MEMORANDUM

TO: Mayor and Council

FROM: Sara Boone Hartley, Acting Director Mike Personett
Watershed Protection Department FOR

DATE: January 29, 2019

SUBJECT: Annual Report to U.S. Fish and Wildlife Service

In September 2014, the U.S. Fish and Wildlife Service ("Service") amended the City of Austin's Endangered Species Act Section 10(a)(1)(B) Permit and renewed it for a period of 20 years. This permit covers incidental take of the federally protected Barton Springs and Austin Blind salamanders that may occur during operation and maintenance of Barton Springs Pool and the adjacent springs located in Zilker Park (Eliza, Old Mill/Sunken Garden, and Upper Barton springs).

In compliance with the measures set forth in the Habitat Conservation Plan, the Watershed Protection Department (WPD) has submitted to the Service the report for year 2018. This report details the City's compliance with the 45 measures listed in the permit. A requirement of the annual reporting measure in the permit is to provide a copy of the annual report to the City Manager, Mayor and City Council.

If you have need additional information, please contact me at (512) 974-1444, or Nathan Bendik, WPD Environmental Scientist Senior, at (512) 974-2040.

Attachments: 10(a)(1)(B) Permit Report

cc: Spencer Cronk, City Manager
Rey Arellano, Assistant City Manager
Mike Personett, Assistant Director, WPD
Tanya Sommer, U.S. Fish and Wildlife Service
Nathan Bendik, WPD

Annual Report January, 2018 – December, 2018
Endangered Species Act Section 10(a)1(B) Permit for the Incidental Take of the Barton Springs Salamander (*Eurycea sosorum*) and Austin Blind Salamander (*Eurycea waterlooensis*) for the Operation and Maintenance of Barton Springs Pool and Adjacent Springs
Permit # TE 839031-2

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Summary of Compliance

Table 1. Summary of compliance for each HCP measure.

HCP Measure	Compliance Status
6.1.1.1 The City will develop written habitat management plans for each spring site. These plans will include ongoing activities to improve the quality of aquatic habitat and ecosystem health. This includes but is not limited to introduction of native aquatic plants and maintenance of adequate tree canopy cover. Habitat management plans will be provided to the Service for review within one year of permit issue. The City will revise these plans with the written or verbal approval of the Service as necessary.	☒ Full Compliance ☐ Partial Compliance ☒ Measure Completed ☐ Measure Needs Amendment Notes: Plans were submitted to the Service at the one-year anniversary of permit issuance.
6.1.1.2 With the verbal or written approval of the Service, the City will redraw the footprint of protected salamander habitat in Barton Springs Pool (Figure 16) to include more habitat that is and can be maintained as suitable for salamander residence and exclude unsuitable habitat based on monitoring data and habitat condition. The total square footage of protected habitat in Barton Springs Pool will not be less than that delineated in the 1998 Habitat Conservation Plan.	☒ Full Compliance ☐ Partial Compliance ☒ Measure Completed Notes: Figure 16 in HCP delimits the footprint.
6.1.1.3 The City will be responsible for the management of aquatic and riparian habitats of: a. Barton Springs Pool and Parthenia Spring (fissures, springs, and Beach habitat; Figure 1), b. Eliza Spring (spring pool, outflow pipe and/or stream; Figure 1), c. Old Mill Spring (spring pool and outflow stream; Figure 1), d. Upper Barton Spring (spring and outflow streams; Figure 1).	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.1.4 The City will continue improvement and maintenance of suitable substrates in salamander habitat. If replacement of rocky substrate of salamander habitat is necessary, the City may use only limestone gravel or cobble in order to maintain the natural groundwater buffering of karst aquifers.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed ☐ Measure Needs Amendment

6.1.1.5 The City will make visual inspections of all protected habitat areas (spring sites when flowing) at least four days a week. City Parks and Recreation Department staff will be present at Barton Springs Pool when it is open and will visually inspect Parthenia Spring daily. Inspections will note any problem conditions such as vandalism, trash, debris, introduction of exotic fish or animals or disturbance of habitat. If problems are discovered, the City will take appropriate action to protect salamanders and their habitat. Appropriate actions may include but are not limited to repairing damage from vandalism, removal of trash, and removal of introduced exotic fish or animals	☒ Full Compliance ☐ Partial Compliance ☒ Measure Completed Notes: Staff continues to remove trash and restore substrate to Upper Barton Spring following disturbances by park visitors. We are currently developing signage to reduce disturbance in front of the springs during high-use periods at Barton Springs Pool.
6.1.1.6 The City will prohibit the following activities to reduce harassment of *Eurycea sosorum* and *Eurycea waterlooensis* and protect associated habitat: a. unauthorized, deliberate disturbance of salamander habitat, including substrate, aquatic vegetation, algae, and leaf litter or woody material from terrestrial vegetation, b. unauthorized, deliberate disturbance or alteration of flow regime, c. introduction of non-native flora or fauna into any salamander habitat or Barton Springs Pool, d. unauthorized SCUBA in salamander habitat or Barton Springs Pool.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: Upper Barton Spring continues to see disturbances from recreating park visitors who build rock dams and leave trash at the site. A new sign was installed in this area in 2016 to give notice that these practices are prohibited. During a weekday in August, over 200 disturbance events occurred from approximately 40 individual swimmers touching salamander habitat in front of the main spring outlets in Barton Springs pool. We are currently developing signage to reduce disturbance in front of the springs during high-use periods at Barton Springs Pool.
6.1.1.7 a. The City will clean salamander habitat as necessary to keep at least the upper 2-3 inches of habitat from becoming embedded with sediment. Easily observable or measurable characteristics of physical habitat (*e.g.*, embeddedness, sediment depth or percent sediment cover) will be used as benchmarks for determining when to clean. **b. All salamander habitats will be cleaned with the spring water of Barton Springs** at pressures not to exceed 30 lb/in^2 at the substrate and/or suspend rocks larger than 4 inches in diameter. Water for cleaning may be obtained by recirculation through submersible pumps, or other methods acceptable to the Service.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Salamander habitat around the spring outlets are surveyed on a quarterly basis and the upper layer of habitat is searched and flushed of sediment during surveys to reduce embeddedness. Sedimentation continues to be a problem at Old Mill Spring, where the water depth and slow velocities make it very difficult to alleviate the embeddedness without destroying all habitat. Therefore, we have been allowing mosses and other plants to re-establish themselves in the hope that this provides adequate cover in lieu of non-embedded rocky substrate on

	the bottom of the pool. The shallow areas along the sides continue to be flushed of sediment during surveys.
6.1.1.8 The City may remove woody debris from aquatic habitat if necessary by hand or any methods approved by the Service through verbal or written correspondence. All debris removed from salamander habitat will be visually inspected for salamanders and their prey before and after removal. Live salamanders will be noted and returned to the water. Live prey will be returned to the water as much as is feasible.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed No woody debris was removed from habitat during 2018.
6.1.1.9 Sediment, algae and debris disturbed or collected during routine cleaning of the Pool will not be disposed of in, allowed to settle in, or otherwise adversely affect aquatic habitat.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed A silt fence is installed within the pool to prevent excess sediment disturbed by cleaning activities from traveling into salamander habitat.
6.1.1.10 The City will minimize the detrimental impacts of withdrawal of spring water from Barton Springs Pool for irrigation and aquatic habitat cleaning by taking the following actions. The City will locate the intake for the pump inside Barton Springs Pool against the downstream dam but outside of habitat areas. The intake will be sufficiently baffled to reduce velocities and the likelihood of entrapment of salamanders on intake screens. Water withdrawn from Barton Springs Pool for irrigation will be used in a manner consistent with the other conservation measures of this plan, and irrigation water will not be allowed to runoff from the grounds back into the Pool. Withdrawal of water for irrigation will be limited to no more than 100 gallons/minute (0.2 ft^3/s) and no more than 6,006,000 gallons will be withdrawn annually. This amount is equivalent to 0.2% of the total annual discharge from Barton Springs calculated using the lowest ever recorded instantaneous discharge value of 9.6 ft^3/s applied for an entire year. Water withdrawn from Barton Springs Pool will be used for irrigation of only areas inside the fence surrounding Barton Springs Pool. The City will observe all watering restrictions applicable under City of Austin regulations when irrigating with water withdrawn from Barton Springs Pool.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed The intake pump for irrigation and pool cleaning was replaced in 2017, as the original pump had failed.
6.1.2.1 The City will reduce loadings of petroleum hydrocarbons, heavy metals and sediments to Barton Springs from current development and other activities located within the Barton Springs Zone in areas subject to the City's jurisdiction. This reduction	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed

in loadings will be achieved through the measures set out in the City's Stormwater Management Plan as required by the City's Texas Pollutant Discharge Elimination System (TPDES) storm water permit. The City's TPDES Stormwater Management Plan includes specific monitoring and protection measures for the Barton Springs Zone to protect the water quality of Barton Springs.	
6.1.2.2 The City will control local surface water runoff around Barton Springs Pool, Eliza Spring, Old Mill Spring, and Upper Barton Spring to the maximum extent practical. Runoff of storm water can carry sediment and potential pollutants directly into Barton Springs Pool and adjacent springs, which could adversely affect aquatic life. Stormwater may be diverted away from Barton Springs Pool or treated using structural best management practices prior to entering Barton Springs Pool. Runoff protection improvement projects will not have adverse effects on salamanders or their habitat. These controls do not include storm water runoff collecting in Barton Creek that causes basin-wide flooding that can inundate the springs.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.3.1 The City will restore and maintain more natural flow regimes in Barton Springs Pool, Eliza Spring, and Old Mill Spring by modifying, replacing or removing existing infrastructure. Restoration of free-flowing spring pools and overland streams at Eliza and Old Mill springs will improve and enlarge surface salamander habitat and improve habitat quality (see section 3.3.3). Restoration of a more natural flow regime in Barton Springs Pool by modification and/or replacement of dams, modification of the bypass culvert infrastructure, and suitable changes in management activities will improve aquatic habitat quality and ecosystem stability, as well as provide maximum operational flexibility. The City will develop plans for these restoration projects and, with concurrence of the Service, implement restoration. Flow regime improvements will not compromise water quality during baseflow.	☐ Full Compliance ☒ Partial Compliance ☐ Measure Completed Notes: See notes below for Eliza and Old Mill projects.

6.1.3.2 The City will allow floodwater to pass through Barton Springs Pool as unimpeded as is feasible to restore or maintain a more natural disturbance regime, which includes increased water velocities that inhibit excess settling of sediment and debris within the Pool confines. This will also reduce the need for dredging or other removal of accumulated flood debris from the Pool, thereby reducing potentially detrimental impacts of such projects on salamanders or their habitat. Some floodwater may continue to enter the bypass culvert and pass around the Pool. Prior to opening the gates in the downstream dam in preparation for potential flooding, Pool staff will confirm with City biologists that Eliza Spring is properly prepared according to the Drawdown Plan. In the event of a flash flood or potential flash flood, Pool staff will prepare the Pool grounds for flooding and coordinate with City salamander biologists in conducting flood-related drawdowns. The City may open dam gates for all floods according to procedures described in the Drawdown Plan.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Please refer to Table 3 below.
6.1.3.3 The City, with concurrence of the Service, will develop and implement a plan for routine silt and gravel removal from the deep channel of the Pool downstream of Parthenia Spring that does not compromise the continued survival of covered species. The Pool is bounded by upstream (southwest) and downstream (northeast) dams across Barton Creek. These dams cause accumulation of aquifer-borne silt as well as flood-borne silt and gravel within the Pool confines, altering flow regime and natural geomorphic processes. Removal of this material from the deep channel of the Pool has been and will continue to be necessary until the dams are modified, replaced, or removed. The plan will describe when the removal of material will occur and focus on vacuum dredging or other minimally invasive methods approved by the Service. The plan will be submitted to the Service within one year of the issuance of this permit and may be revised as necessary with the verbal or written approval of the Service.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: Plan was submitted to the Service at the one-year anniversary of issuance and is planned for execution for early March 2019.
6.1.3.4 The City will maintain a Drawdown Plan, which will provide standard operating procedures for use when Pool water elevation is drawn down. This plan requires the approval of the Service and will be submitted to the Service prior to issuance of this permit. The Drawdown Plan will be updated as needed with concurrence of the Service.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: A revised drawdown plan was submitted to the Service and approved July 16, 2016.

6.1.3.5 The City will not conduct a full drawdown of the water level in Barton Springs Pool if the combined discharge of the Barton Springs complex is less than 54 ft^3/s without consultation and verbal or written concurrence of the Service. This measure is intended to prevent dewatering of surface habitat of Eliza Spring. When discharge is equal to or greater than 54 ft^3/s, water can be maintained in surface habitat of Eliza Spring during a full drawdown, based on current substrate elevation. The 54 ft^3/s threshold can be revised with the verbal or written approval of the Service if habitat restoration or changes in substrate elevation allow maintenance of wetted surface habitat at lower discharges.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.3.6 Approval from a City Salamander Conservation Program salamander biologist is necessary before the water level in Barton Springs Pool may be drawn down under any flow conditions.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.3.7 When water level in Barton Springs Pool is drawn down for cleaning and maintenance, trained and permitted City salamander biologists and staff under their direct supervision will visually inspect all exposed habitat for stranded salamanders before cleaning and maintenance activities in those areas begin. Any stranded salamanders will be moved to permanent water. Water level in Eliza Spring will be inspected to ensure that water is retained in surface habitat of the spring pool.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: see comments below on results of drawdown searches.
6.1.3.8 A minimum of two City salamander biologists will be present when a full drawdown is conducted for cleaning and maintenance, and a minimum of one City salamander biologist will be present when a partial drawdown is conducted for cleaning and maintenance.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed

6.1.3.9 The City may conduct 4 full drawdowns per year exclusive of floods, when the combined Barton Springs complex discharge is at least 54 ft^3/s at the time of drawdown. Exposed habitat will be kept wetted with spring water or creek water while staff searches for stranded salamanders. The City will maintain water over the fissures area during drawdown for cleaning in order to minimize the stranding of salamanders. After the fissures area has been searched for stranded salamanders, the area may be allowed to dry and be cleaned.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.3.10 The City may conduct eight partial drawdowns per year exclusive of floods when the combined Barton Springs complex discharge is equal to or greater than 54 ft^3/s. If the discharge is less than 54 ft^3/s, partial drawdowns will only be conducted in consultation with the Service. The water depth over the beach will be maintained at greater than or equal to 12 inches and surface habitat in the adjacent perennial springs (Eliza and Old Mill) would not be allowed to go dry. This measure will minimize the impact of low aquifer levels at the adjacent perennial spring sites.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.4.1 Eliza Spring flow regime improvement will be implemented to the maximum extent feasible to recreate historical salamander habitat by restoring the surface outflow stream. Presently, the outflow from the spring is routed through an underground pipe into the Barton Springs Pool bypass culvert and ultimately into Barton Creek downstream of Barton Springs Pool; there is no surface stream. The underground pipe is proposed to be "daylighted" and a natural surface stream created in its place. The new stream will be protected salamander habitat and access will be restricted. To fully recreate a free-flowing spring-fed stream system, the natural elevation and composition of the substrate in the spring pool will be restored to the maximum extent feasible. This will eliminate hindrance of aquifer flow to surface habitat and provide wetted surface habitat during low aquifer discharge conditions and drawdowns without hindering outflow from the spring pool. A natural substrate will also provide abundant avenues for movement to and from subterranean habitat, reducing the potential for stranding salamanders during drawdowns. The current outflow pipe may be repaired as necessary until the stream is restored. All restoration activities will be submitted to the Service and receive verbal or written approval before implementation. The City	☒ Full Compliance ☐ Partial Compliance ☒ Measure Completed The project is complete.

will determine the feasibility of this restoration activity and submit an estimate of when construction activities may occur, if feasible, to the Service within 3 years of permit issuance.	
6.1.4.2 Old Mill Spring habitat restoration will be implemented to the maximum extent feasible to eliminate permanent, immovable obstructions and hindrances to free outflow from the spring pool to its stream. Infrastructure associated with the plugged outflow pipe on the Tier 1 stone wall (immediately surrounding the spring pool) will be removed within 3 years of permit issuance if feasible. The elevation of the outflow streambed may be lowered to ensure free water flow from the spring pool to its stream. A community of native aquatic vegetation will be established, which will help mitigate effects of low spring discharge by releasing oxygen into the water. Canopy cover vegetation will be maintained or increased to provide shade over the spring pool and stream, which will help mitigate increased surface water temperature during seasonal periods of high air temperature. Remaining stone walls of the amphitheater outside of aquatic salamander habitat and the supporting riparian habitat (Tiers 2 – 4) may be rehabilitated or stabilized as necessary to ensure safety in publicly accessible areas. Plans will be submitted to the Service and receive verbal or written approval before implementation.	☐ Full Compliance ☒ Partial Compliance ☐ Measure Completed Notes: Due to the complexity of this project, which involves historical structures and crosses several jurisdictional boundaries, and the timing of the Eliza stream restoration, this project is taking longer than originally anticipated. Additionally, the plan has expanded from the original scope, and includes removing a portion of the downstream wall around Old Mill to allow a wider outflow for spring water. COA is currently working on developing a scope of work for a preliminary engineering review to examine adjustments to the upstream dam in the pool, structural rehabilitation of the Eliza amphitheater, structural rehabilitation of the Old Mill historical walls, and Old Mill salamander habitat restoration.
6.1.4.3 The City will restore and permanently maintain groundwater flow and light penetration to the maximum extent feasible in salamander habitat of the fissures of Parthenia Spring. The City will not artificially obstruct groundwater flow or artificially inhibit light penetration in the fissures habitat area. Restoration will include permanent removal of concrete in the natural fissures transmitting groundwater to the surface in Parthenia Spring. Small areas of concrete may be removed gradually using underwater hand tools. Large areas may be removed at one time during drawdown, which would allow use of larger construction tools and foster retreat of salamanders from work area. Removal methods will be chosen to minimize harassment of resident salamanders and subject to verbal or written approval of the Service	☐ Full Compliance ☒ Non-Compliance ☐ Measure Completed ☒ Measure Needs Amendment Notes: City biologists have examined the concrete obstructions in the pool bottom and have concluded that viable habitat could not be created in these areas. Spring water does not appear to issue from these fissures and they are surrounded by unfractured bedrock without cover for salamanders. Most salamanders observed occur near the spring outlets and use gravel and cobble for cover. Because this area receives high velocities during floods, any cover added would be washed away. Therefore, COA believes it is not beneficial to proceed with removing concrete from these fissures.

6.1.5.1 The City may move salamanders among spring sites or release salamanders born in captivity according to a Service-approved plan to maintain genetic diversity of the species. The four spring sites do not harbor genetically unique populations based on current genetic information. Transfer of individuals between sites will not adversely affect the genetic integrity of those populations and will maintain the genetic integrity of the species.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: This has not been implemented yet. The City continues to pursue necessary scientific investigations to facilitate development of a plan for submission to the Service for approval.
6.1.6.1 The City may manually trim and remove aquatic vegetation (macrophytes, bryophytes and algae) as necessary. Vegetation management will not adversely affect habitat or compromise ecosystem health. Only City biologists listed under current federal Endangered Species Act 10(a)(1)(A) and state scientific permits are authorized to manage vegetation in salamander habitat areas.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed The City did not remove any vegetation from habitat, although several were planted in Barton Springs Pool outside of salamander habitat.
6.1.6.2 Specific areas will be designated for the fueling and maintenance of equipment and vehicles used in maintaining the springs and surrounding areas. Fueling and maintenance areas will be at least 25 feet away from the water to avoid the chance of detrimental impacts on the spring habitats or aquatic life. Absorbent pads will be used underneath or around all equipment, supplies, and vehicles containing toxic components during all operations, fueling and maintenance activities.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.6.3 The City will clean the shallow end of Barton Springs Pool without full drawdown of water level in the entire Pool. Adjustable gates in dams or similar water control devices may be used to conduct partial drawdowns that expose only the shallow end for cleaning.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.6.4 The City will use spring water for cleaning in Barton Springs Pool to the maximum extent feasible. The City will install an electrically powered pump system that provides spring water from Barton Springs Pool for cleaning of the Pool. The pump system may also be used to provide spring water for the fissures areas during Pool drawdown.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.1.6.5 The City will prohibit use of toxic chemicals for cleaning of the Pool.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed

6.1.7.1 The City will monitor salamander populations and habitat. Salamander population surveys will be conducted at perennial Parthenia, Eliza, and Old Mill springs and at intermittent Upper Barton Spring when flowing at least bimonthly throughout the year or other interval sufficient to determine the status of the species and population dynamics as deemed appropriate by a City salamander biologist and approved by the Service. The City will develop and maintain a written monitoring plan. The City will ensure that all people surveying for salamanders are properly trained. Surveys can include methods to elucidate life history characteristics of both species. Methods will be evaluated by the Service and conducted under the terms and conditions of a valid federal Endangered Species Act 10(a)(1)(A) scientific permit issued to the City.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: Monitoring plans were emailed to the Service February 25, 2016. The City continues to survey all four spring sites on a quarterly basis, capturing as many salamanders as possible, and photographing them for individual identification. In 2018, the City ceased attempting to photograph individuals from Barton Springs Pool during dive surveys; these surveys seemed to have a higher mortality rate than other surveys and result in more individuals with gas bubble trauma.
6.1.7.2 Eliza Spring and Old Mill Spring will be used as outdoor educational facilities for the study of the biology and ecology of Central Texas springs.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed School groups regularly visit Eliza Spring as part of the City's ongoing education programs.
6.1.7.3 The City will ensure that Barton Springs Pool lifeguards and maintenance staff including seasonal employees are knowledgeable about the protected salamander species. At a minimum, staff will be trained yearly about the protected salamanders, resident aquatic wildlife and flora and the ecology of Edwards Aquifer springs. Training will include contaminant spill and response protocols, proper containment techniques, and remediation. An inventory of necessary containment and remediation equipment will be conducted by Pool staff annually and after the use of equipment in response to any spill. City Parks and Recreation Department Aquatics supervisors will direct and document all cleaning procedures at the Pool.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Training of life guard temporary staff was completed in July.
6.1.7.4 The City will ensure that all people conducting salamander and habitat monitoring are properly trained. All monitoring and surveys will be conducted under the terms and conditions of a current federal Endangered Species Act 10(a)(1)(A) scientific permit issued to the City of Austin.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed

6.1.7.5 The City of Austin will form the Barton Springs Scientific Advisory Committee, which will include local and regional experts. The committee may be divided into subcommittees that focus on specific areas of expertise and will meet at least annually to discuss and refine Barton Springs' maintenance and environmental management activities. A variety of interests including swimming, biology, hydrogeology, and captive breeding may be represented on this committee. In addition, this committee will periodically review this Plan and make suggestions for needed amendments as deemed necessary. The Advisory Committee will also be responsible for helping identify potential revisions to the Plan and suggest adaptive management strategies. The City will be responsible for implementation of adaptive management strategies with verbal or written approval of the Service.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed The third annual meeting occurred 9/10/2018; the meeting notes are included with this report.
6.2.1 Access to Eliza Spring and Old Mill Spring will be restricted to ensure no unauthorized disturbance of salamander habitat and/or its supporting riparian habitat. Unsupervised access to these sites is limited to individuals holding valid federal Endangered Species Act 10(a)(1)(A) and state scientific permits. Recreational access to Barton Springs Pool will continue to be permitted. Public access to Upper Barton Spring is not prohibited. Upper Barton Spring lies within the Barton Creek Greenbelt, and because of its location within the floodplain of Barton Creek it cannot be feasibly isolated from public access.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.2.2 The City will maintain a plan and necessary equipment and training for responding to, and mitigating the effects of catastrophic contaminant spills that threaten protected salamanders or their habitat. Should a catastrophic spill threaten to extirpate *E. sosorum* or *E. waterlooensis* in the wild, the City may conduct a full or partial drawdown as necessary to rescue salamanders. The City will notify the Service in the event of a catastrophic spill. Trained and permitted City staff will search all exposed habitat area for salamanders.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed

6.2.3 The City will maintain viable, evolutionarily fit captive breeding populations of *Eurycea sosorum* and *Eurycea waterlooensis*. The City will designate a staff biologist and dedicate a minimum of \$28,000 annually to the development and maintenance of this program. This program may provide captive salamanders suitable for reintroduction into the wild if catastrophic events that compromise or cause extirpation of wild populations were to occur. This program may provide a refugium facility for salamanders collected in response to contaminant spills or other immediate threat that could cause extirpation of the species in the wild. The program will develop and maintain a captive population of each species that represents the genetic diversity of wild populations without compromising their size or fate by permanently removing individuals from the wild. This program is also intended to support research that contributes to elucidation of biology, life history and natural history of both species. The City will develop and maintain written plans for population management, reintroduction, and husbandry. These plans will be updated as necessary.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.2.4 Under conditions when decreased dissolved oxygen concentrations may be harmful to salamanders, the City may supplement dissolved oxygen in Eliza, Old Mill, and Parthenia springs using air pumps, water recirculation, or other method approved by the Service.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: This was not necessary during 2018.
6.3.1 The City of Austin will set up a fund for conservation and research efforts for *Eurycea sosorum* and *E. waterlooensis*. The City will deposit \$53,000 annually (for the term of the permit) into this fund from the revenues generated by Barton Springs Pool. This fund will also be open to donations from any group or private individual. A committee of technical representatives will determine the allocation of money from this fund. At a minimum, the committee will consist of one technical representative from the City and one technical representative from the Service. These technical representatives must be knowledgeable and experienced in salamander biology. Other committee members could include state, county, university representative or other qualified biologists and karst aquifer hydrogeologists, and swimmer/stakeholder representatives. The City and the Service would both retain "veto" power in deciding how the money is allocated. The funds would be used for study of salamander biology, captive breeding, refugium	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed With approval from the Service, the City is now directly managing the conservation fund and has solicited applications both locally and nationally. Applications are due January 30th and will be provided to the Service upon receipt.

development, reintroduction, watershed related research, improved cleaning techniques for natural water bodies, education and/or land acquisition.	
6.3.2 The City will continue to support research projects designed to gather and evaluate data applicable to wild or captive populations of the Barton Springs Salamander, *E. sosorum*, and the Austin Blind Salamander, *E. waterlooensis*. These projects would be in addition to the regular monitoring already conducted under the permit and would be approved by the Service when applicable.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed
6.3.3 The City will continue to provide educational programs to enhance public awareness and community support for *Eurycea sosorum*, *Eurycea waterlooensis*, Barton Springs, and the Edwards Aquifer. The SPLASH! Into the Edwards Aquifer Exhibit at Barton Springs Pool will continue to be a major focus of this effort. The mission of the SPLASH! Exhibit is to foster stewardship of the Barton Springs Segment of the Edwards Aquifer and Barton Springs through public education. The City of Austin Parks and Recreation Department will dedicate a minimum of $10,000 annually from the revenues generated by Barton Springs Pool to the development and maintenance of this exhibit. The City of Austin Watershed Protection Department will make available at least $35,000 annually for the support of exhibits and events, and maintaining museum operating hours at the SPLASH exhibit. Outdoor educational displays will emphasize the biology and ecology of Barton Springs and the Edwards Aquifer with an emphasis on the Barton Springs Salamander, *Eurycea sosorum*, and the Austin Blind Salamander, *Eurycea waterlooensis*.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Splash statistics (fiscal year 2018): 68,287 visitors 1,810 Public program participants 1,707 Special Event participants
6.3.4 The City will cooperatively develop a memorandum of understanding with the Barton Springs Edwards Aquifer Conservation District to formalize collaborative efforts to protect the Barton Springs Salamander, *Eurycea sosorum*, the Austin Blind Salamander, *Eurycea waterlooensis,* and the Barton Springs Segment of the Edwards Aquifer. The memorandum of understanding will be adopted by the City within one year of permit issuance.	☐ Full Compliance ☒ Partial Compliance ☐ Measure Completed Notes: The City and the District have finalized a draft MOU document, which is currently being reviewed by legal counsel.

6.3.5 The City will participate in regional water resource planning that may affect the Barton Springs Segment of the Edwards Aquifer and advocate for protection of water quality and quantity adequate to protect the Barton Springs Salamander, *Eurycea sosorum*, and the Austin Blind Salamander, *Eurycea waterlooensis*.	☒ Full Compliance ☐ Partial Compliance ☐ Measure Completed Notes: The City continues to participate in a wide variety of regional water quality protection initiatives including regular meetings with the Barton Springs Zone Regional Water Quality Protection Plan working group. In this reporting period, Austin continued to actively engage with the Texas Commission on Environmental Quality to protest the insufficiency of the City of Dripping Springs proposed wastewater discharge to Onion Creek. Austin continued evaluation, inspection and collaboration regional partners regarding Texas Department of Transportation projects that may impact the Barton Springs Segment of the Edwards Aquifer. Austin continued working with local groundwater districts to perform dye tracing to better understand the complex interaction of surface and groundwater in the Trinity Aquifer in the contributing zone of the Barton Springs Edwards Aquifer. Austin participated with regional non-governmental entities in discussions leading to the development of a new Hill Country Conservation Network dedicated to public outreach, land acquisition, spring flow protection, and water quality management for the greater Edwards Aquifer region. Austin collaborated with the Hill Country Alliance and the Barton Springs Edwards Aquifer Conservation District to begin planning for the 2018 Kent Butler Memorial Summit on regional water planning issues, scheduled for April 4, 2018.

For attachments, please go to: https://austintexas.box.com/s/9uqxtjs7ssivd30iexihbdxjoj6c0wax

Incidental Take

Table 2. Salamanders collected from the wild (N=41 *E. sosorum*, N=2 *E. waterlooensis*). Salvaged individuals were killed or injured during surveys, or otherwise found dead. Individuals that were collected alive were done so to serve as voucher specimens. All collected individuals have been deposited in the Biodiversity Collections at the University of Texas at Austin (TNHC) except for hatchlings and small juveniles that are being used to examine gut contents (marked NA).

Museum No.	Species	County	Locality	Date	Notes
NA	*sosorum*	Travis	Eliza Spring	2/12/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/12/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/12/18	Injured during survey; salvaged for gut contents
NA	sosorum	Travis	Eliza Spring	2/12/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/12/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/12/18	<1" TL, collected with injuries, recovering in captivity
NA	*sosorum*	Travis	Eliza Spring	2/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/14/18	Injured during survey; salvaged for gut contents
NA	*waterlooensis*	Travis	Eliza Spring	2/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/14/18	<1" TL, collected with injuries
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	2/16/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Parthenia Spring	2/26/18	1-2" total length, found dead during drawdown
TNHC 108522	*tonkawae*	Travis	SAS Canyon	3/1/18	collected for voucher/genetics
NA	*sosorum*	Travis	Eliza Spring	5/8/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/8/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/8/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/8/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*waterlooensis*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	5/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Old Mill Spring	5/23/18	accidental collection in invertebrate collection
NA	*sosorum*	Travis	Eliza Spring	8/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/14/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/17/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/17/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/17/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/17/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	8/20/18	Injured during survey; salvaged for gut contents
NA	*sosorum*	Travis	Eliza Spring	11/5/18	Injured during survey; salvaged for gut contents

Table 3. Drawdown and flood event details for Barton Springs Pool and number of observed salamanders.

Date	Full or Partial Drawdown	Purpose	Total Barton Springs Discharge prior to drawdown (cubic feet/second)	Salamanders observed
2/26/2018	Partial	Pool cleaning	52	0
5/4/2018	Full	Flood	64	Not searched
9/23/2018	Full	Flood	60	Not searched
11/15/2018	Full	Pool cleaning	95	1; returned to water unharmed
12/27/2018	Full	Flood	99	Not searched

Salamander Status and Biological Data Analysis

In this section we report counts of *E. sosorum* and *E. waterlooensis* from salamander surveys, present abundance estimates for *E. sosorum* at Eliza Spring, as well as data on size distribution and gravidity in *E. sosorum*.

Table 4. Barton Springs and Austin Blind salamander counts from 2018.

Date	Site	Number *E. sosorum*	Number *E. waterlooensis*
2/9/2018	Old Mill Spring	8	0
2/9/2018	Upper Barton Spring	5	0
2/12/2018	Eliza Spring	418	5
2/14/2018	Eliza Spring	498	8
2/16/2018	Eliza Spring	452	10
2/26/2018	Barton Springs	98	0
5/8/2018	Eliza Spring	365	1
5/11/2018	Eliza Spring	308	3
5/14/2018	Eliza Spring	313	4
5/17/2018	Barton Springs	211	0
5/23/2018	Old Mill Spring	9	0
5/23/2018	Upper Barton Spring	5	0
8/9/2018	Old Mill Spring	5	0
8/14/2018	Eliza Spring	427	0
8/17/2018	Eliza Spring	336	0
8/20/2018	Eliza Spring	312	0
8/21/2018	Upper Barton Spring	0 (no flow)	0 (no flow)
8/23/2018	Barton Springs Pool	165	0
10/30/2018	Eliza Spring	43	0
11/2/2018	Eliza Spring	38	0
11/5/2018	Eliza Spring	42	1
11/7/2018	Old Mill Spring	3	0
11/7/2018	Upper Barton Spring	6	0
11/15/2018	Barton Springs	9	0

In 2018 we observed some of the largest abundances of Barton Springs salamanders of the past 10 years (Tables 4 & 5). This population boom was observed at all sites, with the exception of Upper Barton Spring, indicating the likelihood of a common driver, such as beneficial aquifer conditions. We note that this ‘bonanza’ followed a declining discharge pattern, and that abundance was markedly lower following a spike in discharge from early fall rains.

Even though counts remained low at Upper Barton Spring and Old Mill through 2018, the nine Barton Springs salamanders observed at Old Mill Spring were the most seen since the population boom of 2008, where abundances reached over 100, but quickly dropped down into the single digits during the drought of the same year. We observed the most salamanders at Eliza Spring during the first three quarters of the year, due in part to a large influx of juveniles early in the year, as indicated by histograms of body size (Figure 1).

After observing a large mosquitofish (*Gambusia affinis*) attempting to consume a juvenile salamander, we seined mosquitofish out of Eliza Spring and examined their gut contents. After dissecting 45 *Gambusia affinis* collected at Eliza Spring on 3/5/18, there were no Barton Springs Salamanders (*Eurycea sosorum)* found in the gut contents of the fish. The most common gut content was ant alates that likely fell on the surface of the water during mating flights. Adult and larval water penny beetles, trichoptera adults, adult and larval black flies, adult and larval chironomids, adult dytiscid beetles, amphipods, *Rhagovelia*, adult springtails, spiders and a single water mite were also found (listed in order of most to least prevalent). Invertebrates found in the gut of the *Gambusia* are predominately located at the surface or are exclusively terrestrial.

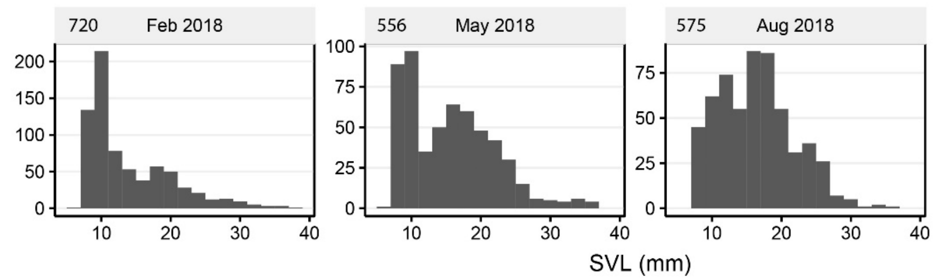


Figure 1. Size histograms of Barton Springs salamanders captured at Eliza Spring from Feb. thru Aug., 2018.

Table 5. Estimates of abundance ($\widehat{N}$) and standard deviation (SD) for four capture-recapture surveys at Eliza Spring in 2018.

Period	$\widehat{N}$	SD
Feb-18	960	27
May-18	874	34
Aug-18	766	23
Nov-18	205	33

We have continued to photograph salamanders at Old Mill and Upper Barton springs, although recapture rates at these sites are very low, precluding any formal statistical modeling. As our photographic database increases in size, we cross-checked photos between sites for evidence of migration and have yet to find any.

In general, our knowledge of *E. waterlooensis* ecology continues to be constrained by it being a primarily subterranean-only dwelling organism. Occasional “accidentals” occur at the surface, and in the past juveniles had been observed in abundance at Old Mill Spring. Unfortunately, a large abundance of juveniles no longer occurs at Old Mill and occurrence of *E. waterlooensis* at the surface in general remains a sporadic event (Table 4).

Using the available gravidity data from each site, we found that gravidity is not strongly seasonal (Figure 2). This is consistent with prior observations of juvenile counts suggesting that reproduction is generally non-seasonal, which is in contrast to *E. tonkawae*. Similar to *E. tonkawae*, we found that the probability of finding a gravid female increases with size (Figure 3).

Additional analyses on *E. sosorum* growth and population demographics are forthcoming, and will be detailed in separate reports.

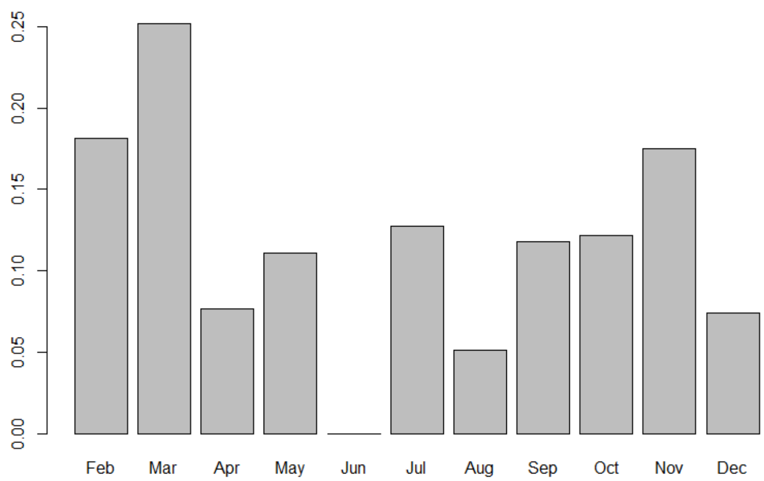


Figure 2. Proportion of gravid Barton Springs salamanders by month (excluding juveniles) from 2014 through 2018 across all spring sites. Note that survey effort was not equal across months.

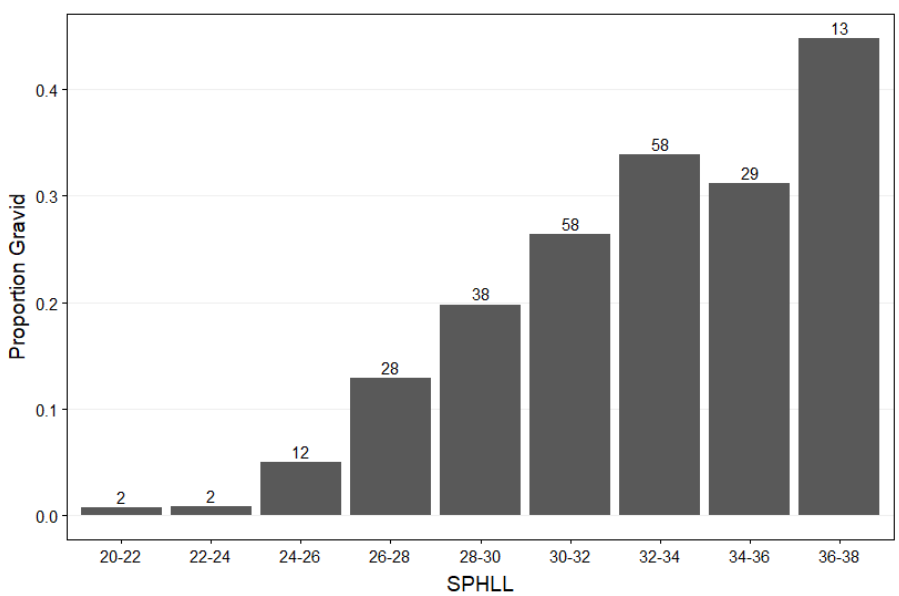


Figure 3. Proportion gravid vs. body size (mm) (snout to posterior hind limb length; SPHLL) for Barton Springs salamanders.

Management Activities: Adaptive Management

Adaptive management (AM) is a framework in which management decisions are made following a scientific approach to decision making. The term “adaptive management” is used in many ways. The steps for AM put forth in the HCP (Dries et al., 2013) are as follows: 1) Consider various actions to meet management objectives; 2) Predict the outcomes of these management actions based on what is currently known; 3) Implement management actions; 4) Monitor to observe the results of those actions; 5) Use the results to update knowledge and adjust future management actions accordingly. Whether AM is appropriate for a specific conservation problem may depend on six key conditions : 1) A real management choice is to be made; 2) There is an opportunity to apply learning; 3) Clear and measurable management objectives can be identified; 4) The value of decision making is high; 5) Uncertainty can be expressed as a set of testable models; 6) A monitoring system can be established to reduce uncertainty (USFWS 2018). Upon further review of AM in the HCP, several of the actions and hypotheses listed either do not meet the AM framework, or do not make sense based on existing management practices. Below, we summarize our review of the questions and hypotheses posed in the AM section of the HCP (Dries et al., 2013 pp 120–132) for each topic based on the above guidelines. Pages numbers cited below refer to relevant sections of the HCP.

6.5.1.1 Drawdowns

The ITP covers drawdowns for cleaning and flood management of Barton Springs Pool (BSP), although drawdowns are not part of habitat management (City of Austin, 2018) or conservation measures (pages 109–120) meant to improve salamander habitat. Therefore, it is not appropriate to evaluate the effectiveness of the drawdowns as a means of improving salamander habitat, as stated on line 3666–3668 (page 121) of the HCP, because this does not constitute a management choice. The questions listed under “Hypotheses” may be better evaluated in the context of whether drawdowns harm salamanders and their habitat, or not.

6.5.1.1.1. Cleaning Drawdowns

H1 asks “Is there a difference in observed take of protected salamanders before and after implementation of new drawdown regime?” for which analysis is supposed to occur annually. This has not been done annually because the drawdown regime has not been changed from what was originally proposed in the HCP. If the drawdown regime changes in the future, it would be hard to know whether or not changes in a drawdown regime would reduce take after only a single year of data are collected. Take may vary as a function of salamander abundance at the surface, which may vary dramatically within a year (Bendik & Dries, 2018; Dries & Colucci, 2018). Similarly, H4 asks whether a difference in abundance results from implementation of a new drawdown regime. Because of the wide variation in salamander abundance from month to month, it would be very difficult to separate the effects of a drawdown with population changes, particularly given the very low frequency of drawdowns.

Based on data reported in the HCP and HCP annual reports since 2003, most drawdowns result in 4 or fewer stranded salamanders, most of which are released unharmed. A single drawdown on 2/26/2016 resulted in 9 stranded salamanders (released unharmed) at Eliza Spring, when the water level was elevated and we were unable to control the water level. With the addition of an adjustable gate at Eliza Spring as part of the Eliza Daylighting project, we are now able to control the water level to prevent salamander strandings completely at this site. Additionally, more than half of all drawdowns at either site do not result in stranded salamanders.

H2 and H3 refer to sedimentation rates. We have not examined the effects of drawdowns on sedimentation because a new drawdown regime has not been implemented with which to compare differences in sedimentation.

The criteria for evaluating success of management states "The plan will include analysis of hypothesis 1 annually and analysis of hypotheses 2 – 4 every 5 years. Statistically significant changes in sediment cover and depth, and rate of accumulation or change in observed Take warrant consideration of a change in habitat management." New drawdown regimes for cleaning have not been implemented or proposed and are not required to be implemented as part of this ITP and HCP. Given the low mortality and low incidence of salamander strandings observed, it is our opinion that the current drawdown regime does not cause significant harm to the salamander population, and therefore a new drawdown regime does not need to be proposed or evaluated.

6.5.1.1.2. Flood Drawdowns

Floods are defined as water flowing over the top of the upstream dam at BSP. The primary purpose of flood drawdowns is to reduce sediment deposition within the deep end of the pool, outside of salamander habitat. High sediment loads must be removed periodically by pool staff to maintain an appropriate depth for swimmers.

Drawdowns may also reduce sediment deposition in salamander habitat in front of the springs, but it is difficult to distinguish between sediment deposited from the flood and sediment introduced from the aquifer. Aquifer storm flow coincides with floods overtopping the dam and storm flow increases the sediment load coming out of the springs (Mahler & Lynch, 1999). The questions posed in this section ask whether there are differences in debris, sediment and take when gates are open. Unfortunately, these questions are impractical to evaluate because the pool needs to be drawn down for cleaning after floods because of the sediment and debris, preventing a comparison to keeping the gates closed after a flood ends.

However, we have adjusted our response to small floods when a drawdown may not be warranted. Small floods bring relatively little sediment into the pool, negating the potential benefits of a drawdown, while increasing the probability of stranding salamanders. Therefore, we have implemented a plan to eliminate drawdowns after small floods (< 750 ft^3/s at the upstream USGS gage), particularly those that are artificially caused by clogging of the Barton Creek bypass tunnel grate.

6.5.1.1.3 Drawdown Discharge Threshold

The proposed threshold for conducting drawdowns (54 ft^3/s) is intended to ensure that water does not recede from surface habitat in the Eliza spring pool. The Eliza stream daylighting project included the installation of an adjustable gate, allowing the City to adjust the level of water within the stream and pool in response to high and low flow conditions. Currently the concrete floor remains in place at Eliza Spring, and it may not be advisable to remove it given that Eliza Spring consistently contains the largest population of salamanders (see attached memo). With or without the concrete floor in Eliza Spring, there is little benefit to performing a series of experimental drawdowns, as suggested here. While we may gain information, full drawdowns during a drought (for example) would increase, rather than decrease, the chances of stranding salamanders, while providing no benefit to salamanders. Therefore, we do not recommend keeping experimental drawdowns as a part of an adaptive management program. However, we can report our observations during non-experimental drawdowns. During a partial drawdown on 2/26/2018, BS discharge was near 52 ft^3/s, and we did not encounter any problems keeping water in Eliza

Spring and stream with the newly installed adjustable gate. No salamanders were found stranded at Eliza or within BSP during this event.

6.5.1.2 Flood Debris Dredging

No dredging has occurred under the renewed permit yet, but it is planned for early March, 2019. Because this is not a regularly occurring event, and occurs far outside of salamander habitat, it is not a problem suitable for an adaptive management approach, although the dredging will be monitored by biologists.

6.5.1.3 Catastrophic Spill Response

Spill response events occur at irregular intervals, are unpredictable, but offer an opportunity to learn. However, it is difficult to see how the uncertainty of these spill events and our response to them can be expressed as a set of testable models or what the clear and measurable management objectives would be. This is partly due to the extremely high level of uncertainty associated with spills- uncertainty in their timing, duration, composition and toxicity. However, we can address specific questions related to spills to better inform our currently established response plan.

This year we identified one area of uncertainty related to the toxicity of chlorine from a treated water spill- one of the most frequent types of spills that occur in the Austin area. Our spill response thresholds are based on toxicity to neotenic *Eurycea* of a simple chemistry consisting of only free chlorine, while treated water consists of a monochloramine-based chemistry, necessitating a re-evaluation of the toxicity thresholds for neotenic *Eurycea*. We have included evaluation of chloramines and their byproducts as a high priority for funding through the Barton Springs Salamander Conservation Fund.

Here, we summarize spill events from 2018 and our corresponding response.

1) On March 26, 2018, 500 gallons (estimated) of diesel fuel spilled at Barton Creek Mall from a generator at the mall. The spill was contained in a storm drain pipe that led to a water quality pond and was subsequently cleaned up that night before any rain occurred. Barton Springs discharge was 45 ft^3/s, indicating a possible Tier 2 or Tier 3 response could have been necessary if a heavy rain had occurred and washed the petroleum out of the stormwater controls. In preparation, we filled the captive breeding back-up tank with well water (in case the well became polluted) and prepared temporary holding tanks to hold rescued salamanders. A rescue was not necessary because the spill was cleaned up, although staff were prepared to follow through.
2) On July 29, 2018, a 2” treated water main broke in the Barton Hills neighborhood, prompting a response by the WPD spills team to determine if the level of predicted chlorine would pose a potential threat to salamanders at the springs. Predicted levels of total chlorine based on modeling the expected decay rate and flow time to the springs indicated that a salamander rescue was not warranted. Later, we obtained hits of total chlorine at the springs using field test kits, and this was later confirmed by taking a sample to Austin Water Utility’s laboratory and performing a titration. Measurements of total chlorine at the springs were not high enough to indicate that free chlorine would pose a threat to salamanders.
3) Between December 18th and 20th, City of Austin staff observed and investigated three separate, discrete discharges of sediment to Barton Springs Pool, directly from the Edwards Aquifer. The discharges caused unusual turbidity, but no acute, adverse impacts to the aquatic life of the pool, nor long-term harm to the water quality of Barton Springs Pool. City of Austin staff worked with the Barton Springs Edwards Aquifer Conservation District (BSEACD) to identify the source of sediment as geothermal/heat pump well drilling activity that was permitted by the District. The wells are located in the confined zone just east of the recharge zone, about a mile from the

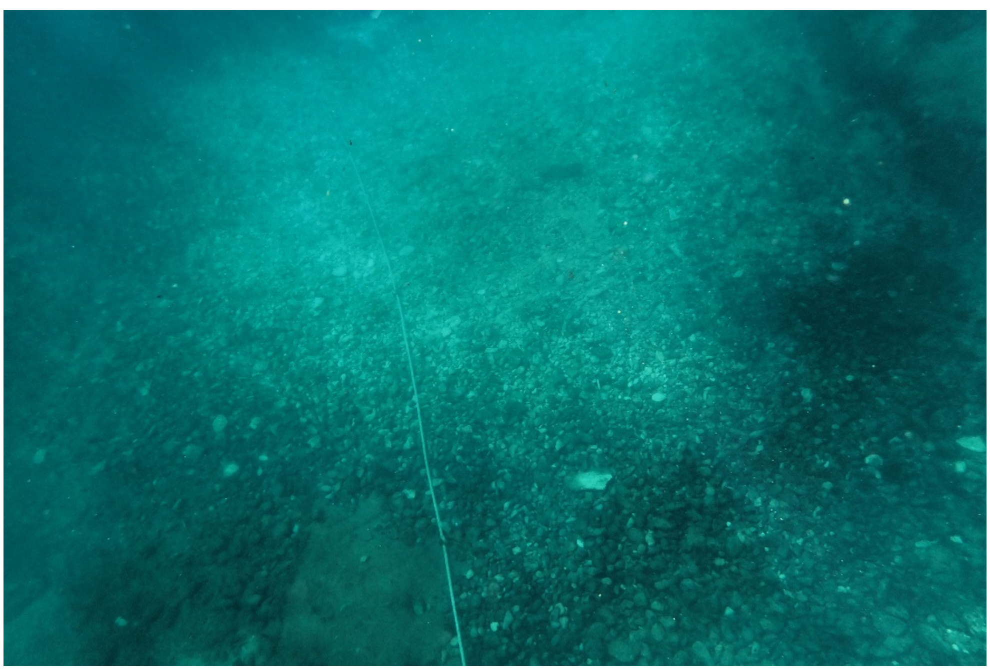
springs, and the timing of the well-drilling activity coincided with the appearance of the turbidity at the springs and the known travel time of water in the aquifer. The City of Austin issued a citation under Austin City Code Title 6, Chapter 5 for a pollutant discharge to a waterway. The wells were completed and closed on Friday, December 28th, 2018, under supervision from BSEACD. We do not anticipate any further discharges from this activity. The City and the District are working together to develop criteria to address these types of wells in the future, or otherwise avoid impacts to conduits to the springs. This is a new phenomenon, so the impact was not anticipated. Now that it has happened once, the two entities can adjust our respective protocol to ensure more preventive practices and increased inspection and monitoring for this type of activity.

6.5.2 Recreation

An adaptive management process is not prescribed in this section (p 125). Below, we comment on the typical types of disturbance observed due to recreation and some observations from this year.

Swimmers occasionally are observed disturbing salamander habitat in front of the spring outlets, particularly Side Spring. Pool staff have been notified of the patterns of disturbance and they notify patrons that disturbance of habitat is prohibited, however this has not been enough to eliminate the disturbance.

Staff performed a single disturbance survey in front of Parthenia spring during a busy Wednesday afternoon from 1–4pm on 8/15/2018. 38 different people were observed diving below the surface of the water in this area, of which 29 pushed off of the bottom disturbing the rocks where salamanders live. Additionally, 14 of these people were observed disturbing the rocks with their hands. In total, 207 instances of habitat disturbance were recorded during this short period. The photo below shows the type of disturbance that occurs from these activities- the rocks are disturbed so much that the algae is scraped off and/or the rocks are overturned. This happens to be the area with the highest densities of salamanders within Barton Springs Pool. We have not directly observed any salamander mortalities as a result, although that would be difficult to do unless we had a way to constantly monitor the area.



Our current plan is to increase the signage to educate swimmers on the sensitivity of salamander habitat, possibly installing signs below water and floating in the vicinity of where the disturbance occurs.

We have, since 2013, observed several dead salamanders at Upper Barton Spring, and have noted frequent disturbance at this site. A permanent sign was installed here, which seems to have abated some of the disturbance, although park patrons still build dams within the habitat and wade here. Police and park rangers are aware this is a problem, but the parks are large and it is difficult to consistently enforce the rules here. Staff continue to check the site several times a week to remove dams and report habitat disturbance to park rangers or the police, if warranted.

6.5.3 Habitat Restoration

A project is underway to evaluate the colonization of invertebrates and salamanders in the newly constructed Eliza stream. A copy of this plan is attached. The opportunities for reevaluation for AM are limited because few elements of the project can be changed, although one aspect we are in the process of planning is a change to the habitat substrate in the Eliza stream. The substrate installed in the stream is large to help reduce velocities at the substrate, but the interstitial spaces are large and inhabited by crayfish rather than salamanders. We will be looking into adding more gravel substrate to provide better microhabitat for salamanders in the downstream sections of the stream. How the change in substrate influences salamanders and invertebrates will be evaluated according to our sampling plan. We anticipate the salamander colonization will increase once more suitable substrate is placed.

6.5.3.1 Modification of Dams

Dams have not been modified, although we are in the early stages of a project to add adjustable gates to the upstream dam at BSP to allow inflow from Barton Creek (including Upper Barton Spring). The gates are anticipated to be open when Barton Creek is flowing and water quality is high. This will offer an opportunity to examine whether the change in flow regime has any impact on salamander habitat or water quality within the pool.

6.5.4 Wild Population Monitoring

One of the key aspects of habitat management at Barton Springs involves sediment abatement. The rate of sediment deposition varies naturally according to hydrologic conditions. Typically, sediment load is higher during and after large storm events. Where water velocity is low, fine sediments readily accumulate, resulting in a lack of interstitial space habitat for salamanders. Salamanders are infrequently observed in areas with high embedded cover. At Parthenia and Eliza spring, sediment abatement involves disturbance and loosening of gravel and cobble sediments during surveys, resulting in the maintenance of available cover as interstitial spaces between gravel and cobble sized rocks. Prior to the construction of the Eliza stream, sediment management also involved occasional flushing with low-pressure garden hoses. This was necessary because sediment deposition in the Eliza pool was periodically exacerbated during high flow conditions due to water backing up from the outflow pipe, resulting in deeper conditions than were ideal.

A natural question is whether the effect of sedimentation (as managed) has a negative influence on salamanders. To answer this question, we modeled monthly counts of Barton Springs salamanders from 2004–2014 in three different size classes (<1”, 1–2”, and >2”) to compare the effect of percent sediment cover (visually estimated) along with other environmental and demographic drivers of population abundance using multivariate autoregressive state-space models. We found that percent sediment cover had a negative association with abundance of all size classes, and this effect was larger than any effects of algae cover, seasonality, or discharge. The analyses and results are summarized in Bendik & Dries (2018). Thus, while monthly removal of sedimentation does not appear to have eliminated its effects on salamander abundance, it is reasonable to conclude that efforts to remove excess sediment should continue.

Since our permit was renewed, we implemented several changes to our habitat management and monitoring. Since 2014, we have reduced the frequency of our surveys from monthly to quarterly, thereby also reducing the frequency with which we remove sediment by hand. We have also started collecting capture-recapture data at all sites except Parthenia. At Eliza Spring, the new stream and sluice gate (September 2017) allow us to much more easily control the depth of the water, particularly during periods of high flow. This potentially reduces the amount of sediment deposition during floods, when the water level in Eliza can be kept at a much lower level than was previously possible.

We have also changed the way we quantify sedimentation. We now take photographs of 10 quadrats longitudinally from downstream to upstream, roughly evenly spaced, at each site. We then overlay a digital grid to quantify the percent embeddedness (gravel or cobble > 50% covered in fine sediments [clay, silt, and sand]) and other quantities of interest. Therefore, we will be able to more accurately quantify changes in sedimentation within each site, and continue to evaluate the relationship between abundance and sediment in relation to management and monitoring. Contingent upon these results we will evaluate whether sediment management needs to be adjusted.

Regarding the current adaptive management question in the HCP, "Changes in sample size proposed in this Plan result in no reduction in statistical power for population growth, salamander abundance and recruitment, and habitat quality analyses," we believe this hypothesis is overly simplistic for use in adaptive management. Statistical power is a function of sample size and effect size, and is a useful concept for null hypothesis testing and study planning. However, in monitoring animal populations, null hypothesis testing is of limited value, as we are typically concerned with quantifying demographic parameters and evaluating their relationship with extrinsic factors, i.e., by quantifying effect sizes and comparing different models using multimodel inference. The sampling program should have specific aims that link to an adaptive management framework (e.g., sediment management, as outlined above).

6.5.4 Ecosystem Resilience

The Eliza Stream Daylighting project incorporated salamander habitat requirements within the constraints of existing infrastructure and construction limits. The project aimed to create a shallow, wide stream, that would be resilient to its location in the 100-year floodplain. Engineering the stream within these constrains prevents natural changes to the stream path over time. Velocity in the channel is also higher in the keyway than is optimal because the spring flow is constrained by the historical amphitheater to a 3-foot width. Reduction in velocity at the substrate occurs with the use of larger limestone rock. Native riparian vegetation was planted along the stream, but is a narrow riparian zone due to the surrounding pool lawn.

For the new stream to increase salamander abundance at Eliza Spring, the new habitat must be good quality. Monitoring is underway to evaluate the quality of the new stream by measuring variables known to influence the abundance of *E. sosorum*, as well as general measures of stream quality including sediment, vegetation, invertebrates, and diatoms, and comparing the stream habitat to the spring pool. Currently data has been collected over 6 sampling events that from September 2017–November 2018. Salamanders were first observed in May 2018.

Salamander numbers remain low in the stream despite colonization by vegetation and invertebrates. The larger substrate in the stream (compared to the spring pool) is one possible cause of the lower observed salamander abundance. We will be looking into adding more gravel substrate to provide better microhabitat for salamanders in the stream.

The hypotheses in this section focus on aquatic and riparian habitat management, comparing the ecosystem before and after habitat changes as well as comparing Barton Springs to other creeks and rivers. Most management actions are one-time events (e.g., removing predatory fish or adding plants). It would be difficult to assess the effects of these events beyond their obvious additions or removal of animals from the ecosystem. These types of changes are also unlikely to affect much of the data proposed for collection here: flow velocity, DO, water temperature, and canopy cover. Sediment removal, which is discussed above, is the management strategy that occurs most often, and its effects are also relatively well known.

6.5.5 Scientific Research

We recently published a time series analysis of *E. sosorum* population dynamics (Bendik & Dries, 2018), documented new *E. sosorum* localities (Devitt & Nissen, 2018), and are currently investigating invertebrate diversity in the springs and aquifer, genetic diversity of *E. sosorum* in the aquifer, and what factors influence changes in body condition of *E. sosorum* at Eliza Spring. However, this section does not appear to address any specific AM aims.

6.5.6 Captive Salamander Program

Population management

The population management plan (attached) addresses many of the questions raised in sections 6*.5.6.1 Captive Population Demographic Management* and *6.5.6.2 Captive Population Genetic Diversity Management*. Namely, it establishes demographic and gene diversity goals, and how those goals can be reached, given what we know about population growth and pedigrees of *E. sosorum* in captivity. Additionally, we have attached a memo outlining a summary of recommendations to protect the captive colony from Bsal.

Augmenting the captive population

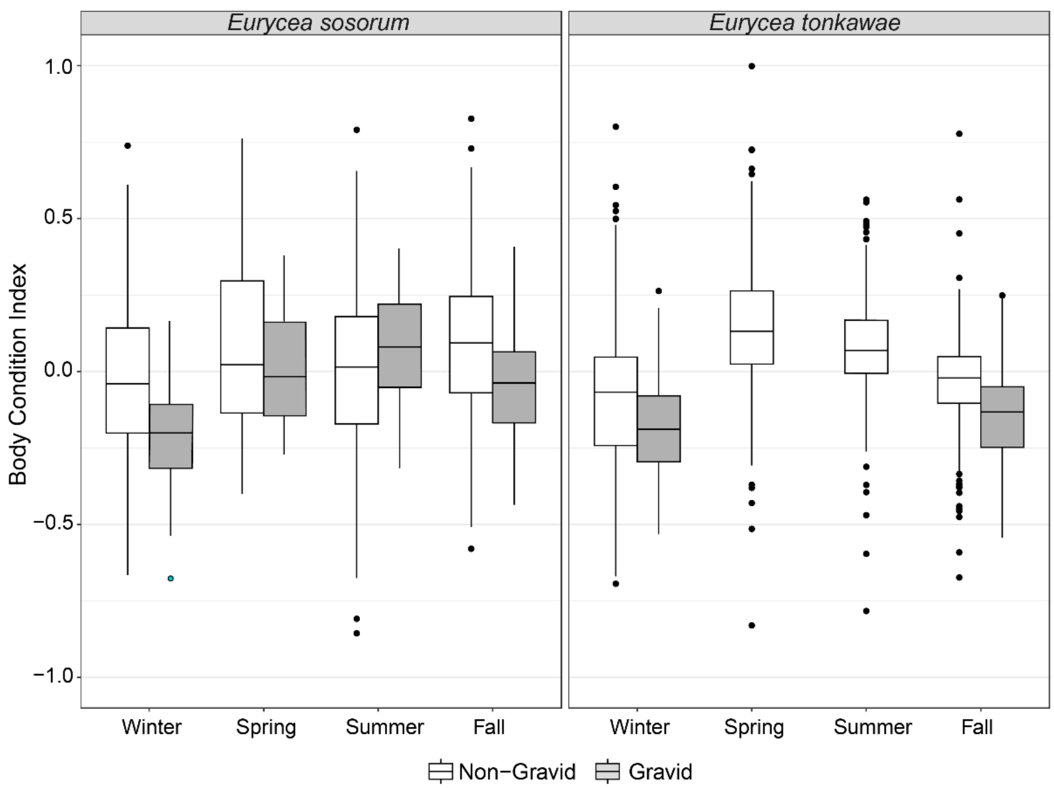
Augmenting the captive population with wild individuals will help achieve the genetic management goals set in the population management plan. In our analysis of *E. sosorum* population dynamics, we demonstrated a pattern of density-dependent population growth (Bendik and Dries, 2018). This indicates, that following periods of high population growth, the population will decline in response to density. Some individuals will die naturally and will comprise the harvestable surplus. Although we did not calculate the amount of compensatory mortality, it stands to reason that some individuals may be periodically harvested to augment the captive population, particularly following population booms, without negatively affecting the population growth rate. Thus, it should be sustainable to collect a small number of individuals from the wild each year. Collected individuals should be small juveniles, which are often the most abundant size class.

6.5.6.3 Reintroduction/Repatriation

In general, this section does not outline a specific AM plan for repatriation, but lists some specific management goals, possible studies, and things to consider in the event that repatriation is required. Thus, AM may be appropriate if repatriation is required, such that the success of reintroduction can be evaluated, and strategies adjusted. We are currently laying the groundwork for comparisons outlined here, such as differences between the wild and captive populations, and how released animals could compare to the wild (extirpated population). We do not plan on releasing captive individuals into the wild except under a scenario where the species is believed to have gone extinct in the wild or extirpated from the Barton Springs sites, threatening extinction, although it is important to be prepared for such an occasion.

Evaluating body condition

One way to evaluate the suitability of captive individuals for release into the wild, in the event of extirpation or extinction, could involve monitoring their body condition. We have recently quantified the average body condition of wild *E. sosorum* (and *E. tonkawae*) over the course of three years based on measurements of tail width relative to body size. This provides a baseline for comparison to captive and released individuals. For example, the figure below shows how body condition changes with gravidity and season.



This approach provides a way for us to compare the body condition captive and wild salamanders without harming them or performing additional surveys beyond our standard monitoring protocol.

Evaluating the success of released individuals

Staff have been performing capture-recapture surveys at the springs, and general methods for capture-recapture are well established. These data allow for estimation of survival, temporary emigration, detection and abundance, providing a baseline with which to compare to a future cohort of released animals. As above, captures from these events can also be measured and assessed for body condition, but also gravidity and individual growth. Without this information, we would have little basis for comparison with a released cohort.

The hypotheses listed in this section are unlikely to be met by a captive management program. There are several reasons why the physical health of the captive population may be different than the wild population (H1). The captive population may live longer due to lack of predators and lack of competition for food availability. As a result of an older average age, the captive population would be expected to have more age-related ailments. This is not a problem as long as the captive population can produce offspring fit for reintroduction. It is unlikely that the life history of wild and captive salamanders can be compared meaningfully (H2), beyond potential characteristics from recaptured individuals (growth, fecundity). For H3, there is no way to compare the reproductive success of the wild population to the captive population, given that reproduction of the wild population is never directly observed. The wild population may reproduce at a higher rate than the captive population. However, this is also an intentional management strategy; the captive population needs to have limited reproduction due to space and staffing constraints. The mortality of the wild offspring is very likely higher than that of the captive population. It is more important that the captive population be able to produce enough salamanders for successful reintroduction than it is for it to match the reproductive characteristics of the wild population. This is addressed in the population management plan.

References

Bendik, N. F., & Dries, L. A. (2018). Density-dependent and density- independent drivers of population change in Barton Springs salamanders. *Ecology and Evolution*, 1–12. https://doi.org/10.1002/ece3.4130

City of Austin (2018). Habitat Management Plan for the Barton Springs and Austin Blind Salamanders. City of Austin, Watershed Protection Department, Austin, Texas. SR-14-17 version 3 updated July 2018. 8 pp.

Devitt, T. J., & Nissen, B. D. (2018). New occurrence records for *Eurycea sosorum* Chippindale , Price & Hillis , 1993 (Caudata , Plethodontidae) in Travis and Hays counties , USA. *Check List*, *14*(2), 297–301. https://doi.org/https://doi.org/10.15560/14.2.297

Dries, L. A., & Colucci, L. A. (2018). Variation in abundance in the Barton Springs salamander associated with flow regime and drought. *Herpetological Conservation and Biology*, *13*(1), 302–316.

Dries, L. A., Herrington, C., Colucci, L. A., Bendik, N. F., Chamberlain, D. A., Johns, D. A., & Peacock, E. (2013). *Major amendment and extension of the habitat conservation plan for the Barton Springs Salamander (Eurycea sosorum) and the Austin Blind Salamander (Eurycea waterlooensis) to allow for the opperation and maintenance of Barton Springs and adjacent springs*. Austin.

Mahler, B. J., & Lynch, F. L. (1999). Muddy waters: Temporal variation in sediment discharging from a karst spring. *Journal of Hydrology*, *214*(1–4), 165–178. https://doi.org/10.1016/S0022-1694(98)00287-X

U.S. Fish and Wildlife Service (2018). Adaptive Management: Structured Decision Making for Recurrent Decisions - ALC3176. https://training.fws.gov/courses/ALC/ALC3176/resources/index.html [*accessed* 12/20/2017]

Batrachochytrium salamandrivorans Captive Treatment and Biosecurity Memo

Donelle Robinson July 2018

Summary of Recommendations

Due to the predicted likelihood of a future *Bsal* epidemic in the United States, measures should be taken to protect the salamander population at Austin Salamander Conservation Center (ASCC). This memo outlines the current state of knowledge for *Bsal*, and includes recommendations for biosecurity and treatment of salamanders at ASCC once *Bsal* is identified in the United States. The following recommendations are made:

- Test salamanders with potential symptoms of *Bsal* to evaluate possible infections
- If *Bsal* is confirmed, first use heat treatment to resolve the infection
- Maintain a supply of voriconazole and polymyxin E to treat *Bsal* if heat treatment is not effective
- Maintain equipment for use exclusively at ASCC (is current practice)
- Disinfect equipment used to transfer salamanders or other organic materials to ASCC (is current practice)
- Quarantine all organisms in a separate room or facility before exposing to the captive population

Because *Bsal* was first identified in 2013, there is still much to be learned about this disease. Treatments and the need for treatments should be reevaluated after more treatments are identified and after information is learned regarding the susceptibility of central Texas *Eurycea.* Additional considerations in the future would be a management plan for *Bsal* in wild populations to prevent potential extinction in the wild.

Background and Need

Batrachochytrium salamandrivorans (*Bsal*) is a fungus that infects the skin of salamanders and causes skin necrosis, often leading to a deadly infection. *Bsal* was first described in 2013 (Martel et al.) as the fungus responsible for the decline of *Salamandra salamandra* in Europe. Several other European salamanders are also susceptible to infection. Susceptibility to *Bsal* varies across salamander species from resisting infection to 100% lethal for all individuals. *Bsal* has the potential to be as devastating for salamanders as the closely related *Batrachochytrium dendrobatidis* (*Bd*) is for frogs. Approximately 200 species of frogs are extinct as a result of *Bd* (Skerratt et al. 2007). While *Bsal* has not yet been detected in the United States, models predict that the fungus will eventually spread to the United States (Grant et al. 2016, Richgels et al. 2016). The United States is considered highly susceptible because it has more salamander species than any other country.

The pet trade is most likely responsible for transferring *Bsal* from its native southeast Asia (Martel el al. 2014). From 2010–2014, 99% of salamanders imported into the U.S. either originated or were transferred via Asia (Yap et al. 2015). U.S. Fish and Wildlife Service has responded by banning the import of 201 salamander species that could transmit the disease under the Lacey Act. Canada has also restricted the salamander trade. Tests of wild salamanders in southern China detected *Bsal* in 33 of 1,143 animals (2.9%) (Yuan et al. 2018). With a prevalence this high, *Bsal* may already be present in pet salamanders in the U.S. While testing of pet salamanders in the United States did not detect *Bsal* (Klocke et al. 2017), even a low prevalence in the pet trade would represent a risk to adjacent wild populations given the ability for the fungus to persist in the environment (Stegen et al. 2017). If *Bsal* is not already in the United States, it may be enter through the illegal salamander trade, the legal salamander trade in other parts of the Americas, accidental release from clinical research involving the fungus, travelers and researchers recently in contact with *Bsal*, and the legal import of carrier species such as anurans. Some salamander and frog species that are asymptomatic can still be carriers of *Bsal* and cause infections in susceptible species (Stegen et al. 2017). Environmental transmission of zoospores is also possible as encysted spores can survive without a host in soil and water (Stegen et al. 2017). Survival in soil appears to be temperature dependent, with *Bsal* still detected after 15 days at 15 °C and for at least 28 days at 4 °C. Survival in pond water was possible for at least 31 days.

No central Texas *Eurycea* have been tested to determine susceptibility. *Eurycea wilderae* from Appalachia is susceptible to *Bsal* (Figures 1, 2, https://ag.tennessee.edu/fwf/bsalproject/Pages/default.aspx). However, *E. wilderae* is not neotenic and thus may not be representative of the impact to central Texas *Eurycea*. Neotenic *Siren intermedia*, *Ambystoma maculatum*, and *A. opacum* are either resistant to infection or tolerant of infections (Martel et al. 2014). However, these species are distantly related to *Eurycea*, so the response in *Eurycea* may differ. It's possible that neotenic species may be less susceptible to infection because they typically have less keratin for zoospores to infect. Alternatively, the presence of *Bd* infections previously found on *E. sosorum* could indicate that the species will be susceptible to *Bsal*. Currently, Paul Crump at TPWD is leading an effort to test *E. sosorum* and *E. nana* for *Bsal* infection risk. Until better data is available, it is more cautious to assume that *Bsal* could have a devastating impact on local populations. In the case of a *Bsal* outbreak, protecting and treating captive populations will be the most straightforward way to protect local species from extinction.

Identification of *Bsal* Infections

White et al. (2016) states that "A clinically compatible case includes amphibians with skin ulcers and lethargy, leading to a typically high mortality rate. Weak or erratic swimming may also occur… Lesions due to *Bsal* infection may occur at any site on the head, body, limbs, or tail of infected individuals." *Bsal* can spread easily at 15–20 °C, indicating it could thrive at the temperatures at the Austin Salamander Conservation Center (ASCC) and in central Texas springs and aquifers. Salamanders showing skin lesions would need followup histopathology or PCR to confirm the presence of *Bsal* (White et al. 2016) because other pathogens and environmental conditions can also cause skin lesions. Histopathology can determine the presence of chytrid zoospores, can distinguish *Bsal* from *Bd,* and may be able to determine the severity of infection. Duplex PCR has been developed by Blooi et al. (2013) to test for *Bsal* and *Bd* simultaneously. Labs that test for *Bsal* are listed here: http://www.salamanderfungus.org/resources/labs/. This list does not include Texas labs. Transferring tissues preserved in ethanol, rather than live animals, should reduce the risk of spreading *Bsal*. Currently the Lacey Act does not include central Texas *Eurycea* because they are not in the pet trade and their susceptibility has not been tested. However, if *Bsal* is found in the United States, the list of species under the Lacey Act could expand. Species included in the Lacey Act require a permit to transfer both live specimens and tissues out of state for testing.

Figure 1. Hemorrhaging and lesion on head of *Eurycea wilderae* infected with *Bsal.* Photo from: https://ag.tennessee.edu/fwf/bsalproject/SliderLibrary/Slider3.jpg

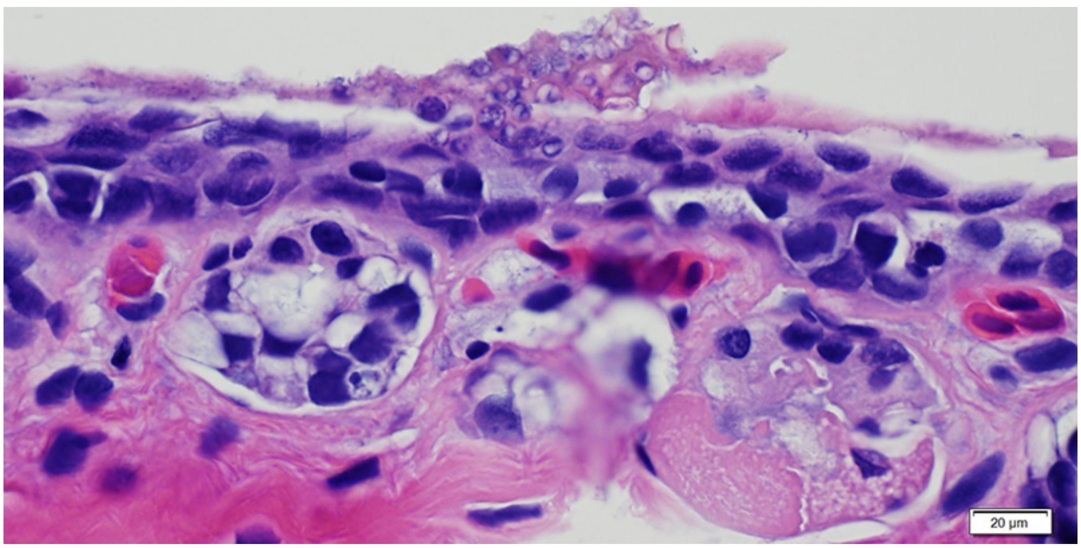


Figure 2. Histopathology image of lesion on *Eurycea wilderae* infected with *Bsal*. Photo from: https://ag.tennessee.edu/fwf/bsalproject/SliderLibrary/Slider4.jpg

Treatment Options

Because of the recent discovery of *Bsal*, only two successful treatment options have been determined so far:

1-Incubating salamanders at 25 ˚C for 10 days (Blooi et al. 2015a).

2-Treating salamanders twice daily by submersing in a bath of polymyxin E (2000 IU/ml) for 10 minutes, then spraying with voriconazole (12.5 µg/ml) at 20 ˚C (Blooi et al. 2015b). Salamanders were treated for 30 days but infections were not detected after 10 days.

Both of these treatments were tested in fire salamanders, *Salamandra salamandra*, which are especially susceptible to *Bsal*. It is still unknown if these treatments will work in all salamander species. However, cultures of *Bsal* are killed at 25 °C, indicating this is its thermal maximum (Blooi et al. 2015a) and that this temperature should be effective across species. Salamander survival was highest from the heat treatment when treated soon after infection, since *Bsal* caused high mortality within 30 days at 15 °C and within 50 days at 20 °C.

If effective for *E. sosorum* and *E. waterlooensis*, heat treatment would be the simplest method of treating salamanders at ASCC. This treatment could be implemented by changing the thermostat on the HVAC system of the building and changing the tank systems to recirculate water within the tanks for the 10-day period. If effective, this would treat all salamanders at the facility for *Bsal*. This temperature should not harm salamanders for the duration needed to eliminate *Bsal*. *Eurycea sososum* has been housed at 25 °C in the past (D.A. Chamberlain personal communication). Critical thermal maxima also were tested in both *E. nana* and *E. sosorum* and found to be greater than 30 °C for both juveniles and adults (Berkhouse and Fries 1995, Crow et al. 2016). Even if the heat treatment is not effective at eliminating *Bsal*, it should reduce the spread of the disease while other treatments are implemented. This temperature may not be effective against the encysted spores that can persist in the environment, so equipment disinfection is important to prevent reinfection of salamanders. The constant incorporation of colder groundwater into tanks at ASCC (for the purpose of maintaining appropriate water chemistry) would prevent the tank systems from being maintained at 25 °C long- term. However, alternative tank setups may allow for warmer temperatures to be maintained.

It would be useful to maintain a supply of voriconazole and polymyxin E in case heat treatment is not effective. Several other medications were unsuccessful at clearing *Bsal* infections in *Salamandra salamandra* at 15 °C. It's possible that some of these other medication combinations would have been effective if they were tested at 20 °C or at higher concentrations. For example, the combination of voriconazole and polymyxin was successful at 20 °C but not 15 °C. The 15 °C temperature was likely chosen by researchers because it was the optimal growth temperature for *Bsal* (Blooi et al. 2015a, Martel et al. 2013). Unsuccessful medications at 15 °C included a twice daily treatment of voriconazole spray (12.5 µg/ml), a combination of voriconazole spray and polymyxin E bath (2000 IU/ml for 10 min) itraconazole spray (0.6 µg/ml), and a combination of itraconazole spray and polymyxin E bath (Blooi et al. 2015b). The lack of effectiveness of itraconazole is surprising because it is commonly used to treat *Bd*. These medications were also able to inhibit the *in vitro* growth of *Bsal*. In the future other treatment options are likely to become available. Eventually a vaccine or antifungal microbes could be used to combat *Bsal* (Woodhams et al. 2016), but these options have not been developed yet. However, salamanders that do not mount an immune response to *Bsal*, such as *Salamandra salamandra*, would not benefit from a vaccine (Stegen et al. 2017).

Biosecurity to Prevent *Bsal* at ASCC

Until *Bsal* is present in the Americas, there isn't an urgent need to change biosecurity procedures at ASCC, though having established biosecurity protocols prepared will make biosecurity easier when *Bsal* is detected. If *Bsal* is identified in the Americas, biosecurity measures should be implemented to reduce the risk of transferring *Bsal* from the field to ASCC before it is detected locally. The detection of *Bsal* will always occur after *Bsal* is already present; thus it is important to implement biosecurity prior to local detection. If *Bsal* is known to occur in Texas, or wild populations exhibit symptoms indicative of *Bsal*, more conservative measures would be necessary, such as the temporary halting of all transfers of organisms into ASCC until a biosecure setup is established to protect the existing captive population.

Biosecurity should include the following:

1-disinfecting equipment and clothing to prevent the transfer of *Bsal*, 2-maintaining equipment used exclusively at ASCC that does not enter the field,

3-establishing a quarantine room.

Disinfection

Van Rooij et al. (2017) recommends using one of the following treatments to disinfect equipment and prevent the spread of *Bsal*, *Bd,* and *Ranavirus:*

- 1% Virkon S for 5 minutes
- 70% ethanol for 1 minute
- 4% sodium hypochlorite (bleach) for 1 minutes.

Currently, either bleach or quaternary ammonium is used to sanitize equipment for use in the field and at ASCC. Quaternary ammonium was found to be effective against *Bsal* (Van Rooij et al. 2017) and against *Bd* (Johnson et al. 2003). However, quaternary ammonium is not generally considered appropriate for sanitizing equipment as it is not as widely effective against some viruses, fungi, bacterial spores, and mycobacteria (McDonnell and Russell 1999). It has been tested on enveloped *Ranavirus* but not non-enveloped *Ranavirus*, and its effectiveness on nonenveloped *Ranavirus* has been questioned (Smith et al. 2017, Van Rooij et al. 2017). The CDC generally considers quaternary ammonium effective on enveloped but not nonenveloped viruses (https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html).

Virkon S is readily available through vendors, such as Fisher Scientific, in tablet and powder form to prepare large volumes of disinfectant. Virkon S is also considered safe for equipment, but requires a respirator when used in powder form. Ethanol is kept in stock at WPD but is not practical to use for large pieces of equipment. Sodium hypochlorite is found in commercially available bleach at concentrations of 8.25% in concentrated bleach and 5.25–6.15% in regular bleach depending on the brand. Sodium hypochlorite also may be difficult to use to disinfect large pieces of equipment.

Several alternatives may exist to chemical sterilization of equipment, though these alternatives have not been tested in *Bsal*. While alternatives have been tested in *Bd*, the encysted spores in *Bsal* should be more resilient to surviving without a host than *Bd* (Stegen et al. 2017). Most microorganisms, including *Bd,* will die after being exposed to temperatures of 140 °F or greater. Steam cleaners, clothes dryers with a sanitize cycle, boiling water, ovens and autoclaves can all be used to achieve this temperature.

Exclusive use of equipment at ASCC to prevent disease spread

Current practices that restrict use of equipment to ASCC should continue to prevent the spread of *Bd* and other diseases. Because zoospores can survive without a host, it is important that only a limited set of equipment should be used for collections and transfers that is not used for other purposes at the facility. This equipment is sanitized after each use in the field to prevent the spread of diseases. Clothing also should not be exposed to the field before entering ASCC. Shoes are especially problematic as they could encounter *Bsal* during normal use. Disinfecting mats (e.g., SaniStride™, https://www.qcsupply.com/sanistride-disinfectant-mat.html) could be used to enter and exit the facility to prevent the spread of *Bsal* from shoes. These mats control the spread of other diseases, such as white nose syndrome in bats.

Water handling at ASCC should already prevent *Bsal* from entering and exiting the facility. Groundwater at ASCC is treated with 0.5 μm filters prior to entering tanks. This is small enough to remove zoospores. Water from tank systems is disposed into the sewer system for treatment, which should reduce the chances of diseases from ASCC spreading to local populations. Currently, some equipment is dried outdoors and this practice would need to stop unless that equipment has been sanitized with one of the above disinfectants.

Quarantine

Currently, salamanders brought into ASCC are quarantined. However, a separate quarantine room would be preferred to reduce the risk of disease spread to the healthy captive population; this setup could be created using the storage room or part of the office at ASCC, or could be in a separate building. A stand of tanks with equipment used exclusively for those tanks should be designated to quarantine and treat at 25 °C all organisms brought in from the field or other captive facilities. If *Bsal* could be present, quarantine would need to expand to all organic materials brought into ASCC as any organic matter could harbor *Bsal*. This includes, but is not exclusive to, salamanders, aquatic vegetation, and invertebrates. Gloves should be worn during handling and hands washed to prevent transfer of zoospores to other tanks in the facility. Because the maximum amount of time zoospores can survive without a host is unknown but is at least 31 days in water (Stegen et al. 2017), heat treatment of all organisms and their water at or above 25 °C for a minimum of 10 days is the best method to prevent *Bsal* from spreading to the captive population. This is the thermal maximum for *Bsal* (Blooi et al. 2015a). Once it is determined how long *Bsal* zoospores can survive without a host, a longer quarantine period may be possible for organisms that are unable to undergo heat treatment.

Even if *Bsal* hasn't been detected in the Americas yet, salamanders at ASCC that exhibit symptoms consistent with *Bsal* should be quarantined until symptoms resolve or followup testing confirms that it is not *Bsal*. If multiple salamanders appear to have a potential *Bsal* infection, treatment should be considered even before test results are received to prevent high salamander mortality.

References

Berkhouse, C.S. and J.M. Fries. 1995. Critical Thermal Maxima of Juvenile and Adult San Marcos Salamanders (*Eurycea nana*). *The Southwestern Naturalist* 40:430-434.

Blooi, M., F. Pasmans, J. E. Longcore, a. Spitzen-Van Der Sluijs, F. Vercammen, and a. Martel. 2013. Duplex Real-Time PCR for Rapid Simultaneous Detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in Amphibian Samples. *Journal of Clinical Microbiology* 51: 4173–77.

Blooi, M, A. Martel, F. Haesebrouck, F. Vercammen, D. Bonte, and F. Pasmans. 2015a. Treatment of Urodelans Based on Temperature Dependent Infection Dynamics of *Batrachochytrium salamandrivorans*. *Scientific Reports* 5:8037. doi: 10.1038/srep08037

Blooi, M., F. Pasmans, L. Rouffaer, F. Haesebrouck, F. Vercammen, and A. Martel. 2015b. Successful Treatment *of Batrachochytrium salamandrivorans* Infections in Salamanders Requires Synergy Between Voriconazole, Polymyxin E and Temperature. *Scientific Reports* 5:11788. doi: 10.1038/srep11788

Crow, J., Forstner, M., Ostrand, K., & Tomasso, J. (2016). The role of temperature on survival and growth of the Barton Springs Salamander (*Eurycea sosorum*). *Herpetological Conservation and Biology*, *11*(2), 328–334.

Grant, E.H.C., Muths, E., Katz, R.A., Canessa, S., Adam, M.J., Ballard, J.R., Berger, L., et al. 2016, Salamander chytrid fungus (*Batrachochytrium salamandrivorans*) in the United States—Developing research, monitoring, and management strategies: U.S. Geological Survey Open-File Report 2015–1233, 16 p., doi: 10.3133/ofr20151233

Johnson, M. L., L. Berger, L. Philips, and R. Speare 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms 57*:255–260. doi: 10.3354/dao057255

Klocke, B., M. Becker, J. Lewis, R.C. Fleischer, C.R. Muletz-Wolz, L. Rockwood, A.A. Aguirre, and B. Gratwicke. 2017. *Batrachochytrium salamandrivorans* not Detected in U.S. survey of Pet Salamanders. *Scientific Reports* 7:13132. doi:10.1038/s41598-017-13500-2

Martel, A., A. Spitzen-van der Sluijs, M. Blooi, W. Bert, R. Ducatelle,M.C. Fisher, A. Woeltjes, et al. 2013a. *Batrachochytrium salamandrivorans sp*. Nov. Causes Lethal Chytridiomycosis in Amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 110 (38): 15325–29.

Martel, A., M. Blooi, C. Adriaensen, P. Van Rooij, W. Beukema, M. C. Fisher, R. A. Farrer, et al. 2014. Recent Introduction of a Chytrid Fungus Endangers Western Palearctic Salamanders. *Science* 346:630-631. doi:10.1126/science.1258268

McDonnell, G. and A. D. Russell. 1999. Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev.*12: 147–179.

Richgels, K.L.D., R. E. Russell, M.J. Adams,C. L.White, E.H.C. 2016 Spatial variation in risk and consequence of *Batrachochytrium salamandrivorans* introduction in the USA. *R. Soc. Open Sci.* 3: 150616. doi: 10.1098/rsos.150616

Skerratt, L.F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. *Ecohealth* 4: 125-134. doi: 10.1007/s10393-007-0093-5

Smith, T.C., A.M. Picco, R. Knapp. 2017. Ranaviruses Infect Mountain Yellow-legged Frogs (*Rana muscosa* and *Rana sierrae*) Threatened by *Batrachochytrium dendrobatidis*. *Herpetological Conservation and Biology* 12:149–159.

Van Rooij, P., F. Pasmans, Y. Coen, and A. Martel. 2017. Efficacy of Chemical Disinfectants for the Containment of the Salamander Chytrid Fungus *Batrachochytrium salamandrivorans.* PLOS One, 12: e0186269. doi:10.1371/journal.pone.0186269

White, C.L., M.J. Forzán, A.P. Pessier, M.C. Allender, J.R. Ballard, A. Catenazzi, H. Fenton, et al. 2016. Amphibian: A Case Definition and Diagnostic Criteria for *Batrachochytrium salamandrivorans* Chytridiomycosis. *Herpetological Review* 47:207-209.

Woodhams, D.C., M. Bletz, J. Kueneman, and V. McKenzie. 2016. Managing Amphibian Disease with Skin Microbiota. *Trends in Microbiology* 24 (3): 161-164.

Yap, T.A., M.S. Koo, R.F. Ambrose, D.B. Wake, and V.T. Vredenburg. 2015. Averting a North American biodiversity crisis. *Science* 349(6347):481-482. doi:10.1126/science.aab1052

Yuan, Z., A. Martel, J. Wu, S.V. Praet, S. Canessa, and F. Pasmans. 2018. Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. *Conservation Letters* e12436:1-8. doi: 10.1111/conl.12436

Eliza Stream Monitoring Quality Assurance Project Plan (Experimental Design 3/4) **Project #:** 594
Project Manager: Robinson, Donelle
Data Manager: Porras, Abel

Experimental Methods

Describe sampling scheme with data collection methods, sampling locations, parameters, number of samples, sampling schedule, and QC specifications. Include special conditions of the sampling scheme (e.g., only during base flow) and maps of the sampling locations.

Salamander Surveys-

The goal of the salamander surveys is to:

1- Assess successful colonization of the stream by salamanders by using monthly quadrat sampling.
2- Assess the time for the density of salamanders in the stream to be comparable to the Eliza spring pool by using a quarterly drive survey.

Quadrats

To determine whether salamanders have colonized the stream, 10 quadrats (0.25 m^2) will be placed throughout the stream and thoroughly searched for salamanders. Quadrats will avoid areas where sediment fills all interstitial spaces. Quadrats will not be placed within the 5' outside of the amphitheater to provide a buffer area for salamanders that may briefly leave the amphitheater. If any salamanders are found in the quadrats, salamanders will be captured using hand nets for photographs and data will be recorded for each salamander as detailed in the QAPP for Project #587 (page 9: species, gravidity, location, and size based on photograph). For salamanders that are missed, surveyors will categorize the missed salamanders by species and size class (<1", 1-2", >2"). This will continue every two weeks until the first drive survey occurs. After the drive survey, we will reevaluate the continued use of the quadrats.

Drive Survey

Drive survey methods will require two people to exhaustively search the stream habitat and will follow methods in the QAPP for Project #587 (page 8) for the Eliza Spring amphitheater. The cumulative survey effort (time) will be recorded. The sampling will coincide with sampling the amphitheater habitat so that all salamanders are accounted for in the capture-recapture design. This design includes an exhaustive survey that repeats three times during the week. Each salamander will be caught using hand nets for photographs and released after the survey. Gravidity and stream section of capture will also be recorded. For salamanders that are missed, surveyors will categorize the missed salamanders by species and size class (<1”, 1-2”, >2”). Drive surveys will occur quarterly beginning in November 2017 and will occur at the same time as the Eliza Spring survey for project #587.

Salamander Habitat Suitability Assessment

The goal of the stream habitat suitability assessment is to ensure that key parameters allow the stream to be habitable by salamanders: stream velocity, sediment cover, and possible salamander loss to the bypass tunnel.

Stream Velocity Profile-The velocity at the substrate will be measured with a Marsh McBirney flow meter. Velocity will be measured at every 5 ft beginning at the amphitheater keyway and ending at the bypass tunnel. At every 5 ft, three measurements will be taken-one measurement at the center of the stream channel, and one measurement 6” from each stream bank. The velocity is primarily expected to vary with spring discharge and with the amount that the downstream gate is open. Thus, velocity will not be assessed on a regular schedule. Instead, it will be assessed initially and then subsequently when the discharge from Eliza Spring increases or decreases by more than 3 cfs. Measurements will occur with the gate ¼ open, ½ open, ¾ open, and completely open, or for as many of these gate positions are feasible for a given flow rate. This information will be useful in the future to assess whether changing the position of gate improves stream velocity for salamanders.

Discharge will be measured with a Marsh McBirney flow meter using methods in the WRE Standard Operation Procedure manual. It will be measured at the amphitheater keyway and at the end of the stream to determine whether there is water gained or lost in the stream run. Measurements will be repeated three times in each location to determine measurement accuracy. This will be measured quarterly.

Substrate, Vegetation, Sediment Deposition-Ten quadrats (0.25 m^2) will be photographed and evaluated according to the QAPP for Project #587 (page 10) for substrate size classes, filamentous algae, leaf/woody debris, macrophytes, and sediment deposition. This will be evaluated quarterly.

Bypass Tunnel Capture-The stream run terminates at a junction box that connects to the Barton Springs Pool bypass tunnel. Salamanders that enter the junction box are lost from the population. When the stream is constructed, substrate will not be placed within 3 ft of the junction box to deter salamanders from the vicinity. To assess the success of deterrence and whether an additional deterrent is needed, a net will be placed in the downstream gate to catch salamanders that would otherwise be caught in the bypass tunnel. The net will be placed for five days and will be checked at the beginning and end of each work day for salamanders. This sampling will begin as soon as the Eliza stream is completed and will continue as needed. Additional samplings may occur later as flow or salamander density changes.

Stream Habitat Monitoring

The goal of the stream habitat monitoring is to:

1- Determine what, if any, changes and additional management are needed to improve stream quality by assessing riparian functionality and the macroinvertebrate and diatom community, using methodology from the aquatic sub-index score of the Environmental Integrity Index.
2- Assess time for stream habitat to be established.

For both of these goals, the Eliza Spring amphitheater will also be assessed and used as baseline data for comparison, with the exception of the riparian habitat. The riparian habitat will be assessed using a riparian functional assessment, but cannot be compared to the amphitheater due to the lack of riparian zone. Habitat will not be assessed during the 2 months after the stream begins flowing to allow for some colonization prior to sampling.

Photodocumentation

A photo of the stream will be taken quarterly to document changes in the stream appearance. Two photos will be taken: one looking upstream and one looking downstream. Exact locations to take photos will be determined after the stream is completed.

Environmental Integrity Index

A subset of the City of Austin's Environmental Integrity Index (EII) will be used to assess the ecological integrity of the Eliza stream habitat. A comprehensive EII will not be used, because not all indices are expected to vary in informative ways in a groundwater dominated stream. Macroinvertebrate and diatom diversity will provide information regarding whether the stream is good habitat for sensitive species. The EPA habitat assessment will look at the physical integrity of the channel.

<u>Macroinvertebrate Assessment</u>

A Hess sampler and Hester Dendy artificial substrate (H-D) will be used to collect benthic macroinvertebrates to compare in the stream and amphitheater using methods outlined in the WRE Standard Operation Procedure manual. H-D will be placed in the stream and amphitheater in four locations of cobble habitat each in the stream and the amphitheater. They will be placed at least six weeks prior to the first sampling. The H-D will be attached to bricks to prevent downstream movement and to suspend above the substrate. The Hess sampler will be used in four locations of cobble habitat each in the stream and the amphitheater, within 1 foot of each H-D sampler if possible. Locations will be selected to maximize habitat diversity. Each sample will be preserved separately and in its entirety (i.e. no subsampling). Additional samples will not be taken if invertebrate abundance is low; this is expected, especially early in stream sampling. H-D will be returned to the same locations to recolonize for the next sampling.

Sampling will occur quarterly the week before salamander surveys. Because the addition of the stream may also improve the invertebrate diversity in the amphitheater, the data for the amphitheater will also be compared to sampling prior to the start of the QAPP from Pete Diaz, USFWS.

<u>Diatoms</u>

Diatoms will be sampled quarterly using procedures in the WRE Standard Operation Procedure manual. Diatom rock scrapings will be collected from rocks in three locations in the stream and Eliza Spring amphitheater. Locations will be selected to maximize habitat diversity.

Sampling will occur quarterly. The addition of the stream may also improve the diatom diversity in the amphitheater. Subsequent diatom samples from the same quarter as the first sample from the amphitheater will be compared to determine if there has been an improvement. Samples will also be compared to the EII reference sites on Barton Creek at Shield Ranch and Barton Creek at Hwy 71.

Riparian Functional Assessment

The riparian functional assessment (RFA) will follow methods in the QAPP for Project #540-City of Austin Riparian Functional Assessment. An initial assessment will occur in 2017, a second assessment in 2019 for the purpose of corrective action due to establishment failure, and a final assessment will occur in 2022. Due to the length of time for plant establishment, assessing the riparian vegetation prior to 2022 would not be useful other than to assess plant mortality. A landscaping contract will replace plants that die during the first year. The results of the riparian functional assessment will be used to determine changes over the five year span, as well as to compare the site condition to degraded and healthy riparian zones. . RFA typically uses six 10 m x 10 m plots for each site. However, because of the small stream size, a full inventory of the stream riparian area inside the fence will be performed. Based on a report evaluating the variables that differed between undisturbed and disturbed urban sites (Richter and Gonzalez 2015), the following variables will be assessed:

-<u>Soil compaction</u>-Measured with a Humboldt Corps of Engineers Cone Penetrometer at 9 points (3 on the west side of the stream, 3 on the bench adjacent to the stream, and 3 on the upper slope of the east side)
-<u>Plant demography</u>-Visually estimate for each vegetation layer for the entire stream: canopy (vegetation at ≥ 5 m), understory (0.5 m$<$x$<$5 m), and ground cover ($<$0.5 m). All species and number of trees present within each demographic category: mature, sapling, or seedling, are recorded.

Snags and woody debris are excluded because plantings could not become large enough within a 5 year period to contribute to these categories.

Fish survey

The presence of some fish species may negatively impact salamanders by preying on salamanders or changing salamander behavior. Fish species and abundances will be recorded quarterly using methods from project #587 (page 10-11) by using a visual assessment from above the water. This information will allow us to know if management is needed to remove species that may negatively affect the salamander population. Smaller fish (*Astyanax*, *Gambusia*, ciprinids) are expected to be more common in the stream than large predator fish (bass and sunfish).

Water Quality

A minisonde will be used quarterly to collect data on dissolved oxygen, conductivity, temperature, and pH. Data will be collected at the end of the stream run near the bypass tunnel.

Promoting Stream Colonization

If after 1 year there is little establishment of plants and invertebrates in the stream, the stream may be seeded to facilitate their establishment. The effects of seeding the stream will not be analyzed, because there is not a reference site to compare the rate of stream colonization in the absence of seeding. The dates and quantities used to seed the stream will be recorded for reference.

Leaf packs (10 packs) will be made by placing leaves into nylon bags with 1/16" mesh size and added throughout the stream to provide organic matter for macroinvertebrates. These will be replaced as needed if they are lost or if the leaves have degraded.

Moss (2 square feet) will be transplanted from the amphitheater to the stream and anchored with rocks. Prior to transplanting, the moss with be checked for salamanders. Additional aquatic vegetation will be transplanted from other salamander spring sites based on availability.

Table 1: Aquatic species available for transplant

Species	Source Location
Water Hyssop (*Bacopa monnieri*)	Barton Creek, Sunken Gardens
American waterwillow (*Justicia americana*)	Barton Creek, Sunken Gardens
Eel grass (*Vallisneria americana*)	Parthenia Springs (Barton Springs Pool)
Delta Arrowhead (*Sagittaria platyphylla*)	Parthenia Springs (Barton Springs Pool)
Maidenhair Fern (*Adiantum capillus-veneris*)	Sunken Gardens
Water primrose (*Ludwigia repens*)	Sunken Gardens

Rocks will be used to seed the stream with periphyton and diatoms. Rocks will be transferred from the Eliza stream pool and Upper Barton Spring. Approximately 1 square foot of rocks will be moved from each site.

If establishment is unsuccessful, reseeding may occur as needed.

QAPP Termination

This QAPP will terminate when the stream is fully colonized. At this time, monitoring the Eliza stream will be included in monitoring for Project #587.

Two elements are expected to extend beyond the other elements of this QAPP: 1-the riparian functional assessment in 2022, and 2-the stream velocity profiles, which will depend on the timing of the next drought.

Sample Monitoring Schedule This schedule assumes 6 months before switching to the drive survey, and about 2 years for the stream to be fully colonized.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2017									A, C, D, F	A, E	A, B, F	
2018		B, F			B, F	C**		B, F, G*			B, F	
2019		B, F	C**		B, D***, F			B, F		C**, E	B, F	
2020		B, F			B, F							
2021												
2022										E		

*timing depends on stream colonization status
** timing depends of change in stream discharge
***timing depends on change in stream density

A Salamander Quadrat Survey
B Salamander Drive Survey
C Stream Velocity Profile
D Bypass Tunnel Capture
E Riparian Functional Assessment
F Quarterly sampling: substrate, sediment, aquatic vegetation, fish survey, macroinvertebrates, diatoms, water quality, discharge, photodocumentation
G Seed Stream

References

Bendik, N.F. (2015) Barton Springs Salamander and Austin Blind Salamander Population Capture-Recapture Monitoring Project #587. Austin.

City of Austin (2010) WRE Standard Operation Procedures Manual.

City of Austin. EII (NCR, PI, AQL) Quality Assurance Project Plan Project #13, 14, 15, 55.

Gonzalez, A. City of Austin Riparian Functional Assessment Project #540. Austin.

Revisiting the Removal of the Concrete Floor at Eliza Spring

Donelle Robinson

Summary

The restoration of natural spring flow conditions at Eliza Spring is included in the Barton Springs Habitat Conservation Plan (HCP) as two projects. The first project, the Eliza Spring Daylighting Project, was completed in 2018 and recreated an overland stream that increased the amount of salamander habitat by approximately 250 square feet. The second project is removal of a concrete floor that was poured over natural substrate inside the Eliza Spring amphitheater during the 1950's. This memo discusses the original goals of removing the concrete floor, assesses benefits and downsides of concrete removal, and what alternatives may be used to achieve the goals listed in the HCP. Some of the goals listed in the HCP are no longer needed, while others are already achieved from the completion of the Eliza Daylighting Project. The goal that is most likely to benefit the salamander population at Eliza Spring is increasing suitable habitat by distributing water inflow and removing flow localization in the spring pool. However, the concrete floor removal may have a detrimental impact to habitat quality. The removal itself is very risky given that it requires construction in the best Barton Springs salamander habitat known. We recommend foregoing removal of the concrete floor and to pursue less invasive options to improve salamander habitat in the Eliza Spring amphitheater.

HCP Goals

The following goals are mentioned in the HCP for concrete floor removal. Appendix 1 includes specific language from the HCP regarding Concrete Floor Removal. In general, most of these goals are no longer needed and are unlikely to benefit the salamander population.

1. Increase suitable habitat by distributing water inflow, remove localization/restore natural water flow
2. Restore natural surface substrate to increase interstitial spaces available
3. Allow plants to establish
4. Less cleaning needed
5. Improve ability for salamanders to move between surface and aquifer
6. Prevent Eliza going dry during drawdowns/may allow pool drawdowns to occur at lower discharge levels

Below each of these goals is discussed along with possible alternatives to achieve the goal:

1. Increase suitable habitat by distributing water inflow, remove localization/restore natural water flow

This is the goal with the greatest likelihood of benefitting the salamander population at Eliza Spring. While water movement inside the amphitheater has already improved due to the shallower water that now occurs after the Eliza Daylighting project, there are still some areas within the amphitheater where flow is localized, creating areas where sediment continues to settle more heavily. The concrete floor may facilitate cleaning of the spring pool by allowing sediment to be flushed out easily by hand. An option to distribute water flow within the amphitheater without removing the concrete floor is to alter the water flow path at the vents and diffuse water flow from vents into a broader area. This would increase the amount of useable surface habitat at the spring pool by reducing the area where sediment settles, allowing natural substrate to be added to these areas for salamanders. If this is not effective, a partial concrete removal to create additional vents in the northern portion of the amphitheater may be considered to reduce localization and would be less risky than a complete floor removal. The feasibility of removing portions of the concrete without impacting adjacent areas would need to be investigated. **This goal is still needed but removing the concrete floor may not be the best option for success.**

2. Restore natural surface substrate to increase interstitial spaces available

We cannot know whether recovery of the natural surface substrate is possible. The natural substrate may be heavily cemented into the concrete and difficult to separate. Additional limestone substrate could be added to the amphitheater to enhance habitat regardless of whether the concrete floor is removed.

The idea of restoring natural surface substrate relates to the concern that concrete is unsuitable habitat for salamanders. However, the Eliza Spring amphitheater has the highest density of Barton Springs salamanders known. This would not be the case if the concrete floor made this location unsuitable. Although the concrete is not natural salamander habitat, the concrete floor in the amphitheater serves a function similar to bedrock. In this way, the concrete present on the floor is not be detrimental to salamanders as long as other important habitat components are present, including limestone substrate and water flow. The surrounding concrete enclosing the spring is a larger issue than the concrete floor because a failure of the amphitheater could threaten the salamander population if it destroys salamander habitat. The amphitheater also prevents establishment of a riparian zone surrounding the spring that would otherwise provide shade and nutrients to the aquatic community. **This goal is not needed.**

3. Allow plants to establish

When salamanders are found in plants, they are typically found in moss, which this does not require deeper water to grow. Including other aquatic plants would benefit the salamander population by feeding their invertebrate prey. However, including a large number of vascular plants could negatively impact the salamander population in the amphitheater if it decreases the amount of habitat available for salamanders. Bendik and Dries (2018, https://doi.org/10.1002/ece3.4130) demonstrated density dependent dynamics at Eliza Spring, indicating that the population size is likely to be determined by habitat availability when salamander density is high. Even with the concrete floor, Eliza Spring has contained more plants in the past. The City has been successful using potted plants in Eliza and could continue these efforts. Some plants also are currently established on the floor of the amphitheater. **Concrete removal is not needed to achieve this goal.**

4. Less cleaning needed

The Eliza Daylighting project has reduced the need for cleaning in the amphitheater. The lower depth in the amphitheater helps naturally flush out sediment from most areas where it previously accumulated. No cleaning in the amphitheater has been needed since fall 2017 beyond the regularly scheduled surveys; however more data may needed as spring flows change with droughts and heavy rains. In addition, the concrete bottom may actually facilitate cleaning of the amphitheater by making the surface habitat shallower and by facilitating habitat cleaning by hand. **Removal of concrete may not achieve this goal.**

5. Improve ability for salamanders to move between surface and aquifer

Based on capture-recapture studies done at Eliza Spring (N. Bendik unpublished data), salamanders are able to move between the surface and aquifer habitats even with the concrete floor present. **This goal is not needed.**

6. Prevent Eliza going dry during drawdowns/May allow pool drawdowns to occur at lower discharge levels

Currently, the city agrees not to perform full drawdowns at lower discharge levels to prevent the concrete floor area at Eliza Spring from going dry. The gate installed during the Eliza Daylighting project man be used to maintain water in Eliza Spring during pool drawdowns at lower spring discharge rates, and so can sandbags in the keyway if needed. A test drawdown could be performed to determine whether the gate would be effective at maintaining the water level during drawdowns at low discharges. However, if the gate is not effective, then the city can continue to avoid full drawdowns at lower discharge levels to avoid causing the amphitheater floor to go dry. **This goal is not needed.**

Possible Negative Consequences of Concrete Floor Removal

Removing the concrete floor is a high stakes change to the best habitat known for Barton Springs salamanders. While the change should theoretically create more natural habitat, it's possible that such a major change could decrease the habitat quality and decrease the population overall. For example, removing the concrete may make the habitat undesirably deep (1-2 feet deeper), which could increase sediment deposition and predatory fish. Both of these issues are currently seen with the deeper habitat at Sunken Gardens.

Lowering the elevation of the spring pool may change the dynamics with the stream, such as increasing the velocity at the entrance of the keyway, since water would need to flow over a higher elevation to exit. Closing the gate some may mitigate this effect, though this will also raise the water level and decrease the velocity in the amphitheater, which would increase sediment deposition.

These are long term possible consequences of floor removal. Construction may also impact salamander habitat and is discussed below.

Construction Challenges of Concrete Floor Removal

Concrete removal will be challenging due to salamander protections needed during construction, spring water present in the area, structural concerns with the historical amphitheater, and permitting required. There is a high risk to the salamander population in the amphitheater if contractors perform work in salamander habitat. Ideally the project would be timed to reduce the impacts of other environmental stressors on the population, such as extreme droughts, or changes in water quality due to spills. Concrete removal would have to be done by hand given the high sensitivity of the habitat and amphitheater. The HCP states that the concrete removal could be phased. This would be necessary in order to move salamanders out of the construction area and prevent excessive take. However, the amphitheater floor is a very small area. In reality, it may be difficult to prevent construction impacts from extending past individual phased sections, which could impact adjacent habitat. If salamander density is high, then construction would reduce the habitat available temporarily and could lead to greater competition for habitat and resources. The extent of salamander habitat below the concrete is also unknown. It would be difficult to protect and move salamanders that are underneath the concrete slab. While some take is accounted for in the HCP, the take that is included in the permit could be exceeded, negatively impacting the population and leading to construction delays. It may also be difficult to remove the concrete following the sections defined in the HCP. Construction debris may move downstream with the spring water exiting the floor. While water may be controlled in some areas, it seems unlikely that the areas where concrete is removed could be dewatered. Water flows out of the ground in 4 of the 5 areas to be phased, and additional water may move out from underneath the concrete slab as it is removed. Dewatering would have to be restricted to small areas given the adjacent salamander habitat.

While the safest option for the salamander project would be to have the work performed slowly within WPD, the volume of concrete alone could make this difficult. At approximately 800 square feet of concrete floor with a thickness of 6-8 inches, this would be 400-530 cubic feet of concrete. At a typical weight of 145 pounds per cubic foot, this would be 14,500-19,285 lbs of concrete to remove. Removing a square yard of concrete floor in the amphitheater would weigh 650-865 lbs.

Removing the concrete floor while protecting the concrete amphitheater steps that connect to it would also be challenging to perform internally. Because the concrete floor is not part of the original structure, we believe it is unlikely that the Texas Historical Commission would require mitigation for its removal. However, protections for the amphitheater would need to be included in the design. Unintended damage to the amphitheater would require repairs, which may be require a specialty contractor to match repairs to the historical structure. This also would require the use of chemicals inside the amphitheater for concrete repair on the walls and steps. There is an above average risk of spills into habitat given that all construction work would occur in salamander habitat. Gravity will move spills from the steps to the amphitheater floor or voids underneath. Materials also could not enter the water that runs underneath the steps. The plans would need to add restrictions to how contractors perform repairs, which will increase the cost of the project over typical construction techniques.

A structural assessment is needed to determine whether it is safe to remove the concrete floor. Funding for this is being pursued by PARD as of June 2018. It's possible that concrete cannot be removed without destabilizing the amphitheater, though this may not be a problem given that the concrete was not original to the structure. A structural report for the Eliza Daylighting Project mentioned several aspects of the amphitheater's condition that would be relevant for concrete remove:

- The concrete slab may function to minimize erosion that could undermine the amphitheater's subgrade
- Ferroscanning should be used to determine whether reinforced steel is present in the walls (which would seem unlikely given the age of the amphitheater) and to determine whether the walls need to be strengthened to accommodate future modifications around the amphitheater
- A more detailed examination of the cracks is needed for any future amphitheater renovation beyond the scope of the daylighting.
- This report missed the presence of large voids underneath the amphitheater steps, and did not evaluate the base and underpinnings of the amphitheater.

If the removal of the concrete floor requires other structural support for the amphitheater, this would add to the project cost and time. For example, micropiles and a shotcrete wall were required for the Eliza Daylighting Project to protect the amphitheater. This added approximately $250,000 to the project cost and 2 months of construction time. If structural work was needed in wetted areas, this could make the project much more challenging. Additional structural support, based on the Eliza Daylighting project, could require concrete work and/or drilling to be performed to support the amphitheater. Challenges of structural work would include performing work in salamander habitat, requiring dewatering of portions of the amphitheater, using chemicals in water if dewatering is not feasible (unlikely to be feasible near vents), and possibly impacting the alluvial sediments.

Permitting from the Texas Historical Commission would be required for the project due to modifications to the historical structure. TCEQ permitting would likely be needed since permitting is required when construction alters/disturbs geologic characteristics of the site and has the potential to contaminate the Edwards Aquifer. Army Corps of Engineers may be needed if concrete removal is considered a construction activity, since a permit is required for construction in the Nation's navigable waters and was required for the Eliza Daylighting Project. Consultation with Development Review would be needed to determine whether a City site plan or SOS Amendment are needed. This would likely depend on whether historical mitigation or structural work is required to be incorporated into the scope of the concrete removal, and whether this mitigation would be considered a maintenance activity versus development in the city code.

Costs and Benefits of Concrete Removal

The recently completed Eliza Daylighting project provided valuable perspective of the costs versus benefits of removing the concrete floor from the amphitheater. High construction costs are expected due to endangered species habitat, significant water inflows, historical structure protection, limited work area, the highly visible work site, and many unknown construction conditions. It is not clear that removal of the concrete floor will improve the habitat quality to the benefit of the salamander population, and it could potentially decrease habitat quality. It is only clear that concrete removal would create a more natural habitat.

Appendix 1: HCP references to concrete floor and its removal:

- P.22 In the 1950s, free water flow into the spring pool was altered with the construction of a concrete floor in the amphitheater; the resulting higher elevation of surface substrate requires obstruction of free water flow from the spring pool to maintain water in surface habitat under most aquifer conditions.
- P. 25 Efforts to reintroduce native aquatic vegetation to Eliza Spring have been hampered by the concrete floor; vegetation cannot become well established even when planted in sediment pockets.
- p. 49 The natural surface habitat of the spring pool is covered by a 6 to 8-inch thick concrete floor. The elevation of rocky substrate beneath the concrete floor is 1 to 2 feet lower than the top of the floor. The elevation and topography of limestone bedrock and spring openings are unknown, although a large rock outcrop is visible in photographs taken before the concrete floor was constructed.
- P. 49 When water depth in Barton Spring Pool decreases, hydraulic pressure exerted by surface water against the spring openings also decreases according to Bernoulli's principle (Prasuhn 1938). Consequently, hydraulic head pressure in Eliza Spring is insufficient to push water up through the concrete floor into surface habitat. This redirection of groundwater occurs until Barton Springs' discharge exceeds 75 ft^3/s (City of Austin unpublished data), when presumably hydraulic head pressure is high enough that re-direction does not occur or is undetectable.
- P. 50 Surface habitat in the spring pool is maintained as a layer of gravel and cobble, one to two rocks deep lying on top of the concrete floor. Since 2003, the water depth has been maintained at 1 to 2 feet except during isolated events (e.g., storms, Barton Springs Pool drawdowns). Both of these strategies help minimize sediment accumulation by increasing flow velocity at any given discharge and reducing obstructions that capture suspended materials. Rocky substrate beneath the concrete floor is generally sediment-laden gravel and cobble.
- P. 54 Dissolved oxygen concentrations in surface habitat of Eliza Spring are slightly higher than those from immediately below the concrete floor.
- P. 60 Since 2003, the major goal of restoration has been to temporarily or permanently reconstruct more natural stream-like flow regimes in Eliza, Parthenia, and Old Mill springs
- P. 80 In the 1940s, a concrete floor was laid over the natural substrate of the spring pool.
- P. 97 Incidental take estimates in HCP for restoration projects for Barton Springs salamanders.

Table 18. Estimates of *E. sosorum* Incidental take from discrete habitat restoration projects in this Plan in Eliza, Old Mill, and Parthenia Spring. Summary statistic in each habitat section is the mean density plus one standard deviation (SD). Take is estimated as the product of density and affected habitat area; Conservation Measures are assumed to be 90% effective in reducing lethal take.

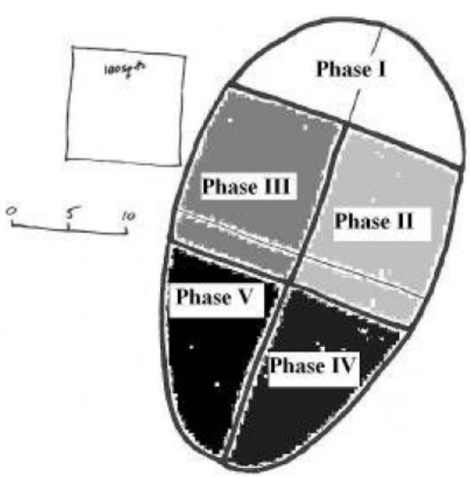
Eurycea sosorum						
Activity	**Area** (ft^2)	**Density + 1 SD** (no./ft^2)	**Lethal Take** (no.)		**Harassment Take w/ CMs** (no.)	**Total Take** (no.)
			No CMs	**With CMs**		
Eliza Spring						
Stream Reconstruction						
Quads III & IV	350	0.86	301	**30**	**271**	**301**
Concrete Floor Removal						
Phase I (Quad I)	75	0.94	71	7	64	71
Phase I (Quad II)	75	0.81	61	6	55	61
Phase II (Quad I)	150	0.94	141	14	127	141
Phase II (Quad IV)	25	0.86	22	2	20	22
Phase III (Quad II)	150	0.81	122	13	110	122
Phase III (Quad III)	25	0.85	21	2	19	21
Phase IV (Quad IV)	150	0.86	129	13	116	129
Phase V (Quad III)	150	0.85	128	13	115	128
Floor Removal Subtotal			**696**	**70**	**626**	**696**
Total Eliza Spring				**100**	**897**	**997**
Old Mill Spring						
Habitat Reconstruction						
All Habitat	1800	0.03	54	**6**	**48**	**54**
Total Old Mill Spring				**6**	**48**	**54**

- P. 123 During drawdowns of Barton Springs Pool when discharge is below 54 ft^3/s, water recedes from Eliza's concrete floor deeper into the aquifer, leaving dry surface habitat. Restoration of the natural substrate elevation and composition by removing this concrete floor is a management activity proposed in this Plan. Lower elevation of surface substrate in Eliza Spring should result in wetted surface habitat during drawdowns at lower discharge values. Conducting drawdowns at lower discharges would allow for restoration of more natural flow regimes during a wider range of aquifer conditions.
- P. 227 Since the early 1900s, the natural flow regime of Eliza Spring has been successively altered with construction of an amphitheater, diversion of the outflow stream into a buried pipe, and addition of a concrete floor into the amphitheater.. In the 1950's, a concrete floor was poured over natural substrate of the spring pool. From this point on, the only inflow from the aquifer into Eliza Spring has been through seven small round holes and 15 rectangular vents in the base of the riser to lowest bench of the amphitheater… Flow regime restoration in Eliza Spring requires two separate projects, reconstruction the overland outflow stream and removing the concrete floor in the amphitheater.
- P. 228 In addition to daylighting the outflow stream, habitat reconstruction will also include removing the concrete floor in the spring pool, which will restore the natural substrate in surface salamander habitat and improve flow regime. The concrete of the floor is not suitable substrate for salamander residence. The

suitable surface habitat is the clean interstitial spaces in the layer of rocks on top of the concrete. The localized inflow of water through the concrete floor limits suitable surface habitat in the spring pool to the areas around these points of inflow. The concrete floor also hinders salamander movement to subterranean habitat when water recedes or for courtship and breeding. Beneath the concrete floor is a natural substrate of limestone bedrock, cobble and gravel (Figure B7). Although this natural substrate is presently laden with sediment it can be easily cleaned once the concrete is removed providing abundant interstitial space for salamander occupation.

- P. 229 The concrete floor also restricts flow of aquifer water into the spring pool and its elevation requires that elevation of water level in Eliza Spring be maintained at approximately 433 feet msl at a minimum. The elevation of water in Barton Springs Pool is maintained at approximately 433.4 feet msl (SAM 2009). Removal of the concrete floor will lower the elevation of the substrate and allow for lower elevation of water in surface habitat. If the elevation were lowered, the hydraulic head between Eliza Spring and Barton Springs Pool would equilibrate or be reversed requiring less pressure to maintain wetted salamander habitat in Eliza Spring. Removal of the concrete floor will make surface habitat more resilient to changes in water elevation in Barton Springs Pool, allowing for drawdowns in a wider range of aquifer conditions without exposing surface habitat in Eliza Spring.
- P. 229 Concrete removal would be a phased project (Figure B8) to localize the potential detrimental impacts on resident salamanders to particular areas of the spring pool. The project could progress from upstream to downstream, shallowest to deepest water, and highest velocities to lowest velocities at the substrate directly in front of vents (Figure B8). The phases could also progress from downstream to upstream. The goal is for each section of substrate exposed by removal of concrete to be cleaned and allowed to transition into suitable salamander habitat before continuing to the next project phase.
- P. 230 Ideally, removal of the concrete floor would be conducted after the surface outflow stream is reconstructed simply because the stream would provide suitable surface habitat into which salamanders can retreat from activities within the spring pool. However, removal of the concrete floor is an important component of habitat reconstruction independent of stream reconstruction. The improvements in habitat in the spring pool and resilience to variation in water depth in Barton Springs Pool are significant benefits that do not rely on overland stream flow. Concrete floor would be removed even if the outflow stream cannot be reconstructed.
- P. 230 Phases for Concrete Floor Removal

Figure B8. Aerial diagram of the spring pool of Eliza Spring showing the footprint of each phase of concrete floor removal if conducted upstream to downstream.



Appendix 2: Photos from 1953 showing natural substrate in Eliza amphitheater.

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

January 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.

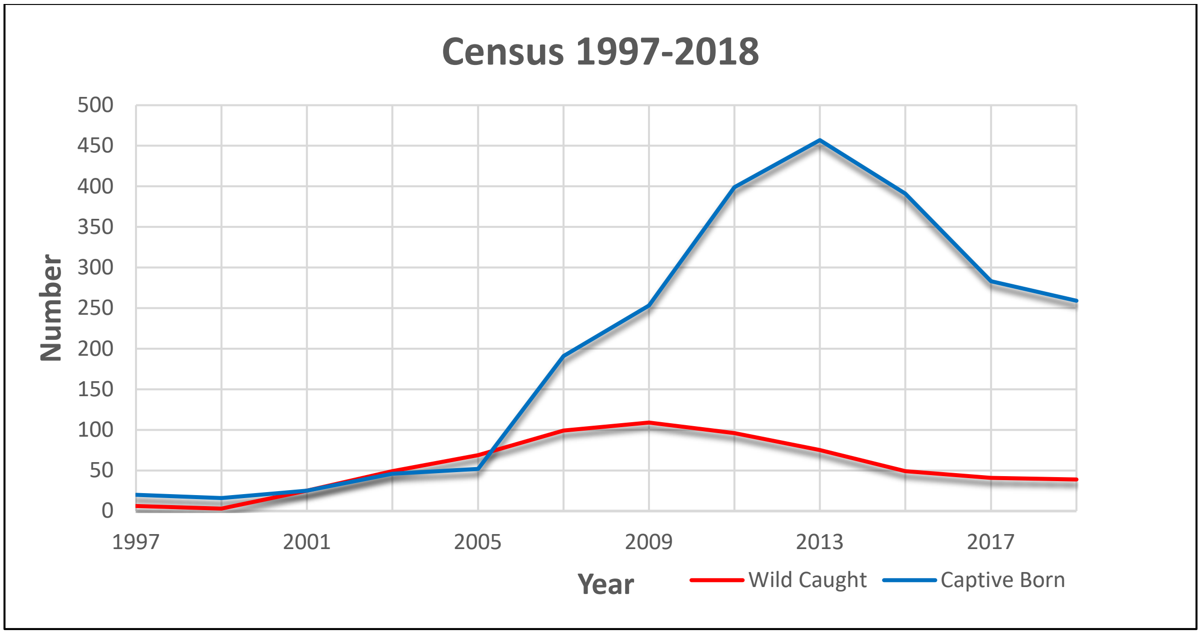

Figure 1. Census of *Eurycea sosorum* captive population 1997[1]-2018.
[1]Prior to 1999, captive-born salamanders consisted of donations by the Dallas Aquarium.

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 (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 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 database, 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 (λ), 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 generation time.

To estimate the maximum potential population growth rate required for population projections, we used the maximum λ (lambda, annual population growth rate), assuming population sizes $\geq$ 100 individuals, based on the set of λ calculated from the census for each year from 1998 to 2018 (Appendix B). For comparison, we also calculated the average λ 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). While, technically, the average mean kinship in the population is the equivalent of the proportional gene diversity loss in the captive-bred population relative to the population from which the founders were sampled, PMx calculates it 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 $GD_t/GD_0 = (1\text{-}1/(2N_e))^{\wedge t}$ (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 was housed in eight reproductive groups, according to spring site of origin (Appendix C). 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 (λ), 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 λ, 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 the spring site population 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 λ, 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 (λ), N_e/N, survivorship (L_x), and gene diversity (*GD*). The population growth rate (λ) was calculated from the average of the five highest λ, 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 λ, 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.

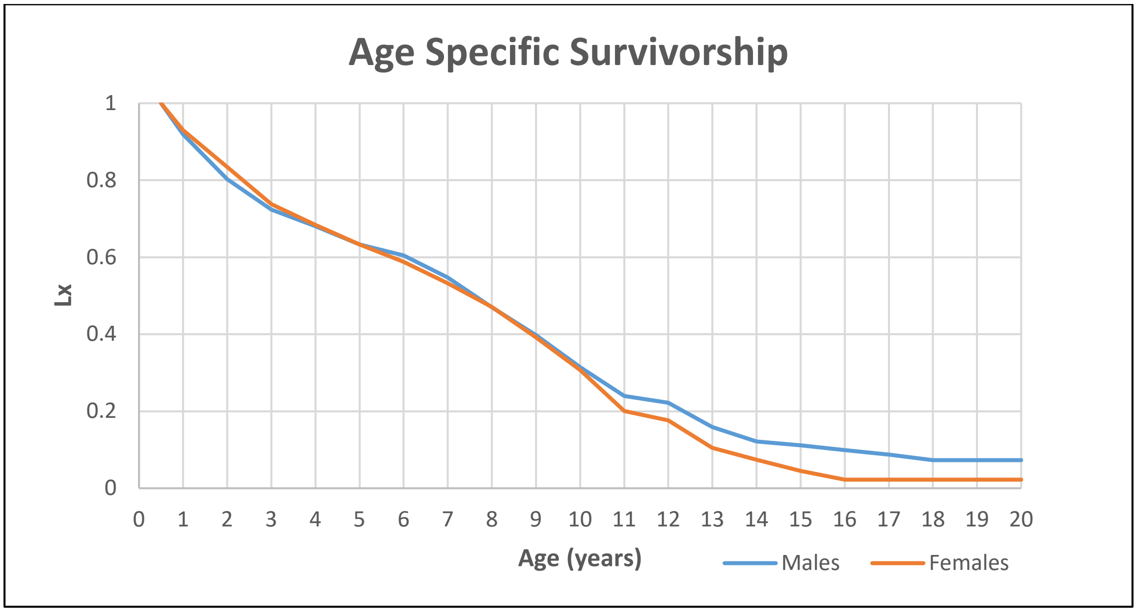

Figure 2. Barton Springs Salamander age specific survivorship, assuming individuals survive to 6-months post-hatch**.** Individuals of unidentified sex are divided equally between males and females. L_x is the probability that an individual will be alive at the beginning of age class x.

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 3). 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 3. Founder reproduction: minimum and potential maximum number of breeders.

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 (λ), and N_e/N (Table 4) generated from the demographic and genetic analyses in population projections to evaluate the effects of management strategies on gene diversity.

Table 4. Demographic and genetic statistics used in population projections.

Variable	Value
Mean generation time (T, years)	3.7
Maximum potential λ	1.63
N_e/N	0.32

<u>Combining spring lineage populations into a single population</u>

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.

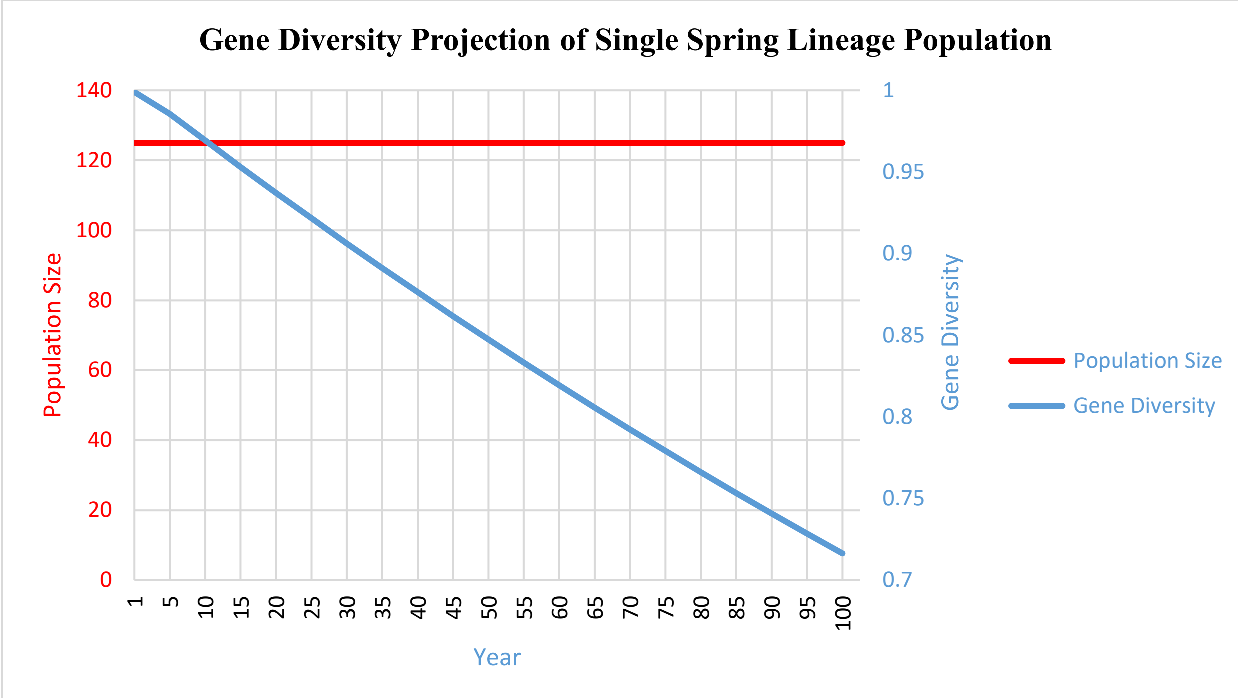

Figure 3. Projection of 90% gene diversity of a single spring lineage population.

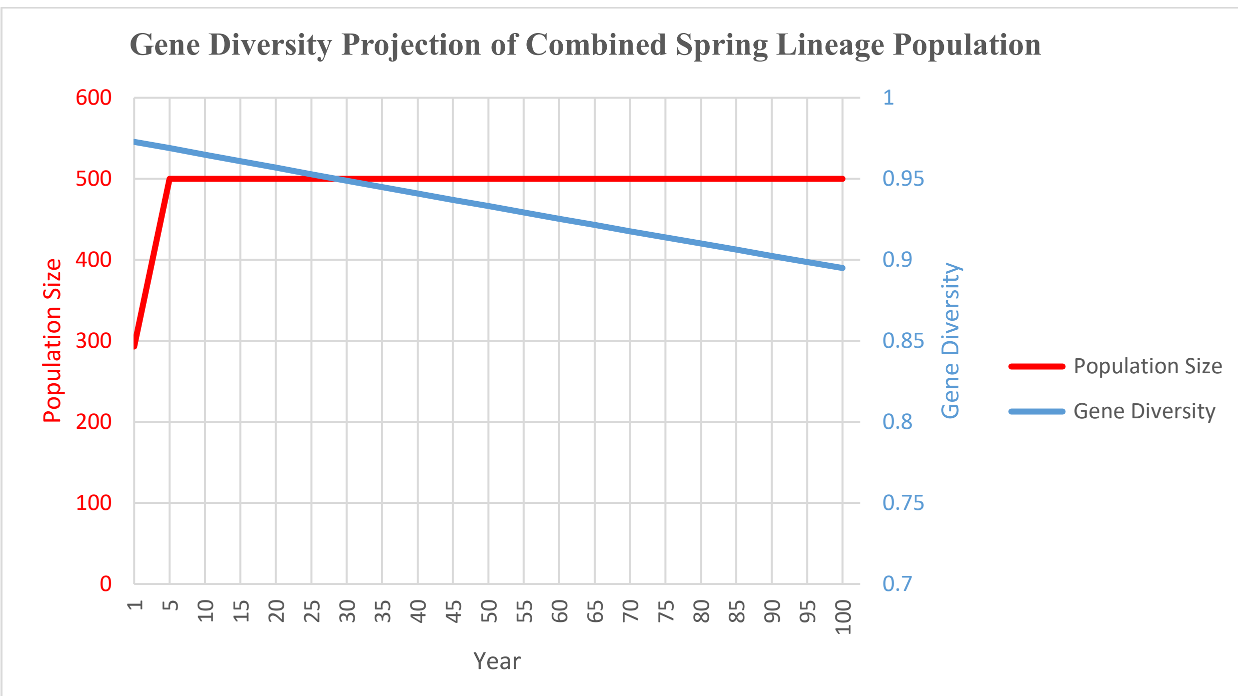

Figure 4. Projection of 90% gene diversity of the current population of combined spring lineages.

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 5, 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 5).

Table 5. 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

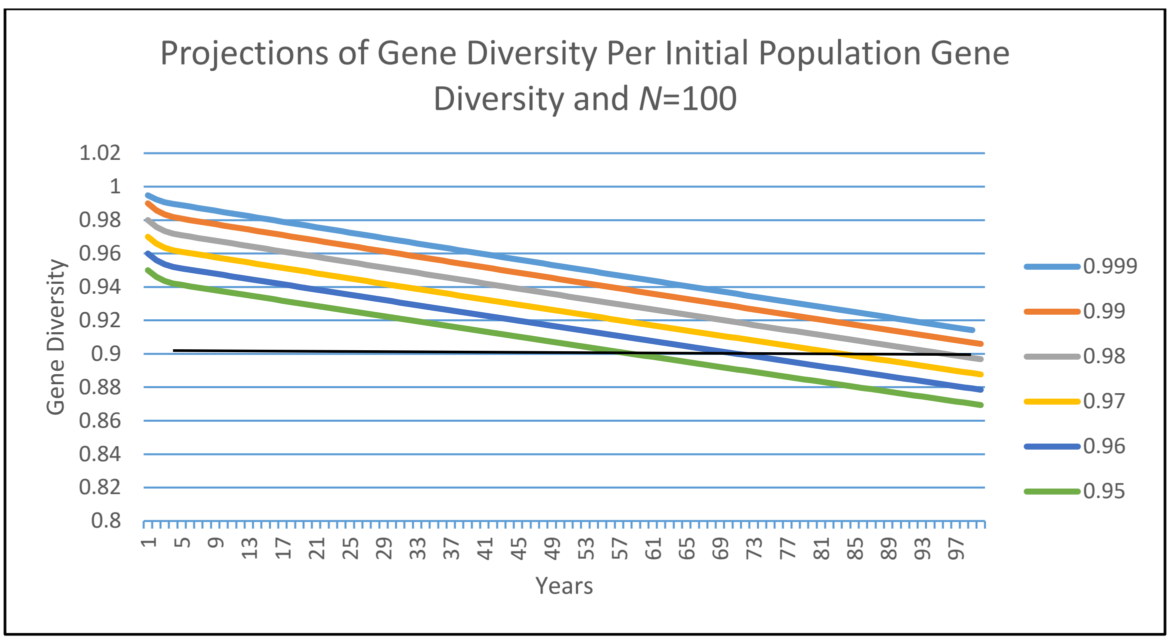

Figure 5. Projections of gene diversity per initial core population gene diversity and $N = 100$

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 7). 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 unexpected mortalities. 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 7. 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 5). 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 λ), then 520 offspring could be produced in a year. If the population λ 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 8) 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 8. Demographic and genetic statistics used in population projections.

Variable	Value

Generation time (T, years)	6.3
Maximum potential λ	1.22
N_e/N	0.11
Current gene diversity	92.4%

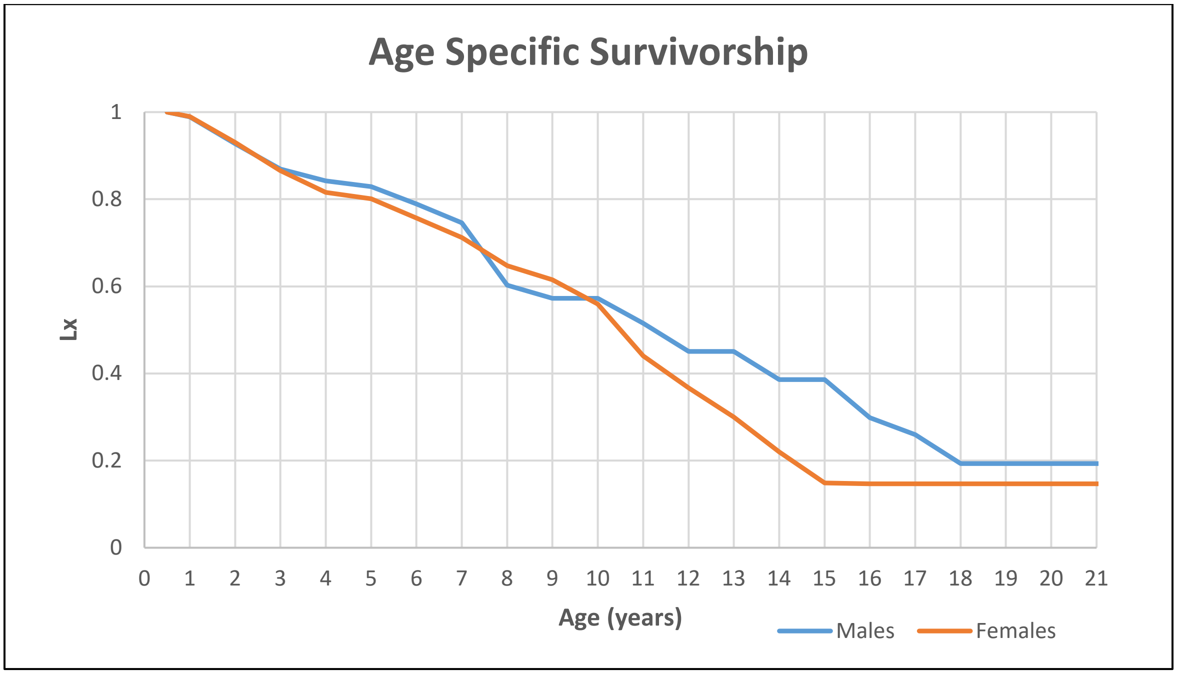

Figure 6. Austin Blind Salamander age specific survivorship assuming individuals survive to 6-months post-hatch**.** Individuals of unidentified sex are divided equally between males and females. L_x is the probability that an individual will be alive at the beginning of age class x.

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 9). 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 9. 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 λ 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 λ 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% of the population. 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.

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Literature Cited

Ballou JD, Lacy RC. 1995. Identifying genetically important individuals for management of genetic variation in pedigreed populations. *In* Ballou JD, Gilpin M, Foose TJ, eds., Population management for survival and recovery. Analytical methods and strategies in small population conservation. New York: Columbia University Press.

Ballou JD, Lacy RC, Pollak JP. 2018. PMx: Software for demographic and genetic analysis and management of pedigreed populations (Version 1.5.20180324). Chicago Zoological Society, Brookfield, Illinois, USA.

Ballou JD, Lees C, Faust LJ, Long S, Lynch C, Bingaman Lackey L, Foose TJ. 2010. Demographic and genetic management of captive populations. *In* Kleiman DG, ed, Wild mammals in captivity: principles and techniques for zoo management. Chicago: University of Chicago Press.

Bendik NF, Dries LA. 2018. Density-dependent and density independent drivers of population change in Barton Springs salamanders. Ecology and Evolution, 2018:1–12.

Cantu V, Crow J, Ostrand K. A comparison of two non-invasive spawning methods to genetically manage captive Barton Springs Salamanders, *Eurycea sosorum*. Herpetological Review 47(1): 59-63.

City of Austin. 2013. Major amendment and extension of the habitat conservation plan for the Barton Springs Salamander (*Eurycea sosorum*) and the Austin Blind Salamander (*Eurycea waterlooensis*) to allow for the operation and maintenance of Barton Springs and adjacent springs. City of Austin Watershed Protection Department.

Dries, LA, Colucci LA. 2018. Variation in abundance in the Barton Springs Salamander associated with flow regime and drought. Herpetological Conservation and Biology, 13:302-316.

Fernandez J, Toro MA, Caballero A. 2004. Managing individuals' contributions to maximize the allelic diversity maintained in small, conserved populations. Conservation Biology, 18(5):1358–1367.

Foose TJ, de Boer L, Seal US, Lande R. 1995. Conservation management strategies based on viable populations. *In* Ballou JD, Gilpin M, Foose TJ, eds., Population management for survival and recovery. Analytical methods and strategies in small population conservation. New York: Columbia University Press.

Hillis DM, Chamberlain DA, Wilcox TP, Chippindale PT. 2001. A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea*: *Typhlomolge*) from Austin, Texas, and a systematic revision of central Texas paedomorphic salamanders. *Herpetologica* 57: 266–280.

ISIS. 2013. Single population animal records keeping system (SPARKS version 1.6). Apple Valley, Minn.: International Species Information System.

Lacy RC. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy 78: 320–335.

Lacy RC. 2012. Extending pedigree analysis for uncertain parentage and diverse breeding systems. Journal of Heredity 103:197–205.

Lacy RC, Ballou JD, Pollak JP. 2012. PMx: Software package for demographic and genetic analysis and management of pedigreed populations. Methods in Ecology and Evolution 3:433–437.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA 70:3321–3323.

Putnam AS, Ivy JA. 2014. Kinship-based management strategies for captive breeding programs when pedigrees are unknown or uncertain. Journal of Heredity, 2014: 105(3):303–311.

Schad K, editor. 2008. Amphibian population management guidelines. Amphibian Ark Amphibian Population Management Workshop; 2007 December 10-11; San Diego, CA, USA. Amphibian Ark, 31p.

Traylor-Holzer K. (ed.). 2011. PMx Users Manual, Version 1.0 IUCN SSC Conservation Breeding Specialist Group, Apple Valley, MN, USA.

U.S. Fish and Wildlife Service (USFWS) and City of Austin. 1998. Final environmental assessment/habitat conservation plan for issuance of a section 10(a)(1)(B) permit for incidental take of the Barton Springs salamander (*Eurycea sosorum*) for the operation and maintenance of Barton Springs Pool and adjacent springs. Austin, Texas, USA. October 1998.

U.S. Fish and Wildlife Service (USFWS). 1997. Endangered and threatened wildlife: final rule to list the Barton Springs Salamander and endangered. Federal Register 62: 23377–23392.

U.S. Fish and Wildlife Service (USFWS). 2013. Endangered and threatened wildlife and plants; determination of endangered species status for the Austin Blind Salamander and threatened species status for the Jollyville Plateau Salamander throughout their ranges; final rule. Federal Register 78: 51278–51326.

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 (λ) 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

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

Habitat Management Plan for the Barton Springs and Austin Blind Salamanders

City of Austin
Watershed Protection Department
SR-14-17; September 2014
Version 2: April 2016
Version 3: July 2018

INTRODUCTION

This document outlines the habitat management plans for each of the four spring sites that comprise the Barton Springs complex, home to the endangered Barton Springs and Austin Blind salamanders (*Eurycea sosorum*, and *Eurycea waterlooensis*, respectively). Development of this plan by the City of Austin (hereafter, "City") and its provision to the U.S. Fish and Wildlife Service ("Service") fulfills measure 6.1.1.1 of the Barton Springs Pool Habitat Conservation Plan ("HCP"; COA 2013) and condition I of the associated Incidental Take Permit TE 839031-1 ("ITP"). Measure 6.1.1.1 states the following:

> The City will develop written habitat management plans for each spring site. These plans will include ongoing activities to improve the quality of aquatic habitat and ecosystem health. This includes but is not limited to introduction of native aquatic plants and maintenance of adequate tree canopy cover. Habitat management plans will be provided to the Service for review within one year of permit issue. The City will revise these plans with the written or verbal approval of the Service as necessary.

Background information, such as the historical condition of the springs, their current physical and biological characteristics, as well as salamander population information can be found in the HCP (see Section 3.2 for a description of each spring site, and sections 3.3 and 3.4 for detailed information on the status of each species). The purpose of this document is to outline the specific management actions intended to improve habitat for the Barton Springs and Austin Blind salamanders. However, because it is difficult to access subterranean habitat, these actions are anticipated to directly affect mostly *E. sosorum*, with the possibility of incidental or indirect benefits to *E. waterlooensis* (a subterranean species found on the surface only occassionally).

The objective of this plan is to restore and/or maintain surface habitat, which is highly modified at Barton Springs. Impoundments influence the hydrology of Barton Creek and the springs which in turn, affects the structure and function of the Barton Springs ecosystem. The actions outlined here strive to enhance, restore, or maintain the ecological integrity of the spring ecosystem in order to benefit salamanders by either counteracting the persistent effects of the modified hydrologic setting (e.g., through maintenance) or by permanently changing the modified conditions (e.g., through habitat restoration projects).

This document is divided into two main sections. In the first section, we describe our overall goals for habitat management, including the habitat characteristics and restoration practices believed to be important for establishing and maintaining suitable habitat for *E. sosorum*. These goals are based on a combination of ecological theory, knowledge of central Texas *Eurycea* salamander (*Paedomolge,* sensu Hillis et al. 2001) biology, habitat associations, prior experience with habitat modifications in Barton Springs, personal observations by City biologists, and guidelines in the HCP and Barton Springs Salamander Recovery Plan (USFWS 2016). In the second section, we outline the specific actions intended to meet management goals, which include general actions common to all sites as well as management plans that are site-specific. These include a range of activities from routine maintenance to major habitat improvement projects. This list will likely be updated frequently as projects are completed or as new information is gained.

Management Goals

Eurycea sosorum and *E. waterlooensis* were listed as endangered species on May 30, 1997 (62 FR 23377-23392), and September 19, 2013 (78 FR 51278-51326), respectively. This habitat management plan furthers recovery efforts by identifying goals and actions to improve aquatic habitat for *E. sosorum* and restore ecosystem function, thereby facilitating resiliency of the target species to ongoing habitat degradation and future environmental change. Three primary goals will contribute to these improvements.

I. *Provide and maintain non-embedded cover objects in epigean habitat to maximize availability of interstices for salamanders and macroinvertebrates.* Rocks such as gravel, cobble and boulders help provide cover and physical space in interstices for salamanders. Several studies have documented the use of gravel and cobble sized rocks (4–256 mm) as cover in *Paedomolge* (*E. nana*: Diaz et al. 2015; *E. sosorum*: Dries 2012), with a preference toward coarse gravel and larger (> 16 mm) when size was considered (*E. tonkawae*: COA 2001; Bowles, Sanders, and Hansen 2006; *E. naufragia*: Pierce et al. 2010). The relationship between rock size and interstitial space is an important determinant of aquatic community structure, and likely shapes predator-prey interactions in other paedomorphic *Eurycea* species (see Martin et al. 2012). Mosses also provide cover for salamanders and their prey. Excess sedimentation may negatively impact salamander habitat when interstices become filled by fine sediment (see Wood and Armitage 1997; Welsh and Ollivier 1998).

II. *Restore and maintain shallow, flowing water near springs to provide less embedded cover, more vegetation, and fewer predators.* Studies examining habitat associations of *Paedomolge* salamanders have shown a positive correlation between abundance and proximity to spring outlets and nearby areas of flowing water (Sweet 1982; Nelson 1993; Bowles, Sanders, and Hansen 2006; Diaz 2010; Pierce et al. 2010). Occupancy of *Eurycea tonkawae* has a strong positive correlation with low temperature variation (an indication of groundwater influence) and a negative correlation with water depth (Bendik et al. 2016). Similar patterns seem to hold for *E. sosorum*: the highest densities are found in areas with higher flow velocities, in shallow water (Dries 2012), and near spring outlets (City, unpublished data). Salamanders may prefer to be within close proximity of the aquifer outlets because subterranean habitat may be used for refugia (Bendik and Gluesenkamp 2013; City, unublished data), egg deposition (Tumlison, Cline, and Zwank 1990; Nelson 1993; Roberts, Schleser, and Jordan 1995; Fries 2002) or other aspects of their life history. Additionally, shallow, flowing spring water may also be preferable to *Paedomolge* salamanders due to higher concentrations of DO, less embedded cover (providing microhabitat for salamanders and their prey), and fewer predatory fish.

III. *Reduce habitat disturbance from humans*. Signs of physical disturbance by humans in the springs, such as building rock dams, moving substrate, and wading, are common during the summer months when Zilker Park and Barton Springs Pool visitation is high. Disturbance is anticipated and accounted for in the ITP at Parthenia Spring and Upper Barton Spring, which are open to the public, by allowing take for recreation. However, habitat disturbance also occurs at Old Mill Spring and Eliza Spring by trespassers, despite being fenced from public access and posted. While habitat disturbance from humans has not been studied, it contributes to the largest component of non-lethal take allocated in the City's incidental take permit and HCP.

Planned Management Actions

1 <u>The Barton Springs Complex (all sites)</u>

1-1. *Flush sediment periodically by hand to prevent the upper 2–3 inches of cover from becoming embedded.* The negative effects of sedimentation in lotic environments are well documented (reviewed in Wood and Armitage 1997). Specific threats to *E. sosorum* and *E. waterlooensis* caused by sedimentation in Barton Springs include filling/covering of habitat, declines in aquatic macrophytes, transport and concentration of contaminants and decline of benthic macroinvertebrate prey (Mahler and Lynch 1999; Service 2005; Geismar 2005). Abundance of salamanders at Eliza Spring was significantly lower prior to restoration of a more natural flow regime and removal of the extensive sediment buildup within the spring pool (Dries 2012). Sediment accumulation has occurred for several reasons in Barton Springs. First, impoundment of Barton Creek to create Barton Springs Pool and the impoundments surrounding Eliza and Old Mill springs encourage deposition of fine sediment. Second, the highly modified morphology of these springs and the surrounding areas has resulted in a loss of natural processes (e.g. storm flow and flood events) that disturb the substrate (Service 2005; p. 1.6-25). Eliza and Old Mill Springs only partially experience this natural disturbance during 100-yr flood events and Parthenia Spring experiences varying degrees of disturbance with flood events. Upper Barton Spring becomes inundated by storm flow when the creek exceeds average wetted width, providing the most frequent natural disturbance. Third, sediment moving through the aquifer and emerging at the springs is increasing over time (Mahler and Lynch 1999). Ultimately, improving flow regime of the springs will reduce the need to manually remove sediment and will help maintain more available cover for salamanders and macroinvertebrates. However, the goal is not to completely remove all sediment present, but to reduce the embeddedness of necessary cover for salamanders. Sediment at most of Old Mill Spring is too pervasive to maintain unembedded substrate in most deep areas until the impoundment is removed from the spring pool. This action will be achieved by (1) suspending sediment by-hand during quarterly salamander population surveys, and (2) using low-pressure (<30 lb/in^2) spring water delivered via handheld garden hoses to flush sediment from habitat in between salamander surveys. We note that although official habitat areas are indicated by the HCP, our sediment management efforts will be focused primarily on areas that may provide suitable habitat and those that can be remediated to regularly support salamanders. This management action addresses goal I and helps fulfill measure 6.1.1.7 of the HCP.

1-2. *Maintain native vegetation both in within the springs and within the riparian zone.* Maintaining native vegetation within the springs and in the riparian zone has several potential benefits. Within the spring pool, aquatic vegetation, including mosses and vascular plants, can provide habitat for both salamanders and macroinvertebrates. Riparian vegetation can provide canopy cover, detritus input, habitat for salamanders and invertebrates, and it can help filter local storm water before it reaches salamander habitat. Detritus input from terrestrial sources is an important source of food and habitat for many invertebrates and providing this resource may contribute to a diverse and abundant prey base for salamanders. Shade from canopy cover may provide some shelter from UV rays and helps prevent water temperature from rising, while shaded cover may provide suitable habitat for some invertebrates. This action will be achieved by (1) planting native riparian hardwood and understory trees, (2) maintaining native vegetation at the water interface, (3) transplanting aquatic flora in the spring and stream habitats from other springs within the Barton Springs complex, including vascular plants and mosses, and (4) removing non-native vegetation (e.g. *Arundo* spp. and *Ligustrum* spp.) with hand tools or a combination of girdling and direct application of acetic acid in the riparian zone of salamander habitat. This management action addresses goals III and helps fulfill measure 6.1.4.2 of the HCP.

1-3. *Periodic removal of predatory fish and crayfish in the springs.* Impoundments of Barton Creek, creating Barton Springs Pool, and of Eliza Spring and Old Mill Spring have created more lentic environments that are favorable for some species of fish (e.g., *Lepomis spp.*, *Herichthys cyanoguttatus*, *Micropterus salmoides*, *Gambusia affinis*, *Astyanax mexicanus*) that may prey upon salamanders. The frequency of salamander predation by fish in the springs is unknown, but laboratory experiments have shown that chemical cues from predatory fish result in antipredator behavior in captive-hatched *E. sosorum* (Desantis, Davis, and Gabor 2013). Salamander predation has been observed in the field (Owen et al. 2016), as well as through gut-content examination of fish (Owen and Devitt 2016). Crayfish abundance was reduced in Eliza Spring after restoration, and this may have helped facilitate the increased abundance of salamanders later observed there (USFWS 2005). In addition to predation on salamanders, fish and crayfish may be competing with salamanders for macroinvertebrate prey. Relocation of mosquitofish (*Gambusia affinis*) and crayfish from Eliza Spring appears to have improved conditions for salamanders there (Service 2005). The high predatory fish densities in Barton Springs Pool and Old Mill Spring may make salamanders more susceptible to predation. This action will be achieved by (1) collecting and/or translocating fish using hand-held seines, rod and reel, minnow traps, gill nets, and/or polespears in cooperation with the Texas Parks and Wildlife Department and the University of Texas as needed, and (2) adding screens to prevent fish from migrating into the pool at Old Mill Spring. This management action addresses goal III and helps fulfill measures 6.3.2 and 6.5.3 of the HCP.

2 Eliza Spring

Restore uninhibited spring flow to the spring pool as much as is feasible. While water movement inside the amphitheater has already improved due to the shallower water that now occurs after the Eliza Daylighting project, there are still some areas within the amphitheater where flow is localized, creating areas where sediment continues to settle more heavily. An option to distribute water flow within the amphitheater without removing the concrete floor is to alter the water flow path at the vents and diffuse water flow from vents into a broader area. This would increase the amount of useable surface habitat at the spring pool by reducing the area where sediment settles, allowing natural substrate to be added to these areas for salamanders. If this is not effective, a partial concrete removal to create additional vents in the northern portion of the amphitheater may be considered to reduce localization and would be less risky than a complete floor removal. The feasibility of removing portions of the concrete without impacting adjacent areas would need to be investigated. While a complete concrete floor removal is included in the HCP, some goals listed in the HCP are no longer needed, while others are already achieved from the completion of the Eliza Daylighting project. The concrete floor removal may have a detrimental impact to habitat quality. The removal itself is very risky given that it requires construction in the best Barton Springs salamander habitat known.This management action addresses goals I and II.

2-1. *Prevent destruction of the amphitheater by removing woody plants growing out of the concrete.* Although woody vegetation currently growing within the amphitheater provides some amount of shade and allochthonous inputs to the lotic system, they are also believed threaten the stability of the amphitheater walls. Without intervention, this may lead to a potentially hazardous condition both for people and for salamanders if the walls were to become unstable. Additionally, because the amphitheater is a historic structure, our partner department PARD is interested in preserving the site as much as is feasible. Therefore, WPD agreed to remove woody plants from the site and continually maintain the structure free of large, potentially damaging plants, in spring 2016. Furthermore, new recruits will be removed periodically during regular site visits.

3 Parthenia Spring

3-1. *Remove accumulated flood debris in salamander habitat at Parthenia Spring by hand as necessary to help reduce accumulation of flood debris over time.* Impoundment of Barton Creek to create Barton Springs Pool causes debris (gravel, sediment, downed wood, etc.) to accumulate following floods. Debris can divert flow directly in front of the springs, cause hazards for swimmers, and make it difficult to survey for salamanders. This action will be achieved by removing all trash and some woody debris by hand that are hazards or impediments. This management action addresses goals I and II and fulfills measure 6.1.1.8 of the HCP.

3-2. *Investigate how modification of upstream and downstream dam can improve ecosystem integrity and flow regime of Barton Springs.* The City intends to determine whether modifications to the upstream dam to allow flood waters to pass freely, or a more consistent input of Barton Creek waters into the pool would improve the conditions for the salamander at Parthenia Spring. Additionally, we are also evaluating whether modifications to the downstream dam would also improve flood throughput and reduce debris and sediment deposition in Parthenia Spring. This placeholder will be replaced by recommended actions once they are determined. This management action addresses goals I and II and helps fulfill measures 6.1.3.1 and 6.1.3.2 of the HCP.

4 Old Mill (Sunken Garden) Spring

4-1. *Modify the Old Mill spring pool and stream to maintain the spring pool at a shallower depth and reduce water velocities in the stream.* Old Mill Spring has been impounded since the late 19th century and salamander habitat within the spring pool and stream has varied greatly (City 2013). Currently, the spring pool is less than two meters deep and water velocity within the spring pool is minimal (City, unpublished data). As it exits the spring pool, the elevation of the stream increases and then decreases until it reaches the manmade waterfall at Barton Creek. Shallower water in the spring pool could facilitate sediment removal, increase DO by increasing water velocity through the spring pool, and discourage large predatory fish from inhabiting the spring. Reducing the stream water velocity will provide a more conducive environment for salamanders to move from the stream back into the spring pool and may reduce one-way migration into Barton Creek. This action may be achieved by (1) widening the outflow in the impoundment from the spring pool to the stream, (2)reconstructing or altering the stream channel to remove any positive grades within the stream channel and reduce the overall negative grade of the existing channel, and (3) widening the stream channel to provide additional salamander habitat and reduce flow velocities.. The City is currently investigating engineered solutions to these problems. This management action addresses goals I, II and III and fulfills measure 6.1.3.1 of the HCP.

5 <u>Upper Barton Spring</u>

5-1. *Discourage and provide remediation for habitat disruption and vandalism*. Construction of temporary dams or any other alteration of spring flow is detrimental to salamander habitat at the spring. Disturbance of cover objects and foot traffic within the spring can cause salamander injuries and mortalities (City, unpublished data). Prohibition and remediation of these activities will be achieved by (1) manually removing dams built immediately upon discovery, (2) redistributing substrate within the spring pool, (3) removing obstructions from the spring pool, (4) having signs to educate the public about which activities are prohibited, (5) requesting increased patrols in this area by law enforcement and park rangers. This management action addresses goals II and III, and helps fulfill measure 6.1.1.6 of the HCP.

References

Bendik NF, Gluesenkamp AG. 2013. Body length shrinkage in an endangered amphibian is associated with drought. J. Zool. 290:35–41.

Bendik NF, McEntire KD, Sissel BN. 2016. Movement, demographics, and occupancy dynamics of a federally-threatened salamander: evaluating the adequacy of critical habitat. PeerJ 4:e1817 https://doi.org/10.7717/peerj.1817

Bowles BD, Sanders MS, Hansen RS. 2006. Ecology of the Jollyville Plateau Salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization. Hydrobiologia 553:111–120.

[COA] City of Austin. 2001. Jollyville Plateau water quality and salamander assessment. City of Austin Unpubl. Rep. COA-ERM 1999-01.

[COA] City of Austin. 2013. Major amendment and extension of the habitat conservation plan for the Barton Springs Salamander (*Eurycea sosorum*) and the Austin Blind Salamander (*Eurycea waterlooensis*) to allow for the operation and maintenance of Barton Springs and adjacent springs. Austin, Texas, USA.

[COA] City of Austin. 2014. Annual Report U.S. Fish and Wildlife Service Scientific Permit (TE-833851). Austin, Texas, USA.

Desantis DL, Davis DR, Gabor CR. 2013. Chemically mediated predator avoidance in the Barton Springs Salamander (*Eurycea sosorum*). Copeia 69:291–297.

Diaz, P. H., Fries, J. N., Bonner, T. H., Alexander, M. L., & Nowlin, W. H. 2015. Mesohabitat associations of the threatened San Marcos salamander (*Eurycea nana*) across its geographic range. Aquatic Conservation: Marine and Freshwater Ecosystems, 25(3), 307–321. http://doi.org/10.1002/aqc.2559

Dries LA. 2012. Variation in abundances of *Eurycea sosorum* and *Eurycea waterlooensis* (Plethodontidae: Hemidactyliini: *Eurycea: Notiomolge*), with examination of influences of flow regime and drought. Austin, Texas. City of Austin Unpubl. Rep. SR-12-16.

Fries JN. 2002. Upwelling flow velocity preferences of captive adult San Marcos salamanders. N. Am. J. Aquac. 64:113–116.

Geismar E. 2005. Sediment Accumulation in the Barton Springs Complex. City Austin Unpubl. Rep. SR-05-05.

Gillespie JH. 2013. Application of stable isotope analysis to study temporal changes in foraging ecology in a highly endangered amphibian. PLoS One 8:e53041.

Hillis DM, Chamberlain DA, Wilcox TP, Chippindale PT. 2001. A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea: Typhlomolge*) from Austin, Texas, and a systematic revision of central Texas paedomorphic salamanders. Herpetologica 57:266–280.

Mahler BJ, Lynch FL. 1999. Muddy waters: temporal variation in sediment discharging from a karst spring. J. Hydrol. 214:165–178.

Martin SD, Harris BA, Collums JR, Bonett RM. 2012. Life between predators and a small space: substrate selection of an interstitial space-dwelling stream salamander. J. Zool. 287:205–214.

Nelson JM. 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River. Texas State University. San Marcos, Texas, USA.

Owen JD, Devitt TJ. 2016. *Eurycea waterlooensis* (Austin Blind Salamander). Predation. Herpetol. Rev. 47:275–276.

Owen JD, Devitt TJ, Colucci LA, Bendik NF. 2016. *Eurycea sosorum* (Barton Springs Salamander). Predation and Diet. Herpetol. Rev. 47:275.

Pierce BA, Christiansen JL, Ritzer AL, Jones TA. 2010. Ecology of Georgetown Salamanders (*Eurycea naufragia*) within the flow of a spring. Southwest. Nat. 55:291–297.

Roberts DT, Schleser DM, Jordan TL. 1995. Notes on the captive husbandry and reproduction of the Texas Salamander *Eurycea neotenes* at the Dallas Aquarium. Herpetol. Rev. 26:23–24.

Sweet SS. 1982. A distributional analysis of epigean populations of *Eurycea neotenes* in central Texas, with comments on the origin of troglobitic populations. Herpetologica 38:430–444.

Tumlison R, Cline GR, Zwank P. 1990. Surface Habitat Associations of the Oklahoma Salamander (*Eurycea tynerensis*). Herpetologica 46:169–175.

[USFWS] United States Fish and Wildlife Service. 2016. Recovery plan for the Barton Springs Salamander, *Eurycea sosorum* amended to include Austin Blind Salamander (*Eurycea waterlooensis*). Albuquerque, NM, USA. 148 pp.

Welsh H, Ollivier L. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. Ecol. Appl. 8:1118–1132.

Wood P, Armitage P. 1997. Biological effects of fine sediment in the lotic environment. Environ. Manage. 21:203–17.