# Barton Springs Salamander (*Eurycea sosorum)* and Austin Blind Salamander (*Eurycea waterlooensis*) Captive Breeding Population Management Plan

SR-20-03, April 2019

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## ABSTRACT

The City of Austin (COA) manages a captive breeding program for the federally endangered Barton Springs Salamander (*Eurycea sosorum*) and Austin Blind Salamander (*E. waterlooensis*) established to produce salamanders for reintroduction if the species is extirpated from COA sites and/or threatened with extinction. Since 1998, COA has tracked the pedigree of 921 *E. sosorum* and 88 *E. waterlooensis* and the current populations consist of 293 *E. sosorum* segregated by spring site lineages and 45 *E. waterlooensis*. To determine strategies to maintain gene diversity in the captive populations over decades after extirpation, we analyze population data, including gene diversity based on pedigree, and then project gene diversity over time to determine the following: 1) the effects of managing spring site lineages separately versus combining them into a single population; 2) initial gene diversity and population size needed to be able to increase the population and maintain 90% gene diversity for decades in the event of extirpation; and 3) the effects that yearly additions of 5, 10, 15, and 20 wild stock would have on the current population. We also estimate the number of hatchlings that could be produced for reintroduction in a year if the captive population were increased to the facility capacity of 500 individuals. Results for *E. sosorum* show that, at capacity, each of the four spring site lineage populations under ideal conditions with all wild-caught could maintain 90% gene diversity for only 31 years, while the population of combined spring site lineages could maintain 90% gene diversity for 92 years. Without an increase in size to capacity, 90% gene diversity and the current gene diversity of 97.3% could be maintained for 54 years and one year, respectively. The *E. waterlooensis* population could maintain 90% gene diversity for one year without an increase in size. Modeling indicates that a population of approximately 150 individuals could meet our goals under conditions in which collections from the wild are possible to boost gene diversity. Projections further indicate that ten *E. sosorum* wild stock additions per year could result in maintenance of 98% gene diversity and additions of 15 *E. waterlooensis* wild stock per year for five years could result in an increase in gene diversity to 97%. We estimate that the *E. sosorum* population at the capacity size of 500 could produce at least 315 offspring for reintroduction per year and the *E. waterlooensis* population could produce at least 110. Recommendations are: 1) combine the *E. sosorum* spring site lineage populations into a single population; 2) maintain a total of at least 150 individuals with as high gene diversity as possible; and 3) collect 10 *E. sosorum* and 15 *E. waterlooensis* wild stock per year. Breeders should be prioritized according to mean kinship to maximize gene diversity. If extirpation were to occur, each species population should be increased to the capacity of 500, which would be maintained to preserve gene diversity and produce offspring for reintroduction.

# Introduction

The City of Austin (COA) Watershed Protection Department (WPD) maintains a captive breeding program for the Barton Springs Salamander (*Eurycea sosorum*) and the Austin Blind Salamander (*Eurycea waterlooensis*), neotenic species listed as federally endangered in 1997 (USFWS 1997) and 2013 (USFWS 2013), respectively. *E. sosorum* spends part of its life in spring outlets and the associated aquifer in the Barton Springs segment of the Edwards Aquifer and is sympatric with *E. waterlooensis*, primarily an aquifer-dweller rarely found at the spring outlets (Hillis et al 2001). One of the primary threats to these species is the possibility of a contaminant spill on the watershed that passes through the aquifer to the spring sites and extirpates the species in the wild (USFWS 1997). The captive breeding program fulfills a requirement of COA's federal 10(a)1(B) incidental take permit and Habitat Conservation Plan (HCP) (USFWS and City of Austin 1998, City of Austin 2013). The primary goal of the program is to maintain a population that can be used to produce offspring that represent the genetic diversity found in the wild for reintroduction if the species is threatened with extinction.

Gene diversity is crucial for the long-term survival of a species. A common goal of captive breeding programs is to maintain 90% gene diversity for 100 years (Foose et al. 1995) or the duration of a program (Schad 2008). In this case, gene diversity is analogous to the expected heterozygosity, relative to the gene diversity of the founder population (Nei 1973, Lacy 2012). A population with high genetic variation will have a greater chance to recover from selection pressures, such as environmental changes (Lacy 1997, Ballou et al. 2010). A loss of heterozygosity and variability can result in lower fitness, lower resilience, higher rates of infections and parasites, higher rates of mortality, and reduced adaptability to changing or stressful environments (Lacy 1997, Fernandez et al. 2004). If individuals are subject to selection pressures and those individuals have the same alleles due to low heterozygosity, then it is possible that an uncommon factor such as disease or environmental events would affect each individual in the same manner and the species would not be able to survive (Lacy 1997).

Small populations are more likely to lose gene diversity through generations over time due to factors such as inbreeding, genetic drift, and random changes that result in deleterious mutations that become fixed in a population (Ballou et al. 2010). Inbreeding can result in the inheritance of identical alleles from each parent, which results in lower heterozygosity, reduced resiliency to disease and stressors, lower reproductive rates, developmental problems, and higher mortality (Lacy 1997). Captive populations are typically small compared to wild populations and are highly susceptible to factors resulting in loss of gene diversity, so it is important to manage the population with strategies to maximize this diversity (Foose et al. 1995, Fernandez et al. 2004, Ballou et al. 2010).

The most efficient approach to managing gene diversity is to selectively breed individuals according to pedigree (Putnam and Ivy 2014), which requires tracking parentage of individuals. In a tracked population, individuals are prioritized for breeding based on mean kinship (Ballou and Lacy 1995, Ballou et al. 2010), a measure of an individual's relatedness to the members of the living population. In this approach, not only pairs, but groups comprised of more than a single male and female can be established for reproduction provided that all of the potential parents are tracked for the pedigree in cases where the exact parent is not known (Lacy et al. 2012). An alternative to pedigree management is generalized group management. This approach is typically used when it is impossible to distinguish individuals (Schad 2008). Group management requires about twice as many individuals to be maintained in captivity with more uncertainty in gene diversity over time compared to the pedigree approach (Schad 2008).

COA has been tracking individuals in the *Eurycea sosorum* and *E. waterlooensis* captive breeding populations since the program's inception in 1998. The captive *E. sosorum* population is currently comprised of 293 salamanders (Table 1), 39 of which are wild-caught and 254 are captive-raised individuals. The population consists of salamanders from four spring site lineages and individuals have been housed and bred according to spring site/lineage of origin, as per the requirements of COA's federal scientific permit (TE-833851) issued by the U.S. Fish and Wildlife Service (USFWS). These four spring sites, which are found in close proximity to each other within Zilker Park, in Austin, Texas, are represented in the captive breeding program because they have been the focus of COA's *E. sosorum* monitoring and habitat management activities required by the HCP. With the exception of a few individuals collected in 2017 and 2018, all of the founders were collected from these sites in 1996-2008. The captive population of *E. waterlooensis* consists of a total of 45 (6 wild-caught, 26 F1's, and 13 F2's) salamanders. This species is rarely found in the wild, so collections have been limited.

**Table 1.** Current *Eurycea sosorum* population in captivity*

| **Spring Site** | **No. Wild-Caught** | **No. Captive-Bred F1** | **No. Captive-Bred F2** |
|---|---|---|---|
| Parthenia Spring | 7 | 25 | 11 |
| Sunken Garden Spring | 5 | 94 | 46 |
| Eliza Spring | 27 | 55 | 18 |
| Upper Barton Spring | 0 | 0 | 5 |
| Total | 39 | 174 | 80 |

*In addition, the program also houses one F2 and one F3 from a lineage of salamanders from the Dallas Aquarium that COA accepted for educational purposes in 1998. The wild stock for the population at Dallas was collected from Parthenia Spring and Sunken Garden Spring and mixed together; therefore, the spring site of origin is not known.

The Barton Springs Salamander captive population reached its highest density in 2013, which was driven by reproduction in captivity rather than collections from the wild (Fig. 1). By this point, the population had increased to over 500 salamanders (Fig. 1) despite management practices, such as culling eggs and separating males from females, already initiated to decrease the population growth rate. A population of this size presents challenges, particularly when spring site lineage populations are maintained separately, and may be larger than necessary if collections from the wild are available to boost gene diversity.

Because a catastrophic event could extirpate the species without warning, threatening extinction of the species in the wild, it is important to maintain as high a level of gene diversity as possible so that genetically diverse offspring could be produced even decades after extirpation or possible extinction. Given that there are healthy populations of the Barton Springs Salamander in the wild (Bendik and Dries 2018, Dries and Colucci 2018), with collections possible, we consider the approach of maintaining a smaller core population of high gene diversity that could be increased in size to the facility capacity if collections from the wild were to become no longer possible. The capacity population would then be used to maintain gene diversity for decades without collections and to produce offspring for reintroduction.

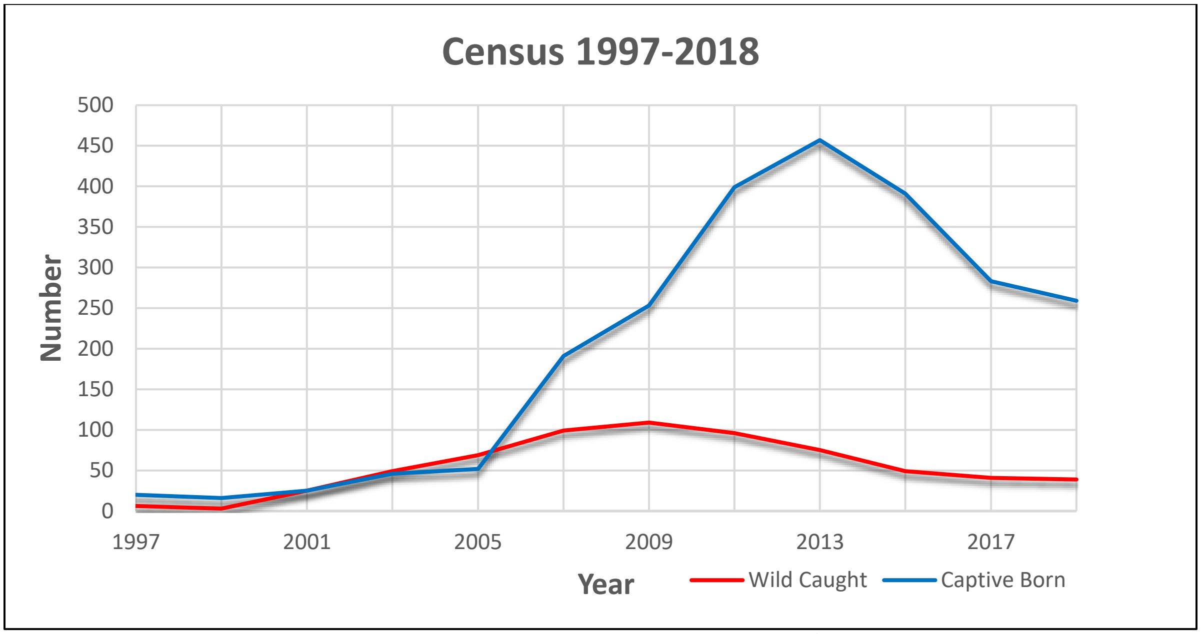

**Figure 1.** Census of *Eurycea sosorum* captive population 1997[1]-2018.
[1]Prior to 1999, captive-born salamanders consisted of donations by the Dallas Aquarium.

In this document, we evaluate strategies to meet our objectives of maintaining as high a level of gene diversity as possible in the captive population that would then be used to produce offspring for reintroduction in the event of extirpation or extinction. Extirpation from sites under COA's purview may threaten the species with extinction; therefore, we use extirpation from all four spring sites, in addition to extinction, as a cause for concern and trigger for increased efforts to protect the species in captivity. Because of the larger dataset, we focus primarily on the Barton Springs Salamander and conduct an abbreviated analysis on the Austin Blind Salamander population. We first calculate population statistics required for population projections, such as generation time, population growth rate, and gene diversity (based on pedigree), and $N_e/N$ (ratio of effective population size to the total population; i.e., percent of successful breeders in the living population) of the captive population. Using this information, we evaluate the following: 1) the effects on gene diversity of maintaining spring lineage populations separately versus combining them into a single population; 2) the population size and gene diversity needed for the "core" ("core" = captive population used to preserve gene diversity when the species has not been extirpated) population in order to be able to increase the population to the capacity size ($N_c$) to maintain gene diversity over decades and produce offspring for reintroduction if the species were extirpated or threatened with extinction from the wild; 3) the effect that collections would have on gene diversity of the current population; 4) the time-frame needed to reach capacity ($\mathrm{N_c}$); and 5) the estimated number of offspring that could be produced for reintroduction with the capacity population. We also analyze the small population of Austin Blind salamanders in captivity and determine the effects that additions of wild stock would have on the gene diversity of that population. We then apply the results and provide recommendations for both species within the context of our gene diversity goal of maximizing the preservation of gene diversity over time.

# Methods

## *Individual and Pedigree Data*

Individual salamanders were tracked over time using photographs by visually matching melanophore and iridophore patterns. Information recorded on individuals includes hatch date, death date, sex, parentage (to the extent known), and spring site of origin (if wild-caught) or spring site of origin of ancestors (if captive-raised). Hatch date of captive-raised individuals was recorded as 1-month post-oviposition date. The hatch dates of wild-caught individuals were estimated based on size at collection, with a maximum age of 1.5 years at collection (Appendix A). All wild-caught salamanders as well as captive-bred salamanders that reached an age of 6-months post-hatch were entered into the population database using Sparks (ISIS 2013), assigned a studbook number, and tracked over time with regard to social group (to track pedigree).

Because salamanders were often housed in groups of more than two individuals, we assigned parents to offspring in one of several ways to record the pedigree. If only one female or one male was present in the tank at the time of the oviposition, or if a female was observed ovipositing, we assigned the offspring to that individual. In cases with multiple potential dams/sires, we recorded every potential dam/sire as having an equal probability of parentage. Finally, in rare cases, if observations indicated that a specific individual was more likely to be the parent, then that individual was assigned a higher probability of being the parent.

To calculate current gene diversity, it was necessary to address the uncertainty in the parental assignments to offspring in cases in which multiple potential parents were recorded. We had two options: 1) assign each potential parent an equal percentage of the offspring, which results in weighted mean individual and population statistics (Lacy et al. 2012; Traylor-Holzer 2011), or 2) assign an individual (e.g., using the lowest studbook number) to be the parent. If option 2 is chosen, and if there were multiple ovipositions resulting from one reproductive group with the same composition of potential dams or sires, then a single dam/sire was assigned as the dam/sire of all of the offspring resulting from that group. Option 1 could overestimate gene diversity while option 2 would likely underestimate gene diversity. The difference in gene diversity of the two settings used with the entire current Barton Springs Salamander population of 293 individuals was 1%: 98.3% assuming equal parentage among possible parents and 97.3% assuming single parentage. The gene diversity difference in the Austin Blind Salamander population was 2% (94.4% assuming equal parentage and 92.4% assuming single parentage). We chose to use option 2 to calculate gene diversity to avoid overestimating gene diversity and option 1 for demographic statistics to calculate an average age of the potential parents for generation time.

We tracked information on pedigree and individual statistics using SPARKS (ISIS 2013) studbook software. For the analyses of the Barton Springs Salamander population, we used data from 921 (171 wild-caught, 750 captive-raised) individuals that had been housed in captivity since the program's inception in 1998 for the demographic analysis and data on the living population for the genetic analysis. Similarly, we used data from 88 Austin Blind salamanders (24 wild-caught, 64 captive-raised) that had been housed in captivity for the demographic analysis and the living population for the genetic analysis. Demographic and genetic statistics as well as projections of gene diversity and population size were calculated with the program PMx v1.5 (Ballou et al. 2018), unless otherwise noted.

## Barton Springs Salamander

### *Population Statistics*

Because the age, fate, and parentage were known for each individual (with varying degrees of certainty; see preceding section), we were able to calculate generation time (*T*), survivorship ($L_x$), the maximum potential annual population growth rate ($\lambda$), and gene diversity (*GD*). We also used reproduction data to determine the reproductive age range and to generate an approximation of $N_e/N$, a metric used in population modeling to indicate the ratio of the number of living proven breeders to the total living population size. In addition, we also calculated the mean number of hatchlings per oviposition to estimate the number of offspring that could be produced in a year when the population is at capacity.

#### Demographics

Mean generation time (*T*), which is the average age at reproduction (averaging males and females), was calculated from the average age of parents at oviposition, including every known parent, as well as every potential parent in cases with multiple potential parents. For comparison, we also calculated the average age of reproduction using the subset of only known parents. Age specific survivorship ($L_x$), which is the percentage of individuals that survive to the beginning of a specified age class, was calculated using the dataset of all of the individuals that survived to 6-months of age since the program's inception. We used survivorship and data on reproductive age range to determine a practical and representative target generation time to breed salamanders before they are at high risk of death.

To estimate the maximum potential population growth rate required for population projections, we used the maximum $\lambda$ (lambda, annual population growth rate), assuming population sizes $\geq$ 100 individuals, based on the set of $\lambda$ calculated from the census for each year from 1998 to 2018 (Appendix B). For comparison, we also calculated the average $\lambda$ for program years during which population sizes $\geq$ 100 individuals and measures were not taken to reduce reproduction.

To determine the expected number of hatchlings that could be produced per oviposition, we calculated the mean number of hatchlings per oviposition using data from previously tracked clutches.

#### Gene Diversity Based on Pedigree

Gene diversity indicates the expected heterozygosity (relative to allele frequencies of the founders) of offspring produced by random mating and is often expressed as a proportion relative to the wild population. It is based on the concept that two alleles at a given locus, sampled at random from a population, are not identical by descent from a common ancestor. For these analyses, it is calculated as 1 minus the average mean kinship of the population (Lacy 2012; Traylor-Holzer 2011); the average mean kinship in the population is the equivalent of the proportional gene diversity loss in the captive-bred population relative to the founders. Kinship calculations are based on the relatedness of each individual to all of the individuals in the living population and individual kinship values are recalculated as the set of individuals in the population changes (Lacy et al. 2012). We calculated gene diversity and the mean kinship of the current population.

### Estimating $N_e/N$

$N_e/N$ is the ratio of the effective population size (i.e., the number of individuals that contribute offspring to the next generation) to the total population size. The effective population size ($N_e$) is the size of a randomly breeding population that would lose gene diversity (through inbreeding and genetic drift) at the rate that has occurred in the captive population and is calculated as $GD_t/GD_0 = (1\text{-}1/(2N_e))$ ^$^t$ ($t$ = average number of generations that have elapsed since the founders) (Nei 1973). $N_e/N$ is typically used as a management metric in captive population gene diversity projections to indicate the percent of the living population that are proven breeders and can be estimated as $(4{*}N_f{*}N_m)/(N_f{+}N_m)$ where $N_f$ and $N_m$ are the number of proven female and male breeders (J Ballou, pers. comm.). $N_e/N$ for our current population is biased low because we reduced reproduction via population management and we did not remove individuals with overrepresented genetics from the total population. To estimate $N_e/N$ during unrestricted reproduction we calculated the percent of successful breeders of a subset of wild-caught salamanders. This subset of 85 salamanders consisted of wild-caught individuals that had been collected from four spring sites over the previous ten years. Upon being transferred to ASCC at the start of the facility, the salamanders were placed in eight reproductive groups as follows: two groups consisting of salamanders originally collected from Parthenia Spring, two groups with salamanders from Sunken Garden Spring, three groups from Eliza Spring, and one group of salamanders collected from Upper Barton Spring (Appendix C). Each group was housed in a separate 65-gallon tank plumbed onto a recirculating water system maintained separately by spring site of origin. Over five months, there were multiple ovipositions in some groups; given that some of the clutches were oviposited days apart within a single group, this likely represented reproduction by more than a single female and possibly more than a single male. Therefore, we calculated the mean of the potential minimum and maximum number of breeders resulting in offspring surviving to 6 months to approximate $N_e/N$. In addition, for this analysis, we assume a single sire for a given clutch of eggs.

## ***Gene Diversity and Demographic Projections***

We projected gene diversity to evaluate strategies that could be employed to maximize gene diversity over time. To conduct these projections, the following population variables are required: generation time ($T$), maximum potential population growth rate ($\lambda$), ratio of effective population size to the total population size ($N_e/N$), current/core population size ($N$), current/initial gene diversity ($GD$), and the maximum allowable population size at the facility (i.e., capacity), ($N_c$). $N_c$ refers to the population used to preserve gene diversity; it does not include individuals produced for reintroduction. For each projection, we assumed the same generation time ($T$), maximum potential $\lambda$, and $N_e/N$, each generated as described above. Current/core population size ($N$) and current/core gene diversity ($GD$) are specified based on the population to be modeled. The % gene diversity goal and number of years to maintain the % gene diversity is either specified or projected as stated for the scenario. We used number of years to maintain 90% gene diversity as a measure to evaluate strategies.

### Combining Spring Lineage Populations Into a Single Population

To evaluate the strategy of combining the four spring site lineage populations versus maintaining them separately, we took into account the maximum allowable population size ($N_c$) based on the facility capacity. The current configuration of tanks will hold approximately 2300 liters for each species, with 25% of the capacity designated for tanks to house eggs and juveniles for reintroduction, 35% to house pairs and small groups for reproduction, and 40% for maintenance tanks for individuals used to preserve gene diversity and not housed in breeding tanks and not planned for release into the wild. This may change somewhat as tank systems are modified. For closed systems, the maintenance tanks are stocked at a maximum of approximately 0.5 adult salamander/L or less, and the reproductive tanks are stocked at various densities. Given this, the capacity for the population used to maintain gene diversity (not including individuals for reintroduction), is approximately 500.

Using this information, we projected the number of years that a single spring site lineage population capped at the population size of 125 (25% of $N_c$, the facility population capacity, of 500) as well as the combined population capped at 500 salamanders could maintain 90% gene diversity during conditions in which the species is thought to be extirpated. To illustrate this, we assumed a best-case scenario for the spring lineage population of all wild-caught individuals with an initial population gene diversity of 99.9%. For the combined population, we used the current population of 293 with gene diversity of 97.3%. Given the facility population capacity of 500, the maximum population size for each of four spring site populations would remain at 125 and the maximum population size for the current population of 293 would be 500. We used the number of years that 90% gene diversity could be maintained as a measure of effectiveness. We then discussed the results in the context of the facility capacity and the goal of maximizing the population gene diversity over time.

### *Gene Diversity and Population Size Needed for Current/Core Population*

To evaluate the current population, assuming combined spring lineage reproductive management, we projected the number of years that 90% gene diversity as well as the current gene diversity could be maintained with and without an increase in size to the facility population capacity ($N_c$) of 500, without additions of wild stock.

To evaluate the population size and gene diversity needed in the core population to meet our gene diversity goal of maximizing gene diversity over time, we conducted the following projections using initial population sizes of 50-500 (in increments of 50 individuals) and initial gene diversity of 96.0%, 97.0%, 98.0%, 99.0%, and 99.9%:

1) The length of time that 90% gene diversity could be maintained if the core population were increased to the facility population capacity ($N_c$) of 500.
2) The gene diversity once the population size reaches $N_c$ that could then be used to produce offspring for reintroduction.
3) The number of years necessary to increase the population to $N_c$.

### *Effect of Wild Stock Additions on Gene Diversity of Current Population*

We determined the effect of yearly importations of 5, 10, 15, and 20 wild stock on gene diversity of the current population after five years. We looked at this timeframe to evaluate an effective strategy to maintain the gene diversity at the current level or higher.

### *Reproductive Output of Genetically Diverse Offspring for Reintroduction*

We estimated the number of genetically diverse offspring that could be produced in a year from the facility population capacity ($N_c$) of 500 individuals, assuming $N_e/N$ as calculated above, an even sex ratio, one oviposition per breeding pair, a single sire for a given clutch of eggs, and the mean number of hatchlings per oviposition (as determined above). We also estimated the number that the population would be increased by based on $\lambda$, as calculated above, for the output for one year.

## Austin Blind Salamander

Using methods described above, we conducted an abbreviated analysis using the Austin Blind Salamander dataset.

### *Population Statistics (Demographics, Gene Diversity Based on Pedigree, and $N_e/N$)*

Using data on the population of individuals tracked over time, we calculated population statistics of generation time ($T$), maximum potential population growth rate ($\lambda$), $N_e/N$, survivorship ($L_x$), and gene diversity ($GD$). The population growth rate ($\lambda$) was calculated from the average of the five highest $\lambda$, assuming a population size of at least 20 individuals (Appendix D). We also calculated the mean number of hatchlings per oviposition using data from previously tracked clutches.

### *Gene Diversity and Demographic Projections*

#### Gene Diversity Projections of Current Population

To evaluate the current population, we projected the number of years that 90% gene diversity as well as the current gene diversity could be maintained, with and without an increase in population size to $N_c$ of 500, without wild stock additions.

#### Effect of Wild Stock Additions on Gene Diversity of Current Population

Using the modeling variables as calculated in the previous section, we projected gene diversity to determine the effects of annual additions of 5, 10, 15, and 20 wild stock on the gene diversity of the population over five years. We then evaluated the results within the context of our gene diversity goal of maximizing gene diversity in the population that would then be used to produce offspring for reintroduction if the species were extirpated from the wild.

#### Reproductive Output of Genetically Diverse Offspring for Reintroduction

We estimated the number of genetically diverse offspring that could be produced for reintroduction in a year from the facility population capacity (Nc) of 500 individuals, assuming the calculated $N_e/N$ (expected percent of successful breeders), an even sex ratio, one oviposition per breeding pair, a single sire for a given clutch of eggs, and the mean number of hatchlings per oviposition (see demographics section). We also use $\lambda$, as calculated above, as another estimate for the output for one year based on the projected increase in population.

# Results and Discussion

## Barton Springs Salamander

### *Population Statistics*

#### Demographics

The generation time in cases in which the exact parents are known, which resulted in 9% of the offspring, is 7.7 years. Based on known parentage, the earliest reproductive age for females and males is 11 months and the oldest age is approximately 12.5 and 15.5 years for females and males, respectively. The generation time of all of the parents, including cases in which multiple potential parents were possible, is 3.7 years.

Although a longer generation time will result in a longer retention of gene diversity, managing for a generation time of 7.7 years may result in mortalities prior to reproduction given that 50% are expected to die by 7.6 years of age (Fig. 2). A generation time of 3.7 years, which we use for the population projections, will provide time to attempt to breed individuals before they are at a high risk of dying.

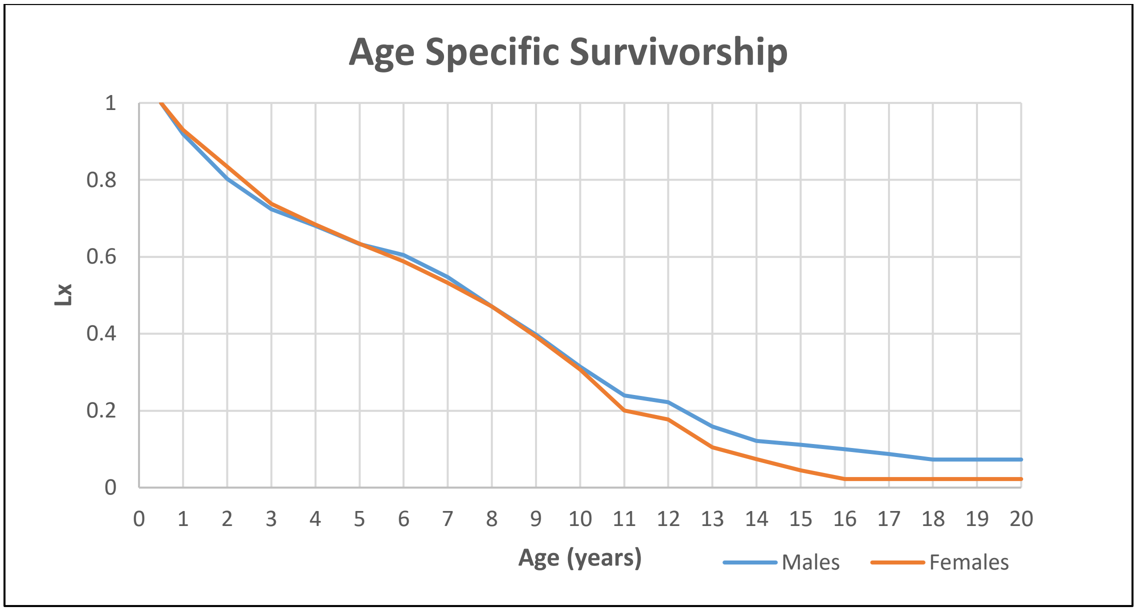

**Figure 2.** Barton Springs Salamander age specific survivorship, assuming individuals survive to 6-months post-hatch**.** Individuals of unidentified sex are divided equally between males and females. $L_x$ is the probability that an individual will be alive at the beginning of age class $x$.

The average annual population growth rate during a four-year period in which $N \geq 100$ and actions were not taken to slow the population growth was 42% (where $\lambda = 1.42$). This would be an underestimate for the maximum potential growth rate, however. The highest annual population growth rate during the course of the program was 63% ($\lambda = 1.63$), which we use for the population projections.

Using data on previously tracked ovipositions, the mean number of hatchlings per oviposition is 6.5 (SD 6.98, *N*=251, range 0–40).

### *Gene Diversity Based on Pedigree*

The gene diversity of the current population of 293 individuals is 97.3%, with an average mean kinship of 0.027. Given this, removing higher mean kinship individuals would result in a population with higher gene diversity. Therefore, selecting lower mean kinship individuals as priority breeders could result in maximizing gene diversity over time and higher gene diversity of the offspring for reintroduction.

### *Estimating $N_e/N$*

Of the wild-caught subset (Appendix C) of 85 salamanders that were set up for reproduction and that we used to estimate $N_e/N$, all eight groups reproduced within two months and some groups reproduced multiple times within five months. Given that all eight groups reproduced successfully, this represents a minimum of 16 breeders out of 85 individuals, or 18.8%. Assuming a unique pair of salamanders for each oviposition, the maximum number of potential breeders is 39 out of 85, or 45.9% (Table 2). The average of the minimum and maximum is 32%, which we use as an approximation of $N_e/N$ for population projections. For comparison, a study on pairwise reproduction in *Eurycea sosorum* found that 9 out of 60 pairs, or 15%, reproduced successfully within two months (Cantu et al. 2016). Because 15% to at least 19% reproduced in two months, additional time would likely result in reproduction of more individuals, increasing the ratio. Therefore, an average $N_e/N$ of 32% may be conservative. In the event that reproduction for reintroduction is necessary, an increased number of tanks would be dedicated for both pairwise and group breeding to maximize $N_e/N$.

**Table 2**. Founder reproduction: minimum and potential maximum number of breeders. P denotes salamanders originally collected from Parthenia Spring, SG, from Sunken Garden Spring, E, from Eliza Spring, and UBS, from Upper Barton Spring.

| **Group** | **No. Salamanders (Male.Female)** | **No. Ovipositions with Offspring** | **Minimum No. Breeders** | **Maximum No. Breeders** |
|---|---|---|---|---|
| P1 | 12 (5.7) | 1 | 2 | 2 |
| SG1 | 10 (6.4) | 4 | 2 | 8 |
| E1 | 13 (7.6) | 4 | 2 | 8 |
| UBS | 9 (6.3) | 1 | 2 | 2 |
| SG2 | 10 (2.8) | 3 | 2 | 5 |
| P2 | 17 (6.11) | 4 | 2 | 8 |
| E2 | 7 (2.5) | 2 | 2 | 4 |
| E3 | 7 (4.3) | 1 | 2 | 2 |

## *Gene Diversity and Demographic Projections*

We used the estimates of generation time ($T$), maximum potential population growth rate ($\lambda$), and $N_e/N$ (Table 3) generated from the demographic and genetic analyses in population projections to evaluate the effects of management strategies on gene diversity.

**Table 3.** Demographic and genetic statistics used in population projections.

| **Variable** | **Value** |
|---|---|
| Mean generation time ($T$, years) | 3.7 |
| Maximum potential $\lambda$ | 1.63 |
| $N_e/N$ | 0.32 |

### *Combining spring lineage populations into a single population*

Given our facility population capacity ($N_c$) of 500 salamanders for this population, each of four spring site lineage populations would be capped at 125, which would result in maintaining 90% gene diversity by the end of 31 years (Fig. 3). In contrast, the current population of combined spring site lineages and 293 individuals increased to the facility population capacity of 500 individuals would be able to maintain 90% gene diversity by the end of 92 years (Fig. 4).

Therefore, combining the spring lineage populations would result in a higher gene diversity over time.

Furthermore, maintaining multiple subpopulations requires extra resources and the resulting small population sizes may make it difficult to avoid inbreeding as generations are reproduced. Given that the goal of the captive breeding program is to maintain the gene diversity that represents the genetic diversity of the species in the wild, including rare alleles if possible, any additional genetic variability obtained from different spring sites could result in greater resiliency (Lacy 1997), protecting the species as a whole.

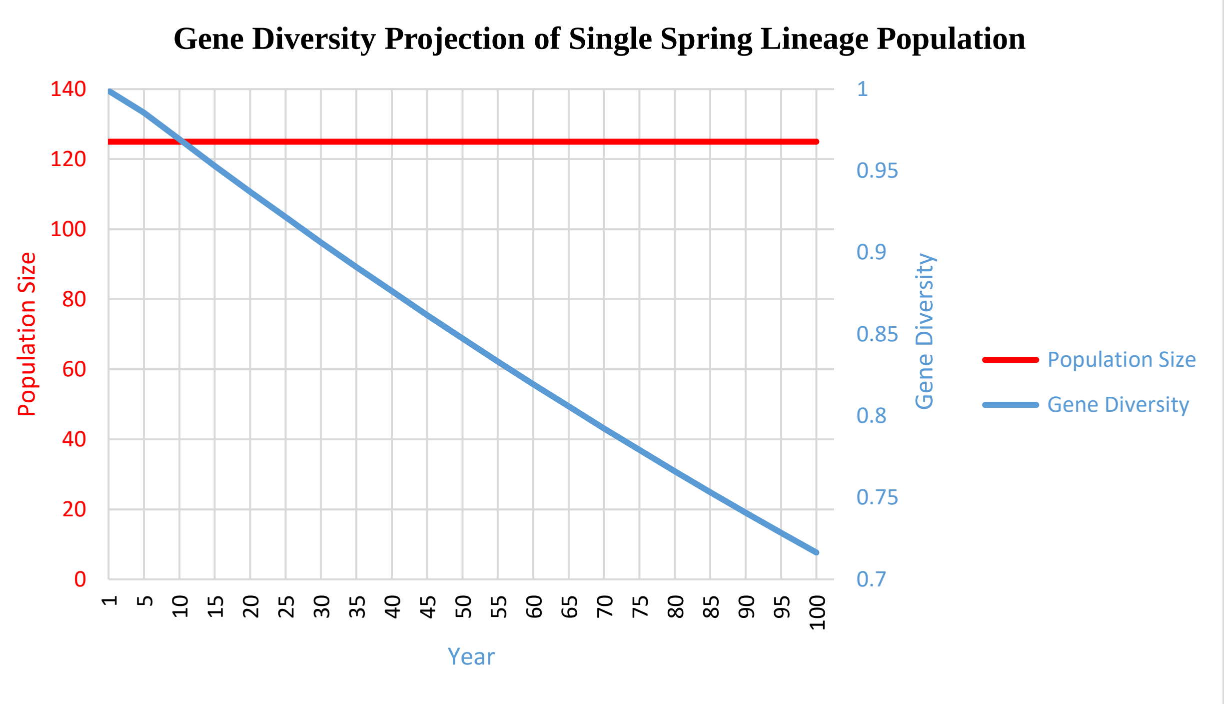

**Figure 3**. Projection of 90% gene diversity of a single spring lineage population.

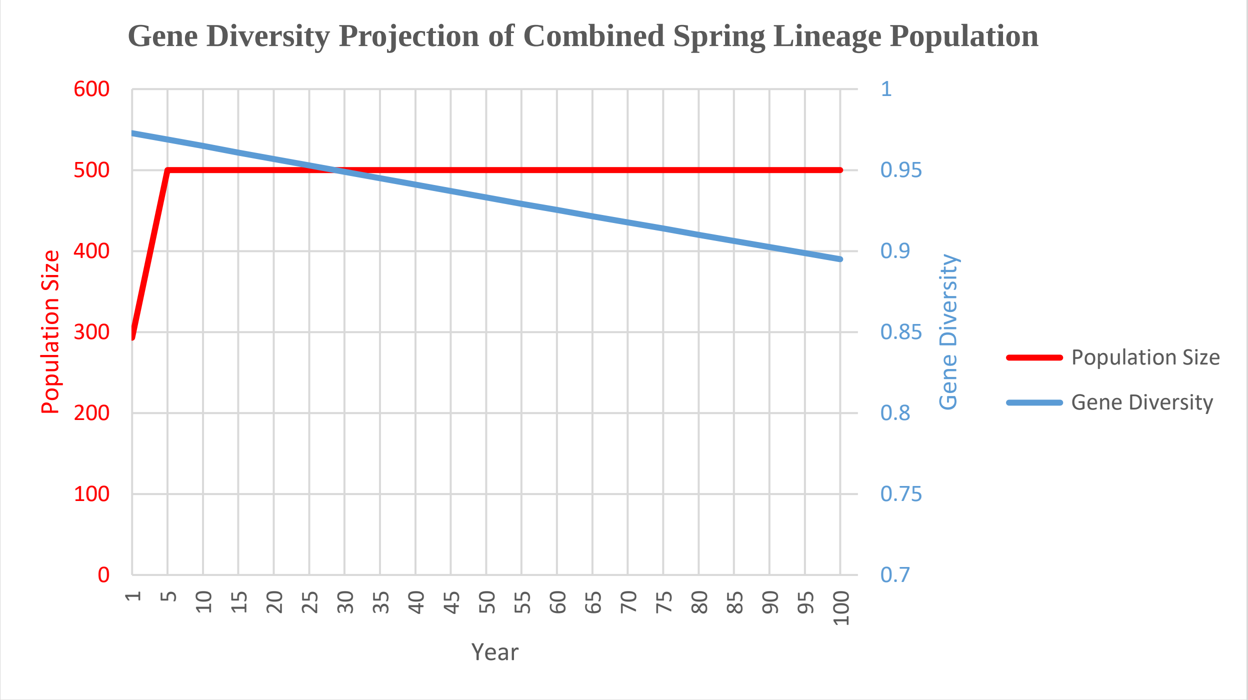

**Figure 4.** Projection of 90% gene diversity of the current population of combined spring lineages.

*Gene Diversity and Population Size Needed for Core Population*

We evaluated the current population and then modeled theoretical populations of various sizes and gene diversities to determine an optimal population size under conditions in which collections are possible and there is no need to increase the population to the facility population capacity ($N_c$).

With an increase to the facility population capacity of 500 and no wild stock additions, the current population ($N$ = 293, $GD$ = 97.3%), consisting of combined spring lineages, could maintain 90% gene diversity for 92 years and the current gene diversity of 97% for two years. With no increase in size and no wild stock additions, the current population could maintain 90% gene diversity for 54 years and the current gene diversity of 97% for one year.

As can be seen in Table 4, the higher the gene diversity of the initial core population (prior to expansion to the facility population capacity), the higher the gene diversity will be over time (Fig. 5). For example, a population of 100 individuals with gene diversity 99% compared to a population of 100 with gene diversity 96% would result in a gene diversity 3% higher when increased to the facility population capacity of 500 and be able to maintain 90% gene diversity for 36 years longer (Table 4).

**Table 4.** Gene diversity ($GD$) and population size projections of initial or "core" population sizes ($N_i$) of 50-500 (in 50 individual increments) with gene diversity of 96%, 97%, 98%, 99%, and 99.9%. ($N_c$ = facility population capacity of 500)

| $N_i$ | **No. Years $GD \geq 90\%$** | **No. Years to reach $N_c$** | **$GD$ when reach $N_c$** |
|---|---|---|---|
| 99.9% Initial *GD* | | | |
| 50 | 106 | 5 | 97.9 |
| 100 | 117 | 4 | 99.0 |

| | | | |
|---|---|---|---|
| 150 | 121 | 3 | 99.3 |
| 200 | 122 | 2 | 99.6 |
| 250 | 123 | 2 | 99.6 |
| 300 | 124 | 1 | 99.8 |
| 350 | 124 | 1 | 99.8 |
| 400 | 124 | 1 | 99.8 |
| 450 | 124 | 1 | 99.8 |
| 500 | 124 | 0 | 99.9 |
| 99.0% Initial *GD* | | | |
| 50 | 95 | 5 | 97.0 |
| 100 | 106 | 4 | 98.1 |
| 150 | 110 | 3 | 98.5 |
| 200 | 111 | 2 | 98.7 |
| 250 | 112 | 2 | 98.7 |
| 300 | 113 | 1 | 98.9 |
| 350 | 113 | 1 | 98.9 |
| 400 | 113 | 1 | 98.9 |
| 450 | 113 | 1 | 98.9 |
| 500 | 114 | 0 | 99.0 |
| 98.0% Initial *GD* | | | |
| 50 | 83 | 5 | 96.1 |
| 100 | 94 | 4 | 97.1 |
| 150 | 98 | 3 | 97.5 |
| 200 | 99 | 2 | 97.7 |
| 250 | 100 | 2 | 97.7 |
| 300 | 101 | 1 | 97.9 |
| 350 | 101 | 1 | 97.9 |
| 400 | 101 | 1 | 97.9 |
| 450 | 101 | 1 | 97.9 |
| 500 | 101 | 0 | 98.0 |
| 97.0% Initial *GD* | | | |
| 50 | 70 | 5 | 95.1 |
| 100 | 82 | 4 | 96.1 |
| 150 | 85 | 3 | 96.5 |
| 200 | 87 | 2 | 96.7 |
| 250 | 88 | 2 | 96.7 |
| 300 | 88 | 1 | 96.9 |
| 350 | 89 | 1 | 96.9 |
| 400 | 89 | 1 | 96.9 |
| 450 | 89 | 1 | 96.9 |
| 500 | 89 | 0 | 97.0 |
| 96.0% Initial *GD* | | | |
| 50 | 58 | 5 | 94.1 |
| 100 | 70 | 4 | 95.1 |
| 150 | 73 | 3 | 95.6 |
| 200 | 75 | 2 | 95.7 |
| 250 | 75 | 2 | 95.7 |
| 300 | 76 | 1 | 95.9 |

| | | | |
|---|---|---|---|
| 350 | 76 | 1 | 95.9 |
| 400 | 76 | 1 | 95.9 |
| 450 | 77 | 1 | 95.9 |
| 500 | 77 | 0 | 96.0 |

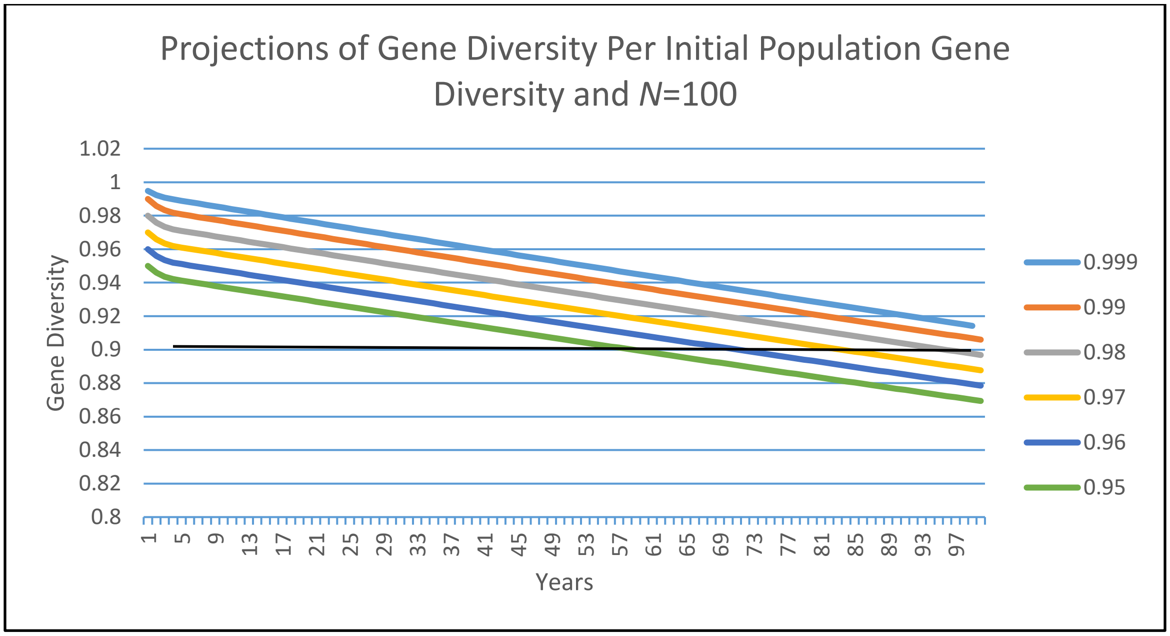

**Figure 5.** Projections of gene diversity per initial core population gene diversity and $N = 100$

In addition, as the initial population size ($N_i$) increases beyond 200, there are diminishing returns for maintenance of gene diversity. For example, $N_i$ from 250–500 provides only a 0.1–0.3% higher gene diversity when grown to capacity compared to $N_i = 200$ at the same initial gene diversity. However, at lower $N_i$ the trade-off between $N_i$ and gene diversity when increased to $N_c$ (facility population capacity) can be more pronounced. For example, at $N_i = 50$ gene diversity is up to 1.7% lower once capacity is reached, compared to $N_i = 200$. Additionally, it takes longer to reach the facility population capacity of 500 at lower $N_i$ and unexpected mortalities could have a much larger, negative impact on future gene diversity, particularly if they occur early on. For these reasons, we believe an optimal size for $N_i$ would be an intermediate value, between 150 and 200 individuals during conditions in which collections are possible to boost gene diversity.

### *Effect of Wild Stock Additions on Gene Diversity of Current Population*

Projections indicate that annual additions of 10 wild stock per year would result in 98% gene diversity in the current population (Table 5). Collections larger than 10 would not greatly increase the projected gene diversity and collections smaller than 10 may not result in having a buffer from mortalities that would diminish the stock of wild-caught genes that could be added to the population. For example, if we collect only five individuals, then we would have only three living individuals to attempt to breed at approximately the average generation time, given a 68% chance of survival to 4 years of age (Fig. 2). If we collect 10 individuals, then, we would have at least six, which would likely represent multiple pairs, assuming both males and females are collected. Without collections, the current population could maintain the current gene diversity of 97% for only one year. Therefore, collections of 10 per year would help maintain high gene diversity over time that could then be used, in the event of extirpation, to preserve gene diversity for production of offspring for reintroduction.

**Table 5.** Projected *GD* resulting from five years of annual additions of 5, 10, 15, and 20 wild-stock (starting *GD* = 97.3, $N$ = 293, $N_{max}$ = 293)

| 5/year | 10/year | 15/year | 20/year |
|---|---|---|---|
| 97.7% | 98.3% | 98.6% | 98.8% |

### *Reproductive Output of Genetically Diverse Offspring for Reintroduction*

The population at the capacity of 500 salamanders could be reached in 5 years or less, depending on the size of the $N_i$ population (Table 4). Once 500 is reached, this population would be maintained and used to produce offspring for reintroduction. Assuming an equal sex ratio, one oviposition per pair, 6.5 hatchlings per oviposition, a sufficient number of tanks for pairings, and reproduction of 32% ($N_e/N$) of the population (and a resulting increase in $\lambda$), then 520 offspring could be produced in a year. Considering our estimated range for $N_e/N$ of 18.8%-45.9% (see above section "Estimating $N_e/N$"), calculated using only five months of reproduction, the number that could be produced would be 306-746. While we do expect the number produced over the course of an entire year to be higher, we use the average of 32% and 520 offspring to err on the side of caution. If the population $\lambda$ of 1.63 is the limiting factor, then at least 315 offspring could be produced per year.

## Austin Blind Salamander

### *Population Statistics*

#### *(Demographics, Gene Diversity Based on Pedigree, $N_e/N$)*

Even though the dataset is limited, we were able to calculate statistics (Table 6) to use in population projections. Generation time ($T$), including potential parents in cases in which multiple potential parents were used, was 6.3 years. Based on known parentage, the earliest reproductive age for females and males is three and four years, respectively, and the oldest age is 11 and 14 years, respectively. Because of the small dataset, information on the reproductive age range is limited and the ranges reflect reproduction of individuals that were collected as adults, so the actual ages at reproduction may have been higher. Given that salamanders have a 50% chance of surviving to 10.9 years of age (Fig. 6), a generation time of 6.3 years would provide time to attempt to reproduce individuals before they reach a high risk of mortality.

**Table 6.** Demographic and genetic statistics used in population projections.

| Variable | Value |
|---|---|
| Generation time ($T$, years) | 6.3 |
| Maximum potential $\lambda$ | 1.22 |
| $N_e/N$ | 0.11 |
| Current gene diversity | 92.4% |

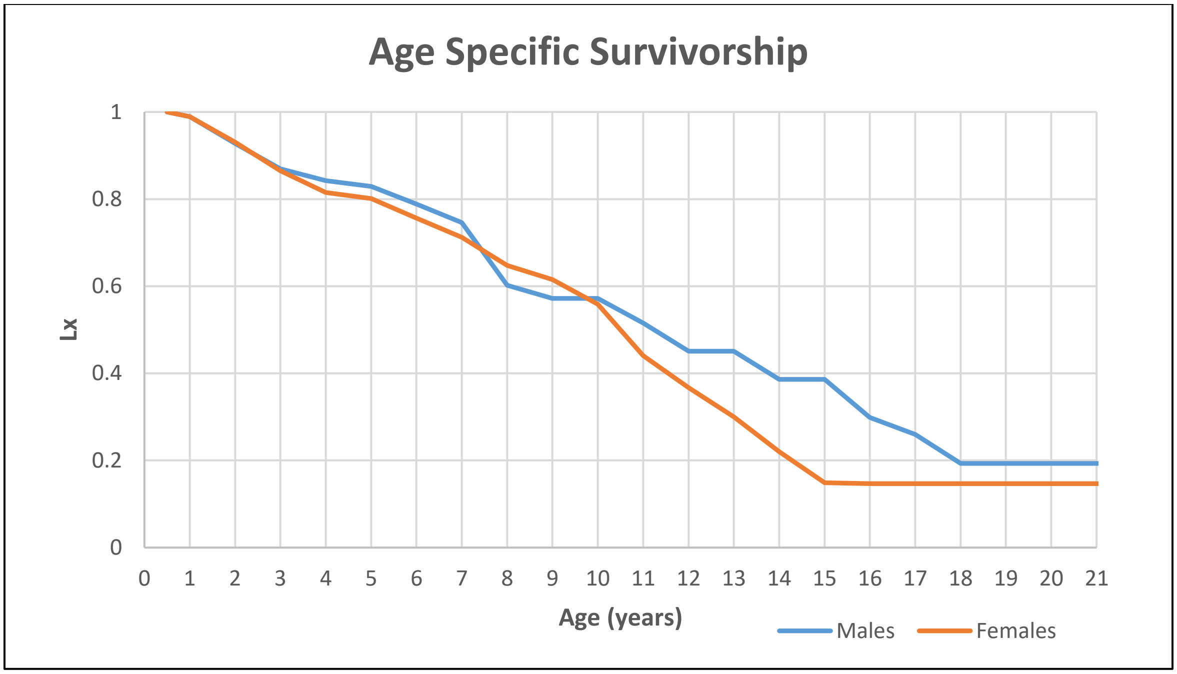

**Figure 6.** Austin Blind Salamander age specific survivorship assuming individuals survive to 6-months post-hatch**.** Individuals of unidentified sex are divided equally between males and females. $L_x$ is the probability that an individual will be alive at the beginning of age class *x*.

The mean number of hatches from oviposition events in captivity was calculated as 5.2 (*N* = 28, SD 6.66, range 0–26).

### *Gene Diversity and Demographic Projections*

#### *Gene Diversity Projections of Current Population*

With and without an increase to the facility population capacity of 500 and no wild stock additions, the current population (*N* = 45, *GD* = 92.4%) could maintain 90% gene diversity for one year and the current gene diversity for less than one year. Therefore, additional founders are needed to maintain a higher gene diversity.

#### *Effect of Wild Stock Additions on Gene Diversity*

Results indicate that collections of at least 15 per year for five years would result in an increase in *GD* to 97% (Table 7). If only 10 were collected for five years, the population gene diversity would not increase above 96%. In comparison, without collections, the current gene diversity of 92.4% could be maintained for less than one year. Annual collections will be necessary to increase the gene diversity of the population, but large numbers are not found in the wild. A goal of 15 collections annually may require efforts such as drift nets at the spring outlets for most of the collections.

**Table 7.** Projected *GD* resulting from five years of annual additions of 5, 10, 15, and 20 wild-stock (starting *GD* = 92.4, *N* = 45, $N_{max}$ = 150)

| **5/year** | **10/year** | **15/year** | **20/year** |
|---|---|---|---|
| 95.1% | 96.7% | 97.4% | 97.8% |

*Reproductive Output of Genetically Diverse Offspring for Reintroduction*

If the population were to be increased to a size of 150 in the short-term, then the facility population capacity of 500 could be reached in six years, if needed, assuming a $\lambda$ of 1.22. Given the assumptions of an equal sex ratio, one oviposition per successful pair, 5.2 hatchlings per oviposition, a sufficient number of tanks for pairings, reproducing 11% ($N_e/N$) of the population, the capacity population of 500 could produce 143 offspring for reintroduction in a year. If we assume the projection is based on the calculated $\lambda$ of 1.22, then the population would be increased by 110. It is important to note, however, that our demographic estimates may have more uncertainty associated with them due to the smaller sample size compared to the Barton Springs Salamander population. As more information is learned about this species, the $N_e/N$ and $\lambda$ might increase, resulting in a higher reproductive yield.

## Recommendations

In this document, we evaluated strategies to maintain high gene diversity in captive populations of *Eurycea sosorum* and *E. waterlooensis* that could be increased to a capacity of 500 in the event that the species is extirpated from the wild to produce offspring for reintroduction and preserve 90% gene diversity for many decades. Based on the results, we recommend the following:

1. *Combine spring lineage populations*
Splitting resources between several populations rather than combining them into a single population interferes with program gene diversity goals in the event of extirpation from the wild. Because combining spring lineage populations results in maintenance of 90% gene diversity for approximately 60 years longer than if the population consisted of four separate spring lineage populations, we recommend combining spring site lineages into a single population.

2. *Maintain minimum population size of 150*
We recommend maintaining a minimum of 150 individuals during conditions in which there are healthy populations in the wild. This size will provide a sufficient number of individuals to successfully reproduce to increase the population to the facility population capacity of 500 in the event that the species is extirpated from the wild. Regarding *Eurycea waterlooensis*, the population should be increased to at least 150 via reproduction and collections. $N_e/N$ should be increased, if possible. Excess individuals as well as individuals with low genetic value (high mean kinship) can be shifted out of the managed population for other purposes, such as research and education.

3. *Supplement maintenance of high gene diversity with wild stock*
Even though the current *Eurycea sosorum* population could maintain 90% gene diversity for 92 years, it could maintain 97% for only 1 year (without collections). Given that there are healthy populations in the wild, we recommend collecting an average of ten salamanders per year to maintain high gene diversity on an on-going basis in this population. In addition, we recommend collecting an average of 15 *E. waterlooensis*, of any size class, per year, for the next five years to increase the gene diversity of the population. This may require drift nets given that *E. waterlooensis* are rarely observed at the surface of the springs. Collection needs should be reevaluated in 5 years. The majority of the *Eurycea sosorum* collections will be of salamanders estimated to be less than one year in age, based on size. Given that it may not be possible to determine the sex of small juveniles, we may need to re-evaluate the size class for collection if we determine later that we are not obtaining an equal sex ratio. In addition, occasionally,

individuals are found injured during field surveys and are collected for recovery and entered into the captive population if they survive. Many of these salamanders are <1” total length; small juveniles collected from the same site on the same day may be related, and some of the individuals that are found injured also exhibit gas bubble trauma and may be more susceptible to this condition. To collect unrelated, healthy individuals, no more than 10% of the collections associated with this plan will include recovered (after six months) salamanders <1” total length found injured during field surveys and collected for recovery.

*Extirpation from the Wild*

In the event that the species is thought to be extirpated from the four spring sites managed by COA, threatening extinction of the species in the wild, the top priority will be to protect the core population that contains the gene diversity. As soon as a crisis occurs and COA and USFWS determine that an event may threaten the survival of the species in the wild, the core population that has been maintained during normal conditions will be increased to the facility population capacity of 500. Mate pairs and reproductive groups should be prioritized according to mean kinship to maximize gene diversity and grow the core population to the capacity population size, attempting to breed at least 32% and 11% of the *Eurycea sosorum* and *E. waterlooensis* populations, respectively. The population of 500 will be maintained to preserve 90% gene diversity for as long as possible or necessary. Any reintroduction efforts will be conducted in consultation with FWS. After the population is increased to the capacity size, genetically diverse offspring can be produced for reintroduction. The pedigree of the offspring should be tracked in order to estimate the gene diversity of the groups used for reintroduction.

## Acknowledgements

I thank Nathan Bendik for helpful discussions and for patiently providing many productive comments throughout the development of the plan. I also thank Donelle Robinson and Tom Devitt for providing edits and comments.

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# APPENDIX A

**Age estimates used in database for wild-caught individuals**

| **Size Class (total length)** | **Estimated Age** |
|---|---|
| ≤17mm | 1.5 months |
| 18mm–24mm | 4 months |
| 25mm–51mm | 9 months |
| ≥52mm | 1.5 years (considered a minimum) |

# APPENDIX B

**Annual population growth rate from census of *Eurycea sosorum* population**

| **Year** | **Annual Population Growth Rate ($\lambda$) from Census** | **Total N** |
|---|---|---|
| 1995 | 0.000 | 2 |
| 1996 | 3.500 | 7 |
| 1997 | 3.714 | 26 |
| 1998 | 0.923 | 24 |
| 1999 | 0.792 | 19 |
| 2000 | 1.263 | 24 |
| 2001 | 2.083 | 50 |
| 2002 | 1.680 | 84 |
| 2003 | 1.131 | 95 |
| 2004 | 1.116 | 106 |
| 2005 | 1.142 | 121 |
| 2006 | 1.628 | 197 |
| 2007 | 1.472 | 290 |
| 2008 | 1.441 | 418 |
| 2009 | 0.866 | 362 |
| 2010 | 1.221 | 442 |
| 2011 | 1.120 | 495 |
| 2012 | 0.988 | 489 |
| 2013 | 1.088 | 532 |
| 2014 | 0.921 | 490 |
| 2015 | 0.898 | 440 |
| 2016 | 0.875 | 385 |
| 2017 | 0.842 | 324 |
| 2018 | 0.920 | 298 |

# APPENDIX C

**5-Month period of founder reproduction at facility.** P denotes salamanders originally collected from Parthenia Spring, SG, from Sunken Garden Spring, E, Eliza Spring, and UBS, from Upper Barton Spring.

| Date | Group | No. Offspring Surviving to 6 Months Post-Hatch |
|---|---|---|
| 30-Dec-07 | E2[1] | 11 |
| 18-Jan-08 | E1 | 0 |
| 22-Jan-08 | SG2 | 13 |
| 27-Jan-08 | P2 | 2 |
| 31-Jan-08 | SG1 | 13 |
| 05-Feb-08 | P1 | 3 |
| 21-Feb-08 | UBS | 5 |
| 25-Feb-08 | SG2 | 3 |
| 26-Feb-08 | E1 | 3 |
| 07-Mar-08 | SG1 | 7 |
| 07-Mar-08 | P2 | 2 |
| 07-Mar-08 | E3 | 1 |
| 12-Mar-08 | E1 | 2 |
| 14-Mar-08 | E2 | 1 |
| 16-Mar-08 | SG1 | 15 |
| 16-Mar-08 | E1 | 12 |
| 16-Mar-08 | P2 | 3 |
| 28-Mar-08 | P2 | 9 |
| 29-Mar-08 | E1 | 0 |
| 25-Apr-08 | UBS | 0 |
| 02-May-08 | SG2 | 11 |
| 09-May-08 | E1 | 0 |
| 13-May-08 | E1 | 1 |
| 14-May-08 | SG1 | 6 |
| 22-May-08 | E1 | 0 |

[1] This group was established approximately 10 days earlier than the other groups.

# APPENDIX D

**Annual population growth rate from census of *Eurycea waterlooensis* population**

| **Year** | **Annual Population Growth Rate (*λ*) from Census** | **Total N** |
|---|---|---|
| 1998 | 0.000 | 1 |
| 1999 | 3.000 | 3 |
| 2000 | 2.000 | 6 |
| 2001 | 2.000 | 12 |
| 2002 | 1.250 | 15 |
| 2003 | 1.133 | 17 |
| 2004 | 1.294 | 22 |
| 2005 | 1.091 | 24 |
| 2006 | 0.958 | 23 |
| 2007 | 1.130 | 26 |
| 2008 | 1.538 | 40 |
| 2009 | 1.075 | 43 |
| 2010 | 1.116 | 48 |
| 2011 | 1.063 | 51 |
| 2012 | 1.020 | 52 |
| 2013 | 0.962 | 50 |
| 2014 | 0.900 | 45 |
| 2015 | 1.000 | 45 |
| 2016 | 0.867 | 39 |
| 2017 | 1.231 | 48 |
| 2018 | 1.000 | 48 |