Insights on habitat chemistry from *in situ* voltammetry

RIDGE Theoretical Institute on Interactions at Deep-Sea vents, September 11, 2007

George W. Luther, III,
College of Marine and Earth Studies, University of Delaware, Lewes, DE

Acknowledgments

University of Delaware:

students: Tommy Moore, Shufen Ma, Katherine Mullaugh, Mustafa Yucel, Heather Nees, Brian Glazer

postdocs: Martial Taillefert, Tim Rozan, Edouard Metzger, Tim Waite, Jeffrey Tsang

colleagues: Craig Cary

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nonUD colleagues: Rich Lutz, Tim Shank, Costa Vetriani, Chuck Fisher, James Childress, Maya Tolstoy and their groups

Outline

***In situ* techniques are necessary to study the environment – voltammetry as a good *non-selective* analytical method**

Comparison between 9 N EPR and Lau Basin – integrated studies with biology and geophysics

How fast and how long do we need to collect data to understand an organism's response to chemistry?

How do organisms respond to the absence or presence of O_2, H_2S and S / Fe species in the environment?

A look at chemosynthesis

Patterns of organism distribution

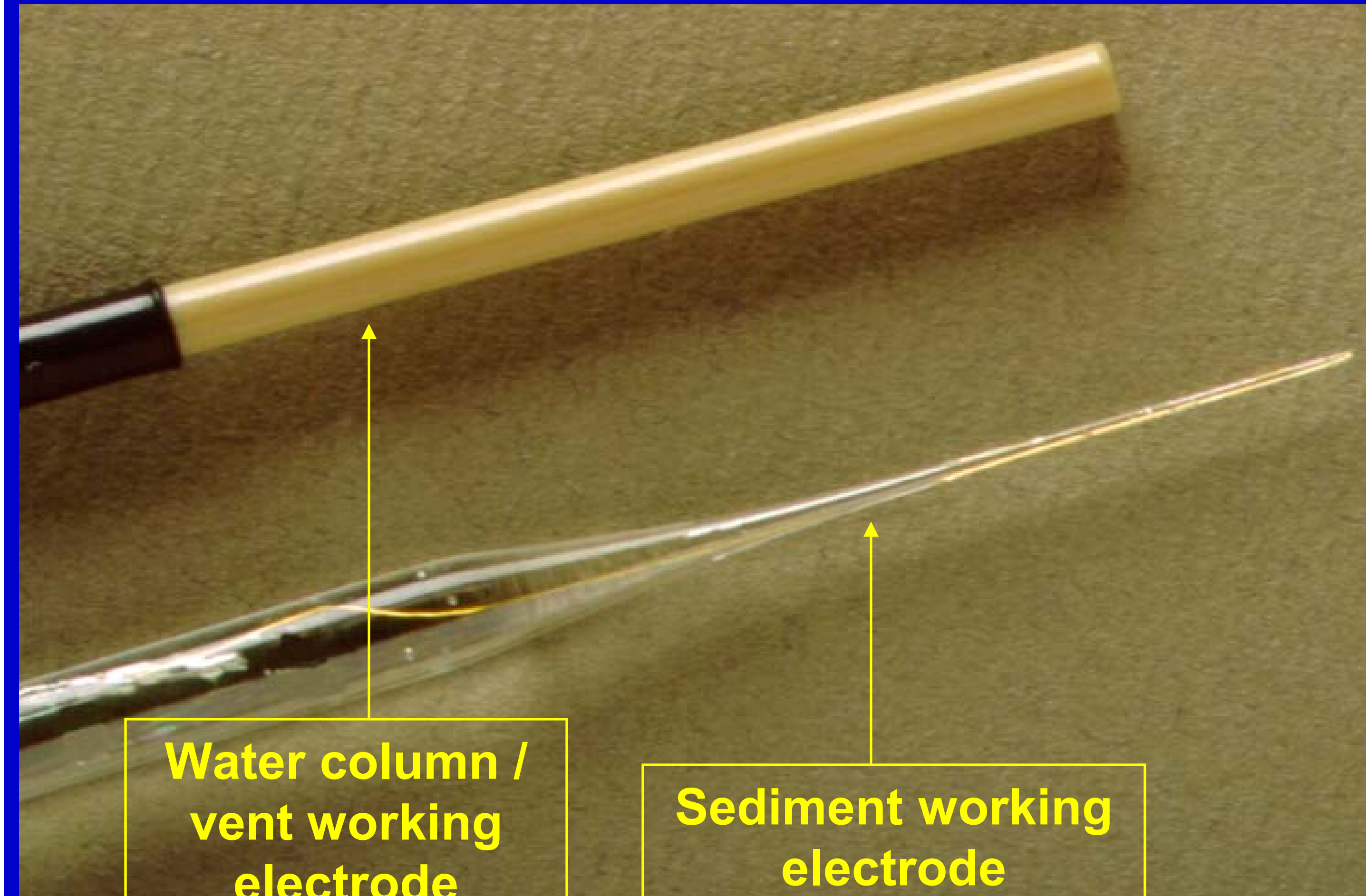
At vents ultimate source of energy = vent fluids

- Therefore, primary productivity positively correlates with environmental stressors/indicators (high temperatures and "toxic" chemistry)

Factors influencing faunal distribution patterns:

- Abiotic environment
- Positive and negative species' interactions
  - Important in areas of high primary productivity but relatively low environmental stress

PEEK & Glass encased electrodes in marine epoxy

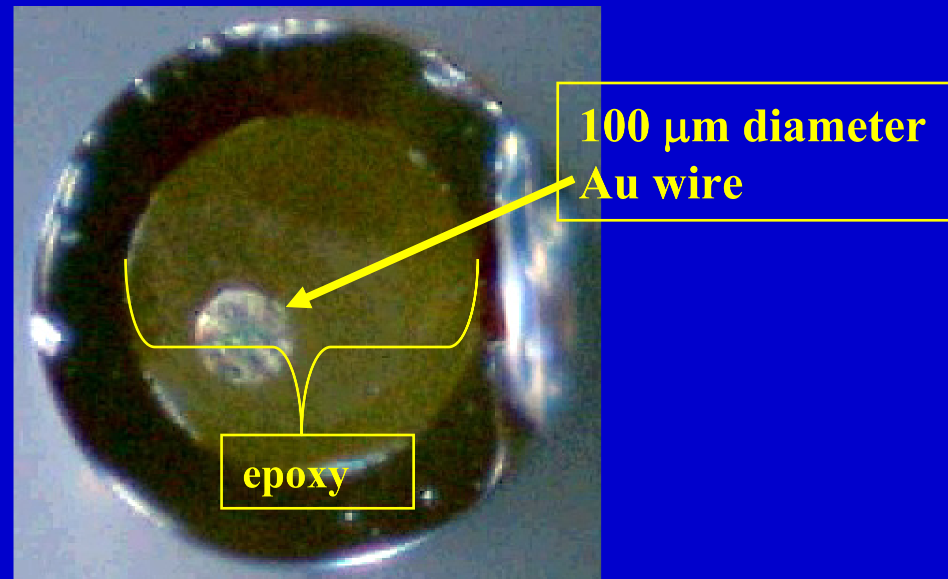


O_2, Fe^{2+}, Mn^{2+}, H_2S, H_2O_2, I^-, S_x^{2-}, $S_2O_3^{2-}$, FeS_{aq}, Fe(III) are all measurable in one scan, if present

Tested to 2600 m and 120 ^oC

Gold Electrode Tip Preparation

After epoxy injection before sanding and polishing - GLASS

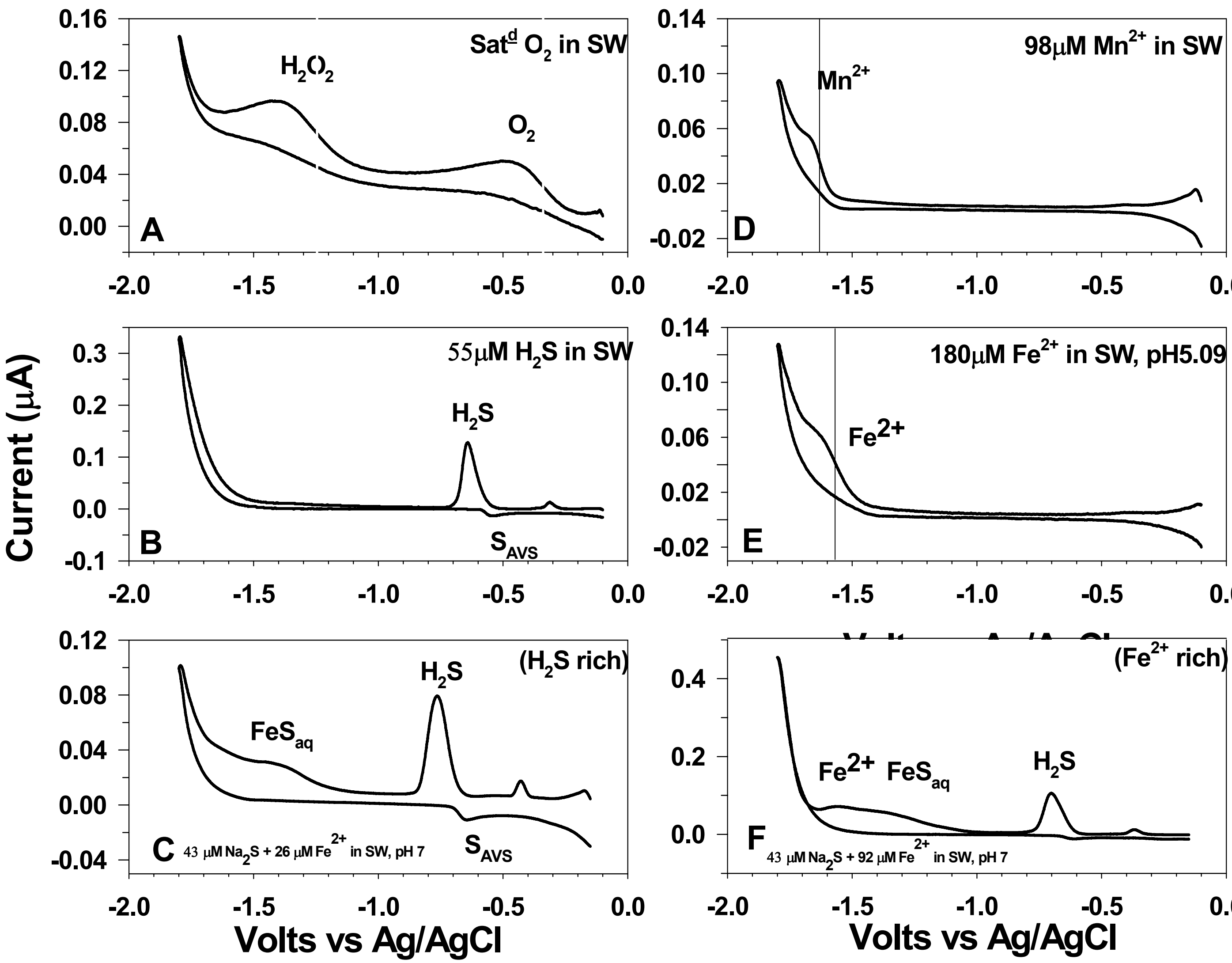


Final polish (GLASS)– 0.24 micron

After polarization (PEEK)– note H_2 gas evolving

VOLTAMMETRY

I vs E plots [similar to A vs λ plots]



Multi-analyte sensor

Au/Hg Electrode Reactions of Interest
Oxygen and sulfur species

Reaction (- scan; 200 mV/s; 25 ^{o}C) — *Ep (V) vs SCE*

1a) $O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$ — -0.30

1b) $H_2O_2 + 2H^+ + 2e^- \rightarrow H_2O$ — -1.30

2a) $HS^- + Hg \rightarrow HgS + H^+ + 2e^-$ — adsorption onto Hg <-0.62

2b) $HgS + H^+ + 2e^- \leftrightarrow HS^- + Hg$ — -0.62

3a) $S(0) + Hg \rightarrow HgS$ — adsorption onto Hg <-0.62

3b) $HgS + H^+ + 2e^- \leftrightarrow HS^- + Hg$ — -0.62

4a) $Hg + S_x^{2-} \rightarrow HgS_x + 2e^-$ — adsorption onto Hg <-0.62

4b) $HgS_x + 2e^- \leftrightarrow Hg + S_x^{2-}$ — -0.62

4c) $S_x^{2-} + x H^+ + (2x-2) e^- \rightarrow x HS^-$ — -0.62 (varies with v)

5) $2 RSH + Hg \leftrightarrow Hg(SR)_2 + 2H^+ + 2e^-$ — < -0.62

6) $2 S_2O_3^{2-} + Hg \leftrightarrow Hg(S_2O_3)_2^{2-} + 2e^-$ — -0.15

Au/Hg Electrode Metal Reactions of Interest

Reaction (+ scan; 200 mV/s; 25 ^{o}C)		*Ep (V) vs SCE*
	Redox metals measurable	
1)	$FeS + 2e^- + H^+ + Hg \rightarrow Fe(Hg) + HS^-$	-1.15
2)	$Fe^{2+} + Hg + 2e^- \leftrightarrow Fe(Hg)$	-1.43
3)	$Mn^{2+} + Hg + 2e^- \leftrightarrow Mn(Hg)$	-1.55
4)	$Fe^{3+}(organic) + e^- \rightarrow Fe^{2+}(organic)$	-0.2 to -0.9
	Trace metals measurable	
5)	$Cu^{2+} + Hg + 2e^- \leftrightarrow Cu(Hg)$	-0.18
6)	$Pb^{2+} + Hg + 2e^- \leftrightarrow Pb(Hg)$	-0.46
7)	$Cd^{2+} + Hg + 2e^- \leftrightarrow Cd(Hg)$	-0.62
8)	$Zn^{2+} + Hg + 2e^- \leftrightarrow Zn(Hg)$	-1.05

Solid state (micro)electrodes for the analysis of biologically relevant compounds and ions

Chemistry Drives Biology

Rationale for design and use

Fine scale resolution - mm in sediments; μm in biofilms and mats

determine sediment heterogeneity vs. homogeneity

use to prospect for life forms and understand ecosystem health

Use in sedimentary porewaters of wetlands, bays, oceans and lakes

in water column; e.g., Chesapeake Bay, Black Sea

at Hydrothermal Vents, Yellowstone hot springs,

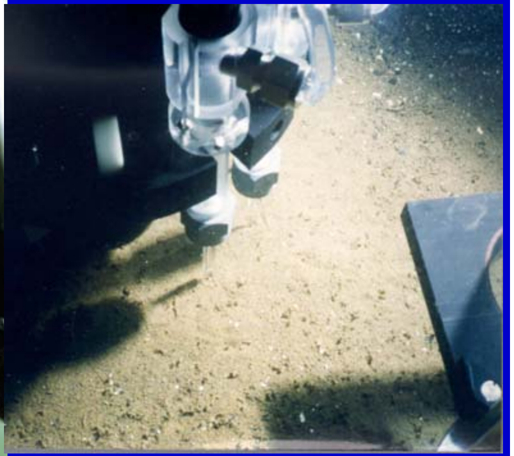
in corrosion studies

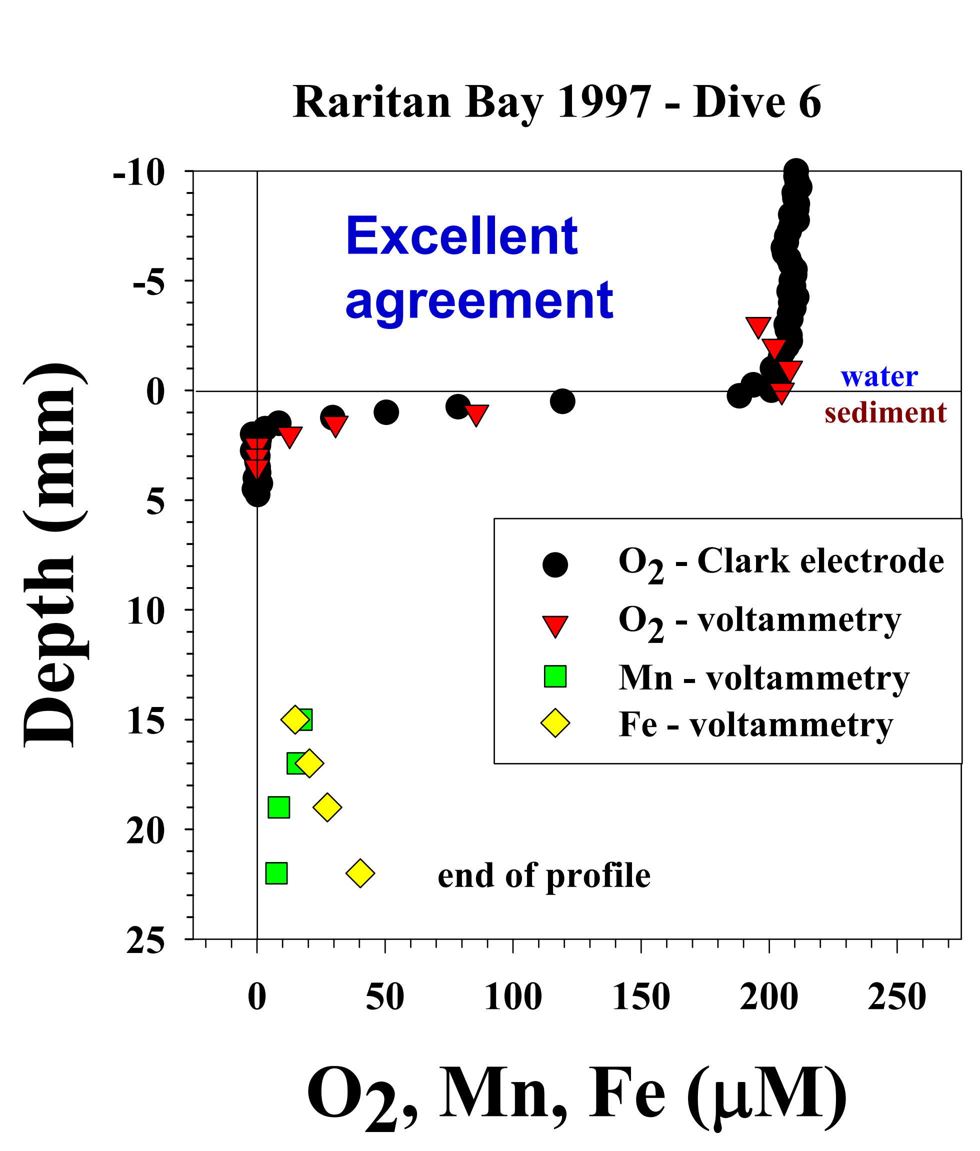
ELECTRODE STANDARDIZATION

- **Electrodes standardized in matrix of interest for each species.**
- **Current is independent of pH (4-8) for O_2, H_2S, Fe, Mn.**
- **Current is dependent on Temperature for all species; the diffusion coefficient depends on temperature.**
- **Current is independent of Pressure.**
- **Current depends on [flow rate]$^{1/2}$. Above 1.68 cm/s, there is NO flow rate dependence on 100 μm diameter electrodes.**
- **Validation *via* discrete samples and *in situ* Clark O_2 electrodes.**

In situ comparison of O_2 Clark vs voltammetric Au/Hg in sediments from a ROV

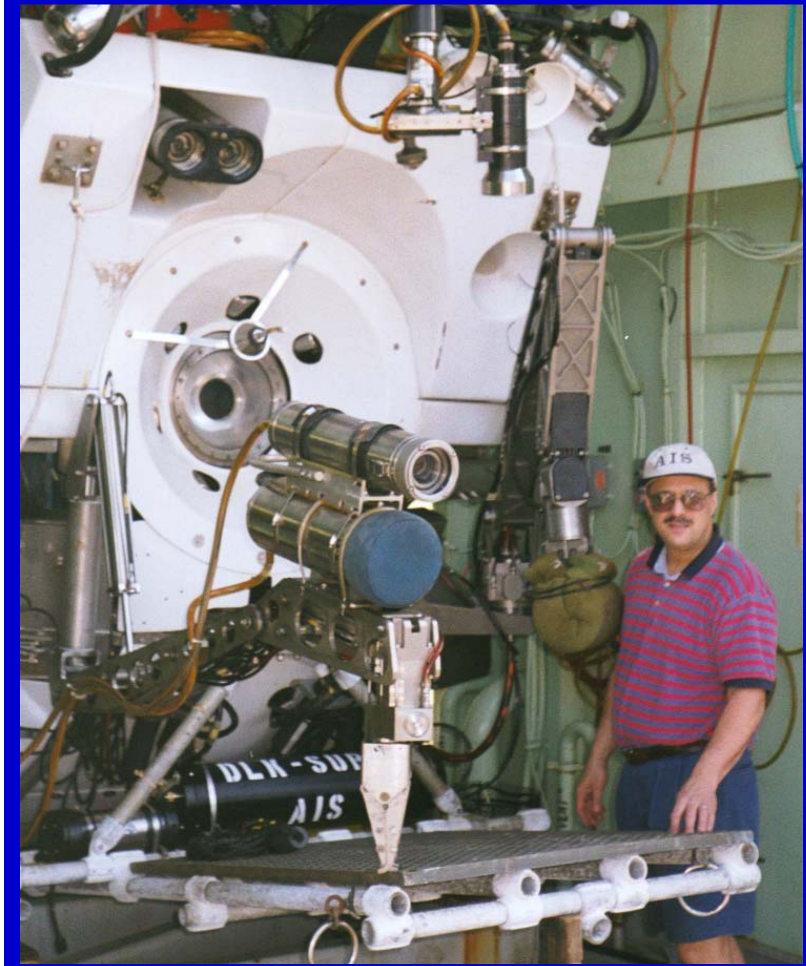
***R**eal time voltammetry of porewaters*



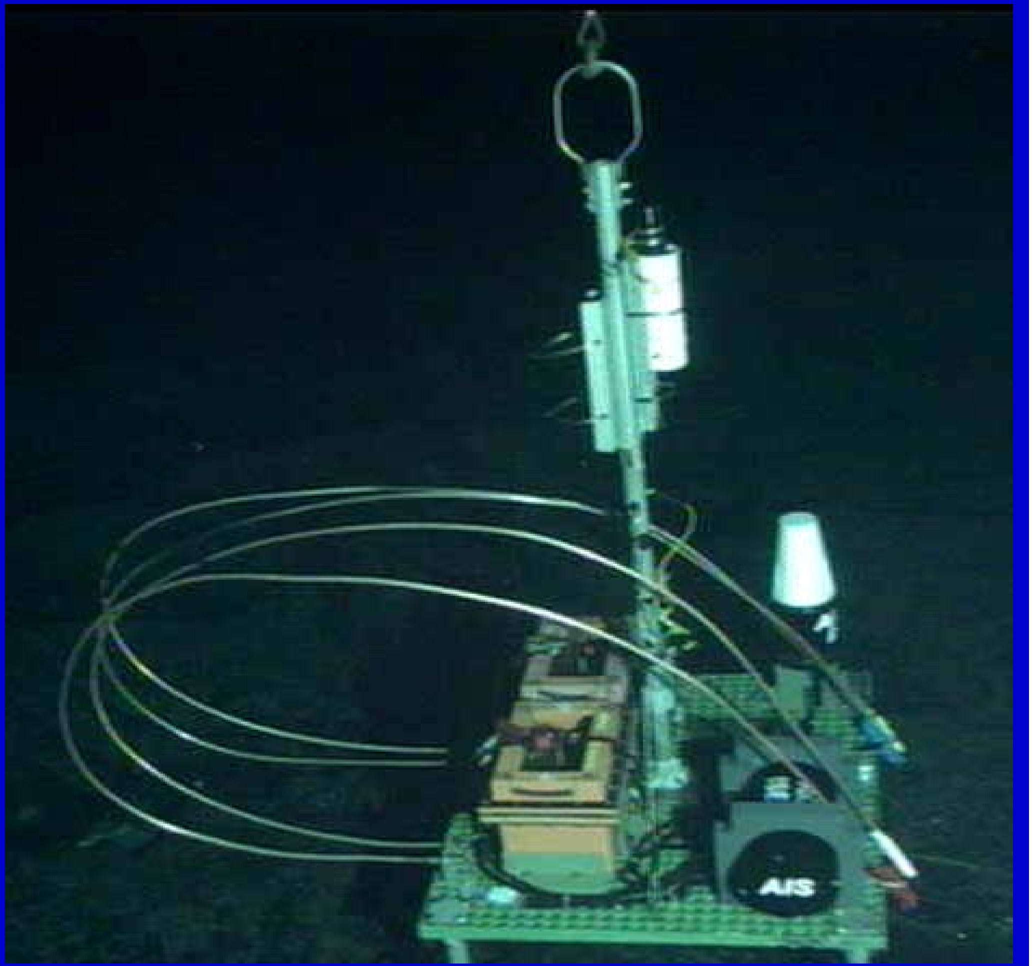


What ranges and variability in chemistry do organisms experience?

Tools for diffuse flow area studies



Unit used from *Alvin* or *JasonII*

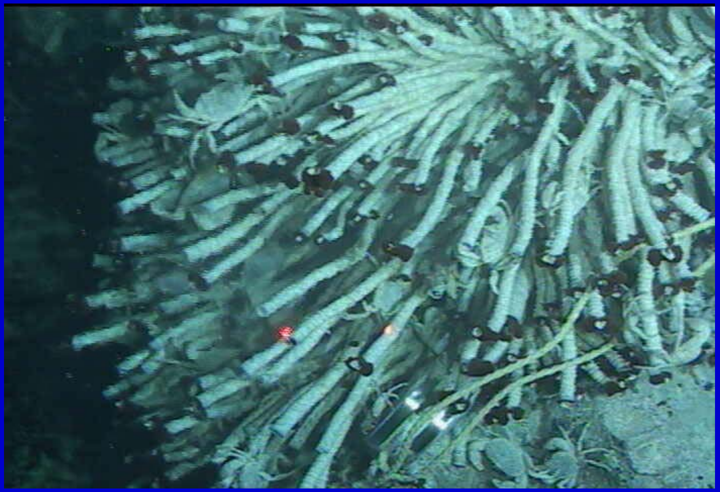


Unattended system (ISEA or INSECT)

9 N EPR Foundation Organisms with endosymbionts

distribution controlled by local physical and chemical environment

Riftia **tubeworm; mussel**

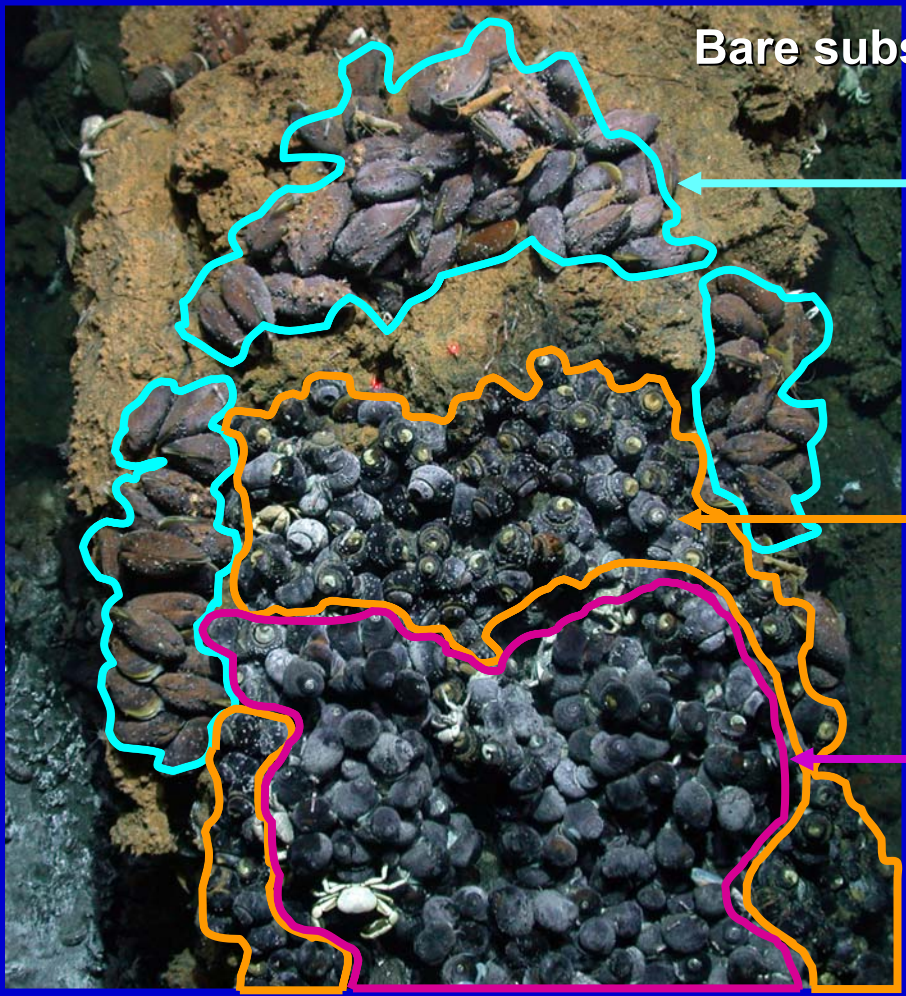


Tevnia **tubeworm**

Basalt surface – glass like

Lau Basin Foundation Organisms with endosymbionts

Less reduced



Mussels - *Bathymodiolus brevior*

Snail "Ifremeria"

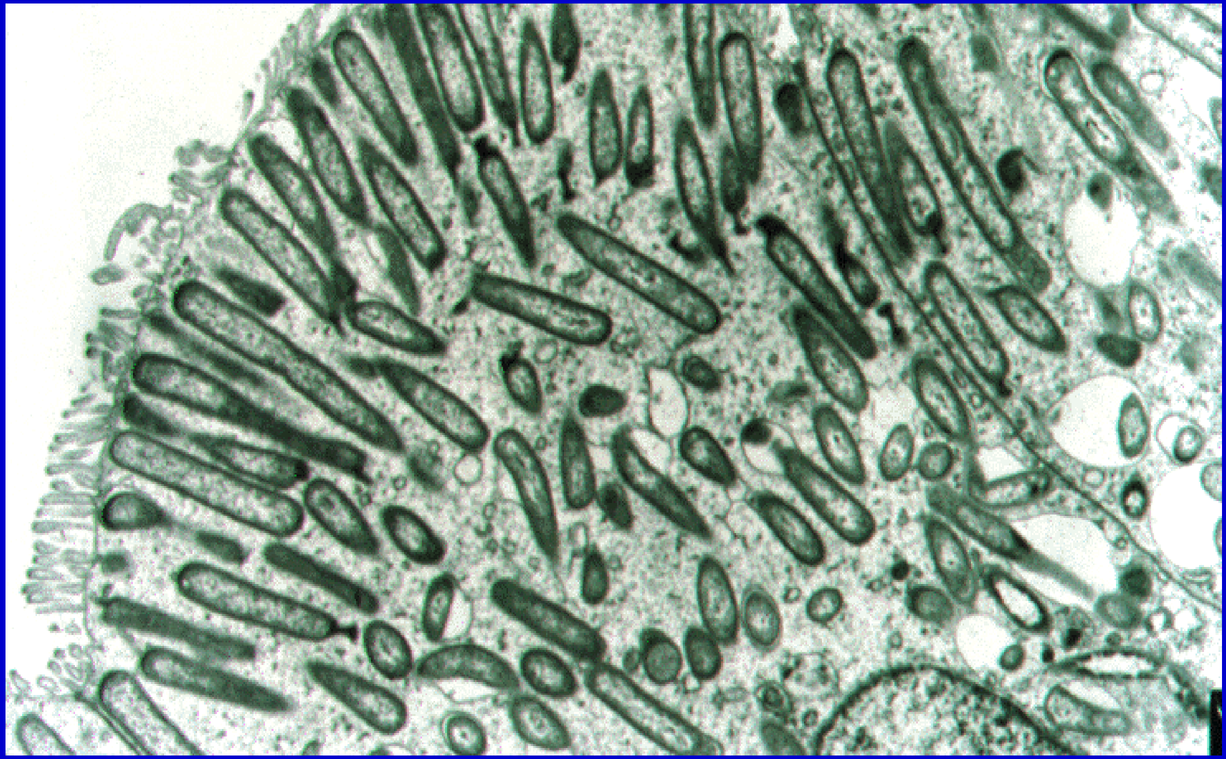
Snail "Alviniconcha"

reduced

Basalt or andesite surface – friable with high surface area

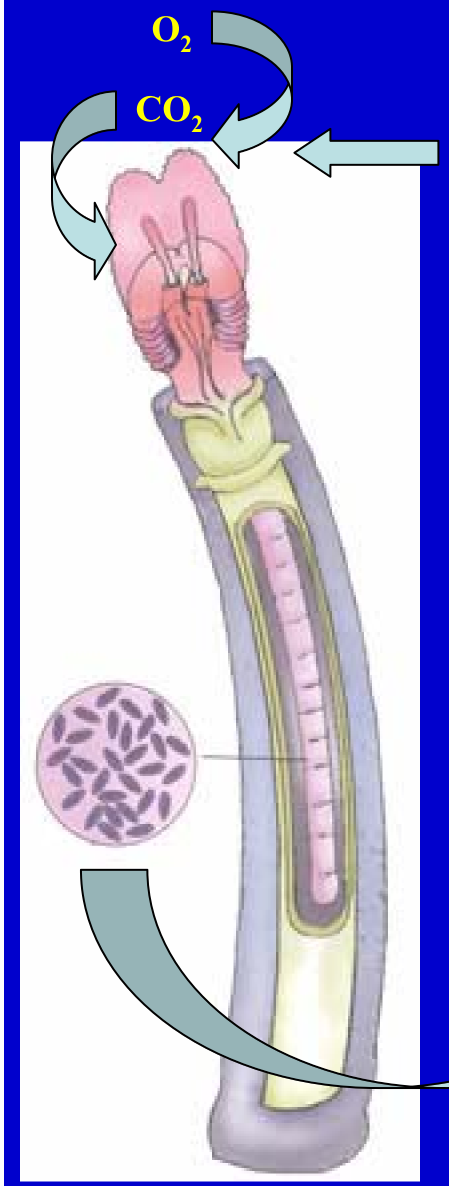
Bacterial Symbionts of Vent Organisms

- **Chemolithotrophic**
- **Endosymbionts**



Requiring co-occurrence of Sulfide (H_2S,HS^-), O_2, and CO_2

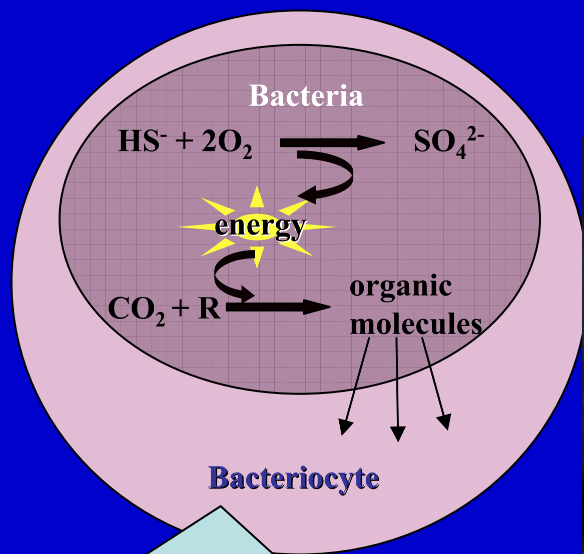
Chemosynthesis or Chemautotrophy

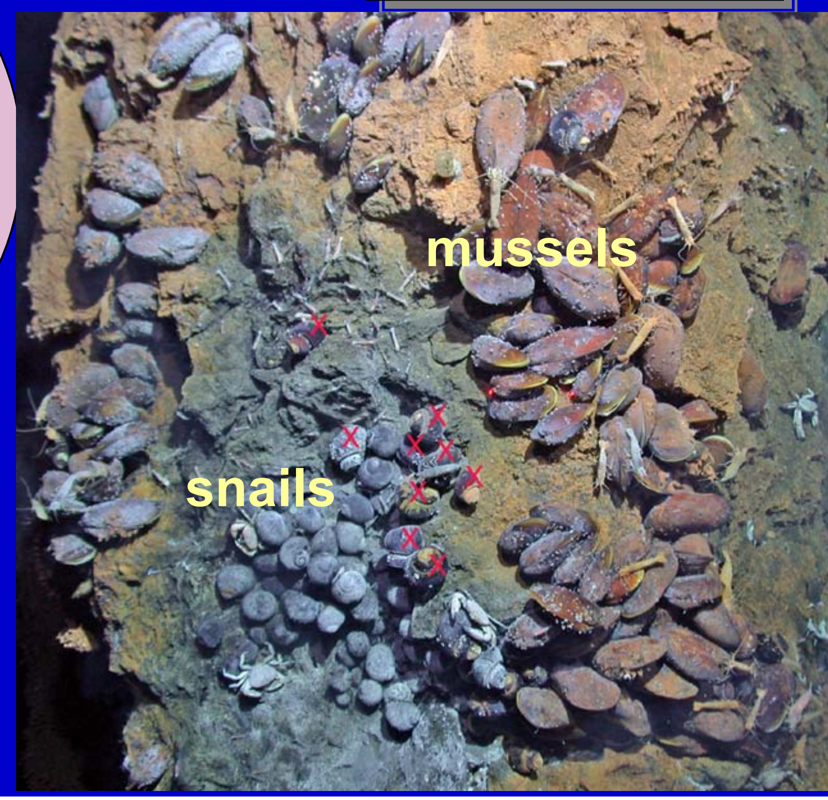


HS^- (binds to tubeworm hemoglobin – red blood)

Mussels and clams have symbionts and red blood too!

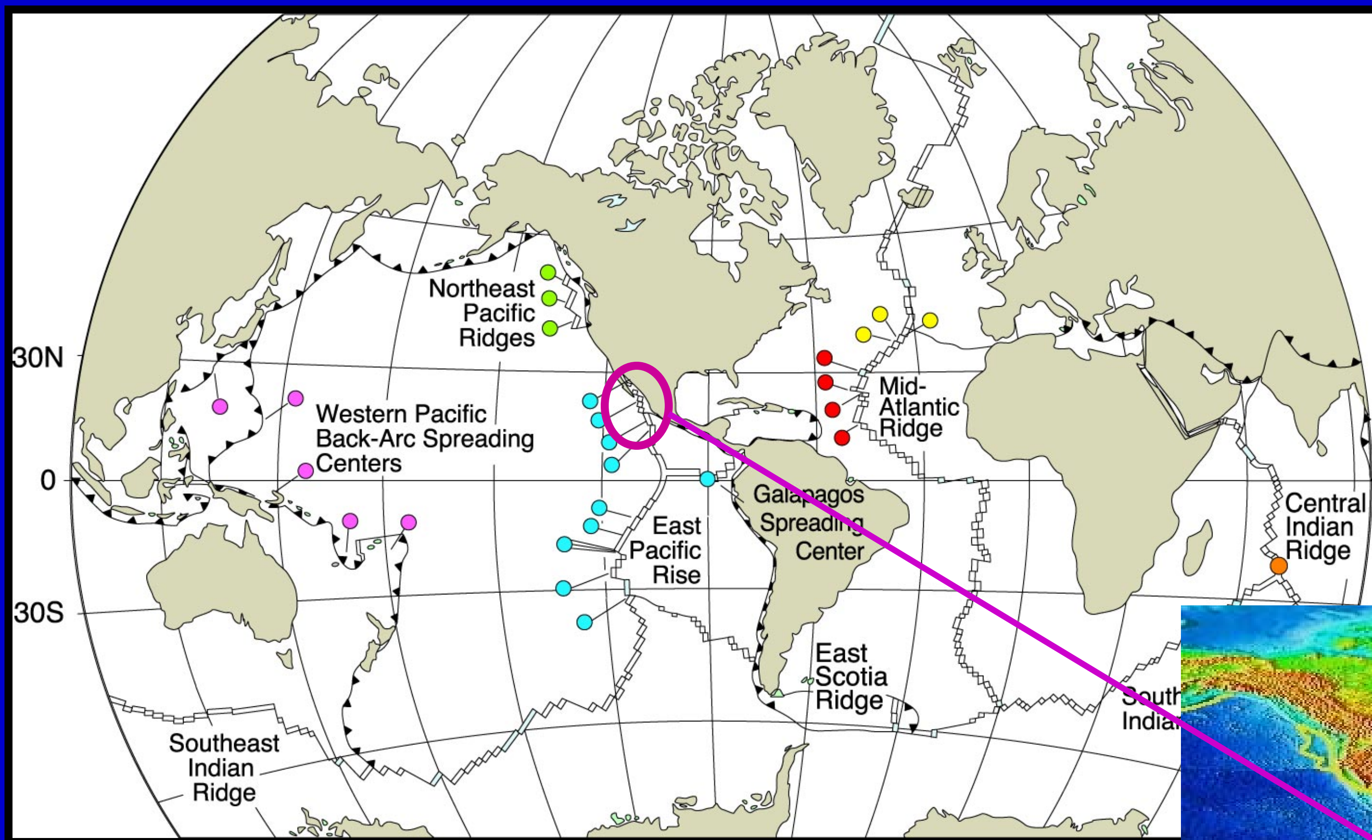
Tubeworms have symbionts



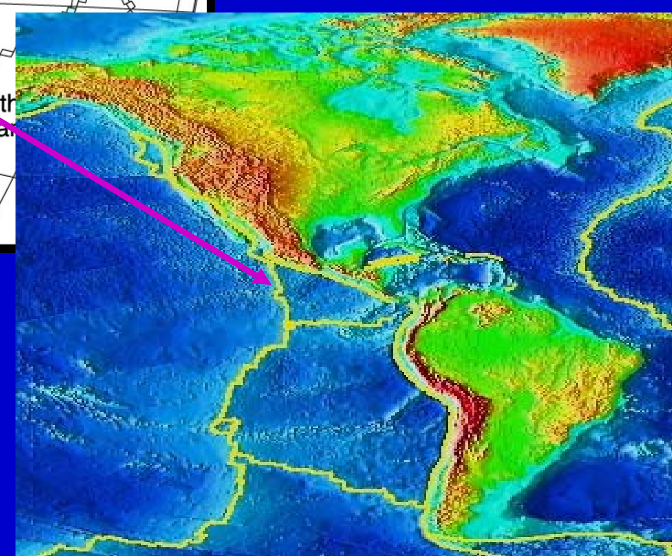


Snails have symbionts and blue blood!

Distribution of hydrothermal vents



North East Pacific Rise



Fe, S chemistry
Origin of Life, of organic compounds and a source of H_2 at HYDROTHERMAL VENTS

$$FeS + H_2S \rightarrow FeS_2 + H_2$$

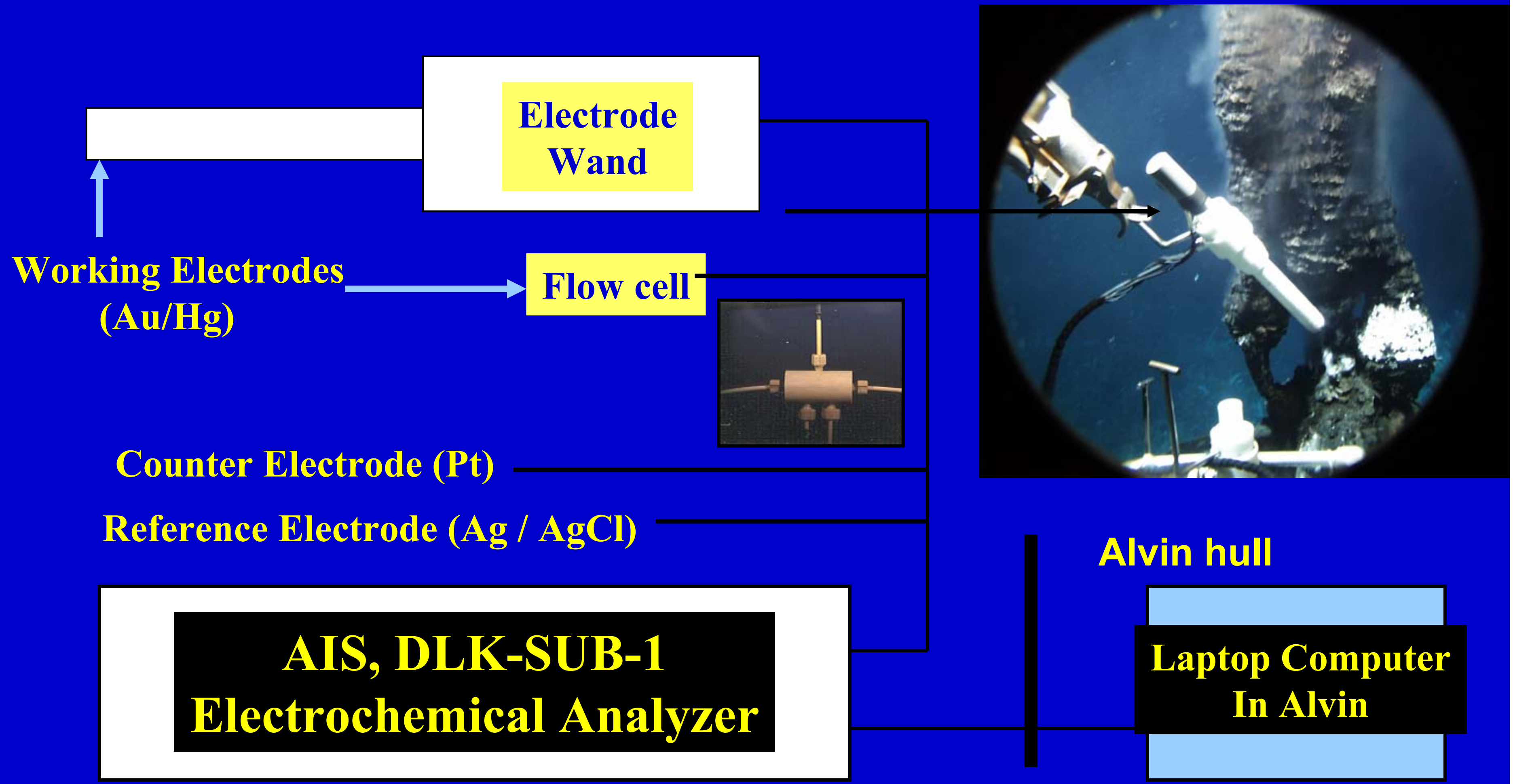
Wachterhauser's hypothesis (1988) BUT first noted by Berzelius!!

Voltammetry can measure FeS_{aq} (molecular clusters) and H_2S

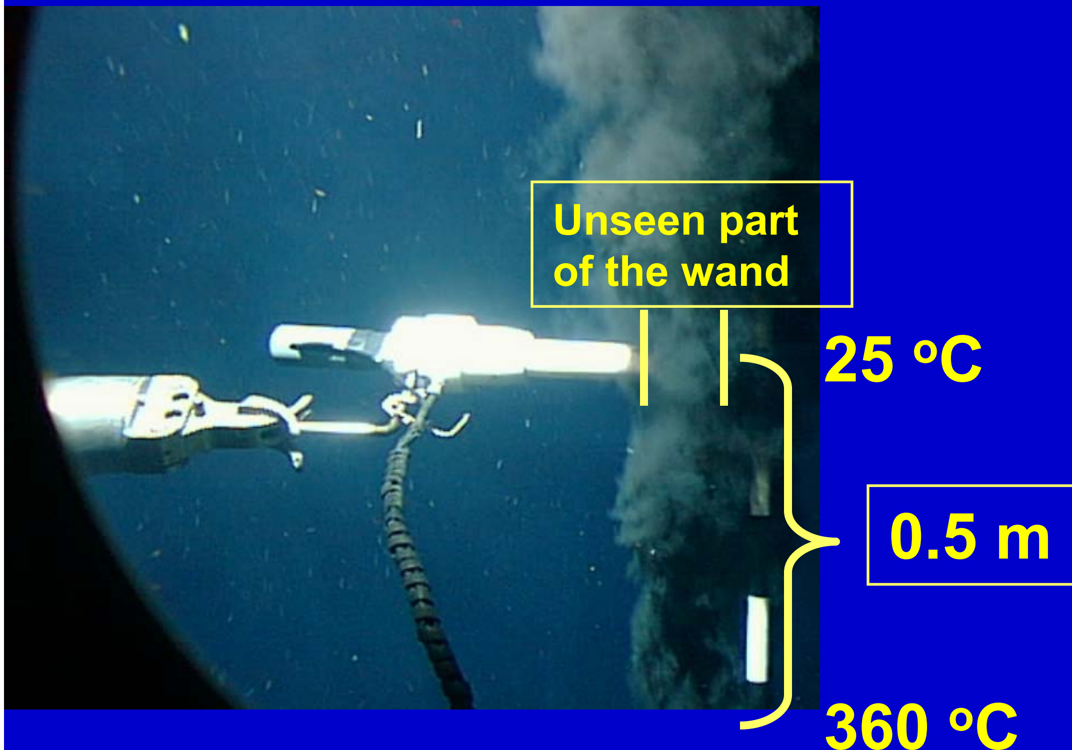
Apply *in situ* solid state electrodes to look for (micro)organisms that can benefit from this reaction or the products of this reaction

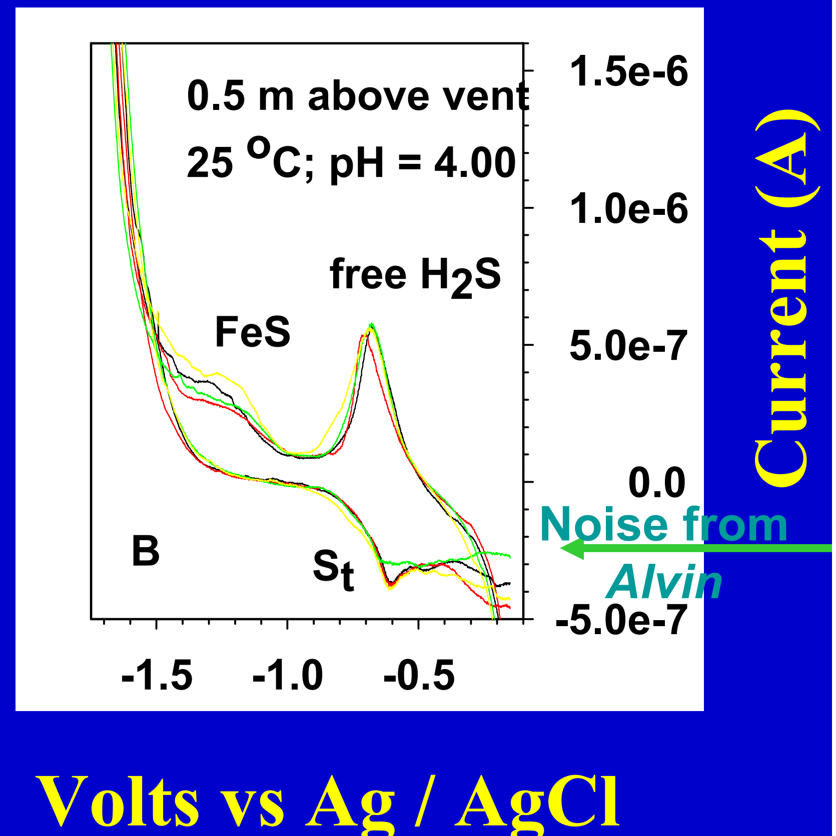
Apply *in situ* solid state electrodes to understand the chemical reason why organisms live in different ecological niches

General Block Diagram of IN SITU submersible Electrochemical Instrument



Black Smoker Voltammetry Speciation Data- 0.5 m above vent chimney

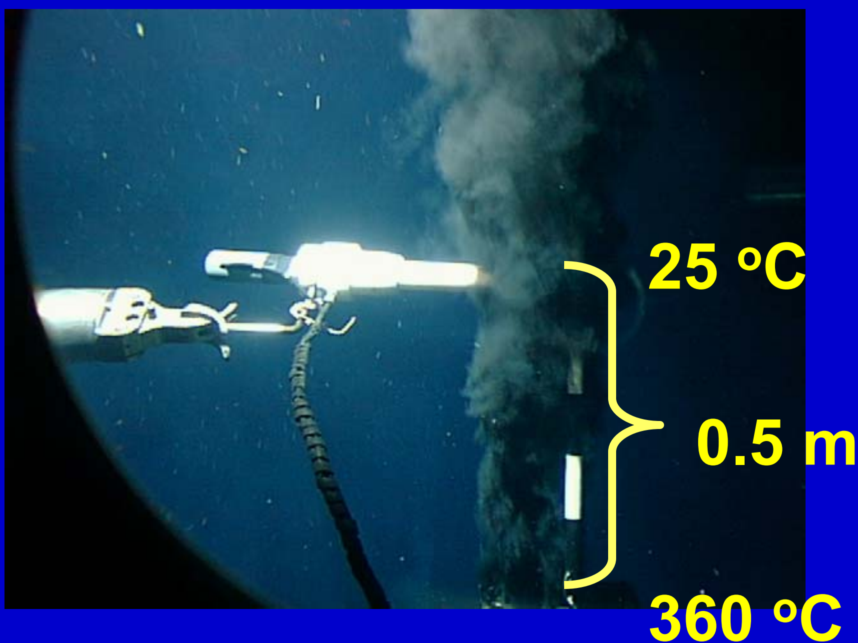


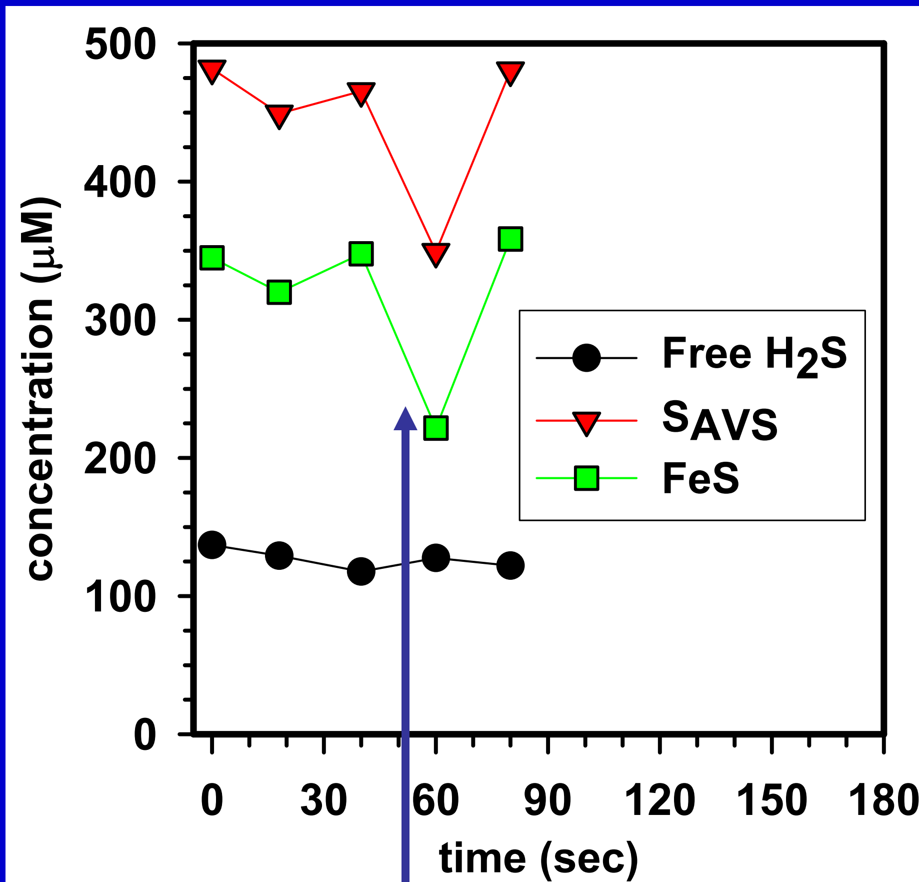


- Major signals for Free H_2S and FeS_{aq}
- O_2 not detected

$$S_t = S_{AVS} = FeS_{aq} + H_2S$$

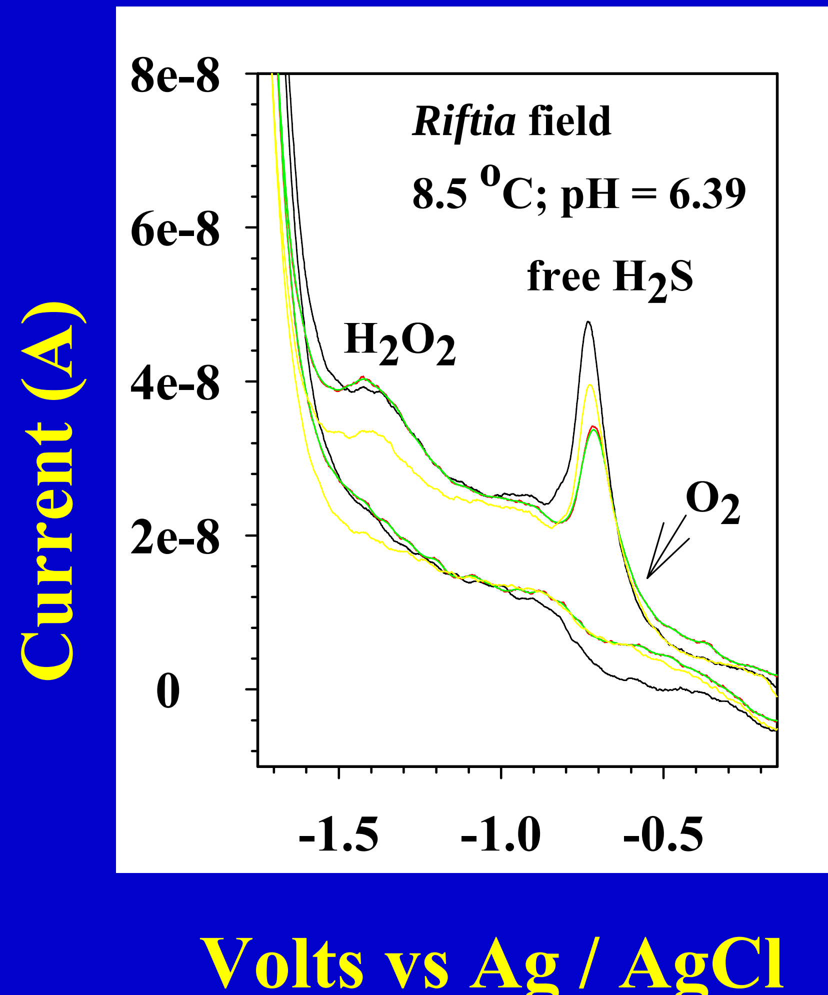
Sulfur chemistry 0.5 m above a Black Smoker





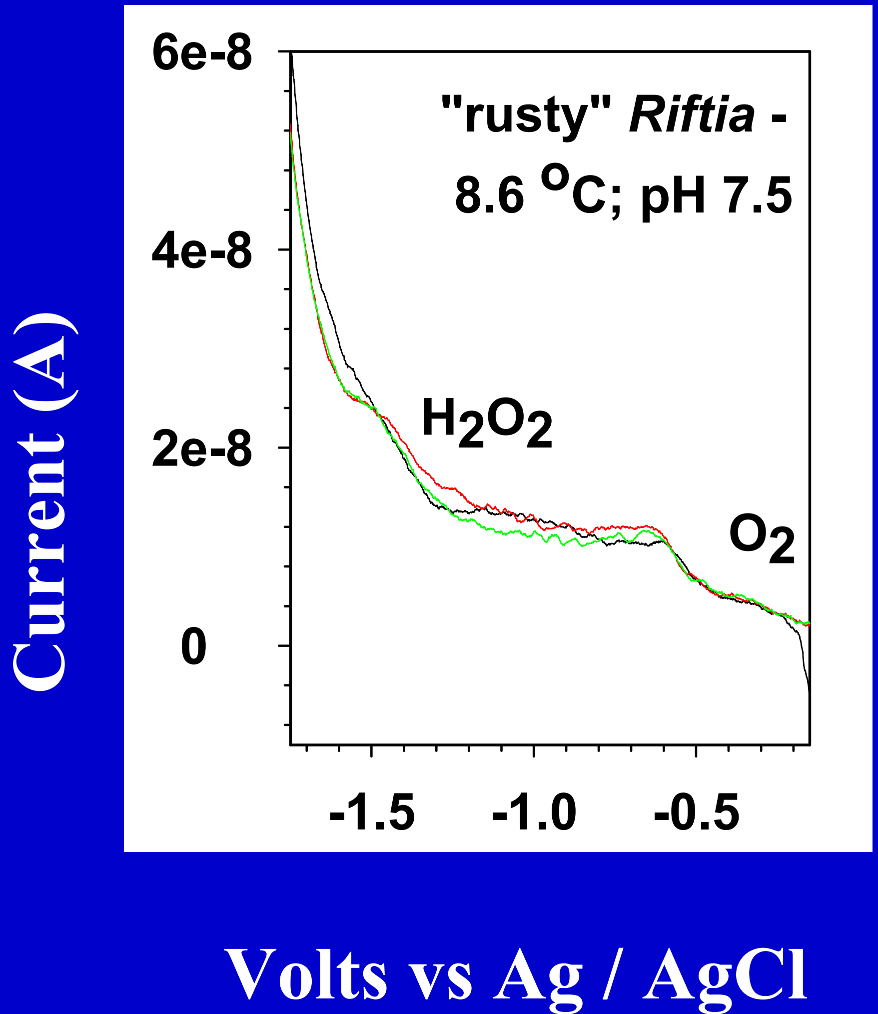
Electrical noise from *Alvin*

Near Plume of *Riftia*



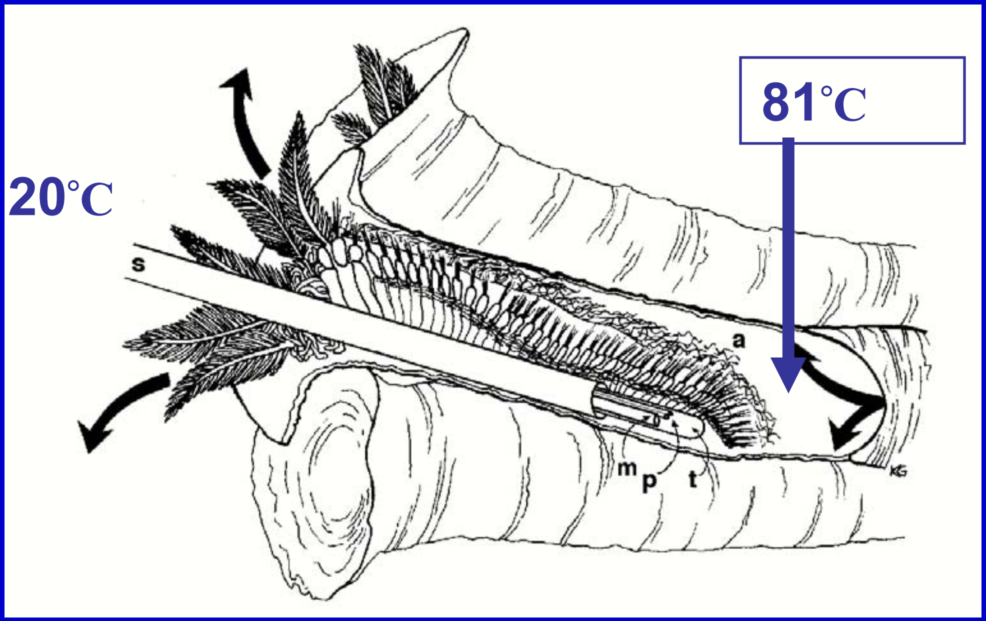
- H_2S/HS^- and O_2 only
- No FeS_{aq}
- polysulfides can be present
- chemoautotrophs require H_2S

"Rusty" Riftia

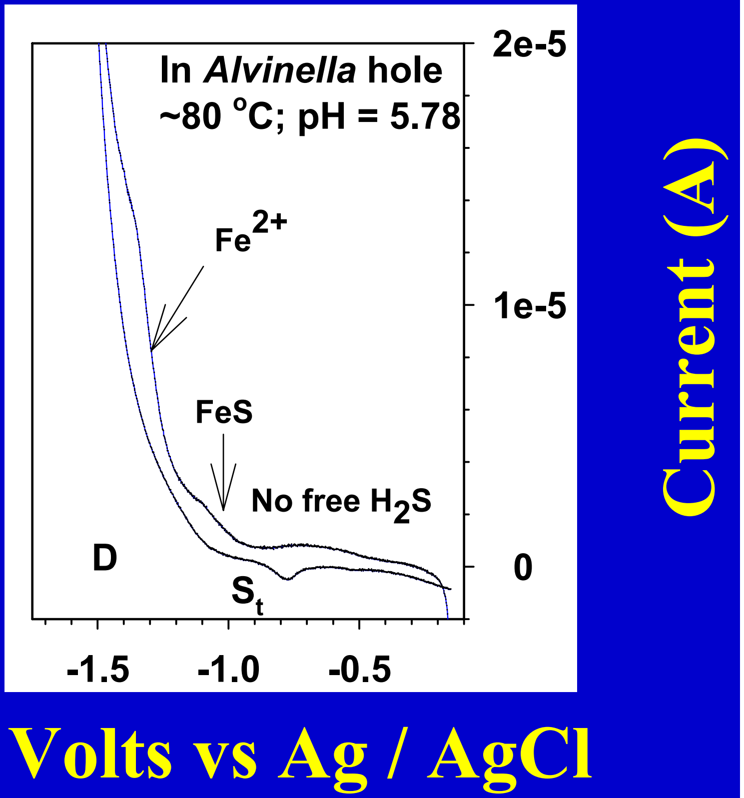


- Near ambient conditions
- O_2 only dominant signal
- Tubes encrusted with Fe (III)
- NO LIVING TUBEWORMS

Pompeii Worm Habitat Characterization

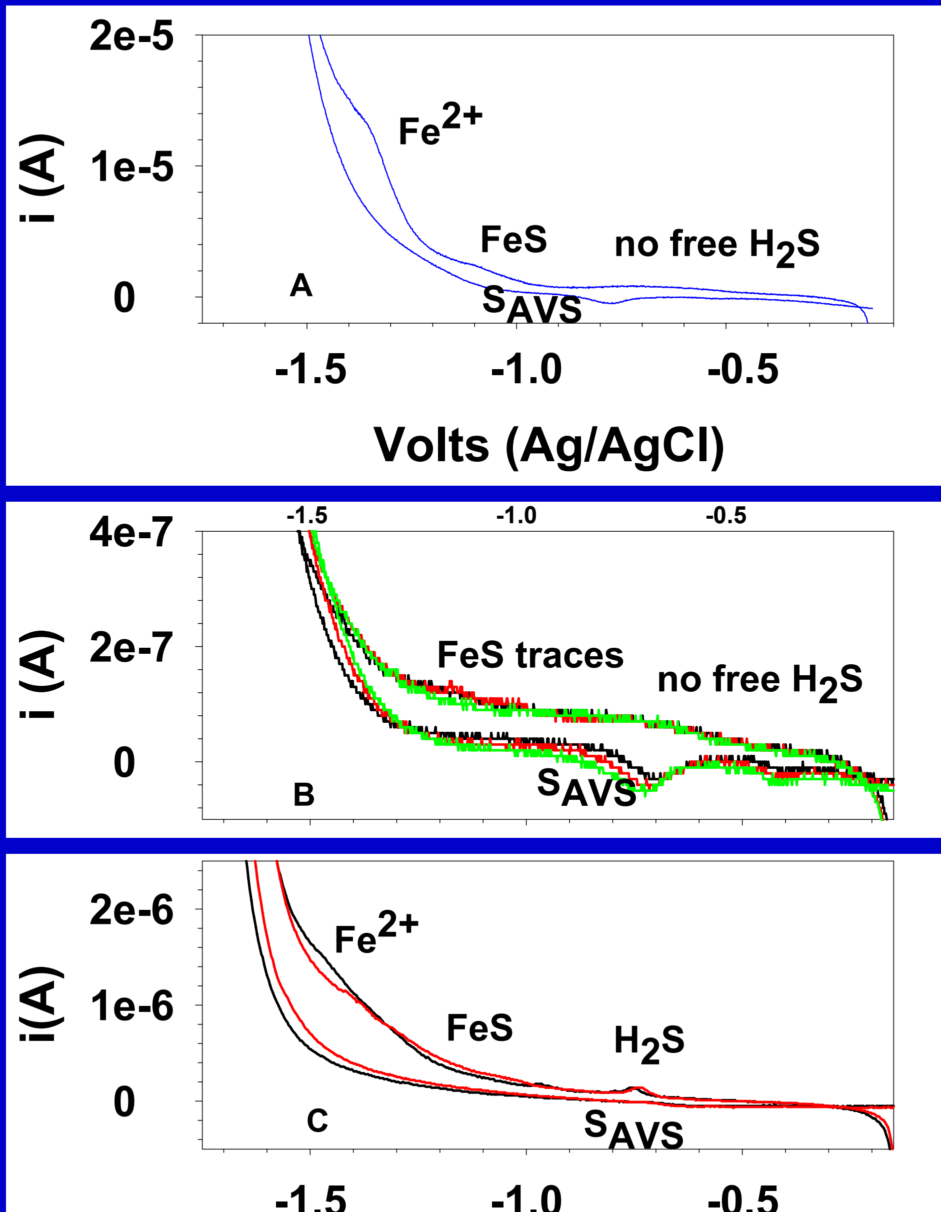


- Major signal due to FeS_{aq} + Fe^{2+}
- Free H_2S/HS^- was not detected
- O_2 not detected
- Epibionts not chemoautotrophic



Electrode indicates in what chemical environment life forms reside

Change in chemical speciation at hydrothermal vents



$H_2S + Fe^{2+} \leftrightarrow FeS_{aq} + 2H^+$

In *Alvinella* tube, 80 ± 20 °C - 250 atm

In flow cell; 2 °C 250 atm

Aboard ship lab 22 °C 1 atm

$FeS_{aq} + 2H^+ \leftrightarrow H_2S + Fe^{2+}$

Shift in equilibrium

LeChatelier's principle

Important Fe/S Chemistry

H_2S oxidation **pH > 6 (near *Riftia*)**

$$O_2 + Fe^{2+} \rightarrow Fe^{3+}$$

$$Fe^{3+} + H_2S \rightarrow Fe^{2+} + S(0)$$ as S_8 and S_x^{2-} ($S_2O_3^{2-}$ also)

Fe(III) ad Mn(III,IV) solid phases react with H_2S also

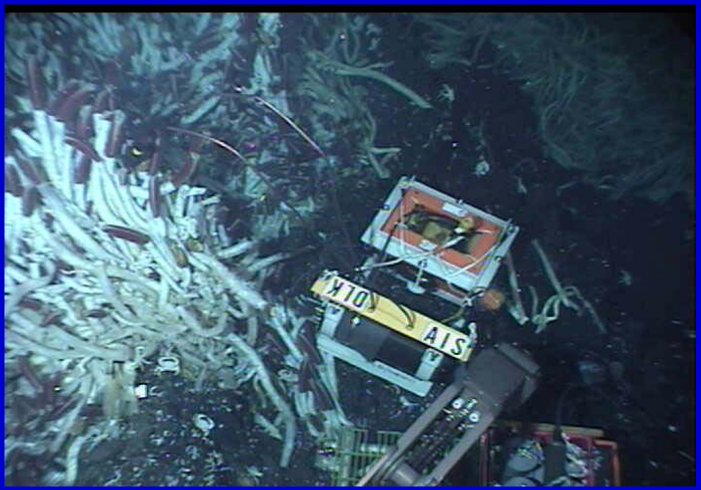
- ***FeS formation and dissociation*** **(near *Alvinella*)**

$$Fe^{2+} + H_2S \leftrightarrow FeS_{aq} + 2\,H^+$$

(FeS_{aq} formation is enhanced with increasing temperature; Rickard, 1997)

- ***Pyrite formation***

$$FeS_{aq} + H_2S \rightarrow FeS_2 + H_2$$



ISEA = *In Situ* Electrochemical Analyzer

The future is in situ sensors?

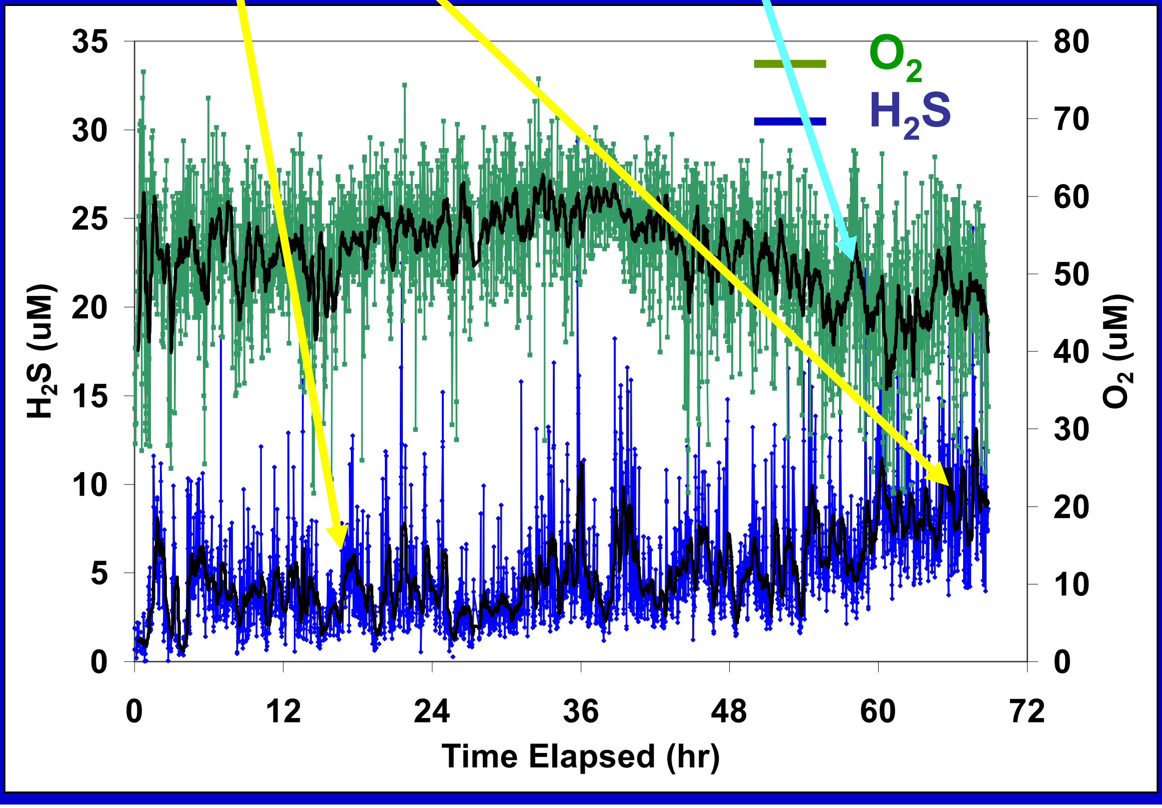
Moored Systems including Hydrothermal vent applications

This area was destroyed in 2006 by an undersea eruption

Riftia at TICA in 2003

O_2 and H_2S data generally anti-correlate but sometimes correlate above *Riftia*

H_2S varies 2 orders of magnitude as O_2 varies 50 %

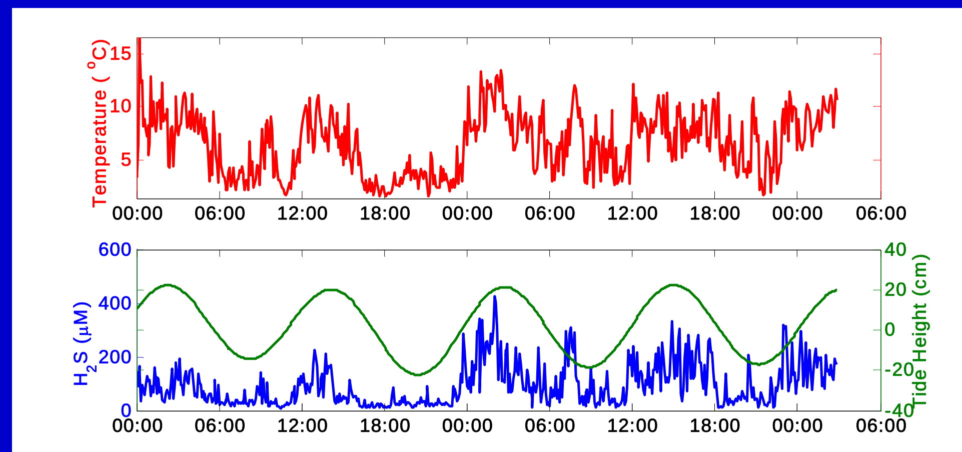


East Wall - 2005

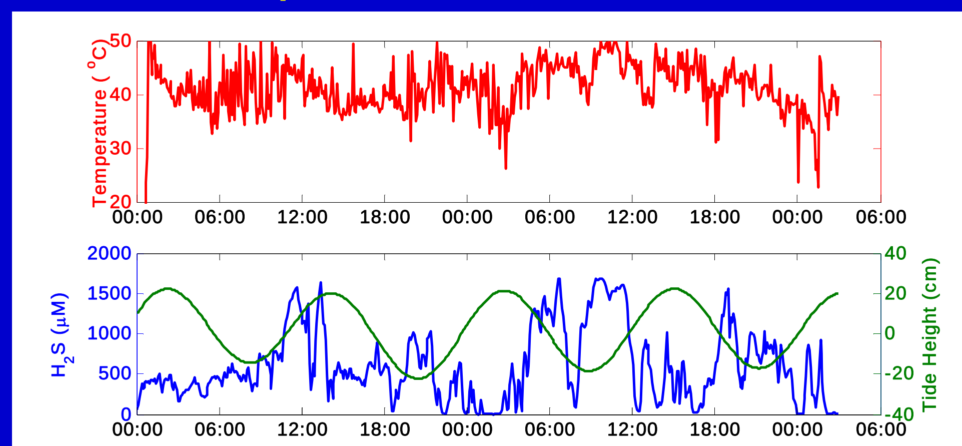
Moore et al, unpublished

4900 scans per electrode over 2.25 days

A) Mussels / *Riftia*

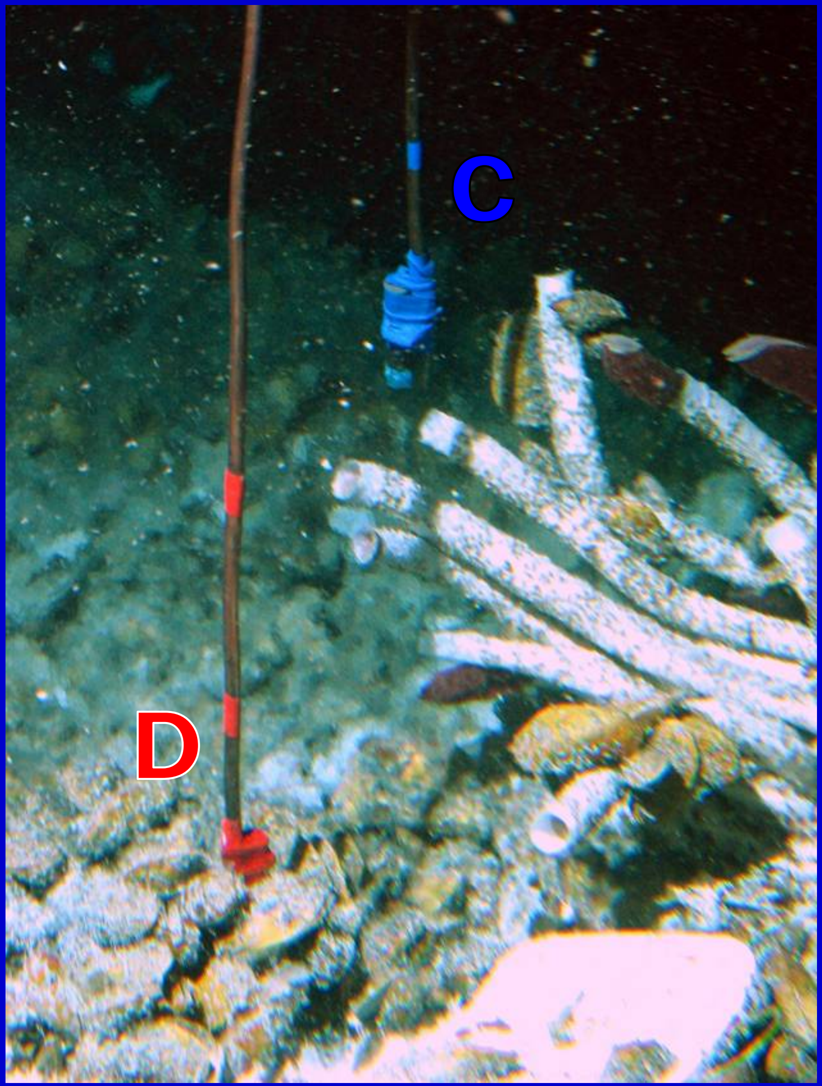


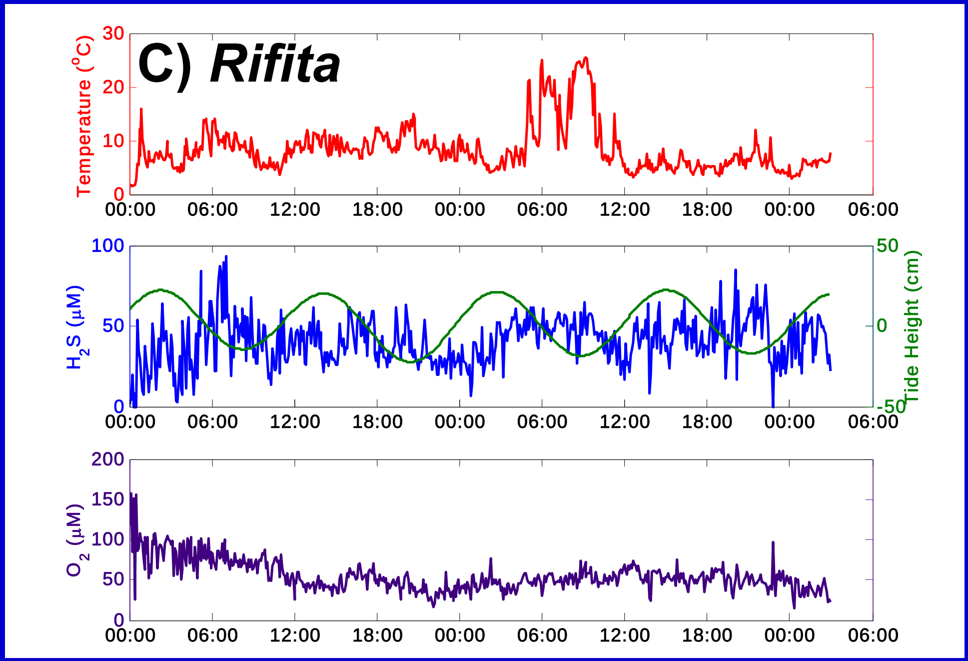
B) Source waters

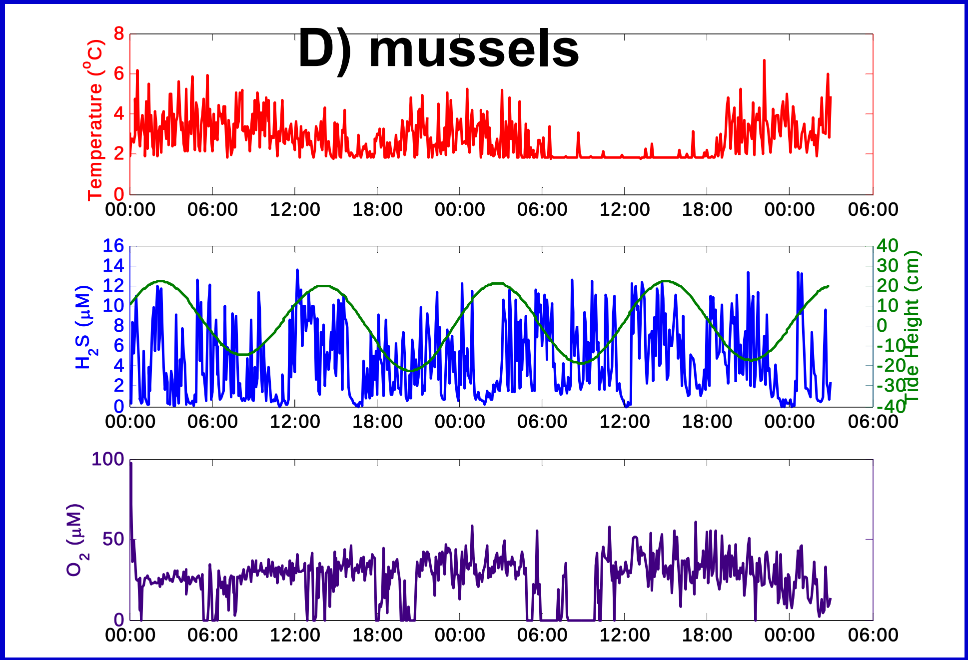


East Wall - 2005

4900 scans per electrode over 2.25 days







Continuous wavelet transforms (WT) of H_2S and cross-wavelet transforms (XWT) of H_2S and tide height for electrodes A and B. Hot colors indicate high wavelet power. Arrows on the XWT indicate phase relationships, arrows pointing to the right are in phase and left are out of phase. Both electrodes have a strong tidal signal (red band at a frequency of .5 on the WT's). Electrode A is in phase with the tides and B is out of phase.

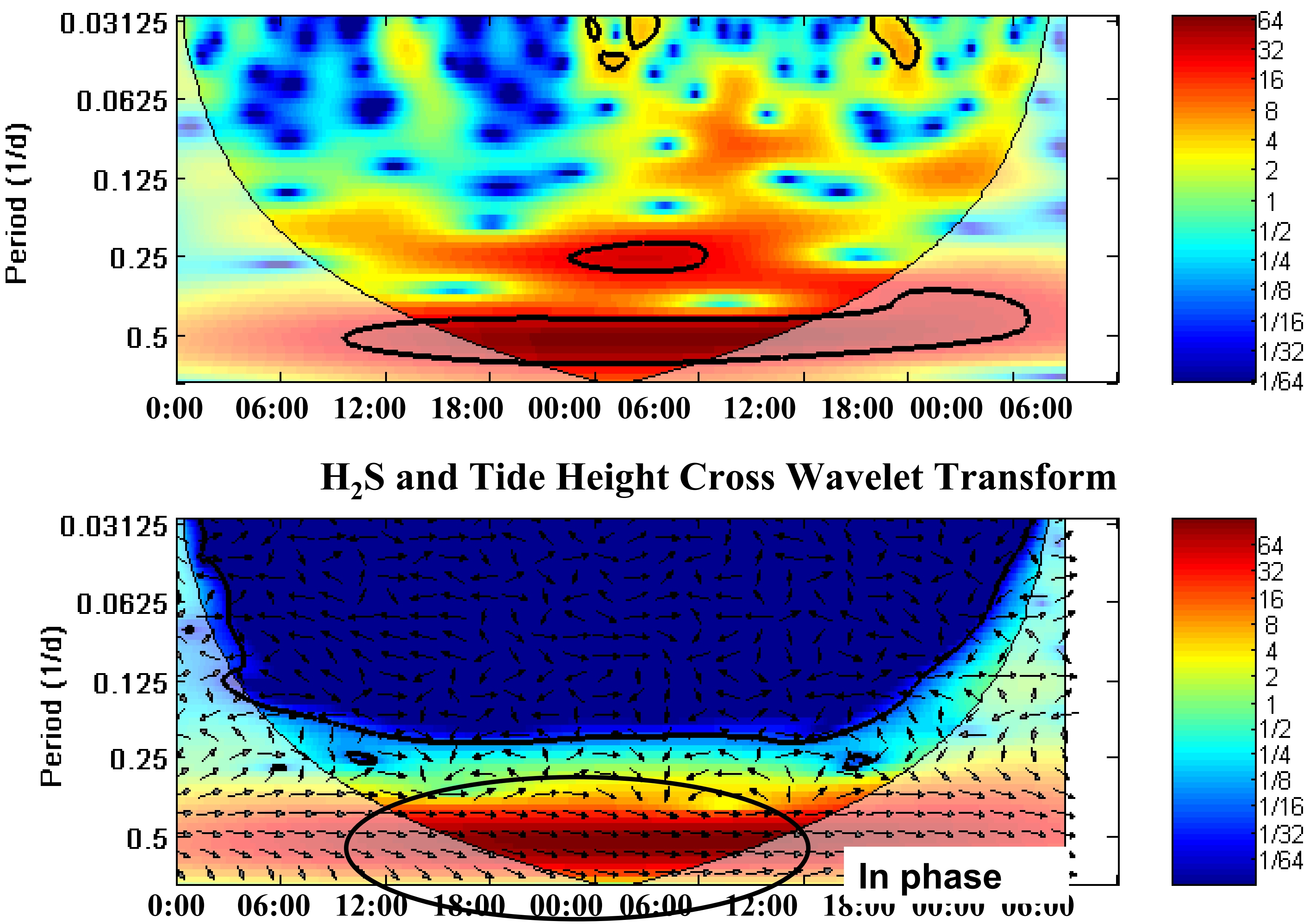
Wavelet Analysis

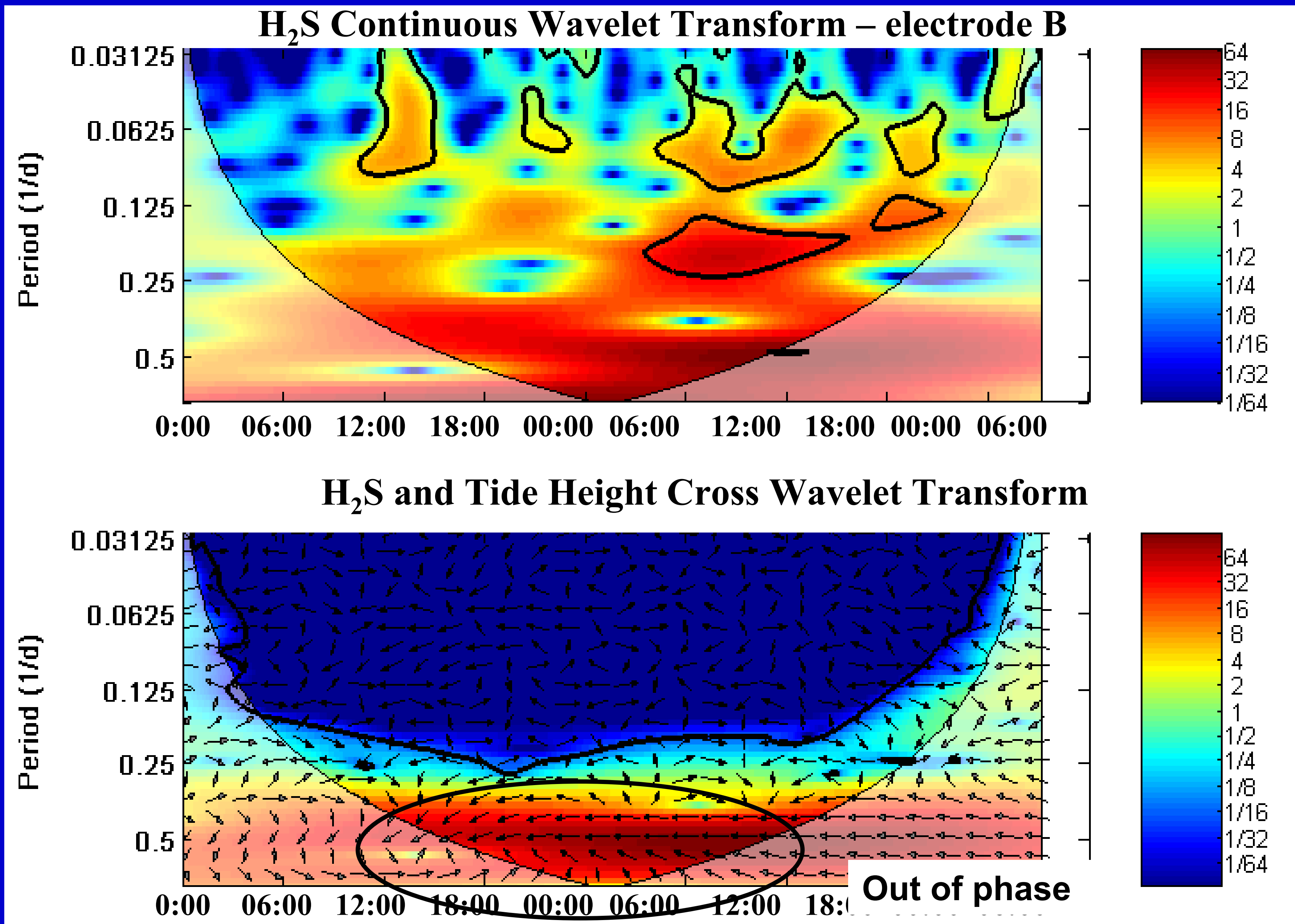
Continuous wavelet analysis, cross-wavelet and wavelet coherence analysis were conducted using Matlab code developed by Aslak Grinsted, and is available at:

http://www.pol.ac.uk/home/research/waveletcoherence/

Continuous wavelet analysis expands time-series data into frequency space. The process is similar to a Fourier transform, and is performed by applying scaleable waveforms to the data at each time-step. The cross-wavelet transform finds regions of high common power in the time series.

H_2S Continuous Wavelet Transform – Electrode A




H2S Continuous Wavelet Transform – electrode B
Period (1/d)
0.03125
0.0625
0.125
0.25
0.5
0:00 06:00 12:00 18:00 00:00 06:00 12:00 18:00 00:00 06:00
64 32 16 8 4 2 1 1/2 1/4 1/8 1/16 1/32 1/64
H2S and Tide Height Cross Wavelet Transform
Period (1/d)
0.03125
0.0625
0.125
0.25
0.5
0:00 06:00 12:00 18:00 00:00 06:00 12:00
64 32 16 8 4 2 1 1/2 1/4 1/8 1/16 1/32 1/64
Out of phase

Some conclusions on high data collection

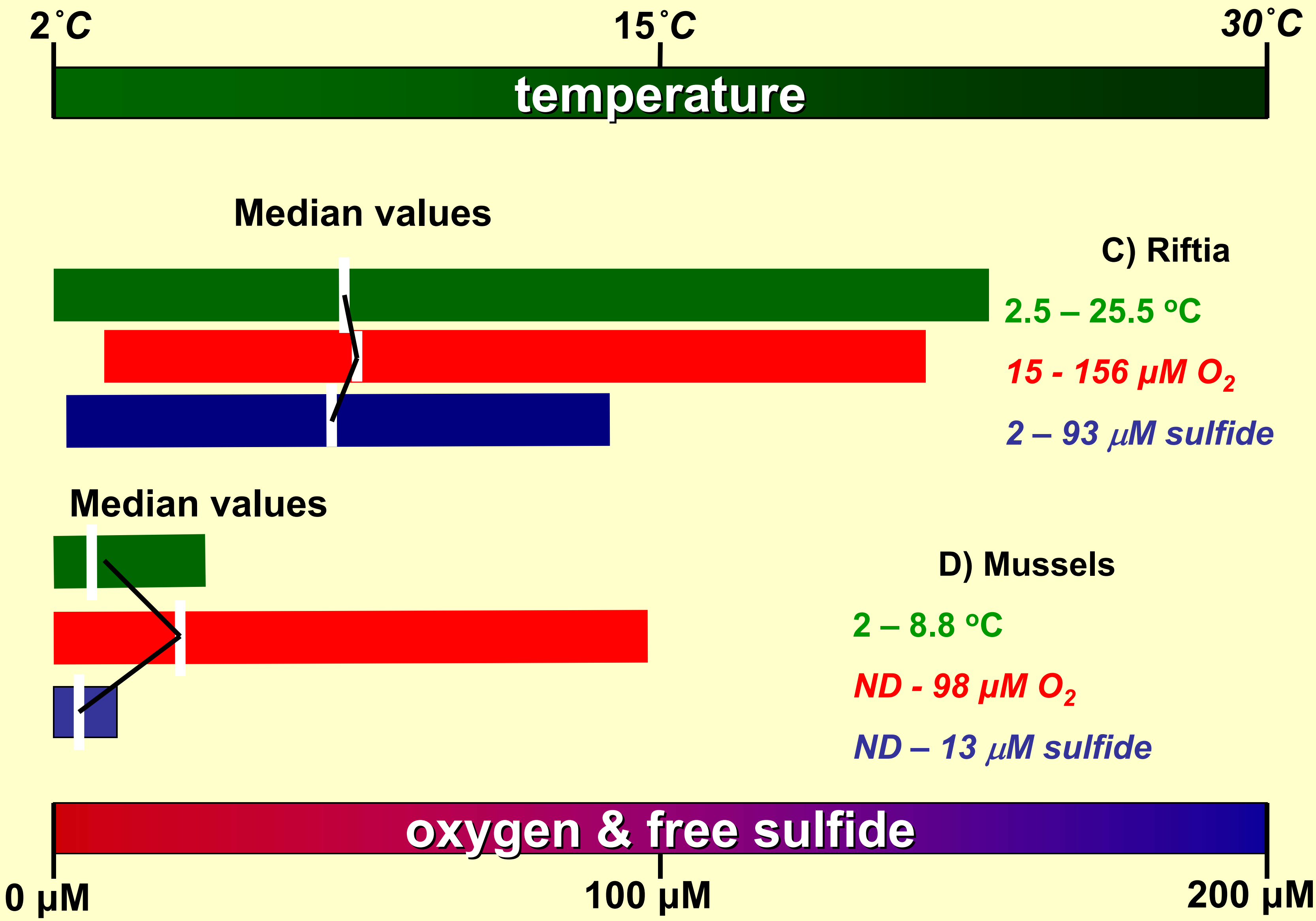
Electrodes A and B both vary with the tides -- A is in phase and B is out of phase. Since these electrodes are positioned within the same plume of shimmering water, the difference in phases may reflect a change in local currents on a tidal frequency.

Additionally, Tolstoy and Waldhauser have found that siesmicity increases at high tide, which could also be influencing diffuse flow chemistry.

Obviously complicated physical supply of chemicals to organisms

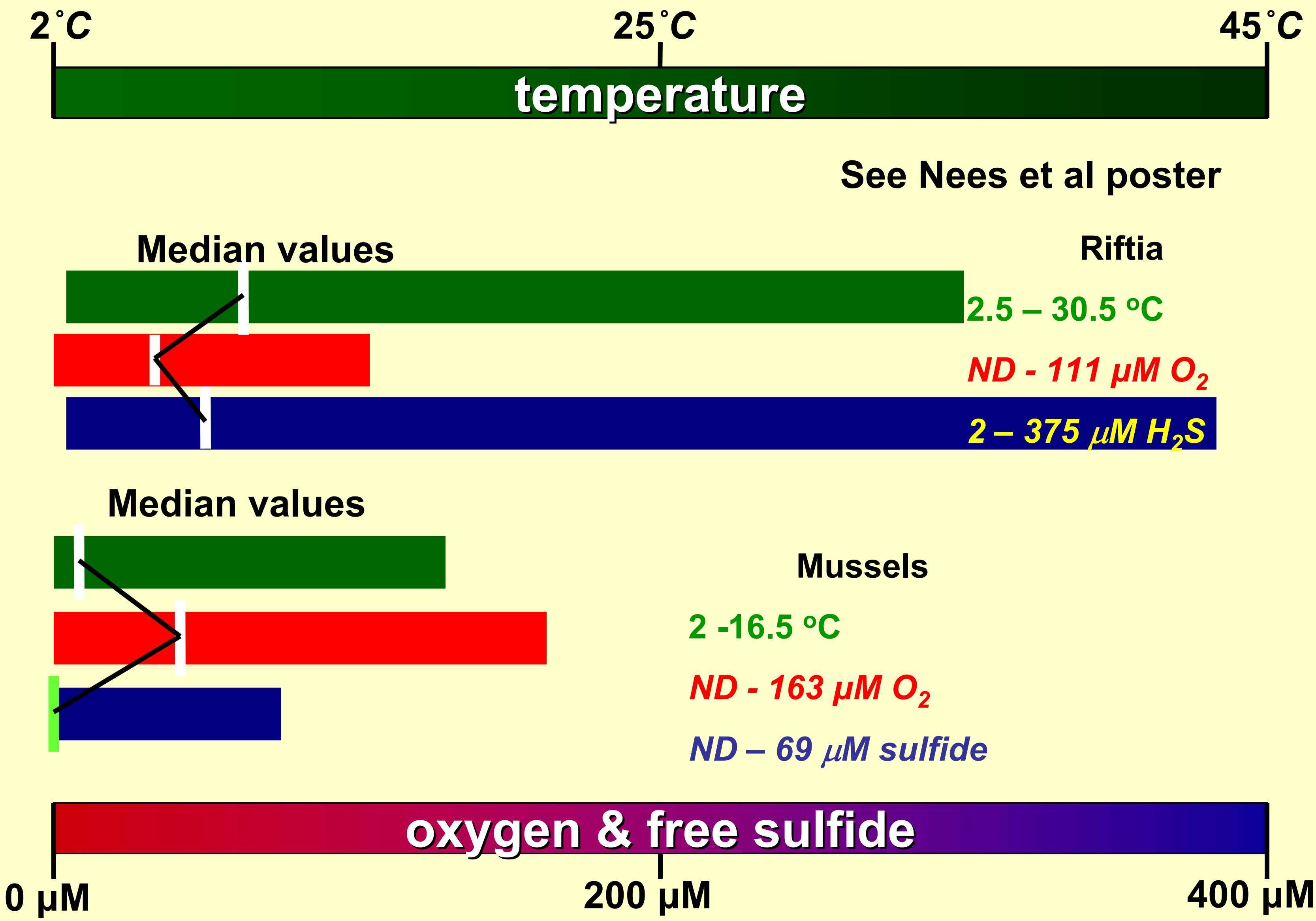
2005 – East Wall 9^0 50’ N East Pacific Rise

4900 scans; 5 scans once every 4 minutes taken at each organism/site

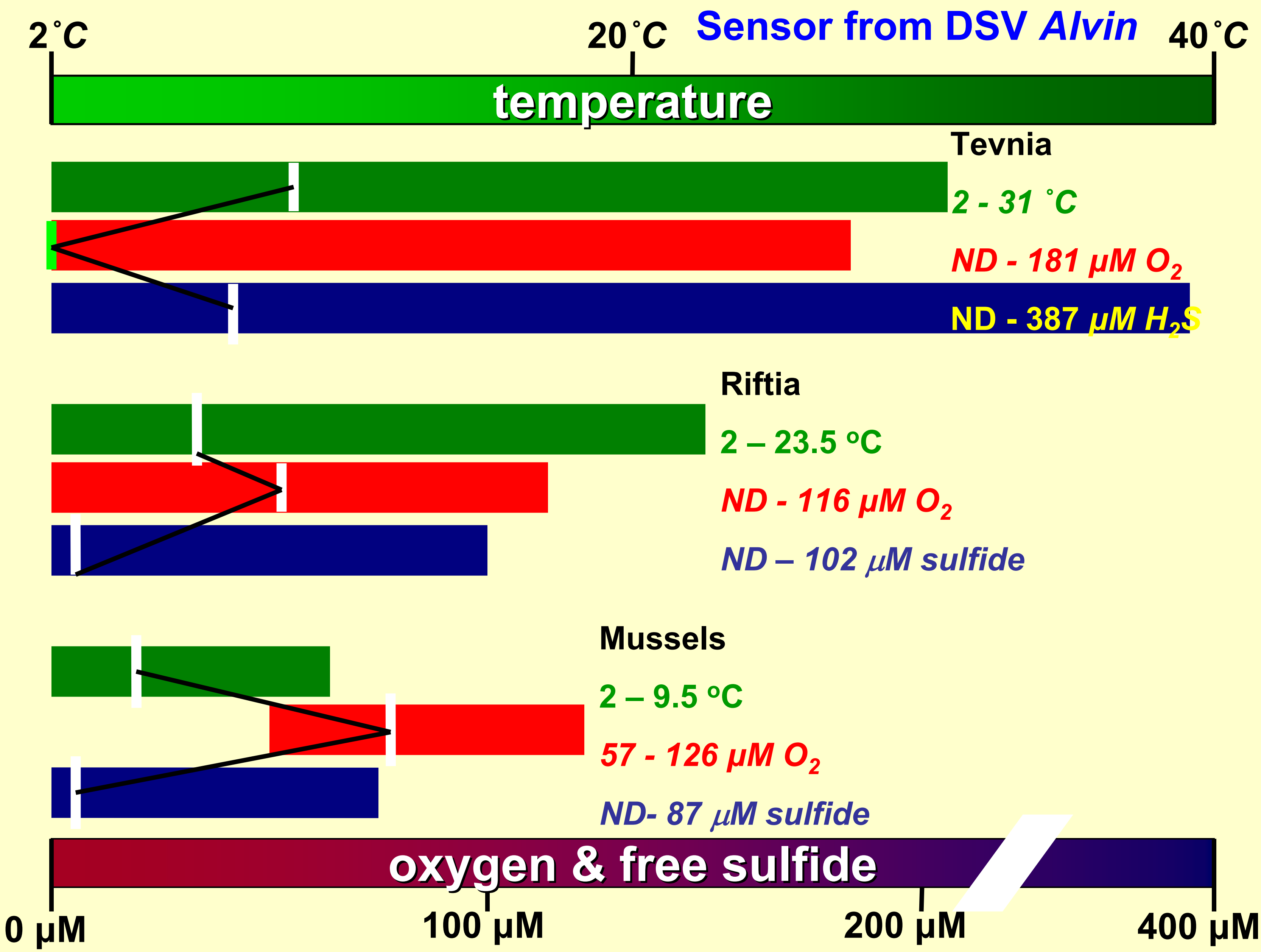


2004 - 9^0 50’ N East Pacific Rise (all data)

Sensor from DSV *Alvin*



2007 - 9° 50’ N East Pacific Rise (all data)



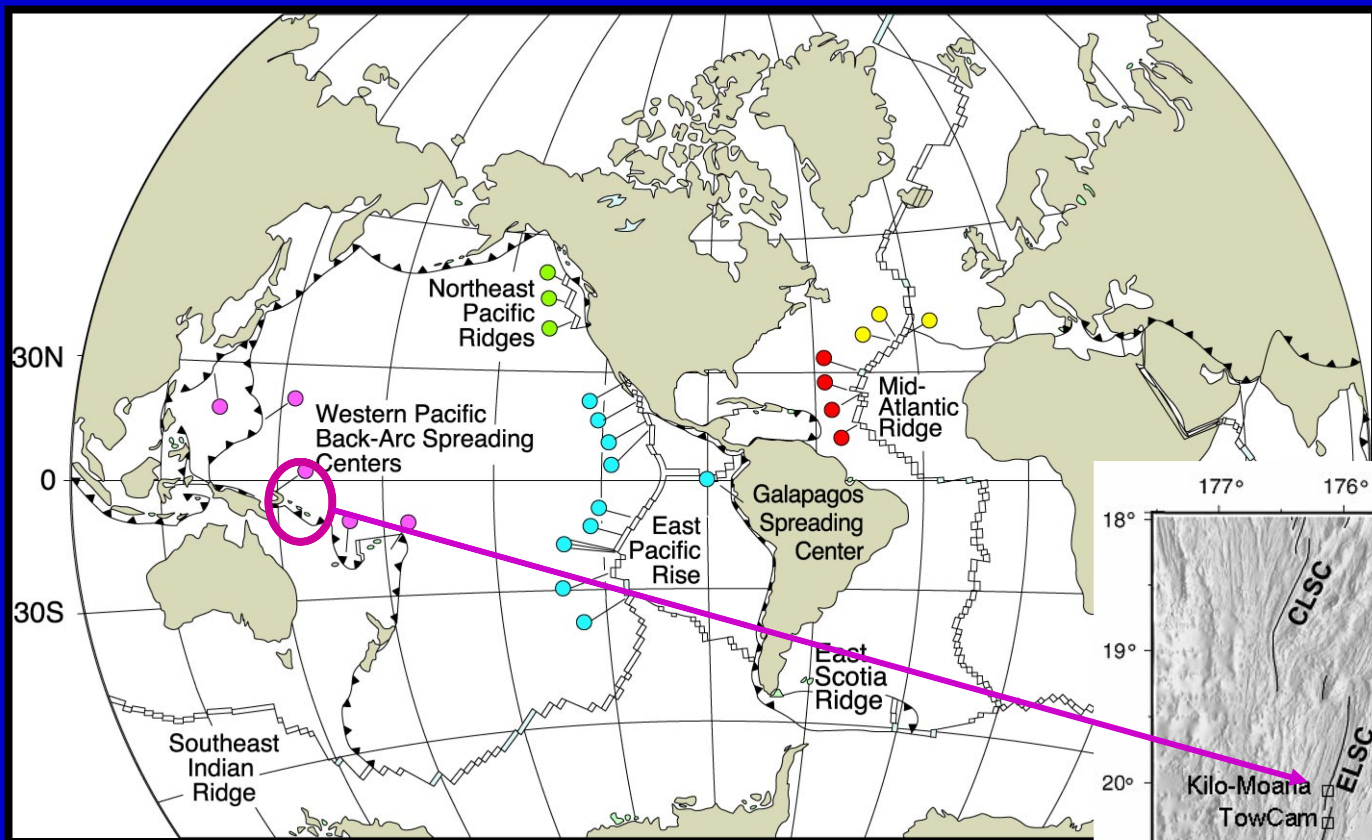
Amphipod swarm – what do they react to?

And how fast?

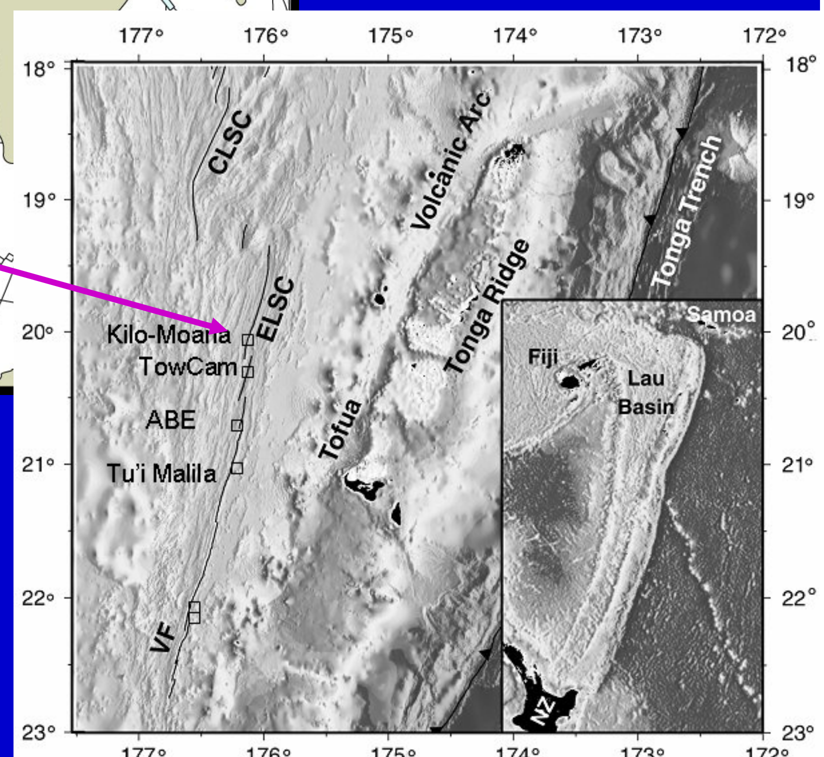
Sensor from DSV *Alvin*

Moore, Shank et al, unpublished

Distribution of hydrothermal vents



Lau Basin

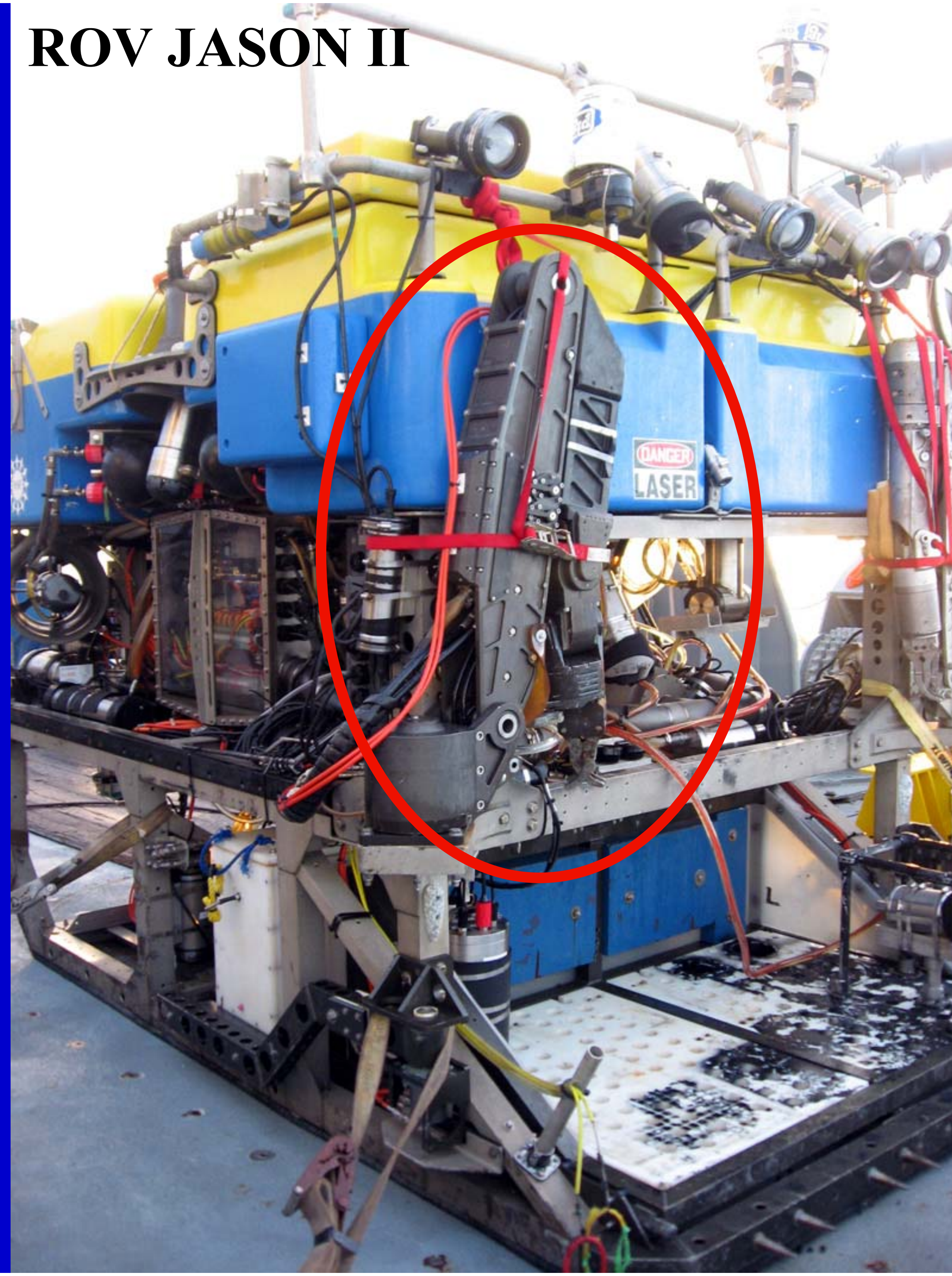


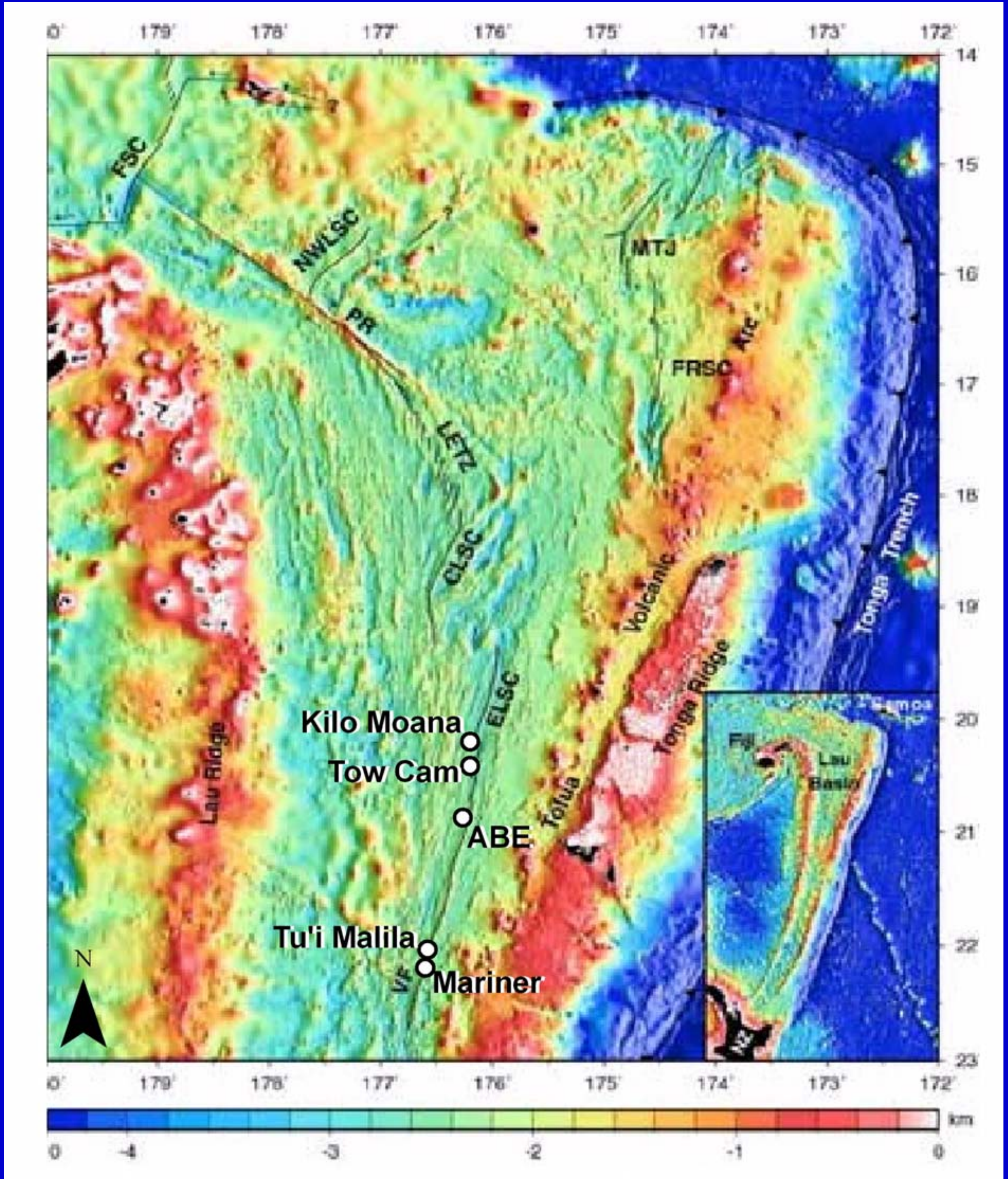
Data Collection

June 2005

- **Imagery:**
    - **JASON II**
- **Chemistry:**
    - ***in-situ* voltametric chemical analyzer**

ROV JASON II





Lau Basin

See Ma et al poster which will show significant Mn^{2+} and $Fe^{2+,3+}$ at Mariner

H_2S/T ratios N→S

5.5 (KM)

5 (TC)

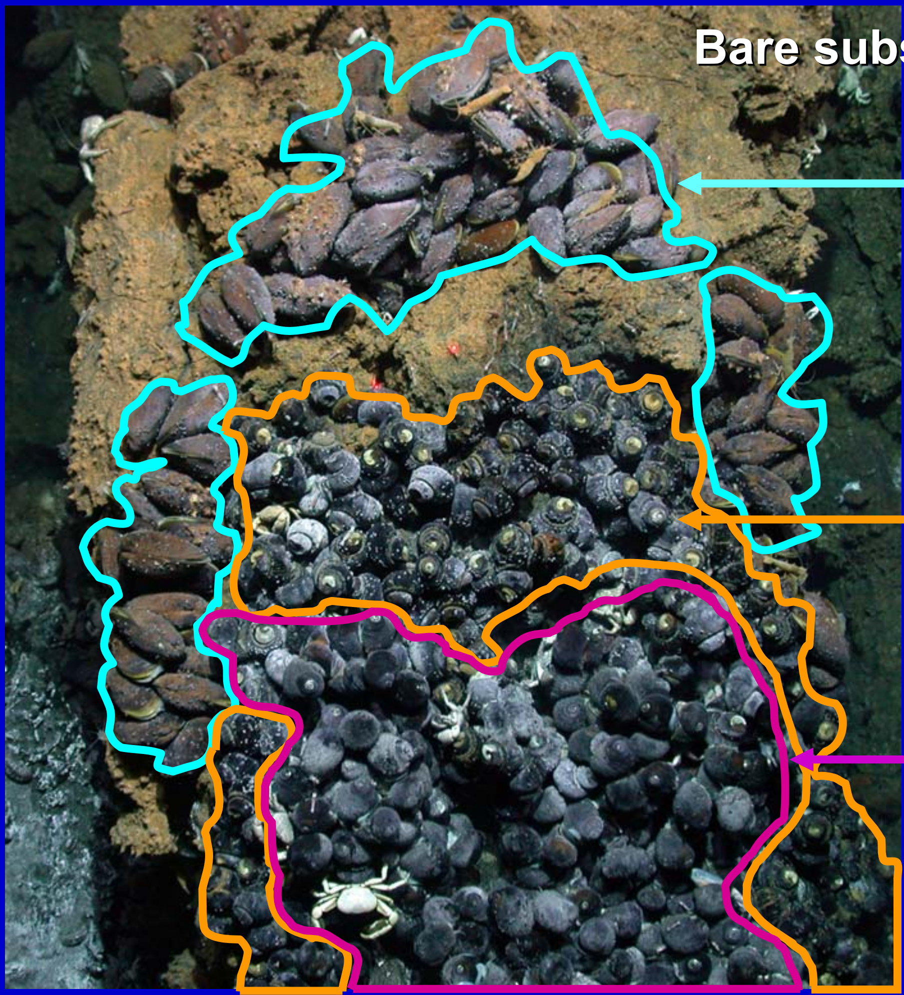
3 (Abe)

2 (Tu'i)

<1 (Mariner)

Kilo Moana *(Dive J2-235)*: Marker E

Less reduced



Mussels have symbionts and red blood (Fe hemoglobin)!

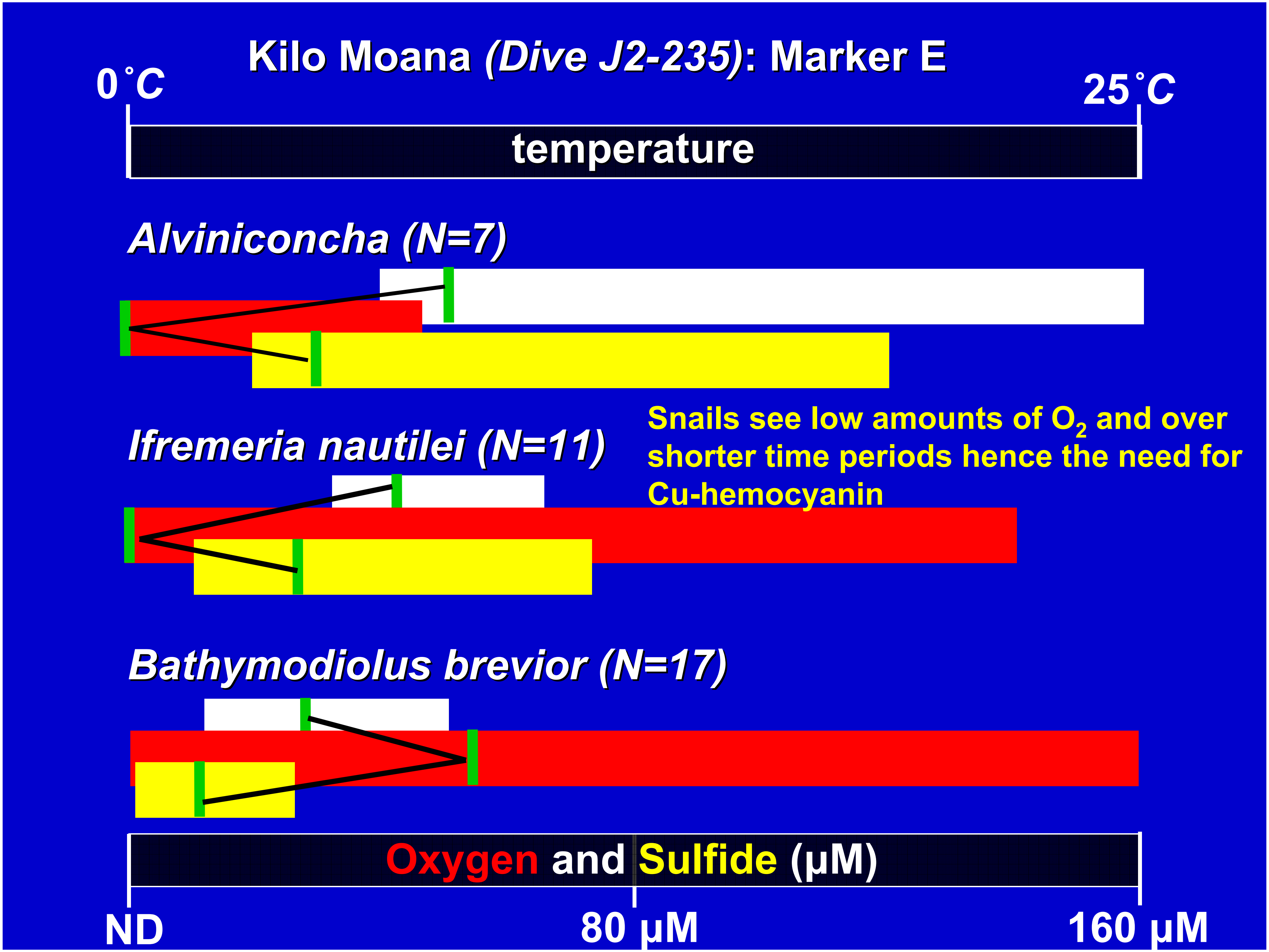
Mussels - *Bathymodiolus brevior*

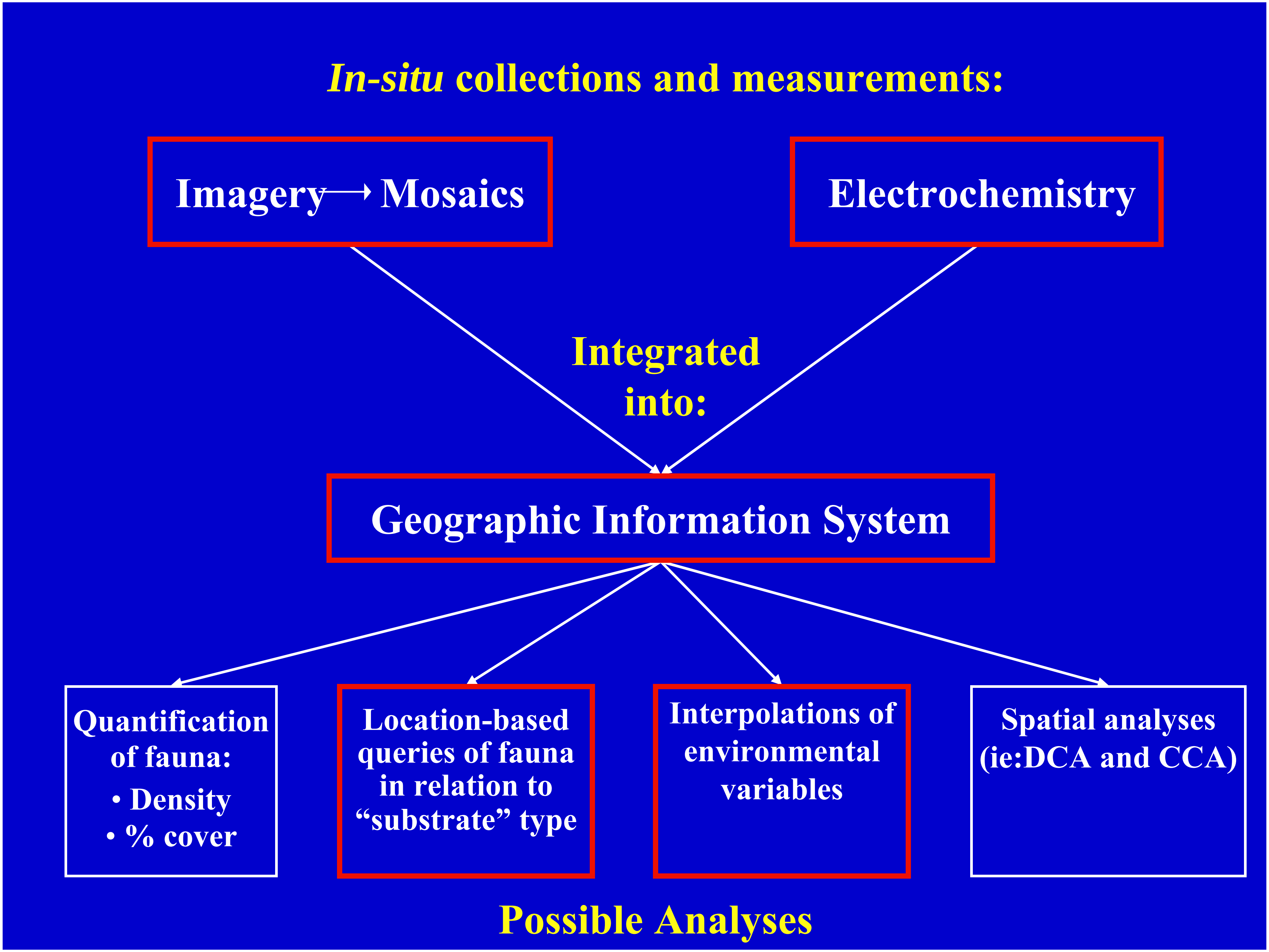
Snail "Ifremeria"

Snail "Alviniconcha"

Snails have symbionts and blue blood (Cu hemocyanin)!

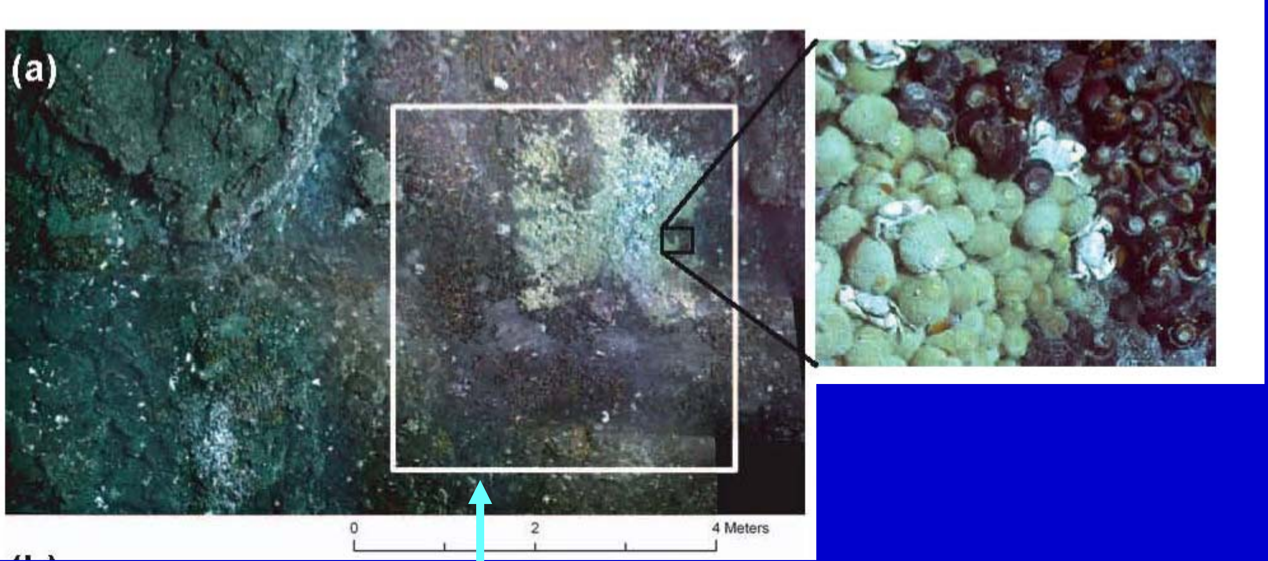
reduced

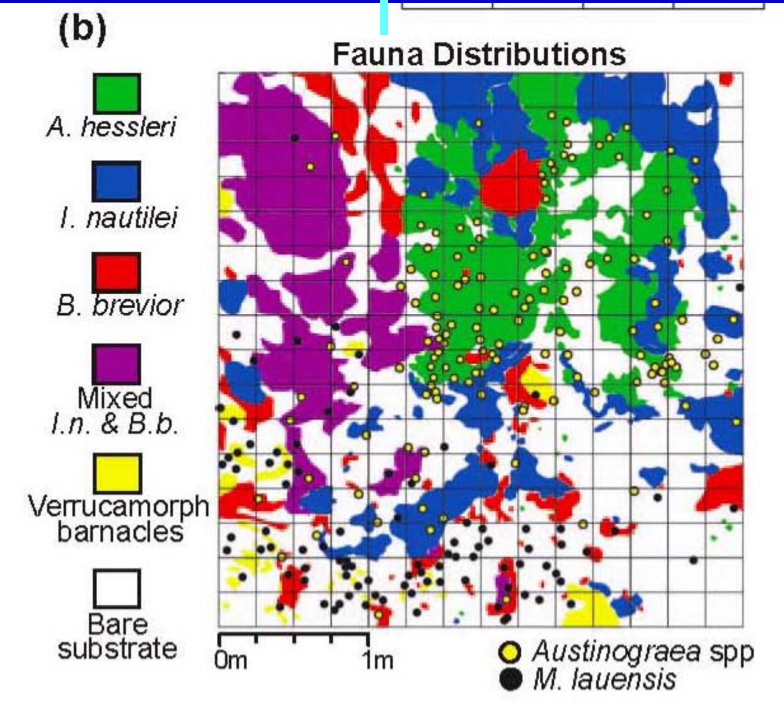

Kilo Moana (Dive J2-235): Marker E
0 °C
25 °C
temperature
Alviniconcha (N=7)
Snails see low amounts of O_2 and over shorter time periods hence the need for Cu-hemocyanin
Ifremeria nautilei (N=11)
Bathymodiolus brevior (N=17)
Oxygen and Sulfide (µM)
ND
80 µM
160 µM

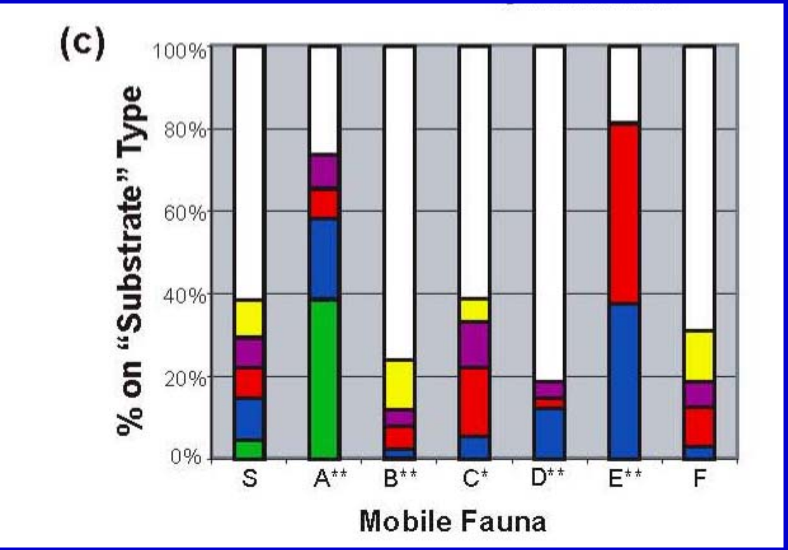

In-situ collections and measurements:
Imagery → Mosaics
Electrochemistry
Integrated into:
Geographic Information System
Quantification of fauna:
• Density
• % cover
Location-based queries of fauna in relation to “substrate” type
Interpolations of environmental variables
Spatial analyses (ie:DCA and CCA)
Possible Analyses

Mosaics from ABE1 in 2005

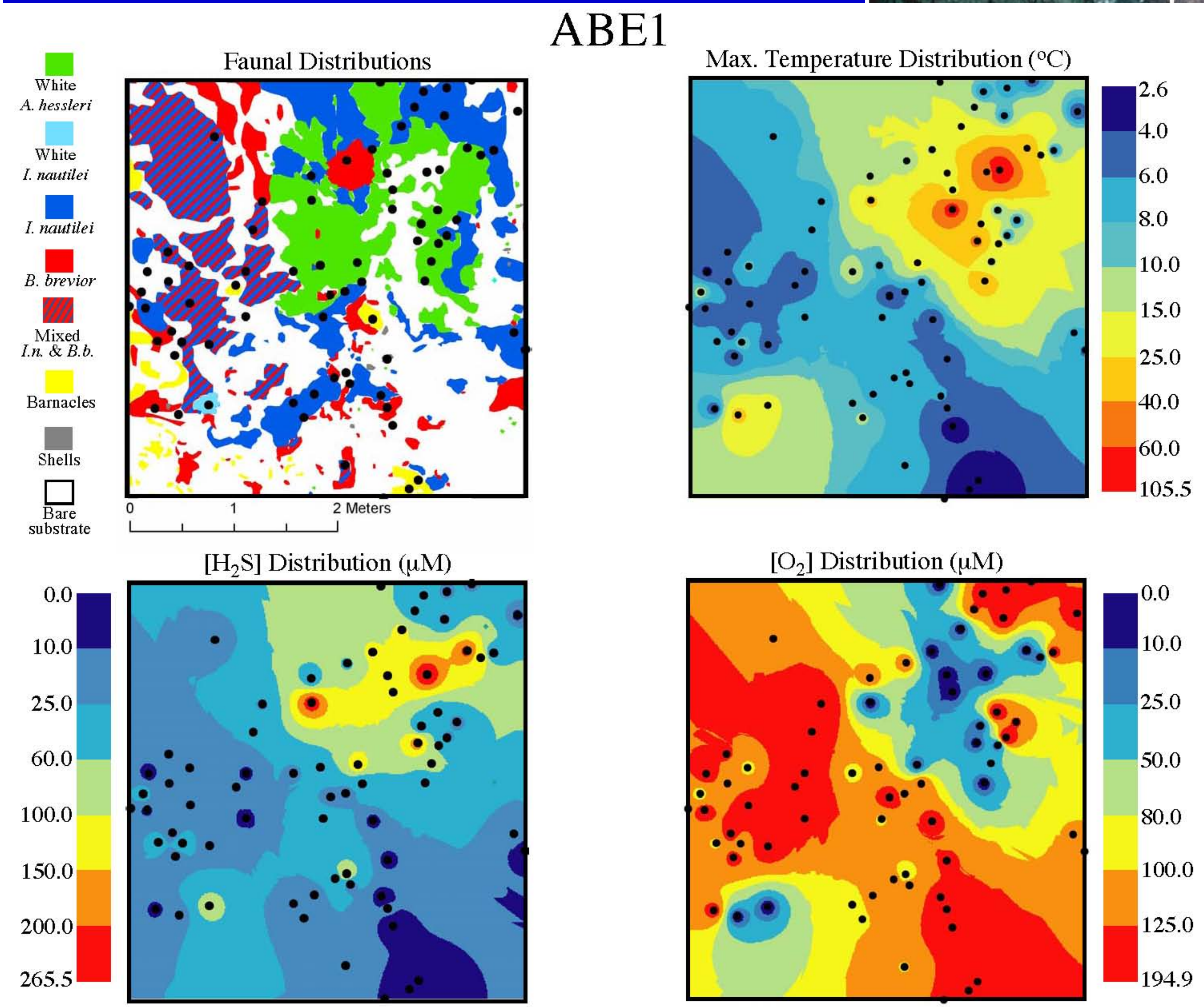
See Podowski and Becker posters

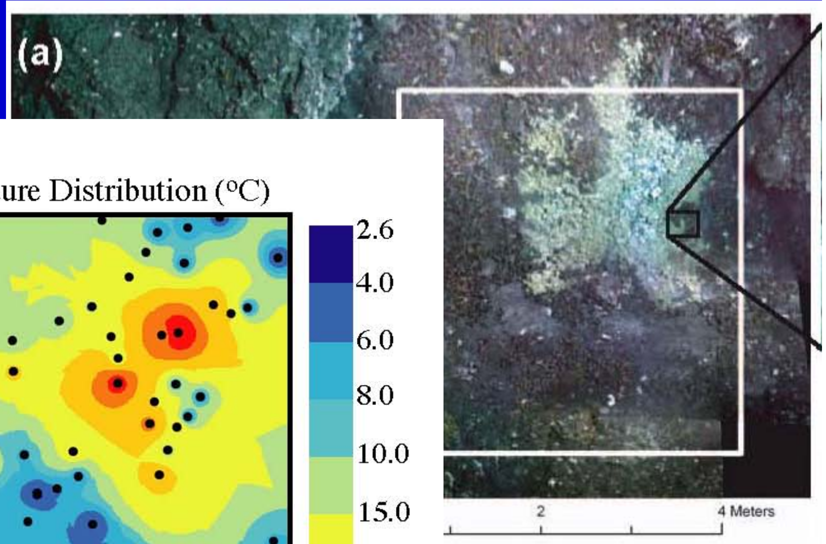






Mosaics from ABE1 in 2005





Additional Redox Indicators

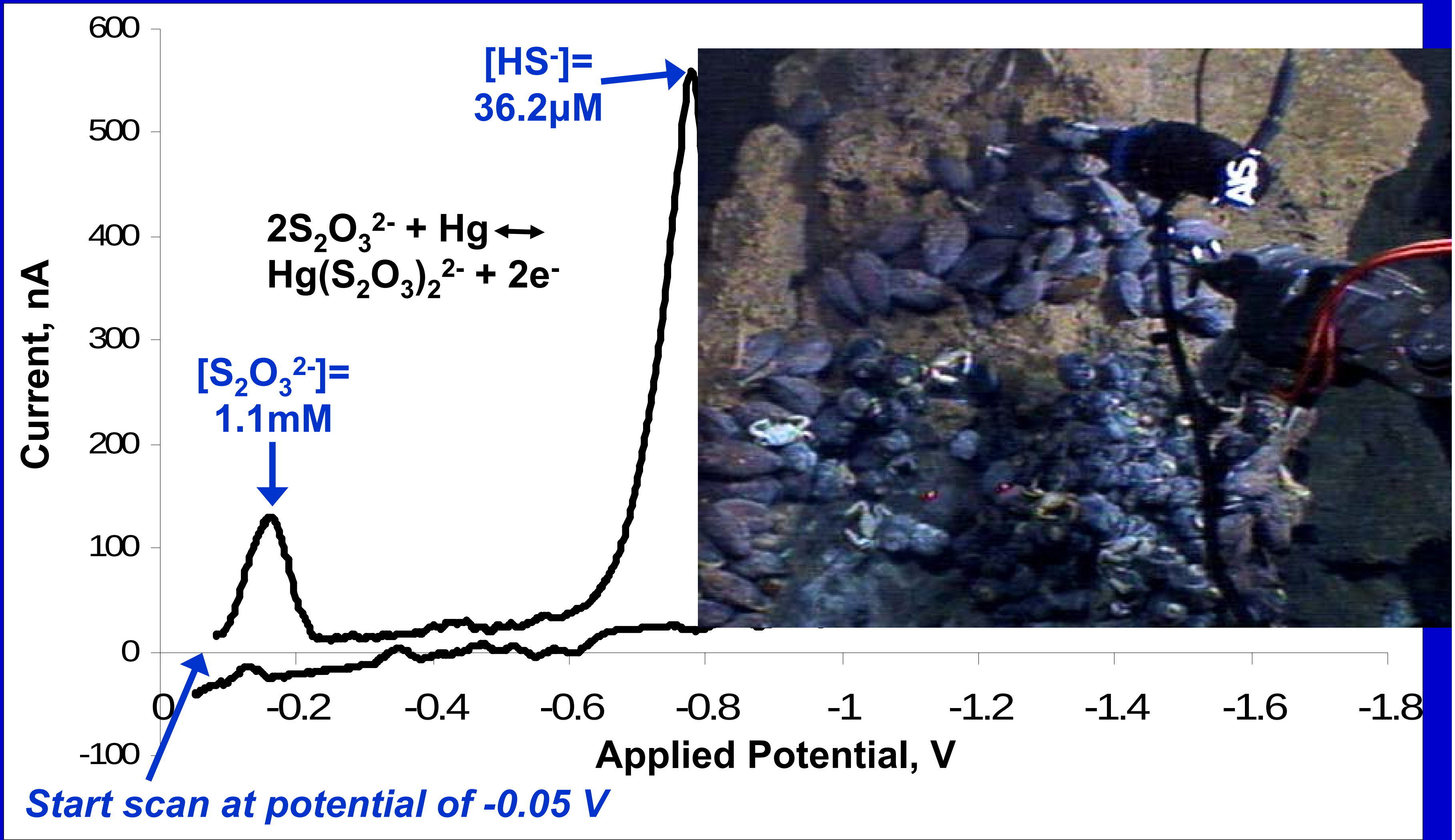
See Mullaugh et al poster

- Measuring H_2S and O_2 only reflects two extremes between reduced and oxidized conditions
- Additional (sulfur) species can be used to characterize intermediate redox environments

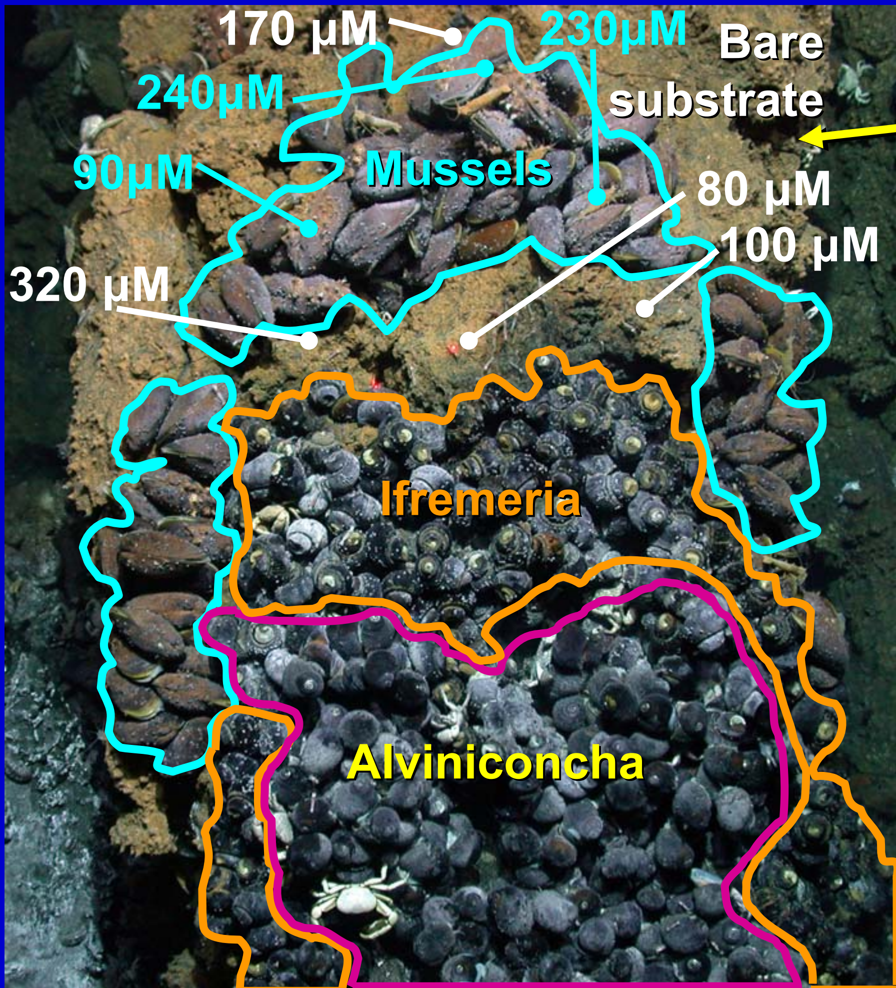
Vent (source)						*Ambient seawater*
S species:	H_2S FeS_{aq}	S_x^{2-}	S_8	$S_2O_3^{2-}$	SO_3^{2-}	SO_4^{2-}
S ox. state:	*(-II)*		*(0)*	*(+II)**	*(+IV)*	*(+VI)*

reduced ← · — · — · — · → **oxidized**

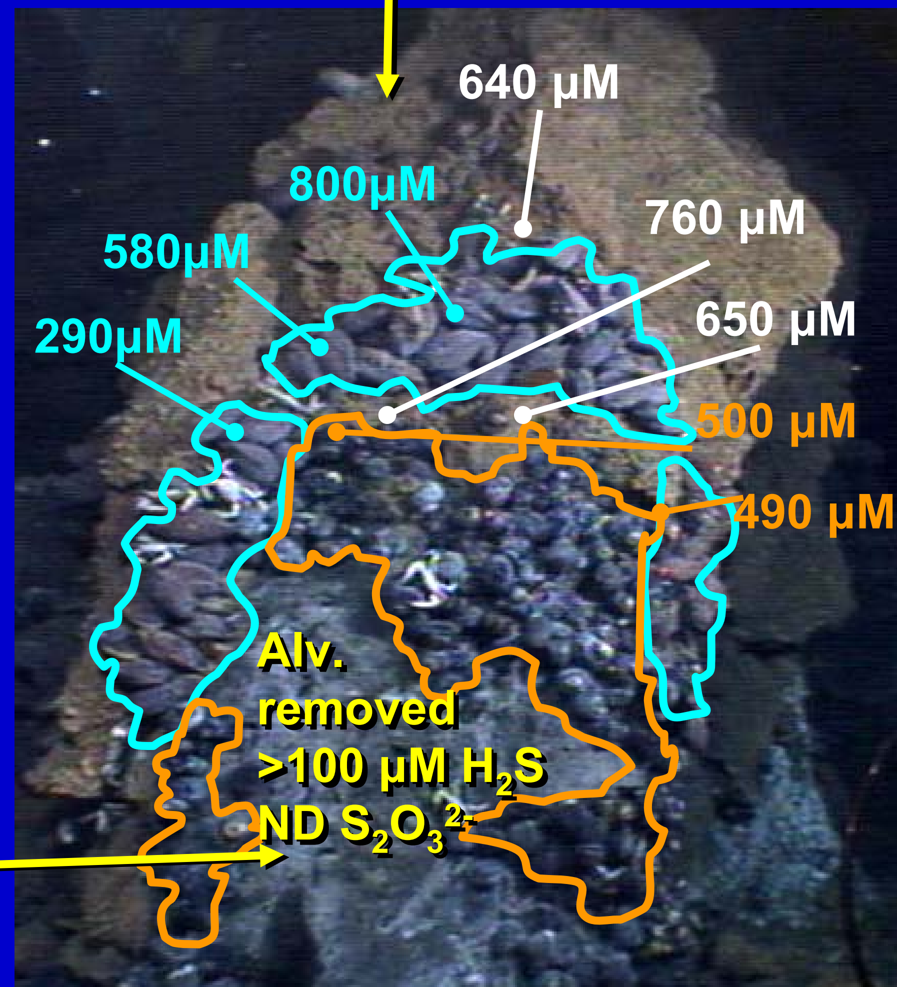
Kilo Moana: Marker E



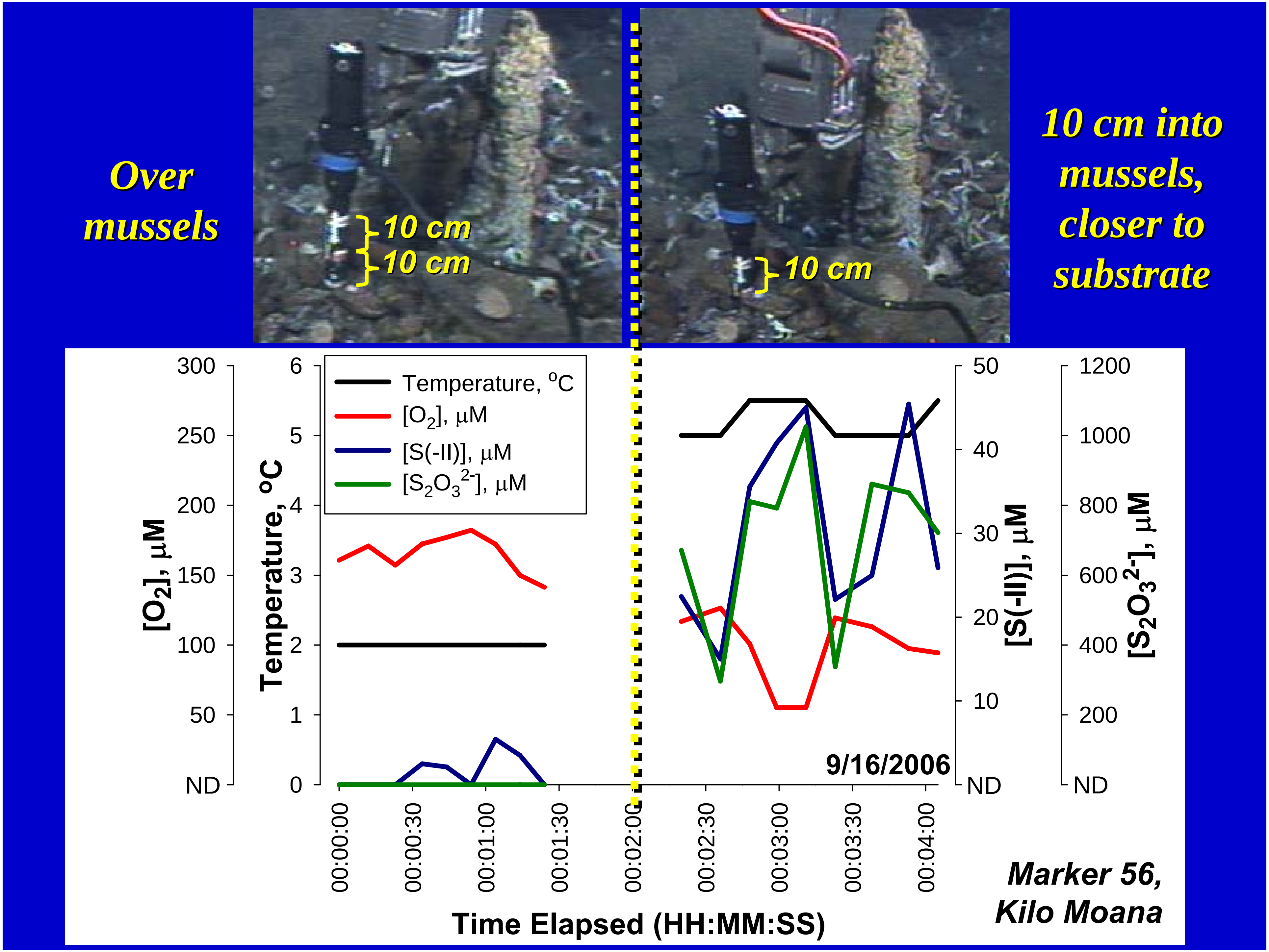
Kilo Moana, Marker E: Thiosulfate



“rusty” (oxidized) substrate



gray (reduced) substrate


Over mussels
10 cm
10 cm
10 cm
10 cm into mussels, closer to substrate
Temperature, °C
[O2], µM
[S(-II)], µM
[S2O3 2-], µM
[O2], µM
Temperature, °C
[S(-II)], µM
[S2O3 2-], µM
9/16/2006
Time Elapsed (HH:MM:SS)
Marker 56,
Kilo Moana

$S_2O_3^{2-}$ can be formed by abiotic and biotic pathways

Incomplete abiotic oxidation by Fe(III) amd Mn(III,IV) minerals present in the substrate

$$Fe(III) / Mn(III,IV) + H_2S \rightarrow S_x^{2-} + S_8 \rightarrow S_2O_3^{2-}$$

Incomplete biotic oxidation during chemosynthesis

$$H_2S \rightarrow S_x^{2-} + S_8 \rightarrow S_2O_3^{2-}$$

At some times increased amounts of $S_2O_3^{2-}$ over the H_2S coming from the diffuse flow source are likely due to active pumping or excretion of this H_2S oxidation byproduct

Conclusions

Voltammetry is an excellent *in situ* tool to study redox species and kinetics in real time; removal experiments show that H_2S is higher after removal indicating its consumption

Organisms can respond on a variety of time scales starting from seconds (based on cultures / mats; amphipods) to ?

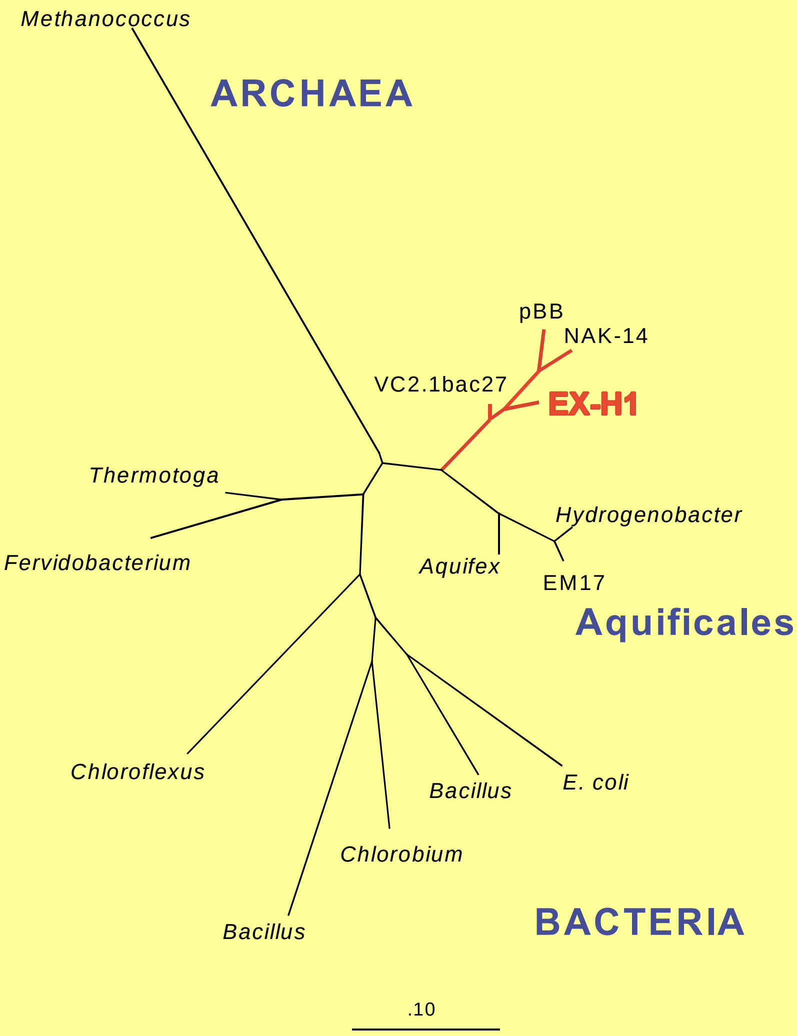
Organisms occupy ecological niches based on chemistry

Mussels at Lau and EPR "appear" to reside in similar diffuse flow (H_2S), but at Lau $S_2O_3^{2-}$ is a prevalent species – due to friable and high surface area substrate (do microbes use $S_2O_3^{2-}$?)

Snails and *Tevnia* live in microaerophilic regions so O_2 transport carrier for *Tevnia* needs further study

Need to combine with other tools/data to better understand physics, chemistry and their role on biology: temperature / salinity, pH, seismicity, etc.

Microaerophilic, H_2 oxidizing, thermophilic and chemolithotrophic eubacterium (EX-H1)



"*Persephonella* spp."

Best growth at 70-75 ^{0}C

Electron donor - H_2, S^0

Electron acceptor - O_2 (microaerophilic),

NO_3^- , $S_2O_3^{2-}$, S^0

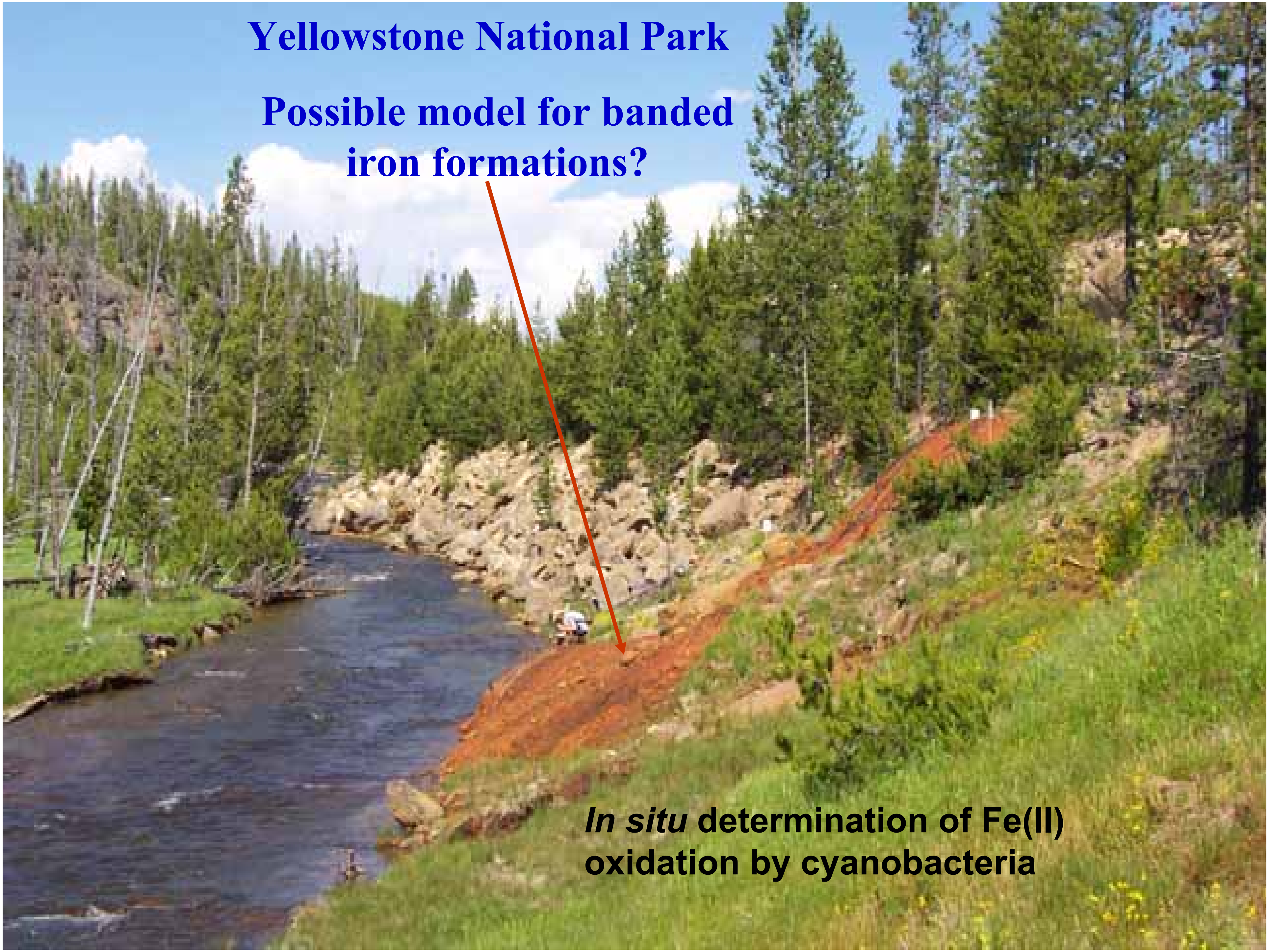
Carbon source - CO_2

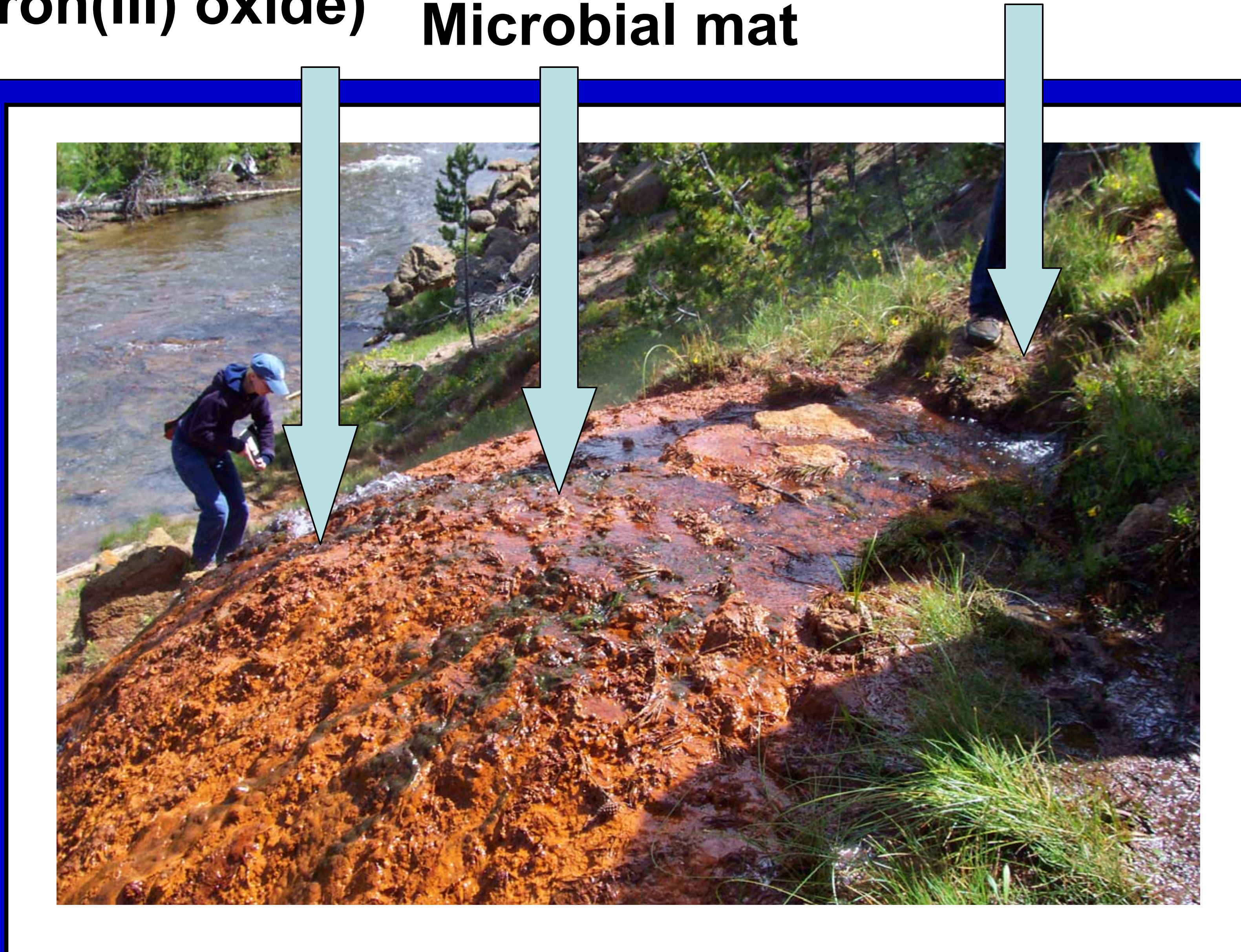
85% similar to *Aquifex*

93 % to Mid Atlantic 16S rRNA sequence

Reysenbach et al. 2000. Nature 404:835
Gotz et al., in press. IJSEM

Electrode helps prospect for life forms


Yellowstone National Park
Possible model for banded iron formations?
In situ determination of Fe(II) oxidation by cyanobacteria

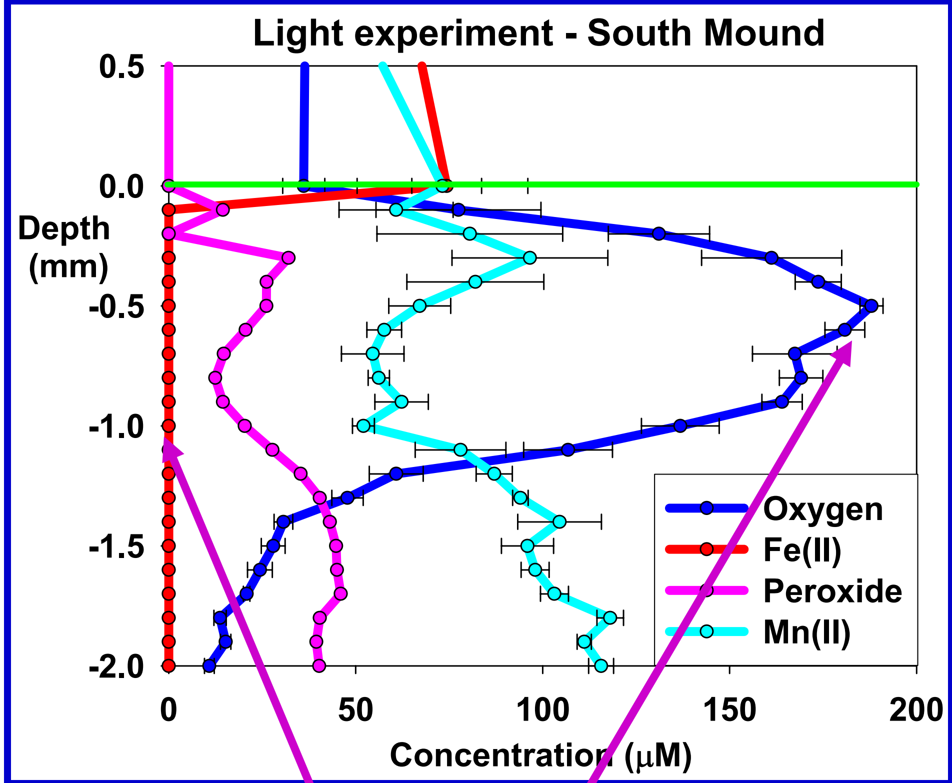

Ferrihydrite
(Iron(III) oxide)
Microbial mat
Hot spring
(source)

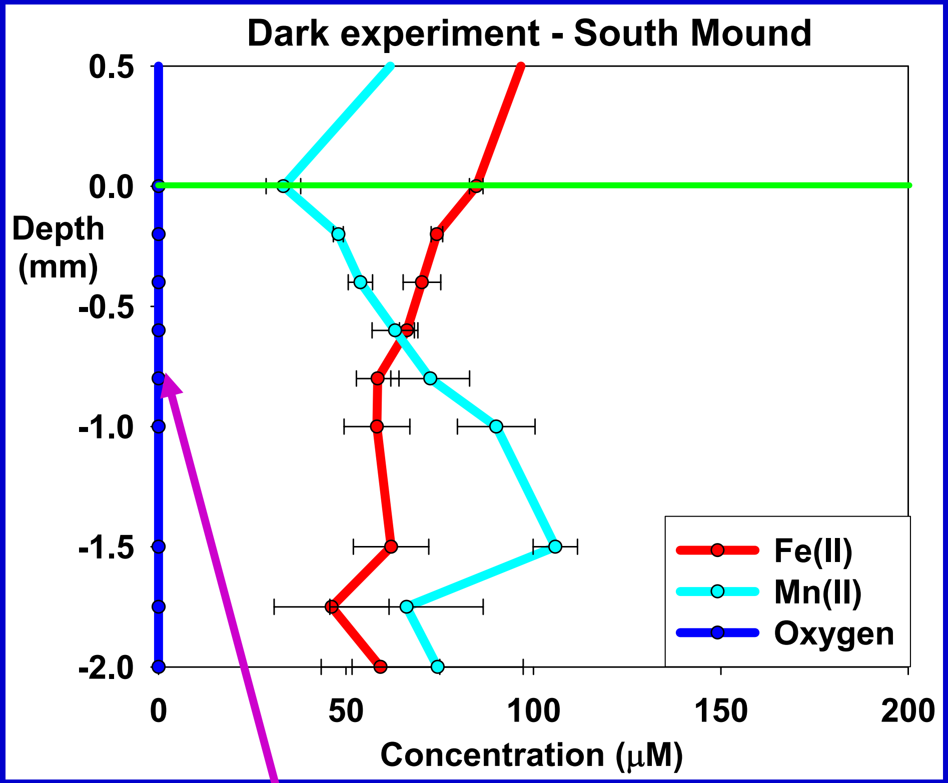
Chocolate Pots – Yellowstone National Park
55°C source; Fe(II) and Mn(II) but no H_2S, O_2, pH ~ 6

Electrodes in mat – light experiment

dark or light filter experiments

Chocolate Pots – South mound profiles





O_2 produced by cyanobacteria oxidizes Fe^{2+} but not Mn^{2+}

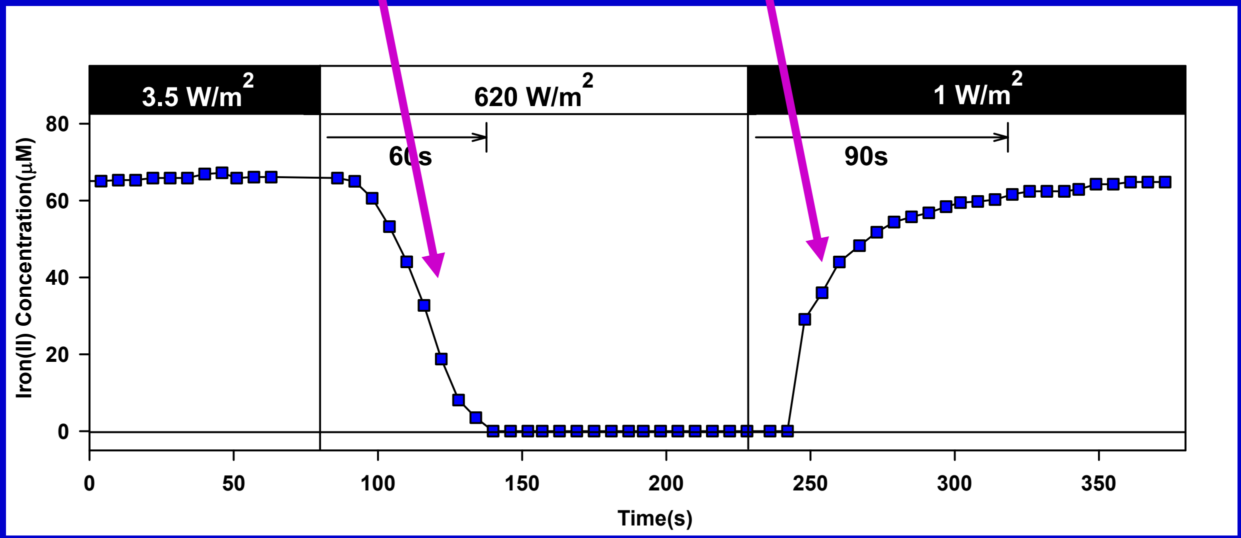
No O_2 production, Fe^{2+} and Mn^{2+} do not oxidize – atmospheric O_2 unimportant

Dark vs Light Fe(II) Kinetics – *in situ*

Electrode located at 0.5 mm below the mat/water interface – where maximum O_2 is produced

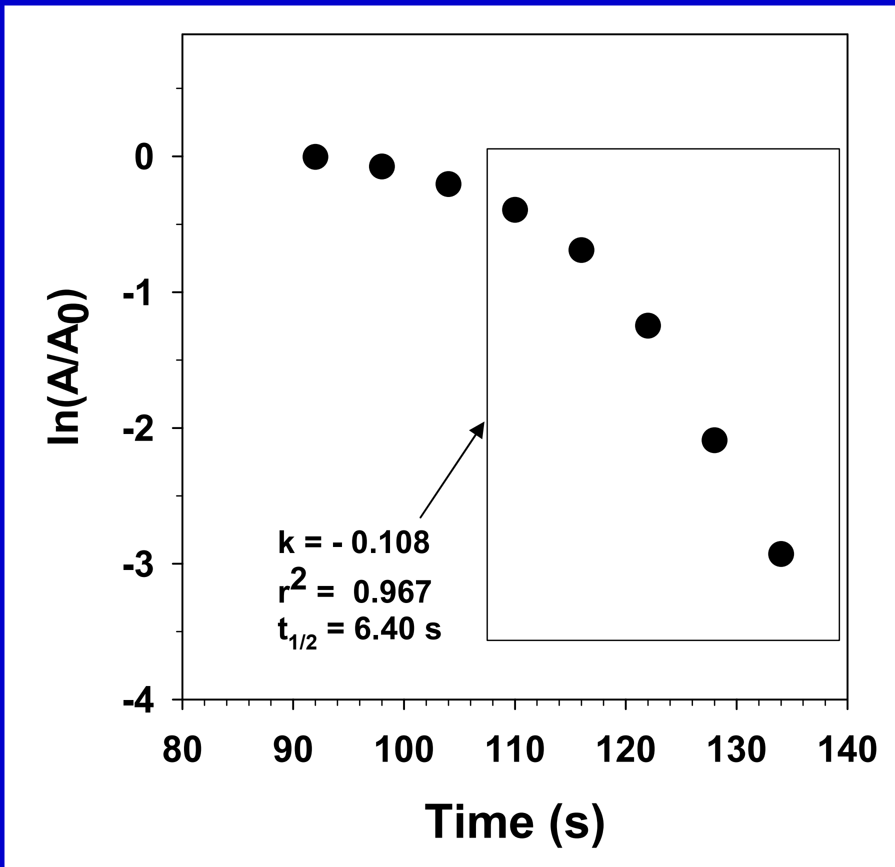
Fe(II) oxidation

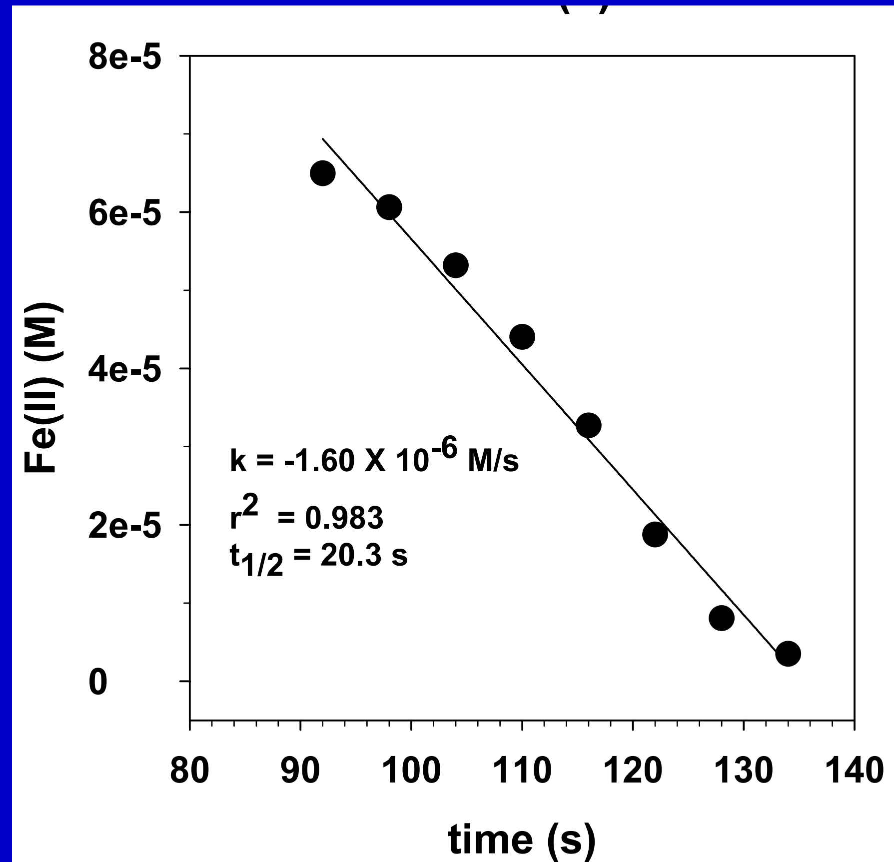
Fe(II) returns to mat



Fe(II) oxidation rates from these data indicate that the reaction is abiotic (inorganic).

Kinetic analysis of the Fe(II) decay region





Pseudo first order plot

Zeroth order plot

A zeroth order reaction is consistent with light being the primary limiting factor as the concentration of O_2 is dependent on light intensity and photosynthesis.