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**SINGLE AND INTERACTIVE EFFECTS OF LOW pH AND LEECH PREDATORS
ON FROG EMBRYOS AND ADULTS**

by

GINA SCHALK

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

June 1997

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**Single and interactive effects of low pH and leech predators
on frog embryos and adults**

submitted by Gina Schalk
in partial fulfillment of the requirements for
the degree of Master of Science

Chair

Supervisor

Supervisor

ABSTRACT

I used an experimental and field approach to assess single and interactive effects of leech predators and pH on frog embryos and adults. Experiments on embryos indicated that leeches (*Macrobdella decora*) and low pH affected hatching success, hatch timing and hatch length of wood frog (*Rana sylvatica*) and green frog (*R. clamitans*) embryos. However, effects of the leech on hatch length and timing did not occur in low pH. The loss of a presumably adaptive trait at low pH could have important fitness consequences for hatchlings.

I conducted a field study testing for negative associations or interactive effects between *M. decora* abundance or pond pH and abundance of adult bullfrogs (*R. catesbeiana*), green frogs and leopard frogs (*R. pipiens*). Only green frog abundance was negatively associated with *M. decora*. There were no relationships between frog abundance and pH or pH-*M. decora* interactions. This is due to the non-acidity of study ponds ($\text{pH} \geq 5.45$) and does not preclude finding relationships at more acidic sites.

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GENERAL INTRODUCTION

Recent research indicates that abiotic factors such as pond drying time, low pH or high metal concentration all have direct effects on freshwater organisms, but they also can alter predator/prey or competitive associations among species in freshwater ecosystems (Wilbur 1987, Dunson and Travis 1991, Jung and Jagoe 1995, Pehek 1995, Weis and Weis 1995). These interactive effects, in turn, may alter species' distributions and abundances. Therefore it may be important to understand interactive effects if we are to understand how communities are structured.

My purpose was to investigate effects of anthropogenic low pH in conjunction with a natural factor, leech predation, on embryonic and adult frogs. Although low pH can occur naturally in some ecosystems such as sphagnum bogs (Pierce and Sikand 1985), many water bodies are anthropogenically acidified, and aquatic organisms living in these environments are evolutionarily naive to this stress (Freda and Dunson 1985). The effects of low pH on frogs are well known, and adult frog abundance and distribution are often negatively correlated with low pH (e.g., Clark 1986, Freda 1986). This may result from high mortality during the susceptible embryonic stage (Freda 1986). Additionally, sublethal effects of low pH include deformities and alterations of hatch length and hatch

timing which may have important consequences for avoidance of predators or for competitive interactions among tadpoles (e.g., Bradford et al. 1992, Warkentin 1995, Brodie and Formanowicz 1983).

Predators are thought to play a major role in structuring amphibian communities through both lethal and sublethal effects (Morin 1983). In particular, predation on early-life stages of amphibians is high (Wilbur 1980), and recent research indicates that predators also may affect size at hatching and the timing of hatching in embryos (Sih and Moore 1993, Warkentin 1995). The addition of a factor, such as low pH, to any predator-prey relationship may alter the importance of predation in shaping population size or species assemblages (Sredl and Collins 1992). An examination of both lethal and sublethal interactive effects of low pH and predation on frogs may further our understanding of the effects of human-induced acidity on amphibians.

I used both an experimental and field-based approach to examine interactive effects of low pH and leech predators on frog embryos and adults, respectively. In Chapter 1, I document experiments that investigate the separate and combined effects of low pH and predation by the leech, *Macrobdella decora*, on embryos of two frog species (*Rana sylvatica*, *R. clamitans*). This allows me to assess how predation (or the threat of predation) and low pH interact to determine hatching success, hatch timing and hatch length of the two frog species. From this understanding, predictions may be made and tested in future studies investigating higher-order interactions, interactions of low pH with other factors

(e.g., competition, other stresses) or the effects of these interactions in natural communities.

In Chapter 2, I report on a field study of the separate and combined effects of pond pH and leech abundance on adult abundance of three frog species (*R. clamitans*, *R. catesbeiana*, *R. pipiens*). This correlational approach allows me to test whether the outcomes observed at an experimental level translate into effects in natural amphibian populations. Thus, I am able to test whether the experimental results hold true in a complex natural system by examining whether known lethal effects at early-life stages are predictive of adult abundance and additionally, whether any sublethal effects of the two factors (sublethal low pH or predation risk) affect individual fitness so as to produce observable population level effects.

CHAPTER 1

Interactive effects of low pH and a leech predator on hatching success, hatch length and hatch timing of two frog species

Introduction

Research on a potentially stressful agent often begins with an examination of direct, lethal effects of that factor on the organism. However, research that is not extended to examine higher-order lethal and sublethal interactions may misrepresent or underestimate the effects of this factor in natural communities (Fauth 1990, Sadinski and Dunson 1992). Despite the broad base of knowledge on the negative effects of low pH on amphibians (see review in Freda 1986), researchers only have begun to investigate the effects of low pH in combination with community factors or various anthropogenic factors, and this research is proving important in understanding how amphibians may respond to anthropogenically-induced low pH in natural communities. For example, Long et al. (1995) found that leopard frog (*Rana pipiens*) embryos showed high hatching success in either moderate acidity or at moderately high levels of UV-B radiation. However, when these factors were combined, hatching success was decreased by almost 50 %. Warner et al. (1993) demonstrated that the outcome of interspecific interaction

between tadpoles of two hylid species was altered in low pH, and Rowe et al. (1994) demonstrated that predatory caddisflies (*Ptilostomis postica*) were more tolerant of low pH than their amphibian prey. It has been demonstrated that exposure of crayfish (*Cambarus bartoni*) and fish (*Pimephales promelas*) to low pH inhibits normal responses to feeding stimuli (Lemly and Smith 1987, Allison et al. 1992). This impairment of chemosensory-dependent activities such as feeding or predator detection could influence fitness. Together, this research provides compelling evidence that interactions with low pH, even when not lethal, may be common and could have important consequences for amphibian populations.

In this study, I experimentally investigated the separate and combined effects of low pH and the presence of the leech, *Macrobdella decora*, on embryos and tadpoles of two frog species (*Rana sylvatica*, *R. clamitans*). This experimental approach allowed me to examine the nature of potential interactions by comparison of combined effects to the effect of each factor in isolation. My goal was to use the results of this necessarily simple experiment to identify potentially important interactions that could then be investigated in more complex natural settings. Examination of interactions between anthropogenic factors and ecological factors may be particularly critical if we are to understand and minimize the detrimental effects of human activities on animal populations.

The two factors used in my experiments were chosen for specific reasons. I chose the leech, *M. decora*, as the predator in my experiments because it is common in ponds that amphibians frequent, it preys on amphibian embryos and

young tadpoles, and it is a parasite of adults (Howard 1980, Klemm 1990, Bendell and McNicol 1991). Given the obviously detrimental effects of predation, there also exists the possibility that predation risk induces adaptive plastic responses in embryos (Sih and Moore 1993, Warkentin 1995). Thus, although there is potential for *M. decora* to have substantial effects on amphibians, very little research has been done on the impacts of this leech (or other leeches) on amphibians. I chose low pH as an anthropogenic stress because although amphibian habitat can be naturally acidic, recent declines in pH of some ponds have been attributed to human activities (Freda and Dunson 1985). Embryos used in experiments were collected from circum-neutral ponds (pH 6.8-7.6), and, thus, exposing these embryos to low pH would represent the effects of anthropogenic acidification. Also important in my choice is that negative effects of low pH on amphibian embryos have been well studied, which enabled me to choose a pH for experiments that would cause sublethal stress to most individuals. Research on sublethal effects of low pH on embryos is relatively uncommon, but sublethal effects may be as important as lethal effects in determining population sizes and species assemblages (Werner 1991). I also chose to study effects of low pH and leech predation because lethal and sublethal interactive effects between predators and low pH may be expected because each factor can have effects on hatching success, hatch timing and hatch size of embryos (e.g., Pierce and Sikand 1985, Bradford et al. 1992, Sih and Moore 1993, Warkentin 1995) and because *M. decora* appear fairly resistant to low pH (Bendell and McNicol 1991, Grantham

and Hann 1994). By using two species of frog in experiments, I was able to determine if responses to pH and leech treatments were repeatable among *Rana*, a common genus of frogs in North America.

Methods

Natural history of prey and predator in relation to study site

The wood frog breeding season occurs early in spring (March - April) and lasts only a few days. Usually, all clutches in a pond (over 200 in this study) are laid within a very small area (ca. 1 m²) (Howard 1980). Each clutch has 2,000 - 3,000 eggs; development is completed within 11 to 18 days (Wright 1914). In contrast to wood frogs, green frogs have a fairly extended breeding season from late May to early August. Clutches are distributed around the margin of the pond (or lake). Clutch sizes range from 3,500 - 4,000 and hatching occurs quickly, only 3 - 5 days after eggs are laid (Wright 1914). Although new hatchlings are capable of swimming, they generally remain immobile by attaching to a solid substrate with secretions from adhesive glands. These glands, located on the ventral surface of the head, gradually disappear as the lips and denticles of the mouth form. Feeding begins a few days after hatching (Gosner 1960).

The predator used in my study was the leech, *M. decora*. These large leeches (up to 15 cm extended length) feed on embryos and hatchlings by attaching to a solid surface with their back sucker and grasping their prey with their front sucker. Tadpoles are consumed whole, and embryos are consumed

after the leech gains access to the embryo by slicing through the egg membrane with its jaws.

Experimental design and procedures

Experiments were conducted from April - June, 1996 at the Queen's University Biological Station (45° 34' N and 76° 13' W), in eastern Ontario, Canada. Frog embryos and leeches were collected from local ponds.

Rectangular tanks lined with polyethylene sheeting were arranged in an unheated, windowed laboratory. Tanks within a treatment condition were set at either one (green frogs) or two (wood frogs) heights above the floor and filled with 45 litres of well water 5 - 10 days before addition of embryos. Because embryonic development is highly dependent on temperature (Wright 1914, Berven et al. 1979), and differences in tank height might lead to differences in temperature, I measured temperature in each tank daily for the duration of experiments. pH of each tank was recorded daily using a CanLab pH meter. Sulphuric acid or potassium hydroxide was added to tanks when necessary to maintain a pH of 4.5 ± 0.05 (wood frogs) or 4.4 ± 0.05 (green frogs) for the low pH treatment. These pHs were chosen from published values of species' tolerances such that 70 - 90 % of the embryos were expected to survive the treatment (Freda 1986, Pierce 1985). The pH of "neutral" tanks was not altered; it averaged 8.10 (range 7.75 - 8.45) and 7.85 (range 7.18 - 8.10) across all tanks in the wood frog and green frog experiments, respectively. Except for small amounts

of water added to the tank with acid or base, water was not added or removed from tanks once the experiment was begun, to minimize disturbance to embryos.

I collected 10 clutches of wood frogs from a single pond on 17 April 1996 and six clutches of green frogs from two ponds between 11 June and 4 July 1996 (two clutches from one pond, four from another pond). Embryos were in early developmental stages (wood frogs: stage 7 - 8; green frogs: stage 4 - 9; Gosner 1960). For wood frogs, four groups of 60 (± 2) embryos were separated from each clutch and each group was randomly assigned to one of four treatment conditions in a 2 X 2 factorial design: 1) neutral pH, no leech predator (Control) 2) neutral pH, leech predator (Neu-Le) 3) low pH, no leech predator (Low-NoLe) and 4) low pH, leech predator (Low-Le). Thus, for wood frogs, there were 10 replicates of each treatment condition. Half of the embryos from each group were placed in a nylon mesh bag (ca. 25 x 15 x 20 cm) suspended in each tank, and half of the embryos were placed outside of the mesh bag. Embryos placed inside the mesh bag were protected from leeches (protected embryos), which allowed me to assess the effect of leech presence without losing these embryos to leech predation. For green frogs, groups of 50 (± 2) embryos (eight groups per clutch from the first two clutches - location one, four groups per clutch from the remaining four clutches - location two) were separated from each clutch and distributed randomly, but equally, among the four treatment conditions, for a total of eight replicates of each treatment condition. All 50 green frog embryos in each treatment condition were placed inside the mesh bag.

M. decora leeches were collected on several occasions from one pond and weighed on an AE 100 Mettler balance. Leeches were paired by mass (average difference of 0.1 g). Each pair was randomly assigned to a set of treatment conditions within 12 hours of embryo addition (beginning of experiment). For each set, one leech of the pair was placed outside of the mesh bag in the Neu-Le and Low-Le treatment conditions.

Embryos were checked for hatching every 12 hours. The proportion of the original number of embryos that hatched (hatching success) was calculated separately for protected and unprotected embryos. Thus, for unprotected embryos, hatching success could be influenced both by low pH and predation. To measure hatch length, I preserved the first 10 protected embryos that hatched in each tank in 10 % formalin and measured the total length of each embryo (to the nearest 0.25 mm) using a Wild dissecting microscope and a stage micrometer. Length was measured within a two day period approximately one month after preservation. I calculated the hatch timing for protected hatchlings as the time (to the nearest 12-hour period) from the beginning of the experiment to hatching for each of the first 10 embryos to hatch (to correspond to the first 10 embryos for which length was measured). In the wood frog experiment, I recorded the number of unprotected embryos that were eaten by the leech (Neu-Le and Low-Le conditions) to assess impacts of low pH on leech feeding and to assess whether embryo responses were dependent on leech feeding activity.

Statistical analyses

All analyses were conducted using SAS software (SAS Institute 1989). I used Type III sums of squares (SS) from 2X2 factorial analyses of variance to determine whether the pH or leech treatments affected hatch timing, hatch length or hatching success of wood frogs and green frogs. Clutch was used as a blocking variable to control for inter-clutch variability. I used the mean temperature each embryo (or group of embryos for hatching success) experienced over the course of the experiment as a covariate in wood frog analyses. The green frog experiment was designed to control for temperature differences within a clutch (by placing all treatment conditions within a set at the same height). There was no difference between mean tank temperatures within a clutch ($P = 0.97$), thus, I did not use temperature as a covariate in green frog analyses.

I began each wood frog analysis by including all 2 and 3-way interactions between the 2 main effects (low and neutral pH, presence/absence of a leech) and mean tank temperature, and pooled non-significant interaction terms with the error term if $F < 2 F_{50}$ (Paull 1950, Kleinbaum et al. 1988). For the green frog analyses, mean tank temperature was not included as a covariate, and thus, only one degree of freedom was used in interaction terms (pH-leech interaction). This term was left in the model regardless of the F -value.

A three-way interaction between the pH and leech treatments and the covariate (mean tank temperature) indicated that the pH and leech treatments interacted differently depending on temperature. When this occurred, I used

Tukey tests for pairwise comparisons among slopes (Zar 1984) to compare the responses to temperature in each of the 4 conditions. When a two-way interaction between the pH and leech factors was significant, I first adjusted means for the effect of the covariate (Huitema 1980) and then assessed the nature of the interaction using pairwise t-tests on adjusted means. I used the sequential Bonferroni procedure to correct *P*-values for multiple comparisons (Rice 1989). In comparing means or slopes, I conducted 5 pairwise comparisons, although 6 comparisons were possible. The comparison between the Neu-Le and the Low-NoLe conditions was not relevant to my study.

I also examined whether hatching success, hatch length and hatch timing of protected wood frog embryos related to the proportion of unprotected embryos eaten by leeches, using a series of regression analyses and controlling for differences in mean tank temperature, clutch and tank pH (i.e., Type III SS for proportion eaten). I conducted a separate one-way analysis of variance to examine whether tank pH explained variation in the proportion of embryos eaten by leeches.

Assumptions of analyses were checked by visual examination of residual plots and/or by testing for a significant linear relationship between the squared residuals versus predictor or predicted values (SAS Institute 1989).

Transformations were used where they were necessary.

Results

Lethal effects

I examined whether hatching success differed depending on pH or leech treatments both for unprotected and protected embryos. For unprotected wood frog embryos, hatching success was dependent on an interaction between pH and leech treatments ($F_{1, 26} = 5.17$, $P = 0.032$; Fig. 1.1). Multiple comparisons indicated that a feeding leech decreased hatching success regardless of the pH of the water (Control vs. Neu-Le and Low-NoLe vs. Low-Le; Table 1.1; Fig. 1.1). Low pH led to a decrease in hatching success only in the absence of a leech (Control vs. Low-NoLe); low pH had no significant effect in the presence of a leech (Neu-Le vs. Low-Le). Compared to the control, there was a 74 % decrease in hatching success when embryos were raised in low pH in the presence of a leech (Control vs. Low-Le; Table 1.1; Fig. 1.1; hereafter, all multiple comparisons resulting from pH-leech interactions are described in the same order as above). Compared to neutral pH, 46 % fewer wood frog embryos were eaten by leeches in low pH ($F_{1, 18} = 6.77$, $P = 0.018$).

Low pH decreased hatching success of protected wood frog embryos by 45 % and green frog embryos by 21 % (wood frogs: $F_{1, 26} = 35.41$, $P = 0.0001$; green frogs: $F_{1, 23} = 6.71$, $P = 0.016$; Fig. 1.2). The presence of a leech had no effect on hatching success for either frog species (both $P > 0.5$) and there was no interaction between the pH and leech treatments (both $P > 0.25$).

Non-lethal effects

Hatch length of wood frog embryos was dependent on a 3-way interaction between pH and leech treatments and mean tank temperature. Hatch length increased significantly with increasing temperature in the Neu-Le and Low-NoLe treatment conditions (both $P < 0.0005$), but was not affected by temperature in the Control and Low-Le treatment conditions (both $P > 0.05$). Pairwise comparisons among slopes indicated significant differences: only the Control vs. Low-Le comparison was non-significant ($P > 0.50$). Thus, I conducted two separate analyses of variance (using clutch as a blocking variable) to test for effects of the pH and leech treatments at temperatures higher than and lower than mean tank temperature (6.2 °C). As a preliminary, separate analyses (controlling for mean tank temperature and blocking by clutch) at each of the two temperature levels indicated no significant three-way interaction between the pH and leech treatments and temperature (both $P > 0.15$). Interpretation of the main effects (or two-way interactions) is thus possible within each of the two temperature levels.

At lower temperatures (5.58 - 6.18 °C), low pH led to a significant decrease in wood frog hatch length ($F_{1, 192} = 140.28$, $P = 0.0001$), whereas the leech treatment had no effect on hatch length ($P = 0.27$) and there was no interaction between the pH and leech treatments ($P = 0.63$).

At warmer temperatures (6.25 - 7.00 °C), there was a significant interaction between pH and leech treatments ($F_{1, 122} = 13.95$, $P = 0.0003$; Fig. 1.1). The presence of a leech led to a significant increase in hatch length compared to the

control (nearly 1/2 mm; Table 1.1; Fig. 1.1), whereas when embryos were raised in low pH, the presence of a leech did not influence hatch length. This indicates that the predator-induced increase in hatch size does not occur in low pH. Low pH led to a reduction in hatch length compared to neutral pH in both the absence of a leech (about 1/2 mm) and presence of a leech (about 1 mm). The combined effect of both low pH and the presence of a leech resulted in significantly smaller embryos compared to the control (2/3 mm; Table 1.1; Fig. 1.1).

Qualitatively similar results were obtained for green frogs: hatch length was dependent on an interaction between pH and leech treatments ($F_{1, 279} = 8.96$, $P = 0.003$; Fig 2). Again, embryos raised in neutral water hatched at a larger size in the presence of a leech (1/5 mm) and this predator-induced plasticity was lost in low pH water (Table 1.2; Fig. 1.2). In contrast to wood frogs, low pH did not influence hatch length compared to the control, however, embryos raised in the presence of a leech hatched at smaller size in low pH. Embryos raised in the control did not differ in size from those raised in low pH with a leech present (Table 1.2; Fig. 1.2).

For both frog species, timing of hatching was influenced by the pH and leech treatments. For wood frogs, hatch timing was influenced by a significant pH-leech interaction ($F_{1, 317} = 8.87$; $P = 0.0031$; Fig. 1.1). Hatch timing was not significantly altered by the presence of a leech in either neutral or low pH (Table 1.1; Fig. 1.1). Timing of hatching was not influenced by low pH alone, but in the

presence of a leech, low pH resulted in earlier hatching. Embryos raised with a leech in low pH hatched about 8/9 of a day sooner than control embryos (Table 1.1; Fig. 1.1). A significant interaction between mean tank temperature and pH ($P = 0.009$) suggested that although the time to hatch was negatively correlated with temperature, this relationship was stronger in low pH.

For green frogs, hatch timing of embryos also was influenced by an interaction between the pH and leech treatments ($F_{1, 286} = 15.27$, $P = 0.0001$; Fig. 1.2). Embryos raised in neutral water delayed hatching in the presence of a leech by about 1/5 of a day, but those raised in low pH did not delay hatch timing in response to a leech, again indicating loss of plasticity in low pH (Table 1.2; Fig. 1.2). In contrast to wood frogs, low pH led to an approximate 1/2 day delay in hatching when embryos were raised without a leech, but when a leech was present, there was no effect of pH treatment on hatch timing. Hatching occurred about 1/3 day later when embryos were raised in low pH with a leech present compared to the control (Table 1.2; Fig. 1.2).

The proportion of unprotected wood frog embryos eaten by leeches had no effect on protected embryo hatching success, hatch length or hatch timing (Table 1.3).

A summary of the main results is presented in Table 1.4.

Table 1.1. The proportion of wood frog embryos hatched, hatch length and hatch timing were influenced by interactions between the pH and leech treatments. Shown are *P*-values associated with multiple comparisons among the four treatment conditions: neutral pH-no leech present (Control), neutral pH-leech present (Neu-Le), low pH-no leech present (Low-NoLe) and low pH-leech present (Low-Le). Numbers in bold type are significant after sequential Bonferroni correction.

Comparison	Proportion Hatching	Hatch Length (mm)	Hatch Timing (# 1/2 day periods)
Control vs. Neu-Le	0.0001	0.0001	0.0613
Low-NoLe vs. Low-Le	0.0015	0.2314	0.0213
Control vs. Low-NoLe	0.0126	0.0001	0.0857
Neu-Le vs. Low-Le	0.5950	0.0001	0.0001
Control vs. Low-Le	0.0001	0.0001	0.0001

Table 1.2. Green frog hatch length and hatch timing were influenced by interactions between the pH and leech treatments: the *P*-values resulting from multiple comparison tests are shown below (see Table 1.1 for comparison abbreviations). Numbers in bold type are significant after sequential Bonferroni correction.

Comparison	Hatch Length (mm)	Hatch Timing (# 1/2 day periods)
Control vs. Neu-Le	0.0036	0.0007
Low-NoLe vs. Low-Le	0.1800	0.0320
Control vs. Low-NoLe	0.8236	0.0001
Neu-Le vs. Low-Le	0.0001	0.1452
Control vs. Low-Le	0.2651	0.0001

Table 1.3. ANCOVAs examining whether responses of wood frog embryos protected from leech predation were dependent on the proportion of unprotected wood frog embryos eaten by leeches*.

Response	F_{eaten}	df_{error}	$P\text{-value}$
Proportion Hatching	0.01	7	0.9379
Hatch Length	1.03	146	0.3127
Hatch Timing	0.61	148	0.4367

* Type III SS for proportion eaten were examined after blocking by wood frog egg clutch and controlling for differences in mean temperature and pH between tanks.

Table 1.4. Summary of responses of the two frog species to the leech and pH treatments. Protected embryos were safe from leech predation inside a mesh bag; unprotected embryos were directly exposed to leech predation.

Species	Response	Leech	pH	pH-Leech Interaction
Wood Frog				
Protected	hatching success	no	↓	no
	hatch length (warmer temp.)	↑	↓	no response to leech in low pH
	hatch timing	no	no	earlier hatching when pH and leech combined
Unprotected	hatching success	↓	↓	leech-pH effect predicted by leech effect

Species	Response	Leech	pH	pH-Leech Interaction
Green Frog				
Protected	hatching success	no	↓↑	no
	hatch length	↑↑	no	no response to leech in low pH
	hatch timing	↑↑	↑↑	no response to leech in low pH

Figure 1.1. Mean (± 1 SE and 1.96 SE) proportion of wood frog embryos hatched, hatch length (for embryos raised above 6.2 °C, see text) and hatch timing. The four conditions in which embryos were raised were neutral pH-no leech (Control), neutral pH-leech present (Neu-Le), low pH-no leech present (Low-NoLe) and low pH-leech present (Low-Le). Proportion of embryos hatched is shown for embryos raised in contact with the leech predator (unprotected embryos). The means and SE's were back-transformed from the following transformations: proportion hatching, arcsine $\sqrt{X}$; hatch length, $(X)^3$; hatch timing, $1/\sqrt{X}$.

Figure 1.2. Mean (± 1 SE and 1.96 SE) proportion of green frog embryos hatched (embryos were protected from predation; responses of protected wood frog embryos were similar), hatch length and hatch timing. See Fig. 1.1 for treatment condition abbreviations. Means and SE's were back-transformed from the following transformations: proportion hatching, arcsine $\sqrt{X}$; hatch length, no transformation; hatch timing, $1/(X)$.

Discussion

Lethal effects of leech and low pH treatments

Both leech predation and low pH led to decreased hatching success of embryos. In neutral water, a feeding leech decreased hatching success of unprotected wood frog embryos by 79 %. Thus, a single leech can easily consume a significant proportion of 30 embryos. Further evidence of the voracity of *M. decora* is provided by Howard (1978) who found that on average, *M. decora* consumed 64 % of embryos in bullfrog (*R. catesbeiana*) egg masses ($n = 8$ egg masses with 12,000 - 20,000 embryos per egg mass, Wright 1914) and Cory and Manion (1953) reported that *M. decora* virtually destroyed a population of wood frog embryos (the number of egg masses was not reported). These observations suggest that leeches can be important sources of embryonic mortality in natural situations.

A reduction in hatching success due to low pH is well established (Gosner and Black 1957, Freda 1986). In this study, both species suffered reductions in hatching success from low pH, although wood frog embryos were more severely affected by the low pH used in experiments. The effect of low pH for wood frog embryos raised outside the mesh bag varied depending on whether a leech was present. There was a reduction in hatching success for embryos raised in low pH without a leech. However in the presence of a leech, there was no additional reduction in hatching success due to low pH because hatching success was already very low. Thus, hatching success for this species given low pH and direct

contact with a leech was predictable from the effect of the predator alone, but not from the effect of low pH alone.

I found that leeches ate fewer unprotected wood frog embryos in low pH compared to leeches in neutral water, which may indicate that leeches were somehow adversely affected by low pH. However, the low pH treatment was not harmful enough to cause death, even when leeches were maintained in low pH for over 20 days. Reduced feeding in low pH could also result if embryos exposed to acidity are less palatable than those raised in neutral water. To my knowledge, this has not been tested. Although fewer embryos were eaten by leeches in low pH, embryonic mortality in this treatment condition was also caused by low pH. Thus, embryos raised with a leech in low pH suffered a reduction in hatching success that was comparable in magnitude to the reduced hatching success of embryos raised with a leech in neutral pH.

Non-lethal effects of leech predator

To my knowledge, plastic developmental responses by amphibian embryos to a predator have been documented in only two previous studies (Sih and Moore 1993, Warkentin 1995). In my study, embryos of both species hatched at larger size in the presence of a leech predator. Green frog embryos also significantly delayed hatching in response to the predator. Such predator-induced developmental plasticity is assumed to be adaptive (Smith-Gill 1983). For example, research indicates that larger hatchlings are less susceptible to

predation than smaller hatchlings (Jung and Jagoe 1995, Warkentin 1995), potentially because larger size allows for faster, more agile swimming (Brown and Taylor 1995, Jung and Jagoe 1995). This advantage may exist for wood frog and green frog hatchlings. The delayed hatching of green frog embryos may allow these embryos to grow relatively safely to larger size within the protective embryonic membrane and surrounding jelly matrix (e.g., Howard 1978, Duellman and Trueb 1986) although the relative vulnerabilities of embryos versus hatchlings remains to be tested.

Plastic responses are maintained within a population when fitness gains associated with the altered phenotype outweigh some of the fitness costs when predators are present, but not when they are absent (Harvell 1990). Thus, it may be expected that larger hatch size or delayed hatching (green frogs) carry costs in the absence of predation. Costs associated with increased hatch length may exist because there is a finite amount of materials and energy in an egg for growth, differentiation, maintenance and storage (Perrin and Sibly 1993, see also McCollum and Van Buskirk 1996). Increasing hatch size presumably leaves fewer resources for storage or differentiation. Hatchlings are sustained by yolk reserves; they do not feed during the first few days of life while their mouthparts undergo modification (Gosner 1960). Thus, hatchlings that exhaust stored reserves may go hungry until they are capable of feeding, and this may reduce the probability of survival. With respect to differentiation, a developmental trade-off between tail size (correlated with swimming ability, Parichy and Kaplan 1995), and

development of feeding structures (e.g., viscera, mouthparts) may enhance predator avoidance capability at the cost of decreased feeding efficiency (e.g., Smith and Van Buskirk 1995, K.M. Warkentin, personal communication). Changes in body proportions supporting a resource-allocation trade-off under the risk of predation have been shown in the growth of tadpoles (*Agalychnis callidryas*, K.M. Warkentin, personal communication) and fish (*Carassius carassius*, Brönmark and Miner 1992), and may apply for the two ranid frogs in my study. The predator-induced hatching delay for green frogs also may carry costs in the absence of a predator: as suggested by Sih and Moore (1993), later-hatchers may be unable to escape the confines of the egg in time to avoid such hazards as predation, desiccation, fungal infection or low oxygen concentration (Licht 1974, Petranka et al. 1982, Beattie et al. 1991, Warkentin 1995).

Importantly, plastic responses of embryos were apparent regardless of whether leeches were allowed to feed on embryos (wood frog vs. green frog experiments), and were not affected by the feeding activity of the leech in the wood frog experiment. This suggests that embryos can respond to the mere presence of a predator (e.g., Petranka et al. 1987), as opposed to requiring predator-feeding cues (e.g., Cowl and Covich 1990) and that in the wood frog experiment, the degree of response was not dependent on the number of embryos eaten.

Non-lethal effects of low pH

In contrast to the presumed adaptive plastic responses to a predator, plasticity in hatch length or timing in response to a toxin such as low pH is not necessarily adaptive, and may be maladaptive. This is because in low pH an embryo may have no alternative but simply to respond passively to the environment: physiologically, it may be unable to respond otherwise (phenotypic modulation, Smith-Gill 1983). Wood frog embryos hatched at smaller size in low pH; this appears to be a typical response to low pH (Beattie and Tyler-Jones 1992, Bradford et al. 1992, Griffiths and DeWijer 1994). These smaller hatchlings may be more vulnerable to predators or less able to compete effectively. In contrast, hatch size of green frog embryos was not altered by low pH. My results suggest that green frog embryos may have compensated for the effects of low pH on hatch length by altering hatch timing. The lack of a similar delay in hatch timing in low pH by wood frogs may reflect an inability of these embryos to respond physiologically at the low pH used in this experiment or could indicate differential mortality of later-hatchers in low pH.

Interactive effects

For wood frogs, I found interactions of the pH and leech treatments with temperature. At warmer temperatures only, hatch length of wood frog embryos was affected by the presence of a leech. Similarly, while wood frog hatch length was not affected by temperature in the control, the presence of a leech led to

increased hatch length with increasing temperature. Both of these results suggest that wood frog response to leeches increases with temperature, and the relatively narrow range of temperature over which these responses occur may indicate that a certain threshold temperature is required for induction of plastic responses. The mechanism of this response is unknown, but there may be a lack of flexibility at cooler temperatures if all available energy is channelled into maintenance (= survival) (Perrin and Sibly 1993).

Importantly, predator-induced plasticity in hatch length and hatch timing did not occur when embryos were raised in low pH. The mechanism of the altered response to the combined factors compared to the factors in isolation is unknown. However, fish and crayfish exhibit a decreased ability to respond appropriately to chemosensory cues in low pH, potentially because of increased mucous secretions (Lemly and Smith 1987, Allison et al. 1992). A mechanism similar to this may explain the embryos' apparent lack of response to the leech predator in low pH. The lack of a presumably adaptive trait under conditions of pH stress can represent a sublethal effect of human-induced acidity on these species and could have important consequences for individual fitness and for amphibian populations.

It is clear that lethal and sublethal interactions among community processes and anthropogenic factors such as low pH are common, but that they will not necessarily be obvious from single-factor effects. One type of interaction that can occur is when each factor alone has an effect compared to the control,

but the combined response is predicted by the response to only one of the two effects in isolation. Fauth (1990) found that the response of *Hyla* tadpoles to newt (*Notophthalmus viridescens*) and crayfish (*C. bartoni*) predators was predicted by the response to newts alone. I found this type of interaction for unprotected wood frog hatching success (leech alone) and wood frog hatch length (warmer temperatures; pH alone). These results demonstrate that even within a simple system, the factor that is predictive of the interactive effect changes depending on the response being examined.

Another type of interaction occurs when, in isolation, only one factor has an effect compared to the control but when the two factors are combined, this effect is "lost" (i.e., the combined effect is not different from the control). For example, only leech presence induced changes in green frog hatch length; low pH had no effect on hatch length. However, when both of these factors were combined, hatch length was indistinguishable from that of control hatchlings. For the sublethal traits measured in my experiment, both of the above interaction types indicated a loss of phenotypic plasticity in low pH.

A third type of interaction can occur whereby neither factor has an effect relative to the control, but when both factors are considered together, there is a significant effect. I found this type of an interaction for wood frog hatch timing: neither low pH nor the presence of a leech had a significant effect in isolation, but when combined, embryos hatched sooner compared to the control. Carey (1993) proposed an interaction of this sort whereby a sublethal "stress" acts to

cause immunosuppression, leading to a fatal attack by the normally benign bacteria, *Aeromonas*. Long et al. (1995) described this type of interaction between low pH and UV-B radiation for hatching success of leopard frogs. All of the interactions described above have the potential to have serious detrimental effects on amphibian populations. Study of sublethal interactions within amphibian communities warrants further investigation in light of recent awareness that amphibian populations may be declining globally and that isolation of single causative agents for mortality is proving difficult (Wake 1991, Blaustein et al. 1994).

CHAPTER 2

Frog abundance in relation to pond pH and leech abundance

Introduction

Recent concern over potential declines of amphibian populations has focused attention on factors that affect amphibian distribution and abundance. Many species exhibit strong natal philopatry (Oldham 1967, Dole 1968, Blaustein et al. 1994), and thus, factors that cause a decrease in survival of embryos, tadpoles or adults may be expected to limit local population sizes (Wilbur 1980). Less obviously, abundance and distribution of amphibians also may depend on factors that impose some stress on the animals without directly causing mortality (e.g., low food supply, sublethal pH, parasitism). This stress may alter the outcome of competitive or predator-prey interactions, which may subsequently affect a species' distribution or its local abundance (Warner et al. 1993, Clark 1986).

The distribution and abundance of amphibians has been negatively associated with predators such as fish or larval dragonflies (Wilbur 1980, Werner and McPeck 1994). Leeches also may negatively affect amphibian populations given that several species of leeches are predators, parasites and /or vectors for

parasites of amphibians (Duellman and Trueb 1986, Davies 1991, Siddal and Dessler 1992); leeches also can be very common in the ponds that amphibians frequent (Sawyer 1986, pers. obs.). For example, *Desserobdella picta* is a small parasitic leech that can kill a *Bufo americanus* tadpole in only a few days (Brockelman 1969) and parasitism by this leech also may reduce the fitness of adult amphibians (Gill 1978). *D. picta* also acts as a vector for the blood parasite *Trypanosoma diemyctyli* in the newt, *Notophthalmus viridescens* (Gill and Mock 1985) and these trypanosomes are thought to be an important selective force in newt populations (Gill and Mock 1985). Another leech, *Macrobdella decora*, is common in ponds of North America (Davies 1991). This leech preys on amphibian embryos and newly hatched tadpoles, and parasitizes adult amphibians (Howard 1980, Klemm 1990, pers. obs.). Although effects of *M. decora* on older tadpoles or adults are unknown, predation by *M. decora* on embryos or young tadpoles can significantly reduce clutch survival (Howard 1980). Despite this, the impact of leeches such as *D. picta* or *M. decora* on amphibian populations is unknown. I examined the relationship between frog abundance and leech abundance to determine whether effects of leeches might translate into negative effects on frog populations.

I also examined the possibility that relationships between frog abundance and leech abundance were dependent upon pond pH. Such interactive effects are expected to be common in amphibian communities (Van Buskirk 1988, Sadinski and Dunson 1992). For example, Warner et al. (1993)

found that the outcome of interspecific competition among two frog species was affected by low pH. An examination of competition at one level of pH would not fully describe this interactive relationship. Similarly, potential interactive effects of low pH with *M. decora* predation may be expected based on the known negative effects of low pH on amphibians (Freda 1986) and the tolerance of *M. decora* of acidic conditions (Grantham and Hann 1994). In addition, interactive effects may be expected because low pH can alter the effects of leeches on frog phenotypes, and because mortality caused by *M. decora* can obscure the negative effect of low pH on frog hatching success (Chapter 1).

In this study, I examined abundance of bullfrogs (*Rana catesbeiana*), green frogs (*R. clamitans*) and leopard frogs (*R. pipiens*) in relation to abundance of *M. decora* leeches and pond pH in eastern Ontario, Canada. These frog species were the most common frogs encountered during summer sampling (see Results) and, as mentioned, this common leech species is a known predator of frog eggs and tadpoles and parasite of adults. For each frog species, I tested for possible interactive effects of pond pH and *M. decora* abundance. Additionally, I tested for an effect of two common species of leech, *Erpobdella punctata* and *Placobdella ornata*, and their possible interactions with pond pH, on the abundance of bullfrogs, green frogs and leopard frogs. Tests using these latter two leech species were exploratory and not based on any expectations of relationships because little is known about effects of these leeches on amphibians. *E. punctata* is a potential predator of embryos and tadpoles, and *P. ornata* is a parasite of

adults (Klemm 1990, Siddal and Desser 1992).

Methods

Data collection

Ponds surveyed were within 8 km of the Queen's Biological Station (45° 34' N; 76° 13' W) in eastern Ontario (Figure 1). In each of two years, frog abundance was surveyed once at each study pond in the summer (late June - early August). Surveys were conducted in 1995 (13 ponds) and 1996 (18 ponds). Before sampling each pond for frogs, a starting point was chosen within presumably suitable frog habitat (i.e., rocky or otherwise non-vegetated shorelines were avoided), and a continuous line of 10, 20-m transects was marked along the shoreline. At some smaller ponds, fewer than 10 transects were laid out (minimum $n = 3$ transects). On sunny warm days (>20 °C), transects were walked slowly. Adult frogs which commonly bask along the shoreline were identified to species and counted within 1.5 m of either side of the transect (Crump and Scott 1994). To maximize sightings and accurate identification of species, transects were walked by two people: the first person watched for frogs, and the second recorded data. For each year, I obtained an estimate of frog abundance in each pond by determining the mean number of frogs of each species per transect.

Leech abundance was surveyed at each pond within 33 (1995) or 20 (1996) days of the frog surveys. I used funnel traps (a maximum of six traps /pond in 1995 and eight traps /pond in 1996) spaced 30-m apart (Bendell and

McNicol 1991). If the pond was too small for the full number of traps, a smaller number was deployed (minimum $n = 3$ traps). Traps were placed in the water along the same portion of the pond used for frog transects. Each trap consisted of a 1-L glass mason jar fitted with an inverted plastic funnel. The narrow end of the funnel was 1 cm in diameter. Each trap was baited with $30 (\pm 2)$ g beef liver, filled with water and submerged to rest horizontally at depth of 0.5 m for $24 (\pm 1)$ hours. An air bubble was left in each trap to allow oxygen exchange. In the laboratory, I killed trapped leeches using 50 % ethanol (Pennak 1953) and immediately identified leeches to species using a dissecting microscope. To estimate leech abundance in each pond in each year, I determined the mean number of each leech species per trap.

Water pH was measured using a CanLab pH meter. pH was sampled concurrently with frog surveys. Average pH was calculated for each pond based on an average of two laboratory- (1995) or three field-determined (1996) pH values. Each sample was taken from a separate location within each pond (≥ 30 m apart).

Statistical analyses

I used Type III sums of squares (SS) from regression analyses to investigate the effect of leech abundance, average pond pH and year of sampling on bullfrog, green frog and leopard frog abundance. Nine separate analyses were conducted: one for each of the three frog species with each of the three most common leech

species (*M. decora*, *E. punctata*, *P. omata*; other leech species were relatively uncommon and were excluded from analyses). I was primarily interested in the three regression models involving *M. decora* because this leech is a known predator and parasite of frogs. The analyses involving the other two leech species were exploratory in nature, and, accordingly, I chose to be conservative in interpretation of these results.

For each model, I included all interactions among independent variables to investigate a potential dependence of frog abundance on the joint effects of two or more variables. These interaction terms were removed from the model (i.e., pooled with unexplained variation) if $F < 2F_{50}$ (Paull 1950) using step-down procedures (Aiken and West 1991). This method has the advantage of increasing power when sample size is relatively low. By employing a more stringent condition for dropping interaction terms (i.e., simple lack of significance at $P < 0.05$ did not necessarily mean a term was dropped), I was able to lower the chance of Type II error (Jaccard et al. 1990). Pooling was not employed in two-factor models (only one interaction term) because there was a potential gain of only one degree of freedom even though the risk of making a Type II error remained. Where pooling was employed, only reduced models are presented.

Collinearity is a problem in regression models containing interaction terms because an interaction term (AB) is often highly correlated with the original independent variables (A, B). When investigating the significance of individual terms (i.e., Type III SS), collinearity can reduce the probability of finding statistical

significance, even if the term(s) is actually important (Jaccard et al. 1990).

Centering of the original independent variables (i.e., adjusting the mean of each independent variable to zero) can substantially reduce collinearity between independent variables and their interaction terms (Neter et al. 1989, Aiken and West 1991). Thus, all continuous independent variables were centered before interaction terms were generated.

Two additional sets of analyses were used to test for the presence of potential confounds in determining whether frog abundances were related to leech abundance or pond pH. I examined the effect of pond pH on *M. decora* abundance. A significant relationship (i.e., a correlation among "independent" variables used in subsequent analyses) would indicate that the effects of leech abundance on frog abundance were inseparable from the effects of pond pH.

I also addressed potential spatial dependence among data. Spatial dependence could exist if ponds located close together were more similar in species abundances or pH than ponds far apart. For example, some other factor such as vegetation or soil type that affects the variables of interest may occupy a region that extends over >1 pond rather than a over a single pond (cf. Odland 1988). Each non-independent pond would then contribute a degree of freedom to statistical tests, whereas each should be represented by some fraction of a degree of freedom (cf. Hinch et al. 1994), thereby leading to an increased chance of detecting a significant relationship between two variables when, in fact, no relationship exists (Type I error, Legendre and Fortin 1989). I assessed potential

lack of independence by categorizing ponds into six groups based their relative locations (Fig. 2.1) and testing for differences among groups of ponds in abundances of bullfrogs, green frogs, leopard frogs, *M. decora* and average pond pH, after controlling for sampling year. Significant differences among groups of ponds for a variable would indicate some degree of dependence. However, if only one variable is affected by spatial dependence and another is not, correlations should not suffer an increased risk of Type I error. If there is an indication of spatial dependence for both variables, then correlations between variables may occur due to an over-representation of the relationships between these variables with a third, spatially patchy factor.

In all analyses, assumptions of homogeneity of residual variance and normality were checked through visual examination of residual plots and by checking for a significant linear relationship between squared residuals versus independent variables or predicted values (SAS Institute 1989). Transformations of response variables were conducted as necessary.

Results

Frog abundance, leech abundance and pond pH

Green frogs were the most abundant species at ponds in 1995, but were approximately equal in abundance with bullfrogs in 1996; leopard frogs were the third most abundant species in both years (Table 2.1). Other frog species were infrequently encountered during transect samples (*Bufo americanus*, one pond in

1995; *Hyla versicolor*, one pond in 1996; *Rana palustris*, three ponds in 1995; *R. sylvatica*, one pond in 1995, one pond in 1996). Summer shoreline transect sampling may not be an effective method for assessing abundances of these other species. Pond pH averaged just above neutral in both years; the lowest pHs were marginally acidic (Table 2.1). Unlike other leech species, *M. decora*, *E. punctata* and *P. omata* were present in traps in both years (Table 2.1, Appendix 1). *E. punctata* was the most abundant leech in 1995 traps, whereas *P. omata* was most abundant in 1996 (Table 2.1).

Tests for potential confounds

I first tested for a relationship between *M. decora* abundance and pond pH, controlling for sampling year. *M. decora* abundance was not related to pond pH ($F_{1,27} = 2.29$, $P = 0.14$), nor did the relationship between *M. decora* abundance and pond pH differ between the two summer seasons (i.e. pH-year interaction term, $F_{1,27} = 2.88$, $P = 0.10$). Thus, *M. decora* and pond pH were uncorrelated when used as independent variables in subsequent analyses.

I next tested for independence of ponds. There was no significant difference in bullfrog or green frog abundances among groups of ponds, and the group-year interaction terms were not significant (Table 2.2). However, leopard frog abundance was affected by a group-year interaction (Table 2.2). I determined the effect of pond grouping on leopard frog abundance in each of the two years by conducting separate one-way analyses of variance for each year.

Leopard frog abundances were significantly different among pond groups in 1995 but not in 1996 ($F_{1,6} = 33.45$, $P = 0.0003$; $F_{1,9} = 0.67$, $P = 0.65$, respectively). *M. decora* abundances were marginally significantly different among groups (Table 2.2). There were no pH differences among pond groups, nor was the interaction term significant (Table 2.2). Thus, there is indication of spatial dependence for some of the variables used in analyses. Spatial dependence for both leopard frog abundance (1995) and *M. decora* abundance may be especially problematic because apparent relationships between these variables may be due to their independent relations with some third, patchily-distributed factor. This problem will be re-considered in interpreting any significant correlations between these two variables.

Frog abundance in relation to M. decora abundance and pond pH

Bullfrog abundance was not related to pond pH or *M. decora* abundance; however, more bullfrogs were counted during transect samples in 1995 compared to 1996 (Table 2.3). Bullfrog abundance was not dependent on interactions among these variables (Table 2.3). Green frog abundance also was higher in 1995 compared to 1996, and, additionally, green frog abundance decreased as *M. decora* abundance increased ($B_1 = 0.148$, transformed response: $1 / \sqrt{(\text{green frog abundance} + 0.5)}$) (Table 2.3). Thus, fewer green frogs were counted with increasing number of leeches trapped. No interaction terms were significant. More leopard frogs were counted at transects in 1995 than in 1996. Leopard frog

abundance was predicted by an interaction between year and *M. decora* abundance (Table 2.3). I determined the effect of leech abundance on leopard frog abundance in each of the two years by conducting separate regression analyses for each year. There was no significant relationship between leopard frog abundance and *M. decora* abundance in either year, although in 1995 there was a nearly significant positive relationship between the two variables (1995: $F_{1,11} = 4.11$, $P = 0.068$; 1996: $F_{1,17} = 0.14$, $P = 0.71$).

In summary, neither bullfrog nor leopard frog abundance was related to *M. decora* abundance. However, green frog abundance decreased with increasing *M. decora* abundance. There were no direct effects of pond pH on any of these three frog species, and the relationships between frog abundance and *M. decora* abundance were not dependent on pond pH.

Frog abundance in relation to E. punctata abundance

Bullfrog abundance was not related to the abundance of *E. punctata*, nor were any interaction terms significant (Table 2.4). Green frog abundance was dependent on a three-way interaction between *E. punctata*, pond pH and sampling year (Table 2.4). In 1996, there was no relationship between green frog abundance and the independent variables ($F_{3,14} = 0.08$, $P = 0.97$). In contrast, 1995 sampling indicated that, at average pH, green frogs were less abundant when *E. punctata* were more abundant ($B_1 = 0.39$, transformed response:

$1/\sqrt{(\text{green frog abundance} + 0.5)}$), but that this relationship also was dependent on an interaction between average pH and *E. punctata* abundance ($F_{1,9} = 5.12$, $P = 0.05$).

Further post hoc tests were conducted for the 1995 data to examine the nature of the pond pH-*E. punctata* interaction for green frogs. I used simple slope analysis at three values of pH (mean pH, one standard deviation below mean pH, one standard deviation above mean pH, Jaccard et al. 1990, Aiken and West 1991). The negative relationship between green frog abundance and *E. punctata* abundance was significant at mean pH (uncentered mean pH = 7.66, $F_{1,9} = 5.41$, $P = 0.045$) and at one standard deviation (SD) above the mean (back-transformed mean pH + 1 SD = 8.46, $F_{1,9} = 5.28$, $P = 0.047$) but not significant at one standard deviation below the mean (back-transformed mean pH - 1 SD = 6.85, $F_{1,9} = 3.80$, $P = 0.083$). These results suggest that in 1995, green frog abundance decreased with increasing *E. punctata* abundance overall, and particularly at higher pH (7.66, 8.46); at lower pH (6.85), the relationship was not significant. I have no explanation for these apparent differences within this relatively narrow range of pH. Further tests, with perhaps higher and lower pHs, would be useful to determine whether these relationships indicate general pH-dependent trends.

Leopard frog abundance also was explained by a three-way interaction between *E. punctata* abundance, pond pH and sampling year (Table 2.4). Separate analyses for each year indicated no relationships of *E. punctata*

abundance or pond pH with leopard frog abundance. However, the two years were quite different from each other: in 1996, all terms were highly non-significant ($p > 0.50$), but in 1995, all terms in the model had a significance level near to 0.10 (e.g., Type III SS for *E. punctata*: $F_{1,9} = 3.26$, $P = 0.10$; $B_1 = -1.78$, transformed response = $1/(\text{leopard frog} + 0.5)^2$).

Frog abundance in relation to P. ornata abundance

Neither bullfrog abundance nor green frog abundance was related to the abundance of *P. ornata*, nor were any interaction terms significant (Table 2.5). I was unable to find a transformation of leopard frog abundance that would satisfy the assumption of homogeneity of variance when testing for effects of *P. ornata* on leopard frog abundance. However, by removing the sampling year term from the model, all assumptions were satisfied (removal of only the pH term was initially preferred because pond pH did not influence frog abundance in any previous model, but this did not eliminate the heterogeneity of variance). Thus, for this analysis, any relationship between independent and dependent variables that was dependent upon year was not assessed. This modified model showed no relationships between leopard frog abundance and *P. ornata* abundance, nor was the *P. ornata*-pond pH interaction term significant (Table 2.5).

Table 2.1. Descriptive statistics for each of the variables used in analyses (upper value based on 13 ponds in 1995; lower value based 18 ponds in 1996). Within each pond, mean pH was calculated based on a mean of two (1995) or three (1996) samples taken at the time of frog censusing. For each frog species (bullfrog, green frog, leopard frog), abundance at each pond was determined from the mean number of individuals /10 m transect /pond (usually $n = 10$ transects/ pond, see text for exceptions). Mean leech (*M. decora*, *E. punctata*, *P. ornata*) abundance for each pond was determined from the mean number /trap /pond (usually $n = 6-8$ traps /pond, see text for exceptions).

Variable	Mean	Minimum	Maximum	Std. Deviation
pH	7.65 7.36	6.55 5.78	9.50 9.85	0.80 1.01
Bullfrog	2.88 0.24	0.00 0.00	16.30 1.10	4.39 0.33
Green Frog	13.43 0.20	0.20 0.00	60.60 1.25	21.50 0.30
Leopard Frog	0.96 0.04	0.00 0.00	5.50 0.50	1.58 0.12
<i>M. decora</i>	0.24 0.31	0.00 0.00	1.67 2.00	0.47 0.52
<i>E. punctata</i>	1.65 0.93	0.00 0.00	19.00 8.38	5.24 2.52
<i>P. ornata</i>	0.09 1.11	0.00 0.00	0.33 7.33	0.13 2.25

Table 2.2. Analysis of variance tests for spatial dependence among ponds for each of the variables used in analyses (frog abundances, *M. decora* abundance, pond pH), after controlling for sampling year (1995, 1996). Ponds were separated into six spatial groups (Fig. 1.1), and these groups were used as a class variable in analyses. Significant differences indicate that the average variability within pond groups is small compared to the variability between pond groups, and that spatial dependence among ponds may exist. Interaction terms are denoted by a dash between terms. The following transformations were used in analyses: bullfrog, $1/(X + 0.5)$; green frog, $1/\sqrt{(X + 0.5)}$; leopard frog, $1/(X + 0.5)^2$; *M. decora*, $1/(X + 0.5)^2$; pond pH, ranked (Zar 1984).

Response	df_{error}	Independent Variable	F	P
Bullfrog	15	Pond Group	2.76	0.058
		Year	14.89	0.0015
		Pond Group-Year	0.97	0.47
Green Frog	15	Pond Group	2.21	0.11
		Year	48.03	0.0001
		Pond Group-Year	2.28	0.099

Table 2.2 (cont.)

Response	df_{error}	Independent Variable	F	P
Leopard Frog	15	Pond Group	13.86	0.0001
		Year	29.81	0.0001
		Pond Group-Year	6.95	0.0015
<i>M. decora</i>	15	Pond Group	3.17	0.038
		Year	0.19	0.67
		Pond Group-Year	1.05	0.42
Pond pH	15	Pond Group	1.10	0.40*
		Year	0.48	0.50*
		Pond Group-Year	0.35	0.87*

* Similar results were obtained using the χ^2 approximation of the Kruskal-Wallis H-statistic (appropriate for ranked data; Zar 1984).

Table 2.3. Overall model and Type III sums of squares from regression analyses testing for a relationship between frog abundance (mean number /transect /pond) and the independent variables: pond pH, *M. decora* abundance (mean number / trap /pond) and sampling year (1995, 1996). The continuous variables, pond pH and *M. decora*, were centered around their means prior to analyses and all interaction terms were initially included in the model, but hierarchically removed if $F < 2F_{50}$ (see text). The final model is shown, and interaction terms are denoted by a dash between independent variables. The following transformations were used in analyses: bullfrog, $1/(X + 0.5)$; green frog, $1/\sqrt{(X + 0.5)}$; leopard frog, $1/(X + 0.5)^2$.

Response	df_{error}	Factor(s)	F	P	R^2
Bullfrog	26	Overall Model	4.44	0.0072	0.41
		pH	0.96	0.34	
		<i>M. decora</i>	0.05	0.83	
		Year	16.71	0.0004	
		<i>M. decora</i> -Year	1.17	0.29	

Table 2.3 (cont.)

Response	df_{error}	Factor(s)	F	P	R^2
Green Frog	25	Overall Model	17.80	0.0001	0.78
		pH	2.33	0.14	
		<i>M. decora</i>	8.99	0.006	
		Year	64.11	0.0001	
		pH-Year	1.29	0.27	
		<i>M. decora</i> -Year	1.74	0.20	
Leopard Frog	23	Overall Model	2.63	0.037	0.45
		pH	0.80	0.38	
		<i>M. decora</i>	5.13	0.03	
		Year	8.93	0.007	
		pH- <i>M. decora</i>	1.79	0.19	
		pH-Year	1.61	0.22	
		<i>M. decora</i> -Year	5.61	0.027	
		pH- <i>M. decora</i> -Year	1.77	0.20	

Table 2.4. Overall model and Type III sums of squares from regression analyses testing for a relationship between frog abundance (mean number/ transect/ pond) and the independent variables: pond pH, *E. punctata* abundance (mean number/ trap/ pond) and sampling year (1995, 1996). See Table 2.3 for details of analysis and transformations of the response variables.

Response	df_{error}	Factor(s)	F	p	R^2
Bullfrog	26	Overall Model	6.39	0.0010	0.50
		pH	1.94	0.18	
		<i>E. punctata</i>	1.71	0.20	
		Year	22.86	0.0001	
		pH- <i>E. punctata</i>	1.41	0.25	
Green Frog	23	Overall Model	11.63	0.0001	0.78
		pH	4.13	0.054	
		<i>E. punctata</i>	7.40	0.012	
		Year	3.48	0.075	

Table 2.4 (cont.)

Response	df_{error}	Factor(s)	F	p	R^2
Leopard Frog	23	pH- <i>E. punctata</i>	7.24	0.013	0.45
		pH-Year	3.52	0.073	
		<i>E. punctata</i> -Year	7.94	0.010	
		pH- <i>E. punctata</i> -Year	7.41	0.012	
		Overall Model	2.69	0.034	
		pH	4.61	0.043	
		<i>E. punctata</i>	5.78	0.025	
		Year	11.11	0.003	
		pH- <i>E. punctata</i>	4.72	0.040	
		pH-Year	5.24	0.032	
		<i>E. punctata</i> -Year	5.29	0.031	
		pH- <i>E. punctata</i> -Year	4.98	0.036	

Table 2.5. *F*-values and *P*-values associated with overall model and Type III sums of squares from regression analyses testing for a relationship between frog abundance (mean number/ transect/ pond) and the independent variables: pond pH, *P. omata* abundance (mean number/ trap/ pond) and sampling year (1995, 1996). See Table 2.3 for details of analysis and transformations of the response variables. A modified model (i.e., no year term) was used to relate leopard frog abundance to *P. omata* abundance because otherwise it was not possible to satisfy the assumption of homogeneity of variance.

Response	df_{error}	Independent Variable	<i>F</i>	<i>P</i>	R^2
Bullfrog	27	Overall Model	5.69	0.0037	0.39
		pH	0.52	0.48	
		<i>P. omata</i>	0.36	0.55	
		Year	16.74	0.0003	
Green Frog	23	Overall Model	9.79	0.0001	0.75
		pH	3.23	0.086	
		<i>P. omata</i>	1.70	0.21	
		Year	10.35	0.0038	

Response	df_{error}	Independent Variable	F	P	R^2
Leopard Frog	27	pH- <i>P. omata</i>	2.25	0.15	0.03
		pH-Year	3.44	0.077	
		<i>P. omata</i> -Year	2.00	0.17	
		pH- <i>P. omata</i> -Year	2.26	0.15	
		Overall Model	0.24	0.87	
		pH	0.02	0.88	
		<i>P. omata</i>	0.64	0.43	
		pH- <i>P. omata</i>	0.09	0.76	

Figure 2.1. Location of summer study ponds in relation to the Queen's Biological Station (marked by a star). Dots at the edges of water bodies denote the location of sampling; dots with no water shown are small, ephemeral ponds. UD, LD and ND refer to Upper Dowsley, Lower Dowsley and North Dowsley, respectively. LLSM refers to Lindsay Lake South Marsh and CIM refers to Cow Island Marsh. For analyses of spatial dependence (see text), ponds were grouped as follows: (1) Otter, Beaver, Poole (2) Barb's, Top (3) UD, LD, ND (4) Pat's, LLSM, Yezerinac (5) Mica, Telephone (6) Deadlock, Pothole. Skycroft, CIM and Salamander were spatially distinct and were not grouped or used in spatial dependence analyses.

Scale 1:50 000

Discussion

Frog abundance in relation to M. decora abundance

Green frog abundance was negatively associated with *M. decora* abundance, as predicted, but neither bullfrog nor leopard frog abundance was related to abundance of this leech. The significant relationship between green frog abundance and *M. decora* abundance could occur for two main reasons. First, it is important to recognize that significant correlations may not reflect a direct causal relationship. For example, spatial dependence may lead to apparent relationships between two factors, when these factors are actually correlated with a third, spatially-patchy factor. Although there was indication of spatial dependence of *M. decora* abundance among pond groups, I did not find spatial dependence of green frog abundance. Thus, the significant green frog-*M. decora* relationship seems unlikely to be biased by strong spatial dependence.

A second way in which correlations may occur is through direct effects of *M. decora* on frog abundances. That is, within the range of *M. decora* abundances at sample ponds, *M. decora* may have negative impacts on green frogs. This negative relationship could occur through predation, parasitism, or adult choice of *M. decora*-free breeding sites within a pond (Howard 1978, cf. Hopey and Petranksa 1994). These hypotheses are not mutually exclusive and also may operate in conjunction with the indirect associations between species described above.

Assuming direct effects of *M. decora* on frog abundance, it seems

reasonable to expect that negative effects of *M. decora* are most pronounced during the embryo or early tadpole stages because *M. decora* prey on very young frogs (and "only" parasitize older animals). This early predation may result in population size varying with leech abundance (cf. Wilbur 1980, Semlitsch 1988). Differences in the nature of the relationship between frog species may reflect differences in clutch/ hatchling mortality due to *M. decora* predation. For both leopard frogs and bullfrogs, the expected negative relationship between frog abundance and *M. decora* abundance did not occur. However, leopard frogs were not sampled during their breeding season. Leopard frogs breed early in the spring, and tend to move from their breeding ponds during the summer (Merrell 1977). Thus, summer abundances of adult leopard frogs at ponds may not reflect the potential for leopard frog embryos/hatchlings to survive in these ponds. In contrast, bullfrogs and green frogs were sampled during their breeding season, and therefore negative relationships between frog abundances and *M. decora* abundance may be expected. The lack of a negative relationship between bullfrog abundance and *M. decora* abundance may be due to the very large clutches of bullfrogs (12,000 - 20,000 eggs, Wright 1914); adult population size may not depend on the relatively small proportion of embryos eaten by leeches. In contrast, green frogs produce smaller clutches ($\leq 4,000$ eggs, Wright 1914), so given the same number of leeches, a greater proportion of embryos will be eaten. Based on this interpretation, I would predict a negative relationship between bullfrogs and *M. decora* when *M. decora* abundance is much higher than

observed in this study. This remains to be tested.

Interactions between M. decora and pond pH

The absence of direct effects of pH or interactive relationships of pH with *M. decora* in influencing frog abundances perhaps is not surprising considering that the "low" pHs observed at my study ponds were not close to lethal or sublethal pH for these frog species. The lowest pH I measured at a pond was 5.45 (in spring 1996), whereas the pH below which hatching success is reduced (compared to that of neutral water) is generally 3.9 - 4.25 for the species studied herein (Freda 1986). Even so, it is still possible that frog abundance decreases with decreasing pH if sublethal negative effects of low pH occur at values above these critical values. For example, in a study of frog distribution in relation to pond pH, Clark (1986) found that even when pond pHs did not reach critical levels for embryos (mortality of embryos was not related to pond pH), there was still a positive relationship between frog density and pond pH. Her results suggest that some factor associated with pond acidity indirectly affected the distribution of frog breeding activity. However, in her study, the lowest "non-lethal" pond pH (4.44) was an order of magnitude lower than in this study. Future study of interactive effects between predator abundance and pH on frog abundances should ideally concentrate on ponds such as the ones in Clark's (1986) study, with sublethal but potentially stressful pH.

Frog abundance in relation to E. punctata and P. ornata abundance

Neither bullfrog nor leopard frog abundances were negatively related to abundance of the leech, *E. punctata*. However, the indication of a negative relationship between adult green frog abundance and *E. punctata* abundance (in 1995) suggests that, in conjunction with the observed negative effect of *M. decora* abundance on green frog abundance, embryos or tadpoles of this frog may be particularly affected by leech predation. This remains to be tested experimentally. The similarity of the green frog-leech relationships are not a simple consequence of strong correlations between abundance of the two leech species ($P > 0.25$ in both years).

Abundances of bullfrogs, green frogs and leopard frogs were not influenced by the abundance of *P. ornata*. An effect of this leech species on frog fitness (and therefore frog abundance) might be expected because it is a parasite of tadpoles and frogs (Sawyer 1986, but it is unlikely an embryo/tadpole predator, unpubl. data). The absence of a relationship could thus indicate that this leech does not influence fitness of its frog hosts (relatively benign parasite), that negative fitness effects on hosts do not translate into observable population-level effects (cf. Anderson and May 1986), or that negative effects of these leeches were not apparent for the range of *P. ornata* abundance found at my study ponds.

Frog abundances in the two sampling years

There was a strong indication of large differences in bullfrog, green frog

and leopard frog abundances between years, which may be due to differences in censusing tactics in the two years—frog censuses in 1995 and 1996 were done by two assistants, and by myself and an assistant(s), respectively. In 1995, there were some very high counts of average frog abundance. It is possible that metamorphosing frogs were included in some of the censuses of adult frogs because metamorphosing frogs are usually very abundant, and potentially difficult to distinguish from adults (in 1996, I minimized this problem by categorizing frogs as adult only if I was able to confirm the absence of a tail). This potential censusing difference accounts for some, but not all, of the difference between years. For example, even by pooling 1996 adult frog counts with 1996 metamorphosing frog counts (tabulated in 1996 but not used in analyses), abundances in 1995 were still much higher at some ponds: there was a large difference (≥ 4 frogs/ transect) between years at 2 ponds for bullfrogs, 6 ponds for green frogs and 2 ponds for leopard frogs. Thus, it appears that some other factor also may be responsible for differences in frog abundances between years. Large annual population fluctuations are common in amphibian populations due to differences in recruitment or adult survival (Pechmann et al. 1991, Berven 1990). Thus, differences between years also may be attributable to inter-annual population fluctuations.

Limitations of study

Two possible limitations of this study require attention. First, all the

variables measured in this study were essentially point measures. Given the limited time and personnel, it was not possible to obtain replicate measures of frog or leech abundance or pond pH within the summer season. This reduces the accuracy and generalizability of the results. Further investigation of the repeatability of the observed relationships between or within years would be necessary to obtain a more detailed understanding of the influence of leeches on frog abundance.

Second, there are potential limitations in using leech trapping data to draw inferences about relationships between frog and leech abundances. Estimates of leech abundance were biased towards those leeches that were attracted to the baited traps. Although there are reasons to believe that leeches common in my traps (especially *M. decora*) may have an important influence on the distribution or abundance of amphibians, leeches that were not attracted to traps may be abundant and may have important influences on frog abundance. Also, indirect effects of leeches on frogs may be important but not captured by testing for simple relationships between leech and frog abundances. For example, the blood parasite, *T. diemyctyli*, has been implicated as a selective force in newt populations (Gill and Mock 1985), and newts in some of my study ponds were infected by this blood parasite (unpubl. data). The newts probably had been parasitised by *D. picta* as this leech is the only known vector of *T. diemyctyli* (Mock and Gill 1984). However, I trapped this leech very infrequently, and although this leech could have an impact on amphibians through its role as a

parasite vector, the importance of this relationship is probably under-represented by simply enumerating *D. picta* leeches. Thus, the relationships (or lack thereof) between frog and leech abundances that I examined in this study do not investigate all potentially important leech-frog associations.

Conclusion

I found a significant negative relationship between green frog abundance and *M. decora* abundance, as would be expected based on the known negative effects of this leech on embryonic and tadpole survival. I did not find a negative relationship between either bullfrog or leopard frog abundances and *M. decora*. These differences may relate to differences in reproductive biology between species. Interactive effects between low pH and *M. decora* abundance on frog abundances were expected based on known interactions from experiments, but were not observed. This result may be due to the absence of critically low pHs at study ponds, and does not necessarily indicate that interactive effects would not exist at lower pond pHs.

SUMMARY AND CONCLUSION

In this study, I examined the separate and combined effects of low pH and the leech, *M. decora*, on embryos and adult frogs. In the embryo experiments, I found that predation by *M. decora* decreased hatching success of wood frog embryos. Embryos not preyed upon showed phenotypic plasticity: wood frog and green frog embryos hatched at larger size in the presence of *M. decora*. The presence of a leech did not significantly affect hatch timing of wood frog embryos but green frog embryos delayed hatching when a leech was present. The mortality caused by leeches may be an important factor affecting the distribution and abundance of amphibians, although the presumably adaptive responses of embryos to the presence of a predator may increase the probability of avoiding leech predation.

I also found that low pH decreased hatching success of wood frog and green frog embryos. For those embryos that survived the low pH treatment, the size and timing of hatching were affected by low pH. Wood frogs hatched at smaller size, but hatch timing was unchanged. In contrast, green frog hatch size was unaffected by low pH, but hatching was delayed. These results suggest that low pH can have both lethal and sublethal effects on embryos.

Interactive effects were common. When wood frog embryos were raised in

low pH and in direct contact with a leech predator, hatching success was as low as mortality due to leech predation alone. In contrast, the combined effect of low pH and leech predation risk was predicted by the effect of low pH alone: embryos raised with a leech in low pH hatched at a length and time similar to those embryos raised in low pH. That is, the presumably adaptive response to the presence of the leech predator did not occur in low pH water. This interactive effect of low pH and predation risk could have negative impacts on fitness of frogs.

The lethal and sublethal interactive effects may translate into observable population-level effects. Therefore, based on the results from Chapter 1, I tested the general predictions (Chapter 2) that frog abundance would be negatively correlated with leech abundance and low pH, and that these two factors would interact to determine frog abundance. I found that green frog abundance was negatively associated with leech abundance as expected, but this trend was not apparent for either leopard frogs or bullfrogs. The differences between species may relate to differences in clutch size and timing of breeding in relation to timing of sampling. I was unable to test my prediction that frog abundance was negatively associated with low pH or that interactive effects between low pH and *M. decora* abundance would exist, because the pH of ponds was not acidic enough for even sublethal effects of low pH.

The main purpose of this thesis was to understand how an anthropogenic factor interacts with a natural community factor, predation, to

affect frog hatching traits and population abundance. I was interested in this topic for two main reasons. First, although many researchers have addressed the effects of such factors in isolation, a logical next step is to address potential interactive effects of factors. Some researchers have successfully done this for other factors using "mesocosm" experiments, in which a natural community is simulated, and replicated, in large tanks (review in Rowe and Dunson 1994). This approach has the advantage of simulating natural pond communities, while permitting control over the variables of interest. However, one potential drawback of this method is that a factor such as food availability may not be expressly manipulated in the study, yet it can vary according to treatments. In a mesocosm experiment, the nature of interactions between this factor and the factors of interest are not accounted for: only the outcome is observed (Sadinski and Dunson 1992).

By experimentally documenting the effects of two factors in isolation and in combination (Chapter 1), I traded the realism afforded by a semi-natural experiment in favour of documenting the nature of the pH-leech interaction. This allows predictions for future studies that investigate effects of low pH (or other toxins) in conjunction with other natural environmental factors, as well as for studies that examine higher-order interactive effects. An additional advantage of this approach relates to my second, more specific, purpose for conducting this study: unexplained amphibian declines. To date, researchers have been unable to determine a single factor that can explain the worldwide and synchronous declines of amphibian populations. It has been suggested (e.g., Hayes and

Jennings 1986, Blaustein and Wake 1990) but rarely tested (Carey 1993, Long et al. 1995) that interactive effects are to blame for the declines. I am not suggesting that an interaction between low pH and leech predators is responsible for amphibian declines. However, by examining the separate and combined effects of these factors experimentally, I was able to demonstrate that they interact. Whether the observed lethal and sublethal interactive effects translate into observable effects on populations remains to be tested. Study of interactions among factors may be the key to understanding the mechanisms, and ultimately, the causes of amphibian declines; and may provide a better understanding of how communities are structured.

LITERATURE CITED

- Aiken, L.S. and S.G. West. 1991. Multiple regression: testing and interpreting interactions. Sage Publications, Newbury Park, California, USA.
- Allison, V., D.W. Dunham and H.H. Harvey. 1992. Low pH alters response to food in the crayfish *Cambarus bartoni*. *Canadian Journal of Zoology* **70**:2416-2420.
- Anderson, R.M. and R.M. May. 1986. The invasion, persistence and spread of infectious diseases within animal and plant communities. *Philosophical Transactions of the Royal Society of London, Series B.* **314**:533-570.
- Beattie, R.C., R.J. Aston and A.G.P. Milner. 1991. A field study of fertilization and embryonic development in the common frog (*Rana temporaria*) with particular reference to acidity and temperature. *Journal of Applied Ecology* **28**:346-357.
- Beattie, R.C. and R. Tyler-Jones. 1992. The effects of low pH and aluminum on breeding success in the frog *Rana temporaria*. *Journal of Herpetology* **26**:353-360.
- Bendell, B.E. and D.K. McNicol. 1991. An assessment of leeches (Hirudinea) as indicators of lake acidification. *Canadian Journal of Zoology* **69**:130-133.

- Berven, K.A. 1990. Factors influencing population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* **71**:1599-1608.
- Berven, K.A., D.E. Gill and S.J. Smith-Gill. 1979. Countergradient selection in the green frog, *Rana clamitans*. *Evolution* **33**:609-623.
- Blaustein, A.R., D.B. Wake and W.P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**:60-71.
- Blaustein, A.R. and D.B. Wake. 1995. The puzzle of declining amphibians. *Scientific American* **273**:52-57.
- Bradford, D.F., C. Swanson and M.S. Gordon. 1992. Effects of low pH and aluminum on two declining species of amphibians in the Sierra Nevada, California. *Journal of Herpetology* **26**:369-377.
- Brockelman, W.Y. 1969. An analysis of density effects and predation in *Bufo americanus* tadpoles. *Ecology* **50**:633-644.
- Brodie, E.D., Jr. and D. Formanowicz, Jr. 1983. Prey size preference of predators: differential vulnerability of larval anurans. *Herpetologica* **39**:199-204.
- Brönmark, C. and J.G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. *Science* **258**:1348-1350.
- Brown, R.M. and D.H. Taylor. 1995. Compensatory escape mode trade-offs between swimming performance and maneuvering behavior through larval ontogeny of the wood frog, *Rana sylvatica*. *Copeia* **1995**:1-7.

- Carey, C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* 7:355-362.
- Clark, K.L. 1986. Distributions of anuran populations in central Ontario relative to habitat acidity. *Water Air and Soil Pollution* 30:727-734.
- Cory, L.C. and J.J. Manion. 1953. Predation on eggs of the wood frog, *Rana sylvatica*. *Copeia* 1953:66.
- Crowl, T.A. and A.P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail. *Science* 247:949-951.
- Crump, M.L. and N.J. Scott Jr. 1994. Visual encounter surveys. *In*: W.R. Heyer, M.A. Donnelly, R.W. McDiarmid L.C. Hayek and M.S. Foster, editors, *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington, DC, USA.
- Davies, R.W. 1991. Annelida: leeches, polychaetes and acanthobdellids. Pages 437-479 *in*: J.H. Thorp and A.P. Covich, editors, *Ecology and classification of North American freshwater invertebrates*. Academic Press Inc., California, USA.
- Dole, J.W. 1968. Homing in leopard frogs, *Rana pipiens*. *Ecology* 49:386-399.
- Duellman, W.E. and L. Trueb. 1986. *Biology of amphibians*. McGraw-Hill Book Company, New York, New York, USA.
- Dunson, W.A. and J. Travis. 1991. The role of abiotic factors in community organization. *American Naturalist* 138:1067-1091.

- Fauth, J.E. 1990. Interactive effects of predators and early larval dynamics of the treefrog *Hyla chrysoscelis*. *Ecology* **71**:1609-1616.
- Freda, J. 1986. The influence of acidic pond water on amphibians: a review. *Water Air and Soil Pollution* **30**:439-450.
- Freda, J. and W.A. Dunson. 1985. The influence of external cation concentration on the hatching of amphibian embryos in water of low pH. *Canadian Journal of Zoology* **63**:2649-2656.
- Gill, D.E. 1978. The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecological Monographs* **48**:145-166.
- Gill, D.E. and B.A. Mock. 1985. Ecological and evolutionary dynamics of parasites: the case of *Trypanosoma diemyctyli* in the red-spotted newt *Notophthalmus viridescens*. Pages 157-183 *In*: D. Rollinson and R.M. Anderson, editors. *Ecology and Genetics of Host-Parasite Interactions* Academic Press Inc., London, England.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**:183-190.
- Gosner, K.L. and I.H. Black. 1957. The effects of acidity on the development and hatching of New Jersey frogs. *Ecology* **38**:256-262.

- Grantham, B.A. and B.J. Hann. 1994. Leeches (Annelida: Hiridinea) in the Experimental Lakes Area, northwestern Ontario, Canada: patterns of species composition in relation to environment. *Canadian Journal of Fisheries and Aquatic Sciences* **51**:1600-1607.
- Griffiths, R.A. and P. DeWijer. 1994. Differential effects of pH and temperature on embryonic development in the British newts (*Triturus*). *Journal of Zoology* **234**:613-622.
- Harvell C.D. 1990. The ecology and evolution of inducible defenses. *Quarterly Review of Biology* **65**:323-340.
- Hayes, M.P. and M.R. Jennings. 1986. Decline of ranid frog species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? *Journal of Herpetology* **20**:490-509.
- Hinch, S.G., K.M. Somers and N.C. Collins. 1994. Spatial autocorrelation and assessment of habitat-abundance relationships in littoral zone fish. *Canadian Journal of Fisheries and Aquatic Sciences* **51**:701-712.
- Hopey, M.E. and J.W. Petranka. 1994. Restriction of wood frogs to fish-free habitats: how important is adult choice? *Copeia* **1994**:1023-1025.
- Howard, R.D. 1978. The influence of male-defended oviposition sites on early embryo mortality in bullfrogs. *Ecology* **59**:789-798.
- Howard, R.D. 1980. Mating behaviour and mating success in woodfrogs, *Rana sylvatica*. *Animal Behaviour* **28**:705-716.

- Huitema, B.E. 1980. The analysis of covariance and alternatives. Wiley & Sons, New York, New York, USA.
- Jaccard, J., R. Turrisi and C.K. Wan. 1990. Interaction effects in multiple regression. Sage Publications, Newbury Park, California, USA.
- Jung, R.E. and C.H. Jagoe. 1995. Effects of low pH and aluminum on body size, swimming performance, and susceptibility to predation of green tree frog (*Hyla cinerea*) tadpoles. Canadian Journal of Zoology 73:2171-2183.
- Kleinbaum, D.G., L.L. Kupper and K.M. Muller. 1988. Applied regression analysis and other multivariable methods. PWS-Kent Publishing Company, Boston, Massachusetts, USA.
- Klemm, D.J. 1990. Hirudinea. Pages 398-415 In: B.L. Peckarsky, P.R. Fraissinet, M.A. Penton and D.J. Conklin Jr., editors. Freshwater macroinvertebrates of northeastern North America. Cornell University Press, Ithaca, New York, USA.
- Lemly, A.D. and R.J.F. Smith. 1987. Effects of chronic exposure to acidified water on chemoreception of feeding stimuli in fathead minnows (*Phimephales promelas*): mechanisms and ecological implications. Environmental Toxicology and Chemistry 6:225-238.
- Legendre, P. and M.-J. Fortin. 1989. Spatial pattern and ecological analysis. Vegetatio 80:107-138.

- Licht, L.E. 1974. Survival of embryos, tadpoles, and adults of the frogs *Rana aurora aurora* and *Rana pretiosa pretiosa* sympatric in southwestern British Columbia. *Canadian Journal of Zoology* **52**:613-627.
- Long, L.E., L.S. Saylor and M.E. Soulé. 1995. A pH/UV-B synergism in amphibians. *Conservation Biology* **9**:1301-1303.
- Merrell, D.J. 1977. Life history of the leopard frog, *Rana pipiens*, in Minnesota. Occasional Paper No. 15. Bell Museum of Natural History, University of Minnesota, Minneapolis, Minnesota, USA.
- McCollum, S.A. and J. Van Buskirk. 1996. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**:583-593.
- Mock, B.A. and D.E. Gill. 1984. The infrapopulation dynamics of trypanosomes in red-spotted newts. *Parasitology* **88**:267-282.
- Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. *Ecological Monographs* **53**:119-138.
- Neter, J., W. Wasserman and M.H. Kutner. 1989. Applied linear regression models. Irwin Inc., Homewood, Illinois, USA.
- Odland, J. 1988. Spatial autocorrelation. Sage Publications Inc., Newbury Park, California, USA.
- Oldham, R.S. 1967. Orienting mechanisms of the green frog, *Rana clamitans*. *Ecology* **48**:477-491.

- Parichy, D.M. and R.H. Kaplan. 1995. Maternal investment and developmental plasticity: functional consequences for locomotor performance of hatchling frog larvae. *Functional Ecology* **9**:606-617.
- Paull, A.E. 1950. On a preliminary test for pooling mean squares in the analysis of variance. *Annals of Mathematical Statistics* **21**:539-556.
- Pechmann, J.H.K., D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.J. Vitt and W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural populations. *Science* **253**:892-895.
- Pehek, E.L. 1995. Competition, pH, and the ecology of larval *Hyla andersonii*. *Ecology* **76**:1786-1793.
- Pennak, R.W. 1953. Freshwater invertebrates of the United States. The Ronald Press Co., New York, New York, USA.
- Perrin, N. and R.M. Sibly. 1993. Dynamic models of energy allocation and investment. *Annual Review of Ecology and Systematics* **24**:379-410.
- Petranka, J.W., L.B. Kats and A. Sih. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour* **35**:420-425.
- Petranka, J.W., J.J. Just and E.C. Crawford. 1982. Hatching of amphibian embryos: the physiological trigger. *Science* **217**:257-259.
- Pierce, B.A. 1985. Acid tolerance in amphibians. *BioScience* **35**:239-243.

- Pierce, B.A. and N. Sikand. 1985. Variation in acid tolerance of Connecticut wood frogs: genetic and maternal effects. *Canadian Journal of Zoology* **63**:1647-1651.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**:223-225.
- Rowe, C.L. and W.A. Dunson. 1994. The value of simulated pond communities in mesocosms for studies of amphibian ecology and ecotoxicology. *Journal of Herpetology* **28**:346-356.
- Rowe, C.L., W.J. Sadinski and W.A. Dunson. 1994. Predation on larval and embryonic amphibians by acid-tolerant caddisfly larvae (*Ptilostomis postica*). *Journal of Herpetology* **28**:357-364.
- Sadinski, W.J. and W.A. Dunson. 1992. A multilevel study of effects of low pH on amphibians of temporary ponds. *Journal of Herpetology* **26**:413-422.
- SAS Institute. 1989. SAS user's guide: statistics. SAS Institute Inc., Cary, North Carolina, USA.
- Sawyer, R.T. 1986. Leech biology and behaviour, vol. 2: feeding biology, ecology and systematics. Oxford University Press, New York, New York, USA.
- Siddal, M.E. and S.S. Desser. 1992. Alternative leech vectors for frog and turtle trypanosomes. *Journal of Parasitology* **78**:562-563.
- Sih, A. and R.D. Moore. 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist* **142**:947-960.

- Semlitsch, R.D. 1988. Allotopic distribution of two salamanders: effects of fish predation and competitive interactions. *Copeia* 1988:290-298.
- Smith, D.C. and J. Van Buskirk. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. *American Naturalist* 145:211-233.
- Smith-Gill, S.J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. *American Zoologist* 23:47-55.
- Sredl, M.J. and J.P. Collins. 1992. The interaction of predation, competition, and habitat complexity in structuring an amphibian community. *Copeia* 1992:607-614
- Van Buskirk, J. 1988. Interactive effects of dragonfly predation in experimental pond communities. *Ecology* 69:857-867.
- Wake, D.B. 1991. Declining amphibian populations. *Science* 253:860.
- Warkentin, K.M. 1995. Adaptive plasticity in hatching age: a response to predation risk tradeoffs. *Proceedings of the National Academy of Science* 92:3507-3510.
- Warner, S.C., J. Travis and W.A. Dunson. 1993. Effect of pH variation on interspecific competition between two species of hylid tadpoles. *Ecology* 74:183-194.

- Weis, J.S. and P. Weis. Swimming performance and predator avoidance by mummichog (*Fundulus heteroclitus*) larvae after embryonic or larval exposure to methylmercury. *Canadian Journal of Fisheries and Aquatic Sciences* **52**:2168-2173.
- Werner, E.E. 1991. Non-lethal effects of a predator on competitive interactions between two anuran larvae. *Ecology* **75**:1709-1720.
- Werner, E.E. and M.A. McPeck. 1994. Direct and indirect effects of predators on two anuran species along an environmental gradient. *Ecology* **75**:1368-1382.
- Wilbur, H.M. 1980. Complex life cycles. *Annual Review of Ecology and Systematics* **11**:67-93.
- Wilbur, H.M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. *Ecology* **68**:1437-1452.
- Wright. 1914. North American anura: life histories of the anura of Ithaca, New York. Carnegie Institution of Washington, Washington, DC, USA.
- Zar, J.H. 1984. Biostatistical analysis, 2nd edition. Prentice-Hall Inc., Engelwood Cliffs, New Jersey.

Appendix 1: Descriptive statistics for other leeches found in traps (excluding leeches used in analyses; see Table 2.1).

Upper value based on 13 ponds in 1995; lower value based 18 ponds in 1996. Mean leech abundance at each pond was determined from the mean number /trap /pond (usually, $n = 6-8$ traps /pond, see text for exceptions).

Family	Species	Mean	Minimum	Maximum	Std. Deviation
Glossiphoniidae	<i>Desserobdella picta</i>	0.00	0.00	0.00	0.00
		0.04	0.00	0.38	0.10
	<i>Helobdella triserialis</i>	0.00	0.00	0.00	0.00
		0.43	0.00	5.50	1.31
	<i>Placobdella hollensis</i>	0.00	0.00	0.00	0.00
		0.25	0.00	0.33	0.91
Haemopidae	<i>Placobdella parasitica</i>	0.01	0.00	0.17	0.05
		0.00	0.00	0.00	0.00
	<i>Haemopsis grandis</i>	0.37	0.00	4.83	1.34
		0.00	0.00	0.00	0.00
	<i>Percymoorensis marmoratis</i>	0.00	0.00	0.00	0.00
		0.05	0.00	0.88	0.20

IMAGE EVALUATION TEST TARGET (QA-3)

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