L02a: Metabolic Diversity and Ecophysiology of Marine Microbes

0. Ethics in science, plagiarism and AI

1. Microbes in the Marine Environment

2. Metabolic Diversity and Ecophysiology of Marine Microbes

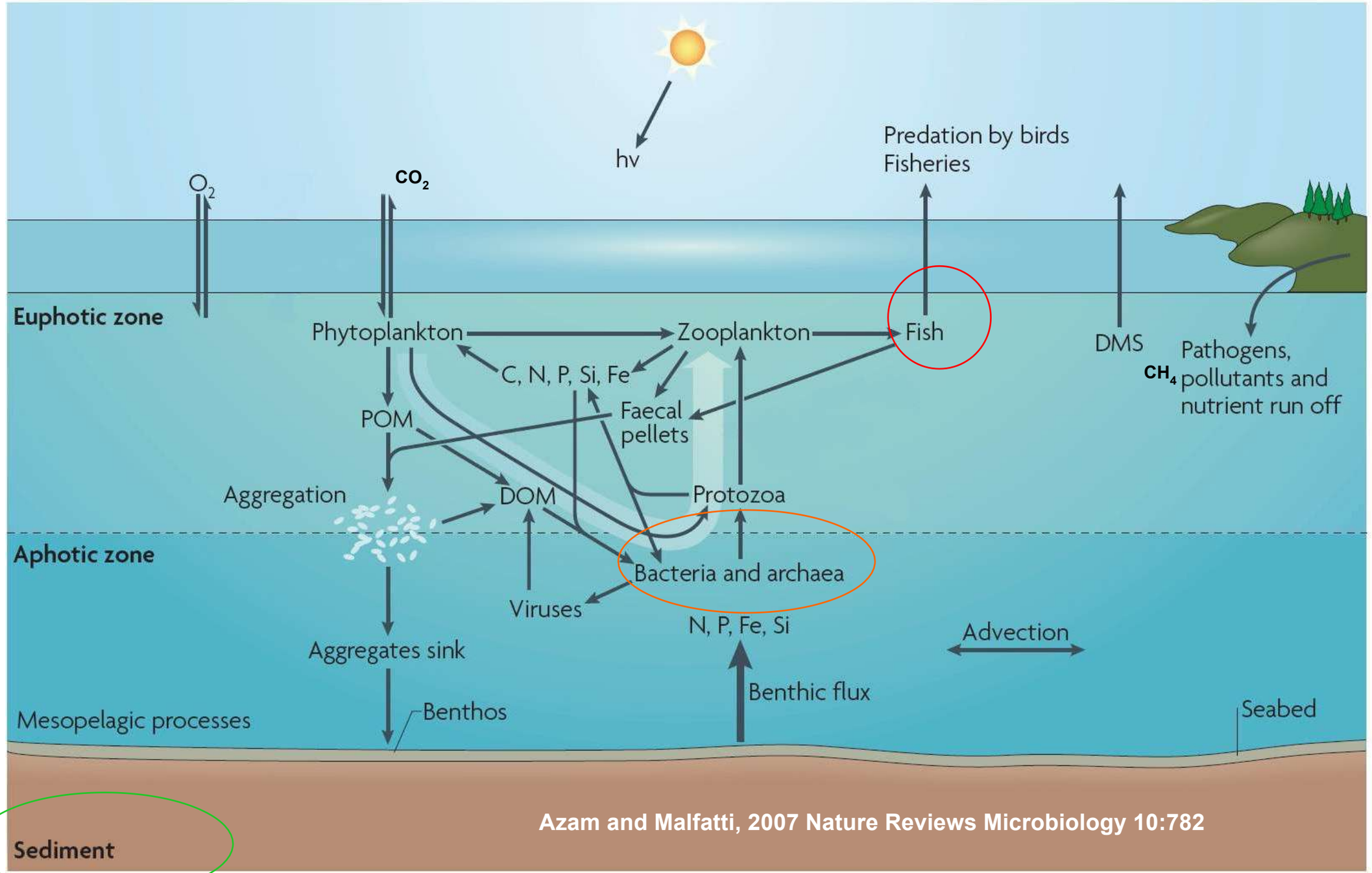
3. Diversity of Marine Bacteria, Archaea, Viruses and unicellular Eukaryotes

4. Microbes in Ocean Processes: Carbon and other nutrients

5. Marine Microbial Symbioses and Diseases

6. Marine Microbes, Climate and Society

Marine Microbial Carbon Biogeochemical cycle

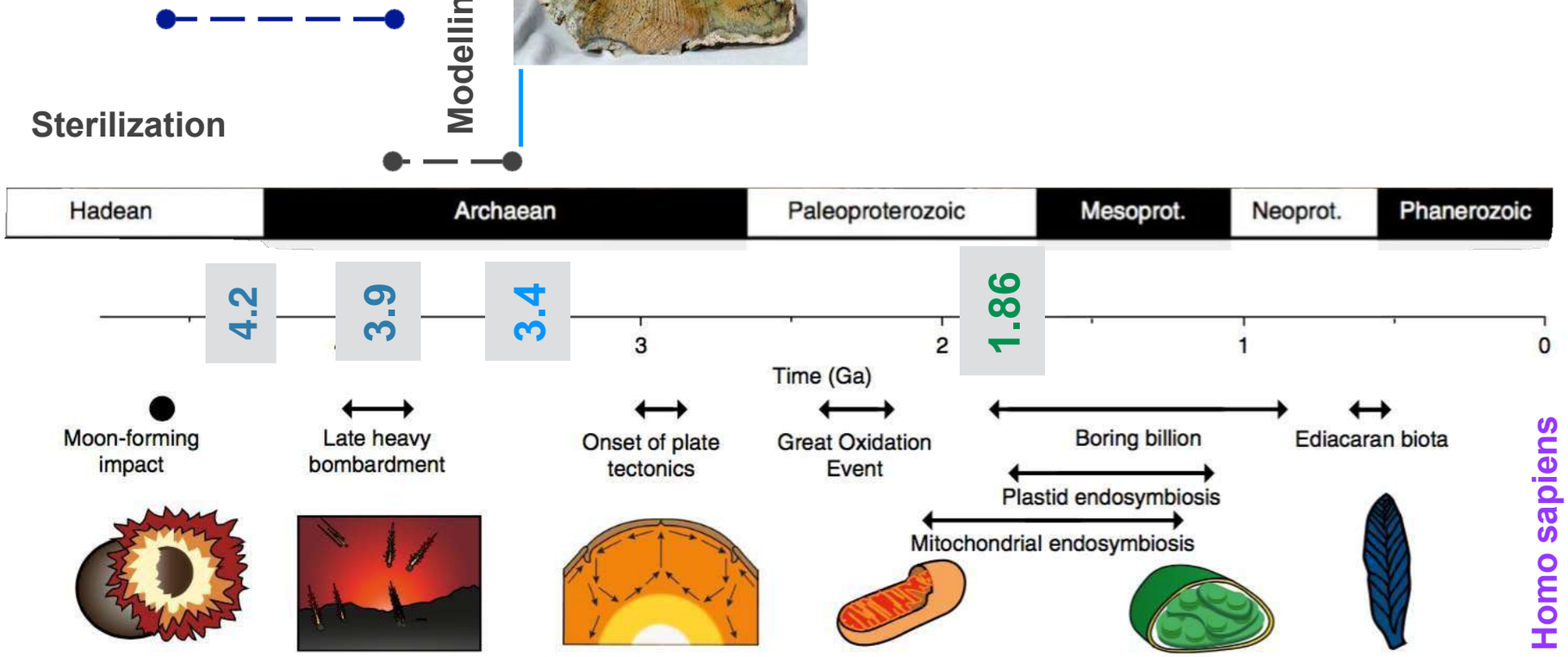


Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

Life on Earth and in the Ocean



Strelley Pool Formation, stromatolites, Australia

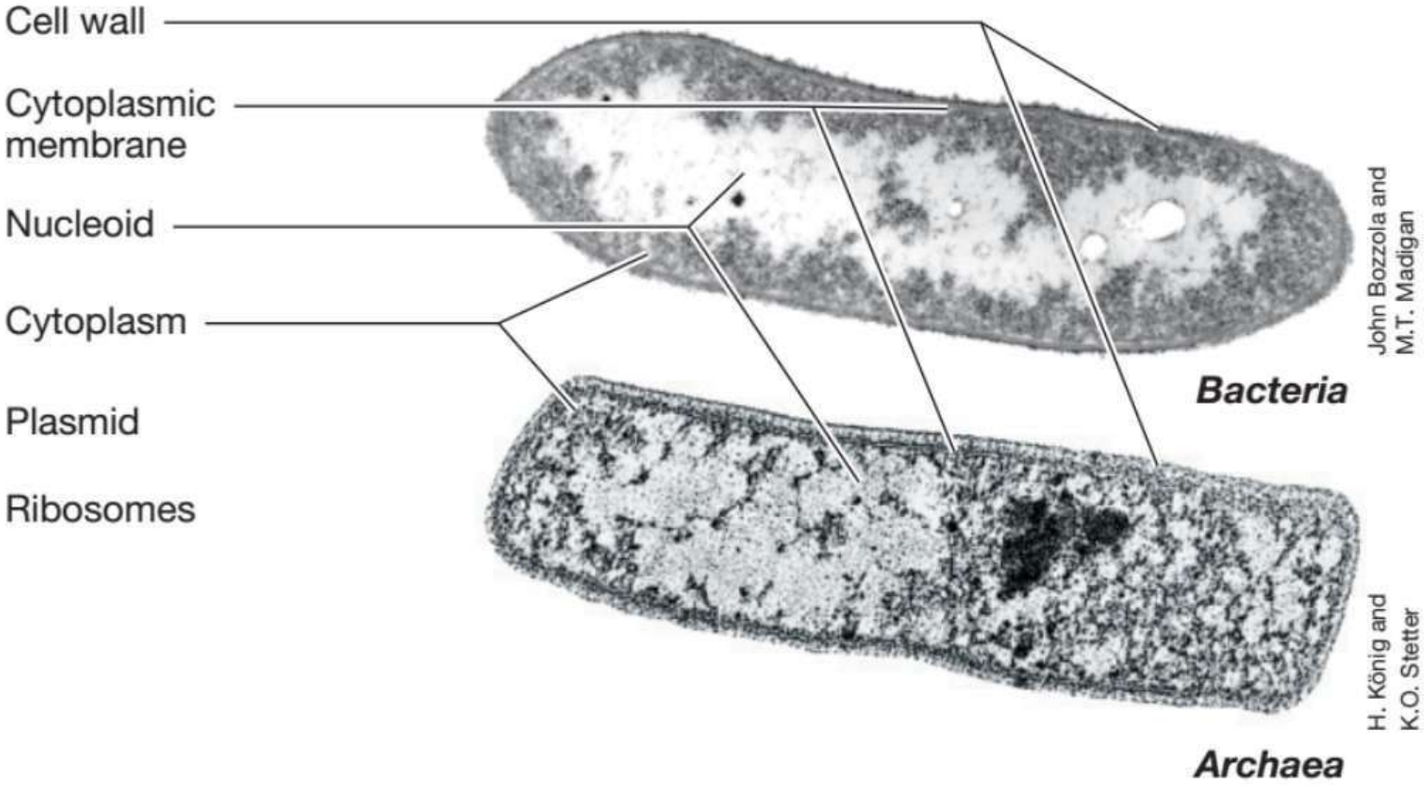


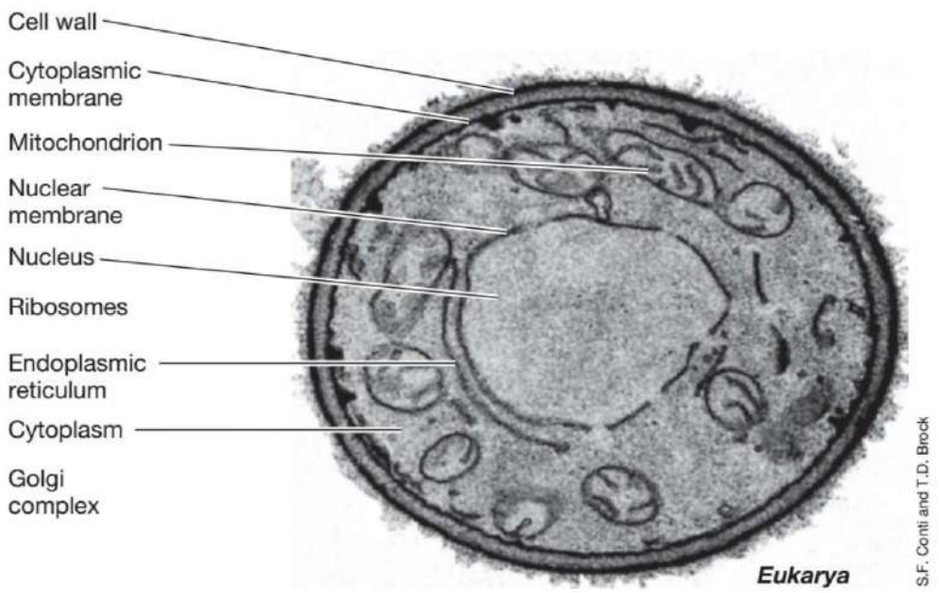
Betts et al., 2018
Moody et al., 2024

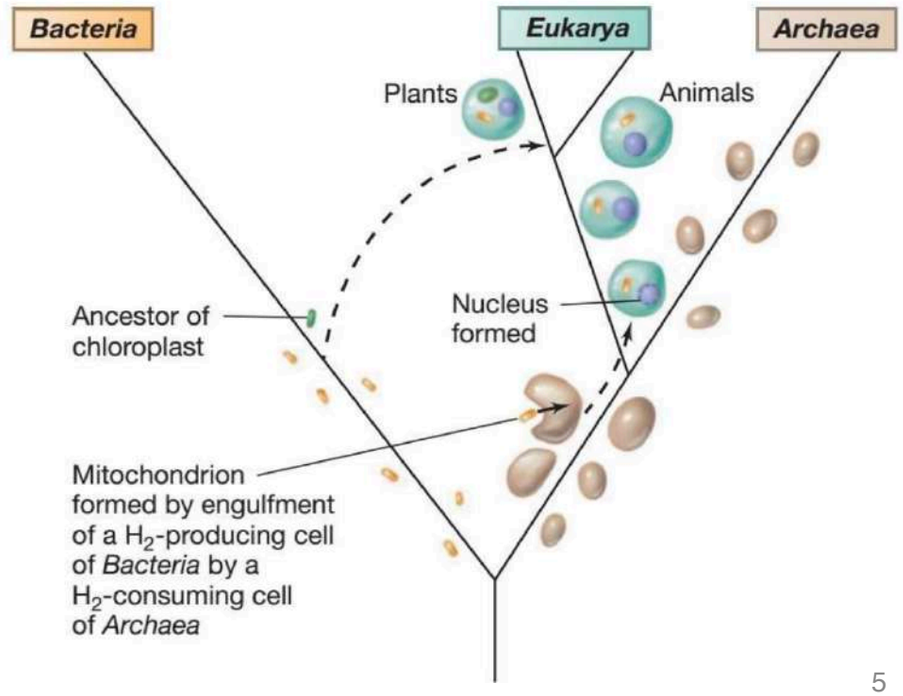
LUCA: Last Universal Common Ancestor

Earth and Ocean were very different than today

Bacteria, Archaea & Eukarya





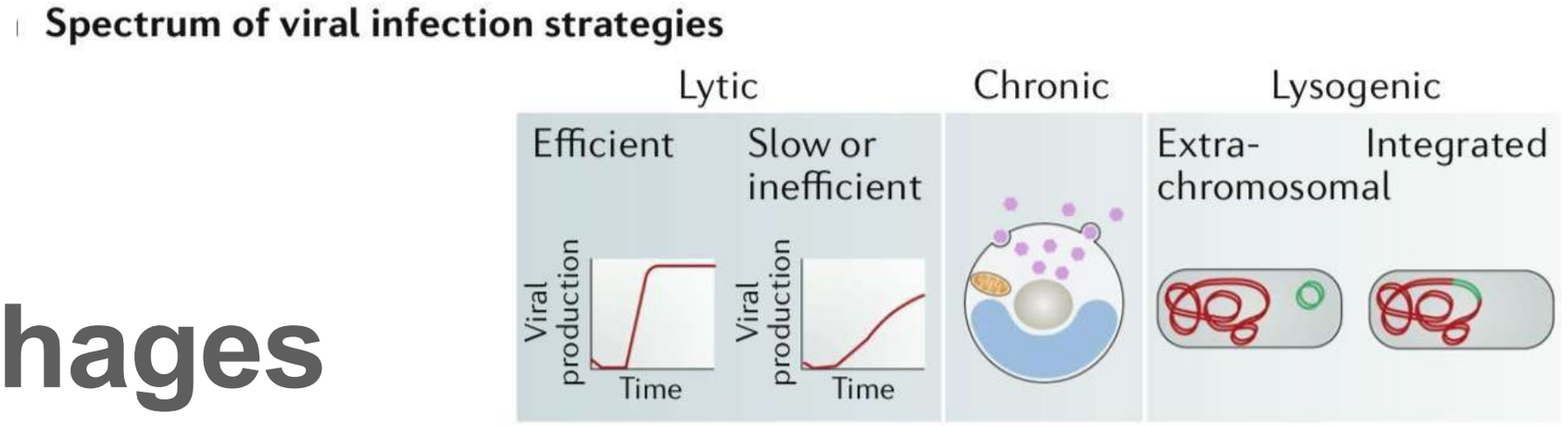


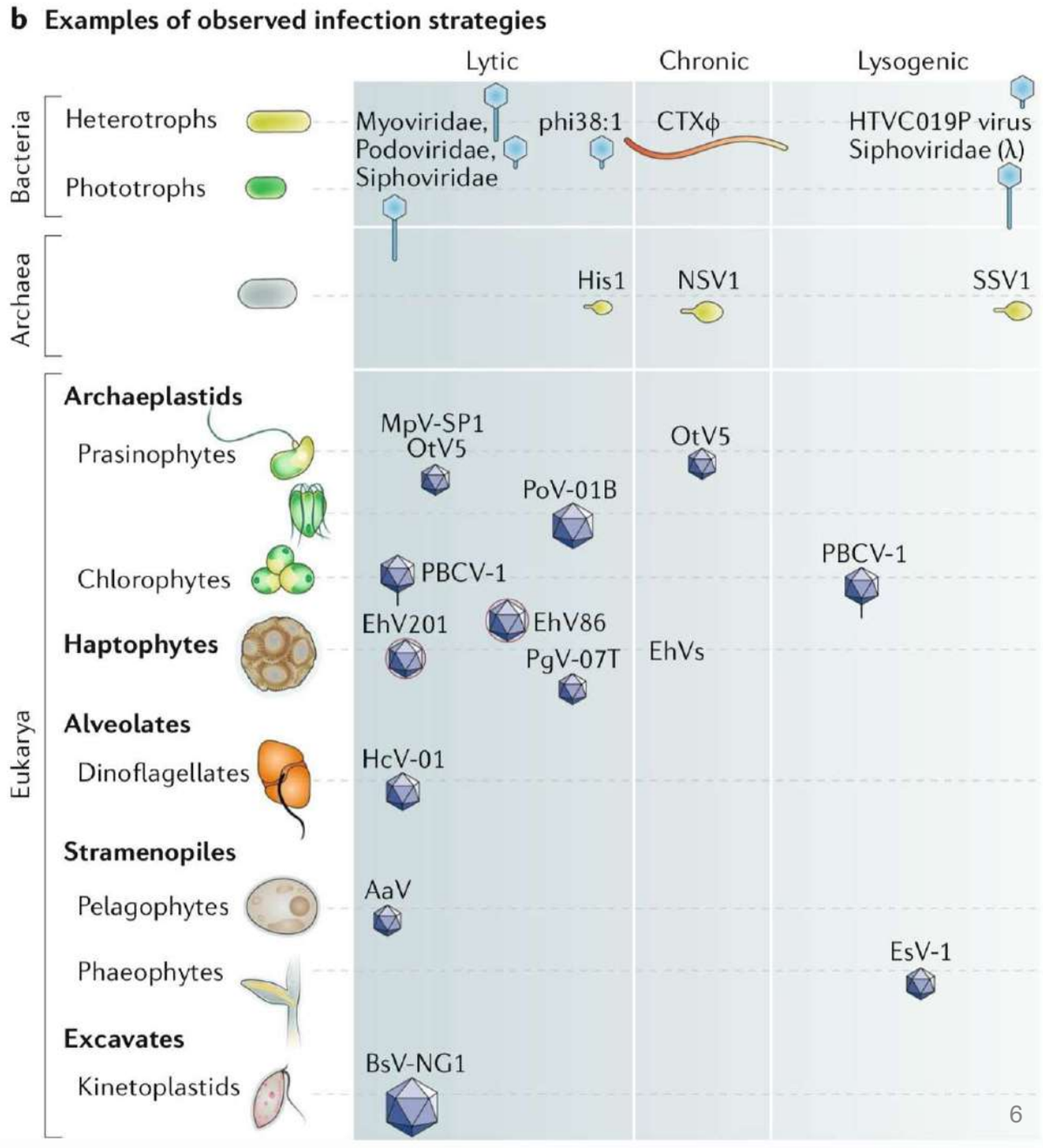
Madigan et al. 2018

Viruses/Phages

- **Moving genetic information**
- **Role in evolution**
- **RNA - DNA**
- **Diverse life strategies**
- **Interaction with every domain of life**
- **Interaction with other viruses**
- **Cells can become resistant**

Zimmerman et al. 2020

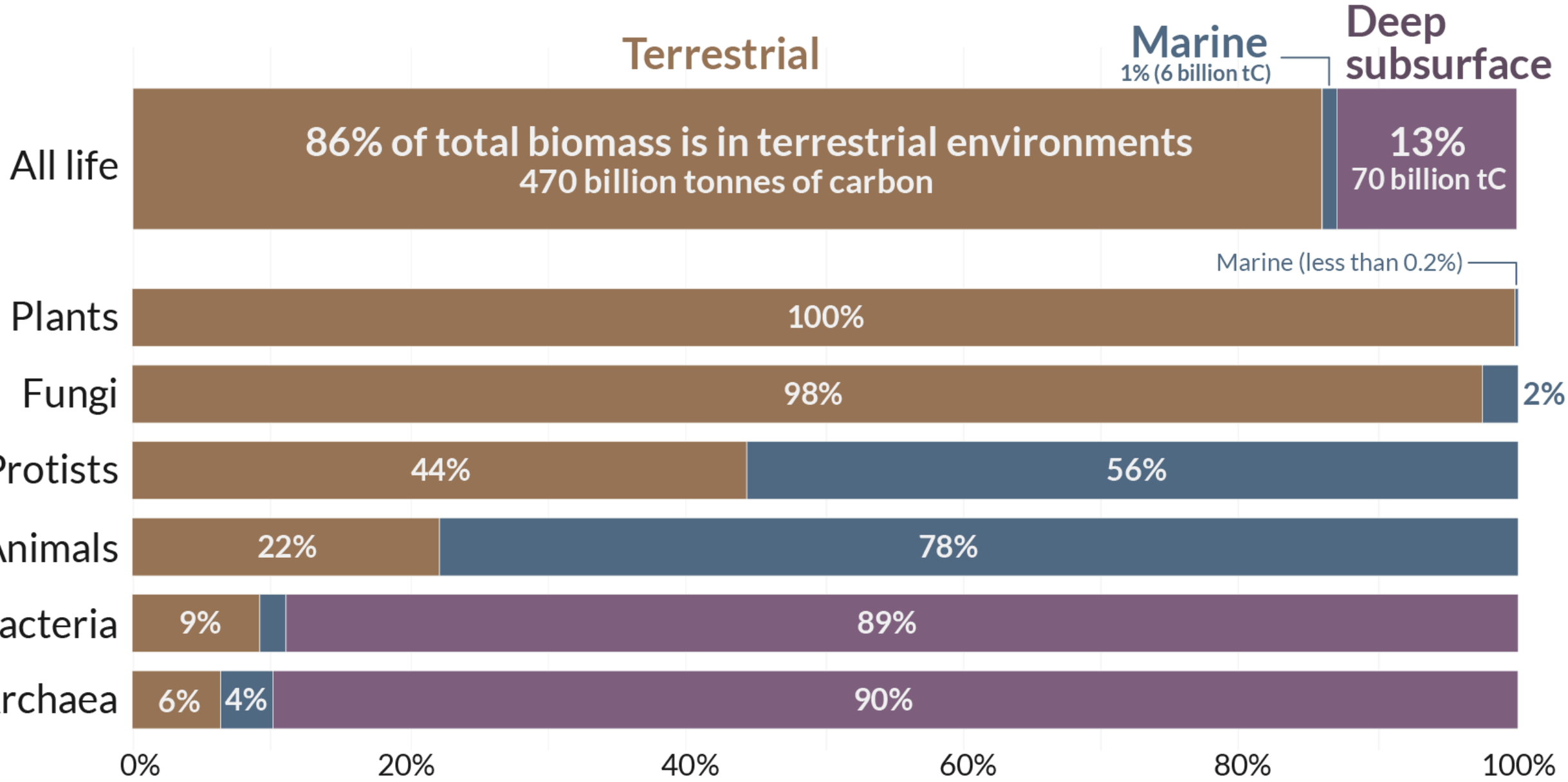




Where do we find life on Earth?

Distribution of global biomass across the world's environments. Biomass is measured in tonnes of carbon.

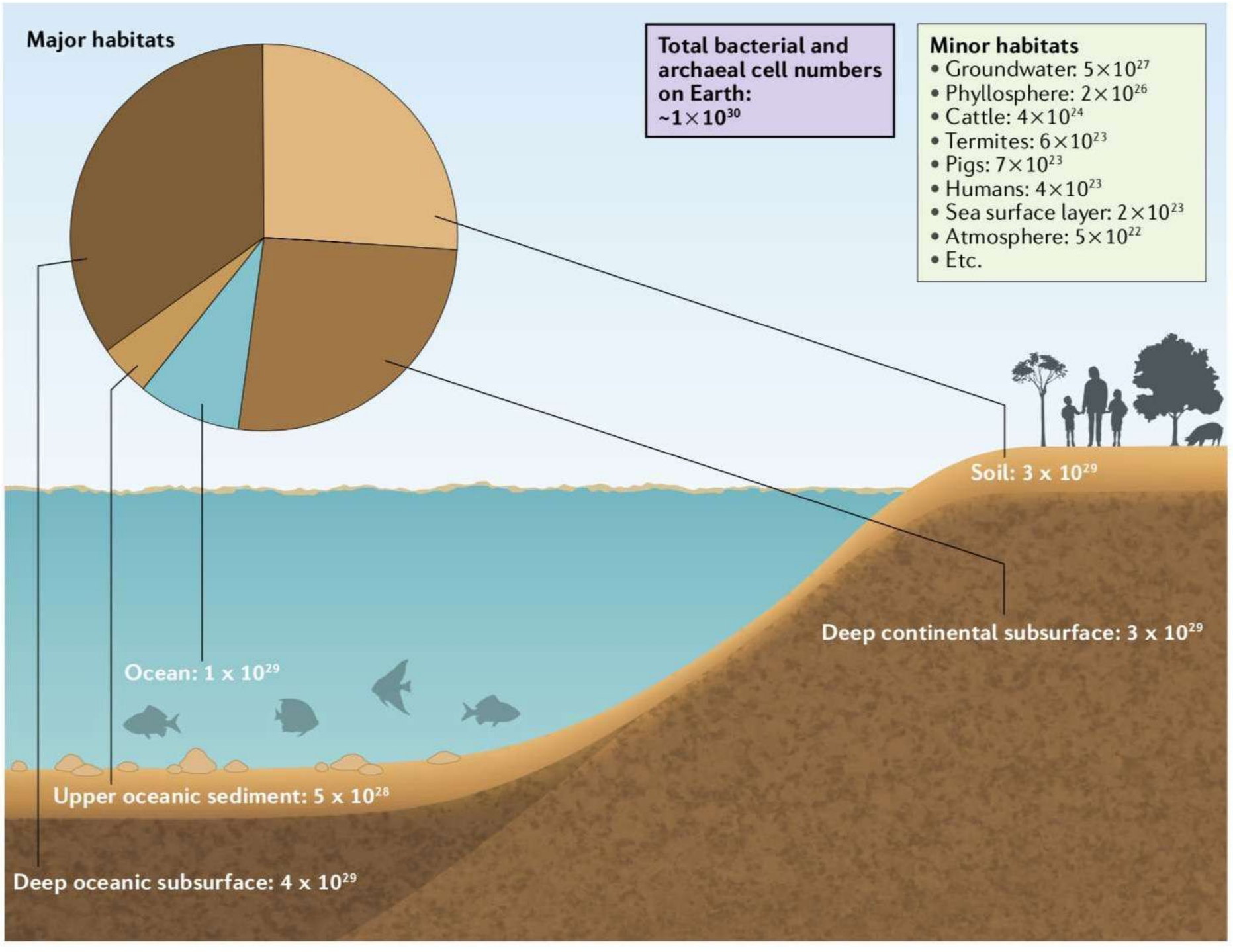
Our World in Data



Data source: Bar-On, Y. M., Phillips, R., & Milo, R. (2018). The biomass distribution on Earth. *Proceedings of the National Academy of Sciences.*

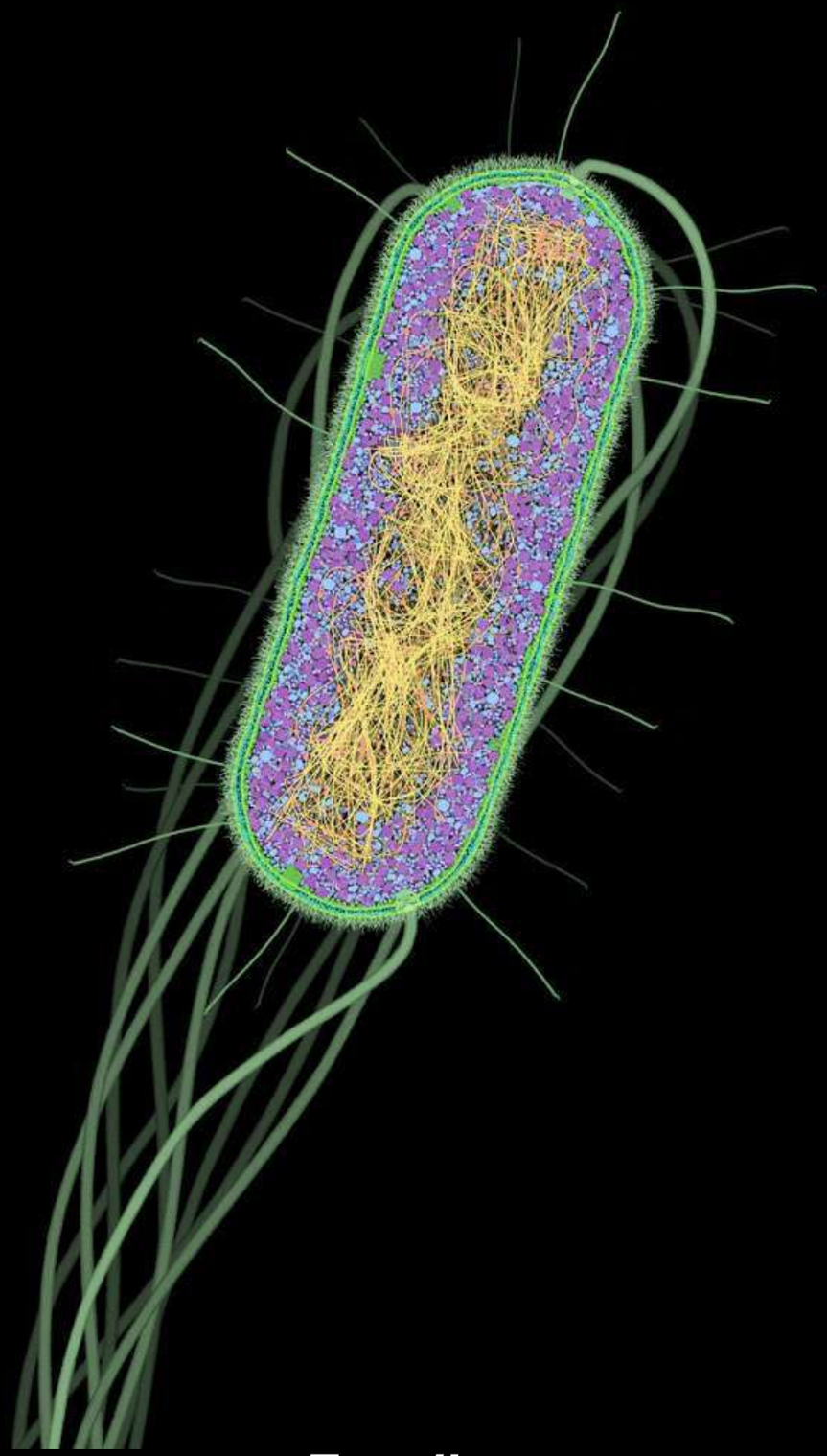
OurWorldinData.org – Research and data to make progress against the world's largest problems. Licensed under CC-BY by the authors Hannah Ritchie and Max Roser.

Abundance of bacteria and archaea in different habitats on Earth



Flemming & Wuertz, 2019

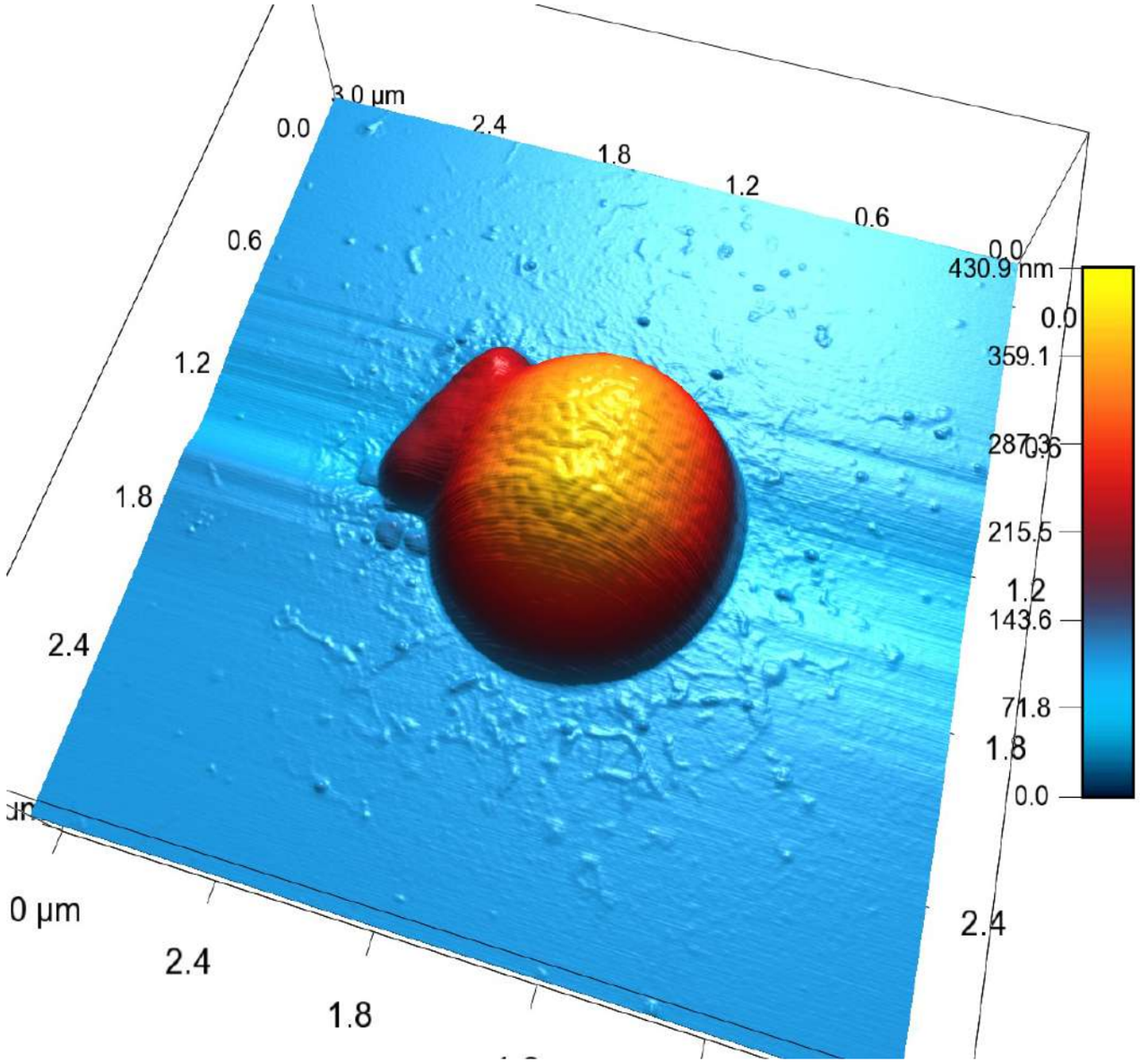
From micro to nanoscale



David S. Goodsell

E. coli

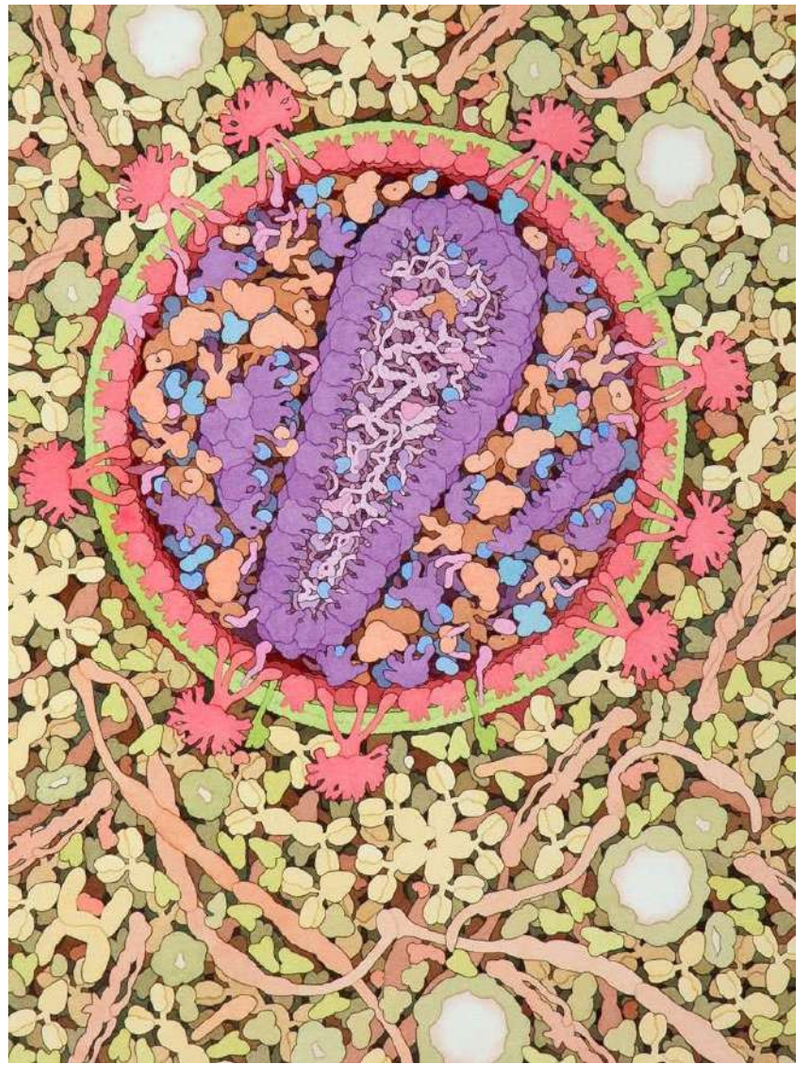
Synechococcus & heterotrophic bacterium



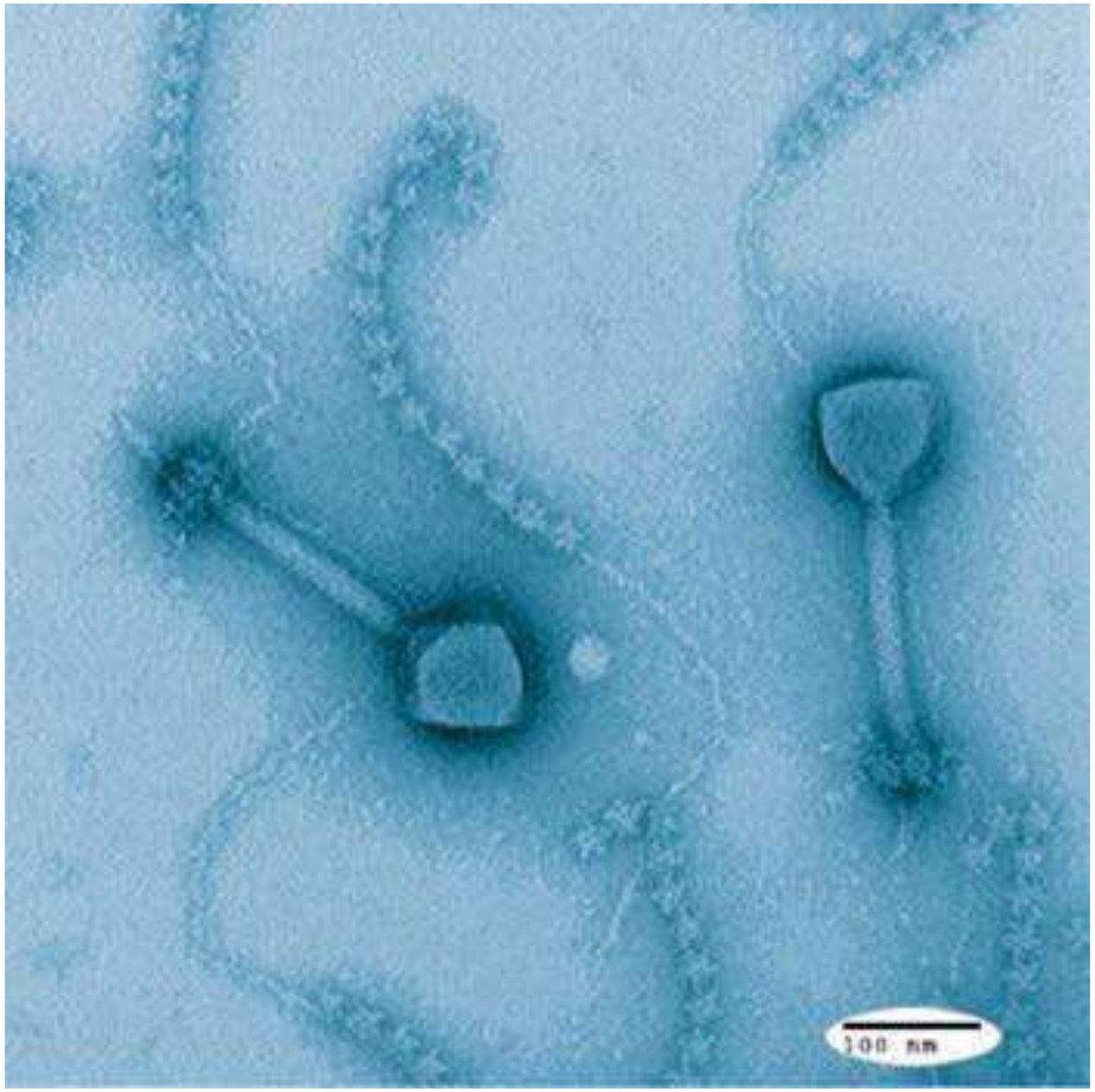
F. Malfatti

Viruses/Phages

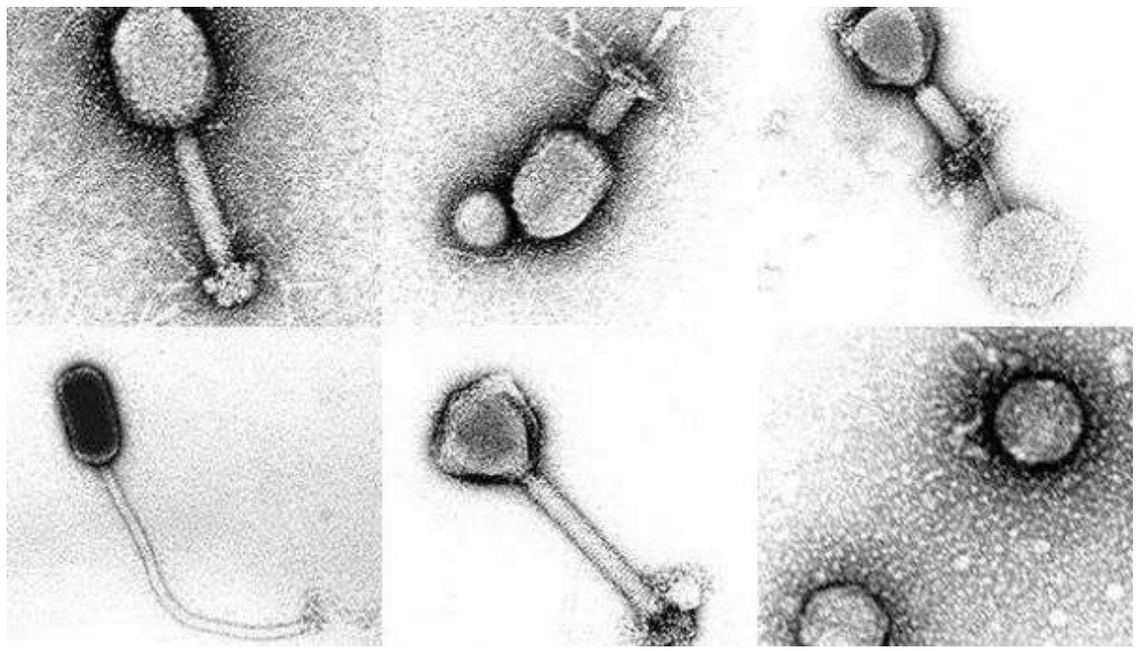
Nucleic Acid
Proteins
Some lipids



David S. Goodsell

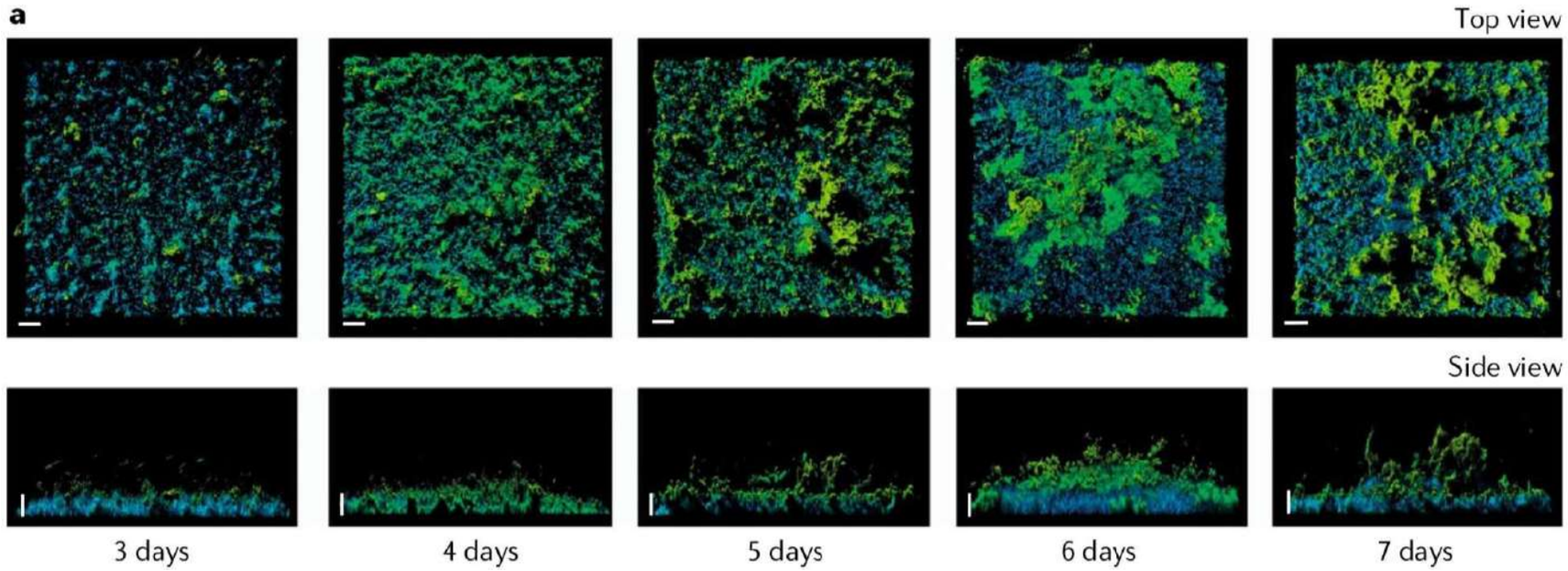


Jennifer Brum, Tucson Marine Phage Lab

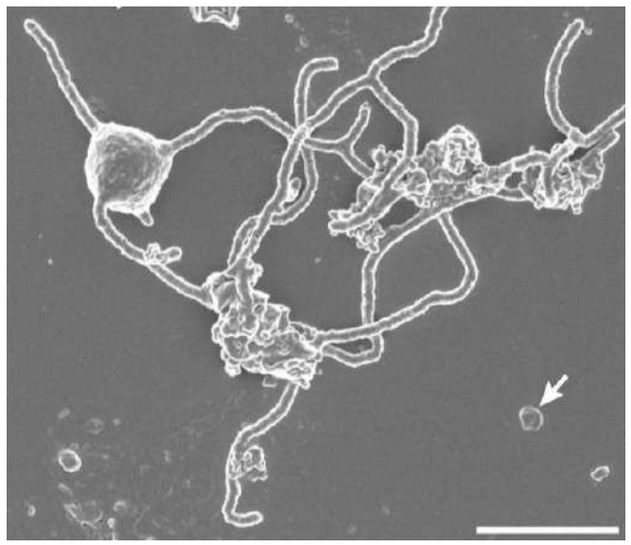


Sullivan lab Lab

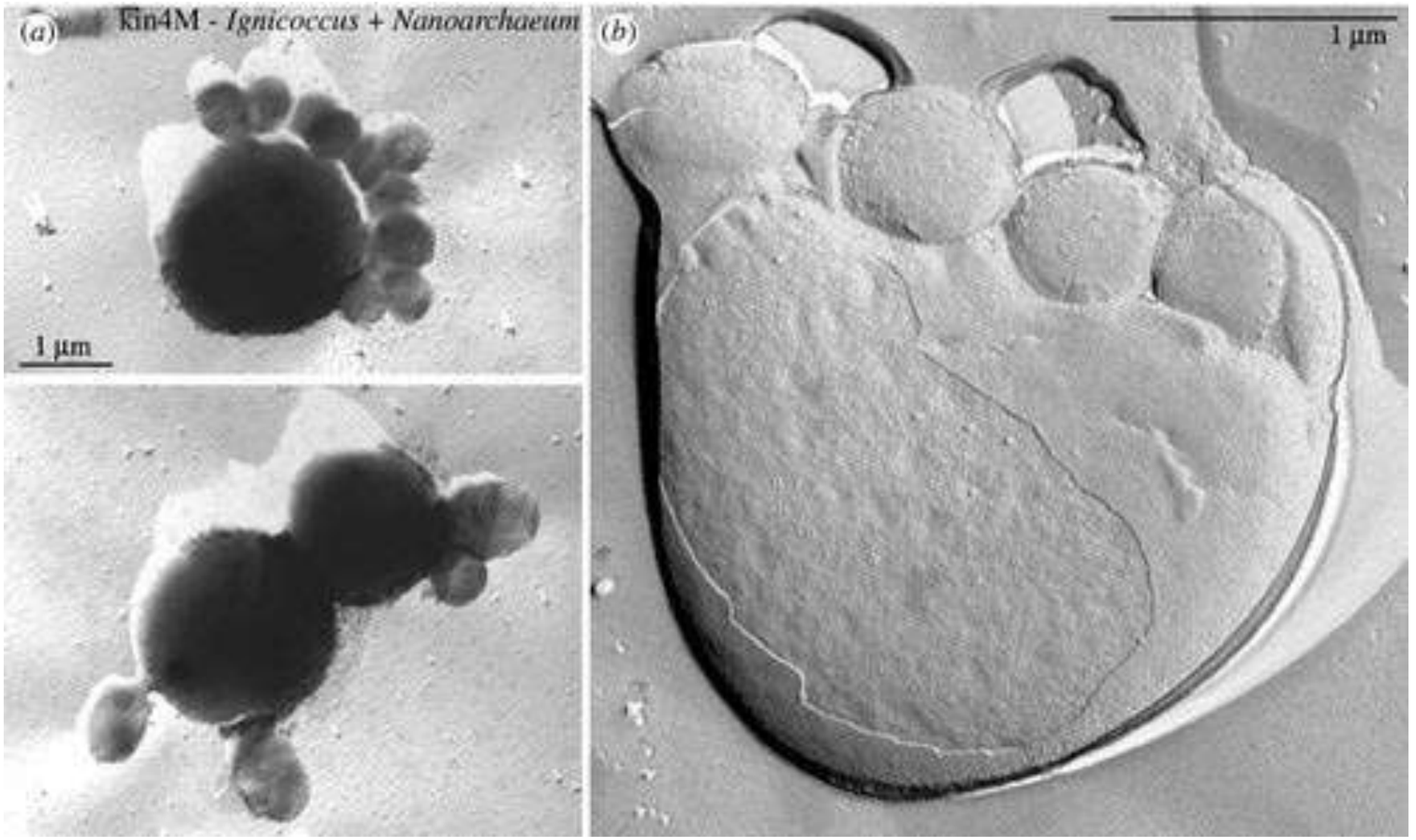
Archaea



van Wolferen et al., 2018



Imachi et al. 2020



Hyperthermophiles in the history of life, Volume: 361, Issue: 1474, Pages: 1837-1843, 2006

Bacterial cities

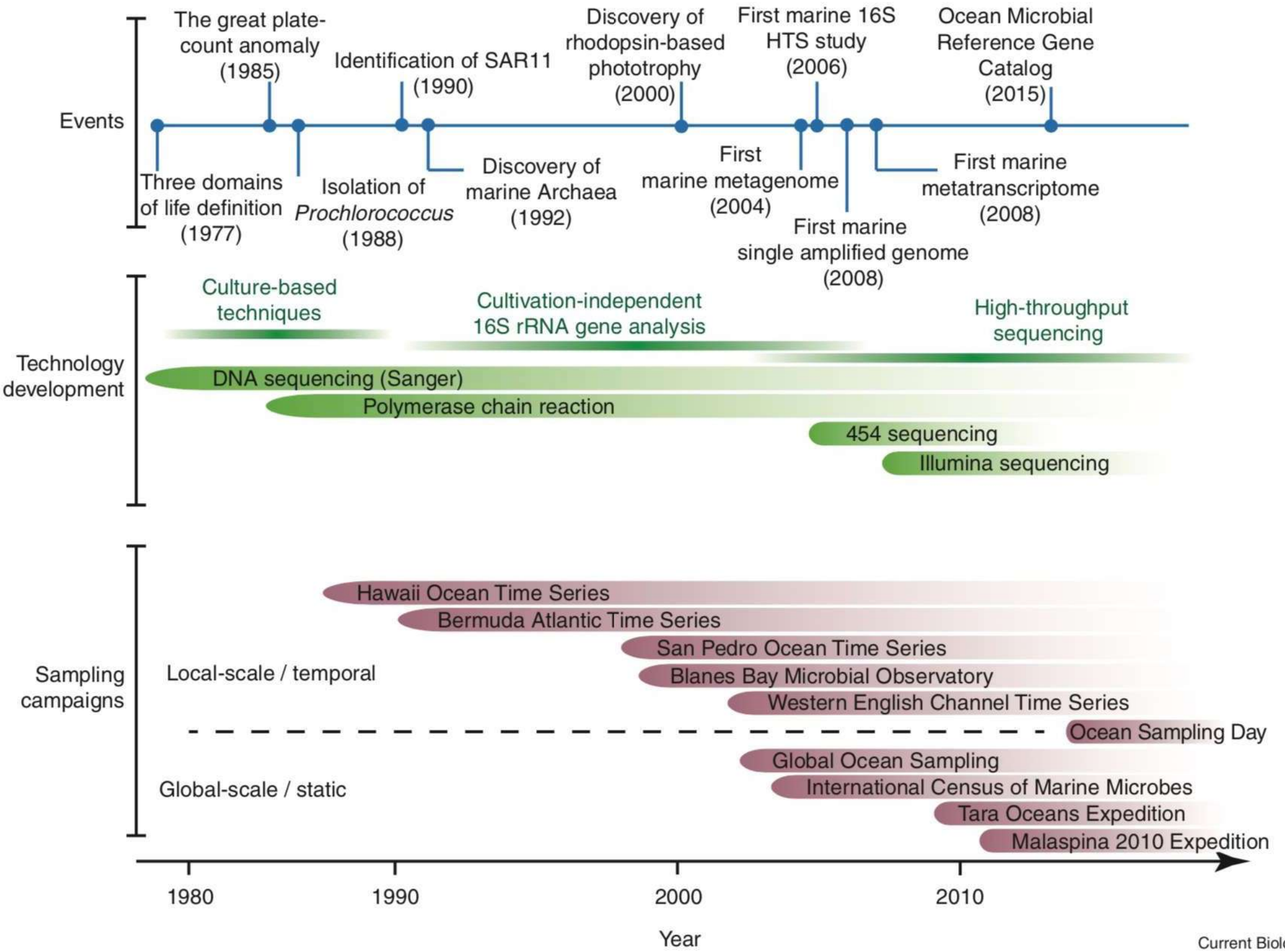
Marine bacteria colonies growing on an agar plate



Lophelia II 2012 Expedition, NOAA-OER/BOEM

- **The Ocean is a desert**
- **The Ocean is too cold**
- **The Ocean is too dark**
- **There are only 1000 bacteria per mL of SW… what can they do?**

Technology development boots marine microbial ecology



Salazar & Sunagawa, 2017

The Age of Discovery

- 1977 Bacteria 10^6 mL^{-1} (10^3 x cfu: great plate anomaly)
- 79/80 High bacterial growth & C demand (dynamic populations)
- 84 Protozoa (10^3 mL^{-1}) major predators on bacteria
- 79-90 Viruses abundant (10^7 mL^{-1}) & major predators on bacteria
- 79 *Synechococcus* 10^3 -10^5 mL^{-1}
- 88 *Prochlorococcus* 10^4-10^5 mL^{-1}
- 90 Widespread Archaea throughout the oceans (10^4-10^5 mL^{-1})

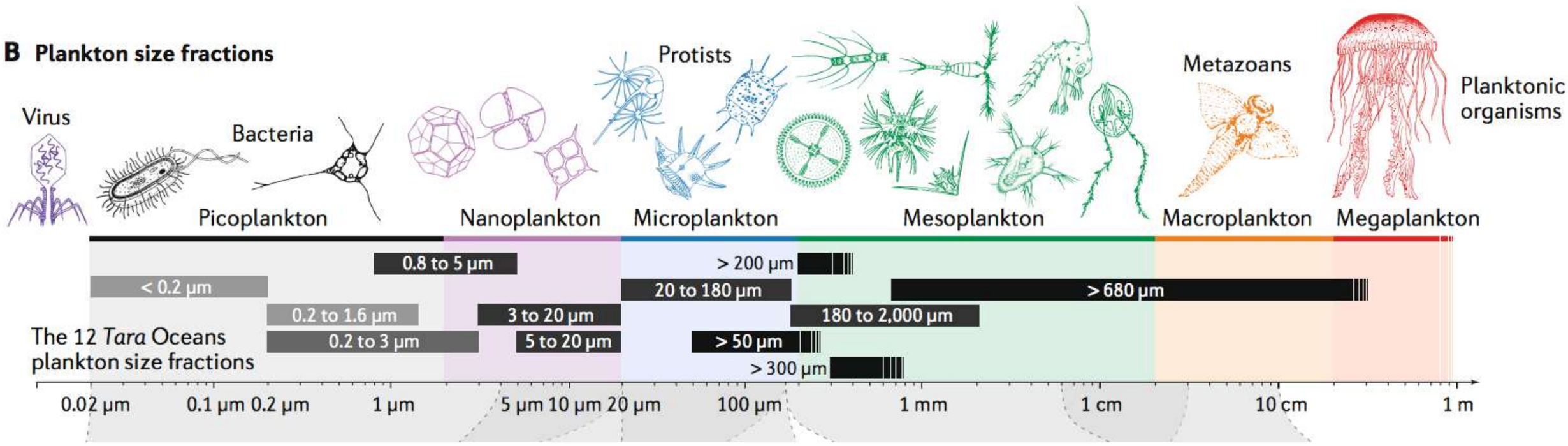
Form Farooq Azam

The Age of Discovery

Form Farooq Azam

- 80-90-00 Microscale ecology
- Discovery of anoxygenic photosynthetic bacteria
- Rise of molecular ecology
- Massive marine genomics and metagenomics-- providing constrains for diversity and ecosystem function
- Culturing the “unculturable”
- Widespread picoeukaryotes
- Holistic approach: new chemicals, new way to design nano materials, detoxification
- OneHealth and Micorbes in Ocean-Climate models

Sizing the Microbial Ocean

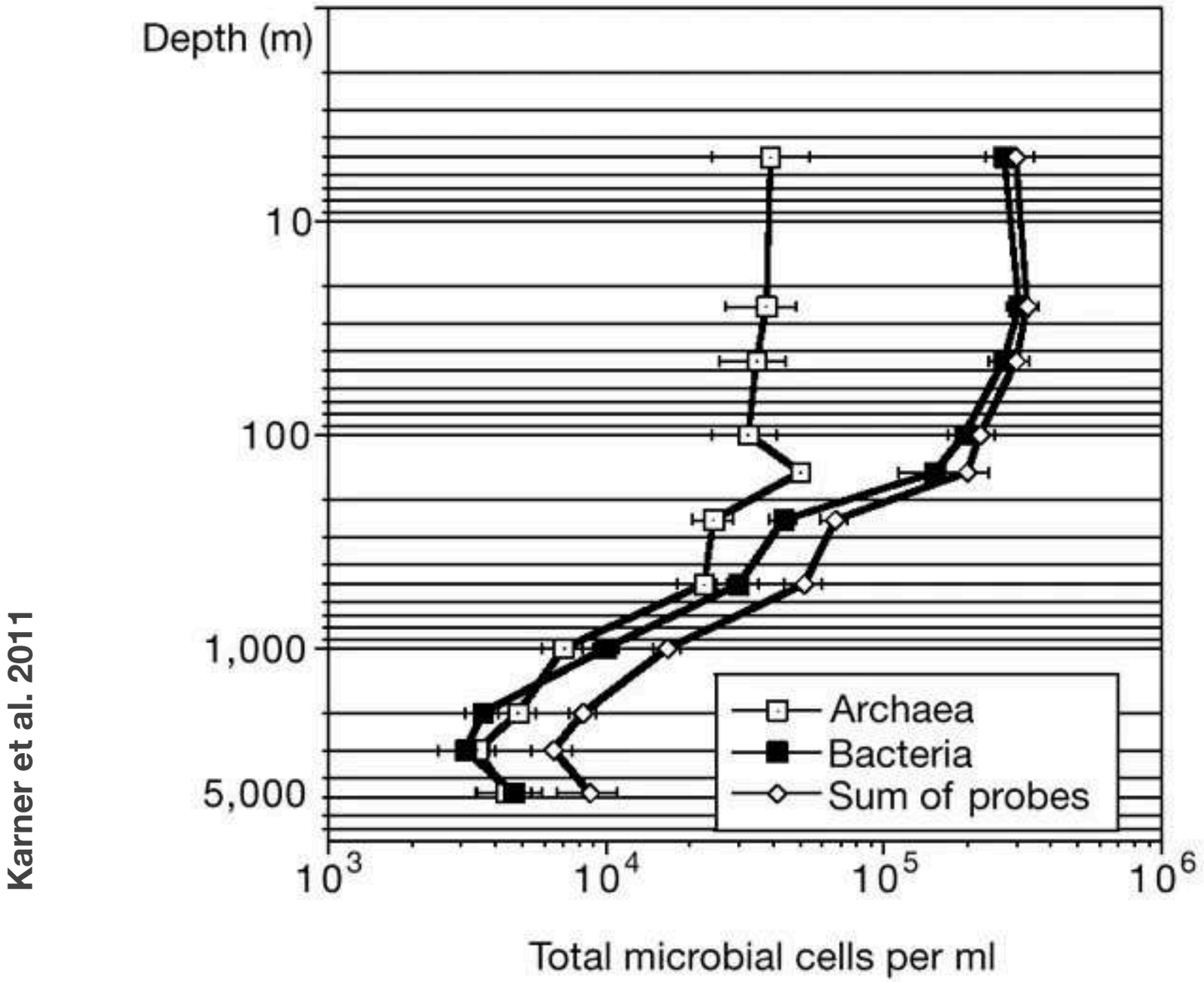


Phytoplankton

Zooplankton

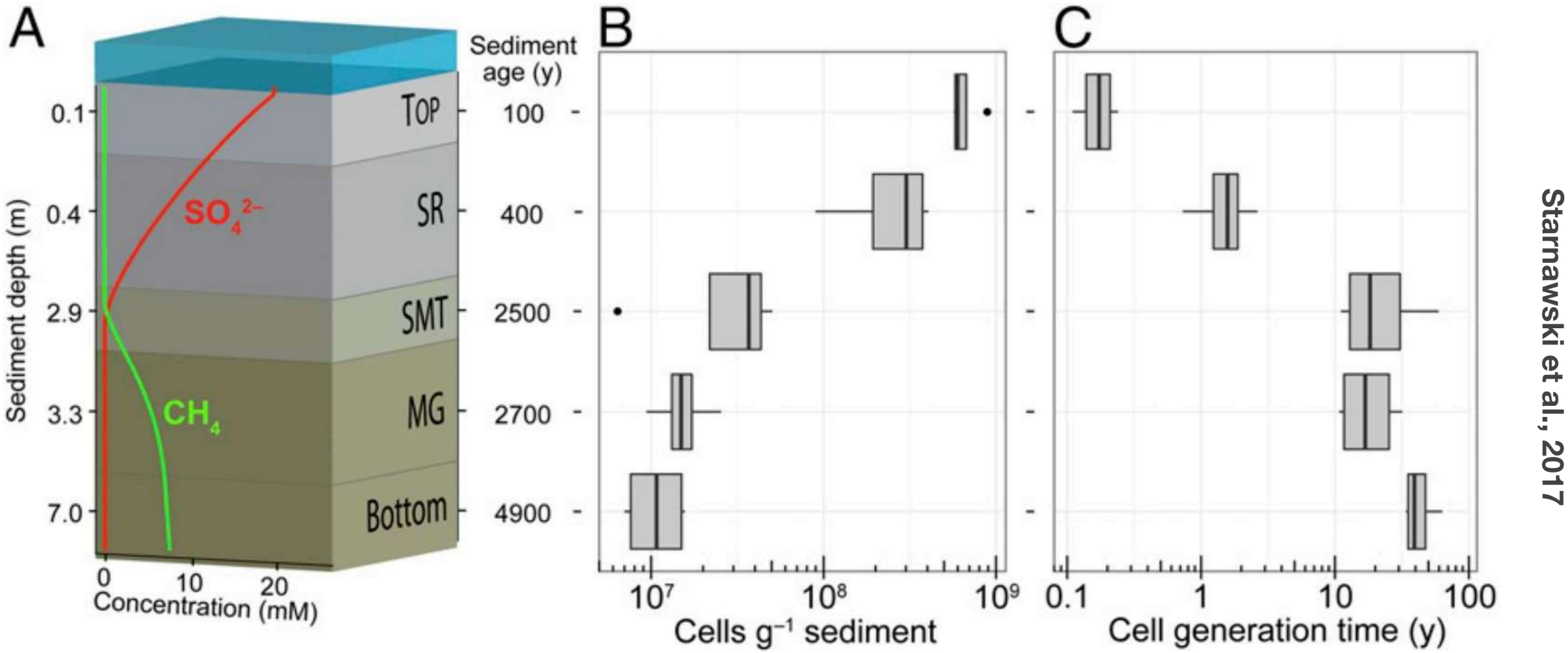
Sunagawa et al., 2020

SW Depth profiles of microbial domain cell abundance: Bacteria and Archaea



Data are averages of up to 14 roughly monthly samplings over a 1-yr period at the Hawai'i Ocean Time-series station, ALOHA

Sediment depth profiles of microbial cell abundance



Starnawski et al., 2017

Microbial (Bacteria-Archaea) abundance across the atmosphere-ocean-sea sediment:

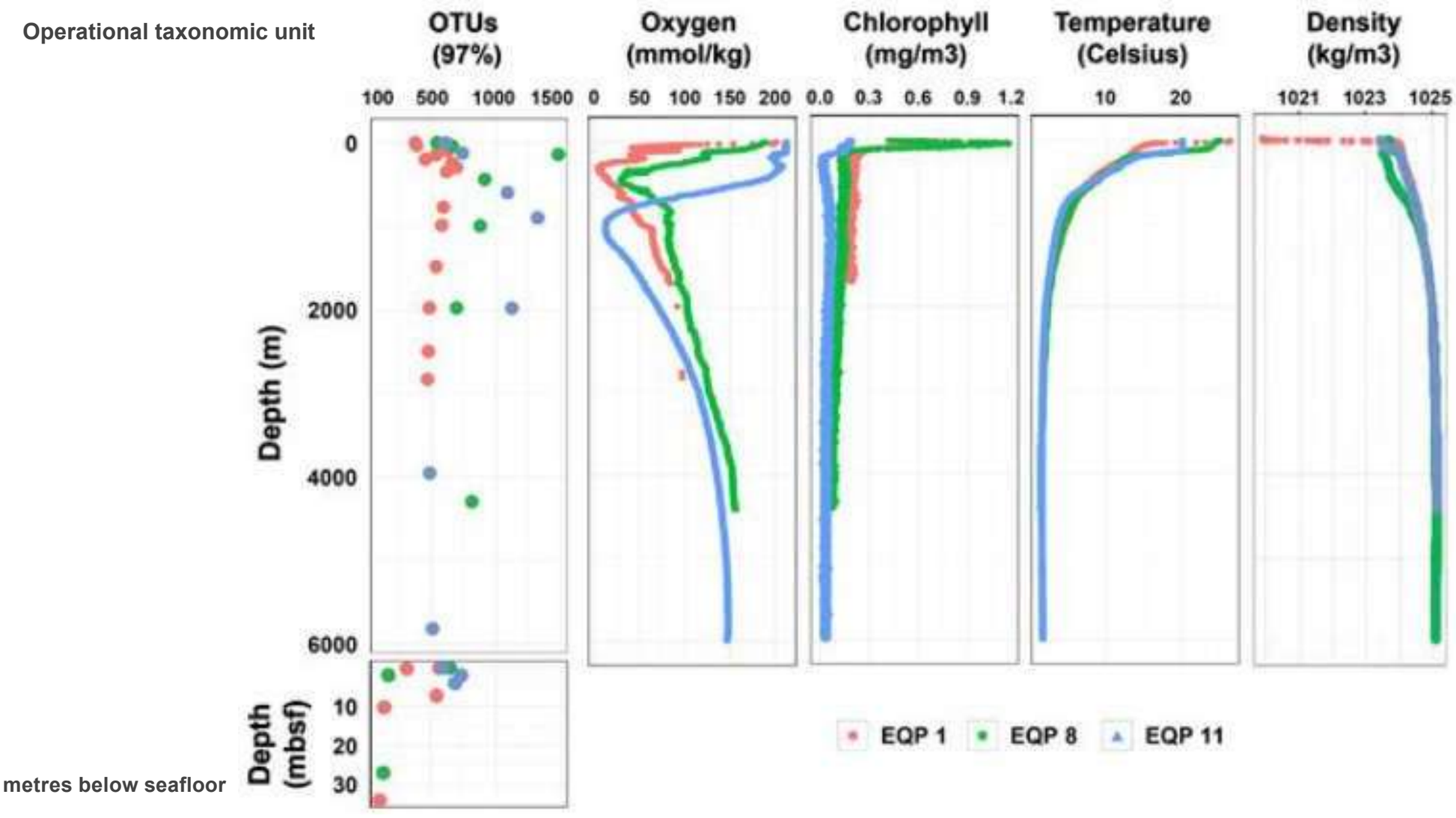
Sea-spray aerosol: 10^2-10^5 cells L^{-1} of air

Sea Surface Microlayer: 0.5-3 10^9 cells L^{-1}

Water column: < 0.01-1x 10^9 cells L^{-1} (>5 x 10^9 cells L^{-1}); L: 20000 “species”

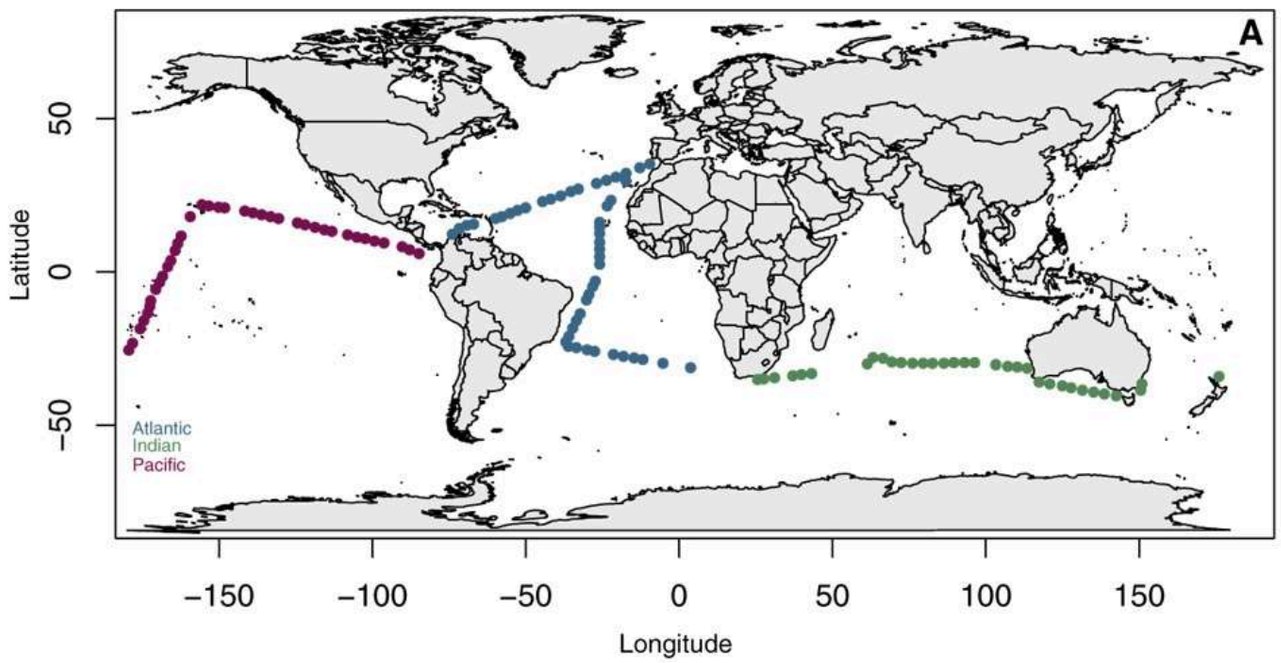
Surface sediment: ~0.001-1x10^{10} cells cm^{-3}; g: 5000-19000 ”species”

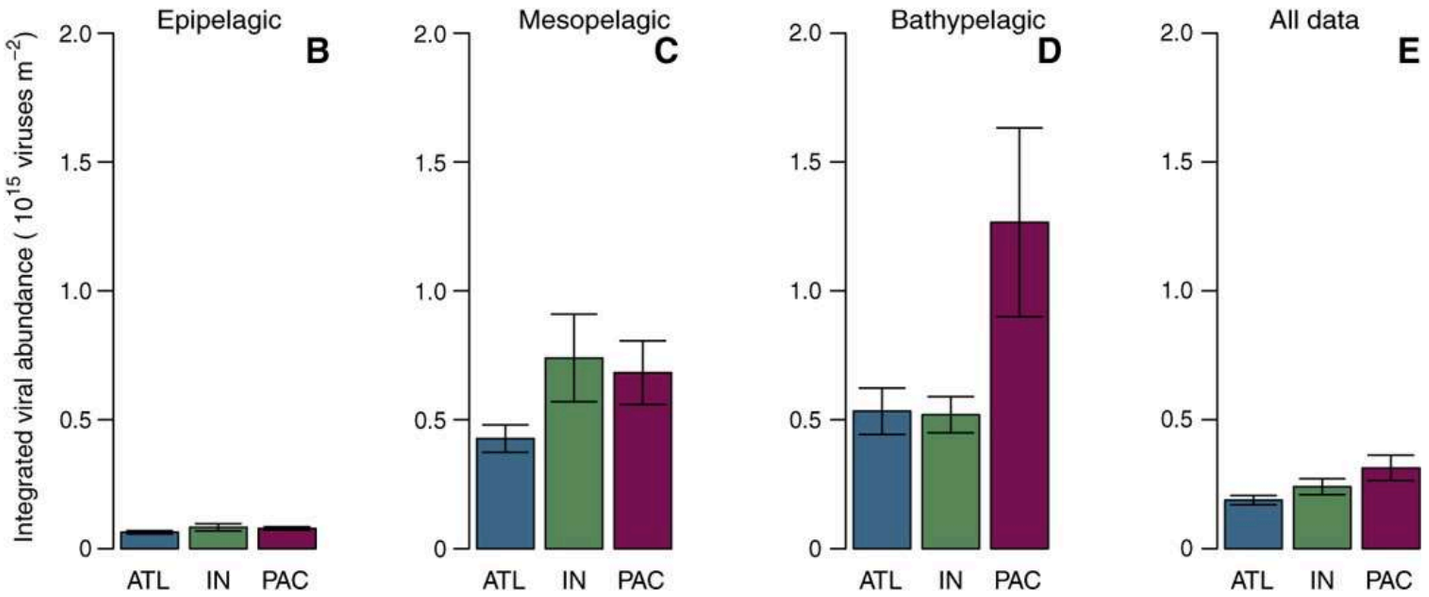
Deep subsurface sediment: ~0.001-1x10^{10} cells cm^{-3}



Walsh et al., 2016

Viral depth profiles in the ocean





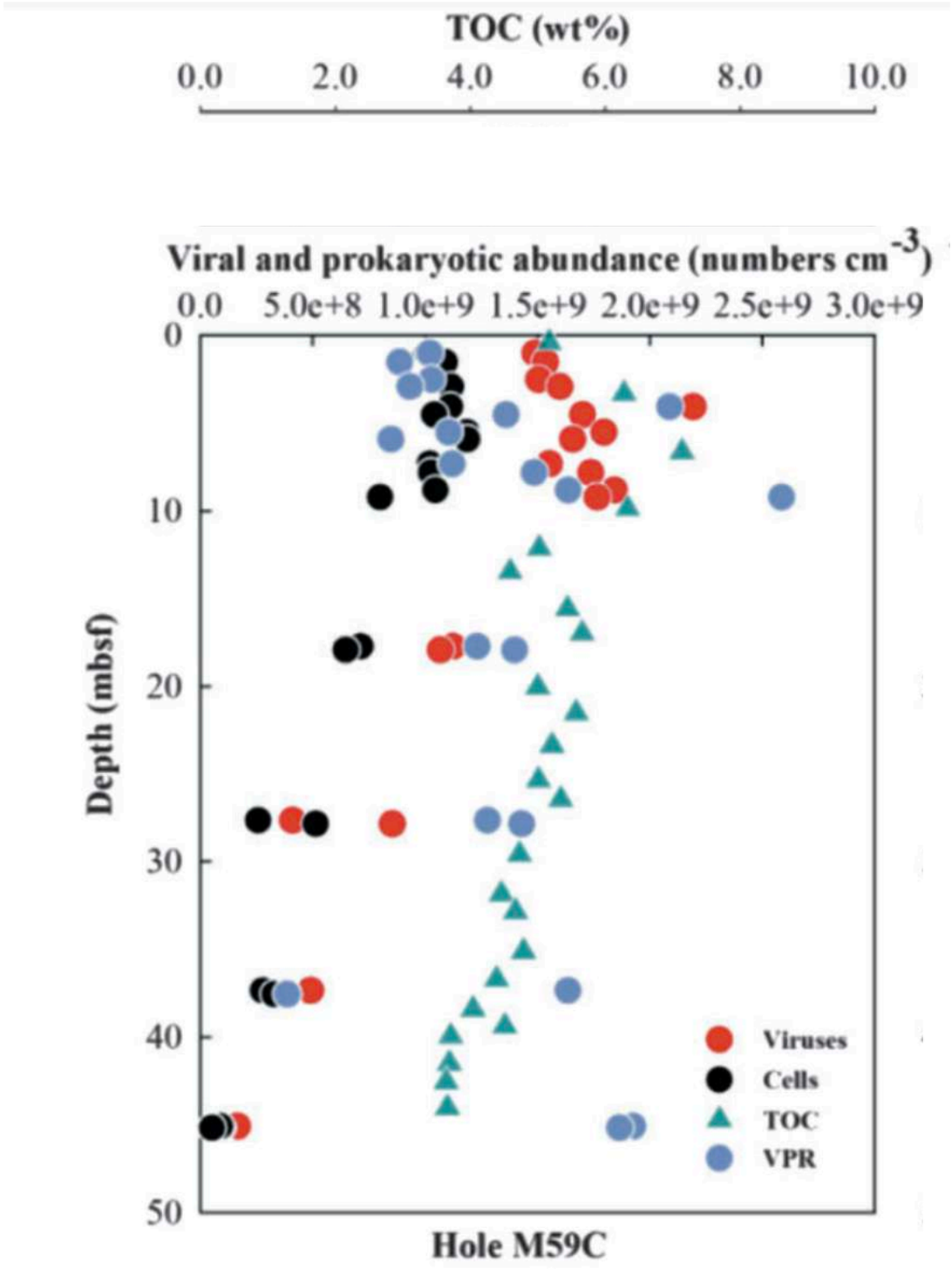
Lara et al. 2017

- Malaspina

- 10X change from 7.76 × 10^6 viruses mL^{-1} in the epipelagic layer to 0.62 × 10^6 viruses mL^{-1} in the bathypelagic layer

(**B**) epipelagic (0 to 200 m)
(**C**) mesopelagic (200 to 1000 m),
(**D**) bathypelagic (1000 to 4000 m) layers

Virus depth profiles in the sediment

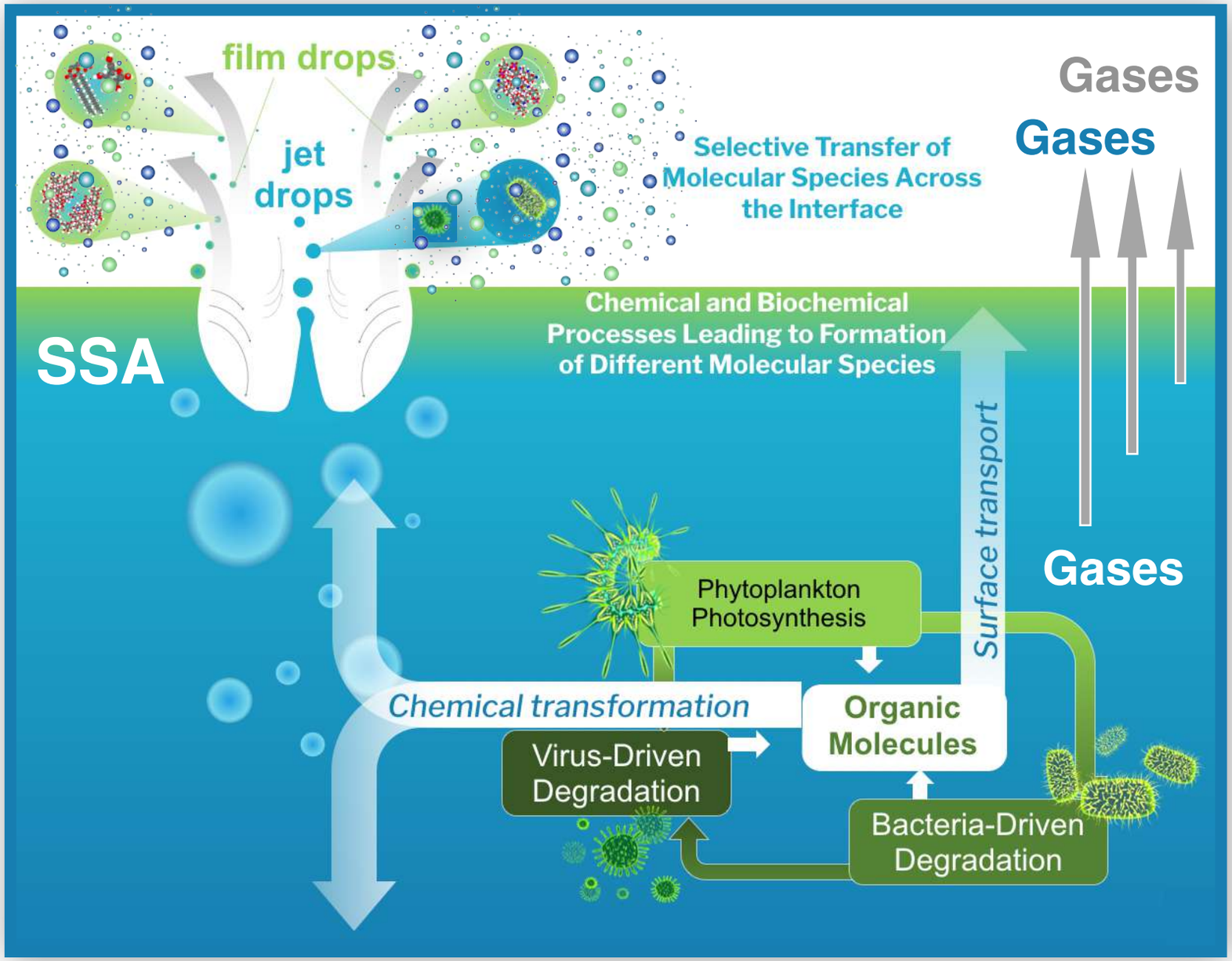


Cai et al. 2019

- Baltic Sea sub-seafloor biosphere harbors highly abundant viruses with densities up to 1.8×10^{10} viruses cm^{-3}

- High potential viral production down to 37 meters below seafloor in ca. 6000-years-old sediments

Microbial atmospheric structuring



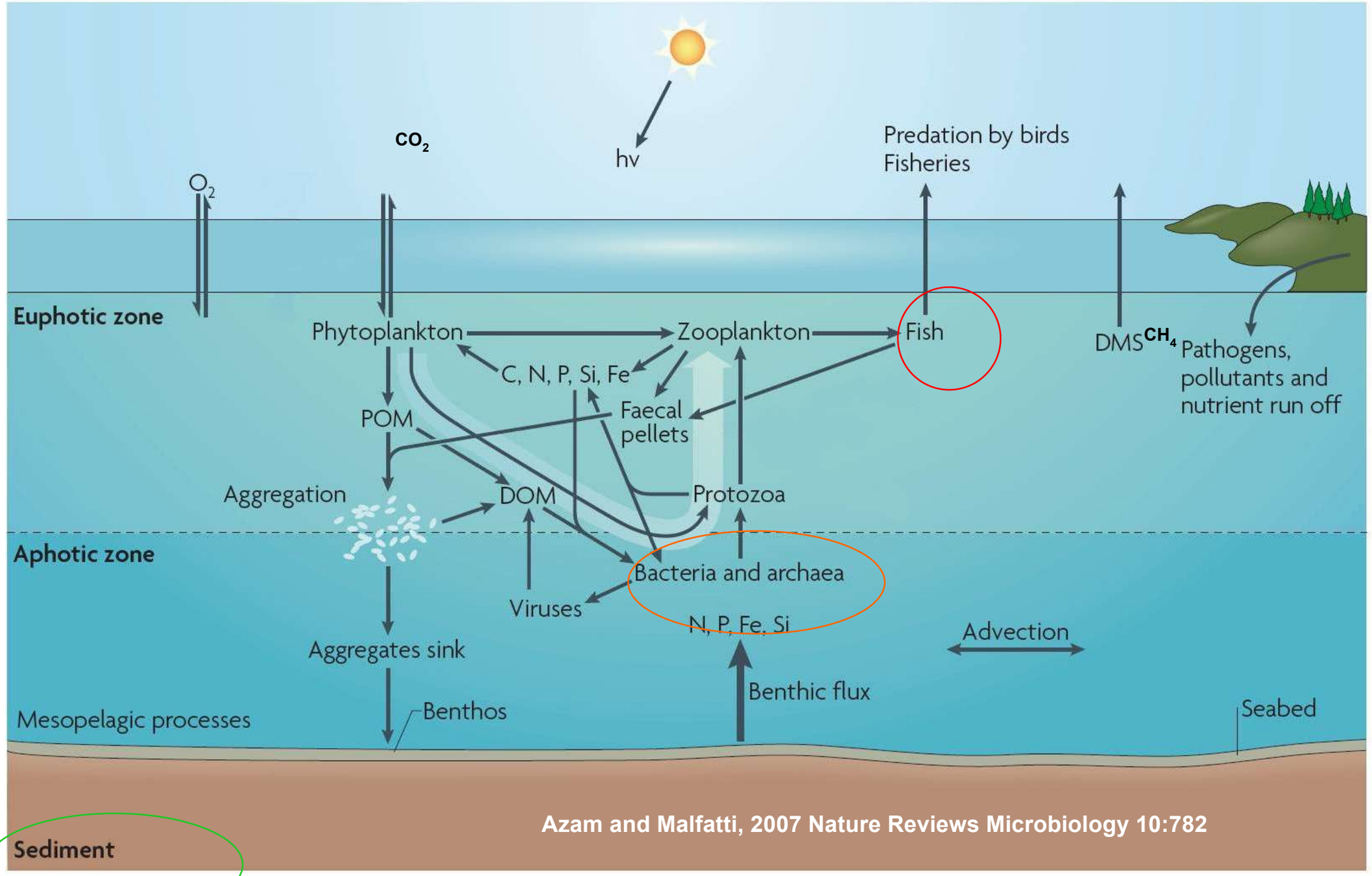
- ~2000-10,000 Tg/yr of SSA is ejected from ocean globally
- **SSA has effect on climate and atmosphere**
- Ocean-atmosphere exchange ~ millions of microbes per m^2 every day

CAICE center for aerosol impacts on chemistry of the environment

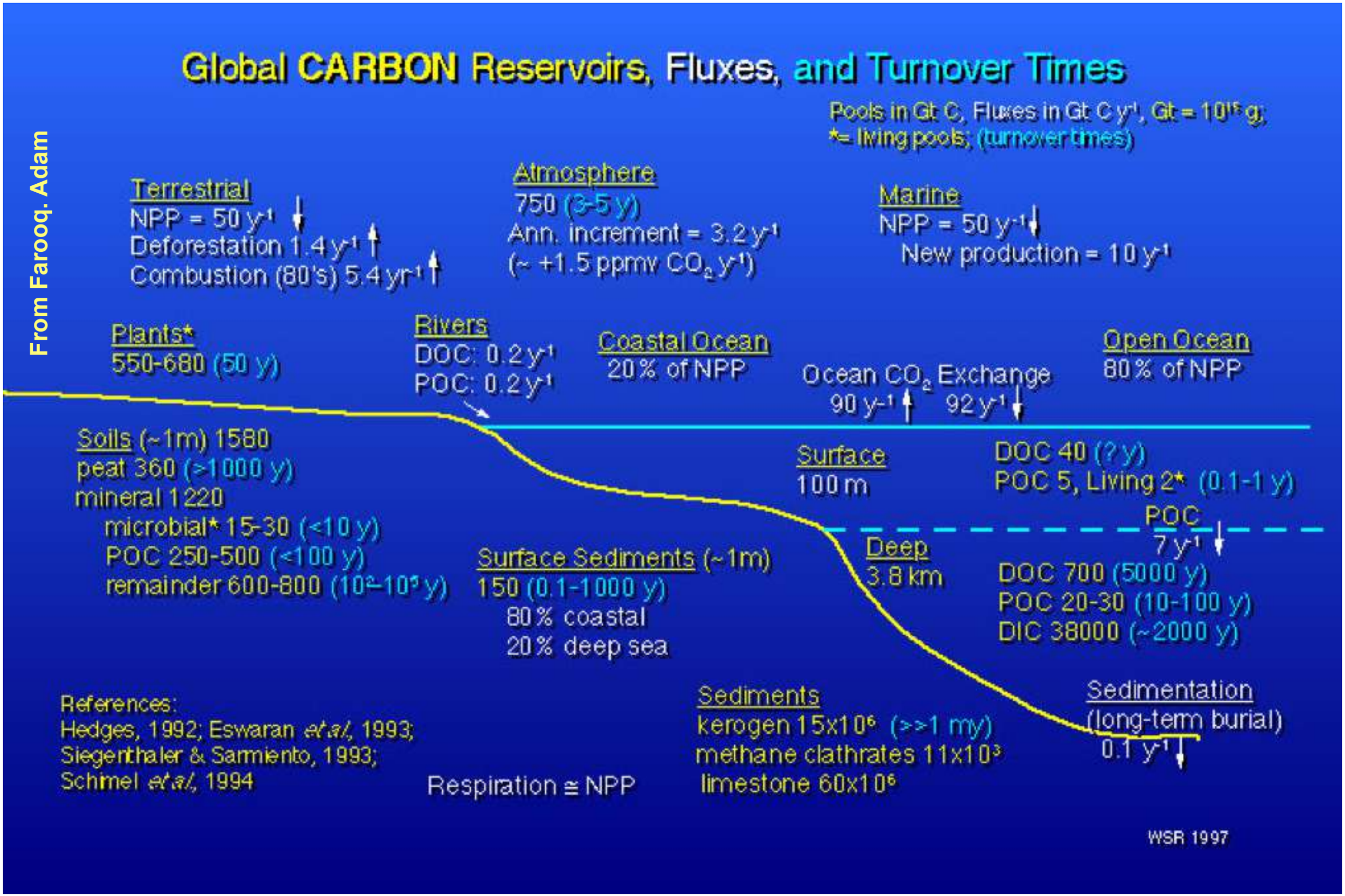
Microbial activities, biotransformation, influence ocean and atmosphere chemistry

Healthy Ocean <—> Functioning Ocean

Functioning Ocean <—> Functioning C biogeochemical cycle



Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782



Global carbon reservoirs, fluxes, and turnover times. Major reservoirs are underlined, pool sizes and fluxes are given in Gt (1015 g) C and Gt C yr-1. Turnover times (reservoir divided by largest flux to or from reservoir) are in parentheses . To convert Gt C to moles C, multiply by 8.3 x 1010.

Microbial growth I

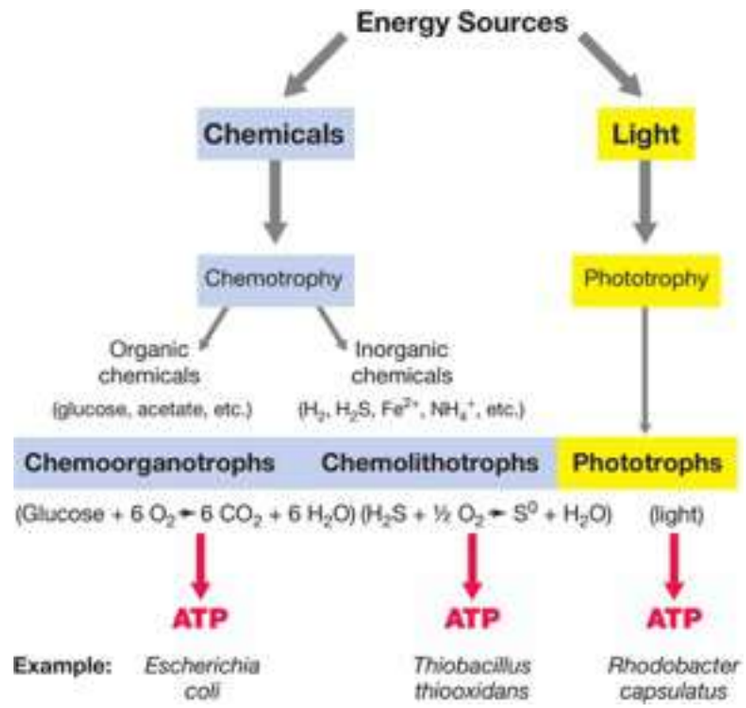
1. **Energy source to generate ATP**
2. **Carbon source of assembling cellular building blocks**
3. **For maintenance of existing cells/ for growth of new cells**

– > Successful exploitation well-defined energy and carbon source
– > Physical, biological and chemical properties vary in space and time

Carbon source	Energy source		
	Chemical, organic	Chemical, inorganic	Light
Fixed organic	**Chemosynthetic organoheterotroph** (Example: humans, fungi, *Pseudomonas*)	**Chemosynthetic lithoheterotroph** (Example: *Beggiatoa* sp.)	**Photosynthetic heterotroph** (Example: purple and green bacteria; *Rhodospirillum*)
Gaseous CO_2		**Chemosynthetic lithoautotroph** (Example: ammonia-, hydrogen-, and sulfur-oxidizing bacteria; *Nitrosomonas, Aquifex*)	**Photosynthetic autotroph** (Example: plants, algae, *Prochlorococcus*)

Terminology:
- Autotroph: carbon from CO_2 fixation
- Heterotroph: carbon assimilated from (fixed) organic compounds
- Photosynthetic: energy from light
- Chemosynthetic: energy from oxidizing reduced chemicals
- Chemolitho: energy from oxidizing inorganic reduced chemicals
- Chemoorgano: energy from oxidizing organic reduced chemicals.

Madsen, 2016



Madigan et al. 2018

Metabolism & Growth in a limited environment

Habitat characteristics and nutrient limitations faced by three physiological classes of microorganisms

Habitat type	Photoautotroph	Chemolithotroph	Chemoorganoheterotroph
Ocean water	Daily light cycle, light penetration depth; scarce iron	Flux of reduced inorganic compounds, especially NH_3, H_2S, H_2, or CH_4 from nutrient turnover and hydrothermal vents	Carbon flux from phototrophs, dead biomass, and influent waters
Lake water	Daily light cycle, light penetration depth; scarce phosphorus	Flux of reduced inorganic materials, especially NH_3, H_2, and CH_4 from nutrient turnover	Carbon flux from phototrophs, dead biomass and influent waters
Sediment (freshwater and oceanic)	Daily light cycle, light penetration depth	Flux of reduced inorganic materials, especially NH_3 and H_2 from nutrient turnover or H_2, H_2S, or CH_4 from hydrothermal vents	Flux of organic carbon from phototrophs and dead biomass; flux of final electron acceptors to carbon-rich anaerobic strata
Soil	Daily light cycle, light penetration depth	Flux of reduced gaseous substrates, especially methane from nutrient turnover by anaerobes	Slow turnover of soil humus, dead biomass, plant root exudates; leaf fall from vegetation
Subsurface sediment	No light	Flux of reduced inorganic materials, especially H_2 and CH_4 from geothermal origin	Carbon flux from nutrient turnover

Madsen, 2016

Microbial Growth II

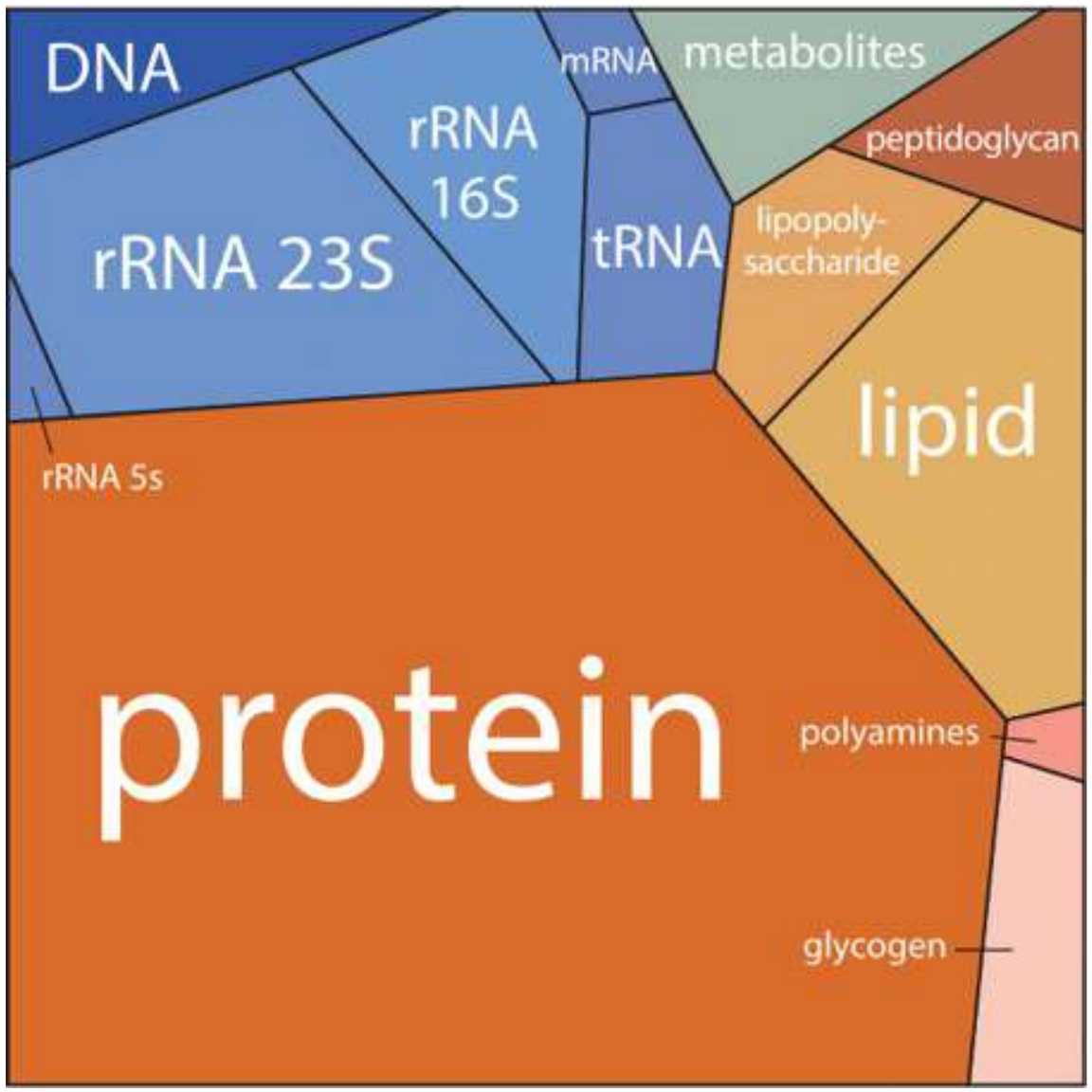
Growth rate (μ): the per-capita change in abundance (N) over time, or the slope of ln(N) versus time in the absence of mortality

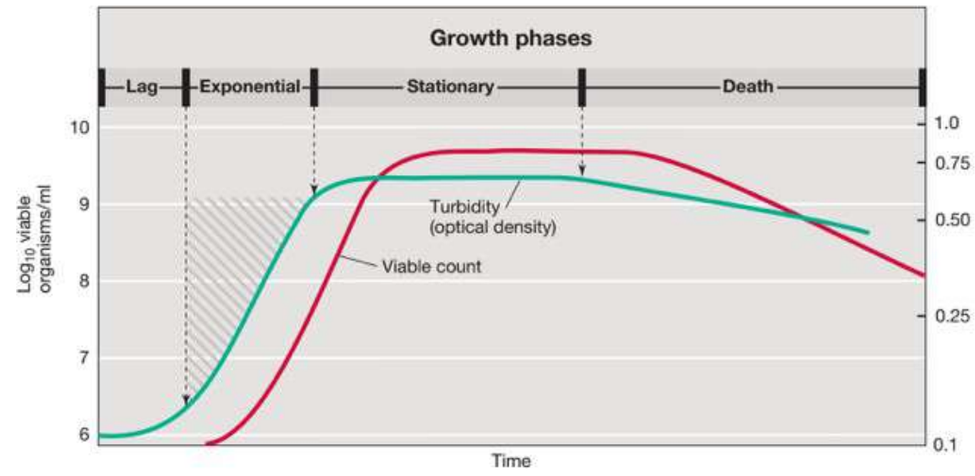
Generation time (g): the time necessary for a population to double [g = ln(2)/μ], also referred to as doubling time; turnover time is mathematically equivalent to generation time

- ***Vibrio natriegens*** is capable of doubling every 7 min equivalent to a growth rate of over 140 d^{-1}
- ***Pelagibacter ubique***, a representative of the most abundant bacterial clade in the ocean, SAR11, has a generation time of nearly 2 days and growth rates of 0.4–0.6 d^{-1} in laboratory pure cultures

Microbial Growth III

A Voronoi tree diagram of *E.coli* composition



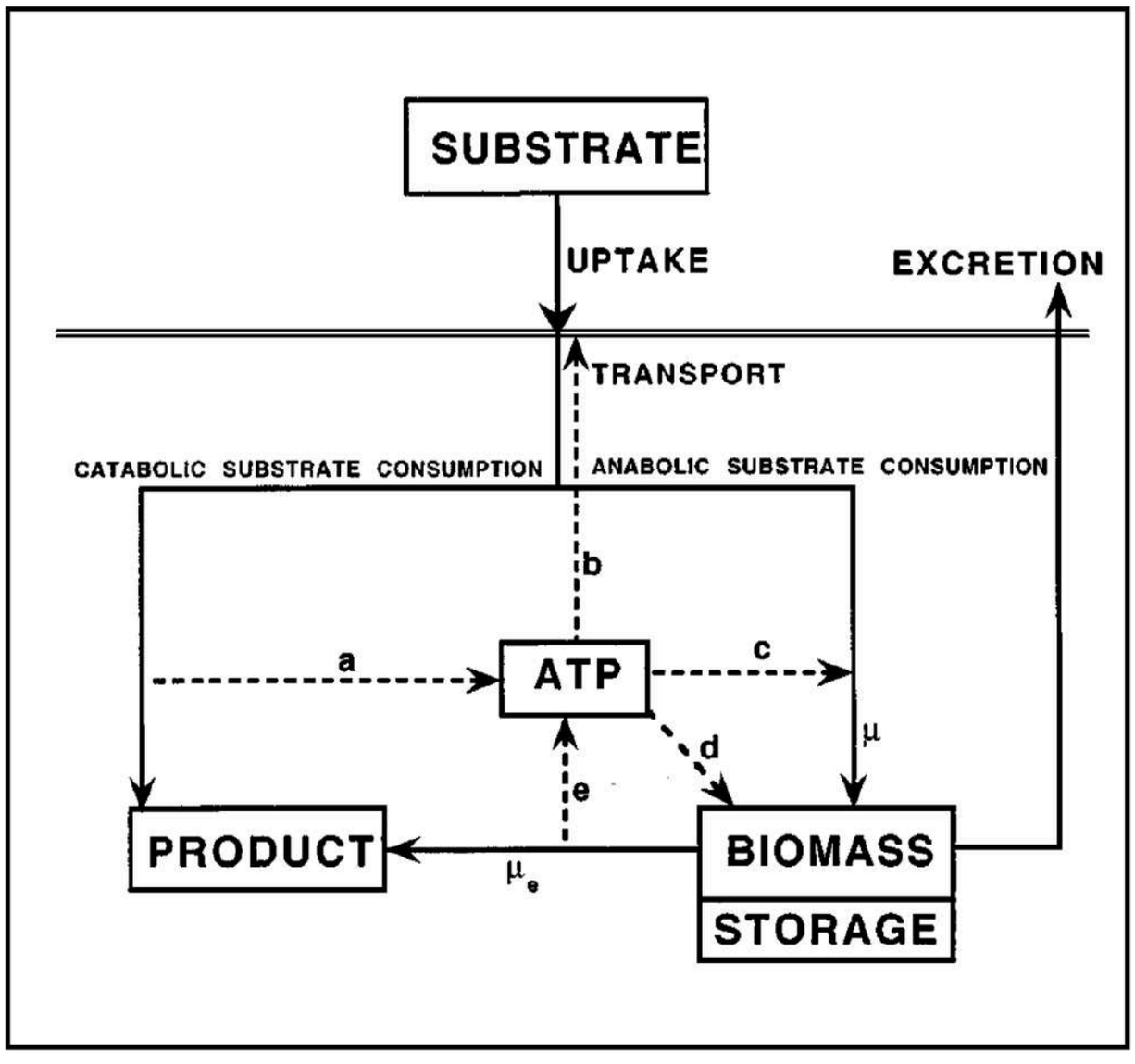


Madigan et al. 2018

40 min

- Each polygon area is the relative fraction of the corresponding cellular constituent (dry mass)
- Similar colors = related functional role
- Steady-state mean cell size (large circles) scales exponentially with nutrient-determined growth rate

Microbial Growth IV



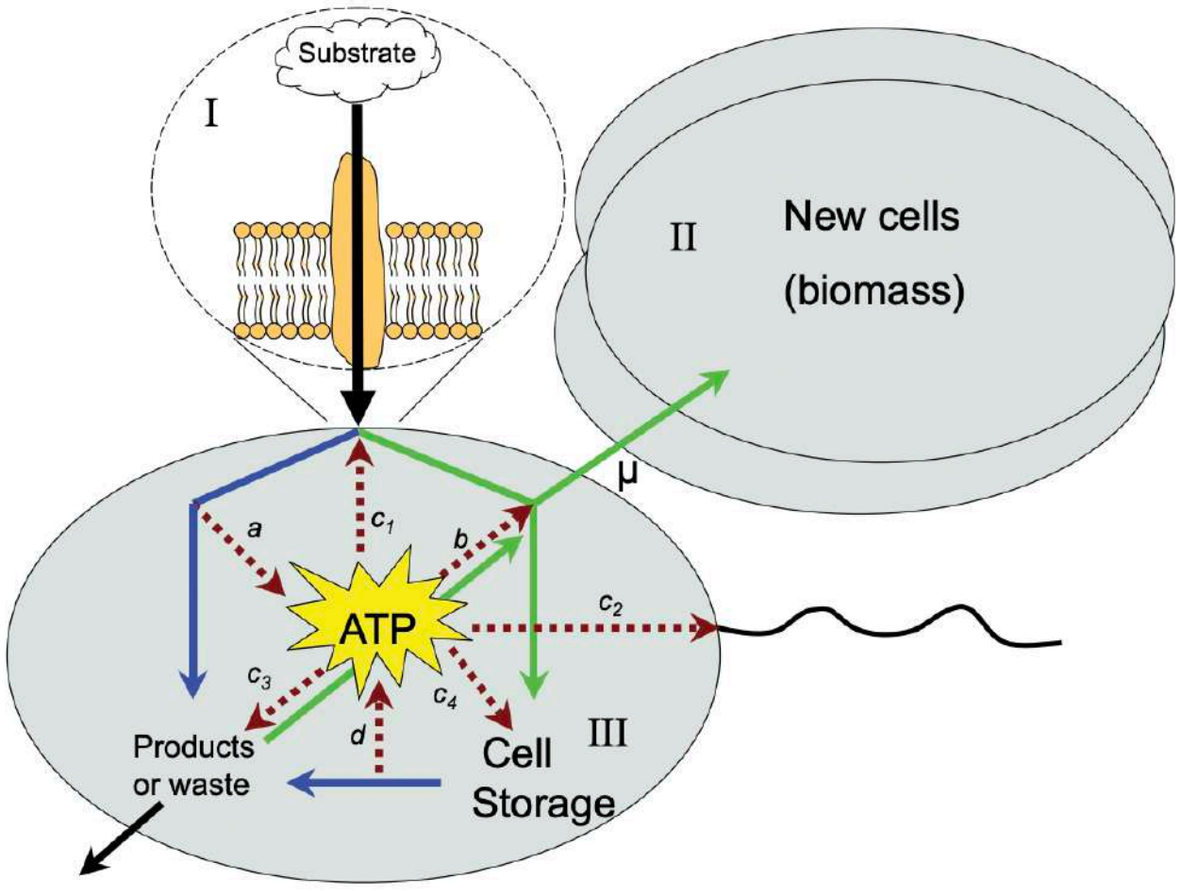
del Giorgio & Cole, 1989

- The oxidation of organic compounds contributes to the energy pool as ATP at a rate ***a***
- Active transport of substrates into the cell requires energy from this ATP pool at a rate ***b***
- Anabolic reactions utilize ATP at a rate ***c*** and result in a growth rate ***μ***
- The anabolic pathways result not only in increases in biomass but also in storage products and organic compounds that may be excreted back to the medium
- Maintenance expenditures consume ATP at rate ***d***
- In the absence of exogenous substrates, minimum maintenance energy requirements must be supported by degradation of biomass through endogenous metabolism (***μe***), which supplies ATP at a rate ***e***

Endogenous metabolism is defined here as the state when no growth is possible, and by definition BGE is 0 under these conditions

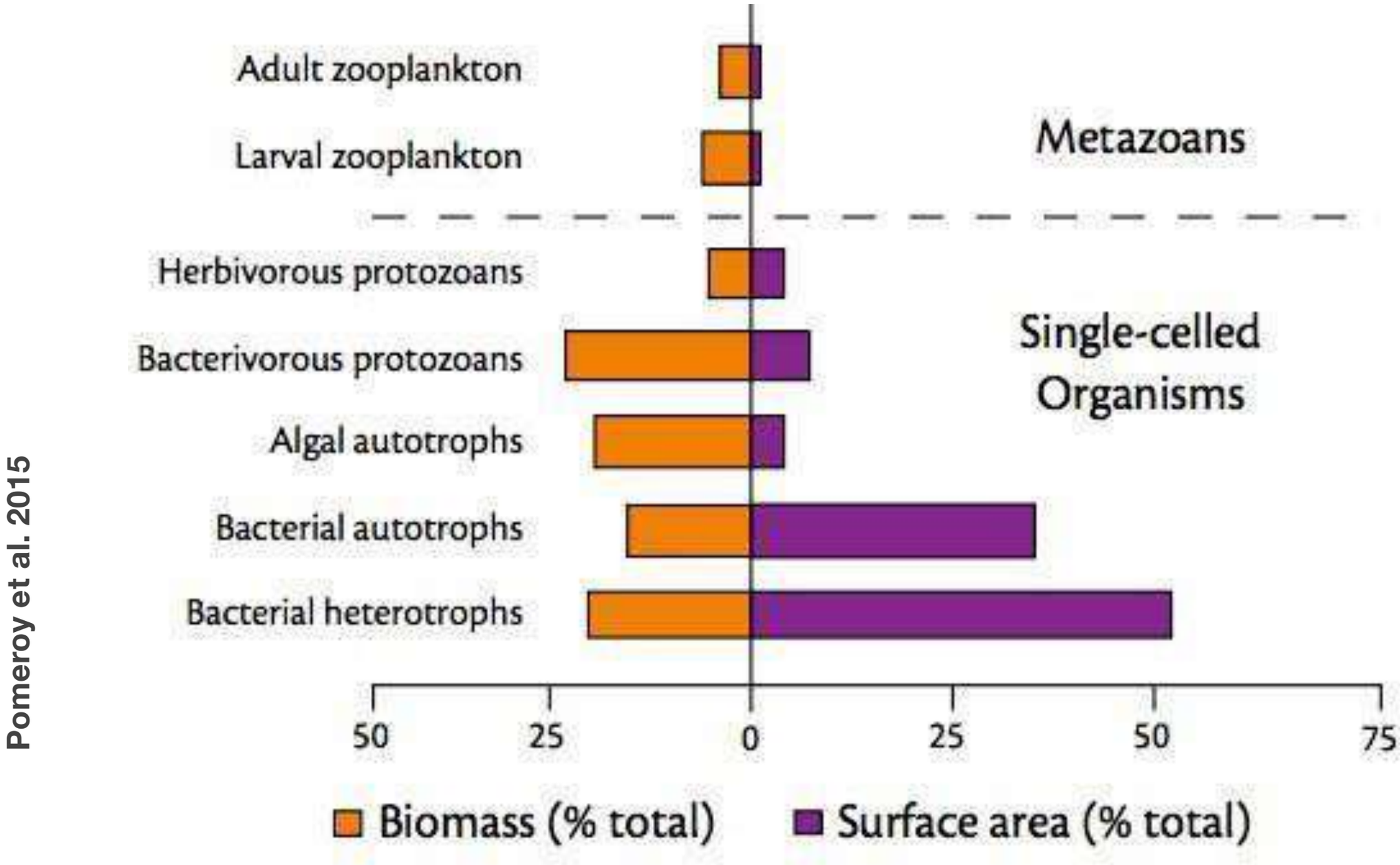
Microbial Growth V

- Substrate < 700 Da are actively taken up via membrane proteins (I)
- As the substrate enters the cell via active uptake, it either enters into catabolic pathways (blue lines) or anabolic pathways (green lines)
- Monomers for anabolism can come preformed from the environment or as products of catabolism
- The red-hashed lines represent the flow of energy to and from these metabolic pathways. **Energy is conserved via substrate catabolism and ATP is produced at a rate a**
- As ATP is hydrolyzed, energy is released and utilized at rate b to drive anabolic processes such as production of new cells (growth; II) and production cell storage products (III)
- Energy is also utilized at various rates to support processes that are independent of anabolism. This maintenance energy is used at rates c1 to activate uptake systems, c2 to fuel cell motility, c3 to actively eliminate waste, and c4 to repair cellular machinery
- In the absence of exogenous organic substrates, the cell can yield ATP at rate d by catabolizing storage material (endogenous substrates)



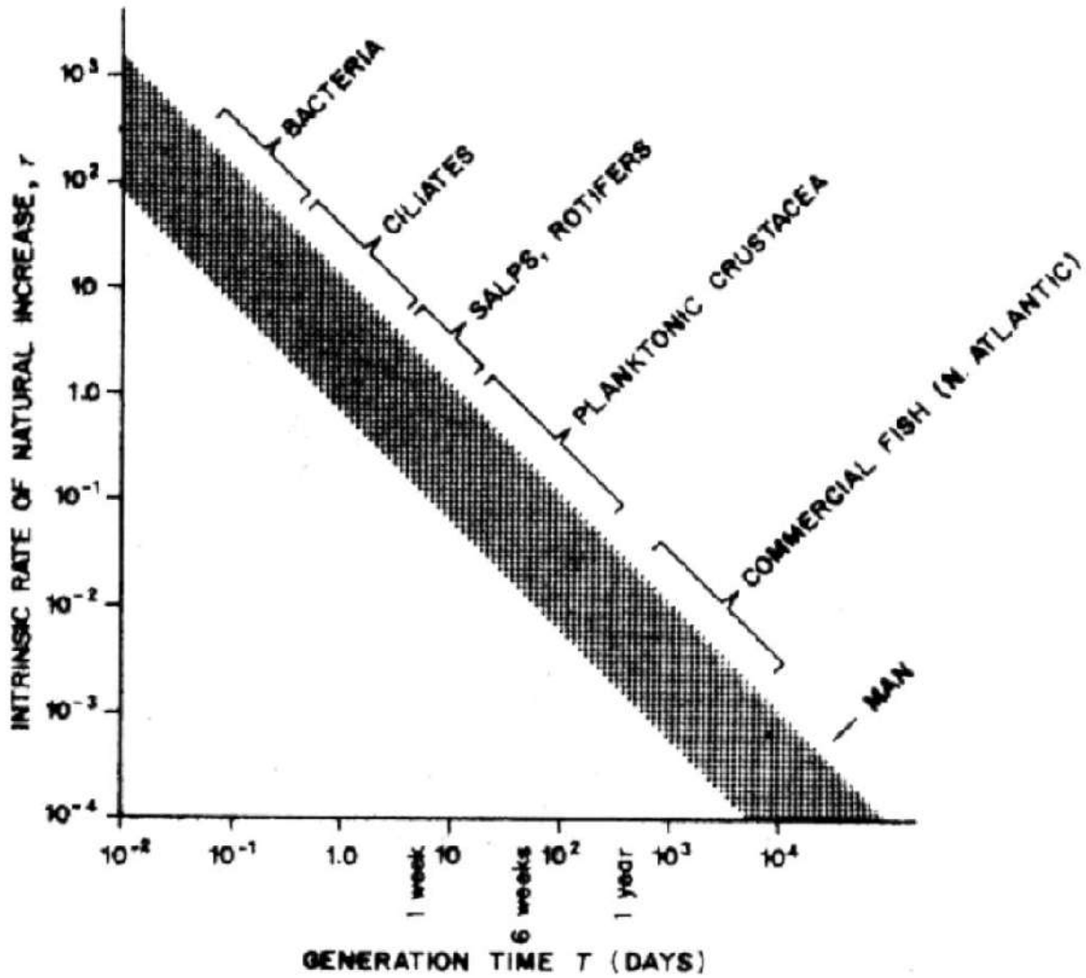
Carlson et al. 2007

Bacteria & Archaea biomass



- Microbes represent 90% of the total biomass in the ocean

Bacteria and Archaea growth



- Fast generation time
- Slow generation time
- Diverse strategies to survive in the marine environments

Figure 12B The intrinsic rate of population increase r, per day as a function of generation time. (Redrawn after Heron, 1972; in Anderson, 1981)

Bacteria & Archaea respiration

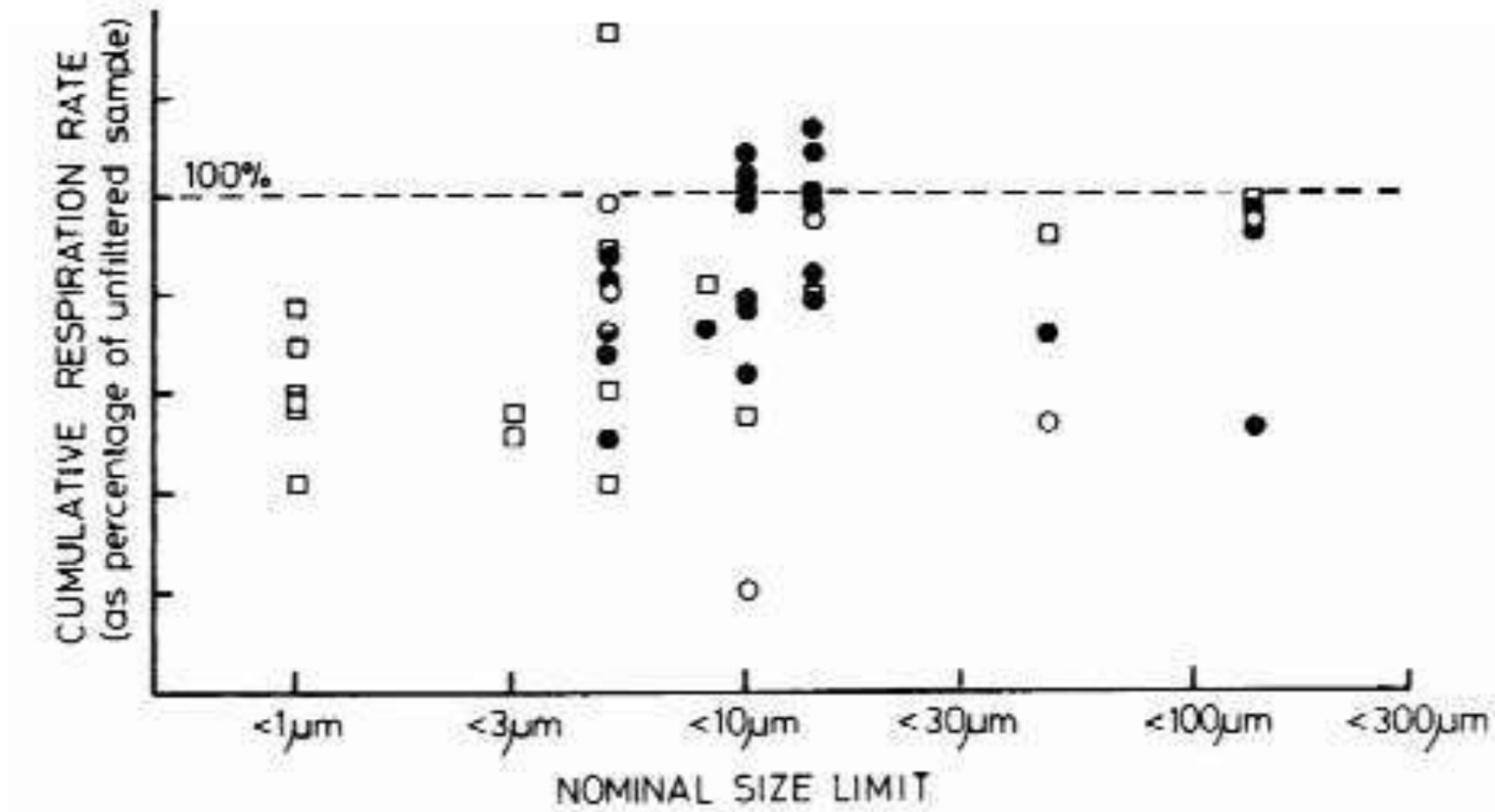
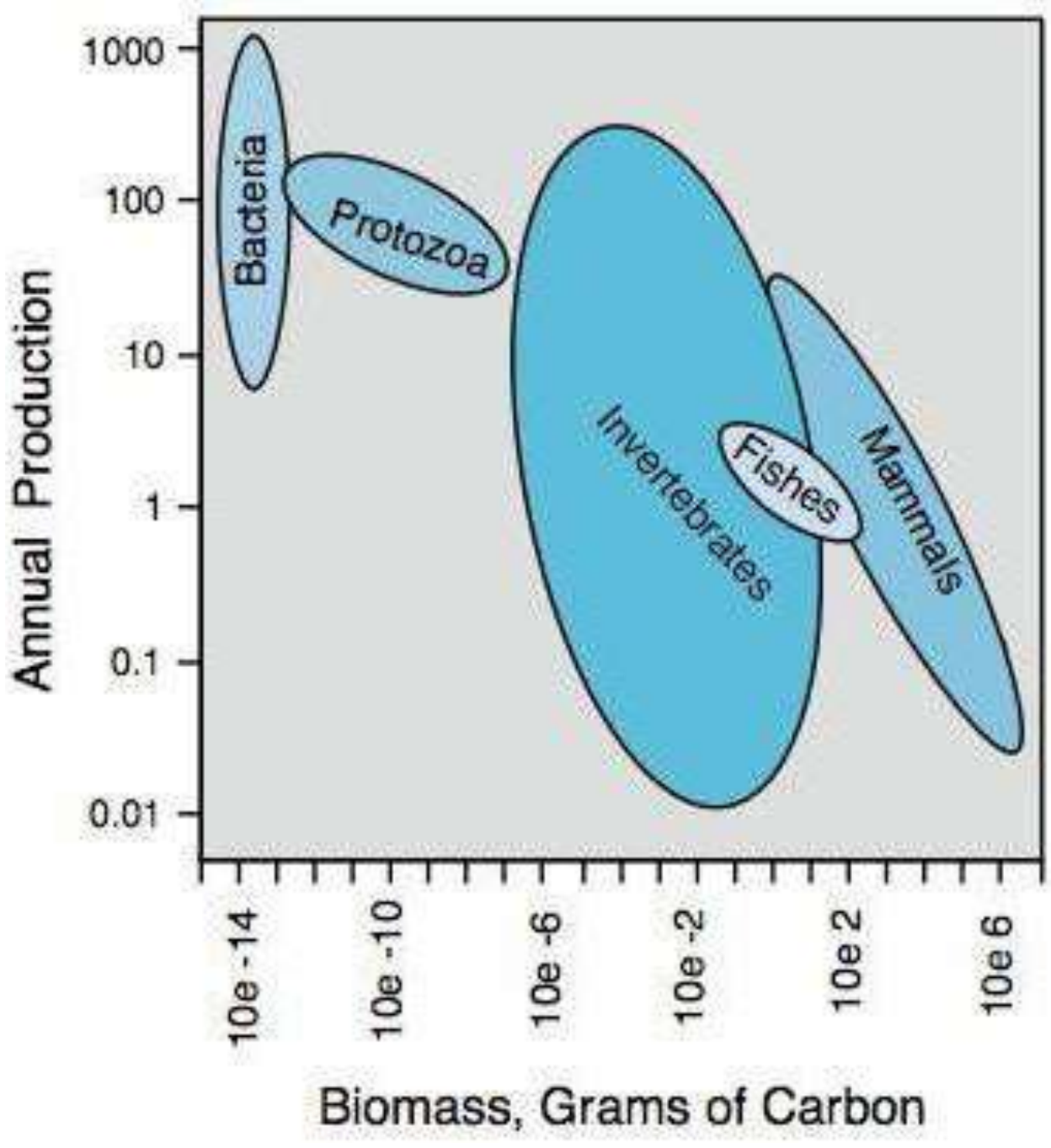


Figure 6. Distribution of respiratory activity with size. (□) CEPEX, samples from bag; (○) Loch Ewe, samples from bag; (●) Loch Ewe samples from outside bag. Data are expressed as cumulative respiration up to various size limits, normalized against the rate in the unfiltered sample. All the data points are for a single size horizon and are not replicates.

Williams (1984)

- Microbes contribute up to 90% to biota respiration

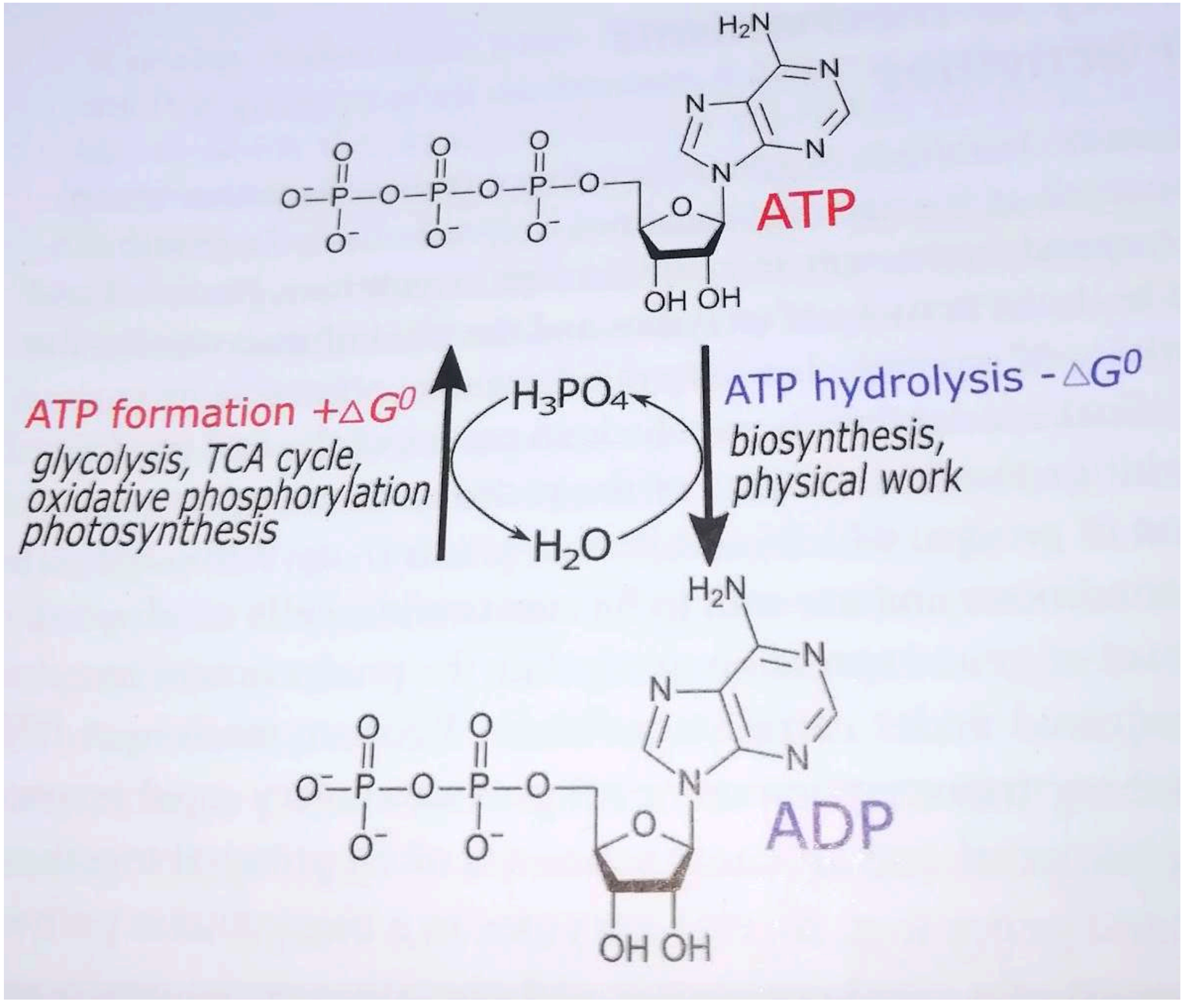
Bacteria & Archaea production



Pomeroy et al. 2015

•Microbes are the most productive organisms in the ocean

To sum: growth in a few words



Munn, 2020

Prokaryotic growth measurements

Methods for measuring bacterial growth in cultures

- **Viable count (plate count)**
- **Turbidimetry**
- **Total count (microscopic)**
- **Electronic counting**
- **Dry weight**

From Farooq Azam

Challenges of measuring bacterial growth in the sea

- **Simultaneous growth and death**
- **Growth probably not balanced (not all components can be used as indicators)**
- **Sparse populations**
- **Low growth rates**
- **Bacteria co-occur with other organisms and with detritus**

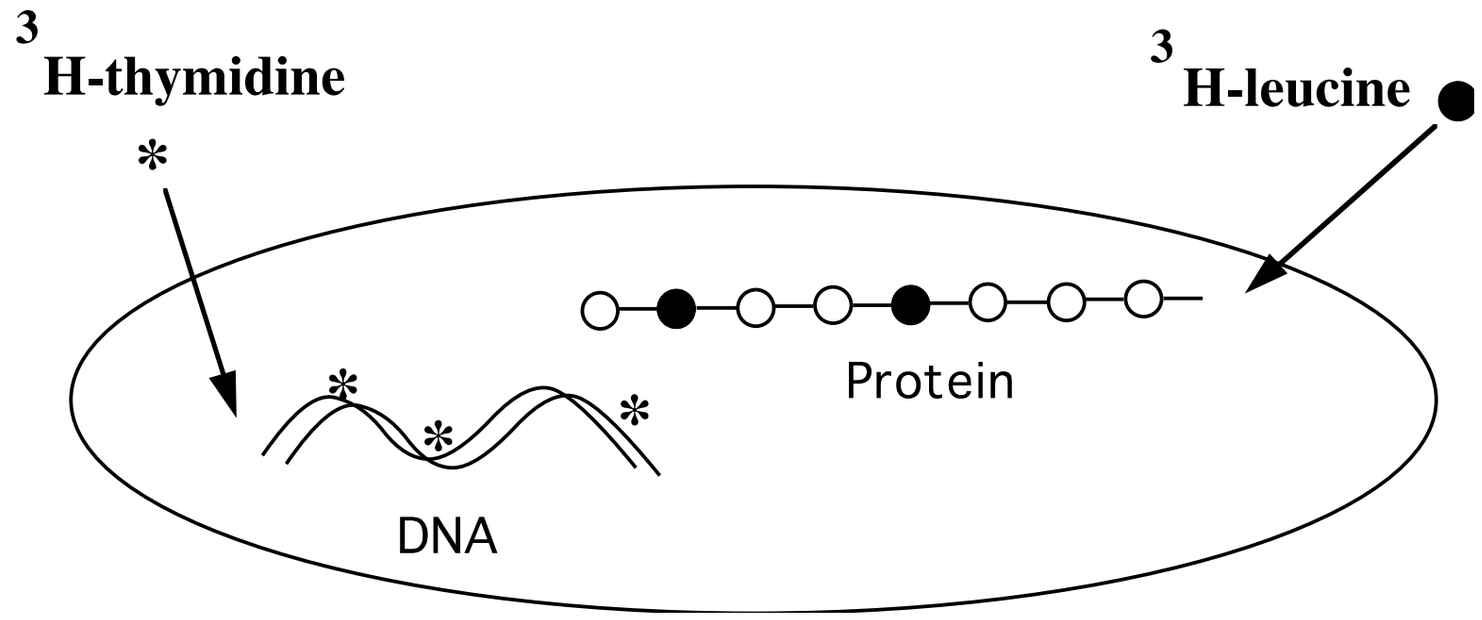
From Farooq Azam

Prerequisites of a method to measure growth

- should be specific for bacteria
- should not alter growth rate
- sensitive enough for use in seawater samples
- rapid to minimize changes during incubations
- independent calibration should be possible
- (you shouldn't need to know the growth rate to measure it)

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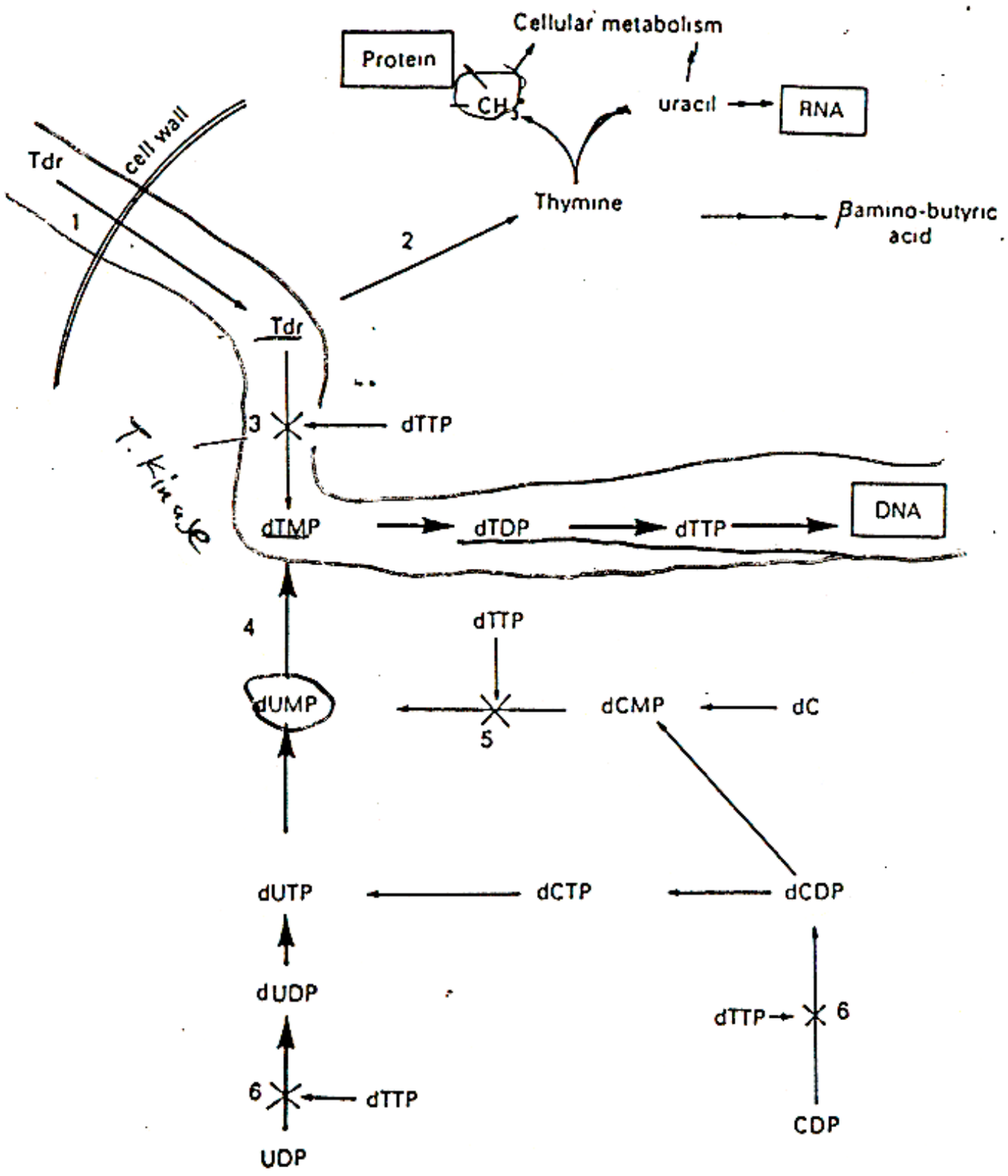
Radiotracer methods for growth rate measurements

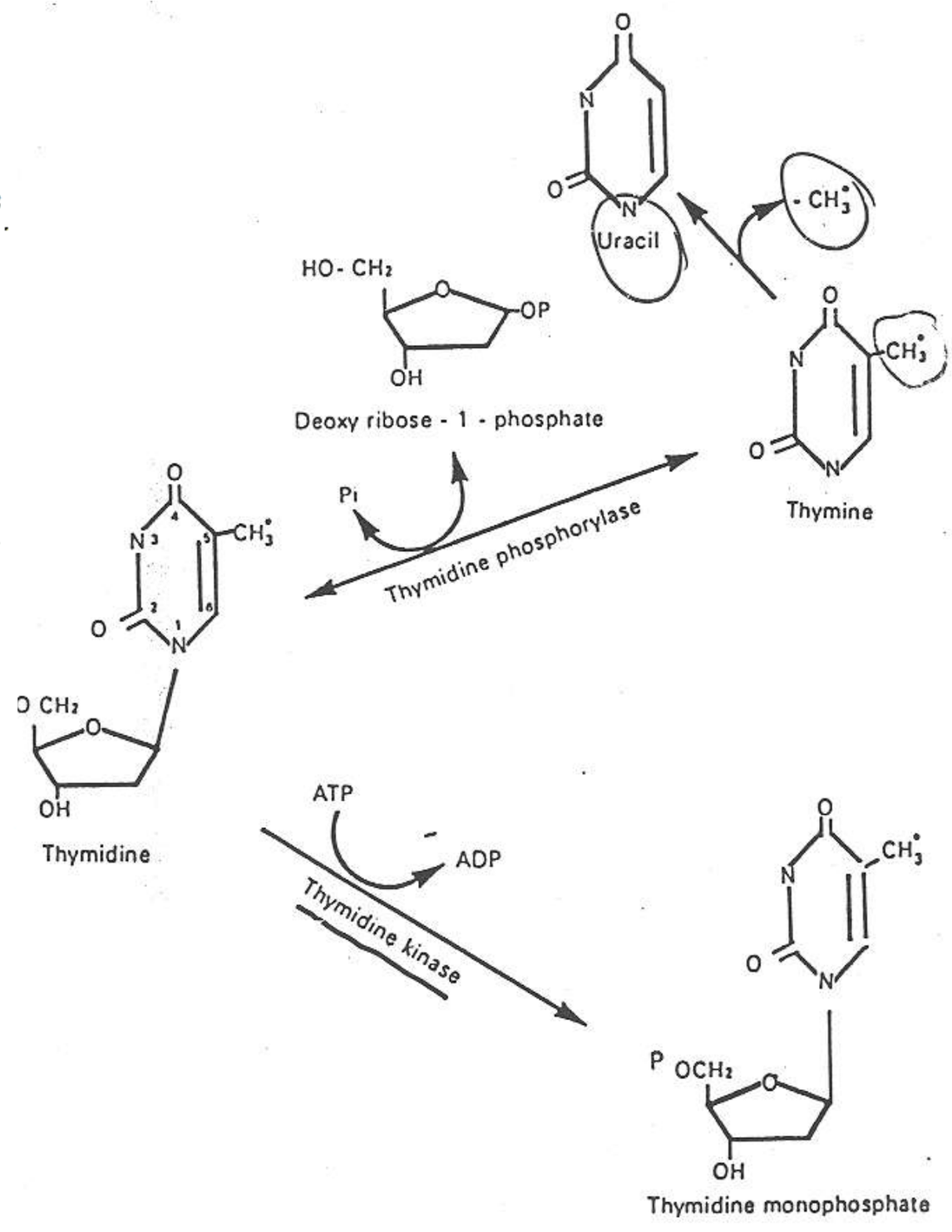


- Add nM ^{3}H-TdR or ^{3}H-leu
- Taken up by Bacteria (Archaea) high affinity salvage systems
- At nM, other plankton don't significantly take up (μ-autoradiography)
- Calibrated w/ seawater cultures (assumes no cell death)

From Farooq Azam

Pathway of exogenous Thymidine incorporation



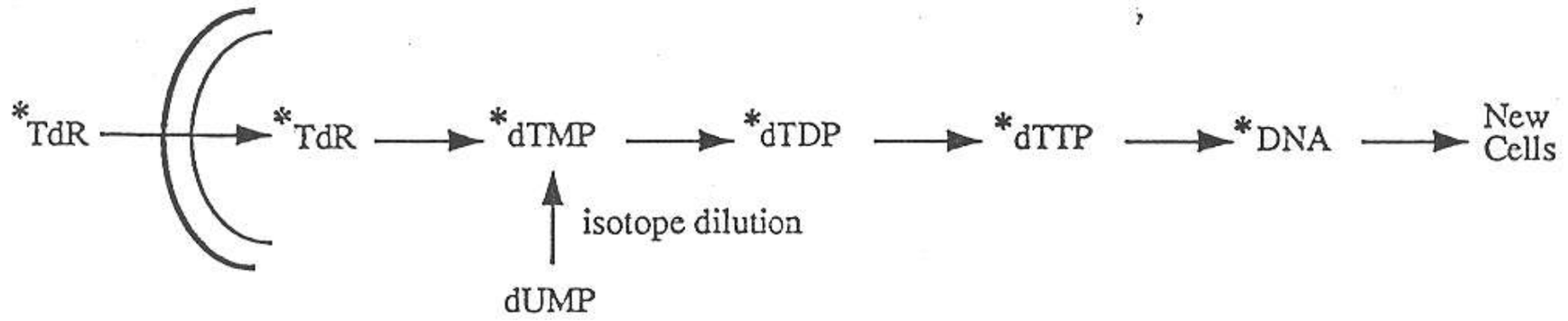


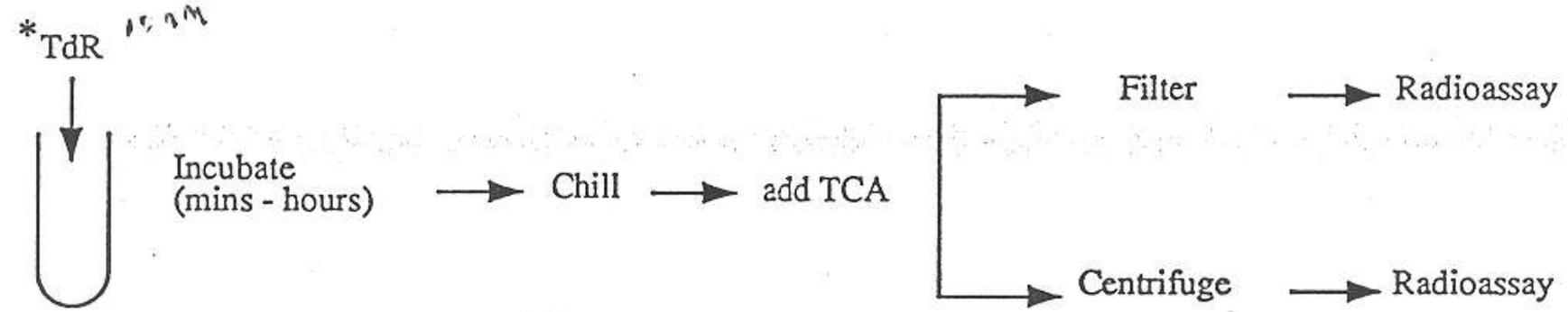
From Farooq Azam

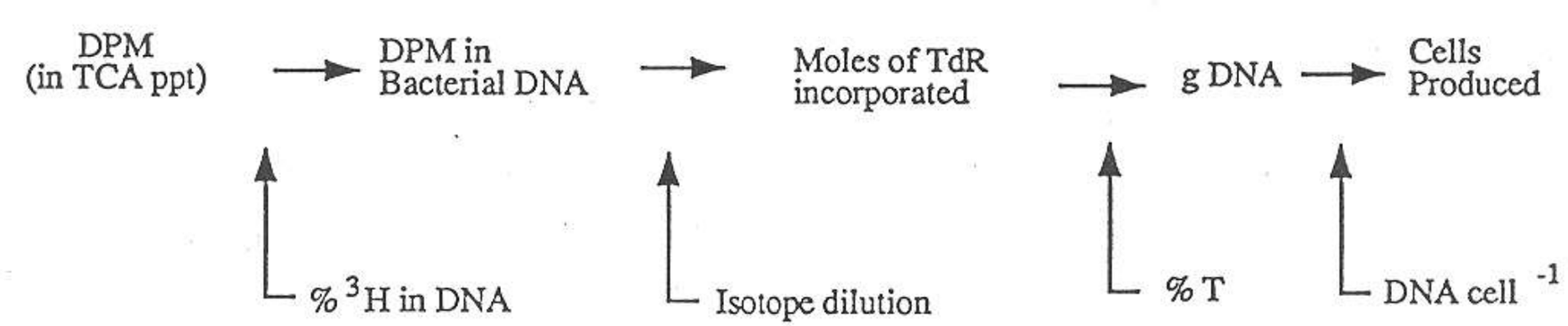
Moriarty, D. J. W. 1986. Adv. Microb. Ecol. 9: 245-292.

methyl ^{3}H-Thymidine Incorporation

Fuhrman & Azam 1980, 82
Ducklow & Carlson, 1993







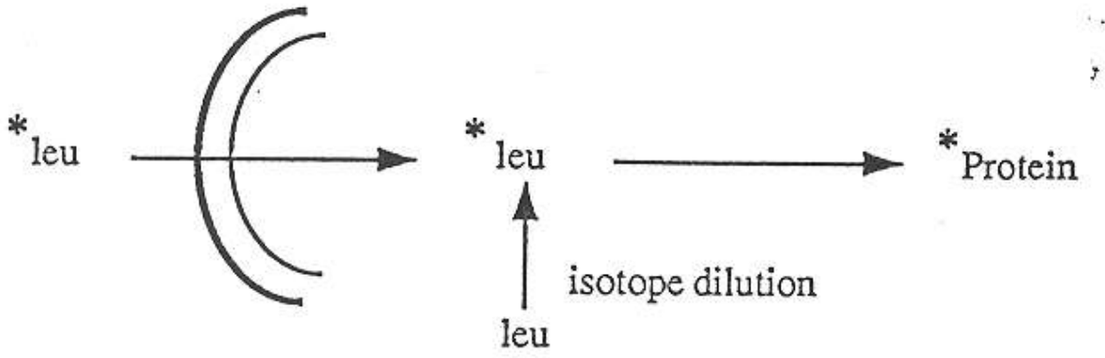
Empirical Relationship: 1- 3 x 10^{18} cells/mole TdR incorporated (but controversial)

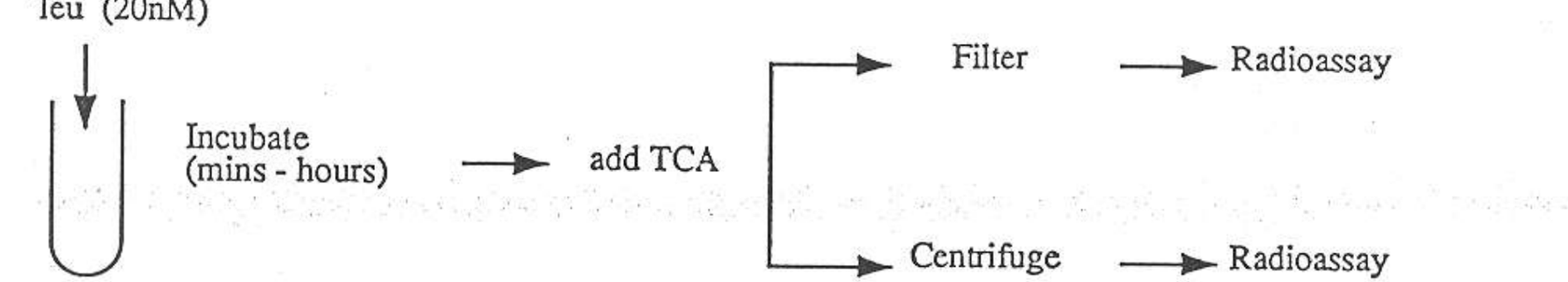
- Not all bacteria take up thymidine (anaerobes)
- Conversion of cells produced to carbon produced requires measurements of the volumes of the new cells produced and carbon/volume factors

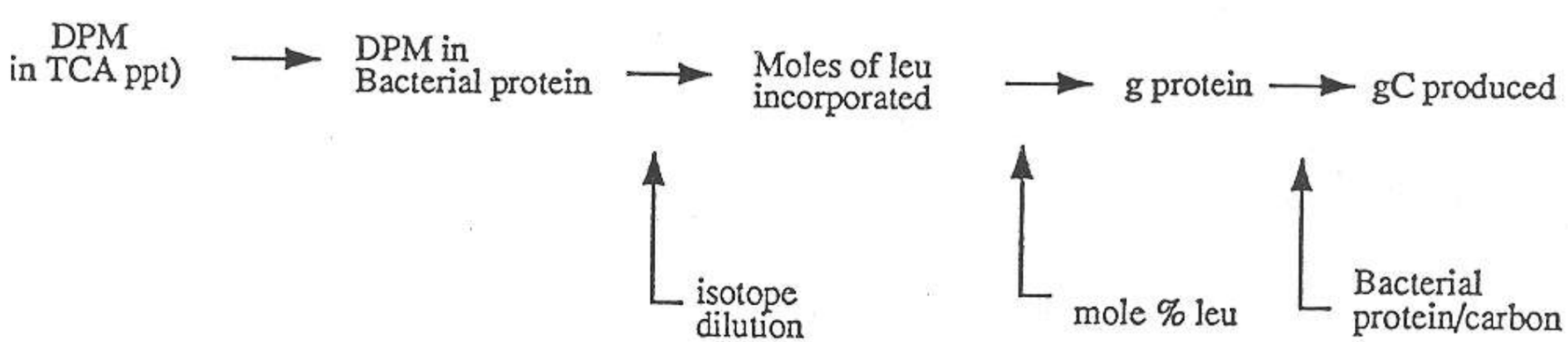
From Farooq Azam

^{3}H-Leucine Incorporation

Kirchman *et al.*, 1985
Simon & Azam, 1989
Smith & Azam, 1992







From Farooq Azam

Converting leu incorporation to BCP

Empirical Relationships determined in Seawater Cultures

Isotope dilution = 2x
Mole % leu = 7.3%
Carbon = (protein)(0.86)

Bacterial Protein Production = (mol leu incorporated)(3584)

- Direct conversion to carbon production (no need for cell volume or C/vol)
- An order of magnitude more sensitive than ^{3}H-thymidine
- Less likelyhood of discrimination for uptake by different bacteria

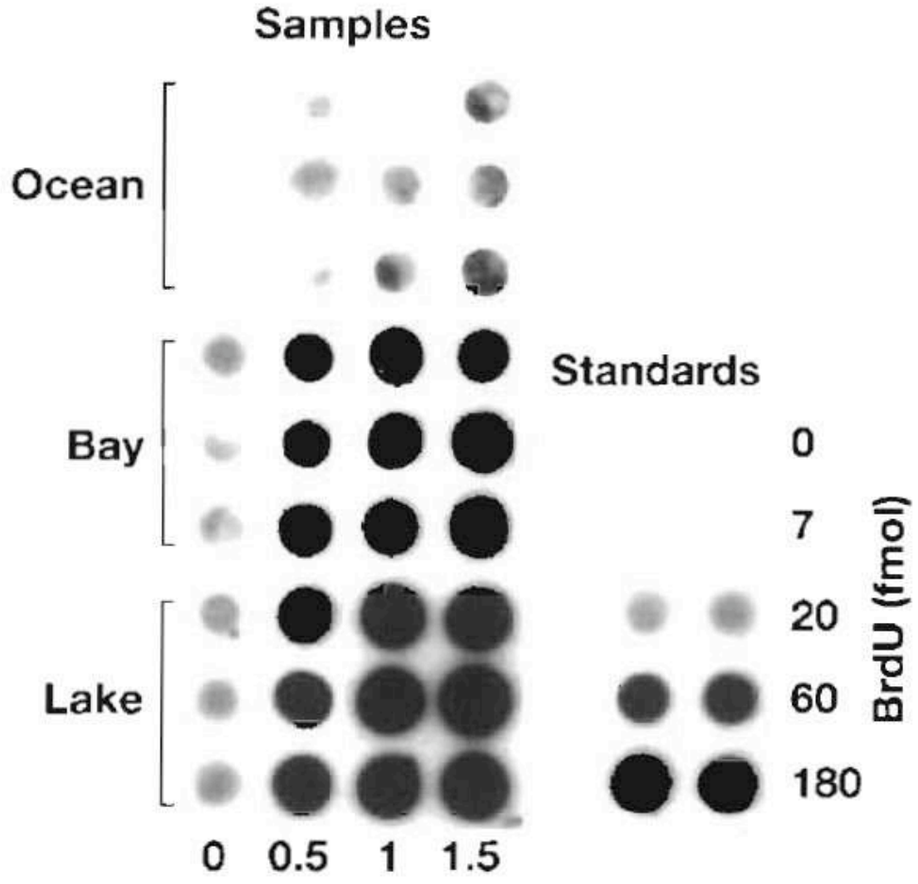
likelihood

- Leu incorp/TdR incorp yields carbon per new cell

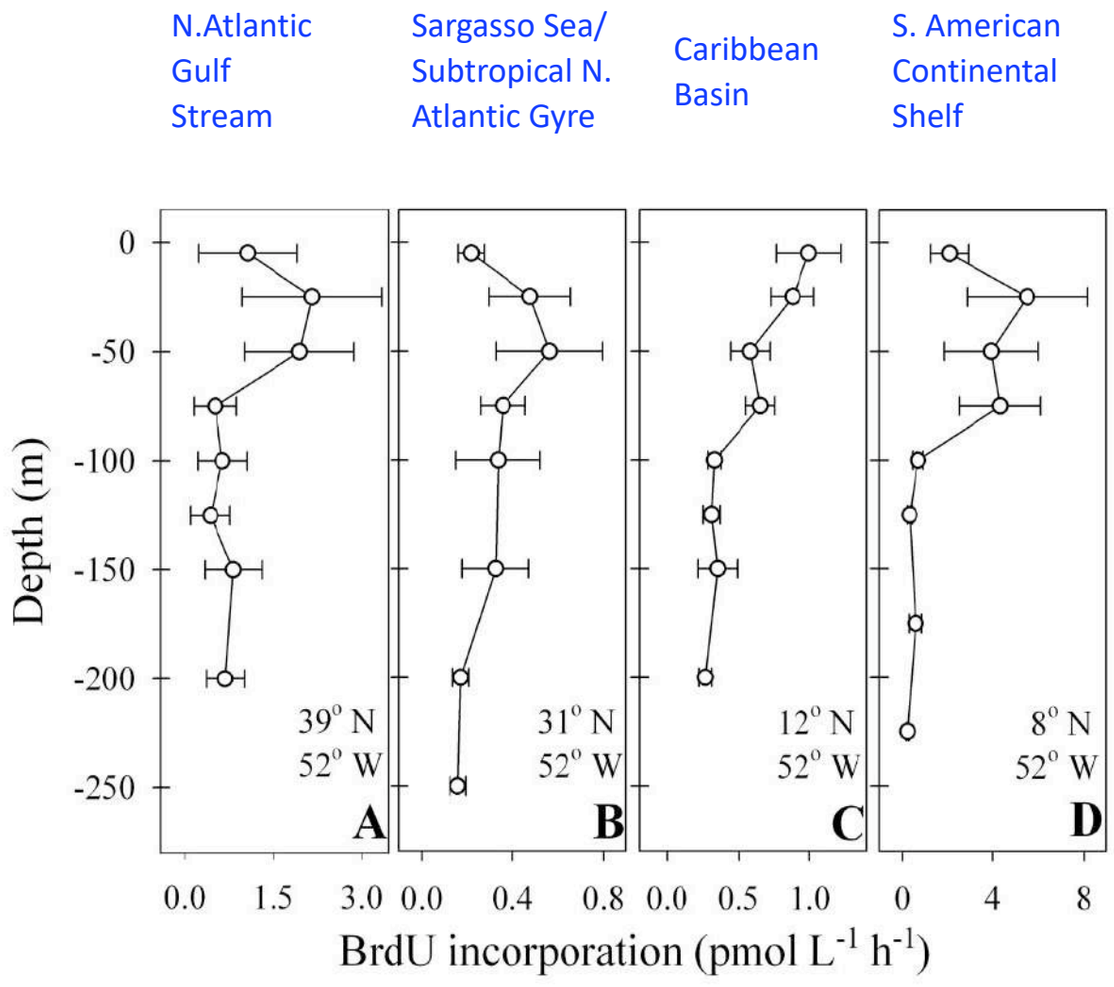
From Farooq Azam

BrdU as alternative to measure ^{3}H-TdR bacterial community productivity

1. BrdU incorporation
2. Immobilize cells on nylon membrane
3. Cell lysis, DNA binding to membrane
4. Anti-BrdU binding
5. HRP-secondary-antibody binding
6. Luminol visualization



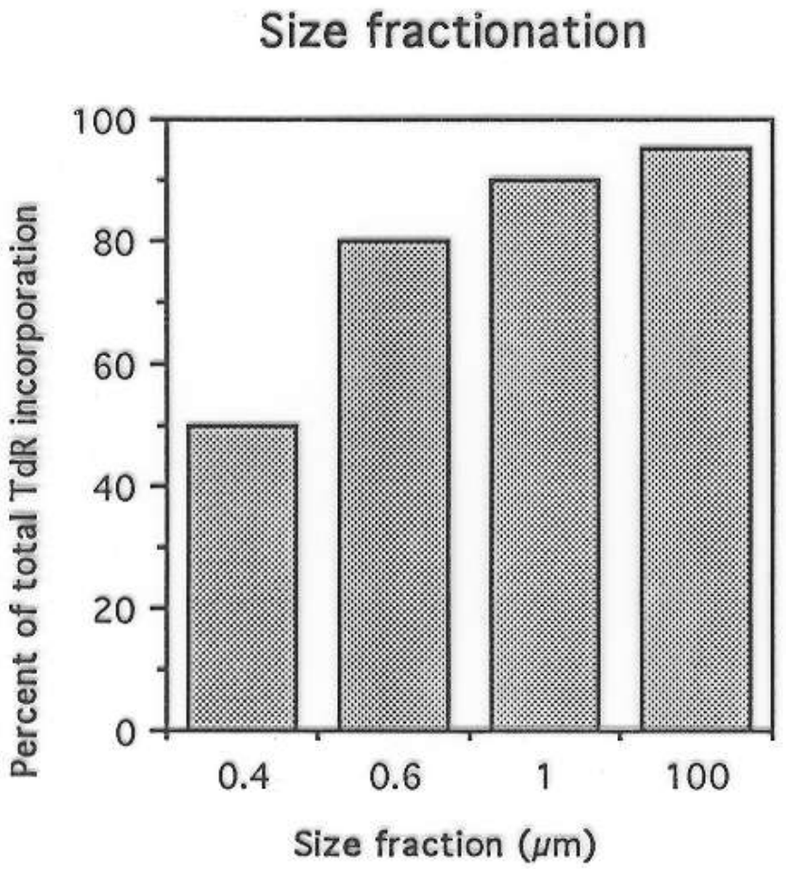
Steward & Azam, 1999 (Aquat Microb Ecol 19: 57-66)



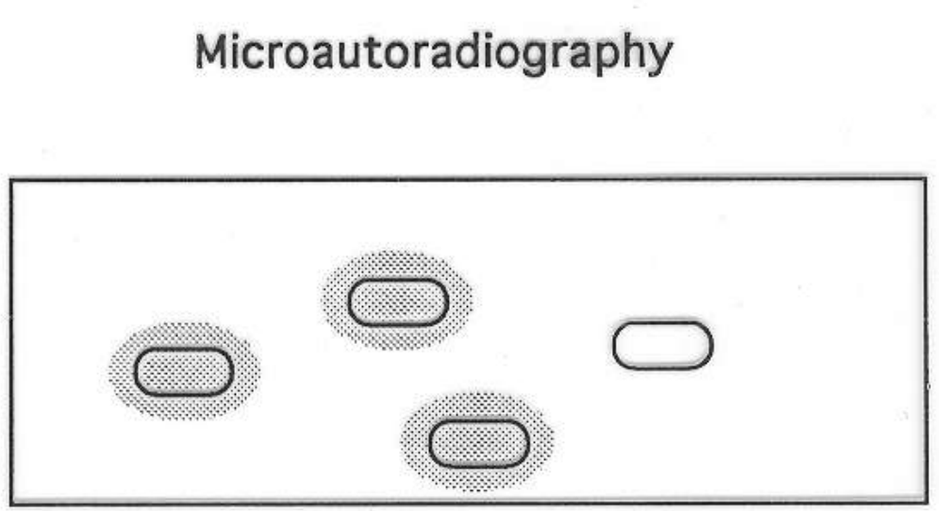
Nelson & Carlson, 2005 (Limnol. Oceanogr.: Methods 3, 211–220)

From Farooq Azam

Is most of the growth due to attached bacteria?

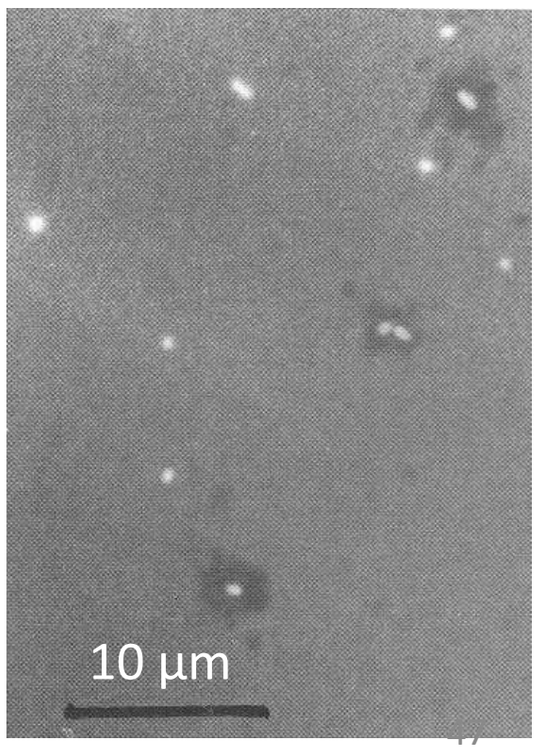


Azam, F. and Hodson, R. E. 1977. Limnol. Oceanogr. 22: 492-501



Fuhrman, J. A. and Azam, F. 1982. Mar. Biol. 66: 109-120

- Size fractionation (^{3}H-TdR)
- Autoradioagraphy (^{3}H-TdR & ^{3}H-Leu)
- ~90% due to free-living--but!!
- DOM → "Free-living" bacteria ->->? dominant pathway??



From Farooq Azam



17.2.1 Incorporazione di timidina

La 3 H-timidina viene incorporata nel DNA cellulare per azione dell'enzima timidino-chinasi, in quantità proporzionale alla velocità di riproduzione delle cellule batteriche.

Nella modalità illustrata, che prevede basse concentrazioni di timidina aggiunta e brevi tempi di incubazione, il metodo è specifico per i procarioti che sono gli unici a disporre dei meccanismi enzimatici che permettono l'incorporazione diretta della timidina nel materiale genetico.

Con coefficienti riportati in letteratura o determinati sperimentalmente è poi possibile convertire la quantità di 3 H-timidina incorporata in numero di cellule batteriche prodotte, e queste ultime in biomassa (espressa come quantità di carbonio per unità di volume).



17.2.2 Incorporazione di leucina

Incubando i procarioti in presenza di leucina marcata (3 H-leucina) è possibile determinare sperimentalmente la velocità di incorporazione della molecola nel materiale proteico cellulare e stimare quindi la velocità di sintesi proteica.

Quest'ultima, in base al rapporto relativamente costante tra proteine e biomassa, pari al 60% del peso secco (Simon e Azam, 1989), potrà essere convertita in produzione di biomassa totale, espressa anche in termini di carbonio.