



31st Annual Meeting

**New England Association of Environmental
Biologists**

**Grand Summit Resort Hotel
Mount Snow, Vermont
March 14, 2007**

Using BioSim2 to identify statistically- valid sectors of indicator taxa

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Outline

- Introduction
 - Index of Biotic Similarity – Brief Review
 - What is a Sector?
- VTDEC Ambient Biomonitoring Program
 - Small, Moderate Gradient (Wadable) Streams
 - Macroinvertebrate sampling
- Results
 - Macroinvertebrate % Composition Data
 - Environmentally Reasonable Sectors
 - Statistically Valid Sectors
- Conclusions

Index of Biotic Similarity

(Pinkham-Pearson Index)

$$B = \frac{1}{k} \sum_{i=1}^k \frac{\text{Min} (X_{ia}, X_{ib})}{\text{Max} (X_{ia}, X_{ib})}$$

Barbour *et al.* (1992) in a systematic comparison of the metrics proposed in EPA's rapid bioassessment protocol (Pfalkin *et al.*, 1989), concluded that B "may be the most appropriate metric to serve as a measure of community similarity."

Index of Biotic Similarity (Pinkham-Pearson Index)

$$B = \frac{1}{k} \sum_{i=1}^k \frac{\text{Min} (X_{ia}, X_{ib})}{\text{Max} (X_{ia}, X_{ib})}$$

	Taxon 1	Taxon 2	Taxon 3	Taxon 4	Taxon 5	Taxon 6	
Site 1	10	0	0	5	30	2	
Site 2	10	0	0	5	30	2	
Site 3	0	10	20	0	0	0	
Site 4	1	50	2	3	15	12	
Site1w	$\frac{10}{10}$	$+$ $\frac{0}{0}$	$+$ $\frac{0}{0}$	$+$ $\frac{5}{5}$	$+$ $\frac{30}{30}$	$+$ $\frac{2}{2}$	$= 1$
Site 2	6						
Site 2	1	$+$ 1	$+$ 1	$+$ 1	$+$ 1	$+$ 1	$= 1$
	6						
Site1w	$\frac{0}{10}$	$+$ $\frac{0}{10}$	$+$ $\frac{0}{0}$	$+$ $\frac{0}{5}$	$+$ $\frac{0}{30}$	$+$ $\frac{0}{2}$	$= 0$
Site 3	6						
Site 3	0	$+$ 0	$+$ 0	$+$ 0	$+$ 0	$+$ 0	$= 0$
	6						
Site1w	$\frac{1}{10}$	$+$ $\frac{0}{50}$	$+$ $\frac{0}{2}$	$+$ $\frac{3}{5}$	$+$ $\frac{15}{30}$	$+$ $\frac{2}{12}$	$= 0.23$
Site 4	6						
Site 4	0.1	$+$ 0	$+$ 0	$+$ 0.6	$+$ 0.5	$+$ 0.17	$= 0.23$
	6						

Matrix of B's Between 11 Habitat Parameters

	SiltRating	PcBoulder%	PcCobble%	PcCoarseGravel%	PcGravel%	PcSilt%	PcClay%	Embeddedness	Canopy%	Width	FilamentousGreen%
SiltRating	1	0.4531	0.5797	0.5264	0.4447	0.1188	0.1122	0.568	0.5953	0.3926	0.2854
PcBoulder%	0.4531	1	0.5296	0.4035	0.4725	0.0701	0.1184	0.444	0.3985	0.4775	0.3455
PcCobble%	0.5797	0.5296	1	0.6305	0.4283	0.0661	0.0198	0.7029	0.5629	0.4591	0.3132
PcCoarseGravel%	0.5264	0.4035	0.6305	1	0.4844	0.0868	0.0521	0.6775	0.6	0.5009	0.2734
PcGravel%	0.4447	0.4725	0.4283	0.4844	1	0.1437	0.0397	0.5284	0.4558	0.5761	0.3737
PcSilt%	0.1188	0.0701	0.0661	0.0868	0.1437	1	0.7736	0.1345	0.0905	0.0832	0.3793
PcClay%	0.1122	0.1184	0.0198	0.0521	0.0397	0.7736	1	0.0305	0.0483	0.0411	0.3153
Embeddedness	0.568	0.444	0.7029	0.6775	0.5284	0.1345	0.0305	1	0.653	0.459	0.2853
Canopy%	0.5953	0.3985	0.5629	0.6	0.4558	0.0905	0.0483	0.653	1	0.4149	0.2304
Width	0.3926	0.4775	0.4591	0.5009	0.5761	0.0832	0.0411	0.459	0.4149	1	0.3314
FilamentousGreen%	0.2854	0.3455	0.3132	0.2734	0.3737	0.3793	0.3153	0.2853	0.2304	0.3314	1

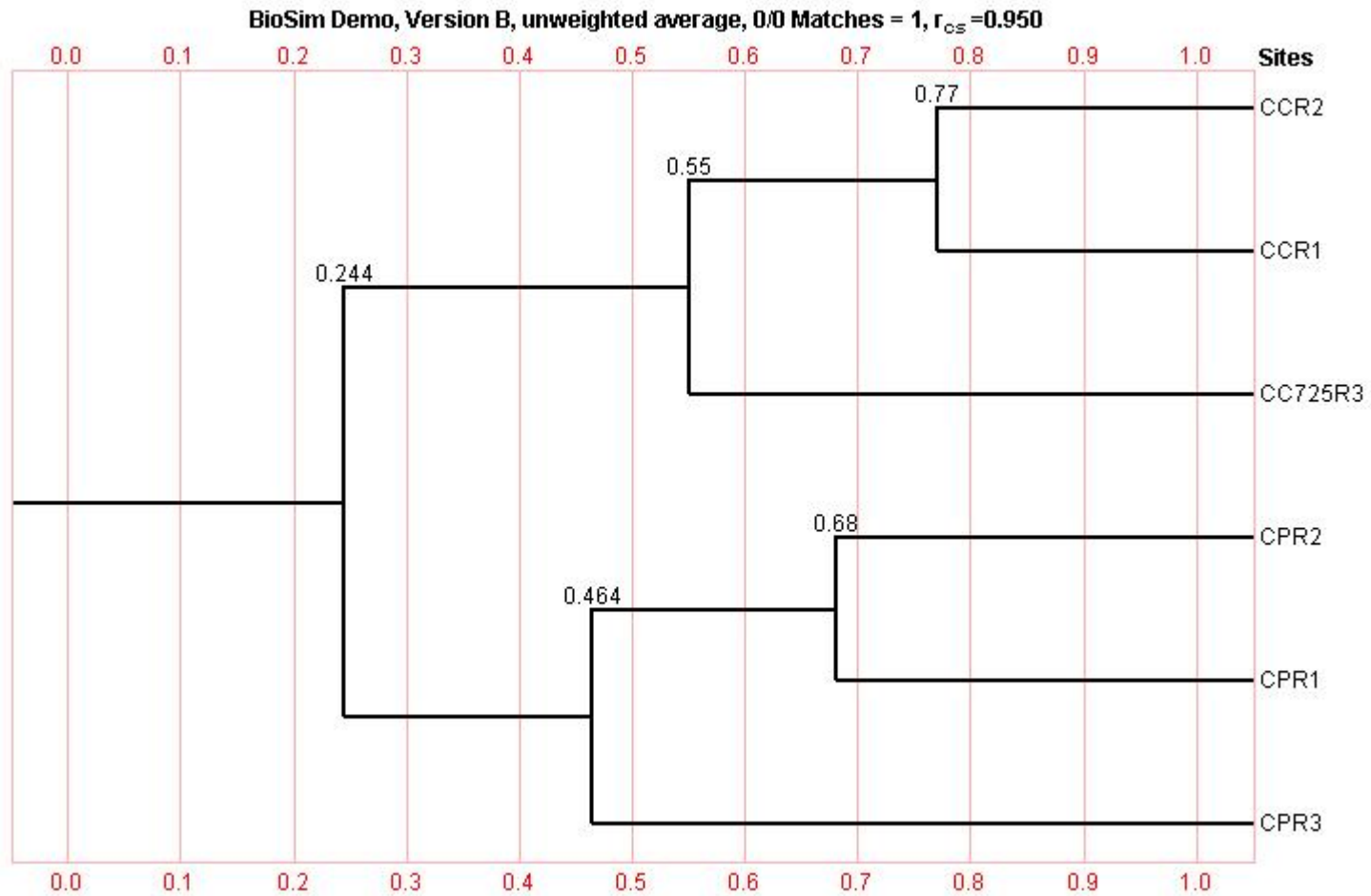
What is a Sector?

original data matrix, rows coded

sites/taxa	A	B	C	D	E	F	G
CPR1	3.8	0.2	0.9	15.7	0.7	6.9	0
CPR2	3.8	0.7	0.7	12.5	6.4	8.7	0
CPR3	8.4	0.5	2	22	3.8	9.6	1.5
CCR1	5.7	3.2	0	0	1.8	0	0
CCR2	7.7	2.1	0	1.1	1.8	0	0
CCR3	9.5	4.7	0	0.5	5	0	0.2

What is a Sector?

Row Dendrogram



What is a Sector?

original data matrix

sites/taxa	A	B	C	D	E	F	G
CPR1	3.8	0.2	0.9	15.7	0.7	6.9	0
CPR2	3.8	0.7	0.7	12.5	6.4	8.7	0
CPR3	8.4	0.5	2	22	3.8	9.6	1.5
CCR1	5.7	3.2	0	0	1.8	0	0
CCR2	7.7	2.1	0	1.1	1.8	0	0
CCR3	9.5	4.7	0	0.5	5	0	0.2

Original data matrix rearranged in order of the row dendrogram

sites/taxa	A	B	C	D	E	F	G
CCR2	7.7	2.1	0	1.1	1.8	0	0
CCR1	5.7	3.2	0	0	1.8	0	0
CCR3	9.5	4.7	0	0.5	5	0	0.2
CPR2	3.8	0.7	0.7	12.5	6.4	8.7	0
CPR1	3.8	0.2	0.9	15.7	0.7	6.9	0
CPR3	8.4	0.5	2	22	3.8	9.6	1.5

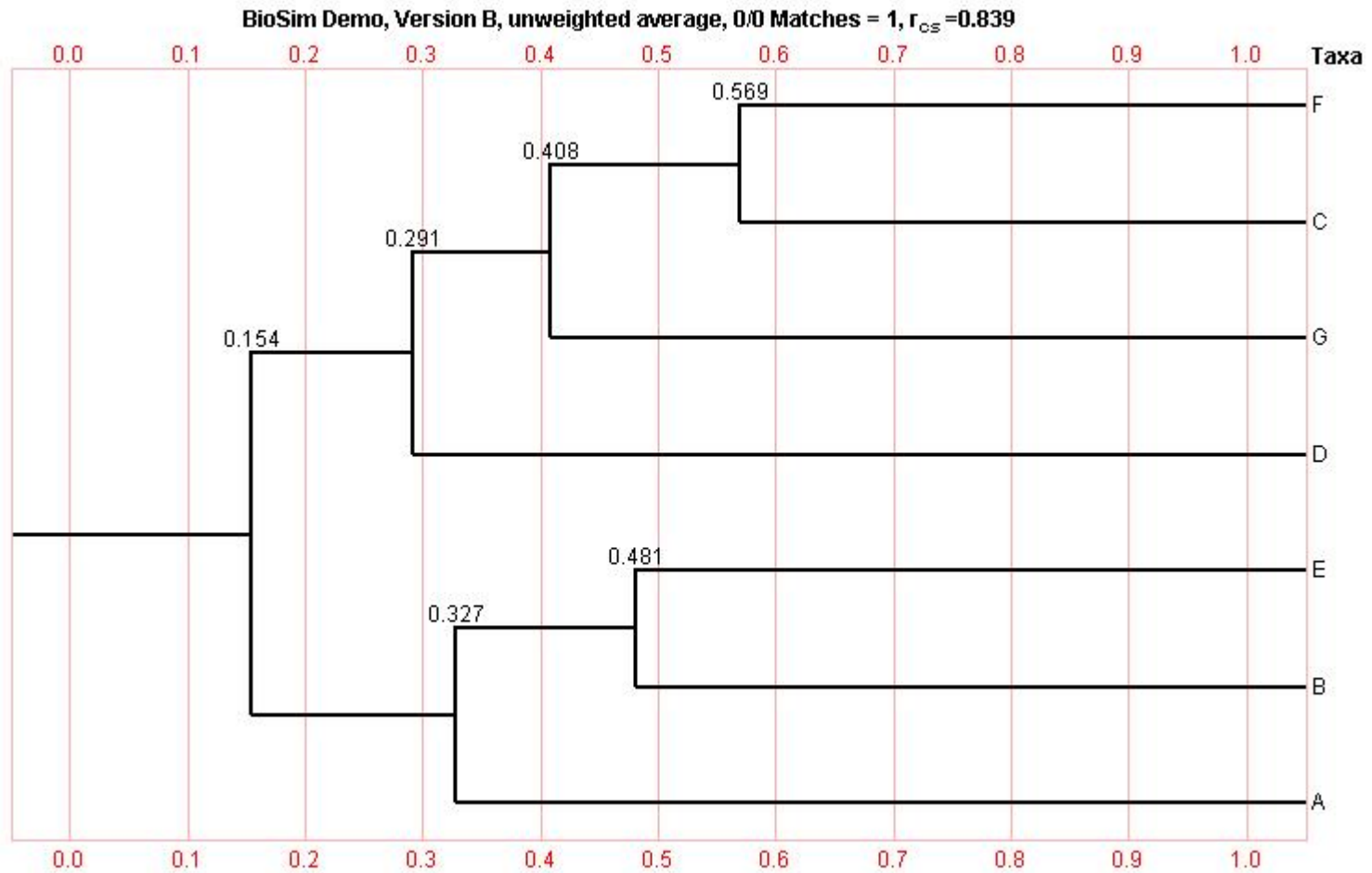
What is a Sector?

Original data matrix rearranged
in order of the **row dendrogram**,
taxa coded

sites/taxa	A	B	C	D	E	F	G
CCR2	7.7	2.1	0	1.1	1.8	0	0
CCR1	5.7	3.2	0	0	1.8	0	0
CCR3	9.5	4.7	0	0.5	5	0	0.2
CPR2	3.8	0.7	0.7	12.5	6.4	8.7	0
CPR1	3.8	0.2	0.9	15.7	0.7	6.9	0
CPR3	8.4	0.5	2	22	3.8	9.6	1.5

What is a Sector?

Taxa Dendrogram



What is a Sector?

Original data matrix rearranged
in order of the **row dendrogram**,
taxa coded

sites/taxa	A	B	C	D	E	F	G
CCR2	7.7	2.1	0	1.1	1.8	0	0
CCR1	5.7	3.2	0	0	1.8	0	0
CCR3	9.5	4.7	0	0.5	5	0	0.2
CPR2	3.8	0.7	0.7	12.5	6.4	8.7	0
CPR1	3.8	0.2	0.9	15.7	0.7	6.9	0
CPR3	8.4	0.5	2	22	3.8	9.6	1.5

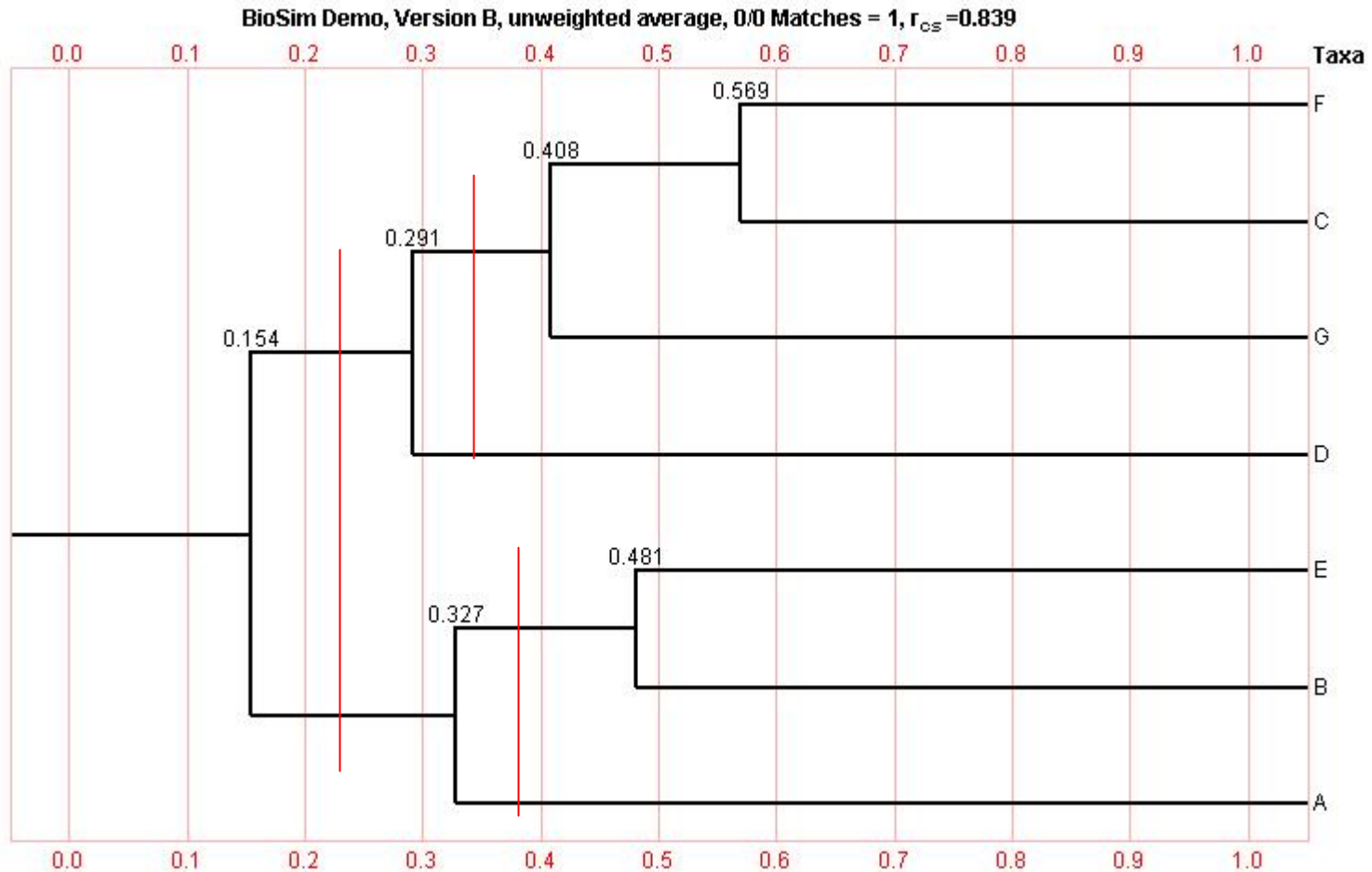
Rearranging original data matrix in **double-dendrogram order**

	F	C	G	D	E	B	A
CCR2	0	0	0	1.1	1.8	2.1	7.7
CCR1	0	0	0	0	1.8	3.2	5.7
CCR3	0	0	0.2	0.5	5	4.7	9.5
CPR2	8.7	0.7	0	12.5	6.4	0.7	3.8
CPR1	6.9	0.9	0	15.7	0.7	0.2	3.8
CPR3	9.6	2	1.5	22	3.8	0.5	8.4

What is a Sector?

Taxa Dendrogram

Identifying environmentally reasonable sectors



What is a Sector?

Establishing environmentally reasonable sectors

	F	C	G	D	E	B	A	
CCR2	0	0	0	1.1	1.8	2.1	7.7	
CCR1	0	0	0	0	1.8	3.2	5.7	
CCR3	0	0	0.2	0.5	5	4.7	9.5	0.55
CPR2	8.7	0.7	0	12.5	6.4	0.7	3.8	0.244
CPR1	6.9	0.9	0	15.7	0.7	0.2	3.8	
CPR3	9.6	2	1.5	22	3.8	0.5	8.4	0.464

0.291 0.154 0.327

VTDEC Ambient Biomonitoring Program

- The Vermont Department of Environmental Conservation (VTDEC) is charged with assessing the biological integrity of wadable stream sites throughout Vermont.
- Specific data are being collected to better refine the biological expectation of **very small** (<1-15 km²), **low elevation** (118-550 ft), **eurhythmic**, **wadable** streams of **moderate gradient** in Vermont.
- These data will be used to evaluate the response of these streams to current agricultural and storm-water management practices, within their watersheds.

Small, Moderate Gradient (Wadable) Streams

- From this Program 27 streams were selected, 26 in the Champlain Valley, One in the Connecticut Valley
- One stream was sampled in 2 successive years to provide a reference for the technique.
- One stream was sampled at two points
- Thus data from 29 streams were used in this study.



Deer Creek



Engelsby Brook



Moorehouse Brook



Reference

Macroinvertebrate Sampling



- Collected in late summer-early Fall index period of Sept to Mid Oct
- Collected from representative locations in a riffle in the stream.
- Substrate in an area about 1 square meter upstream of a 500 micron mesh, D net is thoroughly disturbed by hand.
- Four composite samples collected each sample lasting about 30 seconds
- Two replicates done per stream; preserved in 75% alcohol.

Macroinvertebrate Processing



- 1) Sample washed and spread evenly over a white, gridded tray with 24 squares
- 2) Starting with random grid, it and the next 5 consecutive squares are picked clean of macroinvertebrates using a 3 diopter magnifying light.
- 3) Process is continued if necessary until 300 organisms are picked.
- 4) Total Number of squares picked is recorded.
- 5) Picked macroinvertebrates are preserved in 75% alcohol
- 6) Macroinvertebrates are identified to species or genus, except Oligochaetes (Family)

Macroinvertebrate Results

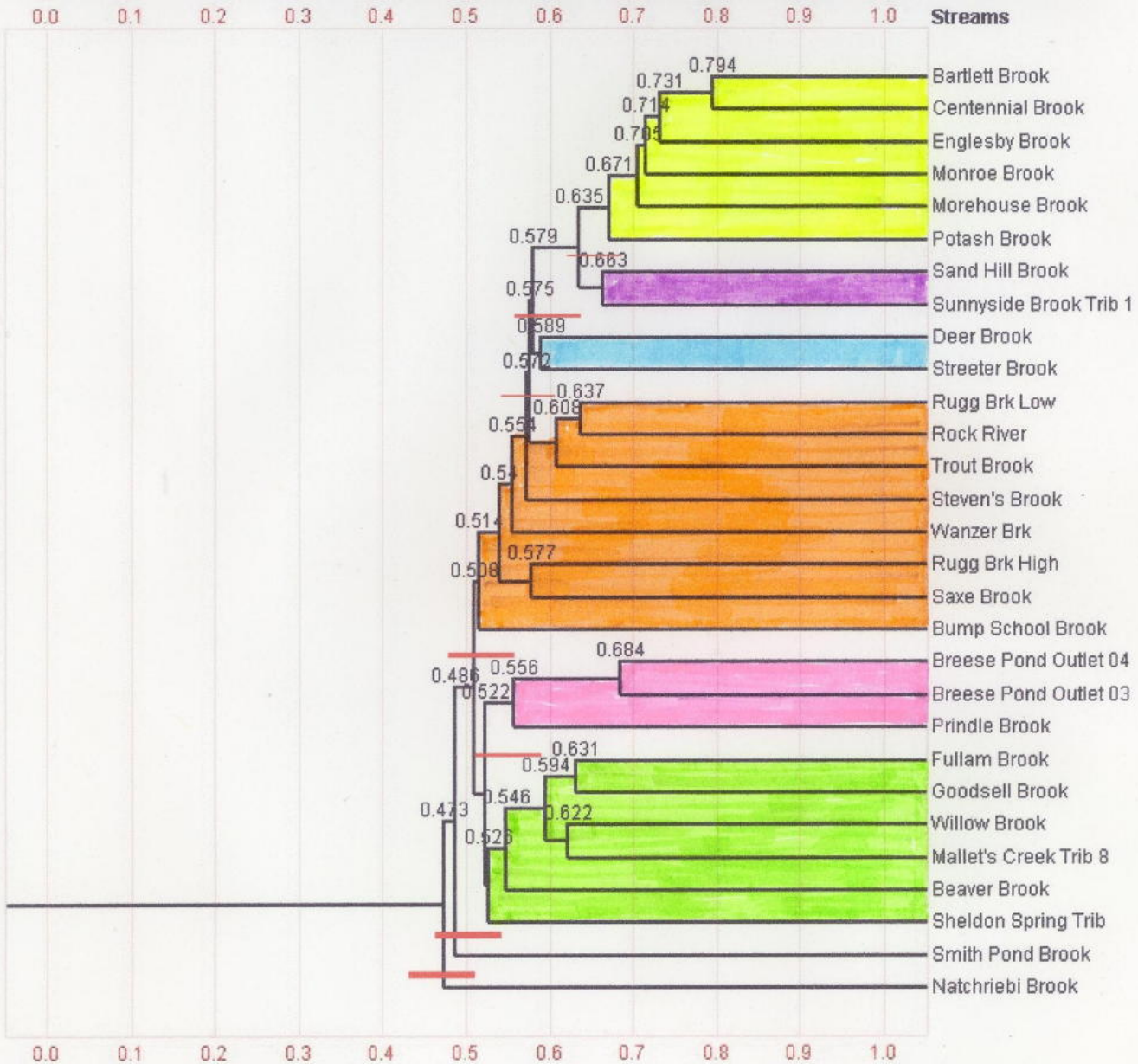
- 170 taxa collected at 29 “Sites”
- Compressed to 91 taxa at 29 Sites by eliminating
 - taxa which appeared in only one site with a % composition < 1.25% (38)
 - taxa which appeared in only two sites with a maximum % comp < 1.2% (23)
 - taxa which appeared in only three sites with a maximum % comp < 1.1% (11)
 - taxa which appeared in only four sites with a maximum % comp < 1% (7)

RESULTS

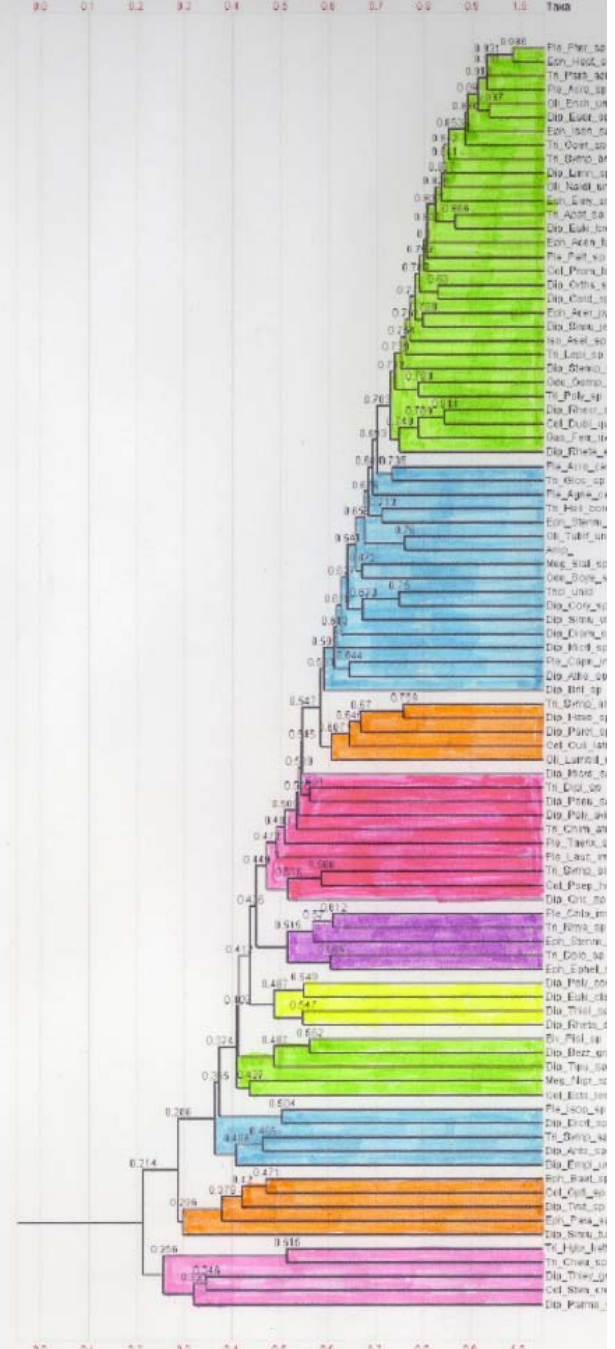
MACROINVERTEBRATE DATA

- Why % Composition
- Stream Dendrogram
- Taxa Dendrogram
- Reordered Data Matrix – 0's Included
- Reordered Data Matrix – 0's Removed
- Reordered Data Matrix - Coded
- Synthesis

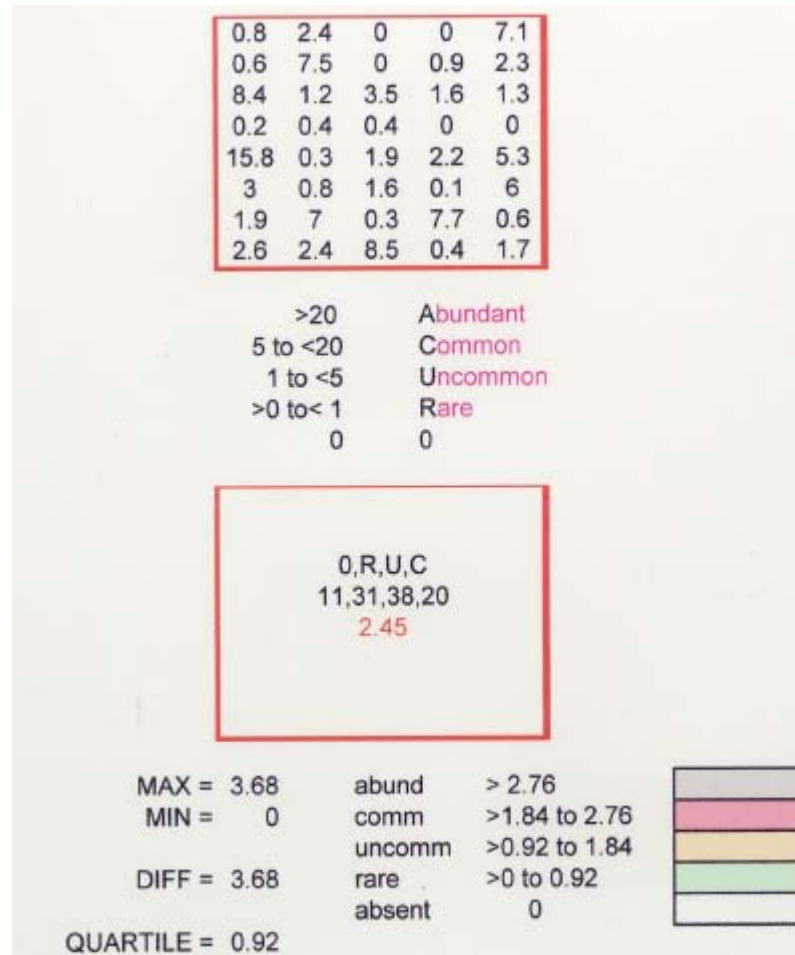
Small, Moderate Gradient, VT Streams-%Comp-Compr91, Version B, unweighted average, 0/0 Matches = 1, $r_{CS}=0.773$



Small, Moderate Gradient, VT Stevans-5 Comp Comp 91, Western IL, unweighted average, 90 Matches = 1, 1, 00-0915



Coding The Reordered Data Matrix



Stream Name	PLA_Pier_sp	Eph_Hepr_sp	Tri_Para_apic	PLA_Acro_sp	Oli_Ench_untd
Barrett Brook	SRU 02.1			SRUC 03.3	
Centennial Brook					
Englesby Brook					
Monroe Brook					
Morehouse Brook					
Potash Brook					
Sand Hill Brook					
Sunnyside Brook Trib 1					
Deer Brook					
Streeter Brook					
Rugg Brk Low					
Rock River					
Trout Brook					
Steven's Brook					
Wanzer Brk					
Rugg Brk High					
Saxe Brook					
Bump School Brook					
Breese Pond Outlet 04					
Breese Pond Outlet 03					
Prindle Brook					
Fuller Brook					
Goodsell Brook					
Willow Brook					
Mallet's Creek Trib 8					
Beaver Brook					
Sheldon Spring Trib					
Smith Pond Brook					
Natchiebi Brook					

Stream Name	PLA_Pier_sp	Eph_Hepr_sp	Tri_Para_apic	PLA_Acro_sp	Oli_Ench_untd
no zeros is below this one					
Barrett Brook					
Centennial Brook					
Englesby Brook					
Monroe Brook					
Morehouse Brook					
Potash Brook					
Sand Hill Brook					
Sunnyside Brook Trib 1					
Deer Brook					
Streeter Brook					
Rugg Brk Low					
Rock River					
Trout Brook					
Steven's Brook					
Wanzer Brk					
Rugg Brk High					
Saxe Brook					
Bump School Brook					
Breese Pond Outlet 04					
Breese Pond Outlet 03					
Prindle Brook					
Fuller Brook					
Goodsell Brook					
Willow Brook					
Mallet's Creek Trib 8					
Beaver Brook					
Sheldon Spring Trib					
Smith Pond Brook					
Natchiebi Brook					

Monroe Brook | Stream in Urban Setting

Sand Hill Brook | Stream in Suburban Setting

Trout Brook | Stream in Agricultural Setting

Breese Pond Outlet 03 | Reference Stream

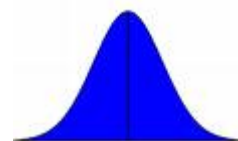
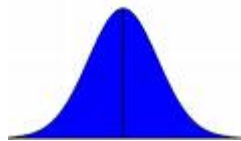
Goodsell Brook | Presumed Reference Stream

Statistical Analysis

- Assumptions
 - The measurements in each creek are independent
 - The relative abundance of taxa follow a normal distribution

Group 1	taxon 1	taxon 2	→	taxon k
Creek 1				
Creek 2				
↓				
Creek n				

↓ Independent



Statistical Analysis

- Calculations

Group 1	taxon 1	taxon 2	→	taxon k
Creek 1				
Creek 2				
↓				
Creek n				
	$\bar{x}_{1,1}$	$\bar{x}_{1,2}$		$\bar{x}_{1,k}$
	$s_{1,1}$	$s_{1,2}$		$s_{1,k}$

Group 2	taxon 1	taxon 2	→	taxon k
Creek 1				
Creek 2				
↓				
Creek m				
	$\bar{x}_{2,1}$	$\bar{x}_{2,2}$		$\bar{x}_{2,k}$
	$s_{2,1}$	$s_{2,2}$		$s_{2,k}$

→
$$d_i = \frac{\bar{x}_{1,i} - \bar{x}_{2,i}}{\sqrt{\frac{s_{1,i}^2}{n} + \frac{s_{2,i}^2}{m}}}$$

Statistical Analysis

- Calculations

H_0 : There is not a significant difference between the percent composition of taxa in Group 1 and Group 2 creeks.

H_a : There is a significant difference between the percent composition of taxa in Group 1 and Group 2 creeks.

Given H_0 is true then

$$d_i : N(0,1)$$

$$\text{so } \sum_{i=1}^k d_i^2 : \chi^2(k).$$

The p -value is calculated using the chi-square distribution.

no zeros is below this one		Dip_Hexe_sp	Dip_Parcl_sp	Col_Oull_lati	Oli_Lumbd_unid	Dip_Micrs_sp	Tri_Dip_sp	Dip_Pseu_sp	Dip_Poly_avic	Tri_Chim_ater	Ple_Taenx_sp	Ple_Leuc_imim	Tri_Symp_sios	Col_Psep_herr	Dip_Cric_sp	Ple_Chlo_imim	Tri_Rhya_sp	Eph_Stenm_lute	Tri_Dolo_sp	Eph_EpheI_sp	Dip_Poly_conv	Dip_Euki_clar	Dip_Thiel_sp	Dip_Rheta_dist	Eph_Baet_sp	Col_Optl_sp	Dip_Tvet_sp	Eph_Para_sp	Dip_Sim_u_tube	Tri_Hybr_bett	Tri_Cheu_sp	Dip_Thiel_grp	Col_Sten_cren	Dip_Parme_sp	
Urban	Bartlett Brook	0	0	0	0	0	2.2	0.7	0	1.1	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.8	29.2	2.5	14	2.9
	Centennial Brook	0	0	0	0	0	0	0.1	0	0	0	0	0	0	3.6	0	0	0	0	0	1.2	0.4	0	0	0	0	0	0	0	0	39.3	31.6	2	1.4	1.5
	Englesby Brook	0	0	0	0	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.7	0.5	0.4	0	0	0.4	0	0	0	1.1	1.9	44.3	0.7	0.7
	Monroe Brook	0	0	0	0	0	0	0	0	0	0.2	3.4	0	0	0	0	0	0	0	0	0.9	3	4	0	2.1	0.2	0.2	0	34.9	18.9	25.7	0	6	0.6	
	Morehouse Brook	0	0	0	0	0.3	9.2	0	0	0	0	14.9	0	0	0	0	0	0	0	0	0	35.5	0	0	0	0	0	0	12.5	3.5	0	1	0	13.9	
	Potash Brook	0	0	0	0	0	0	0	0.1	0	0	0	12.6	0	15.9	0	0	0	0	0	0	0	4	0	0.1	0	0	0	0.9	23.9	0	9.1	0	0	
Agricultural	Rugg Brk Low	0	0	0	0	0	0	0	0	1.8	0.1	2.3	4.2	1.5	44.3	0	0	0	0	0	2	0.2	0	0.1	0.8	0.4	0	0	7.1	10.2	9	0	2.9	0	
	Rock River	0	9	0	0	0	0	0	0	0.3	1.5	0	0	1	14.2	0	0	1.4	0	0	4	0.4	4	0.4	0.6	0.5	0	0.9	2.3	15.7	22	1	4.5	0.1	
	Trout Brook	0	0	0	0	0	0	0	0	21.4	0	0.5	1	0	0	0	1.3	0	0	1.3	0.3	0.3	0.3	0	8.4	1.2	3.5	1.3	12.7	16.1	1	0	0.1	0.8	
	Steven's Brook	0	0	0	0	2	0	0	0	25.9	0	1.6	6.9	0	0	0.1	0	0	0	0	0	0	0	0	0.2	0.4	0.4	0	8.9	4.8	14.7	12.2	1.2	0	
	Wanzer Brk	1.3	0	0	0	0	0	0	3.5	0	0	11	10.7	10.7	0.9	0	1	0.3	0.3	0	0.9	1.6	0.3	15.8	0.3	1.9	2.2	5.3	0	3.5	0	4.1	2.2		
	Rugg Brk High	0	0	0	0	0.8	0	0	0.5	0	0	2.8	0.9	0	0.3	0	0	0	0	0	5	8.6	1.2	0.3	3	0.8	1.6	0.1	6	16.5	21.2	5	2	1.5	
	Saxe Brook	0	0	0	0	0.2	0	0.2	0	7.3	2	0.1	0	0	0.5	0	0	0	0	0	0	0.2	0	0.3	1.9	1	0.3	7.7	0.6	25	11.4	4	4.7	0.9	
	Bump School Brook	0	0	0	0	0.9	4.1	2.6	0	0	0	0	0	0	0	5.2	1	0	4.6	0.4	0	0.3	4	8.7	2.6	4	0	0.4	1.7	3	0	1	0.2	6.1	
Reference	Breese Pond Outlet 04	0.1	0.1	0.2	0	0	6	0.5	0	0	0	0.3	0	0	0	0.9	5	0.4	6.3	24.9	0	0.1	1.3	0.3	1.4	0.6	5.3	0.2	1.9	15.3	2.5	0	0	0	
	Breese Pond Outlet 03	0.1	0.1	0	0	0.1	3.3	0.3	0.3	0	0	0	0	0	0	1.7	2.3	2.3	9.9	28.7	0	0.1	0	0	0	2	2.5	26	0.6	2	2.3	0	0	0.4	
	Prindle Brook	0	1.1	0.2	0	2.1	5.4	0.8	0.6	10.1	0	0	0	0	0	0	0.7	0	2.2	3.2	0	0	0	0	3.6	2.2	5.7	2.9	1.4	9.1	4.3	0	1.5	0.2	
	Fullam Brook	1.5	0.8	0.5	0	0	24.3	0	2.8	0	0	9.7	0	0	0	2	1.2	0	11	0	0	0	0	0	0.8	0.3	5.4	6.6	0.3	0	0	0	0.3	0	
	Goodsell Brook	1.8	0.4	0.9	0.9	0	0	0	1.8	0	0	0	0	0	0	4.5	3.1	0.9	9.4	42.9	0	0	2.2	0	2.7	0.9	3.6	0.9	4.5	0	0.4	0	1.3	1.3	
	Willow Brook	0.7	0.3	3.3	0.1	0.3	0	0	0	0	5.7	0	7.2	0.1	0.1	1.5	2.9	1.5	7.4	20.6	0	0	0	0	0.8	9	2.9	19.3	0	0.5	5.1	0.3	0	0.1	
	Mallet's Creek Trib 8	2.5	1.7	4.5	0.6	0	0	0.3	0.8	0	31.3	3.9	3.4	0.8	0	2.5	2	1.7	9.5	2.2	0	0	0	0	0.8	1.1	0.8	2	0	0.6	0	0.3	0.6	1.1	
	Beaver Brook	1.2	0	0	0	0	0	0	3	0	0	0	4.7	14.7	0.2	13.6	0.6	0	5.4	5	0	0	0.2	5.3	1.1	0.5	0.3	10	0	1.8	0	0	2.4	0.6	
Sheldon Spring Trib	0	0.1	0.6	1.2	0	0	0	0	23.7	2.9	0.1	0.3	0	0.2	0	1.3	0.7	0	7.2	0	0	0	0	1	1.1	4.2	7.7	0	10.7	20.2	2	2.8	1		

Urban vs Agricultural	p-value	0.11	p-value	0.03	p-value	0.02	p-value	0.06	p-value	0.00	p-value	0.36
Urban vs Reference	0.00	0.07	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	
Agricultural vs Reference	0.08	0.37	0.00	0.01	0.01	0.01	0.00	0.00				

S: There is a not a significant difference between the relative abundance of this group of taxa within each classification of creeks

D: There is a significant difference between the relative abundance of this group of taxa within each classification of creeks

Conclusion

		Dip_Hexe_sp	Dip_Parcl_sp	Col_Ouili_lati	Oil_Lumbd_unid	Dip_Micrs_sp	Tri_Dipl_sp	Dip_Pseu_sp	Dip_Poly_avic	Tri_Chim_ater	Ple_Taenx_sp	Ple_Leuc_imm	Tri_Symp_stos	Col_Pseep_herr	Dip_Cric_sp	Ple_Chlo_imm	Tri_Rhya_sp	Eph_Stenn_lute	Tri_Dolo_sp	Eph_Ephel_sp	Dip_Poly_conv	Dip_Euki_dlar	Dip_Thiel_sp	Dip_Rheta_dist	Eph_Bael_sp	Col_Opt_sp	Dip_Tvel_sp	Eph_Para_sp	Dip_Simu_tube	Tri_Hybr_bett	Tri_Cheu_sp	Dip_Thiel_grp	Col_Sten_cren	Dip_Parme_sp		
Urban	Bartlett Brook	0	0	0	0	0	2.2	0.7	0	1.1	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.8	29.2	2.5	14	2.9		
	Centennial Brook	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0	0	0	0	39.3	31.6	2	1.4	1.5			
	Englesby Brook	0	0	0	0	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.7	5	0.4	0	0	1.1	1.9	4.4	0.7	0.7				
	Monroe Brook	0	0	0	0	0	0	0	0	0	0.2	3.4	0	0	0	0	0	0	0	0	0	0	0	0	2.1	0.2	0.2	18.9	25.7	0	6	0.6				
	Morehouse Brook	0	0	0	0	0.3	9.2	0	0	0	0	14.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	3.5	0	1	0	13.9			
	Potash Brook	0	0	0	0	0	0	0	0	0.1	0	0	12.6	0	15.9	0	0	0	0	0	0	0	0	0	0.1	0	0	23.9	16	0	9.1	0				
Agricultural	Rugg Brk Low	0	0	0	0	0	0	0	0	0	0.3	0.1	2.3	4.2	1.5	44.3	0	0	0	0	0	0	0.1	0.8	0	0	0	7.1	10	9	0	2.9	0			
	Rock River	0	0	0	0	0	0	0	0	0	0.3	1.5	0	0	1	14.2	0	0	1	0	0	0	4	0.4	4	0.4	0.6	0	0.9	2.3	15.7	22	1	4.5	0.1	
	Trout Brook	0	0	0	0	0	0	0	0	0	21.4	0.2	0	0.5	1	0	0	1.3	0	0	0	0	0.3	0.3	5	0	8.4	1.2	3.5	1.6	1.3	12.7	16.1	1	8.1	0.8
	Steven's Brook	0	0	0	0	2	0	0	0	25.9	0	0	1.6	6.9	0	0	0.1	0	0	0	0	0	0	0	0.2	0.4	0.4	0	0	8.9	4.8	14	0	12.2	1.2	
	Wanzer Brk	1.3	0	0.8	0	0	0	0	0	3.5	0	0	11	10.7	10.7	0.9	0	1	0.3	0.3	0	0.9	6	0	15.8	0.3	1.9	2	0.4	0	3.5	1	4.1	2.2		
	Rugg Brk High	0	0	0	0	0.8	0	0	0	0.5	0	0	2.8	0.9	0	0.3	0	0	0	0	0	5	8.6	2	0.3	3	0.8	1.6	0.1	6	16.5	21.2	5	2	1.5	
Reference	Saxe Brook	0	0	0	0	0.2	0	0.2	0	7.3	2	0.1	0	0	0.5	0	0	0	0	0	0	0.2	0	0.3	1.9	0.3	0.7	7.7	0.6	25	11.4	4	4.7	0.9		
	Bump School Brook	0	0	0	0	0.9	4.1	2.6	0	0	0	0	0	0	0	5.2	13	0	0	0	0	0	4	8.7	2.6	4	0	0.4	1.7	3	1	0	0.2	6.1		
	Breese Pond Outlet 04	0	0	0	0.2	0	6	0.5	0	0	0	0.3	0	0	0	0	0.5	0.5	0	6.3	24.9	0	0	0.3	0.3	1.4	0	2.1	0.2	1.9	15	2.3	0	0	0	
	Breese Pond Outlet 03	0.1	0.1	0	0	0.1	3.3	0.3	0.3	0	0	0	0	0	0	0	1.7	2.3	2	9.9	28.7	0	0.1	0	0	0	2	2.5	26	0.6	2	2.3	0	0	0.4	
	Prindle Brook	0	1.1	0.2	0	2.1	5.4	0.8	0.6	10	0	0	0	0	0	0	0	0.7	0	2.2	3.2	0	0	0	0	3.6	2.2	5.7	9	1.4	9.1	4.3	0	1.5	0.2	
	Fullam Brook	1.5	0.8	0.5	0	0	24.3	0	2.8	0	0	9.7	0	0	0	2	1.2	0	11	0	0	0	0	0	0.8	0.3	5.4	6.6	0.3	0	0	0	0	0.3	0	

		Dip_Hexe_sp	Dip_Parcl_sp	Col_Ouili_lati	Oil_Lumbd_unid	Dip_Micrs_sp	Tri_Dipl_sp	Dip_Pseu_sp	Dip_Poly_avic	Tri_Chim_ater	Ple_Taenx_sp	Ple_Leuc_imm	Tri_Symp_stos	Col_Pseep_herr	Dip_Cric_sp	Ple_Chlo_imm	Tri_Rhya_sp	Eph_Stenn_lute	Tri_Dolo_sp	Eph_Ephel_sp	Dip_Poly_conv	Dip_Euki_dlar	Dip_Thiel_sp	Dip_Rheta_dist	Eph_Bael_sp	Col_Opt_sp	Dip_Tvel_sp	Eph_Para_sp	Dip_Simu_tube	Tri_Hybr_bett	Tri_Cheu_sp	Dip_Thiel_grp	Col_Sten_cren	Dip_Parme_sp		
Urban	Bartlett Brook	0	0	0	0	0	2.2	0.7	0	1.1	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.8	29.2	2.5	14	2.9		
	Centennial Brook	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0	0	0	0	0	39.3	31.6	2	1.4	1.5		
	Englesby Brook	0	0	0	0	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.7	5	0.4	0	0	1.1	1.9	4.4	0.7	0.7				
	Monroe Brook	0	0	0	0	0	0	0	0	0	0.2	3.4	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1	0.2	0.2	18.9	25.7	0	6	0.6			
	Morehouse Brook	0	0	0	0	0.3	9.2	0	0	0	0	14.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	3.5	0	1	0	13.9			
	Potash Brook	0	0	0	0	0	0	0	0	0.1	0	0	12.6	0	15.9	0	0	0	0	0	0	0	0	0	0	0.1	0	0	23.9	16	0	9.1	0			
Agricultural	Rugg Brk Low	0	0	0	0	0	0	0	0	0	0.3	0.1	2.3	4.2	1.5	44.3	0	0	0	0	0	0	0.1	0.8	0	0	0	7.1	10	9	0	2.9	0			
	Rock River	0	0	0	0	0	0	0	0	0	0.3	1.5	0	0	1	14.2	0	0	1	0	0	0	4	0.4	4	0.4	0.6	0	0.9	2.3	15.7	22	1	4.5	0.1	
	Trout Brook	0	0	0	0	0	0	0	0	0	21.4	0.2	0	0.5	1	0	0	1.3	0	0	0	0	0.3	0.3	5	0	8.4	1.2	3.5	1.6	1.3	12.7	16.1	1	8.1	0.8
	Steven's Brook	0	0	0	0	2	0	0	0	25.9	0	0	1.6	6.9	0	0	0.1	0	0	0	0	0	0	0	0	0.2	0.4	0.4	0	0	8.9	4.8	14	0	12.2	1.2
	Wanzer Brk	1.3	0	0.8	0	0	0	0	0	3.5	0	0	11	10.7	10.7	0.9	0	1	0.3	0.3	0	0.9	6	0	15.8	0.3	1.9	2	0.4	0	3.5	1	4.1	2.2		
	Rugg Brk High	0	0	0	0	0.8	0	0	0	0.5	0	0	2.8	0.9	0	0.3	0	0	0	0	0	5	8.6	2	0.3	3	0.8	1.6	0.1	6	16.5	21.2	5	2	1.5	
Reference	Saxe Brook	0	0	0	0	0.2	0	0.2	0	7.3	2	0.1	0	0	0.5	0	0	0	0	0	0	0.2	0	0.3	1.9	0.3	0.7	7.7	0.6	25	11.4	4	4.7	0.9		
	Bump School Brook	0	0	0	0	0.9	4.1	2.6	0	0	0	0	0	0	0	5.2	13	0	0	0	0	0	4	8.7	2.6	4	0	0.4	1.7	3	1	0	0.2	6.1		
	Breese Pond Outlet 04	0	0	0	0.2	0	6	0.5	0	0	0	0.3	0	0	0	0	0.5	0.5	0	6.3	24.9	0	0	0.3	0.3	1.4	0	2.1	0.2	1.9	15	2.3	0	0	0	
	Breese Pond Outlet 03	0.1	0.1	0	0	0.1	3.3	0.3	0.3	0	0	0	0	0	0	0	1.7	2.3	2	9.9	28.7	0	0.1	0	0	0	2	2.5	26	0.6	2	2.3	0	0	0.4	
	Prindle Brook	0	1.1	0.2	0	2.1	5.4	0.8	0.6	10	0	0	0	0	0	0	0	0.7	0	2.2	3.2	0	0	0	0	3.6	2.2	5.7	9	1.4	9.1	4.3	0	1.5	0.2	
	Fullam Brook	1.5	0.8	0.5	0	0	24.3	0	2.8	0	0	9.7	0	0	0	2	1.2	0	11	0	0	0	0	0	0.8	0.3	5.4	6.6	0.3	0	0	0	0	0.3	0	

Urban vs Agricultural	p-value	0.11	p-value	0.03	p-value	0.02	p-value	0.06	p-value	0.00	p-value	0.36
Urban vs Reference	p-value	0.00	p-value	0.07	p-value	0.00	p-value	0.02	p-value	0.00	p-value	0.00
Agricultural vs Reference	p-value	0.08	p-value	0.37	p-value	0.00	p-value	0.01	p-value	0.01	p-value	0.00

Acknowledgements

The authors wish to thank:

- The EPSCoR Baccalaureate College Summer Research Program under NSF Grant Number, EPS-0236976
- The Norwich University Faculty Development Program for funding
- The Vermont DEC for permission to use their data in this presentation

Questions?

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