Diversity and biogeochemical functions of microbial communities at pelagic redoxclines of the central Baltic Sea

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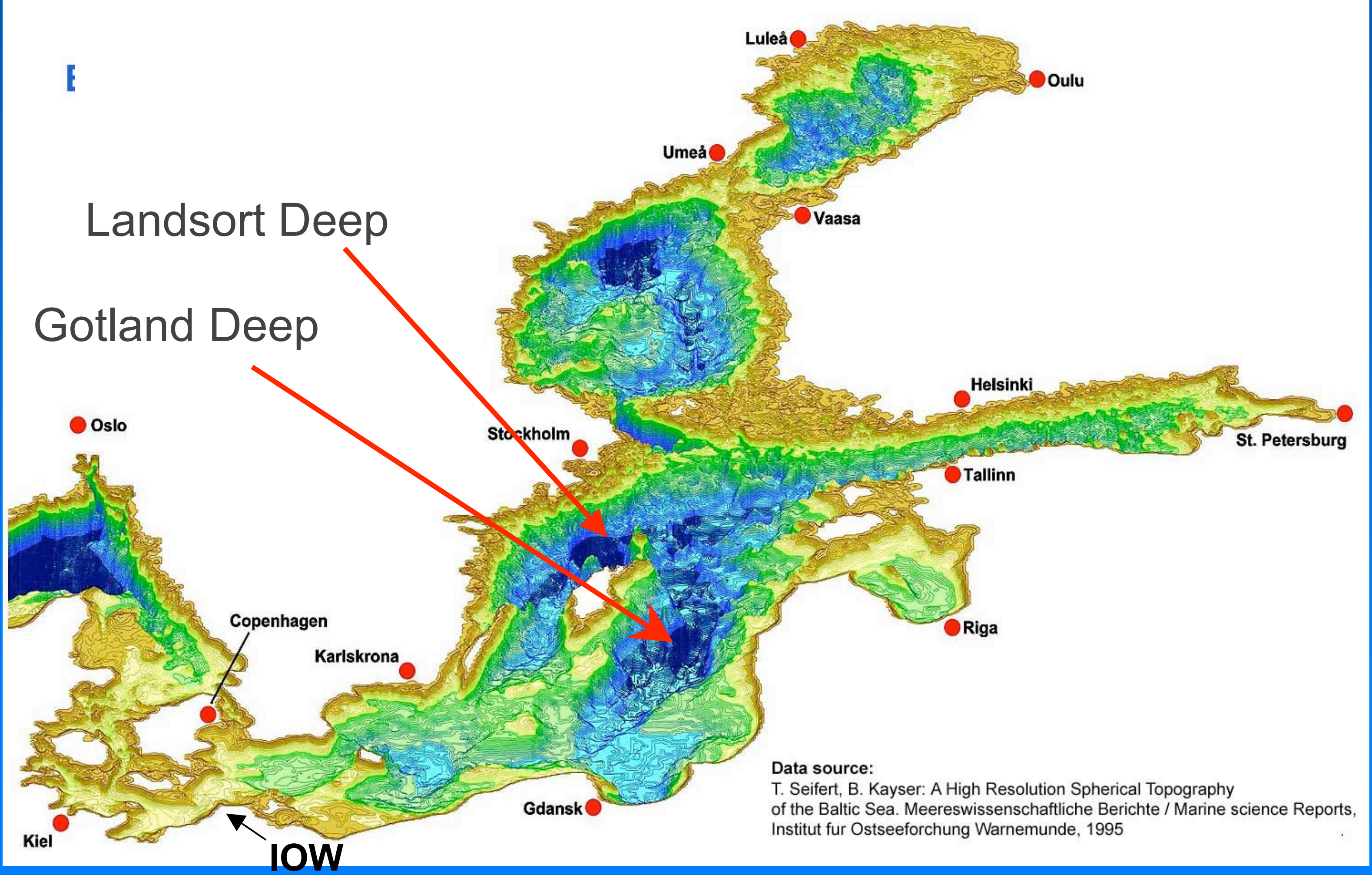
Overview

- Research topic “Pelagic Redoxclines“
- Examples for microbially mediated biogeochemical processes at redoxclines:
- manganese shuttle
- dark CO_2 fixation – chemolithoautotrophic bacteria
- nitrogen transformations
- approaches to identify key organisms

Area of investigation: Baltic Sea

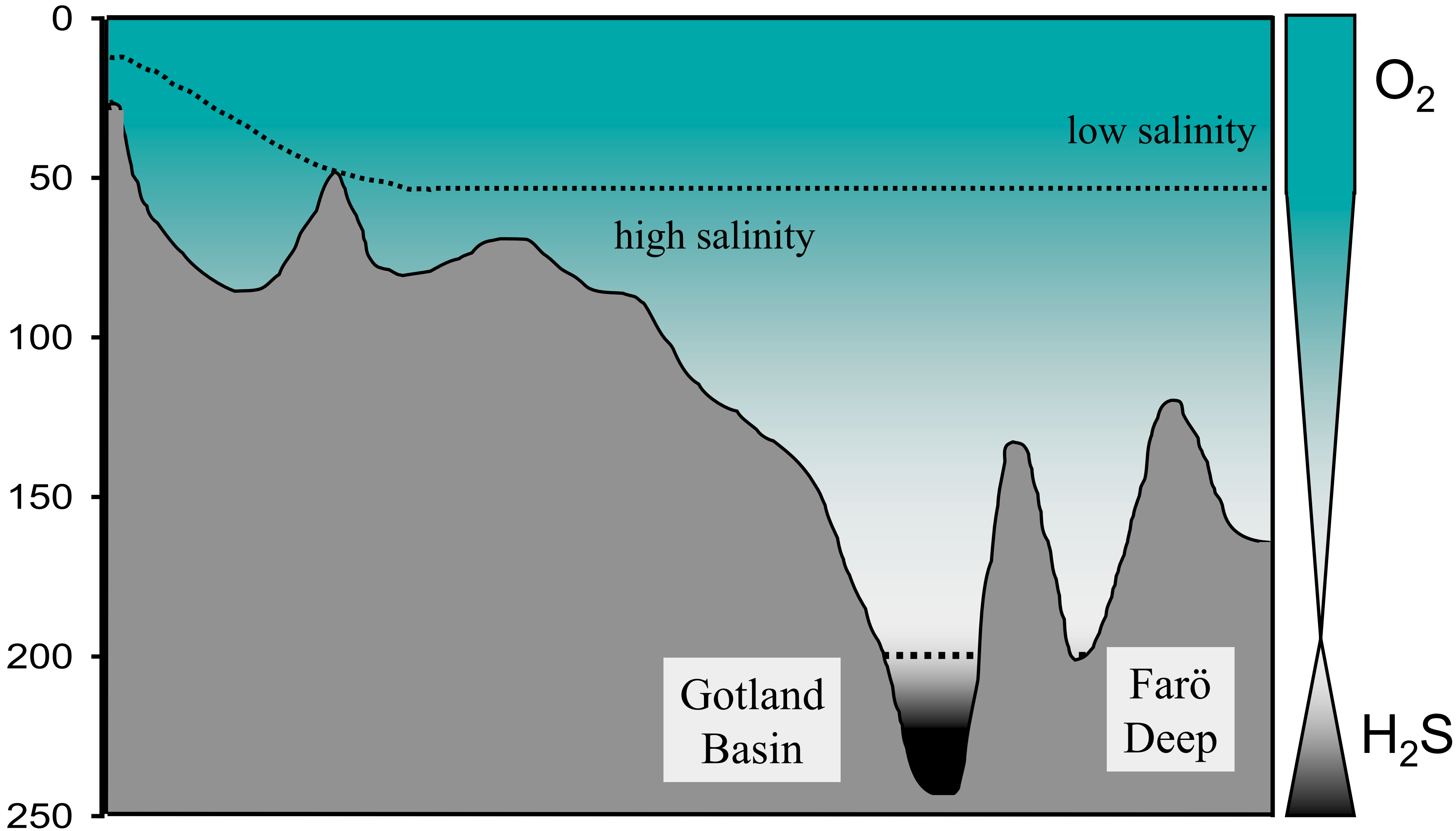


Alexander v. Humboldt

Professor Albrecht Penck

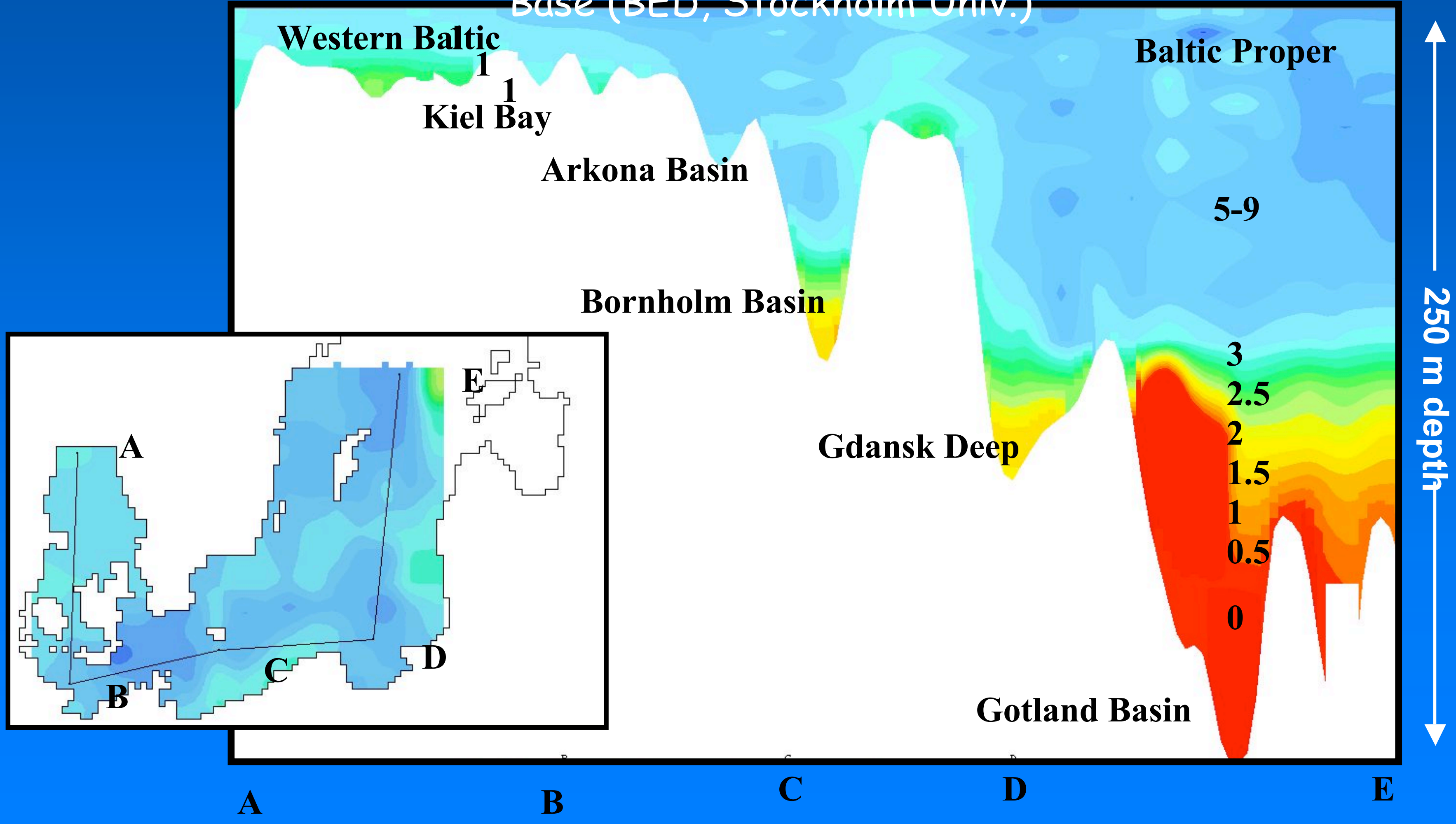

MARIA S.MERIAN

Stratified basins of the central Baltic Sea

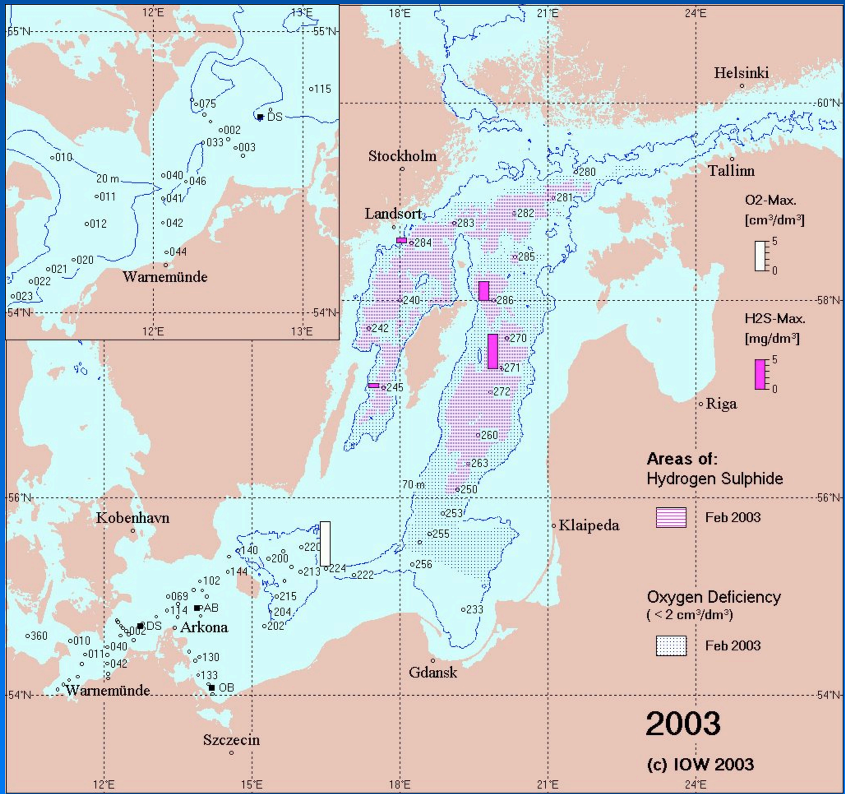


Basin structure and oxygen distribution of the Baltic Sea

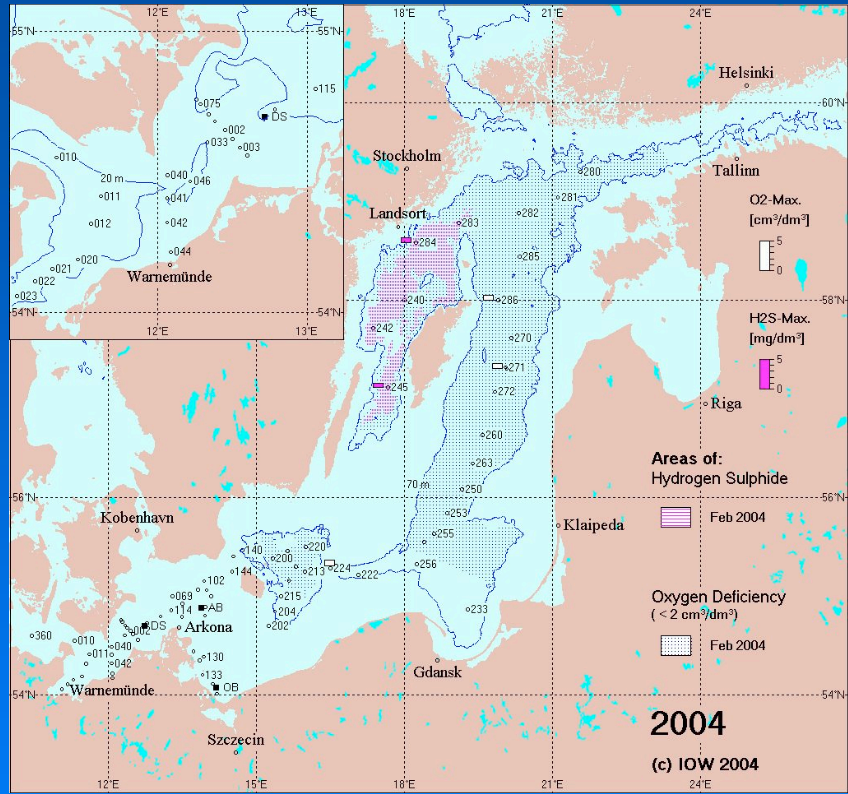
- mean O_2 values 1987-1990 (ml l^{-1})
- Data source: Baltic Environmental Data Base (BED, Stockholm Univ.)



Extension of sulphidic bottom water in the central Baltic Sea



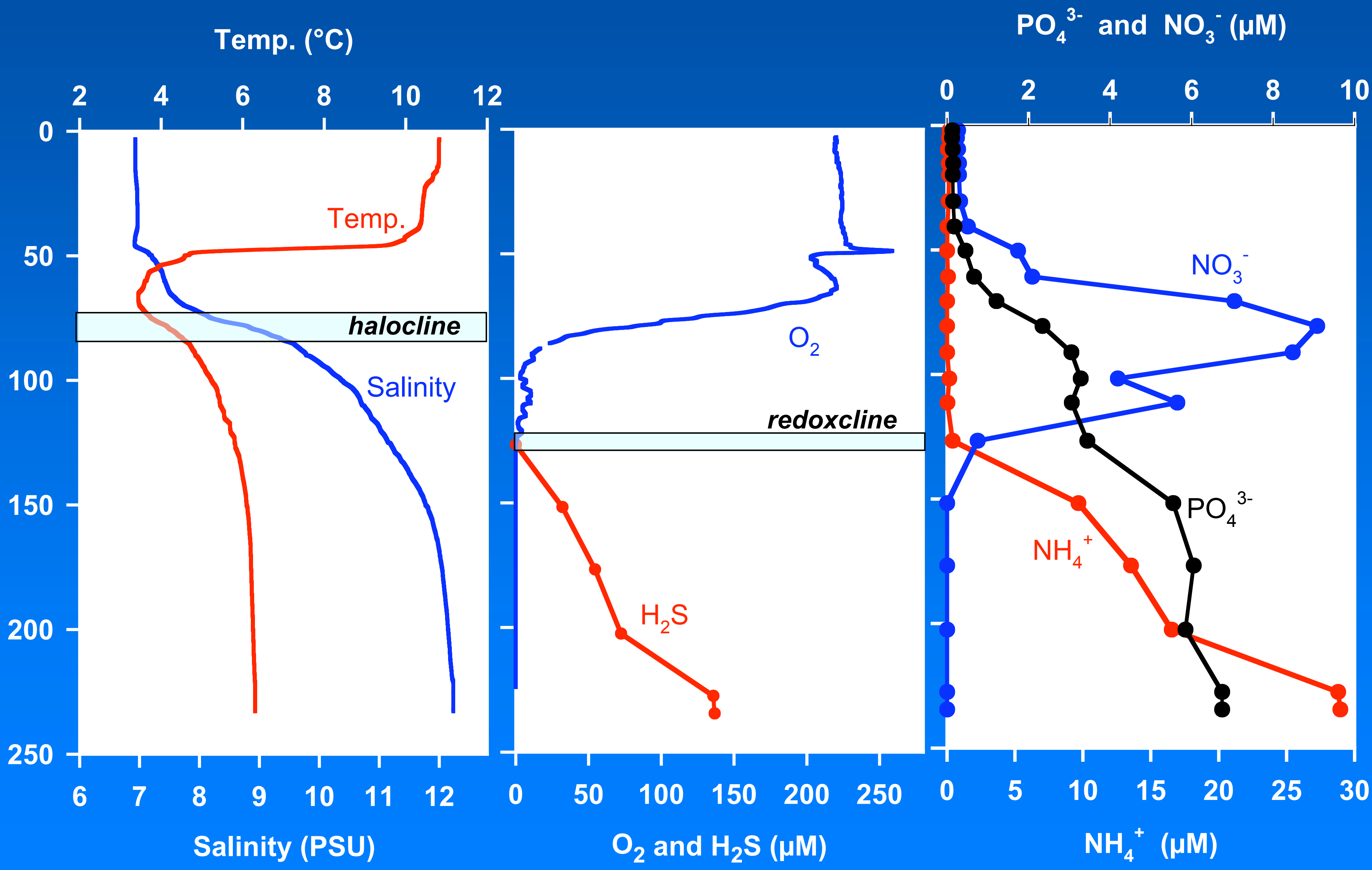
February 2003



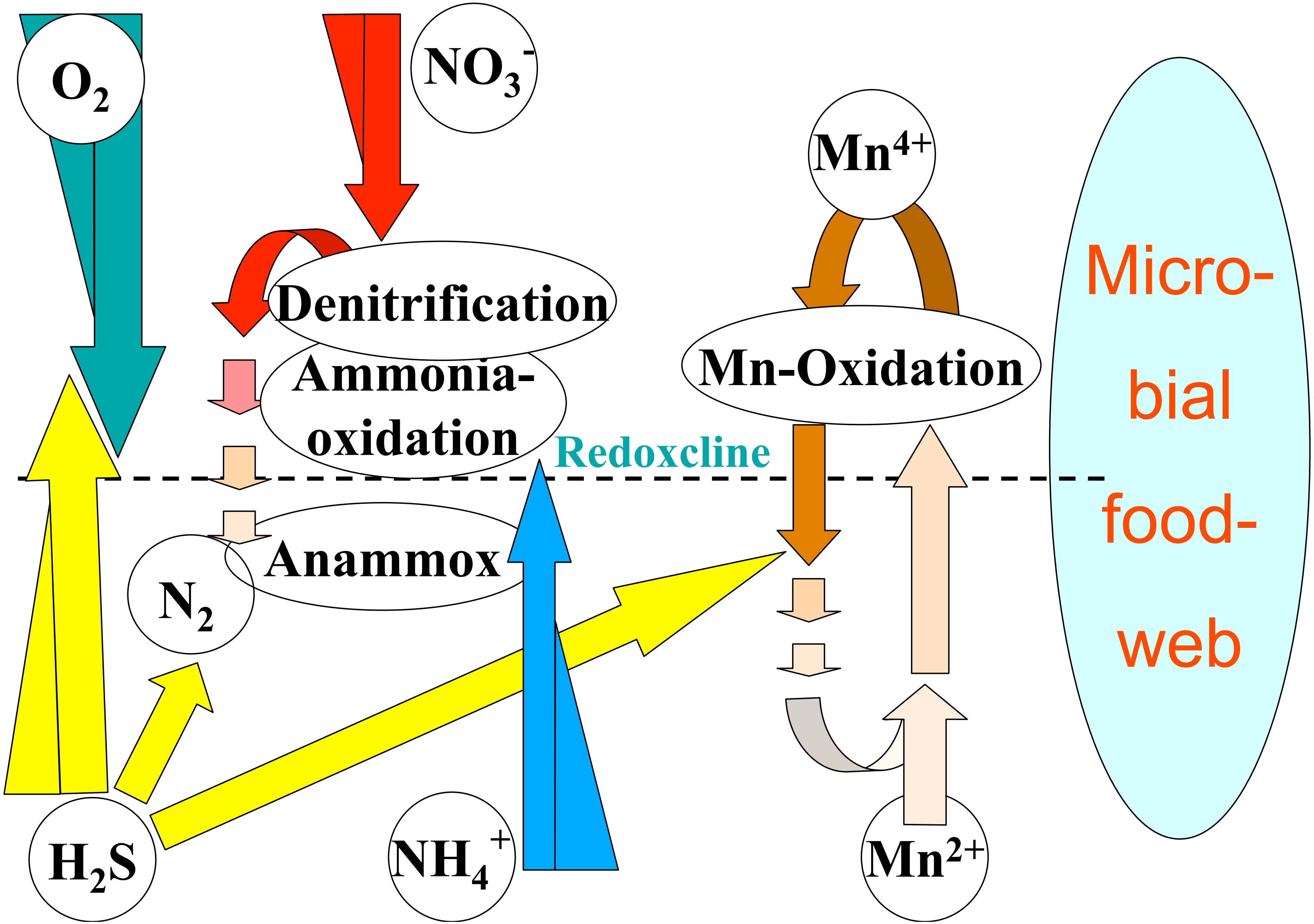
February 2004

Typical physico-chemical profiles in the Gotland Sea

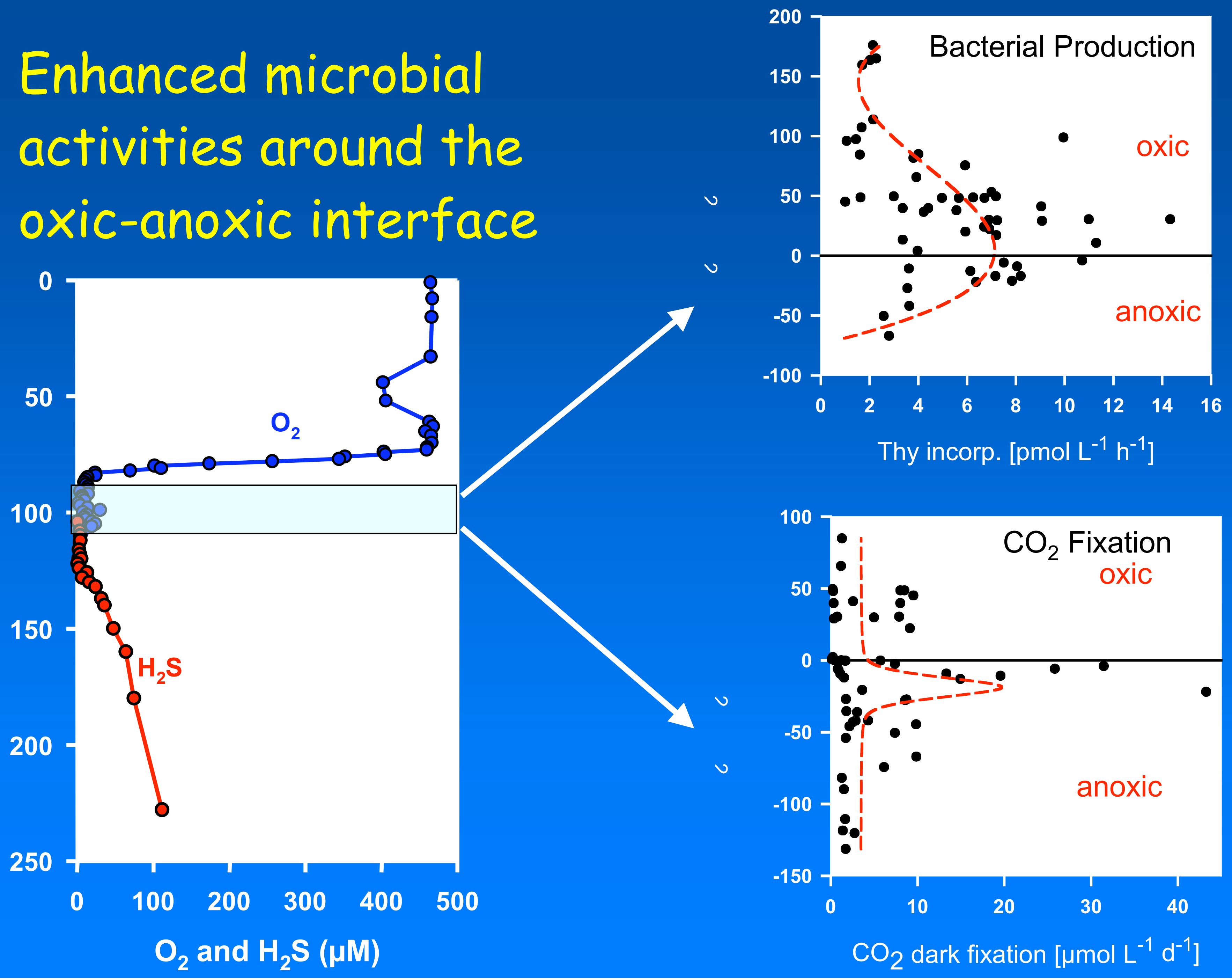
(Example: Nov. 2000)



Potential biogeochemical transformations at the redoxcline



Enhanced microbial activities around the oxic-anoxic interface



Research Goals

- Quantifying rates of element transformation, particle formation and transport across the redox gradient
- Understanding the regulating abiotic and biotic mechanisms for the major transformation processes
- → Baltic Sea redoxclines as a general model system for an understanding of processes at redox gradients

Interdisciplinary Research at Pelagic Redoxclines:

Biology: **- Key organisms, biodiversity**
- Linking microbial diversity with specific functions

Chemistry: - Metal speciation, distribution and fluxes of dissolved and particulate forms

Geology: - Mineralic compounds and particle formation dynamics

Physics: - Modelling of mixing processes by turbulent diffusion

Examples for biological-biogeochemical couplings and insights into redoxcline microbial community structure and functions

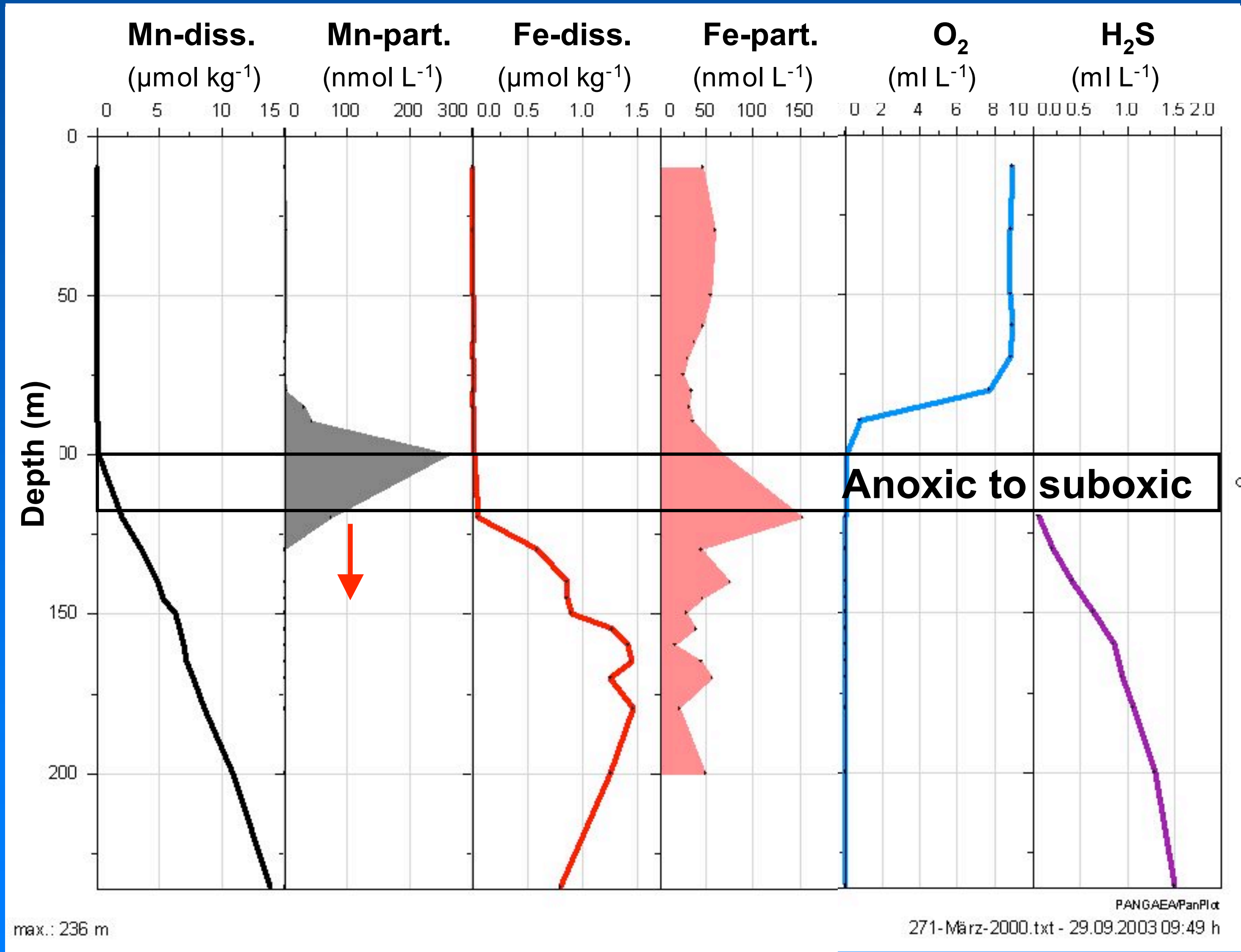
1. Manganese shuttle
2. Chemolithoautotrophic bacteria
3. Nitrogen transformations (nitrification, denitrification, anammox)

Manganese shuttle in the Gotland Deep

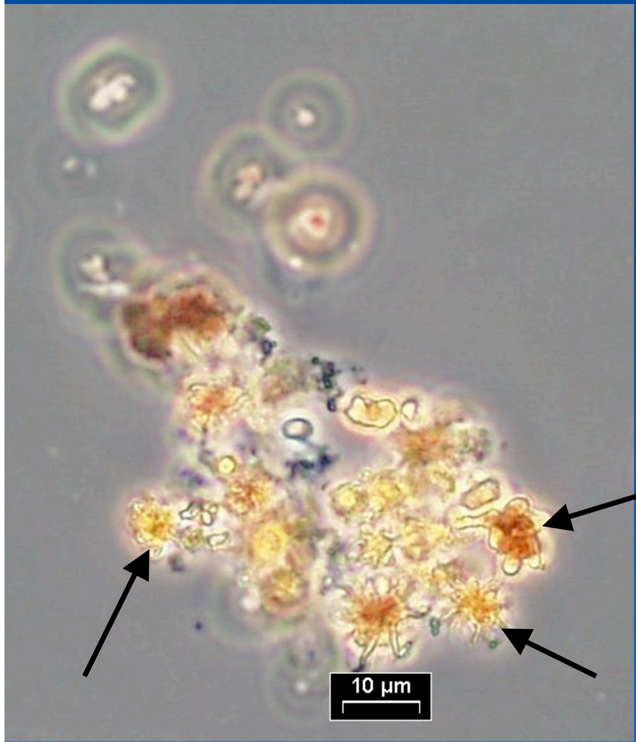
(Neretin et al. 2003, Mar. Chem. 82: 125-143)

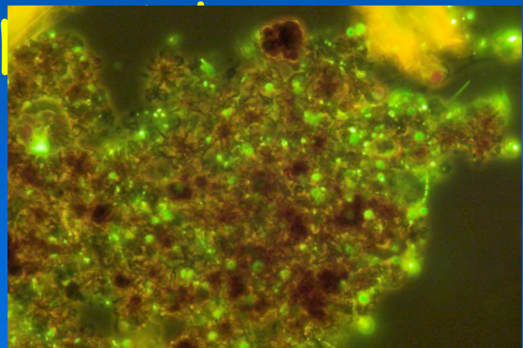
- Mn: important role as electron donor and acceptor in redox processes
- Dissolved Mn (II) – insoluble oxides, oxyhdroxides (Mn III, IV)
- High Mn concentrations in the Baltic Sea :
  up to 3.5 % dw in bottom sediments

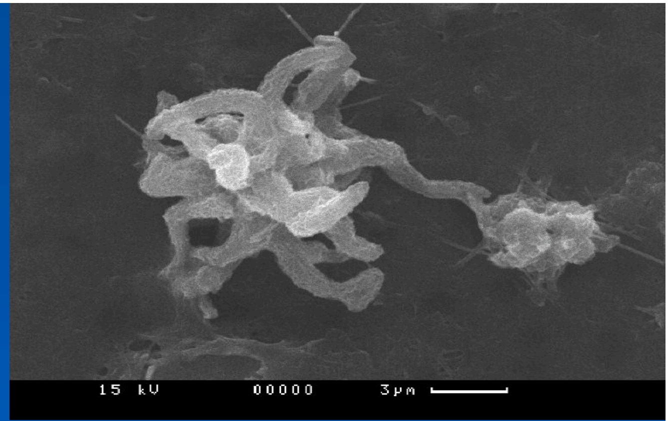
Manganese/iron reduction in the Gotland Deep

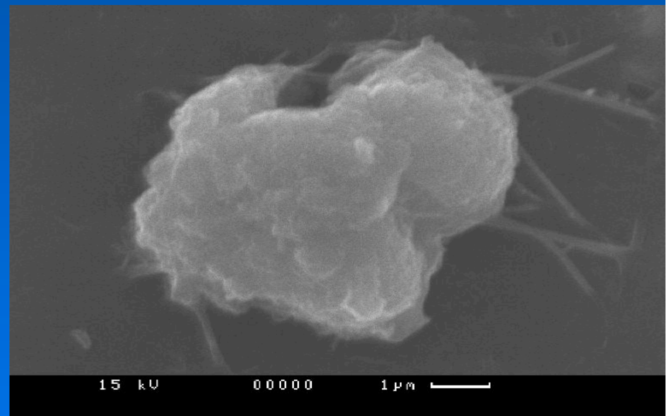


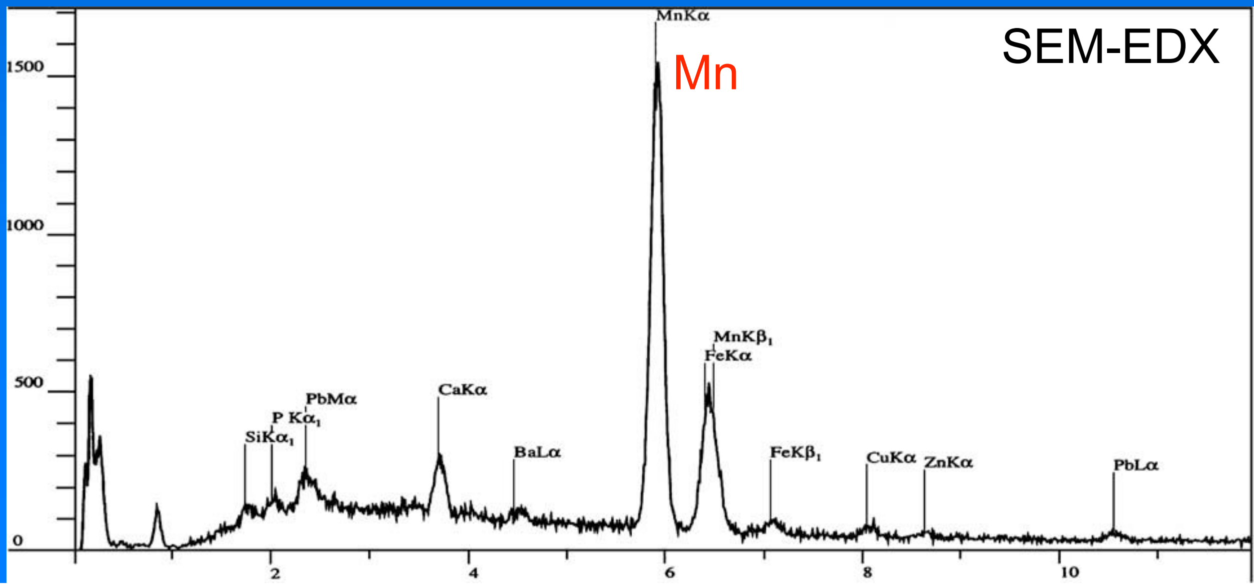
Aggregates with manganese oxide







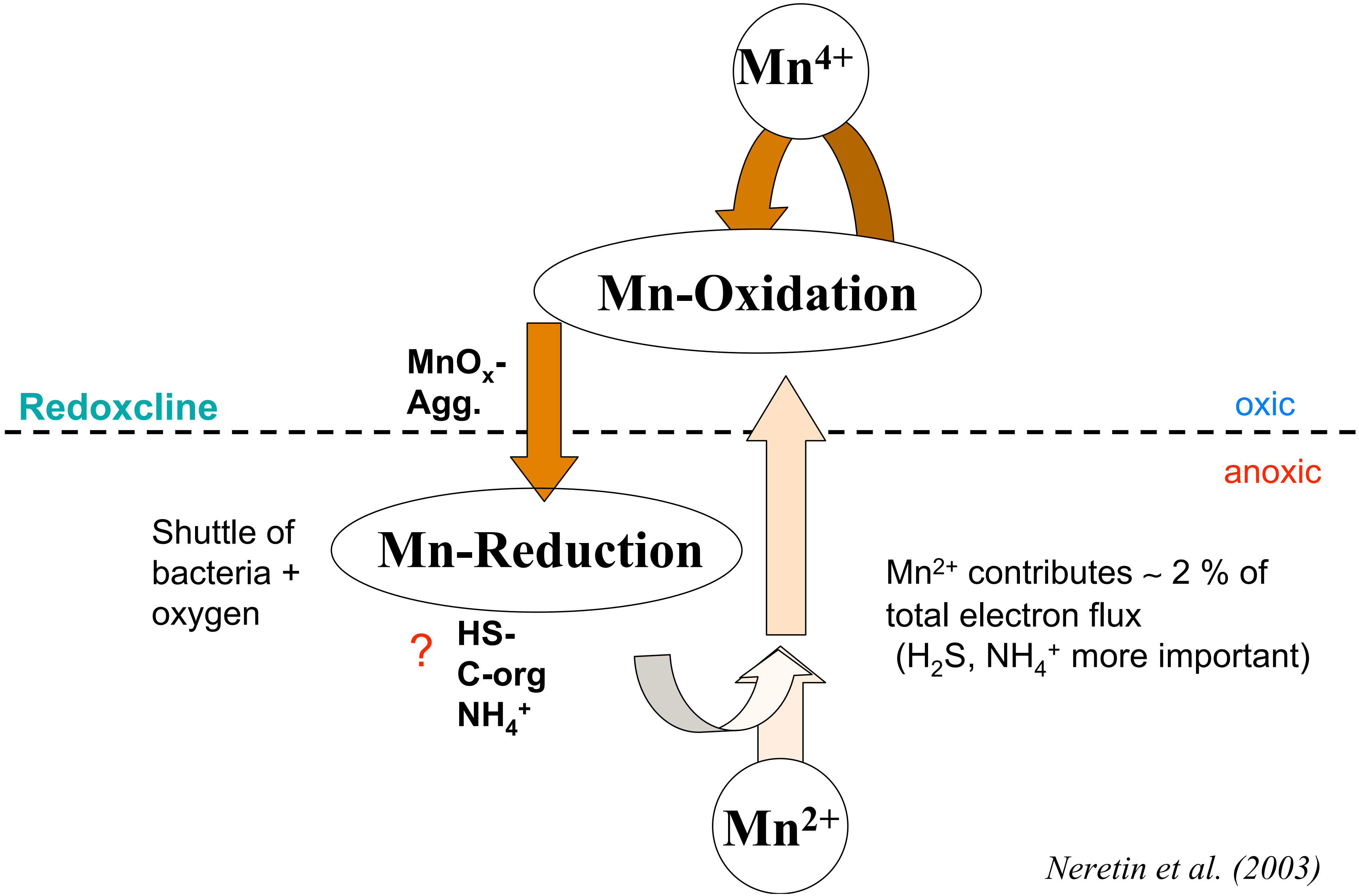




Mn-oxide particles formed in different layers of the suboxic part can sediment into the anoxic zone

- Up to 200,000 aggregates l^{-1} in the MnO_x-rich layer
- Most Mn-rich particles (ca. 70 %) are embedded in organic aggregates; 20 – 100 µm equivalent spherical diameter
- EDX spectra: Mn accounts for > 60 % of elemental composition
- Calculated settling velocities of Mn-aggregates: 1 m d^{-1} (→ downward flux)
- Upward flux of Mn (II): estimated from concentration gradient and vertical eddy diffusion

Mn-shuttle at the redoxcline: Vertical transport of oxygen



Neretin et al. (2003)

Chemolithoautotrophic bacteria

→ important questions

- Which redox reactions are driving chemoautotrophic bacterial production?
- What are the underlying reactions for the CO_2 fixation peak in the anoxic zone ?
- Which are the responsible organisms and how abundant are they?
- What is the fate of the chemoautotrophic bacterial production?

Which electron donors are supporting chemoautotrophic production?

- Problem: about 20 µmol fixed CO_2 per µmol H_2S diffusion
- Sulfide oxidation to account for integrated CO_2 fixation exceeds the necessary flux of H_2S more than 100fold !
  → H_2S recycling mechanisms are necessary

Which electron acceptors are supporting chemoautotrophic production?

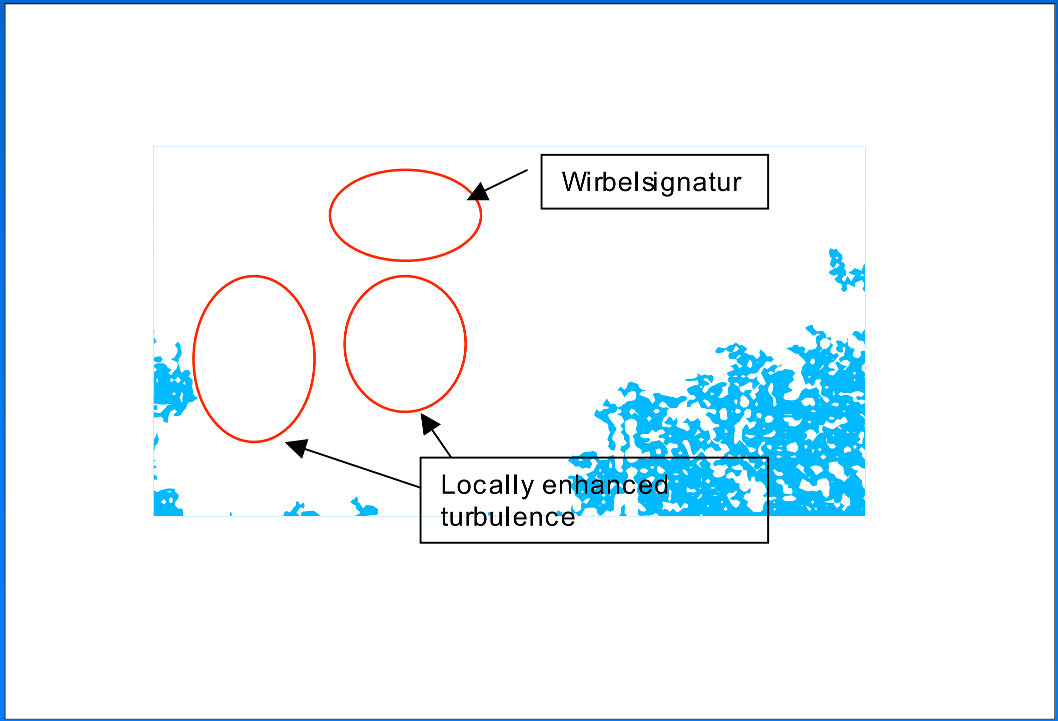
- Downward flux of MnOx: ~ 50 µmol m^{-2} day^{-1}
- Mn dependent oxidation of sulfide to thiosulfate: 7.5 mol H_2S per mol CO_2 fixed; 2 MnO_4 per H_2S oxidized
- 80 – 120 mmol MnOx necessary for 6 mmol CO_2 day^{-1} fixed (integrated anoxic zone)

→ identity of actual electron acceptors unknown!

Solution: Help form the Physical Oceanographers

- Physical processes which result in a mixing of the stratified water layers could enhance microbially mediated redox reactions:
- Internal waves: small scale turbulence events
- Later advective transport of water bodies of different chemical composition (eddies): mesoscale mixing processes (e.g. nitrate into sulphidic zone)

Example: small-scale dissipation events on the order of hours in deeper water of the Gotland Basin



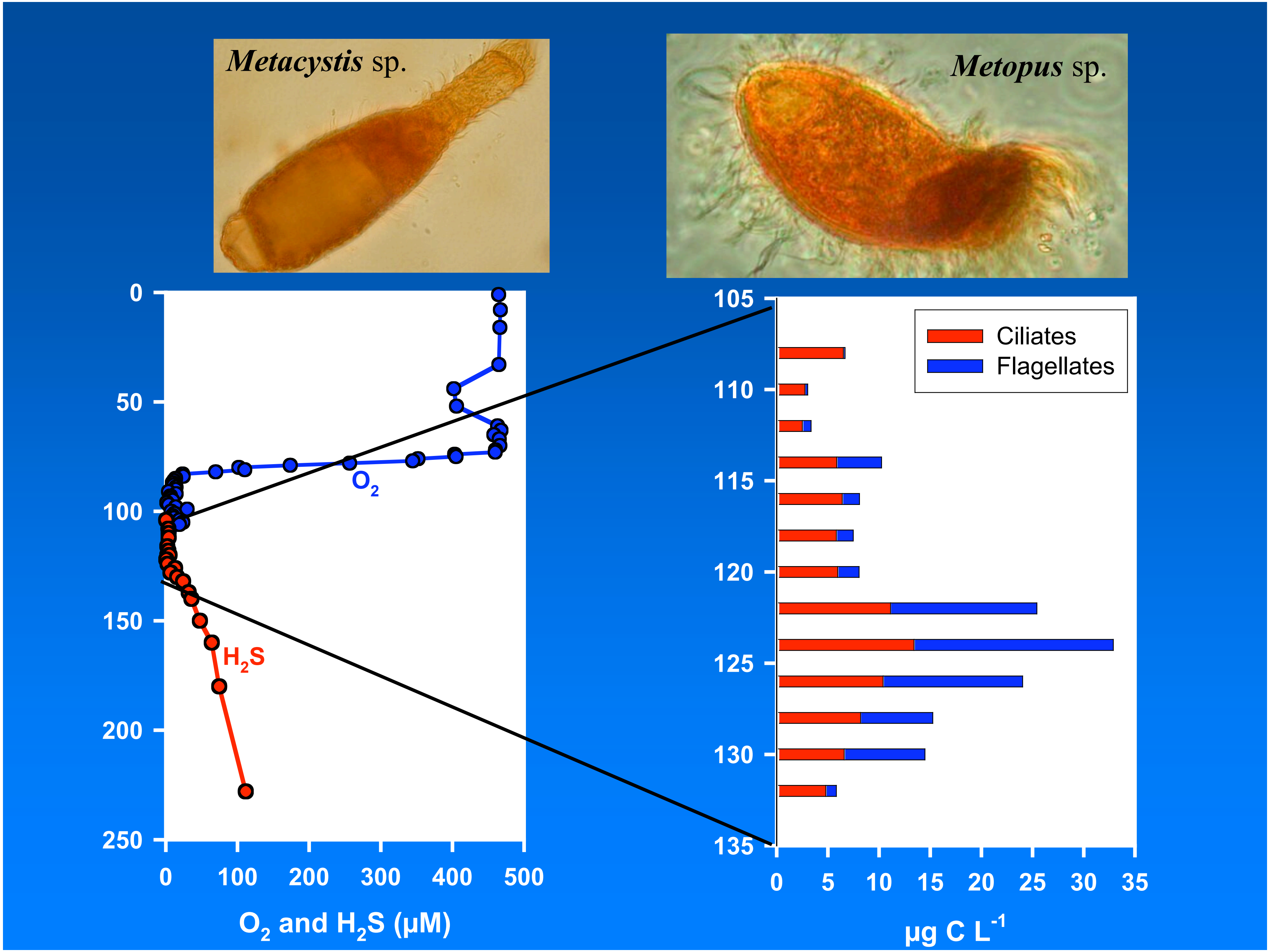
Lass et al. (2003)

Estimation of chemolithoautotrophic production:

- ~ 50 % of total bacteria are chemolithoautotrophic
- 10 – 20 fg C-fixation $cell^{-1}$ d^{-1}
  → ~ 1-2 doublings per day

What is the fate of chemoautotrophic bacterial production ?

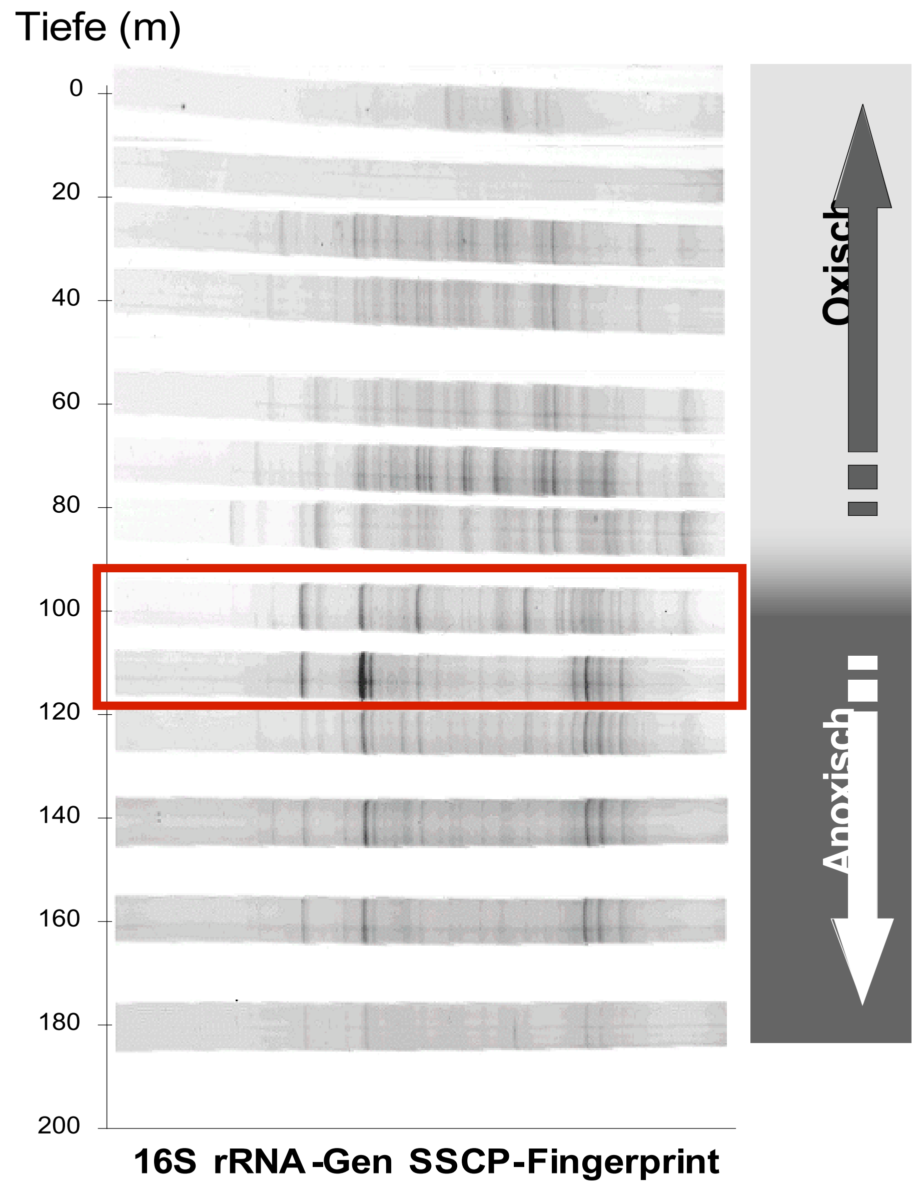
Base of an anaerobic food web?



Metacystis sp.
Metopus sp.
O2
H2S
O2 and H2S (μM)
Ciliates
Flagellates
μg C L-1
0
50
100
150
200
250
0
100
200
300
400
500
105
110
115
120
125
130
135
0
5
10
15
20
25
30
35

Identification of key organisms in the Baltic Sea redoxclines

16S rcDNA SSCP fingerprints of prokaryotic diversity



Sequenzierte 16S rRNA-Gen/rRNA Banden

Euphotische Zone
- *Synechococcus* sp. MW10#1 (97%)
- *Pelagibacter ubique* (97%)
- Uncultured marine Crenarchaote ARCT1-54 (99%)

Winterwasser
- *Synechococcus* sp. MW10#1 (97%)
- *Nitrosospira* sp. Nsp65 (88%)
- *Flavobacterium ferrugineum* (93%)
- ,*Owenweeksia hongkongensis*' (90%)

Suboxische Zone
- *Nitrosospira* sp. Nsp65 (88%)
- *Maorithyas hadalis* thioautotropher Symbiont (98%)

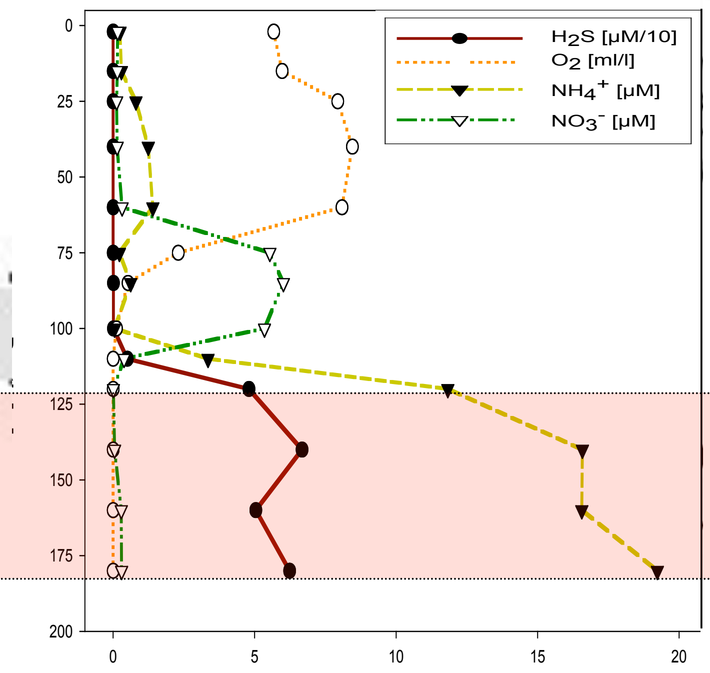
Redoxkline
- *Maorithyas hadalis* thioautotropher Symbiont (98%)
- *Sulfurimonas autotrophica* (93%)
- *Polaribacter franzmannii* (93%)

Sulfidische Zone
- *Sulfurimonas autotrophica* (93%)
- *Maorithyas hadalis* thioautotropher Symbiont (98%)
- Uncultured CFB (97%)
- *Achromobacter xylosoxidans* (94%)
- *Zooglea resiniphila* (86%)
- *Desulfobacula toluolica* (97%)

Tiefenverteilung erster identifizierter Bakterien

Labrenz, Jost & Jürgens (in press)

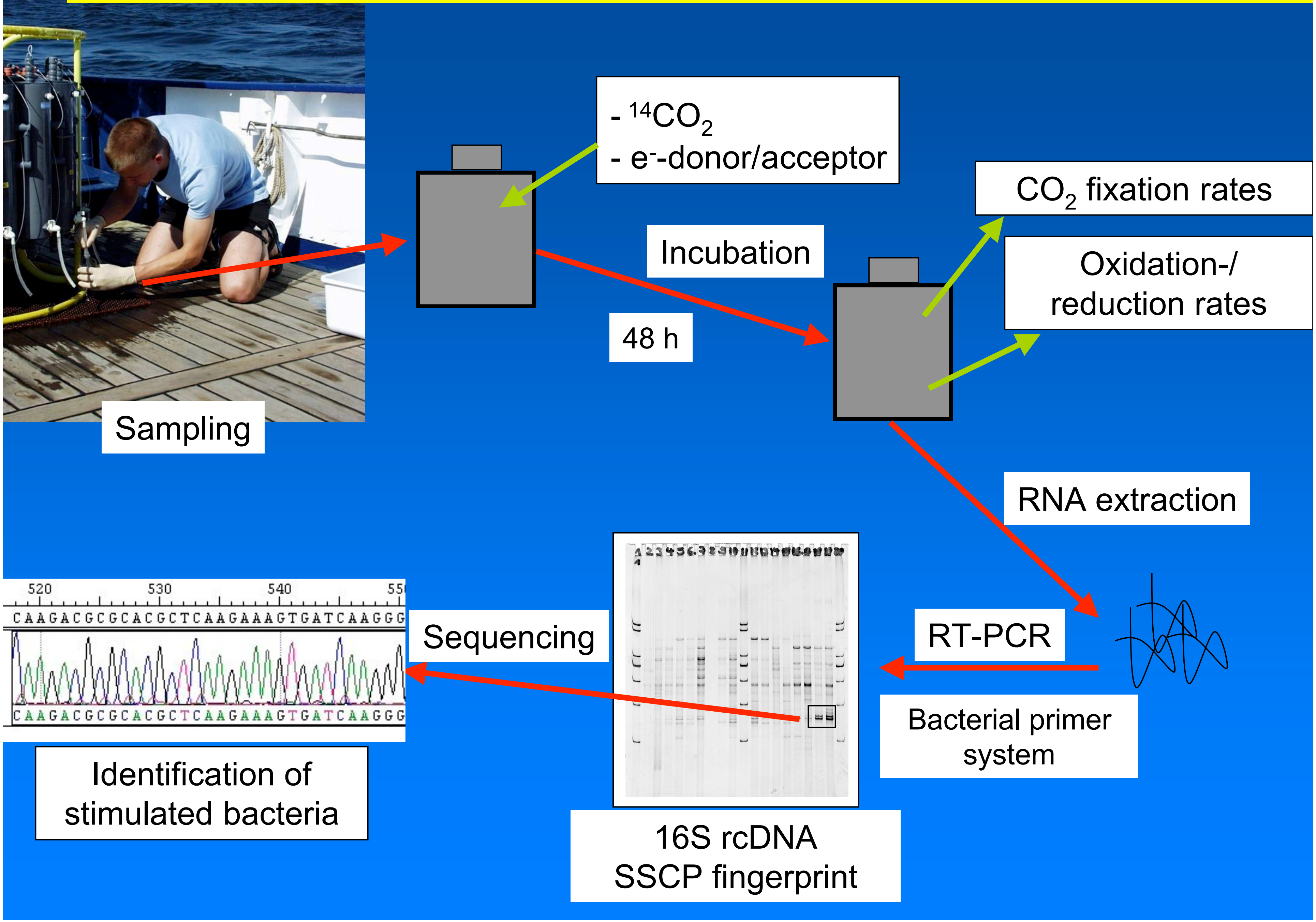
Quantitative distribution of dominant taxa determined by *in situ* 16S rcDNA SSCP/DGGE fingerprinting



Relative amount of 16S rcDNA bands (%)

Electron acceptor?

Experimental design: Enrichment of chemolithoautotrophs



CO_2 fixation in stimulation experiments

Labrenz et al. 2005: AEM 71:6664

Gotland Deep (station 271)

e^--donor/-acceptor (100 µM)	CO_2 fixation rate [µmol L^{-1} d^{-1}]
NH_4/Fe^{3+}	0.02
Fe^{2+}/Mn^{4+}	0.02
$MetOH/Fe^{3+}$	0.03
Glc/Fe^{3+}	0.29
Glc/Mn^{4+}	0.33
$Na_2S_3O_3/Mn^{4+}$	1.38
$Na_2S_3O_3/Fe^{3+}$	2.58
$Na_2S_3O_3$	3.78

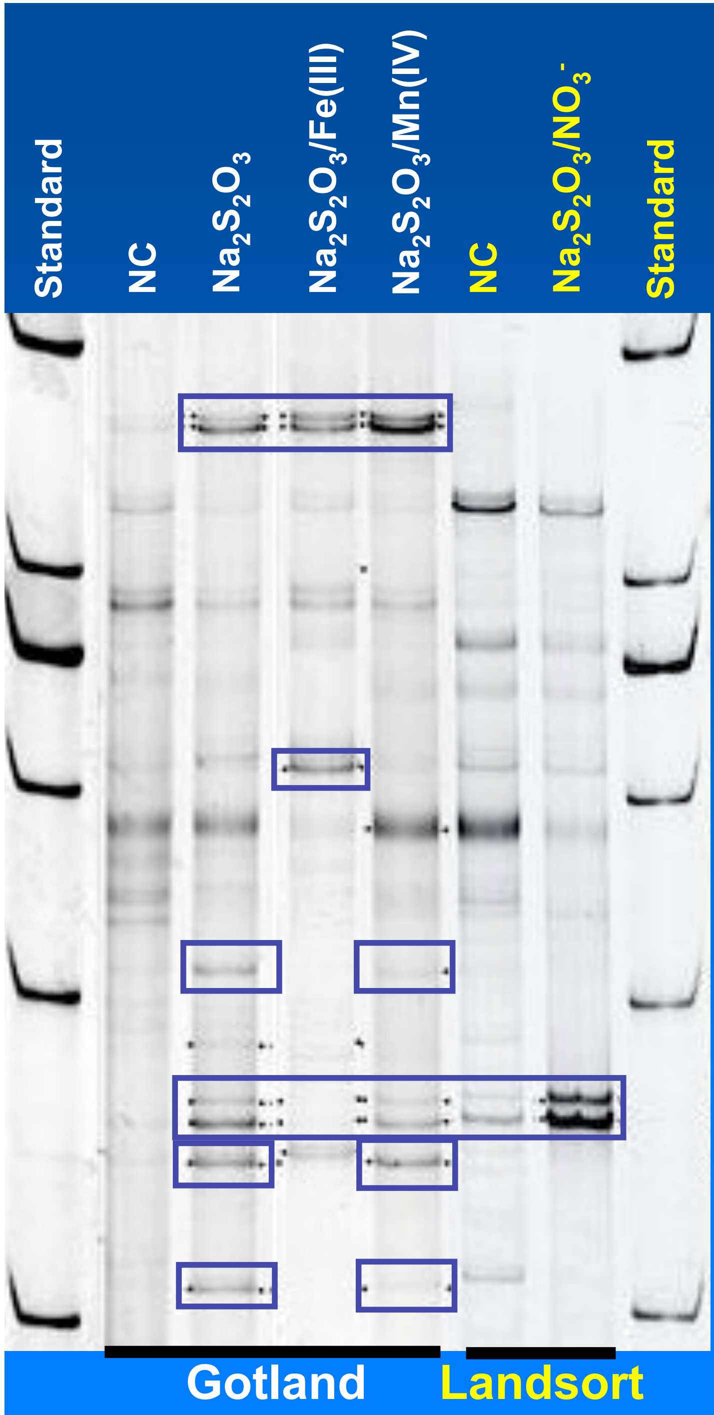
No measurable Mn(IV)/Fe(III) reduction

Landsort Deep (station 284)

e^--donor/-acceptor (100 µM)	CO_2 fixation rate [µmol L^{-1} d^{-1}]
Mn^{2+}/NO_3	0.02
$MetOH/NO_3$	0.03
NH_4/NO_3	0.05
Glc/NO_3	0.13
Na_2S/NO_3	0.24
$Na_2S_3O_3/NO_3$	4.87

Reduction of e^--acceptor 20.2 µmol L^{-1}

16S rRNA stimulation after 48 h of incubation (with thiosulfate)



Stimulated autotrophic bacteria also *in situ* detected

Thiomicrospira psychrophila **SVAL-D (99 %)**

- Uncultured *Helicobacteraceae* stimulated with all redox combinations
- Stimulated in Landsort- and Gotland water

Pseudomonas **sp. He (99 %)**

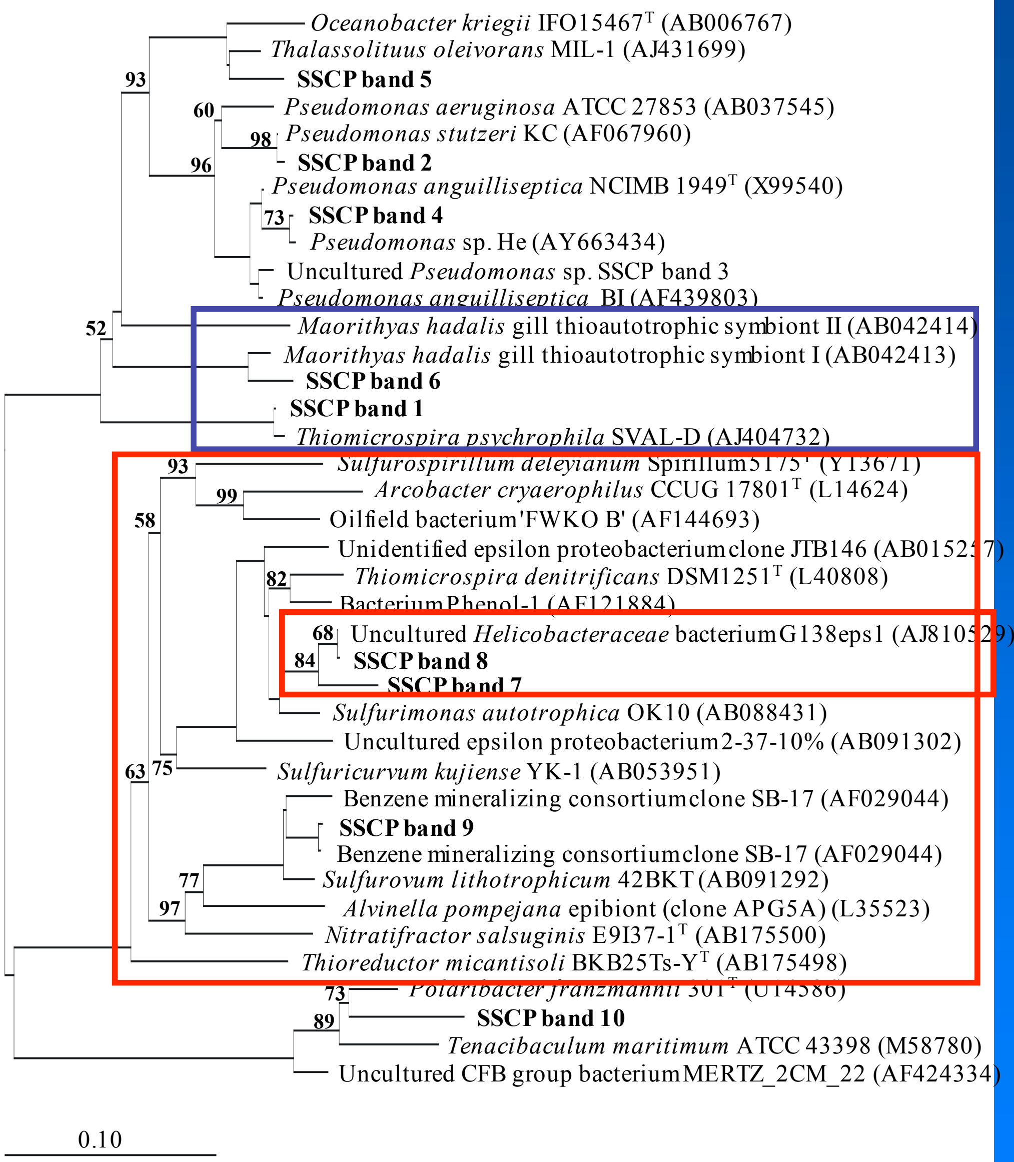
Maorithyas hadalis **gill thioautotrophic symbiont (99 %)**

Uncultured *Helicobacteraceae* G138eps1 (100 %)

Uncultured Epsilonproteobacterium clone Nubeena319 (99 %)

Uncultured CFB group bacterium MERTZ_2CM_22 (94 %)

16S rRNA phylogeny



Gammaproteobacteria

Aerob autotroph

Probe for uncultured Epsilon-Proteobacterium (+isolate)

Epsilonproteobacteria

Mainly anaerob autotroph

Bacteroidetes

Abundance of *Epsilonproteobacteria* in redoxclines of Cariaco Trench and Black Sea

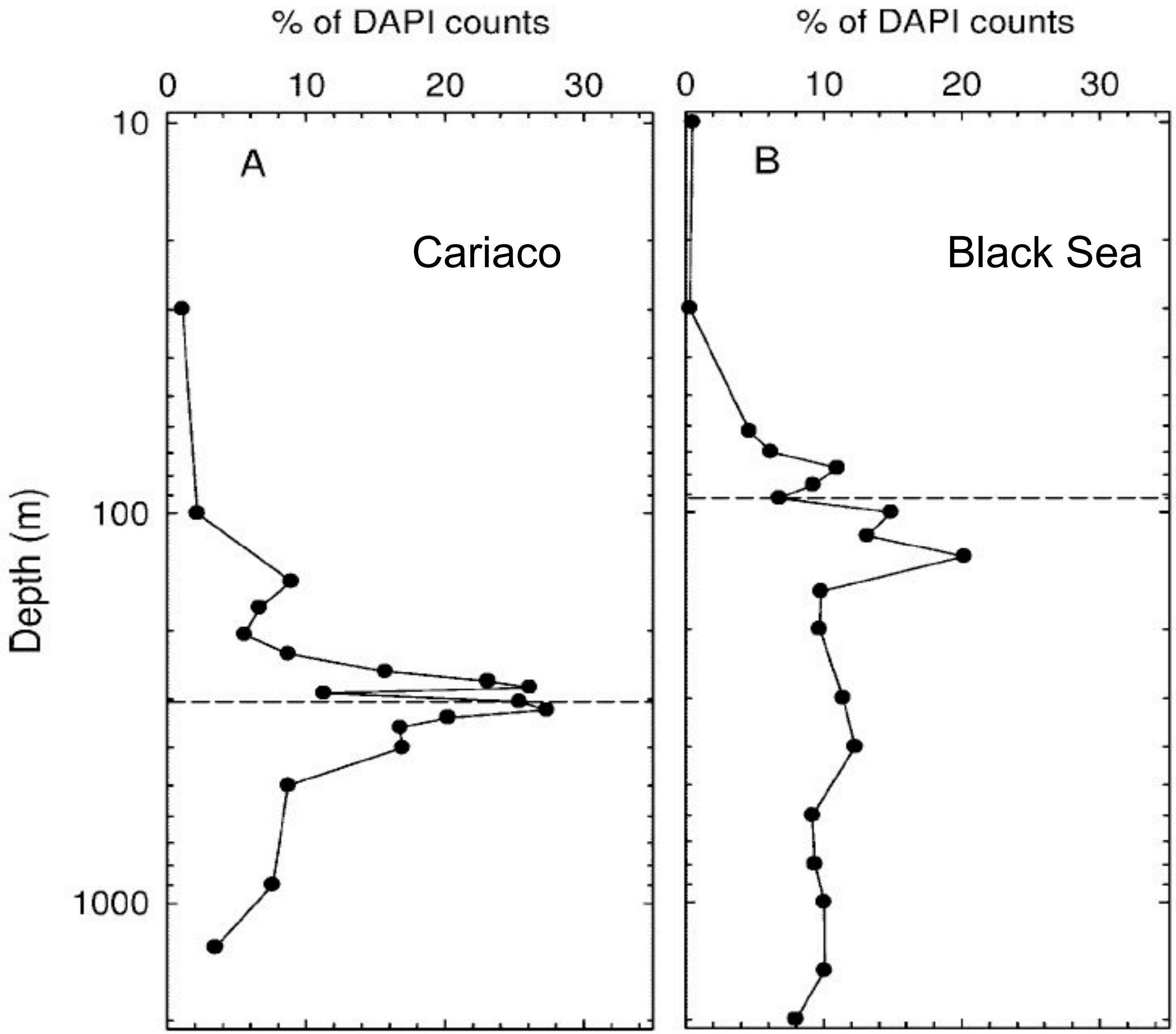
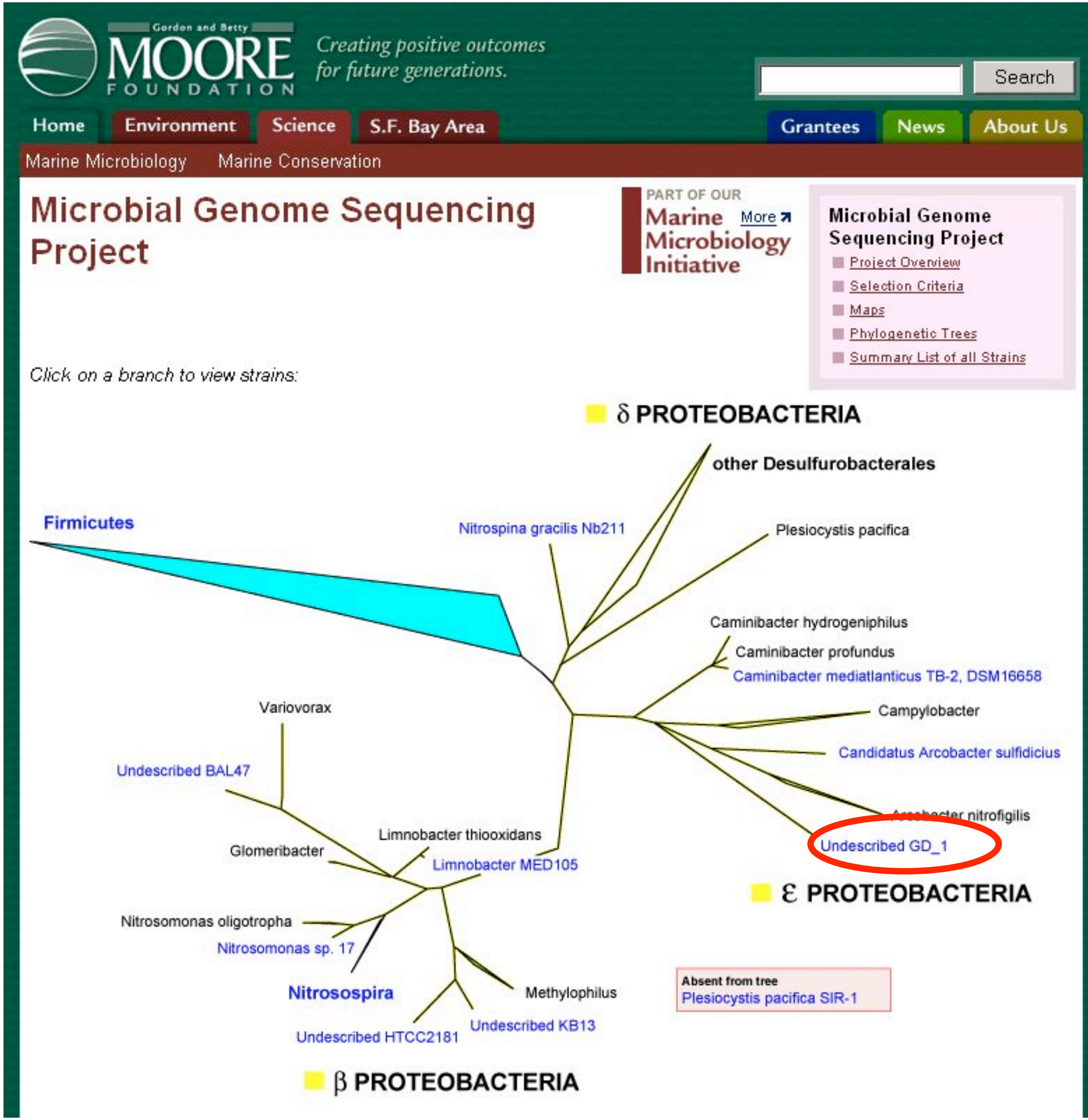


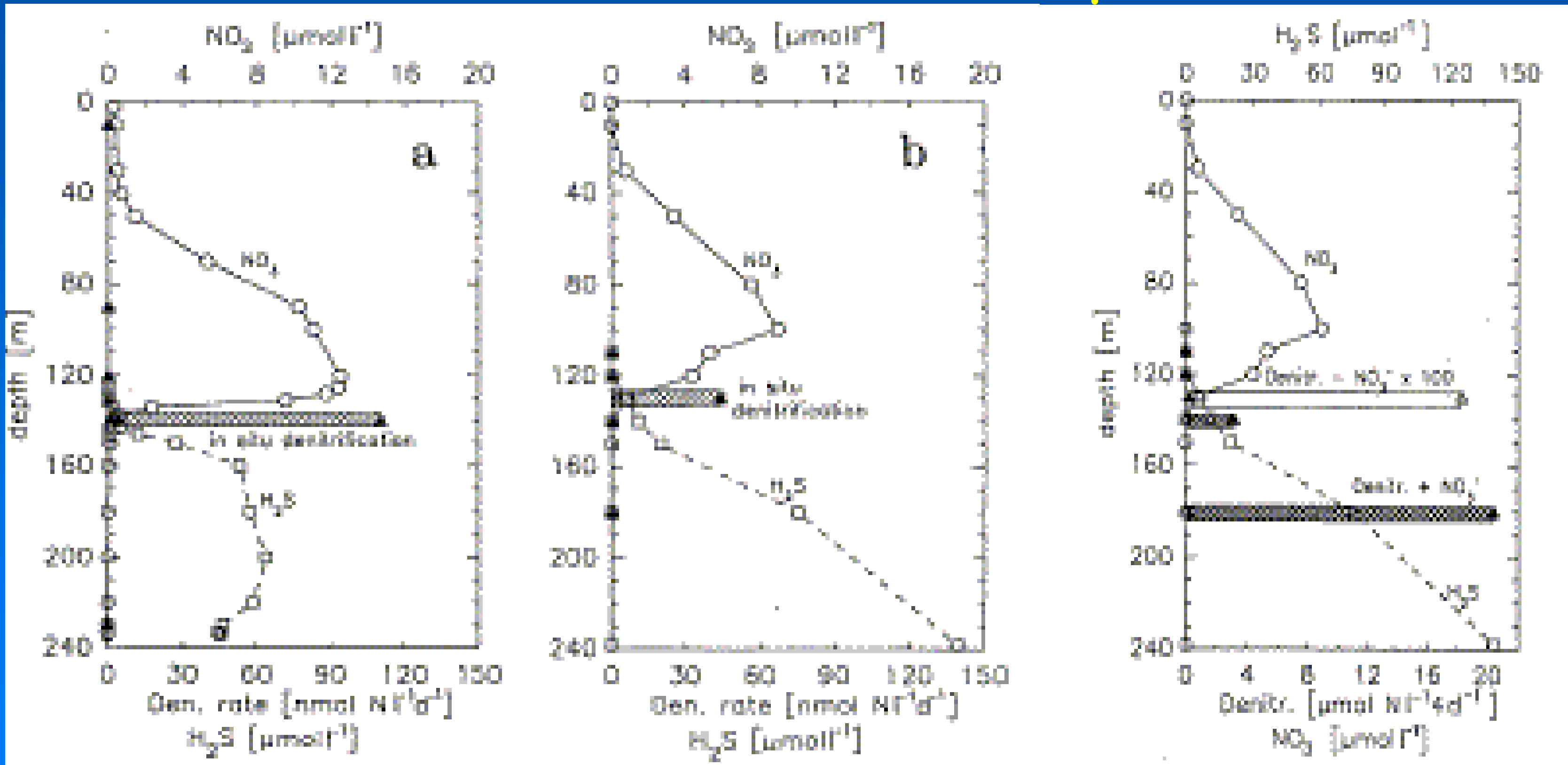
FIG. 4. Vertical distributions of ε-proteobacterial cells (EPS549 positive) relative to total DAPI-positive cells in the Cariaco Basin (A) and the Black Sea (B). Dashed horizontal lines indicate the shallowest appearances of sulfide.

Lin et al., AEM 2006

Genom-sequenzierung



Autotrophic Denitrification (by Epsilon-Proteobacteria): Characteristic for Gotland Deep redoxcline



Acetylen inhibition:
In situ denitrification rates

„Potential“ denitrification rates after **addition of NO_3^- to sulfidic water**

(Brettar and Rheinheimer, 1991)

Goals

Denitrifier communities

- structure and composition of denitrifying communities in the whole water column

➲ fingerprinting method by functional gene analysis

Activity of denitrifying bacteria

- NO_3^- consuming processes in the predicted denitrification zone

➲ *in situ* incubations with labelled ^{15}N-compounds

➲ stimulation experiments

^{15}N-labelling incubation experiments

- **isotope pairing technique**
  - very sensitive method to detect pathways and products in the nitrogen cycle; e.g. denitrification or anammox
- **^{15}N-labelled NO_3^- or NH_4^+**
  - approaches in the suboxic-sulphidic interface of the Gotland Deep (2002, 2004 and 2005)

Denitrification:

$^{15}NO_3^- \longrightarrow {}^{15}N^{15}N\ ({}^{30}N_2)$

$^{15}NO_3^- + {}^{14}NO_3^- \longrightarrow {}^{15}N^{15}N\ ({}^{30}N_2) + {}^{15}N^{14}N\ ({}^{29}N_2) + {}^{14}N^{14}N\ ({}^{28}N_2)$

Anammox:

$^{15}NO_3^- + {}^{14}NH_4^+ \longrightarrow {}^{15}N^{14}N\ ({}^{29}N_2)$ (via $^{15}NO_3^- \longrightarrow {}^{15}NO_2^-$)

$^{14}NO_2^- + {}^{15}NH_4^+ \longrightarrow {}^{14}N^{15}N\ ({}^{29}N_2)$

Summary denitrifier communities

- ➲ relatively low level of denitrifier diversity by T-RFLP analysis
  - **minimum** in the predicted denitrification zone
- ➲ new *nirS* Baltic Sea clusters
  => clusters group according the **oxygen status** of the water column

Summary

^{15}N-labelled incubation experiments:

- no N_2 production in the suboxic zone
- stimulation experiments with organic and NO_3^-
  => no N_2 production after 48 h of incubation
  => early $^{15}NH_4^+$ production, NO_2^- and N_2O intermediates, limiting factors unclear
- autotrophic denitrification in the suboxic-sulphidic interface important process when NO_3^- and H_2S coexist

Change of the suboxic-sulphidic interface

- first evidence for anammox in summer 2004
- Regulating factors? (NO_2^-, NH_4^+, no H_2S ?)

Pelagic Redoxclines: Many open questions... and future issues

- Some of the major redoxprocesses and their interactions are not clear yet (CO_2 fixation, MnOx, denitrification, anammox)
- Seasonal variation; development after inflow events
- Interaction of abiotic and biotic controlling mechanisms (food web processes)
- Biochemical modelling of vertical structure and large scale effects
- Impact of turbulence and lateral intrusions