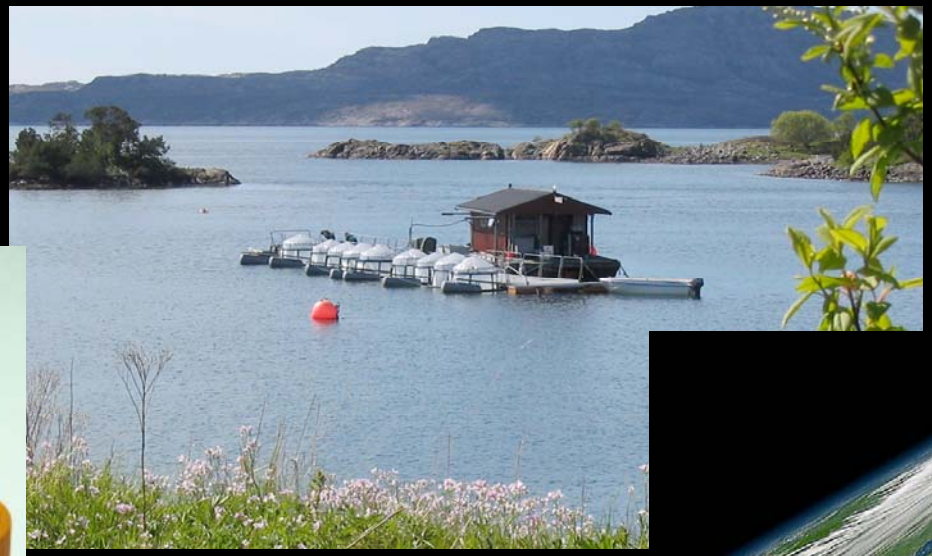
Algal culturing

UNIVERSITY OF Southampton
School of Ocean and Earth Science



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UNIVERSITY OF SOUTHAMPTON AND NATURAL ENVIRONMENT RESEARCH COUNCIL

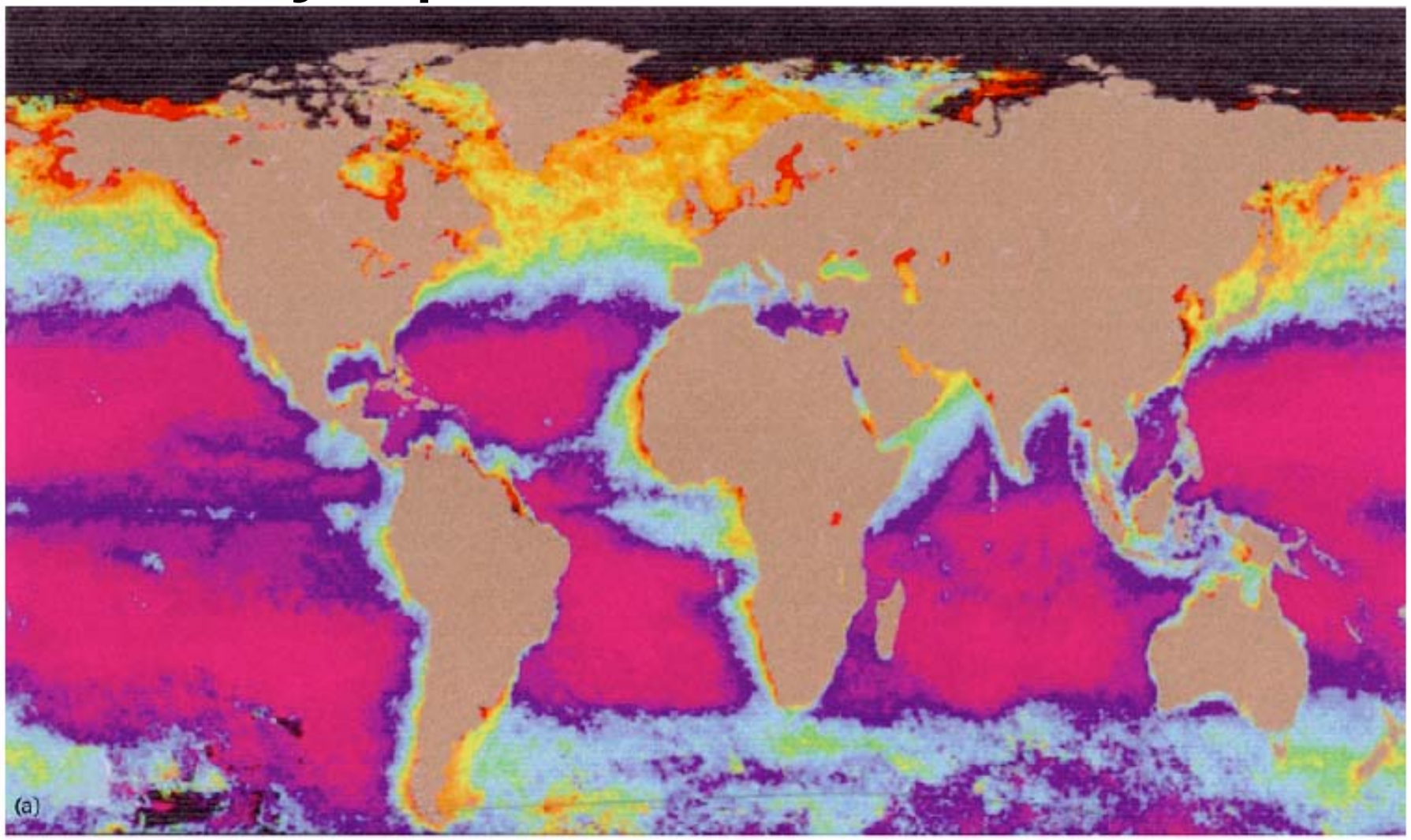
Talk outline

- Phytoplankton
- The basics in culturing algae
- Knowing your organism
- Method of manipulation
- Sampling considerations
- Evolutionary considerations

Phytoplankton

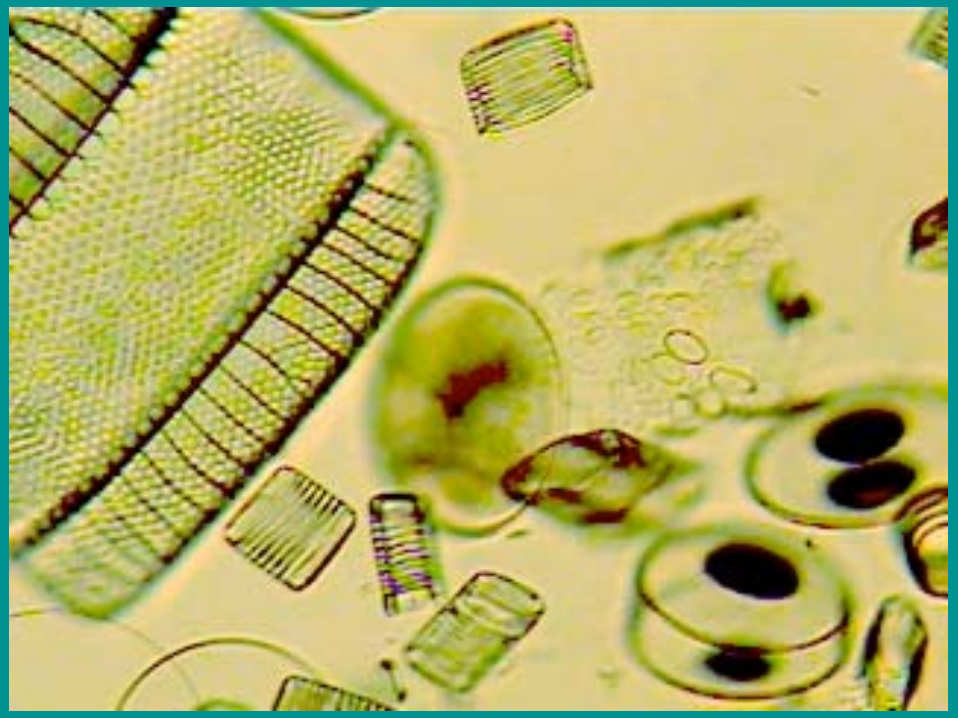
- Eukaryotic and prokaryotic species present in freshwater and marine environments.
- Phytoplankton live in the upper layer of the water column.
- The structure and abundance of the phytoplankton populations are controlled by inorganic nutrients (N, P, Si, Fe).
- Some species form blooms.
- On short time-scales, phytoplankton growth and division are tightly linked to the diel cycle.

Phytoplankton distribution



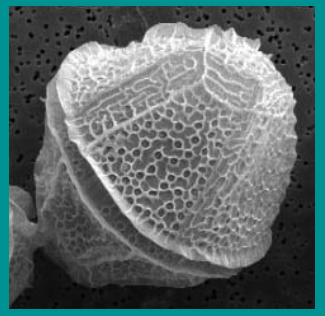
Vaulot, 2001.

Phytoplankton Size Structure and Ecosystem Function



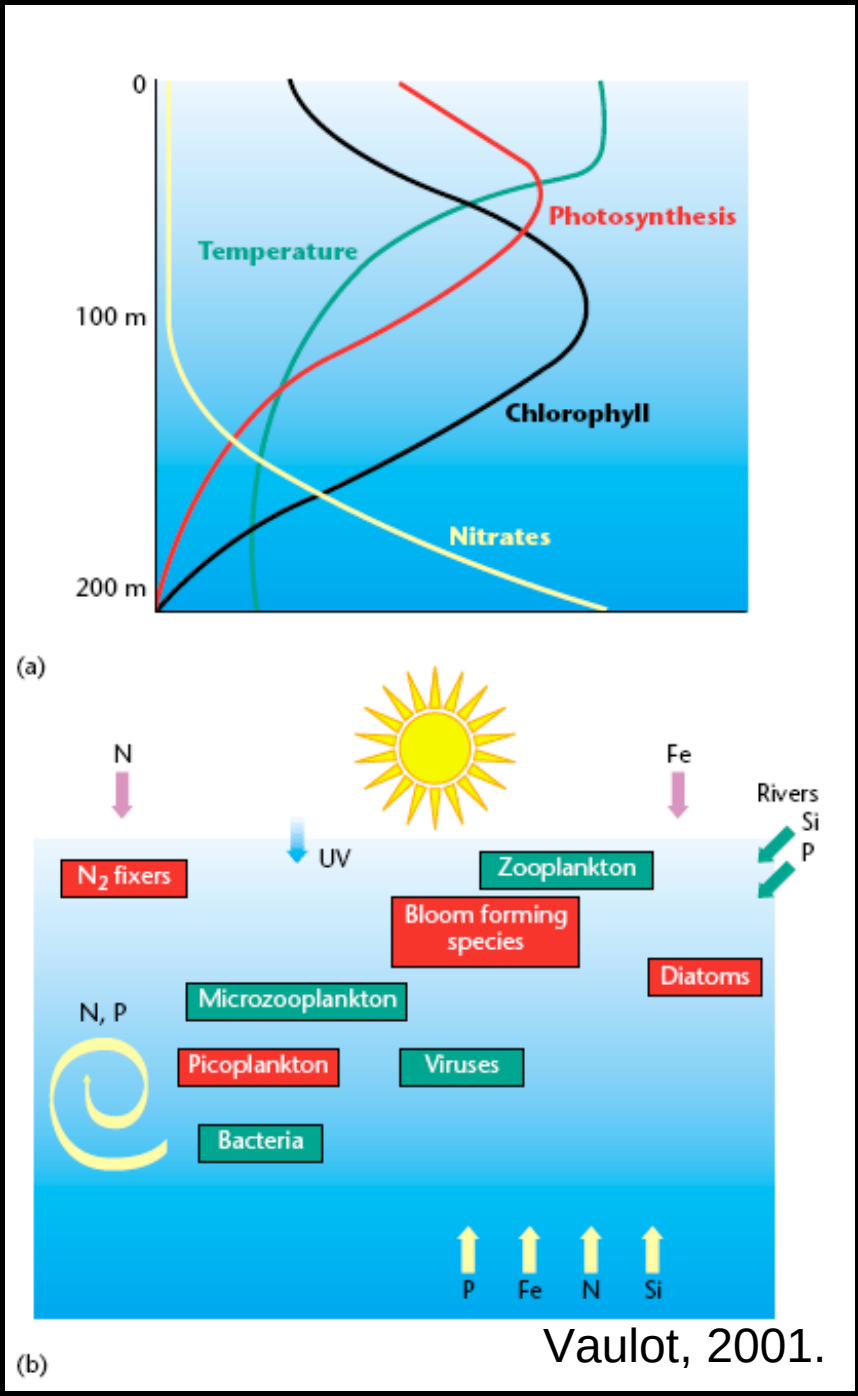
COCCOLITHOPHORES

DINOFLAGELLATES



Factors controlling primary production in the oceans

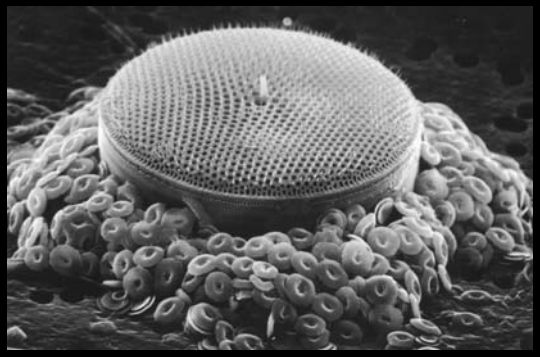
- Light
- Nutrients (C, N, P, Si, trace metals, vitamins)
- Temperature: more important in selecting for species
- Physical processes (e.g., eddies, vertical mixing)

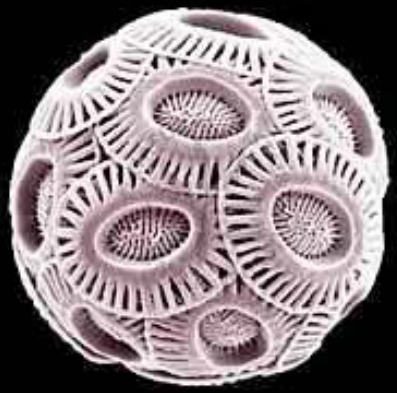


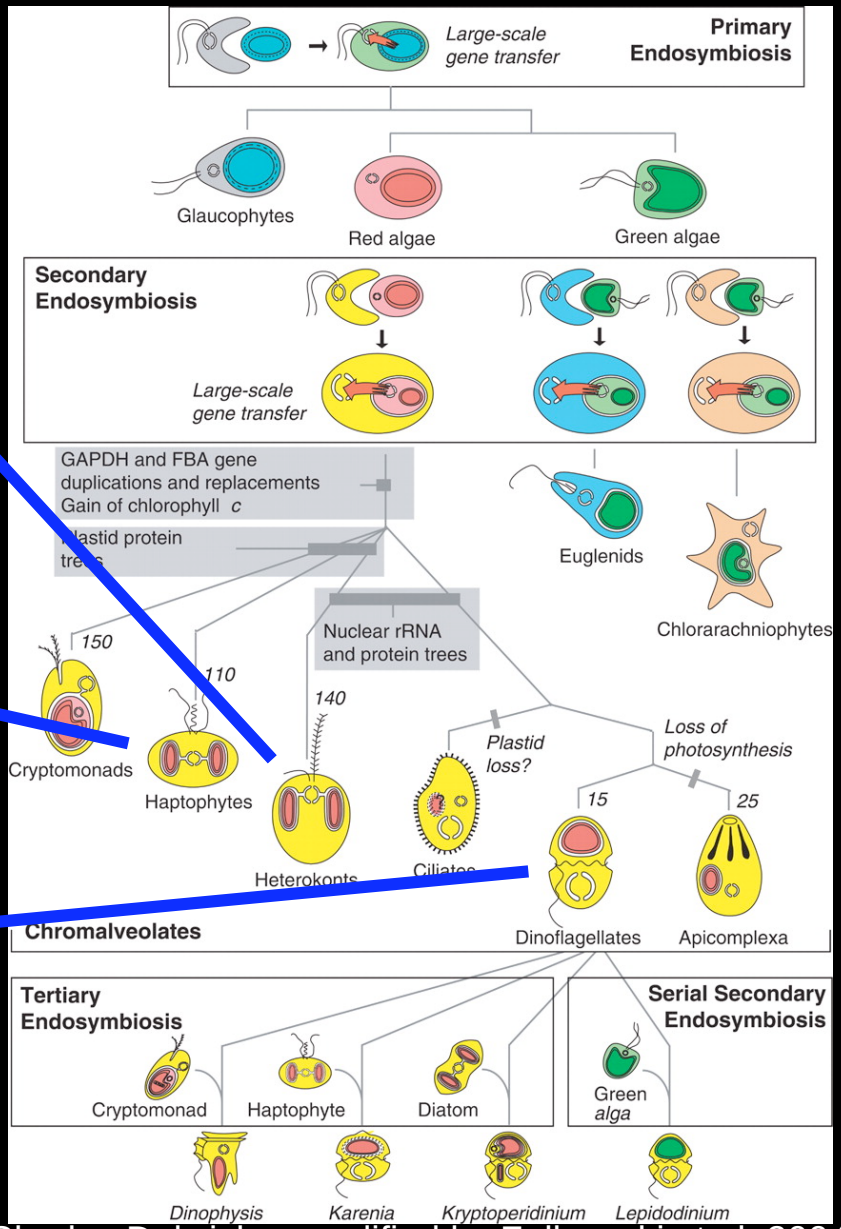
Vaulot, 2001.

Phytoplankton functional groups and global biogeochemical cycles

Algal evolution and the origin and spread of plastids by endosymbiosis







Diatom,: www.palomar.edu/ oceanography/iron.htm,
Coccolithophore image courtesy of Jeremy Young, Natural History Museum, London.Dinoflagellate: http://marinebio.org/Oceans/TheForests/

Charles Delwiche, modified by Falkowski et al, 2004.

Generation times in functional groups (autotrophs and heterotrophs)

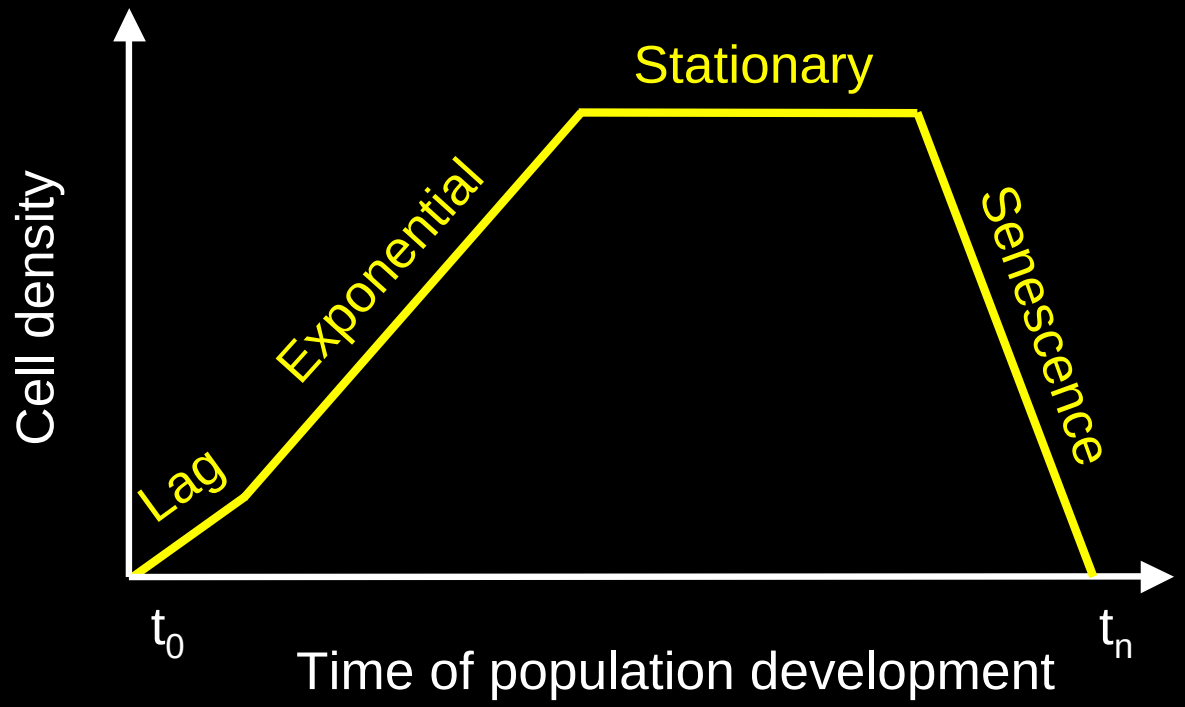
Organism	Generation time
Coccolithophores (autotrophic)	Days
Foraminifera (heterotrophs)	Weeks
Pteropods (heterotrophs)	Months

The basics in culturing algae

- Decide on type of culturing approach (batch *versus* semi-continuous *versus* continuous)
- Decide on the variables to monitor
- Do you have sufficient information about your model organism?
- Will you be able to compare your data with the relevant published results?
- Before getting started pick your colleagues' brains!

Batch cultures

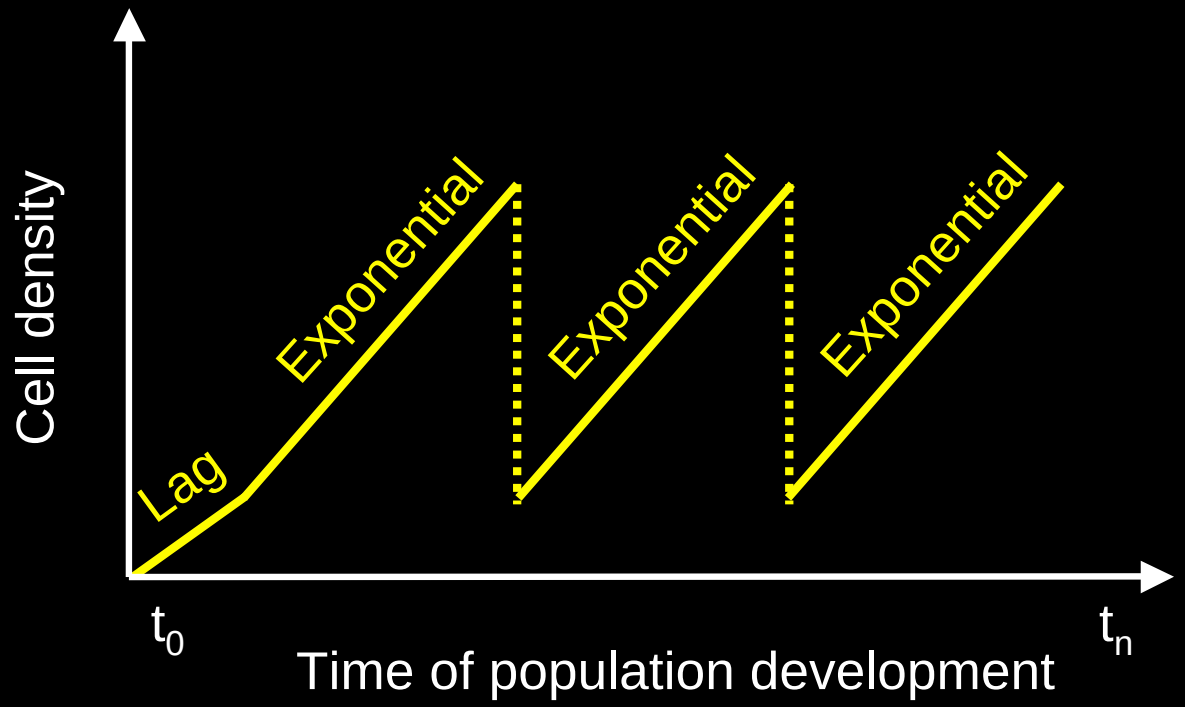
Cultures start with known physico-chemical conditions that evolve over a time period without additional manipulation



Conditions at $t_0 \neq$ conditions at t_n

Semicontinuous cultures

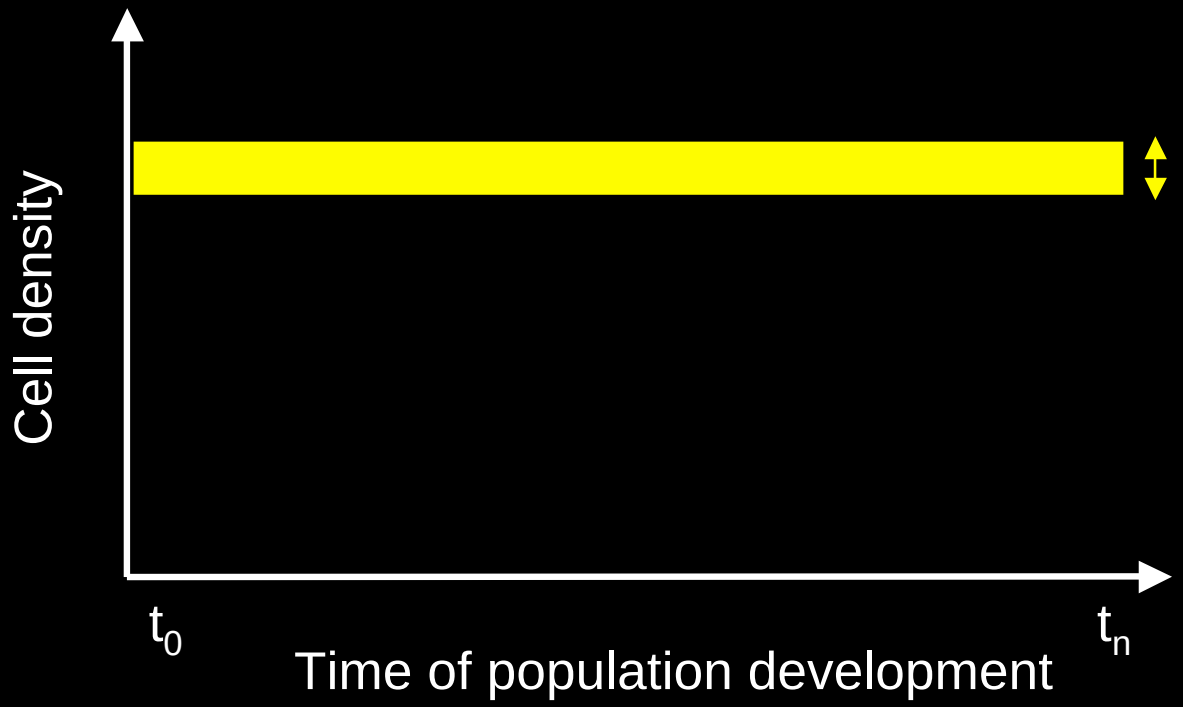
Cultures are kept exponentially-growing by subculturing within a few generations



Conditions at $t_0 \neq$ conditions at t_n but range of change is constant

Continuous cultures

Cultures are kept at ~constant conditions



Conditions at $t_0 \approx$ conditions at t_n

Effect of climate-relevant variables on physiology (calcification)

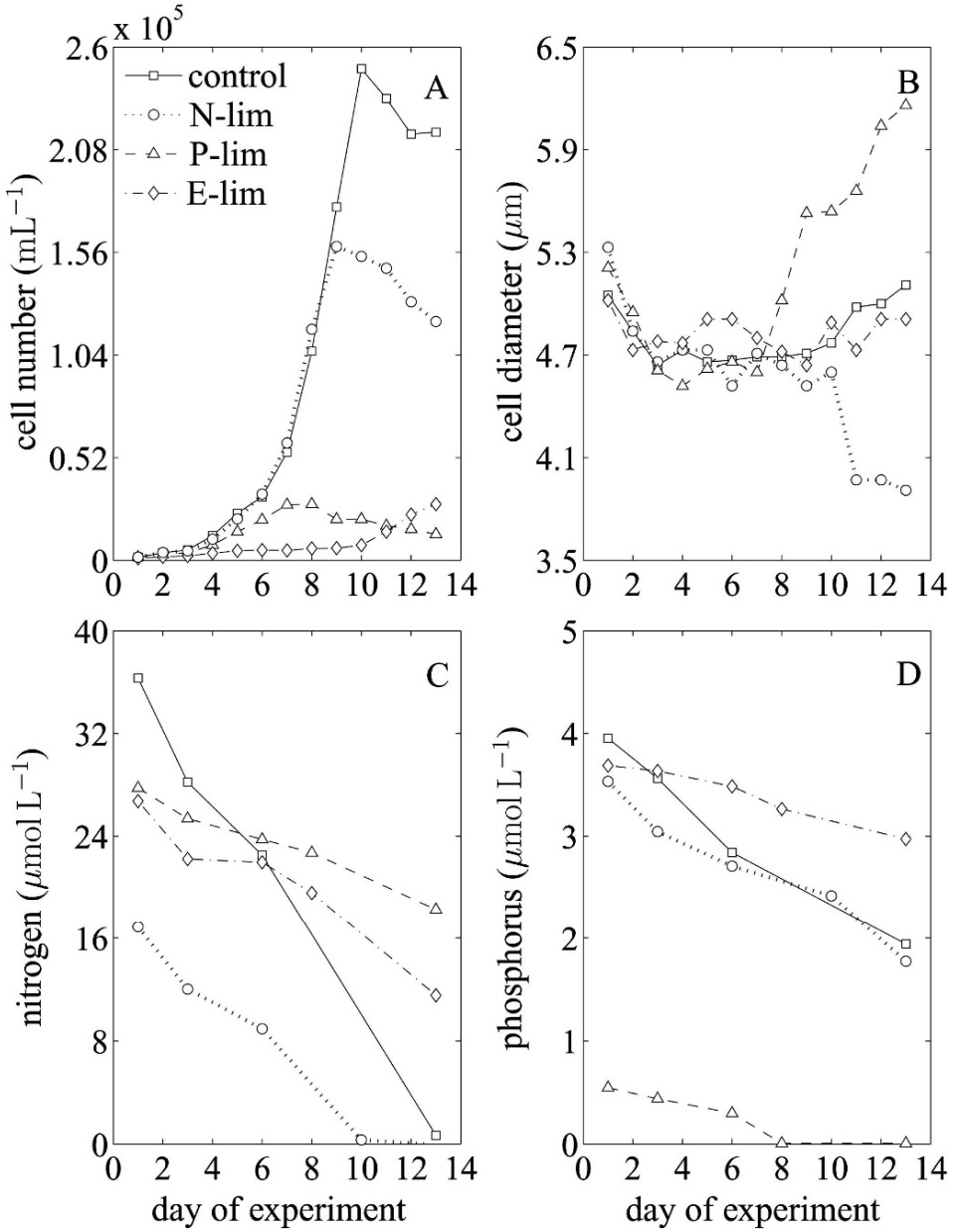
- Lab cultures (phycological research over the last century and recent work, e.g., Riebesell et al., 2000; Langer et al., 2006; Iglesias-Rodriguez et al., 2008; Shi et al., 2009).

- Shipboard experiments (e.g., Tortell et al., 2002; Engel et al., 2005)

- Mesocosms (e.g., DeLille et al., 2005)

How long should my experiment be?

- How long is a long-term experiment?
- How many changes can be detected during the acclimation phase?
- Continuous *versus* batch approaches
- Different questions require different approaches



Müller et al., 2008.

Decide on the variables to monitor

- Adjust volumes and bubbling rates accordingly and ensure headspace is kept relatively constant

- Account for changes in irradiance as a result of changes in cell density and volume

- Conduct trials to ensure conditions are known during experiment and/or calculate uptake rates and threshold for limitation of growth and physiological performance (e.g., cell quota calculations, light limitation, optimal irradiance)

Monitor growth and assess stage

- Knowledge on cell quota - assess the growth stage of the culture

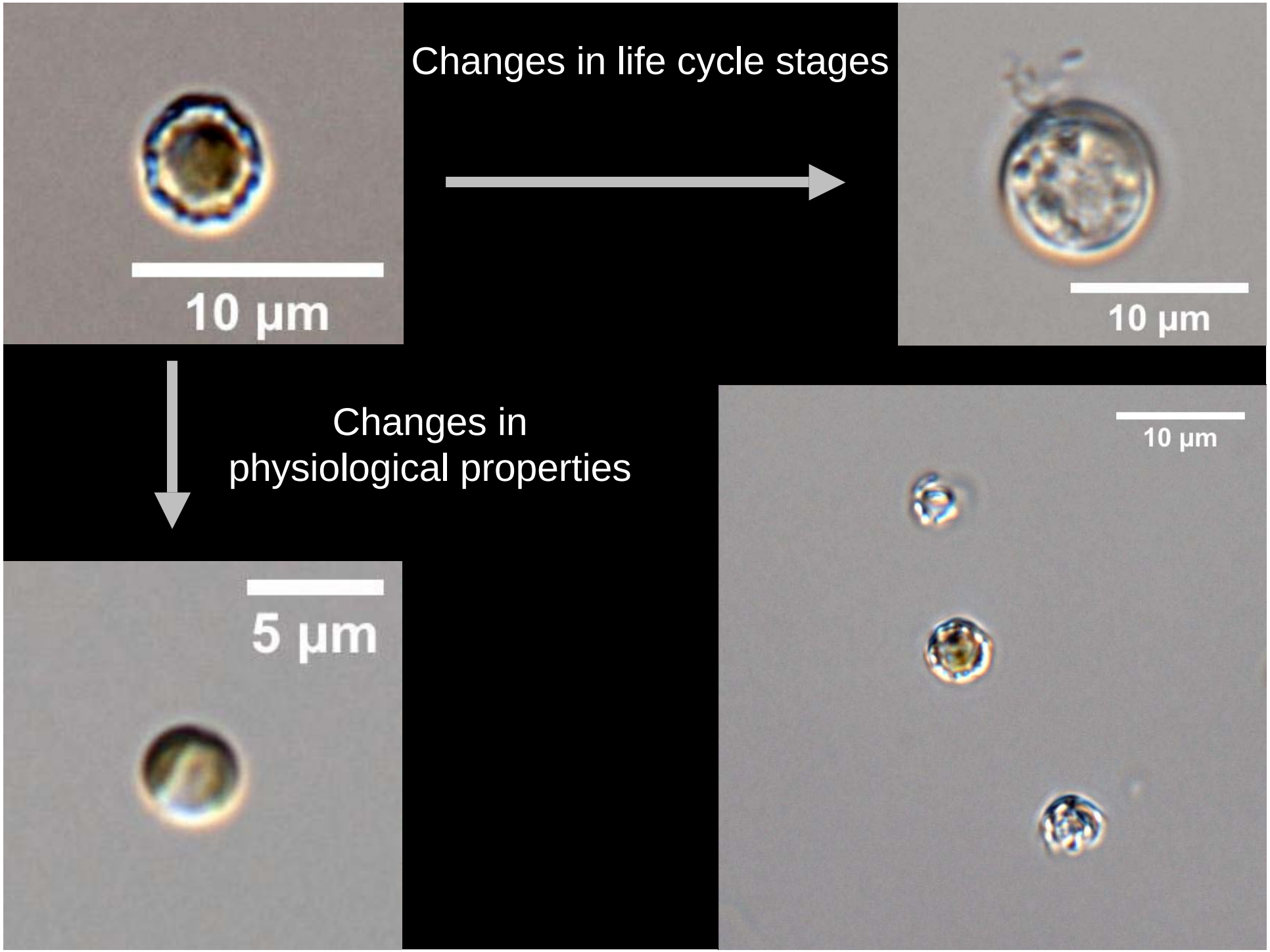
- Monitor nutrient changes during growth.

Example: if testing the effect of ocean acidification under nutrient replete conditions in batch cultures, test under exponential growth phase several times during growth

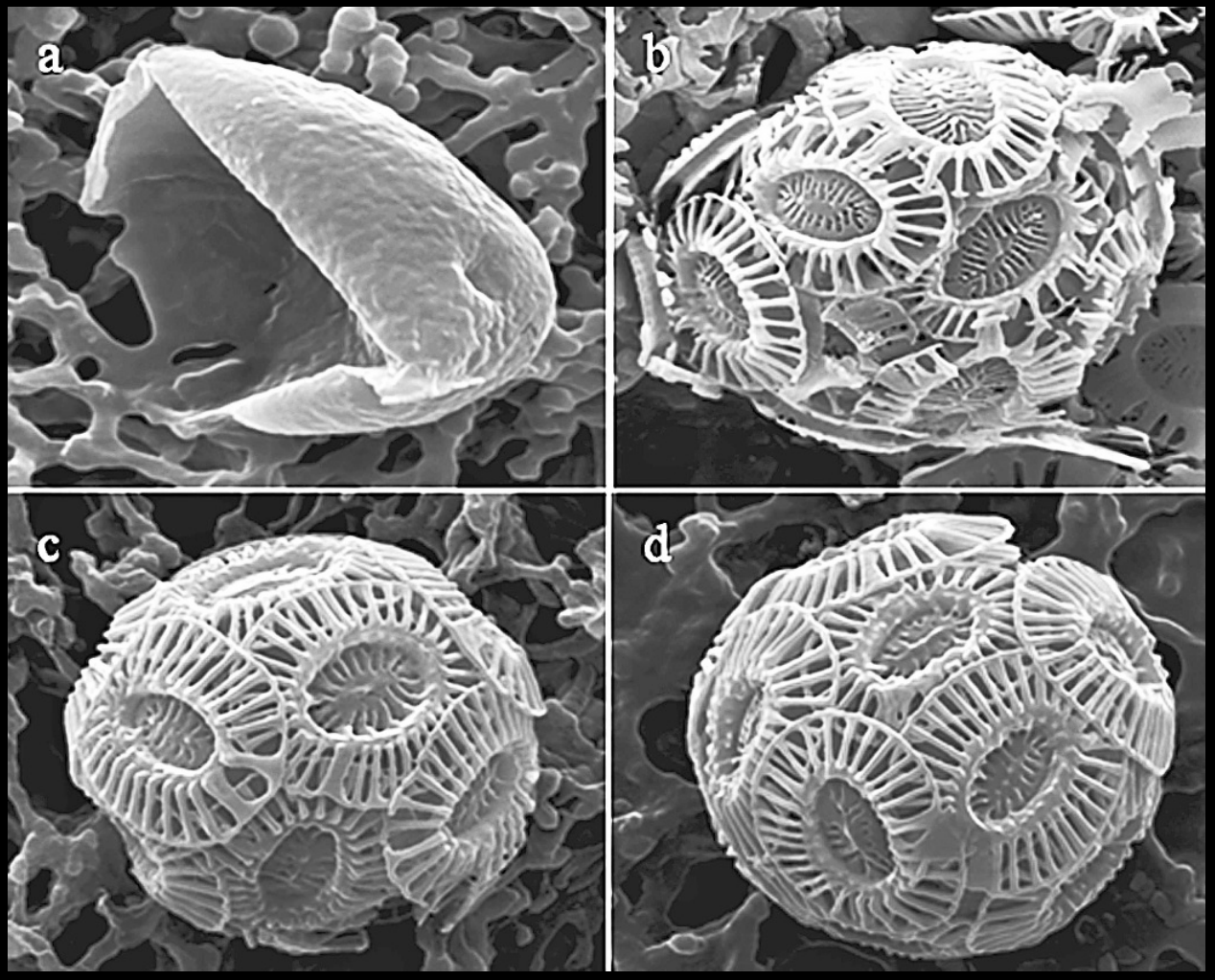
Knowing your model organism?

- What do you know about its cell biology? (e.g., life cycle, reproductive patterns, cyst formation, cellular quotas for nutrients)

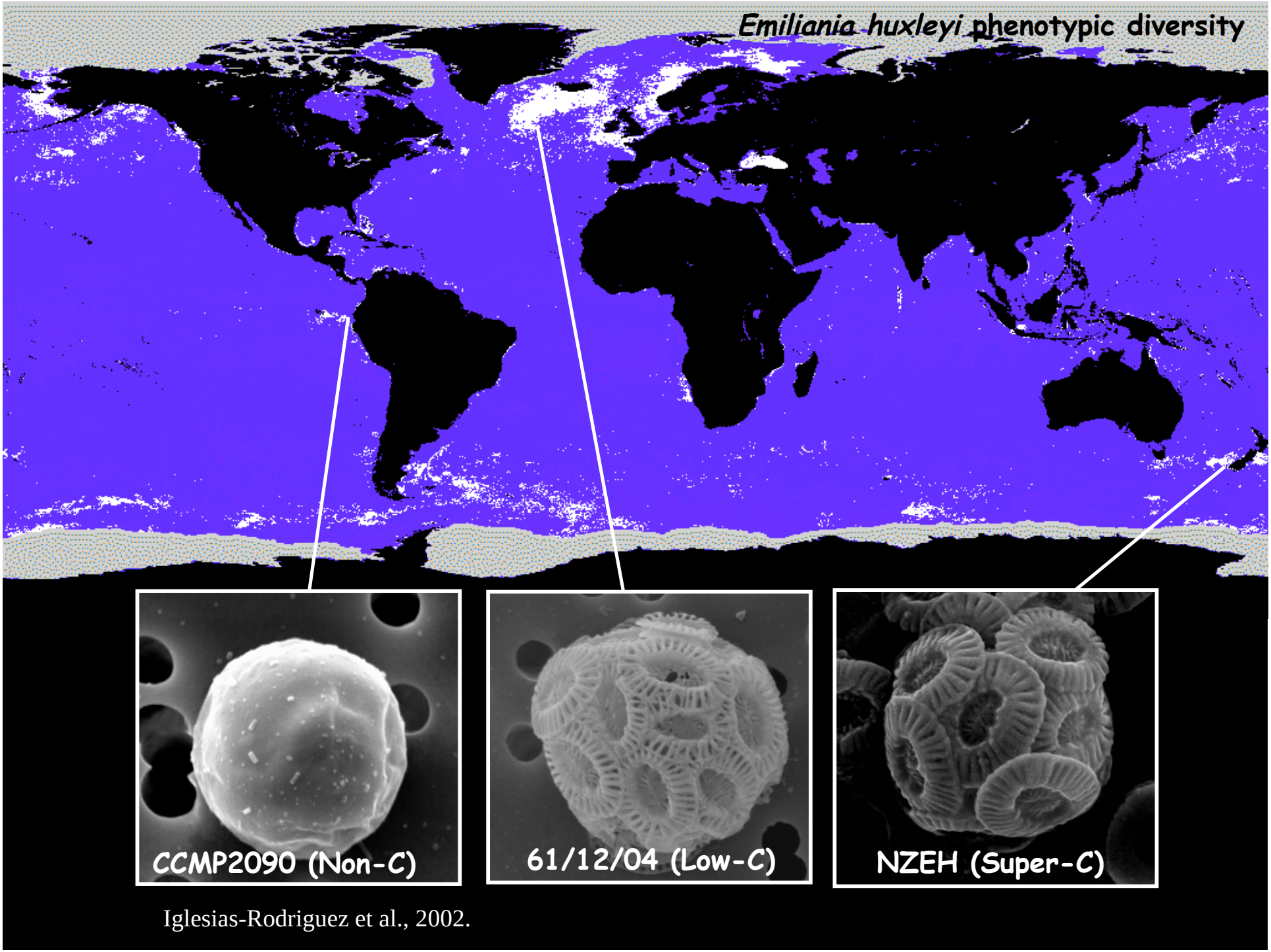
- Always remember to check under the microscope - what you ordered from the culture collection may have changed/may be contaminated/may have undergone changes in life cycle stages


Changes in life cycle stages
10 µm
10 µm
Changes in
physiological properties
5 µm
10 µm

How representative are these types in the population?

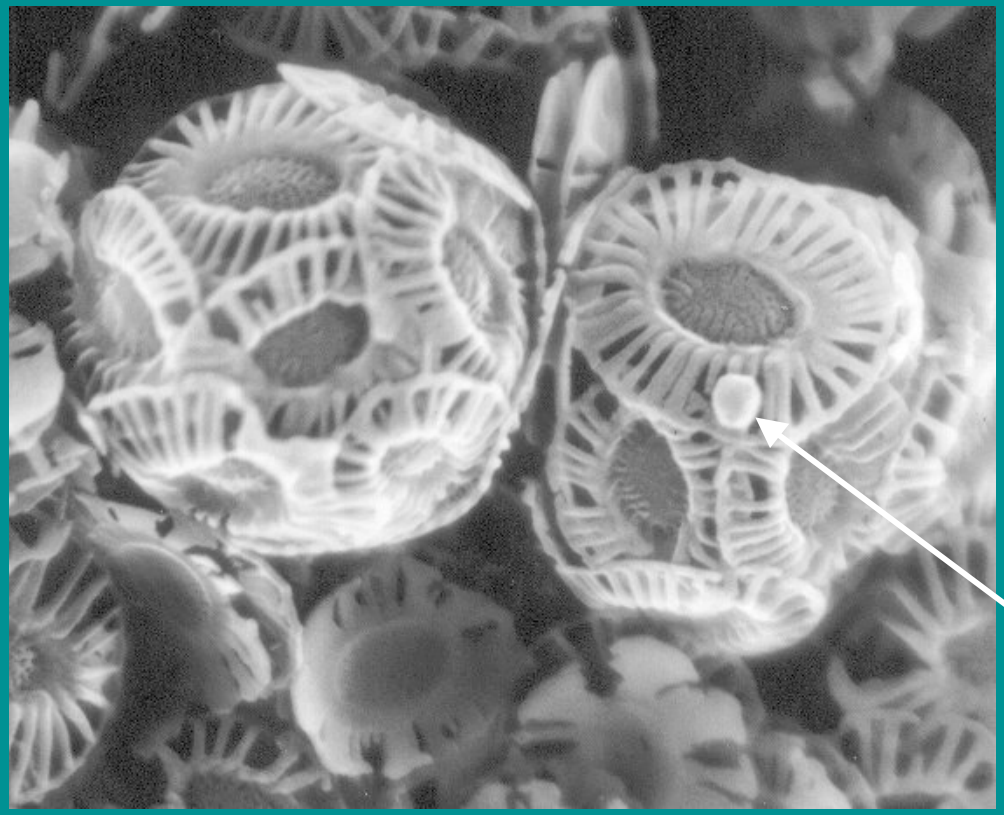


Trimborn *et al*., 2006.



Iglesias-Rodriguez et al., 2002.

Viruses



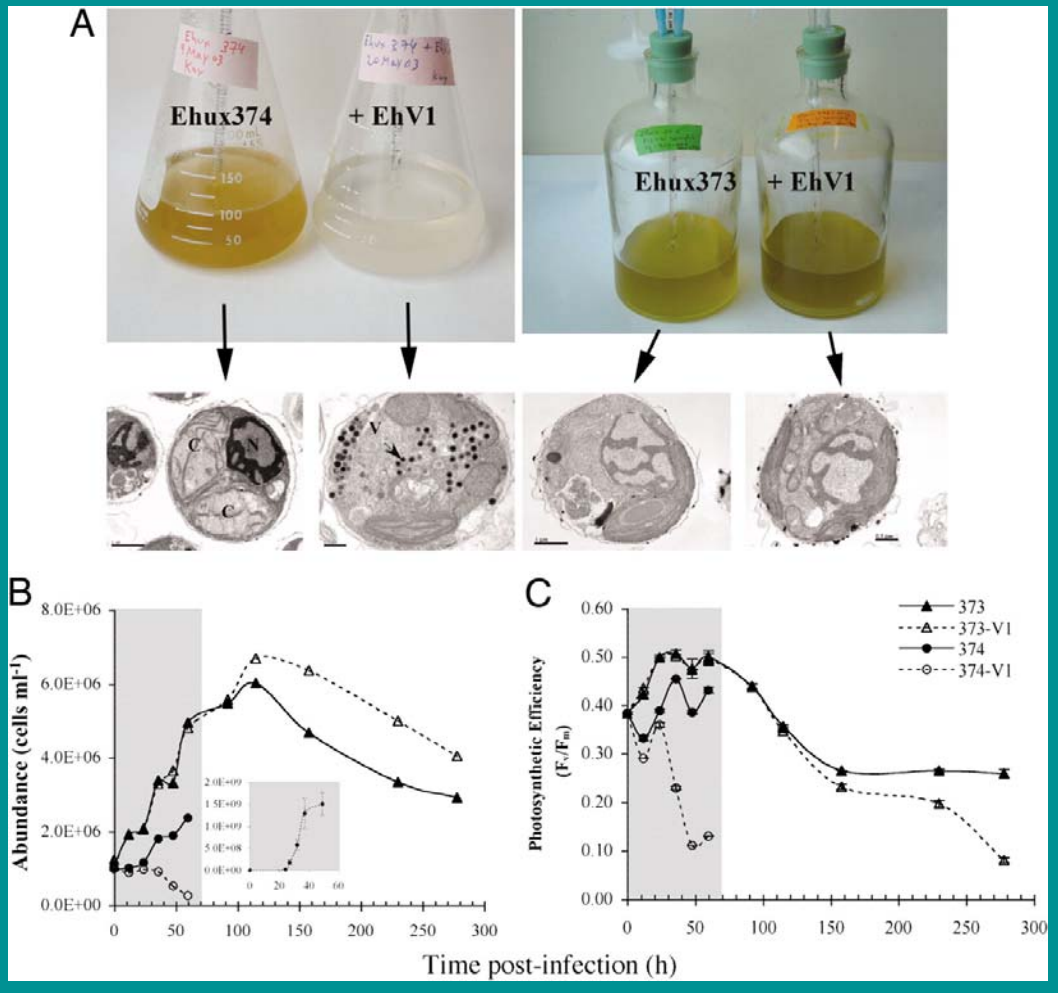
Emiliania huxleyi

- Contains biosynthetic genes for ceramide, a known inducer of PCD via a sphingolipid pathway

- Infection of Ehux374 with EhV86 triggered caspase activation.

Emiliania huxleyi virus

EhV1 infection of “sensitive” Ehux374 and “resistant” Ehux373



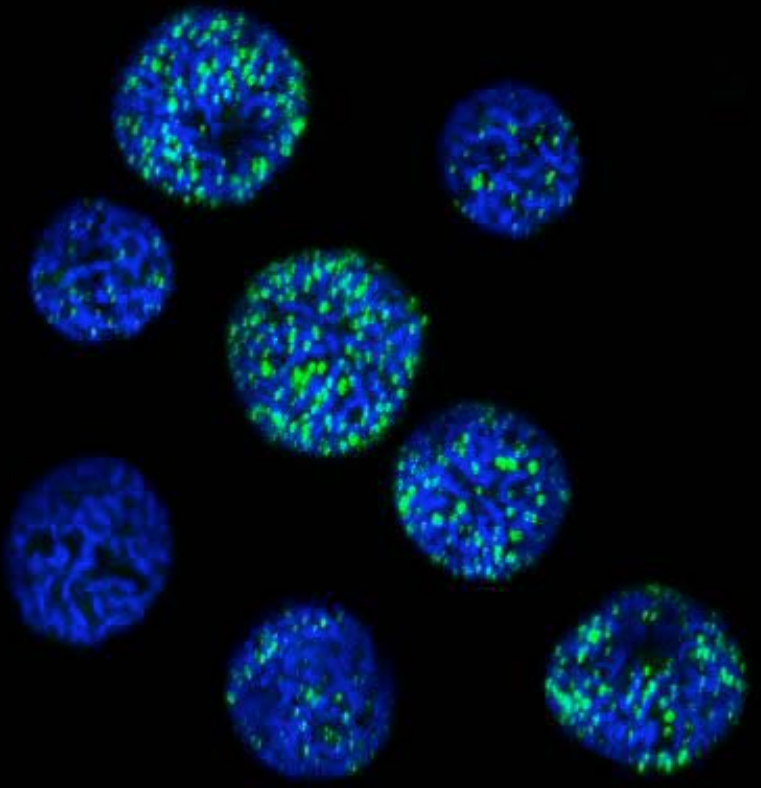
Bidle K D et al. PNAS 2007;104:6049-6054

PNAS

Intraspecific and intraclonal variability

Example: scintillon numbers by confocal microscopy

- Clonal culture *Lingulodinium polyedrum*
- 14% of cells do not contain scintillons (green)
- Only 20% have more than 10 scintillons
- Literature values of 300 scintillons per cell
- Reports of BL and non BL strains of same species



Blue = chlorophyll

Green = luciferin

Iain Dickson, pers. com.

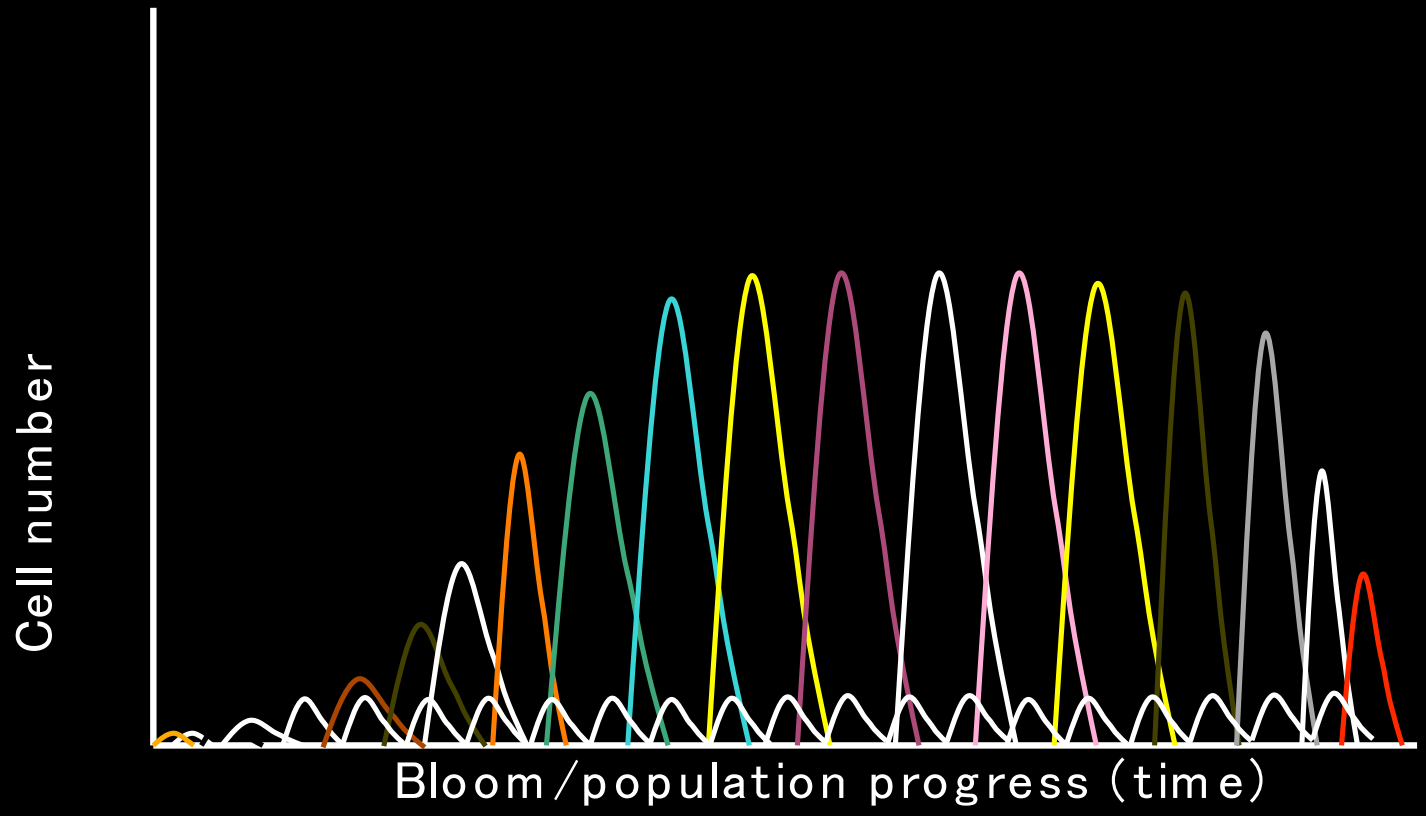
Variability in cell synchrony

• Synchronization of cell population under constant conditions (Pascual & Caswell 1997) was explained by the fact that nutrient assimilation and division are consecutive processes in the cell cycle, the latter process taking place after the completion of the former (Vaulot et al. 1987).

• Inherent variability within and between independent experiments.

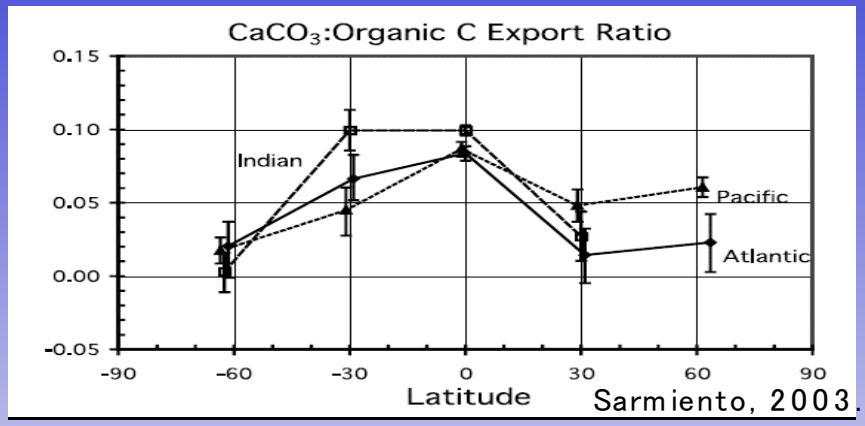
• When reporting on morphological traits via the use of images, provide numerical values, e.g. contribution of a phenotype to the total.

Changes in population structure ?



Diversity and the carbon cycle

Bloom vs non-bloom populations



What is the composition of blooms?

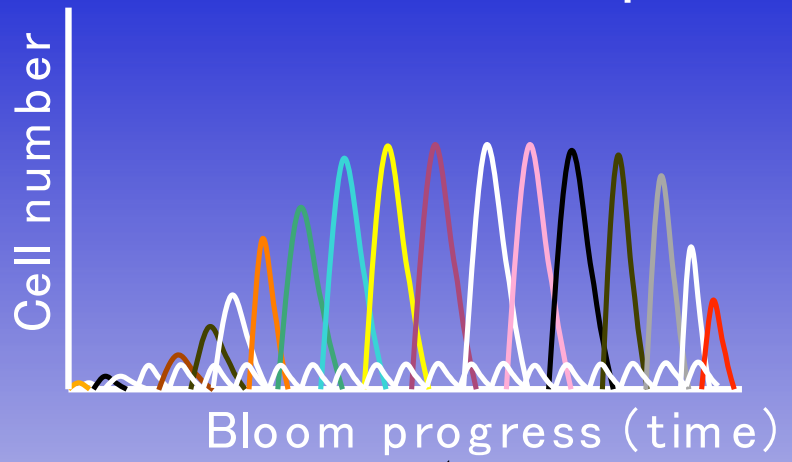
- Blooms are not clonal (more than one type)

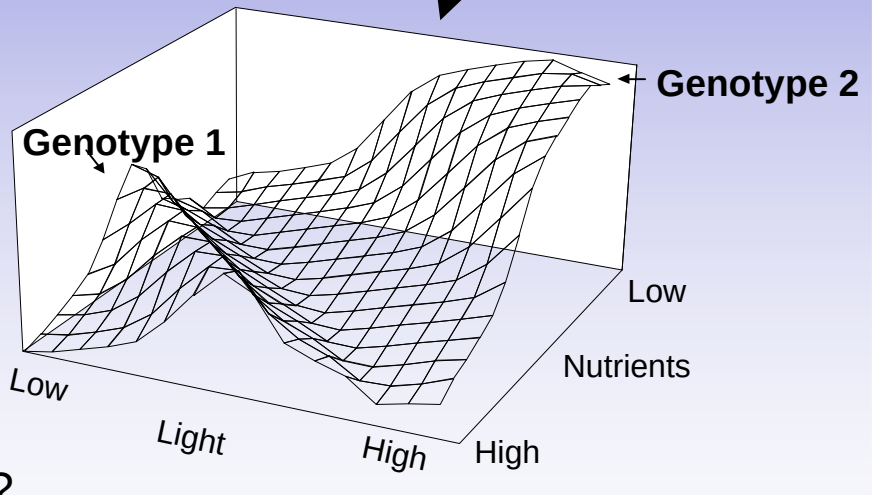
- Pops are highly diverse (Iglesias-Rodriguez 2002, 2006, Baker *et al*., 2008; Frommlet and Iglesias-Rodriguez, 2008).

How do blooms impact upon carbon chemistry?

$$Ca^{2+} + 2HCO_3^- \Leftrightarrow CaCO_3 + H_2O + CO_2$$
$$6CO_2 + 6H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2$$

Coccolithophores





How representative are clones of natural populations?

Associated changes in long-term cultures

- Thousands- millions in a liter of water
- Several generations in a year
- When microorganisms evolve, how do we find them?

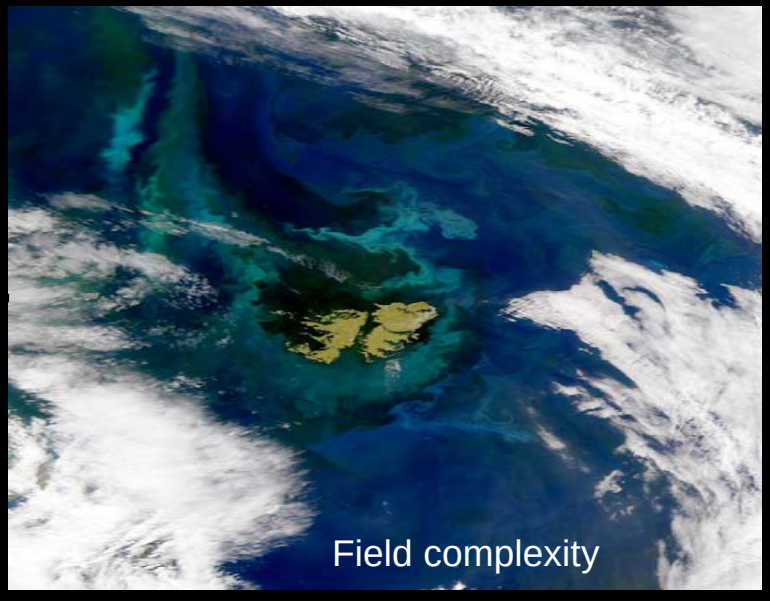
From lab cultures to the field

From the lab to the field

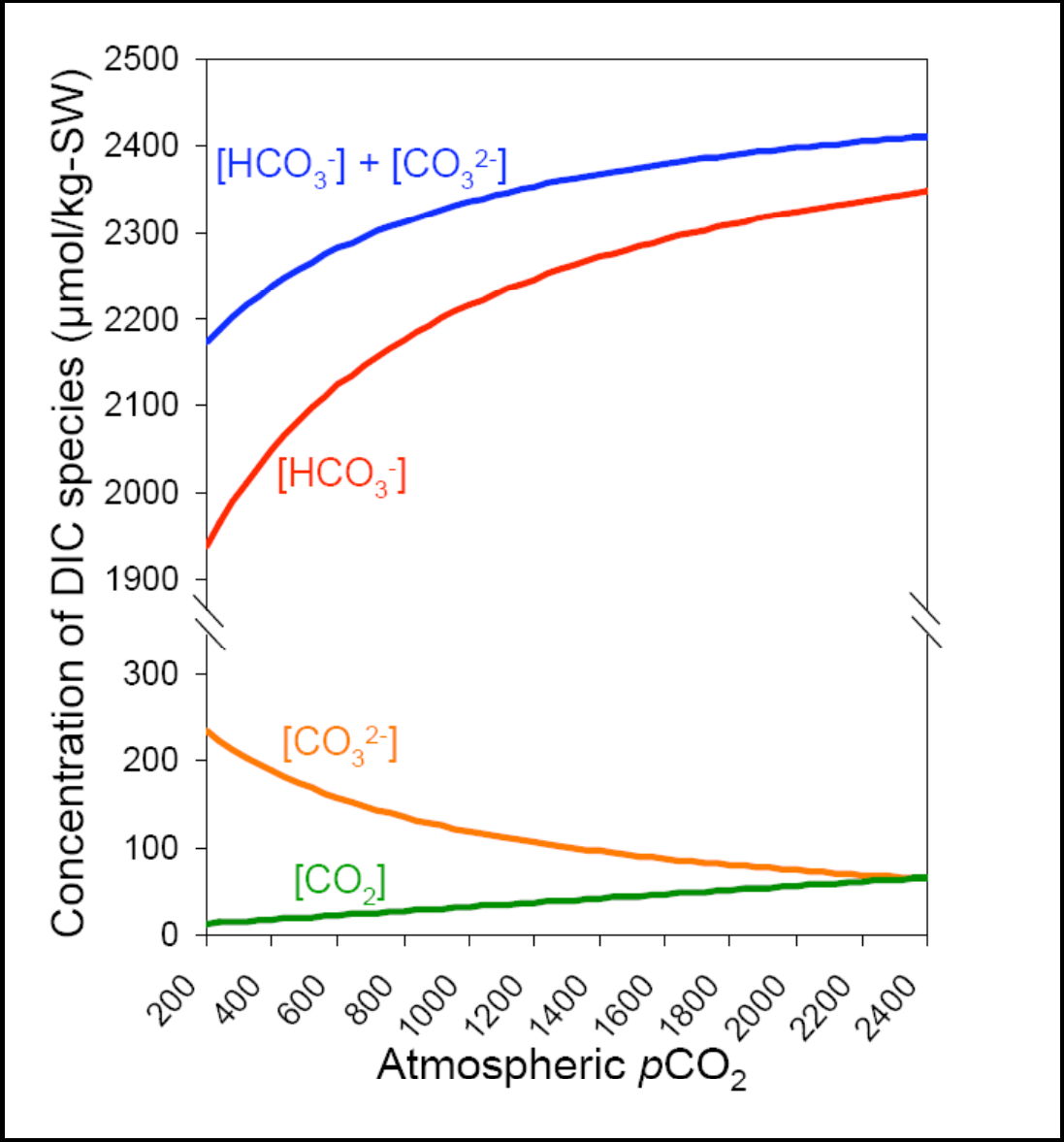

Mid-scale incubations


Monocultures

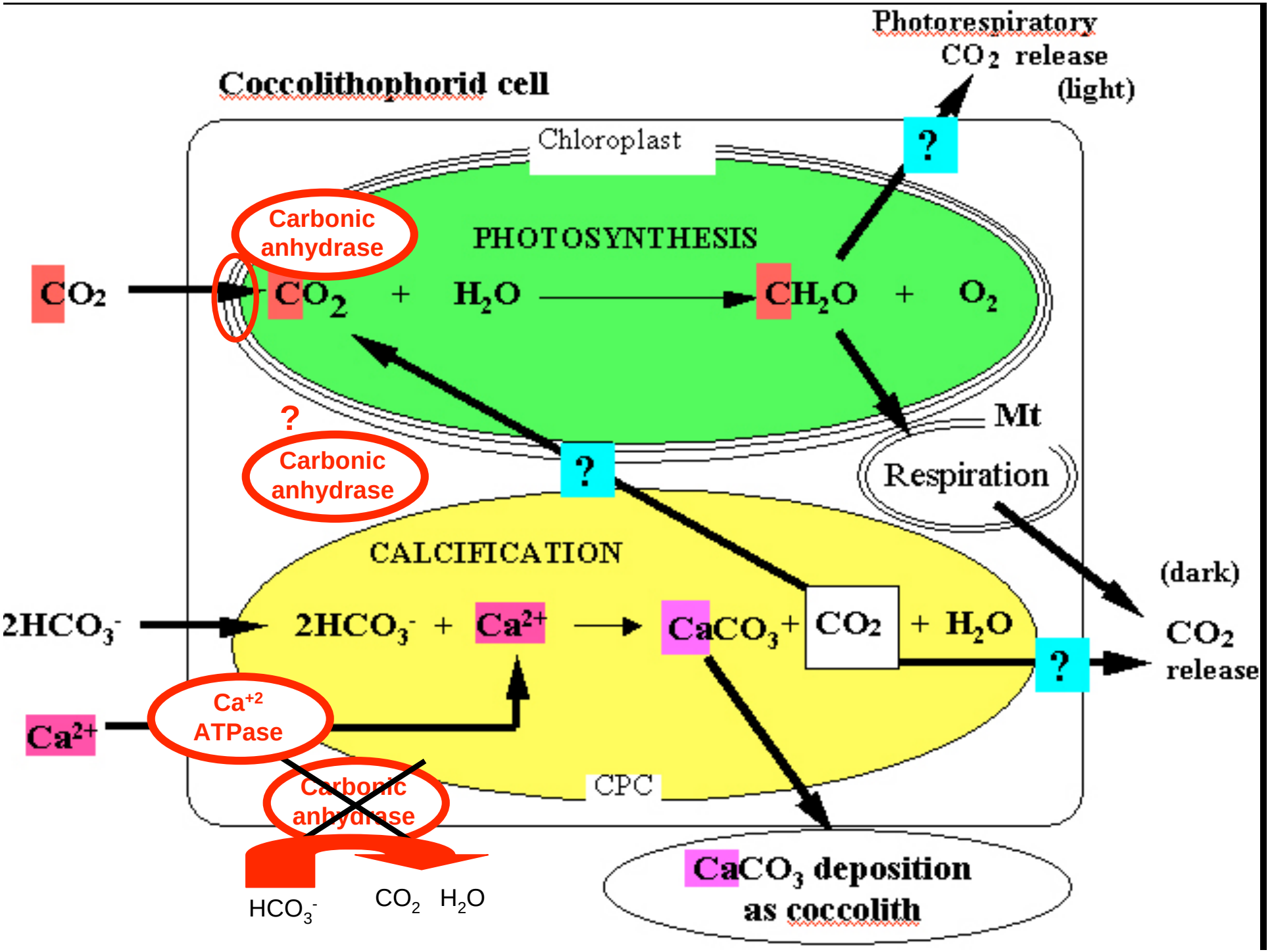

Photobioreactors

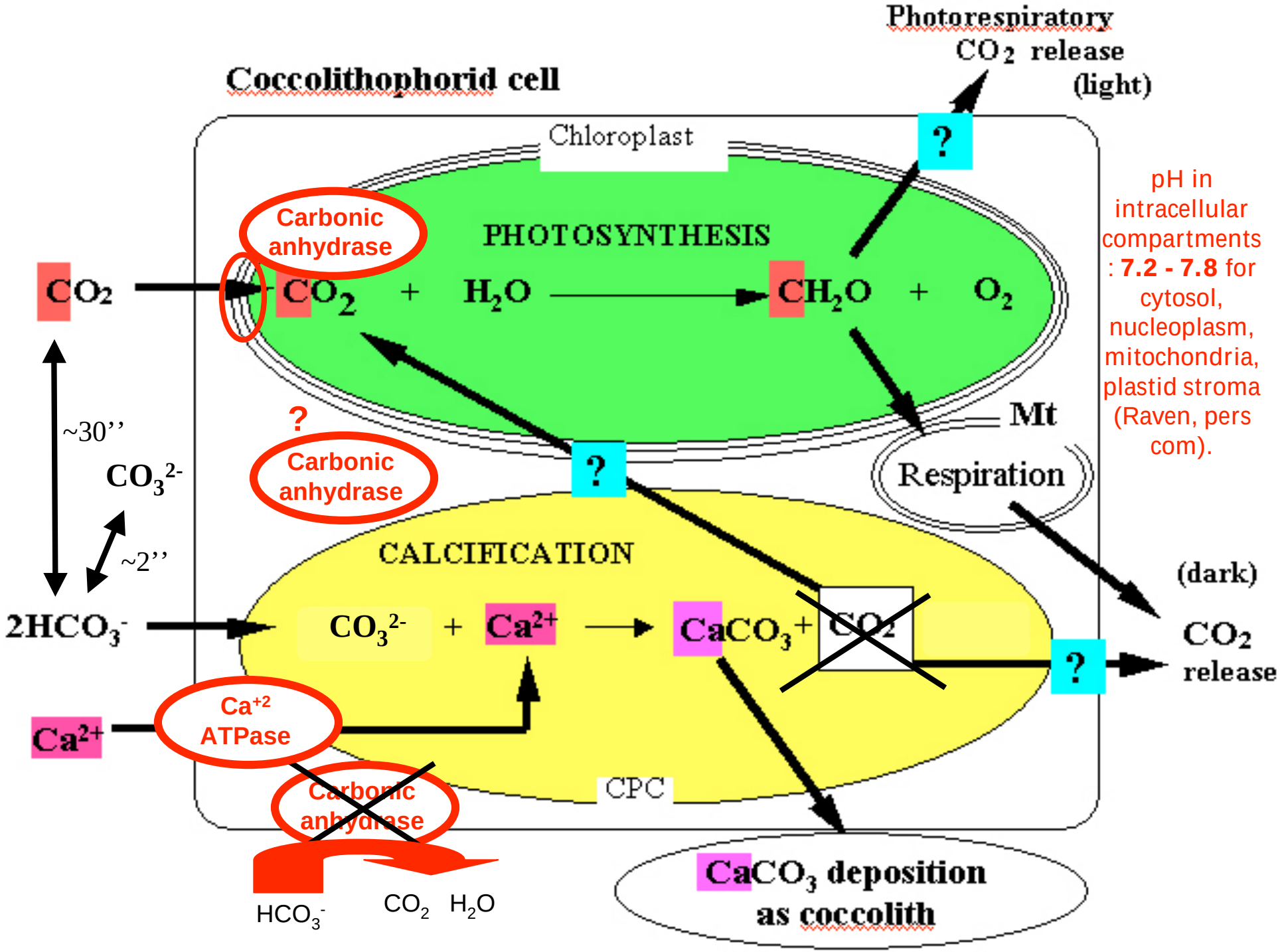

Field complexity

Method of manipulation



Iglesias-Rodriguez ,Buitenhuis, Gibbs, Lampitt, Lebrato, Raven, Ries, Schofield, in prep.


Coccolithophorid cell
Photorespiratory
CO_2 release
(light)
Chloroplast
?
Carbonic anhydrase
PHOTOSYNTHESIS
CO_2
CO_2 + H_2O
CH_2O + O_2
Mt
?
Carbonic anhydrase
?
Respiration
CALCIFICATION
(dark)
$2HCO_3^-$
$2HCO_3^-$ + Ca^{2+}
$CaCO_3$ + CO_2 + H_2O
?
CO_2 release
Ca^{2+}
Ca^{+2} ATPase
Carbonic anhydrase
CPC
HCO_3^-
CO_2 H_2O
$CaCO_3$ deposition as coccolith



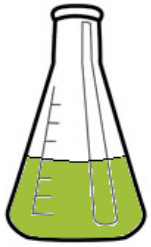
pH in intracellular compartments : **7.2 - 7.8** for cytosol, nucleoplasm, mitochondria, plastid stroma (Raven, pers com).

Bubbling considerations

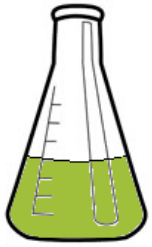
- Potential mechanical effect of bubbling (Shi et al., 2009).
- Measure flow rate, monitor pH.
- Use blanks an check t_0 conditions an how these evolve through and end of experiment.

CO_2 incubation experiments

385ppmv CO_2



1500ppmv CO_2



Decide on replicas (at least three)

Cell concentration maximum (e.g., 50,000/mL)

14.7 L cultures

Integration of results with complementary physiological and chemical measurements

Mechanisms behind the response to ocean acidification

Considerations

- Bubbling must be gentle (Shi et al., 2009) and cells must be checked for physiological stress (e.g., measure maintenance of photosynthetic health (Fv:Fm) using FRRF, check cells under microscope.

- Do you know your organism's physiology?

- In calcifying organisms, Mg tends to substitute for Ca in the lattice. Does your organism form "low-Mg calcite" (%$MgCO_3$ < 4) or "high-Mg calcite" (> 4)?

- Since calcite solubility increases with Mg substitution (Morse et al, 2006) - what is the mineralogy of your calcifier? (Lebrato et al., in review).

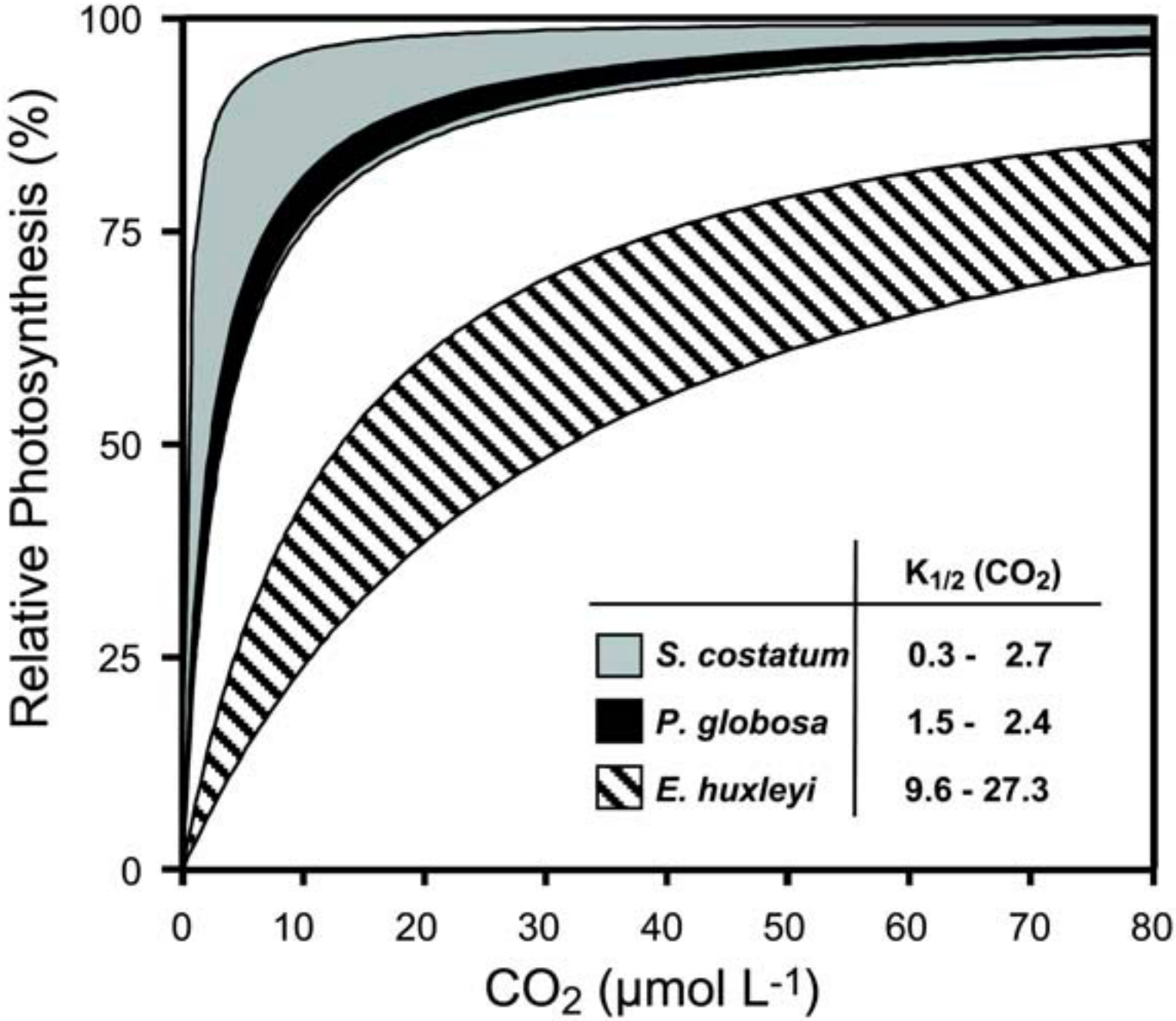
Physiological unknowns in calcifiers

- Calcification generates H^+ if using bicarbonate (no advantage and potential dissolution effect)

- Calcification does not generates H^+ if using carbonate (more susceptibility to decreasing pH??)

- Proton pumps: push protons in and out of membranes (energy cost)

- Ca^{+2} ATPases: controled by changes in Ca^{+2} availability and calcification rates in the calcification ‘vesicle’


Relative Photosynthesis (%)
100
75
50
25
0
0
10
20
30
40
50
60
70
80
CO2 (µmol L-1)
K1/2 (CO2)
S. costatum
0.3 - 2.7
P. globosa
1.5 - 2.4
E. huxleyi
9.6 - 27.3

Will you be able to compare your data with the relevant published results?

- Can you justify using similar conditions? (e.g., medium, light irradiance, temperature)

- Weight improving existing methodology *versus* data comparison with previous work

Meta-approaches

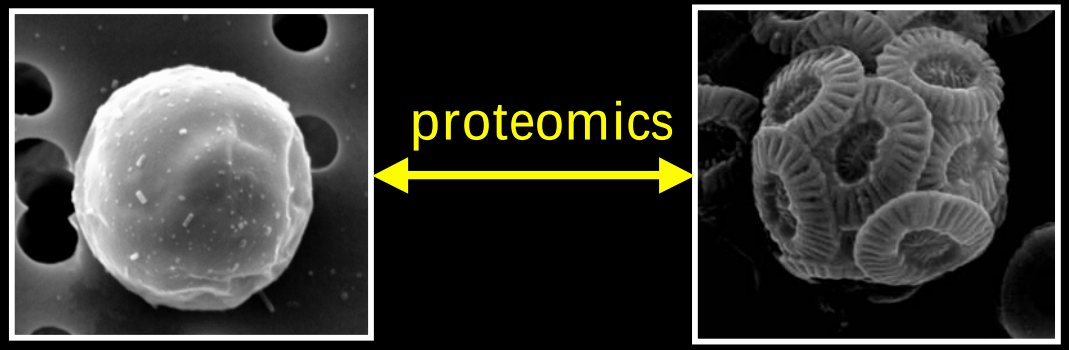
Diversity of marine microbial communities: ‘metagenomics’ (Venter et al. 2004, Delong et al. 2006, Sogin et al. 2006)

Functional properties of marine communities: ‘proteomics’ (Jones, Edwards, Skipp, O’Connor, Iglesias-Rodriguez, 2009)

If cells are in the water, what are they doing?

Genome → Genes → Static

Proteome → Function → Evolving



Before getting started pick your colleagues' brains!

- Particularly important in ocean acidification manipulations - check with the chemists, biologists, geologists

- Not such a thing as too much planning!

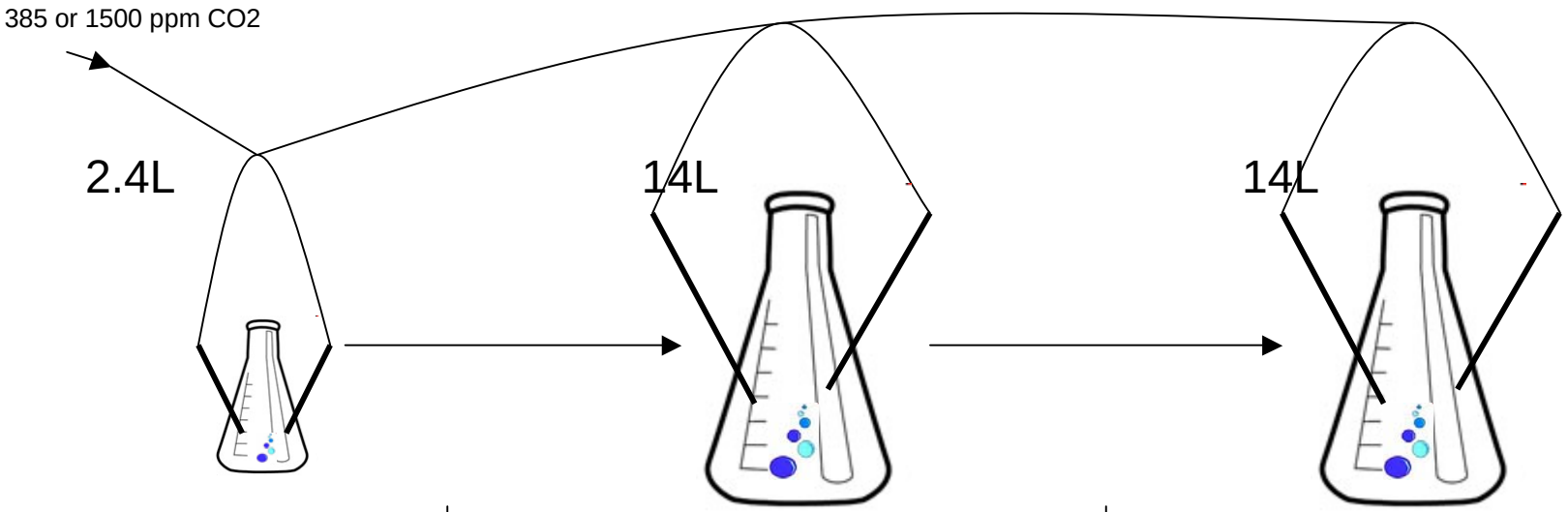
- Back up plan - e.g., collect samples for SEM to check whether there are any changes in cell morphology, volume, shape. Check what 'easy' extra-sampling you can do that will save you time

Sampling considerations

- Ensure there is sufficient replication (at least three)

- Time of sampling: implications of harvesting during day/night, be consistent

- Consider if the time length of sampling will impact upon your measurement, e.g., centrifugation time, moving cultures to a room with different temperature for harvesting - think about how harvesting time may affect the outcome



2-3 days in exponential

5/6 generations

3/4 generations

Sampling:

Before adding culture

Before transfer to 14L culture

- SEM
- Nutrients
- pH
- Salinity
- Temperature

Sampling:

Before starting bubbling

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature

Before adding culture

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature

Before transfer to 14L culture

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature
- PIC
- POC
- SEM
- FRRF

Sampling:

Before starting bubbling

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature

Before adding culture

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature

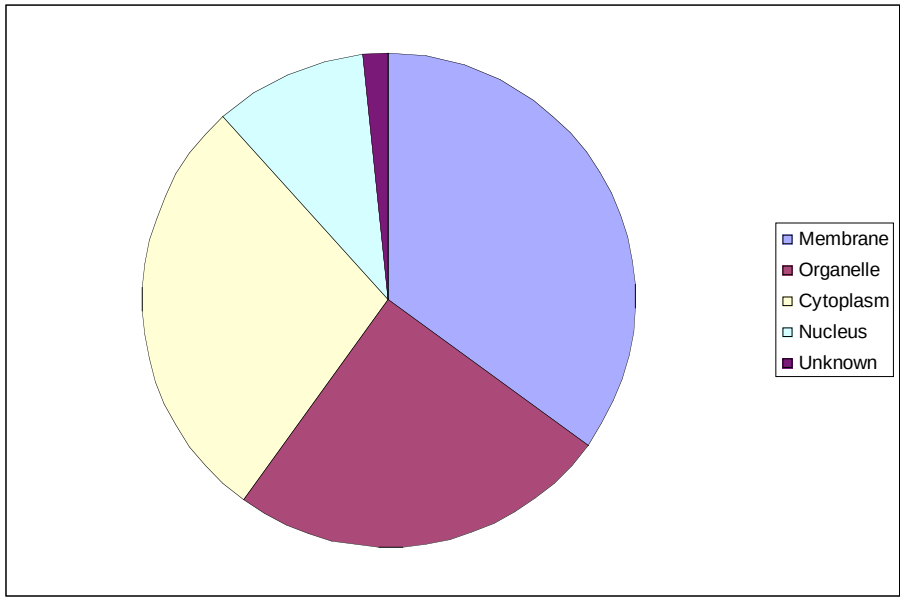
Final harvest

- DIC/Alk
- Nutrient
- pH
- Salinity
- Temperature
- PIC
- POC
- SEM
- FRRF
- Proteins for iTRAQ

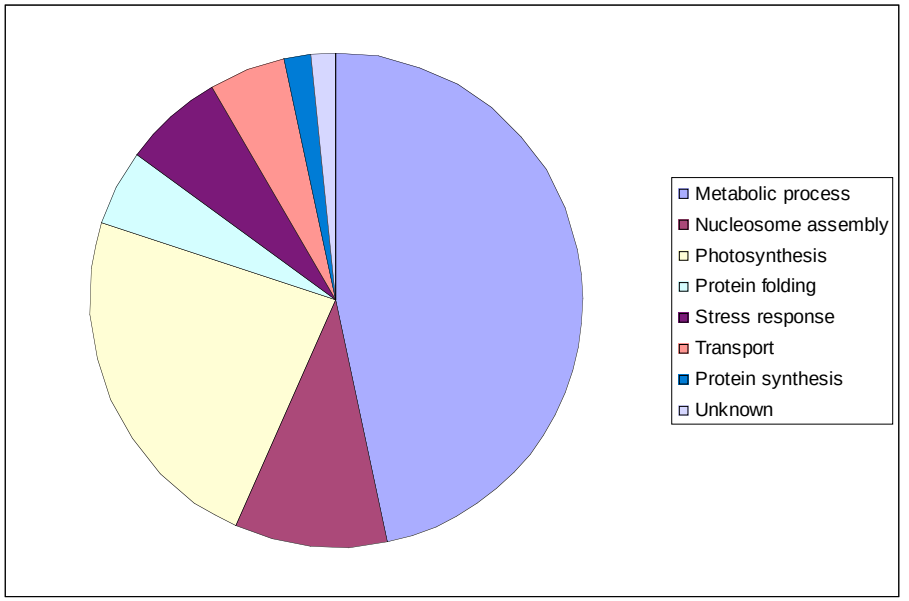
Jones, pers. com.

About ten generations of evolution!!

OA impact on coccolithophores



Subcellular location by protein cluster



Biological process by protein cluster

Jones et al., Proteomics, in review.

Sample preparation and number

- Crucial to obtaining relevant results

- Can influence downstream applications ($\Rightarrow$ it requires thorough experimental planning to save time and money!)

- Assess qualitative (list of parameters) *versus* quantitative (up and down regulation of processes) patterns

- Statistical considerations

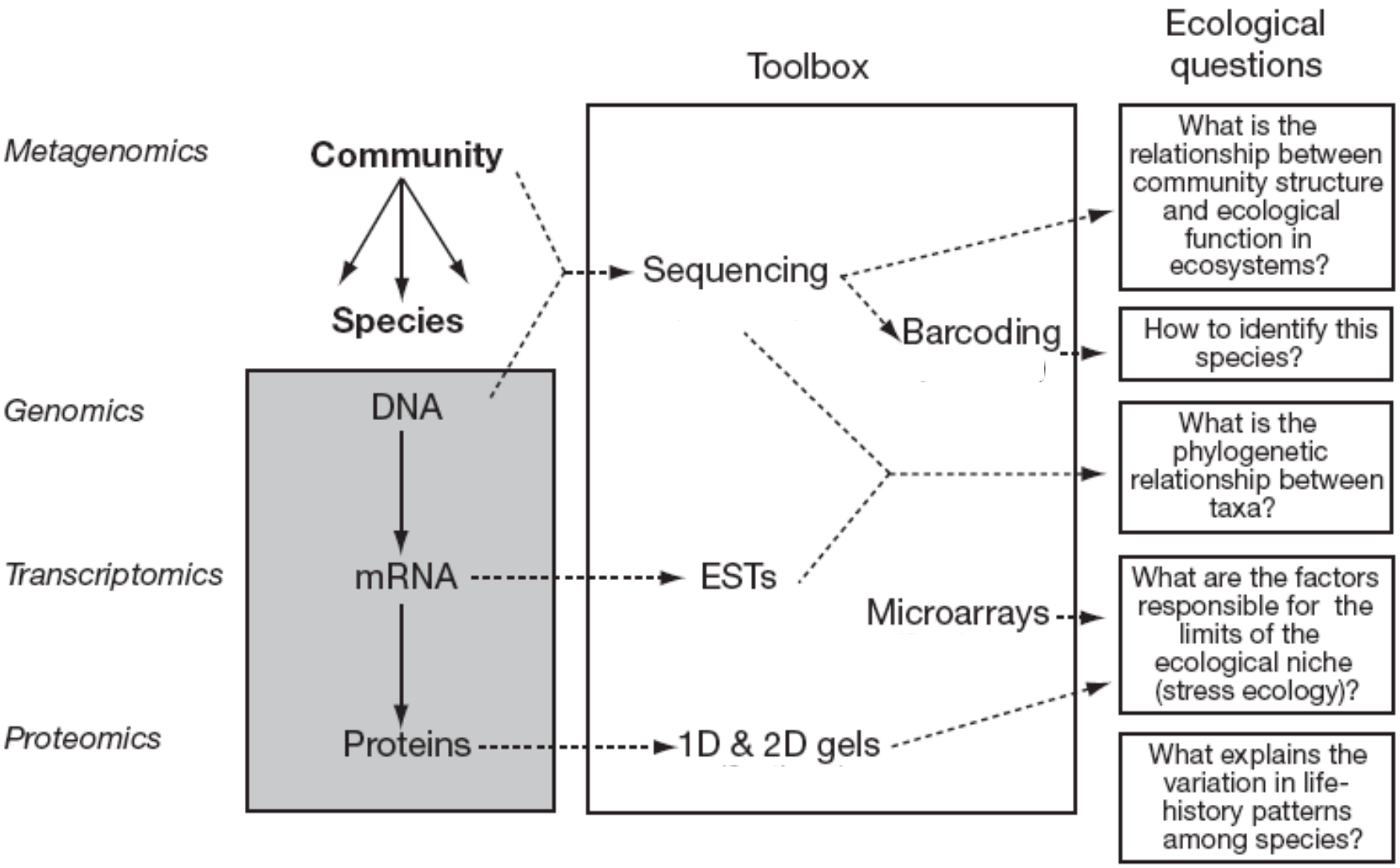
Preserving the *in vivo* properties - do you need to halt the process? Case sudy: proteomic analysis.

- Eukaryote protein synthesis inhibitors - geneticin (G418) and cycloheximide)

- Snap/flash freezing in liquid nitrogen

- Storage facilities important; often -80 ^{o}C with molecular samples

New '-omics' approaches



Dupont et al., 2008.

Molecular considerations

- A gene may remain present in the clone kept in the lab, but is either silent (not expressed) or expressed but producing an inactive product.
- A single mutation may activate the gene (no longer silent) or result in an active product.
- Further mutations can then make the microbe better at using new nutrient conditions.

Case study

Lenski's group (Michigan State University) has evolved 12 *E. coli* cultures in low nutrient broth, transferring daily, since 1988. He has achieved 40,000 generations of evolution - what has he discovered?

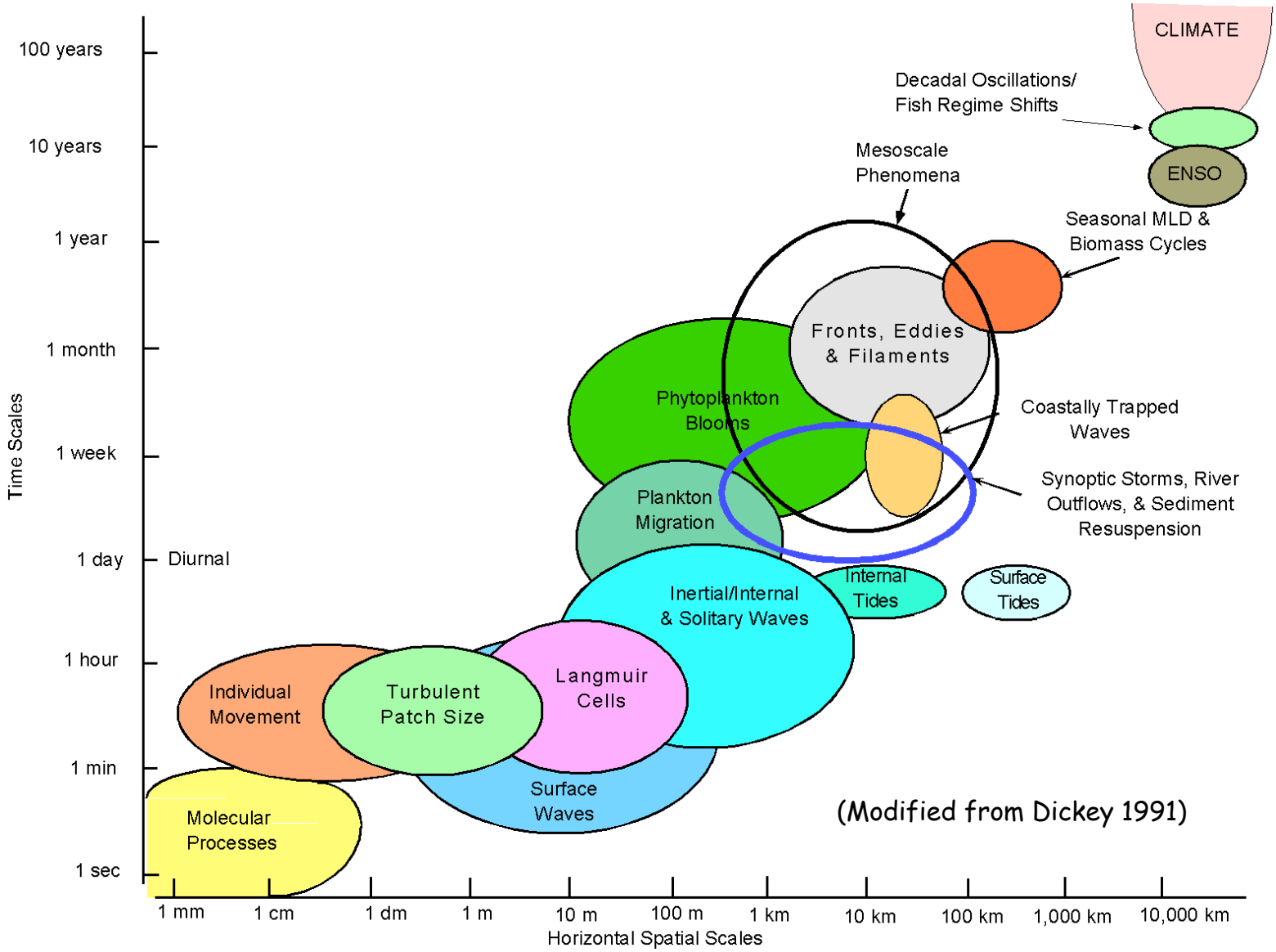
Lenski's results

- Bacteria become fitter within the first 2000 generations
- Cells become bigger
- Most of the gain comes from five different genes that have mutated
- After 20,000 generations, his group sequenced 918,700 bases from 50 isolates- they found 10 changes, all in ones with a "mutator" phenotype

Evolutionary considerations

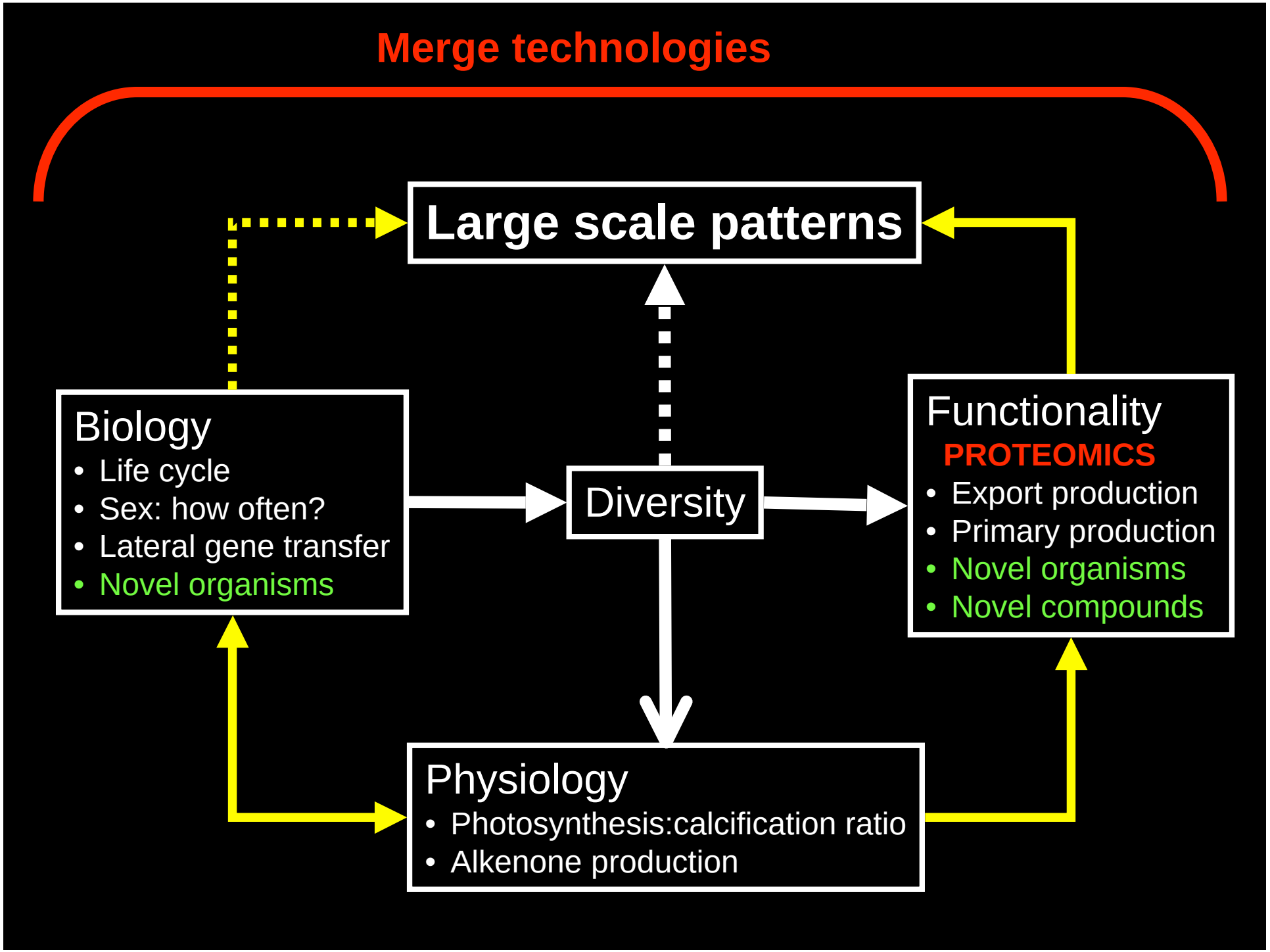
- Can we assess evolutionary adaptation?
- How old is your strain?
- How much has your strain changed in culture?

How do we link different levels of organization, e.g., biological-geological, regional-global?



Acclimation - how?

Adaptation - who wins?


Merge technologies
Large scale patterns
Biology
• Life cycle
• Sex: how often?
• Lateral gene transfer
• Novel organisms
Diversity
Functionality
PROTEOMICS
• Export production
• Primary production
• Novel organisms
• Novel compounds
Physiology
• Photosynthesis:calcification ratio
• Alkenone production

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