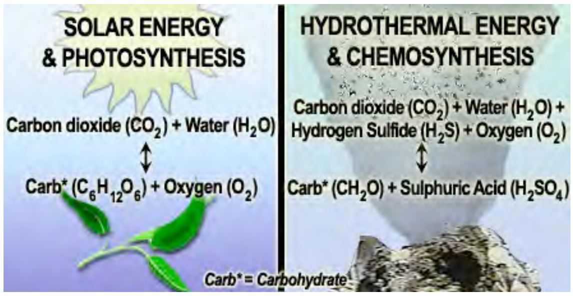
Symbiosis in the Deep Sea

Primary Production

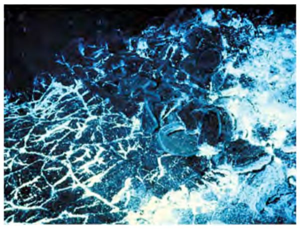
Both processes often fix carbon using the Calvin Cycle



Chemoautotrophy

- Chemolithoautotrophy - A process limited to prokaryotic microbes that get their energy from the oxidation of reduced (inorganic) chemical compounds and use the energy to fix inorganic carbon

  (Chemoautotrpohy or Chemosynthesis for short)



Chemoautotrophy

- Free-living sulfur oxidizing chemoautotrophs
  - Autotrophic growth of *Beggiatoa* using sulfide first demonstrated ~25 years ago
  - Needs both H2S and O2
  - Microhabitats are very important – suitable habitats are limited to interfaces or dynamic environments

Primary Production

Process	Energy Source	Carbon Source
Photoautotrophy	Sunlight	CO2
Chemoautotrophy	Reduced Chem's	CO2
Methanotrophy	CH4	CH4

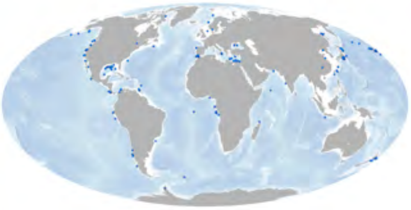
Chemoautotrophy

$H_2S + 2O_2 \rightarrow H_2SO_4$	$\Delta G'_0 = -714$ kJ
$S^0 + 3/2O_2 + 2\,H_2O \rightarrow H_2SO_4$	$\Delta G'_0 = -502$ kJ
$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$	$\Delta G'_0 = -497$ kJ

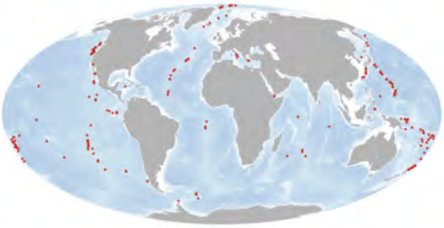
* There are other energy sources for chemoautotrophy, none yet documented for chemoautotrophic symbioses.

- Need to release enough energy to produce ATP (and NADH) to be used in the Calvin cycle

  ADP + Pi --> ATP $\Delta G'_0 = 35$ kJ



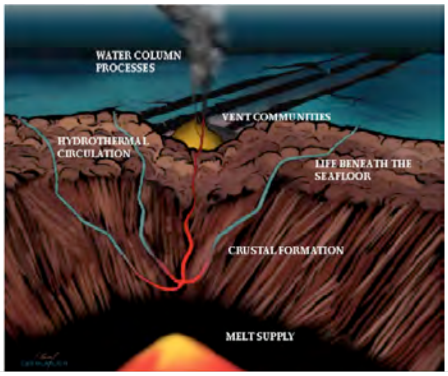
Seeps



Vents

Chemosynthetic Based Ecosystems

Planetary renewal supports life in the deep sea!



Chemoautotrophy

- Annelida
  - Polychaeta: Siboglinidae (all Vestimentiferans, Pogonophorans, and Osedax) and Alvinellidae (genus Alvinella, Paralvinella)
  - Oligochaeta: Phallodrilinidae (all of sub-family Phallodrilinae)
- Mollusca
  - Bivalvia (all Vesicomyidae, Lucinidae, Solemyidae, most Mytilidae)
  - Gastropoda (some snails and some limpets)
- Nematoda (several species, interstitial)
- Platyhelminthes (several turbellarian species, interstitial)
- Crustaceans (shrimp, crabs, and barnacles)
- Ciliates, and some others…
- What's that word again for multiple, independent origins of a phenomenon??

Chemosynthetic Based Ecosystems

Challenges to life at vents

- High pressure & no light
- Low/no oxygen
- Toxicity, particularly sulfide

(also heavy metals, radiation)

- High temperatures
- Extreme environmental variations in time and space

Chemosynthetic Based Ecosystems

- Water goes deep into crust
- Reacts with hot (400C+) rocks
- Rises back to seafloor rich in reduced chemicals and heavy metals
- Emerges at a range temperatures, mixes with
- cold, oxygenated seawater

Chemoautotrophy

- These are animals, not tiny microbes
- The symbioses are nutritive, with the autotroph feeding the heterotroph.
- **So, they need a lot of both sulfide and 02**
- **H_2S is toxic** to most animals, animal tissues
  - Poisons aerobic respiration: Cytochrome C
  - Poisons many hemoglobins
- H_2S is very unstable, spontaneously combine with 02 (i.e. they **do not "normally" co-occur)**

Chemoautotrophy

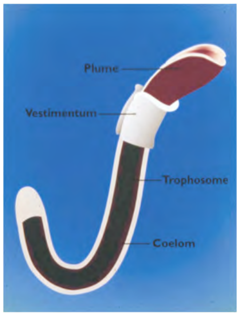
Toxicity aside for now, how could an animal with chemoautotrophic symbionts get enough of both oxygen and sulfide?

- Live where O_2 and H_2S co-occur: Most interfaces too small for "real animals", exception areas where fluids dynamically mix (i.e. vents & seeps)
- Spatially separate acquisition of O_2 and H_2S
  - Bridge the interface with their bodies
- Temporally separate acquisition of O_2 and H_2S
  - Move back and forth across the interface
  - Accumulate one and wait for the other

  (Either of the above requires storing at least one of the two)

Case Study: *Riftia pachyptila*

- Big! 1-2 meter-long tubes
- No GI tract
- Trophosome



Case Study: *Riftia pachyptila*

Determining a symbiosis

1) Tissue stable isotope values ($\partial^{13}C$ and $\partial^{15}N$)

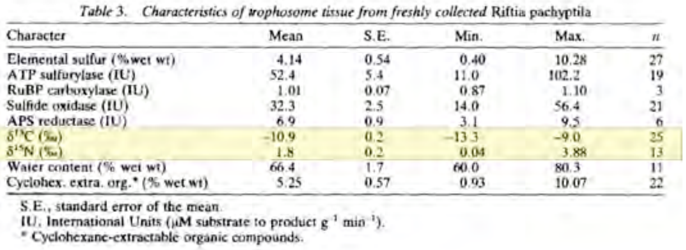


Table 3. Characteristics of trophosome tissue from freshly collected Riftia pachyptila

Character	Mean	S.E.	Min.	Max.	*n*
Elemental sulfur (%wet wt)	4.14	0.54	0.40	10.28	27
ATP sulfurylase (IU)	52.4	5.4	11.0	102.2	19
RuBP carboxylase (IU)	1.01	0.07	0.87	1.10	3
Sulfide oxidase (IU)	32.3	2.5	14.0	56.4	21
APS reductase (IU)	6.9	0.9	3.1	9.5	6
$\delta^{13}C$ (‰)	−10.9	0.2	−13.3	−9.0	25
$\delta^{15}N$ (‰)	1.8	0.2	0.04	3.88	13
Water content (% wet wt)	66.4	1.7	60.0	80.3	11
Cyclohex. extra. org.* (% wet wt)	5.25	0.57	0.93	10.07	22

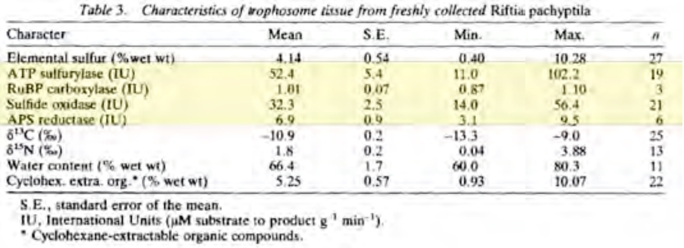
S.E., standard error of the mean.
IU, International Units (µM substrate to product g^{-1} min^{-1}).
* Cyclohexane-extractable organic compounds.

Fisher et al. 1988

Case Study: *Riftia pachyptila*

Determining a symbiosis

2) Enzyme concentrations of sulfur bacteria? Carbon fixation?



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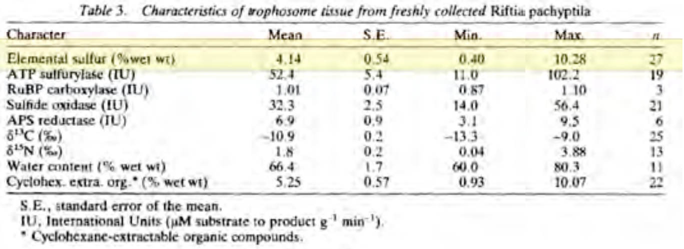
Fisher et al. 1988

Case Study: *Riftia pachyptila*

Determining a symbiosis

3) Observed elemental sulfur in trophosome



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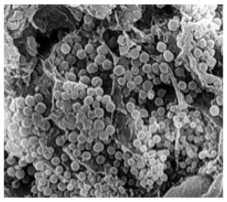
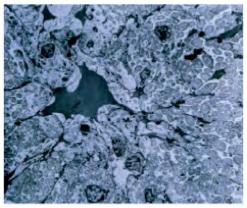
Fisher et al. 1988

Case Study: *Riftia pachyptila*

4) Microscopy of the trophosome

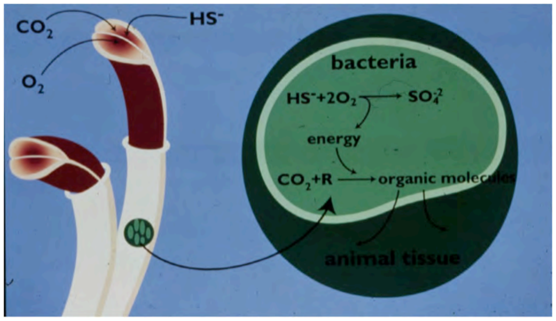
- DAPI staining for DNA
- Transmission Electron Microscopy (TEM)





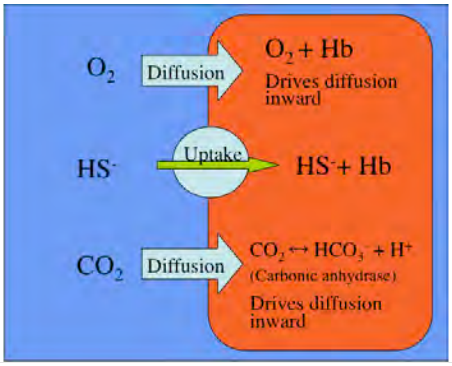
Case Study: *Riftia pachyptila*

CONCLUSION: Symbionts are sulfur oxidizing chemoautotrophic bacteria!



Case Study: *Riftia pachyptila*

Nutrient uptake



O_2 Diffusion $O_2 + Hb$

Drives diffusion inward

HS^- Uptake $HS^- + Hb$

CO_2 Diffusion $CO_2 \leftrightarrow HCO_3^- + H^+$

(Carbonic anhydrase)

Drives diffusion inward

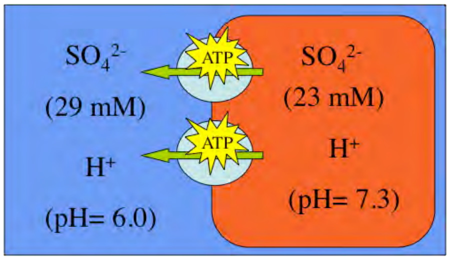
Case Study: *Riftia pachyptila*

- Major physiological adaptations to forming a symbiosis with sulfur-oxidizer
  - Plume
  - Vascularization
  - Hemoglobins

Case Study: *Riftia pachyptila*

Waste removal

- Against the gradient! (requires energy)
- HS- + O2 → SO4(2-) + H+ (← Waste products)



SO_4^{2-} (29 mM) ATP SO_4^{2-} (23 mM)

H^+ (pH= 6.0) ATP H^+ (pH= 7.3)

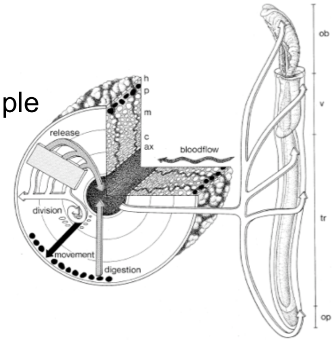
Case Study: *Riftia pachyptila*

- Hemoglobins – Giant extracellular proteins
- Bind O2 and H2S at <u>separate</u> sites, with high <u>affinity</u> and high <u>capacity</u>
  - Avoids sulfur toxicity
  - Can acquire both at low environmental concentrations
  - Provide symbionts with ideal environment
- Vascular blood transports HS-, O2 and HCO3- from plume to trophosome and food to the animal & wastes back to the plume or excretory organs

Case Study: *Riftia pachyptila*

How does the worm get food from symbionts?

- Symbionts release a significant amount of simple sugars after fixation
- Symbionts are also digested to get extra nutrition (amino acids, lipids, etc.)



Case Study: *Riftia pachyptila*

The result?

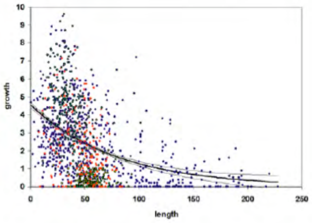
- Adaptation: grow fast and reproduce quickly
- Re-colonization of bare rock observed by scientists:
  - One year community had worms over 1 m tall!
- Vent environments can be short-lived
- Re- colonize new vent sites when old ones die

Contrast *Riftia* with *Lamellibrachia*

- *Lamellibrachia luymesi,* Gulf of Mexico oil seeps → harbors similar sulfur-oxidizing symbionts
- 1-2 meters long, >1000 individuals/aggregation
- 'Bush'-like habitat structure

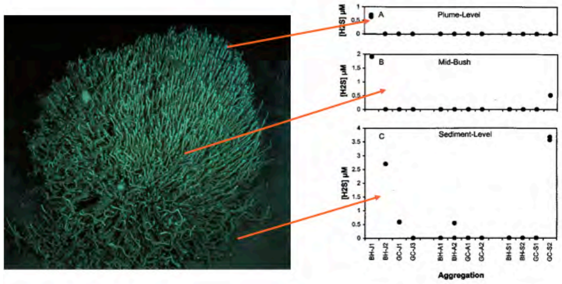
Contrast *Riftia* with *Lamellibrachia*

- Seeps are stable environment: Different life history strategy than *Riftia*
- Slow-growing and long-lived
  - 170-250 years old



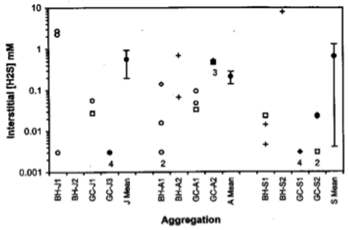
Lamellibrachia: How does it get H2S?

- Plumes 1-2 meters above sediment
- Low sulfide levels near plume
  - WHY?? (think back to last lecture)



Lamellibrachia: How does it get H2S?

- Order of magnitude more sulfide in mud
  - Millimolar vs micromolar concentration near plumes



Lamellibrachia: How does it get H2S?

How to get at H2S in sediment??

Lamellibrachia: How does it get H2S?

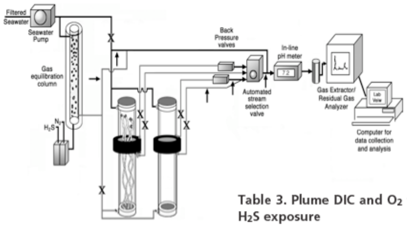


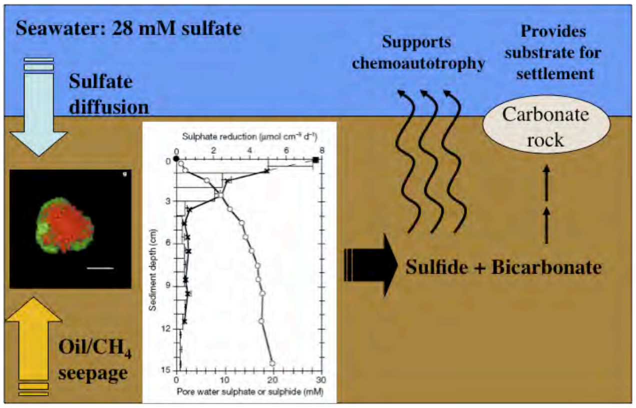
Table 3. Plume DIC and O_2 flux prior to, during, and after root H_2S exposure

Experimental conditions	DIC flux, $\mu mol \cdot g^{-1} \cdot h^{-1}$	O_2 flux, $\mu mol \cdot g^{-1} \cdot h^{-1}$
Before H_2S exposure	−2.4 ± 0.8 (*n* = 73)	6.2 ± 0.9 (*n* = 73)
800 μM H_2S in posterior chamber	2.1 ± 1.0 (*n* = 78)	8.8 ± 0.9 (*n* = 78)
After H_2S exposure (17 h)	−4.9 ± 1.8 (*n* = 38)	4.7 ± 2.3 (*n* = 38)

All data are given as mean ± 1 SD. Positive numbers indicate consumption and negative numbers indicate production. Each *n* represents the number of independent calculations of flux rate for that condition as described in the text.

Freytag et al. 2001

Where does H2S come from at gas/oil seeps?



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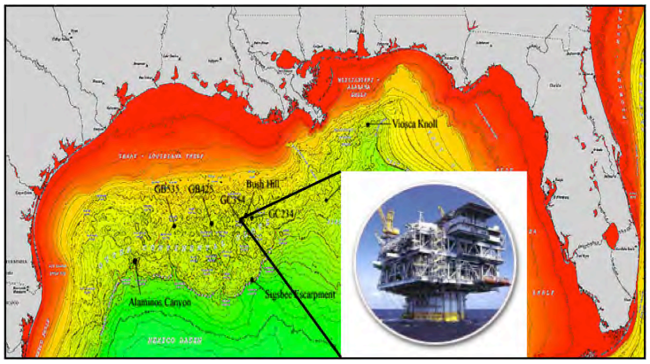

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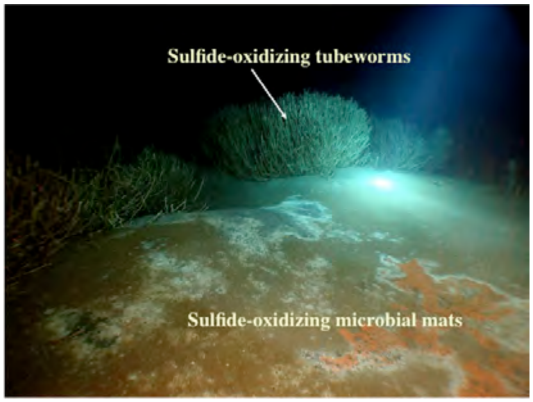
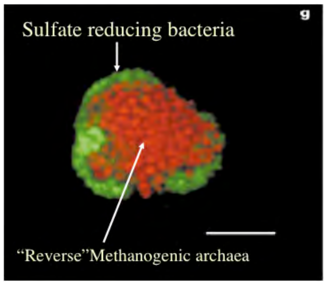


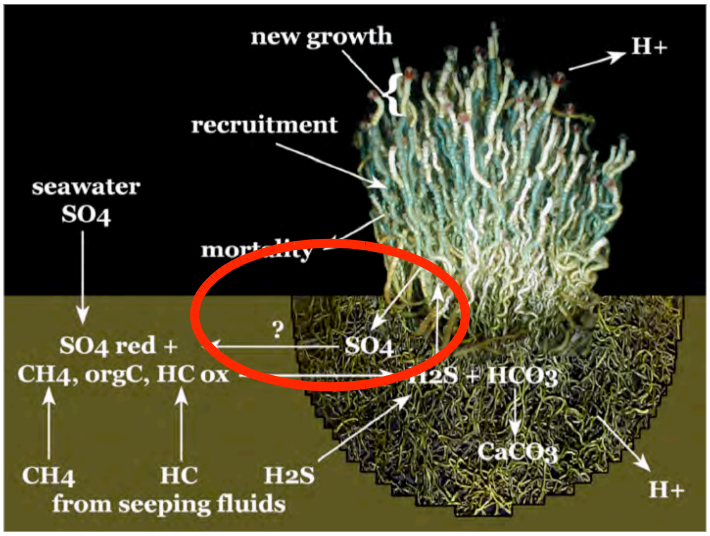
Where does H2S come from at gas/oil seeps?

- Sulfide produced by sulfate reduction coupled with methane oxidation

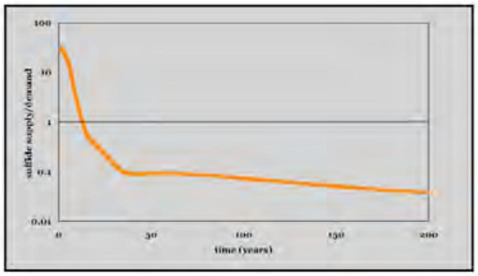
$SO_4^{2-} + CH_4 = HCO_3^- + HS^- + H_2O$

- Microbial Consortia





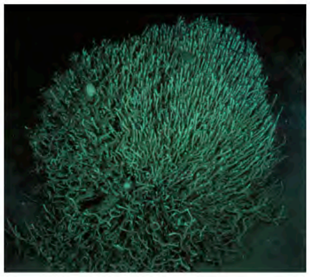
Lamellibrachia: Long Lives, Deep Roots



Modeling H2S supply:demand by advection and diffusion alone

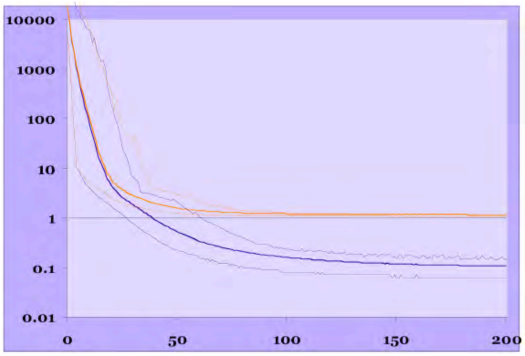
Lamellibrachia: Long Lives, Deep Roots

Young tubeworms:
Can get sulfide across
plumes

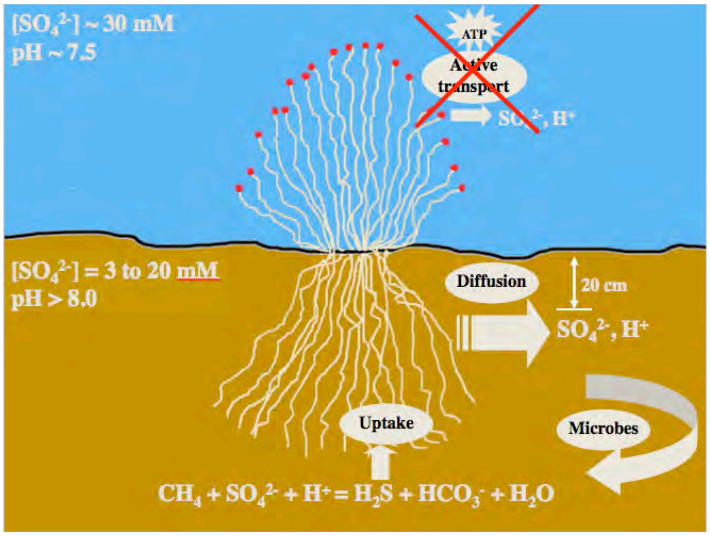


Older tubeworms: Usually no sulfide
across plume: must get sulfide across
root

Lamellibrachia: Long Lives, Deep Roots



Modeling supply:demand with sulfate excretion
constrained by tubeworm H2S oxidation rates



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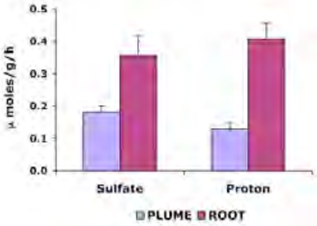
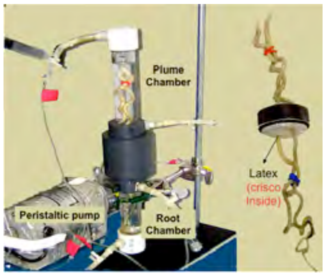




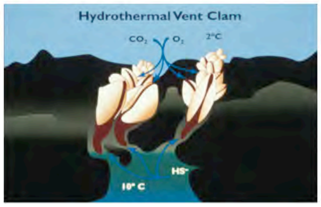
Riftia vs *Lamellibrachia*: Differences

- Ephemeral vent habitat
- Extremely fast growing; short life-span (measured in years)
- Primary gas exchange organ: plume
- Live with plume positioned in sulfide-oxygen mixing zone

- Stable seep habitat
- Slow growing; extremely long life-span (measured in centuries)
- Primary gas exchange organs: plume and root
- Sulfide and oxygen acquisition spatially separated
- “Prime” the ecosystem with sulfate release into sediments

Vesicomyid Clams

- Live in very specific vent microhabitat
- In areas of very low flow rate
- Spatially separate the acquisition of sulfide and oxygen
  - Sulfide across the foot
  - Oxygen directly across their gill



- A lot of red blood
- Blood Binds Sulfide
  - With high affinity
  - With high capacity
  - Reversibly
- But not to hemoglobin (Convergent evolution)
  - Hbs bind O_2, separate protein in serum for H_2S

Chemoautotrophic snails

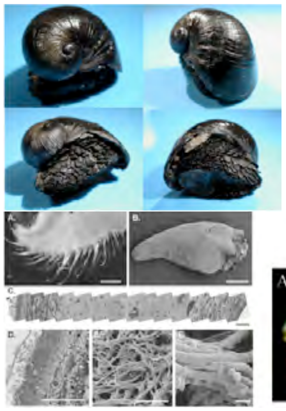
3-4 hydrothermal vent species in the Western pacific
Can be biomass dominants in some microhabitats
Symbionts are intracellular in gills
Some may have multiple symbionts
Methanotrophic and chemoautotrophic

Methanotrophy: Mussels on gas

Bathymodiolus childressi

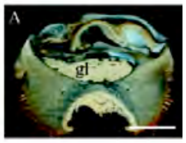
- Gulf of Mexico seeps, sometimes found with Lamellibrachia spp.
- Intracellular methanotrophic symbionts in gills
  - Methane uptake via diffusion
- Symbionts use CH4 as energy AND carbon source

Chemoautotrophic snails



"Scaly-foot" gastropod
Chrysomallon squamiferon

- Scales composed of iron sulfides (pyrite FeS2) and gregite Fe3S4), ferrimagnetic
- Reduced digestive system
- Endosymbionts on esophageal gland

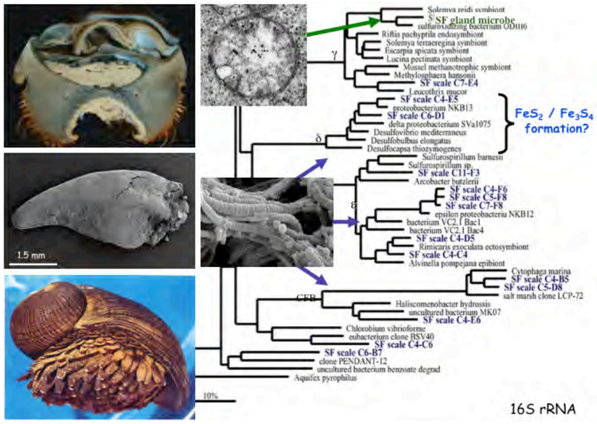


- Ectosymbionts on scales...

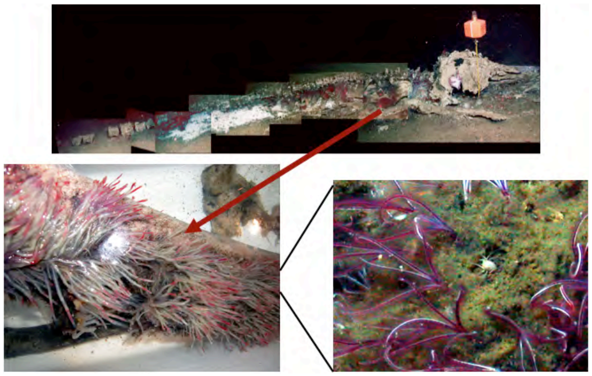
Chemosynthetic Bivalves

- Chemoautotrophic symbiosis is important in five bivalve families
  - Occur in one or two more
- Evolved independently 4 different times and each time symbiosis was likely a driving force in the evolution of the family.
  - Vesicomyidae (Family)
  - Bathymodioline (sub-Family)
  - Solemyidae (Family)
  - Lucinaceanae (Super Family:Lucinidae and Thysiridae)
    - Secondary evolutionary loss in some Thyasiridae
- Symbionts are in (or "on") gills
- GI tracts are reduced or absent
- Likely digest symbionts and "milk" them
- Some Bathymodioline mussels have methanotrophic symbionts
  - Some have both

Chemoautotrophic snails



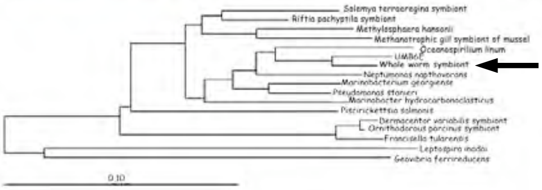
Osedax: Whale bone devourer



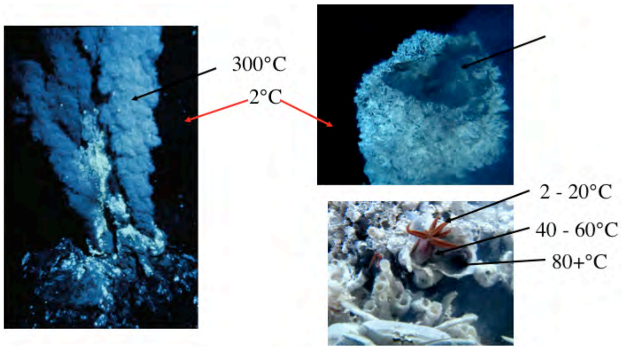
Osedax: Whale bone devourer

- Siboglinid polychaete with symbiont in 'root'-like tissue and brood pouch
- Dissolves bone and symbiont extract energy and carbon source from lipids (aerobically)
- Dwarf, parasitic males

Osedax: Whale bone devourer

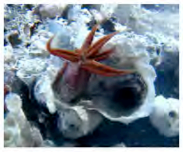


Alvinella: bacterial fur coats



Alvinella: bacterial fur coats

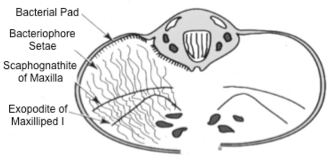
Lives on hydrothermal chimneys
Extreme Temp gradient along body
Can feed (has mouth and gut)
Posterior end is covered with bacteria
It's a consortium
    Heterotrophs and chemoautotrophs
Function?
    Protection from sulfide?
    Protection from Temp?
    Nutrition?

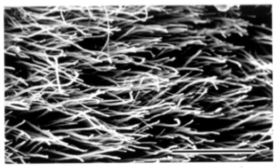


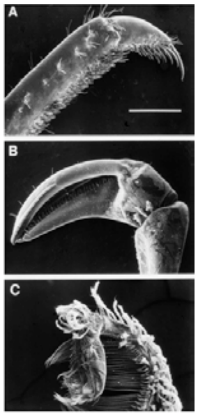
Rimicaris: Bacterial farmers

Rimicaris exoculata

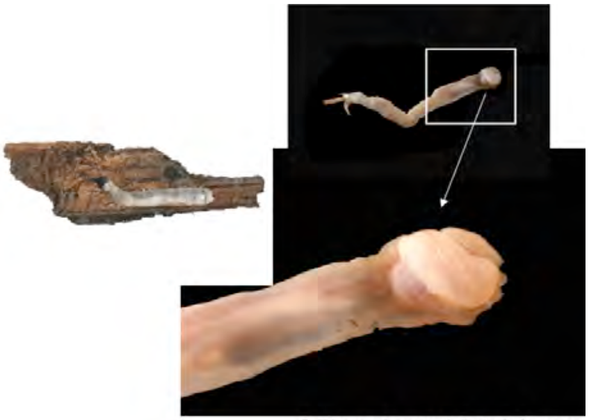
Rimicaris: Bacterial farmers







Teredinidae: Termites of the sea



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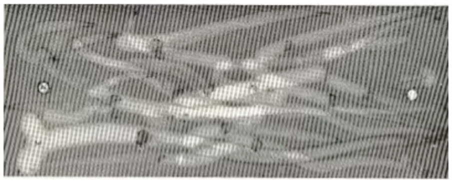
Teredinidae: Termites of the sea

- Family of wood-boring bivalves aka ship worms
- X-ray of submerged wood block, 3 months in deep sea



Teredinidae: Termites of the sea

- Abundant intracellular symbionts in a “digestive gland”, **Gland of Deshayes**
- Symbionts are cellulolytic N2 Fixers