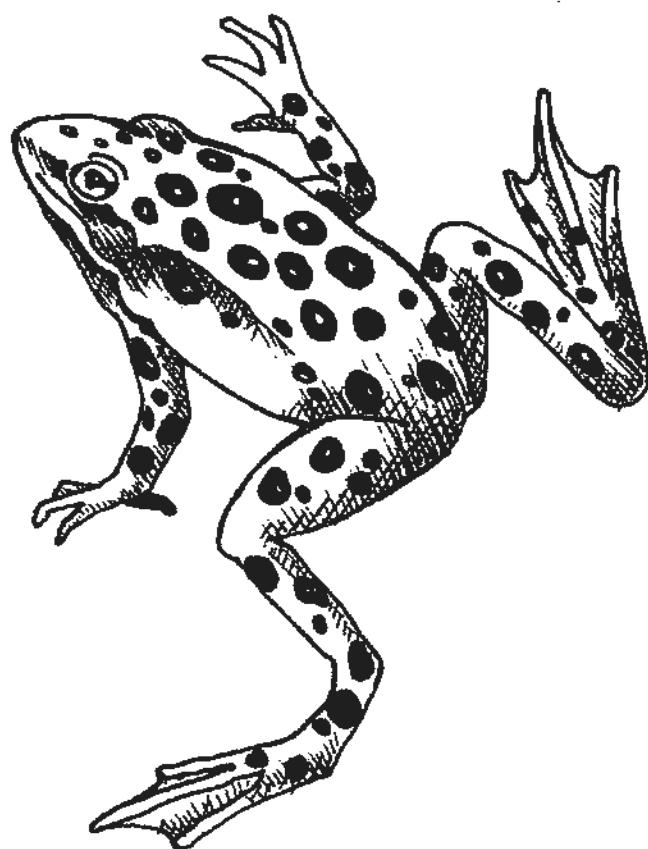


# EFFECTS OF LIVESTOCK GRAZING ON THE INVERTEBRATE PREY BASE AND ON THE SURVIVAL AND GROWTH OF LARVAE OF THE COLUMBIA SPOTTED FROG, *RANA LUTEIVENTRIS*

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Invertebrate Prey Base and on the  
Survival and Growth of Larvae of the  
Columbia Spotted Frog, *Rana luteiventris***

**Final Report on  
research funded by  
Challenge Cost-share Agreements between  
U.S. Bureau of Land Management and  
Boise State University**

**BLM Orders #DBP990049 and #1422D010P980056  
BSU Account #00066128 and #00066013**

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**November 26, 2000**

## **Summary**

This report discusses results primarily from the second of two field seasons in which two aspects of grazing were examined for possible effects on Columbia spotted frogs (*Rana luteiventris*). First, exclosures were used to prevent grazing on portions of the streams and ponds to ascertain the effects of grazing on the invertebrate prey base utilized by the frogs. Although we found no statistically significant effect of grazing on either biomass or diversity of invertebrate prey, care must be taken in the interpretation of these results. While it is possible that there was no effect of the specific grazing regimes of these sites on the invertebrate community, the small sample size, the very general taxonomic identification used, and weaknesses in study design may have masked any true differences. Adult spotted frogs were apparently not actively feeding during late August to late September. Metamorphs and subadults, however, would need to forage at that time to accumulate necessary fat reserves and would therefore be affected by changes in the invertebrate community. Further work is needed to more solidly document the effects of grazing on invertebrates.

Second, spotted frog larvae were raised in microcosms located at the Mudflat Guard Station and were subjected to four levels of cattle waste. During the first year, survival of larvae was very low and growth was stunted, indicating that the experimental design needed modification for the second year's experiment. During the second year, we found that addition of waste negatively affected survival rate. We also found that cattle waste does not appear to be directly toxic, nor does the decreased survival seem to be due to decreased dissolved oxygen levels. The cause of decreased survival is probably an indirect effect of addition of waste, such as an increased ammonia concentration. We also found that addition of waste led to an increased growth rate of larvae. Further study is needed to determine whether, in the more natural conditions of the field, cattle waste affects survival and growth in the same way as was observed in the microcosms.

## **Introduction**

Kauffman and Kreuger (1984) noted that the greatest impact on small streams and riparian areas in the west is often due to cattle grazing. Livestock use riparian areas disproportionately more (Armour et al. 1991, Clary and Booth 1993, Gillen et al. 1985, and others), preferring to graze in riparian areas because of their relatively level terrain, cooler temperatures, and abundance of palatable vegetation. Platts and Nelson (1985) observed that cattle took an average of 29 percent and as much as 40 percent more vegetation from the riparian zone than from the rest of the available rangeland. Cattle grazing in riparian areas has been shown to alter vegetation structure, reduce plant biomass, and alter plant community composition. The depletion of riparian vegetation and the compaction of soil caused by grazing results in increased runoff and sedimentation, which alters water quality. Cattle urination and defecation in or near streams may alter nutrients in the water as well as water chemistry (Gary et al. 1983). Overuse by cattle has resulted in appreciable damage to the riparian ecosystems throughout the west (Armour et al. 1991 and Holechek 1980). Over the long term, overgrazing can alter the vegetation and hydrology of a riparian area and thus cause the loss of critical spotted frog habitat: slow moving water, ponds, oxbows, and meadow vegetation. Repeated season-long grazing can cause the loss of the shrub component.

Additionally, bank-stabilizing rushes, sedges, and willows may be replaced by weedy species and less protective grasses (Tueller 1988, Platts 1990, and Clary 1995). Overgrazing also leads to exposed soils which are subjected to erosion by trampling, wind, and runoff. Increased runoff and decreased water storage capacity leads to reduced water filtration and reduced deposition of sediment necessary for building stream banks, wet meadows, and floodplains (Chaney et al. 1993). With further overuse, channel down-cutting can take place, lowering the water table and drying adjacent wet meadows and oxbows. In time, these areas will be invaded by various species of sagebrush (Platts 1991) leading to further fragmentation of habitat via the loss of movement corridors.

The Owyhee Mountains of southwestern Idaho support an isolated population of Columbia spotted frog (*Rana luteiventris*). This population is part of the portion of the range that is classified by the U.S. Fish and Wildlife Service as a candidate for threatened and endangered status. As a species of concern, it is important to study the potential threats that grazing may present to spotted frogs.

Every life history stage of *Rana luteiventris*, from embryo to adult, has the potential to be affected by cattle grazing. Spotted frogs deposit floating egg masses that are not dependent on the support of vegetation (Nussbaum et al. 1983), so the simple removal of aquatic and riparian vegetation by cattle is unlikely to affect egg masses. However, egg masses can be damaged or stranded as a result of trampling, and water chemistry changes and introduced bacteria in feces have the potential to do harm as well. As larvae, spotted frogs are restricted to the same aquatic habitats that serve as the primary watering source for cattle in this area. Particularly important to larvae are changes in water quality that result from cattle urination and defecation as well as physical disturbance of the water. Larvae could be positively affected by enhancement of food supply that might result from increased nutrient input. Metamorphs may be particularly susceptible to trampling because they are not able to swim well enough to escape in deep water, and they occur only in moist areas next to water bodies, the same place that cattle are concentrated. As adults, spotted frogs depend heavily on riparian vegetation for cover and as a resource for their insect prey. Therefore, the removal of vegetation by grazing might make them more vulnerable to predators and deplete their available food source. Conversely, removal of a portion of the vegetation by grazing might lead to a higher availability of basking sites.

Direct evidence on the effects of cattle on spotted frogs is scanty and often weak:

1. Surveys conducted in the Owyhees that looked for positive or negative association of grazing and presence of adult frogs (Munger et al. 1997 and 1998) detected either a modest negative effect or no observable effect. These analyses relied on relatively imprecise measures of cattle usage, and are therefore not as reliable as experimental studies in which the level of grazing is tightly controlled.
2. Bull and Hayes (2000) used similar survey techniques to assess differences between grazed and ungrazed sites in northeastern Oregon. They found no significant effects of grazing on numbers of spotted frog egg masses, but did find that grazed sites had larger egg masses (suggesting greater food availability at grazed sites). Unfortunately, their study confounded potential effects of cattle with possible elevational effects and was conducted in an area much different from the Owyhees.
3. We have observed that frogs often disappear from ponds that are heavily impacted by cattle. It is not known whether frogs leave the ponds for better habitat, are killed, or simply bury

- themselves deep in the mud to wait for less turbulent times.
4. We have noticed that in some situations, frogs are somewhat easier to find in moderately grazed patches than in adjoining ungrazed patches. We do not know whether this difference reflects an actual difference in numbers or is simply caused by increased visibility of frogs in the more open habitat.

## **Focus and Overview of Project**

Because of the time constraints of a master's thesis, we focused our efforts on two aspects that could be examined in a reasonable time frame:

- a) The impacts of cattle grazing on the food resource (primarily insects) available to spotted frogs.
- b) The effects of cattle grazing on the growth and mortality of the larval stage.

### Impacts on resources

Because grazing is widespread and historically has had impact on the land in the study area, it was necessary to construct exclosures that would allow creation of cattle-free areas for the duration of the experiment. Construction of these exclosures was conducted in the spring and summer of 1998, generally before any grazing had taken place. Riparian areas that have been affected by grazing have been documented to recover fairly quickly when livestock are removed (Huber et al. 1985). At the time of the work described in the present study (1999 field season), exclosures had been in place for two seasons.

Adult spotted frogs are opportunistic feeders, feeding on a variety of terrestrial invertebrates (Turner 1958) that often are closely associated with vegetation. Consequently, invertebrate community composition and overall abundance is likely to vary between areas that have been grazed and areas that have not been grazed. We designed a protocol to compare the biomass and diversity of insects in grazed areas with those in ungrazed areas.

### Larval experiments

Aquatic environments are important in all stages of the frog's life history. Livestock can potentially affect the water quality of streams and ponds in a variety of ways. Sedimentation can reduce the dissolved oxygen and raise the water temperature (Holecbeck 1980). High ammonia concentrations, which can result from the introduction of cattle urine, also have been shown to reduce dissolved oxygen (Meehan and Platts 1985) and can cause decreased survival and growth (Jofre and Karasov 1998). Spotted frog larvae feed on aquatic vegetation, detritus, and algae (Turner 1958), resources that may be increased by the addition of nutrients from cattle waste.

## **Materials and Methods**

### Impact of grazing on the invertebrate prey base

Study sites were comprised of federal, state, and private land holdings that were known to have good resident frog populations. Specific locations of research were chosen based on past survey

work (Munger et al. 1997) and in consultation with members of the BLM that had knowledge of these areas and their respective land-use practices. Sites were chosen first based on the presence of frog populations, then on accessibility and agreement of permittees and landholders. Three streams and nine ponds were chosen for study. Streams chosen for study were Stoneman Creek, Cottonwood Creek, and Long Tom Creek. At each stream, we chose four segments, each forty meters in length, for treatments. Two of these segments were unobstructed to allow for the normal grazing regime that the area would experience and the other two were excluded from grazing with either electric or barbed-wire fencing. Exclosures on streams were constructed to protect an area approximately 40 meters x 20 meters to adequately surround the riparian area. Ponds of interest were also fenced to exclude cattle. Ponds included three ponds on the Collett Ranch, five ponds in the Sam Noble Springs area, and Circle Pond, which had an existing enclosure. Landholders and permittees were concerned with excluding cattle from drinking water, so fencing was only permitted on a subset of ponds and most fencing included only half of a pond. Fencing around ponds was constructed to include the riparian area of one half and fencing was extended across the middle of the pond to completely bisect it preventing cows from disturbing the vegetation on one half. The position of the fence through the pond was adjusted to divide each pond so that somewhat similar physical characteristics and vegetation types were represented on each side.

To include the majority of invertebrates that a frog may encounter during its activity period, we sampled throughout the majority of the frog's activity period (8:00 a.m.-8:00 p.m.), resulting in six two-hour sampling periods in a single day. Invertebrate sampling consisted of a combination of active sampling by sweep netting and passive sampling with sticky traps. The pond sites were sampled once before and once after grazing, and the stream sites were sampled once after most of the grazing had occurred.

Invertebrate sampling dates	
Site	Date
Long Tom	August 30, 1998
Cottonwood	August 16, 1998
Collett Before	June 18, 1998
Collett After	September 5, 2000
Stoneman Creek	July 8, 1999
Long Tom	August 19, 1999
Cottonwood	August 21, 1999
Collett Before	July 21, 1999
Collett After	September 25, 1999
Sam Noble Before	July 16, 1999
Sam Noble After	September 6, 1999
Circle Pond	August 29, 1999

Sweeping: During each of the two-hour periods, we conducted a series of sweep sets throughout each experimental unit. A sweep set consisted of taking five one-meter long sweeps with a sweep net at each sampling point within an experimental unit. A total of 10 sweep sets were taken at even intervals along each of the stream segments and 8 sweep sets were taken at

even intervals around the perimeter of each pond for every time period. All invertebrates caught in the sweeps were collected in plastic bags and euthanized, then later identified to the ordinal level.

Sticky traps: Passive sampling was used to collect those insects that were difficult to obtain actively or effectively transfer from the net to the storage container. Each sticky trap was constructed by coating a 7-1/2 inch diameter yellow plate with a thin film of Tanglefoot® pest barrier. Sticky traps were placed on the ground near the shoreline as well as attached to stakes that suspended the plates approximately 25-30 centimeters above the bank. Sticky traps were left out to collect insects for the entire twelve-hour sampling period. A total of six staked plates and six ground plates were placed in each experimental unit (both pond and stream segments). Trap type was alternated. A trap was placed approximately every five meters on both sides of the stream, and at even intervals around the perimeter of each pond. At the end of the day, plates were collected and sealed in plastic bags. Captured insects were identified to the ordinal level and counted.

#### Stomach Flushing

Stomach flushing is a method of collecting dietary data without the need to sacrifice study individuals. Frogs were captured at the same time as invertebrate sampling was conducted for the Cottonwood, Circle Pond, Sam Noble and Collett Ranch sites. At each site, four to seven adult frogs were captured for analysis. Captured frogs were first anesthetized using an aqueous solution of 0.02% benzocaine. Stomach contents were then collected using the stomach flushing protocol described by Leclerc and Courtois (1993). A 60 cc syringe fitted with a 15 mm length of 2 mm diameter flexible vinyl tubing was filled with filtered water. Each frog was held and its mouth was opened by gentle pressure. The tube was inserted until the tip reached the pyloric end of the stomach. When the tube was in place, the frog was inverted and held with the mouth open over the collection container. The entire contents of the syringe were gently flushed into the stomach and any contents that were forced out were caught in the container for identification.

Stomach flushing dates				
Treatment	Site	Pond Number	Date	Number flushed
Ungrazed	Sam Noble	1	September 6, 1999	4
Ungrazed	Collett Ranch	2	September 25, 1999	4
Ungrazed	Circle Pond	1	August 29, 1999	7
Grazed	Sam Noble	2	September 6, 1999	6
Grazed	Sam Noble	4	September 6, 1999	4
Grazed	Collett Ranch	3	September 25, 1999	3
Grazed	Cottonwood	la	August 21, 1999	7

#### Larval Experiments

To determine the effects of water quality on tadpole growth and development, we set up a series of 20-gallon plastic tanks located at the Mud Flat Guard Station. This location provided an opportunity to study larvae in a place where they would be exposed to nearly natural environmental variables. Waste treatments consisted of a combination of cattle feces and urea.

For the 1998 experiments, four levels of waste (control, low, medium, high) were designed to mimic no grazing, light, moderate, and high grazing. Agitation was meant to mimic the physical effects of cattle in the water and increase in turbidity. A total of 48 aquaria, comprised of six replicates of the following eight treatments, were set up:

1. control - no waste or agitation
2. no waste + agitation
3. low waste - no agitation
4. low waste + agitation
5. medium waste - no agitation
6. medium waste + agitation
7. high waste - no agitation
8. high waste + agitation

We placed a homogeneous layer of pond sediment (approximately 2 inches deep) into each tank and then filled each tank with 10 gallons of pond water to inoculate the tank with algae and detritus. Camouflage netting covered the entire experimental area to control temperature and decrease evaporative water loss. Tanks were allowed to equilibrate and then, 25 tadpoles that were raised from eggs collected in early May were placed into each tank. On June 12, the treatments were randomly assigned and waste was added. The amounts of waste that comprise each treatment are as follows:

High Waste	6 fluid oz feces	10 grams urea
Medium Waste	3 fluid oz feces	5 grams urea
Low Waste	1.5 fluid oz. feces	2 grams urea
Control	Nothing added	Nothing added

Tanks receiving agitation as part of their treatments were raked with a small hand rake twice daily four times a week. Mass measurements were terminated when the tadpoles developed hind limbs. Additional pond water was added to the tanks to keep them at a constant level as necessary. The mass of random samples of 10 tadpoles from each tank were measured approximately at three-week intervals (June 25, July 10, August 4) to determine growth rate. At metamorphosis, tadpoles were measured and released into their natal ponds. The time of metamorphosis of these tadpoles ranged from August 14 to September 2 when the experiment was terminated.

For the 1999 experiments, several modifications were made from the 1998 research to improve survival rates. Agitation was eliminated to simplify the treatments, the number of larvae was reduced from 25 per tank to 10 in response to the stunted growth observed in the 1998 trials, the

number of replicates of each treatment was increased, treatments were introduced slightly later in the season to better represent the grazing regime in this area, and additional measures were taken to deter potential predators.

Four levels of waste (control, low, medium, high) were designed to mimic no grazing, light, moderate, and high grazing. A total of 40 aquaria, comprised of 10 replicates of each of the four waste levels, were set up. We placed a layer of pond sediment (approximately 2 inches deep) into each tank and then filled each tank with 10 gallons of pond water to inoculate the tank with algae and detritus. Camouflage netting covered the entire experimental area to control temperature and decrease evaporative water loss, and the entire tank array was surrounded by aluminum flashing to prevent potential predators, such as garter snakes, from entering. Tanks were allowed to equilibrate and then on June 10, 10 larvae that were raised from eggs collected in early May were placed into each tank. On June 30, the treatments were randomly assigned and waste was added. The amounts of waste that comprised each treatment are as follows:

High Waste	6 oz feces	10 grams urea
Medium Waste	3 oz feces	5 grams urea
Low Waste	1.5 oz feces	2 grams urea
Control	Nothing added	Nothing added

Additional water from a nearby spring was added as necessary to the tanks to keep them at a constant level. The masses of random samples of five larvae from each tank were measured at approximately three-week intervals (July 1, July 24, August 14) to determine growth rate. Mass measurements were terminated when the larvae developed forelimbs. At metamorphosis, larvae were measured and released into their natal ponds. The time of metamorphosis of these larvae ranged from August 14 to September 10, the latter date being when the experiment was terminated. At termination, the tanks were strained and all remaining larvae were counted to get an overall estimate of survivorship.

#### Field Cages

In 1998, we also performed preliminary tests on a protocol and cage specifications needed to determine growth rates of tadpole in a field situation. Experimental cages were constructed of a 16 inch square PVC frame suspended from steel posts that were sunk into the pond substrate. Attached to the square frame was an 18x32 inch bag made of 1 mm fiberglass screening; the bag was suspended at a height that immersed most of the bag in the water and provided approximately the same volume as the plastic tanks used at the guard station. The mesh cages also received the same number of tadpoles as the tanks. The field cages were installed in a pond located at the Collett Ranch that was completely fenced off; the field cages were monitored the same way as the controlled tanks.

#### Fecal Coliform Bacteria

Because the waste levels of the microcosm portion of this experiment were arbitrarily chosen, fecal coliform analysis was used to gauge the appropriateness of these waste levels by comparing counts from microcosms to counts from local grazed ponds. Fecal coliform bacteria are enteric to mammals and their presence in water is indicative of fecal pollution (Kunkle and Meiman 1967). Manure from cattle grazing can dramatically increase fecal coliform concentrations in

runoff from grazed pastures (Edwards et al. 1997). Water samples for bacteriological analysis were collected using sterile glass dilution bottles. Analysis was conducted on a subset of the experimental aquaria that included four randomly chosen microcosms of each treatment type and nine natural ponds in southern Idaho that supported larvae and received late-season light to moderate grazing. Samples from microcosms were collected from the middle of the aquarium approximately 8 cm under the surface. Sampling was conducted on July 1, 1998, approximately four weeks after the initiation of treatments during the 1998 season. In the field, all samples were taken approximately 15 cm under the surface and at least 1.5 m from the edge. Field samples were taken approximately 4 weeks into the grazing treatment. All samples were held on ice and assayed within 24 hours of collection. Standard membrane filtration techniques (Greenberg, Clesceri, and Eaton 1992) were used to generate and count fecal coliform colonies. Due to high sediment content, 15 ml of water was passed through each membrane for analysis; this was repeated three times for each site and an average count for each site was used for comparison.

#### Acute Toxicity Tests

Due to the unexpectedly high mortality rates in the experimental aquaria during the 1998 season, acute toxicity tests were conducted to determine if waste levels were themselves directly toxic. On July 5, 1998, spotted frog larvae were collected from the same breeding ponds in southwestern Idaho as the eggs used in the microcosm experiment. One hundred and sixty-eight larvae measuring between 9 mm and 15 mm total length were selected to most closely mimic the developmental stage of larvae when treatments were added in the microcosm experiment. These larvae were transported to a controlled laboratory setting at Boise State University. A series of 1.5 liter food-quality plastic containers were set up and each filled with one liter of fresh stream water. Two larvae were placed in each container and allowed to acclimate for 3 days before treatments were initiated. On July 9, 1998, treatments were randomly assigned to each of the containers. The effects of urea and feces were examined separately. Seven different urea and four fecal levels were determined based on the quantities used in the microcosm experiments. A series of 84 containers were established to include a total of seven replicates of each of the 11 treatments and controls. Larvae were all fed Purina® pelletized rabbit chow *ad libitum* and were agitated with a wooden dowel for 45 seconds twice daily to maintain high dissolved oxygen. The laboratory was kept at a temperature of 25°C. The containers were surveyed twice daily for a total of 11 days to count and remove any dead individuals.

Acute toxicity treatments	
Urea levels	Feces Levels
1 0.01 g/l	1 14 g/l
2 0.03 g/l	2 20 g/l
3 0.05 g/l	3 40 g/l
4 0.1 g/l	4 60 g/l
5 0.2 g/l	
6 0.3 g/l	
7 0.4 g/l	

## Results

### Invertebrates

In 1998, invertebrate sampling was conducted on both Cottonwood Creek and Long Tom Creek, and on the three Collett ponds before and after grazing. In 1999, invertebrate sampling was conducted at all sites, once at each stream site and before and after grazing at each pond because the pond experimental units were not as well matched. Grazing at all stream sites occurred in the early fall (August and September), with the exception of Stoneman Creek, which has an earlier grazing regime (May-June). The grazing regime at Collett ponds extended from the last week of August until late September. Grazing before invertebrate censuses in the Sam Noble Springs area was only for 6 days during the third week in August. Grazing even at this relatively low intensity noticeably affected vegetation. Ponds were the most affected, vegetation height around the perimeter of the ponds was cropped as low as within 1 inch of the ground. Streams were also affected, but grazing pressure was less than at ponds, and stubble height at the grazed stream segments was between 2 and 4 inches, with some undisturbed patches of grasses and sedges. Although cattle occasionally got into a couple of the exclosures during summer and early fall, only minor changes in vegetation occurred. At Sam Noble Springs, however, substantial heavy grazing occurred in a second bout late in the 1998 season and again after the invertebrate censuses of 1999. At those times, enclosure fences were not being maintained and some grazing occurred within exclosures, reducing the long-term effects of decreased grazing on those plots.

All invertebrates collected were identified to the ordinal level. Counts from both sweeps and sticky traps were pooled to find an overall number of individuals of each order found at each experimental unit. From each experimental unit, 50 insects of each order were randomly chosen and dried in an incubator until desiccated, then weighed to obtain a mean dry weight estimate for each order. These dry weights were used to estimate a total biomass collected in each individual experimental unit. In 1998, small sample size prevented a statistical comparison of grazed to ungrazed portions of ponds (Appendix 1). Comparison of grazed to ungrazed stream segments did not yield significant differences, however, sample size was small here as well (Tables 1 and 2).

Biomass values for each of the areas surveyed in 1999 were calculated and are shown in Tables 3 and 4, and Figure 1. Statistical analyses found no significant differences in biomass between grazed and ungrazed segments of stream or pond (Tables 3 and 4, stream  $P=0.1919$ , pond  $P=0.384$ ). The Shannon-Wiener Function was used to estimate diversity at the ordinal level that existed in each segment. No significant differences in invertebrate diversity were found between the ungrazed and grazed areas in streams or ponds (pond:  $P=0.619$ ; stream:  $P=0.829$ ) (Tables 5, 6, Figure 2). High variability and low sample sizes characterized the analyses of biomass and diversity.

### Stomach Flushing

We flushed the stomachs of a total of 37 frogs that were captured at four grazed sites and three ungrazed sites. None of these individuals were found to have any stomach contents. To confirm this observation, five of these individuals were sacrificed and their stomachs excised and opened. Of these five individuals, 3 were collected from Cottonwood Creek, 1 from Circle Pond, and 1 from the Collett Ranch site on the same days as post-grazing invertebrate sampling occurred for each respective site. Examination revealed that all of the stomachs were, in fact, empty.

### Larval Results

The 1998 larval experiments were continued until late August when a number of individuals had successfully metamorphosed. Of the 48 original tanks, tadpoles in only 26 tanks survived to the termination of the experiment. Of the survivors, only 101 (of a total of 1,200) reached metamorphosis before the termination of the experiment. Because of this very low survival rate, statistical analyses were not performed on the 1998 data.

In the 1999 experiment, larvae were weighed at approximately 3-week intervals from July 1 to August 14, at which time we observed the development of forelimbs. Mass measurements were terminated at that time because at this point in metamorphosis, larvae begin to lose weight. Tanks were monitored from this point on to measure and release metamorphosed individuals. The experiment was terminated on September 10 when many individuals had metamorphosed and freezing night-time temperatures were regular. Of the 40 original tanks, 30 tanks had larvae that survived throughout the duration of the experiment. Of the survivors, 103 reached metamorphosis before the termination of the experiment and an additional 67 survived as larvae. Snout-vent lengths of newly metamorphosed frogs varied from 22 to 35 mm with an average size of 30.2 mm. Survival of larvae differed among treatments (Analysis of Variance; Table 7;  $P=0.0013$ ). Tukey contrasts revealed that survival rates of larvae exposed to High waste treatments were significantly lower than those for larvae in the other three treatment types. Additionally, an average overall growth rate was calculated for each tank. An Analysis of Variance (Table 8) indicates that significant differences existed among treatments ( $P=0.0079$ ), and contrasts indicate that larvae in High waste treatments had significantly faster growth rates than those of larvae in other treatments. We found no significant difference in size at metamorphosis (Table 9;  $P=0.2912$ ). Additionally, an Analysis of Covariance was used to assess the relationship between survivorship and growth rate, the thought being that aquaria with low survival might have less competition and therefore more resources available, thereby causing a higher growth rate. ANCOVA results indicated no significant relationship among these factors (Table 10;  $P=0.6067$ ).

### Acute Toxicity

No mortalities in any of the eleven treatment types occurred during the first 96-hour period of the experiment. The experiment was allowed to continue for an additional 7 days and only 5 mortalities were observed at this time. There was no apparent pattern of these mortalities: two of the individuals died in the lowest urea treatment, two in the medium urea treatment, and one in the highest urea treatment. No mortalities were observed in any of the fecal treatments during the duration of this experiment.

### Fecal Coliform Bacteria

Fecal coliform counts from ponds subjected to grazing and from artificial microcosm experiments are presented outlined in Table 11. Ponds that were assayed all received light to moderate late-season grazing; counts from these samples ranged from 0.911 colonies/ml to 2.356 colonies/ml with an average of 1.470. The experimental tanks had more variable numbers, with means ranging from 0.900 colonies/ml in the control to 6.767 colonies/ml in the high waste with agitation experiments. The low waste experimental tanks without agitation averaged 1.533 colonies/ml.

## **Discussion**

### Effects on Invertebrate Density and Diversity

Our finding of no statistically significant effects of grazing on either invertebrate density or diversity must be interpreted with caution. The failure to reject a statistical null hypothesis ( $H_0$ : no effect of grazing) should not lead to outright acceptance of that hypothesis (Parkhurst 1984). Instead, it should be realized that the actual lack of an effect is only one of a number of possible reasons for a lack of statistical significance.

First, a lack of statistical significance can be caused by low statistical power, which is typically caused by low sample size and/or high variability in the data. The present study was characterized by low sample sizes of only five to seven per treatment and by quite high variability among those samples, with coefficients of variation ranging from 27% to 96%. The resulting experiment would have had to have had a 50% difference between treatment means to have been easily detectable (i.e., 90% of the time). Contributing to the high degree of variability is that, due to logistical constraints, each experimental plot was only assessed for one day. Multi-day samples would have done a better job of characterizing the insect fauna.

Second, it may be that grazing affected only certain species or genera of invertebrates. Fielding and Brusven (1995) found that the total density and species composition of grasshoppers was higher in ungrazed areas than in grazed areas. Our analysis focused on total biomass because that measure is likely to represent what is available to frogs.

Third, a failure to detect a grazing effect on invertebrates may be due to specific circumstances of this study. We assessed the effects of modest grazing that occurred primarily in late August and early September, when most invertebrate growth and reproduction had been completed; it is likely that the lateness of the loss of vegetation would lessen the effect on invertebrates. Interestingly, another study examining complete insect communities (Rambo and Faeth 1999) found that although they observed a substantial difference in the vegetation (both in plant biomass and diversity) between grazed and ungrazed sites, they observed no significant differences between the insect biomass or diversity between these two areas. Note that the study of Rambo and Faeth also was conducted during late season grazing and had a small sample size.

Fourth, our study design may have had weaknesses. In most situations, our grazed areas were immediately adjacent to ungrazed areas. Given that insects are generally highly mobile, it is likely that some may have traveled between treatments, diluting any effects of treatments. It is interesting to note that at Circle Pond, where the sampled ungrazed area is separated from grazed areas by at least 50 m, the insect density was the highest of any plot sampled.

We regard it as highly unlikely that grazing can have no effect on invertebrates regardless of the timing or intensity of the grazing regime. Intensive grazing regimes have been shown to have dramatic and long-lasting effects on the density, physical structure, and species composition of plant communities. How could long-duration, intense grazing not have a measurable negative effect on invertebrate diversity when the dependence of invertebrates on plant community structure and density has been well documented? It is clear that this portion of our study needs to be repeated

with larger sample sizes, larger plots, and more frequent insect sampling in order to better quantify the effect of grazing on insect density and diversity.

Stomach flushing: The lack of any food items in stomachs of any adult frogs sampled was surprising. Frogs were examined from late August through late September, a period when insects were still abundant. We would expect frogs to capitalize on this availability by feeding heavily to lay down fat reserves prior to hibernation. Previous studies have shown that frogs with more fat reserves better survive hibernation and have increased reproductive output the following year. Very little is known about anuran energetics, but many species are known to acquire the most food in the times of greatest prey abundance and during periods close to their hibernation (Duellman and Trueb 1994).

Two possible explanations of our results follow. First, it may be that frogs at our study sites had already acquired necessary reserves for hibernation and reproduction. Dimmitt and Ruibal (1980) found that many desert anurans feed sporadically throughout the season; certain species are even capable of consuming enough in one feeding to supply individuals with enough energy for an entire year. Species that are dietary specialists are those that have evolved feeding strategies to take maximum advantage of peaks in prey abundance (Duellman and Trueb 1994). Feeding strategies for the Columbia spotted frog, a dietary generalist, may not be as reflective of a peak in prey availability.

Second, it may be that the frogs were not feeding because they were stressed by their environmental conditions. Jaeger (1980) found that some amphibians are unable to feed during certain times of the year due to environmental conditions (such as heat and dryness), and therefore had to exist for long periods on negative energy budgets.

This apparent lack of late-season feeding by Columbia spotted frogs needs to be confirmed with further study. Even if this result is borne out, it is unlikely to apply to younger frogs, which will be eating not only to secure energy for hibernations but also to grow. Newly metamorphosed individuals will be most at risk because they only have the relatively short period between metamorphosis in late summer and hibernation in late fall to acquire the fat reserves needed to survive hibernation. In the present study, metamorphs and subadult frogs were not studied because stomach flushing on such small individuals is dangerous and ineffective, and sacrificing enough individuals to get reasonable dietary information was not regarded as feasible.

#### Effects of grazing on larval survival and growth

Our results and analyses clearly show that spotted frog larvae exposed to higher levels of cattle waste, had lower survival than animals exposed to lower levels of waste. It is important to note that our fecal coliform analyses indicate that our levels of addition of waste at least approximate what would be expected in the field.

What might be the mechanism by which larval survivorship is decreased? One possibility is direct toxicity. However, our acute toxicity trials indicated that even at very high levels, short-term effects of addition of urea and feces are not lethal. A second possible mechanism is a decrease in dissolved oxygen concentrations caused by the increased bacterial action associated with the higher quantity of organic materials found in higher waste treatments. The input of

nutrients stimulates activity of the heterotrophic bacteria that utilize dissolved oxygen in their degradation of organic matter. Excessive levels of waste and the corresponding activity of these bacteria can dramatically reduce the dissolved oxygen content of a pond (Gary et al. 1983). To test whether oxygen might be a factor, we measured the dissolved oxygen levels in the experimental aquaria and in natural ponds that supported larvae. An analysis of variance revealed marginally significant differences in dissolved oxygen concentrations among the treatments ( $P=0.078$ , Table 12), with a trend toward lower dissolved oxygen with increased waste concentrations (Figure 3). However, although dissolved oxygen levels were lower in treatments with waste than in controls, oxygen levels were higher in high waste treatments than in ponds that supported healthy tadpole populations (see Appendix 3). Therefore, it is unlikely that dissolved oxygen alone was the sole cause of increased mortality.

A third possibility is that high waste inputs may cause ammonia concentrations to reach harmful levels. Ammonia is generated naturally by processes such as the decomposition of organic matter by heterotrophic bacteria, and ammonia also derives directly from animal excreta. The direct toxic effects of high environmental ammonia have not been well studied in anurans. Jofre and Karasov (1998) found that some anuran species exhibited lower embryo survival rates, increased deformities, and slowed growth and development when exposed to high concentrations of ammonia. Ammonification of the waste by microbes may be the cause of the mortality observed in the microcosm experiments, but we had not made measurements to assess that possibility. Larvae in the acute toxicity trials may not have been subjected to the same levels of ammonia that would be experienced in the field because conditions in the lab were so unnatural: in the lab, no bacteria from pond sediment were present, temperatures were relatively cool and constant, and light levels were substantially lower. Further study is needed to determine the mechanism by which survival was decreased.

Growth rate of larvae was positively affected by the addition of cattle waste. Further analysis indicated no relationship between number of surviving larvae and growth rate. Therefore, decreased density (and corresponding decreased competition) in high waste treatments was probably not the sole cause of increased growth. Perhaps the higher growth rate of larvae in high waste treatments was simply due to an increased quality and quantity of food caused by increased nutrients. Further studies, focused on food habitats of larvae and on the effects of cattle waste on the food resources of larvae, are needed to fully understand this phenomenon.

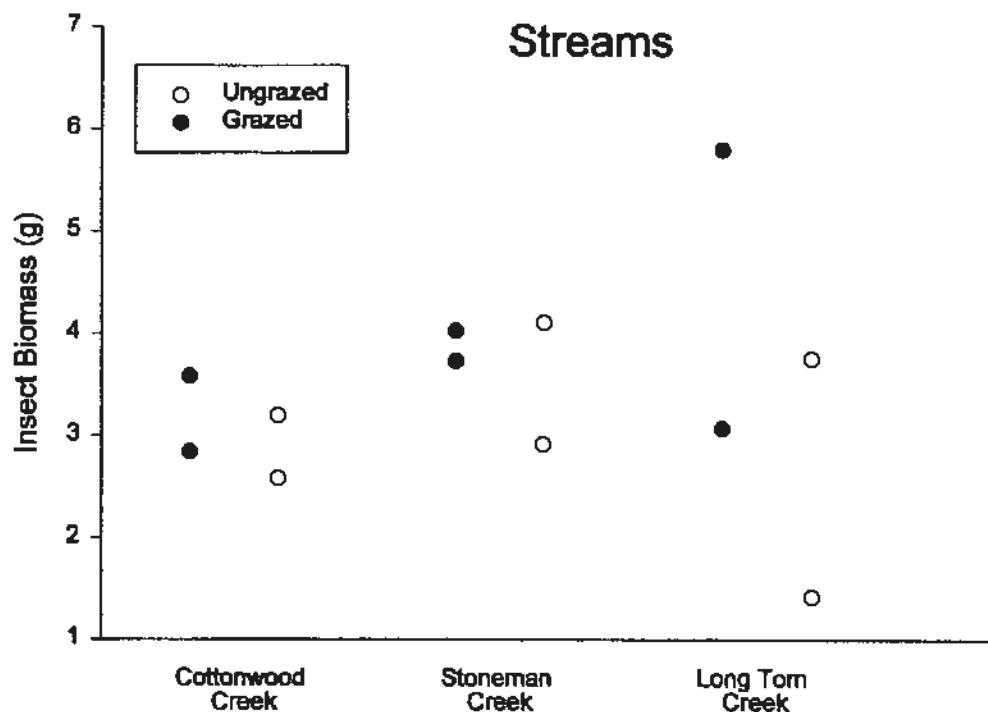
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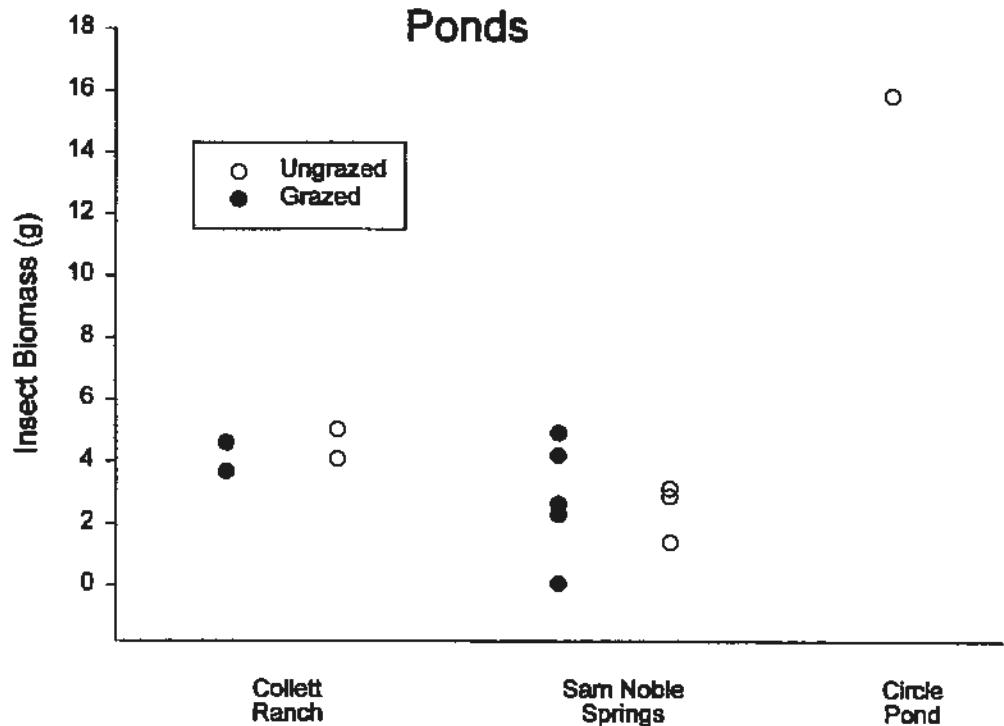
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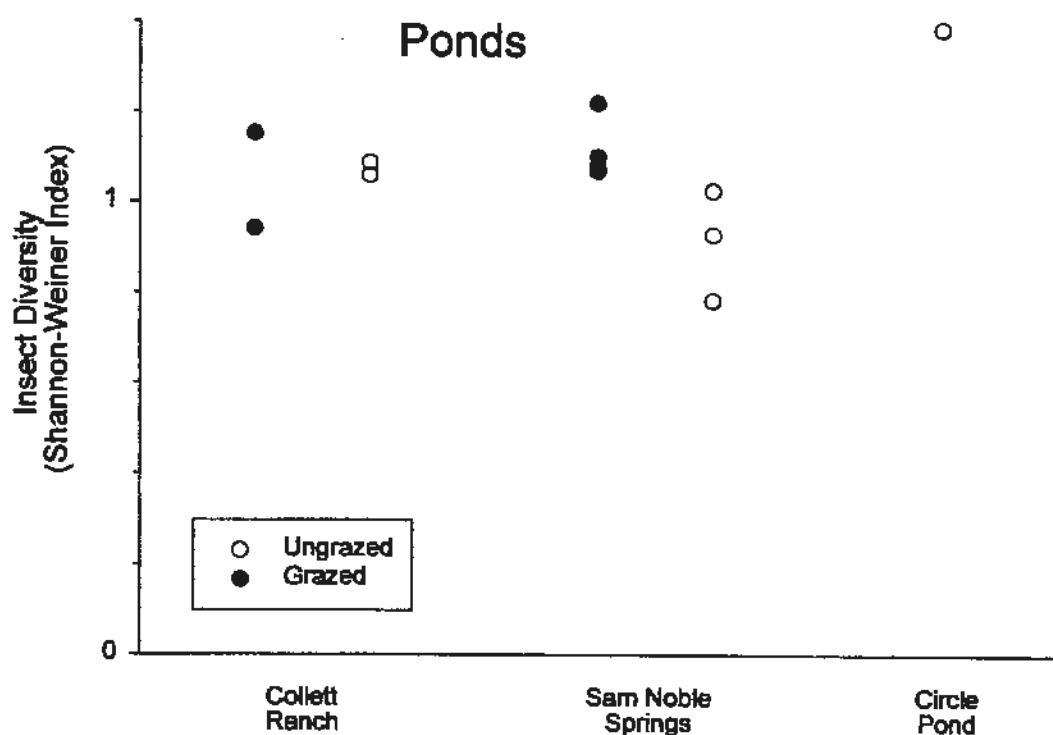
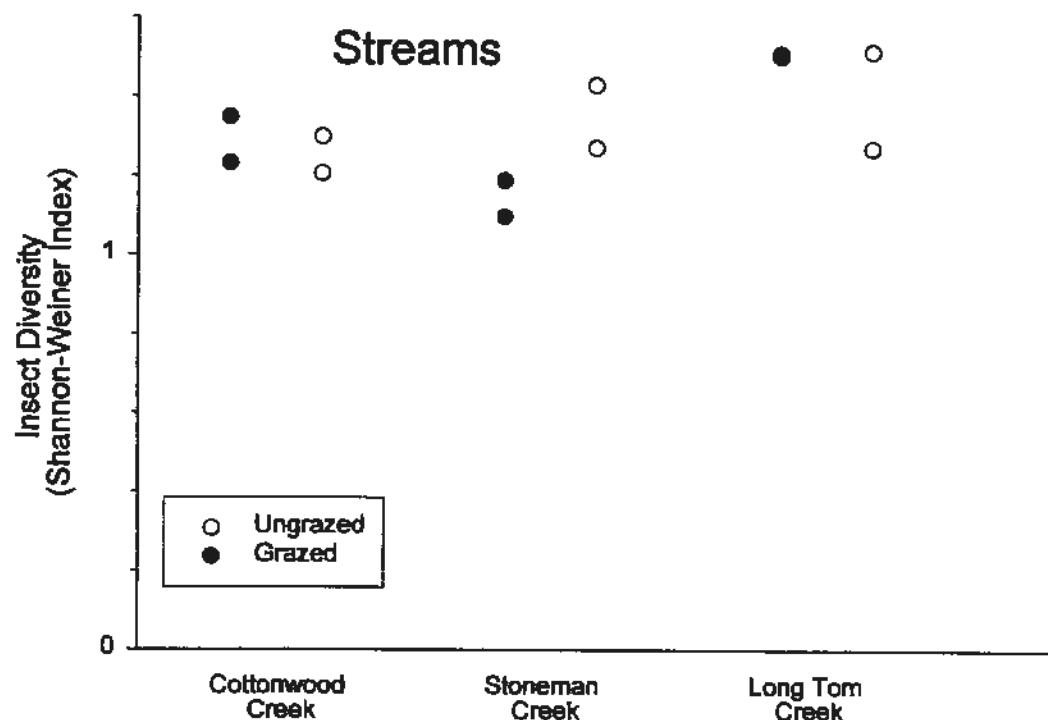
Figure 1.



**Ponds**



**Figure 2.**



**Table 1.**  
**Stream Biomass T-test for 1998**

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>
Grazed	4	2.0587	0.74719
Ungrazed	4	1.9677	0.56563
t	DF	P	
0.0971	6	0.9258	

**Table 2.**  
**Shannon-Wiener T-test results for stream diversity for 1998**

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>
Grazed	4	1.1289	0.0375
Ungrazed	4	1.0811	0.0571
t	DF	P	
0.7002	6	0.5100	

**Table 3.** T-test for invertebrate biomass of streams for 1999

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>	<u>95% Confidence Interval</u>
Grazed	6	3.843	0.4296	2.74 – 4.95
Ungrazed	6	2.998	0.388	2.00 – 4.00
t	DF	P		
1.46	10.0	0.1748		

**Table 4.** T-test for invertebrate biomass of ponds

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>	<u>95% Confidence Interval</u>
Grazed	7	3.197	0.6365	1.64 – 3.20
Ungrazed	5	5.393	2.1485	-0.13 – 10.92
t	DF	P		
-0.98	5.38	0.366		

**Table 5.** T-test results for invertebrate diversity of streams

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>
Grazed	6	1.312	0.069
Ungrazed	6	1.330	0.048
t	DF	P	
-0.2218	10	0.8296	

**Table 6. T-test results for invertebrate diversity of ponds**

<u>Treatment</u>	<u>#</u>	<u>Mean</u>	<u>Standard Error</u>
Grazed	7	1.089	0.032
Ungrazed	6	1.044	0.082
<u>t</u>	<u>DF</u>	<u>P</u>	
0.5207	11	0.6198	

**Table 7. ANOVA table for tadpole survivorship**

<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Treatment	3	130.87	43.620	6.45	0.0013
Error	36	243.50	6.764		

$R^2 = 0.35$

<u>Treatment</u>	<u>Tukey Grouping</u>	<u>Mean</u>	<u>Standard Error</u>
Medium	A	5.700	1.012
Low	A	5.300	0.955
Control	A	4.400	0.622
High	B	1.100	0.618

Means with the same letter are not significantly different.

**Table 8. ANOVA table for tadpole growth rate**

<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Treatment	3	0.001915	0.00064	4.9	0.0079
Error	26	0.00338	0.00013		
$R^2 = 0.36$					

<u>Treatment</u>	<u>Tukey Grouping</u>	<u>Mean</u>	<u>Standard Error</u>
High	A	0.059	0.009
Low	B	0.038	0.003
Medium	B	0.037	0.005
Control	B	0.030	0.003

Means with the same letter are not significantly different

**Table 9. ANOVA table for size at metamorphosis (SVL in mm)**

<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Treatment	3	24.29	8.092	1.260	0.2912
Error	99	634.530	6.409		

$R^2 = 0.37$

<u>Treatment</u>	<u>Number</u>	<u>Mean</u>	<u>Standard Error</u>
Control	9	29.500	0.581
High	3	31.667	0.951
Low	8	30.815	0.351
Medium	8	30.718	0.429

**Table 10. ANCOVA – Survivorship vs. Growth Rate**

<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Treatment	1	0.0011	0.00111	0.287	0.6067
Error	8	0.0310	0.00388		

$R^2 = 0.034$

**Table 11. Average fecal coliform colony counts**

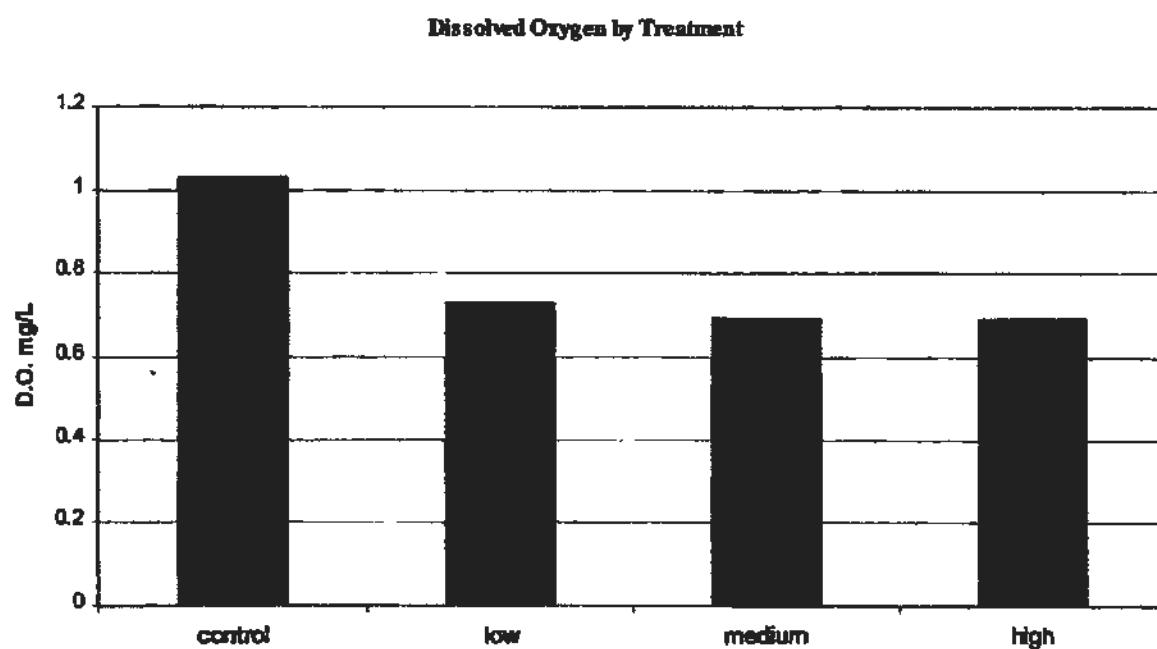
<b>Pond Site</b>	<b>FC Colonies/ml</b>	<b>Treatment</b>	<b>FC Colonies/ml</b>
Sam Noble 1	0.911	High + agitation	6.533
Sam Noble 2	1.178	High, no agitation	6.767
Sam Noble 3	1.356	Medium + agitation	4.000
Sam Noble 4	1.556	Medium, no agitation	3.733
Sam Noble 5	1.156	Low + agitation	2.233
Collett Ranch 1	2.356	Low, no agitation	1.533
Collett Ranch 3	1.600	Control + agitation	1.267
Circle Pond	1.667	Control, no agitation	0.900

**Table 12. ANOVA table for dissolved oxygen**

<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Treatment	3	0.0800	0.26695	2.47	0.0778
Error	36	3.8869	0.10797		

$R^2 = 0.17$

**Figure 3. Mean Dissolved Oxygen**



## Appendix 1. Biomass Data

### 1998 Invertebrate Biomass at Ponds Before and After Grazing

site	Treatment	Total Biomass
#1 Before	Grazed	1.5709
#2 Before	Ungrazed	2.1717
#3 Before	Ungrazed	1.6435
#3 Before	Grazed	0.7388
#1 After	Grazed	2.1914
#2 After	Ungrazed	1.2439
#3 After	Ungrazed	1.0692
#3 After	Grazed	1.1013

### 1998 Invertebrate Biomass at Streams After Grazing

Site	Treatment	Total Biomass
Long Tom #1	Ungrazed	1.1353
Long Tom #2	Grazed	0.9773
Long Tom #3	Grazed	0.7170
Long Tom #4	Ungrazed	0.8587
Cottonwood #1	Ungrazed	3.0639
Cottonwood #2	Grazed	3.9004
Cottonwood #3	Ungrazed	2.8132
Cottonwood #4	Grazed	2.6404

### 1999 Stream Invertebrate Biomass

Stoneman Creek	#1 Ungrazed	#2 Grazed	#3 Grazed	#4 Ungrazed
	1.423	5.799	3.074	3.756
<hr/>				
Long Tom	#1 Ungrazed	#2 Grazed	#3 Grazed	#4 Ungrazed
	2.918	4.030	3.731	4.112
<hr/>				
Cottonwood	#1 Ungrazed	#2 Grazed	#3 Ungrazed	#4 Grazed
	2.585	2.842	3.195	3.584

**1999 Pond Invertebrate Biomass**

Sam Noble	#1 Ungrazed	#1 Grazed	#2 All Grazed	#3 Ungrazed	#3 Grazed	#4 All Grazed	#5 Ungrazed	#5 Grazed
Before grazing	1.643	2.457	1.712	1.834	1.274	3.383	3.105	1.677
After grazing	2.880	2.644	2.295	1.411	0.729	4.188	3.133	4.924

Collett	#1 Grazed	#2 Ungrazed	#3 Grazed	#3 Ungrazed
Before grazing	4.495	3.288	4.493	5.992
After grazing	4.594	5.014	3.666	4.070

Circle Pond	#1 Ungrazed
Never grazed	15.848

**Appendix 2. Shannon-Wiener function of invertebrate diversity**

**1999 Stream Sites**

Site	Condition	S-W diversity
Long Tom 1	Ungrazed	1.2708
Long Tom 2	Grazed	1.1879
Long Tom 3	Grazed	1.0963
Long Tom 4	Ungrazed	1.4293
Cottonwood 1	Ungrazed	1.2042
Cottonwood 2	Grazed	1.2308
Cottonwood 3	Ungrazed	1.2960
Cottonwood 4	Grazed	1.3470
Stoneman 1	Ungrazed	1.26975
Stoneman 2	Grazed	1.5093
Stoneman 3	Grazed	1.5007
Stoneman 4	Ungrazed	1.5137

**1999 Pond Sites**

Site	Condition	S-W diversity
Sam Noble 1	Grazed	1.0796
Sam Noble 1	Ungrazed	0.7827
Sam Noble 2	Grazed	1.0699
Sam Noble 3	Ungrazed	0.9274
Sam Noble 3	Grazed	1.0687
Sam Noble 4	Grazed	1.09971
Sam Noble 5	Ungrazed	1.0237
Sam Noble 5	Grazed	1.21822
Collett 1	Grazed	1.1519
Collett 2	Ungrazed	1.0876
Collett 3	Grazed	0.94157
Collett 3	Ungrazed	1.05907
Circle Pond	Ungrazed	1.38475

### **Appendix 3. Dissolved Oxygen Rates**

#### **Experimental Microcosm Dissolved Oxygen**

<b>Control</b>	<b>Low</b>	<b>Medium</b>	<b>High</b>
1.95	0.85	1.18	0.94
1.25	1.25	0.85	0.95
0.69	0.23	0.32	0.65
0.79	0.74	0.45	0.85
0.8	0.62	0.89	0.42
1.05	0.75	1.23	0.32
1.25	1.31	0.78	0.42
0.98	0.33	0.36	0.85
0.67	0.65	0.5	0.95
0.88	0.59	0.45	0.57

D.O. in mg/l  
Range= 0.23 - 1.95

#### **Natural Pond Dissolved Oxygen (After Grazing)**

0.32	0.18	0.2	0.34
0.21	0.31	0.1	0.23
0.19	0.24	0.23	0.31
0.1	0.32	0.35	0.11
0.31	0.2	0.11	0.24
0.17	0.1	0.36	0.32

D.O. in mg/l  
Range= 0.1 - 0.36

**Appendix 4: Invertebrates captured during 1998.**

Collet Ponds		18-Aug-98								
		Diptera	Lepidoptera	Odonata	Orthoptera	Hymenoptera	Hemiptera	Homoptera	Coleoptera	Spiders
#1	Grazed	429	0	0	0	10	4	195	1	3
#2	Ungrazed	340	0	6	1	34	28	385	8	10
#3	Grazed	124	0	0	0	29	5	91	3	5
#3	Ungrazed	393	0	1	0	16	8	236	3	6

Collet Ponds		05-Sep-98								
		Diptera	Lepidoptera	Odonata	Orthoptera	Hymenoptera	Hemiptera	Homoptera	Coleoptera	Spiders
#1	Grazed	523	0	0	1	19	13	326	3	4
#2	Ungrazed	290	0	2	3	27	24	665	8	16
#3	Grazed	199	0	0	2	18	2	103	6	3
#3	Ungrazed	173	0	1	0	4	5	89	0	2

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Long Tom Creek		06-Sep-98								
		Diptera	Lepidoptera	Odonata	Orthoptera	Hymenoptera	Hemiptera	Homoptera	Coleoptera	Spiders
#1	Ungrazed	148	0	0	6	11	0	140	4	1
#2	Grazed	88	0	0	7	6	9	112	2	3
#3	Grazed	133	0	0	0	16	6	133	3	2
#4	Ungrazed	137	0	0	2	20	3	102	9	1

Cottonwood Creek		11-Aug-98								
		Diptera	Lepidoptera	Odonata	Orthoptera	Hymenoptera	Hemiptera	Homoptera	Coleoptera	Spiders
#1	Ungrazed	440	0	0	13	50	56	268	9	12
#2	Grazed	626	0	1	14	72	27	314	10	18
#3	Ungrazed	563	0	0	7	28	15	294	14	12
#4	Grazed	335	0	0	13	37	28	264	12	12

**Appendix 5: Captures of Invertebrates in 1999 at Streams**

	Long Term	18-Aug-99				Cottonwood				21-Aug-99				Stoneman Creek				08-Jul-99							
		#1		#2		#3		#4		#1		#2		#3		#4		#1		#2		#3		#4	
		Ungrazed	Grazed	Grazed	Ungrazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Ungrazed	Grazed	108	150	0	2	150	150	
Diptera		82	162	135	147		281	214		322		248		77	150		108		150						
Lepidoptera		0	0	0	0		0	1		0		5		0	8		0		2						
Odonata		1	0	0	0		1	1		0		0		0	3		1		1						
Orthoptera		21	32	27	30		5	12		8		11		5	54		13		21						
Hymenoptera		37	26	21	62		79	78		110		151		11	14		42		24						
Hemiptera		10	30	12	41		32	18		48		57		10	17		22		33						
Homoptera		84	217	242	133		197	233		183		272		88	127		151		148						
Coleoptera		0	3	3	8		1	7		8		8		13	18		45		13						
Spiders		12	8	23	45		17	9		8		8		6	13		15		18						
Neuroptera		0	1	0	3		5	0		4		0													
		247	479	463	469		618	573		691		756		220	402		395		409						

**Appendix 6: Captures of Invertebrates at Ponds before Grazing**

Sam Noble	16-Jul-99									Collett Ponds				21-Jul-99			
	#1		#1		#2		#3		#3		#4		#5		#5		
	Ungrazed	Grazed	All	Grazed	Ungrazed	Grazed	All	Grazed	Ungrazed	Grazed	All	Grazed	Ungrazed	Grazed	All	Grazed	Ungrazed
Diptera	51	135	215	35	75	568	71	83	572	275	290	1036	0	2	0	1	
Lepidoptera	0	1	6	0	1	0	2	1	2	3	1	2	0	1	0	1	
Odonata	0	0	5	3	1	3	5	2	2	1	4	1	2	1	2	1	
Orthoptera	0	2	1	3	0	1	5	0	2	1	4	1	2	1	4	1	
Hymenoptera	24	0	30	20	10	35	25	20	36	60	15	62	36	35	22	40	
Hemiptera	1	0	4	3	4	12	37	9	36	35	22	40	185	475	110	643	
Homoptera	88	90	65	50	40	95	90	75	7	75	9	23	7	12	7	8	
Coleoptera	7	15	10	5	0	12	20	10	20	938	458	1816	20	12	7	8	
Spiders	0	1	0	10	0	9	4	3	840	171	244	326	129	131	735	259	203

**Appendix 7: Captures of Invertebrates at Ponds after Grazing**

	Sam Noble		06-Sep-99						Collett Ponds		25-Sep-99		Circle Pond	
	#1 Ungrazed	#1 Grazed	#2 All Grazed	#3 Ungrazed	#3 Grazed	#4 All	#5 Grazed	#5 Ungrazed	#1 Grazed	#2 Ungrazed	#3 Grazed	#3 Ungrazed	#1 Ungrazed	
	73	67	78	83	82	320	52	78	88	223	173	205	358	
Diptera	73	67	78	83	82	320	52	78	88	223	173	205	358	
Lepidoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	
Odonata	0	0	2	0	0	0	2	0	1	0	0	0	0	
Orthoptera	9	6	21	11	3	21	13	24	57	52	12	41	170	
Hymenoptera	7	3	3	2	2	38	3	11	2	3	3	8	6	
Hemiptera	2	1	1	2	2	3	1	1	0	0	0	3	7	
Homoptera	93	232	112	82	42	154	86	107	35	199	154	166	290	
Coleoptera	2	1	2	0	1	3	0	2	1	7	1	1	169	
Spiders	3	0	0	0	3	4	2	1	2	2	2	1	4	
Empheroptera	0	0	0	0	0	2	0	0	188	488	345	427	1024	
	189	310	219	180	115	543	139	224						

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