

Geauga Park District
Research Grant Report

PREPARATOR: Timothy O. Matson, Ph.D.

PROJECT TITLE: Declining Amphibians in Northeastern Ohio
A look at Pathogenic Bacteria, Chytrid, and Potentially Toxic Metals

STATEMENT OF PURPOSE: Purpose of this proposed study is to investigate several agents that may singly or synergistically be causing observed local amphibian population declines.

INTRODUCTION: This project continued our program to monitor status of pond breeding amphibians at selected wetlands in NE Ohio. Metal toxicity to amphibian larvae has been shown to be a factor in limiting the distribution of sensitive species of amphibians such as the Jefferson Salamander and the Wood Frog in Pennsylvania. Both species occur in Ohio and have been observed to have declined in NE Ohio which as a result has diminished amphibian biodiversity. Bacterial infection which causes red-leg disease and other septicemic diseases has been implicated as an agent causing amphibian decline. Chytrid fungus is now considered to be a major cause for global amphibian decline. Toxic metals have been identified in some NE Ohio ponds in concentrations that may be toxic to amphibian embryos and/or larvae. The inconsistency of the metal concentrations observed in some local wetlands indicates that many metal ions become complexed to organic molecules or soil particles and that the source may be sporadic. However, limited testing of collected precipitation indicates the reoccurring atmospheric fallout may be a source for these metals. Red-leg disease has been detected in NE Ohio, but the extent of this disease has not been investigated. Some researchers in Ohio have begun genetic testing for chytrid by sending swabs of amphibians to the few labs conducting the genetic identification tests. Through testing for these three groups of agents that have the potential to cause large scale mortality in multiple species of amphibians we will help delineate problems in NE Ohio and determine if more than one of these agents are present in ponds (which may suggest synergisms).

METHODS: Six seasonal or permanent ponds known to support Wood Frog and/or Jefferson Salamander breeding activities were studied. Ponds selected for study included: two ponds at Big Creek Park in Geauga County (Gauga Park District) and four ponds at the Grand River Terraces in Ashtabula (Cleveland Museum of Natural History). Pond water aliquots were collected on 18 March, 19 May, and 2 July. Water samples from precipitation collectors were collected on those dates if sufficient precipitation had occurred or as soon afterward following precipitation events. All aliquots were placed in laboratory bottles without preservative and transported to BioSolutions, Inc. where they were filtered, preserved, and analyzed. Metals tested included Al, Cu, Ca, Mg, and Zn. Data obtained while at the pond included water temperature, conductivity, and dissolved oxygen.

Several individuals of each species (adults, larvae, and eggs) were swabbed each time water aliquots were drawn. Two types of swabs were required; one was used for bacterial testing and was sent to PetLabs, Inc.; the second type was used to test for chytrid (genetic testing) and was sent to a laboratory at Mount Union College. Amphibians in any of the ponds exhibiting hemorrhaging, edema, crusted skin, or with an estimated recent time death were preserved and placed into the herpetology collections of the Museum. Monitoring of the ponds continued throughout the summer or until all larvae had died or transformed or until the pond dried.

All ponds involved in the study have small to moderate sized populations, therefore we standardized the sample numbers/species. However, the number of specimen samples collected per pond varied from the plan below and was dependent upon the weather conditions, drought, early detection of

mortality, or other unanticipated events.

Bacteriology Testing

	<u>Adults</u>	<u>Egg Mass</u>	<u>Eggs</u>	<u>Larvae 1</u>	<u>Larvae 2</u>
<i>Ambystoma jeffersonianum</i> (Jefferson Salamander)	3	3	3	3	3
<i>A. maculatum</i> (Spotted Salamander)	3	3	3	3	3
<i>Lithobates sylvaticus</i> (Wood Frog)	3	3	3	3	3

Egg Mass = from egg mass surface several days to one week following oviposition

Eggs = about the time the embryos reach the Acomma@ stage

Larvae 1= larvae about 3 weeks post hatch when they can be identified

Larvae 2= larvae about mid-June or when diseased or dead individuals are located

Chytrid Testing

	<u>Adults</u>	<u>Eggs</u>	<u>Larvae 2</u>
<i>Ambystoma jeffersonianum</i> (Jefferson Salamander)	3	2	3
<i>A. maculatum</i> (Spotted Salamander)	3	2	3
<i>Lithobates sylvaticus</i> (Wood Frog)	3	2	3

Chemistry (Metals) testing

Pond Water Ca, Mg, Fe, Cu, Al, total hardness

Precipitation Cu, Al

Modified Gee minnow traps and collapsible minnow traps were used to capture adults to check identifications of amphibian species using the ponds for egg laying, to examine the adults for apparent infections and skin disease anomalies, and for swabbing. Larvae, including tadpoles, were sampled and captured for swabbing by dip-netting.

RESULTS AND DISCUSSION: The trapping period extended 16B20 March. Results of trapping are presented in Table 1. There was no evidence of a >black spot= fungal disease on any salamander trapped or dip-netted at GRT ponds. However, adult Jefferson Salamanders with >black spot= were trapped in both ponds at BCP. In BCP Pond 1, all Jefferson Salamanders caught in one trap on 17 March were thoroughly examined; seven males had the fungus whereas 16 did not and six females evidenced the fungus while 12 did not. Although sample size was small, about 50% of individuals of each sex displayed the >black spot= fungal disease of undetermined identity. In BCP Pond 2 four of 15 Jefferson Salamanders showed >black spot.=

Reproductive success through the larval stage varied between species within and between ponds (Table 2). In most ponds it is difficult to accurately count the actual number of egg masses; however in small ponds with little surface cover of debris or leaf litter or of growth of duckweed or algae accurate counts were possible. Assessing larval numbers is more difficult, especially while larvae are small. Jefferson Salamander larvae fared moderately well in only two ponds, Pond 2 at the GRT and Pond 1 at BCP. In four of the six ponds the mortality from eggs through late larval stages was very high and approached zero survivorship.

Spotted Salamanders laid large numbers of egg masses in most ponds, but survivorship was low in all ponds. The GRT Ponds 2 and 3 were the most difficult to inventory for larvae because of water depth

and extensive amounts of organic matter. The Litt Tract pond on the other had was small, shallow, and tannic but clear and by early June few larvae of any species were alive (Jefferson Salamanders did very well in this pond in 2006-2008). Pond 2 at BCP was also relatively easy to inventory by early June. All Spotted Salamanders died in these two ponds by early July.

Wood Frogs bred in all study ponds but were moderately successful only at GRT Ponds 2 and 6. Wood Frogs typically transform about the first week to 10 days in July in NE Ohio. The apparent total mortality of Wood Frogs in both BCP ponds may in part reflect early transformation. On 2 July over 40 large Wood Frog tadpoles were found dead at GRT Pond 2. Although this is a large number of dead tadpoles the mortality may not be unusually high because the number of tadpoles was high and many tadpoles die during transformation.

In general, the mortality for 13 species observed in all six ponds was high. Predation by invertebrate predators or Jefferson Salamander larvae may account for much of the apparent mortality. Pond 2 of GRT also supports an adult population of predatory Red-spotted Newts (*Notophthalmus viridescens*). Jefferson Salamanders and Wood Frogs were successful in this pond which may indicate that other factors contributed to the high mortality.

Metal concentrations in all precipitation water samples were below the levels of detection by laboratory methods used, that is $[Al] < 50 \mu\text{m/L}$ and $[Cu] < 10 \mu\text{g/L}$. During this study no detectable atmospheric fallout of Al or Cu took place (Table 3).

Pond water analyses are presented in Table 4. All ponds other than BCP Pond 2 would be classified as soft water ponds (total hardness $< 60,000 \mu\text{g/L}$; USGS water hardness scale). BCP Pond 2 would be categorized as moderately hard ($60,000 < \text{hardness} < 120,000 \mu\text{g/L}$). Solubility of metals changes with pH and increases as pH declines toward 5. pH at the GRT ponds varied between 5.3 and 6.0 whereas that of BCP varied between 6.3 and 6.7. Concentrations of Cu were below levels of detection in all samples from each pond. Fe concentrations are consistent within ranges that we find in many ponds in NE Ohio, some of which support breeding populations of these taxa while others support declining populations or no populations. Toxicity of Fe to these taxa at pH above 5.5 and total hardness values observed here is not evident from the literature. Most studies have been conducted at low total hardness levels of many vernal pools in eastern states ($< 1500 \mu\text{g/L}$).

Ca and Mg ameliorate some of the toxic effects of low pH (< 5.5) and some toxic heavy metal concentrations. The ameliorative effects of Ca and Mg at higher pH and higher hardness may be lessened and Ca may become toxic. Many ponds in NE Ohio with Ca and Mg concentrations similar to levels measured in these ponds support healthy populations of these taxa. Amphibians in hard water ponds (total hardness of $120,000$ - $180,000 \mu\text{g/L}$) are more likely to be subjected to Ca toxicity stresses.

Al concentrations may be sufficiently high in some ponds to be of concern. At pHs below 5.5 $[Al]$ of $525 \mu\text{g/L}$ was found to be the lethal limit and non-lethal values as low as $100 \mu\text{g/L}$ in sensitive species (Jefferson Salamanders and the slightly less sensitive Wood Frog) can cause reduced hatching success and reduce both size at hatching and at transformation. At the pHs measured in ponds of this study the amphibians in GRT Ponds 3, 6, and the Litt Tract and BCP Pond 1 may be subjected to sublethal effects of Al toxicity. The pond water chemistry of Al at higher pH and the organic content and organic and inorganic complexes formed within the various ponds determine the level of toxicity to amphibians.

The bacteriological results are most interesting; there are considerable differences between ponds on the GRT and those of BCP (Table 5). At the GRT three genera of bacteria were detected, *Staphylococcus*, *Bacillus* and *Escherichia coli*. *Staphylococcus* was detected on amphibians in several ponds during all the sampling periods and was present in the pond water over some sampling periods. Although *Staphylococcus* was present in some pond waters its detection on some of the animals was probably attributable to field contamination by workers manipulating the specimens during swabbing. All samples exhibited scant to light growth which lends support to the contention that this bacterium was not a cause for elevated rates of mortality. The only occurrence of *Bacillus* was in Pond 3, and it probably had a similar derivation. *Escherichia coli* was not detected until the 2 July sampling with the exception of one adult Wood Frog in March at the Litt Tract pond. Amphibians and water samples supported growths

ranging from scant to heavy, with most larvae supporting heavy growth. In 2006 two dead or dying Jefferson Salamanders collected in Pond 3 supported *Pseudomonas aeruginosa*, a bacterium known to cause red-leg disease in amphibians. This bacterium was not detected in 2009. Similarly, *Klebsiella* and *Pseudomonas* were isolated from a dead Jefferson Salamander in Pond 6, but neither species of bacteria was found in 2009.

The only amphibian egg masses available for bacterial testing on the 19 March date were those of the Jefferson Salamander. Both fresh eggs and those laid several weeks earlier were tested; only scant growth on old eggs from the Litt Tract pond was detected.

In contrast to GRT ponds, we detected six genera of bacteria at the BCP ponds. Adult amphibians in Pond 1 supported heavy growths of *Vibrio alginolyticus* and *Citrobactor braakii* whereas adults in Pond 2 supported heavy growths of *Leclercia adecarboxylata*, *Serratia fonticola*, and *Aeromonas hydrophila*. Egg masses, both fresh and old, in Pond 1 supported heavy growths of the same bacterial species encountered on adults; old eggs in Pond 2 similarly supported heavy growth of bacterial species found on adult amphibians. Larvae in both ponds supported moderate to heavy growths of bacterial species found in their respective ponds. Although predation by Jefferson Salamander larvae may be an important cause for the total absence of Wood Frog tadpoles and Spotted Salamander larvae in the 2 July sample, the presence of Jefferson Salamander larvae may imply that this species has more resistance or tolerance to the bacterial community in the pond (see Table 2).

Several of the bacterial species reported here are fecal coliform bacteria in the family Enterobacteriaceae found in the gastrointestinal tract of homeotherms (primarily endotherms). Environmental occurrences are often associated with fecal contamination and often indicate sewage or animal waste contamination.

Results of the chytrid (fungal) tests are presented in Table 5. Out of the 95 tests over all six ponds for which we have results, nine are positive for chytrid. Although there are numerous other individuals with genome equivalents (ge) <1.0, these are not considered confirmed positive results by the laboratory which performed the PCRs. Some of the standards run with test samples can produce genomic equivalent values < 1.0. Additional testing would be required to determine if the percentage of infected animals is greater than 9.5%. All positive values are low which indicates the infected animals—nearly all infected animals were Spotted Salamanders—were not heavily infected. Chytrid was detected in both Pond 1 (Spotted Salamander) and Pond 2 (Wood Frog) at Big Creek Park and at each of the four sites at the Grand River Terraces. All positive records occurred during the March testing period (on adults) and none in the July testing of larvae. The difference in detection may be attributed to the difference in water temperature since chytrid does poorly at temperatures in the upper 20s and disappears at about 30° C. Swabs were made during three time periods; the May swabs (totaling about 50) of young larvae of all three species were either lost in shipment or at the test laboratory. Although the number of animals tested for which we have results was small, no Jefferson Salamanders tested positive with the infection and few had genome equivalents >0 (<1.0).

Table 1. Trapping data for the six ponds studied at the Grand River Terraces (GRT) and Big Creek Park (BCP) during 2009. Species noted include *Ambystoma jeffersonianum* (A.j), *A. maculatum* (A.m), and *Lithobates sylvaticus* (L.s). The infinity symbol (∞) indicates many individuals captured, but they were not counted.

	Number of Traps	Trap Nights	Number Captured
GRT			
2	4	20	46 A.j, 235 A.m, 13 L.s
3	5	25	23 A.j, 43 A.m, 2 L.s
6	4	20	∞ A.j, 23 A.m, 9 L.s
Litt	4	20	6 A.j, 14 A.m, 25 L.s
BCP			
1	4	20	∞ A.j, 5 A.m, 16 L.s
2	6	30	56 A.j, 4 A.m, 12 L.s

Table 2. Estimated number of egg masses (Em), larvae (L), and tadpoles (T) over time in ponds at the Grand River Terraces and Big Creek Park. Symbols used: ~ means close approximation, ∞ indicates a very large number and could not accurately count, Num = numerous, Mod = moderate number, Few = low number of individuals but not readily countable.

	Grand River Terraces				Big Creek Park	
	Pond 2	Pond 3	Pond 6	Litt	Pond 1	Pond 2
<i>A. jeffersonianum</i>						
April 9-15, 2009	~20 EM	~29 EM	∞ EM	8 EM	∞ EM	∞ EM
June 2, 2009	Num L	Mod L	Mod L	Num L	Mod L	Num L
July 2, 2009	Num L	3 L	4 L	3 L	Mod L	15 L
<i>A. maculatum</i>						
April 9-15, 2009	~80 EM	~90 EM	35 EM	47 EM	21 EM	6-7 EM
June 2, 2009	∞ L	Mod L	Few L	0 L	0 L	7 L
July 2, 2009	3 L	12 L	5 L	0 L	2 L	0 L
<i>L. sylvaticus</i>						
April 9-15, 2009	20 EM	~8 EM	~30 EM	~30 EM	~20 EM	3-5 EM
June 2, 2009	∞ T	Few T	Mod T	Num T	Few T	4 T
July 2, 2009	Num T	Few T	Mod T	9 T	0 T	0 T

Table 3. Concentration of Al and Cu found in precipitation at the Grand River Terraces (GRT) and Big Creek Park (BCP) during 2009. All measurements in $\mu\text{g/L}$.

Site	Date		
	April 7-15, 2009	June 2, 2009	July 2, 2009
GRT			
Al	100	<50	<50
Cu	<10	<10	<10
Litt			
Al	<100	<50	<50
Cu	<10	<10	<10
BCP #1			
Al	<100	<50	<50
Cu	<10	<10	<10
BCP #2			
Al	<100	<50	<50
Cu	<10	<10	<10

Table 4. Pond water metal analysis for the Grand River Terraces (GRT) and Big Creek Park Ponds (BCP) during 2009. All measurements are in µg/L.

Site	Date	Al	Cu	Fe	Ca	Ca as CaCO ₃	Mg	Mg as CaCO ₃	Total Hardness
GRT									
Pond 2									
	March 18, 2009	<100	<10	190	4530	11,325	1450	5974	17,299
	May 19, 2009	<100	<10	670	5570	13,925	2010	8281	22,206
	July 2, 2009	70	<10	550	5530	13,825	1560	6427	20,252
Pond 3									
	March 18, 2009	500	<10	300	4240	10,600	820	3378	13,978
	May 19, 2009	200	<10	590	4380	10,950	870	3584	14,534
	July 2, 2009	170	<10	500	5190	12,975	880	3626	16,601
Pond 6									
	March 18, 2009	840	<10	640	4930	12,325	860	3543	15,868
	May 19, 2009	100	<10	1340	6370	15,925	1280	5274	21,199
	July 2, 2009	230	<10	760	4240	10,600	860	3543	14,143
Litt Tract									
	March 18, 2009	100	<10	220	2140	5350	500	2060	7410
	May 19, 2009	300	<10	950	3340	8350	730	3008	11,358
	July 2, 2009	220	<10	240	3130	7825	620	2554	10,379
BCP									
Pond 1									
	March 18, 2009	280	<10	1180	11,200	28,000	2000	8240	36,240
	May 19, 2009	<100	<10	1040	16,570	41,425	3030	12,484	53,909
	July 2, 2009	180	<10	810	8160	20,400	1420	5850	26,250
Pond 2									
	March 18, 2009	<100	<10	1180	33,300	83,250	7300	30,076	113,326
	May 19, 2009	<100	<10	1050	35,050	87,625	7170	29,540	117,165
	July 2, 2009	60	<10	310	17,200	43,000	550	2266	45,266

Table 5. Bacteria and *Chytrid* found on adults (ad.), eggs (E), and larvae (L) of *Ambystoma jeffersonianum* (A.j), *A. maculatum* (A.m), and *Lithobates sylvaticus* (L.s) in ponds at the Grand River Terraces and Big Creek Park. Bacterial concentrations: no growth (NG), light growth (LG), scant growth (SG), moderate growth (MG), and heavy growth (HG). Numbers in the chytrid columns are genom equivalents (ge); numbers <1.0 may be ambiguous and require retesting. The laboratory recommends having confidence in ge values ≥ 1.0 . When interpreting the table do not consider the animals listed under Species/Stage as having provided the data listed under Bacteria and Chytrid. That particular animal (based upon number, e.g., Aj1/ad) did provide a sample for bacteria and chytrid testing, but the labs concerned did not retain our specimen numbering sequence.

Grand River Terraces Pond 2

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	LG/ <i>Staphylococcus</i>			0		
A.j 2/ad	SG/ <i>Staphylococcus</i>			0		
A.j 3/ad	SG/ <i>Staphylococcus</i>			0		
A.m 1/ad	NG			1.36		
A.m 2/ad	NG			0.651		
A.m 3/ad				2.764		
A.m 4/ad						
L.s 1/ad				0.325		
L.s 2/ad				0.299		
L.s 3/ad				0.410		
A.j/ fresh E				0		
A.j/old E				0.464		
L.s/ E				0		
A.j 1/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0.211
A.j 2/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0
A.j 3/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0
A.m 1/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0
A.m 2/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0
A.m 3/L		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0.289
L.s 1/T		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			0
L.s 2/T		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			
L.s 3/T		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			
Water		LG/ <i>Staphylococcus</i>	HG/ <i>Esherichia coli</i>			

Grand River Terraces Pond 3

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	NG			0		
A.j 2/ad						
A.j 3/ad						
A.m 1/ad	LG/ <i>Staphylococcus</i>			1.769		
A.m 2/ad	NG			5.844		
A.m 3/ad				0		
A.m 4/ad						
L.s 1/ad	SG/ <i>Bacillus</i>			0		
L.s 2/ad						
L.s 3/ad						
A.j/ fresh E						
A.j/old E						
L.s/ E						
A.j 1/L	NG		HG/ <i>Escherichia coli</i>			0
A.j 2/L	NG		HG/ <i>Escherichia coli</i>			0
A.j 3/L			HG/ <i>Escherichia coli</i>			0
A.m 1/L	SG/ <i>Staphylococcus</i>		LG/ <i>Escherichia coli</i>			0.266
A.m 2/L	SG/ <i>Staphylococcus</i>		SG/ <i>Escherichia coli</i>			0
A.m 3/L	SG/ <i>Staphylococcus</i>		HG/ <i>Escherichia coli</i>			0
L.s 1/T	NG		LG/ <i>Escherichia coli</i>			0
L.s 2/T	NG		LG/ <i>Escherichia coli</i>			0
L.s 3/T	NG		HG/ <i>Escherichia coli</i>			0
Water			HG/ <i>Escherichia coli</i>			

Grand River Terraces Pond 6

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	SG/ <i>Staphylococcus</i>			0		
A.j 2/ad	SG/ <i>Staphylococcus</i>			0		
A.j 3/ad	SG/ <i>Staphylococcus</i>			0		
A.m 1/ad	SG/ <i>Staphylococcus</i>			0		
A.m 2/ad				1.449		
A.m 3/ad						
A.m 4/ad						
L.s 1/ad	NG			0.174		
L.s 2/ad	NG			0		
L.s 3/ad				0		
A.j/ fresh E	NG					
A.j/old E	NG					
L.s/ E						
A.j 1/L		SG/ <i>Staphylococcus</i>	SG/ <i>Staphylococcus</i>			0
A.j 2/L		SG/ <i>Staphylococcus</i>	SG/ <i>Staphylococcus</i>			0
A.j 3/L		SG/ <i>Staphylococcus</i>	SG/ <i>Staphylococcus</i>			0
A.m 1/L		SG/ <i>Staphylococcus</i>	SG/ <i>Staphylococcus</i>			0
			MG/ <i>Escherichia coli</i>			0.493
A.m 2/L		NG	SG/ <i>Staphylococcus</i>			0.303
			MG/ <i>Escherichia coli</i>			
A.m 3/L		NG	SG/ <i>Staphylococcus</i>			
			MG/ <i>Escherichia coli</i>			
L.s 1/T		NG	SG/ <i>Staphylococcus</i>			0.401
L.s 2/T		NG	SG/ <i>Staphylococcus</i>			0
L.s 3/T		NG	SG/ <i>Staphylococcus</i>			0
Water	NG	NG	NG			

Grand River Terraces Litt Tract Pond

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	SG/Staphylococcus			0		
A.j 2/ad	SG/Staphylococcus			0		
A.j 3/ad						
A.m 1/ad	SG/Staphylococcus			0		
A.m 2/ad	SG/Staphylococcus			0		
A.m 3/ad	SG/Staphylococcus			0.853		
A.m 4/ad	SG/Staphylococcus			1.839		
L.s 1/ad	SG/Escherichia coli			0		
L.s 2/ad				0.491		
L.s 3/ad				0.800		
A.j/ fresh E	NG					
A.j/old E	SG/Staphylococcus			0.448		
L.s/ E				0		
A.j 1/L		SG/Staphylococcus	SG/Staphylococcus HG/Escherichia coli			0
A.j 2/L		SG/Staphylococcus	SG/Staphylococcus HG/Escherichia coli			0
A.j 3/L		SG/Staphylococcus	SG/Staphylococcus HG/Escherichia coli			0
A.m 1/L						
A.m 2/L						
A.m 3/L						
L.s 1/T		SG/Staphylococcus	SG/Staphylococcus HG/Escherichia coli			0
L.s 2/T		SG/Staphylococcus	SG/Staphylococcus LG/Escherichia coli			0
L.s 3/T		SG/Staphylococcus	SG/Staphylococcus LG/Escherichia coli			0
Water		SG/Staphylococcus	HG/Escherichia coli			

Big Creek Park, Pond 1

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>			0.477		
A.j 2/ad	HG/ <i>Vibrio alginolyticus</i>			0		
A.j 3/ad				0.359		
A.m 1/ad				0.621		
A.m 2/ad				2.001		
A.m 3/ad				1.081		
A.m 4/ad						
L.s 1/ad	HG/ <i>Vibrio alginolyticus</i>			0.277		
L.s 2/ad	HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>			0.919		
L.s 3/ad	HG/ <i>Vibrio alginolyticus</i>			0		
A.j/ fresh E	HG/ <i>Citrobacter braakii</i>					
A.j/old E	HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>					
L.s/ E				0		
A.j 1/L		HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>	HG/ <i>Vibrio alginolyticus</i>			0.129
A.j 2/L		HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>				0
A.j 3/L		HG/ <i>Vibrio alginolyticus</i>				0
A.m 1/L						
A.m 2/L						
A.m 3/L						
L.s 1/T		HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>	HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>			
L.s 2/T		HG/ <i>Vibrio alginolyticus</i>	HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>			
L.s 3/T		HG/ <i>Citrobacter braakii</i>	HG/ <i>Citrobacter braakii</i> HG/ <i>Escherichia coli</i>			
L.s 4/T		HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>				
L.s 5/T		HG/ <i>Vibrio alginolyticus</i> HG/ <i>Citrobacter braakii</i>				
Water	HG/ <i>Serratia fonticola</i>	HG/ <i>Serratia fonticola</i>	HG/ <i>Serratia fonticola</i>			

Big Creek Park Pond 2

Species/Stage	Bacteria			Chytrid		
	March 18, 2009	May 19, 2009	July 2, 2009	March 18, 2009	May 19, 2009	July 2, 2009
A.j 1/ad	HG/ <i>Leclercia adecarboxylata</i>			0		
A.j 2/ad	HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>			0		
A.j 3/ad	HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>			0		
A.m 1/ad				0		
A.m 2/ad				0		
A.m 3/ad						
A.m 4/ad						
L.s 1/ad	HG/ <i>Aeromonas hydrophilia</i> HG/ <i>Leclercia adecarboxylata</i>			1.090		
L.s 2/ad	HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>			0.441		
L.s 3/ad	HG/ <i>Citrobacter braakii</i>			0.412		
A.j/ fresh E						
A.j/old E	HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>			0.183		
L.s/E				0		
A.j 1/L		HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>	MG/ <i>Leclercia adecarboxylata</i> MG/ <i>Serratia fonticola</i>			0
A.j 2/L		HG/ <i>Leclercia adecarboxylata</i> HG/ <i>Serratia fonticola</i>	MG/ <i>Leclercia adecarboxylata</i> MG/ <i>Serratia fonticola</i>			0
A.j 3/L		HG/ <i>Aeromonas hydrophilia</i> HG/ <i>Leclercia adecarboxylata</i>				
A.m 1/L						
A.m 2/L						
A.m 3/L						
L.s 1/T		HG/ <i>Leclercia adecarboxylata</i>				
L.s 2/T						
L.s 3/T						
Water	HG/ <i>Serratia fonticola</i>					

