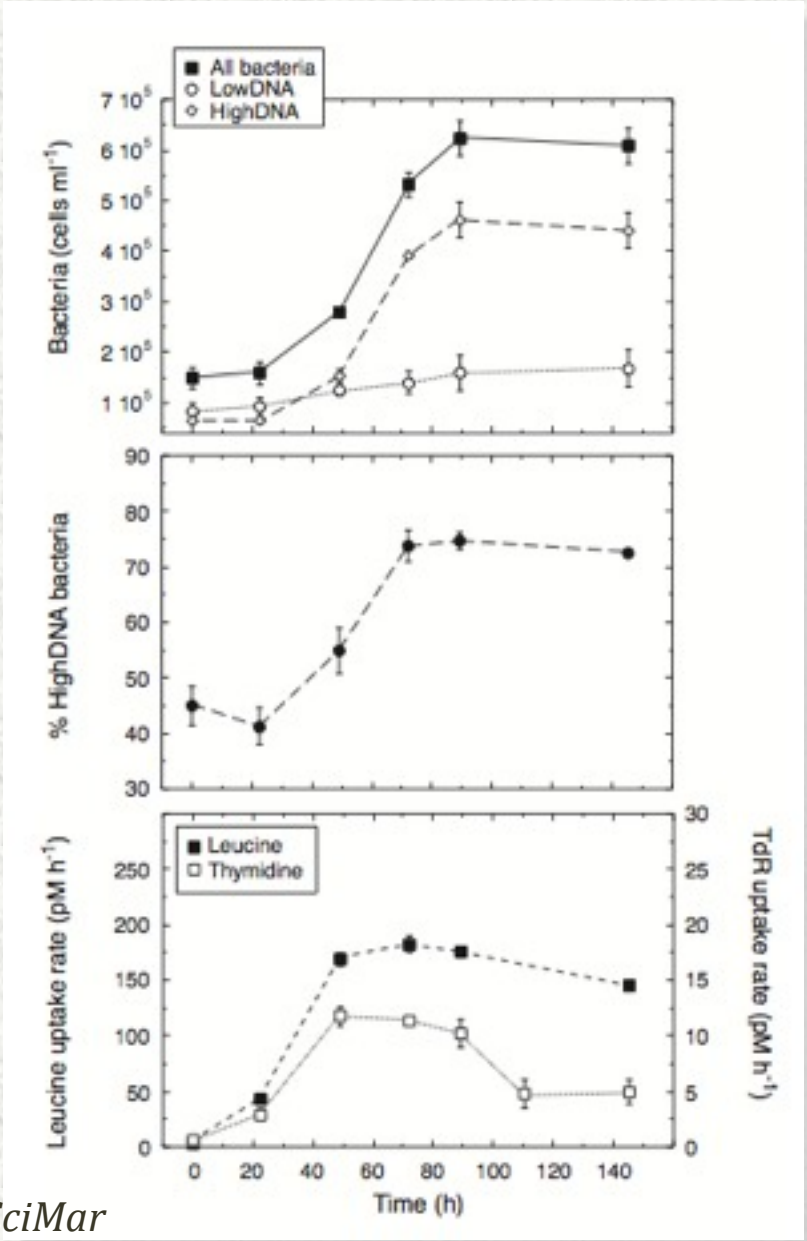
Measuring leucine respiration



less active communities respire most of the added leucine !

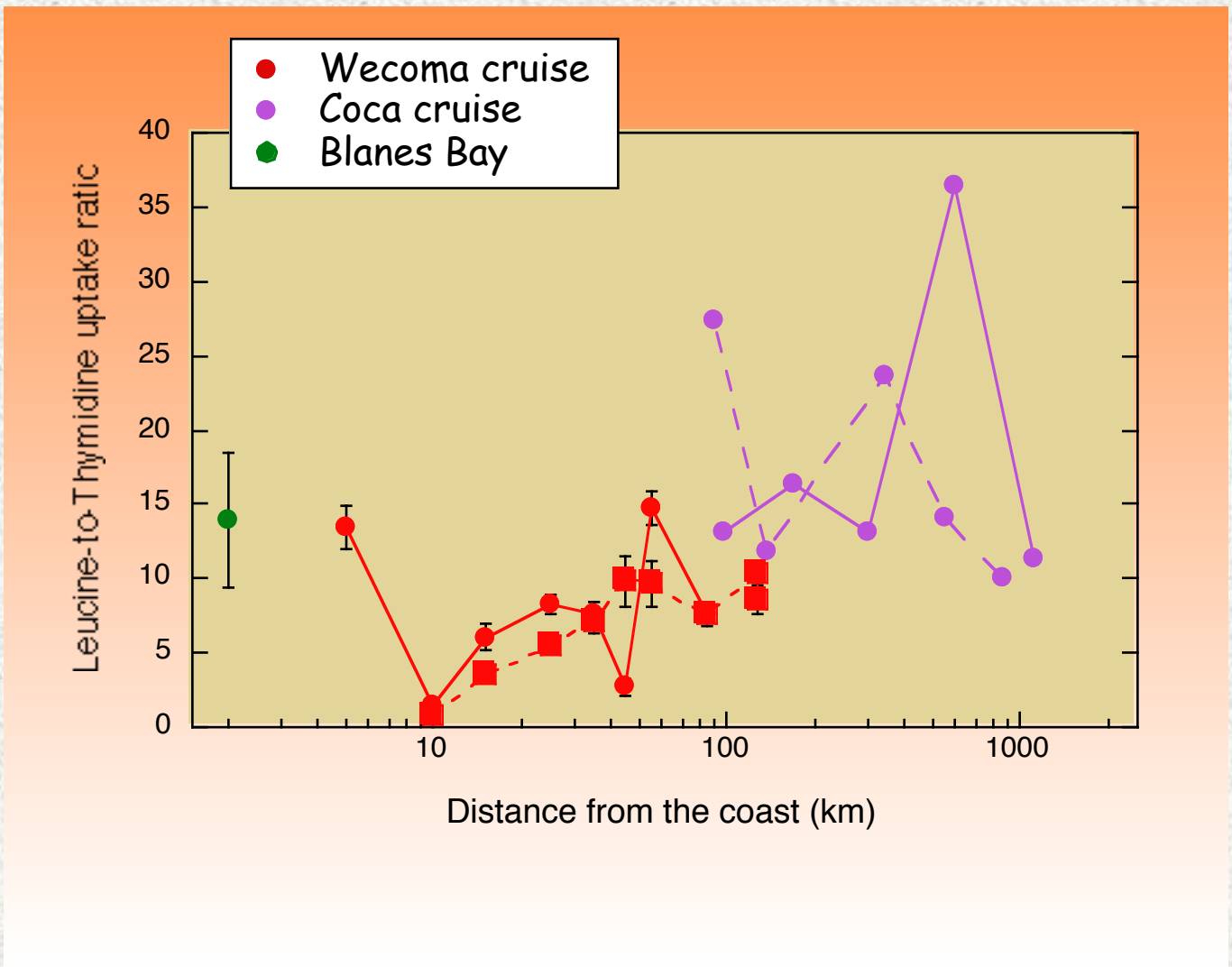
Gasol et al, submitted

TdR vs. Leucine incorporation



Gasol & del Giorgio 2000, SciMar

TdR vs. Leucine incorporation



Gasol et al, submitted

Why such small Leu-to-C CFs?

- **literature patterns in the LCF** — **Average oceanic LCF < 1**
- **why are the LCF so low ?**
  - **protein recycling** — **Not!**
  - **leucine respiration** — **Yes!**
- **why do bacteria respire the leucine ?**

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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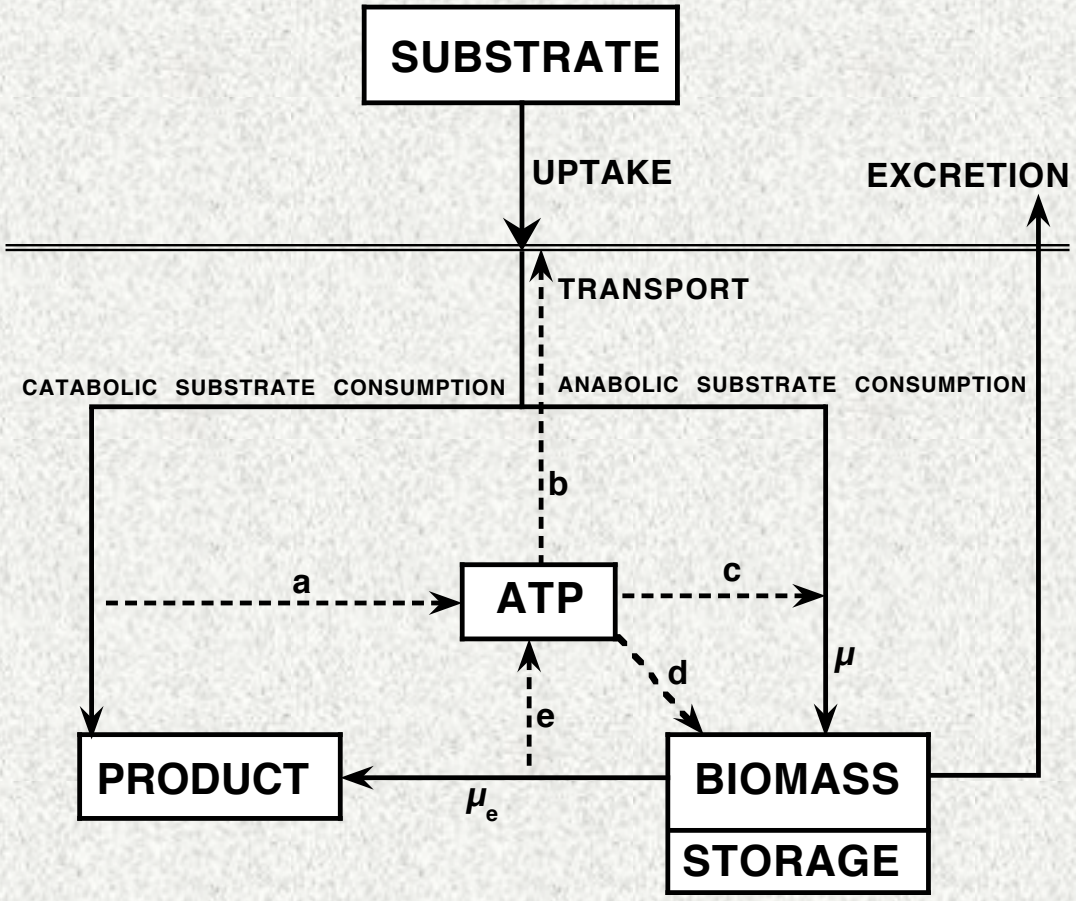
BGE captures the energetic status of a community



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

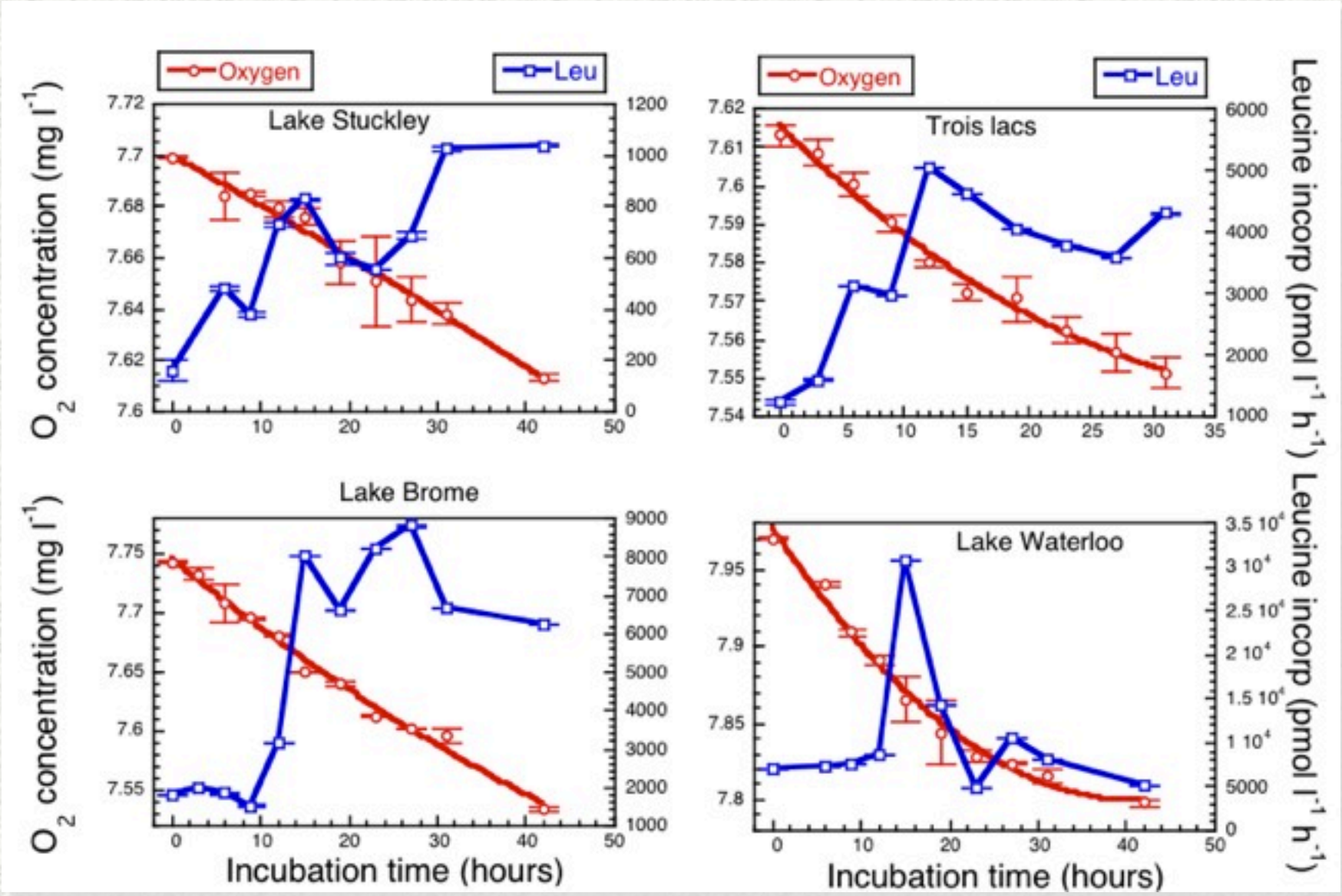
c. ATP utilization for biosynthesis and growth

d. ATP utilization for maintenance

e. ATP production from endogenous metabolism

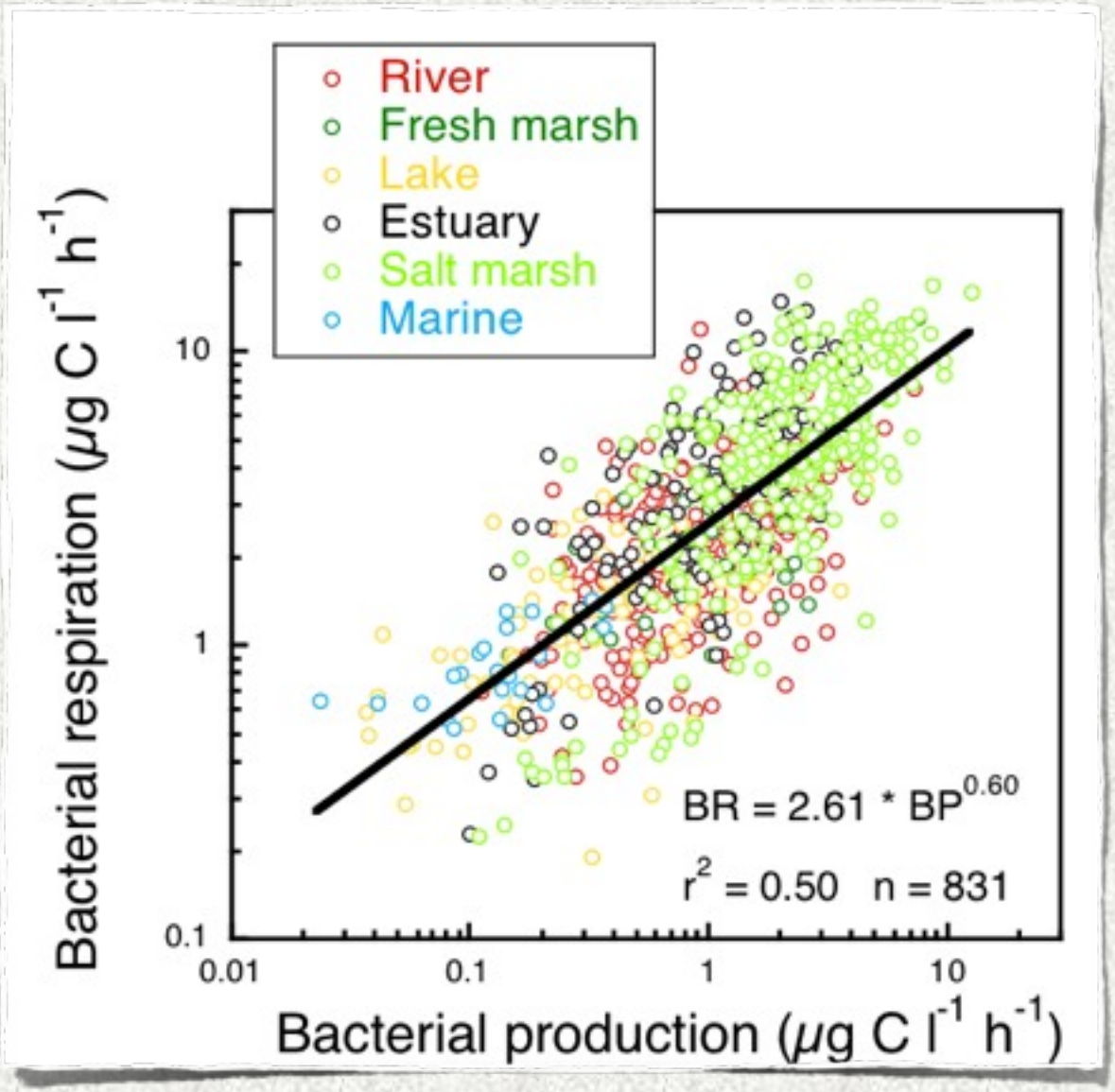
del Giorgio & Cole 1998, Ann. Rev. Ecol. Syst.

Uncoupling between BP and BR in incubations



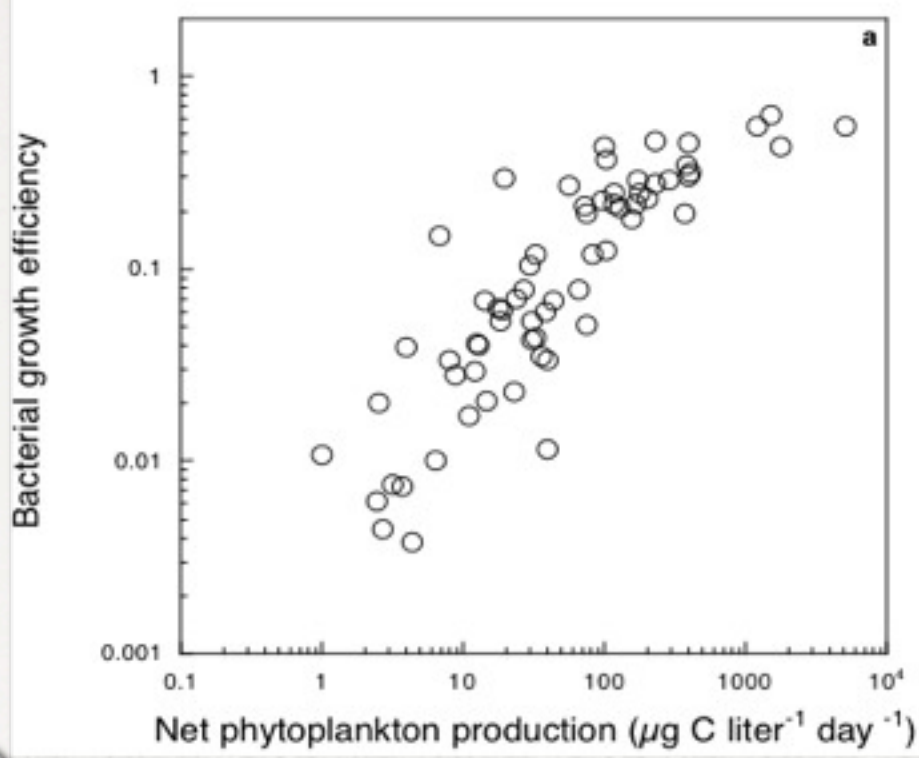
del Giorgio et al. in prep

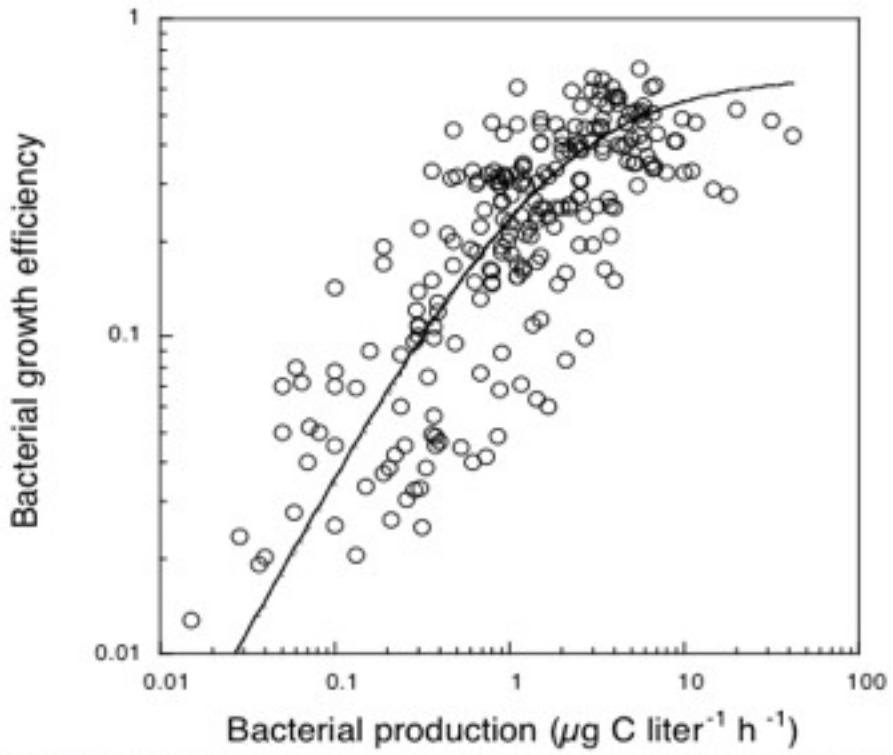
...but large scale covariation



del Giorgio et al. in prep

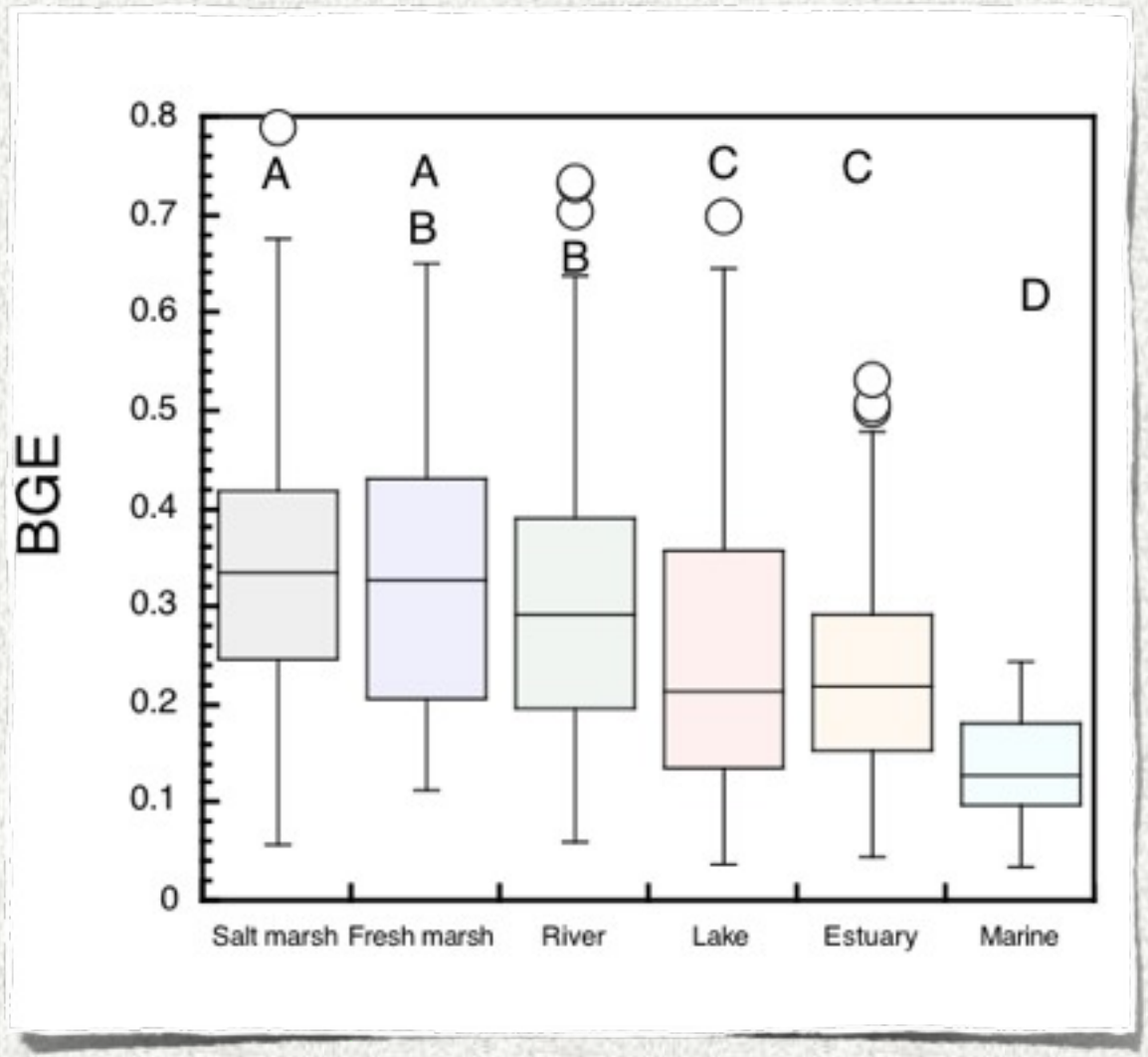
BGE increases with system productivity





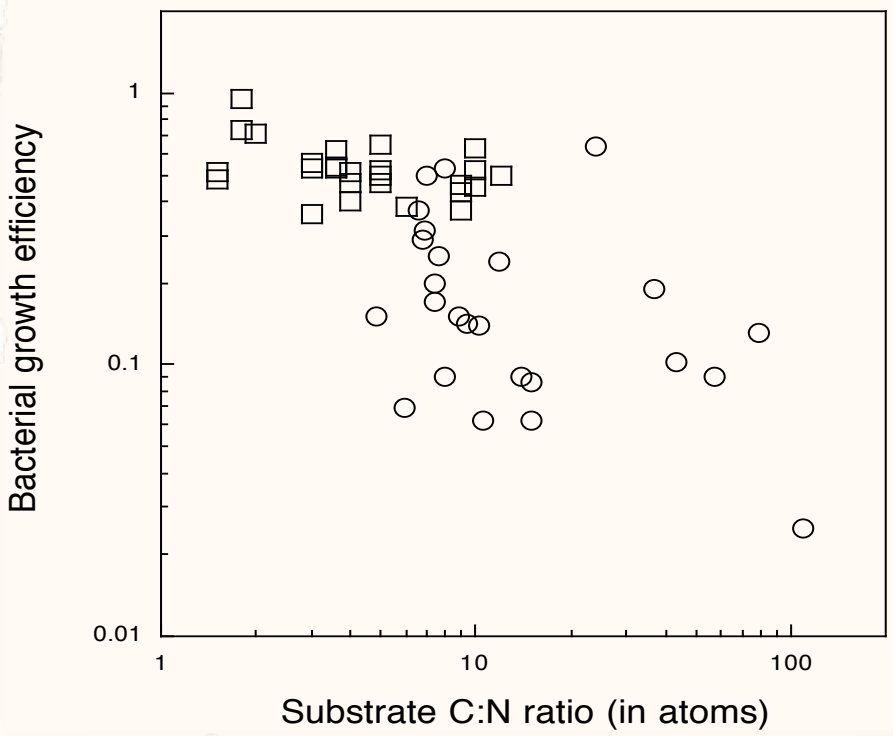
del Giorgio & Cole 1998, Ann. Rev. Ecol. Syst.
del Giorgio & Cole 2000 in Kirchman (ed)

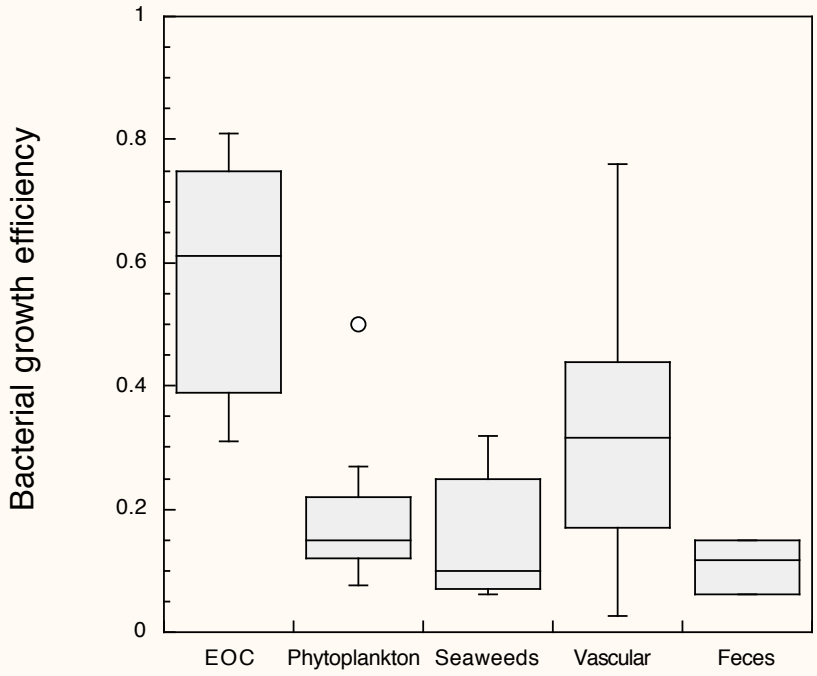
BGE varies according to ecosystem type



del Giorgio et al. in prep

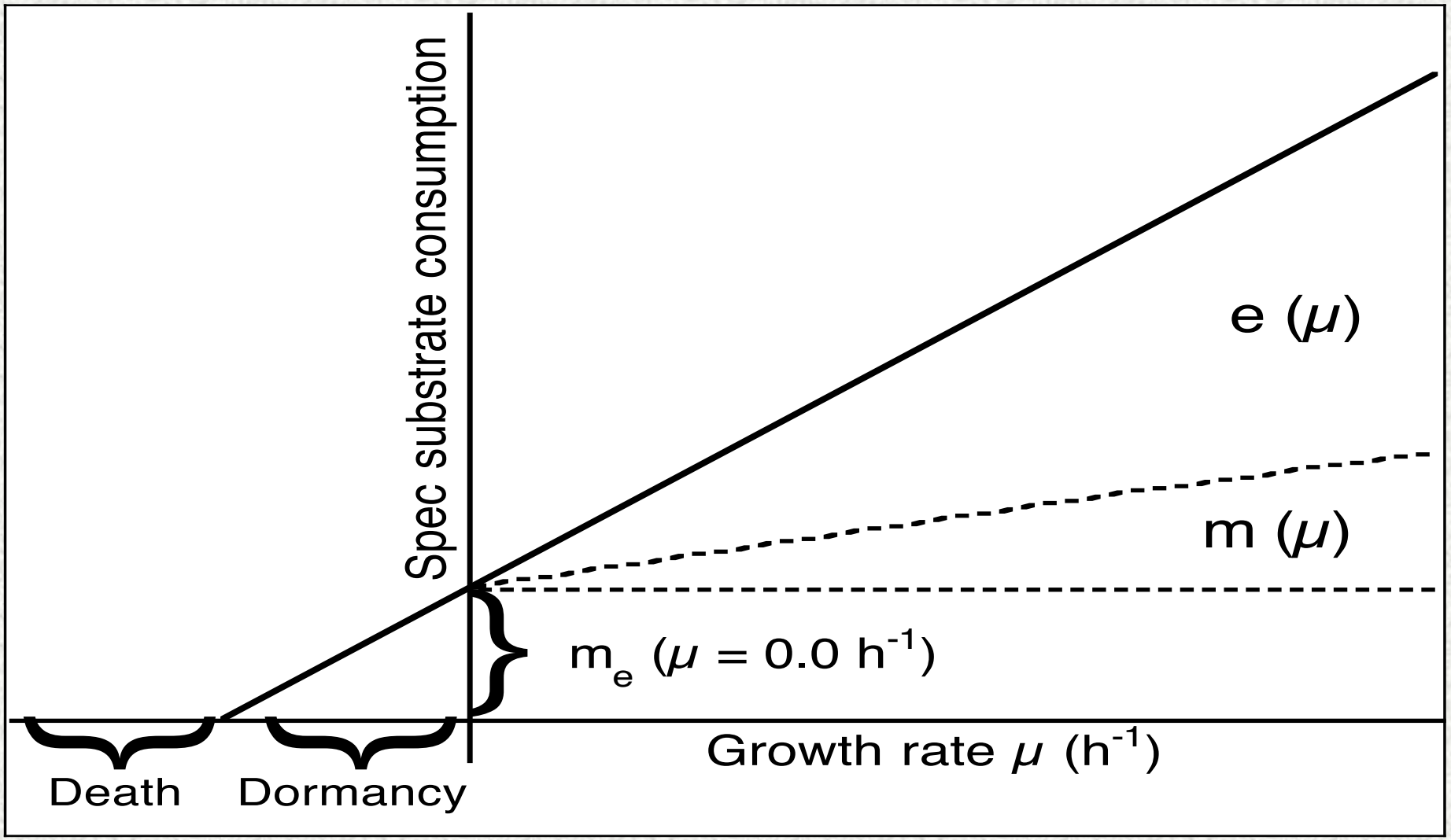
The nature and source of DOC influences BGE





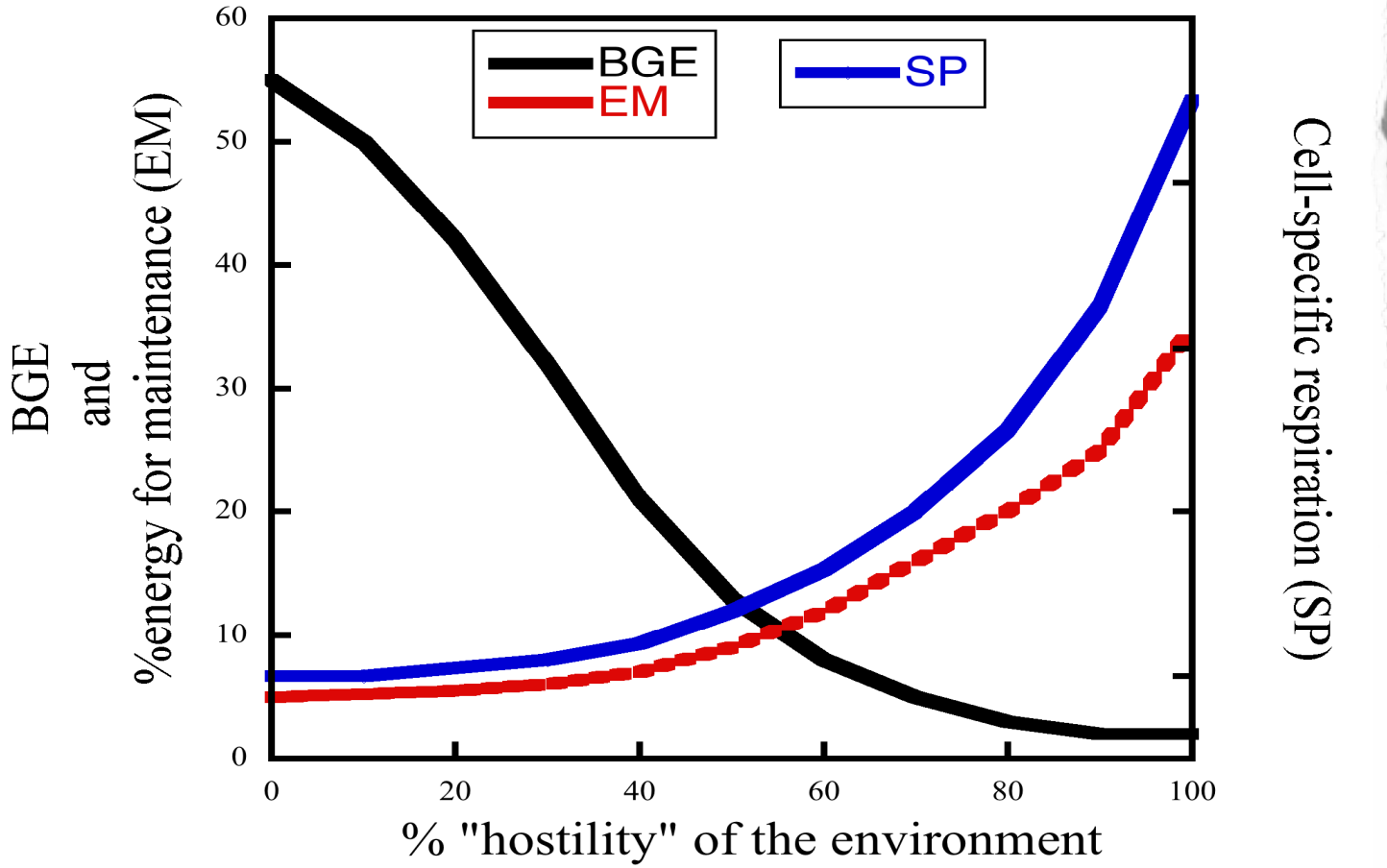
del Giorgio & Cole 1998, Ann. Rev. Ecol. Syst.

Evidence for energy limitation of bacteria



del Giorgio & Cole 2000 in Kirchman (ed)

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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Back to understanding BP

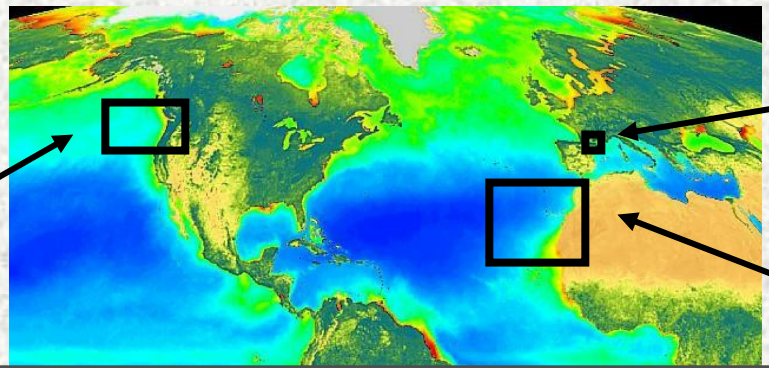
Observation: LCF in oceanic waters are low, very low
Observation: This is not due to protein recycling, but to leucine respiration

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

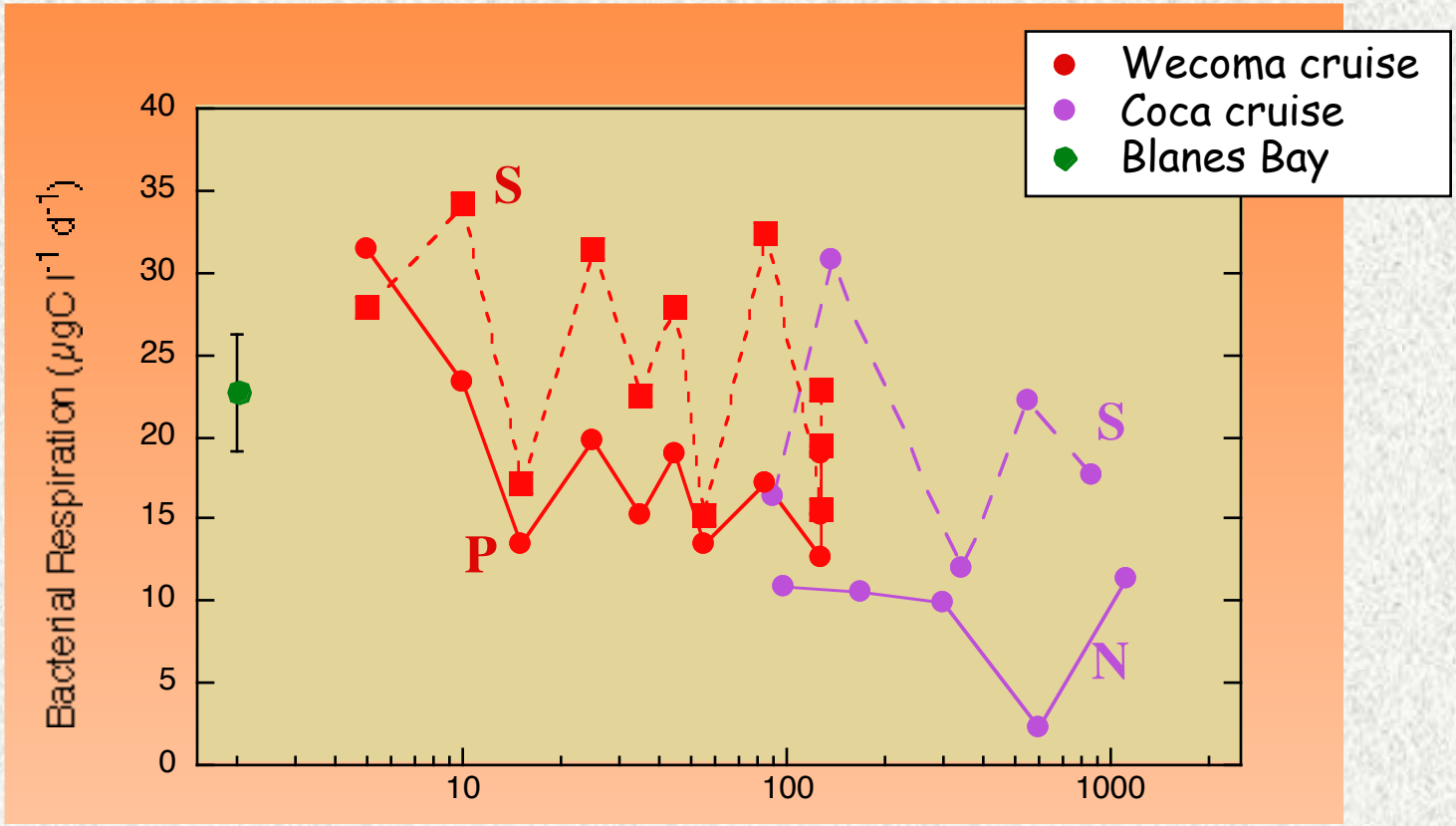
Thus, the LCF (and the respiration of the leucine) should be related to other indicators of physiological status

- 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)



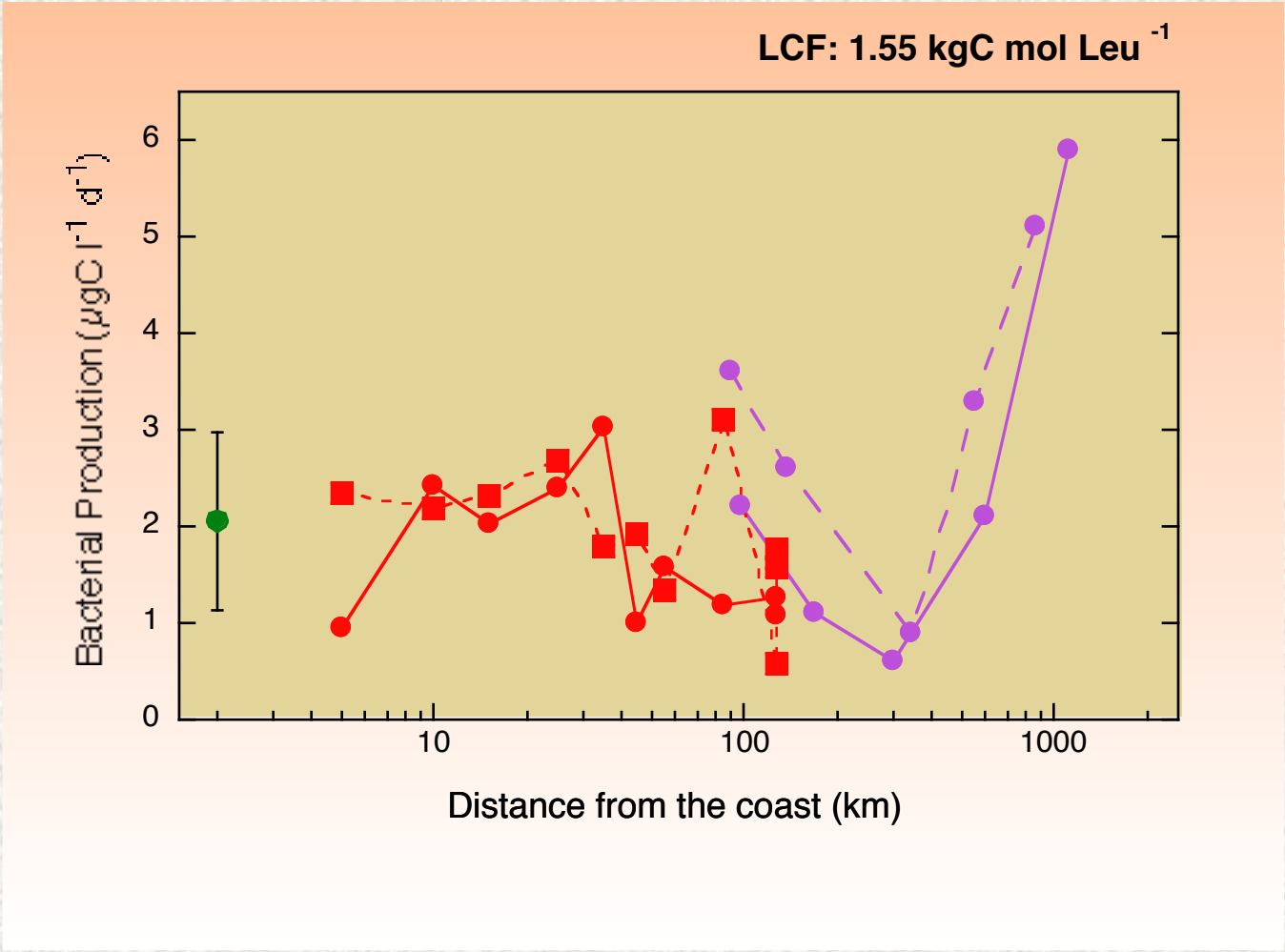
Patterns of C metabolism (BR)



A slight tendency for more respiration in the coastal stations

Gasol et al, submitted

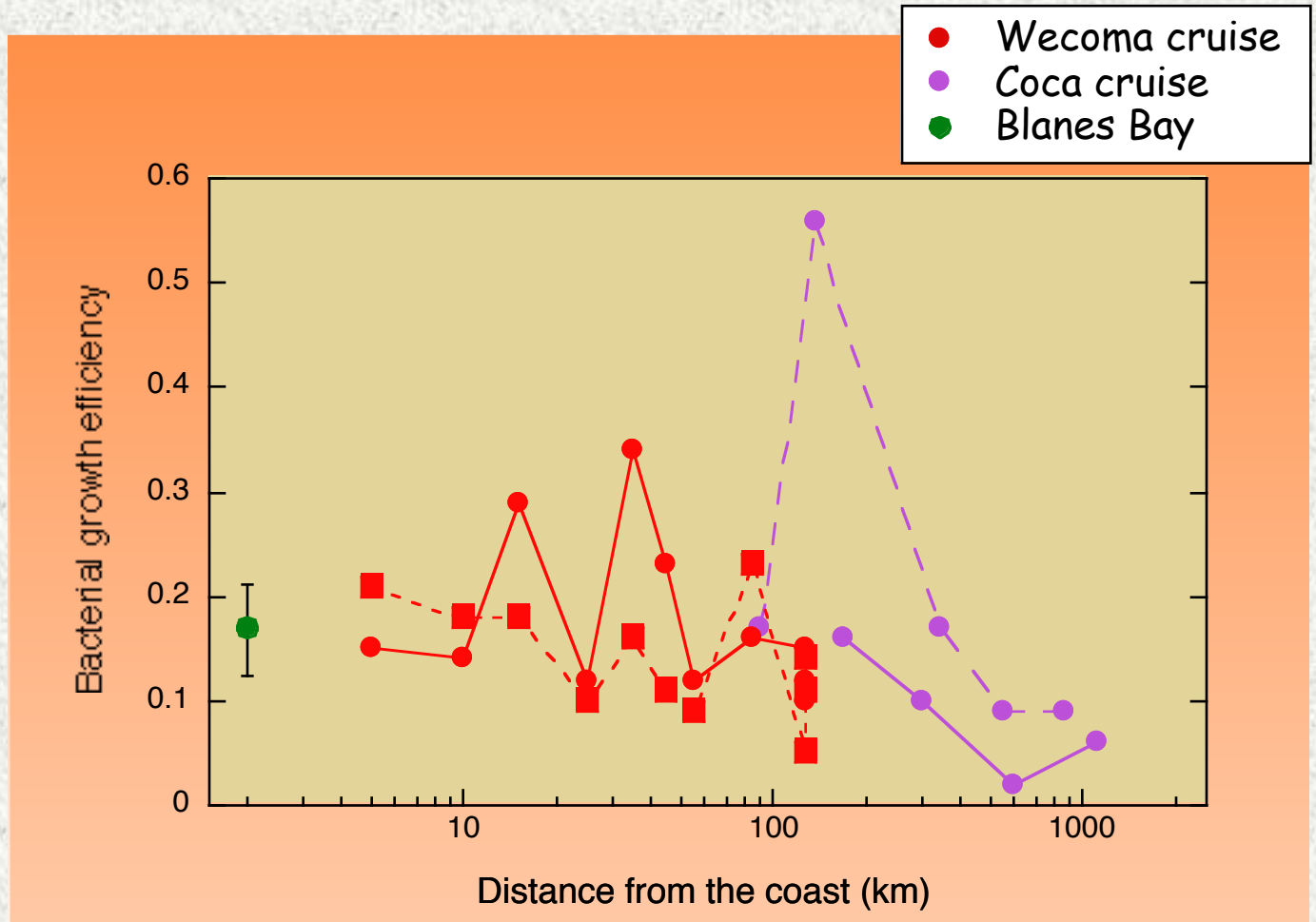
Patterns of C metabolism (BP)



No clear trend for BP (using standard CF)

Gasol et al, submitted

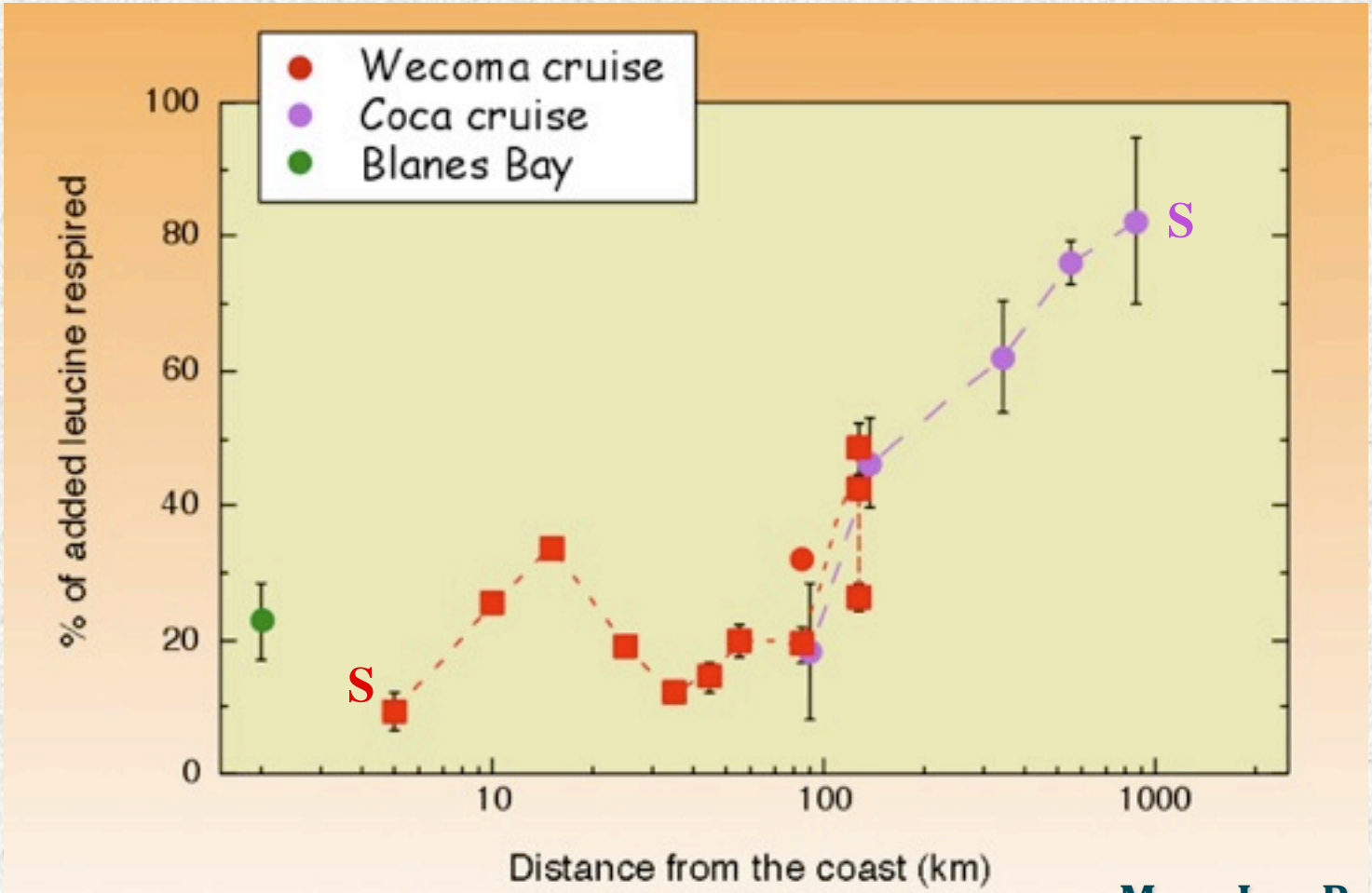
Patterns of C metabolism (BGR)



BGE quite variable, slight tendency to decrease towards offshore
NOT predictable from Temp

Gasol et al, submitted

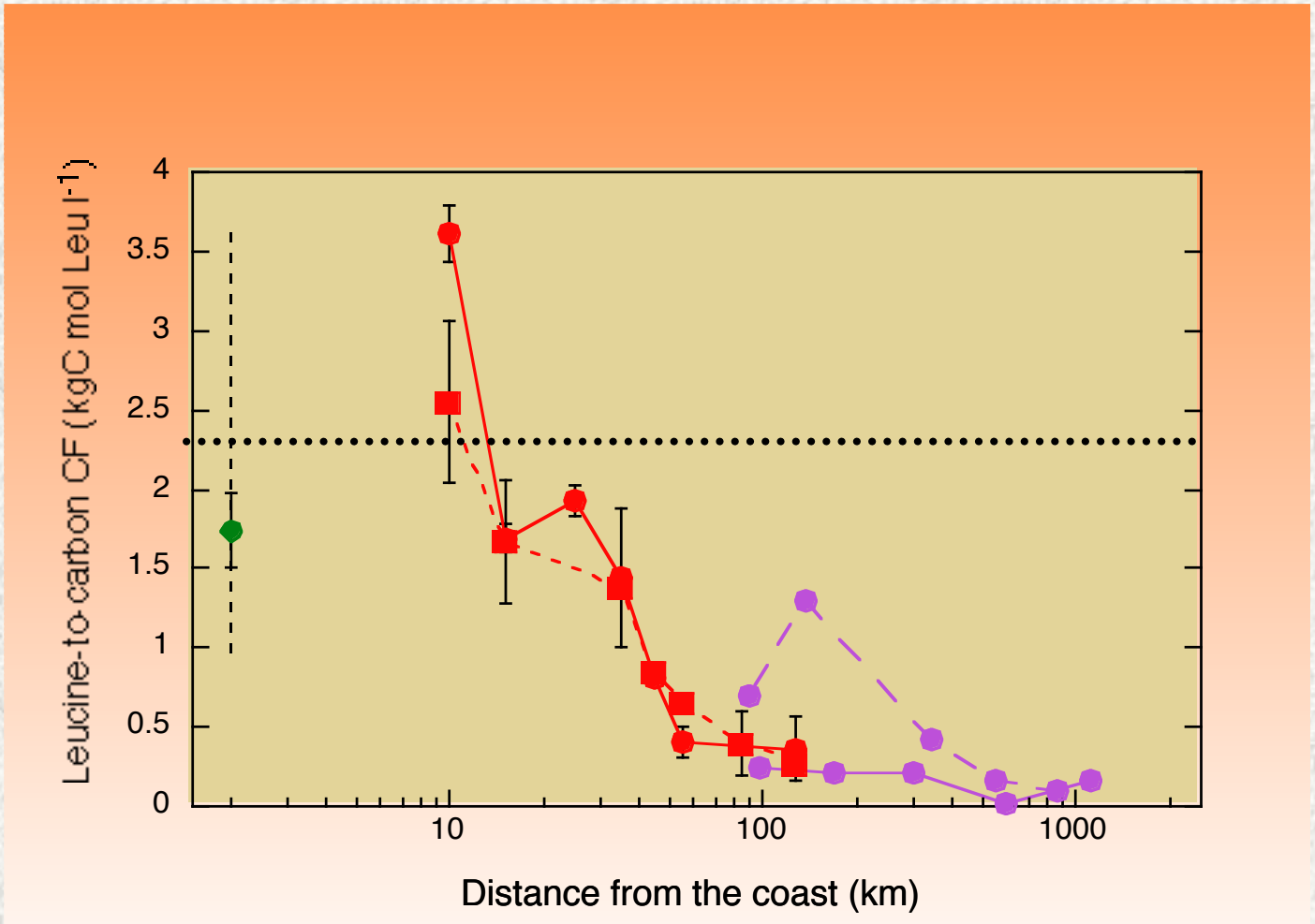
Patterns of C metabolism (Leu resp)



More Leu Resp in offshore waters (at least in surface)

Gasol et al, submitted

Patterns in Leu-to-C conversion factor

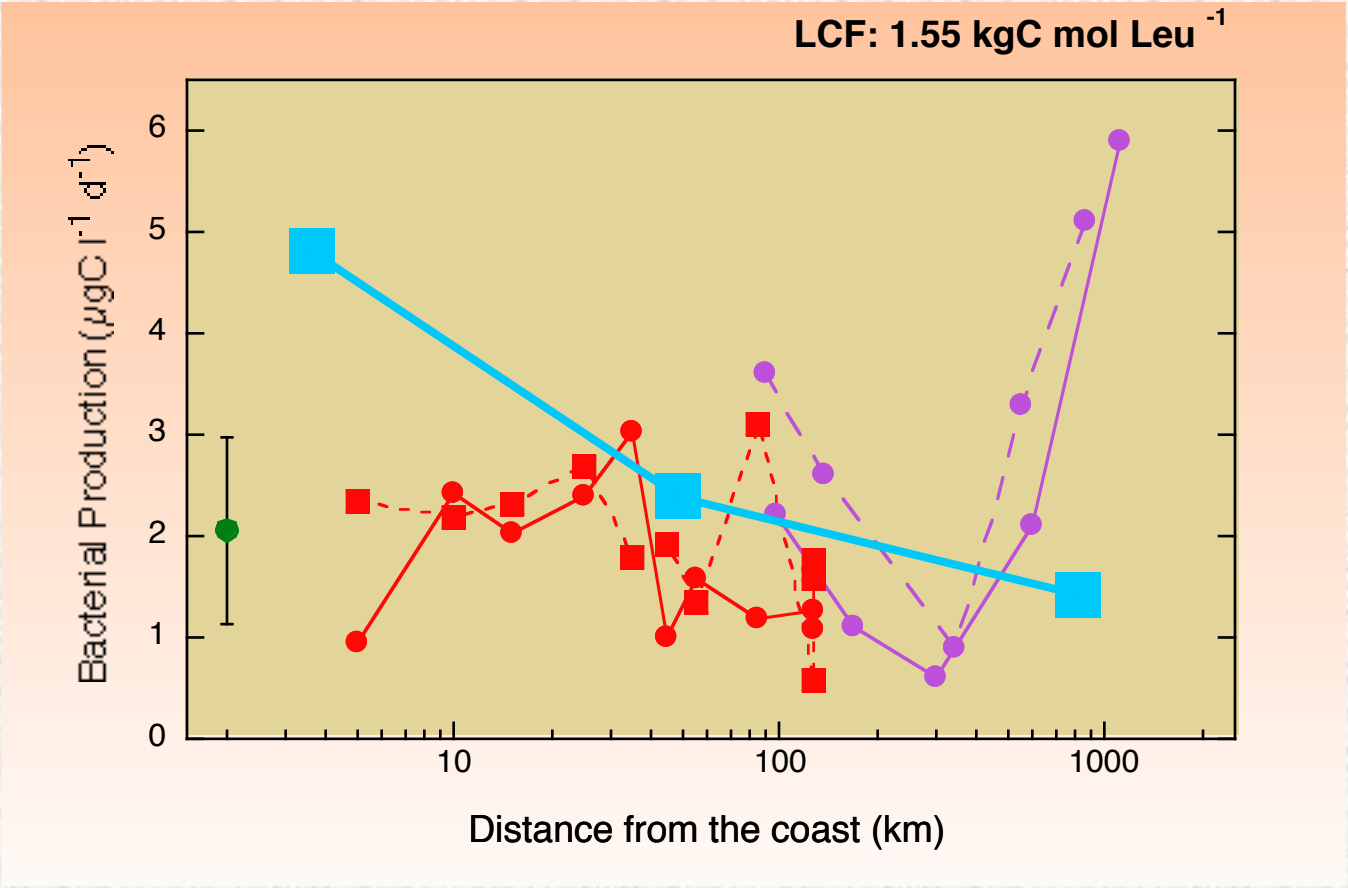


Distance to coast: the best predictor of LCF (not CHL, nor Temp)

Gasol et al, submitted

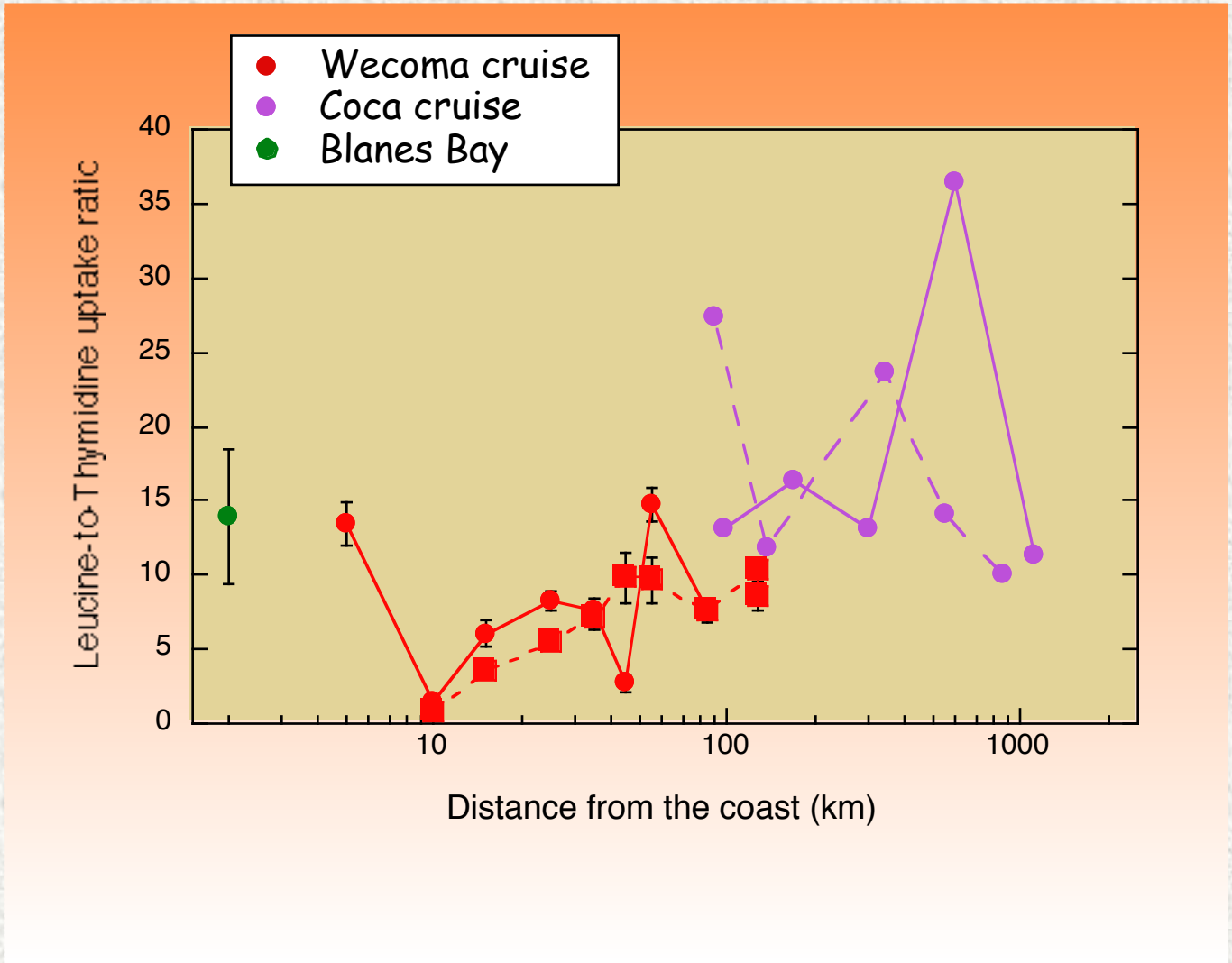
Patterns of C metabolism (BP)

w/o and w EmpCF



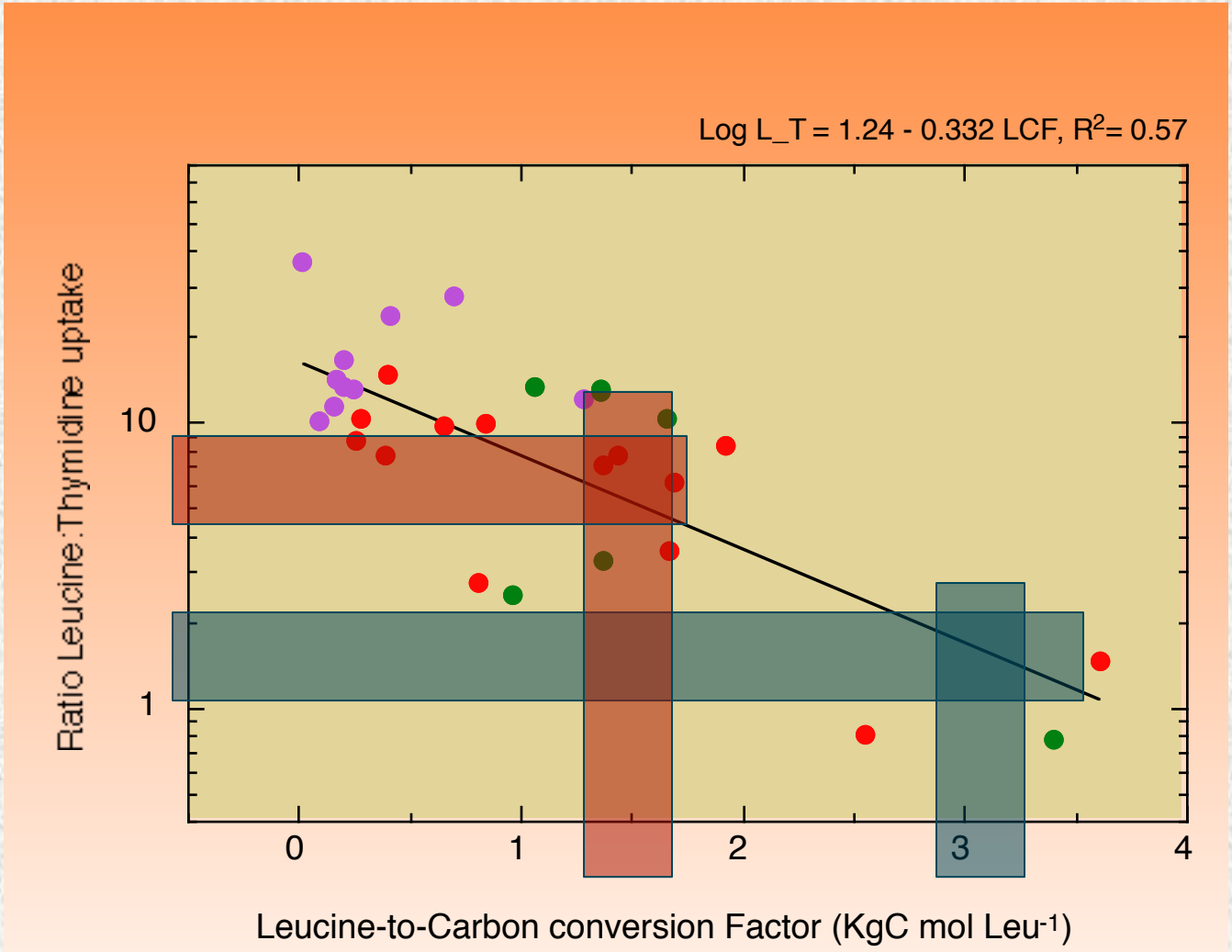
Gasol et al, submitted

Patterns of C metabolism (ratio Leu:TdR)



Gasol et al, submitted

ratio Leu:TdR and LeuCF

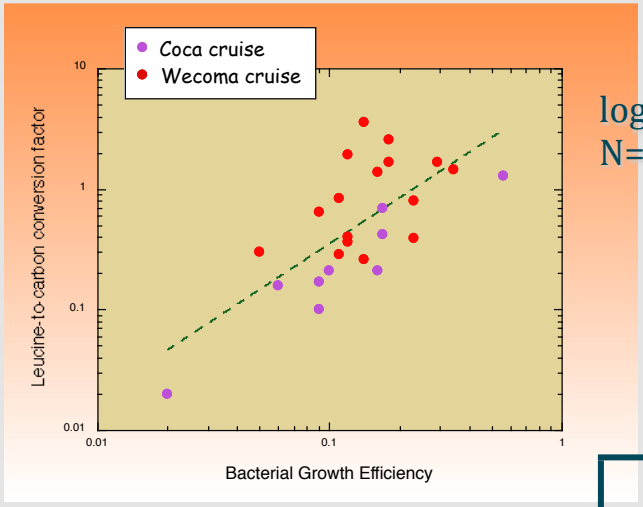


Higher LCF ≈ lower Leu:TdR

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

Gasol et al, submitted

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 LCF = 0.79 + 1.28 LogBGE
N= 25, P <0.001, r^2= 0.48

Matrix of pairwise correlations

	CHL	Emp CF	L:TdR	GR (d-1)	Total Leu inc-	Leu Resp	% Leu Resp	BGE
CHL		0.42			0.82	0.77	-0.29	
Emp CF	0.42		-0.38	0.49		0.55	-0.46	0.45
L:TdR		-0.38		-0.70		-0.33		
GR (d-1)		0.49	-0.70				-0.64	
Total Leu inc-	0.82					0.82	-0.39	
Leu Resp	0.77	0.55	-0.33		0.82			
% Leu Resp	-0.29	-0.46		-0.64	-0.39			-0.34
BGE		0.45					-0.34	

$P < 0.001$

$P < 0.01$

$P < 0.05$

Gasol et al., submitted

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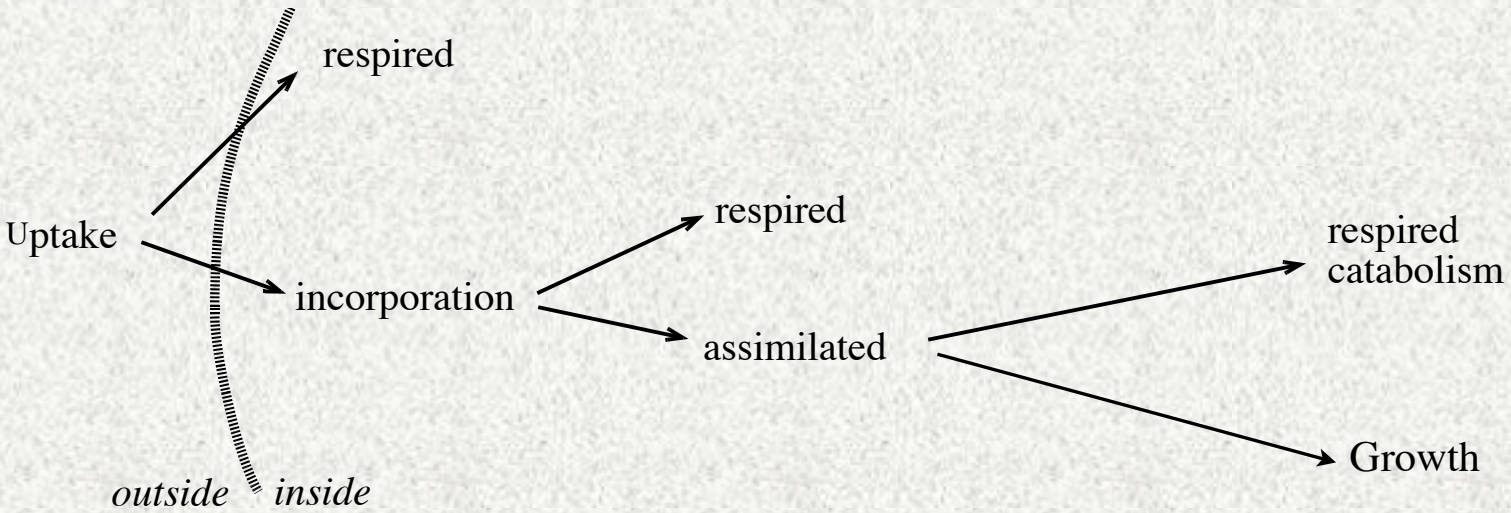
Different processes operating at different scales

Instantaneous	min-to-hr	hr-to-days	>days



Conclusions

- Evidence that BGE is rather variable (more than predicted by some models)
- Open ocean LCFs are << 1 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.

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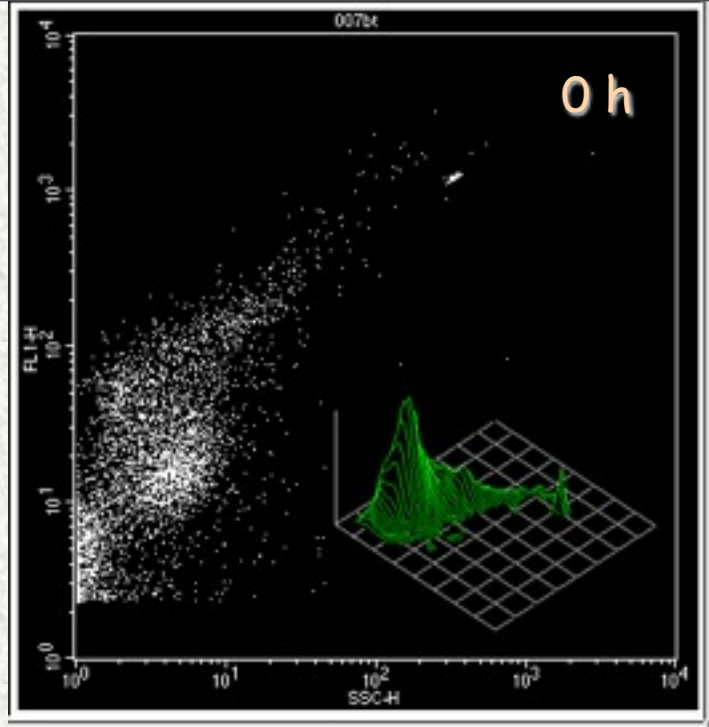
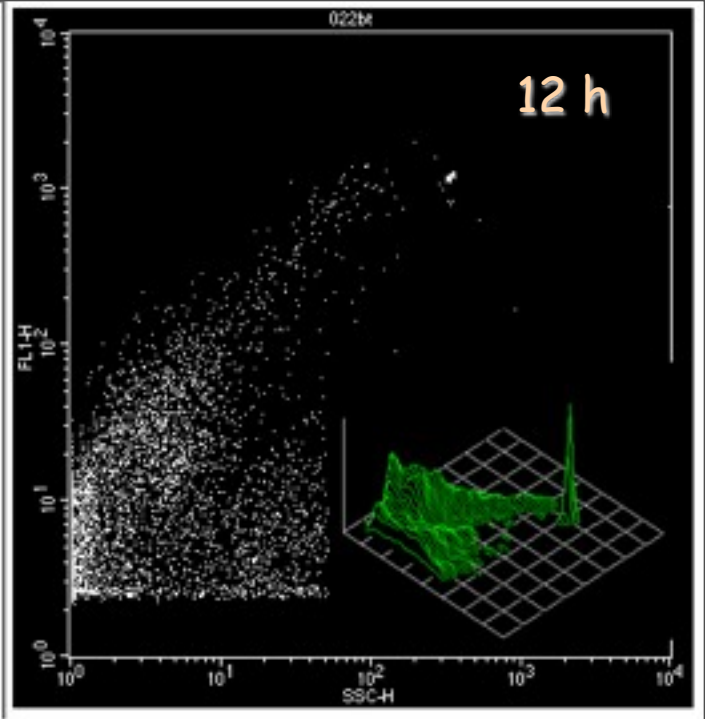
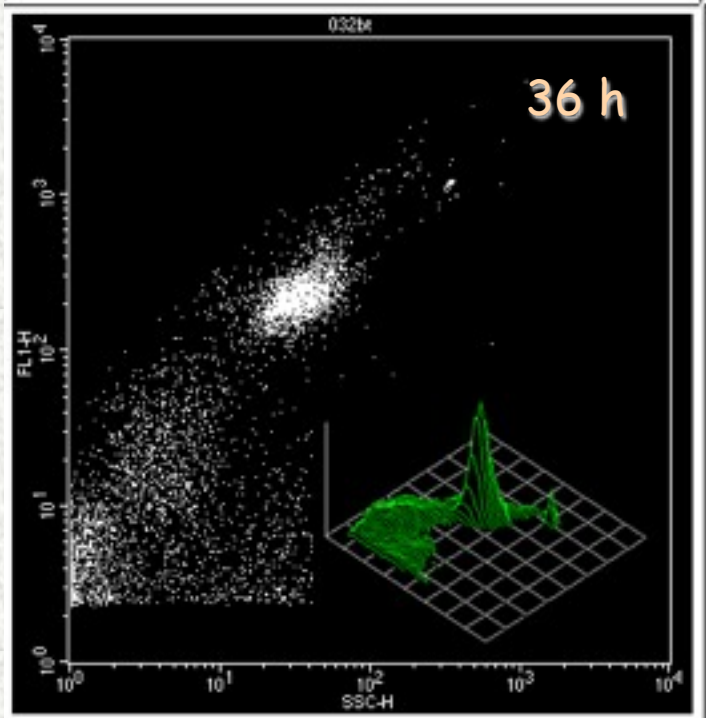
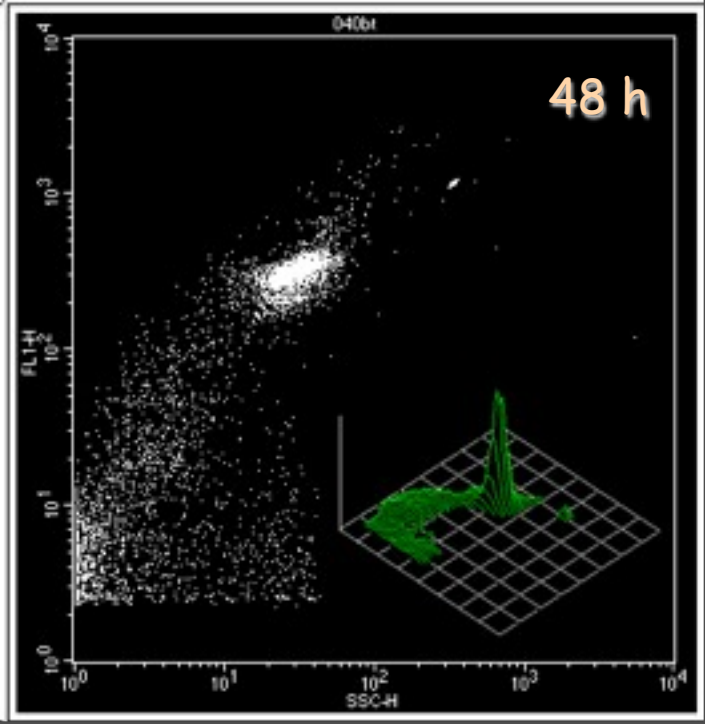
A CF experiment in the Pacific









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

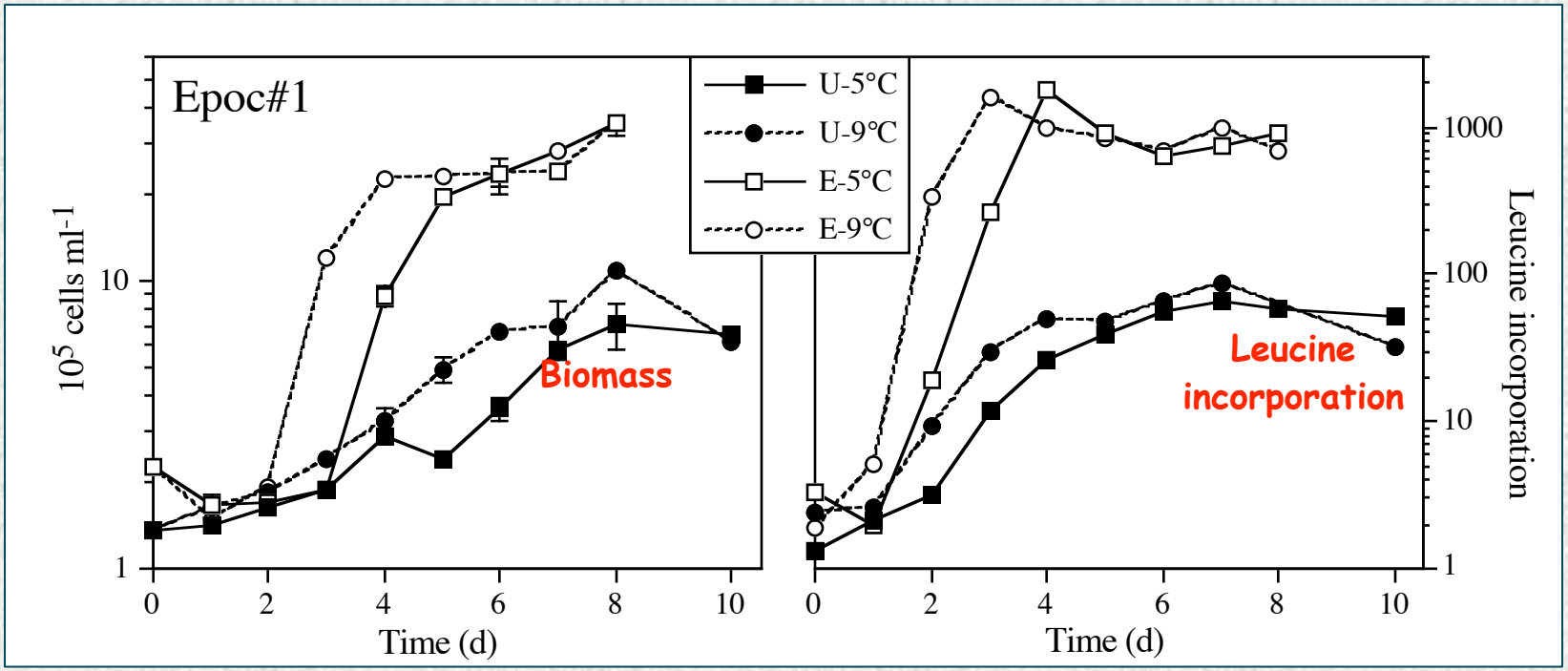
" In dilution experiments, uptake doesn't necessarily mean growth !!!. And even biosynthesis might not mean growth: at the start of starvation protein synthesis is detected at the same time than cells become smaller and smaller" (Güde 1990)

Have the factors obtained in those experiments any ecological sense ?

do they represent phylotype-specific properties or truly ecologically properties ?

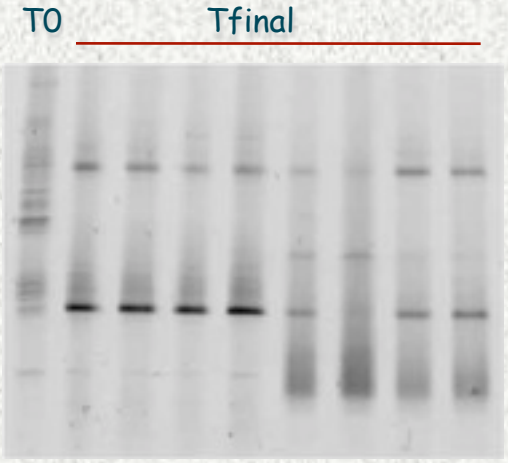
Example 1 - Antarctica

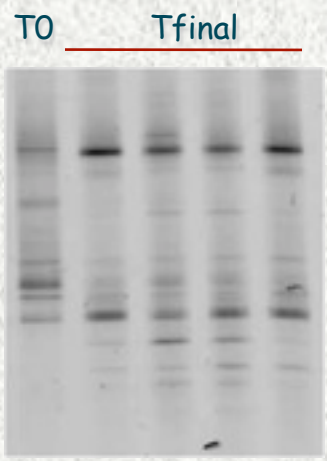
Bacterial diversity changes during incubations

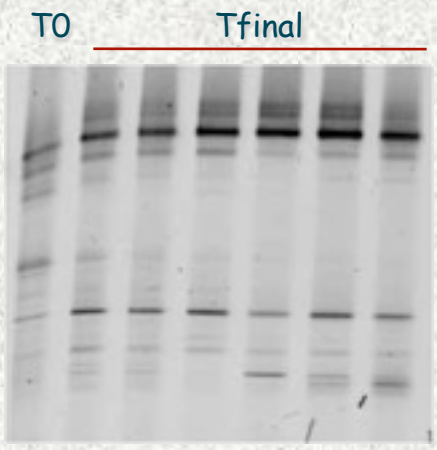


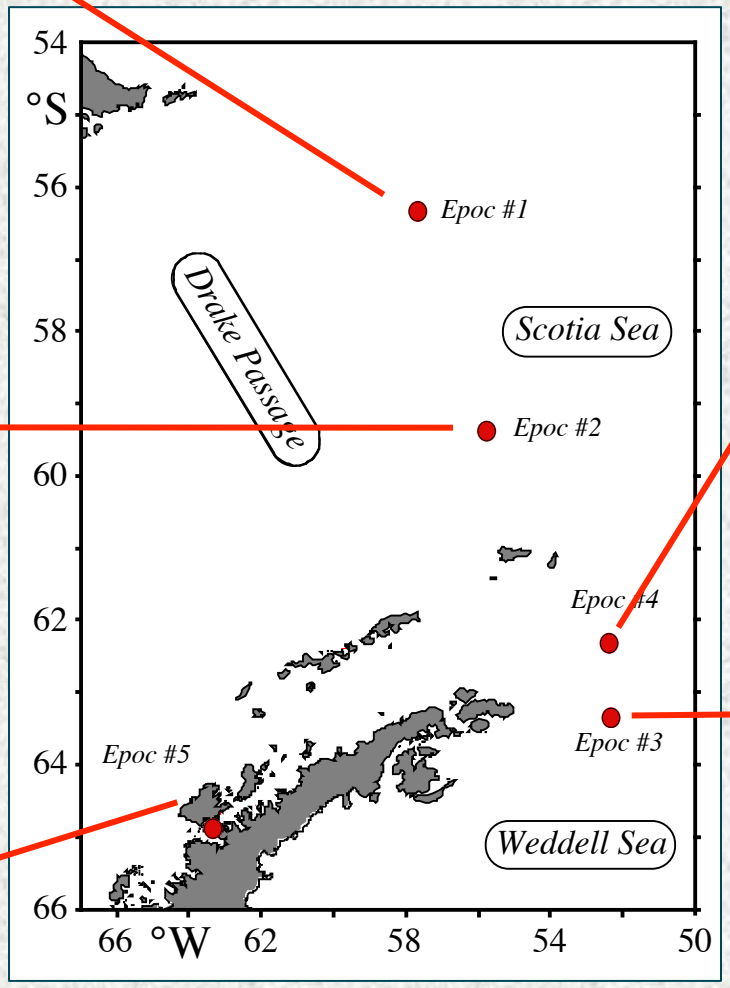
Massana et al. 2001, L&O.

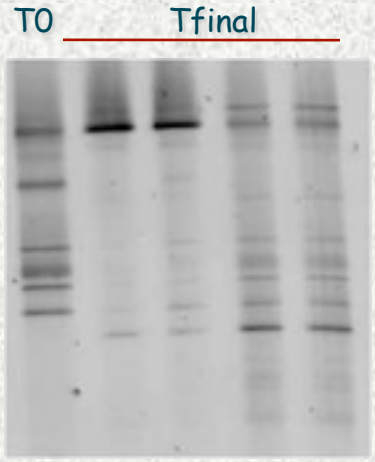
Incubations during cruise DHARMA

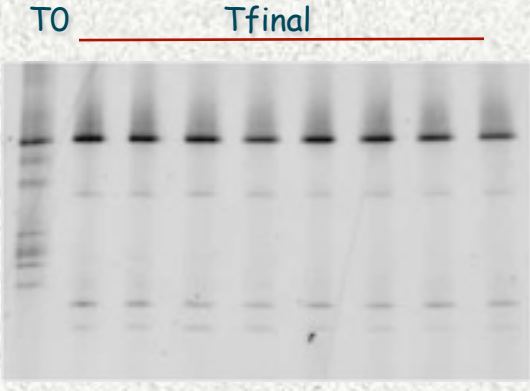






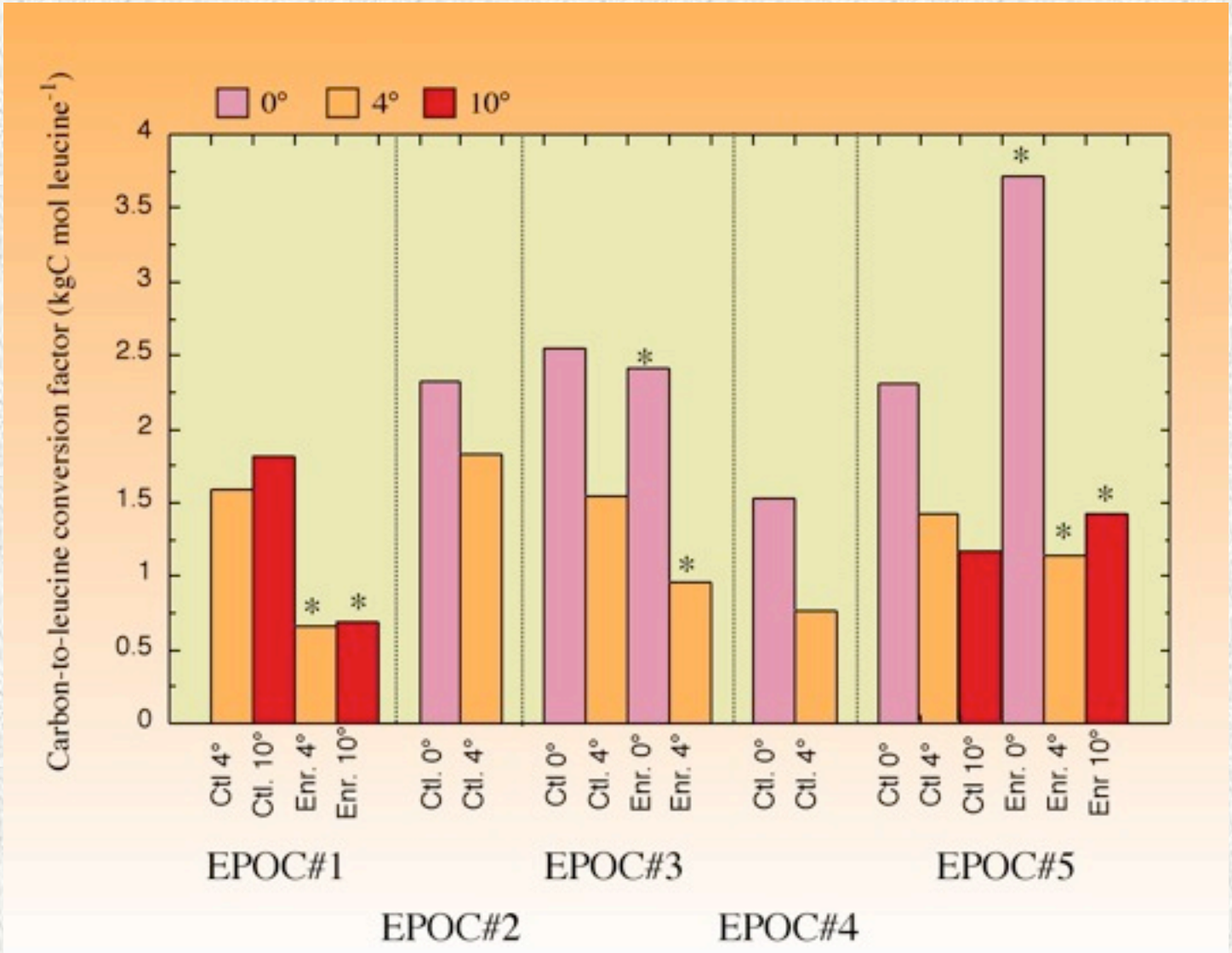






Massana et al. 2001, L&O.

Conversion factors from the Dharma cruise experiments

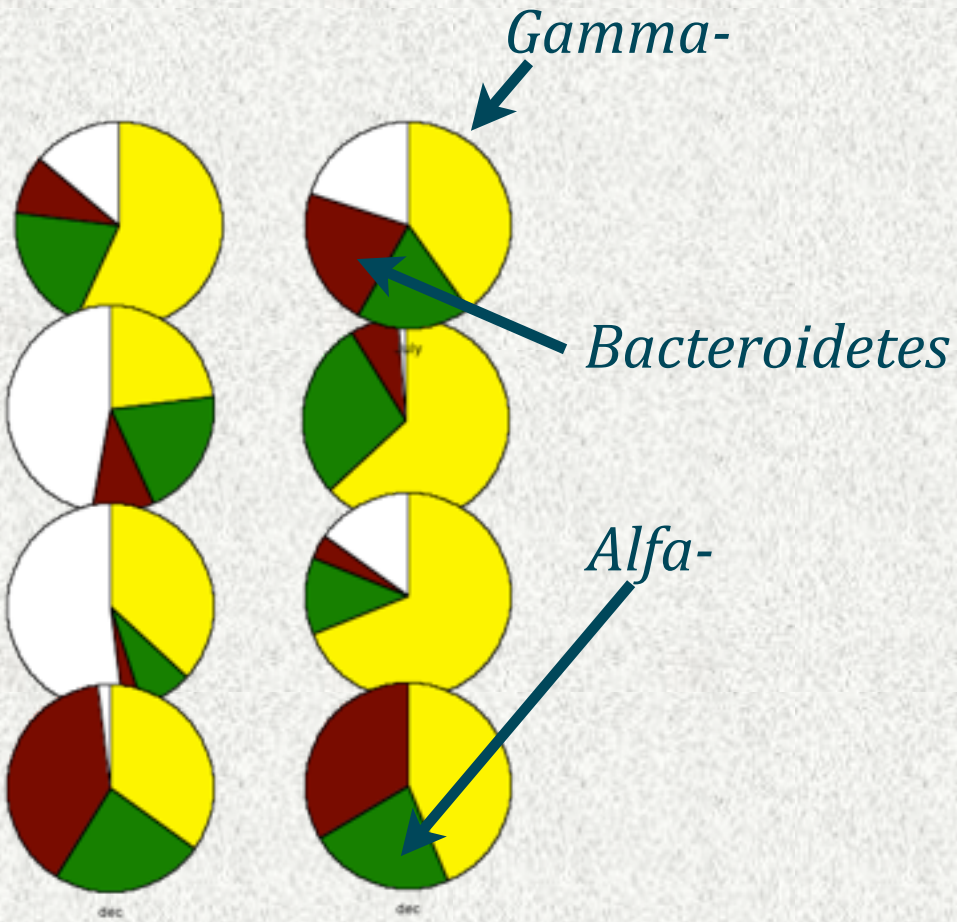


the same bacterial assemblage is selected in each experiment, but the factor does vary systematically with temperature and enrichment

Gasol et al, submitted

Example 2 - Blanes Bay

eCF	Unamm	Amm
July	1.3	1.0
Aug	1.3	0.6
Sep	1.4	0.4
Dec	3.6	1.9



Alonso-Sáez et al. 2010, ENM.

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