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

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)
Phytoplankton functional groups and global biogeochemical cycles
Algal evolution and the origin and spread of plastids by endosymbiosis


### Generation times in functional groups (autotrophs and heterotrophs)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Generation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccolithophores</td>
<td>Days</td>
</tr>
<tr>
<td>(autotrophic)</td>
<td></td>
</tr>
<tr>
<td>Foraminifera</td>
<td>Weeks</td>
</tr>
<tr>
<td>(heterotrophs)</td>
<td></td>
</tr>
<tr>
<td>Pteropods</td>
<td>Months</td>
</tr>
<tr>
<td>(heterotrophs)</td>
<td></td>
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</tbody>
</table>
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

- Lag
- Exponential
- Stationary
- Senescence

Conditions at $t_0 \neq$ conditions at $t_n$
Semicontinuous cultures

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

Conditions at $t_0$ ≠ 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 $t_n$

Cell density

Time of population development

$t_0$ $t_n$
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

Changes in physiological properties
How representative are these types in the population?

Trimborn et al., 2006.
Emiliania huxleyi phenotypic diversity

Iglesias-Rodriguez et al., 2002.
Viruses

- Contains biosynthetic genes for ceramide, a known inducer of PCD via a sphingolipid pathway
- Infection of Ehux374 with EhV86 triggered caspase activation.

*Emiliania huxleyi*  
*Emiliania huxleyi virus*
EhV1 infection of “sensitive” Ehux374 and “resistant” Ehux373

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

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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)


How do blooms impact upon carbon chemistry?

\[
\text{Ca}^{2+} + 2\text{HCO}_3^- \leftrightarrow \text{CaCO}_3 + \text{H}_2\text{O} + \text{CO}_2 \\
6\text{CO}_2 + 6\text{H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2
\]
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

Monocultures

Mid-scale incubations

Photobioreactors

Field complexity
Method of manipulation

Carbonic anhydrase

$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2$

PHOTOSYNTHESIS

Respiration

$2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$

CALCIFICATION

$\text{CaCO}_3$ deposition as coccolith

Photorespiratory $\text{CO}_2$ release (light)

(dark)

$\text{CO}_2$ release

$\text{Ca}^{2+}$ ATPase

$\text{Ca}^{2+}$

$\text{HCO}_3^-$

$\text{CO}_2$

$\text{H}_2\text{O}$
Carbonic anhydrase

$\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2$

$\text{Ca}^{2+}$ ATPase

$2\text{HCO}_3^- \rightarrow \text{CO}_3^{2-} + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{CO}_2$

$\text{pH}$ 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 and check conditions as these evolve through and end of experiment.
CO₂ incubation experiments

385ppmv CO₂  1500ppmv CO₂

Integrate results with complementary physiological and chemical measurements

Decide on replicates (at least three)

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

Mechanisms behind the response to ocean acidification

14.7 L cultures
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” (%MgCO₃ < 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’
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
Diversity of marine microbial communities: ‘metagenomics’ (Venter et al. 2004, Delong et al. 2006, Sogin et al. 2006)


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

Genome → Genes → Static

Proteome → Function → Evolving

proteomics
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
2.4L 14L 14L

2-3 days in exponential

Sampling:
- Before adding culture
  - SEM
  - Nutrients
  - pH
  - Salinity
  - Temperature
- Before transfer to 14L culture
  - DIC/Alk
  - Nutrient
  - pH
  - Salinity
  - Temperature

5/6 generations

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
- Before starting bubbling
  - DIC/Alk
  - Nutrient
  - pH
  - Salinity
  - Temperature

3/4 generations

Sampling:
- Before adding culture
  - DIC/Alk
  - Nutrient
  - pH
  - Salinity
  - Temperature
- Before transfer to 14L culture
  - DIC/Alk
  - Nutrient
  - pH
  - Salinity
  - Temperature
- Before starting bubbling
  - DIC/Alk
  - Nutrient
  - pH
  - Salinity
  - Temperature

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

385 or 1500 ppm CO2

About ten generations of evolution!!

Jones, pers. com.
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 (⇒ 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 study: proteomic analysis.

- Eukaryote protein synthesis inhibitors - geneticin (G418) and cycloheximide
- Snap/flash freezing in liquid nitrogen
- Storage facilities important; often -80 °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.
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?

(Modified from Dickey 1991)
Acclimation - how?
Adaptation - who wins?
Merge technologies

Large scale patterns

Biology
- Life cycle
- Sex: how often?
- Lateral gene transfer
- Novel organisms

Functionality
PROTEOMICS
- Export production
- Primary production
- Novel organisms
- Novel compounds

Physiology
- Photosynthesis: calcification ratio
- Alkenone production

Diversity
Co-authors, NOC team and collaborators

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