Spring 2000

The effects of ultraviolet radiation, highway contamination, and synergism of the two on spotted salamander embryos

Sarah Letitia Turtle
University of New Hampshire, Durham

THE EFFECTS OF ULTRAVIOLET RADIATION, HIGHWAY CONTAMINATION, AND SYNERGISM OF THE TWO ON SPOTTED SALAMANDER EMBRYOS

BY

SARAH LETITIA TURTLE
B. A., Hartwick College, 1991
M. S., University of New Hampshire, 1996

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy

in

Zoology

May, 2000

This dissertation has been examined and approved.

Dissertation Director, Dr. James Taylor
Professor of Zoology

Dr. Kimberly Babbitt, Assistant Professor of Natural Resources

Dr. James Haney, Professor of Zoology

Dr. Thomas Lee, Professor of Plant Biology

Dr. Michael Lesser, Research Associate Professor

Dr. Stanley Sessions, Professor of Biology

15-IV-00
Date

ACKNOWLEDGMENTS

This was not a solo venture.

The spotted salamander provided the endless inspiration and joy that saw me through to the end. My humble thanks to those that died so I could carve out their DNA.

My curiosity in the natural world was encouraged by gifted teachers; Earl Deubler, Sally Farrow, Tom Lee, and Stan Sessions. They got me started and kept me going. I wish to thank my advisor, Jim Taylor, and my doctoral committee; Kim Babbitt, Jim Haney, Tom Lee, Michael Lesser, and Stan Sessions for providing the many challenges of graduate research and the encouragement, support, and guidance to conquer them. Thanks to the many, many graduate students for their camaraderie, understanding, and encouragement over the past six years. I want to especially thank Christy Goodale who offered me a crash course in water chemistry anaylsis, provided the means to conduct 1998 DOC analyses. The New Hampshire Lay Lakes Monitoring Program provided the instruction and materials for the water color analysis and Dr. William McDowell's lab conducted the 1999 water chemistry analyses. I also wish to thank David Allen of the Lee Conservation Commission, Mark West of the Nottingham Conservation Commission and many property owners including the Blichels, Vachons, and Verrettes. They offered friendly advice, assistance, and allowed me to tramp across their fields and ponds at all hours of the day and night.

This work was partially funded by Declining Amphibian Task Force, Sigma Xi, University of New Hampshire Graduate School, and the Department of Zoology.

Thanks especially to my family and friends for believing in me. Especially Bill, Annie, Thatcher, Ellen, Jenny, Jim, Tara, Brook, Scout, and Leta; you all helped remind me of the truly important parts of life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

THE EFFECTS OF ULTRAVIOLET RADIATION, HIGHWAY CONTAMINATION, AND SYNERGISM OF THE TWO ON SPOTTED SALAMANDER EMBRYOS

By

Sarah L. Turtle
University of New Hampshire, May 2000

In this study, I examined the effects of ultraviolet radiation, highway contamination and a synergism of both factors on the spotted salamander, *Ambystoma maculatum* embryos in field and laboratory experiments. There are numerous reports of amphibian population declines from around the world. There are several possible factors causing the decline, including, habitat loss, environmental pollution, disease, and ultraviolet B radiation (UVB, 290 -320 nm). Results of studies conducted to date seem to indicate that the impacts of these factors vary greatly among species and across geography. Amphibians have unique characteristics and life histories that make them more vulnerable to changes in their environment then many other organisms. In particular, *A. maculatum* breeds in temporary wetlands in which embryos and larvae must reach metamorphose before the wetland dries. Factors that prolong development may increase mortality due to pool desiccation. Therefore, habitat degradation may be more harmful for amphibians that breed in this temporary environment.

I conducted field and laboratory experiments to test the effects of roadside runoff on *A. maculatum* embryos. Results indicate that embryo survival is very low roadside vernal pools contaminated with highway runoff. I also tested the effects of UVB on embryos using three radiation treatments. There was no statistically significant effect of

UVB radiation on embryo size at hatching, melanin concentration, or DNA damage in field experiments. Vernal pool water was so darkly colored with dissolved organic material that UVB was quickly attenuated, therefore UVB exposure was very low for the embryos. I also tested the effects of UVB radiation, highway contamination, and the impact of a synergism between these factors in the laboratory. The impact of highway contamination was much clearer than that of UVB radiation. Highway contamination significantly affected membrane diameter, embryo length at hatching and DNA damage. UVB significantly affected embryo wet mass at hatching. Results suggested that the two factors may act synergistically to negatively affect embryos but the trends were not significant.

INTRODUCTION

Amphibians have received much attention in the past ten years because of widespread reports of population declines and disappearances (Crump et al. 1992, Carey 1993, Blaustein and Wake 1995, Fisher and Shaffer 1996, Lips, 1998). Many biologists are concerned with these reports because they consider amphibians to be early indicators of environmental degradation (Blaustein et al. 1994, Hecnar and M'Closkey 1996). Amphibians are especially vulnerable to changes in their habitat for several reasons: their semi-permeable skin, their eggs are unprotected from the environment, and many species require both wetlands and terrestrial uplands to complete their life cycles (Wyman 1990). Reasons for population declines include habitat loss and degradation (Wyman 1990), changes in water chemistry (Tyler-Jones et al. 1989, Rowe et al. 1992, Bradford et al. 1992), disease (Lips 1999), and increased exposure to UV radiation (Blaustein et al. 1994, Blaustein et al. 1995, Anzalone et al. 1998, Lizana and Pedraza 1998). Experimental results indicate that the associations between these factors and amphibian populations are complicated. The effects of different factors vary geographically, interspecifically, and intraspecifically. This complexity suggests that synergism between stressful factors may be a possible explanation for the decline of these vulnerable organisms (Bradford et al. 1992, Kiesecker and Blaustein 1995, Long et al. 1995).

As reports of amphibian declines increased, some biologists began to closely examine the role of environmental factors. Biotic and abiotic factors determine viable ranges of different species (Tilman 1988). The roles of competition and predation have been elegantly described for many freshwater amphibian communities (Wilbur and Collins 1973, Wilbur 1976, Morin 1983). The role of abiotic factors is not as well described. However, abiotic factors determine the physiological performance of a species in a specific habitat (Rowe et al. 1998a) and they interact with biotic factors affecting populations and communities (Wilbur 1987, Warner et al. 1991). Changes in abiotic factors can not only increase percent mortality but at a sublethal level they can decrease fitness, or alter competitive outcomes (Warner et al. 1991, Sadinski and Dunson 1992, Loumbourdis et al. 1999).

Amphibians are most exposed to the abiotic environment during their embryonic and larval stages (Zug 1993). Abiotic factors can affect amphibian distributions directly through mortality and indirectly by increasing environmental stress thus, affecting growth, development, or behavior. Grime (1989) defines environmental stress as the "external constraints limiting the rates of resource acquisition, growth, or reproduction." Any stress that affects the amount of energy organisms can use for growth and reproduction can impact whole populations (Rowe et al. 1998a). Changes in environmental factors, such as pH, can affect time until metamorphosis, size at metamorphosis, resource acquisition, and survival (Freda and Dunson 1986, Rowe et al. 1992). However, the effects of abiotic factors are not limited to the larval stage and metamorphosis. Size at and timing of hatching can greatly affect reproductive success (Wilbur 1976). Therefore, the more subtle indirect effects caused by increased

environmental stress, can have great impacts on amphibian populations (Bradford et al. 1992).

The physical nature of the breeding wetland may increase vulnerability of amphibian populations to changes in the environment. Many of North America's amphibians rely on temporary wetlands for reproduction (Pierce 1985). Four amphibians in New Hampshire, *Rana sylvatica* (wood frog), *Ambystoma maculatum* (spotted salamander), *Ambystoma laterale* (blue-spotted salamander), and *Ambystoma jeffersonianum* (Jefferson's salamander), depend on these fishless wetlands for successful reproduction. Temporary wetlands provide an important amphibian breeding habitat because they are a refuge from fish predation. In temporary wetlands, stressful factors can be even more harmful and increase mortality if they slow growth and delay metamorphosis.

Vernal pools, a type of temporary wetland, are filled each spring by the collection of snowmelt and rainfall in landscape depressions. These pools usually dry annually in late summer or early fall but contain water long enough for embryonic and larval development. Time until metamorphosis for amphibian embryos and larvae is critical because metamorphosis must be achieved before the wetland dries. The vicissitudes of these temporary pools make them risky breeding environment; thus, the effects of any environmental stress may be magnified.

Previously, I examined *Ambystoma maculatum* embryonic survival in roadside and woodland vernal pools (Turtle 2000). Using transplant experiments, I found that embryo survival was very low in roadside vernal pools compared to woodland pools. These roadside pools all had high concentrations of sodium and chloride. Elevated salts

are most likely due to highway winter maintenance. However, in laboratory experiments I found that deicing salt alone had no effect on embryo survival. In the studies reported here, I examined the effects of two abiotic factors on *A. maculatum* embryos. In Chapter 1, I continued to examine the effects of highway runoff on *A. maculatum* embryos in laboratory and field experiments. In Chapter 2, I examined the effects of UV radiation on *A. maculatum* embryo survival, size at hatching, and melanin concentration in field experiments. In Chapter 3, I examined the effects of UV radiation and possible UV radiation/highway runoff synergism on *A. maculatum* embryos. I compared embryo survival, size at hatching, membrane diameter, melanin production and DNA damage between these factors in both field and laboratory experiments.

Ambystoma maculatum

I selected *A. maculatum* for these experiments for several reasons; 1) it is commonly found in eastern North America, 2) it is a pond breeding amphibian and therefore embryos are exposed to UV radiation naturally, 3) it has a longer development period than *Rana sylvatica* (wood frog), another common breeder in temporary wetlands, and therefore may be more vulnerable to sublethal effects during the embryo and larval stages.

A. maculatum is distributed throughout the eastern United States and Canada. As adults, they spend most of their time underground (Kleeberger and Werner 1983). In late winter or early spring adults migrate to breeding wetlands. These migrations usually occur on the first warm rainy nights, the key trigger being the thawing ground (Downs 1989). There is a tendency for *A. maculatum* to use the same route entering and exiting the pools and this trend also appears to be consistent over years (Stenhouse 1985). The

salamanders conduct an intriguing courtship dance (Downs 1989) after which females deposit egg masses of up to approximately 200 eggs within 48 hours. The egg masses are usually attached to stems and twigs (Downs 1989), embryos usually hatch in four to seven weeks, and larvae remain in pools and feed until they reach a critical minimum size for metamorphosis (Wilbur and Collins 1973). Survival can vary greatly among years and among egg masses (Stenhouse 1987).

Contaminated highway runoff

There is a great deal of evidence indicating that highway runoff contaminated with heavy metals and deicing salt is transported to adjacent roadside habitats (Lagerwerff and Specht 1970, Harned 1988, Hofstra and Smith 1984). Gish and Christensen (1974) found elevated levels of cadmium, nickel, lead, and zinc in soils and tissues from earthworms. These levels decreased with increasing distance from road. In winter the highway contamination is stored in snow. Results of chemical analysis of highway snow indicated the presence of deicing salts and polycyclic aromatic hydrocarbons (PAH) (Hautala et al. 1995). In winter, contamination is stored in snow and then in spring it is transported by meltwater to adjacent terrestrial and wetland habitats (Hofstra and Smith 1984). Boxall and Maltby (1997) found that PAHs accounted for 30 – 100% of the toxicity in a stream sediment extract. Crowther and Hynes (1977) documented a substantial increase in sodium chloride in roadside streams and Burbeck et al. (1971) found elevated levels of sodium and chloride in Irondequoit Bay in Rochester, NY.

There is little information describing the impact of highway contamination on wetlands. There is evidence from one study that roadside snow melt can decrease plant

species diversity and richness, plant biomass, and growth (Isabelle et al. 1987). There is also some evidence suggesting that benthic organisms increase drifting behavior during pulses of high salt concentrations (Crowther and Hynes 1977). Little is known about the impacts of highway runoff on amphibians breeding in wetlands adjacent to highways.

Ultraviolet radiation

Ultraviolet radiation (UV radiation) is a stressful factor for organisms so they must avoid it, allocate energy to protect themselves from it, or repair its damage. Organisms evolved a tolerance level to UV radiation approximately equal to the organisms normal exposure level. However, individuals do not appear to have the ability to rapidly adjust tolerance levels to increases in UV radiation irradiance (Calkins and Thordardottir 1980). If UV radiation levels increase an organism has to protect itself at some metabolic expense.

UV radiation forms a portion of the total solar radiation. Solar radiation, the middle portion of the electromagnetic spectrum, contains two invisible parts (to human eyes), ultraviolet radiation and infrared, and one visible part, photosynthetically active radiation (PAR) (Figure I.1). The total ultraviolet wavelength band ranges from 200 - 400 nm wavelengths. The UVC (200 – 280 nm) waveband forms the lowest wavelengths of the UV spectrum but does not reach the Earth's surface. UVB (280 – 320 nm) forms the middle part of the waveband and UVA consists of the longest UV wavebands. The total band that reaches Earth's surface ranges from 290 – 400 nm. The shorter wavelengths of the UV spectrum are the most harmful biologically. Longer UVA wavelengths can cause damage but are also involved in photorepair mechanisms (Smith et al. 1992). The absorption of UV radiation by ozone in the stratosphere

increases exponentially as UV wavelength decreases so the harmful shorter rays make up only a small proportion of the UV radiation that reaches Earth's surface (Cutchis 1974). The decrease in ozone will have a greater effect on the shorter, more harmful wavelengths than on UVA wavelengths and PAR (Smith et al. 1992).

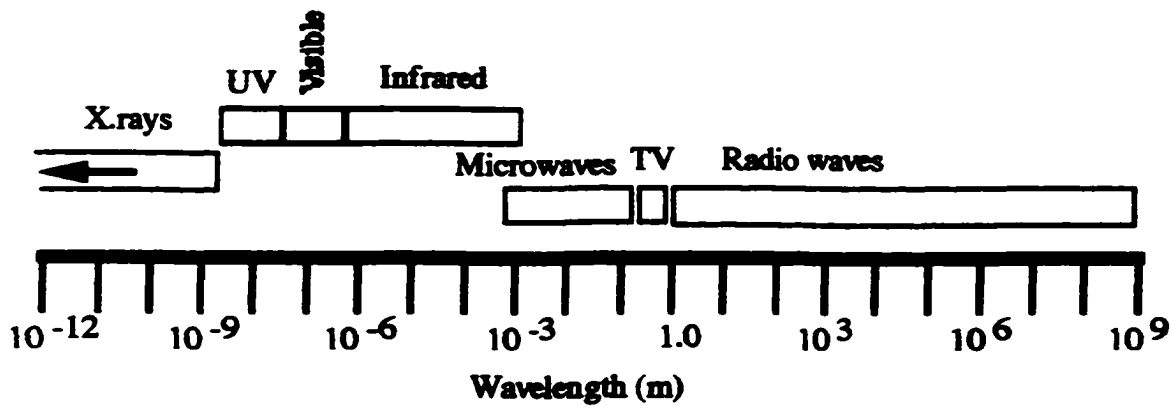

Figure I-1. The electromagntic spectrum. Recreated from Halliday and Resnick's "Fundamentals of Physics".

Reduction in stratospheric ozone in Earth's stratosphere causes an increase in UV radiation at Earth's surface (Smith et al. 1992, Kerr and McElroy 1993). Ozone levels do fluctuate daily, seasonally, and annually, however this fluctuation is cyclic (Cutchis 1974). Human activities, especially the continued use of chlorofluorocarbons, would decrease stratospheric ozone. This human-caused decrease in stratospheric ozone, due to chemical pollution of the air, was predicted to cause a unidirectional depletion of the ozone layer unlike the natural cyclic fluctuations (Cutchis 1974). Therefore, although the amount of ozone fluctuates naturally, the average amount of ozone overall would

decrease causing an increase in average UV radiation irradiance at Earth's surface (Cutchis 1974).

All organisms exposed to UV radiation have evolved several methods of photorepair of damage due to UV radiation and photoprotection against its effects (Calkins and Thordardottir 1980). We do not know the full extent of damage caused by UV radiation at this point, but we are aware of one possible effect. The shorter wavelengths of the UV radiation waveband (UVB) are strongly absorbed by DNA. UV radiation causes the formation of cyclobutane pyrimidine dimers (CPD) among the base pairs, which can disrupt DNA replication and transcription and possibly cause mutation, cancer, and cell death (Sancar 1994). Photoreactivation is the process of repairing such dimers. In this process, photolyase binds to the dimer and absorbs a photon of near UV – visible (300 –500 nm) radiation. This splits the cyclobutane dimer and restores the pyrimidines and then dissociates (Sancar 1994). The photolyase repair mechanism of UV radiation associated DNA damage has been found in all kingdoms, but not all species.

Organisms have evolved several ways to protect themselves from UV radiation by producing UV absorbing compounds. For example, mycosporine like amino acids (MAA) strongly absorb UV radiation in the range 310-360 nm. MAAs are found throughout tropical, temperate, and polar marine algal and invertebrate species (Karentz et al. 1991). The evolution of photosynthesis produced oxygen that formed the protective layer of ozone in the stratosphere. This produced conditions conducive for the evolution of terrestrial organisms. However, land species do not have the additional

protection of water so many have evolved more complex methods, such as pigments and morphological adaptations to protect themselves from UV radiation.

In 1985, Farman et al. reported the first conclusive evidence of a reduction in stratospheric ozone over the Antarctic. They observed that springtime values had declined 40% between 1975 and 1984. Results of field studies in the Antarctic and Arctic indicated that declines in ozone were associated with high levels of chlorine in the atmosphere (Solomon 1990, Anderson et al. 1991, Rex et al. 1997). The most dramatic ozone loss occurs in polar regions due to extremely cold temperatures and the polar vortex formed in winter. These conditions produce polar stratospheric clouds. The cold surfaces of these clouds provide the necessary conditions for several relatively stable species of chlorine (HCl, $ClONO_3$) to be converted into more unstable species that can be photolysed into forms involved in ozone destruction (Solomon 1990, Rex et al. 1997). Reduction in stratospheric ozone causes an increase in the UV radiation irradiance at Earth's surface, particularly within the UVB waveband. However, stratospheric ozone is not the only parameter controlling surface UV radiation irradiances (Smith et al. 1992, Kerr and McElroy 1993, Lubin and Jensen 1995). Clouds, tropospheric ozone, sulphur dioxide, nitrous dioxide and other chemicals can block UVB and reduce the impacts of the decline in ozone (Madronich 1992). The solar zenith angle also affects UVB irradiances. As would be expected, the tropics receive much higher dose of UVB than the temperate and arctic zones (Cutchis 1974).

Reports of declines in the ozone layer have caused concern over the effects of increased UV radiation. As various UV radiation studies were completed it became clear that UVB was a harmful factor for individuals, species, and biological

communities. A majority of early experiments examined the effects of UV radiation on marine systems because of the high transparency of marine water to short wavelengths (Karentz and Lutze 1990, Smith et al. 1992). Smith et al. (1992) found that UVB reached depths of 60 – 70 m during the Antarctic spring when ozone is at its lowest concentration. If UVB irradiance increase with the reduction of ozone, and UVA and PAR irradiances are not affected, the ratio of damaging UVB to UVA and PAR, a component of photorepair, will increase (Smith et al. 1992). Under these conditions UV radiation damage and UV repair mechanisms will not be in balance and organisms may not be able to repair the total extent of the damage (Smith 1992, Buma et al. 1997).

One of the largest ecological concerns about UVB was the potential negative effect on the primary production of the southern oceans. Evidence indicates that UV radiation is stressful to some species of phytoplankton and cyanobacteria. Results of both laboratory and field experiments indicate that UV radiation affects cell growth, reproduction, rates of photosynthesis and enzymatic activity (Jokiel and York 1984, Cullen and Lesser 1991, Smith 1992, Hernld et al. 1993, Lesser et al. 1996). Buma et al. (1997) found that exposure to UVB, at levels lower than found at Earth's surface, produced the majority of CPDs found in *Cyclotella* sp., a marine diatom, and reduced growth rates. However, the effects of UVB are species specific because of different repair mechanisms and protective strategies (Smith et al. 1992, Karentz et al. 1991).

Reports of stratospheric ozone reductions and increased UVB irradiances in temperate latitudes raised interest in and concern for the role of UVB in both terrestrial and freshwater systems. In 1992, Madronich reported that UVB was increasing by at least 20% per decade in north temperate latitudes. Kerr and McElroy (1993) found that

this increase in UVB varied seasonally with levels increasing more in winter months than in summer between 1989-1993 in the Toronto, Canada. They also reported that a downward trend in stratospheric ozone was measured during this period. However, simply associating increasing UVB levels with ozone loss excludes the actual complexity of the issue: tropospheric ozone, sulphate particles, and cloudiness affect UVB irradiance locally (Crutzen 1992). Kerr and McElroy (1993) caution that trends observed during their study should not be extrapolated to predict future levels because results of a few years of data can be influenced by natural cyclic variations.

Increases in UVB at Earth's surface may be an important factor in freshwater ecology. Many freshwater organisms with photoreceptors that are only sensitive to UVA may only respond to changes in UVA and PAR. In this case, they may detect changes in UVB in the water column and therefore will not attempt to behaviorally avoid these harmful environments (Williamson 1995). UVB might also affect freshwater communities by affecting associations of food resources or predators as in Carpenter and Kitchell's (1993) trophic cascade concept (Williamson 1995). It will be difficult to summarize the effects of UVB because the ability to detect UVB is species-specific and therefore, UVB damage will be species specific (Damkaer and Dey 1983, Bothwell et al. 1994).

Evidence is mounting which suggest that UVB is harmful in lakes. Results of several studies indicate that UVB affects freshwater communities at many levels. Williamson et al. (1994) found that mortality increased in some zooplankton species exposed to UVB in oligotrophic lakes but not in eutrophic lakes. Non-mobile species are more vulnerable because they can not reduce exposure behaviorally. Vinebrooke

and Leavitt (1996) found that the development of periphyton is slowed when exposed to UVB, suggesting that UVB can suppress overall primary production in clear water lakes. High levels of UVB are also known to decrease populations of bacterioplankton (Hernld et al. 1993, Ferreyra et al. 1997). The complex feedback loops in freshwater systems make it very difficult to predict the effects of UV on them.

Increases in UVB due to ozone loss are not the only way UVB exposure may increase in lakes. Changes in physical characteristics of lakes may change exposure to UVB (Williamson et al. 1996, Scully and Lean 1994). UVB is more likely to be attenuated in lakes with higher dissolved organic matter, chlorophyll, and other plant pigments (Williamson et al. 1996). Models developed to predict UVB attenuation in lakes from dissolved organic carbon (DOC) have shown that DOC is very important (Williamson et al. 1996). If DOC concentration is an important variable, then factors that change DOC concentrations may have a larger impact on the UVB reaching aquatic organisms than depletion of the ozone layer.

In conclusion, there is substantial evidence indicating that UVB radiation and highway contamination are stressful factors for species exposed to them. These two factors are also of current environmental concern. There is very little information describing their affects on freshwater wetlands in New England. This study will look at these two relevant abiotic factors on a vulnerable amphibian species in southeastern New Hampshire.

CHAPTER I

THE EFFECTS OF DEICING SALT AND HIGHWAY CONTAMINATION ON *AMBYSTOMA MACULATUM* EMBRYOS.

INTRODUCTION

Organisms that exist in roadside habitats are exposed to contaminated highway runoff and air pollution from automobiles. Deicing salt is a major part of highway snow and stormwater runoff (Isabelle et al. 1987). Relatively high levels are found adjacent to roads in soil, streams, lakes, and in plant tissue (Lagerwerrf and Specht 1970, Burbeck et al. 1971, Mudre and Ney 1986). During spring thaws the salt is transported to adjacent environments via snowmelt and rainfall. The distance the salt is transported is increased by plows pushing contaminated snow off the road (Isabelle et al. 1987). Hofstra and Smith (1984) found high salt levels in soil and vegetation 10 m from the roadside. High salt concentrations in roadside wetlands can affect the water chemistry and plant species diversity (Burbeck et al. 1971, Isabelle et al. 1987).

Amphibians have unique characteristics that may make them vulnerable to water-borne pollution: they have semi-permeable skin, their eggs are unprotected from the environment, and they require multiple habitats to complete their life cycles (Wyman 1990). Amphibian embryos and larvae are more sensitive to chemical contamination than adults (Zug 1993). Further, embryos are totally immobile and confined to water so

they can not move to better habitat as larvae and adults may do. Water contamination degrades the quality of breeding habitat and is thought to be a factor in decreased reproductive success at a local scale (Clark and Hall 1985, Freda and Dunson 1985, 1986, Tyler-Jones et al. 1989, Rowe and Dunson 1993). Deicing salt may affect amphibian distributions at the local scale by affecting reproductive success in polluted wetlands.

Results of a field study indicated that *Ambystoma maculatum* (spotted salamander) embryos had lower survival in vernal pools less than two meters from a road than in vernal pools greater than 50 m from a road (Turtle 2000). Results of water chemistry analysis indicated that these roadside pools had significantly higher specific conductance and sodium and chloride concentrations. However, it was not possible to infer a causal relationship between roadside pool water chemistry and low survival from this experimental design. Also, this study only focused on roadside pools with high levels of contamination (specific conductance > 500 $\mu S\ cm^{-1}$). Data collected in 1995 from 20 vernal pools in southeastern New Hampshire indicated that the specific conductance ranges from 40 – 1600 μScm^{-1} (Figure 1-1).

In 1995 I conducted a laboratory experiment to determine the direct effects of deicing salt on *A. maculatum* embryos. I did not observe any lethal effect of deicing salt concentrations of 0, 240, 480, 720, and 960 mg l^{-1} (Turtle 1996). However, there was a significant decrease in the diameter of the outer membrane (the length of the outer membrane at the widest point) as salt concentration increased (Turtle 1996).

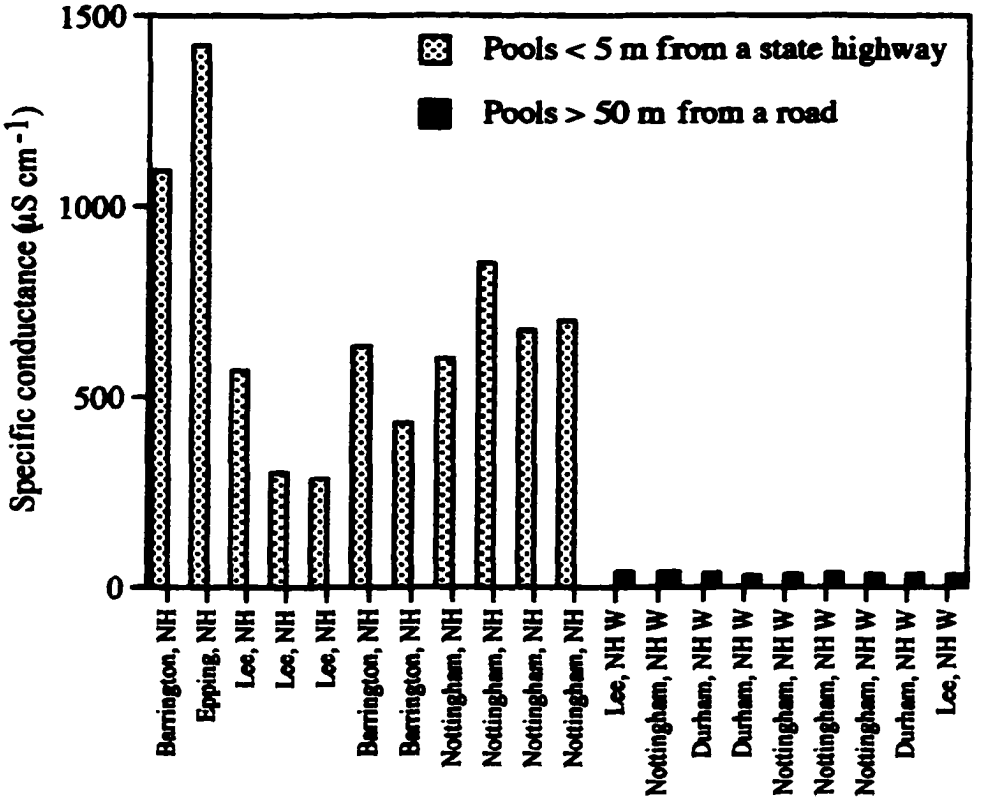

Figure 1-1. Specific conductance measurements taken on April 8, 1995 in vernal pools with roadside edges within 5 meters of a state highway and in vernal pools greater than 50 m from a paved road.

In this chapter I describe laboratory and field studies designed to further examine the effects of deicing salt. In 1996 I repeated the 1995 deicing salt laboratory experiment using higher deicing salt concentrations to determine the direct effects of deicing salt on *A. maculatum* embryos. In 1997 I examined the effects of water from pools < 2m from roads and pools > 50m from a road to more closely mimic the natural roadside environment. I also conducted a field experiment in 1997 to measure the survival of *A. maculatum* embryos in three types of pools; off road pools (pools greater than 50 m from a road), high contamination roadside pools, and low contamination roadside pools.

METHODS

Laboratory experiments

The 1996 laboratory experiment was designed to examine the direct effects of deicing salt. To conduct this experiment, I collected egg masses with all embryos near developmental stage 14 (before gastrulation, Harrison 1969, in Duellman and Trueb, 1986) from a woodland pool and brought them into the laboratory. I divided the egg masses into sections, each containing 1 - 15 embryos, mixed the sections, and then haphazardly placed them into groups of 50. I assigned four replicate groups to each salt treatment (0, 500, 1000, 1500, and 2000 mg l^{-1}). I poured two liters of each concentration, made with deicing salt and filtered pond water, into opaque high-density plastic containers (18 x 32 x 12 cm) and then placed the embryos into the container.

In 1997 I conducted an experiment to determine the effects of water from roadside and woodland pools on *A. maculatum* embryos. To conduct this experiment, I selected three pools in Nottingham, New Hampshire, for the natural water treatments.

Two pools (R1, high contamination and R2, low contamination) were less than two meters from a state highway (Rt. 152), and one off-road pool (W2) was greater than 50 meters from the highway (Table 1-1). I determined pool type with specific conductance data and distance from road. R1 had a very high mean specific conductance (> 1000 μS cm^{-1}), R2 had a low mean specific conductance (< 100 μS cm^{-1}), and W2 had a mean specific conductance of less then 20 μS cm^{-1} (Table 1-1). I collected water weekly (for four weeks) from each pool and brought it back to the laboratory and measured specific conductance and pH in the each pool prior to water collection. Following the procedure I used in 1996, I collected egg masses at developmental stage 14 (Harrison 1969, in Duellman and Trueb 1986) from one pool, divided the masses into sections, mixed the sections, and formed 12 groups of 50 embryos. Four replicate groups were assigned to each water treatment. Water was replaced weekly with fresh water brought in from field.

For both laboratory experiments, I placed treatments in an incubator at 15°C and changed water solutions every two weeks. Survival was monitored weekly throughout the embryonic stage. Just prior to hatching, I measured the outer membrane diameter at developmental stages 45 – 46 (Harrison, in Duellman and Trueb 1986).

For both experiments, I log transformed the membrane diameters to reduce heteroscedasticity and used SYSTAT's ANOVA and Dunnett's test (one-sided) to compare the control means to the treatment means (Wilkinson 1997). When animals hatched, they were released into their natal pools. I calculated means and standard errors for water chemistry data and analyzed these data with the non-parametric Kruskal-Wallis test (N = 4) to determine differences among the pools

1997 Field Transplant Experiments

In 1997 I conducted two replicate field transplant experiments (Transplant A and Transplant B) to determine the embryonic survival in the high contamination roadside pools (specific conductance >500 $\mu S\ cm^{-1}$), low contamination roadside pools (specific conductance < 100 $\mu S\ cm^{-1}$), and off-road pools (specific conductance < 50 $\mu S\ cm^{-1}$) (Table 1-1). I selected a total of six pools to make two replicates of the pool types; each contained one of the three water types. Pools R1, R2, R3, and R4 are roadside pools and specifically Pools R1 and R3 are high specific conductance pools and R2 and R4 are low specific conductance pools. Pools W1 and W2 are off-road pools. I selected the pools according to distance to a state road and specific conductance (Table 1-1). Pool R1, W1, and W2 correspond to pools R1, W1, and W2 in 1995-1996 field study (Turtle 2000).

For Transplant A, I collected eggs in early stages of cleavage from the following pools W2, R1, and R2. Keeping egg masses separate according to their natal pool, I divided the masses into sections and mixed them together. With eggs from each natal pool, I formed nine groups of 50 embryos for each pool and placed each group into a mesh container. I returned three such groups to their natal pool and transplanted three groups to each of the other two pools (Figure 1-2). Transplant B includes pools W1, R3 and R4. I followed the same protocol as for Transplant A (Figure 1-2). For Transplant B, only W1 and R4 contained enough eggs to conduct transplants, therefore, I could not transplant embryos from R3. After completing the transplants, I monitored embryonic survival and water chemistry (temperature, pH, specific conductance, and dissolved oxygen) weekly in each pool until embryos hatched. After the embryos hatched all survivors were released.

I analyzed the survival data using failure time analysis in STATA (Stata Corp. 1997). I used Kaplan-Meier product limit estimates to calculate survival functions and Greenwood's formula as reported in STATA to calculate standard errors (Stata Corp. 1997). I used the Wilcoxon test to test the equality of pool survival functions among embryos that shared pool origin. I used the Kruskal Wallis test in SYSTAT 7.0 to compare the water chemistry parameters (Wilkinson, 1997). A non-parametric test was used because of small sample size.

Table 1-1. Location and water parameters of vernal pools. Mean +/- 1 standard error for water parameter measurements for vernal pools used in 1997 field and laboratory studies. Measurements were taken weekly in each pool throughout the experiments.

1997	N	Location	SPECIFIC CONDUCTANCE ($\mu S\ cm^{-1}$)	TEMPERATURE (C)	DISSOLVED O2 ($mg\ l^{-1}$)	pH
R1	5	43°06.68 N 71°05.38 W	706.53 +/- 76.48	11.94 +/- 0.63	3.74 +/- 0.81	5.7 +/- 0.3
R2	5	43°06.64 N 71°05.28 W	25.25 +/- 1.65	9.00 +/- 0.57	3.53 +/- 0.89	5.3 +/- .09
R3	5	43°06.68 N 71°05.39 W	601.60 +/- 129.78	11.30 +/- 0.62	4.61 +/- 0.81	5.1 +/- 0.21
R4	5	43°05.94 N 71°04.23 W	83.99 +/-12.34	10.40 +/- 0.78	3.09+/- 0.43	5.2 +/- 0.15
W1	4	43°06.73 N 71°05.38 W	18.00 +/- 2	10.32 +/- 0.58	4.12 +/- 0.51	4.9 +/- 0.05
W2	5	43°06.72 N 71°05.35 W	18.00 +/- 1.22	10.40 +/- 0.5	4.02 +/- 0.40	4.8 +/- 0.04

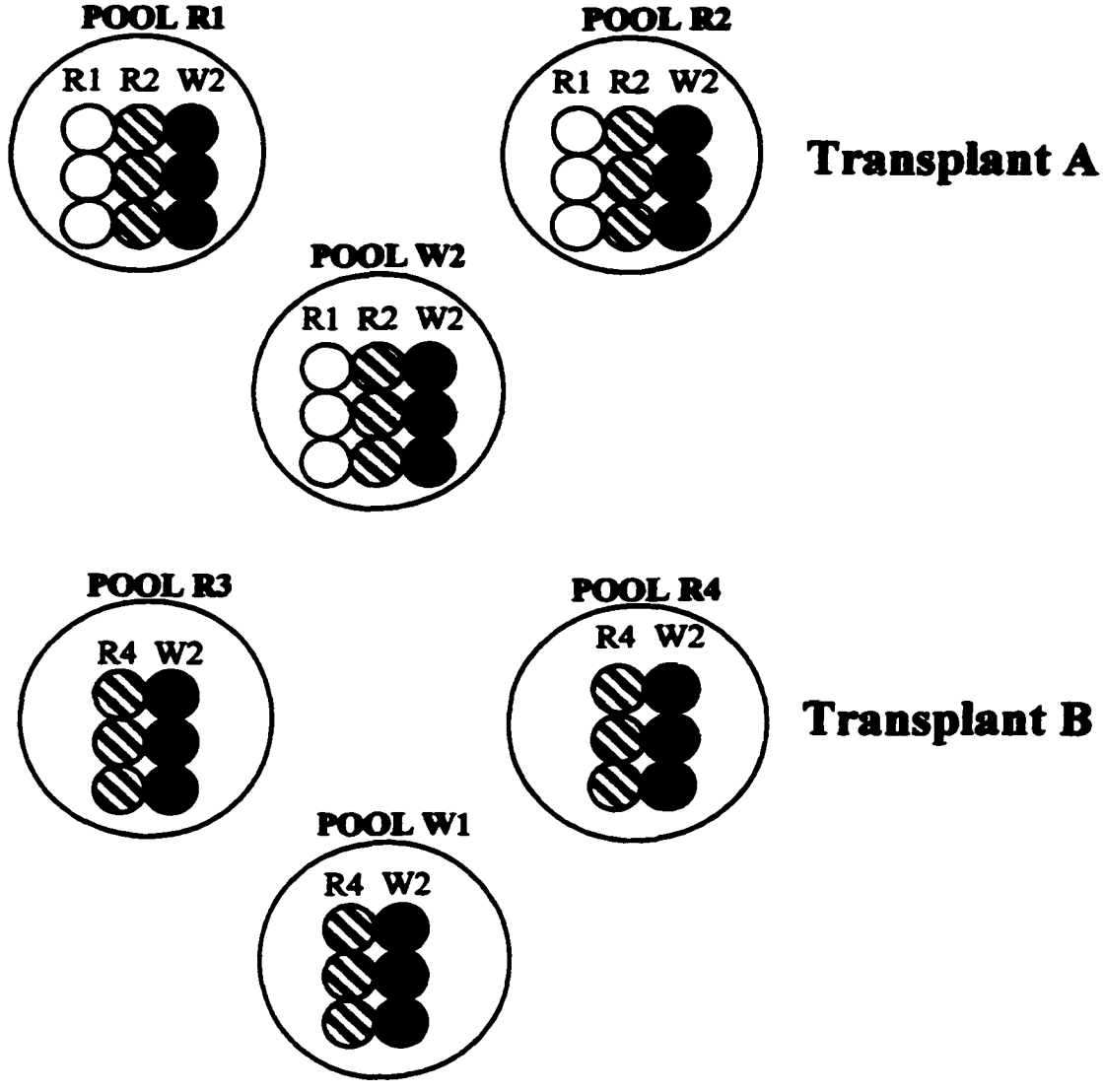

Figure 1-2. Diagram of the field transplants in 1997. Each transplant contained one of each pool type; off road (W1, W2) , high contamination (R1, R3), and low contamination roadside (R2, R4). Egg masses were collected from each pool (except Pool R3), divided into nine groups each containing fifty embryos. Three of these groups were selected to remain in the natal pools three were transplanted to each of the other two pools. Open circles indicate embryos transplanted from a high contamination pool, striped circles indicate embryos transplanted from a low contamination pool, and shaded circles transplanted from a off-road pool. I compared survivorship of embryos that shared natal pools.

RESULTS

Laboratory experiments

In the 1996 laboratory deicing salt experiment survival rate was 98 - 100% for all cases indicating that deicing salt did not significantly affect the survival of *A. maculatum* embryos. Although deicing salt did not have a lethal effect on the embryos, there was evidence of a sublethal effect in the form of different membrane diameters across treatments (Figure 1-3). Mean outer membrane diameters were significantly smaller in the salt treatments than in the control (Dunnett's test, all p's <0.05, Figure 1-3).

Water from roadside pools brought into the laboratory did not influence embryonic survival. All treatments in the 1997 experiment had 98 - 100% embryo survival. There was a significant difference in membrane diameter size (Table 1-2) between all water treatments from the road and the off-road pool (Dunnett's test, all p's <0.05, Figure 1-4). Embryos exposed to water from R1 had smaller membrane diameters than the other two treatments (Figure 1-4). Results of water chemistry measurements indicated that specific conductance and pH were significantly different among the treatments (specific conductance- Kruskal Wallis, $H = 11.73$, $df = 2$ $p < 0.05$; pH Kruskal-Wallis, $H = 10.05$, $p = 0.05$)

Field Transplants

Statistical results of Transplant A suggest that the probability of survival until hatching is significantly different among pool types (1-5; Wilcoxon test, all P's <0.001). In Transplant A, the survival function was lowest (0.10 – 0.60) for all groups in the high contamination pool (R1) regardless of pool origin. The survival function in R2 (low contamination) ranged from 0.68 – 0.72 and the survival function in the W2 (off road)

ranged from 0.60 – 0.70. In Transplant B, the survival trends were similar to Transplant A but they were not as clear (Figure 1-6). The survival function was highest in W1, the off road pool for eggs transplanted from both W1 and R4. Eggs transplanted from R4 had significant differences in survival. However, the survival functions of the eggs transplanted from W1 did not show significant differences among pools.

Table 1-2. Results of ANOVAs comparing membrane diameter among treatments in 1996 and 1997 experiments. A) ANOVA for 1996 deicing salt treatments B) ANOVA for 1997 water type experiments.

A. 1996	SS	df	MS	F	P
Treatment	0.33137	4	0.08284	6.5	0.003
Error	0.19154	15	0.01275		
B 1997					
Treatment	0.29181	2	0.1459	90.1	0.00001
Error	0.01458	9	0.00162		

The analysis of the water chemistry indicated that specific conductance was significantly different among pool types for both transplants (Transplant A – Kruskal Wallis, $H = 11.73$, $df = 2$ $p < 0.05$; Transplant B – Kruskal Wallis $H = 11.01$ $df = 2$ $p < 0.05$). There was a significant difference in pH among pools (Transplant A – Kruskal Wallis, $H = 10.05$, $df = 2$ $p < 0.05$; Transplant B – Kruskal Wallis $H = 7.70$ $df = 2$ $p < 0.05$). The off-road pools in both transplants had the lowest pH levels of all six pools (Table 1-1). In Transplant A, temperature was significantly different (Kruskal Wallis, H

= 6.46, df = 2, $p < 0.05$), but there was no significant difference in temperature for Transplant B (Kruskal Wallis, H = 1.40, df = 2 $p > 0.05$). Dissolved oxygen was not significantly different among pools for the transplants (Transplant A – Kruskal Wallis, H = 3.85, df = 2 $p > 0.05$; Transplant B – Kruskal Wallis H = 1.04 df = 2 $p > 0.05$).

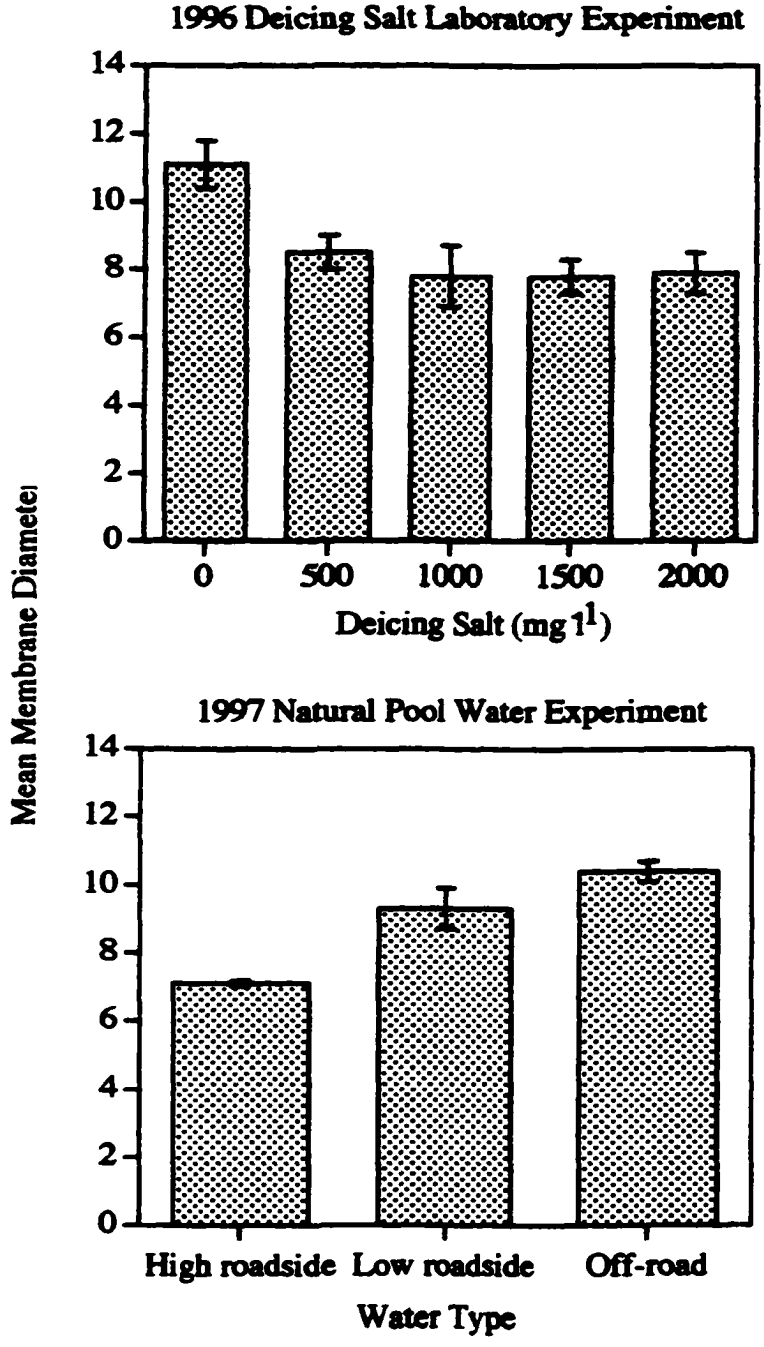

Figure 1-3. Membrane diameter for embryos in 1996 and 1997 laboratory experiments. Means +/- 1 standard deviation are shown.

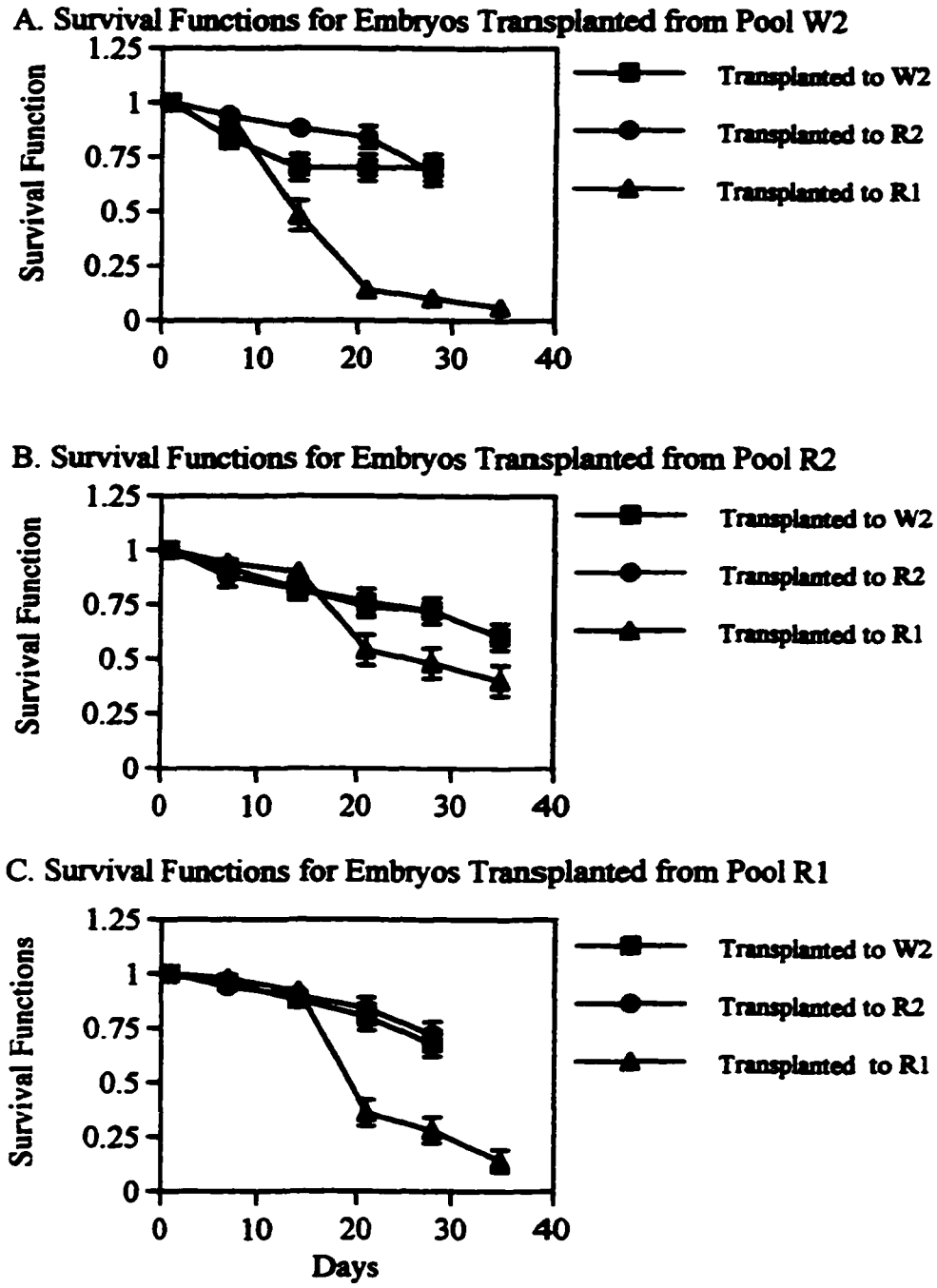

Figure 1-5. Survival functions for *A. maculatum* embryo transplants. Kaplan-Meier probabilities and Greenwood's standard errors were calculated for each census time. Legend indicates pool location of embryos after being transplanted.

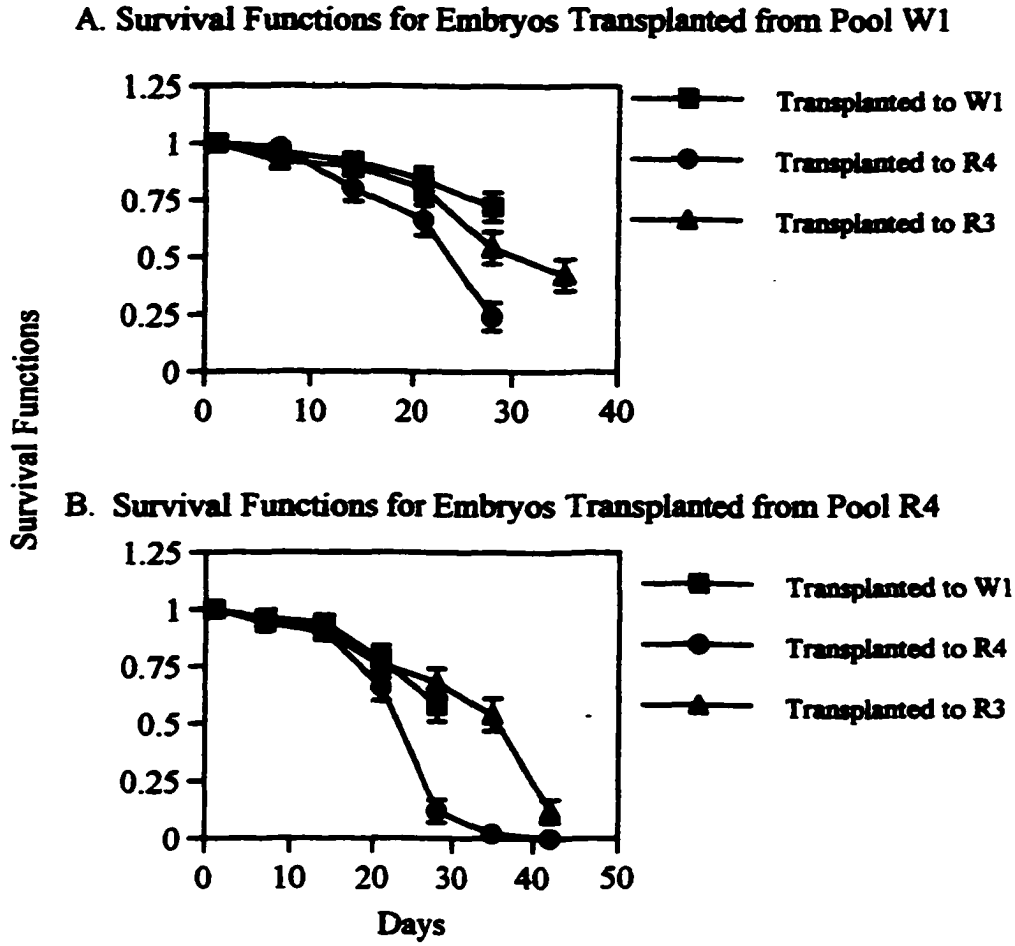

Figure 1-6. Survival functions for *A. maculatum* embryo transplants. Kaplan-Meier probabilities and Greenwood's standard errors were calculated for each census time. Legend indicates pool location of embryos after being transplanted.

DISCUSSION

I did not find evidence to suggest that deicing salt directly caused the low embryo survival that I observed in the 1995 - 1996 field experiments (Turtle 2000). In the laboratory, I exposed animals to salt concentrations an order of magnitude greater than concentrations observed in the field in 1995 and 1996, but observed no effect on survival. This is consistent with results of the 1995 deicing salt experiment (Turtle 1996). It is important to note that there were major differences in environmental conditions between laboratory and field experiments and these could affect the ability to compare results. Embryos in the lab were exposed to constant 15 ° C. water temperatures. Embryos in the field were exposed to a range of temperatures (2 ° C to 18 ° C) (Turtle 2000); amphibians develop faster at higher temperatures (Duellman and Trueb 1986). Embryos exposed to the colder temperatures in the field had longer embryonic periods and were exposed to the aquatic environment for longer time periods. The 1996 laboratory deicing salt experiment used only one possible contaminant found in highway runoff. Highway runoff can also be contaminated with heavy metals (Harned 1988) and polycyclic aromatic hydrocarbons (PAH) (Hautala et al. 1995).

Membrane diameter was smaller for embryos exposed to higher salt concentrations for the 1996 deicing salt experiment. This is consistent with results from the 1995 deicing salt experiment (Turtle 1996). This characteristic effect was first observed in amphibian embryos exposed to low pH and also high salt concentrations (Gosner and Black 1957). Freda and Dunson (1985) also found that *Ambystoma jeffersonianum* embryos exposed to increasing concentrations of salt became tightly curled in later stages of development in laboratory experiments. They suggest that the

hatching enzyme, produced by amphibian embryos, was inhibited by low pH and increased concentrations of Na, Ca, and Mg (Freda and Dunson 1985). The hatching enzyme changes the structure of the perivitelline membrane causing it to expand as the embryo grows. It eventually causes the membrane to rupture when the larvae are ready to hatch (Dunson and Connell 1982). Eggs exposed to low pH and high salt concentrations have smaller volume that causes the embryos to become curled as they grow. This can cause mortality, spinal deformity, and stunted gill development (Robb and Toews 1987).

Water type was also associated with membrane diameter. Membrane diameter was smallest for the embryos that were exposed to water from R1, the pool water with highest specific conductance, and largest for embryos exposed to water from W2, the off-road pool. Membrane diameter of embryos exposed to water from R4, the roadside pool with low specific conductance, were smaller diameter of embryos exposed to W2 but larger than diameters for R1. This suggests that specific conductance levels appear to be related to the decrease in membrane diameter. Results of the water chemistry analysis indicated that specific conductance was the parameter that differed significantly. Deicing salt contamination of highway runoff in the roadside pool most likely caused the difference between specific conductance between the roadside and off-road pools. Results of the previous field studies in 1995 and 1996 indicated that pools with high specific conductance had high levels of sodium and chloride. Other salts, potassium, magnesium, and calcium did not have elevated levels (Turtle 2000). The difference in specific conductance between the two roadside pools was most likely caused by physical differences of the pools. Pool R2 had a temporary roadside stream connected to the

roadside edge. The contaminated runoff may have reached this flowing section of the pool first and been transported downstream before it could enter the pool. This could cause a larger portion of the water in this pool to be collected from runoff from the non-roadside edges. Analysis of water chemistry also indicated that pH was significantly different between pools. Low pH was also found to be associated with small membrane diameters (Gosner and Black 1957). In the current study, membrane diameters did not decrease with decreased pH levels. Pool W1, the pool with low specific conductance, had the lowest pH, but the embryos exposed to this water had the largest membrane diameter. This indicates that pH levels are not the cause of the difference in membrane diameter.

Results of Transplant A in the field experiment indicated that embryonic survival was lowest in roadside pools with highest specific conductance. This was consistent with results of earlier transplant experiments in Turtle (2000). The trends in survival in Transplant B were similar to Transplant A but they were not as clear. Embryos transplanted to W1 had the highest survival functions. However, embryos transplanted to R4 (low contamination) had lower survival functions compared to embryos transplanted to R3 (high contamination). Overall, specific conductance levels appeared to be associated with lower embryo survival. Although there was no significant difference in mean dissolved oxygen levels between pools in Transplant B, R4 does had the lowest levels among all six pools used in this study. The low survival in R4 may be the result of a combination of low oxygen concentration and roadside contamination.

It is also interesting to note that in both transplants all embryos had higher survival functions in off-road pools even if it was not their natal pool. For example, in

Transplant A embryos transplanted from highly contaminated R1 had higher survival functions in pools they were transplanted to (W2 and R4). This adds to evidence collected in 1995 indicating that the embryos have better survival in non-natal pools possibly due to better water chemistry conditions. It also suggests that the disturbance to the embryos by the actual transplant method did not decrease their survival.

Overall, the trend in the deicing salt and the natural water laboratory experiments was similar. Embryos exposed to salt concentrations had smaller membrane diameters compared to the control with no salt and embryos exposed to water from roadside pools contaminated with deicing salt had smaller membrane diameters then embryos exposed to off-road pool water. This indicated that deicing salt contamination is the most likely contaminant causing the sublethal effect observed in this study. Results of the laboratory experiments indicated that deicing salt and roadside contamination alone did not affect survival and therefore were not the cause of the low embryonic survival observed in the field. However, they both produced the same sublethal effect in the form of decreased membrane diameters. Sublethal effects are receiving some attention in recent literature. Rowe et al. (1998a) have found evidence that some stressful environments can cause organisms to increase their allocation of energy to maintenance and decrease allocation of energy to growth and development. For an amphibian in a vernal pool environment, a decrease in the allocation of energy to growth may cause an increase in time needed to reach the critical minimum size to trigger metamorphosis (Wilbur and Collins 1973). Therefore the risk of mortality due to pool desiccation would increase. Recent studies have suggested that synergistic interactions between stressful factors may affect embryonic survival (Clark and Hall 1994; Long et al. 1995;

Kiesecker and Blaustein 1995). Experiments testing the effects of synergistic interactions may be needed to explain the low embryonic survival found in roadside pools in 1995, 1996, and 1997.

CHAPTER II

THE EFFECTS OF ULTRAVIOLET RADIATION ON *AMBYSTOMA MACULATUM* EMBRYOS IN FIELD EXPERIMENTS

INTRODUCTION

Unexplained declines in amphibian populations located in relatively pristine areas have caused some scientists to focus their attention on the effects of recent increases in levels of UVB (ultraviolet B) radiation on amphibians. There are two current hypotheses; 1) UVB directly affects amphibians by increasing mortality or deformities (Blaustein et al. 1994), 2) UVB acts synergistically with other pollutants to increase amphibian mortality (Kiesecker and Blaustein 1995, Long et al 1995).

In 1992 Madronich reported that UVB was increasing by at least 20% per decade in north temperate latitudes. Kerr and McElroy (1993) found that this increase in UVB varied seasonally: UVB (300 nm) levels increased more in winter months and less in summer between 1989-1993 in the Toronto area. They also reported a downward trend in ozone during this period. However, simply associating UVB increases with downward trends in ozone ignores the complexity of the issue; troposheric ozone, sulphate particles, and cloudiness affect irradiance locally (Kerr and McElroy 1993). Although we need more long-term data before we can be sure of these increasing trends, some scientist are already concerned about the direct and indirect effects of UVB on living organisms. It is well established that UVB damages DNA, is associated with cancer, and decreases primary productivity (Larson and Berenbaum 1988).

Amphibians may be especially vulnerable to UVB because adults and larvae have semi-permeable skin and their eggs lack protective shells (Wyman 1990). Pond breeding amphibians have a very vulnerable embryonic stage because embryos are exposed to the environment through the semi-permeable membranes. Also, eggs are laid at shallow water depths and are unable to move to avoid sunlight so they have little protection from UVB (Grant and Licht 1997).

There is evidence that UVB radiation can decrease hatching success of amphibians. Blaustein et al. (1994) found that two of three species of Oregon anurans had lower hatching success when exposed to UVB radiation. They also found photolyase activity was lower in UVB sensitive species (Blaustein et al. 1994). The low tolerance for UVB and low photolyase activity was associated with two species populations thought to be in decline in Oregon's Cascade Mountains (elevations 1190 – 2000 m). Lizana and Pedraza (1998) also observed species-specific UVB tolerance in embryos of two toad species in the mountains of Spain (1920 m). *Bufo bufo* embryos had very low survival when exposed to UVB radiation but there was no difference in survival among UV treatments for *Bufo calamita* (Lizana and Pedraza 1998). These experiments were all conducted at high elevations where exposure to UV radiation is higher. The results of experiments conducted at lower elevations also show species-specific UVB tolerances. Hatching success was lower for *Ambystoma gracile* when exposed to UVB in field experiments at an elevation of 183 m (Blaustein et al. 1995). Anazlone et al. (1998) found that two of three species of anurans had lower embryo survival when exposed to UVB under natural conditions at 290 m above sea level. The effects of UV radiation can also vary within a species for example, in Colorado, *Bufo*

boreas, a species that was sensitive to UVB in Blaustein's et al. experiment (1994), did not show sensitivity to UVB under natural conditions adding a great deal of complexity to the issue (Corn 1998). There is also considerable variation in the effects of UVB on other life history stages of amphibians (Grant and Licht 1995). Collectively, the results of the many experiments indicate that UV tolerance varies among species and sometime even within species (Blaustein et al. 1994, Grant and Licht 1995, Ovaska et al. 1997, Corn 1998).

Most of the research examining amphibians and UV radiation focused on survival of embryos. Observations of decreased survival are then linked to observed population declines. However, many pond breeding amphibians have naturally low hatching success rates and those rates vary between years (Stenhouse 1987). Many lay large numbers of eggs in clusters each year and they put little, if any, parental care into protecting eggs from predation. It may be difficult to predict population effects from embryonic survival studies for species with this life history strategy.

Examination of sublethal effects may be also important in determining how increases in UV may affect populations (Bradford et al. 1992). Several recent studies have concluded that different abiotic factors can cause detrimental effects on populations at a sublethal level. Rowe et al. (1998b) found oral deformities in anuran larvae exposed to coal ash pollution. He speculated that these deformities affected their ability to eat. In another study, Rowe et al. (1998a) found that *Rana catesbeiana* tadpoles in wetlands polluted by coal ash had higher respiration rates than in unpolluted ponds. Other factors have also been known to sublethally affect amphibian larvae. Bridges (1999) found that exposure to sublethal levels of pesticide caused a decrease in activity of *Hyla versicolor*

tadpoles. This decrease in activity decreased the risk of predation but also decreased feeding time and may consequently extend larval period and decrease size at metamorphosis (Bridges 1999).

In temporary wetlands sublethal effects may be even more detrimental than permanent wetlands. Vernal pools, a type of temporary wetland, are formed in the spring by the collection of snowmelt and rainfall and they are usually dry by September depending on annual precipitation. Some species rely on these habitats for embryonic and larval development because they serve as a refuge from fish predation However, there is a tradeoff; to survive amphibian embryos and larvae must metamorphose before the pools dry. Changes in the environment that slow growth or development rates can increase risk of mortality due to desiccation when pools dry. The effects of UVB may therefore be magnified in this stressful aquatic system.

In this study I examined the effects of UV radiation on an obligate vernal pool amphibian, the spotted salamander, *Ambystoma maculatum*. *Ambystoma maculatum* is an obligate vernal pool species commonly found in New Hampshire. In early spring this salamander migrates to vernal pools to breed. Eggs are deposited in late March - mid-April and usually hatch within six weeks. Larvae remain in the pool until they metamorphose in late summer and early fall or sometimes, if pools does not dry, larvae overwinter in the pool.

My goal in this project was to examine lethal and sublethal effects of UV radiation. I examined survival, size at hatching, melanin concentration, and DNA damage in *A. maculatum* embryos in six vernal pools. It is known that variation in factors, such as photolyase activity and egg membrane thickness, explain some of the

variation in UVB effects on amphibians. However, variation in abiotic parameters of breeding sites may also cause variation in UVB effects contributing to overall variation in observed effects of UVB on amphibians. I used vernal pool water chemistry data to determine association between chemical environment and embryo size to determine the factors that affect UV spectral irradiance in each pool.

METHODS

1998 Experiments

I designed a field experiment to examine the effects of UV radiation on spotted salamander embryos in vernal pools. I selected six pools in Durham, Lee, and Nottingham, New Hampshire for the experiment (Table 2-1). Selection was based on presence or absence of spotted salamander breeding activity. I collected egg masses from Pool 2 because this pool had a large number of egg masses laid at approximately the same time so the embryos were all in very early stages of cleavage (Harrison 1969, in Duellman and Trueb, 1986).

Table 2-1. Locations and characteristics of vernal pools used in 1998 and 1999.

	Location	Town	surface area (m^2)	dominant upland tree species	wetland plant species richness	% canopy transmission
Pool 1	43°06.27N 70°58.91W	Lee	120	*Acer rubrum*	3	58%
Pool 2	43°06.04N 70°58.78W	Durham	837	*Pinus strobus*	11	98%
Pool 3	43°06.73N 70°05.38W	Nottingham	230	*Tsuga canadensis*	6	70%
Pool 4	43°06.22N 70°58.93W	Lee	1120	*Acer rubrum*	8	61%
Pool 5	43°07.61N 70°00.00W	Lee	705	*Pinus strobus*	5	100%
Pool 6	43°06.27N 70°58.91W	Lee	5700	*Pinus strobus*	13	67%

I divided the egg masses into sections and mixed sections from all masses to control for genetic diversity. I formed groups of 30 embryos each using a haphazard process. Each

group was placed into a mesh (1.5 mm^2 size mesh) container. The containers were cylindrical in shape, 25 cm high and 25 cm in diameter, and were closed at the bottom. During the experiments, I discovered that predators, such as *Rana sylvatica* larvae, entered containers through the mesh so I placed a muslin bag around each container to protect the embryos. I assigned nine containers to each of the six pools and divided them among three UV treatments; UVO (PAR (photosynthetically active radiation 400-700 nm) only, no UV radiation), UVA (UVA (320 - 400 nm) + PAR), and UVT (UVB (280 - 320 nm), UVA, and PAR) (Figure 2-1). To manipulate UV radiation I used sheets of acrylic and mylar. I used UV opaque acrylic for UVO (90% transmission for wavelengths > 420 nm, 0.001 % transmission for wavelengths < 400 nm), UV transparent acrylic and mylar for UVA (76% transmission for wavelengths > 400 nm, 60% transmission for wavelengths > 340 nm, 0.02 % transmission for wavelengths 320 nm), and UV transparent for UVT (> 80% transmission for wavelengths > 300 nm, 33 % transmission for wavelengths = 290 nm) (San Deigo Plastics Inc.). All acrylic was soaked in tap water for 48 hrs prior to use in vernal pools. For all treatments I cut the acrylic into pieces (60 x 60 cm), and attached each piece to a wooden stand so that they were 15 cm above the water's surface. Each pool contained an acrylic piece for each UV treatment. I hung three containers of eggs underneath each stand so that the bottom 5 cm of the container and all embryos were submerged. I adjusted the containers as pool water levels fluctuated to maintain embryos at a constant depth.

Survival of the embryos was monitored weekly until just prior to hatching (stages 45-46, Harrison stages in Duellman and Trueb 1996). At this time, I brought the embryos into the laboratory, measured their length and froze them for future analyses. I

conducted an embryo melanin assay to look for difference in melanin concentration among treatments (methods modified from Li and Hill 1997). To conduct this assay protein was extracted from entire embryo in a solution of 50 µl of NaOH and 450 µl of double distilled water and sonicated the solution to break up the embryo. The solution was then incubated overnight at room temperature to solubilize the melanin. I centrifuged the solution at 1000 rpm, then diluted 100µl of the solution with 1400µl of double distilled water, and then recorded the absorbance at 410 nm. The absorbance was converted to µg of melanin using a standard curve created from squid melanin (Sigma Corp.). I then used another portion of the protein extraction to quantify the total protein extracted from each embryo. I diluted 10 µl of the protein solution with 990 µl of ddH_2O and recorded absorbance at 235 nm and 280 nm. To calculate mg protein/µl I used the following equation: mg protein/µl = (abs235 – abs 280)/2.51 (Whittaker and Granum 1980). I used the results of the protein analysis to normalize melanin concentration for individual embryos.

1999 Field Experiments

In 1999 I conducted a second field experiment in the same pools used in 1998 (Table 2-1). I followed procedures used in 1998 to create 54 groups of 30 embryos each and then placed each group into an open-topped mesh container. I haphazardly selected nine groups for each pool and, in each pool, divided these groups among the three UV treatments. I used the same UV treatments as in 1998. I changed the design slightly from the 1998 experiment by placing each group under its own section of acrylic to create independent units within the pool. I placed each piece of acrylic in the pool with toggle floats (soaked in tap water for 48 hrs) attached to each corner so that it would

float 10 cm above the water's surface. The containers of embryos were attached below the acrylic so that they were submerged at a depth of 5 cm. I arranged the replicates in a latin-square design (Figure 2-1c) and tethered them to a stake to restrict movement. I measured survival weekly until the embryos reached stages just prior to hatching, removed the embryos from their egg membranes, measured their length and wet mass, and then froze the embryos for the melanin assay according to methods used in 1998.

I isolated DNA (Easy-DNA kit Genomic DNA Isolation, Invitrogen Corp.) from the embryos to examine UV radiation damage represented by the presence of cyclobutane pyrimidine dimers (CPD). After the DNA was isolated I used spectrophotometry to quantify amounts for each sample by measuring the absorbance for each sample at 260 nm and 280 nm. DNA quality was calculated from the ratio of the absorbances (abs 260 nm : abs 280 nm) and then the amount of solution necessary to produce 100 μg of DNA was calculated for each sample. An indirect antibody enzyme linked immunosorbent assay (ELISA) was used to detect cyclobutane pyrimidine dimers (CPD) formed in the DNA by the exposure to UVB (Mori et al. 1991). In the ELISA assay I used the extracted DNA (antigen) to coat microtitre plates (96 wells). The plate was incubated overnight at room temperature and then rinsed with phosphate-buffered saline (PBS)- 0.5% Tween-20 to remove any antigen not bound to wells. The plate was then incubated with 3% Bovine serum in a blocking buffer. The Bovine serum was diluted in a blocking buffer that prevents the nonspecific adsorption of protein to any free sites on the well that are not occupied by DNA (Crowther 1995). Primary antibody (TDM-2) was added at a dilution of 1:1000 and incubated for two hours at room temperature. After incubation the plate was washed three times with PBS – 0.5%

Tween 20. The secondary antibody (Goat anti-mouse IgG conjugated horseradish peroxidase conjugate (1:3000) was added and incubated for two hours at room temperature. After incubation, the plate was washed with PBS – 0.5% Tween 20 three times. PBS – 0.5% Tween 20 was added and incubated the plate for 5 minutes. After this last incubation the plate was emptied and Sigma Fast color reagents (1 urea tablet and 1 hydroxide tablet in 20 mls) were added. Color developed for 30 minutes and then the plate was read in a microplate reader at 405 nm.

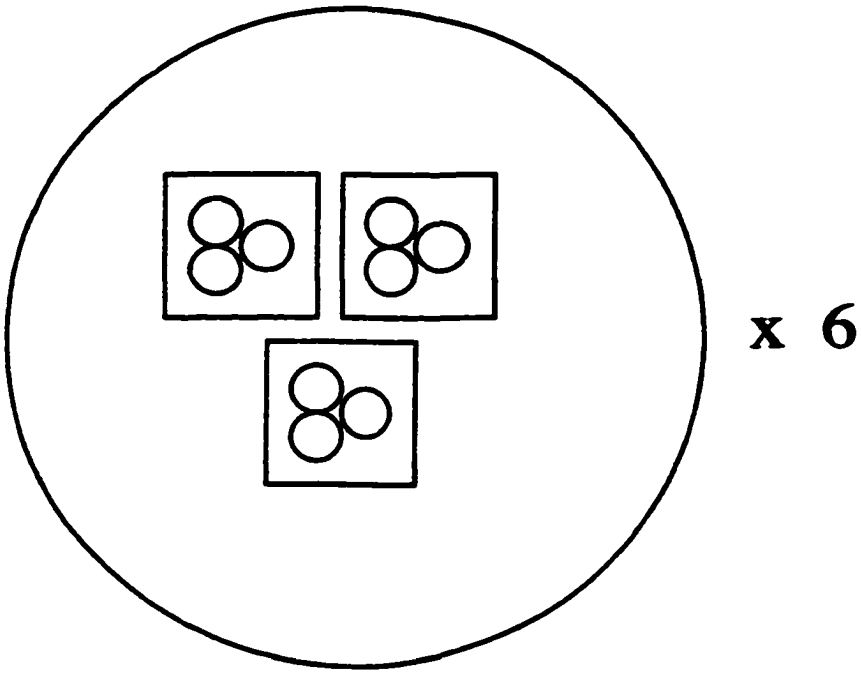

Figure 2-1a. Diagram of experiment set up for embryo field experiments in 1998. Each square represents a UV treatment. The circles represent containers of 30 embryos.

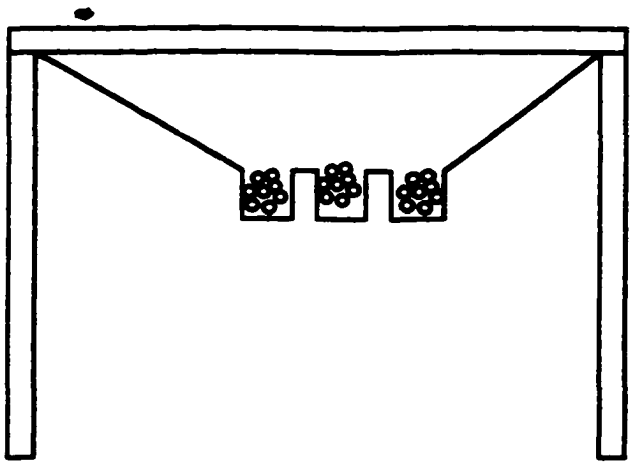

Figure 2-1b. Diagram of set-up for each UV treatment. Baskets were hung so that the bottom of each was approximately 5 cm under the water's surface

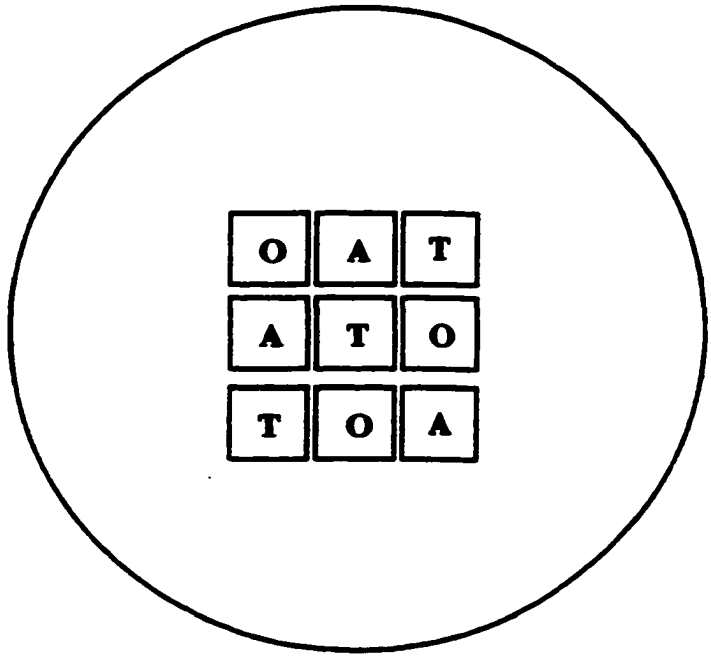

Figure 1c. Diagram of embryo field experiments in 1999. Each square represents a piece of acrylic used to control UV radiation. A container of embryos was placed under each piece of acrylic. I used a latin square design to limit pseudoreplication.

Water Analysis 1998 and 1999

Underwater spectral irradiance was measured at a depth of 5 cm with an underwater spectroradiometer (LI 1800UW). The LI 1800UW scanned wavelengths between 300 and 750 nm at 2 nm intervals. Each measurement was an average of three scans in the specified range. In 1998 measurements were taken in each pool within a two-hour period surrounding solar noon in early and mid April. On May 2, the LI 1800UW was placed in Pool 5 to measure wavelengths between 300 –750 nm hourly from 6 am to 6 PM in Pool 5. In 1999 the LI-1800UW was used to measure underwater UV irradiance three times during the experiments (April 16, April 22, April 30). On April 21, the spectral irradiance was measured under each piece of acrylic in Pool 2. I calculated the total dose of UVB radiation, UV radiation, and PAR by integrating irradiance beneath the spectral curve for the appropriate wavelengths. The total daily dose of UVB radiation, UVA radiation, and PAR for each pool was approximated using irradiance over time measurements from 1998 hourly data from Pool 5 and noon irradiances from sunny days in each pool.

To describe the pool environments temperature, dissolved oxygen, specific conductance (using YSI meters), and pH (Orion meter) were measured weekly for both 1998 and 1999 at a depth of 5 cm. In 1999 alkalinity was measured three times during the field experiment according to methods described in Lind (1985) (titrant 0.002 N H_2SO_4).

In 1998 I collected samples from all pools on April 15 and April 30 to measure dissolved water color. Samples were collected in well-rinsed (double distilled (dd) H_2O) high-density polyethylene (HDPE) bottles and all bottles were rinsed with pool water

immediately prior to collection. Samples were vacuum filtered through Whatman GF/F glass fiber filters into well rinsed (ddH_2O)bottles. In 1999 I collected three samples for dissolved water color (April 22, April 28, May 6). Water samples were collected in syringe filters and filtered water through Whatman GF/F filters into 250 ml bottles. Bottles were rinsed with double distilled water and then with filtered pool water before final collection. I also estimated dissolved water color of unfiltered water for collections made on April 22. To estimate dissolved water color, I measured absorbance at 440 nm with a spectrophotometer (Milton Roy Spectronic 1001, 5 cm pathlength). A standard curve was created using serial dilutions of color standards the regression coefficient from the standard curve was used to estimate color units from sample absorbance.

In 1998 I collected one sample from each pool for dissolved organic carbon (DOC) (April 15). Samples were collected in acid washed and well-rinsed HDPE bottles. Immediately prior to collection, bottles were rinsed thoroughly with pool water. Samples were vacuum filtered through Whatman GF/F filters (ashed 1 hr. at 425°C) into acid washed well-rinsed 30 ml HDPE bottles and frozen for chemical analysis. For the DOC analysis, I thawed samples, acidified them with 10% HCL and sparged them. I used high temperature (680 °C) catalytic (pt) oxidation with a Shimadzu TOC 5000 to determine DOC concentrations. In 1999 I collected four samples (April 22, April 28, May 6, May 10) using a syringe filter. Syringes were rinsed with ddH_2O and pool water prior to collecting sample. Samples were filtered through (ashed 1 hr. at 425°C) Whatman GF/F filters into acid washed well rinsed 30 ml HDPE bottle and frozen for chemical analysis. In 1999 the Water Resources Laboratory (Dr. William McDowell,

Department of Natural Resources, University of New Hampshire, Durham, NH) conducted the DOC analysis using the same protocol used in 1998.

I collected samples for major cations and anions analyses and also heavy metals analyses. For ion analysis, samples were collected on May 6, 1999. I used well-rinsed (ddH_2O) syringe filters to filter water through a 0.2 µl pore size Acrodisc (Gleman Science) into a polyethylene scintillation vial. Samples were refrigerated (at approximately 4°C) until chemical analysis. The Water Resources Laboratory (Dr. William McDowell, Department of Natural Resources, University of New Hampshire, Durham NH) conducted the analysis. They used ion chromatography to estimate the following cations and anions; sodium, potassium, magnesium, calcium, chloride, nitrate, and sulfate. I collected water samples for heavy metals analysis on May 4, 1999. The Analytics Environmental Laboratory Inc (AEL Portsmouth, NH) conducted the analyses. AEL provided high density polyethylene bottles for the collection. Each bottle contained nitric acid for preservation purposes. I collected water without filtration and placed all bottles on ice. Samples were taken to AEL for analysis within two hours of collection.

Pool Surface Area and Vegetation Sampling

To determine pool surface area, I marked the edge of the pool using flags on April 19, 1999. To estimate surface area the length of the pool was measured at its longest distance. Using this line as a reference, the distance to each flag was measured perpendicularly from this line. I used these data to create a representation of each pool's surface area on paper at equal scales. Using the same scale a 10 m x 10 m surface area

model on paper was also created I then estimated the surface area of each pool comparing the mass of the 10 m^2 model to the mass of representative pool model.

I used five,10 m x 10 m quadrats to sample tree abundance in the area surrounding the pool. I placed the non-overlapping quadrats randomly with the first corner at the pool edge. In each quadrat, species and diameter at breast height (dbh) of all trees (dbh > 2.5 cm) was recorded. I calculated relative dominance and created dominance diversity curves to describe the dominant tree types surrounding each pool.

I used the line point transect method to describe the vegetation cover within the pool boundary. I placed ten parallel transects (5 m) randomly inside the pool and I recorded species at 5 cm intervals along each transect for a total of 1000 points. I used these data to estimate percent cover of herbs and shrubs within the pool.

I estimated the percent canopy transmittance using a Sunfleck PAR Ceptometer. (Decagon). I measured PAR in an open canopy area immediately before measuring PAR at the location of the experiment in each pool. All measurements were taken in the two-hour period surrounding solar noon. At each location I took the mean of five readings to estimate PAR photons. To estimate the percent canopy transmittance at each experiment location, I calculated the percent transmittance directly above acrylic treatments in each pool of the total transmittance measured in the open field.

Statistical Analyses

"It does not do to leave a live dragon out of your calculations, if you live near him."
J. R. R. Tolkien

I calculated mean embryo length for each treatment in each pool in each year and I calculated means for each treatment for melanin in 1998 and wet mass in 1999. In 1999 I calculated mean melanin concentration for each container to compare mean

melanin concentration among UV treatments in Pools 2 and 3. I calculated mean absorbance for each treatment in Pools 2 and 5 to compare the production of CPD among treatments. A fixed effects General Linear Model (GLM) ANOVA (Model I ANOVA Sokal and Rohlf 1995) was used in the SYSTAT 7.0 (Wilkinson 1997) to test for the effects of UV treatment, variation due to pool, and year (year was included for length only) on embryo parameters measure in this study. I used to logarithmic transformation for melanin concentration, and CPD data to reduce heteroscedasticity. Although the pools were randomly selected and could be treated as a random effect in a mixed model (Sokal and Rohlf 1995), I was unable to calculate interaction error terms because sample size = 1 in some cells of the model. The independent variable Pool was not significant in any analysis so I removed it and repeated the analysis with only UV treatment. I adjusted the probability of making a type 1 error (α) to 0.01 according to the Bonferroni adjustment to protect the overall experimental alpha of 0.05. I used SYSTAT 7.0 to conduct all these analyses.

I used failure time analysis to test for significant differences in survival between treatments in STATA (Stata Corp. 1997). To conduct these analyses, I used Kaplan-Meier product limit estimates to calculate survival functions and Greenwood's formula to calculate standard errors. I used the log-rank test to test the equality of pool survival functions among UV treatments.

I calculated a correlation coefficient matrix to examine the associations between the water parameters; temperature, dissolved oxygen, dissolved organic carbon, pH, log(UVB spectral irradiance), dissolved water color (absorbance at 440), and the biological variable embryo length for embryos exposed to all UV radiation (UVT). I

reported Bonferonni adjusted probabilities to limit the overall experimentwise error rate. I used Systat 7.0 to conduct the analysis.

RESULTS

There were no significant differences in embryonic survival between UV treatments in 1999. Survival data in 1998 were not interpretable due to heavy predation by *Rana sylvatica* (wood frog) larvae. In 1999 I successfully excluded the anuran larvae but not invertebrates. I analyzed survival from the four pools where predation was not a factor (Pools 1, 2, 3, and 6). In Pool 1, there was no significant difference in survival between UV treatments (Chi square 0.53, $p > 0.05$, Figure 2-2). The UVO treatment had the lowest survival function (UVO = 0.78, UVA = 0.86, UVT = 0.86). There was no significant difference in survival in Pool 2 for UV treatment (Chi square = 0.2, $p > 0.05$, Figure 2-2). Overall, survival function was lowest in Pool 2 compared to the other pools (UVO = 0.61, UVA = 0.68, UVT = 0.63). Pool 3 had the highest overall survival functions (UVO = 0.86, UVA = 0.82, UVT = 0.85), but, again, there was no significant difference in UV treatments (Chi square = 0.0 $p > 0.5$, Figure 2-2). In Pool 6 there was also no significant difference in survival (Chi-square = 1.5, $p > 0.4$, Figure 2-2). The UVO treatment had the lowest survival function of the three treatments (UVO = 0.70, UVA = 0.73, UVT = 0.83).

I did not observe any statistically significant effects of UV radiation on embryo size or melanin concentration. In 1998 the shortest mean embryo length corresponded to the UVT treatment in four of the six pools (Figure 2-3). In 1999 I observed that length was shorter for embryos in UVT treatments in Pools 2 and 4 (Figure 2-4). However, results of statistical analyses indicated that mean embryo length was not

significantly different among UV treatments across all pools in either year (Table 2-2). Mean embryo length was significantly different between pools and between years (Table 2-2).

Table 2-2. Results of analysis of variance (ANOVA) performed on embryo length for UV treatments in six vernal pools in 1998 and 1999.

N = 32 R = 0.87 R2 = 0.75

Source	Sum of Squares	df	Mean Square	F - ratio	P value
UV treatment	0.0004	2	0.0002	0.15	0.86
Pools	0.02	5	0.005	3.6	0.01
Year	0.06	1	0.06	45.4	<0.001
Error	0.03	23	0.001		
Total SS	0.11				

I observed that mass was less for embryos in UVT treatments in which they were exposed to UVB in 4 of 5 pools (Figure 2-5) but there was no statistically significant difference among UV treatments or pools (Table 2-3). When the variable pool was removed from the analysis UV treatment was still non significant ($F_{2,11}$ 0.03, p = 0.96).

Table 2-3. Results of analysis of variance (ANOVA) performed on embryo wet mass for UV treatments in six vernal pools in 1999.

Source	Sum of Squares	df	Mean Square	F - ratio	P value
UV treatment	0.07	2	0.02	1.15	0.4
Pools	0.001	4	0.0008	0.05	0.9
Error	0.11	7	0.02		
Total SS	0.181				

In 1998 melanin concentrations were lower for embryos exposed to UVB radiation in Pools 2, 3, and 4 (Figure 2-6). However, the difference in melanin concentration did not differ significantly among UV treatments across all pools (Table 2-4). There was also no significant difference in melanin concentrations among pools (Table 2-4). Results of one way ANOVA testing the effects of UV treatment alone also indicated no significant differences ($F_{2,15} = 1.51$, $p = .25$). In 1999 I observed no significant differences in melanin concentration among UV treatments within pools 2 and 3 (Table 2-5, Figure 2-7). After the variable pool was removed UV treatment was still not significant ($F_{2,27} = 0.003$, $p = .99$). I also did not observe any trends as in 1998 results (Figure 2-7). I did not observe any significant differences in CPD production for UV treatment or pool variables (Table 2-6, Figure 2-8). Results of one-way analysis indicate that UV treatment did not significantly affect melanin concentration when tested alone ($F_{2,26} = .61$, $p = .55$)

Table 2-4. Results of analysis of variance (ANOVA) performed on melanin concentrations for UV treatments in the six vernal pools in 1998.

Source	Sum of Squares	df	Mean Square	F - ratio	P value
UV treatment	0.63	2	0.31	1.38	0.29
Pools	0.86	5	0.17	0.75	0.6
Error	2.27	10	0.22		
Total SS	3.76				

Table 2-5. Results of analysis of variance (ANOVA) performed on melanin concentrations for UV treatments in the Pools 2 and 3 in 1999.

Source	Sum of Squares	df	Mean Square	F - ratio	P value
UV treatment	0.026	2	0.013	1.006	0.39
Pools	0.0003	1	0.0004	0.029	0.86
Error	0.18	14			
Total SS	0.2063				

Table 2- 6. Results of analysis of variance (ANOVA) performed on absorbance for ELISA for UV treatments in the Pools 2 and 3 in 1999. Absorbance represents cyclobutane pyrimidine dimer production.

Source	Sum of Squares	df	Mean Square	F - ratio	P value
UV treatment	0.24	2	0.12	0.69	0.51
Pools	0.36	1	0.36	2.05	0.61
Error	4.35	25	0.17		
Total SS	4.95				

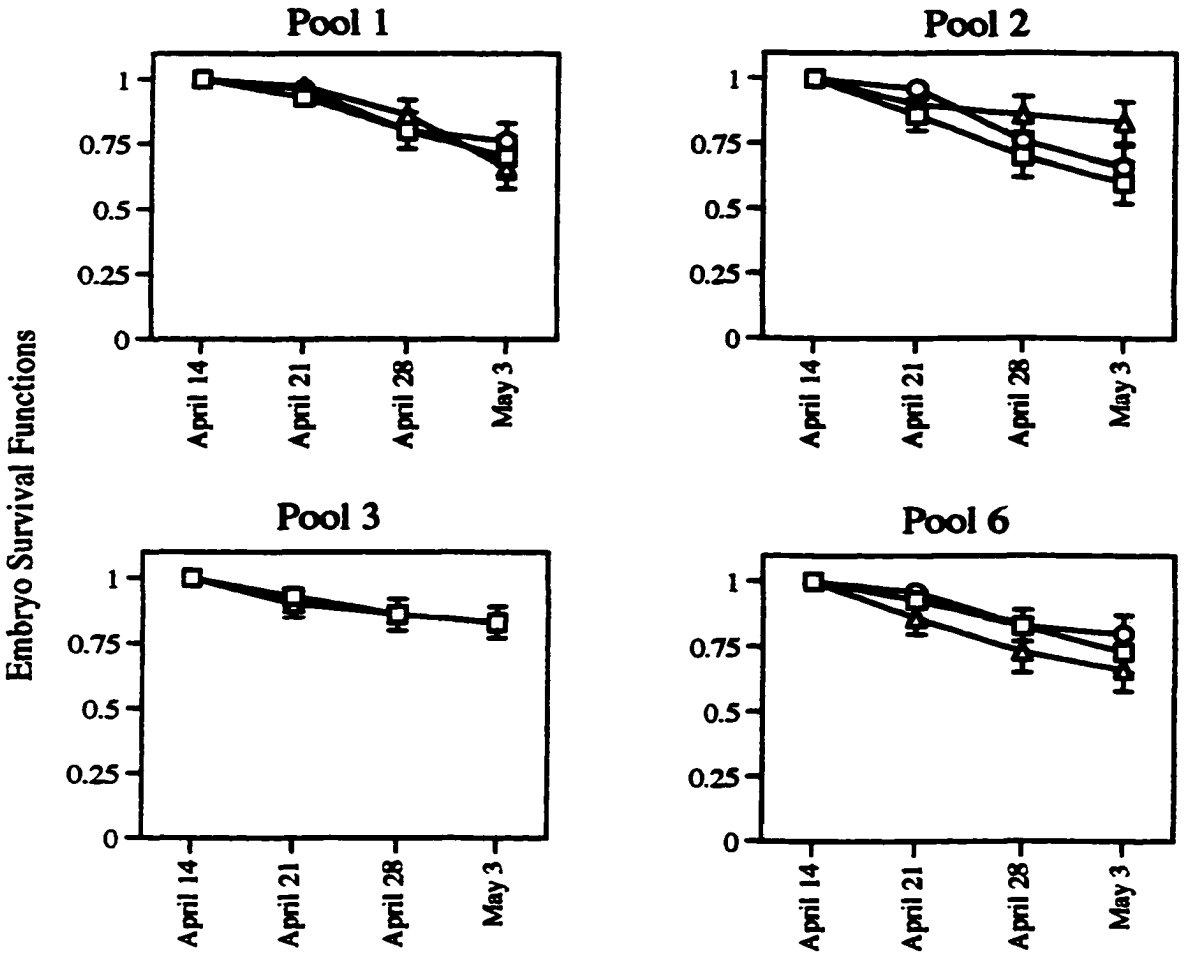

Figure 2-2. Survival functions for four pools in 1999 for all UV treatments. Embryos in pools 4 and 5 were eaten by invertebrate predators. Squares = UVT, triangles = UVA, and circles = UVO.

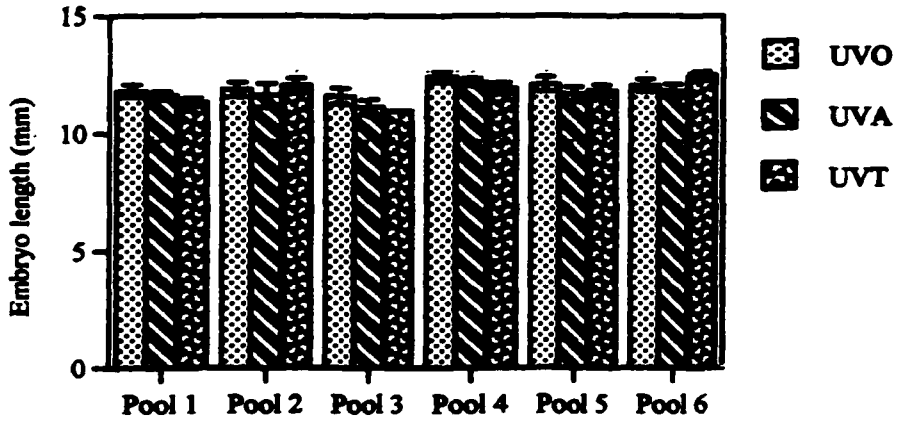

Figure 2-3. Length of embryos for UV treatments in 1998 field experiments. Means +/- 1 standard deviation are shown.

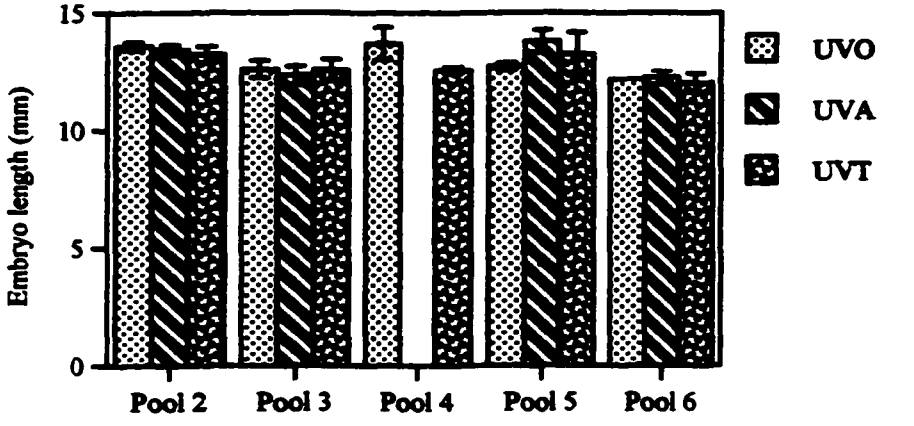

Figure 2-4. Length of embryos at hatching in 1999 field experiments. Means +/- 1 standard deviation are shown.

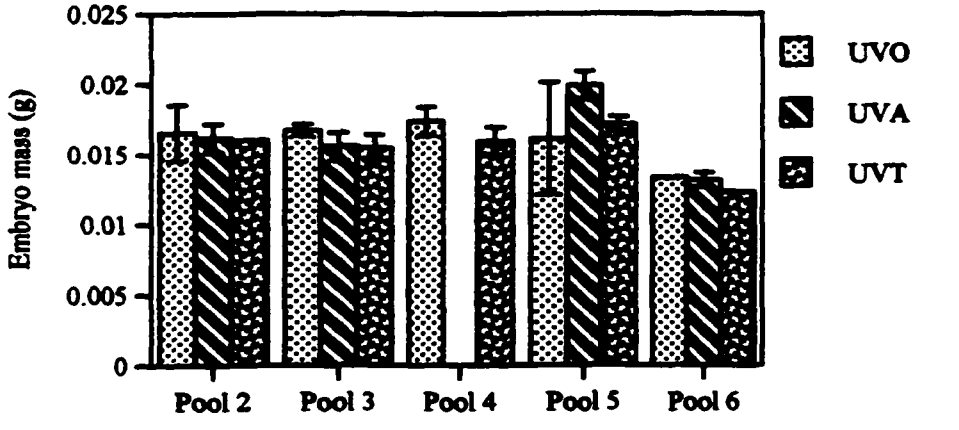

Figure 2-5. Mass of embryos for UV treatments in 1999 field experiments. Means +/- 1 standard deviation are shown.

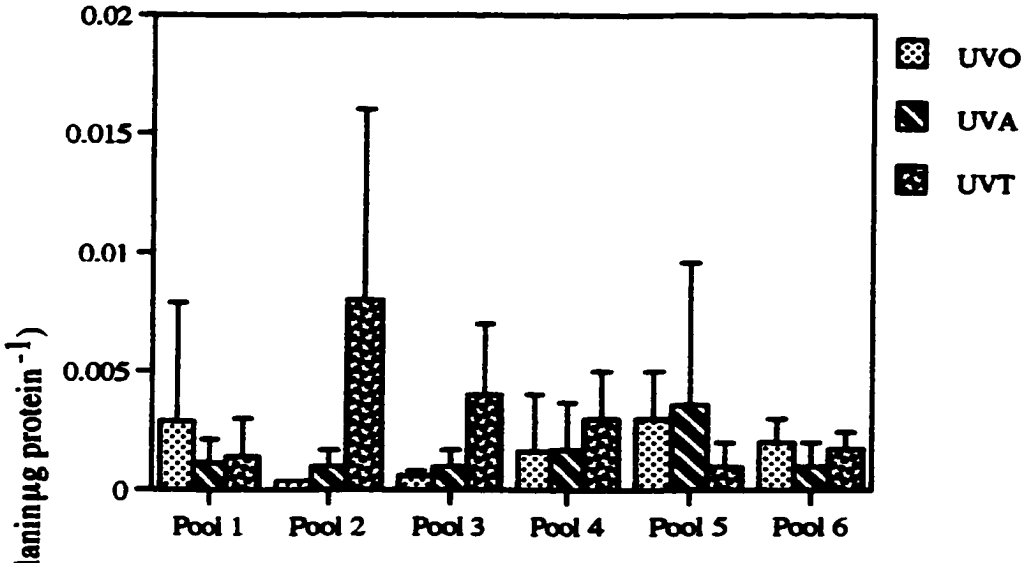

Figure 2-6 Embryo melanin concentrations for field experiments in 1998. Means +/- 1 standard deviation are shown.

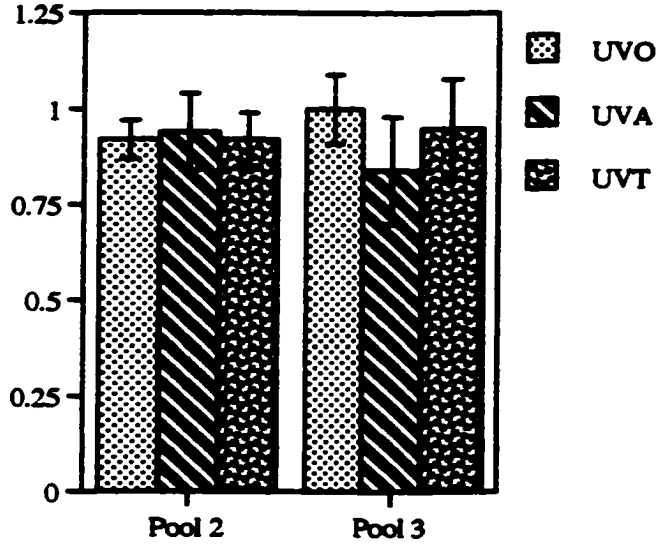

Figure 2-7. Embryo melanin concentrations for 1999 field experiments. Means +/- 1 standard deviation are shown.

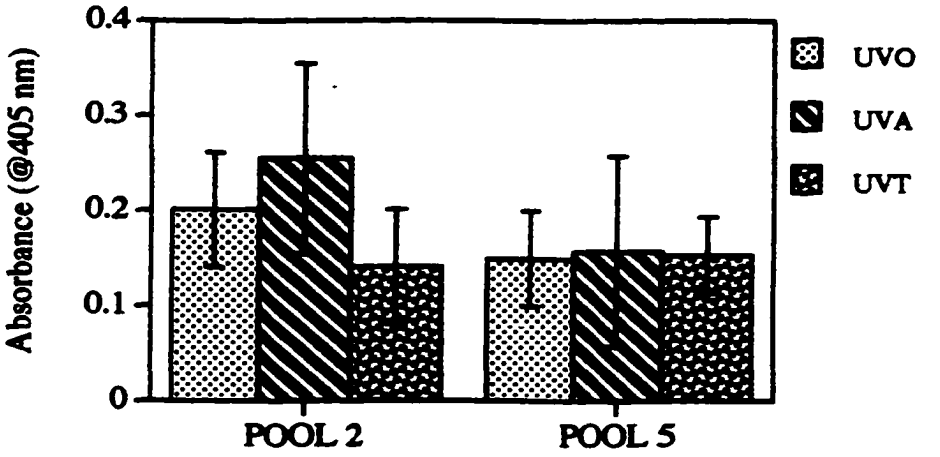

Figure 2-8. Results of enzyme-linked immunosorbent assay (ELISA). Absorbance describes production of cyclo-butane dimers in embryo DNA for the three UV treatments in two vernal pools, Pools 2 and 5.

UVB radiation reached a depth of 5 cm in all six pools. UVB spectral irradiance varied greatly between pools, days, and years (Figures 2-9 and 2-10). However, pools usually ranked in the same order. Pools 2 and 3 had highest overall spectral irradiance in 1998 and 1999. UVB, UVA, and PAR exhibit similar patterns in irradiance among pools (Figures 2-9, 2-10, 2-11, 2-12, 2-13, and 2-14). Spectral irradiance was greatest at noon for Pool 5 but decreased rapidly in the hour before and after solar noon (Figure 2-16). Approximate radiation dose varied greatly among pools in 1999 (Table 2-7). Pools 2 and 3 received the highest dose of radiation. The acrylic affected UV radiation exposure for the UV treatments as measured in Pool 2 (Table 2-8, Figure 2-15). In the UVO treatment embryos were exposed to only 1.0% of the total UVB radiation, 2.0% of the total UVA radiation, and 79% of the total PAR reaching a depth of 5 cm. In the UVA treatment embryos were exposed to 10.0% of total UVB, 60.0% of the total UVA radiation, and 70% of the total PAR reaching 5 cm depth. In the UVT treatment embryos were exposed to 40% of the total UVB radiation, 65% of UVA, and 88% of the total PAR reaching a depth of 5 cm.

Table 2-7. Approximate daily dose of radiation for each vernal pool in 1999.

	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
UVB (kJ m^{-2})	0.12	1.65	1.47	0.50	0.48	0.03
UVA (kJ m^{-2})	26	166	150	82	71	21
PAR (kJ m^{-2})	15127	22260	19677	15549	6363	17987

Table 2-8. Spectral irradiance for UV treatments in Pool 2 on April 21, 1999.

	No acrylic (W m^{-2})	UVT acrylic (W m^{-2})	UVA acrylic (W m^{-2})	UVO acrylic (W m^{-2})
UVB (290 - 320 nm)	0.08	0.03	0.008	0.001
UVR (290 - 400 nm)	8.39	5.47	5.1	0.19
PAR (400 - 750 nm)	1270.63	1125.99	835.4	1104.5

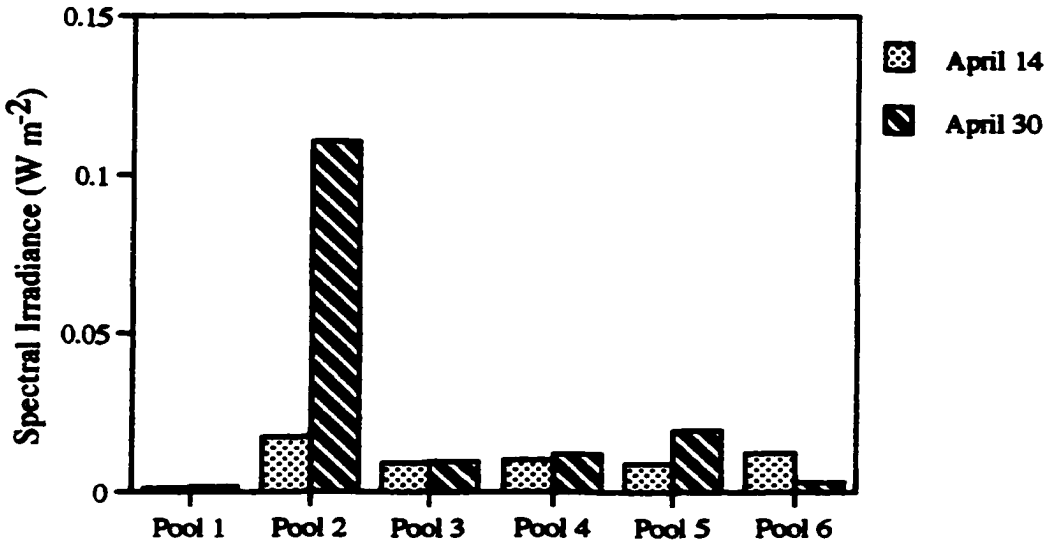

Figure 2-9. UVB spectral irradiance (300 - 320 nm) for pools in 1998. Spectral irradiance was measured in each pool at a depth of 5 cm, within a two hour period around solar noon.

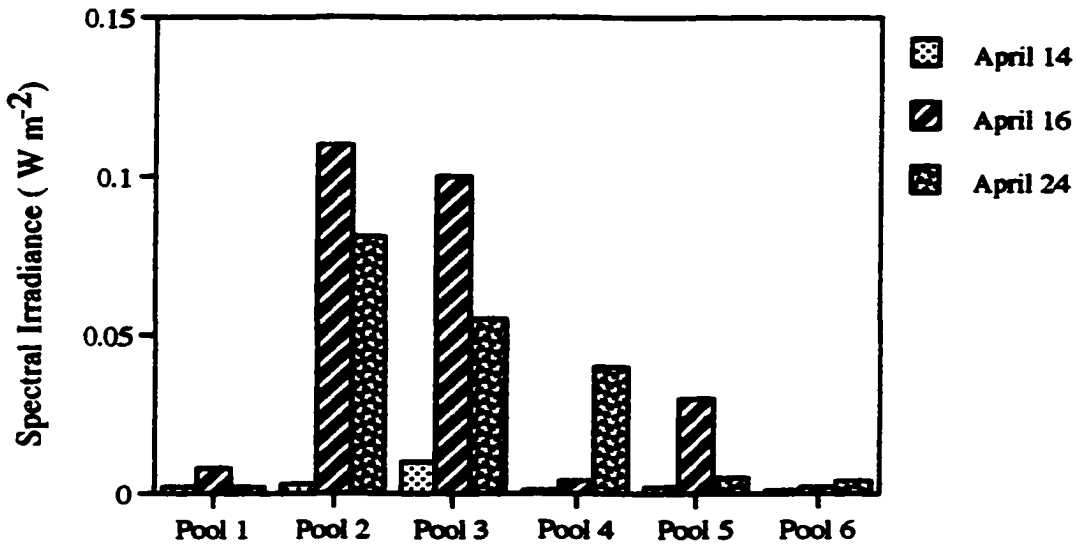

Figure 2-10. UVB spectral irradiance (300 - 320 nm) for pools in 1999. Measurements were taken in each pool at a depth of 5 cm, within a two hour period around solar noon.

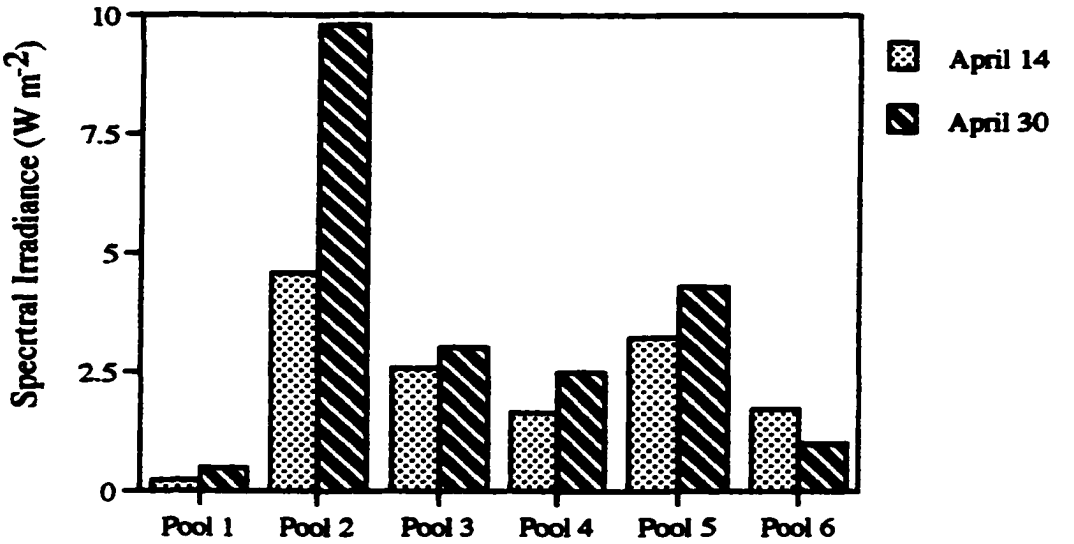

Figure 2-11. UVA radiation (320 - 400 nm) for vernal pools in 1998. Spectral irradiance was measured in each pool at a depth of 5 cm, within two hours before and after solar noon.

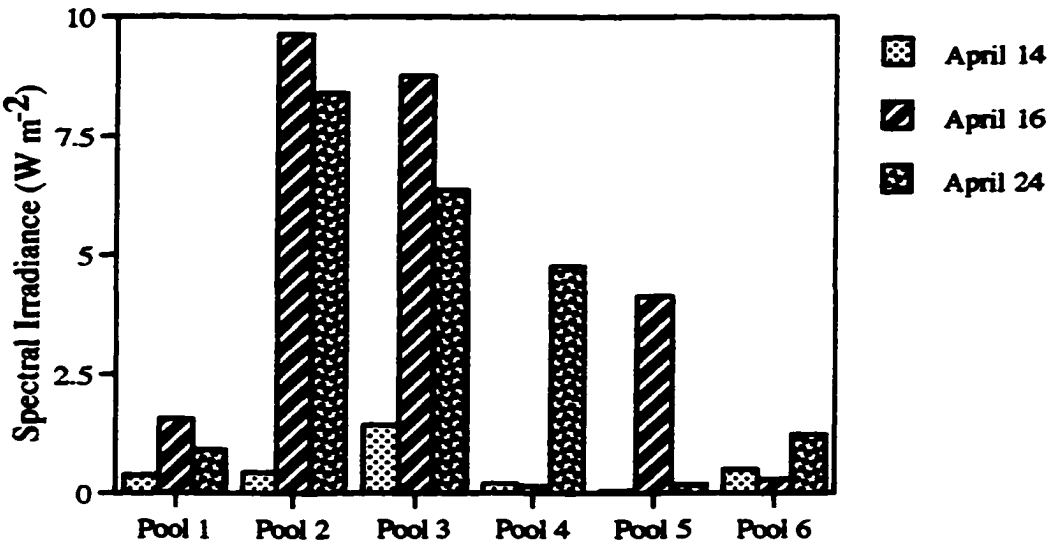

Figure 2-12. UVA radiation (320 - 400 nm) for vernal pools in 1999. spectral irradiance was measured in each pool at a depth of 5 cm, within the two hours around solar noon.

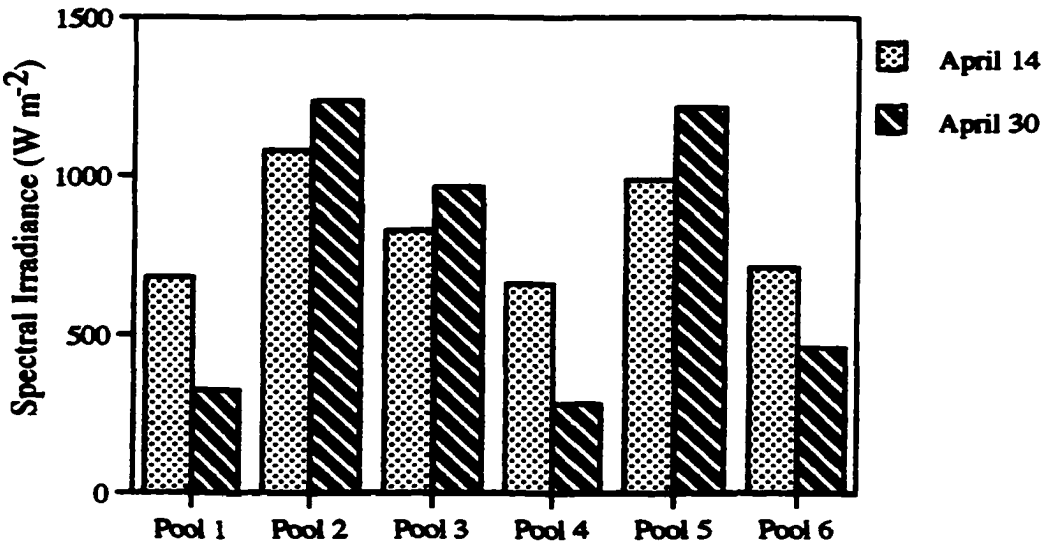

Figure 2-13. Photosynthetically active radiation (PAR) (400 -750 nm) for vernal pools in 1998. Spectral irradiance was measured in each pool at a depth of 5 cm, within the two hour period around solar noon.

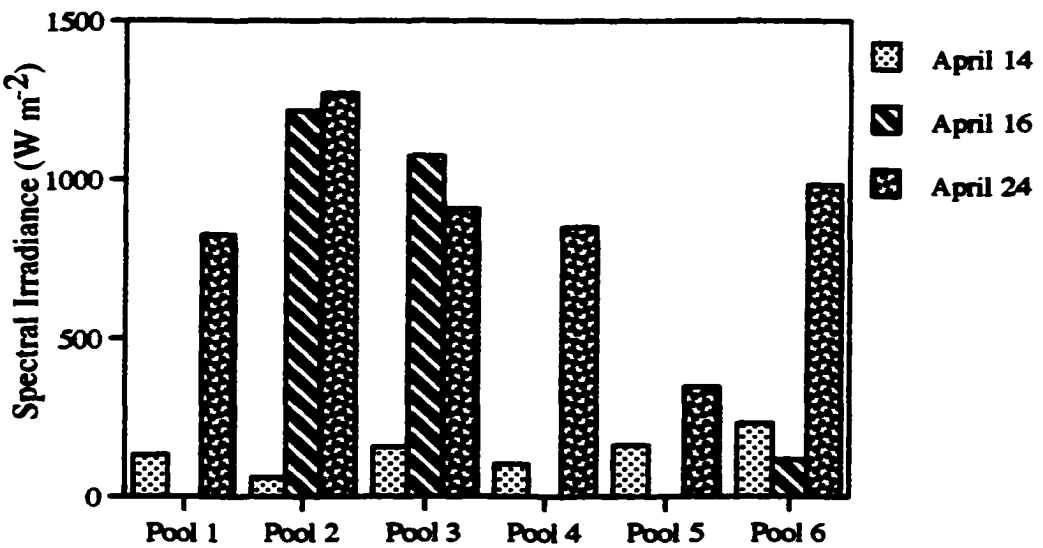

Figure 2-14. Photosynthetically active radiation (PAR) (400 - 750 nm) for vernal pools in 1999. Spectral irradiance was measured in each pool at a depth of 5 cm, within the two hour period around solar noon.

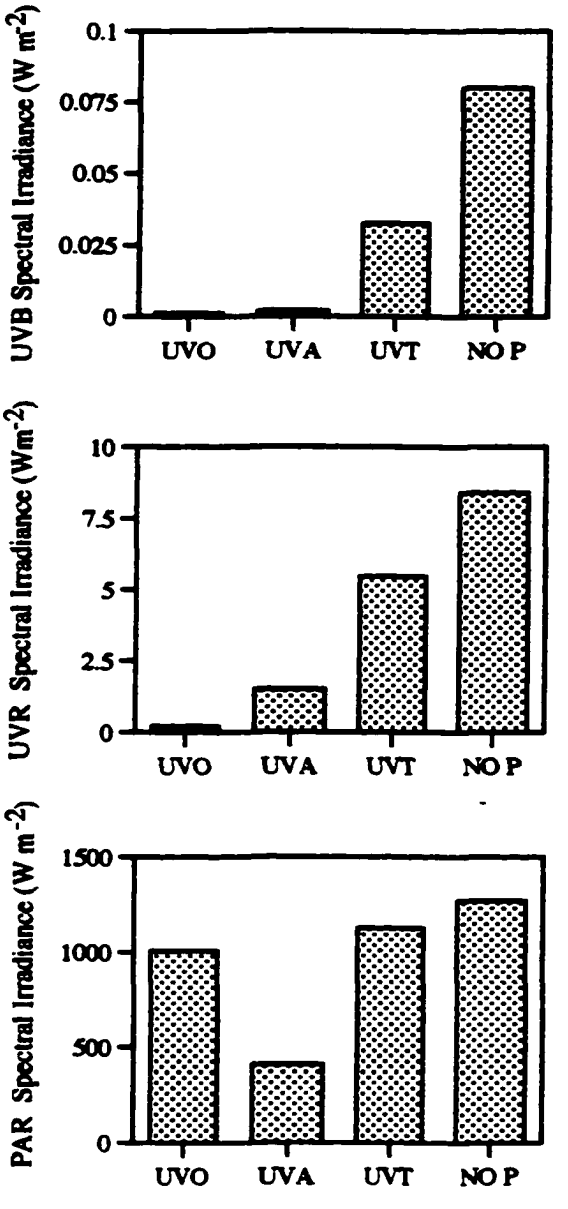

Figure 2-15. Transmittance of acrylic used to control spectral irradiance in UV treatments. These measurements were collected in Pool 2, on April 21, 1999 at 1130 h. All measurements were taken at a depth of 5 cm underneath the UVO acrylic, UVA acrylic, UVT acrylic and with no acrylic (NO P).

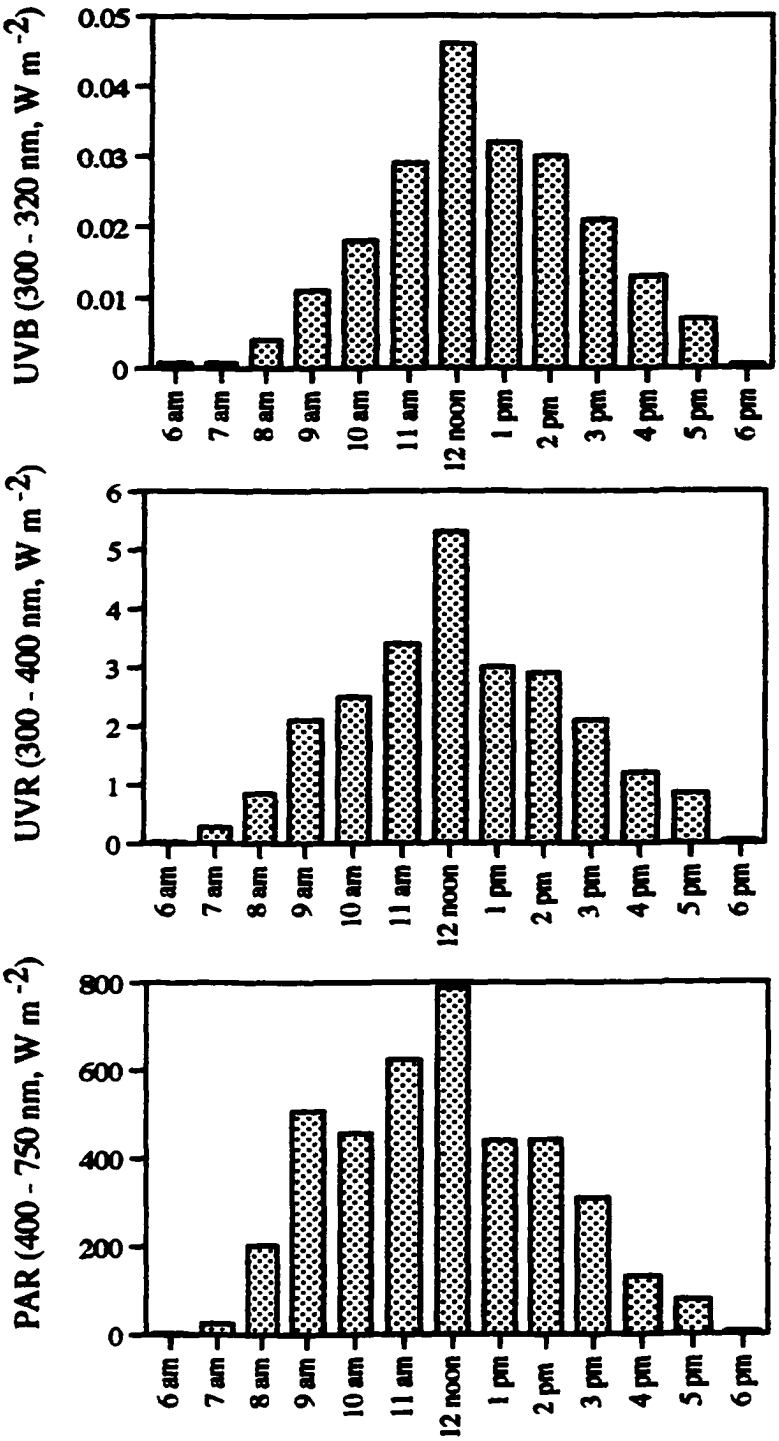

Figure 2-16. Hourly measurements of spectral irradiance for Pool 5 in 1998. Spectroradiometer was placed in the pool for 24 hours and measurements were taken at a depth of 5 cm..

Dissolved water color varied year to year. For most pools color was higher in 1998 meaning that the pool water was darker (Figure 2-17, Table 2-9). In 1999 results indicated that dissolved water color generally increased over the duration of the experiment in all pools (Figure 2-18). Pools 1, 4, and 6 had much higher color levels than the other three pools in both years.

Pool 1 had the highest mean DOC concentrations in both 1998 and 1999 (Table 2-9). However, I did not observe similar trends in the pools rank order of DOC concentrations between years. DOC concentrations increased during the experiment in four of the six pools in 1999 (Figure 2-19).

Temperature, dissolved oxygen, and specific conductance are not directly comparable between the two years as different instruments were used to collect the data. However, in each year the pool ranks were in a similar order for temperature. Pool 4 and 5 had the highest mean temperature both years (Table 2-9). In general, temperature increased over the duration of the spring, although this trend was much more consistent in 1999 than in 1998 (Figure 2-20). Pools 2 and 3 had the highest mean dissolved oxygen concentrations both years and Pool 6 had the lowest dissolved oxygen level in both years (Table 2-9). Dissolved oxygen levels tended to decrease over the duration of the spring. Again this trend was much more consistent among pools in 1999 than in 1998 (Figure 2-21). Pools 1 and 3 had the lowest specific conductance measurements in both years (Table 2-9). I did not observe any consistent trend among pools in specific conductance over the duration of the field experiments (Figure 2-22). Pool 4 had the highest pH both years and Pool 5 had the lowest pH both years (Table 2-9).

Table 2-9. Vernal pool water chemistry for 1998 and 1999. Means +/- 1 standard deviation are shown.

		Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Temperature (°C)	1998	6.2 +/- 0.8	6.3 +/- 1.5	5.2 +/- 2.3	7.0 +/- 0.8	8.3 +/- 0.6	5.3 +/- 0.7
	1999	10.3 +/- 4.1	8.7 +/- 3.3	7.8 +/- 1.8	10.6 +/- 3.8	10.6 +/- 4.4	9.7 +/- 3.0
Dissolved Oxygen (mg L^{-1})	1998	5.2 +/- 0.3	7.23 +/- 0.3	6.7 +/- 0.5	5.1 +/- 0.4	5.8 +/- 0.4	6.1 +/- 0.2
	1999	4.3 +/- 0.9	5.5 +/- 0.4	5.9 +/- 0.6	3.5 +/- 0.8	5.49 +/- 0.4	4.7 +/- 0.7
Specific conductance (µS cm^{-1})	1998	16.7 +/- 11.5	18.3 +/- 2.9	13.3 +/- 5.8	23.3 +/- 11.3	24.3 +/- 4.0	22.3 +/- 3.1
	1999	32.9 +/- 2.4	39.9 +/- 1.0	31.5 +/- 1.1	54.3 +/- 1.1	34.1 +/- 1.0	33.4 +/- 3.8
pH	1998	5.9	4.5	4.9	6.8	4.2	4.9
	1999	5	5.6	4.9	5.9	4.3	5.7
Alkalinity (mg CaCO3 l^{-1})	1999	1.86 +/- .46	3.5 +/- .3	0	11.7 +/- 2.1	0	1.1 +/.05
Water color (Pt. mg l^{-1})	1998	614 +/- 1	264 +/- 51	266 +/- 29	471 +/- 1	314 +/- 29	523 +/- 38
	1999	437 +/- 28	213 +/- 16	224 +/- 9	479 +/- 117	258 +/- 25	445 +/- 57
Dissolved Organic Carbon (mg l^{-1})	1998	34.6	17.4	16	17.4	19.4	12.4
	1999	28.8 +/- 2.9	12.3 +/- 1.1	17.4 +/- 2.5	19.1 +/- 0.9	18.3 +/- 3.2	25.5 +/- 2.7

There was no consistent pattern in pH levels according to these measurements (Figure 2-23). Pool 4 had the highest alkalinity measurements for all samples (Figure 2-24). Pool 3 and Pool 5 had negative alkalinity corresponding to a pH of less than 4.8 (Table 2-9).

The matrix of correlation coefficients indicates that there is strong, negative association between dissolved water color and LOG (UVB) variables ($r = -0.83$, $p < 0.05$). No other correlation was significant (Table 2-10).

Sodium, potassium, magnesium, and calcium did not vary greatly among pools and all were under 5 mg l^{-1} (Table 2-11). Chloride concentrations were all under 5 mg l^{-1} except for Pool 4 (18.5 mg l^{-1}, Table 2-11). Pool 4 also had the highest nitrate and sulfate levels (Table 2-11). Mercury, aluminum, and lead were all below range of accurate detection.

Table 2-10. Matrix of Pearson correlation coefficients and Bonferroni adjusted probablities ($p<0.05$) describing associations among environmental factors and embryo length. UVB spectral irradiance was log transformed to reduce non-normality.

	LOG UVB	Color	DOC	Temperature	Length	pH	Dis. O2
LOG UVB	1.00						
Color	0.83/0.001	1.00					
DOC	-0.56	0.57	1.00				
Temperature	-0.05	-0.10	0.21	1.00			
Length	0.52	-0.35	-0.47	0.62	1.00		
pH	-0.38	0.52	0.19	-0.01	-0.12	1.00	
Dis. O2	0.15	-0.21	-0.04	0.12	0.04	-0.19	1.00

Table 2-11. Results of ion chromatography and metals analysis for vernal pools. ND - not detected, * = estimated but below the range of accurate detection limits.

	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Sodium (mg L-1)	2.56	2.15	3	2.5	1.51	1.69
Potassium (mg L-1)	3.51	1.5	0.6	2.94	0.81	1.48
Magnesium (mg L-1)	0.78	0.8	0.46	1.02	0.53	0.67
Calcium (mg L-1)	2.35	2.45	1.46	4.81	1.41	1.84
Chloride (mg L-1)	4.48	1.75	3.9	18.54	2.81	2.39
Nitrate (mg L-1)	0	0.001	0	0.42	0	0
Sulfate (mg L-1)	0.803	0.87	1.137	6.6	0.295	0.702
Mercury (mg L-1)	ND	ND	ND	ND	ND	ND
Aluminum (mg L-1)	0.1 *	ND	ND	ND	ND	0.1 *
Lead (mg L-1)	ND	ND	ND	.001 *	ND	0.001 *

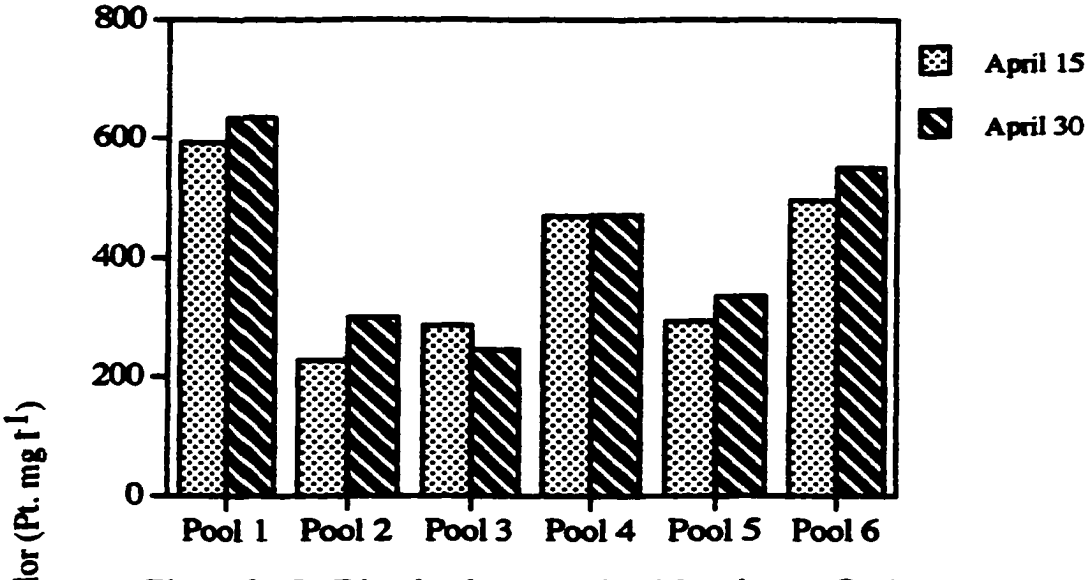

Figure 2-17. Dissolved water color (absorbance @ 405 nm) for vernal pools in 1998.

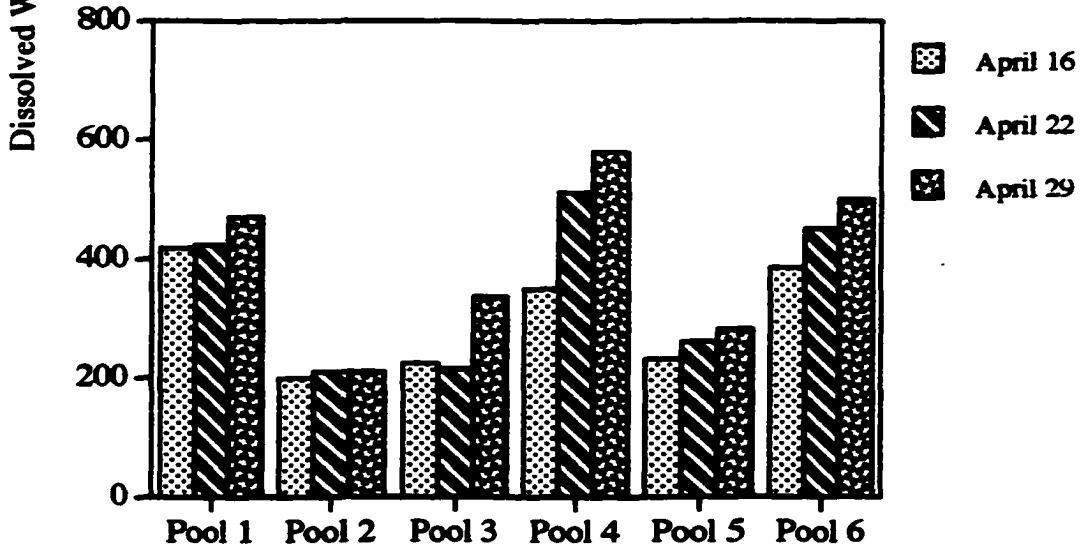

Figure 2-18. Dissolved water color (absorbance @ 405 nm) for vernal pools in 1999.

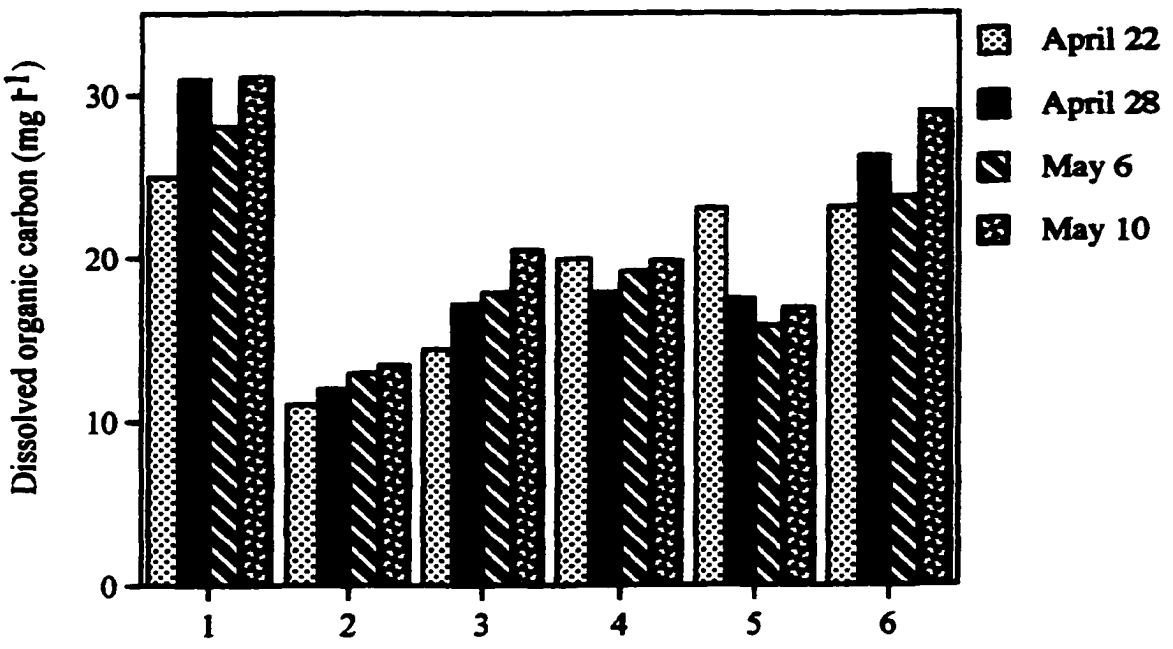

Figure 2-19. Dissolved organic carbon concentrations for vernal pools in 1999. Concentration is shown for each collection in each pool.

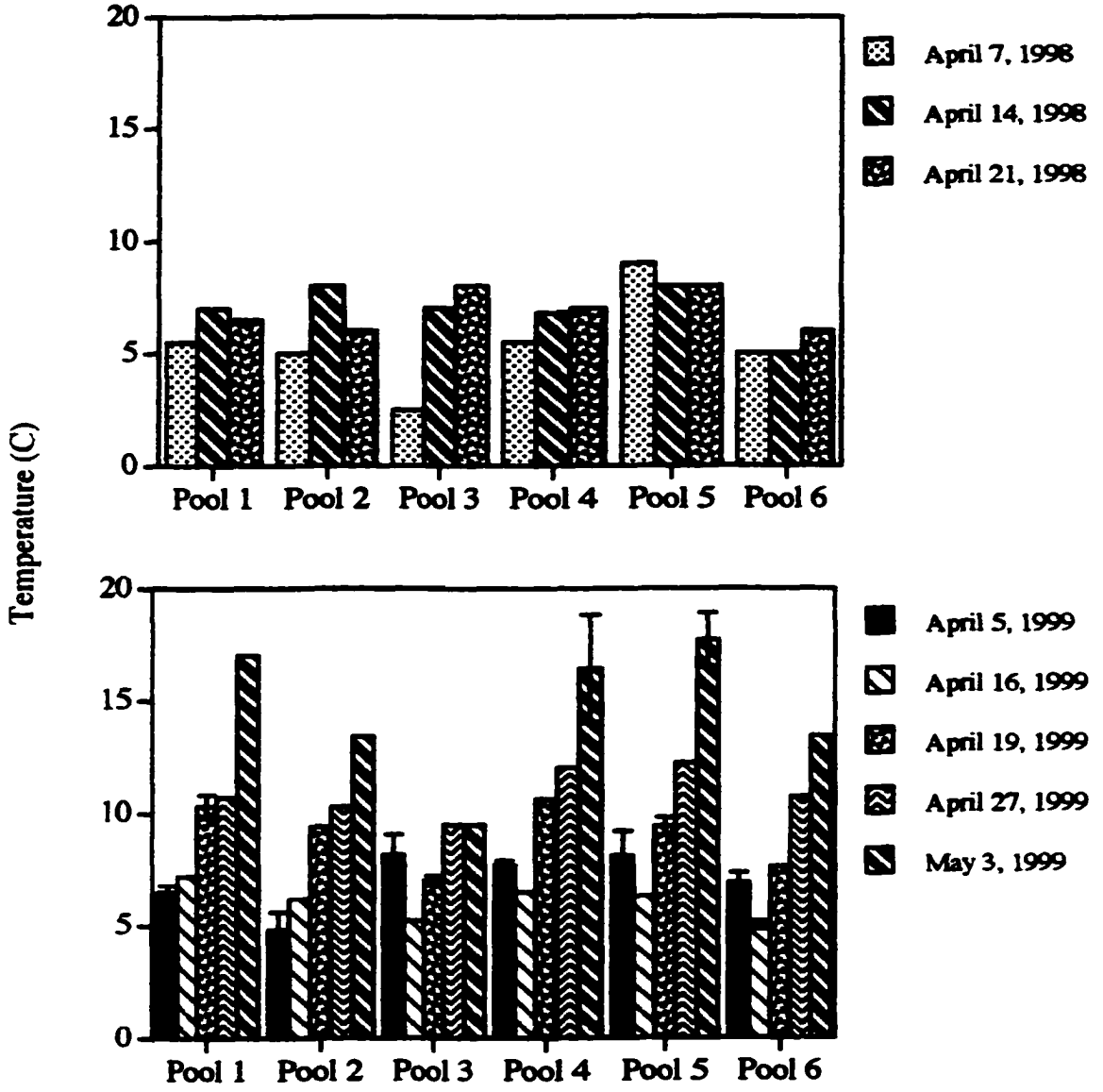

Figure 2-20. Temperature for vernal pools in 1998 and 1999. Mean temperature +/- 1 standard deviation are shown for each collection date.

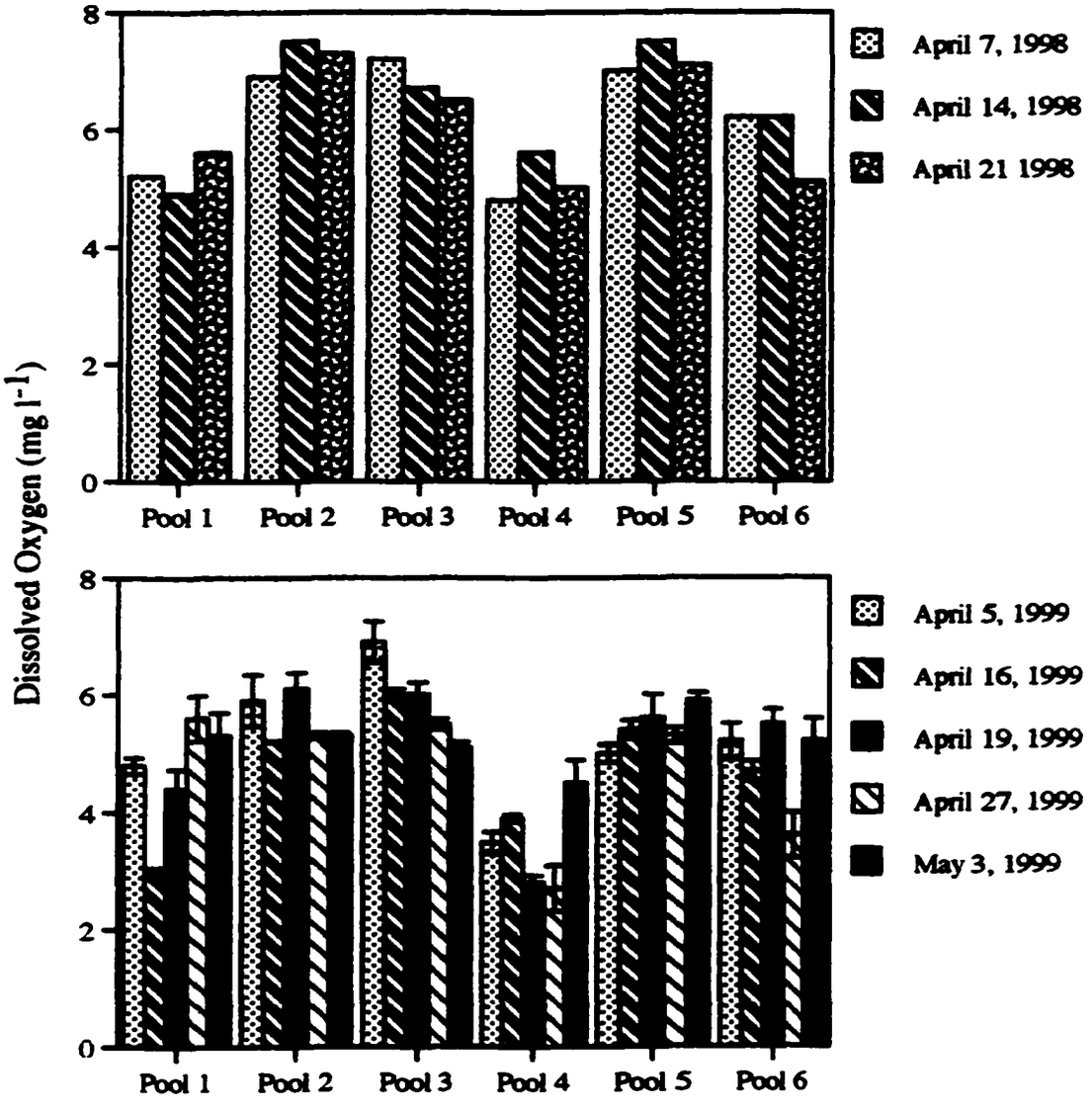

Figure 2-21. Dissolved oxygen for vernal pools in 1998 and 1999. Mean dissolved oxygen +/- 1 standard deviation are shown for each collection date.

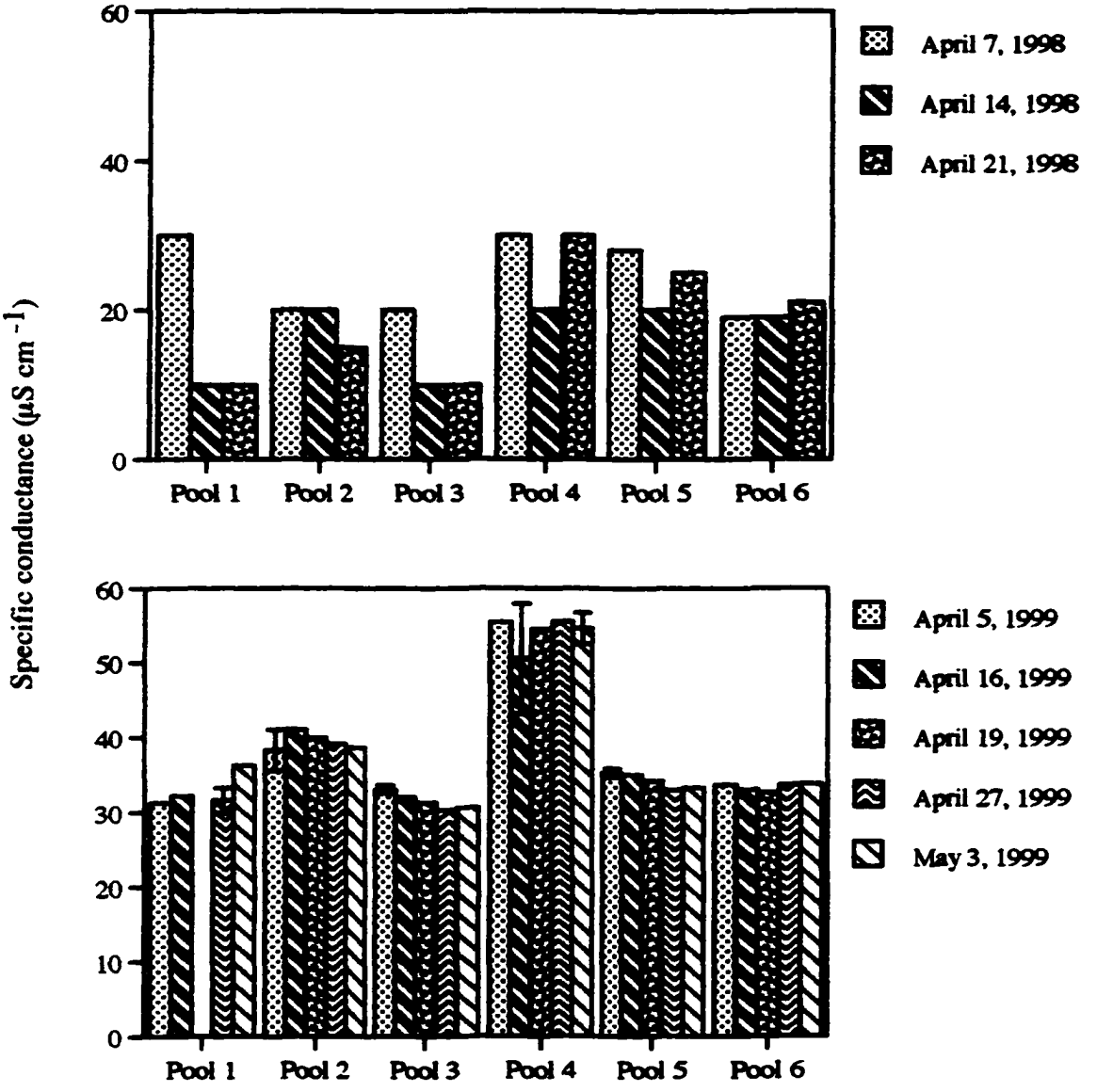

Figure 2-22. Specific conductance measurements for vernal pools in 1998 and 1999. Mean +/- 1standard deviation are shown.

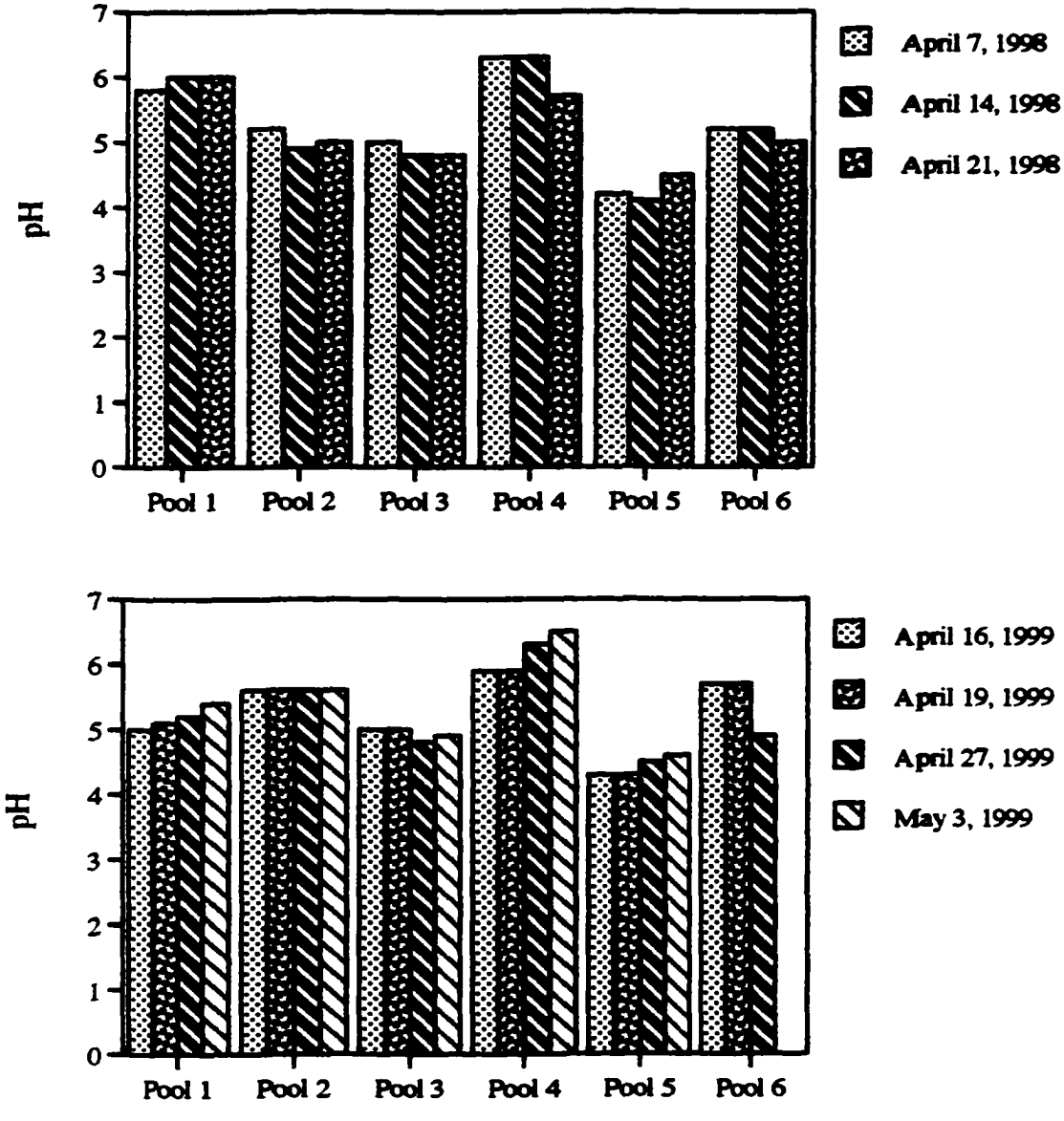

Figure 2-23. Measured pH for vernal pools in 1998 and 1999. Mean pH, calculated from log transformed mean H^+ ion concentrations.

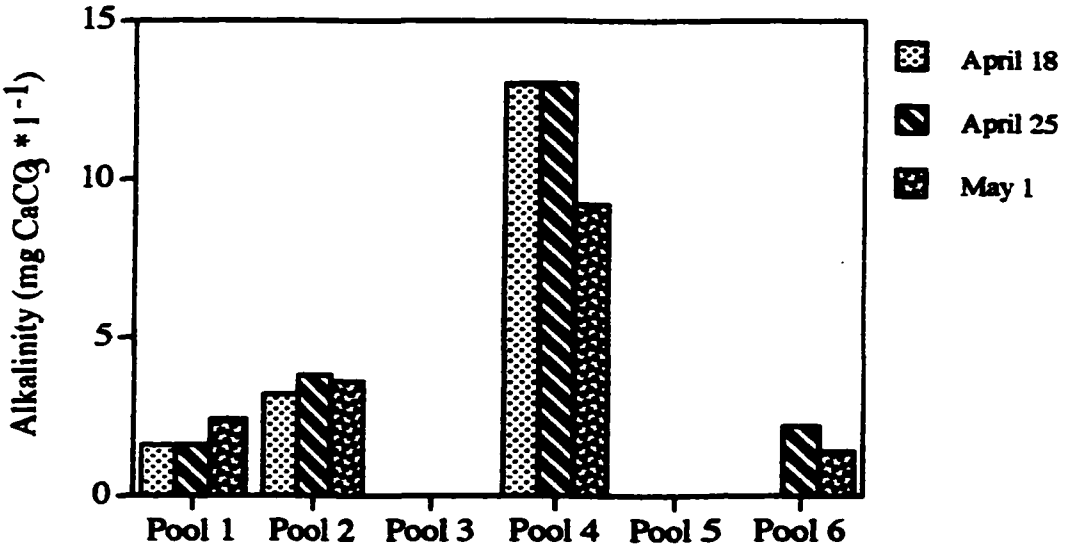

Figure 2-24. Alkalinity in vernal pools in 1999. Pools 3 and 6 had negative alkalinities corresponding to pH < 4.8.

Results of the vegetation survey within the pool boundaries indicate that leaf litter had the greatest percent cover for all pools. It covered over 70% of the pool bottom in all pools except Pool 6 (Figure 2-25). Pool six had the highest estimate of species richness and the lowest percent cover of leaf litter. Pool 1 had the lowest species richness. Species richness was strongly associated with pool surface area (Table 1-1), but this was not tested statistically. The largest pool, Pool 6 had the greatest species richness and the smallest pool had the lowest species richness.

The most dominant tree species in surrounding upland varied among pools. Mean relative dominance of conifers was over 50 % for Pools 2 and 3. Pools 2, 5, and 6 were dominated by *Pinus strobus*. (Figure 2-26). Pools 1 and 4 were dominated by hardwoods, primarily *Acer rubrum* and *Acer saccharum* (Figure 2-26)

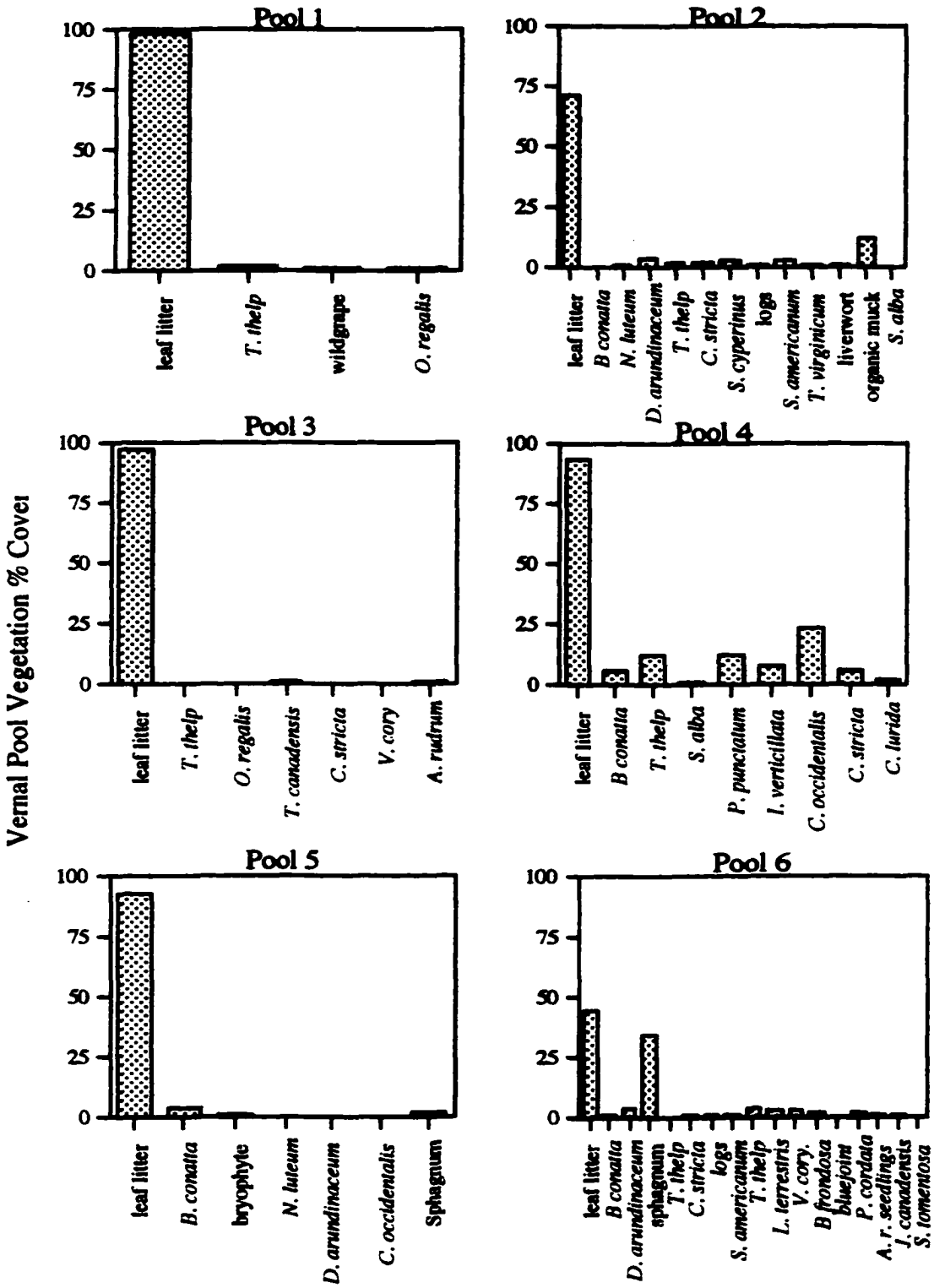

Figure 2- 25. Vegetation percent cover for vernal pools.

Figure 2-26. Dominance diversity curves for vernal pools. I measured relative abundance for all trees (dbl >2.5 cm) in five 10 *10 meter quadrats placed randomly around pool 1 m from edge.

DISCUSSION

Results of this study indicate that the levels of UV radiation in 1998 and 1999 in the northern temperate latitudes were not strong enough to impact *Ambystoma maculatum* populations. There was no consistent trend in UV radiation effects among the pools used in this study.

Current UVB levels did not affect the survival of *A. maculatum* embryos. The four pools in which I successfully tested this hypothesis represented a range of conditions of breeding pools for this species. Although exposure to UVB varied greatly from pool to pool, there was no variation in results of survival analysis; there was no difference in survival at any of the observed irradiances. This is consistent with the *A. maculatum* embryonic survival results from UV experiments in North Carolina (Starnes et al. 2000)

Blaustein et al. (1995) did find that hatching success was lower for *Ambystoma gracile* when they were exposed to natural UVB radiation at low elevations. The inconsistency between the results of Blaustein's et al. (1995) experiment and the results presented here indicates either variation in UVB tolerances within the genus *Ambystoma* or variation in UVB exposure due to experimental design. Both salamanders are known to lay their eggs in shallow, open water areas where exposure to UVB radiation can be high. One might expect with this life history strategy, that UV protection mechanisms would be well developed. It is interesting that for *A. gracile*, photolyase levels are relatively low (Blaustein et al. 1994); for *A. maculatum* photolyase levels are not documented so species tolerances can not be compared on this level. The different experimental designs may cause a difference in exposure to UVB radiation and may

therefore partially explain the different results. I conducted this field experiment in the natural breeding pools used by *A. maculatum*, thereby maintaining the natural level of UV radiation exposure. Blaustein et al. (1995) also conducted the experiment under natural levels of UV radiation, however, they placed the embryos into plastic pools filled with natural pond water. It is not clear if the natural pond water was collected from natural breeding sites. The dose of UV radiation could also differ due to variation in the water chemistry. Williamson et al. (1996) found a strong negative association between UV spectral irradiance and DOC concentrations. It is difficult to decipher whether differences in results among study are due to different tolerances or different exposures because UV spectral irradiance was not reported in either study.

I expected that embryos exposed to UVB radiation would be smaller at hatching, if they needed to allocate more energy to UV repair and protection than to growth. Results in four of six pools did show the expected trend in length in 1998 and in three of five pools in 1999, yet UV treatment was not a significant variable. Also embryo wet mass was less for UVT treatments in four of five pools but, again, the difference was not significantly associated with the UVT treatment.

I expected that embryos exposed to UVB radiation would produce more melanin than embryos in treatments where UVB was blocked. However, I did not observe a significant difference in melanin concentration between UV treatments. I did observe the expected trend in Pools 2 and 3 in 1998. These pools also had high UV spectral irradiance. In 1999 when I conducted the melanin assay for embryos in Pools 2 and 3, I did not observe results that were consistent with 1998.

Embryo size, as measured by length, was significantly associated with pool in which it was located. This association is most likely due to variation in water temperature among pools. Temperature limits growth and development rates (Duellman and Trueb 1986); accordingly, there was a general trend for mean pool embryo length to increase as mean pool water temperature increased. The relationship between temperature and embryo development creates an interesting trade off between the benefits of the warmer water in sun-exposed areas and the exposure to potentially harmful UV radiation. Results of this study indicate that the benefits of warmer temperatures may outweigh any potential harm of UVB radiation at these current levels.

It was not unexpected to find an absence of lethal UV radiation effects and only possible sublethal effects because there are ways embryos are protected from UV damage. Species with life history strategies that include exposure to sunlight, such as depositing eggs in open water, have evolved protective mechanisms to survive the exposure to UVB radiation (Calkins and Thordardottir 1980). *Ambystoma maculatum* deposits eggs in masses of up to approximately 200 eggs (Duellman and Trueb 1986). The eggs of pond breeding amphibians are darkly pigmented with melanin on the dorsal side which may function as protection from UVB radiation (Grant and Licht 1997). The embryo is surrounded by the perivitelline membrane, which is produced by the ovary (Salthe 1965). Several concentric capsules surround the perivitelline membrane. The innermost capsule liquefies and the ovum floats freely in this liquid. The egg mass is held together by a thick matrix of mucopolysacchides and mucoproteins (Salthe 1963). The capsules and jelly-like matrix reduce embryo exposure to UVB radiation. Entire egg masses of *A. maculatum* minus the embryos blocked 16 –77% of UVB radiation

(Grant and Licht 1995), therefore, embryos at the bottom of the egg mass receive a lower does of UVB radiation

Abiotic factors also limit exposure to UVB radiation in freshwater. *Ambystoma maculatum* usually attaches egg masses to vegetation at varying depths in the water but not usually on the surface. Water alone can protect the embryos from high exposure to UV radiation. In one case, *Triturus alpestris* larvae, placed in dishes with clear tap water, had 100% mortality in a laboratory experiment when exposed to UV radiation (Nagl and Hofer 1997). However, mortality was not affected for the larvae at natural breeding sites. There are two possible reasons for this discrepancy: 1) water in natural breeding sites strongly absorbed UVB radiation, 2) larvae reacted to exposure to UV radiation with erratic movements which in nature may move them out of exposed areas (Nagl and Hofer 1997).

During the embryo stage when an organism is immobile, the level of exposure to UV radiation faced by each individual is determined by the depth of the egg mass, the location of the embryo in the egg mass, and the UVB absorbance of the water. Genetic variation and variation in water depth was held constant with the experimental design of this study. Any differences observed in the effects of UVB radiation would have been due to variation in UVB absorbance between pools and variation in thickness of membranes surrounding each embryo. In this study, I divided egg masses into sections consisting of 1 – 15 eggs. This created different sized pieces with different thickness of capsular membranes and the jelly matrix. I more closely mimicked undisturbed egg masses using this method thereby, including the natural variation in UVB exposure due

to an embryos location in the egg mass. Thus, some of the residual variation observed in the statistical analysis may be due to the variation in egg mass thickness.

This study provides some of the first data describing UV spectral irradiance, and factors associated with it, in vernal pools. Currently UVB spectral irradiance is very low. Total daily dose of UV radiation and spectral irradiance varied greatly among pools. Spectral irradiance also varied greatly among days. Irradiance is much lower for cloudy days, such as April 14, 1999, compared to sunny days. Weather condition may therefore greatly affect total dose of UV received by the embryos. It is expected that colored dissolved organic matter (CDOM) strongly influence the UV spectral irradiance (Laurion et al. 1997, Smith et al. 1992, Scully and Lean 1994, Kirk 1994). In this study I measured CDOM in two ways; DOC concentration and dissolved water color (absorbance at 440 nm). Both DOC and dissolved water color were very high in the vernal pools. Absorption of light is usually attributed to water itself, dissolved yellow color, and phytoplankton (Kirk 1994). When photons hit water they can be absorbed, reflected, or scattered. If they are absorbed, they are gone; if they are scattered they can still interact with the biota. The association between CDOM and UVB spectral irradiance is well documented for freshwater systems. Dissolved water color, water depth, and dissolved organic carbon and suspended sediments all affect UV attenuation in water (Laurion et al., 1997, Smith et al. 1992, Scully and Lean 1994).

In this study, only dissolved water color was significantly and negatively related to UVB spectral irradiance. DOC, although it showed the expected trend, was not a significant factor. Color in water is mainly of allochthonous origin coming from dissolved humic substances percolated through the soil (McDowell and Likens, 1988). Humic

substances, created by plants to prevent UVB damage, are released into the soil when plant materials decay, and overland flow of water transports dissolved material to freshwater wetlands (Kirk 1994). Rasmussen (1989) found that dissolved water color in lakes was positively related to drainage ratio and negatively related to watershed slope, mean lake depth, and lake area. Vernal pools appear to fit this model as they are smaller in area and shallower than lakes. Color may also be derived from the breakdown of leaf litter layer covering the pool floors. Breakdown of leaf litter may be more rapid in these temporary wetlands, compared to permanent wetlands, due to the pool bottom's exposure to oxygen during the dry stage.

In this study the two pools with consistently lowest color (absorbance at 440 nm), Pools 2 and 3, had surrounding areas dominated by conifers. Conifer leaves are not as easily broken down and therefore these pools may receive a lower loading of CDOM. As the vegetation changes the importance of UV radiation may change as well. Understanding the association of factors that influence dissolved water color will be important in understanding how future increases in UV radiation will affect vernal pools. Rasmussen et al. (1989) has suggested that vegetation in the watershed may help to explain variation in dissolved water color among wetlands.

In many studies, DOC is also strongly associated with UVB spectral irradiance. Williamson et al. (1996) used data from 65 lakes to determine that DOC is a very important predictor for UV radiation attenuation. The absorption of UVB by DOC increases exponentially with decreasing wavelength (Kirk 1994) and therefore small changes in DOC may cause large changes in UV radiation. Williamson et al. (1996) suggest that this strong association between UV and DOC indicates that environmental

factors that affect DOC may be more important than ozone depletion. Lakes located at higher elevations have lower DOC concentrations most likely due to changes in the ratio of allochthonous to autochthonous DOC inputs (Williamson et al. 1996, Sommaruga and Psenner, 1997). In a survey of 65 lakes in the northeast, DOC ranged from 0.88 mg l^{-1} to 10.68 mg l^{-1} with a mean color of 5.3 mg l^{-1} (Williamson et al. 1996). In the six vernal pools used in this study DOC ranged $12-34$ mg l^{-1}. The absence of a significant association between UVB spectral irradiance and DOC in this study may be due to the very high concentrations.

It is interesting to note that UVB radiation also interacts with DOC and breaks it down. One result of this interaction is that an increase in UVB lowers DOC concentrations thus increasing UVB exposure in wetlands (Williamson et al. 1996). Also, the chemical reaction between UV radiation and DOC produces hydrogen peroxide (Cooper 1996). There is very little information describing the effects of hydrogen peroxides on aquatic life including amphibians.

Transmission of light through the canopy varied greatly among pools. Pools 2 and 5 had the highest light transmission. Both of these pools had large surface areas partially explaining the large canopy gaps. It is interesting that there was no association between underwater spectral irradiance in pools and canopy transmission. However, I recorded spectral irradiance within a two hour period around solar noon when the sun was directly overhead. Differences in canopy cover may have a larger affect on the total daily dose of radiation the embryos received. In pools with small canopy gaps the sun quickly sets behind the canopy decreasing the time of high exposure. The hourly measurements of irradiance in Pool 5 indicate that, even in pools with large gaps,

irradiance quickly declines from its peak at solar noon.

Overall, UV radiation spectral irradiance was very low in vernal pools and therefore there were no effects of UV radiation on *A. maculatum* embryos. It is also important to note that the acrylic used in the UVT treatment reduced UVB transmittance by 60% in1999 from the total UVB. The results of this study are therefore very conservative. The low transmittance of the acrylic was partially due to the fact that this experiment was conducted in the out of doors. Flat objects out of doors soon collect leaf litter and droppings from birds and, in wetlands, provide a lovely sunning surface for frogs, therefore the results of this study are very conservative. "So it goes" (Pilgrim in Vonnegut 1966).

In conclusion, current levels of UV radiation did not have a detectable effect on *A. maculatum* in their natural breeding habitats in the northeastern United States. However, it is possible that UV radiation can locally affect embryos under certain environmental conditions. To understand how populations maybe affected by changes in the environment we need to understand long term variation in water chemistry and interactions between abiotic factors. Overall, UV radiation exposure was very low for these amphibian embryos because water in vernal pools tends to be very darkly colored. Embryos in lighter colored pools are more vulnerable to UV radiation at higher exposures. A decrease in atmospheric ozone is not the only way to increase exposure to UV radiation. The results of this study show the important connection between water chemistry and UVB exposure in vernal pools. Aquatic organisms may be at greater risk to UVB radiation if dissolved water color is altered than if ozone decreases in stratosphere.

CHAPTER III

THE EFFECTS OF A UVB RADIATION, CONTAMINATED HIGHWAY RUNOFF, AND SYNERGISM OF THE TWO ON *AMBYSTOMA MACULATUM* EMBRYOS

INTRODUCTION

In the past few years, reports of declining amphibian populations caused some scientists to investigate how changes in the environment affected amphibians. Amphibian biologists are currently investigating a list of potential natural and anthropogenic causes including habitat loss, environmental contamination, and disease. From results of these studies and population studies it became apparent that the issue of amphibian decline was complex. First, there was no direct evidence to suggest whether observed population declines were caused by natural population fluctuations or due to human impact (Wake 1991). Second, associations between amphibian survival and exposure to various pollutants varied interspecifically and intraspecifically across landscapes, such as for the effects of acid rain described in Pierce (1985). Effects of these factors can also differ between the laboratory and the natural environment. The one point of general agreement is that habitat loss and environmental degradation were important factors affecting amphibian populations (Pough 1976).

Unexplained population declines in relatively pristine environments prompted the investigation of the effects of UV radiation on amphibians. Blaustein et al. (1994)

found that some species of amphibians at high elevations were very sensitive to UVB radiation but that this sensitivity varied among species. Studies at lower elevations indicated that embryo survival was lower for a pond-breeding salamander, *Ambystoma gracile* (Blaustein et al. 1995) when exposed to UVB radiation. However, Grant and Licht did not find significant effects of ambient UV radiation on embryo or larval survival for four species of frogs

In previous experiments, I found that *Ambystoma maculatum* embryos had very low survival in roadside vernal pools (Turtle 2000), but roadside water brought into the laboratory did not affect survival (Chapter 1). However, I did observe sublethal effects. I observed a decrease in egg membrane size for embryos exposed to high concentrations of deicing salt (Turtle 1996) and for embryos exposed to water from roadside vernal pools (Chapter 1). The discrepancy between laboratory and field results was not unexpected. Embryos in the laboratory are exposed to a controlled environment not found in the field. In this laboratory experiment temperature was constant, water was well oxygenated, and light was artificial. Temperature and oxygen fluctuate greatly under natural conditions in vernal pools (Turtle 1996) and embryos are exposed to UVB radiation.

The complicated results of environmental field studies and discrepancy of field and laboratory results prompted some scientists to examine synergism among stressors. Grime (1989) defined environmental stress as the "external constraints limiting the rates of resource acquisition, growth, or reproduction". Natural environments consist of several stressful factors. Multiple stressors may explain the discrepancy between the field and laboratory results in these previous experiments.

Results of some studies suggest that UV radiation is synergistic with other pollutants increasing the damage to amphibian embryos. Long et al. (1995) found that UV radiation and low pH did not significantly affect *Rana pipiens* survival when tested as separate factors but embryos exposed to a combination of the two had significantly higher mortalities in laboratory experiments. Similarly, Kiesecker and Blaustein (1995) found that UV radiation and *Saprolegnia*, a pathogenic fungus, had a greater, non-additive affect on amphibian embryonic survival than either one alone.

During the embryonic stage, amphibians are most vulnerable to the environment for several reasons including: 1) Egg membranes are permeable to the aquatic environment (Salthe 1965) and 2) embryos are immobile and therefore can not move to more favorable habitat during this stage. The embryos are therefore vulnerable to habitat degradation.

Habitat degradation at sublethal levels may be an important factor affecting amphibian populations, although not as obvious as lethal effects. Chemical contamination at a sublethal level in wetlands can slow the growth and development of amphibian embryos and larvae thereby lengthening the time spent in these vulnerable stages (Cummins 1986, Rowe 1998). For amphibians that depend on a temporary environment, sublethal effects of pollutants can become lethal if embryos are not able to metamorphose before pool dries.

Several North American amphibian species use seasonal wetland habitats for reproduction. In New Hampshire four species of amphibians, the spotted salamander (*A. maculatum*), the blue-spotted salamander (*A. laterale*), Jefferson's salamander (*A. jeffersonianum*) and the wood frog (*Rana sylvatica*), depend on these temporary

wetlands for successful reproduction. The unique temporary nature of wetlands provides these amphibians with a refuge from fish predation. Other amphibians use these wetlands but are not dependent on them.

The breeding patterns of these obligate vernal pool amphibians are well adapted to the vicissitudes of these temporary habitats. They take advantage of the pools short hydroperiod by migrating to the pools in early spring (late March – early April) in New Hampshire). The salamanders usually return to their natal pools each year that they breed (Stenhouse 1985). Upon reaching the pools, amphibians court, mate, and then females deposit gelatinous, permeable egg masses.

In this experiment, I examined the stressful nature of roadside pools by examining the effects of UV radiation and highway contamination and a synergism of the two on the growth and development of embryos. *A. maculatum* is an appropriate species for this study of abiotic factors because it is an obligate vernal pool breeder and it is commonly found in New Hampshire. Also, *A. maculatum* deposits egg masses in open water and therefore embryos are exposed to UV radiation.

The investigation of these factors is timely as there is evidence indicating that UV radiation is increasing at Earth's surface in temperate latitudes as well as at the poles (Kerr and McElroy 1994). Also, *A. maculatum* survival appears to be low in roadside pools contaminated with salts from road maintenance (Turtle 2000). These two factors could impact *A. maculatum* populations directly, indirectly, and synergistically by decreasing reproductive success. I examined lethal and sublethal effects of UVB radiation on embryo survival and size at hatching (length and wet mass). I and also investigated two possible mechanisms (melanin and DNA damage).

METHODS

I designed this experiment to investigate the response of *A. maculatum* embryos to UV radiation in a controlled environment and also to investigate a possible synergism between highway contamination and UV radiation. I used a 3 x 3 factorial design with three water treatments and three UV radiation treatments.

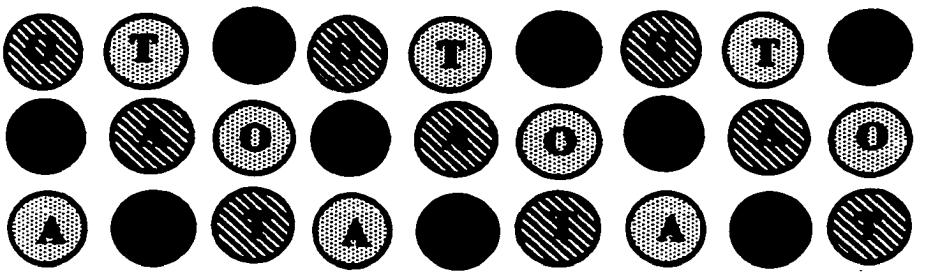

Figure 1. Design for laboratory experiments. UV treatments are indicated by letter. O = no UV, A = UVA + PAR, and T = UVB, UVA & PAR. Water type is indicated by shading. = well water, = off-road water = roadside water.

This experiment consisted of three water treatments (roadside, off-road, and well water) and three UV treatments: UVO (no UV), UVA (UVA and PAR), and UVT (UVB, UVA, and PAR). I collected water from a roadside pool (< 2m from a NH state road) and an off-road pool (> 50 m from a NH state road). These pools correspond to pools R1 and W2 in Chapter 1. Both pools are located along RT 152 in Nottingham, NH (at approximately 43° 06.68 N 71° 05.40). Water was collected weekly at a depth of 10 cm in high-density polyethylene bottles (HDPE) from the pool sources. I obtained oxygenated well water from the AFAIR laboratory at the University of New Hampshire. Bottles were rinsed three times immediately prior to collection. Water was changed

weekly in each treatment. Specific conductance, pH, and dissolved oxygen were measured at the time of water collection in the pools.

To control UV radiation I used sheets of acrylic and mylar. I used UV opaque acrylic for UVO, UV transparent acrylic and mylar for UVA, and UV transparent for UVT. Transmission of these treatments was described in Chapter 2. San Diego Plastics Inc. supplied the acrylic for these experiments. All acrylic was soaked in tap water for 48 hours prior to use.

Egg masses were collected at early stages of cleavage (Harrison 1969, in Duellman and Trueb, 1986) as soon after deposition as possible. I divided the masses in sections, mixed the sections, and formed 27 groups each containing 20 embryos. In the lab, nine dishes were assigned to each water treatment for a total of 27 dishes. Each group of embryos was placed into glass dishes (300 ml) with 50 ml of water from the appropriate source. Within each water treatment, I assigned three dishes to each UVR treatment (UVO, UVA, and UVT) and placed dishes under the corresponding acrylic type (30 cm x 30 cm). The dishes were placed in an environmental chamber (Sherer Chamber, Model CEL 8) at 10 °C with a 12 light /12 dark photoperiod. Two fluorescent UV bulbs, two wide spectrum fluorescent bulbs, and two white fluorescent bulbs were used to create the artificial photo environment in the chamber. I suspended the light source 73 cm above the embryos and used cellulose acetate film (0.13mm thickness) to attenuate the shorter wavelengths of the UV spectrum that are not encountered in nature. The full spectrum in the chamber, and under each treatment type acrylic 73 cm below the radiation source was measured with the LI-1800UW spectroradiometer. The total UVB irradiance, UVA irradiance, and PAR (photosythetically active radiation)

irradiance was calculated by integrating irradiance beneath the spectral curve for the appropriate wavelengths. Temperature was measured in each dish to determine if radiation exposure affected water temperature.

I monitored survival throughout the experiment until embryos were just about to hatch (stages 45-46, Harrison1969, in Duellman and Trueb, 1986). At this point, I removed dishes from the chamber, measured temperature of each dish, and counted surviving embryos. Membrane diameter, the diameter of the outermost membrane at it's widest point was measured using a dissecting scope. Embryos were then removed from their membranes and embryo length and embryo wet mass (Mettler AE 163) were measured. Embryos were then frozen at -20° C.

To analyze melanin, I extracted protein in a solution of 50 µl of NaOH and 450 µl of double distilled water. Embryos were sonicated in solution to break up the larger pieces. The solution was incubated overnight at room temperature to solubilize melanin. I centrifuged the solution at 1000 rpm and then diluted 100 µl of solution with 1400 µl of double distilled water (ddH_20) and recorded absorbance at 410 nm. Absorbance was converted to µg of melanin using a standard curve created from squid melanin (Sigma). I then used another portion of the protein extraction to quantify the total protein extracted from each embryo. I diluted 10 µl of the protein solution with 990 µl of ddH_20 and recorded absorbance at 235 nm and 280 nm. To calculate mg protein/µl I used the following equation: mg protein/µl = (abs235 – abs 280)/2.51 (Whittaker and Granum 1980). I used the results of the protein analysis to normalize melanin concentration for individual embryos.

I isolated DNA (Easy-DNA kit, Genomic DNA Isolation, Invitrogen Corp) to examine UV radiation damage for the treatments. I used spectrophotometry to quantify amounts of the isolated DNA by measuring the absorbance at 260nm and 280 nm. DNA quality was calculated with the ratio of 260:280 nm). I used an indirect antibody enzyme-linked immunosorbent assay (ELISA) to detect cyclobutane pyrimidine dimers formed by the exposure to UVB (Mori et al. 1997). In the ELISA assay, I used extracted DNA (antigen) to coat microtitre plates (96 wells). The plate was incubated overnight at room temperature. The plate was rinsed with phosphate-buffered saline (PBS)- 0.5% Tween-20 to remove antigen not bound to wells. The plate was then incubated with 3% Bovine serum in blocking buffer. The Bovine serum was diluted in a blocking buffer that prevents the nonspecific adsorption of protein to any free sites on the well that are not occupied by DNA (Crowther 1995). Primary antibody (TDM-2) was added at a dilution of 1:1000 and incubated for two hours. After incubation the plate was washed three times with PBS – 0.5% Tween 20 washing buffer. The secondary antibody (Goat anti-mouse IgG conjugated horseradish peroxidase conjugate (1:3000)) was added and the plate was incubated for two hours at room temperature. After incubation, the plate was washed with the PBS – 0.5% Tween 20 and incubated with PBS – 0.5% Tween 20 for 5 minutes. I emptied the plate and added Sigma Fast color reagents (1 urea tablet and 1 hydroxide tablet in 20 mL). The color developed for 30 minutes and then the absorbance for each sample was read in a microplate reader at 405 nm.

Water chemistry

Specific conductance and pH were measured prior to collecting water each week. Water was collected for dissolved water color analysis (April 22) in syringe filters and

then filtered the water through Whatman GF/F filters into 250 ml HDPE bottles. Bottles were well rinsed with dd H_20 and then with filtered pool water before final collection. I also estimated dissolved water color of unfiltered water samples. To estimate water color, I measured absorbance at 440 nm with a spectrophotometer (Milton Roy Spectronic 1001, 5 cm pathlength). A standard curve was created using serial dilutions of color standards and used the standard curve regression coefficient to estimate color units from sample absorbance.

I collected one sample from each pool and from the well water for DOC and ion analysis using a syringe filter. I rinsed filter with dd H20 and appropriate pool or well water. I filtered samples through an Whatman GF/F filters (ashed 1 hr at 425°C) into acid washed well rinsed 30 mL HDPE bottles and froze them until chemical analysis. The Water Resources Laboratory (Dr. William McDowell, Department of Natural Resources, University of New Hampshire. Durham, NH) conducted the DOC analysis (Shimadzu TOC 5000).

I collected samples for ion analyses on May 6, 1999. I used well-rinsed (dd H_20) syringe filters to filter water through a 0.2 pore size Acrodisc (Gleman Science) into a polyethylene scintillation vial. Samples were refrigerated (at approximately 4°C) until chemical analysis. The Water Resources Laboratory (Dr. William McDowell, Department of Natural Resources, University of New Hampshire. Durham, NH) conducted the ion analyses.

I collected water samples for heavy metals analysis on May 4, 1999. The Analytics Environmental Laboratory Inc (AEL, Portsmouth, NH) conducted the analysis. AEL provided high density polyethylene bottles for the collection. Each bottle

contained nitric acid for preservation purposes. I collected water without filtration, placed bottles on ice, and I transported the samples to AEL for analysis within two hours of collection.

Statistical analysis

I used fixed effects ANOVA to test for significant differences between water types and UV treatments and included interaction terms to test for synergism. I adjusted the probability of making a type 1 error to 0.01 to protect the experiment alpha of 0.05 using the Bonfferoni adjustment. I used Tukey's pairwise mean comparisons test to examine specific differences among treatments. Embryo wet mass, melanin concentrations, and CPD production were log transformed to reduce heteroscedasticity in the data. I did not analyze survival data as 93% or greater survived in all treatments.

RESULTS

Embryo survival ranged from 93 - 100 % for all treatments. There was no suggestion that UV radiation or any of the three water type affected embryo survival. Mean embryo length was significantly different among water types but not UV treatments (Table 3-1). Water type explained 31 % of the variation in length (partial $\eta^2 = 0.31$, $p < 0.01$). Results of Tukey mean comparison test indicated that mean length of roadside embryos was significantly smaller then embryos in off-road water ($p < 0.05$). Embryos exposed to roadside water were smaller in length for all three UV treatments then embryos exposed to water from an off-road pool and well water, but differences are minute (Figure 3-2). Embryos exposed to UVB radiation and roadside water had the shortest mean length (Figure 3-2).

Table 3-1. Results of two-way ANOVA performed on embryo length for 1999 laboratory experiments.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	1.22	2	0.61	5.4	0.01
UV treatment	0.37	2	0.19	1.77	0.22
Water type * UV treatment	0.65	4	0.16	1.44	0.26
Error	2.04	18	0.11		
Total SS	4.28				

Table 3-2. Results of two-way ANOVA performed on embryo wet mass for 1999 laboratory experiments.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	0.01	2	0.005	0.93	0.41
UV treatment	0.07	2	0.03	0.07	0.01
Water type * UV treatment	0.04	4	0.01	1.7	0.2
Error	0.11	18	0.006		
Total SS	0.23				

Embryo mean wet mass was significantly different among UV treatments but not water types (Table 3-3). UV treatment explained 40% of the variation in embryo wet mass (partial $\eta^2 = 0.40$, $p < 0.01$). Results of Tukey's mean comparisons indicated that the mass of embryos exposed to UVB (UVT treatment) was significantly less then the mass of embryos exposed to no UV radiation (UVO treatment) ($p < 0.05$). Mean wet mass of embryos for UVT treatments was less then mean wet mass of UVO embryos for all water types (Figure 3-3). Wet mass was greatest for well water, less for off-road water and lowest for roadside water within UVO and UVT treatment but this trend was not significant (Figure 3-3).

Table 3-3. Results of two-way ANOVA performed on the outer membrane diameter for 1999 laboratory experiments.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	8.26	2	4.13	22.4	0.00001
UV treatment	0.17	2	0.08	0.47	0.6
Water type * UV treatment	1.26	4	0.3	1.7	0.2
Error	3.31	18	18		
Total SS	13				

Diameter of the outer membrane was significantly different among water types but not UV treatments (Table 3-4). Water type explained 71 % of the variation in diameter (partial $\eta^2 = 0.71$, $p < 0.01$). Results of Tukey's mean comparisons indicate that mean membrane diameter for embryos in roadside water was significantly lower then mean membrane diameter of embryos in well and off road water ($p < 0.05$). Membrane diameter was smaller for both off-road and roadside water treatments compared to well water (Figure 3-4). Melanin concentration was not significantly different among UV treatments or water types (Table 3-4, Figure 3-5).

Table 3-4. Results of two-way ANOVA performed on embryo melanin concentration for 1999 laboratory experiments.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	0.24	2	0.12	1.7	0.2
UV treatment	0.06	2	0.03	0.46	0.6
Water type * UV treatment	0.01	4	0.003	0.04	0.9
Error	1.3	18	0.07		
Total SS	1.61				

CPD production was significantly different among water types but not UV treatment (Table 3-5). Water type explained 88% of the variation in dimer production (partial $\eta^2 = 0.88$, $p = 0.01$). Within the off-road and roadside water treatments, dimer production increased as UVB exposure increased across the UV treatments (Figure 3-6).

Table 3-5. Results of two-way ANOVA performed on absorbance of the DNA solution according to the protocol for enzyme-linked immunosorbent assay.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	0.53	2	0.26	1.5	0.013
UV treatment	0.004	2	0.001	0.11	0.89
Error	0.069	4	0.02		
Total SS	0.603				

Water Chemistry

Specific conductance was higher in the roadside pool than the off-road pool. The off-road pool water had the lowest pH level (Table 3-6). Dissolved water color was much darker for the natural pools then the well water (Table 3-6). Off-road pool water was darker then roadside pool water. DOC was much higher in the natural vernal pool water then the well water and roadside DOC was higher then off-road DOC (Table 3-6).

Roadside water sodium and chloride were both higher than the detectable limit set in the McDowell water chemistry analysis laboratory. The off-road pool had very low level of all ions analyzed in this study. Well water had higher ion levels (Table 3-6). Heavy metals concentrations were very low or below detectable limits (Table 3-6). Results of ANOVA indicated that UV radiation treatment and water type did not affect the temperature of the water (Table 3-7).

Figure 3-6. Water chemistry parameters for three water types used in the 1999 laboratory experiments. Off road water came from a vernal pool > 50 m form a road and roadside water came from a vernal pool < 2 m from a road.

		Well water	Off road	Roadside
Specific conductance ($\mu S\ cm^{-1}$)	1999		39.9 +/- 1.0	473 +/- 114
pH	1999	8.3	5.6	5.9
Dissolved Water color (Pt, mg l^{-1})	filtered	0	199	132
	unfiltered	0	215	167
Dissolved Organic Carbon (mg * L^{-1})		1.37	13	13.3
Sodium (mg * L^{-1})		14.2	2.2	
Potassium (mg * L^{-1})		6.4	1.5	
Magnesium (mg * L^{-1})		18.3	0.8	0.98
Calcium (mg * L^{-1})		13.7	2.4	3.6
Chloride (mg * L^{-1})		13.7	1.7	
Nitrate (mg * L^{-1})		14.2	0.001	0.2
Sulfate (mg * L^{-1})		0.3	0.8	1.3

Table 3-7. Results of two-way ANOVA performed on temperature of water in experimental dishes for 1999 laboratory experiments.

Source	Sum of Squares	df	Mean Square	F- ratio	P value
Water type	0.09	2	0.04	0.02	0.97
UV treatment	0.17	2	0.08	0.04	0.96
Water type * UV treatment	0.013	4	1	0.47	0.75
Error	37.6	18	2.09		
Total SS	37.873				

UV radiation

Artificial UVB and UVA spectral irradiance were within the range of the ambient UVB radiation spectral irradiance observed in the natural vernal pools described in Chapter 2 (Table 3-8). It is important to note that irradiance was measured out of water in the laboratory so measurements are higher then the actual irradiance underwater in treatments. The spectral irradiance of PAR was much lower in the environmental chamber then observed PAR in the field experiments in Chapter 2 (Table 3-8). Acrylic used to create UV treatments successfully controlled UV radiation (Figure 3-8) for the UV treatments. The UVT (UV transparent) acrylic transmitted 82% of the total UV radiation irradiance and 84% of photosynthetically active radiation in the environmental chamber. The UVA (UVA and PAR only) acrylic transmitted 2% of the UVB radiation and 70% of PAR. The UVO (UV opaque) acrylic transmitted 3% of the UV radiation and 79% of PAR. Total dose of UVB radiation was within the range of observed field doses for sunny days but much higher then doses then cloudy days (Table 3-9). PAR and UVA radiation daily doses were lower in the laboratory then in the field experiments.

Table 3-8. Spectral irradiance for laboratory chamber and range of underwater irradiances for field experiments. All irradiances are shown in W m

	UVB (300-320nm)	UVR (300-400nm)	PAR (400-700nm)
Laboratory	0.017	0.17	34
Pool 1	0.001 - 0.009	0.4 - 1.5	312 - 825
Pool 2	0.003 - 0.11	0.43 - 9.6	59 - 1270
Pool 3	0.02 - 0.1	1.43 - 6.3	156 - 1073
Pool 4	0.0009 - 0.04	0.2 - 4.78	101 - 848
Pool 5	0.002 - 0.03	0.04 - 4.13	161 - 348
Pool 6	0.009 - 0.002	0.3 - 1.2	116 - 981

Table 3-9. Daily doses of UV radiation in laboratory and field experiments (Chapter 2). UVB doses were calculated for both sunny and cloudy days to describe day to day variation.

	Laboratory	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
UVB (kJ day $^{-1}$)	0.80	0.13	1.65	1.47	0.50	0.48	0.03
cloudy UVB (kJ day $^{-1}$)	N/A	0.02	0.03	0.17	0.02	0.03	0.02
UVA (kJ day $^{-1}$)	7.96	25.89	165.66	150.13	82.49	70.75	20.71
PAR (kJ day $^{-1}$)	1582	15127	22260	19677	15549	6363	17987

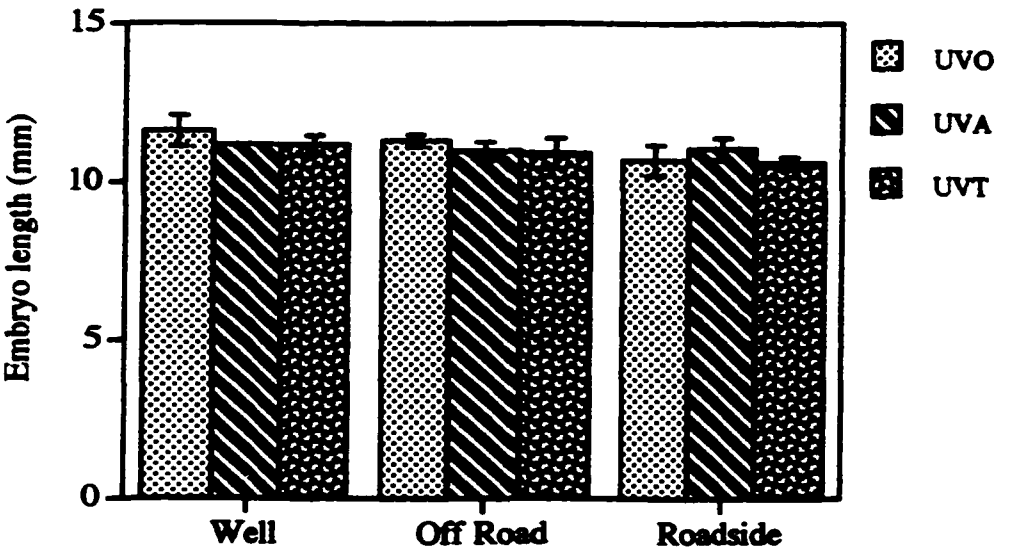

Figure 3-2. Embryo length for UV and water type treatments in laboratory experiments. Means +/- 1 standard deviation are shown.

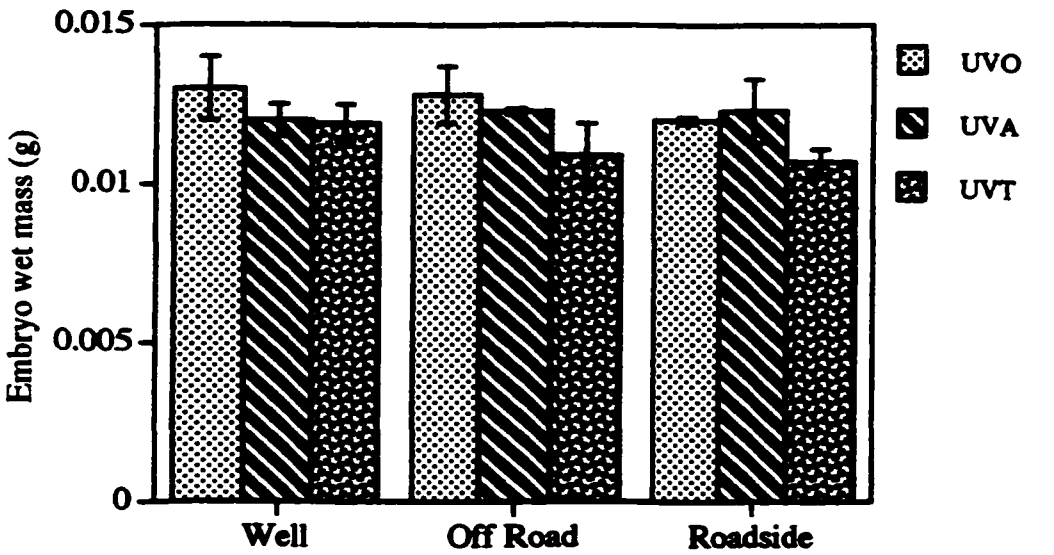

Figure 3-3. Embryo wet mass for UV and water types treatments in laboratory experiments. Means +/- 1 standard deviation are shown.

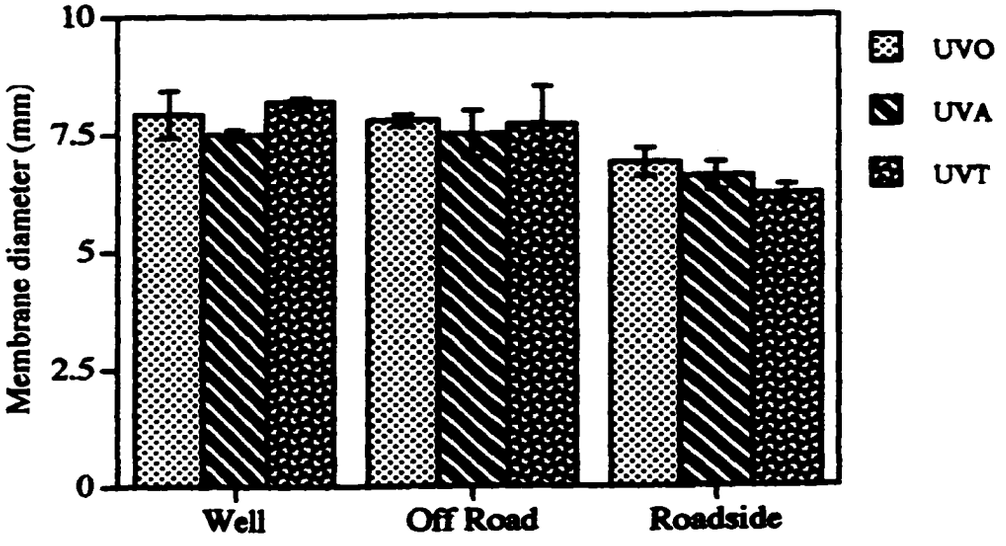

Figure 3-4. Membrane diameter for embryos in UV and water type treatments for laboratory experiments. Means +/- 1 standard deviation are shown.

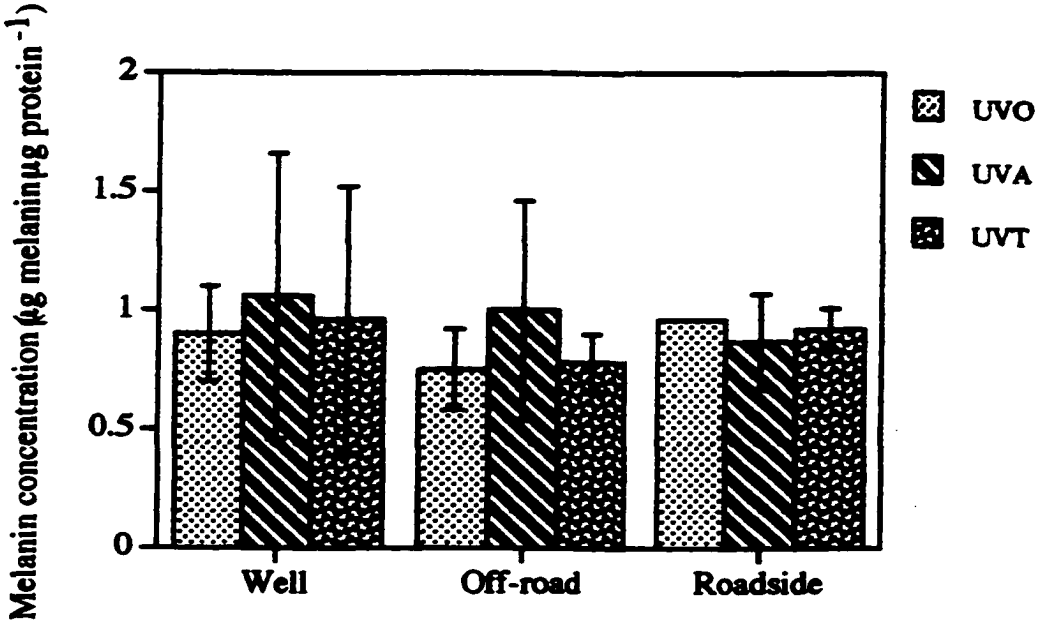

Figure 3.5. Embryo melanin concentrations for UV and water type treatments . Means +/- 1 standard deviation are shown.

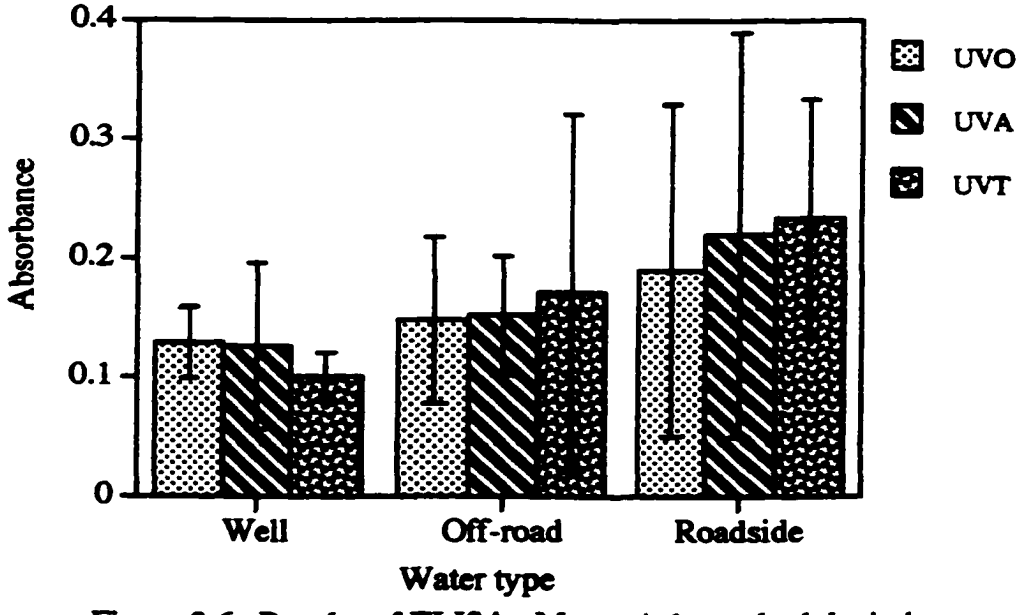

Figure 3.6. Results of ELISA. Mean +/- 1 standard deviation are shown. Absorbance represents production of CPDs for UV treatments and water types.

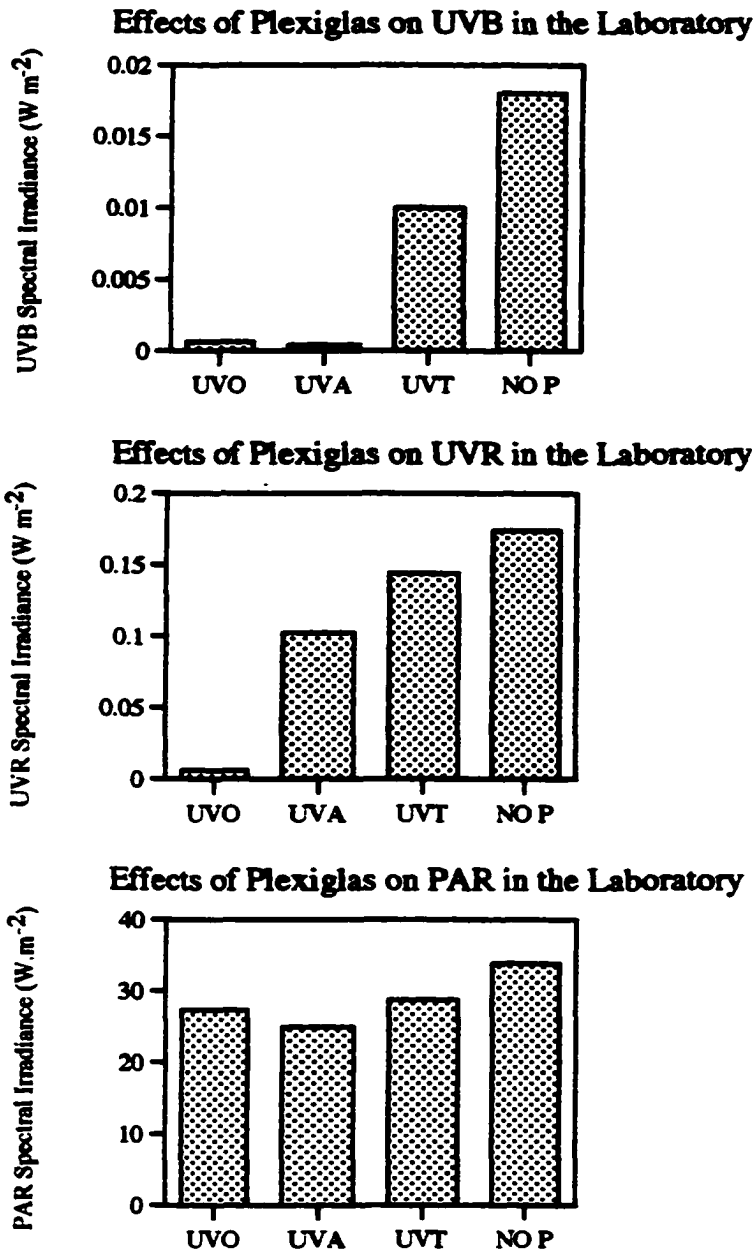

Figure 3-7. Transmittance of acrylic used in laboratory experiments in 1999. Irradiance is described for each acrylic treatment and UVB, UVR, and PAR.

DISCUSSION

Neither UVB nor contaminated highway runoff affected embryo survival alone or synergistically. Starnes et al. (2000) found similar results in field UVB studies with *A. maculatum*. The absence of a lethal effect of roadside water is consistent with results of the 1997 laboratory experiment discussed in Chapter 1. Results of the laboratory experiment suggest that ultraviolet radiation and water type influence different sublethal parameters for *A. maculatum* embryos. I observed that embryo length was smaller for off-road water then for well water and smallest for roadside water overall. Results of other studies also indicate an association between length and water chemistry. Bradford et al. (1992) found that length was smaller for amphibians exposed to lower pH levels. Growth and development can be depressed and even completely arrested in polluted water (Cummins 1986). In this study smaller embryo lengths could be associated with lower pH in the off-road water compared to well water and highway contamination in roadside water. Membrane diameter was smaller for both off-road water and roadside water treatments compared to well water and smallest for roadside water overall.

The association between water chemistry and amphibian embryo outer membrane diameter is well documented in the literature (Gosner and Black 1953, Freda and Dunson 1985, Turtle 1996) and by the results of laboratory experiments discussed in Chapter 1 of this dissertation. Results from other experiments suggest that membrane diameter is affected by both pH and high salt concentrations (Gosner and Black 1953, Freda and Dunson 1985, Dunson and Connell 1986). This was linked to the deactivation of the hatching enzyme by these pollutants (Freda and Dunson 1985). It is interesting to note that, overall, the smallest membrane diameter occurred for embryos exposed to

roadside water treatments and UVB. If this affect also occurs in natural breeding pools embryos in roadside pools may be more at risk then in off-road pools. Deactivation of the hatching enzyme can delay hatching or completely block it. If hatching is delayed embryos are more at risk of predation as their movement is limited when they are in the egg mass. A longer period of immobility in the egg mass also increases risk of desiccation if pool dries quickly and egg mass if left out of water.

Water type clearly affected the production of CPDs. Production of CPD was higher in off-road water and significantly higher for the embryos exposed to roadside pool water then embryos exposed to the well water. CPDs are the major product of the exposure of DNA to UVB. These dimers kill cells by blocking replication a transcription. Photoreactivation prevents the effects of UVB exposure by repairing these lesions. It is also interesting to note that within the off road water and the roadside water, CPD production increased as exposure to UVB radiation. However, this was not significant. The trend does however, suggest a synergism of multiple stressors. It is possible that the repair enzyme photolyase is affected by water chemistry as is the hatching enzyme. Low pH and high salt concentrations may limit its ability to repair DNA. This would explain the trend in increased CPD production across water types and across UV treatments within natural vernal pool water. Larger sample sizes are needed to test this trend, as the effect size of UVB radiation is so small.

Highway contamination can also interact with UV radiation to indirectly affect amphibians. Phototoxic polycyclic aromatic hydrocarbons (PAH), found in petroleum, exist at high levels in aquatic and terrestrial habitat adjacent to highways (Hautala et al. 1995, Christensen et al. 1997). They are phototoxic so the products of their

photochemical reactions could become toxic to amphibians.

Mean wet mass was the only variable affected by UV radiation at a statistically significant level. It is surprising that mass was not significantly less for roadside water, as mass and length are usually strongly associated. Mass is a multidimensional variable and may be more descriptive of embryo size then length. Reduction of embryo wet mass may be associated with elevated maintenance costs. In some ectotherms 80% of the total energy budget is used for maintenance (Congdon et al. 1982). Maintenance costs include basic energy needed for physiological systems to function and to acquire new energy and avoid predators (Rowe et al. 1998). This leaves 20% of the energy budget for growth, storage and reproduction. Stressful environmental conditions may increase the amount of energy allocated to maintenance in the embryo and thereby decreasing energy allocated to growth. This could reduce the mass of the embryo at hatching. Rowe et al. (1998) found that bullfrog tadpoles exposed to environments polluted by coal ash had higher maintenance costs as measured by metabolic rate. They did not observe any association between metabolic rate and tadpole wet mass at the end of the experiment as was expected.

Results of UVO and UVT treatments suggest that mean wet mass was less for embryos exposed to roadside water and overall mean wet mass was lowest for embryos exposed to UVB radiation and roadside water. This suggests a synergism between the factors but the experimental design may not have been powerful enough to detect it.

Results of field experiments indicated that UV radiation is not currently a risk for *A. maculatum*, but there were sublethal effects in the laboratory experiment. The daily dose of UVB was well within the range observed in the field on sunny days. However, it

was much higher then the daily dose on cloudy days. Therefore, the total dose over the entire embryo period may have been higher in the lab where there was no variation in daily dose due to weather conditions. However, it is also important to note that the differences in laboratory and field responses to UV radiation may be due to the low transmittance of the acrylic used in the field experiments. One of the weaknesses of this experiment is the great difference in UVB:UVA ratio between laboratory and field observations, thus the balance of UV damage to UV repair was different; there may have been more damage and less repair in the lab. This may limit the ability of the organism to repair damage caused by UVB radiation (Smith et al. 1992). The results of the laboratory experiments indicate potential affects if UVB irradiance at Earth's surface continues to increase in temperate latitudes. The effects of UVB radiation may be magnified in currently stressful habitats, such as polluted wetlands.

In conclusion, water quality is the greater environmental influence on amphibians of the two factors examined in this study. There is only a suggestion of a synergism between the UV radiation and highway contamination. Clearly, current levels of UVB radiation and highway runoff are not a lethal threat to amphibians. Although in many cases the ecological significance of sublethal effects is not known and it may be substantial (Bradford et al. 1992). It is important to note that embryos exposed to both UVB radiation and roadside runoff had the shortest length, shortest membrane diameter, lowest mass, highest production of CPD. The small sample size used in this experiment may limit the ability to describe the actual trends statistically.

SYNTHESIS AND CONCLUSIONS

In this project, I examined the effects of ultraviolet radiation and roadside runoff on *Ambystoma maculatum* embryos. Changes in the environment, caused by anthropogenic pollution may increase mortality. They may also cause an organism to increase the amount of energy allocated to maintenance reducing the energy budget for growth and reproduction, thereby, reducing overall fitness (Grime 1989).

Embryo survival of *Ambystoma maculatum* was very low in roadside pools, but the exact cause of this is still a mystery. Sodium and chloride concentrations and specific conductance are much higher in roadside vernal pools indicating that these wetlands are contaminated in adjacent highway habitat. These pools are vulnerable to contamination because the water entering these pools has little contact with soil buffering systems in their small watersheds, and there is often little standing water volume to dilute incoming runoff and precipitation (Pough 1976, Gascon and Planas 1986). However, contaminated water alone is not the sole cause of low mortality. Results of laboratory experiments indicate that under controlled conditions water from a contaminated vernal pool does not affect embryo survival. Highway contaminated water did cause sublethal effects, for example, a decrease in membrane diameter and a decrease in embryo length. Multiple stressors are most likely the cause of low survival in the natural environment.

Current UVB exposure in vernal pools is very low. Overall, watercolor and DOC, two factors negatively associated with UVB transmittance under water (Williamson et al. 1996), were very high in these pools. I did not observe any UVB affects on embryo

survival, size at hatching, melanin concentration, or production of cyclobutane pyrimidine dimers. UVB appeared to affect embryos in pools with lower watercolor, but the effects were not significant.

In the field experiments embryo length varied significantly among pools. Variation in temperature most likely explained the variation in embryo length among pools and between years. Warmer temperature increases rates of growth and development (Duellman & Trueb 1996). There is a potential tradeoff between warmer sunny areas where exposure to UVB is higher. At current levels of UVB underwater spectral irradiance exposure is so low that is does not detract from benefits of developing in sun exposed areas.

I did observe sublethal effects of both ultraviolet radiation and highway contamination in the laboratory. The impacts of highway contamination were much stronger. Embryos exposed to roadside water were smaller in length. This may be a result of depressed growth and development or the smaller membrane diameter length. Salts are known to deactivate the hatching enzyme and stunt growth, delay hatching, or cause spinal malformity (Rowe and Dunson 1985). Deicing salt contamination in the field may be one of multiple stressors interacting with the developing embryos, but there are many other contaminants in roadside water (Harned 1988). UVB had a significantly negative affect on the wet mass of the embryos. This is possibly due to decreases in energy allocated to growth caused by the need to allocate more energy to maintenance (i.e., repair or protection). The results of the laboratory experiments also suggest the possibility of synergism between UV radiation and highway contamination but further studies are needed to examine the extent of its impact.

Of the two factors examined in this study, highway runoff is of greater concern. Future work should examine water chemistry and sediments of these polluted roadside pools. It will also be important to examine effects of highway contamination on the larval stage to determine its effects on recruitment of juvenile. Future increases in UV radiation will most likely be more harmful in already stressful environments. It will be important to understand the parameters that naturally protect freshwater organisms from UVB. Changes in these parameters may indirectly have negative effects of organisms by increasing UVB exposure.

Vernal pools are unique and complicated wetland environments. Organisms that breed in these pools trade the risk of fish predation for risk of desiccation. The natural high level of risk of this life history strategy increases the vulnerability of vernal pool amphibian to anthropogenic changes in their environment. In this kind of environment the impacts of contaminants or increased exposure to harmful factors may be subtle when examined at the individual level. However, these same factors may have dramatic long-term impacts on populations.

LITERATURE CITED

Anderson, J. G., D. W. Toohey, and W. H. Brune. 1991. Free radicals within the Antarctic Vortex: The role of CFC's in Antarctic ozone loss. Science 251:39-46.

Anzalone, C.R., L. Kats, and M. Gordon. 1998. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina*, *Hyla regilla*, and *Taricha torosa*. Conservation Biology 12(3) 646-653.

Blaustein, A. R., P. Hoffman, D. G. Hokit, J. Kiesecker, S. Walls, and J. Hays. 1994. UV repair and resistance to solar UV-B in amphibian eggs: A link to population declines? Proc. Natl. Acad. Sci, USA 91:1791-1795.

Blaustein, A. R., B. Edmond, J. Kiesecker, J. Beatty, and D. G. Hokit. 1995. Ambient ultraviolet radiation causes mortality in salamander eggs. Ecological Applications. 5(3) 740-743.

Blaustein, A. R., and D. B. Wake. 1995. The puzzle of declining amphibian populations. Scientific Ameican 272(4)56-61.

Blaustein, A. R., P. Hoffman, J. Kiesecker, and J. Hays. 1996. DNA repair activity and resistance to solar UV-B radiation in eggs of the red-legged frog. Conservation Biology 10(5) 1398:1402.

Bothwell, M. L., M. Darren, J. Sherbot, and C. Pollock. 1994. Ecosystem response to solar ultraviolet-B radiation: Influence of trophic-level interactions. Science 265:97-100.

Boxall, A. B. A. and L. Maltby. 1997. The effects of motorway runoff on freshwater ecosystems: 3 toxicant confirmation. Arch. Environ. Contam. Toxicol. 33:9-16.

Bradford, D. F., C. Swanson, and M. Gordon. 1992. Effects of low pH and aluminum, on two declining amphibian species of amphibians in the Sierra Nevada, California. Journal of Herpetology 26(4):369-377.

Bridges C. 1999. Effects of a pesticide on tadpole activity and predator avoidance behavior. Journal of Herpetology. 33(2) 303-306.

Buma A. G. J., A. H. Engelen, and W. W. C. Gieskes. 1997. Wavelength-dependent induction of thymine dimers and growth rate reduction in the marine diatom *Cyclotella* sp. Exposed to ultraviolet radiation. Mar. Ecol. Prog. Ser. 153:91-97.

Burbeck, R., W. Diment, B. Deck, A. Baldwin, and S. Lipton. 1971. Runoff of deicing salt: effect on Irondequoit Bay, Rochester, New York. Science 172:1128-1132.

Calkins, J., and T. Thordardottir. 1980 The ecological significance of solar UV radiation on aquatic organisms. Nature 283:563-566.
Carey, C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conservation Biology 7:355-362.

Carpenter, S. R. and J. F. Kitchell. 1993. The trophic cascade in lakes. Cambridge.

Christensen, E. R., A.L. Irwan, A. A Razak, P. Rachdawong, and J. F. Karls. 1997. Sources of polycylic aromatic hydrocarbons in sediments of the Kinnickinnic River, Wisconsin. Journal of Great Lakes Research. 23(1): 61-73

Clark, K. L., and R. J. Hall. 1985. Effects of elevated hydrogen ion and aluminum concentration on the survival of amphibian embryos and larvae. Canadian Journal of Zoology 63:116-123.

Cooper, W. J. 1996. Hydrogen peroxide formation: The interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43-75°N gradient. Limnology and Oceanography 41(3) 540-548.

Corn, P. 1998. Effects of ultraviolet radiation on boreal toads in Colorado. Ecological Applications 8(1) 18-26.

Crowther, J. R. 1995. Methods of Molecular Biology ELISA: Theory and practice. Humana Press, Totowa. NJ 07512.

Crowther, R. A. and Hynes, B. N. 1977. The effect of road deicing salt on the drift of stream benthos. Environmental Pollution 14:113-126.

Crump, M. L., F. R. Hensley, and K. L. Clark. 1992. Apparent decline of the gold toad: underground or extinct? Copeia 1992:413-420.

Crutzen, P. J. 1992. Ultraviolet on the increase. Nature 356:104-105.

Cullen, J. J. and M. P. Lesser. 1991. Inhibition of photosynthesisby ultraviolet radiationas a function of dose and dosage rate. Mar. Biol. 111:183-190.

Cummins, C. P. 1986. Effects of aluminum and low pH on growth and development in *Rana temporaria* tadpoles. Oecolgia 69: 248-252

Cutchis, P. 1974. Stratospheric ozone depletion and solar ultraviolet radiation on earth. Science 184:13-19.

Damkaer, D. M and D. B. Dey. 1983. UV damage and photoreactivation potentials of larval shrimp, *Pandalus platyceros*, and adult euphausiids, *Thysanoessa raschii*. Oecolgia 60:169-175

Dohler, G. 1984. Effect of UV-B radiation on the marine diatoms *Lauderia annulata* and *Thalassiosira rotula* grown in different salinities. Mar. Biol. 83:247-253.

Downs, F. L. 1989. Ambystoma maculatum (Shaw). Pages 108 – 125 in Ralph A. Pfingsten and F. L. Downs, editors. Salamanders of Ohio. College of Biological Sciences, The Ohio State University.

Duellman, W. E. and L. Trueb. 1986. Biology of Amphibians. John Hopkins University Press.

Dunson, W. A. and J. Connell. 1982. Specific inhibition of hatching in amphibian embryos by low pH. J of Herp. 16(3)314-316.

Farman, J. C., B. G. Gardiner, and J. D. Shankin. 1985. Large losses of total ozone in Antarctica reveal seasonal ClOx/Nox interaction. Nature 315:207-210

Ferreyra, G. A., S. Demers, P. del Giorgio, and J. Chanut. 1997. Physiological responses of natural plankton communities to ultraviolet -B radiation in Redberry Lake (Saskatchewan, Canada). Canandian Journal of Fisheries and Aquatic Sciences 54:705-714.

Fisher, R. N. and H. B. Shaffer. 1996. The decline of amphibians in California's Great Central Valley. Conservation Biology 10(5) 1387-1397.

Freda, J., and W. A. Dunson. 1985. The influence of external cation concentration on the hatching of amphibian embryos in water of low pH. Can. J. Zool. 63: 2649-2656.

-------, -------. 1986. Effects of low pH and other chemical variables on the local distribution of amphibians. Copeia 1986:454-466.

Frederick, J. E., and H. E. Snell. 1988. Ultraviolet radiation levels during the Antarctic spring. Science 241:438-440.

Gish, C. D. and R. Christensen. 1974. Cadmium, Nickel, Lead, and Zinc, in earthworms from roadside soil. Environmental Science and Technology 4:1060-1062.

Gosner, K. L. and I. H. Black. 1957. The effects of acidity on the development and hatching of the New Jersey frogs. Ecology 38(2):256-262.

Grant, K. P., and L. Licht. 1995. Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. Can. J. Zool. 73:2292-2301.

——, ——. 1997. The effects of ultraviolet radiation on the biology of amphibians. American Zoologist 37:137-145.

Grime J. P. 1989. The stress debate: sympton of impending synthesis? Biol. J. Linn. Soc. 37:3-17.

Harned, D. A. 1988. The effects of highway runoff on the streamflow and water quality of streams in the Sevenmile Creek Basin, a rural area in the piedmont province of North Carolina, July 1981-July 1982. U. S. Geological Survey water supply paper: 2239.

Harrison, R. G. 1969. "Harrison stages and description of the normal development of the spotted salamander, Amblystoma punctatun (Linn.), " Pages 44-66, in R. G. Harrison, editor, Organisation and Development of the Embryo. New Haven Yale University Press.

Hautala, E. L., R. Rekila, J. Tarhanen, and J. Ruuskanen. 1995. Deposition of motor vehicle emissions and winter maintenance along roadside assessed by snow analyses. Environmental Pollution 87:45-49.

Hecnar S. J. and R. M'Closkey. 1996. Regional dynamics and the status of amphibians. Ecology 77(7) 2091-2097.

Herndl, G. J., G. Muller-Niklas, and J. Frick. 1993. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature 361:717-719.

Hofstra, G. and D. W. Smith. 1984. The effects of road deicing salt on the levels of ions in roadside in Southern Ontario. 19:261-271.

Ireland, P. H. 1991. Separate effects of acid-derived anions and cations on growth of larval salamanders of *Ambystoma maculatum*. Copeia 1: 132-137.

Isabelle, P. S., L. J. Fooks, and P. A. Keddy. 1987. The effects of roadside snowmelt on wetland vegetation: an experimental study. Journal of Environmental Management 25:57-60.

Joikel, P. L. and R. H. York. 1984. Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnology Oceanography, 29(1):192-199.

Karentz, D., and L. H. Lutze. 1990. Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnology and Oceangraphy 35(3):549-561.

——, F. S. Mceuen, M. C. Land, and W. C. Dunlap. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Marine Biology 108:157-166.

Kerr, J. B. and McElroy C. T. 1993. Evidence for large upward trends of ultraviolet – B radiation linked to ozone depletion. Science 262:1032-1034.

Kiesecker, J. A. and A. Blaustein 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proc. Natl. Acad. Sci. 92:11049-11952.

Kirk, J. T. 1994. Optics of UV-B radiation in natural waters. Arch. Hydrobiol. Beih Ergebn. Limnol. 43: 17-30.

Kleeberger S. R. and J. K. Werner. 1983. Post-breeding migration and summer movement of Ambystoma maculatum. Journal of Herpetology 17:176-177.

Lagerwerrf, J. D. and A. W. Specht. 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead, zinc. Environmental Science and Technology.

Larson, R. A. and Berenbaum, M. R. 1988. Environmental phototoxicity. Environ. Sci. Technol 22(4) 354-360.

Laurion, I., W. Vincent, D. Lean. 1997. Underwater ultraviolet radiation: Development of spectral models for northern high latitude lakes. Photochemistry and Photobiology 65(1) 107-114.

Lesser, M. P., P. J. Neale, J. J. Cullen. 1996. Acclimation of Antarctic phytoplankton to ultraviolet radiation: ultraviolet-absorbing compounds and carbon fixation. Molec. Mar. Biol and Biotech. 5(4):314-325.

Li, Weixiong and H. Z. Hill. 1997. Induced melanin reduces mutations and cell killing in mouse melanoma. Photochemistry and Photobiology 65(3): 480-485.

Lind, O. T. 1985. Handbook of common methods in Limnology. Kendall/Hunt Publishing Company. Dubuque, IA.

Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. Conservation Biology 12(1) 1-13
-------, 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13(1) 117-125.

Lizana, M., and E, Pedraza. 1997. The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. Conservation Biology 12(3) 703-707.

Long, L., L. Saylor, and M. Soule 1995. A pH/UV-B synergism in amphibians. Conservation Biology 9(5) 1301- 1303.

Loumbourdis, N. S., P Kyriakopoulou-Sklavounou, and G. Zacariadis. 1999. Effects of cadmium exposure on bioaccumulation and larval growth in the frog Rana ridibunda. Environmental Pollution 104:429-433.

Lubin, D., and E. Jensen. 1995. Effects of clouds and stratospheric ozone depletion on ultraviolet radiation trends. Nature 377:710-713.

Madronich, S. 1992. Implications of recent total atmospheric ozone measurements. For biologically active ultraviolet radiation reaching the Earth's surface. Geophysical Research Letters 19: 37-40.

McDowell W. H. and G. E. Likens. 1988. Origin, compositionand flux of dissolved organic carbon in the Hubbard Brook valley. Ecological Monographs 58:177-195.

Mori, T., M. Nakane, T. Hattori, and T. Matsunaga. 1991. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4) photoproduct from the same mouse immunized with ultraviolet irradiates DNA. Photochemistry and Photobiology 54(2) 225-232.

Morin, P. J. 1983. Predation, Competition, and the composition of larval anuran guilds. Ecological Monographs 53(2)119-138.

Mortel, T van de, W. Buttemer, P. Hoffman, J. Hays, and A. Blaustein. 1998. A comparison of photolyase activity in three Australian tree frogs. Oecologia 115:366-369.

Mostajir, B., S. Demers, S. de Mora, C. Belzile, J. Chanut, M. Gosselin, S. Roy, P. Zulema Villegas, J. Fauchot, and J. Bouchard. 1999. Experiments test of the effect of ultraviolet-B radiation in a planktonic community. Limnology Oceanography 44(3):586-596.

Mudre, J. M. and J. J. Ney. 1986. Patterns of accumulation in the sediment of roadside streams. Arch. Environ. Contam. Toxicol. 15:489-493.

Nagl, A. M., and R. Hofer. 1997. Effects of ultraviolet radiation on early larval stages of the alpine newt, *Triturus alpestris*, under natural and laboratory conditions. Oecologia 110:514-519.

Pierce, B. A. 1985. Acid tolerance in Amphibians. BioScience 35(4) 239-243.

Pough, F. H. 1976. Acid precipitation and embryonic mortality of spotted salamanders, Ambystoma maculatum, Science 192:68-70.

Ovaska, K., T. Davis, and I. Flamarique. 1997. Hatching success and larval survival of the frogs *Hyla regilla*, and *Rana aurora* under ambient and artificially enhanced solar ultraviolet radiation. Canadian Journal of Zoology 75:1081-1088.

Rassmussen, J. B., L. Godbout, and M. Schallenberg. 1989. The humic content of lake water and its relationship to watershed and lake morphometry. Limnology and Oceanography 34(7) 1336-1343.

Rex , M., and others. 1997. Prolonged stratospheric ozone loss in the 1995-1996 Arctic winter. Nature 389:835-838.

Robb, L. and D. Toews. 1987. The effects of low ambient pH on perivitelline fluid of *Ambystoma maculatum* (Shaw) eggs. Environmental Pollution 44: 101-107.

Rowe, C. L., W. Sadinski, and W. Dunson. 1992. Effects of acute and chronic acidification on three larval amphibians that breed in temporary ponds. Arch. Envion. Contam. Toxicol. 23:339-359.

-----, and W. Dunson. 1993. Relationships among abiotic parameters and breeding effort by three amphibians in temporary wetlands of central Pennsylvania. Wetlands 13(4): 237-246.

-----, O. Kinney, R. Nagle, and J. Congdon. 1998a. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. Phys. Zool. 71:27-35.

Rowe, C. L., O. Kinney, and J. Congdon. 1998b. Oral deformities in tadpoles of the Bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. Copeia 1:244-246.

Sadinski, W. J., and W. Dunson. 1992. A multilevel study of effects of low pH on amphibians of temporary ponds. Journal of Herpetology 26(4):431-422.

Salthe, S. M. 1963. The egg capsules in the Amphibia. Journal of Morphology 113:161-171.

----- 1965. Increase in volume of the perivitelline chamber during development of *Rana pipiens* Schreber. Physiological Zoololgy 38: 80-98.

Sancar, A. 1994. Structure and function of DNA photolyase. Biochemistry 33:2-9

Scully, N. M., and D. R. S. Lean. 1994. The attenuation of ultraviolet radiation in temperate lakes. Arch. Hydrobiol. Beih Ergeb. Limnol. 43: 135-144

Smith, R. C., B. B. Prezelin, K. S. Baker, R. R. Bidigare, N. P. Boucher, T. Coley, D. Karentz, S MacIntyre, H. A. Matlick, D. Menzies, M. Ondrusek, Z. Wan, and K. J. Waters. 1992. Ozone Depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952-959

Solomon, S. 1990. Progress towards a quantitative understanding of Antarctic ozone depletion. Nature 347:347-354.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, New York.

Sommaruga, R., and R. Psenner. 1997. Ultraviolet radiation in a high mountain lake of the Austrian Alps: Air and Underwater measurements. Photochemistry and Photobiology, 65(6):957-963.

Stata Corporation. 1997. Stata Reference Manual Release 5.College Station TX 77840

Starnes, S. M. C. Kennedy, and J. W. Petranka. 2000. Sensitivity of embryos of southern Applalachian amphibians to ambient solar UV-B radiation. Conservation Biology. 14(1) 277-282.

Stenhouse, S. L. 1987. Embryo mortality and recruitment of juveniles of *Ambystoma maculatum* and *Ambystoma opacum* in North Carolina. Herpetologica 43(4) 496-501.

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton NJ.

Turtle S. L. 1996. The effects of deicing salt on vernal pools and on spotted salamander embryonic survivorship (*Ambystoma maculatum*). Thesis. Masters of Science. University of New Hampshire.

----- 2000. The survival of spotted salamander (*Ambystoma maculatum*) embryos in roadside and woodland vernal pools. J. of Herp. 34:60-67.

Tyler-Jones R., R. C. Beattie, and R. J. Aston, 1989. The effects of acid water and aluminum on the embryonic development of the common frog, *Rana temporaria*. J. Zool. Lond. 219:355-372.

Vinebrooke, R. D. and P. Leavitt. 1996. Effects of ultraviolet radiation on periphyton in an alpine lake. Limnology and Oceanography 41(5) 1035-1040.

Wake, D. 1991. Declining amphibian populations. Science 253:860.

Warner, S. C., W. Dunson, and J. Travis. 1991. Interaction of pH, density, and priority effects on the survivorship and growth of two species of hylid tabpoles. Oecologia 88: 331-339.

Whitaker, J. R. and P., E. Granum. 1980. An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. Analytical Biochemistry 109:156-159.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 18: 1305 – 1314.

Wilbur H. M. 1976. Density-dependent sapects of metamorphosis in *Ambystoma* and *Rana sylvatica*. Ecology 57:1289-1296.

——. 1987. Regulation of structure in complex systems: experimental temporary pond communities. Ecology 68:1437-1452.

Wilkinson L. 1997. SYSTAT: 7.0 Statistics SPSS Inc., Chicago, IL. 60611.

Williamson, C. E., H. Zagarese, P. Schulze, B. Hargreaves, J. Seva. 1994. The impact of short-term exposure to UV-B radiation on zooplankton communities in north temperate lakes. J. of Plankton Research. 16(3):205-218.

Williamson C. E. 1995. What role does UV-B radiation play in freshwater ecosystems? Limnol. Oceangr. 40(2) 386-392.

Williamson C. E., R, Stemberger, D. M. Morris, T. M. Frost, S. Paulsen. 1996. Ultraviolet radiation in North American lakes: Attenuation estimates from DOC measurements and implications for plankton communities. Limnol. Oceangr. 41(5) 1024-1034.

Wyman, R. L. 1990. What's happening to the amphibians? Con. Bio. 4:350-352.

Zug, G. R. 1993. Herpetology: An Introductory Biology of Amphibians and Reptiles. Academic Press, San Diego.

APPENDIX

TABLE 1: List of embryo total mean survival +/- 1 standard deviation and percent survival for all pools used 1995 - 1999. Pool are listed according to location and reference names for different studies and years.

POOL	LOCATION	TOWN	STUDY REFERENCE NAME	YEAR	TOTAL SURVIVAL	PERCENT SURVIVAL
A	43°06.72N 71°05.35W	NOTTINGHAM	POOL W1	1995	28 +/- 4	56
				1996	24 +/- 7.4	47
				1997	33 +/- 5	58
B	43°06.73N 71°05.38W	NOTTINGHAM	POOL W2	1995	42 +/- 83.4	83
				1996	41 +/- 8	81
				1997	37 +/- 5	74
			POOL 2	1998	19 +/- 4.2	65
			POOL 2	1999	25 +/- 3	83
D	43°06.10N 70°[illegible].32W	DURHAM	POOL W4	1995	39 +/- 2.6	78
				1996	35 +/- 13	69
E	43°07.95N 71°00.54W	LEE	POOL W5	1995	9.5 +/- 5	19
F	43°07.61N 71°00.00W	LEE	POOL W6	1996	25 +/- 16	51
				1998	19 +/- 6	63
				1999	15 +/- 5	52
G	43°06.70N 71°05.38W	NOTTINGHAM	POOL R1	1995	10 +/- 7.4	20
				1996	2 +/- 3	3
				1997	27 +/- 15	54
H	43°06.68N 71°05.37W	NOTTINGHAM	POOL R2	1995	21 +/- 4	42
				1996	25 +/- 3	51
I	43°06.04N 71°04.47W	NOTTINGHAM	POOL R3	1995	22 +/- 6.4	45
				1996	0	0
K	43°06.68N 71°05.39W	NOTTINGHAM	POOL R5	1995	3 +/- 4	5
				1996	1 +/- 2.3	3
				1997	14 +/- 11	12
L	43°06.64N 71°05.28W	NOTTINGHAM	VACHON	1997	37 +/- 6	74
M	43°05.94N 71°04.23W	NOTTINGHAM	FARMHOUSE	1997	5 +/- 8	22

TABLE 1: continued

POOL	LOCATION	TOWN	STUDY REFERENCE NAME	YEAR	TOTAL SURVIVAL	PERCENT SURVIVAL
M	43o06.25N 70o58.96W	LEE	Pool 1	1998	23 +/- 4.8	77
				1999	25 +/- 3	84
		DURHAM	Pool 2	1998	28 +/- 3	84
N	43o06.04N 70o52.78W			1999	22 +/- 3	78
O	43o06.22N 70o58.93W	LEE	Pool 4	1998	13 +/- 5	42
				1999	4 +/- 3	12
P	43o06.62N 70o58.91W	LEE	Pool 6	1998	21 +/- 2	70
				1999	24 +/- 3	79

TABLE 2: List of water chemistry data for all pools used 1995 - 1999. Pool are listed according to location and reference names for different studies and years.

POOL	LOCATION	STUDY REFERENCE NAME	YEAR	SPE. COND. ($\mu S\ cm^{-1}$)	TEMP (C)	DO ($mg\ l^{-1}$)	pH	ALKALINITY ($mg\ CaCO3\ l^{-1}$)
A	43°06.72N 71°05.35W	POOL W1	1995	33 +/- 2	10.7 +/- 3.7	4.4 +/- 2		
			1996	24	8.4	2.7	5	1.7
			1997	18 +/- 1	10.3	4.12	5	
B	43°06.73N 71°05.38W	POOL W2	1995	33 +/- 1	12.2 +/- 3.5	5.2 +/- 3		
			1996	23	8.7	3	5	1.1
			1997	18 +/- 2	10.4	4.02	5	
		POOL 3	1998	13 +/- 6	5.2 +/- 2	6.7 +/- 0.5	5	
		POOL 3	1999	31+/- 1	7.8 +/- 2	5.9 +/- 0.6	5	0
D	43°06.10N 70°[illegible].32W	POOL W4	1995		12.2 +/- 3.1	5.2 +/- 3		
			1996	24	9.9	4.7	5	2.3
E	43°07.95N 71°00.54W	POOL W5	1995	34	11.3 +/- 3.1	2.6 +/- 1		
F	43°07.61N 71°00.00W	POOL W6	1996	26	11	5.7	4	0.03
		POOL 5	1998	24 +/- 4	8.3 +/- 0.6	5.8 +/- 0.4	4	
		POOL 5	1999	34 +/- 1	10.6 +/- 4	5.5 +/- 0.4	4	0
G	43°06.70N 71°05.38W	POOL R1	1995	1595	11.3 +/- 2.3	5.5 +/- 2		
			1996	943	9.4	2.9	6	19.5
			1997	706	11.9	3.7	6	
H	43°06.68N 71°05.37W	POOL R2	1995	723	13.2 +/- 2.3	7.3 +/- 4		
			1996	447	9.2	5.4	6	14.2
I	43°06.04N 71°04.47W	POOL R3	1995	664	12.2 +/- 3.6	4.8 +/- 2		
			1996	529	8.6		5	2.3
J	43°06.68N 71°05.39W	POOL R5	1995	613	13	5.4 +/- 3		
			1996	597	9.4	3		
			1997	601	11.3	4.6	5	
K	43°06.64N 71°05.28W	R3	1997	25	9	3.5	5	
L	43°05.94N 71°04.23W	R4	1997	83	10.4	4.6	5	

TABLE 2: continued

POOL	LOCATION	STUDY REFERENCE NAME	YEAR	SPE. COND. ($\mu S\ cm^{-1}$)	TEMP (C)	DO ($mg\ l^{-1}$)	pH	ALKALINITY ($mg\ CaCO3\ l^{-1}$)
M	43o06.25N 70o58.96W	Pool 1	1998	16 +/- 11	6.2 +/- 0.8	5.2 +/- 0.3	6	
			1999	32 +/- 2	10.3 +/- 4.1	4.3 +/- 0.9	5	1.9
		Pool 2	1998	18 +/- 3	6.3 +/- 1.5	7.23 +/- 0.3	5	
N	43o06.04N 70o52.78W		1999	40 +/- 1	8.7 +/- 3.3	5.5 +/- 0.4	6	3.5
O	43o06.22N 70o58.93W	Pool 4	1998	23 +/- 11	7.0 +/- 0.8	5.1 +/- 0.4	7	
			1999	54 +/- 1	10.6 +/- 3.8	3.5 +/- 0.8	6	11.7
P	43o06.62N 70o58.91W	Pool 6	1998	22 +/- 3	5.3 +/- 0.7	6.1 +/- 0.2	5	
			1999	33 +/- 4	9.7 +/- 3.0	4.7 +/- 0.7	6	1.1