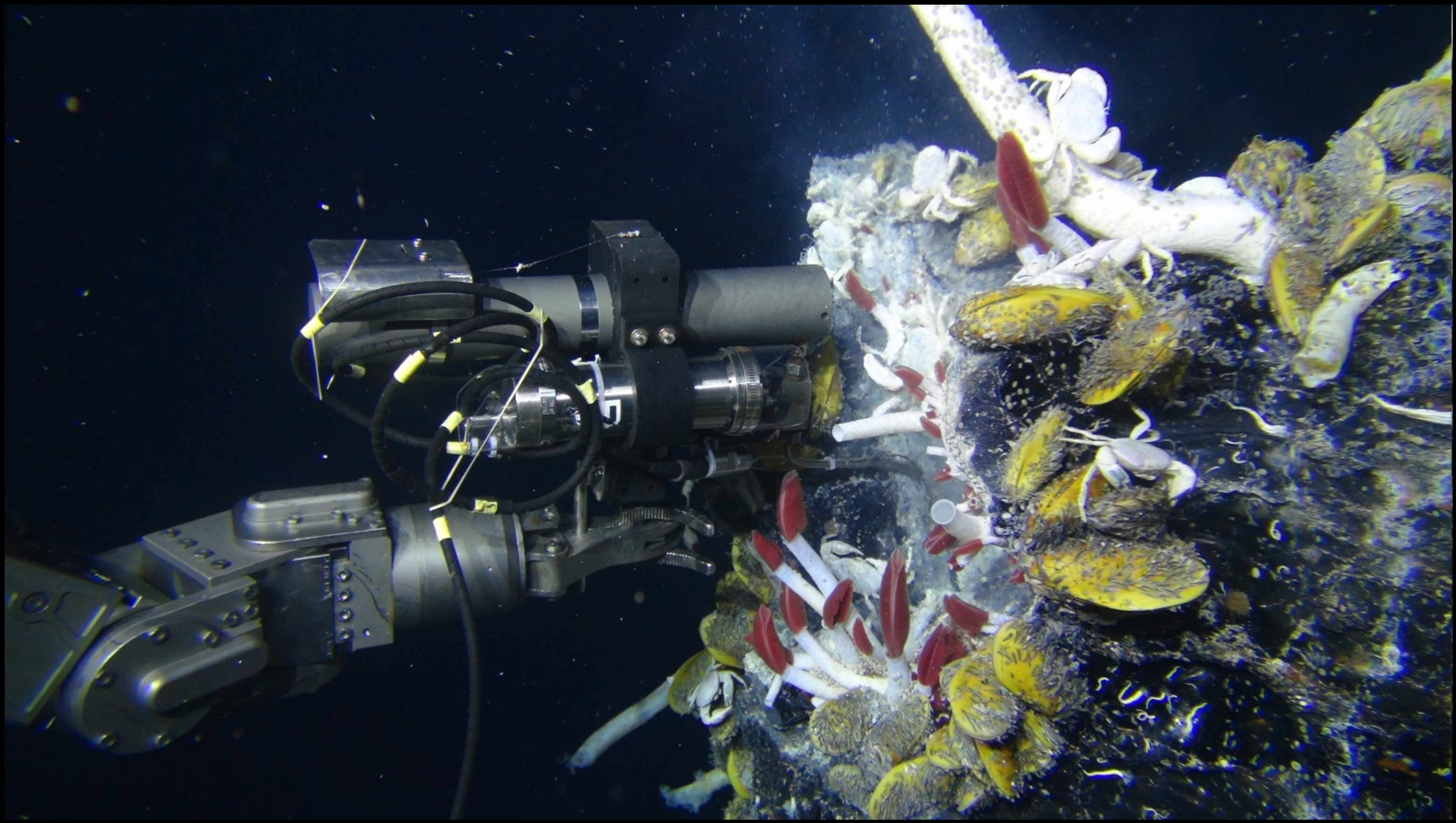
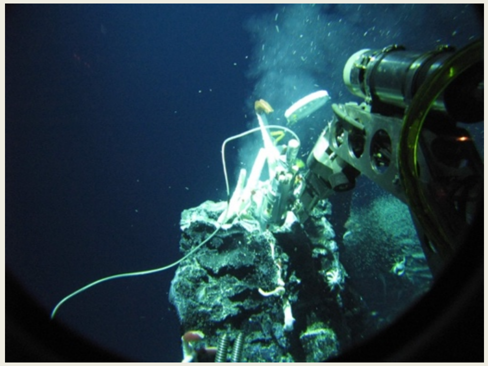
Exploring Life at Deep-Sea Hydrothermal Vents … and what does it have to do with Medicine?



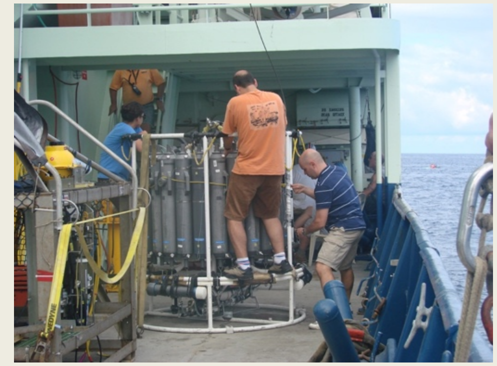
Stefan M. Sievert
Woods Hole Oceanographic Institution
ssievert@whoi.edu
http://www.whoi.edu/people/ssievert

SIEVERT Lab for Dark Life


Salt marsh


Deep-sea vents


Shallow-water vents


Oxygen minimum zones

CHEMOSYNTHESIS

Chemical Energy + Carbon Dioxide

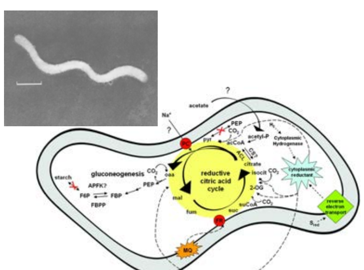
↓

Living cells

Who is there?

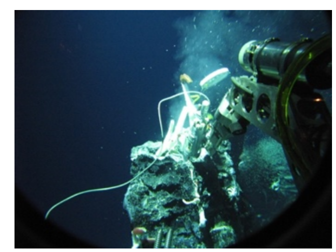
Who is active?

What process(es) are they mediating and at what rate(s)?



Physiology
Biochemistry
Genetics
Genomics
Proteomics

Organism ⟷ **Ecosystem**



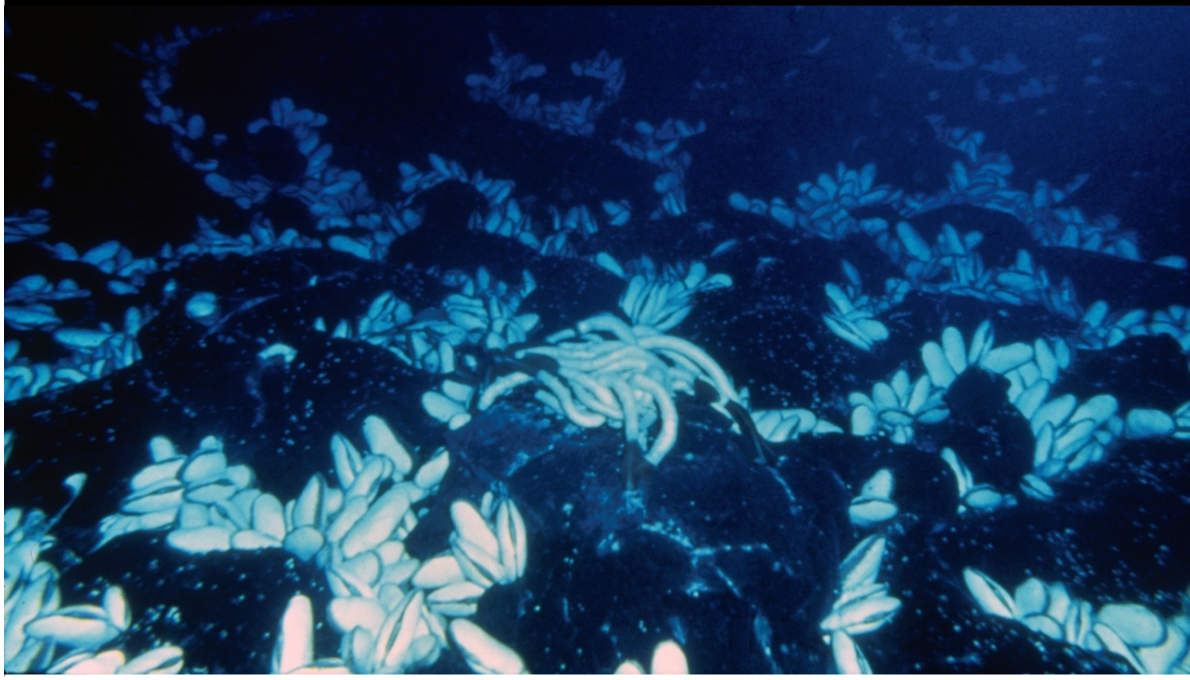
Abundance, Diversity
Functions (Meta 'Omics')
Rates
Geochemistry
Interactions (biotic, abiotic)

http://www.whoi.edu/groups/sievertlab

Outline

- Introduction to Deep-Sea Vents
  - Geological and Physical Setting
  - Chemosynthesis
- Microbial Processes and Symbiosis – Link to Medicine
- Fieldwork at 9^{o}N East Pacific Rise
  - Study site and approach
  - Productivity of subseafloor biosphere
- Synthesis

Discovery of Deep-Sea Hydrothermal Vents only ~40 years ago in 1977



Submarine Thermal Springs on the Galápagos Rift

16 March 1979, Volume 203, Number 4385

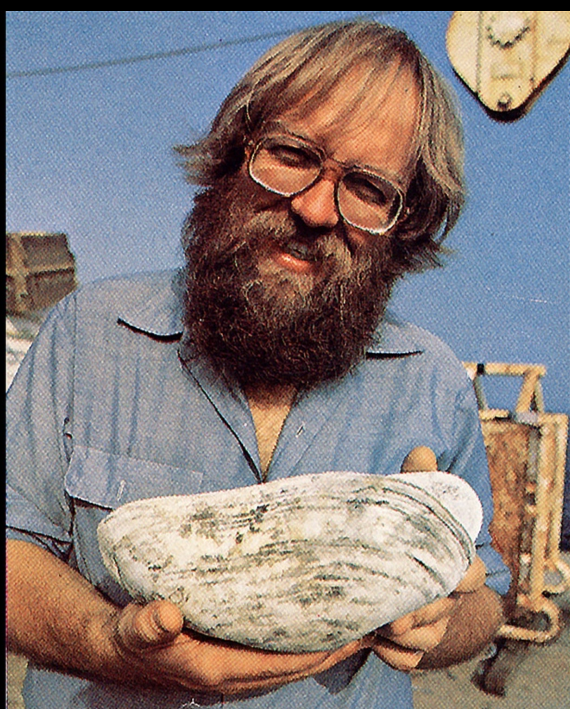
SCIENCE

John B. Corliss, Jack Dymond, Louis I. Gordon, John M. Edmond, Richard P. von Herzen, Robert D. Ballard, Kenneth Green, David Williams, Arnold Bainbridge, Kathy Crane, Tjeerd H. van Andel

John (Jack) Corliss
(OSU)

Bob Ballard
(WHOI)

Discovery of Deep-Sea Hydrothermal Vents only ~40 years ago in 1977



John (Jack) Corliss
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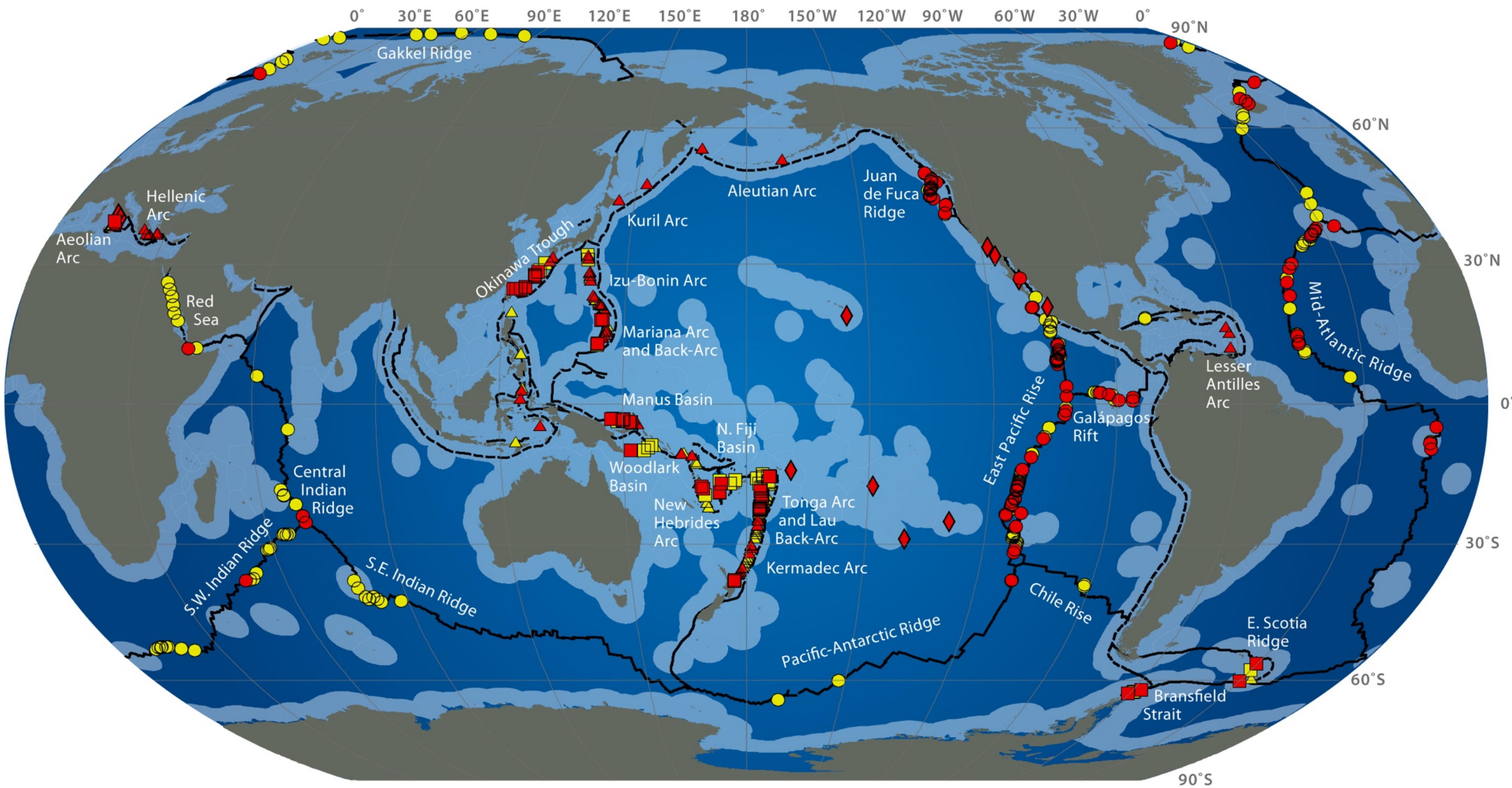
Global Distribution of Hydrothermal Vent Fields

Global Distribution of Hydrothermal Vent Fields

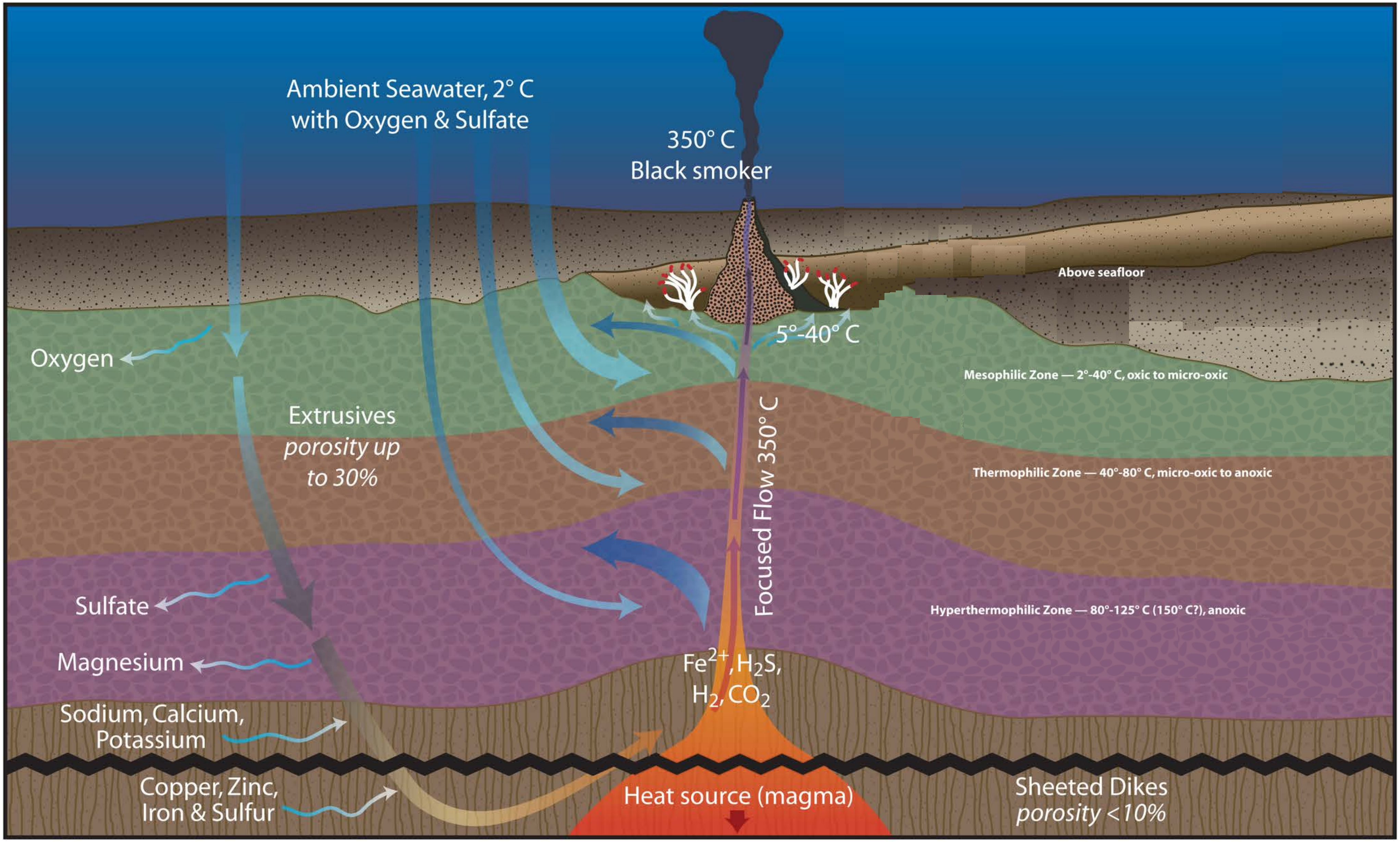


Mid-ocean ridge	Arc volcano	Back-arc spreading center	Intra-plate volcano & Other	
Active	Active	Active		Ridge & Transform
Unconfirmed	Unconfirmed	Unconfirmed	Active	Trench
				Exclusive Economic Zones

INTER RIDGE international cooperation in ridge-crest studies

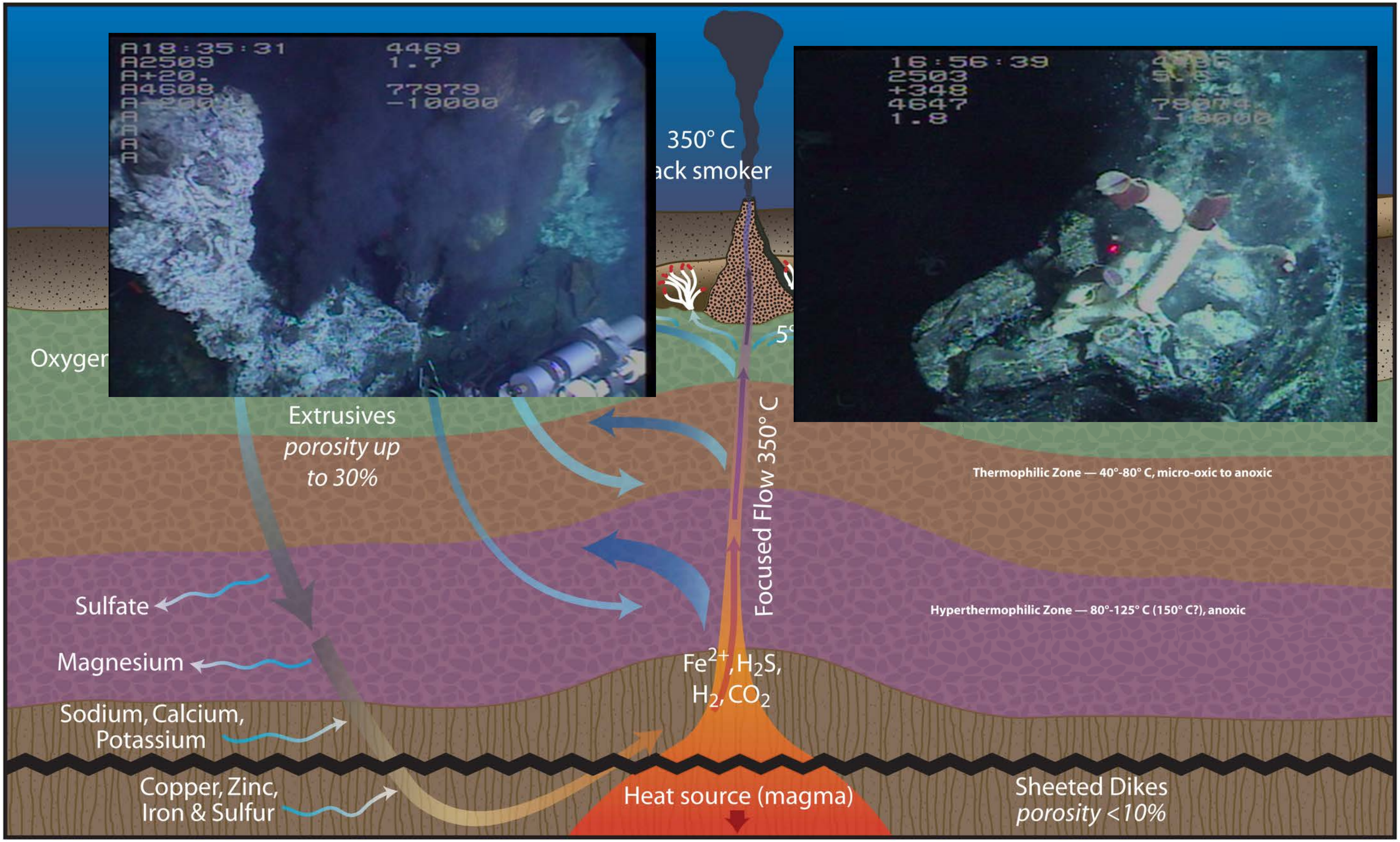
WOODS HOLE OCEANOGRAPHIC INSTITUTION 1930

Physical Setting



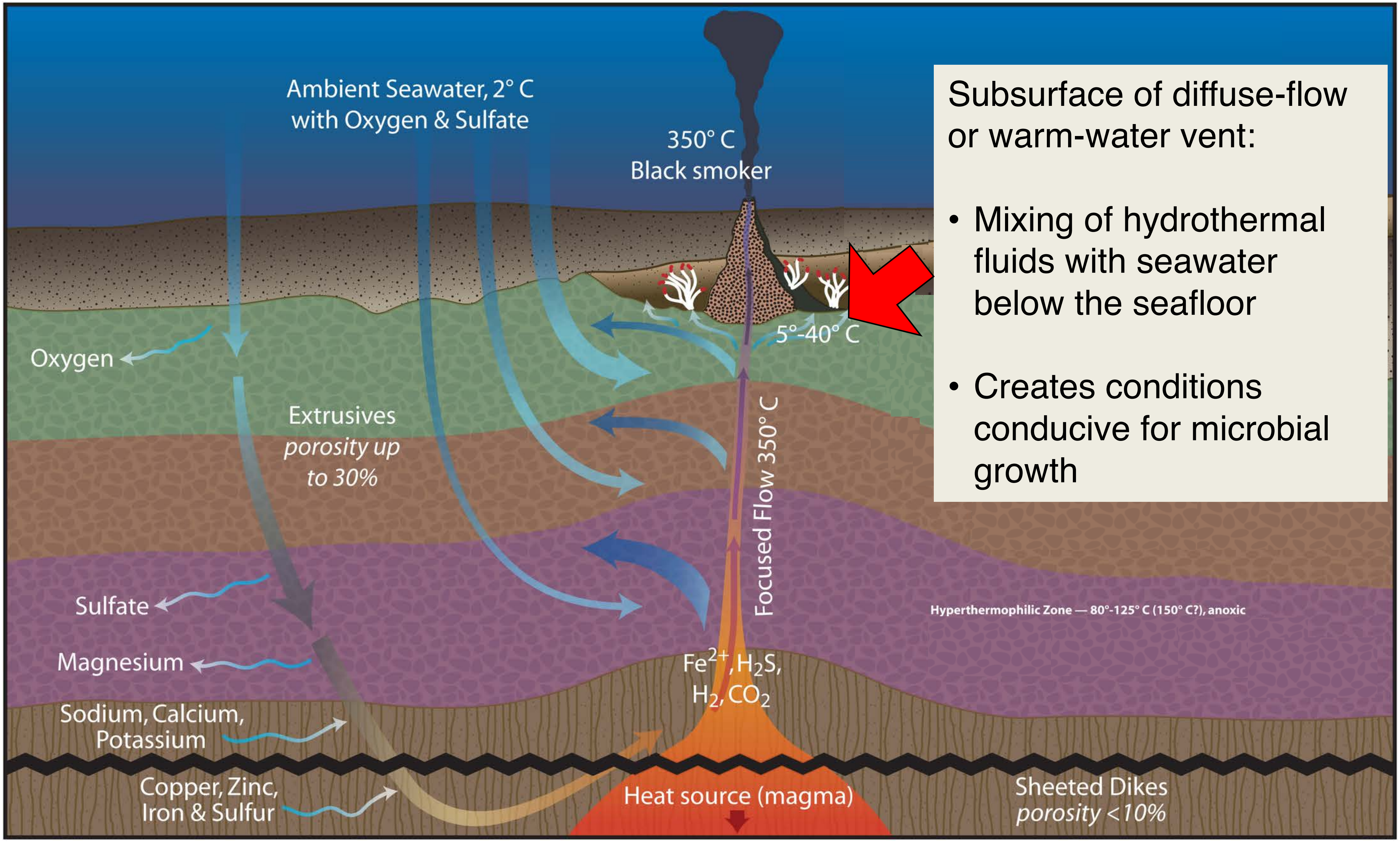
Sievert et al., 2008

Physical Setting



Sievert et al., 2008

Physical Setting



Subsurface of diffuse-flow or warm-water vent:

- Mixing of hydrothermal fluids with seawater below the seafloor
- Creates conditions conducive for microbial growth

Sievert et al., 2008

"Food chain" at Deep-sea vents: Conceptual Framework

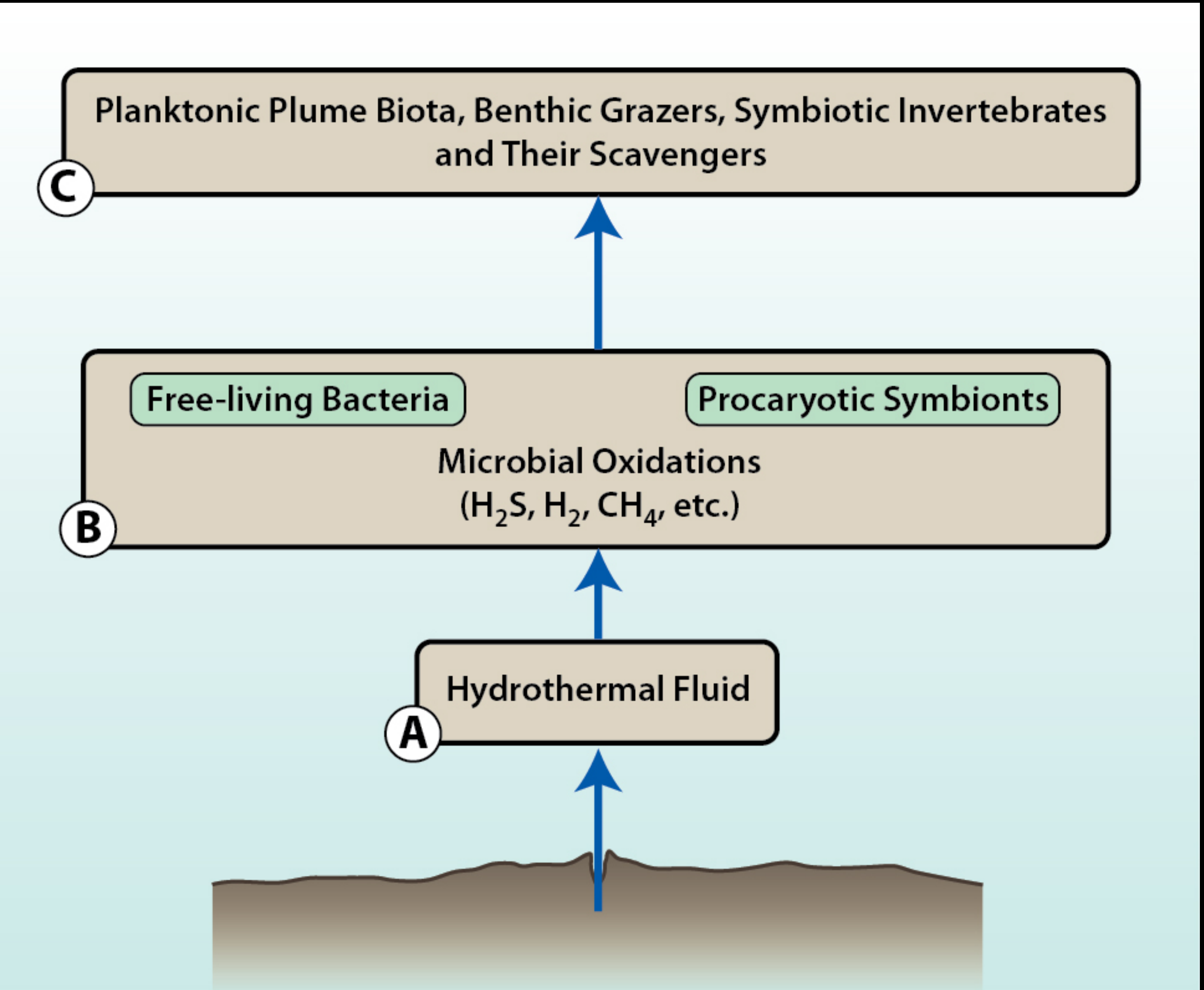


Figure modified from Jannasch, 1995

"FOOD CHAIN" AT DEEP-SEA VENTS: CONCEPTUAL FRAMEWORK

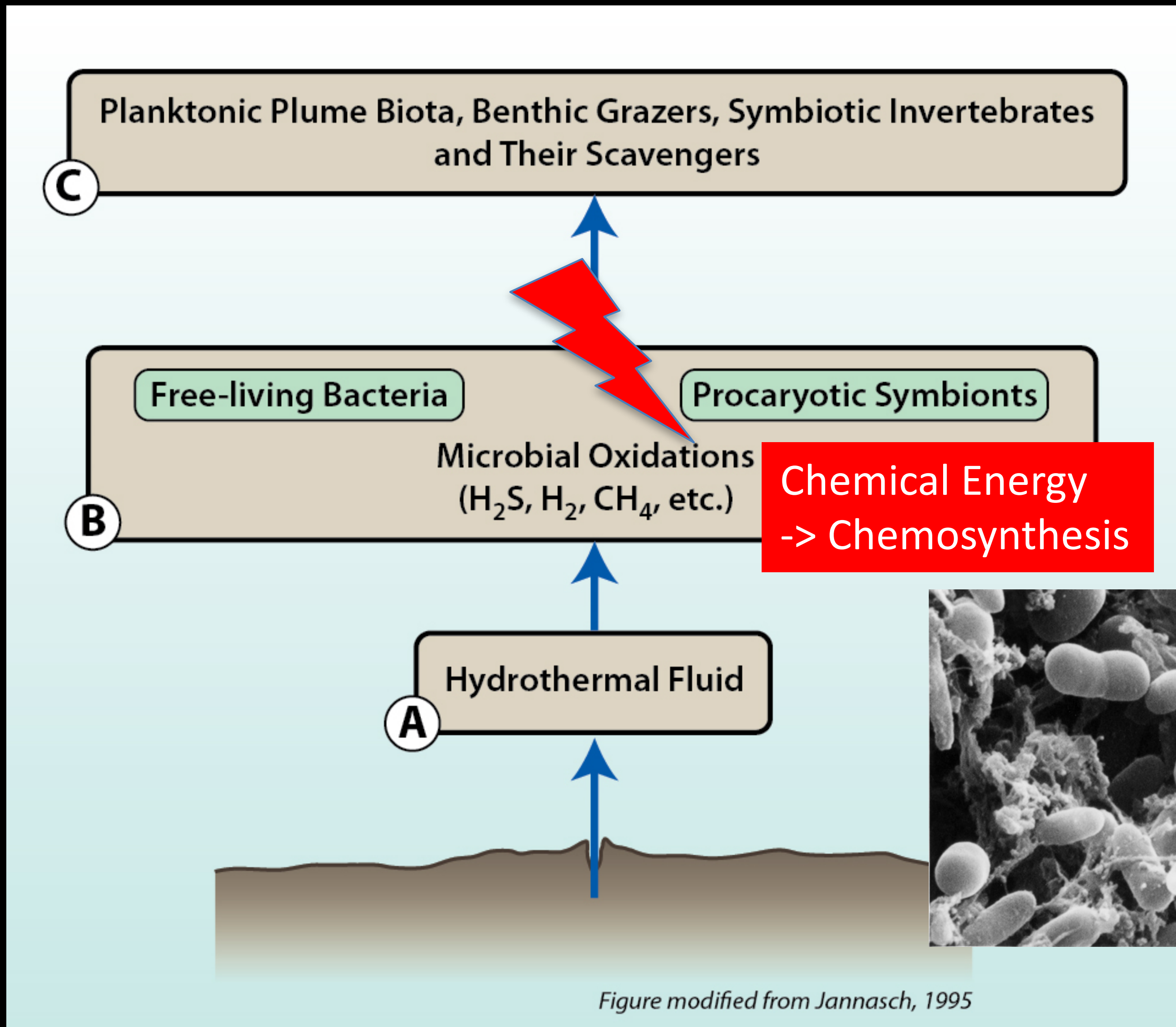
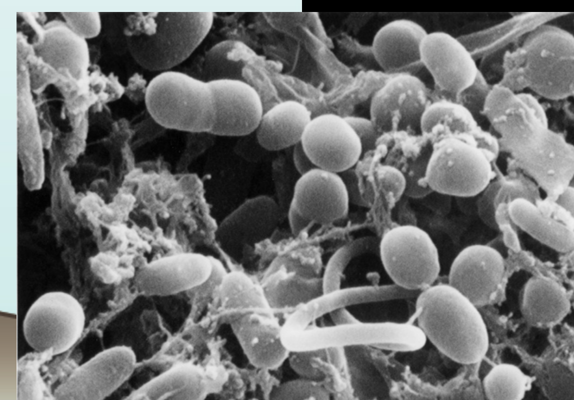


Figure modified from Jannasch, 1995



"FOOD CHAIN" AT DEEP-SEA VENTS: CONCEPTUAL FRAMEWORK

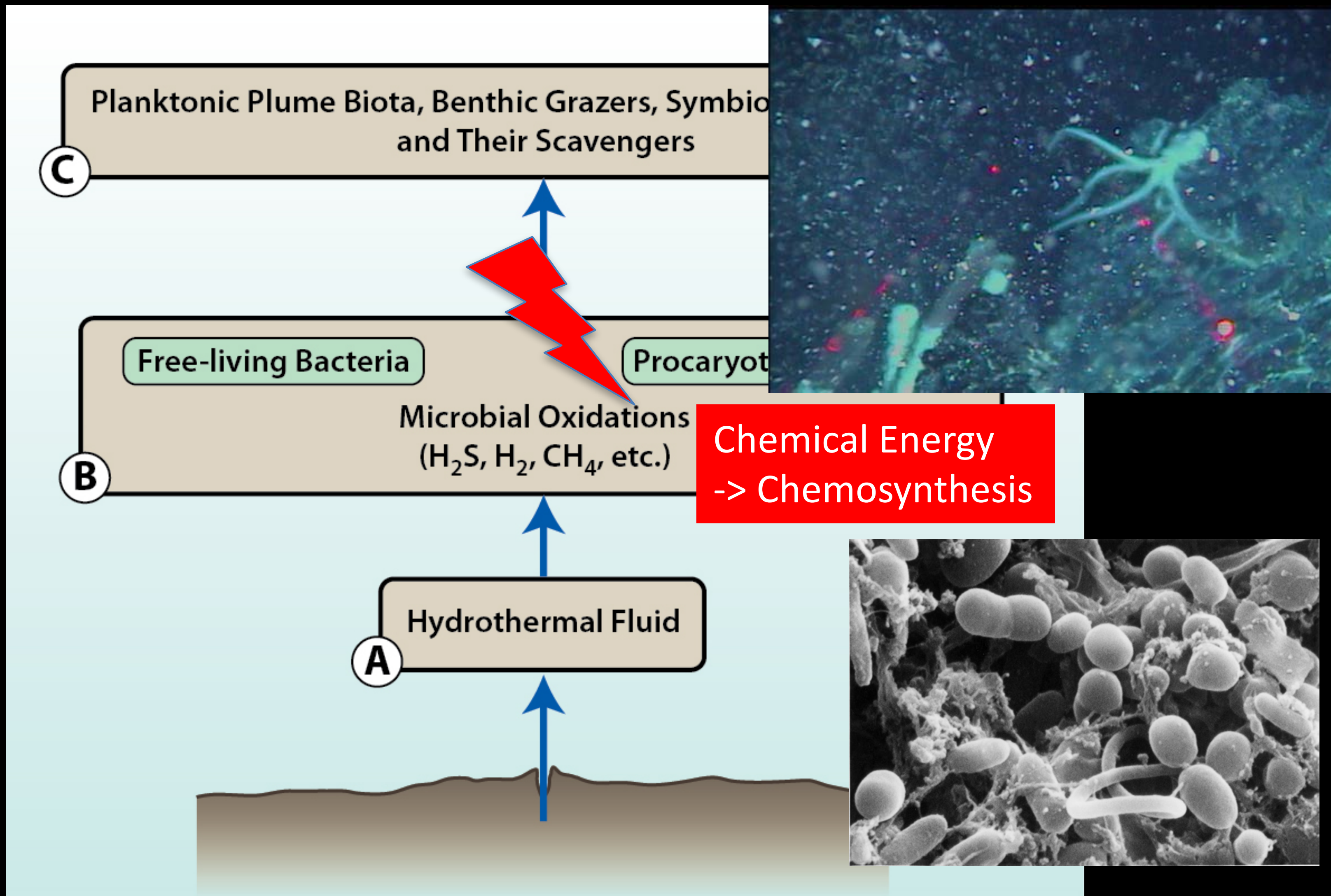
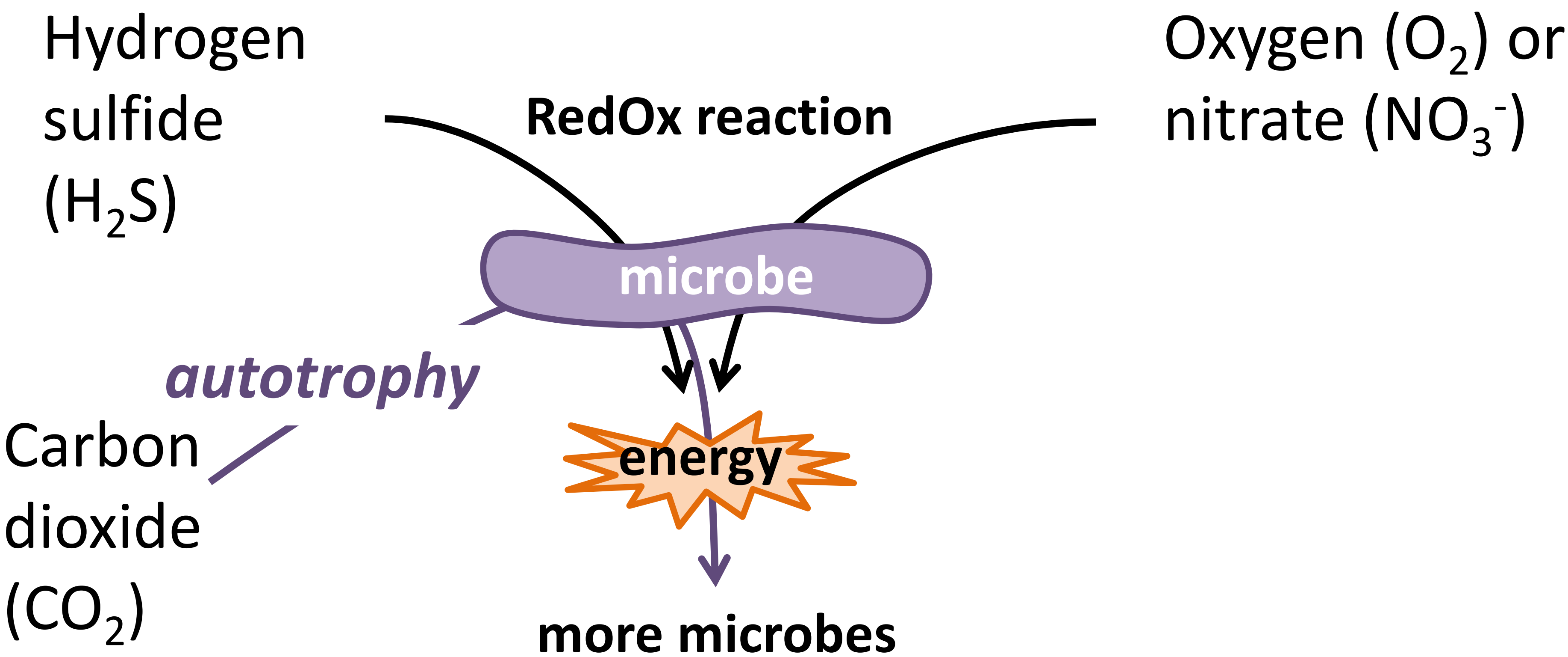


Figure modified from Jannasch, 1995

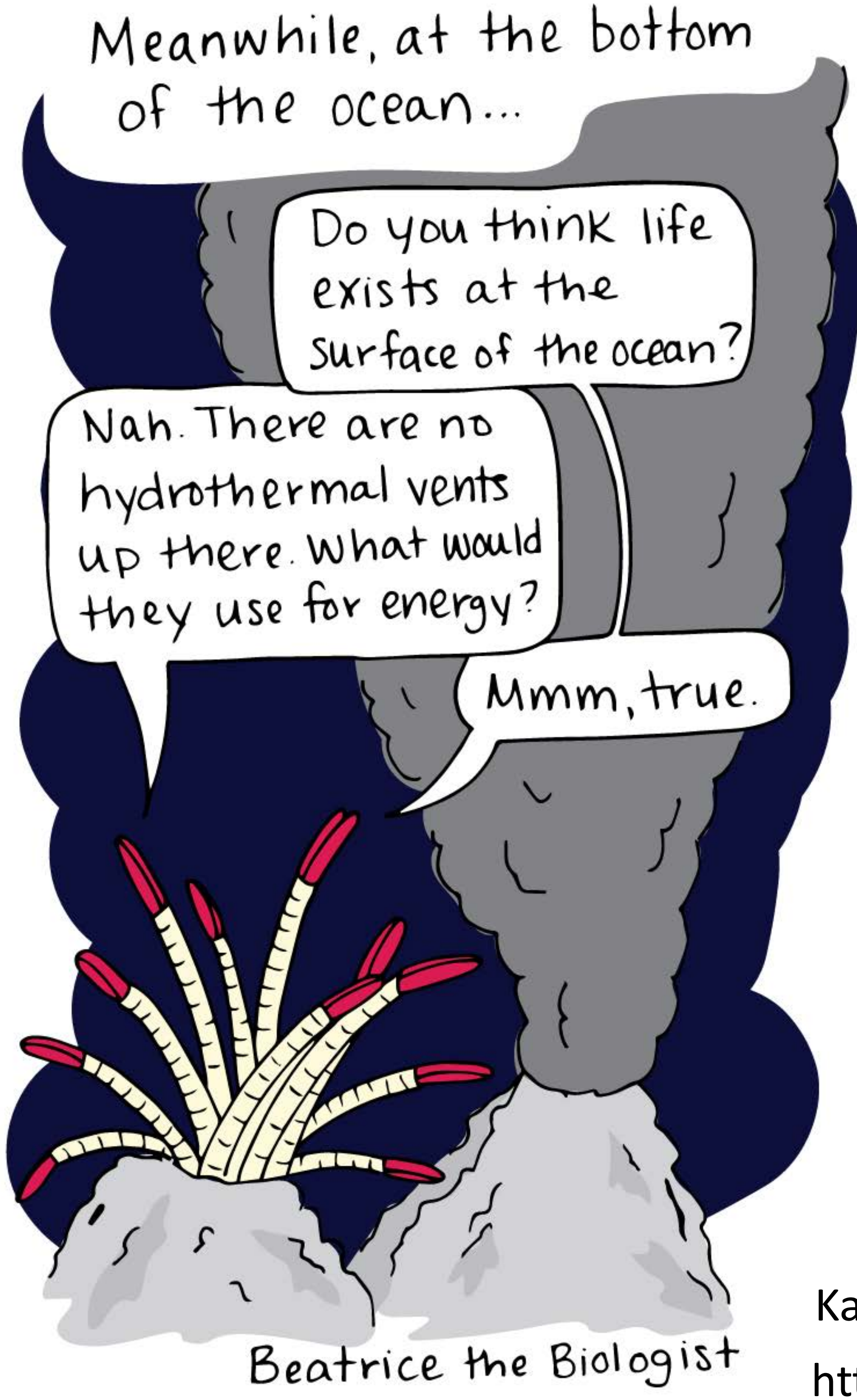
Microbes are at the Base of Ecosystem by Mediating the Transfer of Energy from Geothermal Source to Higher Trophic Levels

Chemosynthesis



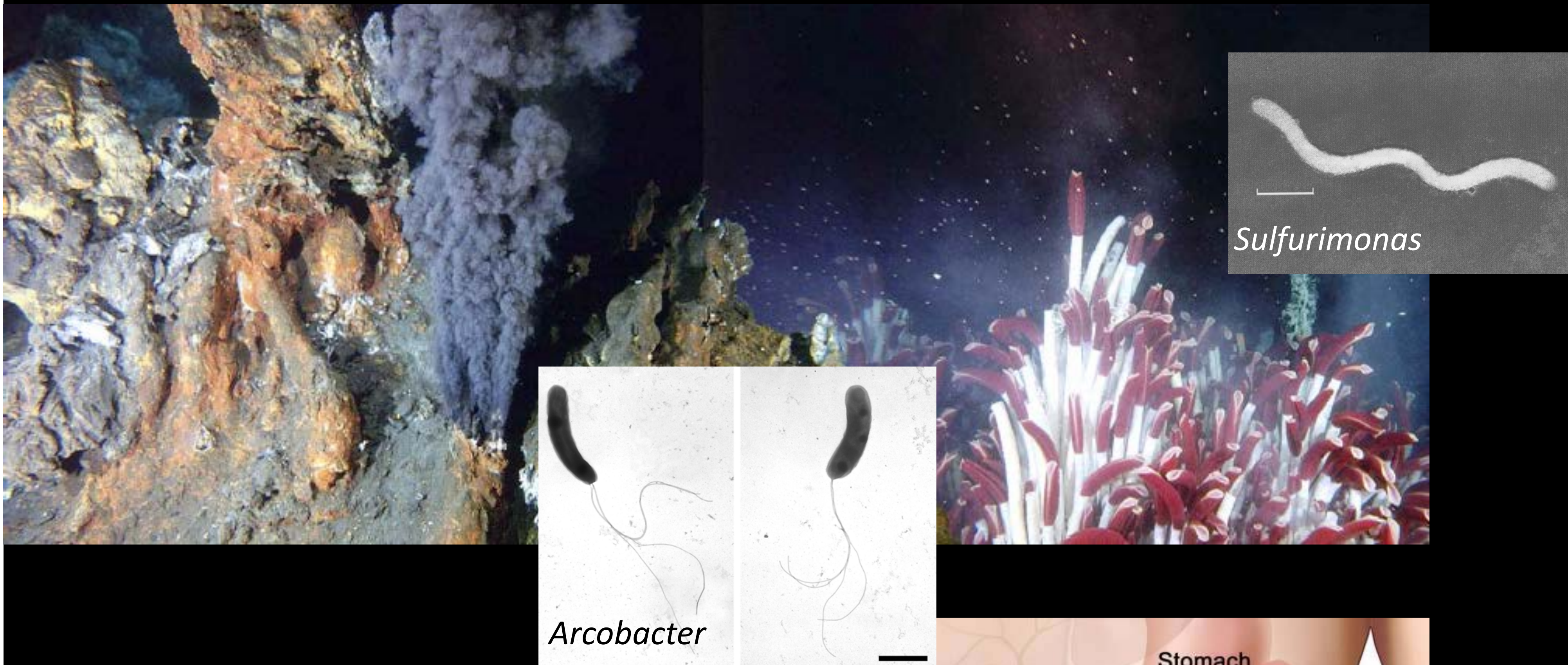
Photosynthesis – Foundation for life on land and surface ocean

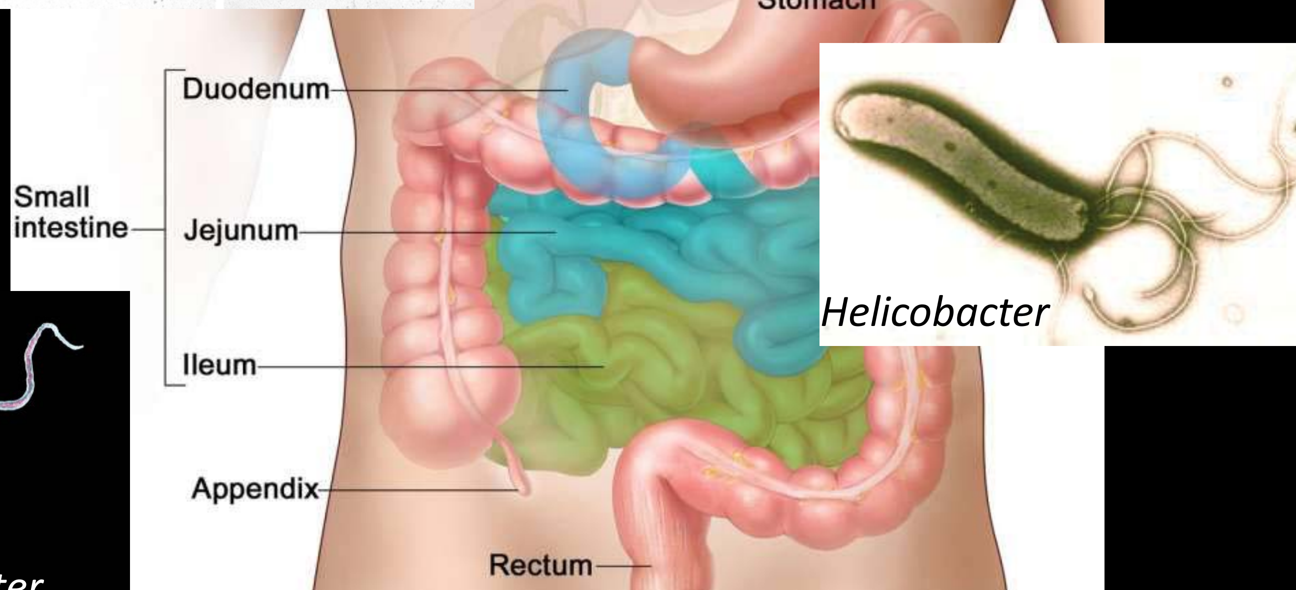
Chemosynthesis – Foundation for life at hydrothermal vents



Katie McKissick

http://www.beatricebiologist.com





What's in common?

Campylobacteria

Formerly known as *Epsilonproteobacteria*

SCIENCE 8 JANUARY 2016 • VOL 351 ISSUE 6269

The 5300-year-old *Helicobacter pylori* genome of the Iceman

Frank Maixner,[1*†] Ben Krause-Kyora,[2†] Dmitrij Turaev,[3†] Alexander Herbig,[4,5†] Michael R. Hoopmann,[6] Janice L. Hallows,[6] Ulrike Kusebauch,[6] Eduard Egarter Vigl,[7] Peter Malfertheiner,[8] Francis Megraud,[9] Niall O'Sullivan,[1] Giovanna Cipollini,[1] Valentina Coia,[1] Marco Samadelli,[1] Lars Engstrand,[10] Bodo Linz,[11] Robert L. Moritz,[6] Rudolf Grimm,[12] Johannes Krause,[4,5‡] Almut Nebel,[2‡] Yoshan Moodley,[13,14‡] Thomas Rattei,[3‡] Albert Zink[1*‡]

Eduard Egarter-Vigl (left) and Albert Zink (right) taking a sample from the Iceman in November 2010. Credit: © EURAC/Marion Lafogler

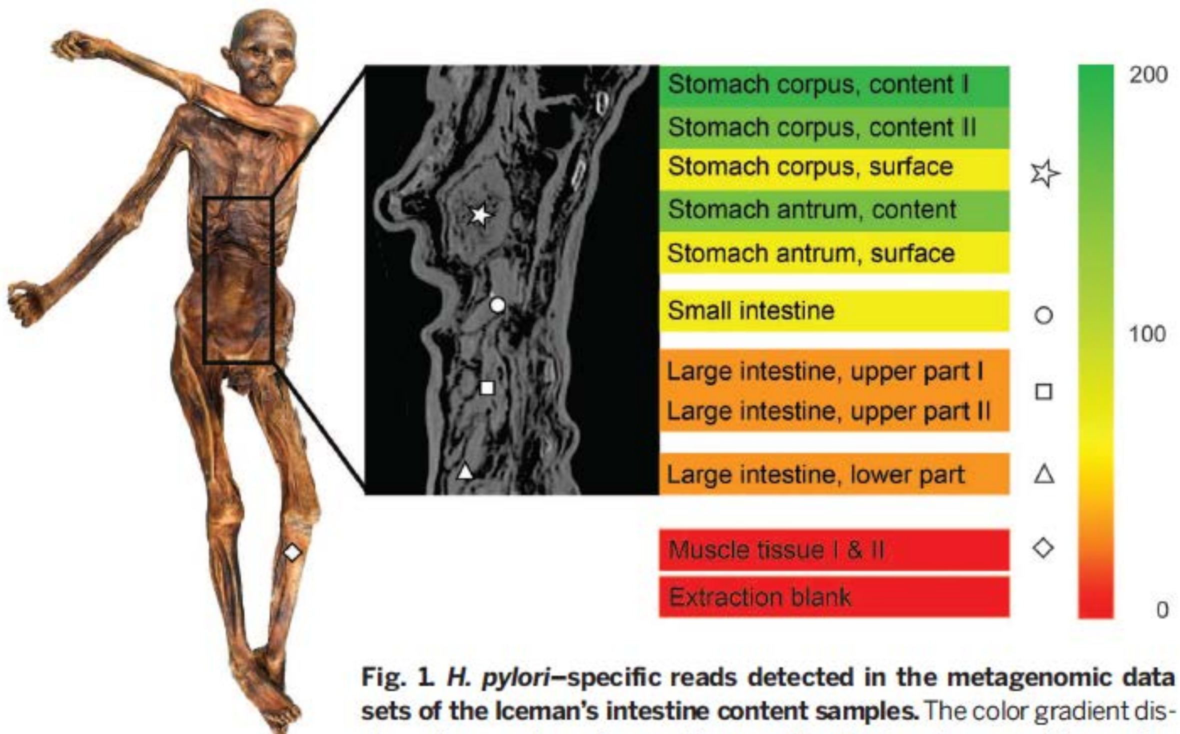
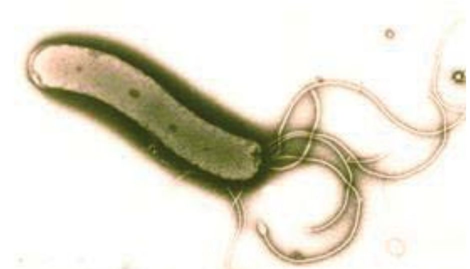


Fig. 1. ***H. pylori*–specific reads detected in the metagenomic data sets of the Iceman's intestine content samples.** The color gradient displays the number of unambiguous *H. pylori* reads per million metagenomic reads. Control metagenomic data sets of the Iceman's muscle tissue and of the extraction blank were included in the analysis. The different intestinal content sampling sites are marked in the radiographic image by the following symbols: asterisk, stomach content; circle, small intestine; square, upper large intestine; triangle, lower large intestine. The sampling site of the muscle control sample is highlighted in the Iceman overview picture (diamond).

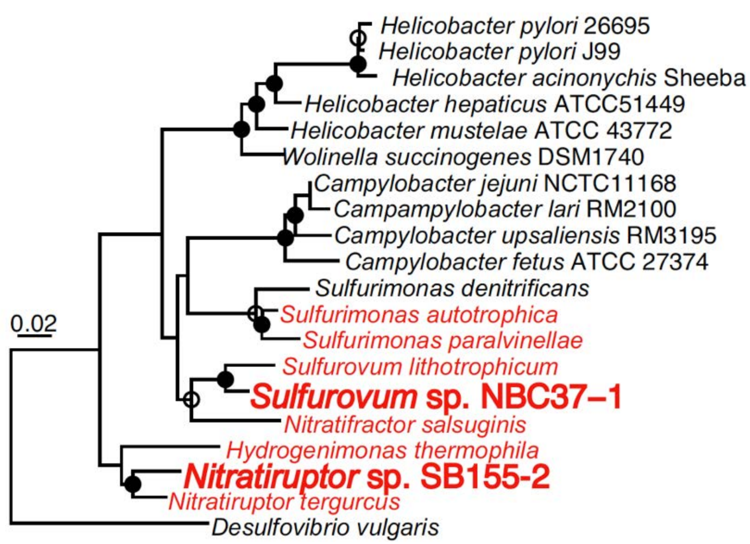


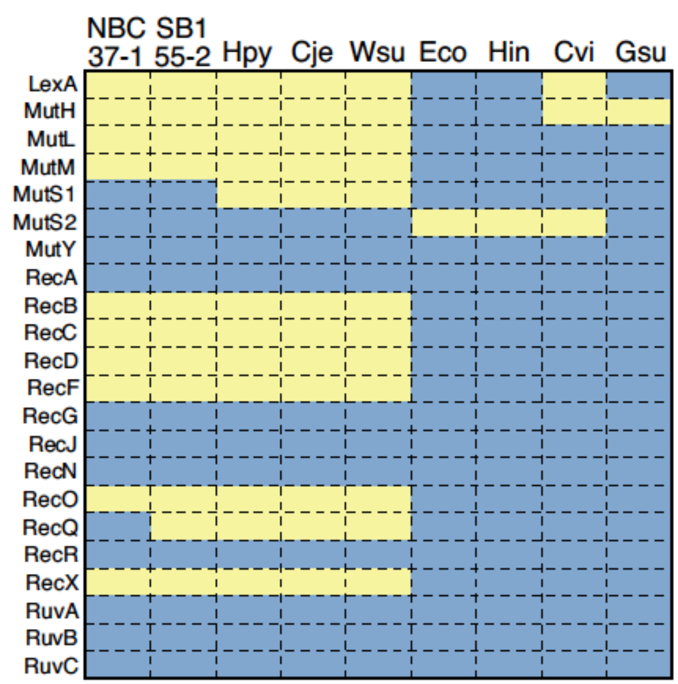
Waite et al., 2017

Deep-sea vent ε-proteobacterial genomes provide insights into emergence of pathogens

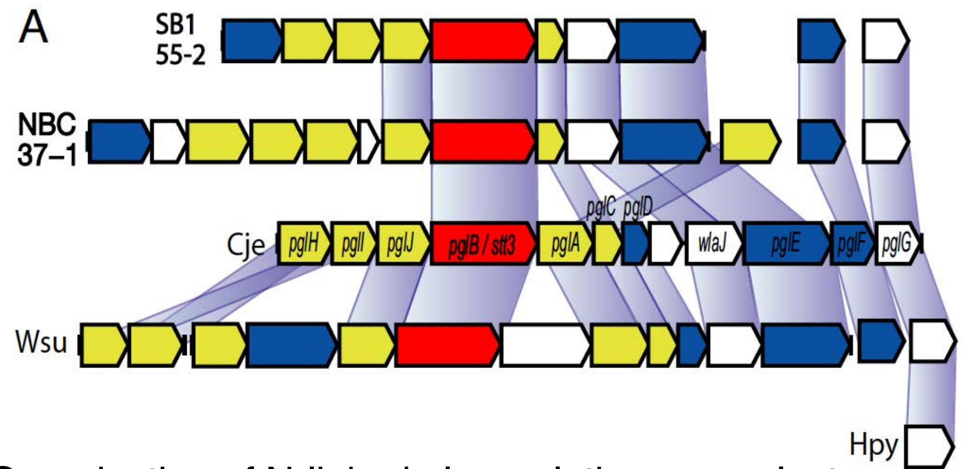
12146–12150 | PNAS | July 17, 2007 | vol. 104 | no. 29

Satoshi Nakagawa[†‡], Yoshihiro Takaki[§], Shigeru Shimamura[§], Anna-Louise Reysenbach[¶], Ken Takai[†], and Koki Horikoshi[†§]

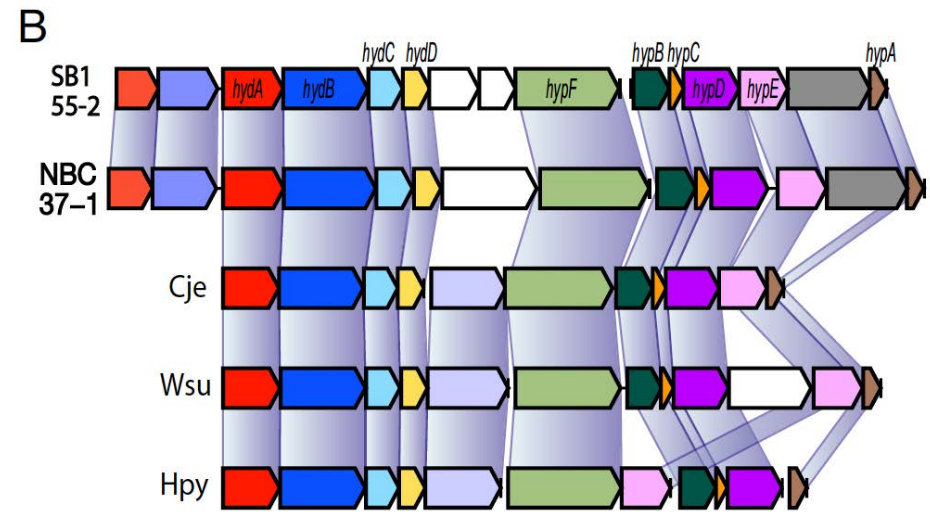




DNA repair genes

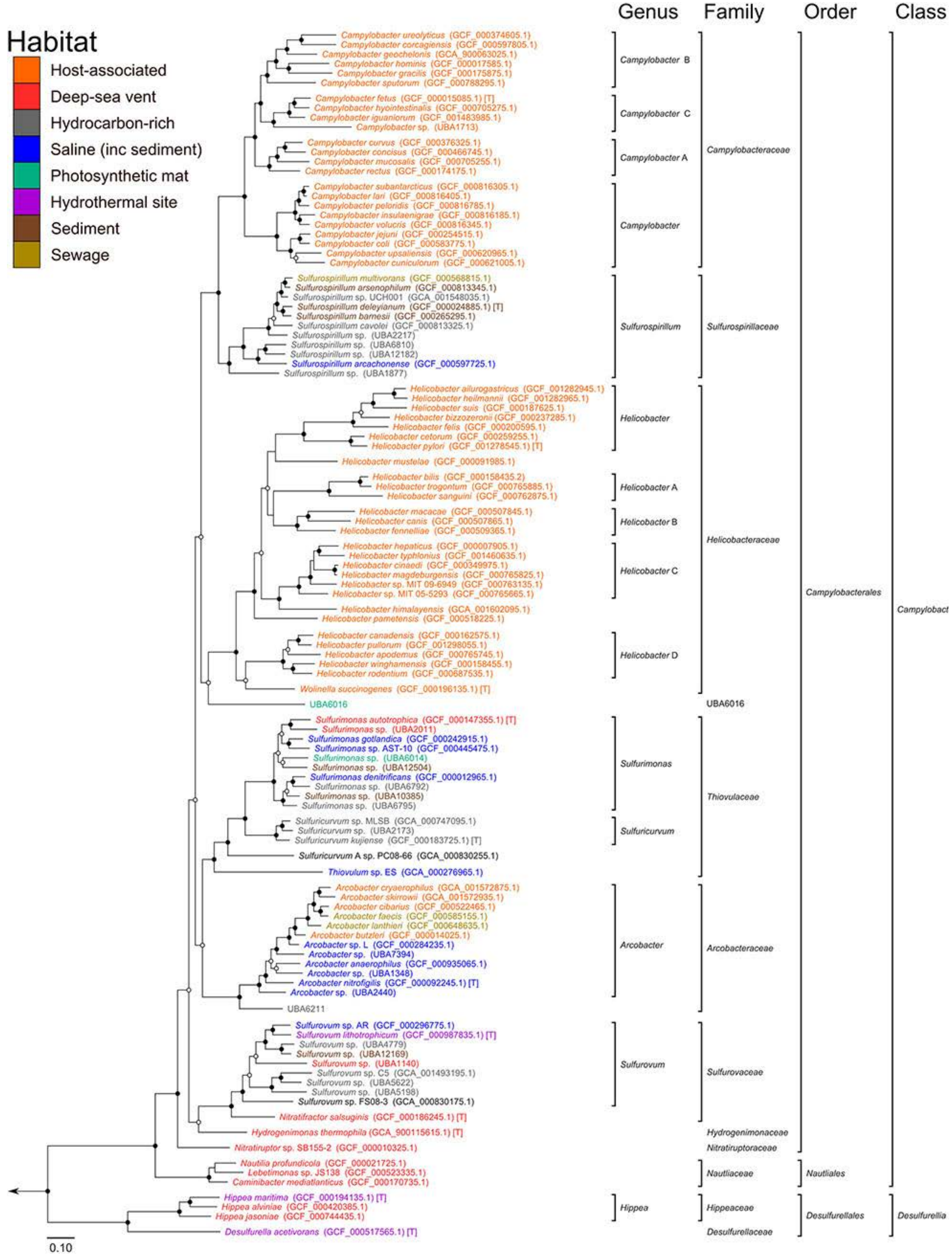


Organization of N-linked glycosylation gene clusters

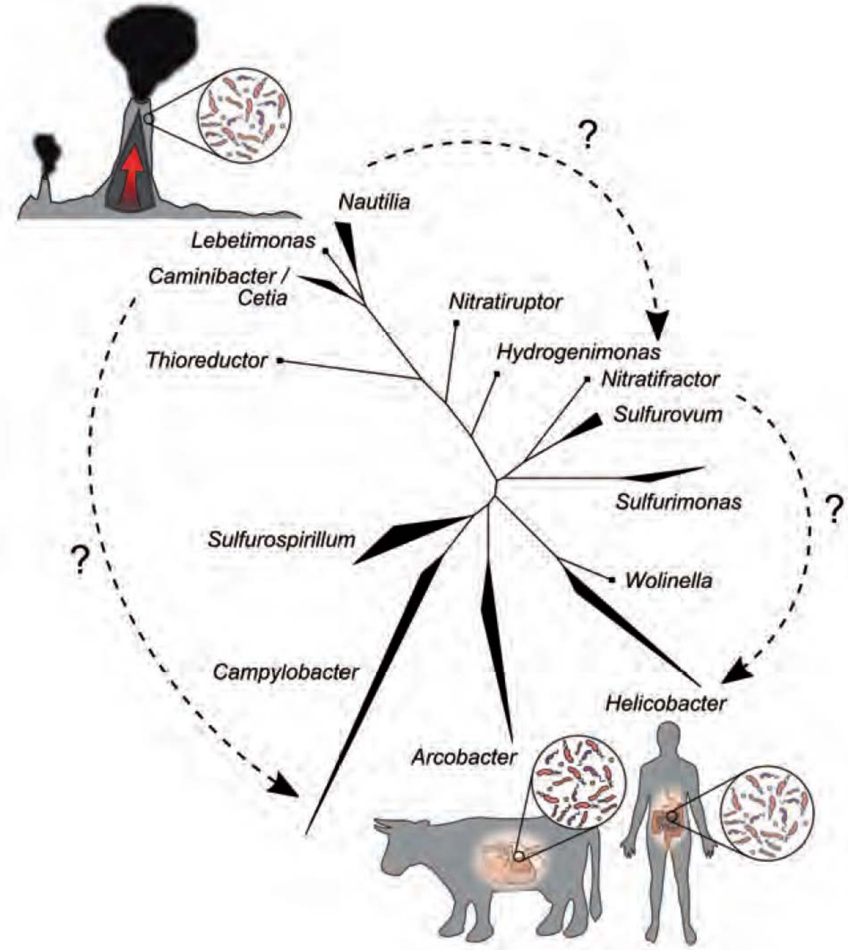


Organization of H_2-uptake and -sensing hydrogenase gene clusters

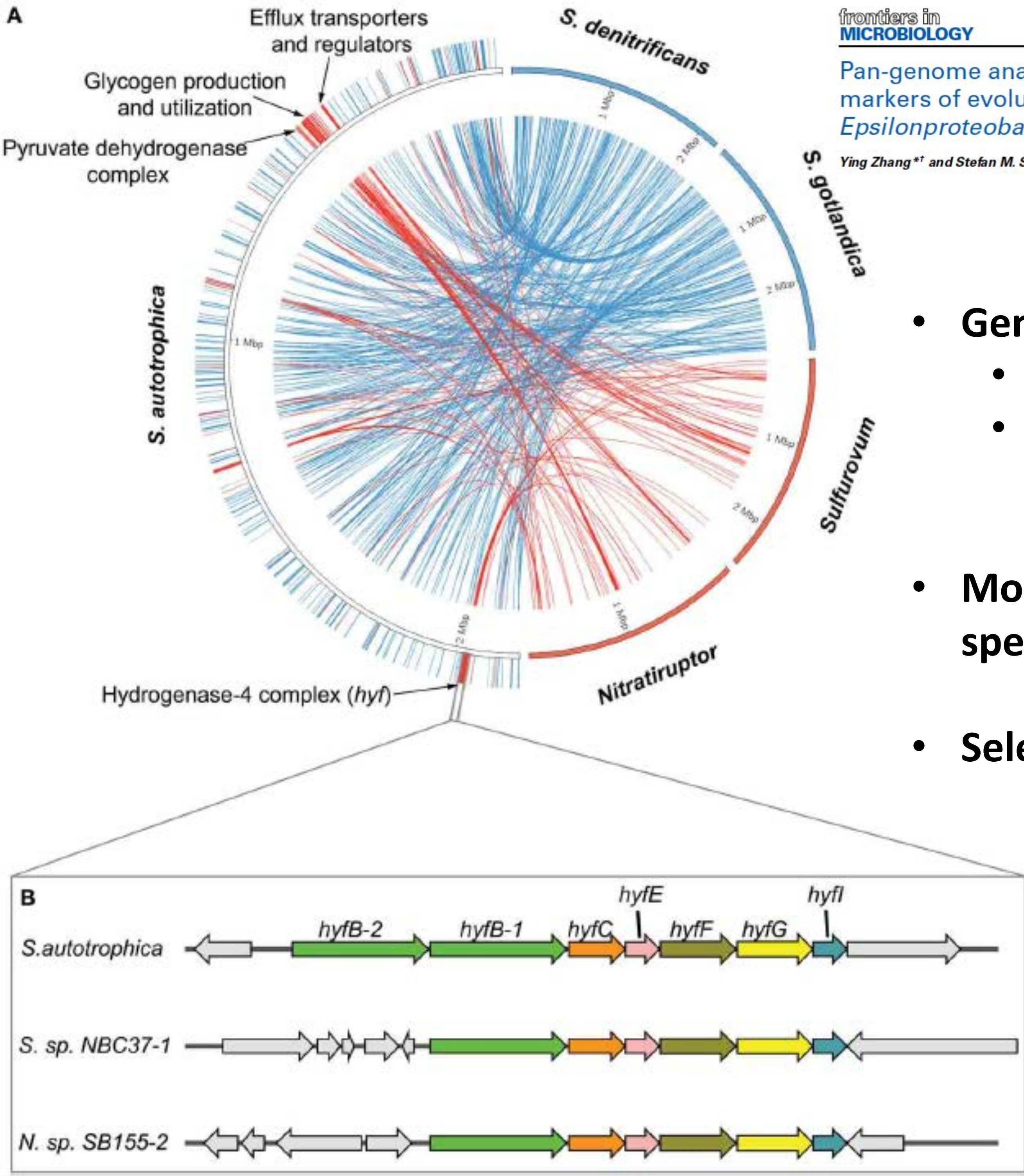
Deep-sea vent chemoautotrophy has provided the core of virulence for important human/animal pathogens!



Waite et al., 2017



Giovanelli & Vetriani, 2017



frontiers in MICROBIOLOGY
ORIGINAL RESEARCH ARTICLE
published: 19 March 2014
doi: 10.3389/fmicb.2014.00110

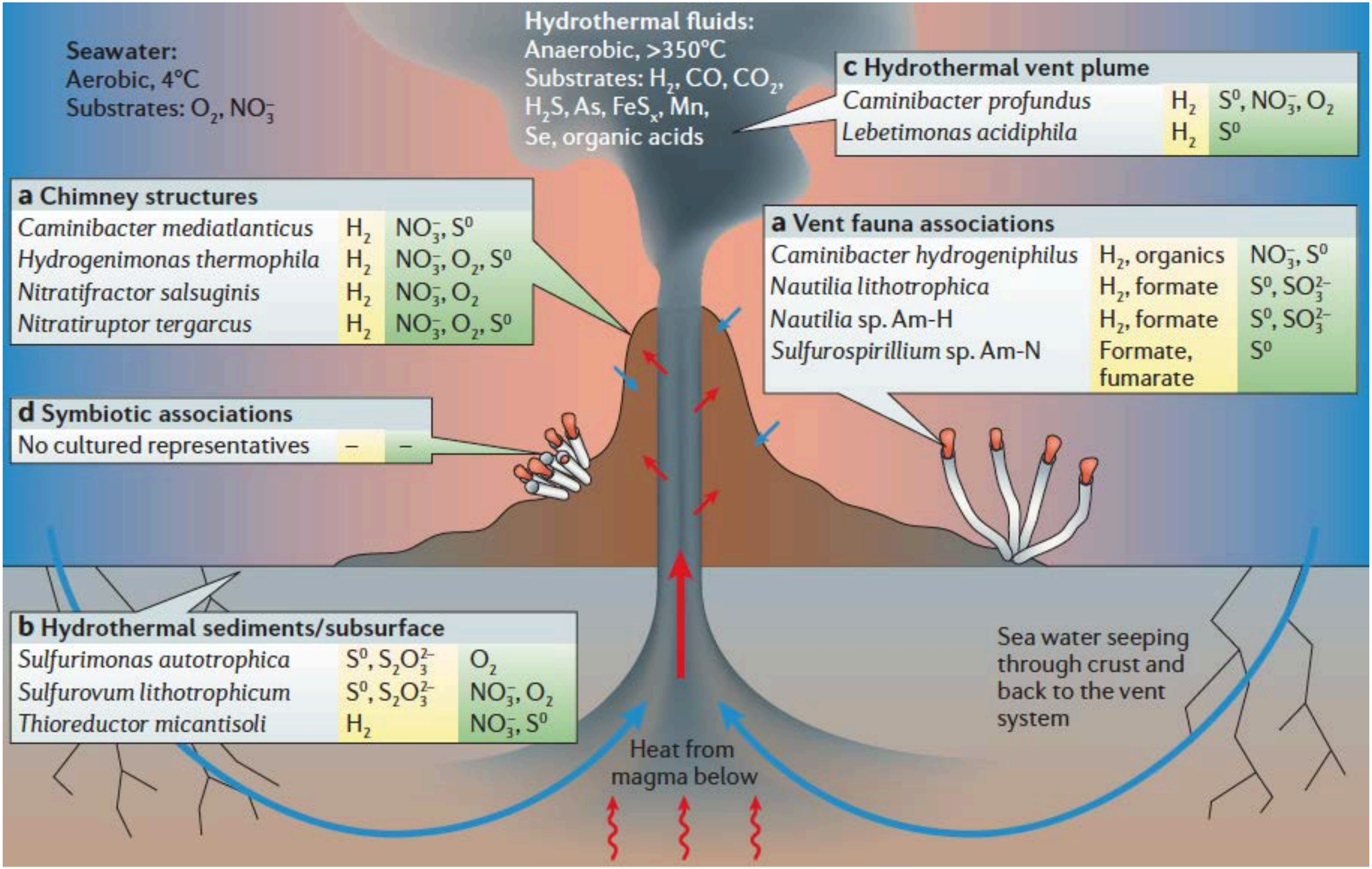
Pan-genome analyses identify lineage- and niche-specific markers of evolution and adaptation in *Epsilonproteobacteria*

Ying Zhang† and Stefan M. Sievert*

- **Genome comparison of**
  - **3 species of same genus**
  - **3 species from different genera, but same habitat**
- **Most genes are lineage specific**
- **Select habitat specific genes**

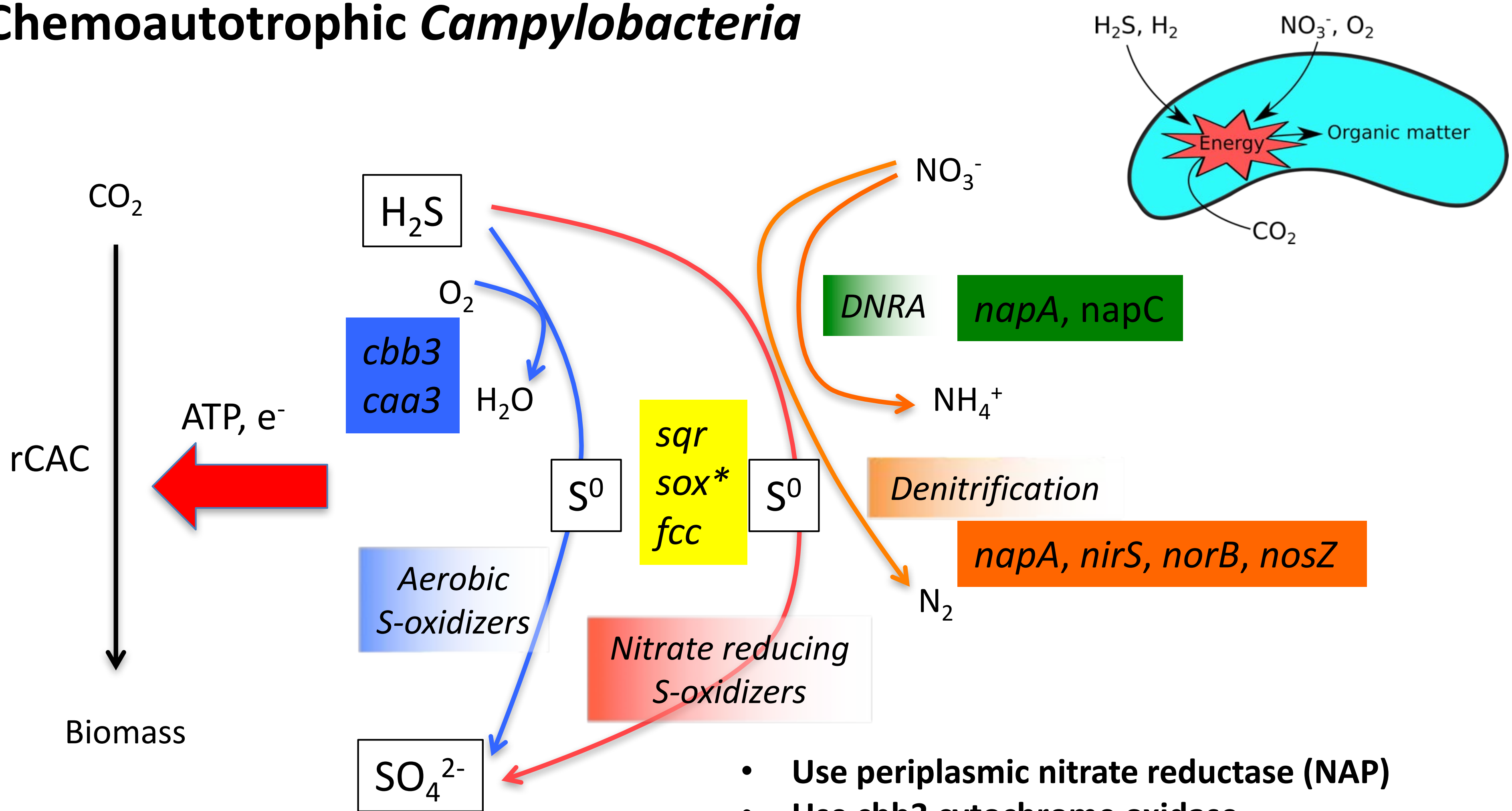
Zhang & Sievert, 2014

Campylobacteria inhabitit a variety of niches at deep-sea hydrothermal vents



Campbell et al., 2006

Oxidation of Sulfide Coupled to Reduction of O_2 and NO_3^- in Chemoautotrophic *Campylobacteria*



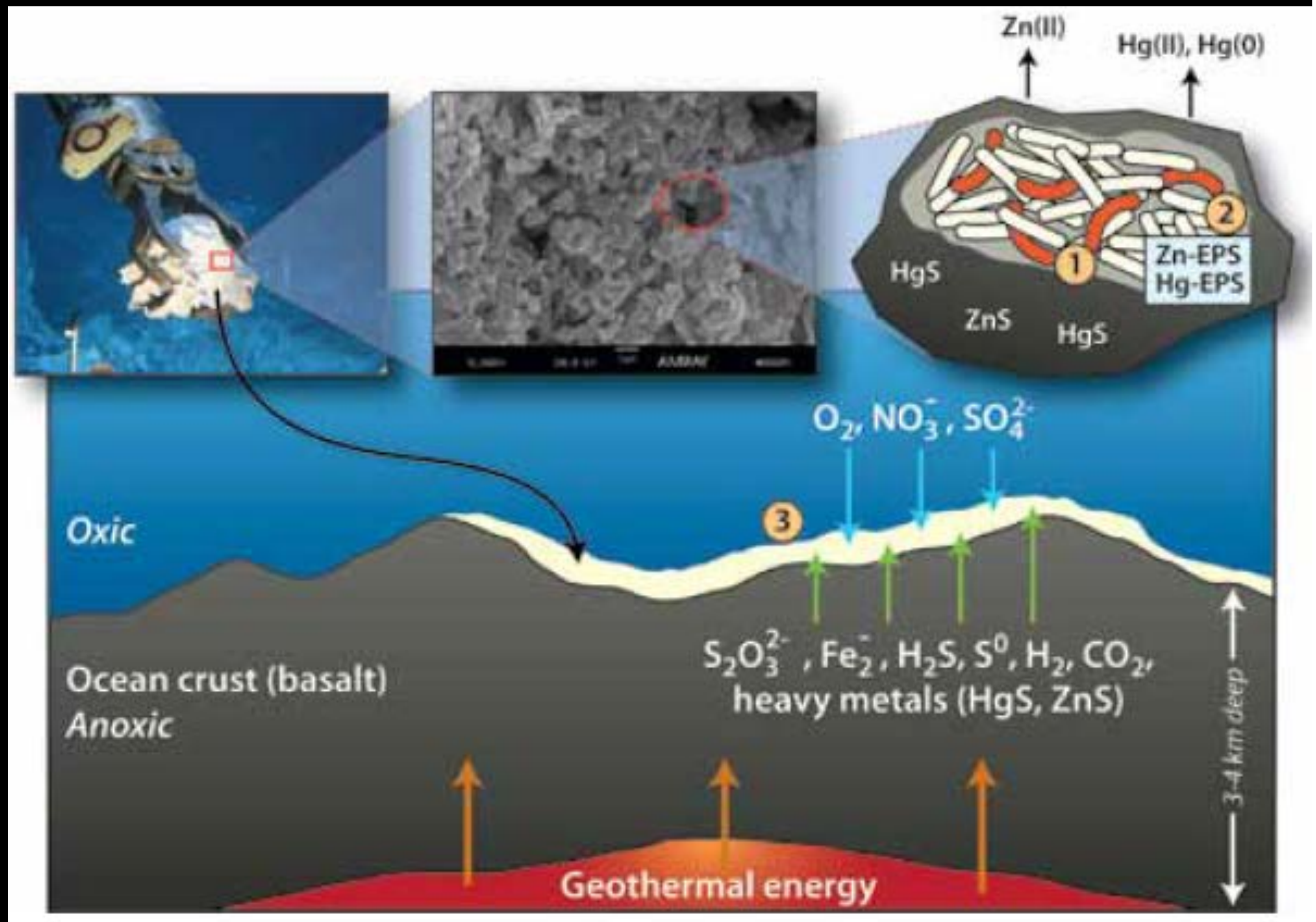
SQR, FCC : S^{2-} -> S^0
SOX : S^{2-} -> SO_4^{2-}

- **Use periplasmic nitrate reductase (NAP)**
- **Use cbb3 cytochrome oxidase**
- **Adapted to low concentrations of nitrate and oxygen**
  - **-> Vents/animal hosts**
- **Citric acid cycle in pathogens utilizes enzymes of reductive citric acid cycle in chemoautotrophs**

*soxABCDXYZ

Colonization of animate and inanimate surfaces at deep-sea vents

- Facilitation of larval settlement
- Trophic interactions
- Symbiotic associations



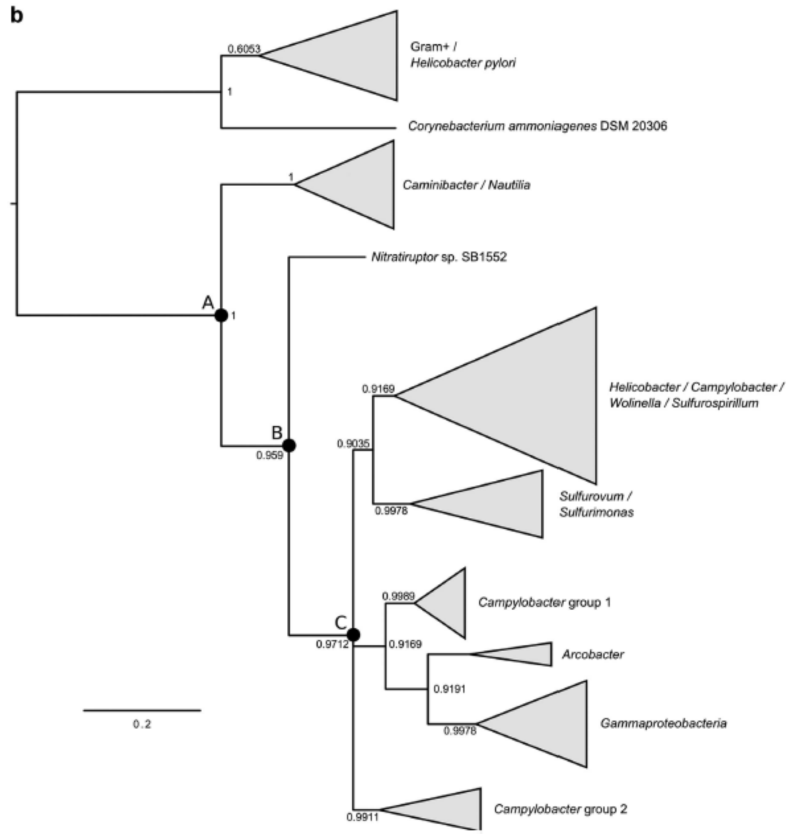
Sievert & Vetriani, 2012

The ISME Journal (2014), 1–13
www.nature.com/ismej

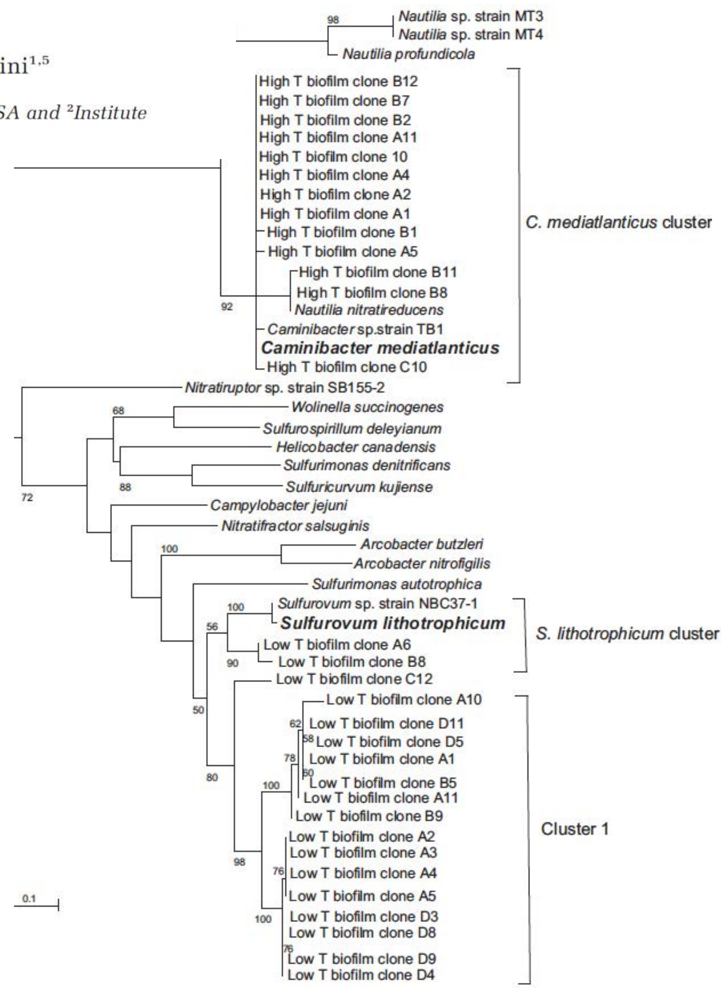
ORIGINAL ARTICLE

From deep-sea volcanoes to human pathogens: a conserved quorum-sensing signal in *Epsilonproteobacteria*

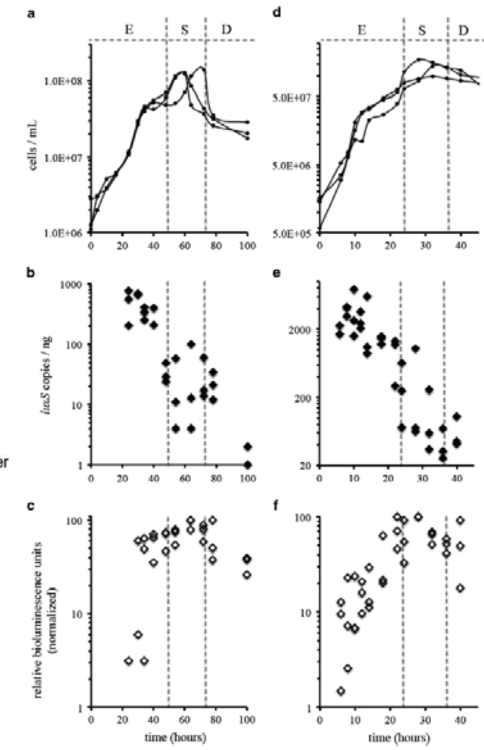
Ileana Pérez-Rodríguez[1,2,3], Marie Bolognini[1,2], Jessica Ricci[1,2,4], Elisabetta Bini[1,5] and Costantino Vetriani[1,2]
[1]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ, USA and [2]Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ, USA



LuxS phylogeny



Expression in vent biofilms



Expression in cultures

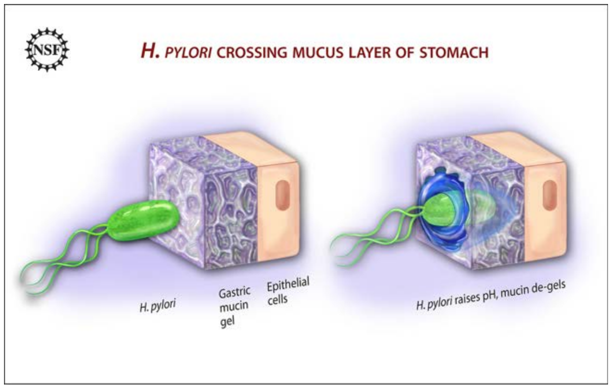
- **Use LuxS/AI-2 as quorum sensing mechanism**
- **Expressed *in situ* in vent biofilms**
- **Stability under harsh conditions?**

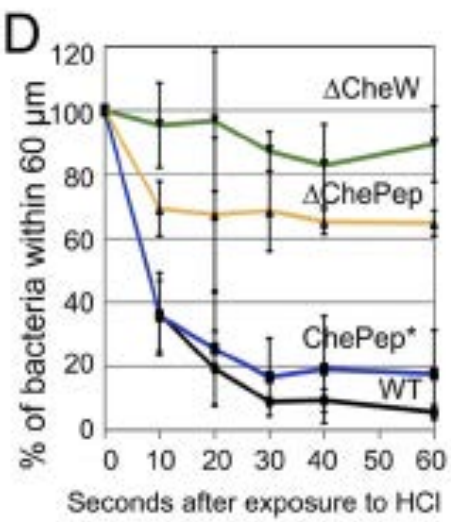
RESEARCH ARTICLE

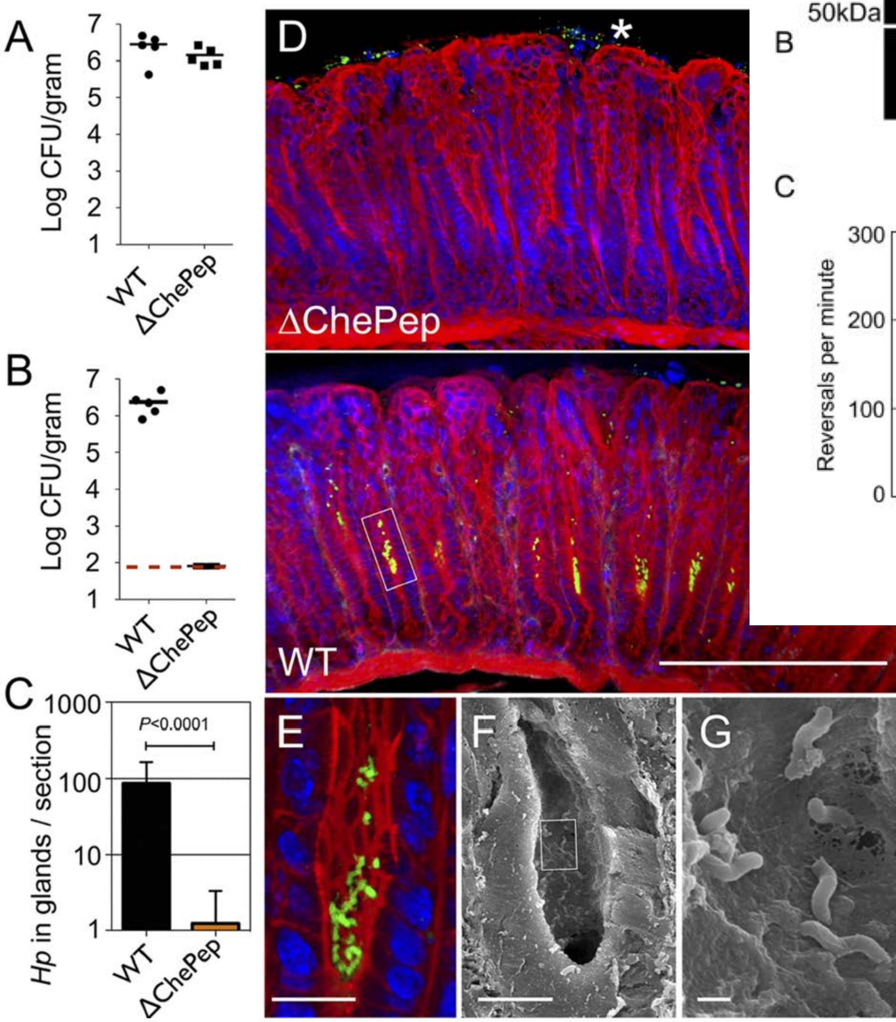
mBio July/August 2011 Volume 2 Issue 4 e00098-11

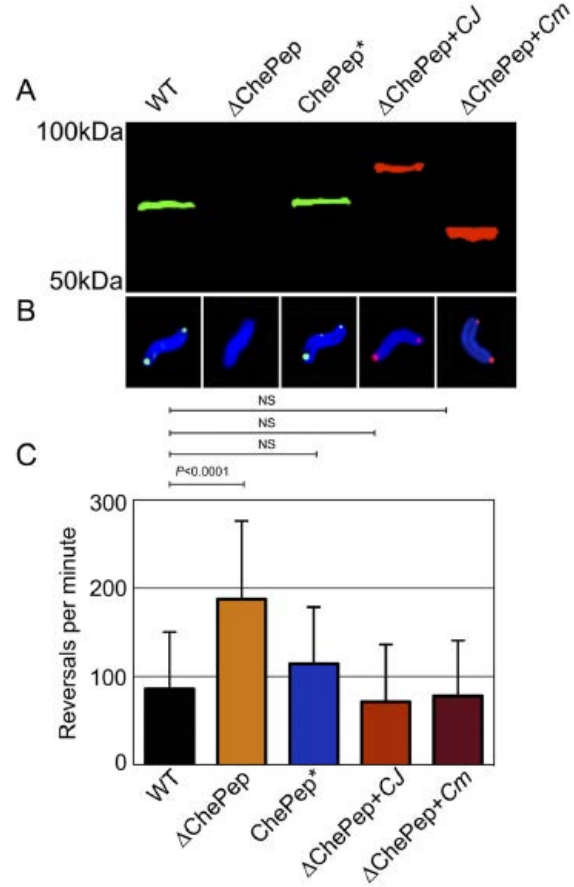
ChePep Controls *Helicobacter pylori* Infection of the Gastric Glands and Chemotaxis in the *Epsilonproteobacteria*

Michael R. Howitt,[a] Josephine Y. Lee,[a] Paphavee Lertsethtakarn,[b] Roger Vogelmann,[a*] Lydia-Marie Joubert,[c] Karen M. Ottemann,[b] and Manuel R. Amieva[a,d]



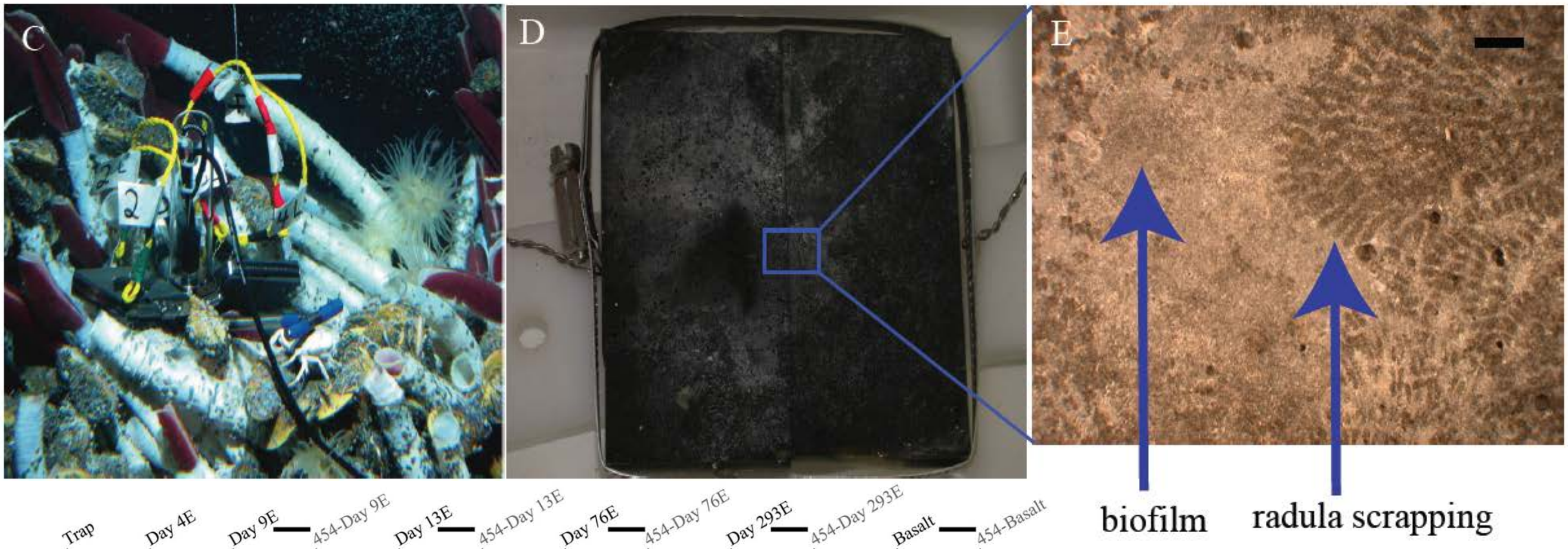


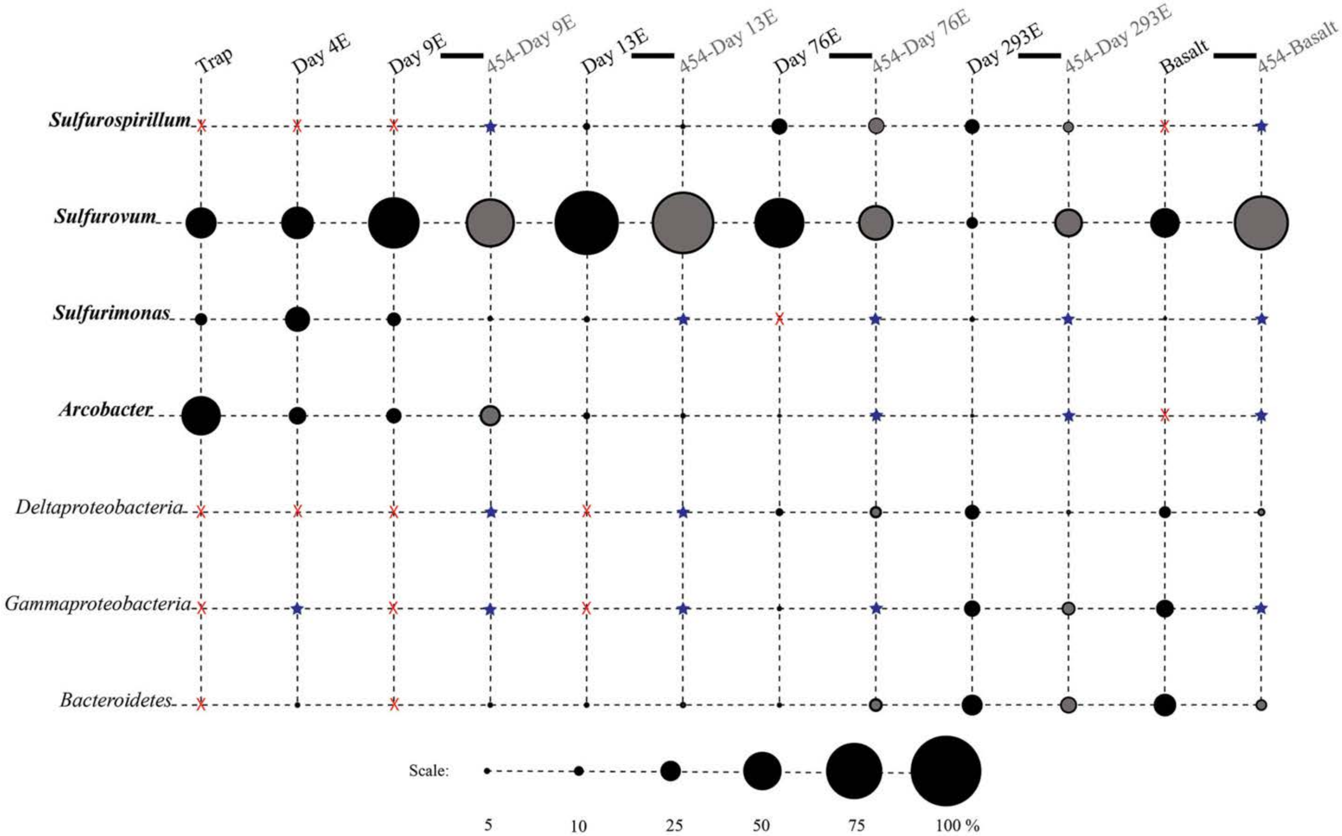




Colonization of gastric glands

Microbial succession on basalt rock: Foundation for Ecosystem



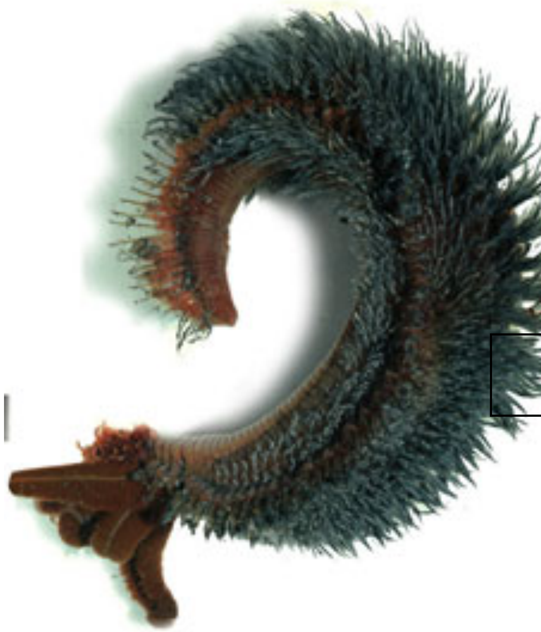


Bacterial diversity and successional patterns during biofilm formation on freshly exposed basalt surfaces at diffuse-flow deep-sea vents

Lara K. Gulmann[1], Stace E. Beaulieu[1], Timothy M. Shank[1], Kang Ding[2], William E. Seyfried[2] and Stefan M. Sievert[1]*

Gulmann et al, 2015

Cooperative Associations:
Alvinella pompejana and *Campylobacteria* episymbionts

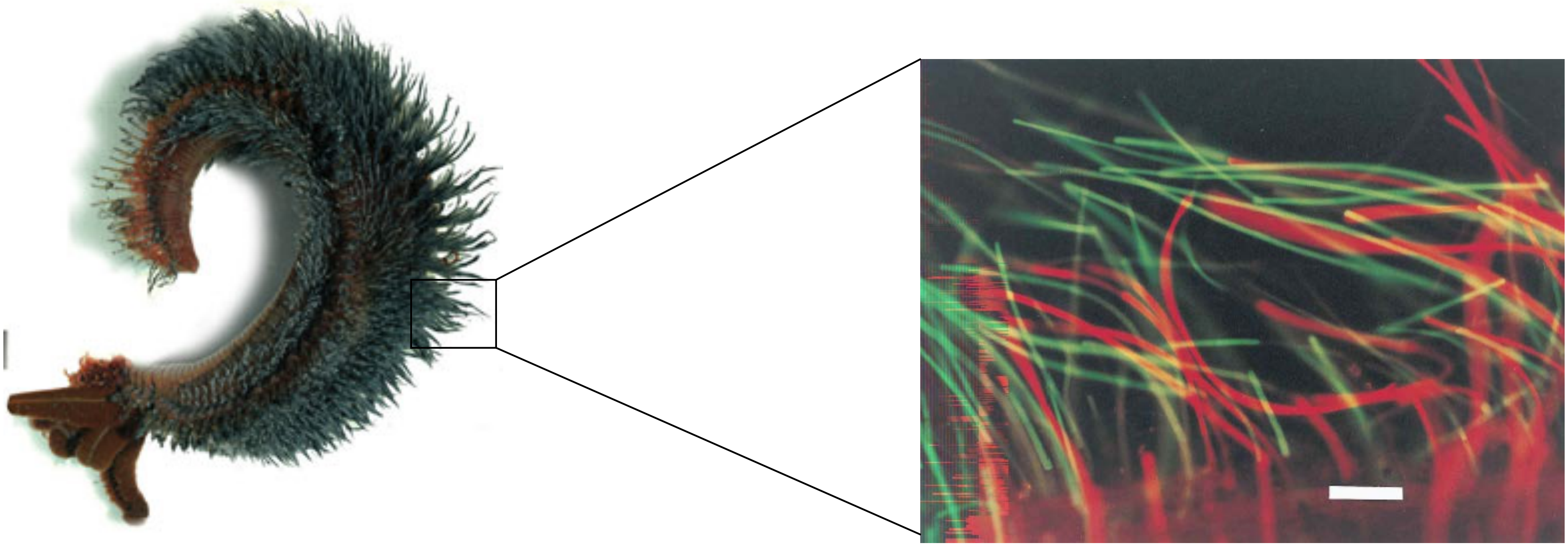


Alvinella pompejana

- **Worms colonize outside of black-smoker chimneys**
- **Most eurythermal animal known, can tolerate 60^{o}C**
- **Colonized by highly specific assemblage of *Campylobacteria***

Cary et al, 1997

Cooperative Associations:
Alvinella pompejana and *Campylobacteria* episymbionts



Alvinella pompejana

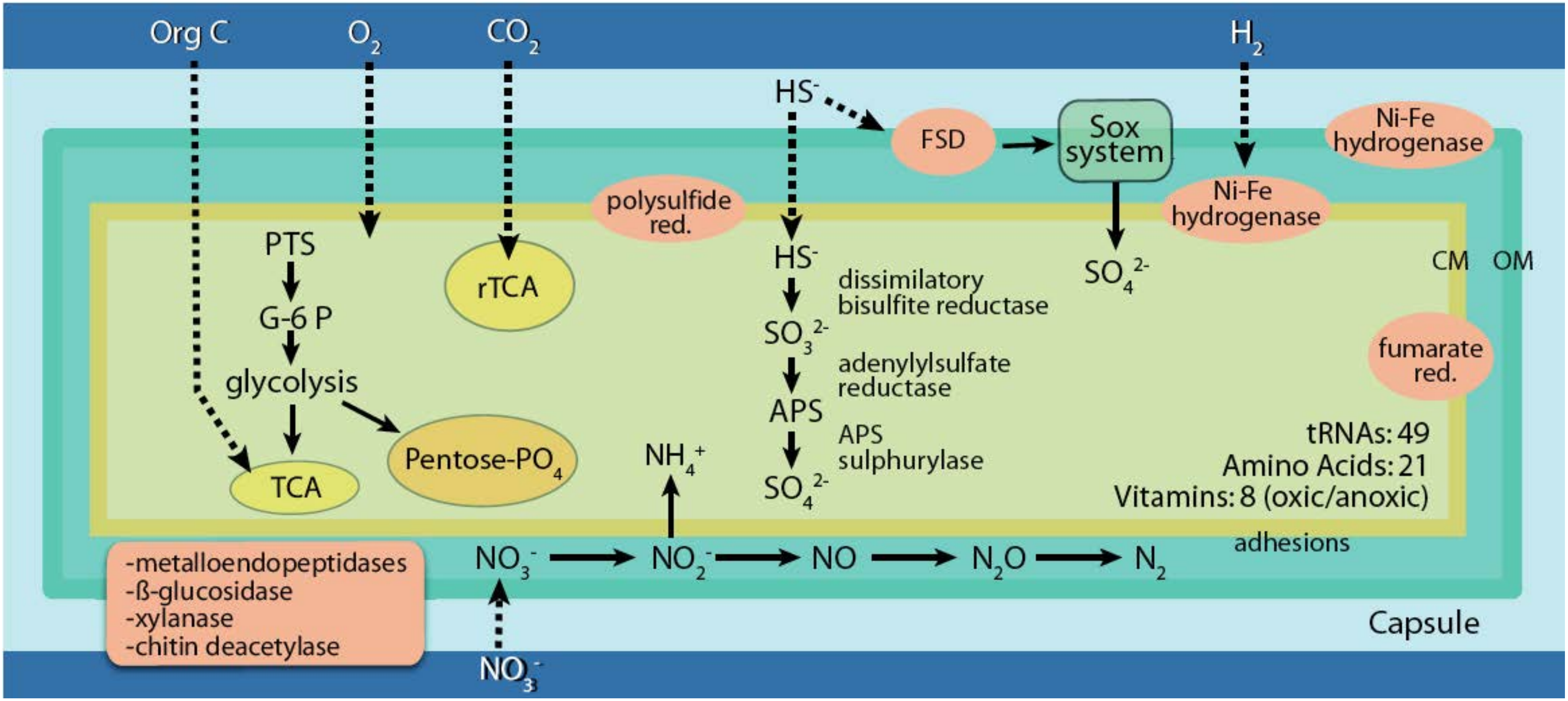
FISH analysis of *Epsilonproteobacteria* attached to the hair structures of *Alvinella pompejana*

Cary et al., 1997. *Appl. Environ. Microbiol.* 63:1124-1130

- **Worms colonize outside of black-smoker chimneys**
- **Most eurythermal animal known, can tolerate 60°C**
- **Colonized by highly specific assemblage of *Campylobacteria***

Cary et al, 1997

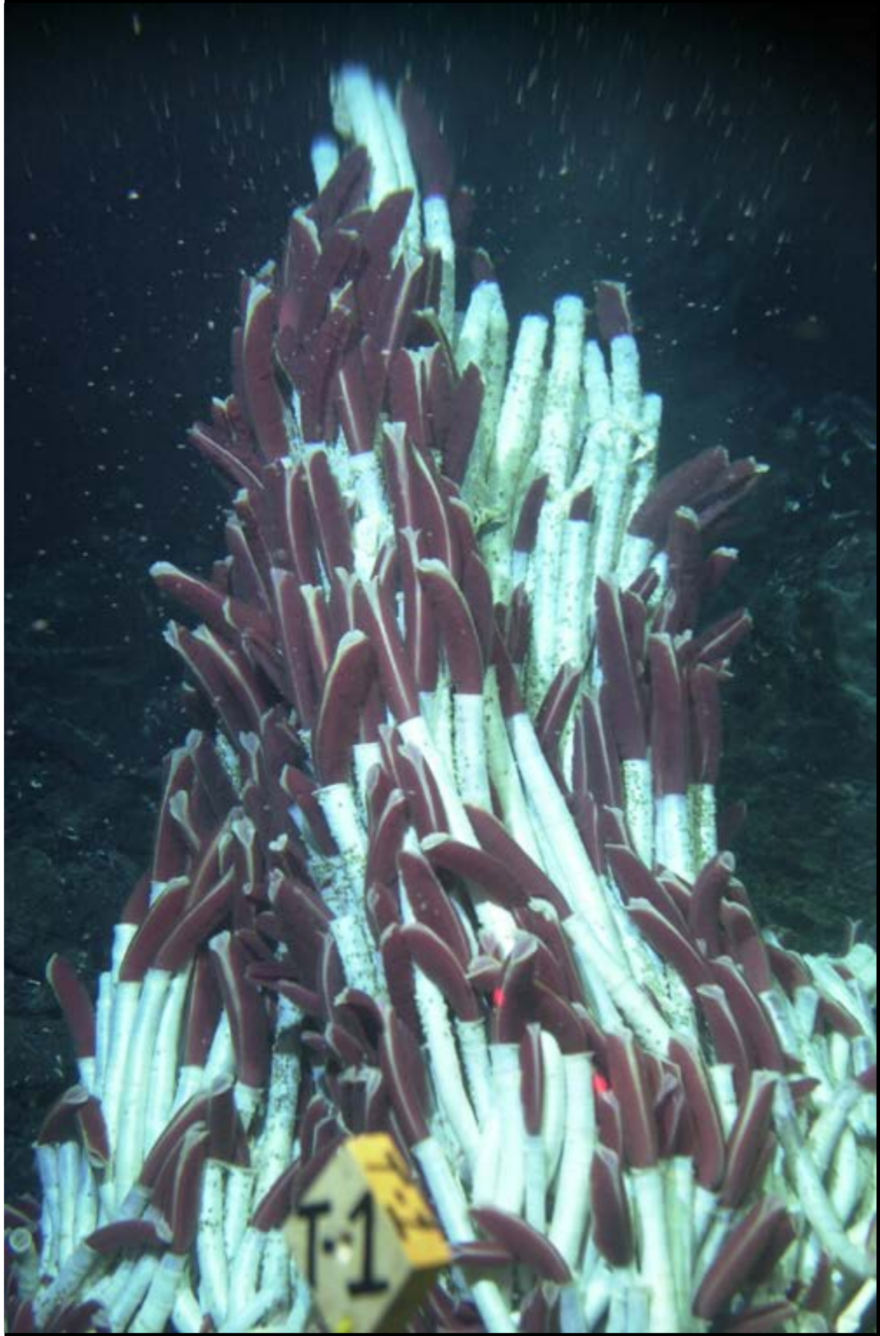
Model of **predicted** metabolic processes in the episymbiont community based on annotation of the episymbiont metagenome



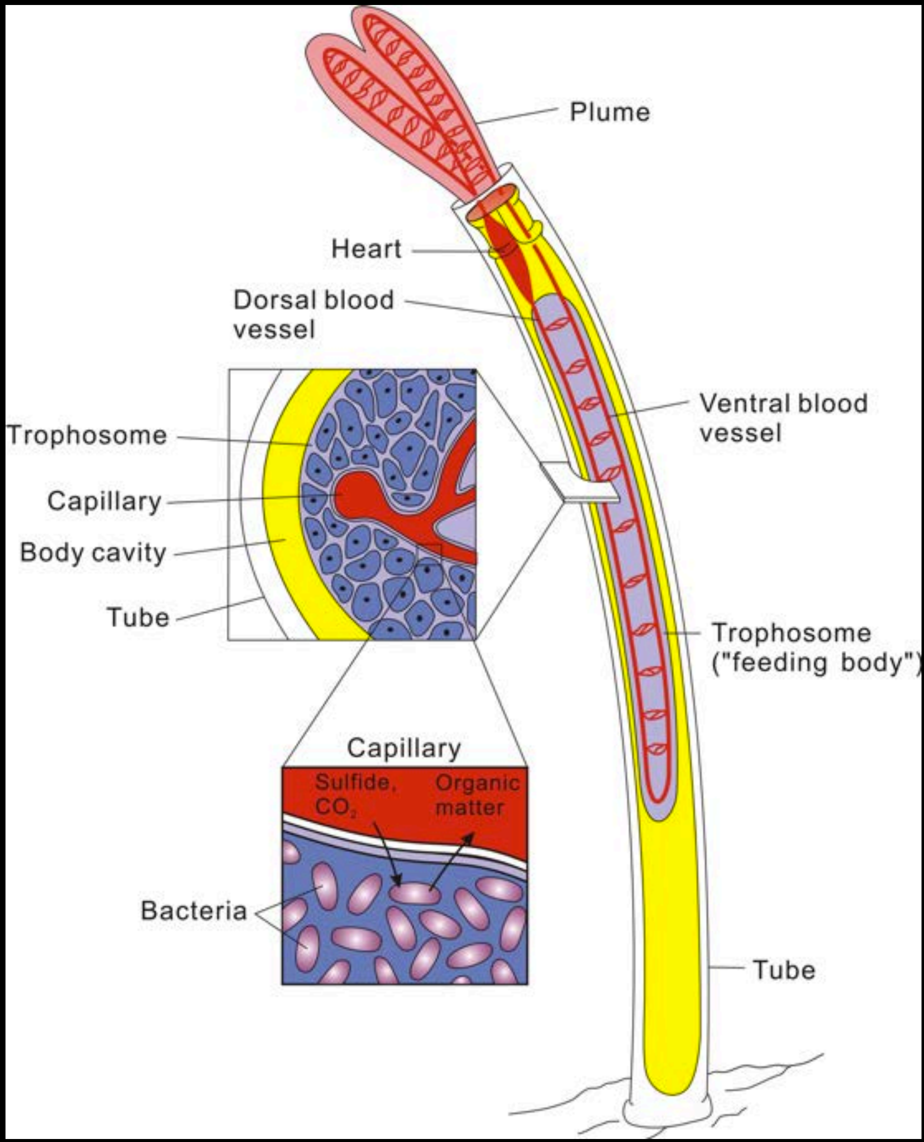
- **Filamentous bacteria provide food and can provide protection from high levels of sulfide and toxic metals (arsenic, cadmium, copper).**
- **Highly specific association, requiring microbe-host recognition**

Grzymski et al., 2008. Proc. Natl. Acad. Sci. USA 105:17516-17521

Riftia pachyptila: A unique symbiosis



Plume: Takes up sulfide, oxygen, and carbon dioxide to deliver to symbiotic chemosynthetic bacteria which produce sugars to feed the worm

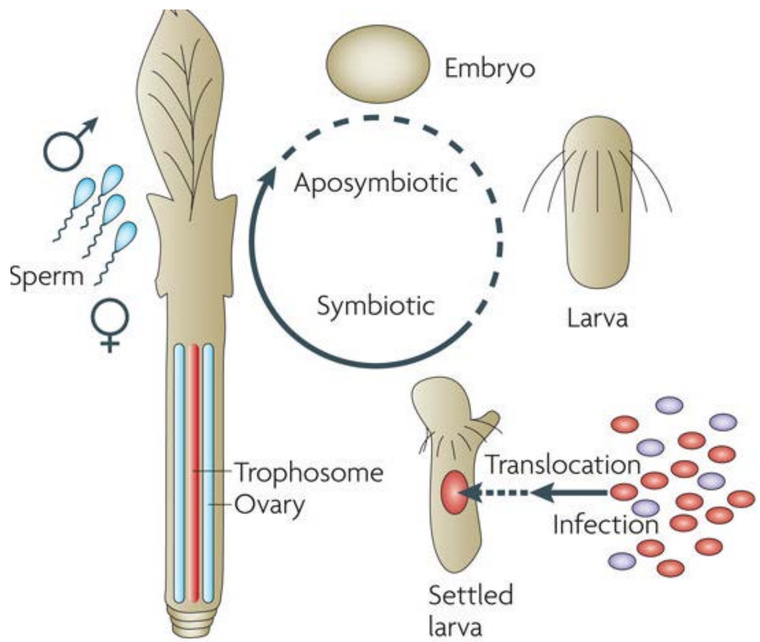


Tube: Provides protection

Throphosome: Houses symbiotic bacteria

Horizontal endosymbiont transmission in hydrothermal vent tubeworms

Andrea D. Nussbaumer[1], Charles R. Fisher[2] & Monika Bright[1]



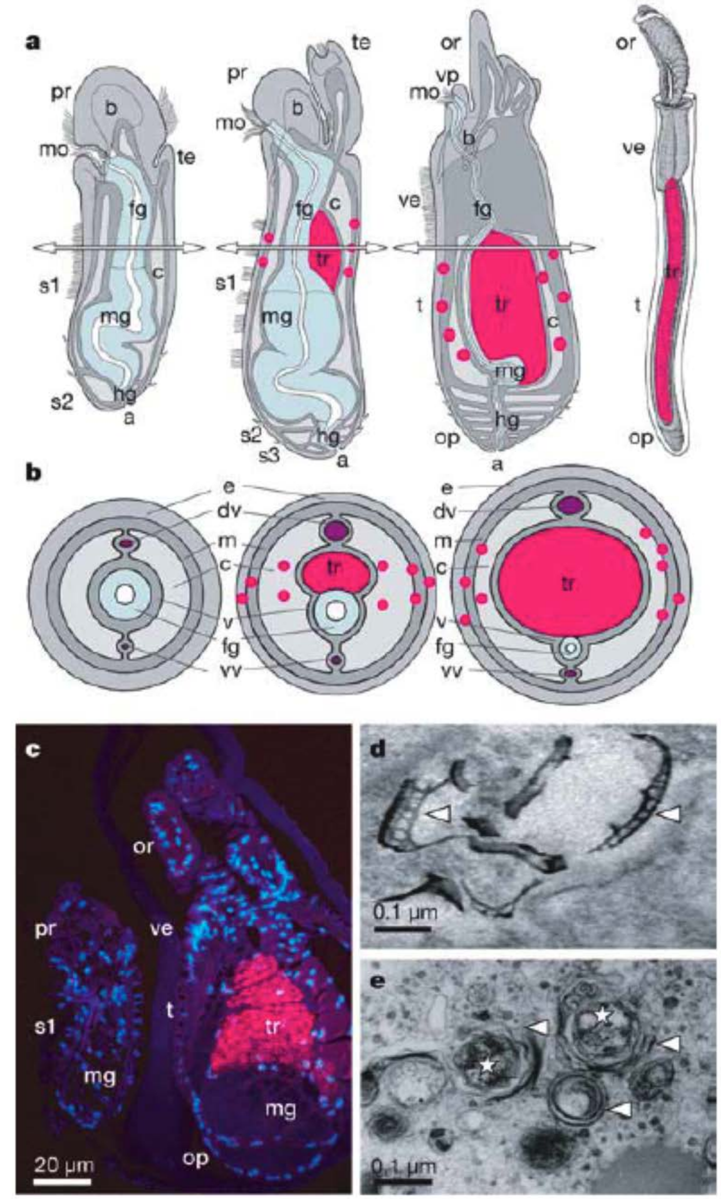


Figure 2 | Symbiont acquisition and early development of recently settled vestimentiferans. Schematic sagittal drawings of animals reconstructed

- **Symbiont is newly acquired from environment w/ each generation**
- **Symbiont acquisition reminiscent of infection**
- **Symbionts penetrate skin of settled tubeworms**
- **'Infected' tissue develops into trophosom**

Activity and Productivity of the Subseafloor Biosphere

Submarine Thermal Springs on the Galápagos Rift

16 March 1979, Volume 203, Number 4385

SCIENCE

John B. Corliss, Jack Dymond, Louis I. Gordon, John M. Edmond, Richard P. von Herzen, Robert D. Ballard, Kenneth Green, David Williams, Arnold Bainbridge, Kathy Crane, Tjeerd H. van Andel

First comprehensive paper on deep-sea vent geology, chemistry, and biology based on 1977 expedition

"This flux of bacteria from the vents must be supported by the ***production of a large population of bacteria living within the rock mass, lining the walls of fissures (and fractures)*** *through which the hydrogen sulfide-laden fluids ascend."*

"and that they may ***significantly influence the chemistry of the system****."*

*"****The generation time*** *for these populations of sulfur-oxidizing bacteria* ***in situ is not known****, but, unlike phytoplankton blooms,* ***their productivity is, presumably, continuous****."*

- Corliss et al, 1979, *Science*, 1979

Deep-Sea Primary Production at the Galápagos Hydrothermal Vents

SCIENCE, VOL. 207, 21 MARCH 1980

D. M. KARL
Department of Oceanography, University of Hawaii, Honolulu 96822
C. O. WIRSEN, H. W. JANNASCH
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

One of first studies to measure primary production at deep-sea vents

In 1980, after follow up study

"In view of the complexity of the entire vent system and the limited amount of sampling possible, **a useful quantification of deep-sea primary production is quite out of reach at this time**."

-Karl, Wirsen and Jannasch, *Science,* 1980

Today

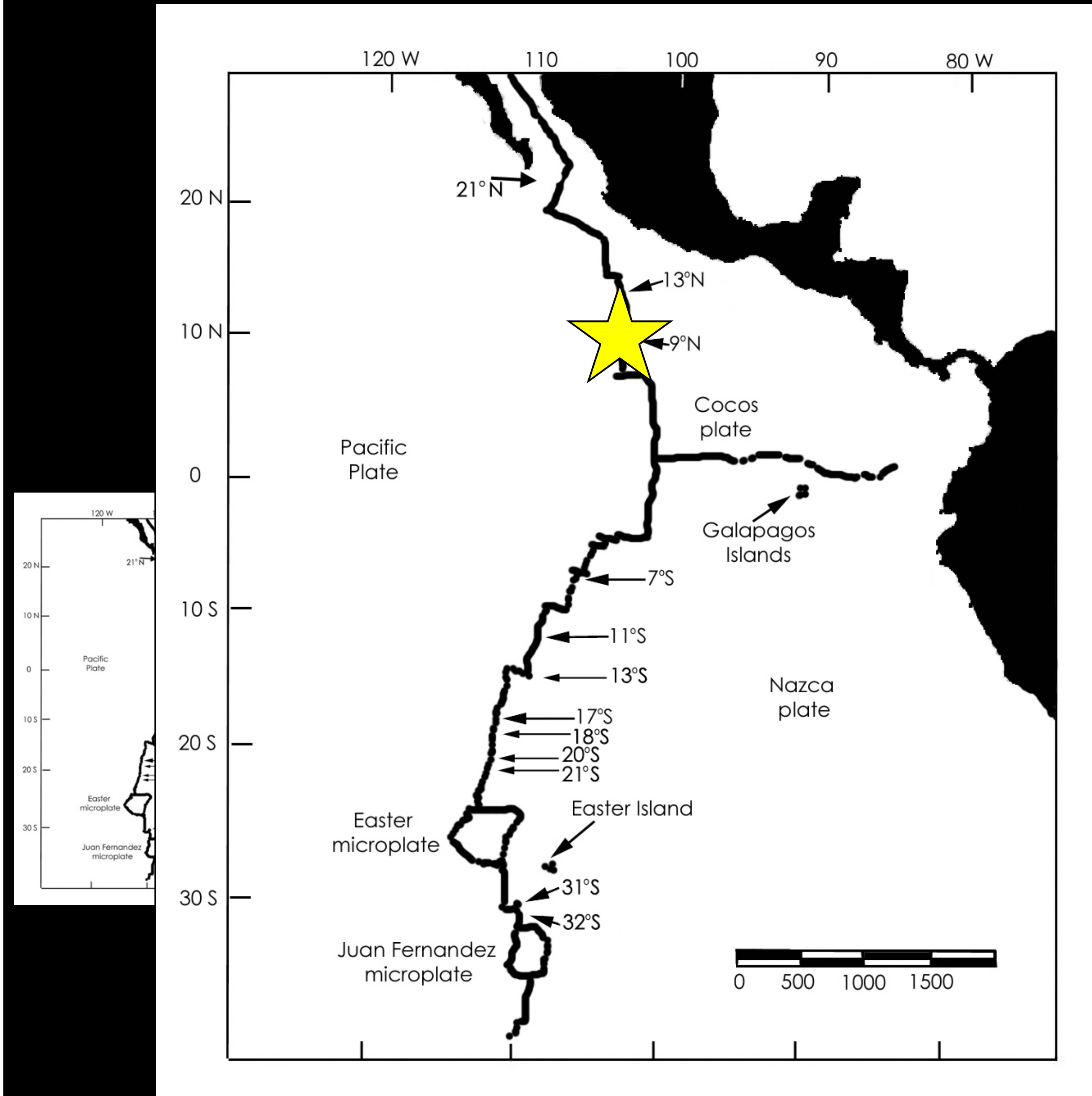
"In view of the complexity of the entire vent system and the limited amount of sampling possible, **a useful quantification of deep-sea primary production is quite out of reach at this time**.

-Karl, Wirsen and Jannasch, *Science,* 1980

This goal is now within reach

'CRAB SPA' AT 9°50'N ON EAST PACIFIC RISE

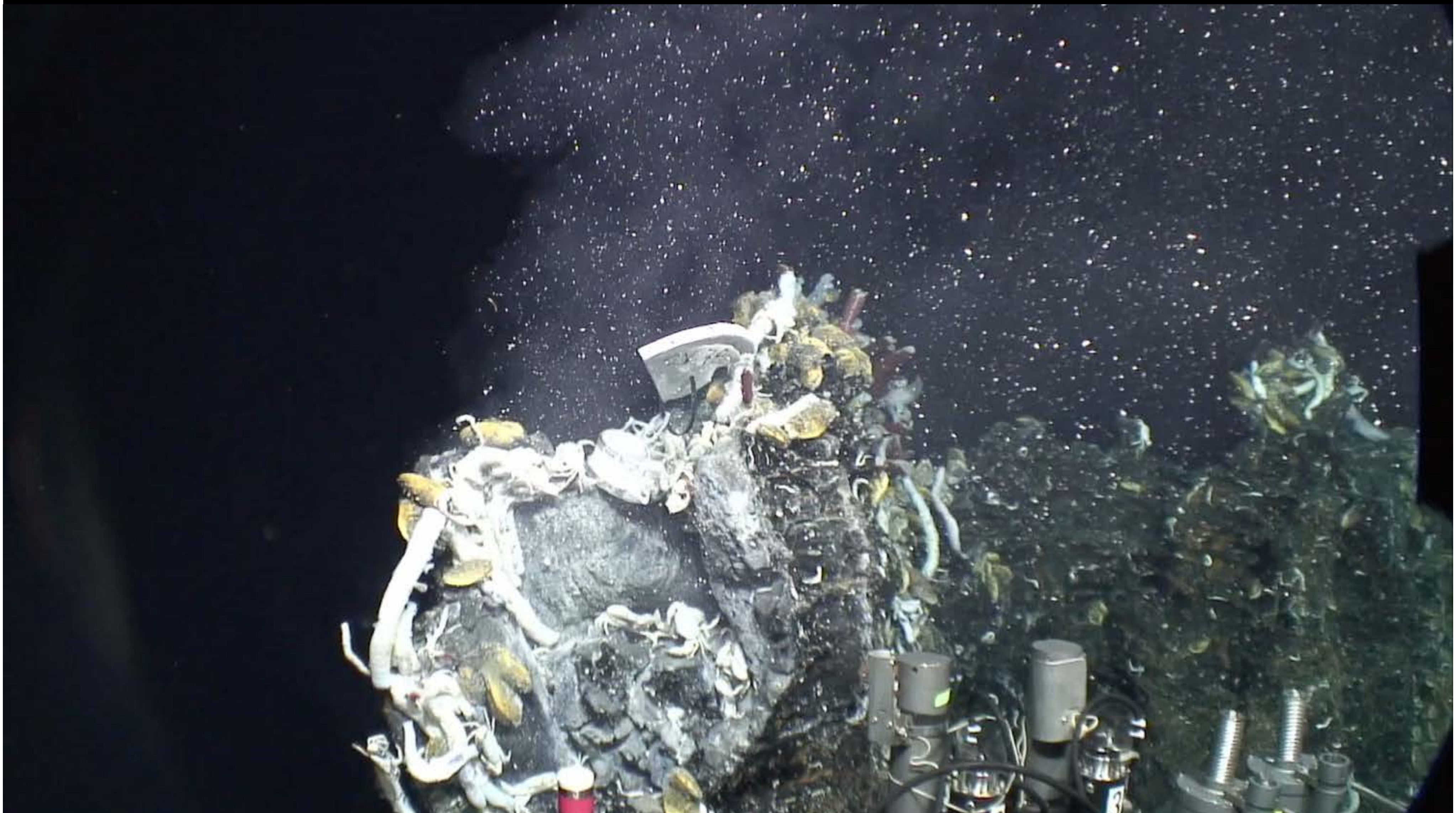
A MODEL SYSTEM TO STUDY CHEMOAUTOTROPHIC PROCESSES AT DEEP-SEA VENTS



<u>Crab Spa at Tica</u>

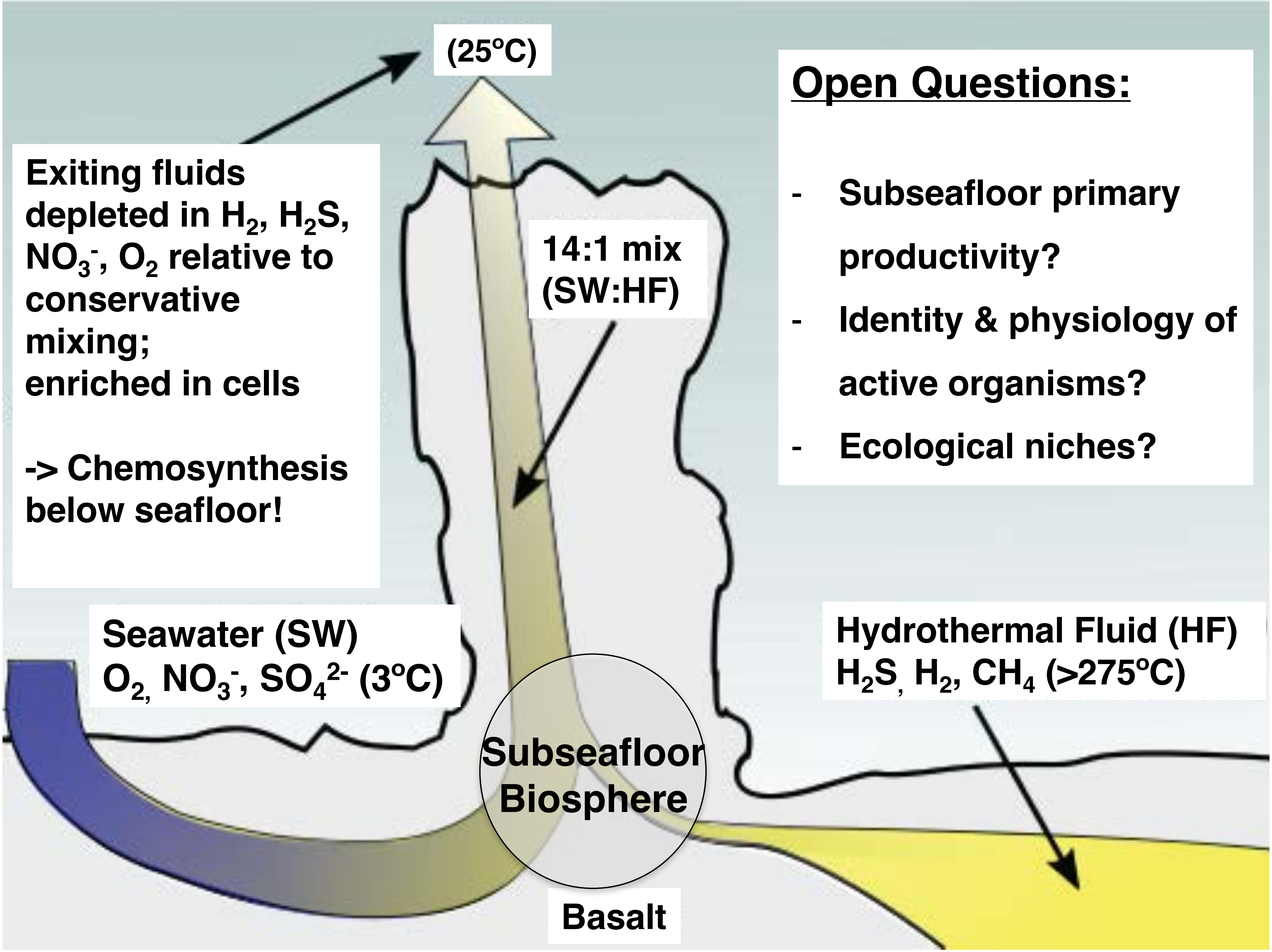
Temp: ~24 (30°C in '07)
H_2S: 300 μM (1 mM in '07)
H_2: ~5 μM
pH: 5.8
O_2: <2 μM, NO_3^{2-}: <7μM
Sampled in Jan '07, Jan '08, Oct '08, May '10, May '12, Jan '14, Nov '14, May '17

National Deep Submergence Facility at WHOI: Alvin, Jason & Sentry

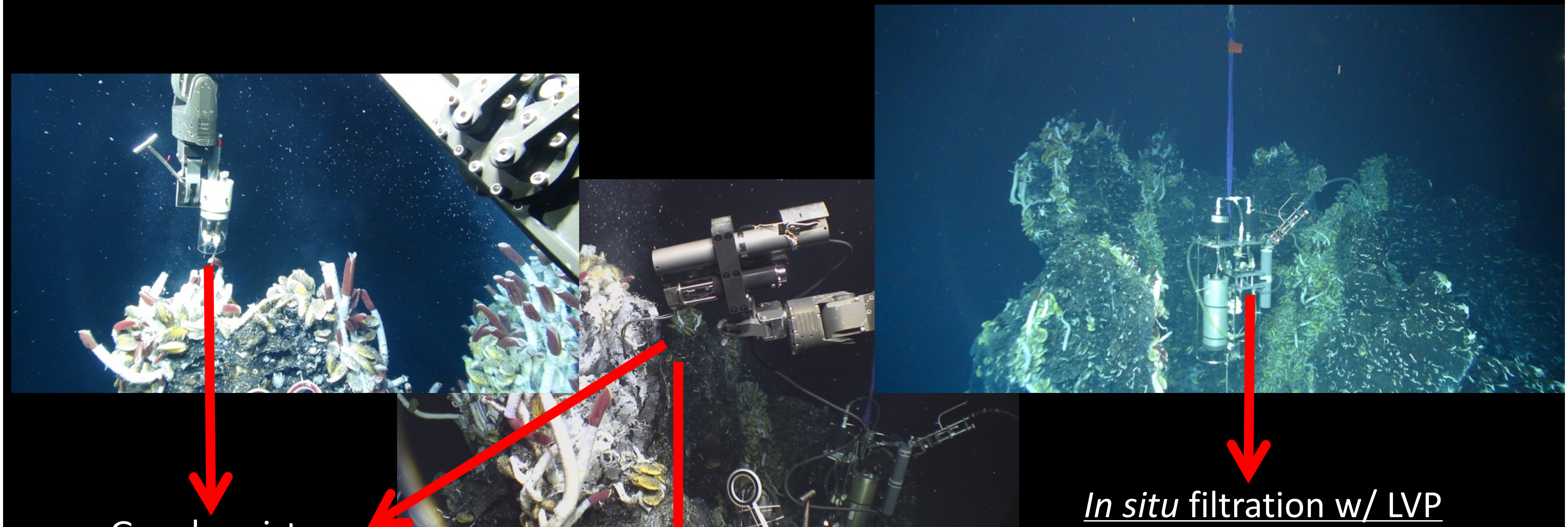


'Crab Spa'

‘Snow Blower’


(25°C)
Open Questions:
- Subseafloor primary productivity?
- Identity & physiology of active organisms?
- Ecological niches?
Exiting fluids depleted in H_2, H_2S, NO_3^-, O_2 relative to conservative mixing;
enriched in cells
-> Chemosynthesis below seafloor!
14:1 mix (SW:HF)
Seawater (SW)
O_2, NO_3^-, SO_4^{2-} (3°C)
Hydrothermal Fluid (HF)
H_2S, H_2, CH_4 (>275°C)
Subseafloor Biosphere
Basalt

Multifaceted Approach to Study Crab Spa Microbiome



<u>Geochemistry</u>

1. *In situ*
2. Lab based

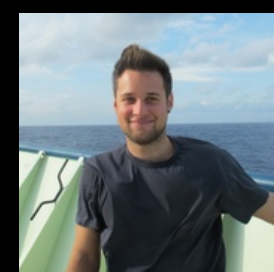
<u>Samples w/ IGT fluid samplers</u>

1. **Shipboard incubations**
2. CARD-FISH
3. Single-cell Amplified Genomes (SAG)

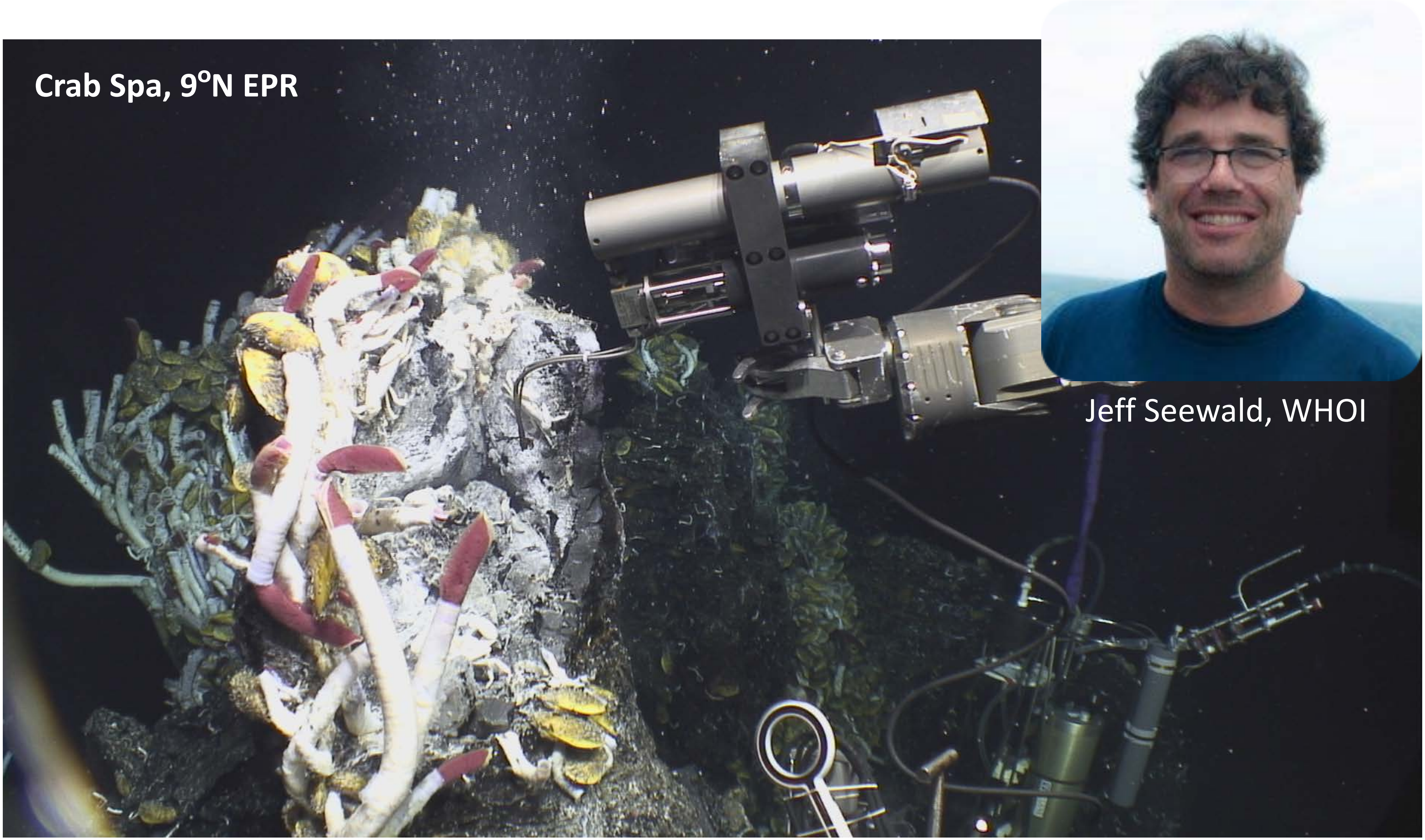
<u>*In situ* filtration w/ LVP</u>

1. 16S rRNA pyrotags
2. Metagenome
3. Metatranscriptome
4. **Metaproteome**
5. Lipids

Jesse McNichol, François Thomas



Florian Götz



Isobaric Gas Tight Sampler (IGT) to collect fluids for chemical analyses and investigating microbial activity at *in-situ* pressure and temperature

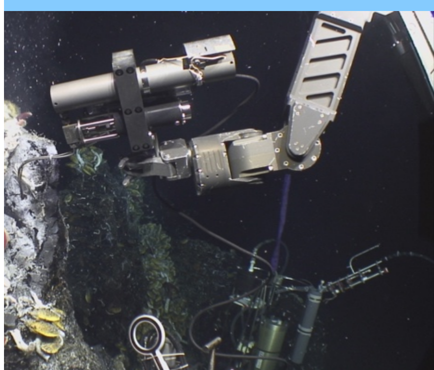
Shipboard Incubations with Hydrothermal Fluids under simulated *in situ* conditions

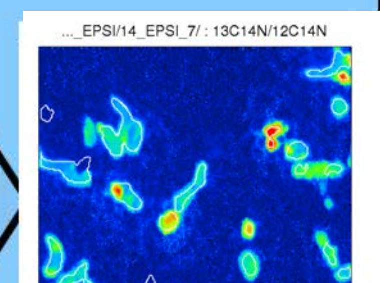
Jesse McNichol

Sample Fluids → Return under pressure → Add substrates → Monitor growth

IGT

nano-SIMS



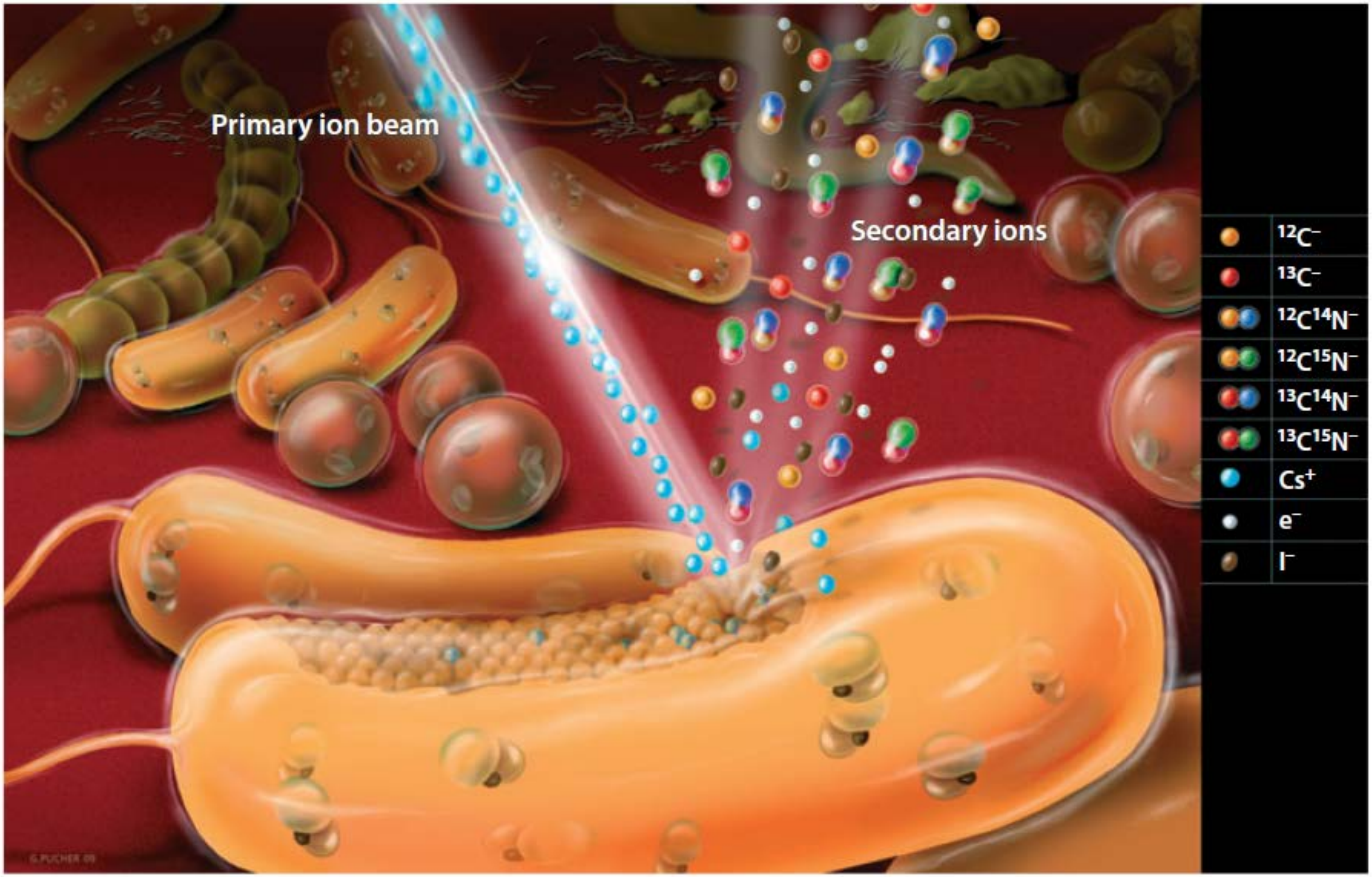


- Added ^{13}C-labeled bicarbonate to incubations conducted at *in situ* pressure, different temperatures and conditions
- Analyses at single cell level with HISH-SIMS: Nanoscale-Secondary Ion Microprobe coupled with Halogen In Situ Hybridization

McNichol et al. 2016 DSR-I, McNichol et al. 2018 PNAS

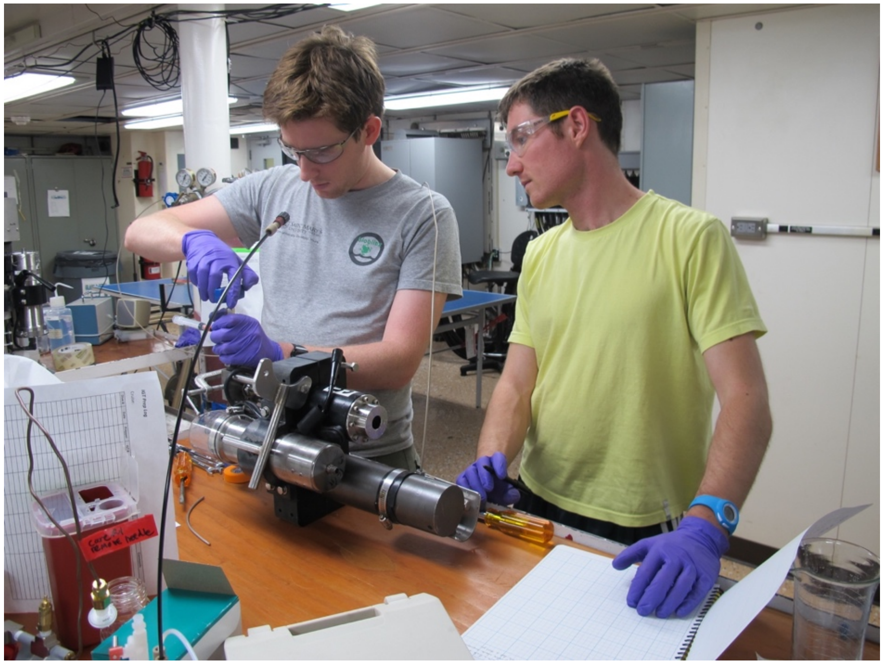
Niculina Musat, UFZ Leipzig

Nano-SIMS is mass spectrometry on individual cells

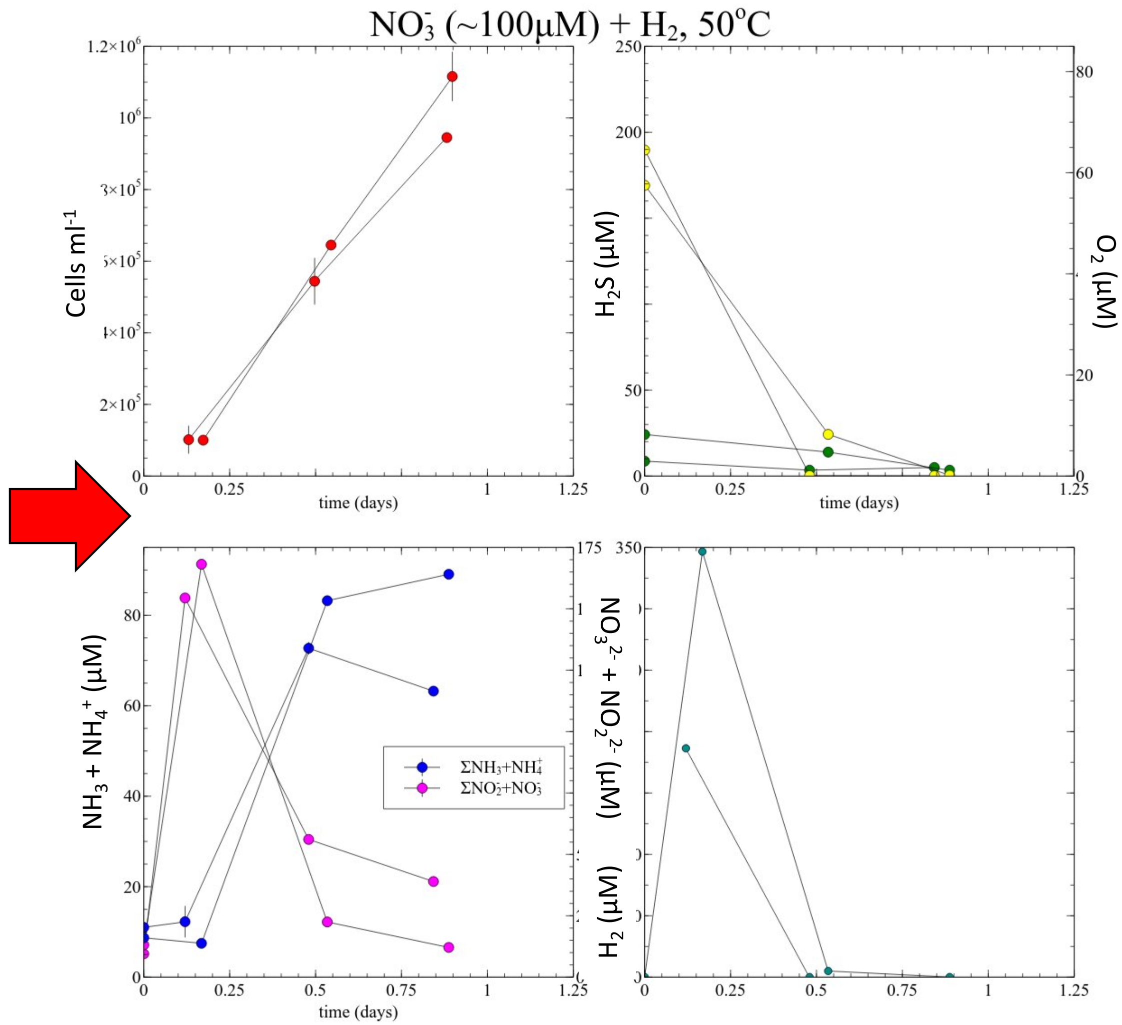


Wagner, 2009, Annual Review of Microbiology

SHIPBOARD GENERATED DATA

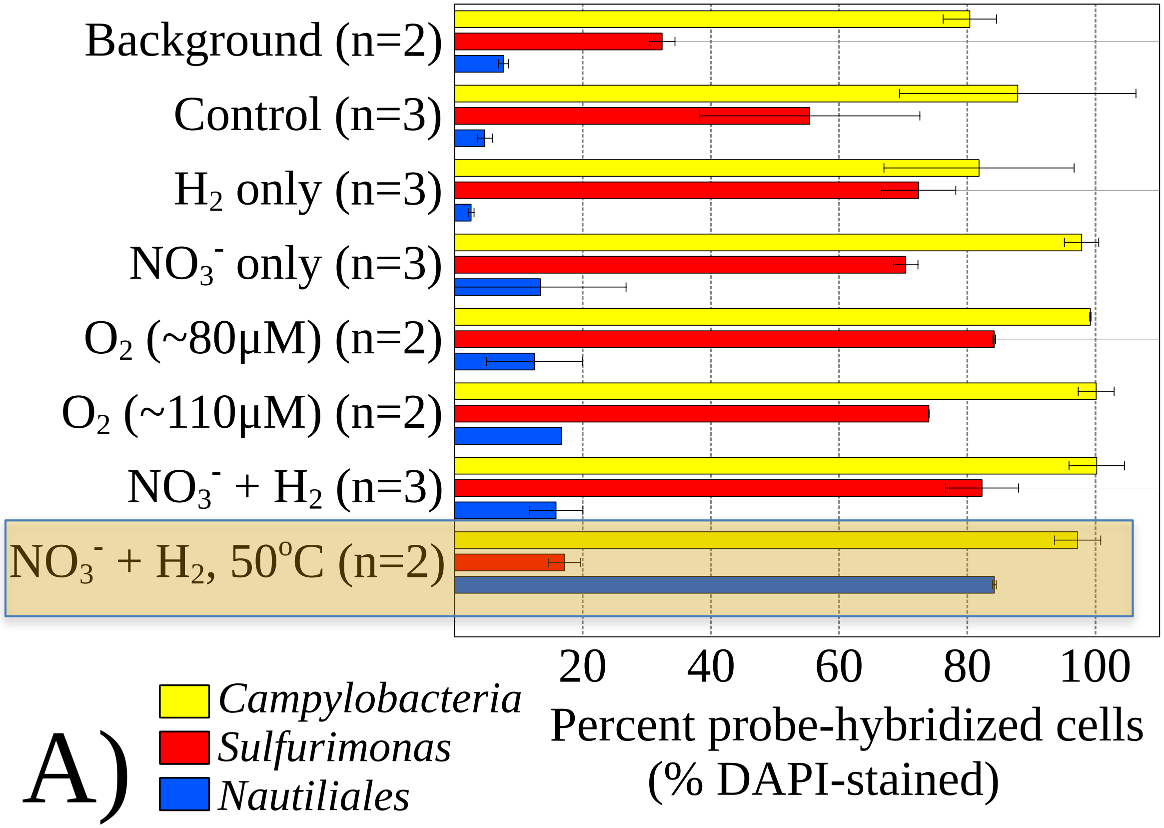


Jesse McNichol & François Thomas

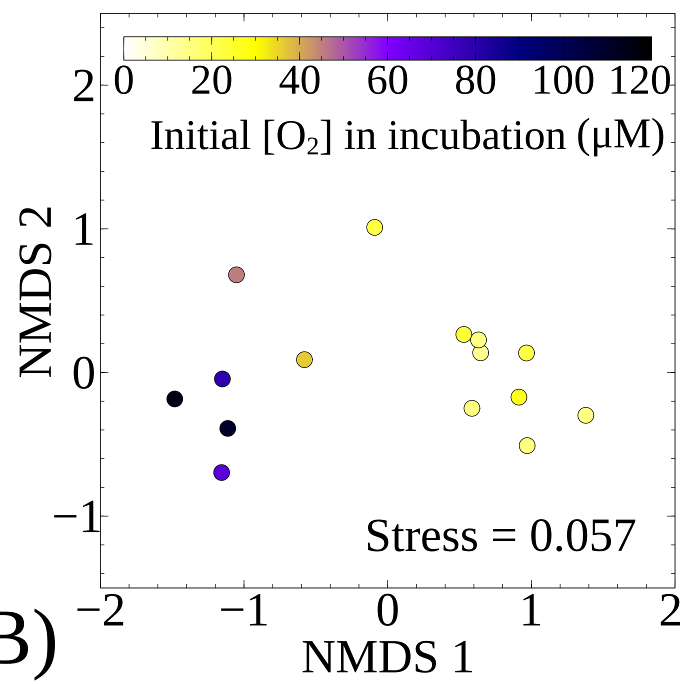


Bacterial community composition during incubations

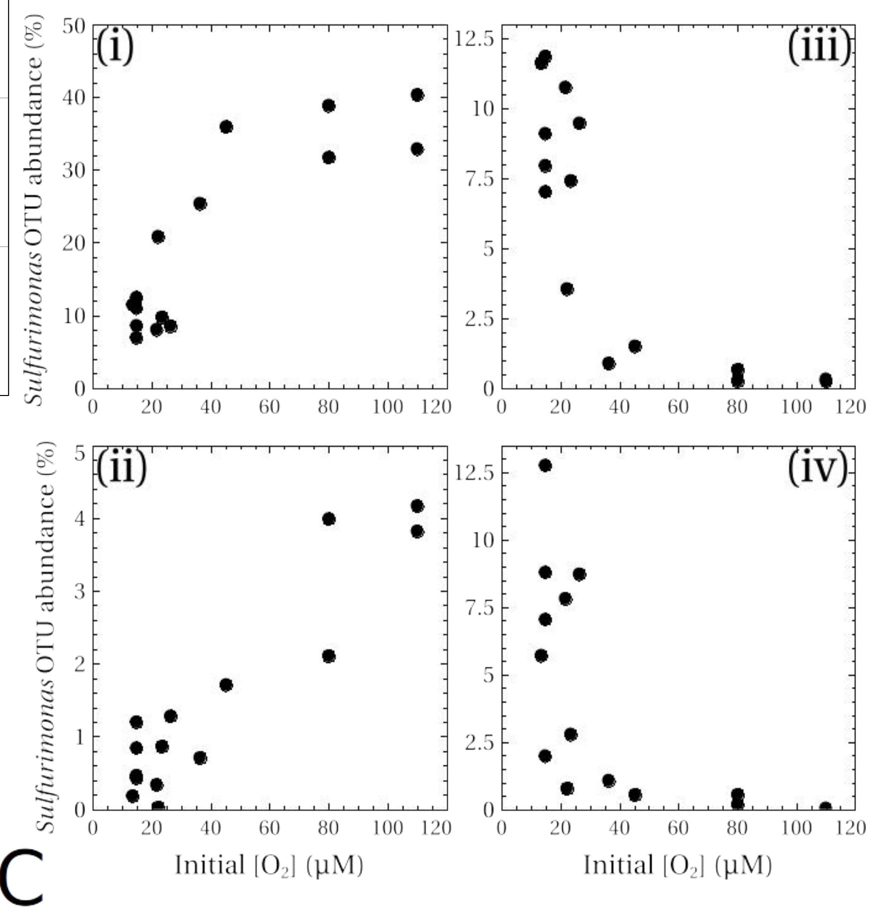
A)



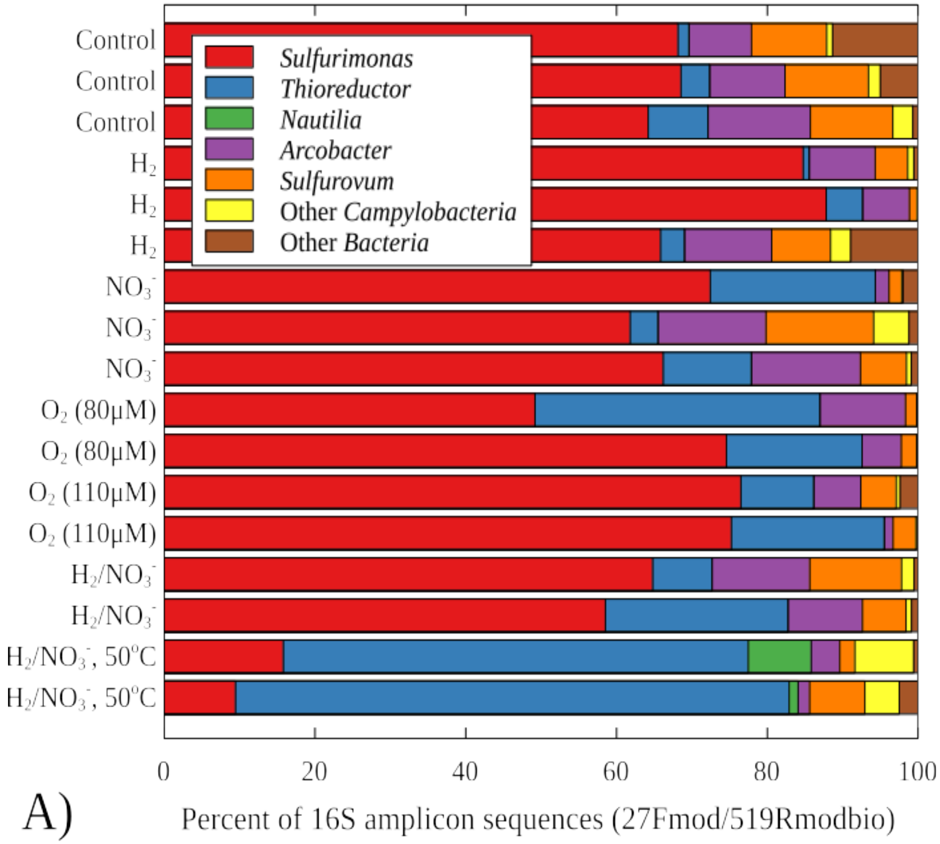
B)



C

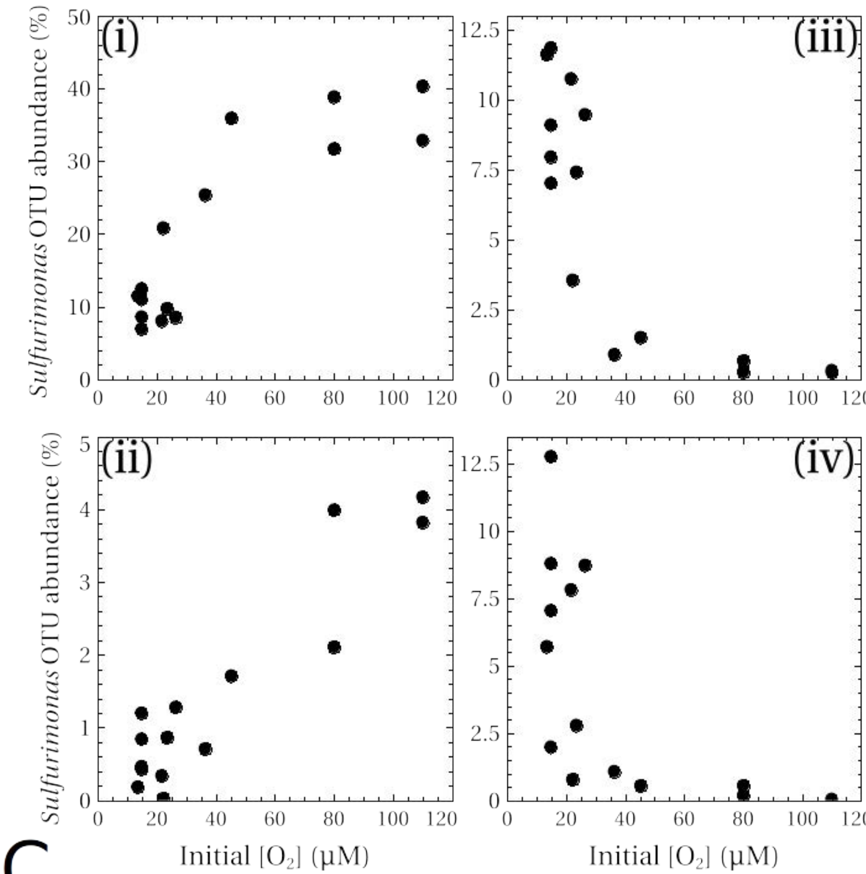


McNichol et al., 2018

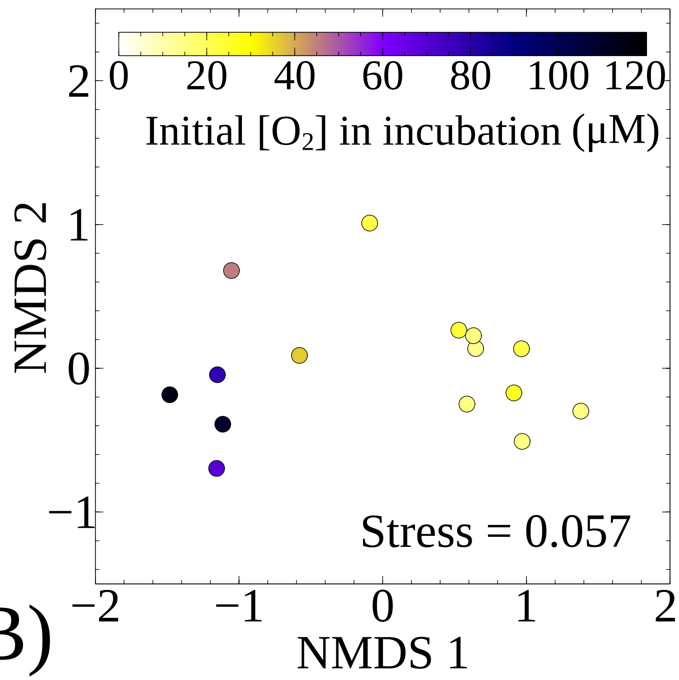


A)

Bacterial community composition during incubations



C

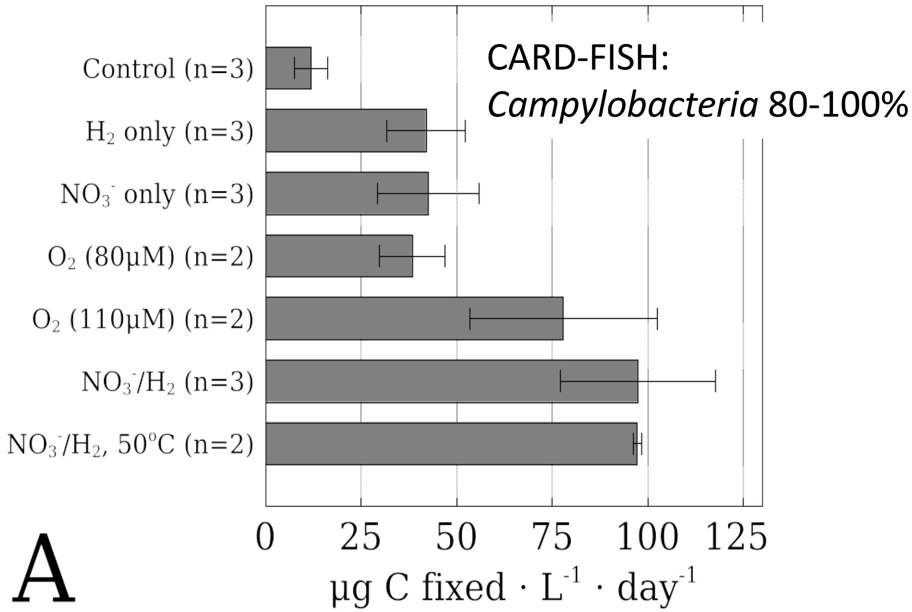


B)

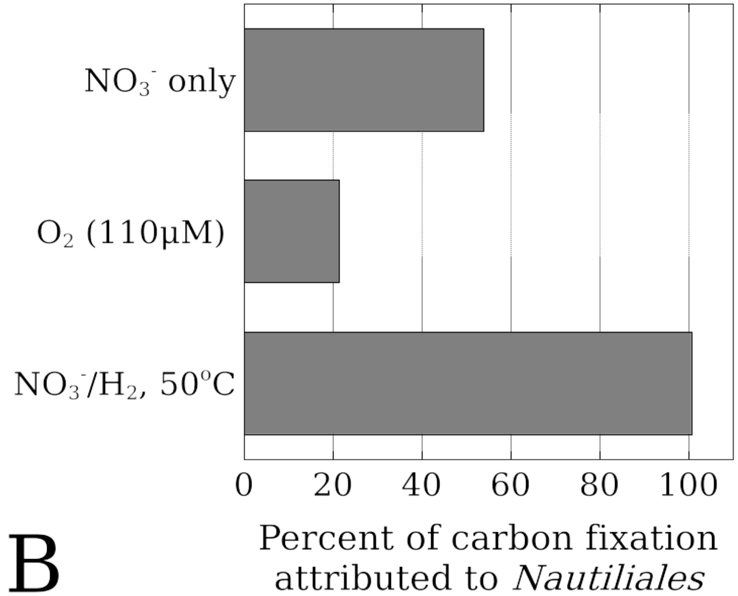
***Sulfurimonas* OTUs respond to differences in O_2-concentration -> niche partitioning?**

McNichol et al., 2018

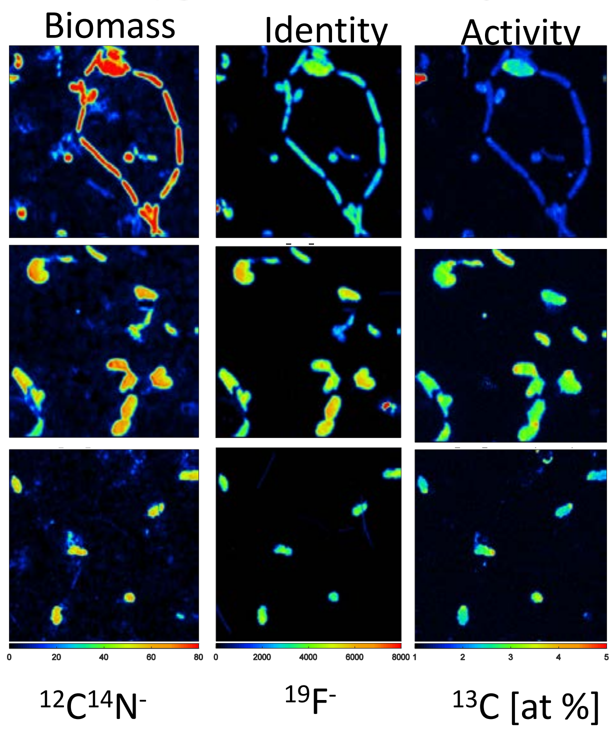
Estimations of Primary Productivity in Incubations of Hydrothermal Vent Fluids at *In Situ* Temperature and Pressure Determined by HISH-SIMS



A



B



- *Campylobacteria* dominate carbon fixation!
- Amendments increased carbon fixation
- Active carbon fixation (and DNRA) also by *Nautiliales* (-> *Thioreductor*) at lower temperatures, w/o H_2, and under oxic conditions
- Evidence for physiological versatility not currently reflected in cultures

McNichol et al., 2018

Constraints on Subseafloor Productivity, Standing Stock, and Turnover from Measurements of CGE

Parameter	Lower bound	Upper bound	Units
Absolute carbon fixation rates[1]	17.3	321.4	$\mu g\ C \circ L^{-1} \circ day^{-1}$
Chemosynthetic growth efficiency[1]	0.06	0.13	Fraction electron equivalents to Carbon fixation
Estimated in situ carbon fixation[2]			
(per L Crab Spa mixed fluid):	104	253	$\mu g\ C \circ L^{-1}$
(per L Crab Spa end-member fluid):	1.4×10^{3}	3.5×10^{3}	
Estimated annual productivity[3] of:			
Crab Spa vent[4]	6.1×10^{3}	1.5×10^{4}	$g\ C \circ y^{-1}$
Surrounding vent field[5]	3.8×10^{6}	9.3×10^{6}	
Global diffuse-flow vents[6]	4.5×10^{10}	1.4×10^{12}	
Standing stock[7], Crab Spa	28.6	NA	g C
Biomass residence time[8], Crab Spa	17	41	hours
Global standing stock[6]	1.4×10^{9}	2.7×10^{9}	g C

McNichol et al., 2018

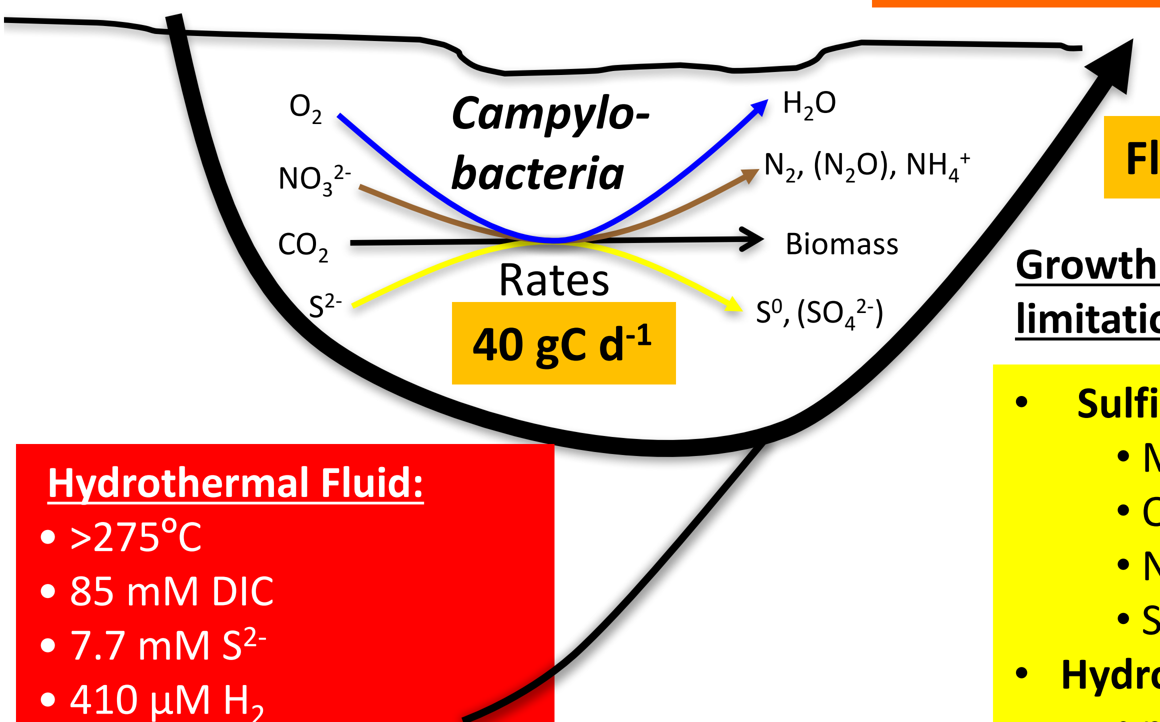
Synthesis

Ambient Deep-Sea Water:
- 2°C
- 2.3 mM DIC
- 115 µM O_2
- 40 µM NO_3^-
- 0 µM S^{2-}, H_2
- 10^4 cells/ml
- ~0% *Campylobacteria*

Diffuse-Flow Fluid:
- 25°C
- 8.2 mM DIC
- < 3.6 µM O_2
- < 6 µM NO_3^-
- ~12 µM NH_4^+
- ~ 200 µM S^{2-}
- < 2 µM H_2
- 2-5 *10^5 cells/ml
- ~80% *Campylobacteria*

Water

Crust

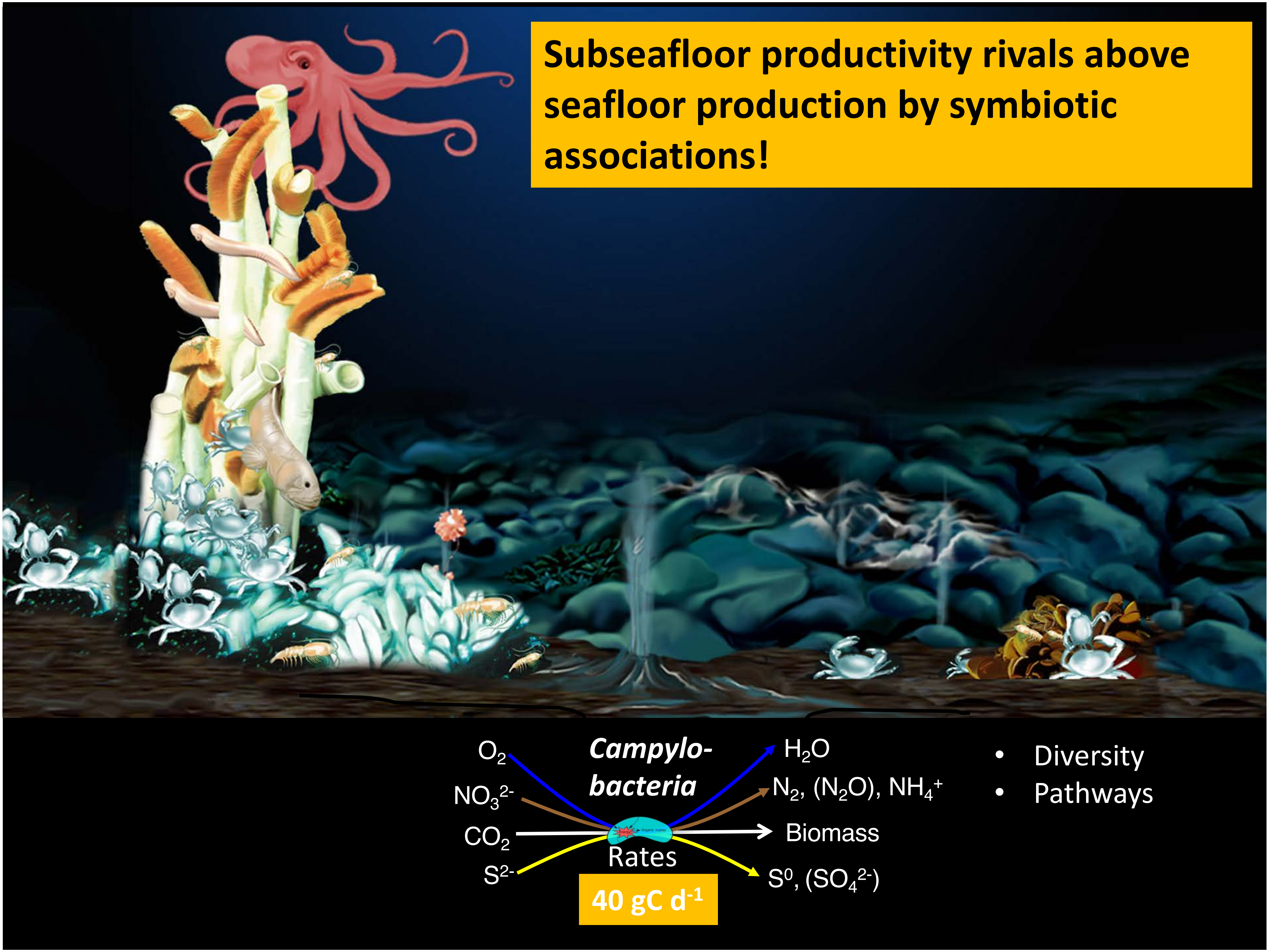


Flow rate: 162 m^3 d^{-1}

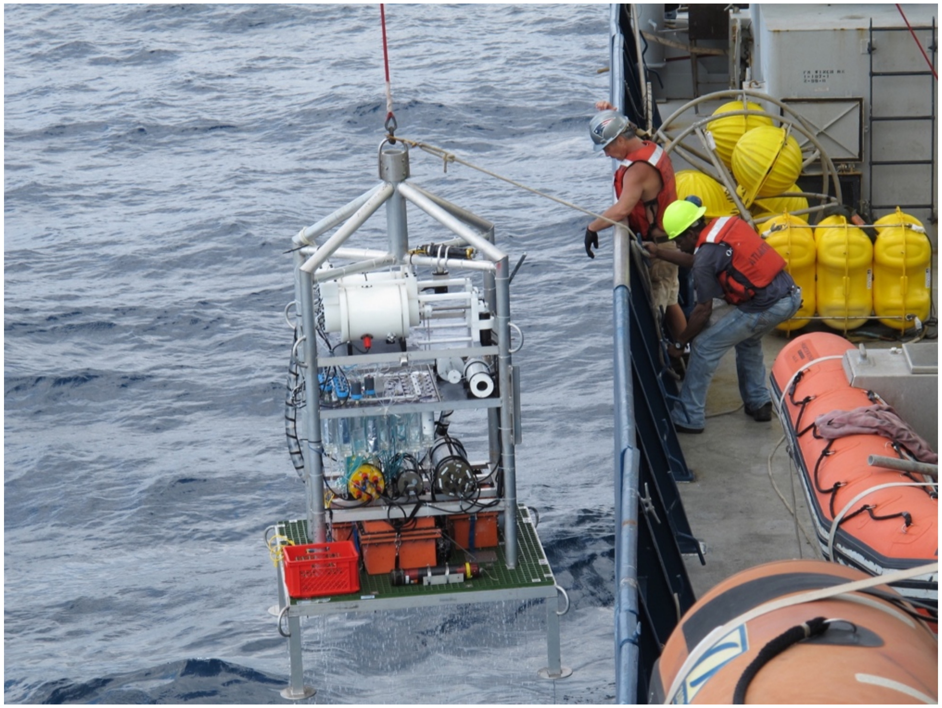
Growth of *Campylobacteria* under e$^-$-acceptor limitation

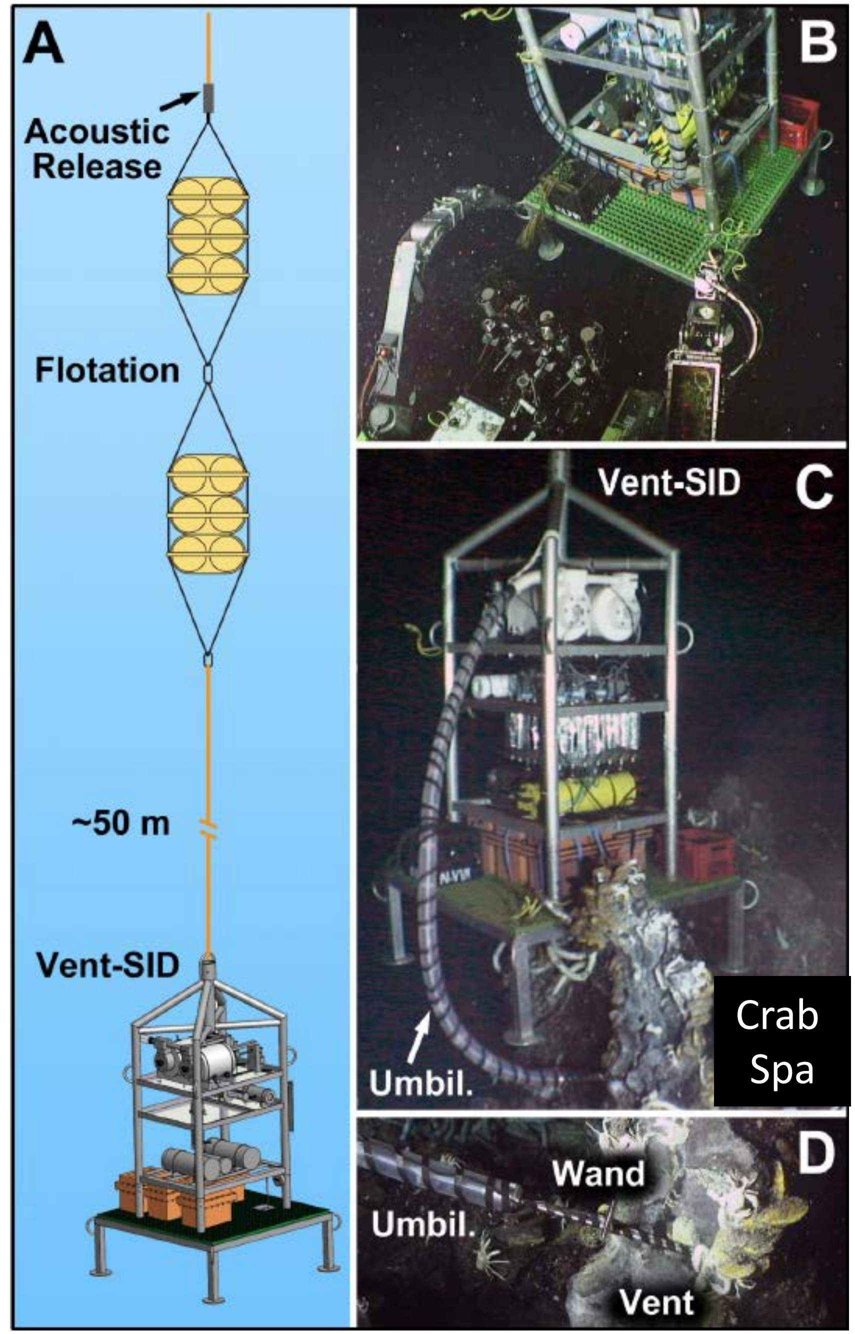
- **Sulfide Oxidation w/ O_2 and NO_3^- to S^0**
  - Mesophiles
  - Oxygen reduction: CBB3
  - Nitrate reduction: **NAP, NIR**, NOR, NOS
  - Sulfide oxidation: **SQR**, SOX (incomplete)
- **Hydrogen Oxidation w/ NO_3^- and S^0**
  - moderate thermophiles, some mesophiles
  - DNRA and denitrification
  - Ni/Fe hydrogenase

Hydrothermal Fluid:
- >275°C
- 85 mM DIC
- 7.7 mM S^{2-}
- 410 µM H_2
- 0 µM O_2
- 0 µM NO_3^-
- 0 Cells


Subseafloor productivity rivals above seafloor production by symbiotic associations!
O_2
NO_3^{2-}
CO_2
S^{2-}
Campylo-bacteria
H_2O
N_2, (N_2O), NH_4^+
Biomass
S^0, (SO_4^{2-})
Rates
40 gC d^{-1}
• Diversity
• Pathways

Next Step: Incubations at the Seafloor





- Deployed and tested at Crab Spa, 9°N EPR (2014, 2017)
- Directly correlate the measurement of these processes with community composition and gene expression
- Can perform repeated incubations

CONCLUSIONS

- *Campylobacteria* are dominant members of microbial communities at deep-sea hydrothermal vents
- Have conserved pathways and mechanisms that have allowed them to colonize a variety of environments, including humans
- Connection between environmental microbiology and medical microbiology, one can inform the other
- Deep-sea vents are potentially rich resource for bioactive compounds: high density, productivity, competition, etc.
- *Campylobacteria* dominate chemoautotrophy at Crab Spa
- Activity and rate measurements indicate very active community
- Processes thought to operate separately co-occur: aerobic/anaerobic, denitrification/DNRA , sulfide/hydrogen oxidation
- First direct estimates of subseafloor productivity, standing stock, and turnover!
- Subseafloor productivity rivals above seafloor production by symbiotic associations

Many thanks to …

SIEVERT lab

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Thank you!
Questions?