

**Effect of habitat alteration and introduced species on endangered/sensitive amphibian populations in Arizona**

Prepared by  
Timothy J. Maret and James P. Collins  
Department of Biology  
Arizona State University  
Tempe, AZ 85287-1501

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## INTRODUCTION

The proposed project involved testing the hypothesis that an interaction between habitat alteration and introduction of exotic species has caused a decline in native amphibian populations in Arizona. We Hypothesized that elimination of native amphibian populations from a habitat occurs in two steps. First, a temporary aquatic habitat is altered to retain water all year. Second, non-native species requiring permanent water invade and displace native amphibians. Because aquatic habitats that retain water all year are rare in the arid West, many temporary habitats have been modified to cause them to hold water for longer periods of time. The most common method of habitat modification is constructing an earthen stock tank at the lower end of a natural marsh. When the marsh dries or freezes, the earthen tank can serve as a refuge for introduced species. When the marsh thaws or refills following snowmelt or rainfall, these introduced species can recolonize the marsh and adversely affect native amphibian larvae.

Our study concentrated on three taxa of native amphibians that are listed as sensitive species by Arizona Game and Fish Department - the Sonora tiger salamander, *Ambystoma tigrinum stebbinsi*, mountain tree frog, *Hyla eximia (wrightorum)*, and native ranid frogs, *Rana chiricahuensis* and *Rana pipiens*. Habitats studied were Plains Interior Marshland in San Rafael Valley [SRV] of southeastern Arizona and Rocky Mountain Montane Marshland on Mogollon Plateau in east-central Arizona. The study involved several components: 1) surveys of habitats for presence or absence of native amphibians and introduced species, including surveys of historical habitats of native amphibians; 2) field experiments to determine effect of introduced species on native amphibian larvae; and 3) laboratory experiments to ascertain palatability and natural defenses of native amphibian larvae to introduced predators. In our study we also used additional field data from prior surveys. These data are in the appendices. This report has three sections devoted to each of the amphibian taxa.

### *Ambystoma tigrinum stebbinsi*

## METHODS

### Study Area and Organism

*A. t. stebbinsi*, a morphologically and genetically distinct subspecies of tiger salamander, is found only in San Rafael Valley (Jones et al. 1988, 1995). Historically, large seasonal cienegas or natural marshes were common along Santa Cruz River, which runs through the valley (Hadley and Sheridan 1995). These cienegas were probably the natural breeding habitat for *A. t. stebbinsi*. In the late 1800's and early 1900's a combination of overgrazing and drought caused extensive erosion and gully formation, which destroyed these natural marshes. Between initiation of cattle ranching and destruction of the cienegas, numerous earthen stock tanks were constructed for watering livestock (Hadley and Sheridan 1995). During this transition, *A. t. stebbinsi* probably became established in these artificial habitats. Reed (1951) reported salamanders quickly colonize new stock tanks in SRV, suggesting *A. t. stebbinsi* has considerable dispersal capability. *A. t. stebbinsi* occurs now only in earthen stock tanks. Many of these artificial habitats also have bullfrogs (*Rana catesbeiana*) and fishes (mosquito fish, *Gambusia affinis affinis*; green sunfish, *Lepomis cyanellus*; bluegill sunfish, *Lepomis*

*macrochirus*; yellow bullheads, *Ameiurus natalis*; largemouth bass, *Micropterus salmoides*) introduced by ranchers and sportsmen. Unlike fish, bullfrogs disperse among habitats, and we found juvenile frogs >1 km from the nearest aquatic habitat.

Like other subspecies of tiger salamander, *A. t. stebbinsi* has a complex life history. Adults breed in ponds during winter and spring. Larvae grow in the aquatic environment until they reach a length of about 7.0 cm snout-to-vent length (SVL), at which time they continue developing in one of two ways. Larvae can metamorphose into a terrestrial morph and leave the aquatic environment returning to the pond to breed, or they can remain in the aquatic environment and mature as branchiate adults. Branchiate individuals may mature within one year of hatching (Collins et al. 1988). The developmental "choice" of adult morphology is at least partially influenced by environmental conditions. In shallow habitats that dry quickly, metamorphosing into a terrestrial adult may be the only option.

### Field Survey

SRV has a history of species introductions. Exotic species of fishes were first introduced into SRV aquatic habitats in the 1950's (Collins et al. 1988), and *R. catesbeiana* was first observed in SRV in 1979 (JPC, personal observation). Field surveys of the distribution of *A. t. stebbinsi* began in 1979 (Collins et al. 1988) when *R. catesbeiana* and fishes were absent or rare in SRV habitats. A "natural experiment" to test the effect of exotic species unfolded as some of these ponds were invaded by *R. catesbeiana*. Some habitats are now inhabited by *R. catesbeiana* and fishes, and some have remained free of introduced species. We surveyed 68 SRV habitats between 1993 and 1995 for salamanders and introduced species (Appendices 1 and 2). We also identified several habitats with salamander populations before 1993 (Appendix 1, Table 1). Salamanders occurred in a total of 26 habitats. Some habitats remain free of introduced species; others now have bullfrogs, introduced fishes, or both. These habitats were all sampled during 1996 to determine the present status of *A. t. stebbinsi*. If introduced species do not affect *A. t. stebbinsi* populations, we would expect the proportion of ponds that presently have salamanders to be the same in all four types of habitats (i.e., ponds free of introduced species, ponds with bullfrogs, ponds with fishes, and ponds with fishes plus bullfrogs). Several habitats where we had not previously found *A. t. stebbinsi*, including three previously unsampled habitats, were also sampled in 1996. Since these habitats had no record of *A. t. stebbinsi* before 1996, they were excluded from statistical analysis.

Unless noted in Appendix 2, surveying consisted of sampling the perimeter of each habitat with a 15 ft x 4 ft seine. If the habitat were shallow enough, we also sampled once across the deepest section of the pond. Salamanders were recorded as present if we found eggs, larvae, branchiate adults, or metamorphosed adults. *R. catesbeiana* were recorded as present if we found larvae or metamorphosed individuals. Any bullfrogs captured in 1996 >10 cm SVL were killed and dissected to determine if they were eating salamanders.

### Field Experiments

We conducted field experiments in 1995 and 1996 in Meadow Valley Flat North Tank to determine effect of fishes (*L. cyanellus*, a locally abundant species) and *R. catesbeiana* on survival and growth of larval salamanders. This habitat was chosen because it already had introduced fish and *R. catesbeiana*, so escapees would not pose any further risk to *A. t. stebbinsi*

populations. We concentrated effort on larval salamanders since this early life history stage should be the most vulnerable to interspecific predation and/or competition. Although we did not expect salamanders and tadpoles to interact directly, tadpoles can reduce standing crop of phytoplankton (Seale 1980), potentially reducing standing crop of zooplankton, primary food of small, larval salamanders.

In 1995 effect of *L. cyanellus* and *R. catesbeiana* tadpoles on growth and survival of larval *A. t. stebbinsi* was tested in the field using 3 m x 5 m enclosures constructed of black plastic walls supported by wooden stakes. Rocks placed along the plastic walls prevented escape of experimental animals. Water depth in enclosures varied from 0.7 m at the beginning of the experiment to 0.4 m at the end of the experiment. Enclosures were stocked on 8 April 1995 with three randomly assigned treatments: 1) *A. t. stebbinsi* larvae; 2) *A. t. stebbinsi* larvae and *L. cyanellus*; and 3) *A. t. stebbinsi* larvae and *R. catesbeiana* tadpoles.

Salamander larvae used in experiments were a mix of laboratory-raised and similarly sized field-caught individuals. Embryos for lab-raised animals were collected from Water Tank Tank and held in the laboratory at 16° C with a light cycle of 10 hr light/14 hr dark. After hatching larvae were fed *ad libitum* newly hatched *Artemia* sp. until the experiment began. Adult salamanders bred in Meadow Valley Flat North Tank in spring 1995, and we found salamander embryos inside and outside the enclosures before the experiment began. Embryos inside enclosures hatched into larvae, which survived because we had removed all *G. a. affinis* from the enclosures (we found no larvae outside of the enclosures, presumably because they were eaten by fish). We used some of these larvae for the field experiment and removed the rest.

Twenty-four salamander larvae (mean SVL = 18.2 mm, SD = 3.63 mm, n = 72) were selected haphazardly and placed in each enclosure. *R. catesbeiana* tadpoles were captured from FS 799 Tank and Meadow Valley Flat North Tank, and *L. cyanellus* were captured from Rosemary Tank. Twelve tadpoles (mean SVL = 45.3 mm, SD = 1.16 mm, n = 10 measured) or six *L. cyanellus* (mean SL = 70.3 mm, SD = 3.56 mm, n = 10 measured) were haphazardly selected and placed in their respective treatments. We replicated treatments three times. The experiment ran until 30 April 1995 when all vertebrates were removed from each enclosure and preserved, and salamanders were counted and weighed.

In 1996 effect of metamorphosed *R. catesbeiana* on growth and survival of larval *A. t. stebbinsi* was tested in 1m x 2m enclosures constructed with black plastic walls and window screen tops and bottoms stapled to a wooden frame. Water depth ranged from 0.4 m to 0.15 m. There were two treatments: 1) salamander larvae and 2) salamander larvae and metamorphosed bullfrogs. Salamander embryos were collected at Meadow Valley Flat South Tank and raised in the laboratory under the same conditions as in the 1995 experiment. Bullfrogs were collected from Earthen Tank.

On 13 April 1996, 20 salamander larvae (mean SVL = 16.7 mm, SD = 1.64, n = 15 measured) were selected haphazardly and placed in each enclosure. Two bullfrogs (mean SVL = 7.3 cm, SD = 0.64, n = 8 measured) were chosen haphazardly and placed in every other enclosure. We replicated treatments four times. On 21 May, enclosures were pulled ashore, all vertebrates removed from enclosures and preserved, and salamanders counted and weighed.

### Laboratory Study

We tested if introduced species were capable of feeding on *A. t. stebbinsi* eggs and larvae in the laboratory. The introduced species and *A. t. stebbinsi* eggs and larvae were collected in the field and maintained in the laboratory before the experiment. Animals were maintained under the same conditions described for salamander larvae in field experiments, except that *R. catesbeiana* tadpoles were fed rabbit chow instead of *Artemia*, and metamorphosed bullfrogs were fed crickets. Food was withheld for 24 hours before the experiment. Salamander embryos or larvae were introduced into a 40 l aquarium with one *A. natalis* (79-140 mm total length), two *L. cyanellus*, (52-80 mm), six *G. a. affinis* (25-43 mm), three *R. catesbeiana* tadpoles (58-143 mm), or three juvenile, metamorphosed *R. catesbeiana* (60-70 mm SVL). Each predator treatment was replicated eight times except the bullfrog treatment, which was replicated three times. Five *A. t. stebbinsi* eggs attached to vegetation or 10 (15 with the metamorphosed *R. catesbeiana*) hatchling *A. t. stebbinsi* larvae (12 mm +/- 2 mm total length) were placed in each aquarium with the introduced species. Uneaten embryos were counted after one week; uneaten larvae after 24 hours.

## RESULTS

### Field Survey

In our 1996 survey of 26 ponds in which *A. t. stebbinsi* were recorded previously, we found *A. t. stebbinsi* in 9 of 11 ponds without introduced species, 8 of 9 ponds with *R. catesbeiana*, 0 of 4 ponds with fish and *R. catesbeiana*, and 0 of 1 pond with only fish (Table 1). One habitat (Game and Fish Tank) was dry. We tested if the present distribution of salamanders were independent of the distribution of introduced species. Salamander distribution was not affected by presence/absence of *R. catesbeiana* ( $X^2 = 0.30$ ,  $P > 0.5$ ), but was affected by the distribution of fishes and *R. catesbeiana* ( $X^2 = 17.98$ ,  $P < 0.001$ ). Only one pond had introduced fishes without bullfrogs, but salamanders were absent from this pond as well. We also found *A. t. stebbinsi* populations in five additional habitats in 1996. Three had *A. t. stebbinsi* alone, one had *A. t. stebbinsi* and *R. catesbeiana*, and one had *A. t. stebbinsi*, *R. catesbeiana* and *G. a. affinis* (Appendix 2). *A. t. stebbinsi* populations were thus identified in 22 habitats in 1996.

Nine large, adult *R. catesbeiana* (> 10 cm SVL) were captured in 1996 at ponds with *A. t. stebbinsi*. We found the remains of a metamorphosed adult salamander in the stomachs of two bullfrogs, while the stomachs of the other seven frogs were empty, or contained insects, smaller bullfrogs, and unidentifiable material.

### Field Experiments

One of the control replicates in 1995 was improperly stocked, leaving us with two replicates for the control. There were no surviving salamander larvae in the *L. cyanellus* treatment, so data cannot meet the assumption of homogeneity of variance required for analysis with ANOVA. Therefore, we conducted t-tests to determine whether larval salamander survivorship in tadpole and control treatments was significantly different from zero (the observed survivorship in *L. cyanellus* treatment) and whether salamander survivorship was different in the tadpole and control treatments. Data were log-transformed to minimize differences in variance. Survivorship in both the control and tadpole treatments was significantly greater than zero

( $t=29.02$ ,  $df=1$ ,  $P<0.05$  and  $t=7.78$ ,  $df=2$ ,  $P<0.05$ , respectively). Survivorship in the control and tadpole treatments was not significantly different ( $t=0.22$ ,  $df=3$ ,  $P>0.5$ ). There was no significant difference between mean mass of surviving salamander larvae in tadpole and control treatments ( $t=0.395$ ,  $df=3$ ,  $P=0.79$ ).

In the 1996 experiment, survivorship of larval salamanders in field enclosures with metamorphosed *R. catesbeiana* was significantly lower than in enclosures without *R. catesbeiana* ( $t = 7.132$ ,  $df = 6$ ,  $P < 0.001$ , Fig. 2). Mean mass of surviving salamanders in enclosures with metamorphosed *R. catesbeiana* was significantly less than in enclosures without *R. catesbeiana* ( $t = 1.269$ ,  $df = 5$ ,  $P = 0.07$ , Fig. 2).

### Laboratory Study

After one week, *L. cyanellus* ate an average of 77.5% (SE = 12.8) of salamander eggs, *A. natalis* 52.8% (SE = 15.6), *G. a. affinis* 15% (SE = 5.0), *R. catesbeiana* tadpoles 12.5% (SE = 8.4), and metamorphosed *R. catesbeiana* 0%. Some eggs died during the one week experiment, and tadpoles only ate eggs already dead.

After 24 hours there were no surviving salamander larvae in aquaria with *L. cyanellus*, *A. natalis*, or *G. a. affinis*. Metamorphosed *R. catesbeiana* ate 68.8% (SE = 5.9) of the salamander larvae, and tadpoles ate 0%.

### DISCUSSION

Our survey data are consistent with the conclusions of Hayes and Jennings (1986), Bradford et al. (1993), Fellers and Drost (1993), and Gamradt and Kats (1996) - introduced fish negatively affect native amphibian populations. During all years of sampling SRV ponds since 1979, *A. t. stebbinsi* was present in only three habitats with introduced fishes. In 1984, branchiate adult salamanders were found together with *Lepomis* sp. in FS 58 Tank (Collins et al. 1988). Despite extensive sampling, no *A. t. stebbinsi* have been found in this habitat since then, although it continues to support fish. In 1995, one branchiate adult salamander and several thousand embryos were found in Meadow Valley Flat North Tank, together with *G. a. affinis* and *R. catesbeiana*. No embryos survived, and the branchiate adult has not been recaptured. In 1996, metamorphosed and branchiate adult salamanders and embryos were found with *G. a. affinis* and *R. catesbeiana* in Willow Springs Canyon Tank. Again, no embryos survived. Adult salamanders are apparently able to survive with some fish species, particularly *G. a. affinis* which is considerably smaller than mature salamanders. Salamander larvae, however, do not appear to be able to survive with fishes, so although adult salamanders may continue to survive and breed in habitats with fish, there is no recruitment.

Data from laboratory and field experiments are consistent with our survey data. *L. cyanellus* reduced survivorship of *A. t. stebbinsi* larvae to zero in field enclosures. *G. a. affinis*, *A. natalis*, and *L. cyanellus* ate all *A. t. stebbinsi* larvae in the laboratory experiment, and *L. cyanellus* and *A. natalis* ate most of the *A. t. stebbinsi* eggs. Although fishes may negatively affect salamanders through competition for resources, the predominant interaction we observed in laboratory and field experiments was predation. Fish appear to exclude *A. t. stebbinsi* from habitats by preying on embryos and larvae.

Metamorphosed *R. catesbeiana* ate larval *A. t. stebbinsi* in laboratory experiments, reduced survivorship and mean mass of *A. t. stebbinsi* larvae in field enclosures, and ate adult *A.*

*t. stebbinsi* in the field. Lower mass of surviving salamander larvae in enclosures with metamorphosed *R. catesbeiana* could result from size selective predation by *R. catesbeiana* on larger larvae, or reduced feeding by larvae in the presence of a potential predator. In either case, in the presence of *R. catesbeiana* salamanders might metamorphose smaller or later thus exposing them longer to predation (Collins 1979), extending time to sexual maturity, and/or reducing reproductive success (Smith 1987, Berven 1990, Scott 1994). Laboratory and field experiments suggest that *R. catesbeiana* tadpoles have no negative effects on *A. t. stebbinsi* embryos or larvae.

Despite negative effects of *R. catesbeiana* on salamanders, *A. t. stebbinsi* occur with *R. catesbeiana* in many habitats (Table 1, Appendix 2). It may be that predation by *R. catesbeiana* is not severe enough to eliminate *A. t. stebbinsi* from a habitat, or perhaps insufficient time has passed since their introduction for *R. catesbeiana* to eliminate salamanders. Many SRV ponds have relatively low densities of *R. catesbeiana* (often only one or two individuals are seen or collected), and a habitat with *R. catesbeiana* in one year may not have bullfrogs the next year (Appendix 3), suggesting that many SRV ponds are marginal habitats for *R. catesbeiana*.

Although *R. catesbeiana* alone may not eliminate *A. t. stebbinsi* from a habitat, in combination with other factors it may threaten salamander populations. Disease periodically spreads among habitats in SRV, killing adult branchiate and larval salamanders in a pond (Collins et al. 1988). Because population structure of salamanders in SRV is skewed toward larvae and adult branchiate animals (Collins et al. 1988), a disease episode may eliminate many individuals in a population. Highest number of metamorphosed individuals ever recorded at a habitat is ten. *R. catesbeiana* does not appear to be affected by the disease, and if *R. catesbeiana* preys on returning metamorphosed individuals, the effect may be to eliminate *A. t. stebbinsi* from that habitat.

The effect of introduced species can not be considered independent of factors facilitating their introduction, survival, and dispersal. Habitat alteration can facilitate establishment of introduced species by altering natural disturbance regime of an ecosystem. For example, Hammerson (1982) reported an increase in the range of bullfrogs in Colorado following creation of permanent water sources. As the bullfrog's range increased, the range of native leopard frogs decreased. Meffe et al. (1983) demonstrated that the range of *G. a. affinis* has expanded in desert habitats that are regulated to reduce flooding. *G. a. affinis* eliminate native Sonoran topminnows (*Poeciliopsis occidentalis*) from these habitats. When regular flooding is not interrupted, however, *G. a. affinis* fail to establish at sufficient densities to exclude *P. occidentalis*.

Land use practices have altered SRV aquatic habitats. Seasonal cienegas have been replaced with earthen stock tanks that often hold water all year (Hadley and Sheridan 1995). Although *A. t. stebbinsi* is adapted to survive in temporary aquatic habitats, introduced fishes and *R. catesbeiana* depend on permanent water. Eliminating natural marshes and constructing earthen stock tanks has facilitated establishment of these introduced species in what was formerly an inhospitable habitat.

Although they often hold water all year, on a longer time scale stock tanks are not permanent since they gradually fill with sediment. After excavation the tank fills with water again with the next heavy rainfall, and persists until it once again fills with sediment. Habitats in SRV are at various stages in this "dig-dry" cycle. Huachuca Tank, which contained catfish in the mid-1980's, has since dried, been excavated, and recolonized by *A. t. stebbinsi*. Meadow Valley

North Tank, site of our field experiments, dried in summer 1996 killing all *G. a. affinis* and *R. catesbeiana*, and may eventually be recolonized by salamanders. Two habitats, Judy and Gypsy Tanks, are unique in that they support salamanders, but are subject to drying every summer because they have not been excavated.

The ability of *A. t. stebbinsi* to recolonize a habitat from which it has been excluded depends on several factors. We estimate the "lifespan" of a stock tank (time from excavation until it begins drying in the summer) at about 20 years, exceeding the estimated life-span of tiger salamanders in the field (L. Allison, pers. comm.). If introduced species replace salamanders in a "young" stock tank, the terrestrial metamorphosed salamanders may die before conditions allow recolonization (i.e., the pond dries, killing the introduced species). The distance of the habitat from other ponds inhabited by *A. t. stebbinsi* will determine whether dispersing, metamorphosed individuals can reach the habitat. Temporary aquatic habitats (such as Judy and Gypsy Tanks) may play an important role as source populations for recolonization of other nearby habitats.

Interactions between *A. t. stebbinsi*, introduced fishes, and *R. catesbeiana* occur against a backdrop of aquatic habitats in a state of constant flux. Persistence of viable populations of *A. t. stebbinsi* in SRV depends on a complex interaction between establishment of introduced species and disturbance. Although introduced species will continue to impact aquatic habitats in SRV, a management plan incorporating disturbance will help insure survival of this subspecies.

### *Hyla eximia*

## METHODS

### Study Area and Organism

*Hyla eximia*, the mountain treefrog, occurs in mountainous habitats in central Arizona and in the Huachuca Mountains in southern Arizona (Stebbins 1985). Terrestrial adults return in summer to temporary aquatic habitats filled by monsoon rains where they breed and deposit eggs. Tadpoles grow and develop quickly, metamorphosing before ponds freeze in winter.

We concentrated on natural aquatic habitats on Mogollon Plateau in east-central Arizona. Many of these habitats have been modified by excavation creating earthen stock tanks to increase their reliability as sources of water for livestock. This habitat modification has facilitated establishment of introduced species, including fathead minnows (*Pimephales promelas*), black bullheads (*Ameiurus melas*), green sunfish (*Lepomis cyanellus*), and crayfish. In addition, habitat modification may have made these habitats accessible to native predators, including larvae of the Arizona tiger salamander, *Ambystoma tigrinum nebulosum*, and dragonfly naiads, *Anax* sp., that require longer aquatic habitat duration than *H. eximia*.

### Field Survey

We surveyed 99 aquatic habitats for *H. eximia* from 1993 to 1996 (Appendices 3 and 4). Surveys were conducted during summer and fall, when adult frogs, embryos, and/or tadpoles would be present. Natural and modified habitats were sampled. Sampling consisted of first walking along the shoreline looking for adult frogs, followed by dipnetting for larvae and embryos in the shallows or sampling the perimeter with a 4 ft x 15 ft seine. Frogs were recorded as present if we found adults, embryos, or larvae. Presence/absence of introduced fishes and

native salamanders was also recorded, and length, width, and maximum depth of the area covered by water was estimated (Appendix 4).

### Laboratory Experiments

Amphibian larvae that occur naturally in habitats with predators often have antipredatory defenses, including chemical defenses that reduce palatability and behavioral defenses that reduce likelihood of capture. (Kats et al. 1988). Because predators are rare or absent in natural *H. eximia* habitats, we predicted that *H. eximia* larvae would not have antipredator defenses. We tested whether introduced and native predators prey on *H. eximia* larvae (i.e., whether tadpoles are palatable to predators), and whether *H. eximia* larvae exhibit behavioral antipredator defenses. *H. eximia* tadpoles and predators were collected from habitats on the Mogollon Plateau and returned to the laboratory. Tadpoles were maintained in aerated buckets of aged tap water and fed a mixture of ground TetraMin fish flakes and alfalfa horse pellets *ad libitum*. To test if aquatic predators ate *H. eximia* larvae, six tadpoles were added to a 40 l aquarium with one of the following: four fathead minnows, one green sunfish, one crayfish, two Arizona tiger salamander larvae, or four dragonfly naiads. All predators were starved for 24 hours before adding tadpoles. Aquaria were checked 24 hours later, and we recorded number of tadpoles eaten. There were six aquaria for each predator treatment, and the experiment was repeated six times for each aquarium.

To determine if *H. eximia* larvae exhibit antipredator defenses, we tested tadpole behavior in the presence of introduced and native predators. Eight tadpoles were placed into a 40 l aquarium with 8 l of aged tap water. A cylindrical cage, 15 cm in diameter made of window screen was placed at one end of the aquarium to house the predator. A 10 cm x 15 cm opaque plexiglass sheet, elevated 1.5 cm above the bottom of the aquarium was placed at the other end of the aquarium to serve as a refuge under which tadpoles could hide (Kats et al. 1988). One hour after tadpoles were placed in the aquarium, a predator (or predators) was placed in the cylindrical cage. Predator treatments consisted of one green sunfish, one crayfish, two salamander larvae, three fathead minnows, four dragonfly naiads, no predators (i.e., control treatment), or one smashed *H. eximia* tadpole (chemical cues of wounded or distressed conspecific). Six hours after adding the predator, observations were made on three antipredator behaviors. Number of tadpoles that were not moving (motionlessness), that were on the opposite side of the aquarium from the predator cage (avoidance), and that were under the plexiglass refuge (hiding) was recorded. Each tadpole was only used for one trial. The experiment was replicated six times.

## RESULTS

### Field Survey

We found *H. eximia* in 27 of the 99 habitats surveyed (Appendix 4). Three habitats with *H. eximia* also had vertebrate predators (Lost Lake and Cart Cabin Tank had salamanders, East Harris Puddle had fathead minnows). Eighteen of the habitats with treefrogs dried at least once during our study. Four more habitats with tree frogs were shallow (< 40 cm), and probably dried during the study. Only five habitats in which treefrogs were found were deeper than 40 cm (Nelson and 27 Mile Lakes, Cattail, Tire, and Cart Cabin Tanks) each time sampled, and

probably held water all year in most years. *H. eximia* is more likely to be found in shallow, drying habitats (i.e., those that dried to <40 cm during this study) than in deep habitats that usually held water ( $G_{adj} = 22.27$ ,  $P < 0.001$ ).

### Laboratory Experiments

Green sunfish ate 95% of *H. eximia* tadpoles, salamander larvae ate 78%, crayfish ate 73%, dragonfly naiads ate 52%, and fathead minnows ate 42%. In the experiment examining antipredator responses, there was no difference among treatments in hiding (ANOVA,  $F_{6,35} = 0.705$ ,  $P = 0.65$ , Fig. 3) or avoidance behavior (ANOVA,  $F_{6,35} = 0.199$ ,  $P = 0.98$ , Fig. 4) by *H. eximia* tadpoles. Although not statistically significant (ANOVA,  $F_{6,35} = 1.869$ ,  $P = 0.114$ ), tadpoles seem to remain motionless around some predators, particularly sunfish (Fig. 5)

### DISCUSSION

Our field survey indicates that although *H. eximia* use aquatic habitats with varying degrees of permanence, they are more likely to inhabit temporary habitats. Permanent aquatic habitats, even those without vertebrate predators, were usually avoided. When a permanent and temporary habitat were close, treefrogs were often found in only the temporary habitat. For example, although *H. eximia* was not present in Crooked, Harris, or Bow Ribbon Tanks (Appendix 4), tadpoles were abundant in temporary puddles only a few yards away (Crooked, East Harris, West Harris, and Northeast Bow Ribbon Puddles).

Data from laboratory experiments indicated that native and introduced predators prey readily on *H. eximia* larvae, and that *H. eximia* has little, if any, antipredator defenses. It appears that treefrogs avoid aquatic predators by breeding in habitats too ephemeral to support predators.

*H. eximia* populations in Arizona depend on suitable aquatic habitats for reproduction. Altering a habitat to increase its permanence appears to render it unusable by *H. eximia*. The long-term viability of this species likely depends on preserving the remaining natural meadows, marshes, and ponds that serve as breeding sites.

### *Rana pipiens* and *Rana chiricahuensis*

### METHODS

#### Study Area and Organism

*Rana pipiens*, the northern leopard frog, is found in mountainous areas in east-central Arizona (Platz 1976). *Rana chiricahuensis*, the Chiricahua leopard frog, is sympatric with *R. pipiens* in the east-central part of the state, and is also found in southeastern Arizona (Platz 1979). Historically, these two species probably occurred in shallow ponds, natural marshes or cienegas, and along shallow streams. Many of these habitats have been excavated to increase the likelihood of holding water for most of the year. Earthen stock tanks often contain water year-round, providing conditions suitable for establishment of introduced fishes and bullfrogs.

Our study concentrated on habitats on Mogollon Plateau in east-central Arizona and SRV. Introduced species that have colonized altered habitats include mosquito fish (*Gambusia affinis affinis*), green sunfish (*Lepomis cyanellus*), bluegill sunfish (*Lepomis macrochirus*),

yellow bullheads (*Ameiurus natalis*), black bullheads (*Ameiurus melas*), largemouth bass (*Micropterus salmoides*), fathead minnows (*Pimephales promelas*), bullfrogs (*Rana catesbeiana*), and crayfish.

### Field Survey

Between 1993 and 1996, we surveyed 76 habitats in SRV and 93 habitats on Mogollon Plateau (Appendices 1-4). Twenty-three habitats had a historic record of occupancy by native ranid frogs (Appendices 1 and 3, Table 2). Sampling consisted of walking along the shoreline looking for adult frogs, followed by sampling by dipnet for larvae and embryos in the shallows or sampling the perimeter with a 4 ft x 15 ft seine. Frogs were recorded as present if we found adults, embryos, or larvae. Presence/absence of introduced fishes and bullfrogs was also recorded, as was maximum depth and either length and width (Mogollon Plateau) or perimeter (SRV) of the area covered by water (Appendices 2 and 4).

### RESULTS

Native ranid frogs were found in six habitats (Tungo, Upper 13, North 14, and South 14 Tanks in SRV, and Hess and East Buckskin Tanks on Mogollon Plateau). None of these habitats had introduced predators (Appendices 2 and 4). Of the 23 historical ranid sites, two still had native ranid frogs (Hess and East Buckskin Tanks). Of 21 historic sites that no longer had frogs, seven had introduced species (Table 2).

### DISCUSSION

Native ranid frogs were only in habitats that did not have introduced fishes or bullfrogs. In addition, seven habitats that formerly had native ranids had introduced species. These results provide weak support for the hypothesis that introduced species negatively affect native ranids. There are, however, many apparently suitable habitats that had neither leopard frogs nor introduced species, including 14 habitats from which native ranids disappeared without establishment of introduced species (Table 2). It is, therefore, unlikely that introduction of exotic species is the sole cause of decline in leopard frog populations in Arizona.

A number of other possible causes for decreases in amphibian populations have been suggested, including acid deposition, increases in ultraviolet radiation due to thinning of the ozone layer, disruption of metapopulation dynamics, and habitat degradation (Blaustein and Wake 1990, Phillips 1990, Hayes and Jennings 1986). Several of these hypotheses can be rejected for native Arizona ranid frogs. It is unlikely that large scale abiotic phenomena such as acid deposition or ultraviolet radiation caused the decline because several "healthy" populations remain. Two remaining hypotheses, disruption of metapopulation dynamics and degradation of shoreline habitat, cannot be rejected and should be studied further.

Small populations of ranid frogs are often connected into a larger "metapopulation" through dispersal of individuals among populations (Gulve 1994). In a functioning metapopulation, small populations that go extinct due to random processes are soon recolonized by dispersal from neighboring populations. If a population is isolated from other populations, immigration will be low or absent and the population will be more likely to go extinct (Gulve 1994, Hanski et al. 1995). In addition, once a population in an isolated habitat goes extinct,

recolonization is unlikely. It is possible that dispersal of leopard frogs across habitats has been disrupted, resulting in small, isolated populations prone to extinction. There are two likely causes of disruption of metapopulation dynamics. First, many aquatic habitats in Arizona have been modified or destroyed by human impacts (AG&FD 1995). Suitable habitats that remain are often isolated. Second, changes in land use have resulted in a dramatic change in vegetational ground cover, particularly in ponderosa pine forests on the Mogollon Plateau. Dispersing adult and juvenile frogs probably rely on grass cover to avoid predators. Although grass cover was extensive historically in ponderosa pine forests, it is now rare or absent (Covington and Moore 1994, Madany and West 1983), which may prevent successful dispersal.

Since adult and juvenile frogs spend a large amount of time in vegetation along or near the shore (hence their common name of grass frog), degradation of shoreline vegetation may be another cause for the decline of leopard frogs. The remaining populations of leopard frogs on Mogollon Plateau and in SRV are exclusively in stock tanks. In most of these habitats, shoreline vegetation is rare or absent, having been removed and trampled by livestock. Interestingly, in the few habitats that still contain frogs, shoreline vegetation is fairly intact. Absence of vegetation may harm leopard frog populations by decreasing availability of prey and/or by exposing frogs to predators.

### CONCLUSION

Two species, *A. t. stebbinsi* and *H. eximia*, fit our proposed model of decline due to habitat alteration and establishment of introduced species. A management plan for these species that incorporates disturbance, particularly the occasional drying of habitats to prevent establishment of introduced species, will help preserve these endangered/sensitive amphibian species. Although habitat alteration and establishment of introduced species may be a factor in the decline of native leopard frogs, *R. pipiens* and *R. chiricahuensis*, it does not appear to be the sole cause of the decline. Other causes of decline, including disruption of metapopulation dynamics and degradation of shoreline habitats, should be investigated in the course of designing a management plan for these species.

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Table 1: Status of *A. t. stebbinsi* and presence/absence of introduced species in 1996 in 26 habitats in which salamander populations were recorded previously.

<u>Locality/Tank</u>	<u>Introduced Species Present</u>	<u>Status of <i>A.t.s.</i></u>
Bog Hole	<i>M. salmoides</i> , <i>L. macrochirus</i> ,	Absent
	<i>R. catesbeiana</i>	
FS 58	<i>I. natalis</i>	Absent
Meadow Valley Flat North	<i>G. affinis</i> , <i>R. catesbeiana</i>	Absent
Hilltop	<i>I. natalis</i> , <i>G. affinis</i> , <i>R. catesbeiana</i>	Absent
FS 799	<i>G. affinis</i> , <i>R. catesbeiana</i>	Absent
Meadow Valley Flat South	<i>R. catesbeiana</i>	Present
Parker Canyon #1	None	Present
Parker Canyon #2	<i>R. catesbeiana</i>	Present
Encino Vista	<i>R. catesbeiana</i>	Present
Out of the Way	<i>R. catesbeiana</i>	Present
Upper Mesa	<i>R. catesbeiana</i>	Present
Campini Mesa North	None	Present
Campini Mesa South	<i>R. catesbeiana</i>	Present
Pylon	<i>R. catesbeiana</i>	Present
Water Tank	None	Present
School Canyon #1	<i>R. catesbeiana</i>	Present
School Canyon #2	None	Present
Earthen	<i>R. catesbeiana</i>	Absent
Sheep Ranch	None	Absent
Meadow Valley	None	Present
Judy	None	Present
Huachuca	None	Present
Inez	None	Present
Bodie Canyon	None	Present
Upper 13 Reservoir	None	Absent
Game and Fish	None	Dry

Table 2: Status of ranid frogs in 1993-96 in 23 historic habitats in Arizona. Abbreviations: SRV - San Rafael Valley; Rim - Mogollon Plateau; *P.p.* - *Pimephales promelas*; *G.a.a.* - *Gambusia affinis affinis*; *L.m.* - *Lepomis macrochirus*; *M.s.* - *Micropterus salmoides*; *R.ca.* - *Rana catesbeiana*.

<u>Site Name</u>	<u>Region</u>	<u>Status of native ranid frogs</u>	<u>Introduced species present</u>
MV Flat N	SRV	Absent	<i>G.a.a.</i> ; <i>R.ca.</i>
Bog Hole	SRV	Absent	<i>G.a.a.</i> ; <i>R.ca.</i> ; <i>M.s.</i> ; <i>L.m.</i>
Flower	SRV	Absent	None
Parker C 1	SRV	Absent	<i>R.ca.</i>
Bear Creek	SRV	Absent	Crayfish
W Buckskin	Rim	Absent/Dry	None
Hess	Rim	Present	None
Middle	Rim	Absent	None
Rocky Draw	Rim	Absent	None
6970	Rim	Absent	None
Cow	Rim	Absent	None
E Buckskin	Rim	Present	None
Jone's	Rim	Absent	None
E Clear	Rim	Absent	Crayfish
Salt	Rim	Absent	None
Brahma	Rim	Absent	None
Brahma Draw	Rim	Absent	None
Potato	Rim	Absent	<i>P.p.</i> ; Crayfish
New	Rim	Absent	None
Maxwell	Rim	Absent	None
Dine's	Rim	Absent	Crayfish
Baker	Rim	Absent	None
Buck Spr	Rim	Absent	None

## FIGURE LEGENDS

Figure 1. Effect of green sunfish and bullfrog tadpoles on survivorship (open bars) and mean mass (shaded bars) of *A. t. stebbinsi* larvae in field enclosures. Error bars indicate  $\pm 1$  SE.

Figure 2. Effect of metamorphosed bullfrogs on survivorship (open bars) and mean mass (shaded bars) of *A. t. stebbinsi* larvae in field enclosures. Error bars indicate  $\pm 1$  SE.

Figure 3. Percent of *H. eximia* larvae hiding under refuge in presence of predators. Error bars indicate  $\pm 1$  SE.

Figure 4. Percent of *H. eximia* larvae on opposite side of aquarium from predators. Error bars indicate  $\pm 1$  SE.

Figure 5. Percent of *H. eximia* larvae moving in presence of predators. Error bars indicate  $\pm 1$  SE.









