

**A Study of the Effects of Dissolved Oxygen Levels on the Growth of**  
*Rana catesbeiana* and *Pimephales promelas*

BIOS 569 – Practicum in Aquatic Biology

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### Abstract

Bioindicators serve to simplify the research involved in identifying large-scale priorities for conservation. The value of amphibians as useful bioindicators depends on their relative sensitivity to environmental factors. One aspect of aquatic environments that has been a matter of concern is decreased oxygen levels due to eutrophication. This study compares the relative sensitivities of bullfrog (*Rana catesbeiana*) tadpoles and fathead minnows (*Pimephales promelas*) to low dissolved oxygen levels. The hypothesis is that growth will be higher in high oxygen conditions than in low oxygen conditions, and that the tadpoles will show a larger difference in growth from the high oxygen and low oxygen conditions than the minnows, thus demonstrating a higher sensitivity to oxygen levels. The study found that DO had a significant effect on the growth of the minnows ( $P = 0.002$ ). The minnows in the low oxygen treatments gained less body weight than those in high oxygen treatments. The effect of DO on tadpoles was not significant ( $P = 0.096$ ), however the trend showed that high oxygen conditions favor a faster growth rate than low oxygen conditions. The relative sensitivities of the minnows and the tadpoles were not significant when compared ( $P = 0.131$ ). However, the minnows showed a larger difference between the high and low oxygen aquaria when comparing the change in mass than the tadpoles. This trends demonstrated in this experiment did not support the use of bullfrog tadpoles as good bioindicators of oxygen stress, but further study is needed before solid conclusions are drawn.

## Introduction

Conservation efforts that depend on understanding the effects of environmental conditions on ecosystems are often limited due to the vast number of species, processes, and interactions that exist compared to the resources available to conduct research and conservation measures. Bioindicators serve to simplify the research involved in identifying large-scale priorities for conservation. A bioindicator is simply a biotic indicator of the overall condition of the environment. Studies have found that the representation of other species is quite high when using bioindicator data (Moore et al., 2003). For example, a study was done on the affects of a road construction project that caused a large input of fine sediments into pristine streams in the Redwood National Park. The study concluded that monitoring stream amphibian densities is an excellent way to gage the ecological stress resulting from fine sediment inputs due to the combination of sensitivity to this stressor and their attributes as highly philopatric, long-lived, and relatively stable populations in undisturbed habitats (Welsh and Ollivier, 1998). A major advantage of using bioindicators is the shortening of the response time to problems by focusing on the quicker responses of sensitive species instead of monitoring the relatively slow response time of the ecosystem as a whole (Rapport, 1992).

Amphibians are good bioindicators for many practical reasons. They are easy to catch and they are suitable for laboratory experiments and simulations (Beebe, 1996). Additionally, amphibians are characteristically more sensitive to their environment than many other organisms due to many factors including: larger constraints in terms of basic needs resulting from their complex life histories requiring both aquatic and terrestrial habitats, a higher vulnerability to water pollution and electrolytes due to the permeability

of their skin, and exposure of their eggs to the environment because they are protected only by a thin gelatinous layer (Vitt et al., 1990). Additionally, there has been speculation over a possible widespread population drop which could indicate a higher relative sensitivity to environmental factors than other aquatic species (Houlahan et al., 2000). The timing of the global trend strongly suggests anthropogenic causes. Using bioindicators could help reveal the consequences of anthropogenic modifications to ecosystems.

The value of amphibians as useful bioindicators depends on their relative sensitivity to environmental factors. One aspect of aquatic environments that has been a matter of concern is decreased oxygen levels due to cultural eutrophication. While amphibian sensitivities and usefulness as bioindicators have been examined for environmental factors such as toxicity, UV-radiation, and ecosystem disturbance, the relative sensitivities of amphibians to dissolved oxygen have been largely unexamined. This study compares the sensitivities of bullfrog (*Rana catesbeiana*) tadpoles and fathead minnows (*Pimephales promelas*) to low dissolved oxygen levels in a controlled laboratory setting. The sensitivity will be measured by comparing the growth of each species in low oxygen conditions relative to that in high oxygen conditions. In general, I predict that growth will be higher in high oxygen conditions than in low oxygen conditions for both species due the physiological stress of the hypoxic environment. Additionally, I hypothesize that the tadpoles will show a larger difference in growth from the high oxygen and low oxygen conditions than the minnows, thus demonstrating a higher sensitivity to oxygen levels. It is likely that late stage bullfrog tadpoles will be

more sensitive because they have a higher critical oxygen tension than fathead minnows (Ultsch et al., 1999).

### **Materials and Methods**

Materials:

- 16 10-gallon glass aquaria
- 8 Air Bubblers
- Tetrafin Tropical Fish Flakes
- Rabbit Chow Pellets
- Blender
- Dip nets
- Electronic scale
- Lake Water
- 80 Fathead minnows
- 40 Bullfrog tadpoles

#### *The Species*

Fathead minnows are a common species native to much of North America from Quebec to Northern Territories, and south to Alabama, Texas, and New Mexico (Page and Burr, 1991). They are also a widely established introduced species across the United States due to their popular use as bait fish (Nico, 2004). Fathead minnows vary in length from 65-70mm. They usually live for less than one year. These minnows are opportunistic feeders, and their diets can include algae, protozoa, plant matter, insects, rotifers, and copepods (Paulson and Hatch, 2002). This species has a wide range of

habitats which includes headwaters, creeks, small rivers, ponds, and lakes. They are notable for their tolerance to many conditions that are unsuitable for most fishes (Page and Burr, 1991). They have a critical oxygen tension ( $P_c$ ) of 31.8 mmHg (Ultsch et al., 1999). This makes them much more tolerant of low-oxygen conditions (Castleberry and Cech, 1992; Robb and Abrahams, 2002; Paulson and Hatch, 2002). This characteristic makes them suitable to test for non-lethal effects of hypoxic conditions.

The native range of bullfrogs stretches across the eastern half of the United States and also into New Brunswick and Nova Scotia. They have been introduced further west as a result of commercial harvesting of frogs' legs. Bullfrog tadpoles range from 10.2 – 17.1 cm (Behler and King, 1979). They usually take 2 – 3 years to mature. The tadpoles feed mostly on algae, along with bacteria and plant material. Bullfrog tadpoles can breathe air as early as metamorphic stage I (staging according to Taylor & Kollros, 1946). Therefore, low-oxygen environments will cause an increased frequency of air-breathing, and will be unlikely to result in death (Ultsch et al., 1999).

### *Methods*

This experiment was designed to compare the effects of high and low oxygen environments on the growth rates of bullfrog tadpoles and fathead minnows. The experiment was conducted in the UNDERC Wet-lab using 16 10-gallon glass aquaria set up on 2 shelving units. Eight were placed on the middle shelves, and eight on the bottom shelves. The tanks were exposed to natural light from the windows; therefore no artificial aquarium lights were used. The aquaria were cleaned, set up, and filled with water from Tenderfoot Lake.

High and low oxygen conditions were achieved by either bubbling with atmospheric air or by having no bubbling. Eight of these aquaria were constantly bubbled with oxygen, while the other eight were not bubbled.

Ninety-two *Rana catesbeiana* tadpoles were caught using dip nets near the dock at Tenderfoot Lake. From this group, forty tadpoles of similar size were selected. The tadpoles were weighed individually by blotting them with paper towels and weighing them in cups filled with water. The mean weight of the tadpoles selected was 23.506g ( $\pm$  3.024g). The weights ranged from 18.96g to 29.99g. The tadpoles were divided into 8 groups, each of similar size, and distributed to the eight aquaria.

Approximately 200 fathead minnows were purchased from Northern Highlands Sports. They were sorted by size into groups of about 25. Two groups of ten were selected and weighed as a group by blotting them with paper towels and weighing them in cups filled with water, and then the remaining five were weighed individually to find the variation. One of the groups was assigned to a high oxygen aquaria and the other was assigned to a low oxygen aquaria. This was repeated 4 times to have 8 aquaria, each with ten minnows. The mean weight of each minnow was 1.330g ( $\pm$  0.251g).

After being placed into the aquaria, the animals were allowed to acclimate for 24 hours before the experiment began. This also allowed the oxygen levels to stabilize with the addition of the animals.

The tadpoles and minnows were fed daily with a blended mix of rabbit chow pellets and Tetramin fish flakes in a 3:1 weight ratio. For the first two days, each tank received 10% of their total body weight in food per day. It was observed that this a large

portion of the food was uneaten and was clouding the water of the aquaria, so starting with day 3, the ration was cut to 5% of the total body weight per day.

The dissolved oxygen and temperature of each tank was measured daily until it stabilized and then was monitored periodically. On day 2, every low oxygen tank received an air bubbling treatment for one hour due to the very low levels of oxygen from the decomposition of the excess food. In the bullfrog aquaria, the DO levels averaged 7.66mg/L ( $\pm 0.221$ mg/L) and 0.48mg/L ( $\pm 0.075$ mg/L) for the high and low oxygen respectively. For the minnow aquaria, the DO levels averaged 8.10mg/L ( $\pm 0.365$ mg/L) and 1.34mg/L ( $\pm 0.273$ mg/L) for the high and low oxygen respectively.

In order to keep the amount of waste from building up, the tanks were siphoned on a regular basis. On day 3, half of the water was changed in each tank. On days 5 and 7, the wastes on the bottom of the tank were siphoned and replaced with water (approximately 1.25 gallons per tank). On day 8, each tank was completely siphoned, cleaned out, and refilled. On days 10 and 12, wastes were siphoned from each tank and the water was replaced.

On day 8 of the experiment, the tadpoles and minnows were weighed by the same methods described earlier. The food rations were adjusted to the new body weights and the experiment was continued for another week.

On day 14, the tadpoles and minnows were weighed for a final time. The response variable to measure growth was the difference between the final and initial mass of all the animals grouped by aquaria. Differences in mass for minnows were calculated by adjusting the initial weight by multiplying it by the percentage of minnows alive on the day they were measured and subtracting the total weight of those minnows from the

adjusted initial weight. Differences between high and low oxygen treatments within species were analyzed with one-way ANOVA and ANCOVA tests while comparisons between species were analyzed with two-way ANCOVA tests. All data was analyzed using SYSTAT 10.

### Results

DO levels had a significant effect on the growth of the minnows ( $P = 0.002$ ). The minnows in the low oxygen treatments gained less body mass than those in high oxygen treatments. The effect of DO on tadpoles was not significant ( $P = 0.096$ ), however the trend showed that high oxygen conditions favor a faster growth rate than low oxygen conditions. While the relative sensitivities of the minnows and the tadpoles were not significant when compared ( $P = 0.131$ ), minnows showed a larger difference between the high and low oxygen aquaria when comparing the change in mass than the tadpoles.

#### *Tadpoles*

On day 8 of the experiment, the tadpoles in high oxygen aquaria had a mean difference in mass of 1.993g with a standard deviation of  $\pm 6.678$ g. The tadpoles in low oxygen conditions had a mean difference in mass of -0.295g with a standard deviation of  $\pm 4.142$ g. One week later, the tadpoles in high oxygen aquaria actually decreased in mean weight to difference of 1.868g ( $\pm 3.339$ g) and the low oxygen tadpoles continued to lose weight with a mean difference of -4.678g ( $\pm 6.934$ g). The changes in weight are shown by percentage of initial mass in Figure 1 and Figure 2.

Figure 1: Percent Difference in Tadpole Mass in High Oxygen Treatments

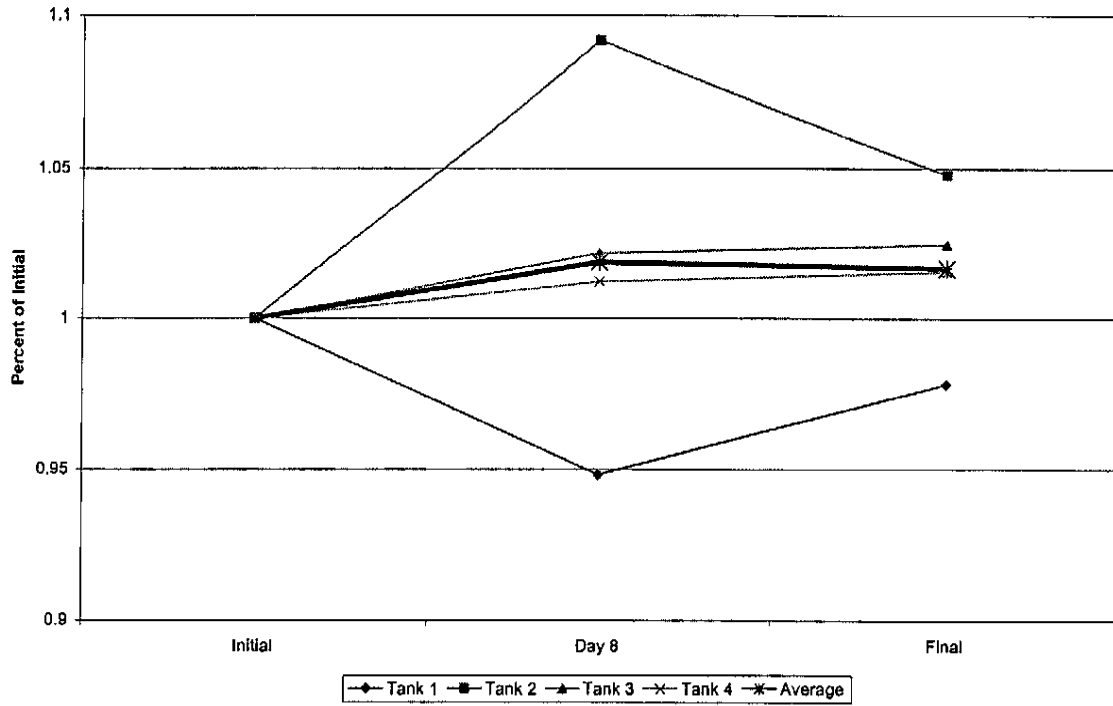
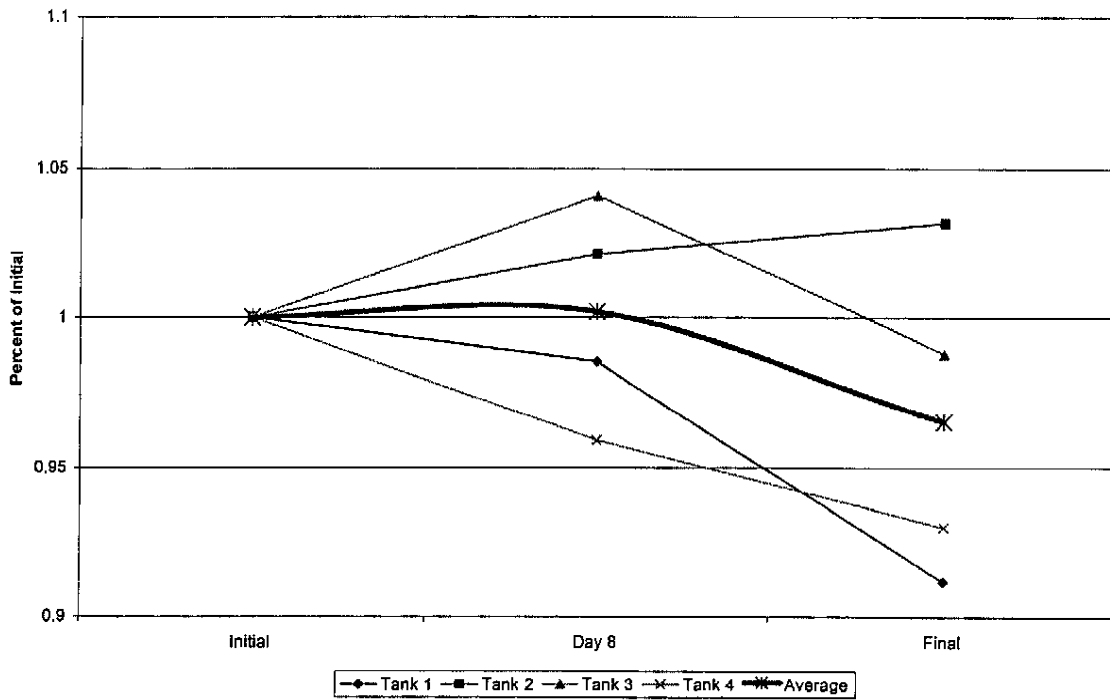


Figure 2: Percent Difference in Tadpole Mass in Low Oxygen Treatments



Analysis of variance showed that the difference in the final mass between the high and low oxygen treatments was not statistically significant ( $P = 0.14$ ). However, there is a possible trend moving towards a significant difference as is shown by the data collected halfway through the experiment. Using ANOVA with the data from day 8 of the experiment, the difference between the high oxygen and low oxygen treatments has a P-value of 0.582. In the second week, the average difference in tadpole mass diverged further as is shown by the considerably lower P-value. ANCOVA tests were used to examine any possible effects of initial mass on the rate of growth. With initial mass as a covariate, ANCOVA produces a P-value of 0.096 comparing the difference in final mass of the high and low oxygen tadpoles.

### *Minnows*

After one week, the minnows in the high oxygen aquaria had an increase in mass per aquarium of 0.429g ( $\pm 0.701$ g). The minnows in low oxygen aquaria had a difference in mass of 0.1055 ( $\pm 0.228$ g). The averages of the final differences in mass for the high and low oxygen minnows were 0.337 ( $\pm 0.152615$ g) and -0.2375 ( $\pm 0.156932$ g) respectively. Figures 3 and 4 show the general trends of changes in mass by percentage of initial mass.

ANOVA tests showed a significant difference between the changes in mass of the minnows in the high and low oxygen treatments at the end of the two-week experiment ( $P = 0.002$ ). The differences between the high and low oxygen minnow weight changes halfway through the experiment were not significant using ANOVA ( $P = 0.414$ ).

Figure 3: Percent Difference in Minnow Mass in High Oxygen Treatments

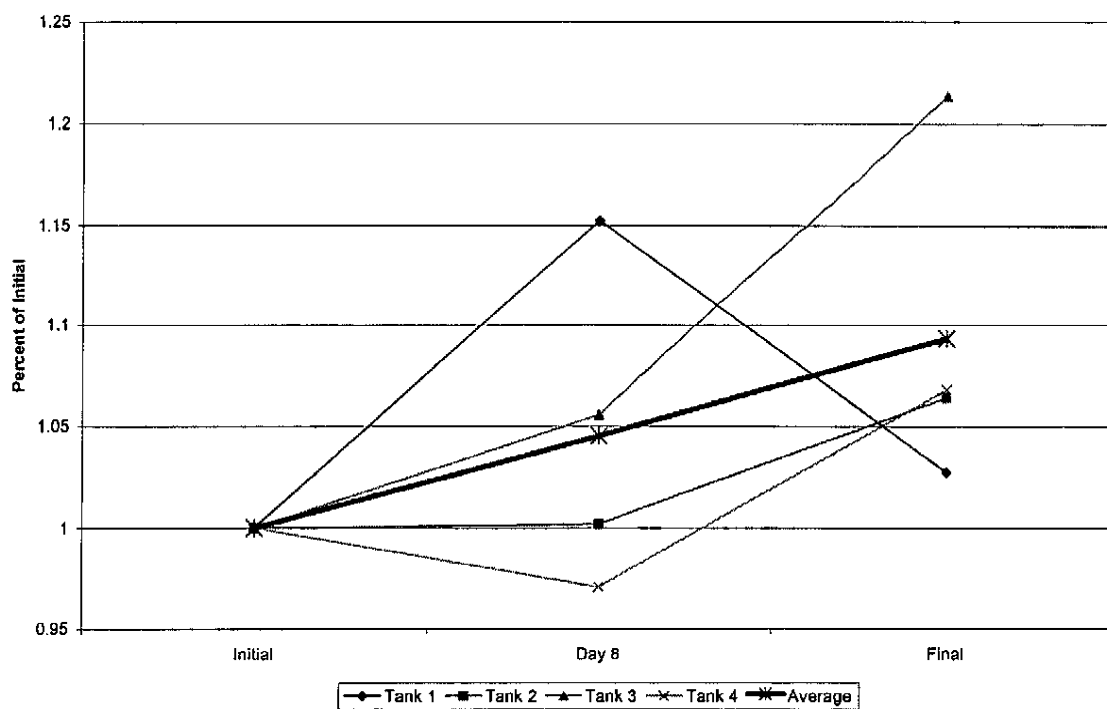
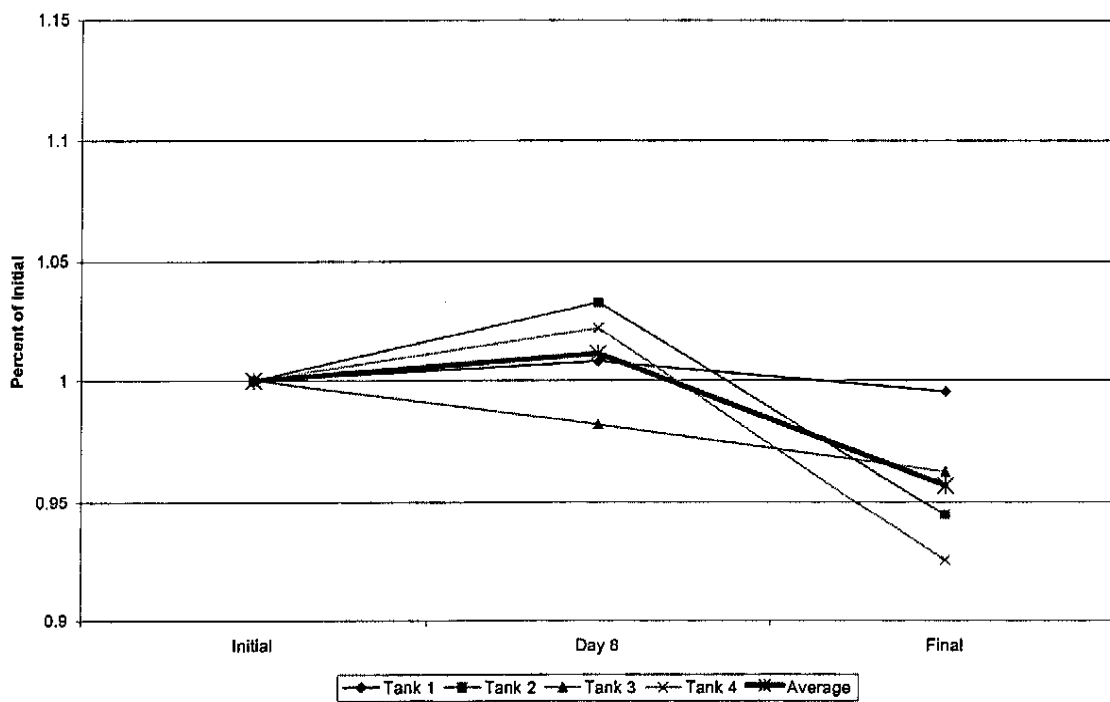


Figure 4: Percent Difference in Minnow Mass in Low Oxygen Treatments



ANCOVA tests comparing high and low oxygen final mass with initial mass as a covariate were significant with a P-value of 0.007.

The majority of the minnows did not survive to the end of the experiment. In the first week, an average of 3 out of 10 minnows died in each high oxygen aquarium, and an average of 2.5 died in each low oxygen aquarium. The second week had an average mortality of 3.25 minnows in each high oxygen aquarium and 3 in each low oxygen aquarium (Figure 5). At the end of the experiment, there was an average survival rate of 37.5% in high oxygen aquaria and 45% in low oxygen aquaria. Analysis of variance did not show a significant difference between the number of deaths in the two types of treatments ( $P = 0.56$ ).

#### *Tadpoles vs. Minnows*

To draw comparisons between the relative sensitivities of the two species, a two-way ANCOVA test compared difference in mass with species and oxygen level as categorical variables, and initial mass as a covariate. The only variable that was significant was oxygen level (Table 1). However, while not statistically significant, minnows showed a greater difference between the change in mass between the high and low oxygen treatments. The difference between percent change in mass of the high and low oxygen levels for the tadpoles was 5.56% compared to that of the minnows which was 11.08% (Figure 6).

Figure 5: Minnow Deaths

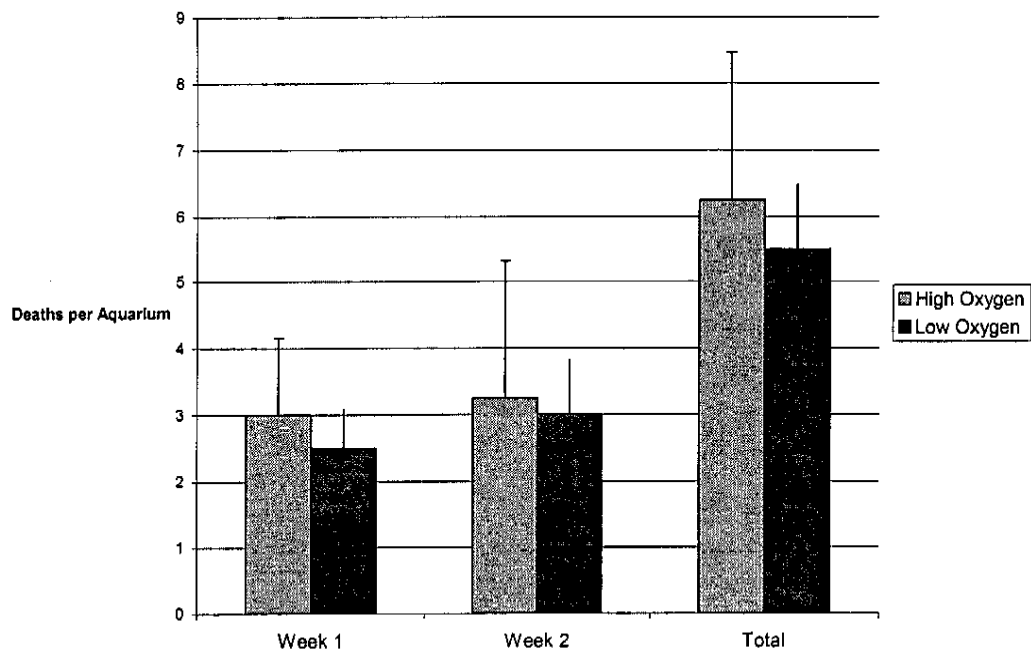
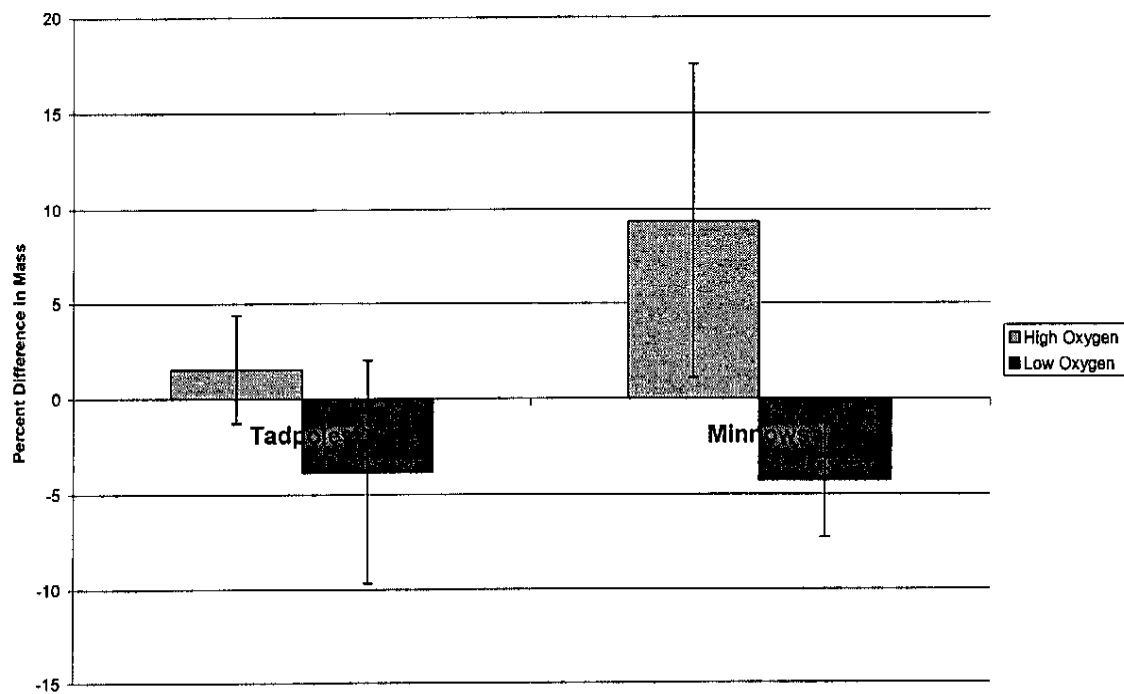


Table 1: ANCOVA test of Difference Between Species Response to Oxygen Levels

Source	df	F-ratio	P
Species	1	1.961	0.189
Oxygen Level	1	14.102	0.003
Species * Oxygen Level	1	2.661	0.131
Initial	1	2.714	0.128

Figure 6: Average Percent Difference in Mass Between Species



## Discussion

In agreement with my first hypothesis, both the Fathead Minnows and the Bullfrog tadpoles were negatively affected by hypoxic conditions as was shown by their decreased growth rate. Non-lethal hypoxia creates the stress of increased physiological costs (Robb and Abrahams, 2002). The effects of this stress were shown in this study through an average loss of mass in low-oxygen aquaria. On average, animals in normoxic aquaria had a net weight gain at the end of the experiment, which as a control group, reinforces the relationship between low oxygen and decreased growth rates.

Behavior modification, induced by low-oxygen conditions, may have contributed to the differences in the growth of the high and low oxygen organisms. When fish are in environments with near critical dissolved oxygen levels, they exhibit behaviors that are not conducive to feeding (Doudoroff and Shumway, 1970; Petit, 1973; Weber and Kramer, 1983). In the short term, they increase activity to increase their probability of finding more oxygenated waters. They also use the relatively oxygen-rich surface waters through aquatic surface respiration (ASR) (Gee et al., 1978). In the long-term, fish will decrease their activity in order to bring down their oxygen demand (Robb and Abrahams, 2002). These behavior changes may have influenced the growth in the fathead minnows. Similar behaviors were qualitatively observed in the bullfrog tadpoles. They were noticeably more lethargic in the low-oxygen aquaria than the high-oxygen aquaria, and they seemed to spend more time near the surface. Being that the food sank to the bottom of the aquaria, this behavior could have reduced their consumption of food.

Contrary to my second hypothesis, the data suggests that the minnows were more affected by the low-oxygen conditions than the tadpoles. Especially at the advanced

stage of the tadpoles, the bullfrog have fairly well-developed lungs (Simard et al., 2003). This allows them to gulp air when dissolved oxygen levels are not sufficient. The minnows, equipped only with gills, are far more reliant on dissolved oxygen. This physiological advantage may have notably contributed to the tadpoles' hypoxia tolerance.

Furthermore, the considerably larger size of the tadpoles must be taken into account as a possible explanation for their weaker response to the oxygen treatments. Larger organisms tend to have a slower rate of growth due their increased demand for resources to simply sustain their current body size. Additionally, a comparative study on bullfrog tadpoles, fathead minnows, and two other fish species showed that fathead minnows had a relatively higher metabolic rate (Ultsch et al., 1999). If the tadpoles had been allowed more time to grow, it is possible that they would have shown further differentiation between high and low oxygen conditions. Food was not likely a limiting factor as all tanks had excess uneaten food on a daily basis.

Minnow mortality rates were quite high in this experiment. This is possibly due to a combination of a short life expectancy and increased stress from handling. The daily rates of mortality did not seem to be affected by syphoning and water changes, therefore it appears to be a likely result of stress and not toxicity levels from decomposing wastes.

There were a number of possible constraints in this experiment that may have affected its outcome. The most notable is that the aquaria had unequal biomass. The increased food, wastes, and resulting toxins, such as ammonium and nitrate, may have introduced an uncontrolled variable into the experiment. Also, it would be interesting to use a younger tadpole that had not yet developed lungs. This would allow for a more accurate comparison between the two groups. With the two groups in this experiment,

direct comparisons are not as strong as they would be if these constraints were corrected for.

In order to draw solid conclusions on the relative sensitivities of minnows and tadpoles, further study is needed. In addition to measuring how growth is affected by hypoxic and normoxic conditions, other factors could be studied such as behavior, predator-prey interactions, and multiple-stress interactions such as toxin exposure. There are many ecological consequences to species sensitivity to oxygen levels. There have been a number of studies that have examined how hypoxia affects habitat choice and predator-prey interactions. They have shown that hypoxia tolerance in prey species may allow them to escape predation by occupying low dissolved oxygen areas of a body of water (McIntyre and McCollum, 2000; Robb and Abrahams, 2002; Ultsch et al., 1999). These interactions should be further explored because if changes in dissolved oxygen levels affects species interactions in addition to the fitness and behavior of individual species, these changes could potentially cause large shifts in community structures.

While this experiment did not support the use of bullfrog tadpoles as good bioindicators of oxygen stress, further study is needed before solid conclusions are drawn. Longer term laboratory and field studies would help to determine their usefulness as bioindicators with more certainty. Additionally, a variety of amphibian and other aquatic species should be tested for relative sensitivities to hypoxic conditions. If the most sensitive species in a community is found, then the difficult task of monitoring and managing aquatic environments will be greatly simplified.

With a firm understanding of organism sensitivity to water with low levels of dissolved oxygen, the results of both natural and anthropogenic mechanisms that change

oxygen levels can be better monitored and understood. If further research determines which species are most sensitive to hypoxia, an intensive survey focus on those species could aid conservationists in catching problem areas early before major damage is done to the community. The use of bioindicators for critical oxygen levels is an important step in conservation as many lakes are undergoing cultural eutrophication which can result in major declines in aquatic populations.

### **Acknowledgements**

I would like to thank Kerry Yurewicz for her guidance and advice for this study, Amy Locke for making tadpole time much more lively, Jamie Peters and Meredith Shepherd for helping clean muck and carry many buckets of water, Al Klein for 'help' in feeding, Lisa Knott for helping me find my way around the wetlab, Neil Vargas for Excel advice and much needed work breaks, James Romanchek for Systat advice, Andy Bordon and Gary Belovsky for answering the numerous last minute questions, Karen Francl for helpful comments on my proposal, and all the rest of the UNDERC students for keeping me sane. I would also like to thank UNDERC for providing me with this opportunity to conduct this study and the Hank Family for their contributions which made this possible.

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