

# Molecular approaches to Marine Microbial Ecology and Biogeochemical cycling of Nitrogen and Carbon

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*Traditional methods of bacterial enumeration, culturing and microscopy are often insufficient to understand some biogeochemical processes – by complex mixed microbial population- the black box*

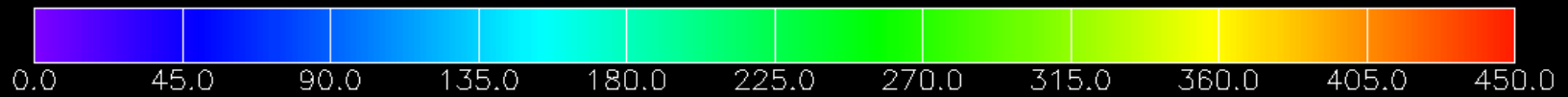
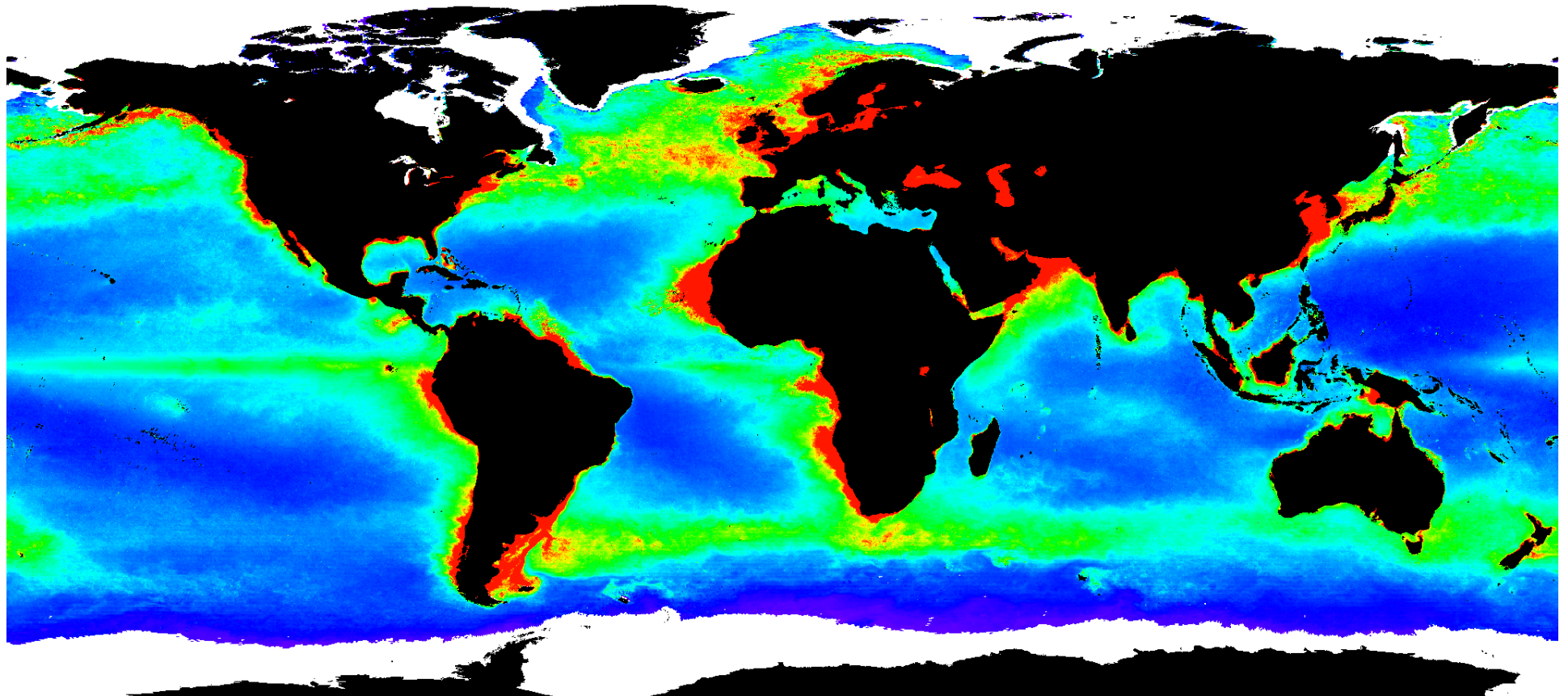
*Insights on the complexity of the microbial population and its interaction with the environment can be probed with molecular tools*

*Molecular tools have been developed and used to understand various groups that catalyze environmental function, its activity and abundance*

*Largely, these techniques rely on PCR amplification of low abundance gene in a mixed soup of abundant other genes*

*Functions and activity of microbial populations can also be understood by using reverse transcriptase coupled with PCR and other techniques*

*Annual Global Primary Production Sept 1998-August 1999*



SeaWiFS: Annual Primary Production (g C/m<sup>2</sup>)

Source: [http://marine.rutgers.edu/opp/swf/Production/results/all2\\_swf.html](http://marine.rutgers.edu/opp/swf/Production/results/all2_swf.html)

# Annual Mean DO at 150m

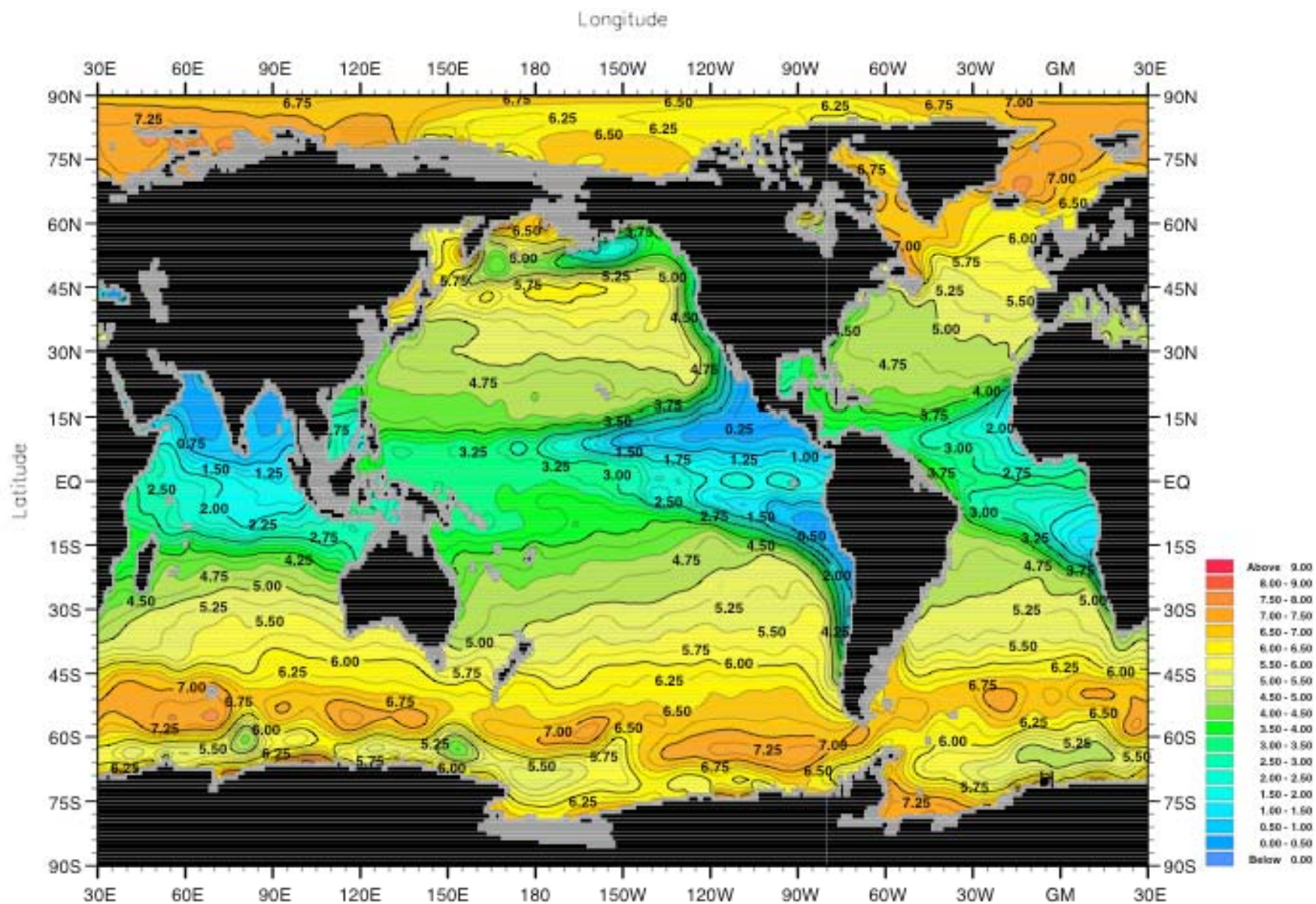


Fig. A2-9. Annual mean oxygen (ml/l) at 150 m. depth.

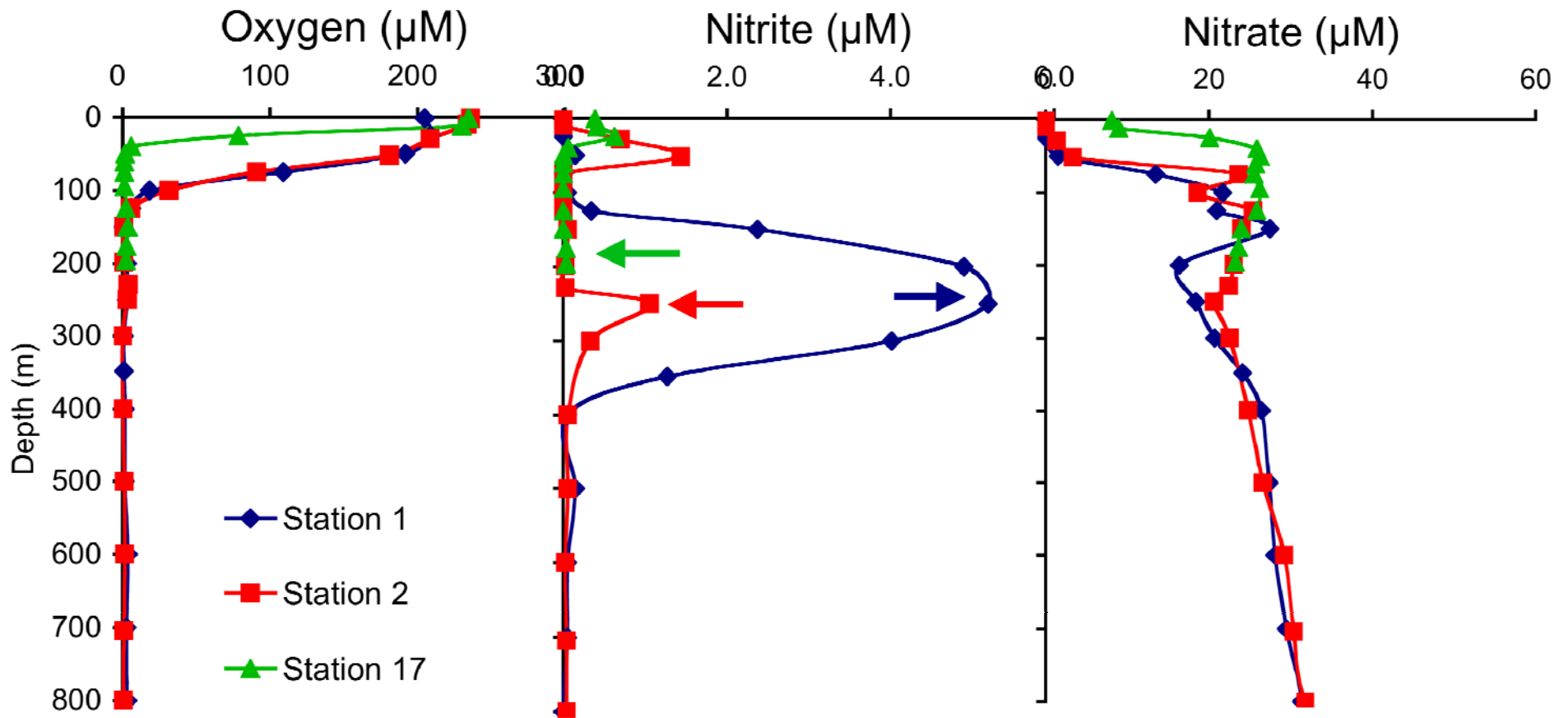
Minimum Value= 0.05

Maximum Value= 8.74

Contour Interval: 0.25



# Arabian Sea nutrient profiles



Naqvi et al. unpublished

# Intermediate Nepheloid Layer and Cell Abundance

The occurrence of pronounced subsurface particle maxima embedded within the core of suboxic layers of Eastern Tropical Pacific (Pak et al 1980, Garfield et al 1983, Spinrad et al 1989)

NAQVI ET AL.: INTERMEDIATE NEPHELOID LAYER IN NORTHWEST INDIAN OCEAN

16,471

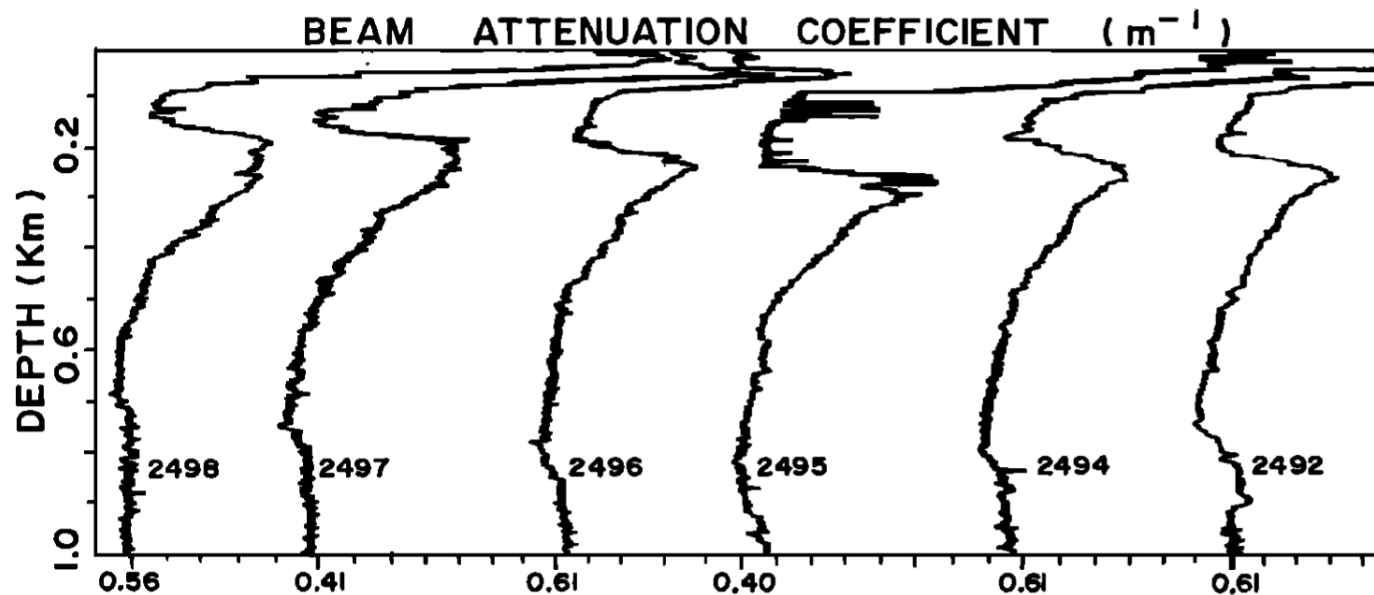
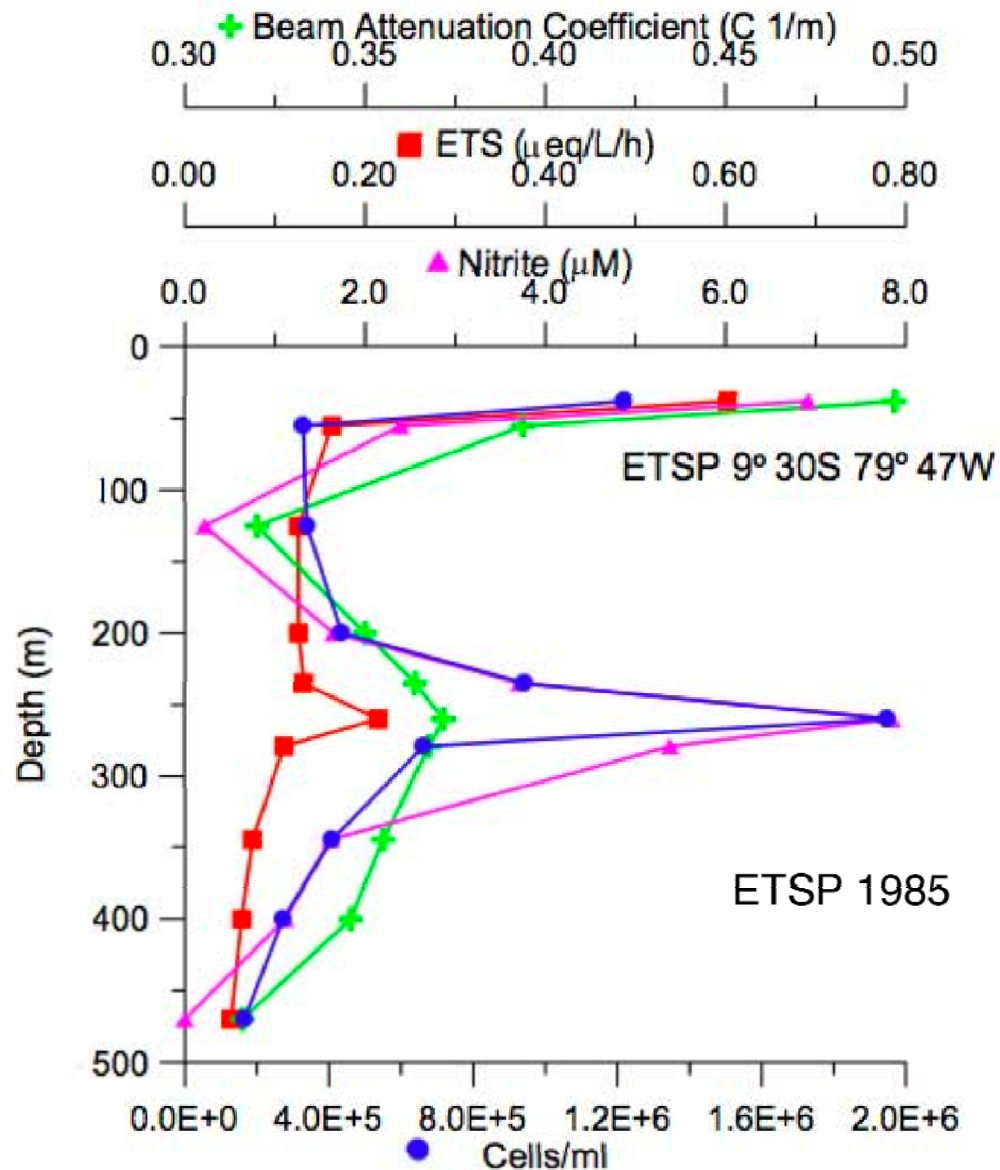


Fig. 2. Vertical profiles of beam attenuation coefficient along the transect perpendicular to the Indian coast. Each division on the transmission scale corresponds to  $0.01m^{-1}$ . The scale for each profile could be read with reference to the number given at the bottom.

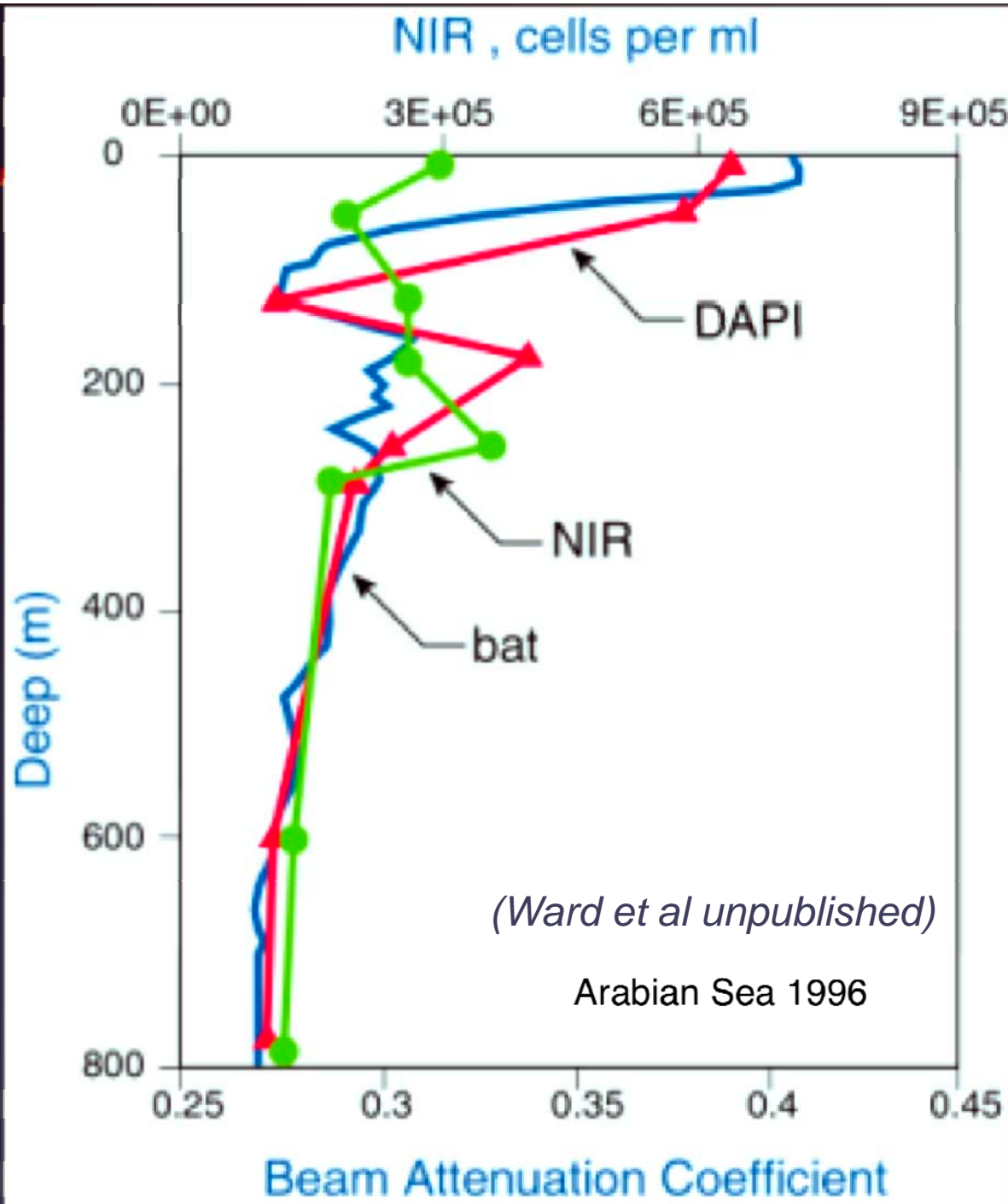
*Arabian Sea, Naqvi et al 1993*



Indicators of denitrification:

Most intense denitrification at SNM

Spinrad et al., 1989





# Key Questions

- What is the composition of the Nepheloid layer
- Are they all denitrifiers or are there others groups as well
- Is there diversity within various functional groups
- If so, who are the key players that contribute to carbon and N cycling in the OMZs

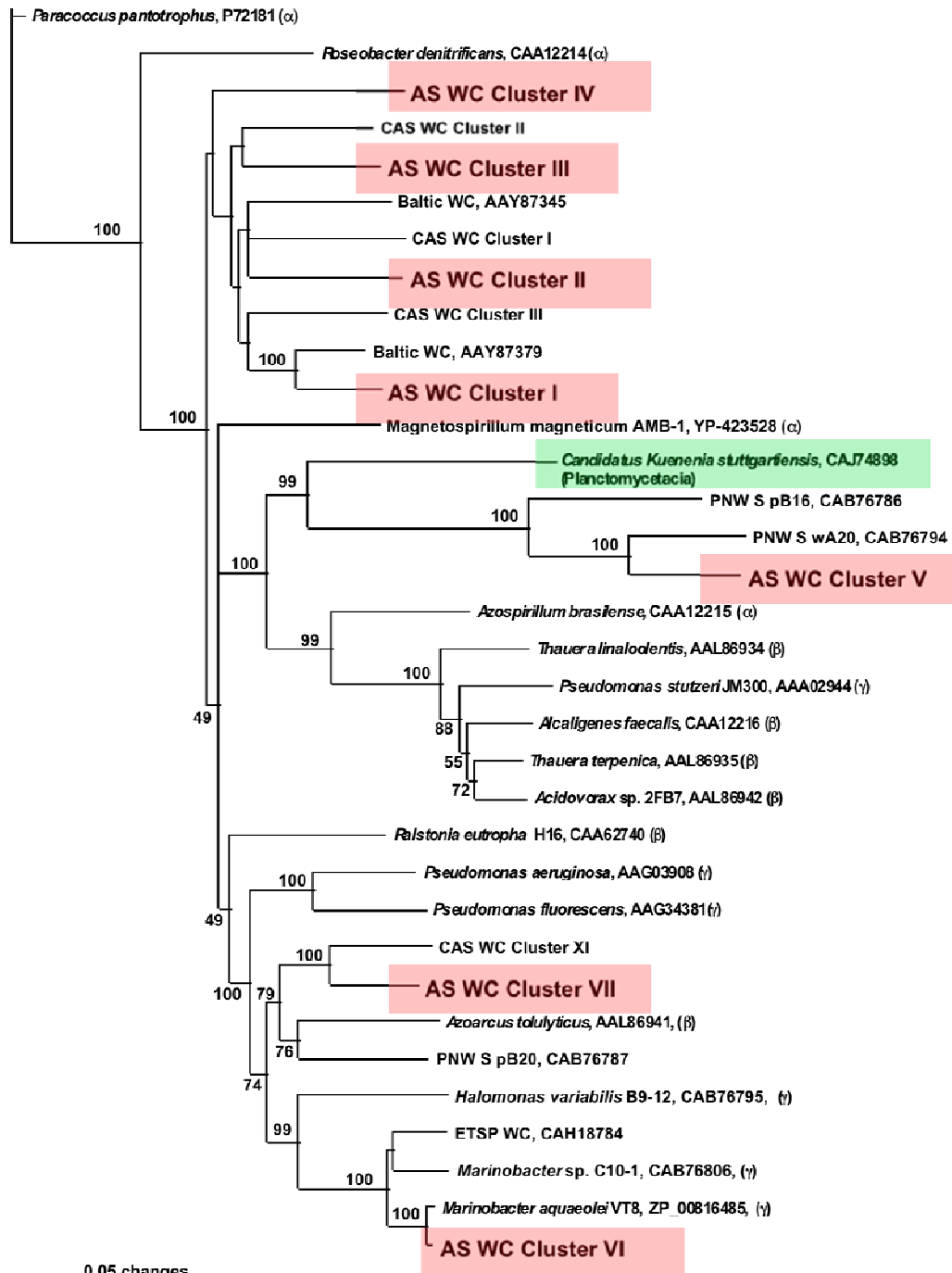
# Nitrate Respiring Microbes

- **Organisms that can remove Oxidized forms of N from the environment include,**
  - **heterotrophic denitrifying bacteria**
  - **anammox bacteria**
  - **archaea and autotrophic nitrifiers**
  - **foraminifera**
  - **thioploca(?)**

# Diversity Studies

- Techniques:
  - Traditional- Isolation, Microscopy
  - Molecular: TRFLP, RFLP, Sequencing, Pyrosequencing.
- Several genes may be targeted depending on the question: e.g. 16S rRNA or a function (e.g.. *nir*, *rbcI*)
- Sequence and Types of Suboxic Respiration (Codispoti et al 2005)
  - $O_2 > IO_3^- > NO_3^- > Mn(IV) > Fe(III) > SO_4^{2-}$
- We have targeted the *nirS*, *nirK* and AMX 16S rRNA

# Arabian Sea *nirS* phylogeny

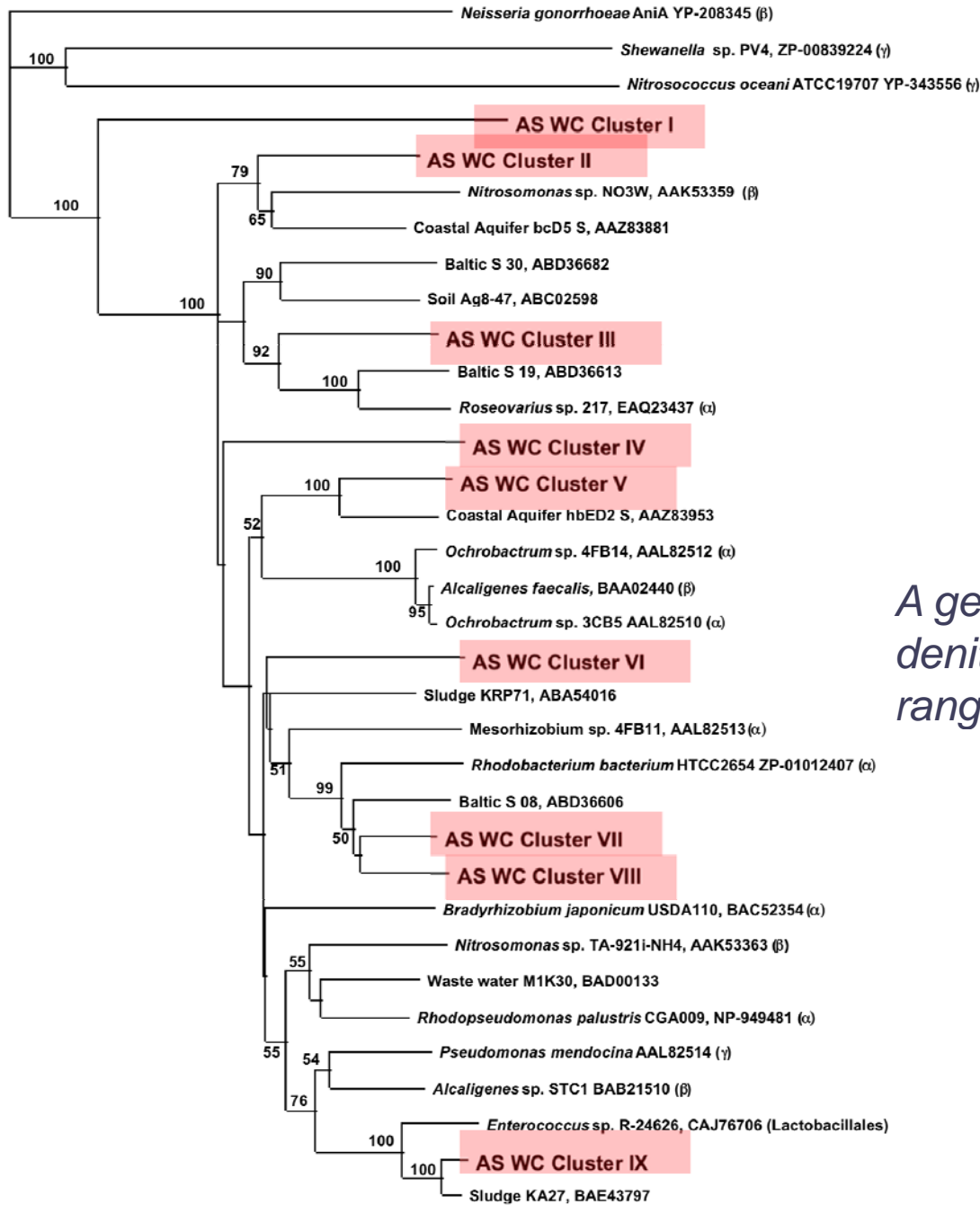


Sample-DNA extraction- 3 stations with varying suboxia/anoxia

PCR amplification- *nirS* 1F-6R (Braker et al 1998)  
-clone library-Sequencing

- Based on 890 bp
- Can compare sequences within as well as from database
- 250 seqs grouped into 7 clusters
- Low nitrite sample- high Diversity
- High nitrite sample- low diversity- Dominance of one group





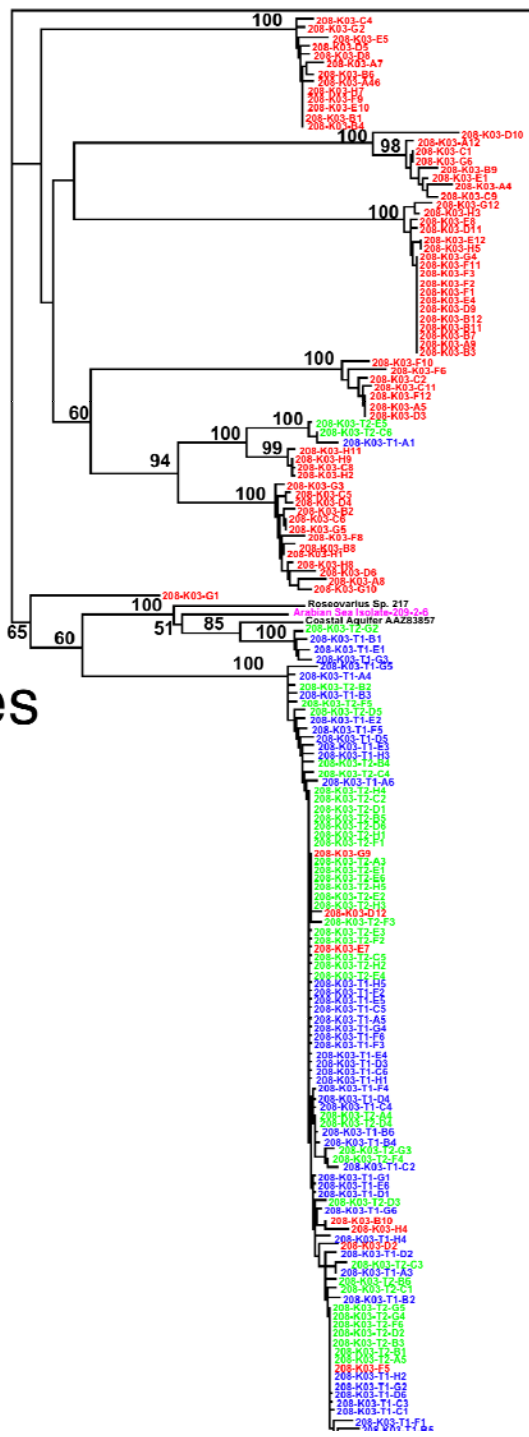
## Arabian Sea *nirk* phylogeny

*A gene found not only in denitrifiers, but also nitrifiers, (wide range of physiological groups)*

0.05 changes

nirK

Coastal (station 17)  
Arabian Sea Incubation  
3 time points (2.5 days)

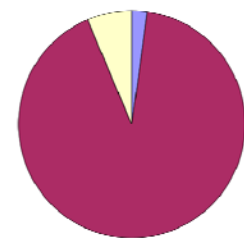


= 0.01 changes  
~156 aa

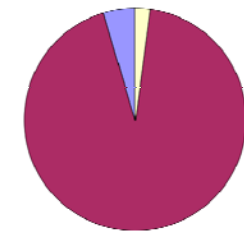
T0



T1

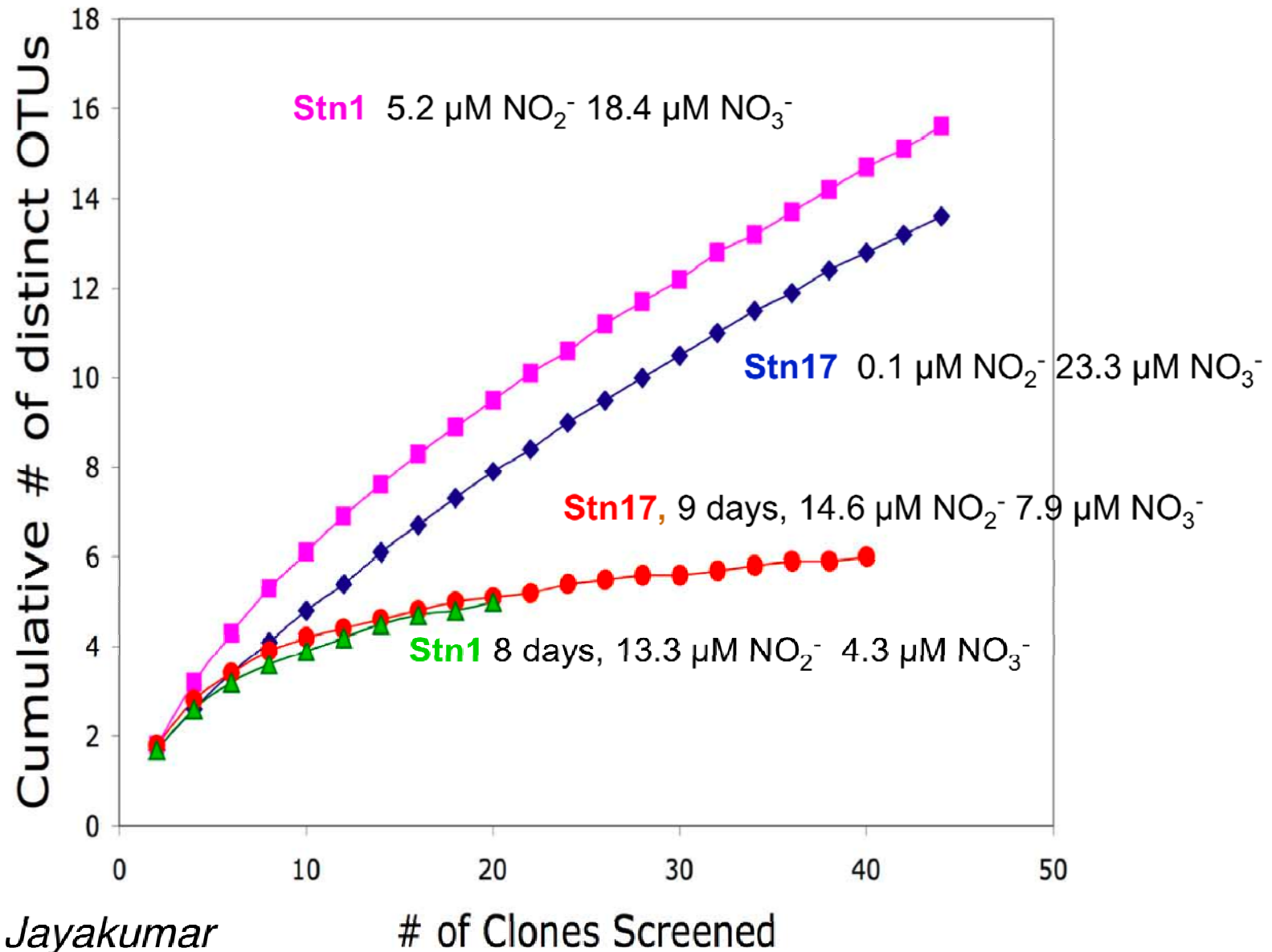


T2



- community shifts towards dominance

# Rarefaction Analysis of *nirK* clones RFLP and sequence data



## ***Phylogeny Summary***

***The overall diversity of nitrite reductase genes are limited in the open Arabian Sea - WRT sedimentary and terrestrial environments and CAS***

***Most nirK or nirS genes from the Arabian Sea do not show any close identities to other published sequences***

***One clone from Coastal AS- 97% identical to waste water organism (Enterococcus )***

***Some clades are unique to the open ocean and some are unique to the coastal regions, only two groups appears to be cosmopolitan***

***Some groups of nirS common to both Open Ocean AS and ETSP***

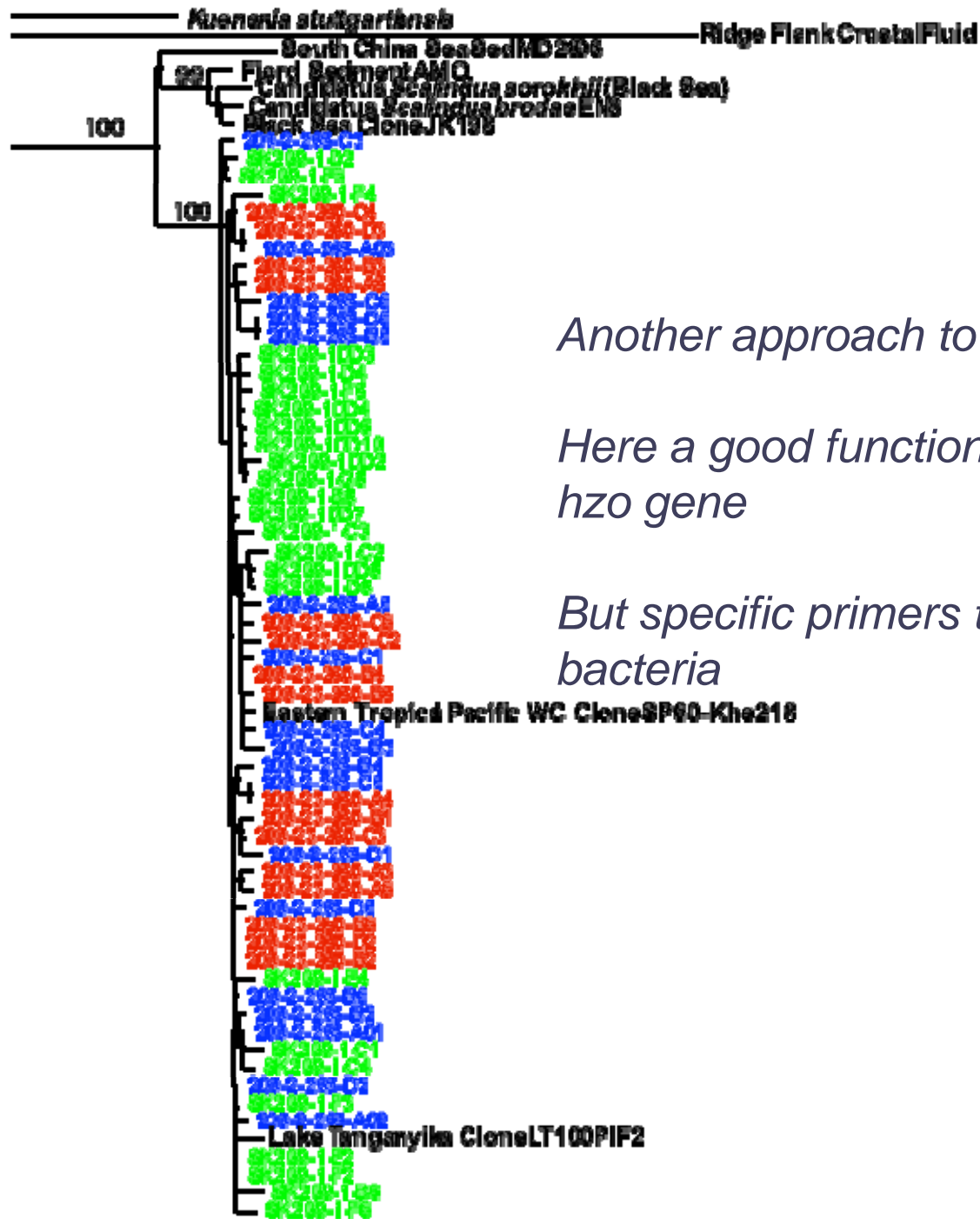
***Cannot usually detect nirS in oxic environments, nirK can be***

***Both nirS and nirK show bloom dynamics***



## Conclusions from phylogenetic studies

- Take home from bloom dynamics—
  - denit are episodic in response to C input, respond rapidly
  - abundant and diverse initially, few phylotypes WIN
  - one winner, DOM *nirS*, was dominant type in two different years, 3 yrs apart, in bags and in water column without incubation
  - DOM *nirS* does not occur in ETSP – so far
  - suspect other strains dominate at other times and places in response to episodic C input



*Another approach to diversity - 16S rRNA*

*Here a good functional gene target would be hzo gene*

*But specific primers targeting only anammox bacteria*

# Diversity studies

## Advantages of sequencing technique

- one can build a data base for other genetic studies
- communities from different environments can be compared (our studies show that the denitrifying communities in AS, ETSP and from CB, other coastal systems are different)

## Disadvantages:

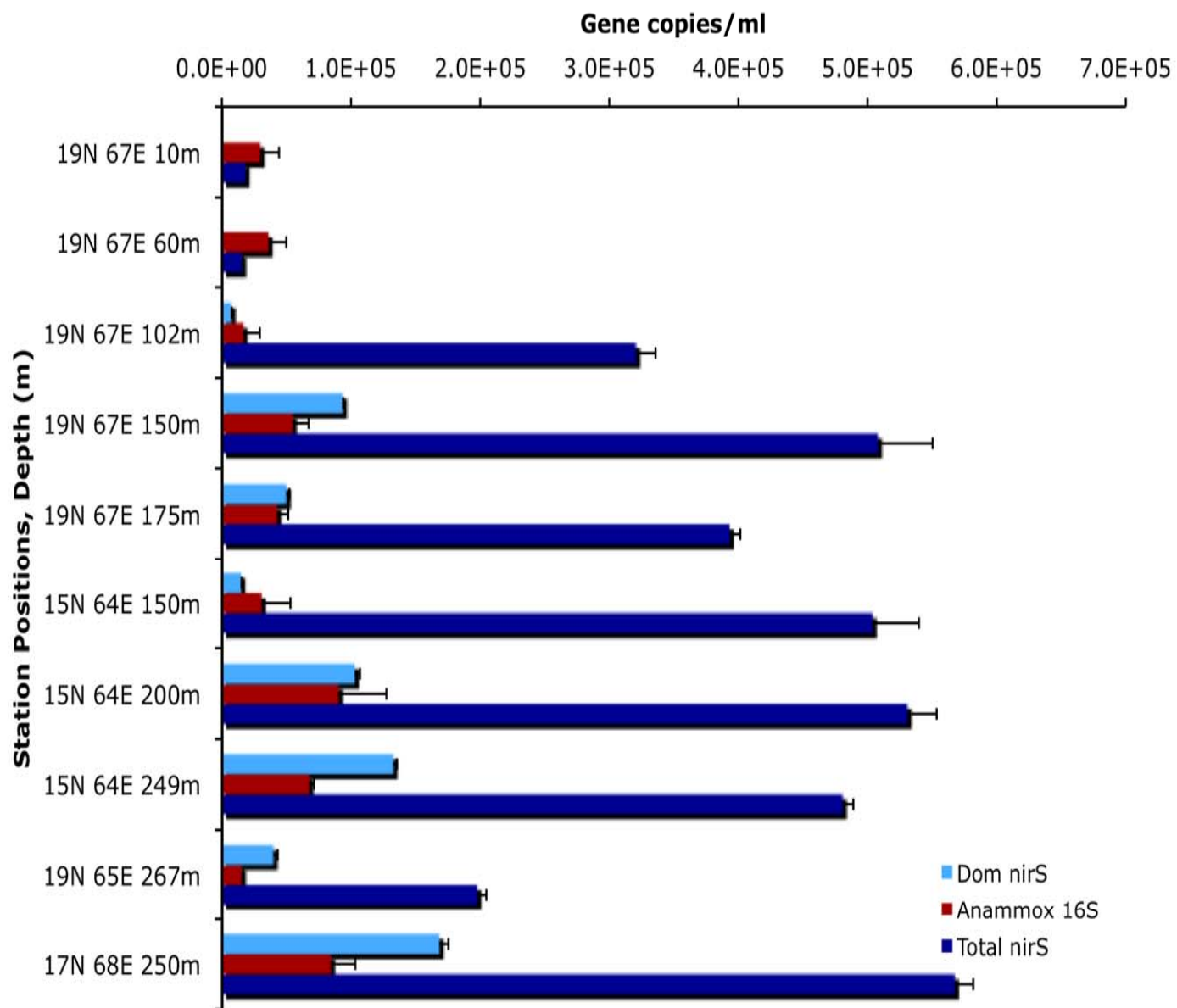
- most common clones are not in culture-might be worth pursuing – do manipulative experiments, especially the key players-genome sequencing
- we do not have any taxonomic information
- selection of appropriate primers very very important
- Labor intensive and expensive

## Different techniques to get the abundance information

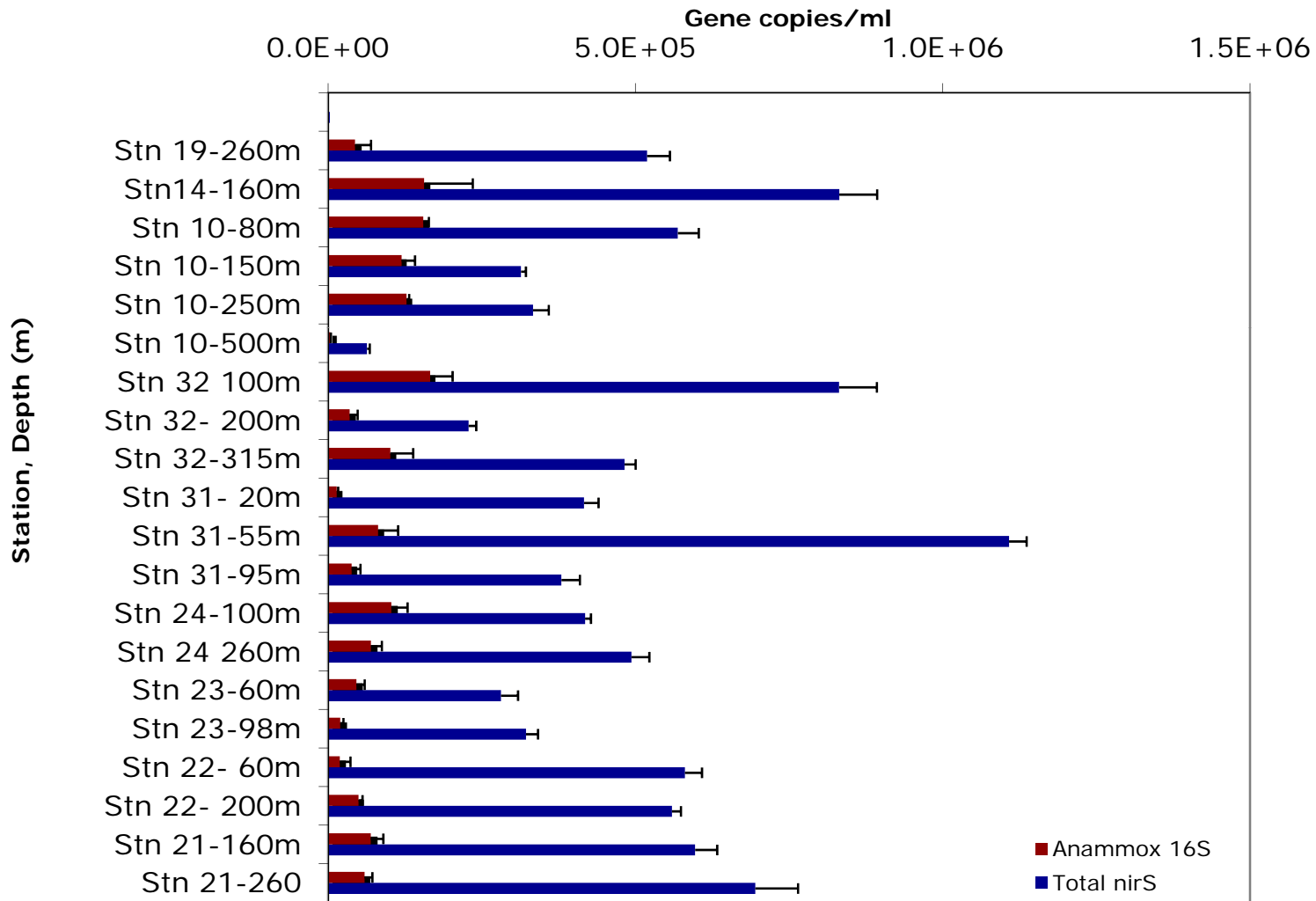
- FISH probes
- qPCR Quantitative info (in contrast to phylogeny)
- Method: This is again a PCR but with fluorescent tag to monitor relative increase in product
- Select primers to amplify <500bp region
- Specific primers to target specific phylogenetic groups
- Standard construction
  
- Micro- arrays



# Arabian Sea OMZ

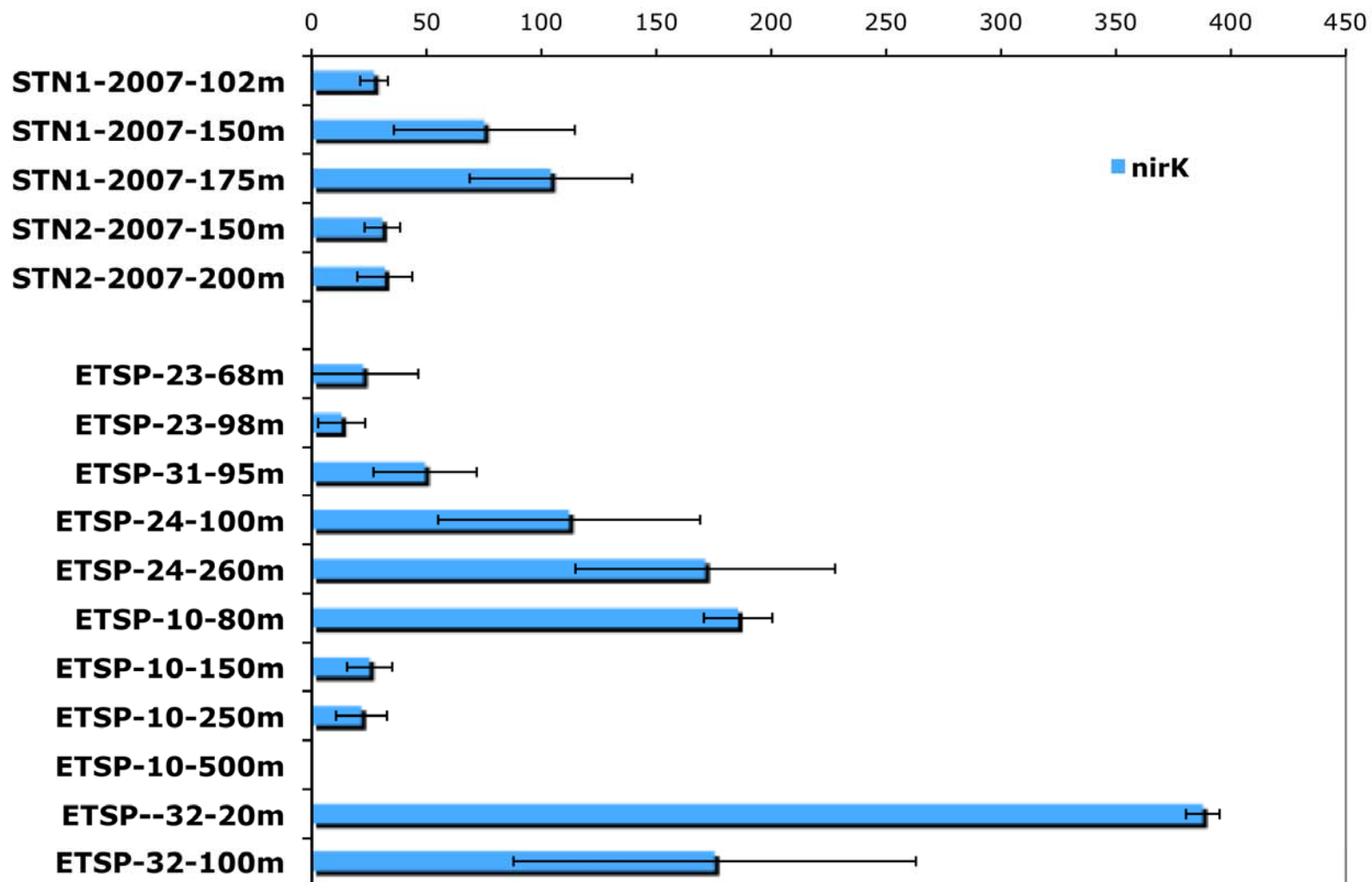


# ETSP OMZ

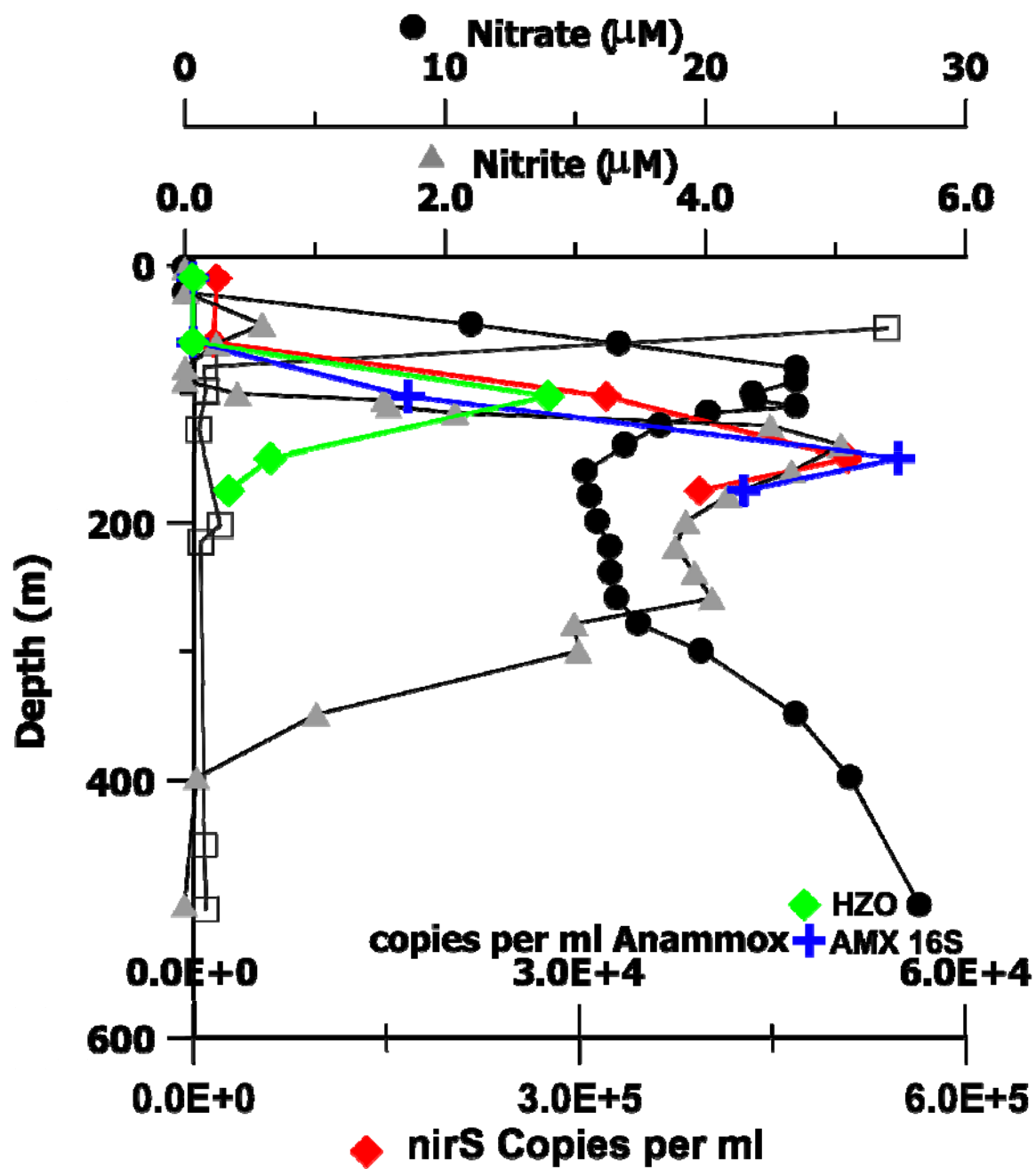


### nirK copies in OMZ

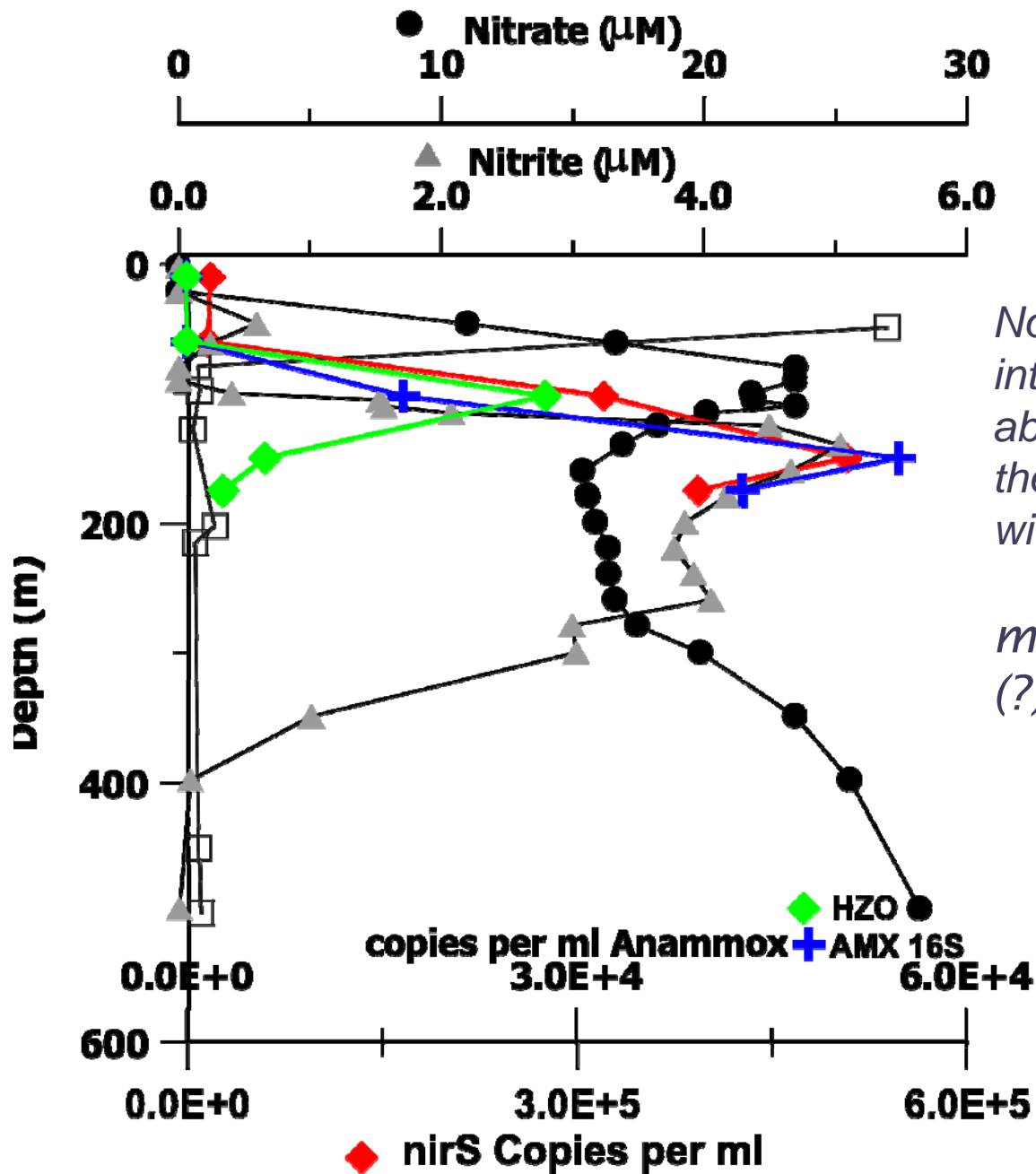
Copies per ml SW



### Station 1 (19° N 67° E) Oct 2007



**Station 1 (19° N 67° E) Oct 2007**



*Now you can see why we're most interested in nirS – much more abundant than nirK (possibly because there is no Copper – it's all bound up with S or other ligands)*

*most abundant = Key players (?) – needs more work*

## Results:

- *nirS* and Anammox seems to be present together in all the environments we have probed, both are not present in oxic waters above and below the OMZ
- *nirK* appears to play a minor role.
- Increasing denitrifying condition leads to increase in abundance of both *nirS* and AMX gene copy numbers
- Abundance of *nirS* gene is always an order of magnitude or so higher than Anammox 16S rRNA gene
- *Dom nirS* in the AS not found in ETSP- community composition differs greatly between the two OMZ regions.
- Abundance appears to be same in AS and ETSP, hence there exists some other major variable that drives the N removal processes, such as supply of substrates, that causes variability in the relative magnitude of the two processes.

## Summary

- *qPCR is a powerful molecular tool to address the change in abundance of various phylogenetic groups-*

- *Downside:*

*Assay that works for one gene may not work for another*

*Optimizing assay conditions*

*make sure that it amplifies only the group of interest  
only one product*

*Standard Preparation*

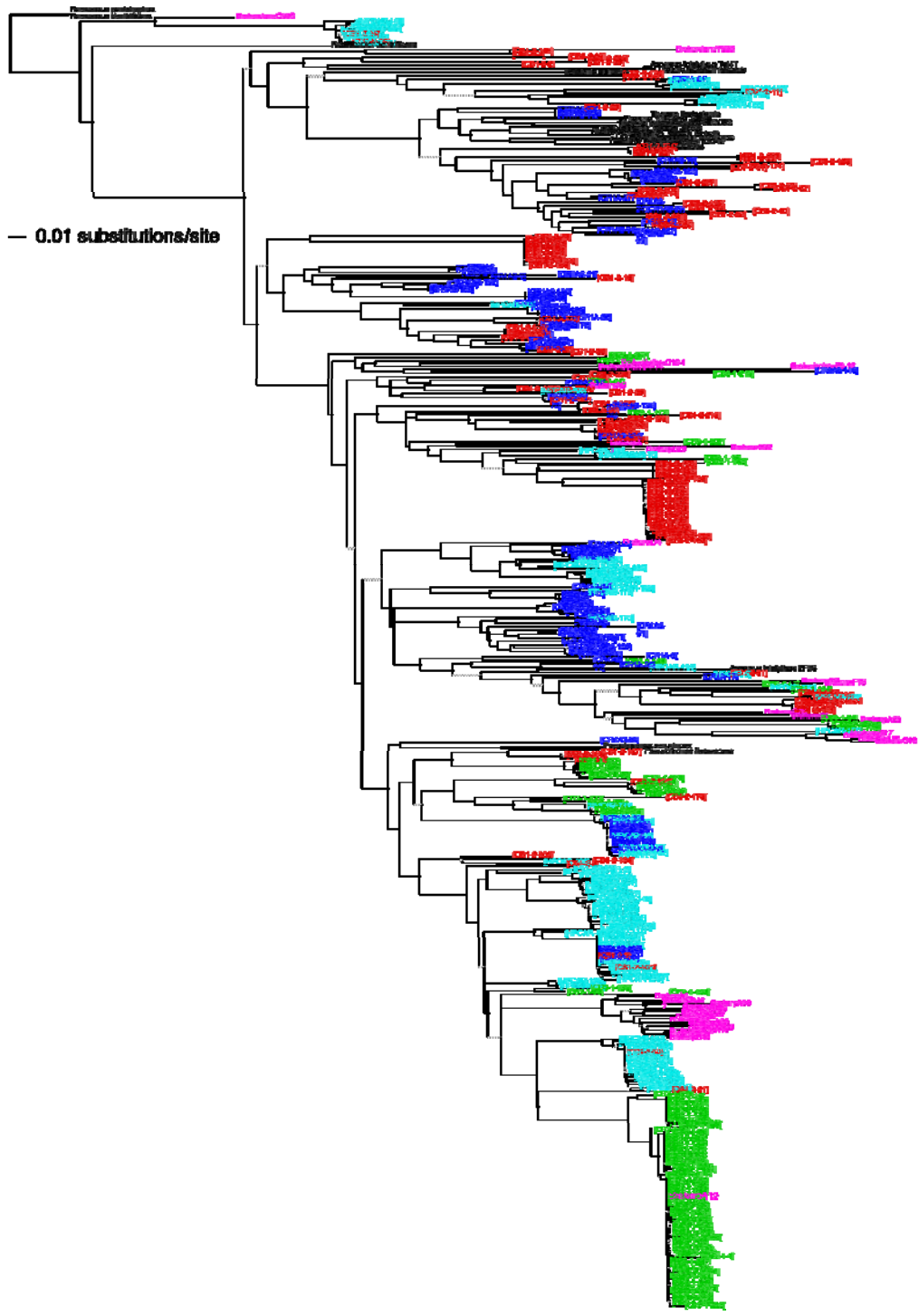
*Selection of primers*

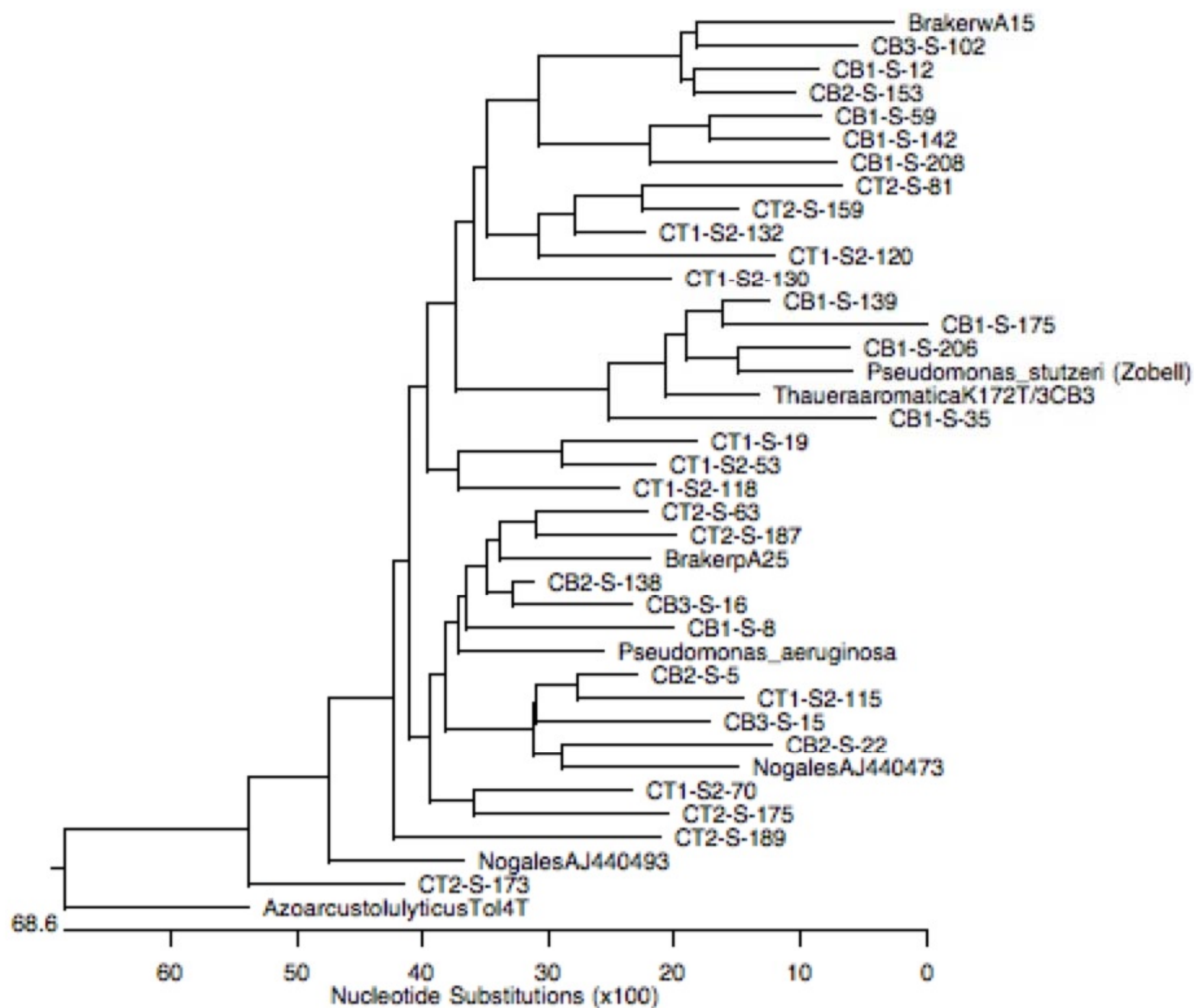
*Expensive equipment and reagents*



# Micro-array

- **relative Abundance.**
- **Chesapeake nirS data**
- **Our current nirS array**





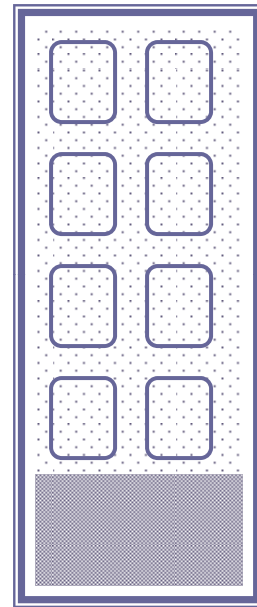
# Oligo array: Internal Standard Universal Oligo

Smith et al. 2000

◆ Definitely not conventional

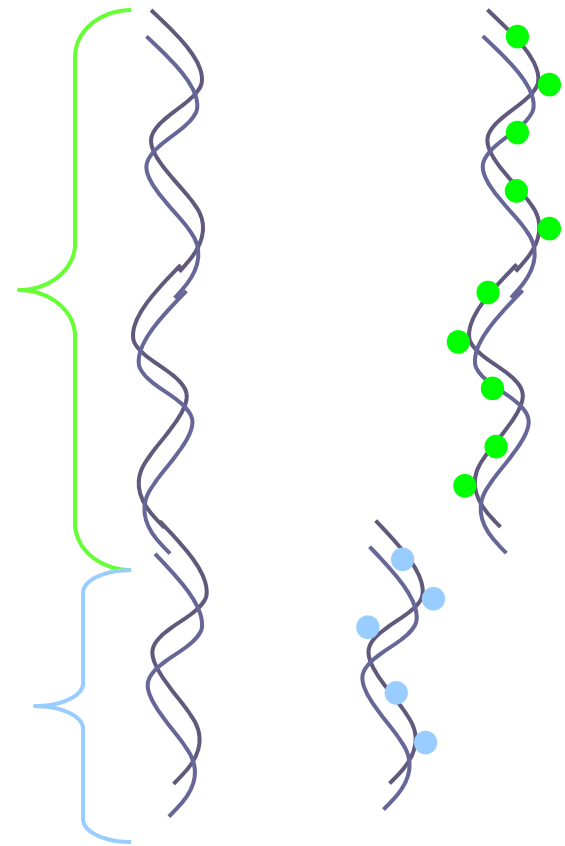
◆ No control

◆

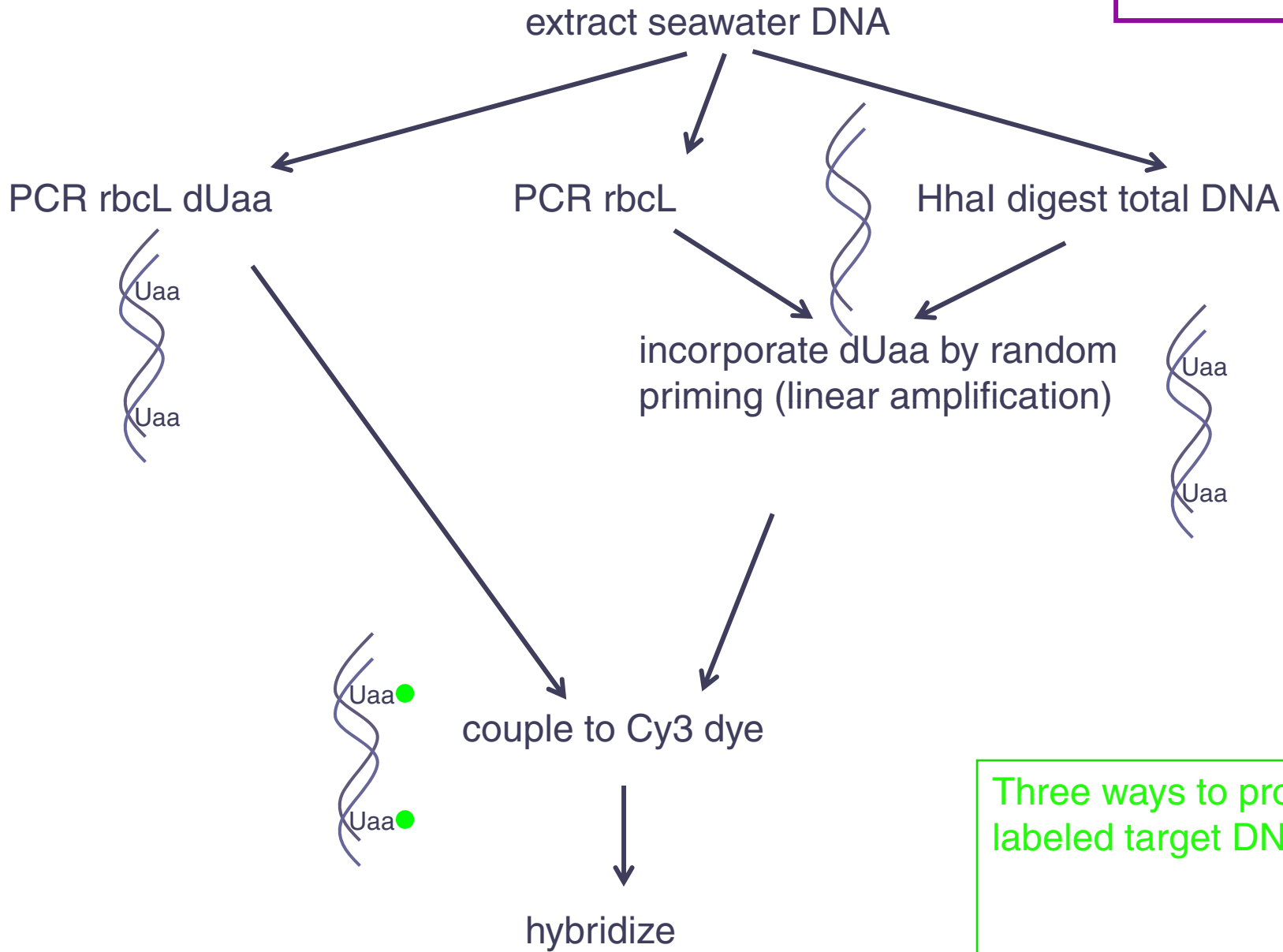


*Gene probe*

*Universal oligo*



PCR: YGWYLF



Three ways to produce labeled target DNA

# Quantification

- 32 blocks, 2 (8) reps
- Sig mean - bk mean
- Background filter
- Cy3 target/Cy5 ref
- Normalize among blocks using mixed probe
- Ave  $\leq$  8 reps
- Compare to std target addition

200 ng NR and 200 ng rbcL dUaa  
seawater target  
BC008\_16

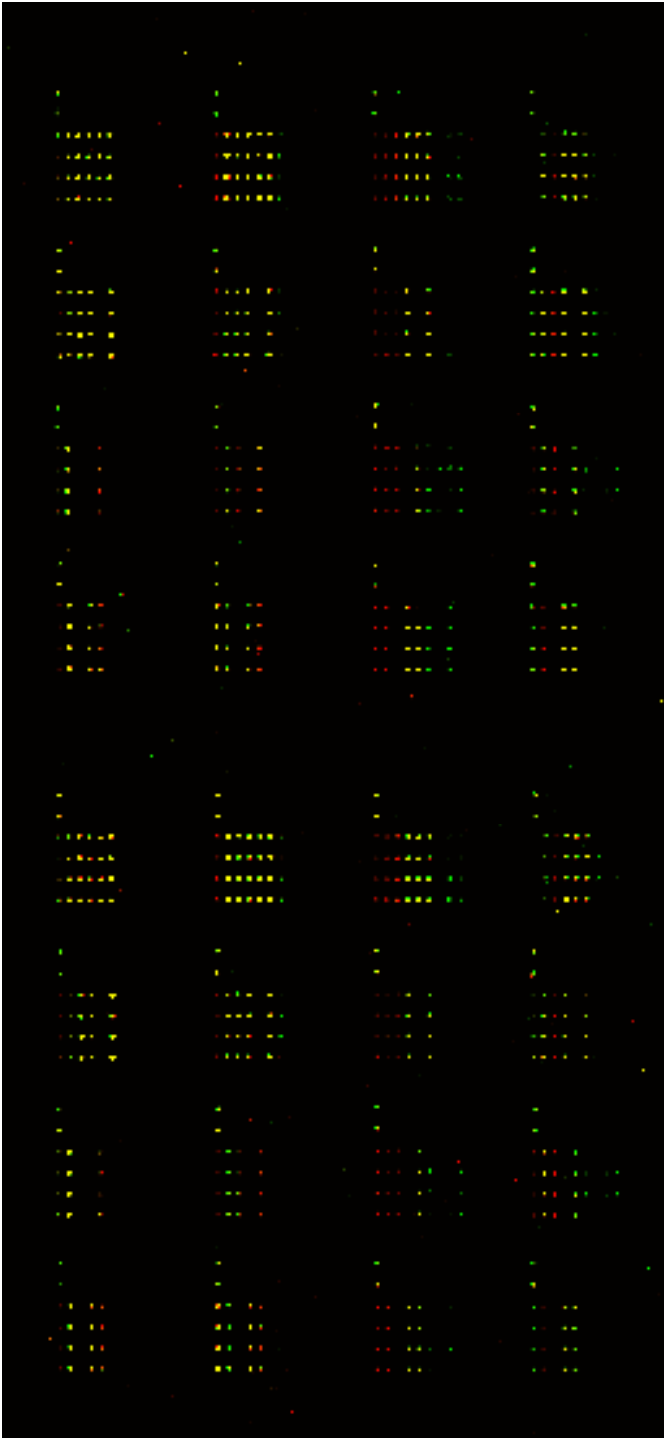


Figure 3a Array to Array

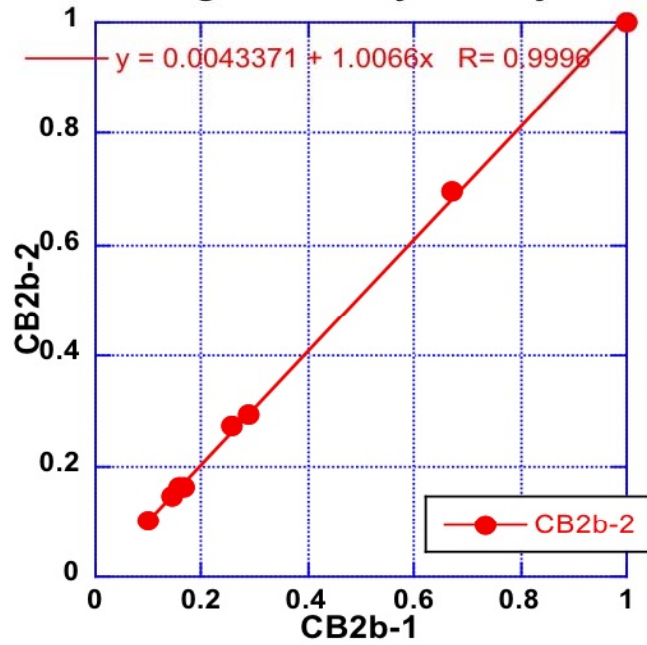


Figure 3b Slide to Slide Comparison

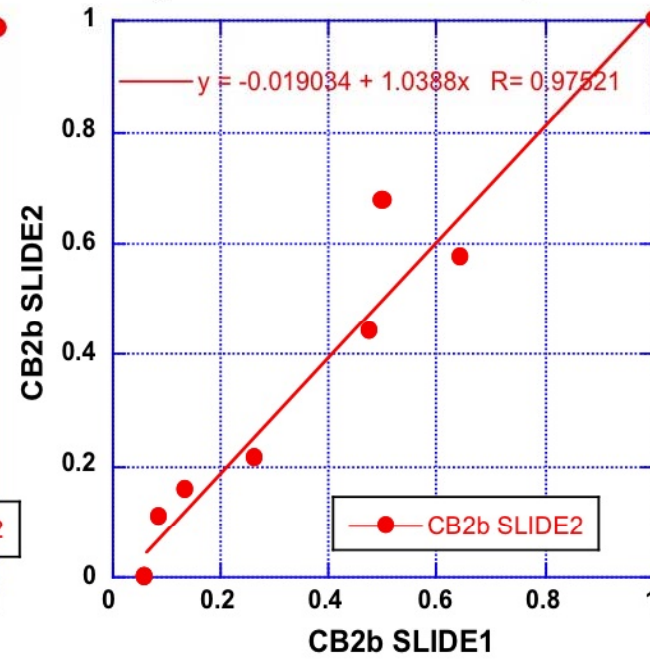


Figure 3c Target to Target Comparison

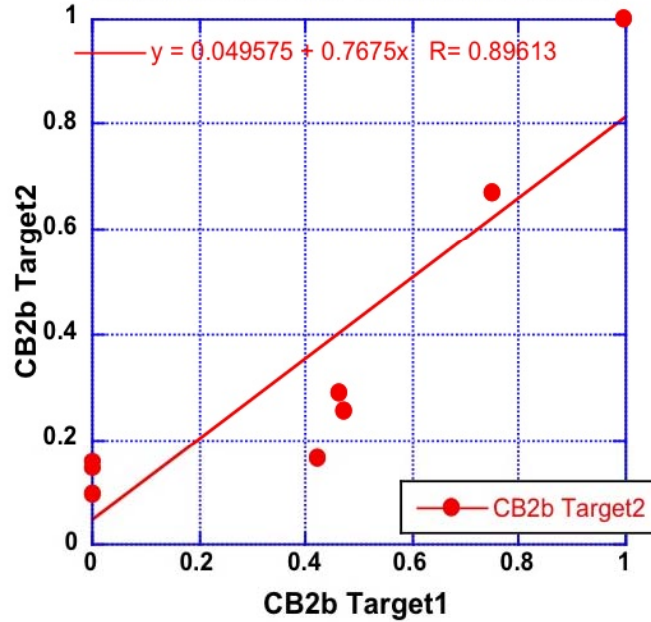
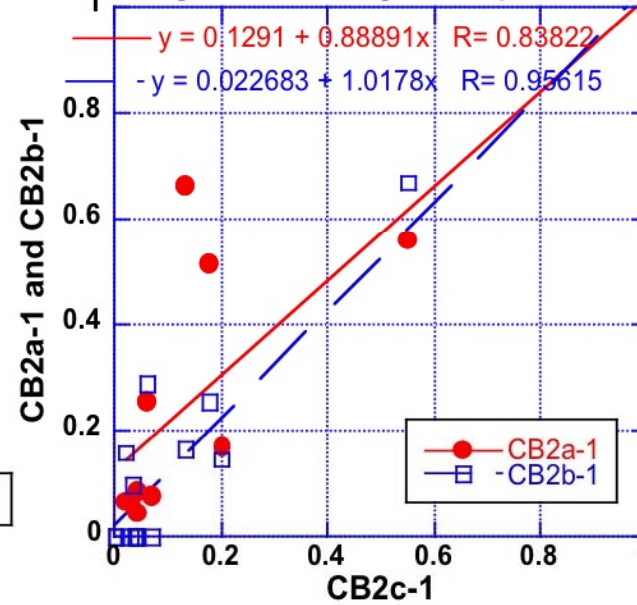
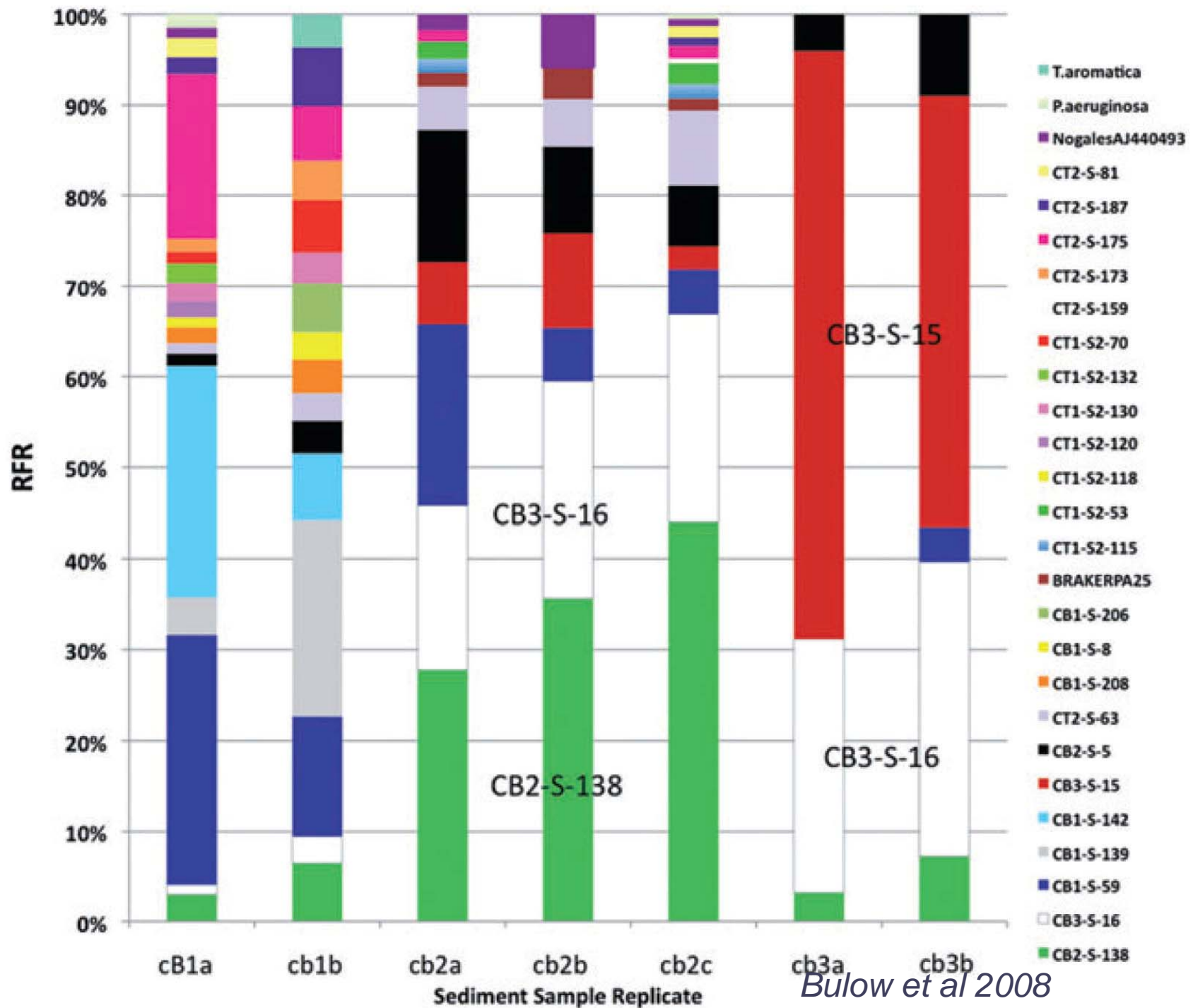
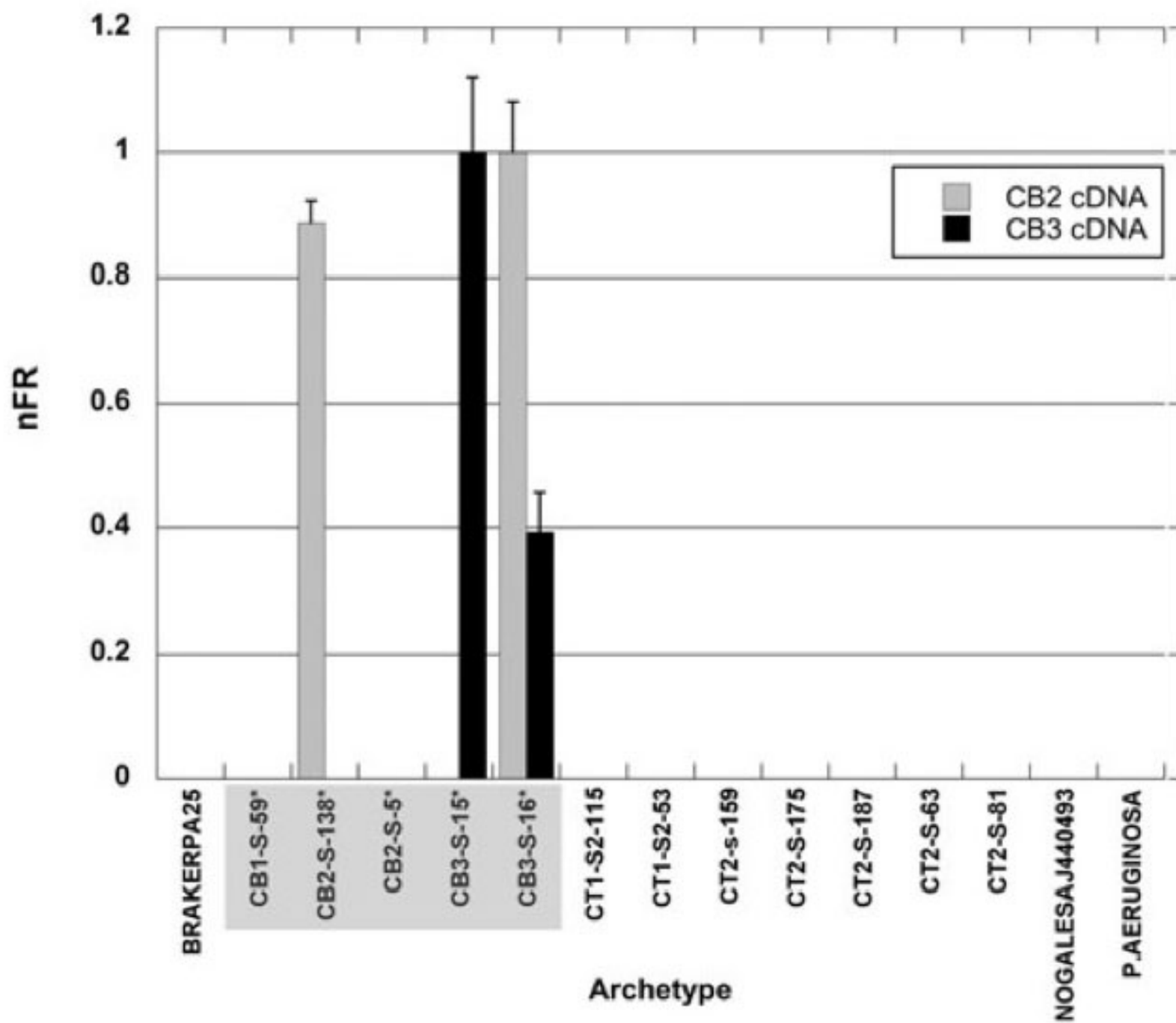


Figure 3d Biological Replicates









# Functional gene microarrays

- Require a good database – not exploratory
- Moderate resolution
- Community composition and gene expression
- Fast, high throughput
- Replication, statistically robust
- Versatile

## Overall summary

***Using some molecular techniques we have shown that there are some powerful tools out there to study the community structure and dynamics***

***Using these tools we have shown that denitrifiers are the major component of the intermediate Nepheloid layer (suspended particles in the OMZs) and are important in carbon and N cycling organisms in the OMZ***

***Anammox organisms and nitrifiers (both groups) are also minor components of the Nepheloid layer.***

***Assuming that there is one copy of nir gene per genome the entire inventory of cell count could be accounted for by denitrifiers***

***There are many more tools available, but choosing the right one is the challenge***

***Along with molecular tools, some geochemical measurements are also required to understand the processes***

## Future directions:

- Does the relative importance of these processes or abundance of genes change temporally and or spatially – more data- so new tools
- What are the environmental factor that drives the pattern of relative importance of one process over the other-Is Carbon the driving force behind nitrogen removal in different OMZ, so that the there are differences in dominant N removal process.
- Can community dynamics and expression of genes provide insights into the processes?
- New *nirS* probes for the next generation array is in progress
- More cruises – Target SNM- more temporal and spatial data

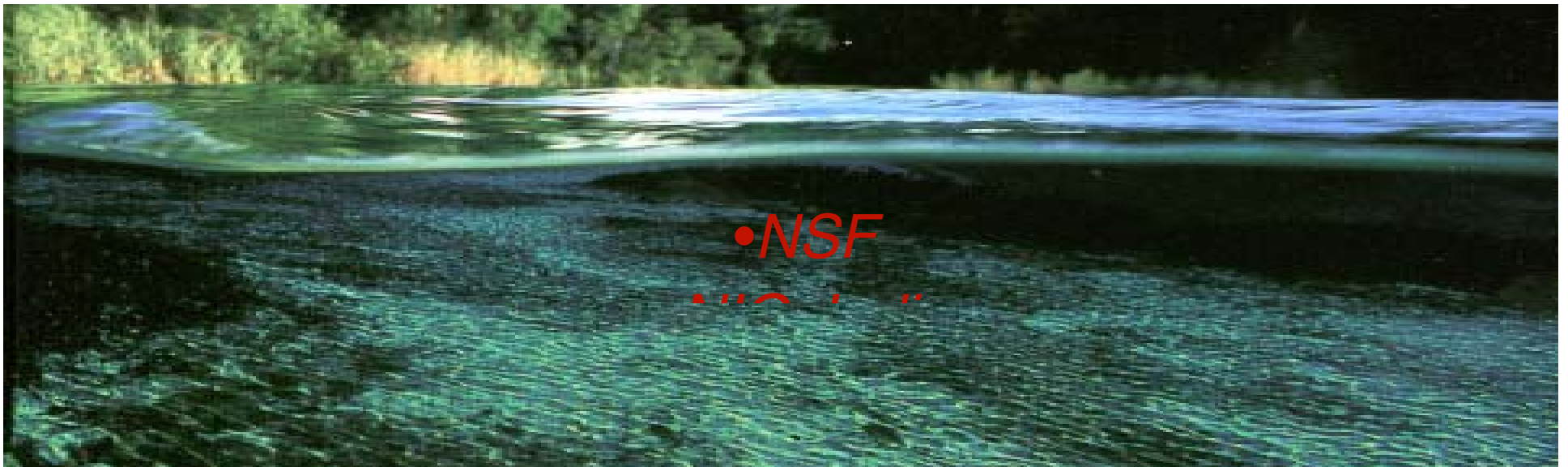


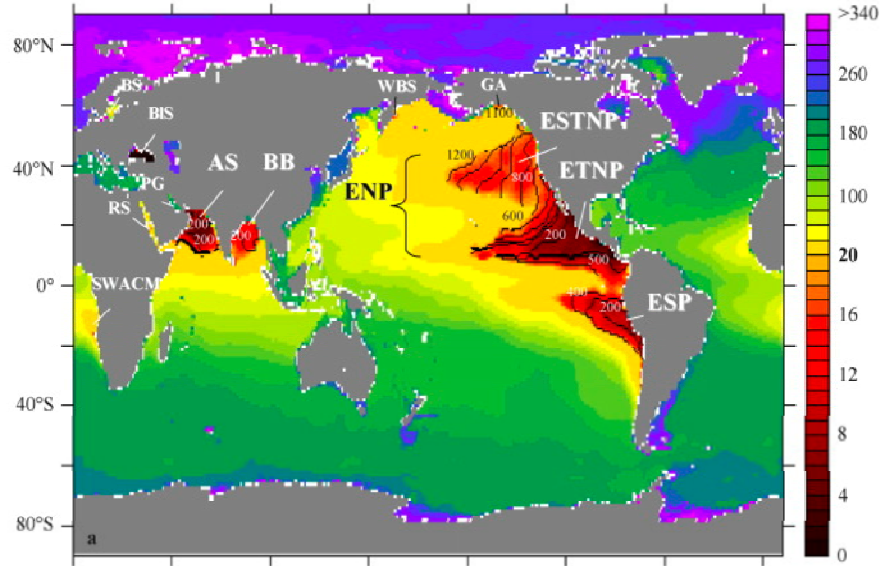
# Acknowledgements



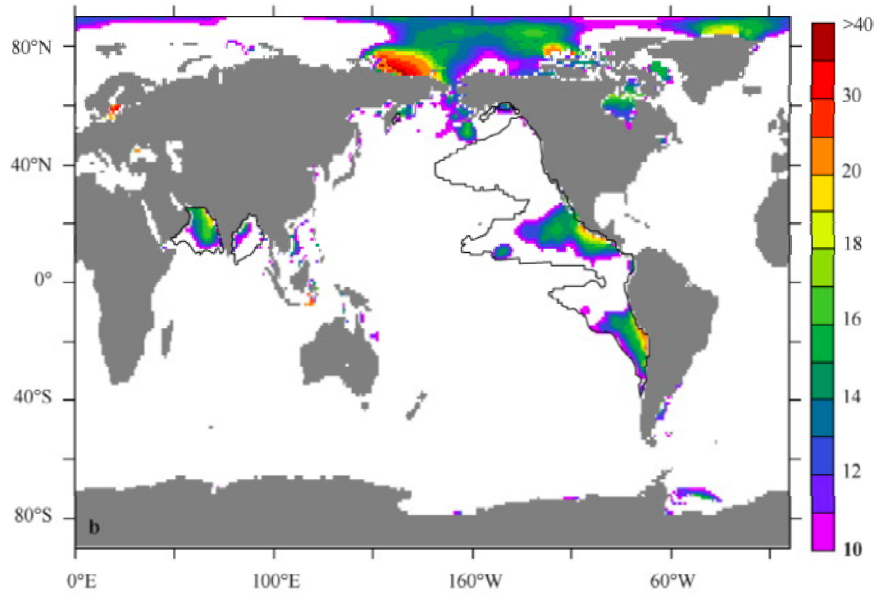
## Collaborators

- Ward Lab Group: Silvia Bulow, Jen Bowen, Sarah Fawcett, Andrew Babbin, Nik Bouskil, Jeremy Rich, Carrie Tuit
- University of Washington: Allen Devol and Bonnie Chang
- UCSD: Jim Moffett and Colleagues
- NIO Goa, India: Wajih Naqvi, and Colleagues





*Minimal DO WAO2005*



*Nitrate Deficit Maximum Zone > 10  $\mu$ M*

*Paulmier and Ruiz-Pino 2009*

# Stn 9

