2066-7

Workshop and Conference on Biogeochemical Impacts of Climate and Land-Use Changes on Marine Ecosystems

2 - 10 November 2009

A walk through the N cycle

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A Walk Through the Nitrogen Cycle (part 2)

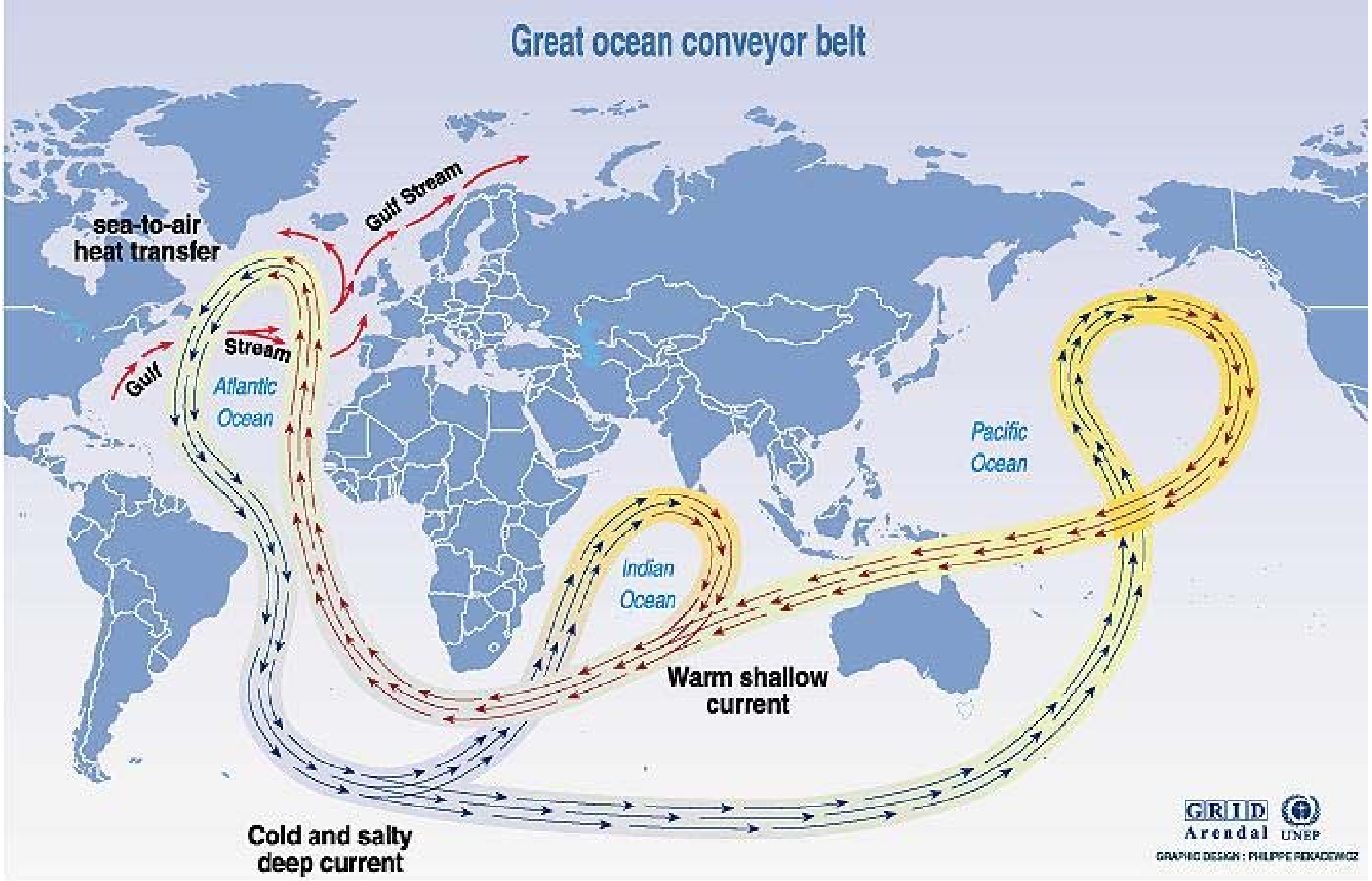
Nitrogen Isotopes: Tracer and Natural Abundance Studies
(and what they tell us about Nutrient Cycles and Food Webs)

Joseph P. Montoya
(ICTP-Trieste, 4 November 2009)

Plan for Today

- Marine N cycle
  - N distribution and N:P stoichiometry (Capone did a lot of this)
  - Oceanic N budget (some today, more on Friday)
- Rate measurements
  - Kinetics
  - Mechanics and examples
- Stable isotopes
  - N (and C) isotope biogeochemistry
  - N_2-fixation and bulk isotopic signatures

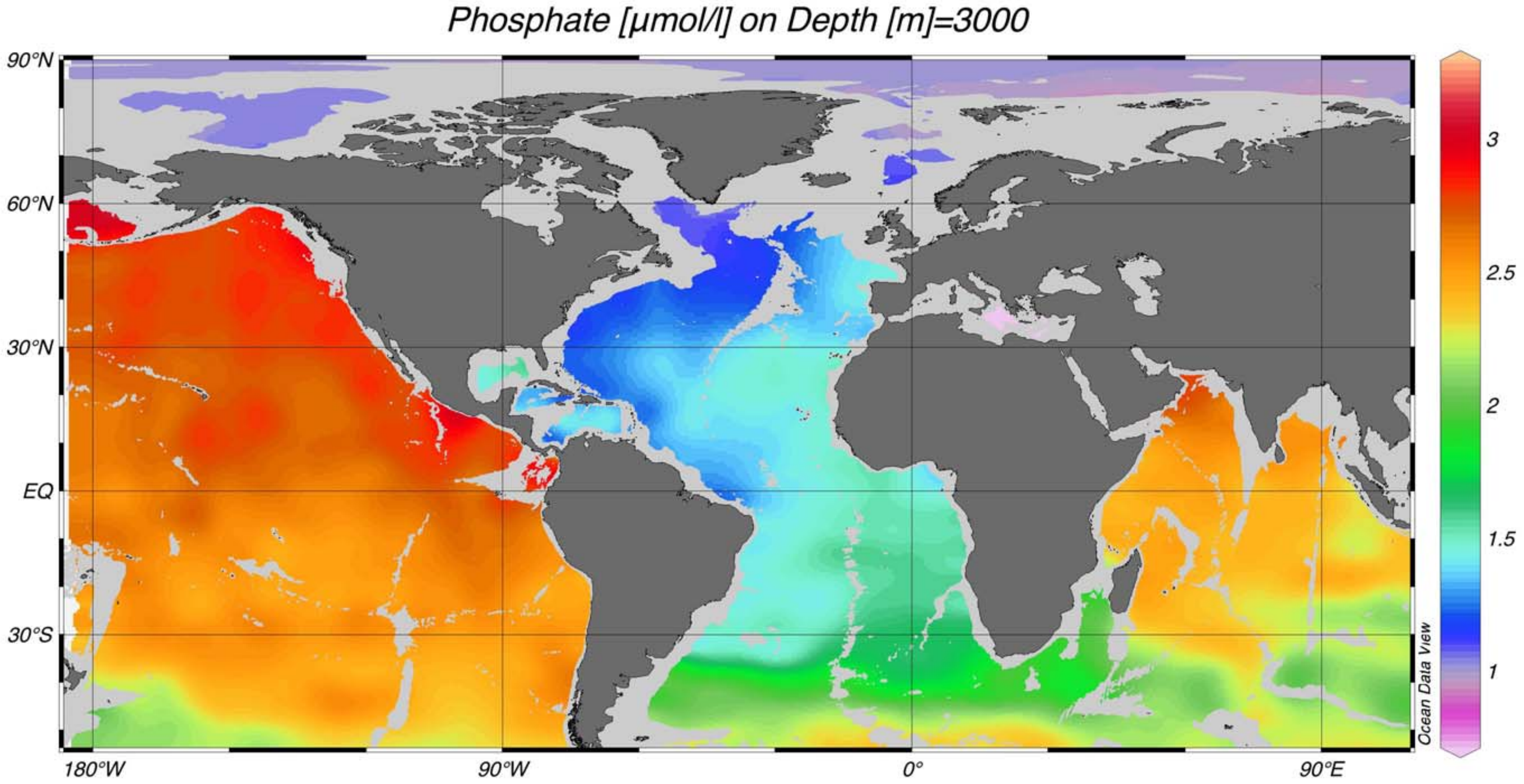
Conveyer Belt



Source: Broecker, 1991, in Climate change 1995, Impacts, adaptations and mitigation of climate change: scientific-technical analyses, contribution of working group 2 to the second assessment report of the intergovernmental panel on climate change, UNEP and WMO, Cambridge press university, 1996.

UN Environment Programme

PO_4^{3-} on 3000 m Horizon



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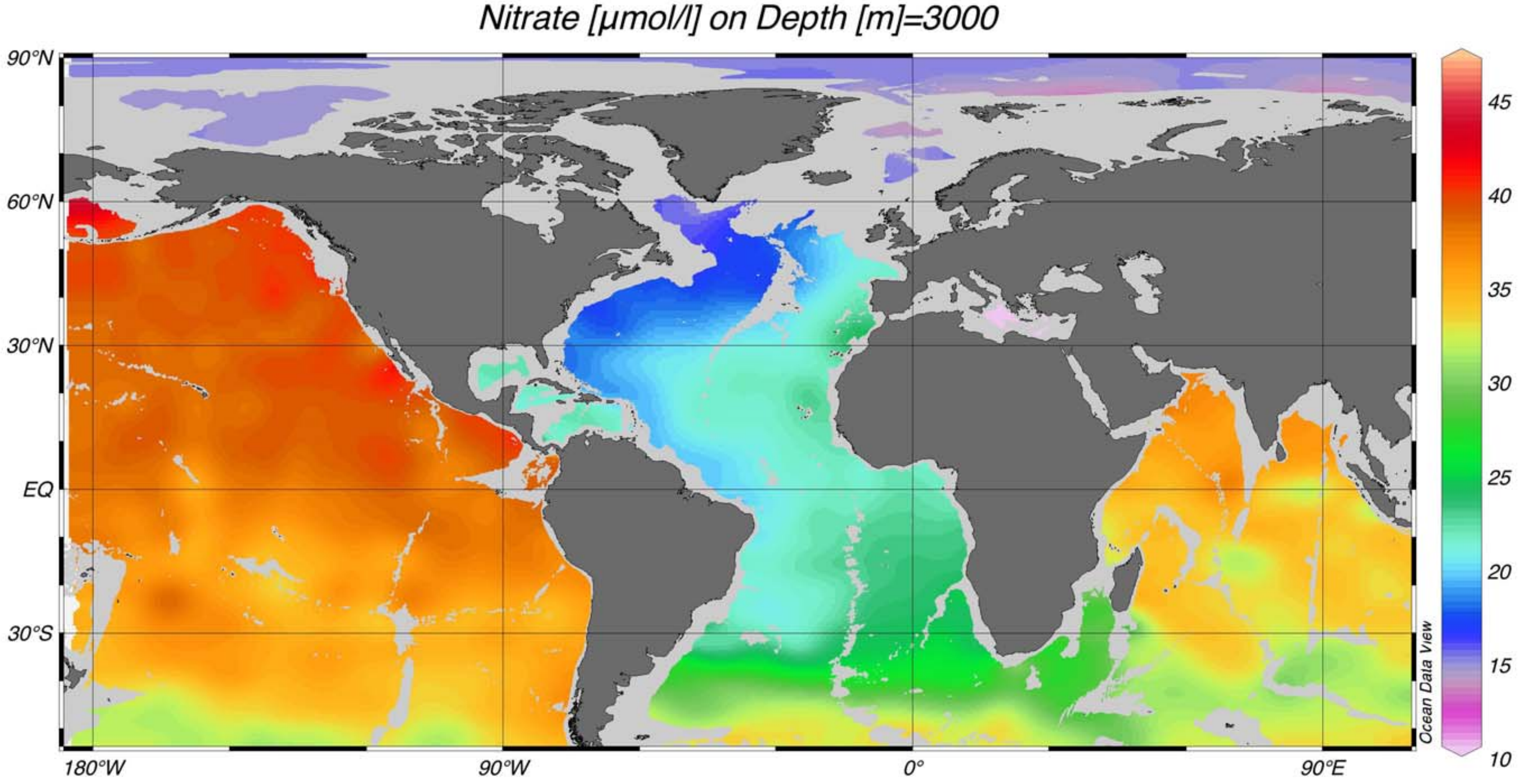
Data Source: World Ocean Atlas 2001. Figure prepared with ODV

NO_3^- on 3000 m Horizon



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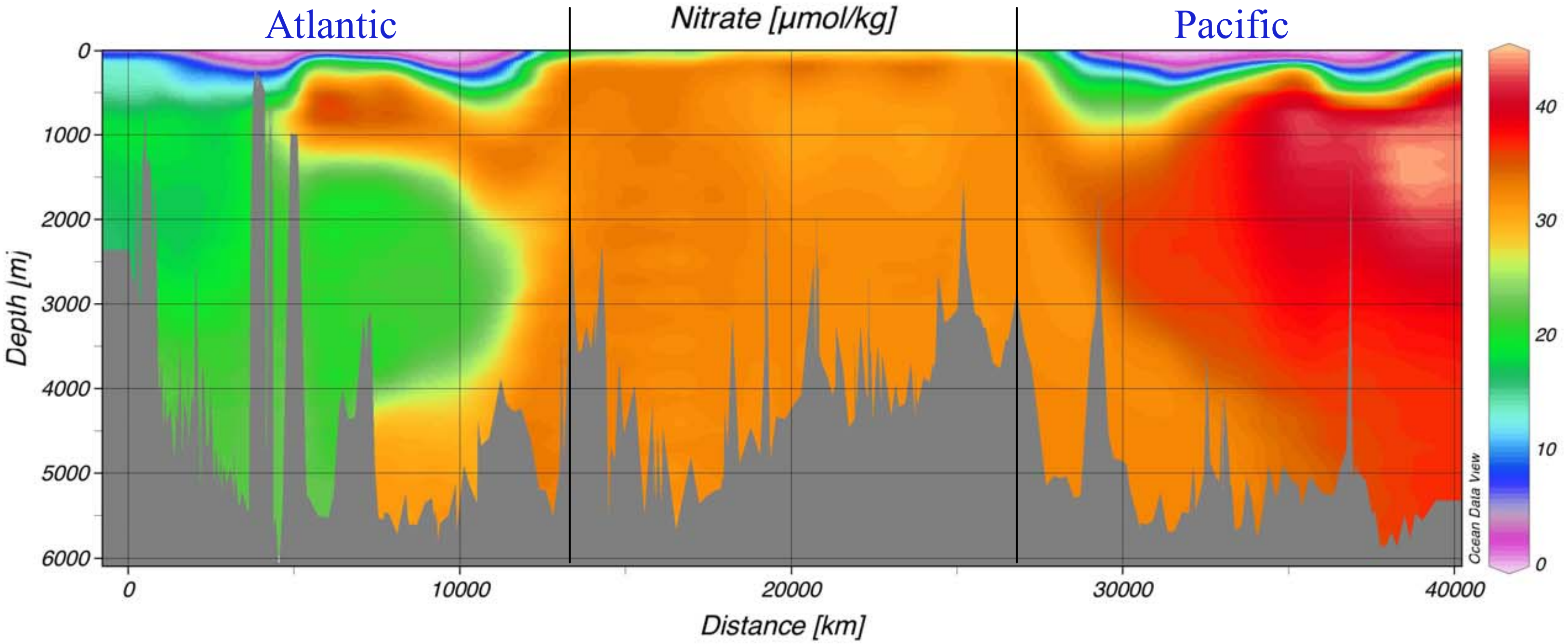
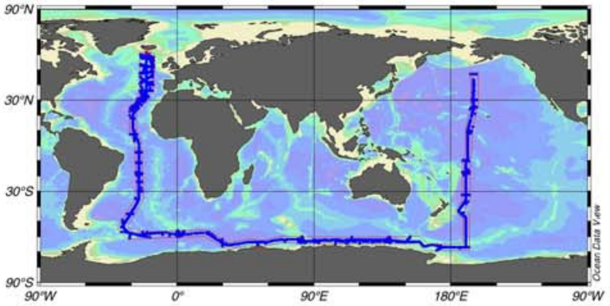
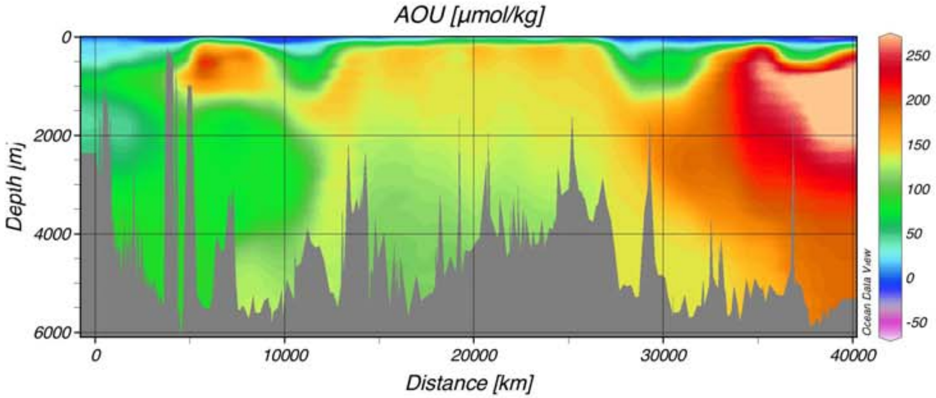
Data Source: World Ocean Atlas 2001. Figure prepared with ODV

Global Ocean NO_3^- & AOU Section







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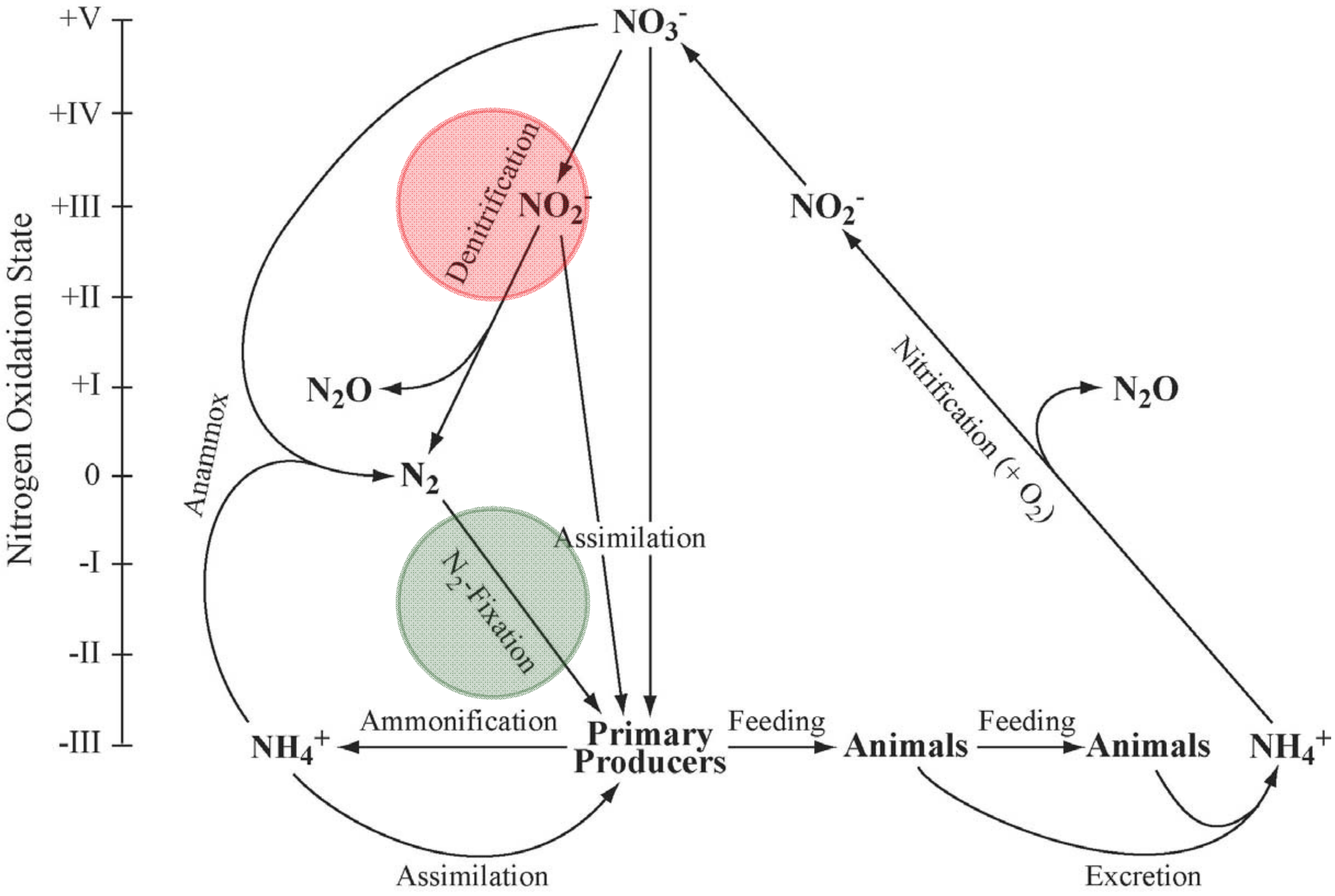
Data Source: World Ocean Atlas 2001. Figure prepared with ODV

N-Cycle as a Redox Web



(Modified from Codispoti 2001and Liu 1979)

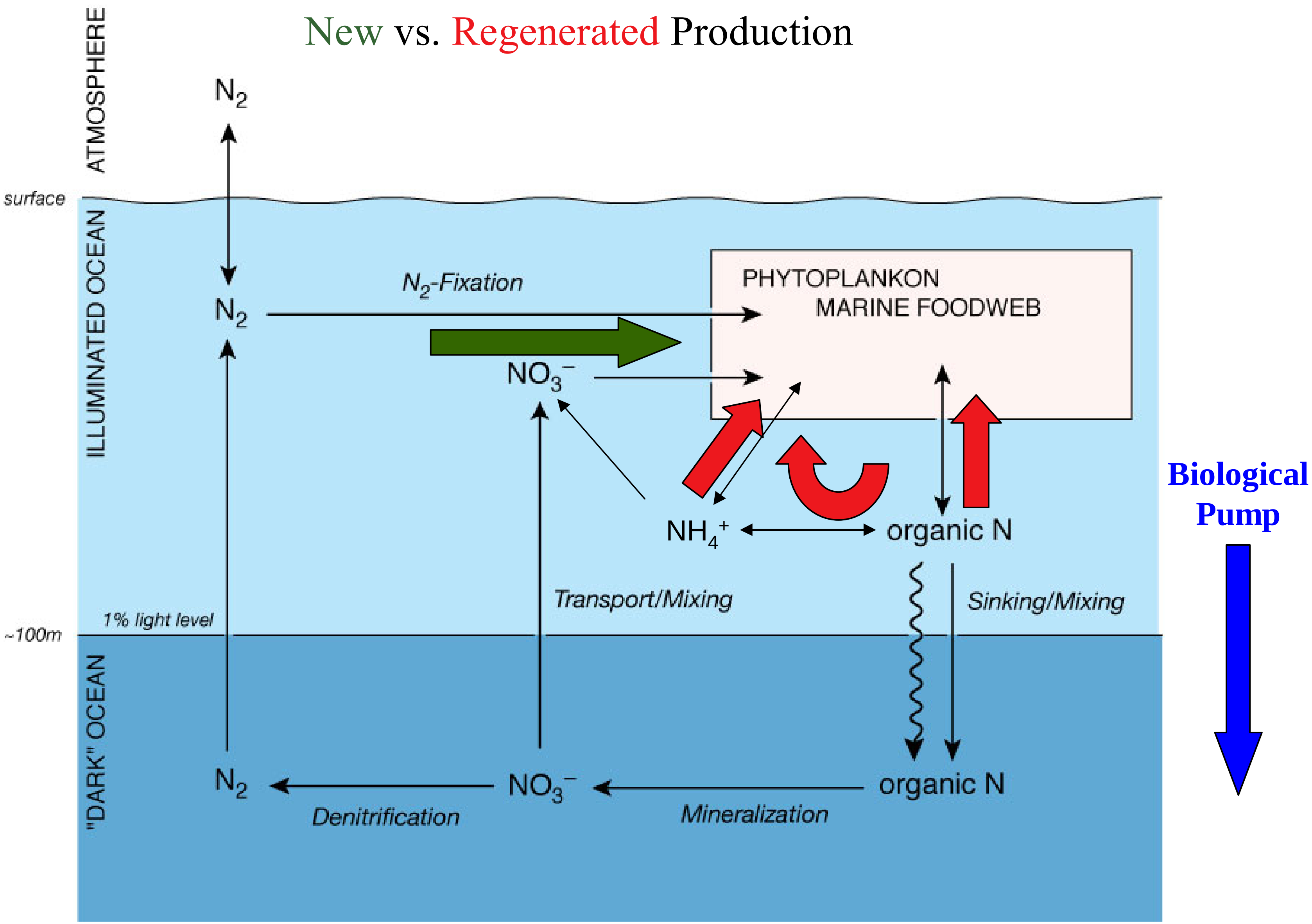
Oceanic Nitrogen Budget Estimates

N Budget Terms **(Tg N y^{-1} = 10^{12} g N y^{-1})**	1970 (Delwiche)	1979 (Liu)	1985 (Codispoti & Christensen)	1997 (Gruber & Sarmiento)	2007 (Codispoti)
Inputs					
atmospheric	4.1	49	40	15	30
runoff	30	17	25	41	78
N_2-fixation	10	30	25	125	135+++
Total Inputs	**44.1**	**96**	**90**	**181**	**243**
Outputs					
pelagic denitrification	40	50	60	85	150++
sedimentary denitrification	0	10	60	85	300+
burial & other	0.2	36	38	19	25
Total Outputs	**40.2**	**96**	**158**	**189**	**475**
(net balance)			**(-68)**		**(-232)**

Who Cares?

- Broad reaches of the ocean are N-limited.
  - Recycling of N within the water column supports biological production, but…
  - Injection of new N into the upper water column is required to support export production.
- The N and C cycles are tightly coupled through biological production of organic matter (C:N $\approx$ 7).
- N_2-fixation plays a key role in regulating the global C cycle and we still don't how much N_2-fixation is occurring in the ocean, who's doing it, and where!

New vs. Regenerated Production



ATMOSPHERE

N_2

surface

ILLUMINATED OCEAN

N_2-Fixation

N_2

PHYTOPLANKON
MARINE FOODWEB

NO_3^-

NH_4^+

organic N

Biological Pump

Transport/Mixing

Sinking/Mixing

1% light level

~100m

"DARK" OCEAN

N_2

NO_3^-

organic N

Denitrification

Mineralization

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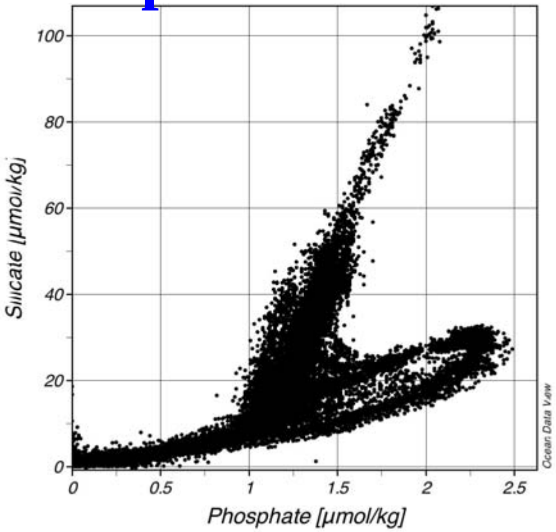
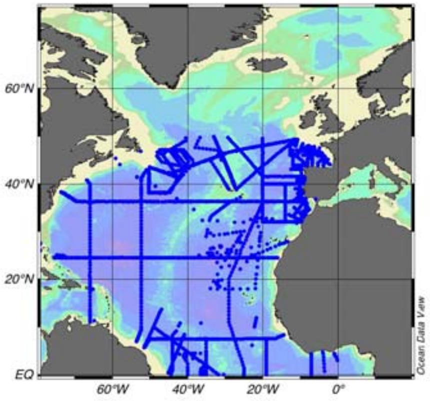
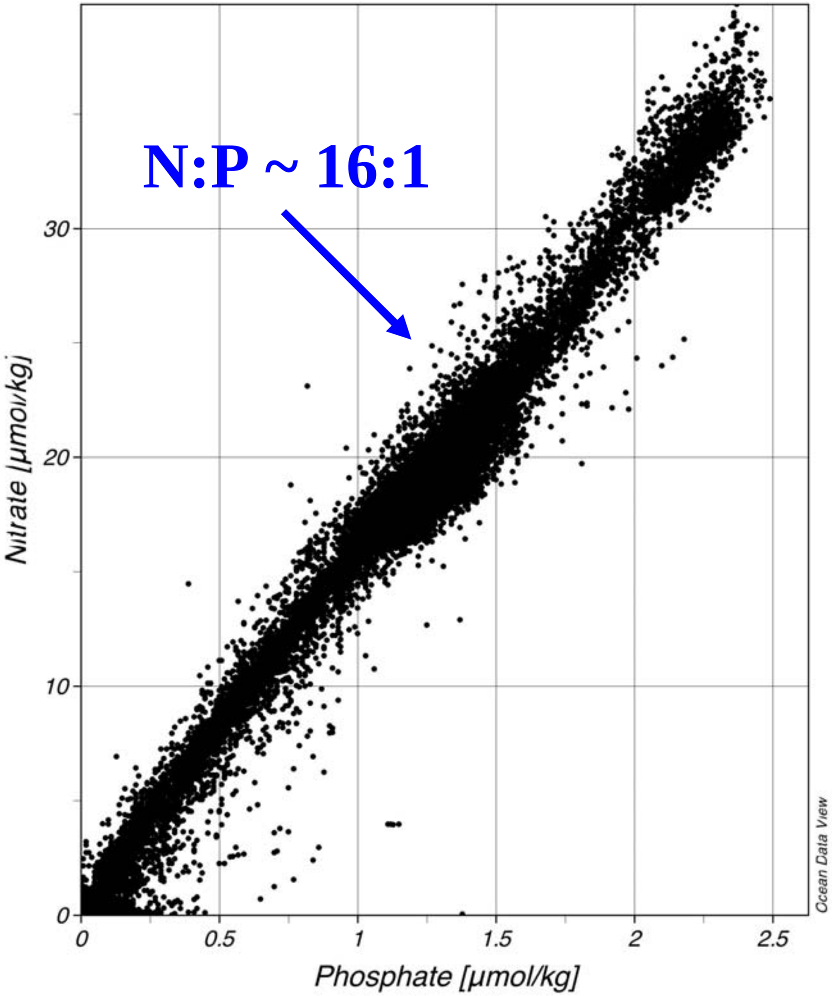
modified from http://www.up.ethz.ch/research/nitrogen_cycle/index

North Atlantic Nutrient Ratios

Si:P is complicated






N:P ~ 16:1

Data: eWOCE. Plot prepared with ODV

R/V Seward Johnson

Nutrient Uptake

- Uptake generally involves an active transport system and typically shows saturation kinetics.
- Measuring uptake
  - Substrate disappearance
  - ^{15}N tracer studies

$$^{15}N\text{-DIN} \rightarrow {}^{15}N\text{-PON}$$

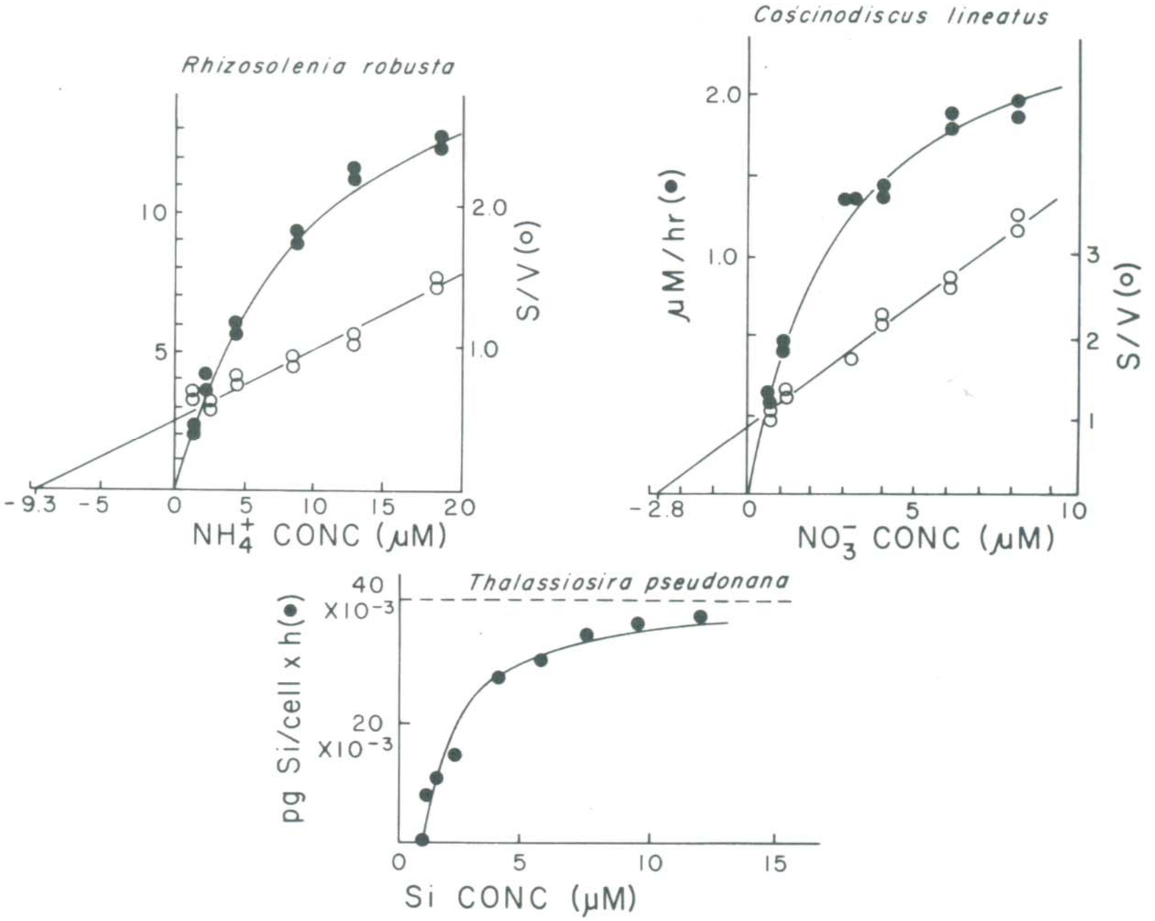
Nutrient Uptake Kinetics: M-M

- Michaelis-Menten equation
  - V = specific uptake rate (t^{-1})
    V_{max} = maximal uptake rate
    S = substrate concentration
    K_s = half satn concentration
  - Saturation behavior with asymptotic approach to V_{max}.
  - K_s provides a rough measure of the affinity of the uptake system for the substrate.
  - Lineweaver-Burke transform:

$$V = \frac{V_{max} S}{K_s + S}$$

$$\frac{1}{V} = \frac{1}{S}\left(\frac{K_s}{V_{max}}\right) + \frac{1}{V_{max}}$$

M-M and L-B Plots for Nutrient Uptake



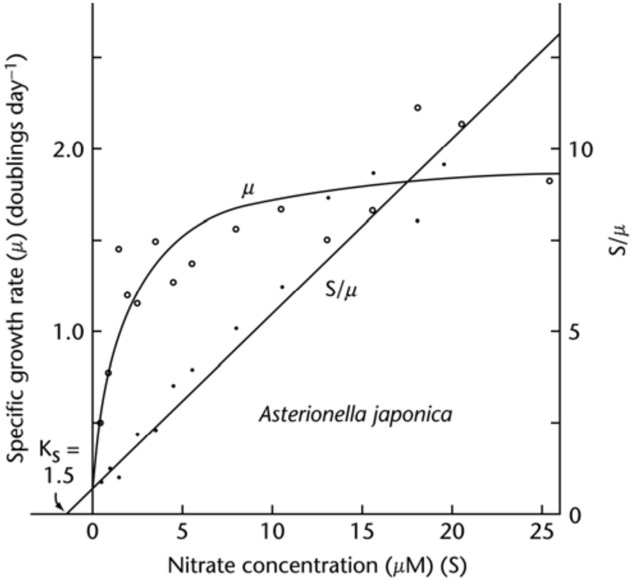
- Michaelis-Menten (M-M) and Lineweaver-Burke (L-B) plots for uptake of ammonium, nitrate, and silicate.
- The x-intercept of the L-B plot provides an estimate of -K_s
- How realistic are the concentrations used in these experiments?

Valiela Fig. 2.16

Nutrient Uptake Kinetics: Monod

- Monod equation
  - μ = specific growth rate (t^{-1})
    μ_{max} = maximal growth rate
    S = substrate concentration
    K_μ = half satn concentration
  - Describes dependence of growth, not uptake, on substrate availability.
  - In general, $K_\mu \neq K_s$

$$\mu = \frac{\mu_{max} S}{K_\mu + S}$$

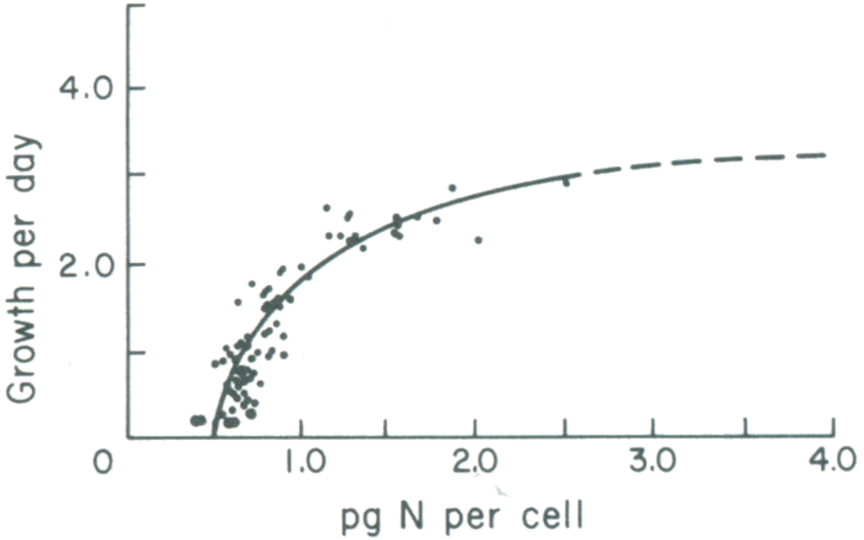


Miller Fig. 3.11

Nutrient Uptake Kinetics: Droop

- Droop equation
  - μ = specific growth rate (t^{-1})
    μ = growth rate when Q
    is very high
    K_Q = minimum viable quota
  - Generates a nicely hyperbolic relationship for nutrients with a large range of Q (B_{12}, P, Fe all show 30 - 100x variation).
  - Does less well in describing growth on nutrients for which Q is less variable (N and Si show only about 5x variation).

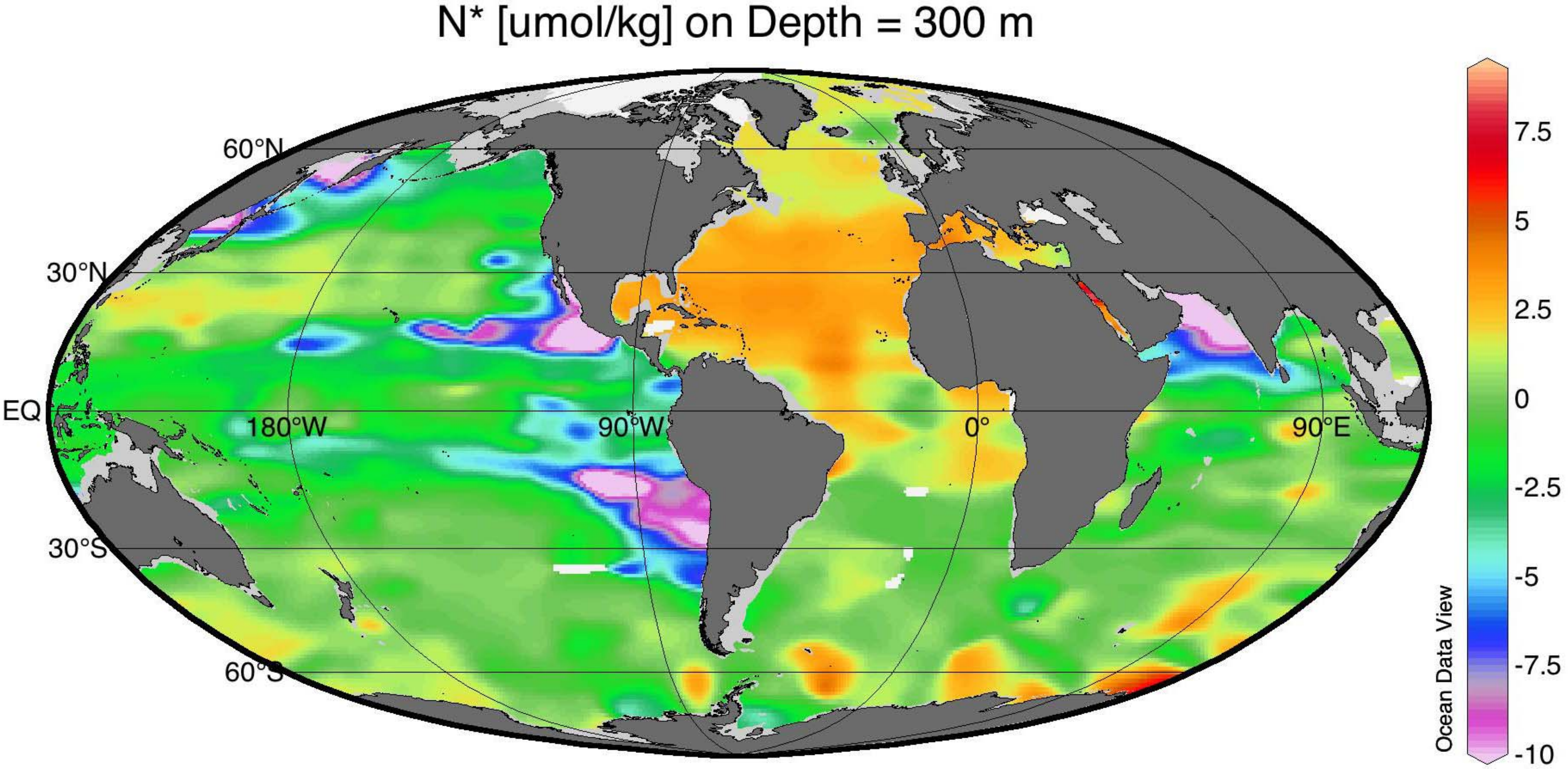
$$\mu = \frac{\overline{\mu}\,(Q - K_Q)}{Q}$$



μ vs. Q for *Thalassiosira pseudonana*.

Valiela Fig. 2.19

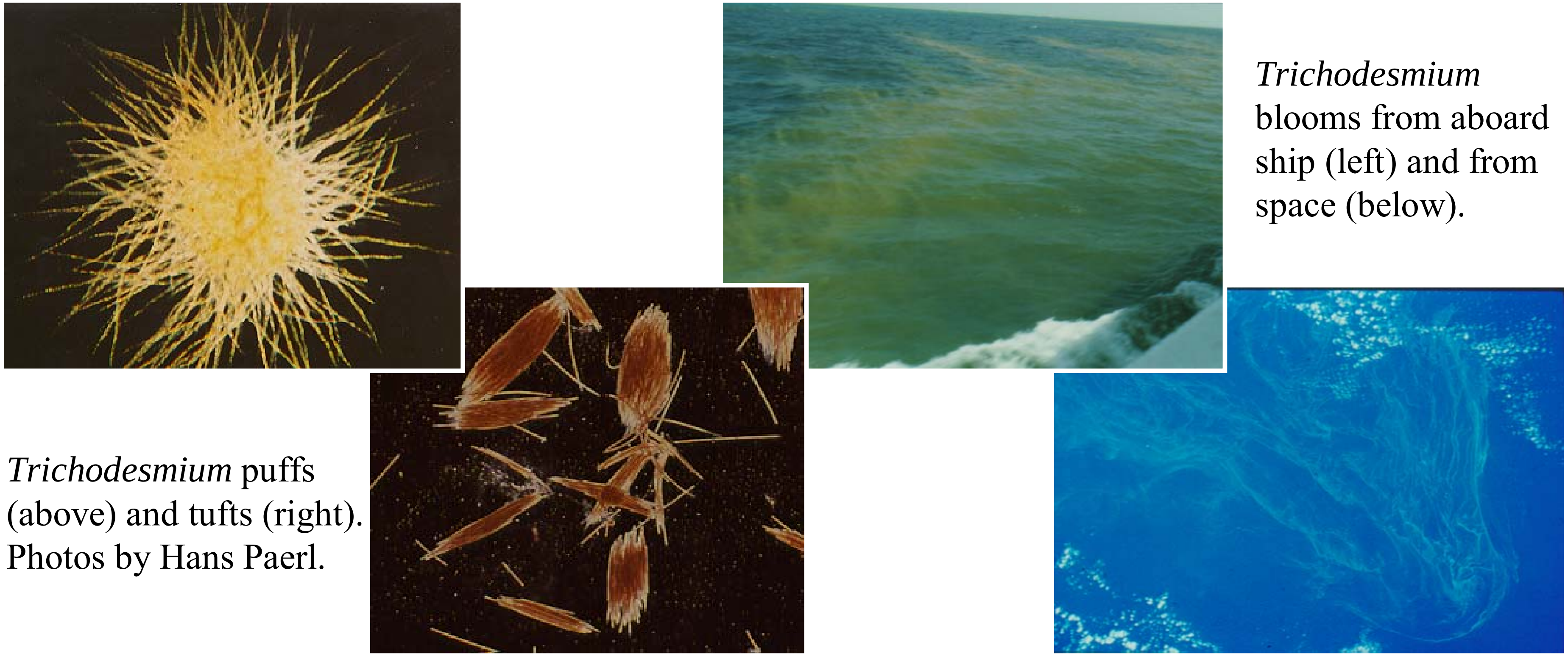
Step Back to the Big Picture:
N* Distribution Shows Interplay Between N_2-Fixation and Denitrification



$N^* = 0.87([NO_3^-] - 16[PO_4^{3-}] + 2.9)$ (Gruber & Sarmiento 1997)

Trichodesmium: the usual suspect

- Diazotrophs, including *Trichodesmium*, are broadly distributed in nutrient poor oceanic waters, but their contribution to the marine N budget remains poorly constrained.



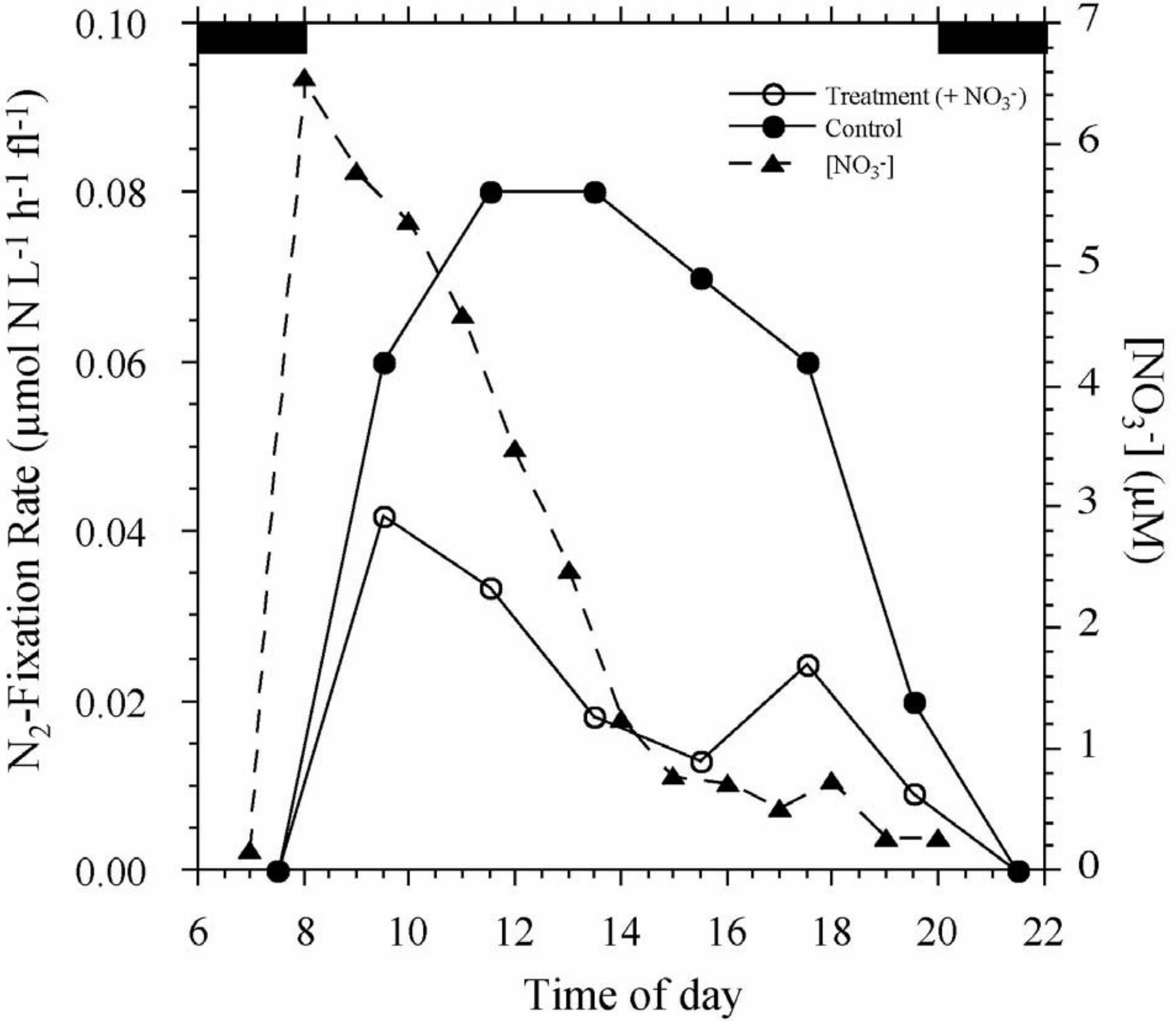
Trichodesmium puffs (above) and tufts (right). Photos by Hans Paerl.

Trichodesmium blooms from aboard ship (left) and from space (below).

Hunting *Trichodesmium*

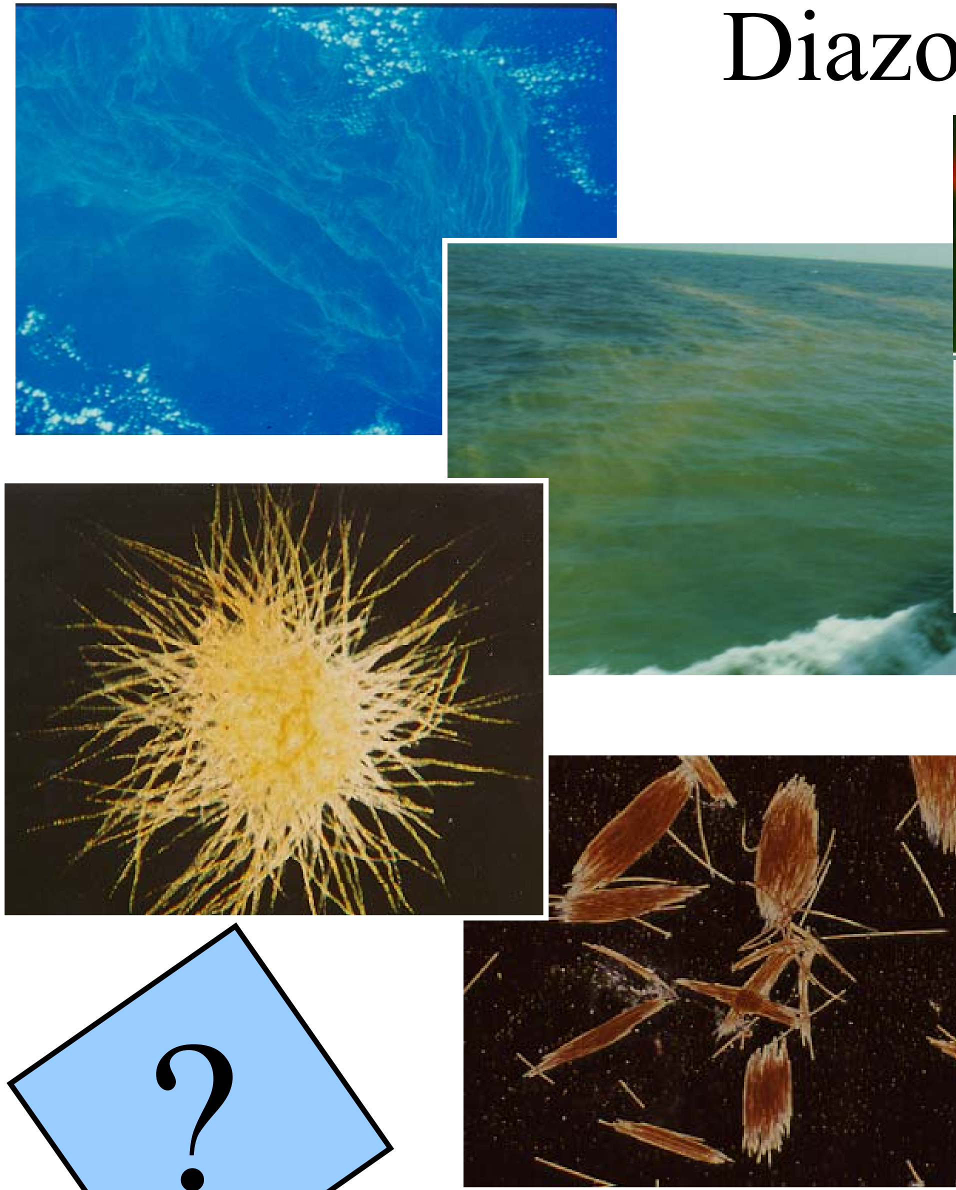
Trichodesmium
Rate Measurements

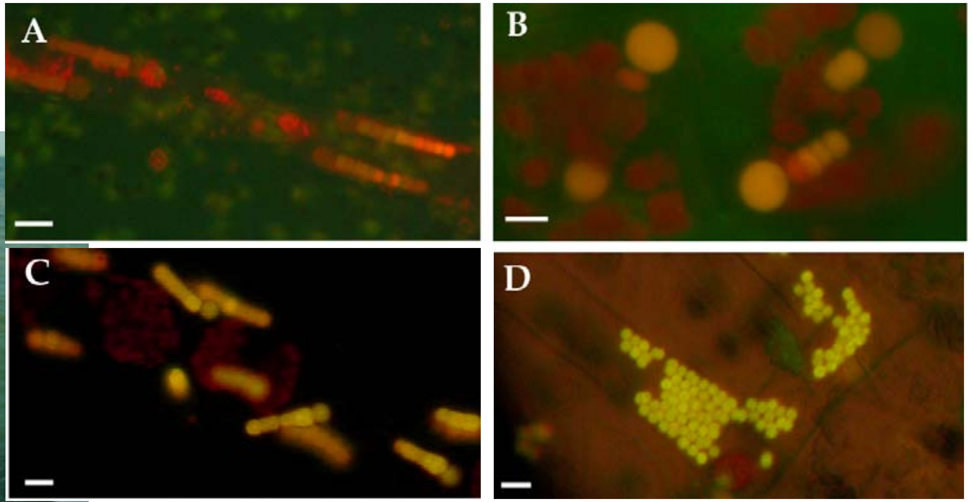
Trichodesmium: NO_3^- Uptake and N_2-Fixation



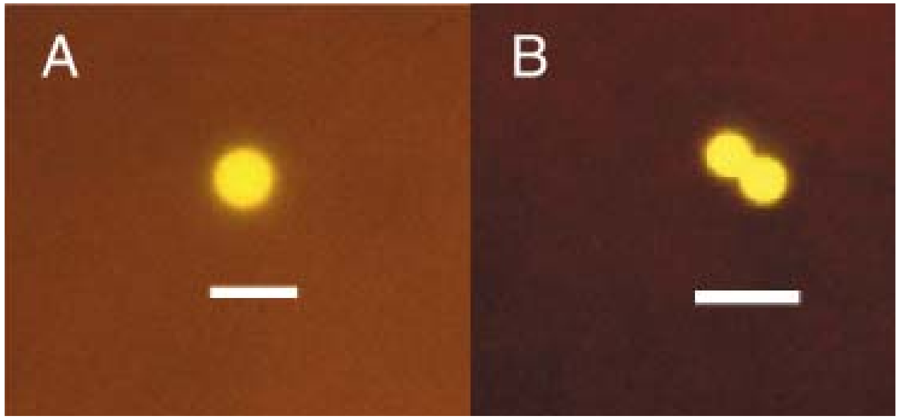
(Holl and Montoya, 2005. J. Phycol. 41: 1178-1183.)

Diazotroph Diversity





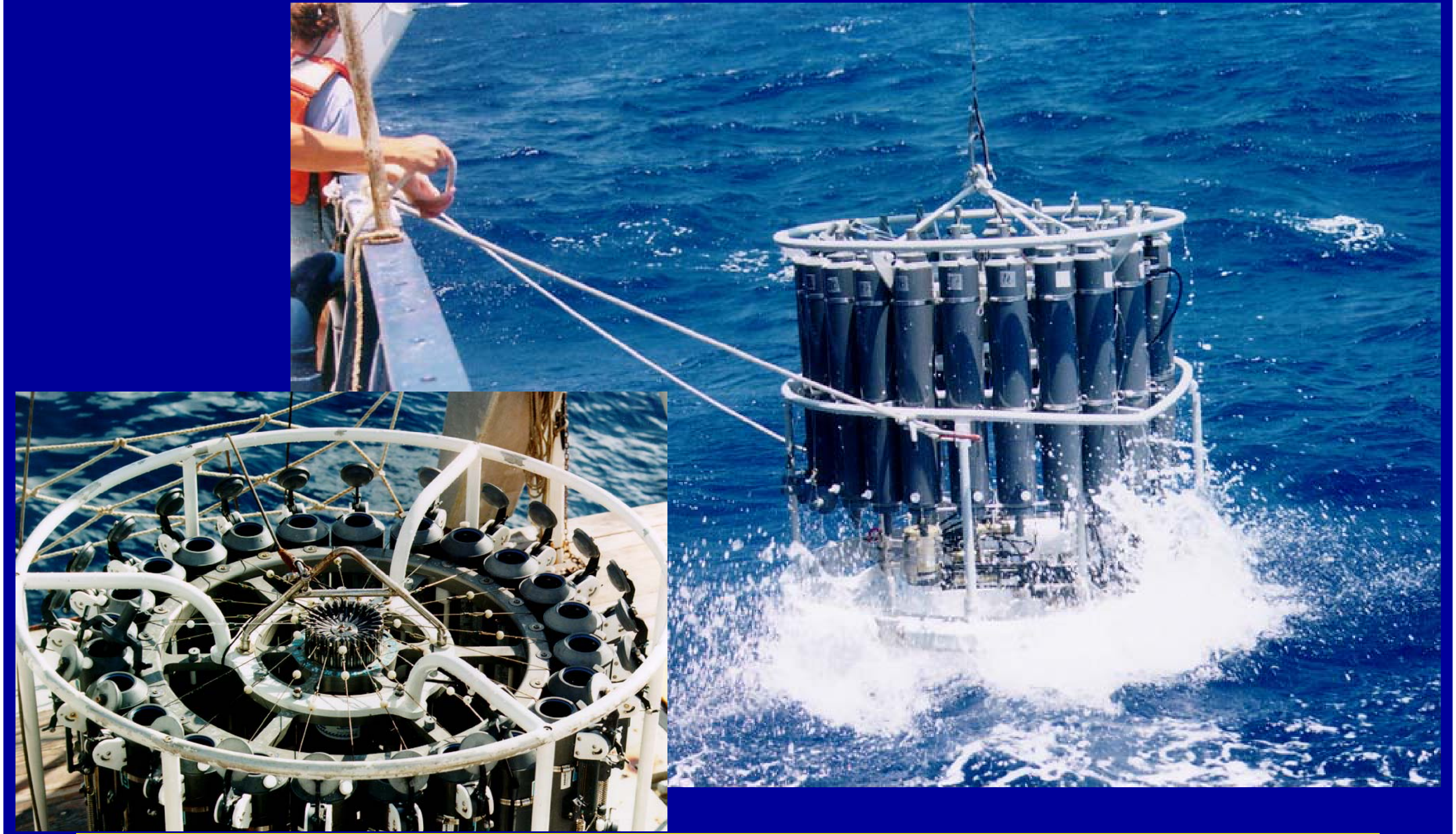
(Images courtesy R. Foster)



(Zehr et al., 2001. Nature 412)

?

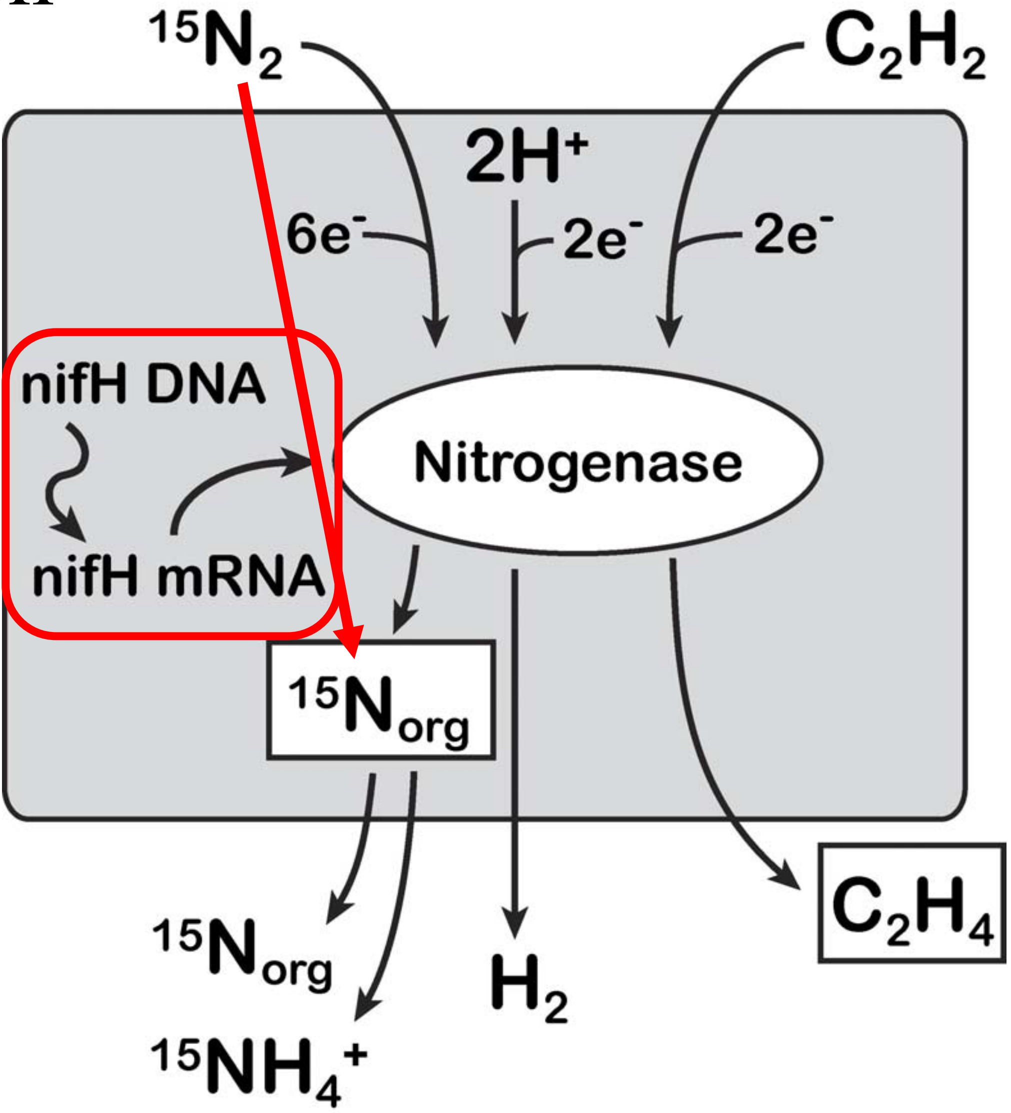
CTD-Rosette



Experimental Approach

- $^{15}N_2$-fixation measures net incorporation of N_2 into organic matter.

- Parallel studies (Zehr Lab) quantify the diversity of diazotrophs (nifH DNA) and their pattern of activity (nifH mRNA).



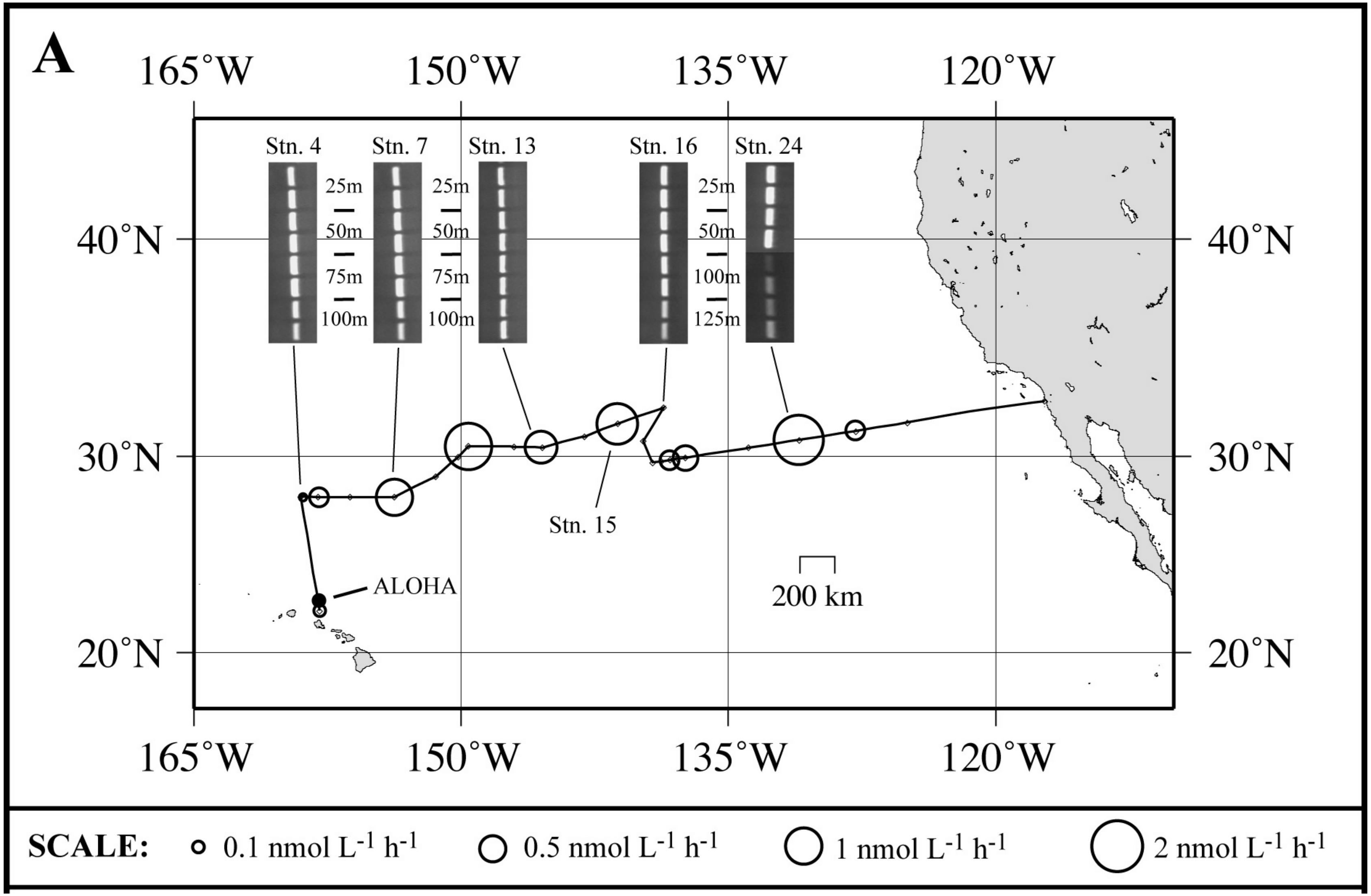
$^{15}N_2$- ^{13}C-Fixation Experiments

- Prefilter water through 110 µm Nitex.
- Add $^{15}N_2$ and $NaH^{13}CO_3$
- Incubate
- Filter (10 µm prefilter)

$^{15}N_2 \rightarrow {}^{15}N\text{-}PON$

$^{13}C\text{-}HCO_3^- \rightarrow {}^{13}C\text{-}POC$

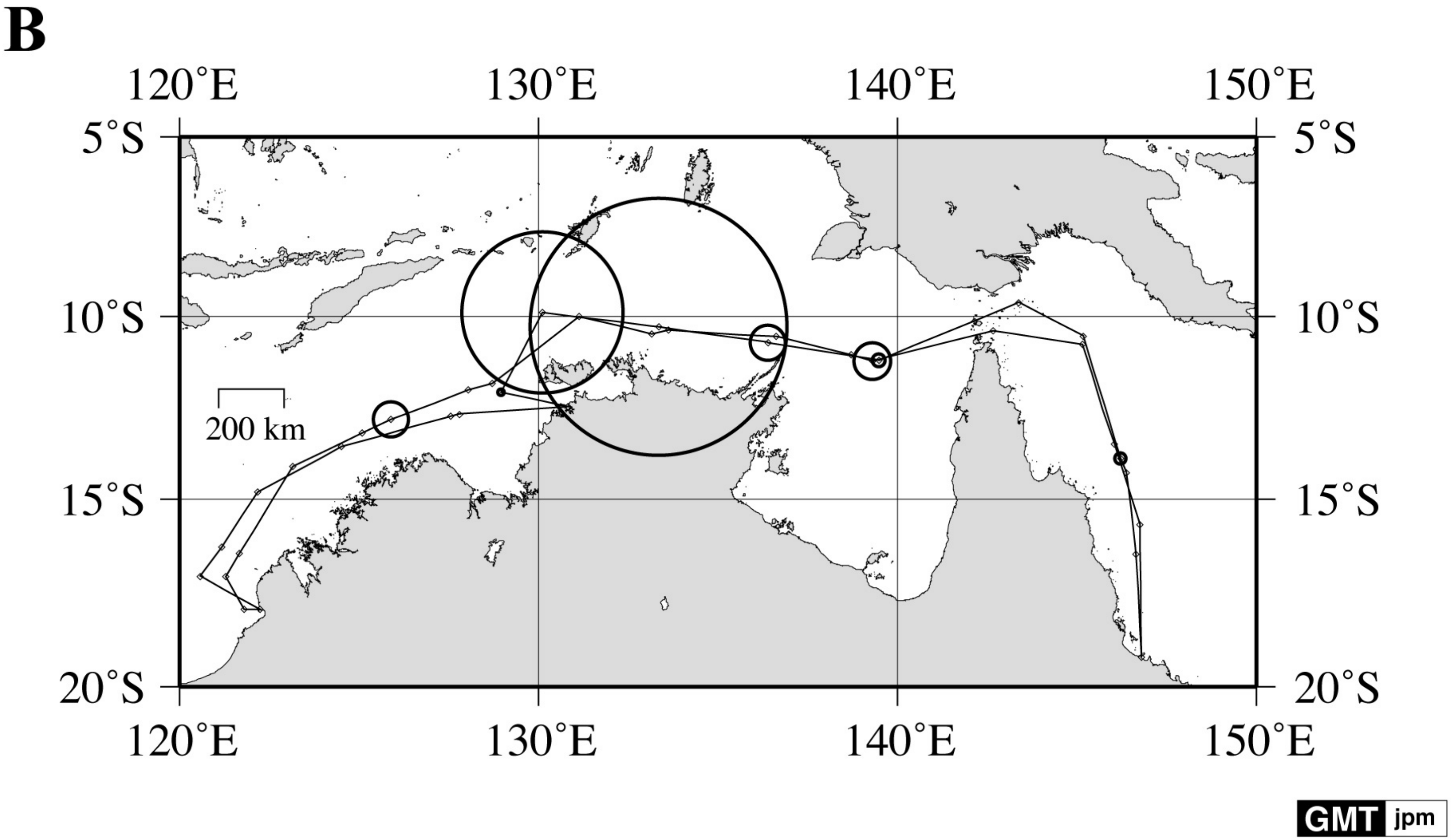
Cook-25: Volumetric $^{15}N_2$-Fixation Rates



(Montoya et al., 2004. Nature 430: 1027-1031)

EW9912: Volumetric $^{15}N_2$-Fixation Rates

SCALE: ○ 0.1 nmol L^{-1} h^{-1} ○ 0.5 nmol L^{-1} h^{-1} ○ 1 nmol L^{-1} h^{-1} ○ 2 nmol L^{-1} h^{-1}



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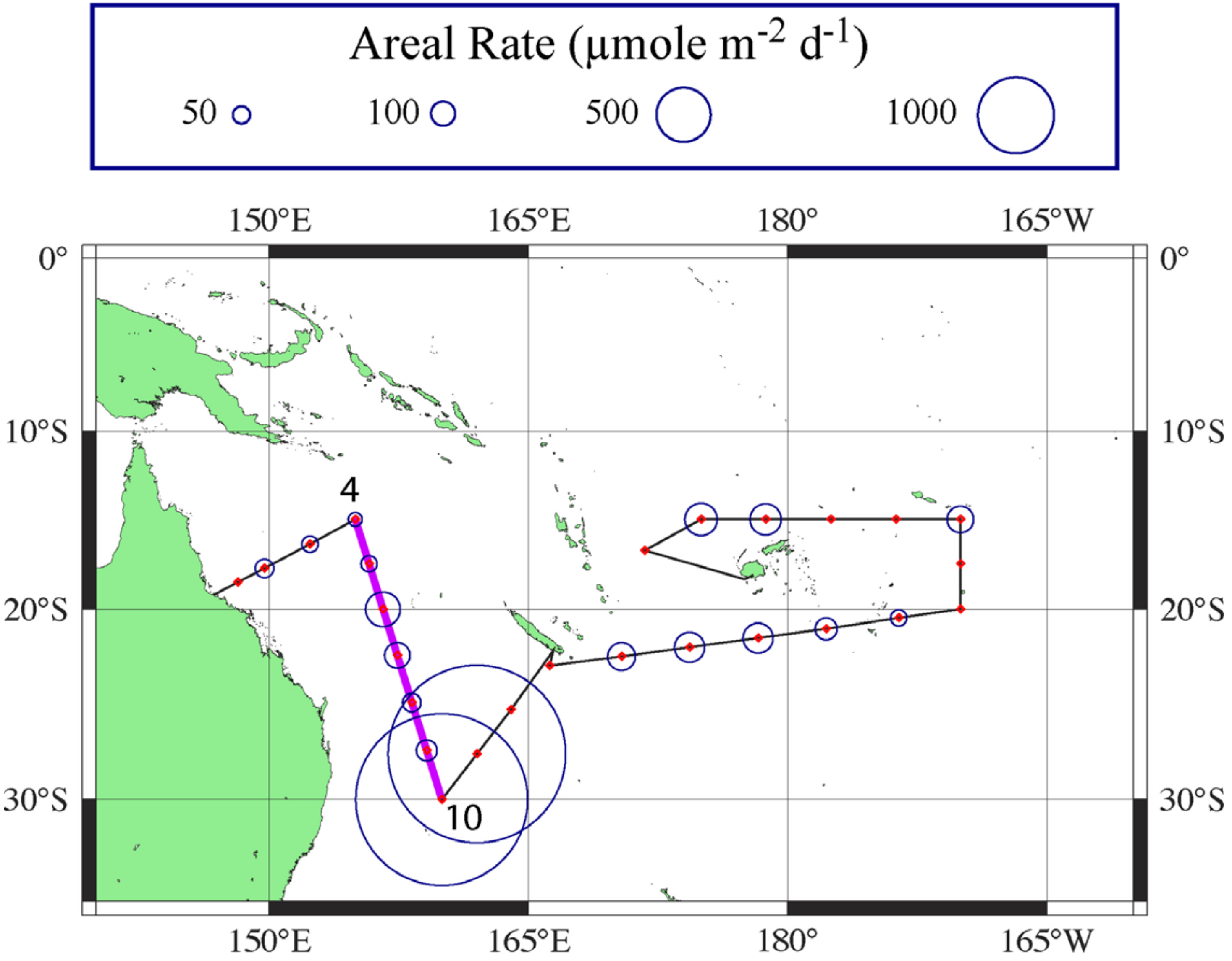
(Montoya et al., 2004. Nature 430: 1027-1031)


Areal Rate (μmole m-2 d-1)
50
100
500
1000
150°E
165°E
180°
165°W
0°
10°S
20°S
30°S
4
10

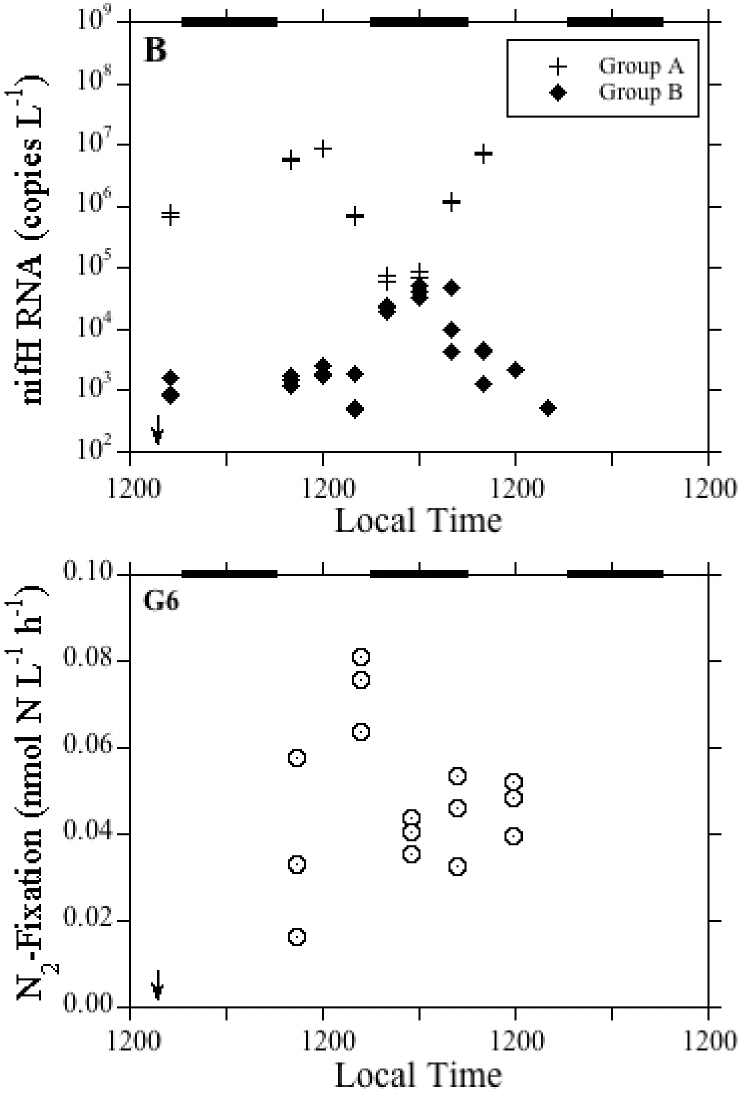
Areal Rates of N_2-Fixation

Location/System	Dates	Areal Rate (μmol N m^{-2} d^{-1})	SE	N
Station ALOHA	2000 Š 2001	66	19	7
Kaneohe Bay	2000 Š 2002	24	6	12
Eastern North Pacific Gyre	Jun Š Jul 2002	505	165	10
Timor - Arafura Š Coral Seas	Nov 1999	126	47	7
Arafura Sea (Stations 26 & 27)	Nov 1999	3955		2
Trichodesmium (range)	1964 - 2001	35 - 283		
Richelia/Hemiaulus (bloom)	Oct 1996	3110		
KM0703 (range)	Mar - Apr 2007	50 Š 5300		

(Refs in Montoya et al., 2004. Nature 430: 1027-1031)

Rate & Molecular Data

- Experiment G6
  - Water collected outside Kaneohe Bay, Hawaii.
  - Time-series of nifH expression (mRNA) and $^{15}N_2$-fixation measurements.
- Major Results
  - N_2-fixation rate relatively constant through the diel cycle.
  - Groups A and B show very different expression patterns with inverse phasing through the light cycle.

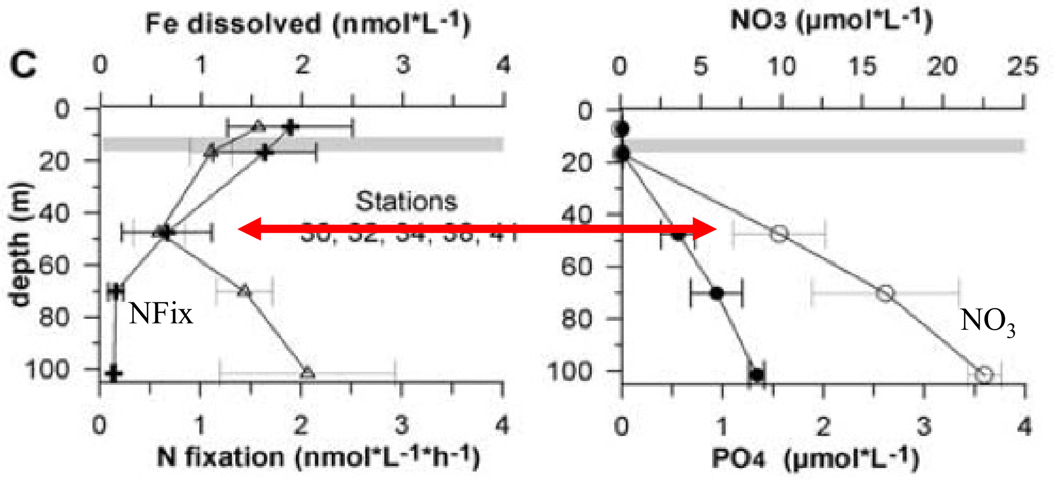


(Zehr et al., 2007. L&O 52: 169-183)

Controls on N_2-Fixation

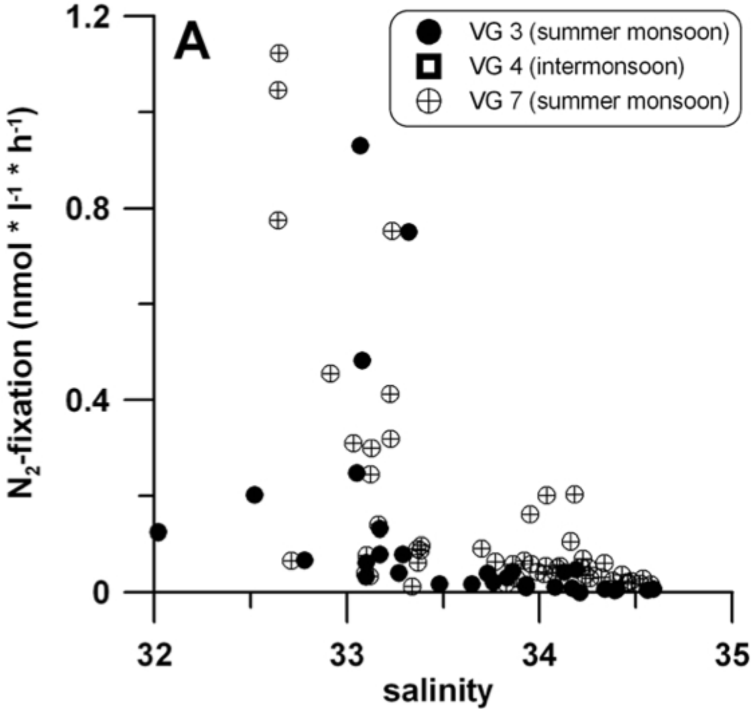
- Eastern Tropical Atlantic
  - Measurable N_2-fixation below the mixed layer in the presence of substantial nitrate.

  (Voss et al. 2004, GRL 31)



- South China Sea
  - N_2-fixation highest at low salinity, which may reflect inputs of nutrients, Fe, organic matter, etc. from the Mekong.

  (Voss et al. 2006, GRL 33)



Tropical Rivers and N_2-Fixation

- Differential consumption, remineralization, and mobilization of N, P, and Fe modify the nature and degree of nutrient limitation in the river plume.
- Different regions/water masses in and around the plume are characterized by different patterns of nutrient/metal limitation and distinct plankton assemblages.
- Changes in nutrient and trace metal loading due to land-use and climate changes will likely alter patterns of nutrient limitation in coastal waters. (e.g., Mekong, Yangtze, Mississippi…)

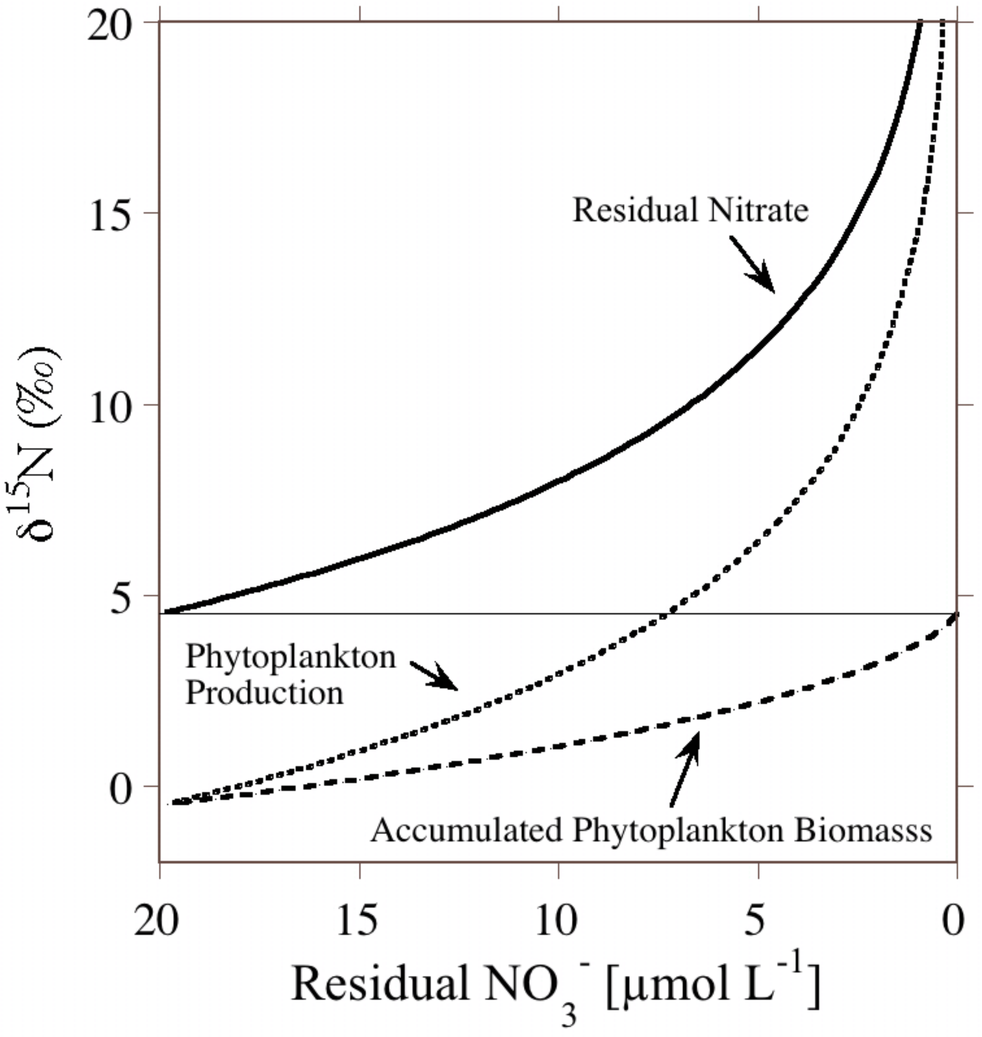
Stable Isotope
Natural Abundance Approaches

Basic Stable Isotope Terminology

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

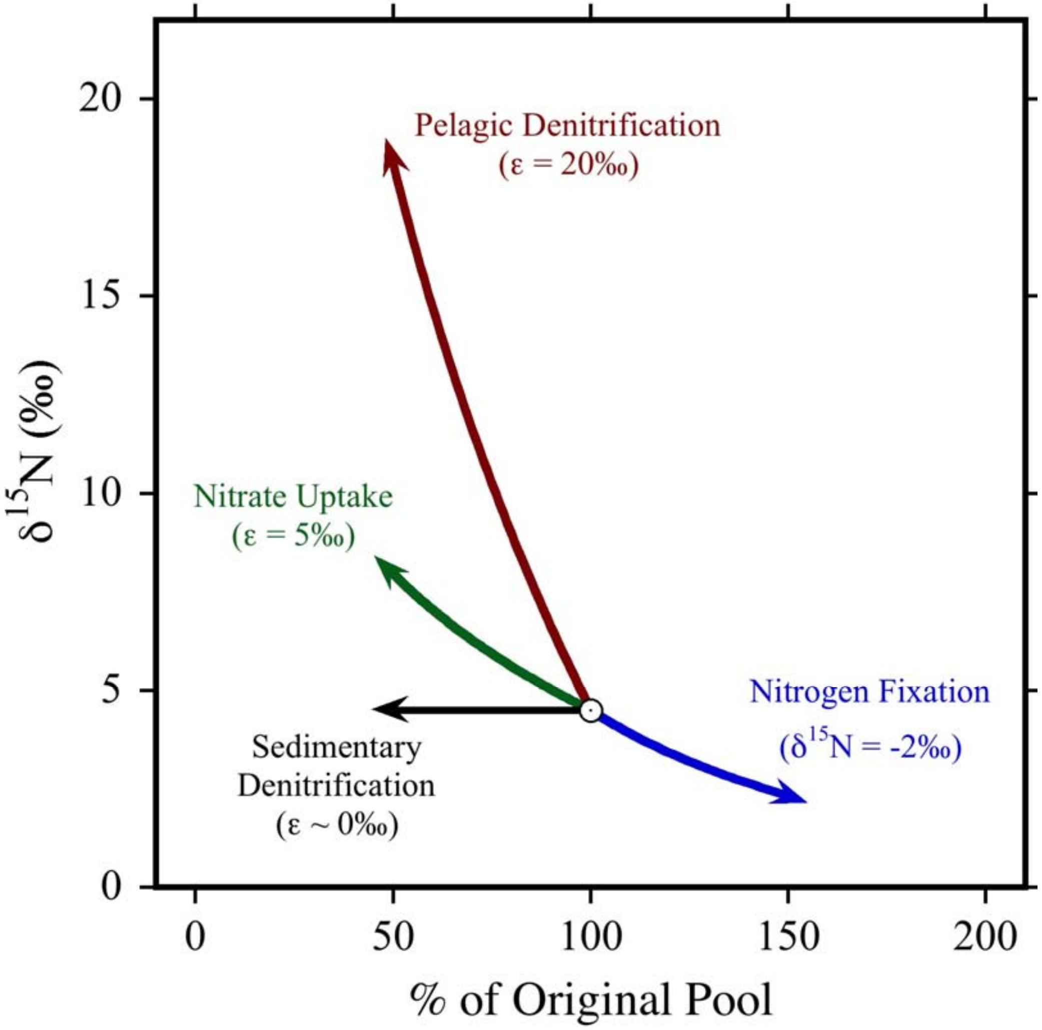
- $X = {}^{15}N$ or ${}^{13}C$
- R = isotope ratio (${}^{15}N{:}{}^{14}N$ or ${}^{13}C{:}{}^{12}C$)
- Standard = atmospheric N_2 or PDB

Isotopic Fractionation

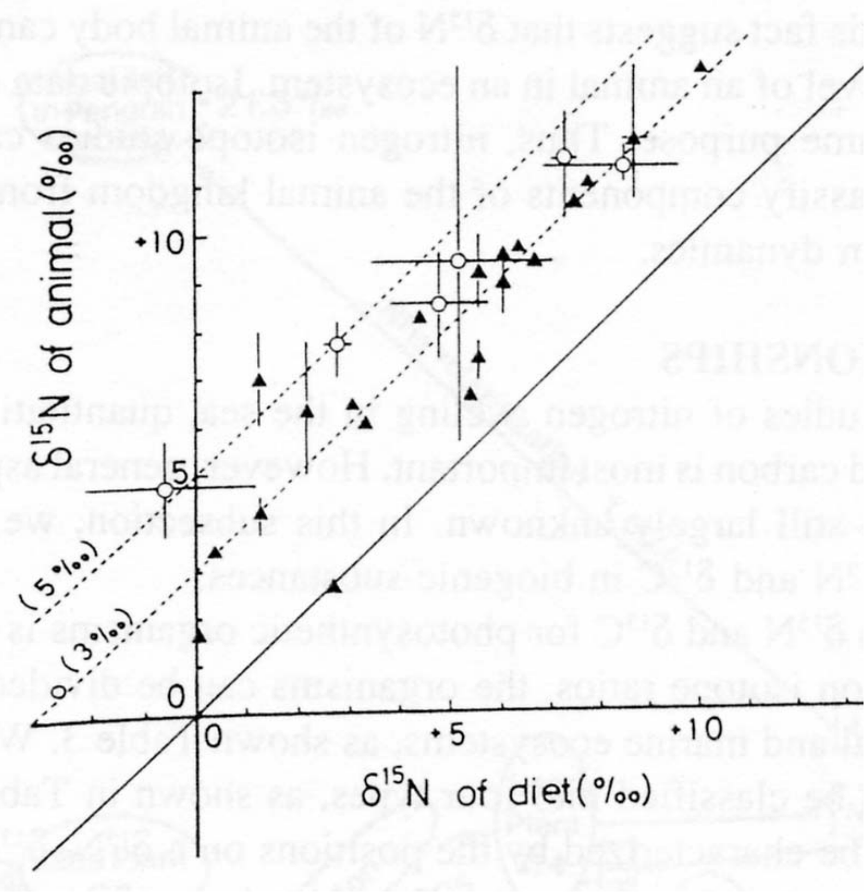


- Many reactions discriminate against the heavy isotope (^{15}N, ^{13}C)
- In a closed system, this will generate predictable changes in isotope abundance in the substrate and product pools.

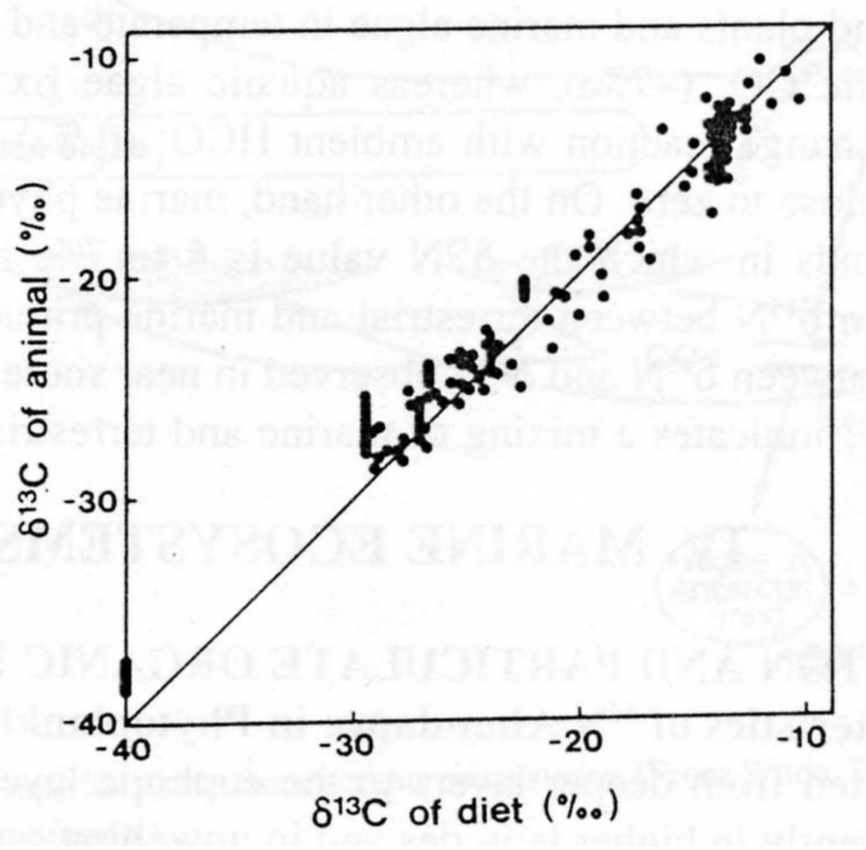
Biological Processes and $\delta^{15}N$ of NO_3^-



In general, δ^{15}N scales with trophic position …



(Minagawa and Wada, 1984, *GCA*)

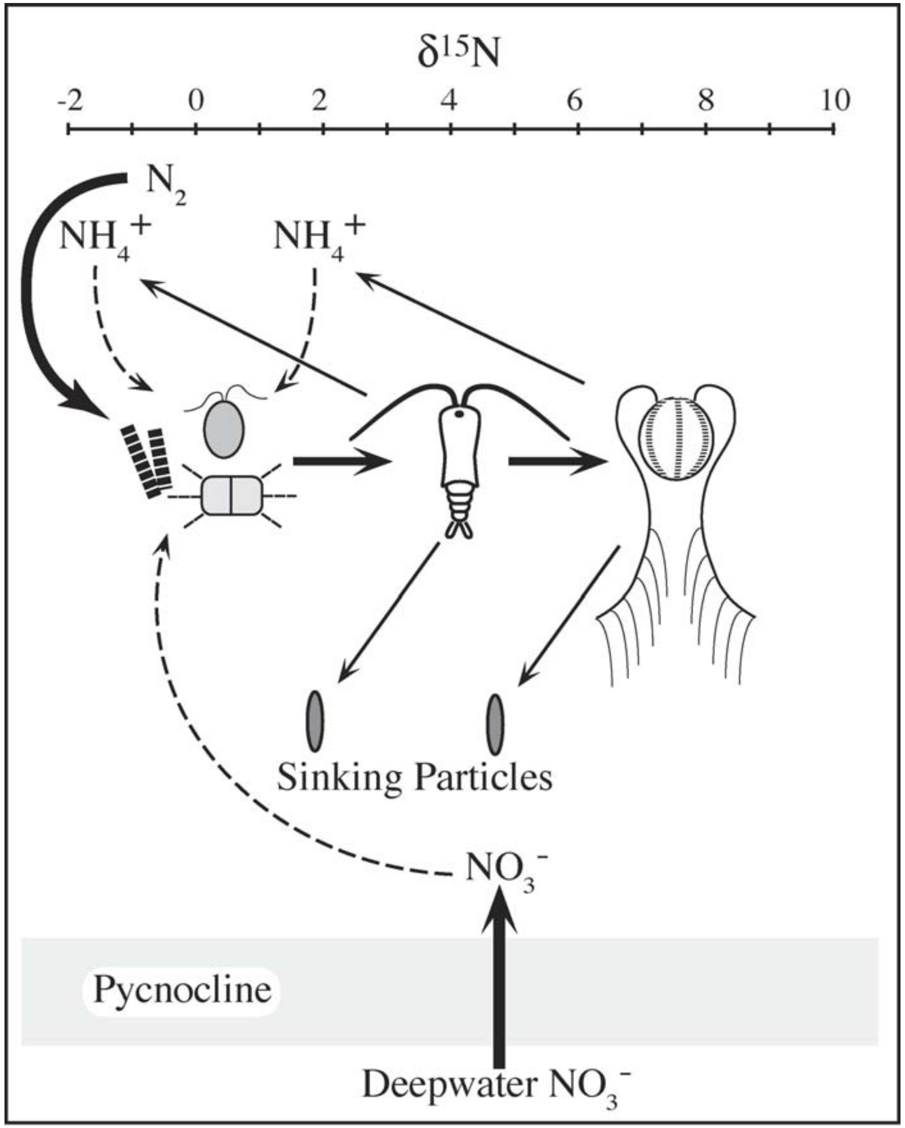


(Fry and Sherr, 1984, *Contrib. Mar. Sci.*)

… but $\delta^{15}N$ also reflects source contrasts

- N_2-fixation can (potentially) alter the isotopic composition of oceanic fixed N.
  - N_2-fixation produces combined N with a low $\delta^{15}N$ (~-2‰).
  - This contrasts sharply with the typical $\delta^{15}N$ of deepwater nitrate (~ 4.5 to 6‰).
- In oligotrophic waters, N_2-fixation injects isotopically depleted N into the upper water column, lowering the $\delta^{15}N$ of the ecosystem.

N Isotopes in the Upper Water Column

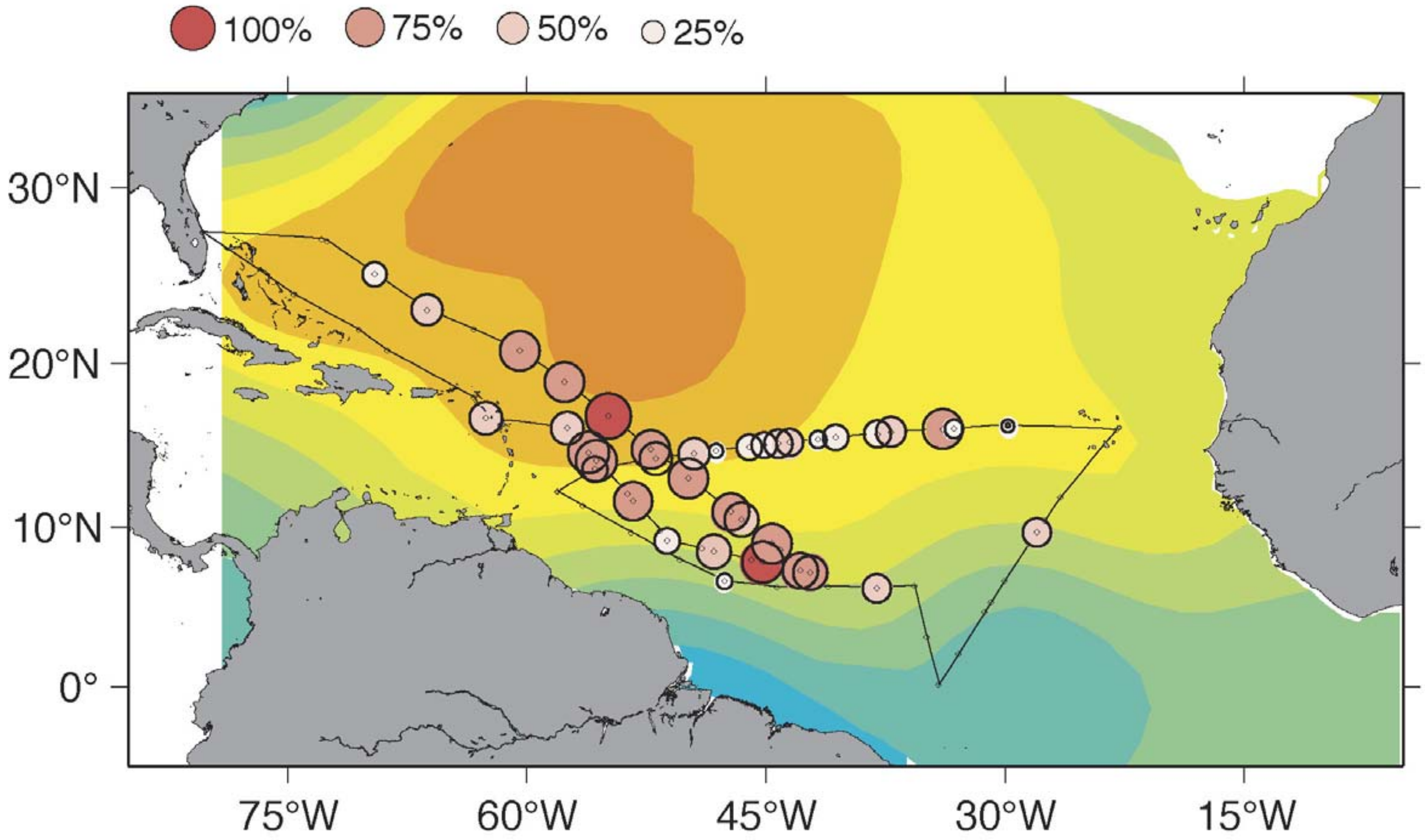


rev: 12/05

- Subsurface NO_3^- has $\delta^{15}N \sim 4.5‰$
- N_2-fixation produces organic matter with a low $\delta^{15}N$ (~ -2‰)
- The $\delta^{15}N$ of organic matter in the upper water column is pulled in opposite directions by upwelled NO_3^- and in situ N_2-fixation.

Plankton Nets

North Atlantic Nutrient Ratios and Isotope Budgets



- Contours show N* on σ_{Θ}=26.5

(Data: Montoya et al., 2002. Limnol Oceanogr. 47: 1617-1228) (Figure courtesy Nicky Gruber)

Gulf of Mexico: Diazotroph Contribution to Zooplankton

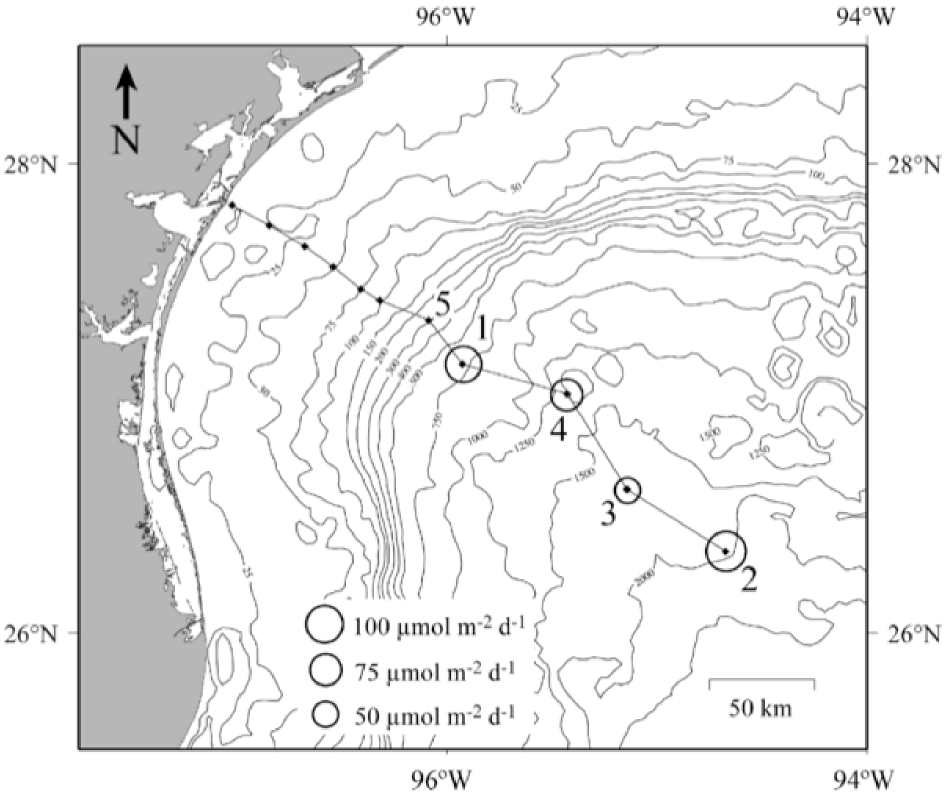


Fig. 1. Station locations and bathymetry for July 2000 cruise. All 10 stations are indicated by small diamonds. Sta. 1–5 are identified with numerical labels. For clarity, the markers for Sta. 6–10 are omitted; these five stations run in sequence from Sta. 5 toward shore. For Sta. 1–4, depth-integrated areal N_2-fixation rates are represented by the open circles. The area of each circle is proportional to the N_2-fixation rate measured at that station.

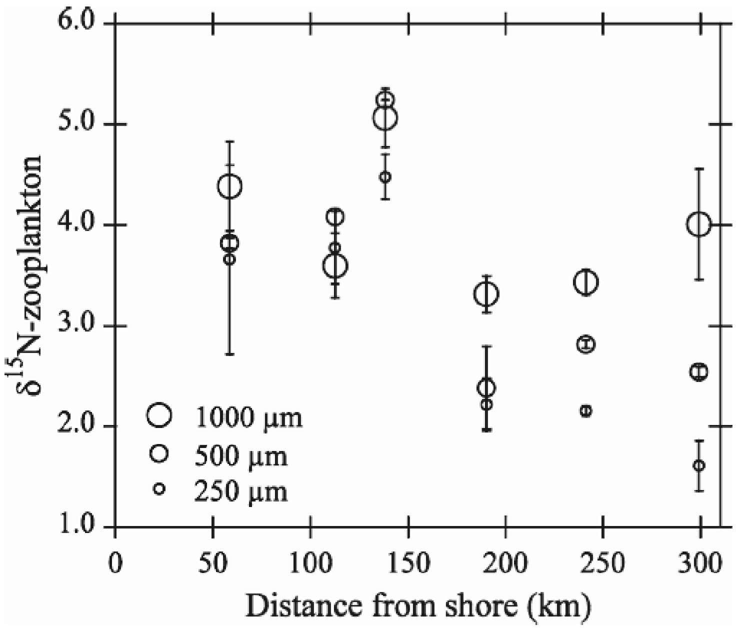
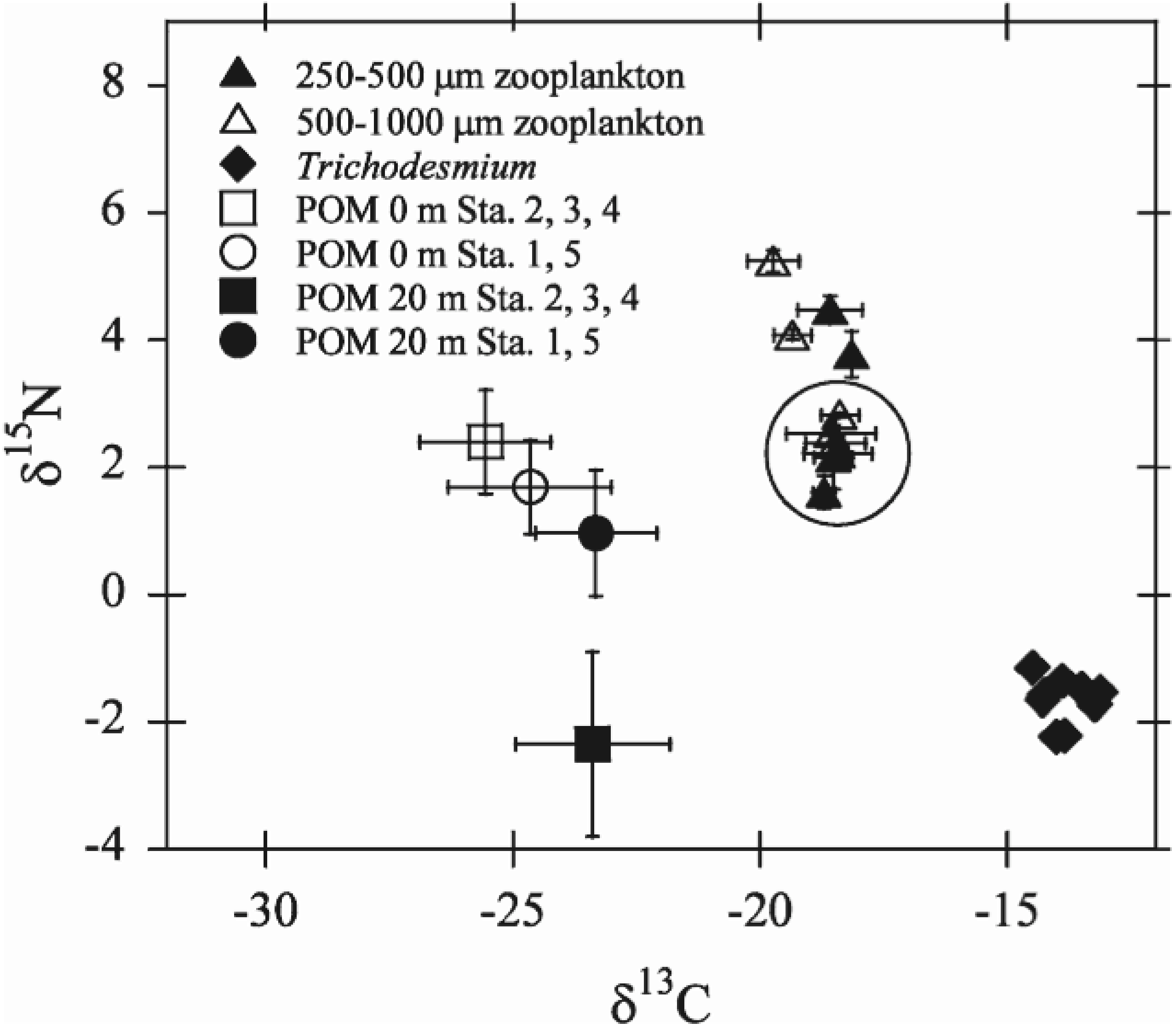


Fig. 9. The δ^{15}N (‰) of the 250-μm, 500-μm, and 1,000-μm zooplankton size fractions as a function of distance from shore (km).

(Holl et al., 2007. Limnol Oceanogr. 52: 2249-2259))



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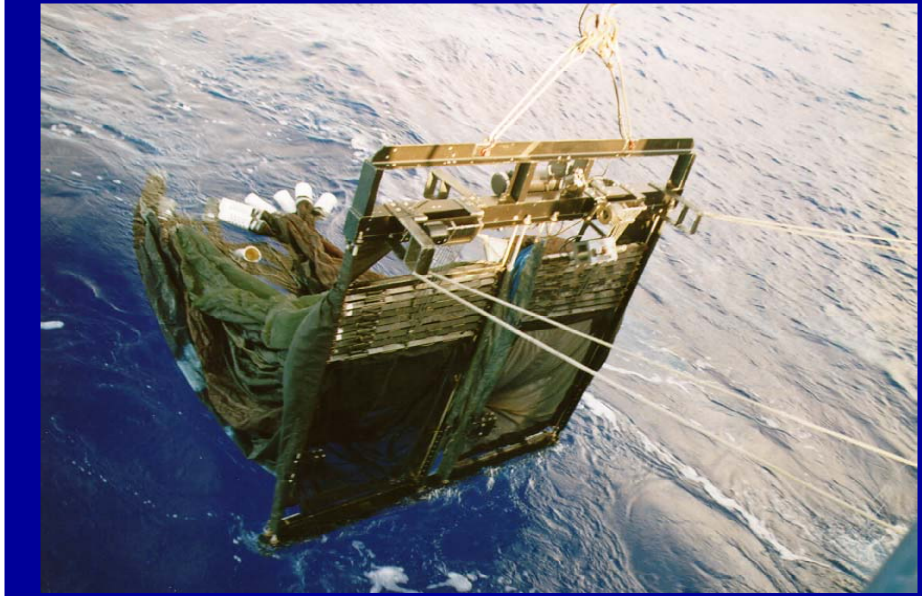
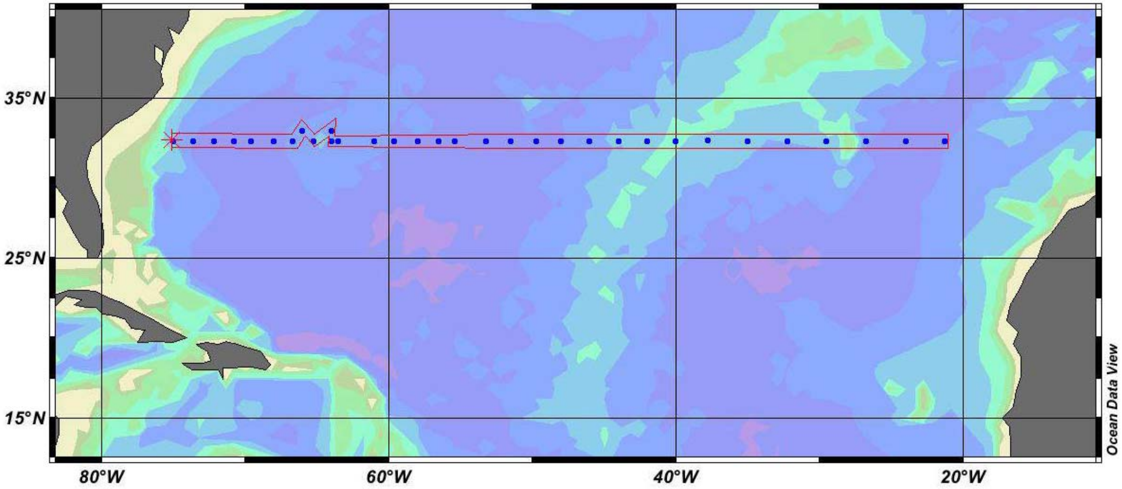
(Holl et al., 2007. Limnol Oceanogr. 52: 2249-2259))



MOCNESS

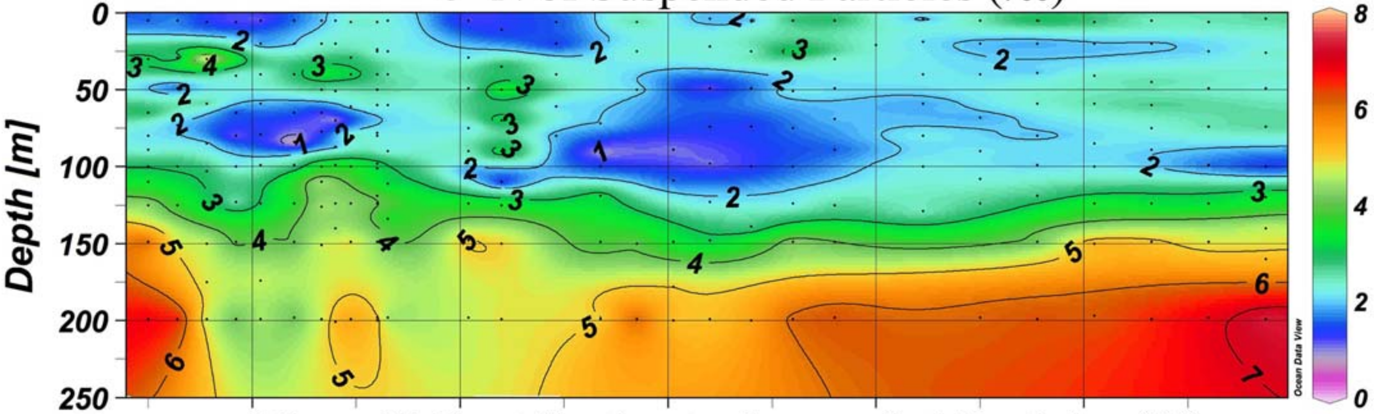
SJ0005: PN Section



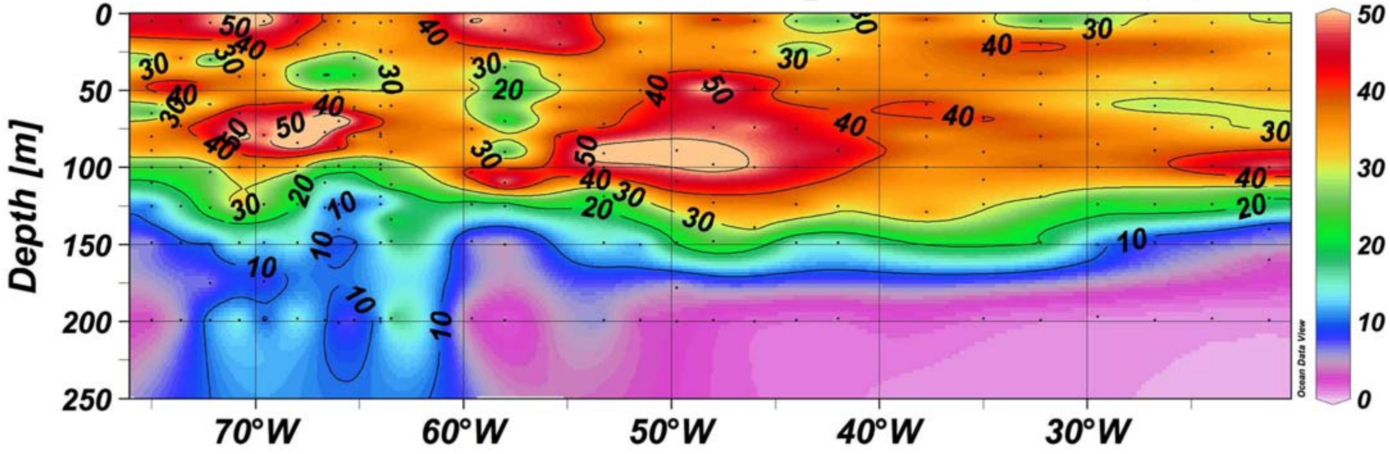
Jason Landrum, in prep

SJ0005: PN Section

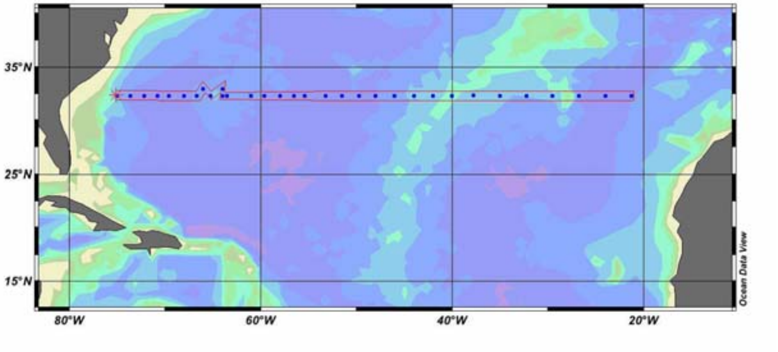
δ15N of Suspended Particles (‰)

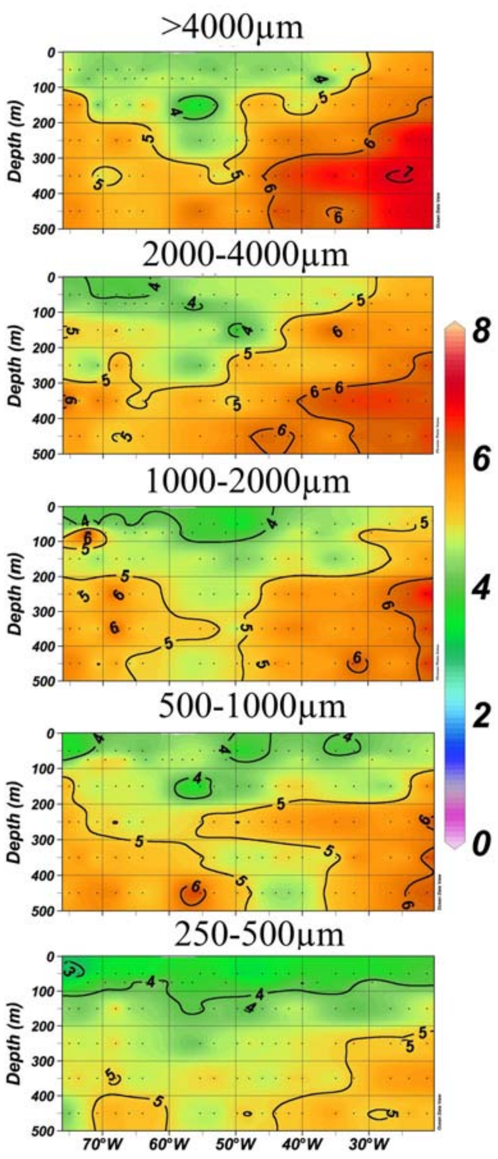


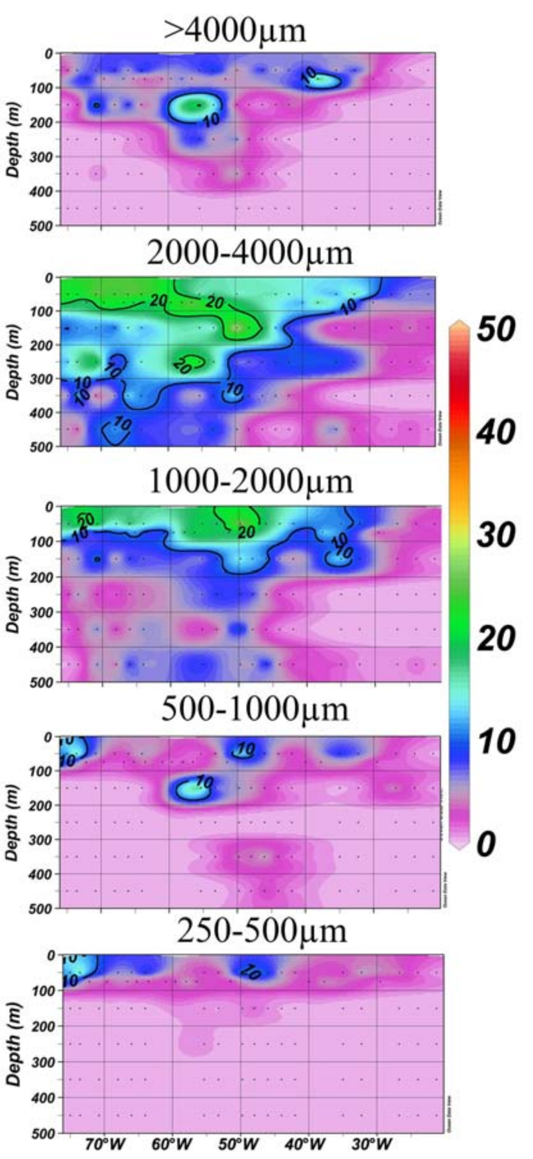
Diazo-N Contribution to Suspended Particles (%)



SJ0005: Zooplankton



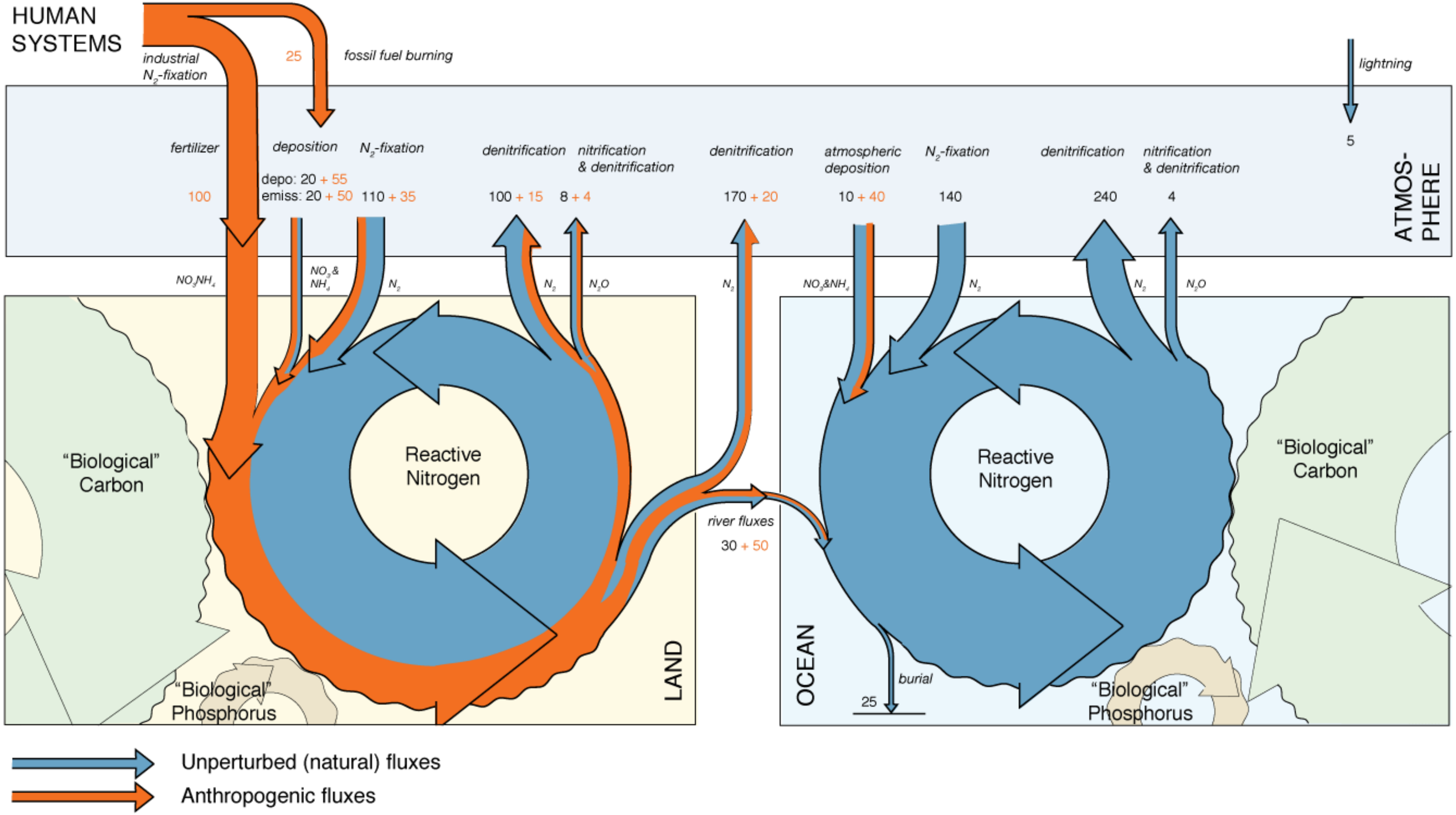

δ15N Zooplankton (‰)


Diazo-N Contribution to Zoops (%)

Jason Landrum, in prep

Fluxes in Tg N y^{-1}

Biogeochemical “Gears”



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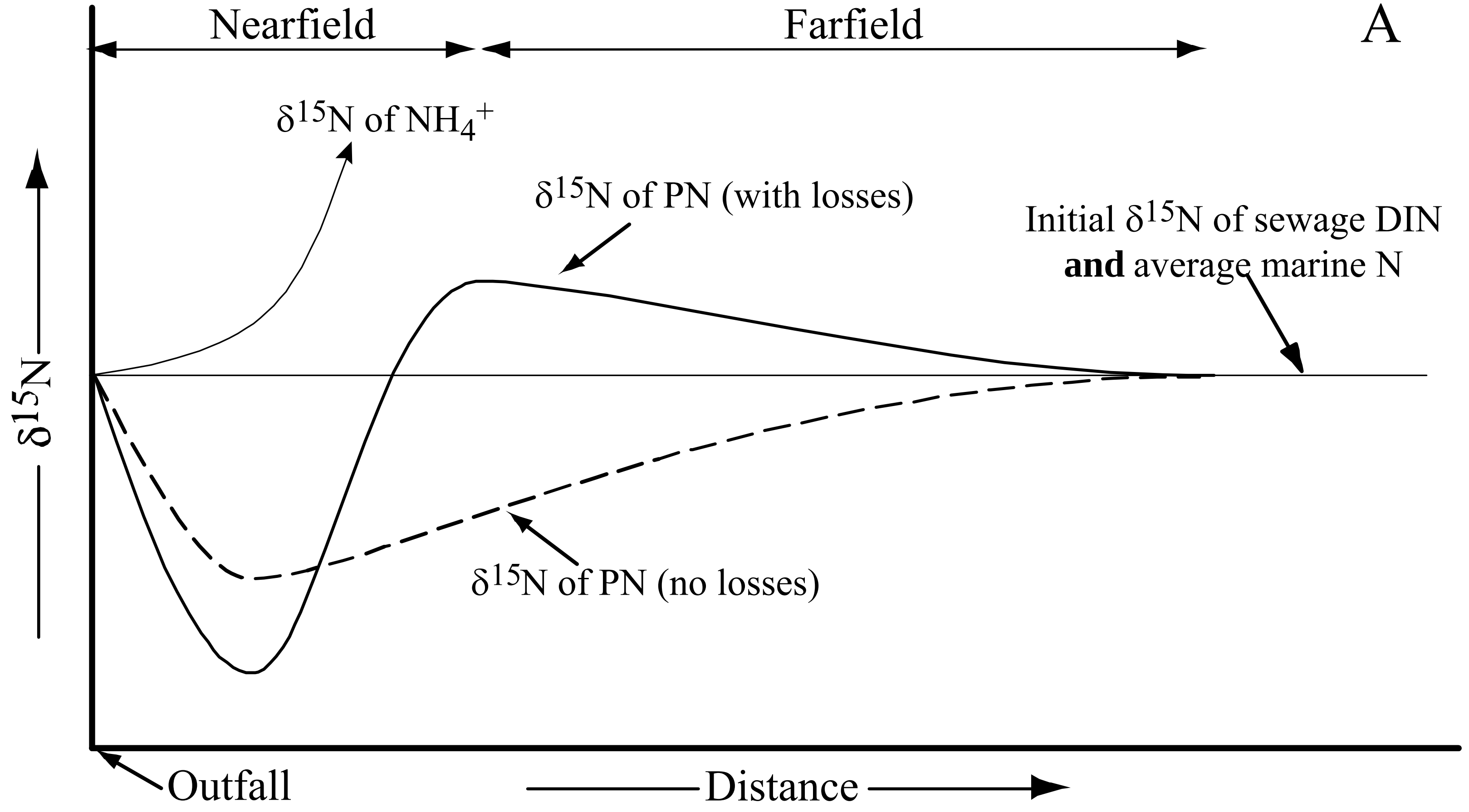
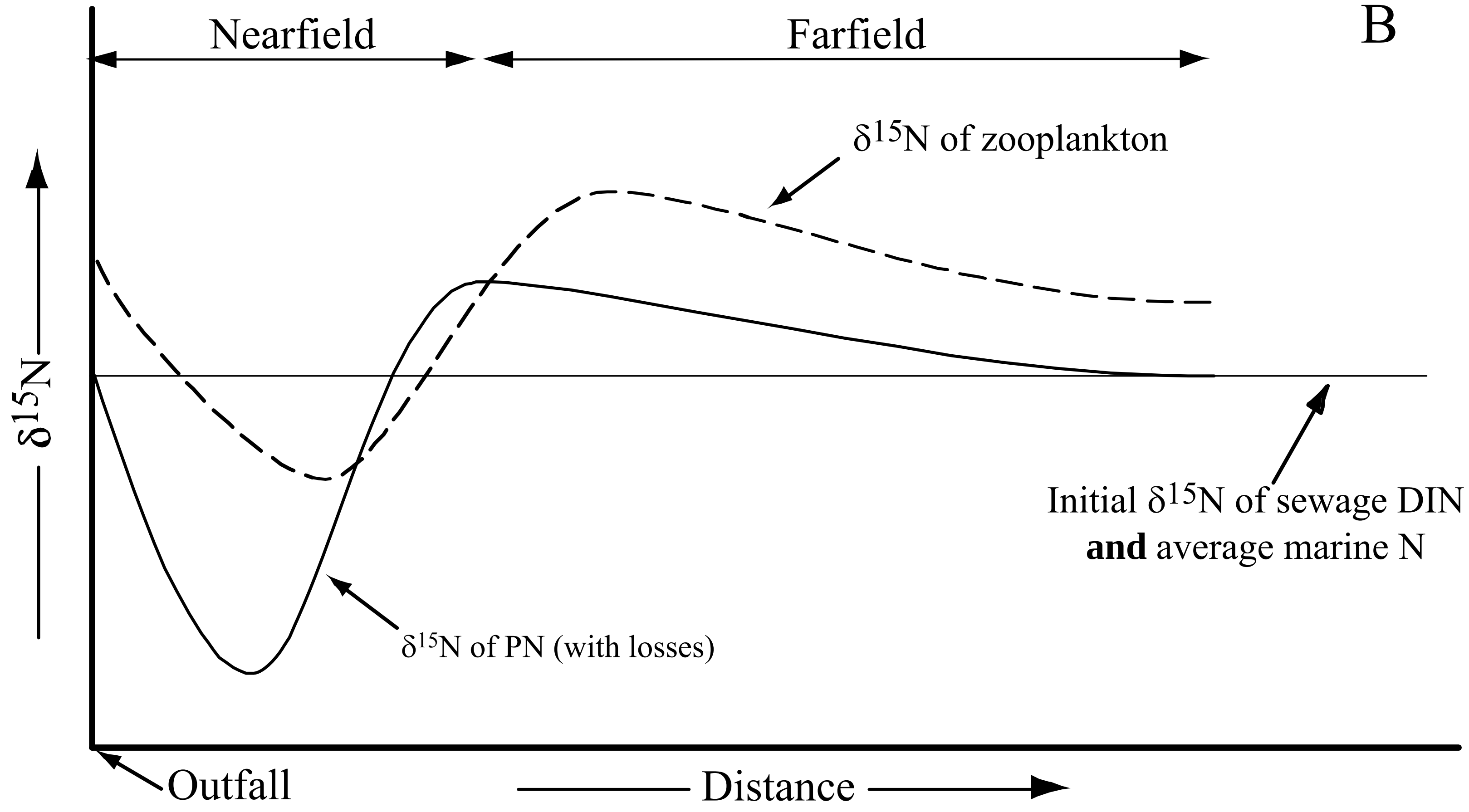
Gruber & Galloway, 2008, Nature

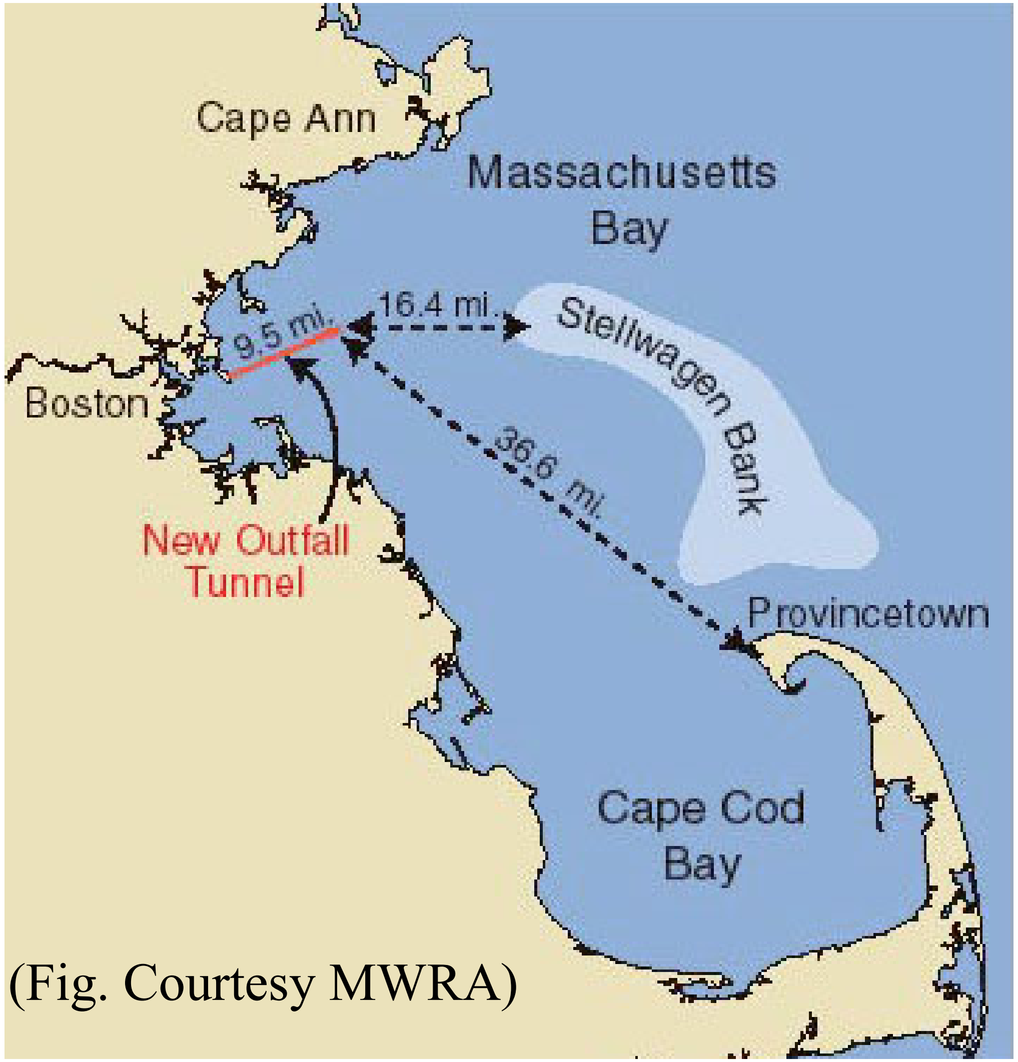
Sewage Produces an Isotopic Signature in PN



The Isotopic Signature of PN Propagates into Zooplankton



Massachusetts Water Resources Authority Outfall

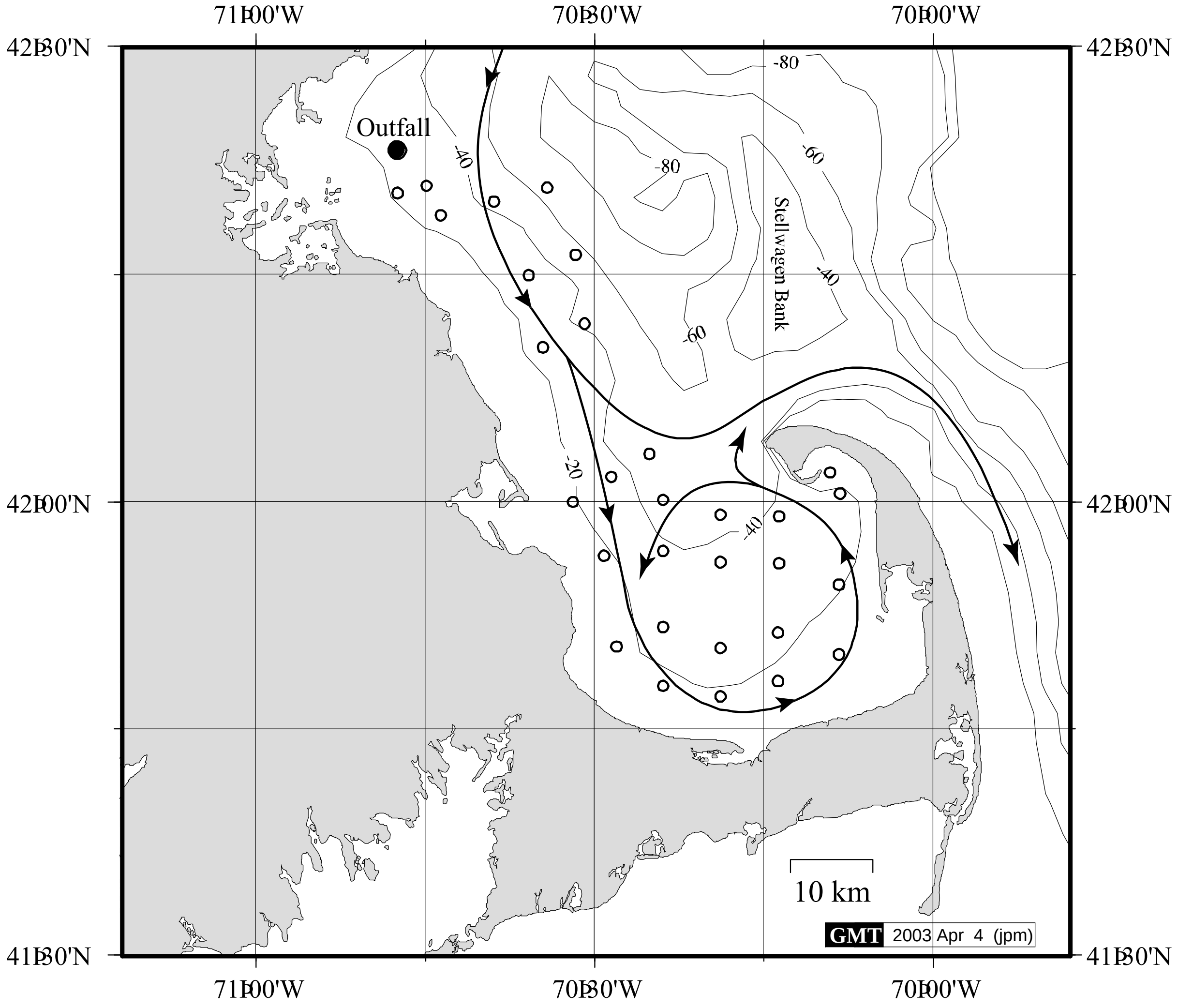


(Fig. Courtesy MWRA)

New MWRA Outfall

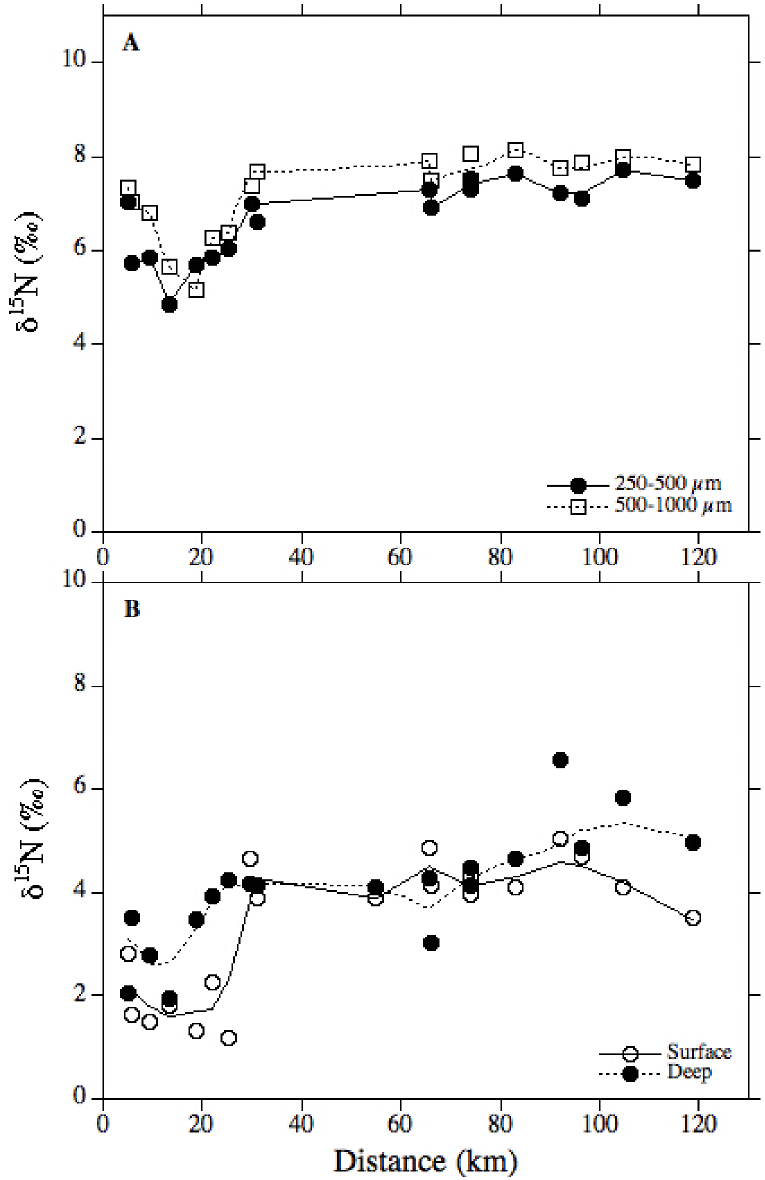
- Operational in Sep 2000
- Sewage transported through a tunnel to a diffuser field outside Boston Harbor.

Center for Coastal Studies Survey Stations



Baseline Isotopic Composition of Sewage

- Deer Island Effluent (Primary Treatment) Samples collected Nov 1994 – Dec 1995.
  - Mean $\delta^{15}NH_4^+ = 7.2 \pm 0.7$ ‰
    Range: 6.1 to 8.3‰ (N=9, Sheats 2000)
- Deer Island Effluent (Secondary Treatment) Samples collected Feb 1999 to Mar 2001 and analyzed at the BU Stable Isotope Lab.
  - Mean $\delta^{15}NH_4^+ = 6.1 \pm 0.2$‰
    Range: 5.0 to 7.7‰ (N=18)
  - Oct 1999 outlier: Mean $\delta^{15}NH_4^+ = 23.6 \pm 1.2$ ‰



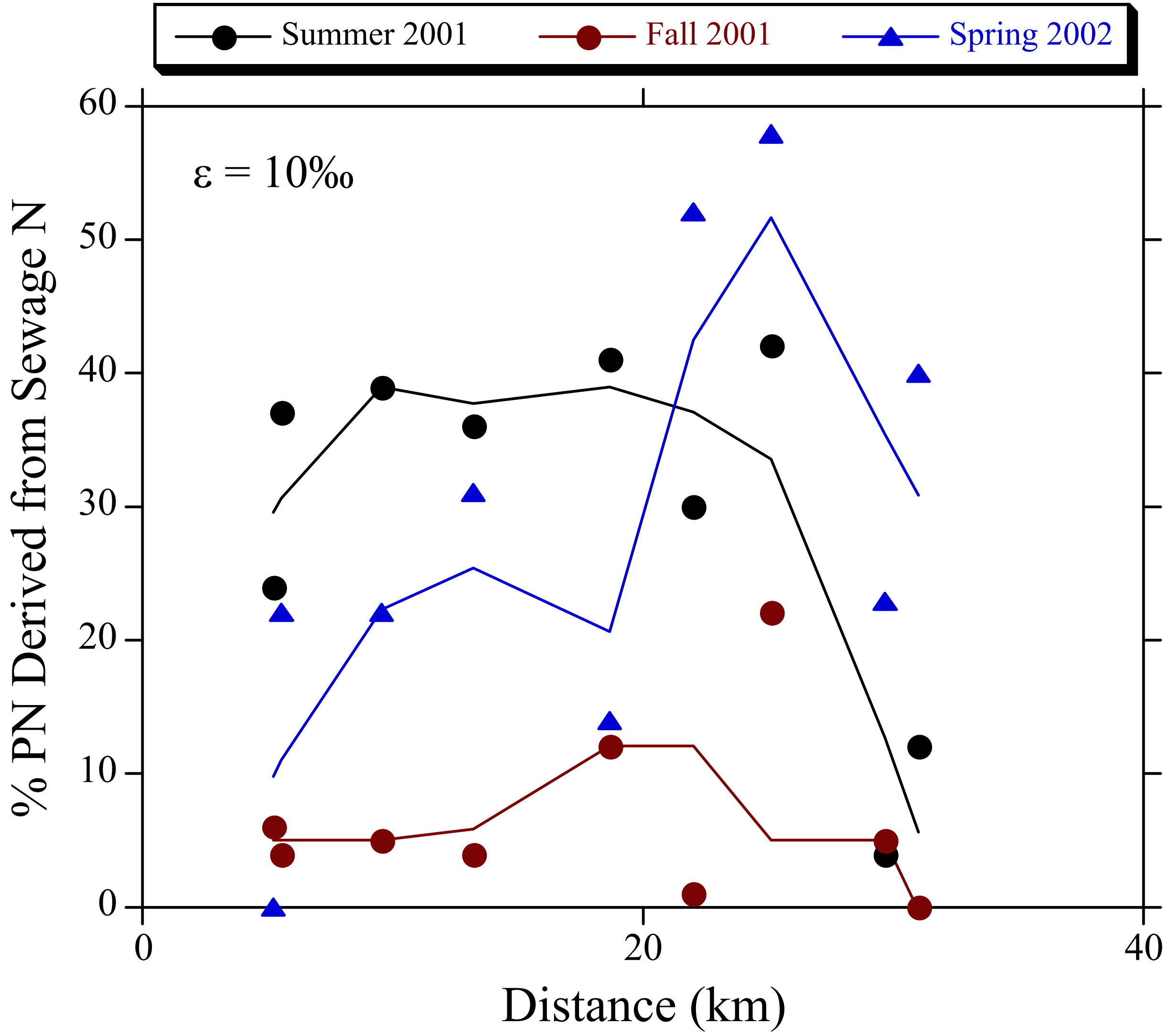
Survey: Summer 2001

δ^{15}N of samples collected between 28 July and 4 August 2001 (cruises SW216, SW217, and SW220) as a function of distance southward from the outfall along the mean flow path through Massachusetts Bay and into Cape Cod Bay. A smoothed trend line is shown for each data set.

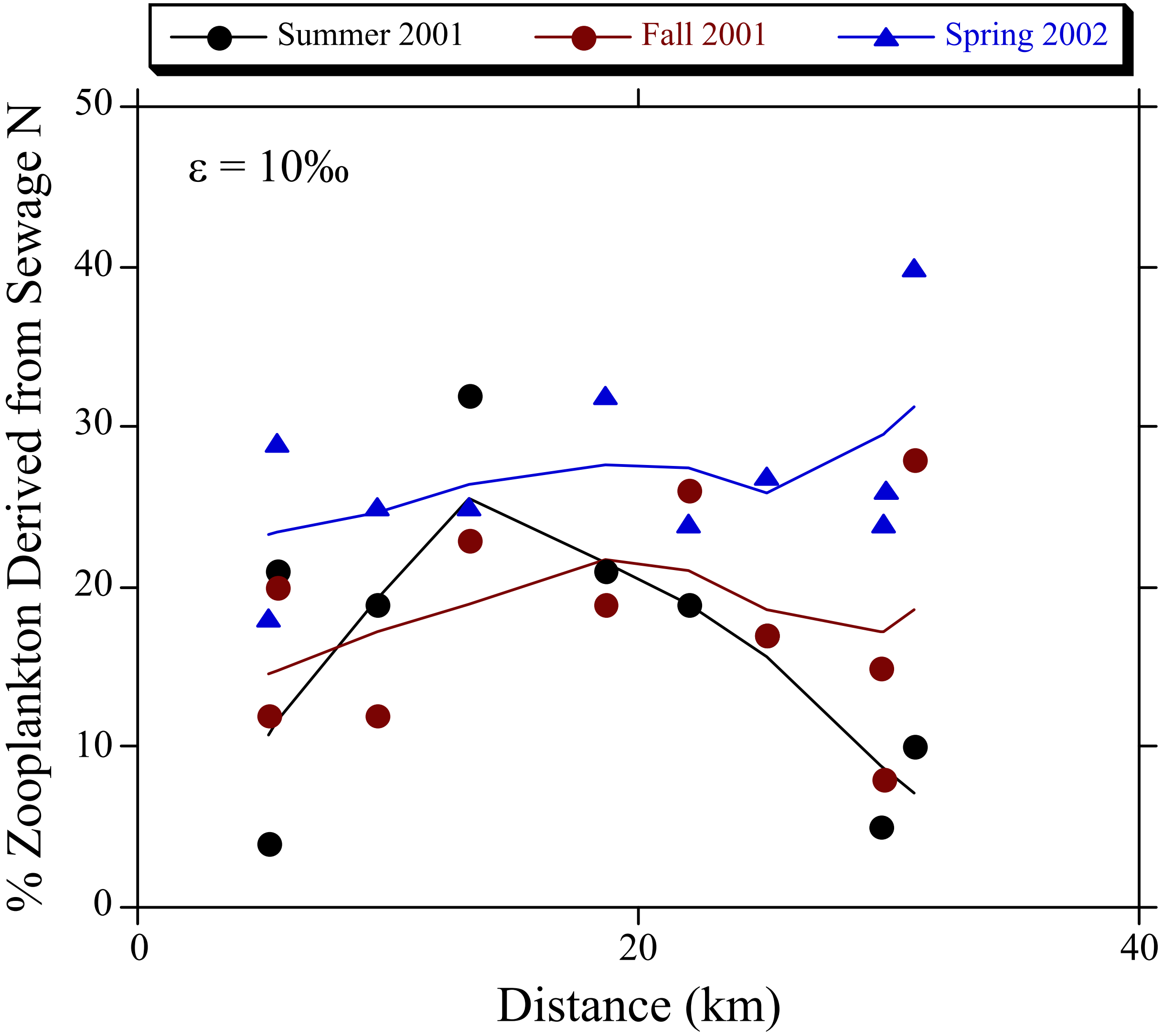
A: zooplankton from the 250 – 500 µm size fraction (circles) and zooplankton from the 500 – 1000 µm size fraction (squares).

B: Surface particulate nitrogen (open circles) and deep particulate nitrogen (filled circles).

Estimated Contribution of Sewage to PN



Estimated Contribution of Sewage to Zooplankton



Thanks for Listening!

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