國立台灣海洋大學 海洋科學與資源學院
海洋環境化學與生態研究所
Institute of Marine Environmental Chemistry and Ecology

Application of molecular probes for detecting nutrient deficiency in marine phytoplankton

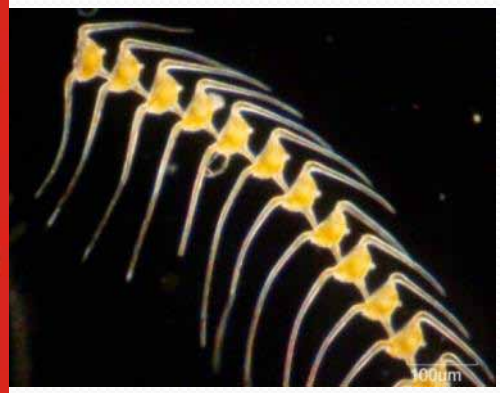
康利國 (Lee-Kuo Kang)
助理研究員

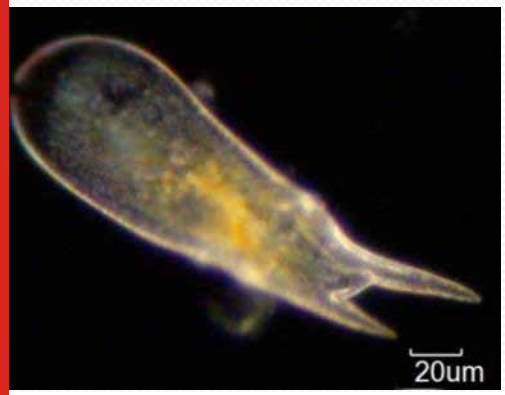
浮游植物

海洋生物全靠它

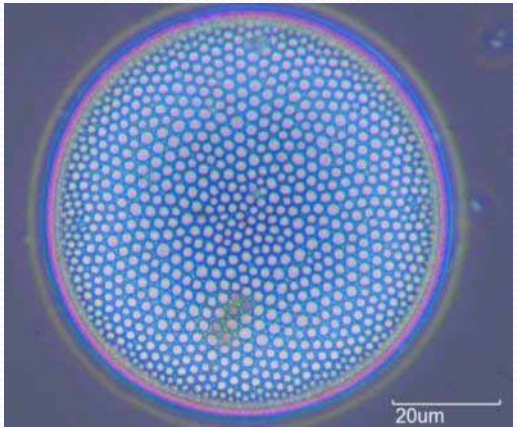
上至鯨魚下至蝦

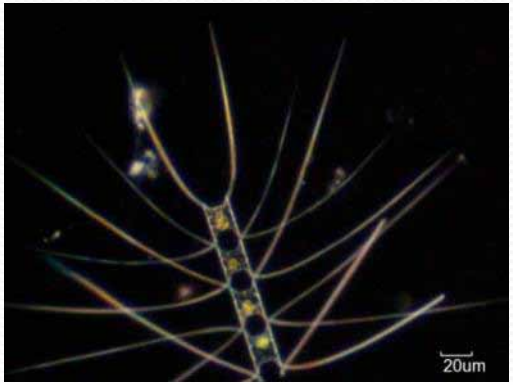
Dinoflagellates



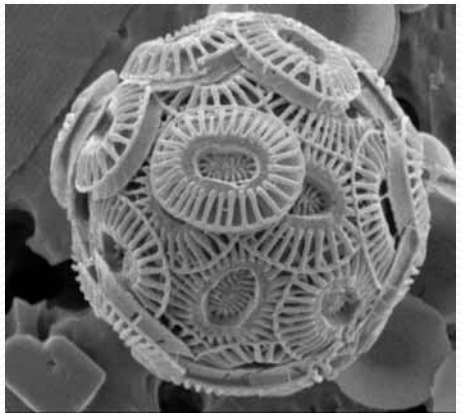


Diatoms



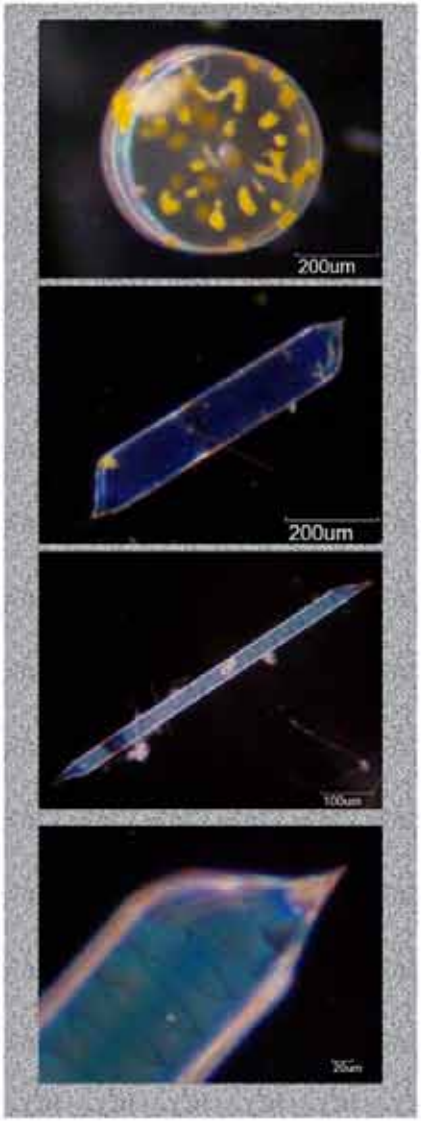


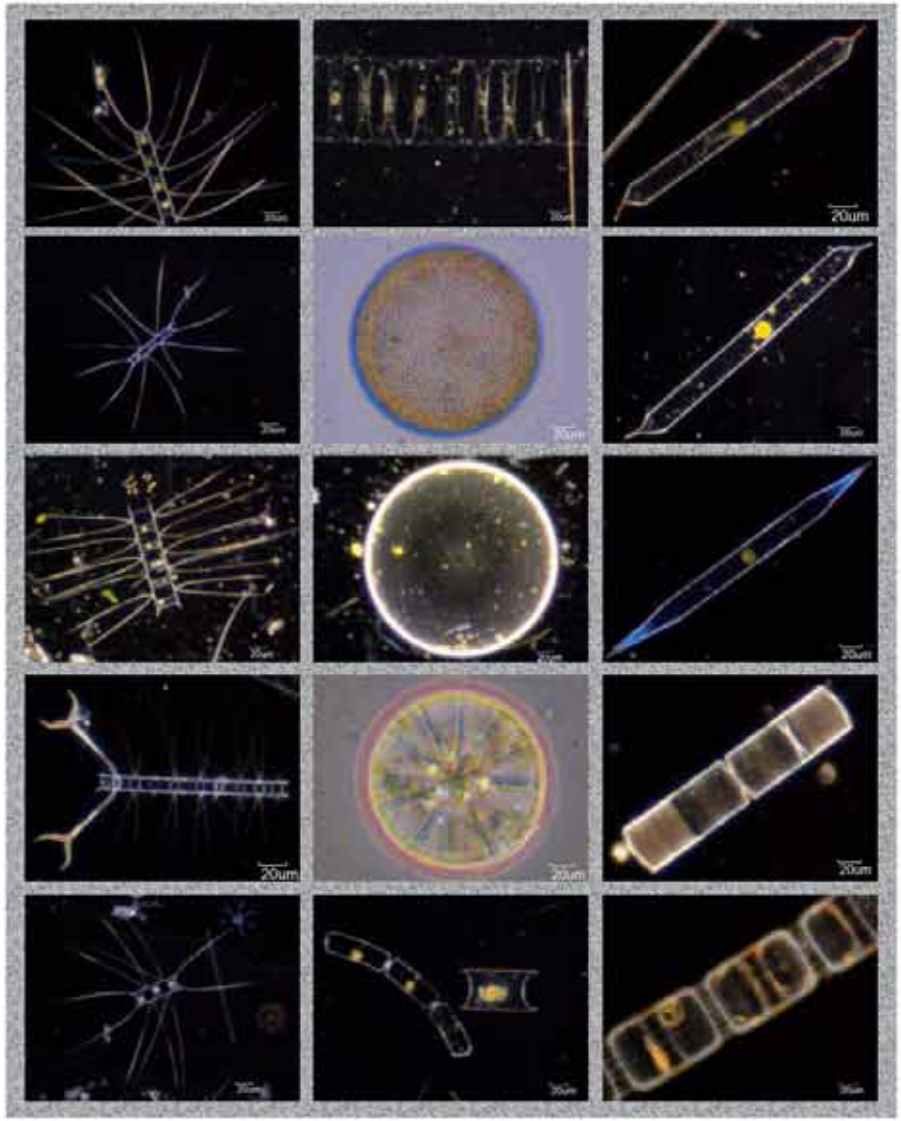
Coccolithophores

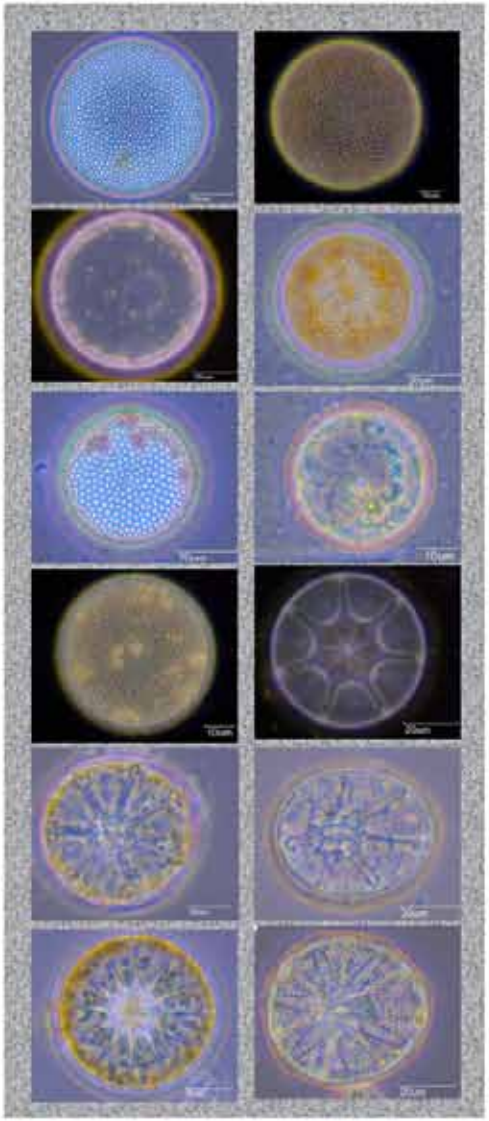


Cyanobacteria

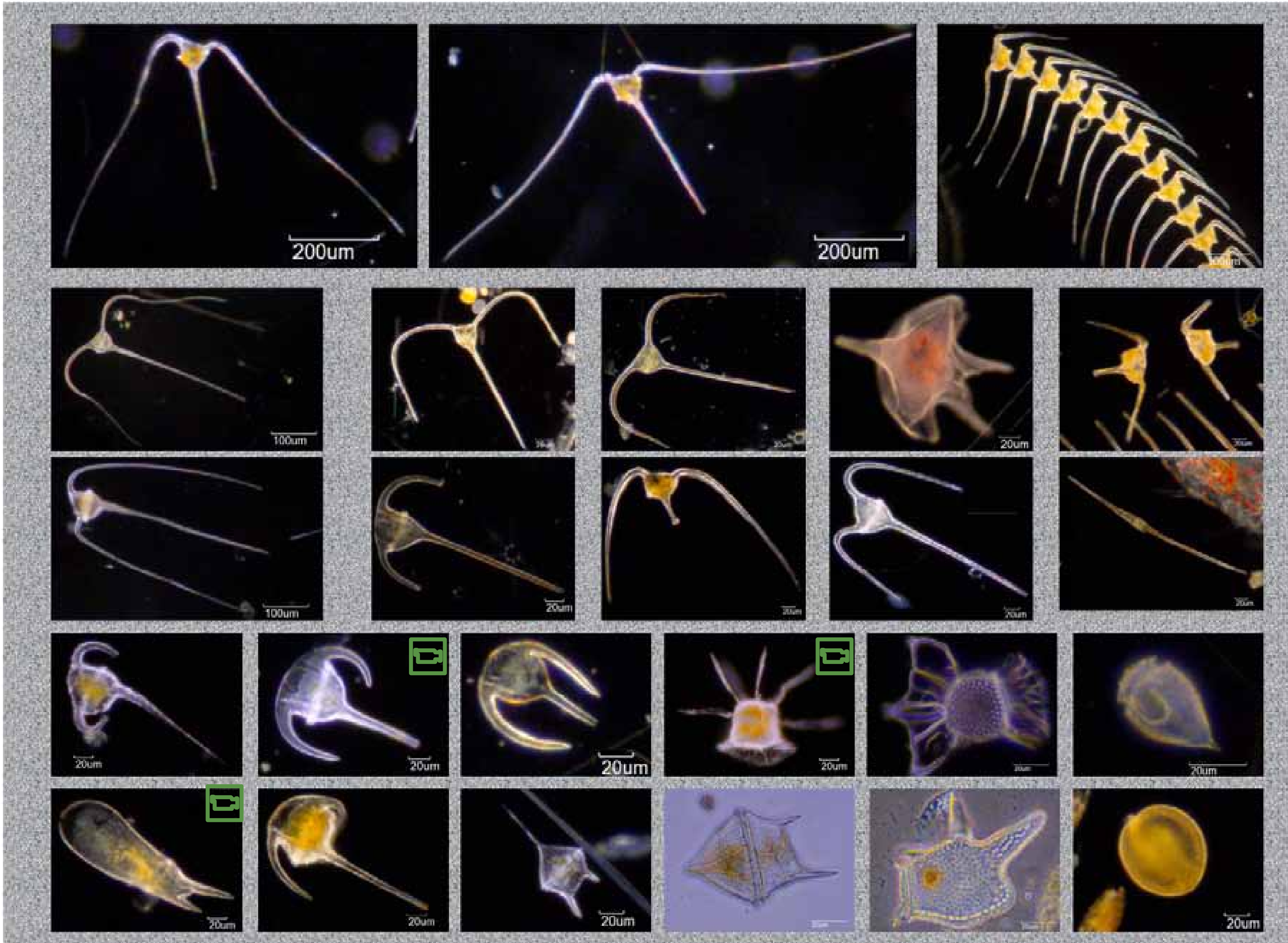
東沙周邊海域的浮游植物(一) 矽藻



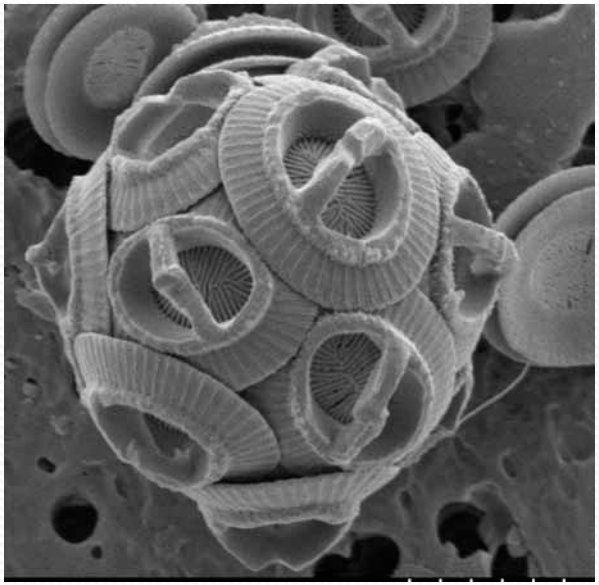
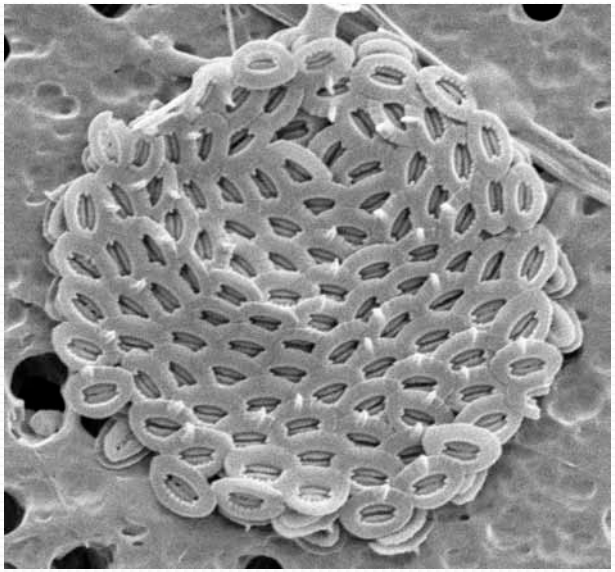
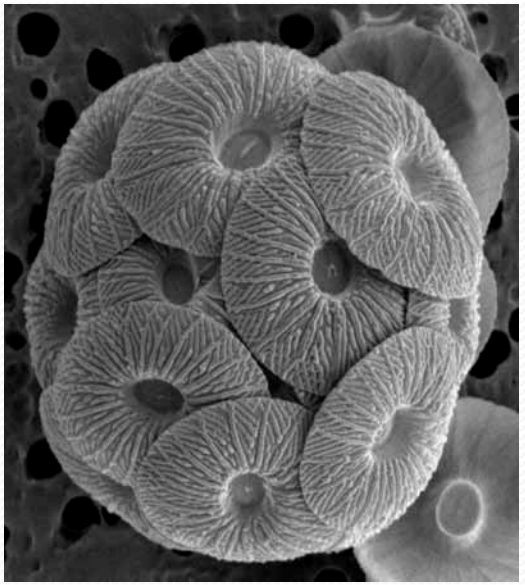


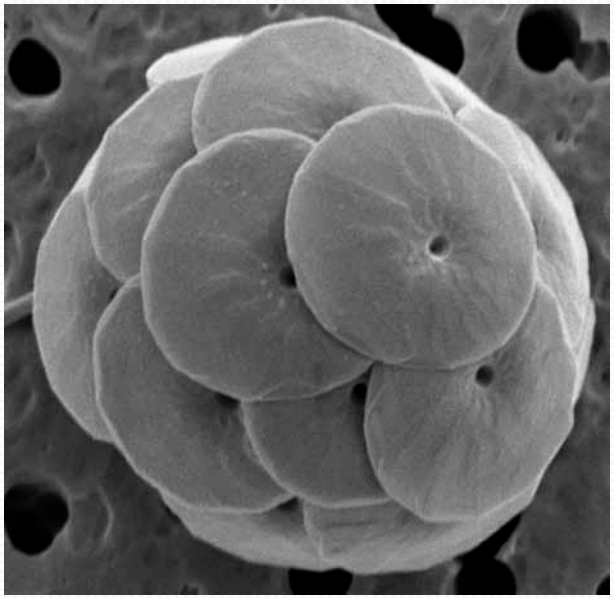


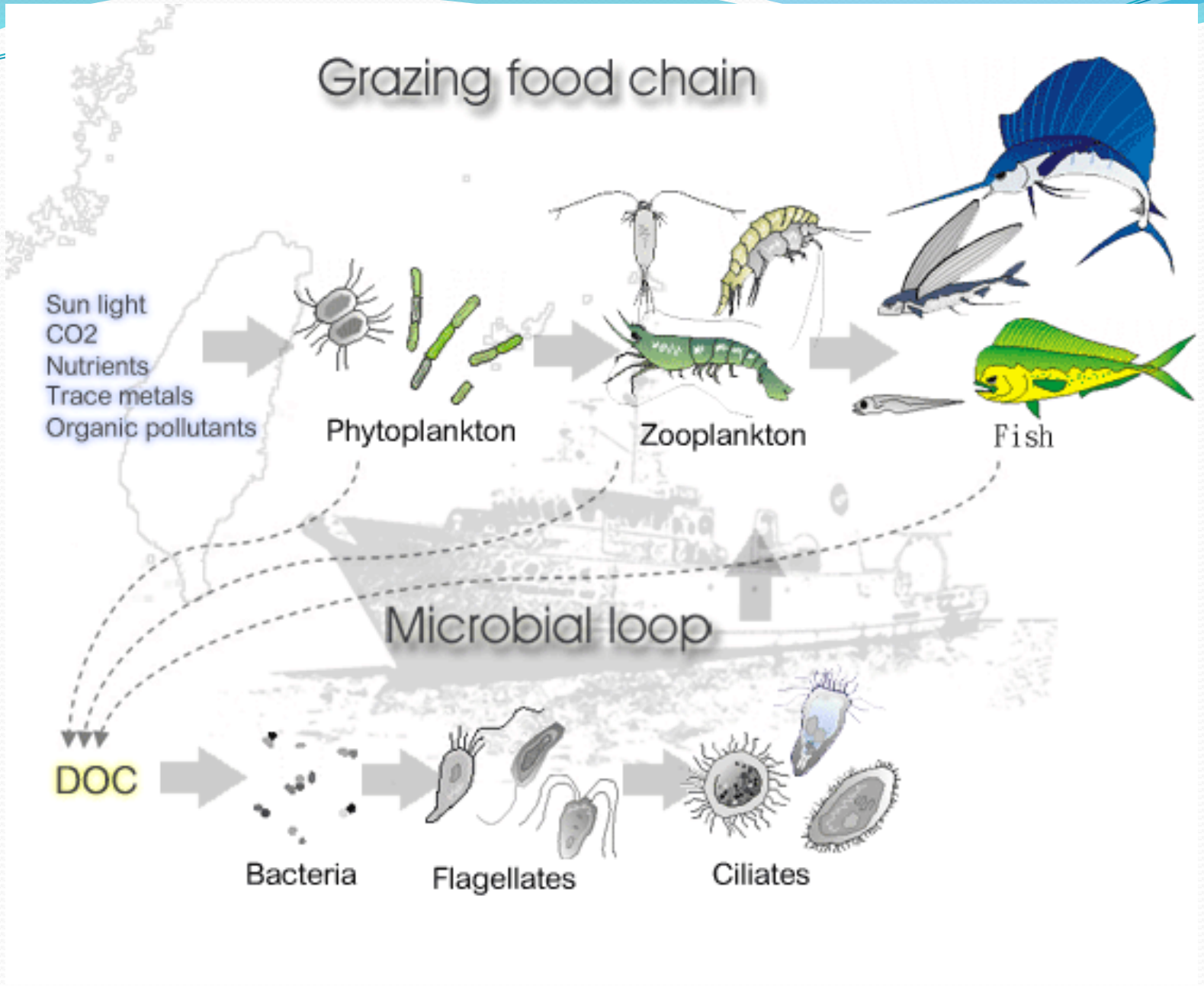
東沙周邊海域的浮游植物(二) 渦鞭毛藻



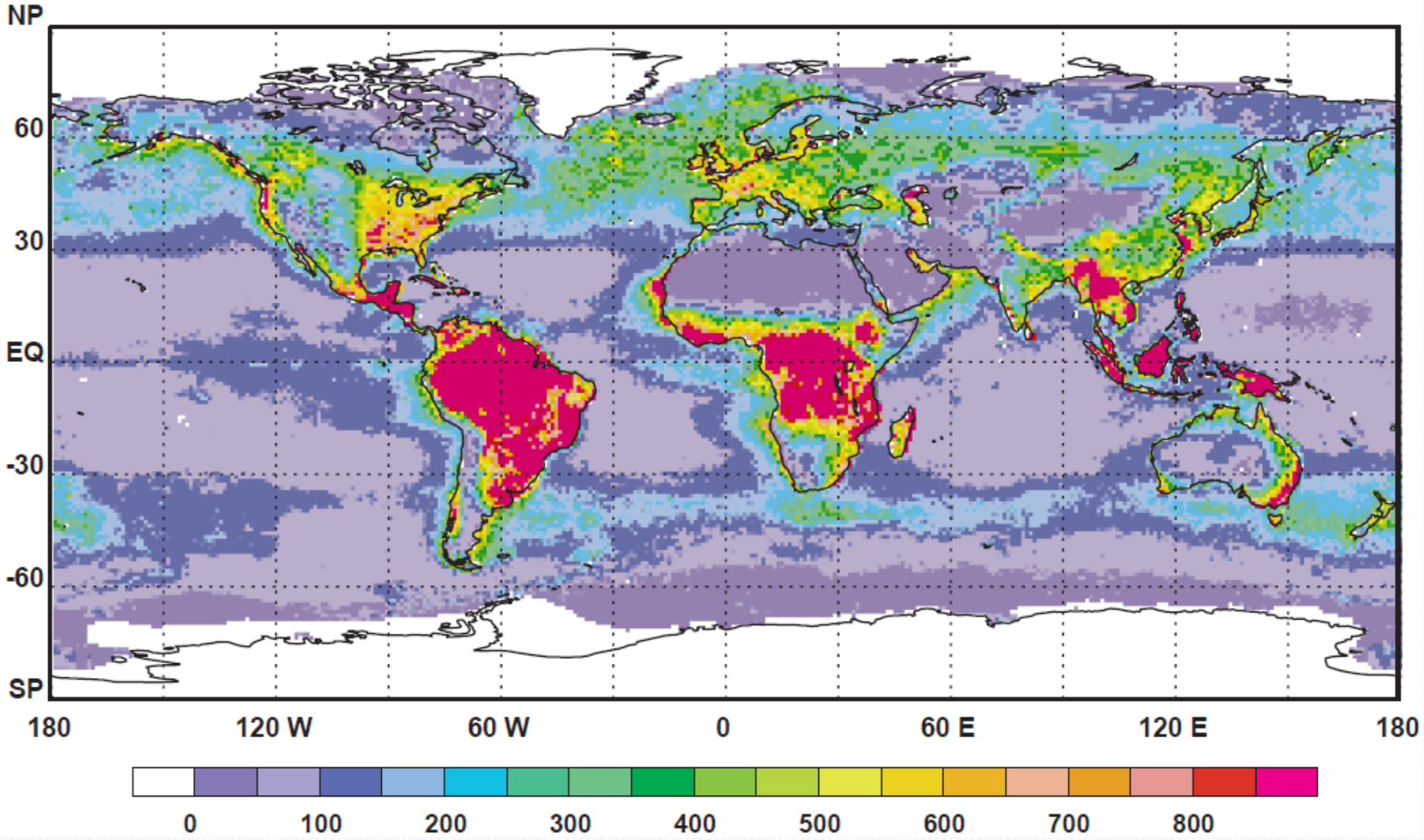
Coccolithophores (East China Sea)





Grazing food chain
Sun light
CO2
Nutrients
Trace metals
Organic pollutants
Phytoplankton
Zooplankton
Fish
Microbial loop
DOC
Bacteria
Flagellates
Ciliates

Global annual NPP for the biosphere



SCIENCE 281, Field et al., 1998

Terrestrial Biomes

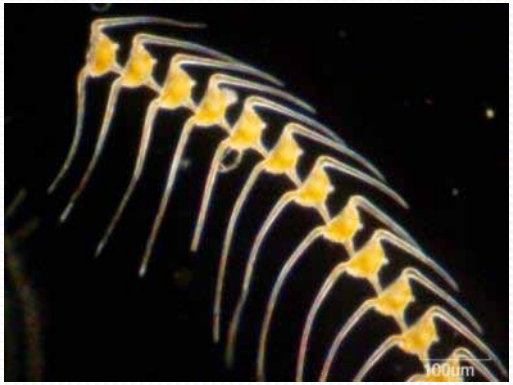
Variation in NPP among Terrestrial Biomes

Biome	NPP (Pg/year)	Terrestrial NPP (%)
Tropical forest	17.8	32
Broad-leaved deciduous forest	1.5	3
Broad-leaved and needle-leaved forest	3.1	5
Needle-leaved evergreen forest	3.1	5
Needle-leaved deciduous forest	1.8	3
Savanna	16.8	30
Grassland	2.4	4
Shrubland	1.0	2
Tundra	0.8	1
Desert	0.5	1
Crops	8.0	14

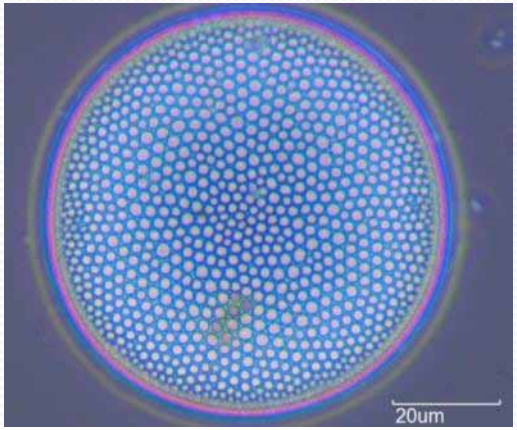
Source: Field et al. 1998.

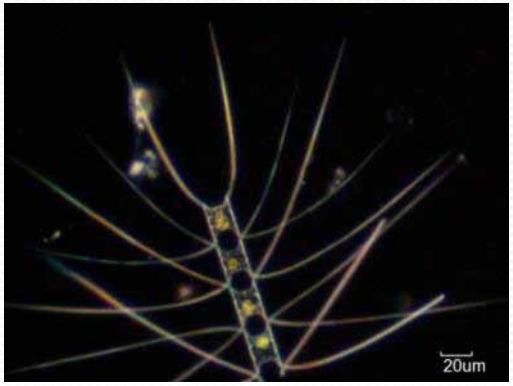
Phytoplankton

Dinoflagellates

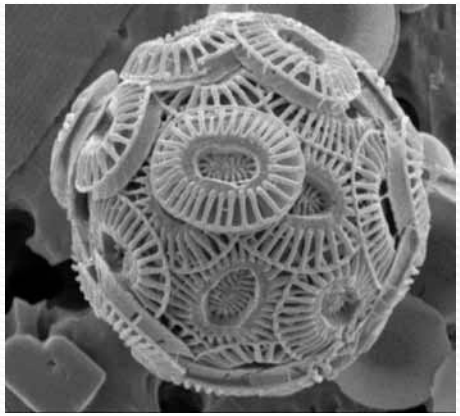


Diatoms



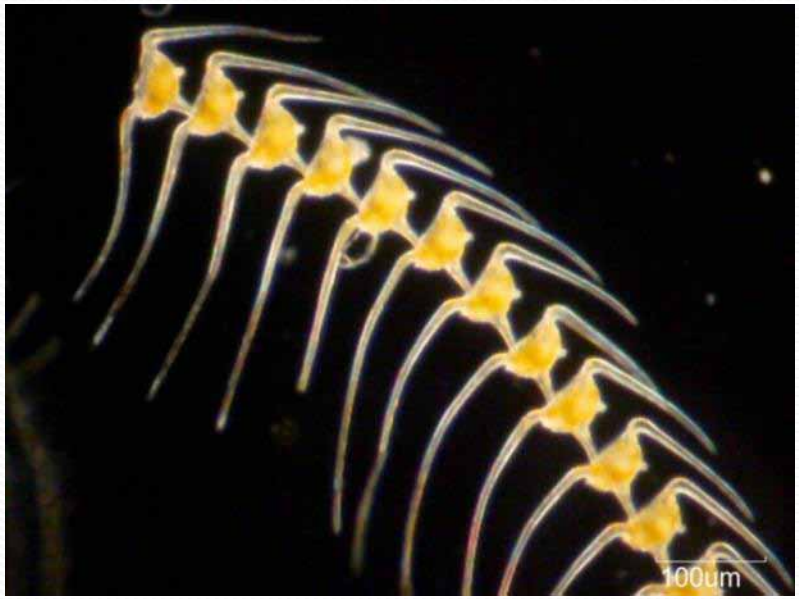


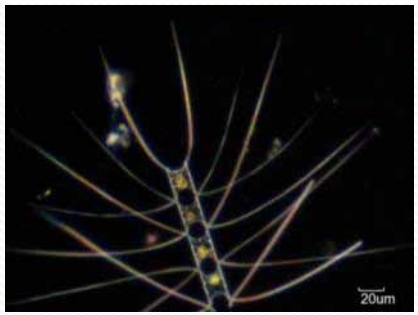
Coccolithophores

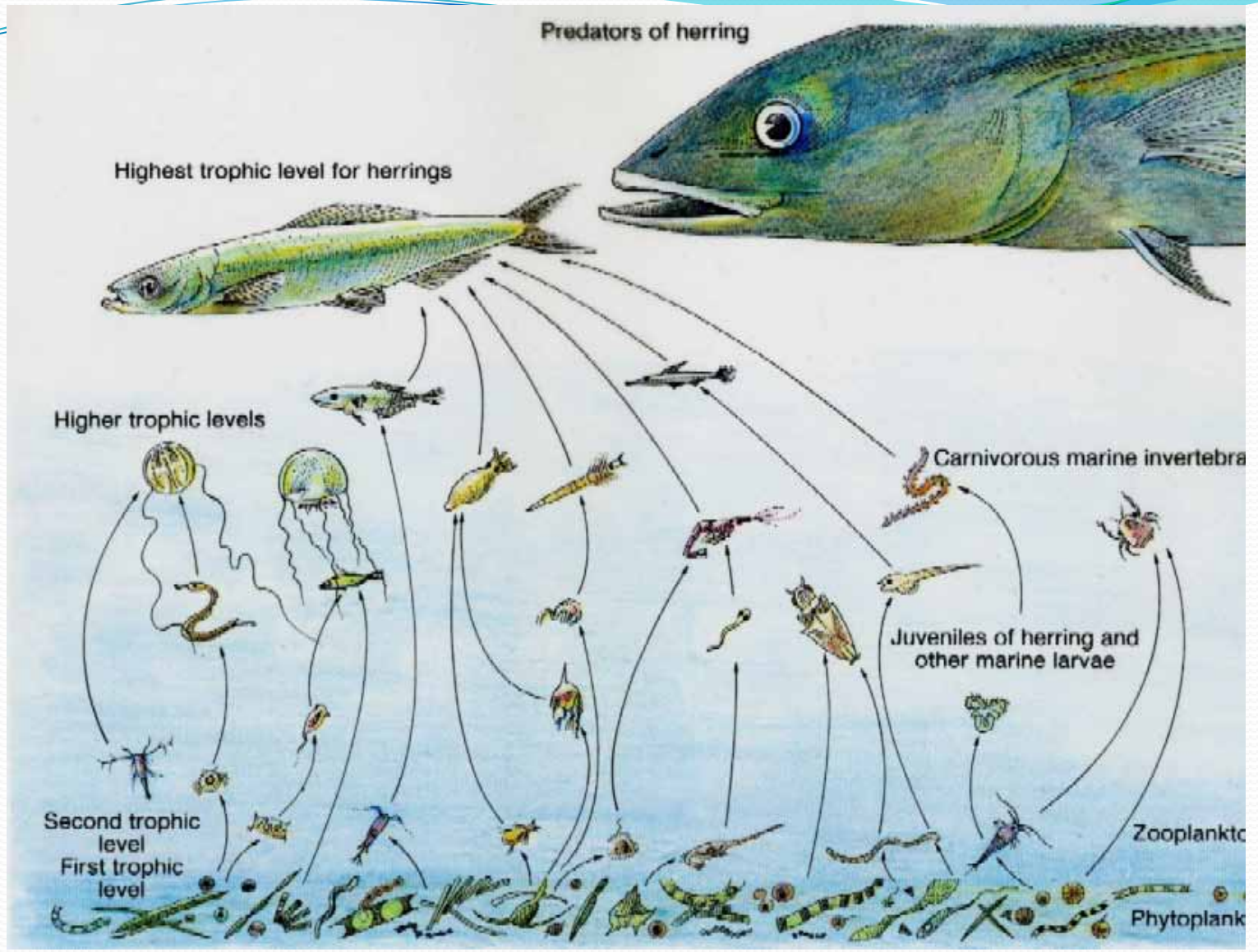


Cyanobacteria

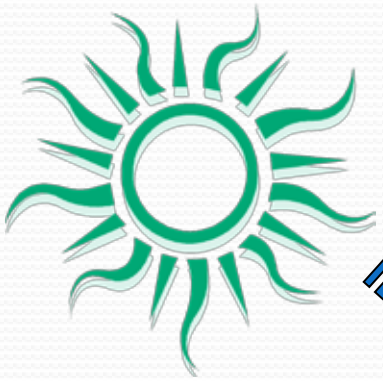
Resolving power of human eye ~0.2mm





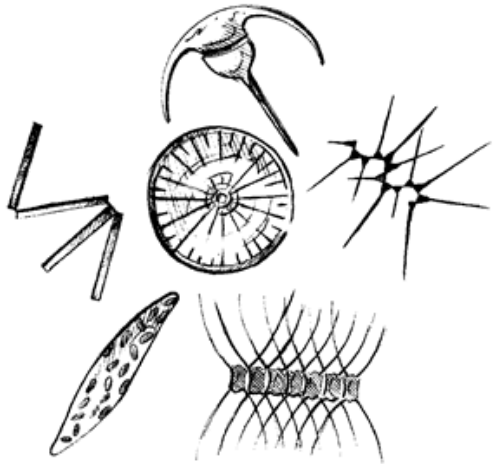

Predators of herring
Highest trophic level for herrings
Higher trophic levels
Carnivorous marine invertebra
Juveniles of herring and other marine larvae
Second trophic level
First trophic level
Zooplankto
Phytoplank

The growth limiting factors



Nutrients :N, P, Fe…

Temperature



Nutrient-enrichment experiments

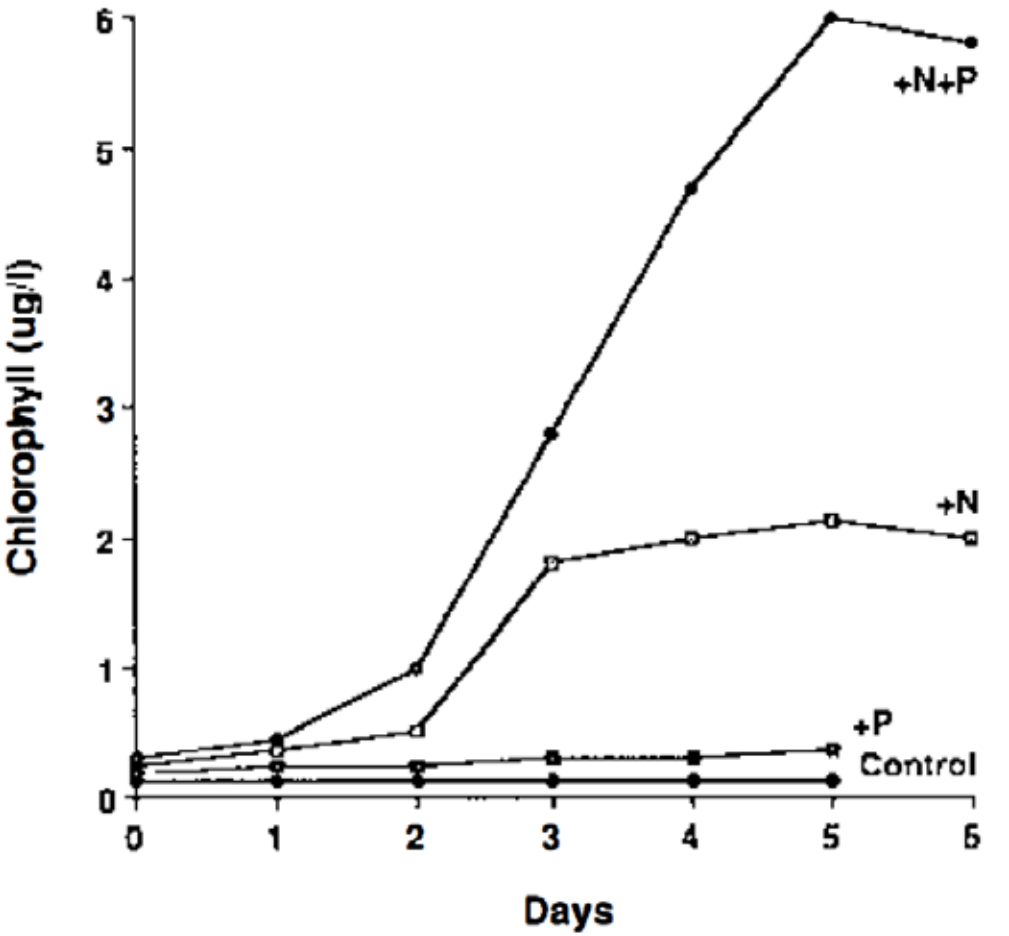
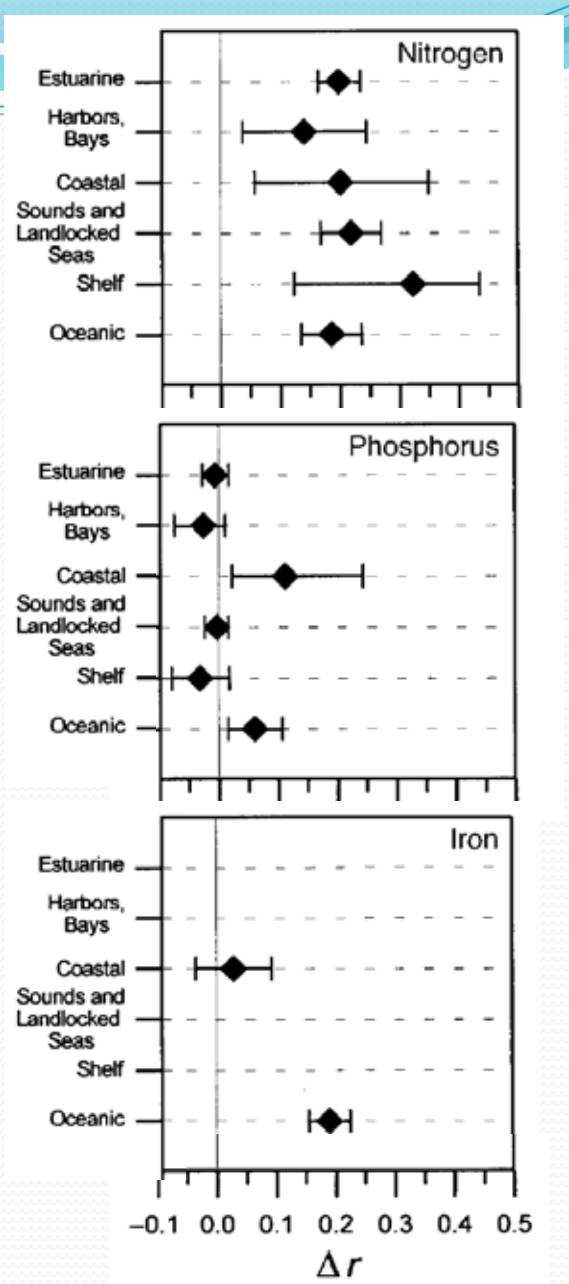
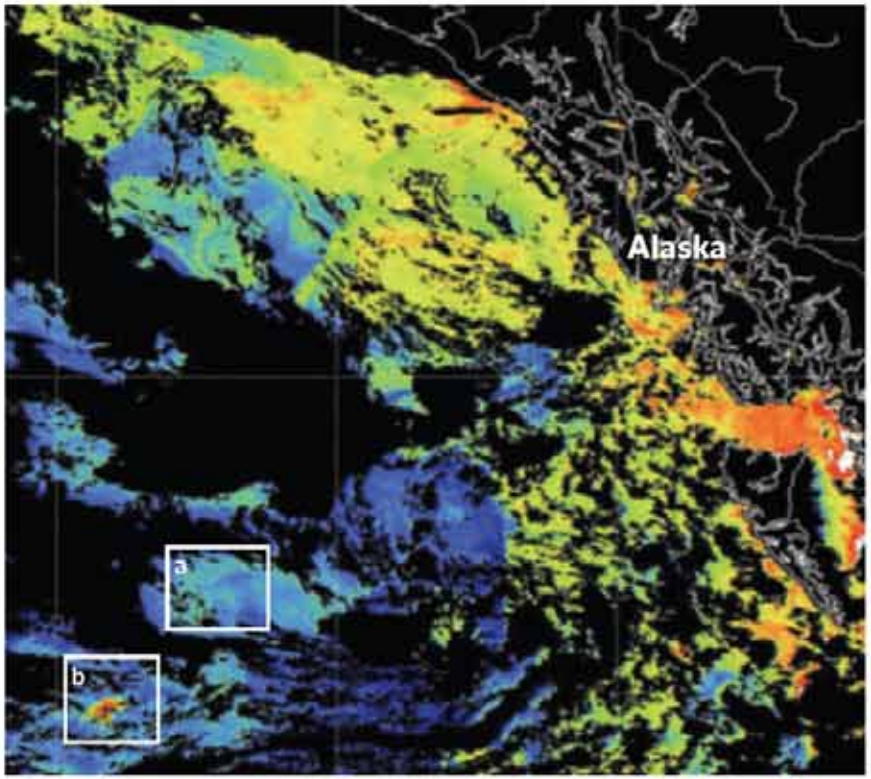


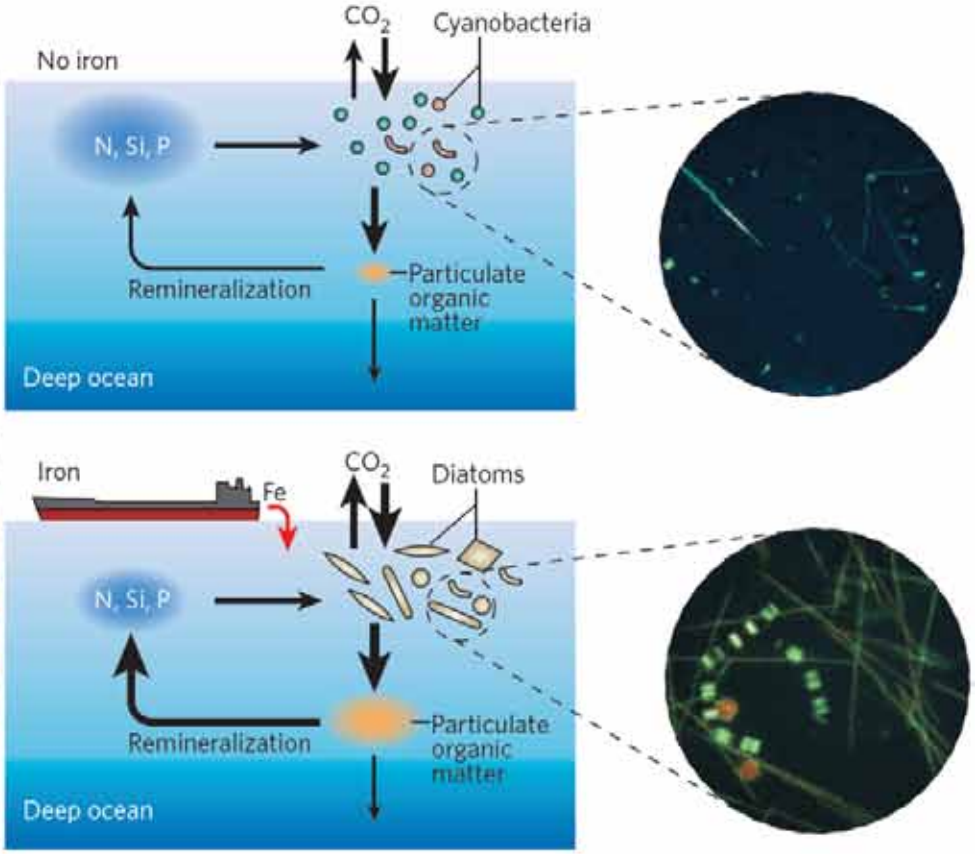
Figure 1 Typical results of a bioassay experiment with seawater from near Woods Hole, MA. Nutrients were added to unfiltered water containing ambient phytoplankton populations. Growth was measured by increase in chlorophyll. Data from Vince & Valiela (1973).



Ecology 80, Downing et al., 1999

The effect of iron fertilization on diatoms



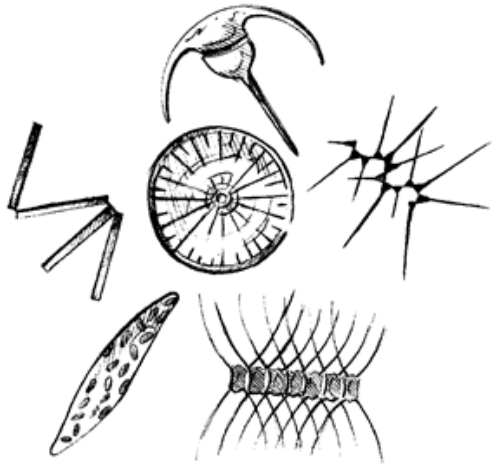


Nature 459, Armbrust, 2009

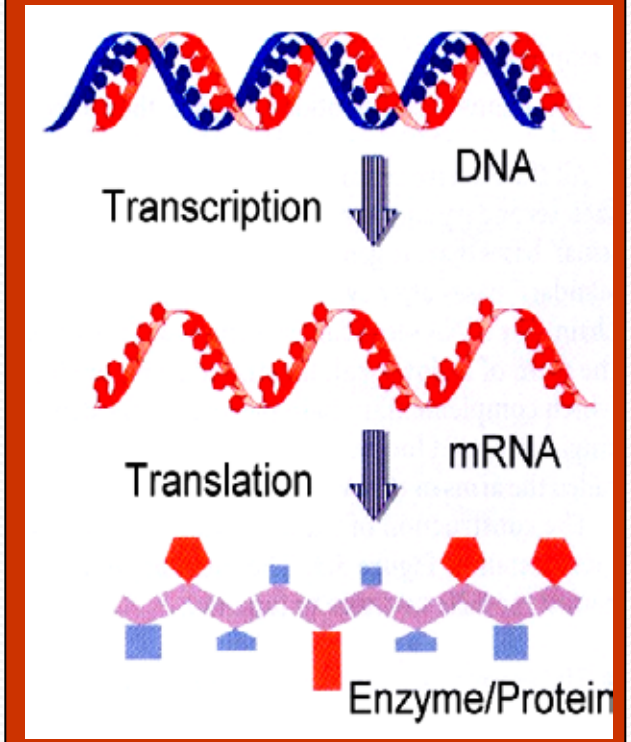
The growth limiting factors

Nutrients :N, P, Fe…

Temperature



Under stress

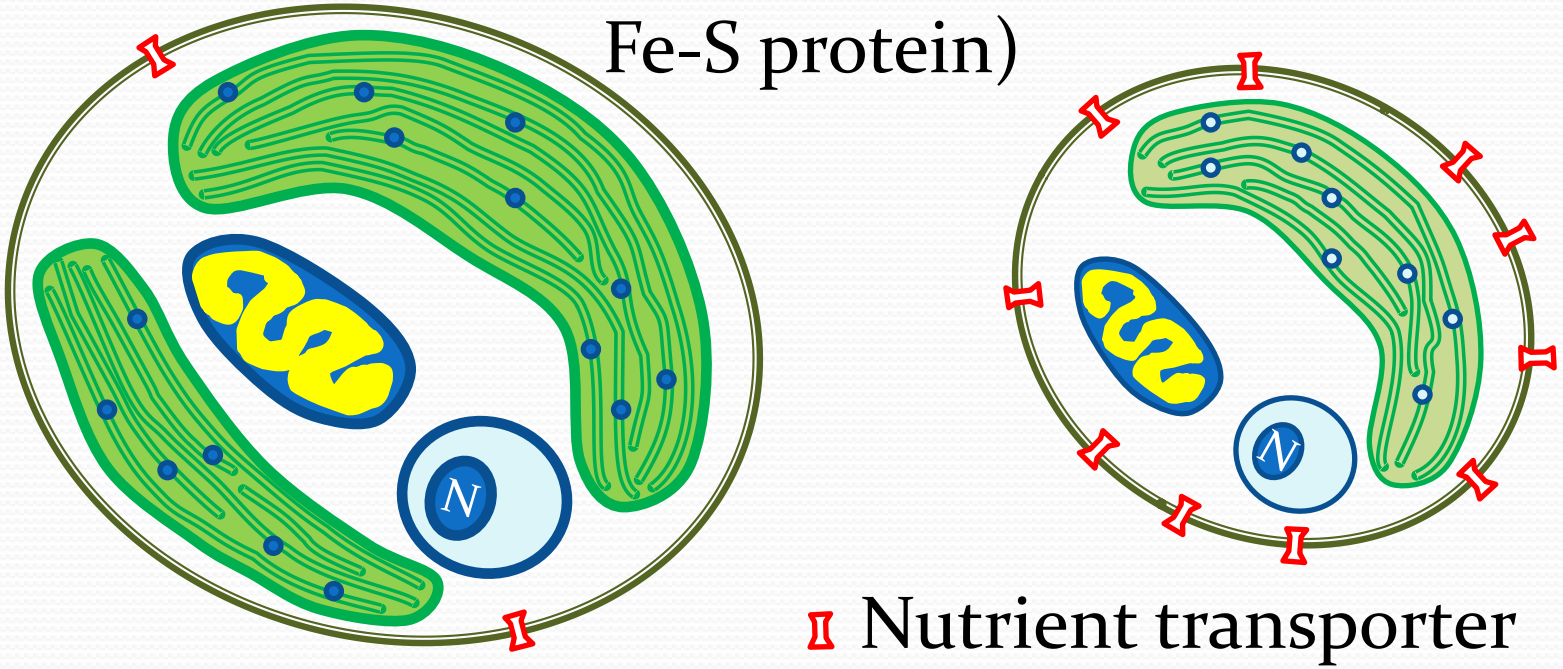


Molecular probe

Phytoplankton exhibit three major responses to stress imposed by nutrient limitation

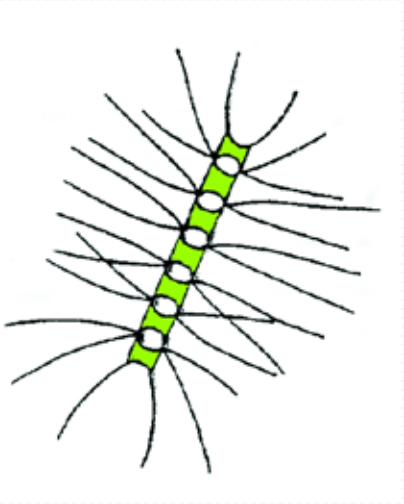
1. Retrenchment or down regulation of physiological rate.
2. Compensation, eg: synthesis of new proteins.
3. Acquisition, eg: development of more efficient uptake systems.

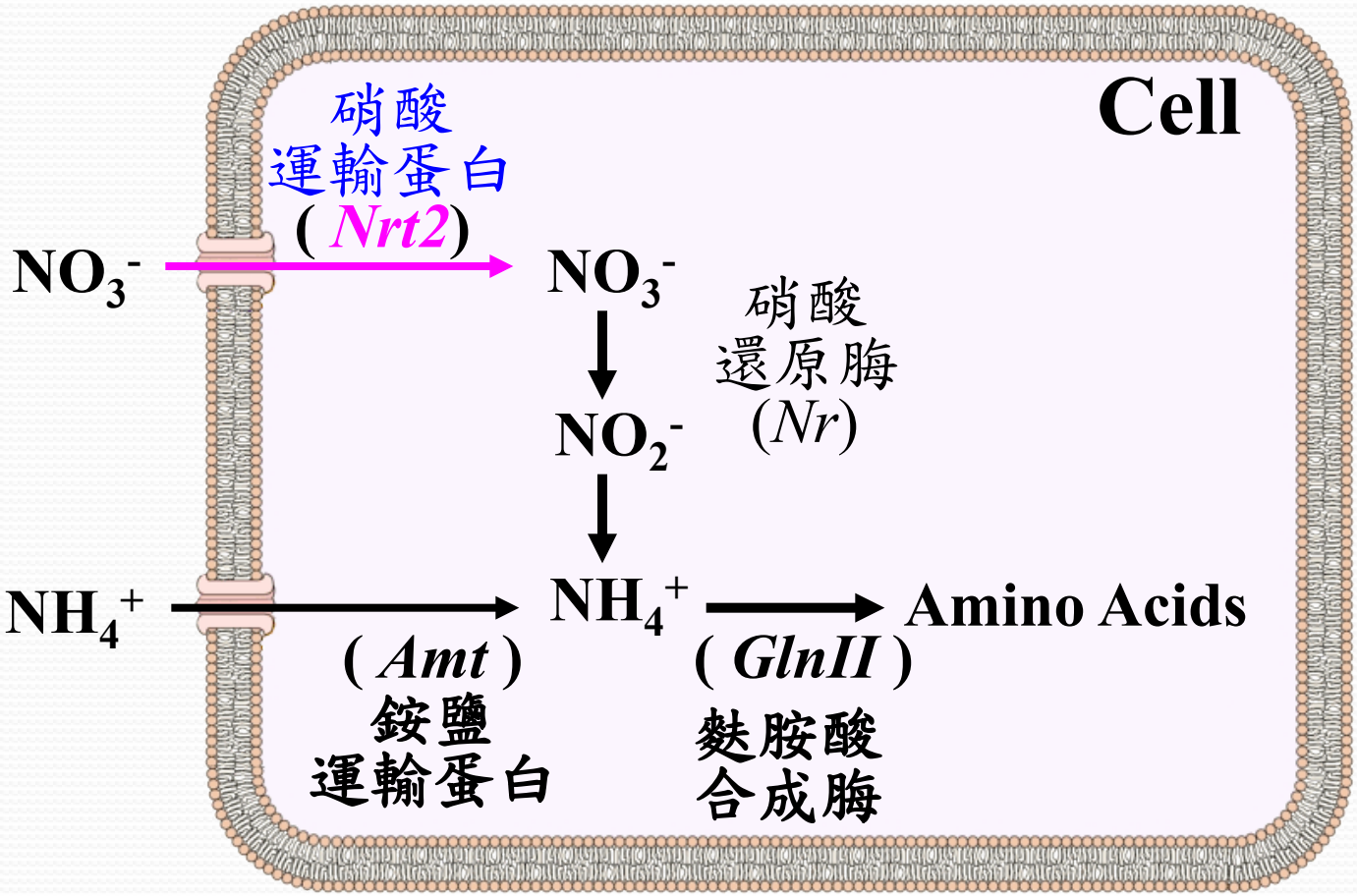
- Ferredoxin Fe-S protein)
- Flavodoxin



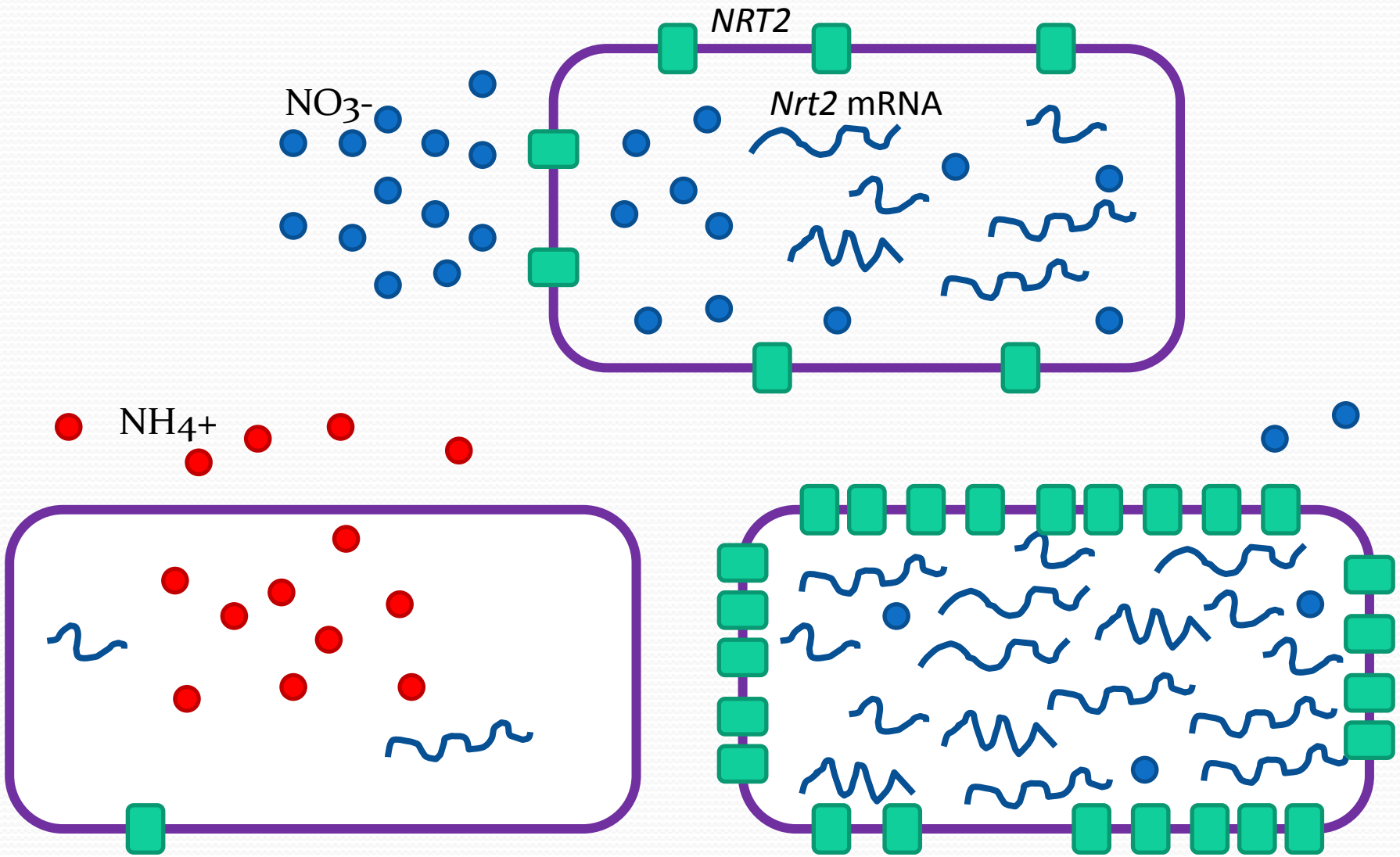
Nutrient transporter

Research goal: Develop a suitable molecular indicator for nitrogen stress

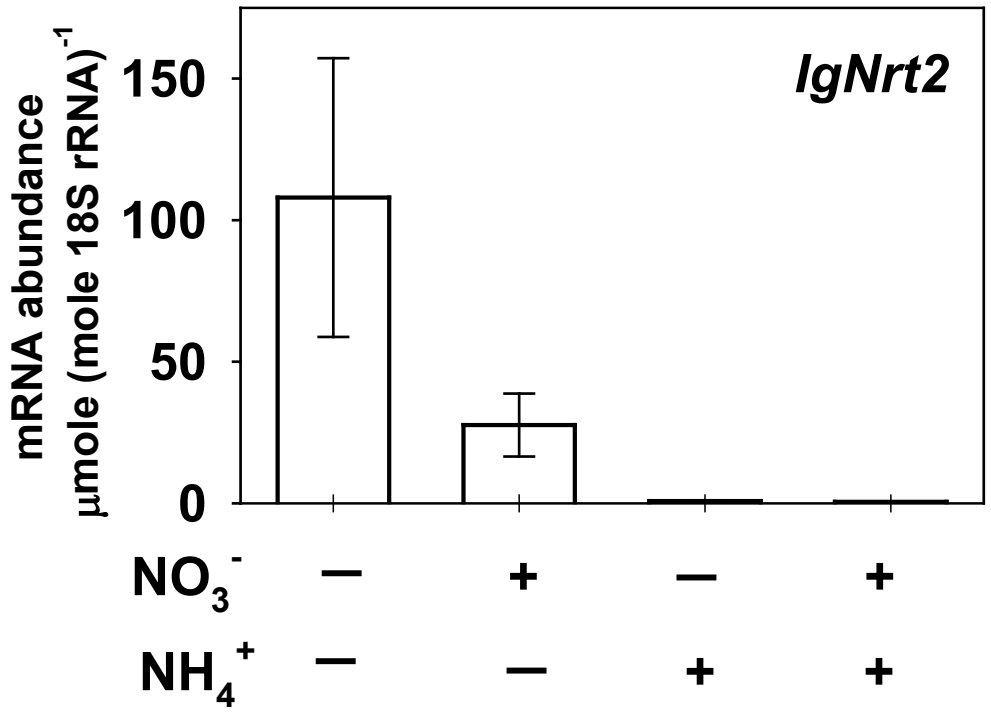




- 硝酸運輸蛋白（nitrate transporter gene；*NRT2*），而且基因的表現量會隨著環境中的氮鹽含量而有所變化



Result : mRNA levels

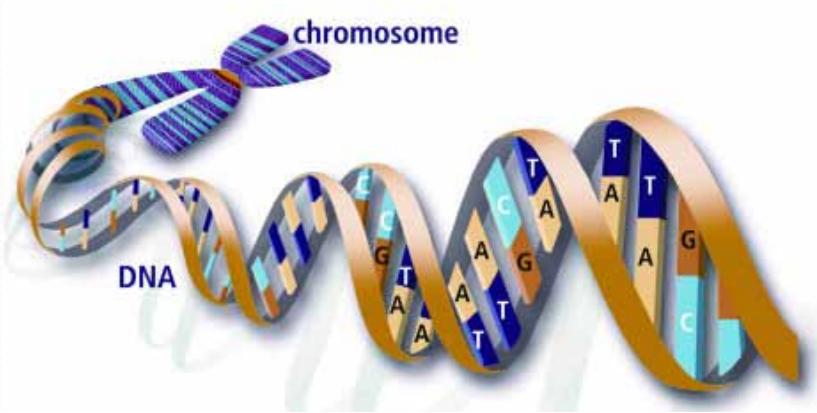


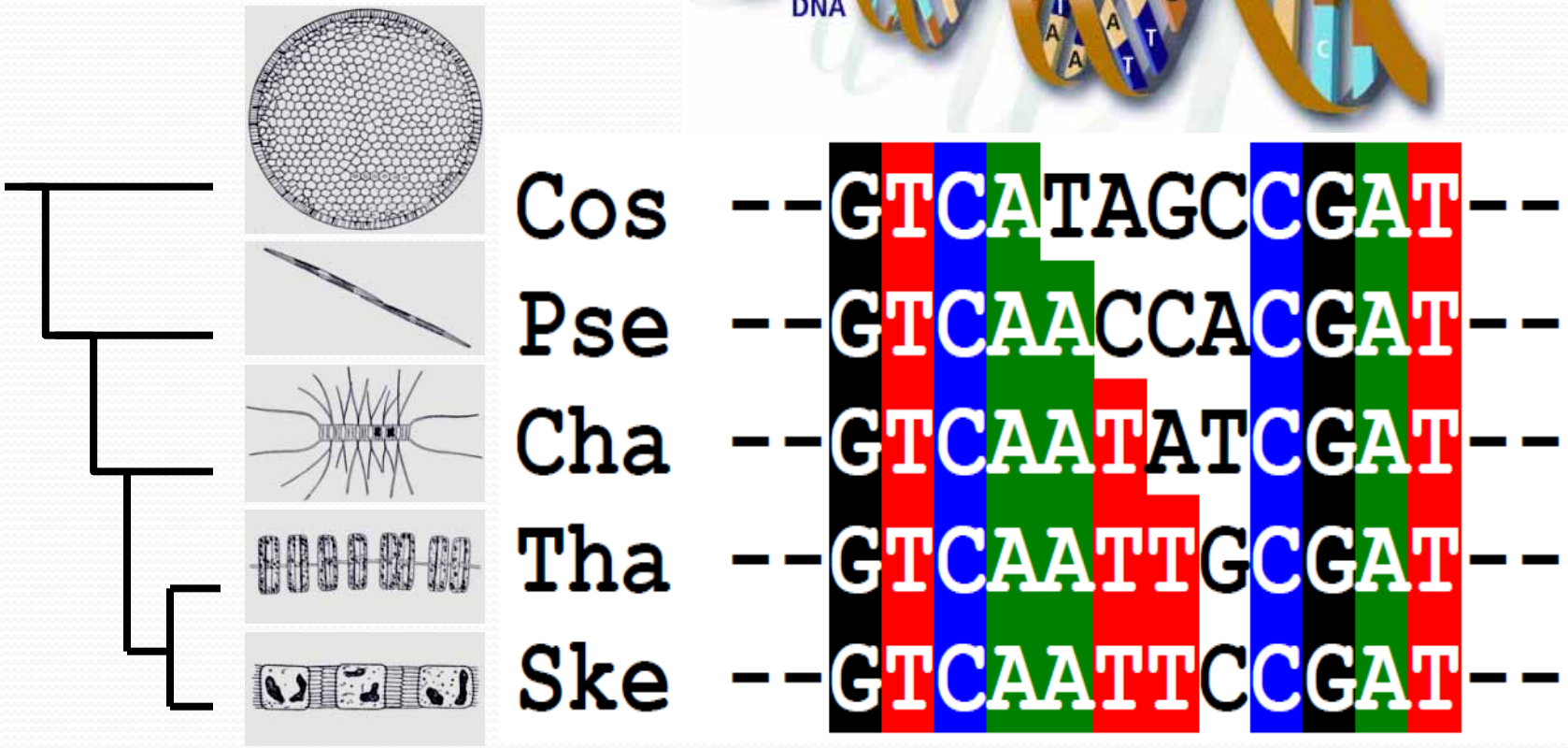
Nrt2 is the first candidate to be a nitrogen marker gene in phytoplankton

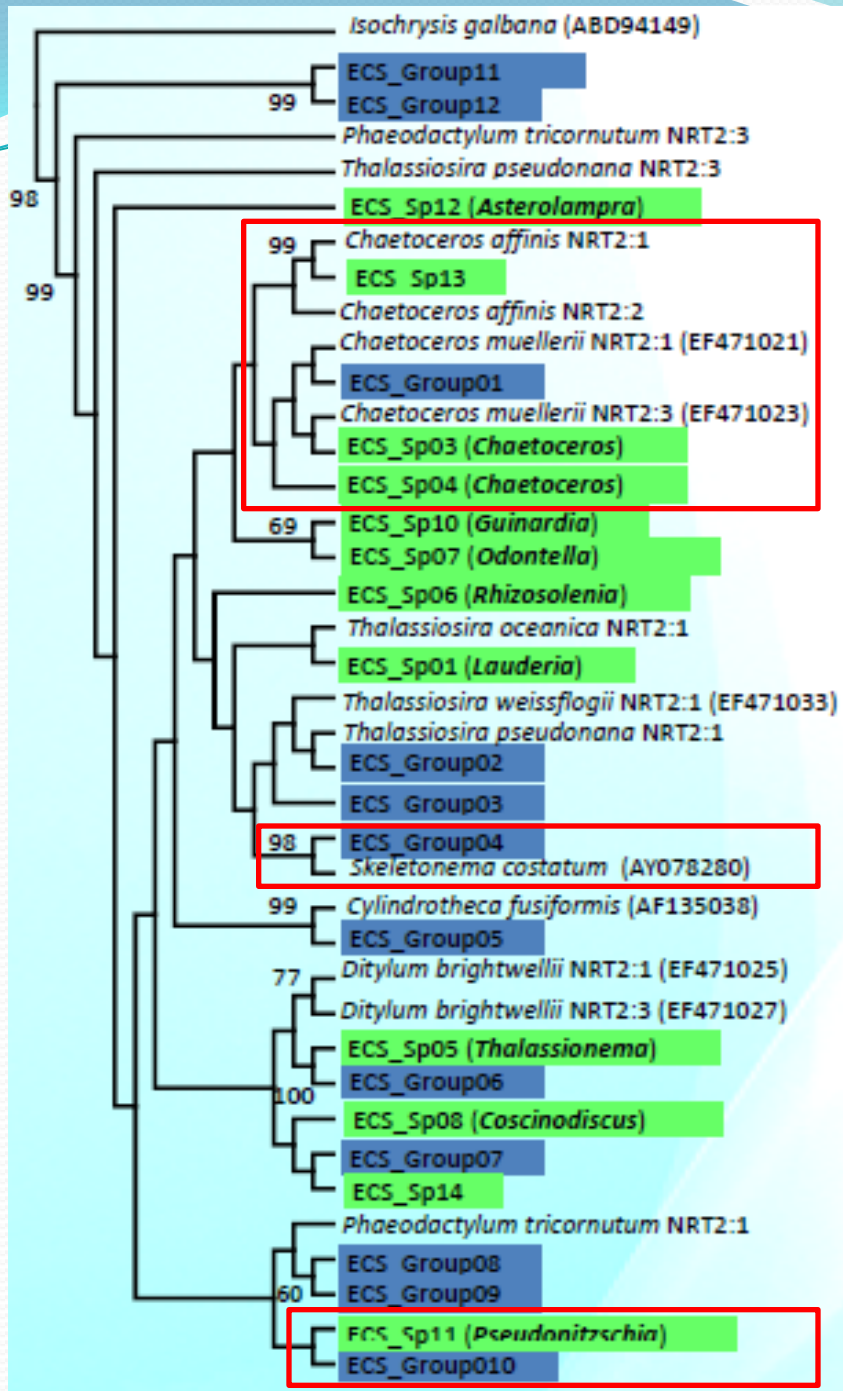
Kang et al., 2007. Phycologia 46: 521

Apply *Nrt2* to detect diatom nitrogen stress in the ocean

The specificity of molecular markers comes from genetic diversity

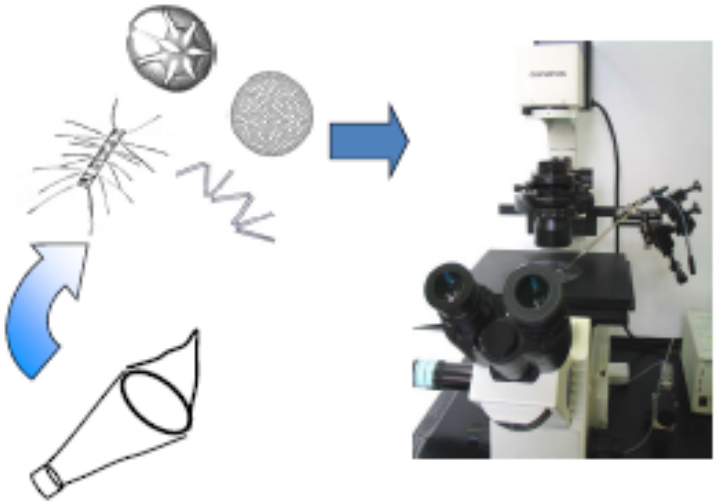






Two methods to obtain *Nrt2* sequences :

1. Single-cell PCR method

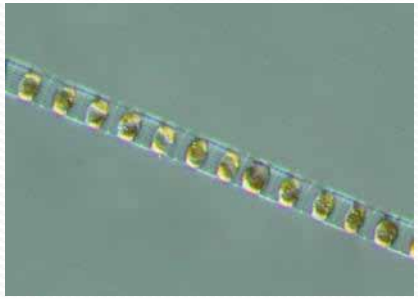


2. Bulk sequencing method

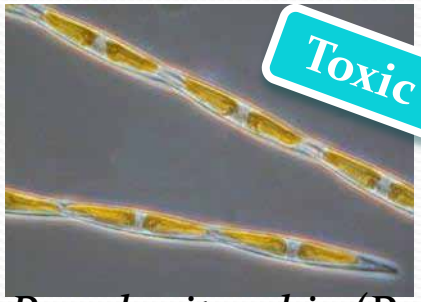
Genomic DNA extracted from field samples

PCR

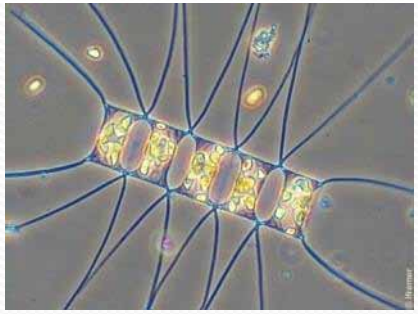
Using Q-RT-PCR to detect diatom *Nrt2* mRNA abundance in the East China Sea



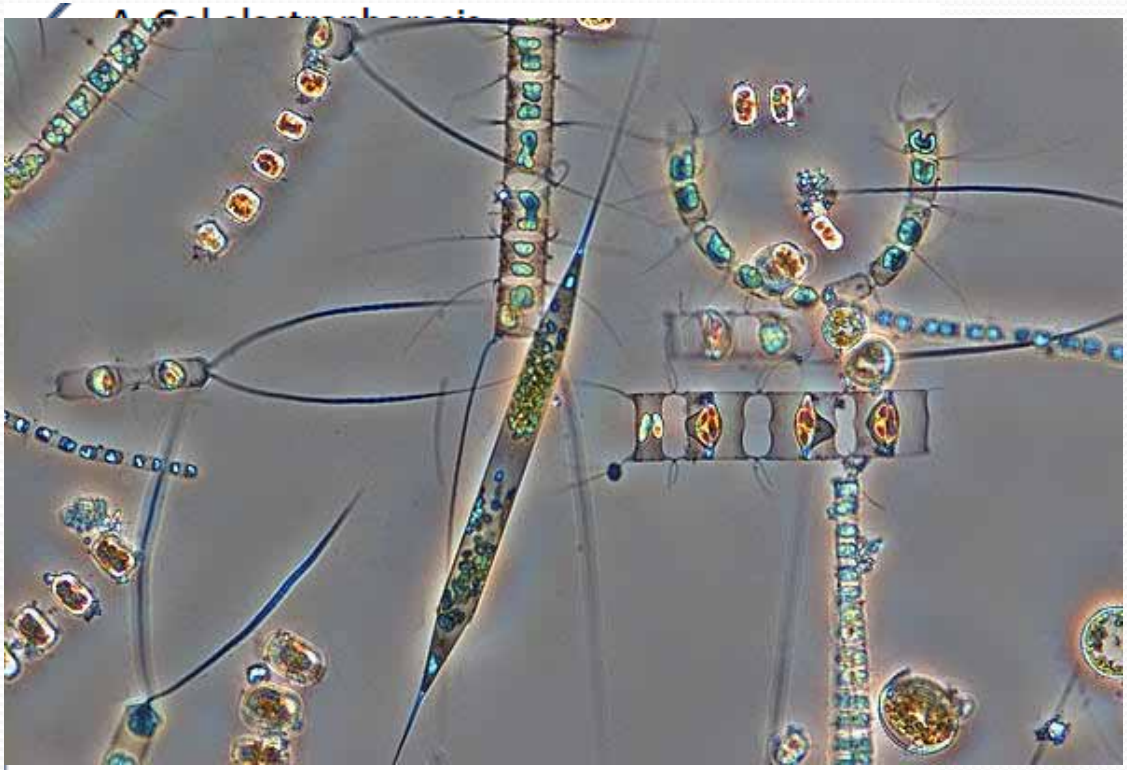
Skeletonema (Ske)

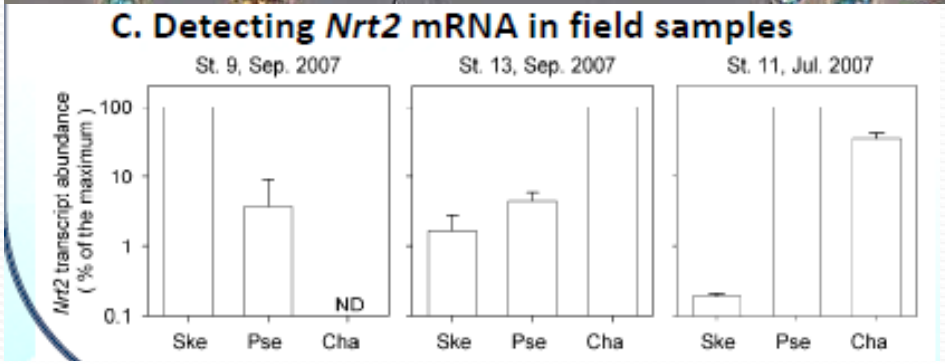


Pseudonitzschia (Pse)



Chaetoceros (Cha)





Kang et al., 2011. AEM 77:122

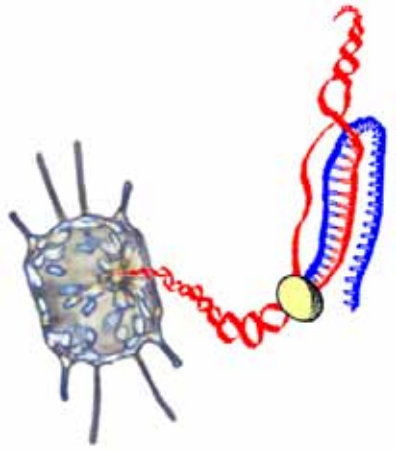
未來的展望是要測量與其他營養鹽吸收利用相關的基因，共同決定浮游植物的生理狀態。

人類的健康檢查

三酸甘油脂 ⇨ 心血管疾病

血糖 ⇨ 糖尿病

矽藻的指標基因

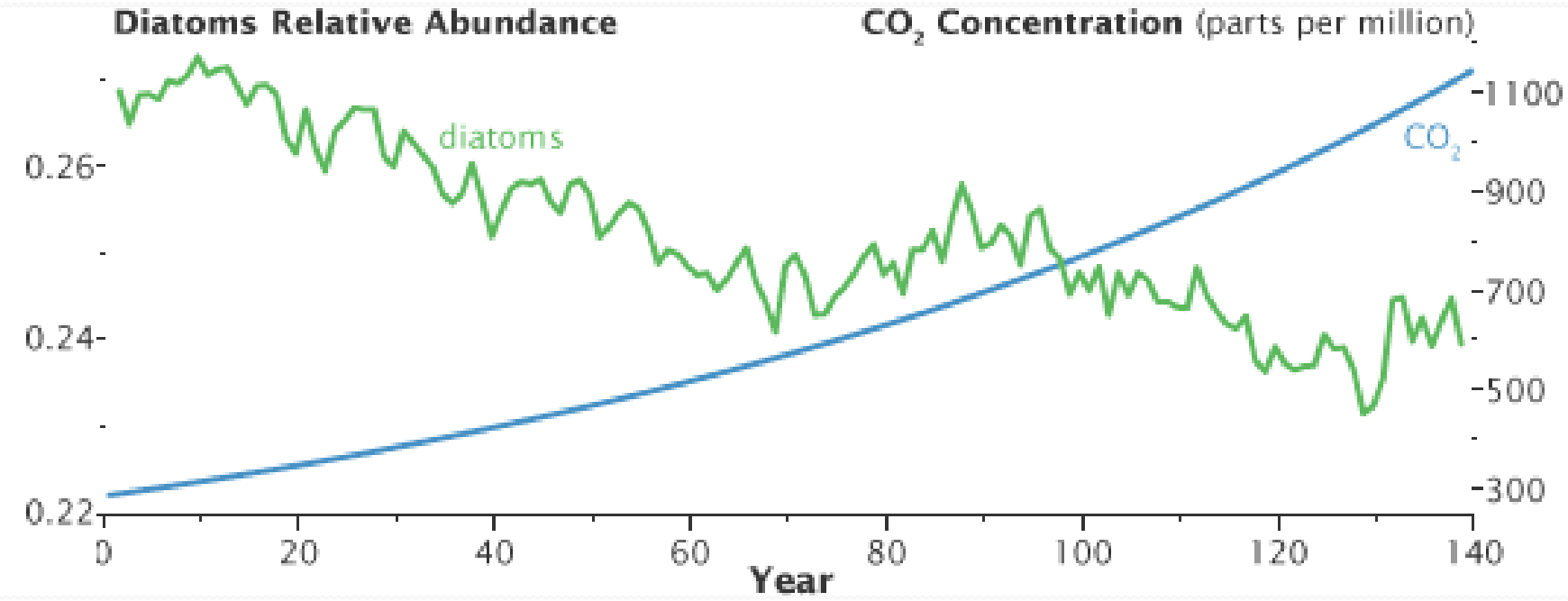


Nrt2 ➡ N stress

PHO ➡ P stress

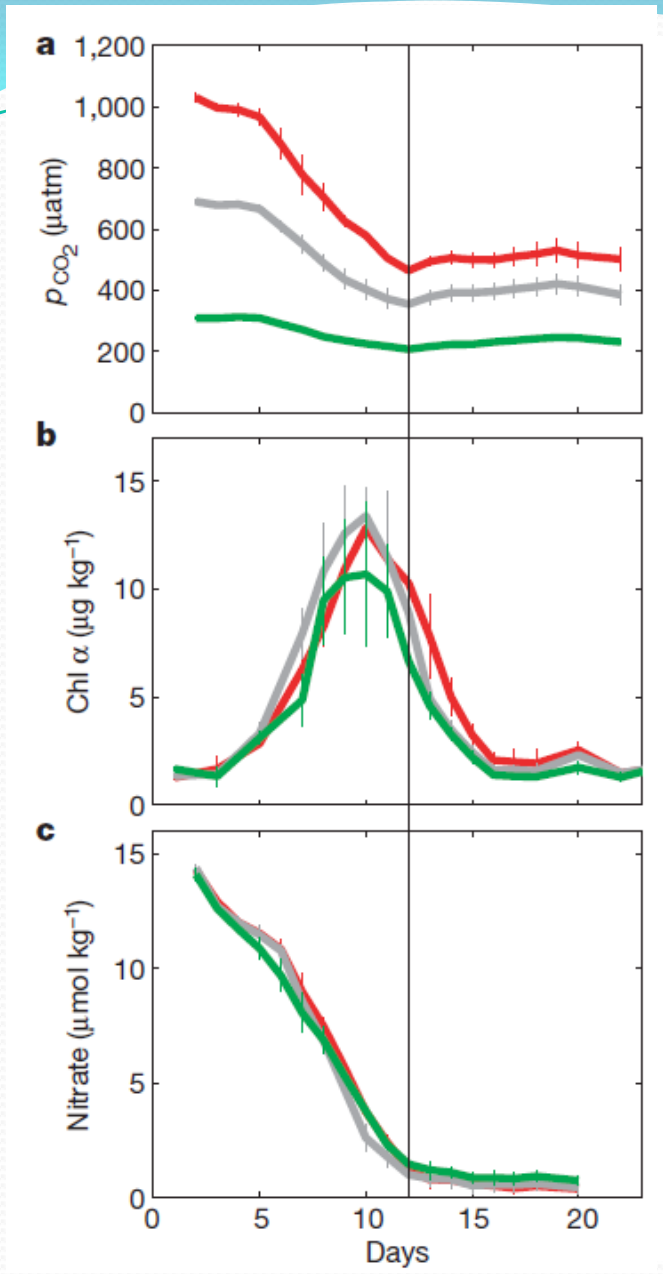
SIT ➡ Si stress

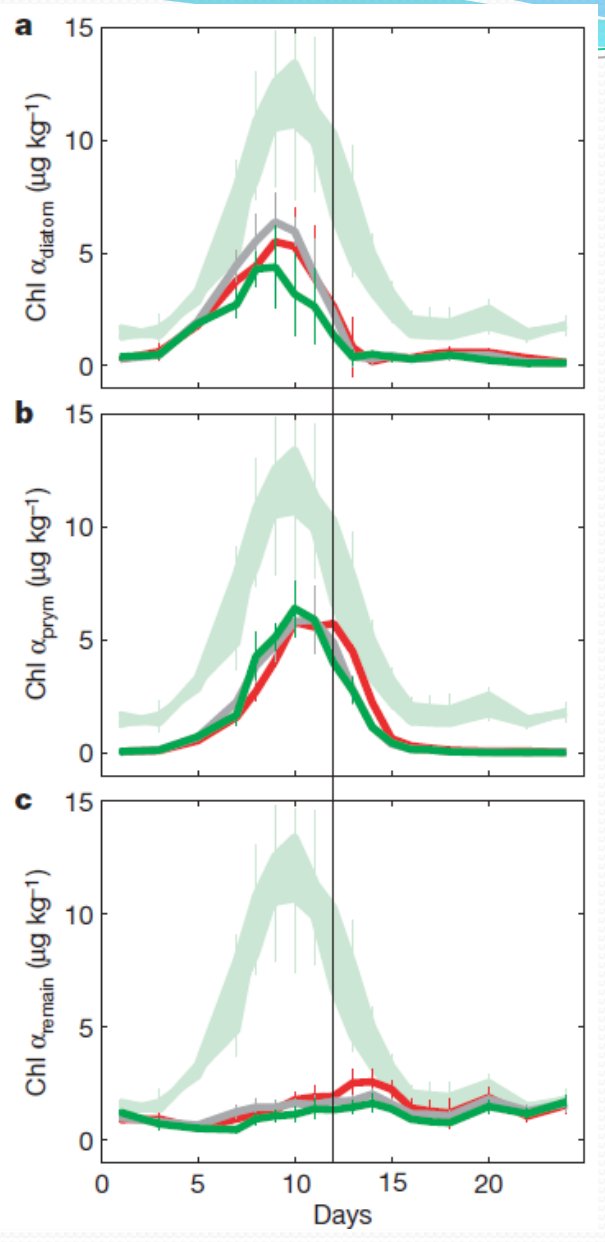
Life will find its way out ! ? !

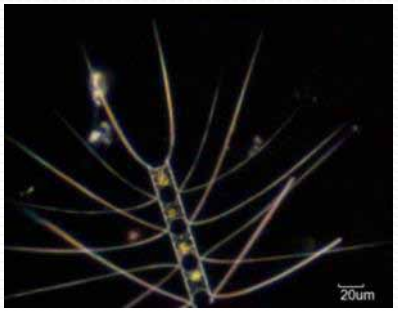


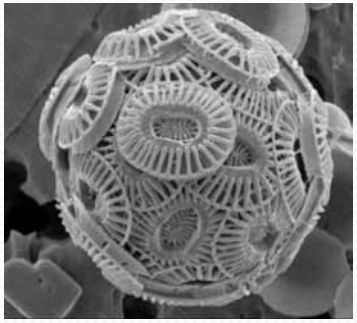
Source: Bopp et al., 2005

Thanks for your attention!









Nature 450, Riebsell et al., 2007