

# Isotopes in Ocean Sciences



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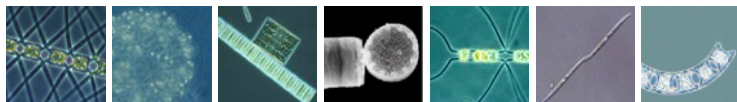
# Overview

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- Brief introduction
- Basic principles of isotope uses in ocean sciences
- Use of isotopes as proxys
- Use of isotopes as tracers

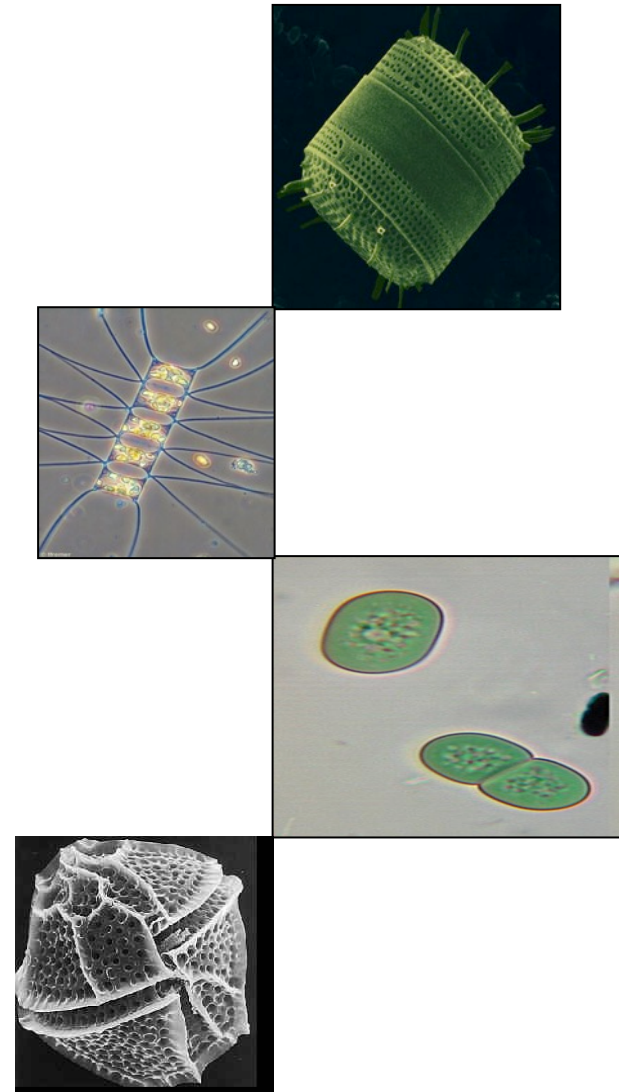
# My research interests:

- The relationship between availability of resources, resource utilization plankton communities, and the ability of these interactions to govern species composition and succession.
  - Cycling of vitamins and trace metals
  - Physiological effects of resource limitation on individual species/groups
- The manner in which climate change processes may alter this relationship.

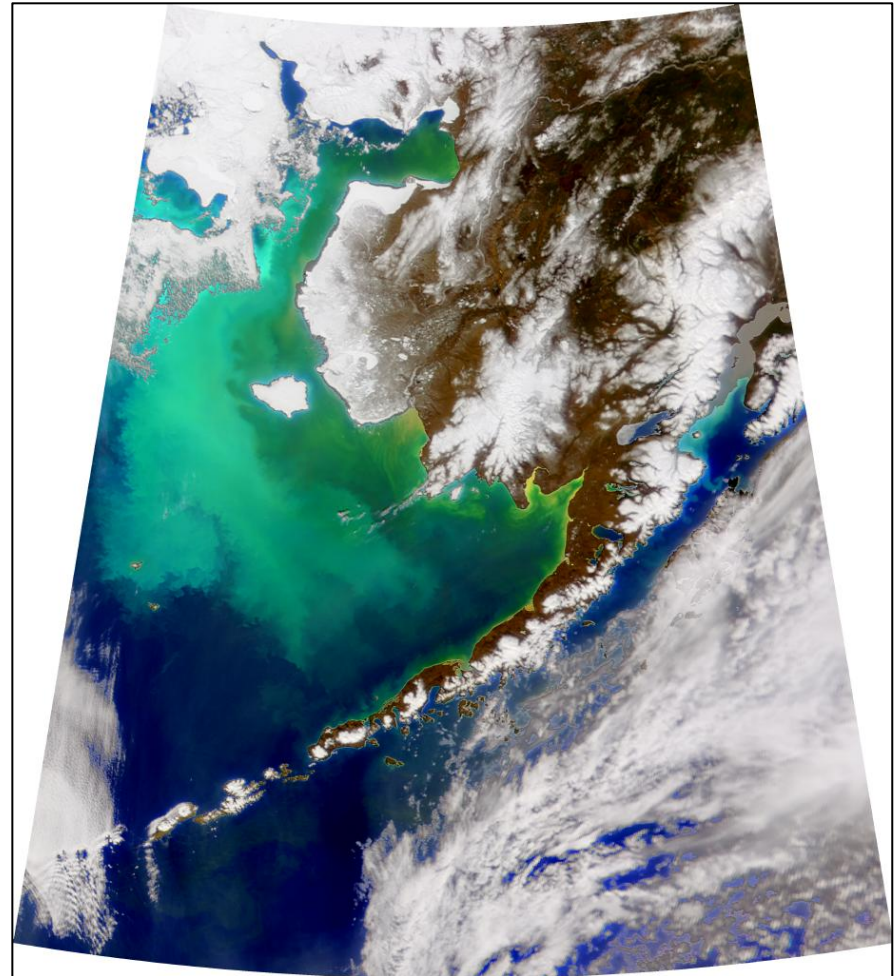
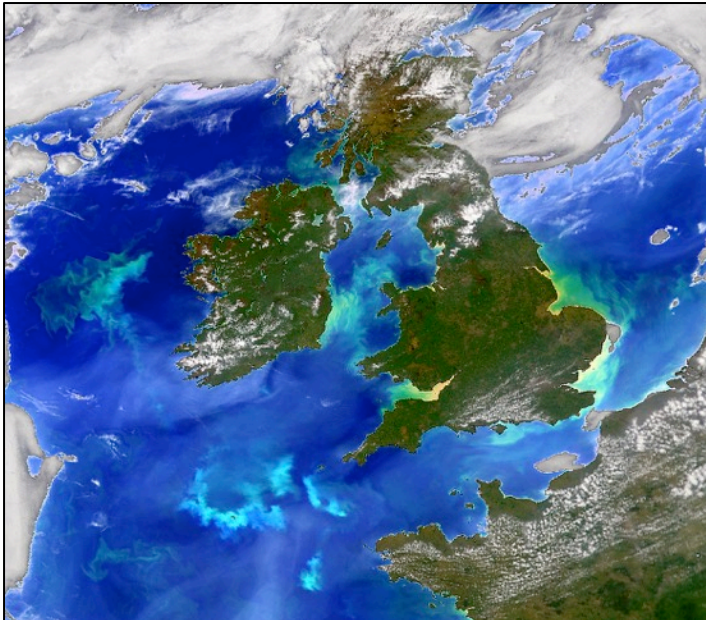
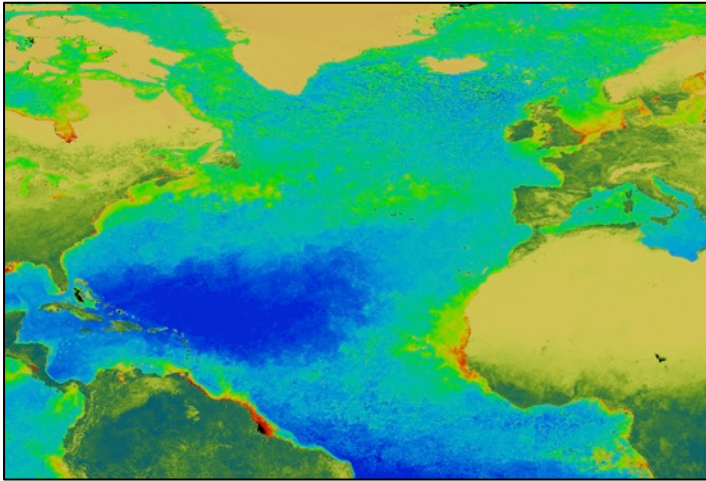


# Phytoplankton: Meadows of the oceans

- Single celled photosynthetic organisms
- Responsible for >50% of the worlds primary production
  - 45 Giga tons of CO<sub>2</sub> to organic carbon
- Primary producers, supporting productive marine foodwebs



# Phytoplankton 'blooms'



# Phytoplankton growth depends on:



- Light and temperature
- Nutrients

- Macronutrients:

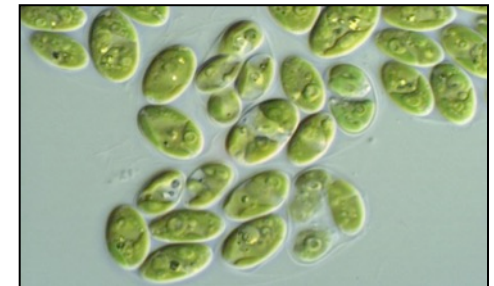
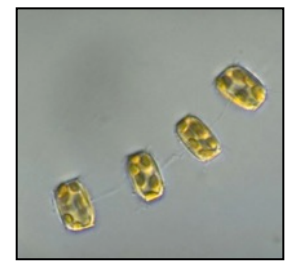
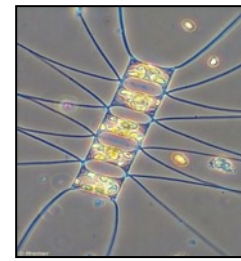
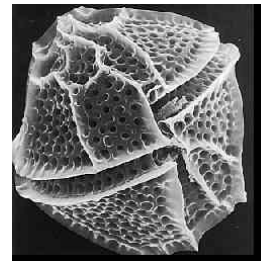
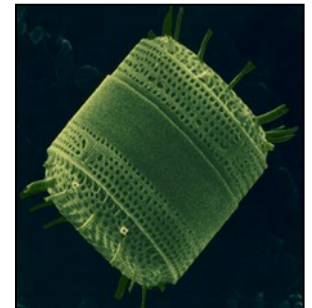
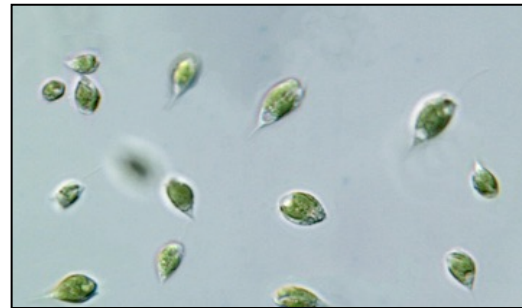
**N, P, Si**

- Micronutrients:

**Fe, Zn, Cu, Mn, Mo, Co**

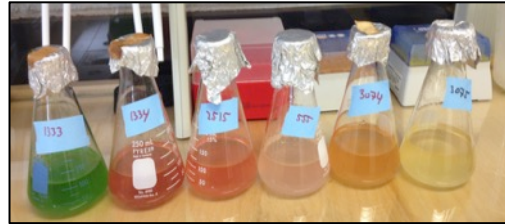
- Vitamins

- B<sub>12</sub> (cobalamin),
- B<sub>1</sub> (thiamin)
- B<sub>7</sub> (biotin)



# A dual approach:

- Lab-based physiological studies



- Field based ecological experiments



# Study sites

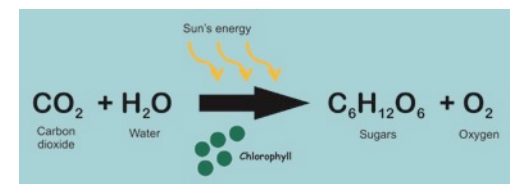
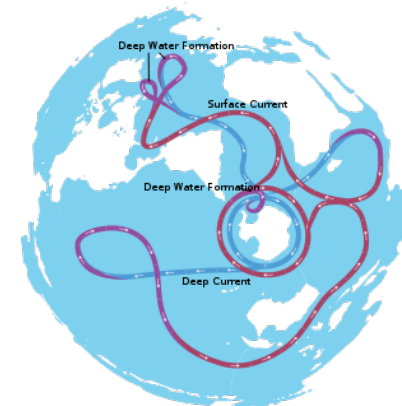
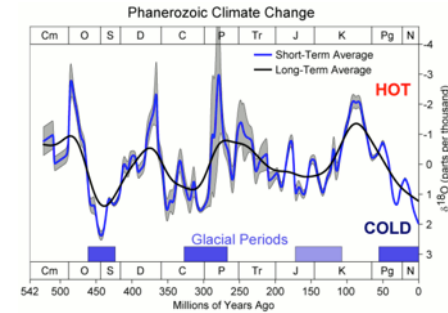




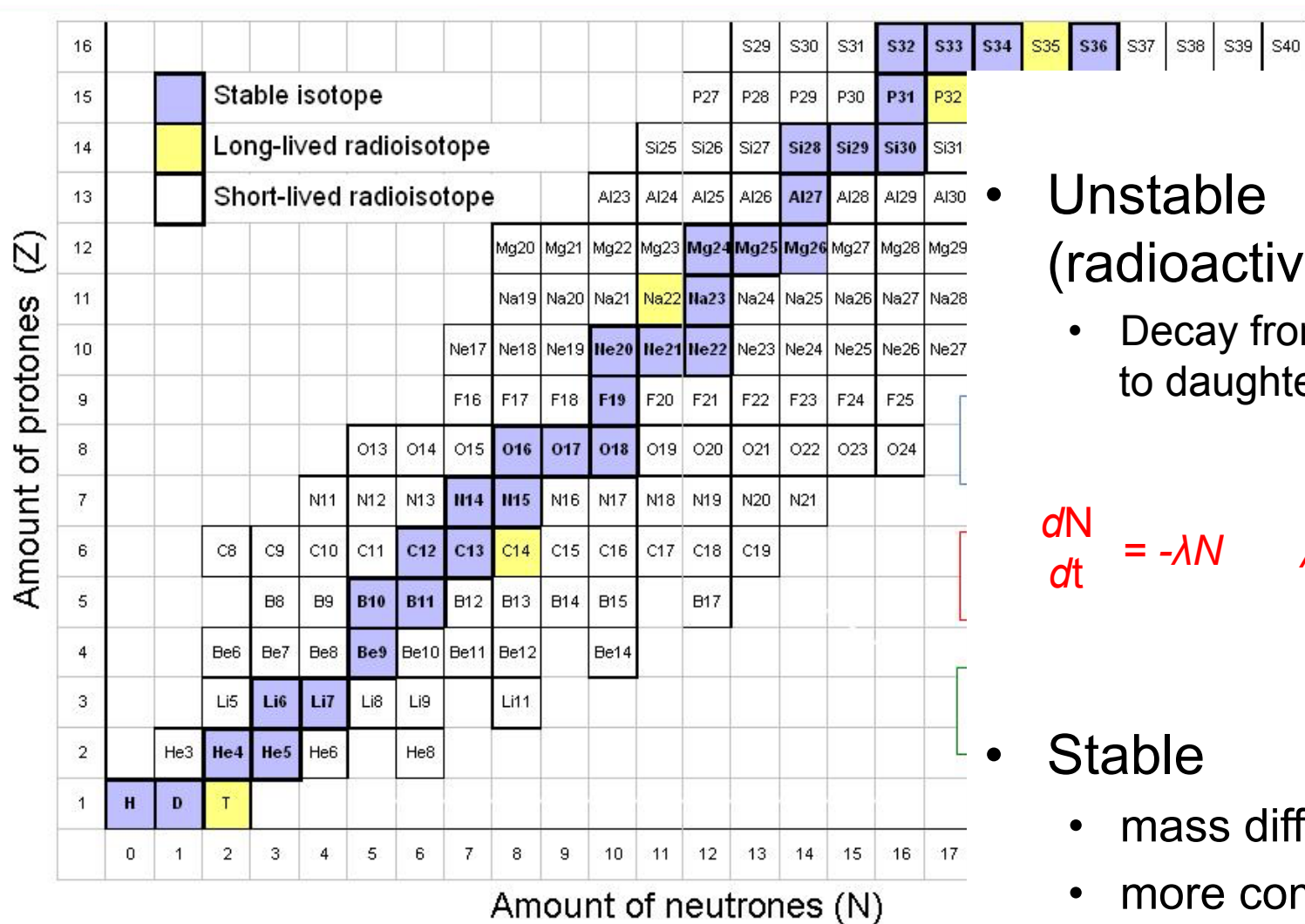
- Brief introduction
- **Basic principles of isotope uses in ocean sciences**
- Use of isotopes as proxys
- Use of isotopes as tracers

# Application of Isotopes in Ocean Sciences

- Used as proxy for past processes
  - Temperatures, pH, thermohaline circulation, sedimentation rates, mixing rates
- Used as tracer of various biogeochemical processes



# Isotopes



- Unstable (radioactive)
  - Decay from parent to daughter isotope.

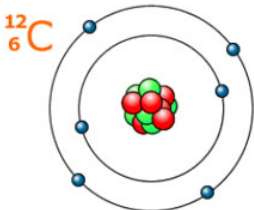
$$\frac{dN}{dt} = -\lambda N \quad \lambda = \text{decay constant}$$

- Stable
  - mass difference
  - more complicated

# Isotopes

- Remember: The **chemical properties** of an element are defined by the number of **protons** in their nucleus.
- Isotopes of an element have different number of neutrons, and therefore different masses.
  - They are **chemically equivalent** but will behave slightly different in chemical reactions due to mass differences (variations on molecular kinetic energy and vibrational energy contained in bonds)

Mass Number and Isotope



$^{12}_6\text{C}$

Proton Neutron Electron

Carbon atoms

Mass Number →  $^{12}\text{C}$ ,  $^{13}\text{C}$

Atomic Number →  $^6_6\text{C}$ ,  $^6_6\text{C}$

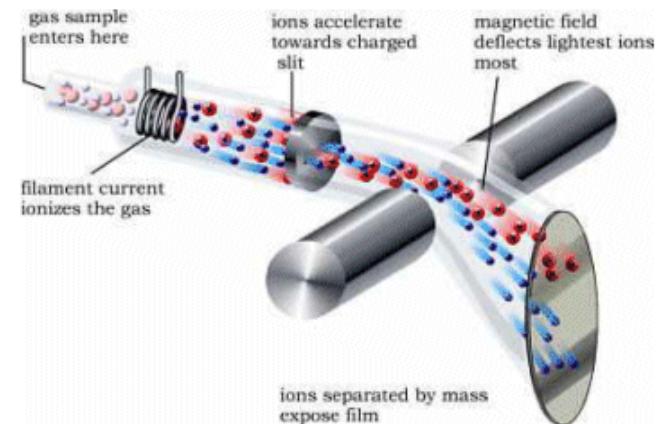
Atomic Number = The number of protons  
= (The number of electrons)

Mass Number = The number of protons + The number of neutrons

Carbon has two isotopes:  $^{12}\text{C}$  and  $^{13}\text{C}$ . Both show the same chemical properties because they have the same atomic number 6. But the mass of  $^{13}\text{C}$  is larger than that of  $^{12}\text{C}$ , because  $^{13}\text{C}$  has one more neutron.

# Isotope fractionation

- It is the slight separation of one isotope from another.
- Results in processes where different isotopes of an element behave differently.
- Causes measurable abundance variation amongst the lighter isotopes in natural materials of **hundredths of a percent to a few percent**.
- Differences can be measured with a mass spectrometer



# Fractionation

- Arises from **mass dependent** energy differences between isotopes
  - The **lighter** the isotope the more **dramatic** the isotope fractionation during a given reaction.

Consider the isotopes of H, C and O:

<u>element</u>	<u>isotope (common name)</u>	<u>mass (amu)</u>
H	${}^1_1\text{H}$ (hydrogen)	~1
	${}^2_1\text{H}$ (deuterium = "D")	~2
	${}^3_1\text{H}$ (tritium = "T")	~3
C	${}^{12}_6\text{C}$	12
	${}^{13}_6\text{C}$	13
	${}^{14}_6\text{C}$ (radiocarbon)	14
O	${}^{16}_8\text{O}$	16
	${}^{17}_8\text{O}$	17
	${}^{18}_8\text{O}$	18

# Fractionation:

- A deuterium atom ( ${}^2_1\text{H}$ ) is twice as massive as a hydrogen atom ( ${}^1_1\text{H}$ )
- ${}^{13}_6\text{C}$  is 1/12 or  $\sim 8\%$  heavier than  ${}^{12}_6\text{C}$
- ${}^{18}_8\text{O}$  is 2/16 or  $\sim 12\%$  heavier than  ${}^{16}_8\text{O}$

THEREFORE WE EXPECT:

H and D fractionation  $\gg$  O-16 and O-18 fractionation  $>$  C-12 and C-13 fractionation

- However this is not true in all cases!
- In addition many elements used have more than two isotopes (N, S, Li, B, Mg, Ca, Fe)

# Fractionation Effects:

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- Due to:
  - **A.** Mass differences in compounds containing different isotopes of the same element.
  - **B.** Those due to mass-dependent bond energy differences.
  - **C.** Those kinetic effects arising from either of the above.



# Fractionation Effects: Mass differences



- There are 5 different molecular masses possible for water molecules:

molecule:	<i>lightest</i> H- <sup>16</sup> O-H	H- <sup>16</sup> O-D	D- <sup>16</sup> O-D
mass:	18	19	20
molecule:	H- <sup>18</sup> O-H	H- <sup>18</sup> O-D	D- <sup>18</sup> O-D
mass:	20	21	22
			<i>heaviest</i>

- The heaviest species is 4/18 or ~22% heavier than the lightest.

## IMPORTANT TO NOTE:

D=~0.015% of H and <sup>18</sup>O ~0.2% of O on earth so water with mass 18 is by far the most abundant!

# Fractionation Effects: Mass differences



- Mass affects the way a gas moves

$$V = \left( \frac{8kT}{\pi m} \right)^{1/2}$$
A red arrow originates from the 'm' in the denominator of the equation and points to the left, indicating that velocity is inversely proportional to the square root of mass.

T=temp, m=mass,  
k=Boltzman constant

V is inversely proportional to the square root of the mass

- Water molecules of **different mass** due to different isotopic composition have **different mean velocities** as a function of temperature. Light molecules move faster!

How large is this effect? (for water molecules mass 18 and 22)

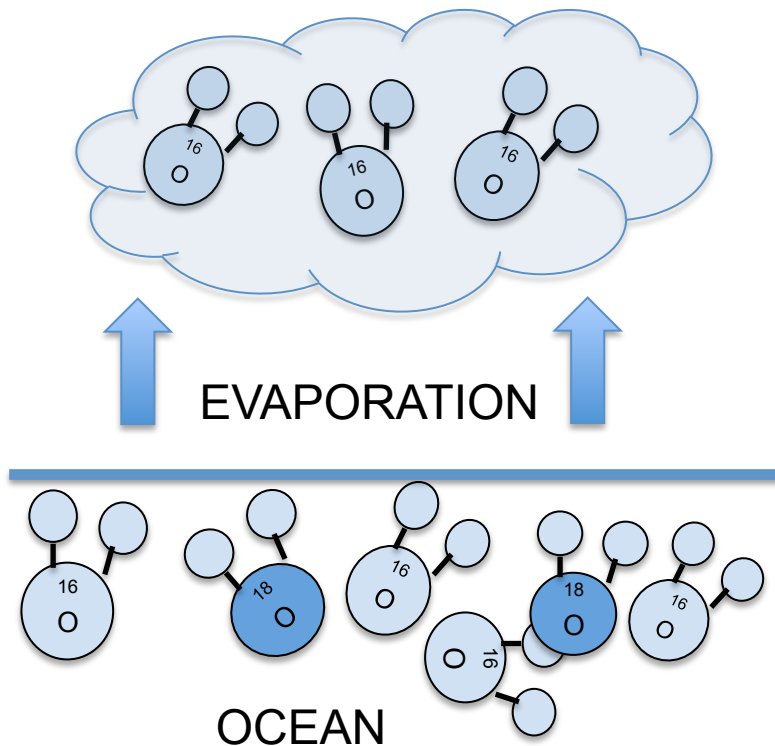
$$\text{For } V_1/V_2 = (m_2/m_1)^{1/2} = (22/18)^{1/2} = 1.106 \text{ or } \sim 11\% \text{ faster}$$

THUS a process like **diffusion** would **allow isotopic fractionation** due to **molecular velocity differences**.

# Fractionation Effects: Kinetic effects

- Can enhance effects due to mass differences

At equilibrium the predicted  $^{18}\text{O}_{\text{gas}} = ^{18}\text{O}_{\text{liquid}}$



- But because  $\text{H}_2^{16}\text{O}$  moves faster than  $\text{H}_2^{18}\text{O}$  it can attain equilibrium faster

Result:

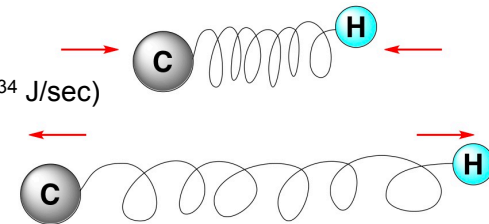
**Evaporate is lighter than source.** Implications? Stay tuned.....

# Fractionation Effects: bonds (B)

- Bonds are like springs and vibrate in proportion to their atom's mass and contained energy.

$$E = \frac{1/2(h)(k)}{2\pi(m)}^{1/2}$$

$h$ =Plank's constant ( $6.63 \times 10^{-34}$  J/sec)  
 $k$ ='spring constant'



If  $E = hv$  or the energy as a function of wavelength then

$$v = \frac{1}{4\pi} \left( \frac{k}{m} \right)^{1/2}$$

= the bond vibrational frequency at  $0^\circ\text{K}$

Both  $E$  and  $v$  are inversely proportional to the square root of the mass!

# Fractionation Effects: bonds (B)

- 'k' is mostly determined by the **chemical properties** of an element (valence electron configuration) the nuclear properties (neutron number) play almost no role.
- **k is the same** for two different isotopes of the same element involved in the same type of chemical bond with another element

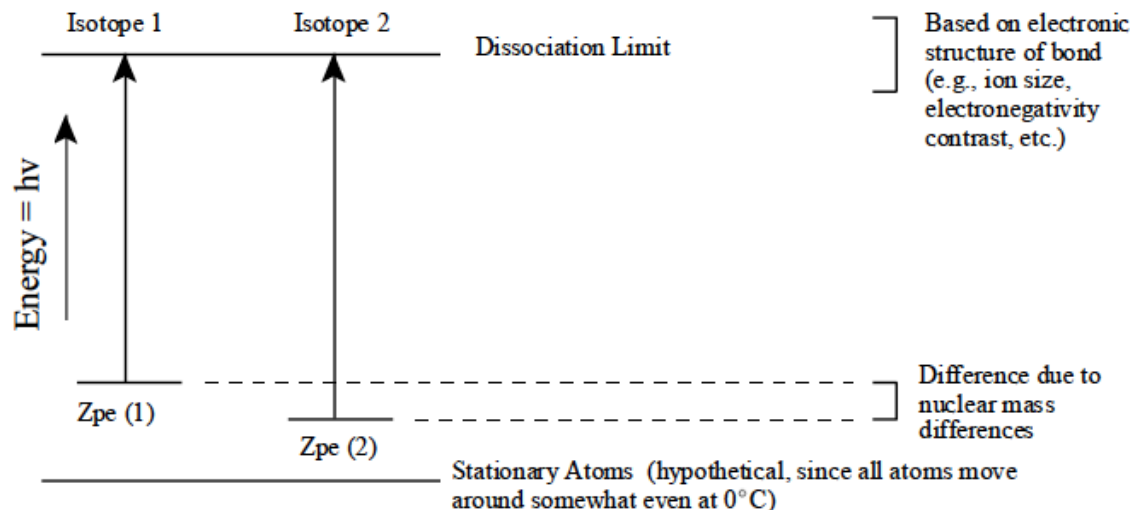
**higher isotope mass = lower bond vibration**

$$V_{\text{heavy bond}} < V_{\text{light bond}} \text{ SO } E_{\text{heavy bond}} < E_{\text{light bond}}$$

- So at 0°K the **heavier isotope** makes the **stronger** (lower v) bond

# Fractionation Effects: bonds (B)

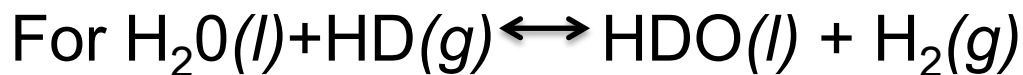
- Dissociation limit is the maximum  $\nu$  at which a bond can exist
  - Independent of mass (same for light and heavy isotope)
- BUT the lighter isotope bond is closer in energy to the E of dissociation and the **heavier isotope** thus makes the **stronger bond**.



# Quantification of isotopic fractionation



- The Isotopic composition of the light stable isotopes are usually expressed as ratios of minor to major isotope.
- Isotopic fractionation between material A and B is expressed by comparing their isotopic composition  $R_A$  ( $^{18}\text{O}/^{16}\text{O}$ ) and  $R_B$  which gives us a **fractionation factor** ' $\alpha$ ' ( $R_A/R_B$ )



Raoult's law

$$K_{\text{eq}} = \frac{a_{\text{HDO}} \times P_{\text{H}_2}}{a_{\text{H}_2\text{O}} \times P_{\text{HD}}}$$

When substituting  $R_{\text{H}_2\text{O}} \sim a_{\text{HDO}}/a_{\text{H}_2\text{O}}$  and  $R_{\text{H}_2} \sim P_{\text{HD}}/P_{\text{H}_2}$

$$K_{\text{eq}} \sim R_{\text{H}_2\text{O}}/R_{\text{H}_2} = \alpha^{\text{H}_2\text{O}}_{\text{H}_2}$$

# The 'Delta notation'

- Isotopic ratios are expressed relative to standard using delta notation:

$$\delta = [R_{\text{sample}}/R_{\text{std}} - 1]$$

$R_{\text{sample}}$  is the R of the sample (i.e.  $^{18}\text{O}/^{16}\text{O}_{\text{sample}}$ )

$R_{\text{std}}$  is the isotopic ratio of a standard or reference material

$R_{\text{sample}} < R_{\text{std}}$  and gives a  $-\delta$  it is said to be *depleted*

$R_{\text{sample}} > R_{\text{std}}$  and gives a  $\delta$  it is said to be *enriched*

**EXAMPLE:**

$$^{18}\text{O}/^{16}\text{O}_{\text{sample}} = 0.00203 \qquad ^{18}\text{O}/^{16}\text{O}_{\text{std}} = 0.00200$$

$$\delta^{18}\text{O} = [0.00203/0.00200 - 1] = 0.015$$

- $\delta$  values are often expressed as 'permil' ( $\text{‰}$ )  
**values are multiplied by 1000**
- For this example  $\delta^{18}\text{O}$  is 0.015 (unitless) or  $15\text{‰}$



# Isotope Ratio Standards

Element	Standard	Abbreviation
H	Vienna Standard Mean Ocean Water	VSMOW
C	PeeDee Belemnite (carbonate)	PDB
N	Air Nitrogen	N <sub>2</sub> (atm)
O	Vienna Standard Mean Ocean Water	VSMOW
S	Trisolite (FeS) from the Canyon Diablo meteorite	CDT

# Isotope Ratio Standards



- These standards are more useful than just referencing the isotopic composition of a sample to a common baseline
- Used for cross calibration between laboratories
  - Instrumental variation etc.
- If five laboratories analyze the same difference and the same reference material any potential ‘lab biases’ are negated since the  $R_{\text{unknown}}$  and  $R_{\text{std}}$  are biased in the same way.

Isotope	Lab 1	Lab 2	Lab 3
$^{18}\text{O}_{\text{sample}}$	0.00205	0.00199	0.00208
$^{18}\text{O}_{\text{std}}$	0.00200	0.00194	0.00203
$\delta^{18}\text{O}$	+25	+25	+25

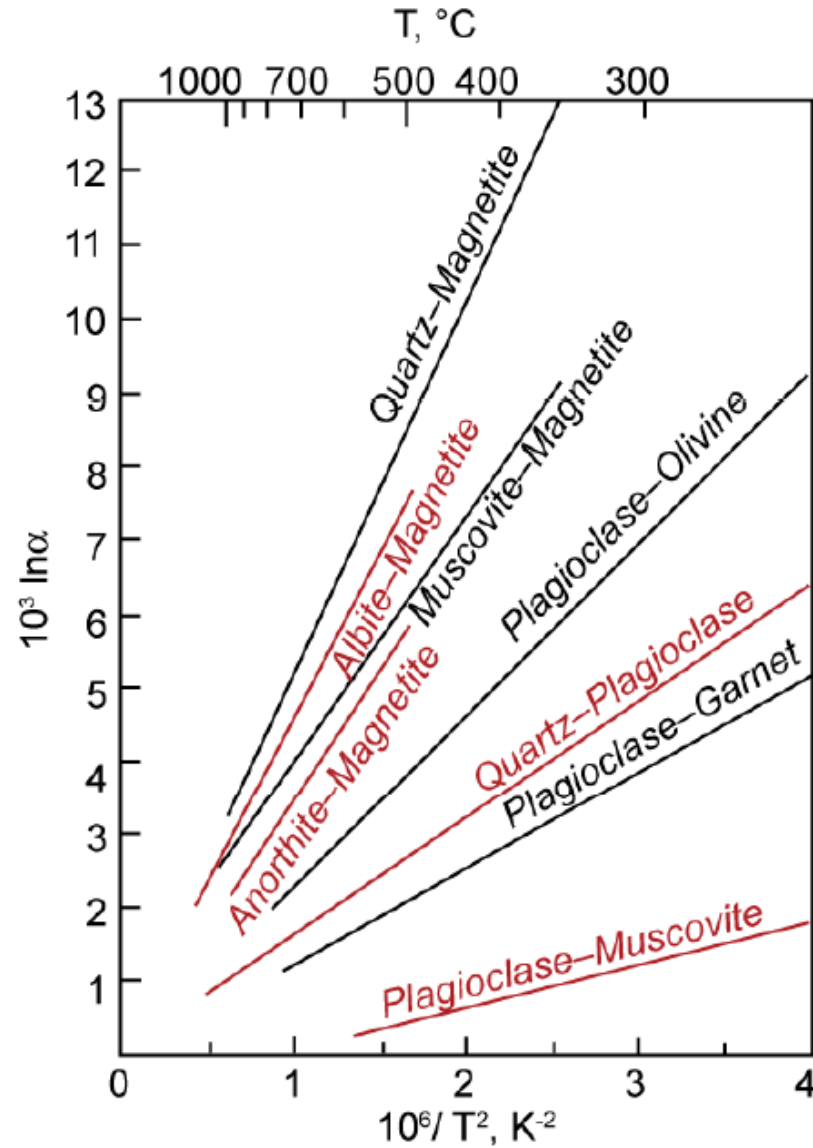
- Isotopic fractionation is temperature dependent.

↑ Temp = ↓ effect (usually)

WHY? Because the system is **more energetic** overall at higher temperatures.

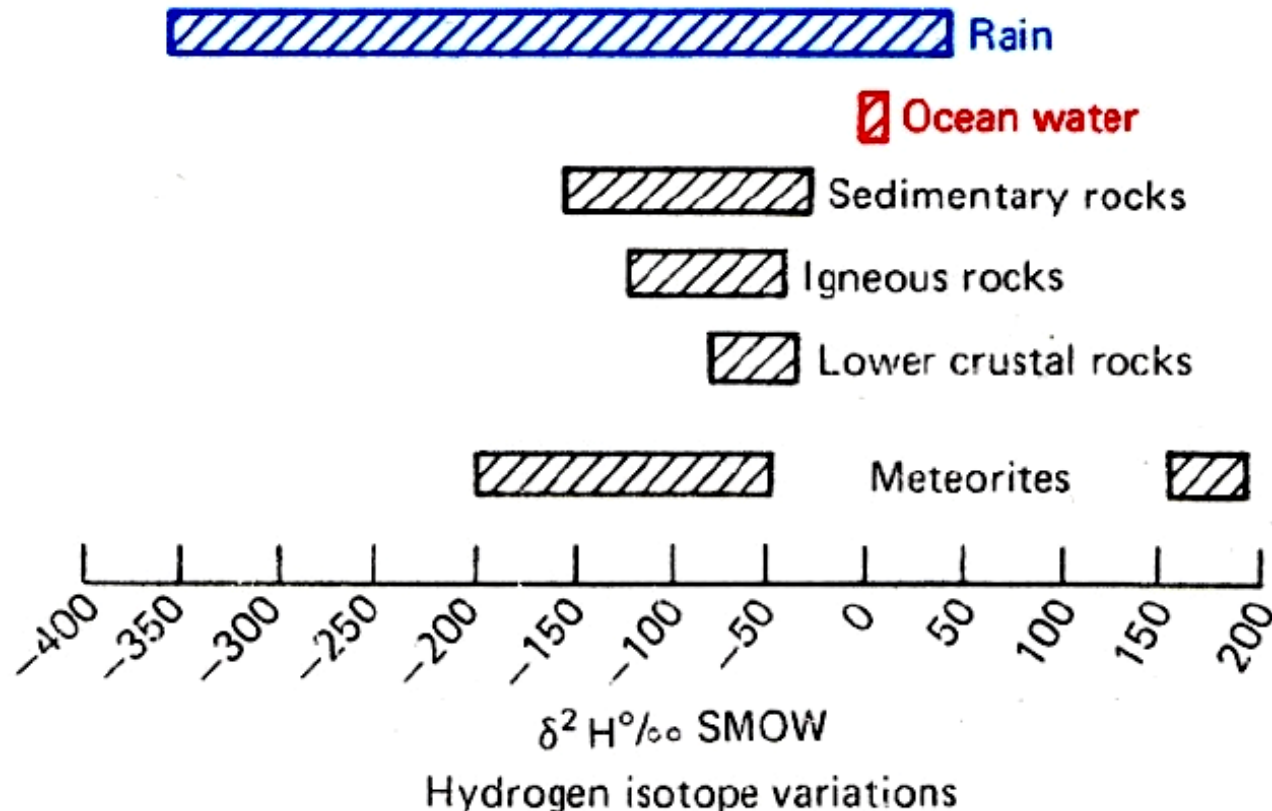
- Therefore the energy differences arising from the isotope mass differences are often a **smaller proportion** of the whole.

# Temperature dependence of fractionation



# Range of isotopic composition on earth

- As expected H shows greatest range, as expected from the large D-H mass difference

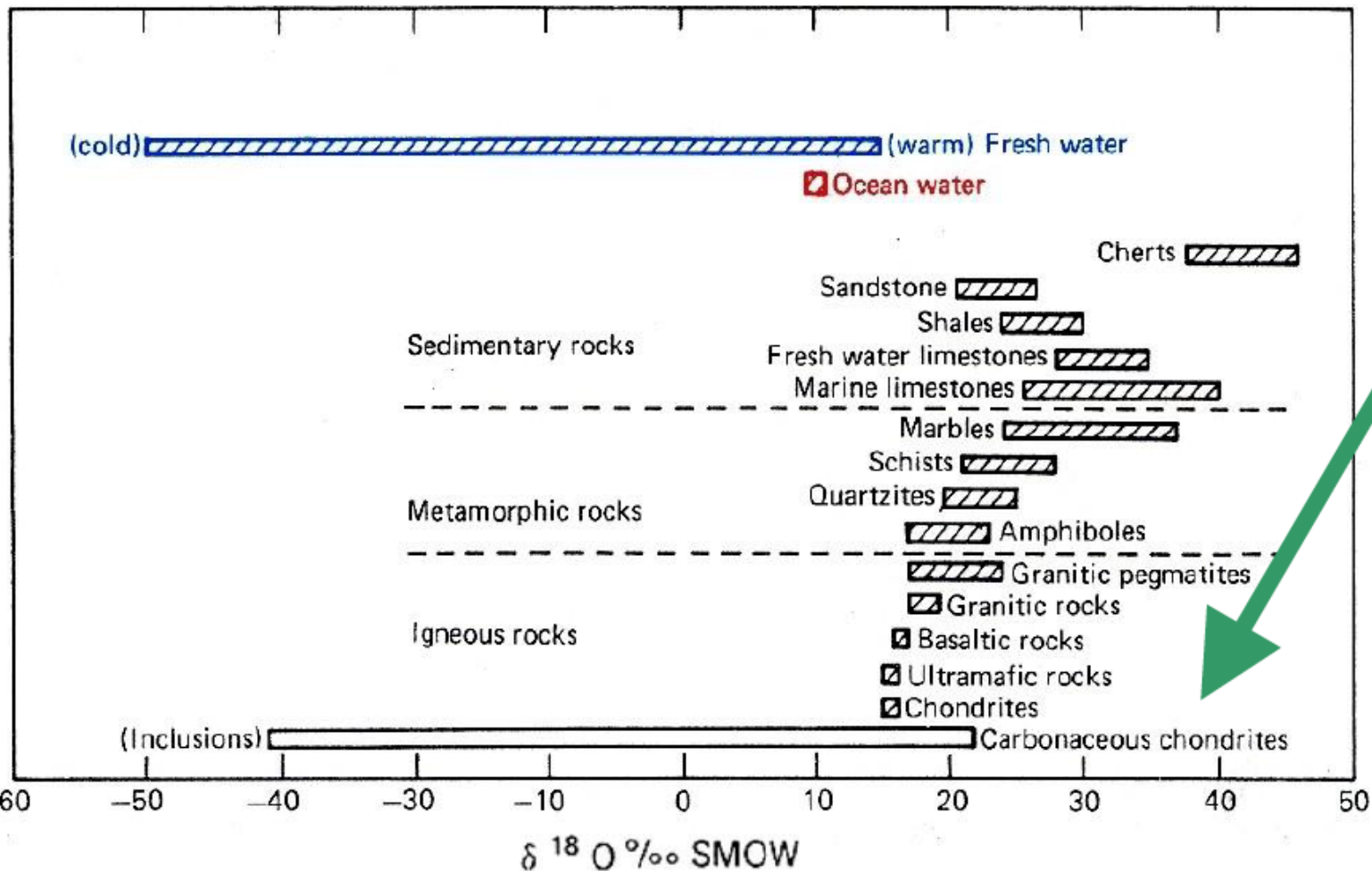


**Figure 7.7** Hydrogen isotope variations.

# Range of isotopic composition on earth



- $\delta^{18}\text{O}$  in rocks **decreases** overall with **increasing** formation temperature.



Generally increasing formation temperature for sedimentary, metamorphic, and igneous rocks.

# Range of isotopic composition on earth

- Inorganic materials have heavier C than organic ones.
- OM is isotopically light because biotic metabolisms are highly tuned to chemical reaction energetics. Favors breaking higher energy (easier to break)  $^{12}\text{C}$ -X bonds.

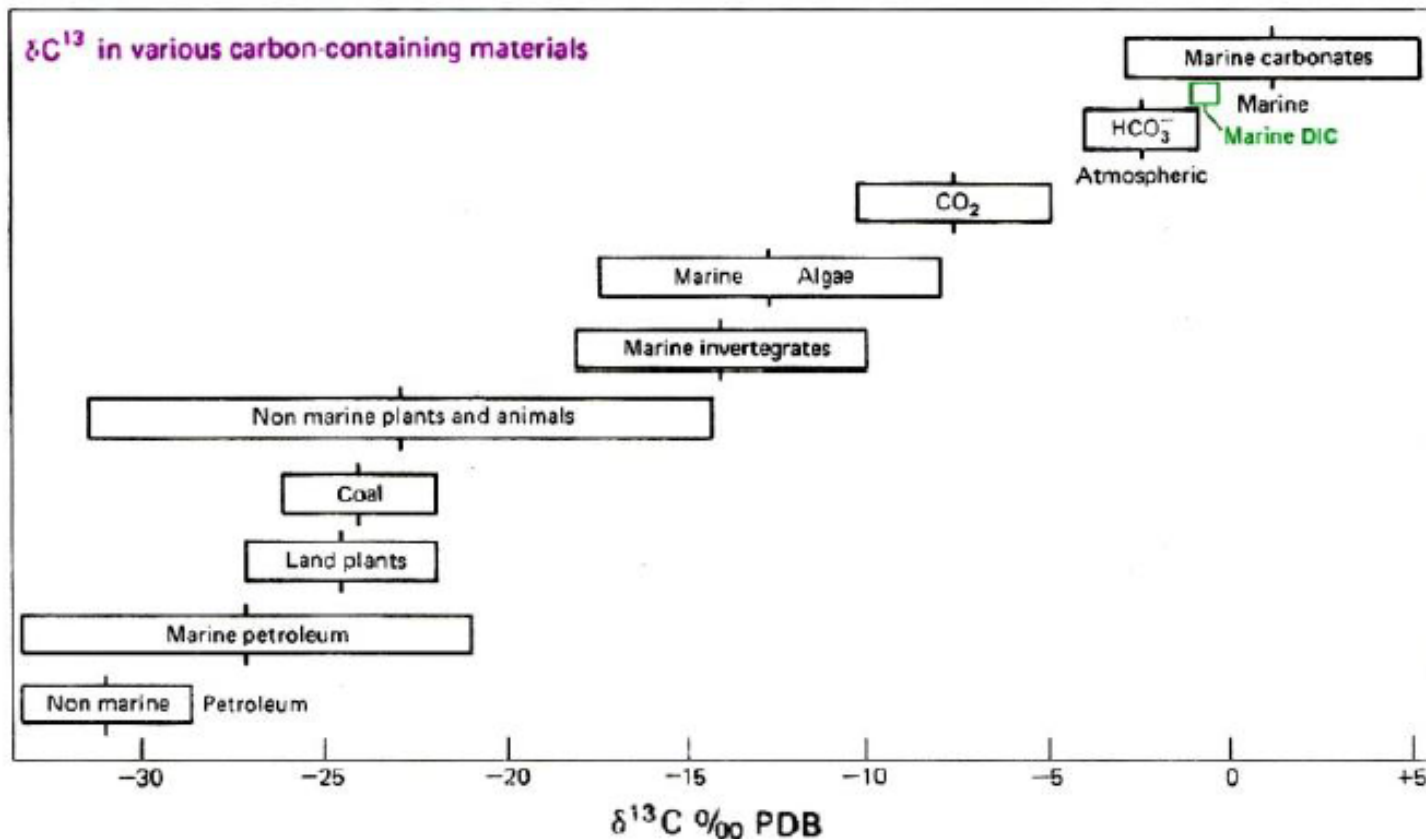


Figure 7.5 Carbon isotope variation in natural carbon-containing materials.

# Range of isotopic composition on earth



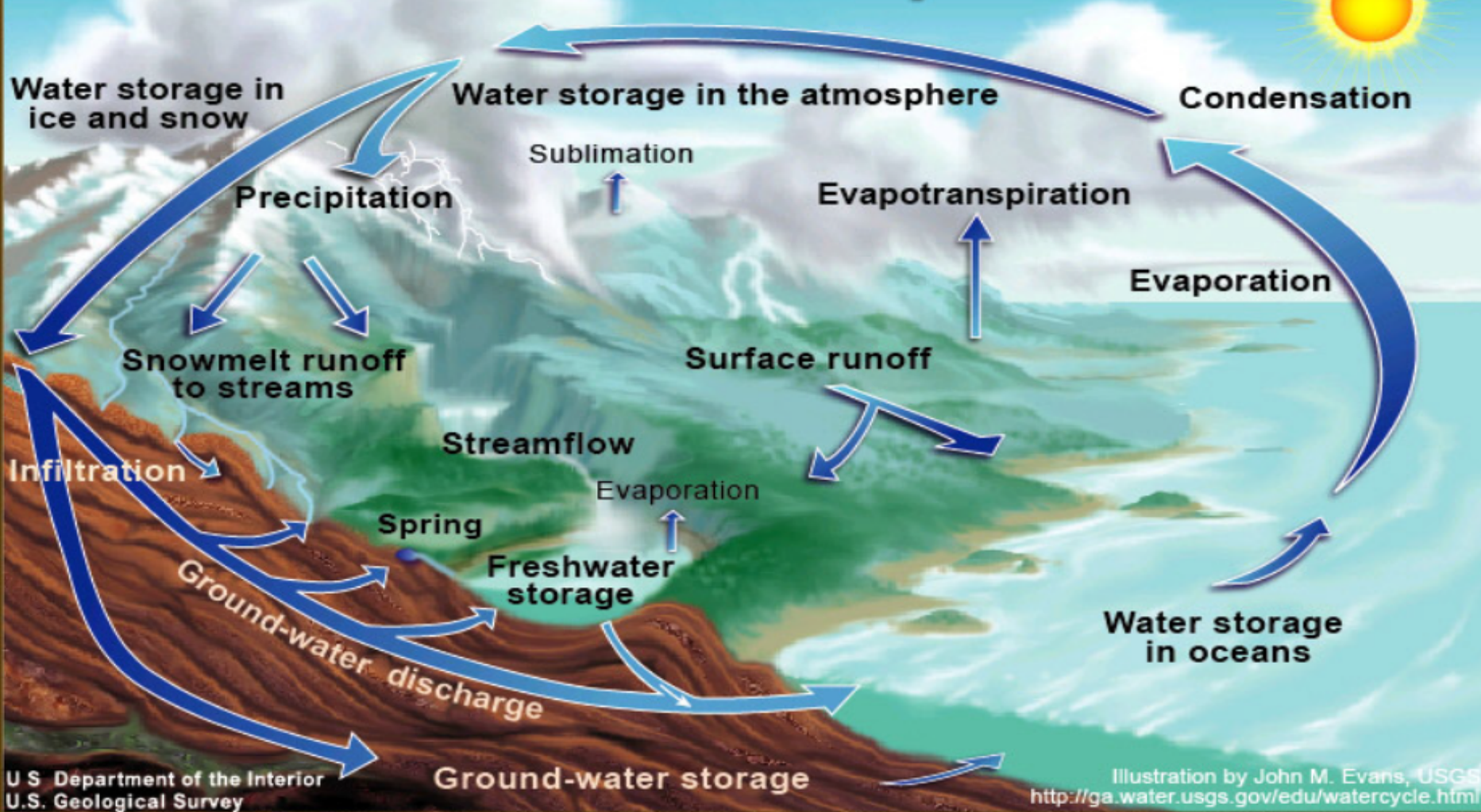
- $\delta^{18}\text{O}$  values relative to SMOW and PDB are the way they are because of the processes that form them (i.e.,  $\delta^{18}\text{O}$  vapor < liquid < solid):
  - SMOW is **liquid water** so most minerals (solid) have positive  $\delta^{18}\text{O}$  relative to it (enriched)
  - PDB is a **shell (solid)** and so should have a greater  $\delta^{18}\text{O}$  than the water in which it was formed.  $\delta^{18}\text{O}$  values of natural waters should therefore be lighter (depleted) relative to PDB.
- $\delta^{13}\text{C}$  values relative to PDB are the way they are because:
  - $\delta^{13}\text{C}$  of **PDB (solid)** should be greater than the DIC of the water in which is formed; the same is true for other shells, which have slightly < or > values compared to PDB.
  - Carbon in atmospheric  $\text{CO}_2$  (gas) is isotopically lighter than shell material and organic matter is light still.



# Isotopic fractionation in the hydrosphere



## The Water Cycle



# Isotopic fractionation in the hydrosphere



- Need to understand Rayleigh fractionation

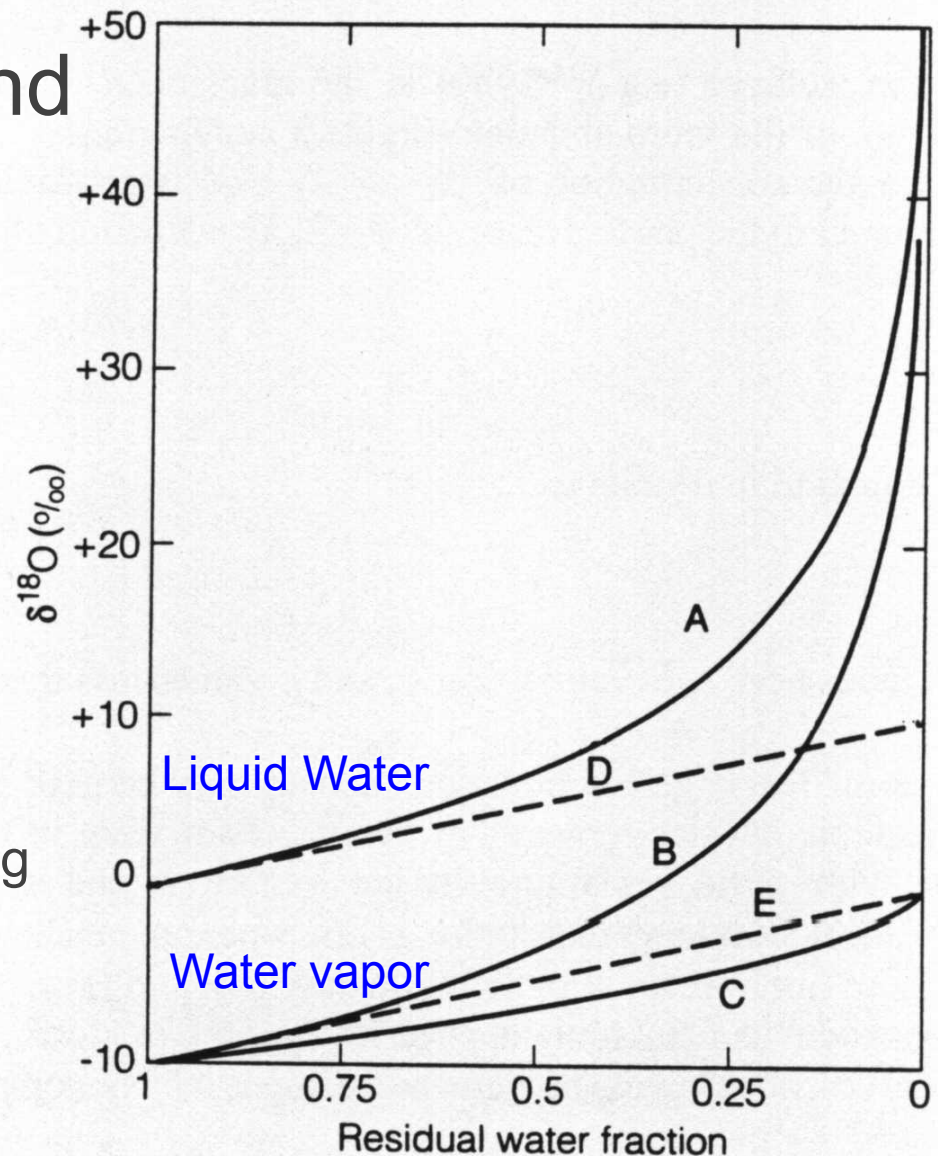
$$R = R_0 f^{(a-1)}$$

R=ratio of isotope in reactant

$R_0$  = initial ratio

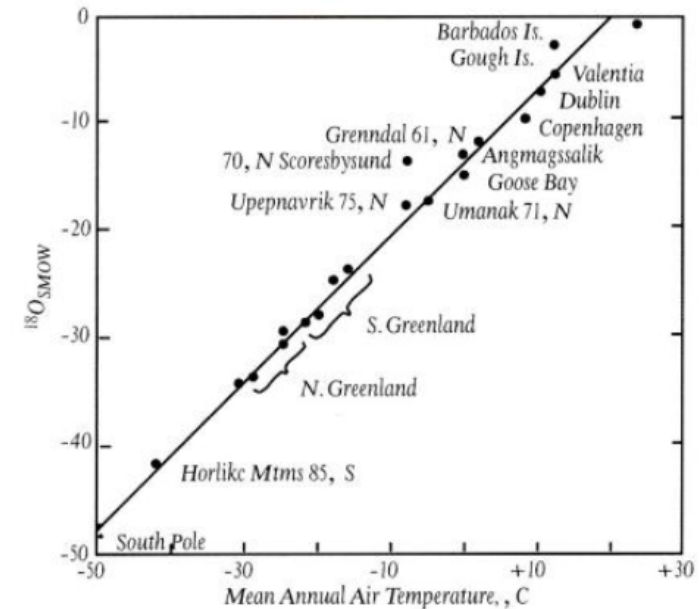
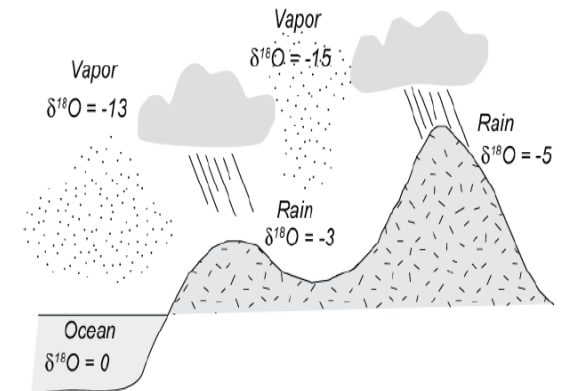
$f$  = fraction of material remaining

$a$  = fractionation factor



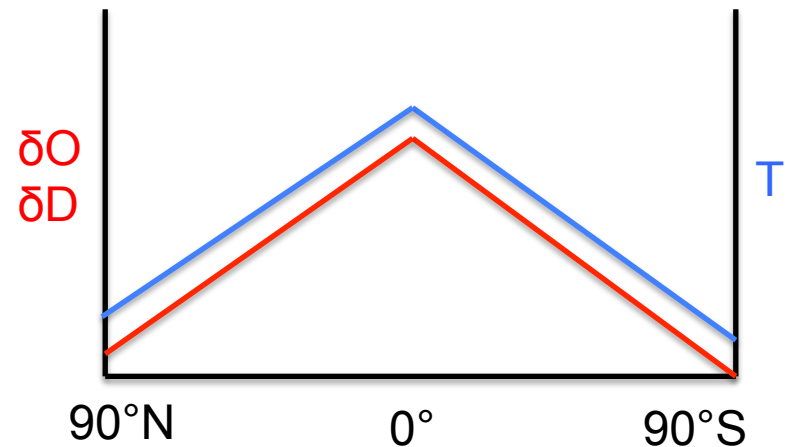
# Isotopic fractionation in the hydrosphere

- $\delta^{18}\text{O}$  in the oceans are nearly constant at 0.
- The hydrological cycle involves many phase shifts (solid-liquid-gas)
- The extent of isotopic variation during liquid-gas is a strong variation of temperature
- On earth this occurs over a wide range of temperatures with evaporation favoring the lighter isotopes at all temperatures but more so at lower temperatures

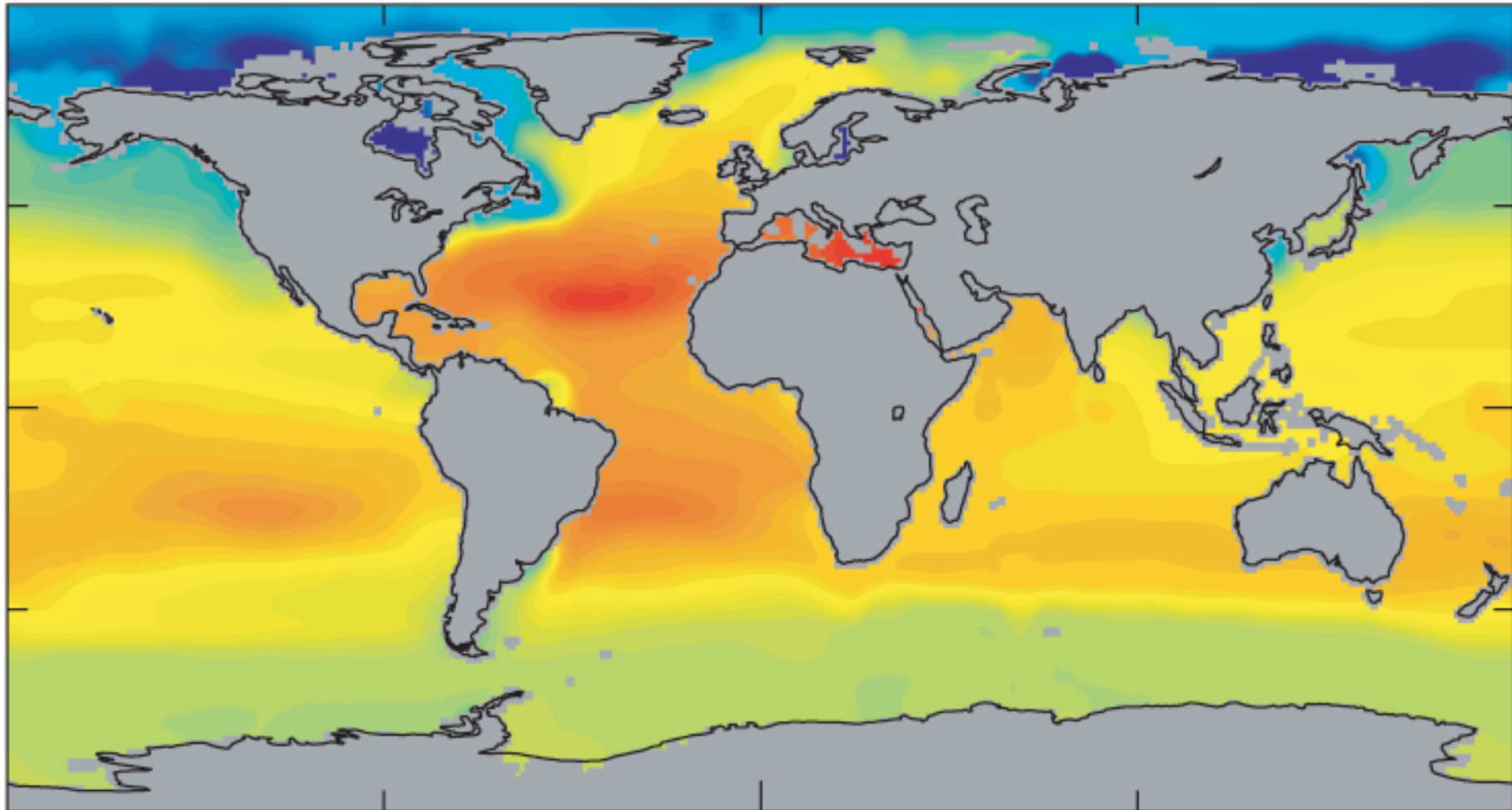


# Isotopic fractionation in the hydrosphere

- Since temperatures are relative cool at the poles, so the **evaporating water** is depleted in heavy isotopes relative to warmer areas.
- Greater than predicted by chemical equilibria alone because the kinetic effects enhance the fractionation and the surface oceans have variation in isotopic composition reflecting the local precipitation
- It is the **temperature gradient** from the equator to poles that causes variation in both **the isotopic ratio of surface waters** and of **water vapor** in the overlying atmosphere



# Isotopic fractionation in the hydrosphere



-3

-2

-1

0

1

2

$\delta^{18}\text{O}$  (‰)

# Isotopic fractionation in the hydrosphere

Meteoric and atmospheric waters around the world follow the  $\delta D$  vs.  $\delta^{18}O$  relationship shown here (meteoric water line)

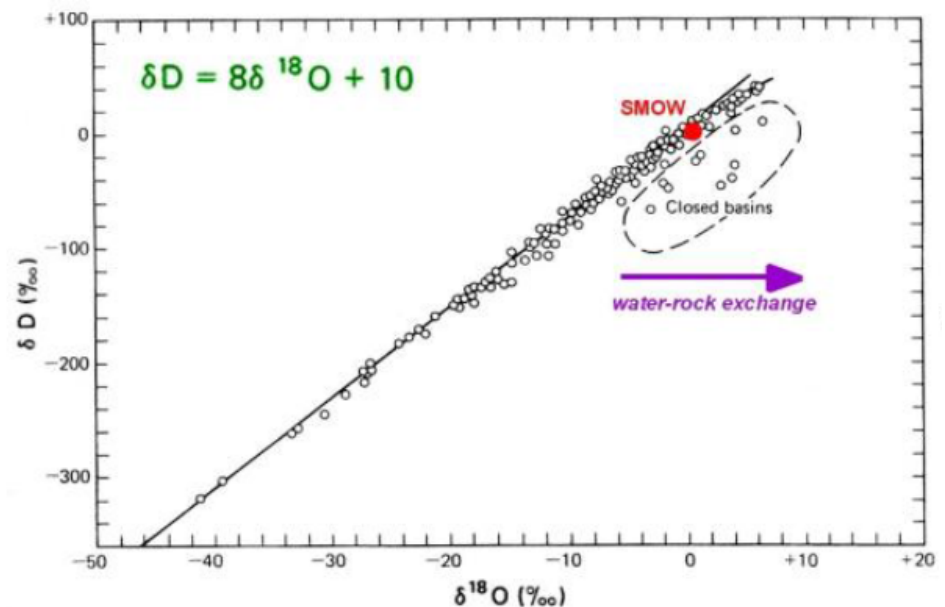
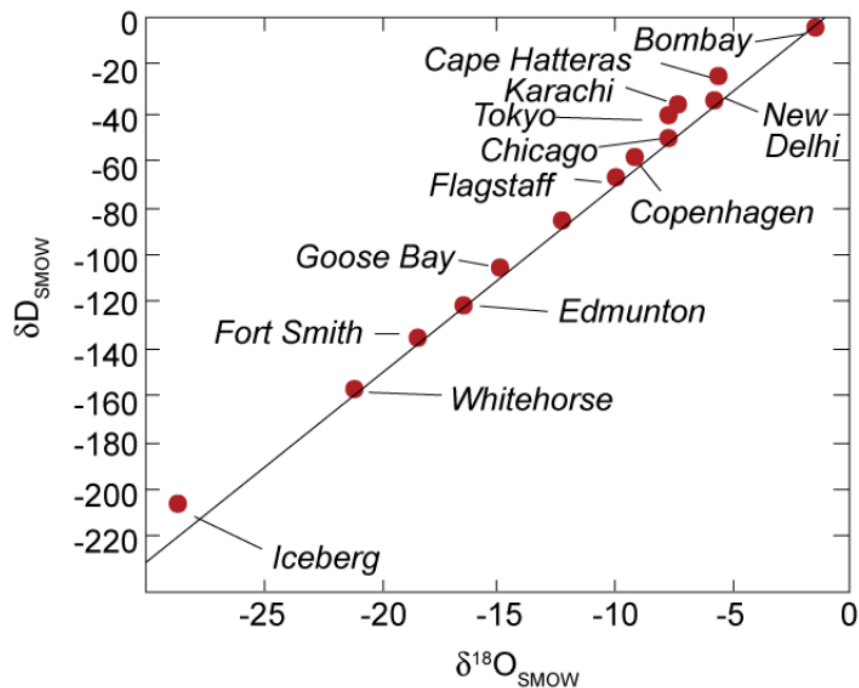
$$\delta D_{SMOW} = 8 \delta^{18}O_{SMOW} + 10$$


Figure 17-1 Deuterium and  $^{18}O$  variations in rivers, lakes, rain, and snow. (Craig, 1961. Copyright 1961 by the American Association of Science.)

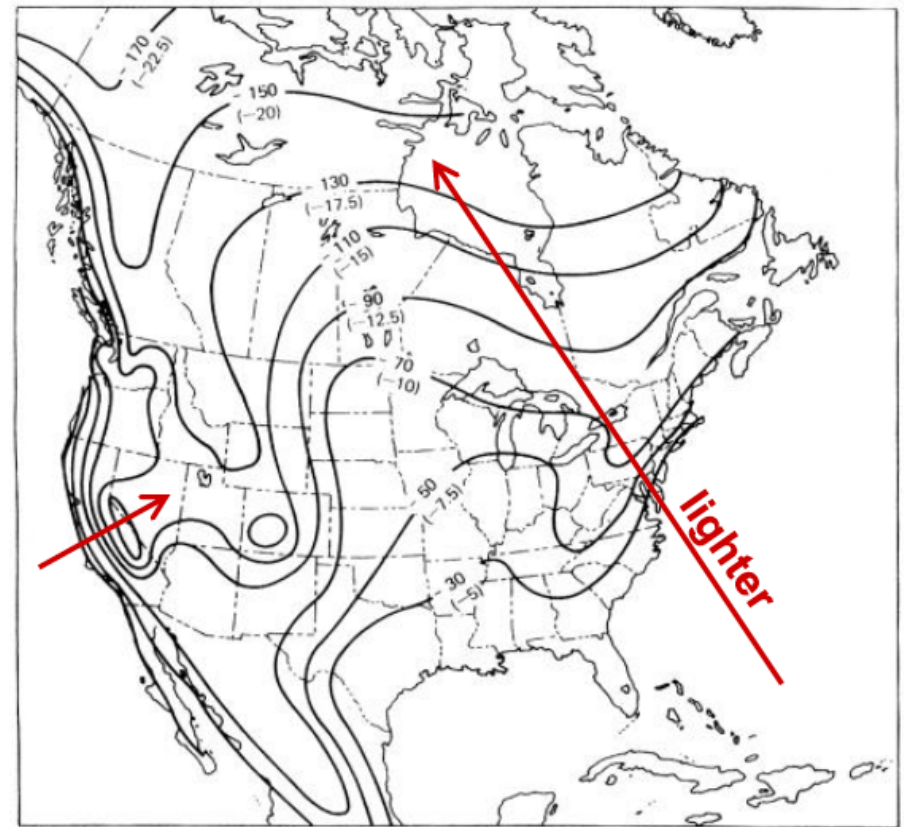
# Isotopic fractionation in the hydrosphere

- This contour map of H and O isotopic composition of precipitation over North America illustrates both the **latitudinal (temp) effect** and the **'rain out' effect**.

## Rain out effect:

Precipitation is enriched in the heavy isotope and thus as an air mass moves inland and produces more and more rain the vapor becomes lighter producing lighter and lighter rain.

Same is true for elevation!

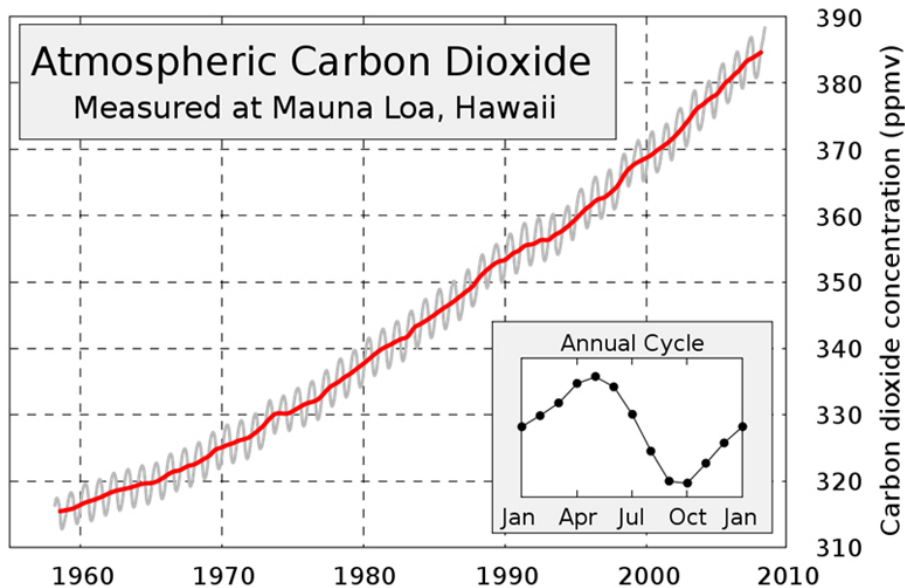


**Figure 17-2** Distribution of  $\delta D$  and corresponding  $\delta^{18}O$  (in parentheses) in meteoric waters in North America (adapted from Sheppard et al., 1969).

# Application: Climate



- Increase in CO<sub>2</sub> from 320 to 390 atm in last 60 years.



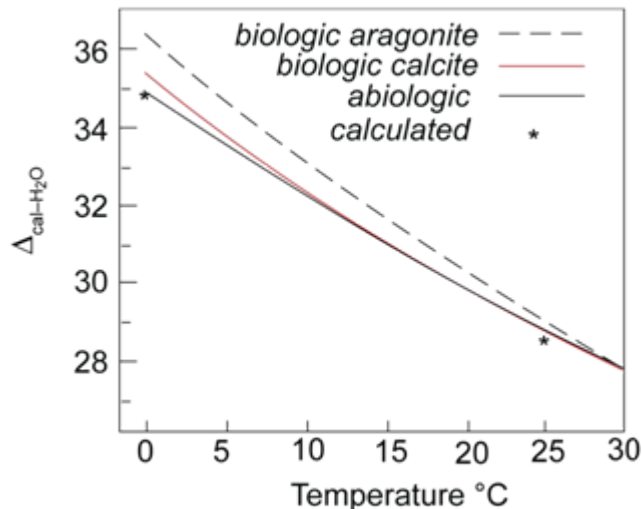
- Is this normal?
- What does this mean for our climate?



# Application: Climate



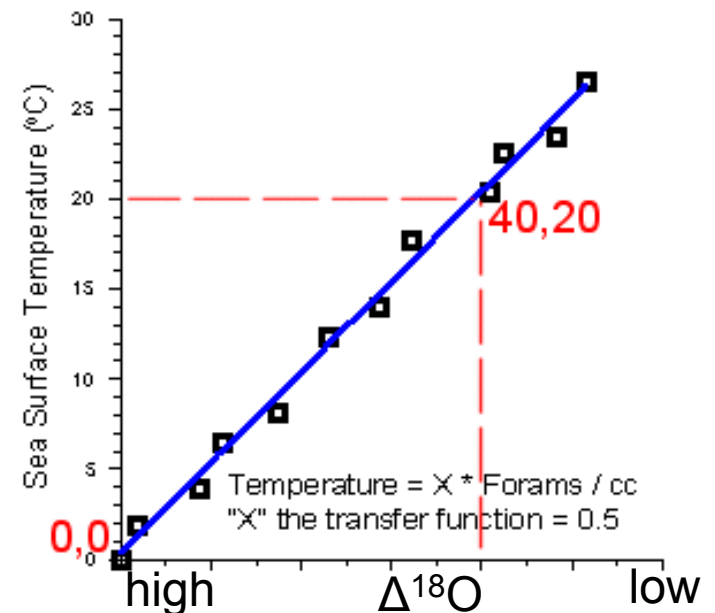
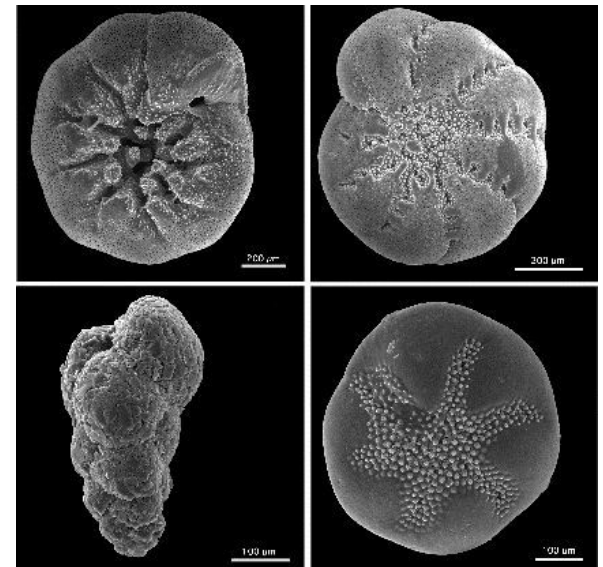
- Harold Urey (1947) laid ground work when he established temperature dependent fractionation factors of various compounds



- $T(^{\circ}\text{C})=16.9-4.2\Delta+0.13\Delta^2$ 
  - where  $\Delta$  is the  $^{18}\text{O}/^{16}\text{O}$  difference between calcite and the water it is precipitated from.
- Made it possible to use isotope fractionation as paleo thermometer

# Application: Climate

- Cesare Emiliani used this principle in benthic foraminifera shells to establish the first paleo temperatures of the ocean from deep sea core.
- Used imperially derived  $^{18}\text{O}/^{16}\text{O}$  ratios from lab and present day specimen growing at different temperatures



# Application: Climate

- Established 'isotope stages'
  - Odd = low  $^{18}\text{O}$  or warm periods (interglacials)
  - Even = higher  $^{18}\text{O}$  or colder periods (glacials)
- Found high correlation with 'Milankovitch cycles'

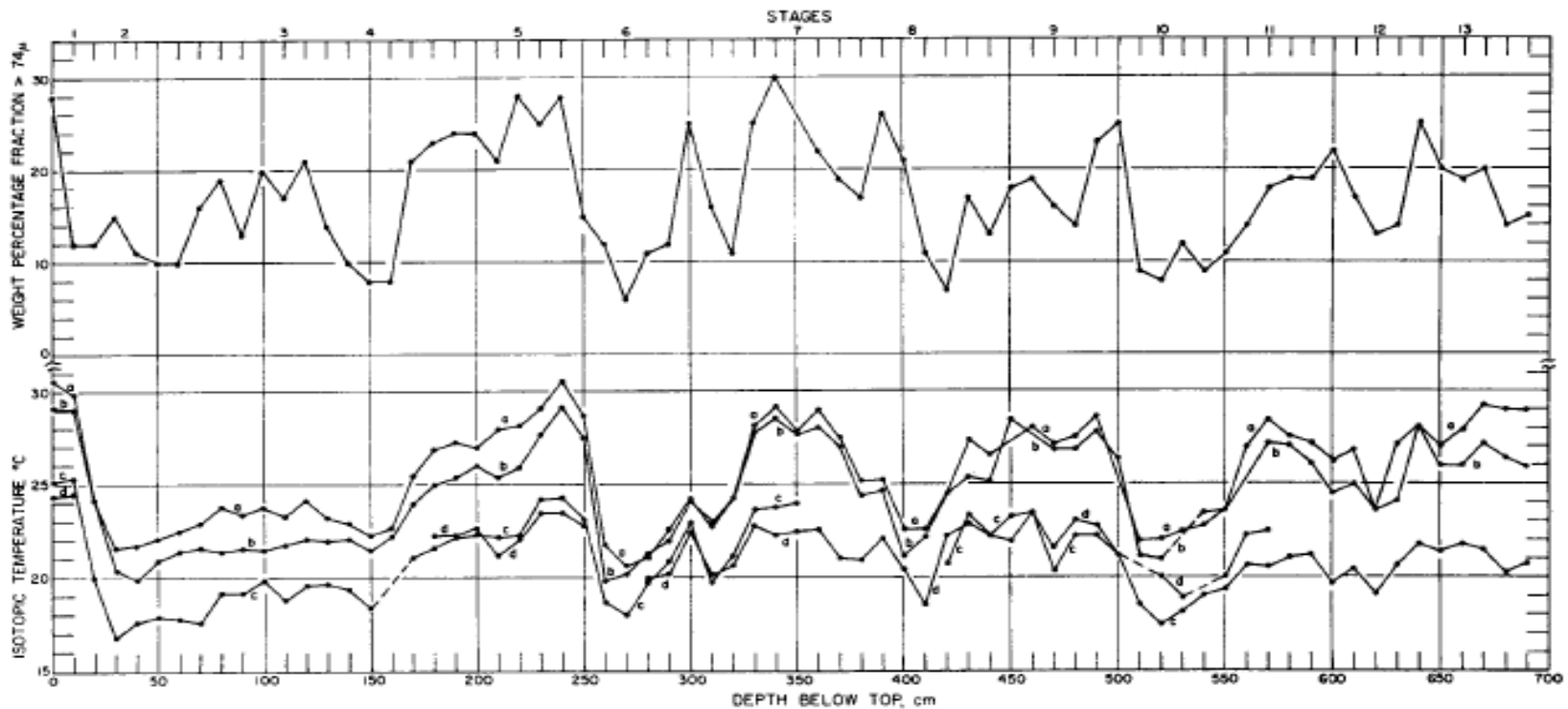
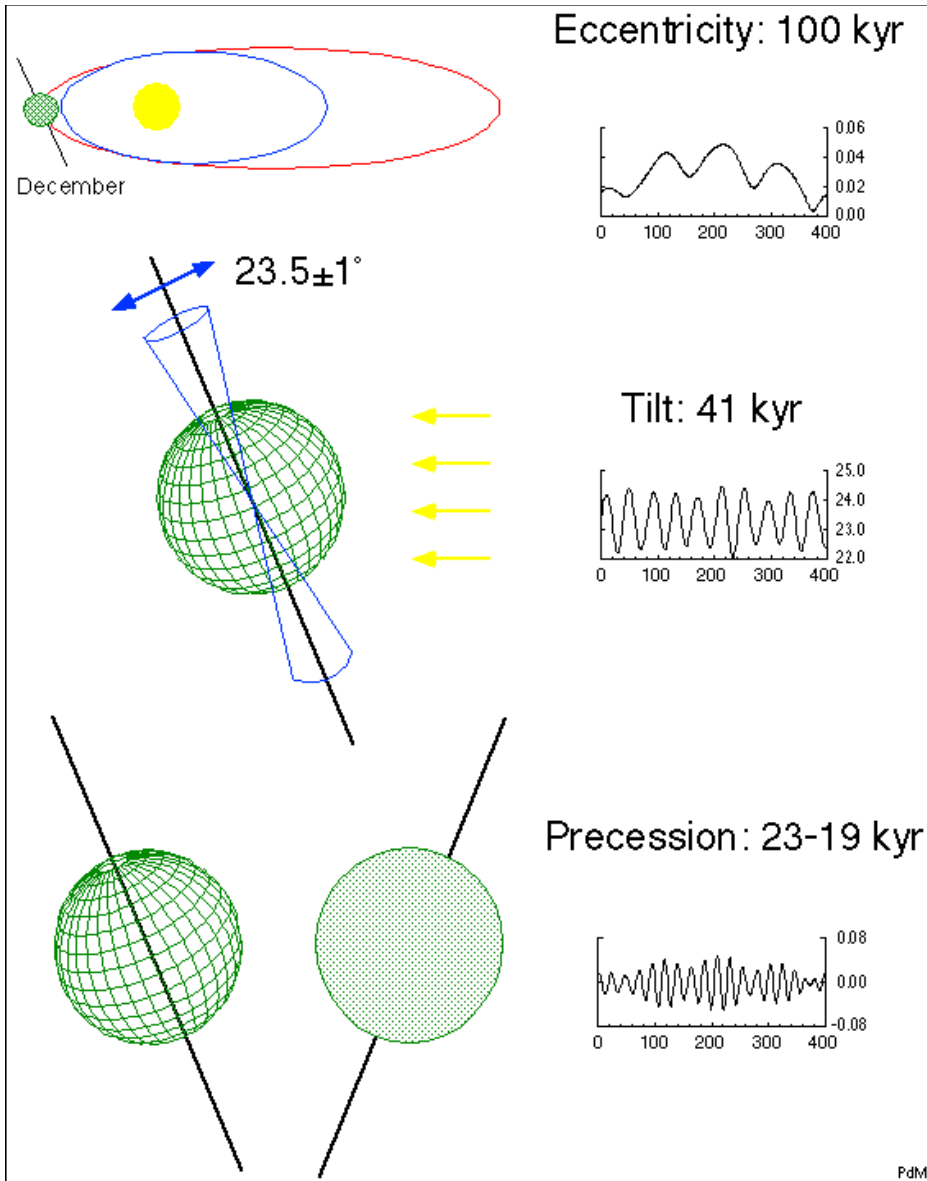


FIG. 2.—Core A179-4: percentages of the fraction larger than 74  $\mu$  and isotopic temperatures obtained from *Globigerinoides rubra* (a), *Globigerinoides sacculifera* (b), *Globigerina dubia* (c), and *Globorotalia menardii* (d).

# Application: Climate



- Cycles can be seen in  $^{18}\text{O}/^{16}\text{O}$  variation.
- Emiliani contributed them mostly to temperature effects on fractionation of calcite
- Temp difference was too large  $6^\circ\text{C}$  between arctic and Caribbean in Atlantic.
- What might also affect the  $^{18}\text{O}/^{16}\text{O}$  signal?

# Application: Climate



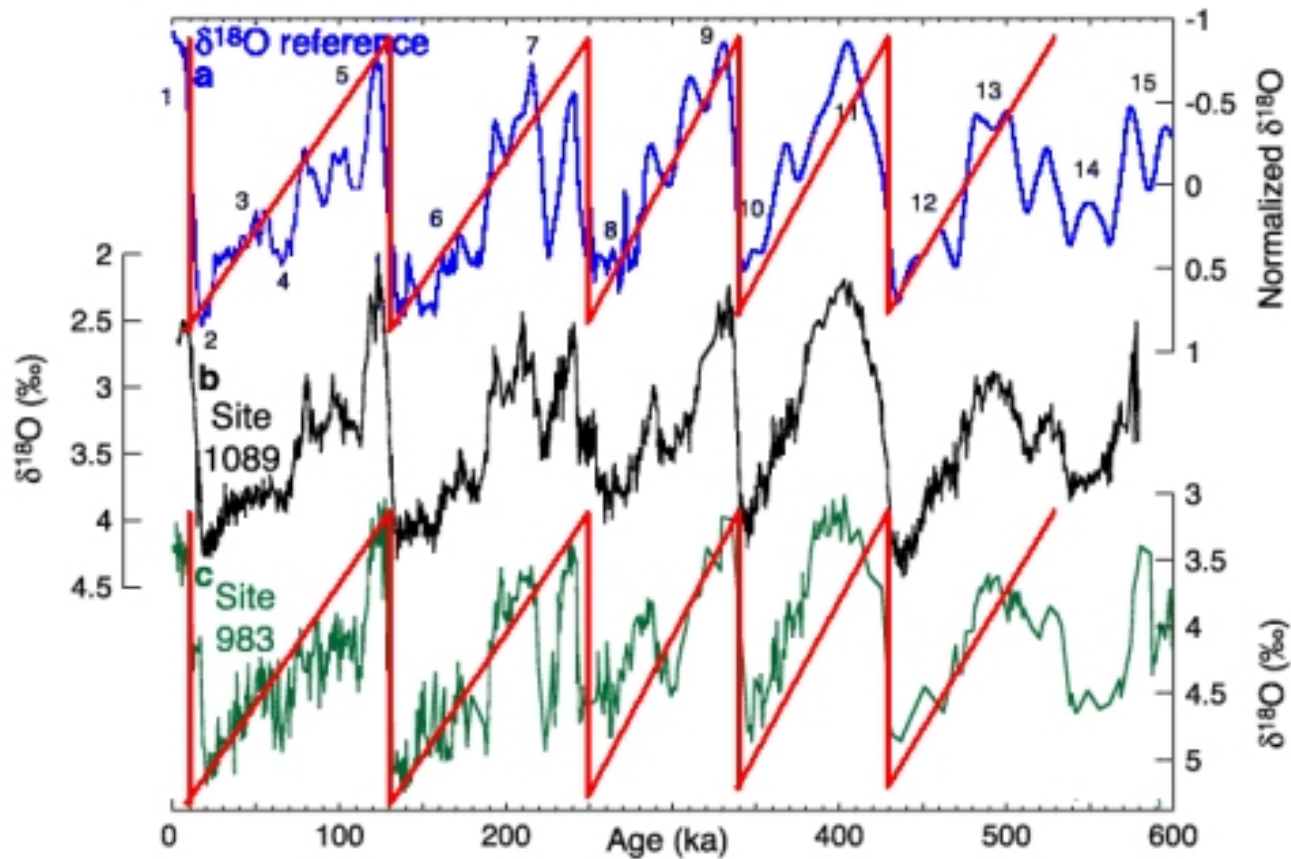
NATURE, VOL. 215, JULY 1, 1967

## Oxygen Isotope Analyses and Pleistocene Temperatures Re-assessed

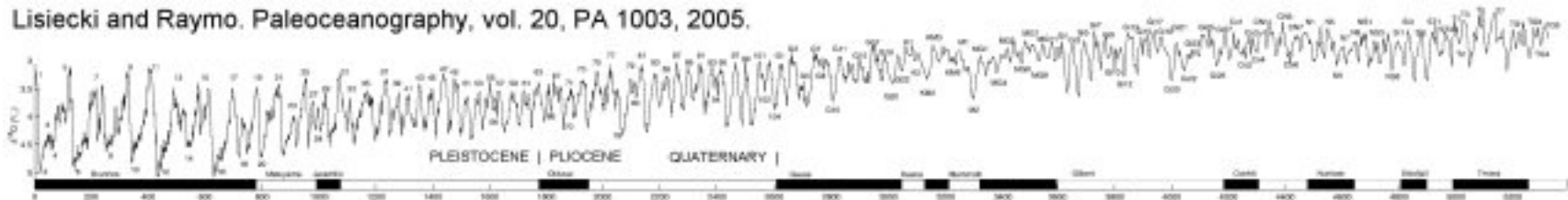
by  
NICHOLAS SHACKLETON  
Sub-department of Quaternary Research,  
University of Cambridge

- Shackleton argued that  $^{18}\text{O}$  values were more heavily influenced by the removal of water and formation of ice sheets than isotope fractionation and revised Emiliana's method.
- Used Sea level estimates from corals

# Application: Climate

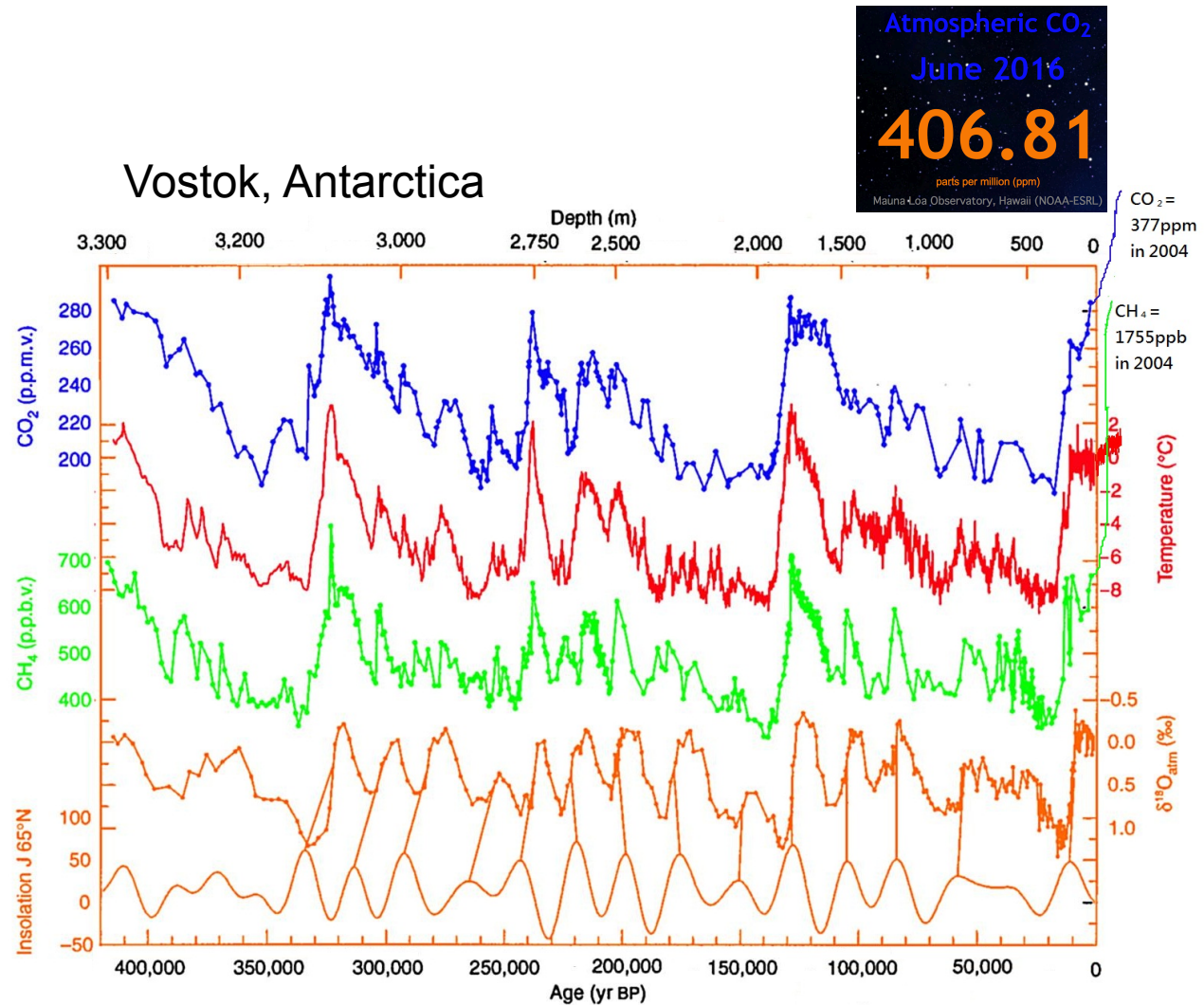


Lisiecki and Raymo. *Paleoceanography*, vol. 20, PA 1003, 2005.



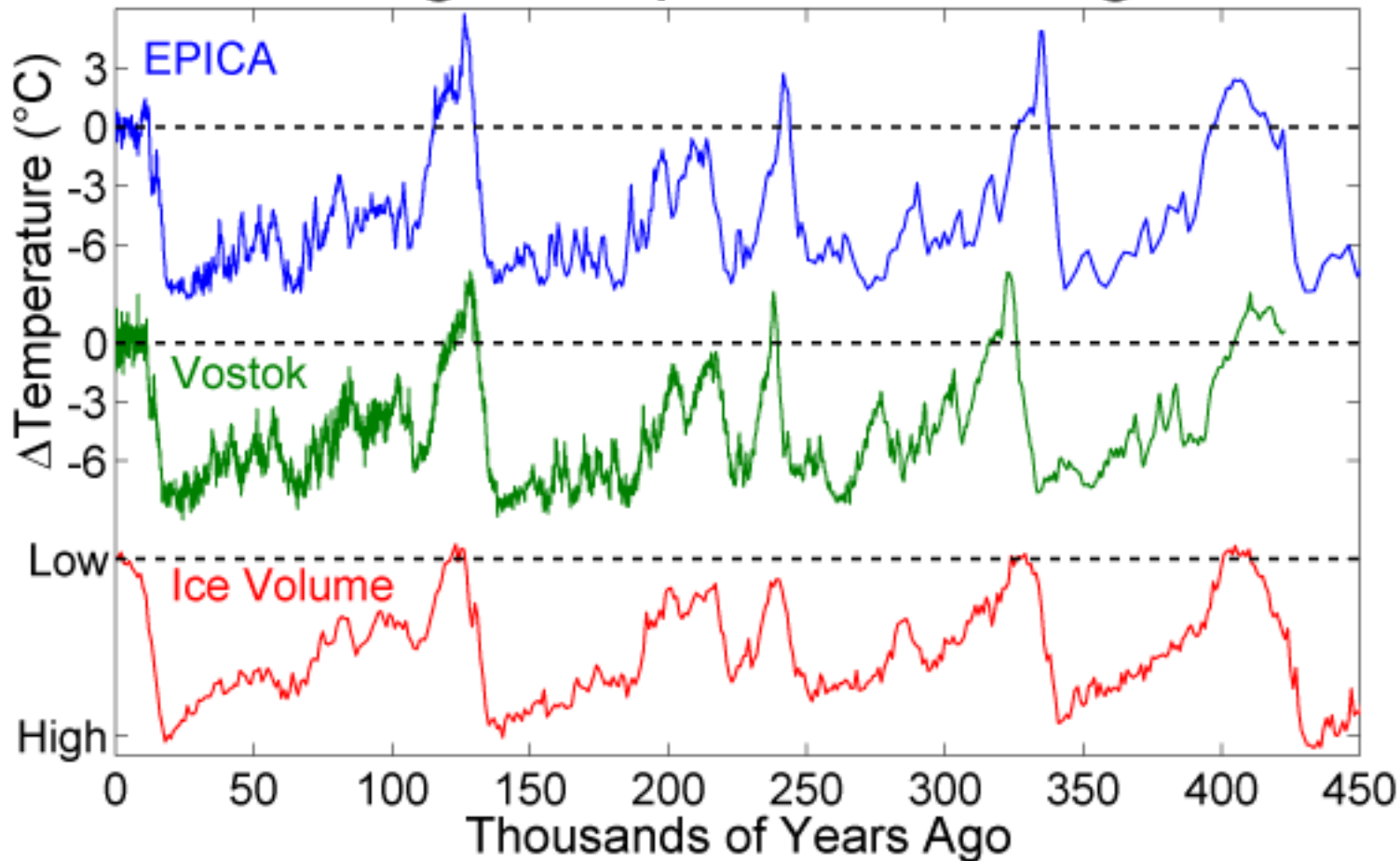
# Application: Climate

- Ice core data
- Accumulation of snowfall
  - Traps air bubbles inside
  - Record of 'ancient' air
- Can establish air temp, precipitation records



# Application: Climate

## Ice Age Temperature Changes



Ice data

Data from Foraminifera



# Isotopic fractionation during biological processes



<u>Substrate</u>		<u>Product</u>
$\text{CO}_2$	$\xrightarrow[\varepsilon \approx 20\text{‰}]{\text{photosynthesis}}$	$\text{C}_{\text{org}}$
$\delta^{13}\text{C}_{\text{CO}_2}$ (-8 to -10‰)		$\delta^{13}\text{C}_{\text{org}}$ (-18 to -23‰)
$\text{NO}_3^-$	$\xrightarrow[\varepsilon \approx 5-10\text{‰}]{\text{nitrate assimilation}}$	$\text{N}_{\text{org}}$
$\delta^{15}\text{N}_{\text{NO}_3}$ (+5‰)		$\delta^{15}\text{N}_{\text{org}}$ (-1 to +5 ‰)
$\text{SO}_4^{-2}$	$\xrightarrow[\varepsilon \approx 2-42\text{‰}]{\text{sulfate reduction}}$	$\text{S}^{-2}$
$\delta^{34}\text{S}_{\text{SO}_4}$ (+20‰)		$\delta^{34}\text{S}_{\text{FeS}_2}$ (-45‰ to +20 ‰)

$\varepsilon$  = isotope effect

# Isotopic fractionation during biological processes

- During photosynthesis  $^{12}\text{C}$  is preferentially taken up
  - Seawater becomes enriched
  - Plankton/Plant matter is depleted
- Differences between C3 (most) and C4 plants (grasses) and algae
  - C3 =  $\delta^{13}\text{C}$  -20 to -30 ‰
  - C4 =  $\delta^{13}\text{C}$  -13 ‰
  - Phytoplankton =  $\delta^{13}\text{C}$  -8 to -18 ‰
    - Take up DIC ( $\text{CO}_2$  and  $\text{HCO}_3^-$ )

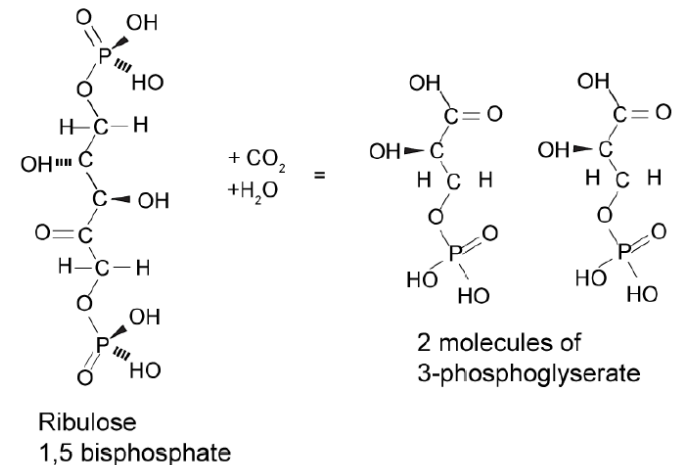


Figure 9.12. Ribulose biphosphate (RuBP) carboxylation, the reaction by which C<sub>3</sub> plants fix carbon during photosynthesis.

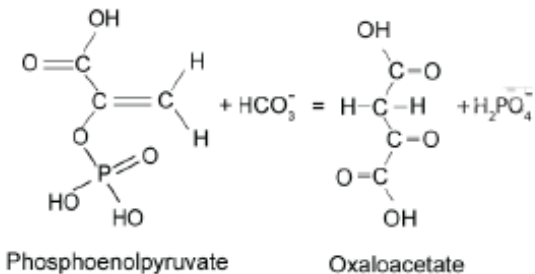
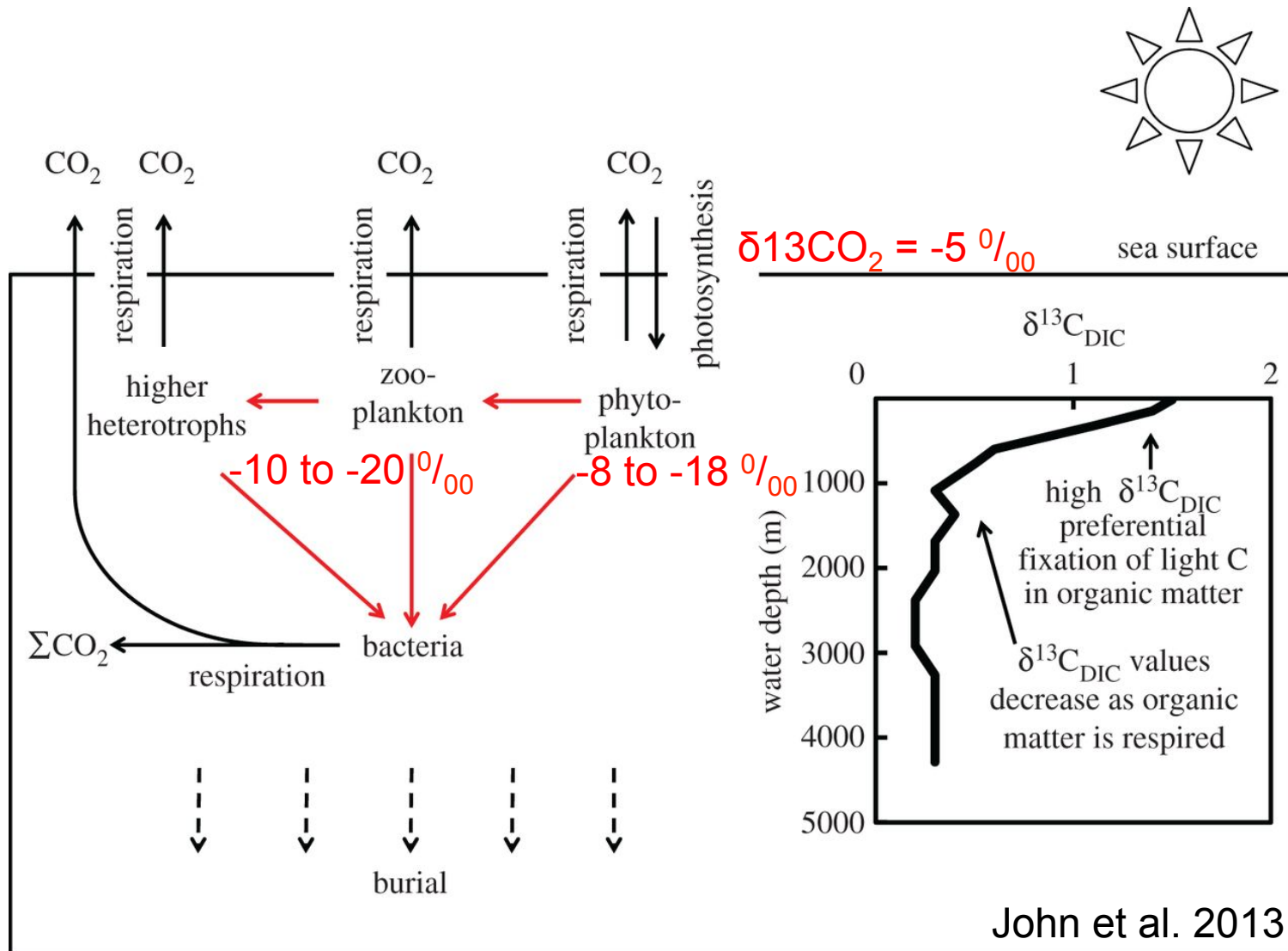


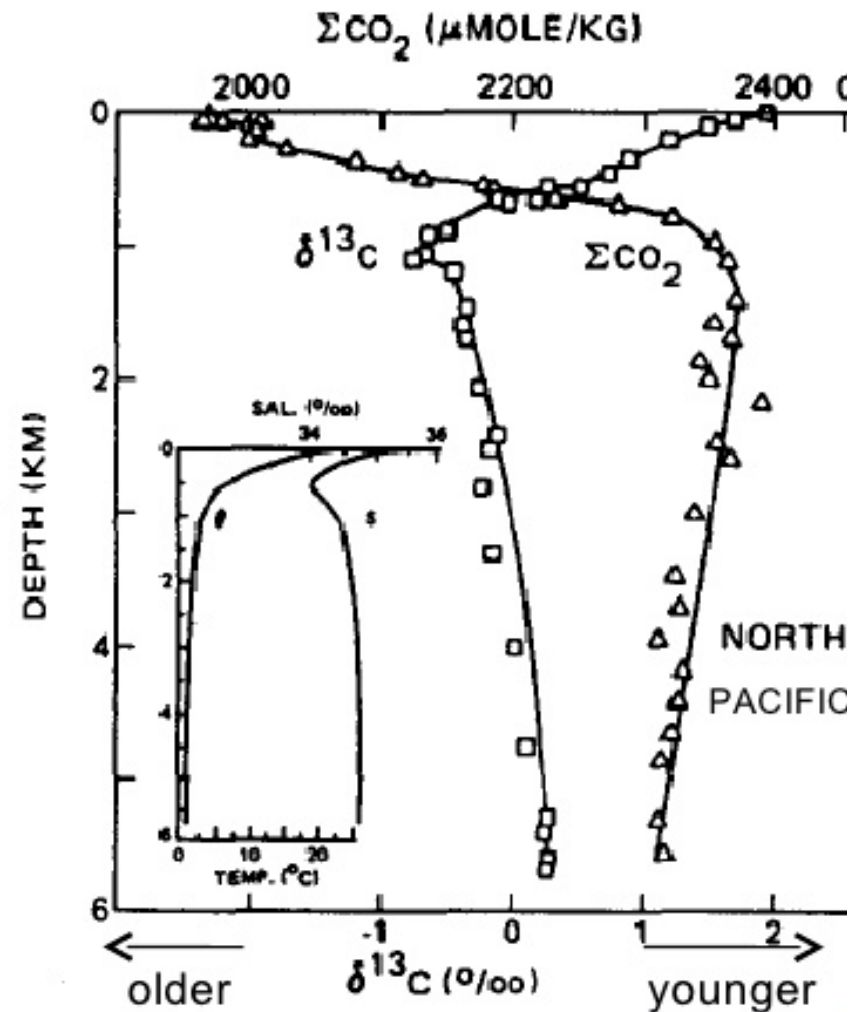
Figure 9.13. Phosphoenolpyruvate carboxylation, the reaction by which C<sub>4</sub> plants fix CO<sub>2</sub> during photosynthesis.

# Isotopic fractionation during biological processes



# Isotopic fractionation during biological processes

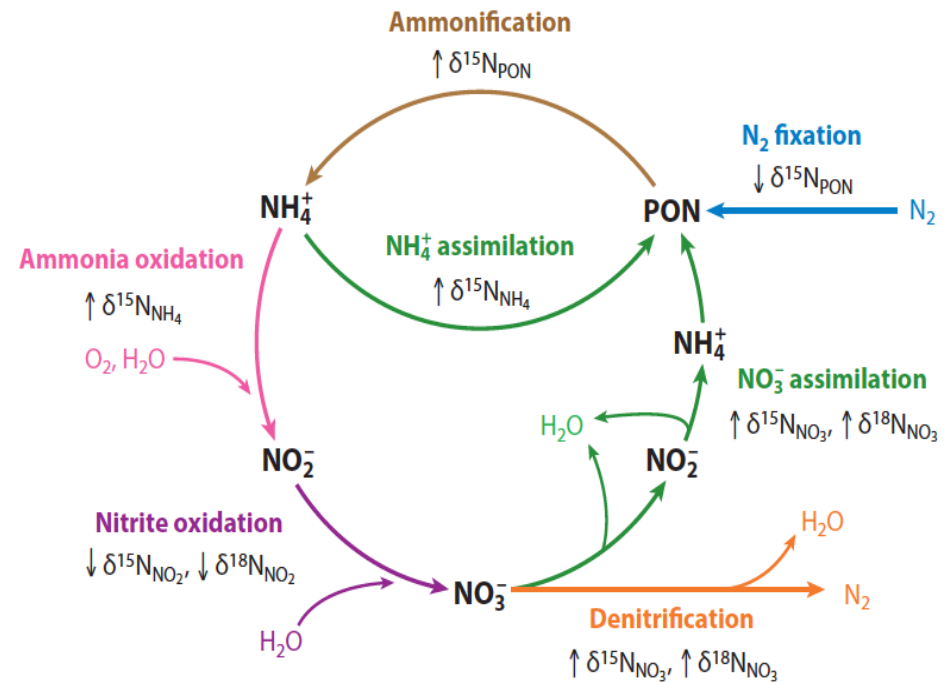
- In ocean this leads to 'vertical profile' and highlights various processes.
- Also since  $\delta^{13}\text{C}$  unlike  $\delta^{15}\text{N}$  is relatively 'independent' of trophic level is indicator of 'food source' and can be used to age water



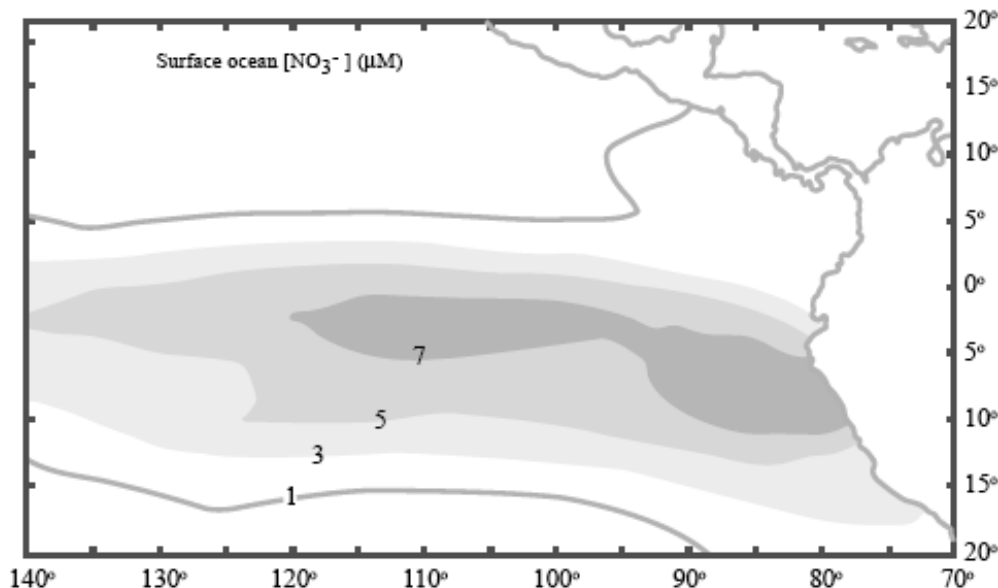
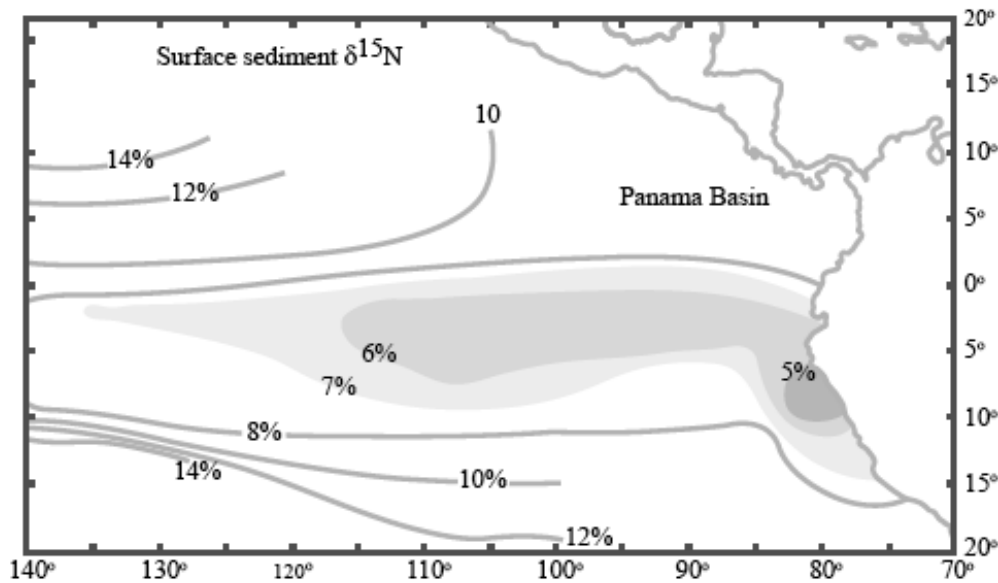
Kroopnik 1985

# Isotopic fractionation during biological processes

- N is important in many biological processes
  - Key component of amino acids, proteins and RNA/DNA.
- Five important inorganic forms
  - $N_2$ ,  $NO_3^-$ ,  $NO_2^-$ ,  $NH_3$  and  $NH_4^+$
- More dynamic than C

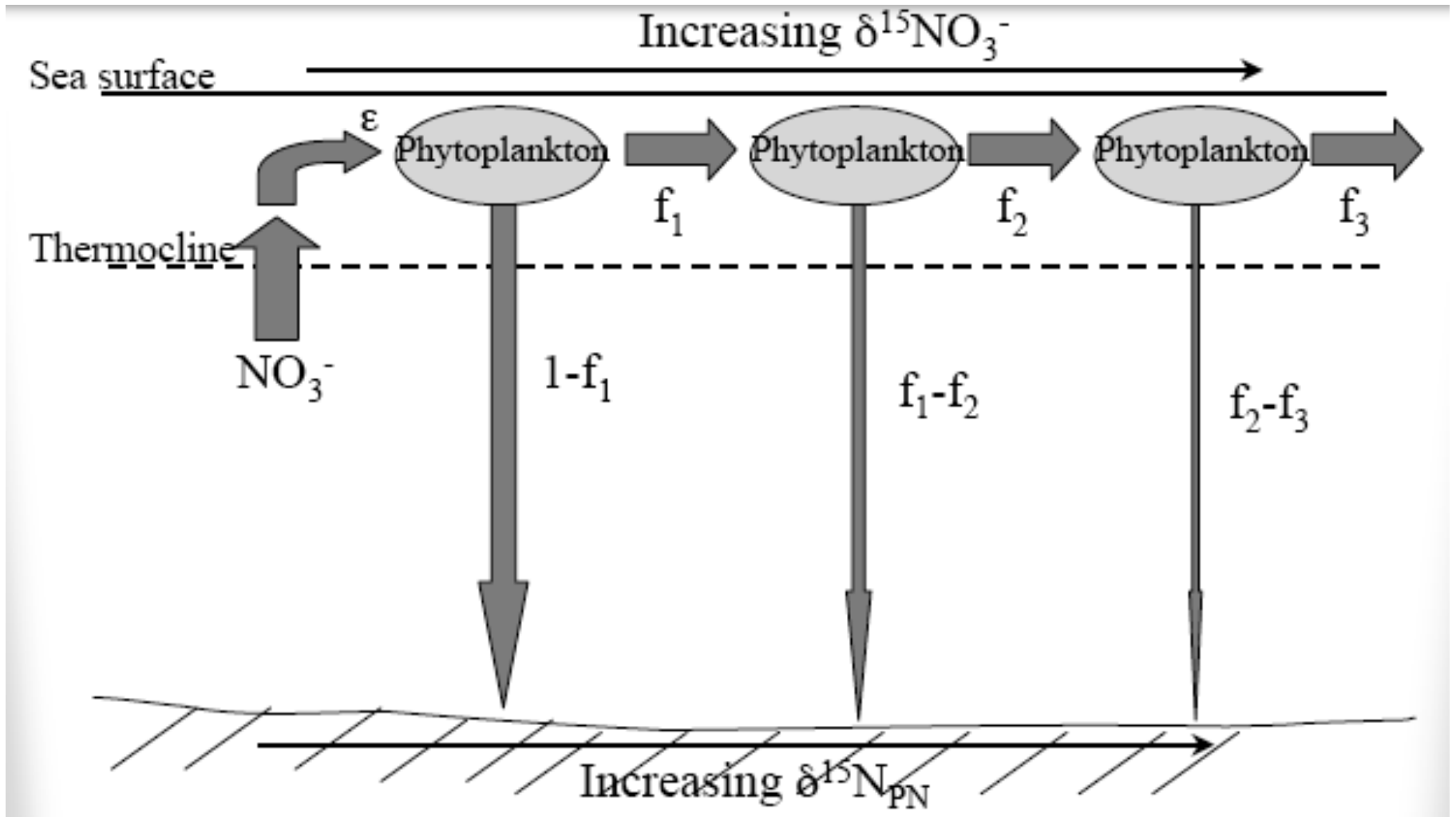


# Isotopic fractionation during biological processes



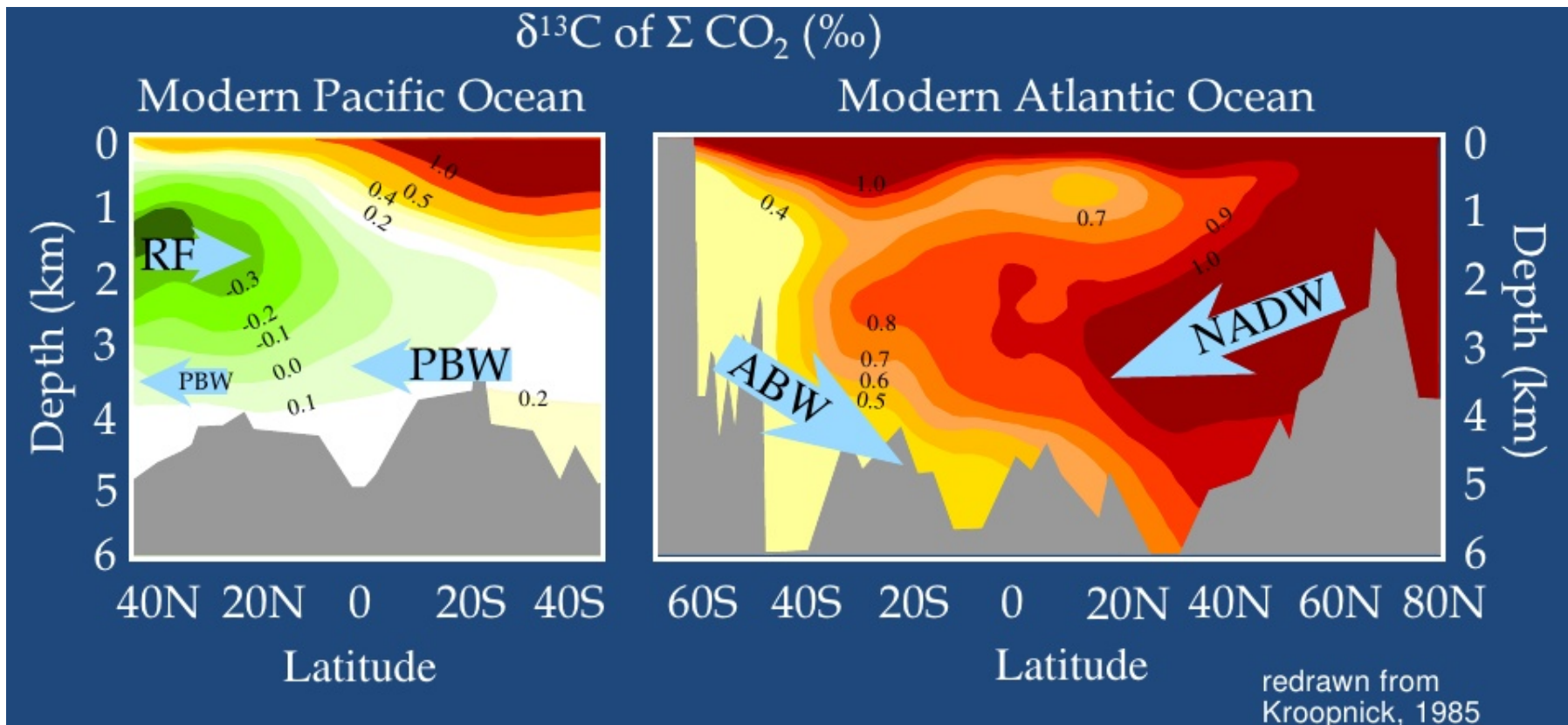
- Indeed explains observations in the ocean:
  - $\text{NO}_3$  rich water is upwelled
  - Fuels Phytoplankton
  - They remove  $\text{NO}_3$
  - POM detritus increases in  $\delta^{15}\text{N}$  away from source

# Isotopic fractionation during biological processes



# Application: Ocean circulation

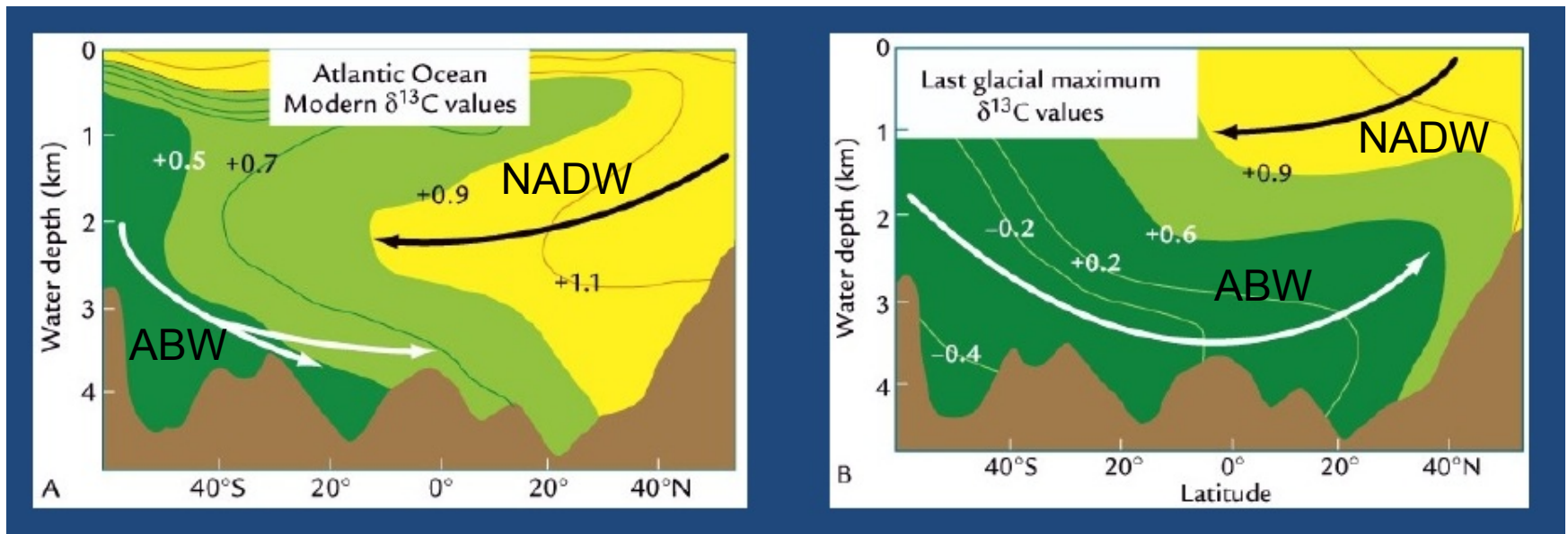
- Using changes in  $\delta^{13}\text{C}$  to age water masses
  - More decay = release of  $^{12}\text{C}$
  - Older water = lower  $\delta^{13}\text{C}$





# Application: Ocean circulation

- Can then use sediment cores to determine past conditions.
- Shoaling of Atlantic Bottom Water during LGM
- Combination of pelagic and benthic foramenifera data.



# Using $^{14}\text{C}$ decay to study ocean mixing

- Created naturally in atmosphere
  - Perfect half life (5.7k years)

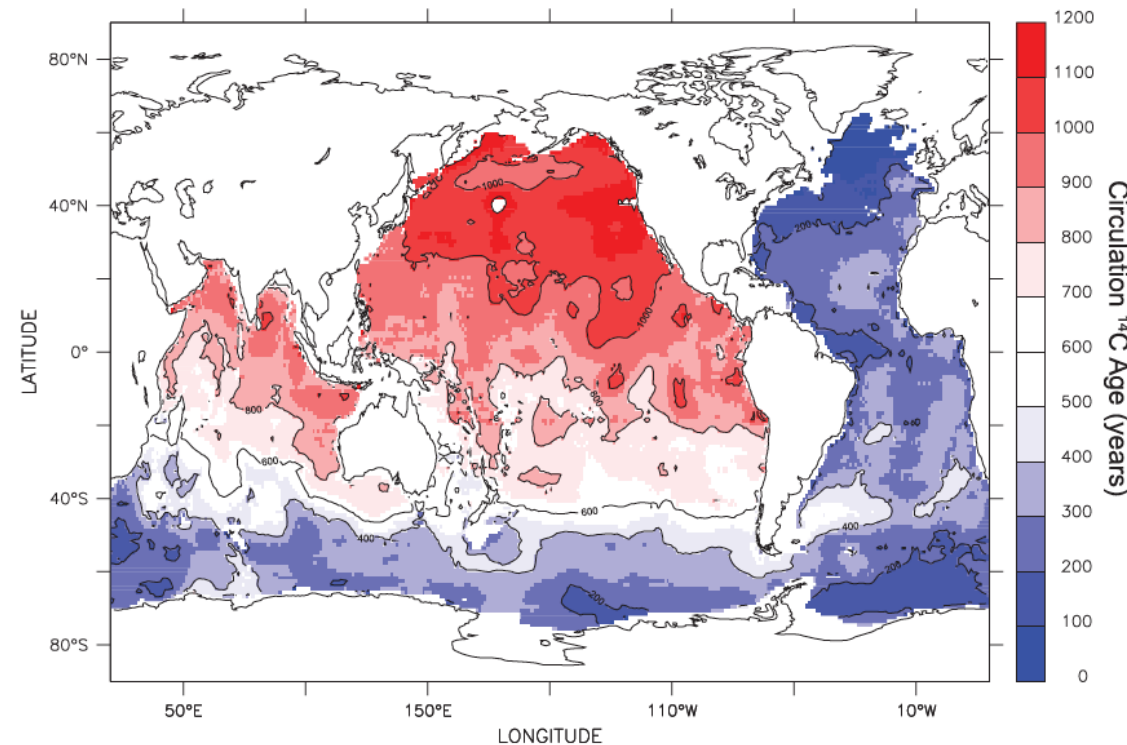
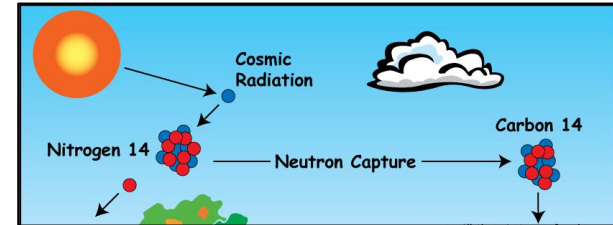
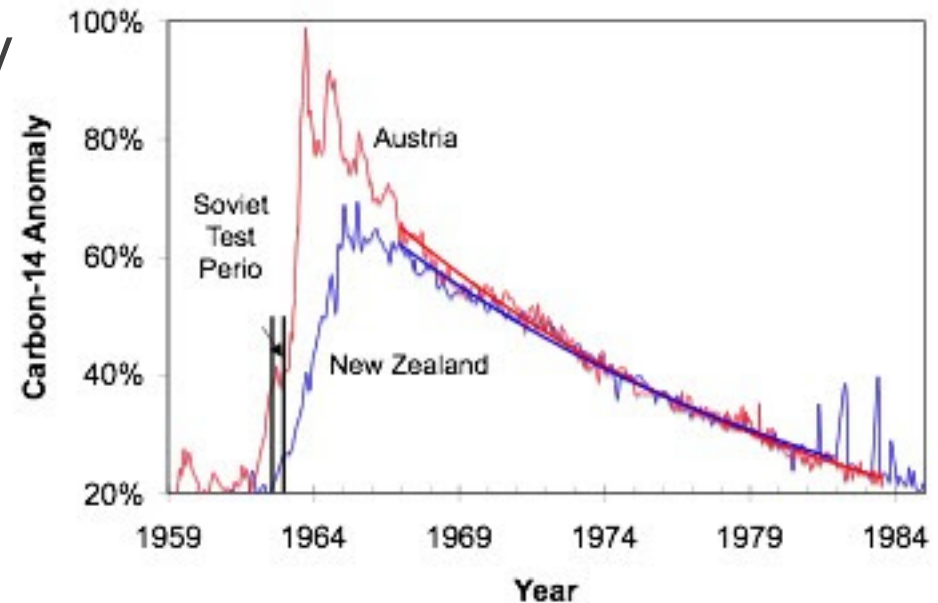


Figure 4. A map of circulation  $^{14}\text{C}$  age below 1500 m. This is equivalent to conventional  $^{14}\text{C}$  age (Figure 1) but accounts for surface ocean  $^{14}\text{C}$  reservoir age and the different sources of deep water. Unit is years.

- From this it was possible to age the different water masses and confirm the general circulation pattern of the ocean currents.
- Anthropogenic contamination! Why?

# Using $^{14}\text{C}$ decay to study ocean mixing

- During the 1940s-50's many atom bombs were tested
  - Lead to huge amounts of added 'anthropogenic'  $^{14}\text{C}$ .
- While this led to complication using natural  $^{14}\text{C}$  dating techniques it represented a 'in situ' experiment allowing for studies of ocean ventilation and mixing rates of surface waters to the deep.



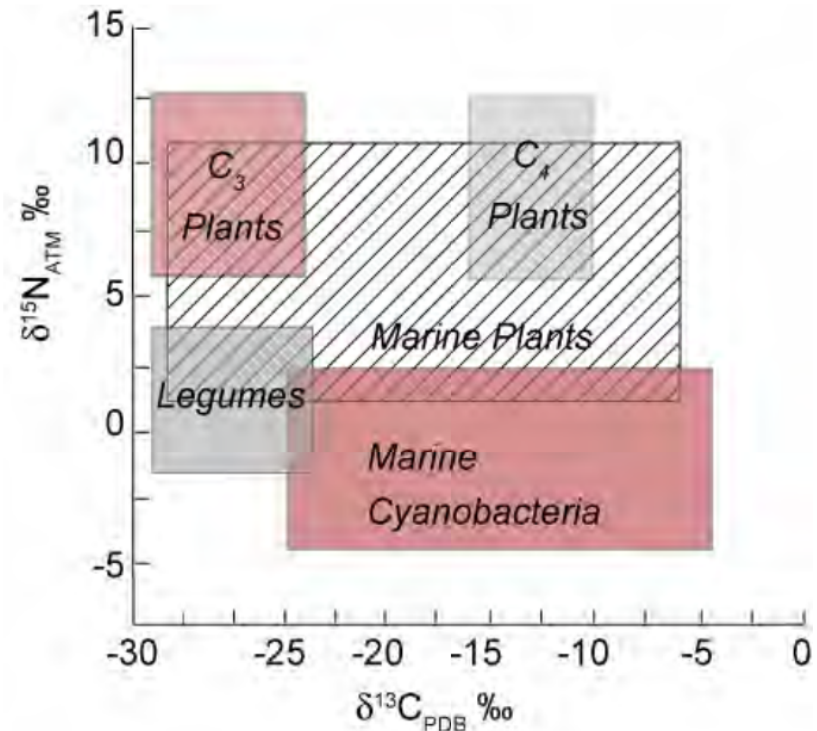
Absorption of  $\text{CO}_2$  after Nuclear Airburst





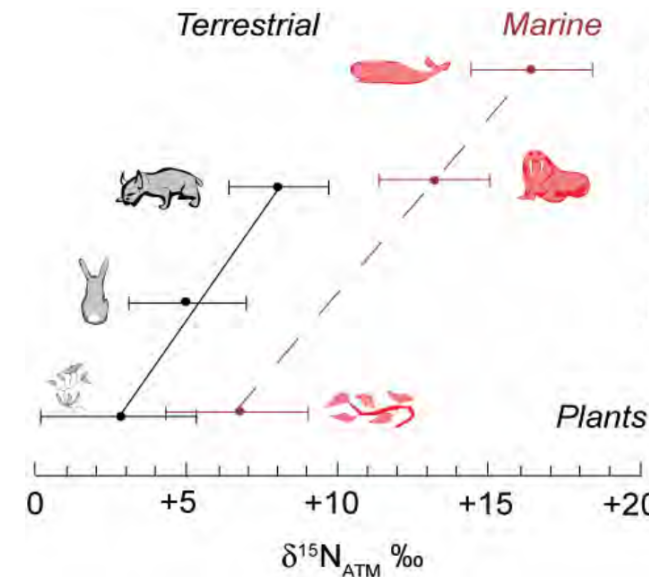
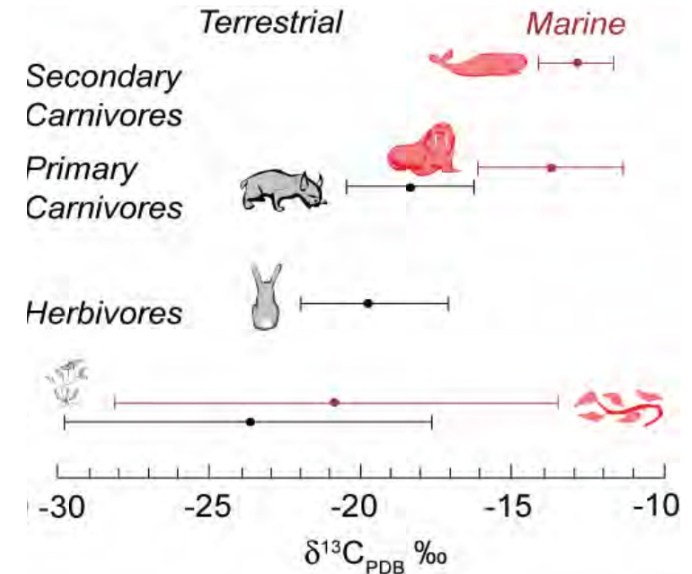
# Application: Isotopic fingerprinting

- Plants can be grouped based on their C and N isotopic composition due to differences in fractionations of marine, C<sub>4</sub> and C<sub>3</sub> autotrophs and nitrogen fixers (cyanobacteria and legumes) and non-nitrogen fixers.

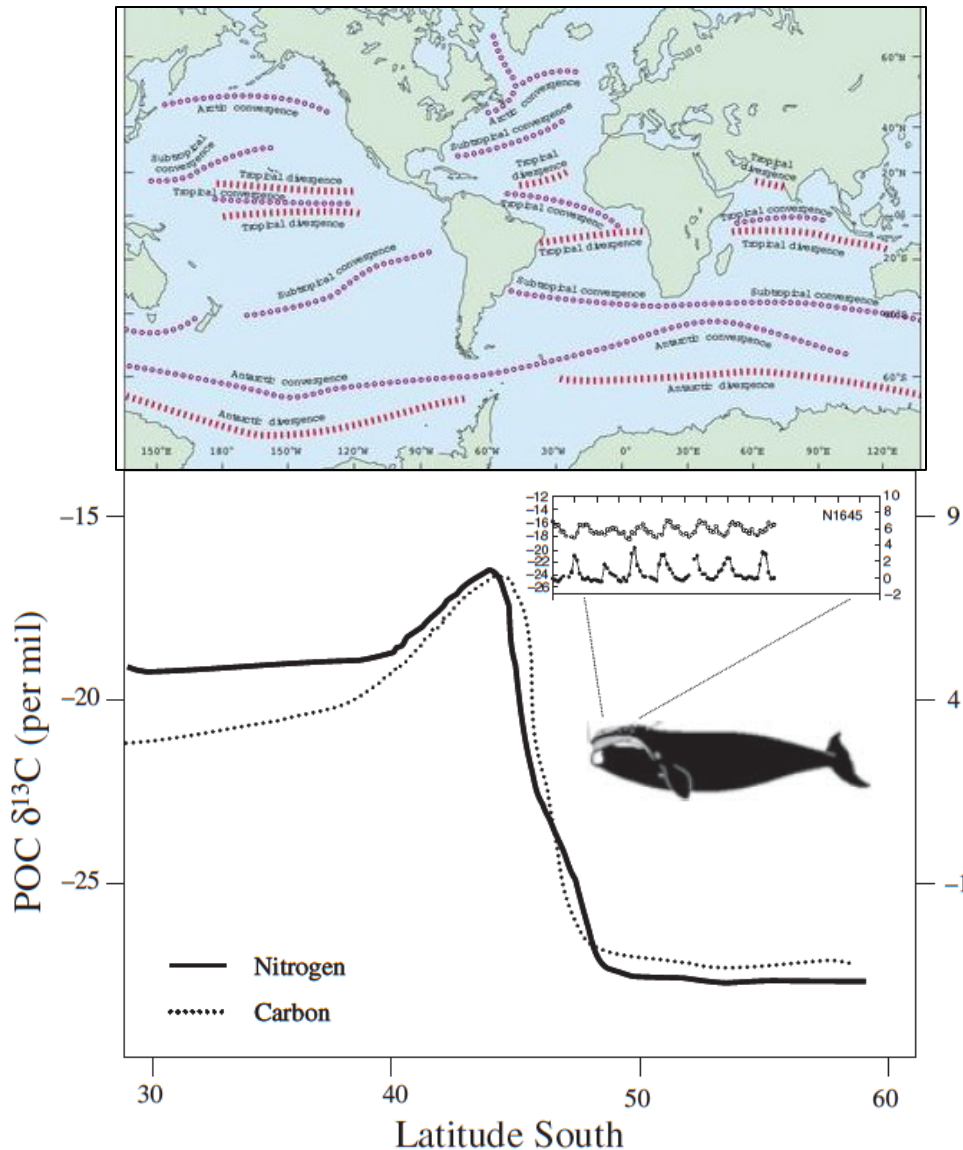


# You are what you eat!

- C isotopes undergo 1-2‰ fractionation with each trophic level. Given the large variation seen in the autotrophs these changes are small.
- N isotopes undergo 1-2‰ fractionation with each step. These are relative large compared to the various autotrophs and thus are good indicators of trophic level.



# Where do the whales go to eat?



- This type can be used to analyze migration patterns
- We know the isotopic POM values of different regions.
- Measure isotopic composition of baleen 'tree rings'

# Application: Harmful Algal Bloom (HAB) ecology



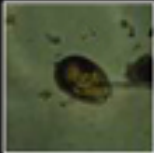


- Growth of an algal species to densities which negatively impact human health or an ecosystem.





# Human Health Syndromes

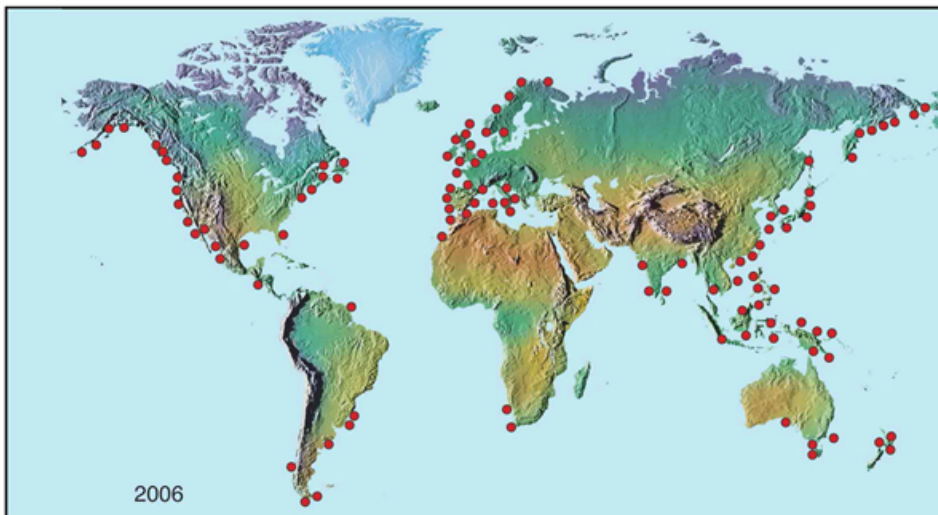
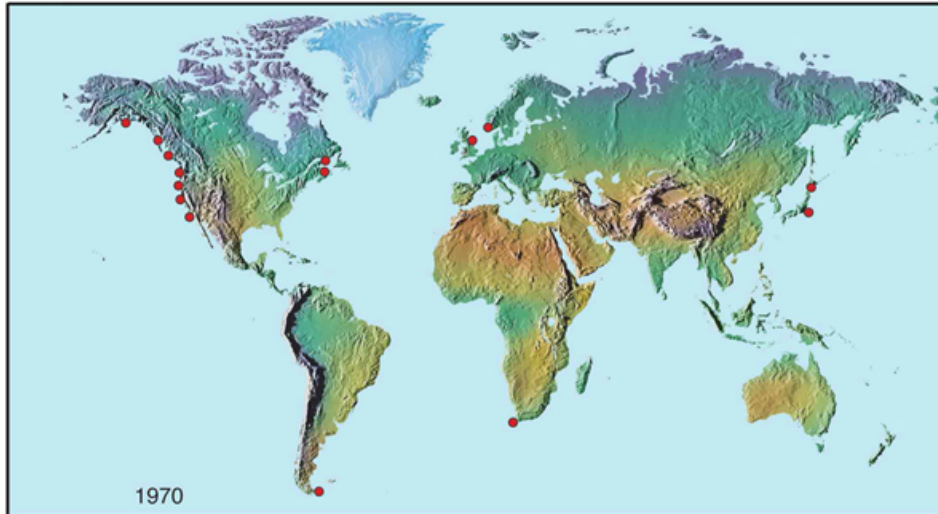
*associated with phytoplankton*

Name of Syndrome	Species and Toxin	Symptoms
Amnesic Shellfish Poisoning (ASP)	<i>Pseudo-nitzschia</i> <b>Domoic acid</b> 	Short term memory loss
Diarrhetic Shellfish Poisoning (DSP)	 <i>Dinophysis</i> <b>Okadaic acid</b>  <i>Prorocentrum lima</i>	Diarrhea Nausea Vomiting
Neurotoxic Shellfish Poisoning (NSP)	 <i>Karenia brevis</i> <b>Brevetoxin</b>	Respiratory problems
Paralytic Shellfish Poisoning (PSP)	<i>Alexandrium</i> <b>Saxitoxin</b> 	Loss of motor control

# The expansion of harmful algal blooms worldwide

HABs cost ~\$50 million annually in the US alone

• PSP

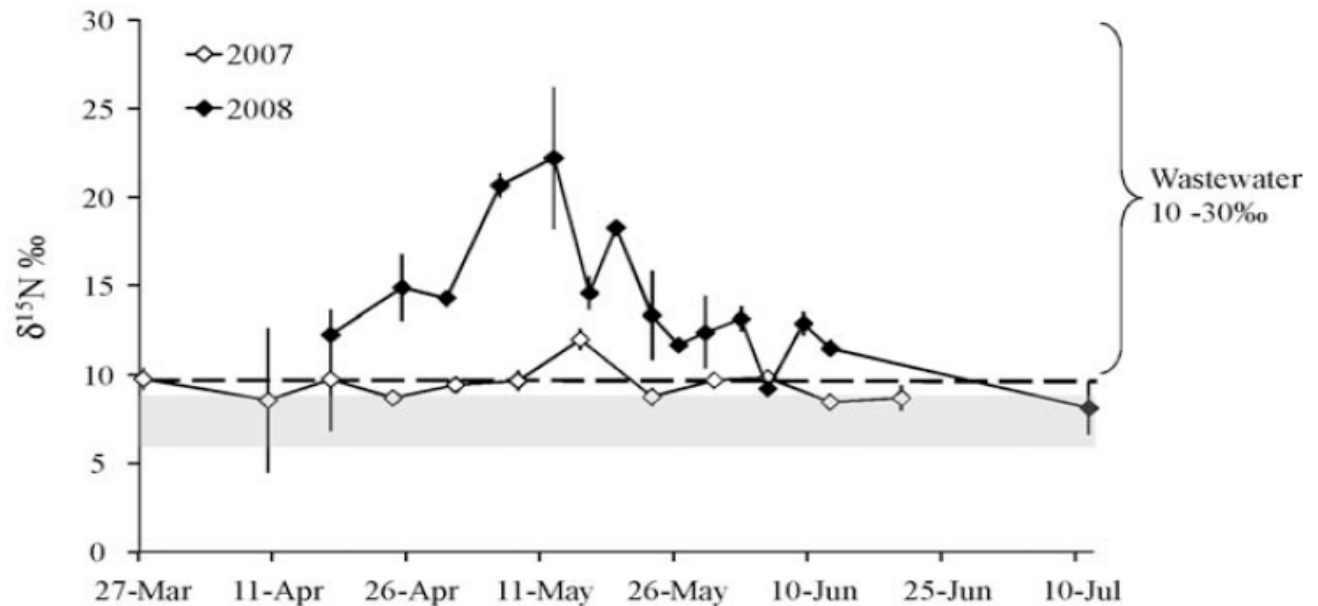
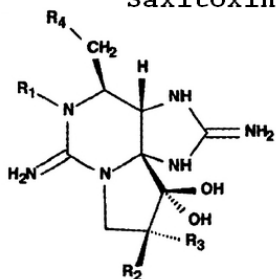


# Application: What sustains harmful algal blooms?

- *Alexandrium tamarenses* is a world wide problem
- What fuels these blooms?
- Isotopic N signature of Wastewater is higher than that of ambient seawater. You are what you eat.....they utilize WW derived N.



Saxitoxin

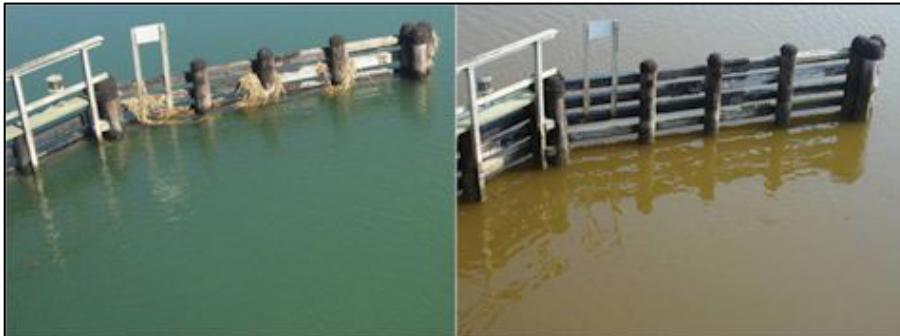


Hattenrath et al. 2010

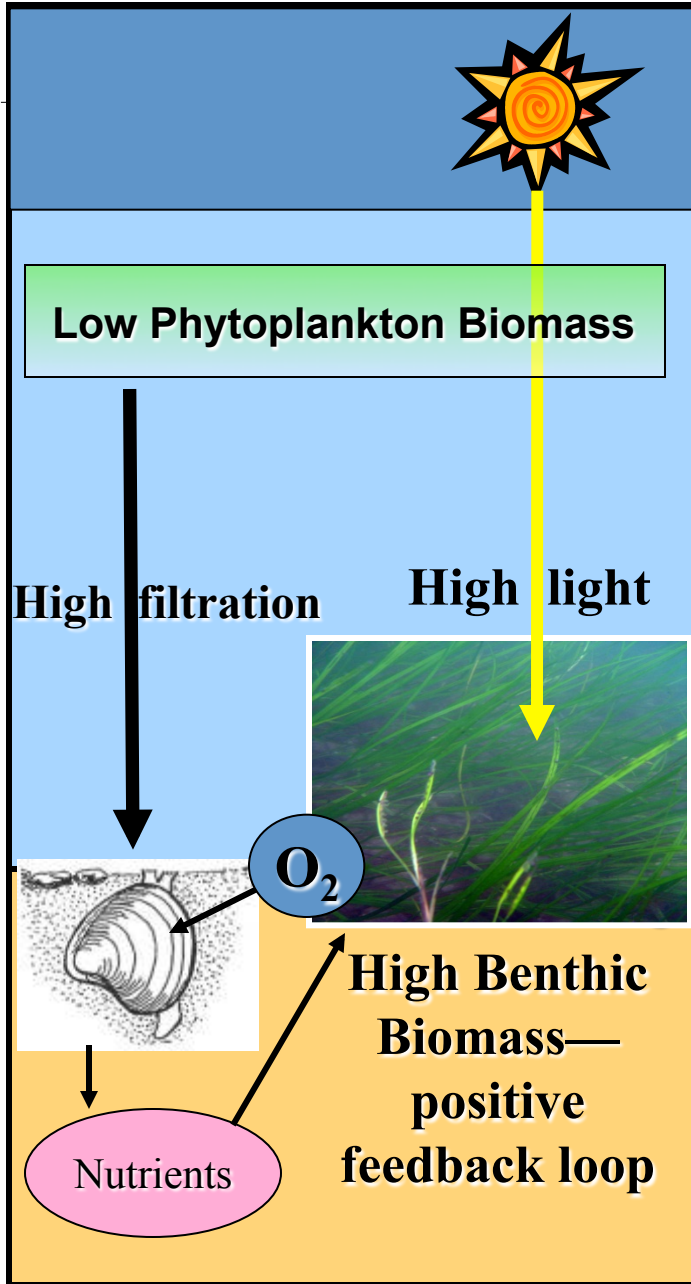
# Another HAB: brown tides



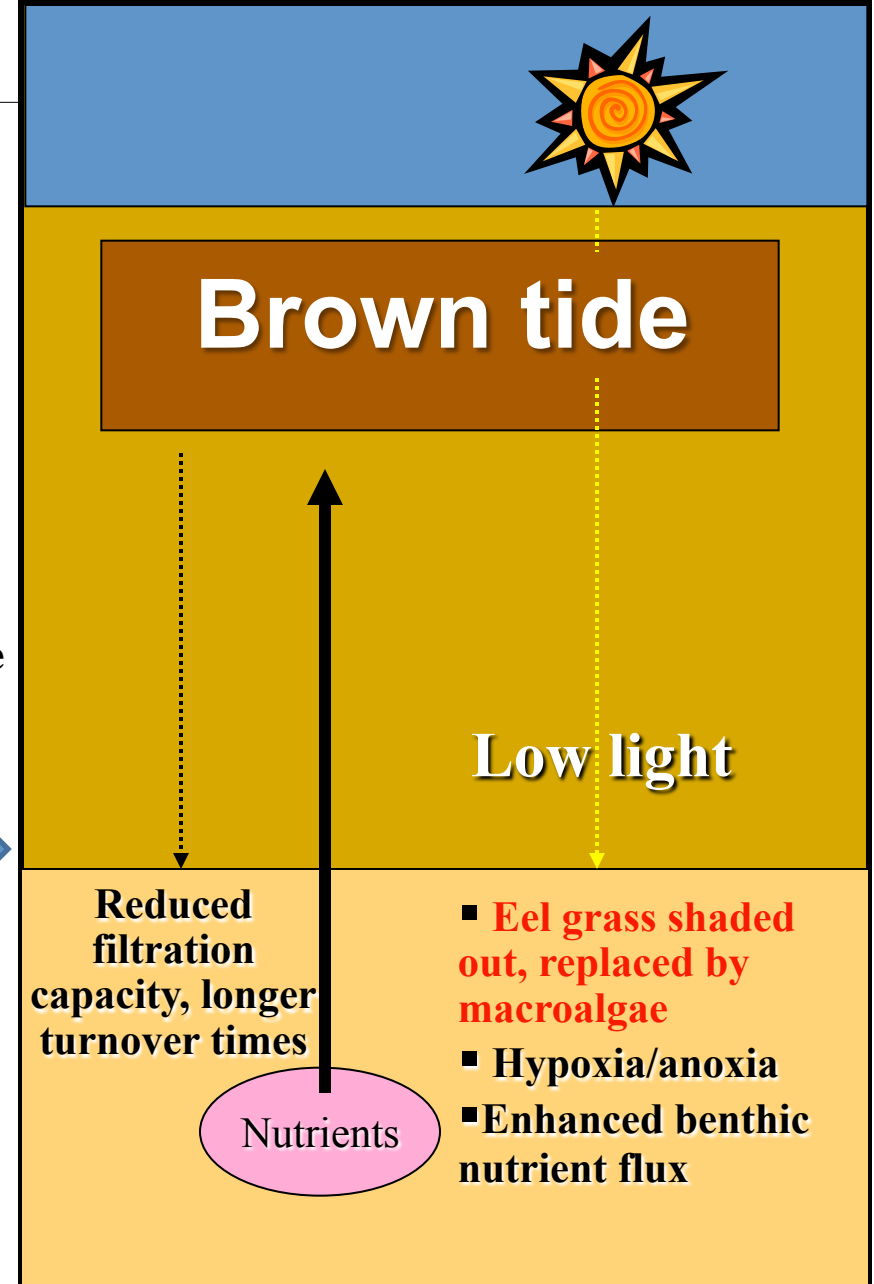
- Do not produce toxins.
- Ecosystem disruptive class of algae.
- Found in Northeastern US, Florida and China



# Normally functioning estuary

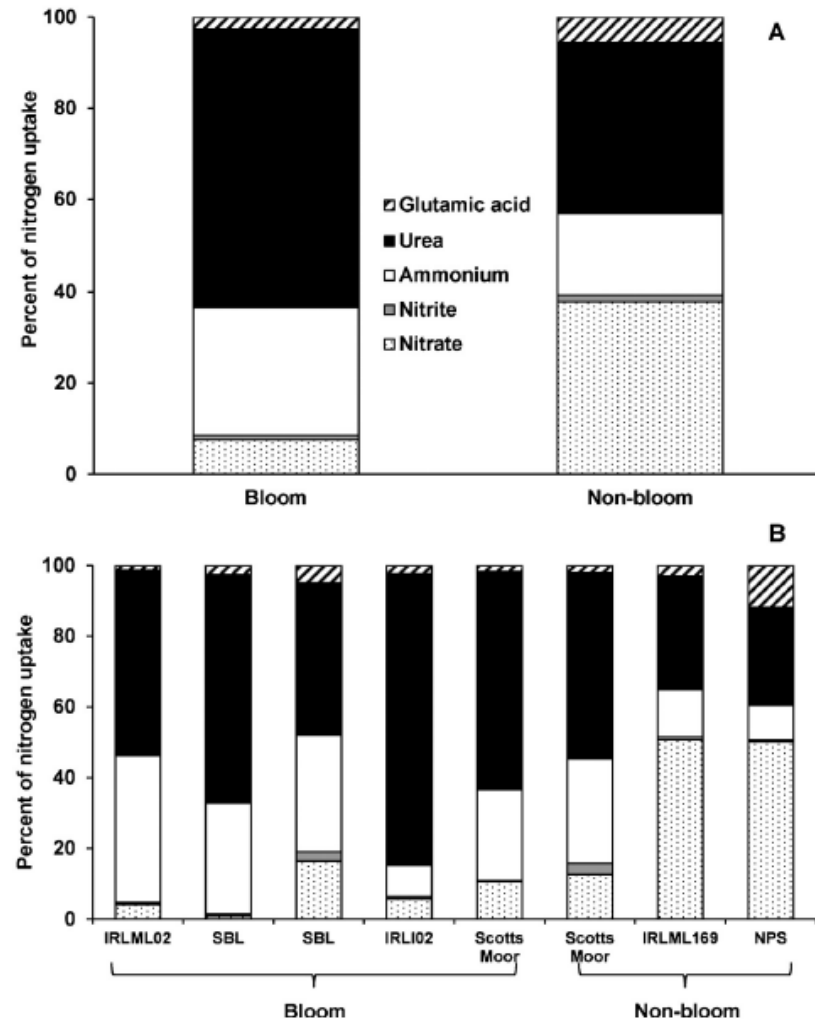


# Estuary with brown tide



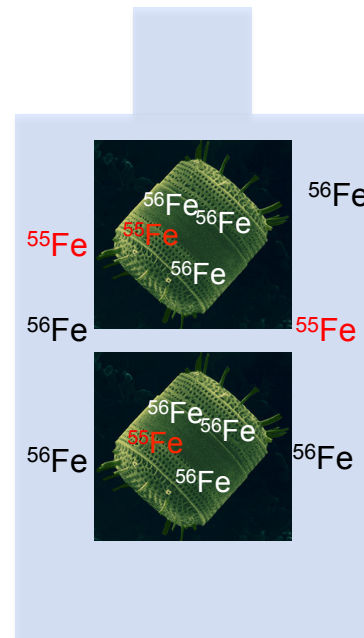
# What fuels these blooms?

- Naturally >99% is  $^{14}\text{N}$
- Create  $^{15}\text{N}$ -labeled compounds (99%  $^{15}\text{N}$ )
- Add to water and measure changing  $^{15}\text{N}/^{14}\text{N}$  ratios to calculate uptake rates.
- Brown tide runs on reduced forms
  - Ammonium and Urea
- Can devise mitigation strategies



# Application: Radioisotopes as tracers

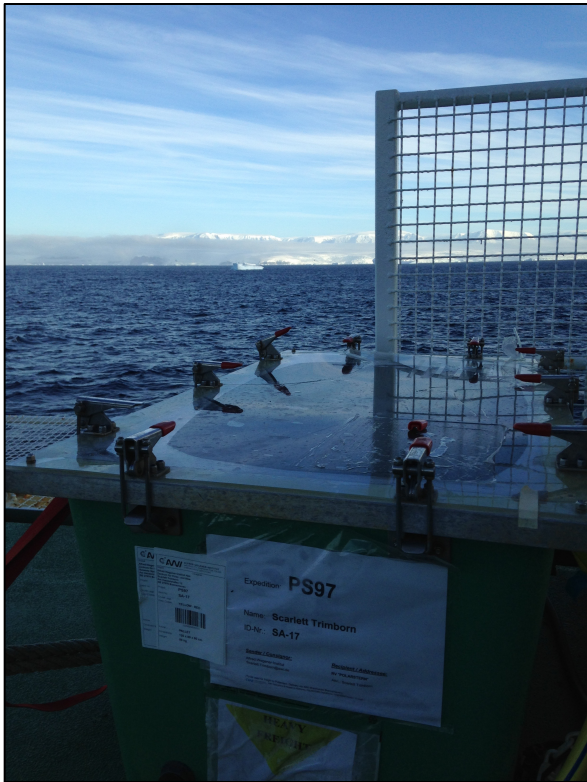
- Besides the dating of processes (through their decay), radioisotopes can be used as a 'tag' to measure uptake of nutrients.
- More sensitive than stable isotopes and more importantly need less material.



$$\frac{A_{55\text{Fe}} \text{ on filter (plankton)}}{A_{55\text{Fe}} \text{ added to water}} \times \frac{[\text{Fediss}] + [\text{Fe added}]}{\text{Incubation time}} = \rho [\text{Fe}]$$

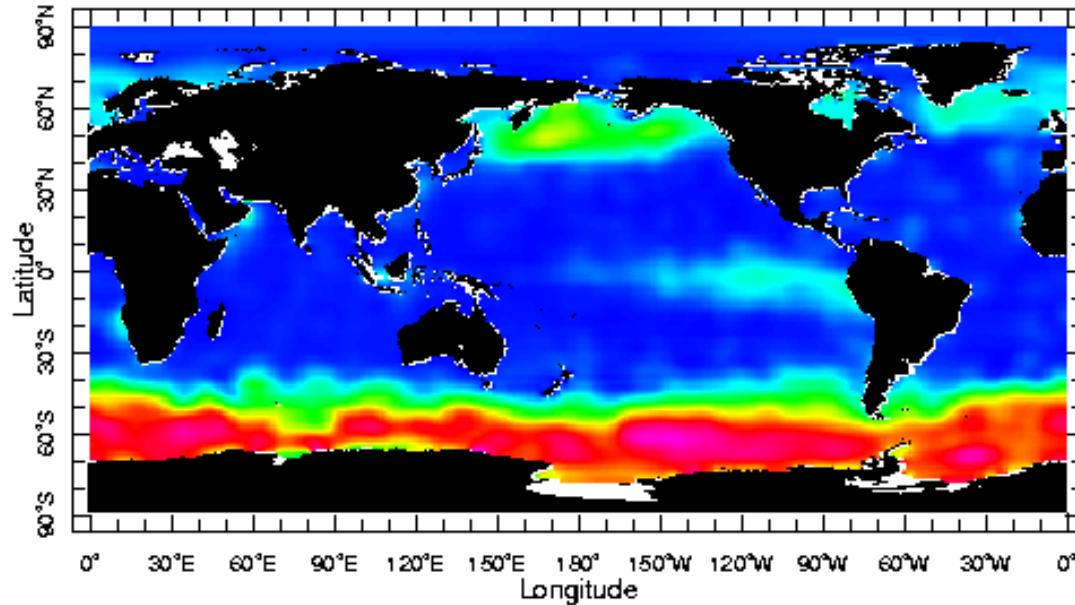


# Using a simple but novel approach to elucidate the dynamics and effects of Iron, Zinc, Cobalt and Vitamin B<sub>12</sub> cycling on the plankton communities in the Polar Ocean





# Using tracers to study trace element cycling in the Southern Ocean



m



- Strong influence on global carbon cycle
- 40% uptake of anthropogenic CO<sub>2</sub>
- 20% of global marine primary production
- In large parts limited by trace elements (Fe, vitamins).

# Goals

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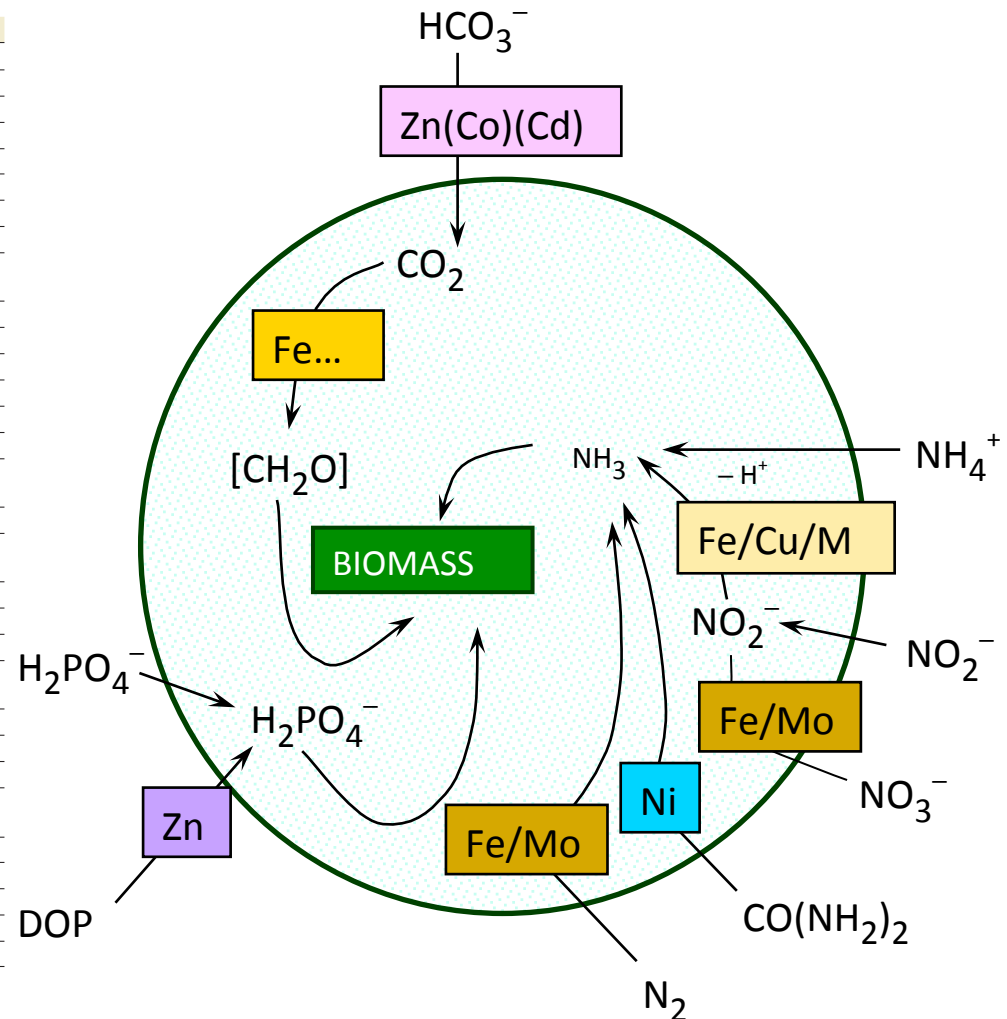
- To investigate the effects of trace metal limitation on the physiology and composition of plankton communities both in the lab and in the field.
- To understand the relative importance of removal and production/recycling mechanisms for Fe, Zn, Co, Vitamin B<sub>12</sub>, and the key players responsible.

This will help explain observed limitations and co-limitations of plankton communities in the field

# Trace metal and vitamin requirement. What is it used for?

Table 1 Common metalloproteins present within marine phytoplankton

Metal	Protein(s)	Function(s)
Fe	Cytochromes	Electron transport in photosynthesis and respiration
	Ferredoxin	Electron transport in photosynthesis and N fixation
	Other Fe-S proteins	Electron transport in photosynthesis and respiration
	Nitrate and nitrite reductase	Conversion of nitrate to ammonia
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
	Catalase	Conversion of hydrogen peroxide to water
	Peroxidase	Reduction of reactive oxygen species
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O <sub>2</sub>
	Zn	Carbonic anhydrase
Alkaline phosphatase		Hydrolysis of phosphate esters
RNA polymerase		Nucleic acid replication and transcription
tRNA synthetase		Synthesis of tRNA
Reverse transcriptase		Synthesis of single-stranded DNA from RNA
Carboxypeptidase		Hydrolysis of peptide bonds
Superoxide dismutase		Disproportionation of superoxide to hydrogen peroxide and O <sub>2</sub>
Mn	O <sub>2</sub> -evolving enzyme	Oxidation of water during photosynthesis
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O <sub>2</sub>
	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Ni	Urease	Hydrolysis of urea
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O <sub>2</sub>
Cu	Plastocyanin	Photosynthesis electron transport
	Cytochrome oxidase	Mitochondrial electron transport
	Ascorbate oxidase	Ascorbic acid oxidation and reduction
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O <sub>2</sub>
	Multicopper ferroxidase	High-affinity transmembrane Fe transport
Co	Vitamin B <sub>12</sub> <sup>a</sup>	C and H transfer reactions
Cd	Carbonic anhydrase <sup>b</sup>	Hydration and dehydration of carbon dioxide
Mo	Nitrate reductase	Conversion of nitrate to ammonia
	Nitrogenase	N fixation



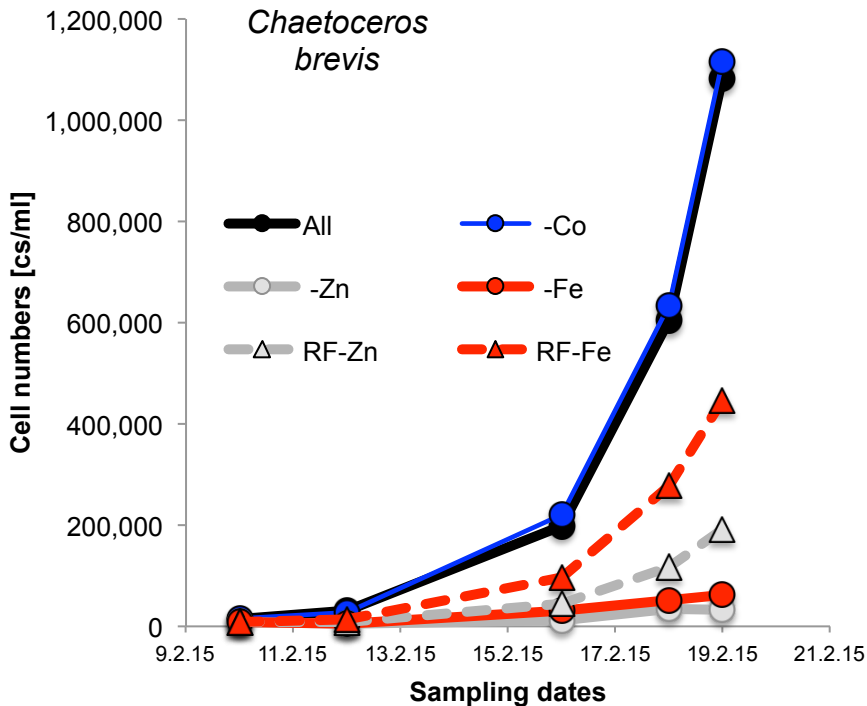
Adapted from Sunda (1988/1989), with additional information from Raven et al. (1999), Frausto da Silva & Williams (2001), and Wolfe-Simon et al. (2005).

<sup>a</sup>Cofactor in a number of enzymes.

<sup>b</sup>Has been found only in diatoms (Price & Morel 1990, Lane & Morel 2000).

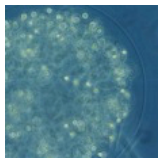
Morel and Price 2003, Michel and Pistorious 2004

# What are the effects of trace metal/vitamin limitation on the physiology of different groups?



## Parameters assessed:

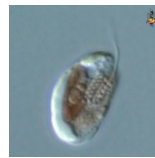
- Photophysiology
- Cellular trace metal contents
- POC/PON
- Pigments
- Biogenic Silica
- Trace metal concentrations and quota
- RNA samples for transcriptomic analysis



*Phaeocystis antarctica*



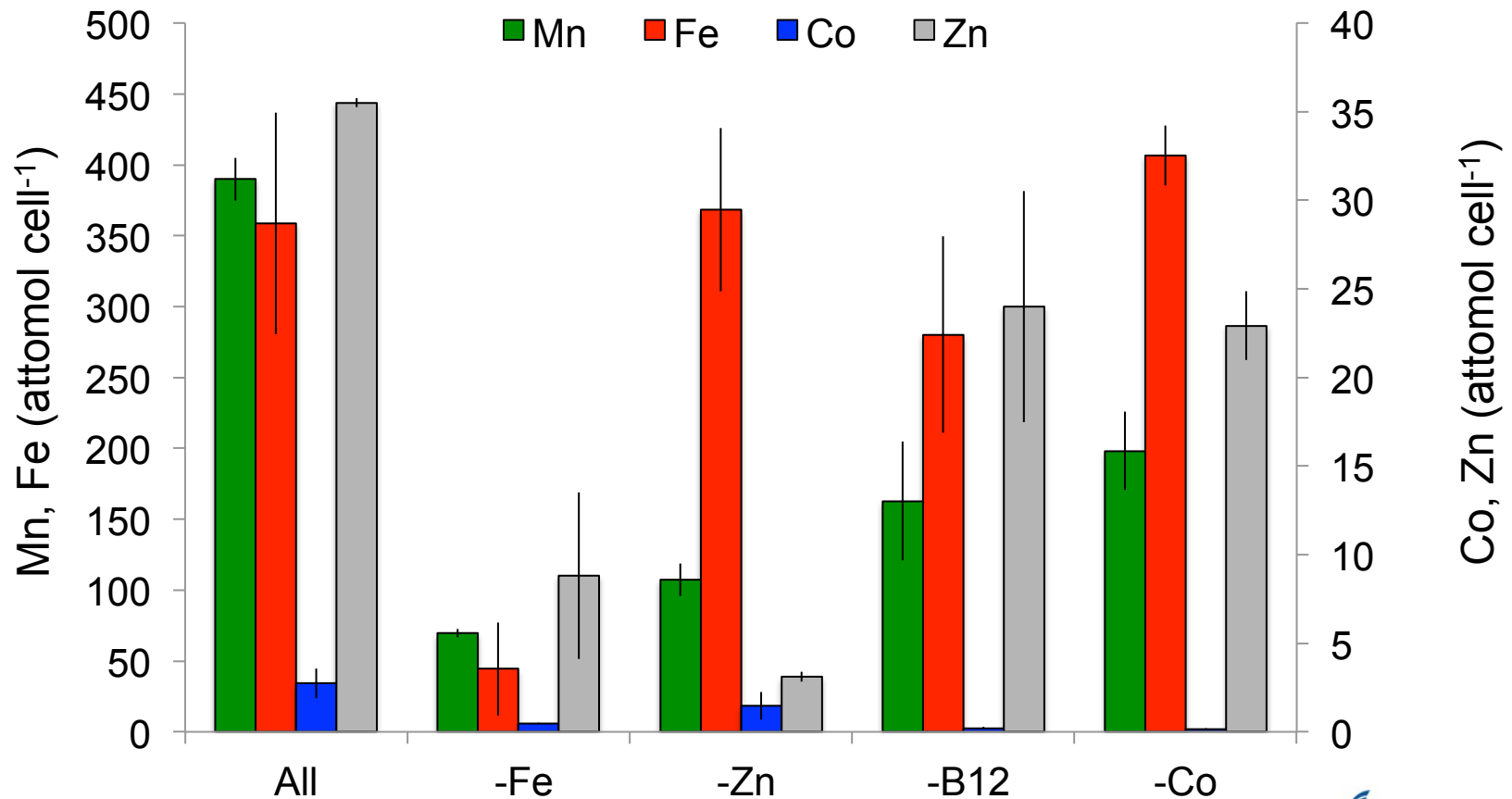
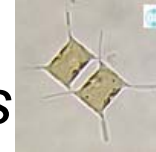
*Chaetoceros brevis*



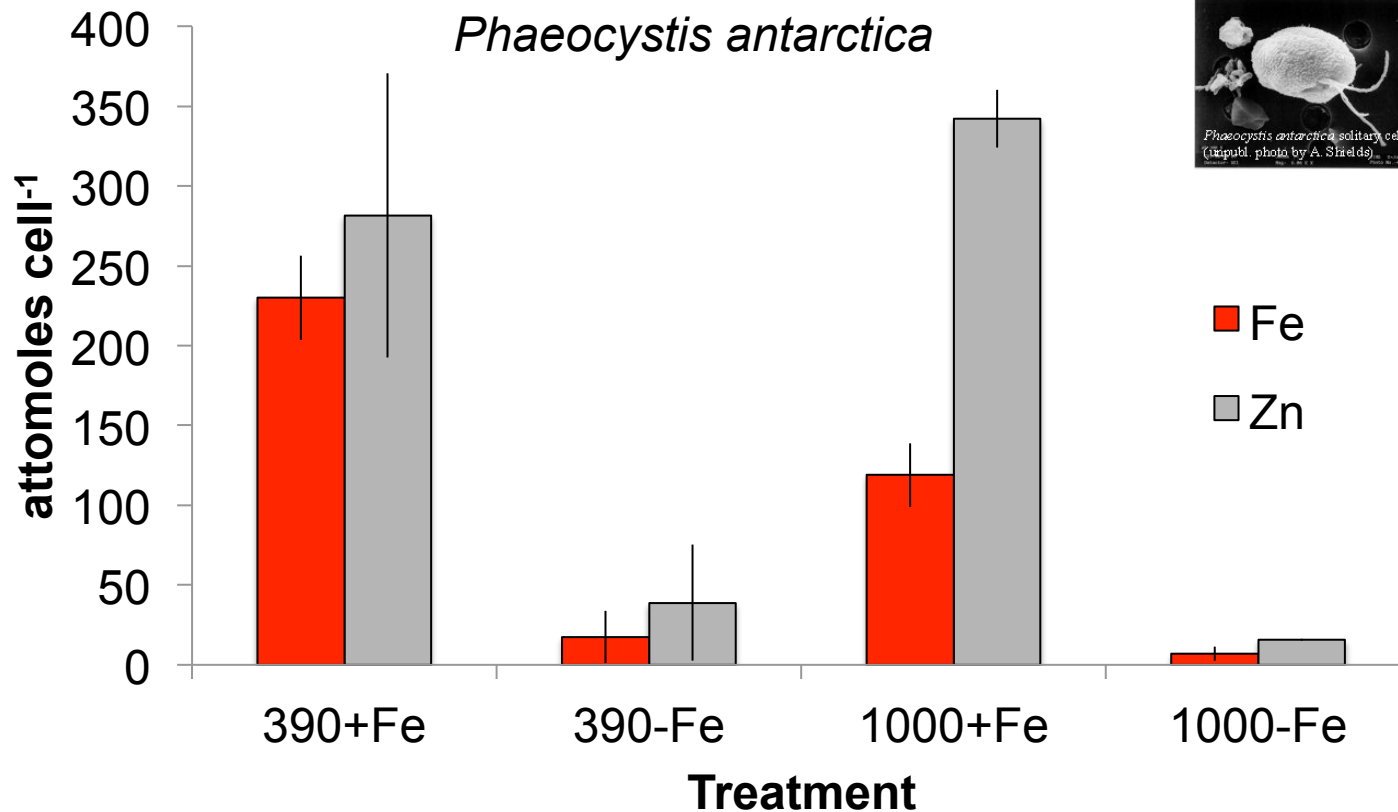
*Geminigera sp.*

# Trace Metal Quota under various limitations

*Chaetoceros brevis*



# What are the effects of trace metal limitation and CO<sub>2</sub> on the physiology of different groups?



# Trace Metals and Vitamins are important in many cellular processes of phytoplankton

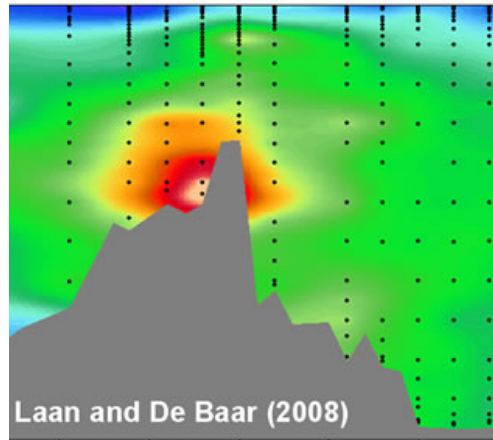


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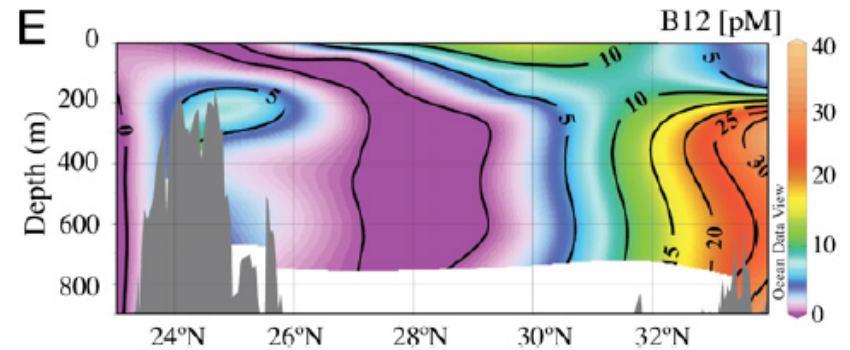
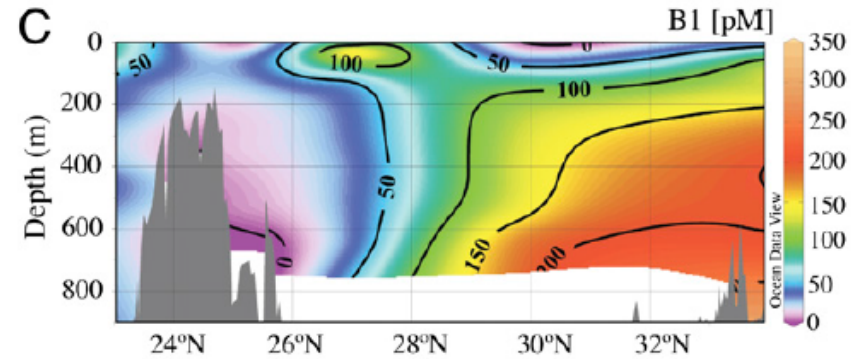
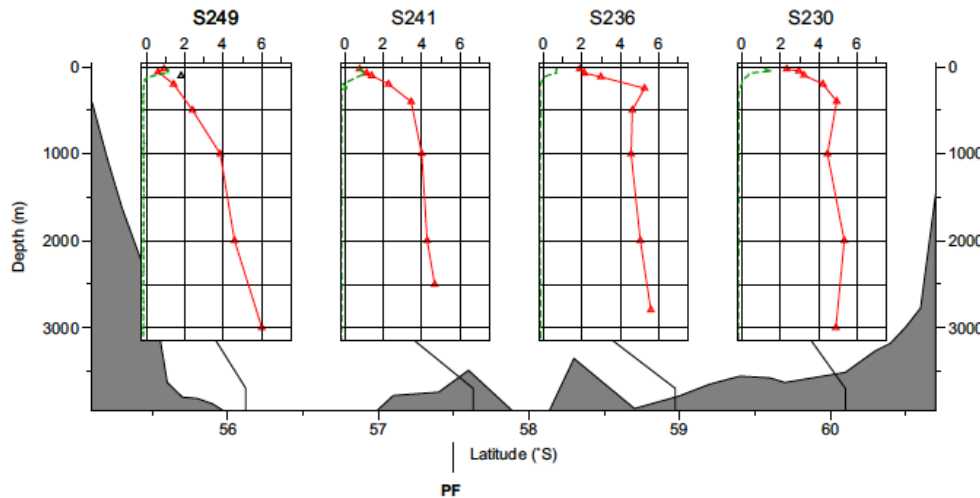
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	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Co	Vitamin B <sub>12</sub> <sup>a</sup>	C and H transfer reactions

# Trace metals/vitamins are present at low concentrations (pM-nM)

*dFe* [nmol/l]



Laan and De Baar (2008)



Sanudo-Wilhelmy et al. 2012

Fig. 5. Distribution of Zn across the Drake Passage during ANTXXIV-3. Croot et al. 2012

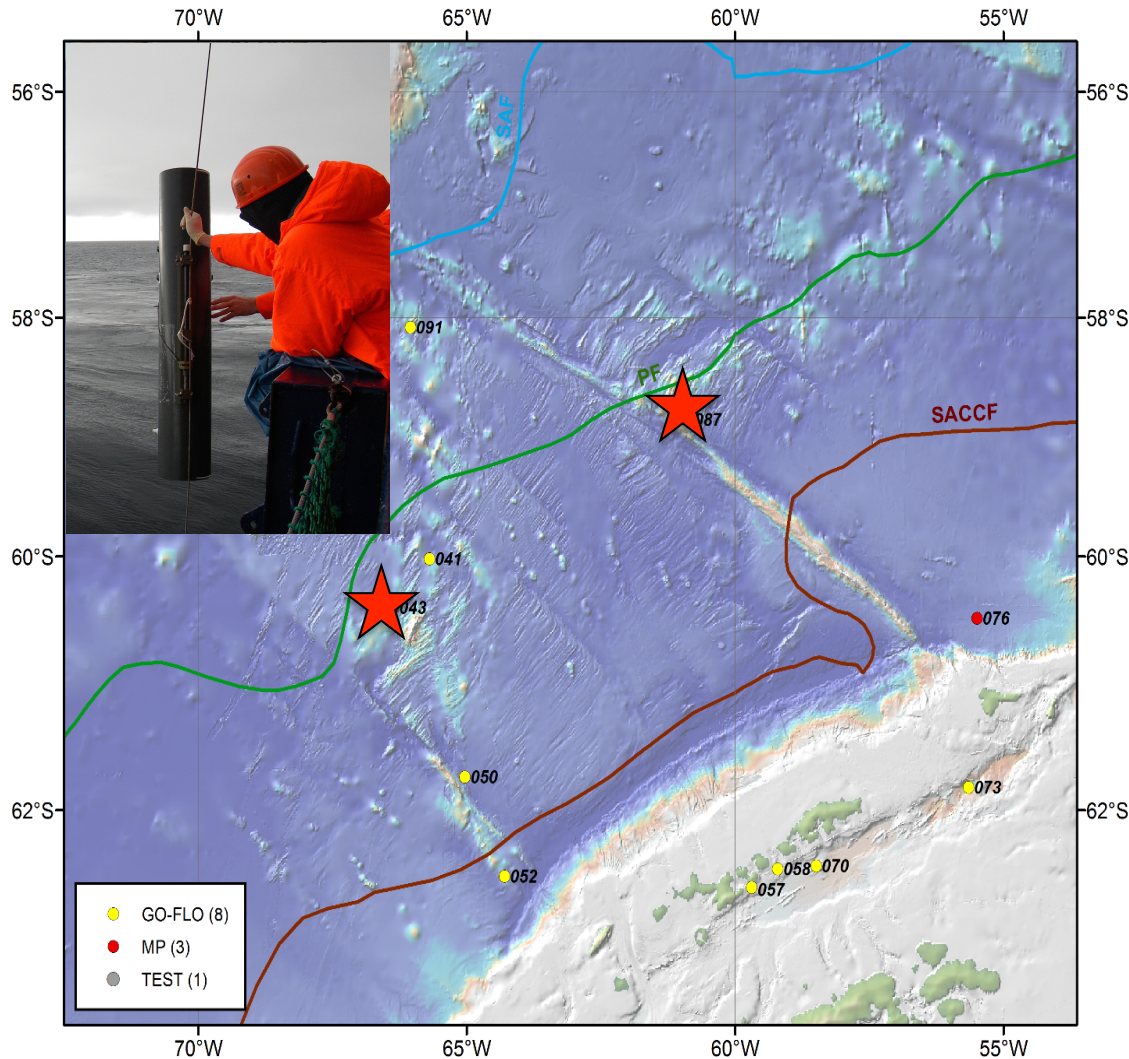


# Trace Metals/Vitamins can limit Primary Productivity



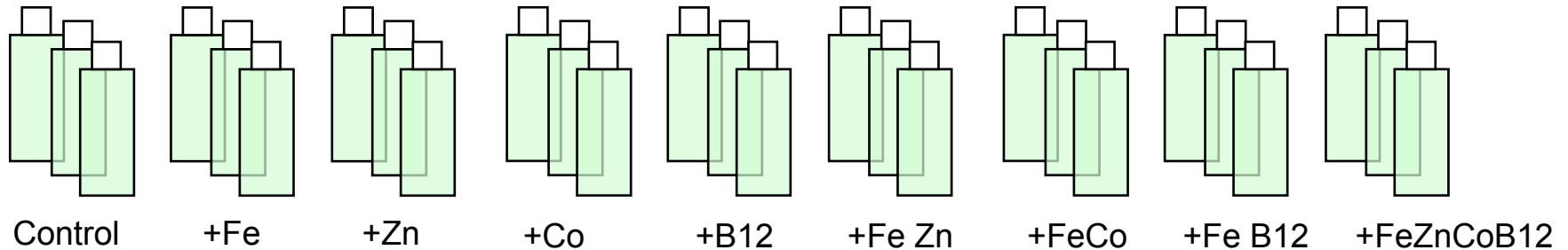
- Fe has been shown to be the primary limiting element in 20% of the world's oceans.
- A few studies found Zn additions to minimally affect biomass/species composition in polar waters Coale et al. 1991, Schareck et al. 1997, Frank et al. 2000, Coale et al. 2003.
- Co implicated in limiting B<sub>12</sub> production in North Atlantic Panzeca et al. 2008.
- B<sub>12</sub> co-limits primary production in the Ross Sea and Antarctic Peninsula (Bertrand et al. 2007, 2014, Panzeca 2006) as well as limiting primary production and shaping community composition in the Gulf of Alaska (Koch et al. 2009) and various coastal ecosystems (Koch et al. 2011, 2012, 2013)

# PS97 (16.2.-8.4.2016) PaleoDrake

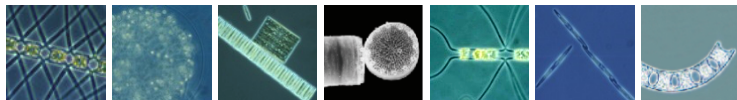
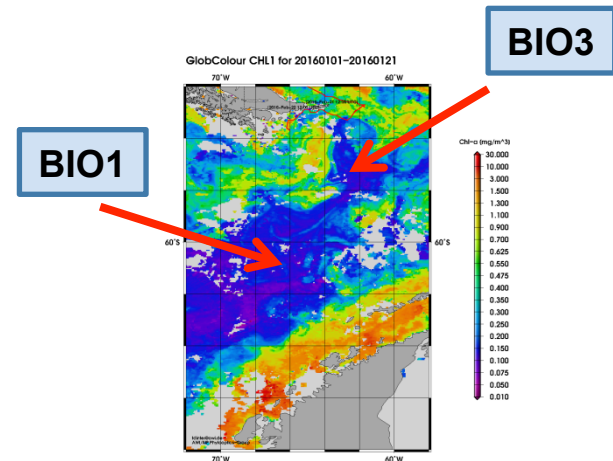
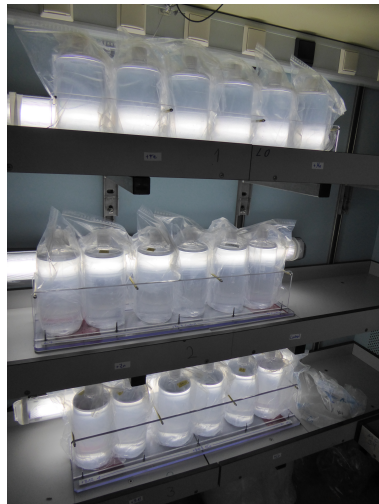


- 11 stations
- 2 long term Incubation Experiments (14 days) ★
- Size fractionated (0.2-2 $\mu$ m and >2 $\mu$ m) uptake of Fe, Zn, Co, B<sub>12</sub>, Primary Productivity.
- Characterization of plankton community
- Cellular TM contents
- T<sub>0</sub> and T<sub>1</sub> of TM/vitamin concentrations

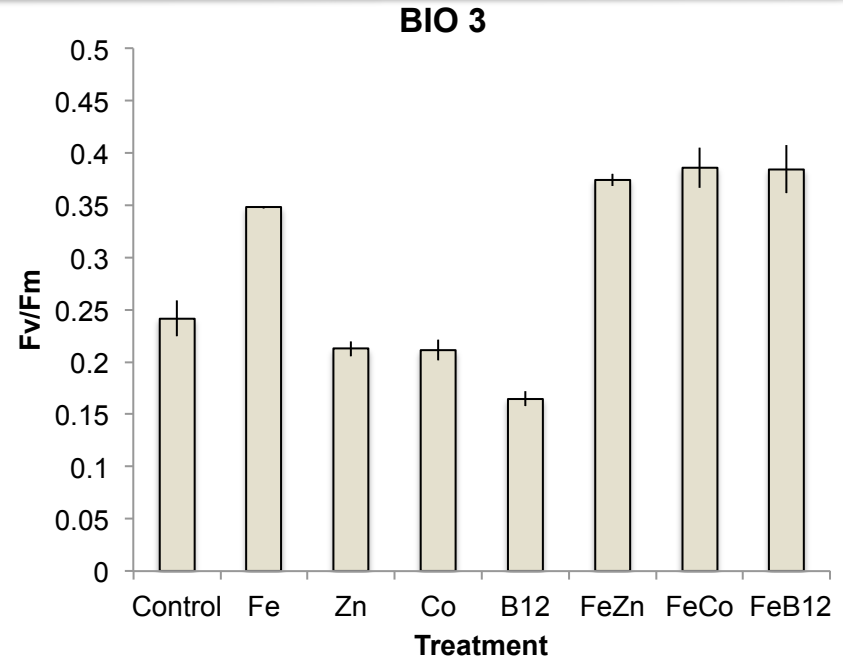
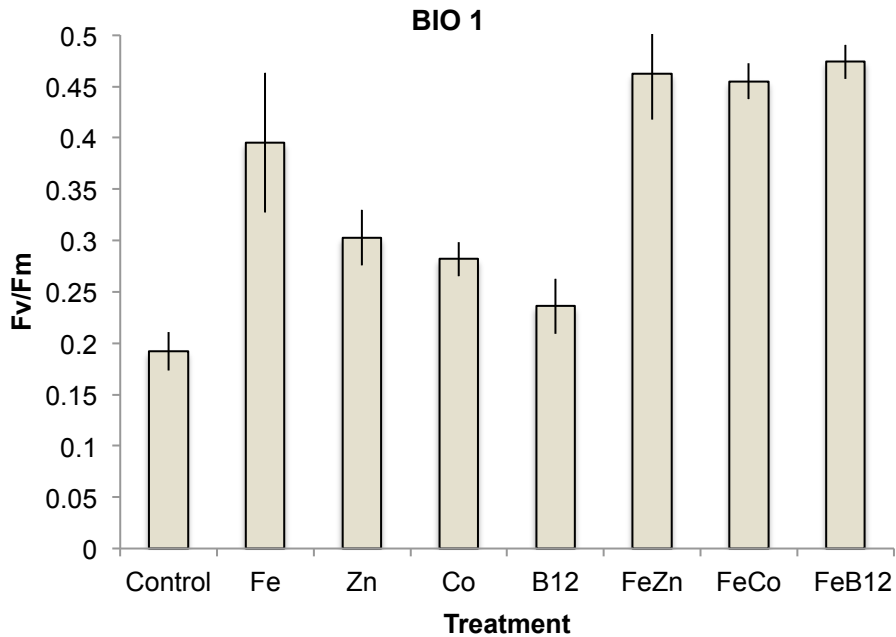
# Potential TM/vitamin limitation of the plankton community



**Incubation experiments with Fe, Co, Zn and vitamin B<sub>12</sub> (10-14 days):**

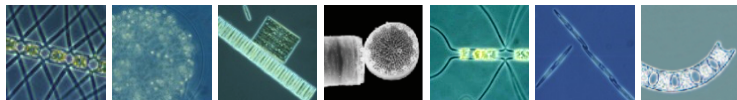
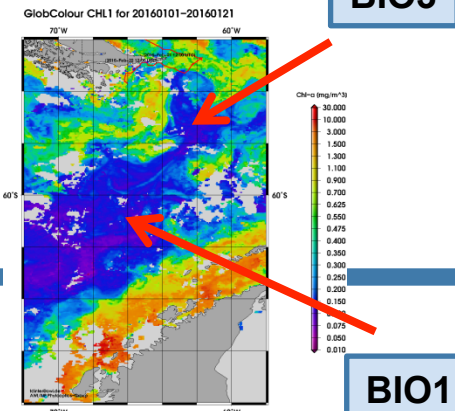


# Potential TM/vitamin limitation of the plankton community



→ Addition of Zn, Co and B<sub>12</sub> raises the yield for BIO 1!

→ Fe is the primary limiting trace metal at BIO 3!



# Goals

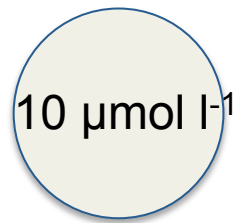
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- To understand the physiological effects of trace metal limitation on key phytoplankton groups.
- To understand the relative importance of removal and production/recycling mechanisms for Fe, Zn, Co, Vitamin B<sub>12</sub>, and the key players responsible.

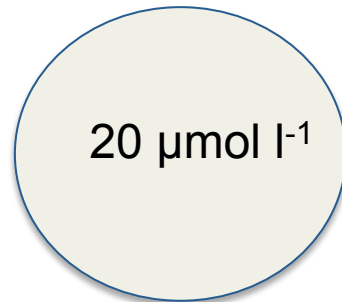
This will help explain observed limitations and co-limitations of plankton communities in the field

# How to measure recycling/production?

## Trace metal/vitamin concentrations (ICP-MS and HPLC-MS/MS)



Day 0

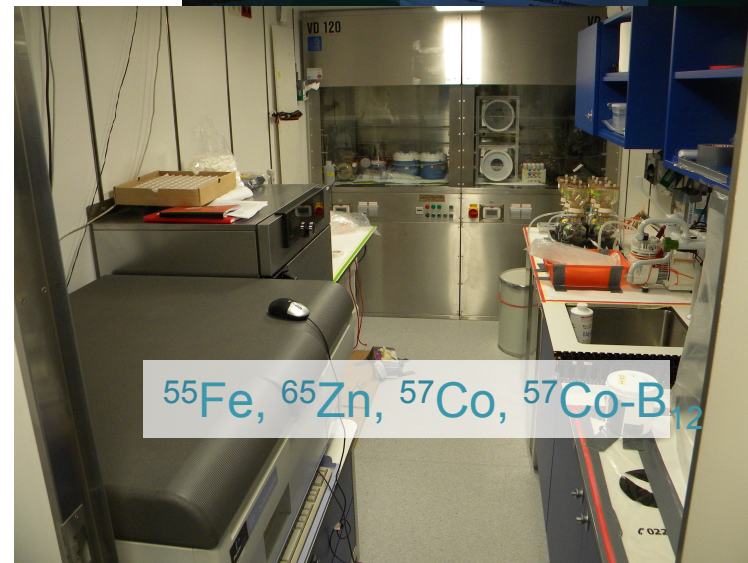
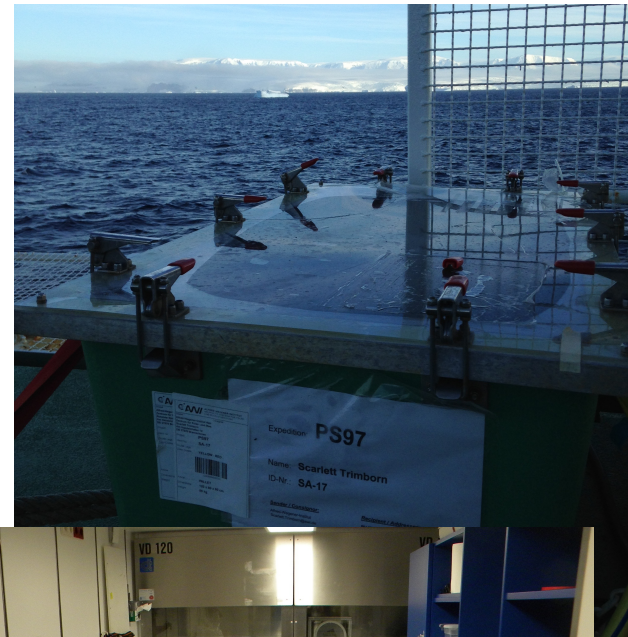
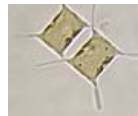
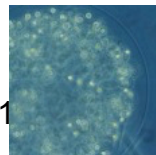


Day 1

## Uptake of metal/vitamin (using radioisotopes)



5  $\mu\text{mol l}^{-1} \text{d}^{-1}$



## Using a mass balance approach:

$$\Delta[\text{TM/vitamin}] \quad 10 \mu\text{mol l}^{-1}\text{d}^{-1}$$

$$+\rho\text{TM/vitamin} \quad 5 \mu\text{mol l}^{-1} \text{d}^{-1}$$

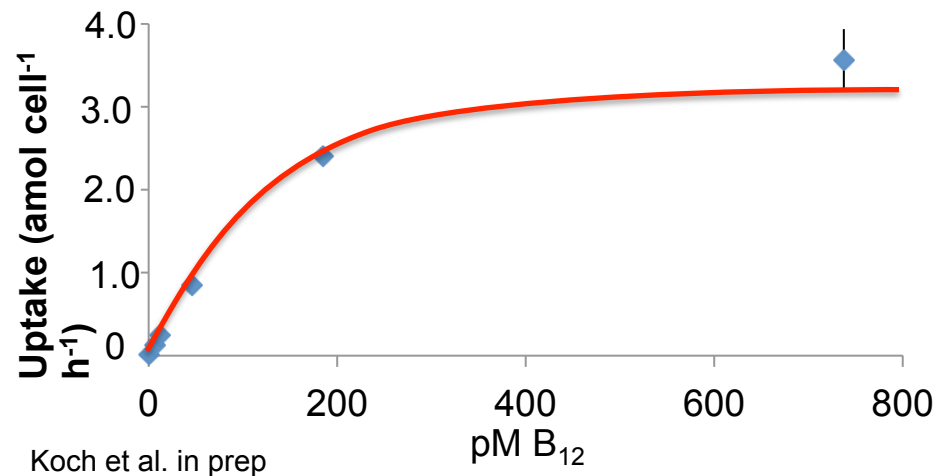
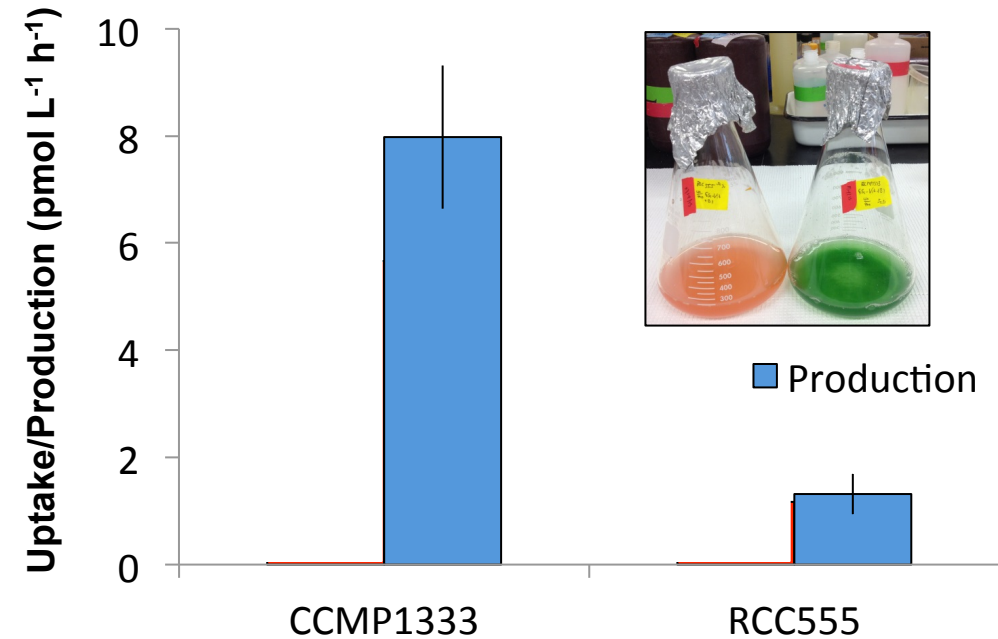
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$$\text{Production/Remineralization} \quad \mathbf{15 \mu\text{mol l}^{-1}\text{d}^{-1}}$$

From this we can obtain uptake and production/recycling rates and calculate turnover times for the various trace metals and vitamins in relation to each other.

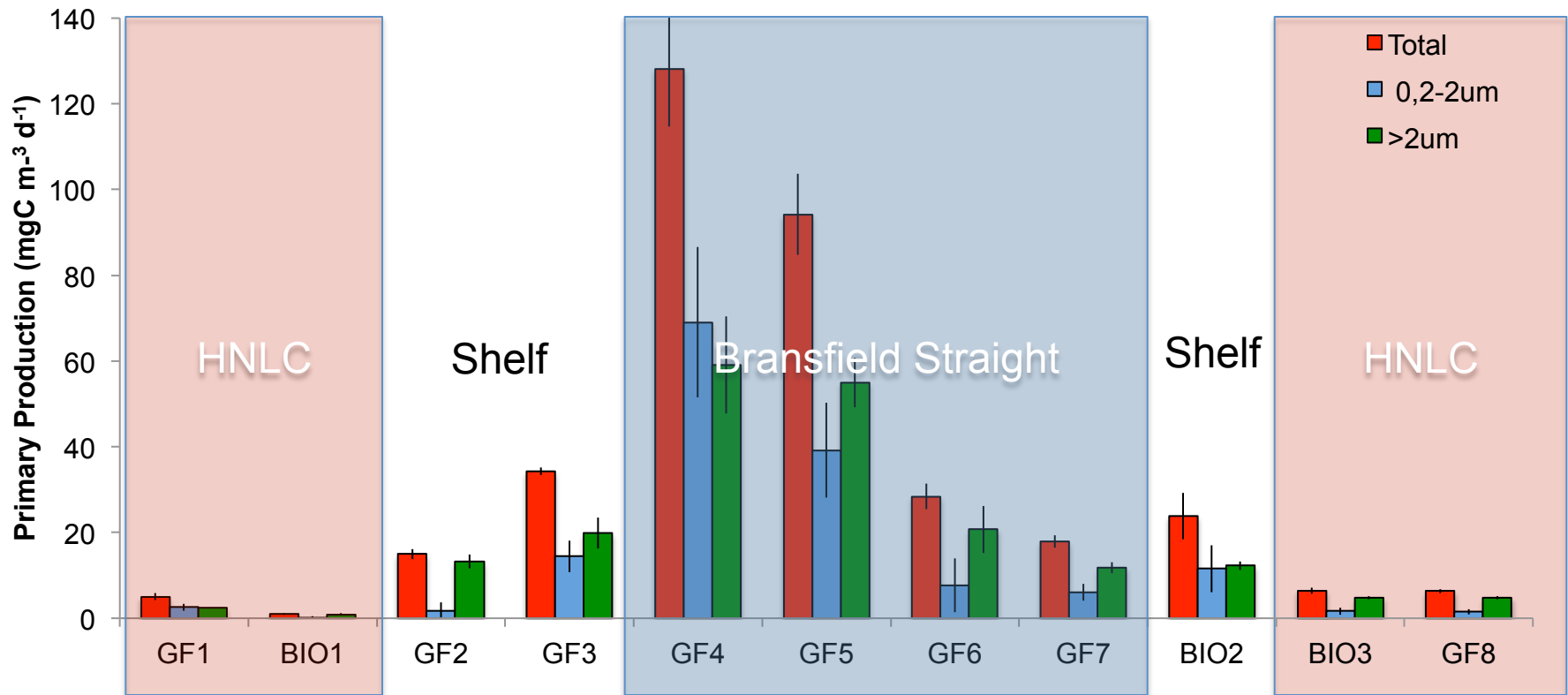
# Production of B<sub>12</sub> by cyanobacteria

- *Synechococcus* sp.
- Production calculated with a mass balance approach
- **Surprise:** They also take it up!
- Follows Michaelis Menten kinetics
- Balance of Production and Uptake = source or sink





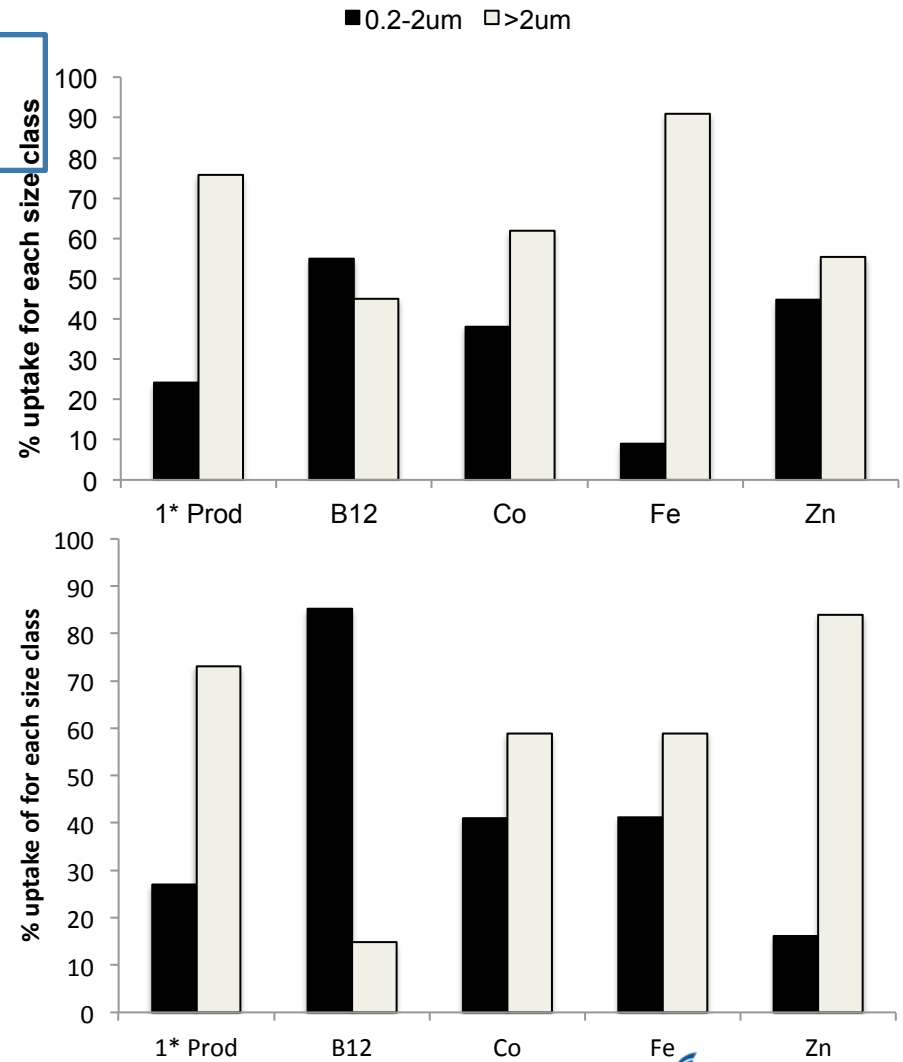
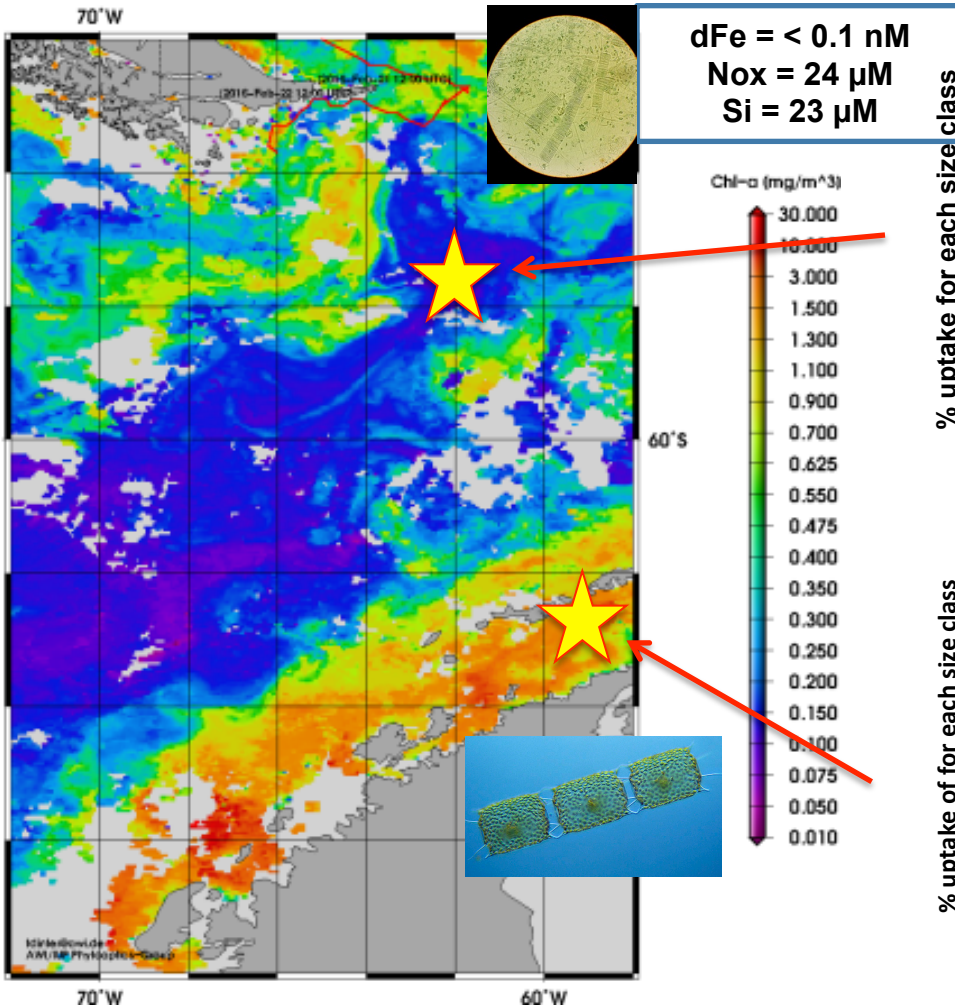
# Primary production PS97



The various regions sampled will shed light on the impacts of the *in situ* plankton community composition on the cycling of essential trace metals and vitamins

# Who is using what?

GlobColour CHL1 for 20160101–20160121



# Questions?

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