



# Past, present, and future Coccolithophores in a changing ocean.

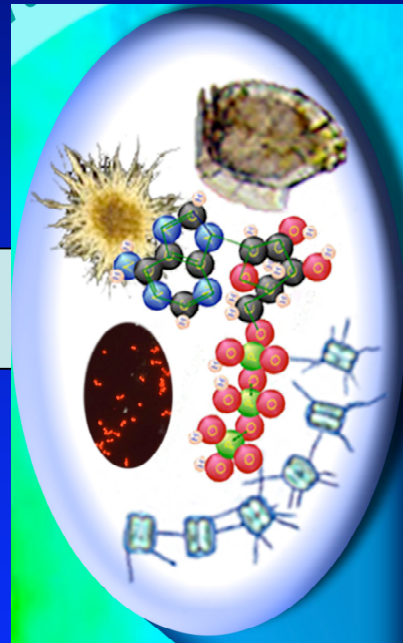
Sonya Dyhrman

Woods Hole Oceanographic Institution

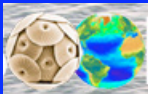
[sdyhrman@whoi.edu](mailto:sdyhrman@whoi.edu)

# A changing ocean - predicting biological and biogeochemical responses

Warming  
Inc CO<sub>2</sub>  
Dec pH  
N and P?



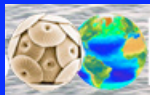
Ecology  
Biogeochemistry  
Climate



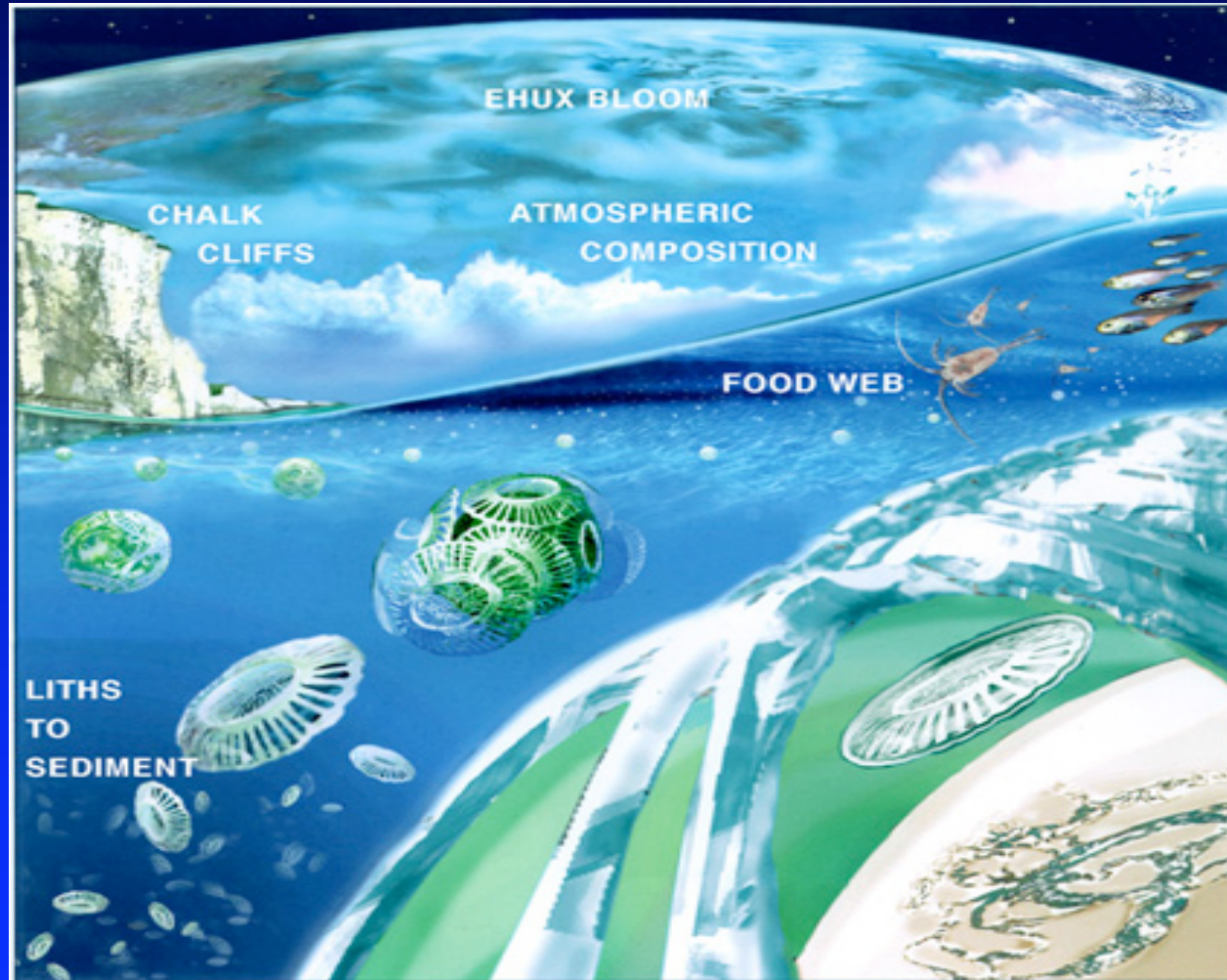


# The take home message...

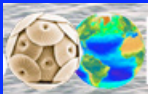
- Coccolithophores are really cool - in part because the dynamics of this one group can dramatically influence biogeochemical cycling
- Of the coccolithophores, *Emiliana huxleyi* is emerging as increasingly important model for studies of ...
  - *Paleo climate reconstruction*
  - *S cycling*
  - *Viral dynamics*
  - *Carbon cycling*
- Anatomy of a eukaryotic genome project “euks are challenging”
- Transcriptome profiling for aiding genome annotation and moving beyond “capacity”
  - *Introduction to SAGE*
  - *N and P scavenging in E. huxleyi*
- What does the future hold? ... The challenges of prediction in microbial oceanography
  - *Two different CO<sub>2</sub> responses*



# Coccolithophores are cool

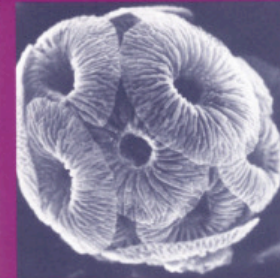
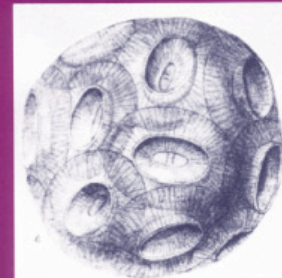
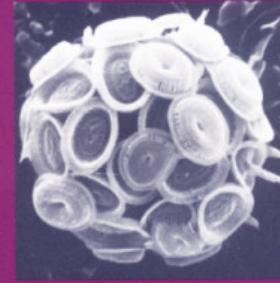
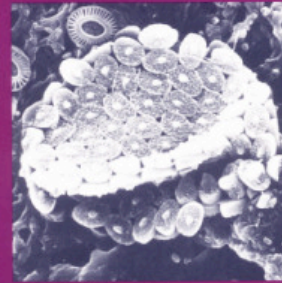


Graphic: [www.noc.soton.ac.uk](http://www.noc.soton.ac.uk)



# Coccolithophores - a rich natural history

## *Coccolithophores*



Published by the Press Syndicate of the University of Cambridge  
The Pitt Building, Trumpington Street, Cambridge CB2 1RP  
40 West 20th Street, New York, NY 10011-4211, USA  
10 Stamford Road, Oakleigh, Melbourne 3166, Australia

© Cambridge University Press 1994

First published 1994

Printed in Great Britain at the University Press, Cambridge

*A catalogue record for this book is available from the British Library*

*Library of Congress cataloguing in publication data*

Coccolithophores / Amos Winter and William G. Siesser, editors.

p. cm.

ISBN 0-521-38050-2

1. Coccolithophores. I. Winter, Amos. II. Siesser, William G.

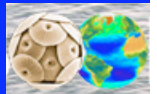
QK569.C63C63 1994

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ISBN 0 521 38050 2 hardback

Edited by  
AMOS WINTER & WILLIAM G SIESSER

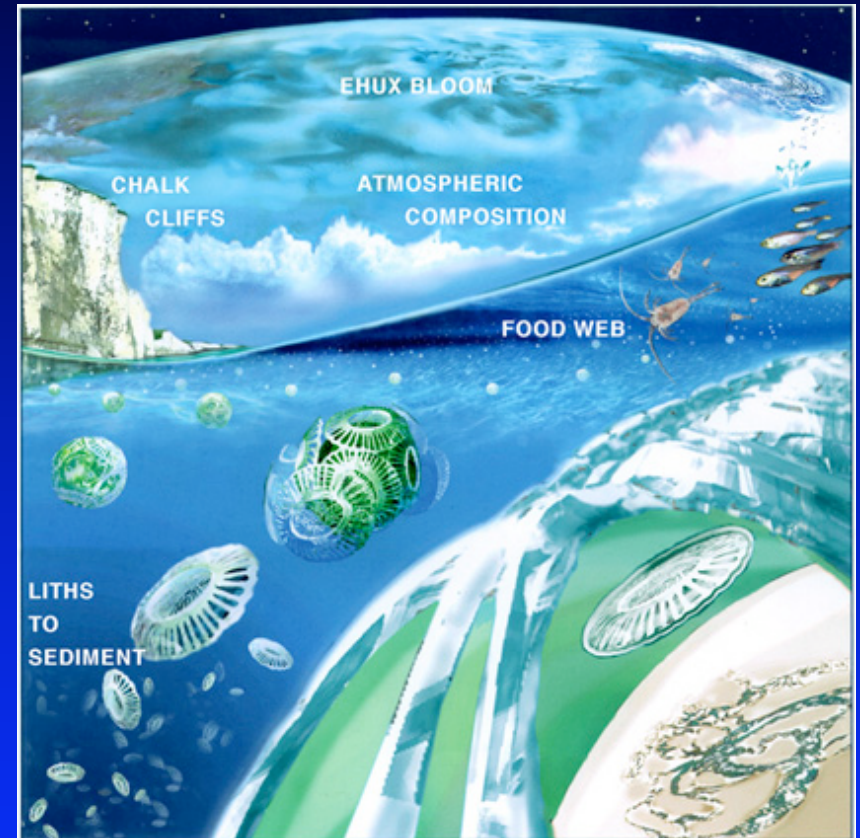
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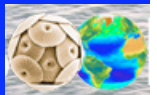


# Coccolithophores - a rich natural history

- Ehrenberg (1795-1876) “the Founder of Micropaleontology”
  - *First to discover and identify fossils of what would be called coccoliths*
  - *1836 First “coccolith” observation in from chalk deposits from the Baltic*
- Thomas Huxley, naturalist and “Darwin’s Bulldog”
  - *1857 coins the term coccolith “spherical stone” for the mineral bodies resembling coccoid cells which he observed in sediments*



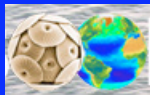
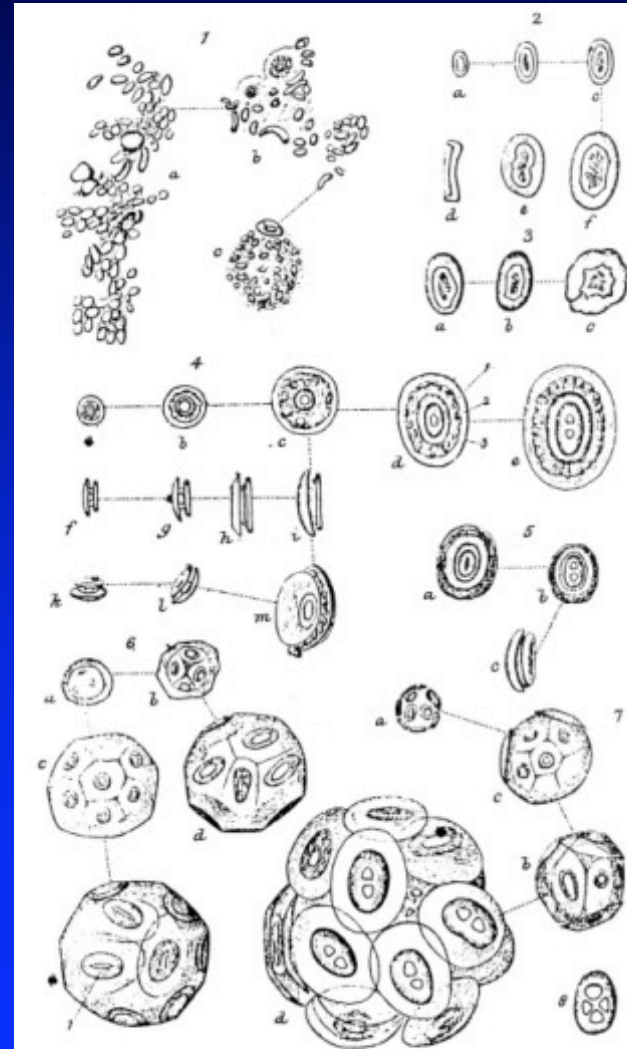
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# Coccolithophores - a rich natural history

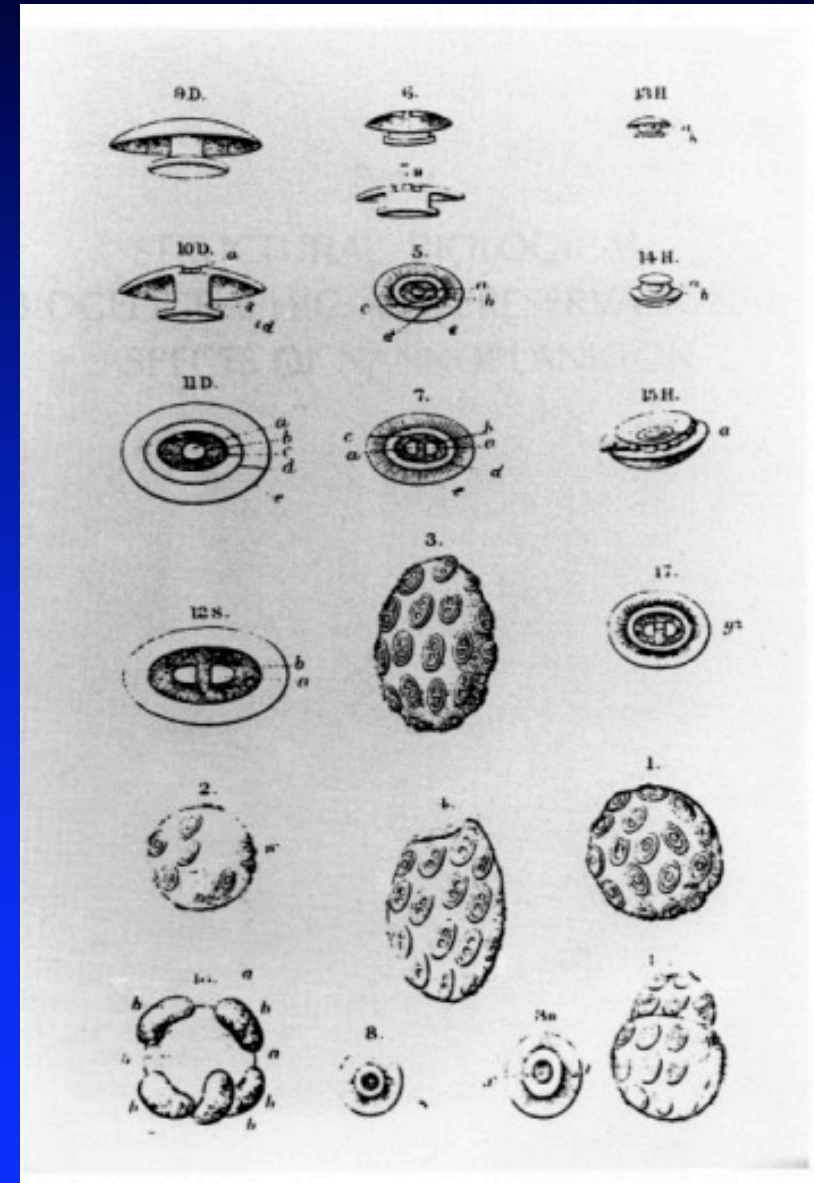
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  - *1857 coins the term coccolith “spherical stone” for the mineral bodies resembling coccoid cells which he observed in sediments*

Fig. 2. Cocospheres and coccoliths from Huxley (1868). Cocospheres of the ‘compact’ type (Fig. 6) and the ‘loose’ type (Fig. 7) are shown. His Fig. 1 shows the gelatinous mass, *Bathybius*. Note the coccoliths enclosed in *Bathybius*.

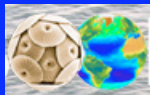


- Wallich on HMS Challenger Expedition  
- 1877 describes association of  
coccoliths with single spherical  
structures (cells) termed  
coccospheres

**Fig. 4.** Coccospheres and coccoliths of *Coccosphaera pelagica* and *Coccosphaera carterii* from Wallich (1877). *Coccosphaera pelagica* is shown as Figs. 1, 2, 5, 8, 9, 10, and 11. *Coccosphaera carterii* is shown as Figs. 3, 4, 6, and 7. Figure 18 is identified as a sporangium of a 'protophyte' from Bengal.

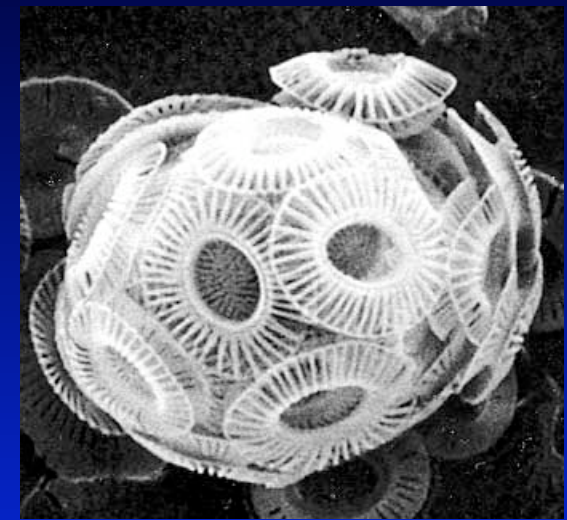
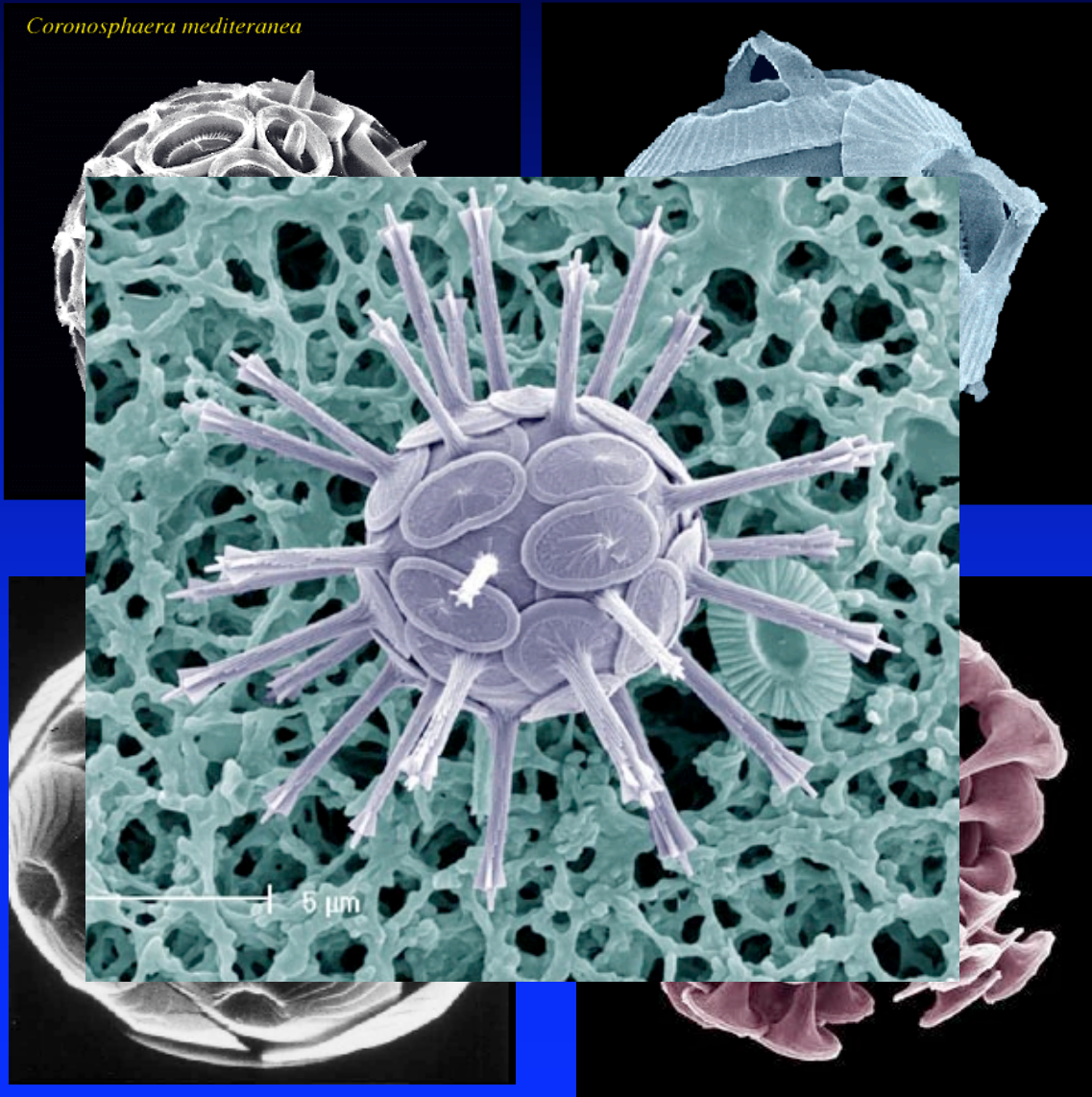


- Lohmann  
-1902 introduces the term...  
coccolithophore

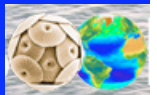




# Coccolithophores - Google image

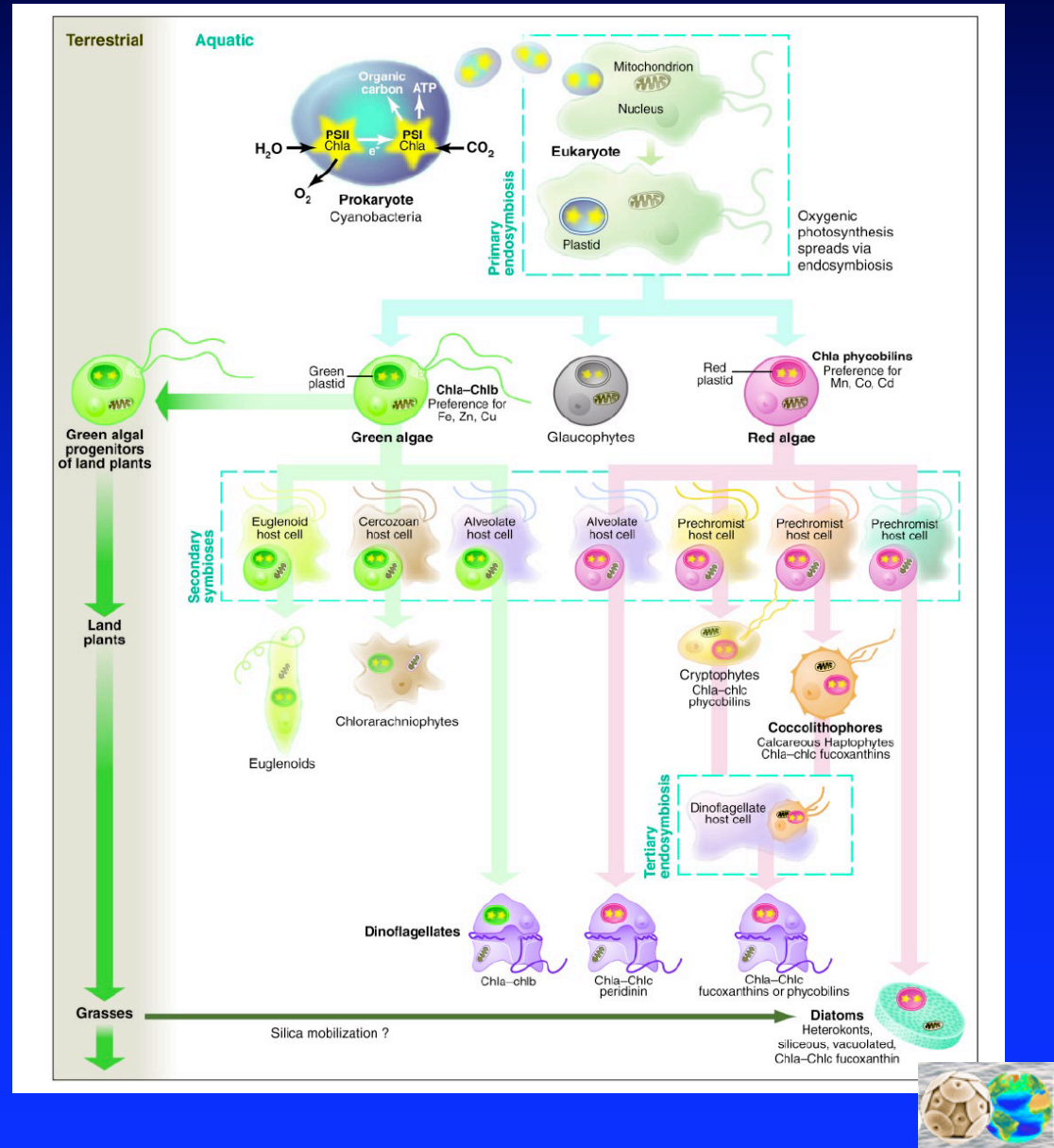


Division:  
Haptophyta



# Haptophyte characteristics

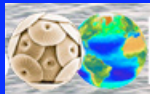
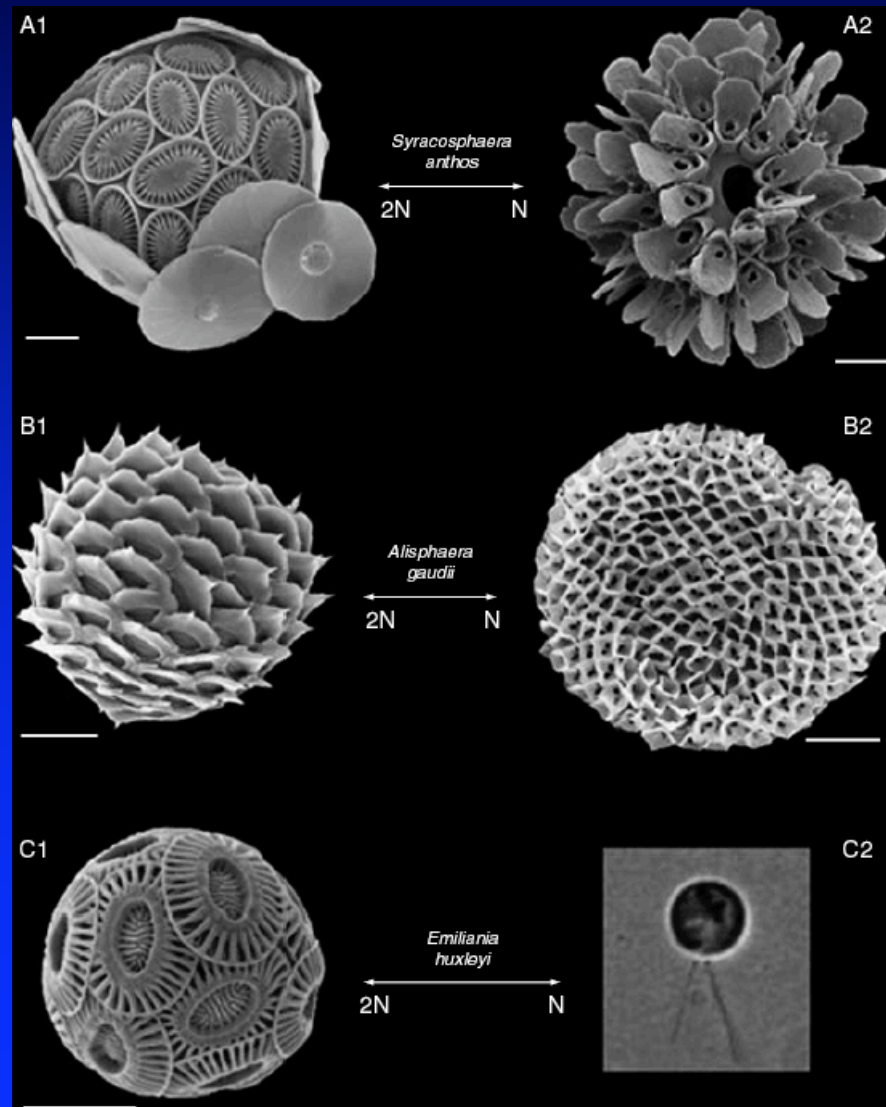
- Molecular genetic data has shown that the Haptophytes are a discrete group from other algal protists
- Diverged during the Pre-Cambrian (>600Ma) protist radiation and acquired chloroplasts subsequently (possibly in the Late Palaeozoic, ca 300-400Ma) as result of secondary endosymbiosis
- Haptophytes are a+c chl containing of the red algal lineage





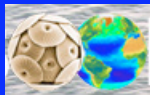
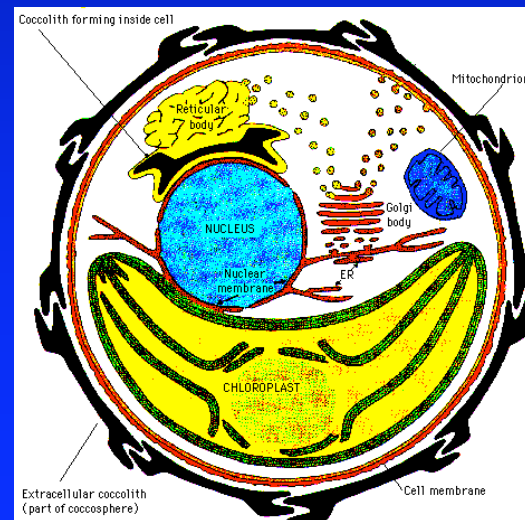
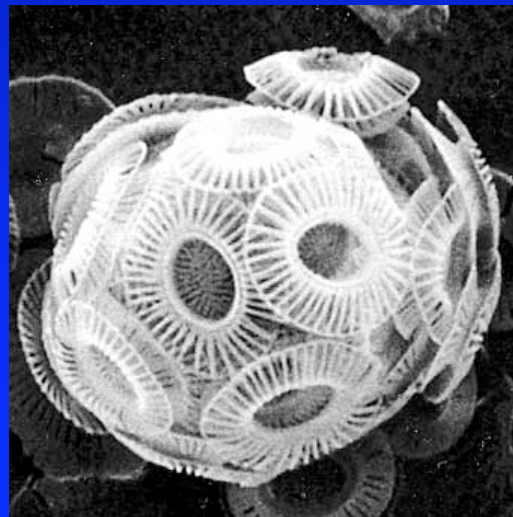
# Haptophyte characteristics

- Haplo-diploid life cycles where both haploid and diploid are capable of asexual reproduction (differs from diploid diatoms, haploid dinoflagellates)
- Morphological diversity between life-history stages



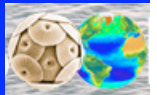
# Haptophyte characteristics

- Emerging monophyletic identity of calcifying haptophytes has no formal name, but subclass calcihaptophycidae was proposed by DeVargas et al. (2006)
- Currently about 280 morphologically distinct genera of calcifying haptophytes or... coccolithophores
- Calcification is the distinguishing feature, but the process is poorly understood

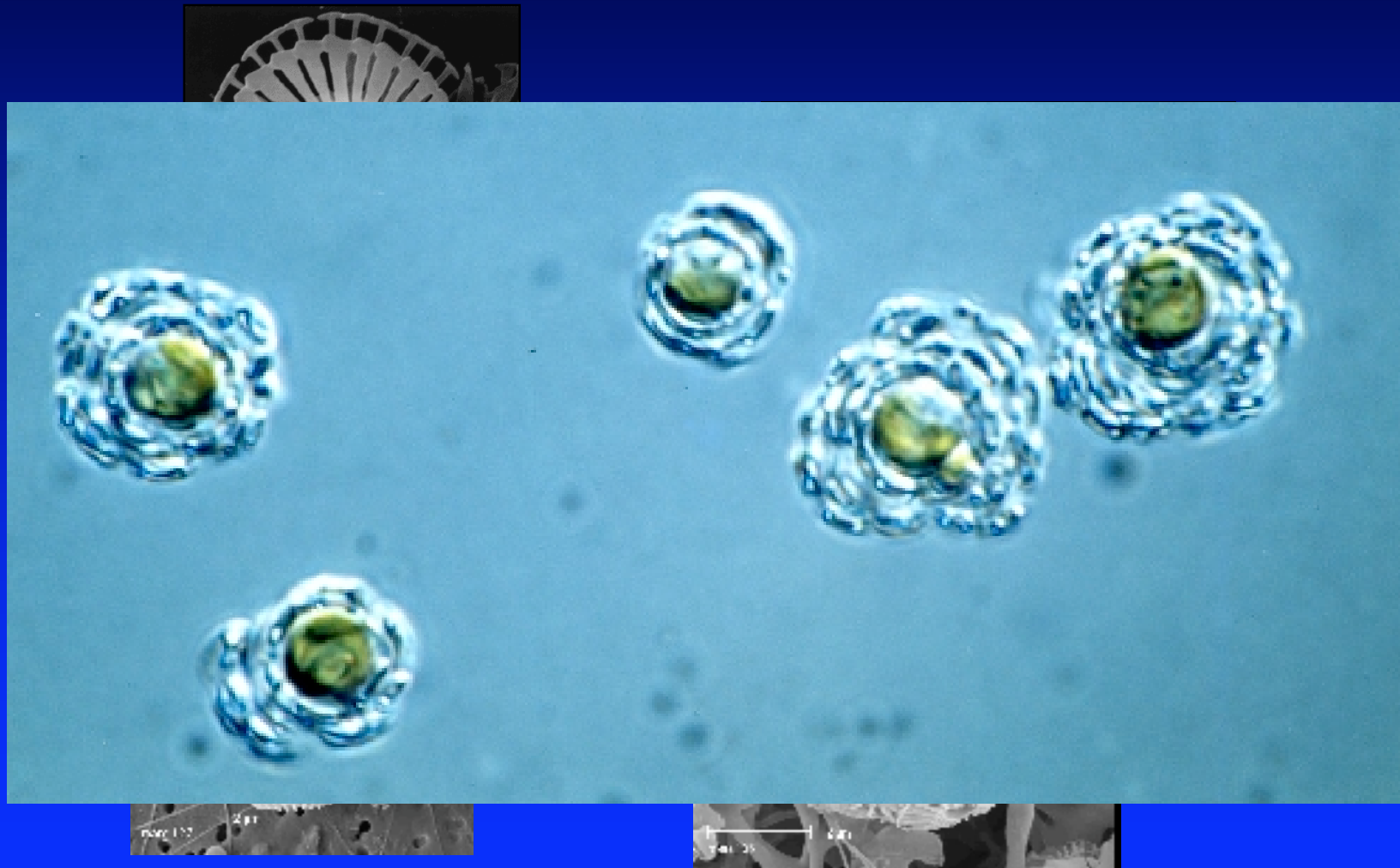


# Haptophyte characteristics

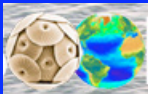
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- Currently about 280 morphologically distinct genera of calcifying haptophytes or... coccolithophores
- Of those ... *Emiliana huxleyi* is an important representative
  - *E. huxleyi* is virtually ubiquitous in surface waters
  - Occurs at temperatures from near freezing to over 30°C
  - Distribution spans equatorial to arctic latitudes
  - Forms large blooms



# *Emiliana huxleyi*

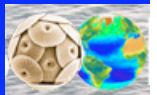


Photos: [www.noc.soton.ac.uk](http://www.noc.soton.ac.uk)



# The take home message...

- Coccolithophores are really cool - in part because the dynamics of this one group can dramatically influence biogeochemical cycling
- Of the coccolithophores, *Emiliana huxleyi* is emerging as increasingly important model for studies of ...
  - *Paleo climate reconstruction*
  - *S cycling*
  - *Viral dynamics*
  - *Carbon cycling*
- Anatomy of a eukaryotic genome project “euks are challenging”
- Transcriptome profiling for aiding genome annotation and moving beyond “capacity”
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- What does the future hold? ... The challenges of prediction in microbial oceanography
  - *Two different CO<sub>2</sub> responses*



# Paleoceanography

GEOCHEMISTRY, GEOPHYSICS, GEOSYSTEMS, VOL. 2, 2001  
[Paper number 2000GC000052]

## Effects of biosynthesis and physiology on relative abundances and isotopic compositions of alkenones

**Elma L. González**

Department of Organismic Biology, Ecology and Evolution, University of  
California, Los Angeles, California 90095

**Ulf Riebesell**

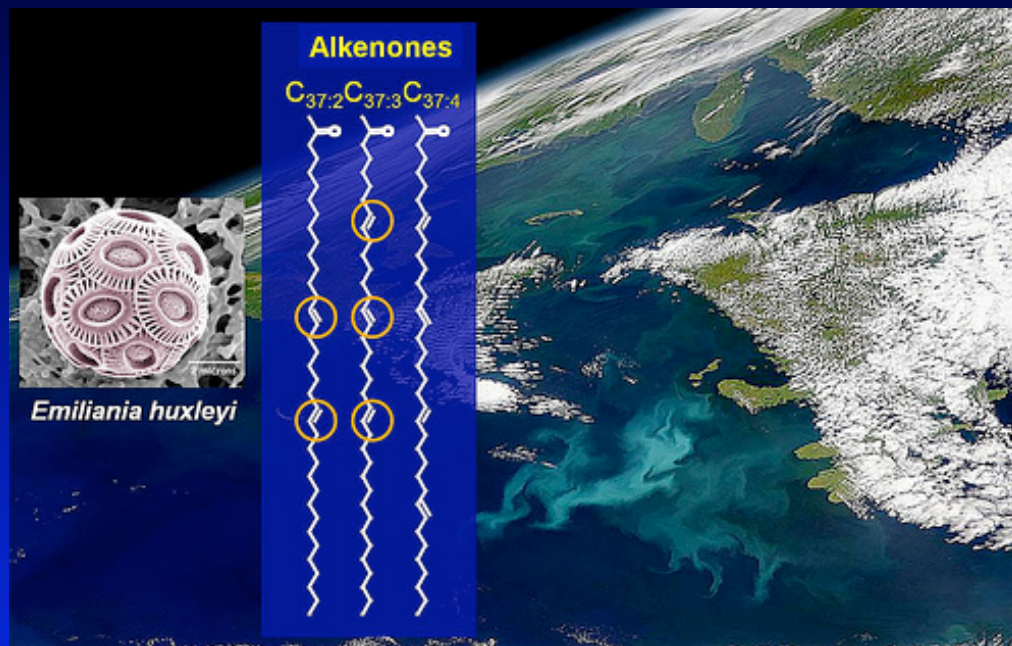
Alfred Wegener-Institute for Polar and Marine Research, Bremerhaven,  
Germany

**John M. Hayes**

Department of Geology and Geophysics, Woods Hole Oceanographic  
Institution, Woods Hole, Massachusetts 02543-1543

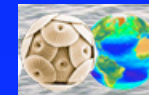
**Edward A. Laws**

Department of Oceanography, University of Hawaii, Honolulu, Hawaii  
96822



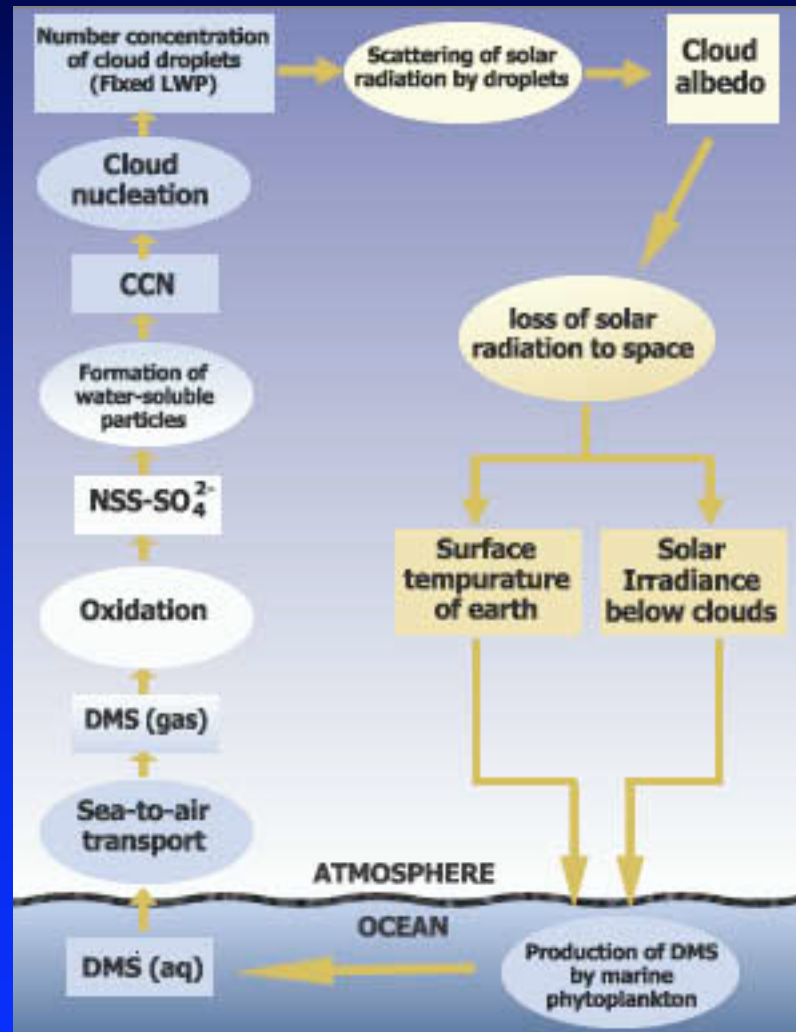
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- Synthesize long-chain lipids = Alkenones
- Degree of saturation is dependent on the ambient temperature at the time of synthesis
- Alkenones are well-preserved in sediments so fossil alkenones can be used as a “thermometer” for ancient sea surface temperature.
- Pathways of biosynthesis not known

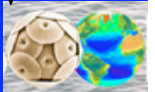




# Sulfur cycle



<http://www.video.bluemicrobe.com/qt/56k/emilianiahuxleyi.mov>



# *Emiliana huxleyi* virus 86

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## The **coccolithovirus** microarray: an array of uses

Michael J. Allen and William H. Wilson

Corresponding author. Michael J. Allen, Plymouth Marine Laboratory, The Hoe, Plymouth, PL1 3DH, UK. E-mail: [mija@pml.ac.uk](mailto:mija@pml.ac.uk)

The *Coccolithoviridae* is a recently discovered family. Here, we review the genomic and transcriptomic characteristics of this family based on the results generated from a **coccolithovirus** microarray. The microarray has been used to aid the annotation of the *Emiliana huxleyi* genome and to investigate the infection process at the transcriptional level and the diversity in genomic content within the family.

**Keywords:** **Coccolithovirus**, *Coccolithoviridae*, *Emiliana huxleyi*

★ Unreviewed, UniProtKB/TrEMBL **Q4A316** (Q4A316\_9PHYC)

Last modified February 5, 2008. Version 12. [History...](#)

[Clusters with 100%, 90%, 50% identity](#) | [Third-party data](#) | [Customize display](#)

[TEXT](#) [XML](#)

[Names and origin](#) · [Ontologies](#) · [Sequence annotation \(Features\)](#) · [Sequences](#) · [References](#) · [Cross-references](#) · [Entry information](#)

### Names and origin

Protein names	<b>Putative phosphate permease</b> [Precursor] <a href="#">EMBL CAI65540.1</a>
Gene names	ORF Names: EhV117 <a href="#">EMBL CAI65540.1</a>
Organism	<b>Emiliana huxleyi virus 86</b> <a href="#">EMBL CAI65540.1</a>
Taxonomic identifier	181082 [NCBI]
Taxonomic lineage	Viruses › dsDNA viruses, no RNA stage › Phycodnaviridae › Coccolithovirus
Protein existence	Inferred from homology.

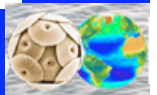
### Ontologies

#### Keywords

Domain [Signal](#)

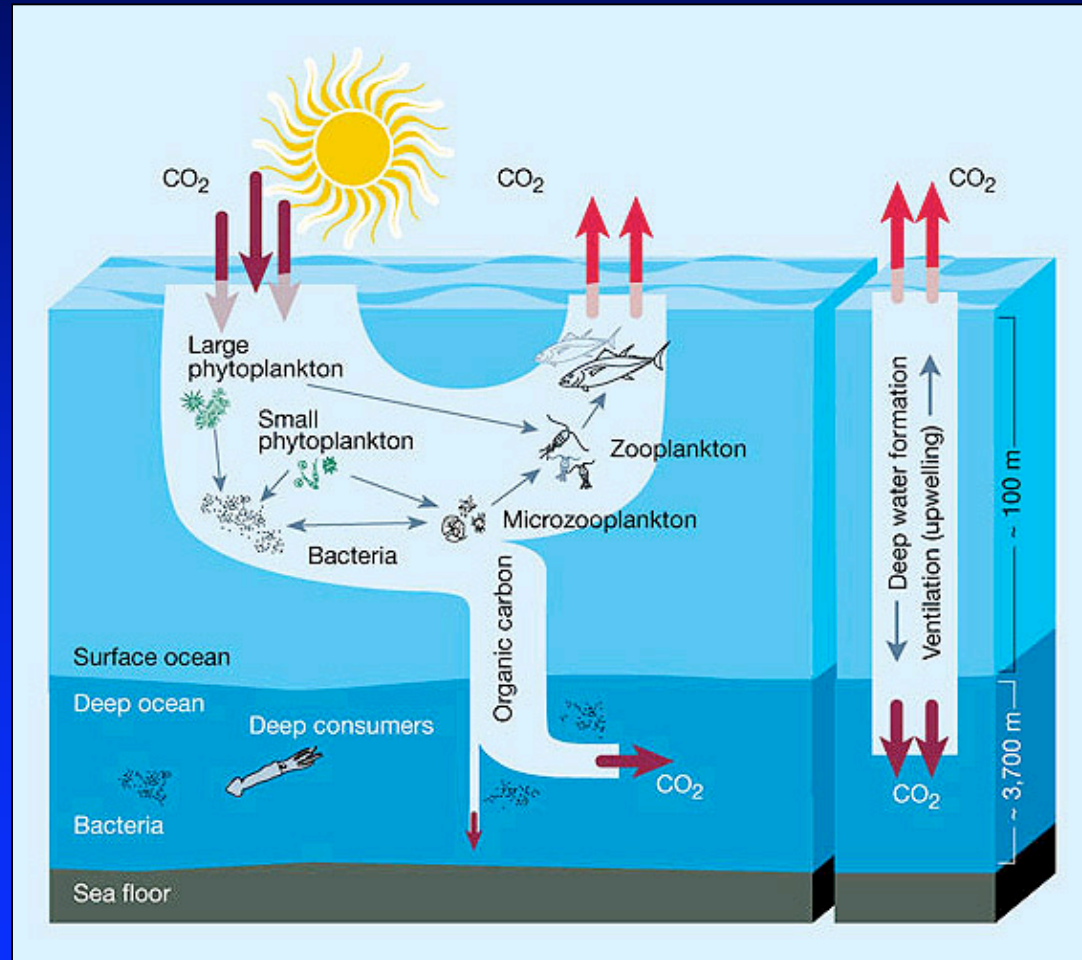
#### Gene Ontology (GO)

Biological process	<a href="#">phosphate transport</a> Inferred from electronic annotation. Source: InterPro
Cellular component	<a href="#">membrane</a> Inferred from electronic annotation. Source: InterPro
Molecular function	<a href="#">inorganic phosphate transmembrane transporter activity</a> Inferred from electronic annotation. Source: InterPro

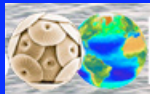


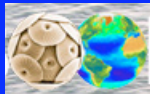
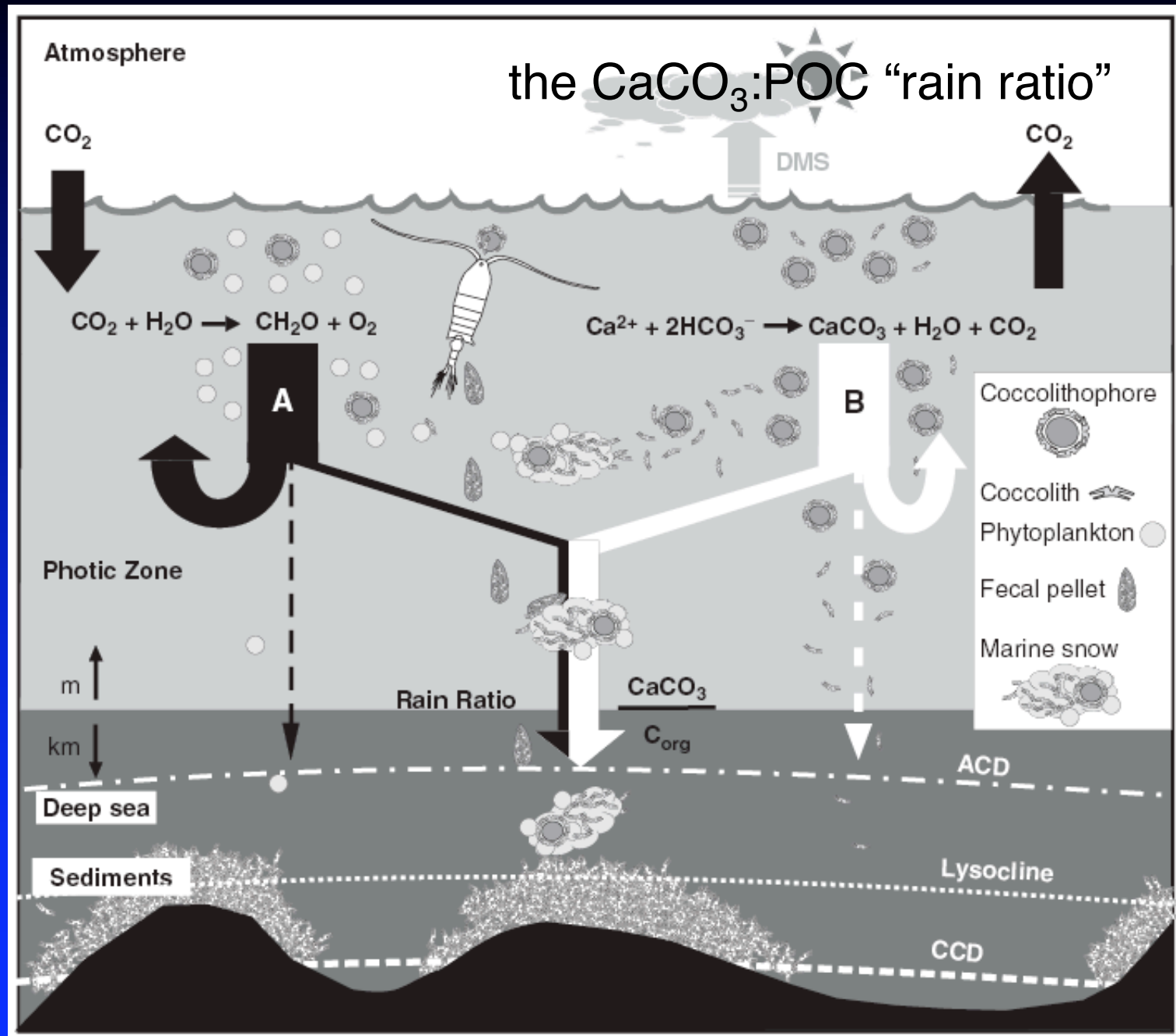


# Carbon cycle

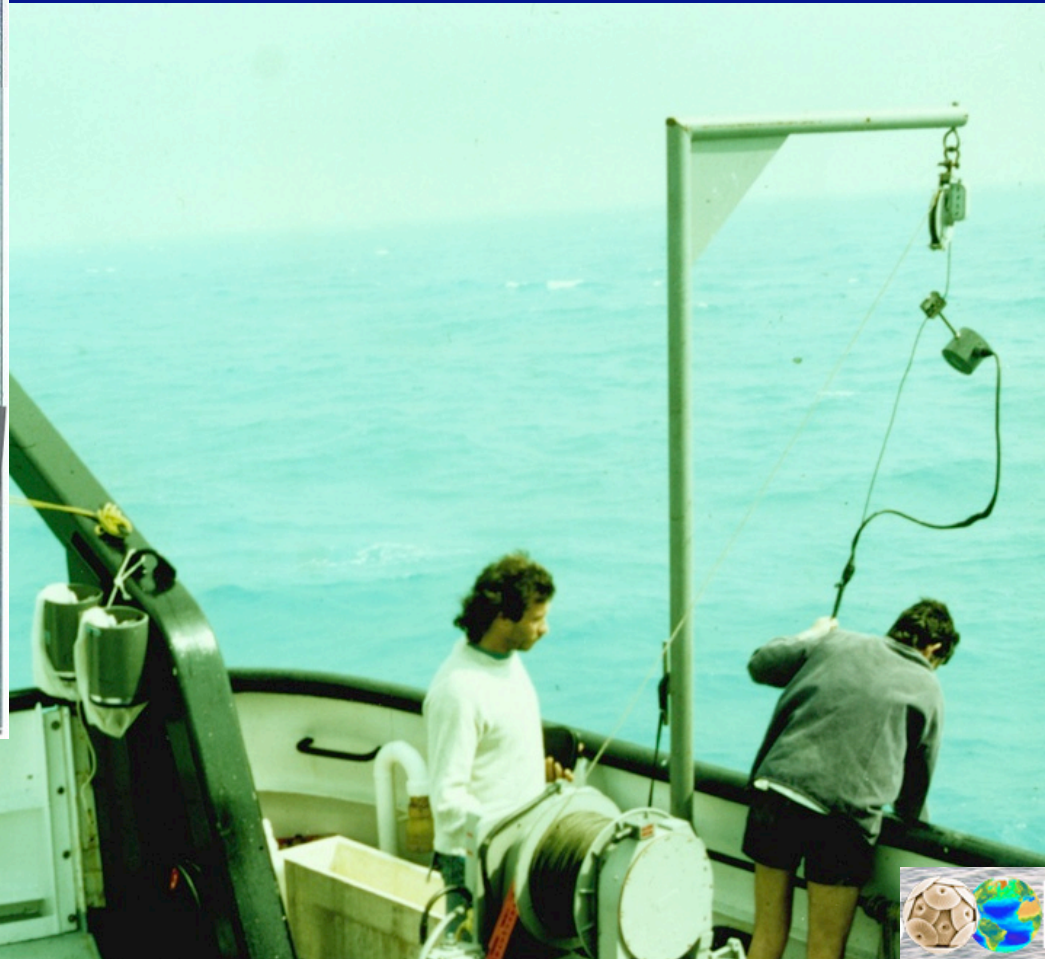


Chisholm 2000



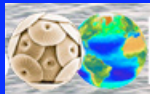
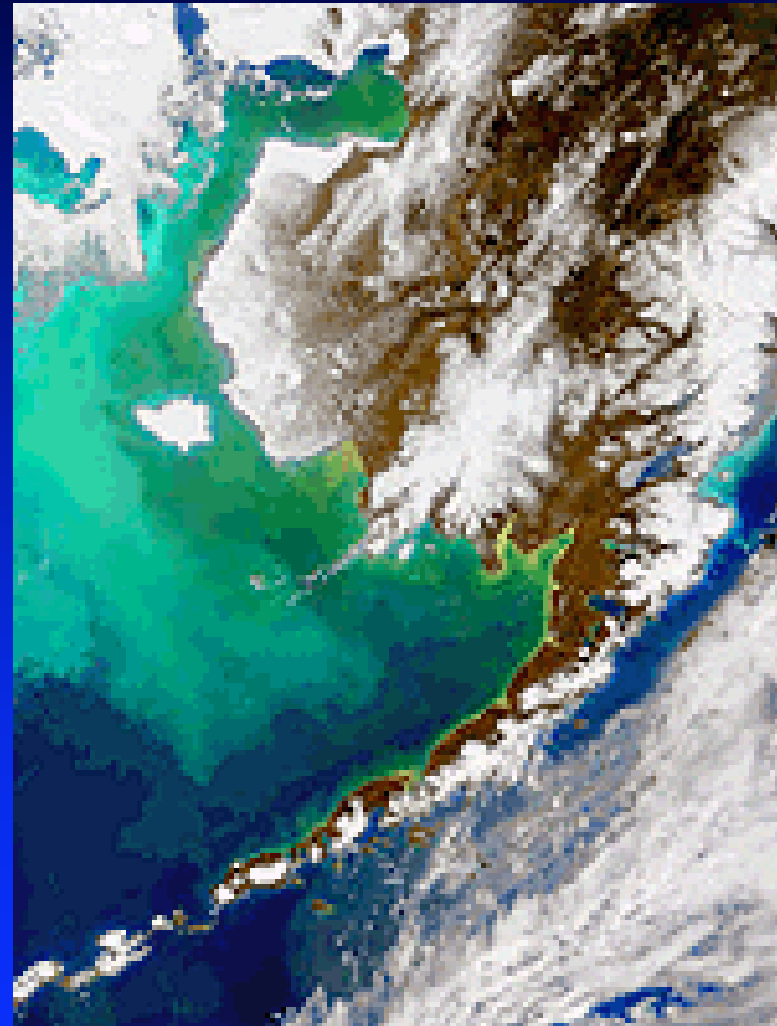
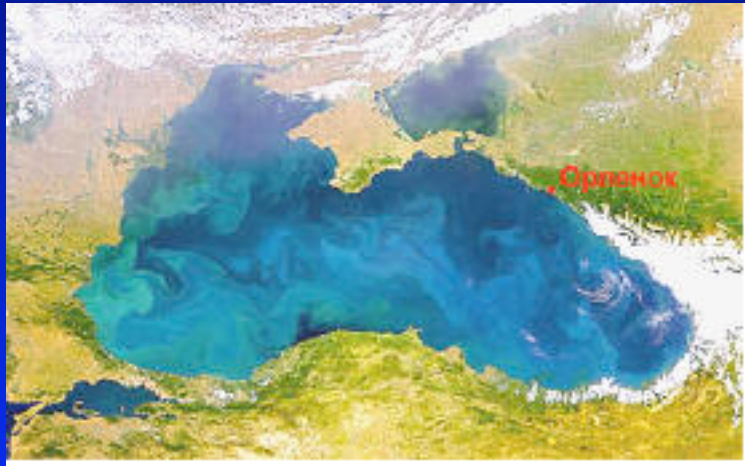


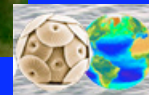
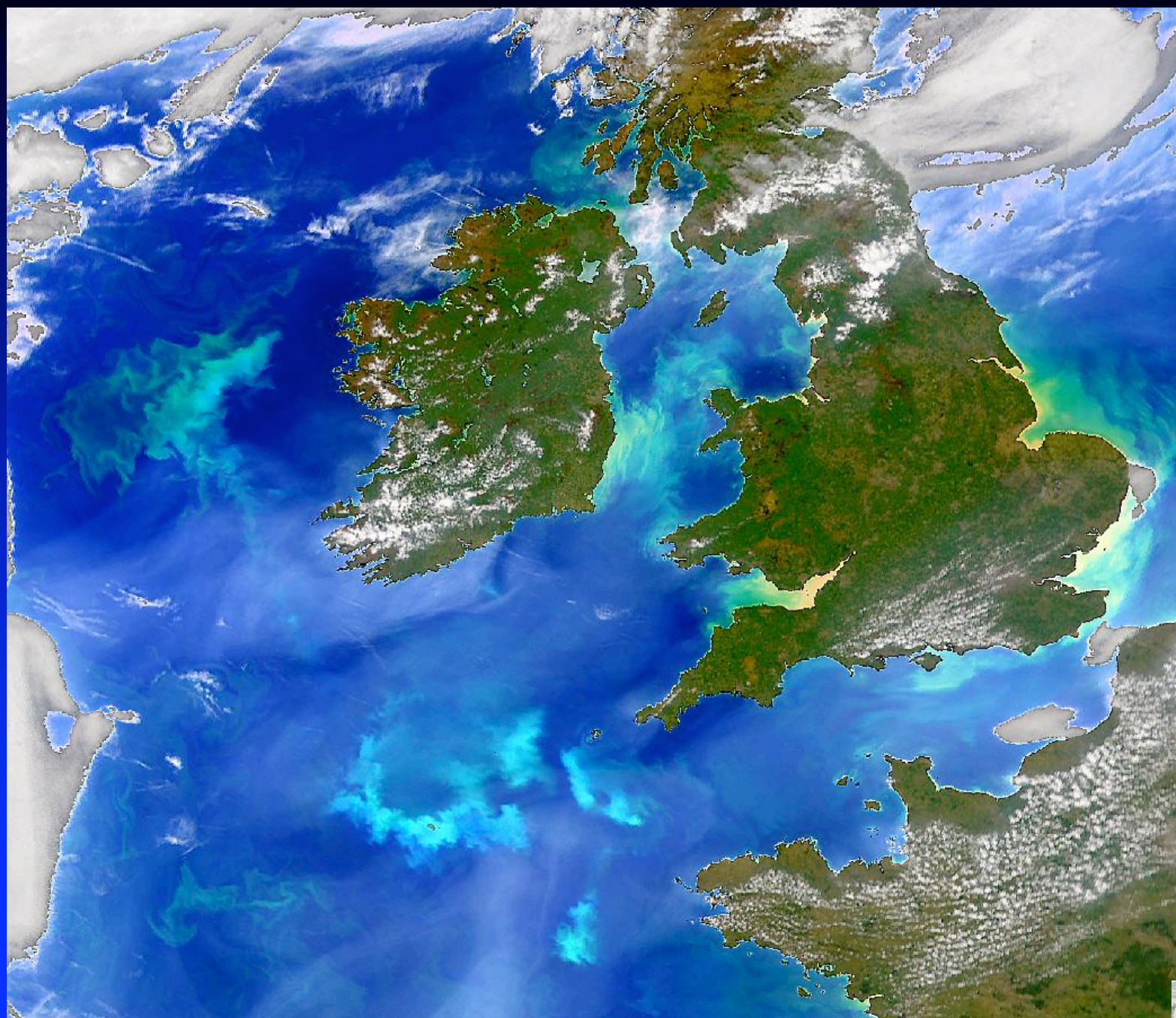
# Large blooms...





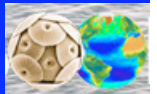
## Large blooms...remotely sensed





# Biomes to genomes

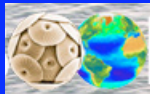
- Despite a growing and broad understanding of coccolithophore evolution, biogeochemistry and ecology, many basic questions remain.
- *What are metabolic pathways and preferred sources of N, P and C?*
- *Biosynthesis and stability of alkenones?*
- *Influence of virus infection on loss terms and the cycling of S, C, N, P ?*
- *How and why to they calcify?*
- *How will all of these processes influence their distribution and activity in a future ocean?*
- These questions and more were used to rationalize the sequencing of a coccolithophore genome sequence.





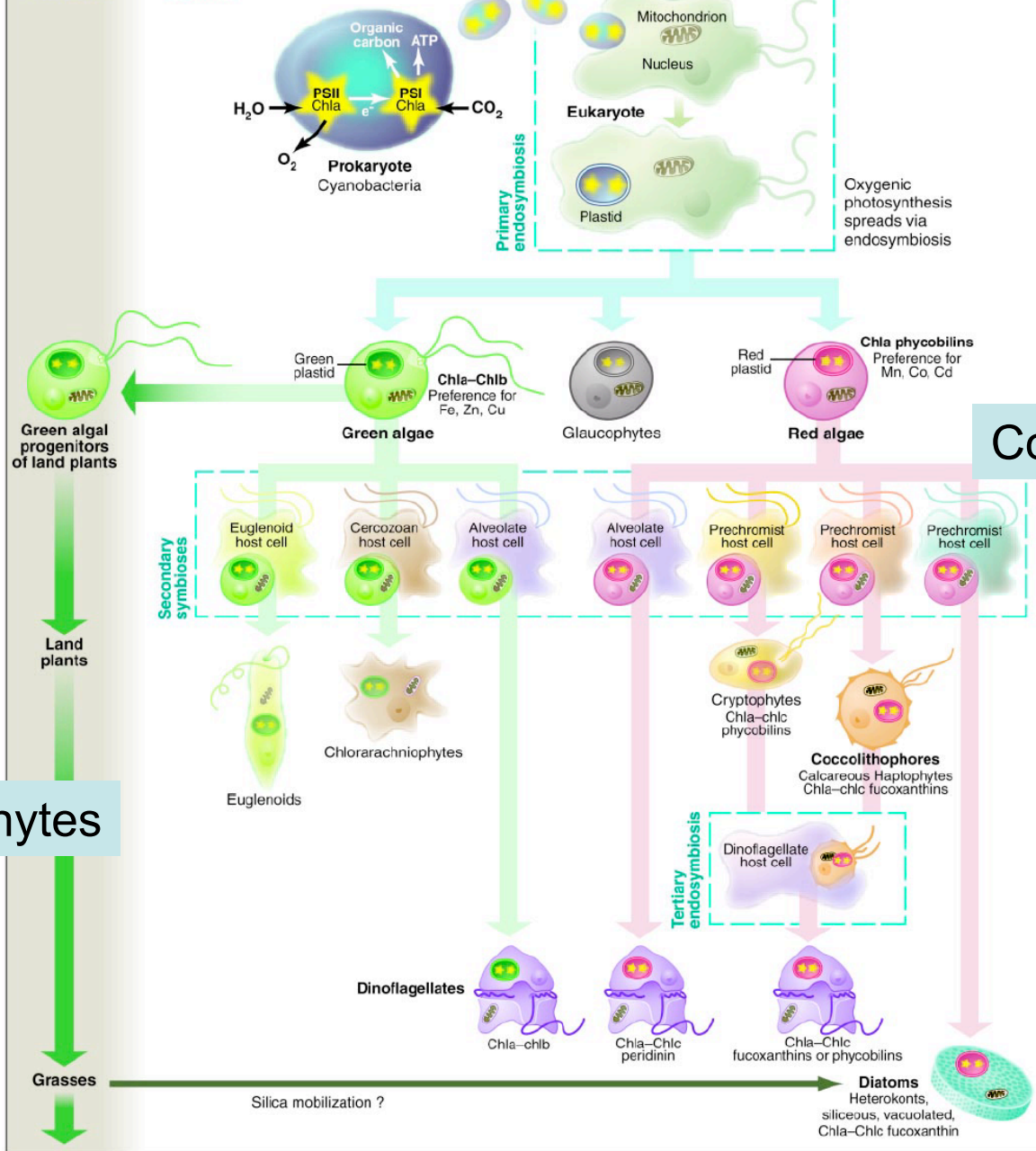
# Selected sequencing efforts with marine eukaryotic phytoplankton

- Diatom: *Thalassiosira* (genome, EST) Armbrust et al. 2004
- Prasinophyte: *Ostreococcus* (genomes) Palenik et al. 2007
- Dinoflagellate:
  - *Karenia* (EST)
  - *Alexandrium* (EST)
- Haptophyte: *Emiliana* (genome, EST) Read and Wahlund
- Pelagophyte: *Aureococcus* (genome, EST) Gobler and Wilhelm
- *Limited genomic infrastructure relative to marine cyanobacteria*



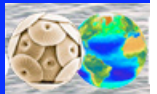
Terrestrial

Aquatic



Coccolithophores

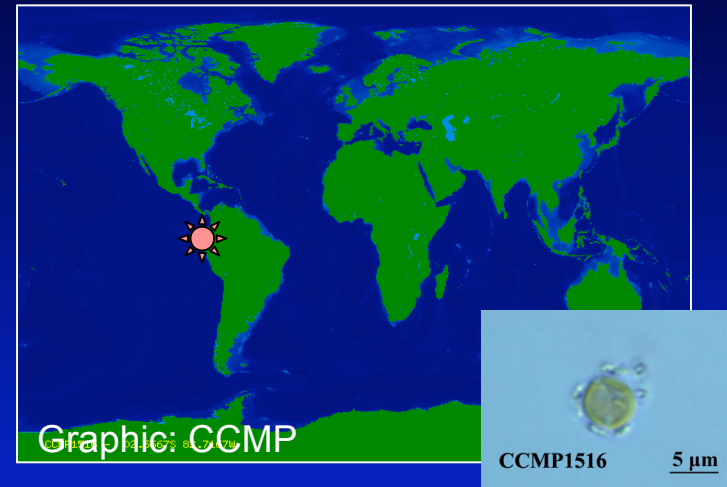
Prasinophytes



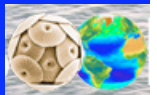


## *E. huxleyi* strain 1516 genome

- Isolated in 1992 from the South Pacific
- Calcifying in f/50
- Relatively slow growth rate



- DOE/JGI has sequenced 80,000 ESTs (e.g. Wahlund et al. 2004) and a draft of the genome (B. Read and T. Wahlund PIs)
- Assembly presented a an unexpectedly huge challenge for the JGI. We were told *E. huxleyi* is the most difficult genome JGI has ever tried to assemble!
- The genome size is ~168 MB with a GC content of 66%. The assembly resulted in 7,809 scaffolds with 94% sequence completeness at 10X coverage



# *E. huxleyi* strain 1516 genome

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## EMILIANA HUXLEYI GENOME PROJECT

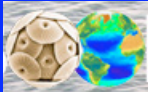


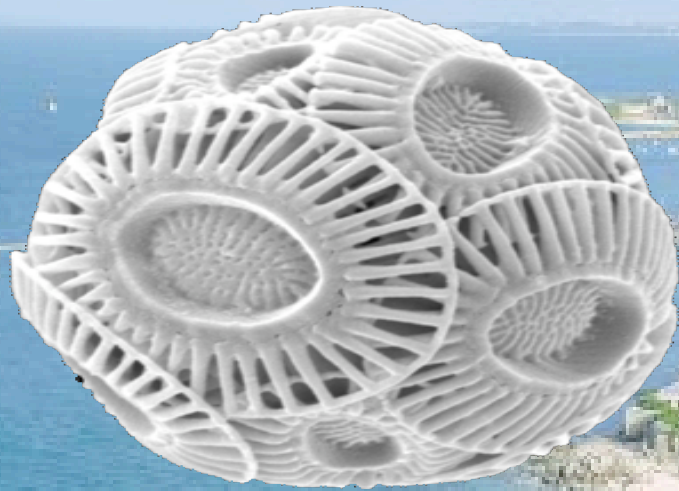
### General Features

Genome Size	168 Mb
Scaffold Number	7809
Coverage	10X
Number of Predicted Genes	39126

### Average Properties

Gene Length	1718.78 nt
Transcript Length	1112.13 nt
Protein Length	340.70 aa
Exon Frequency	3.60 exon/gene
Exon Length	307.27 nt
Intron Length	237.27 nt
Gene Density	233.31 genes/Mb





# *Emiliana huxleyi* Genome Pre-Annotation Jamboree

SBR : Station Biologique de Roscoff

March 19-20, 2007


Organization : Betsy Read  
Host : Colombar de Vargas



# *E. huxleyi* strain 1516 genome

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## EMILIANA HUXLEYI GENOME PROJECT



Roscoff, March 19-20, 2007  
*Emiliana huxleyi* genome - Kickoff meeting

### THE GENOME ANNOTATION TEAM

As a genome-savvy community representing a variety of biological disciplines we intend to promote *E. huxleyi* as a new model species. Our immediate goals are to: 1) improve the computational gene models and interpret genomic data for this model phytoplankton in comparative research, and 2) develop *E. huxleyi* as an important resource for environmental/climatology, biomedical, and material science investigations.

### COCCO EXPRESS

*Coccolithophorids Expressed Sequence Tags (EST) & Microarray Database*

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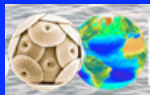
User Name:  Password:  [Login](#) [Register](#)

Select Desired Haptophyte

[Calcidiscus Leptoporus](#)  
[Coccolithus Pelagicus](#)  
[Emiliana huxleyi](#)  
[Gephyracapsa Oceanica](#)  
[Helicosphaera](#)  
[Pleurochrysis](#)  
[Umbilicosphaera](#)

COCCO EXPRESS



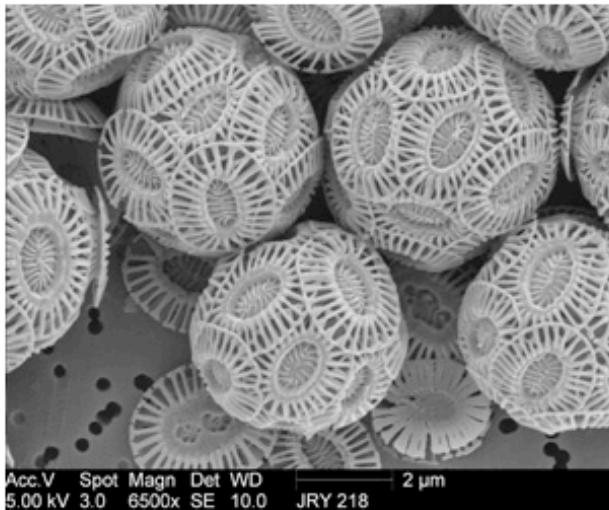


# Manual annotation is underway

**JGI**

[Search](#) | [BLAST](#) | [Browse](#) | [GO](#) | [KEGG](#) | [KOG](#) | [AdvancedSearch](#) | [Download](#) | [Info](#) | [Home](#) **HELP!**

## *Emiliania huxleyi* CCMP1516 v1.0

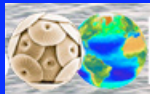


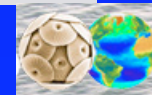
Coccolithophores are marine haptophytes that derive their name from the calcium carbonate coccoliths that blanket the cell. They extend deep into the tree of life and represent the third most abundant group of phytoplankton in today's oceans with some 300 different species. The morphological diversity of their coccoliths is preserved in a continuous and complete fossil record spanning the last 200 Myr, and represents an important tool for dating and correlating strata, reconstructing past climate and oceanic conditions, and dissecting patterns of macroevolution.

*Emiliania huxleyi* (*E. huxleyi*) is the most prominent coccolithophore and has attracted the attention of scientists from fields as diverse as geology, biogeography, paleoclimatology, ecophysiology, material science, and medicine. *E. huxleyi* is distributed throughout the world's oceans and is linked to the

chemical balance between the atmosphere, hydrosphere, and geosphere. Massive blooms of the algae can cover 100,000s of square kilometers and can be detected via satellite imagery due to the reflective properties of its coccoliths. Because of its ecological success and its ability to fix inorganic carbon into both photosynthetic and biomineralized product, *E. huxleyi* has significantly impacted the biogeochemistry of the earth directing carbonate chemistry in surface oceans and exporting large amounts of C to deep water sediments. In addition to playing an important role in global carbon cycling, *E. huxleyi* also contributes to global sulfur cycling. During grazing *E. huxleyi* produces the climatically active trace gas dimethyl sulfide; emissions of which may contribute to marine cloud formation and climate regulation.

*E. huxleyi* is also of interest to those in biotechnology. A group of secondary metabolites known as polyketides that *E. huxleyi* synthesizes possess a wealth of pharmacologically important activities, including antimicrobial, antifungal, antiparasitic, antitumor and agrochemical properties. The ultrastructure and optical features of the coccoliths, on the other hand, are being targeted for applications in nanotechnology relating to biomedical, telecommunications and optoelectronic devices and/or materials.









*Emiliania huxleyi* CCMP1516 v1.0

Search | BLAST | Browse | GO | KEGG | KOG | **AdvancedSearch** | Download | Info | Home | **HELP!**

## GENE/TRANSCRIPT/PROTEIN SEARCH

### Search Terms

alkaline phosphatase

### Options

#### Method

- ☒ Find directly  
☐ Find by homology to related proteins with

E-Value less than

1.0e-20

#### Databases

Emiliania huxleyi CCMP1516  
F. rubripes v3.0  
Generic tutorial  
H. robusta v1.0  
H. sapiens Chr. 16  
H. sapiens Chr. 19 (b34)  
H. sapiens Chr. 5  
L. gigantea v1.0  
Laccaria bicolor  
M. brevicollis v1.0

FASTA file of

### Found

Displaying results 1 to

<input type="checkbox"/>	Relevance	Organism	Assigned Name
<input type="checkbox"/>	1.0	Emihu1	PHO1
<input type="checkbox"/>	1.0	Emihu1	GM1.15500120
<input type="checkbox"/>	0.88	Emihu1	GM1.16200121
<input type="checkbox"/>	0.88	Emihu1	GM1.1038000001
<input type="checkbox"/>	0.88	Emihu1	ESTEXTDG_GENEMARK1.C_91460001
<input type="checkbox"/>	0.88	Emihu1	ESTEXTDG_GENEMARK1.C_2850035
<input type="checkbox"/>	0.71	Emihu1	ESTEXTDG_FGENESH_NEWKGS_KG.C_160033
<input type="checkbox"/>	0.71	Emihu1	FGENESH_NEWKGS_KG.373_4__EST_ALL.FASTA.CONTIG11950
<input type="checkbox"/>	0.62	Emihu1	AP1

## USER ANNOTATIONS

User	Name	Description	DefLine
Louie Wurch	PHO1	DOP metabolism	Putative alkaline phosphatase

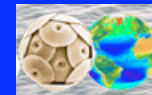
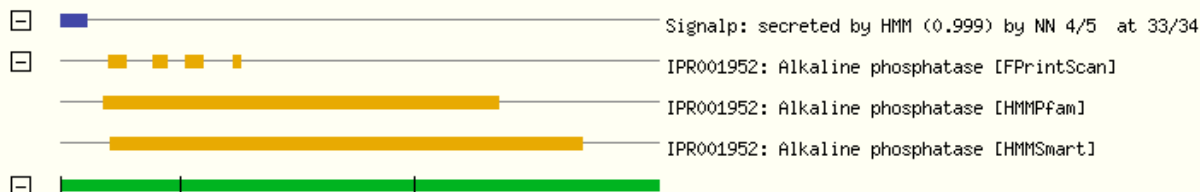
[View/modify manual annotation](#)

[View nucleotide and 3-frame translation](#) [To Genome Browser](#)

[NCBI blastp](#) [Predicted number of transmembrane domains: 1](#)

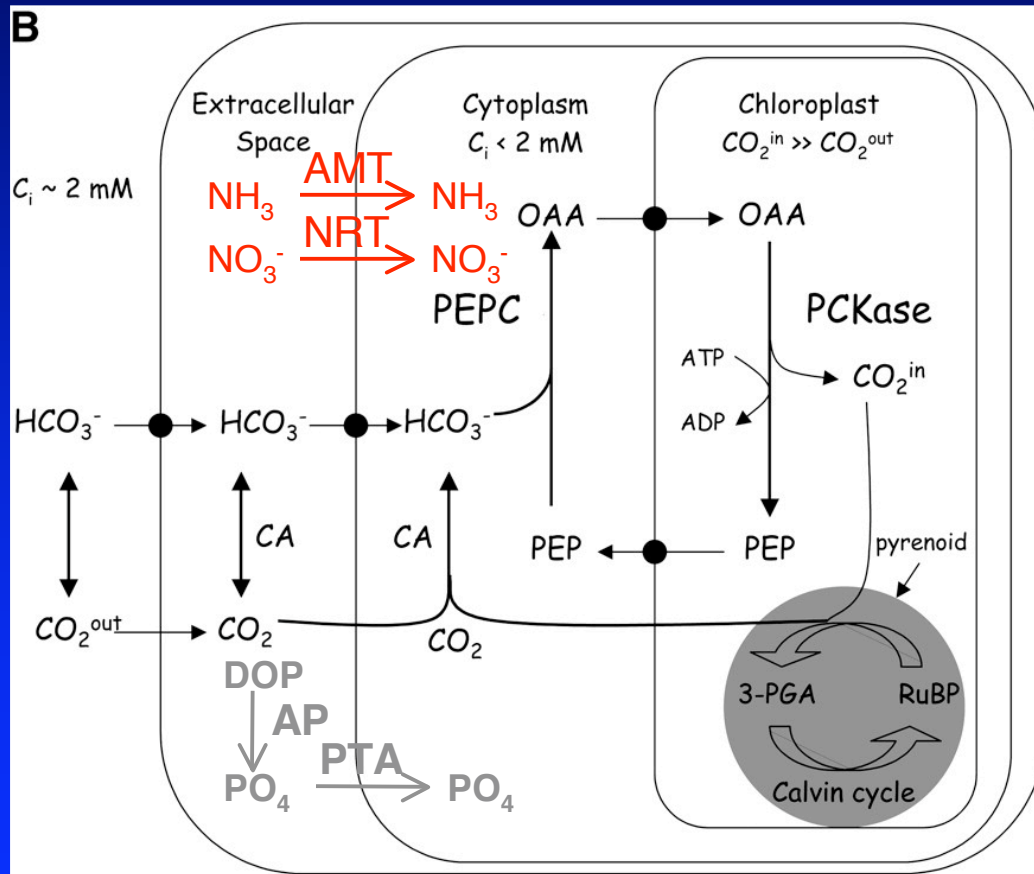
gm1.24600065

[To Genome Browser](#)



# *E. huxleyi* strain 1516 gene annotation

## Current model of CCM function in marine diatoms



### C metabolism:

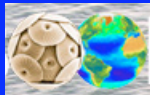
CA  
PEPC  
PCKase  
RuBP

### N metabolism:

AMT  
NRT

### P metabolism:

AP  
PTA





# Comparative genomics

154

*Emiliana huxleyi* Plastid Genome

[Vol. 12,

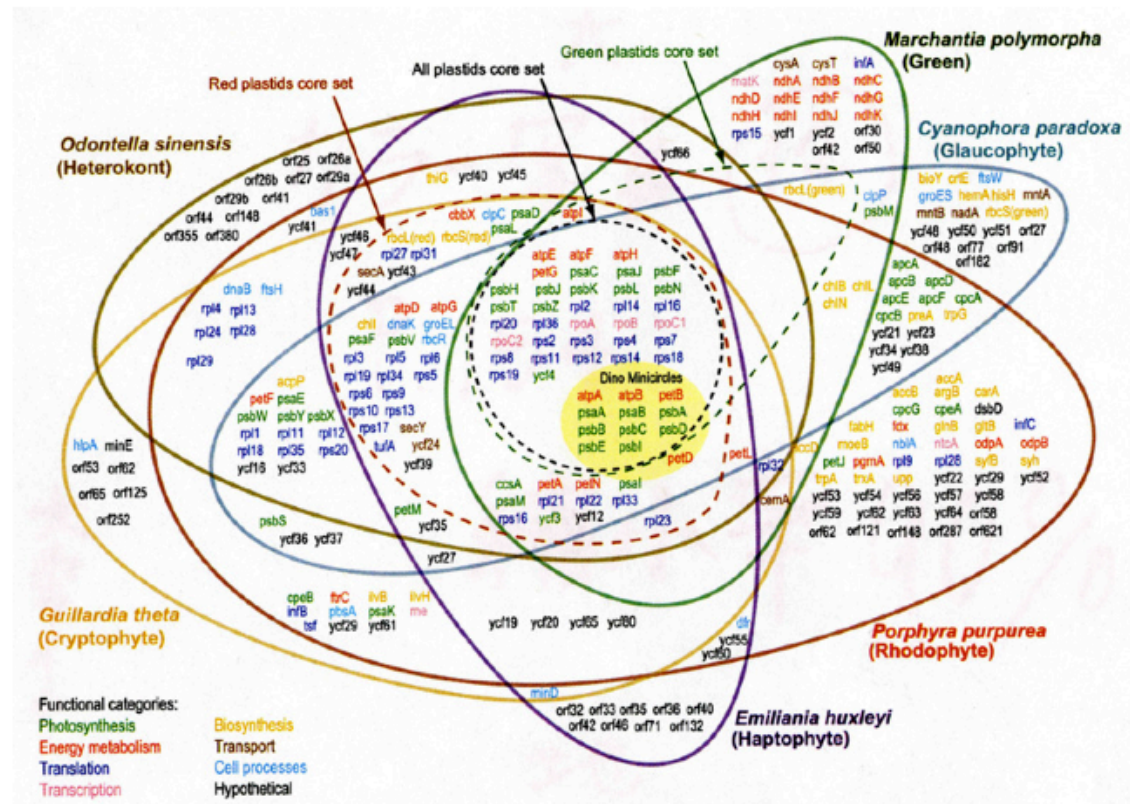
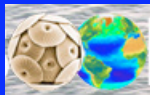


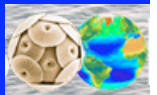
Figure 2. Venn diagram comparing the protein-coding gene content of six plastid genomes. Core sets of genes from all plastids, from red plastids, and from green plastids were inferred from all 36 photosynthetic plastid genomes published to date (Supplemental Information Table 1, [http://www.dna-res.kazusa.or.jp/12/2/07/supplement/supplement\\_t1.html](http://www.dna-res.kazusa.or.jp/12/2/07/supplement/supplement_t1.html)). Genes are colored depending on their functional category.

Supports red algal lineage of the haptophyte plastid Sanchez  
Puerta et al 2005



# Biomes to genomes

- Despite a growing and broad understanding of coccolithophore evolution, biogeochemistry and ecology, many basic questions remain.
- *What are metabolic pathways and preferred sources of N, P and C?*
- *Biosynthesis and stability of alkenones?*
- *Influence of virus infection on loss terms and the cycling of S, C, N, P ?*
- *How and why to they calcify?*
- *How will all of these processes influence their distribution and activity in a future ocean?*
- These questions and more were used to rationalize the sequencing of a coccolithophore genome sequence.



The “oceanic genotype” represents only the potential biological capacity and sets an upper constraint on possible pathways and ecosystem rates. The realized structure and function of marine ecosystems, “oceanic phenotype”, reflects the complex interactions of individuals and populations with their physical and chemical environment and with each other.

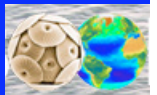
Doney et al. 2004 *Front. Ecol. Environ.*

Capacity?

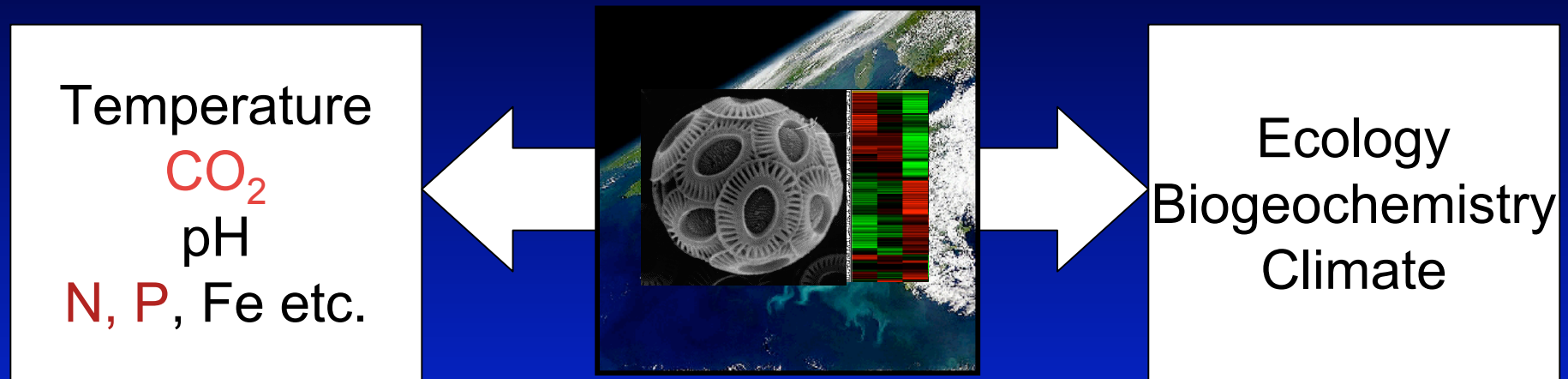
Transcription?

Activity?

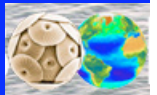
Can studies at the genome level give predictive insight into structure and function of ocean ecosystems?



# A changing ocean - predicting coccolithophore nutritional physiology and ecology



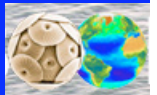
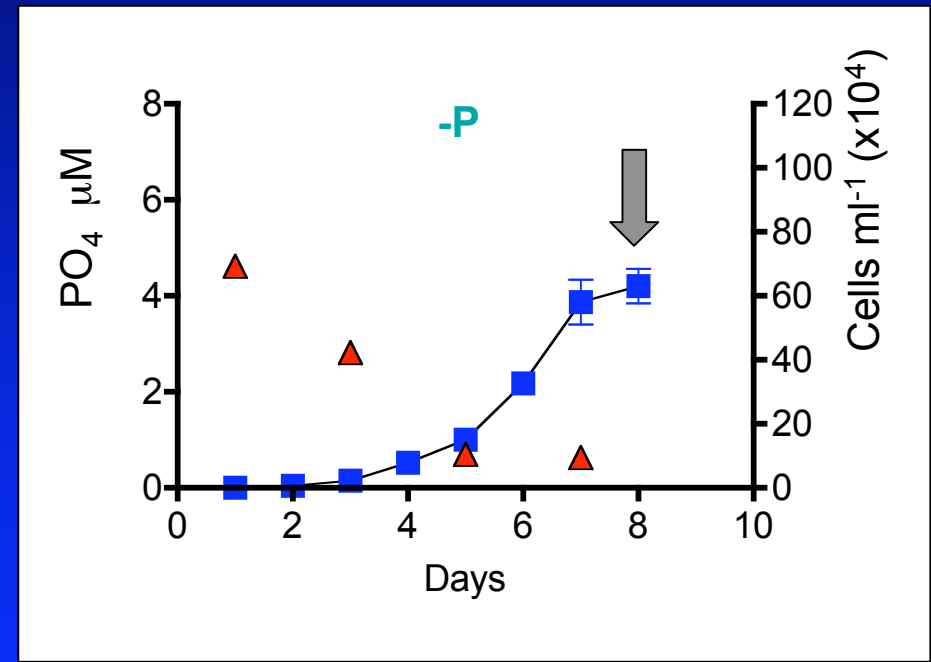
- *Emiliana huxleyi* (1516) responses to P and N deficiency
  - Validity of SAGE for gene expression profiling in the absence of a complete genome sequence.
- Molecular understanding of N, P and C metabolism
- Physiological ecology of field populations



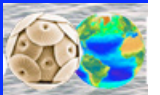
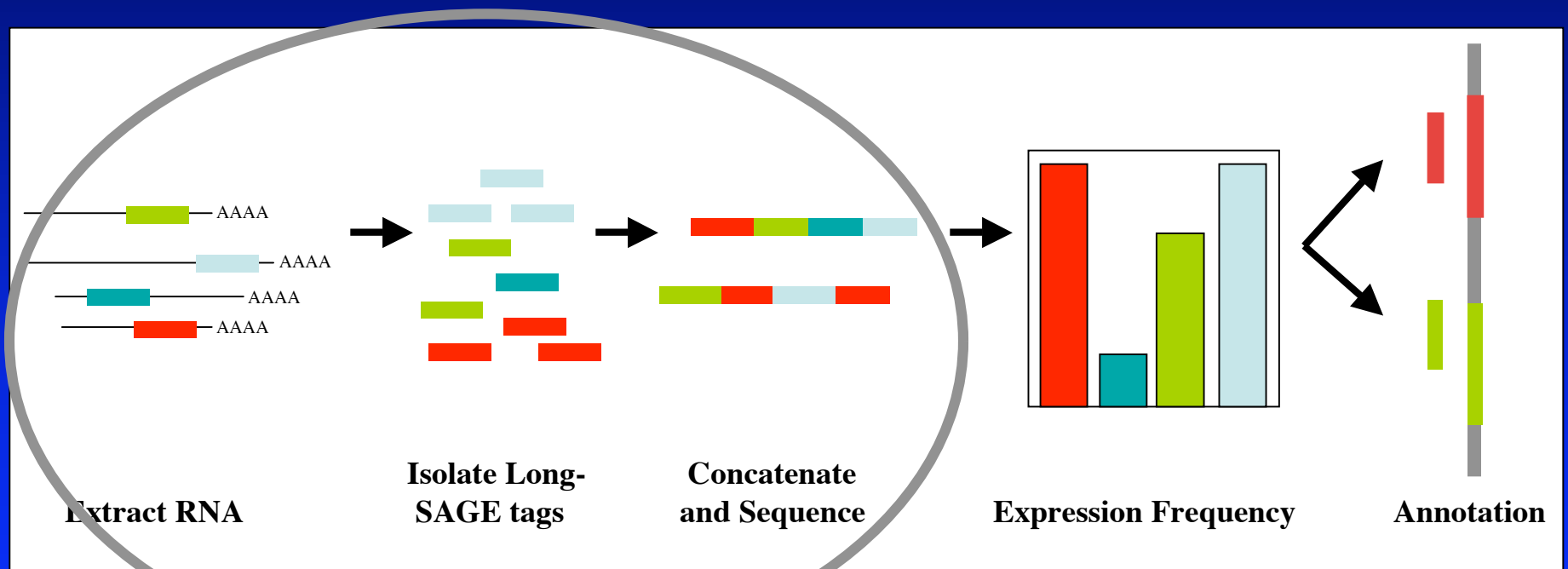


# Global gene expression analyses

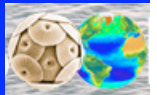
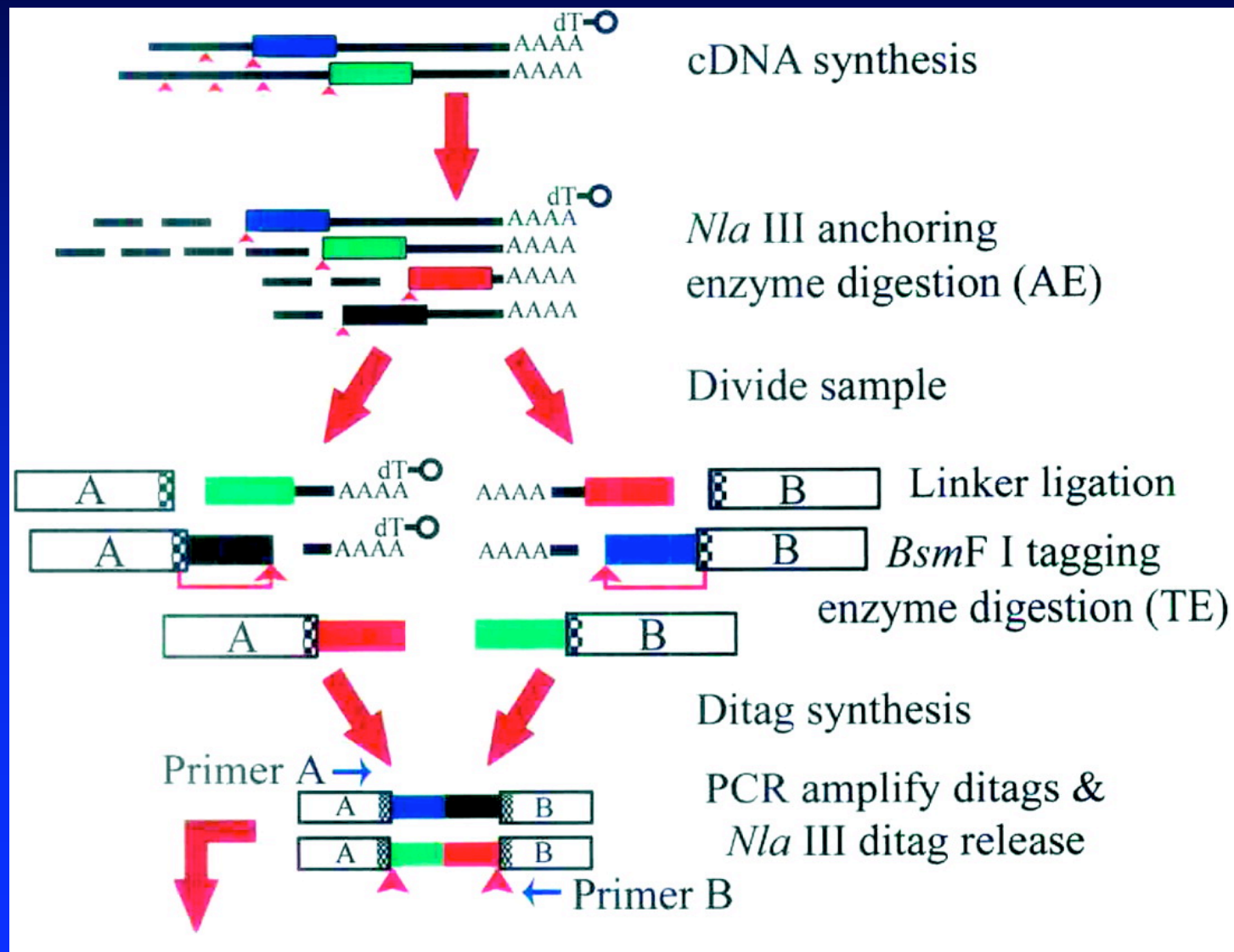
- Serial analysis of gene expression - SAGE
  - Velculescu et al. 1995 (14bp)
  - Saha et al. 2002 (21bp)
  - *Pfiesteria* Coyne et al. 2004
  - *Emiliana* Dyhrman et al. 2006
- Solexa/Illumina approach to SAGE has dramatically influenced through-put and cost.
- Analyses on *Aureococcus* - and diatoms pending
- *E. huxleyi* : N and P stress responses
- Support the genome annotation



# Long-SAGE



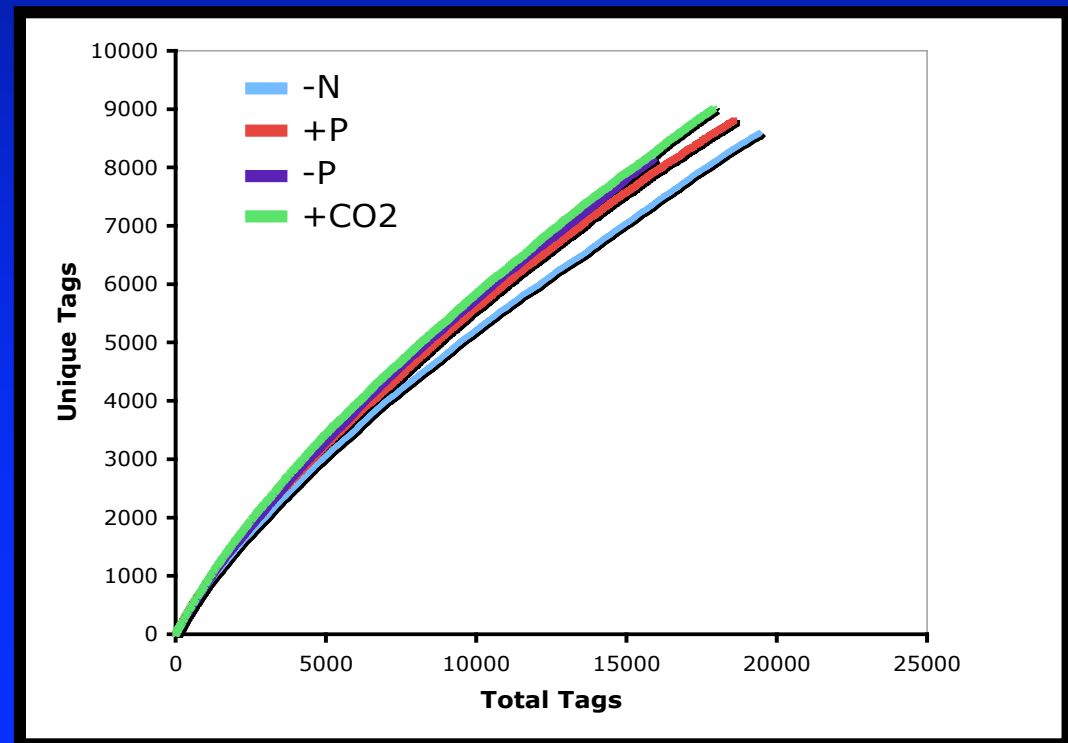
# Long-SAGE: tag isolation



## Long-Sage: library statistics

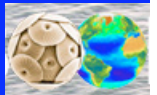
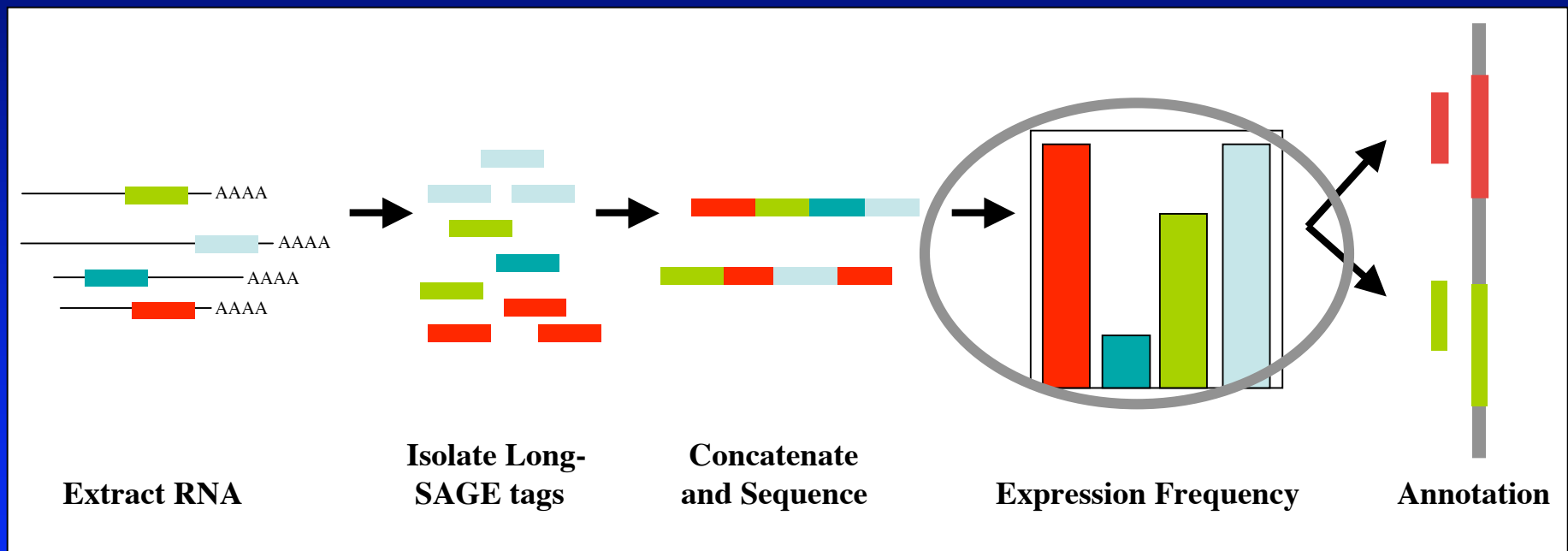
- Total tags sequenced ~ 55,000 across all libraries.
- Roughly 8,000 unique tags.
- Data suggests a unique tag count of ~ 30,000. Relationship of tag count to transcriptome size difficult to predict.

- Multiple tags map to a single gene.
- Single tag maps to multiple genes.
- Gene lacks *NlaIII* site.





# Long-SAGE: expression frequency



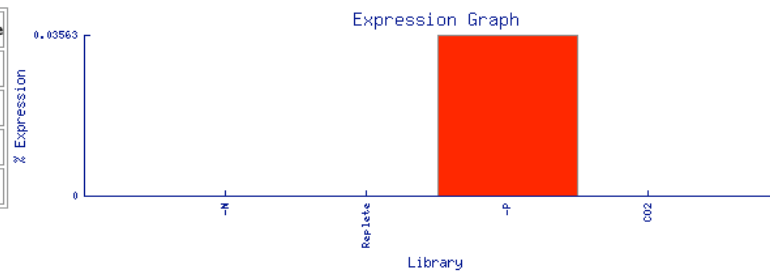
# Long-SAGE: *E. huxleyi* tags up-regulated in low phosphorus conditions

## SAGE Tag 12112

CATGAGGAGAGCGACACTTTG, This tag has not been assigned to an ORF

Library	Raw Count	Percentage
-N	0 of 14647	0.00000%
Replete	0 of 13682	0.00000%
-P	4 of 11225	0.03563%
+CO2	0 of 12787	0.00000%

[\[Details about SAGE libraries and statistics\]](#)

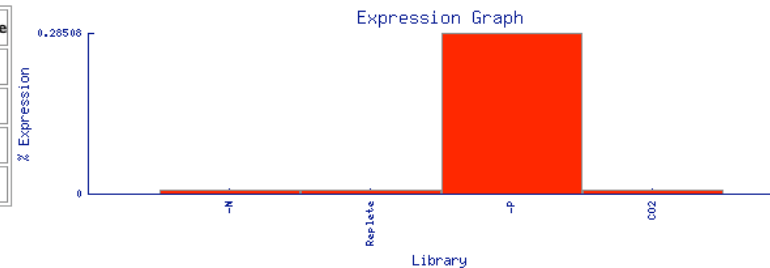


## SAGE Tag 1855

CATGATTGTTAAGAAAGCGCA, This tag has not been assigned to an ORF

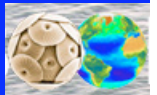
Library	Raw Count	Percentage
-N	1 of 14647	0.00683%
Replete	1 of 13682	0.00731%
-P	32 of 11225	0.28508%
+CO2	1 of 12787	0.00782%

[\[Details about SAGE libraries and statistics\]](#)

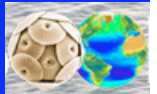
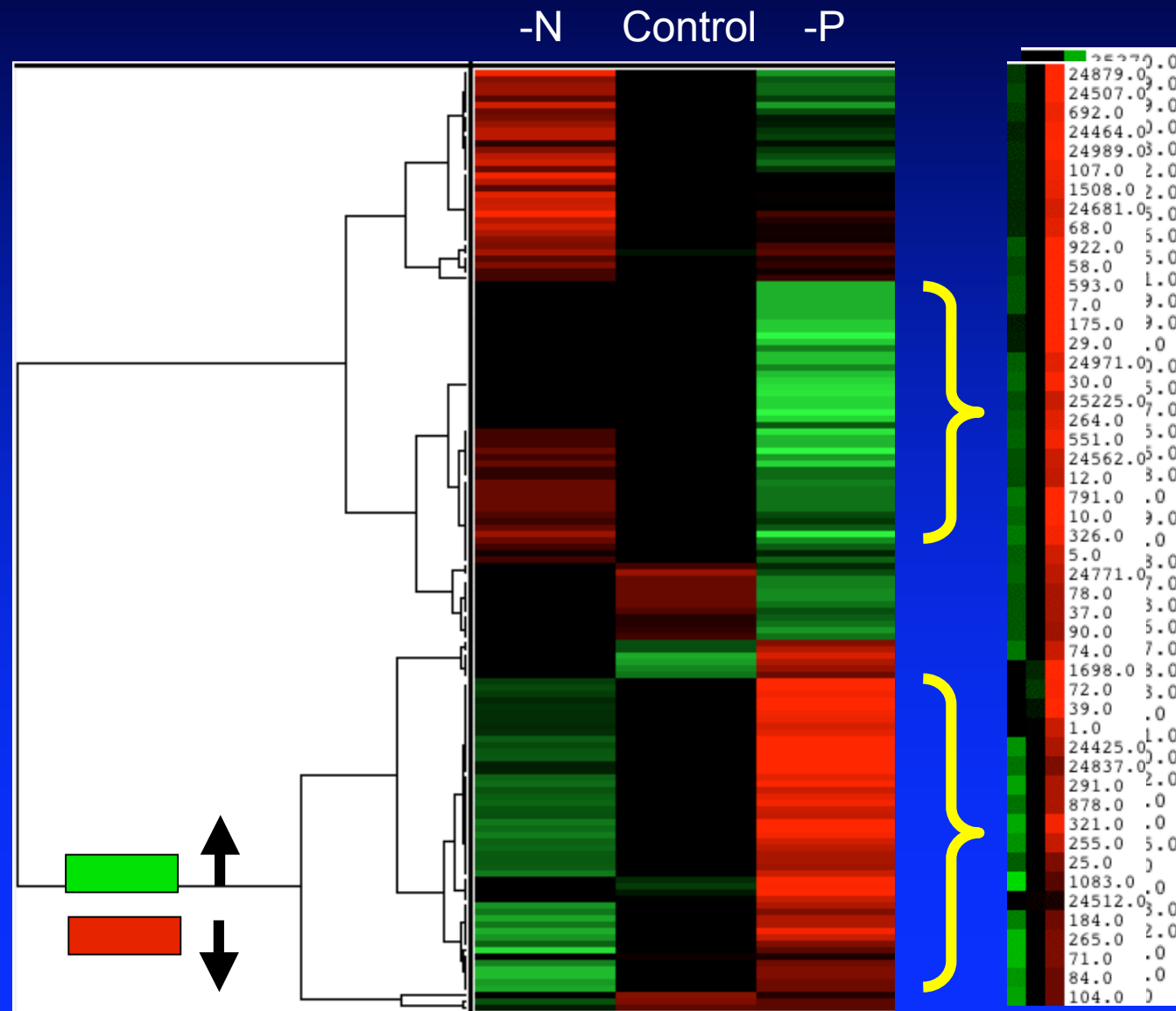


<http://gmod.mbl.edu/perl/site/emiliana04?page=intro>

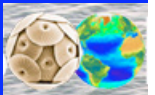
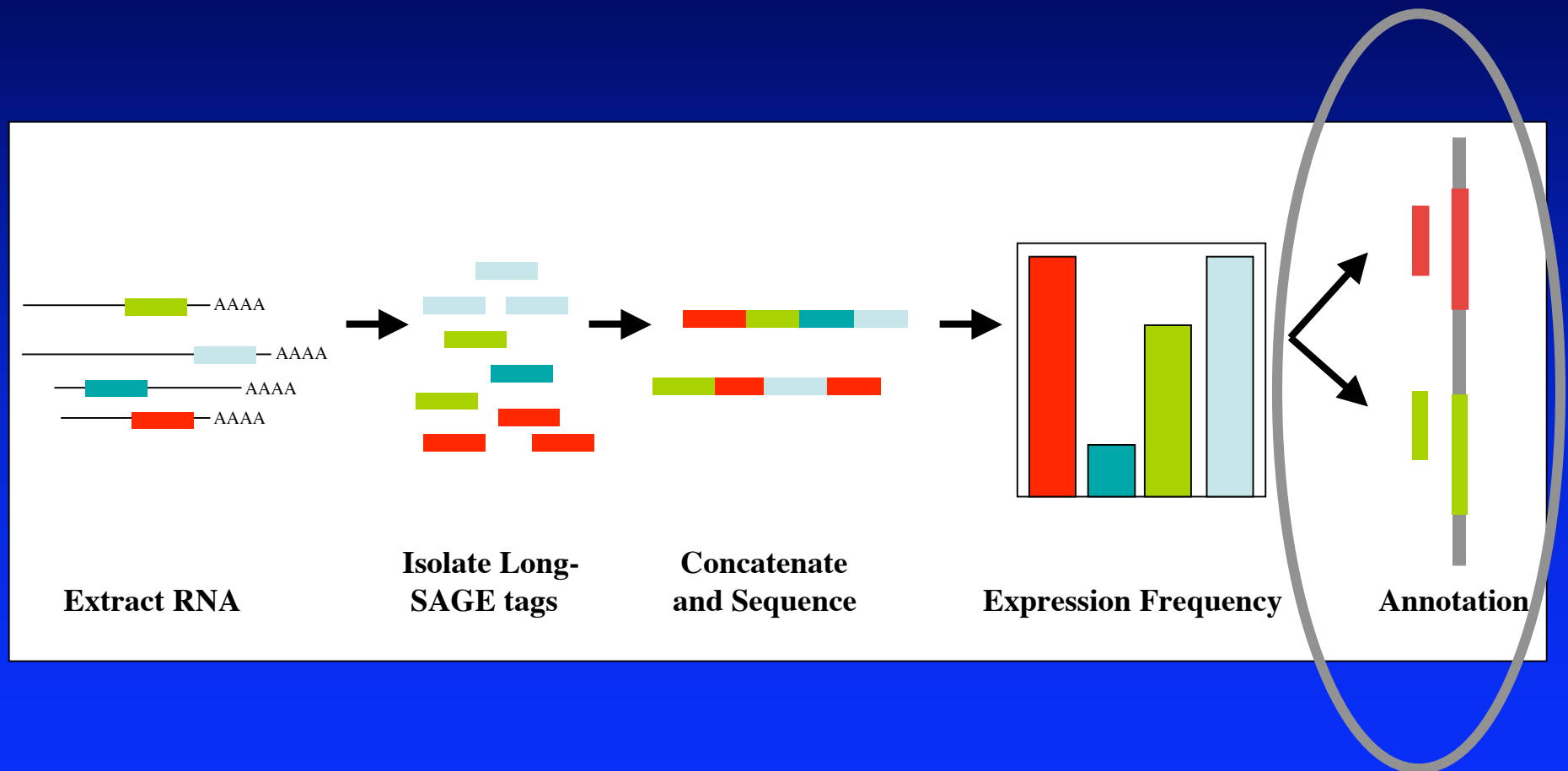
Dyhrman et al. 2006



# Long-SAGE: *E. huxleyi* clustering



# Long-SAGE: annotation





# Long-SAGE: *E. huxleyi* tag annotation

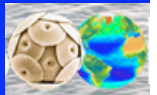
- Map tags to available *E. huxleyi* sequences for annotation
  - Direct match to the most 3' *NlaIII* restriction site

Tag maps to EST or genome sequence with no annotation  
Tag maps to EST or genome sequence with annotation

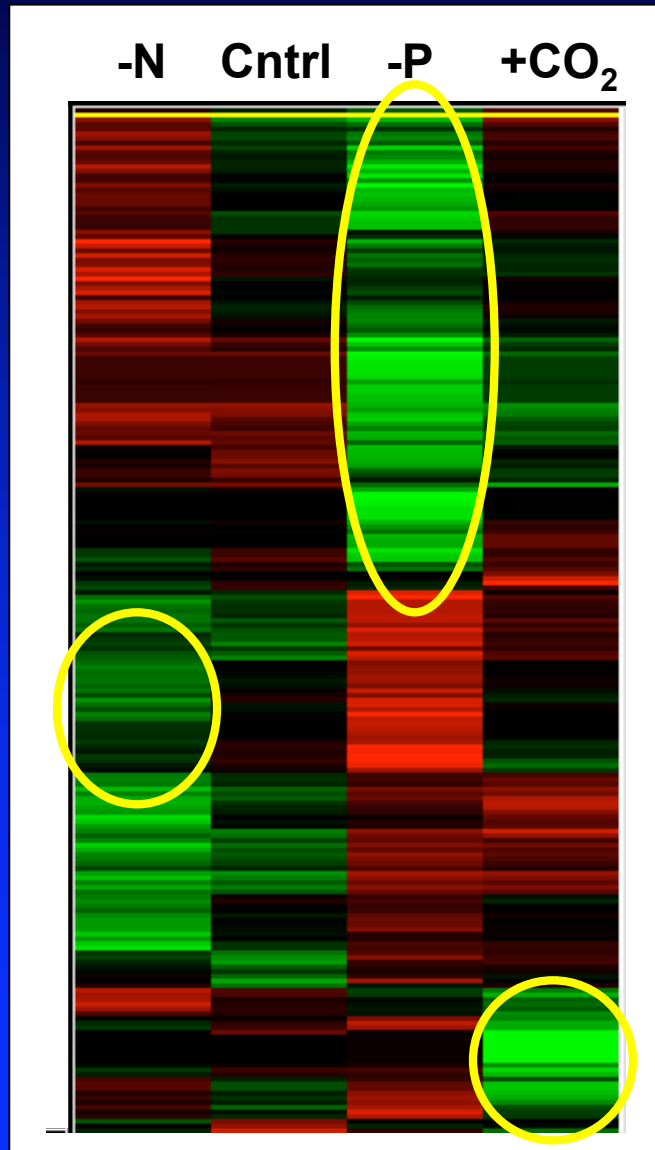
Tag ID	Sequence	Tag Type	OrfID	Description	-N	-P	Replete
<a href="#">12112</a>	CATGAGGAGAGCGACACTTTG	PS	<a href="#">1884</a>	putative inorganic pyrophosphatase [Arabidopsis thaliana] gi 15028285 gb AAK76619.1  putative inorganic pyrophosphatase [Arabidopsis thaliana] gi 7671424 emb CAB89365.1  inorganic pyrophosphatase-like protein [Arabidopsis thaliana] gi 12057177 emb CAC1985	0.000	0.034	0.000

Scientific enquiries should be directed to [sdyhrman@whoi.edu](mailto:sdyhrman@whoi.edu)

This database is hosted by the JBPC [GMOD Server](#). Bug reports and technical problems should be reported to [biocomp@lists.mbl.edu](mailto:biocomp@lists.mbl.edu).



## Long-SAGE: *E. huxleyi* tag annotation



### - Up-regulated by P stress

Alkaline phosphatase\*

Phosphate permease

### - Up-regulated by N stress

High affinity nitrate transporter

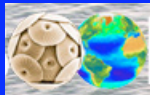
Amino acid transporter

Urease

### - Up-regulated by increased CO<sub>2</sub>

Many tags that map to genes with unknown function

 Up-regulated  
 Down-regulated



# Long-SAGE: *E. huxleyi* tag validation

## P related

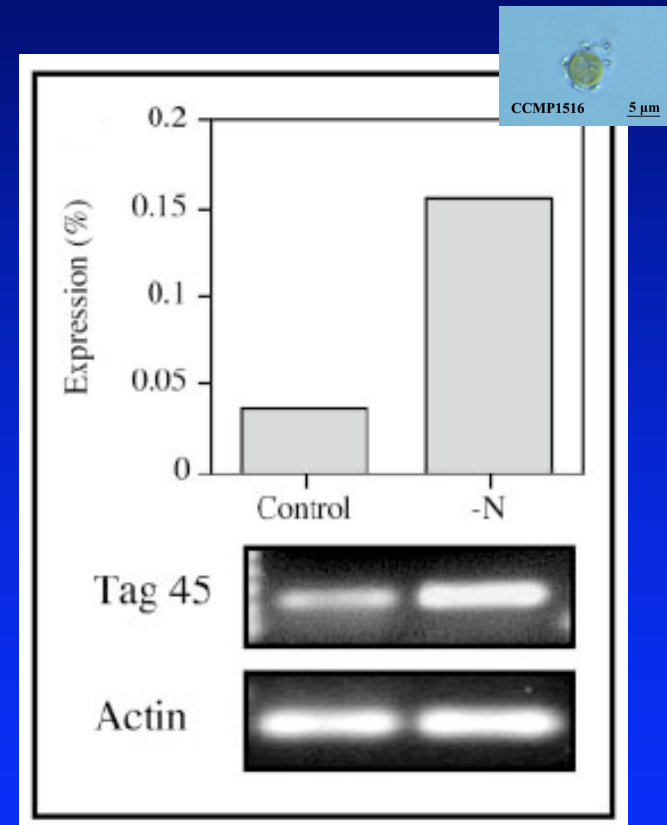
Inorganic pyrophosphatase  
Phosphate-repressible permease  
Polyphosphate synthetase  
Alkaline phosphatase

## N related

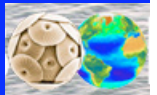
High affinity nitrate transporter\*  
Urease

## C related

Carbonic anhydrase

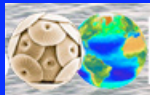


Dyhrman et al. 2006



# Beyond the transcriptome

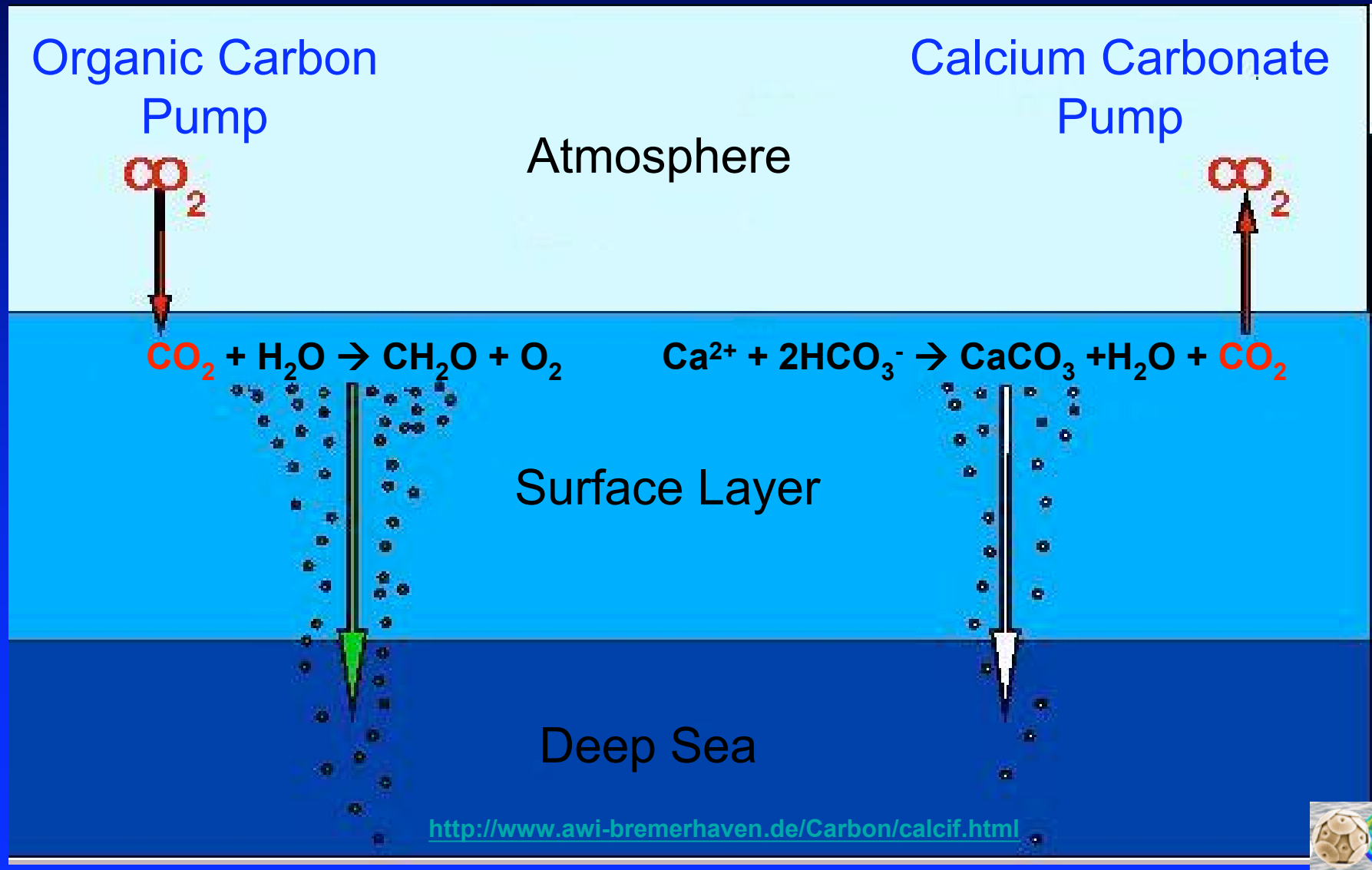
- *What are metabolic pathways and preferred sources of N, P and C?*
- *Biosynthesis and stability of alkenones?*
- *Influence of virus infection on loss terms and the cycling of S, C, N, P ?*
- *How and why to they calcify?*
- How do we get at genes with no known function?
  - *One of the *E. huxleyi* alkaline phosphatases has a very high  $V_{max}$  but no database homology.*
  - *Only identified with molecular/biochemical characterization (Xu et al 2006, Landry et al. 2006)*
- Development of a genetic system may be needed to identify genes involved with calcification etc.
- So - what does the future hold?





# Back to the Future

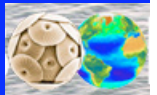
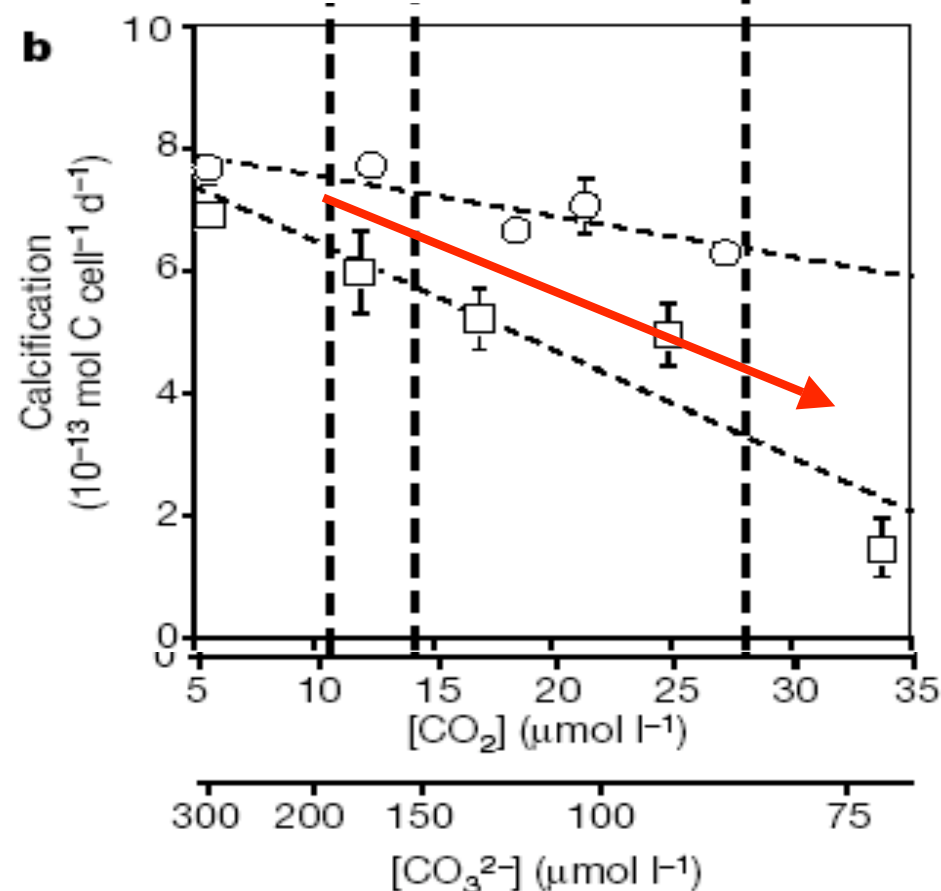
- Coccolithophore responses to high CO<sub>2</sub>

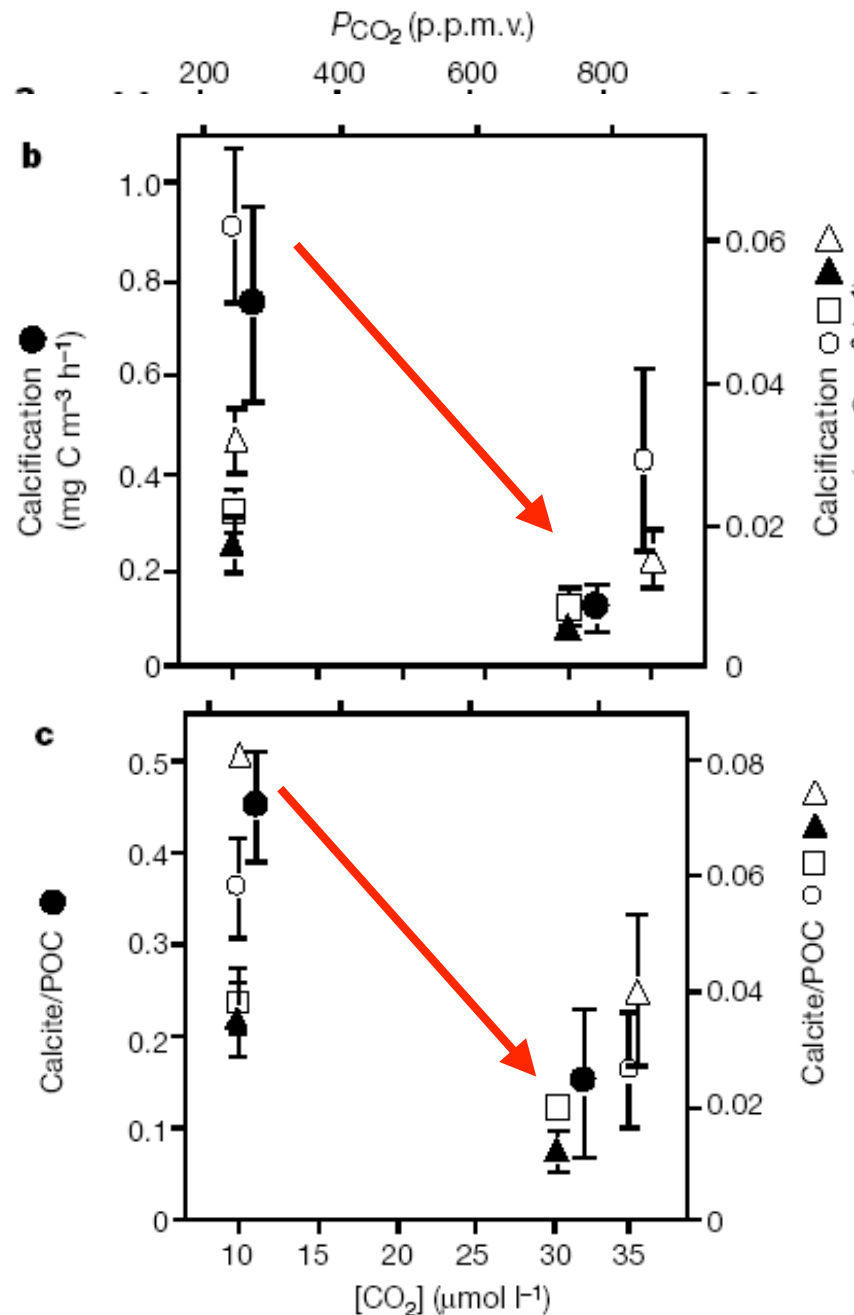


# Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>

Ulf Riebesell \*, Ingrid Zondervan\*, Björn Rost\*, Philippe D. Tortell†,  
Richard E. Zeebe\*‡ & François M. M. Morel†

Culture studies with variable CO<sub>2</sub>  
concentrations generated by  
adding acid

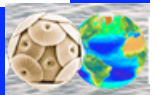




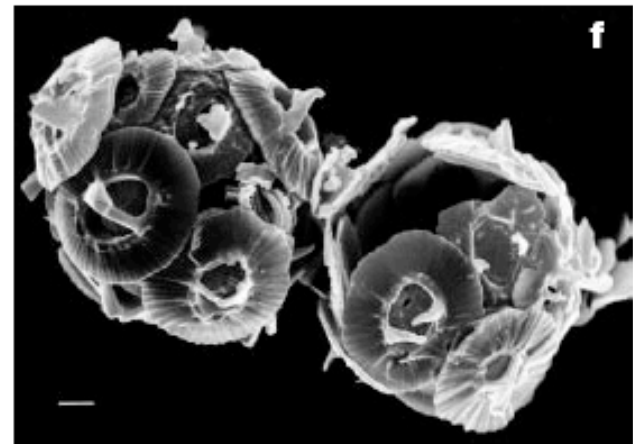
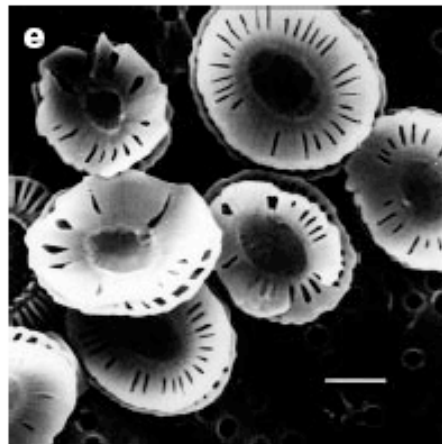
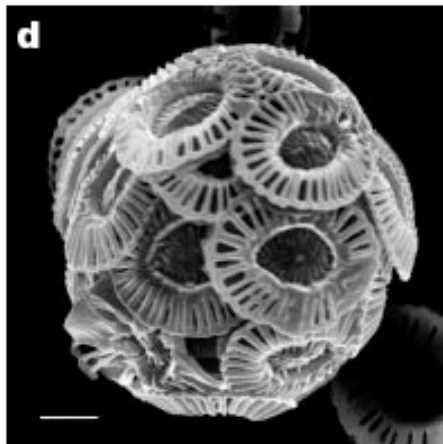
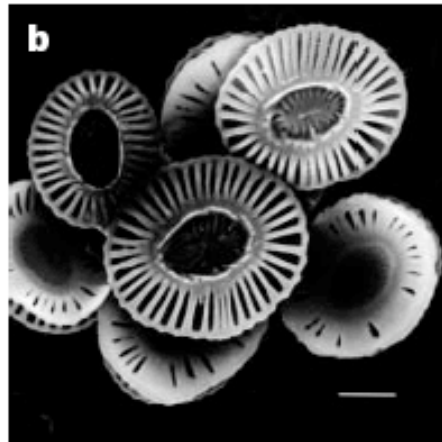
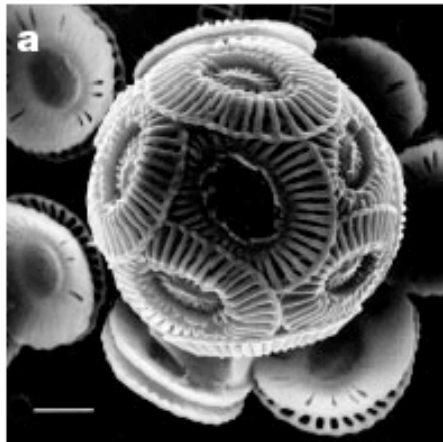
Similar results  
observed for field  
populations in the  
subarctic North  
Pacific.

## Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>

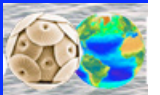
Ulf Riebesell<sup>\*</sup>, Ingrid Zondervan<sup>\*</sup>, Björn Rost<sup>\*</sup>, Philippe D. Tortell<sup>†</sup>,  
Richard E. Zeebe<sup>\*‡</sup> & François M. M. Morel<sup>†</sup>



Normal CO<sub>2</sub>



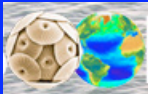
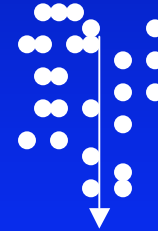
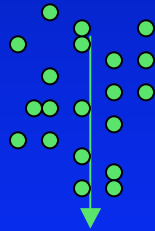
High CO<sub>2</sub>



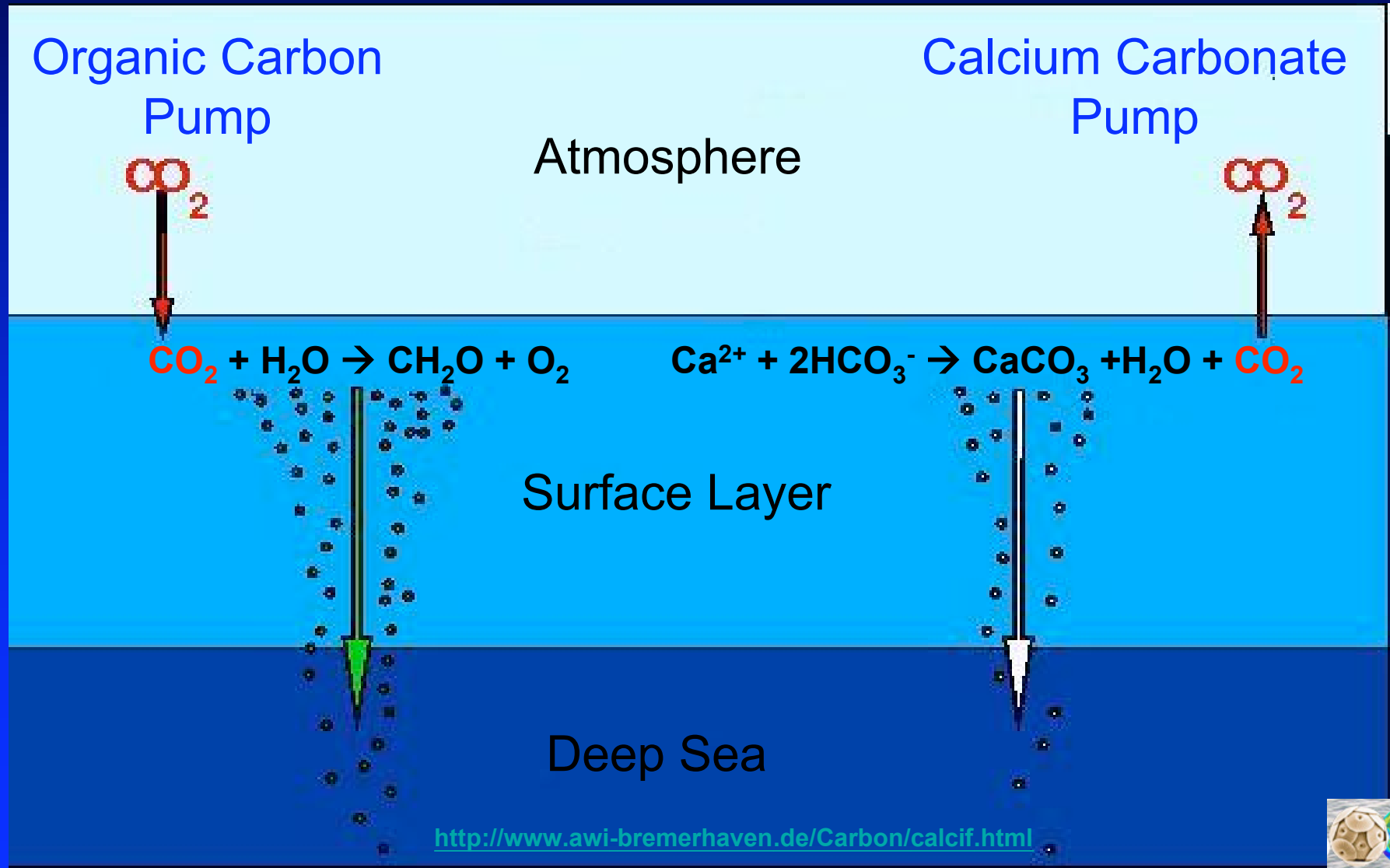


# Back to the Future

- Coccolithophore responses to high CO<sub>2</sub>
- Decrease in calcification observed for *Emiliana huxleyi*, *Gephyrocapsa oceanica*, negligible change observed for *Coccolithus pelagicus*.

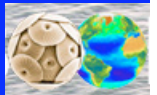
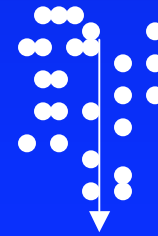
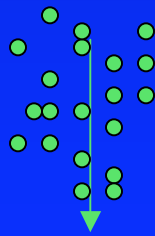


# Negative feedback on CO<sub>2</sub>?



# Back to the Future

- Coccolithophore responses to high CO<sub>2</sub>
- Decrease in calcification observed for *Emiliana huxleyi*, *Gephyrocapsa oceanica*, negligible change observed for *Coccolithus pelagicus*
- But.. The carbonate system was modified by directly adjusting pH
- pH controls the relative proportions of the carbonate species while DIC remains constant.
- Many researchers are suggesting that bubbling CO<sub>2</sub> enriched air through sea water (increasing both CO<sub>2</sub> and DIC) is more realistic.

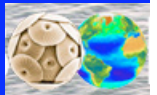


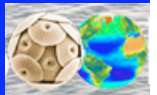
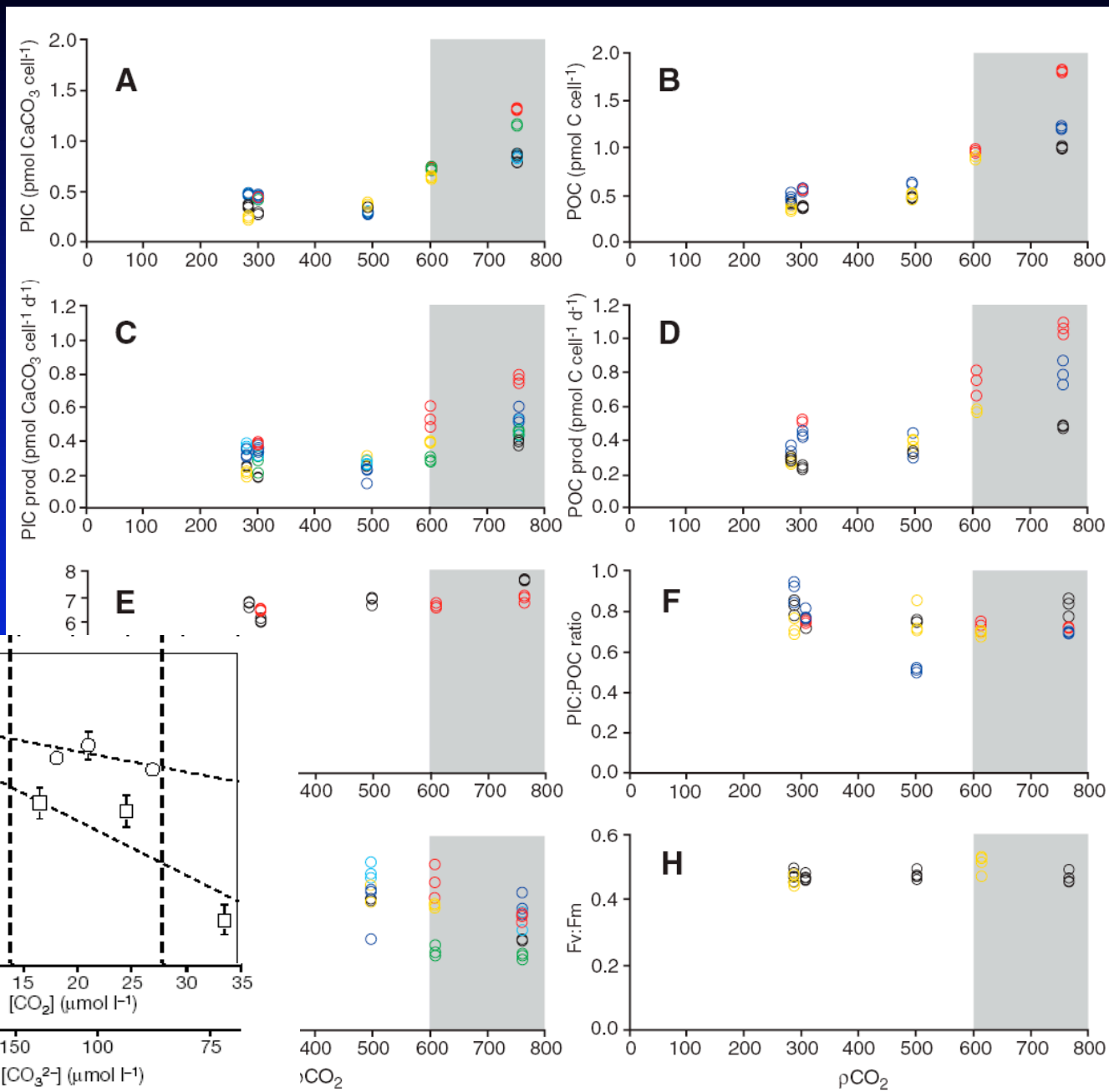
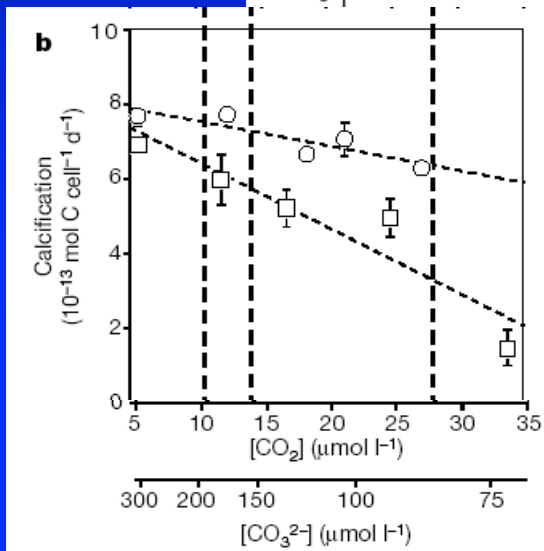
# Back to the Future with CO<sub>2</sub> bubbling...

## Phytoplankton Calcification in a High-CO<sub>2</sub> World

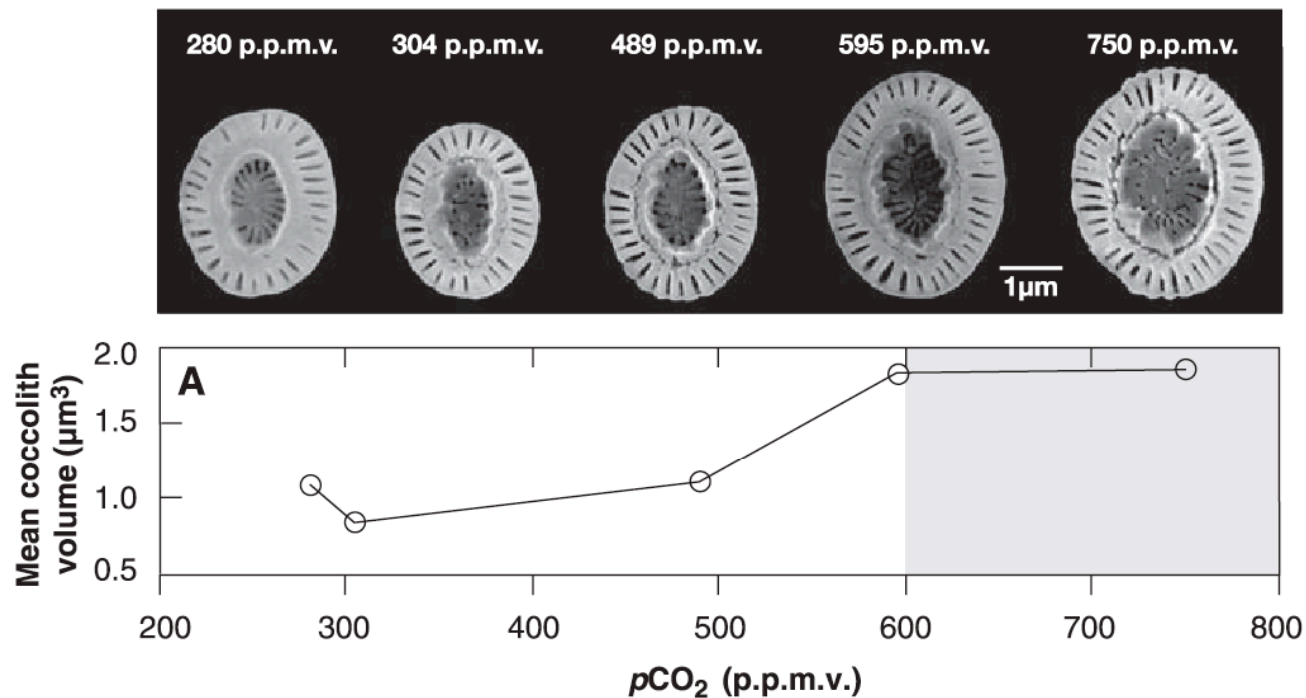
M. Debora Iglesias-Rodriguez,<sup>1\*</sup> Paul R. Halloran,<sup>2\*</sup> Rosalind E. M. Rickaby,<sup>2</sup>  
Ian R. Hall,<sup>3</sup> Elena Colmenero-Hidalgo,<sup>3†</sup> John R. Gittins,<sup>1</sup> Darryl R. H. Green,<sup>1</sup>  
Toby Tyrrell,<sup>1</sup> Samantha J. Gibbs,<sup>1</sup> Peter von Dassow,<sup>4</sup> Eric Rehm,<sup>5</sup>  
E. Virginia Armbrust,<sup>5</sup> Karin P. Boessenkool<sup>3</sup>

Ocean acidification in response to rising atmospheric CO<sub>2</sub> partial pressures is widely expected to reduce calcification by marine organisms. From the mid-Mesozoic, coccolithophores have been major calcium carbonate producers in the world's oceans, today accounting for about a third of the total marine CaCO<sub>3</sub> production. Here, we present laboratory evidence that calcification and net primary production in the coccolithophore species *Emiliania huxleyi* are significantly increased by high CO<sub>2</sub> partial pressures. Field evidence from the deep ocean is consistent with these laboratory conclusions, indicating that over the past 220 years there has been a 40% increase in average coccolith mass. Our findings show that coccolithophores are already responding and will probably continue to respond to rising atmospheric CO<sub>2</sub> partial pressures, which has important implications for biogeochemical modeling of future oceans and climate.

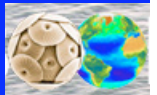




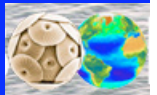
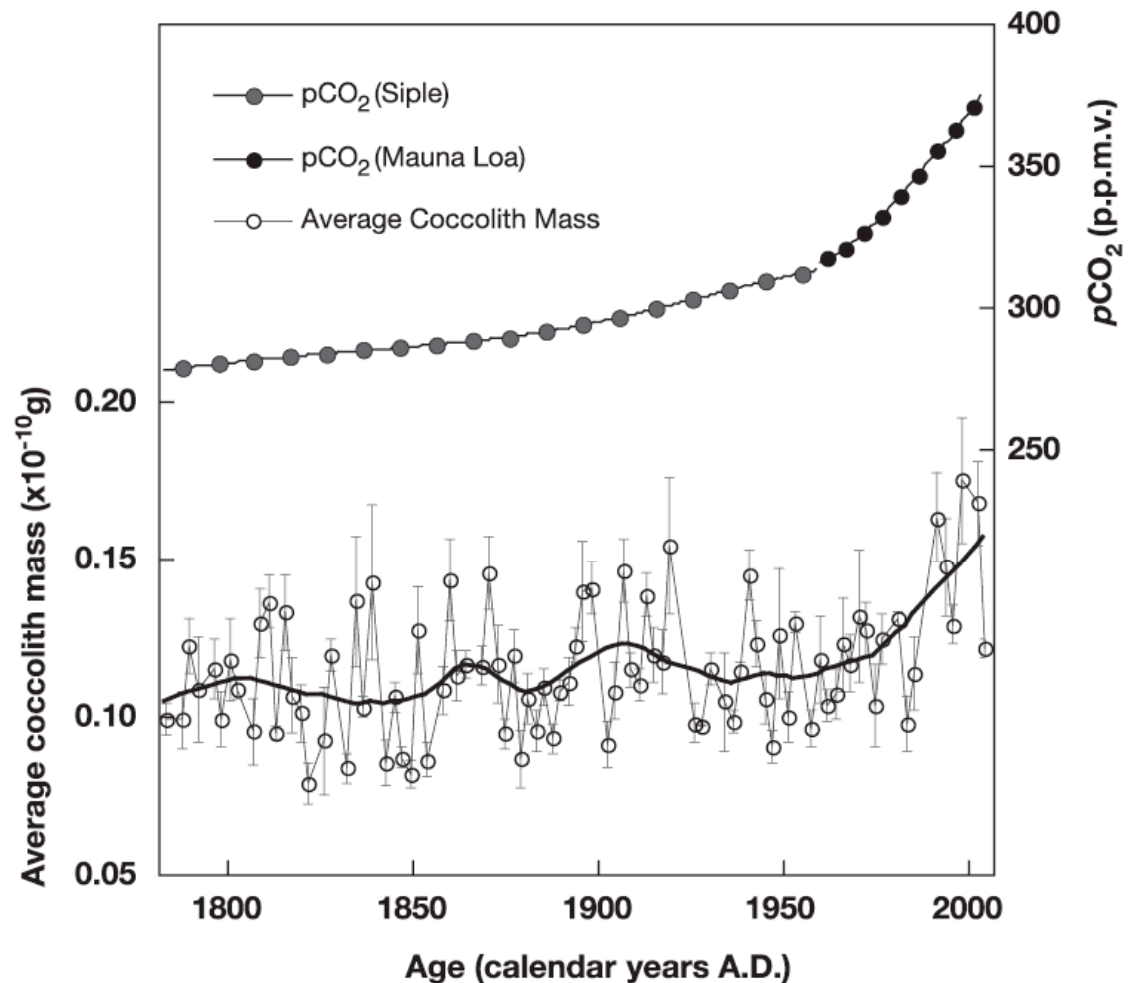




**Fig. 2.** Coccolith volume and CaCO<sub>3</sub> per cell. Increasing coccolith volume is closely coupled with increasing CaCO<sub>3</sub> per cell, indicating down-core measurement of coccolith mass to be representative of CaCO<sub>3</sub> production. Scanning electron microscope (SEM) images show typical coccoliths from each culture with  $P_{CO_2}$  values from 280 to 750 ppmv of CO<sub>2</sub>, of where the measured volume was converted to length using the formula for a heavily calcified coccolith (27).

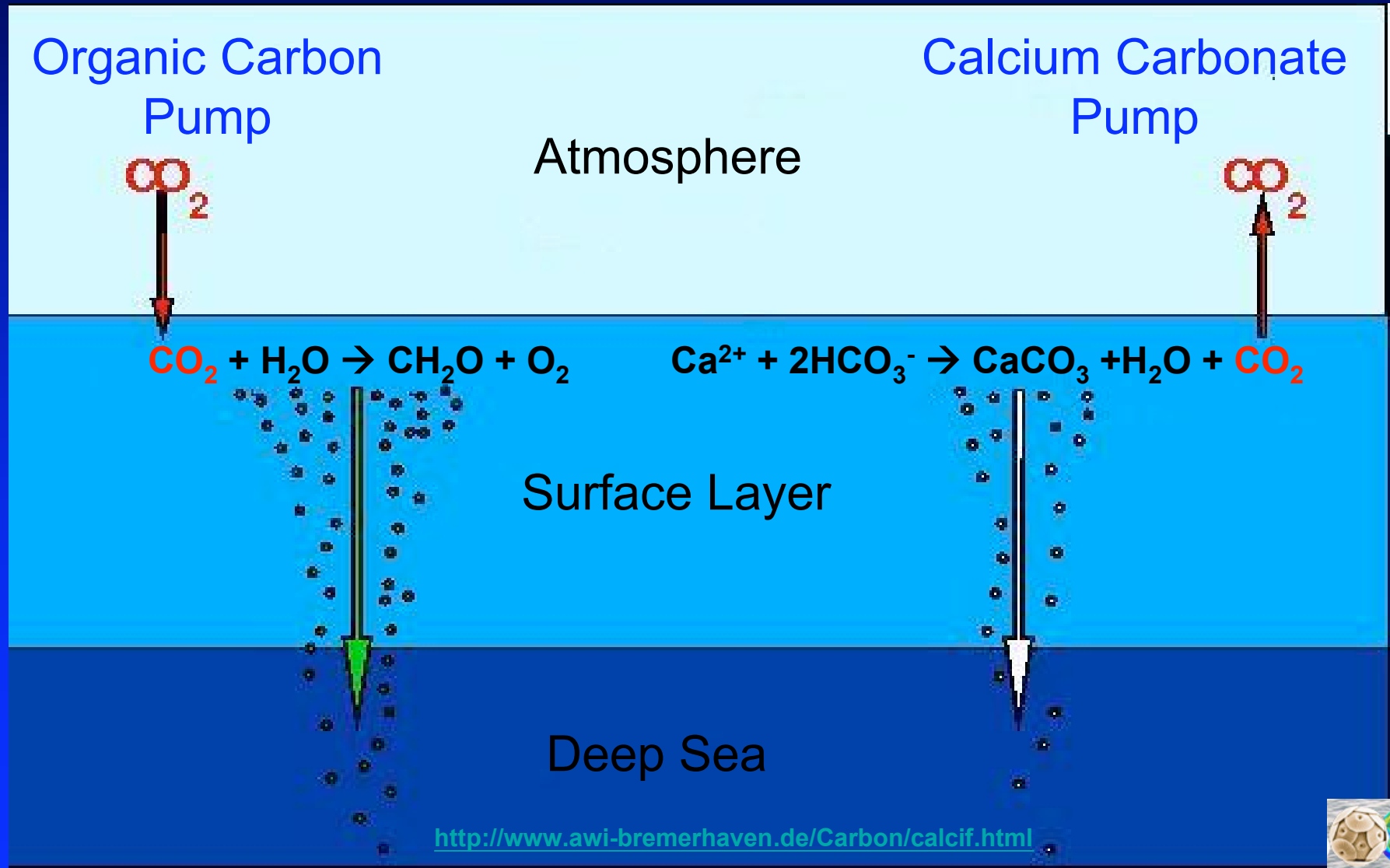


**Fig. 4.** Average mass of  $\text{CaCO}_3$  per coccolith in core RAPID 21-12-B and atmospheric  $\text{CO}_2$ . The average mass of  $\text{CaCO}_3$  per coccolith in core RAPID 21-12-B (open circles) increased from  $1.08 \times 10^{-11}$  to  $1.55 \times 10^{-11}$  g between 1780 and the modern day, with an accelerated increase over recent decades. The increase in average coccolith mass correlates with rising atmospheric  $P_{\text{CO}_2}$ , as recorded in the Siple ice core (gray circles) (26) and instrumentally at Mauna Loa (black circles) (38), every 10th and 5th data point shown, respectively. Error bars represent 1 SD as calculated from replicate analyses. Samples with a standard deviation greater than 0.05 were discarded. The smoothed curve for the average coccolith mass was calculated using a 20% locally weighted least-squares error method.

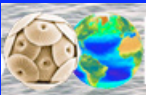
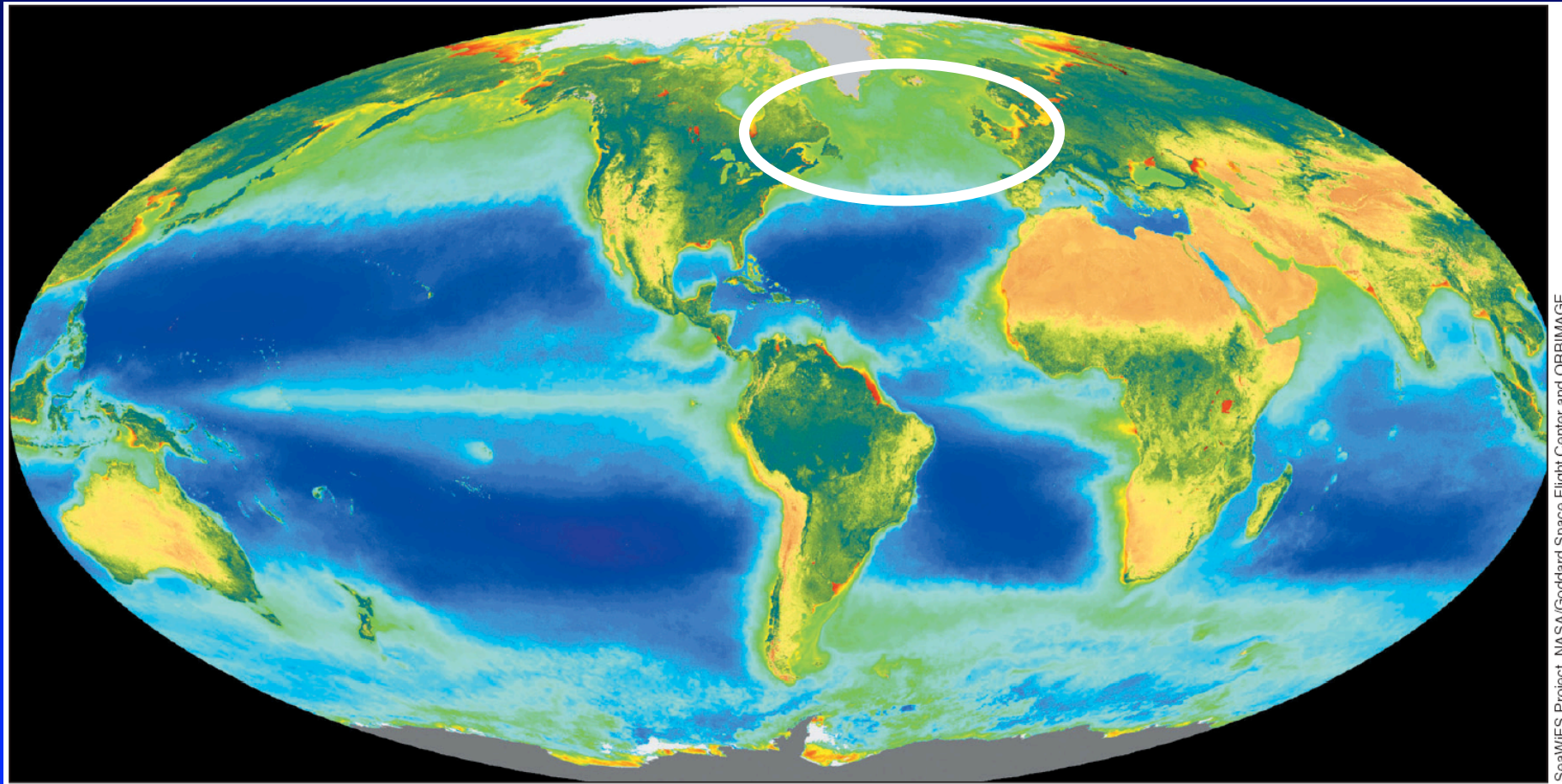


# Negative feedback on CO<sub>2</sub>??????

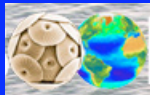
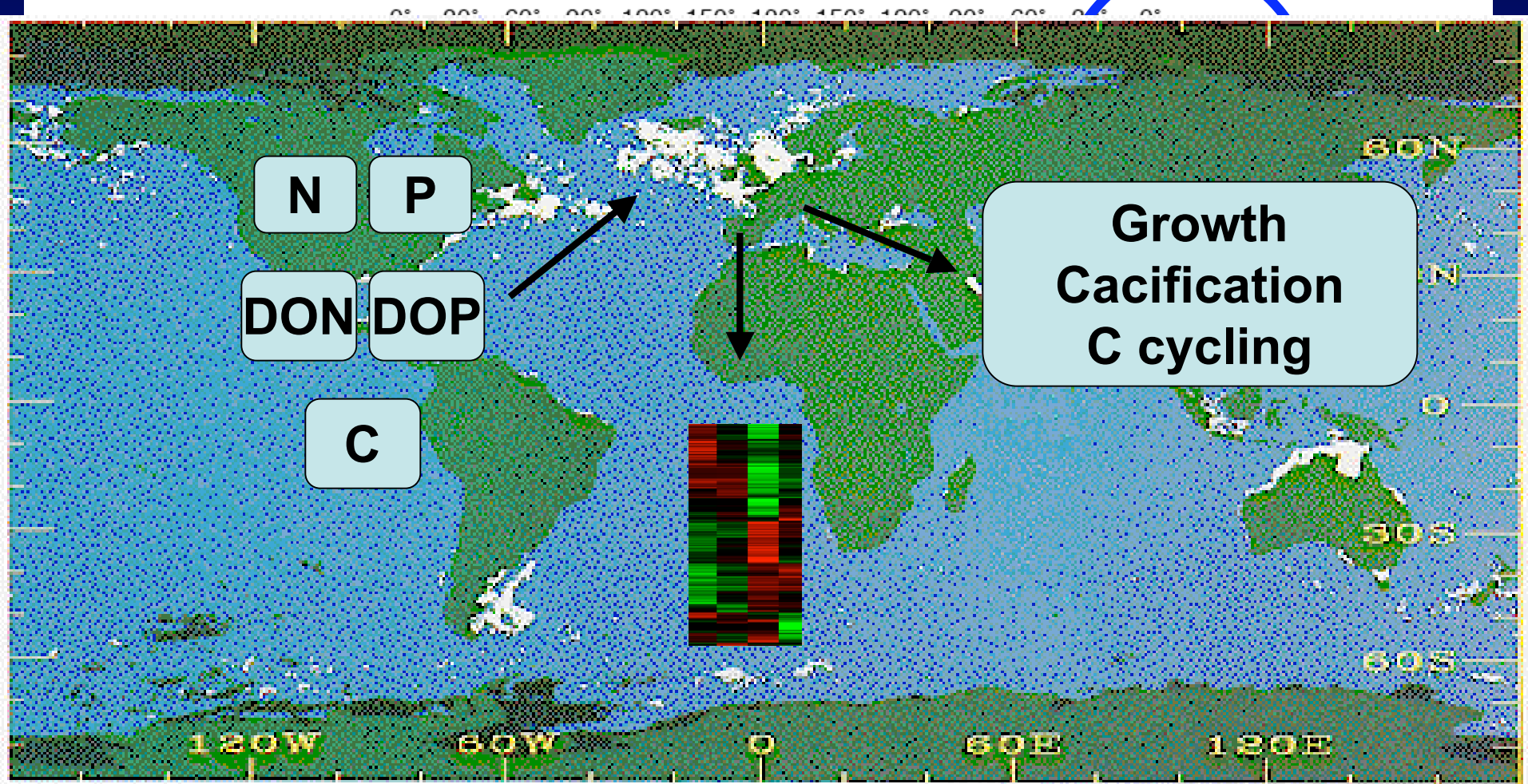
## Or...Positive feedback on CO<sub>2</sub>



# Linking the genome to physiological ecology



# North Atlantic is a CO<sub>2</sub> sink





# Preliminary “ocean phenotype” data

Sample 5m niskins  
Sample incubations



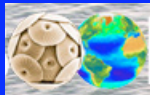
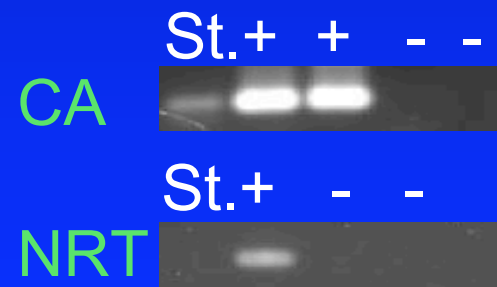
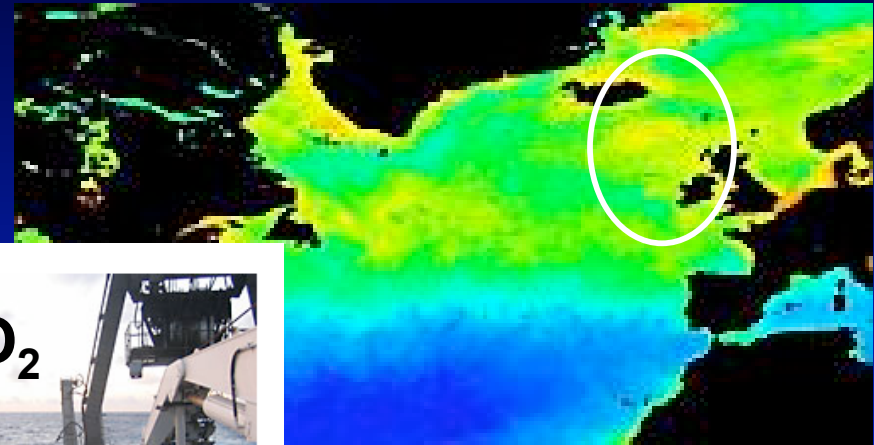
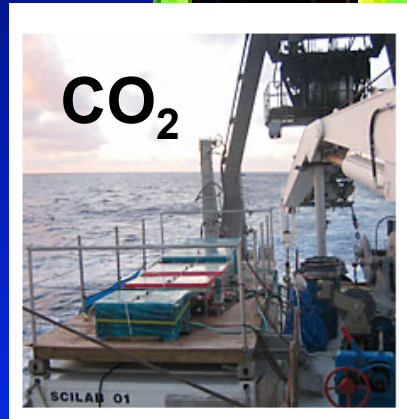
Collect 0.5-0.75L  
onto GF/F













Store in RNA  
extraction buffer





Examine gene  
expression

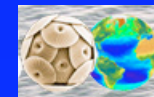


# Gene targets and expression pattern

Transcript	Functional Category	# 33	# 85	# 57 Incubation
Actin	Cell Structure			
Nitrate transporter	N Metabolism			
Pyrophosphatase	P Metabolism			
$\delta$ Carbonic anhydrase	C Metabolism			
O <sub>2</sub> evolving enhancer	Photosynthesis			

 Band Detected  Band Not Detected

Special thanks to Dave Hutchins and Louie Wurch



# The take home message...

- Coccolithophores are really cool - in part because the dynamics of this one group can dramatically influence carbon cycling
- Of the coccolithophores, *Emiliana huxleyi* is emerging as increasingly important model for studies of ...
  - *Paleo climate reconstruction*
  - *S cycling*
  - *Viral dynamics*
  - *Carbon cycling*
- Anatomy of a eukaryotic genome project “euks are challenging”
- Transcriptome profiling for aiding genome annotation and moving beyond “capacity”
  - *Introduction to SAGE*
  - *N and P scavenging in E. huxleyi*
- What does the future hold? ... The challenges of prediction in microbial oceanography
  - *Two different CO<sub>2</sub> responses*

