Microbiological indicators of water quality in submerged karst caves of Wakulla Springs

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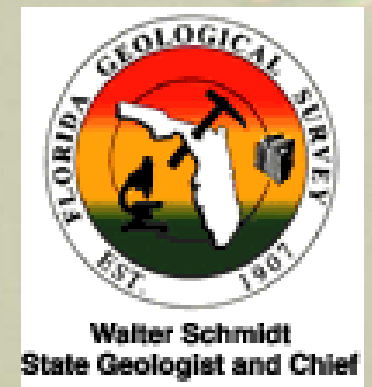
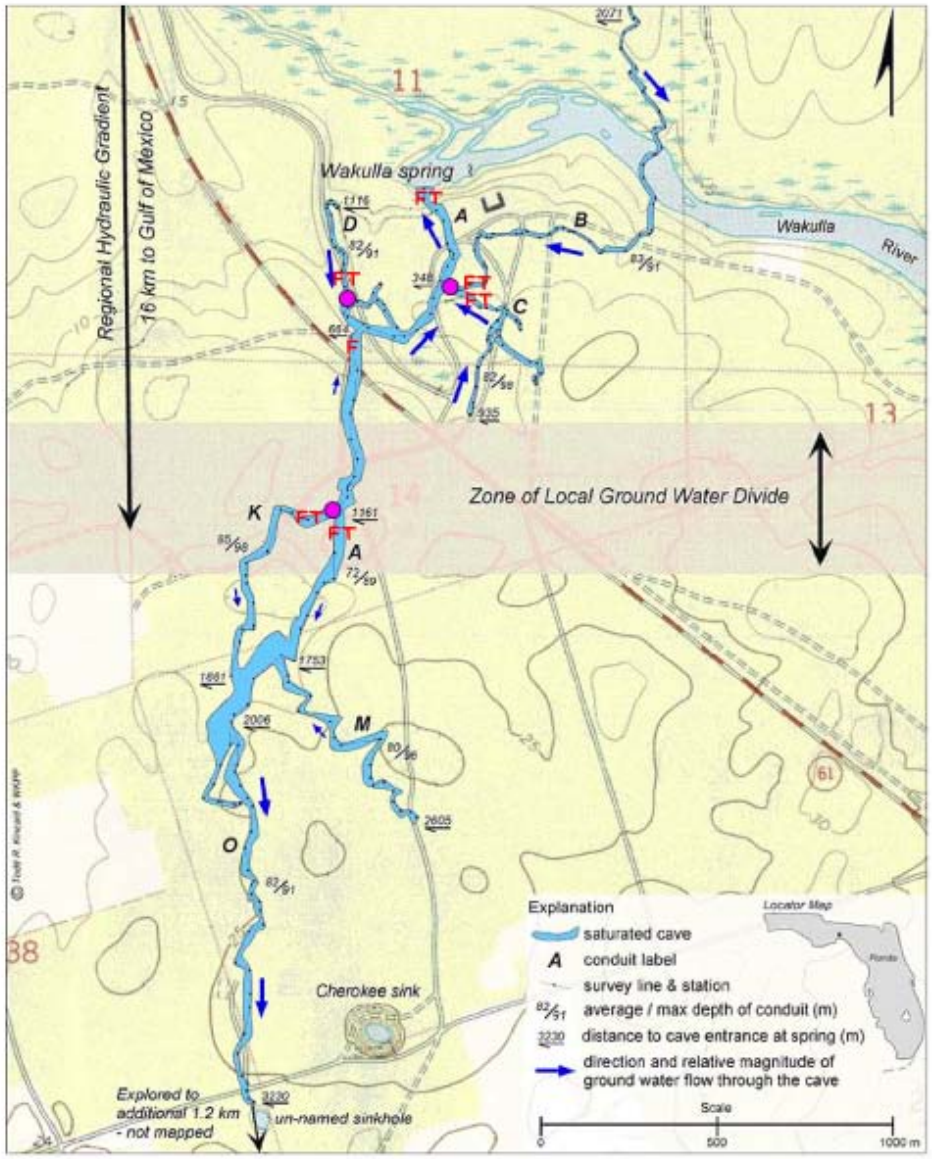


● bore hole FT flow and temperature F flow only



Dr. Todd Kincaid of Hazlett-Kincaid Inc.

Syringe samples taken by divers of cave sediment from walls, nooks and floor__

Molecular analysis Isolation of Amoebae spp

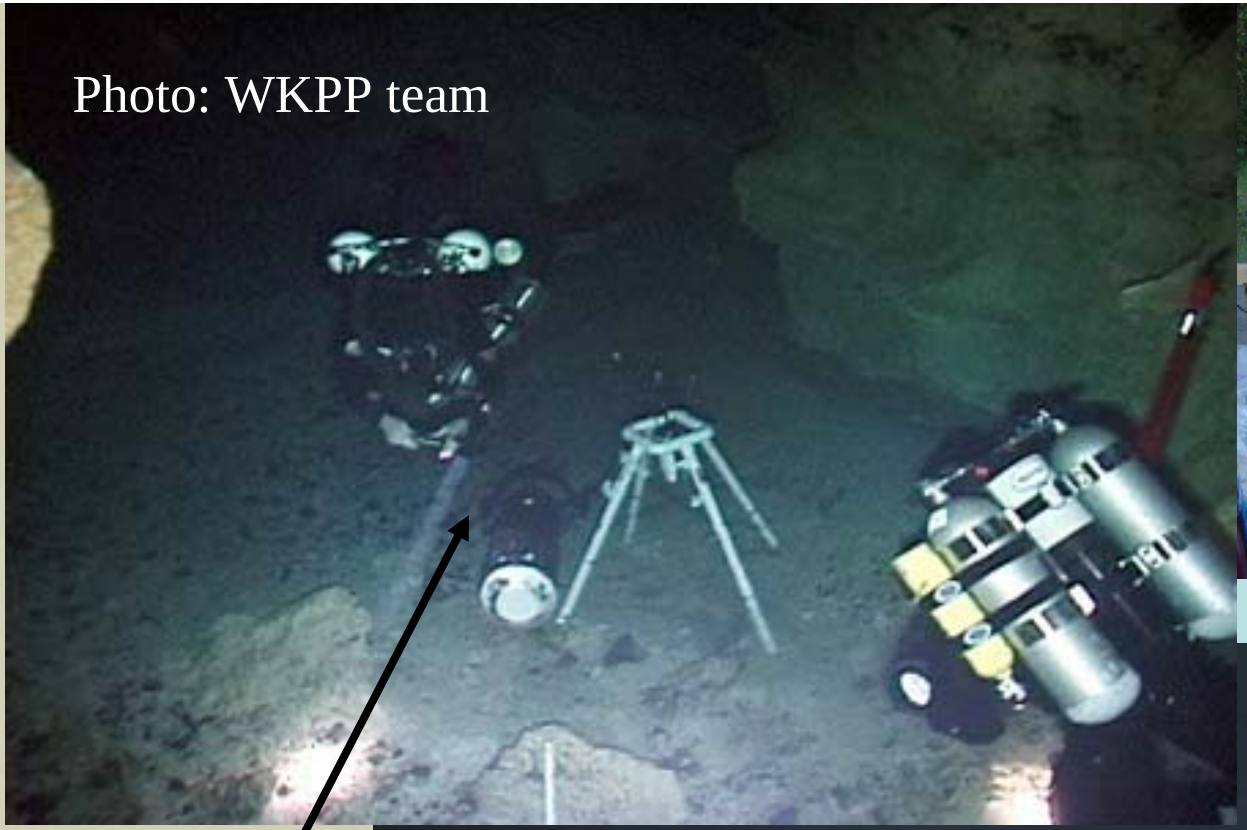
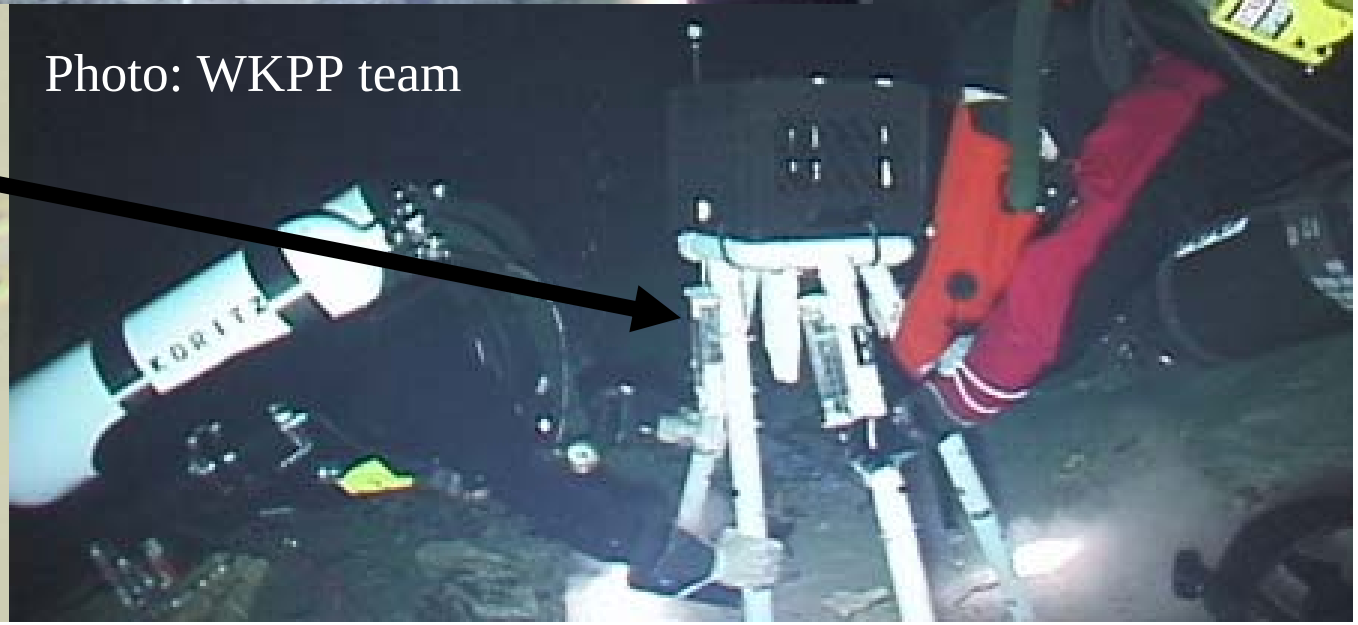


Photo: David Sweetin

Glass slides in acrylic racks affixed to quadpod meter supports for future retrieval



Well tubes purged for minimum 3x the void volume prior to sample collection.

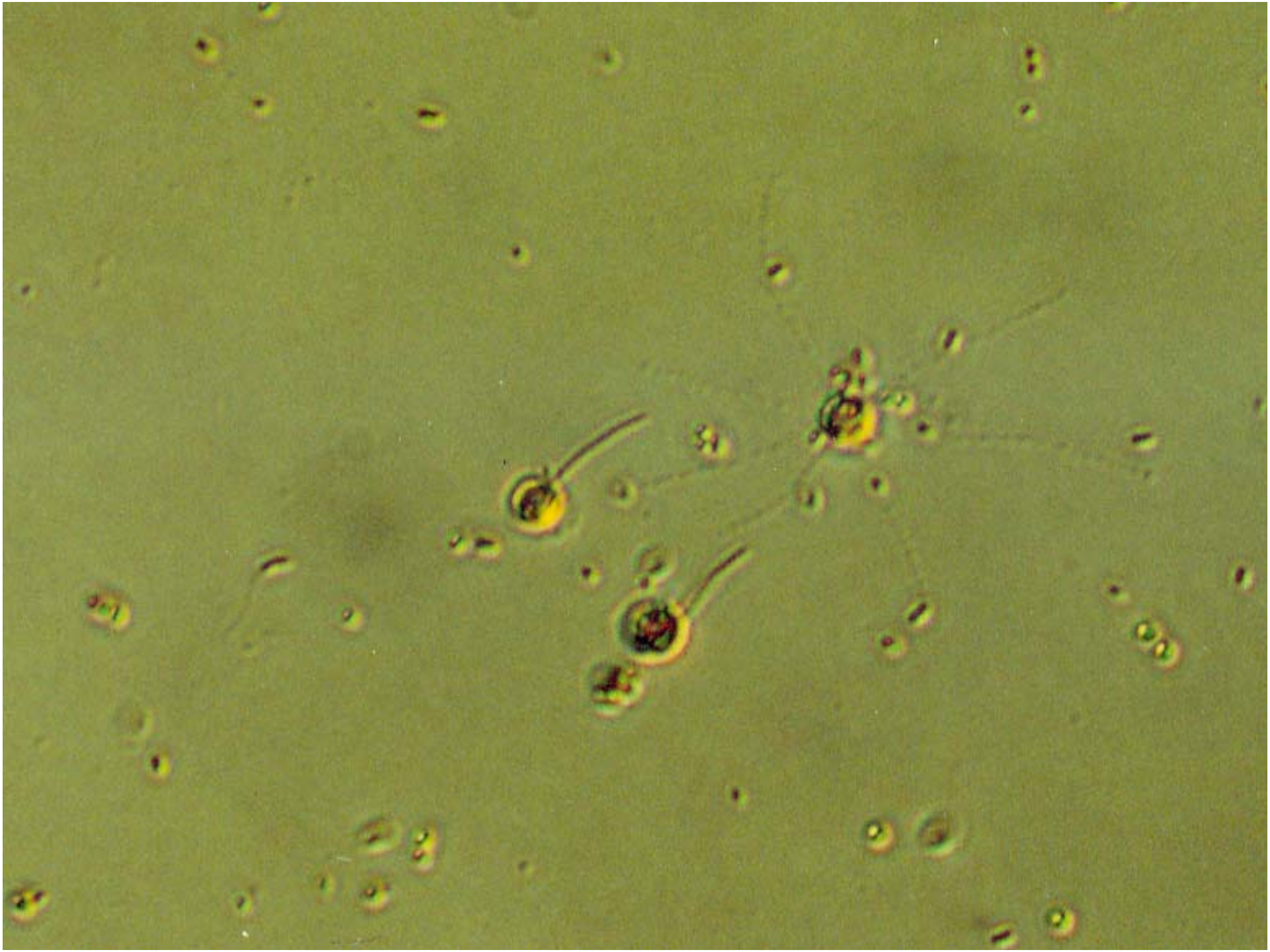
Sample water collected as a 5L composite.

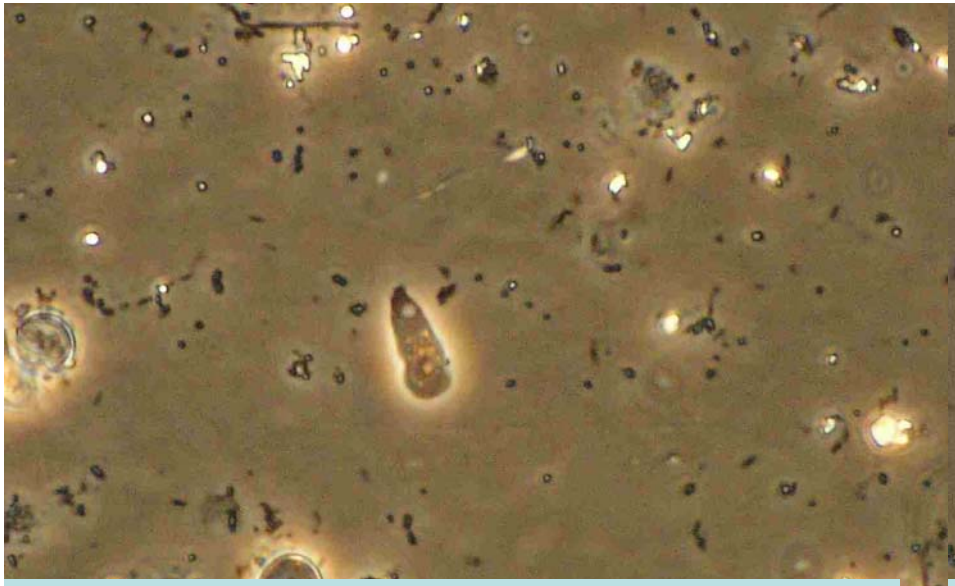
Photo: Andreas Nocker

http://www.gue.com/sites/wkp/overview.shtml

<u>Water samples taken at 4 time points</u>

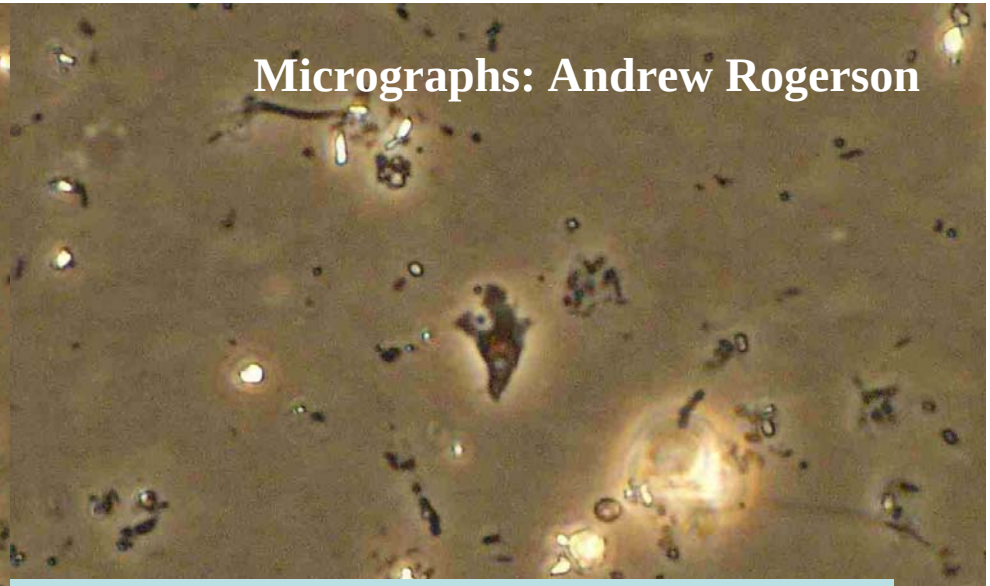
- Bacterial direct count
- Plate counts
  - Enterococcus
  - Total Coliforms and E. coli
  - R2A and TSA
- Molecular analysis
- Elemental analysis of CN
- Major ionic constiuents



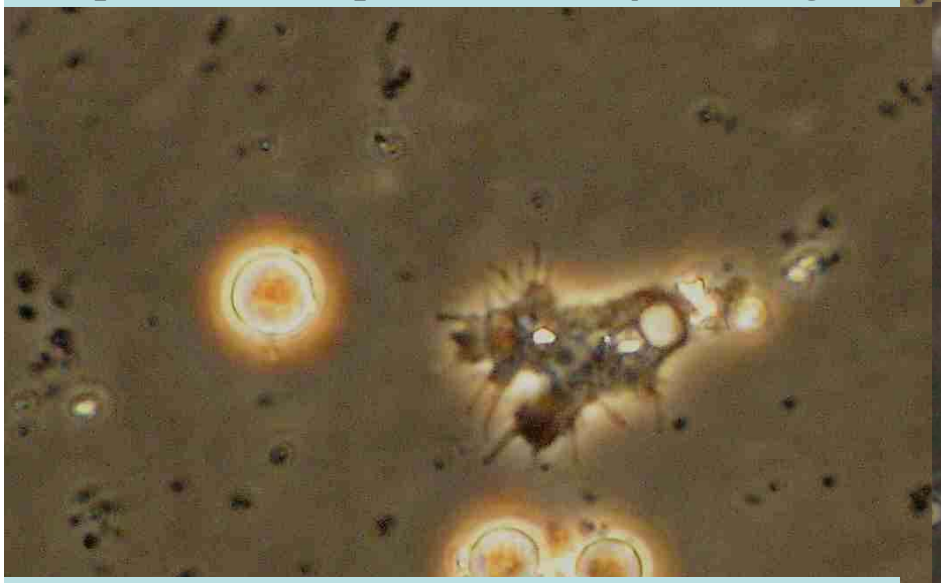


Micrographs: Andrew Rogerson

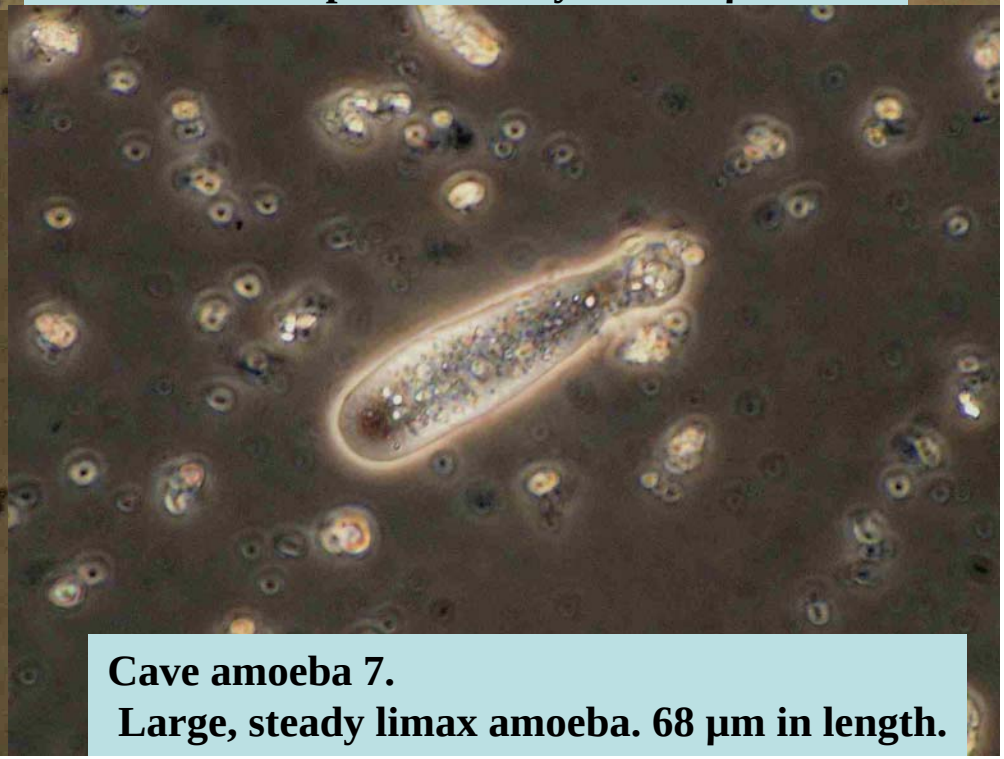
Cave amoeba 1.
Eruptive vahlkampfiid, about 17 µm in length



Cave amoeba 2.
Active, non-eruptive. Usually 10 – 12 µm.



Cave amoeba 6.
Slow moving with occasional eruptive 'bulges'.
Probably *Rhizamoeba*. Up to 34 µm



Cave amoeba 7.
Large, steady limax amoeba. 68 µm in length.

Code	Identity	Size (mean)
Am 1	unidentified	11 µm
Am 2	*Sacchamoeba*	26 µm
Am 3	*Vannella*	14 µm
Am 4	unidentified testate	10 µm
Am 5	unidentified	8.5 µm
Am 6	*Acanthamoeba*	20 µm
Am 7	vannellid	34 µm
Am 8	vahlkampfiid	17 µm
Am 9	*Dactylamoeba*-like	10 µm
Am 10	unidentified	24 µm
Am 11	vahlkampfiid	10 µm
Am 12	*Rhizamoeba*	34 µm
Am 13	*Sacchamoeba*	68 µm
Am 14	unidentified filose	8.5 µm
Am 15	vannellid	20 µm
Am 16	*Gephyramoeba*-like	60 µm
Am 17	vahlkampfiid	20 µm
Am 18	*Vexillifera*	25 µm
Am 19	unidentified	9 µm

Sample event 15 Nov 2003.
B tunnel: Am 3, 8, 9, 10, 11, 12, 13
C tunnel: Am 1, 7

Sample event 5 Jan 2004
A-D tunnel: Am 1, 2, 4
A-D tunnel: Am 1, 2, 3, 5, 6
A-D tunnel: Am 1, 2

Sample event 7 Feb 2004
K tunnel 1: no amoebae
Ktunnel 2: Am 8
D tunnel: Am 4, 6
D tunnel : Am 1
A-K tunnel : Am 2, 9, 19
A-K tunnel : Am 2, 3, 15, 16, 17, 18

Number of Species in each sample

Number of shared Species

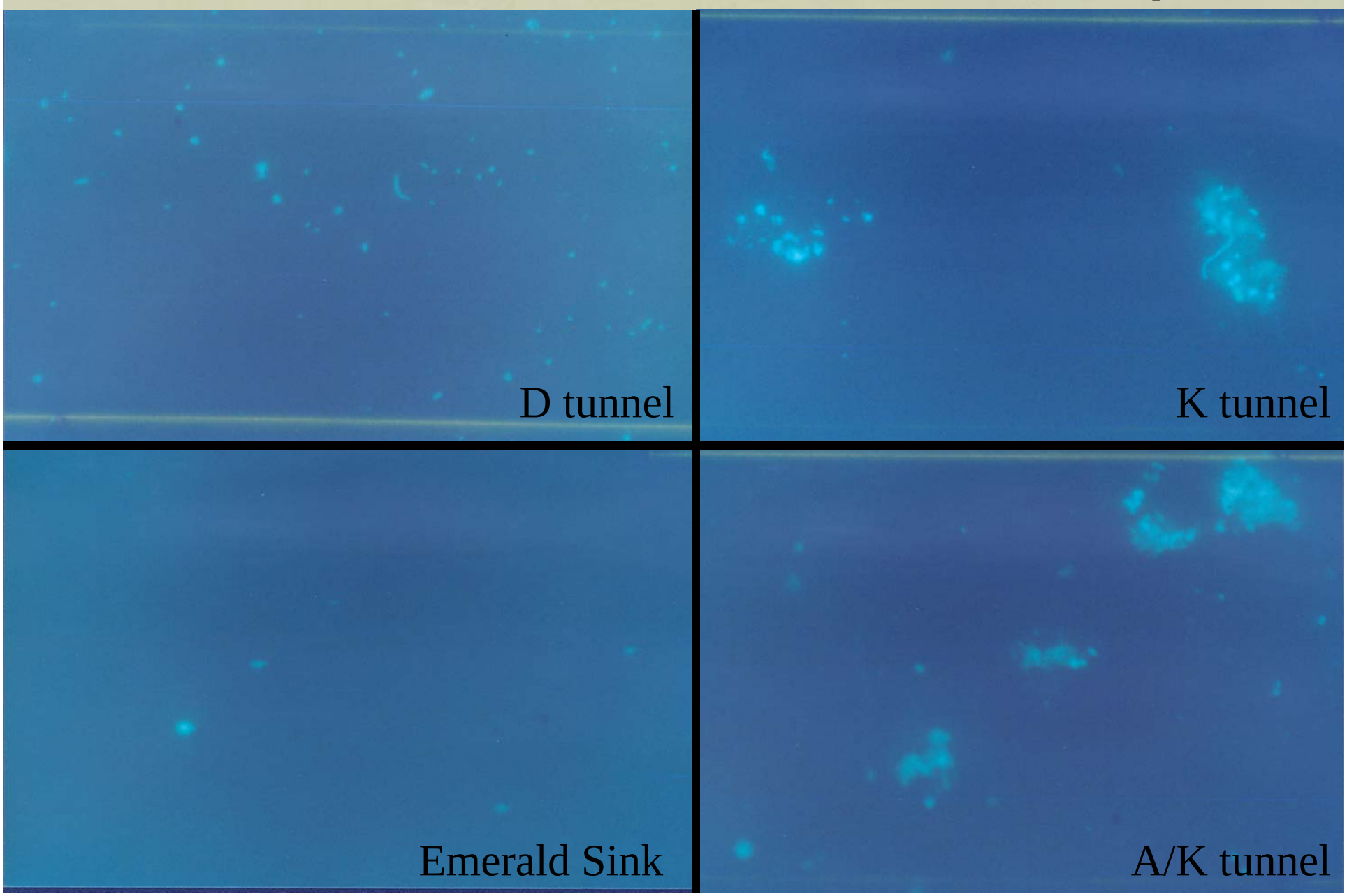
	B	C	D	A / D	K	A / K
B	7	0	0	1	1	2
C		2	1	1	0	0
D			3	3	0	0
A / D				6	0	2
K					1	0
A / K						9

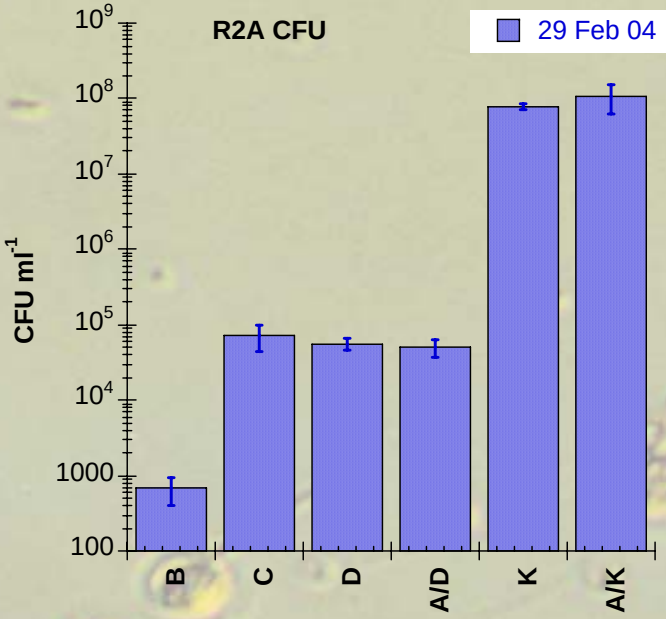
Sorenson's Similarity Coefficient

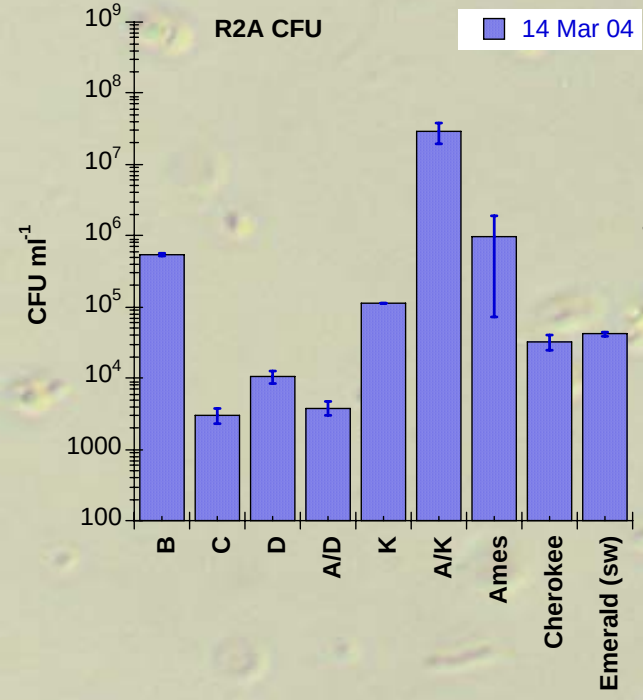
	B	C	D	A / D	K	A / K
B	100%	0.00%	0.00%	15.38%	25.00%	25.00%
C		100%	20.00%	25.00%	0.00%	0.00%
D			100%	66.67%	0.00%	0.00%
A / D				100%	0.00%	26.67%
K					100%	0.00%
A / K						100%

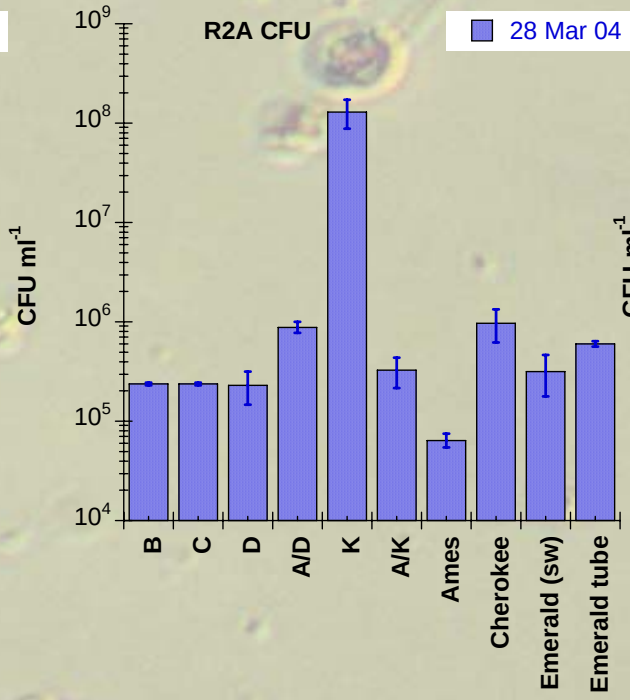
DAPI fluorochrome bacterial direct counts

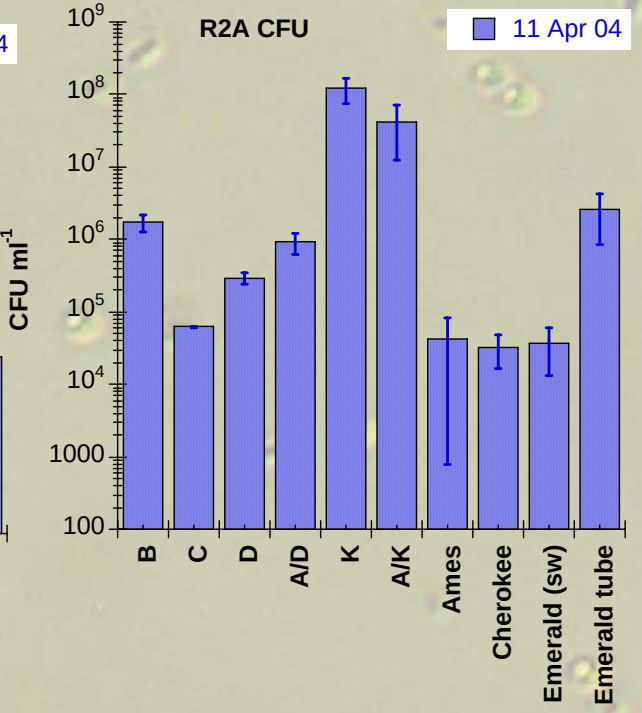
10 Apr 04, 1750 x

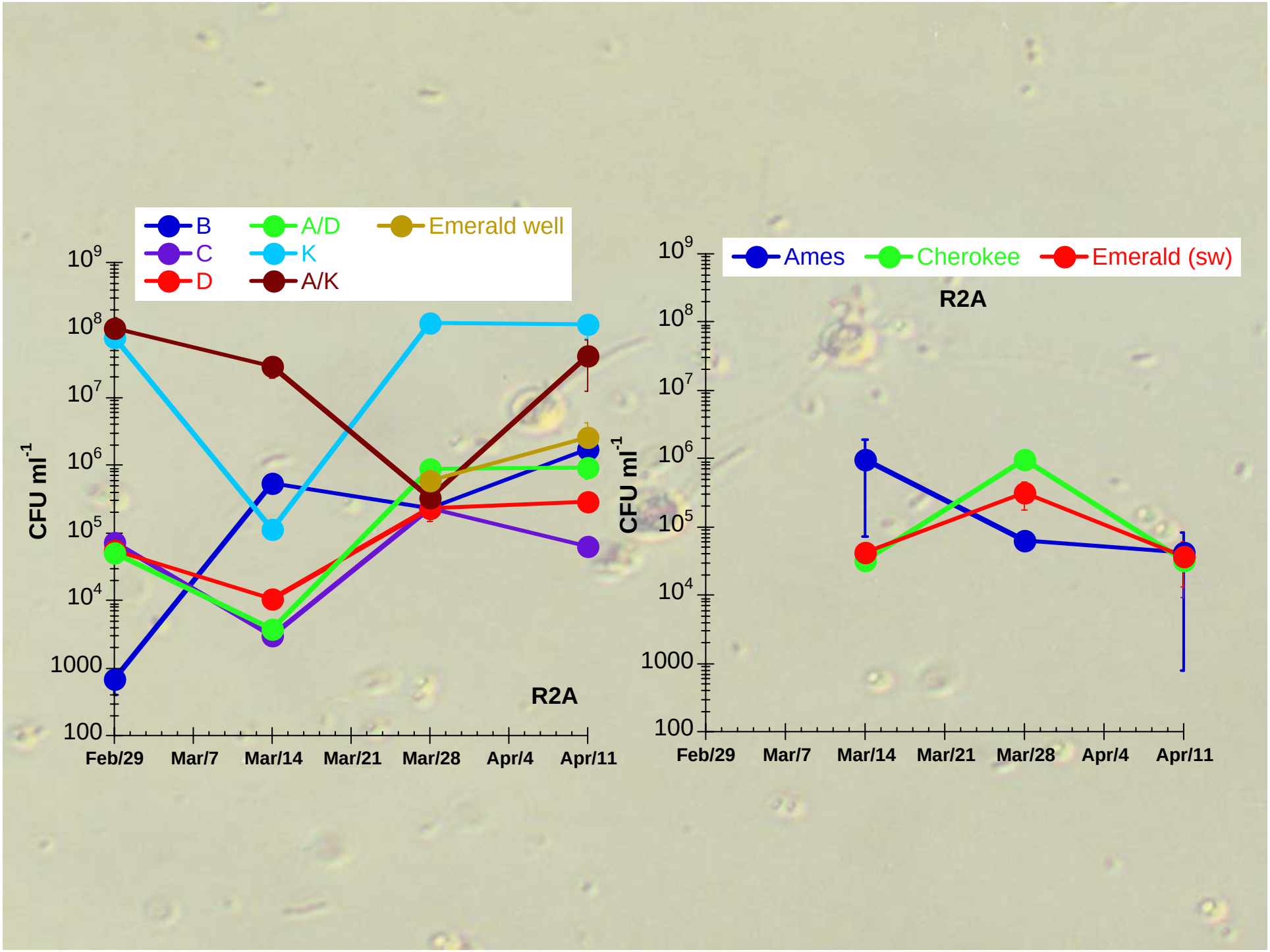


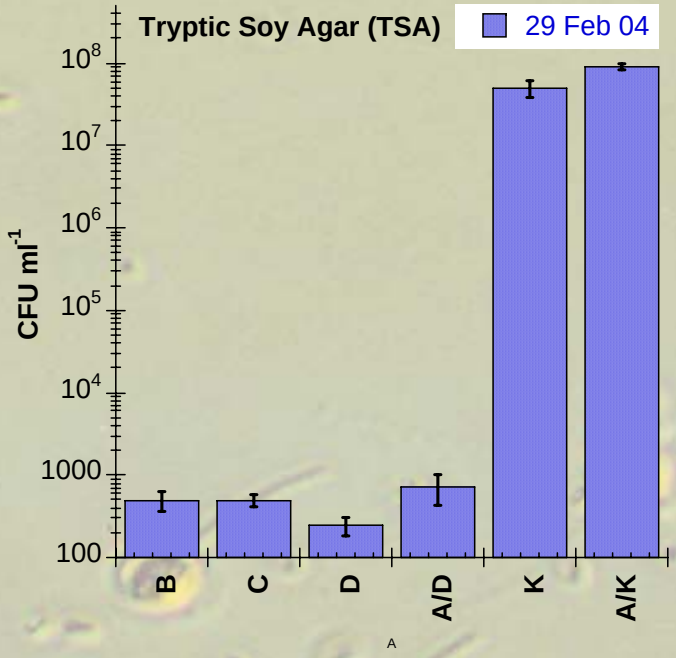

R2A CFU
29 Feb 04
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K

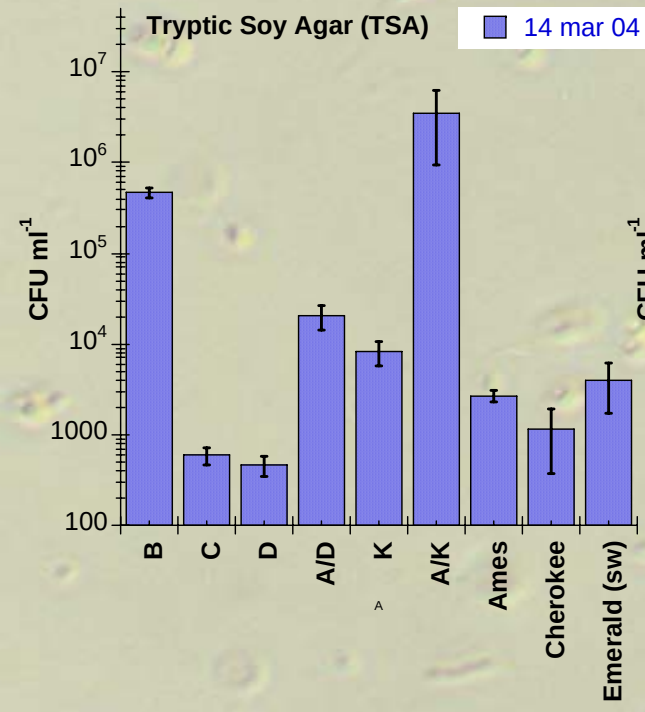

R2A CFU
14 Mar 04
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)

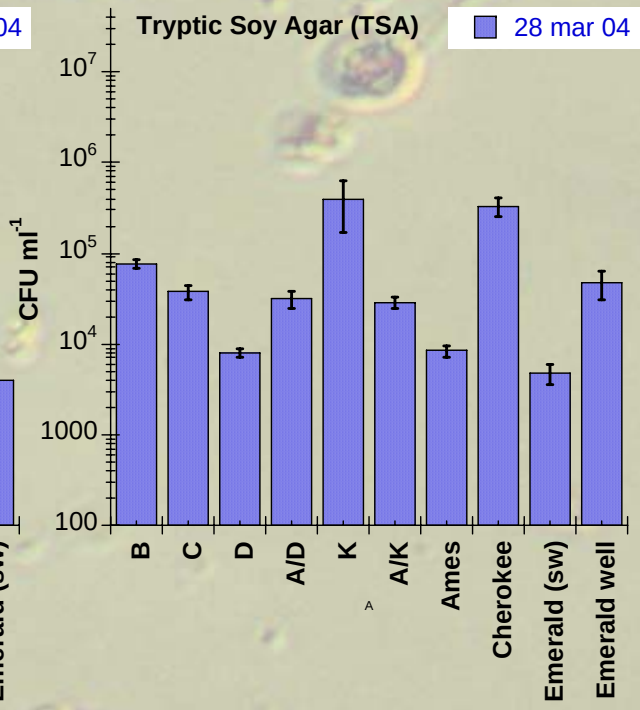

R2A CFU
28 Mar 04
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)
Emerald tube

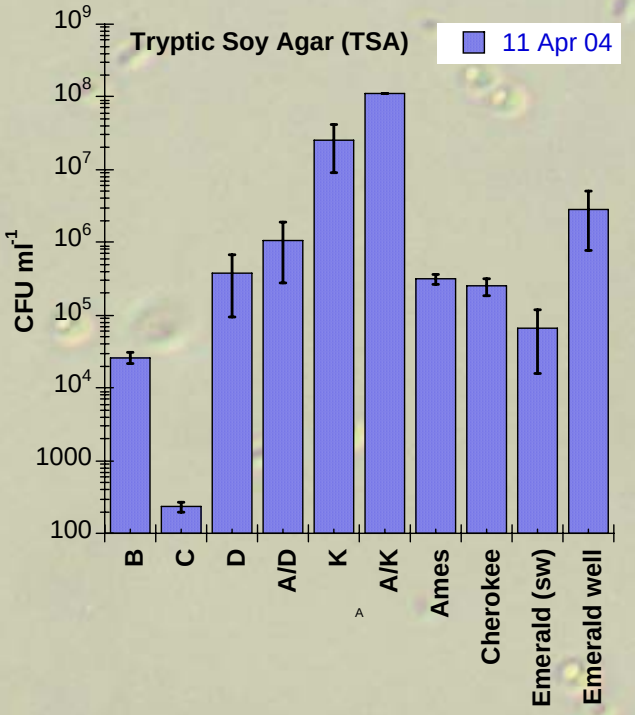

R2A CFU
11 Apr 04
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)
Emerald tube

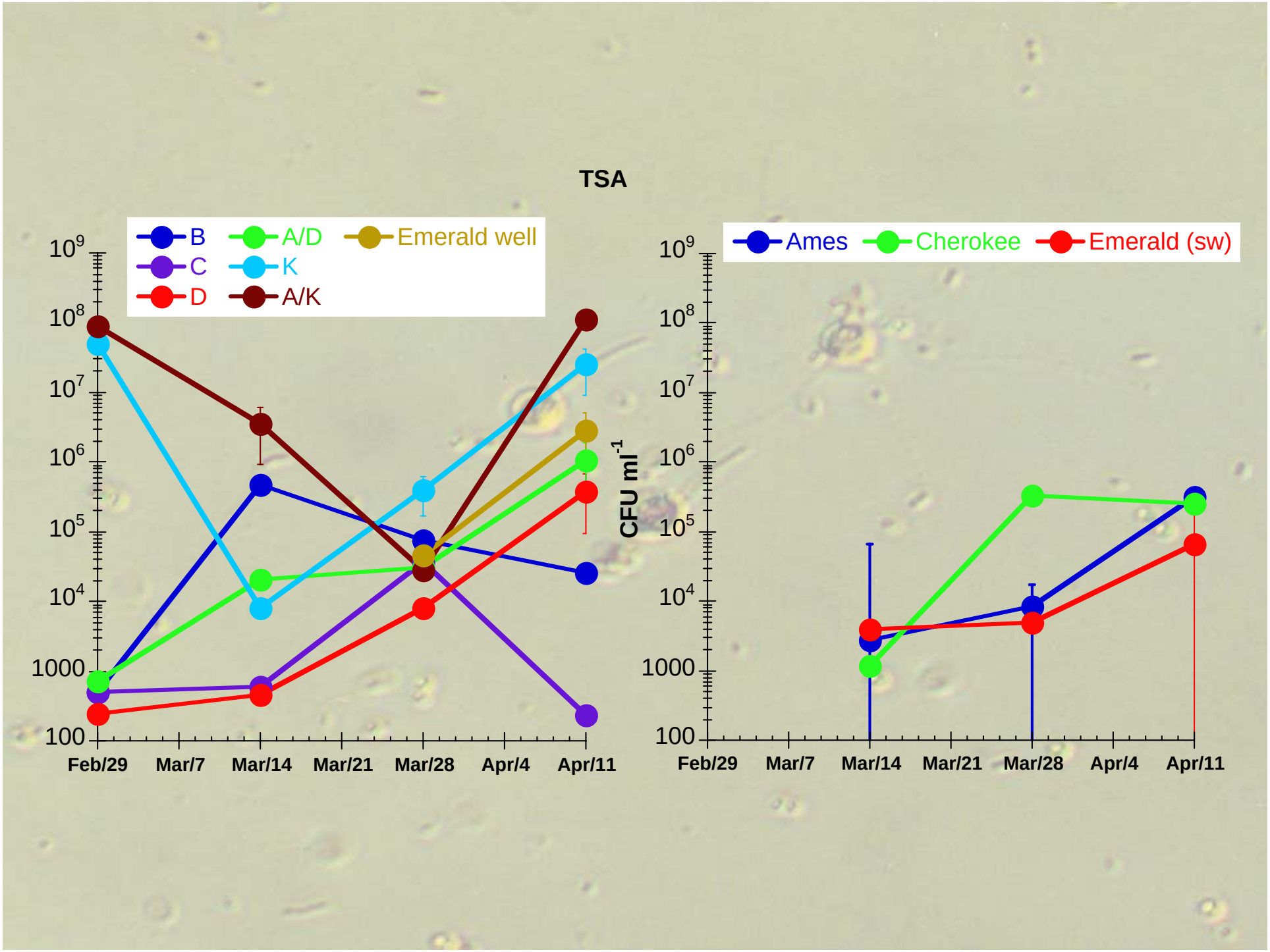

B
A/D
Emerald well
C
K
D
A/K
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
R2A
Feb/29
Mar/7
Mar/14
Mar/21
Mar/28
Apr/4
Apr/11
Ames
Cherokee
Emerald (sw)
R2A
CFU ml-1
Feb/29
Mar/7
Mar/14
Mar/21
Mar/28
Apr/4
Apr/11

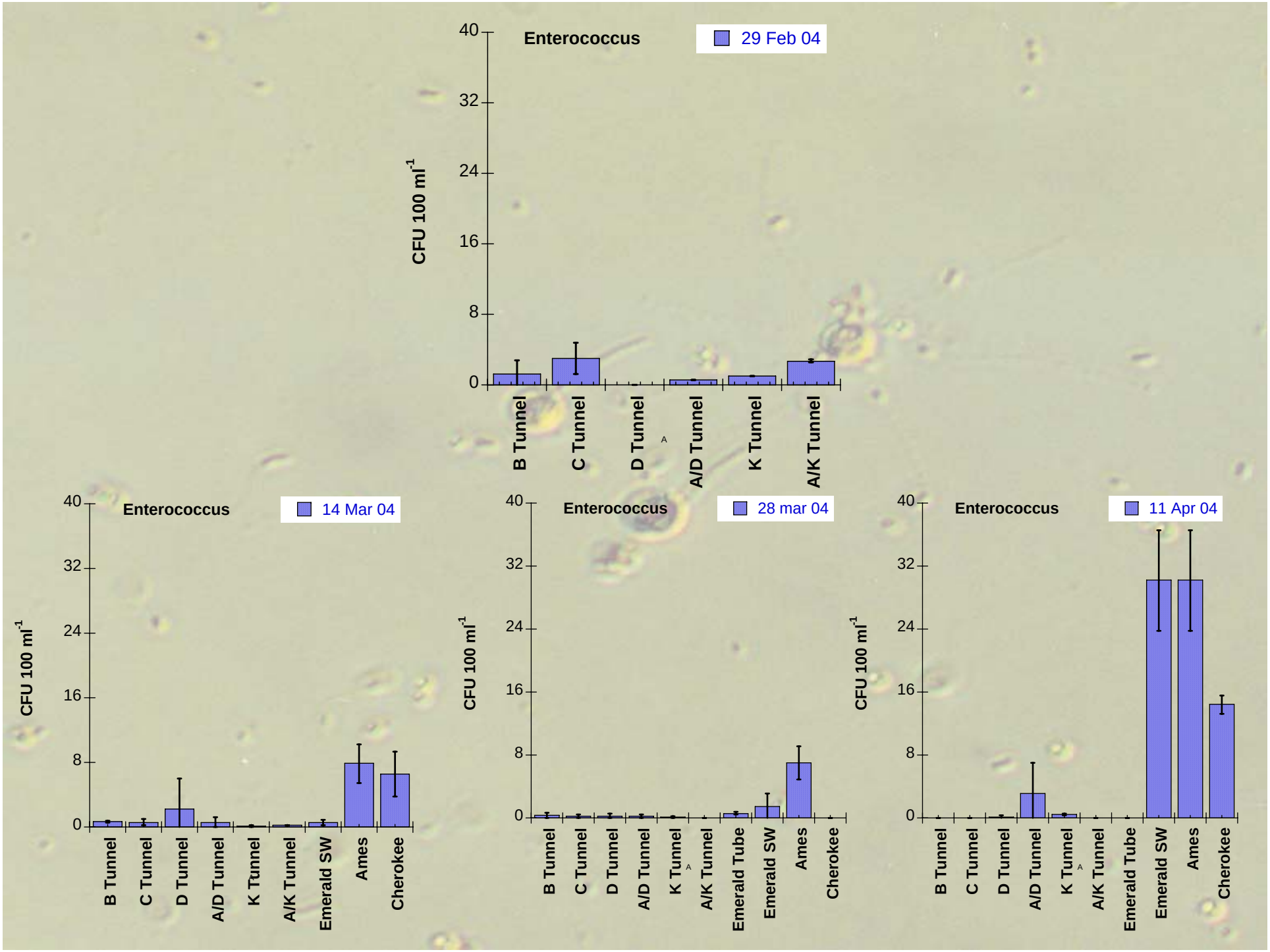

Tryptic Soy Agar (TSA)
29 Feb 04
CFU ml-1
10^8
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
A

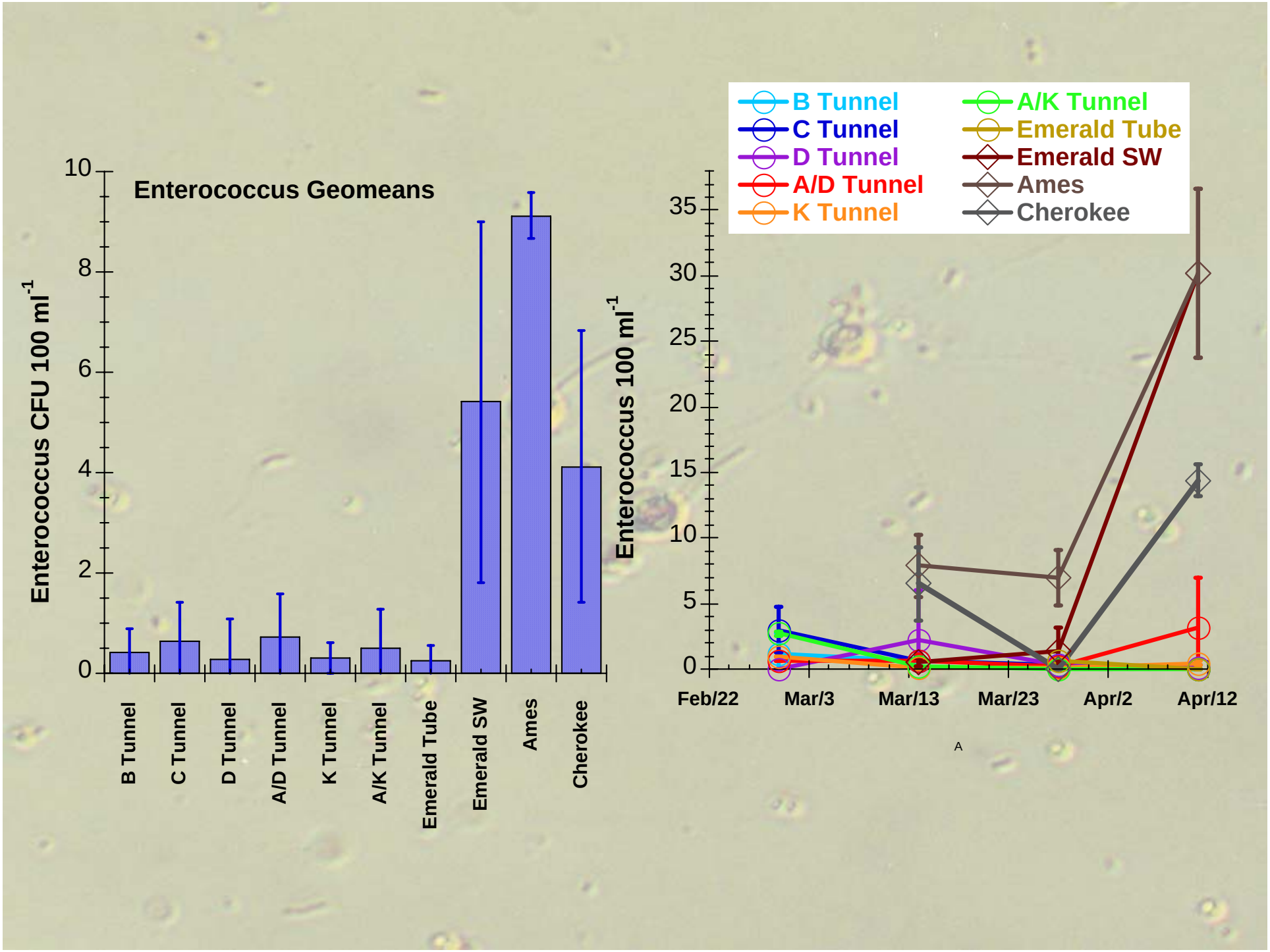

Tryptic Soy Agar (TSA)
14 mar 04
CFU ml-1
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)
A

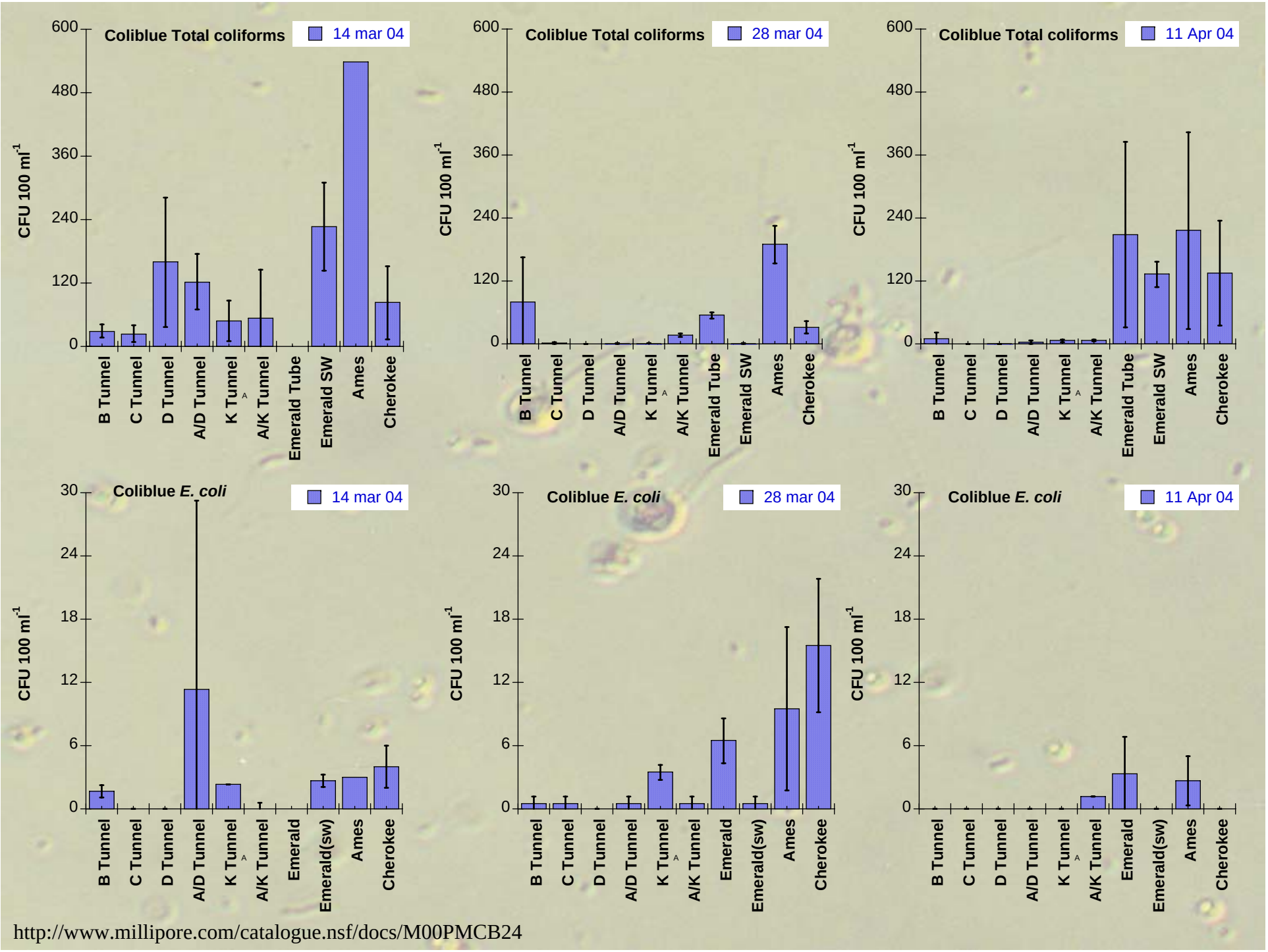

Tryptic Soy Agar (TSA)
28 mar 04
CFU ml-1
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)
Emerald well
A


Tryptic Soy Agar (TSA)
11 Apr 04
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
B
C
D
A/D
K
A/K
Ames
Cherokee
Emerald (sw)
Emerald well
A


TSA
B
A/D
Emerald well
C
K
D
A/K
Ames
Cherokee
Emerald (sw)
CFU ml-1
10^9
10^8
10^7
10^6
10^5
10^4
1000
100
Feb/29
Mar/7
Mar/14
Mar/21
Mar/28
Apr/4
Apr/11

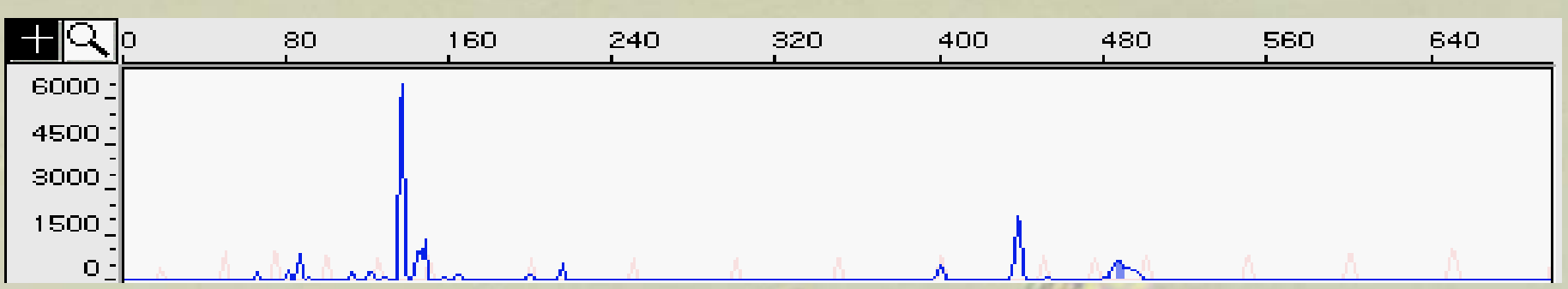

Enterococcus
29 Feb 04
CFU 100 ml-1
40
32
24
16
8
0
B Tunnel
C Tunnel
D Tunnel
A
A/D Tunnel
K Tunnel
A/K Tunnel
Enterococcus
14 Mar 04
CFU 100 ml-1
B Tunnel
C Tunnel
D Tunnel
A/D Tunnel
K Tunnel
A/K Tunnel
Emerald SW
Ames
Cherokee
Enterococcus
28 mar 04
CFU 100 ml-1
B Tunnel
C Tunnel
D Tunnel
A/D Tunnel
K Tunnel
A
A/K Tunnel
Emerald Tube
Emerald SW
Ames
Cherokee
Enterococcus
11 Apr 04
CFU 100 ml-1
B Tunnel
C Tunnel
D Tunnel
A/D Tunnel
K Tunnel
A
A/K Tunnel
Emerald Tube
Emerald SW
Ames
Cherokee


Enterococcus Geomeans
Enterococcus CFU 100 ml-1
10
8
6
4
2
0
B Tunnel
C Tunnel
D Tunnel
A/D Tunnel
K Tunnel
A/K Tunnel
Emerald Tube
Emerald SW
Ames
Cherokee
Enterococcus 100 ml-1
35
30
25
20
15
10
5
0
Feb/22
Mar/3
Mar/13
Mar/23
Apr/2
Apr/12
A

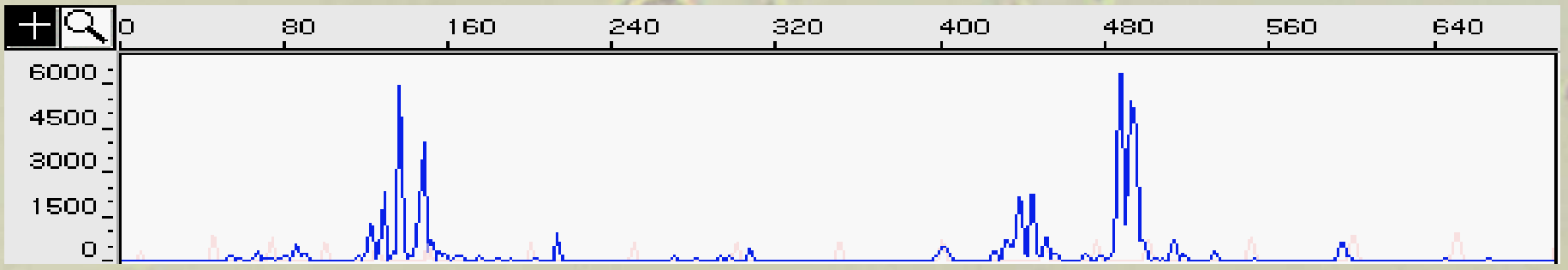


http://www.millipore.com/catalogue.nsf/docs/M00PMCB24

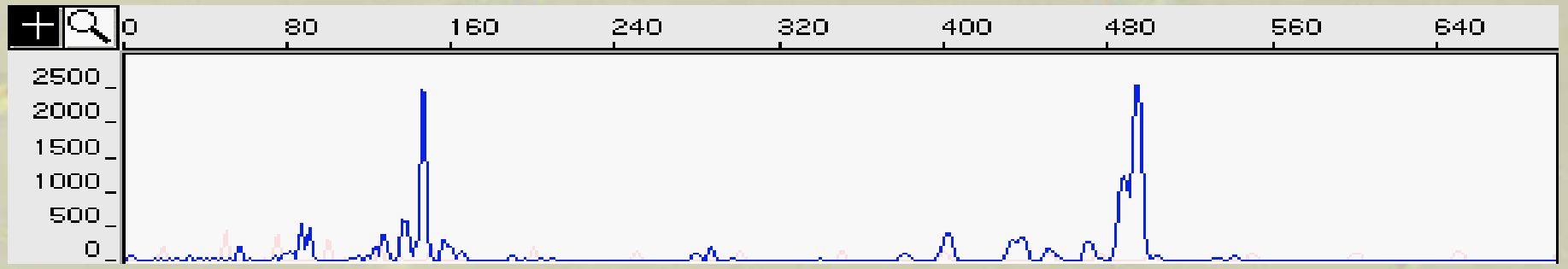
B-tunnel

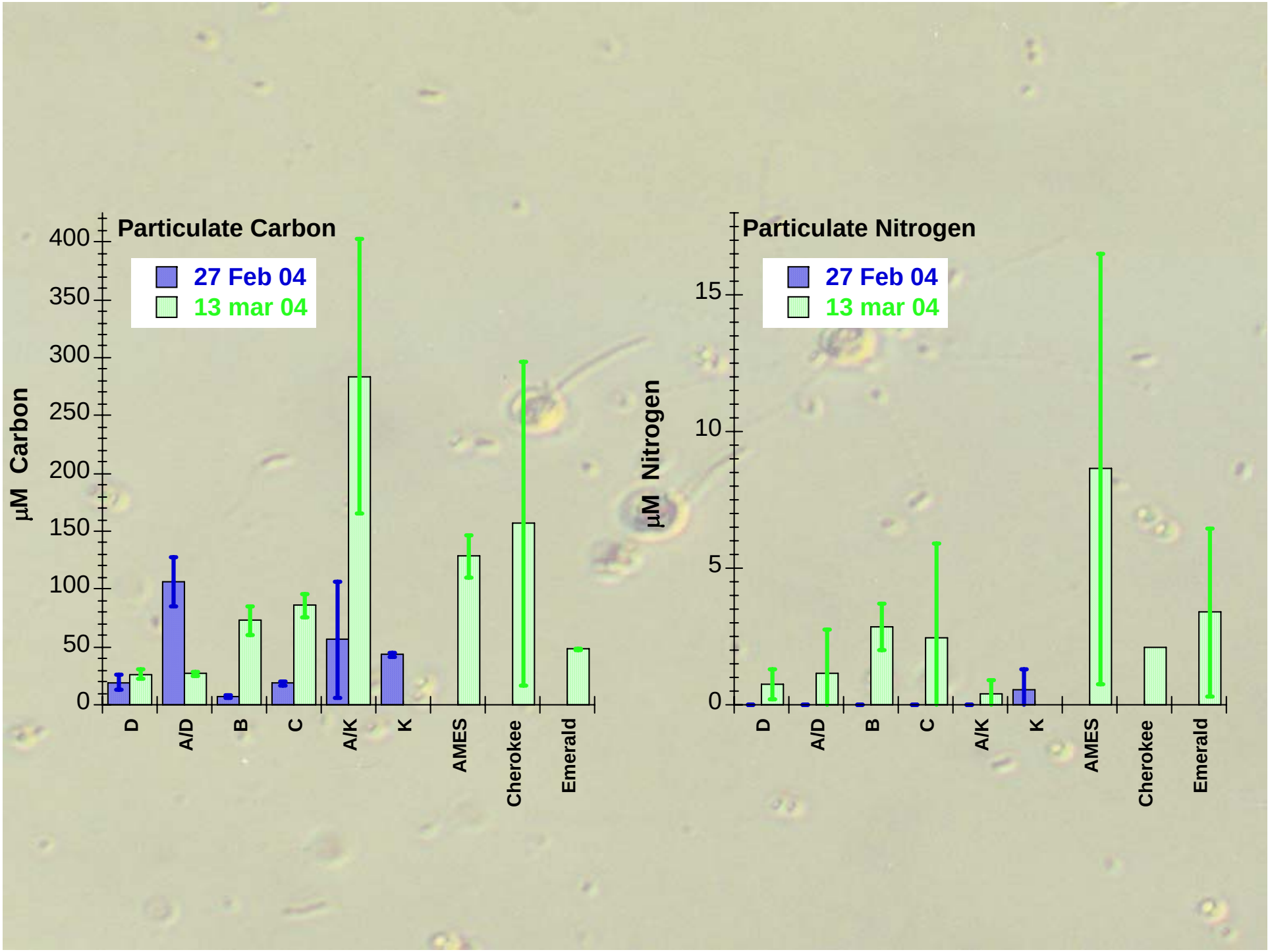


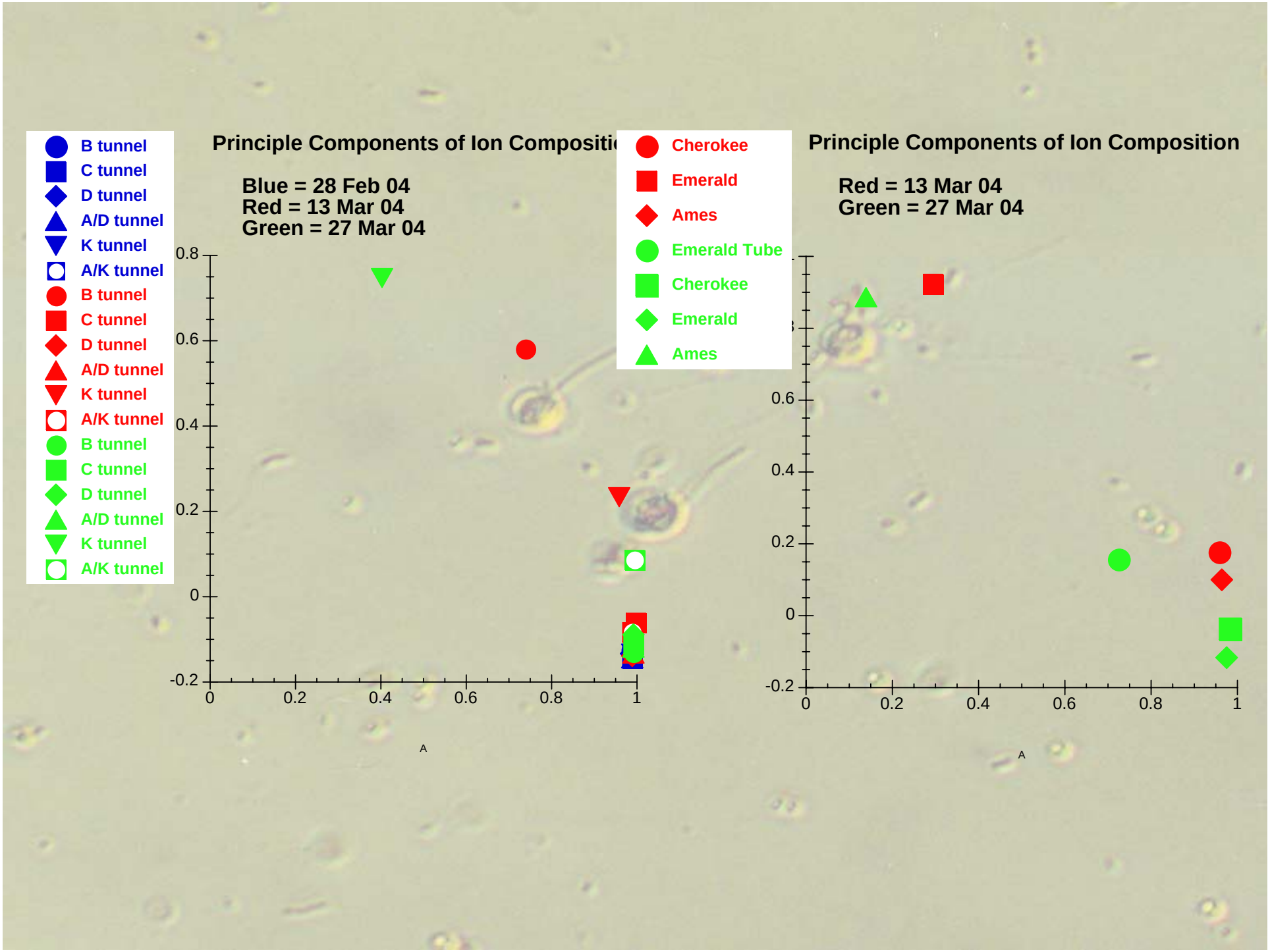
C-tunnel



AK-tunnel




Particulate Carbon
27 Feb 04
13 mar 04
µM Carbon
400
350
300
250
200
150
100
50
0
D
A/D
B
C
A/K
K
AMES
Cherokee
Emerald
Particulate Nitrogen
27 Feb 04
13 mar 04
µM Nitrogen
15
10
5
0
D
A/D
B
C
A/K
K
AMES
Cherokee
Emerald


B tunnel
C tunnel
D tunnel
A/D tunnel
K tunnel
A/K tunnel
B tunnel
C tunnel
D tunnel
A/D tunnel
K tunnel
A/K tunnel
B tunnel
C tunnel
D tunnel
A/D tunnel
K tunnel
A/K tunnel
Principle Components of Ion Compositi
Blue = 28 Feb 04
Red = 13 Mar 04
Green = 27 Mar 04
0.8
0.6
0.4
0.2
0
-0.2
0
0.2
0.4
0.6
0.8
1
A
Cherokee
Emerald
Ames
Emerald Tube
Cherokee
Emerald
Ames
Principle Components of Ion Composition
Red = 13 Mar 04
Green = 27 Mar 04
0.6
0.4
0.2
0
-0.2
0
0.2
0.4
0.6
0.8
1
A

Conclusions

- Conduits have different microbial floras
  - Undescribed species of Amoebae were found
  - Amoebae distribution associated with organic enrichment
  - R2A counts higher than TSA counts
  - TSA counts more reflective of seasonal change
- Organic enrichment increased with the onset of Spring
- Little to no contamination with fecal bacteria
- Potential for using bacterial community structure as water quality indicator

Acknowledgements

GUE Divers

Sandy Cook & crew

Wakulla Springs State Park

James McClean, FL DEP