Microbial Life in the Marine Subsurface:
Tools and Approaches for Detecting Deep, Dark Life

Beth Orcutt, Ph.D.
Bigelow Laboratory for Ocean Sciences

@DeepMicrobe

Photo courtesy WHOI/ROV Jason


IODP
INTEGRATED OCEAN DRILLING PROGRAM
GORDON AND BETTY MOORE FOUNDATION
NSF
EXPLORE • EDUCATE • ENGAGE
C-DEBI
BIGELOW LABORATORY FOR OCEAN SCIENCES
MAINLY MICROBE
MAINLY MICROBE
MAINLY MICROBE
MERELY MICROBE
MAINLY MICROBE
MAINLY MICROBE
MicrobesAreAwesome.com
Photo by Bill Crawford/TAMU

I am Exploring for Life in the **Dark Ocean**

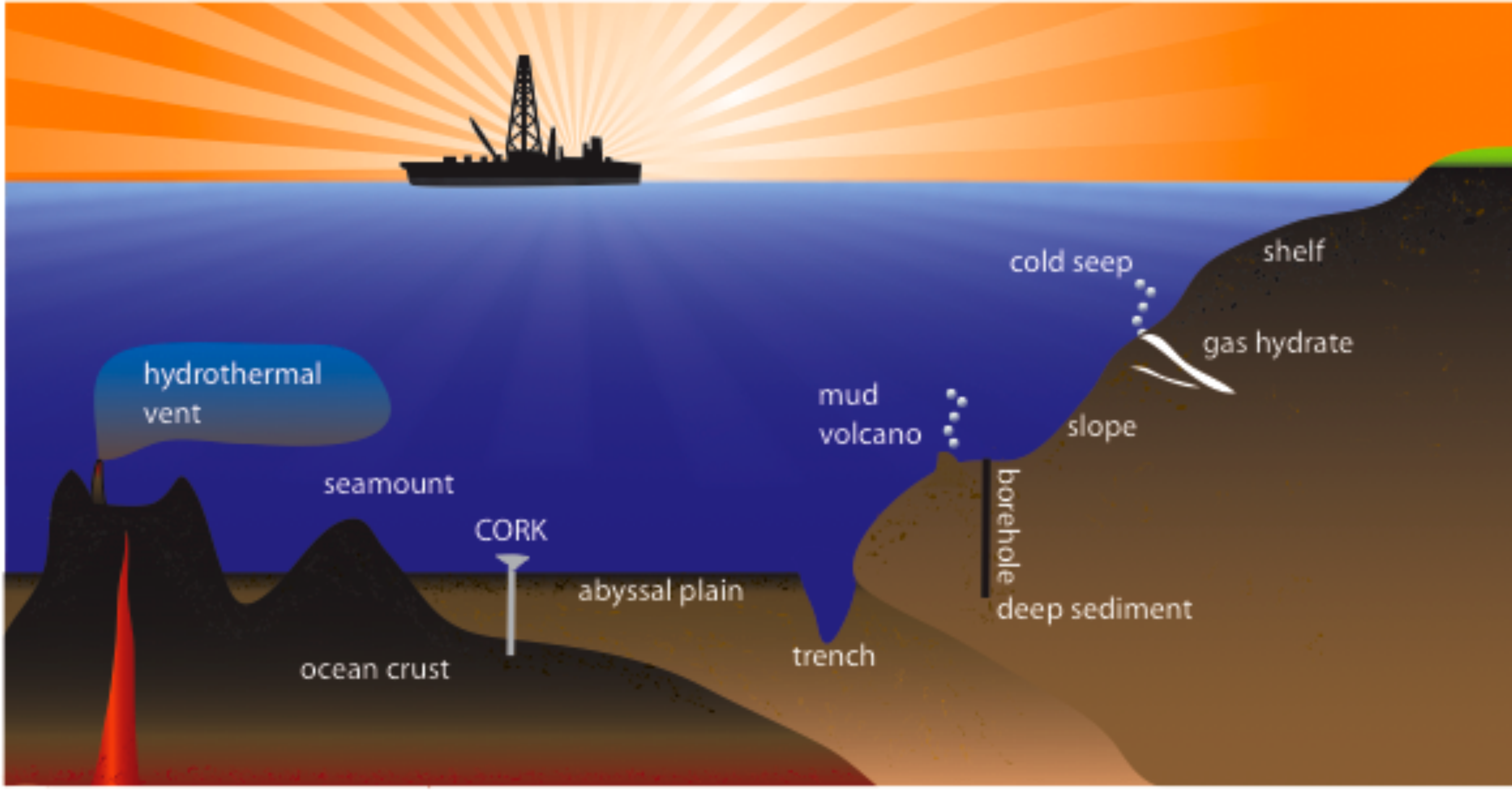


Figure modified from Orcutt et al. Microbiology And Molecular Biology Reviews 2011

Photic zone - 3.0×10^{16} m^3

The Aphotic Zone - 1.3×10^{18} m^3

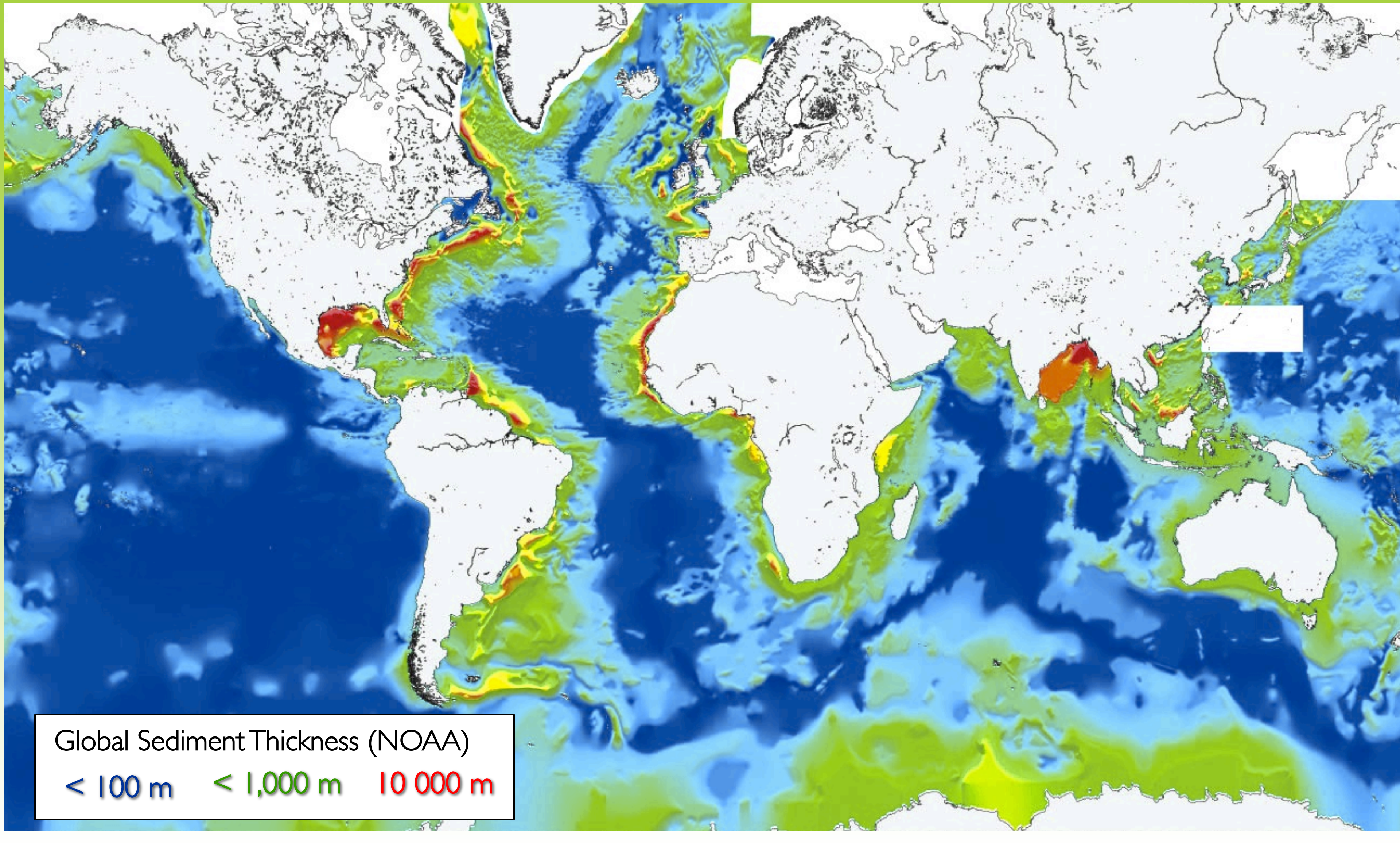
Sediments - 4.5×10^{17} m^3

Upper Oceanic Crust - 2.3×10^{18} m^3

*The majority of habitats in the ocean are in the **DARK***

modified from Orcutt et al. MMBR 2011

70% of the ocean floor is exposed or shallowly buried **oceanic crust**



water moves through the crust - *rapidly*

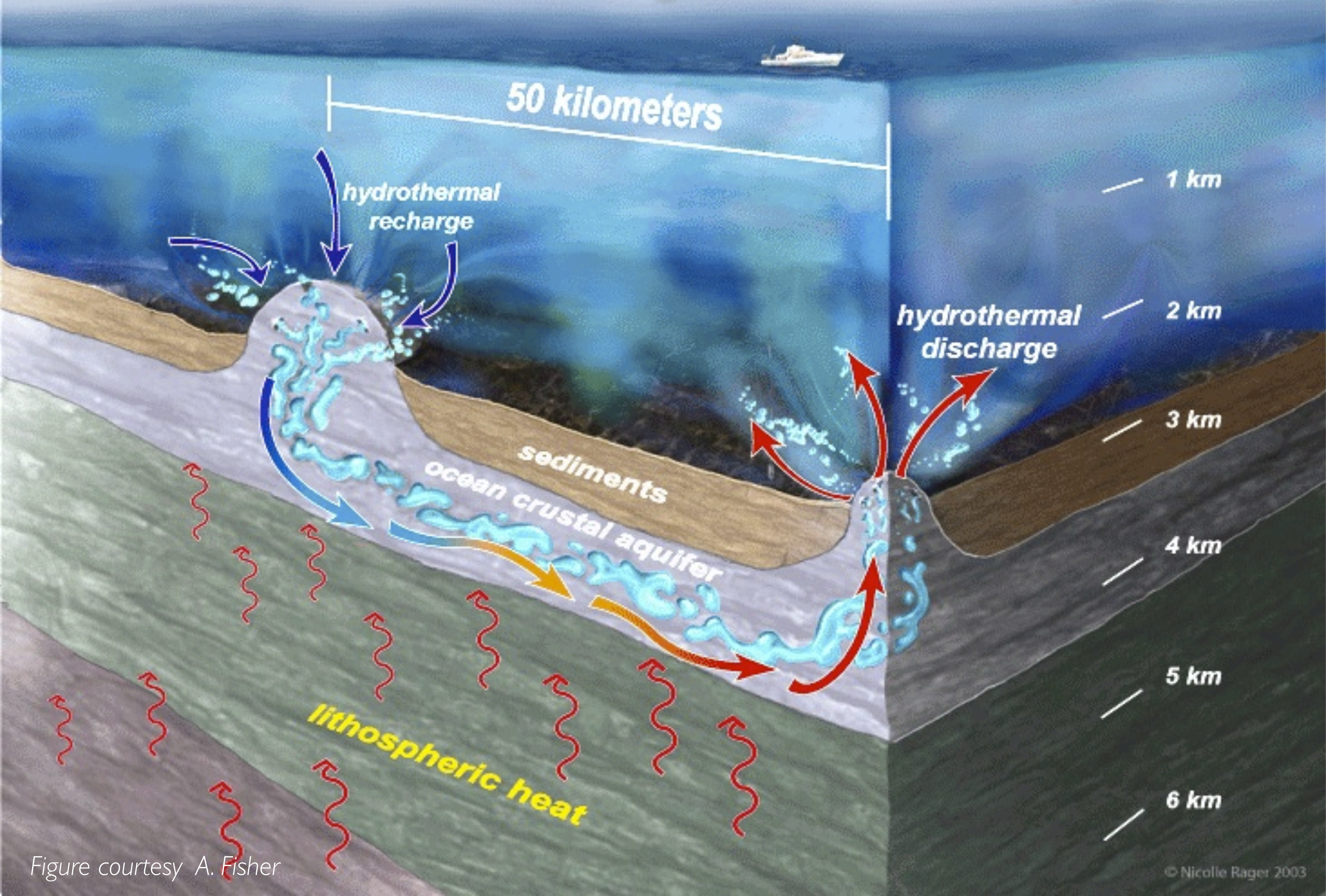
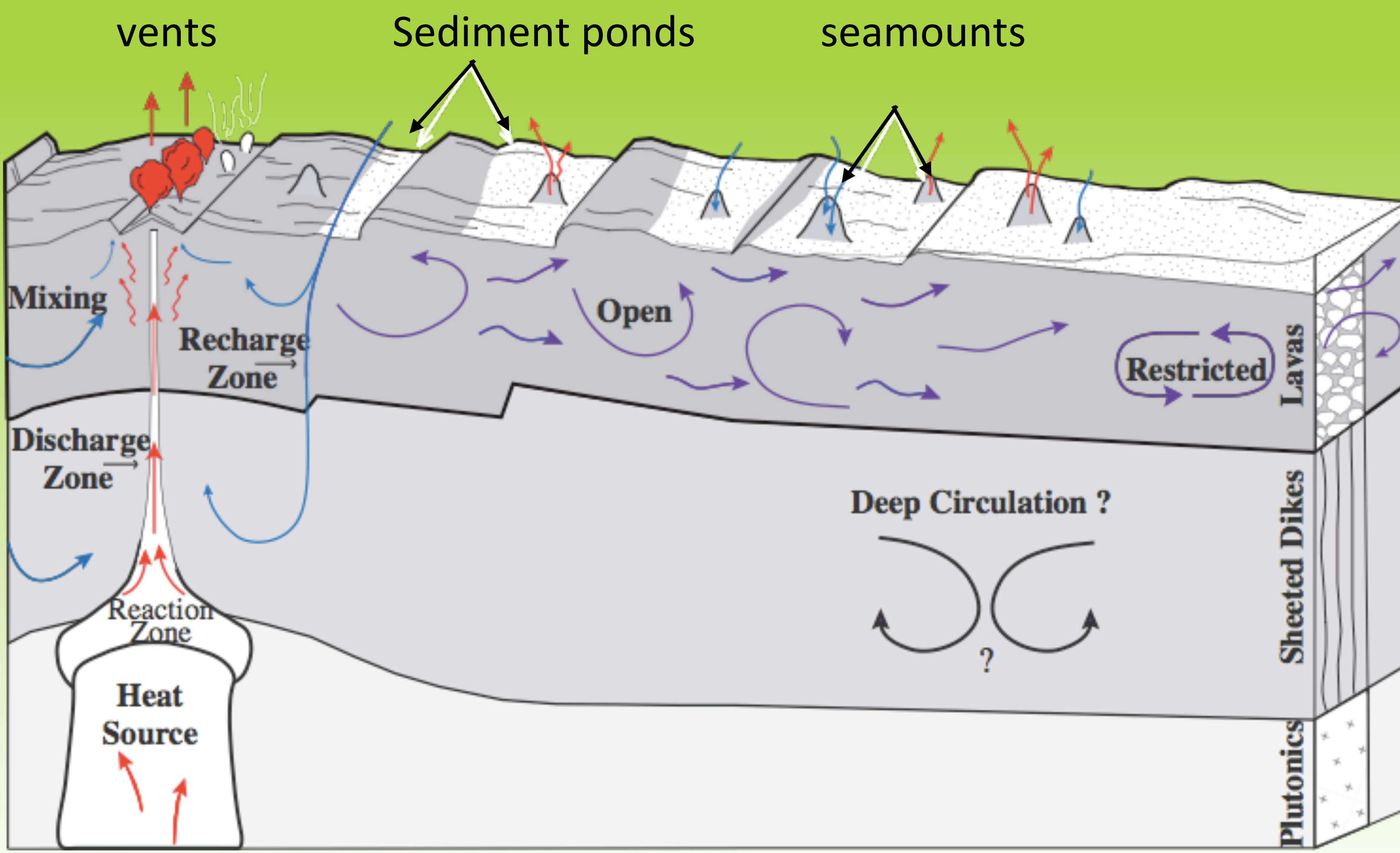


Figure courtesy A. Fisher

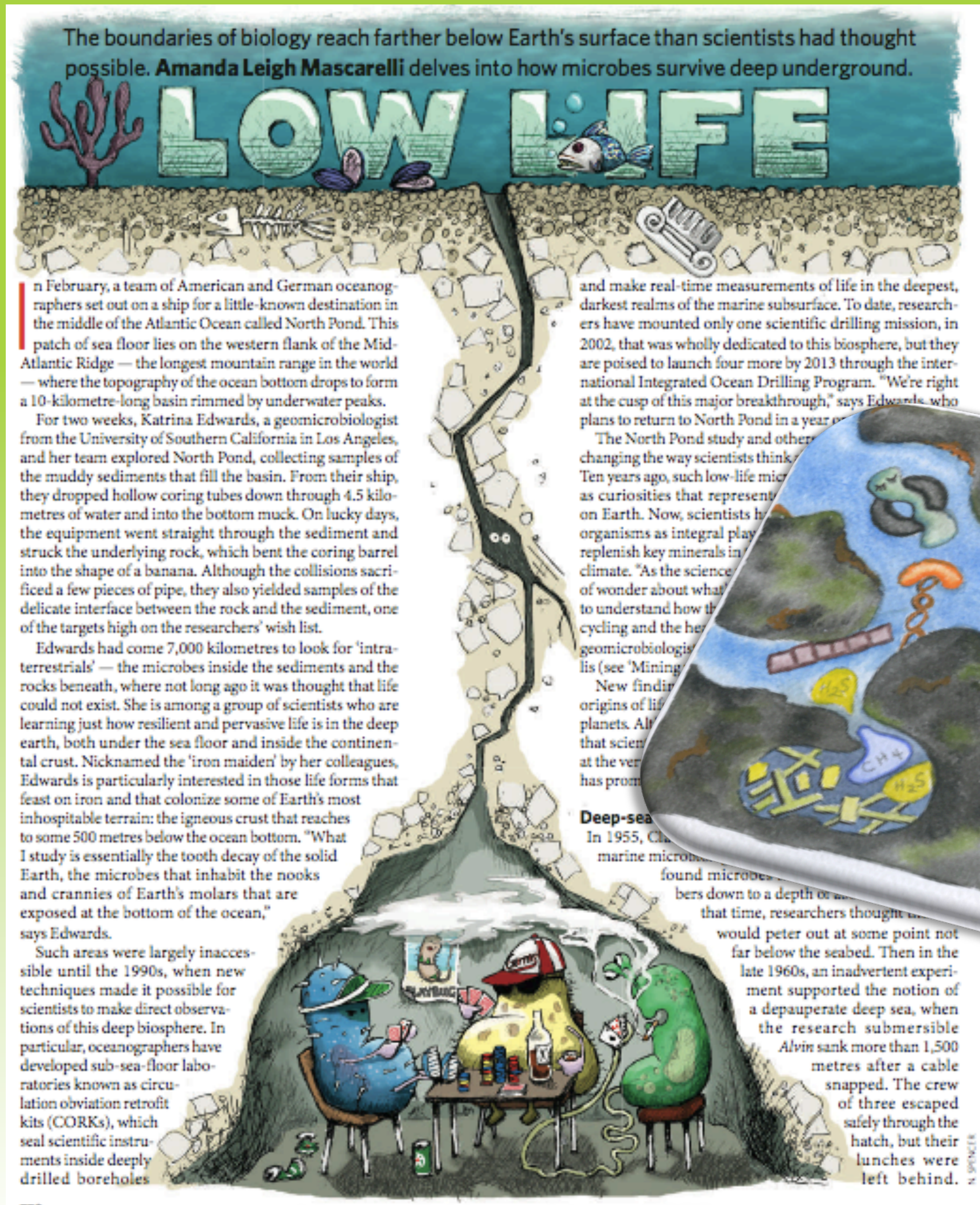
Several habitats in oceanic crust
- axis, ridge flank, seamounts, deeply buried…



Edwards et al., 2005 TRENDS in Microbiol


Is there life in the oceanic crust?
Photo by Beth Orcutt

Exploring the hard rock "intra-terrestrials"



The boundaries of biology reach farther below Earth's surface than scientists had thought possible. **Amanda Leigh Mascarelli** delves into how microbes survive deep underground.

LOW LIFE

In February, a team of American and German oceanographers set out on a ship for a little-known destination in the middle of the Atlantic Ocean called North Pond. This patch of sea floor lies on the western flank of the Mid-Atlantic Ridge — the longest mountain range in the world — where the topography of the ocean bottom drops to form a 10-kilometre-long basin rimmed by underwater peaks.

For two weeks, Katrina Edwards, a geomicrobiologist from the University of Southern California in Los Angeles, and her team explored North Pond, collecting samples of the muddy sediments that fill the basin. From their ship, they dropped hollow coring tubes down through 4.5 kilometres of water and into the bottom muck. On lucky days, the equipment went straight through the sediment and struck the underlying rock, which bent the coring barrel into the shape of a banana. Although the collisions sacrificed a few pieces of pipe, they also yielded samples of the delicate interface between the rock and the sediment, one of the targets high on the researchers' wish list.

Edwards had come 7,000 kilometres to look for 'intra-terrestrials' — the microbes inside the sediments and the rocks beneath, where not long ago it was thought that life could not exist. She is among a group of scientists who are learning just how resilient and pervasive life is in the deep earth, both under the sea floor and inside the continental crust. Nicknamed the 'iron maiden' by her colleagues, Edwards is particularly interested in those life forms that feast on iron and that colonize some of Earth's most inhospitable terrain: the igneous crust that reaches to some 500 metres below the ocean bottom. "What I study is essentially the tooth decay of the solid Earth, the microbes that inhabit the nooks and crannies of Earth's molars that are exposed at the bottom of the ocean," says Edwards.

Such areas were largely inaccessible until the 1990s, when new techniques made it possible for scientists to make direct observations of this deep biosphere. In particular, oceanographers have developed sub-sea-floor laboratories known as circulation obviation retrofit kits (CORKs), which seal scientific instruments inside deeply drilled boreholes and make real-time measurements of life in the deepest, darkest realms of the marine subsurface. To date, researchers have mounted only one scientific drilling mission, in 2002, that was wholly dedicated to this biosphere, but they are poised to launch four more by 2013 through the international Integrated Ocean Drilling Program. "We're right at the cusp of this major breakthrough," says Edwards, who plans to return to North Pond in a year o

The North Pond study and other
changing the way scientists think
Ten years ago, such low-life mic
as curiosities that represent
on Earth. Now, scientists h
organisms as integral play
replenish key minerals in
climate. "As the science
of wonder about what
to understand how th
cycling and the her
geomicrobiologis
lis (see 'Mining

New findin
origins of lif
planets. Al
that scien
at the ver
has prom

Deep-sea

In 1955, Cl
marine microb
found microbes
bers down to a depth o
that time, researchers thought
would peter out at some point not far below the seabed. Then in the late 1960s, an inadvertent experiment supported the notion of a depauperate deep sea, when the research submersible *Alvin* sank more than 1,500 metres after a cable snapped. The crew of three escaped safely through the hatch, but their lunches were left behind.

N. SPENCER

770

Mascarelli, *Nature* News, 2009

How we get samples from the Deep Biosphere

Photo by Bill Crawford/TAMU

Photo by V. Heuer

Photo by Beth Orcutt

IODP
INTEGRATED OCEAN DRILLING PROGRAM

NSF

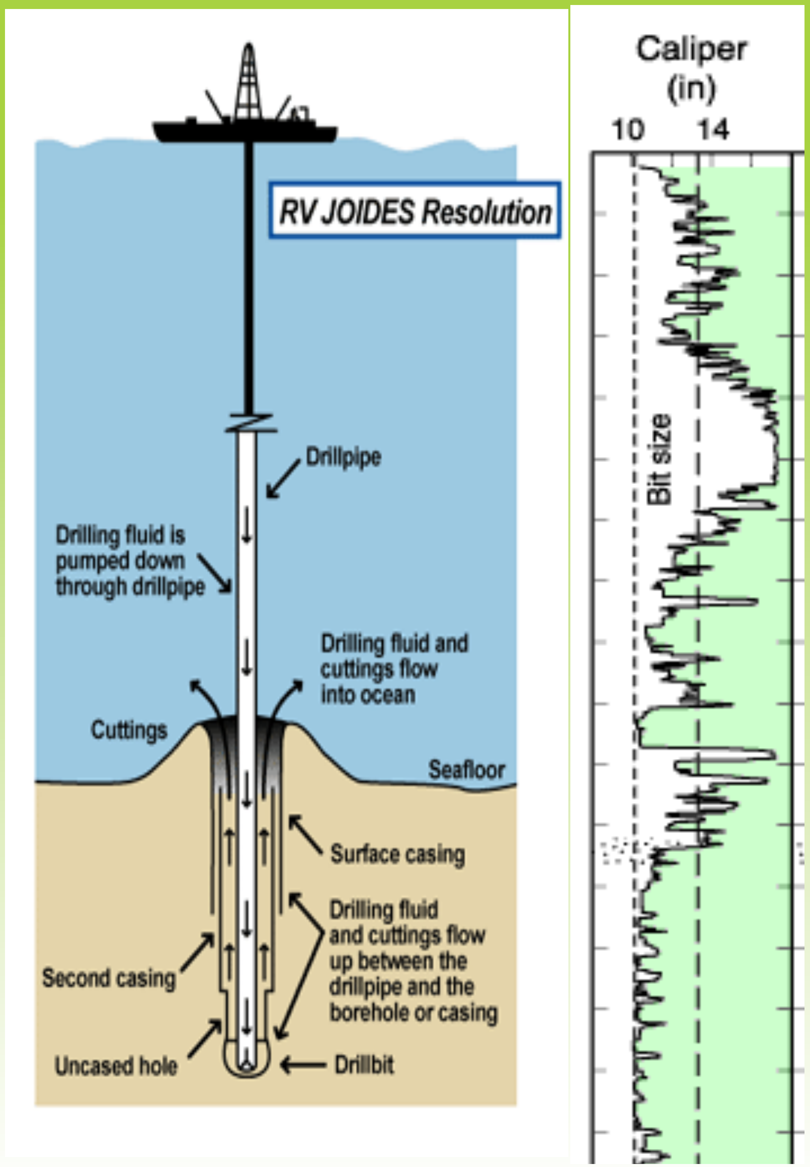
University-National Oceanographic Laboratory System

How we get samples from the Deep Biosphere

Rocks, sediments, hammers…oh my!

Photos courtesy of J. Magnusson, B. Crawford, J. Sylvan

How we get samples from the Deep Biosphere



PROBLEM:

Hard to recover quality rock samples for microbiology (or chemistry) by drilling

What is happening at depth?
Observatories below the seafloor

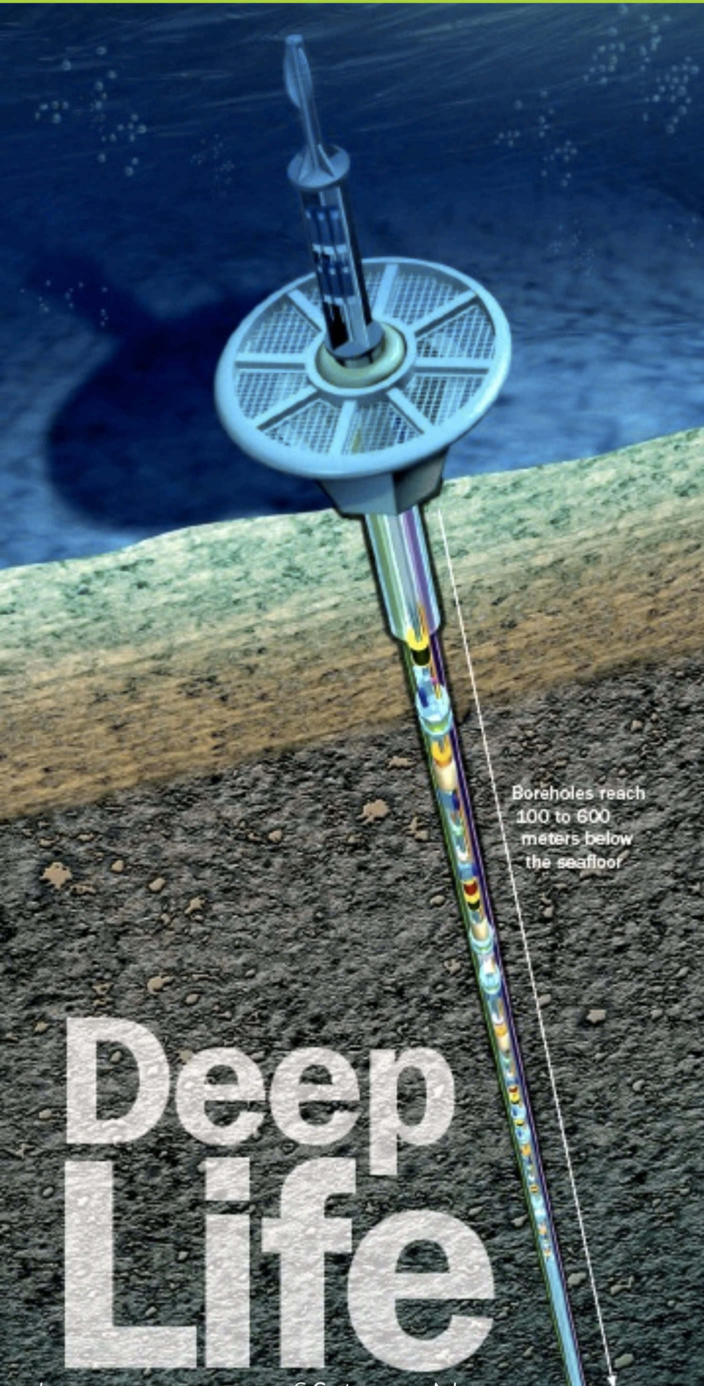


Image courtesy of Science News

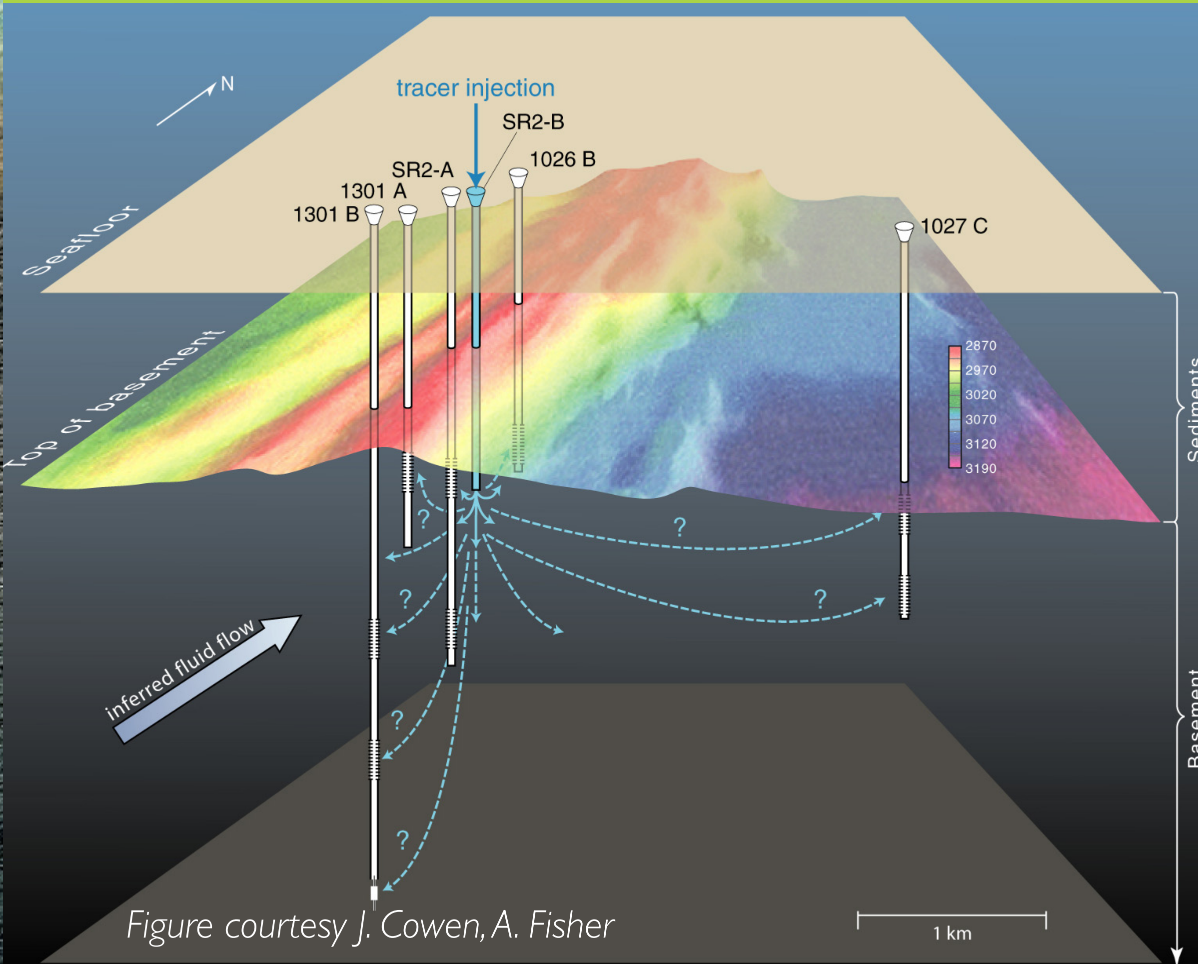
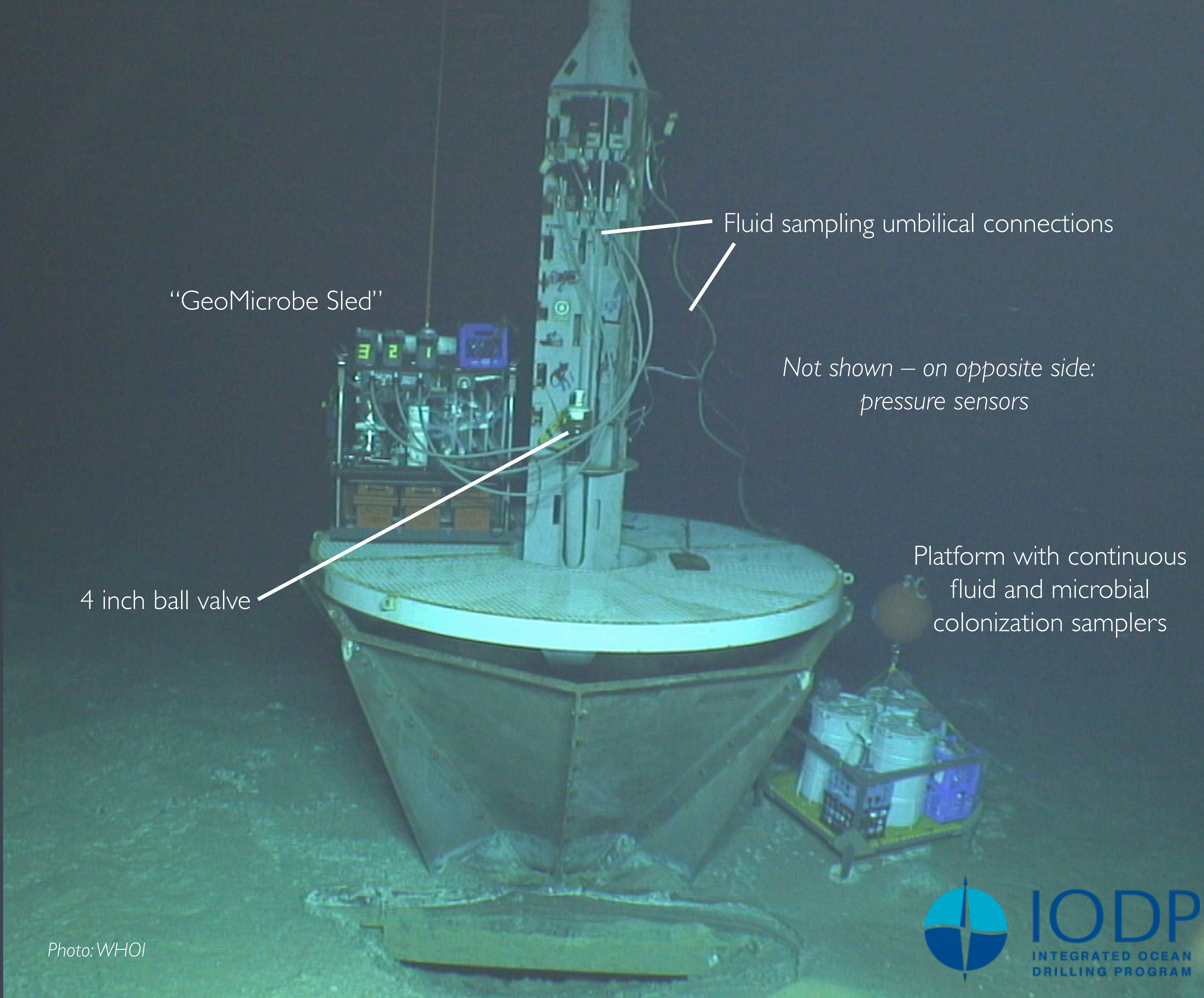
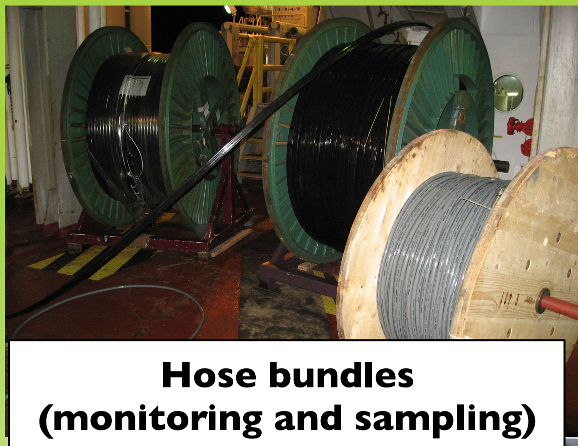


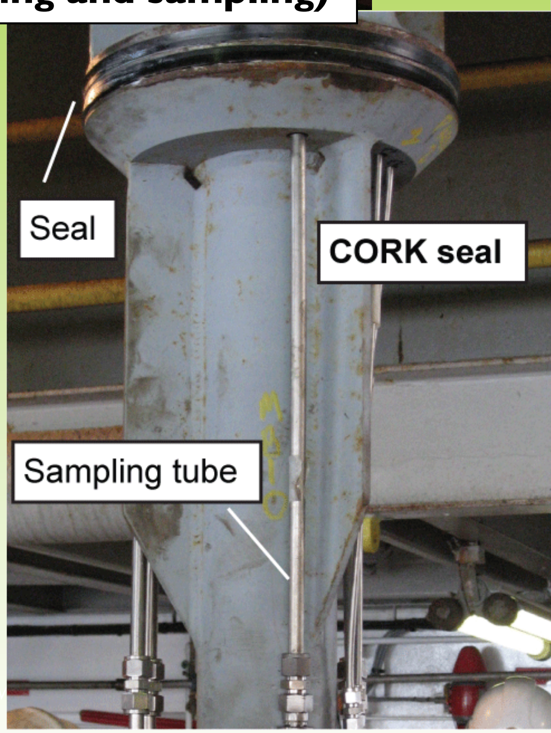
Figure courtesy J. Cowen, A. Fisher

movie

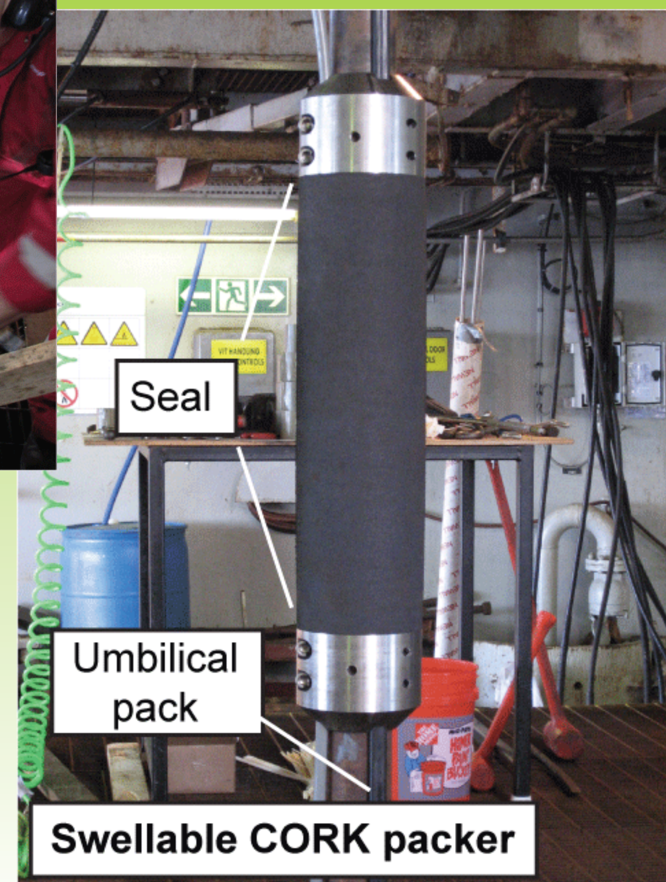

Fluid sampling umbilical connections
"GeoMicrobe Sled"
Not shown – on opposite side: pressure sensors
Platform with continuous fluid and microbial colonization samplers
4 inch ball valve
Photo: WHOI
IODP
INTEGRATED OCEAN DRILLING PROGRAM

Components for sealing, sampling borehole for geochem & microbio

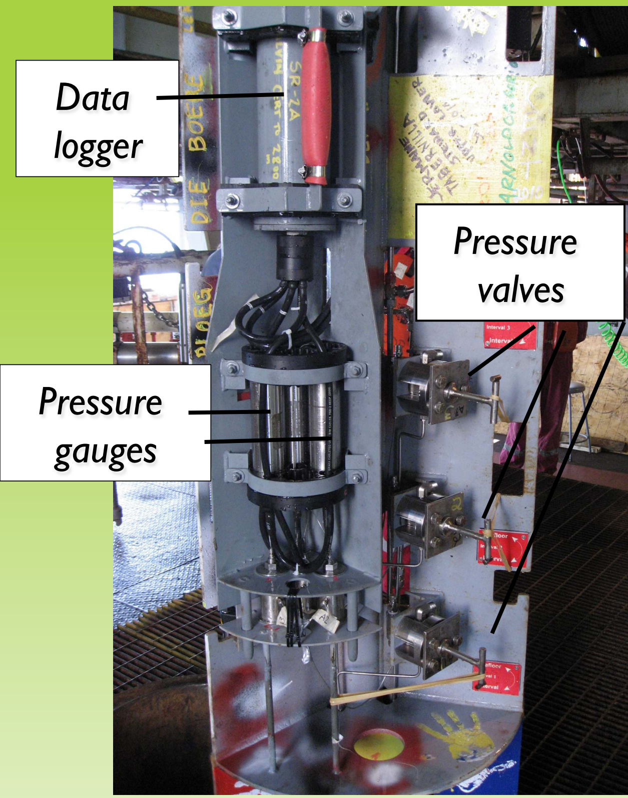




Fisher et al. 2011
X327 volume
Photos: Andy Fisher



Three bays for three kinds of sampling & monitoring



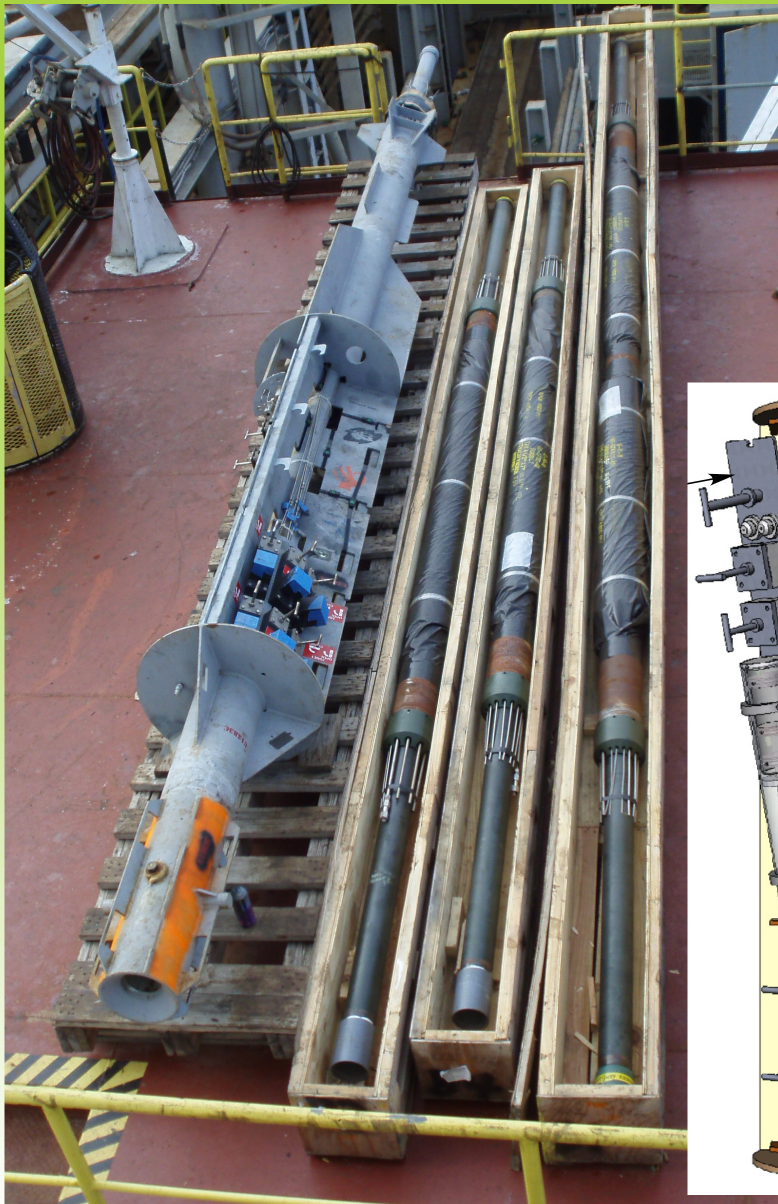
Multilevel Pressure Monitoring

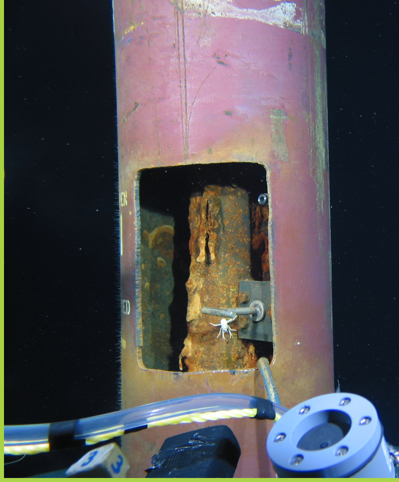
Fluid sampling (Teflon-lined umbilicals) by GeoMicrobe Sled & Free flow valve

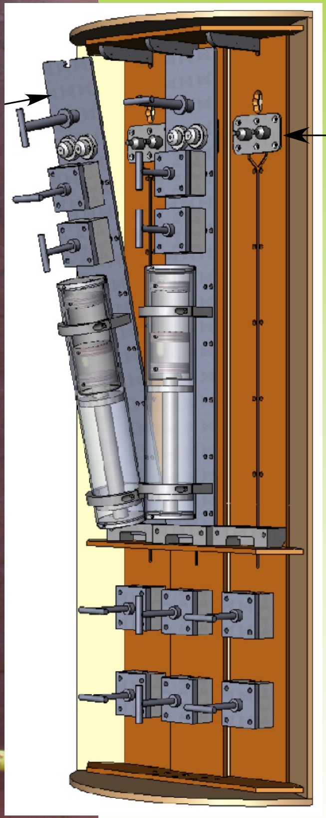
Fluid sampling for microbiology and colonization experiments

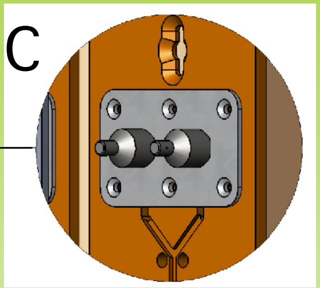
Fisher et al. 2011 X327 volume
Photos: Andy Fisher

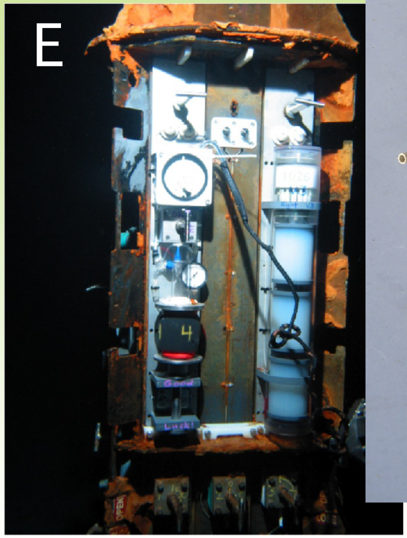
Various ways to connect OsmoSamplers and sleds at the seafloor

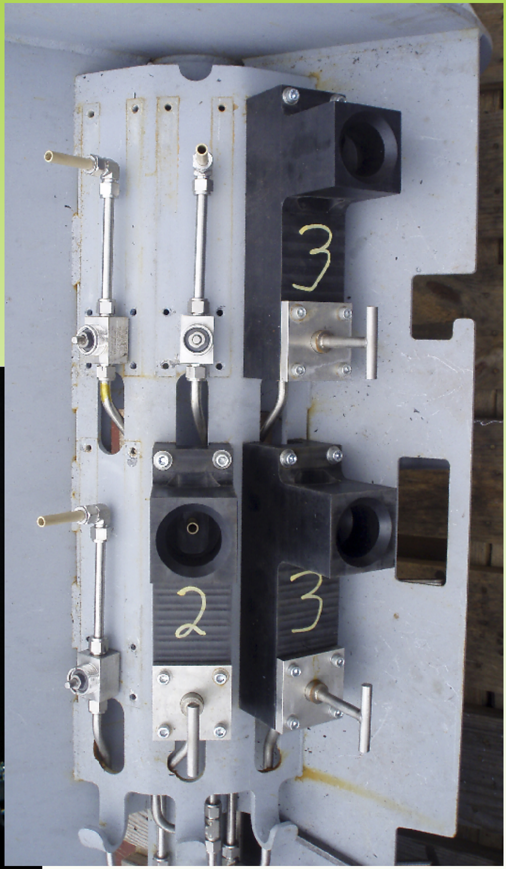




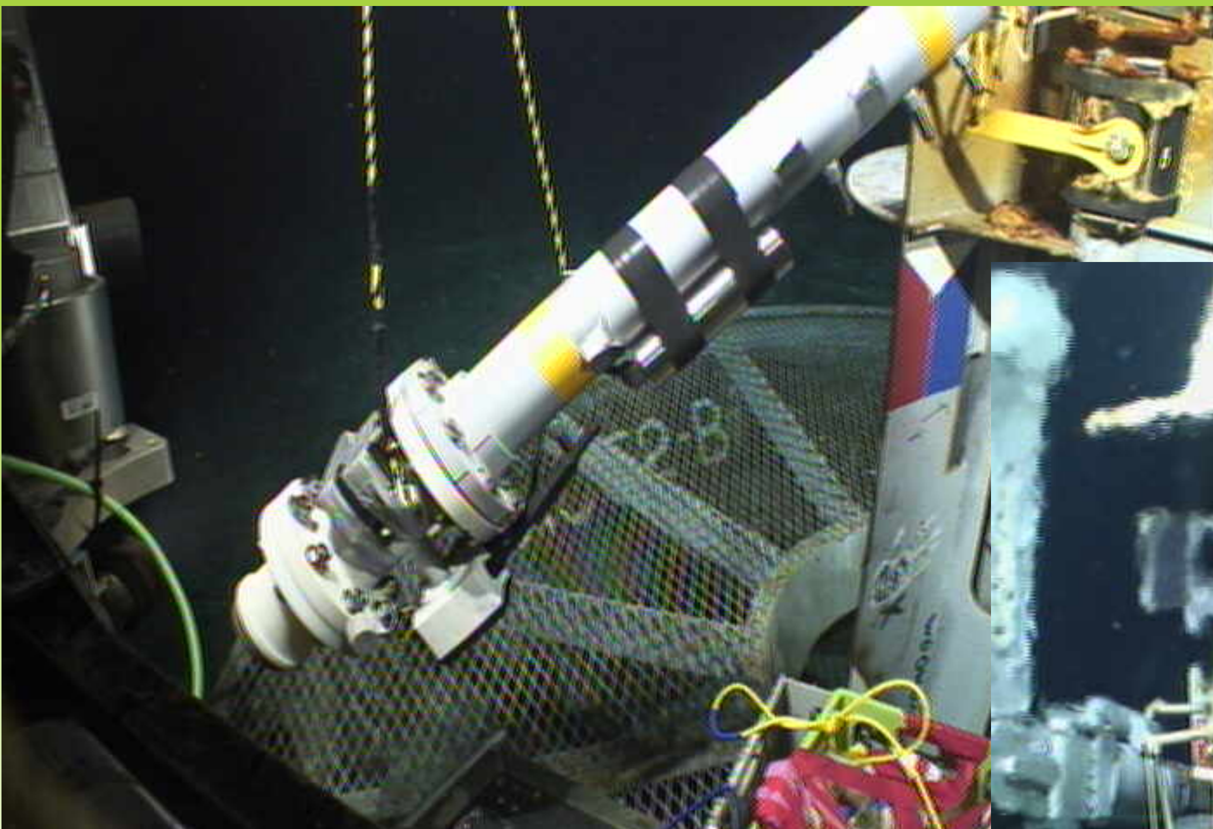




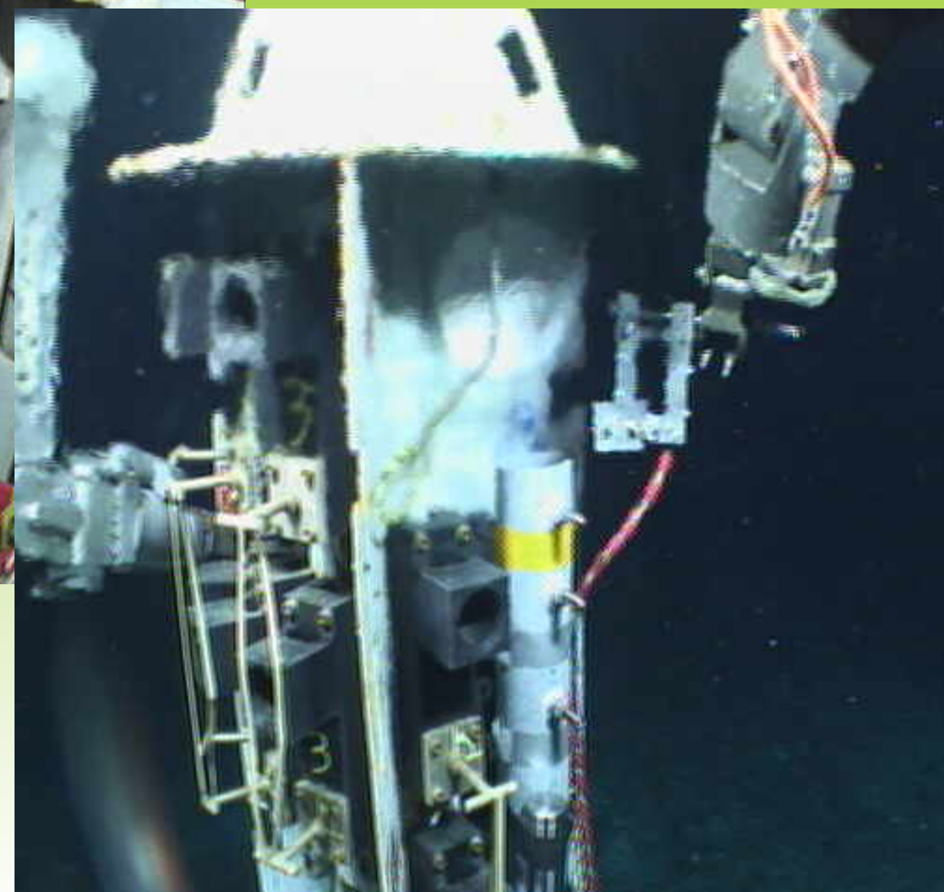




Components for sealing, sampling borehole for geochem & microbio



Fisher et al. 2012
Scientific Drilling
Photos: WHOI



Flow meter installed
on Free flow valve

Downhole OsmoSamplers


Wheat et al. 2011 (IODP X327 volume)
Edwards et al. 2012 (IODP X336 volume)

Photo Geoff Wheat

Photo Geoff Wheat

Many varieties of OsmoSamplers

Regular (Teflon) – major/minor ions

Acid Addition – trace metals

Copper – gases

BOSS – biological preservation

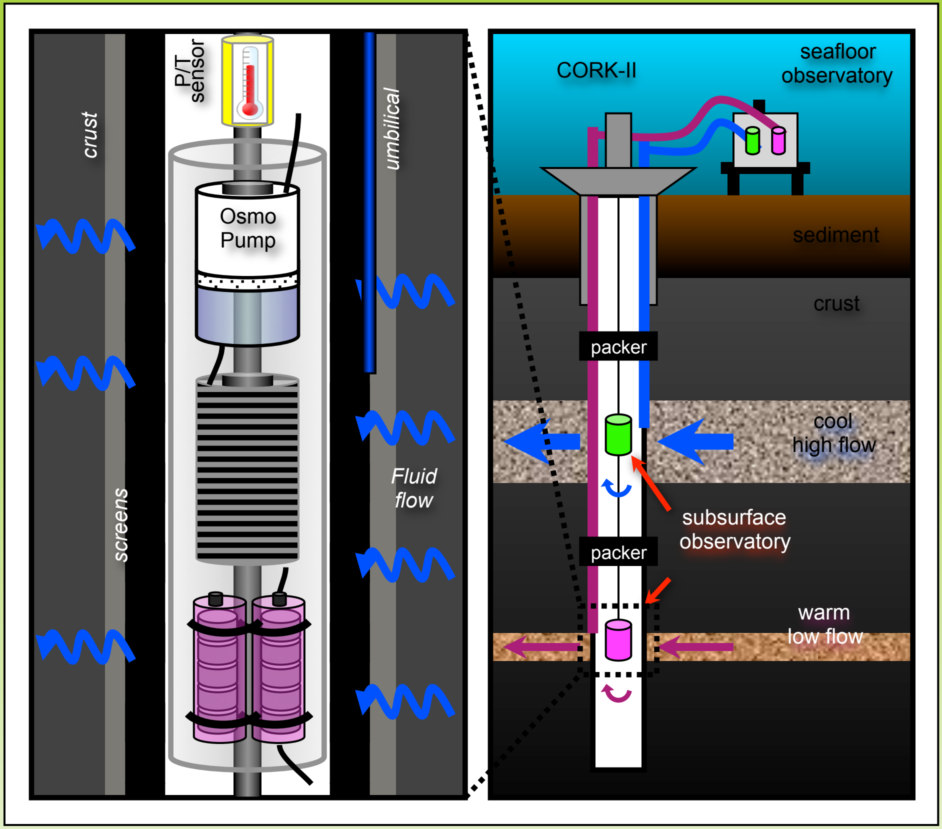
MBIO – microbial colonization experiments

Enrichment – microbial enrichment experiments

Wheat et al. 2011 (IODP X327 volume)

EXAMPLE – Juan de Fuca CORKS (IODP X301/X327)

Using CORKs to explore subsurface microbiology



Subsurface microbial colonization experiments combined with "OsmoSamplers" to pull fluids over substrates for microbial growth

Fisher et al., 2005; Orcutt et al. *ISME J* 2011

EXAMPLE – Juan de Fuca CORKS (IODP X301/X327)

Subsurface microbial observatories

Microbial colonization experiments

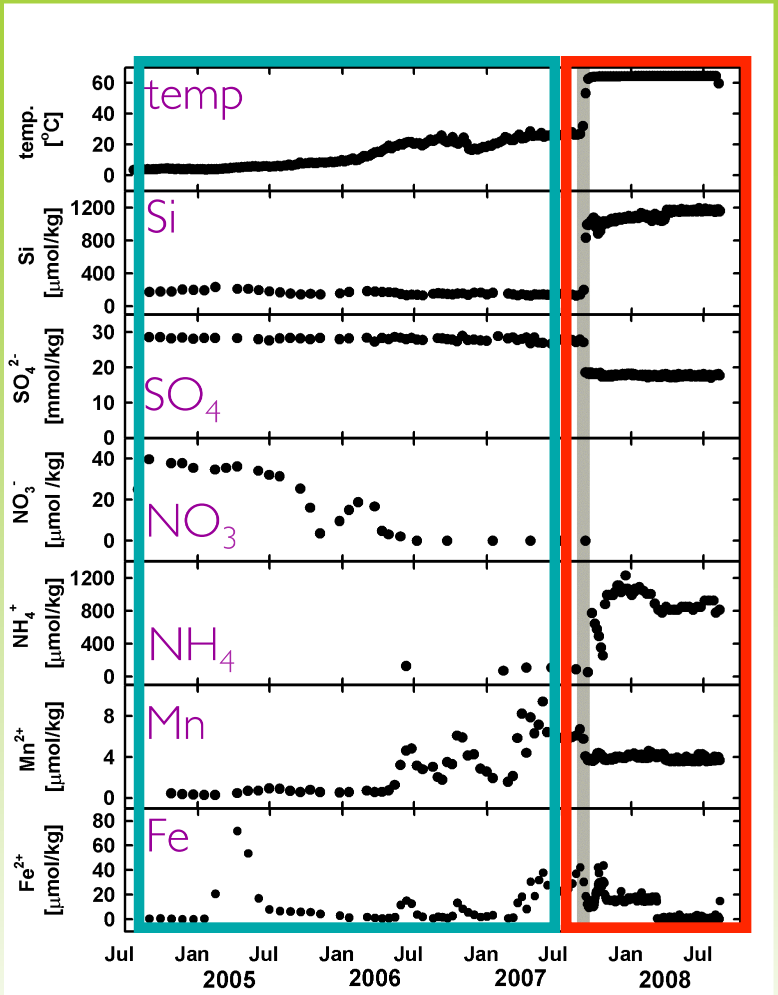
FLOCS = FLow-through Osmo Colonization System

Orcutt et al., Geomicrobiology J 2010

Connected with long-term (~5 year) chemical sensors to track changes in borehole environment

EXAMPLE – Juan de Fuca CORKS (IODP X301/X327)

CORKs on the eastern Juan de Fuca Ridge flank



Continuous temperature and chemical data collection record "re-bound" of hole conditions after ~3 years

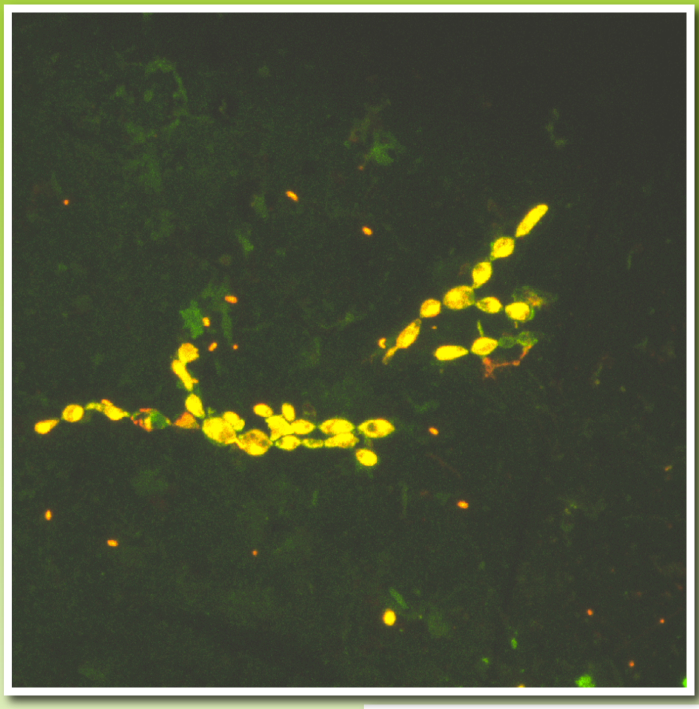
'RECHARGE' - sucking cold seawater into borehole

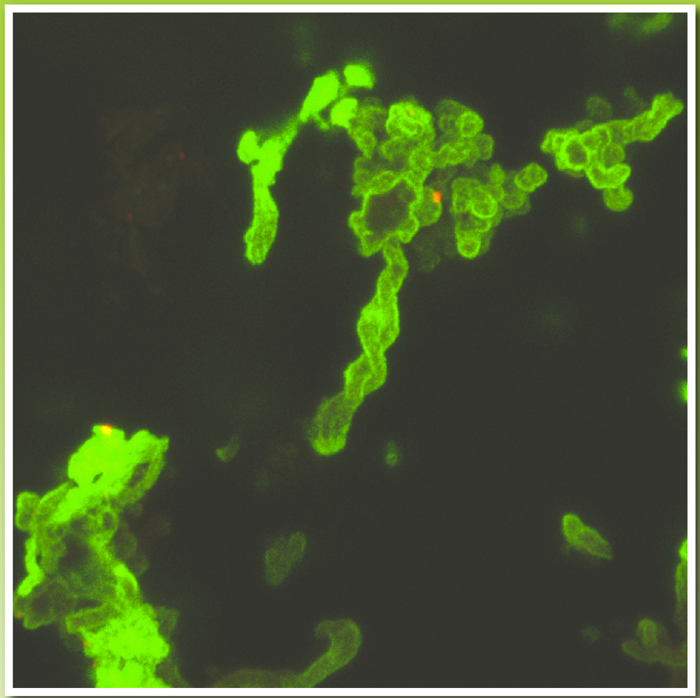
'DISCHARGE' - venting warm, reduced fluids out of crust

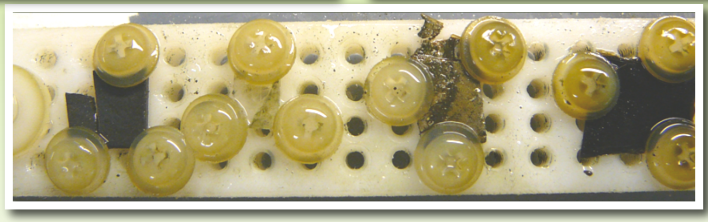
Orcutt et al. *ISME J* 2011; Wheat et al. G^3 2010

EXAMPLE – Juan de Fuca CORKS (IODP X301/X327)

CORKs on the eastern Juan de Fuca Ridge flank







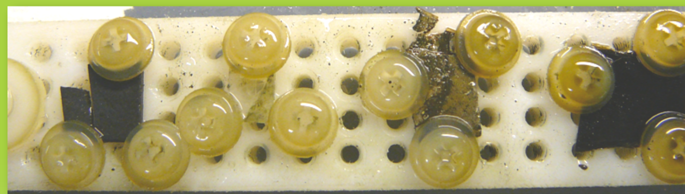
Orcutt et al. *ISME J* 2011

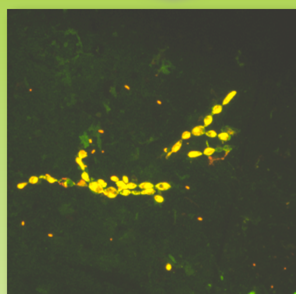
Mineral chips deployed in the subsurface for 4 years were **colonized by cells** with differing morphology

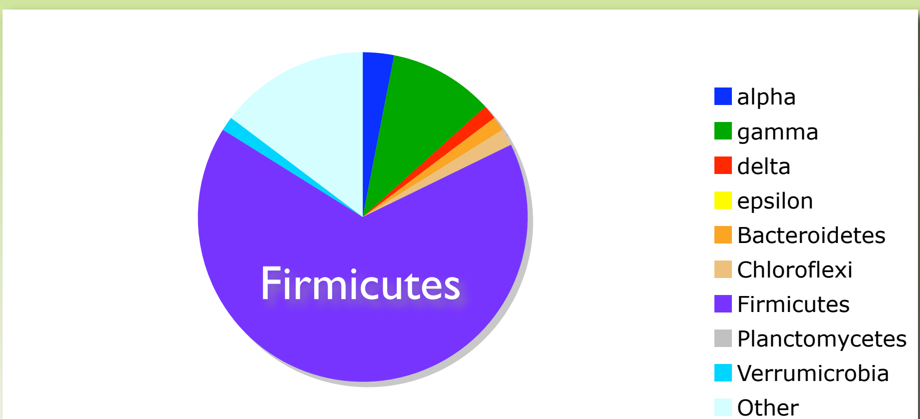
Biogenic-like **twisted stalks** also evident - indication of iron oxidizing bacteria ?

EXAMPLE – Juan de Fuca CORKS (IODP X301/X327)

CORKs on the eastern Juan de Fuca Ridge flank







Percentage of different groups of Bacteria, based on 16S rRNA gene

Majority of borehole mineral chip sequences group within the Firmicutes phyla

- *unclear physiology - N, S cycling ??*

No known iron oxidizers found

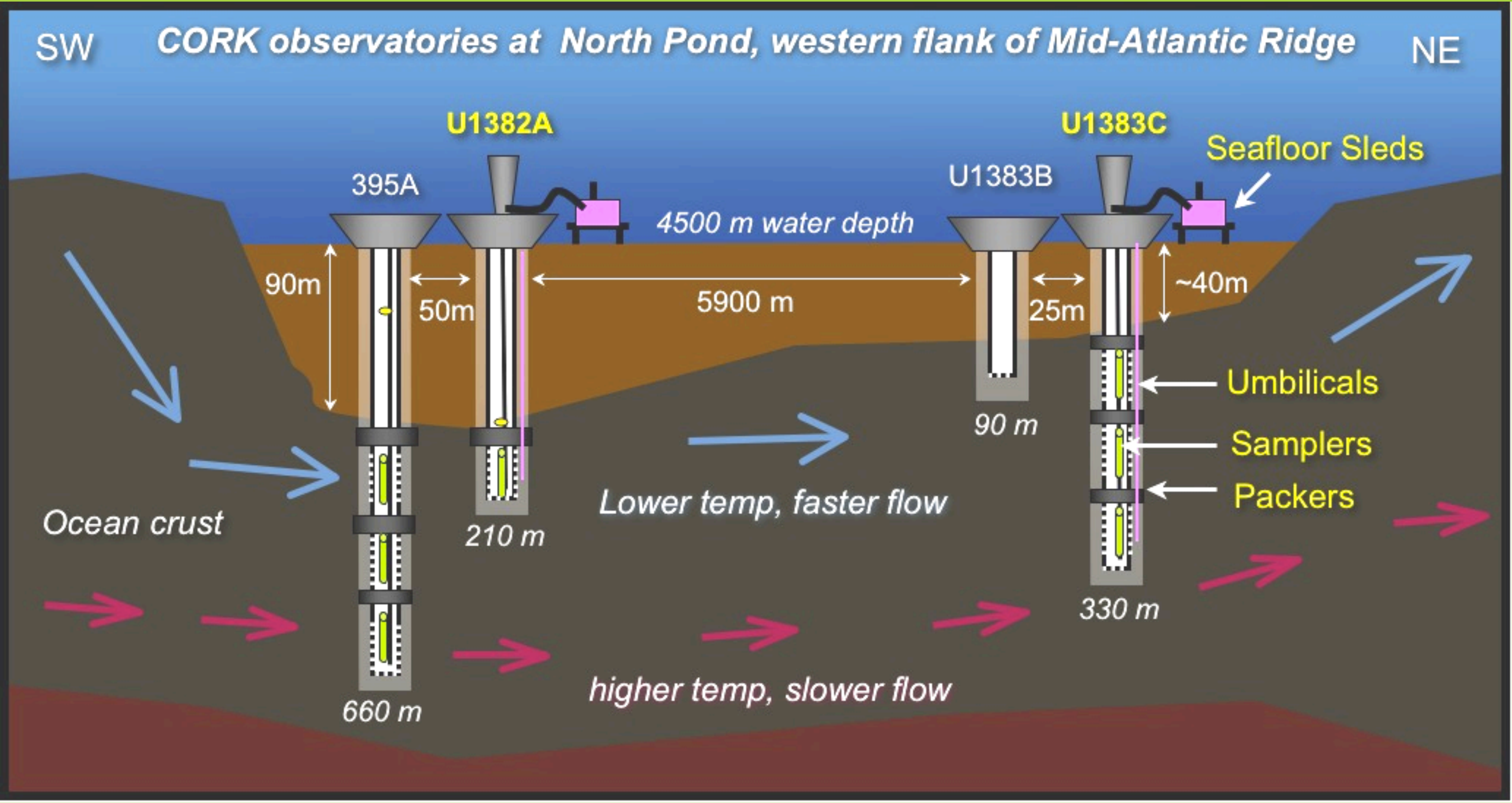
-observed twisted stalks a 'fossil' of life during earlier, cooler borehole conditions

Close relatives include sequences from terrestrial deep biosphere (mines)

- *terrestrial/marine deep biosphere connection ??*

Orcutt et al. *ISME J* 2011

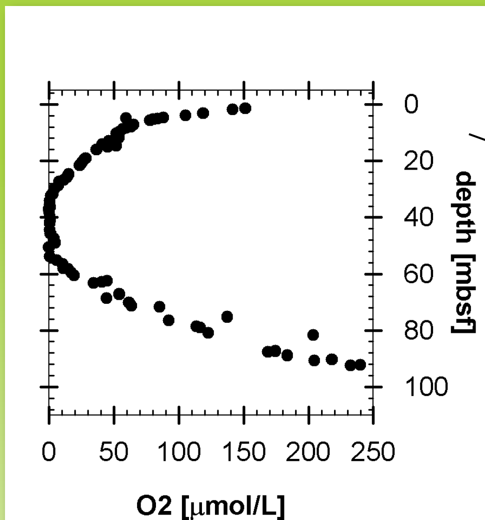
What lives in deep oceanic crust?

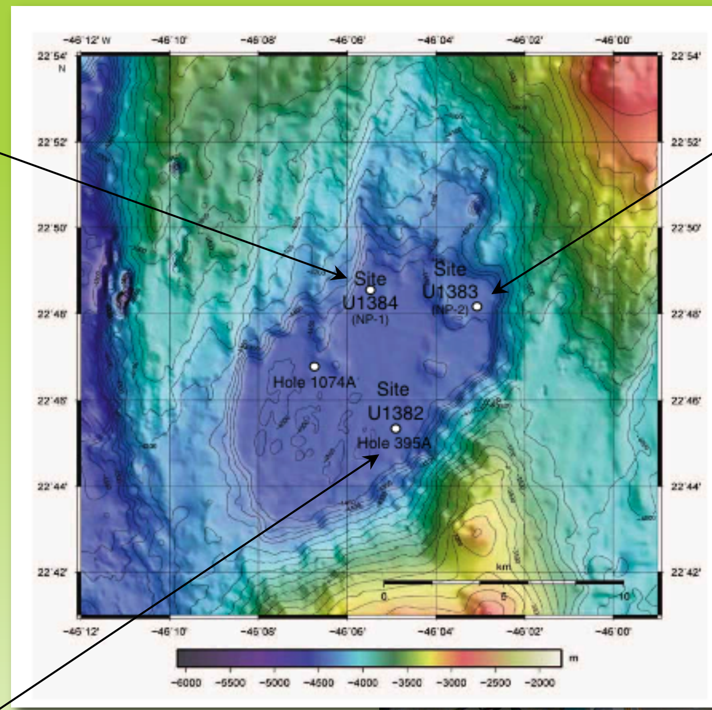


Edwards et al. 2012 IODP X336 volume

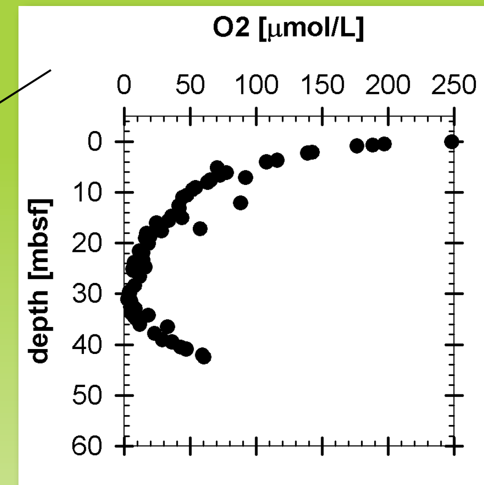
Life in Deep, Young & Cool Crust (X336 North Pond)

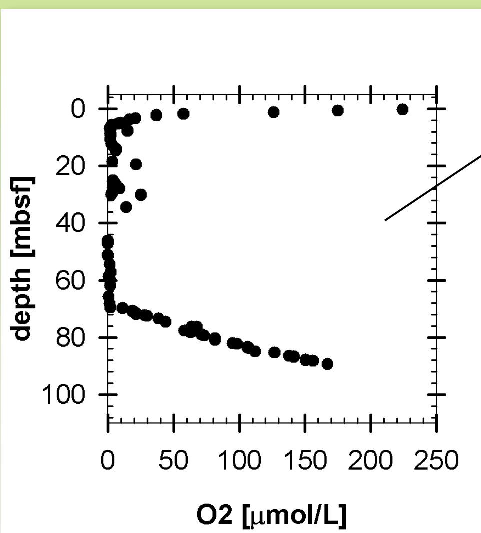
Evidence of **oxygen** diffusing into deep sediments from basement

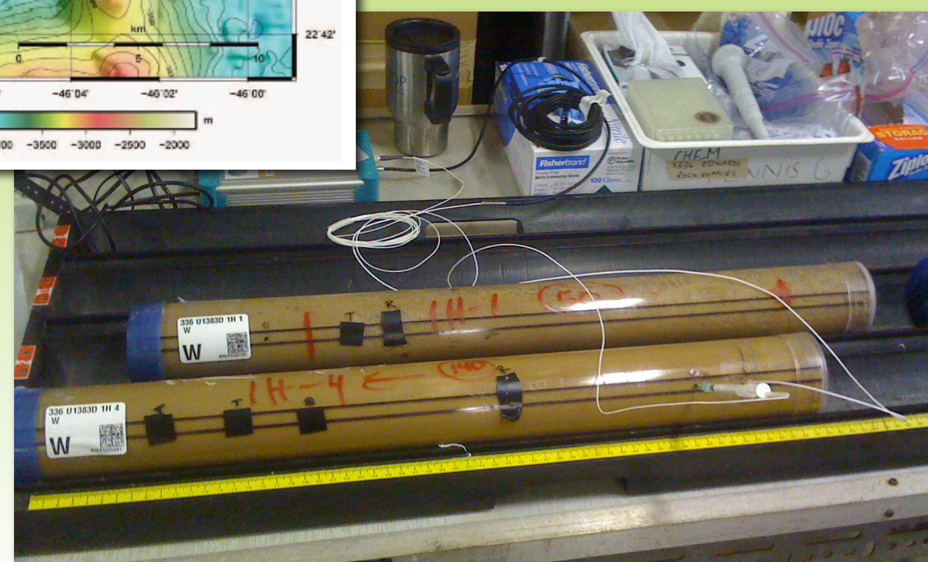




Orcutt et al. in review

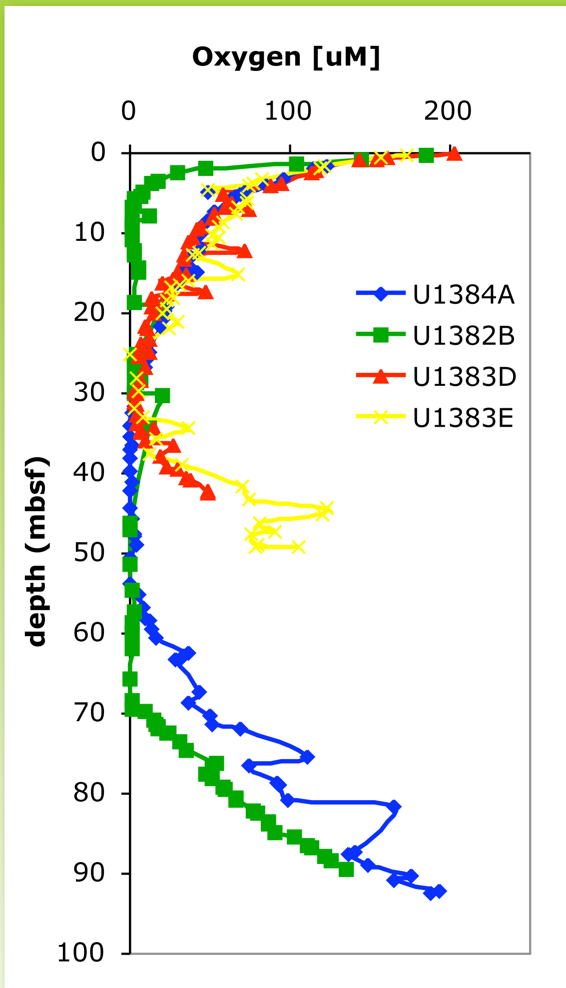




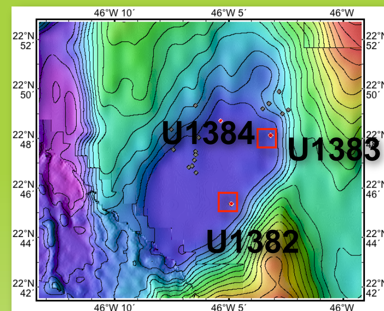


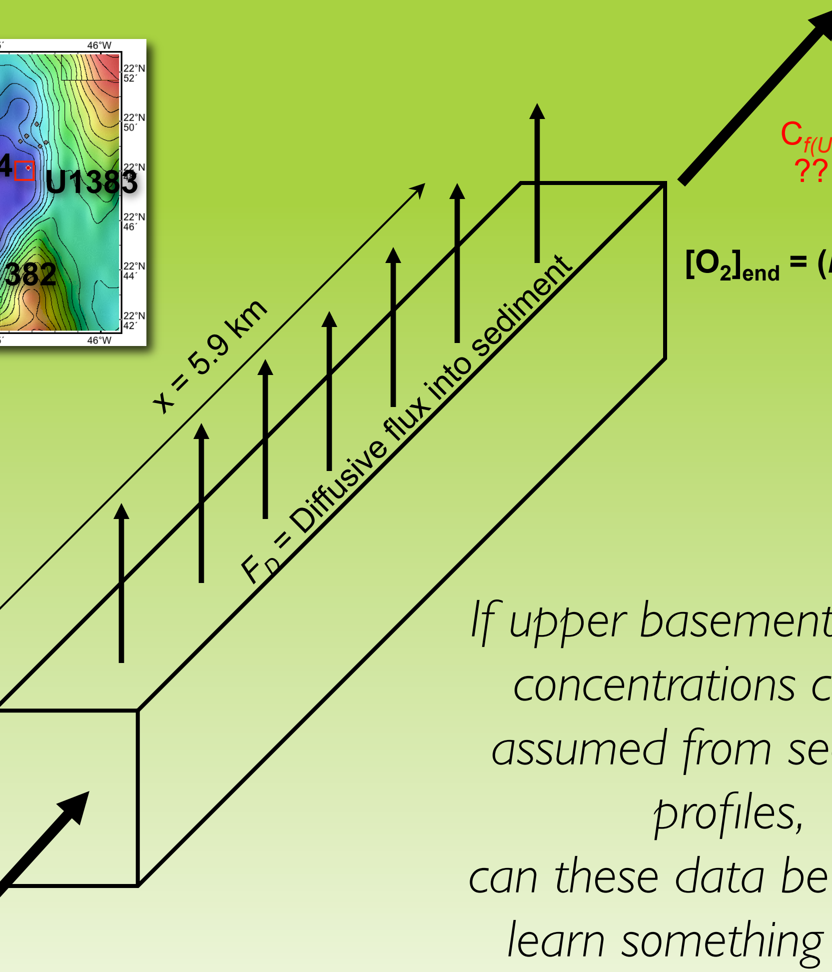
Life in the Deep, Young & Cool Crust

Evidence for life in cool, oxic, oceanic crust ?

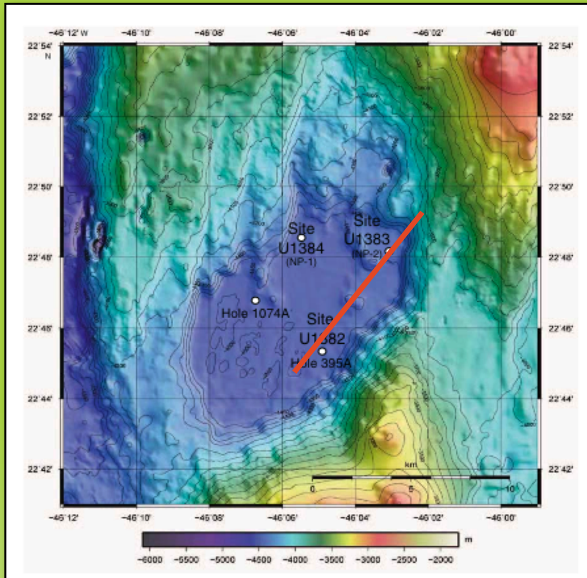


Orcutt et al. in review





If upper basement oxygen concentrations can be assumed from sediment profiles, can these data be used to learn something about oxygen behavior in oceanic crust?

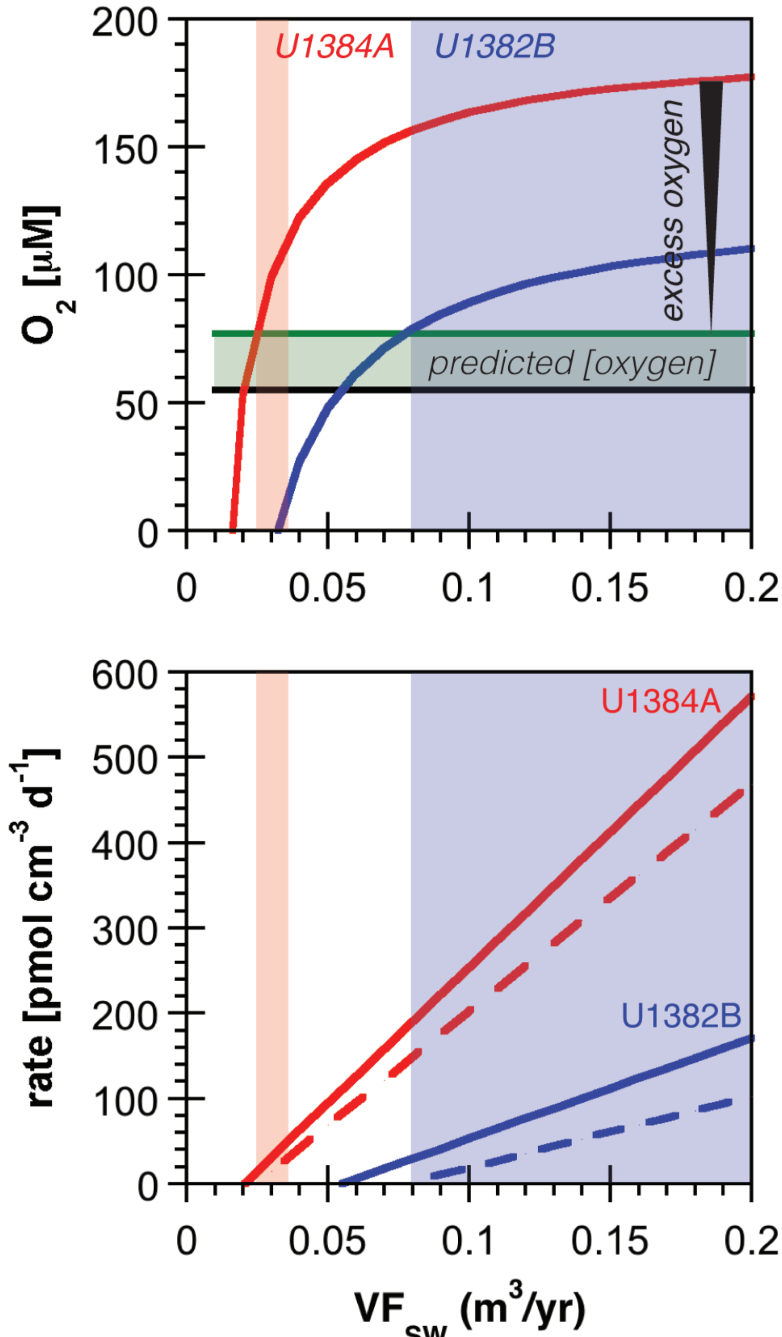


Basement oxygen concentrations can be explained by diffusion alone *only* at **very low fluid flow rates**

Reaction in basement is likely, since fluid flow rates are expected to be higher

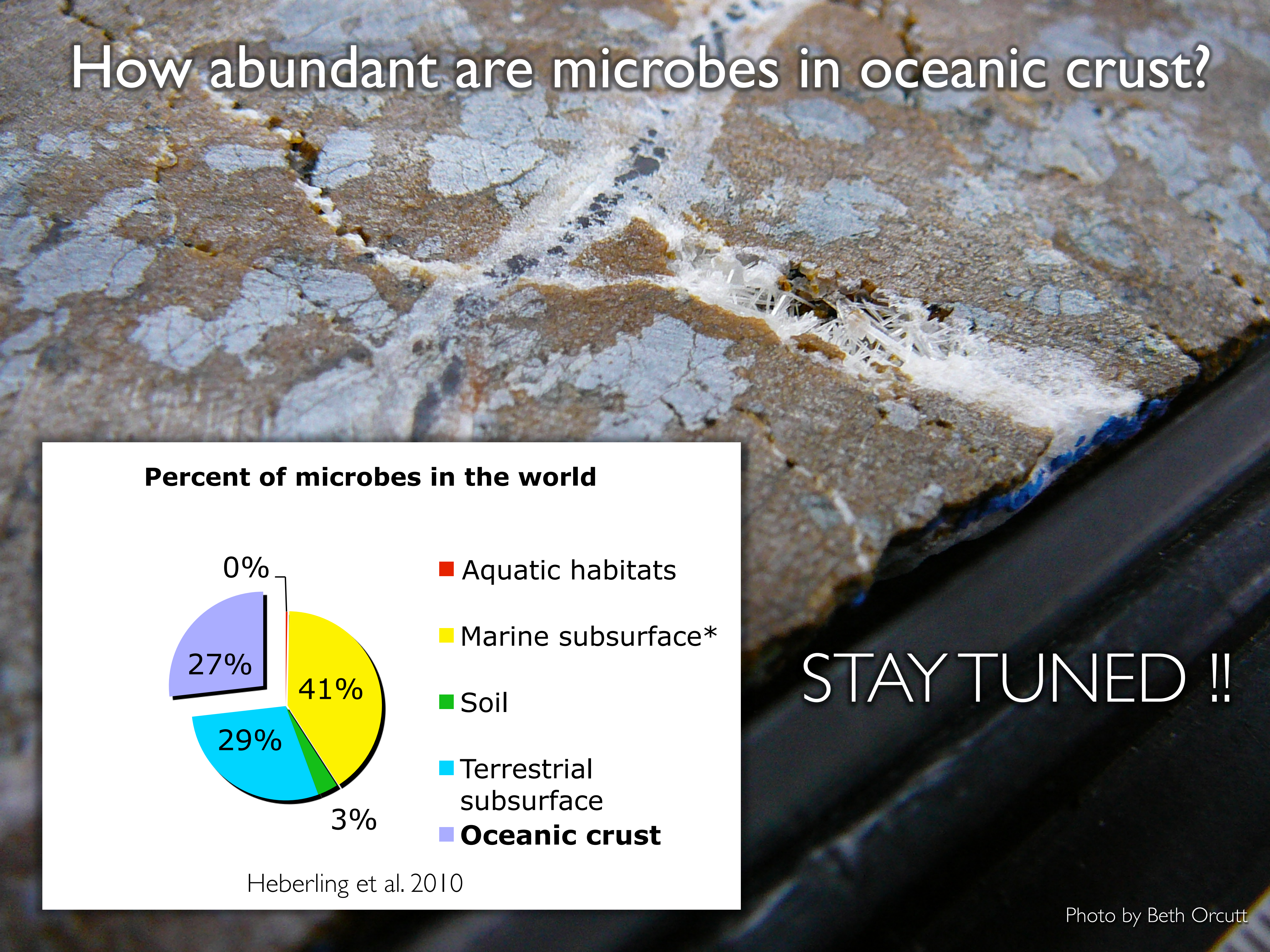
Rates of oxygen consumption in basement may be higher than in sediments

First estimate of oxygen consumption in basement (*but needs refinement!*)



Orcutt et al. in review

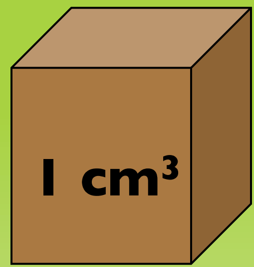
How abundant are microbes in oceanic crust?



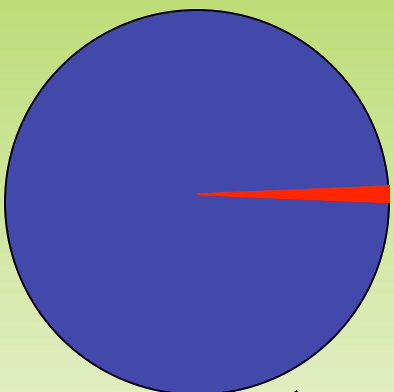
STAY TUNED !!

Photo by Beth Orcutt

How does life in the dark ocean survive ??

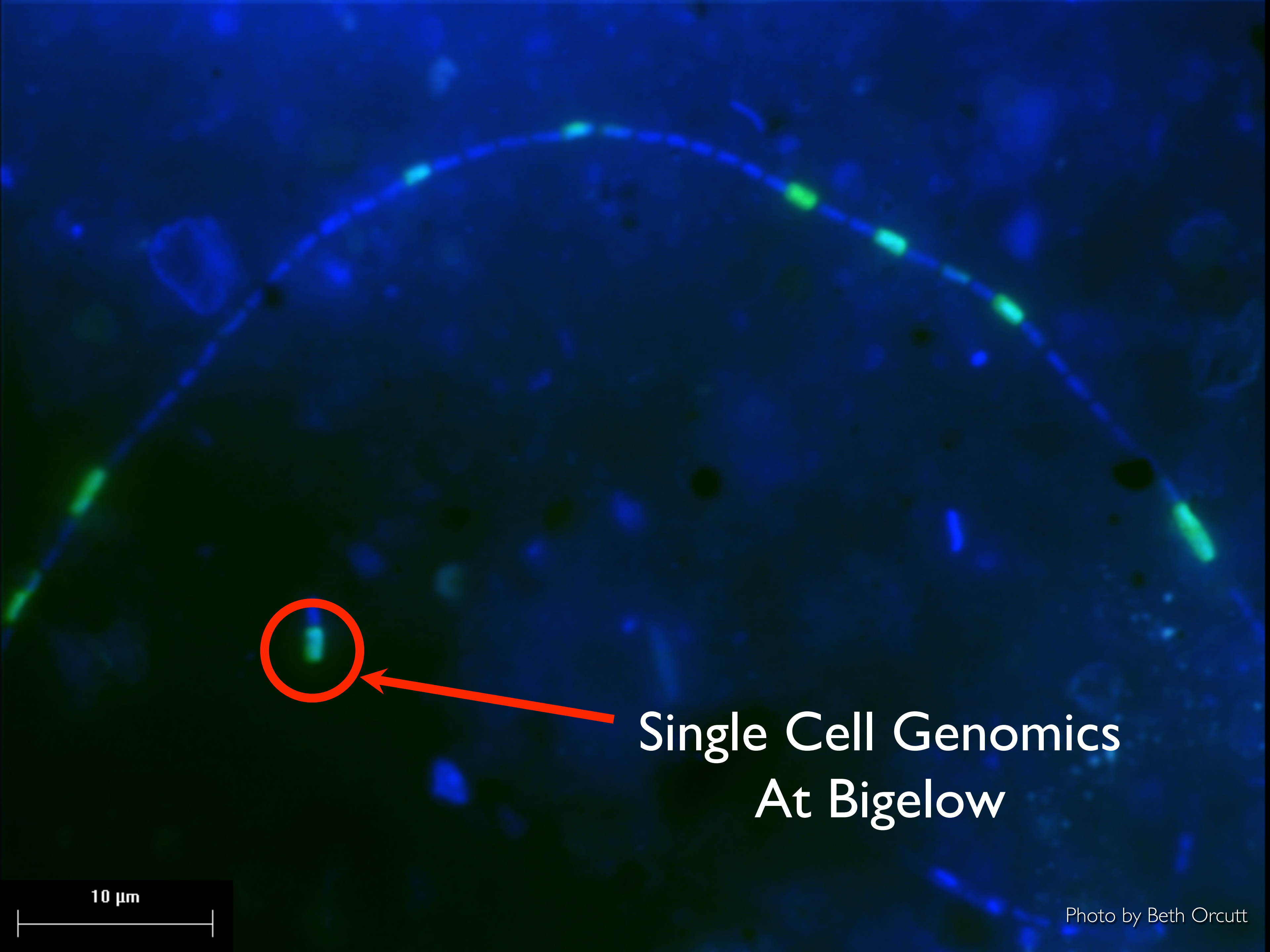


1 cm³ of shallow sediment has
1,000,000 - 1,000,000,000
microbial cells



Less than 1% of the cells
Are known in culture

What is the identity (and function) of microbes in the environment?


Single Cell Genomics
At Bigelow
10 µm
Photo by Beth Orcutt

Life in the Dark Ocean…
Buried Alive

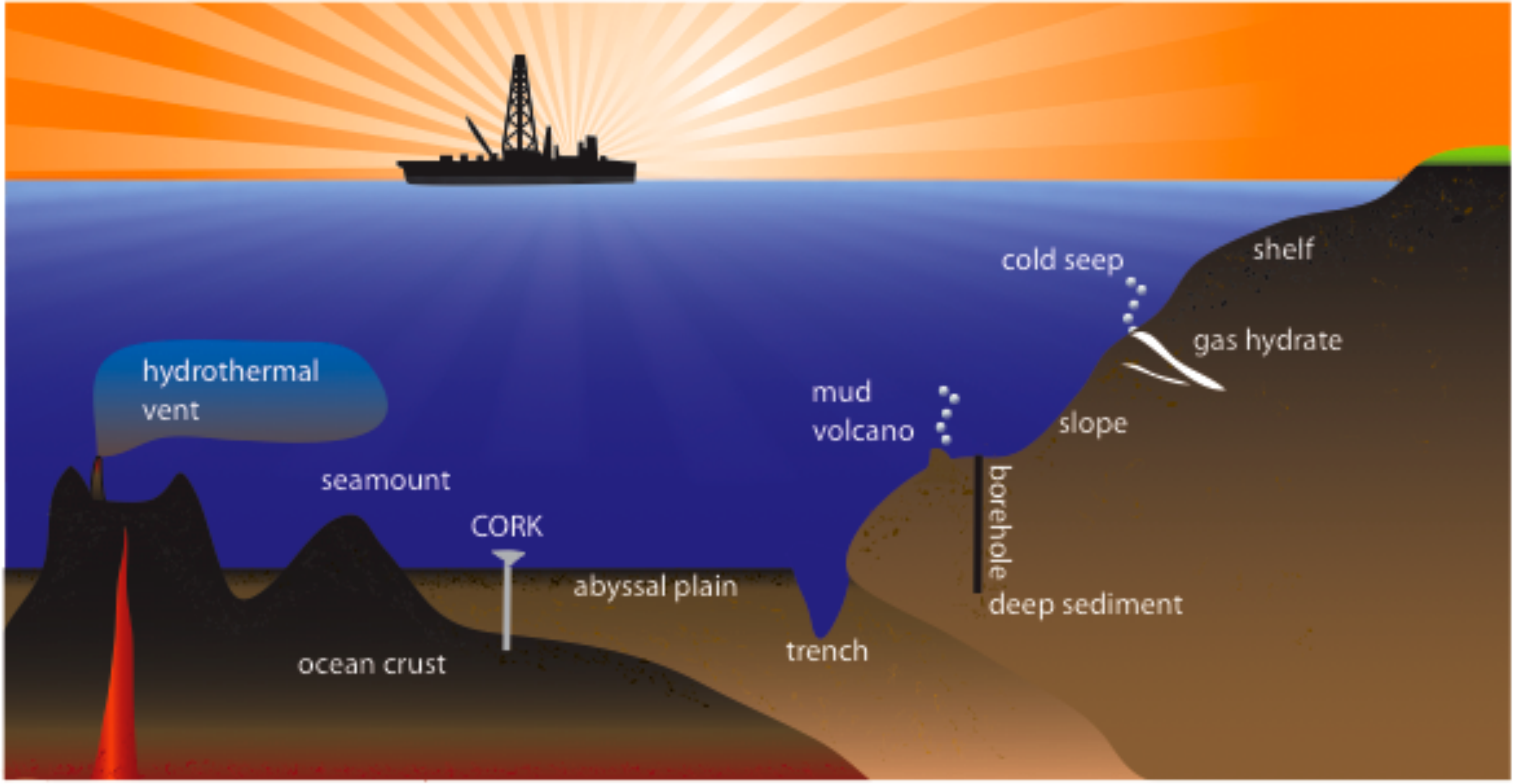


Figure modified from Orcutt et al. Microbiology And Molecular Biology Reviews 2011