The Effects of Mining Effluent on Amphibian Survivorship

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Abstract

Amphibian populations and species diversity are declining at an alarming rate all over the world. In Montana, mining has been suggested as the culprit for this decline. I continued Meis's (1999) study at the Upper Frohner Meadow site after a settling pond had been introduced reduce the heavy metal contamination. I looked for increased survivorship between the two years, using western toad tadpoles (*Bufo boreas*), and determined differences in hardiness by comparing spotted frog tadpoles (*Rana levitrius*), both of which were contained in enclosures in groups of 50. The results showed a 39.4% increase in toad survivorship since the use of a settling pond. The frogs had a 57.6% higher average survivorship than the toads. The consequences of this research suggest settling ponds are effective tools for reducing or eliminating heavy metal contamination, therefore, increasing water quality. It also showed spotted frogs could tolerate the contamination better than western toads and would be most likely to repopulate a bioreclaimated site.

Introduction

In 1990 a Declining Amphibian Population Task Force (DAPTF) was established to bring together amphibian researchers, environmentalist, and others groups to find a reason for the dramatic decrease in amphibian species (Halliday and Heyer 1997). The results of previous research suggest that the decline cannot be attributed to a single factor, but is instead a multitude of individual effects that may act individually or synergistically to affect the viability of amphibian populations.

One factor or causal agent of population decrease may be the increase in UV radiation from the sun due to the deterioration of the ozone layer. Amphibians tend to be very sensitive to this radiation and growth defects and death may result (Blaustein et al. 1994-I). Another factor in the decline may be the increasing use of pesticides and fertilizers in agriculture, causing a variety of changes to the environment. First, excess fertilizers can become part of spring runoffs or soil erosion and end up in waterways. The fertilizers promote rapid growth of aquatic plants which use up the dissolved O_2 in the water. This produces an anoxic environment and amphibian tadpoles are thus unable to survive (Hall and Henry 1991). Second, pesticides have been shown to harm both larva tadpoles and adults in a number of ways. Pesticides decrease the insect population leading to less food for amphibians. It may also affect insect predators. In one case, pesticides killed a specific group of insects that feed on the macroinvertebrate predators of the tadpoles. The predator insects were then uncontrolled and lowered the tadpole population significantly (Hall and Henry 1991). Pesticides also decrease the population by concentrating within the organism's internal system; the permeability of amphibian skin allows absorption into its internal system causing damage or death. There is also the bioaccumulation factor, in which insects containing low levels of pesticides become a problem when amphibians eat them in large numbers. When adult amphibians eat these insects they accumulate all the contaminants from each individual insect. The pesticides may not have an effect in small amounts, but the accumulation of small doses over time becomes fatal (Hall and Henry 1991). Researchers are even finding declining numbers and species in pristine wilderness areas. One reason may be the presence of a fungus causing birth defects or death in frog populations (Blaustein et al. 1994-II). Another factor in declining population is the many toxic pollutants left by humans in some areas that are considered pristine. Many of the pollutants found are contaminates left by early miners during the gold and silver rush of the late 19th and early 20th century (Neufeld 1987; Lefcort et al. 1998). Tailing ponds and traces of foreign chemicals in wilderness streams can be seen in sites scattered across the Northwest United States.

Amphibians are considered vital components of a biotic community because of their trophic links with algae, invertebrates, fish, snakes, birds, and mammals (Freda 1991). Amphibians spend half of their lives in the water as tadpole larva and half their lives as mobile semi-terrestrial adults. In their tadpole stage, amphibians feed off both the substrate and algae, and continuously take in water for respiration, thus their tissues are exposed to a variety of toxins, sediment-bound contaminants, and bioconcentrated metals (Freda 1991; Horne and Dunson 1995). All organisms of this class have highly permeable skin, allowing them to exchange dissolved oxygen, nutrients, and water. The highly permeable skin also makes them more susceptible to slight changes in the water such as changes in pH and temperature (Warner and Dunson 1991). Any of these changes can lead to a variety of negative effects for amphibians, including decreased growth rates, mutations, and death (Warner and Dunson 1991). After metamorphosis the amphibians are able to leave the water; however, they retain their highly permeable skin. This makes them susceptible to the same changes in water quality but also changes to the terrestrial environment. They feed on adult and larva insects, so any disruption to these groups may disrupt the amphibian population. This inherent trait of highly permeable skin and their vital role as the intermediate link in the food chain make them an important indicator species of environmental quality. Amphibians may thus provide an early warning to deteriorating environmental conditions (Harfensit et al. 1989).

Mining was one of the main reasons people came to Montana in the mid-1800s and contributed greatly to the state's wealth. The attraction of gold and silver lead to the rise of many individual mining sites, which are now scattered across Montana's western hills and mountains. Some of the contaminates involved in the mining process include heavy metals such as lead (Pb), zinc (Zn), cadmium (Cd), mercury (Hg), silver (Ag), copper (Cu), arsenic (As), manganese (Mn), molybdenum (Mo), and antimony (Sb), some of which were used to separate the precious metal from the ore and soil (Horowitz et al. 1993). There were few regulations at this time to control mining practices and waste disposal. This lack of ordinance lead to the practice of allowing waste to flow into streams, to be left in piles, or to be disposed of in tailing ponds. These practices lead to the contamination of the ecosystem by direct introduction of toxins, as well as by erosion and leaching. The bill proposed by President Nixon in 1971, together with the Water Quality Act of 1965, established environmental quality standards to "prevent environmental degradation from mining" (McClellan 1971). This made active mine operators responsible for the waste from their mines. However, there were numerous old, abandoned mines which were contaminating the ecosystem but had no owner to hold responsible for clean-up.

Mining began in Jefferson County, Montana, in 1864, with many mines scattered throughout the Elkhorn Mountains. As more and more people came to the state, agriculture became a stable income and so many abandoned their mines in this area (USGS 1963). The Frohner Meadow mine, appoximately 30 miles south of Helena located in upper Frohner Meadow is one of these old mines. Until the fall of 1998, it had a run-off stream, which flowed directly through an abandoned tailings pond and down into the meadow and into a common stream that connected all of the ponds of the meadow (Fig 1). This tailing stream allowed contaminants to flow into the ponds below the confluence, causing damage to the inhabiting species. Research done in the summer of 1998 showed that toad tadpoles were able to survive in the upper ponds but not those located below the tailing stream confluence (Meis 1999). In 1999, the U.S. National Forest Service built a "settling pond" in a bioreclaimation attempt to detoxify the lower ponds and stop any further contamination.

The purpose of my study was to examine the effectiveness of the settling pond. I repeated the study of Meis (1999) using the same western toad species (*Bufo boreas*) and enclosures of the same material to see if the toads would now be able to survive in the lower ponds. I also set up enclosures for spotted frog tadpoles (*Rana luteventris*) in the upper and lower ponds to test for differences in survivorship between these two species.

Materials and Methods

I collected spotted frog and Western toad tadpoles from a natural, uncontaminated pond above the mine tailings confluence in Upper Frohner Meadow (Fig 1). The tadpoles were collected using small aquarium style dip nets, divided into groups of 50 individuals of the same species, and placed in a small bucket containing pond water. The age of the frog tadpoles were one to two weeks old, or 19 on the Gosner scale, and averaged 2.0 cm in total body length (Gosner 1969). The age of the toad tadpoles were one week post hatching, or an 18 on the Gosner scale (1969) and averaged 1.0 cm in total body length. Each group was transported immediately (no longer than 10 minutes out of a natural environment) and placed in one of the research enclosures. Each enclosure was constructed of 2.5 cm x 1.75 cm pine framing measuring 61 cm x 30 cm x 46 cm. A fine fiberglass mesh screen was placed and stapled to the outside of the frame on all but one side. The enclosure was then caulked with silicone along the wood to seal all the small holes and joints. All enclosures were established on the same day, 15 June 1999.

Six enclosures were placed randomly in a pond upstream from the confluence and six were placed in the pond downstream from the confluence. The pond sites were chosen because they were subject to the same weather, temperature, and predator conditions and possessed similar surface area dimensions as well as had similar amounts and types of vegetation both within and around the ponds. The enclosures were submerged by placing two small rocks on the floor of the enclosure on the mesh while being careful no holes were made in the mesh from which the tadpoles could escape. The enclosures were submerged so that 15 to 20 cm were below the water level (10 to 15 cm of the cage above the water line). Three sticks, approximately 60 cm in length were used to “stake” the enclosures to prevent the enclosure from moving or being tipped over by wind or wildlife. Two handfuls of pond vegetation (grass and moss) were pulled out of the pond and placed in each of the 12 enclosures. This allowed a surface medium for algae to grow on to provide food for the tadpoles. Groups of 50 tadpoles were added to each of the three enclosures in the upper pond and to each of the three enclosures of the lower pond for both species. Thus there were three replicates upstream and three downstream for both species.

Each enclosure was checked and living tadpoles were counted on July 2, 1999. When all the tadpoles were considered captured, the stakes were removed from the sides and the cages were lifted out to confirm no more tadpoles were in the enclosures and to make sure no holes had developed in the enclosures. A final count was taken on July 20, 1999. Due to the upper pond drying up, the July 20th data was discarded and the analysis focused on the July 2nd data. This data was used to calculate the mean (x) for each enclosure and the standard deviation (std). Due to experimental error three enclosures were eliminated, frog cage #2 from the upper pond, frog cage #2 from the lower pond, and toad cage #1 of the upper pond. The reason for this elimination was to reduce all outside factors that would misrepresent the results, such as cages that had been dislodged or cages that had been invaded by macroinvertebrate predators.

Results

A significant change in survivorship between 1998 and 1999 was revealed for the toads. Raw data for toad and frogs are presented for 1999 (Table 1) and for 1998 (Table 2: from Meis 1999). The calculated means and standard deviations for both years are in Table 3. The calculations showed there was a significant change in the survivorship for toads between 1998 and 1999, because survivorship increased in both the upstream and downstream ponds in 1999 (Fig 2). The biggest difference came from the toad tadpoles surviving in the downstream pond, a 39.4% survivorship (mean = 14.7) in 1999, while a year earlier no tadpoles (0%) had survived. In the upstream pond there was an 88% survivorship (mean = 44). This is a difference from 1998, in which there was 76.7% survivorship in the upper pond (mean = 23) and no tadpoles had survived in the lower pond.

There was not much difference between the two sites for the frog tadpoles (Fig 3). The percent survivorship for the lower pond was 97% with a small error of 4.2%, while the upper pond had total frog tadpole survivorship (100%) during the study (Table 3).

There was a difference in the variability between locations and between species. Toad survivorship was more variable than frog survivorship, and the downstream location was more variable than the upstream location. This variability can be seen in the lower pond in the average percent survivorship of the two species, with the toads having a 39.4% survivorship (error = 38.6%), while the frogs have a 97% survivorship (error = 4.2%). The difference in the upper pond is much closer, with the average percent survivorship of the toads being 88% (error = 5.7%) while the frogs are 100% (error =

0%). This shows that the frogs have a greater tolerance or hardiness that allowed them to have a higher survivorship in the poor conditions of the lower pond.

Discussion

Overall, my research findings showed the experimental settling pond implemented by the National Forest Service did result in a significant increase in the water quality of the Upper Frohner Meadow, site leading to the ability of some Western toad (*B. boreas*) tadpoles to now survive in the lower ponds. This suggests that bioremediation projects can have a dramatic impact and can result in immediate effects. Western toads are known to return to their same breeding grounds year after year (Hokit 1999 pers comm) and the toads are already known to breed in this meadow. If the downstream ponds were to clean up enough to allow a high survival rate, the population of toads might dramatically increase in the area. The second finding of my study is hat there is a remarkable difference in the hardiness of the spotted frog (*R. luteventris*) tadpoles compared to the toad tadpoles. This suggests that frog tadpoles would be able to live in more contaminated ponds than the toads and that this species would be more likely to re-colonize these polluted downstream ponds if the water quality continues to improve.

One concern that should be addressed in future studies involves the placement of cages in the ponds. One of the toad enclosures of the 1999 study was placed on the other side of the small elbow approximately 61 cm from the other two toad enclosures (Fig 4). This single enclosure was surrounded by a higher amount of aquatic vegetation, and it was this enclosure which had the highest number of survivors, 36. The other two enclosures of the lower pond had a combined total of 6 surviving toads (Table 1). This lead to a high difference in standard deviation (error) on the graphs (Fig 2).

Upper Frohner Meadow is a prime example of a point source pollution site. Although there are other human impacts in this area (cattle grazing, hunting, and logging) that may disrupt the natural ecosystem, the most significant impact is the water quality disturbance from the mine tailings entering the stream which flows through the meadow. This isolated site allowed for monitoring and experimentation to observe the effectiveness of a water and land reclamation project by the National Forest Service, using amphibian tadpoles as an indicator of the water quality.

The water from the tailings pond lead to the significant decrease in the survivorship of the anuran tadpoles in the summer of 1998 (Meis 1999). My study suggested that toad survival increased in the lower pond in 1999. This indicates that the settling pond is an effective tool for ridding the tailing stream of at least some of its harmful pollutants before it enters the meadow. However, the survivorship of the toads also increased in the upper pond. This event is more difficult to interpret since the mine tailings stream never influences the upper pond. There are a few possibilities that might explain this increase in survivorship in 1999 compared with 1998. First, the air temperature before and during the 1999 study was 3-8° C warmer then the previous summer. This increase in air temperature caused the water temperature to be 2-5° C warmer. A warmer water temperature would allow the tadpoles to develop (Hokit 1999 pers comm) and grow at a faster rate than a cold water temperature. This increase in developmental rate might allow the tadpoles to pass through a period of stress quicker and thus allow them to have a higher survivorship number. Second, the weather during the 1999 study was more consistent than the 1998 summer study. The 1999 weather consisted of a lot of sunshine while the weather during the 1998 study period had a little sunshine, with more afternoon thundershowers, a few cold days, and some overcast days. These two reasons would lead to a more consistent developmental period when the tadpoles are in their most susceptible stage of growth. The consistent weather and temperature could possibly result in less stress on the toad tadpoles, aiding in their survival. However, these reasons could also be used to explain the increased survival for the toad tadpoles of the lower pond. Finally, the pH in the pond averaged 7.34 in 1999 versus 6.6 in 1998. Low pH has been associated with decreased growth and survivorship in amphibians (Horne and Dunson 1995).

My results suggest that spotted frogs (Fig 2 & 3), were more resilient to the effects of poor water quality as compared to the toads. This suggests that frogs are more apt to colonize and regenerate in number in these lower ponds, prior to the toads after initial clean-up of contamination has occurred.

The findings of this study brings up a number of other questions which can only be answered by further monitoring or more research. First, the new settling pond may have been effective in eliminating some contaminants. However, we cannot be certain the increase in survival is due to decreased contamination or other enhanced rearing conditions for tadpoles. Furthermore, we cannot be certain that water quality will continue to improve. Further studies are necessary to examine the identity of the water contaminants.

Another issue is whether the contaminates which have settled out in the downstream ponds prior to the placement of the settling pond in 1998 will continue to be a hazard. The rate of water flow slows as it moves from a stream channel into a pond, allowing the heavy metals to settle out onto the bottom of the pond bed. Since the tailing stream has been dumping contaminates into the Upper Frohner Meadow region for many years, there has been a significant and clearly visible amount of sedimentation in the pond bottoms of the lower ponds. The question is whether this sediment will affect the tadpole development and regeneration, the vegetation (which has also been disrupted by the sedimentation), or if there will be no effect because only the turbid pollutants are causing the amphibian tadpole decline. Some studies suggest it is likely that sediments will impact amphibians. A study done in the Silver Valley region of northern Idaho showed that, in the laboratory setting, the presence of lead (Pb) in the sediment in levels similar to those in the river caused mortality to spotted frog tadpoles (Lefcort *et al.* 1998) and that the presence of multiple metals dramatically increased the mortality rate. The possible reasons for this increased mortality rate may be because the tadpoles feed off the substrate (algae and small plants) growing in the sediment or because the movement of the tadpoles on the floor of the ponds causes the contaminants to be somewhat resuspended and inhaled by the tadpoles, both of which would cause the metals to accumulate in the organism.

In order to strengthen my findings and support those of Lefcort, future studies should include analyzing a number of water samples from the upper and lower pond to define what elements are present in the water and the concentration of each. These levels could be compared to a chart produced by the Silver Valley study (Lefcort et al 1998) showing the minimum concentration of the mining contaminants needed to cause death in the spotted frog tadpoles. Regardless, my study suggests that the settling pond may have improved conditions for toad tadpoles and that toads are far more susceptible to mining effluent than are spotted frogs.

Table 1. Raw data for the 1999 survivorship after implementation of the settling pond.

Amphibian Survivorship Data for Upper Frohner Meadow						
	Upper Pond			**Lower Pond**		
Frogs	**Cage 1**	**Cage 2**	**Cage 3**	**Cage 1**	**Cage 2**	**Cage 3**
June 15th	50	50	50	50	50	50
July 2nd	50	0[1]	50	47	0[2]	50
July 20th	0[3]	0[3]	0[3]	45	0[2]	50
Toads						
June 15th	50	50	50	50	50	50
July 2nd	0[1]	46	42	36	6	0
July 20th	0[3]	0[3]	0[3]	31	6	0

[1] – cage blown/knocked over
[2] – ditiscid larvae in the cage
[3] – pond was dried up where cages placed

Table 2. Raw data from the 1998 survivorship study (Meis 1998).

Amphibian Survivorship Data for Upper Frohner Meadow 1998						
	Upper Pond			**Lower Pond**		
Toads	**Cage 1**	**Cage 2**	**Cage 3**	**Cage 1**	**Cage 2**	**Cage 3**
Initial #	30	30	30	30	30	30
Final #	27	18	24	0	0	0

Table 3. Calculated data for the toad and frog sites for 1998 and 1999.

Statistical Calculations of Survivorship at Upper Frohner Meadow					
Site/Year	**Total #**	**Mean**	**Std. deviation**	**% Survivorship**	**% Error**
Toad Upper 1998	69	23	4.58	76.7	15.3
Toad Lower 1998	0	0	0	0	0
Toad Upper 1999	88	44	2.83	88	5.7
Toad Lower 1999	42	20	19.3	39.4	38.6
Frog Upper 1999	100	50	0	100	0
Frog Lower 1999	97	48.5	2.12	97	4.2

Figure 1 -- Upper Frohner Meadow study site

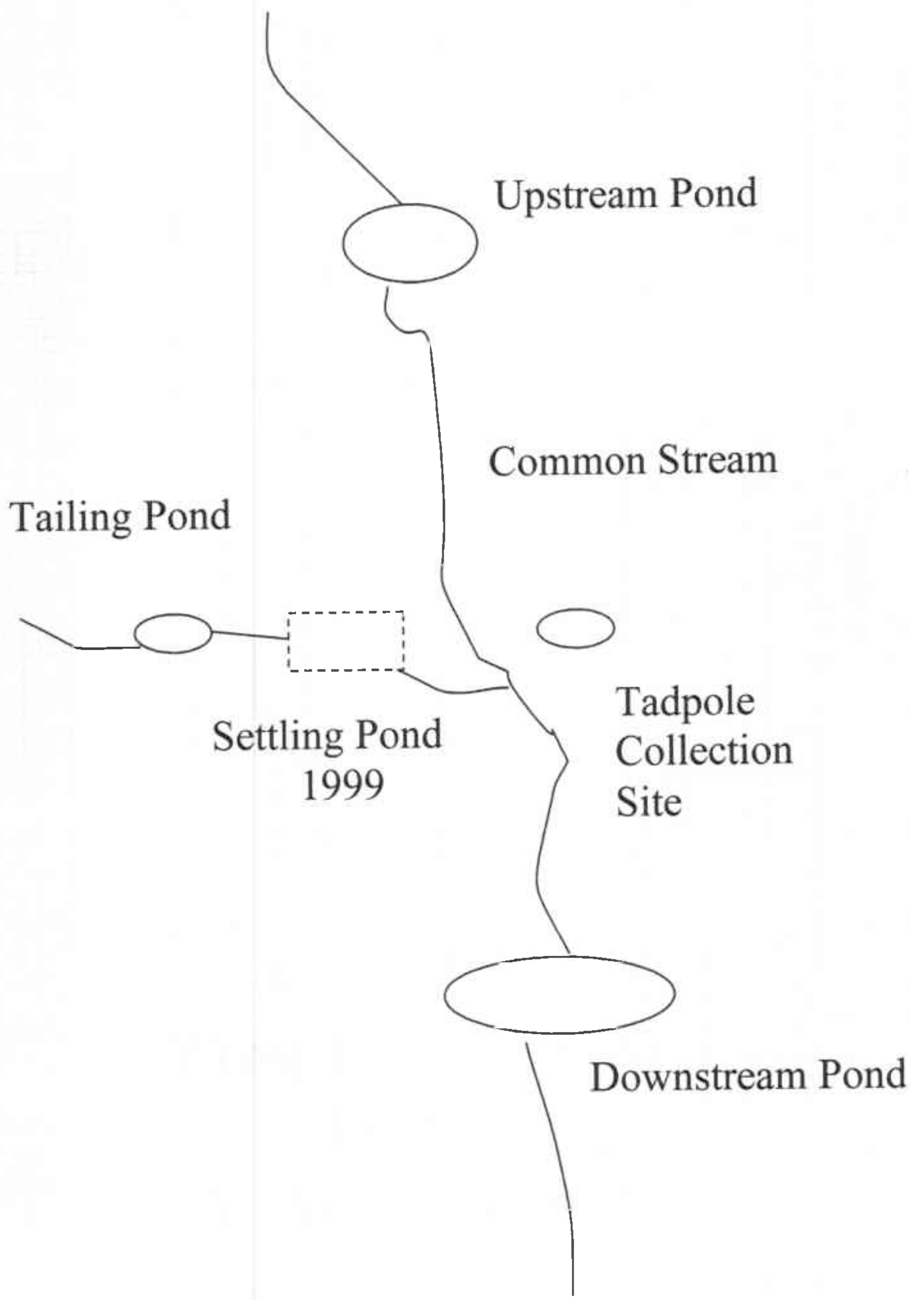


Figure 2. The average survivorship per enclosure for the Western toad tadpoles for the 1999 data.

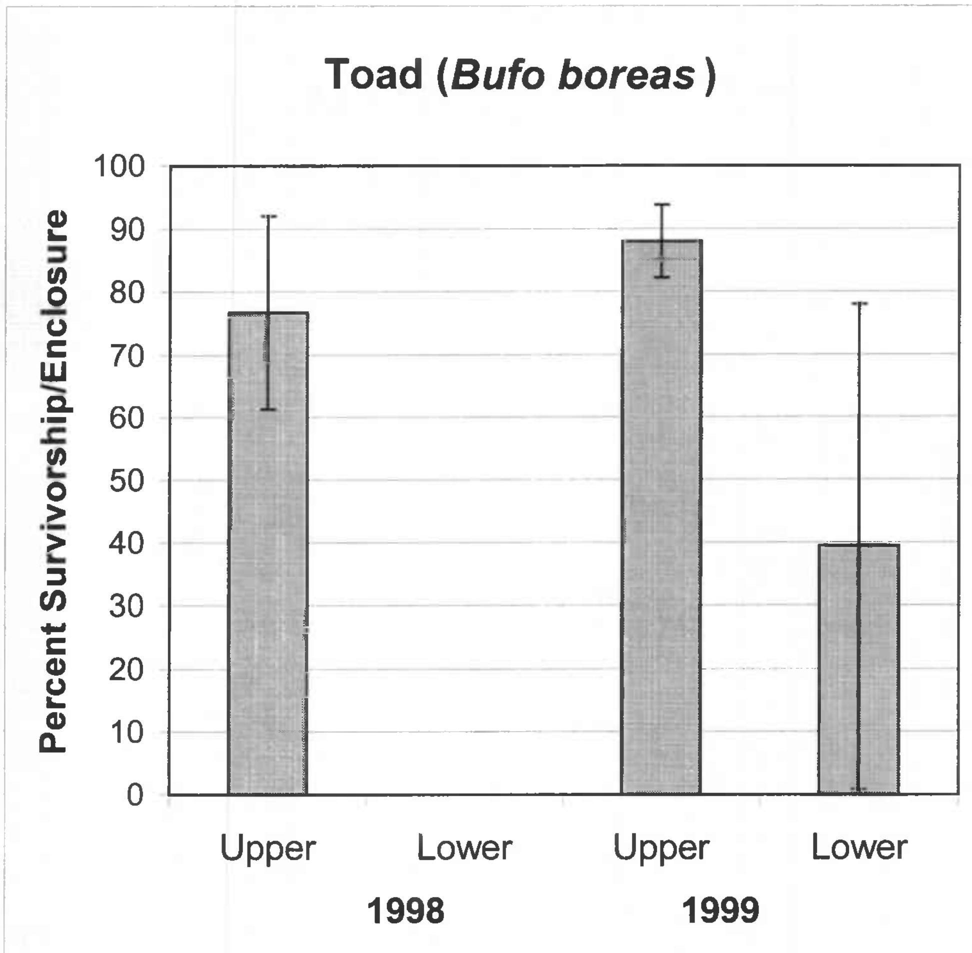


Figure 3. The average survivorship per enclosure for the spotted frog tadpoles for the 1999 data.

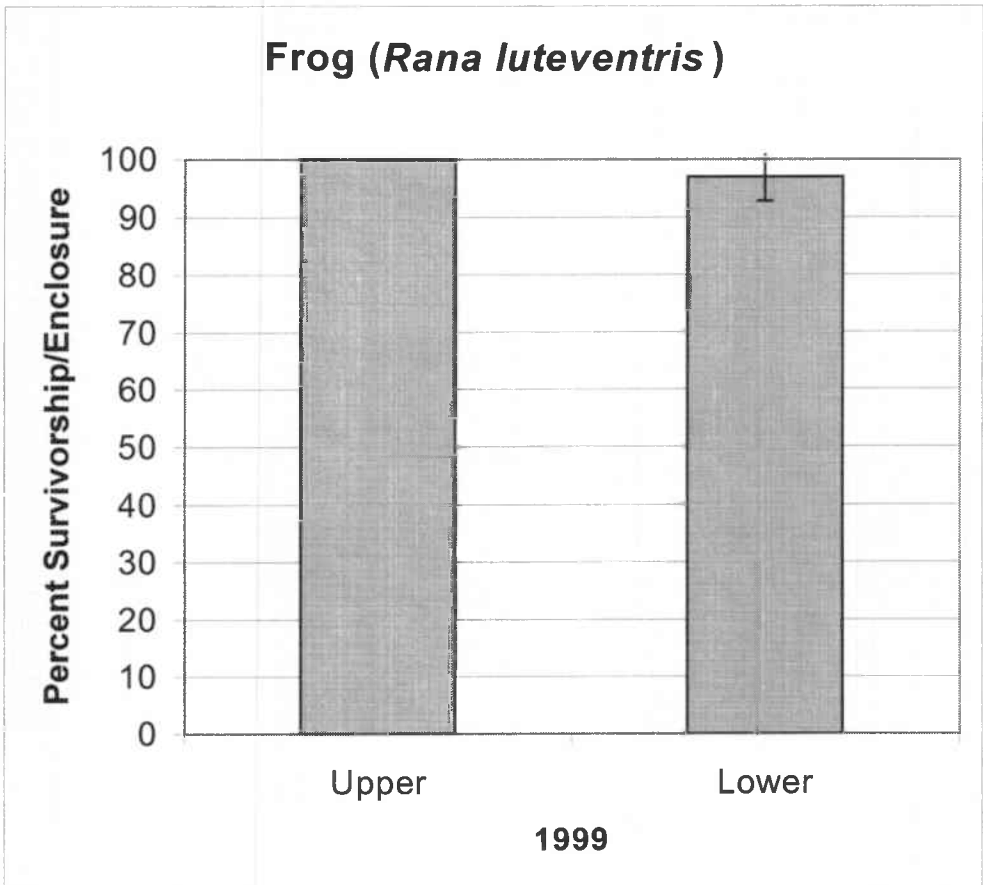
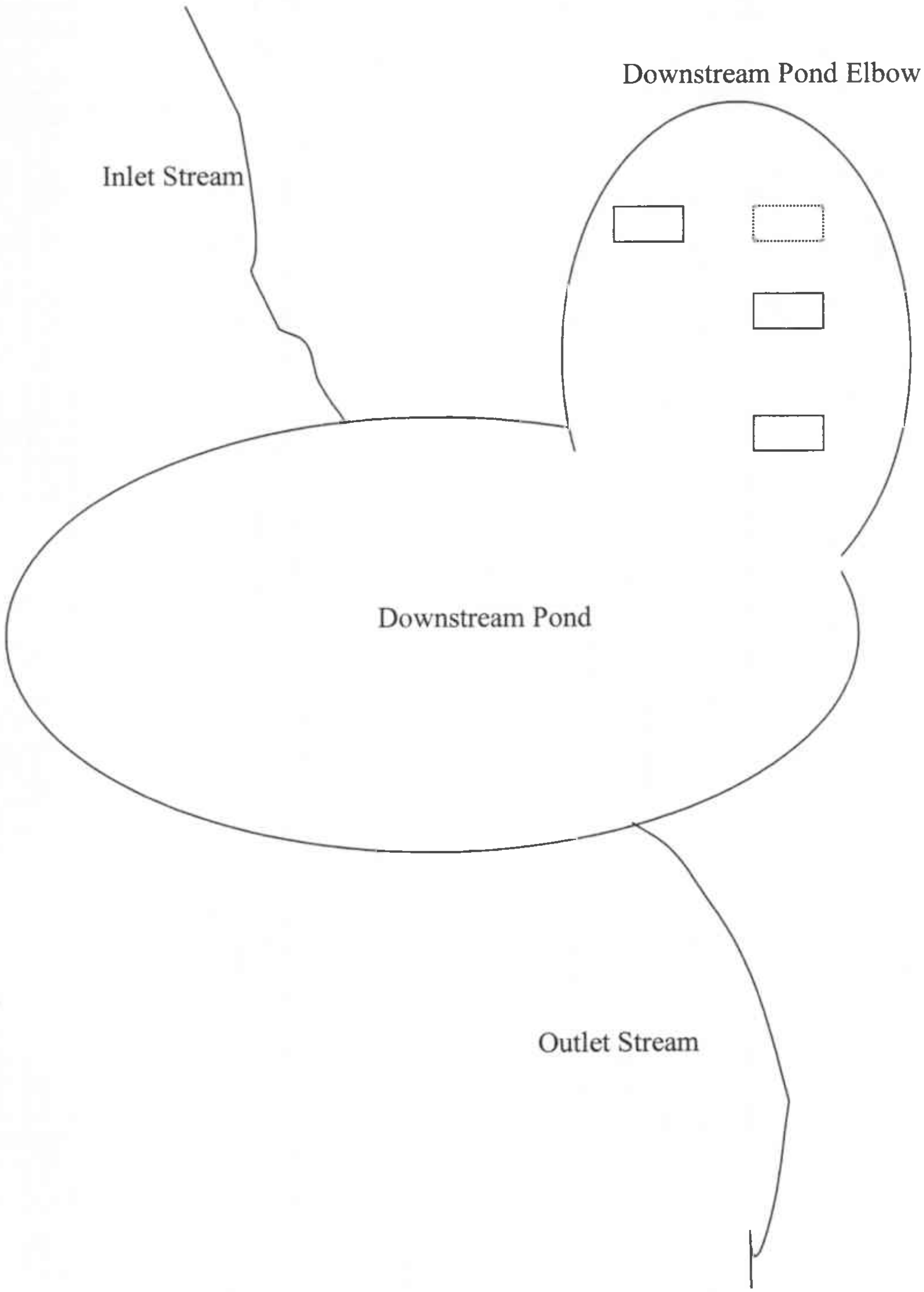


Figure 4 - The tadpole cage arrangement in the downstream pond at Frohner Meadow. The solid boxes represent the 1999 study cage placement, the dotted cage represents where the cage should have been placed to keep the cage data consistent.



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