Investigations into the Causes of Amphibian Malformations in the Lake Champlain Basin of New England

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Final Report
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Project Description: Background

The Vermont Department of Environmental Conservation (VTDEC) first responded to reports of malformed frogs in the Lake Champlain Basin in the summer of 1996. Malformed frogs were reported from twelve sites in five counties within the Lake Champlain Basin in Vermont. The twelve sites reported in 1996 were all close to Lake Champlain or a major tributary to the Lake. The Lake Champlain Basin includes part of VT, NY and Quebec. The total area of the basin is over 8000 mi^2 (20720km^2).

Since the initial reports in 1996, VTDEC has gathered extensive information about the incidence and distribution of amphibian physical anomalies at numerous sites throughout the Vermont portion of the Lake Champlain Basin. The majority of VTDEC activities have focused on characterizing the physical characteristics of metamorphs of the Northern Leopard Frog, (*Rana pipiens*). Since the summer of 1996, biologists from VTDEC have examined over 10,000 *R. pipiens* metamorphs from twenty-two sites throughout the basin. 6.0% of the metamorphs observed had some sort of gross external abnormality. By far the most dominant categories of observed anomalies include missing and partial hind limbs and shortened and missing digits. Rates of anomaly occurrence varied between sites (0% - 45%), between seasons at sites (summer vs. fall), and between years at sites. For example, an initial survey conducted at a site adjacent to the Poultney River in Vermont in the summer of 1997 found abnormalities in 45% of the newly metamorphosed *R. pipiens* collected (n = 121). Repeat sampling in the fall of 1997 found an incidence rate of 18% (n = 67). Subsequent collections have documented ranges of <1% percent to 6%. Large-scale population sampling using drift net methods at this same site has resulted in observation of less than 1 percent abnormalities among thousands of animals examined. These data suggest that the occurrence of abnormalities is highly variable over time and that continued data collection will provide valuable information describing abnormality incidence.

In the spring of 1999, VTDEC collected and examined tadpoles at various stages of development at several sites within the Lake Champlain Basin. Observations at one site where a relatively high rate of hind limb abnormality incidence (6%-21%) has been observed were suggestive of recent hind leg trauma to late stage tadpoles. Of 218 late stage (stage 39-43 Gosner) (Gosner, 1960) tadpoles collected at one site in June of 1999, 5.5% had hind limb truncations. Many of those truncations showed signs of recent trauma as evidenced by redness and discolored pigmentation.

In nearby Quebec researchers have documented elevated amphibian malformation occurrence in active vs. inactive agricultural lands, postulating that agro-chemical use may be a factor in elevated anomaly occurrence (Ouellet et al, 1997). Little work has been done in the Lake Champlain Basin to evaluate the occurrence of pesticides or their derivatives at *R. pipiens* monitoring sites. Although none of the sites historically monitored by VTDEC would be characterized as “farm ponds”, many are adjacent to widely cultivated (primarily corn and/or apples) landscapes where a variety of pesticides are employed. Recent toxicity identification evaluation (TIE) studies have identified a variety of potentially teratogenic pesticide-related compounds in environmental samples from Minnesota (Fort et al.1999a).

A number of researchers, in collaboration with VTDEC, have pursued a variety of investigative approaches in an attempt to describe the mechanism(s) by which the observed anomalies in *R. pipiens* have been produced at selected sites in the Lake Champlain Basin. Environmental samples from Lake Champlain Basin sites with known rates of abnormality incidence have been examined using standard laboratory assay’s procedures to assess potential for inducing primary developmental errors. Recently metamorphosed *R. pipiens* have been examined for a range of pathological and skeletal indicators of malformation potential and occurrence. Some of the findings of these studies are summarized below.

Studies with laboratory frog embryo teratogenesis assays using *Xenopus laevis* (FETAX) have shown that water and sediment extracts from survey sites in several states, including Vermont, support the argument that unknown biogenic factors in the water/sediment extract matrix contribute to the induction of developmental malformations similar to those observed in wild populations of *R. pipiens* . Potential factors include pesticides (including growth regulators) or naturally occurring retinoids or other compounds, which have the potential to induce primary developmental errors; e.g. produce thyroid disruption or acetylcholinesterase inhibition (Ankley et al. 1998; LaClair et al. 1998; Burkhart et al. 1998; Ouellet et al. 1997; Fort et al.1999a; 1999b).

The USGS-BRD National Wildlife Health Center has evaluated *R. pipiens* specimens collected from sites in the Lake Champlain Basin in 1997, 1998, and 1999. Results from 1997 are available at: http://www.nwhc.usgs.gov/research/amph_dc/Vermont_Frog_Report.pdf. Significant findings to date include: 1) No correlation has been found between the incidence of abnormalities and viral/bacterial burdens in newly metamorphosed *R. pipiens*; 2) No correlation has been found between incidence of abnormalities and parasite burden in newly metamorphosed *R. pipiens*. Both normal and abnormal frogs can have heavy or light burdens of cysts. If parasites are a factor in abnormality induction, findings would suggest that induction occurs during early life stages and that parasite burden in adults is not indicative of their potential influence on development; 3) there is little evidence to support the hypothesis that abnormalities observed in adult *R. pipiens* are a result of post-metamorphic trauma.

“Of the Vermont frogs missing entire limbs, x-rays revealed that 73% were also missing bones in the hip, providing evidence that a predator had not removed the limbs, but that developmental errors were to blame.” (USGS News Release, “X-Ray Studies Shed Light on Frog Deformities” March 29, 2000, http://www.usgs.gov). Evidence does not discount the potential of pre-ossification trauma as a causative agent although adult radiography strongly suggests that a wide range of observed hind limb abnormalities are most likely the result of primary developmental errors of undetermined origin.

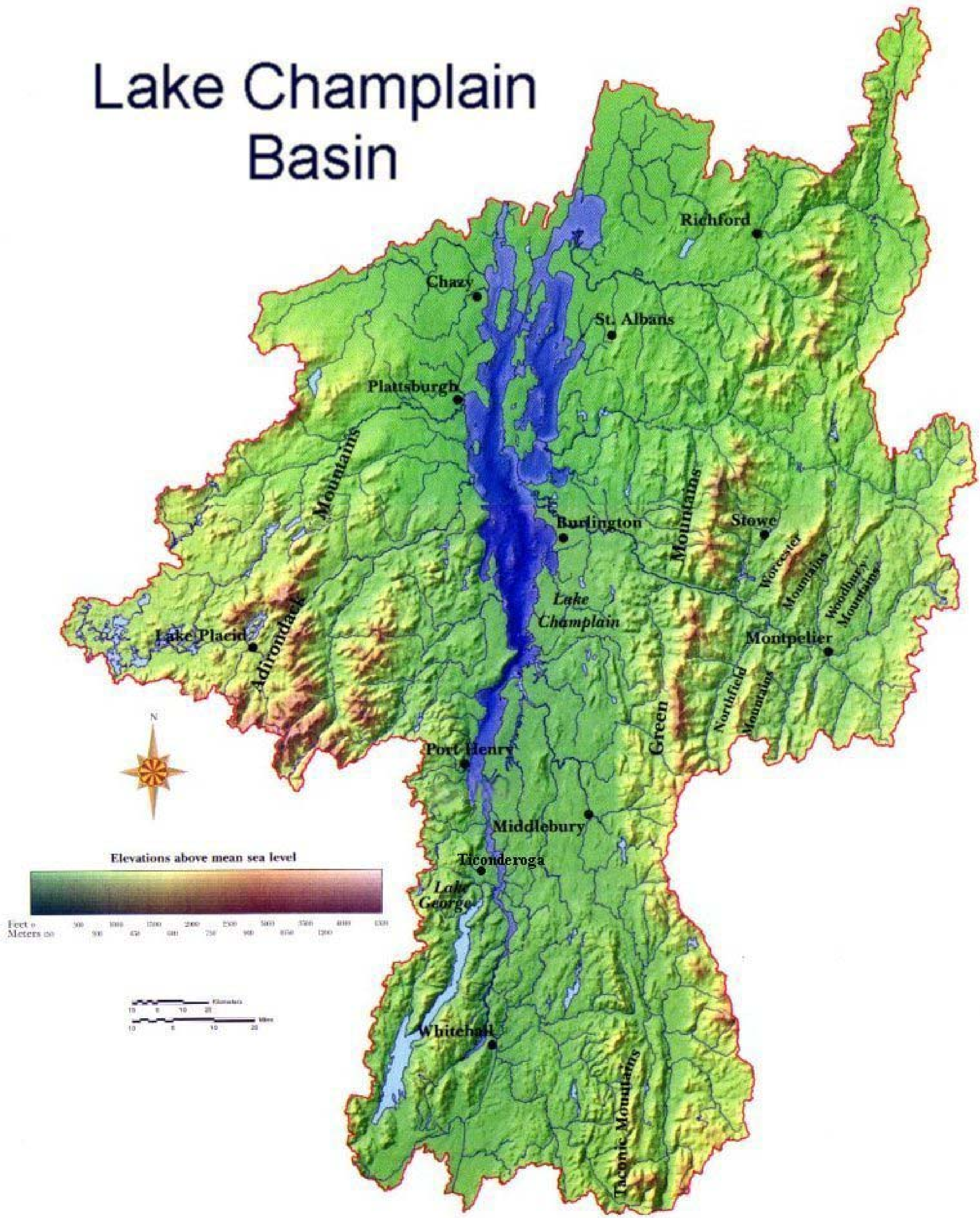
early developmental exposure to materials capable of disrupting normal hind limb development although additional data are needed to assess early stage limb development and the timing of abnormality occurrence.

Objectives

The overall objective of this project was to conduct a series of coordinated field and laboratory tasks that will: characterize the occurrence of *R. pipiens* abnormalities in both sub-samples and broad population level samples over time; evaluate the potential of site sediment and water to induce developmental abnormalities of a type and at a frequency of occurrence observed in the field through laboratory developmental limb bud assays; investigate potential causal agents through field developmental observations, chemical characterization of water and sediment from test sites, and laboratory assays evaluating limb development and response to environmental sample exposure. The five sites chosen within the Lake Champlain Basin of Vermont (Figure 1) support breeding populations of *R. pipiens*, and show a range of gross hind limb abnormality incidence indicative of impact and reference conditions.

Breeding and development of *R. pipiens* was monitored at all sites through adult metamorphosis. Representative sub-samples of developing and metamorphosed *R. pipiens* were collected and examined for the purpose of determining abnormality incidence rate and the relationship of observed anomalies to selected physical characteristics, including evidence of physical trauma. Intensive monitoring of amphibian populations was conducted at the Ward Marsh site to describe multi-species population-level abnormality incidence. Potential pesticide contamination at all sites was evaluated by analyzing representative sediment and water samples for a range of agricultural pesticides and metabolites in common use throughout the Lake Champlain Basin. Developmental limb bud assays were conducted using both *Xenopus laevis* and Ranid species in order to evaluate the test response relationships between the two species and to evaluate endpoints relevant to Lake Champlain findings, specifically hind leg reduction malformations.

Figure 1. Lake Champlain Basin Map



METHODS

Sampling Design Rationale

The objective of this study was to gather field and laboratory information that would further our knowledge regarding the role of biologically active agents (including current use agricultural pesticides) on the occurrence of *R. pipiens* abnormalities in the Lake Champlain Basin and expand current data bases documenting the status and variability of morphological abnormalities among *R. pipiens* populations at historically monitored sites in the Lake Champlain Basin.

Site Selection

Five sites were selected as primary study sites (Table 1, Figure 2). The sites selected were established sites with a minimum of three years of data (VTDEC) describing the frequency of occurrence of abnormalities among *R. pipiens* (Table 8). Two of the five sites (Mud Creek, North Hero) have monitoring data demonstrating a low incidence of abnormality (<3%) and will serve as "control" sites. The remaining three sites have monitoring data demonstrating a high incidence of abnormality (>8%) and will serve as test sites.

All sites are located in the Lake Champlain Basin. Preference has been given to sites where previous research activities have been conducted. One of the primary study sites, the Ward Marsh-Poultney River site, was selected for intensive drift fence sampling. Sampling sites have been selected with the goal of representing *R. pipiens* breeding grounds across a range of geographical locations within the Lake Champlain Basin of Vermont.

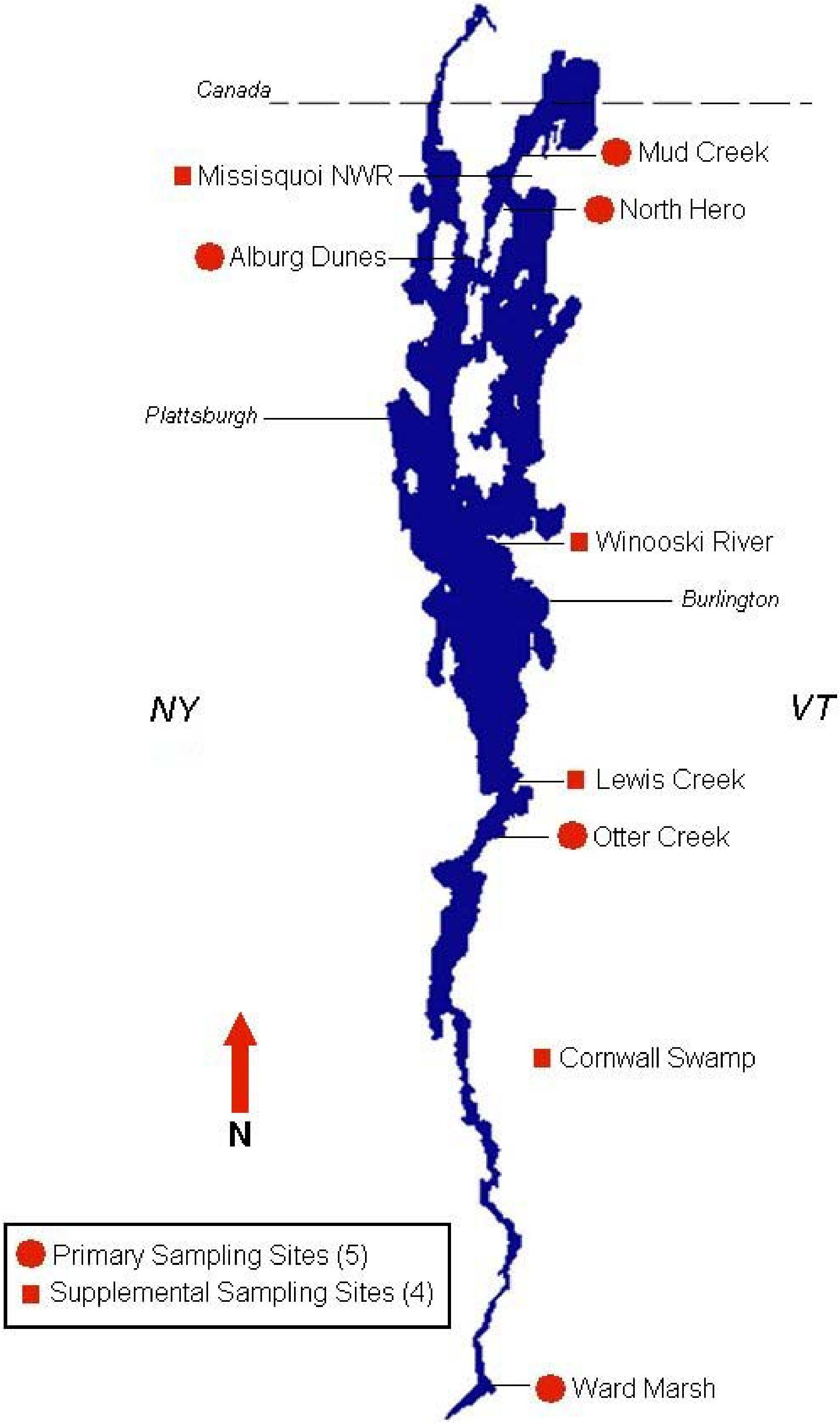
All of the primary study sites are located close to Lake Champlain and as such are affected by the lakes annual fluctuations. The site elevations range from 99 to 100 feet (30m), average annual lake levels fluctuate between 95 and 101 feet (29 –31m).

Table 1. Five Primary Study Sites

				Latitude			Longitude		
Site	Site Code	Town	County	Deg	Min	Sec	Deg	Min	Sec
Otter Creek	OTC	Ferrisburg	Addison	44	13	11.7	73	18	15.9
Mud Creek	MDC	Swanton	Franklin	44	58	3.7	73	16	12.8
Ward Marsh*	WM	West Haven	Rutland	43	34	13.4	73	23	46.0
Alburg Dune	ALB	Alburg	Grand Isle	44	51	50.2	73	17	41.4
North Hero	NH	North Hero	Grand Isle	44	55	13.8	73	14	31.9

*The Ward Marsh site has also been referred to as the Poultney River site (POL) within this report.

Figure 2. Sampling sites.



Site Characterization

Sites were characterized by natural communities using maps at the Nongame and Natural Heritage Program – Vermont Fish and Wildlife Department. Many of the sites were made up of wetland complexes consisting of several natural communities. Since the life history of the *R. pipiens* is likely to encompass much of the wetland complex, we did not attempt to depict one natural community as the type.

Northern leopard frog (*R. pipiens*) surveys

Rana pipiens was chosen as the target species for several reasons: it has been the most reported species with abnormalities in Vermont (and within the Lake Champlain Basin); it is primarily a terrestrial species as an adult; and it can be very abundant locally.

At the five primary study sites, a minimum sample size of 50 (maximum 150 per sampling event) *R. pipiens* metamorphs (<4cm) were targeted for collection using the search and seize method. Frogs were collected in open grassy areas adjacent to breeding areas using hand held nets. The frogs were placed into buckets that held some water and vegetation. Holding the frogs prevented recapture bias and helped keep the frogs in good condition. Frogs collected were measured and weighed to determine overall body condition and examined in the field for gross external abnormalities and characterized using standard nomenclature (Meteyer, 2000a). Uniform classification of malformations using universally accepted terminology allow for better comparison and insight into these syndromes. Two collections were made during the summer of 2001, the first collection occurred in early July as the metamorphs were transforming, the second collection occurred 4 - 6 weeks later. Digital photographs were taken as warranted to detail and catalog specific abnormalities observed.

In addition to the five primary study sites, four supplemental sites shown in Table 2 were surveyed to provide additional data on the frequency and occurrence of abnormalities among newly metamorphosed *R. pipiens*. These sites were also located in the Lake Champlain Basin and have been monitored since 1997 (Table 8). All of the supplemental sites with the exception of Cornwall Swamp are located close to Lake Champlain and as such are affected by the lakes annual fluctuations.

Table 2. Four Supplemental Sites Surveyed for *R. pipiens*

Site	Site Code	Town	County	Latitude			Longitude		
				Deg	Min	Sec	Deg	Min	Sec
Winooksi River Delta	WIN	Colchester	Chittenden	44	32	8.2	73	16	32.0
Lewis Creek	LEW	Ferrisburg	Addison	44	14	42.0	73	15	17.2
Cornwall Swamp	CSW	Salisbury	Addison	43	55	39.0	73	10	28.3
Missisquoi NWR	MSQ	Swanton	Franklin	44	56	30.2	73	09	44.2

Tadpole Surveys

Developing *R. pipiens* were also observed at the five primary sites, from early limb development to metamorphosis. Larval stage tadpoles, Gosner stage 30-45 were collected and examined for gross abnormalities. A minimum of 100 tadpoles were examined from each site, using a dissecting scope (7-60X). Developmental characteristics (e.g. stage) were described and all abnormalities were characterized using a standardized descriptive format. Of particular interest were observations on the occurrence and character of hind limb abnormalities.
A portion of abnormal tadpoles were brought back to the Laboratory for continued observations and rearing. These observations may directly address a data gap in the testing of the hypothesis that observed abnormalities are primary errors in development (malformations) rather than abnormalities caused by mechanical or non-developmental means (deformities). Digital photographs were taken as warranted to detail and catalog specific abnormalities observed.

Statistics for Metamorph and Tadpole Surveys

Frequency of abnormalities – The frequency of morphological abnormalities was calculated for each site as the percentage of abnormal individuals relative to the total number examined (n>50).

Abnormality composition- morphological abnormalities were described by site and life history stage (larval, adult).

Drift Fence Monitoring at Ward Marsh 1998 -2001

In 1997, large numbers of abnormalities were observed in Northern Leopard Frogs (35% abnormal) by the VTDEC at Ward Marsh in West Haven, Vermont. In 1998 seven drift-fences were built in an effort to begin intensive long-term monitoring of all amphibian species present at the site. Amphibian populations have now been monitored at this site from 1998 through 2001. The intensive long-term monitoring of this study has multiple goals: to (1) establish a baseline data set of abundance indices for the amphibian species caught in the fences, (2) monitor year-to-year changes in their abundance indices, (3) monitor changes in the number and type of external abnormalities of Northern Leopard Frogs and other amphibian species, (4) compare conclusions derived from annual small sub-samples (50-150 metamorphs) with this intensive population sampling, in order to evaluate the sub-samples' representativeness in regards to both malformation rates and temporal and spatial variations in amphibian population characteristics, (5) provide information on dates of metamorphosis, average size at metamorphosis, and size classes.
Seven drift-fences were put into the ground in late June 1998, around the southernmost bays of Ward Marsh (Figure 3) in West Haven, Vermont. The marsh is an extensive cattail marsh located next to the Poultney River at the southern tip of Lake Champlain. Large hay fields border the marsh, separated from them only by a barbed-wire fence and a narrow strip of old field in early successional growth. Most of the fences (six of the seven) were placed between the fields and the marsh in the narrow strip of uncut old- field (Figure 4). These fences were built in three side-by-side pairs.

Figure 3. Location of seven drift fences at Ward Marsh in West Haven, Vermont.

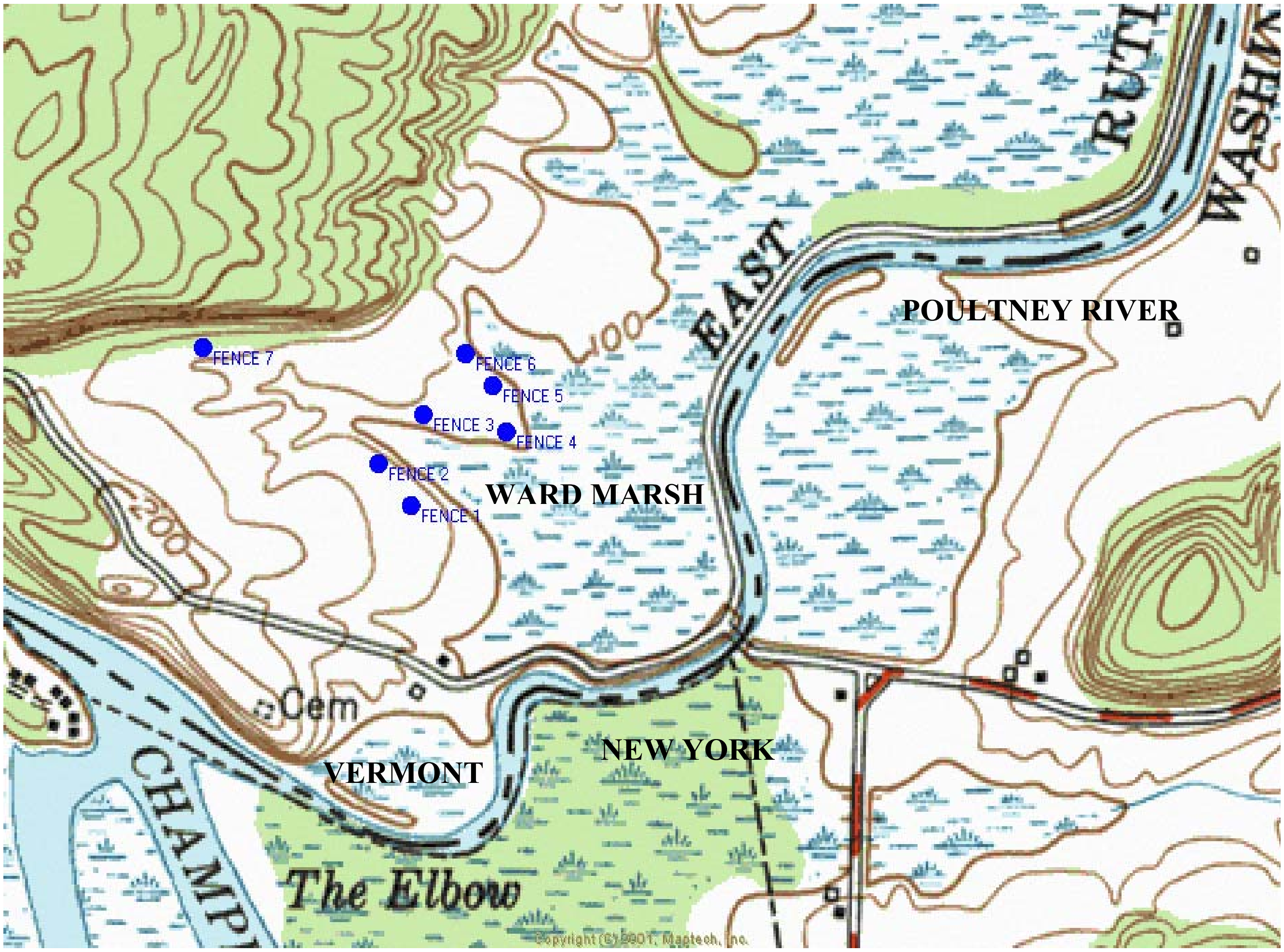
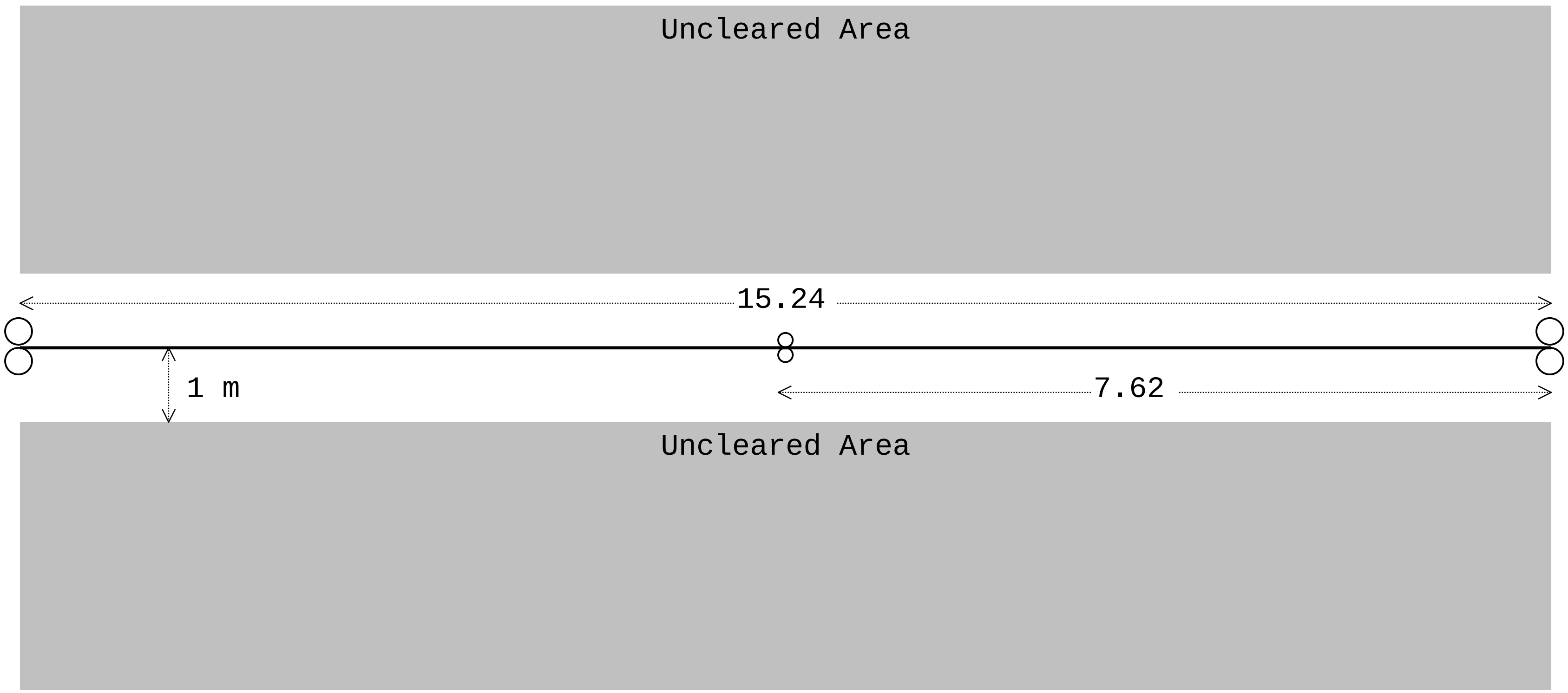


Figure 4. Diagram of the design of the drift-fences at Ward Marsh, West Haven, Vermont. Each fence is 15.24 meters long, with a series of buckets and cans placed flush along it. There is one meter on each side of the fence that is cleared of vegetation.



The old-field habitat immediately surrounding these fences is largely grasses but includes scattered bull thistle, gray-stemmed dogwood, prickly ash, and honeysuckle, along with the occasional red osier dogwood, red cedar, Queen Anne's lace, goldenrod, and white ash (near Fence 6). In the marsh between Fences 1 & 2 and 3 & 4 are large sedges, with cattails near the mouth of the bay. Fences 1 and 2 are 24 meters apart, an average of 22 meters from the high water mark, and 3 meters from the fence line. Fences 3 and 4 are 59 meters apart, an average of 15 meters from the high water mark, and 6.5 meters from the fence-line. Fences 5 and 6 were 34 meters apart, an average of 32 meters from the high water mark, and 4.25 meters from the fence-line. Fence 7 was set apart from the other paired fences in an effort to sample amphibian use of a small pond near the edge of the woods on the uphill edge of the fields. It is 38 meters from the pond, and 16 meters from the edge of the woods. In the woods near Fence 7 are white and red oak, white ash, maples, hop hornbeam, and shagbark hickory. In all, it is estimated that these fences cover 12.5% of the perimeter of the two southernmost bays of the marsh.

All fences were made of 15.24-meter lengths of 51 cm aluminum flashing, buried 10 cm in the ground. This left a 41 cm barrier to amphibian feeding and migratory movement. Tangential with the flashing and buried flush with the ground surface are a series of cans and buckets with lids that can be opened prior to periods of expected amphibian activity. On each side of a fence, there are two 22.75-liter buckets (one on each end), and one 6.2-liter stainless steel can placed halfway between the buckets (Figure 5). The can is 15.5cm in diameter, and 33cm in depth. Each can has a funnel placed into the top of it that has an opening diameter of 10.5cm. This is to prevent escape of animals that could crawl up the sides of the cans. Both the cans and the buckets have holes drilled in them 2.5cm from the bottom, in order to hold some water and prevent dehydration of amphibians. There is 1-meter strip on each side of the fence that is cleared of shrubs and tall grasses.

Figure 5. Drift-fence at Ward Marsh in West Haven, Vermont.

Fences were opened before noon on warm days with rain, or when rain was expected, three times per month from April through October. 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 for population indices are used from only the three most productive nights (greatest number of amphibians caught). Occasionally, 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 seven days), or the end of the previous month (last seven days), and three other heavy rains also occur during that month, then the data are shifted to the dry month. In order to calculate an index of number of captures per trapping, data from four months (July through October) are used. This is due to the fact that in 1998, the fences were not all installed until July, so in order for the four years' data to be comparable, only data from July through October could be included in the index. Traps were left open for the afternoon and evening and checked the following morning, and every individual was measured, checked for external abnormalities, and released. In 1999, 2000, and 2001, the side of the fence that amphibians were captured on was recorded as well.

Data Analyses For Population Trends

We used power analyses to evaluate our ability to accurately observe trends in amphibian populations. The likelihood that an apparent trend reflects a true trend in population numbers is referred to as power. Statistically it is defined as the likelihood of correctly rejecting the null hypothesis (no trend). Our goal is to achieve a power of 90% or greater. The powers of these data sets are dependent upon a number of variables: the length of the series of data-gathering units (at this point 4 years), the number of indices on which variation is based (8, see description below), the number of locations from which data are gathered (in this case one, because although seven fences are used, the data are combined), the variability of the data collected (differs for each species, see below), the starting value of the abundance indices (differs for each species), how small a trend we hope to be able to detect (5% annually), and what statistical level of significance is acceptable: alpha = 0.10 (10% chance of incorrectly rejecting the null hypothesis). The variability (standard deviation) of the data collected for each species is an estimate of how much the index varies. At Ward Marsh, six of the seven fences were set up in pairs side-by-side so that we could calculate standard deviation. One fence in each pair was randomly selected (Fences 1, 4, and 5 grouped together; Fences 2, 3, and 6 grouped together), and an index for both adults and juveniles was calculated. These indices were based on 12 data sets per year (three per month for July through October). Standard deviation was then calculated from these two indices (three fences each). The standard deviations from all four years were averaged to give a single standard deviation value that was more accurate than any one year's value. The standard deviation is therefore based on eight indices (two each year for four years). The starting value used in the power calculation was the index calculated from all seven fences. The power figures shown (Table 16 in the results section) was generated using the Monitor.exe freeware program written by James P. Gibbs and available on the National Biological Survey's Inventory and Monitoring website (http://www. mp1-pwrc.usgs.gov/powcase/). Also available through this site is a more extended discussion of power and the rationale for the power and alpha values used here.

Limb Bud Assays

Three sets of five surface water and sediment samples from five different sites were collected over a 2 month period (Table 3) and shipped overnight via commercial carrier to the laboratory at 4°C. Three sites labeled ALB, POL, and OTC were designated as test sites and two sites labeled MDC and NH were designated as a reference sites (refer to Table 1 for site codes). These designations were based on previous field and laboratory observations. These samples were stored at 4°C upon receipt and throughout the testing period. Each sample was thoroughly mixed before testing. Each set of water samples received was initially analyzed for pH, dissolved oxygen (DO), conductivity, hardness, alkalinity, ammonia-nitrogen, and chlorine. Temperature, pH, and DO were routinely monitored throughout the study.

Test Culture Care and Breeding

Adult *X. laevis* were acquired from *Xenopus* I (Dexter, MI). *Xenopus* adult care, breeding, and embryo collection were performed as described in ASTM E1439-98 (ASTM, 1998), at Fort Environmental Laboratories, Inc. Adult *X. laevis* were fed Salmon Starter pellets, purchased from Zeigler Bros. (Gardners, PA), once per day on alternating days, ad libium.

R. pipiens adult care, breeding, and embryo collection were performed at Carolina Biological Supply Company (Burlington, NC). The collected embryos were shipped overnight via commercial carrier to Fort Environmental Laboratories, Inc. Larval stage specimens were fed Salmon Starter Mash (Zeigler Bros., Gardners, PA) three times daily, ad libium.

Early Embryo-Larval Stage Development (Phase I)

The *X. laevis* larvae were exposed to each of the five water samples plus a laboratory negative control of FETAX solution (ASTM E 1439, 1998) until Gosner stage 26-28 (approximately 4-6 days). In addition, 2 concentrations of 6-aminonicotinamide (5.5 mg/L to approximate a teratogenic concentration and 2,500 mg/L to approximate a predominantly lethal concentration in *X. laevis*) were used as positive controls. Initially, 5 replicates of 10 blastulae (stage 8-9) each were placed in 60 mm Petri dishes containing 10 mL each of test material and incubated at 23° ± 1°C. Five replicates of *R. pipiens* were exposed to 40 mL of each water sample, plus negative and positive laboratory controls, in 100 mm Petri dishes until Gosner stage 26-28 (ca. 10 days). Five replicates of *R. catesbeiana* blastula were set up for the FETAX solution control and water/sediment samples MDC and NH following the same criteria used for *R. pipiens*. Due to limited sample, sample sites ALB, POL, and OTC were not tested using *R. catesbeiana*.

Daily mortality was determined for each replicate dish and an average (representative) stage of development was determined for larvae in each water exposure. At the end of each incubation period (free-swimming stage), all surviving larvae were scored for abnormalities, digitally photographed, and transferred to larger test vessels for the limb development phase of the study.

Limb Development (Phase II)

Each replicate dish of stage 26-28 free-swimming larvae were transferred from the Petri dish to an identically labeled 4-L plastic test vessel containing approximately 750 g sediment sample and 3 L of water sample approximating a 1:4 ratio. Prior to adding larvae, each vessel was fitted with a mesh screen insert positioned at the sediment and water interface to prevent the larvae from becoming lost in the sediment. Dechlorinated (aged) tap water and sterilized sand were used as the negative control, replacing the FETAX solution used in Phase I. FETAX solution was designed specifically for the needs of short-term bioassays and extended periods of exposure to the higher salt concentrations in the FETAX solution was a concern. Each test vessel received continuous aeration throughout Phase II. The test cultures were fed Salmon Starter Mash three times per day. Each test vessel was renewed every 1 to 2 weeks by removing 1 L of test water and replacing that volume with 1 L of fresh water sample from refrigerated storage. Ammonia-nitrogen in the test vessels was periodically measured to monitor possible waste accumulation due to depuration and decomposition of food materials. If ammonia-nitrogen levels approached 10 mg/L, the renewal frequency for that vessel was increased.

Mortality and developmental stage was recorded daily for each replicate test vessel. As the test specimens reached the end of limb development (stage 40-42), each was scored for abnormalities and digitally photographed. The study was concluded at the end of 3 months. Refer to Appendix E for Raw Data on Water Chemistry, Mortality and Malformations.

Water Quality Measurements

All water and sediment collections and measurements were made at site locations within known *R. pipiens* breeding grounds. Locations were considered to be representative of the site.

Field Water Quality Measurements

Field water quality measurements and collections were made prior to sediment collections and any other sampling activities just below the surface of the water where the water depth was less than one foot (30cm). Water column measurements performed on site with field meters included: temperature, pH, conductivity and dissolved oxygen.

Water collections for VTDEC Laboratory analysis included: (13) priority pollutant heavy metals, calcium, magnesium, sodium, potassium, ammonia and nitrate (Table 3).

Water and Sediment Collections for Pesticide Analysis and Limb bud Assays

Representative water and sediment samples for pesticide analysis and limb bud assays were collected from the five primary sites during *R. pipiens* critical developmental exposure periods in breeding areas (April 15 –June 15).

Three water and sediment collections were evaluated for potential effects on hind limb development (limb bud assays). Two water and sediment samples were evaluated for the occurrence of current use pesticides and metabolites (Table 5), the first was collected in late

April and the second was collected in mid-June. These collections coincided with the first and third water and sediment collections for the limb bud assays.

Sediment samples were collected using methods described in USEPA OEME/ECA Sediment Sampling Collection Methods SOP #2.25. A 6-inch Ekman dredge was used to collect sediment samples for pesticide analysis and limb bud assays. Sediments were collected from the upper 4 inches of bottom substrate.

Water and sediment collection methods, sample volumes, containers and preservation are listed in Section 12.9 of the QAPP (VTDEC, 2001).

Table 3. Sampling Schedule of Chemical, Physical Measurements for the Five Study Sites.

Parameter	Schedule
pH, field	each site visit
pH, Lab (stirred)	3X (early May, late May, mid-June)
Alkalinity	3X (early May, late May, mid-June)
Conductivity, field	each site visit
Conductivity, lab	3X (early May, late May, mid-June)
Dissolved Oxygen	each site visit
Color	each site visit
Water Temperature	each site visit
Nitrogen, Nitrate (Ion Chromatography)	3X (early May, late May, mid-June)
Nitrogen-Ammonia	3X (early May, late May, mid-June)
Ca, Mg, Na, K	3X (early May, late May, mid-June)
Priority Pollutant Metals: (13) Antimony Arsenic Beryllium Cadmium Chromium Copper Lead Mercury Nickel Selenium Silver Thallium Zinc	1X (mid-June)
Pesticide Analytes (water and sediment)	2X (early May, mid June)
Limb bud Assay (water and sediment)	3X (early May, late May, mid-June)

Pesticide Analysis

Two water and sediment samples were evaluated for the occurrence of current use pesticides and metabolites, the first was collected in late April and the second was collected in mid-June.

All samples were analyzed utilizing methods developed in the Vermont Department of Agriculture and Food Markets, (VDAFM) laboratory, generally adapted from published methods. Analytes were subdivided by a combination of analytical methods used and host group category (see Tables 4 and 5). The soil and water Method Detection Limits (MDLs) for each analyte are listed in Table 5. The methods for Acid Herbicides in sediment and Metabolites in sediment were not finalized at the time of this study so the MDLs are still in the draft stage. The following is a description of the sample prep method in water and sediment and the analytical procedure for each analyte group (VDAFM Standard Operating Procedures (SOPs) are available upon request).

Table 4. Commercial Pesticide Usage Summary for 2000 by Host Group

Host Group	Pounds of Active Ingredient	Percent of Total
Cooling Towers	807923	71.9%
Corn	239477	21.3%
Wood Treatment	29813	2.6%
Golf Courses	20363	1.8%
Grass, Turf, Poison Ivy, Weeds	5444	0.5%
Electrical Utility	4945	0.4%
Railroads	3956	0.4%
Tree Fruits	3371	0.3%
Structural	2839	0.2%
Ornamentals	2186	0.2%
Highway	1345	0.1%
Small fruits and vegetables	1088	<0.1%
Mosquitoes	552	<0.1%
Nurseries, plant propagation, xmas trees	369	<0.1%
Aquatic	325	<0.1%
Field and forage	186	<0.1%

Table 5. Pesticide Analyte List

Analyte Group	Commercial Use[1]	Percent Of Total[2]	Water Mdl[3] (Mean Recovery)	Soil Mdl[3] (Mean Recovery)
Corn Herbicides				
Atrazine	88878	28.1	0.03 ppb (108%)	7 ppb (76%)
Simazine	22774	7.2	0.03 ppb (96%)	8 ppb (59%)
Alachlor	2966	0.9	0.02 ppb (109%)	17 ppb (107%)
Metolachlor	70102	22.2	0.02 ppb (110%)	5 ppb (82%)
Cyanazine	1636	0.5	0.06 ppb (109%)	10 ppb (79%)
Pendimethalin	31541	10	0.03 ppb (90%)	18 ppb (86%)
Dimethenamid	6534	2.1	0.02 ppb (106%)	4 ppb (71%)
Acetochlor	1581	0.5	0.03 ppb (108%)	16 ppb (74%)
Acid Herbicides				
2,4-D	1891	0.6	0.05 ppb (100%)	5 ppb (68%) [4]
MCPP	970	0.3	0.05 ppb (68%)	5 ppb (82%) [4]
MCPA	1249	0.4	0.05 ppb (80%)	5 ppb (76%) [4]
Dicamba	1420	0.4	0.05 ppb (62%)	5 ppb (94%) [4]
Triclopyr	3441	1.1	0.05 ppb (89%)	5 ppb (85%) [4]
Dacthal	0	0	0.05 ppb (75%)	NA
Insecticides				
Diazinon	77	<0.1	0.06 ppb (75%)	4 ppb (117%)
Chlorpyrifos	1516	0.5	0.05 ppb (71%)	2 ppb (94%)
Malathion	79	<0.1	0.05 ppb (76%)	6 ppb (111%)
Right Of Way				
Imazapyr	30	<0.1	0.06 ppb (112%)	3 ppb (69%)
Flumetsulam	1593	0.5	0.13 ppb (83%)	3 ppb (80%)
Nicosulfuron	226	<0.1	0.11 ppb (62%)	3 ppb (76%)
Metsulfuron methyl	3	<0.1	0.13 ppb (59%)	3 ppb (59%)
Sulfometuron methyl	216	<0.1	0.12 ppb (72%)	3 ppb (99%)
Diuron	2326	0.7	0.16 ppb (101%)	5 ppb (88%)
Primisulfuron	4	<0.1	0.10 ppb (39%)	4 ppb (90%)
Metabolites				
Desethyl atrazine	NA	NA	0.02 ppb (105%)	5 ppb (62%) [4]
Desisopropyl atrazine	NA	NA	0.02 ppb (94%)	5 ppb (58%) [4]
Alachlor ESA	NA	NA	0.05 ppb (65%)	5 ppb (22%) [4]
Alachlor OA	NA	NA	0.02 ppb (41%)	5 ppb (54%) [4]
Metolachlor ESA	NA	NA	0.03 ppb (72%)	5 ppb (21%) [4]
Metolachlor OA	NA	NA	0.02 ppb (45%)	5 ppb (21%) [4]
Acetochlor ESA	NA	NA	0.06 ppb (63%)	5 ppb (37%) [4]
Acetochlor OA	NA	NA	0.02 ppb (41%)	5 ppb (44%) [4]
Total	**241,053**	**76%**		

[1] = POUNDS OF ACTIVE INGREDIENT APPLIED BY COMMERCIAL APPLICATORS IN 2000.
[2] = PERCENT OF TOTAL NON-COOLING TOWER COMMERCIAL APPLICATION IN 2000
[3] = Method Detection Limit
[4] = Draft Method Detection Limit (see text)

Corn Herbicides

<u>Water:</u> 800ml of sample water was measured into a 1000ml erlenmeyer flask, and propazine added at a concentration of 0.77 ppb, as a surrugate to assess extraction efficiency. The sample was then extracted on an Autotrace automatic sample extractor using 'Waters Sep-Pak C18 SPE cartridges (500mg)' conditioned with methanol and eluted with 6 ml of 90% ethyl ether/10% hexane. The organic solvent was evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of ethyl acetate containing anthracene as internal standard. (SOP # VDAFM-PRL-2, modified from EPA method 525.1).

<u>Sediment</u>**:** Each sediment sample was air dried and sieved through a number 10 (2.0 mm) sieve prior to analysis. 50g of sediment was weighed into a 200ml centrifuge bottle, and propazine was added to a concentration of 0.062 ppm as a surrugate to assess extraction efficiency. Sediment was extracted twice with 80% methanol/20% water, for three hours in a 65 C. shaking water bath. After centrifugation the extracts were combined, the volume recorded, and 10 ml. was added to 1490 ml of deionized water. This solution was run through the Autotrace autoextractor as described above for the water samples. (SOP # VDAFM-PRL-5, modified from: Huang and Pignatello,1990).

<u>Analysis</u>**:** Corn herbicide analysis was by Gas Chromatograph/Mass Spectometer (GC/MS) using a Finnigan Polaris Q ion trap GC/MS, 30m x 0.25 mm x 0.25 μ DB-5ms column, 1.5 μl injection, and three point calibration ranging from MDL to 25 times MDL.

Acid Herbicides

<u>Water:</u> 800 ml of sample water was adjusted to pH 12 with NaOH for one hour, to hydrolyze any esters, the sample was then acidified to pH 1.9 with H2SO4. The acidified water was extracted on an Autotrace automatic sample extractor using 'Supelco ENVI-Chrom P SPE cartridges (500mg)' conditioned with methanol and eluted with 6ml of 90% MTBE/ 10% MeOH. Acid herbicides in the MTBE/MeOH extract were then converted to their respective methyl esters using a micromolar diazomethane generator as described in EPA method 515.2. The extract solution was then evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of ethyl acetate containing anthracene as internal standard. (SOP # VDAFM-PRL-4a, modified from EPA method 515.2).

<u>Sediment</u>**:** Each sediment sample was air dried and sieved through a number 10 (2.0 mm) sieve prior to analysis. 20g of sediment was extracted twice with 0.5 N KOH in 10% KCL, in boiling water bath and orbital shaker, the extracts were centrifuged then combined. The pH of the combined extract was adjusted to 1.5 prior to being extracted three times with chloroform. The chloroform extract was evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 5ml of ethyl ether plus 1 ml of methanol prior to diazomethane derivatization as described in EPA method 515.2 (SOP # VDAFM-PRL-10, modified from EPA-NEIC method "Method for Chlorinated Phenoxy Acid Herbicides in Soil and Vegetation").

Analysis: Acid herbicide analysis was by Gas Chromatograph/Mass Spectometer (GC/MS) using a Finnigan Polaris Q ion trap GC/MS, 30m x 0.25 mm x 0.25 μ DB-5ms column, 1.5 μl injection, and three point calibration ranging from MDL to 25 times MDL.

The herbicide Dacthal is listed in Table 2 as an acid herbicide, but it is generally applied as a methyl ester which breaks down very rapidly in the environment to an acid metabolite. It is this acid metabolite which was actually analyzed for during this study.

Insecticides

Water: 1000 ml of sample water was measured into a 2000 ml separatory funnel, and propazine added at a concentration of 0.77 ppb, as a surrugate to assess extraction efficiency. The sample was extracted three times with 15% methylene chloride/ 85% hexane, the extracts were filtered through Na_2SO_4 to remove residual water and combined. The combined extract was then evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 0.50 ml of ethyl acetate containing anthracene as internal standard. (SOP # VDAFM-PRL-18, modified from EPA method 614).

Sediment: Each sediment sample was air dried and sieved through a number 10 (2.0 mm) sieve prior to analysis. 15g. of sediment was weighed into a 33 ml sample cell and propazine was added to a concentration of 0.038 ppm as a surrugate to assess extraction efficiency. Samples were extracted in a Dionex ASE 200 Accelerated Solvent Extractor at 100^{C} and 2000 psi for 15 minutes using 50% acetone/50% hexane (plus 1% acetic acid). The extract was then evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 2.0 ml of ethyl acetate containing anthracene as internal standard. (SOP # VDAFM-PRL-23, modified from EPA method 3545, and Dionex Application Note 319).

Analysis: Insecticide analysis was by Gas Chromatograph/Mass Spectrometer (GC/MS) using a Finnigan Polaris Q ion trap GC/MS, 30m x 0.25 mm x 0.25 μ DB-5ms column, 1.5 μl injection, and three point calibration ranging from MDL to 25 times MDL.

Right of Way

Water: 250 ml of sample water was measured into a 500 ml erlenmeyer flask and 2.5 ml of glacial acetic acid was added to acidify the sample. The acidified water was manually extracted using Waters Oasis HLB SPE cartridges (200 mg) conditioned with methanol and eluted with 10ml of methanol. The extract solution was then evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of 95% water (0.1% acetic acid)/5% acetonitrile. (SOP # VDAFM-PRL-17, modified from: Krynitski 1997).

Sediment: Each sediment sample was air dried and sieved through a number 10 (2.0 mm) sieve prior to analysis. 15g. of sediment was weighed into a 33 ml sample cell and extracted in a Dionex ASE 200 Accelerated Solvent Extractor at ambient temperature and1500 psi for 30 minutes using 70% (0.1 M ammonium carbonate)/30% methanol. The extract diluted with water to 100 ml and the pH was adjusted to 3.3 prior to manually extracting using Waters Oasis HLB SPE cartridges (200 mg) conditioned with methanol and eluted with 10ml of methanol.

The extract solution was then evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of 95% water (0.1% acetic acid)/5% acetonitrile. (SOP # VDAFM-PRL-21, modified from: Krynitski 1997).

Analysis**:** Right of way sample analysis was by High Performance Liquid Chromatograph/Mass Spectrometer (HPLC/MS) using a Finnigan LCQduo ion trap HPLC/MS, 15 cm x 2.1 mm x 5µ Supelco Discovery C18 column, 50 µl injection, and three point calibration ranging from MDL to 25 times MDL. Mobile phase was from 95% water (0.1% acetic acid)/5% acetonitrile, to 20% water (0.1% acetic acid)/80% acetonitrile.

Metabolites

Water**:** 800 ml of sample water was extracted on an Autotrace automatic sample extractor using 'Supelco ENVI-Chrom P SPE cartridges (500mg)' conditioned with methanol and eluted with 6ml of 90% ethyl ether/10% hexane, followed by 6 ml of methanol. The extract was evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of 95% water (0.2% formic acid)/5% acetonitrile. (SOP # VDAFM-PRL-19).

Sediment: Each sediment sample was air dried and sieved through a number 10 (2.0 mm) sieve prior to analysis. 15g of sediment was weighed into a 33 ml sample cell and extracted in a Dionex ASE 200 Accelerated Solvent Extractor at 100^{C} and 2000 psi for 45 minutes using 80% methanol/20% water. The extract was diluted with water to 500 ml and extracted on an Autotrace automatic sample extractor using 'Supelco ENVI-Chrom P SPE cartridges (500mg)' conditioned with methanol and eluted with 6ml of 90% ethyl ether/10% hexane, followed by 6 ml of methanol. The extract was evaporated just to dryness under nitrogen, using an N-evap nitrogen evaporator, and re-dissolved in 1.0 ml of 95% water (0.2% formic acid)/5% acetonitrile. (SOP # VDAFM-PRL-22).

Analysis: Metabolite sample analysis was by High Performance Liquid Chromatograph/Mass Spectrometer (HPLC/MS) using a Finnigan LCQduo ion trap HPLC/MS, 15 cm x 2.1 mm x 5µ Supelco Discovery C18 column (two in series), 50 µl injection, and three point calibration ranging from MDL to 25 times MDL. Mobile phase was from 95% water (0.2 % formic acid)/5% acetonitrile, to 50% water (0.2% formic acid)/50% (38% methanol/62% acetonitrile).

An attempt was made to include the atrazine metabolite hydroxy-atrazine in the Metabolites method, but it was not possible due to analytical problems with this compound.

RESULTS

Site Characterizations

Otter Creek (OTC)

The Otter Creek study site is located in the northwestern town of Ferrisburg within the lower Otter Creek Wildlife Management Area (WMA) (Figure 6). This WMA site comprises over 650 acres and is one of the largest floodplain forest-back swamp marsh complexes in Vermont. Behind the silver maple – dominated levee forest are huge expanses of deep rush and cattail marsh and buttonbush shrub swamp.

The study site is located on the west side of Fort Cassin Road approximately 1 km (3000') from Otter Creek's confluence with Lake Champlain. A dairy farm is located in close proximity to this study site. Cornfields (feed-corn) abut the southeastern portion of the wetland. Dairy cows are grazed and sheltered within 0.5 miles of the study site.

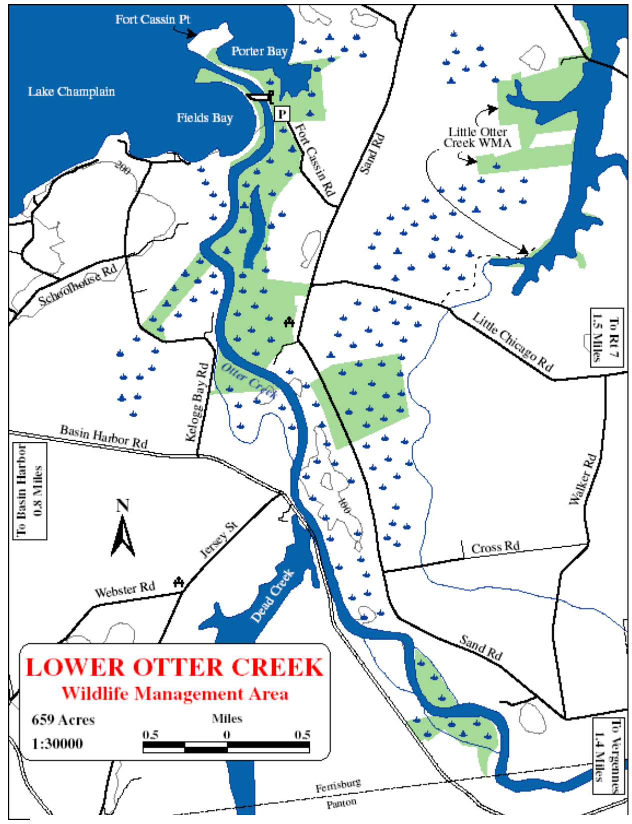


Figure 6. Otter Creek WMA

Figure 6a. Otter Creek Wetland

The majority of floodplain of lower Otter Creek is low and wet (Figure 6a), retaining standing water throughout the year, or at least the soils are supersaturated and the common communities are marsh and shrub swamp. The creek is a very low-gradient slow moving waterway where spring floods raise the creek level over 3' (1m) each spring.

The dominant land cover type in the Otter Creek watershed is forest, covering approximately 60% of the watershed. Agricultural land comprises 23% of the watershed area. (VTDEC, 1998). The lower reaches of Otter Creek and its tributaries are impaired by organic nutrients due to agricultural impacts which are not fully diluted due to the slow moving and impounded waters.

Otter Creek was first surveyed for *R. pipiens* abnormalities in 1996, we observed a moderate to high frequency of abnormalities. Subsequent years of sampling have demonstrated this site to have consistently moderate to high levels (average 4 out of 5 years greater than 10%).

Mud Creek (MDC)

Mud Creek is located in northwest Vermont in the town of Alburg within the Mud Creek WMA (Figure 7) This 1150-acre WMA is a mix of cattail-dominated emergent marsh and forested swamp (red maple/black ash; spruce/fir/tamarack) wetlands and a forested upland of red maple and white cedar. Mud Creek WMA is located within a highly agricultural landscape. Mud Creek flows to Lake Champlain However in the spring or other high lake water periods, the lake water flows into the wetland impoundment. The outlet is located at the southwestern portion of the wetland, and is approximately 1 mile from Lake Champlain.

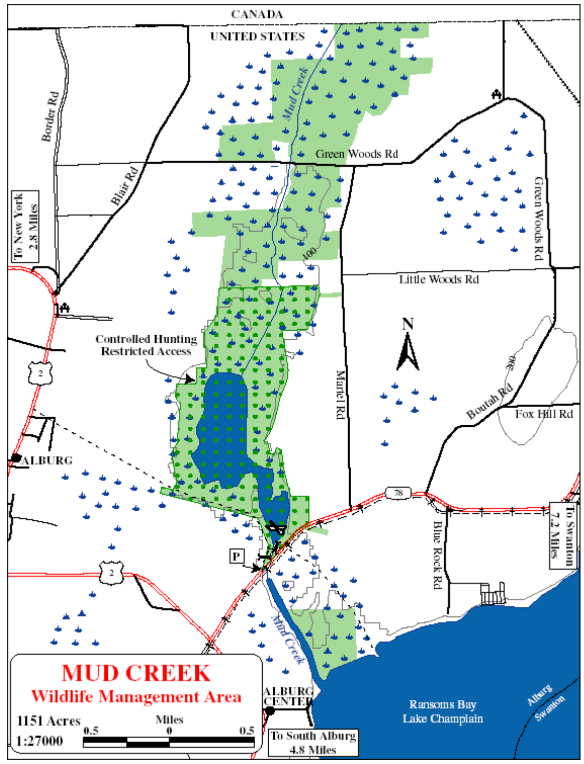


Figure 7. Mud Creek WMA

Water and sediment collections were taken from the southwestern portion of the impounded (dam controlled) wetland, closest to RT 78. Tadpole collections at this site failed and were moved to a northeastern location. Although the project's strategy was to collect water and sediment for analysis from the same location as tadpole collections, the historical aspects of this location justified retaining the established collection site.


Figure 7a. Mud Creek Marsh

The water and sediment sampling location is a cattail-dominated emergent marsh (permanent) (Figure 7a), whereas the tadpole collection location is red maple forested swamp (temporary).

Mud Creek was first surveyed for *R. pipiens* abnormalities in 1997, subsequently used as a control site due to low observed frequency of *R. pipiens* abnormalities and large area of buffered habitat.

Investigations by James LaClair have documented the presence of Methoprene metabolites at this location (LaClair et.al.,1998). Albeit there are no records of widespread Methoprene use in this part of the state.

Developmental bioassays have been conducted on water and sediment samples (Fort 1999a). Concentrations of heavy metals in sediment have been analyzed by Wall (1999).
R. pipiens metamorph examinations have been conducted by USGS NWHC. (Meteyer, 1997 & 2000b) and limb bud assays have been conducted by (LaClair, unpublished).

Alburg Dunes (ALB)

Located in the northwest corner of the state in the town of Alburg within Alburg Dunes State Park (Figure 8). Alburg Dunes was acquired and became a Vermont State Park in 1996. The park comprises 600+ acres along Lake Champlain, and includes a diversity of land types. There is a natural sand beach and dune complex

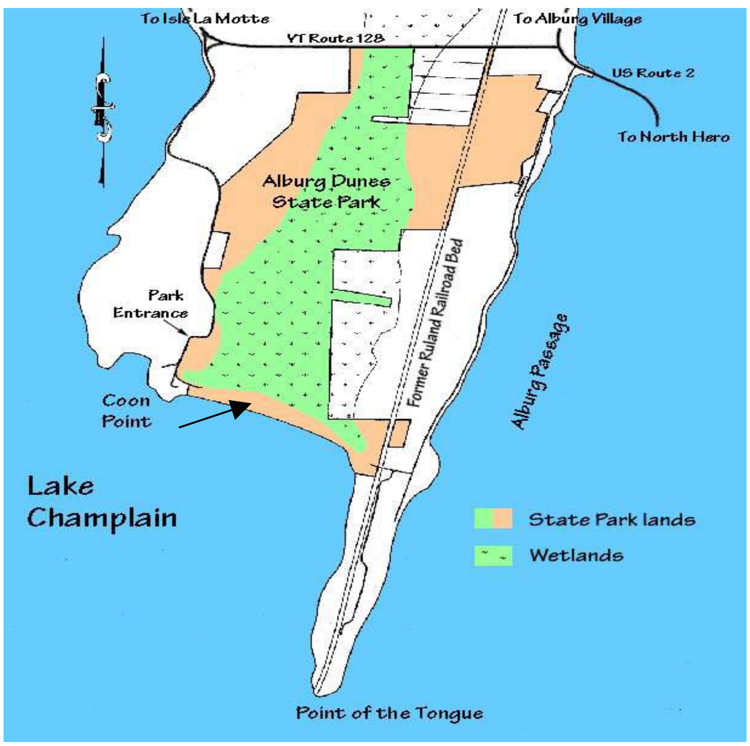


Figure 8. Alburg Dune State Park

Large expanses of productive wetland that are home to uncommon plants and contain a diversity of wetland types including forested wetland and emergent marsh, northern white cedar swamp, red maple-black ash swamp, and a rare black spruce/tamarack bog.

There is virtually no agricultural activity (Figure 9) adjacent Alburg Dunes State Park, the large expanses of wetland continues north of the park boundary encompassing an additional 500 plus acres. A small half-acre tannic pond was the primary focus of study in 2001 and in previous years. Lake Champlain is situated within 50 meters of the south side of the pond, and has the potential to contribute lake water to the pond during high water periods.

Of greater import is the wetlands contribution to this tannic pond. Connecting the north side of the pond to hundreds of acres of wetland is a road culvert. During high water periods the wetland drains into this pond. As the water levels drop the pond becomes isolated further concentrating the relative abundance of dissolved substances.

Figure 9. Aerial Photo of Alburg Site.

Alburg Dunes was first surveyed for *R. pipiens* abnormalities in 1997, we observed moderate to high frequencies (5%-15%) of *R. pipiens* abnormalities.

Laboratory tests conducted by James LaClair, revealed evidence of malformation-inducing acetylcholinesterase inhibition, typical of organo-phosphate pesticides and certain cyanobacterial toxins, caused by exposure to environmental samples from some Vermont sites (LaClair, unpublished).
Developmental bioassays were conducted on water and sediment samples (Fort, 1999a).
R. pipiens metamorph examinations have been conducted by USGS NWHC. (Meteyer, 1997 and 2000b).

North Hero (NH)

Located in the northwest corner of the state in the town of North Hero, at the northernmost tip of North Hero Island (Figure 10). This site is located within North Hero State Park, a 400-acre park. Nearly one third of the land lies below 100 foot elevation(Figure 9a). Lake level on Champlain fluctuates seasonally from about 95 to 101 feet above sea level. So much of the park is subjected to seasonal inundation. Chain pickerel and northern pike spawn and feed in the flooded areas.

Figure 9a. North Hero Floodplain.

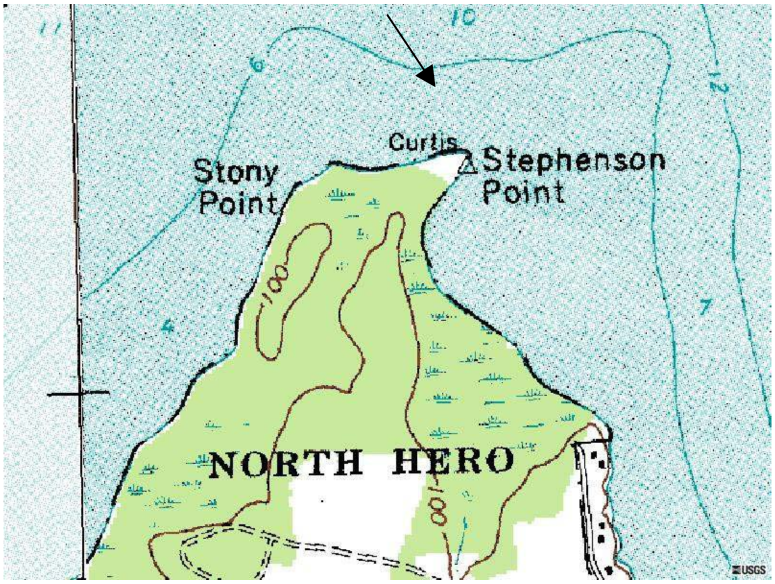


Figure 10. North Hero Study Site

The study sites are located within lakeside floodplain forest. Wetlands between Stephenson and Stony Point are forested wetlands.

There is no agricultural activity adjacent the State Park or study site. *R. pipiens* surveys have been conducted since 1997 and have yielded low numbers of abnormalities (1997-2001 average 2.08%).

Ward Marsh (WM) - Poultney River (POL)

Ward Marsh is located in west central Vermont in the town of West Haven. This Wildlife Management Area (WMA) (Figure 11) is a cattail marsh with a seasonally flooded red and silver maple forest fringe around the wetland and is adjacent to the Poultney River (Figure 11a). As such, the Poultney River's seasonal flooding influences the water quality of Ward Marsh. Approximately 0.5 miles (0.8 km) below the Ward Marsh site, the Lake Champlain Canal in New York joins with Poultney River. From this point, the Poultney River flows northwesterly for 2 miles to its terminus in Lake Champlain, adjacent South Bay.

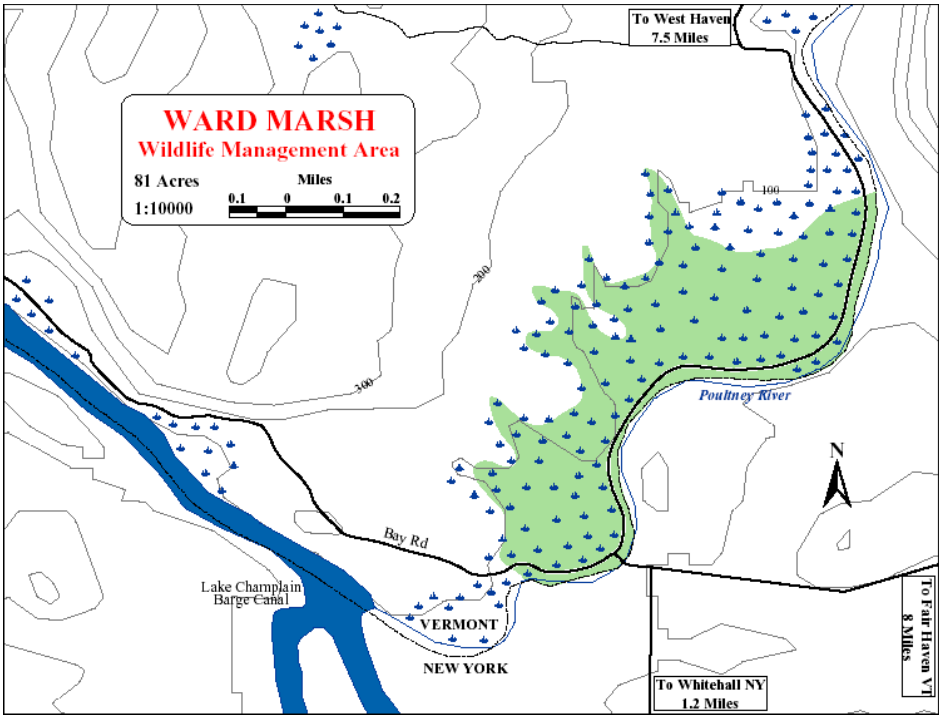


Figure 11. Ward Marsh WMA

The Poultney River drains 236mi^2 (611 km^2) in Vermont and is 40 miles long within and along the borders of Vermont.

The land use information generated through the Vermont Land Cover Classification Project shows that the Poultney River watershed is predominantly forested with 67% of the watershed area either deciduous or evergreen forest. A considerable portion of the watershed land use, 16%, is agricultural. Surface water covers about 8% of the watershed and wetlands comprise 3%. Transportation and other developed land covers about 6% of the watershed.

Figure 11a. Poultney River

Ward Marsh was first surveyed for *R. pipiens* abnormalities in July 1997, and achieved notable recognition due to extremely high frequencies of abnormalities (45%), a second survey in September 97, yielded 17% abnormalities. The combined average for the year was 35% abnormal.

Drift fence monitoring has been conducted at the Ward Marsh site from 1998 - 2001 by James Andrews, Middlebury College.

Developmental bioassays have been conducted on water and sediment samples (Fort, 1999a). Concentrations of heavy metals in sediment have been analyzed (Wall, 1999). *R pipiens* metamorph examinations have been conducted by USGS- NWHC. (Meteyer,1997 and 2000b).

Northern Leopard Frog (*Rana pipiens*) Metamorph Surveys

A total of 2959 *R. pipiens* metamorphs (juveniles) were collected and characterized from the five primary study sites (n = 2439) and the four supplemental sites (n = 520) in 2001.
Overall abnormality rates at the five primary sites and the supplemental sites were 4.2% and 4.0%, respectively. Percent abnormalities ranged from 0% – 8.8% for the primary sites and 0% – 6.5% for the supplemental sites. Table 6 and 7 present the number of abnormal metamorphs observed by site and sampling event at the primary and supplemental sites.

Table 6. Summary of *R. pipiens* Metamorph Surveys by Date at the Five Primary Sites 2001.

Site	Date	Normal	Abnormal	N	%Abnormal	%Abnormal Range
Otter Creek	07/10/01	63	1	64	1.6	
Otter Creek	07/20/01	121	3	124	2.5	
Otter Creek	08/08/01	119	11	130	8.8	
Otter Creek	08/23/01	128	9	137	6.6	
Otter Creek	09/12/01	138	12	150	8.0	
Total		**569**	**36**	**605**	**5.9**	**1.6 - 8.8**
Mud Creek	07/12/01	2	0	2	0.0	
Mud Creek	07/12/01	29	0	29	0.0	
Mud Creek	07/24/01	65	1	66	1.5	
Mud Creek	08/01/01	60	1	61	1.6	
Mud Creek	08/14/01	77	4	81	4.9	
Mud Creek	09/11/01	55	2	57	3.5	
Total		**288**	**8**	**296**	**2.7**	**0 - 4.9**
Ward Marsh	07/09/01	93	0	93	0.0	
Ward Marsh	8/7/2001	98	1	99	1.0	
Ward Marsh	9/6/2001	145	3	148	2.0	
Total		**336**	**4.0**	**340**	**1.2**	**0 - 2.0**
Alburg Dune	07/06/01	154	5	159	3.1	
Alburg Dune	07/24/01	95	4	99	4.0	
Alburg Dune	08/01/01	72	4	76	5.3	
Alburg Dune	08/09/01	51	4	55	7.3	
Alburg Dune	08/29/01	125	7	132	5.3	
Alburg Dune	09/18/01	105	8	113	7.1	
Total		**602**	**32**	**634**	**5.0**	**3.1 - 7.3**
North Hero S.P.	07/17/01	145	0	145	0.0	
North Hero S.P.	08/09/01	85	6	91	7.1	
North Hero S.P.	08/30/01	156	9	165	5.5	
North Hero S.P.	09/19/01	154	9	163	5.5	
Total		**540**	**24**	**564**	**4.2**	**0 - 7.1**
Total 2001		**2335**	**104**	**2439**	**4.3**	**0 - 8.8**

Table 7. Summary of *R. pipiens* Metamorph Surveys at the Four Supplemental Sites 2001.

Site	Date	Normal	Abnormal	N	%Abnormal	%Abnormal Range
Missisquoi -Trails	07/31/01	102	3	105	2.9	
Total		**102**	**3**	**105**	**2.9**	**na**
Lewis Creek	07/25/01	53	0	53	0.0	
Total		**53**	**0**	**53**	**0.0**	**na**
Cornwall Swamp	07/26/01	114	8	122	6.5	
Total		**114**	**8**	**122**	**6.5**	**na**
Winooski River	7/18/2001	110	1	111	0.0	
Winooski River	8/22/2001	120	9	129	6.9	
Total		**230**	**10**	**240**	**4.1**	**0 - 6.9**
Total		**499**	**21**	**520**	**4.03**	**0 - 6.5**

Frequency of abnormalities exceeded 4% at all of the primary study sites except for Ward Marsh. Figure 12 presents the frequency of abnormalities at the five primary sites for all sampling events 2001 (July – September).

Please note within the volume of this report; we will be using the term, "abnormality" as a default way of describing any observed change from the normal. Since the means to characterize and interpret specimens may not be currently available to make a more definitive characterization with regards to "deformation," or "malformation." See Appendix G for definitions.

Figure 12. Percent Abnormal *R. pipiens* Metamorphs at Five Primary Sites 2001 (July-Sept). Survey's 1 thru 6 within graph legend denote season progression. (Site Abbreviations: Otter Creek -OTC, Mud Creek - MDC, Ward Marsh–WM &POL, Alburg Dune – ALB, North Hero – NH).

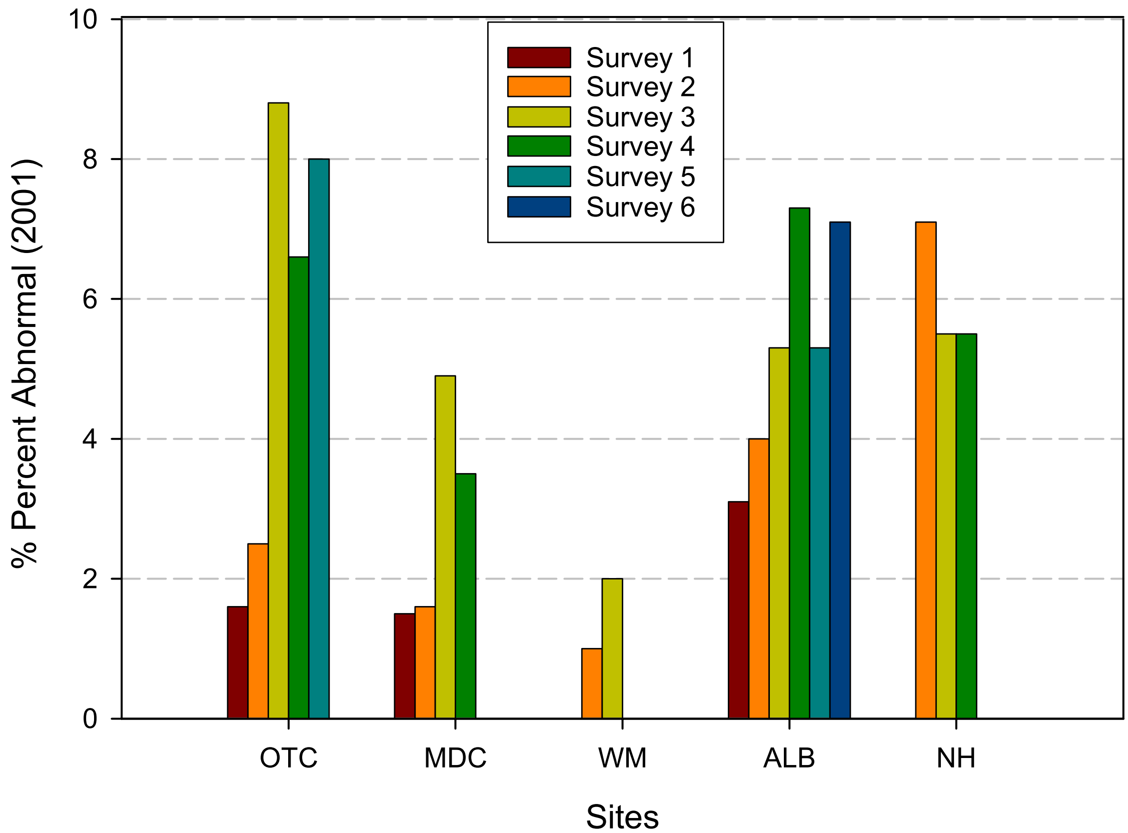
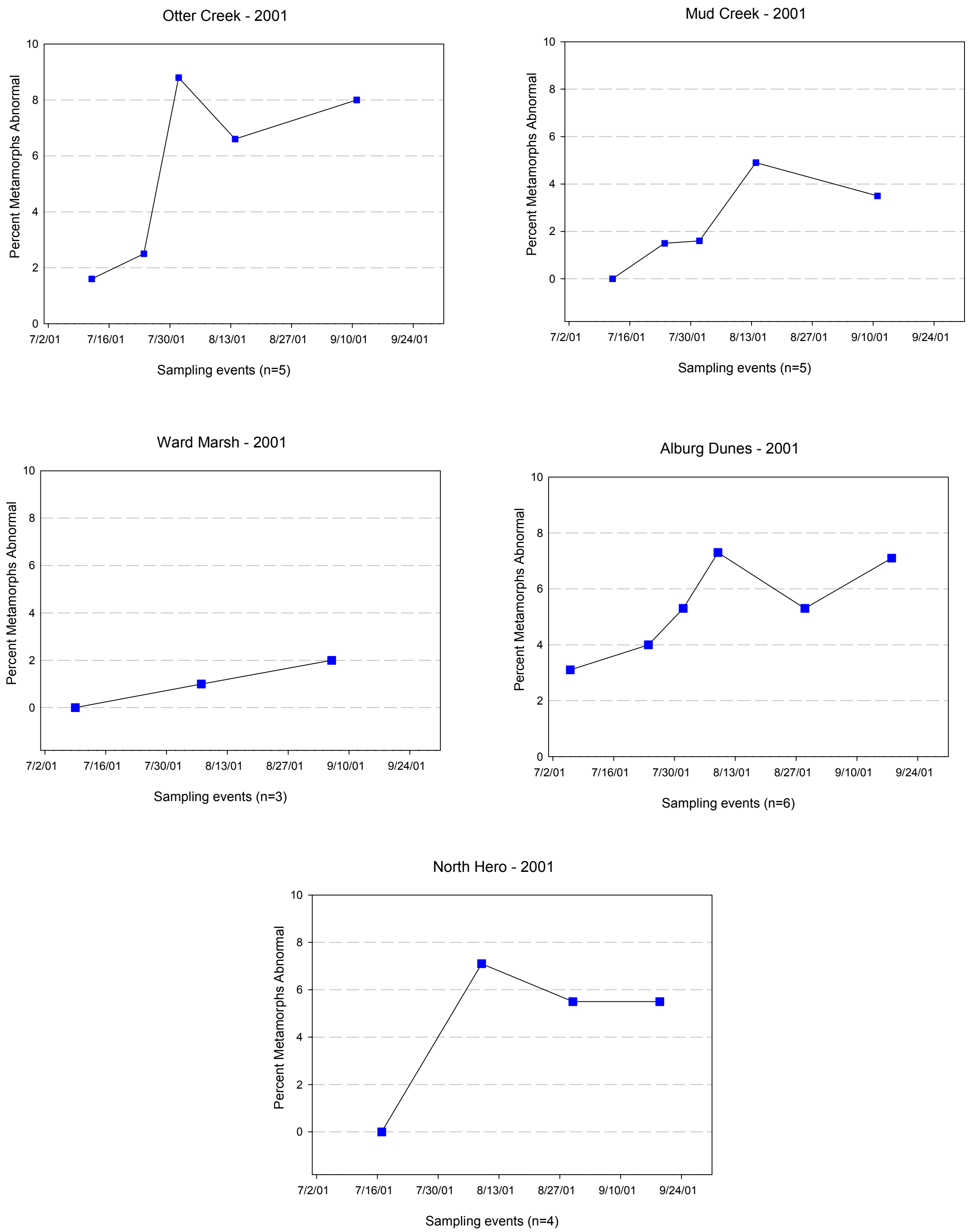


Figure 13 presents the percent abnormal *R. pipiens* at the five primary sites by Sampling event. Note that as the season progresses (July – mid August) all of the sites show an increase in the percent abnormalities observed.

Figure 13. Percent Abnormal *R.pipiens* Metamorphs at the Five Primary Sites by Sampling Event (July- September 2001).



To help depict the levels of abnormalities observed at sites surveyed in 2001, Table 8 includes *R. pipiens* summary data for 1997 - 2001 sampling events for the five primary sites.

Table 8. Summary of *R. pipiens* Metamorph Abnormalities 1997 - 2001 at Five the Primary Sites.

Year	Site	Normal	Abnormal	N	%Abnormal	% Abnormal Range
	Otter Creek	174	13	187	6.9	
	Mud Creek	54	0	54	0.0	
	Ward Marsh	121	67	188	35.1	
	Alburg Dune	163	10	173	5.7	
	North Hero	74	1	75	1.3	
1997		**586**	**91**	**677**	**13.4**	**0 - 35.1**
	Otter Creek	162	27	189	14.2	
	Mud Creek	233	11	244	4.7	
	Ward Marsh	535	19	554	3.4	
	Alburg Dune	228	18	246	7.3	
	North Hero	255	10	265	3.7	
1998		**1413**	**85**	**1498**	**5.7**	**3.4 - 14.2**
	Otter Creek	142	36	178	20.2	
	Mud Creek	71	2	73	2.7	
	Ward Marsh	105	3	108	2.7	
	Alburg Dune	71	4	75	5.3	
	North Hero	1	0	1	0.0	
1999		**390**	**45**	**435**	**10.3**	**0 - 20.2**
	Otter Creek	121	1	122	0.8	
	Mud Creek	70	1	71	1.3	
	Ward Marsh	66	0	66	0.0	
	Alburg Dune	263	9	272	3.3	
	North Hero	82	1	83	1.2	
2000		**602**	**12**	**614**	**2.0**	**0 - 3.3**
	Otter Creek	569	36	605	5.9	
	Mud Creek	286	8	294	2.7	
	Ward Marsh	336	4	340	1.2	
	Alburg Dune	602	32	634	5.0	
	North Hero	540	24	564	4.2	
2001		**2333**	**104**	**2437**	**4.2**	**1.2 - 5.9**
Total 1997 - 2001		**5324**	**337**	**5661**	**6.0**	**0 - 35.1**

The 1997 –2001 *R. pipiens* surveys at the five primary sites characterized 5661 metamorphs, with an overall abnormality frequency of 6.0% (abnormal = 337). The graphical representation of the average annual 1997 – 2001 data shown in Figure 14 clearly shows a decrease in the incidence of abnormal metamorphs after 1998 at three of the five sites. Otter Creek showed a decrease after 1999, while Ward Marsh decreased significantly after 1997. All of the sites except for North Hero were at their lowest frequency of abnormalities in 2000. The 2001 sampling showed an increase at all five sites, though it was barely noticeable at the Ward Marsh site. If it were not for the 1997 data, Ward Marsh would have been considered a low incidence site.

Figure 14 Percent Abnormal *R. pipiens* Metamorphs at the Five primary study sites 1997-2001

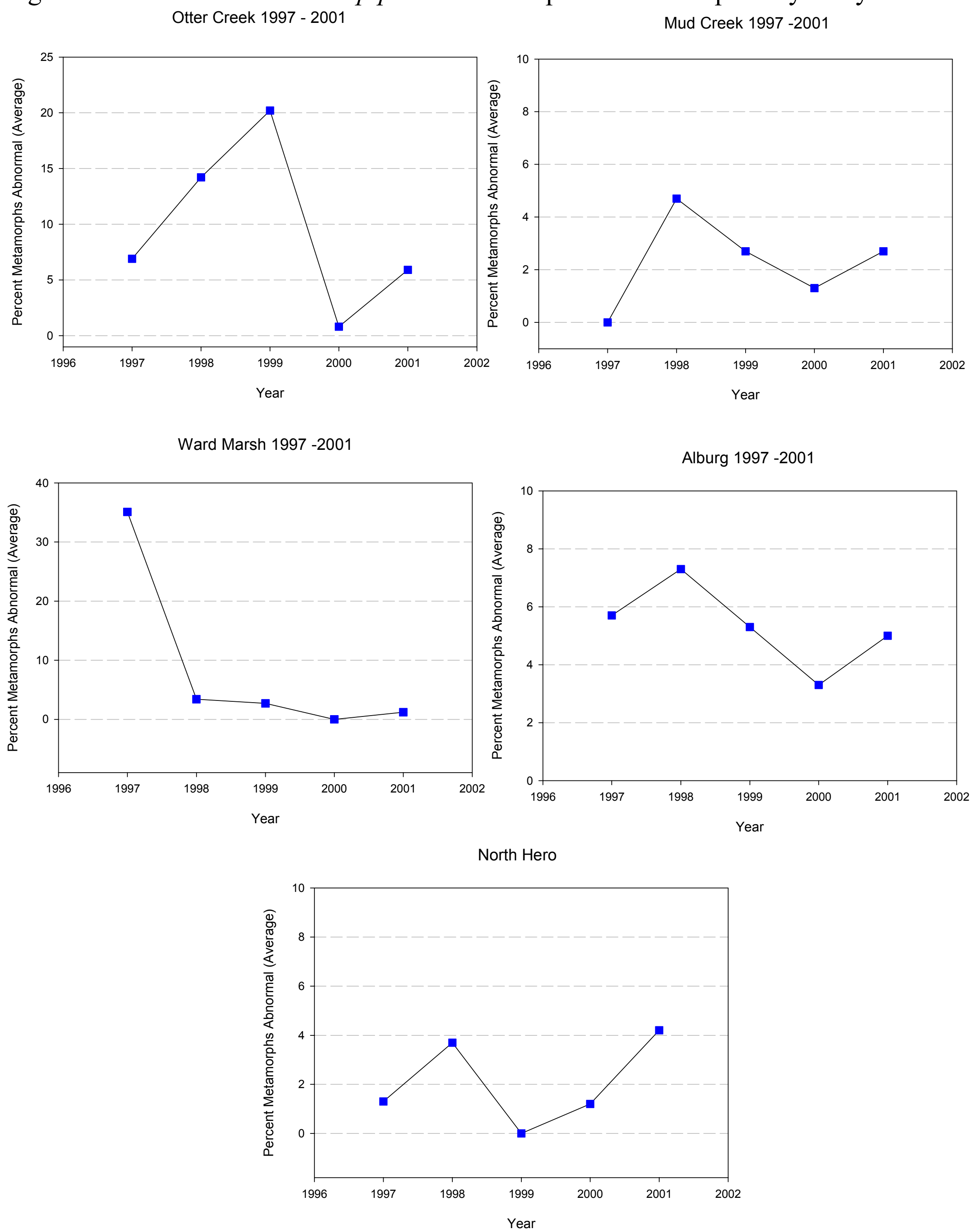
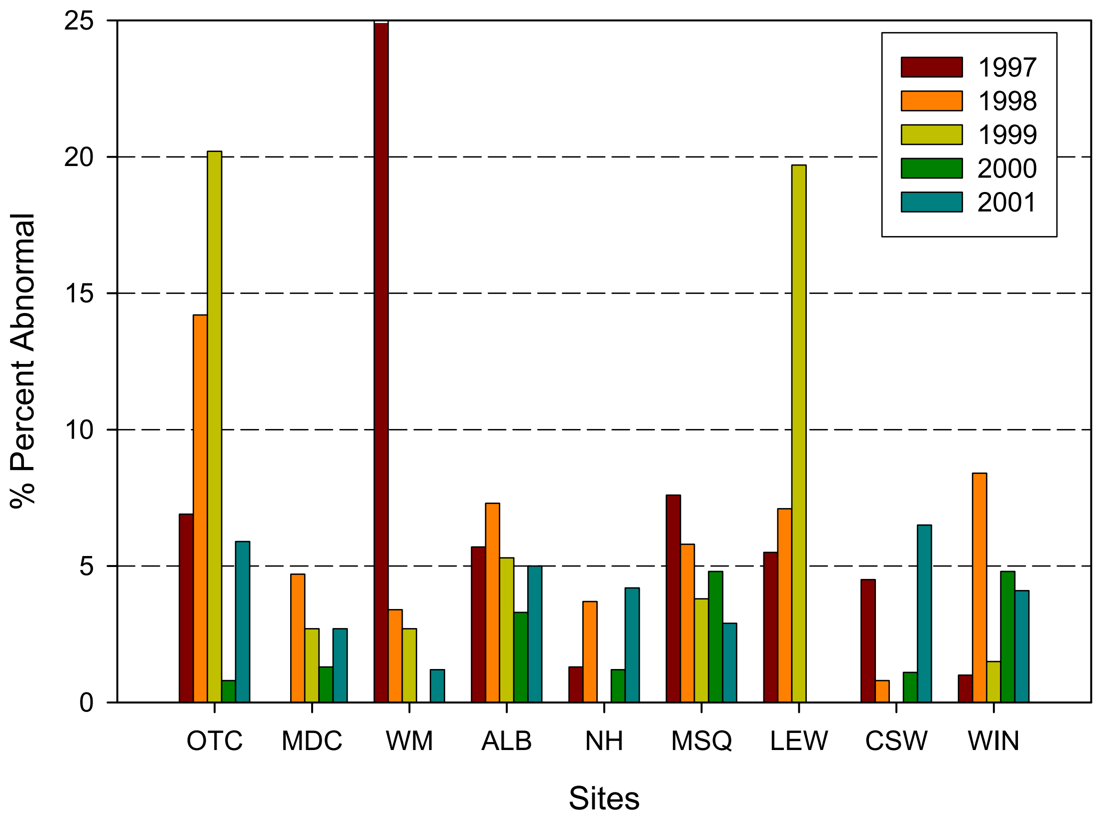


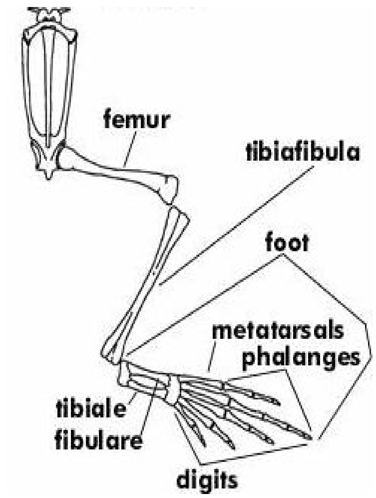
Figure 15 presents the average annual percent abnormal metamorphs at the primary and supplemental study sites from 1997 – 2001. Using the annual "average" will moderate the observed minimum and maximum percentages. The percent abnormal presented at the Ward Marsh site for 1997 has been truncated from 35% to 25% so as not to eclipse other sites. It is notable that the annual average percent abnormalities have exceeded five percent at all but two of the nine sites presented (Mud Creek and North Hero).

Figure 15. Percent Abnormal *R. pipiens* Metamorphs at Nine Study Sites 1997 – 2001.
(Site Abbreviations: OTC – Otter Creek, MDC-Mud Creek, WM- Ward Marsh, ALB- Alburg Dune, NH- North Hero, MSQ- Missisquoi, LEW-Lewis Creek, CSW-Cornwall Swamp



Categories of Metamorph Abnormalities

We characterized 120 abnormalities affecting 103 metamorphs. Metamorph characterization was restricted to external visual examinations and the use of a head mounted 2x magnifier. It should be acknowledged that many abnormalities could go undetected without the use of radiography and histopathology. Previous Vermont studies revealed malformations of the hip that would not have been detected without the use of high detail radiography (Meteyer, 1997). Radiographs, necropsy and histopathology are also important in differentiating trauma from malformation.



Courtesy http://www.npsc.nbs.gov/narcam/

Descriptions of frog abnormalities in this report are based on terminology used within the Biological Science Report USGS/BRD/BSR-2000-0005, " Field Guide to Malformations of Frogs and Toads." (Meteyer, 2000a). Further guidance was obtained from "Hind Limb Malformations in Free-Living Northern Leopard Frogs" (Meteyer, 2000b).

Table 9 presents the categories of *R. pipiens* metamorph abnormalities observed at the five study sites in 2001. Appendix A provides this data by site and sampling date.

Hind limb abnormalities comprised 79% (n = 95 abnormalities).
Forelimb abnormalities and eye abnormalities affected 15% and 5% of the specimen's respectively. Figure 16 presents the major categories of metamorph abnormalities observed.

Figure 16. Major Categories of Metamorph Abnormalities at Five Primary Sites – 2001 (n = 120).

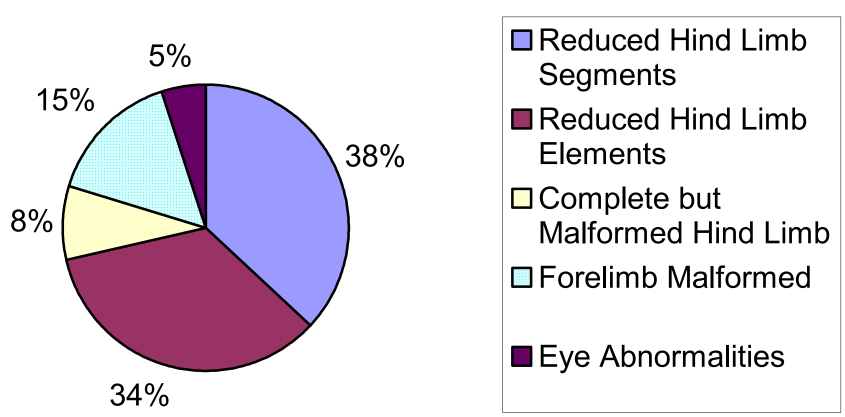
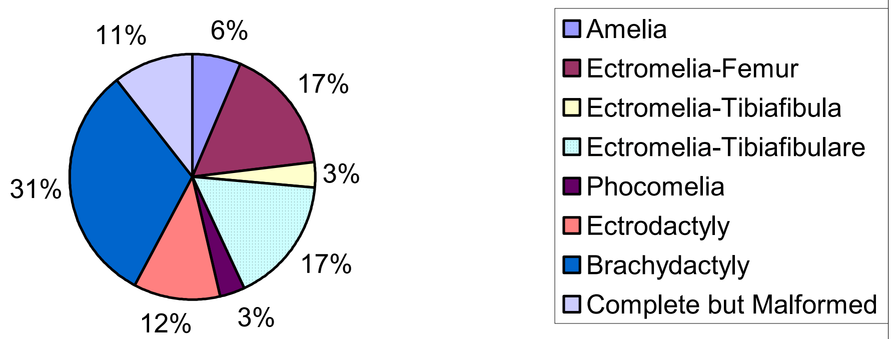


Table 9. Summary By Site: Abnormalities of *R. pipiens* Metamorphs at Five Primary Study Sites – 2001. (Percent values relative to the total number of abnormalities are noted in parenthesis).

	Otter Creek	Mud Creek	Ward Marsh	Alburg Dune	N.Hero	Total
	SUMMARY	SUMMARY	SUMMARY	SUMMARY	SUMMARY	
Hind Limb Malformations						
I. No Limb:						
A. Amelia	2 (4.8)	0 (0)	0 (0)	4 (11.1)	0 (0)	6 (4.9)
II. Reduced hind limb segments						
A. Ectromelia						
1. Femur	8 (19.5)	1 (12.5)	0 (0)	7 (18.9)	0 (0)	16 (13.1)
2. Tibiafibula	0 (0)	0 (0)	0 (0)	3 (8.1)	0 (0)	3 (2.4)
3. Tibiale fibulare	7 (17.0)	1 (12.5)	3 (33.3)	1 (2.7)	4 (15.4)	16 (13.1)
B. Phocoamelia	1 (2.4)	0 (0)	1 (11.1)	1 (2.7)	0 (0)	3 (2.4)
III. Reduced hind limb elements						
A. Ectrodactyly	4 (9.7)	1 (12.5)	2 (22.2)	1 (2.7)	3 (11.5)	11 (9.0)
B. Brachydactyly	7 (17.0)	0 (0)	2 (22.2)	10 (27.8)	11 (42.3)	30 (24.5)
IV. Complete but malformed hind limb						
A. Rotation	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	1 (0.8)
B. Micromelia	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	1 (0.8)
C. Hemimelia	2 (4.8)	2 (25)	0 (0)	0 (0)	0 (0)	4 (3.2)
D. Bone bridge	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
E.Digits Fused	0 (0)	0 (0)	0 (0)	2 (5.4)	0 (0)	2 (1.6)
F. Other	2 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.6)
Fore Limb Malformations						
I.No Limb						
A. Amelia	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)
II. Ectomelia	1 (2.4)	1 (12.5)	1 (11.1)	1 (2.7)	1 (3.8)	5 (4.0)
III. Reduced fore limb elements						
A. Ectrodactyly	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	1 (0.8)
B. Brachydactyly	2 (4.8)	1 (12.5)	0 (0)	2 (5.4)	5 (19.2)	10 (8.2)
IV. Emergence Failure	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)
Spine Malformations						
I. Scoliosis: curvature	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.8)	1 (0.8)
Head Malformations						
I. Eye Abnormalities						
A. Anophthalmia: missing eye	2 (4.8)	0 (0)	0 (0)	2 (5.4)	1 (3.8)	5 (4.0)
B. Small pupil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
C. pupil/iris abnormal	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
D. Red eye	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)
II. Jaw abnormalities						
A. Microcephaly	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
B. Curved Jaw	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Other Abnormalities:						
I.Trauma: Skin/Broken Bone(s)*	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)
II. Abnormal color*	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)
III. Cyst above vent				1 (2.7)		1 (0.8)
Total sample size	605	296	340	635	564	2440
Number of frogs with malformations	34.0	8	4	33	24	103
Total number of malformations	41.0	8	9	37	26	120
% Frogs with malformations	5.6	2.7	1.2	5.1	4.3	4.1
% Malformations	6.8	2.7	2.6	5.8	4.6	4.9
*Trauma and abnormal color not included in total calculations						

Figure17 presents a comprehensive description of the hind limb abnormalities observed.

Figure 17. Hind Limb Abnormalities at Five Primary Sites – 2001 (n = 95).



Amelia –No limb element
Amelic frogs have no evidence of a limb, and comprised 6.3% of the hind limb abnormalities. Alburg and Otter Creek were the only sites where amelia was observed (n = 4 and n = 2, respectively) (Figure18) .

Previous examinations of Vermont frogs, which included radiography, revealed that amelic frogs often had pelvic malformations. This would be suggestive of a very early developmental error. (Meteyer ,1997).

Figure 18.

Figure 19.

Polymelia -Multiple Limb Segments
No supernumerary limbs were observed during the 2001 survey. In fact, only one *R. pipiens* (Figure 19) examined (>10,000) since 1996 has had polymelia. This is particularly enlightening with regards to recent studies linking parasite (*Ribeiroia sp*) infection to amphibian malformations (Johnson, 2002). Johnson's studies revealed that 10% – 43% of morphological abnormalities from fifty-nine sites supporting the trematode *Ribeiroia sp* consisted of polydactyly (extra digit) and polymelia (extra limb).

Figure 20

Sessions (Stopper 2002) used trematode cyst infestation to induce limb deformities in two species of *Rana; R. sylvatica* and *R. pipiens* (Figure 20) . Mirror image duplications comprised 50% of the cyst-induced deformities, hind limb truncations were not observed. Further evidence negating the trematodes playing a significant role in the *R. pipiens* hind limb abnormalities

Ectromelia – Reduced limb segments
An incomplete limb with the lower portion of the leg missing. Ectromelia limbs comprised 46.3% of the hind limb abnormalities. Ectromelic limbs were further classified on the basis of the affected limb segment into three categories, femur, tibiafibula and tibiafibulare. Ectromelia of the femur, tibiafibula (Figure 21) and tibiafibulare comprised 17%, 3%, and 17% percent, respectively of the hind limb abnormalities.

Figure 21.

Figure 22.

Phocomelia
Frogs with phocomelia have small, disorganized, unidentifiable proximal bones to which are attached an abnormal foot which originates close to the body (Meteyer, 2000a).

There were 3 specimens (3.1%) representing three sites that exhibited phocomelia, Otter Creek, Alburg and Ward Marsh (Figure 22).

Ectrodactyly- missing toe
Distinguished from brachydactyly and refers to a completely missing digit including the metatarsal bone and phalanges (Figure 23). There were 11 specimens (12%) with this phenotype representing all five sites. The incidence at Otter Creek and North Hero was the highest, 4 and 3 respectively.

Figure 23.

Brachydactyly – short toe
The normal number of metatarsal bones is present but the number of phalanges (bones in the toe) are reduced. There were 30 specimens (31%) exhibiting this phenotype, representing all the sites except for Mud Creek. The incidence at North Hero and Otter was the highest, 11 and 10 respectively.

The "Complete but malformed hind limbs"
This category consisted of several sub-categories (Table 9), 10 specimens (11%) were included under this category. The incidence at Otter Creek and Alburg was the highest, with 4 affected animals at each site.

Ectromelic forelimbs
Forelimbs develop protected in peribranchial sacs within the branchial chamber and emerge fully developed at stage 42 (Gosner, 1960; Duellman and Trueb 1986). Sessions and Ruth (1990) hypothesized that the pelvic limb buds would be prone to greater exogenous insult than their anterior counterpart. This possible explanation is evidence that hindlimb development in anuran larvae would be more vulnerable to environmental hazards during critical cell-division stages and morphogenesis (Ouellet, 1997).

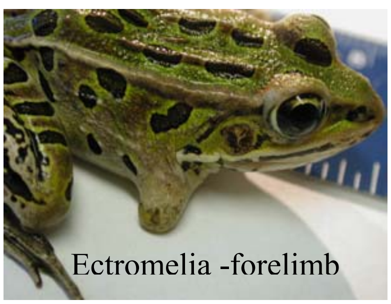


Figure 24.

It is somewhat surprising that forelimb abnormalities (Figure 24) affected 18 metamorphs, comprising 15% of the abnormalities observed. This is significantly higher than observations from previous years. Forelimb abnormalities in previous years (1996 - 2000) comprised roughly 5% of the abnormalities. These observations argue against predation since the forelimbs emerge late in development.

Eye abnormalities
Eye abnormalities affected 6 metamorphs (5%), categories included missing eyes (anophthalmia) (Figure 25) and pupil/iris abnormalities (Figure26). Endocrinologist at the University of New Hampshire examined a frog with a missing eye, and located the eye next to the brain (Babbitt, pers.comm.)

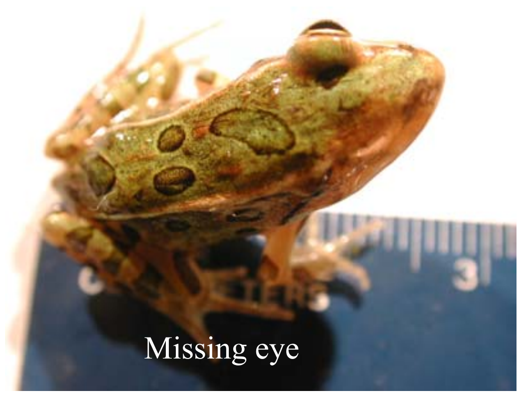


Figure 25.

Figure 26.

Bilateral Abnormalities
Only two animals had bilateral abnormalities, and one of those was from capture injury. The bilateral abnormalities were not symmetrical. Laboratory UV-B studies, exposing *R. pipiens* to UV radiation for 24 days produced rear limb malformations that were primarily bilateral symmetrical truncations (Ankley et. al., 1998), so UV-B by itself may not be an important cause of malformations.

Trauma
Characteristic changes expected in a traumatized limb include swelling, inflammation, or irregularity at the termination of the limb (Meteyer, 1997). Scarring and abnormal pigmentation over the end of the limb would also be indicative of a traumatized limb.
Only one frog was found to have trauma not related to capture injury, that was observable externally, this specimen had a broken hind limb. Two late stage tadpoles (Gosner stage 40) from Otter Creek were inadvertently traumatized during the collection process. Both tadpoles suffered limb "amputations", one a bilateral truncation, ectromelia of the femur on the right side and ectromelia of the tibiafibula on the left side.

Figure 27.

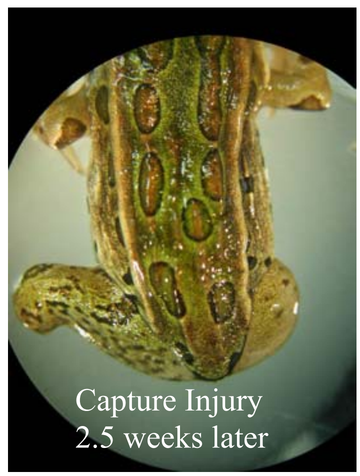


Figure 28.

The second traumatized tadpole endured ectromelia of the right hind limb.
The misfortune of the tadpoles did provide the opportunity to observe the healing process, pigmentation changes and even bone characterization through radiography.
The tadpoles were brought back to the lab and reared until metamorphosis.
Figures 27 and 28 shows the bilateral truncation 1 week and 2.5 weeks after trauma.

Although it was beyond the scope of this project to perform radiographic characterization of abnormal frogs, an opportunity to obtain radiographs of the abnormal frogs presented itself. Dr. Michael J. Lannoo, Professor of Muncie Center for Medical Education at Indiana University provided radiographic images for a total of 81 *Rana pipiens* metamorphs (77 abnormal, 4 normal) collected during the 2001 season. Of the 81 metamorphs submitted for radiography, 73 were obtained from the 5 primary sites, the balance (8 frogs) were from supplemental sites.

Note: Refer to Appendix B for a photo atlas of radiographic images of Vermont frogs.

As previously stated, radiographs can be useful in differentiating trauma from malformation and often reveal other abnormalities. If the radiograph does not reveal obvious inflammation or infection at the end of the limb truncation, and the pigment and skin at the end of the limb is consistent with what is present along the entire limb, the truncation should be considered a malformation (Meteyer, pers. comm.).With this goal in mind, we shared some of the radiographs with researchers and requested their opinion on characterizing the radiographs.

Carol U. Meteyer, DVM, from the National Wildlife Health Center was kind enough to offer her opinion of this radiographic image (Figure 29), before we correlated our radiographic images with our field records. Thus we were unaware that this specimen had been subjected to capture injury. Meteyer described the right femur (which appears on the left side of the radiograph) as follows, "severe proliferative terminal right femur with no obvious cortex that can be defined." It was Meteyer's opinion that trauma with inflammation (osteomyelitis) would be present. Meteyer was careful to point out that external observation and histopathology would be equally important for a proper interpretation.

Figure 29.

Figure 30.

There was disruption of skin pigmentation and pattern at the terminus of the limb truncations, which would provide further evidence of a trauma related truncation (Figure 30). This type of pigment disruption would be consistent with the limb buds being mangled as opposed to simple amputation (Lannoo pers.comm.)

Of particular interest were radiographs that appeared to have "spongiform bone," at the terminus of the truncated limbs. Spongiform bone as described by Lannoo (Lannoo 2003) is expansion of the cancellous bone at the distal tip of ectromelic limbs or associated with traumelias (bony

triangles); expansions are typically terminal, irregulary shaped, and only present on the affected limb. It is Lanoo's opinion that radiographs Figure31 and 32 show spongiform bone, and that this morphology is not associated with trematode infections. The most common hindlimb malformation type (Ouellet, et. al.,1997; Meteyer, et. al., 2000b) was ectromelia, followed by micromelia (proportionally small or short limb) and the presence of spongiform bone (Lannoo 2003).

Lannoo has conducted frog amputation studies; radiographs of the healed amputated limbs have not revealed spongiform bone, or altered pigment patterns proximal to the wound. Suggesting that the presence of spongiform bone is not a positive indication of trauma.


Figure 31.


Figure 32.

Radiographs revealed spongiform bone on a significant number of Vermont specimens with truncated limbs. Hindlimb malformations may also be produced by failed predation attempts, however 74% of the ectromelias in animals sampled in Minnesota by Lannoo (2003) showed spongiform bone.

Meteyer also examined radiographs Figures 31 and 32, and felt that both were likely malformations based on bone observations. The truncated bones were not severely proliferative and there appeared to be pelvic involvement in Figure 31, though the pelvis is tilted in the radiograph. The pigment on these frogs is consistent from the base to the termination and there is no scarring, further suggesting a malformation.

Limb Regeneration
It is well known that newly formed limbs of anuran tadpoles regenerate readily upon amputation, however the capacity to regenerate limbs is lost entirely during metamorphosis (Dent,1960). The stage at which the ability to regenerate limb parts is lost varies among different species (Forsyth, 1946). However it is lost first in the proximal portion of the limb and then, gradually, in more and more distal regions (Harland, 1943).

Although the adult of *Xenopus* always produces a regenerate after a limb is amputated, the regenerative is heteromorphic, consisting of a single spike-like structure. Internally, the spike is made up mainly of cartilage with some dense connective tissue but with scarcely any bone or muscle. Atypical or limited regeneration resulting in a regenerative spike also may occur in adult amphibians following amputation (Ouellet, 2000)

Dr. Stanley Sessions, professor at Hartwick College, New York, provided insight into some of the abnormal *R. pipiens* collected from Vermont study sites in 1996. Several of the cleared and stained abnormal frogs showed a cartilaginous (regenerative) spike on the end of the truncated limb. (Figure 33). The presence of the spike indicates that the limb was lost after it had developed, and is a normal regenerative response for a post metamorphic frog.

Sessions did not find evidence of trematode cysts within the stained and cleared frogs. It is also worth noting that radiographs would not depict a regenerative spike, since the spike is cartilaginous (stained blue in Figure 33).

Figure 33.

Abnormal Pigmentation

The Field Guide to Malformations of Frogs and Toads (Meteyer, 2000a) note pigment malformation on an ectromelic leopard frog. There is abnormal small reticular pattern of brown-black pigment over the truncated limb rather than the large spots of black-brown pigment, which are normal for the leopard frog. "At present, this is considered a malformation of skin pigment pattern. The melanophores that contain pigment are of ectodermal (neural crest) origin and might be another form of inappropriate development in the malformation syndrome" (Meteyer, 2000b).

We did notice this type of skin pattern abnormality (Figure 34) and perhaps of equal or greater importance we noted abnormal skin pigmentation on the ventral side of truncated limbs (Figures 35 and 36).

With regards to our *R. pipiens* observations, any skin pigmentation on the ventral side would be considered abnormal, since there is an absence of skin pigment on the ventral side of "normal" frogs.

Figure 34.

Figure 35.

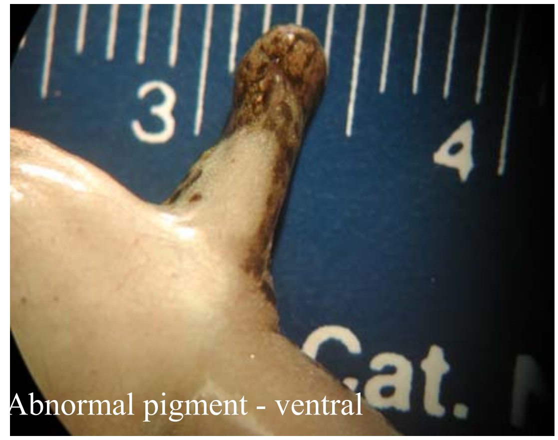


Figure 36.

We shared images of the ventral pigmentation with Lannoo, who believes that ventral pigment is associated with developmental problems. In agreement with Meteyer's description above, it is Lannoo's opinion that this is a sign of developmental breakdown characterized by neural crest tissue going where it should not. "No one has yet to explain to me how a distal "amputation" can affect limb pigment proximally." Minnesota frogs have shown signs of pigment breakdown on hind limbs (Lannoo, pers. comm.).

Summary

Almost 2/3^{rds} of the abnormal metamorphs were obtained from Otter Creek (33%) and Alburg Dune (32%). North Hero metamorphs comprised 23% of the abnormalities observed (Figure 37). However as Figure 37 and Table 9 illustrate the Alburg and Otter site abnormalities were dominated by missing hind limb segments (ectromelia), whereas the North Hero site was dominated by missing hind limb elements (ectro/brachydactyly). In fact 45% (n = 11) of the abnormalities at North Hero were brachydactyly, which could be considered the least severe of abnormalities encountered. Mud Creek and Ward Marsh represented only 4% and 8% of the metamorph abnormalities observed, respectively.
Note: Refer to Appendix C for a photo atlas of metamorph images.

Figure 37. Percent Metamorph Abnormalities by Site – 2001 (n = 103).

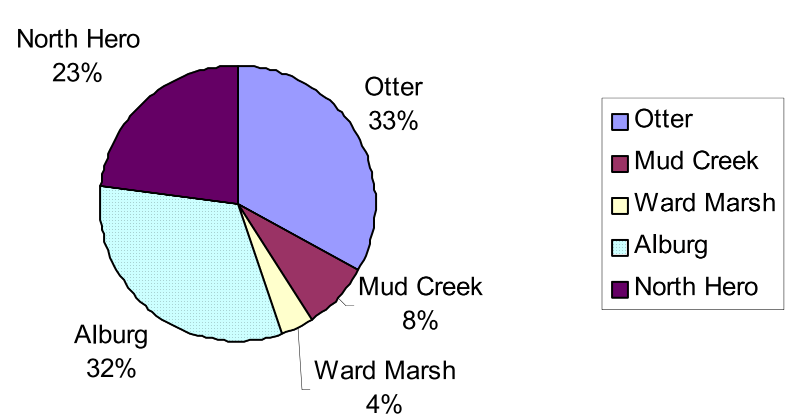
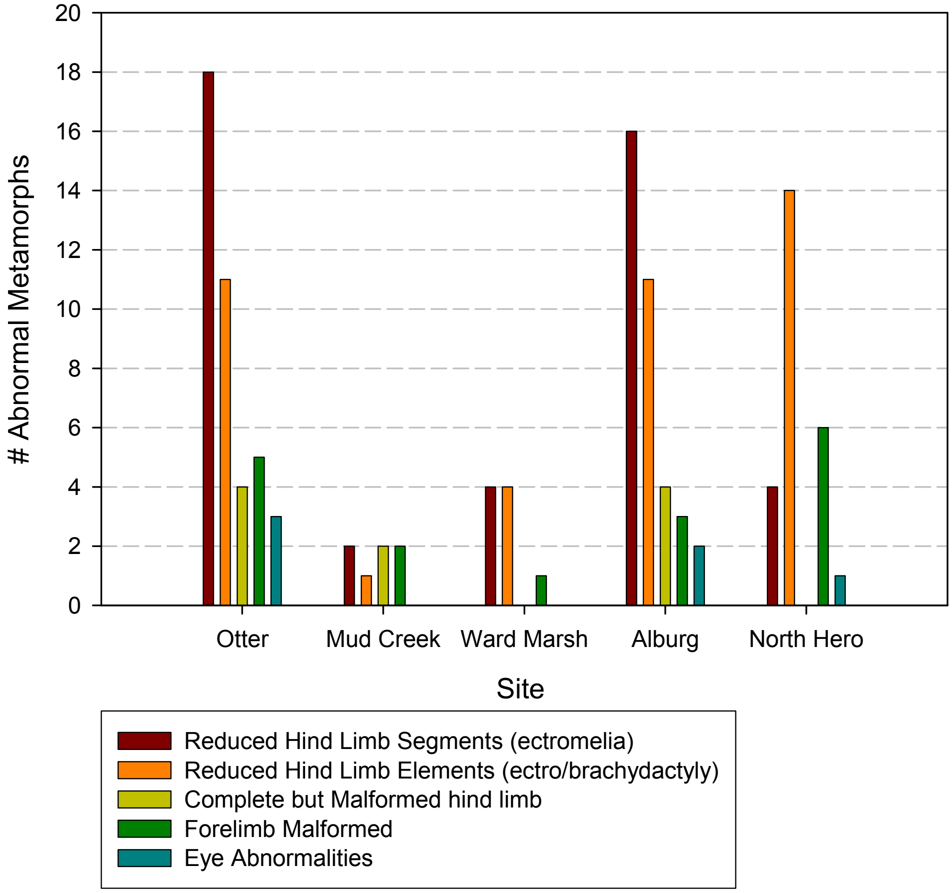


Figure 38. Major Categories of Metamorph Abnormalities by Site (2001).



Rating the severity of hind limb abnormalities (truncations) may provide some insight with regards to distinguishing our study sites. The most severe rating would be assigned to amelia (entire limb missing), followed by ectromelia of the femur, tibia-fibula and tibiafibulare.
The least severe hind limb categories would be ectrodactyly and brachydactyly, with brachydactyly being the most moderate. With this rating system in mind, a closer look at Table 9 reveals that Otter Creek and Alburg Dune monopolized 100% of the most severe truncations (amelia) and 94% (18/19) of the next most severe categories; ectromelia of the femur and ectromelia of the tibiafibula. The Otter Creek and Alburg Dune sites continued to achieve recognition within the anophthalmia (missing eye) category, dominating 4 of the 5 affected metamorphs. The Alburg and Otter sites should be considered the most potent with regards to producing metamorph abnormalities.

Age and Growth – Metamorphs

Size and Condition

Frogs collected were measured and weighed to determine overall body condition. The size of the metamorphs at metamorphosis (early July) and their overall growth through September can be reflective of the health and nutritional status of the frogs. Time to metamorphosis is temperature, and perhaps, density dependent ranging from 60 –90 days and may occur over several weeks at a single site. Size at metamorphosis can affect lifetime fitness (Werner, 1986).

Growth rates were determined using snout to vent measurements (Figure 38a) taken at each sampling event. The average length of metamorphs from the time of metamorphosis (early July) to mid September is presented in Figure 39 and Figure 40. Table 10 presents the minimum and maximum average length, the total growth for the season, and the average growth per day calculated by dividing the total growth by number of days within the sampling season for each site.

The size of frogs at metamorphosis ranged from 2.5 – 3.6 cm. Metamorphs at four out of five sites were under 3.5 cm in length. Most noteworthy was the Alburg Dune site; metamorphs averaged only 2.5 cm in length. This is significantly smaller than the findings of Merrell (1977) in Minnesota, who found the usual size of frogs at metamorphosis to be 3.5-4.0 cm (drought conditions and crowding could explain differences is size observations).

Figure 38a

Table 10. 2001Growth Rates and Average Snout to Vent Measurements at the Five Primary Sites

Site	Avg. Min. (cm)	Avg. Max. (cm)	Total Growth (cm)	# Days	Growth/day (mm)
Otter Creek	3.2	4.3	1.1	64	.17
Mud Creek	3.2	4.8	1.6	60	.23
Ward Marsh	3.6	4.7	1.2	59	.20
Alburg Dune	2.5	3.9	1.4	72	.19
North Hero	3.3	4.0	0.7	60	.11
Range	2.5 – 3.6	3.9 – 4.8	0.7 – 1.6	59 - 72	0.11 – 2.3

The average maximum length at the end of the sampling season (mid September) ranged from 3.9 – 4.8 cm, Mud Creek had the largest metamorphs and the best growth rate (0.23 mm/day). North Hero had the lowest growth rate (0.11mm/day) and the smallest total growth for the season (0.7cm).

There are numerous factors that could explain the very small metamorphs observed at the Alburg site. Werner (1986) theorizes and discusses the optimal size at which to metamorphose and recognized how seasonal effects such as temperature variation, and the timing of metamorphosis, or oviposition, or cohorts of predators can have important effects on mortality and growth. Gromko (1973) studied tadpole crowding effects and observed that the number of days to metamorphosis increased as tadpole density increased. Gromko also found crowded tadpole populations showed greater mortality, smaller size and slower growth rates. Food was not a factor in these experiments. The Alburg site had the greatest tadpole densities as evidenced by the number collected. It is known that the overwintering success of smaller juveniles diminishes significantly due to a lack of fat reserves.

It is possible that environmental conditions such as high temperature may have prompted "stressed" the late stage tadpoles into metamorphosing sooner than was optimal. Temperature measurements at the Alburg site (Figure 51) exceeded all other sites and came within 1 degree of the upper tadpole tolerance value of 31^{o}C (Werner 1986). It is likely that temperatures exceeded our measurements both temporally and spatially.

Ouellet (2000) reported in his Amphibian deformity review paper, that anomalies in forelimb and hindlimb skeletons have been induced artificially in larvae of *Bufo vulgaris formosus* reared at high temperature (Mutto 1969a, 1969b, 1970). Digital malformations involving the metacarpal, metatarsal and phalangeal bones (brachydactyly, ectrodactyly) were commonly observed at 30^{0}C, while the development was normal in control toads raised at 20^{0}C. Further studies revealed that anomalies were obtained at 30^{0}C when the water was aerated by an air pump (Muto 1971), determining that the high temperature was the primary teratogenic agent rather that the associated lower oxygen supply.

Alburg data reveals recorded pond temperatures of 27^{0}C on May 21, 2001 coinciding with very early tadpole limb development (Gosner stage 27-28). In addition water temperatures in adjacent flooded tadpole habitat were even higher, 29.5^{0}C.

Fort (1999a) found that Alburg test site sample increased the rate of tail resorption, and this was reversed with the addition of methimazole, indicating the increased rate of resorption was due to hyperthyroid activity. Hyperthyroidism can lead to premature metamorphosis typically lethal due to rapid onset of adult features and abnormal development (Fort 1999a). This may explain the very small metamorphs observed at the Alburg site.

Figure 39. *R. pipiens* Metamorph Growth Rates at the Five Primary Sites by Sampling Event 2001 (Average Snout To Vent Length (cm)).

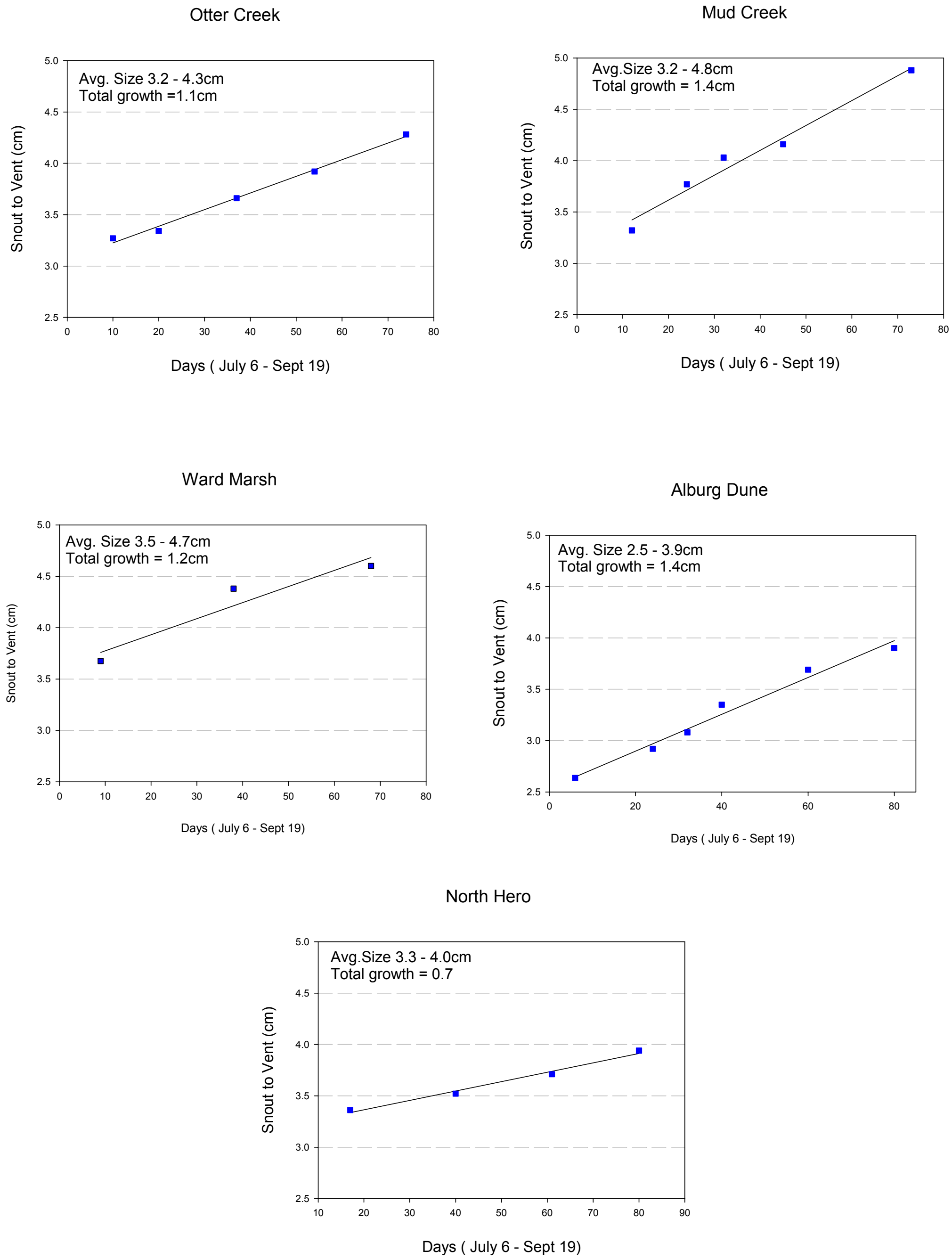
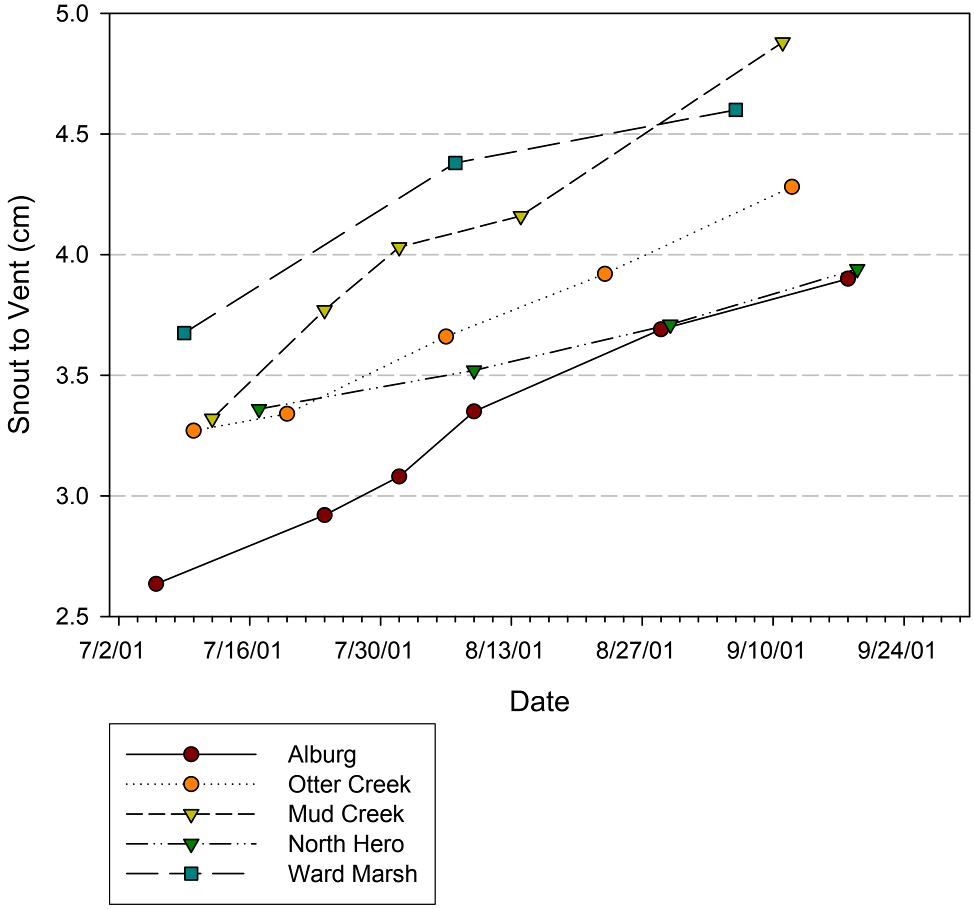


Figure 40. Metamorph Average Length at Five Primary Sites by Sampling Event.

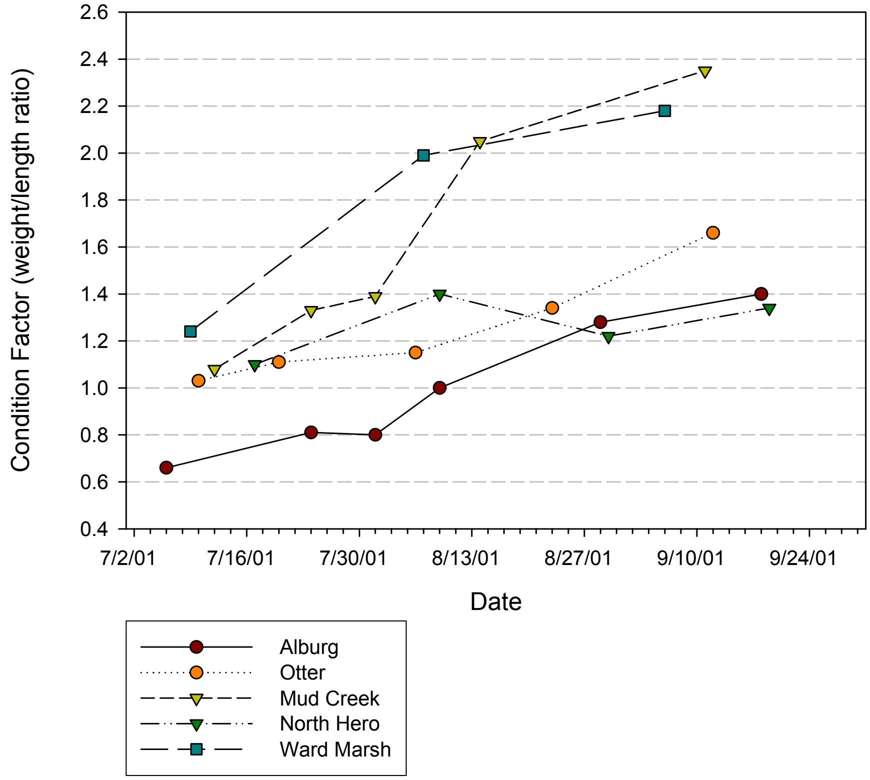


Condition Factor Index

The weight (gm) of the frog divided by the length (cm) of the frog provides an approximation of the nutritional status or body condition as a numerical index value (Meteyer, 1997). As the numerical value increases, so does that condition or nutritional status of the frog.

We calculated the weight/length ratio for each "normal" metamorph examined, and then calculated the overall condition factor for each site, and for each sampling event. Figure 41 presents the average condition factor at the primary sites by sampling event. The Alburg Dune frogs had the lowest condition factor, while Mud Creek and Ward Marsh frogs scored the highest. Note that as the season progresses most of the sites show an increase in the condition index, similar to average length by site (Figure 40). It is likely that frog populations from sites with low condition factors will have less overwintering success.

Figure 41. Metamorph Average “Condition Factor” at Five Primary Sites by Sampling Event.



Presumably there may be a relationship between the condition factor of frogs at a site and the percent abnormal metamorphs at a site. Table 11 presents the average condition factor and the percent abnormal metamorphs by site. The table also includes the range (min/max) for percent abnormal metamorphs and condition factor by site.

Table 11. Condition Factor Index and Percent Abnormal Metamorphs by Site.

Site	Condition Factor (Average)	Metamorphs	Abnormal	ge Condition
Alburg Dune	0.99	5.0	3.1 – 7.3	0.66 – 1.4
Otter Creek	1.25	5.9	1.6 – 8.8	1.03 – 1.66
Mud Creek	1.64	2.7	0 – 4.9	1.08 – 2.35
North Hero	1.26	4.2	0 – 7.1	1.1 – 1.34
Ward Marsh	1.80	1.2	0 – 2.0	1.2 – 2.18

The data set is small and the range of observed abnormalities is low this year, however there does appear to be an inverse relationship with condition factor and percent abnormal metamorphs by site. Alburg and Otter Creek had the lowest condition factors and the highest observed rates of abnormalities. Ward Marsh and Mud Creek had the highest condition factors and the lowest observed rates of abnormalities. Previous years data have shown a more dramatic relationship between condition factor and percent abnormal metamorphs by site.

The 2001 *R. pipiens* metamorph survey was far from exhaustive with regards to data collection and observations. However we do feel that pertinent life history and age and growth data can greatly increase our understanding of the abnormality phenomenon.

When we examine the rates and categories of metamorph abnormalities, together with the age and growth studies a more complete picture may appear. Otter Creek and Alburg Dune both had the highest rates of abnormalities; both sites also had the most severe abnormalities (and very similar). Age and growth studies (condition factor) scored these frog populations lower than any of the other sites. Historical survey data (Table 8 and Figure 14) show a consistent, somewhat parallel level of abnormalities. These observations may be coincidence or they may be indicative of similar causative factors.

Tadpole Surveys - *Rana pipiens*

Leopard frogs (*R. pipiens*) emerge from overwintering sites shortly after ice begins to melt when water temperatures reach 9 ^{o}C to 12^{o}C (Merrell, 1977). Short overland migrations may be made between overwintering sites and breeding ponds. In some instances breeding may occur in overwintering habitats.

In Vermont *R. pipiens* breed in April, they lay about 5,000 eggs, which generally reach metamorphosis by early July. The span from fertilization to hatching is about 8 days and to metamorphosis (in the laboratory at 23^{o}C) is about 75 days. The eggs are normally layed in water at about 15^{o}C and the upper limit of temperature tolerance is about 31^{o}C.

Tadpoles spend about 10 -13 weeks feeding within the floating vegetation and are consuming algae, phytoplankton and detritus (Hine, 1981).

Figure 41a. Staging Tadpoles at Alburg.

A total of 1254 *R. pipiens* tadpoles (Gosner Stage 26-45) were collected and characterized (Figure 41a) from the five primary study sites in 2001. Tadpole collections were not performed at the 4 supplemental sites. Overall tadpole abnormality rates at the five primary sites was 4.4% (55 of 1254), abnormalities ranged from 0% – 16.8% (Table 12). Mud Creek had the highest percent of tadpole abnormalities (average 10.2%), range 0% –16..8%. By contrast sampling events at the other four sites resulted in less than 5% abnormalities (0% – 4.3%).

The actual number of tadpoles collected during each site visit corresponded strongly to our ability to locate tadpoles at the site (Figure 41b). As tadpoles grow their preferred habitats change also. Locating tadpoles at the Ward Marsh site was the most challenging as evidenced by the low numbers examined (n = 93).

In contrast to the Ward Marsh site, the Alburg Dune site (a small tannic pond) provided more tadpoles than we were able to process in a day. Three tadpole collections at the Alburg site yielded 558 tadpoles. Total numbers of tadpoles examined from each site will be important to note when interpreting the observed abnormalities by site.

Figure 41b. Otter Creek Wetland

Table 12. Summary of *R. pipiens* Tadpole Surveys 2001 (Gosner Stage and range indicated).

Site	Date	Normal	Abnormal	N	% Abnormal	% Abnormal Range	Average Stage	Stage Range
Otter Creek	05/24/01	70	2	72	2.8		27.75	26-31
Otter Creek	06/06/01	66	3	69	4.3		32.30	28-35
Otter Creek	06/25/01	27	0	27	0.0		40.22	28-42
Total		**161**	**5**	**168**	**3.0**	**0 – 4.3**		
Mud Creek-T1	05/29/01	82	2	84	2.4		34.40	31-37
Mud Creek-T1	06/07/01	89	18	107	16.8		35.60	33-37
Mud Creek-T2	06/21/01	52	6	58	10.3		39.45	38-42
Mud Creek-T2	07/03/01	5	0	5	0.0		43.20	42-45
Total		**228**	**26**	**254**	**10.2**	**0 – 16.8**		
Ward Marsh-T1	05/31/01	89	2	91	2..2		32.63	31-35
Ward Marsh-T2	06/12/01	1	0	1	0.0		-	-
Ward Marsh-T2	06/20/01	1	0	1	0.0		40.00	40
Total		**91**	**2**	**93**	**2.1**	**0 - 2.2**		
Alburg Dune	06/01/01	208	7	215	3.2		31.24	26-34
Alburg Dune	06/14/01	191	8	199	4.0		36.35	31-42
Alburg Dune	06/26/01	159	4	163	2.5		42.73	34-46
Total		**558**	**19**	**577**	**3.3**	**0.1 - 4.2**		
North Hero -T1	06/04/01	11	0	11	0.0		29.45	29-30
North Hero -T2	06/04/01	58	1	59	1.7		28.07	27-29
North Hero- T2	06/19/01	86	2	88	2.2		34.51	31-36
North Hero- T2	07/03/01	4	0	4	0.0		38.50	38-39
Total		**159**	**3**	**162**	**1.9**	**0 - 2.2**		
Total 2001		**1197**	**55**	**1254**	**4.4**	**0 – 16.8**		

Using a dissecting scope (7-60X), developmental characteristics (e.g. stage) were described and all abnormalities were characterized using a standardized descriptive format. Of particular interest were observations on the occurrence and character of hind limb abnormalities.

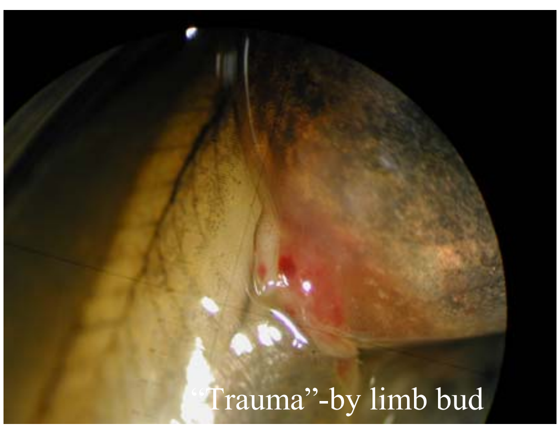
Table 13 presents the categories of *R. pipiens* tadpole abnormalities at the five primary sites 2001. Appendix A provides this data by site and sampling date. It is important to note that since it was not always possible to discern what is "abnormal" from what is "normal" we erred on the conservative side and documented all observations that we considered to be abnormal. It is likely that our classification of tadpole abnormalities will change as the results are shared with peers and more information is obtained.

The most difficult abnormalities to categorize were tadpoles that had signs of "trauma." Trauma related abnormalities comprised over 72% (n = 54) of the total. Trauma related categories included, red spots (hemorrhage) observed on hind limbs, body and tail. Mud Creek and Alburg comprised 88% (n = 48) of the trauma related abnormalities.

The tadpole category table also includes several categories that are not included in the totals, such as tail trauma, or capture injury trauma. Note: Refer to Appendix D for a photo atlas of Tadpole Images.

Some of the "hemorrhage" trauma (Figures 42 and 43) we observed around the limb area appears to look similar to what researchers have seen shortly after trematode (*Ribeiroia*) infection. (Lunde, pers.comm.). Carol Meteyer, DVM agree's with Lunde and feels that cercaria (of any genus) may cause hemorrhage at the site of penetration. Leeches were also a problem for tadpoles at some Vermont sites in 1999. Leeches leave a small circular area of hemorrhage.

However these tadpoles were not examined for parasite burden by dissection or clearing and staining. Categories of tadpole abnormalities are summarized in Table 13.



"Trauma"-by limb bud

Figure 42.

"Trauma" – by tail fin

Figure 43.

Table 13. Summary of Abnormal *R. pipiens* tadpoles at five primary study sites – 2001.

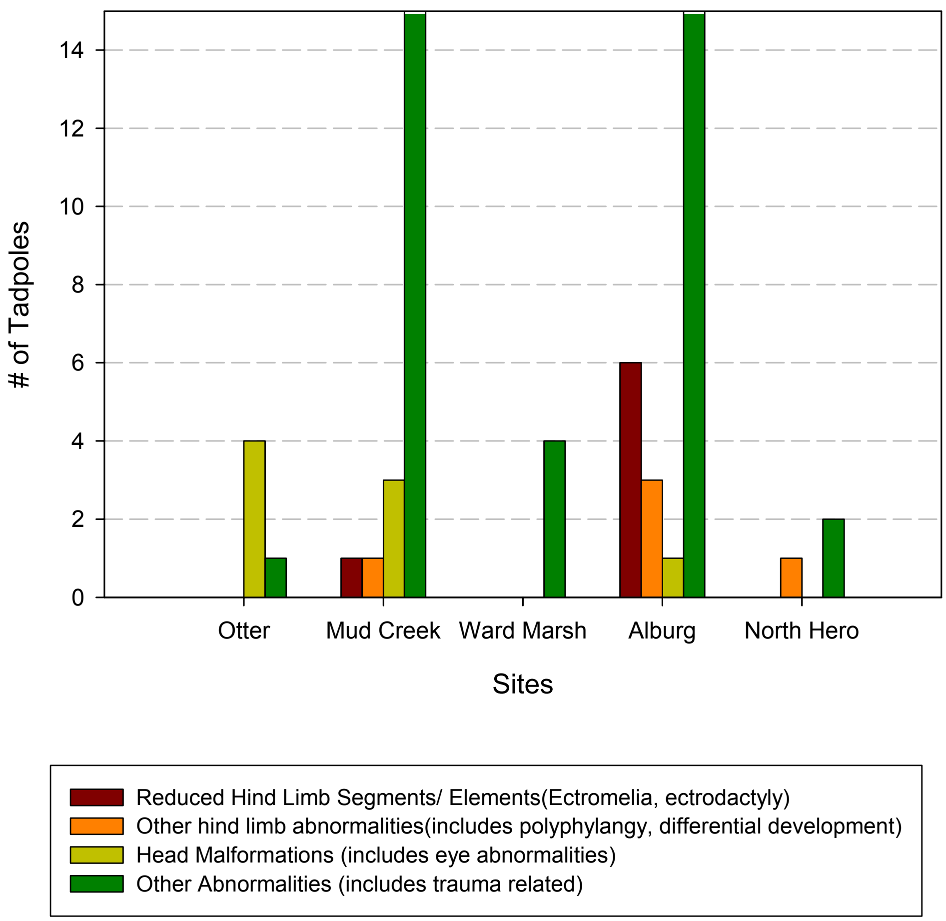
	Otter Summary	Mud Summary	Ward Marsh Summary	Alburg Summary	North Hero Summary	TOTAL
Hind Limb Malformations						
I. No Limb:						
A. Amelia						
II. Reduced hind limb segments						
A. Ectromelia						
1. Femur	2[a]	1		1		4
2. Tibiafibula	1[a]			2		1
3. Tibiale fibulare				1		1
B. Phocoamelia				1		1
III. Reduced hind limb elements						
A. Ectrodactyly				1		1
B. Brachydactyly						
IV. Complete but malformed hind limb						
A. Differential development				2		2
B. Polydactyly		1				1
C. Delayed development				1		1
D. Limb misshapen					1	1
IV.Head Malformations						
1. Contorted head	1	1				
2. Eye Abnormalities						2
A. Small pupil		1				
B. pupil/iris abnormal	1					1
C. Red spot near eye	2	1		1		4
V.Other Abnormalities:						
1. Forked tail		1				1
2. Trauma related[b]						
A.Red spots on hind limb(s)	1	8	2	8		19
B.Red spot(s) on body		10	2	2		14
C.Red spot(s) on the tail		5				5
D. Tail Trauma[c]		26[c]	1[c]	6[c]	4[c]	37[c]
E. Tail short		4		7		11
F. Body Trauma		3			2	5
3.Protozoa -Epistylis[c] (by vent)	1[d]	21[d]				21[d]
4. Bumps on the tail proximal to body						
Total sample size	168	249	91	577	158	1243
Number of tadpoles with abnormalities	5	26	2	19	3	55
Total number of abnormalities	5	37	4	27	3	76
% tadpoles with abnormalities	3.0	10.4	2.1	3.3	1.9	4.4
% Abnormalities	3.0	14.8	4.4	4.7	1.9	6.1

Note: The total number of abnormalities may or may not equal the number of abnormal animals as many specimens had more than one abnormality.
a Trauma-capture injury not included in total(s) or percent calculations
b Trauma - causation uncertain
c Tail Trauma- not included in total(s) or percent calculations
d Protozoa (Epistylis) located by vent not included in total(s) or percent calculations
e Red spots diminished after 24hrs
f Tadpole dead (recent)

Figure 44 presents the major categories of tadpole abnormalities by site. The categories of greatest interest are the hind limb abnormalities, reduced hind limb segments (ectromelia) and elements (ectrodactyly) comprised 9.3% (n = 7) of the total abnormalities observed. Only Alburg Dune and Mud Creek represented this category (n = 6 and n = 1, respectively).

The category "other hind limb abnormalities," comprised 6.6% (n = 5) of the total abnormalities observed. Categories under this heading included: differential development, delayed development and of polypdactyly. Three of the five sites represented this category, Alburg Dunes, Mud Creek and North Hero. It is worth noting that Alburg Dune and Mud Creek monopolized 11 of the 12 hind limb abnormalities observed (n = 9 and n = 2, respectively).

Figure 44. Major Categories of Tadpole Abnormalities by Site – 2001.



Conceivable the most captivating observations made during the tadpole study occurred while rearing a portion of the "abnormal" tadpoles in the laboratory. Digital photographs captured early stage progression of polypdactyly (Figure 45a,b), differential development (Figures 46a,b) ectrodactyly (Figure 47a,b,c), delayed development, and epidermal healing of limb trauma from known capture injury (Figure 27 and 28).

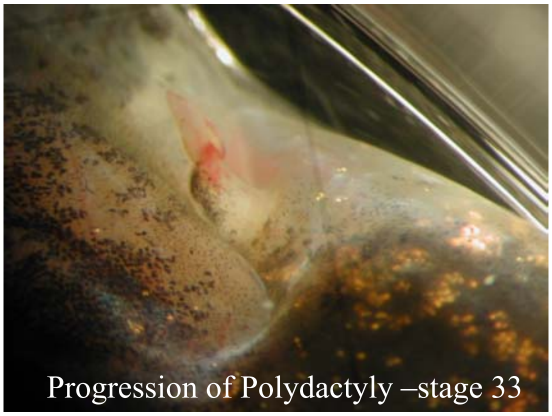


Figure 45a

Figure 45b

The progression documented provides evidence that at least a portion of the hind limb abnormalities observed in the metamorphosed frogs appear to be manifest at early tadpole development and are not a result of late stage trauma from predation or other environmental insult. Ouellet (1997) also observed many tadpoles with abnormal hind limb buds (unequal sizes) during surveys in the St. Lawrence River Valley of Quebec, Canada.

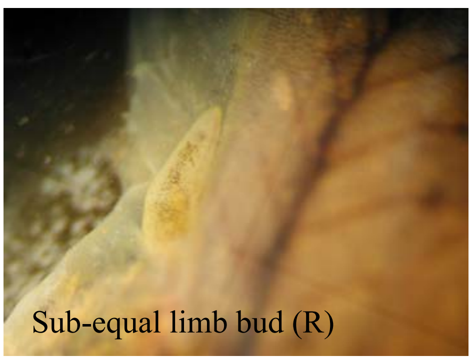


Figure 46a

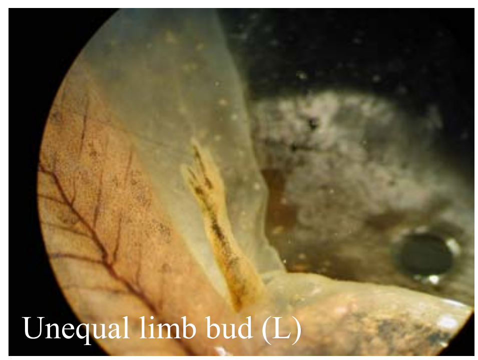


Figure 46b

These observations may directly address a data gap in the testing of the hypothesis that observed abnormalities are primary errors in development (malformations) rather than abnormalities caused by mechanical or non-developmental means (deformities).

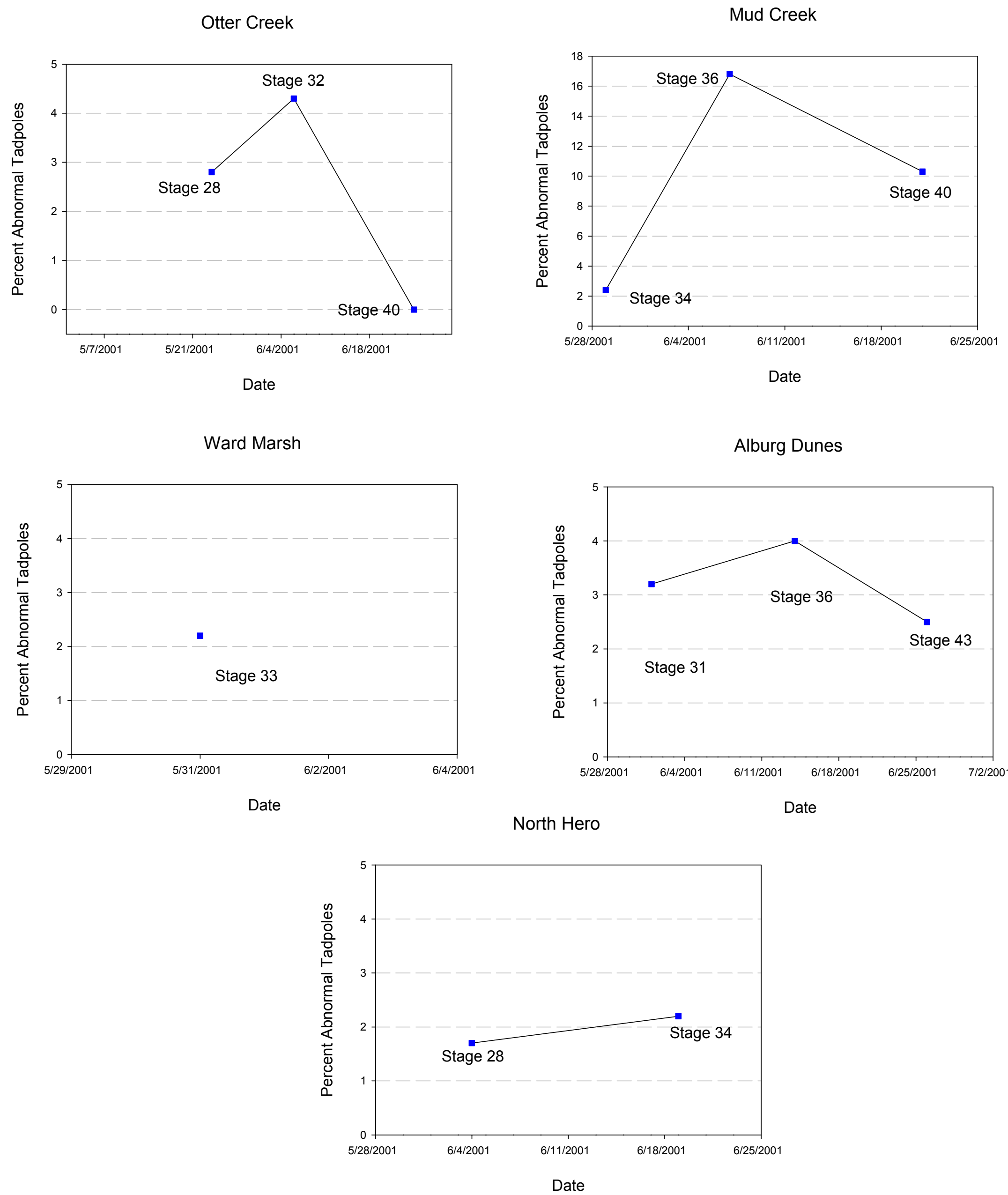
Figure 47a

Figure 47b

Figure 47c

All of the sites with the exception of Ward Marsh (only one valid sampling event) showed an increase in tadpole abnormalities as the season progressed, or more importantly as the tadpole development progressed. The Mud Creek and Alburg abnormalities did decrease at the last sampling event. Figure 48 presents the percent abnormal tadpoles by site and date with average Gosner stage shown.

Figure 48. Percent Abnormal Tadpoles by Site and date with Average Gosner Stage (Gosner Limb Development Stages are 26 – 40)



It is doubtful that the observed increase in tadpole abnormalities is statistically significant, though it would be logical to assume that as the duration of exposure increases and the size of the limb bud increases so does the risk to trauma. In addition, abnormalities from earlier critical exposures may become manifest.

Limb development begins at Gosner Stage 26 and is complete at Stage 40. The developing limb bud at Stage 26 is very small (<1mm), close to the body and more protected than later stages. Late in hind limb development (Gosner Stage 36-40) the hind limb hangs down in a precarious manner, definitely more vulnerable to predation and/or trauma by aquatic insects such as diving predaceous beetles (Dysticus), Back Swimmers (Notonectidae), and Giant water bugs (Belostomatidae).

Licht (1974) observed many aquatic insects (*Notonecta*, *Lethocerus*, *Dysticus*, dragonfly larvae and leeches) feeding on tadpoles and many tadpoles were caught which showed evidence of having been attacked by these invertebrates (tail portion or limb missing).

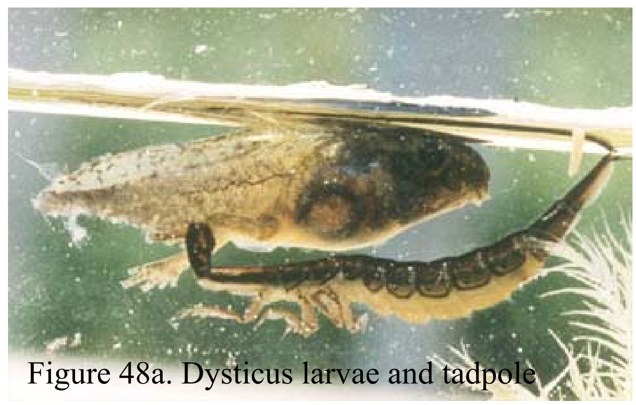


Figure 48a. Dysticus larvae and tadpole

We observed many aquatic insects at each site, especially while sweeping for tadpoles. It was easy to imagine how variations in habitat, water levels, tadpole densities and aquatic insect populations could provide a wide range of opportunities for predation and predation related trauma. The red-maple forested swamp site at Mud Creek had a very large population of diving predaceous beetle larvae (*Dysticus*) present. Each sweep for tadpoles often captured 10 or more dysticus larvae. While tadpole related tail-trauma (Table 13) was quite high (n = 26) at Mud Creek, there was only one tadpole observed with a truncated limb (ectromelia of the femur). Furthermore the metamorph abnormalities at the Mud Creek site ranged from 0%-4.9% (Table 6, in metamorph section), and only 2 specimens had hind limb truncations. Of the 1254 tadpoles examined at the five sites, we did not find any tadpole limb truncations that showed signs of fresh trauma (n =1254). In fact only 6 tadpoles had hind limb truncations (0.5%).

Lannoo (2000) discusses the predation hypothesis in detail, and list several convincing statements negating this hypothesis. 1) One would expect if failed predation caused missing limbs, more herpetologists would be observing large numbers of animals with missing limbs. 2) Missing limbs occur in the absence of predators. 3) Developing limbs cannot be easily pulled from the body by animals with mouthparts designed for swallowing prey whole. 3) Why would predators selectively choose hind limbs? Failed predation on tadpoles by aquatic vertebrates typically result in lost tails. 4) If predation is causal, why are there rarely signs of wound repair in newly metamorphosed animals? 5) Radiographs of missing limbs nearly always show abnormal spongiform-like bone malformations proximal to the sight of the absence. This morphology is inconsistent with known inflammatory responses. 6) Some animals missing whole limbs are also missing portions of the associated pelvic or pectoral girdles.

Growth – Tadpoles

Tadpoles were Gosner staged at each collection, thus providing information on growth rates between the first and the last collection. Figure 49 shows tadpole growth rates with Gosner stage by site.

Taylor and Kollros (1946) staging table for R. *pipiens* provides data on the duration of stage intervals. This data was obtained by rearing tadpoles at 20°C with continuous feeding.

We used the Taylor staging duration table to calculate by site the number of laboratory days needed to pass from our first observed stage to the last observed stage. By comparing the duration (# lab days at 20°C) to the observed number of field days Table 14, we may provide insight with regards to other environmental factors influencing growth rates, particularly temperature.

Figure 49. Tadpole Growth Rates at Five Primary Sites.

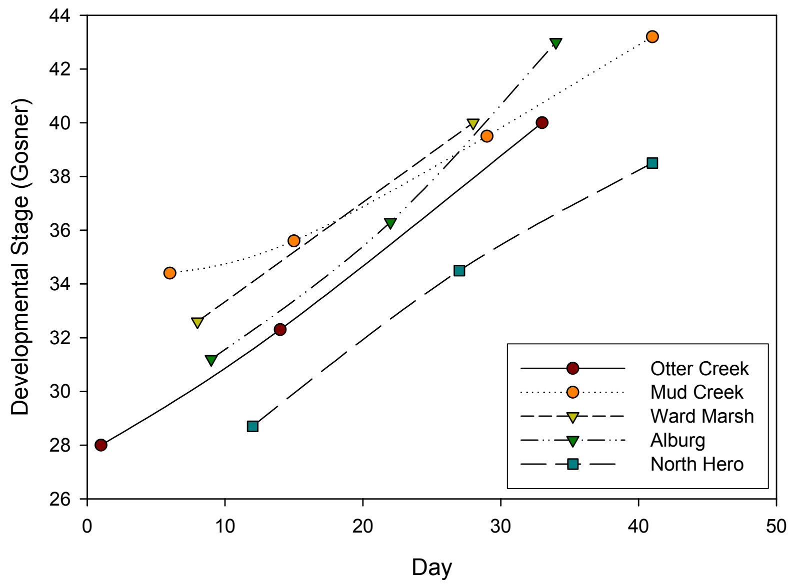


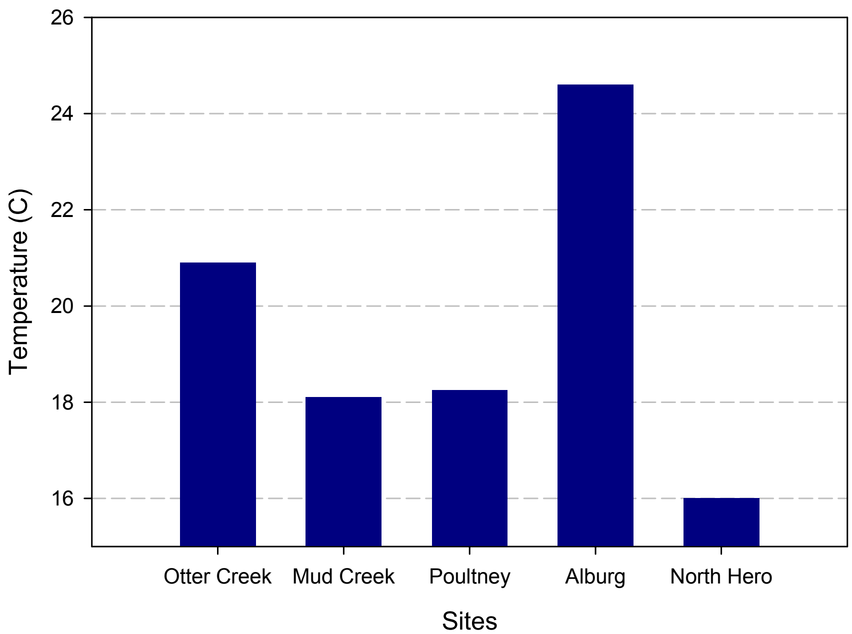
Table 14 compares Taylor growth rates to the observed field growth rates. Average temperature and number of days above 20°C is presented. The number of days above 20°C at sites is likely a more reliable index of the water temperature's influence on tadpole growth than the average temperature alone, presented in Figure 50.

Table 14. Taylor Laboratory and Field Growth Rates with Average Temperature.

Site	Stage (Gosner) 1st Collection	Stage (Gosner) Last Collection	Actual # Field Days	Taylor Lab days at 20°C	Field days/ Taylor days	Field days/ Above 20°C	Average Field Temp °C
Otter Creek	28	40	30	56	0.53	33	21.0
Mud Creek	34	43	36	42	0.85	5	18.0
Ward Marsh	32	40	20	40	0.50	25	18.5
Alburg Dune	31	43	26	53	0.49	45	24.5
North Hero	29	39	28	36	0.77	10	16.0

Water temperature measurements by site, for the entire tadpole development period (April – July) are presented in Figure 51.

Figure 50. Average Water Temperature at Five Primary Sites During Tadpole Development (April – July).

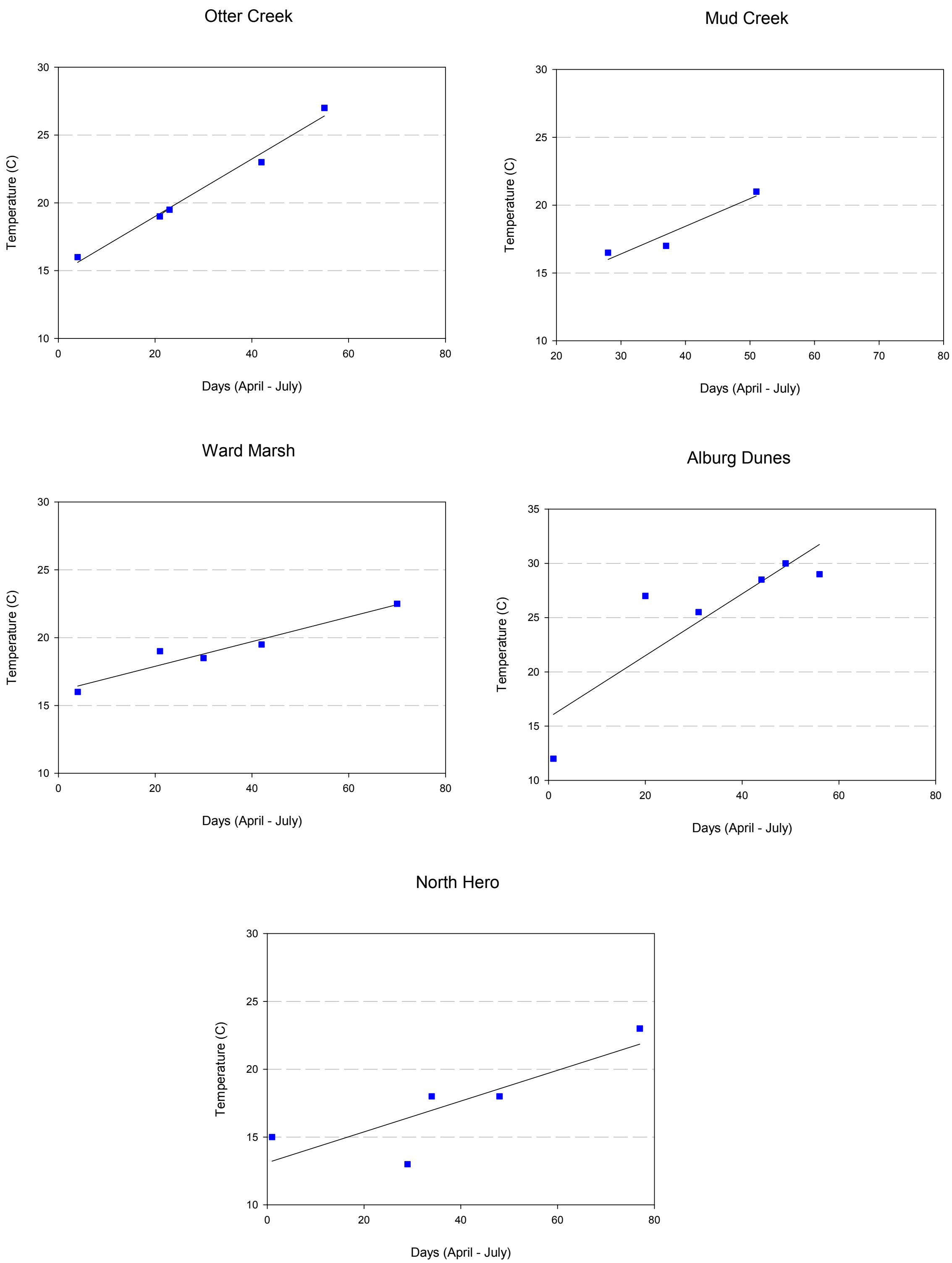


All five sites exceeded the growth rates that were observed in the Laboratory at 20^{o}C. The field days to Taylor ratio shows that our observed field growth rates were substantially greater, roughly twice as fast at three of the five sites. This is probably primarily due to temperature differences and secondarily due to food resources and nutritional difference.

The three fastest growth rates were Otter, Ward Marsh (Poultney) and Alburg, the field days/Taylor ratio ranged from 0.49 – 0.53. The number of days greater than 20^{o}C ranged from 25 – 45 days. The Alburg site with an average temperature of 24.5^{o}C had 45 days above 20^{o}C. It is notable that Alburg's growth rate did not surpass Ward Marsh and Otter Creek's growth by a greater margin.

Mud Creek and North Hero growth rates were the slowest (ratio 0.85, 0.77) and most closely resembled the Taylor growth rates. These two sites also had the lowest average temperatures and more significantly the lowest number of days above 20^{o}C, 5 and 10, respectively. This data does suggest that water temperature plays a dominant role in tadpole growth rates, affecting duration of exposure to anthropogenic and natural conditions. Critical exposure period duration increases as growth decreases.

Figure 51. Water Temperature During Tadpole Development (April – July).



Drift Fence Monitoring at Ward Marsh 1998 -2001

Species

Over the four years of monitoring, eight species of amphibian have been captured in the drift-fences. Of these, the great majority are Northern Leopard Frogs (*Rana pipiens*) (91%, Figure 52). Green Frogs (*Rana clamitans*) are also caught in relatively large numbers (5%, 245 captures over four years). Eastern Newts (*Notophthalmus viridescens*, 3%, 147 caught) and Spotted Salamanders (*Ambystoma maculatum*, 0.4%, 21 caught) are the only two species of salamander we have caught, but this is not surprising considering that the fences are not set in appropriate habitat to catch most salamander species. Newts and Spotted Salamanders are not caught in large numbers, but generally the number of captures is large enough (with the exception of adult Spotted Salamanders) to accurately monitor their populations. Other species that are caught occasionally, but not frequently enough to accurately monitor their populations are Pickerel Frog (*Rana palustris*, 14 caught), American Bullfrog (*Rana catesbeiana*, 7 caught), American Toad (*Bufo americanus*, 4 caught), and Gray Treefrog (*Hyla versicolor*, 2 caught).

Trends

We have only collected four years of data at this point, which makes conclusions about trends in abundance of amphibian species tenuous. However, some possible trends appear using linear regression lines (Figures 53-59). Of the three adult amphibians we can accurately monitor, two (Eastern Newt and Northern Leopard Frog) appear to be increasing (Figures 54 and 56 respectively; Table 15). As shown in Table 16, we have the power (90% or greater) to accurately detect both of these trends. Adult Green Frogs appear to be declining (Figure 55), and we have 100% power to detect this change, but this could be primarily due to the high starting value we began with in 1998 (Table 15). Overall, adult salamanders, frogs, and amphibians appear to be increasing (Figures 57-59; Table 16). We have acceptable power (greater than 90%) to detect these changes.

Table 15. Monitoring results from the seven drift-fences at Ward Marsh in West Haven, Vermont from 1998-2001. Indices were generated from the three most successful trappings per month. Because full data sets in 1998 were not collected until July, the indices for all years were generated using only four months of data (July through October). In 1998, dates used for the generation of the indices were July 5, 9, and 24; August 9, 12, and 24; September 8, 16, and 22; October 8, 15, and 29. In 1999, dates used were June 29 and 30, and July 10; July 25, August 9 and 15; September 8, 11, and 17; and October 1, 11, and 21. In 2000, dates used were July 16, 17, and 31; August 1, 15, and 24; September 13, 16, and 24; and October 6, 18, and 19. In 2001, dates used were July 11, 18, and August 5; August 11, 18, and 21; September 1, 21, and 22; and September 26, October 15, and 24. Not included in the table are American Bullfrog, American Toad, Gray Treefrog, Pickerel Frog, and adult Spotted Salamanders, as they were not caught in large enough numbers to accurately monitor their populations.

Common Name	Scientific Name	Number Of Captures Per Trapping							
		Adults				Juveniles [1]			
		1998	1999	2000	2001	1998	1999	2000	2001
Caudates (Salamanders)									
Spotted Salamander	*Ambystoma maculatum*	NA	NA	NA	NA	0.50	0.00	0.25	0.00
Eastern Newt	*Notophthalmus viridescens*	0.33	0.67	0.25	1.50	1.42	0.58	2.67	0.33
Group totals		0.33	0.67	0.33	1.50	1.92	0.58	2.92	0.33
Anurans (Frogs and Toads)									
Green Frog	*Rana clamitans*	1.75	0.25	0.42	0.25	2.75	5.75	2.92	1.25
Northern Leopard Frog	*Rana pipiens*	18.83	4.33	11.17	21.08	130.75	30.50	39.75	53.17
Group totals		20.83	4.75	11.83	21.33	133.67	36.75	42.67	54.67
Amphibian totals		21.17	5.42	12.17	22.83	135.58	37.33	45.58	55.00

[1]For each species, individuals under a given total length were considered juveniles. The chosen length was based on the timing of the appearance, gaps in their size continuum, and records in the literature. The cutoff sizes used were A. maculatum(70 mm), N. viridescens(45 mm), R. clamitans(44 mm), and R. pipiens(41 mm).

Table 16. Power analyses of the Ward Marsh (West Haven, Vermont) data from 1998 through 2001 using the Monitor.exe freeware program. Percentages in bold are generated with a power greater than 90% after four years of monitoring. Because full data sets in 1998 were not collected until July, the indices for all years were generated using only four months of data (July through October). Not included in the table are American Bullfrog, American Toad, Gray Treefrog, Pickerel Frog, and adult Spotted Salamanders, as they were not caught in large enough numbers to accurately monitor their populations.

Common name	**Starting value (1998)** [1]		**SD** [2]		**Power 5% decline** [3]		**Power 10% decline** [4]		**Power (x%)** [6]		**Annual change** [6]		**Annual % change**	
	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)	Adults (A)	Juveniles (J)
Caudates (Salamanders)														
Spotted Salamander	NA	0.50	NA	0.01	NA	1.00	NA	1.00	NA	1.00 (-10%)	NA	-0.125	NA	-25.00%
Eastern Newt	0.33	1.42	0.06	0.22	0.49	0.58	0.90	0.96	1.00 (10%)	0.87 (-8%)	0.308	-0.117	92.40%	-8.24%
Group totals	0.33	1.92	0.06	0.21	0.52	0.86	0.91	1.00	0.99 (10%)	1.00 (-10%)	0.317	-0.242	95.01%	-12.61%
Anurans (Frogs and Toads)														
Green Frog	1.75	2.75	0.07	0.37	1.00	0.67	1.00	0.99	1.00 (-10%)	0.99 (-10%)	-0.433	-0.733	-24.76%	-26.67%
Northern Leopard Frog	18.83	130.75	1.72	4.71	0.93	1.00	1.00	1.00	1.00 (7%)	1.00 (-10%)	1.358	-22.350	7.21%	-17.09%
Group totals	20.83	133.67	1.77	4.99	0.95	1.00	1.00	1.00	0.93 (4%)	1.00 (-10%)	0.858	-23.108	4.12%	-17.29%
Amphibian totals	21.17	135.58	1.77	4.85	0.97	1.00	1.00	1.00	1.00 (6%)	1.00 (-10%)	1.175	-23.350	5.55%	-17.22%

[1]This is the number caught per trapping in 1998 at all seven fences. It is used in the Monitor.exe freeware program to calculate power.

[2]This standard deviation (SD) is based on two indices, each of which used three fences (one from each pair of fences).

[3]This is the power to detect a 5% annual population decline after four years of monitoring.

[4]This is the power to detect a 10% annual population decline after four years of monitoring.

[5]This is the power to detect the percent change indicated in the parentheses after four years of monitoring. This percent change is equivalent to the value in the column "Annual % Change" (rounded to the nearest whole number), except when the annual percent change is greater than 10%. Because the Monitor.exe program does not calculate power for an annual change greater than 10%, the power in these cases is equal to the power at 10% increase or decrease.

As expected, we generally catch more juveniles than adults over the course of the year. Although the numbers of juveniles for the four most abundant species (Spotted Salamander, Eastern Newt, Green Frog, and Northern Leopard Frog) appear to be declining (Figures 53-56), we catch too few Spotted Salamanders to take the trend seriously and we do not have the power to accurately detect this trend for Eastern Newts (Table 16). The juvenile Newt population fluctuates quite a bit from year to year, making it difficult to see trends with so few years of data (Figure 54). We captured our lowest numbers ever of juvenile Green Frogs in 2001 (1.25 per trapping). This trend is strong (-26% annual change), and we have 99% power to detect it. Juvenile Northern Leopard Frogs started out with very large numbers per trapping (131) in 1998, dropped to only 30.5 in 1999 and have increased gradually since then (Table 15). Over the four years this trend is a strong decline, however it appears this decline (-17%) is simply a factor of starting with a very successful breeding year, thereby producing large numbers of metamorphs, and appearing as a population decline. Overall, juvenile salamanders, frogs, and combined amphibians all appear to be declining (with 100% power in all cases to detect the decline, Figures 57-59). Both frog and amphibian trends are driven primarily by Northern Leopard Frog captures, and therefore follow their trends.

At this point in time, it is not possible to draw any strong conclusions about the increase or decline of any amphibian species at these fences. Additional years of monitoring would allow us to more accurately detect changes in abundance. They do show however that although the numbers of adult Northern Leopard Frogs have stayed fairly stable, breeding success was at a peak when we first sampled this site in 1998 and dropped quickly afterward.

Abnormalities

Abnormality data comes from the entire year of trapping, including days that were not considered successful and therefore not included in the abundance indices. Overall abnormality rates were very low for all species caught in significant numbers over the entire four-year period. Since Northern Leopard Frogs *(Rana pipiens)* were the species most frequently caught at the site, the vast majority of abnormalities seen were in this species, however, the relative percentage of abnormalities was essentially the same for Green Frogs (*Rana clamitans*). Northern Leopard Frogs had a total abnormality rate of 0.88% (38 out of 4313) versus 1.22% (3 out of 245) for Green Frogs.

Summaries of the types of abnormalities that occurred in Northern Leopard Frogs are found in Table 17 and Figure 60. Abnormalities were categorized using the North American Reporting Center for Amphibian Malformation's online guide and key (NARCAM, 1997) (http://www.npwrc.usgs.gov/narcam/). Figures 60a and 61a are included for readers not familiar with the technical terminology. If an amphibian had more than one abnormality, it was counted separately; for example, if an amphibian had both brachydactyly and ectrodactyly, it was put under this category, and not included in the separate brachydactyly or ectrodactyly categories. In Table 17, Northern Leopard Frog abnormalities were separated into malformities (probably caused by a problem with development) and deformities (probably caused by predation or injury after normal development).

Table 17. Summary of abnormality results for Northern Leopard Frogs (*Rana pipiens*) from the seven drift-fences at Ward Marsh, West Haven, Vermont, from 1998 to 2001. Data used are from all trap efforts (20 in 1998, 28 in 1999, 28 in 2000, and 30 in 2001). Based on the type and location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured.

Type Of Abnormality	# Abnormal [1]	% Abnormal of Species Total	# Abnormal Adults	% Abnormal Adults	# Abnormal Juveniles	% Abnormal Juveniles	% Occurring In Rear Legs
Ectromelia of the femur	2	0.05%	1	0.10%	1	0.03%	100%
Ectromelia of the tibiafibula	7	0.16%	0	0.00%	7	0.21%	86%
Ectromelia of the tibiale/fibulare	1	0.02%	0	0.00%	1	0.03%	0%
Hemimelia of the tibiale/fibulare	1	0.02%	0	0.00%	1	0.03%	100%
Amelia	5	0.12%	0	0.00%	5	0.15%	60%
Micromelia	1	0.02%	0	0.00%	1	0.03%	0%
Unspecified malformity of foot	1	0.02%	0	0.00%	1	0.03%	100%
Malformities of the legs	18	0.42%	1	0.10%	17	0.52%	72%
Ectrodactyly	3	0.07%	0	0.00%	3	0.09%	100%
Brachydactyly	4	0.09%	2	0.19%	2	0.06%	100%
Syndactyly	2	0.05%	0	0.00%	2	0.06%	0%
Polydactyly	1	0.02%	1	0.10%	0	0.00%	100%
Malformities of the toes	10	0.23%	3	0.29%	7	0.21%	80%
Hemimelia of the tibiafibula & brachydactyly	1	0.02%	1	0.10%	0	0.00%	100%
Hemimelia of the tibiale/fibulare & brachydactyly	2	0.05%	0	0.00%	2	0.06%	100%
Hemimelia of the tibiale/fibulare, brachydactyly, & ectrodactyly	1	0.02%	1	0.10%	0	0.00%	100%
Brachydactyly & ectrodactyly	1	0.02%	0	0.00%	1	0.03%	100%
Multiple types of malformities	5	0.12%	2	0.19%	3	0.09%	100%
Total malformities	33	0.77%	6	0.58%	27	0.82%	79%
Trauma - Missing toes	1	0.02%	1	0.10%	0	0.00%	100%
Trauma - Missing a foot	2	0.05%	0	0.00%	2	0.06%	100%
Injury to trunk [1]	1	0.02%	0	0.00%	0	0.00%	NA
Injury to trunk and eye	1	0.02%	0	0.00%	1	0.03%	NA
Trauma-related deformities	5	0.12%	1	0.10%	3	0.09%	100%
Total abnormalities	38	0.88%	7	0.68%	30	0.91%	82%
Total number of captures	4313						
Total number of adult captures	1030						
Total number of juvenile captures	3283						

[1]No measurement was taken on this animal (caught in 1998), as the rear half of the frog was missing due to predation. Therefore, it is not listed in this table as either adult or juvenile, and is only included in the total count.

Malformities were further subdivided into malformities of the legs, of the toes, and multiple types of malformities. Most malformities occurred in the legs, followed by the toes. The most common malformity was ectromelia of the tibiafibula, missing leg(s) below the knee (7 captures, 18% of the abnormalities). Amelia [missing entire leg(s)] occurred 5 times (13%). Brachydactyly [short toe(s)] (11%) occurred 4 times by itself. Ectrodactyly occurred 3 times (8%). Other abnormalities occurred in only 1 or 2 individuals. The overall percent malformed was barely higher for juveniles than adults with 0.82% of juveniles malformed and 0.58% of adults malformed (Table 17). The largest difference in malformity rates between adults and juveniles was in malformities of the legs (0.1% adult versus 0.5% juveniles), which might be expected considering that these malformities are probably more lethal or serious than are other external malformities. It appears that a larger percentage of juveniles with malformities of the legs versus other types do not survive to adulthood. We only caught 1 adult with a malformed leg (ectromelia of the femur). Overall, the majority of malformities for Northern Leopard Frogs occurred in the rear legs (72%).

Since 1998 had the highest numbers of Northern Leopard Frogs overall, it also had by far the most abnormalities and malformities (Figure 61, Table 18). Still, the percentage of abnormalities was no greater than in 1999 (tied with 1999 at 1.1%). The malformity rate of 1.0% was just barely less than the 1.1% of 1999. The majority of these malformities occurred in the legs (ectromelia of the tibiafibula and amelia being the two most common malformities). Interestingly, amelia only occurred in frogs in 1998, and has not reappeared since. Similarly, ectrodactyly has not reappeared since 1998 in our trapping efforts. In 2000 we had both the lowest number of abnormalities and the lowest percent abnormal (3, 0.4%). One of these was a trauma-related deformity, so the percent malformed was only 0.3%. In two of the four years, the adult percentage of malformities was slightly higher than the juvenile percentage (Table 19). Despite being low to begin with, the percentage of malformed adult Northern Leopard Frogs has declined slowly but steadily over the four years and although slightly more variable the trend in malformed juveniles has also been toward lower percentages.

Table 18. Abnormality results for all Northern Leopard Frogs (*Rana pipiens*) from the seven drift-fences at Ward Marsh, West Haven, Vermont, from 1998 to 2001. Data used are from all trap efforts (20 in 1998, 28 in 1999, 28 in 2000, and 30 in 2001). Based on the type and location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured.

Type Of Abnormality	# Abnormal				% Abnormal Of Species Total				% Occurring In Rear Legs			
	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
Ectromelia of the femur	0	1	1	0	0.0%	0.2%	0.1%	0.0%	NA	100%	100%	NA
Ectromelia of the tibiafibula	5	0	1	1	0.3%	0.0%	0.1%	0.1%	100%	NA	100%	0%
Ectromelia of the tibiale/fibulare	0	0	0	1	0.0%	0.0%	0.0%	0.1%	NA	NA	NA	0%
Hemimelia of the tibiale/fibulare	1	0	0	0	0.1%	0.0%	0.0%	0.0%	100%	NA	NA	NA
Amelia	5	0	0	0	0.3%	0.0%	0.0%	0.0%	60%	NA	NA	NA
Micromelia	0	1	0	0	0.0%	0.2%	0.0%	0.0%	NA	0%	NA	NA
Unspecified malformity of foot	1	0	0	0	0.1%	0.0%	0.0%	0.0%	100%	NA	NA	NA
Malformities of the legs	12	2	2	2	0.6%	0.4%	0.3%	0.2%	83%	50%	100%	0%
Ectrodactyly	3	0	0	0	0.2%	0.0%	0.0%	0.0%	100%	NA	NA	NA
Brachydactyly	3	1	0	0	0.2%	0.2%	0.0%	0.0%	100%	100%	NA	NA
Syndactyly	0	1	0	1	0.0%	0.2%	0.0%	0.1%	NA	0%	NA	0%
Polydactyly	0	1	0	0	0.0%	0.2%	0.0%	0.0%	NA	100%	NA	NA
Malformities of the toes	6	3	0	1	0.3%	0.6%	0.0%	0.0%	100%	67%	NA	0%
Hemimelia of the tibiafibula & brachydactyly	0	0	0	1	0.0%	0.0%	0.0%	0.1%	NA	NA	NA	100%
Hemimelia of the tibiale/fibulare & brachydactyly	0	1	0	1	0.0%	0.2%	0.0%	0.1%	NA	100%	NA	100%
Hemimelia of the tibiale/fibulare, brachydactyly, & ectrodactyly	1	0	0	0	0.1%	0.0%	0.0%	0.0%	100%	NA	NA	NA
Brachydactyly & ectrodactyly	1	0	0	0	0.1%	0.0%	0.0%	0.0%	100%	NA	NA	NA
Multiple types of malformities	2	1	0	2	0.1%	0.2%	0.0%	0.2%	100%	100%	NA	100%
Total malformities	20	6	2	5	1.0%	1.1%	0.3%	0.5%	90%	67%	100%	40%
Trauma - Missing toes	0	0	1	0	0.0%	0.0%	0.1%	0.0%	NA	NA	100%	NA
Trauma - Missing a foot	0	0	0	2	0.0%	0.0%	0.0%	0.2%	NA	NA	NA	100%
Injury to trunk	1	0	0	0	0.1%	0.0%	0.0%	0.0%	NA	NA	NA	NA
Injury to trunk and eye	1	0	0	0	0.1%	0.0%	0.0%	0.0%	NA	NA	NA	NA
Trauma-related deformities	2	0	1	2	0.1%	0.0%	0.1%	0.2%	NA	NA	100%	100%
Total abnormalities	22	6	3	7	1.1%	1.1%	0.4%	0.6%	82%	67%	100%	57%
Total number of captures each year	1952	543	727	1091								

Table 19. Abnormality results for Northern Leopard Frogs (*Rana pipiens*) separated by size into adults and juveniles (<41 mm), from the seven drift-fences at Ward Marsh, West Haven, Vermont, from 1998 to 2001. Data used are from all trap efforts (20 in 1998, 28 in 1999, 28 in 2000, and 30 in 2001). Based on the type and location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured.

Type Of Abnormality	# Abnormal Adults				% Abnormal Adults				# Abnormal Juveniles				% Abnormal Juveniles			
	1998 [1]	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
Ectromelia of the femur	0	0	1	0	0.0%	0.0%	0.5%	0.0%	0	1	0	0	0.0%	0.3%	0.0%	0.0%
Ectromelia of the tibiafibula	0	0	0	0	0.0%	0.0%	0.0%	0.0%	5	0	1	1	0.3%	0.0%	0.2%	0.2%
Ectromelia of the tibiale/fibulare	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	0	0	1	0.0%	0.0%	0.0%	0.2%
Hemimelia of the tibiale/fibulare	0	0	0	0	0.0%	0.0%	0.0%	0.0%	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Amelia	0	0	0	0	0.0%	0.0%	0.0%	0.0%	5	0	0	0	0.3%	0.0%	0.0%	0.0%
Micromelia	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	1	0	0	0.0%	0.3%	0.0%	0.0%
Unspecified malformity of foot	0	0	0	0	0.0%	0.0%	0.0%	0.0%	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Malformities of the legs	0	0	1	0	0.0%	0.0%	0.5%	0.0%	12	2	1	2	0.7%	0.5%	0.2%	0.3%
Ectrodactyly	0	0	0	0	0.0%	0.0%	0.0%	0.0%	3	0	0	0	0.2%	0.0%	0.0%	0.0%
Brachydactyly	2	0	0	0	0.8%	0.0%	0.0%	0.0%	1	1	0	0	0.1%	0.3%	0.0%	0.0%
Syndactyly	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	1	0	1	0.0%	0.3%	0.0%	0.2%
Polydactyly	0	1	0	0	0.0%	0.7%	0.0%	0.0%	0	0	0	0	0.0%	0.0%	0.0%	0.0%
Malformities of the toes	2	1	0	0	0.8%	0.7%	0.0%	0.0%	4	2	0	1	0.2%	0.5%	0.0%	0.0%
Hemimelia of the tibiafibula & brachydactyly	0	0	0	1	0.0%	0.0%	0.0%	0.2%	0	0	0	0	0.0%	0.0%	0.0%	0.0%
Hemimelia of the tibiale/fibulare & brachydactyly	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	1	0	1	0.0%	0.3%	0.0%	0.2%
Hemimelia of the tibiale/fibulare, brachydactyly, & ectrodactyly	1	0	0	0	0.4%	0.0%	0.0%	0.0%	0	0	0	0	0.0%	0.0%	0.0%	0.0%
Brachydactyly & ectrodactyly	0	0	0	0	0.0%	0.0%	0.0%	0.0%	1	0	0	0	0.1%	0.0%	0.0%	0.0%

Type Of Abnormality	# Abnormal Adults				% Abnormal Adults				# Abnormal Juveniles				% Abnormal Juveniles			
	1998 [1]	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
Multiple types of malformities	1	0	0	1	0.4%	0.0%	0.0%	0.2%	1	1	0	1	0.1%	0.3%	0.0%	0.2%
Total malformities	3	1	1	1	1.2%	0.7%	0.5%	0.2%	17	5	1	4	1.0%	1.3%	0.2%	0.6%
Trauma - Missing toes	0	0	1	0	0.0%	0.0%	0.5%	0.0%	0	0	0	0	0.0%	0.0%	0.0%	0.0%
Trauma - Missing a foot	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	0	0	2	0.0%	0.0%	0.0%	0.3%
Injury to trunk [1]	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	0	0	0	0.0%	0.0%	0.0%	0.0%
Injury to trunk and eye	0	0	0	0	0.0%	0.0%	0.0%	0.0%	1	0	0	2	0.1%	0.0%	0.0%	0.3%
Trauma-related deformities	0	0	1	0	0.0%	0.0%	0.5%	0.0%	1	0	0	2	0.1%	0.0%	0.0%	0.3%
Total abnormalities	3	1	2	1	1.2%	0.7%	1.0%	0.2%	18	5	1	6	1.1%	1.3%	0.2%	0.9%
Total number of adults caught	251	146	206	427												
Total number of juveniles caught	1701	397	521	664												

[1]No measurement was taken on this animal (caught in 1998), as the rear half of the frog was missing due to predation. Therefore, it is not listed in this table as either adult or juvenile, and is only included in the total count.

Abnormalities also occurred in Spotted Salamanders and Green Frogs from 1998 to 2001, though in relatively low numbers (2 abnormal Spotteds, 3 abnormal Green Frogs, Table 20). Over all four years, the percent abnormal was 9.5% for Spotteds, and 1.2% for Green Frogs. The Spotted percent abnormal was much higher than that of Northern Leopard Frogs and Green Frogs, but the overall sample size was too low (21 captures) for an adequate comparison. If one of these abnormalities was trauma related the percentage would drop to 5%, if 2, then 0%. All of the abnormalities for both species occurred in the rear legs. Of the Spotted Salamander abnormalities, 50% occurred in juveniles, and of Green Frogs abnormalities, 33% occurred in juveniles. The types of abnormalities that occurred were generally similar to those of Northern Leopard Frogs, with the addition of polyphalangy (an additional digit attached to the rear toe of a Spotted Salamander) and anophthalmia (a Green Frog missing an eye).

Table 20. Abnormality results for all Spotted Salamanders *(Ambystoma maculatum)* and Green Frogs *(Rana clamitans)* from the seven drift-fences at Ward Marsh, West Haven, Vermont, from 1998 to 2001. Data used are from all trap efforts (20 in 1998, 28 in 1999, 28 in 2000, and 30 in 2001). Based on the type and location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured.

Common Name	Type Of Abnormality	# Abnormal					% Abnormal Of Species Total					% Occurring In Rear Legs				
		1998	1999	2000	2001	1998-2001 (Age class) [2]	1998	1999	2000	2001	1998-2001	1998	1999	2000	2001	1998-2001
Spotted Salamander	Hemimelia of tibiale/fibulare, ectrodactyly, & brachydactyly	0	0	1	0	1 (J)	0.0%	0.0%	14.3%	0.0%	4.8%	NA	NA	100%	NA	100%
	Ectrodactyly, brachydactyly, & polyphalangy	0	0	0	1	1 (A)	0.0%	0.0%	0.0%	14.3%	4.8%	NA	NA	NA	100%	100%
	Total abnormalities [1]	0 / 6	0 / 1	1 / 7	1 / 7	2 / 21	0.0%	0.0%	14.3%	14.3%	9.5%	NA	NA	100%	100%	100%
Green Frog	Brachydactyly & syndactyly	1	0	0	0	1 (A)	1.6%	0.0%	0.0%	0.0%	0.4%	100%	NA	NA	NA	100%
	Brachydactyly & ectrodactyly	1	0	0	0	1 (A)	1.6%	0.0%	0.0%	0.0%	0.4%	100%	NA	NA	NA	100%
	Anophthalmia	0	0	1	0	1 (J)	0.0%	0.0%	1.3%	0.0%	0.4%	NA	NA	NA	NA	NA
	Total abnormalities [1]	2 / 64	0 / 78	1 / 75	0 / 28	3 / 245	3.1%	0.0%	1.3%	0.0%	1.2%	100%	NA	NA	NA	100%

[1] The number to the left of the slash is the total number of abnormalities; the number to the right is the total number of captures each year.
[2] In this column, the letters next to the numbers designate the age class of the abnormal individual. J is equivalent to juvenile, and A is equivalent to adult.

The only other amphibian species caught in significant numbers was the Eastern Newt (*Notophthalmus viridescens*). Interestingly, of the 147 Eastern Newts captured none had abnormalities that could be considered malformities. Two had traumatic injuries to their tails.

Recaptures

Beginning in 1999, the side of the fence on which amphibians were captured was recorded. It was hypothesized that, especially for juveniles moving out of the pond, they would pass by the fences once, and therefore by looking at only abnormalities on the marsh side of the fence we could be more sure that we were not recapturing individuals. As it turns out, on the peak movement day and surrounding days, frogs are caught on both sides of the fence, suggesting that movement occurs in both directions (Figures 62-65). However, in examining the abnormality results from both sides of the fences, it appears that none of the abnormal amphibians were recaptured (based on the type and location of the abnormality, the size of the amphibian, and the location of capture). This suggests that there are very few if any recaptures in the general population as well.

Length of sampling period

We also examined the one day each year that had the highest number of captures of amphibians, then looked at the abnormality percentages on that day. Although this rules out the possibility of recaptures, it also limits the length and size of the sample. We then checked to see if the abnormality percentages were similar to those from the entire year's sampling. The percentages of abnormal Northern Leopard Frogs on the one day with the largest amphibian movement (Table 22) were very close to the percentages over the entire year (within 0.6%) but were consistently lower and did not show the same directional movement from year to year. They do match the relative sizes of the juvenile populations well. This makes sense since these peaks are made up almost entirely of metamorphs.

Table 21. Abnormality results for all Spotted Salamanders *(Ambystoma maculatum),* Green Frogs *(Rana clamitans),* and Northern Leopard Frogs *(Rana pipiens)* from the marsh side of the seven drift-fences at Ward Marsh, West Haven, Vermont, from 1998 to 2001. Data used are from all trap efforts (20 in 1998, 28 in 1999, 28 in 2000, and 30 in 2001).

Common Name	# Abnormal [1]				% Abnormal Of Species Total			
	1999	2000	2001	1999-2001	1999	2000	2001	1999-2001
Spotted Salamander	0 / 1	1 / 5	1 / 5	2 / 11	0.0%	20.0%	20.0%	18.2%
Green Frog	0 / 51	1 / 46	0 / 11	1 / 108	0.0%	2.2%	0.0%	0.9%
Northern Leopard Frog	3 / 321	2 / 482	4 / 557	9 / 1360	0.9%	0.4%	0.7%	0.7%
Total	3 / 373	4 / 542	5 / 573	12 / 1479	0.8%	0.7%	0.9%	0.8%

[1] The number to the left of the slash is the number of abnormalities on the marsh side of the fences; the number to the right is the total number of captures each year on the marsh side of the fences.

Table 22. Abnormality results from the seven drift-fences at Ward Marsh, West Haven, Vermont, on the day each year when the highest number of amphibians were caught. All abnormalities on those days occurred in Northern Leopard Frogs (*Rana pipiens*).

Type Of Abnormality	# Abnormal				% Abnormal Of Amphibian Total			
	7/5/1998	6/29/1999	7/31/2000	7/11/2001	1998	1999	2000	2001
Ectromelia of the tibiafibula	2	0	1	0	0.2%	0.0%	0.7%	0.0%
Hemimelia of the tibiale/fibulare	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Amelia	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Unspecified malformity of foot	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Malformities of the legs	5	0	1	0	0.4%	0.0%	0.7%	0.0%
Brachydactyly	1	0	0	0	0.1%	0.0%	0.0%	0.0%
Syndactyly	0	0	0	1	0.0%	0.0%	0.0%	0.3%
Malformities of the toes	1	0	0	1	0.1%	0.0%	0.0%	0.3%
Hemimelia of the tibiale/fibulare & brachydactyly	0	1	0	1	0.0%	0.5%	0.0%	0.3%
Multiple types of malformities	0	1	0	1	0.0%	0.5%	0.0%	0.3%
Total malformities	6	1	1	2	0.5%	0.5%	0.7%	0.5%
Trauma - Missing a foot	0	0	0	1	0.0%	0.0%	0.0%	0.3%
Trauma-related deformities	0	0	0	1	0.0%	0.0%	0.0%	0.3%
Total abnormalities	6	1	1	3	0.5%	0.5%	0.7%	0.8%
Total number of amphibian captures on each date	1198	220	139	374				

Timing of metamorphosis

Figures 62-65 give us important information about the timing of metamorphosis for Northern Leopard Frogs. Every year, peak metamorphosis occurred in July, but ranged from as early as July 5 (1998) to July 31 (2000). Size at metamorphosis ranged from 25 to approximately 38 mm, with a peak at 32 mm (Figure 66). The largest of the frogs reached 81 mm in snout to vent length. Although we experimented with a variety of graphs of limited time periods we were unable to see clear size-classes of Northern Leopard Frogs in any of them.

Comparisons

In comparing our abnormality percentages to those found by the VTDEC, ours are slightly lower every year, but follow a similar trend. In 1998, VTDEC found 3.4% abnormal; in 1999, 2.7%; in 2000, 0%; in 2001, 1.17%. We similarly had our highest percentage in 1998 (1.1%), and our lowest in 2000 (0.4%). This suggests that the sub-sampling method used by DEC is comparable to the drift fence monitoring. However, if abnormal frogs are dispersing a shorter distance (from breeding site), the drift fence monitoring may be biased to normal frogs.

In 1998, when we both had relatively large numbers of malformities, ectrodactyly or brachydactyly had the largest number of malformities in both surveys. The next most abundant abnormality in the DEC surveys were ectromelia of the femur and abnormal eye, while ours were ectromelia of the tibiafibula and amelia. This suggests that the types of malformities we document are not necessarily in the same proportion or even the same types every year.

Comparisons to other monitoring locations

The percent of abnormal amphibians at Ward Marsh is slightly higher than that of upland amphibian species at our two other drift-fence monitoring locations elsewhere in the state. At Mt. Mansfield in northern Vermont, the percent abnormal was 0.75% from 1998-2001. At the Lye Brook Wilderness Region in southwestern Vermont, the average percent abnormal was 0.17%. Compared to the 0.88% at Ward Marsh, these other values are very similar. None of the values are of great concern, as they are well within expected levels.

Summary

Baseline indices of populations have been established at Ward Marsh. Although it is difficult to accurately detect trends after four years of monitoring, we have the power (90% or greater) to see these trends in three species each for adults and juveniles, and for groups (salamanders, frogs, and amphibians) in both adults and juveniles. Numbers of juvenile Northern Leopard Frogs dropped drastically between 1998 and 1999 but have been increasing gradually ever since. Green Frog juveniles appear to be declining, and adult Eastern Newts and Northern Leopard Frogs appear to be on the increase. The overall malformity rate for Northern Leopard Frogs was 0.77%, slightly lower than that observed in subsampling of the population by the VT DEC. The overall percent malformed was barely lower (0.24%) in adults than in juveniles and in half the years was slightly higher. Compared with other types of malformities, it appears that a larger percentage of Leopard Frogs with malformities of the leg do not survive to adulthood. The greatest malformity rate occurred in 1999, with the lowest in 2000 but the total range was very limited, ranging from a high of 1.1% to a low of 0.3%. Abnormalities were also observed in Spotted Salamanders (9.5%) and Green Frogs (1.2%), but the sample size for Spotteds was very low. No malformities were seen in Eastern Newts. Rates of recaptures at the drift-fences appear to be very low. Data generated from single-visit samples of Northern Leopard Frogs at metamorphosis (DEC samples) give very similar levels of malformities as those of the juveniles caught at the fences. Metamorphosis of Northern Leopard Frogs over the four years has peaked at different dates throughout July. Metamorphs range in size from 25 to 38 mm with a peak at 32 mm. The rate of malformities at Ward Marsh is currently very similar to those of other amphibian species at two other drift-fence locations (Lye Brook Wilderness and Mt. Mansfield). However, given that DEC estimates for 1998-2001 parallel ours it strongly suggests that their very high abnormality rate of 35% in 1997 would have been verified by drift-fence data had fences been in place at Ward Marsh. However, abnormality percentages at the two higher elevation sites were not abnormally high in 1997. It would have been interesting to see if the higher percentages of Northern Leopard Frog malformities occur simultaneously with higher levels of breeding success. High floodwater conditions in Lake Champlain in the spring of 1997 would seem to have facilitated greater breeding success and may also have provided conditions facilitating malformity production. If monitoring is maintained we may be able to establish some correlations in the future.

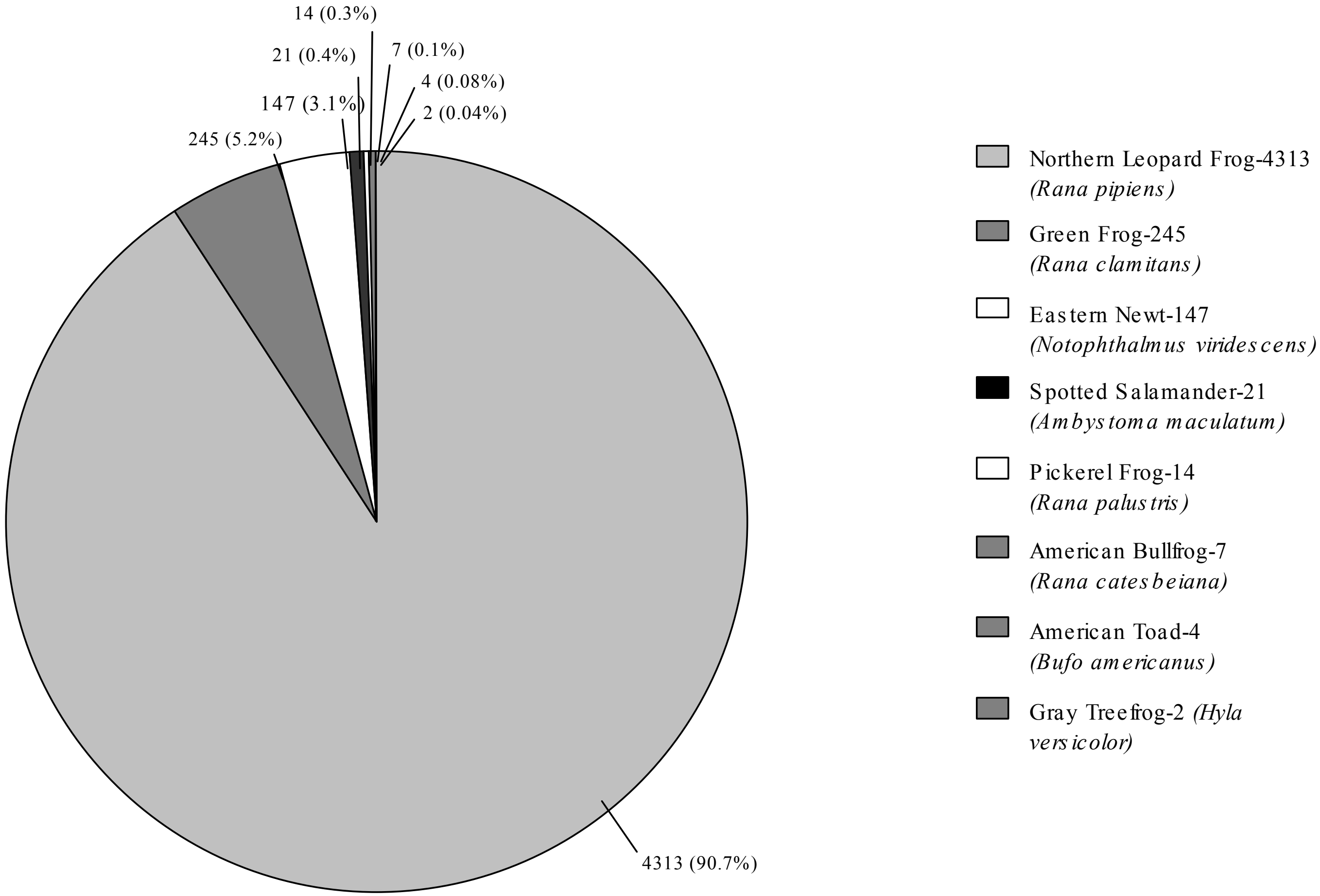


Figure 52. Total numbers of each species captured at Ward Marsh, West Haven, Vermont, from 1998-2001. The total number of captures is listed, with the percentage of the total number of captures in parentheses.

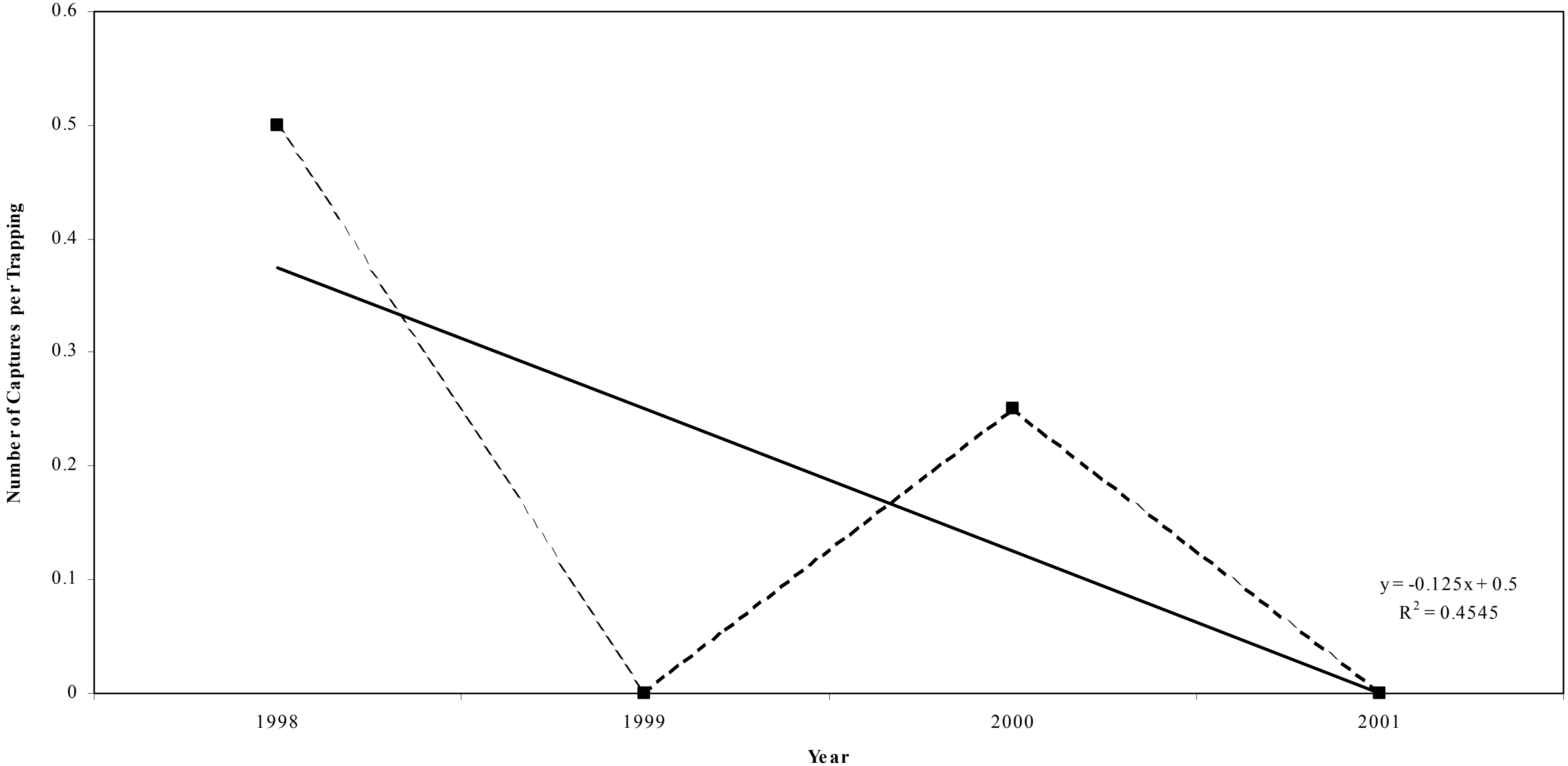


Figure 53. Number of juvenile (<70 mm) Spotted Salamander *(Ambystoma maculatum)* captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. Indices were generated from four months of data, July through October.

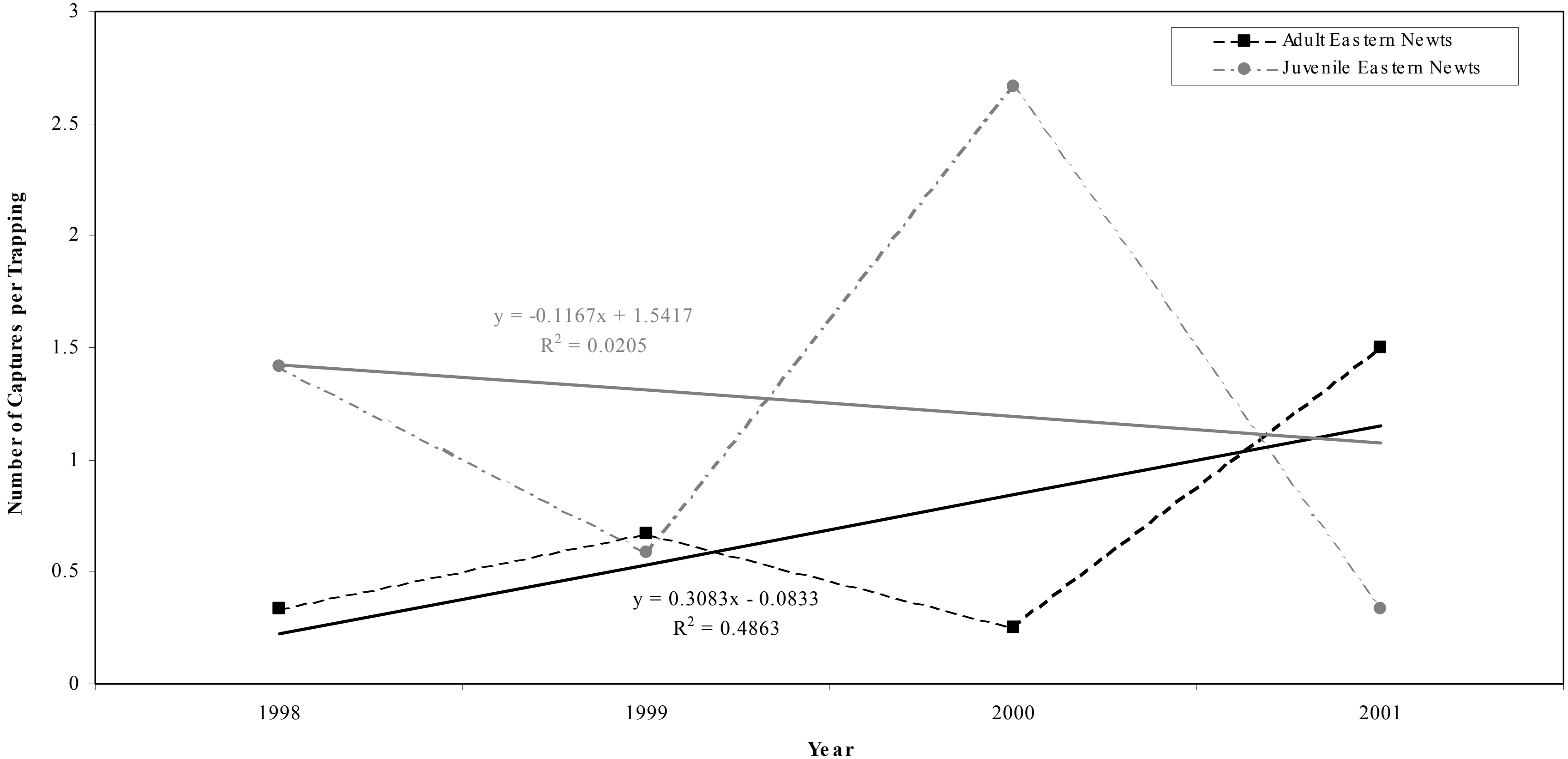


Figure 54. Number of adult and juvenile (<45 mm) Eastern Newt *(Notophthalmus viridescens)* captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. Indices were generated from four months of data, July through October.

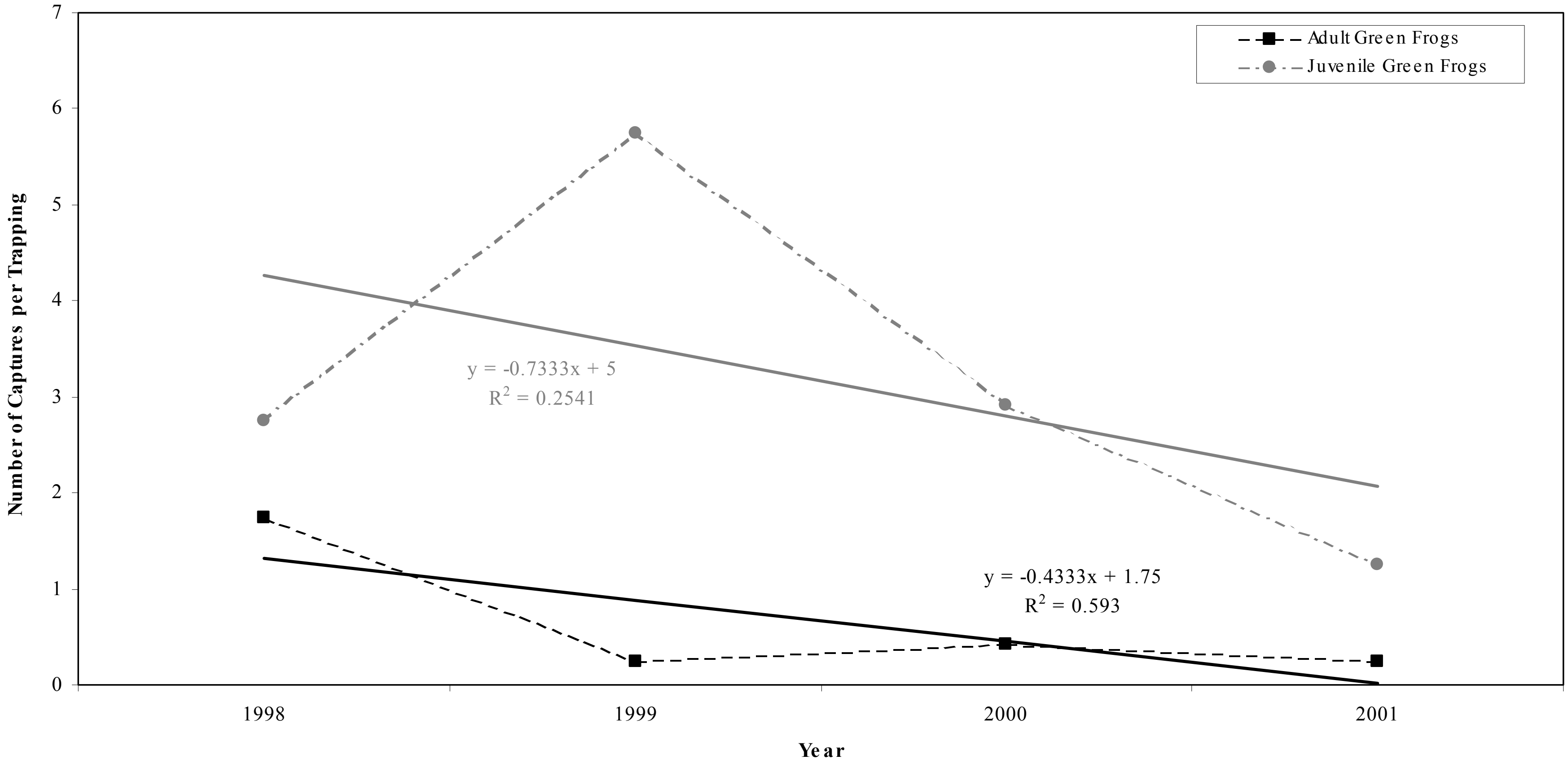


Figure 55. Number of adult and juvenile (<44 mm) Green Frog *(Rana clamitans)* captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. Indices were generated from four months of data, July through October.

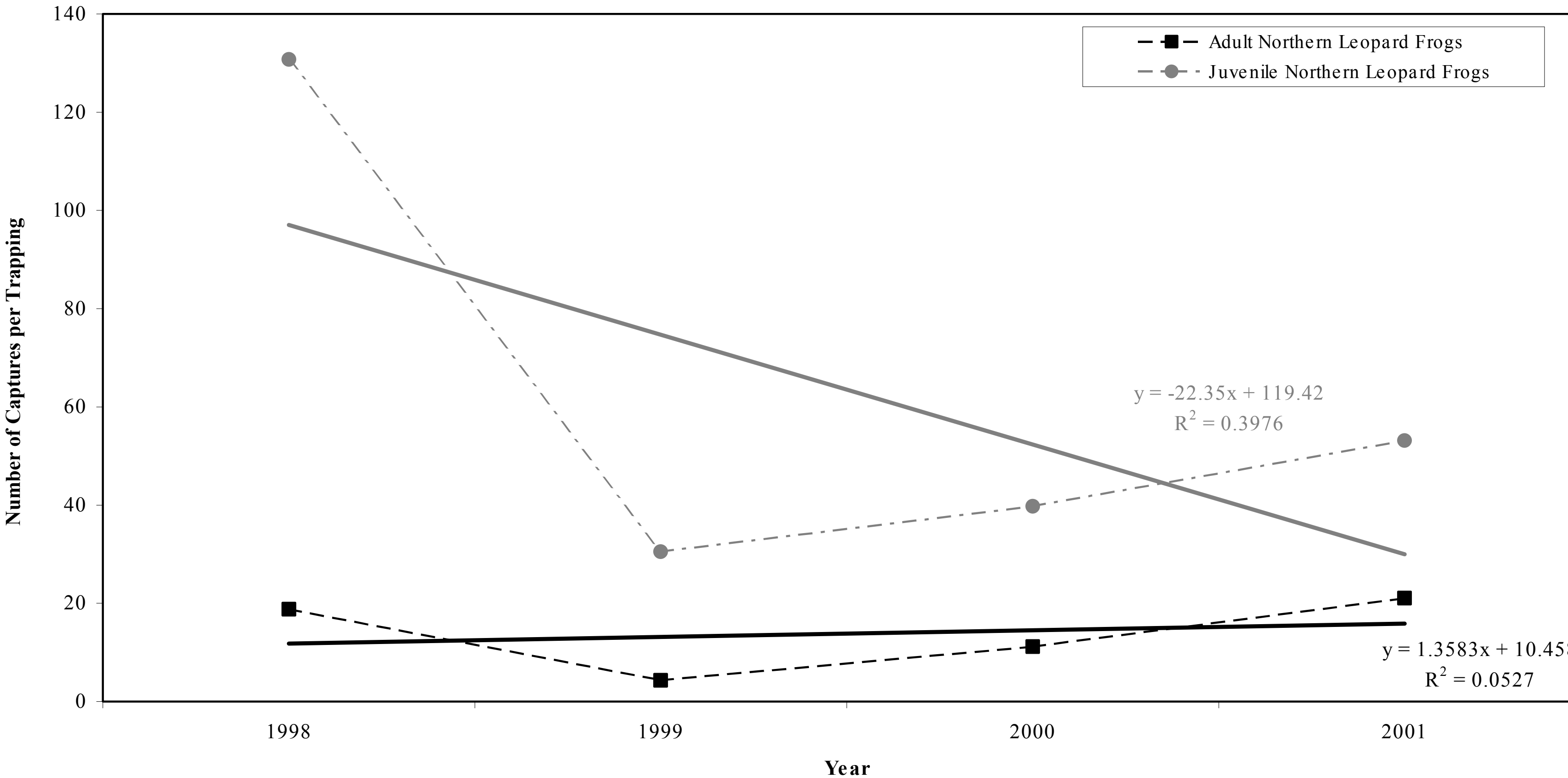


Figure 56. Number of adult and juvenile (<41 mm) Northern Leopard Frog *(Rana pipiens)* captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. Indices were generated from four months of data, July through October.

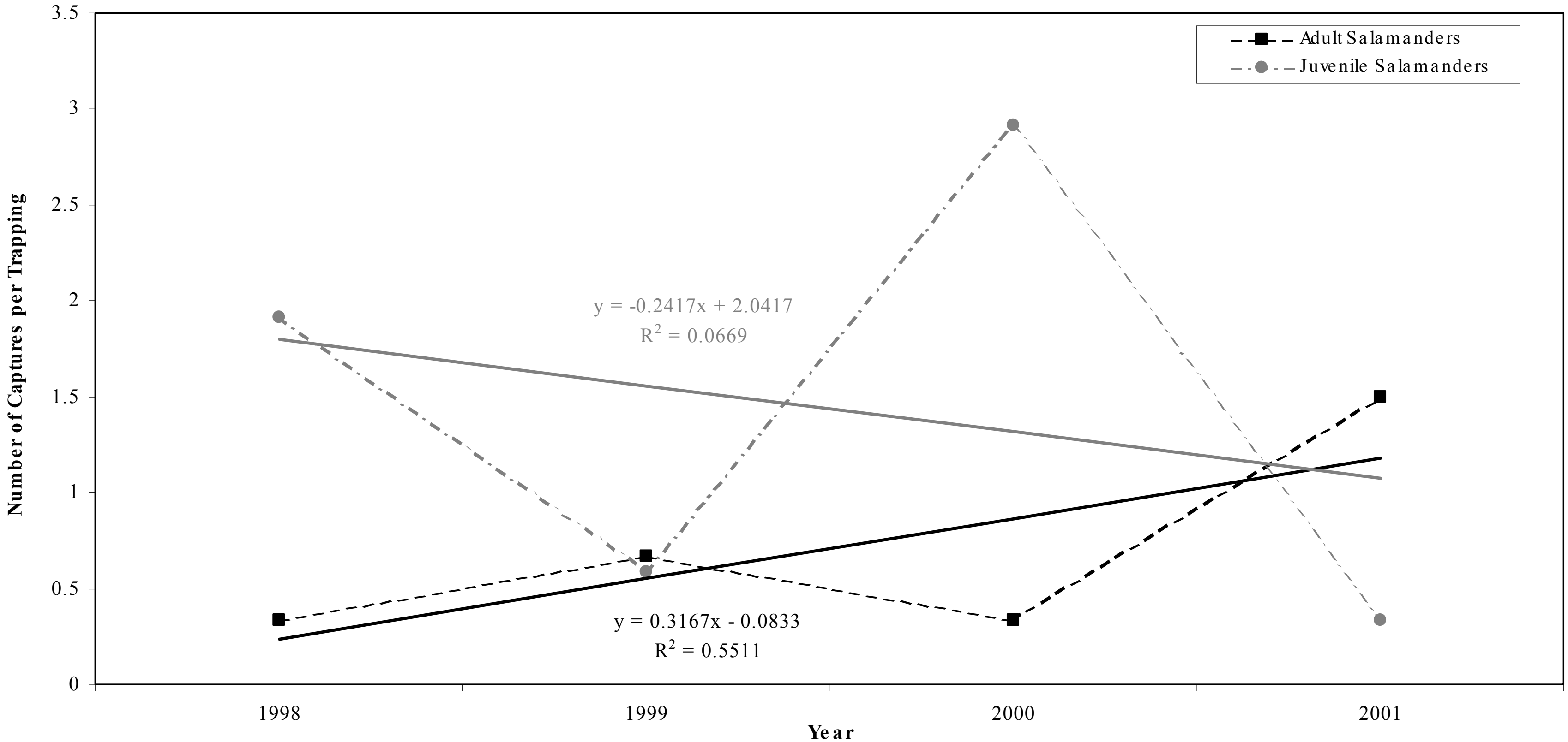


Figure 57. Number of adult and juvenile salamander captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. These numbers include adult Spotted Salamanders *(Ambystoma maculatum)* that are not included in Table 7. Indices were generated from four months of data, July through October.

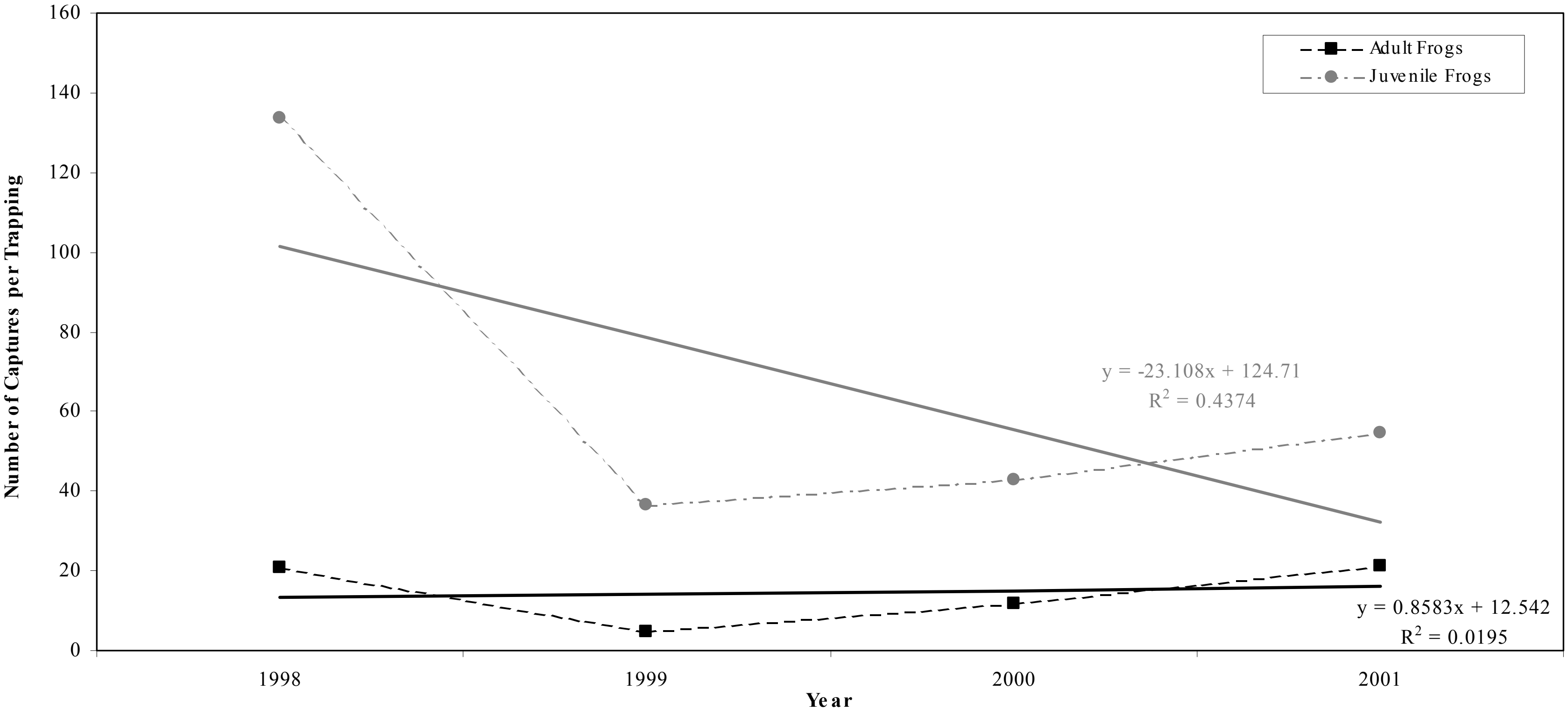


Figure 58. Number of adult and juvenile frog captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. These numbers include captures of Pickerel Frogs *(Rana palustris)*, American Toads *(Bufo americanus)*, Gray Treefrogs *(Hyla versicolor)*, and American Bullfrogs *(Rana catesbeiana)* that are not included in Table 7. Indices were generated from four months of data, July through October.

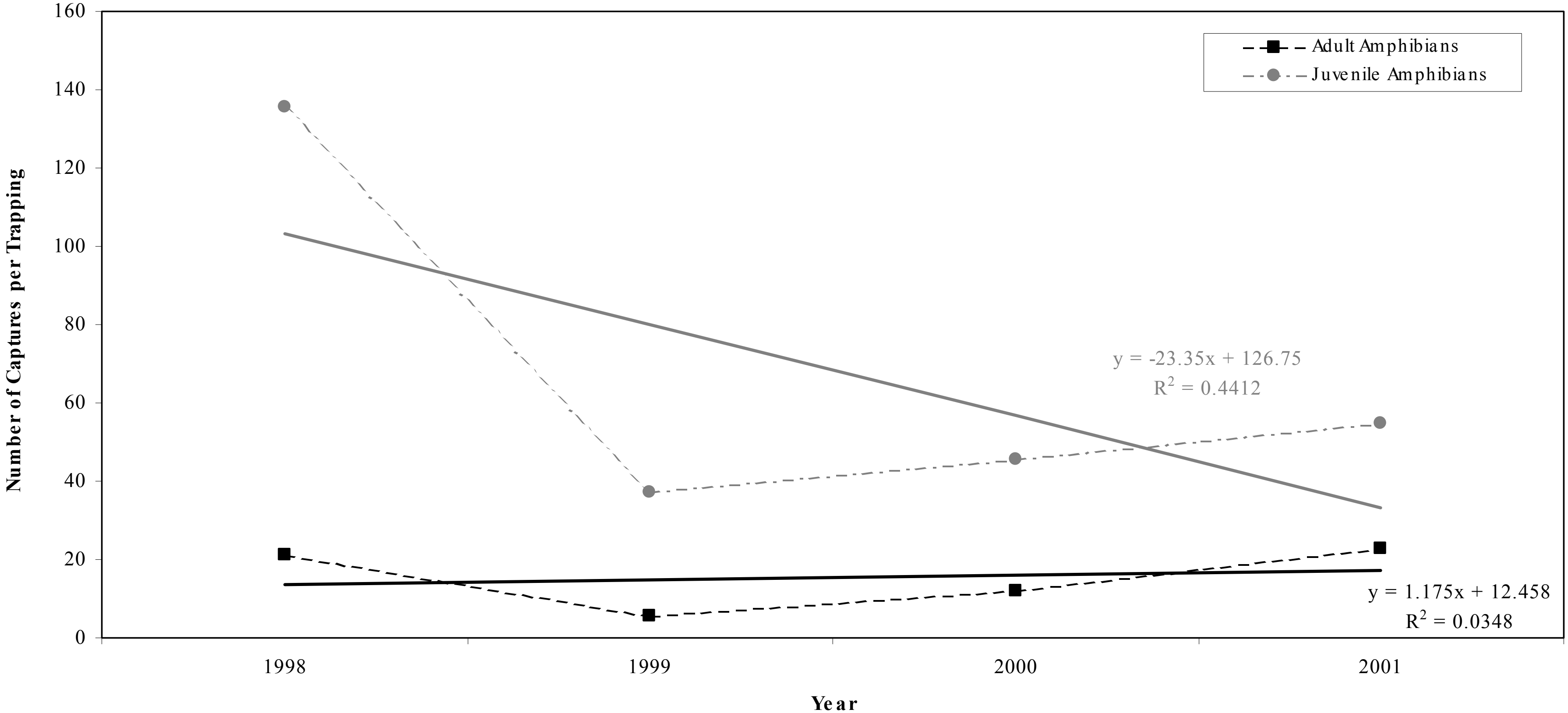


Figure 59. Number of adult and juvenile amphibian captures at the seven Ward Marsh drift-fences, West Haven, Vermont, from 1998-2001. These numbers include captures of Pickerel Frogs *(Rana palustris),* American Toads *(Bufo americanus),* Gray Treefrogs *(Hyla versicolor),* and American Bullfrogs *(Rana catesbeiana),* and adult Spotted Salamanders *(Ambystoma maculatum)* that are not included in Table 7. Indices were generated from four months of data, July through October.

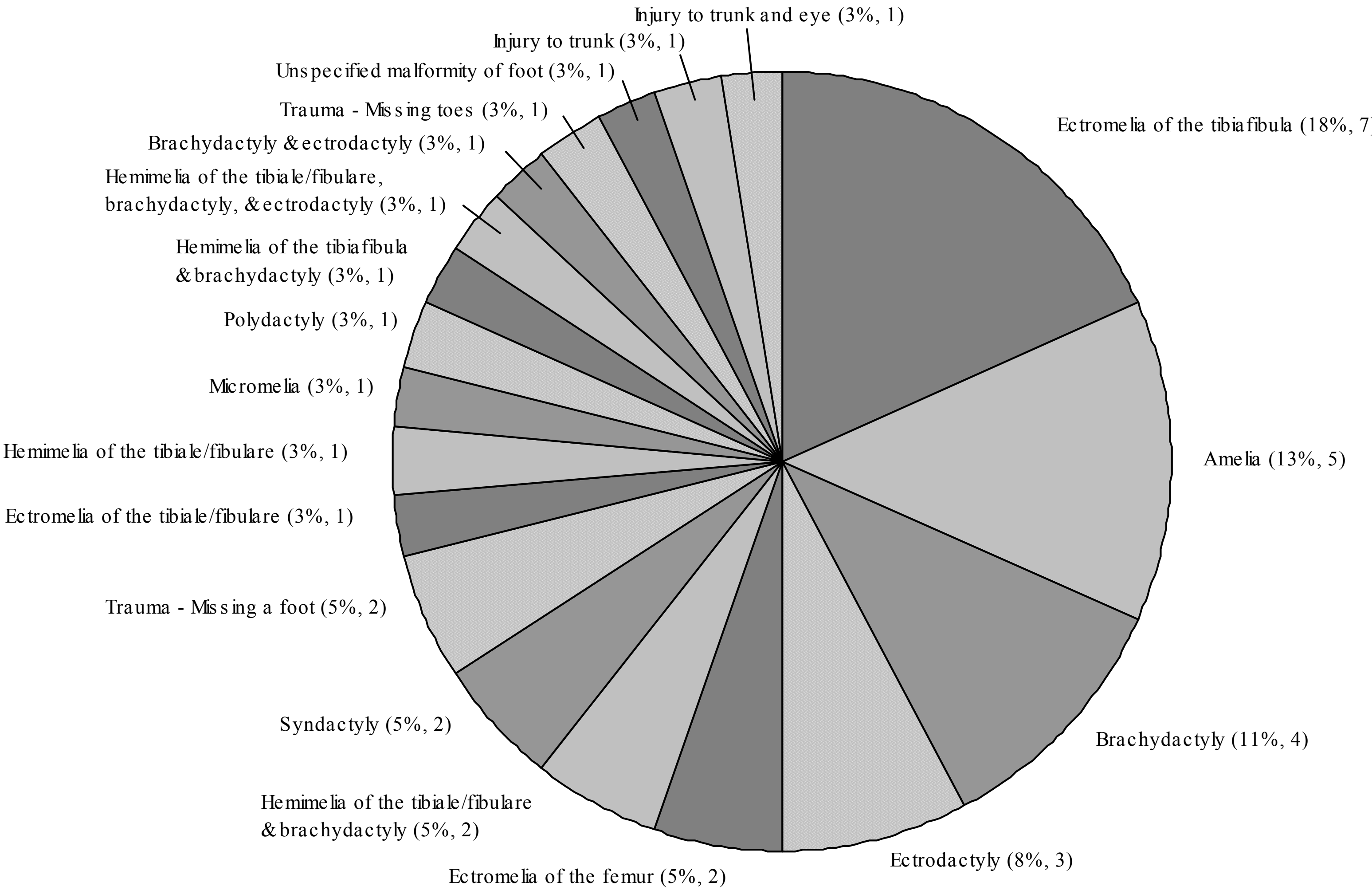


Figure 60. Summary of abnormality results for Northern Leopard Frogs *(Rana pipiens)* at Ward Marsh, West Haven, Vermont, from 1998-2001 of abnormality is followed by the percentage of the total number of abnormalities, and the number of each type of abnormality. Based on the t location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured. Of the abnormalities, three (one each of brachydactyly, syndactyly, and micromelia) were symmetrical. All others were asymmetrical. The total per abnormalities was 0.88% (38 / 4313 captures).

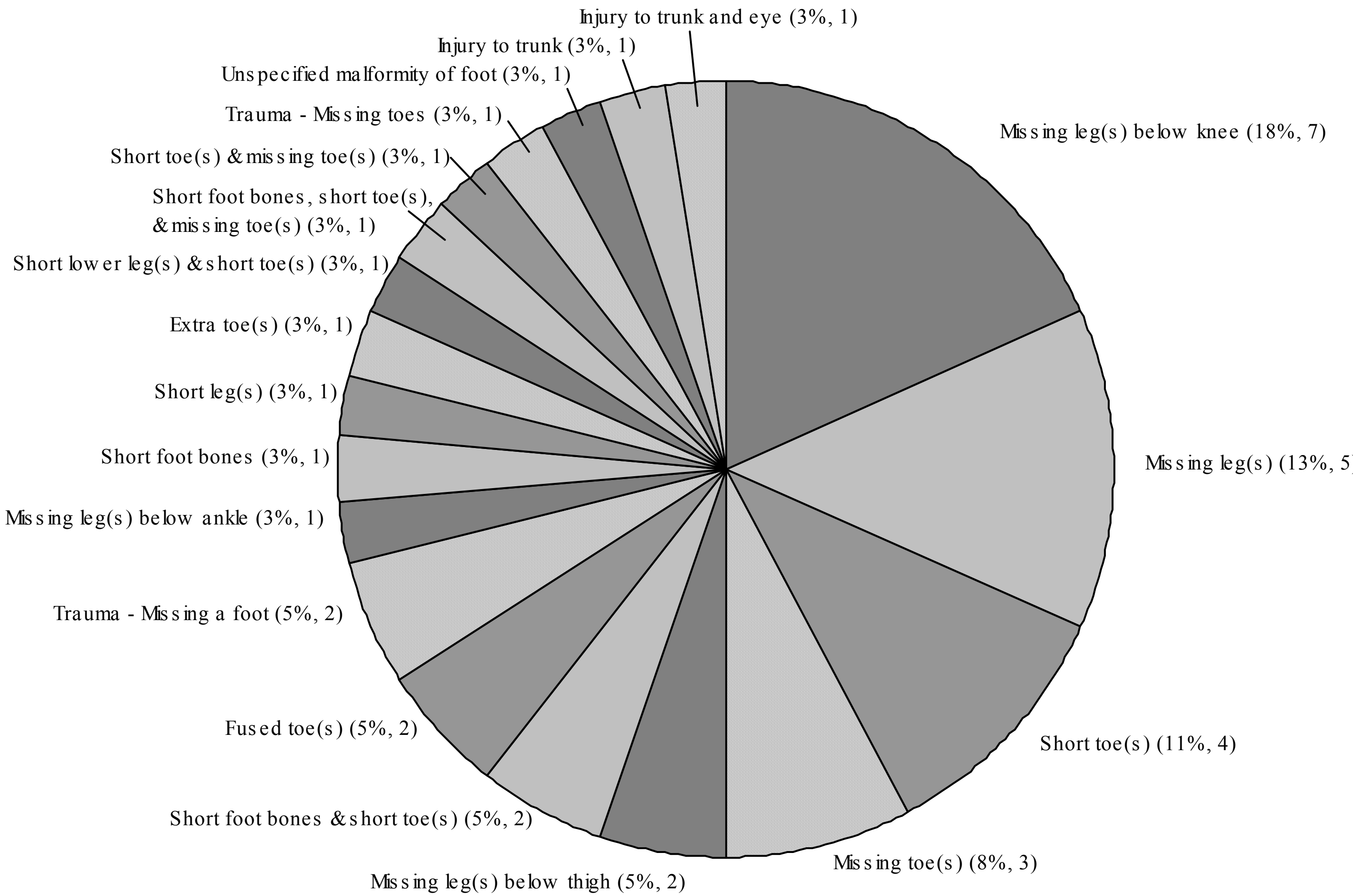


Figure 60a. Summary of abnormality results for Northern Leopard Frogs *(Rana pipiens)* at Ward Marsh, West Haven, Vermont, from 199
of abnormality is followed by the percentage of the total number of abnormalities, and the number of each type of abnormality. Based on
location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured. Of
abnormalities, three [one each of short toe(s), fused toe(s), and short leg(s)] were symmetrical. All others were asymmetrical. The total
abnormalities was 0.88% (38 / 4313 captures).

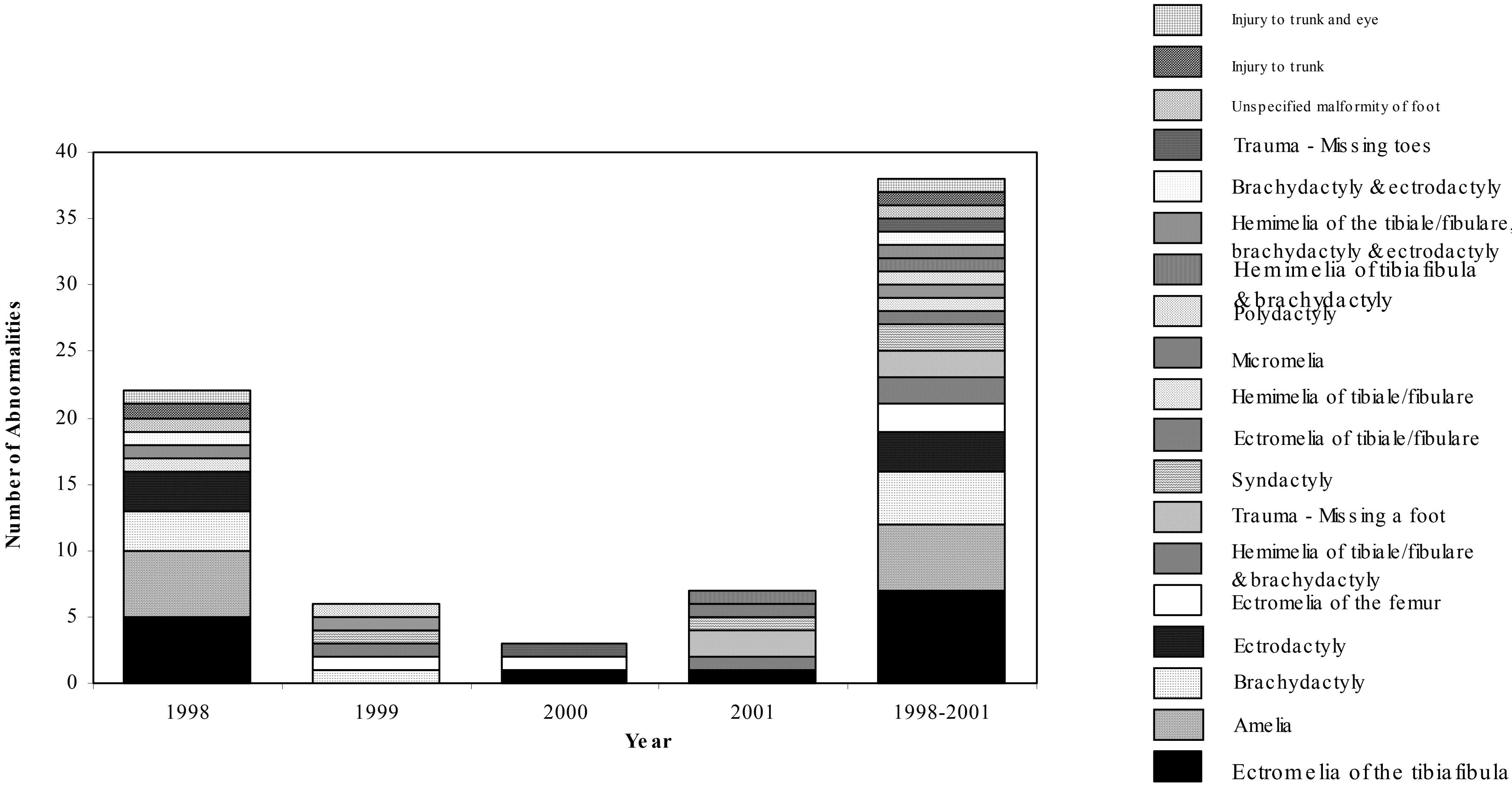


Figure 61. Abnormality results for Northern Leopard Frogs *(Rana pipiens)* at Ward Marsh, West Haven, Vermont from 1998-2001, showing each individual year and results from the combined four years. Based on the type and location of the abnormality, the size of the amphibian, and the location of capture, none of the abnormal amphibians were recaptured. Of the abnormalities, three (one each of brachydactyly, syndactyly, and micromelia) were symmetrical. All others were asymmetrical. The total percentage of abnormalities was 0.88% (38 / 4313 captures).

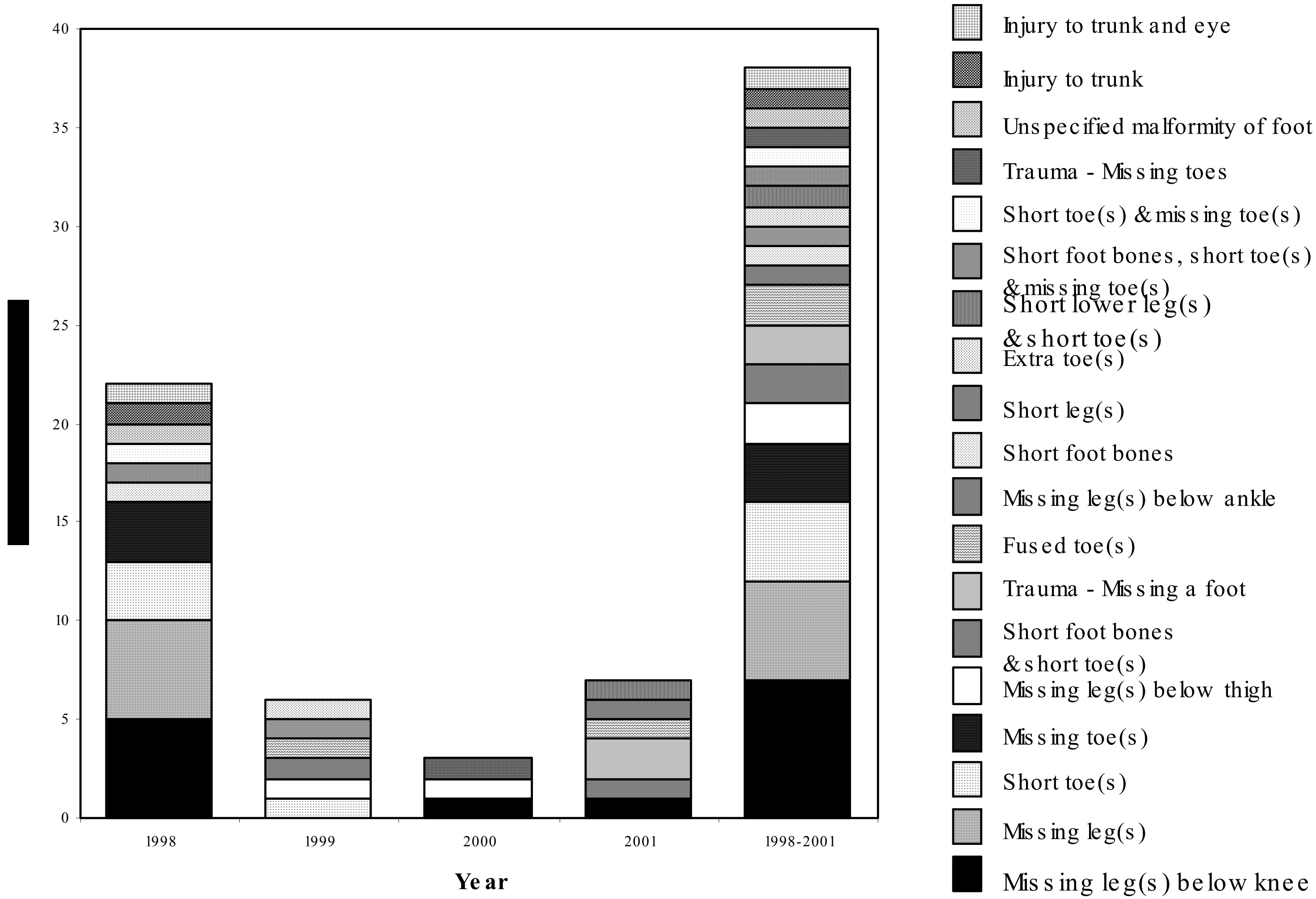


Figure 61a. Abnormality results for Northern Leopard Frogs *(Rana pipiens)* at Ward Marsh, West Haven, Vermont from 1998-20
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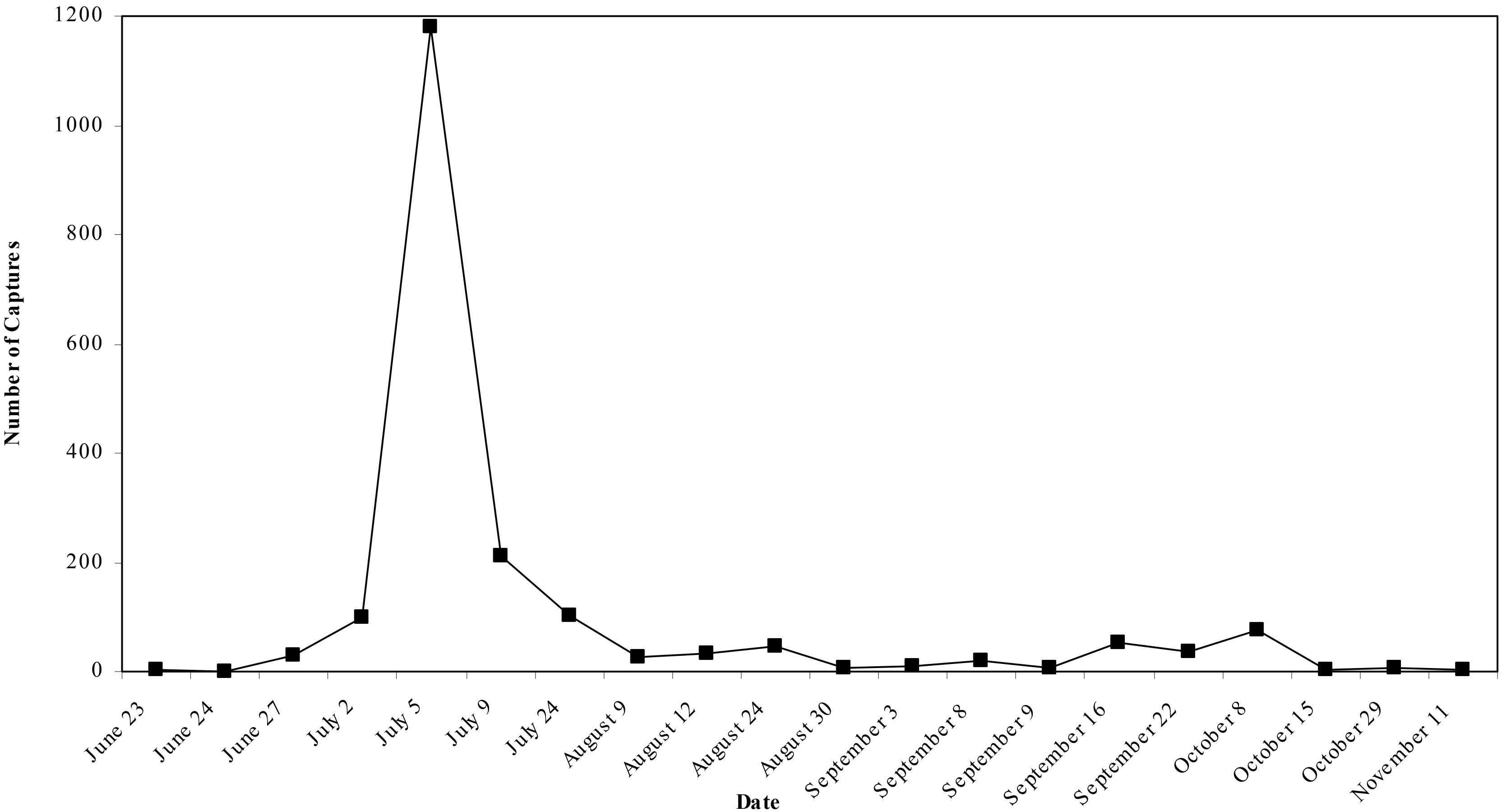


Figure 62. Northern Leopard Frogs *(Rana pipiens)* captured in 1998 at the seven drift-fences at Ward Marsh, West Haven, Vermont. Fences were not all installed until July 2, 1998. The side of the fence an amphibian was caught on was not recorded in 1998. A total of 1952 captures were made over 20 trapping efforts.

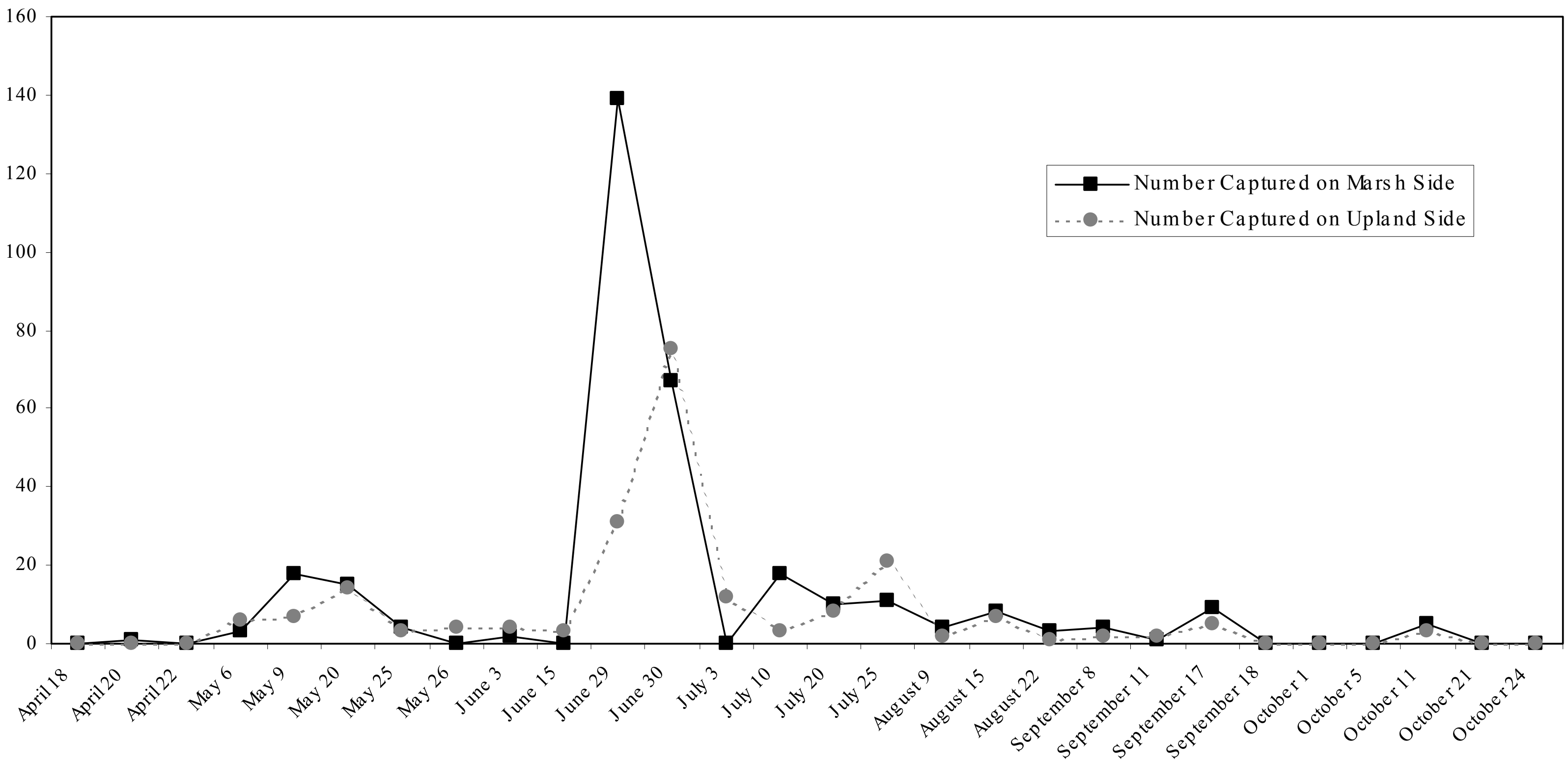


Figure 63. Northern Leopard Frogs *(Rana pipiens)* captured in 1999 at the seven drift-fences at Ward Marsh, West Haven, Vermont. A total of 543 captures were made over 28 trapping efforts.

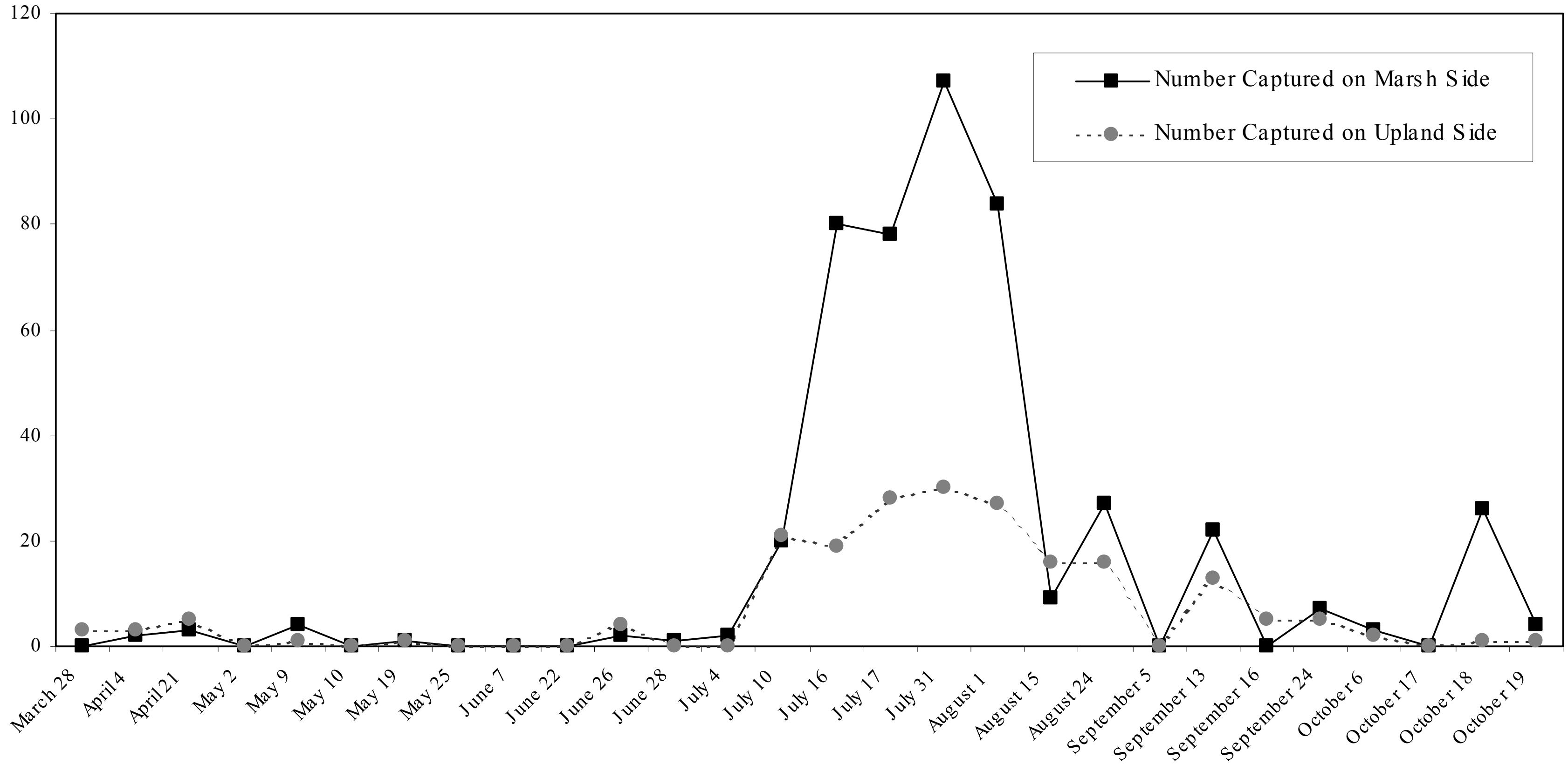


Figure 64. Northern Leopard Frogs *(Rana pipiens)* captured in 2000 at the seven drift-fences at Ward Marsh, West Haven, Vermont. A total of 727 captures were made over 28 trapping efforts.

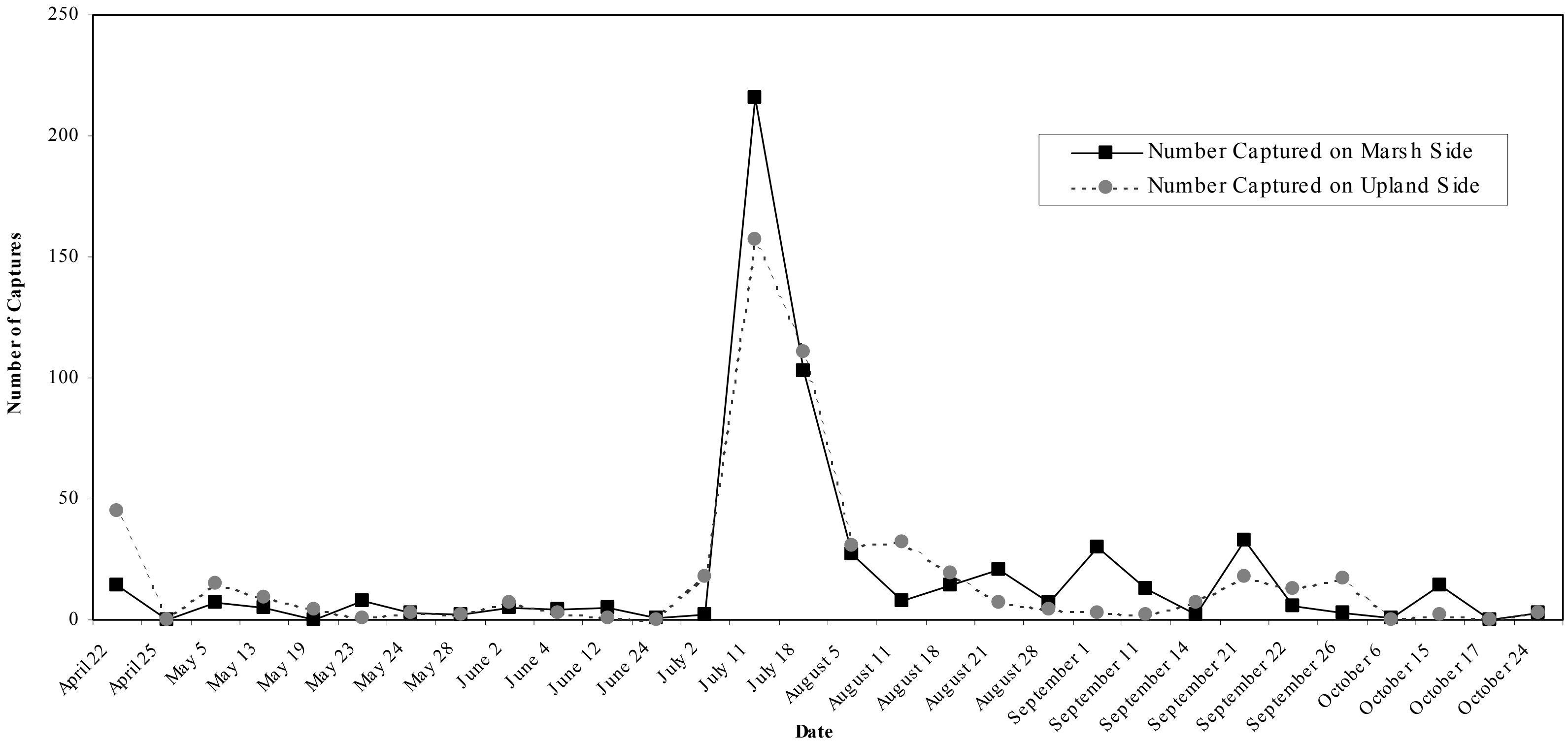


Figure 65. Northern Leopard Frogs *(Rana pipiens)* captured in 2001 at the seven drift-fences at Ward Marsh, West Haven, Vermont. A total of 1091 captures were made over 30 trapping efforts.

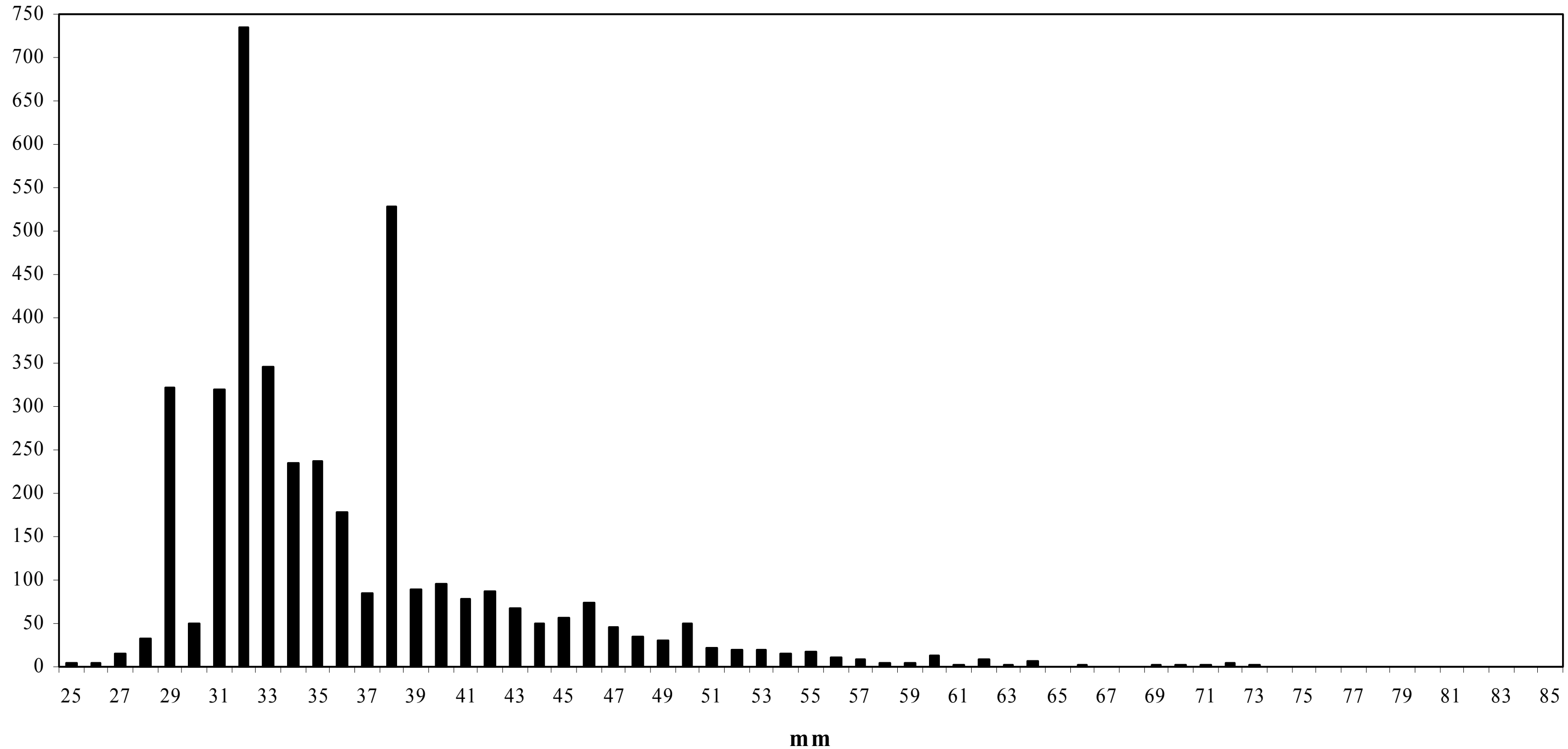


Figure 66. Size of Northern Leopard Frogs *(Rana pipiens)* captured at Ward Marsh, West Haven, Vermont, from June 22 to November 11, 1998-2001.

Limb Bud Assay's

In this report, we describe a study designed to evaluate potential effects on limb development in frogs exposed to site-specific environmental waters and sediments. The study attempted to determine the effects of site-specific environmental water and sediment exposure to larval stage amphibians by evaluating mortality, morphological development (with emphasis on limb development), and teratogenesis (malformations) between the five sites and the three species of frogs. The Gosner Index (Gosner, 1960) was used to standardize developmental staging and facilitate comparisons between test species. The study was divided into two phases, early stage development and limb development. Phase I consisted of short-term (4-10 days) embryo-larval exposure (Frog Embryo Teratogenesis Assay—*Xenopus* [FETAX]) described in ASTM E1439-98 (ASTM, 1998), modified to accommodate ranid species. Phase II comprised a long-term exposure assay (ca. 90 days) designed to observe hind limb and forelimb development during pre-metamorphosis.

The raw data for this study are presented in Appendix E as tables and spreadsheets containing general water chemistry and culture mortality, developmental stage, and malformation data. DO, pH, conductivity, hardness, alkalinity, ammonia-nitrogen, and chlorine levels of the water samples initially received were within acceptable limits to support aquatic life. Data on rates and normalcy of metamorphosis were collected and are presented with the raw data. However, these results are not discussed in this report, since they were outside the scope of this study. Temperature, pH, and DO were routinely monitored throughout the study and were maintained with acceptable ranges.

Laboratory Controls

The negative FETAX solution and positive 6-aminonicotinamide controls met the criteria established in ASTM E1439-98 (ASTM, 1998), for mortality and malformation endpoints for each species evaluated. Mortality and malformation for the FETAX solution control with *X. laevis* were $\leq$ 14.0 % at Gosner stage 26. Mortality and malformation rates for 6-aminonicotinamide with *X. laevis* were 32.0 % and 50.0 %, respectively, for the 5.5 mg/L concentration. The 2,500 mg/L concentration induced complete embryo lethality to *X. laevis*.

Mortality and malformation rates for *R. pipiens* and *R. catesbeiana* FETAX solution controls were both 0 % at Gosner stage 26. The 5.5 mg/L 6-aminonicotinamide control induced 0 % mortality and 20.0 % malformation, while the 2,500 mg/L 6-aminonicotinamide concentrations induced 100 % mortality in *R. pipiens*.

Site Abbreviations

Otter Creek – OTC, Mud Creek – MDC, Ward Marsh – WM or POL
Alburg Dune – ALB, North Hero - NH

Mortality

Mortality rates during early embryo-larval development (Gosner stage 26-28) for each test species are presented in Figure 67. For *X. laevis*, reference samples MDC and NH induced 20.0 and 12.0 % mortality, respectively. Test site samples ALB, POL, and OTC induced 32.0, 14.0, and 22.0 % mortality, respectively. For *R. pipiens*, reference samples MDC and NH induced 4.0 and 18.0 % mortality, respectively. Test site samples ALB, POL, and OTC induced 16.0, 20.0, and 10.0 % mortality, respectively. No mortality in *R. catesbeiana* cultured in the MDC and NH reference site waters and sediments was observed.

Mortality rates during limb development (Gosner stage 30-42) are also presented in Figure 67. For *X. laevis*, both reference samples MDC and NH induced 26.0 % mortality. Test site samples ALB, POL, and OTC induced 36.0, 18.0, and 54.0 % mortality, respectively. For *R. pipiens*, reference samples MDC and NH induced 54.0 and 62.0 % mortality, respectively. Test site samples ALB, POL, and OTC induced 18.0, 36.0, and 42.0 % mortality, respectively. *R. catesbeiana* had mortality rates of 5.0 and 10.0 %, respectively, for MDC and NH reference sites.

Developmental Stages

For the present study, stages 8-25 were considered the early embryo-larval developmental phase. The limb development phase, marking the beginning of hind limb development, started at stage 26 and continued to approximately stage 40. Forelimbs appeared at stage 42. The rates of development of the three test species for each water/sediment combination are compared in Figures 68-73. A comparison of stage of development between the five water/sediment samples for each test species is presented in Figures 74-76. In this study, R. pipiens developed at a faster rate and reached a greater developmental stage than *X. laevis*, with the fastest rates occurring in MDC reference and NH water/sediment, followed by POL, OTC, and ALB. The rate of development for *R. catesbeiana* was slower in all water/sediment samples tested (MDC and NH) and never advanced past stage 33.

Malformation

No malformation in any of the species exposed to the MDC reference samples was detected at either the early embryo-larval stage or the limb development stage (Figure 77). The effect of the samples on early embryo-larval malformation is presented in Figures 78-80. The NH samples induced 4 % malformation in *X. laevis* and no malformation in R. pipiens or *R. catesbeiana* during the early embryo-larval development phase. The NH samples induced 6.1 %, 5.3 %, and 0 % in *X. laevis*, *R. pipiens*, and *R. catesbeiana*, respectively, during the limb development phase. Samples from ALB, POL, and OTC induced malformation rates of 46.0, 37.8, and 26.8

% in *X. laevis*, respectively, at the early embryo-larval development stage. ALB, POL, and OTC samples caused malformation in 42.0, 32.0, and 18.8 % of R. pipiens tested during early embryo-larval development. At the limb development stage, ALB, POL, and OTC samples induced malformation in 54.6, 43.2, and 47.6 % of the *X. laevis*, and 35.7, 43.8, and 26.7 %, of the *R. pipiens* exposed, respectively.

The malformation syndromes observed in each water/sediment sample for the three amphibian species tested by developmental phase are presented in Figures 81-88. Common malformations included mal-development of the mouth, tail, forelimb, hind limb, face, eye, brain, fin, notochord, and gut. Hemorrhaging and edema were also noted, but at a lesser rate. Overall, the malformation syndromes induced by each test site sample were reasonably consistent between *X. laevis* and *R. pipiens*.

Conclusion

Results from the present study indicated that samples from ALB, POL, and OTC were capable of inducing early embryo-larval mal-development, as well as malformation at limb development stages in *X. laevis* and *R. pipiens*. Samples from NH were weakly capable of inducing abnormal development and samples from MDC (reference site) did not induce malformation at either developmental stage. In terms of species comparison, *R. pipiens* developed more quickly than *X. laevis*, which showed signs of developmental delay. In terms of mortality and malformation endpoints, both species were reasonably consistent. Overall, *X. laevis* appeared to be slightly more sensitive to abnormal development induced by exposure to the test site samples than *R. pipiens*. Both hind limb and forelimb malformation were observed in each of the test site samples ALB, POL, and OTC. A few cases of limb malformation were observed in R. pipiens, but not *X. laevis*, exposed to NH samples. A slightly greater incidence of limb malformation was observed in R. pipiens compared to *X. laevis*. However, the overall malformation syndromes induced by the developmentally toxic samples (ALB, POL, and OTC) were consistent between species. Additional work will be required to determine if a particular species is more susceptible to specific malformations caused by other stressors, and thus, show a predisposition to certain anomalies. Increased rates of abnormal development in *X. laevis* compared to *R. pipiens* may be the result of increased exposure to developmental stressors during critical periods of development due to slower development at critical points.

More work will be required to further evaluate this potential relationship. Results with *R. catesbeiana* were not surprising, since the bullfrog has been found to be a tolerant species compared to both *X. laevis* and *R. pipiens*.

FIGURE 67
VERM01-00004
VERMONT EPA RARE PROJECT
MORTALITY COMPARISON BY SPECIES AND STAGE

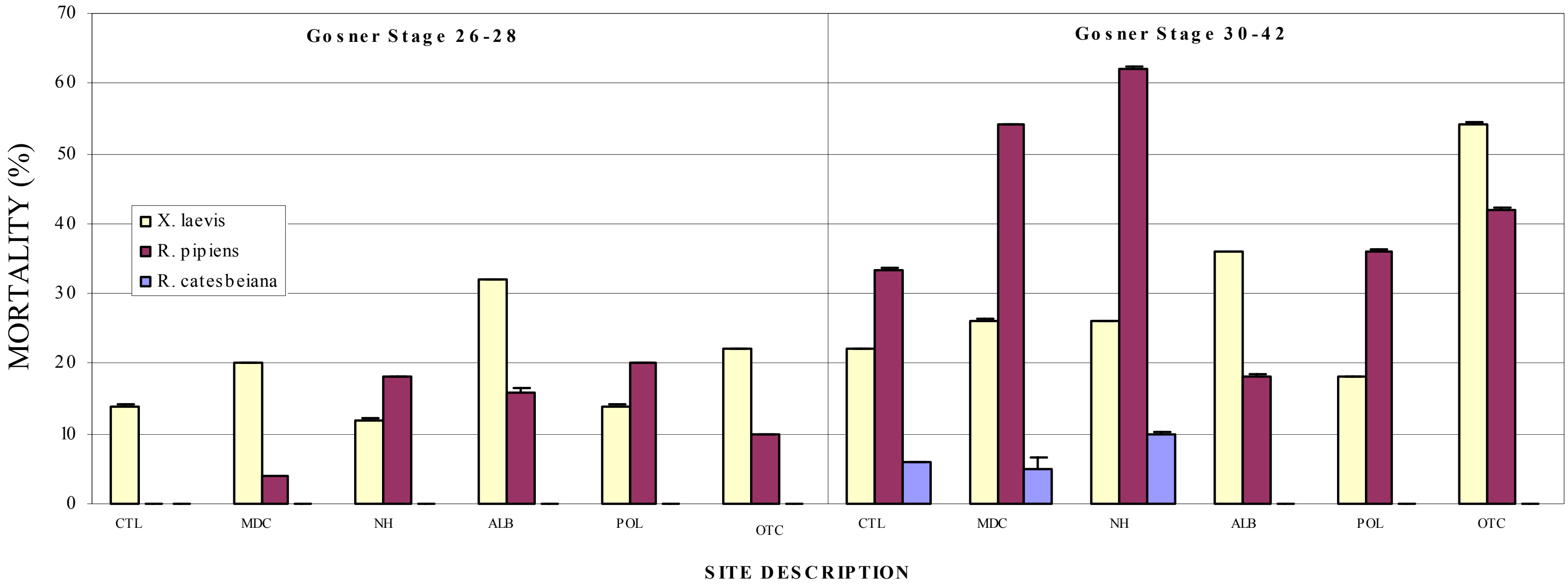


FIGURE 68
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
LABORATORY WATER/SAND CONTROL

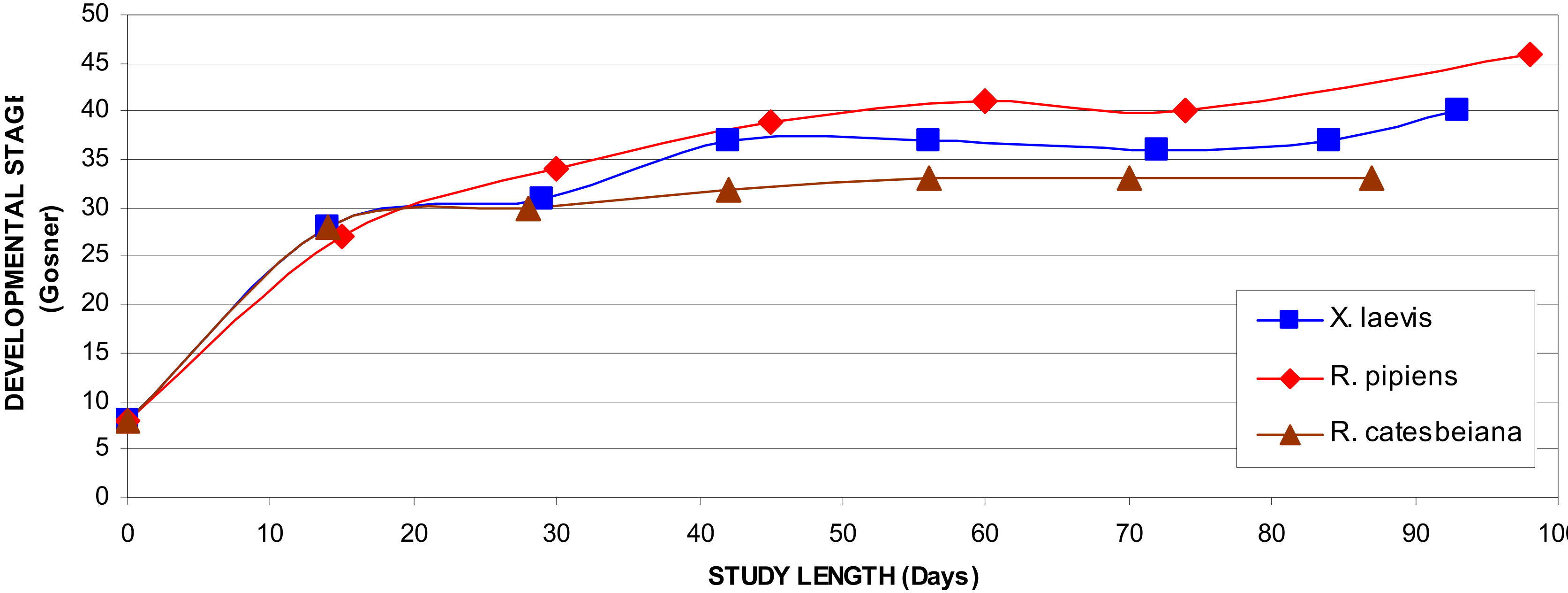
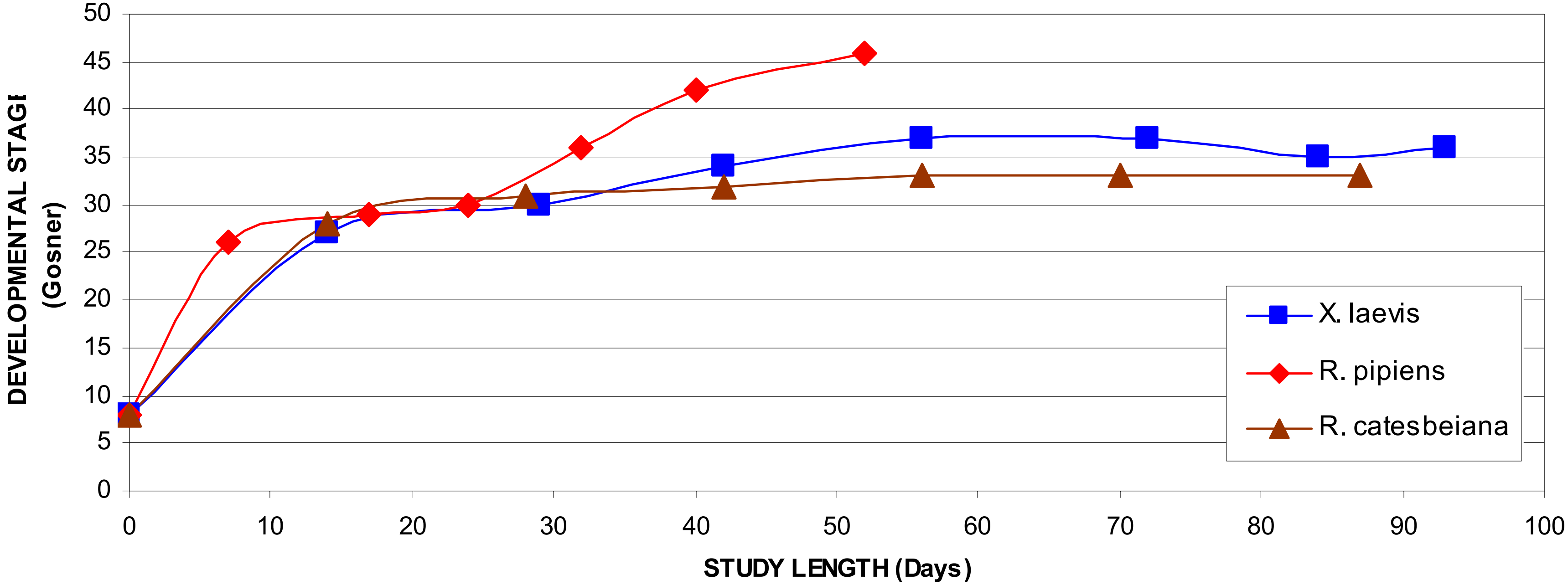


FIGURE 69
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
MDC REFERENCE SITE WATER/SEDIMENT



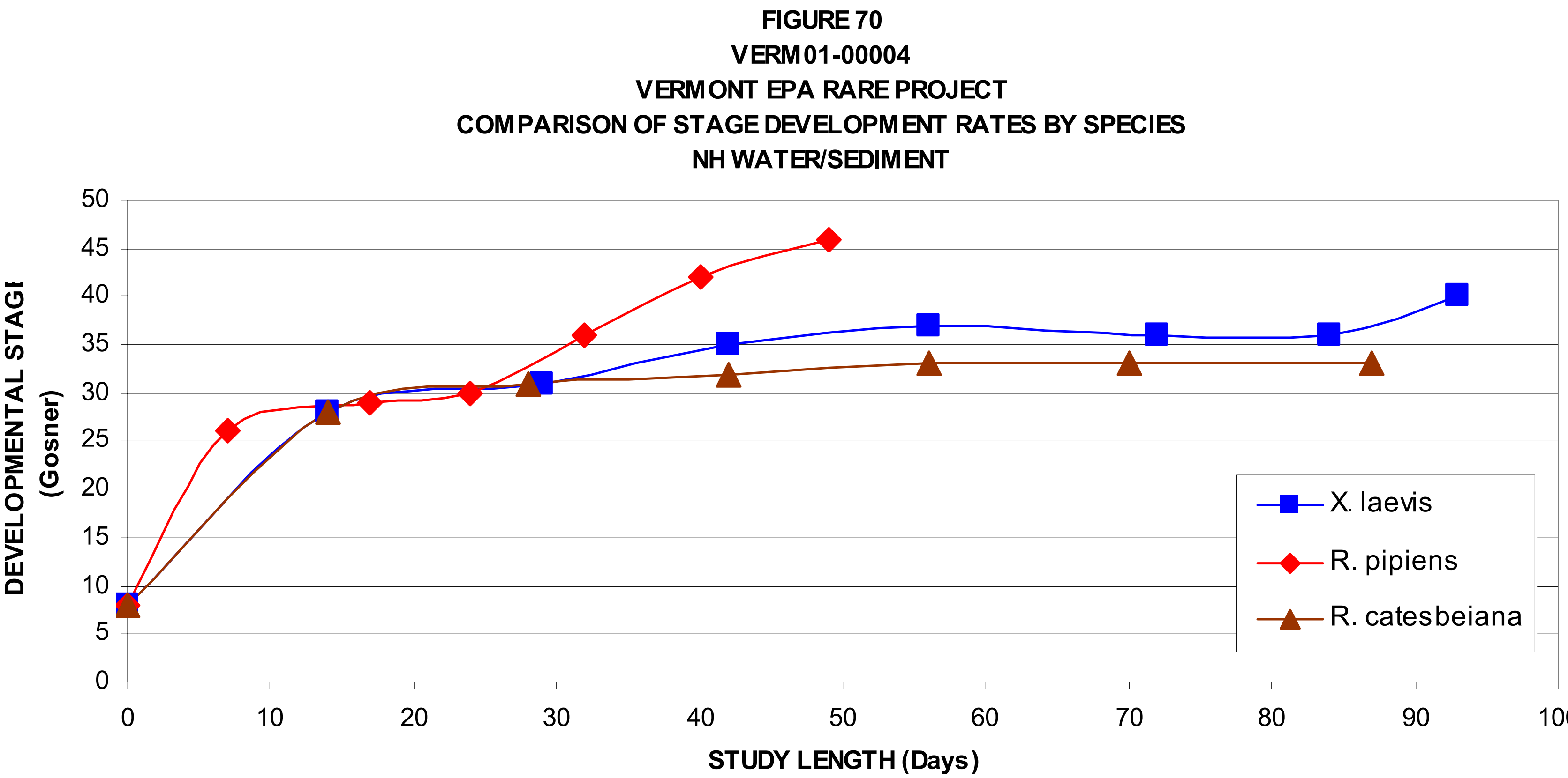


FIGURE 70
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
NH WATER/SEDIMENT
DEVELOPMENTAL STAGE (Gosner)
STUDY LENGTH (Days)
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100
X. laevis
R. pipiens
R. catesbeiana

FIGURE 71
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
ALB WATER/SEDIMENT

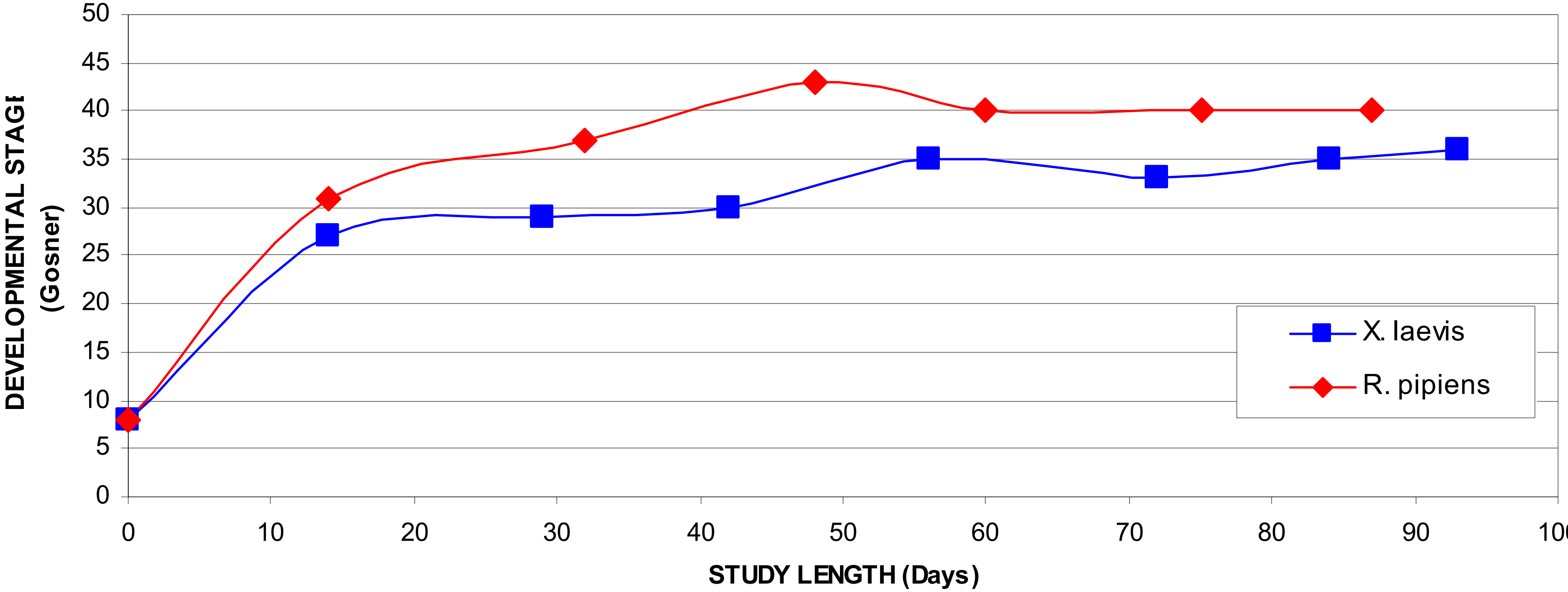


FIGURE 72
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
POL WATER/SEDIMENT

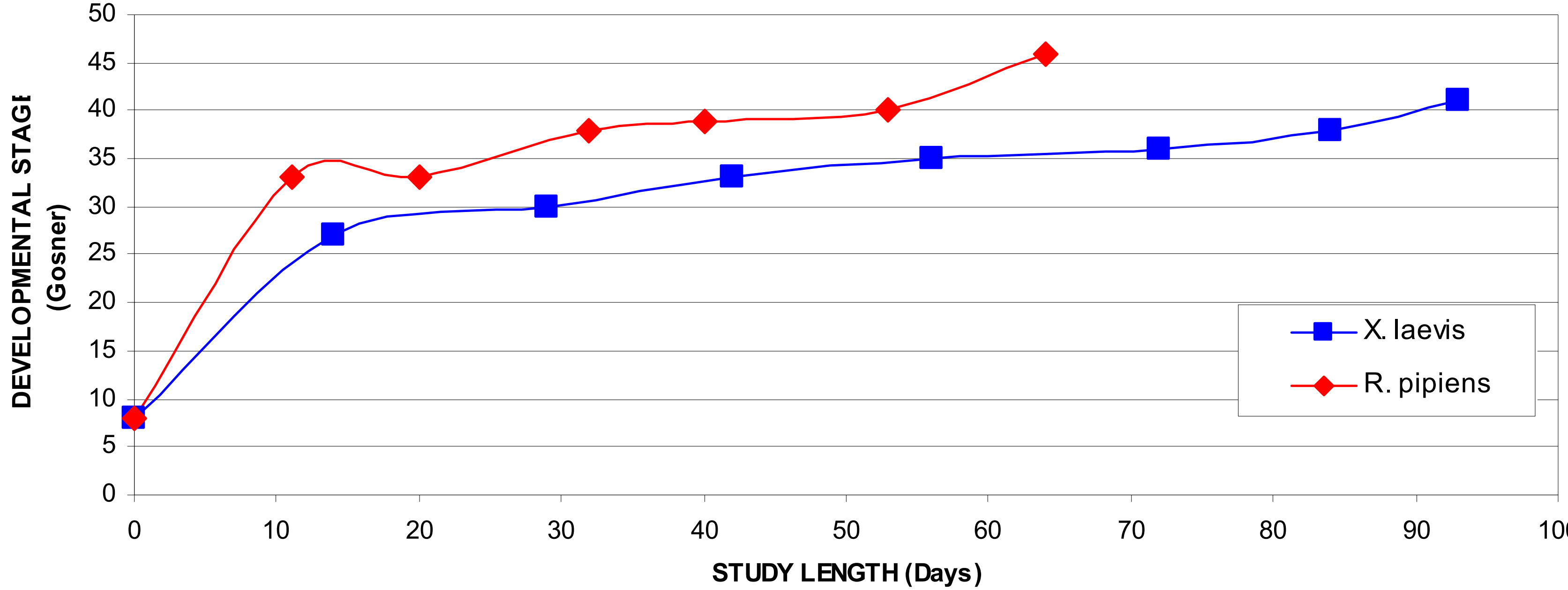


FIGURE 73
VERM01-00004
VERMONT EPA RARE PROJECT
COMPARISON OF STAGE DEVELOPMENT RATES BY SPECIES
OTC WATER/SEDIMENT

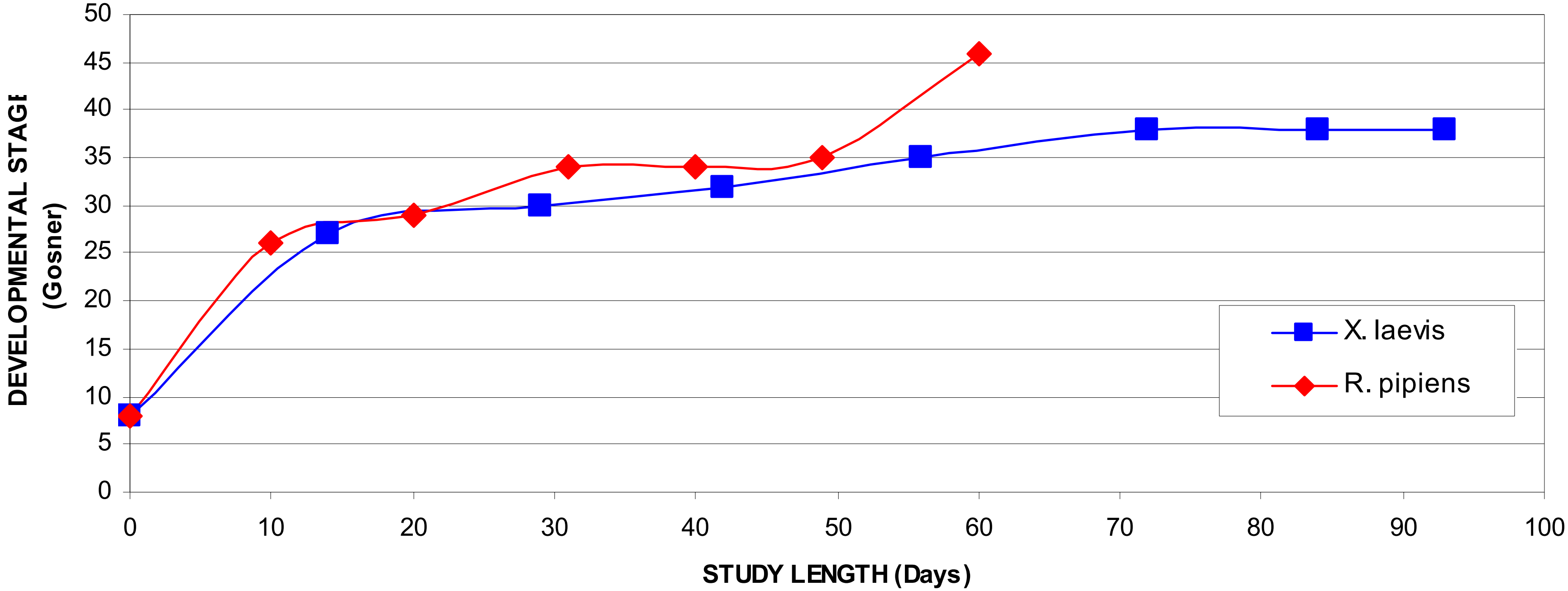


FIGURE 74
VERM01-00004
VERMONT EPA RARE PROJECT
Xenopus laevis STAGE DEVELOPMENT RATE

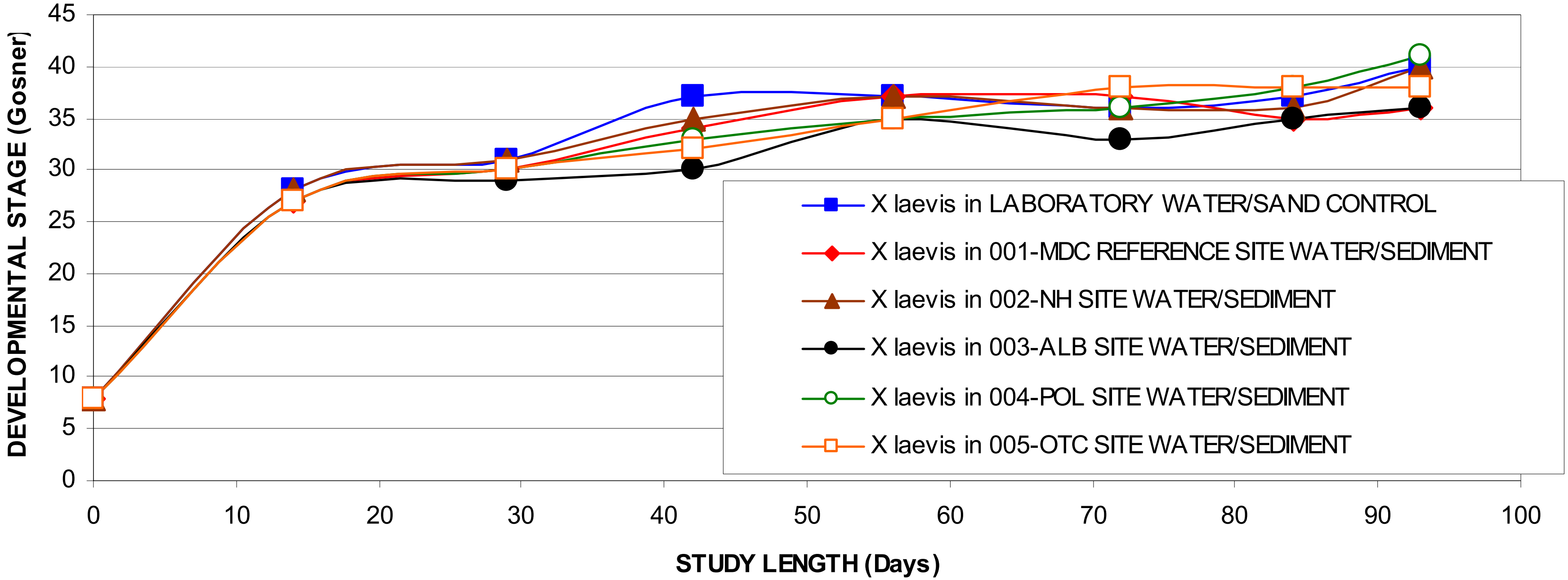


FIGURE 75
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* STAGE DEVELOPMENT RATE**

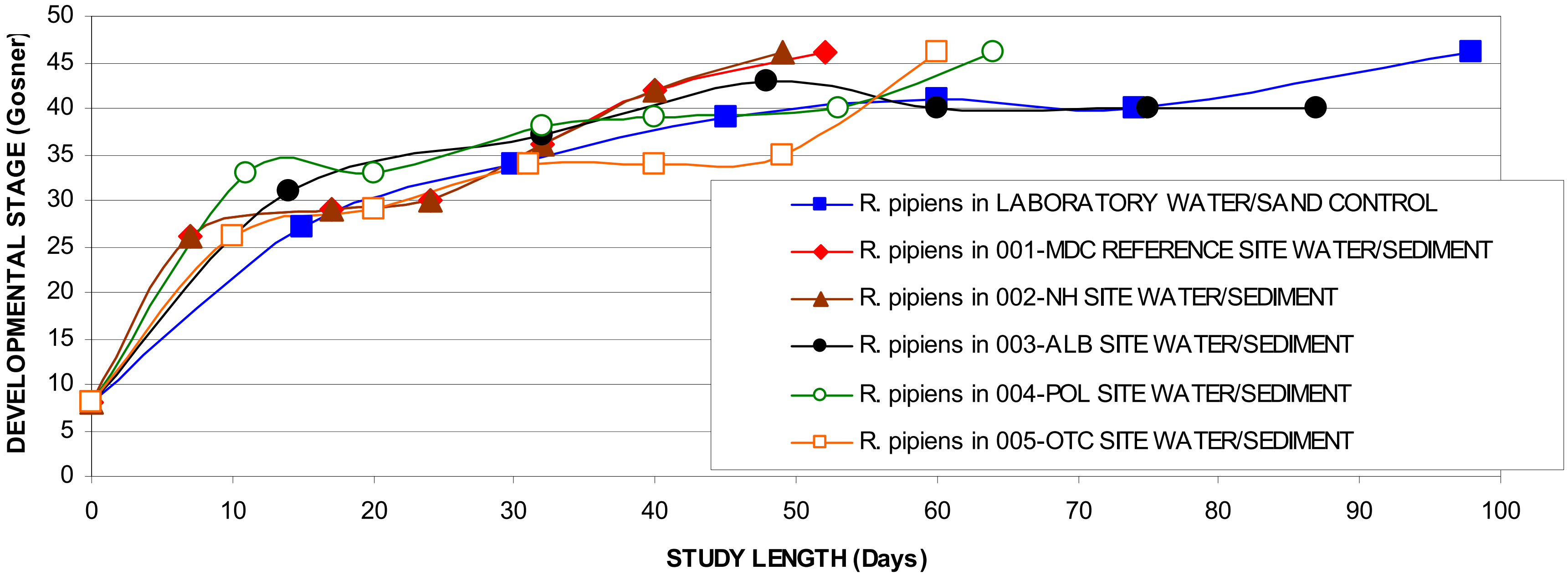


FIGURE 76
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana catesbeiana* STAGE DEVELOPMENT RATE**

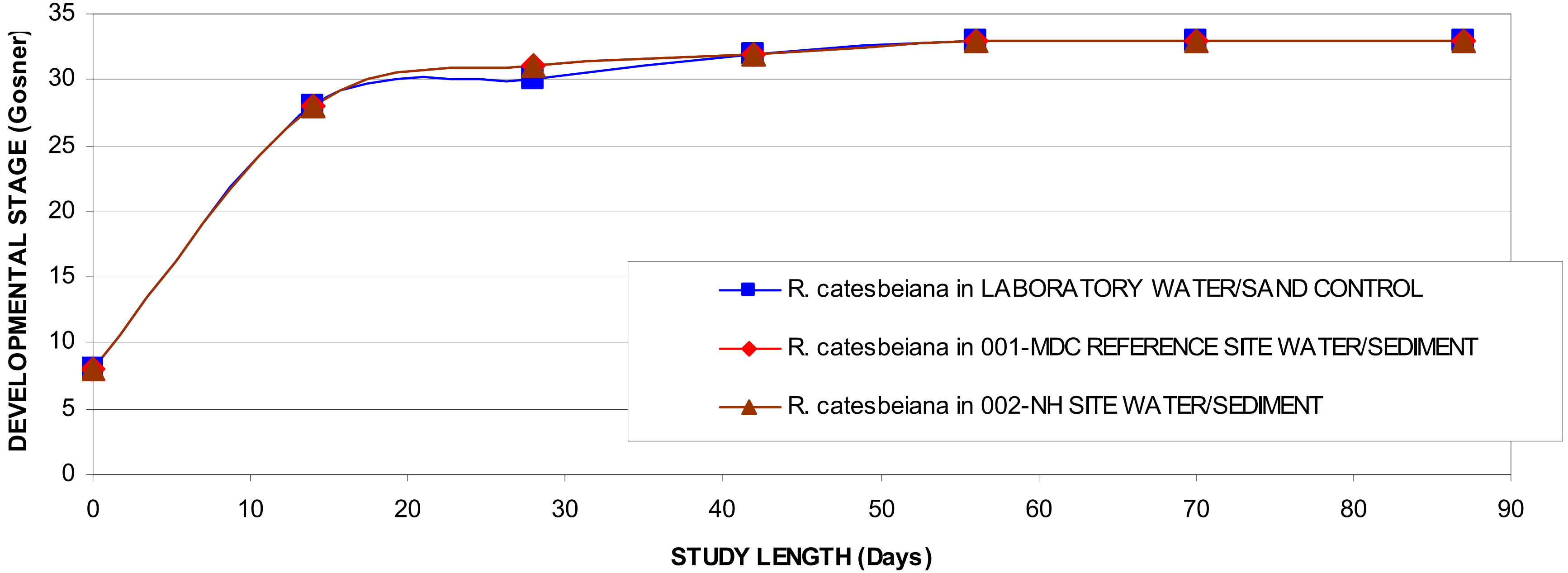


FIGURE 77
VERM01-00004
VERMONT EPA RARE PROJECT
MALFORMATION COMPARISON BY SPECIES AND STAGE

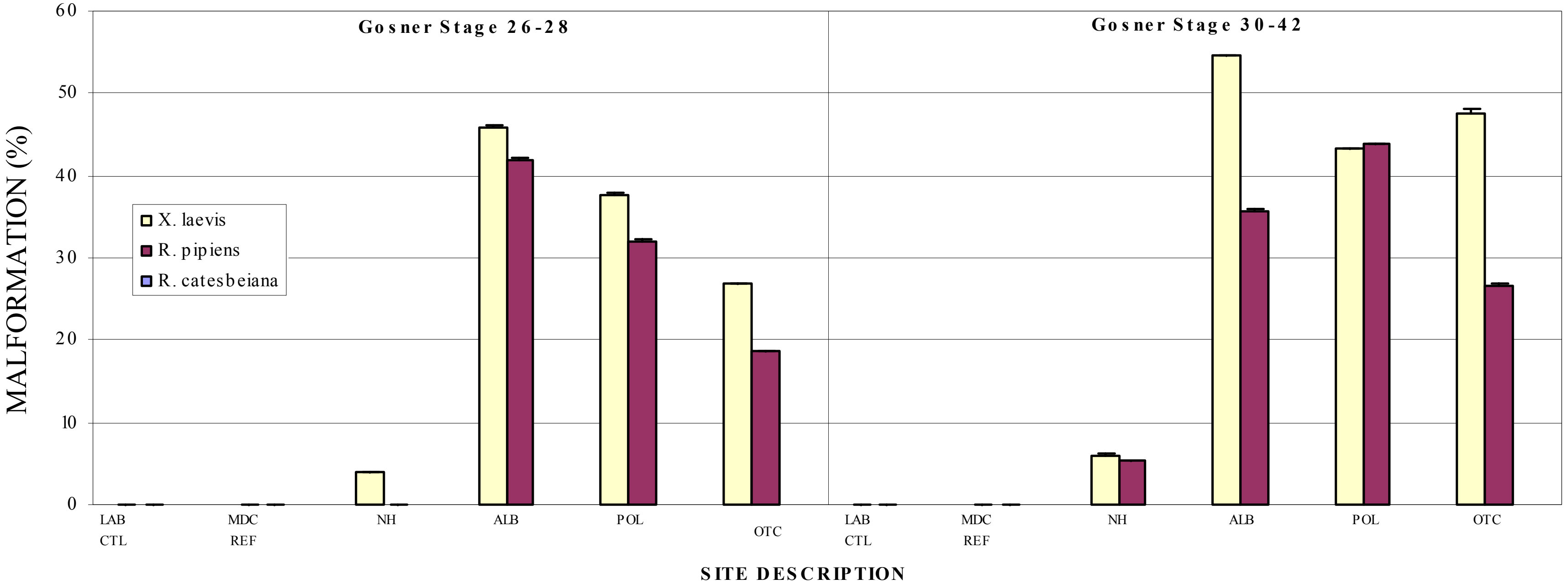


FIGURE 78
VERM01-00004
VERMONT EPA RARE PROJECT
***Xenopus laevis* TOTAL MALFORMATIONS BY SITE**

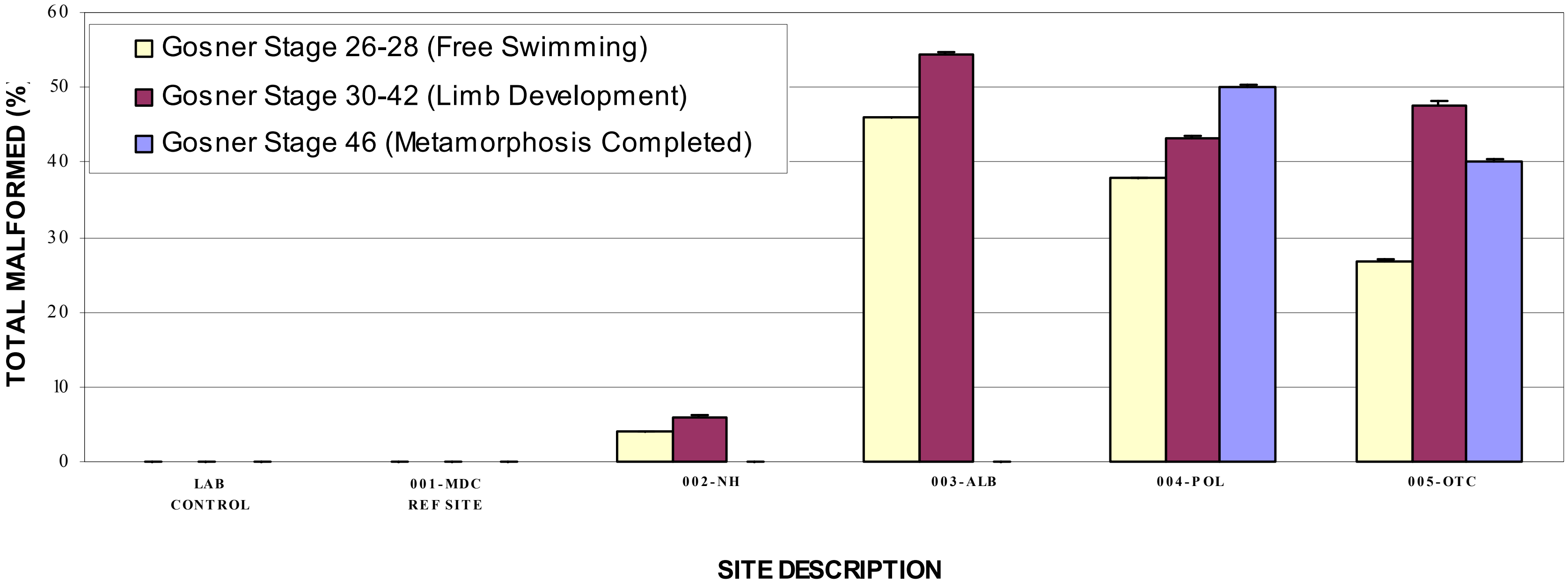


FIGURE 79
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* TOTAL MALFORMATIONS BY SITE**

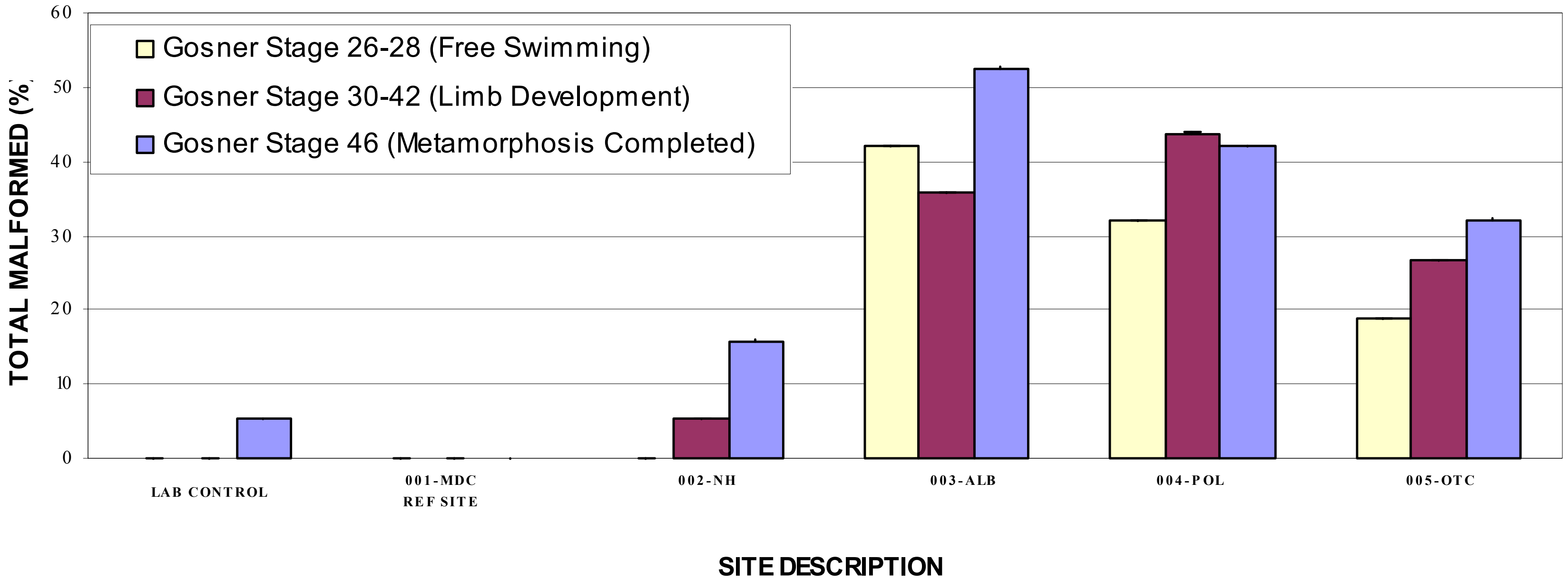


FIGURE 80
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana catesbeiana* TOTAL MALFORMATIONS BY SITE**

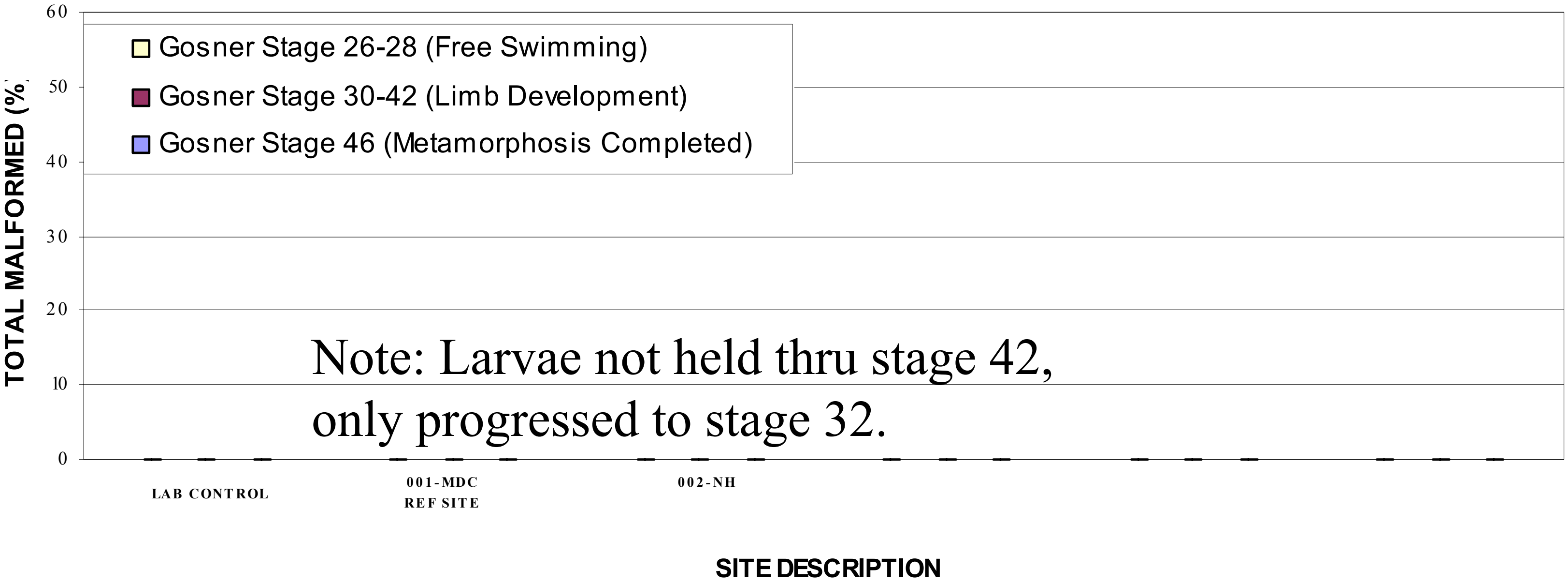


FIGURE 81
VERM01-00004
VERMONT EPA RARE PROJECT
***Xenopus laevis* CUMULATIVE MALFORMATION BY TYPE**
SITE NH

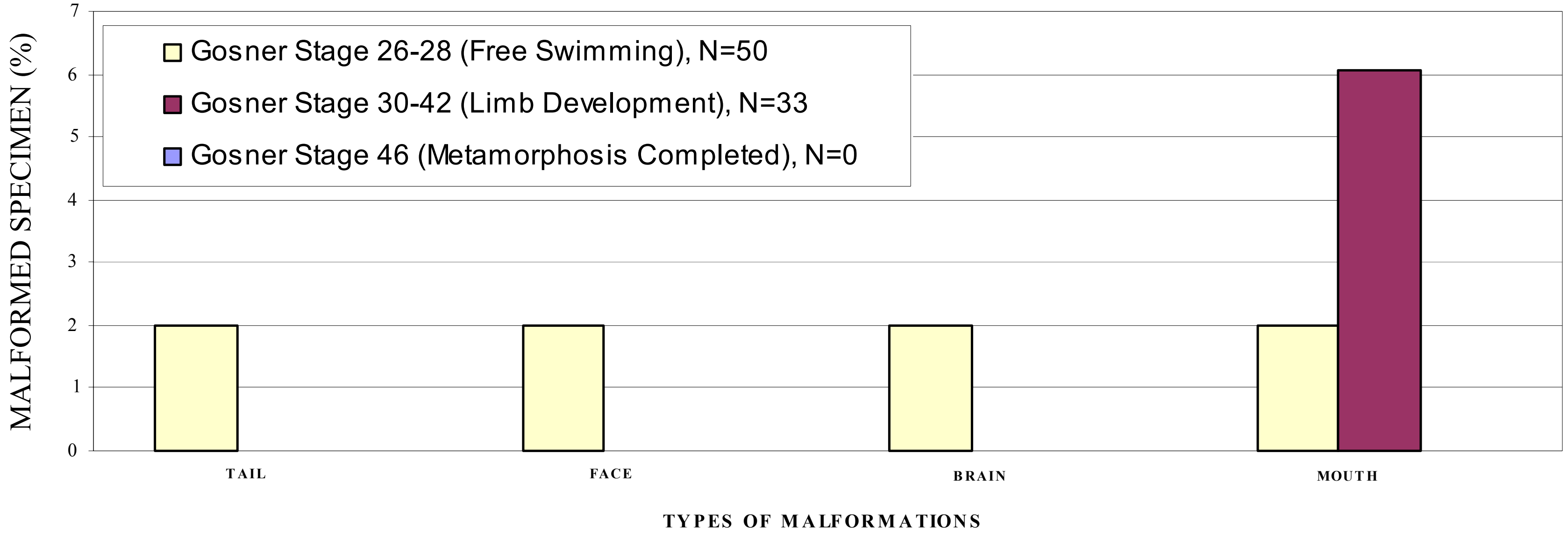


FIGURE 82
VERM01-00004
VERMONT EPA RARE PROJECT
***Xenopus laevis* CUMULATIVE MALFORMATION BY TYPE**
SITE ALB

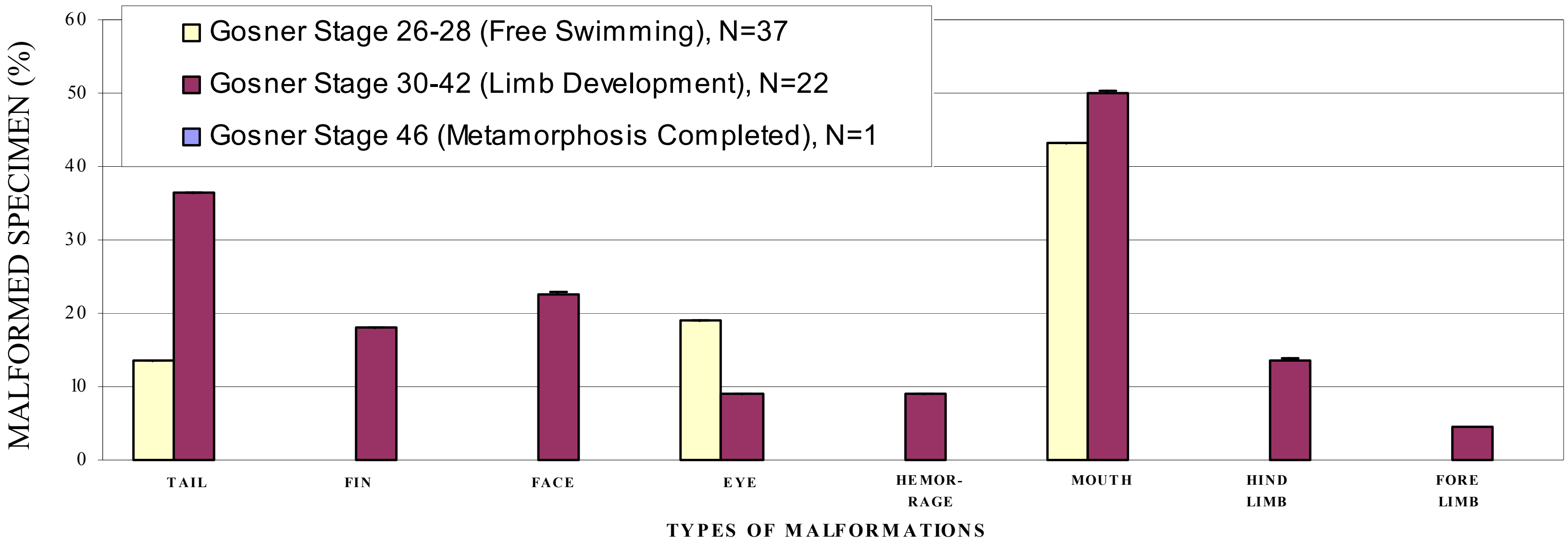


FIGURE 83
VERM01-00004
VERMONT EPA RARE PROJECT
***Xenopus laevis* CUMULATIVE MALFORMATION BY TYPE**
SITE POL

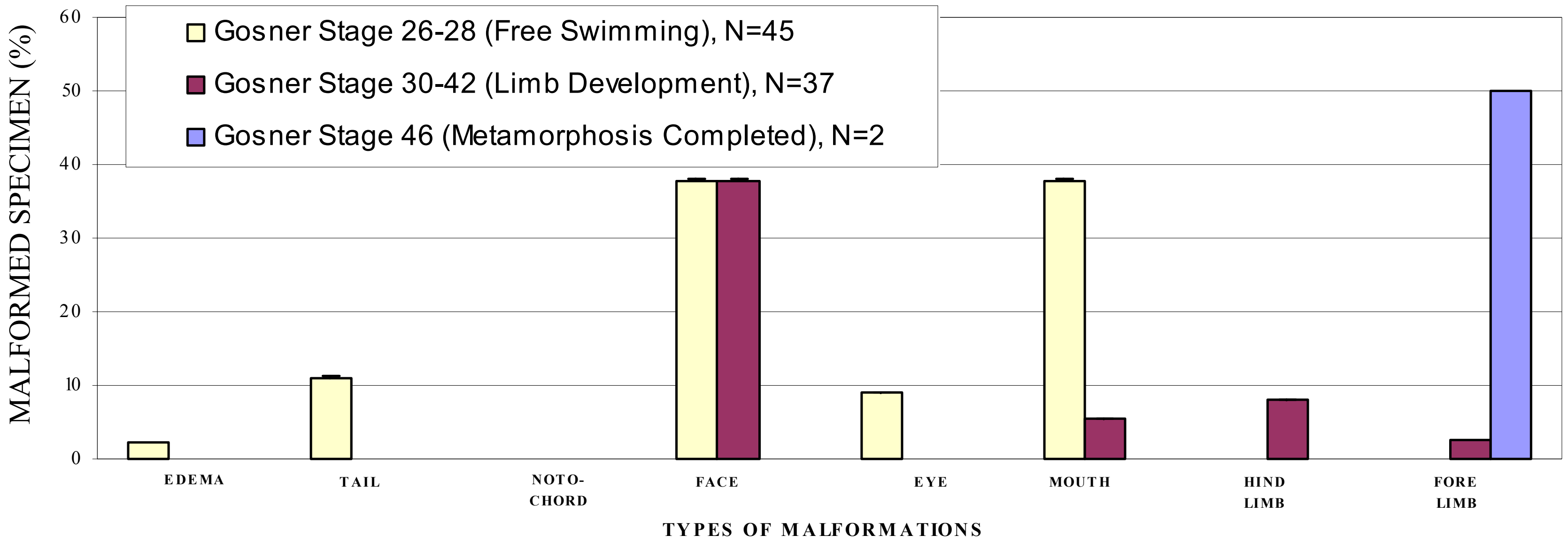


FIGURE 84
VERM01-00004
VERMONT EPA RARE PROJECT
***Xenopus laevis* CUMULATIVE MALFORMATION BY TYPE**
SITE OTC

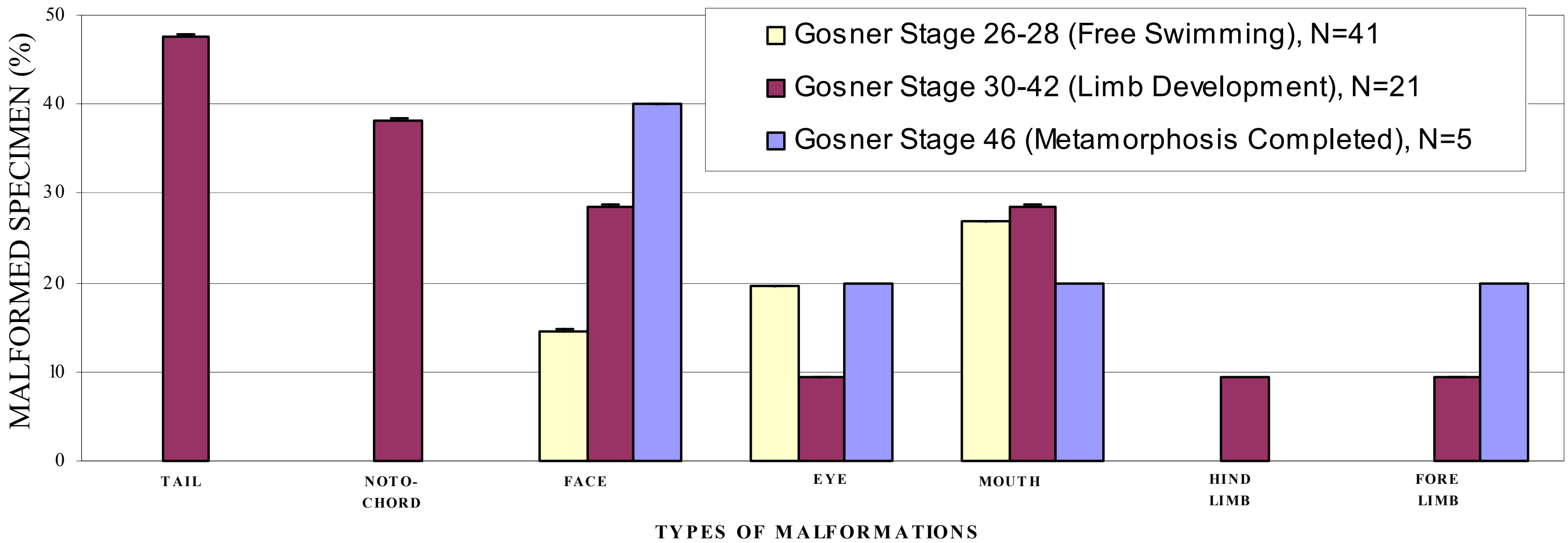


FIGURE 85
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* CUMULATIVE MALFORMATION BY TYPE**
SITE NH

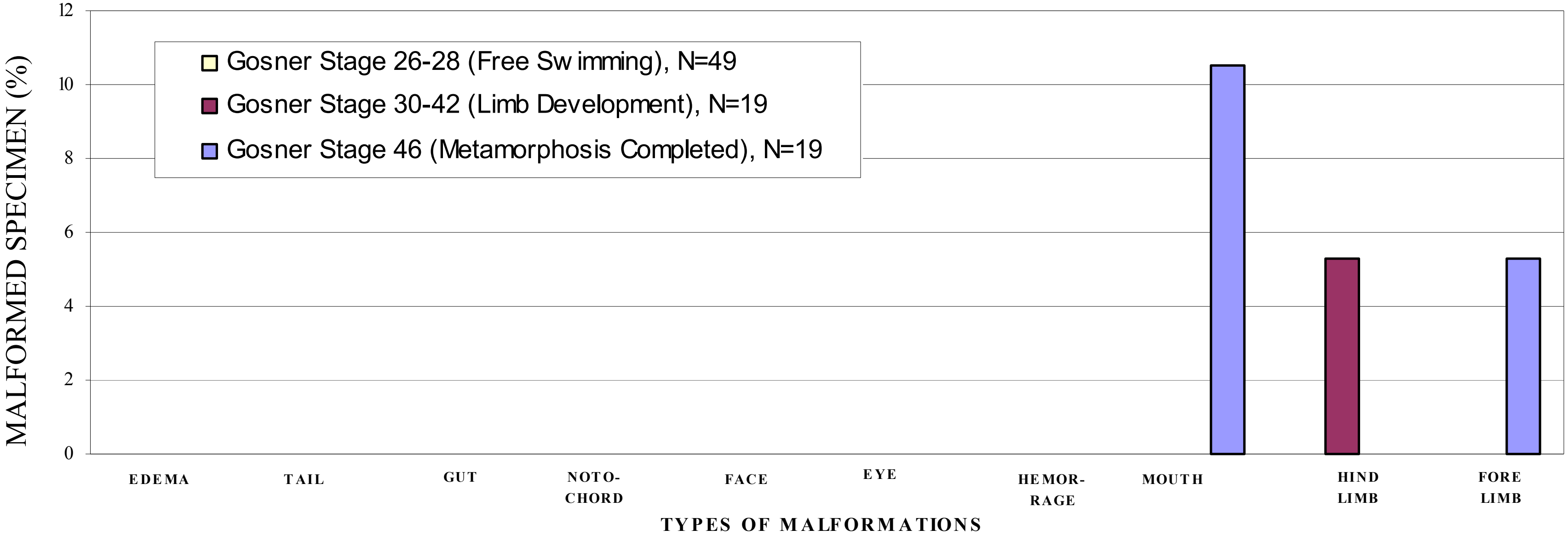


FIGURE 86
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* CUMULATIVE MALFORMATION BY TYPE**
SITE ALB

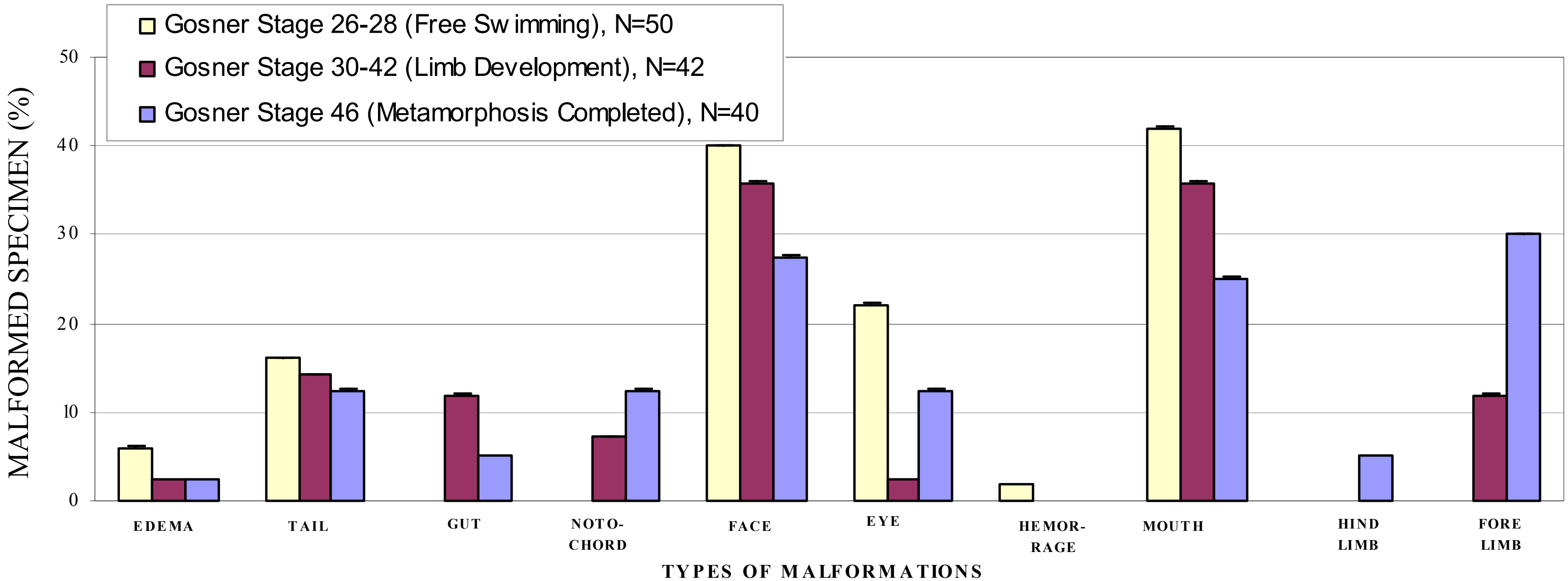


FIGURE 87
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* CUMULATIVE MALFORMATION BY TYPE**
SITE POL

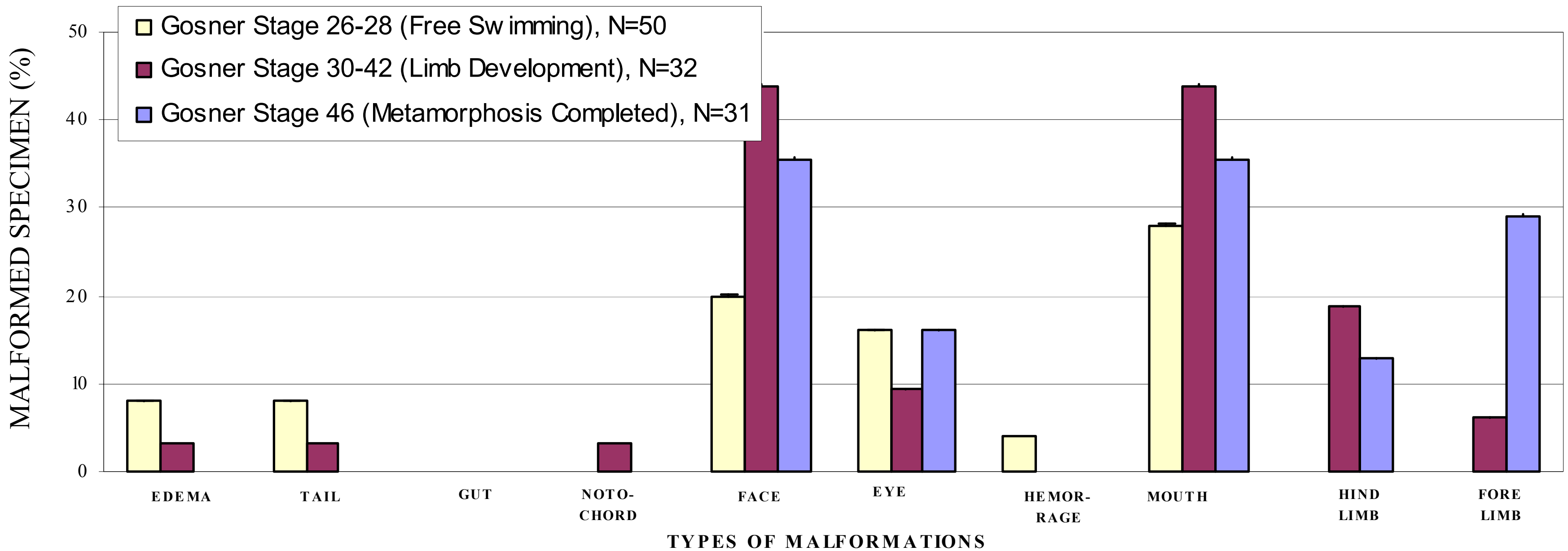
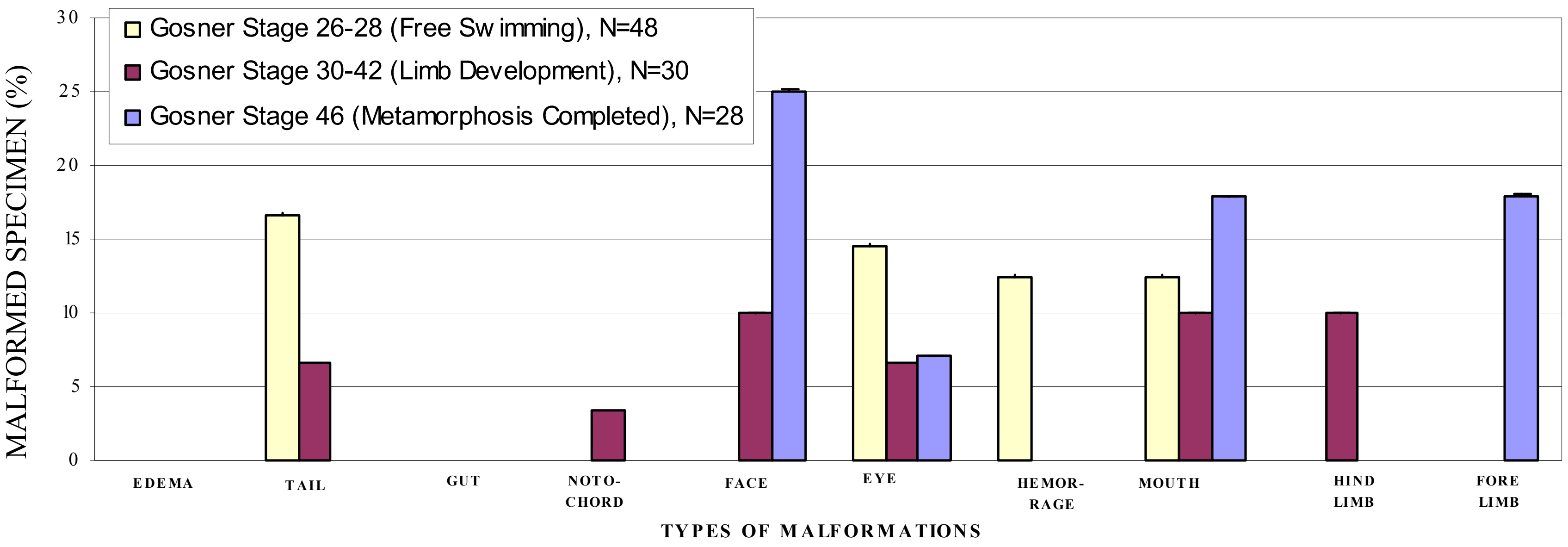


FIGURE 88
VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* CUMULATIVE MALFORMATION BY TYPE**
SITE OTC



Water Quality Measurements - Field

Water Temperature, pH, Conductivity, Dissolved Oxygen, Color

Field water quality measurements were made prior to other activities just below the surface of the water where the water depth is less than 1 foot (30cm). Measurements that were performed on site with field meters include: water temperature, pH, conductivity, dissolved oxygen, and color. (Note "J" values were discarded due to meter error)

Results for water temperature, pH, conductivity, dissolved oxygen and color are presented in Table 23 and in Table 24 with minimum, median and maximum range values.

Dissolved Oxygen

Dissolved Oxygen ranged from extremely low concentrations of less than 1.0 mg/l (Mud Creek, North Hero) to greater than 7.0 mg/l (Otter Creek, Ward Marsh and Alburg Dune). Many of the sites showed a steady decrease in dissolved oxygen as the season progressed (water levels dropped, temperature increased). Alburg Dune had the most pronounced seasonal decrease in dissolved oxygen, from 8.4 mg/l to 1.6 mg/l. Diurnal variation of oxygen in shallow ponds can be expected, maximum concentrations would occur late in the day, minimum dissolved oxygen would occur very early in the day. These fluctuations would be attributed to photosynthesis and the quantity of oxygen removed at night by community respiration (Williams, 1987).

It is unclear what affect the low dissolved oxygen had on the tadpoles; one would presume this would be dependent on the developmental stage. Most of the low dissolved oxygen measurements were recorded late in the season, which would correspond with later developmental stages. We observed late stage tadpoles at many sites surfacing for air.

Temperature

Temperature ranged from a high of 37^{o}C (96^{o}F) to a low of 10^{o}C, like dissolved oxygen, temperature values increased as the season progressed. The Alburg study site, a small tannic pond had the highest value recorded (37 ^{o}C) on August 8th. The threshold temperature criterion for tadpoles is 31^{o}C. A considerable diurnal fluctuation would also be expected at most of the study site. The highest temperatures recorded corresponded with late afternoon measurements.

pH

The lowest pH recorded was 6.50 (Alburg), the highest value recorded was 7.71 at Ward Marsh. Many of the study sites are influenced by Lake Champlain water (average pH 7.4) especially during high water events. The pH values recorded are all close to neutral and as such should not present a risk to tadpole development.

Conductivity

Conductivity values ranged from 96 – 742 us/cm at the five study sites. Otter Creek had the lowest value and North Hero had the highest value. Most sites showed an increase in conductivity as the season progressed. Sites that exhibited the greatest decrease in water level had the highest conductivities. The North Hero floodplain site and the Mud Creek red-maple swamp habitats had the highest conductivities, 742, and 525 us/cm. These values do correspond to the last measurements of the season and the lowest water levels at each of the sites. Williams

(1987) observed a four-fold increase in the conductivity of a temporary pond in Ontario, from the time that the pond filled in early spring to just before it dried up.

Color

Color in water may result from the presence of natural metallic ions (iron and manganese), humus and peat materials, plankton, weeds, and industrial wastes. (Standard Methods 1992). Color was measured at each site with a portable Hanna color meter; Alburg Dune had the highest and most consistent color readings (>500pcu). The color values at the North Hero and Mud Creek sites, increased as the season progressed. The evaporation of site water coupled with the leaching of leaf material most likely contributed to the increased color at these shallow sites. The color of the water is important with respect to temperature, highly colored water heats up to a greater extent near the surface, whereas a clear pond will absorb much heat in its bottom mud leading to a more uniform water temperature (Williams 1987). It should also be noted that colored water will block more ultraviolet radiation than transparent water.

Table 23. Field Water Quality Results

Site	Date	Time	Water Temp (^{O}c)	pH (Std Units)	Cond (Us/Cm)	D.O.(Mg/L)	Hanna Color (Pcu)
Otter Creek	05/01/01	13:30	16.0	7.13	96.5	-	-
Otter Creek	05/22/01	14:15	19.1	7.12	55	-	78
Otter Creek	05/24/01	13:50	19.5	-	-	-	-
Otter Creek	05/30/01	12:15	20.5	6.99	J	-	65
Otter Creek	06/06/01	13:20	21.0	7.20	184	-	90
Otter Creek	06/12/01	10:15	23.0	7.28	189	-	80
Otter Creek	06/25/01	14:15	27.0	7.28	198	7.3	66
Otter Creek	07/10/01	14:30	20.0	7.20	145	6.3	166
Otter Creek	07/20/01	12:00	26.0	-	-	5.8	-
Mud Creek	04/27/01	9:30	10.0	-	-	-	-
Mud Creek	05/21/01	10:00	21.0	7.06	-	J	200
Mud Creek	05/29/01	11:00	19.0	7.12	187	-	203
Mud Creek	06/07/01	14:15	19.5	7.52	182	-	240
Mud Creek	06/11/01	13:15	22.5	7.23	179	-	187
Mud Creek	07/03/01	14:15	22.0	J	176.2	-	190
Mud Creek	07/12/01	13:15	23.0	J	J	7.0	170
Mud Creek	07/24/01	10:30	22.5	7.02	193.4	5.8	160
Mud Creek	08/01/01	12:50	27.5	7.51	202	7.8	169
Mud Creek	08/14/01	15:10	28.0	7.30	202	6.8	161
Mud Creek	09/11/01	13:20	-	7.49	223	5.9	J
Mud Creek T1	05/29/01	12:15	16.5	7.05	J	-	435
Mud Creek T1	06/07/01	15:10	-	6.84	230	-	460
Mud Creek T1	06/21/01	12:00	21.0	6.71	271	0.7	>500
Mud Creek T2	06/21/01	13:50	26.0	6.74	253	0.8	312
Mud Creek T2	07/03/01	14:15	20.0	6.80	188.7	2.3	220

Site	Date	Time	Water Temp (^{O}c)	pH (Std Units)	Cond (Us/Cm)	D.O.(Mg/L)	Hanna Color (Pcu)
Mud Creek T2	07/12/01	11:45	20.5	7.36	525	-	190
Ward Marsh	05/01/01	9:30	16.0	-	-	-	-
Ward Marsh	05/22/01	11:00	19.0	7.27	244	-	85
Ward Marsh	05/31/01	12:00	18.5	7.30	288	-	132
Ward Marsh	06/12/01	10:45	19.5	6.97	164	-	283
Ward Marsh	06/20/01	14:10	26.0	7.47	264	5.6	152
Ward Marsh	07/09/01	15:15	22.5	7.71	380	5.8	161
Ward Marsh	08/07/01	15:10	28.0	7.36	341	9.5	95
Ward Marsh-T1	06/20/01	13:05	32.0	7.48	163.6	4.5	170
Ward Marsh-T1	07/09/01	15:00	32.0	7.03	206	4.8	>500
Alburg Dune	04/27/01	11:00	12.0	-	-	-	-
Alburg Dune	05/21/01	13:00	27.0	7.25	128	-	475
Alburg Dune	06/01/01	15:00	25.5	7.20	163	-	437
Alburg Dune	06/11/01	14:00	20.5	7.18	156	-	>500
Alburg Dune	06/14/01	13:00	28.0	7.18	127	8.4	>500
Alburg Dune	06/19/01	14:25	30.0	7.29	125	8.0	>500
Alburg Dune	06/26/01	11:15	29.0	7.19	104	7.0	>500
Alburg Dune	07/06/01	14:00	18.5	7.07	225	6.0	>500
Alburg Dune	07/24/01	12:00	28.0	J	76	4.0	>500
Alburg Dune	08/01/01	15:15	30.5	6.50	75.8	4.5	>500
Alburg Dune	08/08/01	14:50	37.0	6.90	238	2.0	>500
Alburg Dune	08/29/01	12:00	25.0	6.59	243	1.6	440
North Hero-T1	04/27/01	13:30	15.0	-	-	-	-
North Hero-T1	05/21/01	-	-	6.98	46	-	195
North Hero-T1	05/30/01	14:30	13.0	6.55	J	-	348
North Hero-T1	06/04/01	11:00	18.0	6.66	154	-	420
North Hero-T1	06/11/01	15:30	18.0	6.57	175	-	>500
North Hero-T2	06/04/01	12:00	17.5	6.66	J	-	375
North Hero-T2	06/19/01	13:45	18.0	6.88	180	0.7	215
North Hero-T2	07/03/01	12:15	17.0	6.79	176.2	0.3-2.8	190
North Hero-T2	07/17/01	13:50	23.0	6.77	742	-	420

Table 24. Field Water Quality Results
(Minimum, Median, Maximum and Range values)

SITE	DATE	Water Temp (^{o}c)	Ph (Std Units)	Cond (Us/Cm)	D.O.(Mg/L)	Hanna Color (Pcu)
Otter Creek	Minimum	16.0	6.99	96.5	5.8	65
	Median	20.5	7.20	184	6.3	80
	Maximum	27.0	7.28	198	7.3	166
	Range	16.0-27.0	6.99-7.28	96.5-198	5.8-7.3	65-166
Mud Creek	Minimum	10.0	7.02	176.2	5.8	160
	Median	22.5	7.23	187	6.8	170
	Maximum	28	7.52	223	7.8	240
	Range	10.0-28.0	7.02-7.52	176.2-223	5.8-7.8	160-240
Mud Creek T1	Minimum	16.5	6.71	230	.07	435
	Median	-	6.84	-	-	460
	Maximum	21.0	7.05	271	.07	>500
	Range	16.5-21.0	6.71-7.05	230-271	-	435->500
Mud Creek T2	Minimum	20.0	6.74	188.7	0.8	190
	Median	20.5	6.80	253	-	220
	Maximum	26.0	7.36	525	2.3	312
	Range	20.0-26.0	6.74-7.36	188-525	0.8-2.3	190-312
Ward Marsh	Minimum	16.0	6.97	164	5.6	95
	Median	19.5	7.30	288	5.8	152
	Maximum	28.0	7.71	380	9.5	283
	Range	16.0-28.0	6.97-7.71	164-380	5.6-9.5	95-283
Ward Marsh T1	Minimum	32.0	7.03	163.6	4.5	170
	Median	-	-	-	-	-
	Maximum	32.0	7.48	206	4.8	>500
	Range	-	-	-	-	-
Alburg Dune	Minimum	12.0	6.50	75.8	1.6	437
	Median	27.0	7.18	127	4.5	>500
	Maximum	37.0	7.29	243	8.4	>500
	Range	12.0-37.0	6.50-7.29	75-243	1.6-8.4	437->500
North Hero T1	Minimum	13.0	6.55	46	-	195
	Median	15.0	6.57	157	-	348
	Maximum	18.0	6.98	175	-	>500
	Range	13.0-18.0	6.55-6.98	46-175	-	195->500
North Hero T2	Minimum	17.0	6.66	176.2	0.3	190
	Median	17.5	6.77	180	0.7	215
	Maximum	23.0	6.88	742	2.8	420
	Range	17.0-23.0	6.66-6.88	176.2-742	0.3-2.8	190-420

Water Quality Measurements - Laboratory

Calcium, magnesium, potassium, sodium, total nitrogen, nitrate, ammonia, alkalinity, conductivity and pH were all determined using USEPA approved methods contained in the VTDEC Laboratory Quality Assurance Plan (1999). All laboratory samples presented in Table 25-27 were analyzed at the VTDEC laboratory.

Major cations

Major cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) were measured at the primary study sites; results are presented in Table 25 and in Table 26 with minimum, median and maximum values. Concentrations of major ions can play an important role in the growth and development of amphibians. Tietge (2000) conducted a series of experiments on four test solutions with varying concentrations of major ions. Results strongly suggested that deficient ionic concentrations alone could adversely affect growth and development in standard developmental toxicity test (FETAX). Limitations in Potassium, a key physiological cation necessary for normal cellular function may have contributed to some of the adverse effects observed (Tietge et al., 2000).

Most of the sites showed an increase in the major cations measured from early May to mid-June. This would be expected as the site water evaporates the concentration of total dissolved salts would increase. As the calcium carbonate and calcium sulphate precipitate out, the loss of calcium ions is accompanied by relative increases in sodium and magnesium ions (Williams, 1987).

The concentration and relative abundance of dissolved substances in temporary waters vary more than in most permanent waters. The primary reason for this is due to evaporation, concentrating the chemical ions dissolved in the water.

With regards to cation concentrations we did not have the opportunity to observe the full seasonal range of ionic concentrations, since only three measurements were taken, the last one in June. We are uncertain how the cation concentrations observed relate to requirements for proper tadpole development and growth. Tietge (2000) studies were suspect of low potassium levels at one site (0.7 mg/l), these concentrations are similar to potassium concentrations observed at the Alburg and Otter site; 1.19 and 1.13 mg/l K^+ respectively.

Nitrate, Nitrogen, Ammonia

Surface waters normally contain trace amounts to 1 mg/l of nitrate, but concentrations above 5 mg/l reflect anthropogenic contamination (Hecnar,1995). Nitrate, nitrogen and ammonia levels (Table 25 and Table 26) ranged from trace to < 1.0 at all the sites except for North Hero. Nitrate + nitrite (NOX) levels at North Hero ranged from <0.05 to 4.62 mg/l, and ammonia (NH_3)levels ranged from <0.05 –1.36 mg/l.

These levels are substantially less than acute and chronic amphibian values reported by Hecnar (1995). Hecnar conducted acute and chronic toxicity of ammonium nitrate fertilizers to several amphibians, including *R. pipiens*. In the chronic experiments Chorus and leopard frogs experienced increased mortality in the 10-mg/l NO_3-N treatment.

In areas of intensive agriculture in southern Ontario, nitrate concentrations commonly exceed the drinking water limit of 10 -mg/l NO_3-N (Hecnar, 1995).

Based on the three measurements taken in this study, it appears that nitrogen concentrations at four of the five (<1.0 mg/l) study sites are not adversely affecting tadpole development.
The Nitrate + Nitrite value 4.62 mg/l at the North Hero site may merit further investigation, since Nitrite is reported to induce methemoglobinemia in larvae of *Rana catesbeiana* by oxidizing Fe^{2+} to Fe^{3+} (Allran et al 2000).

It is probable that Nitrogen concentrations have exceeded the values we observed, the timing of collections with regards to rain events and anthropogenic activities can have a significant effect on associated water quality values.

Alkalinity

Alkalinity values ranged from a low of 36 mg/l recorded at Mud Creek to a high of 101 mg/l at Ward Marsh. All five sites showed a significant increase in alkalinity as the season progressed. In fact 3 of the 5 sites showed a two-fold increase, evaporation and concentration of dissolved substances would be the most likely explanation for the observed increases.

Conductivity and pH

Conductivity and pH were also measured as part of the Field Water Chemistry (previous section) and as such have already been discussed. The laboratory analysis of pH and Conductivity provided additional quality control for these field meter measurements.

Table 25. Laboratory Water Quality Results
Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Nitrate + Nitrite (NOX),
Nitrate (NO3I), Nitrogen, Ammonia (NH3), Alkalinity (ALK), Conductivity (COND) and pH.

SITE	DATE	CA (mg/l)	MG (mg/l)	K (mg/l)	NA (mg/l)	NOX (mg-n/l)	$N0_3$ (mg-n/l)	NH_3 (mg-n/l)	ALK (mg/l)	COND (umhos/ cm)	PH
Otter Creek	5/1/2001	11.9	3.63	1.13	3.91	0.8	< 0.10	< 0.05	40.9	112.00	7.36
Otter Creek	5/22/2001	20	6.4	1.56	6.71	< 0.05	< 0.10	< 0.05	71.6	184.00	7.19
Otter Creek	6/12/2001	22.3	7.2	1.92	8.32	< 0.05	<0.10	< 0.05	80.9	200.00	7.44
Mud Creek	4/27/2001	14.6	2.85	1.88	4.89	0.8	0.85	< 0.05	36.8	166.00	7.68
Mud Creek	5/21/2001	23.8	3.42	2.79	5.91	< 0.05	< 0.10	< 0.05	70.1	187.00	7.38
Mud Creek	6/11/2001	27.8	4.14	1.92	6.62	< 0.05	< 0.10	< 0.05	78.9	193.00	7.38
Ward Marsh	5/1/2001	23.9	5.00	1.14	6.70	0.19	< 0.10	< 0.05	70.7	191.00	7.15
Ward Marsh	5/22/2001	30.8	7.70	1.74	9.97	< 0.05	< 0.10	< 0.05	101	255.00	7.31
Ward Marsh	6/12/2001	14.4	7.55	2.08	7.89	< 0.05	0.02 (J)	0.05	43.4	171.00	7.06
Alburg Dune	4/27/2001	17.9	3.74	1.46	7.95	0.19	0.19	< 0.05	51.2	166.00	-
Alburg Dune	5/21/2001	0.00	0.00	0.00	0.00	< 0.05	< 0.10	0.05	55.1	-	7.68
Alburg Dune	6/11/2001	30.7	2.23	1.19	5.64	< 0.05	< 0.10	< 0.05	72.7	162.00	7.3
North Hero S.P.	4/27/2001	20.5	3.35	2.07	5.33	0.26	0.26	< 0.05	55.4	162.00	7.41
North Hero S.P.	5/21/2001	< 0.05	20.0	3.53	2.62	4.62	< 0.10	0.08	59.4	156.00	6.97
North Hero S.P.	6/11/2001	27.9	4.32	5.3	10	< 0.05	< 0.10	1.36	76.0	191.00	6.68

Table 26. Laboratory Water Quality Results
(Minumum, Median, Maximum and Range values)

SITE	DATE	CA (mg/l)	MG (mg/l)	K (mg/l)	NA (mg/l)	NOX (mg-n/l)	N0$_3$ (mg-n/l)	NH$_3$ (mg-n/l)	ALK (mg/l)	COND (umhos/cm)	PH
Otter Creek	Minimum	11.9	3.63	1.13	3.91	< 0.05	< 0.10	< 0.05	40.9	112.00	7.19
	Median	20	6.4	1.56	6.71	< 0.05	< 0.10	< 0.05	71.6	184.00	7.36
	Maximum	22.3	7.2	1.92	8.32	0.8	<0.10	< 0.05	80.9	200.00	7.44
	Range	11.9-22.3	3.63-7.2	1.13-1.92	3.91-8.32	< 0.05-0.8	<0.10	< 0.05-< 0.05	40.9-80.9	112.0-200.0	7.19-7.44
Mud Creek	Minimum	14.6	2.85	1.88	4.89	< 0.05	< 0.10	< 0.05	36.8	166.00	7.38
	Median	23.8	3.42	1.92	5.91	< 0.05	< 0.10	< 0.05	70.1	187.00	7.38
	Maximum	27.8	4.14	2.79	6.62	0.8	0.85	< 0.05	78.9	193.00	7.68
	Range	14.6-27.8	2.85-4.14	1.88-2.79	4.89-6.62	< 0.05-0.8	< 0.10-0.85	< 0.05-< 0.05	36.8-78.9	166.0-193.0	7.38-7.68
Ward Marsh	Minimum	14.4	5.00	1.14	6.70	< 0.05	< 0.10	< 0.05	43.4	171.00	7.06
	Median	23.9	7.55	1.74	7.89	< 0.05	< 0.10	< 0.05	70.7	191.00	7.15
	Maximum	30.8	7.70	2.08	9.97	0.19	0.02 (J)	0.05	101	255.00	7.31
	Range	14.4-30.8	5.0-7.7	1.14-2.08	6.7-9.97	< 0.05-0.19	< 0.10-< 0.10	< 0.05-0.05	43.4-101	171.0-255.0	7.06-7.31
Alburg Dune	Minimum	17.9	2.23	1.19	5.64	< 0.05	< 0.10	< 0.05	51.2	162.00	7.3
	Median	na	na	na	na	< 0.05	< 0.10	< 0.05	55.1	162.00	7.3
	Maximum	30.7	3.74	1.46	7.95	0.19	0.19	0.05	72.7	166.00	7.3
	Range	17.9-30.7	2.23-3.74	1.19-1.46	5.64-7.95	< 0.05	< 0.10-0.19	< 0.05-0.05	51.2-72.7	7.68-166.0	7.3
North Hero S.P.	Minimum	< 0.05	3.35	2.07	2.62	< 0.05	< 0.10	< 0.05	55.4	156.00	6.68
	Median	20.5	4.32	3.53	5.33	0.26	< 0.10	0.08	59.4	162.00	6.97
	Maximum	27.9	20.0	5.3	10	4.62	0.26	1.36	76.0	191.00	7.41
	Range	< 0.05-27.9	3.35-20.0	2.07-5.3	2.62-10	< 0.05-4.62	< 0.10-0.26	< 0.05-1.36	55.4-76.0	156.0-191.0	6.68-7.41

Heavy Metals

Heavy metals have often been associated with toxicity in water and sediments.
Frogs exposed to Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} in FETAX assays have shown these metals to be teratogens, causing malformities and mortality. The malformities of frogs exposed to these metals include retinal depigmentaion and pelvic and hind limb malformities (Luo,1993; Plowman et. al., 1994).

Water samples were collected and analyzed for 13 priority pollutant metals.
Results from these test are presented in Table27, none of the water samples analyzed exceeded the Laboratory detection limits.

Concentrations of Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} were determined in sediment samples from Ward Marsh and Mud Creek using ICAP analysis, as part of an undergraduate thesis (Wall, 1999).
Wall found that concentrations of Cu, Ni, Cr and Fe in sediment from Ward Marsh significantly exceeds sediment quality criteria established by research done in Collingwood Harbour, Lake Heron, Ontario. Mud Creek sediment samples only exceed the quality criteria from Cu and Ni. Samples from Ward Marsh were found to contain statistically higher concentrations of Zn^{2+}, (mean= 103 mg/kg), Co^{2+} (mean= 18.3 mg/kg), Ni^{2+} (mean=39.4 mg/kg), and Cr^{2+}(mean=43.4 mg/kg) than Mud Creek. Metals concentrations at the Ward Marsh site indicate a trend ranging from low concentrations in soils farther from East Bay to higher concentrations in sub-aqueous sediment closer to the bay (Wall, 1999).

Table 27. Laboratory Water Quality Results
Priority Pollutant Metals (13)

TEST	Alburg Dune 6/11/2001	Mud Creek 6/11/2001	North Hero S.P. 6/11/2001	Otter Creek 6/12/2001	Ward Marsh 6/12/2001
Antimony (ug/L)	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
Arsenic (ug/L)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Beryllium (ug/L)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium (ug/L)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Chromium (ug/L)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Copper (ug/L)	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5
Lead (ug/L)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Mercury (ug/L)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Nickel (ug/L)	<12.5	<12.5	<12.5	<12.5	<12.5
Selenium (ug/L)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Silver (ug/L)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
Thallium (ug/L)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Zinc (ug/L)	< 25.0	< 25.0	< 25.0	< 25.0	< 25.0

Data Usability

In compliance with the quality assurance/quality control guidelines outlined in the QAPP (VTDEC et.al., 2001), field duplicates were collected for selected tests during each round of sampling (n = 5.4%). These samples were collected at the same location within the designated sites and were analyzed as unique samples. Field duplicates represent how variable the sampling method was. Sampling precision was quantified by calculating relative percent difference between duplicate analyses (Table 28).

Additionally, approximately 10% of all samples processed by VTDEC were analyzed as laboratory duplicates. Laboratory duplicates represent how precise the analysis was. Laboratory precision was quantified by calculating relative percent difference between duplicate analyses (Table 29).

All reported compounds from the duplicate samples met the relative percent difference goals established in the Quality Assurance Project Plan. The relative percent difference (RPD) for both field and laboratory duplicates was calculated using the following equation:

RPD= (count a - count b) / (count a + count b) / 2 x 100

Table 28. Mean Relative Percent Differences for 2001 Field Duplicate Samples.

Sample Type	Symbol	Mean RPD %	# of Duplicate Pairs
Calcium- Water	WCA	2.7	3
Magnesium – Water	WMG	5.5	2
Potassium - Water	WK	1.0	2
Sodium - Water	WNA	1.5	2
Nitrate - Filtered	NO3I	0.0	1
Nitrate + Nitrite - Water	NOX	0.0	1
pH	pH	1.0	1

Table 29. Mean Relative Percent Differences for 2001 Laboratory Duplicate Samples.

Sample Type	Symbol	Mean RPD %
Calcium- Water	WCA	1.0
Magnesium – Water	WMG	1.0
Potassium - Water	WK	1.0
Sodium - Water	WNA	1.0
Nitrate - Filtered	NO3I	4.0
Nitrate + Nitrite - Water	NOX	1.0
Silver - Water	WAG	2.0
Arsenic - Water	WAS	2.0
Beryllium - Water	WBE	2.0
Cadmium - Water	WCD	2.0
Chromium - Water	WCR	2.0
Copper - Water	WCU	10.0
Mercury - Water	WHG	1.0
Nickel - Water	WNI	3.0
Lead - Water	WPB	3.0
Antimony - Water	WSB	2.0
Selenium - Water	WSE	3.0
Thallium - Water	WTL	3.0
Zinc - Water	WZN	2.0

Pesticides

Of the 32 pesticides and metabolites analyzed during this study, the only compounds detected in water were atrazine and it's desethyl metabolite, and metolachlor along with its Ethanesulfonic acid (ESA) and Oxanilic acid (OA) metabolites (Table 30). No pesticides or metabolites were detected in any of the sediment samples (Table 31). The Method Detection Limit (MDL) and mean recoveries for all analytes are listed in Table 5. Because of the exploratory nature of this study, all detections above the MDL, which could be qualitatively confirmed, are reported, even though the quantitation may be approximate near the MDL.

Table 30. Pesticide Results: Water

	Alburg		Otter Creek		Mud Creek		North Hero		Ward Marsh	
Analyte Group	**04/27/01**	**06/11/01**	**05/01/01**	**06/12/01**	**04/27/01**	**06/11/01**	**04/27/01**	**06/11/01**	**05/01/01**	**06/12/01**
Corn Herbicides										
Atrazine	ND	ND	ND	0.13 ppb	0.07 ppb	0.09 ppb	ND	ND	ND	ND
Simazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	ND	0.11 ppb	0.04 ppb	0.05 ppb	0.02 ppb	ND	ND	ND
Cyanazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pendimethalin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dimethenamid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetochlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acid Herbicides										
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCPP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dacthal	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Insecticides										
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Right Of Way										
Imazapyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flumetsulam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nicosulfuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metsulfuron methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfometuron methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Primisulfuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolites										
Desethyl atrazine	0.02 ppb	ND	ND	0.02 ppb	0.03 ppb	ND	0.02 ppb	ND	ND	ND
Desisopropyl atrazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor ESA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor OA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor ESA	0.05 ppb	ND	0.03 ppb	0.05 ppb	0.10 ppb	0.07 ppb	0.08 ppb	ND	0.05 ppb	ND
Metolachlor OA	0.02 ppb	ND	ND	0.02 ppb	0.04 ppb	0.02 ppb	0.03 ppb	ND	ND	ND
Acetochlor ESA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetochlor OA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 31. Pesticide Results: Sediment

Analyte Group	Alburg		Otter Creek		Mud Creek		North Hero		Ward Marsh	
	04/27/01	06/11/01	05/01/01	06/12/01	04/27/01	06/11/01	04/27/01	06/11/01	05/01/01	06/12/01
Corn Herbicides										
Atrazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Simazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyanazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pendimethalin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dimethenamid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetochlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acid Herbicides										
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCPP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dacthal	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]	N/A [1]
Insecticides										
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Right of Way										
Imazapyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flumetsulam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nicosulfuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metsulfuron methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfometuron methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Primisulfuron	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metabolites										
Desethyl atrazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Desisopropyl atrazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor ESA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alachlor OA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor ESA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor OA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetochlor ESA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acetochlor OA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1] not analyzed (see text).

Atrazine was detected in three out of ten water samples with one detection above 0.1 ppb, at Otter Creek in the June 12, 2001 sample. Metolachlor was detected in four of the ten water samples analyzed, again with the only sample above 0.1 ppb being the Otter Creek June sample. Desethyl atrazine was detected in four of ten samples, all below 0.1 ppb. Desethyl atrazine was the only metabolite of atrazine detected during this study, and the major metabolite detected during other surface water investigations (Kolpin et. al., 1999 and Thurman et. al., 1992). Metolachlor ESA was the major metolachlor metabolite detected, being found in seven of ten water samples, with one at 0.1 ppb. The metolachlor OA metabolite was also detected, in five of ten water samples, all below 0.05 ppb.

These levels are far below any current regulatory aquatic life criteria, although some studies have reported effects on algae at the low ppb concentration range. For instance Torres et. al.1976, reported a Lowest Observable Effect Level (LOEL) for algae of 1.0 μg/L (Tierney et. al., 1999). The Canadian aquatic life criteria for atrazine is 1.8 ppb and for metolachlor is 7.8 ppb, while the EPA has a draft ambient aquatic life criteria of 12 ppb for atrazine. With no pesticide detections above 0.13 ppb, the data from this study indicates levels well below any regulatory criteria.

Atrazine and metolachlor are the two most widely used pesticides in Vermont, after the cooling tower pesticides, together accounting for over 50% of commercial use in 2000. Because these compounds are highly water-soluble they have been found to easily leach into groundwater and also are often contaminants of surface water where they are used. The levels found during this study are lower than those often found in rivers and streams in the mid-west (Thurman et. al., 1992), probably reflecting the smaller amount of corn grown in Vermont. Thurman et. al., (1992) found median, base flow atrazine concentrations of greater than 0.2 ppb in watersheds throughout the midwest, with peak runoff concentrations as much as 100 times as high. Due to the limited sampling during the current study, peak runoff concentrations in Vermont are unknown.

There are very little data available on the relative toxicities of the various corn herbicide metabolites, compared with the parent compounds. Therefore it is often assumed that the toxicities are similar (EPA 2002) and that is what will be assumed here. With this assumption in mind, the metabolites were converted back to atrazine and metolachlor equivalents using the molecular weights of the various compounds and the fact that one molecule of each metabolite originates from one molecule of the parent compound. Table 32 summarizes the pesticides detected during this study, converted back to atrazine and metolachlor equivalents. It can be seen in Table 32 that pesticide concentrations tend to decrease from early sampling to late at Alburg Dunes, North Hero, and Ward Marsh, while levels are essentially constant at Mud Creek, and increase at Otter Creek. The early sampling date, around the end of April, is before most spring agricultural pesticide applications, while the late sampling was during or after the height of agricultural pesticide use. While it is difficult to extrapolate from only two sets of data points, it appears that the detections at Otter Creek are due to spring runoff from this large, highly agricultural watershed. Mud Creek is a much smaller watershed, with the wetland sampled being surrounded by agriculture, so there appears to be some chronic low-level contamination present at this site. The other three sites are more difficult to interpret because at the time of the early sampling these sites were apparently under the direct influence of Lake Champlain flood waters (Levey, pers. comm.). An indication of what is happening can be found by looking at the relative contribution of the metolachlor metabolites in these samples (Table 32). In all early season water samples, at least 75% of the metolachlor equivalents comes from metabolized metolachlor. There is little of the parent compound present, only metabolites. This trend is also present with atrazine, although not as clear. This data, in combination with the fact that the early samples were collected before most corn herbicide applications had occurred for the 2001 season, suggests that the herbicide detections found are remnants from previous years' applications which have run off into Lake Champlain and caused low level lake wide contamination. This possibility is being investigated by the Vermont Department of Agriculture in 2002.

Table 32. Pesticides Results Summary

	Atrazine Plus Metabolites [A] (percent from metabolites)	**Metolachlor Plus Metabolites [B] (percent from metabolites)**
Alburg Dunes		
4/27/01	0.02 PPB (100%)	0.06 PPB (100%)
6/11/01	ND (0%)	ND (0%)
Otter Creek		
5/1/01	ND (0%)	0.03 PPB (100%)
6/12/01	0.15 PPB (15%)	0.17 PPB (35%)
Mud Creek		
4/27/01	0.10 PPB(30%)	0.17 PPB (76%)
6/11/01	0.09 PPB (0%)	0.13 PPB (62%)
North Hero		
4/27/01	0.02 PPB (100%)	0.12 PPB (83%)
6/11/01	ND (0%)	ND (0%)
Ward Marsh		
5/1/01	ND (0%)	0.05 PPB (100%)
6/12/01	ND (0%)	ND (0%)

Studies of herbicide concentrations in the Great Lakes (Rygwelski et. al., 1999, Schottler and Eisenreich 1994), have found chronic low-level atrazine and metolachlor present in the Great Lakes. The half-life of atrazine on fields is reported to be about 60 days (Hornsby et. al., 1996), while half-life estimates for atrazine in Lake Michigan range from two years (Tierney et. al., 1999), to 14 years (Schottler and Eisenreich 1994) to being essentially inert, with no breakdown at all (Rygwelski, et. al., 1999). These studies found atrazine levels of around 0.03 to 0.04 ppb throughout Lake Michigan with a homogeneous distribution both vertically and horizontally. Rygwelski et. al., (1999) concluded that atrazine was not breaking down in the cold dark waters of Lake Michigan and, with a water residency time of 99 years, the atrazine concentrations would continue to rise even without an increase in input. The Lake Michigan atrazine concentration was predicted to stabilize in about 300 years at approximately 0.160 ppb (Rygwelski et. al., 1999). Because the residency time of the water in Lake Champlain is about 2.5 years, compared with 99 years for Lake Michigan, it is not expected that herbicide levels would continue to rise over the same time span as the Great Lakes, but it is not known whether Lake Champlain herbicide concentrations have reached equilibrium yet or not.

Based on previous years' frog data, the Mud Creek and North Hero study sites were designated as low deformity or reference sites at the start of this study, while the Alburg Dunes, Otter Creek, and Ward Marsh sites were designated high malformity sites. There is no clear relationship between these designations and the pesticide results found. In fact, based on the data in Table 34, the reference sites appear to have more chronic low level herbicide contamination than the high malformity sites. Given that the pesticide levels found during this study are significantly below any regulatory aquatic life criteria, and are of similar concentrations at high and low malformation sites, it appears that these pesticides are not likely to be the cause of the leopard frog malformations observed in Vermont. If these pesticides are contributing to the frog malformation problem in Vermont it must presumably be due to either unusually high sensitivity to toxins during some phase of development in leopard frogs, or a synergistic effect with other, unknown environmental parameters. A recent report by Hayes et. al., (2002) found, in a lab study, that the endocrine system in male *Xenopus laevis* was disrupted by as little as 0.1 ppb of atrazine during development, causing hermaphroditism. Although these results were published very recently, and no one has yet reported being able to repeat them, they suggest that the levels of pesticides as found during this study have the potential to be causing developmental problems in leopard frogs via some unknown route. It should be noted that the malformations observed by Hayes et. al., are not the same as those seen in leopard frogs in the Champlain Valley of Vermont. Although the significance of the Hayes study is not yet clear, it might be prudent to assess the toxicity of atrazine, metolachlor, and the various metabolites at sub parts per billion levels via the leopard frog toxicity test developed by Fort et al., in a similar manner to the Hayes et al study. This might help to determine if the low concentrations of herbicides and metabolites detected during this study could be contributing to the leopard frog malformations observed in Vermont.

DISCUSSION

This report marks the culmination of a five-year investigation into the causes of *Rana pipiens* abnormalities. Numerous multidisciplinary collaborations have contributed to this study. The 2001 study will be the focus of this discussion, though it would be remiss not to include pertinent observations from previous years.

We have collected and examined over 10,000 *R. pipiens*, and have found hind limb truncations at more than 20 sites, spanning 120 miles of Lake Champlain. The common denominator of these sites is the Lake Champlain Basin and more specifically, the Lake Champlain lowlands. The rates of abnormalities have been highly variable, both seasonally and annually. However only a few sites have consistently maintained low rates of abnormalities (less than 4%).

This investigation conducted a series of coordinated field and laboratory tasks that were designed to provide data that would help point the way to the cause of the northern leopard frog abnormalities. The life cycle of *R. pipiens* helps illustrate the complexity of this endeavor. The leopard frog encompasses three environments: the breeding wetland, the feeding landscape of the adult frogs and the overwintering site.

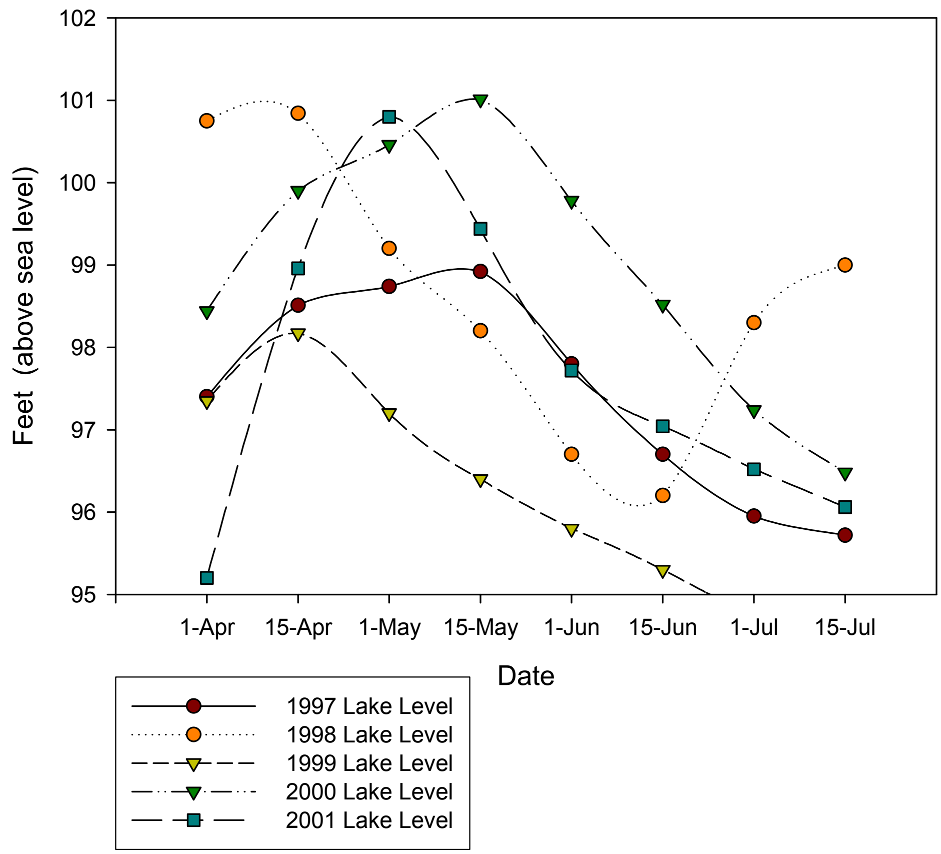
The potential for biological and chemical exposure are as plentiful as the environments of the leopard frog. Being a lowland species only adds to the elixir. Breeding in the floodplains of the Champlain lowlands exposes *R. pipiens* to large drainage areas and associated pollutants. *R. pipiens* populations breeding near the mouth of the Otter Creek are developing at the bottom of a 900 square mile drainage area. Their overwintering habitat may be in the river, lake or nearby permanent pond.

Perhaps one of the most significant variables of the breeding success and development of *R. pipiens* in the Lake Champlain lowlands is Lake Champlain's fluctuating water levels. Water level in the adjacent floodplains can affect temperature, tadpole densities, predator densities, predator-prey relationships, ionic composition, contaminant concentrations, parasite densities, ultraviolet radiation penetration, and disease. All of the primary sites, and three of the four supplemental sites are directly affected by Lake Champlain water levels.

The lake level variability presented in Figure 89 is impressive; it is easy to appreciate the impact that these varying water levels can have on developing tadpoles. A high water spring can create more breeding habitat, which may increase the overall breeding success of *R. pipiens*. However, we have observed high lake levels in the spring, and drought conditions by early June, leading to massive mortality. Furthermore, drought conditions in 1999 may have been a leading factor in tadpole trauma observed at the Otter Creek site.

Figure 89. Presents Lake Champlain water levels (1997 – 2001) for the *R. pipiens* developmental period (April – July). Note annual lake level fluctuates from 95 – 101 feet above sea level.

Figure 89. Lake Champlain Water Levels (1997-2001).



High Lake levels may maintain cooler water temperatures at sites, extending the duration of critical developmental periods. Conversely high lake levels may flood more low grassy land and actually create shallow swampy areas in which frogs and tadpoles develop. These shallow wetlands may be warmer than in lower water years.

Lake level affects a multitude of factors directly and indirectly related to the life history and microenvironments of *R. pipiens.* The annual variability in abnormalities we have observed may very well be connected to these fluctuating water levels.

Morphological Fingerprints

Lannoo (2000b) discussed the idea that morphology can give clues to malformation causes. There may be morphological signatures that uniquely identify malformation causes. This concept is especially intriguing when we recognize that most malformed frogs are observed following metamorphosis, and that hind limb development has occurred weeks or even months earlier.

Lannoo (2000b) points out that this temporal disparity may be sufficient to allow whatever caused the malformation to leave, or be diluted to the point that it is undetectable.
With this concept in mind it may be insightful to address the three main hypotheses for amphibian malformations (1) trematode infestation, (2) xenobiotic chemicals and (3) UV-B radiation exposure.

Each of these causes has been determined to produce amphibian malformations in laboratory studies. The question at large is, can these causes produce the types and frequencies of malformations that have been recently observed in Vermont?

Utilizing the data and observations obtained during this investigation and the "morphological signature" premise, we will address these causes, following Lannoo's (2000a) careful examination of the three main causes.

Parasite infestation:
1) parasite infestation causes limb and pelvic malformities only
2) multiple limbs or mirror image limbs predominate
3) there is a correlation between parasite infestation and the presence of a malformity (within older tadpoles and newly metamorphosed animals).

The following Vermont observations would appear to exclude this hypothesis:
Forelimb abnormalities were observed at all of the study sites, eye and pigment abnormalities were observed at many of the sites (2001). Over 10,000 *R.pipiens* metamorphs examined (1997-2001) have yielded only 1 specimen with a supernumerary limb (Figure 19). Several radiographs of ectromelic frogs have revealed spongiforme bone, which is not consistent with parasite infestation (Lannoo, 2000b). A subset of frogs examined by the National Wildlife Health Center showed no correlation between those frogs that had malformations and those that had metacercaria. (Meteyer, 1997).

UV-B Radiation:
1) limb malformity consisting of bilaterally symmetrical ectromelias (Ankley, 1998)
2) animals exposed to UV-B do not show spongiforme bone (Lannoo, 2000b)

This hypothesis can be excluded based on the "symmetrical" morphological signature of UV-B radiation. To date there have been no symmetrical limb truncations observed in Vermont. Furthermore many of the unilateral ectromelic frogs radiographed in Vermont do show spongiforme bone.

Xenobiotic chemical:
This hypothesis, predicts a wide suite of malformities involving the limbs, a variety of organ systems, and a number of biochemical/physiological processes (Lannoo, 2000a).
This hypothesis is consistent with the range of abnormalities we have observed in the Lake Champlain Basin.

A fourth hypothesis that receives a fair amount of attention is the failed predation hypothesis, or amputation by predators. We address this hypothesis for both metamorphs and tadpoles.

Amputation- Failed Predation – Metamorphs:

1) Limb will show signs of swelling, inflammation or irregularity at the termination of the limb (Meteyer, 1997).
2) Scarring and abnormal pigmentation over the end of the limb would also be indicative of a traumatized limb.
3) Scarring and abnormal pigmentation anywhere on the body

This hypothesis can be excluded based on the dearth of trauma related observations. No metamorphs were observed with swelling or inflammation, or signs of scarring anywhere on the body (except for known capture injuries). Meteyer (1997) found trauma related abnormalities to be very rare. Radiographs of ectromelic limbs often showed spongiforme bone, which is inconsistent with trauma related amputations (Lannoo, 2003). Furthermore, we have observed abnormal ventral pigmentation on many of the ectromelic limbs, which is associated with developmental problems (Meteyer, 2000a; Lannoo, 2003). Many specimens missing whole limbs also had abnormal pelvic structures; this supports an early developmental error rather than amputation (Meteyer, 2000b).

Amputation –Failed Predation –Tadpoles:

1) Developing limb buds would show signs of swelling, inflammation
2) Scarring and abnormal pigmentation would be present over the end of the limb
3) Would expect to see damage adjacent tissue or the whole tadpole.

This hypotheses appears to be an unlikely candidate for explaining limb truncations based on the following 2001 observations: 1) of 1254 tadpoles examined, there were no limb truncations that showed signs of trauma. 2) early stage progression of ectrodactyly, polydactyly and differential development were documented, providing evidence that at least a portion of phenotypes are not the result of trauma from predation.

However, in 1999 we observed late stage tadpole trauma at the Otter Creek site.
Of 218 tadpoles examined, 5.5% had hind limb truncations; many showed signs of recent trauma. Drought conditions had created severe crowding. It is not known what caused the trauma to these tadpoles, but it might be reasonable to assume that trauma from aquatic insects or other tadpoles had been exacerbated by crowed conditions.

Our findings from the 2001 Vermont investigation show a strong dissociation between the parasite hypotheses, the UV-B radiation hypotheses and the failed predation hypotheses. However the xenobiotic chemical hypotheses has been reported to produce the range of abnormalities that we have observed in Vermont.

It should be noted, the exclusion of the hypotheses above; parasite infestation, UV-B radiation and failed predation does not mean that these hypotheses do not explain any of the observed abnormalities. What we are saying is that it is highly unlikely that these hypotheses are the sole causation of the observed hind limb abnormalities.

Malformation vs. Deformation

The characterization and interpretation of *R. pipiens* (metamorphs and tadpoles) has provided significant evidence that malformations are occurring in metamorphs and tadpoles. In contrast, there has been a general lack of evidence supporting deformations.

If we are observing a predominance of malformations, we will still need to determine whether these malformations are due to anthropogenic causes or natural causes. Even within the volume of this report we recognize that there are many biotic factors such as high water temperatures or deficient ionic concentrations that can play a role in malformations.

The water chemistry screening conducted during this investigation has provided some data with regards to ionic concentrations, nutrients, common use pesticides and priority pollutant metals. We must recognize that this is a small data set, limited spatially and temporally.

Additionally our interpretation of "critical concentrations" is becoming more difficult as we learn more about the complexities of exposure, whether acute or chronic. Conventional toxicity data may have limited value for interpreting effects in the field. Bridge (2000) studied the effects of chemical exposure during different life stages on the long-term growth and development of the Southern leopard frog. Many acute and chronic toxicity studies fail to observe mortality or growth and development problems that occur after the test period.

Pesticides

The two most widely used pesticides in Vermont, atrazine and metolachlor, were detected as parent compound or metabolite in water samples from all of the study sites. The concentrations (parent compound) were far below any current regulatory aquatic life criteria.

Although atrazine has been widely detected in surface waters, concentrations in freshwater rivers and streams rarely exceed 20 to 25 ppm atrazine (Solomon 1996). These concentrations are less than the lowest-observable-adverse-effect concentration (LOAEC, 1.1 mg atrazine/L) for deformities in acute (96-h) exposure testing in Xenopus laevis (Morgan 1996).

The no-observed effect concentration [NOEC] is 200 ppm atrazine/L for larval growth and metamorphosis in the northern leopard frog (Allran 2001).

However there is little data available on the relative toxicities of the metabolites. Recent studies have found concentrations of atrazine (0.1ppb) similar to our findings capable of endocrine disruption, inhibiting testosterone and inducing estrogen secretion in amphibians (Hayes et. al., 2002). Although the Hayes study did not relate to hind limb abnormalities, another study has linked limb malformations with abnormal sex hormone concentrations in New Hampshire frogs (Sower et al., 2000).

Bioconcentration of atrazine by *R. pipiens* larvae has been reported to be six times the concentration in the water (Allran et al., 2000). Atrazine is also capable of interacting synergistically with other agricultural chemicals to decrease survival of amphibian larvae (Howe et al., 1998). In addition to the active ingredients in pesticides, a number of pesticides also contain solvents as inert ingredients that may contribute to potential developmental toxicity.

High lake levels appear to have contributed some of the pesticide residues observed. Alburg, North Hero and Ward Marsh had a high percentage of metabolite present during the early spring sampling event (before application). These findings illustrate that there is a continuous contaminant burden, with unknown consequences.

Future work should include looking for gonadal malformations in the field; lab tests with atrazine and its metabolites should be carried out to limb development to see if there are any effects.

Synergism

Recent field and laboratory studies have shown that environmentally realistic concentrations of agricultural pesticides decrease the ability of developing amphibians to elicit an immune response that would prevent parasite infection (Christin et al.2003, Kiesecker 2002). Kiesecker's studies linked increased trematode infection, and increased limb deformities, to pesticide exposure. Christin (2003) showed agricultural pesticides modulated immune responses and increased the sensitivity of *Rana pipiens* to parasites.

These convincing studies in synergism reveal that a multitude of natural and synthetic variables may work together, affecting developing amphibians with unknown consequence.

Drift Fence Monitoring

Baseline indices of populations have been established at the Ward Marsh site. The number of *R. pipiens* metamorphs dropped drastically between 1998 and 1999, this appears to correspond well with the lake level data shown in Figure 89. The breeding period (early-mid April) in 1998 had lake levels over 100 ft (30m) above sea level, whereas in 1999 the breeding period lake level was less than 98 ft (29m) above sea level.

The drift fence monitoring data corresponded well with the smaller scale *R. pipiens* metamorph surveys. Rates of abnormalities were similar, though the overall rates of abnormalities were low (0 –3.4%) for the 1998-2001 period.

Laboratory Limb Bud Assays and Field Observations

The objective of the laboratory developmental limb bud assays was to evaluate the potential of site sediment and water to induce developmental abnormalities of a type and frequency of occurrence observed in the field.

Results from limb bud assays indicated that samples from Alburg, Ward Marsh and Otter Creek were capable of inducing embryo-larval mal-development, as well as malformation at limb development stages in *X.laevis* and *R.pipiens*.

Table 33 presents the mortality and malformation data for *R. pipiens* in the limb bud assays. High *R. pipiens* mortalities were observed at the North Hero and Mud Creek sites during the limb development stage, 62 and 54 percent respectively. The control mortality was 36% within the limb development stage.

General water chemistry data reported for the limb bud assays in Appendix E show diminished water quality conditions for the North Hero samples on 6/22/01. Dissolved oxygen was 2.4 mg/l, a pH of 6.0 and 8.40 NH3-N mg/l. Field water chemistry values (Table 23) at the North Hero site also diminished as the season progressed with the lowest values recorded in late June.
It is possible the diminished water quality at the North Hero site contributed to the high mortality observed, however high mortality during limb development was also observed at Mud Creek (54%) where water quality values were not impaired.

Table 33. Mortality and Malformation Data for *R. pipiens* Limb bud Assay
(Percent values relative to the total number of animals used in test are noted in parenthesis)

Sites	Mortality (n=50)		Malformation (n=50)		
	Embryo-larval	Limb Development	Embryo-larval	Limb Development	Gosner 46 (Metamorphosis Complete)
Control*	-	11 (36)	-	-	1 (5)
Mud Creek	2 (4)	27 (54)	-	-	-
North Hero	9 (18)	30 (62)	-	1 (5.3)	3 (16)
Alburg	8 (16)	8 (16)	21(42)	15 (36)	21 (52)
Ward Marsh	10 (20)	18 (36)	16(32)	14 (4)	13 (41)
Otter Creek	5 (10)	18 (42)	9 (19)	8 (27)	9 (32)

*Control n=30 (3 replicates of 10)

Malformations during the limb development stage were highest for the Alburg and Otter Creek samples (Table 33); this correlates with observed metamorph abnormalities at the Alburg and Otter sites during field surveys (Figure 37). Alburg samples also had the highest malformation rate (52%) within the Gosner 46 period; Ward Marsh and Otter Creek followed with 41 and 32 percent malformed. The Ward Marsh limb bud assay results (41% malformed) did not overlay well with the 2001 field results, of 4% abnormal (Figure 37).

A comparison of the abnormalities (malformations) observed during limb bud assays and the metamorph field surveys are presented in Table 34.
Only the Gosner stage 46 category of the limb bud assay was used for this comparison since earlier stage categories used in the limb bud assay are not in alignment with the field metamorph categories.

Table 34. Comparison between select *R. pipiens* field abnormalities and observed Limb Bud Assay Lab abnormalities for *R .pipiens* (Gosner 46).
(Percent values relative to the total number of abnormalities observed are noted in parentheses.)

Site	Control	Mud Creek		North Hero		Alburg		Ward Marsh		Otter Creek	
	Lab	Lab	Field	Lab	Field	Lab	Field	Lab	Field	Lab	Field
Hind Limb abnormal	0	0	5 (62.5)	0	18 (69.2)	2 (5.0)	31 (83.7)	4 (12.9)	8 (88.8)	0	38 (80)
Forelimb abnormal	1 (5.2)	0	3 (37.5)	1 (5.2)	6 (23.0)	12 (30)	3 (8.1)	9 (29)	1 (11.1)	5 (17.8)	5 (12.0)
Eye abnormal	0	0	0	0	1 (3.8)	5 (12.5)	2 (5.4)	5 (16.1)	0	2 (7.1)	3 (7.3)
Total no. abnormal animals	1 (5.2)	0	8	3 (15.7)	24 (4.3)	21 (52)	33 (5.1)	13 (41.9)	4 (1.2)	9 (32.1)	34 (5.6)
Total no. abnormalities	1	0	8	3	26	54	37	29	9	19	41
No. abnormalities per abnormal animal	1	0	1	1	1.1	2.5	1.1	2.2	2.25	2.1	1.2

Field surveys documented high frequencies of hind limb abnormalities at all 5 sites, ranging from 62 –88 percent. In contrast limb bud assays observed low frequencies (5 –13%) of hind limb abnormalities were observed at only the Alburg and Ward Marsh sites within the Gosner 46 stage.

Forelimb abnormalities were observed at all of the sites during the field surveys, ranging from 8 –37 percent. Limb bud assays observed forelimb abnormalities at all of the sites except Mud Creek, frequencies ranged from 0 –30 percent.

Eye abnormalities were observed at three of the five sites for both the field surveys and limb bud assays. Rates ranged from 0 –16.1 percent in the limb bud assays and 0- 7.3 percent in the field surveys.

Tadpole abnormalities observed during field surveys were compared to select abnormalities observed during the Gosner stage 30-42 limb bud assays (Table 35).

Tadpole hind limb abnormalities were observed at 3 of the 5 sites, frequencies ranged from 5 –33 percent. Hind limb abnormalities were also observed at 3 of the 5 sites during limb bud assays, frequencies were lower, ranging from 5 –18 percent.

The number of abnormalities induced (all categories) during the limb bud assays (Gosner stage 30-42) was highest at the Alburg and Ward Marsh site, 51 and 42 respectively.
This reflected 3.4 and 3.0 abnormalities per animal at the Alburg and Ward Marsh sites.
The highest number of abnormalities observed per animal during the tadpole surveys was 2, at the Ward Marsh site.

Table 35. Comparison of hind limb and eye abnormalities between Limb bud assay's (Gosner stage 30-42) results and observed abnormalities from tadpole surveys.

Site	Control	Mud Creek		North Hero		Alburg		Ward Marsh		Otter Creek	
	Lab	Lab	Field	Lab	Field	Lab	Field	Lab	Field	Lab	Field
Hind Limb abnormal	0	0	2 (5.4)	1 (5.2)	1 (33)	0	9 (33.3)	6 (18.7)	0	3 (10)	0
Eye abnormal	0	0	2 (5.4)	0	0	1 (2.3)	1 (3.7)	3 (9.3)	0	2 (6.6)	3 (60)
Total no. abnormal Animals	0	0	8	1	3 (1.9)	15 (35.7)	19 (3.3)	14 (43.7)	2 (2.1)	8 (26.6)	5 (3.0)
Total no. abnormalities	0	0	8	1	3	51	27	42	4	14	5
No. abnormalities per abnormal animal	0	0	1	1	1	3.4	1.4	3.0	2.0	1.7	1

Site sediment and water did induce developmental abnormalities of a type observed during the tadpole and metamorph field surveys. The frequency of abnormalities did not always correlate very well, most notably the high frequencies of *R .pipiens* hind limb abnormalities observed during field surveys were not observed within the Gosner stage 46 limb bud assays.
Future work of this kind would benefit from a more enhanced overlay of laboratory abnormalities with field abnormalities. A more detailed description of limb abnormalities within the limb bud assays would have allowed for more detailed comparison, perhaps providing greater insight.

Summary

In summary we feel that this multidisciplinary approach is on target with regards to providing a greater understanding of the biotic and abiotic factors that are playing a role in the *Rana pipiens* abnormalities. While a definitive answer is still at large, we feel that we are significantly closer to understanding this phenomenon.

Future work should include continued monitoring at study sites, characterizing *Rana pipiens* metamorphs and tadpoles during early developmental stages, drift fence monitoring at the Ward Marsh site, and pesticide monitoring at study sites. Laboratory amphibian developmental toxicity test with atrazine and its metabolites should be carried out to limb development. Field specimens should also be examined for gonadal malformations. Field studies utilizing enclosures would be helpful in providing more information on the role parasites, predators and other environmental factors play in the observed abnormalities.

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Appendix A

Rana pipiens
Metamorph and Tadpole Data
Vermont 2001

Appendix A1. Abnormalities of *Rana pipiens* Metamorphs by Date at the Five Primary Study Sites - 2001

	Otter Creek					Otter		Mud Creek					Mud
	7/10/2001	7/20/2001	8/8/2001	8/23/2001	9/12/2001	**SUMMARY**		7/12/2001	7/24/2001	8/1/2001	8/14/2001	9/11/2001	**SUMMARY**
Hind Limb Malformations													
I. No Limb													
A. Amelia		1		1		2							
II. Reduced hind limb segments													
A. Ectromelia													
1. Femur	1	1	6			8						1	1
2. Tibiafibula													
3. Tibiale fibulare			2	3	2	7			1				1
B. Phocoamelia			1			1							
III. Reduced hind limb elements													
A. Ectrodactyly				2	2	4					1		1
B. Brachydactyly				2	5	7							
IV. Complete but malformed hind limb													
A. Rotation													
B. Micromelia													
C. Hemimelia				2		2				1	1		2
D. Bone bridge													
E. Digits fused													
F. Other			1	1		2							
Fore Limb Malformations													
I. No Limb													
A. Amelia			1			1							
II. Ectomelia					1	1						1	1
III. Reduced fore limb elements													
A. Ectrodactyly											1		1
B. Brachydactyly					2	2					1		1
IV. Emergence Failure					1	1							
Spine Malformations													
I. Scoliosis: curvature													
Head Malformations													
I. Eye Abnormalities													
A. Anophthalmia: missing eye			1		1	2							
B. Small pupil													
C. pupil/iris abnormal													
D. Red eye				1		1							
A. Microcephaly													
B. Curved Jaw													
Other Abnormalities:													
I.Trauma-Skin/ Broken Bone(s)		1											
II. Abnormal color													
III. Cyst above vent													
Total sample size	64	124	130	137	150	605		31	66	61	81	57	296
Number of frogs with malformations	1	3	11	9	12	36		0	1	1	4	2	8
Total number of malformations	1	3	12	12	14	42		0	1	1	4	2	8
% Frogs with malformations	1.6	2.4	8.5	6.6	0.1	6.0		0.0	1.5	1.6	4.9	3.5	2.7
% Malformations	1.6	2.4	9.2	8.8	9.3	6.9		0.0	1.5	1.6	4.9	3.5	2.7

Appendix A1. con't.

	Ward Marsh			Ward				Alburg Dune					Alburg
	7/9/2001	8/7/2001	9/6/2001	**SUMMARY**		6/1/2001	7/6/2001	7/24/2001	8/1/2001	8/9/2001	8/29/2001	9/18/2001	**SUMMARY**
Hind Limb Malformations													
I. No Limb													
A. Amelia							3				1		4
II. Reduced hind limb segments													
A. Ectromelia													
1. Femur							1	2	1			3	6
2. Tibiafibula							1	1	1				2
3. Tibiale fibulare		1	2	3					1				1
B. Phocoamelia			1	1							1		1
III. Reduced hind limb elements													
A. Ectrodactyly		1	1	2							1		1
B. Brachydactyly		1	1	2						3	3	4	10
IV. Complete but malformed hind limb													
A. Rotation											1		1
B. Micromelia									1				1
C. Hemimelia													
D. Bone bridge													
E. Digits fused											1	1	2
F. Other													
Fore Limb Malformations													
I. No Limb													
A. Amelia													
II. Ectomelia			1	1						1			1
III. Reduced fore limb elements													
A. Ectrodactyly													
B. Brachydactyly											2		2
IV. Emergence Failure													
Spine Malformations													
I. Scoliosis: curvature													
Head Malformations													
I. Eye Abnormalities													
A. Anophthalmia: missing eye						1	1	1					1
B. Small pupil													
C. pupil/iris abnormal													
D. Red eye													
A. Microcephaly													
B. Curved Jaw													
Other Abnormalities:													
I.Trauma-Skin/ Broken Bone(s)													
II. Abnormal color													
III. Cyst above vent						1							
Total sample size	93	99	148	340		1	159	99	76	55	132	113	475
Number of frogs with malformations	0	1	3	4		1	5	4	4	4	7	8	27
Total number of malformations	0	3	6	9		1	6	4	4	4	10	8	30
% Frogs with malformations	0.0	1.0	2.0	1.2		na	0.0	4.0	5.3	7.3	5.3	7.1	5.7
% Malformations	0.0	3.0	4.1	2.6		na	0.0	4.0	5.3	7.3	7.6	7.1	6.3

Appendix A1. con't.

	North Hero S.P.				NH
	7/17/2001	8/9/2001	8/30/2001	9/19/2001	**SUMMARY**
Hind Limb Malformations					
I. No Limb					
A. Amelia					
II. Reduced hind limb segments					
A. Ectromelia					
1. Femur					
2. Tibiafibula					
3. Tibiale fibulare		1	2	1	4
B. Phocoamelia					
III. Reduced hind limb elements					
A. Ectrodactyly			2	1	3
B. Brachydactyly		2	6	3	11
IV. Complete but malformed hind limb					
A. Rotation					
B. Micromelia					
C. Hemimelia					
D. Bone bridge					
E. Digits fused					
F. Other					
Fore Limb Malformations					
I. No Limb					
A. Amelia					
II. Ectomelia			1		1
III. Reduced fore limb elements					
A. Ectrodactyly					
B. Brachydactyly		3		2	5
IV. Emergence Failure					
Spine Malformations					
I. Scoliosis: curvature				1	1
Head Malformations					
I. Eye Abnormalities					
A. Anophthalmia: missing eye				1	1
B. Small pupil					
C. pupil/iris abnormal					
D. Red eye					
A. Microcephaly					
B. Curved Jaw					
Other Abnormalities:					
I.Trauma-Skin/ Broken Bone(s)					
II. Abnormal color					
III. Cyst above vent					
Total sample size	145	91	165	163	564
Number of frogs with malformations	0	6	9	9	24
Total number of malformations	0	6	11	9	26
% Frogs with malformations	0.0	6.6	5.5	5.5	4.3
% Malformations	0.0	6.6	6.7	5.5	4.6

Appendix A2. Abnormalities of *Rana pipiens* Metamorphs by Date at the Four Supplemental Study Sites - 2001

	Winooski River		**Winnoski**		**Lewis Creek**		**Cornwall Swamp**		**Missisquoi N.W.R[a]**		**Missisquoi N.W.R**
	7/18/2001	8/22/2001	**SUMMARY**		7/25/2001		7/26/2001		7/16/2001		7/31/2001
Hind Limb Malformations											
I. No Limb											
A. Amelia											
II. Reduced hind limb segments											
A. Ectromelia											
1. Femur											
2. Tibiafibula		1	1								
3. Tibiale fibulare	1		1						1		
B. Phocoamelia											
III. Reduced hind limb elements											
A. Ectrodactyly		1	1								
B. Brachydactyly		5	5								
IV. Complete but malformed hind limb											
A. Rotation											
B. Micromelia											
C. Hemimelia											
D. Bone bridge											
E. Digits fused											
F. Other											
Fore Limb Malformations											
I. No Limb											
A. Amelia											
II. Ectomelia		1	1				1**T**				1
III. Reduced fore limb elements											
A. Ectrodactyly											
B. Brachydactyly		1	1								
IV. Emergence Failure											
Spine Malformations											
I. Scoliosis: curvature											
Head Malformations											
I. Eye Abnormalities											
A. Anophthalmia: missing eye											
B. Small pupil							7				
C. pupil/iris abnormal		1	1								
D. Red eye											
A. Microcephaly											1
B. Curved Jaw											
Other Abnormalities:											
I.Trauma-Skin/ Broken Bone(s)									1		1
II. Abnormal color											
III. Cyst above vent											
Total sample size	111	129	240		53		122		101		104
Number of frogs with malformations	1	9	10		0		8		1		3
Total number of malformations	1	10	11		0		8		1		3
% Frogs with malformations	0.9	7.0	4.2		0.0		6.6		0.9		2.9
% Malformations	0.9	7.8	4.6		0.0		6.6		0.9		2.9

Appendix A3. Abnormalities of *Rana pipiens* Tadpoles by Date at the Five Primary Study Sites in Vermont in 2001

	OTC 5/24/2001	OTC 6/6/2001	OTC 6/25/2001	OTC Summary	MDC 5/29/2001	MDC 6/7/2001	MDC 6/21/2001	MDC SUMMARY	WM 5/31/2001	ALB 6/1/2001	ALB 6/14/2001	ALB 6/26/2001	ALB SUMMARY	NH 6/4/2001	NH 6/19/2001	NH SUMMARY
Hind Limb Malformations																
I. No Limb:																
A. Amelia																
II. Reduced hind limb segments																
A. Ectromelia																
1. Femur			2[a]	2[a]			1	1				1	1			
2. Tibiafibula			1[a]	1[a]								2	2			
3. Tibiale fibulare												1	1			
B. Phocoamelia												1	1			
III. Reduced hind limb elements																
A. Ectrodactyly												1	1			
B. Brachydactyly																
IV. Complete but malformed hind limb																
A. Differential development											2		2			
B. Polyphylangy					1			1								
C. Delayed development											1		1			
D. Limb misshappen														1		1
IV.Head Malformations																
1. Contorted head		1		1		1		1								
2. Eye Abnormalities																
A. Small pupil							1	1								
B. pupil/iris abnormal		1		1												
C. Red spot near eye	2[e]			2		1		1			1		1			
V.Other Abnormalities:																
1. Forked tail						1		1								
2. Trauma related[b]																
A.Red spots on hind limb(s)		1		1		7	1	8	2	3	5		8			
B.Red spot(s) on body					1	9		10	2	1	1		2			
C.Red spot(s) on the tail					1	4		5								
D. Tail Trauma[c]					1[c]	20[c]	5[c]	26[c]	1[c]	6[c]			6[c]		4[c]	4[c]
E. Tail short							4	4		7			7			
F. Body Trauma					1	2[f]		3							2	2
3.Protozoa -Epistylis[c] (by vent)		11[d]		1[d]		20[d]	1[d]	21[d]								
4. Bumps on the tail proximal to body																
Total sample size	72	69	27	168	84	107	58	249	91	215	199	163	577	70	88	158
Number of tadpoles with abnormalities	2	3	0	5	2	18	6	26	2	7	8	4	19	1	2	3
Total number of abnormalities	2	3	0	5	5	25	7	37	4	11	10	6	27	1	2	3
% tadpoles with abnormalities	2.7	4.3	0.0	3.0	2.3	16.8	10.3	10.4	2.1	3.2	4.0	2.4	3.3	1.4	2.2	1.9
% Abnormalities	2.7	4.3	0.0	3.0	5.9	23.3	12.0	14.8	4.3	5.1	5.0	3.6	4.6	1.4	2.2	1.9

Note: The total number of abnormalities may or may not equal the number of abnormal animals as many specimens had more than one abnormality.

[a]Trauma-capture injury not included in total(s) or percent calculations
[b]Trauma - causation uncertain
[c] Tail Trauma- not included in total(s) or percent calculations
[d]Protozoa (Epistylis) located by vent not included in total(s) or percent calculations
[e]Red spots diminished after 24hrs
[f]Tadpole dead (recent)

Appendix B

Photo Atlas:
Radiographs of *Rana pipiens*
Vermont 2001

Figure B1(a&b). Ward Marsh: Sept 6, 2001 Phocomelia. **B:** Close up

Figure B2.Alburg: July 24, 2001(A) Ectromelia.

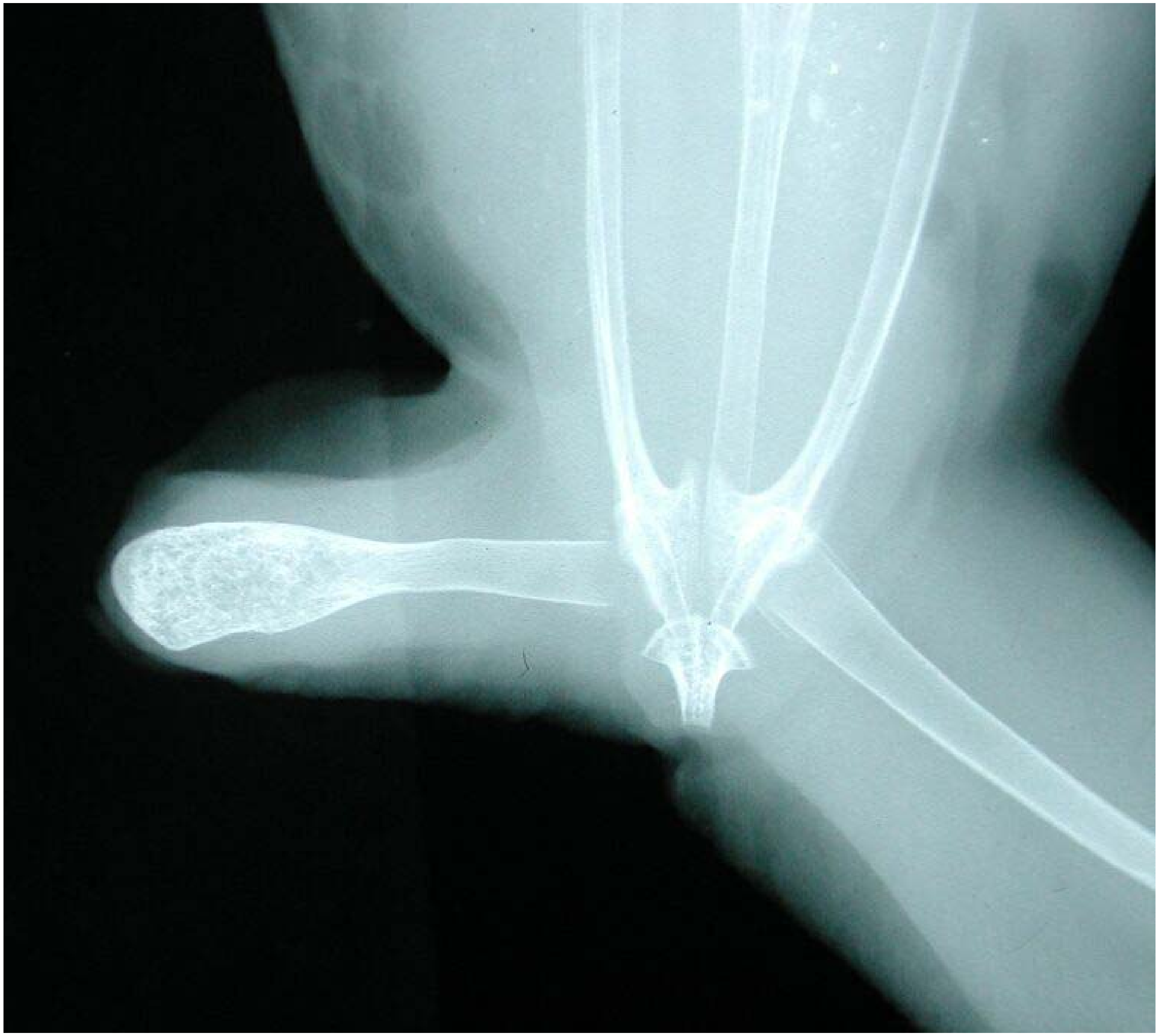

Figure B3. Ectromelia.

Figure B4. Otter Creek: June 25, 2001(B) Capture injury, bilateral truncation.

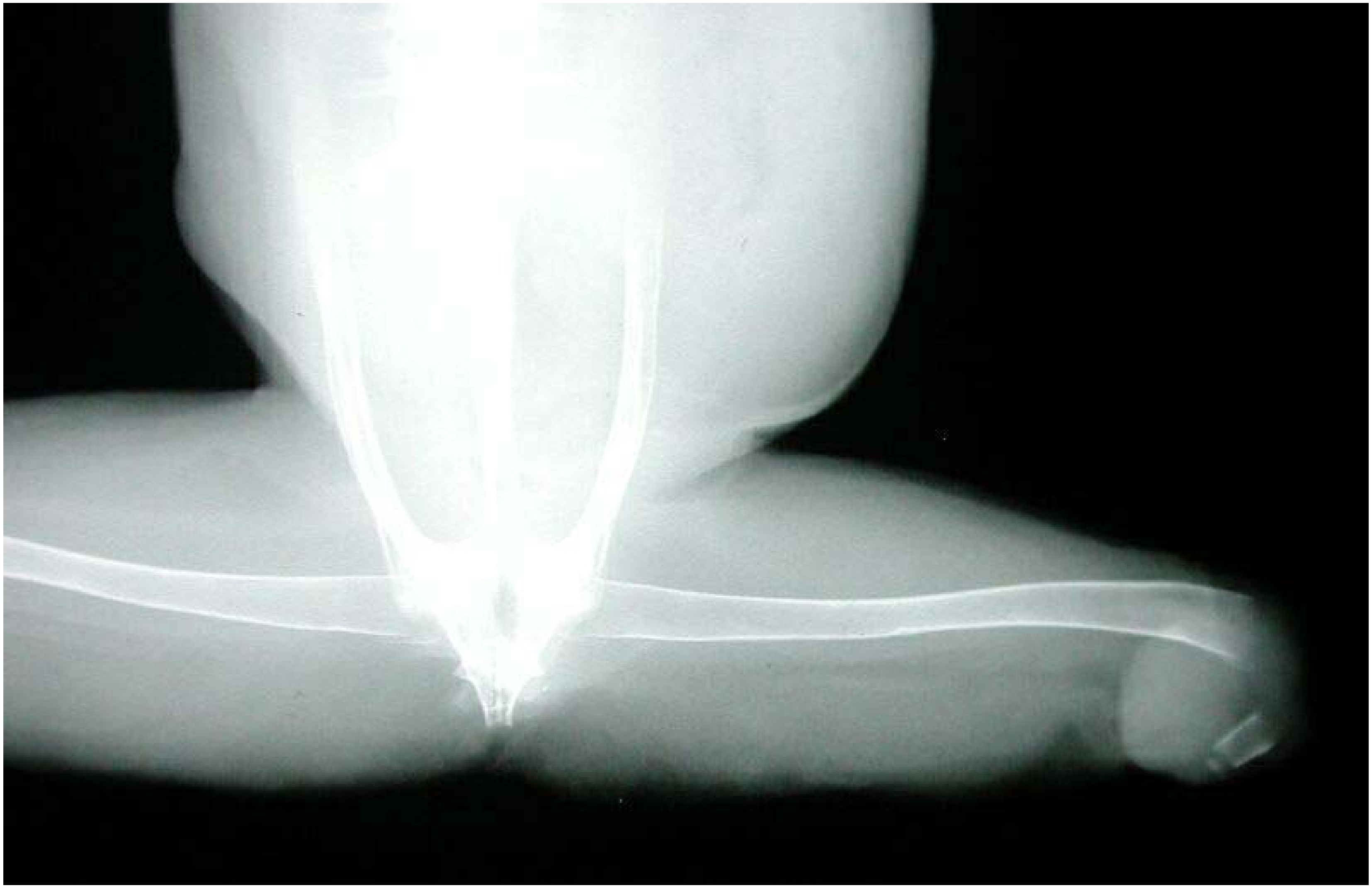

Figure B5. Ectromelia.

Figure B6. Otter Creek: Sept. 9, 2001(G) Ectromelia – forelimb.

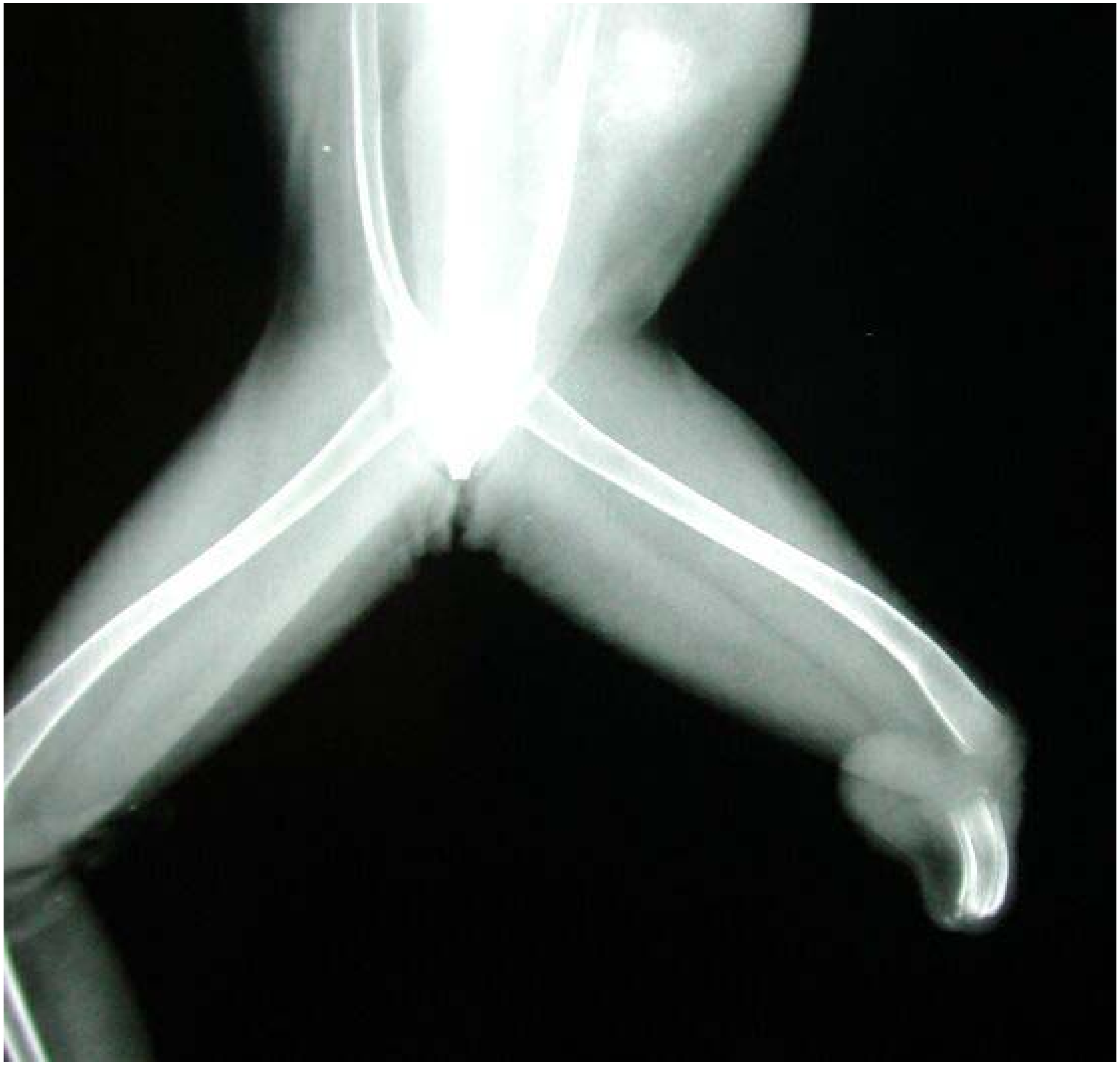

Figure B7. Ectromelia

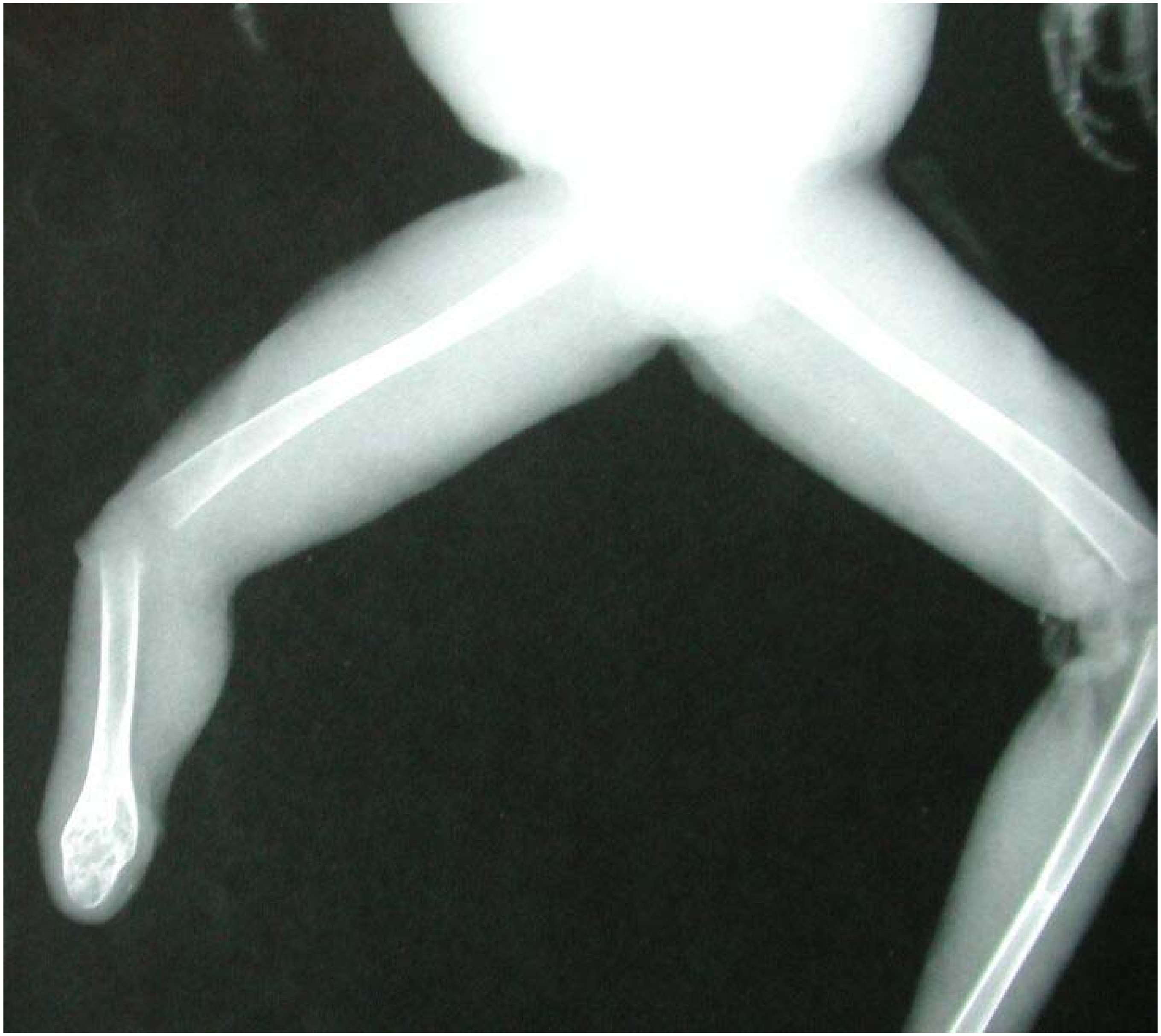

Figure B8. Ectromelia.

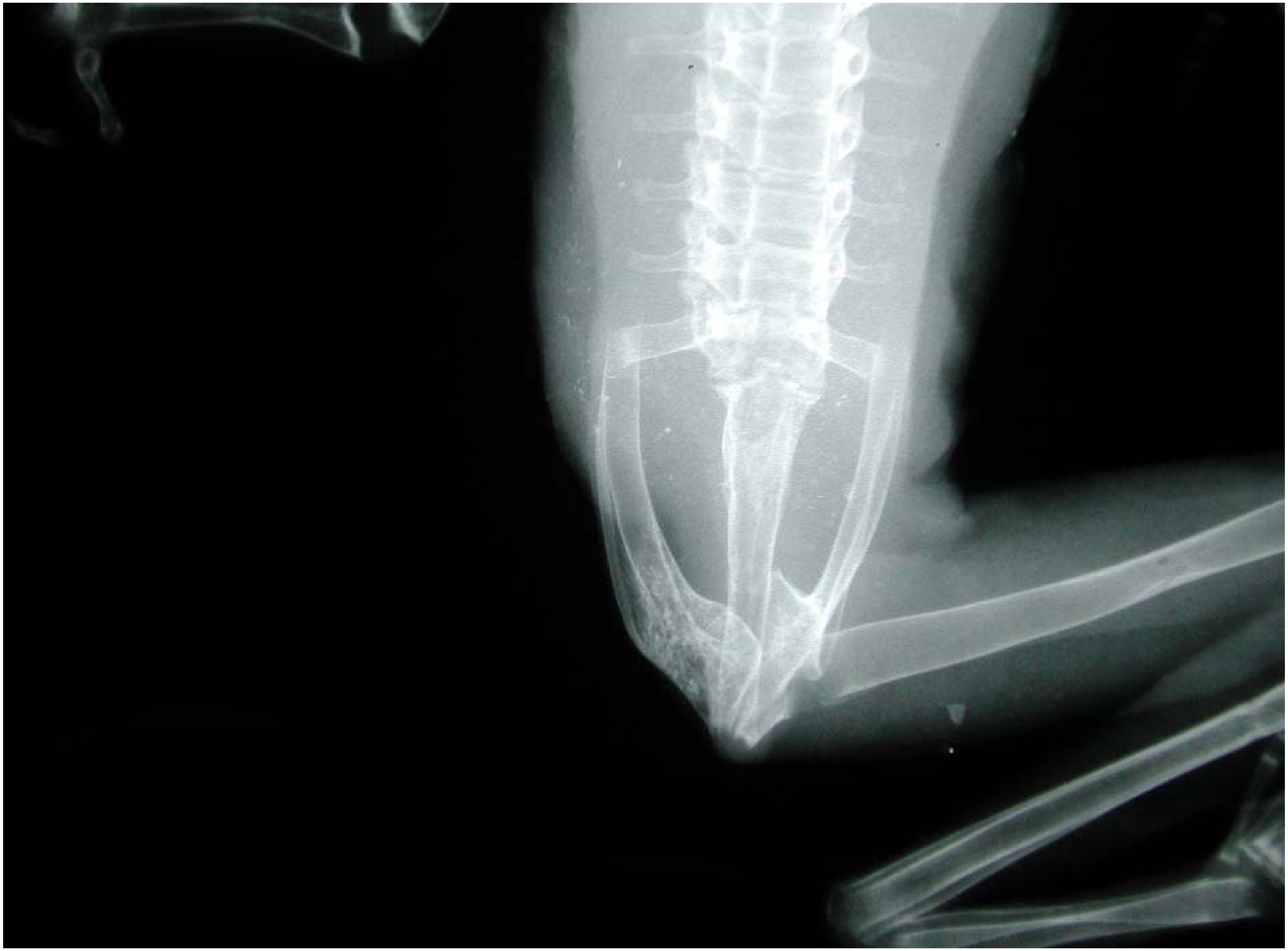

Figure B9. Alburg: August 29, 2001(C). Amelia.

Figure B10. Ectromelia.

Figure B11. Alburg: July 6, 2001(D) Bilateral truncation.

Appendix C

Photo Atlas:
Rana pipiens – Metamorphs
Vermont 2001

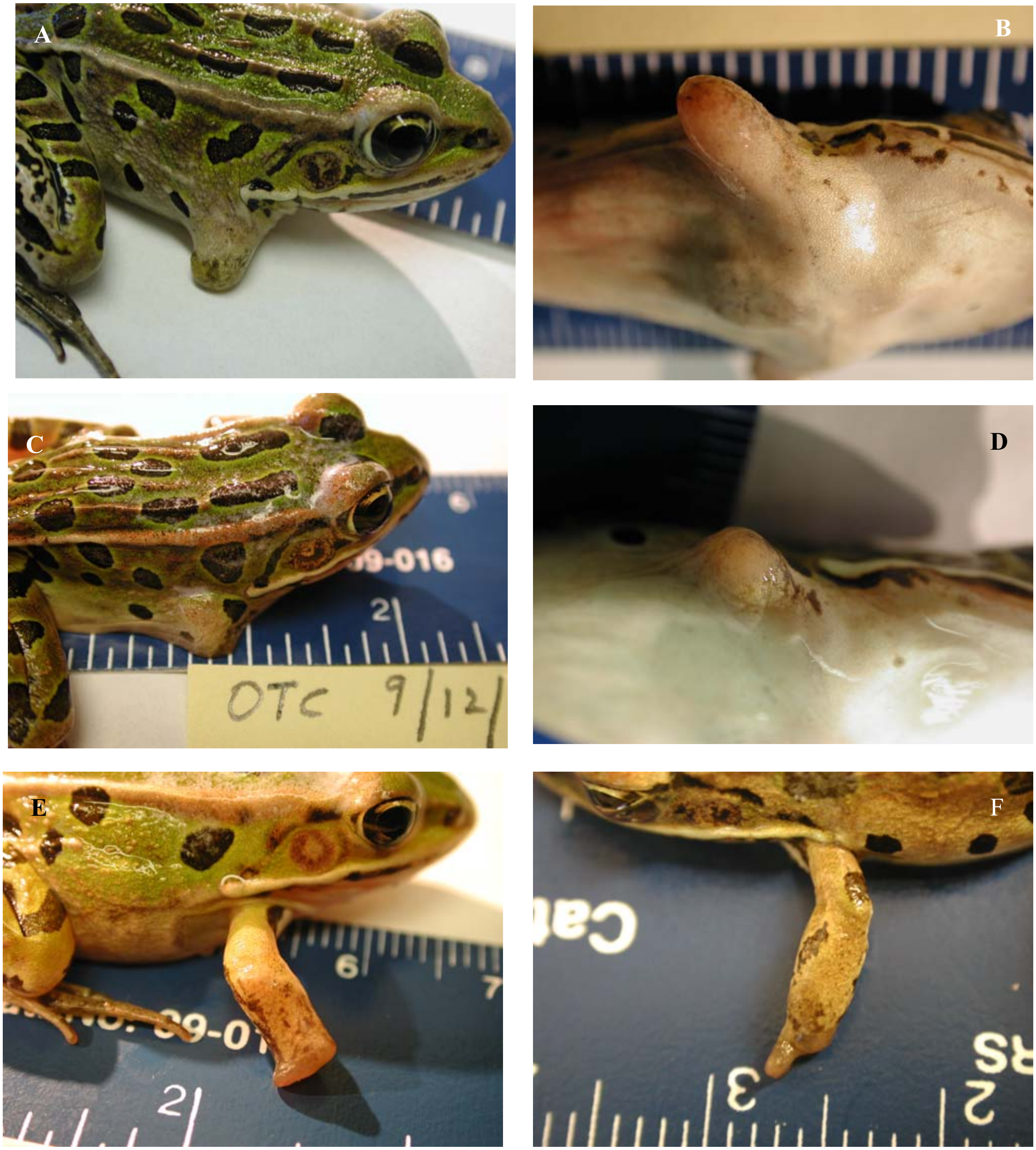

Figure C1. Ectromelic forelimbs (A-F). **A,B:** Otter Creek September, 1201G. **C,D:** Otter Creek September, 1201B. **E:** Winooski August, 2201B. **F:** Otter Creek August, 0801C.

Figure C2. Amelia (A-D). **A,B:** Alburg Dune September, 1801B, amelia of right hind limb. **C,D:** Otter Creek August, 2301F, amelia of left hind limb. Note: Radiographs may reveal specimens are not truly amelic.

Figure C3. Ectomelia of tibiafibula and abnormal pigmentation, Alburg Dune June, 2601E (A-D). **A,B:** Showing truncated limb and dorsal view of pigmentation. **C,D:** Ventral view of truncation and abnormal pigmentation. Note: Pigmentation is normally absent from ventral side.

Figure C4. Ectromelia and abnormal pigmentation (A-D). **A,B:** Winooski August, 2201D. **A:** Ectromelia of the tibiafibula. **B:** Ventral view showing abnormal pigmentation by truncation. **C,D:** Ward Marsh September, 0601A. **C:** Ectromelia of the left hind tibiafibula, note abnormal pigmentation pattern by terminus of truncation. **D:** Ventral view showing abnormal pigmentation of truncated limbs.

Figure C5. Phocomelia and abnormal pigmentation (A-E). **A-E:** Ward Marsh September, 0601C, right hind limb phocomelia. **A,B:** Phocomelia right hind limb. **C,D:** Showing abnormal pigmentation on ventral side of phocomelic limb. **E:** Radiograph of right hind limb.

Figure C6. Eye abmormalities (A-D). **A:** Alburg Dune July, 2401D, right eye anophthalmia. **B:** Otter Creek September, 1201F, left eye anopthalmia . **C:** Winooski August, 2201A, left eye large pupil “black eye?” **D:** Winooski July, 1801A, left eye “pin pupil.”

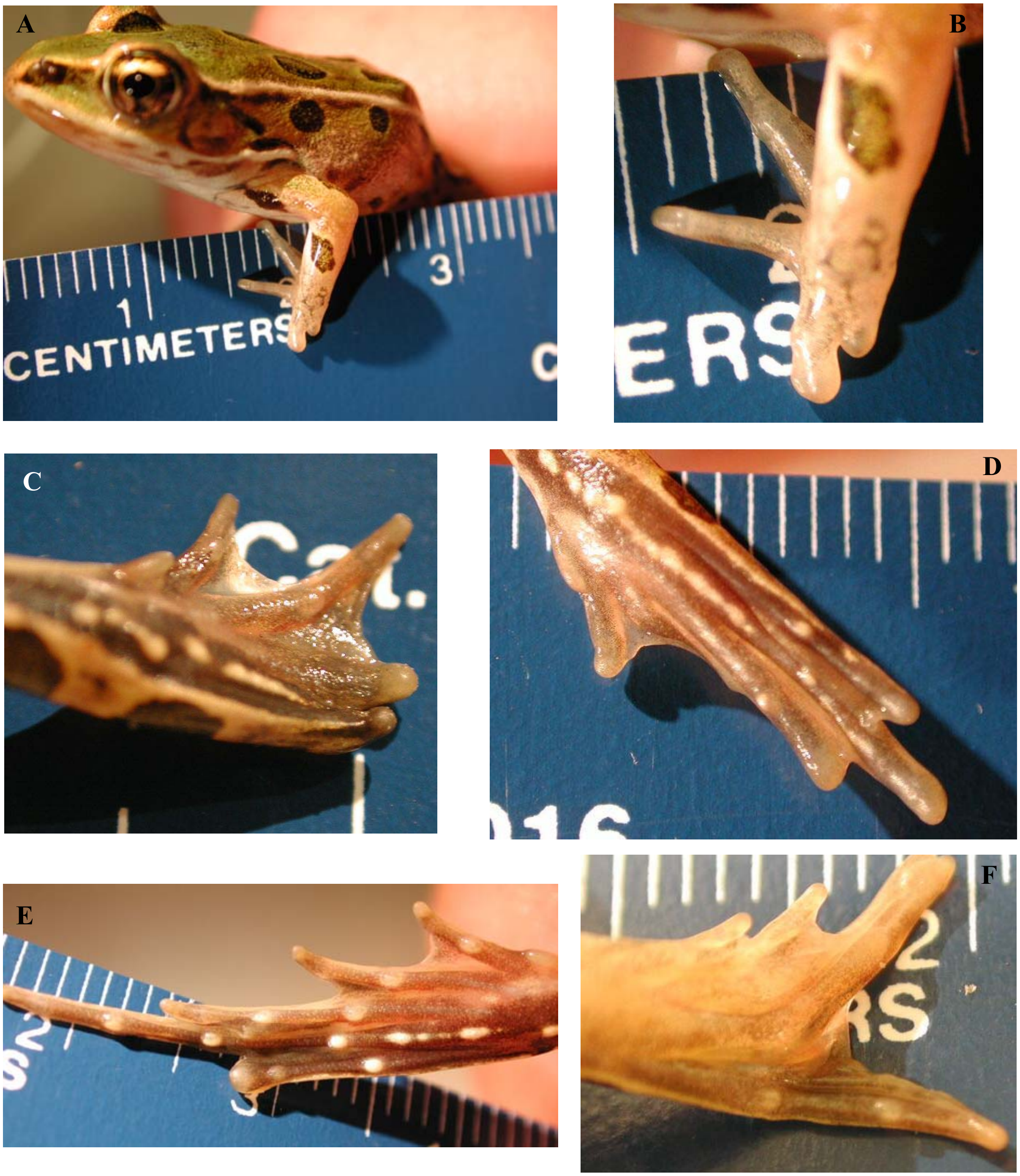

Figure C7. Ectro/brachydactyly (A-E). **A,B:** North Hero August, 0901D, left forelimb ectro/ brachydactyly. **C:** North Hero August 3001E, left hind limb ectro/ brachydactyly. **D:** North Hero August, 0901A, right hind limb ectrodactyly. **E:** Alburg Dune August, 0901B, right hind limb brachydactyly. **F:** Alburg Dune August, 2901A, left hind limb brachydactyly, 3rd and 4th digit fused.

Appendix D

Photo Atlas:
Rana pipiens – Larvae
Vermont 2001

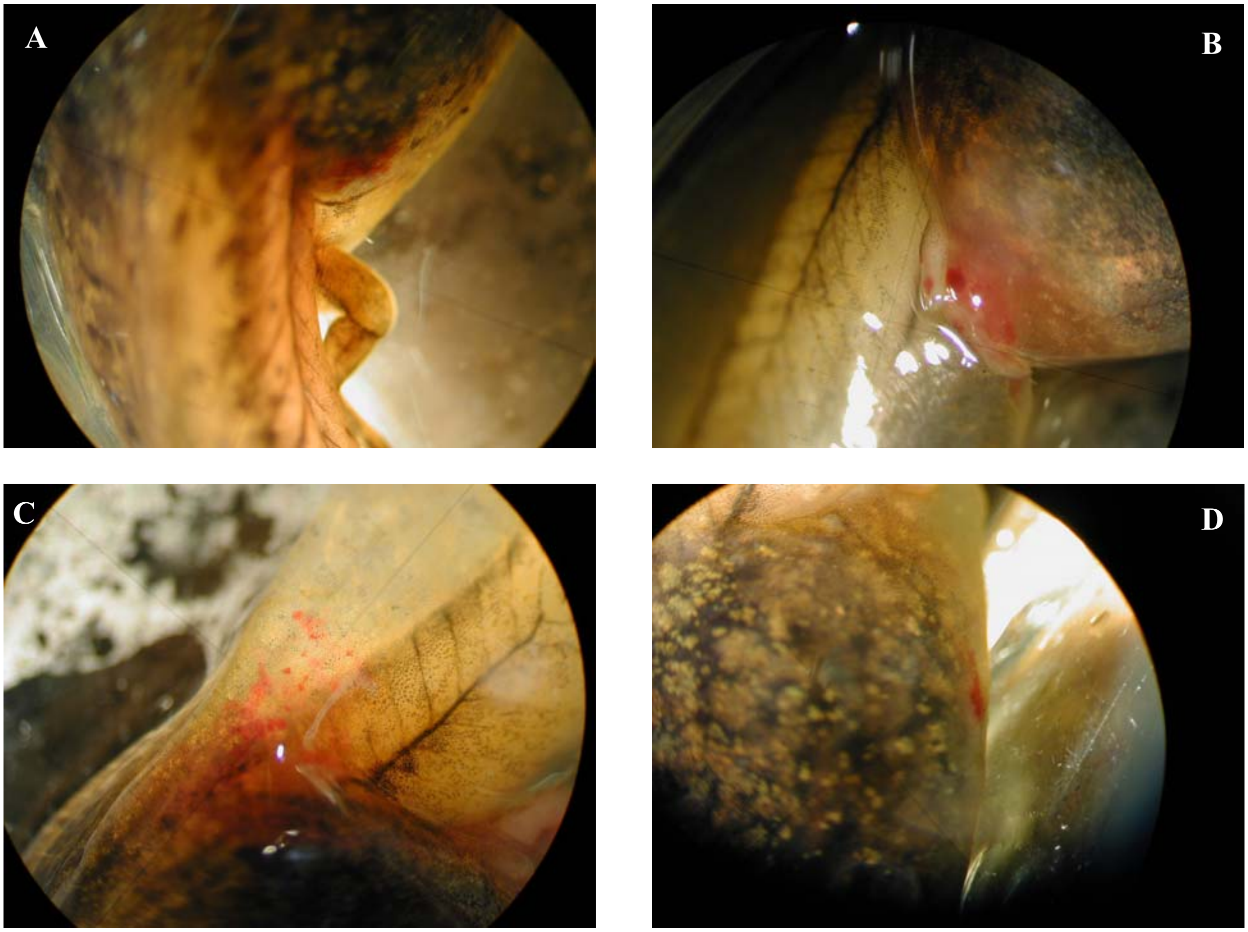

Figure D1. Tadpole trauma showing red spots and hemorrage in close proximity to hind limb (A-D). **A:** Alburg Dune June, 1401C. **B:** Alburg Dune June, 0101A. **C:** Alburg Dune June, 0101C. **D:** Mud Creek May, 2901B.

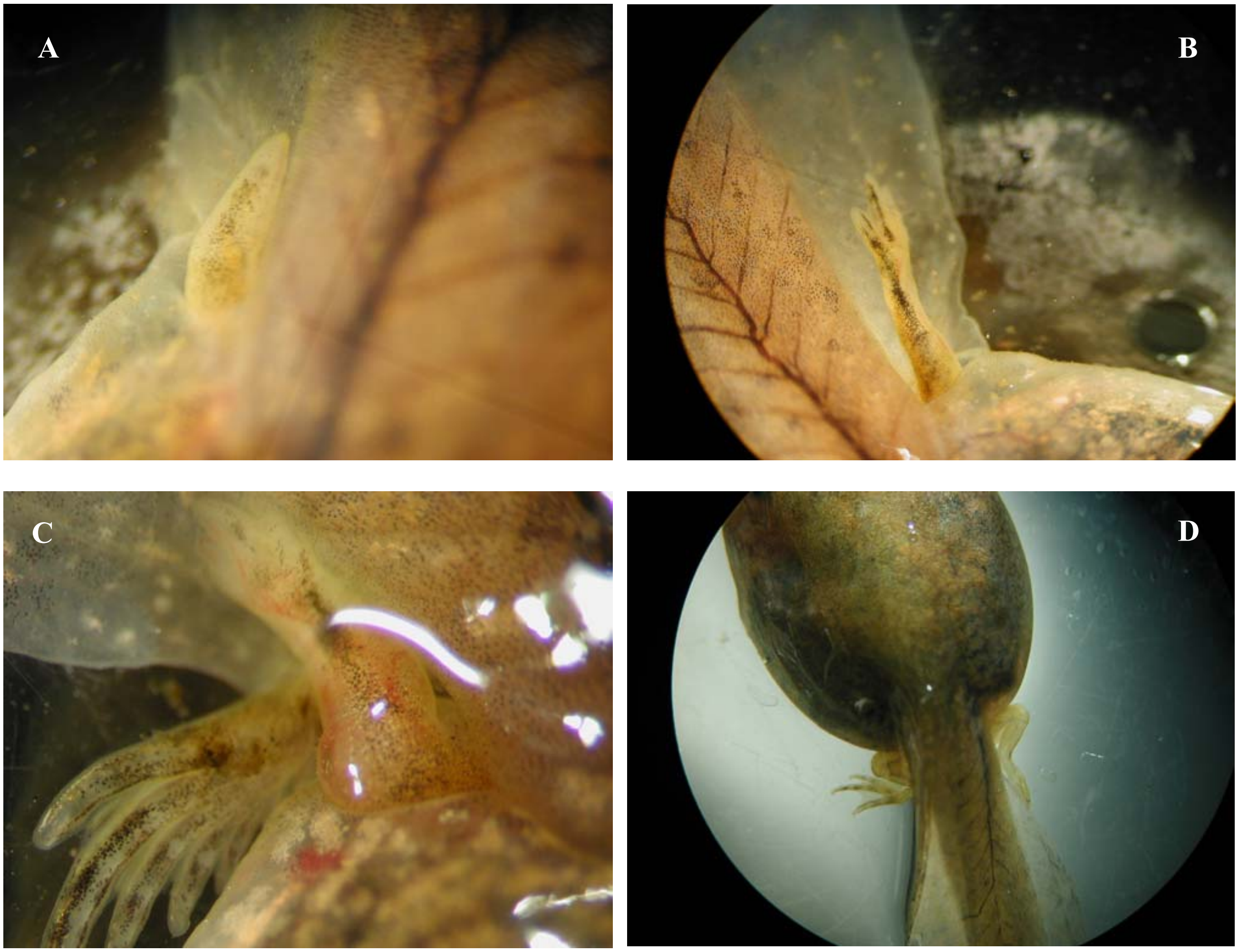

Figure D2. Progression of differential development. (A-D). **A-D:** Alburg Dune June, 1401A. **A,B:** Photos taken on same day. **A:** Left hind limb, Gosner stage 29. **B:** Right hind limb, Gosner stage 36. **C,D:** photos taken one week later. **C:** Left limb, Gosner stage 34, right limb Gosner stage 37. **D:** Left limb Gosner stage 37, right limb, Gosner stage 40.

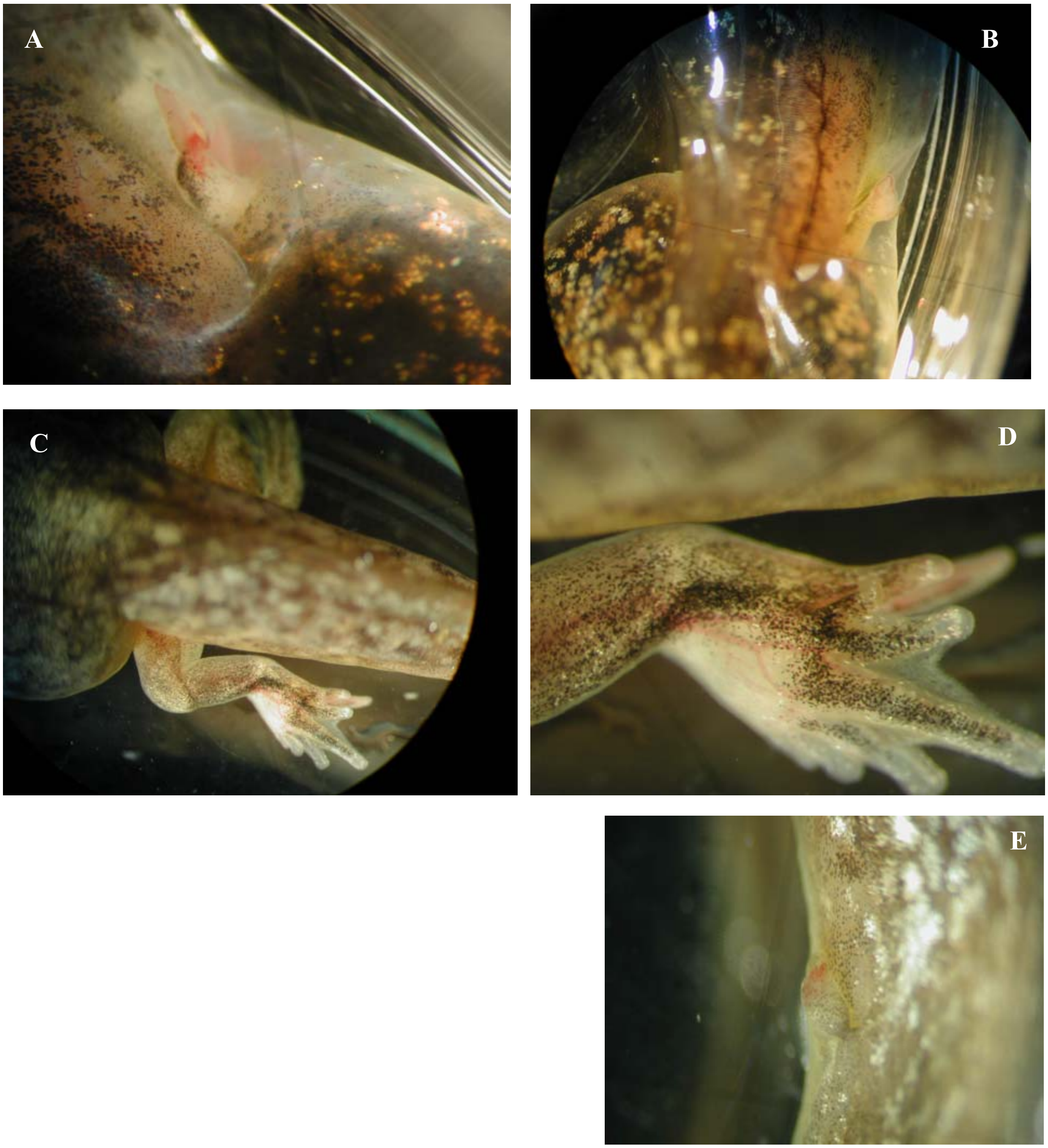

Figure D3. Progression of polydactyly, Mud Creek May, 2901B (A-E). **A:** Gosner stage 33, showing constrictions and redness. **B:** Gosner stage 34. **C,D:** Gosner stage 37, showing multiple extra digits, two weeks later. **E:** Abnormal growth on tail.

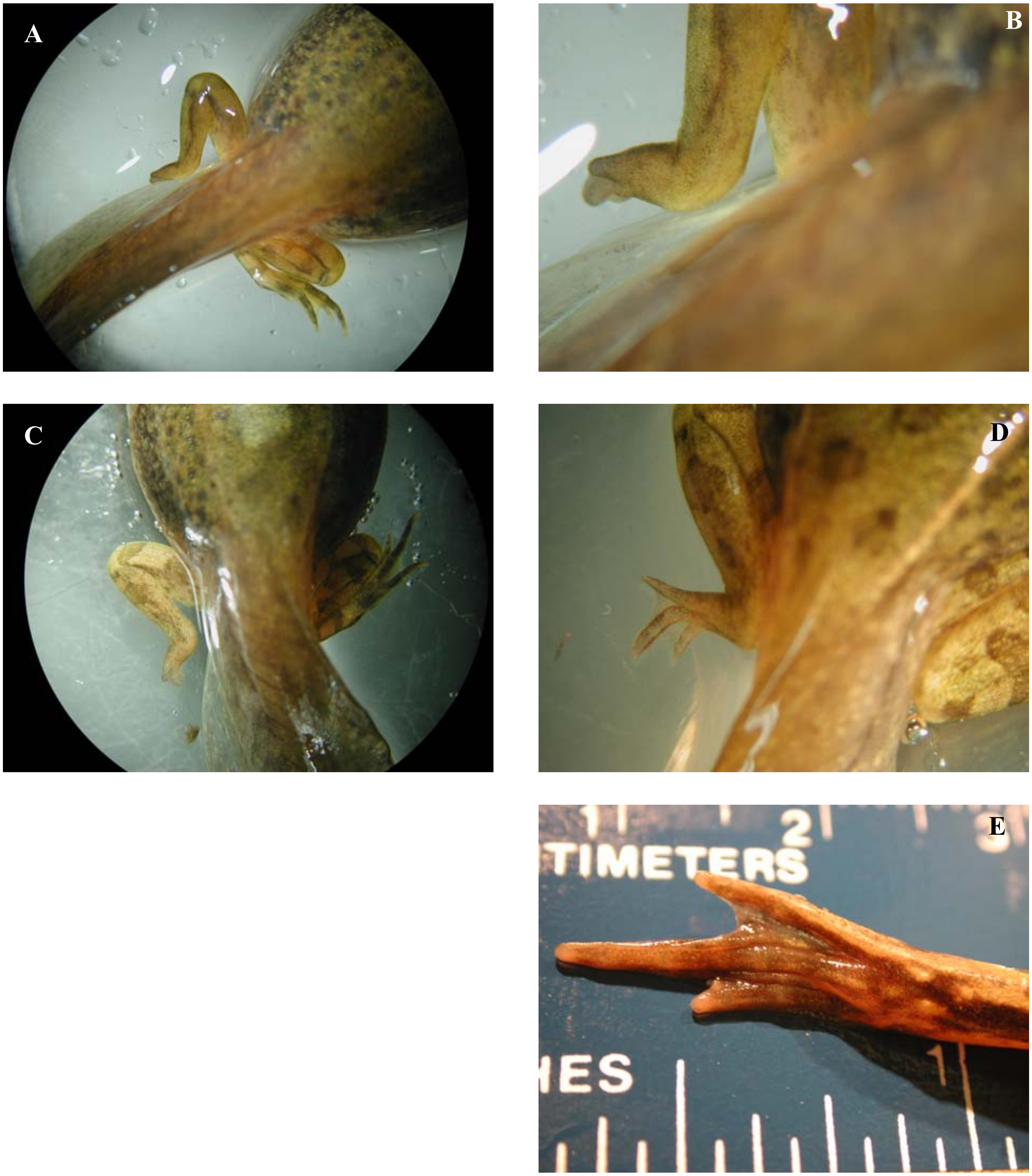

Figure D4. Progression of differential development and ectrodactly, Alburg Dune June, 2601B (A-E). **A:** Right limb, Gosner stage 40; left limb Gosner stage 35. **B:** Close up of left limb, Gosner stage 35. (note only 3 digits). **C,D:** Showing progression of growth on left limb, Gosner stage 36-37. **E:** Metamorph reveals left limb with ectrodactly.

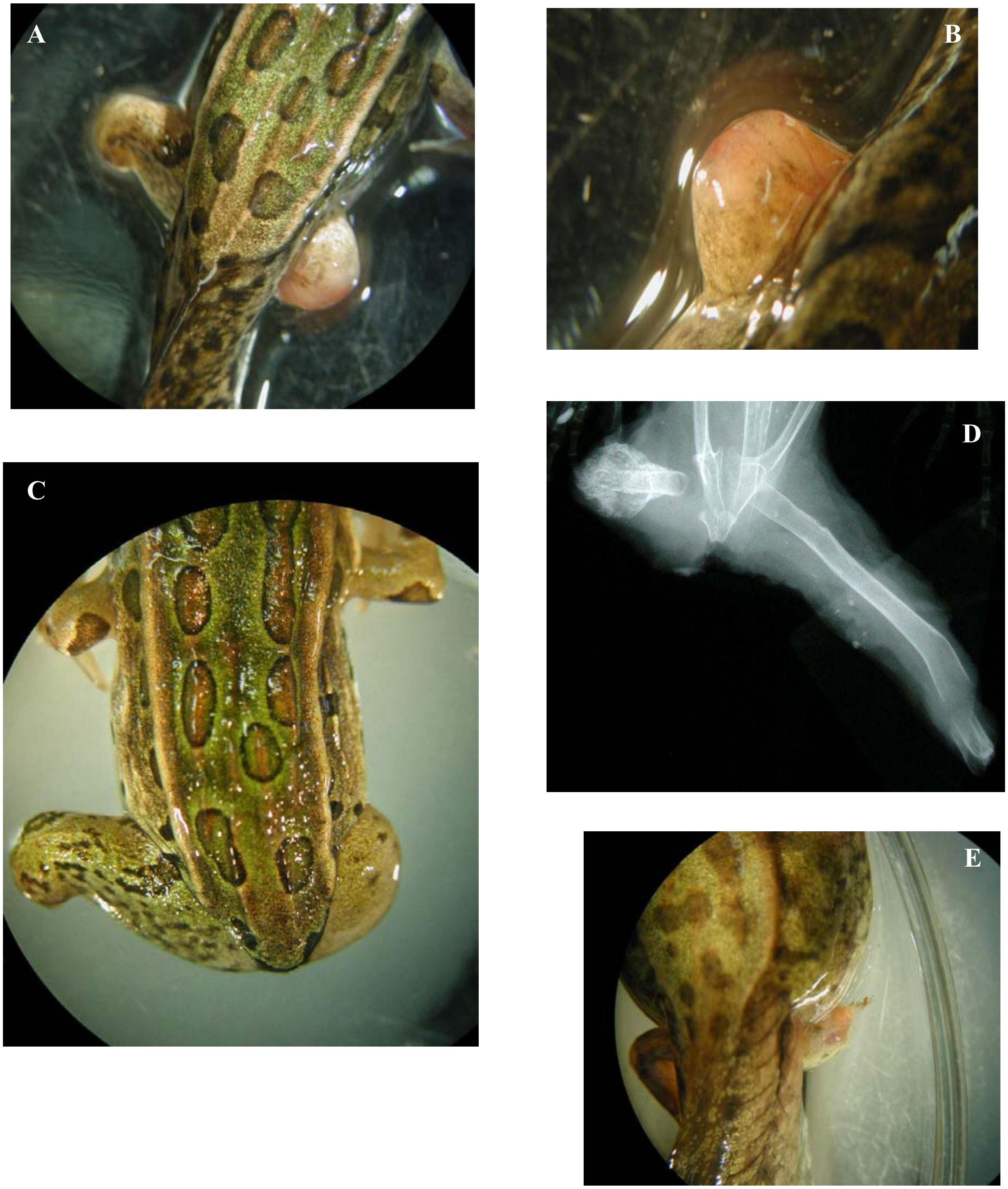

Figure D5. Capture injury trauma (A-E). **A-D:** Otter Creek, June 2501B, Bilateral hind limb truncation. **A:** Bilateral hind limb truncation (1 week after trauma). **B:** Close up of right hind limb truncation (1 week after trauma). **C:** Bilateral hind limb truncation, note pigmentation (2.5 weeks after trauma). **D:** Radiograph of right hind limb truncation showing severe proliferative terminal right femur (after metamorphisis). **E:** Otter Creek, June 2501A; right hind limb truncation (1 week after trauma).

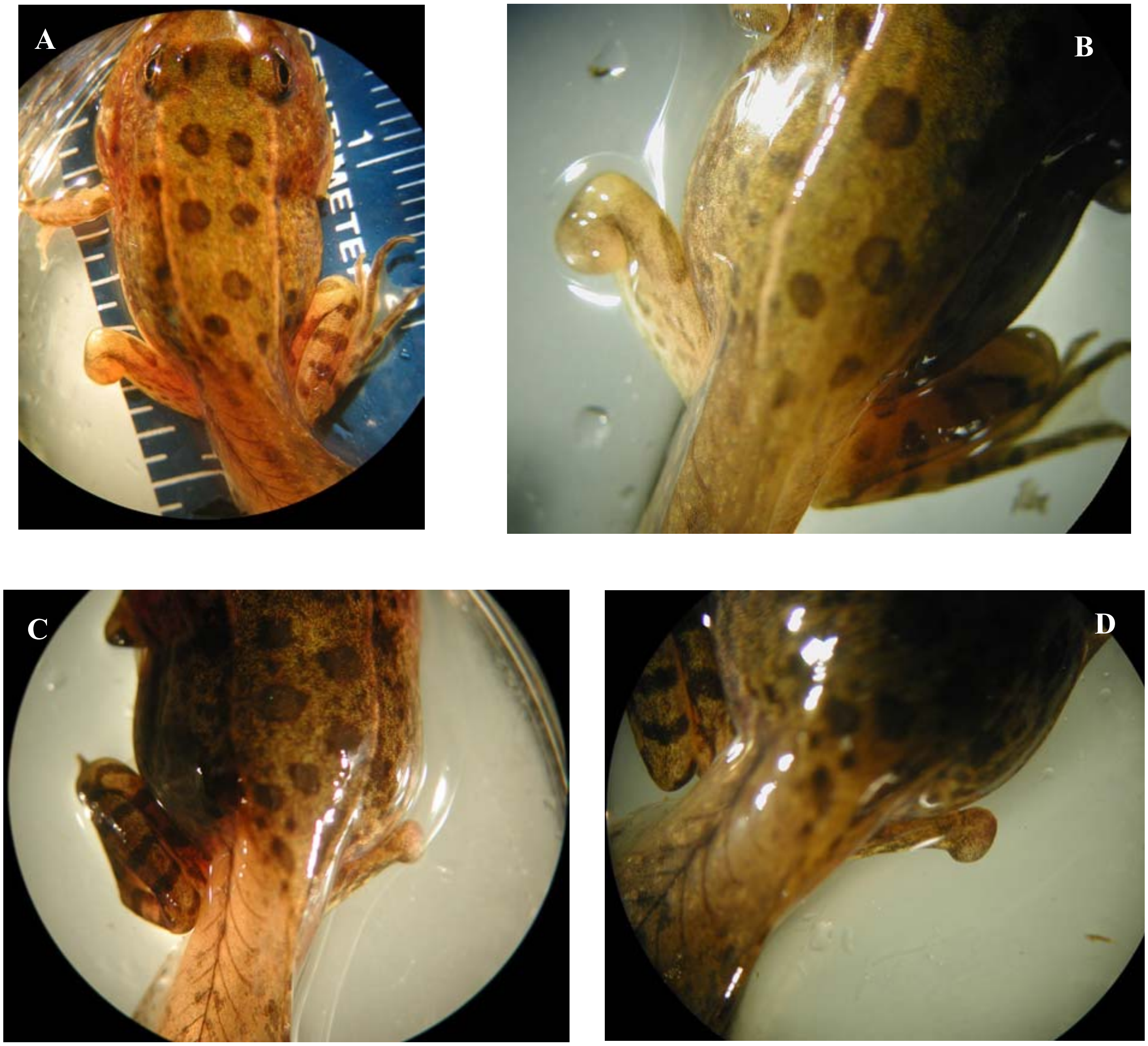

Figure D6. Late stage (Gosner 42) hind limb ectromelia (A-D). **A,B:** Alburg Dune June, 2601A, ectromelia of left tibiafibula (Gosner stage 42). **C,D:** Alburg Dune June, 2601C, ectromelia of right tibiafibula (Gosner stage 42).

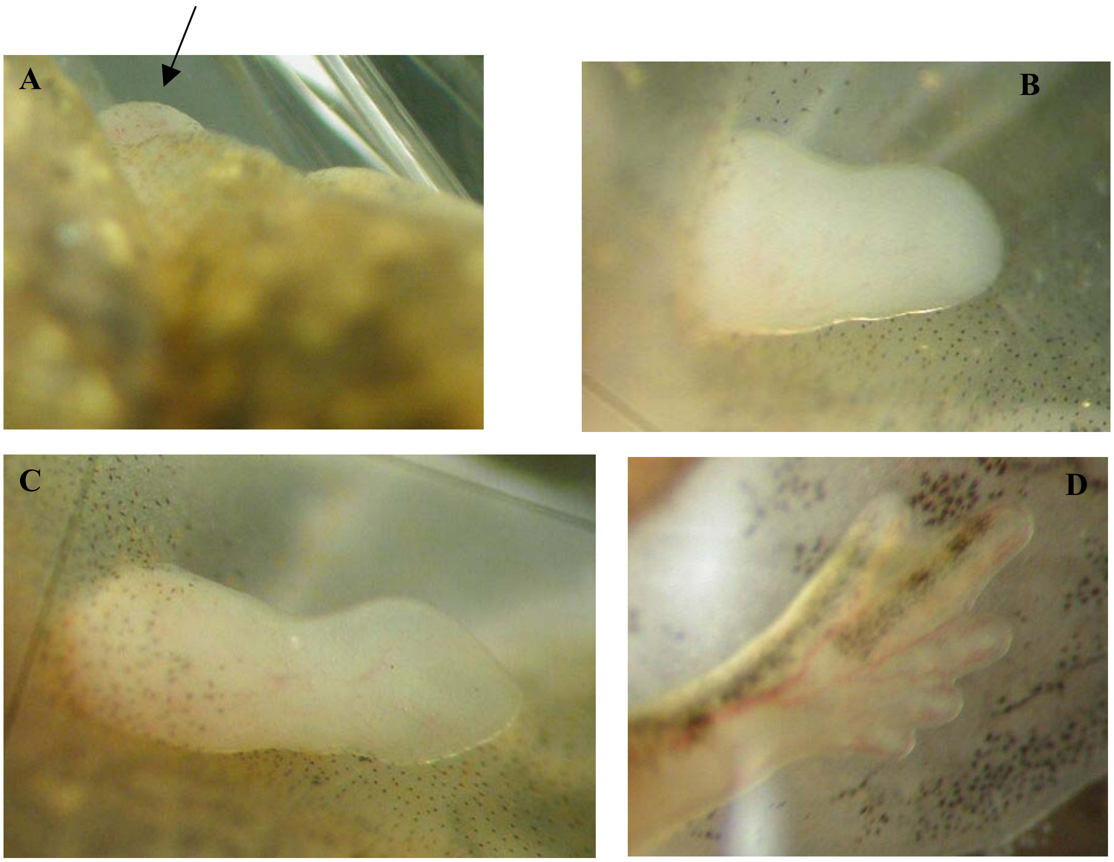

Figure D7 . Progression of limb development (A-E). **A:** Gosner stage 26. **B:** Gosner stage 29. **C:** Gosner stage 31. **D:** Gosner stage 36. **E:** Gosner stage 37, note vent growth – protozoa (epistylis).

Appendix E

Raw Data:

General Water Chemistry
Mortality
Developmental Stages
Malformations

CLIENT NAME: VDEC CLIENT/PROJECT No.: VERM01 W.O. No.: 00004

Date	Tech Initials	Sample ID	Sample No.	Temp (C)	pH (su)	DO (mg/L)	Conduct. (µS/cm)	Hardness (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Chlorine (mg/L)
05/03/01	PWP	MDC Water	001		6.0	9.0	94.1	60.0	32.0	0.24	0.06
		NH Water	002		6.3	7.5	109.6	65.0	46.0	0.41	0.06
		ALB Water	003		6.5	9.3	114.8	57.5	47.0	0.10	0.05
05/04/01	PWP	FETAX Solution	-		7.2	7.6	1415.0	100.0	60.0	<0.06	0.04
05/05/01	PWP	MDC Water	001		5.9	9.2	97.5				
		NH Water	002		6.1	8.1	122.5				
		ALB Water	003		6.2	9.4	126.2				
		POL Water	004		6.2	8.7	145.8				
		OTC Water	005		6.3	9.3	83.2				
05/06/01	PWP	MDC Water	001		6.0	9.0	95.3				
		NH Water	002		6.1	8.0	115.4				
		ALB Water	003		6.2	9.3	124.8				
		POL Water	004		6.1	8.9	143.6				
		OTC Water	005		6.3	9.1	85.1				
05/07/01	PWP	MDC Water	001		6.2	7.8	102.0				
		NH Water	002		6.2	7.3	125.6				
		ALB Water	003		6.2	8.0	129.0				
		POL Water	004		6.3	7.7	148.8				
		OTC Water	005		6.3	8.0	85.3				
05/08/01	PWP	MDC Water	001		6.2	7.5	103.7				
		NH Water	002		6.2	7.2	127.3				
		ALB Water	003		6.2	7.6	128.9				
		POL Water	004		6.4	7.6	148.5	80.0	66.0		0.02
		OTC Water	005		6.4	7.6	85.3	60.0	36.0		0.09
05/09/01	PWP	MDC Water	001		6.0	9.1	104.6				
		NH Water	002		6.1	8.8	127.0				
		ALB Water	003		6.2	9.0	128.3				
		POL Water	004		6.3	9.0	148.6			0.22	
		OTC Water	005		6.3	9.1	85.0			0.14	

CLIENT NAME: VDEC CLIENT/PROJECT No.: VERM01 W.O. No.: 00004

Date	Tech Initials	Sample ID	Sample No.	Temp (C)	pH (su)	DO (mg/L)	Conduct. (µS/cm)	Hardness (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Chlorine (mg/L)
05/11/01	PWP	MDC-A	001	20.0	6.2	4.4					
(Sediment and		MDC-C	001	19.0	6.3	4.7					
aeration added)		MDC-E	001	19.0	6.4	3.7					
		NH-A	002		6.6	2.2					
		NH-C	002		6.7	1.7					
		NH-E	002		6.7	2.7					
		ALB-A	003		6.5	4.6					
		ALB-C	003		6.4	5.1					
		ALB-E	003		6.4	2.8					
		POL-A	004		6.5	2.1					
		POL-C	004		6.5	5.4					
		POL-E	004		6.4	6.3					
		OTC-A	005		6.2	2.8					
		OTC-C	005		6.1	6.4					
		OTC-E	005		6.1	5.9					
05/14/01	PWP	MDC-B	001	22.0	6.4	6.5					
		NH-D	002	21.5	6.6	6.4					
		ALB-C	003	21.5	6.8	6.2					
		POL-A	004	22.0	6.4	6.6					
		OTC-E	005	21.0	6.5	5.9					
05/16/01	PWP	MDC-C	001	21.5	6.5	6.4					
		NH-E	002	21.0	6.7	5.8					
		ALB-D	003	21.5	6.9	5.8					
		POL-B	004	21.5	6.5	6.8					
		OTC-A	005	22.0	6.5	5.8					
05/18/01	PWP	FETAX Solution	-	22.0	7.0	7.9					
		MDC-D	001	21.5	6.8	6.5					
		NH-A	002	22.0	7.0	5.6					
		ALB-E	003	21.3	7.1	4.8					
		POL-C	004	21.5	7.1	5.3					
		OTC-B	005	21.0	6.9	7.3					
05/21/01	PWP	FETAX Solution	-	21.0	7.4	7.4					
		MDC-E	001	21.0	6.9	6.5				4.64	
		NH-D	002	21.0	7.3	6.5				3.64	
		ALB-A	003	21.0	7.1	7.1				0.44	
		POL-D	004	21.0	7.0	5.5				0.48	
		OTC-C	005	21.0	7.1	5.8				9.92	

CLIENT NAME: VDEC CLIENT/PROJECT No.: VERM01 W.O. No.: 00004

Date	Tech Initials	Sample ID	Sample No.	Temp (C)	pH (su)	DO (mg/L)	Conduct. (µS/cm)	Hardness (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Chlorine (mg/L)
05/23/01	PWP	FETAX Solution	-	21.0	7.0	3.5					
		MDC-A	001	21.3	7.1	6.1					
		NH-C	002	20.7	7.1	2.9					
		ALB-B	003	21.3	7.4	6.5					
		POL-E	004	20.7	7.4	5.4					
		OTC-D	005	21.1	7.0	3.7					
05/25/01	PWP	FETAX Solution	-	20.6	7.4	7.1					
		MDC-B	001	21.1	6.9	4.9					
		NH-D	002	20.5	7.2	6.4					
		ALB-A	003	21.0	7.4	6.9					
		POL-A	004	21.5	7.2	4.8					
		OTC-E	005	20.5	7.1	5.1					
05/30/01	PWP	MDC	001		6.2	8.0	127.9	80.0	56.0	0.57	0.07
		NH	002		6.1	7.7	107.0	65.0	54.0	0.79	0.11
		ALB	003		6.0	6.4	103.7	75.0	50.0	1.33	0.05
		POL	004		6.4	6.0	190.2	95.0	98.0	0.24	0.06
		OTC	005		6.2	8.0	116.6	80.0	71.0	0.19	0.11
06/01/01	PWP	FETAX Solution	-	21.0	7.4	8.7					
		MDC-A	001	21.0	6.9	6.9					
		NH-B	002	21.0	7.0	5.8					
		ALB-G	003	21.5	7.2	7.5					
		POL-H	004	21.0	7.2	6.8					
		OTC-E	005	21.0	7.0	7.0					
06/04/01	PWP	FETAX Solution	-	22.8	7.7	6.8					
		MDC-B	001	22.8	7.3	5.8					
		NH-C	002	21.2	7.2	2.5					
		ALB-H	003	22.5	7.5	6.7					
		POL-I	004	20.5	7.5	6.5					
		OTC-A	005	23.9	6.9	5.0					
06/05/01	PWP	NH	002		6.0	3.1	135.9	80.0	68.0	1.15	<0.01
		OTC	005		6.4	5.6	146.3	70.0	70.0	0.25	0.04
06/08/01	PWP	FETAX Solution	-	21.2	7.5	7.6					
		MDC-C	001	21.6	6.9	6.8					
		NH-D	002	21.5	6.9	6.3					
		ALB-I	003	20.2	7.3	6.8					
		POL-A	004	20.5	7.3	7.2					
		OTC-B	005	21.9	6.9	6.4					

CLIENT NAME: VDEC CLIENT/PROJECT No.: VERM01 W.O. No.: 00004

Date	Tech Initials	Sample ID	Sample No.	Temp (C)	pH (su)	DO (mg/L)	Conduct. (µS/cm)	Hardness (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Chlorine (mg/L)
06/20/01	PWP	FETAX Solution		20.5	7.2	7.9					
		MDC-C	001	20.6	7.0	7.7					
		ALB-D	003	20.5	7.4	7.8					
		POL-B	004	20.5	7.4	7.5					
		OTC-E	005	20.5	7.2	7.4					
06/22/01	PWP	MDC	001		6.2	5.5	151.8	95.0	75.0	0.60	0.05
		NH	002		6.0	2.4	182.2	90.0	84.0	8.40	<0.01
		ALB	003		6.3	5.1	116.7	100.0	58.0	1.92	<0.02
		POL	004		6.3	5.2	135.5	75.0	42.0	0.43	0.10
		OTC	005		6.4	5.9	154.6	85.0	76.0	0.30	0.05
07/11/01	PWP	FETAX Solution	-	21.2	7.4	7.5					
		FETAX Solution	-	21.7	7.5	7.5					
		MDC-C	001	21.7	7.1	7.3					
		NH-A	002	22.1	6.8	6.5					
		ALB-D	003	21.4	7.4	6.7					
		POL-H	004	21.7	7.2	6.7					
		OTC-B	005	21.9	6.9	6.8					
08/14/01	DM	DeCL2 Water	-	22.2	8.3	7.5				0.08	
		DeCL2 Water	-	22.1	8.2	7.3				0.08	
		DeCL2 Water	-	22.0	8.2	7.2				0.21	
		MDC-D	001	21.6	7.4	6.8				0.49	
		MDC-G	001	21.7	7.6	7.2				0.60	
		NH-H	002	21.8	8.1	7.1				0.96	
		ALB-E	003	21.5	8.2	7.1				0.80	
		POL-F	004	22.5	7.9	6.1				0.35	
		OTC-B	005	22.2	6.2	6.7				0.80	

VERM01-00004
VERMONT EPA RARE PROJECT
SPECIES DATA COMPARISON SUMMARY
(METAMORPH DATA BASED ON INITIAL SPECIMEN COUNTS)

Gosner Stage 26-28 (Free Swimming)

Species		LAB CTL	001-MDC	002-NH	003-ALB	004-POL	OO5-OTC
X. laevis	% MORTALITY	14.00	20.00	12.00	32.00	14.00	22.00
	SEM	0.19	0.24	0.15	0.06	0.16	0.15
	% MALFORMATION	0.00	0.00	4.00	45.95	37.78	26.83
	SEM	0.00	0.00	0.08	0.09	0.17	0.07
R. pipiens	% MORTALITY	0.00	4.00	18.00	16.00	20.00	10.00
	SEM	0.00	0.08	0.27	0.37	0.20	0.00
	% MALFORMATION	0.00	0.00	0.00	42.00	32.00	18.75
	SEM	0.00	0.00	0.00	0.18	0.15	0.06
R. catesbeiana	% MORTALITY	0.00	0.00	0.00	-	-	-
	SEM	0.00	0.00	0.00	-	-	-
	% MALFORMATION	0.00	0.00	0.00	-	-	-
	SEM	0.00	0.00	0.00	-	-	-

Gosner Stage 30-42 (Limb Development)

Species		LAB CTL	001-MDC	002-NH	003-ALB	004-POL	OO5-OTC
X. laevis	% MORTALITY	22.00	26.00	26.00	36.00	18.00	54.00
	SEM	0.21	0.31	0.19	0.08	0.15	0.42
	% MALFORMATION	0.00	0.00	6.06	54.55	43.24	47.62
	SEM	0.00	0.00	0.16	0.02	0.14	0.44
R. pipiens	% MORTALITY	33.33	54.00	62.00	18.00	36.00	42.00
	SEM	0.38	0.13	0.25	0.35	0.44	0.25
	% MALFORMATION	0.00	0.00	5.26	35.71	43.75	26.67
	SEM	0.00	0.00	0.10	0.22	0.23	0.16
R. catesbeiana	% MORTALITY	6.00	5.00	10.00	-	-	-
	SEM	0.08	1.58	0.32	-	-	-
	% MALFORMATION	0.00	0.00	0.00	-	-	-
	SEM	0.00	0.00	0.00	-	-	-

Gosner Stage 46 (Metamorphosis Completed)
(% Mortality based on total number of specimens)

Species		LAB CTL	001-MDC	002-NH	003-ALB	004-POL	OO5-OTC
X. laevis	% MORTALITY	28.00	44.00	40.00	52.00	24.00	62.00
	SEM	0.18	0.50	0.48	0.15	0.13	0.35
	% METAMORPH	10.00	14.00	0.00	4.00	4.00	10.00
	SEM	0.17	0.16	0.00	0.08	0.08	0.14
	% MALFORMATION	0.00	0.00	-	0.00	50.00	40.00
	SEM	0.00	0.00	-	0.00	0.50	0.26
R. pipiens	% MORTALITY	36.67	54.00	62.00	20.00	38.00	44.00
	SEM	0.28	0.13	0.25	0.41	0.43	0.29
	% METAMORPH	38.00	46.00	38.00	50.00	36.67	56.00
	SEM	0.51	0.13	0.25	0.43	0.39	0.29
	% MALFORMATION	5.26	0.00	15.79	52.50	41.94	32.14
	SEM	0.13	0.00	0.21	0.17	0.16	0.16
R. catesbeiana	% MORTALITY	14.00	5.00	10.00	-	-	-
	SEM	0.08	0.16	0.32	-	-	-
	% METAMORPH	0.00	0.00	0.00	-	-	-
	SEM	0.00	0.00	0.00	-	-	-
	% MALFORMATION	-	-	-	-	-	-
	SEM	-	-	-	-	-	-

VERM01-00004
VERMONT EPA RARE PROJECT
***X. laevis* MORTALITY/METAMORPH DATA**
(metamorph data based on initial number of specimen)

X laevis in LABORATORY WATER/SAND CONTROL

	Replicate 1						Replicate 2						Replicate 3						Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	8	0.00	0.00	0.00	#DIV/0!	0.00	0.00	0.00	#DIV/0!
14	3	3	7	0	30.00	0.00	2	2	8	0	20.00	0.00	2	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	28	14.00	1.80	0.19	9.58	0.00	0.00	0.00	#DIV/0!
29	0	3	7	0	30.00	0.00	2	4	6	0	40.00	0.00	0	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	2	2	8	0	20.00	0.00	31	22.00	2.20	0.21	6.74	0.00	0.00	0.00	#DIV/0!
42	0	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	0	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	37	22.00	2.20	0.21	6.74	0.00	0.00	0.00	#DIV/0!
56	0	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	0	2	8	1	20.00	10.00	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	37	22.00	2.20	0.21	6.74	2.00	0.20	0.06	22.36
72	1	4	6	0	40.00	0.00	0	4	6	0	40.00	0.00	0	2	8	2	20.00	20.00	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	36	24.00	2.80	0.24	6.97	4.00	0.80	0.13	22.36
84	0	4	6	1	40.00	10.00	0	4	6	1	40.00	10.00	1	3	7	2	30.00	20.00	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	37	26.00	2.80	0.24	6.44	8.00	0.70	0.12	10.46
93	0	4	6	1	40.00	10.00	0	4	6	1	40.00	10.00	0	3	7	3	30.00	30.00	1	1	9	0	10.00	0.00	0	2	8	0	20.00	0.00	40	28.00	1.70	0.18	4.66	10.00	1.50	0.17	12.25

X laevis in 001-MDC REFERENCE SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3						Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	8	0.00	0.00	0.00	#DIV/0!	0.00	0.00	0.00	#DIV/0!
14	5	5	5	0	50.00	0.00	2	2	8	0	20.00	0.00	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	27	20.00	3.00	0.24	8.66	0.00	0.00	0.00	#DIV/0!
29	0	5	5	0	50.00	0.00	2	4	6	0	40.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	30	24.00	3.80	0.28	8.12	0.00	0.00	0.00	#DIV/0!
42	0	5	5	0	50.00	0.00	1	5	5	0	50.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	34	26.00	4.80	0.31	8.43	0.00	0.00	0.00	#DIV/0!
56	0	5	5	1	50.00	10.00	0	5	5	0	50.00	0.00	0	1	9	0	10.00	0.00	0	1	9	1	10.00	10.00	0	1	9	0	10.00	0.00	37	26.00	4.80	0.31	8.43	4.00	0.30	0.08	13.69
72	0	5	5	2	50.00	20.00	0	5	5	0	50.00	0.00	0	1	9	1	10.00	10.00	0	1	9	1	10.00	10.00	0	1	9	1	10.00	10.00	37	26.00	4.80	0.31	8.43	10.00	0.50	0.10	7.07
84	0	5	5	2	50.00	20.00	5	10	0	0	100.00	0.00	3	4	6	1	40.00	10.00	1	2	8	3	20.00	30.00	0	1	9	1	10.00	10.00	35	44.00	12.30	0.50	7.97	14.00	1.30	0.16	8.14
93	0	5	5	2	50.00	20.00	0	10	0	0	100.00	0.00	0	4	6	1	40.00	10.00	0	2	8	3	20.00	30.00	0	1	9	1	10.00	10.00	36	44.00	12.30	0.50	7.97	14.00	1.30	0.16	8.14

X laevis in 002-NH SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3						Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	8	0.00	0.00	0.00	#DIV/0!	0.00	0.00	0.00	#DIV/0!
14	0	0	10	0	0.00	0.00	2	2	8	0	20.00	0.00	2	2	8	0	20.00	0.00	2	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	28	12.00	1.20	0.15	9.13	0.00	0.00	0.00	#DIV/0!
29	1	1	9	0	10.00	0.00	2	4	6	0	40.00	0.00	1	3	7	0	30.00	0.00	0	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	31	20.00	2.50	0.22	7.91	0.00	0.00	0.00	#DIV/0!
42	0	1	9	0	10.00	0.00	0	4	6	0	40.00	0.00	0	3	7	0	30.00	0.00	0	2	8	0	20.00	0.00	1	1	9	0	10.00	0.00	35	22.00	1.70	0.18	5.93	0.00	0.00	0.00	#DIV/0!
56	1	2	8	0	20.00	0.00	0	4	6	0	40.00	0.00	1	4	6	0	40.00	0.00	0	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	37	26.00	1.80	0.19	5.16	0.00	0.00	0.00	#DIV/0!
72	0	2	8	0	20.00	0.00	0	4	6	0	40.00	0.00	0	4	6	0	40.00	0.00	0	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	36	26.00	1.80	0.19	5.16	0.00	0.00	0.00	#DIV/0!
84	0	2	8	0	20.00	0.00	2	6	4	0	60.00	0.00	5	9	1	0	90.00	0.00	0	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	36	40.00	11.50	0.48	8.48	0.00	0.00	0.00	#DIV/0!
93	0	2	8	0	20.00	0.00	0	6	4	0	60.00	0.00	0	9	1	0	90.00	0.00	0	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	40	40.00	11.50	0.48	8.48	0.00	0.00	0.00	#DIV/0!

X laevis in 003-ALB SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3						Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	8	0.00	0.00	0.00	#DIV/0!	0.00	0.00	0.00	#DIV/0!
14	3	3	7	0	30.00	0.00	4	4	6	0	40.00	0.00	3	3	7	0	30.00	0.00	3	3	7	0	30.00	0.00	3	3	7	0	30.00	0.00	27	32.00	0.20	0.06	1.40	0.00	0.00	0.00	#DIV/0!
29	0	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	1	4	6	0	40.00	0.00	0	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00	29	34.00	0.30	0.08	1.61	0.00	0.00	0.00	#DIV/0!
42	0	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	0	4	6	0	40.00	0.00	1	4	6	0	40.00	0.00	0	3	7	0	30.00	0.00	30	36.00	0.30	0.08	1.52	0.00	0.00	0.00	#DIV/0!
56	0	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	0	4	6	0	40.00	0.00	0	4	6	0	40.00	0.00	0	3	7	0	30.00	0.00	35	36.00	0.30	0.08	1.52	0.00	0.00	0.00	#DIV/0!
72	1	4	6	1	40.00	10.00	0	4	6	0	40.00	0.00	1	5	5	0	50.00	0.00	0	4	6	0	40.00	0.00	0	3	7	0	30.00	0.00	33	40.00	0.50	0.10	1.77	2.00	0.20	0.06	22.36
84	0	4	6	1	40.00	10.00	0	4	6	0	40.00	0.00	1	6	4	0	60.00	0.00	0	4	6	0	40.00	0.00	1	4	6	0	40.00	0.00	35	44.00	0.80	0.13	2.03	2.00	0.20	0.06	22.36
93	0	4	6	1	40.00	10.00	2	6	4	1	60.00	10.00	0	6	4	0	60.00	0.00	0	4	6	0	40.00	0.00	2	6	4	0	60.00	0.00	36	52.00	1.20	0.15	2.11	4.00	0.30	0.08	13.69

VERM01-00004
VERMONT EPA RARE PROJECT
***X. laevis* MORTALITY/METAMORPH DATA**
(metamorph data based on initial number of specimen)

X laevis in 004-POL SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
14	0	0	10	0	0.00	0.00	2	2	8	0	20.00	0.00	3	3	7	0	30.00	0.00
29	0	0	10	0	0.00	0.00	1	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00
42	1	1	9	0	10.00	0.00	0	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00
56	0	1	9	0	10.00	0.00	0	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00
72	1	2	8	0	20.00	0.00	0	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00
84	1	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00	0	3	7	1	30.00	10.00
93	0	3	7	0	30.00	0.00	0	3	7	0	30.00	0.00	0	3	7	1	30.00	10.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	**27**	**14.00**	**1.30**	**0.16**	**8.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
29	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	**30**	**16.00**	**1.80**	**0.19**	**8.39**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
42	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	**33**	**18.00**	**1.20**	**0.15**	**6.09**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
56	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	**35**	**18.00**	**1.20**	**0.15**	**6.09**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
72	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	**36**	**20.00**	**1.00**	**0.14**	**5.00**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
84	1	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	**38**	**24.00**	**0.80**	**0.13**	**3.73**	**2.00**	**0.20**	**0.06**	**22.36**
93	0	2	8	1	20.00	10.00	0	1	9	0	10.00	0.00	**41**	**24.00**	**0.80**	**0.13**	**3.73**	**4.00**	**0.30**	**0.08**	**13.69**

X laevis in 005-OTC SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
14	2	2	8	0	20.00	0.00	1	1	9	0	10.00	0.00	2	2	8	0	20.00	0.00
29	1	3	7	0	30.00	0.00	0	1	9	0	10.00	0.00	1	3	7	0	30.00	0.00
42	1	4	6	0	40.00	0.00	0	1	9	0	10.00	0.00	0	3	7	0	30.00	0.00
56	2	6	4	0	60.00	0.00	0	1	9	0	10.00	0.00	5	8	2	0	80.00	0.00
72	2	8	2	0	80.00	0.00	0	1	9	1	10.00	10.00	0	8	2	0	80.00	0.00
84	0	8	2	1	80.00	10.00	1	2	8	1	20.00	10.00	0	8	2	0	80.00	0.00
93	0	8	2	2	80.00	20.00	1	3	7	1	30.00	10.00	0	8	2	0	80.00	0.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	2	2	8	0	20.00	0.00	4	4	6	0	40.00	0.00	**27**	**22.00**	**1.20**	**0.15**	**4.98**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
29	1	3	7	0	30.00	0.00	0	4	6	0	40.00	0.00	**30**	**28.00**	**1.20**	**0.15**	**3.91**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
42	2	5	5	0	50.00	0.00	0	4	6	0	40.00	0.00	**32**	**34.00**	**2.30**	**0.21**	**4.46**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
56	3	8	2	0	80.00	0.00	0	4	6	0	40.00	0.00	**35**	**54.00**	**8.80**	**0.42**	**5.49**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
72	0	8	2	0	80.00	0.00	0	4	6	1	40.00	10.00	**38**	**58.00**	**10.20**	**0.45**	**5.51**	**4.00**	**0.30**	**0.08**	**13.69**
84	0	8	2	0	80.00	0.00	0	4	6	2	40.00	20.00	**38**	**60.00**	**8.00**	**0.40**	**4.71**	**8.00**	**0.70**	**0.12**	**10.46**
93	0	8	2	0	80.00	0.00	0	4	6	2	40.00	20.00	**38**	**62.00**	**6.20**	**0.35**	**4.02**	**10.00**	**1.00**	**0.14**	**10.00**

VERM01-00004
VERMONT EPA RARE PROJECT
***R. pipiens* MORTALITY/METAMORPH DATA**
(metamorph data based on initial number of specimen)

R. pipiens in LABORATORY WATER/SAND CONTROL

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
15	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
30	1	1	9	0	10.00	0.00	5	5	5	0	50.00	0.00	4	4	6	0	40.00	0.00
45	0	1	9	3	10.00	30.00	0	5	5	0	50.00	0.00	0	4	6	1	40.00	10.00
60	1	2	8	4	20.00	40.00	0	5	5	4	50.00	40.00	0	4	6	4	40.00	40.00
74	0	2	8	5	20.00	50.00	0	5	5	5	50.00	50.00	0	4	6	5	40.00	50.00
98	0	2	8	8	20.00	80.00	0	5	5	5	50.00	50.00	0	4	6	6	40.00	60.00

		Replicates 1-3 Cumulative Mortality Statistics				Replicates 1-3 Cumulative Metamorph Statistics			
DAY	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
15	**27**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
30	**34**	**33.33**	**4.33**	**0.38**	**6.24**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
45	**39**	**33.33**	**4.33**	**0.38**	**6.24**	**13.33**	**2.33**	**0.28**	**11.46**
60	**41**	**36.67**	**2.33**	**0.28**	**4.17**	**40.00**	**0.00**	**0.00**	**0.00**
74	**40**	**36.67**	**2.33**	**0.28**	**4.17**	**50.00**	**0.00**	**0.00**	**0.00**
98	**46**	**36.67**	**2.33**	**0.28**	**4.17**	**63.33**	**2.33**	**0.28**	**2.41**

R. pipiens in 001-MDC REFERENCE SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
7	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
17	5	5	5	0	50.00	0.00	5	5	5	0	50.00	0.00	7	7	3	0	70.00	0.00
24	0	5	5	0	50.00	0.00	0	5	5	0	50.00	0.00	0	7	3	0	70.00	0.00
32	0	5	5	0	50.00	0.00	0	5	5	0	50.00	0.00	0	7	3	0	70.00	0.00
40	0	5	5	2	50.00	20.00	0	5	5	0	50.00	0.00	0	7	3	0	70.00	0.00
52	0	5	5	5	50.00	50.00	0	5	5	5	50.00	50.00	0	7	3	3	70.00	30.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
7	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	**26**	**4.00**	**0.30**	**0.08**	**13.69**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
17	4	5	5	0	50.00	0.00	4	5	5	0	50.00	0.00	**29**	**54.00**	**0.80**	**0.13**	**1.66**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
24	0	5	5	0	50.00	0.00	0	5	5	0	50.00	0.00	**30**	**54.00**	**0.80**	**0.13**	**1.66**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
32	0	5	5	0	50.00	0.00	0	5	5	0	50.00	0.00	**36**	**54.00**	**0.80**	**0.13**	**1.66**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
40	0	5	5	3	50.00	30.00	0	5	5	0	50.00	0.00	**42**	**54.00**	**0.80**	**0.13**	**1.66**	**10.00**	**2.00**	**0.20**	**14.14**
52	0	5	5	5	50.00	50.00	0	5	5	5	50.00	50.00	**46**	**54.00**	**0.80**	**0.13**	**1.66**	**46.00**	**0.80**	**0.13**	**1.94**

R. pipiens in 002-NH SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
7	2	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00	1	1	9	0	10.00	0.00
17	2	4	6	0	40.00	0.00	3	3	7	0	30.00	0.00	5	6	4	0	60.00	0.00
24	1	5	5	0	50.00	0.00	0	3	7	0	30.00	0.00	1	7	3	0	70.00	0.00
32	0	5	5	0	50.00	0.00	1	4	6	0	40.00	0.00	1	8	2	0	80.00	0.00
40	0	5	5	2	50.00	20.00	0	4	6	3	40.00	30.00	0	8	2	1	80.00	10.00
49	0	5	5	5	50.00	50.00	0	4	6	6	40.00	60.00	0	8	2	2	80.00	20.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
7	5	5	5	0	50.00	0.00	1	1	9	0	10.00	0.00	**26**	**18.00**	**3.70**	**0.27**	**10.69**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
17	3	8	2	0	80.00	0.00	5	6	4	0	60.00	0.00	**29**	**54.00**	**3.80**	**0.28**	**3.61**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
24	0	8	2	0	80.00	0.00	0	6	4	0	60.00	0.00	**30**	**58.00**	**3.70**	**0.27**	**3.32**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
32	0	8	2	0	80.00	0.00	0	6	4	0	60.00	0.00	**36**	**62.00**	**3.20**	**0.25**	**2.89**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
40	0	8	2	0	80.00	0.00	0	6	4	1	60.00	10.00	**42**	**62.00**	**3.20**	**0.25**	**2.89**	**14.00**	**1.30**	**0.16**	**8.14**
49	0	8	2	2	80.00	20.00	0	6	4	4	60.00	40.00	**46**	**62.00**	**3.20**	**0.25**	**2.89**	**38.00**	**3.20**	**0.25**	**4.71**

R. pipiens in 003-ALB SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
14	6	6	4	0	60.00	0.00	2	2	8	0	20.00	0.00	0	0	10	0	0.00	0.00
32	0	6	4	0	60.00	0.00	0	2	8	4	20.00	40.00	1	1	9	8	10.00	80.00
48	0	6	4	2	60.00	20.00	0	2	8	8	20.00	80.00	0	1	9	9	10.00	90.00
60	0	6	4	3	60.00	30.00	0	2	8	8	20.00	80.00	0	1	9	9	10.00	90.00
75	0	6	4	3	60.00	30.00	0	2	8	8	20.00	80.00	0	1	9	9	10.00	90.00
87	1	7	3	3	70.00	30.00	0	2	8	8	20.00	80.00	0	1	9	9	10.00	90.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**31**	**16.00**	**6.80**	**0.37**	**16.30**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
32	0	0	10	8	0.00	80.00	0	0	10	6	0.00	60.00	**37**	**18.00**	**6.20**	**0.35**	**13.83**	**52.00**	**11.20**	**0.47**	**6.44**
48	0	0	10	10	0.00	100.00	0	0	10	10	0.00	100.00	**43**	**18.00**	**6.20**	**0.35**	**13.83**	**78.00**	**11.20**	**0.47**	**4.29**
60	0	0	10	10	0.00	100.00	0	0	10	10	0.00	100.00	**40**	**18.00**	**6.20**	**0.35**	**13.83**	**80.00**	**8.50**	**0.41**	**3.64**
75	0	0	10	10	0.00	100.00	0	0	10	10	0.00	100.00	**40**	**18.00**	**6.20**	**0.35**	**13.83**	**80.00**	**8.50**	**0.41**	**3.64**
87	0	0	10	10	0.00	100.00	0	0	10	10	0.00	100.00	**40**	**20.00**	**8.50**	**0.41**	**14.58**	**80.00**	**8.50**	**0.41**	**3.64**

VERM01-00004
VERMONT EPA RARE PROJECT
***R. pipiens* MORTALITY/METAMORPH DATA**
(metamorph data based on initial number of specimen)

R. pipiens in 004-POL SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
11	3	3	7	0	30.00	0.00	3	3	7	0	30.00	0.00	0	0	10	0	0.00	0.00
20	5	8	2	0	80.00	0.00	0	3	7	0	30.00	0.00	1	1	9	1	10.00	10.00
32	1	9	1	0	90.00	0.00	0	3	7	3	30.00	30.00	0	1	9	8	10.00	80.00
40	0	9	1	0	90.00	0.00	0	3	7	5	30.00	50.00	0	1	9	8	10.00	80.00
53	0	9	1	1	90.00	10.00	0	3	7	7	30.00	70.00	0	1	9	9	10.00	90.00
64	0	9	1	1	90.00	10.00	0	3	7	7	30.00	70.00	0	1	9	9	10.00	90.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
11	1	1	9	0	10.00	0.00	3	3	7	0	30.00	0.00	**33**	**20.00**	**2.00**	**0.20**	**7.07**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
20	1	2	8	0	20.00	0.00	0	3	7	0	30.00	0.00	**33**	**34.00**	**7.30**	**0.38**	**7.95**	**2.00**	**0.20**	**0.06**	**22.36**
32	0	2	8	1	20.00	10.00	0	3	7	5	30.00	50.00	**38**	**36.00**	**9.80**	**0.44**	**8.70**	**34.00**	**10.30**	**0.45**	**9.44**
40	0	2	8	5	20.00	50.00	0	3	7	6	30.00	60.00	**39**	**36.00**	**9.80**	**0.44**	**8.70**	**48.00**	**8.70**	**0.42**	**6.14**
53	1	3	7	6	30.00	60.00	0	3	7	7	30.00	70.00	**40**	**38.00**	**9.20**	**0.43**	**7.98**	**60.00**	**9.00**	**0.42**	**5.00**
64	0	3	7	7	30.00	70.00	0	3	7	7	30.00	70.00	**46**	**38.00**	**9.20**	**0.43**	**7.98**	**62.00**	**9.20**	**0.43**	**4.89**

R. pipiens in 005-OTC SITE WATER/SEDIMENT

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
10	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00
20	0	1	9	0	10.00	0.00	2	3	7	0	30.00	0.00	4	5	5	0	50.00	0.00
31	1	2	8	0	20.00	0.00	0	3	7	0	30.00	0.00	0	5	5	0	50.00	0.00
40	0	2	8	2	20.00	20.00	0	3	7	1	30.00	10.00	0	5	5	0	50.00	0.00
49	0	2	8	8	20.00	80.00	0	3	7	7	30.00	70.00	1	6	4	2	60.00	20.00
60	0	2	8	8	20.00	80.00	0	3	7	7	30.00	70.00	1	7	3	3	70.00	30.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
10	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00	**26**	**10.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
20	3	4	6	0	40.00	0.00	5	6	4	0	60.00	0.00	**29**	**38.00**	**3.70**	**0.27**	**5.06**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
31	0	4	6	0	40.00	0.00	0	6	4	0	60.00	0.00	**34**	**40.00**	**2.50**	**0.22**	**3.95**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
40	0	4	6	0	40.00	0.00	0	6	4	0	60.00	0.00	**34**	**40.00**	**2.50**	**0.22**	**3.95**	**6.00**	**0.80**	**0.13**	**14.91**
49	0	4	6	5	40.00	50.00	0	6	4	4	60.00	40.00	**35**	**42.00**	**3.20**	**0.25**	**4.26**	**52.00**	**5.70**	**0.34**	**4.59**
60	0	4	6	6	40.00	60.00	0	6	4	4	60.00	40.00	**46**	**44.00**	**4.30**	**0.29**	**4.71**	**56.00**	**4.30**	**0.29**	**3.70**

VERM01-00004
VERMONT EPA RARE PROJECT
***R. catesbeiana* MORTALITY/METAMORPH DATA**
(metamorph data based on initial number of specimen)

R. catesbeiana in LABORATORY WATER/SAND CONTROL

	Replicate 1						Replicate 2						Replicate 3					
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
14	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00
28	0	0	10	0	0.00	0.00	1	1	9	0	10.00	0.00	1	1	9	0	10.00	0.00
42	1	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00
56	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00
70	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00
87	1	2	8	0	20.00	0.00	0	1	9	0	10.00	0.00	0	1	9	0	10.00	0.00

	Replicate 4						Replicate 5							Replicates 1-5 Cumulative Mortality Statistics				Replicates 1-5 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	#DIV/0!	0	0	10	0	0.00	#DIV/0!	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	0	0	10	0	0.00	#DIV/0!	0	0	10	0	0.00	#DIV/0!	**28**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
28	1	1	9	0	10.00	#DIV/0!	0	0	10	0	0.00	#DIV/0!	**30**	**6.00**	**0.30**	**0.08**	**9.13**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
42	0	1	9	0	10.00	#DIV/0!	2	2	8	0	20.00	#DIV/0!	**32**	**12.00**	**0.20**	**0.06**	**3.73**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
56	0	1	9	0	10.00	#DIV/0!	0	2	8	0	20.00	#DIV/0!	**33**	**12.00**	**0.20**	**0.06**	**3.73**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
70	0	1	9	0	10.00	#DIV/0!	0	2	8	0	20.00	#DIV/0!	**33**	**12.00**	**0.20**	**0.06**	**3.73**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
87	0	1	9	0	10.00	#DIV/0!	0	2	8	0	20.00	#DIV/0!	**33**	**14.00**	**0.30**	**0.08**	**3.91**	**0.00**	**0.00**	**0.00**	**#DIV/0!**

R. catesbeiana in 001-MDC REFERENCE SITE WATER/SEDIMENT

	Replicate 1						Replicate 2							Replicates 1-2 Cumulative Mortality Statistics				Replicates 1-2 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**28**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
28	0	0	10	0	0.00	0.00	1	1	9	0	10.00	0.00	**31**	**5.00**	**0.50**	**0.16**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
42	0	0	10	0	0.00	0.00	0	1	9	0	10.00	0.00	**32**	**5.00**	**0.50**	**0.16**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
56	0	0	10	0	0.00	0.00	0	1	9	0	10.00	0.00	**33**	**5.00**	**0.50**	**0.16**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
70	0	0	10	0	0.00	0.00	0	1	9	0	10.00	0.00	**33**	**5.00**	**0.50**	**0.16**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
87	0	0	10	0	0.00	0.00	0	1	9	0	10.00	0.00	**33**	**5.00**	**0.50**	**0.16**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**

R. catesbeiana in 002-NH SITE WATER/SEDIMENT

	Replicate 1						Replicate 2							Replicates 1-2 Cumulative Mortality Statistics				Replicates 1-2 Cumulative Metamorph Statistics			
DAY	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	NO. DEAD	CUMUL. DEAD	CUMUL. LIVE	CUMUL. METAM.	% DEAD	% METAM.	Gosner Stage	(%)	VAR(S2)	SEM	CV(%)	(%)	VAR(S2)	SEM	CV(%)
0	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**8**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
14	0	0	10	0	0.00	0.00	0	0	10	0	0.00	0.00	**28**	**0.00**	**0.00**	**0.00**	**#DIV/0!**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
28	0	0	10	0	0.00	0.00	2	2	8	0	20.00	0.00	**31**	**10.00**	**2.00**	**0.32**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
42	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	**32**	**10.00**	**2.00**	**0.32**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
56	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	**33**	**10.00**	**2.00**	**0.32**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
70	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	**33**	**10.00**	**2.00**	**0.32**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**
87	0	0	10	0	0.00	0.00	0	2	8	0	20.00	0.00	**33**	**10.00**	**2.00**	**0.32**	**14.14**	**0.00**	**0.00**	**0.00**	**#DIV/0!**

VERM01-00004
VERMONT EPA RARE PROJECT
SPECIES DATA COMPARISON SUMMARY
DEVELOPMENTAL STAGE RATES

	LAB CTL		001-MDC REF		002-NH		003-ALB		004-POL		OO5-OTC	
Species	DAY	AVG. STAGE (Gosner)	DAY	AVG. STAGE (Gosner)	DAY	AVG. STAGE (Gosner)	DAY	AVG. STAGE (Gosner)	DAY	AVG. STAGE (Gosner)	DAY	AVG. STAGE (Gosner)
X. laevis	**0**	8	**0**	8	**0**	8	**0**	8	**0**	8	**0**	8
	14	28	**14**	27	**14**	28	**14**	27	**14**	27	**14**	27
	29	31	**29**	30	**29**	31	**29**	29	**29**	30	**29**	30
	42	37	**42**	34	**42**	35	**42**	30	**42**	33	**42**	32
	56	37	**56**	37	**56**	37	**56**	35	**56**	35	**56**	35
	72	36	**72**	37	**72**	36	**72**	33	**72**	36	**72**	38
	84	37	**84**	35	**84**	36	**84**	35	**84**	38	**84**	38
	93	40	**93**	36	**93**	40	**93**	36	**93**	41	**93**	38
R. pipiens	**0**	8	**0**	8	**0**	8	**0**	8	**0**	8	**0**	8
	15	27	**7**	26	**7**	26	**14**	31	**11**	33	**10**	26
	30	34	**17**	29	**17**	29	**32**	37	**20**	33	**20**	29
	45	39	**24**	30	**24**	30	**48**	43	**32**	38	**31**	34
	60	41	**32**	36	**32**	36	**60**	40	**40**	39	**40**	34
	74	40	**40**	42	**40**	42	**75**	40	**53**	40	**49**	35
	98	46	**52**	46	**49**	46	**87**	40	**64**	46	**60**	46
R. catesbeiana	**0**	8	**0**	8	**0**	8	-	-	-	-	-	-
	14	28	**14**	28	**14**	28	-	-	-	-	-	-
	28	30	**28**	31	**28**	31	-	-	-	-	-	-
	42	32	**42**	32	**42**	32	-	-	-	-	-	-
	56	33	**56**	33	**56**	33	-	-	-	-	-	-
	70	33	**70**	33	**70**	33	-	-	-	-	-	-
	87	33	**87**	33	**87**	33	-	-	-	-	-	-

VERMONT EPA RARE PROJECT
Xenopus laevis DEVELOPMENTAL MALFORMATION DATA
LABORATORY WATER/SAND CONTROL

Gosner Stage 26-28 (Free Swimming), N=45

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	7	0	0.00											
2	8	0	0.00											
3	10	0	0.00											
4	10	0	0.00											
5	10	0	0.00											
TOTAL NUMBER	45	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=31

SAMPLE ID	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	5	0	0.00											
2	5	0	0.00											
3	4	0	0.00											
4	9	0	0.00											
5	8	0	0.00											
TOTAL NUMBER	31	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=5

SAMPLE ID	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	1	0	0.00											
2	1	0	0.00											
3	3	0	0.00											
4	0													
5	0													
TOTAL NUMBER	5	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

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VERMONT EPA RARE PROJECT
***Xenopus laevis* DEVELOPMENTAL MALFORMATION DATA**
001-MDC REFERENCE SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=41

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	6	0	0.00											
2	8	0	0.00											
3	9	0	0.00											
4	9	0	0.00											
5	9	0	0.00											
TOTAL NUMBER	41	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=24

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	3	0	0.00											
2	1	0	0.00											
3	5	0	0.00											
4	7	0	0.00											
5	8	0	0.00											
TOTAL NUMBER	24	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=7

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	2	0	0.00											
2	0													
3	1	0	0.00											
4	3	0	0.00											
5	1	0	0.00											
TOTAL NUMBER	7	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

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VERMONT EPA RARE PROJECT
***Xenopus laevis* DEVELOPMENTAL MALFORMATION DATA**
002-NH SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=50

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	1	10.00		1									
2	10	1	10.00					1		1		1		
3	10	0	0.00											
4	10	0	0.00											
5	10	0	0.00											
TOTAL NUMBER	50	2		0	1	0	0	1	0	1	0	1	0	0
TOTAL % MALFORMED			4.00	0.00	2.00	0.00	0.00	2.00	0.00	2.00	0.00	2.00	0.00	0.00
Var (S2)			0.30											
SEM			0.08											
CV (%)			13.69											

Gosner Stage 30-42 (Limb Development), N=33

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	0	0.00											
2	4	0	0.00											
3	4	2	50.00									2		
4	8	0	0.00											
5	9	0	0.00											
TOTAL NUMBER	33	2		0	0	0	0	0	0	0	0	2	0	0
TOTAL % MALFORMED			6.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.06	0.00	0.00
Var (S2)			0.80											
SEM			0.16											
CV (%)			14.76											

Gosner Stage 46 (Metamorphosis Completed), N=0

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1														
2														
3			**NO SPECIMEN COMPLETED METAMORPHOSIS**											
4														
5														
TOTAL NUMBER	0	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED														
Var (S2)														
SEM														
CV (%)														

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VERMONT EPA RARE PROJECT
Xenopus laevis DEVELOPMENTAL MALFORMATION DATA
003-ALB SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=37

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	7	3	42.86		3				2			2		
2	7	4	57.14		1				1			4		
3	7	3	42.86						1			3		
4	8	3	37.50						2			3		
5	8	4	50.00		1				1			4		
TOTAL NUMBER	37	17		0	5	0	0	0	7	0	0	16	0	0
TOTAL % MALFORMED			45.95	0.00	13.51	0.00	0.00	0.00	18.92	0.00	0.00	43.24	0.00	0.00
Var (S2)			0.30		1.33				0.30			0.70		
SEM			0.09		0.19				0.09			0.14		
CV (%)			1.19		8.54				2.90			1.93		

Gosner Stage 30-42 (Limb Development), N=22

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	5	3	60.00		2			3	1			3		
2	3	1	33.33					1	1			1	2	
3	4	2	50.00		2			1				1	1	
4	6	4	66.67		4	2					1	4		
5	4	2	50.00			2					1	2		1
TOTAL NUMBER	22	12		0	8	4	0	5	2	0	2	11	3	1
TOTAL % MALFORMED			54.55	0.00	36.36	18.18	0.00	22.73	9.09	0.00	9.09	50.00	13.64	4.55
Var (S2)			1.30		1.33	0.00		1.33	0.00		0.00	1.70	0.50	
SEM			0.24		0.25	0.00		0.25	0.00		0.00	0.28	0.15	
CV (%)			2.09		3.18	0.00		5.08	0.00		0.00	2.61	5.19	

Gosner Stage 46 (Metamorphosis Completed), N=2

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	1	0	0.00											
2	1	0	0.00											
3														
4														
5														
TOTAL NUMBER	2	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)														
SEM														
CV (%)														

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VERMONT EPA RARE PROJECT
***Xenopus laevis* DEVELOPMENTAL MALFORMATION DATA**
004-POL SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=45

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	3	30.00					3	1			3		
2	8	2	25.00					2				2		
3	9	5	55.56		3			5	1			5		
4	9	4	44.44	1	2			4				4		
5	9	3	33.33					3	2			3		
TOTAL NUMBER	45	17		1	5	0	0	17	4	0	0	17	0	0
TOTAL % MALFORMED			37.78	2.22	11.11	0.00	0.00	37.78	8.89	0.00	0.00	37.78	0.00	0.00
Var (S2)			1.30		0.50			1.30	0.33			1.30		
SEM			0.17		0.11			0.17	0.09			0.17		
CV (%)			3.02		6.36			3.02	6.50			3.02		

Gosner Stage 30-42 (Limb Development), N=37

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	7	2	28.57					2						
2	7	4	57.14					3				1		
3	7	3	42.86					2					1	1
4	7	3	42.86					3				1	1	
5	9	4	44.44					4					1	
TOTAL NUMBER	37	16		0	0	0	0	14	0	0	0	2	3	1
TOTAL % MALFORMED			43.24	0.00	0.00	0.00	0.00	37.84	0.00	0.00	0.00	5.41	8.11	2.70
Var (S2)			0.70					0.70				0.00	0.00	
SEM			0.14					0.14				0.00	0.00	
CV (%)			1.93					2.21				0.00	0.00	

ENLARGED THYROID SEEN IN MOST STAGE 30-42 SPECIMENS

Gosner Stage 46 (Metamorphosis Completed), N=2

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1														
2														
3	1	1	100.00											1
4	1	0	0.00											
5														
TOTAL NUMBER	2	1		0	0	0	0	0	0	0	0	0	0	1
TOTAL % MALFORMED			50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.00
Var (S2)			0.50											
SEM			0.50											
CV (%)			1.41											

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VERMONT EPA RARE PROJECT
Xenopus laevis DEVELOPMENTAL MALFORMATION DATA
005-OTC SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=41

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	TYPE OF MALFORMATION/DELTS OBSERVED										
				EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	2	25.00					2	1			2		
2	9	2	22.22						2			2		
3	9	3	33.33					1	2			3		
4	8	2	25.00					1	1			2		
5	7	2	28.57					2	2			2		
TOTAL NUMBER	41	11		0	0	0	0	6	8	0	0	11	0	0
TOTAL % MALFORMED			26.83	0.00	0.00	0.00	0.00	14.63	19.51	0.00	0.00	26.83	0.00	0.00
Var (S2)			0.20					0.33	0.30			0.20		
SEM			0.07					0.09	0.09			0.07		
CV (%)			1.67					3.95	2.81			1.67		

Gosner Stage 30-42 (Limb Development), N=21

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	TYPE OF MALFORMATION/DELTS OBSERVED										
				EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	4	0	0.00											1
2	6	4	66.67		4		4	2	1			2		
3	3	2	66.67		2		1	1				1		1
4	2	0	0.00											
5	6	4	66.67		4		3	3	1			3	2	
TOTAL NUMBER	21	10		0	10	0	8	6	2	0	0	6	2	2
TOTAL % MALFORMED			47.62	0.00	47.62	0.00	38.10	28.57	9.52	0.00	0.00	28.57	9.52	9.52
Var (S2)			4.00		1.33		2.33	1.00	0.00			1.00		0.00
SEM			0.44		0.25		0.33	0.22	0.00			0.22		0.00
CV (%)			4.20		2.42		4.01	3.50	0.00			3.50		0.00

Gosner Stage 46 (Metamorphosis Completed), N=5

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	TYPE OF MALFORMATION/DELTS OBSERVED										
				EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	2	1	50.00					1	1			1		1
2	1	0	0.00											
3	0													
4	0													
5	2	1	50.00					1						
TOTAL NUMBER	5	2		0	0	0	0	2	1	0	0	1	0	1
TOTAL % MALFORMED			40.00	0.00	0.00	0.00	0.00	40.00	20.00	0.00	0.00	20.00	0.00	20.00
Var (S2)			0.33					0.00						
SEM			0.26					0.00						
CV (%)			1.44					0.00						

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VERMONT EPA RARE PROJECT
Rana pipiens DEVELOPMENTAL MALFORMATION DATA
LABORATORY WATER/SAND CONTROL

Gosner Stage 26-28 (Free Swimming), N=30

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	0	0.00											
2	10	0	0.00											
3	10	0	0.00											
TOTAL NUMBER	30	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=19

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	0	0.00											
2	5	0	0.00											
3	6	0	0.00											
TOTAL NUMBER	19	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=19

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	1	12.50											1
2	5	0	0.00											
3	6	0	0.00											
TOTAL NUMBER	19	1		0	0	0	0	0	0	0	0	0	0	1
TOTAL % MALFORMED			5.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.26
Var (S2)			0.33											
SEM			0.13											
CV (%)														

VERM01-00004
VERMONT EPA RARE PROJECT
Rana pipiens DEVELOPMENTAL MALFORMATION DATA
001-MDC REFERENCE SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=50

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	10	0	0.00											
2	10	0	0.00											
3	10	0	0.00											
4	10	0	0.00											
5	10	0	0.00											
TOTAL NUMBER	50	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=23

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	5	0	0.00											
2	5	0	0.00											
3	3	0	0.00											
4	5	0	0.00											
5	5	0	0.00											
TOTAL NUMBER	23	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=23

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	5	0	0.00											
2	5	0	0.00											
3	3	0	0.00											
4	5	0	0.00											
5	5	0	0.00											
TOTAL NUMBER	23	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* DEVELOPMENTAL MALFORMATION DATA**
002-NH SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=49

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	9	0	0.00											
2	10	0	0.00											
3	10	0	0.00											
4	10	0	0.00											
5	10	0	0.00											
TOTAL NUMBER	49	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=19

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	5	0	0.00											
2	6	0	0.00											
3	2	0	0.00											
4	2	0	0.00											
5	4	1	25.00										1	
TOTAL NUMBER	19	1		0	0	0	0	0	0	0	0	0	1	0
TOTAL % MALFORMED			5.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.26	0.00
Var (S2)			0.20											
SEM			0.10											
CV (%)			8.50											

Gosner Stage 46 (Metamorphosis Completed), N=19

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	5	1	20.00											1
2	6	2	33.33									2		
3	2	0	0.00											
4	2	0	0.00											
5	4	0	0.00											
TOTAL NUMBER	19	3		0	0	0	0	0	0	0	0	2	0	1
TOTAL % MALFORMED			15.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.53	0.00	5.26
Var (S2)			0.80											
SEM			0.21											
CV (%)			5.66											

VERM01-00004
VERMONT EPA RARE PROJECT
Rana pipiens DEVELOPMENTAL MALFORMATION DATA
003-ALB SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=50

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	3	30.00		1			3	1			3		
2	10	4	40.00		2			4	1			4		
3	10	3	30.00		1			3	2			3		
4	10	6	60.00	2	1			5	3			6		
5	10	5	50.00	1	3			5	4		1	5		
TOTAL NUMBER	50	21		3	8	0	0	20	11	0	1	21	0	0
TOTAL % MALFORMED			42.00	6.00	16.00	0.00	0.00	40.00	22.00	0.00	2.00	42.00	0.00	0.00
Var (S2)			1.70	0.50	0.80			1.00	1.70			1.70		
SEM			0.18	0.10	0.13			0.14	0.18			0.18		
CV (%)			3.10	11.79	5.59			2.50	5.93			3.10		

Gosner Stage 30-42 (Limb Development), N=42

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	4	2	50.00	1	2		2	2	1			2		1
2	8	1	12.50					1				1		1
3	10	4	40.00		1		1	4				4		2
4	10	4	40.00		1	2		4				4		1
5	10	4	40.00		2	3		4				4		
TOTAL NUMBER	42	15		1	6	5	3	15	1	0	0	15	0	5
TOTAL % MALFORMED			35.71	2.38	14.29	11.90	7.14	35.71	2.38	0.00	0.00	35.71	0.00	11.90
Var (S2)			2.00		0.33	0.50	0.50	2.00				2.00		0.25
SEM			0.22		0.09	0.11	0.11	0.22				0.22		0.08
CV (%)			3.96		4.04	5.94	9.90	3.96				3.96		4.20

Gosner Stage 46 (Metamorphosis Completed), N=40

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	3	3	100.00						2					3
2	8	4	50.00		1		1	3				1		2
3	9	4	44.44		2		2	2				2		2
4	10	4	40.00		1		1	2	1			3	2	3
5	10	6	60.00	1	1	2	1	4	2			4		2
TOTAL NUMBER	40	21		1	5	2	5	11	5	0	0	10	2	12
TOTAL % MALFORMED			52.50	2.50	12.50	5.00	12.50	27.50	12.50	0.00	0.00	25.00	5.00	30.00
Var (S2)			1.20		0.25		0.25	0.92	0.33			1.67		0.30
SEM			0.17		0.08		0.08	0.15	0.09			0.20		0.09
CV (%)			2.09		4.00		4.00	3.48	4.62			5.16		1.83

VERM01-00004
VERMONT EPA RARE PROJECT
Rana pipiens DEVELOPMENTAL MALFORMATION DATA
004-POL SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=50

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	4	40.00					3	1			3		
2	10	2	20.00		1			1	2			1		
3	10	2	20.00	2	2			1	1			2		
4	10	4	40.00	2	1			4	2			4		
5	10	4	40.00					1	2		2	4		
TOTAL NUMBER	50	16		4	4	0	0	10	8	0	2	14	0	0
TOTAL % MALFORMED			32.00	8.00	8.00	0.00	0.00	20.00	16.00	0.00	4.00	28.00	0.00	0.00
Var (S2)			1.20	0.00	0.33			2.00	0.30			1.70		
SEM			0.15	0.00	0.08			0.20	0.08			0.18		
CV (%)			3.42	0.00	7.22			7.07	3.42			4.66		

Gosner Stage 30-42 (Limb Development), N=32

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	1	1	100.00					1				1	1	
2	7	4	57.14	1				4	1			4	2	1
3	9	3	33.33		1		1	3	1			3	1	1
4	8	2	25.00					2				2	1	
5	7	4	57.14					4	1			4	1	
TOTAL NUMBER	32	14		1	1	0	1	14	3	0	0	14	6	2
TOTAL % MALFORMED			43.75	3.13	3.13	0.00	3.13	43.75	9.38	0.00	0.00	43.75	18.75	6.25
Var (S2)			1.70					1.70	0.00			1.70	0.20	0.00
SEM			0.23					0.23	0.00			0.23	0.08	0.00
CV (%)			2.98					2.98	0.00			2.98	2.39	0.00

ENLARGED THYROID SEEN IN MOST STAGE 30-42 SPECIMENS

Gosner Stage 46 (Metamorphosis Completed), N=31

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	1	1	100.00					1				1	1	1
2	7	3	42.86					3	2			3	1	3
3	9	3	33.33					2	1			2	1	2
4	7	3	42.86					3	2			3		2
5	7	3	42.86					2				2	1	1
TOTAL NUMBER	31	13		0	0	0	0	11	5	0	0	11	4	9
TOTAL % MALFORMED			41.94	0.00	0.00	0.00	0.00	35.48	16.13	0.00	0.00	35.48	12.90	29.03
Var (S2)			0.80					0.70	0.33			0.70	0.00	0.70
SEM			0.16					0.15	0.10			0.15	0.00	0.15
CV (%)			2.13					2.36	3.58			2.36	0.00	2.88

VERM01-00004
VERMONT EPA RARE PROJECT
***Rana pipiens* DEVELOPMENTAL MALFORMATION DATA**
005-OTC SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=48

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	9	2	22.22		1				2		2	1		
2	9	2	22.22		2				1		1			
3	10	1	10.00		1						1	1		
4	10	2	20.00		2				2		2	2		
5	10	2	20.00		2				2			2		
TOTAL NUMBER	48	9		0	8	0	0	0	7	0	6	6	0	0
TOTAL % MALFORMED			18.75	0.00	16.67	0.00	0.00	0.00	14.58	0.00	12.50	12.50	0.00	0.00
Var (S2)			0.20		0.30				0.25		0.33	0.33		
SEM			0.06		0.08				0.07		0.08	0.08		
CV (%)			2.39		3.29				3.43		4.62	4.62		

Gosner Stage 30-42 (Limb Development), N=30

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	3	37.50		2		1	1				1	1	
2	7	1	14.29					1				1		
3	5	1	20.00										1	
4	6	2	33.33					1	2			1		
5	4	1	25.00										1	
TOTAL NUMBER	30	8		0	2	0	1	3	2	0	0	3	3	0
TOTAL % MALFORMED			26.67	0.00	6.67	0.00	3.33	10.00	6.67	0.00	0.00	10.00	10.00	0.00
Var (S2)			0.80					0.00				0.00	0.00	
SEM			0.16					0.00				0.00	0.00	
CV (%)			3.35					0.00				0.00	0.00	

Gosner Stage 46 (Metamorphosis Completed), N=28

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	GUT	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	2	25.00					2	1			1		1
2	7	3	42.86					2	1			1		3
3	3	1	33.33											1
4	6	2	33.33					2				2		
5	4	1	25.00					1				1		
TOTAL NUMBER	28	9		0	0	0	0	7	2	0	0	5	0	5
TOTAL % MALFORMED			32.14	0.00	0.00	0.00	0.00	25.00	7.14	0.00	0.00	17.86	0.00	17.86
Var (S2)			0.70					0.25	0.00			0.25		1.33
SEM			0.16					0.09	0.00			0.09		0.22
CV (%)			2.60					2.00	0.00			2.80		6.47

VERM01-00004
VERMONT EPA RARE PROJECT
***Rana catesbeiana* DEVELOPMENTAL MALFORMATION DATA**
LABORATORY WATER/SAND CONTROL

Gosner Stage 26-28 (Free Swimming), N=50

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	0	0.00											
2	10	0	0.00											
3	10	0	0.00											
4	10	0	0.00											
5	10	0	0.00											
TOTAL NUMBER	50	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=43

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	8	0												
2	9	0	0.00											
3	9	0	0.00											
4	9	0	0.00											
5	8	0	0.00											
TOTAL NUMBER	43	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=0

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	**NUMBER SCORED**	**NUMBER MALFORMED**	**% MAL.**	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1														
2														
3			**NO SPECIMENS COMPLETED METAMORPHOSIS**											
4														
5														
TOTAL NUMBER	0	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED														
Var (S2)														
SEM														
CV (%)														

VERM01-00004
VERMONT EPA RARE PROJECT
Rana catesbeiana DEVELOPMENTAL MALFORMATION DATA
001-MDC REFERENCE SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=20

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	10	0	0.00											
2	10	0	0.00											
TOTAL NUMBER	20	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=19

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1	10	0	0.00											
2	9	0	0.00											
TOTAL NUMBER	19	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=0

REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
				TYPE OF MALFORMATION/DELTS OBSERVED										
1														
2	**NO SPECIMENS COMPLETED METAMORPHOSIS**													
TOTAL NUMBER	0	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED														
Var (S2)														
SEM														
CV (%)														

VERM01-00004
VERMONT EPA RARE PROJECT
Rana catesbeiana DEVELOPMENTAL MALFORMATION DATA
002-NH SITE WATER/SEDIMENT

Gosner Stage 26-28 (Free Swimming), N=20

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	0	0.00											
2	10	0	0.00											
TOTAL NUMBER	20	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 30-42 (Limb Development), N=18

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1	10	0	0.00											
2	8	0	0.00											
TOTAL NUMBER	18	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00											
SEM			0.00											
CV (%)														

Gosner Stage 46 (Metamorphosis Completed), N=0

				TYPE OF MALFORMATION/DELTS OBSERVED										
REPLICATE NUMBER	NUMBER SCORED	NUMBER MALFORMED	% MAL.	EDEMA	TAIL	FIN	NOTO-CHORD	FACE	EYE	BRAIN	HEMOR-RHAGE	MOUTH	HIND LIMB	FORE LIMB
1														
2			**NO SPECIMENS COMPLETED METAMORPHOSIS**											
TOTAL NUMBER	0	0		0	0	0	0	0	0	0	0	0	0	0
TOTAL % MALFORMED														
Var (S2)														
SEM														
CV (%)														

Appendix F

Photo Atlas:

Xenopus laevis

Rana pipiens

APPENDIX F
EVALUATION OF THE EFFECT OF ENVIRONMENTAL SAMPLES FROM THE LAKE CHAMPLAIN BASIN IN *XENOPUS laevis* AND *RANA pipiens*
PHOTO ATLAS
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Figure 1. *X. laevis* in Laboratory Reference Water/Sand

Figure 2. *X. laevis* in Laboratory Reference Water/Sand

Figure 3. *X. laevis* in Laboratory Reference Water/Sand

Figure 4. *X. laevis* in Laboratory Reference Water/Sand

Figure 5. *X. laevis* in Site ALB Water/Sediment

Figures 6 and 7. *X. laevis* in Site POL Water/Sediment

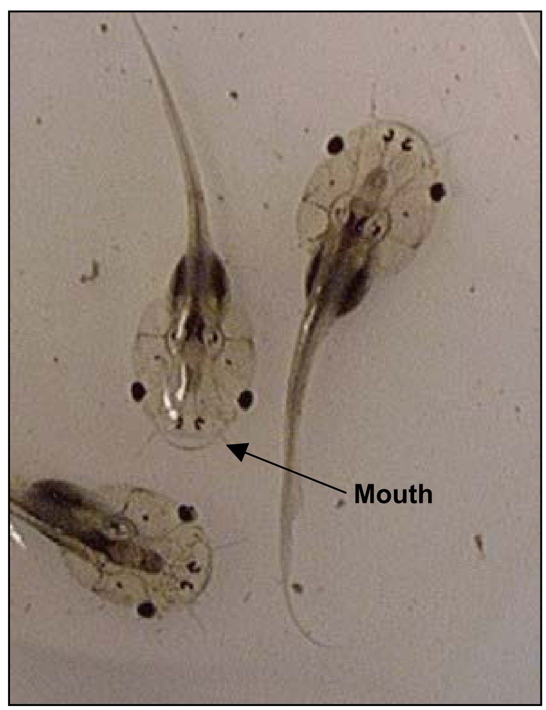


Figure 8. *X. laevis* in Site POL Water/Sediment

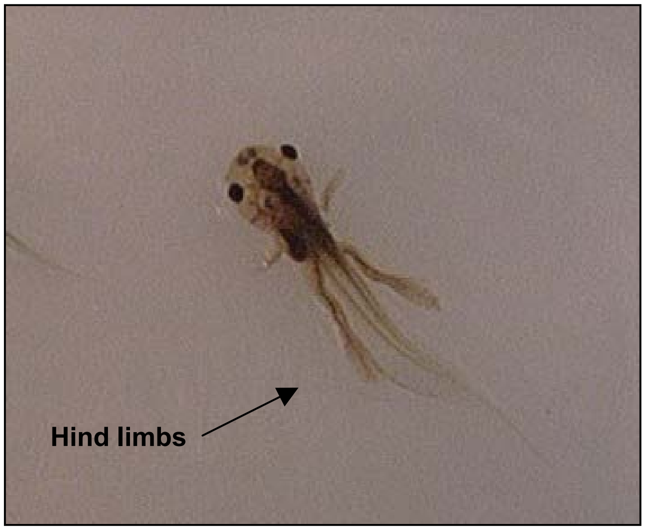


Figure 9. *X. laevis* in Site POL Water/Sediment

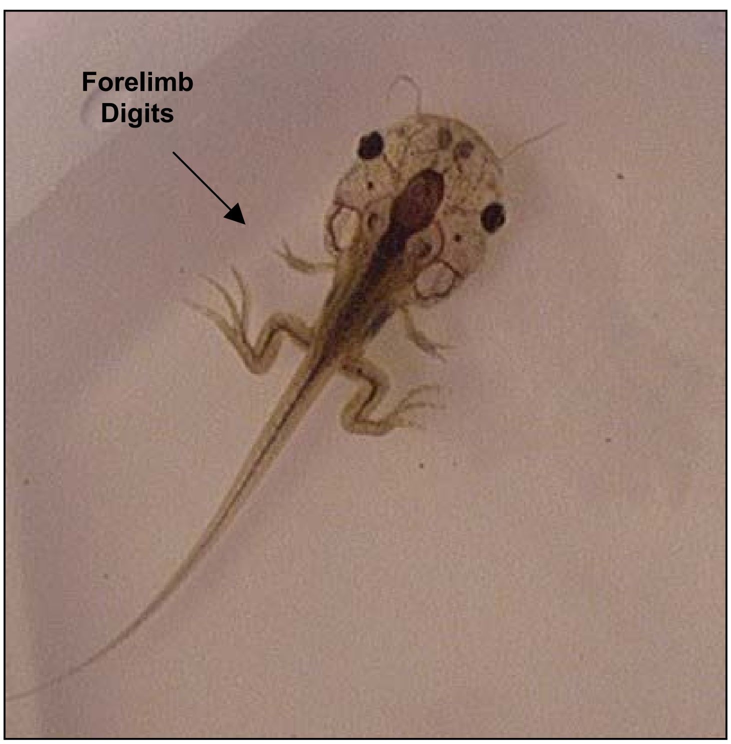


Figure 10. *R. pipiens* in Laboratory Reference Water/Sand

Figure 11. *R. pipiens* in Laboratory Reference Water/Sand

Figure 12. *R. pipiens* in Reference Site MDC Water/Sediment

Figure 13. *R. pipiens* in Reference Site MDC Water/Sediment

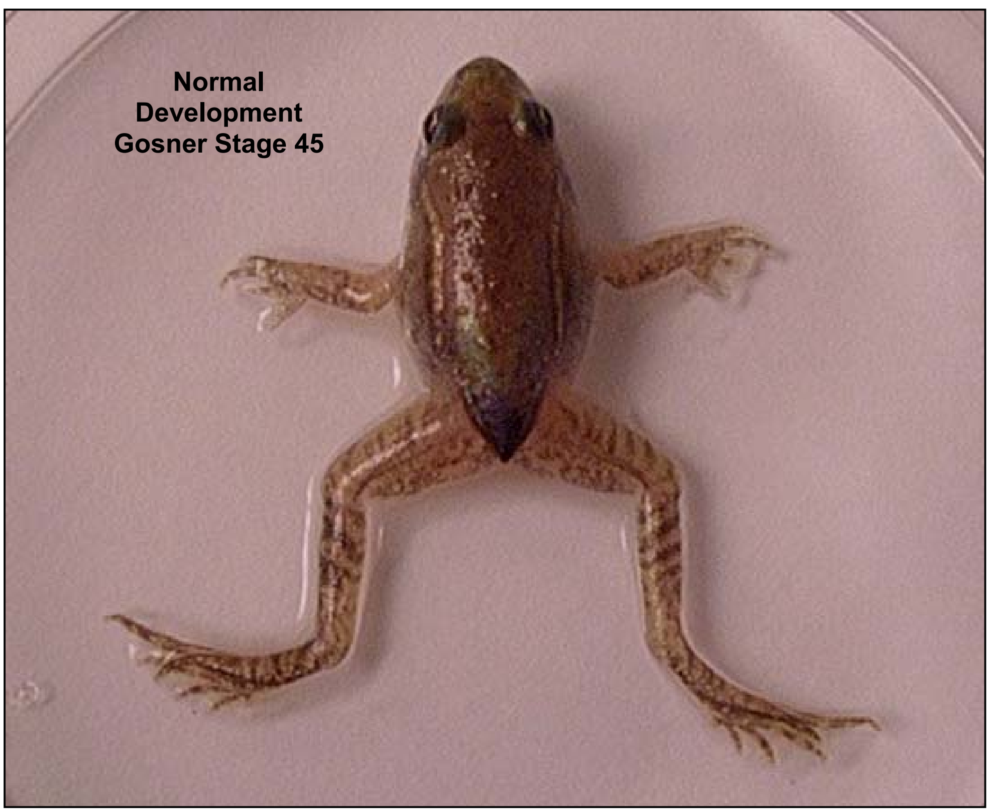


Figure 14. *R. pipiens* in Site ALB Water/Sediment

Figure 15. *R. pipiens* in Site ALB Water/Sediment

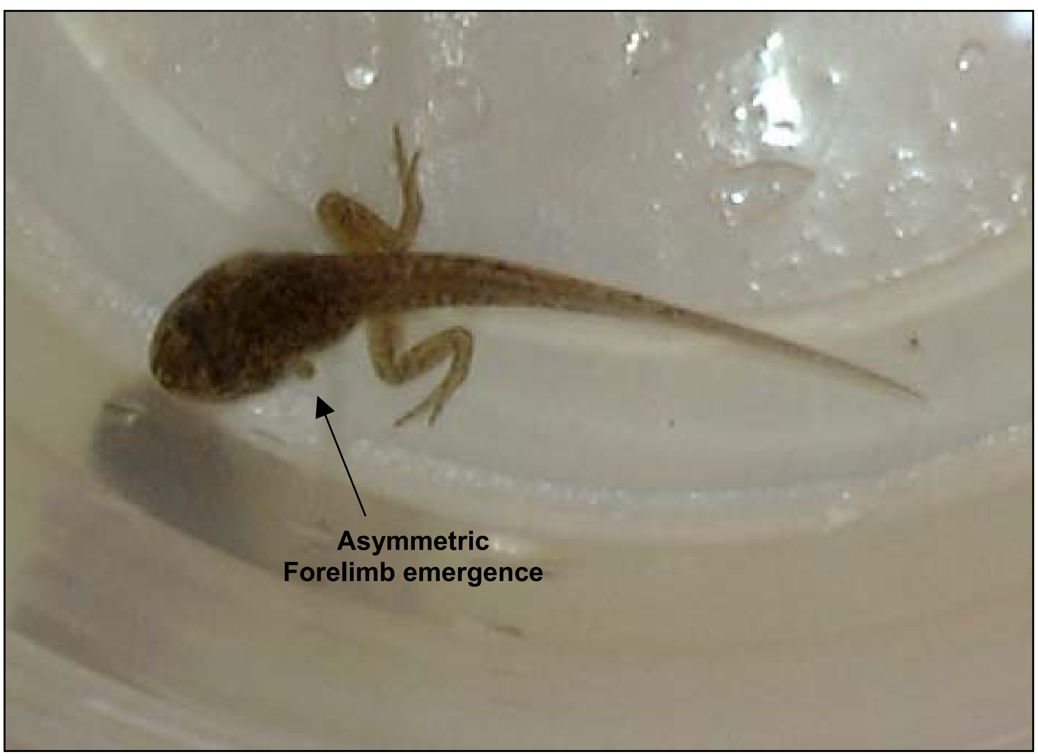


Figure 16. *R. pipiens* in Site ALB Water/Sediment

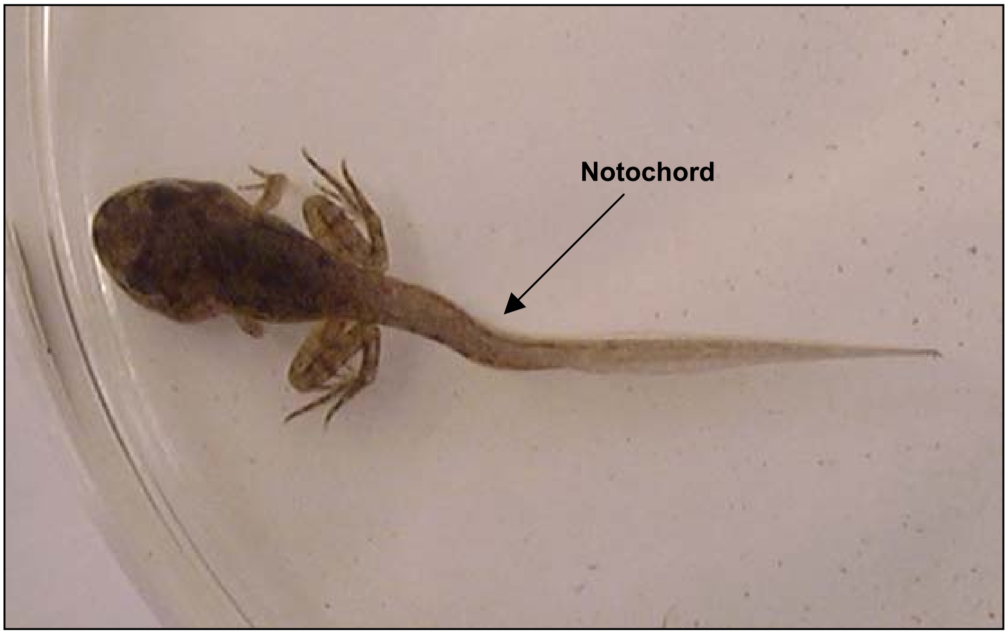


Figure 17. *R. pipiens* in Site ALB Water/Sediment

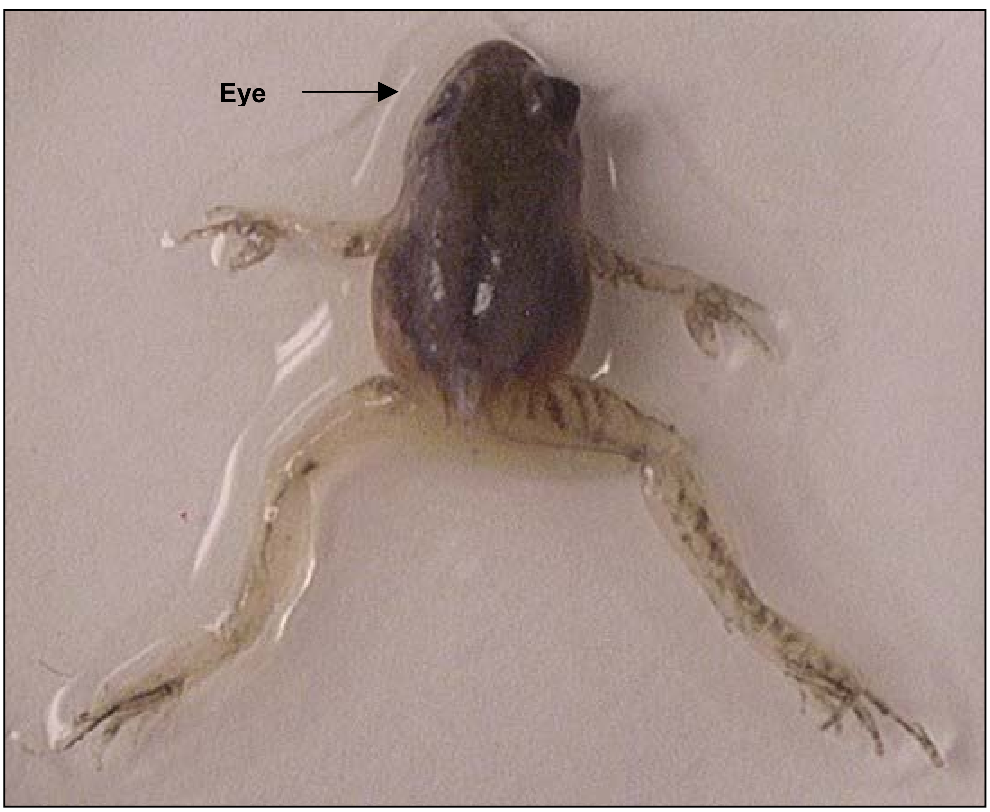


Figure 18. *R. pipiens* in Site ALB Water/Sediment

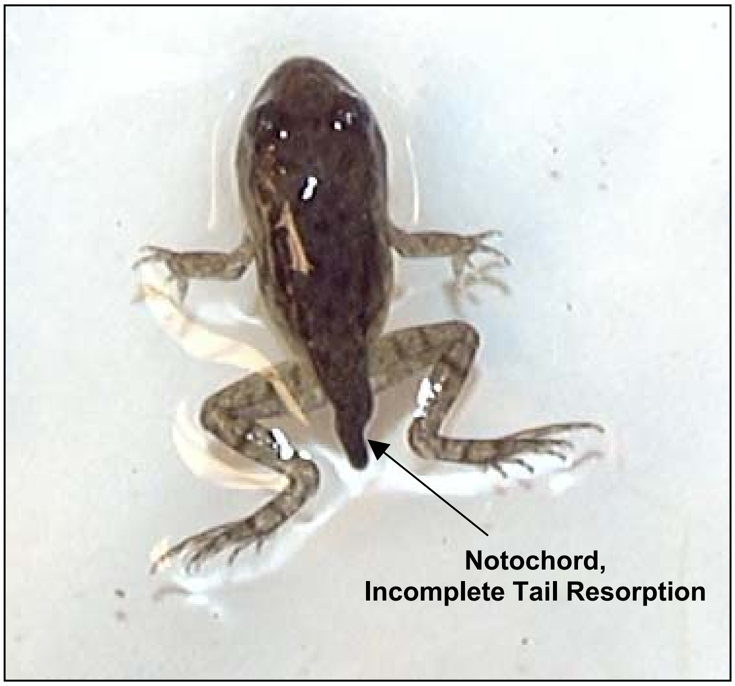


Figure 19. *R. pipiens* in Site POL Water/Sediment

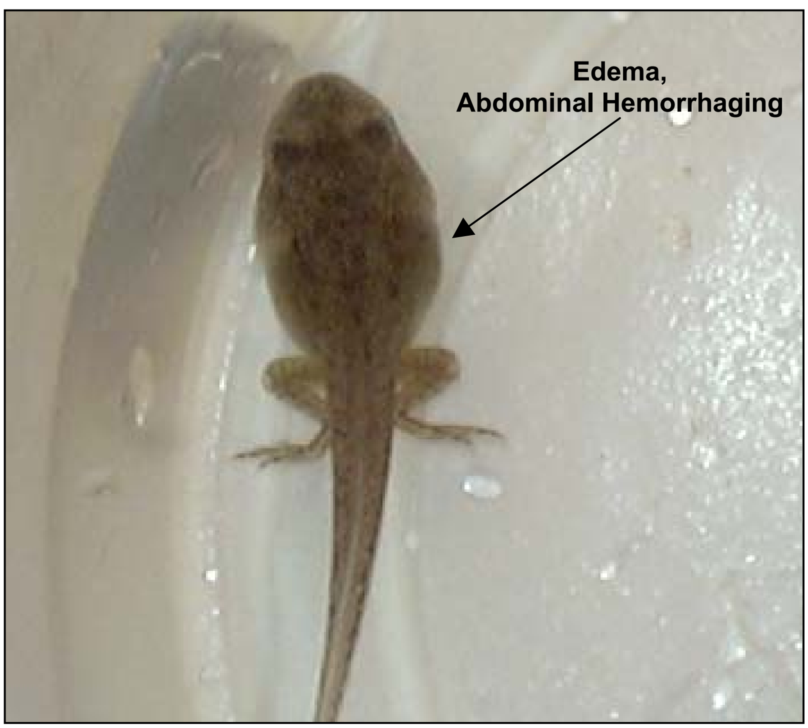
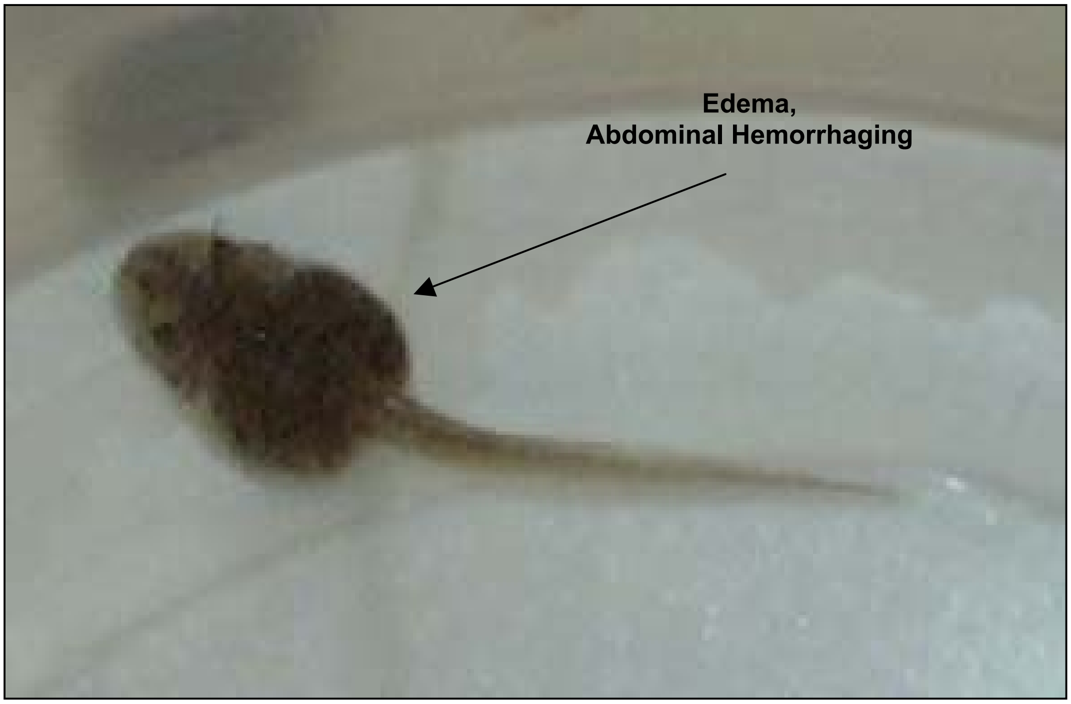


Figure 20. *R. pipiens* in Site OTC Water/Sediment



Appendix G

Definitions:

APPENDIX G: DEFINITIONS

(Taken mostly from :Field Guide to Malformations of Frogs and Toads, U.S. Department of the Interior,U.S. Geological Survey Biological Science Report, USGS/BRD/BSR–2000–0005, http://www.nwhc.usgs.gov/research/amph_dc/frog.pdf)

Descriptions of frog malformations in this report are based on terminology used in human literature (Bolande, 1979; Robbins, et al., 1989), developmental biology (O'Rahilly, et al., 1996; Carlson, 1994; Gilbert, 1997), and teratology (Wise, et al., 1997). Many of these terms were originally used to describe abnormalities in the mammalian fetus at birth. However, application of common terminology may allow comparison of similar conditions in related specialties and across species and bring new interest and collaboration to the issues involving malformed frogs and toads.

Amelia: No evidence of a limb, the hip region is smooth and the pigment pattern is not disrupted.

Anophthalmia: Missing eye.

Abnormality : Any change from the normal, does not imply any cause or that the organ was ever normal.

Aplasia (agenesis): Lack of development of an organ or tissue often resulting from failure of appearance of the primordium of an organ in embryonic development. For example, amelia is aplasia or agenesis of a limb.

Brachydactyly: Short toe; The normal number of metatarsal bones are present but the number of phalanges (bones in the toe) are reduced.

Brachygnathia: Abnormal shortness of lower jaw; same as mandibular micrognathia.

Bilaterally symmetrical rear limb malformations refer to the occurrence of the same type of malformation in both rear limbs.

Bilateral rear limb malformations refer to the occurrence of malformations of any type in both rear limbs.

Bone bridge: A bone structure that spans the space between two margins of bent bone. This bone structure appears radiographically as a plane of linear rays of bone that extend from the margins of bent bone and fill the angle between the bone margins.

Complete but malformed limb: All bones of the limb are present, but the limb is still abnormal e.g., rotation, bone bridge, skin web, micromelia.

Deformations: Deformations arise later in fetal life and represent alterations in form or structure resulting from mechanical factors such as amputation. A deformation does not involve an intrinsic defect in morphogenesis and impacts a structure that otherwise developed normally.

Digits: Toes; identified by the number of phalanges and relative position on the foot or hand (Fig. 1C page 4).

Ectoderm: The embryonic layer from which epidermal tissues (skin, hair, etc.), mucous membranes, nervous tissue, and external sense organs (eye, ear, etc.) are derived.

Ectrodactyly: Missing toe; Distinguished from brachydactyly and refers to a completely missing digit including the metatarsal bone and phalanges.

Ectromelia: An incomplete limb with the lower portion of the leg missing. Types of ectromelia refer to the last identifiable bone e.g., ectromelia of the femur, ectromelia of the tibiafibula, and ectromelia of the tibiale and fibulare. Phocomelia and amelia are also considered types of ectromelia.

Hemimelia: Short bone; The affected bone is short but distal limb and foot are present, e.g., hemimelia of the tibiafibula, means the tibiafibula is short but the foot is present.

Hypoplasia: Incomplete development of an organ.

Hock joint: Ankle.

Kyphosis: Abnormally convex (hunchback) thoracic spine.

Malformations: Primary errors in any phase of morphogenesis including cell proliferation, cell migration, differentiation, programmed cell death or regression of larval structures.

Mesoderm: The embryonic layer from which connective tissue, bone, cartilage, muscle, blood, vasculature, notochord, pleura, pericardium, peritoneum, kidney, and gonads are derived.

Microcephaly: Small head, blunt snout.

Micromelia: Proportionaly small or short limb.

Microphthalmia: Small eye.

Morphogenesis: The development of highly organized and specialized tissues through cell division and proliferation, cell migration, cell differentiation, and programmed cell death all of which are orchestrated through chemical communication within and between cells.

Pathogenesis: Cellular events and tissue reactions that occur in the progression of disease.

Phocomelia: Absence of the proximal portion of a limb, with the foot attached very close to the body and proximal bones that cannot be identified.

Phalanges: Bones of the toe.

Polydactyly: More than the normal number of metatarsal bones are present with or without a complete set of phalanges.

Polymelia: More than two forelimbs or more than two rear limbs are present. The extra limb needs to have identifiable major segments (e.g. femur and tibiafibula) to be classified as a multiple limb.

Polyphalangy: The normal number of metatarsal bones are present at the tibiale-fibulare-metatarsal joint but with duplicate sets of phalanges.

Rotation: Distortion of the direction of bone growth in such a way that the orientation of the limb and foot is abnormal. **Primary** rotation is the misdirection of bone growth without a predisposing cause such as a fracture, a bone bridge or a skin web. A **secondary** rotation also has abnormal orientation of the foot, but it is due to the formation of a bone bridge, skin web or fracture.

Scoliosis: Lateral deviation (either left or right) in the normally straight line of the spine.

Skin web: A band of skin crossing a joint and restricting motion of that limb.

Spongiforme bone: Expansion of the cancellous bone at the distal tip of ectromeliac limbs or associated with traumelias; expansions are typically terminal, irregularly shaped, and only present on the affected limb.

Stifle joint: Knee.

Teratogen: An agent or factor that causes malformations.

Teratogenesis: Abnormal development that gives rise to malformations