Population Size, Distribution, and Life History of *Eurycea nana* in the San Marcos River.

Thesis

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By

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ABSTRACT

During 1991, the population size of the federally-listed as threatened San Marcos salamander (*Eurycea nana*) in the San Marcos River, Hays County, Texas, was estimated to be 54,000 (range 10,854 - 105,210). In 1968, the population was estimated to be 21,000, but in 1968 only a portion of the salamander's current range was sampled. Current distribution of *E. nana* is from the northernmost reach of Spring Lake, where about 60% of the population resides, down through the lake to 150 m downstream of the Spring Lake dam.

Eurycea nana less than 16 mm in total length was collected from Spring Lake in each calendar month during either 1991 or 1992, substantiating the belief that *E. nana* spawns year-round. Small salamanders were transported to the laboratory and reared in aquaria. Salamanders 14 - 15 mm in total length were started and reared on *Artemia* and/or *Ceriodaphnia dubia* until they were approximately 27 mm in total length, at which point they were fed aquatic annelids. The growth rate at 20 - 21^{o}C in aquaria, from larvae to adults, was approximately 1.25 mm per week. Photoperiod, water temperature, water exchange rate, and spawning substrate were manipulated in attempts to spawn the salamanders. Females developed mature eggs but they were reabsorbed.

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INTRODUCTION

Duellman and Trueb (1986) list 11 Eurycea spp. and report their range to be restricted to the eastern half of North America. Five of the Eurycea listed by Duellman and Trueb (1986) inhabit Central Texas. Their distribution and taxonomy is currently being examined. Chippindale et al. (1991), in an interim report reviewed current information on the Texas Eurycea and the taxonomic changes that are expected. The taxonomy and distribution of E. nana, the San Marcos salamander, are not in question.

Historically, E. nana was found only in Spring Lake, the headwaters of the San Marcos River, San Marcos, Texas (Baker 1961). This limited distribution and the threat of reduced spring flow necessitated the federal listing of E. nana as a threatened species (45 FR 47355; July 14, 1980). Flow from the San Marcos Springs depends on regional annual rainfall and water pumpage from the Edwards Balcones Fault Zone (Edwards Aquifer). Regional population growth and the resulting increase in use of groundwater is expected to cause a cessation of flow from San Marcos Springs around the year 2000 (U. S. Fish and Wildlife Service 1984). Urban growth around San Marcos Springs has increased the number of potential local threats. These threats include urban storm runoff, urban pollutants, introduced exotic species, and recreational use of the river (U. S. Fish and Wildlife Service 1984).

The San Marcos salamander has generally been collected in the vicinity of the springs which are a fairly constant 21°C, 7.1 pH, 220-232 mg/l methyl orange alkalinity, and a dissolved oxygen of 3.1 mg/l (Tupa and Davis 1976). Norris et al. (1963) found that the oxygen consumption rate of E. nana was affected by temperature. When tested at 20, 25, and 30°C, the salamander's highest rate of oxygen consumption was at 25°C. Norris et al. (1963) and Tupa and Davis (1976) both found that the upper temperature limit for E. nana held in the laboratory was near 30°C.

In addition to the clean, clear water associated with the springs, E. nana appears to require a substrate of sand, gravel, and rock; vegetative cover; and a food supply of living organisms (U. S. Fish and Wildlife Service 1984). Tupa and Davis (1976) reported the usual organisms found in the stomach and intestines of E. nana to be tendipid larvae and pupae, and amphipods.

Eurycea nana was originally believed to spawn in June and possibly in the fall (Mackay 1952). Studies conducted by Bogart (1967) and Tupa and Davis (1976) indicate that E. nana probably spawns year around. To date, there has been no published report on the laboratory spawning of E. nana nor have eggs been collected from the wild. Bogart (1967) was successful in spawning E. neotenes in the laboratory and found that 24 h after courtship eggs were deposited on plant material, stones, and the bottom of a glass bowl. The eggs hatched after 25 d of incubation. Jordan et al. (1992) were also successful in spawning *E. neotenes* and their results were similar to Bogarts(1967).

Only one population estimate has been made for *E. nana* and that was done by Tupa and Davis (1976). They estimated the population to be 20,880 salamanders restricted to the upper most reaches of Spring Lake. Tupa and Davis also reported that the population structure, ratio of juveniles to males and gravid females, was stable throughout the year.

The objectives of this study were (1) to estimate the population size and distribution of *E. nana*, (2) to obtain life history information through observations made in the laboratory and field, and (3) to develop laboratory culture techniques.

METHODS AND MATERIALS

Study Area

The San Marcos Springs discharge from several large and numerous small openings scattered throughout Spring Lake. Between 1957 and 1991, average recorded flow from the springs was 4.6 m^3/s and has ranged from 1.3 m^3/s to 12.1 m^3/s (Buckner and Shelby 1991). The alkaline water emerging from the springs is nearly a constant 21^{o}C year-round. Spring Lake water temperatures annually range from 20.4 to 25.5^{o}C. Spring Lake is approximately 3.9 surface ha and has a maximum depth of 9.2 m. Water clarity permits heavy growth of aquatic vegetation throughout the lake. Below Spring Lake

Dam, the San Marcos River is between 5 and 15 m wide and up to 4 m deep. The substrate above and below the dam ranges from silt in areas of low flow and aquatic vegetation, to sandy areas, to cobble substrate, and to large rocks where the springs are located. Large limestone boulders are found throughout Spring Lake.

Much of the rocky and concrete substrate at the north end of the lake is colonized by the aquatic moss Leptodictyium riparium, and the filamentous blue-green algae, Lyngbia spp.. The latter coats the sandy substrate and floats in masses. Spirogyra spp. and the carnivorous angiosperm Utricularia gibba are present in low densities. Rooted aquatic macrophytes include Sagittaia platyphylla, Myriophyllum brasiliense, Ludwigia repens, and Vallisneria americana which exist in the shallow (1-3 m) areas; and Cabomba caroliniana, Egeria densa and Hydrilla spp. which exist in association with the muddy-detrital benthos (U.S. Fish and Wildlife Service 1984).

The quality and quantity of water emerging from the San Marcos Springs is believed to have attracted people since they first arrived in Central Texas (Brune 1981). Currently Spring Lake is the main attraction of Aquarena Resort, which hosts glass bottom boat rides and an underwater show with human and other performers. Approximately a quarter of a million people visit Spring Lake each year. Aquarena Resort personnel remove vegetation from various parts of Spring Lake throughout the year. The removal of vegetation and general maintenance of equipment related to the underwater show necessitates a large amount of underwater work by Aquarena Springs gardeners. These gardeners reported, in an interview, that salamanders were often seen in the areas around the spring openings. Eight exploratory SCUBA dives were made throughout Spring Lake to identify potential habitat for E. nana.

Population Estimate

Two methods were used to estimate the population sizes of the salamanders in the different habitat types. In the rocky areas, a Time-Constraint sampling procedure was used (Campbell and Christmam 1982). One SCUBA diver kept time while a second diver turned over rocks in search of salamanders for a 5-minute period. The number of rocks turned and the number of salamanders observed were recorded.

The area of each site where E. nana was found was measured, in the following manner. Two ropes with knots tied every 60 cm were laid out parallel to each other. The knots on the ropes insured that all measurements across the site were parallel and made at 60 cm intervals. The area within each 60 cm wide rectangle was determined and then all rectangles within a site were added together to determine the total area of the site. The outer edge of each site was the interface between the rocks and silt.

The average number of rocks in each site was estimated using a square made of rebar, 0.5 m on a side. The 0.25 m^2 square was thrown three times in each habitat area. The number of rocks within the 0.25 m^2 area was recorded. The numbers of rocks within the sample were then averaged to determine the mean number of rocks per m^2. The mean number of rocks per m^2 was multiplied by the total area of the site to estimate the total number of rocks within the site. Only the top layer of rocks was estimated.

The number of rocks estimated for each site and the number of salamanders observed under a known number of rocks during the 5-minute observation periods were used to estimate the number of salamanders inhabiting at each of the spring sites. Each site was sampled three times during April to September 1991. These sites were divided into two categories; above the dam and below the dam. The population was estimated at every site each time an area was sampled and confidence intervals were determined. Transformed data was used to calculate confidence intervals of the population estimate. The transformation was done by adding 0.5 to population estimates of each site and taking the square root of the sum. Standard deviations were obtained with the transformed data. Confidence intervals were calculated following methods described in Zar (1984). Reported confidence intervals were calculated by squaring the transformed confidence intervals and subtracting 0.5. The means were totaled to estimate the total population. The confidence intervals were also totaled for each site to calculate a total confidence limit. The number of salamanders per m^2 was estimated.

The number of salamanders inhabiting the filamentous algae at the north end of the lake was estimated using techniques outlined by Tupa and Davis (1976). Two pieces of wire were inserted into the cloth binding of an aquatic sampling net. Each of the two pieces of wire was bent to form a 90^{o} angle. The wires were joined by interlocking loops at the end of each wire to form a square which measured 15 cm on a side. This device provided a sampling bag which would open fully, close tightly, and sample a 225 cm^2 area (Tupa and Davis 1976). The device was used to sample the area described by Tupa and Davis (1976) on three occasions during August 1991. Eight locations were sampled each sampling day. The same location was not sampled twice. A sample was obtained by placing the opened end of the sampler on the water surface and then rapidly pulling the sampler straight down through the water column to the bottom of the lake. The hinged mouth of the device was closed at the lake bottom. Only the area from the lake surface to the bottom of the lake was sampled. Salamanders found under rocks in this area were not included in the estimate. Salamanders under the rocks were sampled by the Time-Constraint method. The contents of the sampler were placed in a white tray, and all vegetation was removed. Salamanders were counted and returned to the lake. The mean number of salamanders per 225 cm^2 was found (Zar 1984). Confidence intervals were determined in the same manner as described above. The mean was multiplied by the 180 m^2 area studied by Tupa and Davis

(1976). The number of salamanders per m^2 was also estimated. Vegetation type and abundance along with the number of *Etheostoma fonticola* (Fountain darter) captured were recorded.

Range Determination

The range of *E. nana* was determined by making seven SCUBA dives (6 h of dive time) in the area from Spring Lake Dam to Rio Vista Dam, approximately 4 river km. All rocky areas within the river between the two dams were inspected.

Collection of Life History Information

During the exploratory dives, the dives to estimate the population size, and the dives to determine the salamander's range, notes were made on habitat characteristics in the areas where the salamanders were found. Information recorded included substrate characteristics, vegetation type, and presence of fountain darters and crayfish. Eggs were searched for during all dives.

During the 1950's, Aquarena Resort installed a large pipe on top of one of the major springs, Diversion Spring (Figure 1), in the bottom of Spring Lake, to divert the outflow from the spring to the Submarine Theater (Mike Campbell, personal communication). This was done to keep the water in the show area clear. During the late 1970's, Dr. Glenn Longley fitted a sampling net over the discharge end of the diversion pipe to collect subterranean organisms present in the outflow from Diversion Spring (Glenn Longley, personal communication). The Diversion Spring net is cone-shaped and

74 cm in diameter at its opening. The collection end is approximately 15 cm in diameter and has a removable collecting vessel (Figure 1). Over the years, erosion has caused openings to form at the spring opening/pipe interface. These openings caused a suction, venturi, to develop. Bottom lake water, aquatic organisms, plant material and some bottom sediment are now drawn through the diversion pipe along with the spring discharge. Because small and subadult E. nana were routinely collected in the Diversion Spring net, it was decided to collect information on when, during the year, E. nana was reproducing. Sampling of the net began 26 February 1991. The net was left on the pipe continuously until 19 April 1991. During this period, the contents of the net were removed once daily. From 19 June 1991 to 14 August 1991, the net was installed once a week for a 24 to 36 hour period and the contents were removed about every 2 to 4 h during the daylight hours. From 15 October 1991 through 18 August 1992, the net was installed once every 4 weeks for 24 hours or until juvenile E. nana and a Typhomolge rathbuni (Texas Blind Salamander) were collected. Total length was determined for each specimen collected. Notes were kept on relationships between total length and developmental stage of a representative sample of yolk sac bearing salamanders collected.

On four occasions, different types of vegetation and algae from Spring Lake were collected and examined for E. nana eggs. A single collection consisted of approximately 2 to 3 l of water, vegetation, and some limestone particles. Each sample was returned to the laboratory and examined for eggs. Vegetation and algae were removed from the same areas that adults were found. Diversion Spring was examined thoroughly because young were being routinely captured in the net placed over the Diversion Spring pipe.

To gain information on the nocturnal habits of _E_. _nana_, four nighttime SCUBA dives were made in Spring Lake. Search efforts were concentrated around spring openings, particularly Diversion Spring. Infrared lighting (two 36 LED Infrared II illuminators, Fuhrman Diversified Inc. La Porte, TX) and a video camera (Sony SSC D-7) were also used to record salamanders held in an aquarium in the laboratory under nighttime conditions.

Laboratory Culture

Collection of salamanders

Eurycea nana used for developing culture techniques and used to construct a growth curve were collected in the Diversion Spring collection net, from vegetation in front of the hotel using dip nets, and at Deephole Spring by SCUBA divers using aquarium dip nets. After capture, the salamanders were transported to the National Fish Hatchery and Technology Center (NFHTC), San Marcos, where all laboratory work related to this project was conducted.

Breeding studies

The salamanders were maintained under three different conditions: in static aquaria in environmental chambers, in static aquaria at room temperature, and in flow through aquaria. All water used to maintain the salamanders was untreated well water taken from the Edwards Aquifer. Other than temperature the well water used was the same as water flowing from the springs into Spring Lake.

Environmental chambers--Four ten-gallon (38 L) aquaria were placed in environmental chambers (Model 1-35LL, Percival Manufacturing, Boone, Iowa). Each aquarium was equipped with a sponge filter aeration system. A 180-day summer/fall/winter/spring photoperiod cycle was run from October 1990 until June 1991 after which a daily 12-h light/12-h dark cycle was maintained. Aquaria were kept at 20-22°C. A variety of spawning substrates were provided. Originally two spawning mops (Brandt et al. In press), a 10 by 10 by 2 cm Spawntex™ mat (Blocksom and Co., Michigan City, Indiana), and a 1.2 or 2.5 cm in diameter 7 to 10 cm long piece of PVC pipe cut in half along its longitudinal axis were placed in each aquarium. Later on, Ceratophyllum spp. collected from Spring Lake was added to all aquaria. The Ceratophyllum spp. was thoroughly rinsed in well-water to minimize contamination before being placed in the aquaria. Periphyton-covered rocks, collected from Spring Lake, were added to two of the aquaria. Rocks were rinsed in the same manner as the vegetation. The final substrate in one aquarium consisted of a layer of limestone sand, several small rocks covered with periphyton, some Cabomba spp. and some Ceratophyllum spp.

Six salamanders were originally placed in each aquarium. When the project started, eggs could not be seen in any of the salamanders, but as time passed females developed eggs. On 14 October 1991, all salamanders were removed from the aquaria, sexed, and restocked into the aquaria to try to establish a sex ratio of four females to two males per aquarium. For the duration of the study, an attempt was made to maintain this same ratio. Salamanders were determined to be females if eggs were present and presumed to be male if eggs were not present. The salamanders were removed from the aquaria and examined periodically. Between 14 October 1991 and 26 June 1992, the salamanders were removed from the aquaria and examined at 4 week intervals. When examined, a salamander was placed in a petri dish, measured (total length), photographed, and presence or absence of eggs recorded.

Photographs were taken in an attempt to record egg development within the salamanders. The quickest method found to photograph the eggs was to place an individual salamander in a girded clear glass petri dish containing water, along with a clear ruler, and a piece of 1.3 cm in diameter PVC pipe which had been cut in half along its longitudinal axis. The salamander, when under the pipe, would at times remain motionless, allowing a photograph to be taken. The petri dish was placed on a ring stand and the salamander was photographed from below. When present, eggs were visible through the ventral side of the animal. A second ruler placed beneath the petri dish was used to obtain total length of the salamander. Egg counts were made visually, as was condition of the eggs.

Care for the salamanders followed a weekly schedule. On Mondays, Wednesdays, and Fridays the salamanders were fed small crustaceans (consisting mostly of amphipods obtained from hatchery ponds) or aquatic annelids (obtained from Aqua Life Co., Fresno, CA), depending on which was available. On Tuesdays, Thursdays, and Saturdays the aquaria were cleaned by siphoning solids off of the bottom along with approximately one quarter of the water. Well water was used to replace siphoned water. As needed to maintain clean glass through which to view the salamanders, the front and bottom of each aquaria was scrubbed just before siphoning the tank. To limit the transfer of pathogens, the siphon tube was always dipped in a Roccal II™ (National Laboratories, Montvale, NJ) solution and thoroughly rinsed after each tank was cleaned. All equipment associated with providing air, maintaining temperature, and providing light/dark cycle was checked daily. Records were kept on daily water temperatures, organisms fed, cleaning activities, mortalities, presence of spawned eggs, and other events.

Aquarium room--Eight salamanders of unknown sex were kept in a 15-gallon (57 L) aquarium at room temperature. These salamanders were kept on a 180-day summer/fall/winter/spring photoperiod at 24 to 31°C. The aquarium was equipped with a sponge filter/aeration system, two spawning mops, a 10 by 10 by 2 cm piece of Spawntex™, and either a 1.2 or 2.5 cm in diameter 7 - 10 cm long pieces of PVC pipe cut in half along its longitudinal axis. The same procedures described above for feeding, cleaning, and record keeping were also followed with this group of salamanders.

Nursery room--Salamanders previously held in the environmental chambers were stocked into two 30-gallon (114 L) flow-through aquaria, six salamanders per aquarium. In the beginning, a presumed female to male ratio of 4:2 was maintained. Eventually in one of these two aquaria a sex ratio of 1:1 was attempted, but before being placed together the salamanders were kept separated for about a month. In a third aquarium, six 42 to 54 mm in total length salamanders collected from the north end of Spring Lake were stocked. Eggs could not be detected in any of the salamanders collected. Eighteen to twenty-four °C well water was flowed through the aquaria to prevent the possible build-up of hormones or other substances which might inhibit breeding. The salamanders were maintained on a 144-day summer/fall/winter/spring photoperiod cycle. Two aquaria were equipped with spawning mops, periphyton-covered rocks, and weighted-stacked-sponge filters. The sponge filters were stacked on top of each other because while holding salamanders in aquaria the salamanders often crawled under the filters presumably for cover. By loosely stacking the filters it was thought that the salamanders may use the area between the filters for cover and possibly for depositing their eggs. The third aquarium contained limestone sand, gravel, periphyton covered rocks, and *Cabomba* spp., to simulate a natural environment. At 4 week intervals, all salamanders were removed from the aquaria, measured, photographed, and examined for the presence of eggs. The same procedure for feeding, cleaning, and record keeping as described above was followed.

Growth curve

The first two small *E. nana*, 13 and 15 mm total length, brought to the NFHTC were collected from Diversion Spring. The salamanders were placed in an environmental chamber in a shallow plastic dish with a lid. One week after collection, the salamanders were offered *Ceriodaphnia dubia*. Approximately 4 weeks after collection, aeration and a small piece of PVC pipe were added to the container.

In November and December of 1991, 31 *E. nana*, between 9 and 23 mm in total length were collected from the Diversion Spring net and from the algal mats in the north end of Spring Lake. They were kept in 7.5 L aquaria with five to eight individuals per aquarium until week 20 at which time they were moved to 38 L aquaria with seven individuals per tank. The aquaria were housed in an environmental chamber to maintain temperature at approximately 21^{o}C. Each aquarium was equipped with a 1.2 or 2.5 cm in diameter piece of PVC pipe and a bubble aeration system. First feeding and early juvenile salamanders had their tanks cleaned daily and were fed daily either brine shrimp or *C. dubia*. As soon as the salamanders were large enough to consume annelids, they were fed annelids and zooplankton was no longer offered. All salamanders were measured (total length), and examined for eggs every 4 weeks.

RESULTS AND DISCUSSION

Population Estimate

Eurycea nana was found in all the spring openings scattered throughout Spring Lake, Figure 2. In the same area in the northernmost portion of Spring Lake where Tupa and Davis (1976) sampled, salamanders were found in algal mats. Salamanders were also found in the rocky areas just downstream of the Spring Lake dams.

Substrate, water discharge rate, and size of rocky area varied for the different spring openings inhabited by salamanders in Spring Lake, Table 1. Estimated number of salamanders, using Time-Constraint procedures, inhabiting the spring opening sites reflected the physical variability of the sites. Estimates ranged from 3 at sites 3 and 4, two of the smaller sites, to 7,625 at Site 2, the largest site, Table 2. Linear regressions comparing number of salamanders to number of rocks and comparing number of salamanders to site size were determined. Total number of rocks present at a site seems to have a stronger positive correlation with the number of salamanders present than did the size of the site, r^2 values of 0.831 and 0.671, respectively. Unfortunately spring discharge rate was not measured, but I believe that it influenced the number of salamanders inhabiting a site.

Confidence intervals for the rocky areas above and below Spring Lake dam are large. This is partly because each site was sampled only three times, and the number of salamanders and habitat characteristics may vary within a given area. Density of salamanders at the various sites also was quite variable, 0.4 to 50.5 salamanders/m^2, Table 2. Regressions were also calculated comparing salamander density to both site size and number of rocks present at a site. Values for r^2 for both determinations were low, 0.063 and 0.159, respectively, and it appears that both factors had very little influence on density.

The floating algal mats in the north end of Spring Lake were sampled using the same procedure as followed by Tupa and Davis (1976). They estimated that the mats were inhabited by 20,880 salamanders at a density of 116/m^2 compared to 23,200 at a density of 128.9/m^2 in this study, Table 2. The populations inhabiting the algae mats were similar the two years that the estimates were made. Considering the constancy of the Spring Lake environment, the agreement of these two estimates is understandable.

The Time-Constraint procedure was used to estimate the number of salamanders inhabiting the rocky areas just below the two Spring Lake discharges. Sites 21 and 22 contained an estimated 4,730 $\pm$ 1351 and 483 $\pm$ 1475 at a density of 12.5 and 5/m^2, respectively, Table 2. Of the three study areas, floating algal mats, rocky spring openings above dam, rocky areas below dam, the estimate made for the floating algal mats is believed to be the most accurate. The area covered by the algal mats was easy to define and the salamanders present were easy to capture and accurately count. The rocky areas was not accurately defined because only the top layer of rocks could be sampled. The salamanders are known to inhabit the rocks deeper than the first layer, but how deep is not known. The ability of the observer to spot the salamanders when the rocks were turned over was influenced by many factors including water clarity, salamander size, speed and direction the salamander swam to escape. The estimates made for the rocky areas are believed to be low.

All density determinations were made on a m^2 basis. This was done to be compatible with Tupa and Davis (1976) and because the rocky areas were two dimensional since only the top layer of rocks was sampled. Density determinations were based on the population estimates so the algal mat determinations were the more accurate for reasons stated above. Density of the salamanders in the floating mats was 2.5 times the highest density of the salamanders in a rocky area, Site 14. If volume instead of area had been used for determining salamander density and assuming average depth of the algal mat area to be 1.4 m and average depth of the rocky substrate sampled to be 0.2 m, about 33% of the rocky sites would have had a higher density than the algal mats site.

Current Range

Tupa and Davis (1976) reported E. nana to be restricted to the north end of Spring Lake, but they did not thoroughly search the areas covered in this study (W. K. Davis, personal communication). Considering the habitat stability of the upper San Marcos River it is assumed that the salamanders were inhabiting the same areas when Tupa and Davis did their work. The current range of E. nana was found to include all areas in Spring Lake where rocks were associated with spring openings and up to 150 m downstream of the Spring Lake dam. The downstream range ends where the continuous rocky substrate below the dam ends. Many rocky areas exist downstream of the rocky areas associated with the dam, but only one salamander was found in these areas. Since the estimated number of salamanders below the dams is roughly 5,000 (Appendix 1) and both a gravid female and juvenile (> 20 mm) were captured below the dam it is assumed the salamanders are reproducing throughout their range.

Life History

The rocky habitats where E. nana was found were strongly influenced either by water flow from spring openings or by flow of water over the dam. In these areas, water velocity was sufficient to prevent the build up of silt on the substrate. Salamanders were never found in areas where silt covered the substrate. Salamanders were found under, and between silt free rocks and were observed burrowing into the substrate in the vicinity of spring openings. The filamentous algae where E. nana was found was located near several spring openings in the north end of the lake. Salamanders were not found in the macrophytes. Tupa and Davis (1976) examined the biota living in the algal mats and found an abundance of tendipids larvae, amphipods, leeches, and planaria along with some snails and water mites. Current necessary to prevent the deposit of silt, cover to prevent predation (primarily by fish), and a food source appears to be provided by both the rocks and algal mats where the salamanders are found.

During this study, 330 salamanders were collected (Appendix 2). Two hundred and sixty-seven of the salamanders collected were captured in the Diversion Spring net over a 19 month period, Figure 3. Salamanders from 8 to 58 mm were captured in the net indicating that several life stages co-exist in the same area. Salamanders ranging from small to large were also observed in the same habitats while SCUBA diving. Since the small salamanders are present and have limited mobility there is a high probability that eggs are also present in the same areas. A possible explanation for eggs not being collected is that the eggs are located under rocks or down in the gravel where they would not be influenced by the venturi created by the spring discharge through the diversion pipe. This would also explain why only one 8 mm larvae was collected. Possibly when the larva emerges from the egg it remains protected in the gravel for a period of time. At some point, the salamander emerges from the gravel. Since 78% of the salamanders collected in the net were 11 to 15 mm in total length this is probably the size of the salamanders when they leave the gravel. Salamanders are weak swimmers, when this size, and probably cannot avoid being pulled by the venturi into the pipe and then into the net. As salamander size increases above 15 mm the probability of it being able to swim out of the venturi might increase. Only 8 of the 267 salamanders captured in the net were larger than 25 mm. Eurycea nana larger than 25 mm were probably strong enough swimmers to avoid being pulled into the net.

The area around Diversion Spring was examined several times but no eggs were found. Other sites were also examined for eggs. Depending on the site, rocks were turned over, or substrate, vegetative, and algal samples were removed from the lake and examined in the laboratory, but no eggs were found. It is suspected that the eggs are present in the bottom substrate but deeper than what was examined during this study.

Bogart (1967) and Tupa and Davis (1976) both proposed that E. nana spawned year-round. Since salamanders between 11 and 15 mm in total length were collected in the Diversion Spring net during all months, Figure 3, the findings of this study also support the proposal of year-round spawning. Like Tupa and Davis (1976) only one presumed hatchling size salamander was collected during this study. The description of the hatchling collected is slightly different from the one collected in this study, Table 3. Characteristics of larval development were noted from E. nana collected in the diversion net, Table 3.

Tupa and Davis (1976) reported that the fountain darter was frequently observed in the same habitat as E. nana. That was quite often the case in this study along with crayfish. Tupa and Davis (1976) suspected that crayfish may have preyed on E. nana. No evidence supporting consumption of salamanders by crayfish was found during this study. Like Tupa and Davis reported, fish were seen consuming salamanders in this study and are suspected to be the major predator of salamanders.

Laboratory Culture

Breeding studies

Over the 2 years that E. nana was held in aquaria no spawning occurred. Photoperiod whether maintained at 12L/12D or cyclical through the four seasons did not seem to affect the salamanders. Temperature whether held constant or changing also did not trigger a response. The various substrates - rocks, gravel, sand, vegetation, spawning mops, Spawntex™, PVC pipe, and sponge filters were present for the salamanders to use for spawning but were not used. The plastic mesh backing on the Spawntex™ mats caused an unexpected problem. A salamander became entangled in the 4 mm^2 mesh. To prevent this from happening again all mats were removed.

Once eggs were detected in a salamander the eggs would enlarge until they reached about 2 mm in diameter. Eventually eggs in some salamanders were reabsorbed.

During the course of this study, five females died and were dissected. The females contained between 23 to 37 eggs, which were between 1 to 2 mm in diameter. The smallest live female in which eggs were detected was 58 mm in total length. Bishop (1941) reported that both male and female E. nana are mature at a total length of 41 mm. Only salamanders in which the eggs could be seen in externally were dissected in this study. Smaller females, containing undeveloped eggs which could not be seen externally, were presumed to be juveniles and thus were not examined.

Even though all aquaria used in this study had internal lips, several salamanders crawled out of the tanks. Eight of the salamanders which crawled out did not contain eggs and were presumed to be males. The bodies of all other salamanders which crawled out were either not found or had deteriorated to such a point that sex could not be determined. For the eight salamanders which did not contain eggs, all crawled out of aquaria which contained between two and six egg containing females. In three aquaria, which each contained two males and two to four females, all six of the males crawled out while all of the females remained in the tanks. When aquaria were stocked with just one salamander, the salamanders did not crawl out. One possible explanation for this observation is that the egg containing females drove the males from the tanks. Further research is needed to verify this.

It was noticed that all the salamanders in an aquarium were missing toes. This occurred when eggs were developing in the salamanders. When five salamanders missing toes were each stocked into separate aquaria, one salamander per aquarium, all toes were regenerated. Unrelated to the episode with the toes, one salamander which lost a leg regenerated the leg within 2 months. Aggression between *Eurycea* spp. has been studied with varying results. Jaeger (1988) in a laboratory study found that male *Eurycea longicauda* did not exhibit territorial aggressive behavior towards other males. Grant (1955) also working in the laboratory found that *Eurycea bislineata* confronted and would assault other males which wandered into their territory.

Literature dealing with aggression between female *Eurycea* and between females and males was not found. In this study, the presumed female-male and other aggressive behavior is not believed to be due to food or home range competition because excess food was almost always present, salamanders were never observed trying to defend an area, and salamanders were often observed together under a sponge filter or in a piece of PVC pipe. It is assumed the aggression is related to development of eggs and mating. Explanations for the observations made during this study are preliminary in nature and should be viewed as such.

Growth curve

The 13 and 15 mm in total length salamanders were offered live *C. dubia* 1 week after the salamanders were collected. Nine days after collection, a 15 mm salamander was observed consuming the zooplankton. This salamander was the smallest observed consuming food during this study. Both salamanders died 4.5 weeks after being collected. They died on the same day, 3 days after aeration and a piece of PVC pipe were added to their rearing container. Cause of death was unknown.

The 31 salamanders collected in November and December from Spring Lake were used in the development of a growth curve. As with the first two salamanders, these salamanders readily consumed live *C. dubia*. The young salamanders were also fed brine shrimp which was readily accepted. Between 25 to 30 mm in total length the salamanders were offered aquatic annelids, which were also readily consumed. During week 16 of the study, all salamanders were measured, the tanks were cleaned, and the salamanders were fed a heavier than normal amount of food. Fifteen deaths occurred shortly thereafter. Because of this incident, salamanders were not fed immediately before or after being measured. Survival of the salamanders over the 45 weeks of this study was 32%, Table 4. Juvenile salamanders appear to be more sensitive to handling and water quality related stresses than adults. The salamanders grew approximately 1.25 mm per week, Figure 4. The equation for the curve developed from the data collected is: $Y = 14.280 + 0.74121x + 4.9066e{-2}x^2 - 9.3745e{-4}x^3$. Where Y = total length in mm, and X = time in weeks. Even though uniform size salamanders were not initially collected, r = 0.966.

Bishop (1941) reported that male and female _E_. _nana_ mature at 41 mm in total length. Jordan et al. (1992) reported that _E_. _neotenes_ was 7 mm in total length at hatching. In this study the smallest _E_. _nana_ captured was 8 mm. Jordan et al. (1992) reported that _E_. _neotenes_ started feeding on brine shrimp when 15 mm long and about 30 days old. The smallest _E_. _nana_ in this study observed consuming zooplankton was also 15 mm long. Assuming _E_. _neotenes_ and _E_. _nana_ grow at the same rate, _E_. _nana_ are about 5 weeks old when they start feeding at 15 mm. If 5 weeks is added to 20 weeks, the time required for _E_. _nana_ to grow from 15 to 41 mm in this study, Figure 4, _E_. _nana_ could conceivably be sexually mature at 6 months if size alone determines maturity.

RECOMMENDATIONS

1. Vegetation control

A. Manual

Eurycea _nana_ benefits from the clearing of the vegetation from spring areas by Aquarena personnel. If the vegetation were allowed to cover an area, siltation would increase, thus reducing available habitat.

B. Mechanical

There is possible harm to *E. nana* from the harvester boat if algal mats in front of the hotel are cut and harvested. Salamanders are always found in algal mats. Future harvesting of algal mats is not recommended. Inspection of harvested macrophytes turned up no *E. nana*. This indicates that harvesting of the macrophytes does not affect the population significantly.

2. Archaeological work

There is probable harm to *E. nana* individuals from the underwater archaeological work being conducted in Spring Lake. Displacement of salamanders occurs, during water suction activity, making them vulnerable to predation. An incidental take permit is recommended. Silt displaced by the suction is fouling other habitat areas. This is probably having minimal affect on total population numbers.

3. Spring flow

Proper management of the aquifer is vital to the survival of the species. The aquifer should be managed so that natural spring flow occurs sufficient to allow the natural incubation of the young in the spring areas.

4. Culture techniques

Development of culture techniques should be continued. Based on recent discussions with Dave Schlesser of the Dallas Aquarium who is working on the Comal Springs salamanders we suspect that *E. nana* may be laying eggs in the substrate of the springs where they incubate and spend several weeks of their early life.

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Appendix 1. Number of rocks moved, <u>Eurycea nana</u> observed, and estimated number of salamanders inhabiting each site.

Site no.	Est. no. of rocks in site	Sample 1 Rocks moved	Sample 1 No. obs.	Sample 1 Est. no.	Sample 2 Rocks moved	Sample 2 No. obs.	Sample 2 Est. no.	Sample 3 Rocks moved	Sample 3 No. obs.	Sample 3 Est. no.	Est. mean	Std	SE	C.L. (95%)
	Spring Lake - under rocks													
1	367	18	0	0	9	2	82	13	3	85	56	4.9	2.8	33 - 339
2	22795	22	6	6217	13	3	5260	14	7	11398	7625	18.2	10.5	1664-17239
3	81	3	0	0	22	1	4	15	1	5	3	0.9	0.5	0 - 15
4	29	11	2	5	14	1	2	12	1	2	3	0.4	0.3	0 - 8
5	32	6	2	11	3	1	11	10	1	3	8	0.9	0.5	0 - 24
6	116	9	0	0	9	1	13	10	0	0	4	1.7	1.0	6 - 35
7	508	18	10	282	13	12	469	14	8	290	347	2.7	1.6	137 - 640
8	2128	20	3	319	22	3	290	22	3	290	300	0.5	0.3	260 - 342
9	4795	19	3	757	25	6	1151	15	11	3516	1808	16.8	9.7	2 -6724
10	2080	28	2	149	12	4	693	16	9	1170	671	11.14	6.4	11 -2698
11	7128	18	5	1980	14	4	2037	14	8	4073	2697	11.0	6.3	570 -6150
12	8407	17	5	2473	15	2	1121	16	3	1576	1723	8.2	4.7	425 -3762
14	10491	16	14	9180	19	17	9387	18	4	2331	6966	27.8	16.0	129-22284
15	2480	22	3	338	16	7	1085	16	8	1240	888	9.1	5.3	38 -2654
16	229	12	13	248	15	1	15	9	7	178	147	6.3	3.6	19 - 703
17	6243	17	5	1836	18	2	694	17	5	1836	1456	9.5	5.5	187 -3724
18	1106	15	0	0	20	3	166	15	4	295	154	8.6	4.9	120 - 992
19	1033	21	7	344	20	0	0	18	3	172	172	9.2	5.3	142 -1126
20	2262	24	1	94	19	2	238	21	2	215	182	4.2	2.4	14 - 598
	Total											25238		
	Below Spring Lake dams - under rocks													
21	17434	31	9	5062	33	8	4226	32	9	4703	4723	3.2	1.8	3674 -5904
22	4657	13	0	0	18	2	517	20	4	931	483	15.5	8.9	417 -3183
	Total											5213		
	Total under rocks										30451			

Appendix 2. Lengths of Eurycea nana captured in Spring Lake between March 1, 1991 and October 23, 1992.

Collection site	Date collected d-mo-yr	Number Collected	Total length (mm)
7	1 Mar 91	2	15**, 58**
7	2 Mar 91	2	14**, 15**
7	3 Mar 91	1	15**
7	5 Mar 91	3	9*, 13*, 13*
7	8 Mar 91	5	14***, 14*, 14*, 15*, 15*
7	9 Mar 91	2	15*, 17*
7	13 Mar 91	6	12***, 12***, 13***, 14*, 15*, 21*
7	15 Mar 91	5	11***, 13***, 13***, 15*, 18*
7	17 Mar 91	3	12***, 14***, 15*
7	19 Mar 91	12	11***, 11***, 12***, 12***, 13***, 13***, 13***, 13***, 13***, 13***, 14***, 14***
7	20 Mar 91	10	8***, 11***, 13***, 13***, 13***, 13***, 13***, 14***, 14***, 15***
7	21 Mar 91	9	10***, 10***, 11***, 12***, 13***, 14***, 15***, 16***, 11*
7	22 Mar 91	5	12***, 13***, 14***, 14***, 15*
7	23 Mar 91	6	9***, 11***, 14*, 14*, 15*, 15*
7	27 Mar 91	4	9***, 9***, 10***, 11***
7	28 Mar 91	1	14***
7	1 Apr 91	4	13***, 13***, 14***, 14***
7	3 Apr 91	3	13***, 14*, 56*
7	4 Apr 91	1	14***
7	7 Apr 91	1	14***
7	8 Apr 91	2	11***, 13***
7	10 Apr 91	7	11***, 11***, 12***, 12***, 13***, 13***, 15***
7	11 Apr 91	1	10*** (tail cut)
7	13 Apr 91	1	11***
7	16 Apr 91	2	10***, 12***
7	17 Apr 91	1	10***

Appendix 2. Continued.

Collection site	Date collected d-mo-yr	Number Collected	Total length (mm)
7	18 Apr 91	9	10***, 11***, 12***, 12***, 12***, 16***, 8*** (tail cut), 21***, 13*
7	19 Apr 91	5	11***, 16***, 13**, 15**, 15*
7	20 Jun 91	1	13***
7	9 Jul 91	3	50**, 52**, 56**
16	10 Jul 91	7	14***, 8*** (tail cut), 15**, 15**, 15**, 15**, 35**
7	18 Jul 91	1	15**
7	19 Jul 91	2	15**, 16**
7	25 Jul 91	1	9**
7	26 Jul 91	10	13**, 13**, 13**, 13**, 14**, 14**, 14**, 15**, 15**, 16**
7	1 Aug 91	3	13**, 14**, 15**
7	8 Aug 91	3	14**, 14**, 18**
7	14 Aug 91	1	14**
7	16 Oct 91	2	15**, 47**
algal mats	23 Oct 91	7	42**, 43**, 45**, 50**, 52**, 52**, 54**
7 and algal mats	14 Nov 91	17	13**, 14**, 15**, 15**, 15**, 15**, 16**, 16**, 16**, 17**, 17**, 18**, 18**, 19**, 20**, 23**, 53**
7	12 Dec 91	17	12***, 13***,, 13***, 13***, 13***, 14***, 15***, 16***, 14**, 14**, 15**, 15**, 15**, 16**, 16**, 16**, 60*
algal mats	13 Dec 91	7	14**, 17**, 19**, 20**, 20**, 21**, 21**
7	13 Jan 92	1	9*
7	5 Feb 92	3	12***, 15*, 42*
7	6 Feb 92	3	14*, 16*, 16*
7	7 Feb 92	2	14*, 15*
7	8 Feb 92	2	15*, 16*
7	4 Mar 92	1	15*
7	1 Apr 92	4	14*, 15*, 15*, 16*
7	29 Apr 92	1	
algal mats	11 May 92	26	15**

Appendix 2. Continued.

Collection site	Date collected d-mo-yr	Number Collected	Total length (mm)
algal mats	11 May 92	6	45**
7	27 May 92	1	10*
7	24 Jun 92	2	15*, 17*
7	22 Jul 92	1	15*
7	2 Sep 92	1	15*
7	5 Sep 92	1	16*
7	6 Sep 92	2	14*, 14***
7	8 Sep 92	3	10*, 16*, no length
7	11 Sep 92	2	14*, 16*
7	13 Sep 92	2	13*, 14*
7	14 Sep 92	2	14*, 15*
7	15 Sep 92	4	15*, 15*, 15*, 15*
7	17 Sep 92	1	16*
7	19 Sep 92	6	11*, 14*, 14*, 15*, 15*, 17*
7	21 Sep 92	2	16*, 16*
7	23 Sep 92	1	13*
7	24 Sep 92	1	13*
7	26 Sep 92	4	9*, 13*, 14*, 15*
7	29 Sep 92	2	15*, 16*
7	1 Oct 92	3	14*, 14*, 15*
7	2 Oct 92	3	15*, 15*, 16*
7	4 Oct 92	2	12*, 13*
7	5 Oct 92	3	14*, 15*, 16*
7	6 Oct 92	1	56*
7	7 Oct 92	3	13*, 15*, 15*
7	9 Oct 92	1	15*
7	10 Oct 92	4	15*, 15*, 15*, 15***
7	12 Oct 92	2	15*, 16*
7	13 Oct 92	4	14*, 15*, 15*, 16*
7	14 Oct 92	1	15*
7	16 Oct 92	1	15*
7	17 Oct 92	4	15*, 15*, 16*, 17*
7	18 Oct 92	1	17*
7	20 Oct 92	1	14*
7	21 Oct 92	2	15*, 14***
7	22 Oct 92	3	15*, 15*, 50*
7	23 Oct 92	1	15*
7	24 Oct 92	2	14*, 15*
7	25 Oct 92	1	16*
7	26 Oct 92	1	11*
7	27 Oct 92	1	15*
7	29 Oct 92	2	15*, 16*
7	31 Oct 92	2	15*, 17*

* Released, ** Alive and transported to NFHTC, ***Collected dead

Table 1. Site descriptions for areas within Spring Lake and the San Marcos River where *Eurycea nana* were found.

Site number	Site size (m^2)	Estimated number of rocks	Site Description
1	7.1	367	One medium to high volume discharge spring, with rocks and silt over spring. Next to the spring was sandy substrate with rocks on top. Some green and black filamentous algae occurred in the area.
2	316.6	22,795	Several large and many small springs discharged in this area. Substrate ranged from large limestone boulders to cobble, rocks were covered with periphyton, and the entire area had abundant green and black filamentous algae.
3	4.0	81	Low pressure springs discharged into sandy area with a few rocks on top of the sand, and there was silt surrounding the spring.
4	7.4	29	Low pressure springs discharged into sandy/silt area.
5	4.0	32	One medium pressure spring was present. The spring area was "V"- shaped with some sand and large rocks but mostly was silt.
6	5.8	116	One medium to high pressure spring was present. The spring area is "V"- shaped with silt on sides and rocks and sand on the bottom
7	14.0	508	A large spring , which was diverted, discharged in this area. The area had medium-to-large rocks over a cobble/sand substrate, rocks covered with periphyton.
8	19.0	2,128	Many low pressure springs emitted into a sandy substrate with some small rocks.
9	41.3	4,795	Many low to medium pressure springs were present, medium to small rocks over a cobble substrate. It was part of the old river channel.
10	32.5	2,080	Several medium to low pressure springs, several logs and medium to large rocks over a cobble/sand substrate with silt at the edges.
11	99.0	7,128	Many low pressure springs, large to medium rocks covered with periphyton over smaller rocks on top of cobble substrate.
12	125.6	8,407	High water flow occurred in this area from a diversion pipe. The area had medium-to-large rocks over a cobble/sand substrate, rocks covered with periphyton
14	138.0	10,491	Many low to medium pressure springs, large to medium rocks over a cobble substrate with some sand. It was the old river channel
15	32.6	2,480	A few high to medium pressure springs, medium-size rocks on sandy substrate.

Table 1. Continued.

Site number	Site size (m^2)	Estimated number of rocks	Site description
16	19.1	229	Two high pressure and some low pressure springs discharge in this area. Part of the area had large-to-medium rocks on cobble substrate, and part of the area had rocks on loose sand.
17	111.5	6,243	Many low to medium pressure springs, large-to-medium rocks on top of other rocks.
18	23.0	1,106	Many low to medium pressure springs, large-to-medium rocks on top of other rocks with some silt.
19	16.1	1,033	Many low to medium pressure springs, medium rocks on top of other rocks with some silt.
20	29.8	2,262	Few low pressure springs, medium rocks on top of rocks with some sand and silt.
21	379.2	17,434	There was a series of riffles and pools. The area had a rocky bottom with a cobble substrate.
22	97.0	4,657	There was a high rate of flow under the falls. The area had a rocky bottom with cobble substrate.

Table 2. Estimated number and density of *Eurycea nana* at each of the inhabited sites in Spring Lake and the San Marcos River.

Site	Estimated number	Confidence limits (95%)	Estimated density (m^2)	Confidence limits (95%)
	Spring Lake - under rocks			
1	56	33 - 339	7.8	1.6 - 42.7
2	7625	1664 - 17239	24.1	5 - 54.4
3	3	0 - 15	0.8	0 - 3.2
4	3	0.2 - 8	0.4	0 - 1.1
5	8	0.2 - 24	2.1	0 - 6.1
6	4	6 - 35	0.7	0 - 5.1
7	347	137 - 640	24.8	9.6 - 45.7
8	300	260 - 342	15.8	13.7 - 18.0
9	1808	1.7 - 6724	43.8	0 - 162.4
10	671	11 - 2698	20.6	0 - 81.9
11	2697	570 - 6150	27.2	5.7 - 61.9
12	1723	425 - 3762	13.7	3.2 - 29.9
14	6966	129 - 22284	50.5	0.6 - 160.8
15	888	38 - 2654	27.2	0.9 - 80.8
16	147	19 - 703	7.7	0 - 35.1
17	1455	187 - 3724	13.1	1.5 - 33.3
18	154	120 - 992	6.7	1.4 - 37.1
19	172	142 - 1126	10.7	4.0 - 62.4
20	211	14 - 598	7.1	1.0 - 14.5
Total	25238			
	Below Spring Lake dams - under rocks			
21	4730	3674 - 5904	12.5	8.8 - 15.5
22	483	417 - 3183	5.0	0.6 - 26.9
Total	5213			
	Spring Lake - in algal mats*			
Total	23200	3006 - 26066	128.9	

*Habitat sampled by Tupa and Davis (1976)

Table 3. Characteristics of larval *Eurycea nana* as it develops.

Total length (mm)	Developmental characteristics
8	No pigment, gill buds present, prominent yolk sac, no leg buds.
9	Brown pigment on dorsal surface present for remainder of development, gills were present for the remainder of development, prominent yolk sac, front legs were elongated buds, rear leg buds.
10	Yolk sac present, front legs forming, rear legs forming.
11	Yolk sac present, front legs well developed, rear legs forming.
12	Yolk sac present, rear legs formed.
13	Yolk sac not as prominent, toes forming on front and rear legs.
14	Yolk sac not as prominent, toes further developed.
15	Yolk sac reduced, toes completely formed, one salamander was observed consuming *Ceriodaphnia dubia*.
16	Yolk sac reduced.
17	Yolk sac reduced.
18	Yolk sac absent, food in gullet.

Table 4. Number, size range, and survival of *Eurycea nana* collected from Spring Lake, San Marcos, Texas, and used to develop a growth curve.

Group number	Number collected	Range in total length when collected (mm)	Number surviving
1	6	18 - 23	0
2	5	15 - 17	0
3	5	13 - 15	0
4	8	14 - 16	6
5	7	14 - 21	4

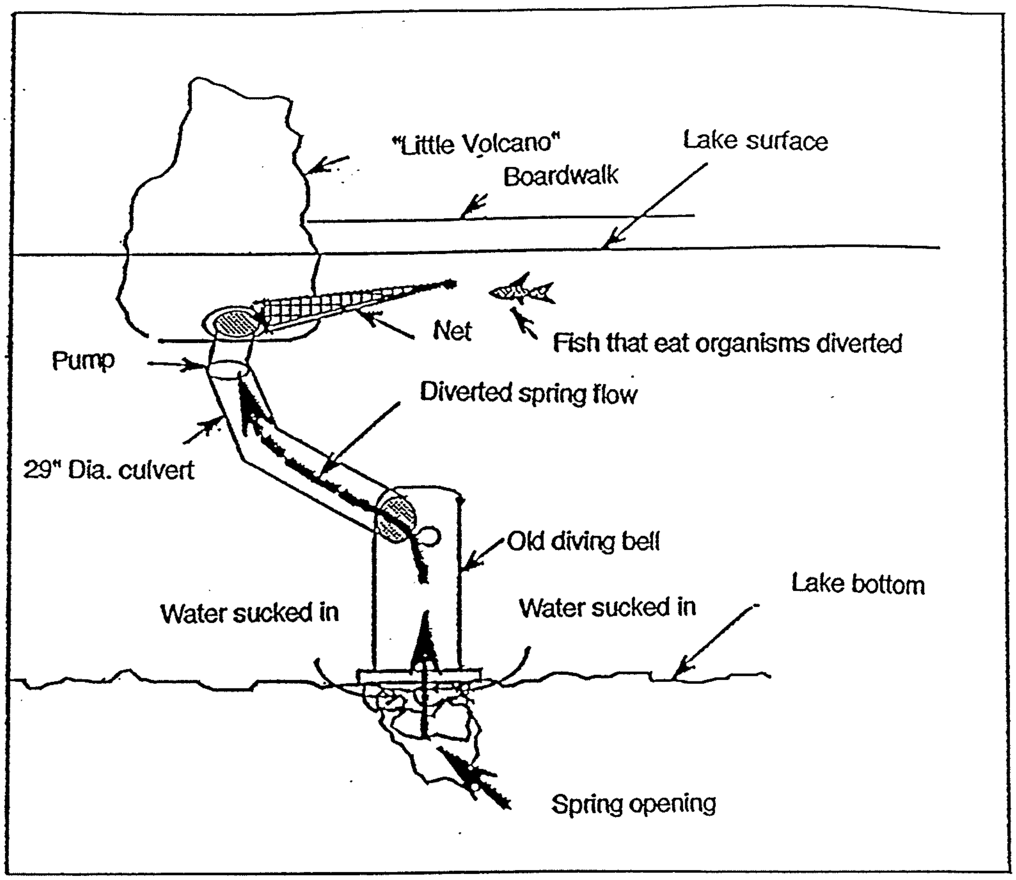

Figure 1. Diagram depicting location of collection net placed on structure installed to divert flow from Diversion Spring to underwater show area in Spring Lake.

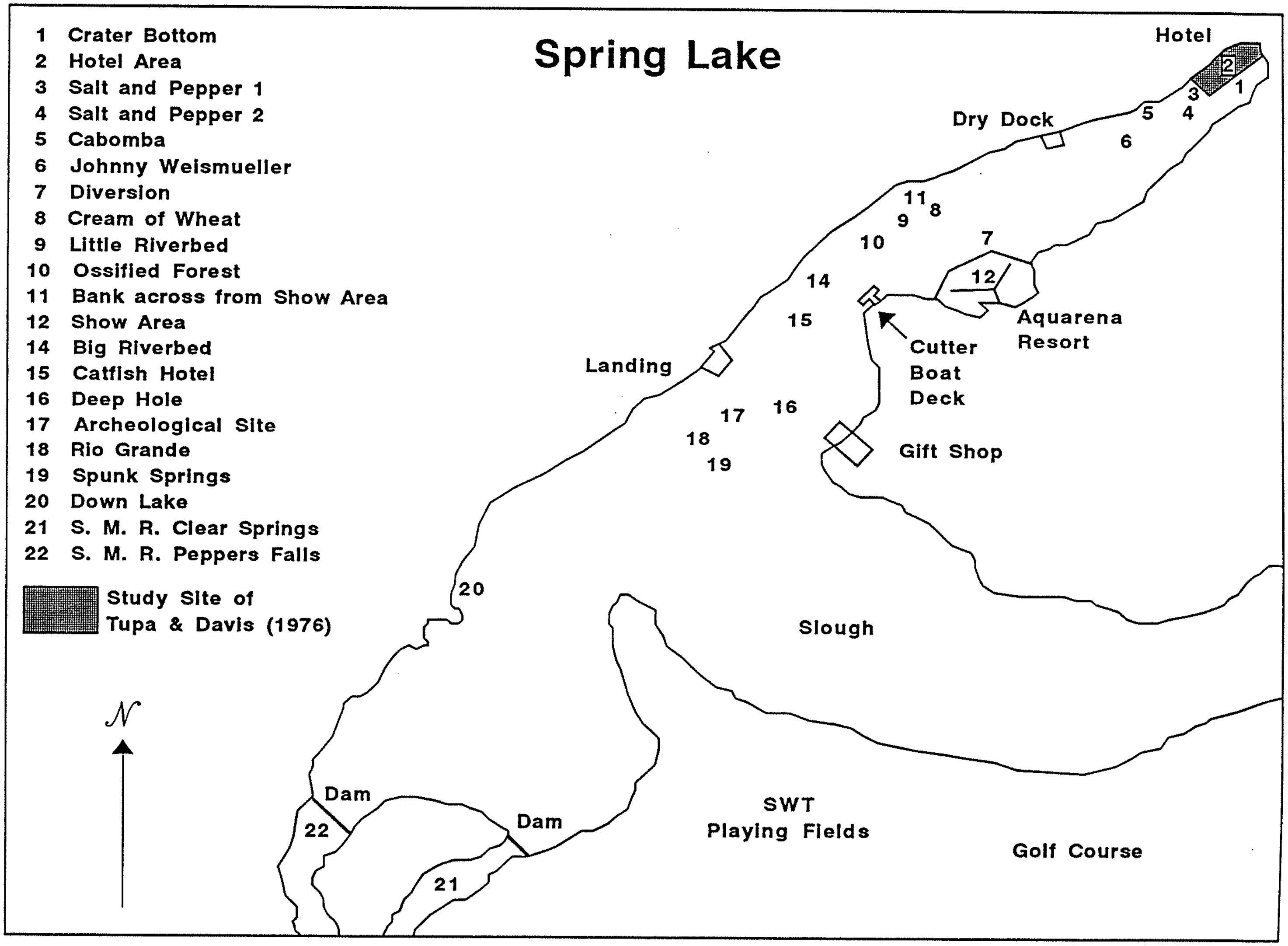

Figure 2. Upper San Marcos River sites inhabited by Eurycea nana.

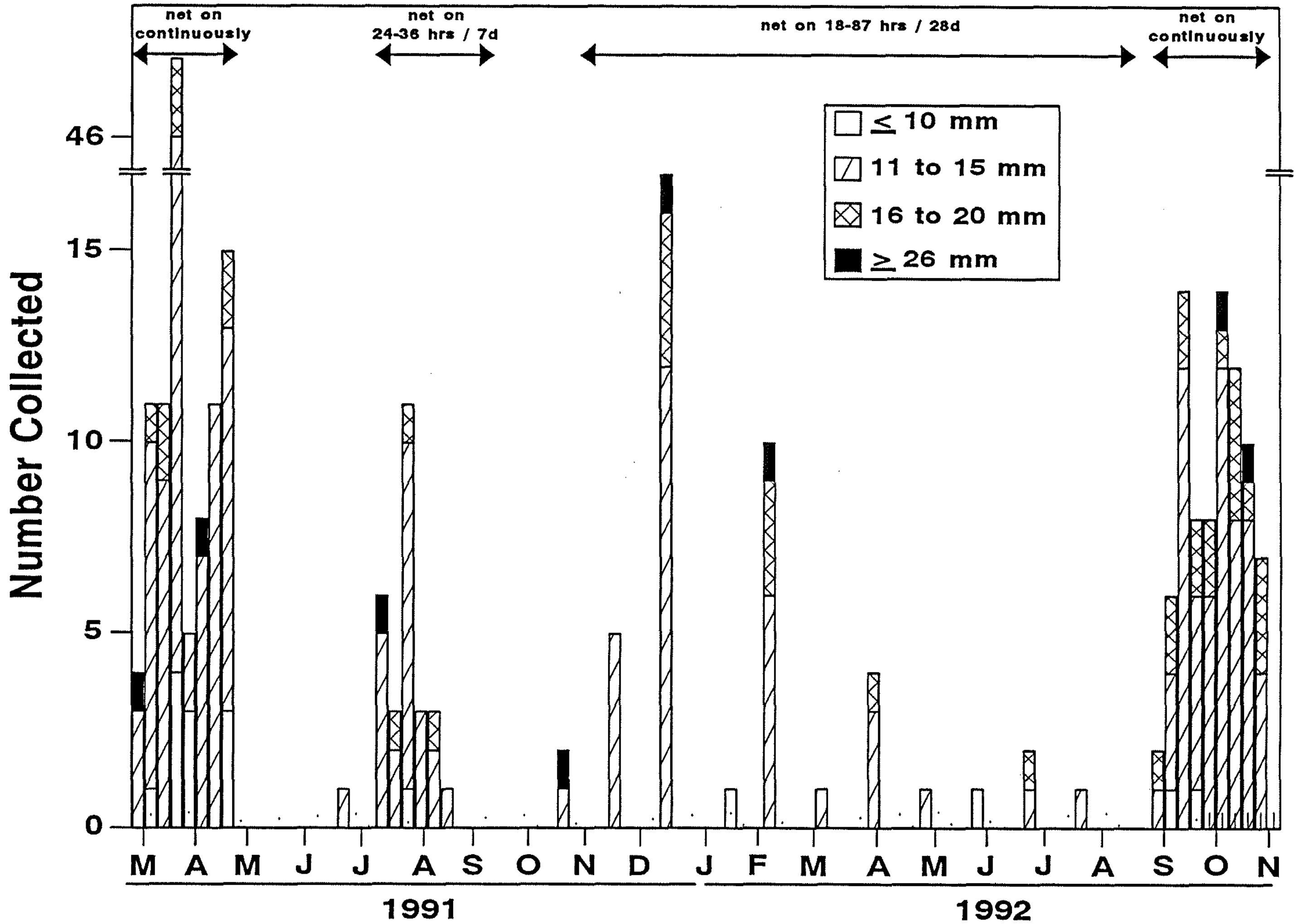

Figure 3. Number and lengths of Eurycea nana collected weekly from Diversion Spring, Spring Lake, San Marcos, Texas during 1991 and 1992.

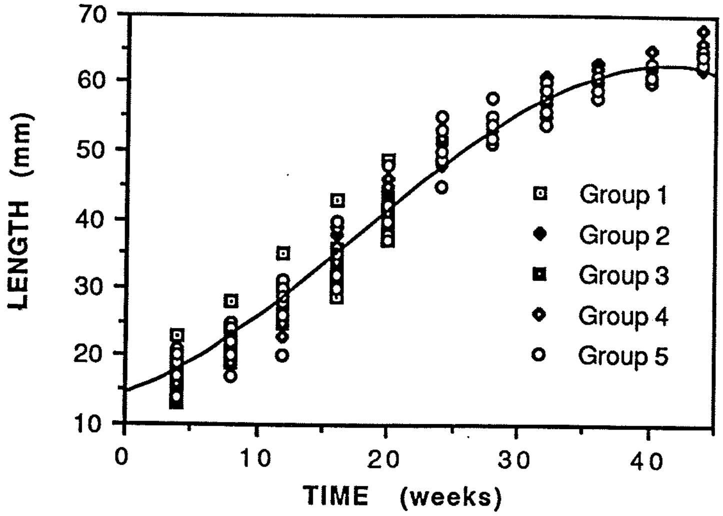

Figure 4. Growth curve for Eurycea nana reared in the laboratory.