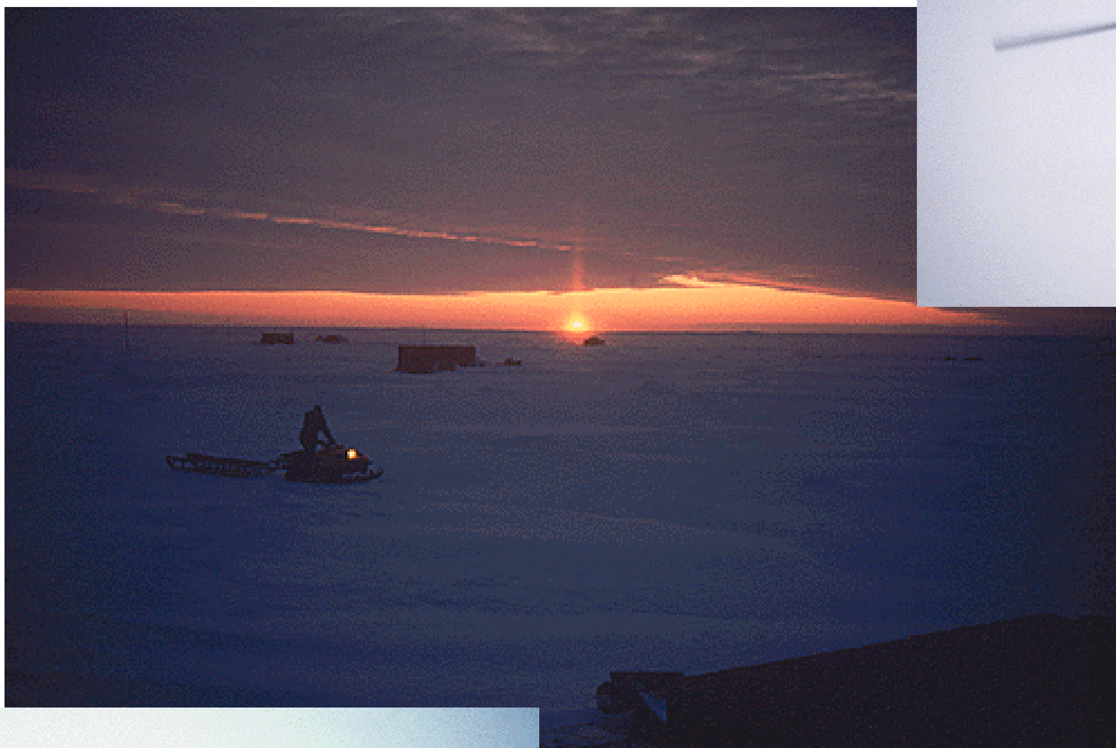
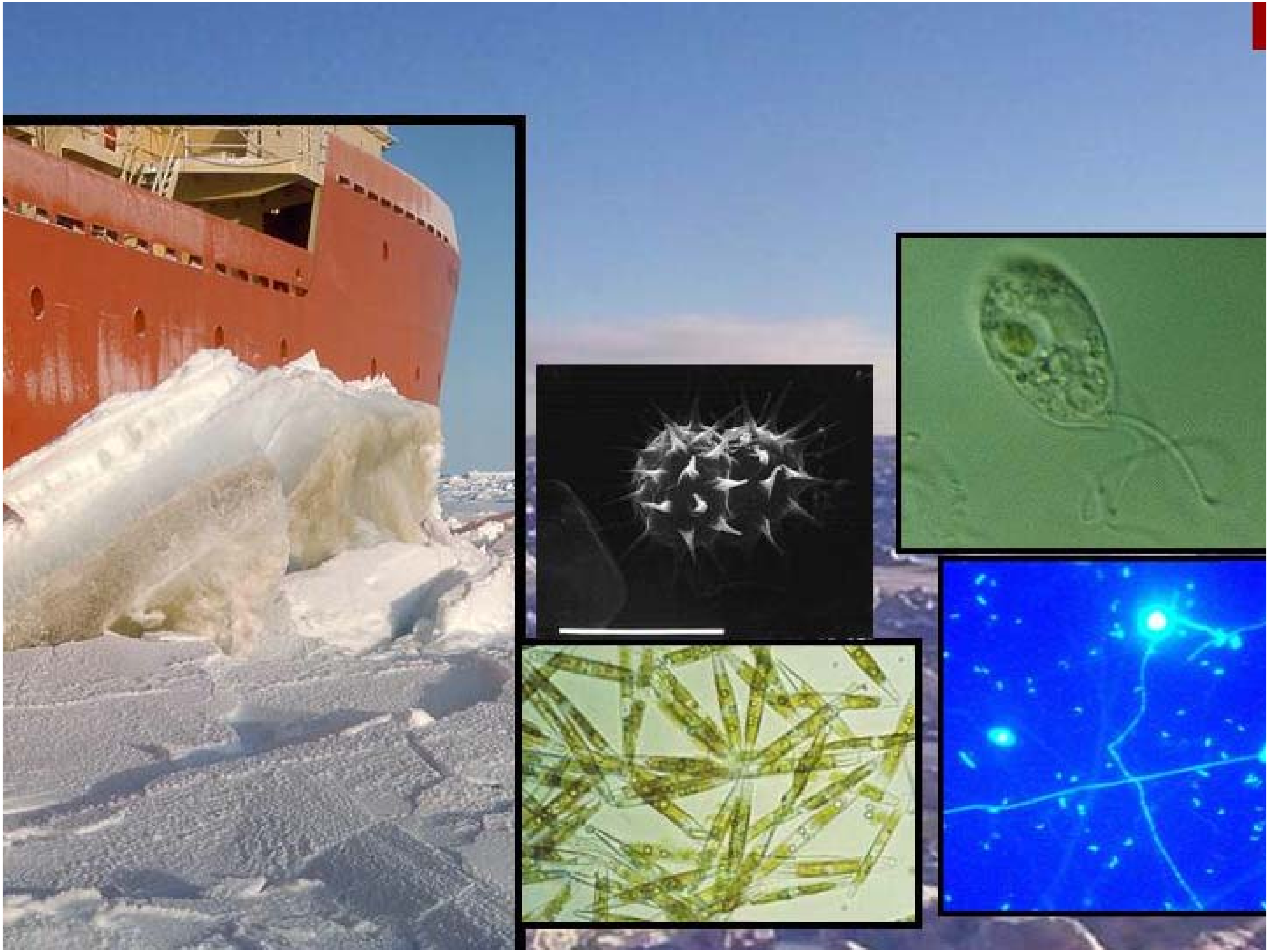
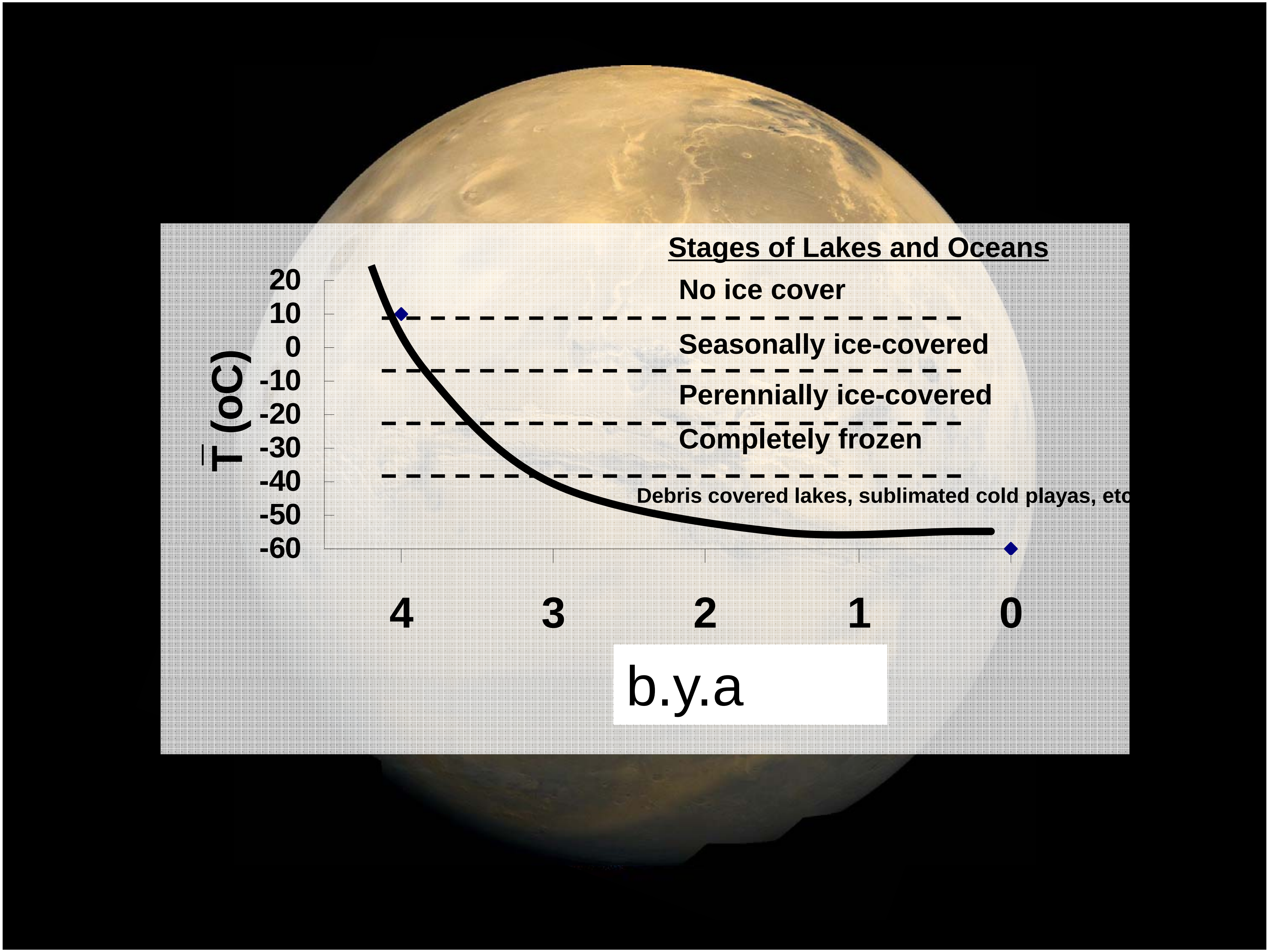
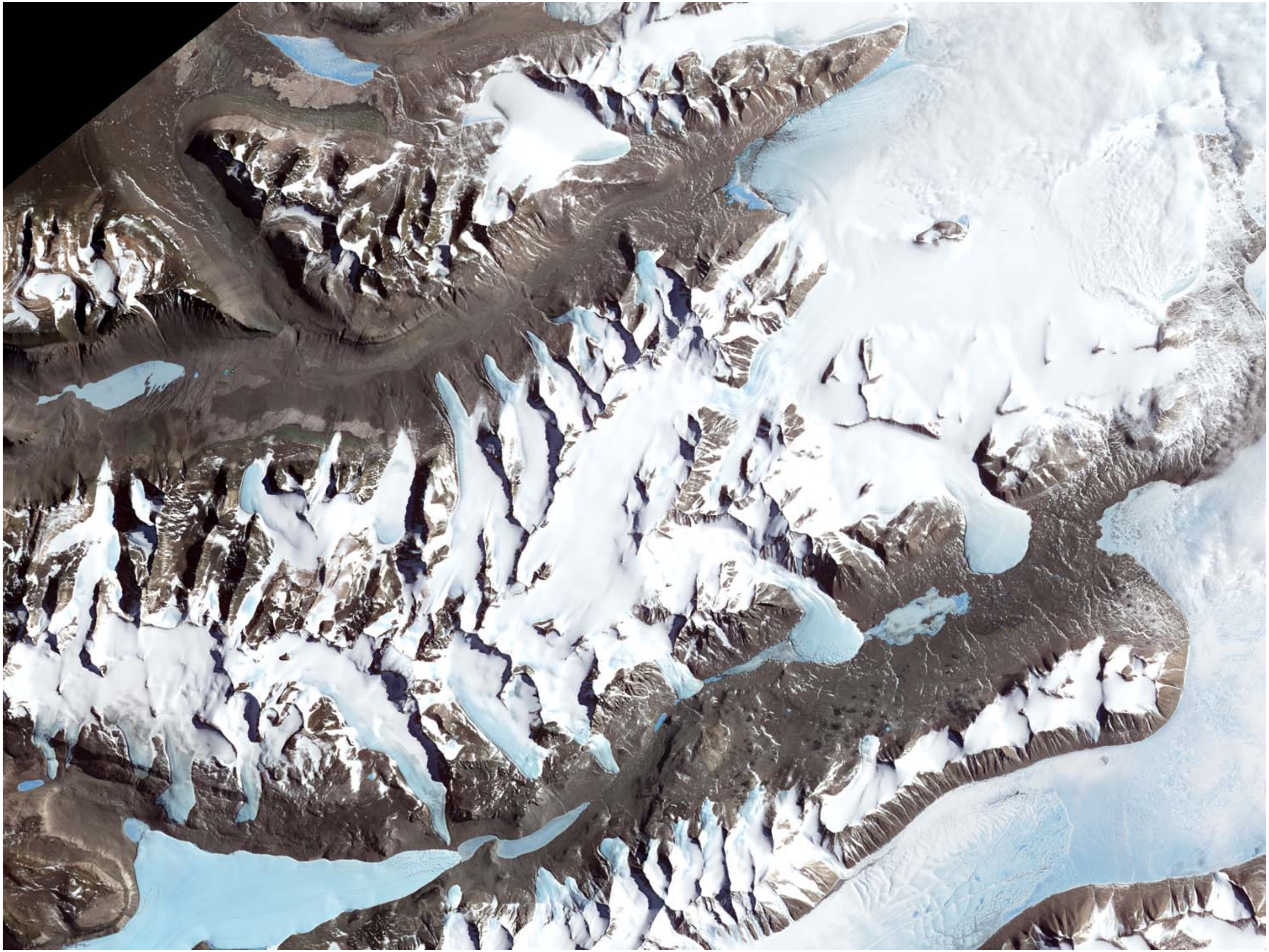


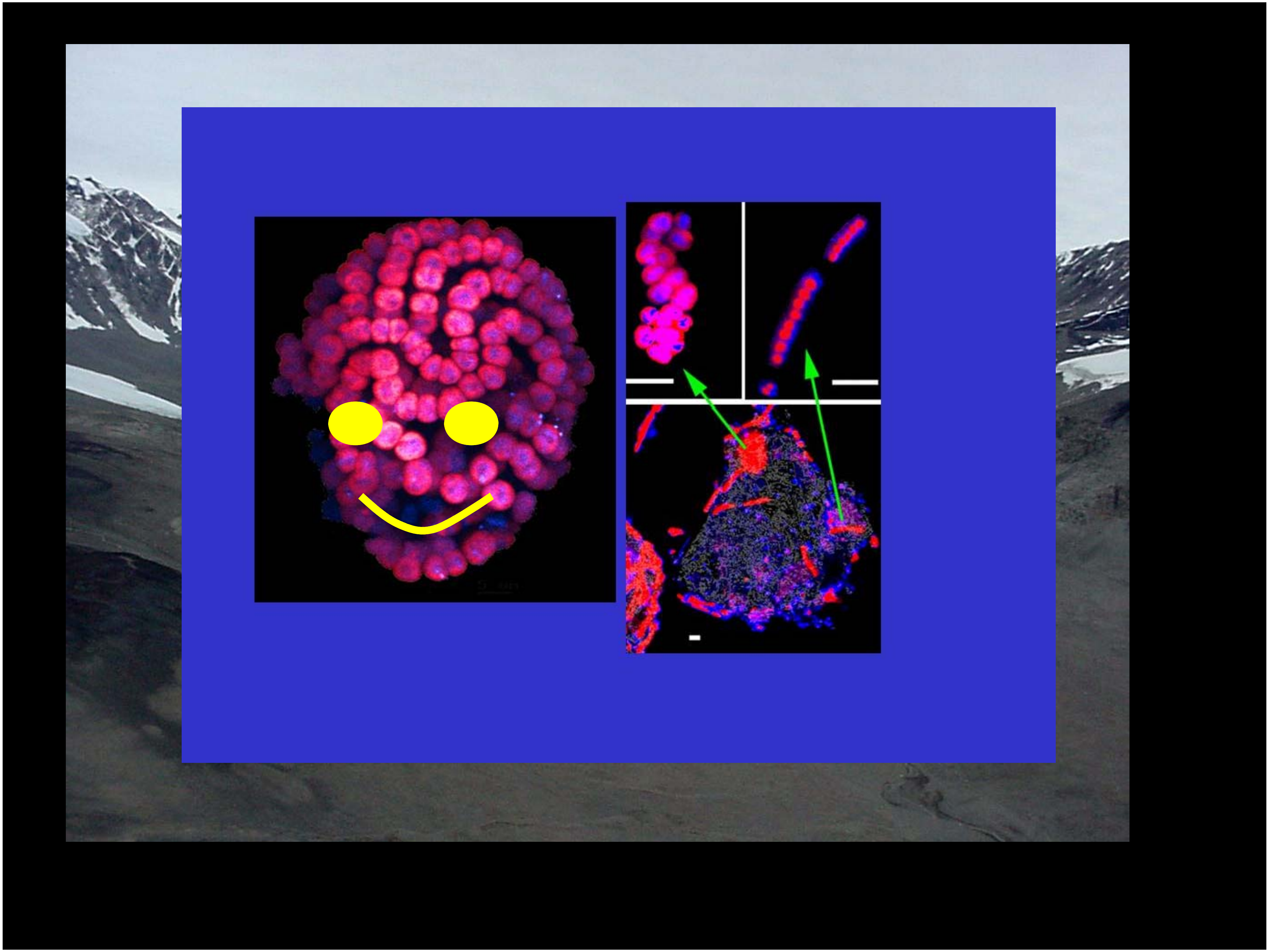


Microbe Hunting in and below Antarctic



Stages of Lakes and Oceans
No ice cover
Seasonally ice-covered
Perennially ice-covered
Completely frozen
Debris covered lakes, sublimated cold playas, etc
20
10
0
-10
-20
-30
-40
-50
-60
T (oC)
4
3
2
1
0
b.y.a





Lakes of the Dry Valleys

- Perennially ice covered
- Glacial melt water flows beneath ice covers through seasonal moats and sustains floating ice covers over liquid water column
- Extremely valuable systems for comparative ecology, adaptation, physical-biological landscape linkages etc...
-These lakes are also thought to provide varied insights into plausible type of lakes that could have existed on Mars at some time in its past

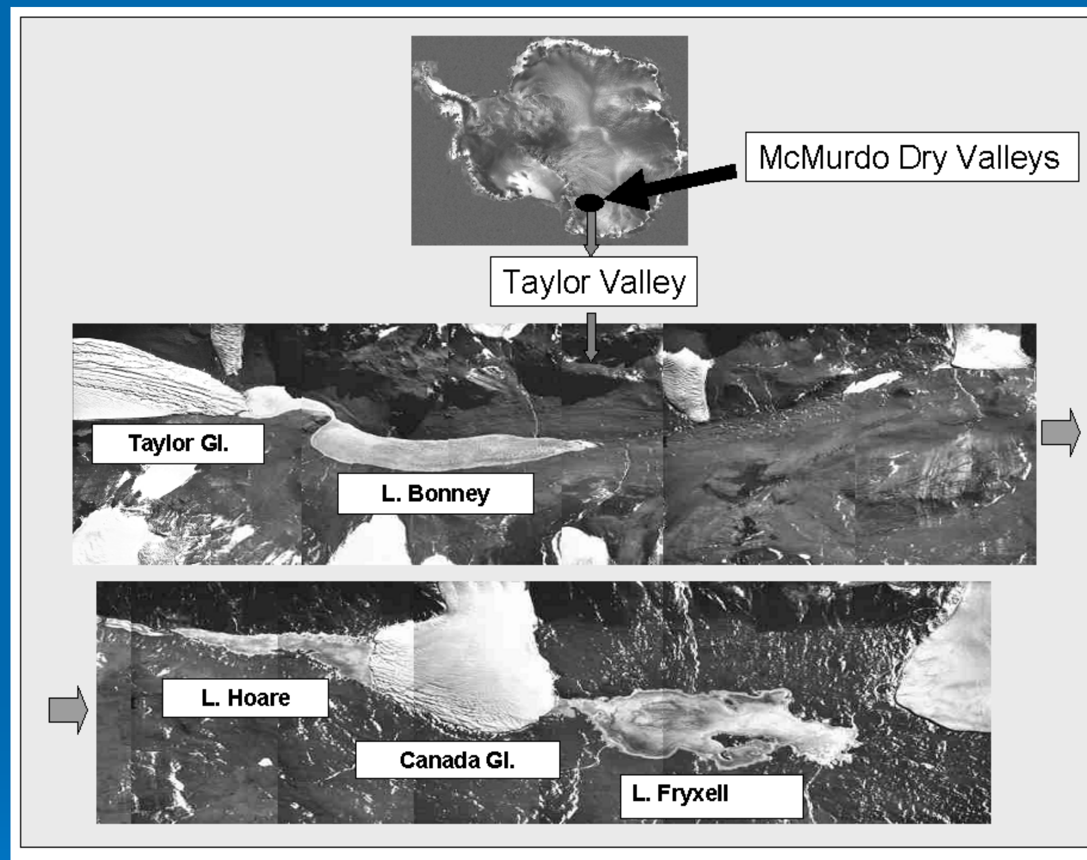
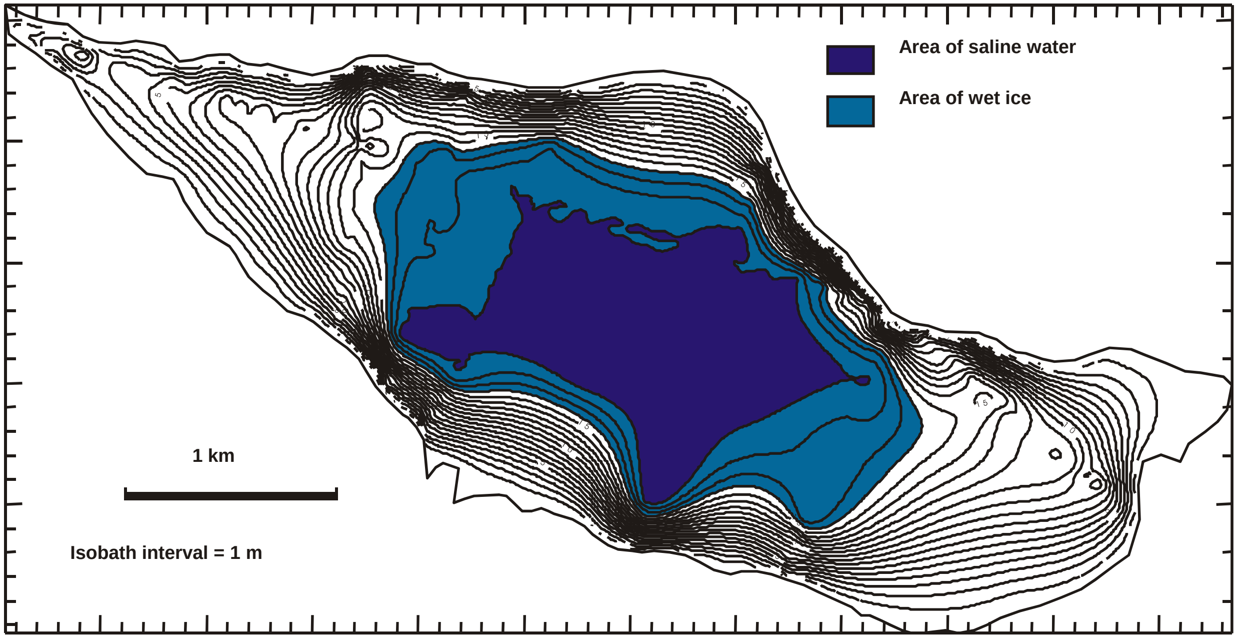


Fig. 1. Priscu et al.



Temperature ~ -12^{o}C
Salinity ~ 180 ppt

- *Kpar* = 0.82 to 1.1 m^{-1}
- 0.1% Irradiance @ ~ 7m

- Therefore, the euphotic zone is in the ice cover and the cold brine exists in the aphotic zone which would preclude photoautorophic energy input

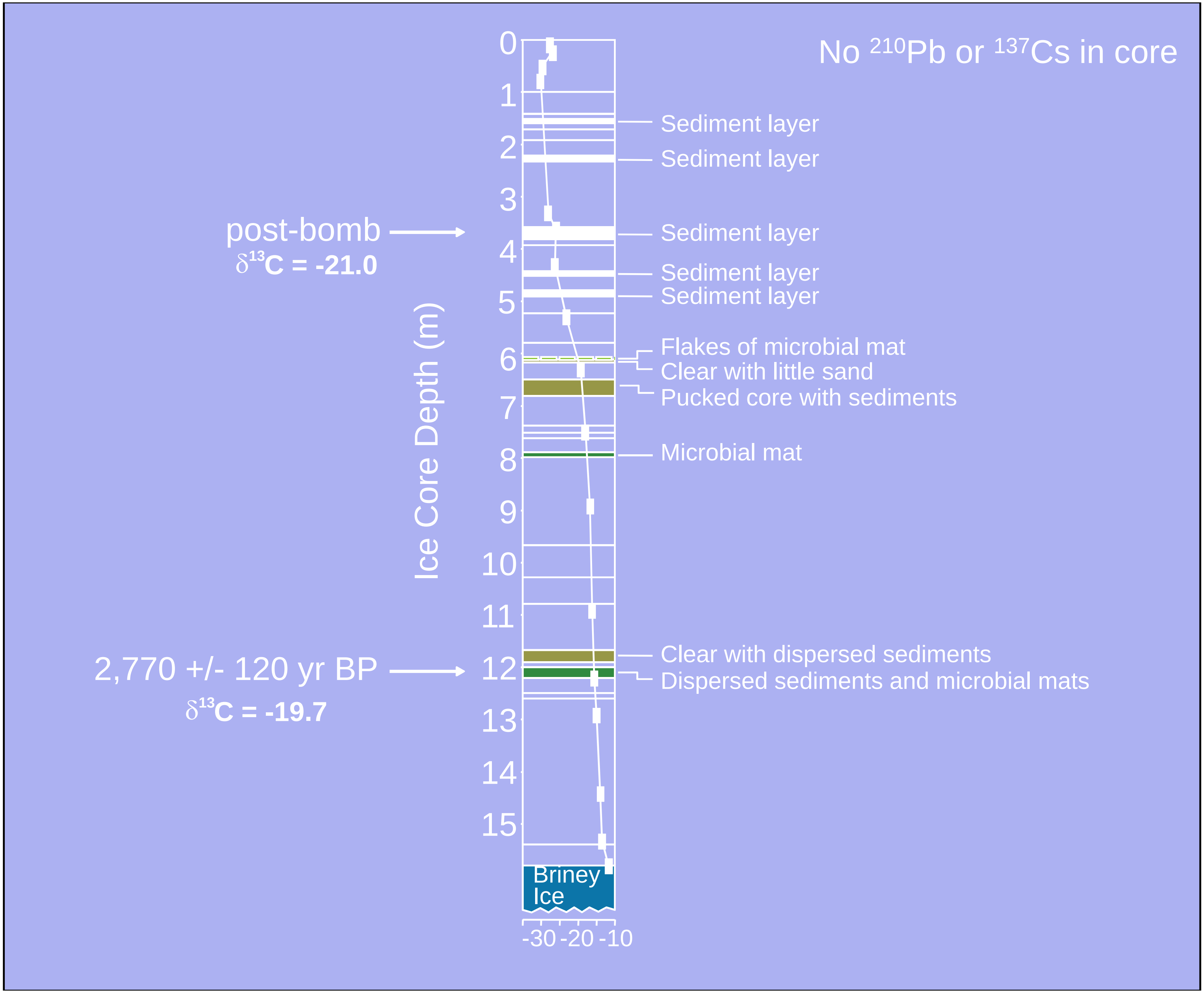
- Moreover, any energy potentially derived in the euphotic zone in this lake is not likely to be linked or coupled to the aphotic brine due to the physical ice barrier

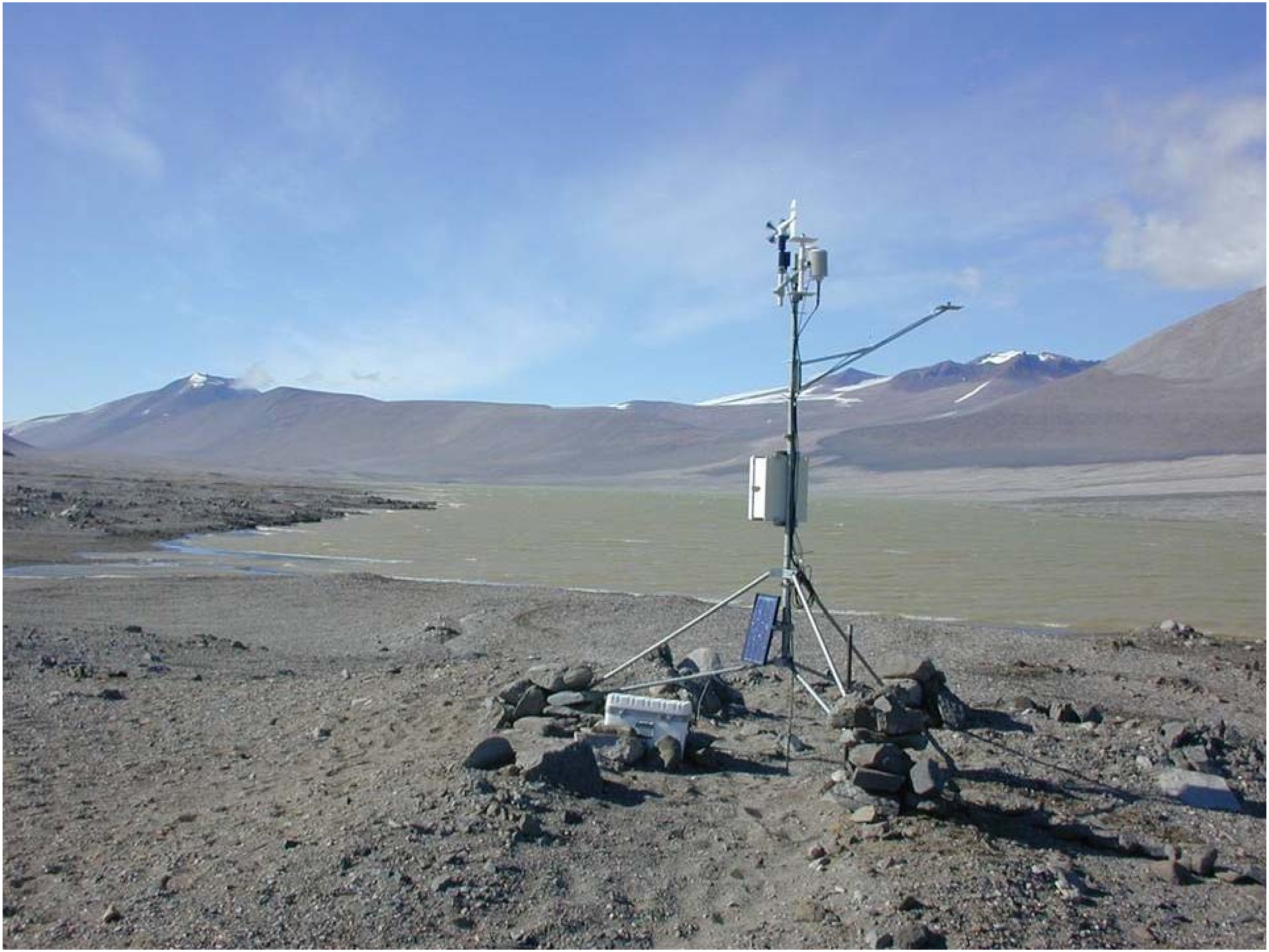
- This represents a new type of lake in a in a cooling progression

- Are there microbiota, present, active and/or growing? Or is this system devoid of life because of its isolated nature or "extreme" conditions?

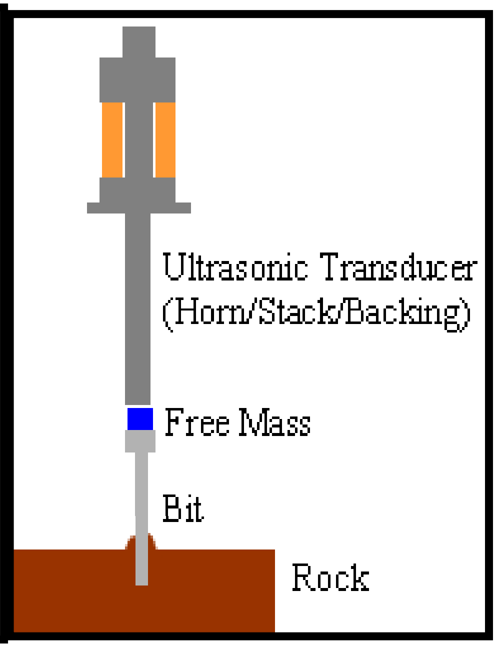

N40340
NATIONAL SCIENCE
FOUNDATION

Lake Vida, Antarctica
October ‘96

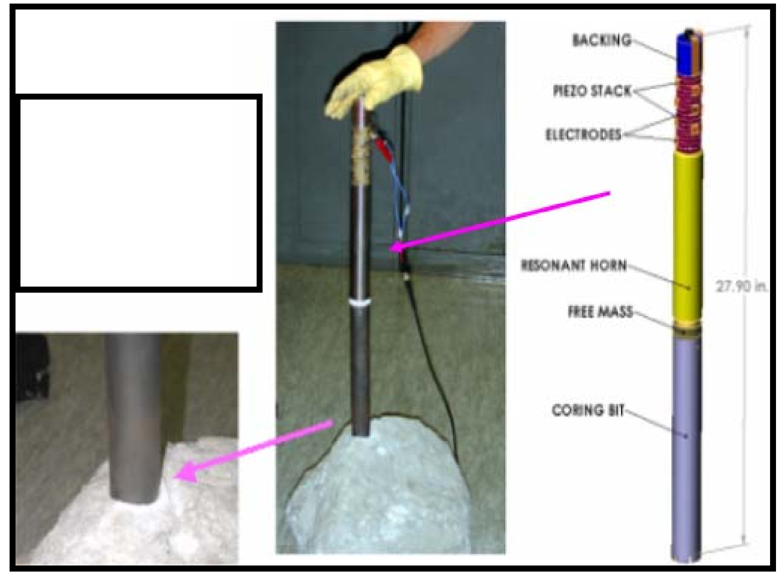

No ^{210}Pb or ^{137}Cs in core
post-bomb
δ^{13}C = -21.0
2,770 +/- 120 yr BP
δ^{13}C = -19.7
Ice Core Depth (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Sediment layer
Sediment layer
Sediment layer
Sediment layer
Sediment layer
Flakes of microbial mat
Clear with little sand
Pucked core with sediments
Microbial mat
Clear with dispersed sediments
Dispersed sediments and microbial mats
Briney Ice
-30 -20 -10



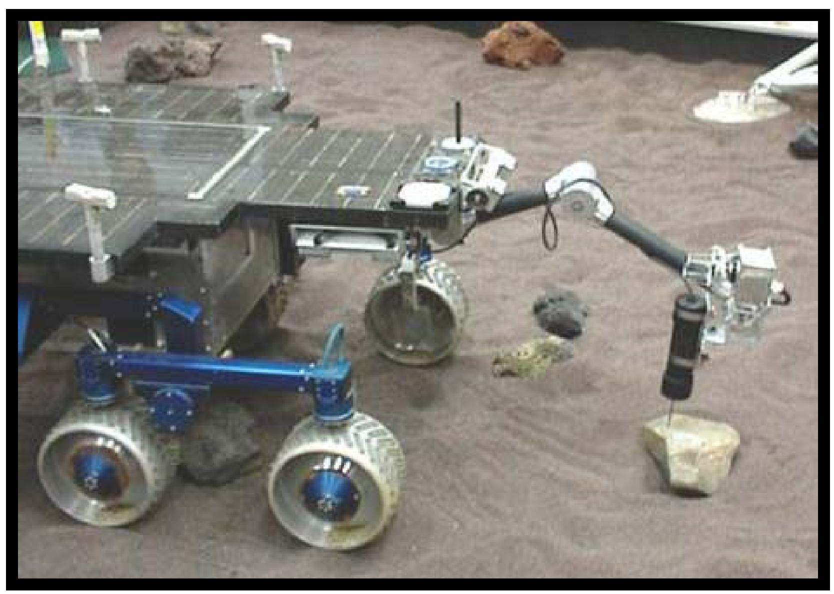

ICE GOPHER



Ultrasonic Transducer
(Horn/Stack/Backing)
Free Mass
Bit
Rock



BACKING
PIEZO STACK
ELECTRODES
RESONANT HORN
FREE MASS
CORING BIT
27.90 in.

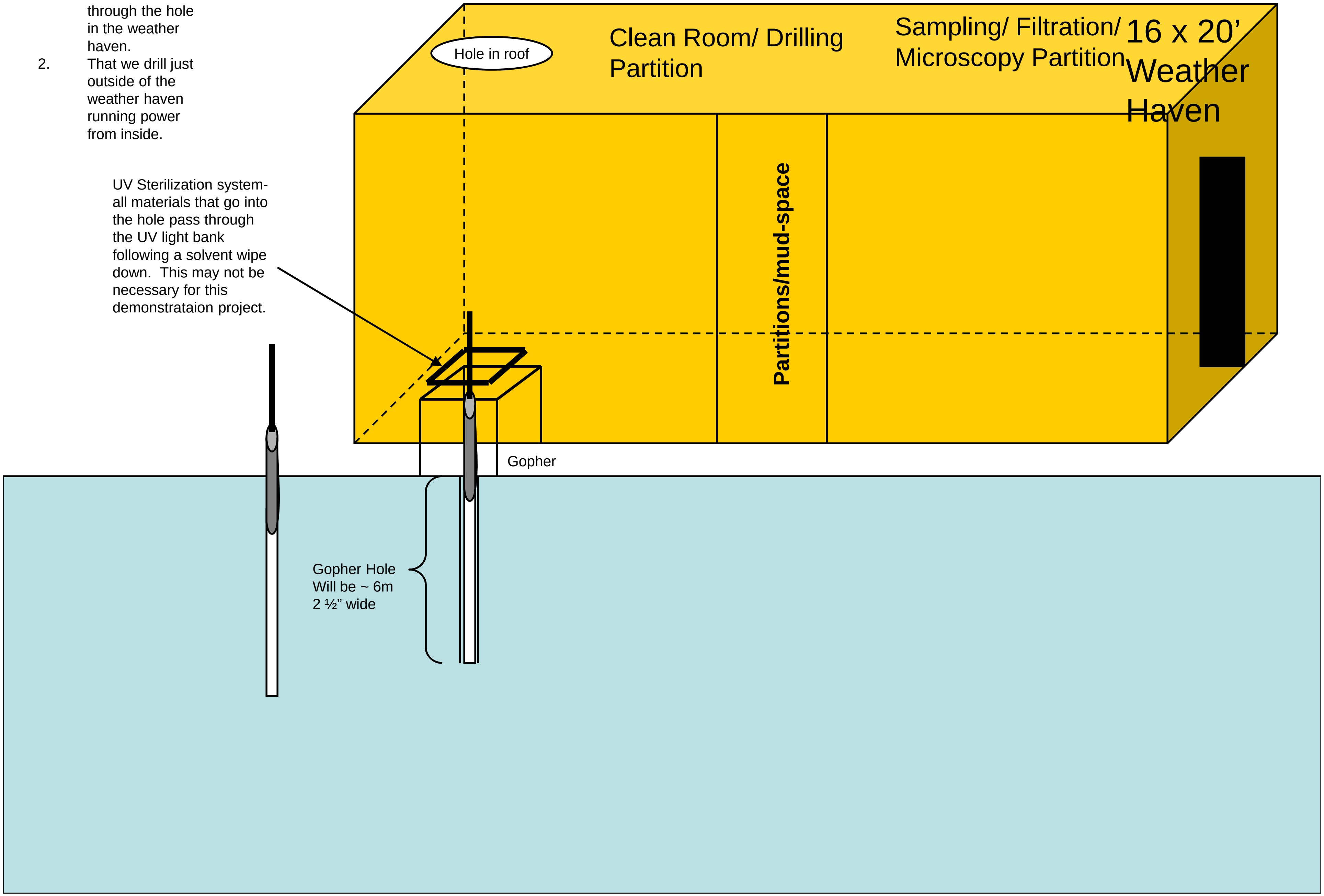



600V WATER RESISTANT (UL)

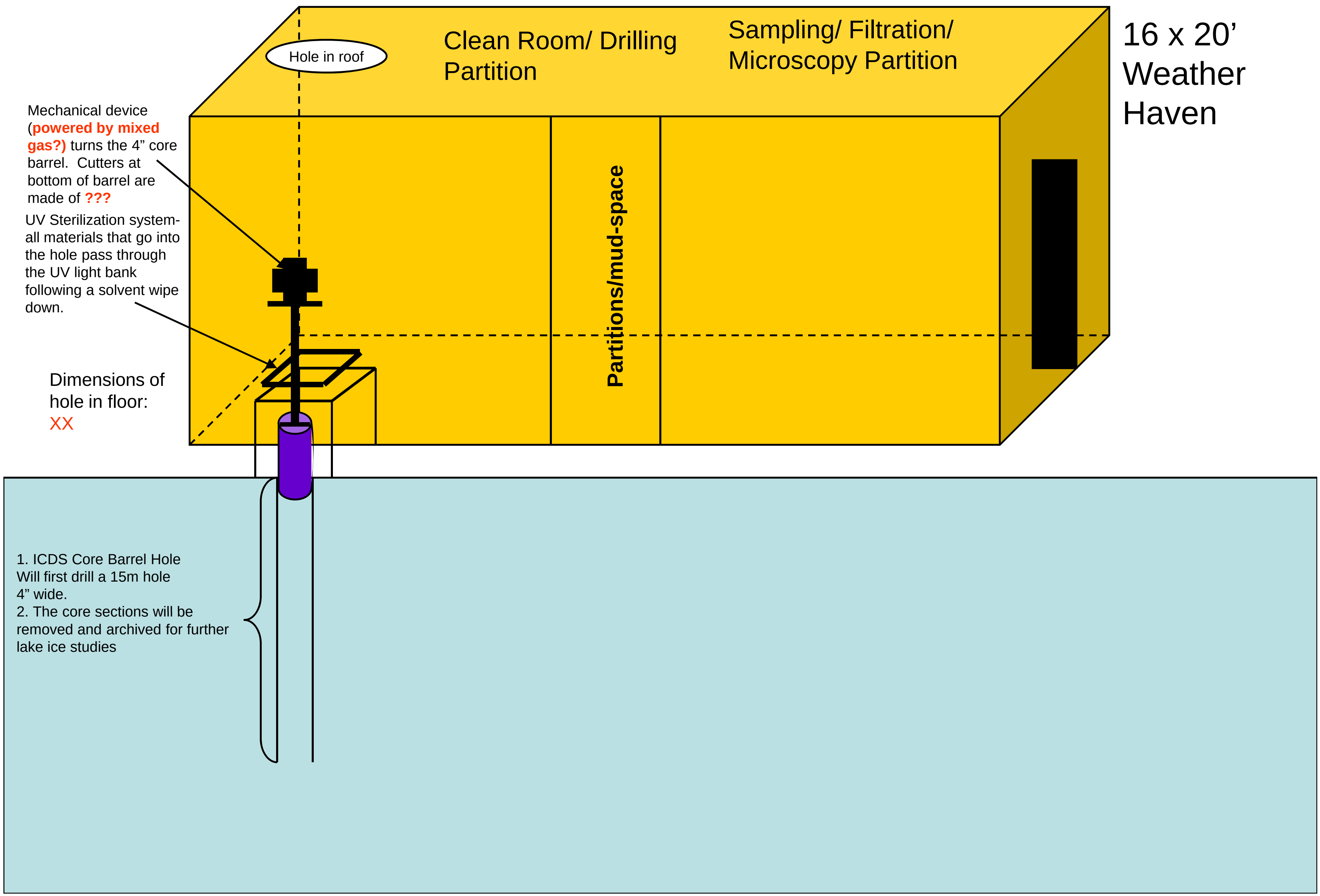
Lake Vida Field Camp Layout – Drilling Part 1: Demonstration of the Ultrasonic Gopher

Two scenarios

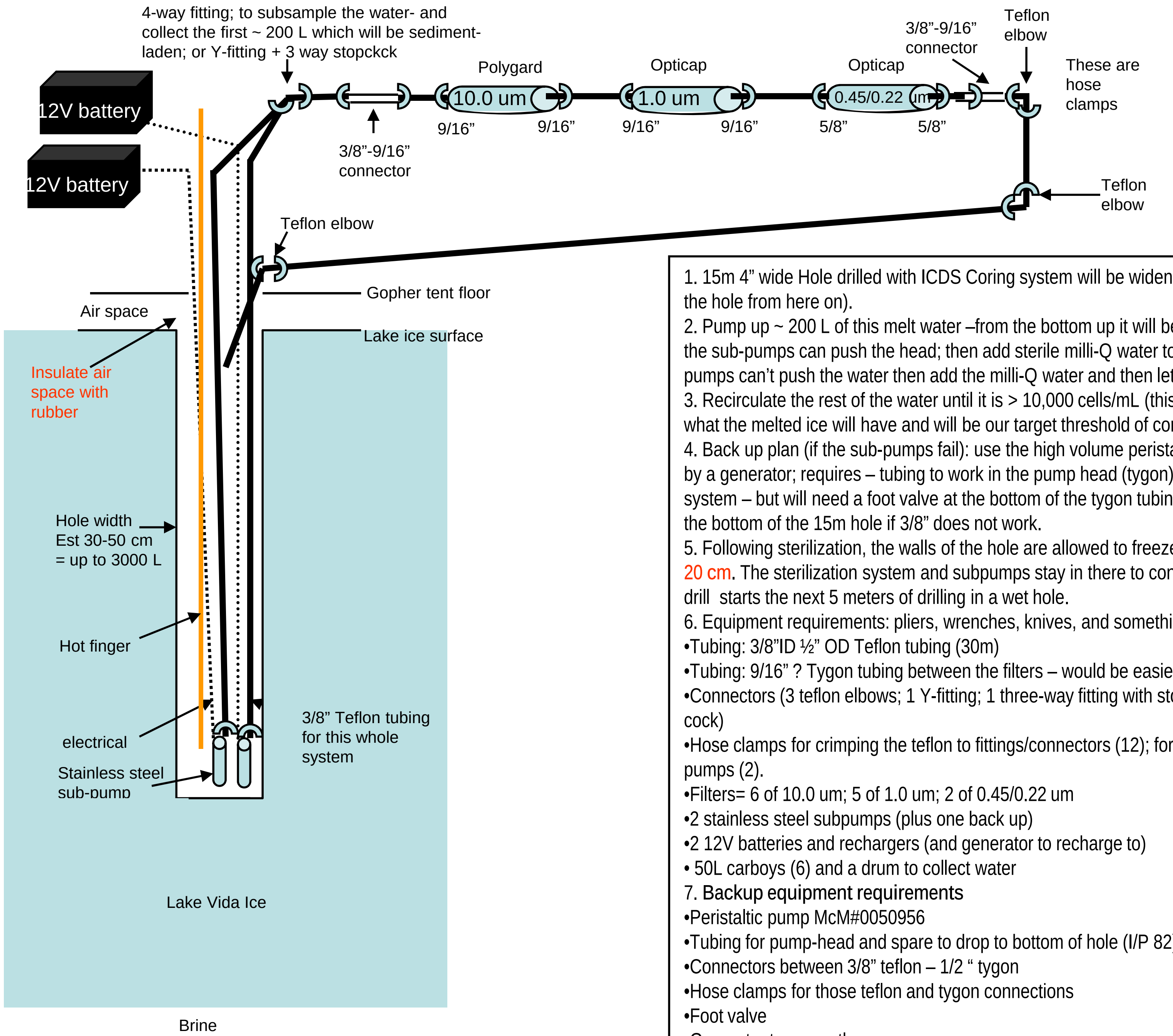
1. That we drill through the hole in the weather haven.
2. That we drill just outside of the weather haven running power from inside.



Lake Vida Field Camp Layout – Drilling Part 2: ICDS Supported Science Hole



Hole Cleaning – purpose to sterilize the water in the 15m hole

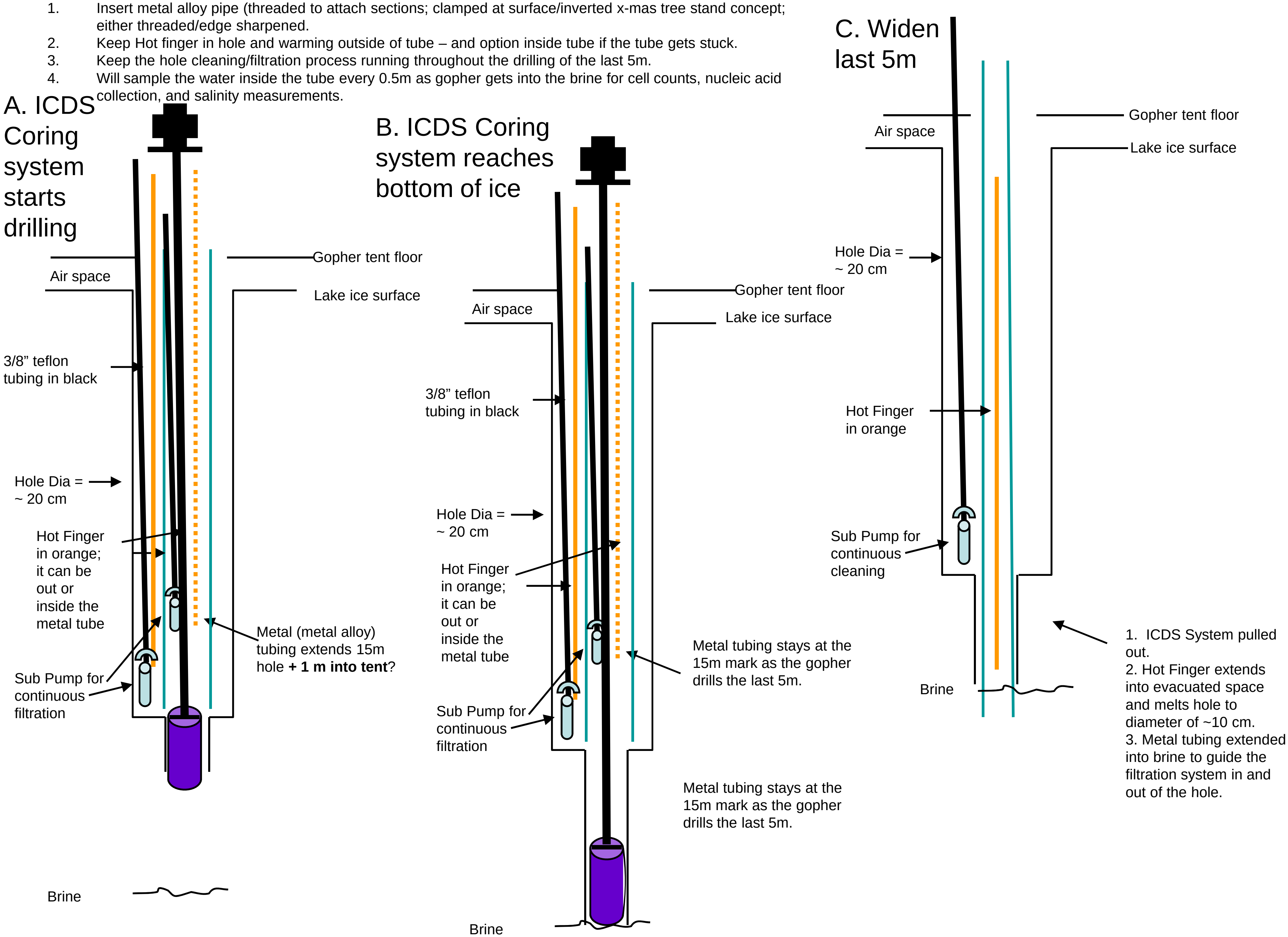


1. 15m 4" wide Hole drilled with ICDS Coring system will be widened with hot finger (hot finger stays in the hole from here on).
2. Pump up ~ 200 L of this melt water –from the bottom up it will be the most sediment-laden waterif the sub-pumps can push the head; then add sterile milli-Q water to near surface of the hole (or if pumps can't push the water then add the milli-Q water and then let settle and pump up the water).
3. Recirculate the rest of the water until it is > 10,000 cells/mL (this is 2 orders of magnitudebelow what the melted ice will have and will be our target threshold of contamination).
4. Back up plan (if the sub-pumps fail): use the high volume peristaltic pump (~ 12L /minute) powered by a generator; requires – tubing to work in the pump head (tygon). Can use the rest of the tubing system – but will need a foot valve at the bottom of the tygon tubing. Alternate also to drop tygon to the bottom of the 15m hole if 3/8" does not work.
5. Following sterilization, the walls of the hole are allowed to freeze back to final internal diameter of 20 cm. The sterilization system and subpumps stay in there to continue hole cleaning while the ICDS drill starts the next 5 meters of drilling in a wet hole.
6. Equipment requirements: pliers, wrenches, knives, and something to cut the teflon
•Tubing: 3/8"ID ½" OD Teflon tubing (30m)
•Tubing: 9/16" ? Tygon tubing between the filters – would be easier to clamp and switch out. (2m)
•Connectors (3 teflon elbows; 1 Y-fitting; 1 three-way fitting with stopcock; 1 4-way fitting with stop-cock)
•Hose clamps for crimping the teflon to fittings/connectors (12); for tygon to filters (6); teflon to sub-pumps (2).
•Filters= 6 of 10.0 um; 5 of 1.0 um; 2 of 0.45/0.22 um
•2 stainless steel subpumps (plus one back up)
•2 12V batteries and rechargers (and generator to recharge to)
• 50L carboys (6) and a drum to collect water
7. Backup equipment requirements
•Peristaltic pump McM#0050956
•Tubing for pump-head and spare to drop to bottom of hole (I/P 82)
•Connectors between 3/8" teflon – 1/2 " tygon
•Hose clamps for those teflon and tygon connections
•Foot valve
•Generator to power the pump

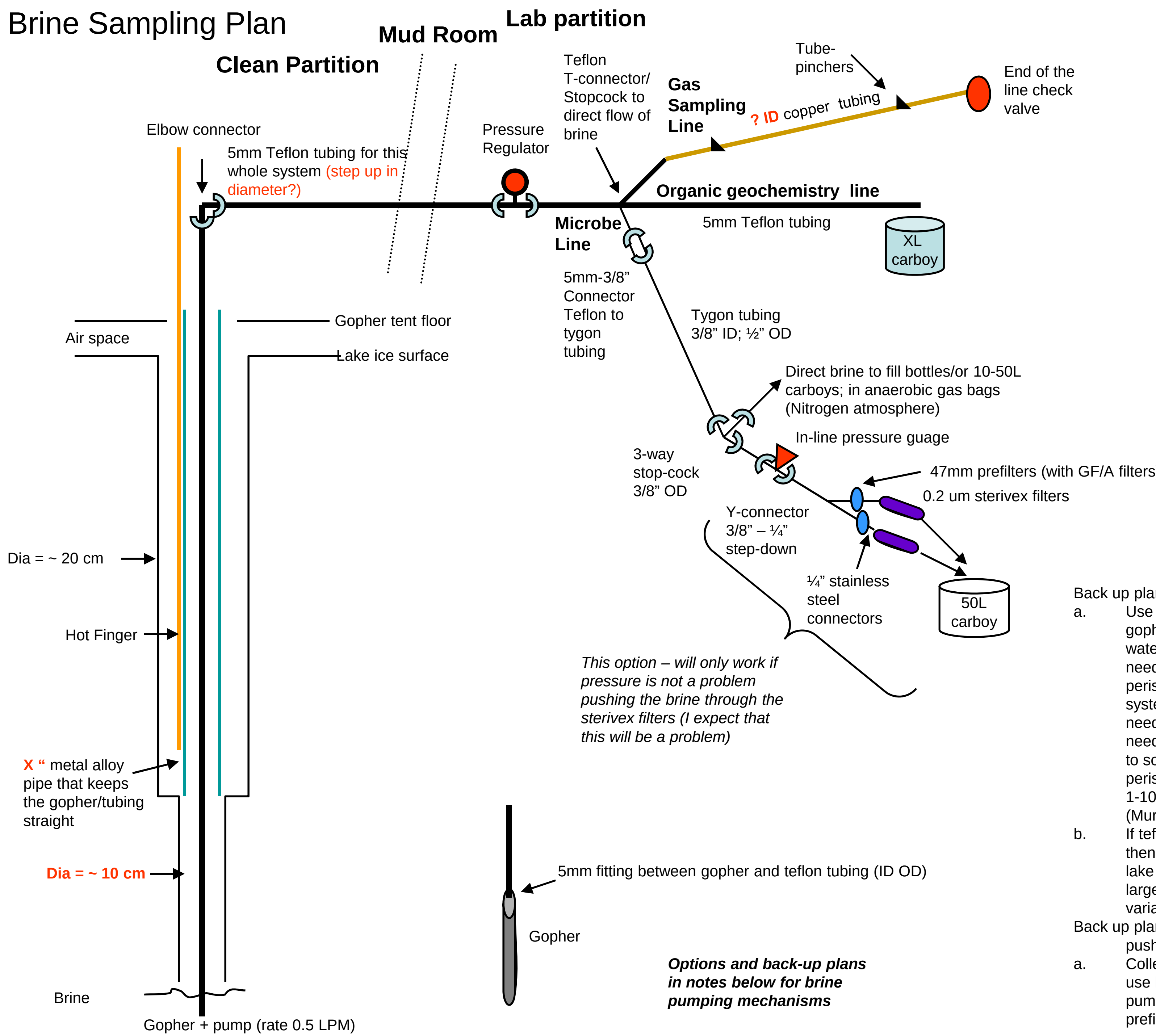
Hole Extending Plan past 15m to the brine with metal alloy tubing

Steps following hole cleaning

1. Insert metal alloy pipe (threaded to attach sections; clamped at surface/inverted x-mas tree stand concept; either threaded/edge sharpened.
2. Keep Hot finger in hole and warming outside of tube – and option inside tube if the tube gets stuck.
3. Keep the hole cleaning/filtration process running throughout the drilling of the last 5m.
4. Will sample the water inside the tube every 0.5m as gopher gets into the brine for cell counts, nucleic acid collection, and salinity measurements.



Brine Sampling Plan



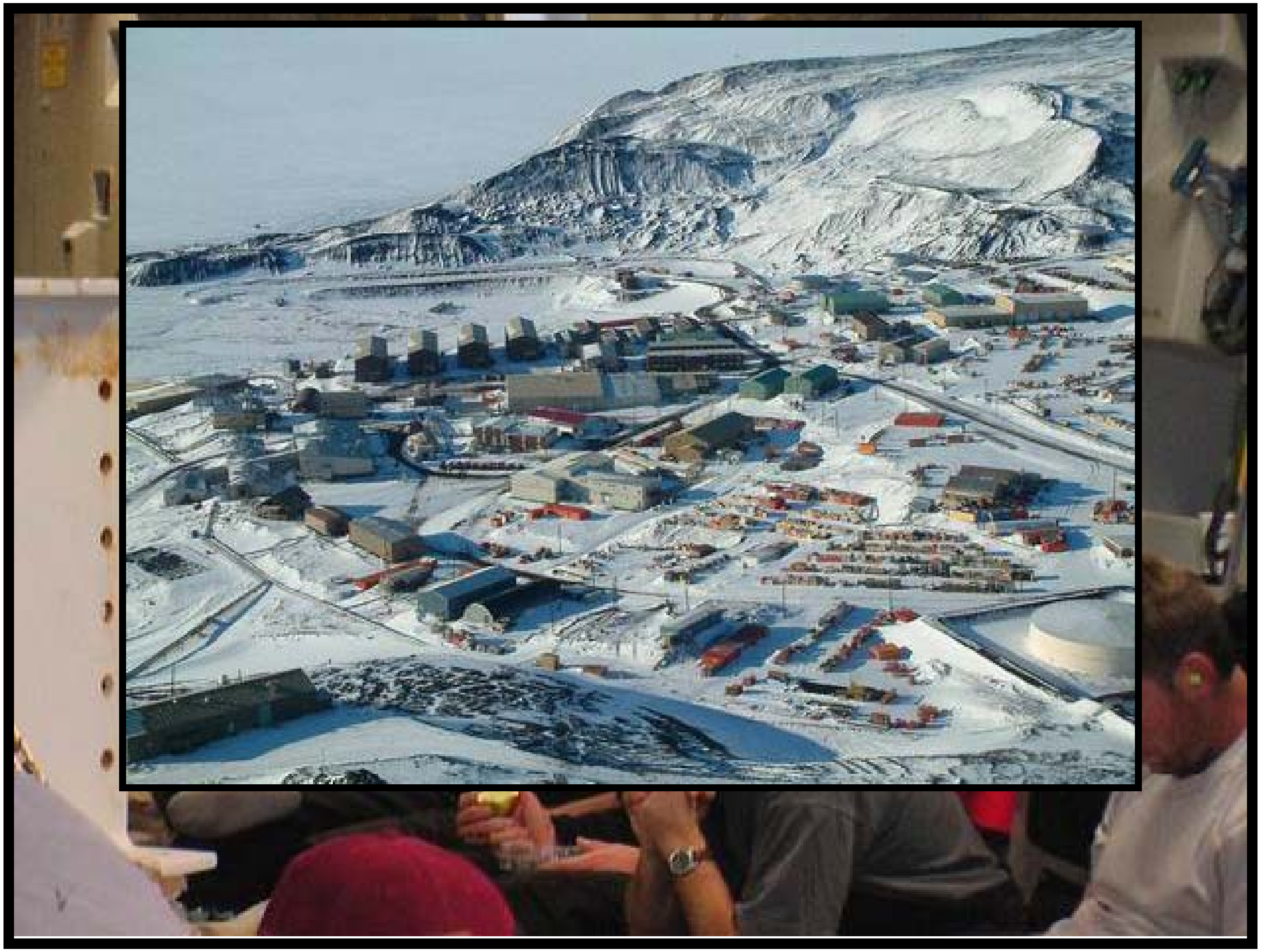
Back up plan if gopher pump fails:

a. Use peristaltic pump in the gopher tent to pump up the water through the teflon line/ need connectors and tubing for peristaltic pump. Use basic system as drawn here. Will need to bring the connectors needed to go from 5mm tubing to something that can fit in the peristaltic pump head. Use the 1-100 rpm variable speed pump (Murray lab) probably.

b. If teflon tubing is a problem then drop tygon tubing into the lake and pump up with the large scale peristaltic pump variable speed.

Back up plan if gopher pump can't push water through the filters:

a. Collect water in carboys and use low volume peristaltic pump to push brine through the prefilter/sterivex filter



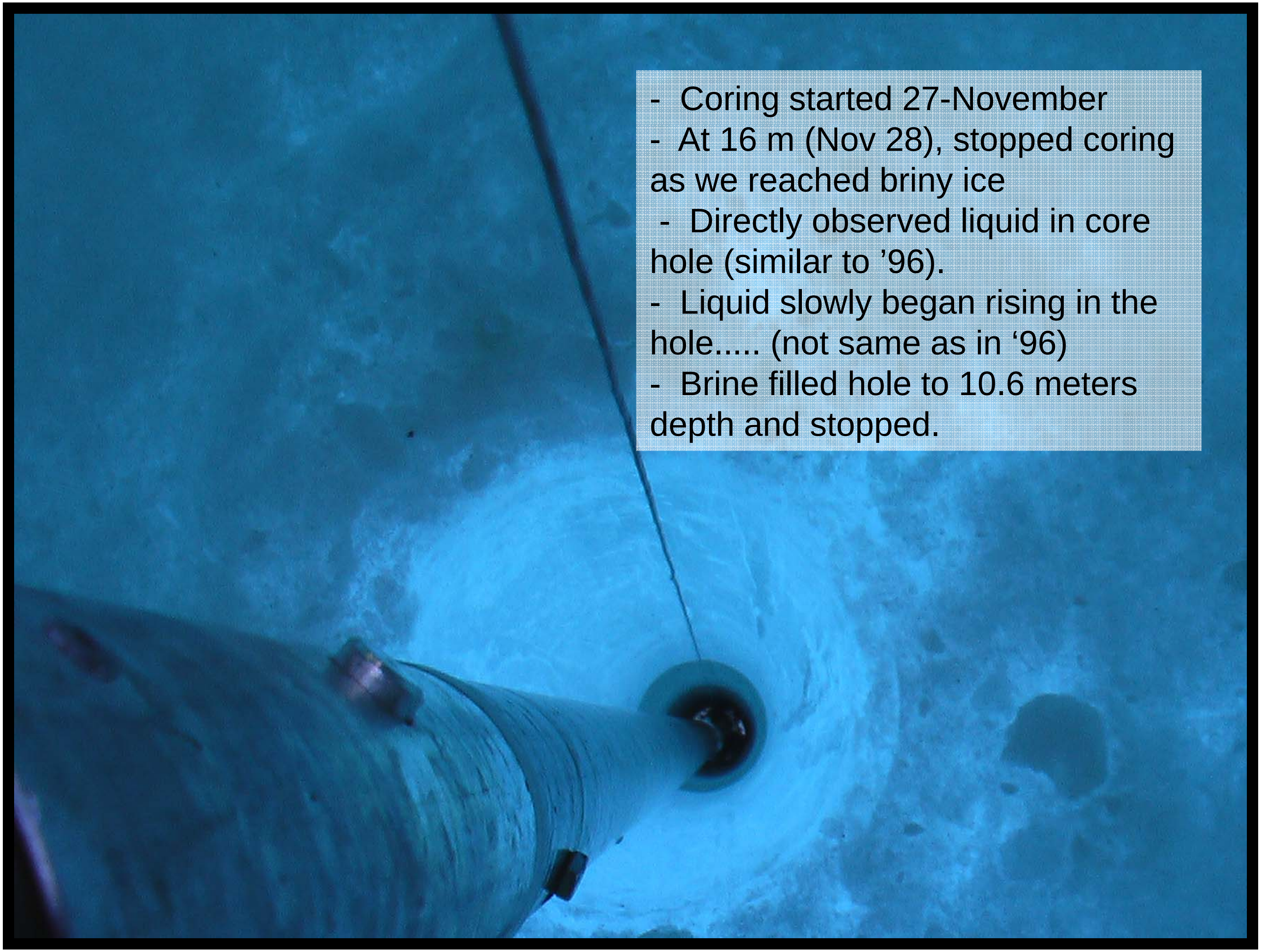
"Given the delay in preferred start date, I'm concerned with the prospect of overall success. Will the team feel 'pushed' to move more quickly-and potentially less safely from the environmental point of view due to the possibility of an early melt? "

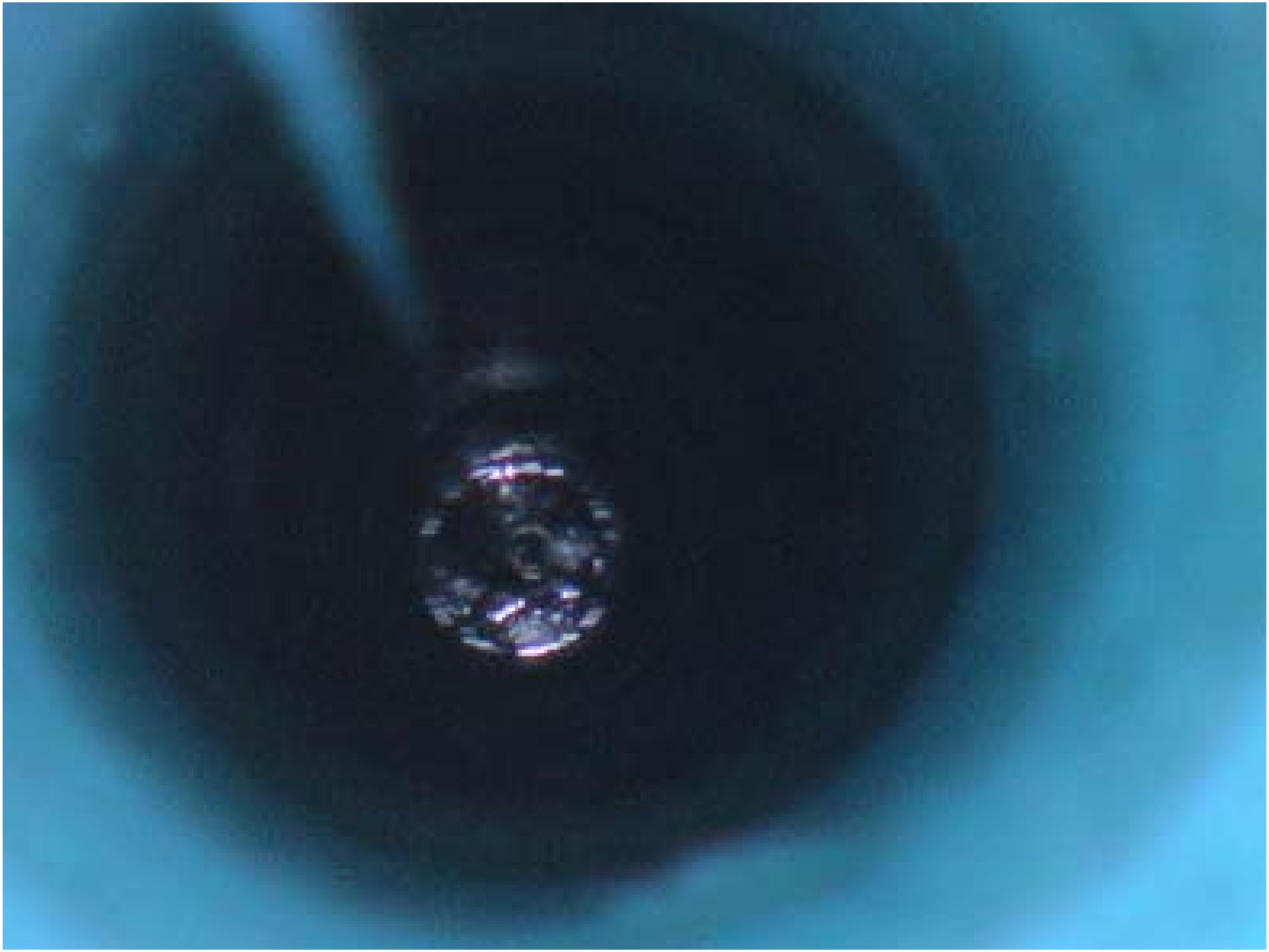
“Don’t screw it up for the rest of us”

- Anonymous scientist and life long critic.....
Nov 20th, 2005

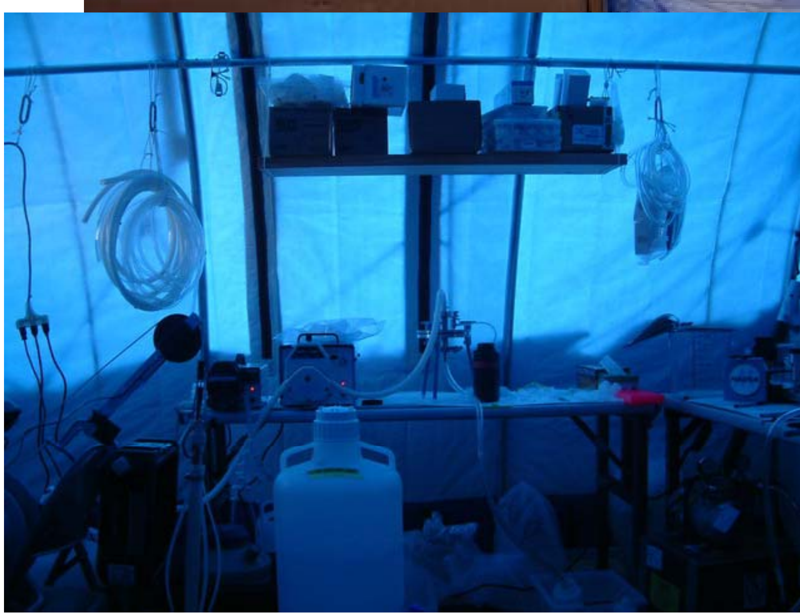

Nov 24

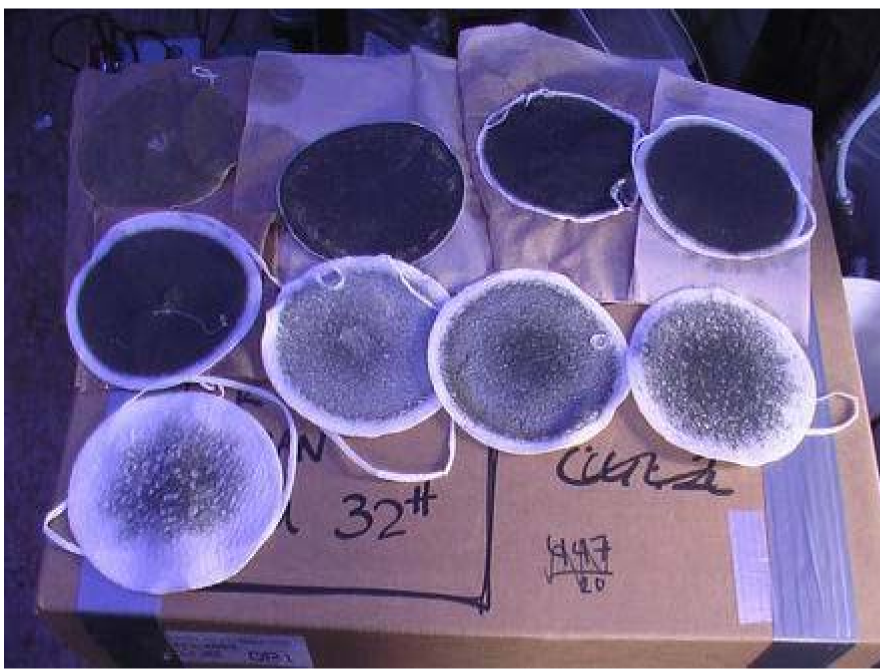

Nov 27

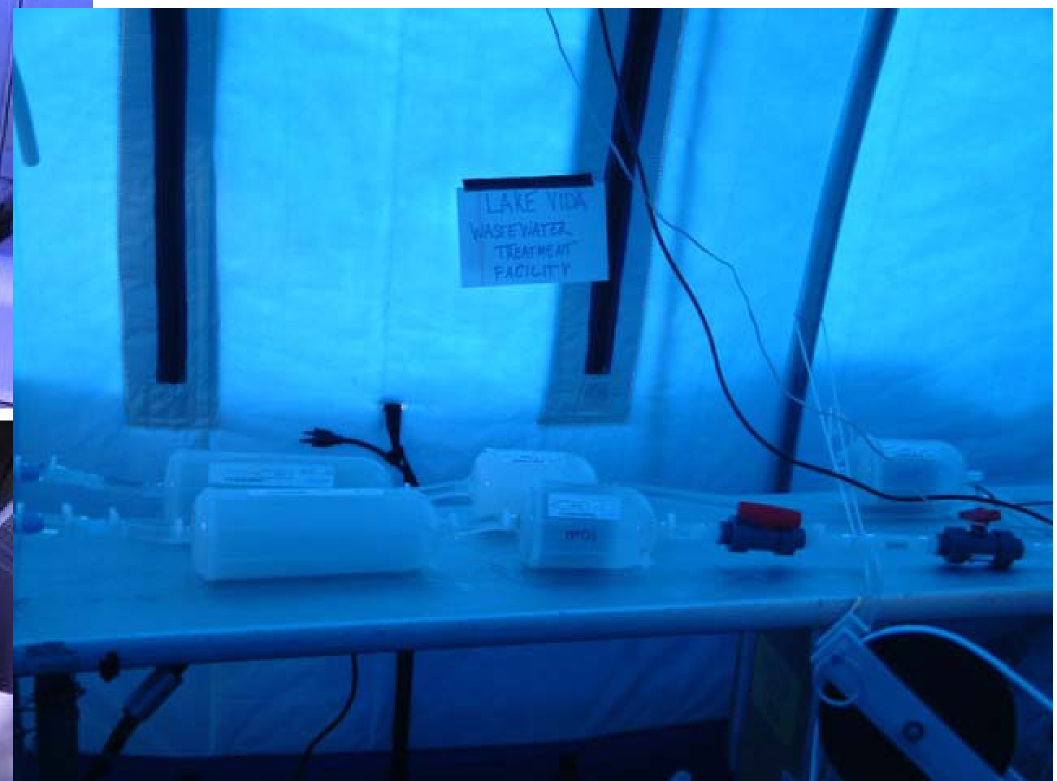

- Coring started 27-November
- At 16 m (Nov 28), stopped coring as we reached briny ice
- Directly observed liquid in core hole (similar to ’96).
- Liquid slowly began rising in the hole..... (not same as in ‘96)
- Brine filled hole to 10.6 meters depth and stopped.



November 28, 2007






LAKE VIDA
WASTEWATER
"TREATMENT"
FACILITY

[redacted]

Hole cleaning procedures were employed to minimize risk of introducing microbes into the brine\water column

Hole was cleaned to targets of ~10^4 cells per ml on two separate occasions.

Next Stage…..

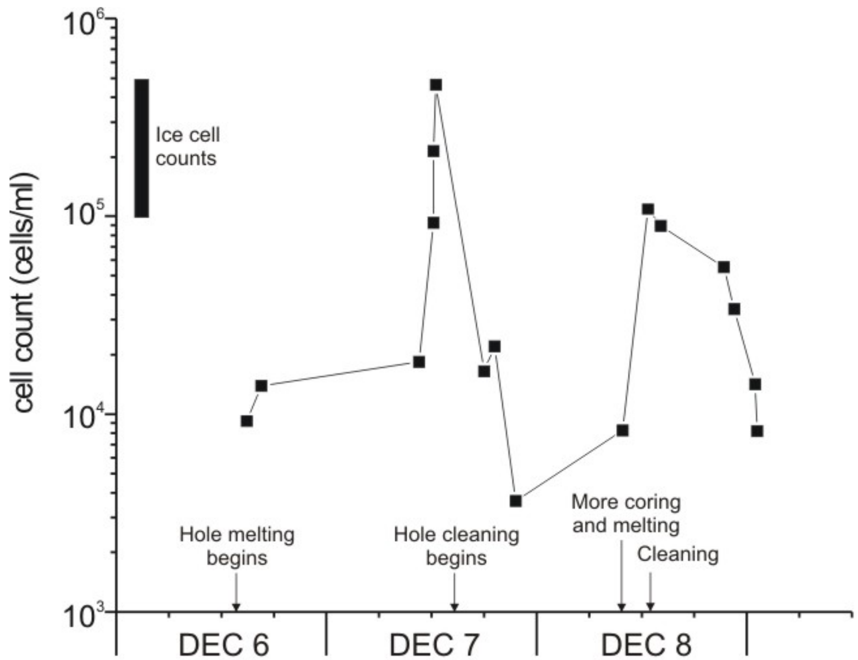


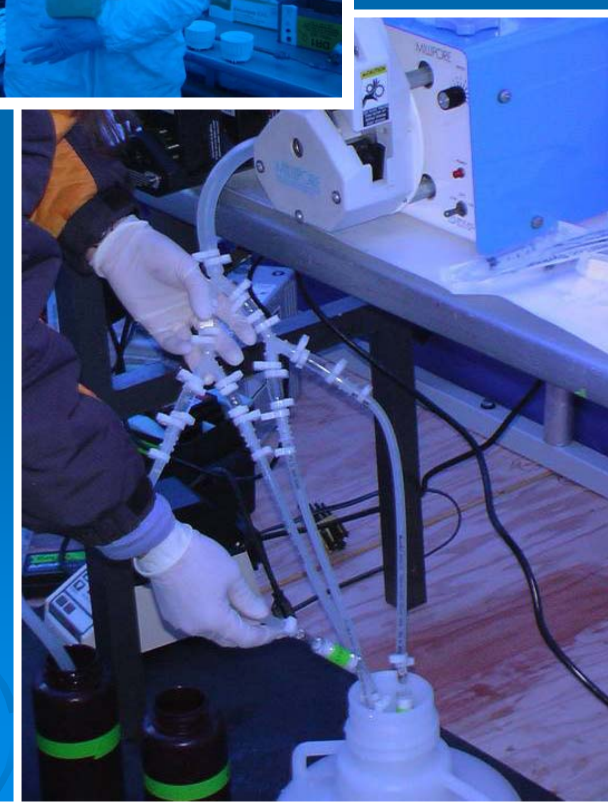
Figure 5: Microbial cell concentrations in water in the hole over time during hole making and cleaning operations. Water in the hole at the start of melting was deionized in the lab before being transported to the lake. The values for the ice cell counts comes from a previous study (Mosier et al. 2005)

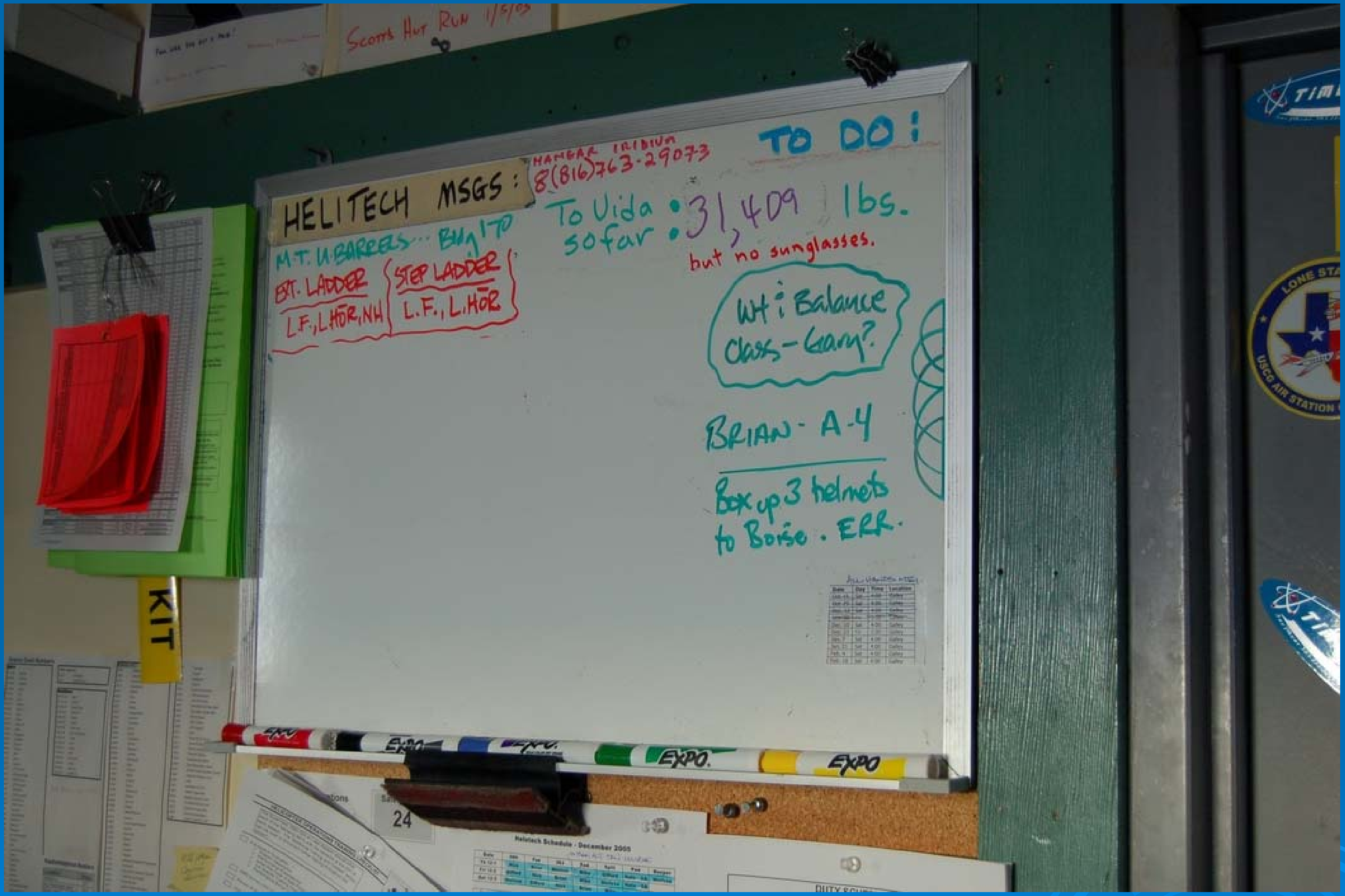
Sampled/Pumped all brine out of hole (range of samples taken- in anaerobic glove bags, copper tubes, carboys, etc...).

Observed the brine flowing into the side of the ice hole at ~15.9 meters depth.

Layered on Milli-Q water (to 10 meters) and let water freeze, thus sealing brine at depth

After freezing of Milli-Q we proceeded with original plan of widening and cleaning hole (above 15 meters so brine would not come into hole) but... ran out of time to complete the access plan (impending stream flow and flooding of site).

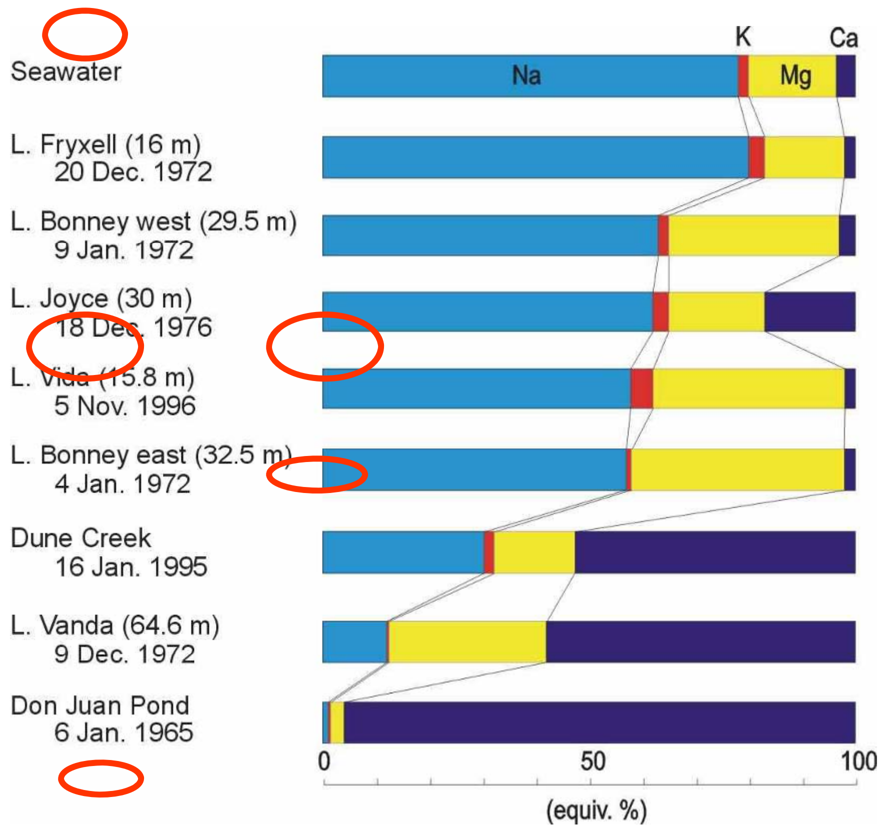


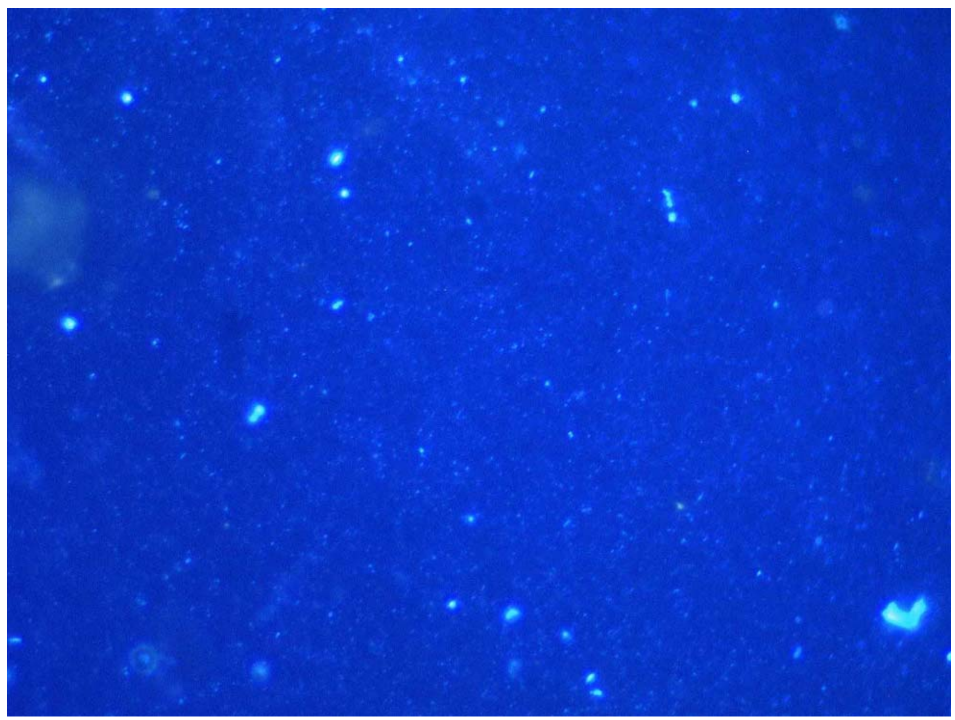


Scotts Hut Run 1/5/05
HELITECH MSGS:
HANGAR IRIDIUM
8(816)763-29073
TO DO!
M.T. U.BARRELS... Bldg 170
EXT. LADDER
L.F., L.HOR, NH
STEP LADDER
L.F., L.HOR
To Vida so far: 31,409 lbs.
but no sunglasses.
Wt & Balance Class - Gary?
BRIAN - A-4
Box up 3 helmets to Boise. ERR.
KIT
Helitech Schedule - December 2005
24

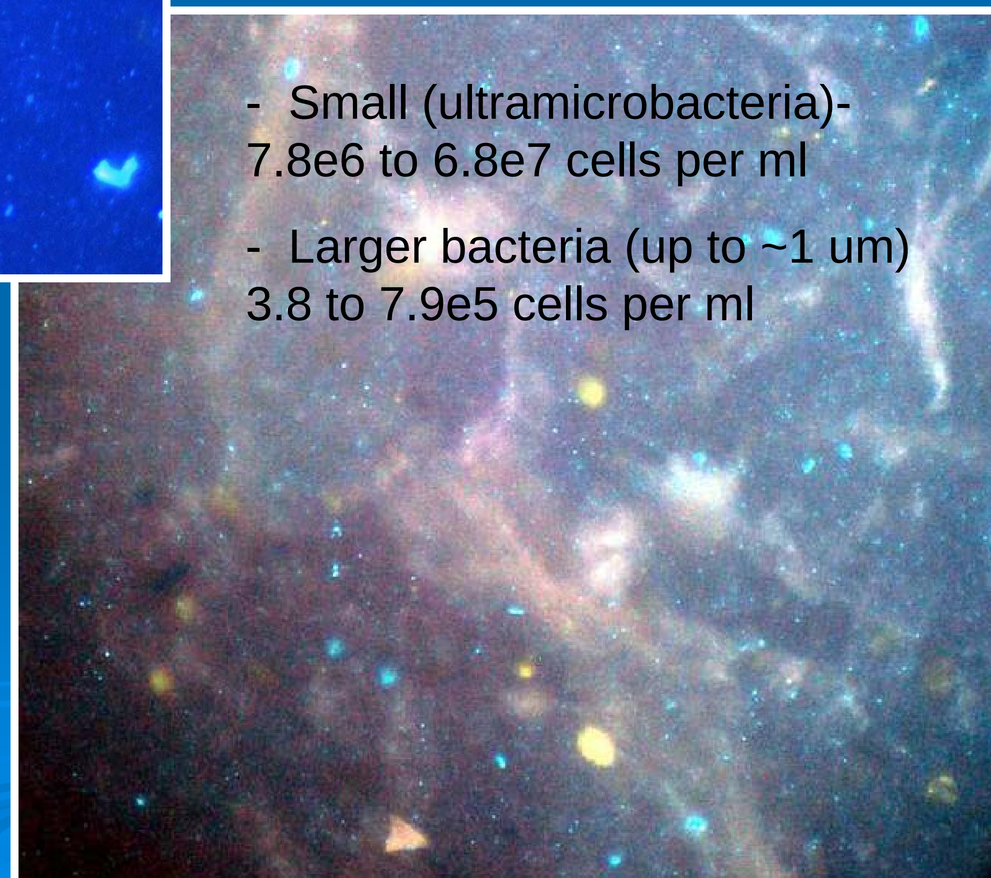
Brine Geochemistry

Constituent	Lake Vida
pH	**6.2**
Temp	**-12.8**
DIC (mg/l)	730
DOC (mg/l)	580
Oxygen	**suboxic**
Major Ions (mM)	
Na	1910
K	82
Ca	30
Mg	666
Cl	3312
SO4	58
HCO3 + CO3	61
Nutrients (µM)	
NH3-N	3884
NO3-N	277
PO4-P	4.8
Micro Elements (µM)	
Sr	445.10
Fe	291.88
Mn	82.46
Zn	12.54
U	0.63
Pb	0.58
Ba	0.52
As	0.89
Ni	1.14
Mo	0.34
Co	0.54
Gases/ Sulfur cmpds (uM)	
N_2O	59
MeSH	0.21
DMS	0.07
DMSO	25
H_2S	ND





Samples were a challenge to filter and prepare for microscopy (due to iron precipitates if exposed to air).

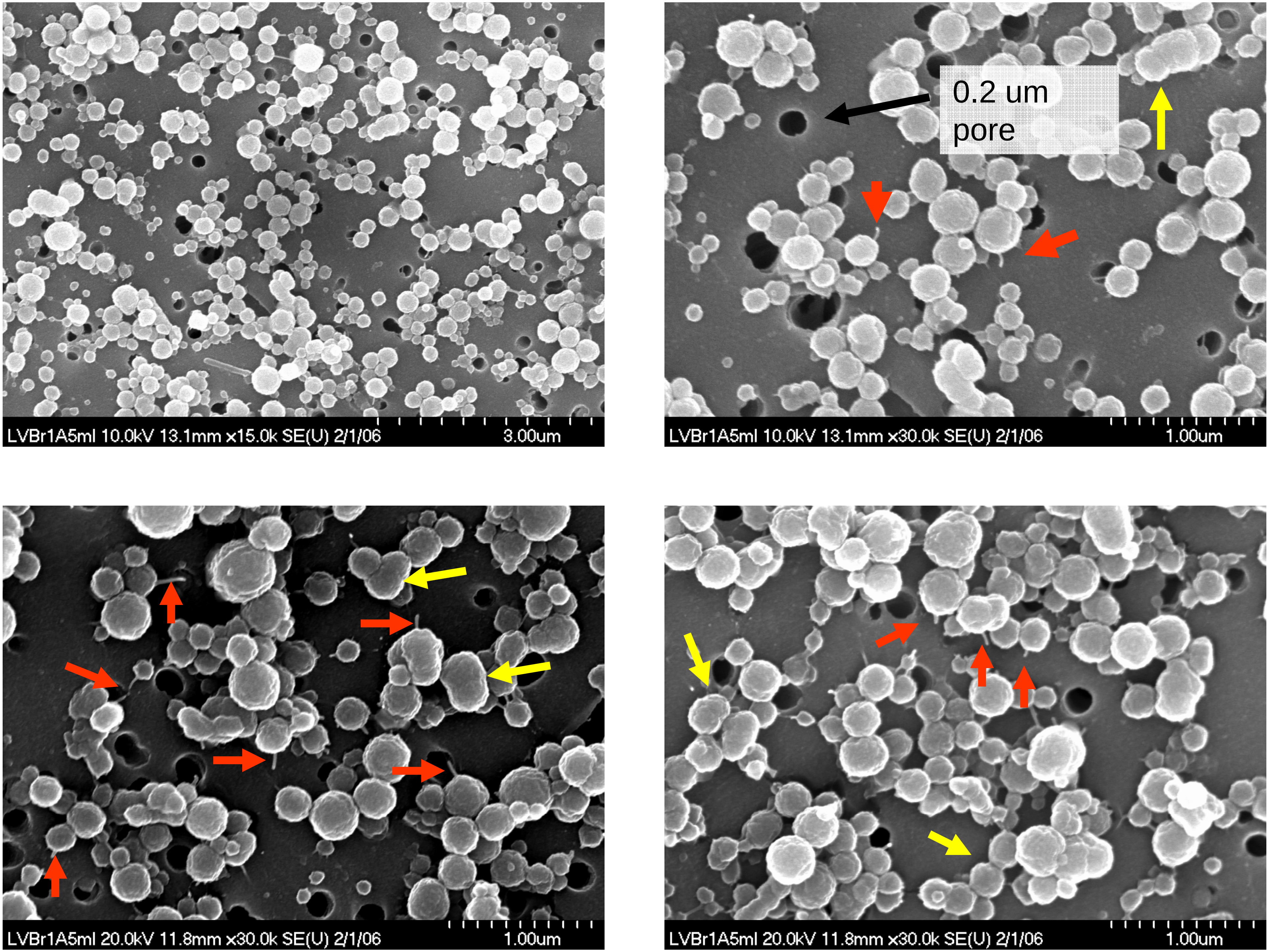


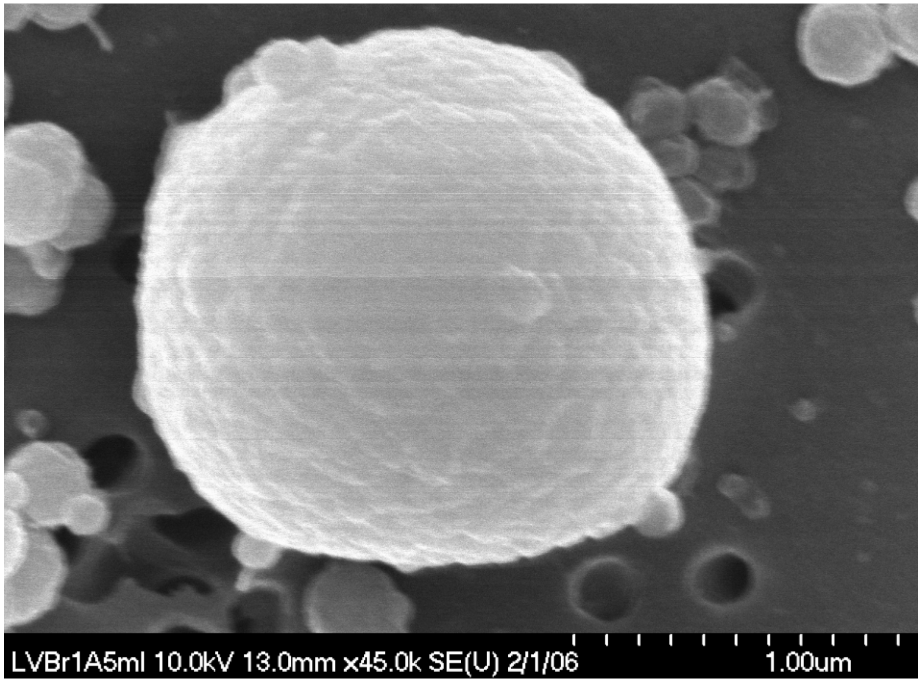
- Small (ultramicrobacteria)- 7.8e6 to 6.8e7 cells per ml
- Larger bacteria (up to ~1 um) 3.8 to 7.9e5 cells per ml

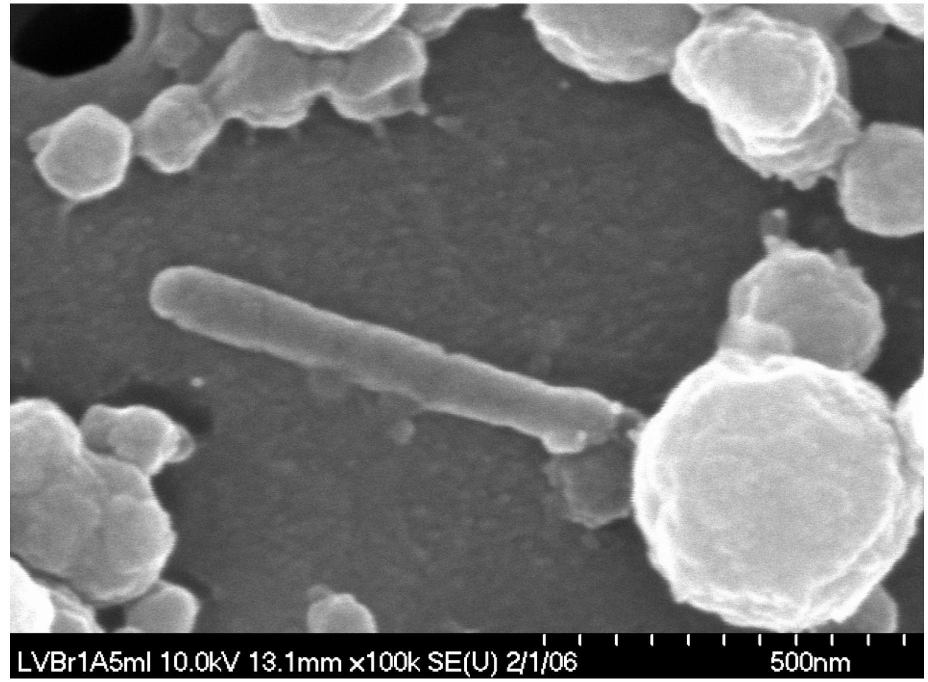
Reminiscent of....

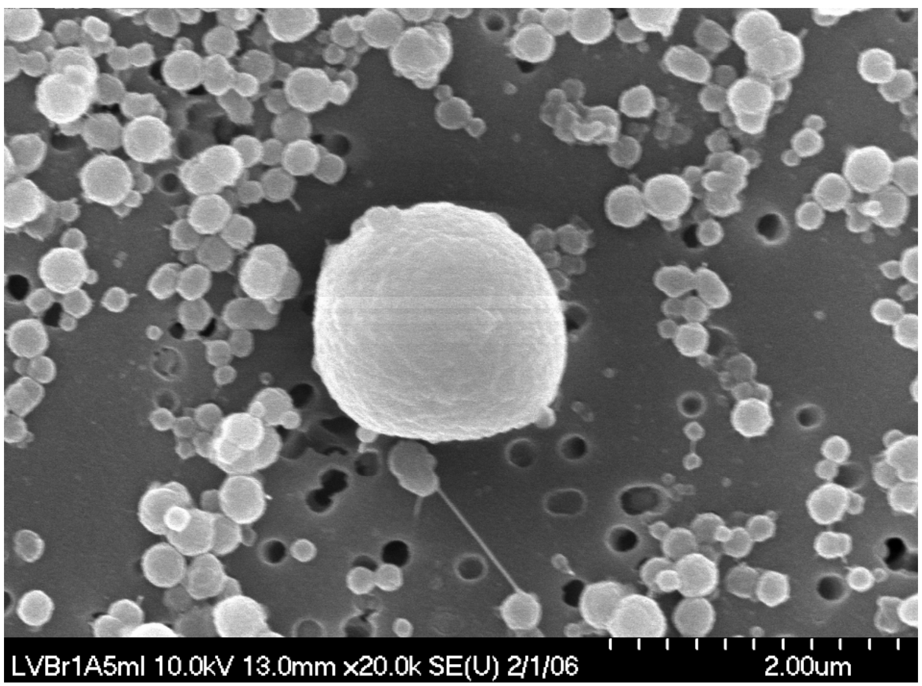
a. **Ultramicrobacteria reported in other extreme environments-**
   e.g. Miteva and Brenchley 2005. AEM
   e.g. Baker et al. 2006. Science

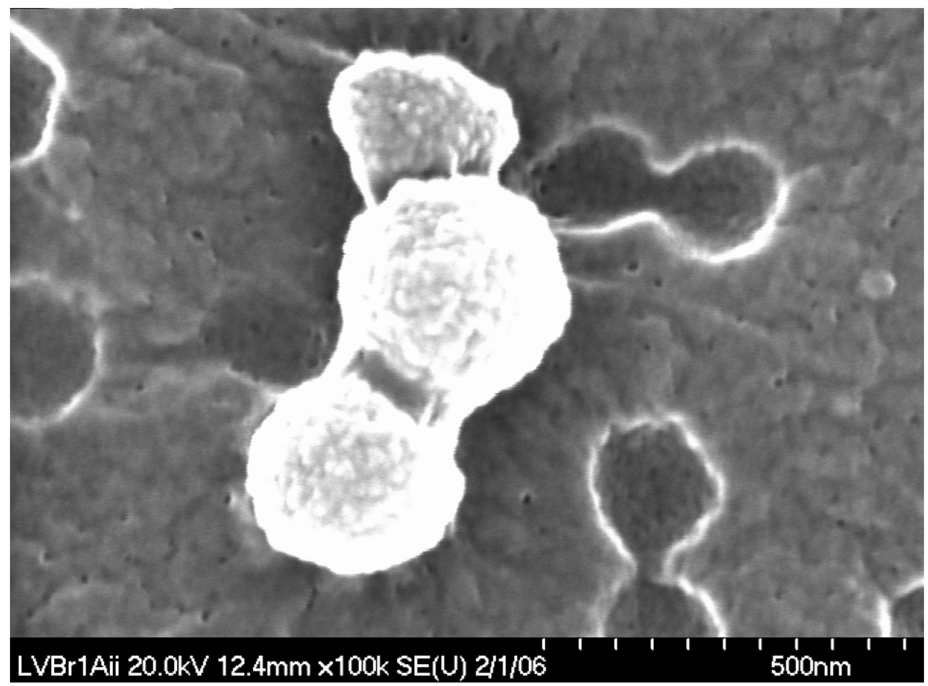
b. **High abundances reported in saltern environments where grazing losses are low or absent (Maturrano et al. 2006 AEM).**


LVBr1A5ml 10.0kV 13.1mm x15.0k SE(U) 2/1/06
3.00um
0.2 um
pore
LVBr1A5ml 10.0kV 13.1mm x30.0k SE(U) 2/1/06
1.00um
LVBr1A5ml 20.0kV 11.8mm x30.0k SE(U) 2/1/06
1.00um
LVBr1A5ml 20.0kV 11.8mm x30.0k SE(U) 2/1/06
1.00um

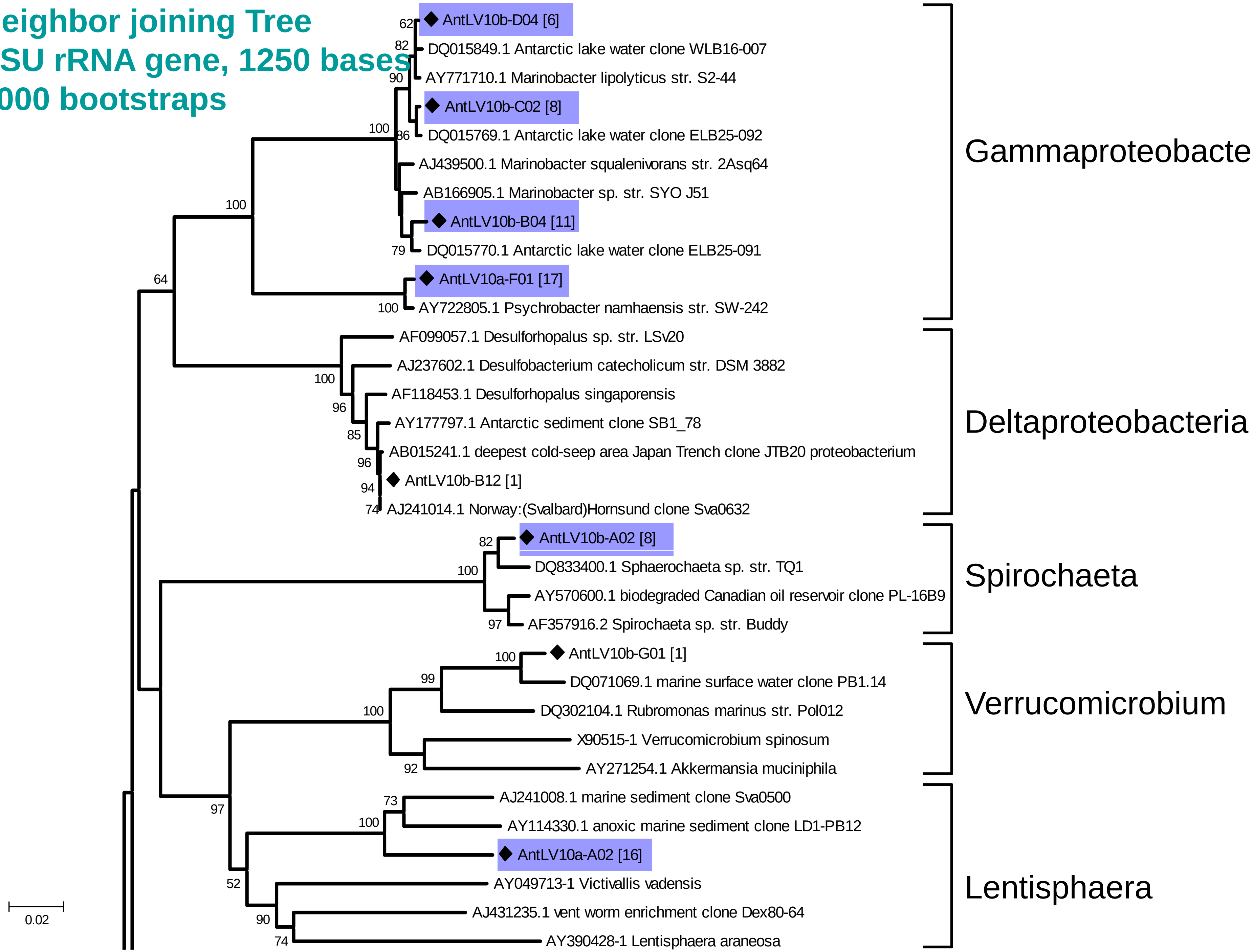

LVBr1A5ml 10.0kV 13.0mm x45.0k SE(U) 2/1/06
1.00um

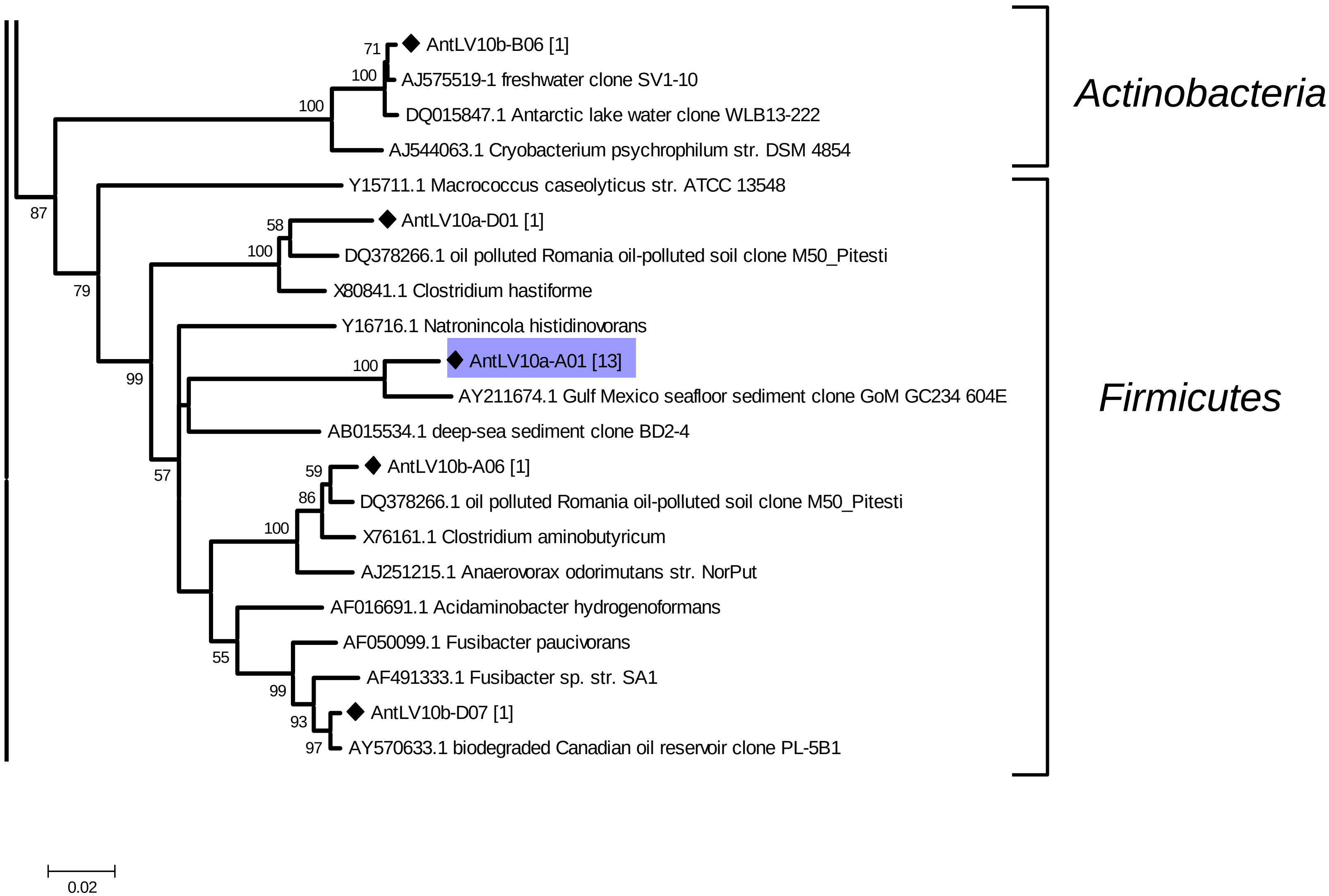

LVBr1A5ml 10.0kV 13.1mm x100k SE(U) 2/1/06
500nm


LVBr1A5ml 10.0kV 13.0mm x20.0k SE(U) 2/1/06
2.00um


LVBr1Aii 20.0kV 12.4mm x100k SE(U) 2/1/06
500nm

Neighbor joining Tree
SSU rRNA gene, 1250 bases
1000 bootstraps

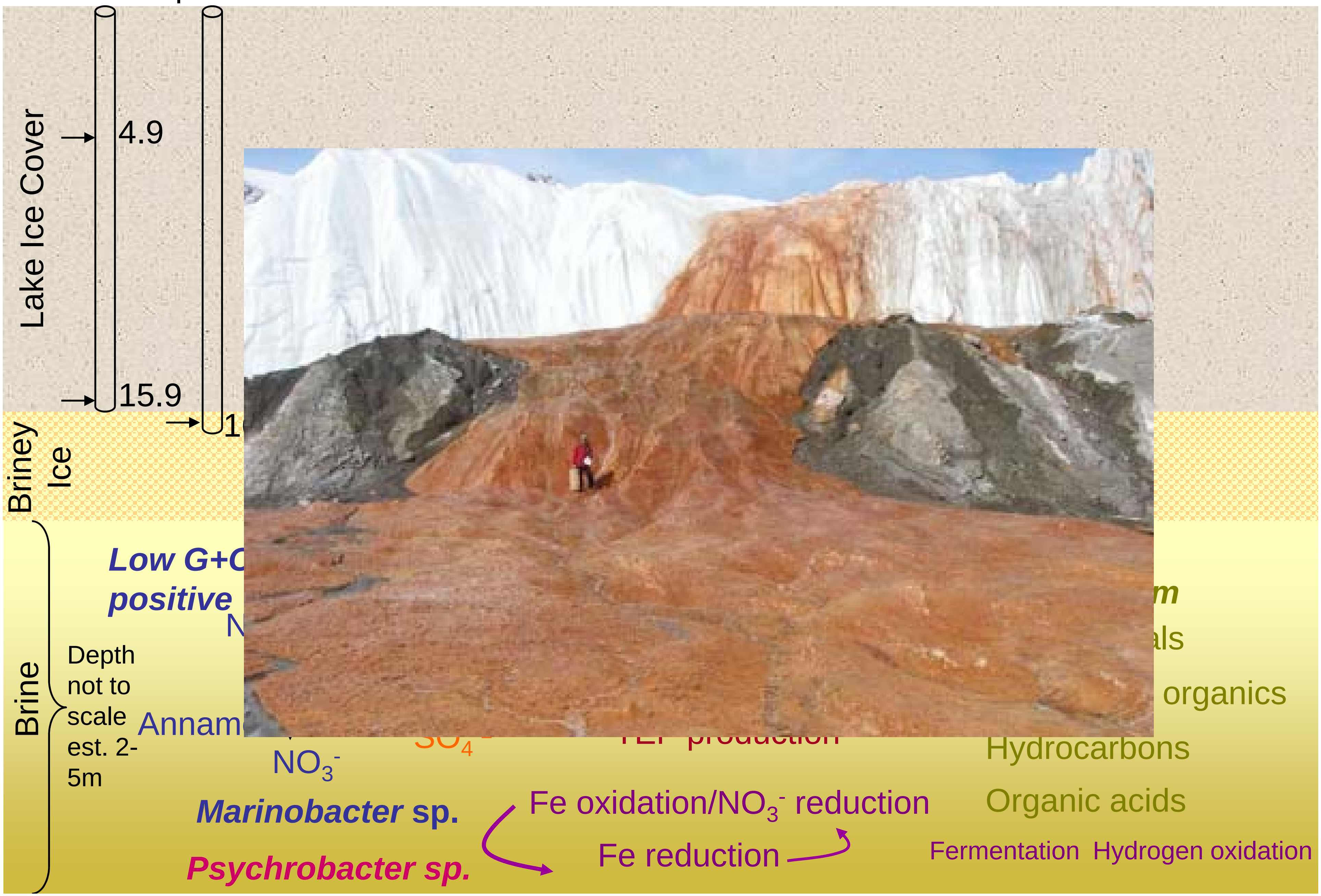




AntLV10b-B06 [1]
AJ575519-1 freshwater clone SV1-10
DQ015847.1 Antarctic lake water clone WLB13-222
AJ544063.1 Cryobacterium psychrophilum str. DSM 4854
Y15711.1 Macrococcus caseolyticus str. ATCC 13548
AntLV10a-D01 [1]
DQ378266.1 oil polluted Romania oil-polluted soil clone M50_Pitesti
X80841.1 Clostridium hastiforme
Y16716.1 Natronincola histidinovorans
AntLV10a-A01 [13]
AY211674.1 Gulf Mexico seafloor sediment clone GoM GC234 604E
AB015534.1 deep-sea sediment clone BD2-4
AntLV10b-A06 [1]
DQ378266.1 oil polluted Romania oil-polluted soil clone M50_Pitesti
X76161.1 Clostridium aminobutyricum
AJ251215.1 Anaerovorax odorimutans str. NorPut
AF016691.1 Acidaminobacter hydrogenoformans
AF050099.1 Fusibacter paucivorans
AF491333.1 Fusibacter sp. str. SA1
AntLV10b-D07 [1]
AY570633.1 biodegraded Canadian oil reservoir clone PL-5B1
Actinobacteria
Firmicutes
0.02

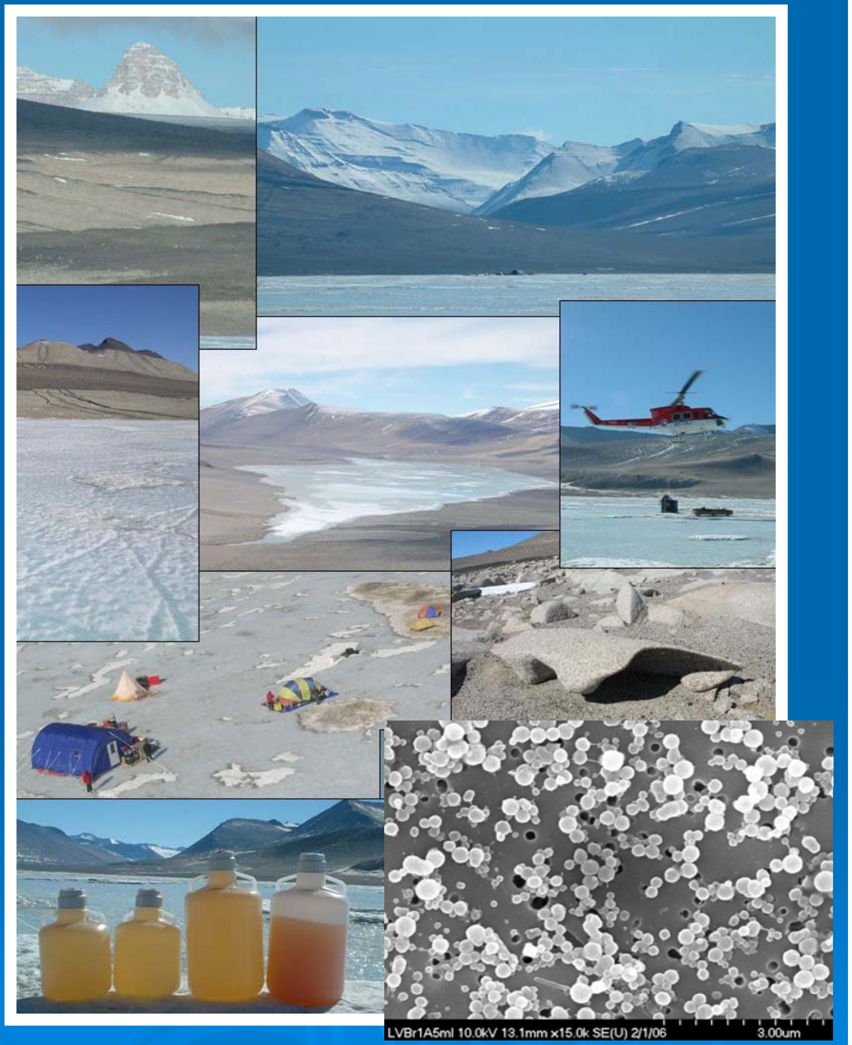
HYPOTHETICAL MODEL
of microbial metabolisms in Vida Brine

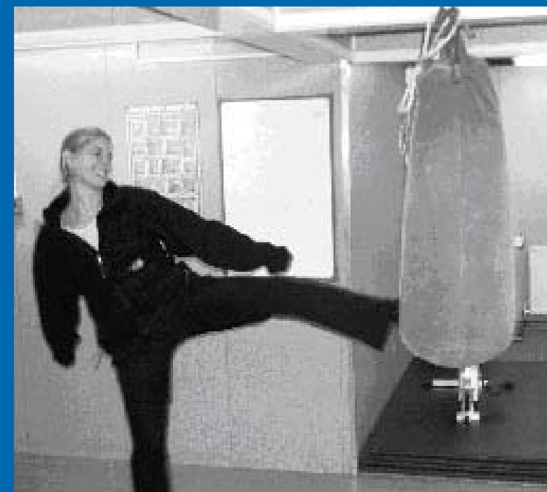
Lake Vida
vertical profiles



We have collected brine from Lake Vida (a first)

- The brine is in the aphotic zone of the lake where it is cold, slightly acidic, has low oxygen, high Fe, extremely high N, DOC and has abundant small cells.
- From this information we conclude that this particular ice-sealed environment is one that does not preclude microbial activity and an ecosystem's existence
  - (also see Murray et al. presentation at 1100 hrs- this session).
- Important Note: The collected brine only represents the uppermost level of brine in the lake system.
- More work is ongoing (e.g. culturing efforts at DRI).
- We can access the brine and conduct a full characterization of the lake's isolated ecosystem.

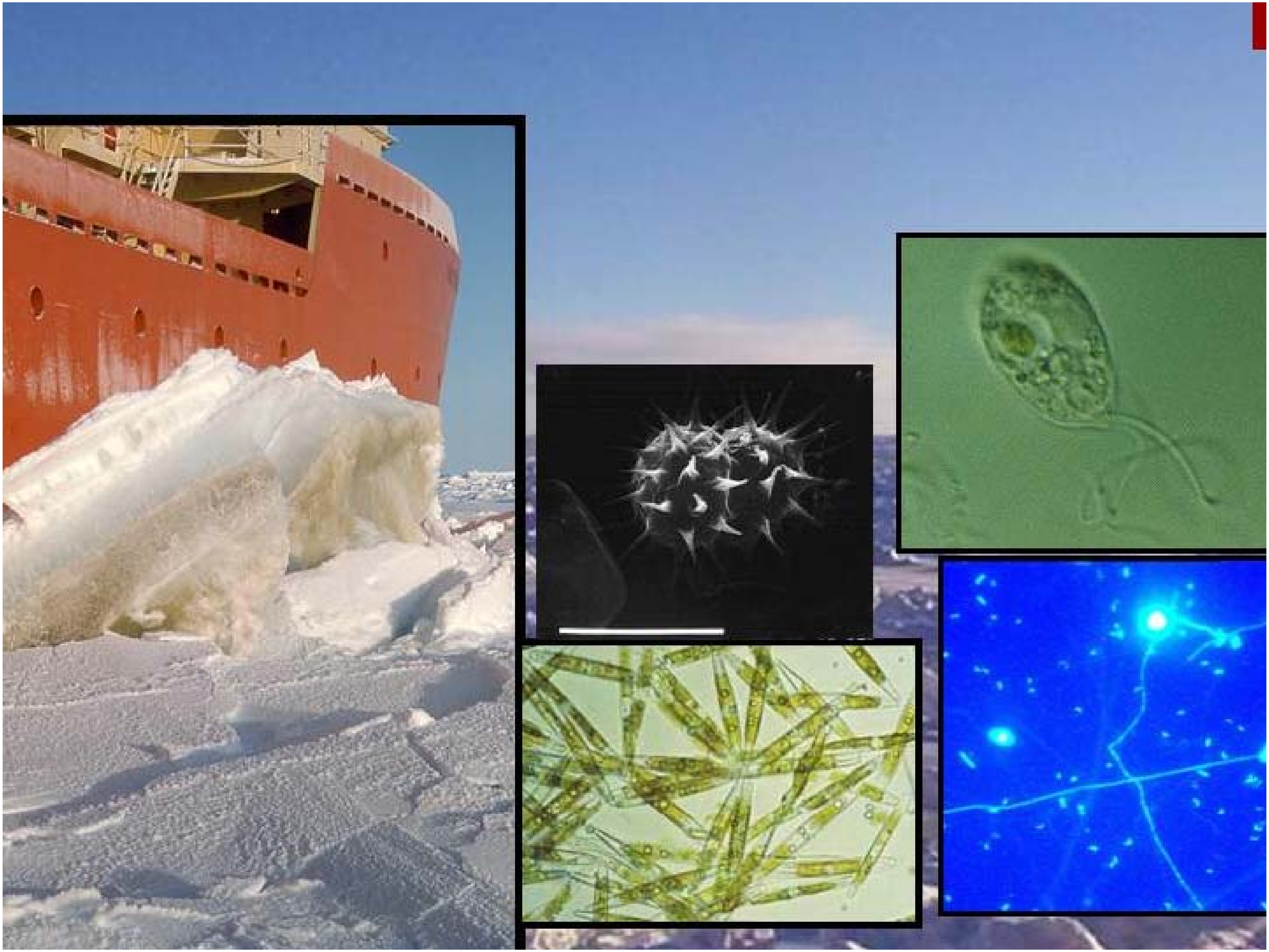




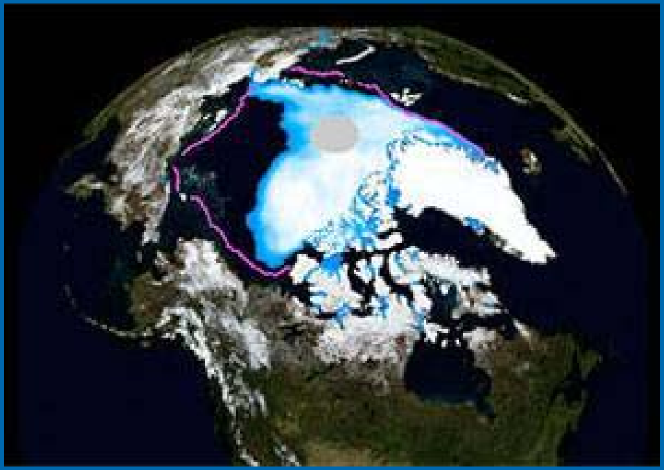
NASA

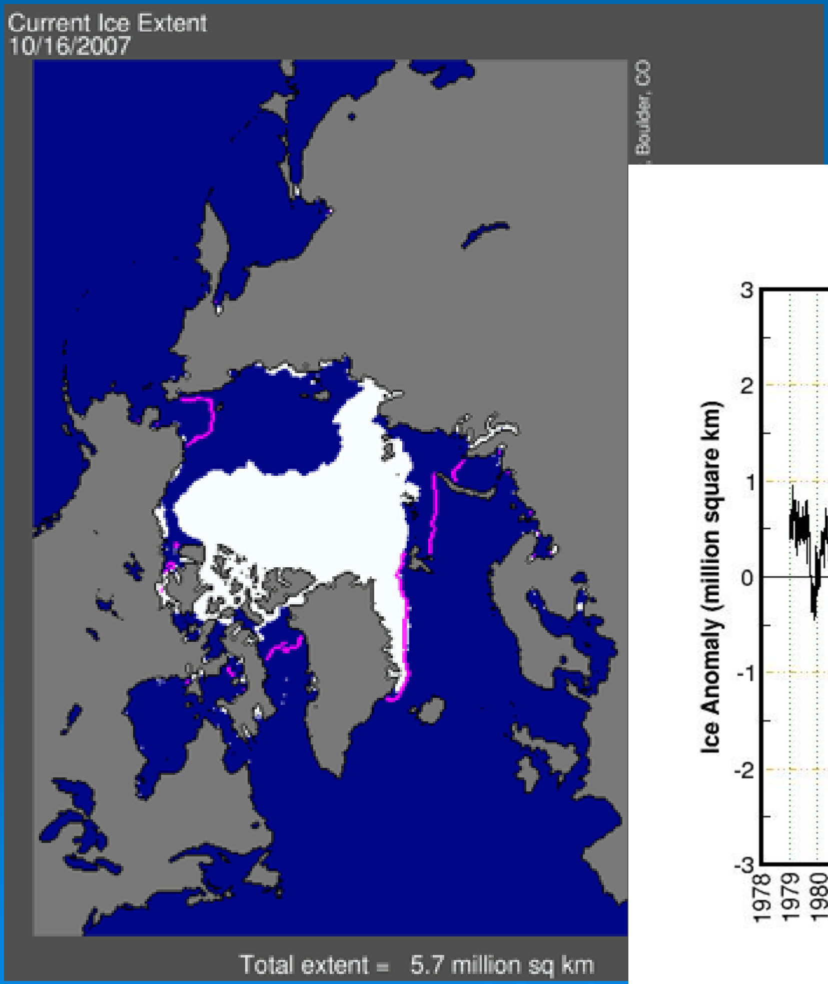
DRI
Desert Research Institute

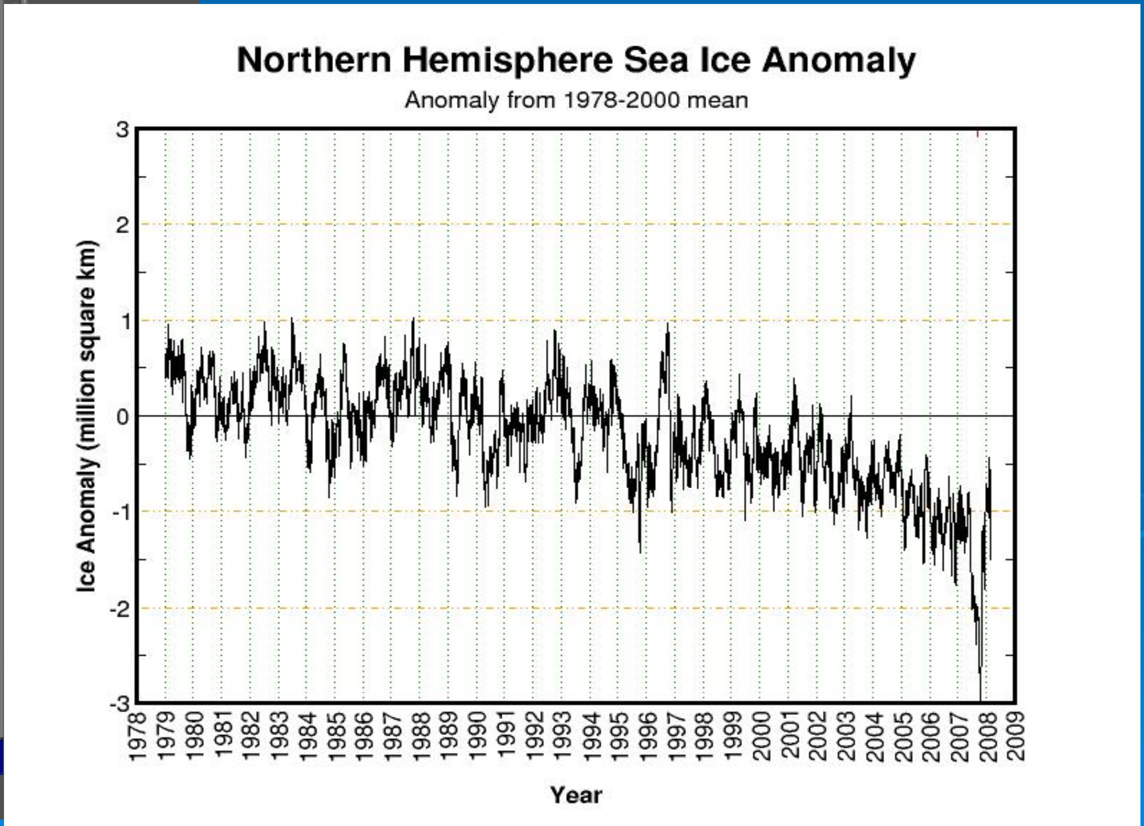
UNITED STATES ANTARCTIC PROGRAM
NATIONAL SCIENCE FOUNDATION



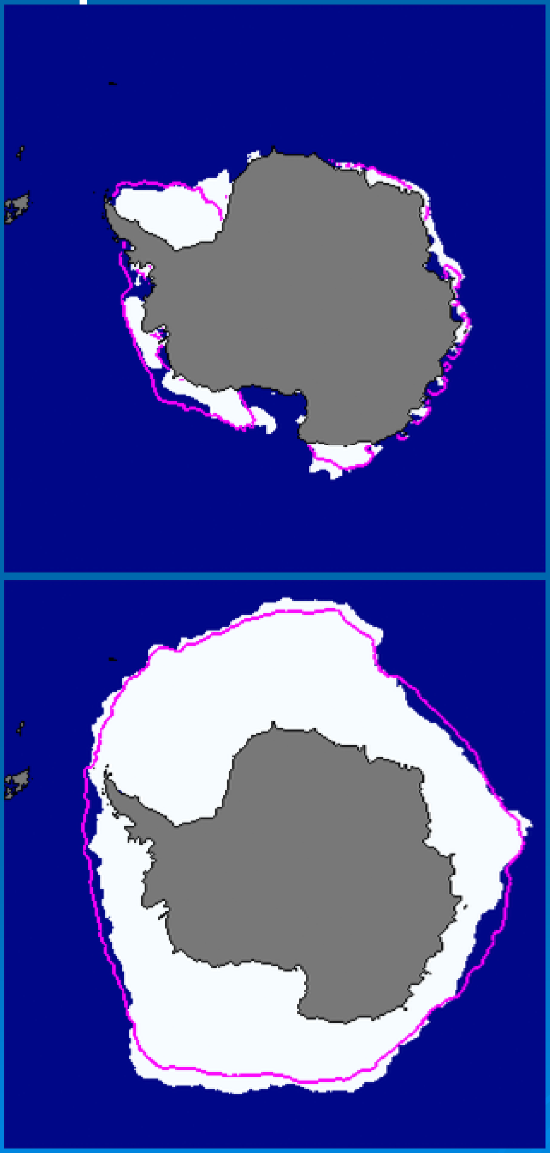
Arctic Sea ice is decreasing.

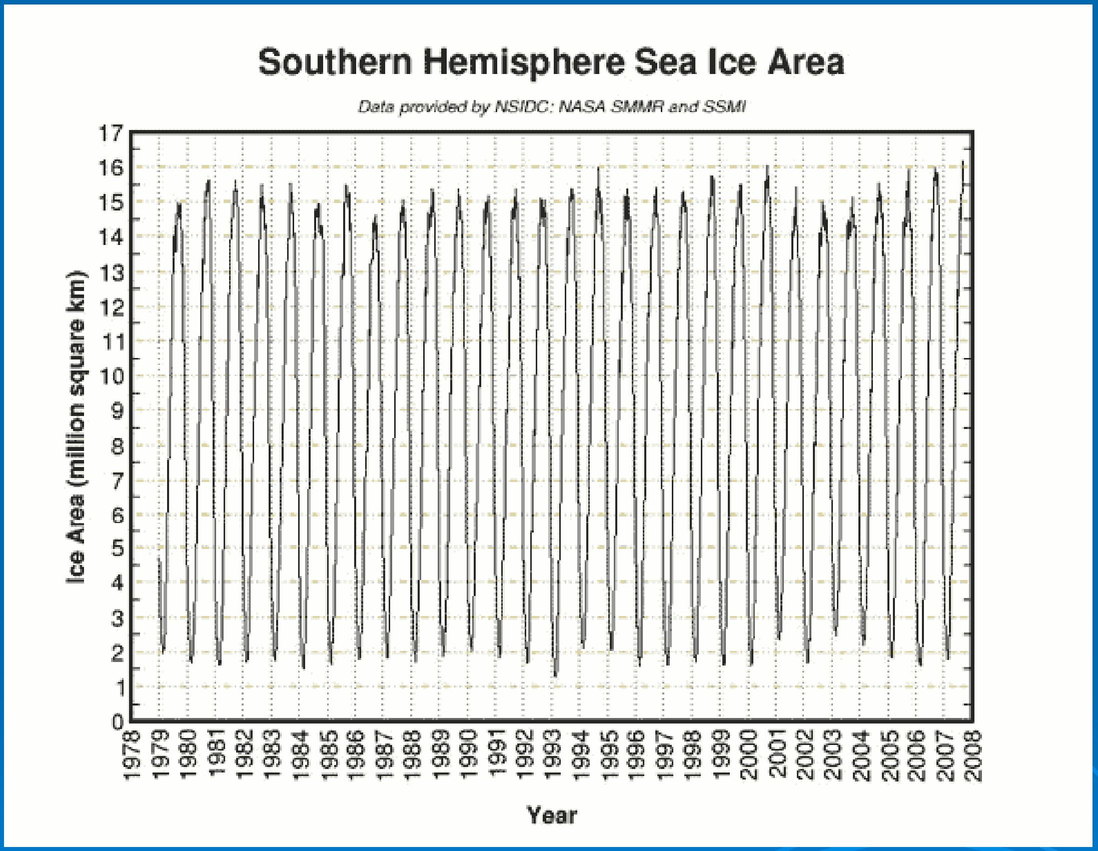


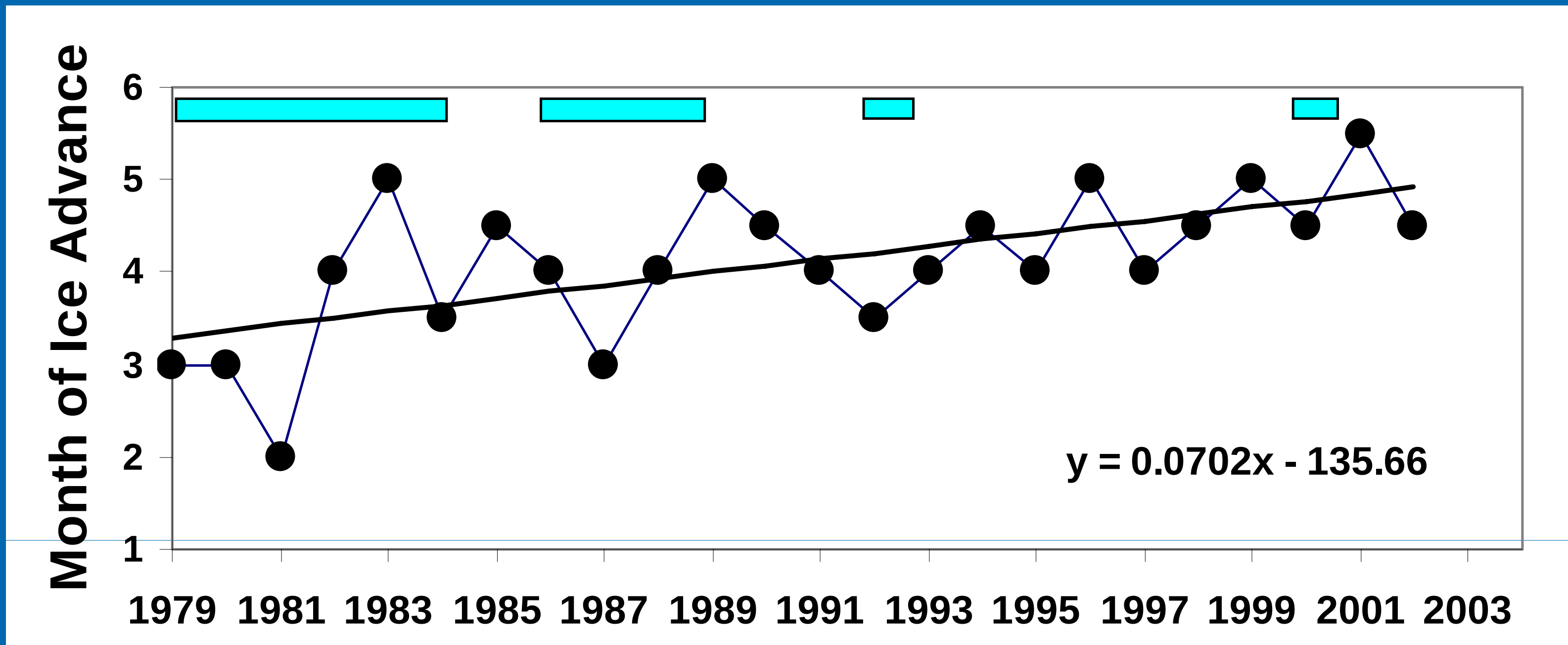




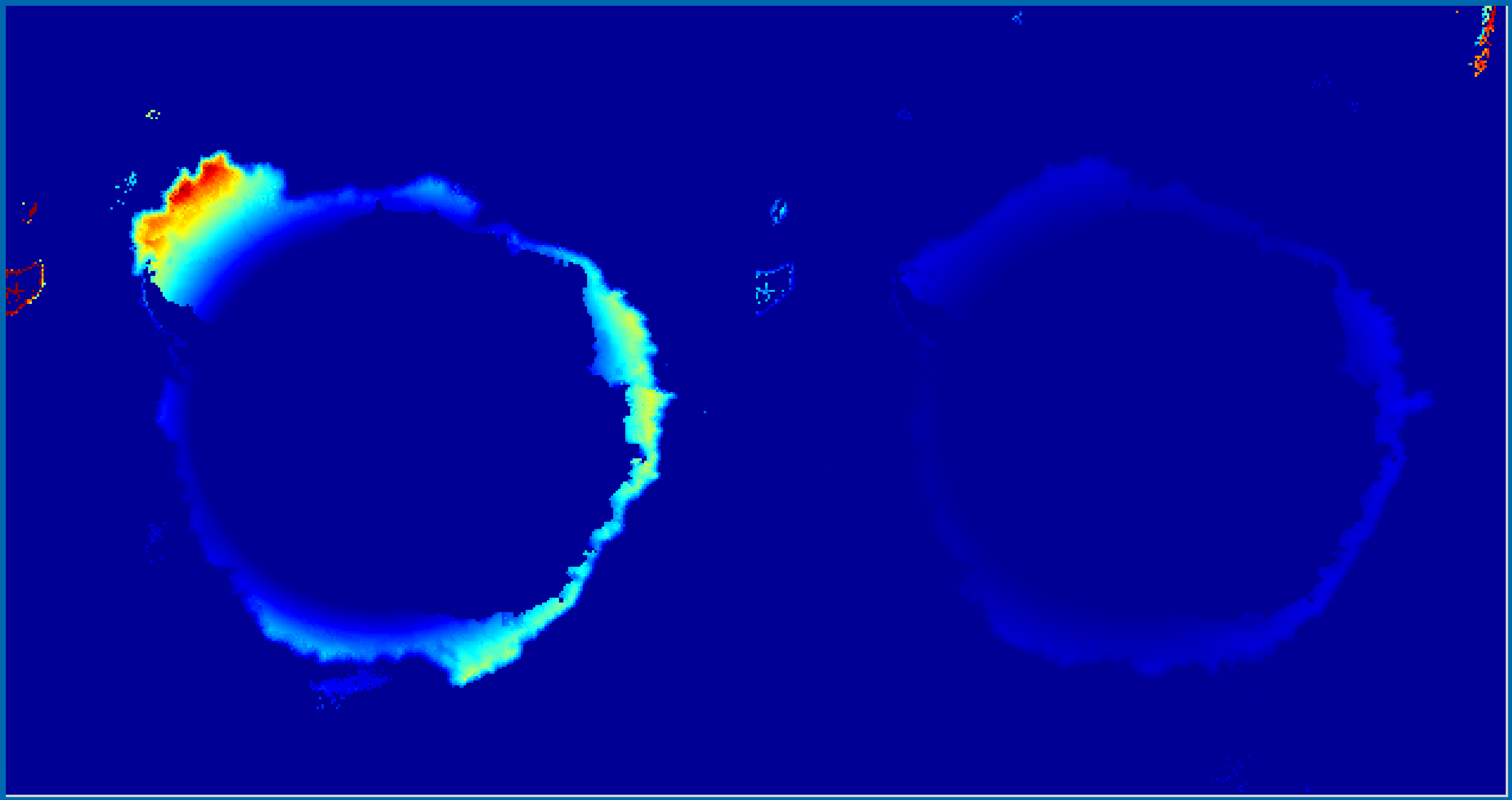
Antarctic Sea ice: The story appears to be less







-Presence of Second Year ice also appears to be decreasing (illustrated by bars in graph below)



The changes observed in the marine ecosystem of the western Antarctic Peninsula (WAP) region to date are the most likely to have had a direct impact on the marine fauna, principally through shifts in the extent and timing of habitat for ice-associated biota. - *Clarke et al. 2007*