# Inventory and Monitoring of Amphibian Biodiversity in the Lye Brook Wilderness Region of the Green Mountain National Forest March 1993-October 1995

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#### Abstract

An inventory of amphibians in the Lye Brook Region of the Green Mountain National Forest in Bennington County was begun in 1993 and completed in 1995. Monitoring of resident amphibians began in 1994. The goals of this inventory and monitoring effort were to determine which amphibians were present in the region and begin monitoring selected species. Six species of salamander (Dusky salamander, Eastern newt, Northern two-lined salamander, Redback salamander, Spotted salamander, Spring salamander) and seven species of frog (American toad, Bullfrog, Gray treefrog, Green frog, Pickerel frog, Spring peeper, Wood frog) were located. The most abundant species of salamander in this region is the Eastern newt. The most abundant species of frog appears to be the Spring peeper. The species found in this region are typical of what has been found at other mid- to high-elevation sites in the central Green Mountains. A monitoring scheme has been developed for this region using drift-fences, egg-mass counts, and stream surveys. One year of monitoring data has been gathered at drift-fences. Two years of monitoring data have been gathered using egg-mass counts and stream surveys.

# Introduction

# **Overview**

This report presents the results of three field seasons of work in the Lye Brook Wilderness (LBW) Region of the Green Mountain National Forest in Vermont. The results are reported and discussed in two sections: a review of that work done in 1993 and reported on in a preliminary report (Andrews 1994) and that work done in 1994 and 1995. The intent of this study was to do an inventory of the amphibians located in the region and begin long-term monitoring of its amphibian populations. Concurrent with this effort was an inventory and monitoring program at another site within the main range of the Green Mountains in the Abbey Pond / Beaver Meadows (AB / BM) Region outside of Middlebury, Vermont. In the preliminary report (Andrews 1994) I compared the inventory data from these two sites. That comparison is included here in a slightly altered form.

# Study site

The region surveyed spans roughly 40 mi<sup>2</sup> and is located in the towns of Manchester, Sunderland, Stratton, and Winhall in Bennington County, Vermont. Most of the region consists of a plateau at about 2500 ft. in elevation. It has been logged heavily in the past, but is now protected. The plateau forest consists primarily of a mix of of northern hardwoods and conifers with many bogs, swamps, small lakes, beaver dams, and old beaver meadows. The water bodies and the soils of the plateau tend to be acidic. The region drains primarily to the west, dropping in elevation to approximately 800 ft. in elevation. The west facing slopes hold a higher percentage of the northern hardwood forests and a number of fast moving streams. The high point of the region reaches close to 3000 ft.

# Methods

The methods used in 1993 were designed to inventory the amphibians of the region and to lay the groundwork for gathering amphibian monitoring data in 1994. A limited number of amphibian inventory methods were continued in 1994 at selected sites. Since no additional species were located in 1994, the inventory results from that year were not combined with the 1993 totals. Monitoring began in 1994 using two methods and in 1995 three methods were used to gather baseline data for long-term monitoring.

# Methods used during 1993

In 1993 six different inventory methods were used in the LBW Region.

<u>Active searches</u> are a concentrated effort in a predetermined area to locate amphibians in the leaf litter, under rocks and logs, within rotten logs or under bark. These searches usually lasted 1.5 hours.

<u>Canoe searches</u> are used to do a visual search of a lake or pond margin from the water.

Drift fences are semi-permanent structures built to interrupt the feeding and migratory movements of amphibians on rainy nights or nights immediately after rains. The three used are constructed of 30 m lengths of aluminum flashing (Figure 1). Tangential with the flashing and buried flush with the ground surface are a series of cans and buckets that can be opened prior to evenings of expected amphibian activity. In addition traps that roughly resemble minnow traps made out of window screening are placed parallel to and abutting the fence. A small piece of plywood is used to provide shade for any reptiles and amphibians trapped in them. The traps are only opened during, after, or in anticipation of, rainy weather. They are always checked the following day when all amphibians are identified, recorded, and released. Three fences were built in the area. I refer to the drift-fence locations as Falls Access, LBW Fence #1, and LBW Fence #2 (Figures 2 & 3). The Falls Access fence is located at 800 ft. in elevation in a second growth hardwood stand. It is over 200 meters from the nearest standing water. LBW Fence #1 is located at 2400 ft. and LBW Fence # 2 is at an elevation of 2700 ft. Both of these upper elevation sites are entirely wooded with a mix of northern hardwoods and some spruce and fir. The upper elevation sites are located close enough to standing water to interrupt breeding movements of pond-breeding amphibians. LBW #1 is located on an upland ridge within foraging distance of two breeding locations.

<u>Night-time visits</u> are made to selected sites in an effort to hear the calls of breeding and territorial anurans (frogs). The time of the year and the weather conditions are chosen specifically to locate species not already located using other methods.

<u>Night-time road searches</u> are not possible at most remote sites. They involve driving a set route at a speed of ten to fifteen mph with the vehicle window open to hear calling anurans, and eyes on the road and road margins to see amphibians crossing the route. The method can be very effective in roaded areas.

<u>Site checks</u> are a less localized form of active search that include time spent searching for and traveling between the best microhabitats.

Accidental discoveries are often made while employing a method not intended to locate that specific species or while scouting or working at a site. Individuals located accidentally are identified as such in the data base and in the tables. I often found Eastern newts in large numbers while I was counting egg masses, building drift fences, or using a method

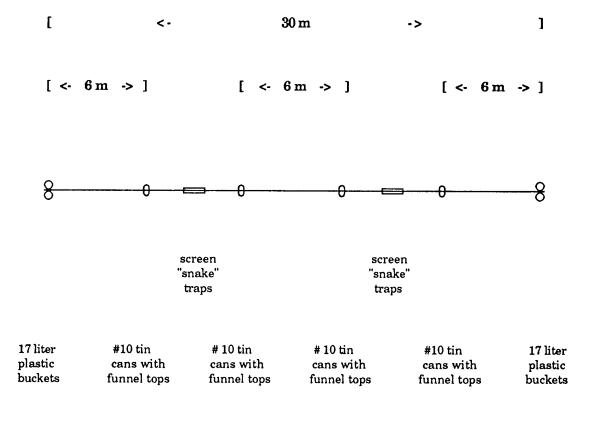


Figure 1. Design of the drift fences used for inventory and monitoring in the Lye Brook Wilderness Region.

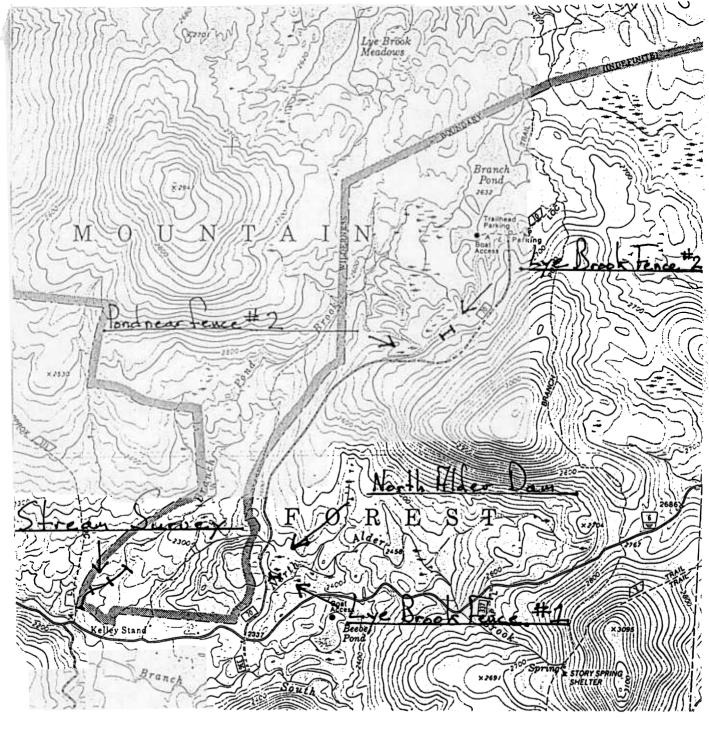


Figure 2. Upper-elevation amphibian-monitoring sites in the Lye Brook Wilderness Region. Two drift-fence sites, two egg-mass count locations, and the stream-survey site are shown.



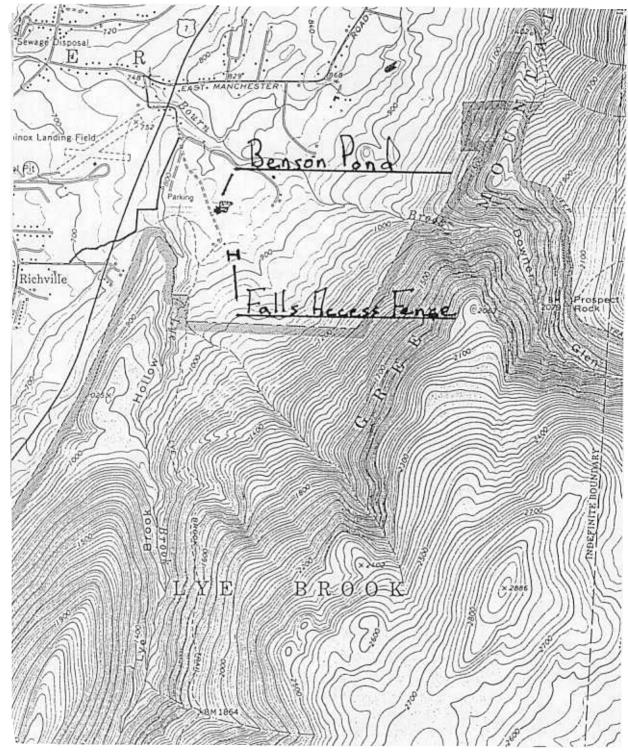


Figure 3. Lower-elevation amphibian-inventory and -monitoring sites in the Lye Brook Wilderness Region. One drift-fence site and one egg-mass count location are shown.

designed to locate a different species. Occasionally these were entered simply as "many" in the database. In the Preliminary Report (Andrews 1994) and in the 1993 data reported here, each reference to "many" was counted as 10 individuals in the data analysis. I have since decided that this was too conservative a treatment of these entries. In all subsequent data "many" was interpreted as fifty individuals.

None of these methods alone will survey all species of amphibian that may occur in an area. A combination of these methods must be employed in an initial inventory effort.

## Other data

<u>Soil and water pH</u> were measured with a Nester Instruments portable pH meter. A planar Duraprobe designed for measuring the pH of surfaces was pushed into the soil one to two cm after the litter was removed. Soil pH was measured in at least three locations at any site where it was recorded. A separate probe was used to measure the pH of breeding ponds in at least three locations in each water body and at a depth of approximately 15 cm. The pH meter was calibrated with 4.0 and 7.0 buffer solutions at least once daily.

<u>Other data</u> were gathered and entered into the data base but are not included in this report. They include natural history notes, weather, time of day, and air and water temperatures.

#### Methods used during 1994 and 1995

During the 1994 field season, site checks, night-time visits and drift-fences were continued as inventory methods. The night-time visits were used specifically to locate Gray treefrogs. Two new methods (egg-mass counts and stream surveys) were added to acquire baseline monitoring data.

In 1995 three methods were used to gather monitoring data and one to gather inventory data. The two upper elevation drift-fences, egg-mass counts, and stream surveys were used for monitoring purposes. Only the low-elevation drift-fence was used to gather inventory data.

<u>Drift-fences</u> were used as an inventory method in 1993 and 1994. In 1995 the upper two fences were used as a monitoring tool. They are more easily standardized than many other methods and hence I feel they are a useful method for this purpose. I followed the protocol in Appendix A. They were opened three times per month from April through October with the exception of August. Since field technicians need to be continually on call for rainy conditions, I decided that there needed to be one month during the summer when they could make other plans. I chose August based on previous survey and drift-fence results which showed it to be a month of limited amphibian activity. Occasionally the fences were opened in anticipation of a rain that did not materialize or during which very little rain fell. When this happened the fences were opened an additional time during the month. If the fences were opened more than three times per month, data are used from only the three most productive nights (greatest number of amphibians caught). Occassionally, heavy rains do not occur three times during a month. In these cases, if a heavy rain occurs at the beginning of the following month (first ten days), or the end of the previous month (last ten days), and three other heavy rains also occur during that month, then the data are shifted to the dry month. The data used from the upper two drift fences in 1995 were gathered on April 19, 28, May 6, 11, 12, 25, June 3, 16, 25, July 2, 8, 18, Sept. 8, 10, 14, 23, and Oct. 6 and 15.

During 1994 a sub-sampling protocol was established (Appendix A) and size-class data were collected for the first time. For each species, individuals under a given total length were considered young of the year. The chosen length was based on the timing of their appearance, gaps in their size continuum (from AP / BM data in 1994) and records in the literature. Although, I realize that the size to which young will grow in the course of one season will differ from year to year, I picked a standard size to use in comparisons. The cutoff sizes used were: Spotted salamander (70 mm), Northern two-lined salamander (60 mm), Eastern newt (45 mm), Redback salamander (32 mm), American toad (30 mm), Spring peeper (20 mm), Green frog (41 mm), Pickerel frog (30 mm), and Wood frog (25 mm).

Egg mass counts took place at three sites that I refer to as near Benson Pond, North Alder Dam, and the pond near fence # 2 (Figures 2 & 3). These counts followed monitoring protocols that were tailored for the sites at which they were used. At all the sites the index that is presented is the highest count of egg-masses on any one day for each of the two species monitored. These counts are not cumulative nor do they have to be from the same day for different species.

At the site near Benson Pond I monitor a small beaver pond that lies approximately 20 m west of the southwest corner of Benson Pond. Benson Pond itself is a large man-made pond. The monitoring site is northwest of the small inlet stream entering Benson Pond and extends into the edge of the woods. The beaver pond measures approximately 10 m across on its longest axis and has a maximum depth of approximately 1 m. The entire pond is searched for egg-masses.

The North Alder Dam is a large beaver dam directly north of driftfence # 1. At this site a four-meter strip around all of the pond except the northern end is surveyed. Due to the very irregular and swampy shoreline along the northern end it would be very difficult to survey.

The pond near drift-fence # 2 is also a large beaver dam. It is located southwest of the drift-fence and is visible from USFS road # 70. At this site a four-meter strip around the entire margin of the pond is surveyed. This site was chosen in 1994 to replace the Kelly Stand Dam site which I had anticipated using. Recent beaver activity at the latter site made it unsuitable for egg-mass surveys.

All surveys are performed under conditions that allow the viewer to see easily into the pond (limited wind, no rain, and adequate light from a high angle). Polarized glasses are sometimes helpful. The counts are designed to take place in habitats where Wood frog and Spotted salamander have been previously located and during or shortly after their breeding period. Eggs were counted on three occasions beginning as soon as the snow and ice melted enough to allow access to the ponds and continuing until all egg laying activity ended in the end of May or beginning of June. At the Benson Pond site in 1995 only two counts were run in 1995. At its low elevation I do not believe that any additional egg-laying activity took place after my last visit on May 12.

<u>Stream surveys</u> were run on one day per year along one brook (Branch Pond Brook, Figure 2). Ideally surveys would take place more frequently along more brooks but funding limitations did not allow it. Branch Pond Brook was chosen because of the relative abundance of Spring salamanders that were found there during the inventory. It had the highest concentration of this species that I was able to locate in the Lye Brook Wilderness Region.

The surveys consist of three contiguous transects of 50 m each. The first transect begins approximately 100 m upstream from the point where the brook is crossed by an old wood-road which runs north from Kelly Stand Road. The width of the transects is defined by the normal high water marks on either side of the brook. These are quite obvious and are marked by a vertical rise of approximately 30 cm. This line is also marked by the appearance of vascular plants. The transects are divided into three regions one-third the width of the stream. Each transect is searched by three people (one per region) for 20 minutes apiece (1 person-hour). Only rocks that are partially or entirely above the water level are turned. The second and third transects take place immediately upstream of and contiguous with the preceding transects.

<u>Water testing</u> was continued during the 1994 and 1995 field seasons. However in 1994 additional samples were sent to Jim Kellogg at the Vermont Department of Environmental Conservation Laboratory to test alkalinity, total color, pH and conductivity using their protocols. I recorded pH and temperature using a new Cole-Parmer hand-held pH meter (microcomputer pH vision model #6009). Three measurements were taken from shore at three widely separated locations at an approximate depth of 15 cm. Calibration was checked at each site using premixed buffer solutions of pH 4.0 and 7.0.

# Results

#### Overview of the Results and Work Effort for the 1993 Field Season

During the first year of this inventory project, 6 salamander species and 7 frog species were located in or near the Lye Brook Wilderness Region of the Green Mountain National Forest (Table 1). Field teams visited ~28 sites in the region (Figures 4a-e) and located 764 individuals of 13 species. In addition, 91 egg Table 1. The results of the 1993 amphibian inventory of the Lye Brook Wilderness Region in Bennington County, Vermont. Six species of salamander and seven species of frog were located. The table shows the combined data gathered using six different methods. Included are all metamorphosed individuals (no eggs or larvae). Chorusing data are not included. Percent individuals are out of a total of 564 caudates and 200 anurans located.

Species	Common name	% of individuals
Caudates (salamanders)		
Notophthalmus viridescens	Eastern newt <sup>1</sup>	53%
Eurycea bislineata	N. two-lined salamander	20%
Plethodon cinereus	Redback salamander	18%
Gyrinophilus porphyriticus	Spring salamander	6%
Ambystoma maculatum	Spotted salamander	2%
Desmognathus fuscus	Dusky salamander	2%
Anurans (frogs and toads)		
Rana clamitans	Green frog	48%
Bufo americanus	American toad	23%
Rana sylvatica	Wood frog	12%
Pseudacris crucifer	Spring peeper	12%
Rana palustris	Pickerel frog	4%
Hyla versicolor	Gray treefrog	0.5%
Rana catesbeiana	Bullfrog	0.5%

<sup>1</sup>I often found this species in large numbers while I was counting egg masses, building drift fences, or using a method designed to locate a different species. Occasionally these were entered simply as "many" in the database. In the data reported here each reference to "many "was counted as 10 individuals in the data analysis.

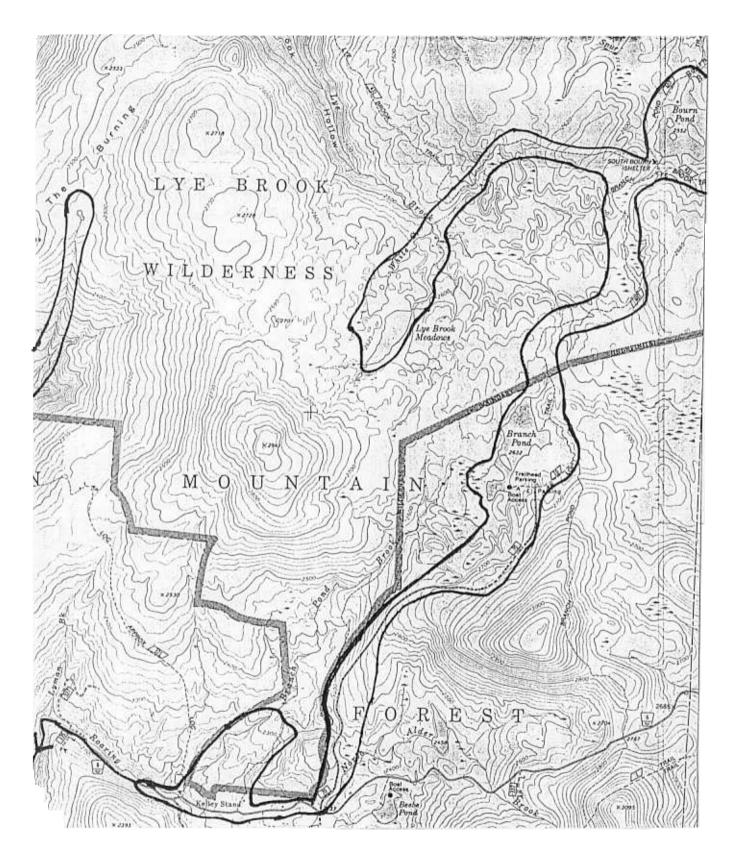


Figure 4a. Areas surveyed during the amphibian inventory of the Lye Brook Wilderness Region.

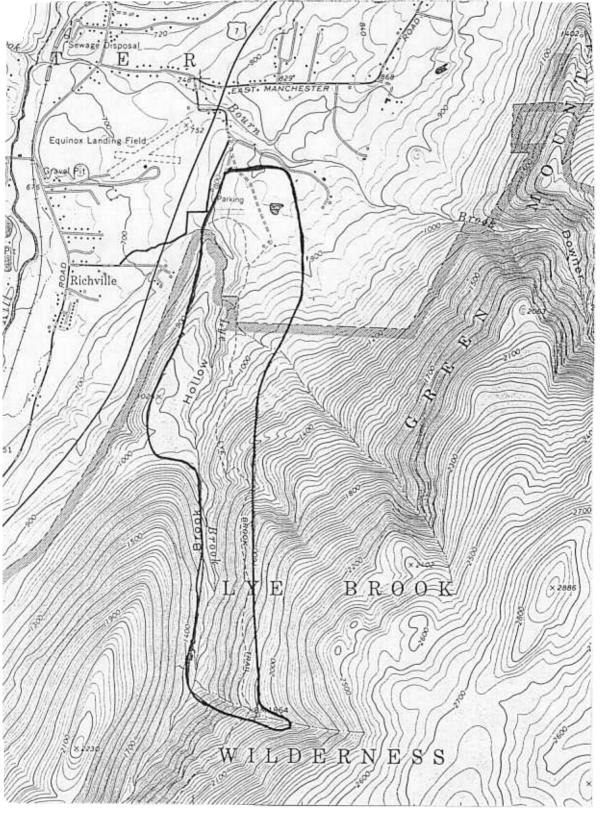


Figure 4b. Areas surveyed during the amphibian inventory of the Lye Brook Wilderness Region.



Figure 4c. Areas surveyed during the amphibian inventory of the Lye Brook Wilderness Region.

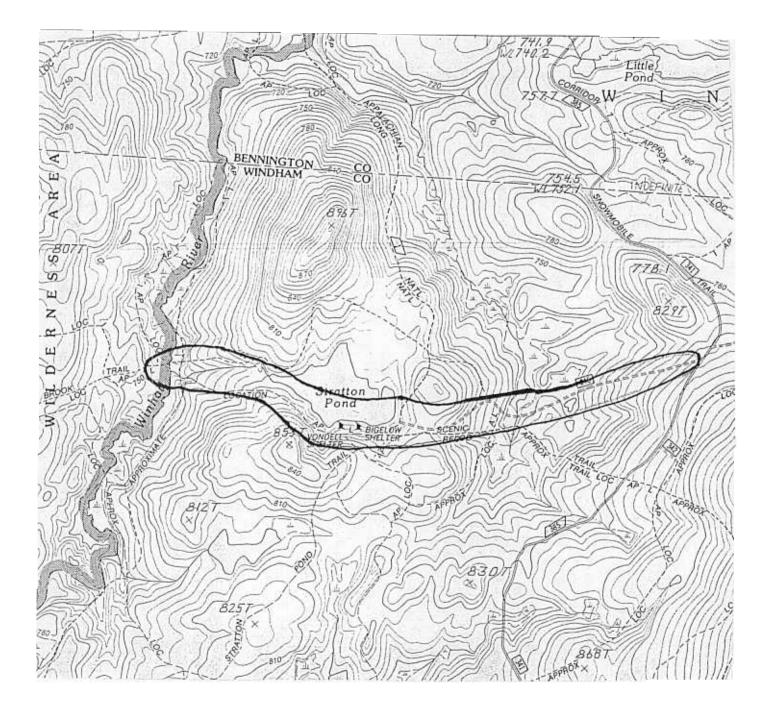


Figure 4d. Areas surveyed during the amphibian inventory of the Lye Brook Wilderness Region.



Figure 4e. Areas surveyed during the amphibian inventory of the Lye Brook Wilderness Region.

masses, 20 choruses of breeding frogs and a variety of amphibian larvae were identified. Aside from scouting and building drift fences, data were gathered on 22 different days, using 6 different methods, producing a total of 55 data gathering efforts between the dates of April 28th and October 31st, 1993.

If amphibian choruses are added into the total number of individuals at the rate of 10 individuals per chorus a more realistic estimate of the relative abundances of the species at this site is generated (Table 2). It is important to keep in mind that these percentages are affected by the relative amounts of effort spent using the different methods. In addition, the time of year during which each method was used also has an effect on which species are most likely to be located. In Appendix B I have shown the results relative to the method, time of year, number of sites and amount of effort. For the purposes of long-term monitoring, results need to be compared between the same methods and standardized for the number of sites and amount of effort.

In addition to the above data gathering efforts, the field teams located sites for and installed three drift fences that are designed to be used as long-term monitoring sites as well as inventory devices. Two of these sites are located on the high plateau south of the wilderness area boundary. A field team of faculty from the Stratton Mt. School were trained to open and check both fences as well as record the data. The third fence is located at an elevation of close to 800 feet in the extreme northwest corner of the region within the National Forest but just outside of the wilderness area. Due to the large size of the region, a separate team of teachers and students from the Burr and Burton Seminary were trained to open and record data from this fence. In order to effectively use drift fences for monitoring, an individual (or group of individuals) needs to be continually on call to make trips to open the fences when the right environmental conditions exist. Although a lot of useful data were collected from the fences in the fall of 1993 and during the 1994 field season, it is inventory data, not baseline monitoring data. In order for data to be useful for monitoring purposes they need to be collected according to a rigid protocol. The 1995 data from the upper two drift fences satisfy this requirement.

Of the many potential breeding sites visited, three sites were selected for long-term monitoring of egg masses. Egg masses of Wood Frog and Spotted salamander were located at these sites. These species are both early spring breeders with obvious and easily identified egg masses. I refer to the original sites selected as Benson Pond, North Alder Dam and Kelly Stand Dam. They are all associated with beaver dams or in beaver meadows, although the Benson Pond site is adjacent to what is apparently a man-made permanent pond. I did not locate sites that meet the criteria of a classic vernal pool in this area. I was not totally satisfied with the sites that I selected, since they were associated with beaver dams or meadows that were subject to manipulation and change over time. Kelly Stand Dam was subsequently inundated due to beaver activity and an alternative site (the pond near drift fence # 2) was selected.

As a result of the need for the long-term monitoring sites to be readily accessible from April through October, under a variety of weather conditions, they were located outside of the wilderness area boundaries. My inventory efforts sampled a wide variety of sites inside the wilderness area and to the best of my knowledge the count sites are representative of the habitats found within it. Table 2. Relative percentages of frogs from the 1993 amphibian inventory of the Lye Brook Wilderness Region in Bennington County, Vermont. Chorusing results are included. Each chorus is counted as 10 individuals and combined with the individual tally. Those species which call for a limited time (Gray treefrog, Woodfrog), under limited conditions (Gray treefrog), or have a weak call (Pickerel frog) are probably under-sampled. The table shows the combined data gathered using six different methods. Included are all metamorphosed individuals (no eggs or larvae). Percent individuals are out of an estimated total of 400 anurans heard or located.

Species	Common name	% of individuals
Anurans (frogs and toad	5)	
Pseudacris crucifer	Spring peeper	41.0%
Rana clamitans	Green frog	29.0%
Bufo americanus	American toad	19.0%
Rana sylvatica	Wood frog	8.5%
Rana palustris	Pickerel frog	2.0%
Hyla versicolor	Gray treefrog	0.3%
Rana catesbeiana	Bullfrog <sup>1</sup>	0.3%

<sup>1</sup>Found only at low elevations.

Table 3. Water test results from the egg-mass counting sites in the Lye Brook Wilderness Region in 1994. A single water sample was taken from each site on the day listed in the table. Water samples were analyzed by Jim Kellogg of the Vermont DEC. The right column shows the mean pH as measured by me for comparison.

Site	Alkalinity - Gran (mg/l) <sup>1</sup>	Color, Total Visual (Pt-Co) <sup>1</sup>	Conductivity (umhos/cm)	pH from DEC	pH from my data
Near Benson Pond					
sample date: 4/26/94	57.0	25	11.5 <sup>1</sup>	7.45	7.3 N = 1
North Alder Dam					
sample date: 5/11/94	0.07	70	. 16.5	5.03	$5.0 \pm 0.3 \text{ SD}$ $N = 2$
Pond near drift-fence #2					
sample date: 5/11/94	0.89	30	19.2	5.86	

<sup>1</sup>According to Jim Kellogg this low conductivity is a result of the "dilutional effect of spring run-off"

Site	Date tested	pH of water, SD	pH of soil, SD
Winhall Brook	5/27/93	$6.7 \pm 0.8 (N = 4)$	
North Alder Brook	7/9/93	$5.2 \pm 0.6 (N = 4)$	
Mill Brook (2nd site)	7/2/93	$5.1 \pm 0.6 (N = 4)$	
The Burning	7/2/93	$4.9 \pm 0.4$ (N = 3)	$4.2 \pm 0.3$ (N = 3)
Small Brook on Trail to Lye Brook Falls	7/8/93	4.9 (N = 1)	$5.8 \pm 0.7 (N = 3)$
Mill Brook (Upper East Branch)	7/2/93	$4.7 \pm 0.4 (N = 4)$	$4.8 \pm 0.8$ (N = 4)
Mill Brook (1st site)	7/2/93	$4.7 \pm 0.4 (N = 4)$	$4.8 \pm 0.8$ (N = 4)
Mud Pond	5/27/93	$4.6 \pm 0.7 (N = 5)$	
Branch Pond	5/11/93	$4.4 \pm 0.8$ (N = 3)	
Small Stream on Branch Pond Trail	5/12/93	$4.0 \pm 0.4$ (N = 5)	$3.7 \pm 0.5 (N = 5)$
Branch Pond Brook	7/1/93	$3.8 \pm 0.5 (N = 8)$	
Lye Brook Falls Brook (above Lye Brook)	7/8/93	$3.6 \pm 0.4 \ (N = 3)$	
Grand mean		$4.7 \pm 0.9$ (N = 12)	$4.7 \pm 0.9$ (N = 5)

Table 4. Water and soil pH values other than those reported previously with the eggmass counts and stream-surveys. Sites are organized in descending order according to the pH of the water.

Table 5. Amphibians caught in 1994 in three drift fences in the Lye Brook Wilderness Region in Bennington County, Vermont. Four species of salamander and five species of frog were caught. Percent individuals are out of a total of 160 caudates and 85 anurans caught. These data are not meant to be used as baseline monitoring data.

Species	Common name	#	% of	% of all
-		caught	group	amphibians
Caudates (salamanders)				,
Notophthalmus viridescens	Eastern newt	62	39%	25%
Ambystoma maculatum	Spotted salamander	49	31%	20%
Plethodon cinereus	Redback salamander	47	29%	19%
Eurycea bislineata	N. two-lined salamander	2	1%	1%
Total caudates		160	100%	65%
Anurans (frogs and toads) Rana clamitans	Green frog	37	44%	15%
Rana sylvatica	Wood frog	35	41%	14%
Pseudacris crucifer	Spring peeper	7	8%	3%
Bufo americanus	American toad	5	6%	2%
Hyla versicolor	Gray treefrog	1	<u>1</u> %	<u>&lt;1</u> %
Total anurans		85	100%	34%
Total amphibians		245		

#### Water and soil tests

Although the buffering potential of the upper two egg-mass sites is very low (alkalinities of 0.07 and 0.89), the buffering potential from the low elevation site near Benson Pond is high (57.0, Table 3). Consequently the pH of the site near Benson Pond (7.3) is well within the safe range for all Vermont amphibians (Freda 1986). The pH of the pond near drift-fence #2 (5.7) is within the safe range for those amphibians presently breeding there, but is approaching the limit for less acid tolerant species found elsewhere in Vermont (e.g., Jefferson salamander). The North Alder Dam egg-mass site, which has essentially no buffering capacity, has a pH of 5.0. This is the pH at which the least acid tolerant species have been shown to be negatively affected. The pH of other surface waters ranged from a low of 3.6 to a high of 6.7 with a mean of 4.7 (Table 4).

The mean pH of all soil measurements was also 4.7 (Table 4). The soil pH was measured at 5 sites and ranged from 3.7 to 5.8. This is higher than the pH measured at other Green Mountain sites in Addison County. The mean pH from three upland sites in the AP / BM Region was  $3.6 \pm 0.0$  SD, N = 3. At another study site of mine in the Green Mountain National Forest in Ripton (Andrews 1995b) the mean pH was  $3.5 \pm 0.4$  SD, N = 60. However, one set of samples was measured in a wetland seepage area in the AP / BM Region. At this site the mean pH was  $6.5 \pm 0.4$  SD, N = 3. The mean soil pH from a study site in the northern Taconic Mountains in Orwell, Vermont was  $4.5 \pm 0.7$  SD, N = 60 (Andrews 1995b). Soil pH values from within the Lake Champlain Basin in Addison County were less acidic. The mean soil pH from one study site on the south end of Snake Mountain in Bridport was  $5.7 \pm 0.9$  SD, N = 45.

#### Inventory results from the 1994 and 1995 field season

## <u>Drift fences</u>

Four species of salamander and five species of frog were caught in the driftfences in 1994 (Table 5). Eastern newt, Spotted salamander and Redback salamander made up 99% of the salamander species caught. Green frog and Wood frog made up 85% of the frogs and toads caught. All species had been found previously in 1993. Drift fences like any method create biases in the relative numbers of each species caught and results should only be compared to other drift-fence data sets collected according to the same protocol. The 1994 data from the drift-fences was not collected according to monitoring protocols (Appendix A) and hence it is not meant to be used as baseline monitoring data nor is it directly comparable to drift-fence data from other sites.

The 1995 results from the low-elevation drift fence (Table 6) show a species of frog (Pickerel frog) that was not seen at any fence in 1994. Also shown is a low percentage of Green frogs and a complete absence of the Northern two-lined salamanders. This fence is much farther away from water than the other two drift fences. I suspect that the latter two species don't travel as far from their breeding sites as the other water-breeding amphibians which were caught. Table 6. Amphibians caught in 1995 in the low-elevation drift fence in the Lye Brook Wilderness Region in Bennington County, Vermont. Three species of salamander and five species of frog were caught. These data are not meant to be used as baseline monitoring data.

Species	Common name	# caught	% of group	% of all amphibians
Caudates (salamanders)				
Notophthalmus viridescens	Eastern newt	87	62%	54%
Plethodon cinereus	Redback salamander	49	35%	30%
Ambystoma maculatum	Spotted salamander	4	3%	2%
Total caudates		140	100%	87%
Anurans (frogs and toads) Rana palustris	Pickerel frog	11	52%	7%
Rana sylvatica	Wood frog	4	19%	2%
Bufo americanus	American toad	4	19%	2%
Pseudacris crucifer	Spring peeper	1	5%	<1%
Rana clamitans	Green frog	1	5%	<1%
Total anurans		21	100%	13%
Total amphibians		161		

Table 7. Combined totals by species from accidental finds, night-time visits, and site checks in the Lye Brook Wilderness Region from April 1994 through September 1995. Individual amphibians may have been counted more than once as a result of repeated visits to egg-mass and drift-fence sites.

Species	# of individuals	# of choruses	# of egg- masses
Salamanders			
Eastern newt	813 <sup>1</sup>	T I	
N. two-lined salamander	5	-	-
Spring salamander	2	T 1	-
Spotted salamander	1		
Frogs and toads			
Green frog	-	6	
Spring peeper		- A	
American toad		_	1
Wood frog	10		
Pickerel frog	4		
Gray og	2		

<sup>1</sup>Total includes references to "many" which were seen in the ponds while counting egg-masses. For this species on this table "many" were interpreted as 50 individuals. I suspect this is a conservative estimate.

 $^{2}$ One reference to "many metamorphs" was interpreted as 20 individuals.

## Other methods and accidentals

Night-time visits were made on June 8 and June 13, 1994 in an effort to locate calling Gray treefrogs. Five species of frog were heard calling: Spring peepers, Green frogs, Pickerel frogs, American toads, and Gray treefrogs. Only two Gray treefrogs were heard. Two other amphibians species were seen: Wood frog and Northern two-lined salamander.

A single site check on July 18 did not locate any additional species. The combined results from these two methods and all accidental finds from both 1994 and 1995 are shown in Table 7.

#### A Spring salamander egg mass

In 1994 a Spring salamander egg mass was found within one of the transects on Branch Pond Brook during the stream survey. Since egg masses of this species are rarely found and have not been described in the literature I will describe it here. On July 18, 1994 a mass of 73 eggs was found attached to the underside of a partially-submerged flat rock in the middle of the brook. The rock was 40 cm x 50 cm x 8 cm. Each egg was individually attached to the rock. The entire group covered an area of 13 x 15 cm. Three eggs were measured and found to be 14 x 14 mm, 14 x 12 mm and 13 x 14 mm. The rest were approximately the same size and shape. Those which were slightly oval were distended along the same axis as the lengths of the embryos inside them. Embryos were creamyyellow, approximately 14 cm long, and yolk sacs were visible. Some, but not all, of them moved when the eggs were touched. An adult was found under the same rock with the egg mass. It stayed within the depression created by the rock. Most adults quickly fled to other cover when disturbed. The site was revisited 8 days later on July 26, 1994. Only 25 of the original 73 eggs remained. The two eggs measured were 18 x 13 mm and 16 x 13 mm. Some of the embryos were tightly curled into a 6 mm ball while others remained straight. An adult was again under the rock with them but this time the adult fled quickly when the rock was disturbed.

#### **Diseased Eastern newts**

Specimens of diseased Eastern newts were originally discovered in 1993 at Upper Abbey Pond in the Green Mountain National Forest in Addison County, Vermont. They were collected in June and sent to Dr. D. Earl Green D.V.M. at the Animal Health Diagnostic Laboratory in College Park, Maryland. Seven specimens that showed external evidence of the disease were sent along with 4 specimens from the site with no apparent symptoms. In addition a control group of 3 newts from a pond approximately 15 miles away in the Lake Champlain Valley were sent as a control group. A copy of his final pathology report is included in its entirety in Appendix C. A paper will be published on this disease in the near future. In brief, on June 28, 1994, five adults were found dead at this pond. Four of them showed the characteristic growths of this disease. On July 14, 1994, 7 adults were found dead at this site. Three of them showed the characteristic signs of this disease. On June 28 and 29, adult newts were both netted and trapped from the pond. Of 72 netted, 12 (17%) showed signs of the

## Monitoring results from the 1994 and 1995 field seasons

# **Drift-fences**

The combined results from the two upper drift-fences can be used both as baseline data for the first full year of monitoring and as a basis for comparison with other sites where the same protocol has been used (Table 8).

The three most frequently caught salamanders in descending order were Eastern newt (52%), Spotted salamander (36%), and Redback salamander (8%).

The three most frequently caught frogs (and toads) were Wood frog (41%), Green frog (34%), and American toad (22%, Table 8).

The most frequently caught amphibians in descending order were Eastern newt (29%), Spotted salamander (20%), and Wood frog (18%). The least frequently caught amphibians in ascending order were Spring peeper and Northern twolined salamander (2% each), and Redback salamander (5%).

At least 67% of all American toads and 63% of all Spotted salamanders caught were young of the year. None of the Northern two-lined or Redback salamanders and very few Wood frogs (4%) were young of the year (Table 8).

#### Egg-mass counts

The most useful figure that can be generated from these counts for monitoring purposes are the maximum counts of egg masses for each of the two species monitored (Table 9). The first two years of monitoring show a large amount of variation in the numbers of egg masses of both species between years and between sites. At the Benson Pond site, the number of egg masses of both species dropped. At North Alder Dam the number of Spotted salamander egg masses increased dramatically (200%) while the numbers of Wood frog egg masses decreased dramatically (99%). At the pond near drift fence #2 the numbers of Spotted salamander and Wood frog egg masses both increased dramatically. The pH values at all of the sites varied little from 1994 to 1995.

#### Stream surveys

I have collected two years of stream survey data (Table 10). Although it is too early to look for any meaningful trends, the number of Spring salamanders dropped from 10 in 1994 to 6 in 1995 while the numbers of Northern two-lined salamanders found dropped from 11 to 1. The pH in both years was higher than that measured in 1993 [ $3.8 \pm 0.5$  (N = 8)], but still very acidic. disease. Of 69 trapped, 9 (13%) showed signs of the disease. One of these was dead in the trap. On July 14, 1994 of 66 netted, six (9%) showed the characteristic growths. Of 66 trapped, 6 (9%) also showed signs of the disease. By October 25, 1994 however, of 183 newts caught at Upper Abbey Pond only 4 (2%) showed signs of the disease.

Although Dr. Green's report states that the swellings are probably the result of a fungus (*Aureobasidium pullulans*), he later informed me that this organism was probably a contaminant. He now believes that the infecting organism is most likely Ichthyophonus or an Ichthyophonus-like fungus. The external symptoms of this disease had been described once in newts from Sleepy Creek Lake in West Virginia by a Dr. Herman, but the cause of the disease was not correctly identified. This fungus was not found in any of the control group of newts from the Lake Champlain Valley, but was located in two of the newts from Upper Abbey Pond which appeared symptom-free in the field. Dr. Green's report suggests that these two newts were recovering from the fungal infection.

A small group of four visibly diseased newts was taken into my lab along with four healthy control newts from the Lake Champlain Valley-site. Three of the four diseased newts died in the lab. All four of the control group survived. Although the small numbers sampled can not generate a statistically significant result, the result does suggest, as do Dr. Green's findings, that while some newts do die as a result of this disease, others can survive it. This disease may be spread by leeches.

I have now located newts with these symptoms in Nebraska Notch on Mt. Mansfield in the north-central Green Mountains, Silver Lake in the central Green Mountains, and in North Alder Dam here in the LBW Region. So far, I have only found newts with these symptoms at elevations above 1,200 ft.

# Other species found during the inventory from 1993 through 1995

While in the field during the course of this inventory, many species other than amphibians were seen and identified. Some of these species were recorded in my notes. I have included a list of those species in Appendix D. It is not meant to be inclusive. Moose tracks and sign were often seen while working in the region. A Common loon was seen on Bourn Pond on May 12, 1993 and an Osprey was fishing on Branch Pond on May 11, 1993. A Blue-gray gnatcatcher was seen at Benson Pond during spring migration on April 26, 1994 and Rusty blackbirds were seen on June 14, 1994 in a boggy area between Branch and Bourn Ponds and on June 9, 1995 at North Alder Dam. The Common garter snake was the only species of reptile seen in the region.

Pitcher plants were found east of Mud Pond and near Branch Pond. Cotton grass was found in The Burning. Early azalea was in bloom near Branch pond on June 16, 1993. Table 8. Monitoring results from the upper two drift-fences in the Lye Brook Wilderness Region during 1995. Three trappings per month in April, May, June, July, September, and October are included (18 trappings).

Species name	# of all ages	# of young of the year	% young of the year <sup>1</sup>	date of first meta- morph <sup>2</sup>	largest adult (total length)	# per trapping <sup>3</sup>	% of group	% of total catch
Salamanders								
Eastern newt	228	384	17%	July 2	98	12.7	52%	29%
Spotted salamander	156	63	40%	Sept. 8 <sup>2</sup>	206	8.7	36%	20%
Redback salamander	36	0	0%		90	2.0	8%	5%
Northern two-lined	15	: 0	0%		95	0.8	3%	2%
Group totals	435	101	23%			24.2	100%	56%
Frogs and Toads								
Wood frog	147	6	4%	Sept. 10 <sup>2</sup>	70	8.2	41%	18%
Green frog	122	39	32%	July 2	81	6.8	34%	15%
American toad	78	67	86%	May 25	86	4.3	22%	10%
Spring peeper	14	2	14%	June 25	35	0.8	4%	2%
Group totals	361	114	32%			20.0	100%	45%
Amphibian totals	796	215	27%			44.2		100%

<sup>2</sup>No trapping took place in August.

<sup>3</sup>Number per trapping are rounded to the nearest 0.1. All other figures are rounded to the nearest whole number.

<sup>4</sup>Three individuals below the 45 mm cut-off length were caught very early in the spring. This suggests that they either overwintered at a very small size or overwintered as larvae and metamorphosed in the spring. They are not included in the young of the year category.

# Discussion

# Discussion of the inventory results

The overall diversity found at this site fits well with a pattern that has emerged as a result of work in Addison, Rutland, Chittenden, and Bennington Counties (Andrews 1988a, b, 1990, 1992, 1994, 1995a, b; Trombulak 1994).

The species list generated (Table 1) is almost identical to that of the two other central Green Mountain sites where I have data sets (Mt. Mansfield and the AP / BM Region). The proportions of each species differ, but the relative percentages are dependent on the mix of methods used for the inventory. A more direct comparison of drift-fence results will be available after the 1995 field season results from all three sites have been compiled.

The Vermont amphibian species which were not found at this site (Table 11) are either very rare, localized in other regions of the state, or found in habitat types which were not found in this region. Compared to sites outside of the Green Mountains, but still in western Vermont, this site has a lower diversity of amphibian species. Whether this is a result of the decreased buffering capacity of the soils and bedrock, habitat types, elevation, or other direct or indirect effects of a change in microclimate is not yet clear.

## Missing frogs

The Western chorus frog has only been located along the northern end of Lake Champlain and even there it has not been located in this decade. The Northern leopard frog appears to be missing from large areas of southern Vermont and never has been found at high elevations in Vermont. It seems to prefer large permanent water bodies with extensive vegetation at low elevations. The Mink frog would have been a remote possibility at this site. It is a northern species that is frequently found within the belt of northern conifers in northeast and north central Vermont. It has not been reported from this far south in Vermont, although the habitat and local climate appear to be suitable.

#### Missing salamanders

The absence of Four-toed salamanders, Jefferson salamanders and Bluespotted salamanders from the three Green Mountain sites I have surveyed suggests that they are entirely limited in distribution to foot hills and floodplains. The Blue-spotted salamander seems to prefer wooded lowland floodplains. The Jefferson salamander seems to prefer less acidic soils and waters in low- to midelevation oak-hickory woodlands. The Four-toed salamander has been found in association with the Blue-spotted and the Jefferson salamander but like them not at high elevations. I had thought that if I was to locate them at all in the Green Mountains, it might be on the south and west flank of the mountains in an area of oak woodlands. Clearly most of this region is not that type of woodland, but I did locate one of the drift fence and egg mass sites at only 800 feet in elevation, on the extreme western edge of this region. In addition, I performed site checks and active searches along the western border but I still did not locate any of these Table 9. Maximum counts of egg masses from monitoring locations in the Lye Brook Wilderness region in 1994 and 1995. At the site near Benson Pond the entire pond is surveyed. At North Alder Dam a four-meter strip around all of the pond except the swampy north end is surveyed. At the Pond Near Drift Fence #2 a four-meter strip around the entire pond is surveyed.

Site	Spotted salamander	Wood frog	Mean pH <sup>2</sup>
Near Benson Pond			
1994 count dates: 4/26, 5/10, 5/25	10	67 <sup>1</sup>	7.3 (N = 1)
1995 count dates: 4/24 <sup>2</sup> , 5/12	3	19	6.8(N = 1)
North Alder Dam			
1994 count dates: 5/11, 5/25, 6/8	97	225	$5.0 \pm 0.3$ SD (N = 2)
1995 count dates: $4/24^2$ , $5/12$ , $6/9$	292	3	$5.1 \pm 0.4$ SD (N = 2)
Pond Near Drift Fence #2			
1994 count dates: 5/11, 5/25, 6/9	6	3	$5.7 \pm 0.3$ SD (N = 2)
1995 count dates: 4/24 <sup>2</sup> , 5/12, 6/9	70	152	$5.6 \pm 0.4$ SD (N = 2)

<sup>1</sup>Hatched by May 10

 $^{2}$ All readings taken on April 24, 1995 were believed to be erroneus and are not included in the mean. Each reading used in the average is itself composed of three measurements taken from different areas of the ponds. All pH means have been rounded to the nearest 0.1.

Table. 10. The 1994 and 1995 combined results of three 50 m stream transects in Branch Pond Brook in the Lye Brook Wilderness Region. Only adult *Gyrinophilus porphyriticus* (Spring salamander) and *Eurycea bislineata* (Two-lined salamander are included in the table. All other species and larvae are excluded.

Year	Spring salamander	Two-lined salamander	pH <sup>1</sup>	Water temp. in °C <sup>1</sup>	Max. water depth <sup>2</sup> in cm
1994	•				-
(7/18/94)	10	11	$4.9 \pm 0.2$ (N = 3)	17.4	20
1995					
(7/24/95)	6	1	$4.4 \pm 0.5$ (N = 5)	17.4	26

<sup>1</sup>Temperature and pH were taken two meters downstream from the downstream end of the first transect. Temperature was taken only once in 1994 and is the average of three measurements in 1995.

 $^{2}$ Reference point is the deepest point between the two large rocks which constrict the channel approximately two meters downstream from the beginning of the first transect.

Table 11. Vermont amphibian species that were not found in the Lye Brook Wilderness Region. With the exception of historical records of the Mink frog (northern Vermont), none of these species have been reported from any mid- to high-elevation site in the Green Mountains.

Species	Common names
Salamanders	
Ambystoma jeffersonianum	Jefferson salamander
Ambystoma laterale	Blue-spotted salamander
Hemidactylium scutatum	Four-toed salamander
Necturus maculosus	Mudpuppy
Frogs	
Pseudacris triseriata	Western chorus frog
Rana pipiens	Leopard frog
Rana septentrionalis	Mink frog

Table 12. A comparison of the relative abundances of amphibians found in the Lye Brook Wilderness Region and the Abbey Pond / Beaver Meadow Region. Data used are from the drift fences, site checks, and active searches during the 1993 inventories of the two regions. The results have been altered in an effort to adjust for differing amounts of effort devoted to each of the three methods. The active search results from the Lye Brook Wilderness Region have been multiplied by 2. Site check results by 1.7 and drift fence-nights x 1.23. This data set was generated only for comparison purposes and should not be interpreted as the actual abundance of the species at each site.

Species	s Lye Brook Wilderness Region		Abbey Pond / Beaver Meadows Region						
Salamanders									
Eastern newt	436	49%	583	61%					
N. two-lined salamander		23%		7%					
Redback salamander		17%	140	15%					
Spring salamander		7%	2	<1%					
Spotted salamander		2%	59	6%					
Dusky salamander	-		102	11%					
Total				~100%					
Frogs	t	l							
Green frog	104	49%	114	53%					
American toad	40	19%	20	9%					
Wood frog	28	13%	54	25%					
Spring peeper	26	12%	22	10%					
Pickerel frog	13	6%	6	3%					
Gray treefrog	0	0%	1	<1%					
Bullfrog	<u> </u>	0%	0	0%					
Total	211	~100%	217	~100%					

species within the LBW Region. I did locate one individual of the Blue-spotted salamander group in the Batten Kill Valley less than 1 km west of the wilderness area.

The Mountain dusky salamander (*Desmognathus ochrophaeus*) has been reported from one site in central Vermont. The individual was an immature specimen that remains the only report of this species in Vermont. It is a species which can tolerate generally drier conditions than its close relative the Dusky salamander. Due to the scarcity of records for this species I have not included it in Table 11. However, there are many records from central New York State and it is a species which may (again?) be found in Vermont. Habitat which appears appropriate for the Mountain dusky salamander does occur within the region.

Mudpuppies would be expected primarily in Lake Champlain, the Connecticut River and their major tributaries, hence not at this site.

## Frogs limited in abundance

Two frog species found within the wilderness area appear to be much more abundant within the Batten Kill floodplain: Bullfrog and Gray treefrog. Only one Bullfrog was found within the wilderness area. It was calling from Benson Pond near the access to Lye Brook Falls. I have not located Bullfrogs above 1200 ft. at any of my Green Mountain Study sites. In 1993 I located a single Gray treefrog inside the wilderness area. Despite efforts designed specifically to locate this species only three more were located during 1994 and 1995. I strongly suspect that there are small populations of this species within the wilderness area that I was unable to locate due to their arboreal habits and limited calling period.

The pickerel frog on the other hand appears to be more abundant in the uplands than in the broad river valleys, but even in the uplands it is one of the rarest frogs at any of my Green Mountain study sites. It was not caught in any of the drift fences in 1993 or 1994 and it was caught in only the Falls Access driftfence in 1995. Outside of this corner of the survey area I located a very few of them using other methods near Stratton Pond, Bourn Pond, and Winhall Brook.

#### Salamanders limited in abundance

The Dusky salamander is not a common salamander anywhere in Vermont. It seems to prefer cool, well-shaded, permanent seepage areas or stream edges with an substrate that contains a lot of organic matter. Although I have occasionally found streams or seepage areas where this species is quite abundant, its distribution within a study region is often spotty. I found a very few scattered pockets of this species in this region. Most of them were found at low elevations near the base of Lye Brook (8 out of 11 individuals), one was found near Stratton Pond, and two along Winhall Brook.

#### Comparisons between this region and the AP / BM Region.

The most responsible comparison that can be made from the 1993 inventory data between the LBW Region and the AP / BM Region involves balancing the relative effort of three methods: drift fences, site checks and active searches. For example if the amount of person-hours spent in active searches in the LBW Region was half that spent in the AP / BM Region, the amphibian numbers should be doubled for that method before they are compared. Once the figures for each method are standardized (active searches x 2, site checks x 1.7 and fence-nights x 1.23) they can then be combined and compared. The results of balancing the effort are shown in Table 12.

At both of the sites the high percentages of Green frog and Eastern newt are noteworthy. Research at other sites in the northeast would lead one to expect that the salamander found in greatest abundance would be the Redback salamander rather than the Eastern newt (Burton and Likens 1975, Wyman pers. comm.). I can think of four possible factors that may be the cause of the results at these sites.

1. The Redback salamander has been shown to be limited by soils with a pH value < 4.0 (Wyman and Hawksley-Lescault 1987) whereas the Eastern newt has been shown to be quite tolerant of a variety of substrate pH levels (Wyman and Jancola 1992). The mean soil pH from five sites in the LBW Region was generally acidic [ $4.7 \pm 0.9$  (N=5)].

2. The Redback salamander has been shown to be most abundant in areas of more mature hardwoods (>70 yrs.) (Petranka et al 1993) and most of this area has been regularly logged, some of it relatively recently.

3. The amount and distribution of permanent and semipermanent standing water in this area, although relatively acidic, provides good breeding sites for Eastern newts. In contrast Redback salamanders breed in or under fallen trees and among tree roots. Many of the large or rotten hardwood trees and branches that might have fallen and provided breeding sites for this species have been removed.

4. The Redback salamander has been shown to prefer the litter of broadleafed forests over coniferous forests (Heatwole 1962). Large segments of this region are covered with dense spruce-fir forests.

Wood frogs are the species of frog that I would expect to be most abundant in the northern hardwood forests of the state. In this region and in the AP / BM Region the apparently higher abundance of Green frog is probably the result of abundant breeding sites with permanent water. The tadpoles of green frogs overwinter in ponds, lakes, and beaver dams. Without permanent water they can not survive. The wood frog tadpole on the other hand is found in temporary pools and metamorphoses by the end of summer. Consequently, the abundance of permanent water in this area is probably one of the main reasons for the higher percentage of Green frogs. Another possibility could be the existence of fish in the larger breeding sites. Predation on Wood frog larvae from fish, either natural or introduced, is presumed to be a large part of the reason that they usually breed in temporary ponds where fish can not survive. Green frogs, on the other hand, are frequently found in lakes, ponds, and streams with fish populations. This suggests that Wood frogs are less tolerant of the presence of fish than Green frogs. Introduction of fish to these ponds may have helped to increase the relative abundance of Green frogs relative to Wood frogs.

Although the American toad is the second most frequently found frog in this comparison (Table 12), I do not believe that it is actually the second most abundant in the region. American toads are more active during the day and in hot weather than both Wood frogs and Spring peepers. Spring peepers are most effectively located during calling surveys. I believe that the site checks and active searches would be biased in favor of American toads relative to Wood frogs and all three methods would be biased in favor of American toads relative to Spring peepers. I believe that the results at the drift fences in 1994 (Table 5), which show the American toad caught at one seventh the rate of Wood frogs, are a less biased comparison. The data do suggest that the American toad is relatively more abundant in the LBW Region than in the AP / BM Region.

Equally surprising in this comparison were the low numbers of the Spotted salamander and the Dusky salamander relative to the AP / BM Region. As expected the drift-fence results from 1994 and 1995 (Tables 5 & 8) show the Spotted salamander as the second most frequently caught salamander. In the case of the Dusky, on the other hand, I have had only limited success in locating breeding sites in the LBW Region.

The area surveyed in the AP / BM Region contains less of the appropriate habitat type for the Northern two-lined salamander than the LBW Region. In the LBW Region the Northern two-lined salamander makes up 23% of the salamanders found. It is second in abundance only to the Eastern newt. While in the AP / BM Region, it makes up only 7% and ranks fourth in abundance using these methods. Once one moves off the central plateau of the LBW Region, there are many fast well oxygenated brooks and streams. These streams were included in the area surveyed in this region. This was not the case in the AP / BM Region. The area surveyed in the AP / BM Region contains only one short section of this habitat type. These streams are the favored habitat of this salamander. The hypothesis that the large numbers of the Northern two-lined salamanders in the LBW Region are a result of the larger percentage of this habitat type in the area surveyed is further supported by the greater relative abundance of the Spring salamander in the LBW Region. In the AP / BM Region it is the least common salamander at only 0.2% while in the LBW Region it comprises 7% and is 4th in abundance. This large stream predator is found in the same habitat type as the Northern two-lined salamander.

Comparisons between two large regions, although useful, are less reliable in showing small proportional differences than comparisons between the same sites over time.

#### Discussion of the monitoring results

It is too early to look for any meaningful trends in any of the monitoring data. I believe that at least three years of monitoring data need to be collected before I can claim to have even a snapshot in time for future comparisons. Year to year variation is to be expected for some species as a result of natural factors such as the amount and distribution of precipitation and overwintering conditions. Ten years of monitoring data would begin to reveal any long-term trends of these populations.

The Eastern newt and the Spotted salamander made up 88% of the salamanders caught at the two drift fences (Table 8). It is possible that the

Redback salamander is under-represented in this sample. Both the Eastern newt and the Spotted salamander breed in water. The young disperse from the breeding sites after metamorphosis from their larval stages. In contrast, the Redback salamander breeds on land. The migration of metamorphs of both the Spotted salamander and Eastern newt away from the breeding site combined with the migration of adults of the Spotted salamander to and from the breeding site may skew the results of these two salamander species relative to that of the Redback salamander moves across the surface less often, it would be less likely to be caught in a drift fence. At one site in Addison County (Andrews 1995b), I saw evidence of fall movement of the Redback salamander. This may or may not be taking place here. If it is, it would help to balance the skewing of the relative abundance figures. This potential bias is not a concern for monitoring purposes since I am primarily interested in year to year comparisons within species rather than comparisons between species in a given year.

Based on the large number of metamorphs caught (Table 8), the Spotted salamander seems to have had a successful breeding year at these sites, despite the early summer drought. Perhaps the increase in the number of egg masses of this species over last year (Table 9) helped compensate for those eggs and larvae which dried up.

Young of the year of the Northern two-lined salamander are not showing up in this sample. Perhaps, unlike the adults, the young remain near their breeding sites (springs and brooks).

The absence of Redback salamanders under 32 mm is a surprise. This suggests that this species did not have a successful breeding year in the LBW Region. In the 1994 results from the AP / BM Region (Andrews 1995c), 10 percent of this species were under 32 mm. Although none of the Redback salamanders caught in the LBW Region this year were under 32 mm, slightly larger salamanders were caught. This could be intrepreted in a couple ways: the young of the year might have been growing faster and achieving a larger size sooner, or there was limited breeding success this year and the slightly larger individuals were last years cohort which grew slowly. Close examination of the data from a series of years will help clarify results such as these.

Based on the number of young caught (Table 8), both the American toad and the Green frog appeared to have had a successful breeding year, while the Wood frog seemed to produce relatively few young. The number of egg masses of the Wood frog counted in 1995 compared to 1994 (Table 9) did not show a consistent trend. Wood frog egg masses decreased near one drift fence and increased near the other. One might expect that the early drought would have limited larval metamorphosis of this species which is an early spring breeder. However, the Spotted salamander is also an early spring breeder and they successfully produced many young.

As a result of its climbing abilities, I believe we catch a small percentage of the number of Spring peepers in the vicinity of the fence. Therefore, I hesitate to make any judgements on its breeding success based on this data.

The relative success of a breeding year for any species at this site will become clearer as the number of years of data increases and comparisons can be made between years. I suspect that the slight drop in the number of Spring salamanders (10-6) found in the stream surveys between 1994 and 1995 (Table 10) is a result of normal sampling variation. However, the drop in the number of Northern two-lined salamanders from 11 to 1 bears watching.

Although the data from the Falls Access drift fence was not collected according to the monitoring protocol (no May data, Table 6), the appearance of 11 Pickerel frogs is remarkable considering none were caught in the previous year at any of the three drift fences (Table 5). All individuals were less than 37 mm in total length and all were caught in September. Apparently this species had a successful breeding season at the Falls Access site.

#### Context

Very little data had been collected on Vermont reptiles and amphibians until the late 1980's. What little had been collected had not been compiled and presented in a document. Earlier this year the first compilation of reptile and amphibian records from Vermont was published (Andrews 1995a). The data collected in this inventory and in inventories of the Abbey Pond / Beaver Meadow Region (Andrews 1995c), Mt. Mansfield (Trombulak 1994), Addison County (Andrews 1990, 1995b) and parts of Franklin (Andrews 1992) and Rutland Counties (Andrews 1988a, 1988b) were all very important components of that report. Additional records of reptiles and amphibians still need to be gathered from many parts of the state to establish the present distribution of these species.

Global amphibian decline has recently become a subject of scientific concern (Blaustein and Wake 1990, Bishop and Pettit 1992, Vial and Saylor 1993). Coordinated monitoring programs have been called for in order to identify the current status and projected trends for this important group throughout their range. In addition to providing the initial amphibian inventory of the LBW region and establishing a monitoring program to track their populations over time, this effort is part of a larger monitoring network in Vermont. Three long-term monitoring regions have been established within the Green Mountains of Vermont (LBW Region, Mt. Mansfield, and the AP / BM Region). Data from all three regions will allow us to see if any amphibian species are declining in the Green Mountains of Vermont. In addition, it will allow us to see if population variations are local or state-wide.

#### **Future plans**

If funding continues, I hope to continue monitoring amphibian populations at the three Green Mountain sites for at least the next three years and hopefully the next eight years. In addition, funds are being sought to inventory reptiles and amphibians in additional areas of the state and gather records from other interested and knowledgable Vermonters. If funded, a state-wide education effort will also be implemented to raise awareness of these species and to encourage as many people as possible to contribute records and play an active role in reptile and amphibian conservation.

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# Appendix A

Protocols

#### Guidelines for the Use of the Drift-fences

#### When

I would like to get the traps open three times per month for the months of April, May, June, July, September and October. In many months you won't get more than three nights of good activity, so you can not afford to pass up the right conditions very often. Having a back-up person who can open the traps when you can't is essential.

Amphibians are most active at night during and immediately after heavy rains. In the early spring it is possible to get activity associated with a wet snow, particularly if the spring is a little later than normal. Even if it stops raining well before dark, if the surface of the ground is still wet, and the soil and vegetation are saturated, activity could take place. Ideal conditions are a solid steady rain that starts during the day and continues well into the night. In the summer, thundershowers that occur during the evening, during the night, or late enough during the day so that the ground and vegetation are still wet can be good. Frequently you have to gamble that good weather conditions will develop after you open the traps. With the help of the weather radio you often need to make an educated guess on whether or not good conditions are likely to occur over night. Generally I don't open the traps if the ground is still dry and the chance of rain is under 70%. You will occasionally open the traps on dry nights by mistake. It is to be expected. Turn in the data sheets for these nights, but try to get an extra trapping in as well.

On occasion I have gone for a whole month without having good trapping conditions. The best you can do in that situation is open the traps in less than ideal conditions. If things have been really dry for a while and it looks like it may continue that way, open the traps after a lighter rain than you normally would. Also if, for example, you trapped successfully only two times in April, get in an extra trapping in early May. Sometimes there is nothing that you can do about it. It just doesn't rain. Remember that the weather at the trap site may be different from the weather where you are located. Often the higher elevation sites get rain or at least moisture that passes over the valleys.

When necessary, spread your trap-nights out. I am not suggesting that you skip a perfect night, but, if in April (for example), you had three successful trappings in the first week, add an extra trapping later in the month. We don't want to entirely miss the relatively short breeding periods of some species.

I have been impressed with the ability of some species to migrate and breed when there are still patches of snow on the ground and ice on the ponds. These early migrations are particularly important to catch. Some of our species have evolved a strategy of breeding in temporary pools that may dry up over the summer. In order for them to produce young, they need to get their eggs in the pond as early as possible. The first rains of spring will bring out spotted salamanders, wood frogs, and a few other early spring breeders that we may not be fortunate enough to trap later in the year. In the Lake Champlain Valley this activity sometimes starts as early as late March. At lower elevations that are exposed, it usually begins by the second week of April. At very high or shaded sites breeding activity may not begin until early May. As soon as some of the ice has melted around the edges of ponds or around the bases of trees in the swamps, breeding could begin. If large areas of ground are free from snow, and moisture can reach the wintering amphibians in the soil, some of them will move. Some of the sites will be hard to get to in the spring. Get there as early as you safely can.

#### How

When you open the traps make sure the bottom of the snake traps (screening) are placed flush with the fence, covered, and weighted down. Plug all other avenues of travel with leaves or soil debris to direct the amphibians into the traps. Spread a few leaves in the entrance to the traps as well.

Remove any branches that have fallen on the fence, as well as vegetation that has grown next to it. We don't want any bridges which might allow amphibians to get over the fence.

Open the traps before dark or very soon after and check them the next day.

It is safer, much more efficient, and more fun, to open and check traps with a partner. If you can't go with a partner, at least let someone know when and where you are checking or opening traps. Many fences are remote and accidents can happen.

When checking the pit-traps check under the funnel rims carefully. In some traps this requires a pencil or sharp stick to clear the area that your fingers are too large to reach. The bottom of the trap needs to be very carefully checked by sight and by feel. Small salamanders such as redbacks can easily swim around your fingers as you feel in the water. Use a cup to bail as much water as possible. In any remaining water move your fingers around in circles while touching the bottom and corner of the can in one direction (say clockwise) than quickly change to the other direction (counter-clockwise). Feel carefully for any momentary contact with anything in the trap. Remove all leaves and debris that your fingers come into contact with. Look for salamanders that may be half way up the sides of the can as well.

In the snake traps very carefully check the corners around the funnels at both ends. Small salamanders curled in these corners are easy to miss. Remove all debris, spiders, slugs, etc. that may accumulate in the traps.

Cover all traps securely, snapping the covers into place tightly. Put a rock on a lid if you think that it might not be secure. Hang the snake traps in the trees by the clips with the port open. We don't want to catch and kill creatures by accident while we are not using the fences.

#### Data

Take down data carefully while you are checking the pit traps. Do not wait until after you have checked a few. Do not risk forgetting or confusing some of the data.

Carefully identify all reptiles and amphibians caught in the traps. If you have any doubt at all about the identity of a species, put it in a plastic container with wet paper towels and take it out so that we can look at it. Store them in a cool dark place. Label the container with a wax pencil so that you will be sure to remember where you found it. Any species that is not listed on the species list as either common or occasional at that site should be put in a plastic container as well. We need to verify any new or unusual species ourselves.

Identify species by the first letter of their genus name (capitalized) and their full specific epithet (not capitalized) for example; A. maculatum. Familiarize yourself with the measuring protocol (see the separate information sheet). Keep a running tally of the numbers of each size group of each species. When you are finished at the site, total up each size group, write the total at the end of the line, and circle it.

Fill out all appropriate blanks on the data sheets; remember, some don't apply to your situation (water temp., person time, specific location, and habitat type). Take the air temperature in the shade. Leave the thermometer in place for at least five minutes. Describe the weather conditions over the last 24 hours with particular emphasis on the timing and amount of rain; for example; (a heavy rain started yesterday around noon and continued all night). If you have some idea of how much rain fell, by all means put it down, but this is not critical.

Create a section near the end of your data sheet titled <u>Accidentals</u>. In this section list all amphibians and reptiles that you found while checking the drift fence that were not in the traps themselves. Individuals that were found in the mouths of traps, along the fence, or that were seen or heard on the way to or from the fences should be listed here along with any accompanying details.

Also create a section at the end of your data sheet titled <u>Other Species</u>. In this section list (or describe) species, other than reptiles or amphibians, that were caught in the traps. With a little practice you should be able to identify the small mammals in the traps (see the separate mammal identification sheet). We will help you learn to identify these. They usually fall into the basic categories of voles, mice, jumping mice, short-tailed shrews, and other shrews. Any unknown or unusual small mammals should be sealed in a plastic bag and frozen until we can look at them. At Mt. Mansfield fences all small mammals should be frozen and labeled with the location of the drift fence they originated from.

At the end of each month <u>make photocopies</u> of your data sheets and send me the originals.

#### Protocol for Sampling and Measuring Amphibians Captured in the Drift-fences

By keeping track of the sizes of amphibians caught we can begin to see a picture of survivorship, reproductive success, and perhaps health of the species as well as obtain data about the size at metamorphosis, maximum size and average adult sizes. However we don't want to have to measure all the amphibians caught, for example on days when 50 N. viridescens metamorphs are found in the traps. At the same time we need a standard method of deciding which ones to measure, so that apparent changes from year to year are not the result of different sampling or measuring methods.

So, measure the first three individuals of each size class of each species at each drift fence. For example if you get a trap full of N. viridescens metamorphs (tiny individuals that have just left the water this year for the first time), measure the first three of those. In a different can, you find a red eft that is much larger and is probably from last years crop of young. Measure that one and the next two of that size range that you find. If you then find an individual that is visibly larger than the efts you already measured, then that is a new size class and you should measure the first three of those as well. What you may be seeing is metamorphs, efts that left the water one year ago, others that left the water two years ago, and some others that are adults. In the case of adult toads and frogs you may also find that some look considerably larger than others. Instead of being a function of age this may be a function of sex. I suspect that most of the time in the late summer and fall you will catch many young of the year and relatively few adults. In the spring you will probably find that very few of the young have survived to be caught. Clearly this method requires some judgement on your part on whether or not an individual belongs to a separate size class. Short of measuring all of the individuals, there is no way around this that I can see. If you have the time and want to measure all of the amphibians, go right ahead. Write on your data sheet whether you measured all individuals or the first three of each size class. If you measure less than all of them, use this system.

All salamanders that you measure should be measured in two ways. You should measure their total length (from the tip of their nose to the tip of their tail) and their snout-vent length (from the tip of their nose to the beginning of their vent (cloaca). In frogs these two measurements are usually the same, unless the frog retains some of its tail immediately after changing from a tadpole. You need to measure only from the tip of their nose to the end of their body and write it in the snout vent box on the data sheet. Measure both lengths with any snakes that you find as well. With turtles measure the length of the shell on the bottom (plastron). Check your field guide on the green end papers for diagrams of these measurements. We differ only in measuring to the beginning of the vent, instead of to the end as shown in the book.

Even though you are only measuring a certain sample of those individuals you catch, you should still group all unmeasured amphibians and reptiles with the others of the same size class, so that I can see for example that there were twenty of a certain size class, three of which you measured. Also continue to write in the notes section whether they were metamorphs, juveniles and adults. Refer to the book if you aren't sure whether or not an individual is adult size.

# Appendix B

Field Efforts and 1993 Results by Method

Month	Active Searches	Canoe Searches	Drift-fence nights	Night- time Visits	Night-time Road Searches	Site Checks	Accidental Discoveries	Total Visitation Days
April	29			28		28,29		2
May	12,27	11		11	11	11,26	11,12	4
June	16			16		17	16,17	2
July	8,9			1,8		1,2,8,9	2,27*	4
August				4				1
September		na 1976 - Santa Santa Roman - Santa Santa	9,18,19			14	14	4
October	12		13,17, 21,22				12,31*	5
Total # of times each method was used	14	2	7 nights, 3 fences, 13 fence- nights	7	1	18		55 data- gathering efforts, on 22 days
# of Sites	12	1	3	5	1 Route	16	10	28*** Sites
Significant Units**	~30 Person- hours	2 Canoe- hours	4 Nights Upper Fences 5 Nights Lower Fence	4 hours	l Route at ~12 km	50 Person- hours at 16 Sites		

Table B1. A summary of field efforts in the Lye Brook Wilderness Region during the 1993 inventory.

\*not counted as a data gathering day (drift fence building or maintenance)

\*\*hours rounded to the nearest quarter-hour

\*\*\*more than one method was used at most sites, hence this number is not the sum of the this row

Species	Active Searches	Canoe Searches	Drift Fences	Night Visits	Night Road Searches	Site Checks	Accidental Discoveries	Totals	% of All Caudates
Ambystoma maculatum Spotted Salamander	4 egg masses	11 egg masses	10		4	21 egg masses	18 egg masses	14 54 egg masses	2%
Desmognathus fuscus Dusky Salamander	6					4	1	11	2%
<i>Eurycea bislineata</i> Two-lined Salamander	65		1			42 6 egg masses	2	110 6 egg masses	20%
Gyrinophilus porphyriticus Spring Salamander	14					18		32	6%
Notophthalmus viridescens Eastern Newt	117	10+	51	20+	1	82+	17+	298+	53%
Plethodon cinereus Redback Salamander	26		22			43	8	99	18%
# of Species	6	2	4	1	2	6	. 5	6	·
# Individuals	228	10+	84	20+	5	189	28	564	
# Egg Masses	4	11	· · ·			27	18	60	
% of Caudate Species	100%	33%	67%	17%	33%	100%	83%	100%	

Table B3. Frogs located in the Lye Brook Wilderness Region during the 1993 inventory.

Species	Active Searches	Canoe Searches	Drift- fence Nights	Night-time Visits	Night-time Road Searches	Site Checks	Accidental Discoveries	Totals	% of All Frogs
Bufo americanus American Toad	4		1	3 2 choruses	13 1 chorus	18 1 tadpole	7	46 3 choruses 1 tadpole	23.0%
		· · · · · · · · · · · · · · · · · · ·				site		site	
Hyla versicolor Gray Tree Frog				1?				1?	0.5%
Pseudacris crucifer Spring Peeper	7	1		6 3 choruses	2 10 choruses	7	1 1 chorus	24 14 choruses	12.0%
Rana catesbeiana Bullfrog				1				1	0.5%
<i>Rana clamitans</i> Green Frog	18	6		20 2 choruses	2	40	10	96 2 choruses	48.0%
<i>Rana palustris</i> Pickerel Frog	3	• • • • • • • • • • • • • • • • • • •				4	1	8	4.0%
Rana sylvatica Wood Frog	4	4 egg masses	4	5	2	9 1 chorus 25 egg masses	2 egg masses	24 1 chorus 31 egg masses	12.0%
# of Species	5	3	2	6	4	5	5	7	
# Individuals	36	7	5	36	19	78	19	200	••
# of Egg Masses	<u></u>	4	9	50	13	25	2	31	••
# of Choruses	N. P. S. S.		<u></u>	7	11	25 1	<u> </u>	20	••
# Tadpole Sites			a all'internette		11	1	1	20	
% of All Frog Species	71%	43%	29%	86%	57%	71%	71%	100%	••

Table B4a. Combined totals of all amphibians found in the Lye Brook Wilderness Region during the 1993 inventory.

# of Species	11	5	6	7	6	11	10	13
# Individuals	264	17+	89	56+	24	267	47	764
<pre># of Egg Masses</pre>	4	15	-			52	20	91
# of Choruses				7	11	1	1	20
# Tadpole Sites						1		1
% of All Amphibian								
Species	85%	38%	46%	54%	46%	85%	77%	100%

Table B4b. Reptiles located in the Lye Brook Wilderness Region during the 1993 inventory.

Thamnophis sirtalis Common Garter	3			5	3	11
Snake						

# Appendix C

Dr. Green's Pathology Report

MARYLAND DEPARTMENT OF AGRICULTURE Animal Health Laboratory FINAL PATHOLOGY REPORT 8077 Greenmead Drive College Park, Maryland 20740 301-935-6074 Accession No. CP4-4083 & CP4-4502 Date: 16 January 1995 94P251 940287 Submission Date: 7 & 28 June 1994 Owner: Jim Andrews Veterinarian: None listed. Department of Biology Middlebury College Middlebury, VT 05753 Species: Amphibian, Caudata, Notophthalmus viridescens (red-spotted newts) Weights & Lengths (see Table A) Age: >7 yrs Sex: 5-F, 6-M No. Submitted: 14 Specimen: Live animals Group size: Unknown Mortality: Unknown Morbidity: Unknown Locations: Two ponds: Upper Abbey Pond, Ripton, VT & Andrews Home Pond, Bridport, VT Collector: Jim Andrews Dates of Necropsies: 7-9 June 1994 Dates of Euthanasias: 7-9 June 1994 28-9 June 1994 28-9 June 1994 Significant Findings and Remarks: HISTORY: For at least the last two breeding seasons (1993 & 1994) red-spotted newts in Upper Abbey Pond have been observed with swellings (lumps) in the muscles of the pelvis. Morbidity and mortality rates were not known. NECROPSY FINDINGS: See Tables A & B (page 2) PARASITE EXAMINATIONS: See Table B (examinations of stained blood smears are in progress). FLUORESCENT ANTIBODY TESTS (F.A.T.): 1. Chlamydia psittaci ("psittacosis"): Negative in 3 of 3 newts. 2. Leptospirosis: Negative in 3 of 3 newts. BACTERIA & FUNGUS CULTURE RESULTS: See Table C (on page 3). CYTOLOGIC EXAMINATIONS & WHITE BLOOD CELL COUNTS: See Table D (on page 3). HISTOLOGIC EXAMINATIONS: See also Tables E & F on page 8. 1. Skeletal muscles: Mycotic granulomatous myositis, subacute to chronic, multifocal to disseminated, minimal to severe, in 9 of 14 newts. Etiology probably <u>Aureobasidium pullulans</u> fungus infection. 2. Small intestines: Protozoal enteritis, multifocal to disseminated, mild, in 4 of 14 newts. Etiology suggestive of coccidiosis (Eimeria, Isospora, or similar apicomplexan protozoa). 3. Small intestines: Protozoiasis, luminal, disseminated, mild to moderate, in 4 of 14 newts. Etiology: luminal protozoan about the size and shape of Hexamita or diplomonads. 4. Small intestines: Verminous enteritis, focal or multifocal, very mild, in 2 of 14 newts. Etiology: small nematodes and flukes. 5. Stomach: Protozoal gastritis, diffuse, mild to moderate, in 1 of 14 newts. Etiology suggestive of Cryptosporidium-like infection. 6. Liver: Hepatitis, eosinophilic, reactive & sinusoidal, diffuse, mild to moderate, in 3 of 14 newis. Etiology not detected but probably reactive to the fungal infection in the muscles. COMMENTS: Fungi were isolated in pure cultures from the muscles of 3 newts submitted on 28 June 1994. Two of these fungal isolates were submitted to the National Veterinary Services Laboratory in Ames, Iowa, and both were identified FINAL PATHOLOGY REPORT, Page 2 CP4-4083 & CP4-4502; Newts

as <u>Aureobasidium pullulans</u>. An exhaustive literature search has yet to be done on this fungus, but at present, I can find no reports of this fungus causing infections in animals or man. Neither can I find any histologic descriptions of fungi resembling <u>Aureobasidium</u> in animals, except for the single report by Herman (1984) in which the organism in newts from West Virginia was described as an Ichthyophonus-like infection. Histologic features of the fungus in Herman's report and your Vermont newts are identical, but are <u>not</u> typical of Ichthyophonus infection. I have contacted Dr Herman by telephone, and he has not done any further work on the disease, and is not aware of any other studies or publications. He does however, think the infection has recurred in newts from his locality (Sleepy Creek Lake, West Virginia) yearly for at least 20 years. I have examined newts from at least 4 ponds in Virginia in the last 5 years and have never seen this condition. So, although the disease cannot be considered "new", the one description in the literature has some errors, incorrect assumptions, is incomplete, and the organism was not isolated in cultures. Hence, this fungal disease is definitely worthy of publication.

This fungal infection has many very unusual features and associations. You submitted 14 newts from 2 ponds, but the infection was detected in only newts from Upper Abbey Pond. At necropsy, gross lesions were observed in 7 of 14 newts (Tables A & B). However, histologic examinations showed that 9 newts had mycotic (fungal) infections of the muscles (Table E). As detected at necropsy, the muscles of the posterior body, pelvic region, hindlimbs, and anterior tail were most heavily affected. Much fewer numbers of fungi were found in the muscles of the tail tip, anterior body, forelimbs, and neck. In two newts, fungal cysts were detected within the skin (epidermis). The fungi were not detected in any internal organs (or tissues) of the body. Ichthyophonus fungi infect a wide variety of internal organs (heart, liver, intestines, etc) so this feature is a major clue that the fungus in the newts was not Ichthyophonus. The cause for the selective (topistic) vulnerability of muscles is unknown, but it is possible that this region of the body was the site of invasion by the fungus. For reasons which are explained below, it is suggested (speculated) that this fungus may be spread by wounds or bites of leeches or some other predator. A key piece of information that might link this infection to leeches would be if leeches prefer to attach to newts in the pelvic region.

It appears based on histologic examinations, that some newts have an intense inflammatory reaction to the fungi in the muscles. However, this inflammatory reaction (granulomatous inflammation) may be slow to develop, because some newts had no inflammatory cells around the infected muscle cells, while some newts had intense inflammation around nearly all fungi. And some newts had both. The two newts in which the fungus infection was not suspected at necropsy had extensive inflammation around the fungi with collapse of the fungi and loss of their internal contents. It is logical to assume these two newts were in the healing, and, perhaps, recovery stages of this infection. Those newts with heavily infected muscle cells but little or no evidence of inflammatory reaction may be those animals which would have succumbed to the infection.

The morphology of the fungi in the muscle cells was most unusual and may be unique. This infection should be easily recognized histologically, and readily distinguished from other fungal infections. One problem which has not been resolved is the proper name or terminology for the fungal elements in the muscle cells. This problem may be resolved when a literature search is completed. Names such as spores, endospores, chlamydospores, sphaerules, spherical forms, FINAL PATHOLOGY REPORT, Page 3 CP4-4083 & CP4-4502; Newts

cysts, pseudocysts, sporangia, and sporangiophores were considered. Although we probably may name the fungal elements in histologic sections whatever we choose, the terms spores, cysts or simply fungal elements are probably best. The shape of the fungal elements in the muscle cells was fusiform or cigarlike. Basically, the fungi seemed to conform to the shape of the muscle cells. In cross sections, however, the fungi were distinctly round. The size of the fungi was measured in unstained fresh crush smears of the infected muscles, and in histologic sections; measurements of the fungi are shown in Table F. The fungi never showed hyphae, budding, daughter spores, or any significant internal structures; absence of such features is important for distinguishing this fungus from other fungal infections. All fungi appeared to have moderately thick walls, and in one newt the fungi appeared to have very thick mucinous capsules. Color of the fungi was variable: unstained fresh smears showed the fungi to be slightly to mildly brown while in stained histologic sections the fungi lost the brown color and took up various colors from the stains. Characteristics of the fungi in special stains are listed in Table G.

The inflammatory reaction to the fungi in the muscles was highly variable. In some newts there was little inflammatory cell reaction despite the presence of massive numbers of fungi. Other newts had intra-muscular fungi with no inflammatory cell reaction, while nearby areas had intense granulomatous inflammation. A few newts had inflammatory reaction around all fungi. In some regions of the pelvic and lower body muscles over 90% of muscle cells were infected by fungi. It is merely assumed that those newts with massive numbers of fungi and little or no inflammation had infections which would have been fatal. Those newts in which all fungi had inflammation probably would have recovered. The prognosis for those newts which had fungi with and without inflammation is uncertain.

The presence of the fungal infection appeared strongly correlated with the presence of trypanosomiasis (Table E). Out of 9 newts with trypanosome infections, 8 (89%) had fungal infections; of the 5 newts without trypanosome infections, 4 (80%) did not have fungal infections. The one newt which had fungal infection without trypansomes, actually may have had trypanosome infec-tion but it was simply not detected. This strong correlation between trypanosomiasis and this fungal infection implies that the leeches may be the proxi-mate cause of the fungal infection. In newts, trypanosomiasis is transitted by leeches. It is possible the fungus enters the newts at the bite wound(s) of leeches. It cannot be determined whether this fungus infection is an opportunistic infection of the leech's bite wound, or whether the fungus is present in the leeches and is transmitted in a method similar to the trypanosomes. It would be very interesting to examine newts from Sleepy Creek Lake (West Virginia) [Herman, 1984] to determine if they have a similar correlation of trypanosomiasis and fungal infections. Many newts which have been examined from ponds in Virginia have had trypanosomiasis but no fungal infection, so the presence of trypanosomiasis or leeches is not the only factor in the transmission of the fungus infection.

The newts from the two ponds in Vermont had a variety of intestinal helminthic parasites (cestodes, trematodes, and nematodes) and at least three species of protozoan parasites. Further identification of some helminthic parasites is being attempted at the Zoological Society of London. A sample of the helminths was submitted in early December; the amount of time necessary for identification of these parasites is unknown, but results will be transmitted to you as soon as they are received. Two of the protozoan parasites were preFINAL PATHOLOGY REPORT, Page 4 CP4-4083 & CP4-4502; Newts

sent entirely in the lumen of the intestines and on the surface of the the intestinal mucosal cells; these protozoa actually may have been part of the normal intestinal fauna. One luminal protozoan was probably a flagellate and the other a ciliate. No specific disease or illness can be attributed to the luminal parasites. However, the third protozoan had invaded the cells of the intestinal mucosa; the parasite resembled the disease of animals called coccidiosis. Coccidia are apicomplexan protozoa, all of which are considered parasites in the strictest sense. Eimeria and Isospora are the most common coccidia of mammals and birds. Three species of Eimeria have been described from newts in North American: <u>E. longaspora</u>, <u>E. megaresidua</u> and <u>E. grobbeni</u>; only the first two species have been documented in red-spotted newts. It was not possible to identify the coccidia of these newts to the genus or species level. All coccidian infections in these newts were considered mild, so it is unlikely they were causing illness. Finally, one newt had unusual tiny structures on the surface of the mucosal cells of the stomach; these structures may have been parasites of the genus Cryptosporidium. These organisms are so small the only certain method of identification is by electron microscopy.

Two potential disease-producing bacteria were isolated from the intestines of 2 newts: <u>Yersinia sp.</u> and <u>Aeromonas hydrophila</u>. Histologic examinations of both newts failed to detect any lesions which could be attributed to infection by these bacteria. Therefore, these two bacteria were probably inocuous in these newts, were transient in the intestines, or, were part of the normal intestinal and/or environmental flora.

SUMMARY & CONCLUSIONS: Nine of eleven newts from Upper Abbey Pond had fungus infections limited to the skeletal muscles. The fungus was cultured from 3 newts and was identified at a reference laboratory as <u>Aureobasidium pullulans</u>, a species which, to my knowledge, has never been described as causing infections in amphibians. However, this fungal infection in newts has been previously described (albeit poorly) and the fungus was mis-identified as an Ichthyophonus fungal infection. Therefore, a publication re-describing this infection and the fungus are warranted.

These newts had at least 4 protozoan parasites, as well as nematode, cestode, and trematode parasites. The blood protozoan was <u>Trypanosoma diemyctyli</u> and one intestinal protozoan may have been either <u>Eimeria longaspora</u> or <u>E</u>. <u>megaresidum</u>. Two protozoa were intraluminal organisms and were probably innocuous; these luminal protozoa could not be identified but probably were a flagellate and a ciliate. Some of the helminthic worms presently are being identified at the Zoological Society of London. None of these parasites were considered serious or life-threatening infections. However, there was a striking correlation between the occurrence of trypanosome infections and the fungus infections, suggesting the two organisms may have had the same vector (ie, leeches). Two potentially pathogenic bacteria (Yersinia and <u>Aeromonas hydrophila</u>) were isolated from the intestines of two newts; histologically, neither newt showed lesions which could be attributed to infection by these bacteria, therefore, disease cannot be attributed to these two bacteria.

FINAL PRIMARY DIAGNOSES:

- 1) Skeletal muscles: Mycotic myositis, in 9 of 14 newts;
  - etiology: Aureobasidium pullulans fungus infection.
- 2) Blood: Internal parasite: <u>Trypanosoma</u> <u>diemyctyli</u> protozoa in 8 of 14 newts.

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FINAL SECONDARY & INCIDENTAL DIAGNOSES:

- 3) Intestines: <u>Yersinia</u> <u>sp.</u> bacterial infection (culture diagnosis only).
   4) Intestines: Internal parasite: Coccidiosis in 4 of 14 newts.
- 5) Stomach & intestines: Internal parasite: Flukes in 5 of 14 newts.
  6) Intestines: Internal parasite: Nematode worms in 4 of 14 newts.
  7) Duodenum: Internal parasite: Tapeworm in 3 of 14 newts.

- 8) Urinary bladder: Internal parasite: Nematode worm in 1 of 14 newts.

At la 34

D. EARL GREEN, D.V.M. Diplomate, A.C.V.P.

CC: J Andrews SR: 56 (1,4)\deg ST: All organs of 14 newts FINAL PATHOLOGY REPORT, Page 6 CP4-4083 & CP4-4502; Newts

TABLE A: Signalments, Morphometry, & Gross Necropsy Findings in 14 Newts

							Necro	psy Lesio	ns
Newt	Date of	Pond				Tail	Muscle	Spleno-	Black
ID No.	Necropsy	<u>Site</u>	<u>Sex</u>	<u>Weight</u>	<u>SVL</u>	<u>Length</u>	<u>Fungi</u>	megaly	<u>Liver</u>
251A	7 June	AHP	М	2.3	ND	ND	0	0	0
251B	7 June	AHP	F	1.45	ND	ND	0	0	0
251C	7 June	AHP	F	2.08	ND	ND	0	+	0
251D	7 June	UAbP	NR	2.62	ND	ND	+++	0	0
251E	8 June	UAbP	М	2.75	47	53	+++	++	++
251F	8 June	UAbP	NR	2.12	44	46	++	0	+++
251G	9 June	UAbp	М	2.48	46	48	++	+++	+++
251H	8 June	UAbp	NR	2.37	46	48	0	0	0
2511	9 June	UAbp	F	2.31	49	55	0	++	+++
251J	9 June	UAbP	F	1.72	41	43	0	0	0
251K	9 June	UAbp	М	1.82	43	46	0	+	+
287A	28 June	UAbP	М	2.5	46	49	+++	+	++
287B	29 June	UAbp	м	2.1	44	46	++++	0	+++
287C	29 June	UAbp	м	2.0	44	44	++++	0	+++

NR- Not recorded; the sex of all newts will be confirmed histologically from the histologic slides at a later date. 
 ND- Not done
 SVL = Snout-vent length (in millimaters)

 Weights are in grams.
 F = Female

 + = minimal
 ++ = mild
 + = minimal ARP = Andrew's home pond UAb? = Upper Abbey pond ++++ = 34¥+24

TABLE B: Parasites in 14 Newts

	Blood	Muscle		Stomach &	Intestine		Bladder
Newt	Trypanosoma	Fungus	Duodenal	Stomach	Intestine	Jejunal	
ID No.	diemyctyli	Spores	Tapeworm	<u>Flukes</u>	Flukes	Nematodes	
251A	0	0	0	0	0	0	
251B	0	0	0	0	0	0	
251C	0	0	0	0	0	0	0
251D	0	+++	0	0	0	0	0
251E	++	+++	0	0	0	4	0
251F	+	++	0	0	0 <sup>°</sup> .	1	0
251G	+++	++	0	0	-Ò	0	1
251H	+++	0	0	0	1	0	0
2511	+++	0	0	2	0	0	0
251J	0	0	0	0	5	1	0
251K	+	0	1	0	2	1	0
287A	++	+++	1	0	0	0	0
287B	0	++++	1	0	0	0	0
287C	++	++++	0	0	0	0	0

Numbers represent the number of helminthic parasites found.  $\Rightarrow = \min a + \Rightarrow = \min a + \Rightarrow = moderate = a$ 

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TABLE C:	Bacteria	a & Fungus Cult	ure Results	
		Rump Skin		
	<u>Si</u>	te & Muscle	<u>Liver</u>	<u>Intest/Colon</u>
0615			5	No
251A	AH		<u>No growth</u>	No growth
251B	AH	IP ND	<u>No growth</u>	No growth
251C	нA	IP ND	<u>*S.liquefaciens</u>	<u>Hafnia alvei</u>
251D	UA	ND ND	No growth	No growth
251E	UA	UD ND	No growth	Hafnia alvei
251F	UA	ND ND	No growth	Hafnia alvei
251G	UA	USP ND	No growth	Hafnia alvei
251H	UA	ND ND	No growth	Hafnia alvei
2511	UA	LOP ND	No growth	Yersinia sp.
				Escherichia coli
		•		**Ps. putida
251J	8Jun UA	LOP ND	No growth	No growth
251K	9Jun UA	LAP ND	No growth	No growth
287A	28Jun UA	bP ****A.p.	No growth	***Aero, hydrophila
287B	29Jun UA	bP ****A.p.	ND	Hafnia alvei
287C	29Jun UA	bP ****	No growth	ND

- S. is the bacterial genus Secratia
 - Ps. is the bacterial genus Pseudomonas
 - Aero. is the bacterial genus Aeromonas

.... = A.p. is the fungue Aureobasidium pullulans

						Differ	ential	WBC Cour	nts	
Newt		Trypan	osomes	Smear	Lymph-	Foamy	Мопо-		Eosin-	Baso-
ID No.	<u>Site</u>	<u>Wet*</u>	Dry*	<u>Type</u>	ocytes	Lymph's	<u>cytes</u>	"PMNs"	<u>ophils</u>	phils
251A	ABP	0	0	Heart	21	0	2	52	6	18
251R	AHP	0	0	Chest	32	6	14	46	õ	2
		-	•			-	-		-	2.
251C	UAbp	0	0	Clot	12	4	12	40	8	24
**251D	UADP	0	1	Tail	24	20	0	48	4	4
**251E	UAbp	++	5	Tail	24	14	· 6-	44	4	4
**251F	UAbP	+	0	Tail	40	10	8	36	0	6
**251G	UAbp	+++	0	Tail	16	10	`2	50	4	18
251H	UAbp	+++	1	Tail	20	36	10	30	0	4
2511	UAbp	+++	0	Tail	20	18	6	44(+2)	8	2
251J	UAbp	0	0	Tail	36	4	12	36	6	6
251K	UAbP	+	0	Tail	20	8	18	46	6	2
**287A	UAbP	++	2	Tail	4	12	20	58	0	2
**287B	UAbP	0	0	Heart	28	0	14	48	4	6
**287C	UADP	++	6	Tail	10	12	2	76	0	0

TABLE D: White Blood Cell Counts in 14 Red-spotted Newts

<u>Wet</u> refers to fresh whole blood examined as a wet mount and unstained; trypanoscmes were easily detected by their movement. <u>Dry</u> refers to air dried, stained blood smears; the number refers to the actual number of trypanoscmes observed during counts of the white blood cells.
 These 7 newts had grossly evident fungal infections in their pelvic muscles.
 TWNs = Jolymorphonuclear cells, also referred to as neutrophils.
 WBC = White blood cells (laucorytes); numbers in the columns are percentages.
 i () = Number in parenthesis refers to bend (Limature) neutrophils.

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	CYTOLOGIC FINDING					HISTOLOGIC FINDINGS MUSCLE FUNGI BY LOCATION							
Newt		Trypan		Smear		MUSC	LE_F	UNGI Clo		OCATI	<u>N</u>	_	
<u>ID No</u>	<u>Site</u>	<u>Wet*</u>	<u>Dry*</u>	<u>Түре</u>	<u>Neck</u> C G	Bo C	dy_ G	<u>/Ru</u> C	mp G	<u>Ta</u>	<u>i1</u>	$\frac{1}{2}$	eq
251A	AHP	0	0 /	Heart		0	0	0	0	<u> </u>	0	0	<u>G</u> 0
251B	AHP	0	0	Chest		0	0	0	0	0	0		-
251C	UAbp	0	0	Clot		0	0	0	0	0	0	0	0
**251D	UAbp	0	1	Tail		+	+	++	+	++	+	+	+
**251E	UAbp	++	5	Tail		-	-	++	++	++	÷	Ó	ò
**251F	UAbp	+	0	Tail		+	++	+	++	+	ò	+	++
**251G	UAbp	+++	0	Tail		Ó	++	ò	+	ò	+	ò	0
2518	UAbp	+++	1	Tail		+	0	ō	ò	ŏ	++	ŏ	ŏ
2511	UAbp	+++	0	Tail		Ó	+	Ō.	+	ŏ	+	ŏ	õ
251J	UAbP	0	0	Tail		õ	ò	ō.	ò	ŏ	ò	ŏ	õ
251K	UAbp	+	0	Tail		ō	ō	õ	õ	ŏ	õ	õ	õ
**287A	UAbp	++	2	Tail		++	++	++	++	Ť	+	ŏ	õ
**2878	UADP	0	ō	Heart		++	0	++	+	++	т +	•	
**287C	UAbP	++	6	Tail		++	++ ·	++	•		-	+	+
			<u> </u>	1017		ŦŦ	τŦ	++	++	+	+	+	+

#### TABLE E: HISTOLOGIC (and CYTOLOGIC) FINDINGS

TABLE F: SIZES OF FUNGAL ELEMENTS IN MUSCLES

Newt No.	FRES	H	HISTOLOG	IC SECTIONS
	DIAMETER	LENGTH	CROSS SECTION	LONGITUDINAL SECTION
94P251D		155.5 (175) [95-360]	74.5 (74.9) [36-135]	236.5 (231.7 [144-302
94P251E	ND	ND	93 (91.1) [43-149]	212 (216.7) 135-351]
94P287A	ND	ND	101.5 (98.4) [59-135]	ND
94P287B	ND	ND	103.5 (104.4) [54-144]	241 (245) [158-315]
94P287C	ND	ND	99 (97.1) [63-135]	ND

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( ) = Mean [ ] = Range All measurements in microns

C = Cysts lacking inflammatory cell reaction G = Granulomatous inflammation around fungal elements \* & \*\* = See Table D.

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# TABLE G: COMPARATIVE MORPHOLOGY & SPECIAL STAINING FEATURES OF AUREOBASIDIUM IN RED-SPOTTED NEWTS AND OTHER AMPHIBIAN INFECTIONS.

	Location			Internal Natural		<u>HISTOLOGIC STAINS</u> Hematoxylin		
AGENT	<u>in Host</u>		<u>Shape</u>			<u>&amp; Eosin (H&amp;E) P</u>	.A.S.	Giems
<u>Aureobasidium pullulans</u>	Skeletal muscle	35-150 x 135-360	Spindle	None	Lt brwn	Lt red or blue	++	Blue
<u>Basidiobolus</u>	Ventral skin	NR	Sphere	None	NR	NR	+	NR
<u>Mucor</u> amphibiorum	MO,liver, bladder	5 to 36	Sphere	Daughter spherules	NR	Blue	++	NR
Ichthyophonus spp.	Hrt,Liv, Kid,MO	4 to 23	Sphere	Multi- nucleate	NR	NR	+	
Chromomycosis	Skin, MO	3 to 17	Hyphae & yeast	Sparingly septate	Brn to black	Brn to black		Brn to blac
Saprolegniasis	Skin, muscle	2 to 4	Nyphae	Sparingly septate	Clear	Clear to lt blue	+	Blue
Dermocystidium spp.	Skin	>250	Sphere, U-shape	Spores	NR	NR	NR	
Dermosporidium spp.	Skin	>250	Sphere	Spores	NR	NR	NR	
<u>Prototheca</u> <u>spp.</u>	NR	2 to 20	Sphere	4 endo- spores	Clear	Clear	+	NR
<u>Pleistophora</u> <u>danilewskyi</u>	Muscle	NR	Spindle	Spores	Clear	Blue	NR	NR
Brn= brown MO = many organs NR = not reported				's reaction microns				_

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# Appendix D

Other Species

Table D1. Other species that were noted in the region. This is not meant to be a comprehensive list. Only those species that were recorded in my field notes for any reason are included. Unusual species are in bold type.

## <u>Mammals</u>

#### Moose

White-tailed deer Black bear Masked shrew Short-tailed shrew Beaver Eastern chipmunk Redback vole Woodland jumping mice Peromyscus sp.

### **Birds continued**

Red-winged blackbird Rusty blackbird White-throated sparrow

### **Reptiles**

Common garter snake

## Birds

Common loon Great blue heron Mallard American black duck Wood duck Broad-winged hawk Osprey Spotted sandpiper Mourning dove Chimney swift Belted kingfisher Pileated woodpecker Downy woodpecker Least flycatcher Tree swallow Black-capped chickadee White-breasted nuthatch Winter wren Swainson's thrush Blue-gray gnatcatcher Golden-crowned kinglet Solitary vireo Magnolia warbler Black-throated blue warbler Yellow-rumped warbler Blackburnian warbler Black-throated green warbler Blackpoll warbler Ovenbird Northern waterthrush Common yellowthroat American redstart

## <u>Fish</u>

#### Bullhead

## **Invertebrates**

Damselflies Dragonflies Crickets Daddy-long legs Water beetles Mosquitoes Black flies Deer flies Ground beetles Grasshoppers Springtail Slugs Crayfish

## <u>Plants</u>

Cotton grass Pitcher plant Early azalea Buckbean Wild sarsaparilla Water arum