Microbial Diversity Associated with *Lophelia pertusa* in the Gulf of Mexico

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U.S. Geological Survey

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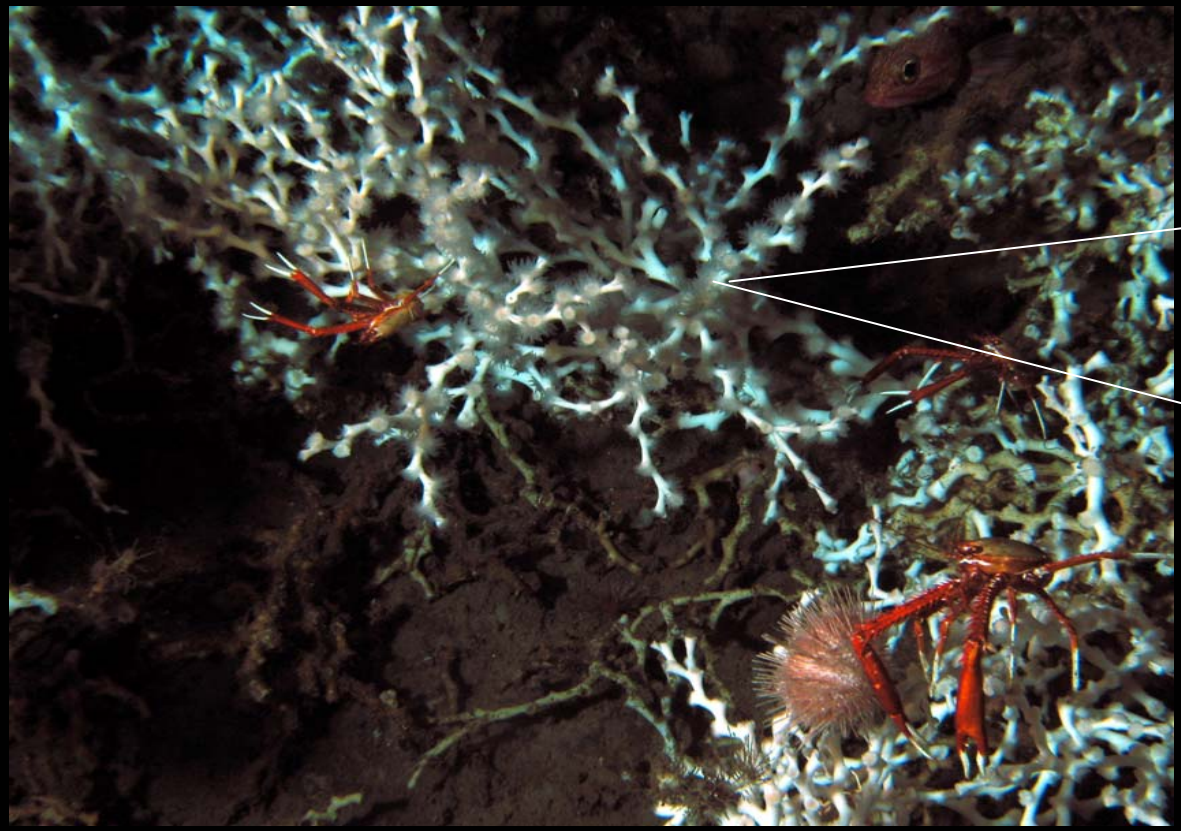
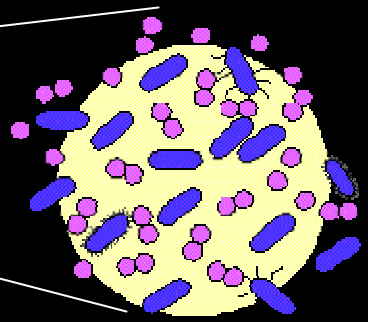
What is coral microbial ecology?





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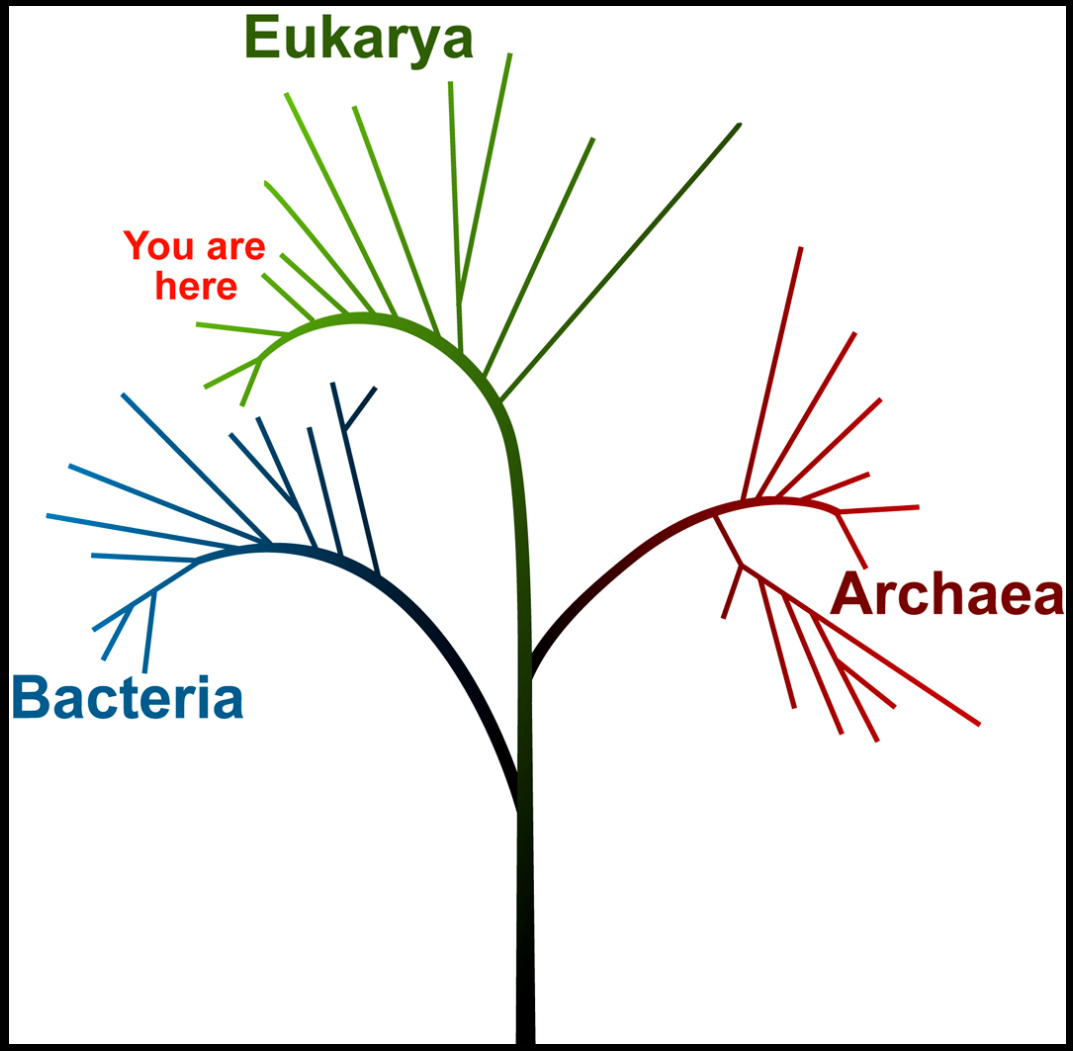
Who are they?



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Where are they?

- Coral surface microlayer … aka mucus
- Tissue
- Skeleton

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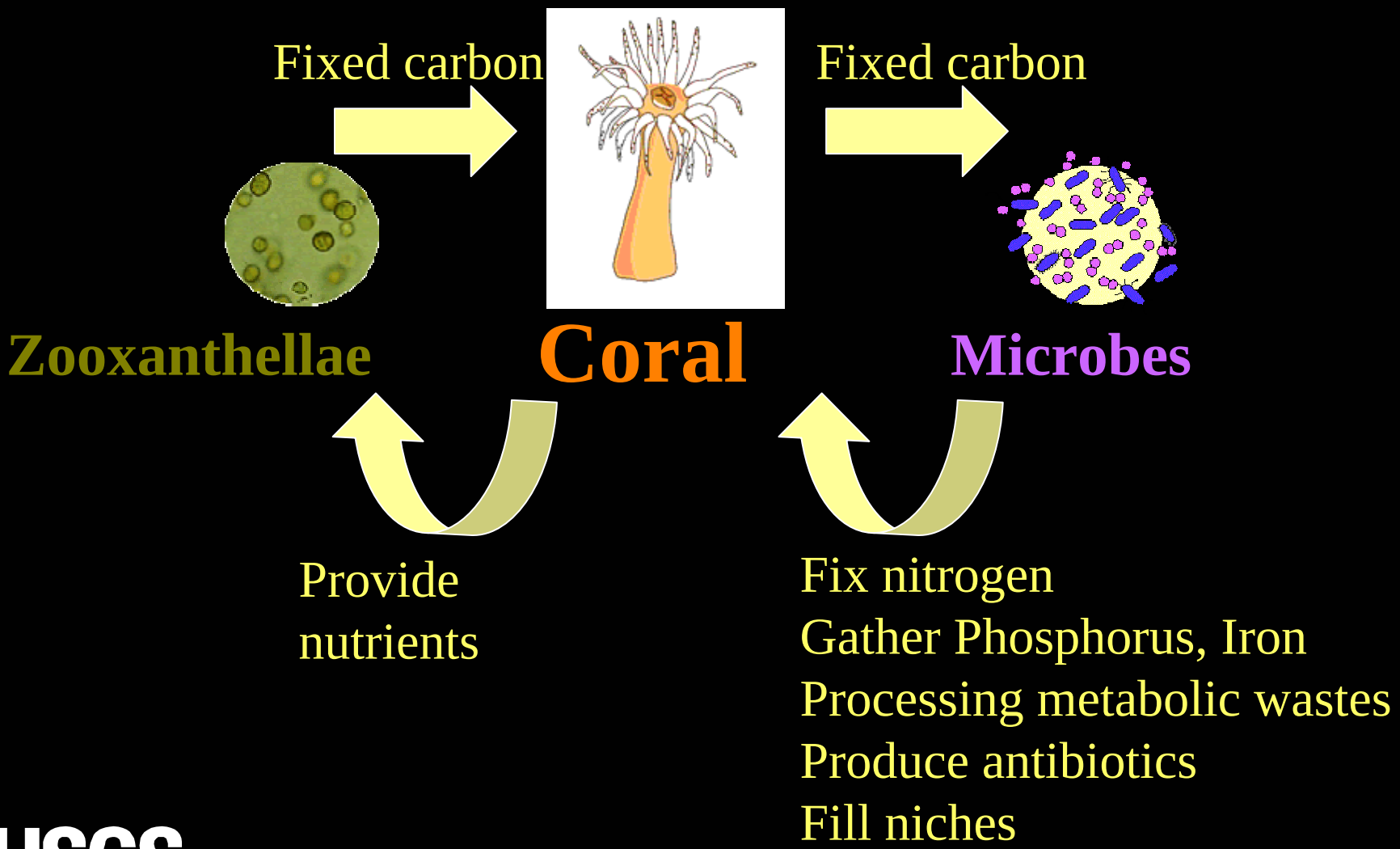

From Rohwer et al. 2002

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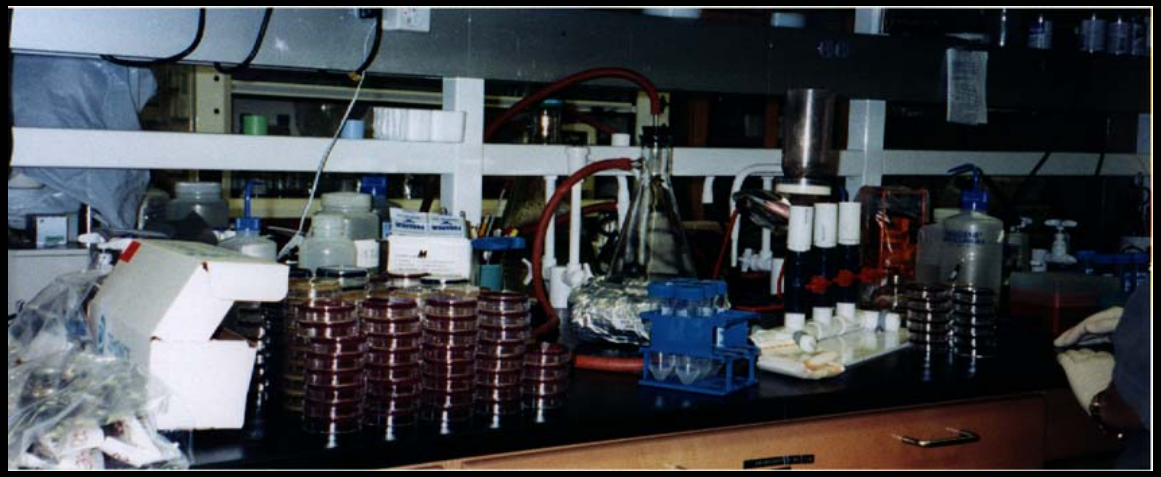
How do we study microbes?

- Culture studies – growing the microbes on nutrient medium

- Molecular techniques – DNA-based methods



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Culture Club

- PROS: You have the bug. You can investigate its genetics, biochemistry, metabolic capabilities, all kinds of experiments.

- CONS: Only ~ 1% of bacteria can be cultured and barely any archaea.

Molecular Techniques

- Extract DNA → PCR → Clone → Sequence → Compare to knowns

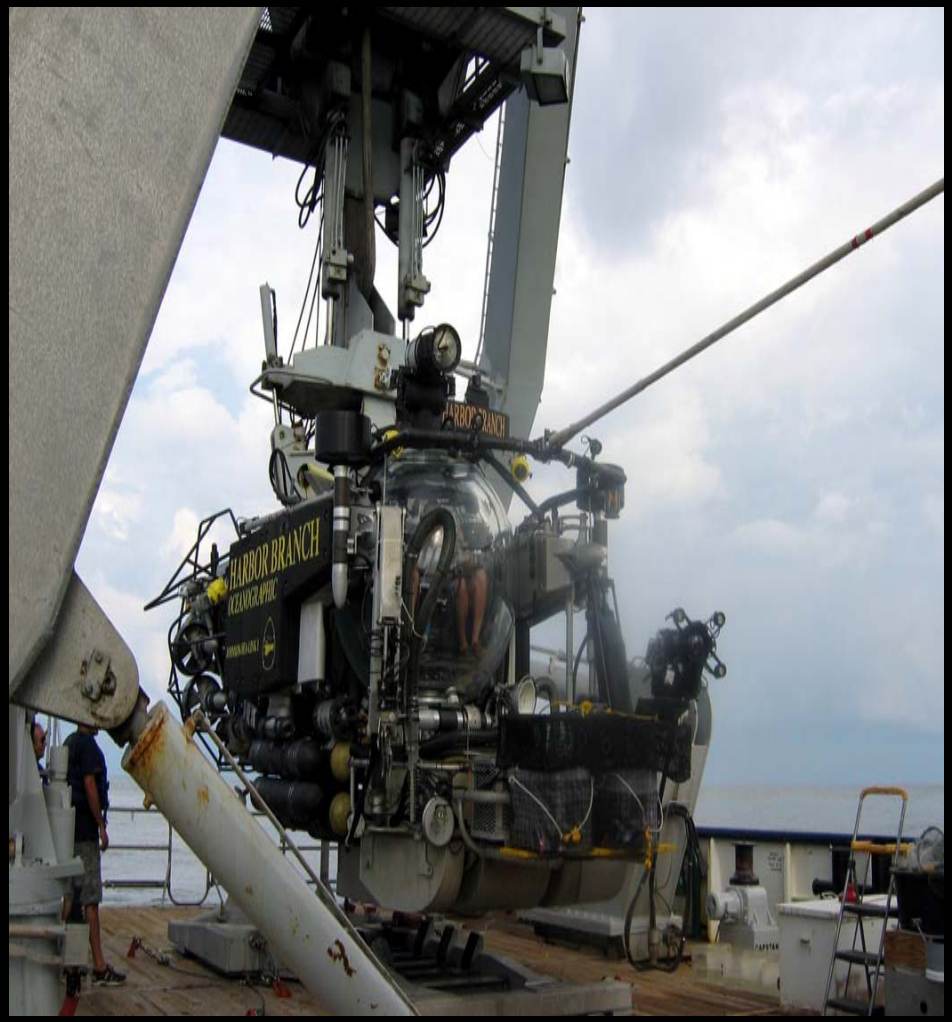
Molecular Techniques

- PROS: You can identify the uncultured 99% of bacteria and archaea – uncovering new diversity left and right!

- CONS: You only have a phone book of DNA sequences. You know who is there, but not what they are doing or are capable of doing.

Methods

- Sample coral (mucus/ tissue/skeleton) – fixed at depth vs. live
- Culture on marine agar
- Extract total DNA
- Amplify DNA using PCR + specific primers
- DGGE, cloning, sequencing



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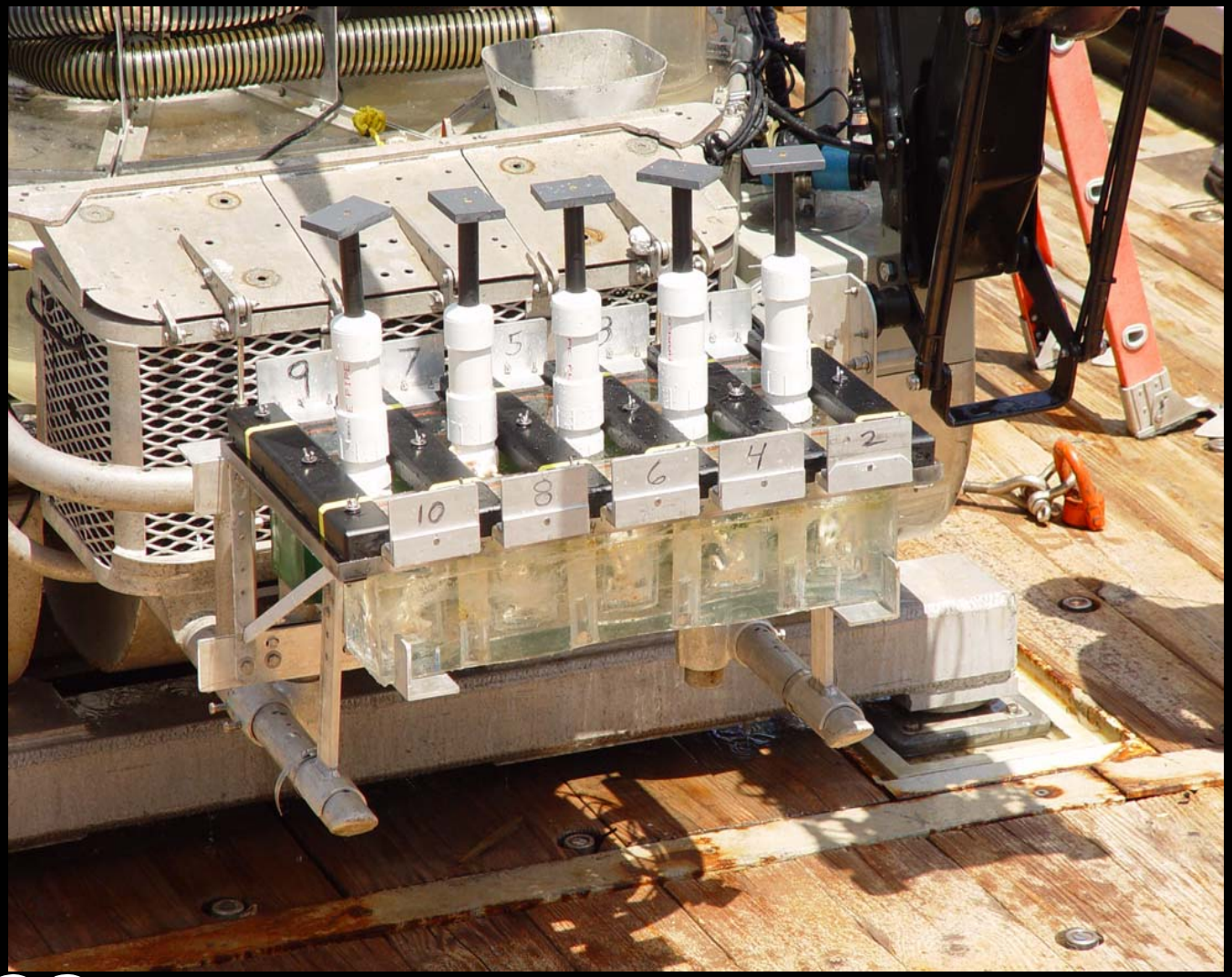
Kellogg Sampling Device



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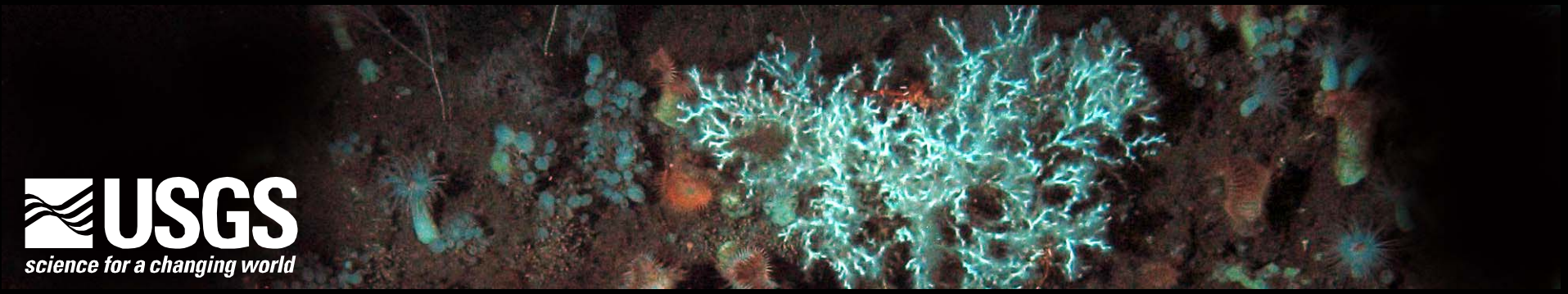
Questions:

- What kind of microbes are associated with *Lophelia*?

- How do these microbial communities compare to those of shallow-water corals or deep-sea gorgonians?



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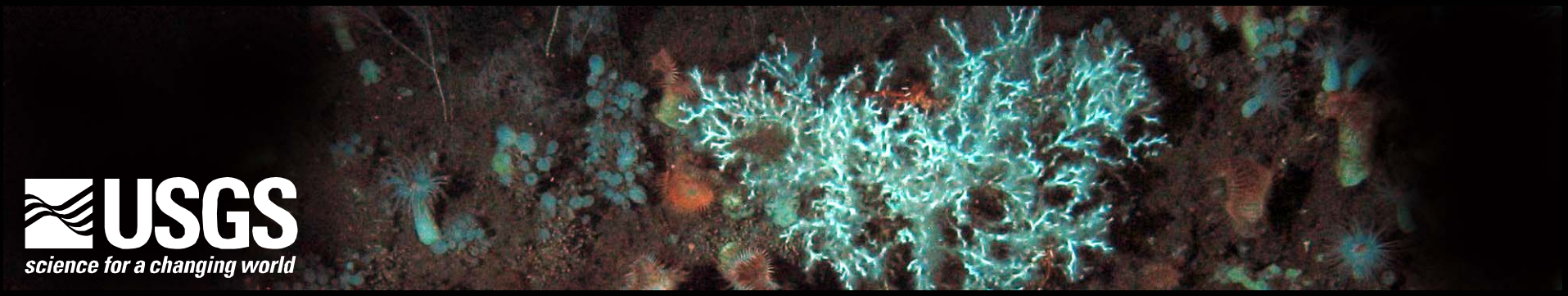
Questions:

- Are these microbes acting as symbionts (since there are no algal symbionts)?
    - Are microbes the link between *Lophelia* and hydrocarbon seeps?

- Is there a difference between samples fixed at depth and those brought to the surface live? (Sampling issues)



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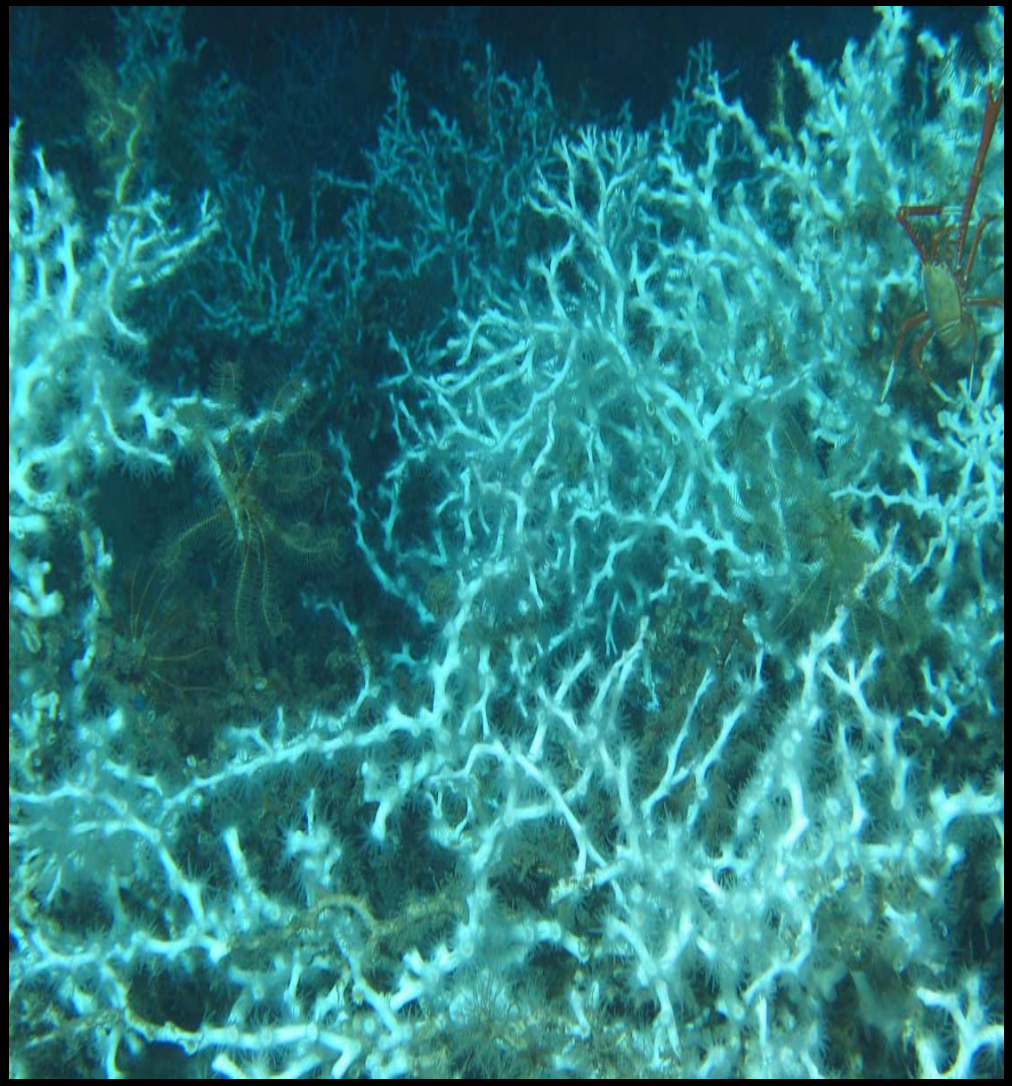
Gulf of Mexico

- Sites
  - Viosca Knoll 862
  - Viosca Knoll 826



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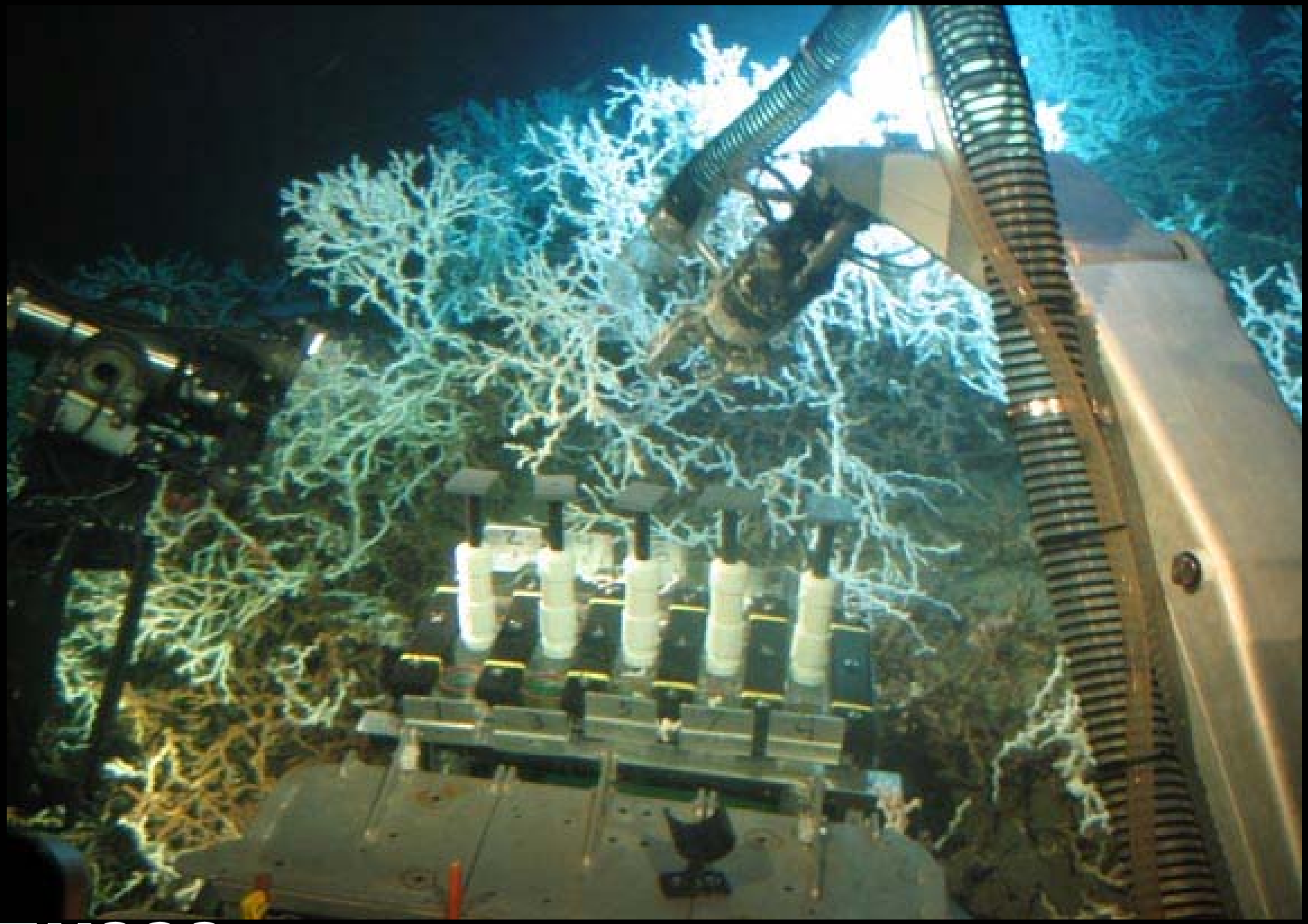

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Lophelia Culture Data

- *Achromobacter* sp. (98%)
- *Alteromonas nigrifaciens* (98%)
- *Photobacterium* spp. (99%)*
- *Pseudoalteromonas* spp. (98%)
- *Pseudomonas stutzeri* (99%)
- *Psychrobacter glacincola* (100%)*
- 7 *Vibrio* spp. (98-99%)

* psychrophilic or cold-loving organisms

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Lophelia Culture Data

- *Cobetia* spp. (96-100%)
- *Colwellia* sp. (93%)*
- *Halomonas* sp. (97-99%)
- *Moritella* sp. (94%)
- *Shewanella* spp. (96-98%)*

* psychrophilic or cold-loving organisms

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Lophelia Culture Data

- Previously uncultured bacterial clone 131851* (99%)
- Unknown *Alteromonadaceae* (85–90%)
- Unknown *Firmicutes* (89%)
- Unknown *Vibrionaceae** (82%)

* psychrophilic or cold-loving organisms

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Fixing Samples at Depth

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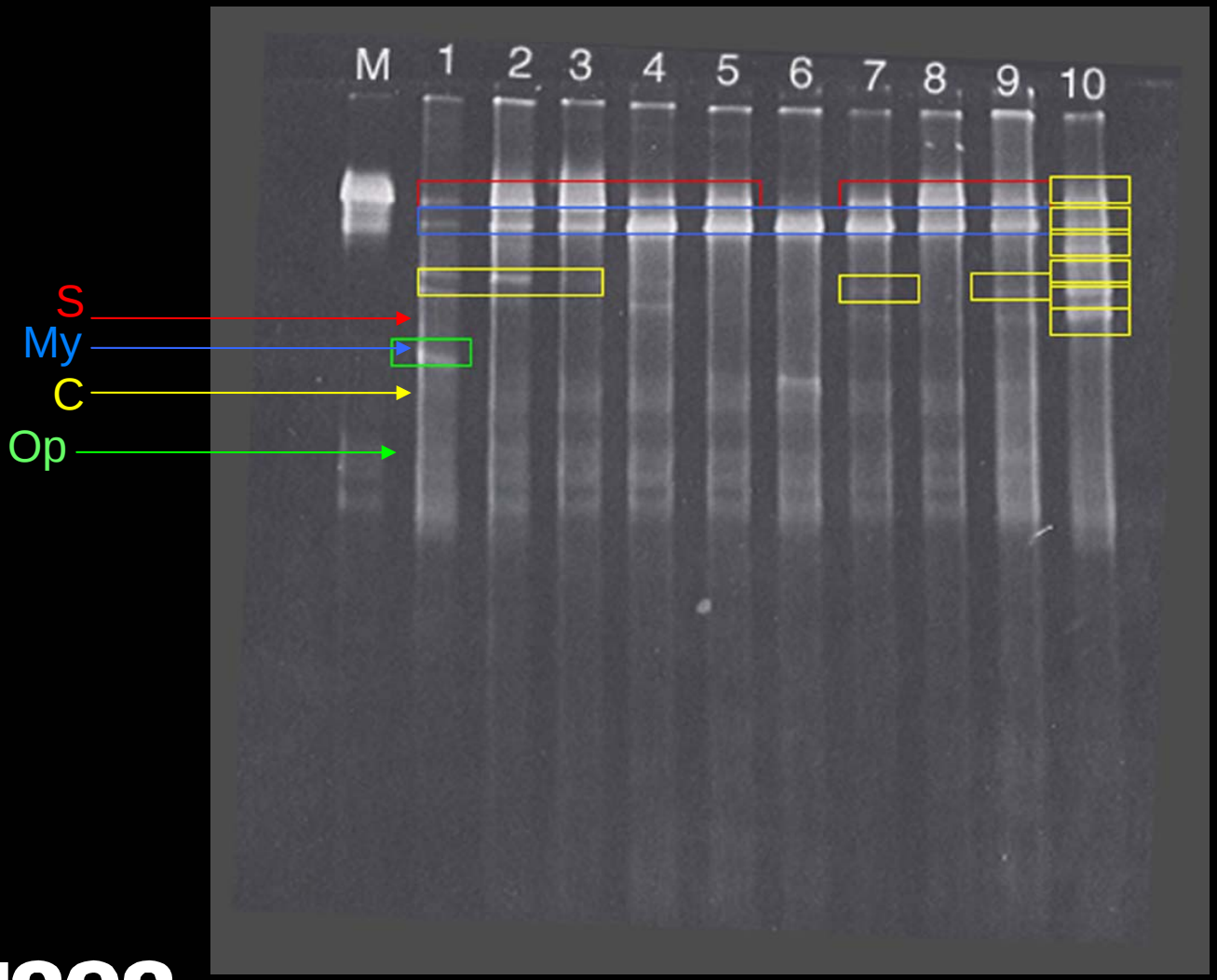
Fixing Samples at Depth



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Lophelia Molecular Data

- 93% of clones were novel (<95%, usually <89% match to known)
  - Seven clones had no real match to anything
- *Colwellia* sequences (97–98%)
- Four clones similar to clones from deep-sea octocorals
  - Two α-Proteobacteria (94–9%)
  - Two unknown *Firmicutes* (84–85%)

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Lophelia Molecular Data

- 51% were unknown *Firmicutes* (mycoplasma-like sequences)
- 27% were related to thiotrophic bacterial symbionts of methane-seep clams
- 39% were γ-*Proteobacteria*

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Lophelia Molecular Data

- VKLP1 ~ a *Lophelia*-specific bacterium?
  - γ-*Proteobacterium*
  - Related to seep clam symbionts
    - Sulfide-oxidizer?
    - Methanotroph?
  - Present in all *Lophelia* samples tested (n=6)
  - Dominant in two libraries from VK862

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Lophelia Molecular Data

- Biogeographic (?) differences between sites:
  - VK862
    - VKLP1, psychrophiles, squid symbionts
    - 325m, temp >10 degrees C, delicate morph, no seep
  - VK826
    - *Firmicutes*
    - 500m, temp <10 degrees C, calcified morph, localized seep, assoc. fish

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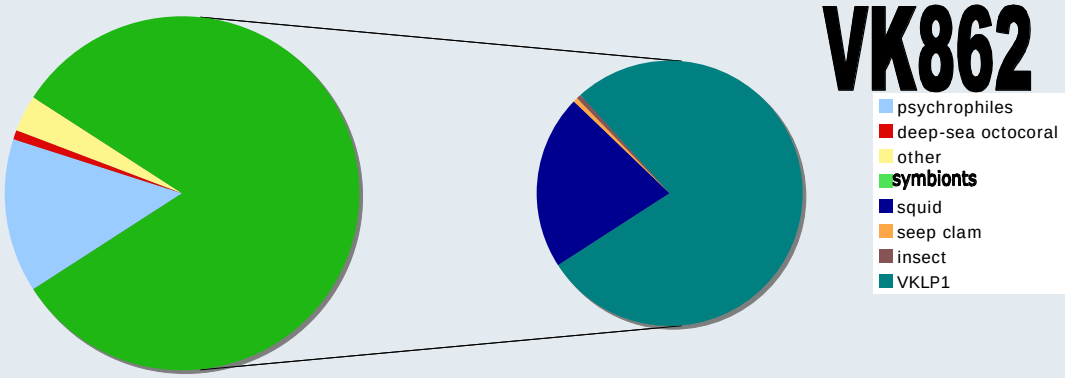

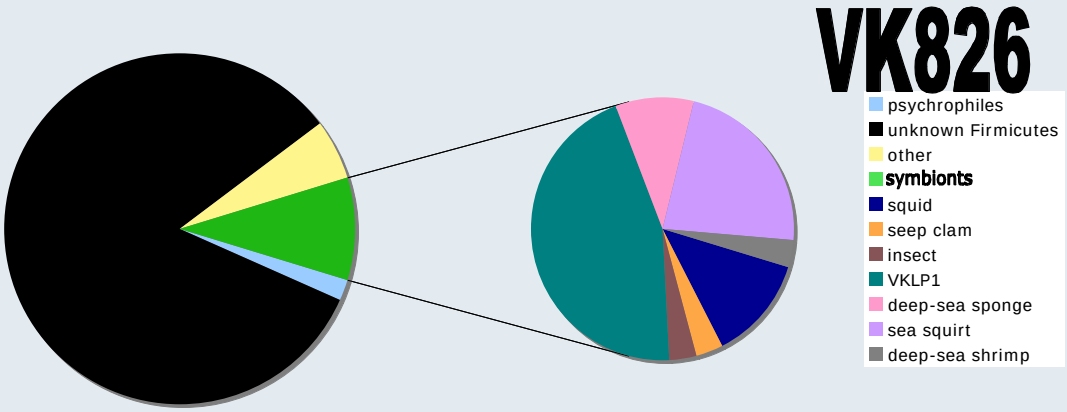

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Lophelia Molecular Data

- Archaea
  - No amplification with two sets of primers

    (Still working on it)

- Fungi
  - *Paecilomyces*/*Acremonium* sp.
  - Non-specifc 18S primers

    (Still working on that, too)

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Summary

- Proper sample collection (minimizing contamination and thermal shock) ***is*** important

- Most of the bacteria associated with *Lophelia* are novel

Summary

- Deep-sea corals (both gorgonian and scleractinian) have in common:
  - Psychrophiles (including *Colwellia* spp.)
  - *Firmicutes* (mycoplasma-like sequences)
  - Many γ-*Proteobacteria*

Summary

- Both molecular and culture data have produced close matches (99%) to microorganisms from shallow-water corals

- Both molecular and culture data include matches to symbionts (of fish, squid, seep-clams, sponges, and insects)

Summary

- VKLP1
  - present in all *Lophelia* samples tested (n = 6)
  - a *Lophelia*-specific bacterium that links the coral to methane seep communities

Summary

- First identification of a fungal-associate of a live deep-sea coral
  - *Paecilomyces/Acremonium*

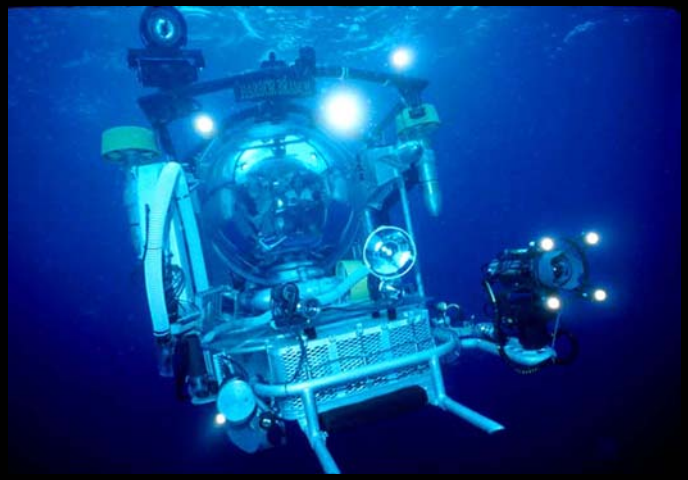
Summary

- Clear difference between VK862 and VK826
  - Biogeography?
  - Evidence of stress?

Future Directions

- Biogeography
  - *Lophelia* samples from northwestern Gulf, West Florida slope, Atlantic slope for comparison

- Targeted culturing
  - Sulfide-oxidizing bacteria (inclu. VKLP1)
  - *Firmicutes*
  - Fungi



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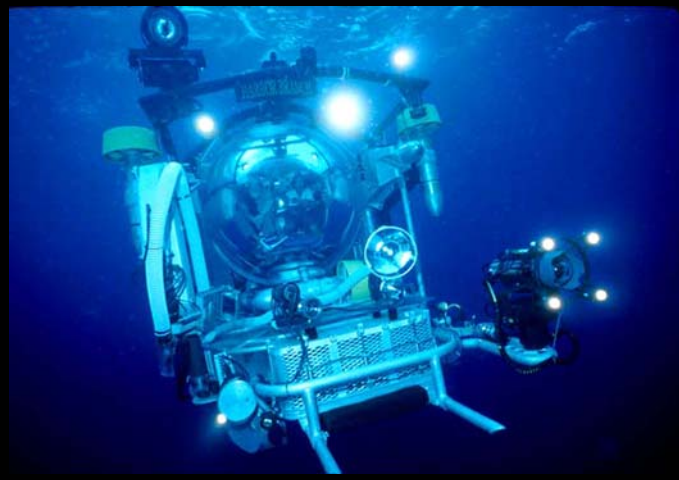
Future Directions

- Microbial investigations of dominant fauna that have direct contact with *Lophelia*
    - Conger eels
    - Scorpaenid fishes
    - Galathaeoid squat lobster
    - Echinoderms
    - Hydroids

(Potential sources of microbial transfer)



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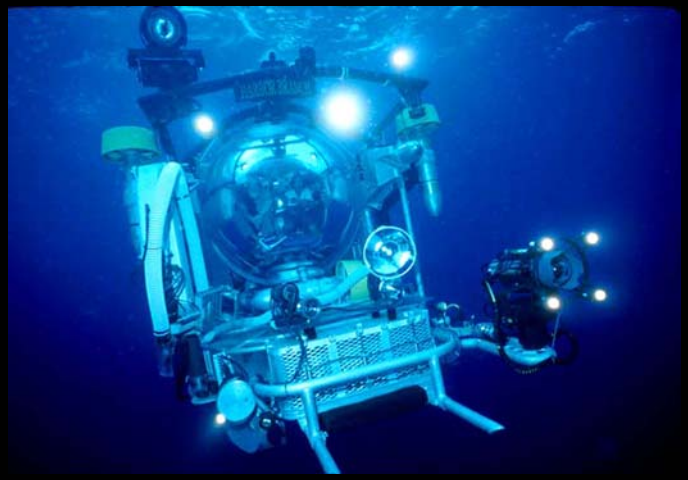
Future Directions

- Microarrays
  - Screening for a broad range and large number of bacterial and archaea taxa
  - Specifically targeting methanotrophs



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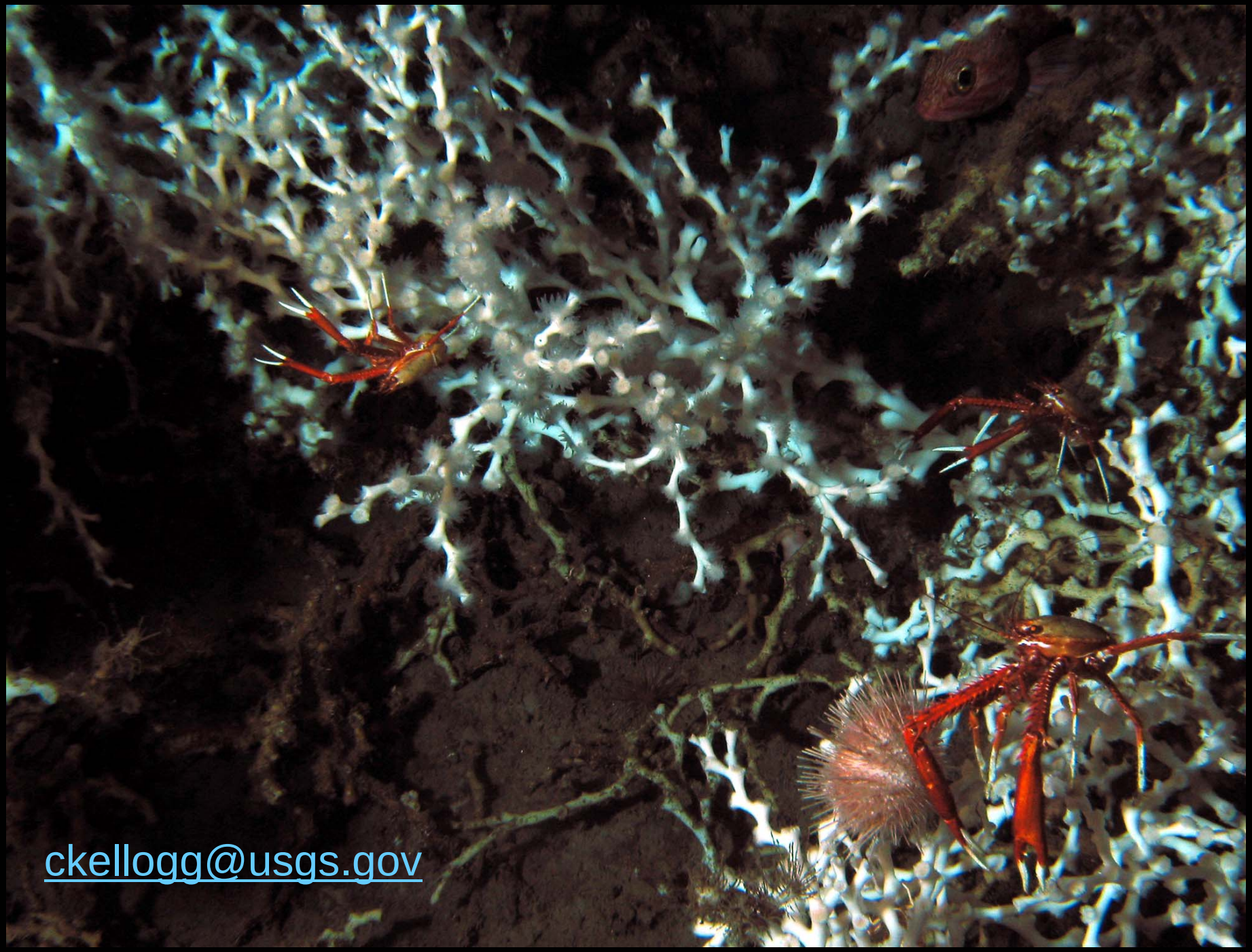

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Reference

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. Marine Ecology Progress Series 243:1–10.