CAN NUTRIENT ADDITIONS MEDIATE GAPE LIMITED PREDATION IN LARVAL *RANA SPHENOCEPHALA*?

By

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Some predators are limited in the size prey item that they can catch and consume, and this is referred to as "gape limited". Prey found with these predators may have the ability to respond by growing rapidly to reach a size too large to be preyed upon, and thus reach a size refuge from predation. Due to decreased competition, these prey may have the additional benefit of experiencing an environment with more consumable resources available.

One factor that may influence the ability of prey to outgrow the gape limitation of their predators is nutrient availability. Providing an influx of limiting nutrients can result in an increase in abundance of primary producers, and allow herbivore prey living in nutrient enriched environments to grow more quickly than those that live in a nutrient-limited environment. Thus, prey living in nutrient rich environments may be more likely to be able to outgrow the gape limitation of a predator, and reach a predator-free size refuge, than those prey living in areas with less nutrient availability.

I was interested in whether nutrient additions can mediate the effects of gape limited predation in an aquatic food web. A mesocosm experiment was performed to evaluate the effects of nutrient influx on *Rana sphenocephala* tadpoles in the presence of two groups of

bluegill fish predators: one that is likely to become gape limited and one that is not. The nutrients added were crystalline $NaNO_3$ and K_2HPO_4, in a 16:1 N:P ratio, in six different amounts ranging from 0.0 to 4.0 mg/l. The experiment ran for four months to evaluate the larval anuran response (survival, mass, time to metamorphosis) after one pulse of nutrient influx. Algal biomass and zooplankton abundance were evaluated at two times during the experiment as zooplankton feed on algae and bluegill can also feed on zooplankton.

The results of this experiment indicate that although nutrient addition was found to significantly impact larval anuran survival, there was no evidence of an interaction between nutrient addition and predation. Thus, there is no strong support for the hypothesis that bottom up forces created by nutrient addition can mediate the top down forces created by predation. Higher abundances of leopard frogs in more nutrient rich environments, as a result of higher survivorship, may change the structure of aquatic communities and have cascading effects on the rest of the food web.

Currently we are experiencing an influx of inorganic nutrients reaching our waterways due to anthropomorphic sources. Worldwide human activities, such as agriculture and development, have increased the amount of nitrogen and phosphorus reaching our aquatic systems. This experiment reinforces the idea that increases in these nutrient levels can cause changes in life history characteristics of populations and in community structure of aquatic and terrestrial systems impacted by nutrient additions.

CAN NUTRIENT ADDITIONS MEDIATE GAPE LIMITED PREDATION IN LARVAL *RANA SPHENOCEPHALA*?

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By

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CHAPTER 1

INTRODUCTION:

Predator-prey interactions are fundamental interactions in ecology. These essential associations result in the removal of individual prey and impact the dynamics of ecosystems (Brooks and Dodson 1956, Paine 1966, Sih *et al*. 1985). Predation can serve as a top-down force, regulating organisms occupying lower trophic levels. Top-down theory (Hairston *et al*. 1960, Paine 1980) predicts that an increase in the abundance of predators will result in a decrease in abundance of the prey of those predators, and an increase in their prey's food resources. For example, a study involving northern pike, lake perch, and zooplankton found that a reduction in lake perch juveniles resulted in more zooplankton (Lennart *et al.* 1996). Another experiment found that the reduction of zooplankton by fish predators resulted in an increase in biomass of algae (Carpenter et al. 1987).

Predation has been found to have both lethal and nonlethal effects on prey. Nonlethal effects of predators on prey populations may include changes in prey behavior, physiology, morphology, and even life history (Kats and Dill 1998, Lima 1998, Tollrain and Harvell 1999, Relyea 2002). One particularly important nonlethal effect predators can have is to reduce prey foraging behavior. This reduction in foraging may decrease encounters with predators and reduce the number of prey being consumed by predators, but includes the risk of growing slowly and reducing future fecundity (Werner and Hall 1988, Werner and Anholt 1993, Relyea and Werner 1999, Urban 2007, Urban 2008). Alternatively, prey may respond to predators by using rapid growth to achieve a body size too great for the handling capacity of a predator (Brooks and Dodson 1965, Zaret 1980, Urban 2007, Urban 2008). Specifically, prey may emphasize rapid growth in the presence of predators that are limited by their own gape size ("gape limited") in the size prey they can consume. In these cases prey risk additional encounters with predators by increasing their foraging activity in order to find enough consumable resources to increase growth rates and optimally reach a size refuge from predation. Faster growth has the additional benefit of inferring greater potential for a juvenile to become a more successful adult because of better resource acquisition and usage (Wilbur *et al*. 1983, Chelgren *et al.* 2006).

There is considerable evidence that gape size limitation is an important factor in some predator-prey dynamics (Brooks and Dodson 1965, Paine 1976). The size of prey a predator consumes is frequently related to the size of the predator, with larger predators capable of consuming larger prey individuals than smaller predators (Brooks and Dodson 1965, Christensen 1996, Truetemper 2005, Arim *et al.* 2010). In other cases, predator mouth size or feeding mechanics instead of predator body size itself is more relevant to a predator's ability to consume prey of a given size (Paine 1976, Zaret 1980, Christensen1996, Persson *et. al* 1996, Nilsson and Brönmark 2000). In any of the cases of gape limitation, prey capable of growing larger than a predator can consume will experience a refuge from predation. The prey that reach such a refuge are often rewarded with increased consumable resources due to lower rates of competition (Paine 1976).

One factor that may influence the ability of prey to outgrow the gape limitation of their predators is nutrient availability. Bottom up theory predicts that resources for the primary producers are the limiting factor in composition of trophic levels and limit the total energy found in a trophic system (Hairston *et al.* 1960, Abrams 1993). Providing an influx of limiting nutrients can result in an increase in abundance of primary producers (Hillebrand 2002, Rabalais 2002, Elser et. al 2007, Gruner et. al 2008). As the energy in the lowest trophic level increases, the amount of energy transferred into the herbivore trophic level also increases. The literature to date, however, shows significant variation in how the addition of nutrients affects primary production, as well as the additional trophic levels found in these ecosystems (Abrams 1993, Warren and Spencer 1996, Rabalais 2002). When looking at one meta-analysis, 36 experiments showed an increase in fisheries yield associated with an increase in primary production from nitrogen increase, but in 47 food web studies it was found that nitrogen increased phytoplankton biomass but the increases did not transfer to higher trophic levels (Rabalais 2002). Some evidence suggests that herbivore prey living in nutrient enriched environments can grow more quickly than those that live in a nutrient-limited environment (Travis 1984, Babbit 2001, Schiesari *et al.* 2006). If so, it is possible that prey living in nutrient rich environments are more likely to be able to outgrow the gape limitation of a predator, and reach a predator-free size refuge, than those prey living in areas with less nutrient availability.

I was interested in whether bottom-up processes (i.e. the response to nutrient additions) can mediate the effects of top-down processes (i.e. gape limited predation) in an aquatic food web. I used food chains with one, two, or three trophic levels, comprised of algae, *Rana sphenocephala* tadpoles, and bluegill fish (*Lepomis macrochirus*). Specifically, I was interested in testing whether *Rana* will have increased success (increased survivorship, larger average mass, shorter time to metamorphoses) in the presence of small gape limited aquatic predators in treatments with increased nutrient levels when compared to those with lower resource levels. An increase in resources may allow prey to outgrow the gape size of predators at an accelerated rate, providing a refuge from predation. This effect should be more pronounced in systems with small gape-sized predators than with large gape predators. In order to evaluate this hypothesis I performed a mesocosm experiment examining predation on larval *Rana sphenocephala* as anuran herbivores by small-gaped and large-gaped predators (*Lepomis macrochirus*) at six different levels of nutrient additions.

METHODS:

Study System:

The herbivores used in our experiment were *Rana sphenocephala*, southern leopard frog, tadpoles. These tadpoles are commonly found in eastern North Carolina as they live in a variety of aquatic environments, from rivers to ephemeral ponds or ditches. Although not all frog species will oviposit where fish occur, *Rana* species will. *Rana* tadpoles mainly graze algae off substrate such as debris and aquatic vegetation, but phytoplankton may be consumed as well. These tadpoles reach a large size and can metamorphose in one summer season, or remain in a pond until the following spring (i.e. “overwinter” in the pond). The large size the tadpole is capable of reaching has been shown to exceed the gape sizes of smaller gaped vertebrate predators in previous experiments (Chalcraft and Resetarits 2003). *Rana* tadpoles were raised from several egg masses collected from a local body of water outside of Greenville, North Carolina.

The predators used are a type of sunfish, *Lepomis macrochirus*, commonly called bluegills. Bluegills are a very common inhabitant of ponds in North Carolina. This species of fish has a long history as an experimental organism both in the lab and in natural environments (Gerking 1962, Werner and Hall 1974, O’Brien *et al.* 1976, Werner *et al.*1981). *Lepomis macrochirus* is a generalist predator that feeds on a variety of invertebrates, but has been found to be very selective with prey size (Werner and Hall 1974, O’Brien *et al.* 1976). In field studies it has been found that larger prey items have higher survival rates than smaller prey items in the presence of bluegill (Werner and Hall 1974).

Experimental Methods:

I performed an experiment in mesocosms located at the West Research Campus of East Carolina University, located in Greenville, North Carolina. Mesocosms used were created from 1136 L cattle tanks. The mesocosms were equipped with standpipes to limit overflows due to precipitation, and the standpipes were covered with window screening to prevent loss of animals from the mesocosm as well as preventing organisms from entering the mesocosm. Window screen covers held in place with bungee cords placed on each tank were also used to exclude unwanted organisms from entering the mesocosms. On June 6, 2012 the mesocosms were filled with aged well water and then 1.8 kg of pine leaf litter was added to each tank to provide refuge for the tadpoles. Five days later a standard inoculation of phytoplankton and zooplankton homogenized from several local sources, with larger invertebrates excluded, were added to each mesocosm.

The experiment crossed four food chain types (plankton only, plankton and 400 tadpoles, plankton + 400 tadpoles + one small gape limited fish, plankton + 400 tadpoles + one large gape limited fish) with six nutrient addition levels (0, 0.25, 0.50, 1.00, 2.00. and 4.00 mg/l of nitrogen and phosphorus at a 16:1 ratio of nitrogen to phosphorus) for a total of 24 treatments (Table 1). These treatments were randomly assigned to mesocosms, in which four of the six nutrient levels were replicated twice and the others were represented once. Nutrient levels were manipulated by adding nitrogen and phosphorus at a 16:1 ratio that is appropriate when mimicking total nitrogen level variation in ponds in eastern North Carolina (Ardon *et al.* 2010, Chescheir 2003) and has been used in other experiments to replicate a variety of watershed systems with agriculture present (Butzler and Chase 2009, Turner 2004). Nitrogen was added in the form of crystalline $NaNO_3$, and the phosphorus was added in the form of crystalline K_2HPO_4. The nutrient addition was added only once at the start of the experiment to imitate the pulse of a large rain event washing excess nutrients from the surrounding landscape into an aquatic ecosystem. One week after the nutrients were added the tadpoles were added, followed by the addition of predators 24 hours later.

Zooplankton and two samples of algal biomass were collected in the middle (August, 2012), and again at the conclusion (October, 2012) of the experiment. Algal samples were collected to evaluate abundance using chlorophyll *a* levels. Phytoplankton present in the water column was collected by using a 1L vessel filled from the middle of the water column. The sample volume was then filtered through glass fiber filters, sealed in foil, and frozen to prevent the breakdown of chlorophyll. The biomass of the algae was estimated using spectrophotometric chlorophyll *a* analysis (Clesceri et. al 1998). Periphyton levels were measured using a periphytometer (sections of flagging tape submerged inside the mesocosm). The periphyton was analyzed by removing the periphyton on a six inch area of the flagging tape, collecting the periphyton via filtration on glass filters, freezing filters, and then ascertaining the level of chlorophyll *a* present using spectrophotometric analysis. Zooplankton were collected by filling an 800 cm^3 vessel from the middle of the water column from four quadrants of each mesocosm, then filtered using plankton nets and preserved in 80% ethanol. The zooplankton abundance was then calculated using 5 ml subsamples of each mesocosm sample.

Mesocosms were checked daily for metamorphosing anurans, larvae with at least one limb emerged. The date at which a tadpole’s tail was no longer visible was recorded as the date of complete metamorphosis for that individual. Mass at metamorphosis was determined by weighing each individual on its day of metamorphosis. The experiment continued until no additional metamorphs were found in the mesocosm (i.e. five days in a row without a metamorph following the peak time of metamorphosis), which occurred on October 7, 2012. At the end of the experiment the tanks were drained and the leaf litter sorted to find any remaining predator or prey individuals. The mass of each overwintering tadpole was carefully measured and recorded. Predator's mass and length were measured and recorded, and then predators and remaining prey were returned to their area from which they were collected.

Statistical Analyses:

All statistical tests were done using Proc GLM in SAS Enterprise guide 4.3®. I conducted separate repeated-measures ANCOVAs to examine changes in periphyton, phytoplankton, and zooplankton abundances over time among the various treatments, in which chlorophyll *a* level (periphyton, phytoplankton) or zooplankton abundance was the response variable, nutrient addition was the covariate, and predation level (four levels, including a no prey- no predator control), time, and all possible two- and three-way interactions were the predictor variables. Four separate analyses of covariance were calculated to examine the effects of nutrients (the covariate), gape limited predation level (three levels), and their interaction on tadpole survivorship, mass of overwintering tadpoles, mass at metamorphosis, and time to metamorphosis. These four response variables were calculated as an average response per mesocosm. Survivorship was calculated as the proportion of metamorphs and overwintering tadpoles found in each mesocosm out of the original number of 400 larval anurans placed in each tank. The amount of variation found in response variables that could be explained by the model were measured as the coefficient of determination (R^2).

RESULTS:

Survivorship of larval *Rana* increased significantly with nutrient addition ($F_{1,29}$=6.08, P=0.0212) (Fig. 1). There was a trend for predation to impact survivorship of larval anurans ($F_{2,29}$= 2.92, P=0.0733). There was no interaction between nutrient additions and predation level in survival of larval *Rana* ($F_{2,29}$= 1.88, P=0.1743). The linear model incorporating nutrient addition and predation level was able to explain 61.8% of the variation found in survivorship. Only 2418 larval anurans (86 metamorphs and 2332 tadpoles) were recovered from the original 12,000 placed in the mesocosms. Only 0.0355% of the surviving larval anurans were metamorphs, while 96.44% percent remained as overwintering tadpoles. Not all treatments had metamorphs or surviving tadpoles at the conclusion of the experiment.

The predator treatment in a mesocosm was found to have a significant influence on tadpole mass, with larger-gaped predator treatments resulting in larger average tadpole mass ($F_{2,25}$=11.92, P=0.0004) (Fig. 2). There was no statistically significant influence of nutrient addition on average mass of the overwintering tadpoles ($F_{1,25}$=2.97, P=0.1002) or the interaction of nutrient addition and predation level ($F_{2,25}$= 1.04, P=0.3705). The linear model incorporating nutrient addition and predation level was able to explain 65.10% of variation in the average mass of tadpoles.

The mass of the *Rana* metamorphs was not found to be effected by nutrient additions ($F_{1,20}$=0.07, P=0.7987), predation levels ($F_{2,20}$= 1.18, P=0.3341), or the interaction of the two factors ($F_{2,20}$=0.82 , P= 0.4584)(Fig. 3). Moreover, the time required to reach metamorphosis was not significantly influenced by nutrient addition ($F_{1,20}$=1.35, P=0.2641), predation level ($F_{2,20}$= 0.10, P=0.9058), or the interaction of the two factors ($F_{2,20}$= 1.06, P=0.3698).

There was significantly less periphyton (as measured by chlorophyll *a*) at the half-way point of the experiment compared to the end of the experiment ($F_{1,78}$= 25.99, P<0.0001) (Fig. 4). Periphyton chlorophyll *a* levels did not significantly differ with nutrient level ($F_{1,78}$=0.03, p=0.8738), predation type ($F_{2,78}$= 0.65, P= 0.5271), or the interaction between predation level and nutrients ($F_{2,78}$=0.20, P=0.8170). There was no significant interaction between nutrients and time ($F_{1,78}$=0.13, P=0.7208), predation and time ($F_{2,78}$= 0.38, P=0.6848), or three-way interaction of nutrients, predation level, and time ($F_{2,78}$=0.44, P=0.6477) on chlorophyll *a* levels in periphyton. The linear model incorporating nutrient addition and predation level was found to explain 49.81% of the variation found in calculated periphyton levels.

Chlorophyll *a* levels derived from phytoplankton did not vary with nutrient additions ($F_{1,79}$=0.06 , P= 0.8128), predation level ($F_{2,79}$= 0.04, P=0.95293), or the interaction between the two ($F_{2,79}$= 0.17, P=0.8409) (Fig. 5). Also, there was no significant difference in phytoplankton chlorophyll *a* levels with time ($F_{1,79}$=0.37 , P=0.5434). There was no significant interactions of nutrient addition and time ($F_{1,79}$= 0.00, P=0.9658), predation level and time ($F_{2,79}$=0.34, P=0.7099), or interaction of nutrient addition, predation, and time ($F_{2,79}$=0.07, P=0.9317). The linear model incorporating nutrient addition and predation levels only explained 4.9% of the variation in chlorophyll *a* level found in phytoplankton.

Time was the only significant factor found to affect zooplankton. The final zooplankton abundance (October) was found to be lower than that at the mid-point (August) of the experiment ($F_{1,79,}$=6.67, P=0.0119) (Fig. 6). Zooplankton abundance was not found to be influenced by nutrient addition ($F_{1,79}$= 0.67, P=0.4155) or predation level ($F_{2,79}$=0.81, P=0.4475). None of the two-way or three-way interactions significantly influenced zooplankton abundance (nutrient* predation: $F_{2,79}$= 0.10, P=0.9015; time*nutrient level: $F_{1,79}$=1.75, P=0.1904; time*predation level: $F_{2,79}$=0.62, P=0.5399; nutrient*predation*time: $F_{2,79}$= 0.74, P=0.4796). The linear model involving nutrient addition and predation level was only able to explain 16.93% of the variation found in zooplankton abundance.

DISCUSSION:

Both nutrient additions and predation type can be important in determining community structure, but these results present no strong evidence for bottom-up forces offsetting the impacts of gape limited predators. Nutrient addition positively affected survival of larval anurans in the absence of a predator and in the presence of a small gape limited predator, but not in the presence of a large gape predator. This difference between predator treatments was not significant, however, as there was no interaction found between nutrient addition and predator gape size. Thus, there is no strong support for our hypothesis that an increase in resources may allow prey to outgrow the gape size of small-gaped predators, providing a refuge from predation, but would not provide the same advantage to prey in the presence of large-gaped predators.

The positive main effect of nutrients on survival of larval anurans seems most likely to have been caused by an increase in the main larval anuran food source, edible periphyton, with increasing nutrient levels. However, there was no resulting increase of primary production found when looking at chlorophyll *a* analysis of either periphyton or phytoplankton with increasing nutrient levels. More algal mats or a reduction of visibility occurred more often in greater nutrient enriched treatments (Z. Aardweg, *personal observation*), but that did not result in statistical differences in chlorophyll *a* levels among nutrient treatments. Other experiments have found relationships between nutrient influx and increases in primary production (Schindler 1978, Hillebrand 2002, Rabalais 2002, Elser et. al 2007, Gruner et. al 2008), but other previous work has found that the presence of herbivores will significantly suppress the growth of algae (Leibold and Wilbur 1992, Wilbur 1997, Werner and Peacor 2006). If the larval anurans in my experiment were increasingly grazing and reducing algal levels present in higher nutrient treatments, it could explain both the higher survivorship with increasing nutrient levels and why I found no significant relationship with nutrients and algal levels.

Predation was not found to be statistically significant when evaluating survival, but there was an indication of decreasing survival with increasing gape sized predator. Some of the large gape predators consumed all the larval anurans placed in the mesocosm. While prey may have been able to outgrow the gape size of small gaped predators, they did not appear to be able to outgrow risk of predation by large gape predators. Alternatively, it is possible that the large gape predator was able to successfully feed on the larval anurans before the prey was capable of reaching a size refuge from predation, and therefore consume all prey found.

While both nutrient level and predation had important impacts on the survival of larval anurans, there was only some support for the hypothesis that increasing nutrients would benefit prey in the presence of no predator or a small-gaped predator more than a large-gaped predator. It is possible that initially there was an increase of consumable algae present, but over time the composition of algal species changed to less palatable species for anurans. It has been shown that in the presence of grazing anurans, periphyton levels may decline and phytoplankton may become more abundant and use more of the available resources (Leibold and Wilbur 1992). When phytoplankton receive increased nutrients, species composition can change from unprotected, more easily grazed species to types of algae that are less palatable to consumers (Leibold 1999, Rabalais 2002). Thus, even with an increase in algae there may not be an increase of consumable resources for herbivores, resulting in a decoupling of the energy transfer from producers to consumers.

Although the average mass of metamorphs was not found to be significantly influenced by predation, the average mass of the overwintering tadpoles was found to be significantly influenced by predation. Many other studies and experiments have shown predation has an effect on tadpole mass (Morin 1986, Werner 1986, Werner 1991, Relyea 2002). Depending on the species of amphibian and the type of predators, there can be a wide range of larval response to the presence of a predator, including both increasing and decreasing mass (Morin 1986, Werner 1986, Werner 1991, Relyea 2002). In this experiment, low tadpole survival with the large gape limited predators provided an environment in which those tadpoles that did survive experienced limited competition. This competitive release may have allowed the larval anurans to reach a larger size, which may have provided a size refuge from predation. It is also possible that even these larger prey were not too large for the gape size of the large-gaped predators, but that tadpole numbers were so low that encounter rates with predators were low enough for some tadpoles to survive.

Neither mass of larval anurans at metamorphosis nor time to metamorphosis was influenced by nutrients, predation, or the interaction of those factors. There are previous experiments that show a wide variety of larval anuran responses to the presence of a predator, including an increase in metamorph mass as a result of reduced competition or smaller mass due to a reduction of feeding activity (Werner 1986, Relyea and Werner 1999, Peacor 2002). Other studies have shown no effect of predation on *Rana* metamorph mass (Babbit 2001). The low numbers of metamorphs in my experiment make it difficult to draw strong conclusions about metamorph mass or larval period. The 0.7% of original tadpoles that reached metamorphosis did not represent all treatment levels and may not have been sufficient to find any variation due to nutrient influx, predation, or any interaction of those factors.

The low number of *Rana* metamorphs collected during the course of my experiment indicates that larval anurans used the life history strategy of overwintering as tadpoles instead of metamorphosing prior to winter. A reduction in predation pressure has been found to lengthen anuran larval periods (Werner 1986). This could indicate that many of the tadpoles had reached a refuge from predation by outgrowing the gape limitation of the bluegill. The tadpoles remaining would have an environment with reduced competition, resulting in increased consumable resources available. There is evidence of larval amphibians with ample resources remaining larvae longer in order to reach a larger size for metamorphosis (Alfred and Harris 1987). The advantage the larval anurans would receive for this delay in metamorphosis could be a larger size at metamorphosis, as larger mass at metamorphosis has been found to be a strong indicator of increased fitness in amphibians (Wilbur *et al*. 1983, Smith 1987).

In this experiment, the larval anurans seemed to be more successful as overwintering tadpoles as opposed to quickly growing tadpoles able to metamorphose in a single growing season. Increases in overwintering tadpoles may contribute to an increase in competition for anurans breeding in early spring. It is also possible that larger numbers of overwintering tadpoles may decrease the quantity or quality of consumable resources for early season tadpoles (Hernandez and Chalcraft 2012). This may have negative impacts on other larval anuran species that reproduce early in the season because the order in which frogs oviposit and tadpoles emerge has been shown to affect the tadpole assemblage found in bodies of water (Wilbur 1997). There is evidence that increased numbers of larval *Rana* may also result in an increase in predation pressures on other species when found in conjunction with a fish predator (Gregoire and Gunzburger 2008).

There was an increase in chlorophyll *a* levels from periphyton in the October sample when compared to the August samples. There are two factors likely responsible for the increase. The first potential reason for an increase in periphyton levels is a decrease in browsing by herbivores. It has been demonstrated that herbivores can significantly reduce periphyton levels (Leibold and Wilbur 1992). There was a reduction in zooplankton found between the same samples and the number of tadpoles present decreased through time and that may have resulted in an increase in periphyton levels. There may also have been a reduction in foraging activity due to temperature decreases in October. A second potential factor in the increase in periphyton may have been an increase in water clarity. There was a noticeable clearing of the water as temperatures dropped in the fall and all the algal mats sank (Z. Aardweg, *personal observation*). Mats of filamentous algae have been found to create a 60-90% reduction in light penetrating the water (Butzler 2002). The resulting increase in light energy reaching greater depths in the mesocosms due to clearer water may have been responsible for a significant increase in chlorophyll *a* levels found on periphytometers in the October sample. Future studies may want to include more sampling periods in order to examine if the algal increase can be correlated with any life history events in the herbivores. In addition, pairing water clarity tests with plankton sampling may reveal a correlation between water clarity and periphyton abundance.

Zooplankton abundance was examined as it can be prey for bluegills as well as potential competitors of larval anurans. Zooplankton abundance was not found to be influenced by nutrient addition, predation level, or the interaction of those variables. There was a significant difference in zooplankton abundance due to time, with less zooplankton found in the October sample compared to the August sample. It is possible this reduction came from a reduction in reproduction due to declining temperatures.

One potential difficulty in interpreting my results is caused by the fact that I did not take measurements of the exact levels of each nutrient-based ion in the water after I added the crystalline forms. It is possible my nutrient additions did not effectively increase usable nutrient availability. Some organisms, such as *Daphnia*, have been found to respond differently to the amount of available nutrients which can result in altered zooplankton and algal compositions (Wilbur and Peacor 1992). There also is the potential for some of the added nutrients to have been washed out of the mesocosms as the result of precipitation events. Had the nutrient levels been monitored, more exact information about the relationship of nutrients and production may have been found. As it was, the increased survivorship in each predation level was the only indicator of a potential increase in consumable resources due to nutrient additions.

Higher abundances of leopard frogs in more nutrient rich environments, as a result of higher survivorship, may change the structure of communities tied to aquatic systems (Berven 1990, Wilbur 1997). Many different terrestrial species depend on aquatic systems for a portion of their diet or habitat for at least one life stage, and that helps to link the food webs of aquatic and terrestrial ecosystems (Finlay and Vredenburg 1997, Wilbur 1997). Nutrients leave aquatic systems as tadpoles develop and metamorphose. With increasing tadpole survival in nutrient rich systems leading to more juvenile frogs, more insects and invertebrates would be consumed. In addition, these frogs would serve as additional food for animals like birds and raccoons that prey on frogs. These changes may have cascading effects on the rest of the food web.

Currently we are experiencing an influx of nonorganic nutrients reaching our waterways due to anthropomorphic sources. Worldwide human activities, such as agriculture and development, have increased the amount of nitrogen and phosphorus reaching our aquatic systems (Abrams 1993, Vitousek *et. al* 1997, Van Buskirk 2000, Rabalais 2002). Agriculture inputs nutrients into areas involved in raising crops and livestock. Housing complexes and golf courses that use fertilizers to encourage grass growth can create nutrient influxes via storm runoff. As evidence in this experiment, increases in these nutrient levels can cause changes in life history characteristics of populations and in community structure of aquatic and terrestrial systems impacted by nutrient additions.

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Table 1. The experimental treatments including nutrient additions from 0.0 to 4.0 mg/l of nitrogen crossed with three levels of predation (no predation, small gape predator, and large gape predator). Phosphorus was also added at a 16:1 N:P ratio for each nutrient addition level. Controls used included no nutrient addition, no prey, and no predator present.

Predation and Prey Level	Nutrient Addition					
No Predator, No Prey	0	0.25*	0.5*	1	2*	4*
No Predator, 400 Prey	0	0.25*	0.5*	1	2*	4*
Small gape predator, 400 prey	0	0.25*	0.5*	1	2*	4*
Large gape predator, 400 prey	0	0.25*	0.5*	1	2*	4*
*= Levels replicated 2 times						

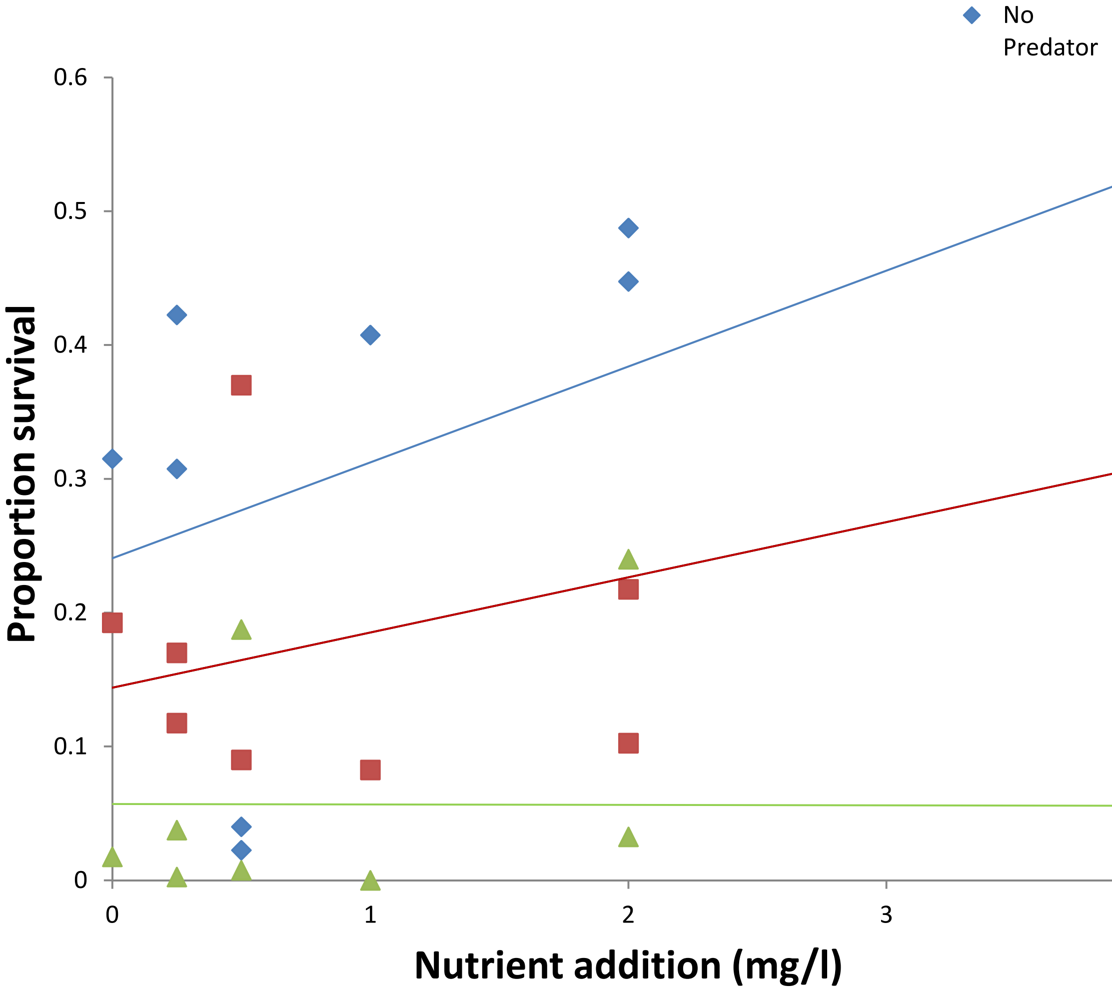

Figure 1. Survivorship of larval *Rana* was found to be significantly affected by nutrient addition ($F_{1,29}$=6.08, P=0.0212), but not predation ($F_{2,29}$= 2.92, P=0.0733), or the interaction of the two factors ($F_{2,29}$= 1.88, P=0.1743). A linear model that incorporates both nutrient addition and predation level explains 61.8% of the variation in larval *Rana* found in survivorship.

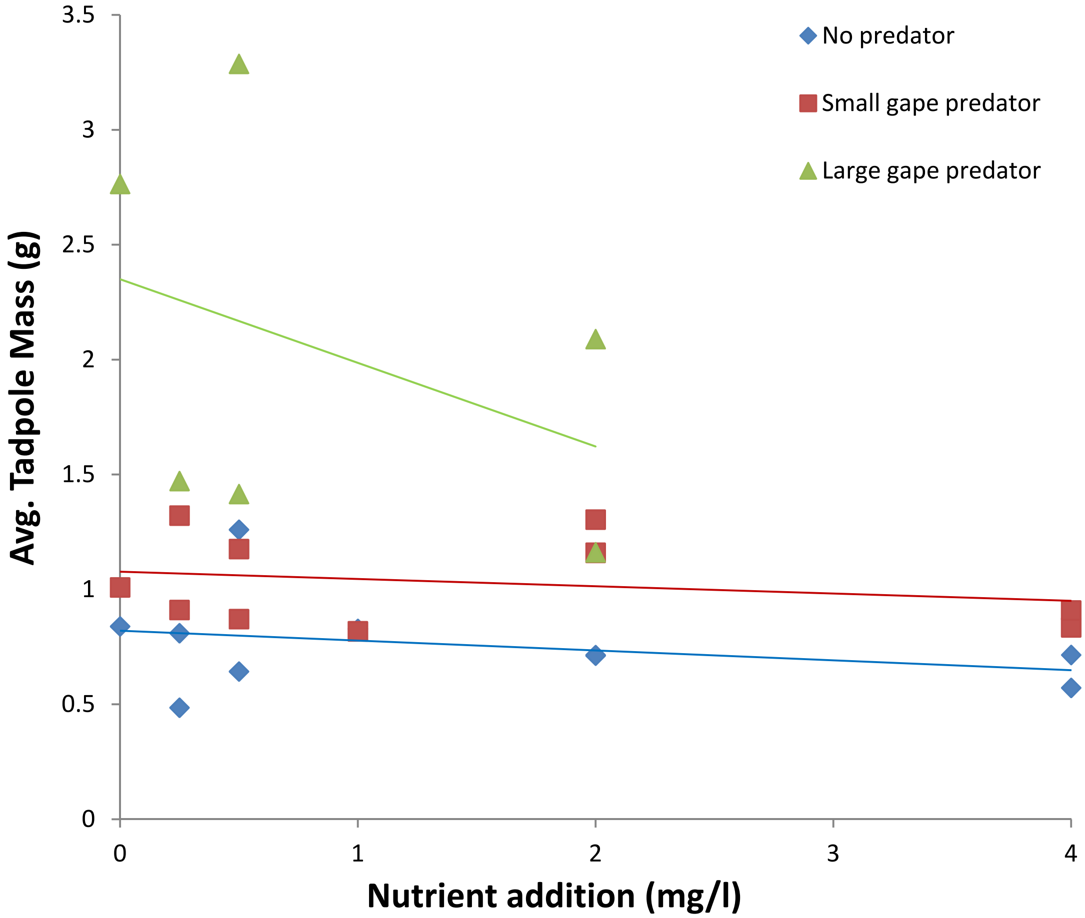

Figure 2. Nutrient addition was not found to influence average tadpole mass ($F_{1,25}$=2.97, P=0.1002), however, predation levels had significant influence on tadpole mass ($F_{2,25}$=11.92, P=0.0004). The interaction of nutrient additions and predation levels was not found to significantly alter the mass of overwintering *Rana* tadpoles ($F_{2,25}$= 1.04, P=0.3705). A linear model incorporating nutrient addition and predation level explained 65.10 % of variation found in the mass of tadpoles.

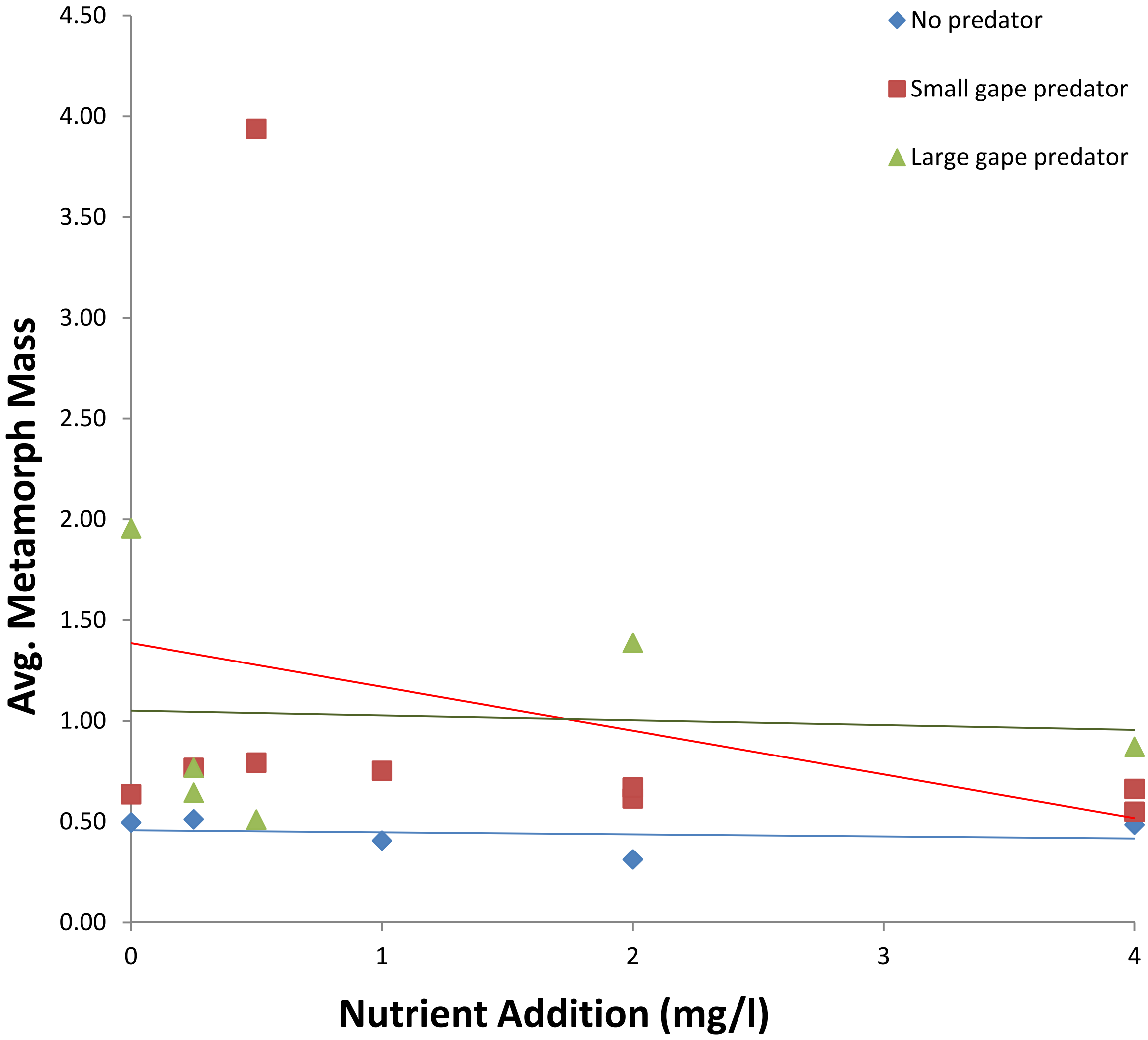

Figure 3. Nutrient addition ($F_{1,20}$=0.07, P=0.7987) and predation level ($F_{2,20}$=1.18, P=0.3341) had no significant influence on metamorph mass. The interaction of nutrient addition and predation level was not found to significantly alter the mass of *Rana* metamorphs ($F_{2,20}$= 0.820, P=0.4584). A linear model incorporating nutrient addition and predation level explained 21.35% of variation found in the mass of metamorphs.

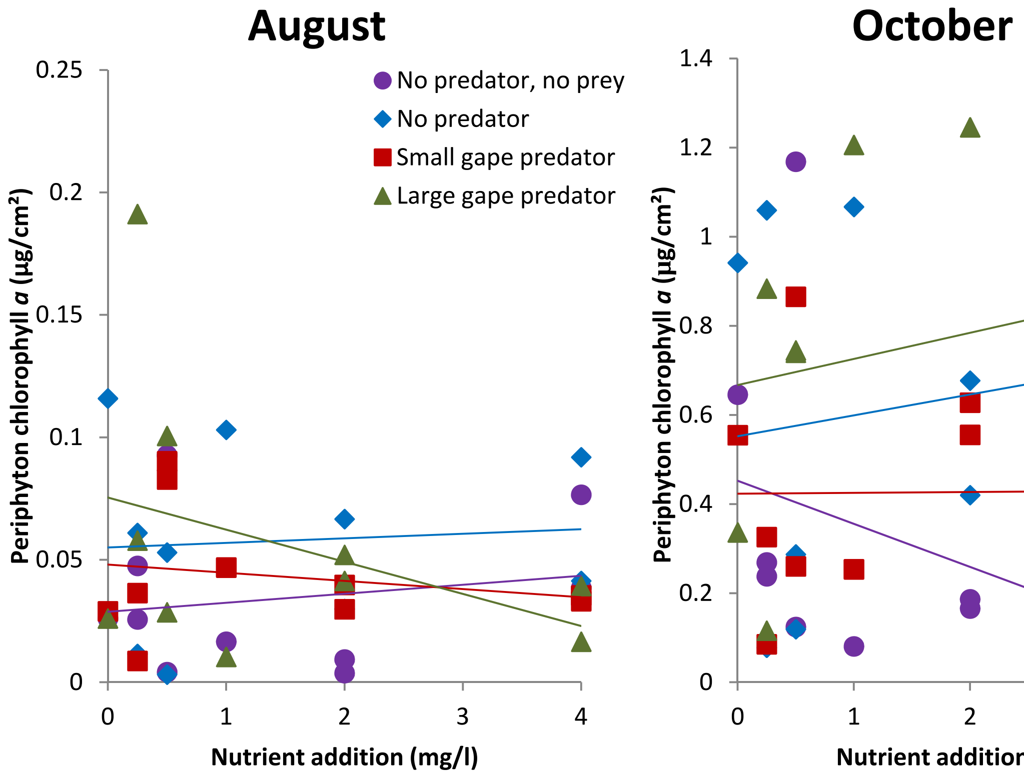

Figure 4. There was no effect of nutrient addition ($F_{1,78}$=0.03, P=0.8738), predation level ($F_{2,78}$= 0.65, P= 0.5271), or the interaction of the two ($F_{2,78}$=0.20, P=0.8170) on chlorophyll *a* levels from periphyton. There was a significant effect of time ($F_{1,78}$= 25.99, P<0.0001), but not of any interactions of time and nutrients ($F_{1,78}$=0.13, P=0.7208), time and predation ($F_{2,78}$= 0.38, P=0.6848), or the interaction of time, nutrient level, and predation ($F_{2,78}$=0.44, P=0.6477). The linear model including nutrient addition and predation explains 49.8% of the variation found in chlorophyll *a* levels derived from periphyton samples.

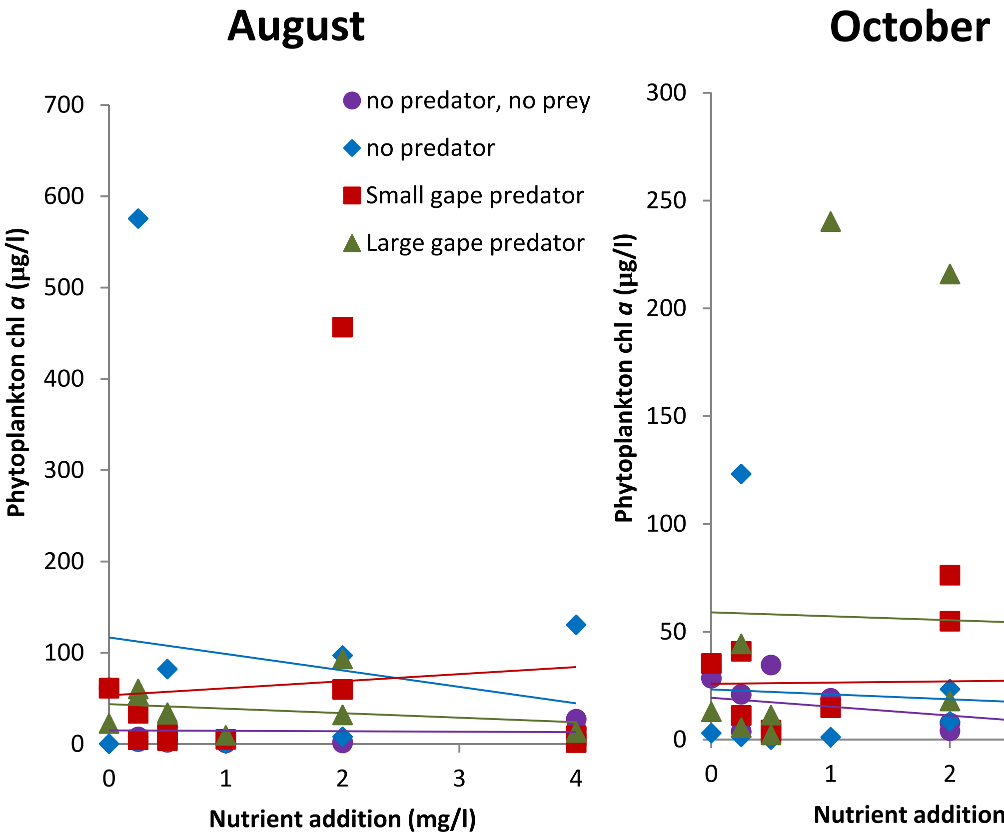

Figure 5. There was no effect of nutrient addition ($F_{1,79}$=0.06, P= 0.8128), predation level ($F_{2,79}$= 0.04, P=0.9593), time ($F_{1,79}$=0.37, P=0.5453), interactions of nutrient addition and predation ($F_{2,79}$=0.17, P=0.8409), nutrient addition and time ($F_{1,79}$= 0.00, P=0.9658), predation level and time ($F_{2,79}$=0.34, P=0.7099), or the interaction of all three ($F_{2,79}$=0.07, P=0.9317) on phytoplankton chlorophyll *a* levels. The linear model was only able to explain 4.9% of the variation found in chlorophyll *a* levels.

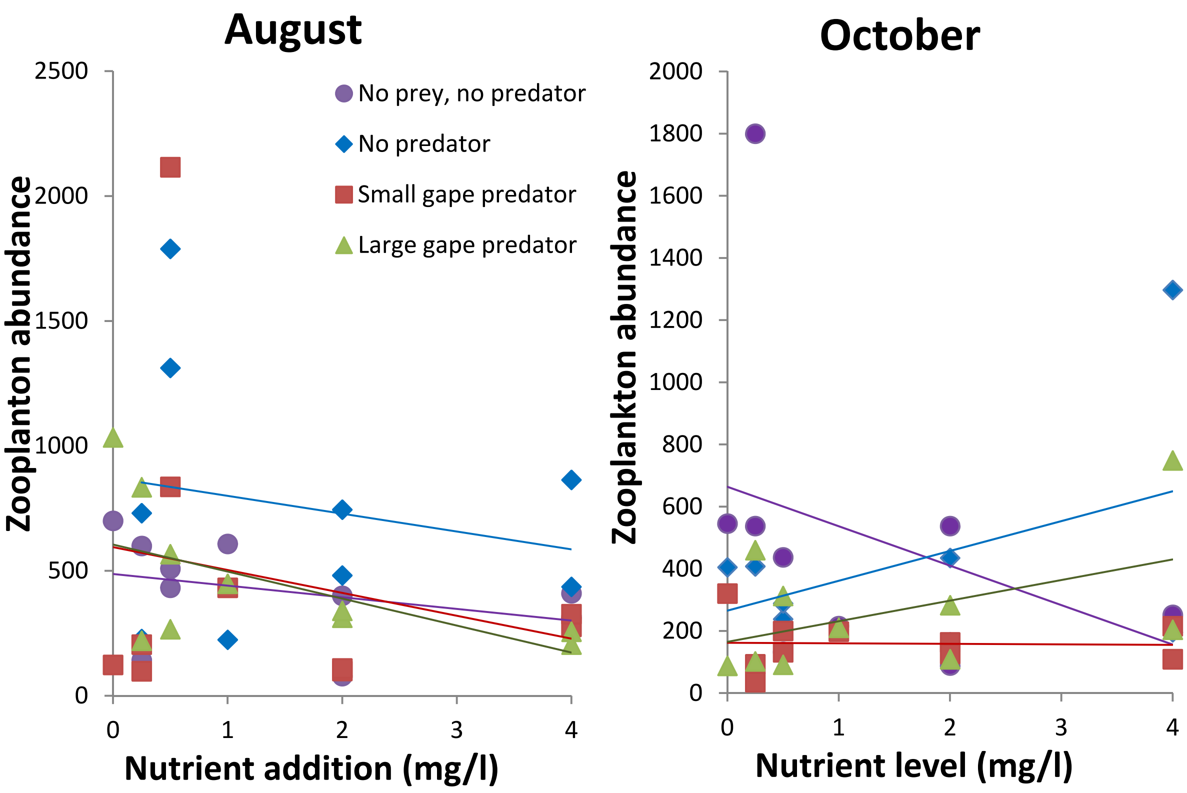

Figure 6. Zooplankton abundance was not found to be influenced by nutrient addition ($F_{1,79}$= 0.67, P=0.4155), predation levels ($F_{2,79}$=0.81 , P=0.4475), or the interaction between nutrient addition and predation level ($F_{2,79}$= 0.10, P=0.9015). Time was found to influence zooplankton abundance ($F_{1,79,}$=6.67, P=0.0119). The interaction of time and nutrient addition was not significant ($F_{2,79}$=1.75, P=0.1904), nor was the interaction of time and predation level ($F_{2,79}$=0.62, P=0.5399), or the interaction of nutrient addition, predation level, and time ($F_{2,79}$= 0.74, P=0.4796). The linear model was only able to explain 16.93% of the variation found in zooplankton abundance.

APPENDIX: ANIMAL USE PROTOCOL APPROVAL

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834
252-744-2436 office
252-744-2355 fax

May 22, 2012

Heather Vance-Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Vance-Chalcraft:

Your Animal Use Protocol entitled, "Nutrient Addition and its Effect on Survival of Larval Anurans in the Presence of Gape Limited Predators" (AUP #D277) was reviewed by this institution's Animal Care and Use Committee on 5/22/12. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure