

**EFFECTS OF GRAZERS ON  
FRESHWATER HABITATS:**

**Interaction Between Salamanders and  
Terrestrial Grazers**

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## ABSTRACT

Amphibians in Arizona frequently breed in shallow ponds that contain easily disturbed bottom sediments. Intense sediment disturbance occurs when large grazing animals wade into amphibian breeding sites to drink water. This study used laboratory and field experiments to examine impacts of sediment disturbance as caused by large grazers (e.g., cattle, deer, elk) on early life history stages of two native salamanders, *Ambystoma tigrinum nebulosum* and *A. t. stebbinsi*. Interaction between larval salamanders and terrestrial grazers is an important topic of research because of the biotic sensitivity of Rocky Mountain Montane Marshland and Plains Interior Marshland communities, in which salamanders and large numbers of grazing cattle co-occur, and because of the endangered status of *A. t. stebbinsi*. Sediment disturbance significantly affected abiotic conditions in study ponds and experimental field enclosures by increasing concentration of suspended sediment, decreasing organic content of suspended sediments, and reducing dissolved oxygen concentration. Sediment disturbance did not affect embryo survival, larval growth rates, or larval survivorship; however, disturbance did significantly affect larval diet and timing of embryo hatching. Sediment disturbance did not significantly affect chlorophyll or bacterial concentrations in field enclosures. We did not assess effects of disturbance on distribution or pathogenicity of a newly discovered viral pathogen known to cause high levels of mortality in tiger salamanders. Overall, indirect effects of sediment disturbance on larval salamanders were weak and likely do not pose a threat to salamander populations. Direct effects of disturbance by grazing animals (e.g., crushing embryos or larvae while wading in a pond) were not assessed, but are also likely not significant. Future research should examine the role that terrestrial grazers might play in transmission and pathogenicity of the new virus.

## INTRODUCTION

Disturbance is typically regarded as a discrete event resulting in removal or death of individuals or populations (Sousa 1984, Pickett and White 1985, Menge and Olson 1990, Power et al. 1996). Disturbance can alter community structure by removing or introducing species (White and Pickett 1985), but disturbance can also modify ecosystem-level processes such as primary and secondary production, nutrient cycling, and energy flow (Sousa 1984). Thus, disturbance can affect linked biological elements on a variety of scales (Pickett et al. 1989). Of primary concern to conservation biologists is the introduction of novel anthropogenic disturbance or human-induced alteration of natural disturbance regimes that might result in loss of threatened or endangered species (e.g., Collins et al. 1981).

Aquatic communities can be disturbed by various mechanisms. Marine intertidal communities can be re-structured by wave impact, shifting of soft substrates, and introduction of novel predators (Thistle 1981, Menge and Olson 1990, Dial and Roughgarden 1998). Floods in stream and river ecosystems can completely alter physical substrates upon which aquatic insect and plant communities develop (Gurtz and Wallace 1984, Power et al. 1996). One consequence of physical disturbance in aquatic ecosystems is resuspension of sediment, a phenomenon especially prevalent in lakes and ponds.

Sediment resuspension is well studied in lotic ecosystems (lakes, ponds, wetlands), but most of these studies focus on inorganic sediments in large lakes which can be resuspended by turbid inflows or wind-induced waves. Walmsley (1978) and Stewart and Martin (1982) describe increases in lake water turbidity following import of inorganic silt from surrounding streams during flooding, and both studies also found significant increases in turbidity following seasonal overturn events. Lake turbidity can also increase due to wind-induced resuspension of

bottom sediments in near-shore areas (Stewart and Martin 1982, Cooper and Bacon 1980).

Experimental studies have detailed effects of sediment suspension on chlorophyll concentrations, primary productivity, responses to fertilization, and zooplankton community structure and behavior in lakes (Cooper and Bacon 1980, Coker 1987, Threlkeld and Soballe 1988), but these studies all focused on inorganic sediments.

Shallow, temporary ponds, like those found throughout arid regions in North America, differ in various ways from larger lakes with respect to sediment characteristics and patterns of sediment suspension (Thames and Ziebell 1988, Williams 1988). Rooted macrophyte production across the basin of a temporary pond leads to high concentrations of organic material in the sediments (Clark 1988). Import of organic material from wading animals, as well as from the surrounding watershed during sudden thunderstorms, can also contribute to organic content of the sediments. The entire basin of a temporary pond can also be disturbed by wind or wave action, unlike larger lakes where this type of disturbance is limited to shallower margins (Stewart and Martin 1982, Bengtsson and Hellstrom 1992). Sediments of a temporary pond may also be disturbed by wading animals such as livestock, elk (*Cervus elephus*), and deer (*Odocoileus virginianus*, *O. hemionus*). When these large animals wade into ponds to drink, large amounts of sediment from several centimeters below the basin surface can be returned to the water column, representing a very different mechanism of sediment suspension from those typically operating in larger lakes (personal observation).

Sediment suspension in shallow, temporary ponds can impact the abiotic and biotic components of aquatic communities. Chronic disturbance and suspension of organically rich pond sediment may present an enormous oxygen demand to the pond ecosystem (Cole and Hannan 1990) as organic material is respired in the water column. Suspended sediments can

also have significant effects on freshwater zooplankton communities either directly by interfering with filter feeding mechanisms (Arruda et al. 1983, Kirk and Gilbert 1990, Kirk 1991, Hart 1992) or indirectly by limiting primary production through limitation of light penetration (Marzolf and Osborne 1972, Cooper and Bacon 1980, Hart 1988). Competitive relationships among zooplankton, notably between cladocerans and rotifers (Kirk and Gilbert 1990, Kirk 1991) and between cladoceran species (Hart 1988, 1992, Kirk and Gilbert 1990, Cuker and Hudson 1992), can also be affected by suspended sediments. At low levels of primary production, organic material adsorbed to suspended sediments may provide an important source of nutrition to zooplankton, especially filter-feeding cladocerans (Arruda et al. 1983, Hart 1988). Thus, sediment suspension may have very complex effects on zooplankton species, which make up the bulk of the diet of freshwater zooplanktivores such as larval salamanders and many species of fish.

Predation by visually oriented zooplanktivores has important effects on freshwater communities by directly affecting zooplankton populations (Hall et al. 1976, McQueen and Post 1988) and indirectly by affecting other trophic levels through trophic cascading interactions (Carpenter et al. 1985, Holomuzki et al. 1994). However, several factors are known to mediate top-down effects of consumers on their resources, including habitat heterogeneity (Holomuzki et al. 1994), high (McQueen et al. 1989) or low (Elser and Goldman 1990) levels of resource productivity, and decline in food edibility or availability (Werner et al. 1983, Harvell 1984). Therefore, given that suspended sediments generally negatively affect zooplankton populations and reduce light penetration into water, and that changes in abiotic conditions can affect the relationship between consumers and their resources (Menge 1978, Robles 1982, Power et al.

1995), sediment disturbance and suspension may affect the influence of zooplanktivores in temporary pond communities.

In this study, we examined the effects of sediment disturbance like that created by large, wading animals on growth and survivorship of larval tiger salamanders (*Ambystoma* spp.) in temporary ponds in Arizona. We focus on amphibians because of recent emphasis on amphibians as indicators of environmental change (Wilbur and Smith 1990), and we focus on tiger salamanders because of their close association with sensitive habitats in Arizona including Plains Interior Marshland and Rocky Mountain Montane Marshland (AGFD 1995). Larval salamanders are also important determinants of temporary pond community structure (Holomuzki et al. 1994). In addition, *Ambystoma tigrinum stebbinsi* (Jones et al. 1995) has recently been classified as endangered under the Endangered Species Act, and studies examining factors regulating populations in this taxon are especially timely. Finally, our study focused on large grazers as the mechanism of sediment disturbance because these species rely on temporary ponds in arid regions of North America (Boyd 1978, Meagher 1978), and because of the increase in the number of cattle grazing in arid regions over the past 100 years. While effects of cattle grazing on streams has been studied (Smith 1989, Schultz and Leininger 1990), little is known about how large grazers impact shallow pond ecosystems. Cattle represent a human-induced change in the sediment disturbance regime in temporary ponds (in terms of timing and severity) as compared with the normal regime induced by elk and deer.

## **METHODS**

### **Field Techniques**

Sites Visited and Utilized. We visited a total of 13 sites in the San Rafael Valley, Santa Cruz County, southern Arizona; we utilized 1 site on the Mogollon Plateau, Coconino County,

central Arizona. We will reference study sites in this report by Site Numbers as presented in Appendix 1.

Field Surveys. We surveyed all sites for all life history stages of *Ambystoma tigrinum stebbinsi* during 12 field trips to the San Rafael Valley between 20 October 1996 and 21 June 1997. Salamander larvae and adults were captured using 5m- to 10m-long X 1.5m high seines (mesh size: 6mm) along the shallow margins of each stock tank. Newly hatched larvae were sampled using fine-meshed dipnets (mesh size: 3mm), and we searched by hand for embryos. All larvae and adults were counted and returned to stock tanks immediately; we estimated embryo number before returning them to the pond. All animals were handled for as short a time as possible to reduce handling stress.

Suspended Sediments. Water samples (25 - 100ml) were taken from mid-water column in several areas of each pond, placed on ice, and returned to the laboratory. To exclude larger suspended particles and zooplankton, all sediment samples were collected from inside an 80  $\mu$ m-mesh zooplankton net suspended in the water. In the lab, sediment samples were filtered through oven-dried Whatman GF/C glass fiber filters (1  $\mu$ m particle retention) and dried overnight at 60E C. After being weighed, filters were then ashed overnight at 500EC in a muffle furnace and weighed again. Filters were cooled in a desiccator after all drying procedures. Weight difference after initial drying was used to calculate suspended sediment concentration in mg/l, and weight difference after ashing was used to calculate percentage of suspended sediments comprised of organic material.

During field surveys and field experiments, we measured suspended sediment concentration and percent organic content before and after sediment disturbance. We collected suspended sediment samples from the water column of ponds or experimental enclosures

(described below) immediately upon arrival at a site and also ten minutes after disturbing sediments with a 2.5cm diameter PVC pipe. A relatively small section of each survey pond (~75 m<sup>2</sup>) was disturbed in this fashion to mimic the disturbance presented by 3 - 5 cattle drinking from the water's edge; sediment and dissolved gas samples (described below) were then only taken from this section. We inserted the pipe several cm into the substrate and then withdrew it rapidly. This mode of sediment disturbance mimicked disturbance induced by large wading animals when drinking water from a pond.

Dissolved Gases. Dissolved oxygen was measured using a YSI 5270 oxygen probe held in the center of the water column; dissolved oxygen concentration is expressed as mg/l. To measure dissolved carbon dioxide, we collected water samples in 60ml syringes and discharged these samples into 250 ml glass jars while holding the tip of the syringe near the bottom of the jar. Several such samples were collected and discharged into the jar, always with the syringe tip near the jar bottom. By allowing water to overflow the jar by several volumes, we were able to prevent exchange between atmospheric and water sample carbon dioxide (Wetzel and Likens 1991). Free carbon dioxide concentration was determined using the phenolphthalein/0.0454 N sodium carbonate titration method described by Lind (1974).

Chlorophyll *a*. To measure concentration of chlorophyll *a* in experimental enclosures, we used 60 ml syringes to sample 30 - 100 ml of water mid-water column in several areas of each enclosure (described below). These samples were then filtered through Whatman GF/C filters. Filters were stored in waterproof, light-tight containers and were frozen until analysis in the laboratory (< 48 hours). In the lab, filters were thawed and extracted at 4E C for 24 hours in 90% buffered acetone (Wetzel and Likens 1991). We measured chlorophyll concentrations in the extract using a model 112 Turner fluorometer; chlorophyll concentration is expressed as □g/l

of pond water. Samples for chlorophyll concentrations were always taken prior to any sediment disturbance.

Bacteria. Water samples for bacteria were collected in sterile, 50 ml glass vials and preserved using 2% formalin previously filtered through 0.2  $\mu$ m Nucleopore filters (Kepner and Pratt 1994). These samples were placed on ice and stored at 4°C until counted. In the lab, subsamples were diluted, filtered through 0.2  $\mu$ m Nucleopore filters and stained using acridine orange (10  $\mu$ g/l) (Kepner and Pratt 1994, Porter and Feig 1980). Bacterial cells were then enumerated using a fluorescent microscope (maximum excitation at 470 nm) at 1000X magnification. Bacterial density is expressed as # cells/ml of pond water.

Zooplankton. Zooplankton were collected from experimental enclosures using a hand-operated bilge pump. This pump had 2.5cm diameter hoses and flapper-style rubber valves; thus, zooplankton were not damaged during transit through the pump. 15 liters of water were pumped from several areas and depths of each enclosure to obtain an integrated sample; water was discharged from the pump through an 80  $\mu$ m mesh zooplankton net with a collecting jar attached. We preserved zooplankton samples in the field using Lugol's solution (Wetzel and Likens 1991).

In the laboratory, zooplankton in two, 10 ml subsamples were enumerated under a dissecting microscope at 120X magnification; body length of up to 20 randomly selected individuals of each taxon encountered was measured to the nearest 0.02 mm using an ocular micrometer. Each zooplankton taxon was identified to at least genus level using Pennak (1989) and Thorp and Covich (1991).

## **Field Experiments**

To examine effects of sediment disturbance such as that caused by large, wading animals on the pond ecosystem and on larval salamander growth and survival, we performed a field experiment at Site 1 (see Appendix 1) in the San Rafael Valley. We carried out the study using twelve, polyethylene barrels (80 cm high X 55 cm diameter) arranged along one side of the pond (Figure 1) on 20 May 1997. Nylon screening (4 meshes per cm) placed over the bottom of each barrel excluded salamanders and predaceous insect larvae from barrels as they were placed in the water; barrels were left open at the top. We then spread approximately 30 l of pond sediments over the bottom of each barrel, being careful to exclude insects and salamanders. Zooplankton collected from the pond using an 80  $\mu$ m mesh zooplankton net were stocked into each barrel to return zooplankton to ambient densities. We then left barrels undisturbed for 8 days to allow sediments to settle and zooplankton and algal populations to recover.

We assigned treatments to individual barrels on 28 May 1997, using a 2 X 2 factorial design with disturbance regime (either disturbed or undisturbed) and salamander predation (two larval salamanders either present or absent) as factors. Each of the four treatment combinations was replicated three times (Figure 1). Salamanders for the study were captured from the pond, matched for size, weighed to the nearest 0.01 g, and assigned to barrels. We used salamander predation as a factor in this study to be able to detect any interaction between salamander presence and disturbance regime on other biotic and abiotic variables in the pond ecosystem.

We stirred sediments in disturbed treatment barrels every three to four days using a 2.5 cm PVC pipe that was pushed into the sediments and withdrawn rapidly. Care was taken to avoid damaging nylon screens. We terminated the study on 21 June 1997 after disturbing barrels on 17 June. Surviving larval salamanders were collected and preserved in 10% formalin. We also collected bacteria, chlorophyll, suspended sediment, and zooplankton samples from each

barrel as described above, and we measured dissolved oxygen and carbon dioxide concentrations in each barrel. After larvae and all samples were collected, we stirred sediments in disturbed treatment barrels and again measured suspended sediment, oxygen, and carbon dioxide concentrations in those barrels.

In the lab, larvae were weighed to determine growth over the course of the study. In addition, we dissected stomachs from preserved larvae to detect treatment effects on feeding rate and prey selection. Stomach contents of each larva were sorted by volume in petri dishes, and prey items were identified as being either large or small insects, zooplankton, benthic worms, or unidentified material (which could include suspended sediments). Stomach contents were then filtered and dried overnight at 60°C to estimate total food mass.

An experiment similar to the one described above was carried out at Site #14 (see Appendix 1) between 30 August 1998 and 1 October 1998. Briefly, the same twelve polyethylene barrels were used as enclosures and were set up in a fashion similar to the San Rafael Valley experiment (Figure 1), and sediment disturbance regime matched that described for the previous study. Effects of sediment disturbance on bacterial, chlorophyll, and zooplankton concentrations and on larval salamander (*A. t. nebulosum*) growth and diet were determined as above; we were unable to determine treatment effects on dissolved gas concentrations or on organic content of suspended sediments.

### **Laboratory Experiments**

Embryo Hatching Success. Embryos of *A. t. stebbinsi* for this experiment were collected from Site #1 and Site #12 (Appendix 1) on 14 February 1997 and returned to the laboratory. We assigned embryos at random in groups of five to 40, 250 ml glass jars containing 200 ml of aged tap water. Half of these jars then received approximately 10 g of sediment collected from Site

#12 while the other half had no sediments added. Embryos were then allowed to develop in an environmental chamber maintained at 10E C on a 12:12 light:dark cycle. Half of the sediment-added jars and half of the no-sediments jars were swirled by hand twice a day; swirling caused sediments to be re-suspended and then slowly settle out over the embryos. Thus, this experiment was a two-factor randomized design, with disturbance regime (either swirled twice a day or undisturbed) and sediment presence/absence as factors. Water levels were maintained throughout the study by adding necessary amounts of aged tap water.

We monitored embryo hatching at least twice per day throughout the study. We recorded the day on which individual embryos hatched in each treatment, and continued the study until all embryos had either hatched or died (5 April 1997). We compared hatching schedule of all 50 embryos in each treatment to determine effects of physical disturbance and sediment re-suspension on embryo survival and hatching success.

Early Larval Growth. Newly hatched (< 3 weeks old) larval *A. t. nebulosum* were collected from Site #14 on the Mogollon Plateau and returned to the laboratory. Larvae were then weighed to the nearest 0.01 g, placed into individual plastic cups containing 250 ml of aged tap water and fed brine shrimp (*Artemia salina*) daily at a concentration sufficient to allow rapid growth beginning 8 August 1998. We used a total of 30 larvae for this study. Twenty cups received about 10 g of sediment from Site #14 while no sediment was added to the other 10 cups. The ten no-sediment cups and 10 of the sediment-added cups were swirled twice per day during the study. Thus, this experiment was a two-factor randomized design, with disturbance regime (either swirled twice per day or undisturbed) and sediment presence/absence as factors. Due to unexpected mortality of larvae in the lab, we were unable to maintain a no disturbance/no

sediment control treatment. Water levels were maintained in the cups as necessary, and water was changed every 7 - 10 days.

We terminated this study on 3 September 1998 after larvae had grown to 3 - 4 times initial weight. All larvae were weighed again to evaluate treatment effects on early larval growth and survival. All surviving larvae from both laboratory experiments were preserved in 10% formalin at the conclusion of each experiment.

### **Statistical Analyses**

Chlorophyll concentration, bacterial concentration, suspended sediment mass, larval salamander growth, and mass of larval stomach contents were log-transformed prior to analyses to better conform with assumptions of statistical tests (Sokal and Rohlf 1995). Organic fraction of suspended sediments and proportions of larval salamander diet components were arc-sine, square-root (angularly) transformed, as these are percentage variables constrained in value between 0 and 1. Relative growth of larval salamanders (total growth divided by initial size), oxygen concentrations, and embryo hatching schedule variables (measured in days) were not transformed.

In general, treatment effects were analyzed using Analysis of Variance (ANOVA) (Sokal and Rohlf 1995). Suspended sediment mass and percent organic content of suspended sediments were compared before and after sediment disturbance in both field experiments and field surveys using one way ANOVA. We did not statistically compare these variables between ponds as we did not have enough replicate samples from each pond for a powerful repeated-measures ANOVA. Treatment effects on chlorophyll and bacterial concentrations, zooplankton density and mean size, and larval salamander growth and survival in field experiments were analyzed using two way ANOVA; two way ANOVA was also used to examine treatment effects on larval

salamander growth and survival in laboratory experiments. Treatment effects on hatching schedule of embryos in the laboratory experiment was analyzed using two methods: two way ANOVA tested for effects on days required for first embryo to hatch as well as total number of days required for replicate groups of five embryos to all hatch, and pairwise Kolmogorov-Smirnov tests (Sokal and Rohlf 1995) tested for treatment effects on the schedule of hatching (computed as the cumulative fraction of all 50 embryos in each treatment to hatch) between treatments.

## **RESULTS**

### **Field Surveys**

Results of SRV field surveys for all life history stages of *Ambystoma tigrinum stebbinsi* completed during this study are presented in Table 1. We surveyed a total of 13 sites on several dates between October 1996 and June 1997. Data presented in Table 1 only represent observations made during this study; other researchers from our laboratory surveyed additional ponds in the SRV. Some sites were difficult to sample quantitatively (e.g., Site 5), and we did not observe any life history stages of *A. t. stebbinsi* at some sites where salamanders had been observed previously (e.g., Sites 8 and 13).

Suspended sediment concentration and organic composition of suspended sediments were both affected strongly by our mechanism of sediment disturbance. Sediment disturbance trials were run in seven survey ponds in the SRV (Sites 2, 3, 7, 8, 9, 10, and 12) on two dates (14 February and 6 April 1997). Concentration of suspended sediments increased on average over 6-fold within 10 minutes of disturbing sediments (Table 2, Figure 2A). Percent organic composition of suspended sediments was significantly decreased by disturbing sediments (Table

3, Figure 2B). These patterns held for both sampling dates, suggesting no interaction between seasonal growth of algae and changes in response variables to sediment disturbance (interaction term not significant in Tables 2 and 3). In survey ponds, sediment disturbance did not significantly affect either dissolved carbon dioxide or dissolved oxygen concentrations 10 minutes after disturbance (ANOVA, both  $P > 0.37$ ).

### **Field Experiments**

In the SRV barrel experiment at Site #1, sediment disturbance significantly affected both suspended sediment concentration and percent organic composition of sediments in a fashion analogous to that seen in survey ponds (Tables 4, 5, Figure 3). Chronic levels of suspended sediment tended to be higher in barrels than in survey ponds (compare Figure 3A to 2A), and disturbance resulted in similar levels of suspended sediments between barrels and survey ponds. Percent organic composition of sediments tended to be higher in barrels than in survey ponds, both before and after sediment disturbance (cf. Figures 3B and 2B, D). Salamander presence did not affect sediment concentration or percent organic composition of suspended sediments.

Dissolved oxygen concentration in barrels did not differ between treatments prior to sediment disturbance, but disturbance significantly lowered oxygen concentration in disturbed barrels 10 minutes after disturbance (Table 6, Figure 4). Salamander presence in itself did not significantly affect oxygen concentration. Interestingly, the interaction between salamander presence and disturbance was significant (Table 6), driven primarily by the larger effect of disturbance on oxygen in barrels that contained salamanders (Figure 4).

Neither chlorophyll *a* concentration nor bacterial concentration were significantly affected by treatments (Table 7, Figures 5, 6). Chlorophyll concentrations were far higher in this experiment than was observed in a series of ponds on the Mogollon Plateau and White

Mountains of Arizona by Holomuzki et al. (1994); we do not know if these very high chlorophyll concentrations represents a general phenomenon in the SRV. The only crustacean zooplankton species present in any appreciable density was the calanoid copepod *Diaptomus nudus*. We identified and quantified both adult and copepodite life history stages (Pennak 1989); there were no significant treatment effects on density of either life history stage (Table 8, Figure 7).

Larval salamanders survived well in this experiment (only one larva died), and disturbance regime had no significant effect on larval growth (one-way ANOVA testing disturbance effect on change in mean weight,  $P > 0.5$ ). Food mass in larval stomachs at the end of the study was also not affected by disturbance regime (Table 9, Figure 8A), but larval diets were affected by sediment disturbance. Larvae in undisturbed barrels had relatively more zooplankton in their diet, whereas larvae from disturbed barrels had a greater proportion of small aquatic insects (e.g., small corixids, notonectids, larval damselflies) in their diet (Table 10, Figure 8B). Even though univariate ANOVAs revealed significant treatment effects on these diet components, the overall effect of sediment disturbance on diet using MANOVA was only marginally significant ( $P = 0.10$ , Table 10).

In the Mogollon Plateau barrel experiment at Site #14, sediment disturbance also greatly increased suspended sediment concentration as in the SRV study (Table 11, Figure 9). Chronic levels of suspended sediment in experimental enclosures tended to be lower at Site 14 than in Site 1 (compare Figure 9 with Figure 3A); however, sediment disturbance at Site 14 resulted in more than double the suspended sediment concentration as at Site 1 (cf. Figures 9 and 3C). There was also a significant effect of larval salamander presence on suspended sediment concentration, especially after sediment disturbance (Table 11).

As in the SRV study, sediment disturbance did not affect chlorophyll *a* or bacterial concentrations at Site 14 (Table 12, Figures 10, 11). Bacterial concentrations observed in the Plateau study were comparable to those observed in the SRV, and chlorophyll concentrations in the Plateau study were much more comparable to those seen in other Mogollon Plateau/White Mountain sites as compared with the SRV study (see Holomuzki et al. 1994).

Several zooplankton species were observed in samples taken from barrels at Site 14, including *Daphnia* sp., *Chydorus* sp., *Alona* sp. (herbivorous cladocerans), *Cyclops* sp. (a predaceous cyclopoid copepod), and *Diaptomus* sp. (a herbivorous calanoid copepod). Larval salamanders prefer to feed on herbivorous cladocerans when they are available (Holomuzki et al. 1994), and there were no significant effects of sediment disturbance on densities of those three species (Table 13A - C). Larval salamanders tended to depress densities of cladocerans, as has been observed in other studies, and salamander presence significantly decreased density of *Alona* ( $P < 0.038$ , Table 13B).

Contrary to the SRV study, sediment disturbance slightly increased larval salamander growth rates over the course of the Plateau study, although this effect was not statistically significant (Table 14). Sediment disturbance did not significantly affect mass of food in larval stomachs (Table 15, Figure 12A), nor did it significantly affect proportional representation of zooplankton or aquatic insects in larval diets (Table 16, Figure 12B).

### **Laboratory Experiments**

We examined effects of presence/absence of sediment and physical disturbance (being swirled twice per day) on hatching schedule of *A. t. stebbinsi* embryos from the SRV raised in the laboratory. Analyzed using ANOVA, data showed no treatment effect on survival of embryos to hatching (Table 17A); however, there was a significant effect of disturbance on total

number of days required for hatching in that disturbed embryos required fewer days for all embryos to hatch after the first embryo hatched (Table 17B). We also used pair-wise Kolmogorov-Smirnov tests to examine differences in cumulative relative frequencies of hatched embryos over time for each treatment; none of these pair-wise comparisons revealed significant differences between disturbance regimes (Table 18, Figure 13).

There were no significant treatment effects of sediment presence or physical disturbance on growth of early larval *A. t. nebulosum* raised in the laboratory. This held whether we examined absolute change in larval mass (Table 19A) or change in mass relative to initial mass (= relative growth; Table 19B, Figure 14). Recall that we were unable to maintain a control treatment in this part of the study (i.e., there was not a No sediments/no physical disturbance@ treatment combination present); thus, we cannot assess whether sediment presence *or* physical disturbance might have independently affected early larval growth.

## DISCUSSION

Ambystomatid salamanders and many other amphibian species breed in shallow, temporary ponds that possess easily disturbed bottom sediments (Duellman and Trueb 1986). Such sediments can be disturbed by wave action (Bengtsson and Hellstrom 1992), by rain water inflow, and especially by activities of large, wading animals that use shallow ponds as water resources (Boyd 1978, Meagher 1978). Interaction between sediment disturbance and amphibian life histories in Arizona is of interest given the endangered status of *Ambystoma tigrinum stebbinsi*, a salamander native to southern Arizona (Jones et al. 1995); the vulnerability of biotic components in Rocky Mountain Montane Marshlands and Plains Interior Marshlands, habitats considered to be sensitive by Arizona Game and Fish Department (AGFD 1995) and

that possess several amphibian species; and the current debate over causes of worldwide amphibian declines (Pechmann and Wilbur 1994). The purpose of this study was to evaluate the impact that large, terrestrial grazers (e.g., cattle, elk, deer), through their activities in using shallow ponds as water resources, might have on native salamander species.

Overall, effects of sediment disturbance on early life history stages of *A. t. stebbinsi* and *A. t. nebulosum* appear to be weak. We found that sediments in ponds supporting both species are easily disturbed, and that sediment disturbance can temporarily alter abiotic conditions in these sites (Table 2, Figures 2, 3, 4). Suspended sediment concentration increases dramatically, percentage organic content of suspended sediment declines, and dissolved oxygen concentration decreases in response to sediment disturbance. However, these effects were not as strong when we disturbed relatively small sections of a natural pond as they were inside experimental enclosures (see Results, Tables 3, 4). Polyethylene barrels, while allowing us to carefully analyze effects of sediment disturbance on salamander growth and other aspects of pond communities, did not allow water in disturbed barrels to exchange with undisturbed areas outside barrels. Our results suggest that dissolved oxygen likely diffuses from undisturbed areas of a natural pond into areas disturbed by wading animals rapidly enough to offset local abiotic effects of sediment disturbance. Thus, our results suggest that disturbance of relatively small sections of shoreline of a large pond by wading animals will not significantly affect early life history stages of salamanders through alterations of abiotic conditions. On the other hand, abiotic conditions may be more generally affected if a large number of grazers utilize small ponds.

Free dissolved carbon dioxide concentration did not increase in response to sediment disturbance, even over relatively short periods of time. This is probably due to the carbonate chemistry of these waters. In hard water (water with high carbonate content), free carbon

dioxide released by bacterial metabolism can be converted rapidly to bicarbonate (Wetzel and Likens 1991). Bicarbonate is not known to be toxic to aquatic vertebrates and is still usable by phytoplankton as a substrate for photosynthesis. In softer waters (e.g., ponds in the White Mountains of Arizona on primarily volcanically-derived soils), carbon dioxide released by respiration of organic sediments may more readily remain in a toxic, free state and correspondingly drive down pH (Wetzel and Likens 1991).

In our laboratory experiments, presence of sediments and sediment disturbance did not significantly affect embryo survival (Table 17A), but disturbed embryos did show less highly variable hatching schedules (Table 17B). In other words, disturbed embryos required fewer total days for all of them to hatch (once the first embryo had hatched) than did undisturbed embryos. This may have been due to an aerating action of disturbance itself. Amphibian embryo development is sensitive to local concentrations of dissolved oxygen (Salthe and Mecham 1974), and some amphibian species even possess parental strategies that include aeration of embryos (Duellman and Trueb 1986). Undisturbed embryos in our laboratory experiment may have depleted dissolved oxygen locally, especially as they were in contact with each other during the study. Sediments may have also decreased local oxygen concentrations at the bottom of our experimental jars (see Methods), and we noted that undisturbed embryos in the presence of sediments took the longest to hatch. Disturbing embryos twice daily thus may have allowed a mild aeration of all embryos in disturbed treatments, even in the presence of sediments, and consequently reduced variability in developmental and hatching rates. Embryos of both *A. t. stebbinsi* and *A. t. nebulosum* are frequently laid in close contact with each other in natural ponds (personal observation), and mild water disturbance by wave action and wading animals may help aerate them during development.

Growth of larvae in laboratory and field experiments was not affected significantly by sediment disturbance (see Results, Table 19). Disturbance did significantly affect diet composition in the SRV experiment, and in both field experiments, larvae from disturbed barrels had a lower fraction of zooplankton, and a greater fraction of insects, in their diet compared to larvae from undisturbed barrels (Figures 8, 12). This diet shift could have been caused by reduced availability of zooplankton in the presence of suspended sediments, as observed by Kirk (1991) and Kirk and Gilbert (1990). Our results do not support this hypothesis, however, as zooplankton densities were not significantly affected by sediment disturbance in either field experiment (Tables 8, 13; Figure 7). We feel it more likely that either zooplankton were not as readily detected by larvae in disturbed barrels, or that aquatic insects were more available to larvae in disturbed barrels due to unstable sediments and a lack of stable perching sites (Merritt and Cummins 1996).

Bacterial concentrations were not affected in either field experiment by sediment disturbance. Our focus on bacterial concentrations is due to observations of salamander mortality due to bacterial infection (*Aeromonas* sp. = Red-leg disease) (Worthylake and Hovingh 1989, Berna 1990) and a possible link between bacterial disease and global amphibian decline (Carey 1983, Laurance et al. 1996). Our results suggest that bacteria are fairly uniformly distributed throughout the water column in SRV and Mogollon Plateau ponds, even in response to sediment disturbance. It is now known that a novel viral pathogen may be playing a role in salamander mortality in Arizona (Jancovich et al. 1997), and it is not known how sediment disturbance might affect transmission characteristics/pathogenicity of this virus. We observed a major die-off of branchiate *A. t. stebbinsi* at Site #4 in the SRV (see Table 1); characteristics of dead animals suggested *Aeromonas* infection as the cause of death. However, *Aeromonas*

infection was likely secondary to infection by the virus described by Jancovich et al. (1997), and stock tanks in the SRV should be monitored closely for incidence of this virus.

## CONCLUSIONS

Sediment disturbance, and its effects on abiotic conditions in shallow ponds, does not appear to significantly affect early life history stages of Ambystomatid salamanders in Arizona. This may not be surprising given the accepted evolutionary history of this taxon. Survivorship and spread of the genus *Ambystoma*, especially in central North America, is believed to have been maintained and facilitated historically by the availability of breeding sites created and maintained by large terrestrial grazers (e.g., buffalo). Thus, species in this genus may already be adapted to disturbance and abiotic conditions created in shallow ponds by activities of large grazers. This study focused on indirect effects of terrestrial grazers; that is, effects of sediment disturbance resulting from activities of wading animals. The potential direct effects of large grazers (e.g., crushing embryos and/or larvae while wading in the pond) are not known, and we do not expect them to be a significant source of mortality.

Future research on interactions between salamanders and terrestrial grazers in Arizona should include focus on how grazers might impact the spread of disease organisms from pond to pond. We have observed that mortality rates from viral and secondary bacterial infections can be very high (Berna 1990, Jancovich et al. 1997), and management of endangered and/or sensitive amphibian populations should be planned in light of the potential role that cattle, elk, antelope, and deer might play in the epidemiology of such diseases.

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**Table 1.** Results of surveys of SRV ponds for *A. t. stebbinsi* from October 1996 to June 1997.

Site # <sup>*</sup>	Embryos <sup>H</sup>	Larvae <sup>I</sup>	Metamorphs <sup>J</sup>	Neotenes <sup>a</sup>
1	1	0	1	1
2	0	1	0	0
3	2A	1	1	0
4 <sup>b</sup>	1	0	0	0
5 <sup>c</sup>	0	0	0	0
6	0	0	0	0
7	4	1	0	1
8	0	0	0	0
9	3	0	0	0
10	2	1	1	0
11	0	0	0	0
12	4	2	0	0
13 <sup>d</sup>	0	0	0	0

<sup>\*</sup> Refer to Appendix 1 for site names and localities.

<sup>H</sup> Embryo densities: 0 = not observed; 1 = < 100 (likely only one clutch); 2 = 100 - 500 (likely only 1 clutch); 2A = 100 - 500 (likely more than one clutch); 3 = 500 - 1000; 4 = > 1000

<sup>I</sup> Larval densities: 0 = not observed; 1 = < 100; 2 = 100 - 500; 3 = > 500

<sup>J</sup> Metamorph (terrestrial adult) densities: 0 = not observed; 1 = < 20; 2 = 20 - 50; 3 = > 50

<sup>a</sup> Neotene (mature branchiate) densities: 0 = not observed; 1 = < 10; 2 = 10 - 30; 3 = > 30

<sup>b</sup> This tank was subject to a major die-off due to disease in December of 1995. We observed ~100 dead mature branchiate salamanders around the edge of this site.

<sup>c</sup> This site has extremely steep sides, making it very difficult to sample for salamanders.

<sup>d</sup> This site has contained healthy populations of *A. t. stebbinsi* in the past; however, during most this study, the pond was nearly dry and its source of water (pumped from a nearby steel cattle watering tank) appeared dry.

**Table 2.** Effects of sampling date and disturbance treatment on concentration of suspended sediments (log-transformed g/liter) in survey ponds in the SRV.

Source	SS	d.f.	MS	F	P
Sampling Date	0.0002	1	0.0002	1.62	> 0.21
Disturbance Regime	0.0031	1	0.0031	20.81	< 0.0002
Sampling Date * Disturbance	0.0015	1	0.0015	1.03	> 0.32
Error	0.00322	22	0.00015		

**Table 3.** Effects of sampling date and disturbance treatment on angularly transformed percent organic composition of suspended sediments in survey ponds in the SRV.

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0435	1	0.0435	1.700	> 0.20
Disturbance Regime	1.0760	1	1.0760	42.03	< 0.00001
Salamander * Disturbance	0.0107	1	0.0107	0.417	> 0.52
Error	0.5632	22	0.0256		

**Table 4.** Treatment effects on suspended sediment concentration in SRV barrel experiment, before and 10 minutes after sediment disturbance.

Before sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0058	1	0.0058	0.4917	> 0.50
Disturbance Regime	0.0016	1	0.0016	0.1357	> 0.72
Salamander * Disturbance	0.0001	1	0.0001	0.0050	> 0.94
Error	0.0945	8	0.0118		

After sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0019	1	0.0019	0.1084	> 0.75
Disturbance Regime	0.2250	1	0.0225	12.604	< 0.008
Salamander * Disturbance	0.0016	1	0.0016	0.0892	> 0.77
Error	0.1428	8	0.0178		

**Table 5.** Treatment effects on percent organic composition of suspended sediments (angularly transformed) in SRV barrel experiment, before and 10 minutes after sediment disturbance.

Before sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0116	1	0.0116	1.651	> 0.23
Disturbance Regime	0.0235	1	0.0235	3.332	> 0.11
Salamander * Disturbance	0.0083	1	0.0083	1.177	> 0.31
Error	0.0565	8	0.0071		

After sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0031	1	0.0031	0.518	> 0.49
Disturbance Regime	0.5890	1	0.5890	97.58	< 0.0001
Salamander * Disturbance	0.0205	1	0.0205	3.397	> 0.103
Error	0.0482	8	0.0060		

**Table 6.** Treatment effects on dissolved oxygen concentration in SRV barrel experiment before and 10 minutes after sediment disturbance.

Before sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	4.320	1	4.320	1.578	> 0.24
Disturbance Regime	7.053	1	7.053	2.577	> 0.14
Salamander * Disturbance	4.813	1	4.813	1.758	> 0.22
Error	21.900	8	2.737		

After sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.241	1	0.241	0.071	> 0.79
Disturbance Regime	102.668	1	102.668	30.345	< 0.0006
Salamander * Disturbance	22.688	1	22.688	6.706	< 0.04
Error	27.067	8	3.383		

**Table 7.** Treatment effects on (A) log-transformed chlorophyll *a* concentration and (B) log-transformed bacterial concentration in SRV barrel experiment.

A. Chlorophyll *a*

Source	SS	d.f.	MS	F	P
Salamander Presence	0.1851	1	0.1851	1.781	> 0.21
Disturbance Regime	0.0130	1	0.0130	0.124	> 0.73
Salamander * Disturbance	0.0482	1	0.0482	0.463	> 0.51
Error	0.8319	8	0.1039		

B. Bacteria

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0002	1	0.0002	0.0426	> 0.84
Disturbance Regime	0.0009	1	0.0009	0.1757	> 0.69
Salamander * Disturbance	0.0105	1	0.0105	1.9842	> 0.19
Error	0.0426	8	0.0053		

**Table 8.** Treatment effects on (A) log-transformed adult copepod densities and (B) log-transformed copepodite densities in SRV barrel experiment (copepod species is *Diaptomus nudus*).

A. Adult copepod densities

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0027	1	0.0027	0.026	> 0.87
Disturbance Regime	0.0001	1	0.0001	0.001	> 0.99
Salamander * Disturbance	0.1215	1	0.1215	1.193	> 0.31
Error	0.8144	8	0.1018		

B. Copepodite life history stage densities

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0361	1	0.0361	0.724	> 0.41
Disturbance Regime	0.0282	1	0.0282	0.566	> 0.47
Salamander * Disturbance	0.0007	1	0.0007	0.013	> 0.91
Error	0.3988	8	0.0498		

**Table 9.** Effects of disturbance regime on food mass (log-transformed) in larval salamander stomachs at the end of the SRV barrel experiment.

Source	SS	d.f.	MS	F	P
Disturbance Regime	< 0.001	1	< 0.001	0.036	> 0.85

Error	0.004	10	0.0004
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**Table 10.** Effects of disturbance regime on angularly transformed proportions of zooplankton and small aquatic insects in diet of larval salamanders at end of SRV barrel experiment (one-way ANOVA tables followed by multivariate test). Remaining diet items were unidentified and their proportions were not significantly affected by disturbance.

Diet Item	Source	SS	d.f.	MS	F	P
Zooplankton	Disturbance Regime	0.230	1	0.230	6.414	< 0.03
	Error	0.359	10	0.036		
Insects	Disturbance Regime	0.301	1	0.301	7.101	< 0.02
	Error	0.423	10	0.042		
Wilk's $\lambda = 0.479$				$F_{3,8} = 2.98$		P < 0.103

**Table 11.** Treatment effects on suspended sediment concentration in Mogollon Plateau barrel experiment, before and 10 minutes after sediment disturbance.

Before sediment disturbance

Source	SS	d.f.	MS	F	P
Salamander Presence	0.006	1	0.006	3.729	> 0.09
Disturbance Regime	0.003	1	0.003	1.791	> 0.21
Salamander * Disturbance	0.0001	1	0.0001	0.121	> 0.73
Error	0.014	8	0.002		

After sediment disturbance

Source	SS	d.f.	MS	F	P
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Salamander Presence	0.084	1	0.084	6.29	< 0.04
Disturbance Regime	2.495	1	2.495	187.4	< 0.001
Salamander * Disturbance	0.050	1	0.050	3.75	> 0.08
Error	0.106	8	0.013		

**Table 12.** Treatment effects on (A) log-transformed chlorophyll *a* concentration and (B) log-transformed bacterial concentration in Mogollon Plateau barrel experiment.

A. Chlorophyll *a*

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0014	1	0.0014	0.667	> 0.43
Disturbance Regime	0.0001	1	0.0001	0.036	> 0.85
Salamander * Disturbance	0.0021	1	0.0021	1.362	> 0.27
Error	0.014	8	0.002		

B. Bacteria

Source	SS	d.f.	MS	F	P
Salamander Presence	0.333	1	0.333	1.08	> 0.32
Disturbance Regime	0.213	1	0.213	0.692	> 0.42
Salamander * Disturbance	0.085	1	0.085	0.270	> 0.61
Error	2.467	8	0.3083		

**Table 13.** Treatment effects on log-transformed densities of (A) *Daphnia* sp., (B) *Alona* sp., and (C) *Chydorus* sp. in Mogollon Plateau barrel experiment.

A.

Source	SS	d.f.	MS	F	P
Salamander Presence	< 0.0001	1	<0.0001	0.00016	> 0.98
Disturbance Regime	0.00044	1	0.00044	0.0228	> 0.88
Salamander * Disturbance	0.0405	1	0.0405	2.102	> 0.18
Error	0.154	8	0.0192		

B.

Source	SS	d.f.	MS	F	P
Salamander Presence	1.239	1	1.239	6.28	< 0.038
Disturbance Regime	0.291	1	0.291	1.48	> 0.25
Salamander * Disturbance	0.019	1	0.019	0.098	> 0.76
Error	1.577	8	0.197		

C.

Source	SS	d.f.	MS	F	P
Salamander Presence	0.0061	1	0.0061	0.151	> 0.70
Disturbance Regime	0.0255	1	0.0255	0.631	> 0.45
Salamander * Disturbance	0.0029	1	0.0029	0.073	> 0.79

Error	0.3229	8	0.0404
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**Table 14.** Effects of disturbance regime on growth (defined as change in mass) of larval salamanders in the Plateau barrel experiment.

Source	SS	d.f.	MS	F	P
Disturbance Regime	1.771	1	1.771	4.375	< 0.064
Error	4.048	10	0.405		

**Table 15.** Effects of disturbance regime on log-transformed food mass in larval stomachs at the end of the Plateau barrel experiment.

Source	SS	d.f.	MS	F	P
Disturbance Regime	0.00014	1	0.00014	0.875	> 0.50
Error	0.0016	10	0.00016		

**Table 16.** Effects of disturbance regime on angularly transformed proportions of zooplankton and small aquatic insects in diet of larval salamanders at end of Plateau barrel experiment (one-way ANOVA tables followed by multivariate test). Remaining diet items were unidentified and their proportions were not significantly affected by disturbance.

Diet Item	Source	SS	d.f.	MS	F	P
Zooplankton	Disturbance Regime	0.165	1	0.165	2.778	> 0.12
	Error	0.594	10	0.059		
Insects	Disturbance Regime	0.195	1	0.195	1.397	> 0.26
	Error	1.395	10	0.139		
Wilk's $\lambda = 0.730$				$F_{3,8} = 1.663$		$P > 0.24$

**Table 17.** Treatment effects on (A) survival of embryos to hatching and (B) total number of days required for embryos to hatch for *A. t. stebbinsi* embryos raised in the laboratory. Treatments included presence/absence of sediments and physical disturbance (swirled twice per day).

A. Embryo survival

Source	SS	d.f.	MS	F	P
Sediment Presence	0.173	1	0.173	0.865	> 0.35
Disturbance Regime	0.011	1	0.011	0.054	> 0.81
Sediments * Disturbance	0.692	1	0.692	3.459	> 0.08
Error	7.00	35	0.200		

B. Days required for hatching

Source	SS	d.f.	MS	F	P
Salamander Presence	10.167	1	10.167	1.472	> 0.23
Disturbance Regime	32.308	1	32.308	4.679	< 0.038
Salamander * Disturbance	4.469	1	4.469	0.647	> 0.42
Error	241.622	35	6.903		

**Table 18.** Matrix of pair-wise Kolmogorov-Smirnov tests comparing cumulative relative frequency of hatched embryos over time (by day) between treatments. Tabled values reflect P-values testing the probability that the two distributions differ only by chance.

	No sediment/ Undisturbed	Sediment/ Undisturbed	No sediment/ Disturbed	Sediment/ Disturbed
No sediment/ Undisturbed	-----			
Sediment/ Undisturbed	0.269	-----		
No sediment/ Disturbed	0.999	0.269	-----	
Sediment/ Disturbed	0.953	0.269	0.953	-----

**Table 19.** Treatment effects on early larval growth of *A. t. nebulosum* in the laboratory. A. Effects on log-transformed change in absolute mass; B. effects on change in mass relative to initial mass. Only three treatments were maintained (no sediment/disturbed, sediments/undisturbed, and sediments/disturbed); no control treatment (no sediment/undisturbed) was present. Thus, this table does not reflect a two-way, fully-crossed design.

A. Change in mass

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Source	SS	d.f.	MS	F	P
Treatment	0.138	2	0.069	3.42	> 0.14
Error	0.483	24	0.0201		

**B. Change in mass relative to initial mass**

Source	SS	d.f.	MS	F	P
Treatment	2.711	2	1.355	1.09	> 0.35
Error	0.48329.843	24	1.243		



Re: *Population Genetics of Huachuca Salamanders* (I96046)

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