Annual Report (January 1 - December 31, 2002):

City of Austin's Captive Breeding Program For the Barton Springs and Austin Blind Salamanders

Barton Springs salamander (*Eurycea sosorum*)

Austin blind salamander (*Eurycea waterlooensis*) © David Hillis

Submitted to the U.S. Fish and Wildlife Service
In Compliance with Permit # PRT-839031

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Barton Springs salamander egg laid in captivity

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Executive Summary

The City of Austin's captive breeding program currently supports 100 salamanders, including 85 Barton Springs salamanders and 15 Austin blind salamanders. Throughout 2002, captive breeding biologists focused efforts on creating environments that would induce females to lay eggs. Biologists set up a 75-gallon glass tank in late January that was designed to provide diverse habitat types and simulate aquifer, spring, and spring run conditions. Twelve clutches were laid in this tank over an 8-month period. Two other Barton Springs salamander tanks were set up based on this prototype in late August/early September and produced 3 and 2 egg-laying events, respectively, totaling 17 egg-laying events at the captive breeding facility in 2002. An additional clutch was laid in the display tank at the Splash! Into the Edwards Aquifer Educational Exhibit. Captive breeding biologists have produced extensive video footage of salamander behavior and activities, including courtship, egg-laying, and egg development. Although several females are in each Barton Springs salamander tank, not all laid eggs. Whether these females would lay eggs with additional space needs to be determined. Additional space is also needed to remove the adults soon after egg-laying to increase survival from the egg to adult stage.

City biologists collected the first Barton Springs salamander egg ever found in the wild during a survey of Sunken Gardens on May 31, 2002. The egg hatched in captivity on June 19, 2002. The juvenile is still being maintained in the captive breeding program.

Based on the development of eggs in the captive-raised young, Barton Springs salamander females mature within 12 to 17 months from hatching and are approximately 50 mm in total length. One young salamander was also observed exhibiting male courtship behavior at one year from hatching.

Two Barton Springs salamanders (1 male, 1 female) in the City's captive breeding program were collected as adults in June 1996. The Dallas Aquarium still has some adult salamanders that were collected as adults in the spring of 1995. Assuming adults were at least a year old when collected, known longevity for Barton Springs salamanders in captivity is at least nine years.

Between January and June 2002, 19 Barton Springs salamanders were found at Upper Barton Springs (17) and Sunken Gardens (2) with a condition known as gas bubble trauma. Several other organisms were also affected. City biologists are still investigating the cause of this unprecedented phenomenon. Of the 19 affected salamanders, 6 recovered and are still alive in captivity (1 later seen courting females, 1 later developed eggs).

During 2002, captive breeding biologists developed a technique to recognize individual salamanders by photographing the unique patterns of melanophores and irdiphores on the head and body. This has enabled biologists to track individual behaviors, movements, and reproductive activity in captivity. Biologists are testing this technique in the field in an effort to develop a mark-recapture program for wild populations.

In the fall 2002, captive breeding biologists received permission from the City of Austin's Parks and Recreation Department to build a permanent salamander facility at the Austin Nature and Science Center. Factors considered in selecting this location include a reliable source of groundwater with water quality similar to Barton Springs, adequate space and infrastructure, and potential to conduct research and public outreach. While the facility will be located at the Austin Nature and Science Center, the Watershed Protection and Development Review Department will assume full responsibility for constructing and maintaining the facility (including permitting, funding, construction, maintenance, staffing, and equipment).

In December 2002, the City contracted with an architectural and engineering firm (Martinez, Wright, and Mendez) to estimate costs associated with building a captive breeding facility at this site. A final report has been prepared. Because the costs will exceed the City's current budget of $154,000, captive breeding biologists are seeking additional sources of funding, including grant funds.

Purpose

Measure 41 of the City's section 10(a)(1)(B) permit (PRT-839031), which authorizes incidental take of the endangered Barton Springs salamander (*Eurycea sosorum*) for the operation and maintenance of Barton Springs Pool and adjacent springs, states:

> "The City of Austin will maintain a viable captive breeding population of Barton Springs salamanders. The City will designate a staff biologist and dedicate a minimum of $20,000 annually to the development and maintenance of this program. The purpose of this program is to provide a contingency plan for the species if a catastrophic event were to occur. Funding and design of the new program will be in place within six months of the issuance of this permit."

The captive breeding program also includes the Austin blind salamander (*Eurycea waterlooensis*), which was discovered as a new species in 2001. In June 2002, the Austin blind salamander was designated as a candidate for classification as endangered (USFWS 2002). The Austin blind salamander is found only at Barton Springs.

In addition to complying with the City's permit, the captive breeding program provides an excellent opportunity to form partnerships with academia, environmental organizations, and government agencies to learn more about the salamanders' biology, habitat requirements, and sensitivity to environmental disturbance and to promote increased awareness of threats to these species and the Edwards aquifer.

Ethics

The City's captive breeding biologists are committed to providing a cruelty-free environment and ensuring the well being of the salamanders. Our goal is to help promote their long-term survival and recovery in the wild through discovery of their biological and ecological requirements, share this information with the human community, foster stewardship of these unique creatures and the Barton Springs watershed, and provide an additional safety net to protect against extinction.

Captive Breeding Facility

In the fall of 2000, captive breeding staff worked with Dr. David Hillis (University of Texas) and Dr. Jerry Fineg (Director of the University of Texas Animal Resources Center) to develop a plan to temporarily house the captive breeding program at the Animal Resources Center (ARC). In February 2001, the salamanders in the captive breeding program were relocated from an office at Two Commodore Plaza to two 75-square foot rooms at the ARC. ARC provides high security and is equipped for aquatic research projects needed for the start-up phase of the captive breeding program. In addition to the ARC, Barton Springs salamanders are on display in a tank at the Splash! Into the Edwards Aquifer Educational Exhibit at Barton Springs Pool.

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In December 2002, the City contracted with an architectural and engineering firm (Martinez, Wright, and Mendez) to estimate costs associated with building a captive breeding facility at this site, and a final report has been prepared. Because the costs will exceed the City's current budget of $154,000, captive breeding biologists are seeking additional sources of funding. In January 2003, biologists submitted a Conservation Action Grant proposal to the Texas Parks and Wildlife Department. Funding sources for this grant program come from the U.S. Fish and Wildlife Service and the Horned Lizard License Plate fund, created by the Texas Parks and Wildlife Department and Texas Department of Transportation. Grant awards will be announced in 2003.

Equipment and Maintenance

The City currently maintains two custom-made artificial spring upwellings and five commercially manufactured (one 60-gallon, four 75-gallon) glass tanks. All aquaria are maintained at 69-71^{o}F by 1/6 hp Delta-star titanium water chillers and filtered by Eheim canister filters. Lighting is provided by full-spectrum and fluorescent ceiling lights that are set on a timer to provide lighting patterns intended to simulate day and night. A Hydrothrustor-Q System 500 pump provides a continuous flow rate of 3-7 gallons per minute through the 60 and 75-gallon tanks. Water cascades into each of the 60 and 75-gallon tanks through an elevated PVC pipe, providing a splash effect to help aerate the water.

<u>Upwelling Tanks (2)</u> - The two upwelling tanks were made by the University of Texas Biology and Chemistry Machine Shop in the spring of 2001 and are based on a design that has been successful in cultivating *Eurycea* salamanders at the Dallas aquarium (Roberts et al. 1995). Each upwelling consists of a 36" long 6" interior diameter acrylic tube with a 5-gallon tank attached to the top of the tube. The tube is filled with rocks and the top tank is filled with rocks and moss. Water is pumped up through the tube at a flow rate of 1.5 gallons per minute and exits through a screened overflow near the top of the aquarium. The tube is covered with black plastic to simulate the darkness of the aquifer. One egg-laying event occurred in one of these upwelling tanks in May 2001. The F1 ("first generation") salamanders from this clutch remained in the upwelling throughout 2002. The other upwelling tank housed wild-caught salamanders from Barton Springs Pool (2 females, 1 male) until September 2002, when they were transferred to the Barton Springs Pool tank. F1s from a December 2001 clutch were subsequently placed in this upwelling.

Barton Springs Pool Tank – On January 31, 2002, captive breeding biologists set up a 75-gallon tank designed to provide diverse habitat types and simulate aquifer, spring upwelling, and spring run conditions. A black Plexiglas platform was placed in one end of the tank, and the area under the platform was kept dark ("aquifer") by wrapping the outside of this section with black plastic or cloth. Large limestone rocks were placed between the edge of the platform and the remainder of the tank. A hole drilled in the aquarium under the platform allowed water to flow upward through a separate pump system (flow rate about 1 gallon per minute) and exit the "aquifer" through several large holes in the platform, which was layered with rocks and moss ("spring"). An elevated PVC pipe provided a waterfall just beyond the platform to simulate a "spring run." Gravel, cobble, large limestone rocks, plastic plants, and moss (*Amblystegium riparium*) were provided throughout this portion of the tank. Captive breeding biologists initially placed 7 adult Barton Springs salamanders from Barton Springs Pool (4 males, 3 females) in this tank and added 4 adults (2 males, 2 females) at the beginning of September 2002. The first egg-laying occurred on Valentine's Day, 14 days after the salamanders were first introduced, culminating in 12 egg-laying events over an 8-month period. In late October 2002, captive breeding biologists began noticing some of the salamanders in this tank with missing digits on their front and back limbs. This may have been due to a bacterial or fungal infection. The tank was disassembled and disinfected on November 25, 2002. Only a few small rocks and moss were placed back in the tank to facilitate tank cleaning and observation of salamander health; the sparse habitat will be maintained until captive breeding biologists are certain that all salamanders are fully recovered. No egg-laying events have occurred in this tank since the tank was reassembled with less extensive habitat.

Sunken Gardens, Upper Barton Springs, and Austin Blind Salamander Tanks - In August and September 2002, captive breeding biologists set up three other tanks based on the Aquifer/Spring/Spring run prototype. Two 75-gallon tanks are similar in design. Ten adult Barton Springs salamanders from Sunken Gardens (4 males, 3 females, 3 undetermined) were introduced to one tank on September 11, 2002, and 15 adult Austin blind salamanders were introduced to the other tank on September 16, 2002. The 60-gallon tank does not have a platform or separate pump system providing water flow from the bottom of the tank, but has gravel, cobble, large limestone rocks, plastic plants, and moss. Barton Springs salamanders from Upper Barton Springs were added to this tank on August 27, 2002. Between August/September through December 31, 2002, 3 egg-laying events occurred in the Upper Barton Springs tank and 2 occurred in the Sunken Gardens tank.

Container Tank – One 75-gallon tank is used to house salamanders separately by size class, sex (for individuals from the same clutch to prevent inbreeding), collection site, or for health reasons. Small (~ 20 mm or less) salamanders are housed in plastic containers without holes to prevent injury or escape. Salamanders that may have a pathogen or other illness are also isolated in containers without holes. Larger (>25 mm) salamanders that appear to be healthy and robust are placed in containers with multiple holes drilled along each side to allow water to flow through. Each container is covered with a tight-fitting plastic lid. An airstone is placed in each container to provide aeration.

Water for all aquaria comes from Barton Springs Pool. To prevent salamanders from climbing out, secure lids are fitted to each tank and the water level is at least several inches from the top. Maintenance is performed on the tanks every week to 10 days. Captive breeding biologists siphon and replace about 10% of the water from each tank, removing algae and vacuuming the bottom to remove excess food, debris, and wastes. Water changes in individual containers without holes are done every one to three days.

Based on observations of wild and captive individuals, both Barton Springs salamanders and Austin blind salamanders are frequently found in *Amblystegium riparium*. This aquatic moss is commonly found growing on bare rock at the spring outlets and appears to be an important habitat component. The moss provides cover and harbors a variety and abundance of aquatic invertebrates for the salamanders to feed on. The aquatic moss is also an important habitat component for the captive breeding program and is provided in each tank and container.

Salamanders are generally fed every two to three days. Adults are fed commercially available live blackworms (*Lumbriculus* sp.) and invertebrates collected from Sunken Gardens and Eliza Springs. Newly hatched salamanders are fed tiny, wild-caught invertebrates daily or every other day. Larger juveniles are fed wild-caught invertebrates and/or small pieces of blackworms every two to three days.

Wild-caught invertebrates from Sunken Gardens and Eliza Springs are maintained with *Amblystegium riparium* and leaf litter in coolers and large plastic tubs filled with spring water. Airstones and sponge filters are used to keep the water aerated and clean. Small water changes are done frequently. Small amounts of fish flakes are occasionally provided. The abundance of tiny amphipods and other invertebrates indicates reproduction of prey species is occurring in this system.

Captive breeding biologists check on the salamanders daily to ensure proper functioning of all equipment and health of each salamander.

Collections

The City is currently housing 100 salamanders (85 Barton Springs and 15 Austin blind) (Tables 1-3). Ninety-five salamanders (80 Barton Springs and 15 Austin blind) are housed at ARC. The Splash! exhibit has 5 Barton Springs salamanders in its display tank. The combined space at ARC and Splash! is at maximum capacity, and additional space is needed to expand the captive breeding program.

Of the 85 Barton Springs salamanders, 33 are wild-caught (12 from Barton Springs Pool, 1 from Eliza Spring, 13 from Sunken Gardens, 7 from Upper Barton Springs) and 52 (43 F1s, 9 F2s) were bred in captivity (Table 1). Of the captive-raised salamanders, 34 are F1 (“first generation”) progeny from Barton Springs Pool founders, 9 were reared at the Dallas Aquarium from Barton Springs Pool and Sunken Gardens founders, and 9 are F2s (“second generation”) from the Dallas Aquarium F1s. All 15 of the Austin blind salamanders are wild-caught (2 from Barton Springs Pool, 13 from Sunken Gardens).

City biologists collected the first Barton Springs salamander egg ever found in the wild during a survey of Sunken Gardens on May 31, 2002 (Table 2). The egg was found on sediment next to a spring upwelling. The egg hatched in captivity on June 19, 2002. The juvenile Barton Springs salamander is still being maintained in the captive breeding program. A second egg was found along a fissure in Barton Springs Pool on December 31, 2002. The well-developed larva hatched within minutes in a dipnet and swam into the fissures area.

Thirty salamanders (23 Barton Springs and 7 Austin blind) were collected in 2002 (Table 2). Of the 23 Barton Springs salamanders, 19 were found with gas bubble trauma at Upper Barton Springs (17) and Sunken Gardens (2). Of these 19 affected salamanders, 7 were found dead, 5 ere died shortly after collection, and 6 recovered (2 from Sunken Gardens and 4 from Upper Barton, including 1 juvenile) and are still alive (1 later seen courting females, 1 developed eggs in captivity); 1 apparently recovered but died 3 months later from unknown causes. Other collections in 2002 include a small juvenile (17mm) Barton Springs salamander found ventral side up due to unknown causes during a survey of Sunken Gardens; this salamander recovered in captivity. Only 3 salamanders were collected alive and healthy in 2002, including the egg collected on May 31 from Sunken Gardens, one adult Barton Springs salamander from Upper Barton Springs, and one juvenile found in moss collected for invertebrates from Sunken Gardens.

To date, Austin blind salamanders, which have never been found at Upper Barton Springs, have not been observed with gas bubble trauma. Of the 7 Austin blind salamanders collected in 2002, four were small juveniles ($\leq$21 mm) that later died in captivity. The other 3 were collected as adults (>67 mm) and are still alive. One of the small juveniles was found during a survey of the beach in Barton Springs Pool, and one adult was found near the main fault in the pool following drawdown for cleaning. The other five Austin blind salamanders were collected from Sunken Gardens (tables 1 and 2).

Salamanders are collected from the wild with small aquarium nets and placed in small plastic containers with water from the collection site. The container is sealed with a lid and placed in a cooler containing spring water to maintain the temperature. Salamanders are immediately transported to the ARC. A battery-operated aerator is used to aerate the water in the event of a time delay. Once the collected salamander arrives at the ARC, the salamander is acclimated to the tank water with small water changes for a period of about 30 minutes. The collected salamander is placed in a container without holes overnight so any fecal pellets can be siphoned out and analyzed for food eaten to determine salamander food items.

The City's federal permit requires the City to maintain a viable captive breeding population of Barton Springs salamanders. Although the permit does not define "viable," a general rule of thumb commonly obtained from conservation biology literature prescribes a minimum short term effective population of 50 individuals to prevent unacceptable inbreeding and a minimum long-term effective population of 500 to maintain overall genetic diversity (Franklin 1980, Soule 1980). Effective population generally refers to individuals that contribute offspring to a population. If only 10% of the individuals in a population reproduce, the 50/500 rule would translate to a short-term minimum viable population (MVP) of over 500 individuals. Other MVP estimates from the literature range from 1,000 to 10,000 adults. MVPs have been the subject of considerable debate, and population genetics research and population viability analyses would be needed to develop better MVP estimates for the Barton Springs and Austin blind salamanders. Regardless, population viability depends on reliable breeding success. Furthermore, although 500 individuals provides a target for the captive breeding program, collecting this number of salamanders could deplete the wild populations. Thus, the City is currently focusing on conducting research needed to ensure a successful captive breeding program, collecting individuals slowly over time, building a larger facility to support larger numbers of salamanders, and providing a contingency plan should an emergency situation (such as a catastrophic spill) require immediate collection of large numbers of salamanders from the wild.

USGS Patuxent Wildlife Research Center has applied for a grant to conduct a population viability analysis as an initial step to help estimate target numbers needed for the captive breeding program. If grant funds are awarded, this work would be conducted later in 2003. Captive breeding biologists hope to initiate population genetics research through the Barton Springs Salamander Conservation Fund.

Individual Identification

In 2002, captive breeding biologists developed a technique to identify individual salamanders based on unique patterns of melanophores and iridiphores on the head and body. This pattern recognition technique is used to identify individuals in the captive breeding program, and the feasibility of applying it in the field for mark-recapture studies is being tested. Photographs are taken of each individual using a Nikon N90S camera with a 105mm macro lens and a Coolpix 995 digital camera. A Sony digital video camera is also used to videotape individual salamanders.

Reproductive Activity

Sexual dimorphism: The only obvious sign of sexual dimorphism in Barton Springs salamanders is the appearance of eggs, which are visible through the translucent skin covering the female's body cavity. However, since adult females may reabsorb eggs and have been observed in what appears to be courtship behavior with other females, the sex of salamanders without eggs is difficult to ascertain. In general, large (i.e., greater than 50 mm) salamanders that have not developed eggs in captivity for long periods of time (for example, a year or more), and/or have been observed exhibiting male courtship behaviors (tail undulation) are assumed to be males. For all other Barton Springs salamanders without eggs, the sex is treated as undetermined.

Age and size at first reproduction: Within the plethodontids (lungless salamanders), species that have aquatic larvae generally mature earlier (0.5-4 years for males and 0.5-5 years for females) than do those having direct development of terrestrial eggs (Duellman and Trueb 1986). Nine of the wild-caught Barton Springs salamanders developed eggs in captivity within 2 to 17 months from the date of collection, but their ages at the time of collection are unknown (Table

2). Four captive-raised salamanders (3 F1s, 1 F2) developed eggs within 11 to 17 months from hatching (clutches hatched May 2001, June 2001, December 2001/January 2002) (Table 3). These salamanders were approximately 50 mm in total length at the time they developed eggs. At least 4 captive-raised salamanders (1 F1, 3 F2s) from clutches hatched in February 1999, May/June 1999, and June 2001 are believed to be males based on size, no visible sign of eggs, and/or observations of male courtship behavior (Table 3). One young salamander was observed exhibiting male courtship behavior at one year from hatching.

In February 2003, one of the 15 Austin blind salamanders in the captive breeding program began developing eggs (Table 2). This is the first Austin blind salamander ever observed with eggs and was collected without eggs on May 2, 2002. No other signs of reproductive activity have been observed in any wild or captive Austin blind salamanders.

Courtship: Courtship behavior has been observed between most male and female pairs of captive Barton Springs salamanders. Courtship consists of the tail-straddling walk described by Arnold (1977), which is unique to plethodontids. The tail-straddling walk is characterized by the female straddling the male's tail and rubbing her chin on the dorsal base of his tail while the male walks slowly forward. At times during the walk, the male undulates the base of his tail laterally, possibly dispersing pheromones or to orient the female in relation to the spermatophore which is deposited on the substrate. Captive breeding biologists have observed pairs engaged in the tail-straddling walk for several minutes to more than an hour. Other courtship behaviors between males and females include the male rubbing his chin across the dorsal area of the female's head and vice versa.

Salamanders may rely on chemical cues to distinguish between sexes and species and to orient themselves during the tail-straddling walk (Arnold 1977). Some plethodontids possess mental glands on their chin and premaxillary teeth, which are used to deliver secretions to the female. Although the Barton Springs salamander does not have glands that are visible to the human eye, captive breeding biologists have observed behaviors that suggest delivery of secretions via mental glands as well as premaxillary teeth consistent with the "head-slap" behavior. The function of this "head-slap" behavior is not well understood but has been suggested as a means of delivering mental gland secretions to the female (Arnold 1977) via premaxillary teeth.

Captive breeding biologists have observed what appears to be sexual interference. On one occasion, a male Barton Springs salamander interrupted a male-female pair engaged in the tail-straddling walk. After delivering what appeared to be a "head-slap" to the female, the intruding male replaced the female's position and engaged in the tail-straddling walk with the original male. Sexual interference has been observed in other salamander species and can be accomplished by stealing a female, disrupting the spermatophore deposition of a rival male, covering the spermatophores of rival males, or duping rival males into unprofitable spermatophore depositions (Duellman and Trueb 1986).

Courtship behaviors in Barton Springs salamanders have been observed in different types of aquaria, from small, 2-liter plastic containers to 75-gallon tanks. A male and female that recovered from gas bubble trauma at Upper Barton Springs have been observed courting.

Individuals also engaged in courtship activity immediately after handling and transfer to the new Upper Barton Springs and Sunken Gardens tanks. In 2002, captive breeding biologists videotaped unusual courtship interactions in the Container Tank, one involving 3 Barton Springs salamanders (1 male, 2 females) in one container and the other between 2 females in a separate but adjacent container. These interactions occurred simultaneously in plastic containers with holes that allow for water exchange. Interactions between two females housed in one container included chin rubbing across the dorsal area of the head and the base of the tail. In one instance an encounter was observed during courtship behavior involving two females and one male in which one female struck the head of the second female with her snout. The female which struck the second female laid eggs on two occasions in the Sunken Gardens tank.

<u>Sperm transfer and fertilization</u>: At some point during the tail-straddling walk, the male deposits a spermatophore (sperm packet attached to a glycoprotein base), which is picked up by the female and stored in a specialized portion of her cloaca, known as the spermatheca. Some species of female salamanders are known to store spermatophores for up to 2.5 years before fertilization and ovulation occur (Duellman and Trueb 1986). Females of some species may also store more than one spermatophore by one or different males (Houck et al. 1985a, 1985b). Substrate may play a role in successful spermatophore transfer; in salamander species that anchor the spermatophore, it may be necessary to have a roughened (not smooth, such as glass) substrate (Wright 2001). To date, captive breeding biologists have not observed a spermatophore or sperm transfer. In most salamanders, fertilization is internal and occurs during oviposition, when sperm are released onto eggs as they pass through the female's cloaca (Sever 2000). In 2001, a female Barton Springs salamander placed in an upwelling tank oviposited viable eggs after one month of being in the tank by herself, thus indicating that Barton Springs salamanders can store sperm for at least this length of time.

<u>Ovipositions (egg-laying)</u>: During 2002, captive breeding biologists focused efforts on creating environments that would induce oviposition. A total of 18 egg-laying events occurred in four tanks (12 Barton Springs Pool tank, 3 Upper Barton Springs tank, 2 Sunken Gardens tank, 1 Splash! exhibit tank) (Table 4). In the Barton Springs Pool tank, 2 of the 3 females laid at least 11 of the 12 clutches over an 8-month period. One female laid 5 of 6 clutches that were 35 to 42 days apart, and the other female laid 5 of 5 clutches 53 to 59 days apart. These two females often laid eggs within a week of each other, with intervals between each clutch ranging from 6 to 53 days (mean = 21.64 days). Four intervals between clutches were 6-7 days apart. In the Upper Barton Springs tank, 2 to 3 females have laid eggs since it was set up on August 27, 2002. Egg-laying events have been 15 to 71 days apart. In the Sunken Gardens tank, 1 of 3 females has laid 2 clutches since it was set up on September 11, 2002. These two events were 72 days apart. A female from the exhibit tank at Splash! laid eggs at the beginning of 2002; the male was subsequently removed, and no further egg-laying has occurred in this tank.

Clutch sizes for all tanks ranged from 11 to 39 eggs (mean = 24.39, standard deviation (std) = 9.38) (Table 4). Clutch sizes ranged from 11 to 39 eggs (mean = 23.75, std = 10.46) for the Barton Springs Pool tank, 17 to 29 eggs (mean = 23.00, std = 6.00) for the Upper Barton Springs tank, and 20 to 27 eggs (mean = 23.50, std = 4.95) for the Sunken Gardens tank. Clutch sizes for the two most prolific females from the Barton Springs Pool tank ranged from 11 to 39 eggs for one female (mean = 25.50, std = 12.86) and 15 to 33 eggs (mean = 22.60, std = 9.07) for the other. Barton Springs salamanders may lay all or only a few of their eggs, and most females quickly (within a few weeks) redevelop their eggs after oviposition. Clutch sizes are likely underestimates because not all areas of the habitat are visible to the biologists.

Based on egg-laying observations during 2002, space and habitat heterogeneity appear to play an important role in influencing ovipositing. Another important factor may be the inclusion of gravel on the bottom of the tanks, which may provide an appropriate substrate for spermatophore deposition and transfer. Although courtship activity has been observed in the Container Tank, egg-laying has never occurred in the small plastic containers. The Barton Springs Pool tank has produced the greatest number of egg-laying events, and egg-laying occurred in the Sunken Gardens and Upper Barton Springs tanks soon after they were set up. The last egg-laying event in the Barton Springs Pool tank occurred on October 10, 2002, before it was disassembled for disinfection on November 25, 2002. Other than a few small rocks and moss, most of the habitat has not been replaced (including the platform and upwelling that provided the "aquifer" and "spring" areas, gravel/cobble substrate, large limestone rocks) to facilitate tank cleaning and observation of salamander health until captive breeding biologists are certain that all salamanders are fully recovered.

Some females may be more successful breeders than others. Clutches were laid by 2 of the 3 females in the Barton Springs Pool tank between February and August 2002 (Table 4). These same 2 females each produced an additional clutch after other females (total of 5 females in the tank) were added to the tank in early September 2002. In the Upper Barton Springs tank, 2 and possibly all 3 of the gravid females laid eggs. In the Sunken Gardens tank, 1 of the 3 females has laid eggs. The tendency for one or a few females to lay eggs could be due to territoriality, and additional space may be necessary for the other females to lay eggs. This is a research priority that will require expansion of the captive breeding program.

Captive breeding biologists have videotaped some of the egg-laying events. Females move about the entire tank and lay eggs singly on a variety of substrates and in different areas of the tank. Laying a single egg occurs in minutes, and the entire egg-laying event takes hours, depending on clutch size. Over half of the eggs observed in 2002 were laid on moss and plastic plants, while the remainder were fairly evenly distributed on gravel, cobble, netting, Plexiglas, and prefilter substrates. Eggs were rarely laid on glass. Eggs were most commonly laid in the "aquifer" section and on the opposite end of the tank, which contains the intake for the pump system. This may be related to flow patterns in the tank and distribution of food and cover. Multiple factors were not quantified that could undoubtedly influence egg loss; such factors include habitat variation in the tank, flow, and salamander pathways. In general, however, preliminary information on egg loss before day 16 (earliest recorded hatching day) indicates that egg loss may be higher on gravel and glass substrates and lower on large limestone rocks in this tank design, but further investigation is necessary.

Egg morphology and development: Like most amphibian eggs (Duellman and Trueb 1986), the salamander egg consists of an ovum surrounded by a series of concentric capsules. Barton Springs salamander ova are white and generally encompassed by three capsules. These protective capsules are semipermeable to allow gas exchange and waste elimination necessary for embryo development (Duellman and Trueb 1986). Eggs are generally about 3 mm in diameter. The embryo in the egg collected from Sunken Gardens on May 31, 2002 was 3 mm in diameter, and the diameter of the outer egg capsule was 7 mm. Embryos begin to develop some pigmentation during the later stages of development. Captive breeding staff has videotaped egg development.

Cannabalism: Cannabalism of juveniles and adults has never been observed in Barton Springs salamanders, and only one adult salamander has only been observed eating an egg on one occasion. An egg (laid by a different female) became stuck to the end of a female's snout while she was moving along the substrate. She appeared to try to shake the egg off before consuming it. Whether this was an intentional or accidental act is unknown. Adults have been observed walking directly next to juveniles without consuming them. Regardless, eggs and young frequently disappear when adults are present in the tank. Of the 285 eggs laid in the Barton Springs Pool tank in 2002, only 12 juveniles (4%) survived through the end of December (Table 4). Eleven of 25 eggs (44%) from the April 2001 upwelling clutch hatched and survived while the female was still in the tank with the small juveniles for a few weeks. Twelve of 21 eggs (57%) from the December 2001 clutch hatched and survived for several months in a tank where the adults were removed soon after the eggs were laid. Additional space is needed to increase survivorship from the egg to adult stage.

Hatching: Overall, hatching of Barton Springs salamanders in captivity has occurred in the range of 16 to 39 days. Because the new tank designs provide many hiding places for salamanders, hatching success is difficult to determine. Thus, if a developing egg disappeared at day 16 or later, it was assumed to have hatched. Therefore, hatching success estimates for the 2002 clutches represent an overestimate. Estimated hatching success for the 18 clutches in 2002 ranged from a maximum of 10 to 100 percent (mean = 44%) (Table 4). Hatching success for the Barton Springs Pool tank ranged from a maximum of 10 to 100 percent (mean = 49%), 15 to 67 percent (mean = 41%) for the Sunken Gardens tank, 23 to 30 percent (mean = 26%) for the Upper Barton Springs tank, and 57% for the Splash! exhibit tank. Most clutches included one or more eggs that did not develop. Some eggs may have been infertile, some developed fungus, which may halt development, or some other factor may have reduced or halted egg development. The maximum length of time from egg-laying to hatching for the 18 clutches in 2002 varied from about 23 to 37 days (Table 4).

Juvenile survival: The City's captive breeding biologists have generally found the first three months following hatching to be a critical period for juvenile survival. Again, because of the multiple hiding places in the new tanks, juvenile survival is not possible to determine without disassembling the entire tank. Of the 285 eggs laid in the Barton Springs Pool tank in 2002, 12 juveniles (4%) survived through the end of December (Table 4). The number of juveniles in the Sunken Gardens and Upper Barton Springs tanks has not been determined, but juveniles are seldom seen, indicating low juvenile survivorship.

Juvenile Development and Growth Rates

Newly hatched Barton Springs salamanders are generally about 13 mm from snout to tail (total length). Growth rates vary considerably. Individuals from the same clutch may be two to three times as long as their siblings, while other siblings be small and/or emaciated. After 4 months, individuals in the December 4, 2001 clutch raised in a 75-gallon glass tank represented a large range in sizes with the smallest individual being approximately one-half inch in total length and being about one-third the length of its largest sibling. In contrast, a group of F2's raised in small containers (mostly plastic) after 5 months were found to be 17-27mm, representing the smaller end of the growth range of that found in the 75-gallon glass tank.

Differences in growth rates warrant further analysis and investigation. If the differences are significant, underlying causes could be due to space, flow, container material (plastic vs. glass), and/or other factors. Captive breeding biologists spend considerable time providing an abundance of small invertebrates (ostracods, copepods, and primarily amphipods) for the juveniles; however, further investigation needs to be conducted to determine if some food items are more effective than others.

In previous years, Austin blind salamander juveniles collected in the wild at about 15mm in total length grew rapidly for about 8 months to about 60 mm in total length, after which growth slowed to about 1 mm per month. All four Austin blind salamander juveniles collected in 2002 failed to grow significantly and died within a few months (Table 2) One juvenile measured 17 mm when it was collected from Sunken Gardens on September 21, 2001, and only 18 mm when it was found dead 132 days later on January 31, 2002. Juveniles initially appeared to feed and do well but eventually weakened and died. The cause for the mortalities is unknown and warrants further investigation.

Mortality

All four juvenile Austin blind salamanders (<21 mm) collected in 2002 died within a few months from unknown causes (Table 2). One of these had been collected in moss from Sunken Gardens and was placed in the Barton Springs Pool tank, but was not seen again. In addition to the 4 Austin blind salamanders collected in 2002, two juveniles (17 mm, 28 mm) collected on September 9, 2001, died in early 2002 from unknown causes.

One of the Barton Springs salamanders in the City's captive breeding collection (male collected June 1996) died from injuries that occurred in the Barton Springs Pool tank (Table 2). The salamander wedged himself between a Plexiglas divider and the divider tract and was not able to move himself out of the small space. The divider was moved, which allowed the salamander to free himself, but its back legs were red and swollen, and it died a few days later (August 9, 2002). The divider, which was intended to help keep the "Aquifer" section of the tank dark, was replaced with large limestone rocks.

A female Barton Springs salamander from Barton Springs Pool died about two months after being removed from an upwelling tank in late August 2002 (Table 2). She was one of 3 salamanders that were moved from this tank to the Barton Springs Pool tank. While the other 2 salamanders would move throughout the upwelling tube and top tank, this female remained on the bottom of the 36" long tube despite several days of trying to encourage her to surface. All of the rocks in the tube were removed one by one before she could be collected. The move appeared to have injured the female's tail, which was bent near the tip. After moving her to the new tank, she became more lethargic and began holding her mouth open continuously. She was removed to the Container Tank for closer observation in late October and died on November 2, 2002.

In addition to wild-caught salamander mortalities, 1 female F1 (Dallas progeny) Barton Springs salamander was found dead in a plastic container in the Container Tank on April 5, 2002 (Table 3). The death is believed to have been due to elevated ammonia levels, although the water was being changed daily or every other day. The other F1 female in the container was revived with water changes and is still alive.

Longevity

Two Barton Springs salamanders (1 male, 1 female) in the City's captive breeding program were collected as adults in June 1996 (Table 2). The Dallas Aquarium still has a few adult salamanders that were collected as adults in the spring of 1995. Assuming adults were at least one year old when collected, known longevity for Barton Springs salamanders in captivity is at least nine years.

Health Problems

In 2002, nineteen salamanders were found at Upper Barton Springs with a condition that appeared to be gas bubble trauma. Twelve of these were found dead or near death. The pathology reports for the Barton Springs salamanders sent to the USGS National Wildlife Health Center showed no parasites or other pathogens as causative agents. The pathologist stated that he could not explain the presence or location of the bubbles in the salamanders and assumed that the cause must be gas bubble trauma. Gas bubble trauma is a noninfectious condition, resulting from uncompensated hyperbaric pressure of total dissolved gases, which produces primary lesions in blood (emboli) and in tissues (emphysema) and subsequent physiological dysfunctions. In animals exposed to supersaturated water, gas bubble trauma results from the formation and accumulation of bubbles under the skin and within the vascular system reducing and/or blocking blood flow, and potentially leading to hemorrhaging. Progressive hemorrhaging may result in large areas of eroding and necrotic skin, making the animal more susceptible to other problems such as fungal infections. Supersaturation occurs when the partial pressures of the dissolved gases exceed their respective partial pressures in the atmosphere. This condition is similar to human decompression sickness ("the bends") experienced by scuba divers. Supersaturated groundwater may result from high pressures and/or increases in temperature. However, City biologists have been unable to find any other report of gas bubble trauma in organisms that are endemic to naturally-occurring supersaturated waters.

Salamanders with mild gas bubble trauma had the appearance of “blisters” in the tail or large bubbles in the throat and body cavity. Seven of the 19 affected individuals (twelve were found dead or near death) later recovered in captivity (Table 2). The increased buoyancy and impaired mobility may make affected salamanders vulnerable to predation, impair feeding, and reduce their chances of survival in the wild. Progressed stages included bubbles throughout the entire body, including the limbs, and hemorrhaging. The skin of some individuals ruptured, and a fungus attacked the ruptured areas. One individual was found still alive the morning after collection in an inverted position with fungus along its ventral side where the skin had split open.

Gas bloat is also observed in captive salamanders, but not to the same degree as those found with gas bubble trauma in the wild. Gas bubble formation in captivity appears to be a physiological response to changes in temperature, aeration levels, flow regimes, and/or stress. The gas bubbles typically appear in the throat and body cavity. Affected individuals often float at the surface of the water in a “comma-shaped” position with the head at the surface and the tail pointing down. Some individuals exhibit gas bubble formation without any obvious signs of environmental change while other salamanders in the same tank appear unaffected, indicating that some individuals may be more prone to this condition than others.

Problems other than gas bubble trauma were observed in 2002. One F1 individual in captivity (December 4, 2001 clutch) was found ventral side up on January 31, 2002. Microscopic examination revealed a live mite in its abdomen. Whether this was the cause of the condition is unknown. Although the mite was gone on February 1, 2002, the salamander was still ventral side up and lethargic. It did not begin moving until February 4, and appeared to be recovered by February 17, 2002.

In summer 2001, captive breeding biologists noticed a bend in the abdomen of one of the F1 juveniles (about 13mm) from the April 2001 clutch. In October 2001, this individual was removed and maintained in the Container Tank for closer observation. Other than the bend in the abdomen, it appeared to feed normally and was placed back in the upwelling in June 2002. In December 2002, captive breeding biologists began noticing physiological problems in all of the salamanders in this upwelling, including what appeared to be partial paralysis in the lower body, inability to move the back legs, and erratic or lethargic swimming behavior. The cause of these conditions is unknown. All of the F1s have since been removed and are being monitored in the Container Tank.

A juvenile Barton Springs salamander was found ventral side up during a survey of Sunken Gardens on June 25, 2002 (Table 2). The salamander was collected and recovered in captivity. This same phenomenon was also observed in 3 Barton Springs salamanders (2 from Sunken Gardens, 1 from Eliza) in 2001, all of which recovered and one of which laid two clutches of eggs (Sunken Gardens tank) in 2002.

In late summer 2002, one of the F1 (Dallas progeny) Barton Springs salamander females in the Splash! display tank developed a growth on one side of her abdomen. The growth continued to enlarge but the salamander engaged in normal behaviors, such as feeding. As of December 31, 2002, the salamander was being monitored closely.

In late October 2002, captive breeding biologists observed several salamanders in the Barton Springs Pool Tank with all or parts of digits missing on the front and/or back feet. This condition affected primarily the front feet, but digits were also missing on the back feet of some individuals. One individual eventually lost all of its toes on one front foot while the others appeared unaffected. The tank was disassembled and disinfected on November 25, 2002, and salamanders were reintroduced on November 27, 2002. Affected individuals regenerated missing digits and appeared to be recovered by December 31, 2002. This condition also appeared in 5 adult salamanders in 2001 (Chamberlain and O'Donnell 2001), and some of the individuals affected in 2001 were also in the Barton Springs Pool tank. The 5 salamanders were in a compartment opposite from where the water cascades into the tank, while individuals in the remainder of the tank appeared to be healthy. While the Barton Springs Pool tank does not contain discreet compartments, some individuals tend to reside in particular areas. Individuals that resided in the end opposite from where the water cascades into the tank also appeared to be most affected, while several individuals observed in the other end of the tank appeared normal. While the cause of digit loss is unknown, captive breeding biologists suspect it is due to bacterial or fungal infection.

Behavior

With the development of the "marking" techniques, captive breeding biologists are able to distinguish and track individual salamanders in captivity and have been observing what appears to be site fidelity in some individuals. An adult female from one of the upwelling tanks remained on the bottom of the tube and had to be physically removed to transfer her to another container. In the Barton Springs Pool Tank, an adult male was seen on top of the platform for the entire period before the tank was disassembled. On the few occasions when the platform had to be removed, the male clung to the platform until it was taken out of the tank. A male in the Sunken Gardens tank has been observed courting females under the platform and is almost always seen in this location. Other salamanders appear to move about the entire tank, including adult males and females. The tendency of one or a few females to be the primary breeders in a given tank could also be due to spacing behaviors.

Personnel

Dee Ann Chamberlain and Lisa O'Donnell, environmental scientists, Watershed Protection and Development Review Department, manage the captive breeding facility and the tanks at the ARC. Michael Adair, exhibit specialist, Parks and Recreation Department, maintains the display tank at Splash! His expertise and assistance continues to be a great asset to the captive breeding program.

Budget

Funding for the captive breeding program was provided in the FY 2002 operating and capital budgets of the City of Austin's Watershed Protection and Development Review Department. The City of Austin provided an additional $154,000 for a captive breeding facility from the sustainability fund in FY 2000-2001. The City remains committed to providing $20,000 annually for the program for the duration of its permit (through FY 2012-2013).

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Table 1. Inventory of salamanders in the City of Austin's captive breeding program, as of December 31, 2002.

	# Wild-caught (founders)	# First Captive Generation (F1s)	# Second Captive Generation (F2s)	TOTAL
E. sosorum				
Barton Springs Pool	12	34	0	46
Eliza Spring	1	0	0	1
Sunken Gardens Spring	13	0	0	13
Upper Barton Springs	7	0	0	7
Dallas Aquarium	0	9*	9**	18
Total	33	43	9	85
E. waterlooensis				
Barton Springs Pool	2	0	0	2
Eliza Spring	0	0	0	0
Sunken Gardens	13	0	0	13
Upper Barton Springs	0	0	0	0
Total	15	0	0	15
Grand Total	**48**	**43**	**9**	**100**

*Founders of the F1s raised at Dallas Aquarium were collected from Barton Springs and Sunken Garden in 1995. In May 1998, the Dallas Aquarium donated the F1s to the Splash! Educational Exhibit.

**From Dallas Aquarium F1 salamander ovipositions at Splash! in 1999, 2001, and 2002.

Table 2. Wild-caught salamanders currently housed at ARC and 2001-2002 collections.

Species/Individual	Collection Site	Collection Date	Total Length (mm)	Sex	Comments
E. sosorum	Barton Springs Pool	06/21/96	> 25	F	Developed eggs in captivity (11/97); laid eggs 04/99; developed eggs again; reabsorbed eggs 04/02; developed eggs 02/03
	Barton Springs Pool	06/21/96	> 25	M	
	Barton Springs Pool	06/21/96	>25	M	Died 08/09/02; wedged body between tank divider and divider tract; injury near back legs
	Sunken Gardens	08/11/00	>25	M	
	Barton Springs Pool (drawdown)	11/07/00	>25	F	Collected gravid; laid eggs 04/01; Died 11/02/02
	Upper Barton Springs	11/30/00	>25	F	Developed eggs in captivity (5/01); laid eggs 12/02
	Upper Barton Springs	11/30/00	>25	F	Collected gravid; laid eggs 10/02
	Sunken Gardens	12/28/00	>25	M	
	Barton Springs Pool (drawdown)	01/12/01	65	F	Developed eggs in captivity; laid eggs at least 6 times in 2002
	Barton Springs Pool (drawdown)	01/12/01	60	F	Collected gravid; laid eggs at least 5 times in 2002
	Barton Springs Pool (drawdown)	01/12/01	47	F	Developed eggs in captivity
	Barton Springs Pool (drawdown)	01/12/01	45	M	
	Barton Springs Pool (drawdown)	01/12/01	35	M	
	Sunken Gardens	02/23/01	> 25	M	
	Barton Springs Pool (drawdown)	02/26/01	> 25	F	Developed eggs in captivity (8/01)
	Barton Springs Pool (drawdown)	02/26/01	> 25	F	Developed eggs in captivity (9/01); laid eggs 12/01
	Barton Springs Pool (drawdown)	02/26/01	> 25	M	
	Barton Springs Pool	03/26/01	> 25	M	

	Sunken Gardens	05/02/01	42	F	Collected gravid
	Sunken Gardens	07/13/01	13	M	
	Sunken Gardens	08/02/01	> 25	F	Found lethargic, ventral side up; recovered in captivity by 11PM; developed eggs in captivity (10/01)
	Sunken Gardens	08/02/01	> 25	F	Found lethargic, ventral side up; recovered in captivity by 11PM; developed eggs in captivity (4/02); laid eggs 10/02 and 12/02
	Eliza	08/02/01	> 25	U	Found lethargic, ventral side up; recovered in captivity by 11PM
	Sunken Gardens	08/10/01	32	M	
	Barton Springs Pool (drawdown)	08/27/01	> 25	M	Found with gas bloat; recovered in captivity a few days later
	Upper Barton Springs	01/28/02	>25	M	Found with gas bubbles in tail
	Upper Barton Springs	02/22/02	>40	U	Found with gas bubble condition; died 02/25/02; preserved and sent to Dr. David Green, veterinary pathologist at USGS National Wildlife Health Center
	Upper Barton Springs	02/22/02	>40	U	Found with gas bubble condition; shipped live to USGS National Wildlife Health Center; salamander was euthanized for pathology work
	Upper Barton Springs	02/22/02	>40	U	Found with gas bubble condition; shipped live to USGS National Wildlife Health Center; salamander was euthanized for pathology work
	Upper Barton Springs	02/23/02	>40	U	Found with gas bubble condition; died 02/25/02; preserved and sent to USGS National Wildlife Health Center
	Upper Barton Springs	02/25/02	>25	F	Found with gas bubble condition; recovered in captivity and developed eggs 09/02
	Upper Barton Springs	03/05/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Upper Barton Springs	03/05/02	>25	U	Collected for captive breeding program
	Upper Barton Springs	03/06/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Sunken Gardens	03/17/02	<25	U	
	Upper Barton Springs	03/19/02	<25	U	Found dead with gas bubble condition; frozen for tissue analysis

	Upper Barton Springs	03/25/02	<25	U	Found with gas bubble condition; recovered in captivity.
	Upper Barton Springs	03/31/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Upper Barton Springs	04/04/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Upper Barton Springs	04/07/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Sunken Gardens	04/11/02	>25	U	Found with gas bubble condition; recovered in captivity
	Upper Barton Springs	04/17/02	16	U	Found with gas bubble condition; recovered in captivity. Died of unknown cause in captivity 07/31/02.
	Upper Barton Springs	05/03/02	>25	F	Found with gas bubble condition; near death on 05/07/02, shipped same day to USGS National Wildlife Health Center
	Upper Barton Springs	05/12/02	>25	U	Found dead with gas bubble condition; frozen for tissue analysis
	Sunken Gardens	05/14/02	>25	U	Found with gas bubble condition; recovered in captivity
	Sunken Gardens	05/31/02	Egg	U	Collected as an egg, the first egg found in the wild; hatched 06/19/02 in captivity.
	Sunken Gardens	06/25/02	17	U	Found ventral side up on sediment during monthly survey; recovered and currently in captivity.
	Upper Barton Springs	06/26/02	>25	U	Found with gas bubble condition
E. waterlooensis	Sunken Gardens	11/19/98	21	U	
	Sunken Gardens	01/22/99	17	U	
	Barton Springs Pool (drawdown)	01/29/99	15	U	Found in fissures area; found ventral side up; recovered later in captivity
	Sunken Gardens	08/23/00	19	U	
	Sunken Gardens	12/28/00	66	U	
	Sunken Gardens	12/28/00	30	U	
	Sunken Gardens	04/30/01	20	U	
	Sunken Gardens	05/02/01	16	U	
	Sunken Gardens	06/07/01	28	U	
	Sunken Gardens	08/02/01	23	U	
	Sunken Gardens	09/21/01	17	U	Died 01/31/02, unknown cause

	Sunken Gardens	09/21/01	24	U	
	Sunken Gardens	09/21/01	28	U	Died 03/14/02
	Sunken Gardens	10/26/01	38	U	
	Sunken Gardens	04/04/02	17	U	Died in captivity 04/05/02
	Sunken Gardens	05/01/02	70	F	Developed eggs 02/03
	Barton Springs Pool	05/02/02	17	U	Died in captivity 07/23/02
	Sunken Gardens	05/31/02	80	U	Missing large portion of tail
	Barton Springs Pool (drawdown)	07/01/02	67	U	Found near main fault
	Sunken Gardens	07/23/02	15	U	Found in moss collected for invertebrates; placed in 75-gallon tank; disappeared
	Sunken Gardens	08/09/02	21	U	Died in captivity 10/02

Table 3. Inventory of captive-bred Barton Springs salamanders.

Species	Dam	Generation in Captivity	Date Eggs Laid	Total Length (Fall 2002)	Sex	Comments
E. sosorum	Dallas F1	F2	01/24/99	~70	M	
	Dallas F1	F2	01/24/99	~70	M	
	BSP 06/96	F1	04/23/99	70	M	
	Dallas F1	F2	05/30/01	>50	F	Developed eggs 10/02
	Dallas F1	F2	05/30/01	>50	M	
	*1	F1	12/01	50	U	One F1 observed with 3-4 eggs on 12/06/02 (moved to Container Tank)
	*1	F1	12/01	42	U	
	*1	F1	12/01	47	U	
	*1	F1	12/01	37	U	
	*1	F1	12/01	48	U	
	*1	F1	12/01	54	U	
	*1	F1	12/01	42	U	
	*1	F1	12/01	52	U	
	*1	F1	12/01	54	U	
	*1	F1	12/01	48	U	
	*1	F1	12/01	56	U	
	*1	F1	12/01	62	U	
	*2	F1	05/01	NA	F	One F1 observed with eggs on 05/23/02; a second F1 observed with eggs on 10/25/02 (both moved to Container Tank); two additional individuals were observed with eggs when they were moved to the Container Tank in 01/03
	*2	F1	05/01	NA	F	
	*2	F1	05/01	NA	F	
	*2	F1	05/01	NA	F	
	*2	F1	05/01	NA	U	
	*2	F1	05/01	NA	U	
	*2	F1	05/01	NA	U	
	*2	F1	05/01	NA	U	
	*2	F1	05/01	NA	U	
	*3	F1	2002	55	U	F1s from 12 clutches in Barton Springs Pool Tank (see Table 4); measured when the tank was disassembled 11/25/02.
	*3	F1	2002	30	U	
	*3	F1	2002	38	U	
	*3	F1	2002	29	U	
	*3	F1	2002	60	U	
	*3	F1	2002	58	U	
	*3	F1	2002	60	U	
	*3	F1	2002	45	U	
	*3	F1	2002	57	U	
	*3	F1	2002	50	U	
	*3	F1	2002	15	U	
	*3	F1	2002	17	U	
	Dallas F1	F2	01/01/02	28	U	

	Dallas F1	F2	01/01/02	32	U	
	Dallas F1	F2	01/01/02	35	U	
	Dallas F1	F2	01/01/02	42	U	
	Dallas F1	F2	01/01/02	44	U	

*1 female collected 02/26/01 from Barton Springs Pool
*2 female collected gravid 11/07/02 from Barton Springs Pool
*3 one of group of females collected 01/12/01 or 02/26/02 from Barton Springs Pool

Table 4. Summary of egg-laying events, 2002.

Dam	Tank	Substrate for Eggs	Other Salamanders in Tank	Date Eggs Laid	# Eggs Laid	Maximum # Days for Eggs to Hatch	Minimum # Eggs That Did Not Hatch *3	Maximum # Eggs That Hatched	# Juveniles Survived	Comments
Dallas Aquarium F1	35-gallon display tank	Plastic plant, gravel	1 male 2 females	01/01/02	38	26-35	16	22 eggs	5 survived to 1 year	Eggs transferred to and hatched at ARC
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Gravel, cobble, moss, netting, Plexiglas	4 males 2 females	02/14/02	39	30	5	34 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #2	*BSP Tank	Moss, Plexiglas prefilter, glass	4 males 2 females	02/20/02	32	26	8	24 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Gravel, cobble, moss, netting, Plexiglas, prefilter, glass	4 males 2 females	03/24/02	37	33	11	26 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #2	*BSP Tank	Gravel, cobble, moss, netting, Plexiglas	4 males 2 females	04/14/02	33	37	21	12 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Gravel, cobble, moss, netting, prefilter	4 males 2 females	05/05/02	11	25	8	3 eggs at most	*1	

Collected from Barton Springs Pool, 01/12/01 Female #2	*BSP Tank	Gravel, moss	4 males 2 females	06/07/02	16	32	5	11 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Gravel, cobble, moss, netting, Plexiglas, prefilter	4 males 2 females	06/13/02	34	30	29	5 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #2	*BSP Tank	Moss, Plexiglas	4 males 2 females	08/05/02	17	27	14	3 eggs at most	*1	
Collected from Barton Springs Pool	*BSP Tank	Moss, prefilter	4 males 2 females	08/11/02	19	31	17	2 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Moss, prefilter, netting	6 males 4 females	09/05/02	11	25	0	11 eggs at most	*1	
Collected from Upper Barton Springs, 11/30/00 (gravid)	*UBS Tank	Moss, prefilter, plastic plant	2 females 1 male 2 unknowns	10/03/02	17	25	13	4 eggs at most	*2	
Collected from Barton Springs Pool, 01/12/01 Female #2	*BSP Tank	Moss, Plexiglas, prefilter	6 males 4 females	10/04/02	15	24	9	6 eggs at most	*1	
Collected from Barton Springs Pool, 01/12/01 Female #1	*BSP Tank	Moss, prefilter, netting	6 males 4 females	10/10/02	21	23	13	8 eggs at most	*1	
Collected from Sunken Gardens, 08/02/01	*SG Tank	Moss, prefilter, plastic plant	2 females 4 males 3 unknowns	10/18/02	20	31	17	3 eggs at most	*2	
Collected from Upper Barton Springs, 11/30/00	*UBS Tank	Moss, Plexiglas, plastic plant	2 females 1 male 2 unknowns	12/13/02	23	25	16	7 eggs at most	*2	

Collected from Upper Barton Springs	*UBS Tank	Gravel, moss, prefilter, plastic plant	2 females 1 male 2 unknowns	12/28/02	29	23	22	7 eggs at most	*2	
Collected from Sunken Gardens, 08/02/01	*SG Tank	Moss, prefilter, plastic plant	2 females 4 males 3 unknowns	12/30/02	27	NA	9	18 eggs at most	*2	

*Barton Springs Pool Tank, 75-gallon tank set-up on January 31, 2002 with diverse habitat and small upwelling; 4 adults (2 females, 2 males) added September 2002; tank disassembled and disinfected November 25, 2002; salamanders reintroduced with moss and small amount of gravel only on November 27, 2002.
*Upper Barton Springs Tank, 60-gallon tank set-up on August 27, 2002 with diverse habitat.
*Sunken Gardens Tank, 75-gallon tank set-up on September 11, 2002 with diverse habitat and small upwelling.
*1 As of 12/31/02, there were 12 surviving juveniles of all clutches combined in this tank.
*2 Due to the fact that there are many hiding places in the tanks, it is too soon to determine if there are small juveniles in the tanks.
*3 Did not develop, developed fungus, or disappeared before day 16.