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Natural History and Habitat Utilization of the Spring Cavefish, *Forbesichthys agassizi*, in Southern Illinois

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Executive Summary

1. Seasonal size distribution and habitat selection of spring cavefish in three springs at LaRue-Pine Hills Research Natural Area, Union County, Illinois, was examined during 6 seasons, between winter 2000 and spring 2001, in order to provide current data on population status.

2. Abundance of spring cavefish was similar to numbers of fish estimated in the early 1970's for Elm Spring but drastically lower for Class Spring and higher for Otter Pond Spring. Decrease in abundance of fish at Class Spring could not be linked to any known factor; however, increase in habitat quality is a probable cause for increased abundance of cavefish at Otter Pond Spring.

3. Deer Spring, potentially a new spring locality for fish at LaRue-Pine Hills, had large numbers of fish in a plunge pool downstream of two culverts. Monitoring of this population is essential since a large portion of the population is stranded in the plunge pool and may not be able to contribute to the reproductive pool.

4. There was a seasonal trend in overall spring cavefish abundance, with highest numbers in the spring (corresponding to appearance of young-of-the-year) and lowest in the fall.

5. Seasonal variation in size distribution occurred in the winter and spring (related to appearance of young and movement of adults back to the surface) and a decrease in cavefish > 40 mm in the summer and fall due to mortality of 2+ post-spawn adults and movement of individuals underground. Movement of adult individuals to the underground environment may occur as early as summer; a much earlier date than previously reported in the literature.

6. Condition of spring cavefish was highest in the spring for Elm Spring and both spring and summer for Otter Pond Spring, corresponding to increases in lipid storage. Reproductively mature individuals in winter exhibit similar condition factors to those in spring.

7. Station was a significant variable affecting overall distribution of cavefish, with highest abundance of cavefish in the Upland Station compared to the Transition and Lowland Stations.

8. Habitat models for adults at both Elm and Otter Pond Spring provided useful information on the seasonal habitat selection of cavefish. Upland Station, Elm Spring, provided important habitat in the form of large cover items and gravel substrate during every season. At Otter Pond Spring, more seasonal variation in habitat use occurred, with the Transition Station providing important habitat in the spring.

9. Habitat models for young-of-the-year (YOY) indicate similar habitat use as adults at Elm Spring. At Otter Pond Spring, YOY were negatively correlated to silt/gravel substrate and found primarily in mud substrate in narrow plots whereas adult cavefish were positively correlated to silt/gravel substrate. Young-of-the-year at Class Spring were found in association with silt substrate.

10. Spring cavefish abundance was positively correlated to abundance of *Eurycea* spp. at all springs, probably related to similarities in life history traits.

11. A total of 39 gravid females were found between 11 November 2000 and 27 February 2001 at Elm Spring, Class Spring, and Cave Spring Cave. No gravid females were observed at Otter Pond Spring.

12. Cave Spring Cave was monitored on a weekly basis beginning 18 December 2000. A total of 154 cavefish were seen on nine trips to the cave, with an average of 22 cavefish per trip.

13. Reproductive behavior and eggs were not found during this study despite intensive monitoring of spring cavefish at Cave Spring Cave. In surface springs, gravid females were only observed at the most upstream areas of the spring, adjacent to the spring head.

14. Fecundity measures were taken in the laboratory for 8 specimens from Elm Spring, Class Spring, and Cave Spring Cave. Total number of eggs was correlated to both female total length and weight. Egg diameter increased from approximately 1.25 mm in mid-November to 1.7 mm in mid-February.

15. We monitored four surface springs at LaRue-Pine Hills RNA for first appearance of YOY using several techniques including visual searches, drift sets, and dipnetting. Growth rates, drift behavior, habitat use, and phototactic response were examined.

16. Young-of-the-year spring cavefish were found at all spring locations, indicating successful reproduction. Young were first found on the surface at Otter Pond Spring on 3 February 2001, Class Spring on 4 February, Deer Spring on 14 February, and Elm Spring on 25 February. These young were metalarvae based on the presence of finfolds and well-developed pectoral fins.

17. Total length of spring cavefish at first appearance was remarkably similar among springs (6.5-10 mm TL). We suggest larval cavefish become susceptible to drift at this stage and dispersal may increase with spring discharge.

18. Young spring cavefish had little to no yolk and had already begun exogenous feeding. Young grew at an exponential rate after appearing on the surface, growing approximately

15 to 20 mm in total length in the first four to five months. Substantial variation in size distribution of young in later samples suggests that although there is a pronounced "spike" in abundance of young, reproduction does not occur at the same time for all individuals in the population.

19. Juvenile spring cavefish were apparent by June and were distinguished by lack of finfold and incomplete vent migration. Adult cavefish were distinguished by complete vent migration.

20. Both larval cavefish and larval *Eurycea* spp. exhibited significant diel fluctuation in drift density, with highest numbers observed at night. Movement of young cavefish from the underground environment is supported by drift of young into nets placed at the spring head.

21. Larval spring cavefish were most abundant in the Lowland Station, as opposed to the Upland Station in post-larval stages. Larval cavefish were often found in groups, indicating settling out of larvae in low flow areas.

22. Larval cavefish were found to be vulnerable to sampling by light traps. Previous researchers have indicated amblyopsids are photonegative and this behavior has not been previously examined for this life stage.

23. Spring cavefish populations at LaRue-Pine Hills RNA, as a whole, appear to be stable. However, due to the sensitive nature of their habitat and relatively small population size at a given location, we recommend the species be added to the Watch List to insure future monitoring. We found a substantial decrease in population size over the last 28 years at Class Spring; therefore, this population should be closely observed.

24. Maintaining the integrity of spring resurgences and allowing movement between surface and subterranean environments is essential for reproduction. Managers and future investigators should consider the impact of roads and associated culverts on the movements of spring cavefish, particularly at Deer Spring and Otter Pond Spring.

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Introduction

"The cave fishes of North America, par excellence, are the Amblyopsidae. All the members of this family, 8 in number, have degenerate eyes; 5 have mere vestiges; 6 permanently live in caves; 1 is known only from a spring and another from open streams." Carl H. Eigenmann (1909)

Family Amblyopsidae, cavefishes and swampfishes, is comprised of four genera and six species found in the eastern United States (Woods and Inger 1957, Lee et al. 1980, Berra 1981, Robins et al. 1991). Since their discovery by W. T. Craige in 1842 and description by DeKay (1842), cavefish have elicited considerable interest from the scientific community. They represent the best documented model of cave adaptation for any known group of cave organisms (Eigenmann 1909, Woods and Inger 1957, Poulson 1963). Cavefishes are found in the southeastern/central United States (Page and Burr 1991). Although the family is widespread within this area, the species appear to have restricted ranges. Specific habitat requirements and the limited range of cavefishes has resulted in federal listing of two of the species (*Amblyopsis rosae* and *Speoplatyrhinus poulsoni*) and state listings for five of the species (*Amblyopsis rosae* – Arkansas, Missouri, and Oklahoma; *Amblyopsis spelaea* – Indiana, Kentucky; *Typhlichthys subterraneus* – Alabama, Georgia, Indiana, Kentucky; *Speoplatyrhinus poulsoni* – Alabama; *Forbesichthys agassizi* – Missouri). Factors most likely to limit or cause decline within cavefish populations are: 1) destruction of habitat through land use practices, alteration of surface drainage, and sedimentation; 2) overcollecting for commercial purposes and scientific research; 3) disturbance by humans, either indirect or direct; and 4) loss of genetic variation (Willis 1989, Pearson and Boston 1995).

Spring cavefish (*Forbesichthys agassizi*) is a nocturnal species distributed from southeastern Missouri to central Tennessee (Pflieger 1997). Spring cavefish are characterized by diminutive eyes (functional only for light detection), enhanced sensory system, absent pelvic fins, and small body size (maximum total length approximately 90 mm) (Woods and Inger 1957, Etnier and Starnes 1993). Although they share many characteristics with their troglobitic (cave–dwelling) relatives, spring cavefish are unique within the amblyopsids because they appear to be in a transitional stage of cave adaptation; reportedly moving into caves during daytime hours and exiting the caves at night to feed in springs (Smith and Welch 1978). Poulson (1963) inferred from measurements of morphology, physiology and life history that this species colonized caves from surface streams.

Spring cavefish appear to be restricted to clear springs and their associated groundwater streams (Burr et al. 1996). This species is listed as "endangered" in Missouri, found only at Benton Hills Swamp, Scott County. In Illinois, spring cavefish are known from 5 counties in the Shawnee Hills (Hardin, Jackson, Johnson, Pope, and Union) (Table 1). Spring cavefish do not currently have a conservation status, but they were listed as a species of "special concern" in 1969 and as "endangered" in the state of Illinois in 1973 (Lopinot and Smith 1973).

Literature Review

Previous studies of spring cavefish in Illinois - Spring cavefish are dependent on clear, cool springs and have been historically abundant in springs along bluffs in the LaRue-Pine Hills Research Natural Area (RNA) in Union County, Illinois (Table 1). Forbes (1882) described specimens from Pine Hills (*Chologaster papillifera*), and it is interesting to note his description of the habitat in a letter he wrote to C. H. Eigenmann (1909):

> *"Doubtless you have received the little Chologaster which I sent you yesterday. The spring in which they are found is in an almost inaccessible part of Jackson County and I drove 17 miles from Cobden, Illinois in a wagon to this place. The spring is a very large one, flowing from the bottom of a 250-foot cliff of flint and limestone. The little fishes were found under stones at the edges of the spring, very close to the bluffs, and when disturbed they swam back under the cliff..."*

The vast majority of research on spring cavefish in Illinois has been conducted at LaRue-Pine Hills RNA. Information concerning reproductive biology, diet, behavior, and demography is available, but most of the data were collected on one visit or during one season. Layne and Thompson (1952) collected 13 and 14 fish (27.6-33.6 mm standard length) from two springs in August, 1950 (probably Pine Hills), but provide limited biological information. Gunning and Lewis (1955) noted that spring cavefish in two springs at Pine Hills appeared to stay underground during the day, coming out onto the surface at night. They noted that individuals were not only found in the spring/stream systems but were also in the swamp. A series of behavioral studies were conducted in the laboratory on a minimum of 106 fish collected from Pine Hills in 1953 (Weise 1957). Weise (1957) examined the stomach contents of 75 fish and found mostly amphipods (*Gammarus*). He qualitatively assessed variation in fish abundance within a spring at Pine Hills (probably Elm Spring) in relation to habitat; more spring cavefish were found in the "rocky" upstream area of the spring/stream than downstream. Increased cover and decreased predation near the spring head were proposed explanations for this pattern. However, water quality parameters such as temperature, pH, dissolved oxygen, and conductivity were not measured on a regular basis, limiting the conclusions Weise (1957) could make about abiotic factors potentially influencing the distribution and abundance of cavefish. Smith and Welch (1978) conducted a mark/recapture study at LaRue-Pine Hills RNA to estimate population sizes and examine connectivity of eight springs. Their estimates of population size ranged from 18 to 302 fish for a particular spring. Marked individuals were never recaptured at another spring location, suggesting each spring is isolated or that individuals have fidelity to their natal spring.

<u>Reproductive biology</u> - Important aspects of reproduction (e.g., reproductive mode, timing, reproductive capacity, critical spawning habitat, and ecological requirements of the young) are incomplete or remain a mystery for this species throughout its range, including Illinois. Some authors (Weise 1957, Hill 1969b) have suggested spring cavefish are dependent on underground spawning habitats to complete their life cycle. Reduced numbers of adults on the surface during winter has led to the conclusion that spawning occurs in winter and only underground (Weise 1957). Courtship behaviors, including how mature eggs are fertilized, have never been observed. These aspects are interesting when considering the suspected environment in which reproduction occurs (the cave) and the jugular position of the anus and urogenital opening in both sexes. Eigenmann (1909) and subsequent authors (Weise 1957, Poulson 1963, Smith and Welch 1978) hypothesized spring cavefish to be gill brooders, based on the discovery by Eigenmann and later Poulson, of either eggs or young in the gill cavity of *A. spelaea*. Gill brooding is unique among teleost fishes, and has never been confirmed for spring cavefish.

The pirate perch, *Aphredoderus sayanus*, the closest living relative of the cavefishes, has its anus and urogenital opening also in the jugular position in adults. Martin and Hubbs (1973) observed that squeezing of females resulted in eggs moving along a groove into the gill cavity. A female pirate perch (museum specimen) was found to have 3 eggs in its gill cavity (Boltz and Stauffer (1986). This evidence, combined with a jugular vent and the observation of gill brooding in *A. spelaea*, has led some to speculate that pirate perch are gill brooders (Martin and Hubbs 1973, Boltz and Stauffer 1986, Pflieger 1997). Jenkins and Burkhead (1993) provided reasonable arguments against assertions that the finding of Boltz and Stauffer (1986) was strong evidence for gill brooding. Eddy and Surber (1947) stated that pirate perch were nest-builders and that both parents assisted with nest-guarding. Katula (1992) observed pirate perch spawning in a shallow depression in an aquarium and concluded the species was not a gill brooder based on his observation of nesting and the size and number of eggs produced. Fontenot and Rutherford (1999) also found no evidence of gill brooding in an aquarium setting. Based on information available for pirate perch, jugular position of the vent is not necessarily correlated with gill brooding and may be a derived character within Amblyopsids.

Age at first reproduction in spring cavefish is thought to be 12 months (Poulson 1963). Weise (1957) found females with eggs every month of the year at LaRue-Pine Hills RNA; however, attempts to spawn these fish in a laboratory setting were unsuccessful. It is unclear if persistent presence of mature gonads reflects year–round spawning or if limited available energy results in formation of mature gonads when food items become available. Estimates of fecundity (80-285 eggs) are based on 12 females and the relationship between female body size (length) and number of eggs is known for only 5 individuals; mature ova range in size from 1.5 - 2.0 mm (Weise 1957, Poulson 1963). Some of the fecundity estimates are based on fish held in the laboratory, and all fish were captured in springs or streams. Therefore, no fecundity data are available from populations that are primarily cave-dwelling.

Even though young have been captured previously, little is known of the biology and ecological requirements of early life stages. Poulson (1963) reported the collection of 350 young (primarily at Rich's Pond, Kentucky) less than 15 mm from January - June. The fact that 78%

of these young were collected prior to March provides evidence for a late winter spawning/hatching period. Hill (1969a) examined the gut contents of Age 0 fish collected from a cave and surface environment; fish on the surface primarily utilized chironomids and oligochaetes, whereas, only 1 out of 1,920 stomachs examined in the cave contained a food item. Newly hatched young are predated upon by older spring cavefish on both the surface and in the cave (Hill 1969a). Ten individuals between 10.9 and 14.6 mm were found at LaRue-Pine Hills RNA in March (Weise 1957), representing the only collection of spring cavefish less than 20 mm in Illinois. No previous research has been conducted on the behavior and habitat use of young (larvae/early juvenile) spring cavefish.

Objectives

Many of the studies conducted to date in Illinois have been primarily qualitative and limited to one time and location. Few specimens were museum-vouchered from previous studies making it difficult or impossible to interpret conclusions, particularly those regarding reproduction. Additionally, most studies considered only factors that may affect adults within a population and not those of younger life stages. Since Smith and Welch (1978), no research on the status of spring cavefish in Illinois has been conducted. In fact, Smith and Welch (1978) were the last researchers to study the ecology of spring cavefish anywhere within its range. The lack of critical biological information for the species (e.g., reproduction and early life history) and of information concerning population status in LaRue-Pine Hills RNA in over twenty years prompted us, in 2000, to initiate a multi-year study in southern Illinois. The Illinois Department of Natural Resources, Wildlife Preservation Fund-Small Grants Program, funded initial research in 1999-2000. Our current research is a continuation of the project initiated in 1999-2000.

The primary goals of this project were to determine the current status of spring cavefish in spring/stream systems at LaRue-Pine Hills RNA and to increase scientific knowledge of their natural history. Specifically, we **1)** examined local abundance by quantifying seasonal changes in fish density, **2)** documented seasonal changes in size distribution and condition, **3)** determined habitat utilization, **3)** studied aspects of reproduction, and **4)** investigated early life history. A cave population of spring cavefish was also studied in Cave Spring Cave, Union County, Illinois.

Methods

Study Area

Spring cavefish were studied in spring/stream systems in the LaRue-Pine Hills Research Natural Area (RNA) located approximately 1.3 km east of La Rue, Union County, Illinois and approximately 4.8 km east of the main channel of the Mississippi River. The Pine Hills (limestone bluffs) and adjacent LaRue Swamp are primary features of the Ecological Area formed through past activities of the Mississippi River and nearby Big Muddy River. LaRue Swamp, along with Otter Pond and Wolf Lake, is within a remnant channel of the Big Muddy River (Evers 1963). Gunning and Lewis (1955), Weise (1957), Evers (1963), Jenio (1972), Smith and Welch (1978) and Ballard (1994) provided reviews of the unique fauna and flora found within LaRue-Pine Hills RNA.

Numerous springs flow from the base of the west-facing, limestone bluffs into LaRue Swamp, and approximately eight contain spring cavefish; five of the springs are commonly known to harbor substantial numbers of spring cavefish (Smith and Welch 1978). In the current study, we intensively studied the species in three springs (Class Spring, Elm Spring, and Otter Pond Spring) and documented abundance and early life history in an additional spring (Deer Spring) (Figure 1 and Table 2). Spring cavefish are reported from Big Spring (Smith and Welch 1978), but we did not include this spring in our study. In general our study springs are narrow (< 1.5 m), shallow (< 0.2 m), and resurge into a spring/stream that flows directly into the swamp. The study springs have low discharge but are perennial. Continuous flow and shading from the bluffs and surrounding vegetation contribute to the springs being relatively stable environments.

Class Spring is in the northern part of the RNA (Section 4), 0.32 km north of the intersection of Levee Road (from Route 3) and Forest Service road 345 (Table 2 and Figure 2). We referred to this spring as "McCann Spring" in a previous report (Adams et al. 2000). Class Spring is a system of three primary springs (there are also some smaller seeps). In the North Branch, water originates from a stone basin and flows 37 m before its confluence with a second spring resurging from a ceramic tile (SpringHead). An early 1950's photograph of the

SpringHead station revealed the tile was in place at that time (Weise 1953). Water then flows under Forest Service road 345 through a culvert and 30 m before the confluence of Watercress Spring (so named because it contained a conspicuous bed of watercress *Rorippa Nasturtium - aquaticum*). From Watercress Spring, water flows approximately 160 m and then empties directly into a backwater of the Big Muddy River. Class Spring has distinct upland and lowland reaches. A gravel substrate and cover in the form of large gravel, cobble, and a bed of Ludwigia sp characterize the Upland Station. A photograph of the SpringHead Station and Upland Plot 3 suggest the physical layout of this portion of the spring has not changed in 50+ years (Weise 1953). The Transition Station begins below the road; here, the spring is a shallow, run/riffle with a gravel bottom overlain with silt in some areas. The remaining portion of the spring (Lowland Station) has soft substrates (organic mud) with occasional beds of watercress and *Ludwigia* sp. Instream woody debris and a high discharge (0.0015 m^3/s; summer baseflow) relative to other springs were conspicuous features of Class Spring. It is not separated from the Big Muddy River by a levee and can be completely consumed by floodwaters in high water years.

Elm Spring is located approximately 1.3 km east of La Rue, Illinois, directly at the south gate on Forest Service road 345 (Table 2 and Figure 3). The spring emerges from the base of the bluffs into a sunken tile (with a metal lid) and flows 15 m before going under the gravel road through a culvert. From the road, the spring flows approximately 30 m before entering the swamp, defined as the highest elevation of seasonal inundation by the swamp. The Upland Station, from the spring head to the gravel road, is characterized by a gravel substrate, continuous flow, and three large boulders. Our description of this stream reach is almost identical to Jenio (1972), including the presence of three large boulders; however, this stream reach was very different in the early 1950's based on written descriptions and a photograph provided by Weise (1953). When Weise visited the stream, he found the upland portion to be covered by an expansive bed of watercress and devoid of large boulders. In the Transition Station, the substrate changes to gravel/silt and silt. This reach of the stream is typically very shallow and devoid of cover. Jenio (1972) found a bed of watercress in this portion of the stream, but we found it to be sparsely vegetated. The Lowland Station is characterized by soft substrates (organic mud), little to no flow, and seasonal stands of lizard's tail *Saururus cernuus*. During late winter and spring, the Lowland Station is engulfed by the swamp. Summer baseline discharge of Elm Spring was 0.00015 m^3/s. Magnitude of change in discharge in this spring following rain events was low relative to other springs.

Otter Pond Spring is located on the unimproved road leading to Otter Pond and the abandoned SIUC Pine Hills Field Station (Table 2 and Figure 4). To get to the spring, we parked our vehicle at the south gate on Forest Service road 345 and walked approximately 300 m down the road. Otter Pond Spring flows from a small stone basin on the east side of the road. Approximately 12 m above the stone basin in the bluffs is a cave (Twilight Cave) from which water flows during spates. Welch (1973) reported the spring flowed under the road through a culvert and into a plunge pool; spring cavefish were seasonally abundant in this pool and could not make it back upstream because of a 15 cm difference in elevation. At the time of our study, no culvert or plunge pool existed, and the spring flowed alongside the road for 14 m and then directly over it. Fifteen meters downstream of the road, Side Spring enters which only flows when the water table is high. From this point, Otter Pond Spring flows approximately 92 m before reaching LaRue Swamp/Otter Pond, designated as the seasonal high water line. The Upland Station has a gravel substrate, and cover is provided by pieces of large gravel and cobble, particularly near the stone basin. The Transition Station is directly below the road where the substrate is gravel with silt. Beginning at the upper end of the Lowland Station, the substrate abruptly changes from gravel/silt to soft organic mud for the remainder of the stream. In the Lowland and Swamp Stations, packs of leaf litter and woody debris were the only available cover. Very little flow is present in the Lowland and Swamp Stations, and the Swamp Station becomes engulfed when the swamp rises. Discharge during baseflow in summer was 0.00015 m^3/s.

Deer Spring is located 1.77 km north of Elm Spring on Forest Service road 345 and 2.17 km south of the north gate (Table 2 and Figure 5). The spring is difficult to see from the road in a vehicle during all seasons but winter. Deer Spring emerges from the base of the bluffs into a shallow pool with a few scattered boulders. Noteworthy is the fact that no artificial structure (tile, sunken culvert, etc.) obstructs flow of the spring from the bluffs. The spring head has substantial flow year-round and is comprised of multiple seeps. From the spring head, water flows 34 m over mostly gravel substrate where a second spring (North Branch) enters. North Branch has a much lower discharge. Deer Spring then flows under the road through two culverts and empties into a large plunge pool; the culverts appear to be a substantial barrier to upstream movement by fish. From the road, the spring flows 60 m and then sinks underground, resurging again 22 m northwest before flowing into the swamp. Through its entirety, Deer Spring has a higher gradient than the other study springs which has resulted in a series of runs, riffles, and pools; the spring has at least three plunge pools. The substrate is mostly gravel with some silt deposition in pools and slow-flowing runs. Baseline summer discharge is 0.00052 m^3/s.

Seasonal Population Sampling

Three populations of spring cavefish were sampled on a seasonal basis beginning in winter 2000 and continuing through spring 2001 (a total of 6 seasons) at LaRue-Pine Hills RNA and included Elm Spring, Class Spring, and Otter Pond Spring. Since one of the primary goals of this study was to provide baseline data on spring cavefish at LaRue-Pine Hills RNA, we also wanted to devise a standard sampling method that could be duplicated in future monitoring efforts. To determine fish abundance and habitat selection, springs were divided into three permanent stations based on distance from the spring emergence and habitat type, particularly distinct changes in substrate (e.g., Figure 2). The stations extended from the spring head to the swamp and included Upland Station, Transition Station, and Lowland Station. An additional station, Swamp Station, was established at Otter Pond Spring for better coverage. Three plots, 1.5 meters long, were established within each station (e.g., Figure 2). An exception was Otter Pond Spring, which had four plots in the Swamp Station. A wax pencil was used to mark rocks along the edge of the spring to assure sampling was conducted in the same location each season. Sampling was conducted during daytime; therefore we examined only those fish that remained on the surface and did not make diel movements underground (sensu Weise 1957). Potential error due to daily movements underground did not effect our estimates in most plots because of the distance from the spring head; however, daytime abundance estimates in plots directly at the spring head were probably conservative. At Class Spring, we made diel counts on abundance of fish in the tile to assess day/night variation in numbers.

Prior to sampling, the upstream and downstream margins of a plot were blocked with a net (1.6 mm mesh) to prevent movement of fish into and out of the plot. To quantify substrate and instream cover, a 20x20 cm square (PVC) was placed in the plot three times along a diagonal (Figure 6). Substrate type and cover were measured at each corner and in the middle for a total of five points per square. This method of quantifying habitat, modified from Vadas and Orth (1998), allowed us to integrate 15 measurements of each variable per plot. Substrate and habitat types are summarized in Table 3. Water depth was measured in the middle of each square with a wooden meter stick. Stream width in a plot was measured with a meter stick or tape measure at three points. Percent canopy cover was measured using a spherical densiometer. Water velocity was determined based on a qualitative visual estimate and placed into one of three categories: 0-5 cm/s, 6-10 cm/s, or 11-15 cm/s. Point-in-time measurements of major water quality parameters (dissolved oxygen, conductivity, temperature, and pH) were taken in each station. Temperature data loggers (Onset StowAway TidbiT Loggers®) recorded water temperature every five minutes (January-May 2001) at the spring heads of Class, Elm, and Otter Pond Springs; additionally, a logger was placed in each station at Elm Spring.

Sampling of cavefish in plots commenced at the most downstream station. All cover objects were removed from a plot. Cavefish and other aquatic organisms were removed from the plot using a standard D-frame dipnet (900 μm mesh) or small aquarium net and placed directly into a bucket filled with ambient spring water. When harvesting a plot an attempt was made to "drive" the fish into the downstream net. In shallow plots it was obvious when all spring cavefish had been captured. In the deeper plots of the Lowland Stations, two people sampled with D-frame nets for fifteen minutes per plot.

Size distribution was determined by measuring weight and length of each cavefish. Weight was measured on a portable balance to the nearest 0.1 gram and length (typically both standard and total length) was measured to the nearest 0.1 mm with dial calipers. Care was taken to assure cavefish remained submerged in water to reduce stress during measurements. Powder-free latex gloves were worn during handling and Stress Coat® was added to the water to reduce damage to the mucous membrane. This method was used successfully and no adverse effects to the fish were observed. Once all cavefish were measured, instream cover placed back into the spring, and block nets positioned in the next plot to be sampled, cavefish were returned to the water. In addition to cavefish, abundance and size measurements were made on other fish species (length and when possible weight), salamanders (snout-vent length), and crayfish (carapace length). Weise (1957) found amphipods comprised a majority of cavefish diets at LaRue-Pine Hills RNA; therefore, we ranked amphipod abundance (low, medium, and high) to provide some measure of food availability.

Data analysis - Three-way analysis of variance (ANOVA) was used to examine the effects of location (spring), season, and station on abundance of spring cavefish. Cavefish abundance was adjusted for the area of the plot and converted to numbers of fish per meter square. Abundance values were $\log_{10}$ X+1-transformed prior to analysis.

Fish condition was calculated for all seasons and each spring. Condition measurements provided seasonal comparisons of relative robustness of individuals within a spring population, which may indicate reproductive readiness. The equation $\log_{10}TW = a + b(\log_{10}TL)$ was used, where a is the Y intercept, b is the slope, TW is the total weight, and TL is the total length (Bogler and Connolly 1989, Trippel and Hubert 1990). Regression lines were compared seasonally using ANCOVA to determine the effect of season on mass, with length as the covariate. Regression analysis also was used to provide a generalized predictive model for the relationship between total length and standard length. Significant pairwise differences were detected using Tukey HSD tests (alpha = 0.05).

Multiple regression was used to determine which habitat variables were the best predictors of spring cavefish abundance. Forward stepwise regression was used to predict abundance of cavefish for each location by season. Only variables with an alpha < 0.05 were included in the model, and data were examined for colinearity and significant correlations among variables (those correlated at > 0.7 were removed). Because of apparent differences in habitat use between adult and young-of-the-year cavefish, individuals were placed into two categories (<36 mm or >36 mm), and these two groups were analyzed independently in models examining the effects of both location and season. These size classes were selected based on size frequency histograms generated from the seasonal sampling method and growth rate data provided in this report and by Poulson (1963). Habitat variables, including dissolved oxygen, conductivity, temperature, pH, mean water depth, and mean stream width, were $\log_{10}$-transformed prior to analyses. Substrate and cover variables were converted into percent values for each plot then arcsine-transformed, the results were expressed in radians. Amphipod abundance was also included in the regression model as a nominal variable (low, medium, and high). Measures of canopy cover and water velocity were not included in the analyses due to missing data. Nonparametric correlation (Spearman's r_s) was used to determine if abundance of cavefish was related to abundance of other vertebrates and invertebrates. Correlation analyses were conducted at the spring level with all seasons combined.

Reproduction and Early Life History

Reproduction-Gonadal development - Beginning in November 2000, study sites at LaRue-Pine Hills (Elm and Class Springs) and Cave Spring Cave were monitored on a biweekly basis for evidence of mature adults. Once gravid females were found, observations were made at approximately weekly intervals in Cave Spring Cave because spawning is thought to occur underground (Weise 1957). Monitoring was accomplished on the surface by turning over large rocks near the spring head and dipnetting adults from the stream. In the cave, fish were usually swimming unwarily in a small pool and were easily dipnetted. Cavefish were examined for presence of mature gonads using a strong light and magnifying glass. Number of adults, sex (if mature gonads were observed), and overall health of the fish were noted. Adults were weighed and their lengths measured before being returned to the water. Between 27 December and 16 February, 10 adults assumed to be females were collected to determine estimates of fecundity in relation to size. These adults were preserved in 10% formalin and measurements were made in the laboratory. Adult blotted wet weight was measured to the nearest 0.0001 gram and standard and total lengths were determined to the nearest 0.1 mm with dial calipers. The ovary (singular in this species) was then removed and blotted wet weight was taken (0.0001 gram). All eggs were removed from the ovary and counted. Egg diameter was measured on 10% of the eggs using an ocular micrometer. Data were analyzed using regression and compared to data collected on 7 fish by Weise (1957), who provided the only other data on spring cavefish.

Cave Spring Cave in Union County, Illinois is designated as a Natural Heritage Landmark, and the landowners (Mr. And Mrs. Edelman) graciously allowed us access to the cave. Cave Spring Cave is located in Union County, Illinois. This cave contained two pools that made monitoring of the population relatively easy. Once inside the cave, the water was followed downstream for approximately 400 m until we reached a small plunge pool. This pool typically harbored between 10 and 20 fish, except during high water. The small plunge pool was connected to a larger pool by a small riffle at a point where the ceiling dropped; observations of fish in this area required a belly crawl. Cavefish in Cave Spring Cave were monitored approximately every week until the end of February. We were especially motivated to monitor the cave population because previous authors have suggested that spawning occurs only underground and those fish that remain on the surface will not be able to reproduce, but must resorb eggs. Monitoring the cave population consisted of counting all visible cavefish, making behavioral observations, and finally removing between 2 and 5 cavefish from the pool to obtain weight and length measurements and examine gonads.

Appearance of larvae - Our first goal, regarding early life history, was to document, in as narrow a time period as possible, when newly-hatched spring cavefish first appeared in the surface springs. Beginning 27 December 2000, we monitored springs for the presence of young cavefish at 4 - 7 day intervals, typically after sunset. Monitoring was accomplished using a variety of techniques (15-20 min visual searches, 1-2 hr drift sets, and dipnetting). Once young were found, we documented growth and aspects of development. We also studied downstream dispersal, habitat use, and phototactic behavior of young (larval/juvenile) spring cavefish.

Growth and development - We measured total length (TL) to the nearest 0.1 mm with dial calipers from a sample of young-of-the-year (YOY) fish taken from each spring over time (typically 20-30 day intervals). Fish were sampled from the Lowland Station on each occasion (fish were always taken downstream of the road at Deer Spring). One exception was that the last set of measurements taken at Elm Spring included fish collected in the Upland Station because few fish could be collected from the Lowland Station.

A series of YOY spring cavefish were preserved in 5% formalin at intervals to document development. Young cavefish were sampled at first appearance in the surface springs and then periodically from February to June 2001. We also studied young that had been collected in February 2000 and preserved in 75% ethanol. Adult specimens collected from November 2000 to February 2001 (10% formalin) were used for comparison to YOY specimens. One adult was a catalogued specimen from the Southern Illinois University at Carbondale Fluid Vertebrate Collection and had been stored in 75% ethanol. Morphometric measurements (Figure 7), based on Snyder and Muth (1990), were made with an ocular micrometer mounted in a WILD Heerburgg microscope and Mitutoyo dial calipers. Morphologically distinct characteristics of the young were noted.

Downstream dispersal - Our previous research at LaRue-Pine Hills indicated young cavefish, with vestiges of the ventral fin-fold, could be captured in the surface springs drifting downstream in the current by placing nets at the spring head and/or at other locations along the stream (Adams et al. 2000). In the present study, we used drift nets (D-frame nets with 250 and 900μm mesh) to monitor the presence or absence of YOY cavefish in the surface springs and to study the abiotic conditions during which YOY cavefish are displaced downstream. Drift samples were taken at multiple stations within Class Spring and Deer Spring.

Once YOY cavefish appeared in the drift at Deer Spring, we determined if drift density differed between night and day. Drift samples were taken at 30-minute intervals beginning approximately 2 hrs prior to sunset and ending 2-3 hrs post-sunset. Data were analyzed from two stations (Figure 5); the AboveCulvert Station was sampled on 15 and 17 February 2001 and the BelowCulvert Station was sampled on 17 February 2001. Nets were positioned in the primary stream flow and encompassed approximately 80% of the stream width at both stations. Depth and velocity measurements taken at each net were used to calculate drift densities following Smock (1996). Day and night drift densities were averaged for each sampling station and date, and the overall means (N = 3 for each treatment) were compared using the Mann-Whitney *U* - Test.

Habitat - We studied habitat use of young spring cavefish at two spatial scales: 1) at the level of a spring/stream system and 2) within a particular reach of spring/stream. Timed searches were conducted at each spring to determine the distribution and macrohabitat use of YOY fish in surface springs from the spring head to the confluence with the swamp. Two researchers, one on each side of the spring, counted fish for 3 minutes within previously established 1.5 m plots located within stations in each spring. Visual searches were conducted after sunset and consisted of fish that could be seen in the open as well as those coerced from cover. At Class Spring, an additional station with three plots was added closer to the swamp. An additional plot was searched in the Lowland Station at Elm Spring for better longitudinal coverage. Stations and plots had not been previously established at Deer Spring for seasonal population estimates; therefore, we searched 1.5-m segments of the stream within major macrohabitat types (e.g., pool, run, and riffle). Major water quality parameters were measured at each station. Searches were conducted twice at Class Spring (11 February 01 and 27 February 01) and once at Elm Spring (2 March 01), Otter Pond Spring (2 March 01), and Deer Spring (27 February 01).

Two-way ANOVA was used to test the hypotheses that numbers of YOY fish differed between stations and springs (Class, Elm, and Otter Pond Spring). Numbers of YOY fish were $\log_{10}$ (X+1)-transformed prior to analysis. Plots within a station were averaged for a particular spring. At Class Spring and Otter Pond Spring, the Swamp and Lowland Stations were combined to represent one lowland station.

We studied microhabitat selection of YOY cavefish on 11 February 01 in a 15-m reach of Class Spring near Lowland Plot 3. This reach was chosen because it contained a high concentration of YOY fish (based on timed searches) and the habitat was relatively heterogeneous regarding depth, velocity, and cover. Sixteen samples were taken after sunset by disturbing an area up to 35 cm immediately upstream of a standard D-frame dipnet having 900 μm mesh. Samples were taken on diagonal transects with one made on each stream bank and one in the thawlweg. Fish were counted and released. Depth was measured with a meter stick. Maximum water velocity was measured with a Swoffer Model 2100 flow meter unless water depth was too shallow, in which case, flow was visually estimated; during data analysis, flow was categorized as slow (0-4 cm/s), medium (5-9 cm/s) and fast ($\geq$ 10 cm/s). Total percent cover was visually estimated for each sample and the proportion of leaf litter, aquatic vegetation, and woody debris was also estimated. Substrate was categorized as either silt or silt with a thin overlaying layer of fine particulate organic matter (FPOM). Stepwise multiple regression was used to determine which habitat variables, if any, were significant predictors of fish abundance. Prior to analysis, depth and fish abundance were $\log_{10}$-transformed, and percentage data were arcsine-transformed.

Phototactic behavior - Although adults are reportedly photonegative (Weise 1957), we previously captured 27 young spring cavefish in traps baited with chemical light sticks (Adams et al. 2000). To further evaluate phototactic behavior and utility of light traps to sample young, we conducted a field experiment using traps baited with and without chemical light sticks. We experimented with light traps in reaches of springs that had the highest concentration of YOY spring cavefish. Six trials were conducted in the lower reach of Elm Spring, near the confluence with the swamp. One trial was conducted in the Lowland Station of Otter Pond Spring. We also sampled with light traps at Deer Spring; however, these data were not included in analyses due to inconsistencies in methodology.

Traps were two-liter plastic bottles with three 15 cm longitudinal slots (10 mm wide). A chemical light stick (Cyalume Yellow - 12 hr) was placed in a smaller tube glued inside the plastic bottle. To conduct a trial, ten traps (5 with light sticks and 5 without light sticks) were positioned 1 m apart in the middle of the stream perpendicular to the bank. At Otter Pond Spring six traps of each treatment were used. A coin flip determined if the most upstream trap received a light stick, then treatments were alternated systematically. Water depth ranged from approximately 6-13 cm. At least two slots of the trap were always inundated. Traps were set prior to sunset and harvested 4-5 hrs post-sunset. Harvesting consisted of lifting the trap while simultaneously engulfing it with a D-frame dipnet (250 μm mesh); contents of both the trap and net were emptied into a pan and enumerated. Temperature was measured after each trial with a

YSI Model 9501 digital meter. The null hypothesis of no difference in mean number of YOY spring cavefish (and other major taxa) captured in traps with and without yellow light sticks was tested with a Mann-Whitney *U* - Test.

Results and Discussion

Seasonal Population Sampling

Population assessments - Location, station, and season were all significant variables influencing abundance of cavefish (all sizes combined) at Pine Hills (three-way ANOVA, Table 4). Elm Spring (mean = 12.95; SE = 2.81) and Otter Pond Spring (mean = 6.14; SE = 2.40) had significantly higher mean number of cavefish compared to Class Spring (mean = 1.74; SE = 2.86), but were not significantly different from one another. Class Spring was consistently lower in abundance throughout the study; however, it appeared numbers were increasing towards the end of the study.

Although we were unable to calculate actual population estimates, we compared abundance numbers collected in this study to number of fish tagged and population estimates provided by Smith and Welch (1978), who used a mark-recapture method. Estimates from Smith and Welch (1978) are difficult to interpret because of the low numbers of fish initially tagged and the reported error associated with the estimates. Using the Forest Service Report by Welch (1973), we were able to match up spring locations at Pine Hills to springs used in our study and to springs in Smith and Welch (1978) who only provided numbers and not locality information. Although specific dates are not reported, it appears their study commenced in January and ended in May; therefore, we used only our winter and spring sampling dates for comparison. Since we are unsure of the portion of the spring run sampled in Smith and Welch (1978), we conservatively assumed they only measured fish in the Upland Stations near the spring head.

Smith and Welch (1978) tagged 31 fish and estimated a population size of 180 ± 123 for Elm Spring (labeled Spring 2 in their paper). Based on our data from winter and spring in the report, abundance appears to be increasing. This spring should be closely monitored in the future.

At Otter Pond Spring we found between 20 and 222 fish in the winter and spring samples. For this location we included both Upland and Transition Stations because Welch (1973) reported most fish found at this spring corresponded to our Transition Station. Welch (1973) estimated the population at 63 $\pm$28, which is considerably lower than abundance estimates we typically obtain in the spring sample (Spring 1). Based on his study, it appears Otter Pond Spring has undergone a dramatic change in habitat. At one time a culvert separated the Upland and Lowland Stations, resulting in restricted upstream movement of fish (Welch 1973); once downstream of the culvert, fish were stranded in a plunge pool. The culvert has since been removed (date unknown to us), allowing adults downstream of the road access to the spring head and underground spawning sites. This may explain the increase in numbers of cavefish at this site. Currently the spring run flows over the gravel road where it is directly impacted by vehicles. Cavefish are rarely seen in this habitat but it may be an essential portion of the spring run, allowing for upstream movement of cavefish and drift of larvae.

We sampled fish at Deer Spring during one sampling trip on 15 May 2001. Although cavefish have been observed throughout the entire spring and spring/stream (except for North Branch), only the large plunge pool located just downstream of the culverts was sampled. The culverts act as barriers to upstream movement and large numbers of fish were easily observed from the pool edge. The plunge pool measured 3.2 m wide by 2.34 m long. We made three seine hauls (3.66-m seine; 1.6-mm mesh) in which we attempted to encompass as much of the pool as possible in each haul. After pulling the seine, fish were kept submerged in the net until transferred to a bucket filled with ambient spring water. Once all fish were collected from the three seine hauls, we recorded lengths and weights and returned fish to the pool. It should be noted that we probably did not deplete the entire pool, but initiated a standard sampling protocol for future monitoring of this site. We examined a total of 181 fish that ranged in size from 14.4 to 72.2 mm total length. We were not able to confidently match this spring with a corresponding spring in Welch (1973). It appears Deer Spring could coincide with Spring 7, 9, 12, or some combination of these springs. Site descriptions do not match with any of Welch's data, but significant changes in habitat may have occurred since his study. Population estimates are provided for Springs 7 and 9 in Welch (1973) (reported as Springs 5 and 6 in Smith and Welch) and were 18 $\pm$ 16 and 250 $\pm$ 539, respectively. However, only 7 and 12 fish were marked from each spring and the high variation in population estimates indicates low reliability of these data. Little information is available for this spring and it is not cited in any other studies of LaRue-Pine Hills. The large concentration of fish in the plunge pool may represent a substantial portion of the population, which is trapped downstream of the spring head.

<u>Seasonal fish abundance and size distribution</u> - We collected data on a total of 1,168 fish during the seasonal sampling periods at Elm, Otter Pond, and Class Springs. Seasonal numbers of cavefish per plot and sampling date are provided for each spring location in Tables 6, 7, and 8. Season had a significant effect on the mean number of cavefish throughout the year (ANOVA, $P < 0.01$) (Table 4). Mean number of cavefish was significantly higher in spring compared to fall and summer, but not winter. Winter values were not significantly different from any season (Figure 8). Cavefish abundance was highest during the spring sample then tended to decrease each season through the fall.

Weise (1957) also reported seasonal variation in abundance for spring cavefish at LaRue-Pine Hills. At Elm Spring he found only 25 cavefish on 23 January but found 104 on 14 April and stated that composition of age classes ruled out the increase in numbers being related to reproduction. He based this conclusion on the assumption that amblyopsids exhibit reduced growth rates compared to other species. In general, we observed a similar seasonal trend in spring cavefish abundance at all three springs at Pine Hills (Figure 8). Our year-round sampling provides a more complete data set upon which to base conclusions compared to previous researchers. At Elm Spring we found 56 and 78 fish for 2000 and 2001 winter samples (January), respectively, but found a total of 276 and 168 fish in spring samples (May). Of the 444 fish caught in the two May samples, 320 were young-of-the-year and ranged from 25.5 to 36 mm total length (Figure 9). Contrary to Weise, we attribute increase in spring cavefish abundance to appearance of young from winter reproduction (based on growth rate data and length-frequency histograms from this study).

Length frequency data are provided for Elm and Otter Pond springs in Figures 9 and 10. Class Spring data were not used in this comparison because of the low numbers of fish observed throughout the study. In general, the length frequency histograms reveal a similar pattern for both springs: a wide distribution of lengths in the winter and spring and a reduction in the frequency of larger adults in summer and fall. Large peaks appear in the graphs in the spring (notice y-axis scale), corresponding to occurrence of young-of-the-year in the sample. Based on growth rate data from Poulson (1963), it appears that increase in mean total length from spring to fall is primarily a result of YOY growth and not movement of adults to the surface. By winter, adults greater than 50 mm TL re-appear in the sample, and by spring, we see the full spectrum of sizes. Spring cavefish are thought to grow to a mean size of 36 mm TL (range 24-44 mm) in their first year (Poulson 1963); therefore, fish greater than 50 mm in the winter sample are most likely adult fish which have migrated back to the surface. The presence of larger adults in the winter and spring sample may reflect a phenomenon described by Hill (1969a), who found age 1+ cavefish appeared on the surface at approximately the same time as larvae drifting from the spring, and 2+ age fish appear some two to three weeks after appearance of larvae. In other words, reappearance of adults on the surface in winter and spring samples may be tied to the end of the reproductive season.

In general, fewer numbers of adults were seen in the springs during summer sampling compared to other seasons. At Elm Spring no individuals > 40 mm TL were observed in summer. Although some larger adults were present in the summer sample at Otter Pond Spring, these were found at the most upstream station directly adjacent to the spring head. In all other plots at Otter Pond Spring there were no fish greater than 40 mm. Reduced numbers of adults in the summer sample could be due to mortality of 1 or 2+ age post-spawn cavefish and/or movement of adults underground. Spring cavefish are reported to live two to three years of age, but it is unknown if they possess the potential to spawn more than once during their lifetime. If mortality of cavefish was the primary explanation for alterations in seasonal size distribution

Table 9. Water quality data at Class Spring at LaRue-Pine Hills RNA, Union County, Illinois, for each station.

Season	Station	pH	Temperature (°C)	Dissolved (mg O_2/L)	Conductivity (µohms)	Width (meters)	Depth (cm)
Winter	Upland	7.5	11.8	10.8	200	1.48	1.3
	Transition	7.8	9.6	11.1	185	1.23	2
	Lowland	7.8	8.8	10.8	165	0.98	3.5
Spring	Upland	7.9	13.1	8.63	180	1.1	2.7
	Transition	7.7	13.6	8.88	220	2.2	3
	Lowland	7.9	13.1	8.63	180	1.13	6.3
Summer	Upland	7.8	14.4	9.22	255	1.27	2.5
	Transition	8.0	15.2	9.73	235	1.07	2
	Lowland	8.0	16.3	9.2	235	0.97	6.2
Fall	Upland	7.7	11.9	9.37	221	1.66	2.2
	Transition	7.8	11.3	9.52	221	1.09	2.3
	Lowland	7.9	11.1	9.25	220	1.19	3.7
Winter 01	Upland	8.0	11.9	12.1	180	1.73	2.3
Spring 01	Upland	8.2	15.6	10.6	260	1.36	1.3
	Transition	8.2	15.6	10.2	255	1.67	1.1
	Lowland	8.3	15.9	7.74	265	0.97	5.8

Table 10. Water quality data at Elm Spring at LaRue-Pine Hills RNA, Union County, Illinois, for each station.

Season	Station	pH	Temperature (°C)	Dissolved (mg O_2/L)	Conductivity (µohms)	Width (meters)	Depth (cm)
Winter	Upland	7.6	12	9.6	95	1.2	1.8
	Transition	7.7	10.4	10.2	135	1.43	2
	Lowland	7.7	7	10.5	120	1.87	1.7
Spring	Upland	8.0	13.9	11.7	159	1.1	3
	Transition	8.2	14.7	11.3	164	1	1
	Lowland	8.0	16.2	9.78	182	2.3	4
Summer	Upland	7.6	14.5	9.49	163	1.1	2
	Transition	7.9	15.5	9.4	178	0.85	1
	Lowland	8.0	18.9	7.55	190	0.63	4.7
Fall	Upland	7.6	12.7	8.91	180	1.06	3
	Transition	7.8	11.9	8.84	189	1.47	2
	Lowland	7.8	9.2	9.15	168	0.5	2.5
Winter 01	Upland	7.7	11.9	9.21	148	0.93	5.5
	Transition	7.9	10.7	9.58	147	1.28	1.7
	Lowland	8.1	8.9	10.1	140	1.7	6.4
Spring 01	Upland	7.2	14.2	8.96	165	0.9	2.5
	Transition	7.2	15	9.37	168	1.33	1.2
	Lowland	7.0	20.8	3.04	140	1.93	15

Table 11. Water quality data at Otter Pond Spring at LaRue-Pine Hills RNA Union County, Illinois, for each station.

Season	Station	pH	Temperature (°C)	Dissolved (mg O_2/L)	Conductivity (μohms)	Width (meters)	Depth (cm)
Winter	Upland	7.5	12.4	9.13	170	0.98	3
	Transition	7.8	11.5	9.65	170	1.02	4.5
	Lowland	7.8	11	9.62	165	0.76	2.8
Spring	Upland	7.7	13.6	8.18	182	0.8	2.7
	Transition	8.0	15.4	9.05	192	0.9	3.2
	Lowland	8.1	14.6	8.25	185	0.78	2.5
	Swamp	8.2	16	8.2	178	0.84	2
Summer	Upland	7.5	14	8.73	181	0.79	4.2
	Transition	8.0	15.8	9.5	198	0.39	2.8
	Lowland	7.9	15.8	9.19	199	0.7	4
	Swamp	7.9	17.4	8.71	212	1.53	5.7
Fall	Upland	7.6	12.1	8.7	165	0.42	3.2
	Transition	7.6	10.2	8.88	150	0.77	3
	Lowland	7.8	8.4	8.13	170	0.81	5.8
	Swamp	7.6	6.5	7.0	160	1.98	5.8
Winter 01	Upland	7.7	11.9	8.84	170	0.61	2
	Transition	7.8	9.9	9.98	165	0.83	2.3
	Lowland	7.8	8.5	9.96	160	0.9	3
	Swamp	7.9	2.5	11.9	135	1.7	5.7
Spring 01	Upland	7.7	13	8.07	155	0.6	1.8
	Transition	7.9	14.2	8.75	165	0.78	1.5
	Lowland	7.9	14.4	8.34	170	0.8	1.7
	Swamp	7.8	15.5	8.12	175	1.75	3.3

Table 12. Habitat models calculated for adult spring cavefish (>36 mmTL) at Elm, Otter Pond, and Class Springs in LaRue-Pine Hills RNA, Union County, Illinois.

Spring	Season	Variable	Std. Beta	P-value	Model R^2
Elm	Winter	Pebble	0.550	0.0001	0.95
		Boulder	0.914	0.0001	
	Spring	Boulder	0.813	0.0001	0.64
	Summer	*	*	*	N.A.
	Fall	Gravel Substrate	0.650	0.0001	0.99
		Boulder Cover	0.530	0.0001	
Otter Pond	Winter	Gravel Substrate	0.492	0.0001	0.823
		Pebble Cover	0.575	0.0001	
	Spring	Amphipod Density	-0.991	0.0001	0.87
		Conductivity	0.220	0.0180	
		Silt/Gravel Substrate	0.325	0.0005	
	Summer	Amphipod Density	-0.723	0.0050	0.522
	Fall	Width	-1.479	0.0029	0.67
		Zero Cover	-0.46	0.0500	
		Depth	1.155	0.0133	
Class	Winter	Dissolved Oxygen	0.455	0.0002	0.94
		Conductivity	-0.448	0.0009	
		Gravel Cover	1.030	0.0001	
	Spring	*	*	*	N.A.
	Summer	*	*	*	N.A.
	Fall	*	*	*	N.A.

Table 13. Habitat models calculated for young-of-the-year spring cavefish (<36 mmTL) at Elm, Otter Pond, and Class Springs in LaRue-Pine Hills RNA, Union County, Illinois.

Spring	**Season**	**Variable**	**Std. Beta**	**P-value**	**Model R^2**
Elm	Spring	Gravel Substrate	0.563	0.0001	0.95
		Boulder Cover	0.621	0.0001	
	Summer	Gravel Substrate	0.927	0.0003	0.86
	Fall	Gravel Substrate	0.980	0.0001	0.96
Class	Spring	Silt Substrate	0.612	0.007	0.37
Otter Pond	Winter	Width of Plot	-0.678	0.0026	0.40
		Silt/Gravel Substrate	-0.439	0.044	

Table 14. Numbers of gravid females and mature males observed during this study at Cave Spring Cave in Union County, Illinois, and Elm and Class Springs in LaRue-Pine Hills RNA, Union County, Illinois. Where possible, total number of fish seen at the location was recorded and corresponds to the Total Number column.

Location	Date	Gravid Females	Mature Males	Total Number
Cave Spring Cave	18 Dec 2000	15	0	40
	27 Dec 2000	4	0	14
	6 Jan 2001	3	1	25
	12 Jan 2001	2	1	N/A
	19 Jan 2001	0	0	30
	30 Jan 2001	0	0	10
	4 Feb 2001	4	2	35
	12 Feb 2001	1	3	N/A
	27 Feb 2001	0	0	0
Elm Spring	11 Nov 2000	5	0	54
	8 Dec 2000	0	0	20
	6 Jan 2001	1	3	N/A
	12 Jan 2001	0	0	15
	7 Feb 2001	0	0	73
Class Spring	30 Jan 2001	4	0	N/A
	27 Dec 2001	2	0	N/A
	16 Feb 2001	2	0	N/A

Table 15. Summary data for eight gravid females collected from Elm and Class Springs at LaRue-Pine Hills RNA and Cave Spring Cave, Union County, Illinois.

Location	Date	Total Weight (g)	Standard Length (mm)	Total Length (mm)	Gonad Weight (g)	No. Eggs	Mean Egg diameter (mm)
Elm Spring	11 Nov 2000	0.52	35.85	44.35	0.083	92	1.39
Elm Spring	11 Nov 2000	0.94	41.65	50.1	0.149	168	1.23
Elm Spring	11 Nov 2000	1.8	50.05	59.21	0.257	292	1.29
Class Spring	27 Dec 2000	2.78	58.17	66.96	0.42	383	1.43
Class Spring	27 Dec 2000	1.46	49.85	60.71	0.152	111	1.41
Cave Spring Cave	04 Feb 2001	4.88	63.22	73.29	0.386	233	1.57
Class Spring	16 Feb 2001	2.57	46.61	54.8	0.593	242	1.70
Class Spring	16 Feb 2001	2.42	47.01	57.64	0.421	174	1.75

Table 16. Dates and methodology employed to determine the initial appearance of young-of-the-year spring cavefish in study sites at LaRue-Pine Hills RNA and Big Creek, Union County, Illinois.

Date	Site	Methodology	YOY?
27 Dec 00	Elm Spring	Visual Search, Drift Net	No
3 Jan 01	Elm Spring	Visual Search, Drift Net	No
6 Jan 01	Elm Spring	Visual Search, Drift Net	No
12 Jan 01	Elm Spring	Visual Search, Drift Net	No
	Class Spring	Visual Search	No
30 Jan 01	Elm Spring	Visual Search, Drift Net	No
	Class Spring	Visual Search	No
3 Feb 01	Otter Pond Spring	Quarterly Sample	Yes
4 Feb 01	Class Spring	Quarterly Sample	Yes
6 Feb 01	Elm Spring	Visual Search, Drift Net	No
	Class Spring	Visual Search, Drift Net	Yes
	Otter Pond Spring	Visual Search, Drift Net	No
7 Feb 01	Elm Spring	Quarterly Sample	No
10 Feb 01	Elm Spring	Visual Search, Drift Net	No
	Otter Pond Spring	Visual Search	Yes*
14 Feb 01	Elm Spring	Visual Search, Drift Net	No
	Otter Pond Spring	Visual Search	No
	Deer Spring	Visual Search, Drift Net	Yes
15 Feb 01	Elm Spring	Visual Search	No
17 Feb 01	Big Creek	Visual Search, Dipnet	No
25 Feb 01	Elm Spring	Visual Search	Yes
	Big Creek	Visual Search, Dipnet	No
26 Feb 01	Otter Pond Spring	Visual Search	Yes*
2 March 01	Otter Pond Spring	Visual Search	Yes
3 March 01	Big Creek	Visual Search, Dipnet	No
10 March 01	Big Creek	Visual Search, Dipnet	No

* Only one young cavefish was observed

Table 17. Morphometric data for spring cavefish in four life history stages collected from LaRue-Pine Hills RNA, Union County, Illinois. Eye diameter and snout-vent lengths are expressed as percentages of standard length. Means (mm) are stated with ranges in parentheses; N = number of specimens measured.

Stage	N	% Eye Diameter (mm) Mean (range)	% Snout-Vent Length (mm) Mean (range)
Larvae	24	6.21 (3.14-7.33)	59.77 (57.09-61.89)
Early Juvenile	1	5.30	61.74
Juvenile	3	4.13 (4.06-4.21)	52.34 (51.45-53.14)
Adult	9	2.61 (1.33-3.84)	20.29 (17.37-25.61)

Table 18. Morphometric data for spring cavefish in four life history stages collected from LaRue-Pine Hills RNA, Union County, Illinois. Means (mm) are stated with ranges in parentheses; N = number of specimens measured.

Stage	N	Standard Length (mm)	Total Length (mm)	Eye Diameter (mm)	Snout-Vent Length (mm)
Larvae	24	6.95	8.61	0.43	4.03
		(5.91-9.09)	(7.61-10.78)	(0.26-0.52)	(3.44-4.90)
Early Juvenile	1	15.95	18.9	0.85	9.60
Juvenile	3	24.57	30.08	1.01	12.83
		(24.10-24.97)	(29.50-30.75)	(*)	(12.40-13.10)
Adult	9	41.01	48.89	1.03	8.13
		(**25.70-52.55)	(31.70-63.20)	(0.65-1.35)	(6.08-9.95)

*All specimens measured had the same eye diameter (1.0140 mm).

**Adult specimen collected 6 December 2000.

Table 19. Summary of drift sampling at three springs at LaRue-Pine Hills RNA, Union County, Illinois. Data include number of young-of-the-year (YOY) spring cavefish (range of total lengths in mm) and *Eurycea* salamanders.

Date	Station	Time (hr)	YOY Spring Cavefish N (Range TL)	YOY *Eurycea* spp. N
	Elm Spring			
18 Feb 00	SpringHead	1200-2130	24 (7.2-8.6)	114
18 Feb 00	BelowCulvert	1500-2130	26 (7.9-10.6)	259
19 Feb 00	SpringHead	1430-2130	14	41
19 Feb 00	BelowCulvert	1430-2130	17	128
27 Feb 00	SpringHead	1530-2030	0	56
27 Feb 00	BelowCulvert	1630-2030	0	136
6 Feb 01	Swamp	1700-2100	0	0
10 Feb 01	SpringHead	1730-2130	0	NA
	Class Spring			
26 Feb 00	SpringHead	1600-2100	5	115
26 Feb 00	BelowCulvert	1600-2100	6	126
6 Feb 01	SpringHead	1930-2030	6 (8.4-9.2)	1
6 Feb 01	BelowCulvert	1930-2030	2 (8.5,9.2)	0
6 Feb 01	NorthBranch	1930-2030	0	0
10 Feb 01	BelowCulvert	1800-2100	0	0
10 Feb 01	WaterCress	1800-2100	0	1
14 Feb 01	WaterCress	1700-2100	0	2
14 Feb 01	SpringHead	1700-2100	3 (7.7-8.4)	2
	Deer Spring			
14 Feb 01	SpringHead	1700-2030	2 (8.4)	21
14 Feb 01	AboveCulvert	1800-2030	40 (8.0-9.6)	72
15 Feb 01	SpringHead	1530-2100	0	102
15 Feb 01	AboveCulvert	1530-2100	47 (8.1-10.1)	457
17 Feb 01	AboveCulvert	1500-2100	16 (8.0-9.6)	38
17 Feb 01	BelowCulvert	1530-2100	20 (7.4-10.5)	49
17 Feb 01	Pool	1700-2100	5 (8.5-8.7)	23

Table 20. Diel drift in young-of-the-year (YOY) spring cavefish and *Eurycea* salamanders at Deer Spring, LaRue-Pine Hills RNA, Union County, Illinois, in February 2001. Day and night treatments represent pre-sunset and post-sunset, respectively. Mean drift densities ($\pm$ 1 SE) were calculated from data collected at 30-min intervals.

Date	Station	Time (hr)	Treatment	Mean Drift Density (# indv/30min/10m^3)
	YOY Spring Cavefish			
15 Feb 01	AboveCulvert	1530-1730	Day	1.68 (0.901)
15 Feb 01	AboveCulvert	1800-2100	Night	3.49 (0.591)
17 Feb 01	AboveCulvert	1500-1730	Day	3.09 (0.813)
17 Feb 01	AboveCulvert	1800-2100	Night	3.40 (0.755)
17 Feb 01	BelowCulvert	1530-1730	Day	0
17 Feb 01	BelowCulvert	1800-2100	Night	10.60 (2.485)
	YOY *Eurycea* spp.			
15 Feb 01	AboveCulvert	1530-1730	Day	5.46 (2.021)
15 Feb 01	AboveCulvert	1800-2100	Night	41.79 (9.72)
17 Feb 01	AboveCulvert	1500-1730	Day	0.88 (0.881)
17 Feb 01	AboveCulvert	1800-2100	Night	13.61 (4.45)
17 Feb 01	BelowCulvert	1500-1730	Day	0.62 (0.618)
17 Feb 01	BelowCulvert	1800-2100	Night	25.43 (6.133)

Table 21. Longitudinal distribution of young-of-the-year spring cavefish on two sampling dates in Class Spring, LaRue-Pine Hills RNA, Union County, Illinois. Stations and Plots (1.5 m long) are listed downstream to upstream. Total numbers of fish counted by two researchers during a 3-min visual search are shown. Water temperature (Temp), dissolved oxygen (DO), conductivity (Cond) and pH were measured once in each Station. Maximum water velocity was estimated in each Station.

Station	Plot	YOY Fish	Temp (^{0}C)	DO (mg/L)	Cond (µmhos/cm)	pH	Velocity (cm/s)
	11 February 2001						
Swamp	1	0	8.4	10.56	161	8.0	
	2	0					
	3	1					
Lowland	1	0	9.0	9.83	180	8.0	
	2	0					
	3	21					
Transition	1	1	10.8	9.78	181	7.8	
	2	1					
	3	0					
Upland	1	4	10.2	9.88	182	7.8	
	2	0					
	4	0					
	27 February 2001						
Swamp	1	0	10.0	10.0	135	7.8	0
	2	0					
	3	0					
Lowland	1	0	10.5	9.52	170	7.7	15-20
	2	17					
	3	29					
Transition	1	8	11.0	9.6	170	7.7	20-25
	2	3					
	3	1					
Upland	1	5	10.9	9.6	170	7.5	20-25
	2	0					
	4	0					

Table 22. Longitudinal distribution of young-of-the-year spring cavefish on 2 March 2001 in Otter Pond Spring, LaRue-Pine Hills RNA, Union County, Illinois. Stations and Plots (1.5 m long) are listed downstream to upstream. Total numbers of fish (YOY and Adult) counted by two researchers during a 3-min visual search are shown. Water temperature (Temp) and dissolved oxygen (DO) were measured once in each Station. Maximum water velocity was estimated in each Station.

Station	Plot	YOY	Adult	Temp (^0C)	DO (mg/L)	Velocity (cm/s)
Swamp	1	2	0	9.3	7.8	0
	2	0	0			
	3	1	0			
	4	9	2	9.1	8.4	1
Lowland	1	9	0	10.5	9.2	5
	2	2	0			
	3	0	1			
Transition	1	0	0	10.9	9.2	5-10
	2	0	0			
	3	0	3*			
Upland	1	0	2	12.1	8.7	5-10
	2	2	1			
Spring Head		1	3	12.3	8.6	5-10
Side Spring		3	0			

* These fish were underneath a boulder that had recently been placed in the plot.

Table 23. Longitudinal distribution of young-of-the-year spring cavefish on 2 March 2001 in Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois. Stations and Plots (1.5 m long) are listed downstream to upstream. Total numbers of fish (YOY and Adult) counted by two researchers during a 3-min visual search are shown. Water temperature (Temp) and dissolved oxygen (DO) were measured once in each Station. Maximum water velocity was estimated in each Station.

Station	Plot	YOY	Adult	Temp (^{0}C)	DO (mg/L)	Velocity (cm/s)
Lowland	1	0	0	9.0	8.0	0
	2	0	0			
	3	7	1			
	4	11	1			
Transition	1	0	0	10.8	9.07	5
	2	0	0			
	3	0	3			
Upland	2	0	2	11.9	8.66	5
	3	0	2			
Spring Head		0	8	12.5	9.0	2-3

Table 24. Longitudinal distribution of young-of-the-year spring cavefish on 27 Feb 2001 in Deer Spring, LaRue-Pine Hills RNA, Union County, Illinois. Sampling plots (1.5 m long) are listed downstream to upstream. Total numbers of fish (YOY and Adult) counted by two researchers during a 3-min visual search are shown. Maximum water velocity was estimated in each Plot.

Plot	YOY	Adult	Velocity (cm/s)	Macrohabitat Type
1a	0	0	5-10	Resurgence
1b	0	0	5-10	Resurgence
2	3	0	0	Pool
3	0	0	20-25	Riffle
4	0	1	5-10	Run
5	3	0	0	Pool
6	0	0	25-30	Riffle
7a	12	0	1-5	Pool/Run
7b	24	2	5-10	Pool/Run
8	0	1	20-25	Run
9	6	0	5-10	Run
10	3	4	5-10	Run

Table 25. Microhabitat data associated with young-of-the-year (YOY) spring cavefish at Class Spring, LaRue-Pine Hills RNA, Union County, Illinois, on 11 February 2001. Flow was categorized as Slow (0-4 cms/s), Medium (5-9 cm/s) and Fast (≥ 10 cm/s). Substrate was either Silt or silt with an overlying layer of fine particulate organic matter (Silt/FPOM).

Station	Flow	Depth (cm)	Substrate	Total Cover (%)	Woody Debris (%)	Leaf Litter (%)	Aquatic Vegetation (%)	YOY Cavefish
13	Slow	0.5	Silt/FPOM	0.5	0	0.5	0	4
14	Med	4.0	Silt	0.3	0	0.03	0.27	1
15	Med	3.5	Silt/FPOM	0.4	0	0	0.4	5
16	Med	4.0	Silt/FPOM	0.9	0	0	0.9	0
17	Med	4.0	Silt/FPOM	0.4	0.04	0	0.36	0
18	Fast	6.5	Silt	0.2	0.2	0	0	1
19	Slow	3.0	Silt/FPOM	0.8	0.16	0.64	0	0
20	Slow	2.0	Silt/FPOM	0.7	0.7	0	0	3
21	Med	6.0	Silt/FPOM	0.9	0.09	0	0.81	1
22	Slow	3.5	Silt/FPOM	0.7	0.63	0.03	0.03	17
23	Fast	6.0	Silt	0.8	0.08	0.32	0.4	1
24	Slow	2.5	Silt/FPOM	0.5	0.45	0.05	0	2
25	Med	4.0	Silt/FPOM	0.4	0.04	0	0.36	5
26	Fast	7.0	Silt	0.1	0.1	0	0	0
27	Slow	3.5	Silt/FPOM	0.2	0.02	0	0.18	6
28	Slow	3.0	Silt/FPOM	0.3	0.03	0.15	0.12	15

Table 26. Taxa captured with light traps at Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois. Traps were baited with either a light stick (yellow, green, red) or received no lightstick (No Light). Total number of individuals are shown. Range of total lengths (mm) are in parentheses for cavefish.

Date	^{0}C	Treatment	# of Traps	YOY Cavefish N (range TL)	Other Vertebrates	*Nepa*	*Gammarus*	Coleoptera	Decapoda	Trichoptera
26 Feb 01	8.8	Yellow	5	3 (8.4-8.8)	3	17	120	0	3	0
26 Feb 01	8.8	No Light	5	0	1	0	37	0	1	0
27 Feb 01	9.0	Yellow	5	11 (8.1-9.0)	1	5	64	4	0	2
27 Feb 01	9.0	No Light	5	1 (8.4)	0	0	45	0	0	0
3 March 01	8.7	Yellow	5	13	0	7	95	0	7	3
3 March 01	8.7	Green	5	20	0	0	70	1	3	0
3 March 01	8.7	Red	2	3	0	0	15	0	2	0
3 March 01	8.7	No Light	5	3	0	1	10	0	0	0
21 March 01	8.6	Yellow	5	13 (9.7-13.5)	0	2	110	1	3	0
21 March 01	8.6	No Light	5	4	0	0	15	0	0	0
24 March 01	6.8	Yellow	5	6	1	0	115	1	3	1
24 March 01	6.8	No Light	5	2	0	0	49	0	0	0
29 March 01	10.2	Yellow	5	4	0	0	180	1	3	1
29 March 01	10.2	No Light	5	4	2	1	27	0	0	0

Table 27. Taxa captured with light traps at Deer Spring and Otter Pond Spring, LaRue-Pine Hills RNA, Union County, Illinois. Traps were baited with either a light stick (yellow, green, red) or received no lightstick (No Light). Total number of individuals are shown. Range of total lengths (mm) are in parentheses for cavefish. Larval *Eurycea* comprised the majority of other vertebrates.

Date	^{0}C	Treatment	# of Traps	YOY Cavefish	Other Vertebrates	*Nepa*	*Gammarus*	Coleoptera	Decapoda	Trichoptera
	Deer Spring									
20 Feb 01	11.5	Yellow	3	2 (8.4, 10.6)	0	0	20	0	0	0
20 Feb 01	11.5	Green	3	0	3	0	21	0	0	0
20 Feb 01	11.5	No Light	3	1 (10.6)	0	0	6	0	0	0
2 March 01		Green	5	4	0	0	2	0	0	2
2 March 01		No Light	5	3	0	0	0	0	0	0
	Otter Pond Spring									
27 April 01	14.9	Yellow	6	6 (19.3-21.8)	30	4	60	0	0	0
27 April 01	14.9	No Light	6	0	9	0	16	0	1	0

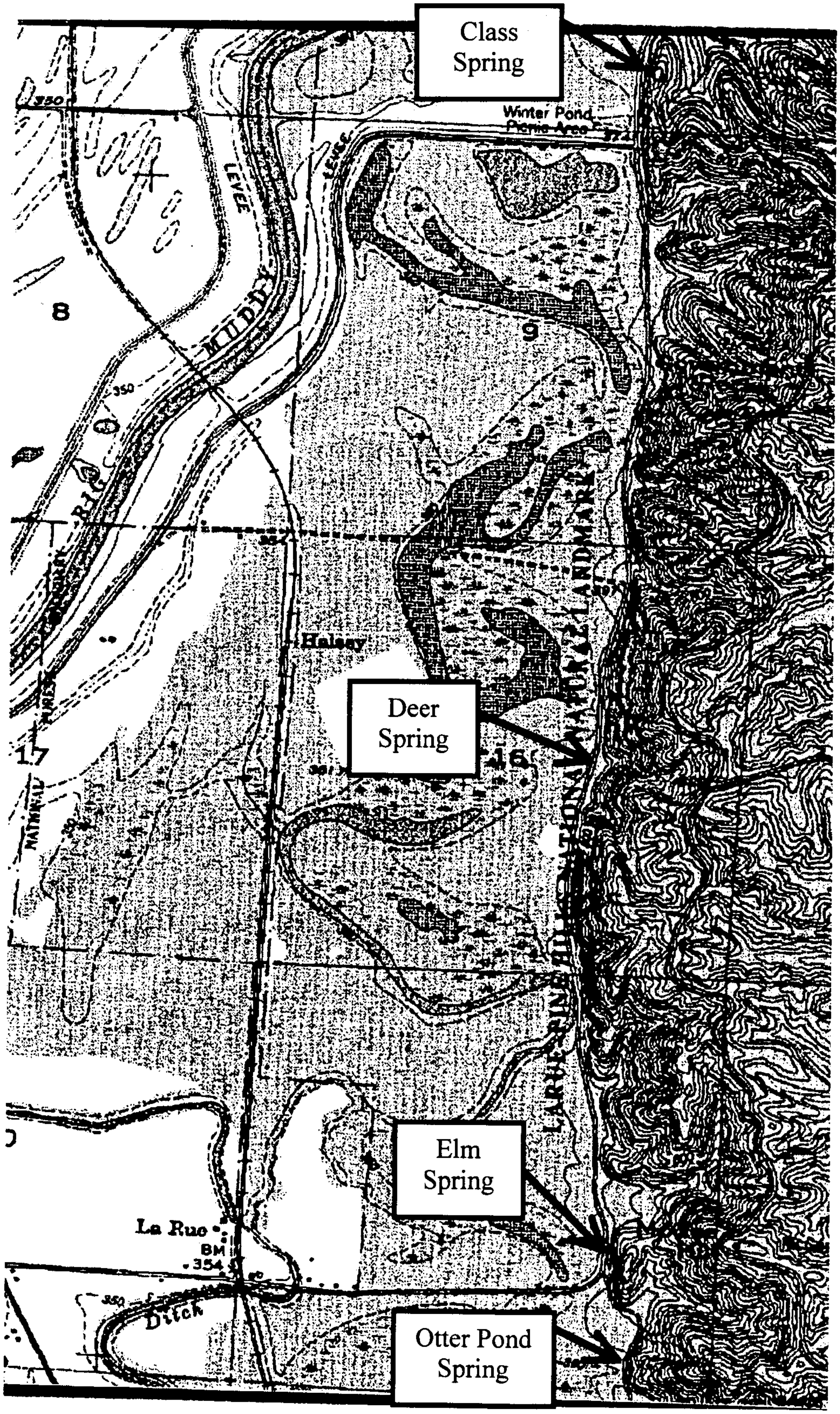

Figure 1. Topographic map depicting locations of the four study springs in the LaRue-Pine Hills RNA, Union County, Illinois.

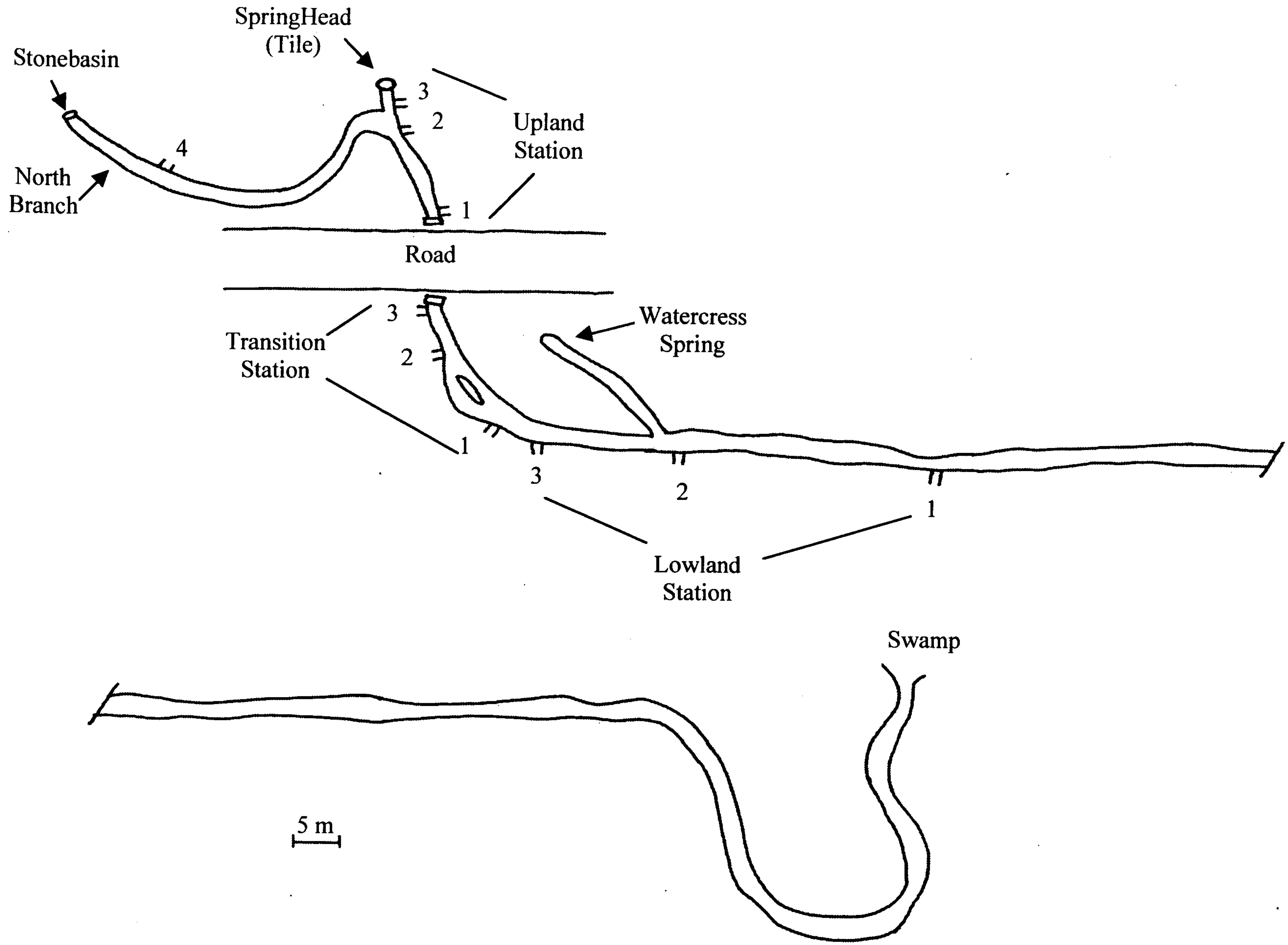

Figure 2. Class Spring, LaRue-Pine Hills RNA, Union County, Illinois. Seasonal sampling stations and plots are shown.

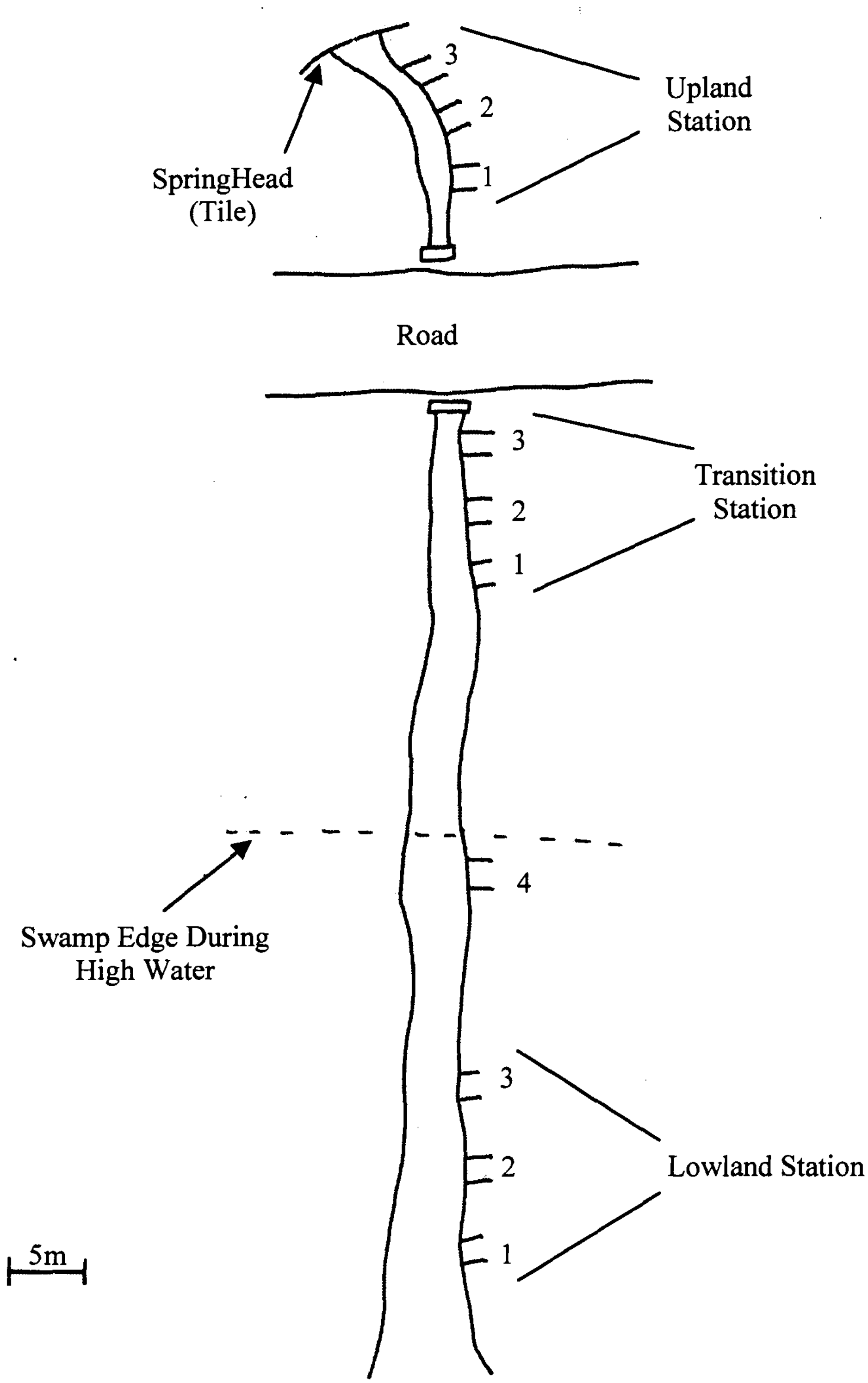

Figure 3. Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois. Stations and plots within each station are depicted.

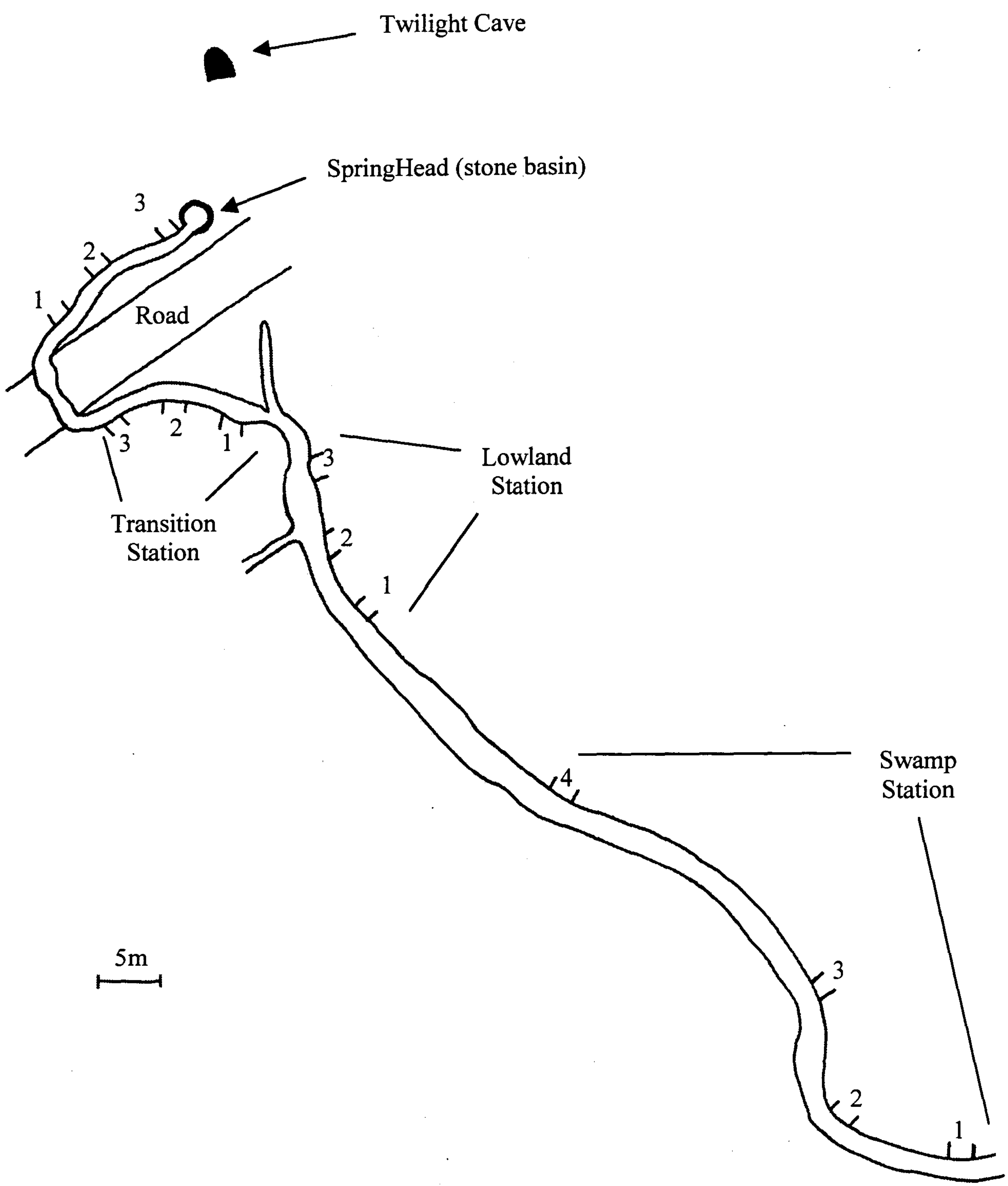

Figure 4. Otter Pond Spring, LaRue-Pine Hills RNA, Union County, Illinois. Seasonal sampling stations and plots are shown.

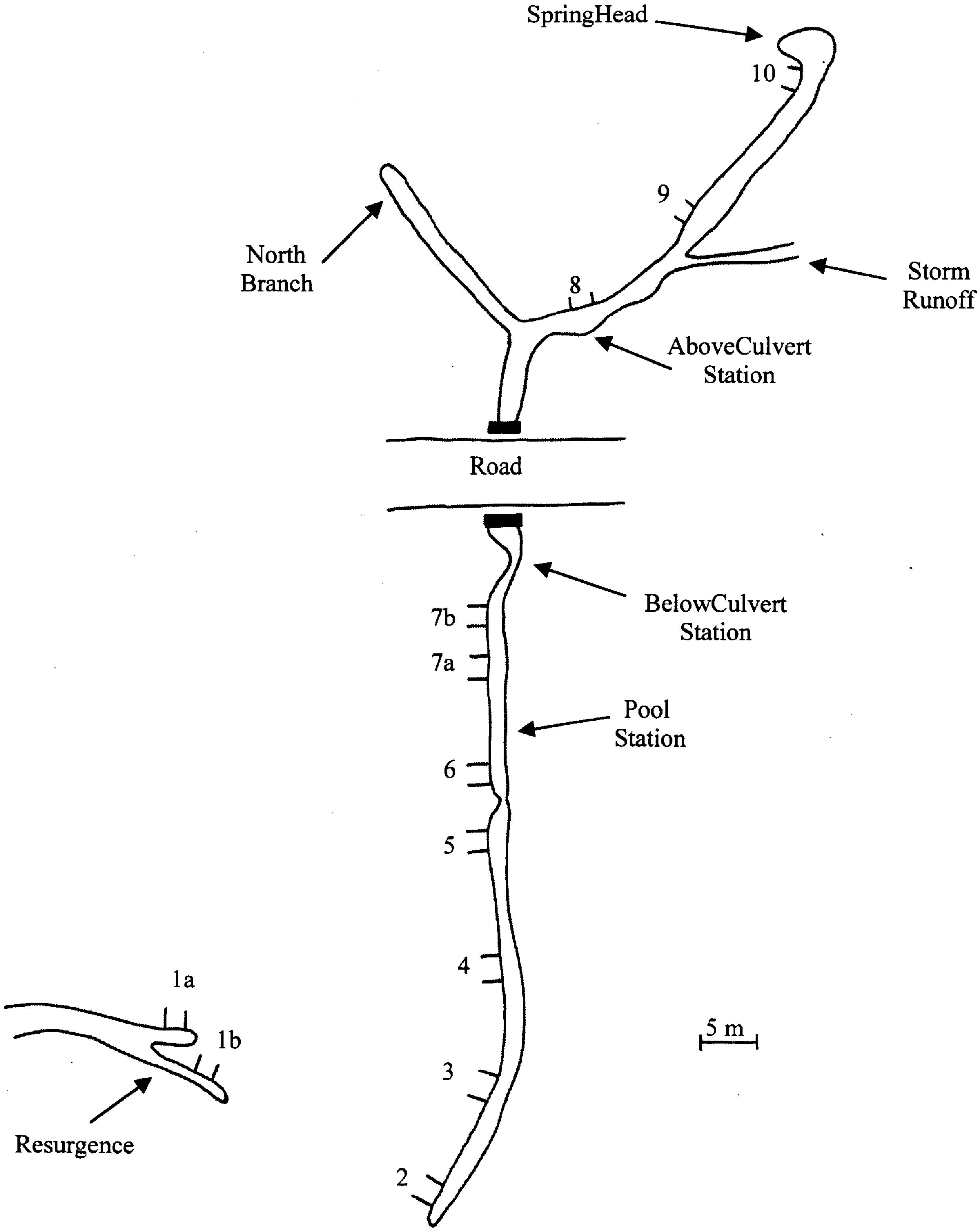

Figure 5. Deer Spring, LaRue-Pine Hills RNA, Union County, Illinois. Stations and plots used for monitoring YOY spring cavefish within the spring/stream are depicted.

Sampling technique for each plot.

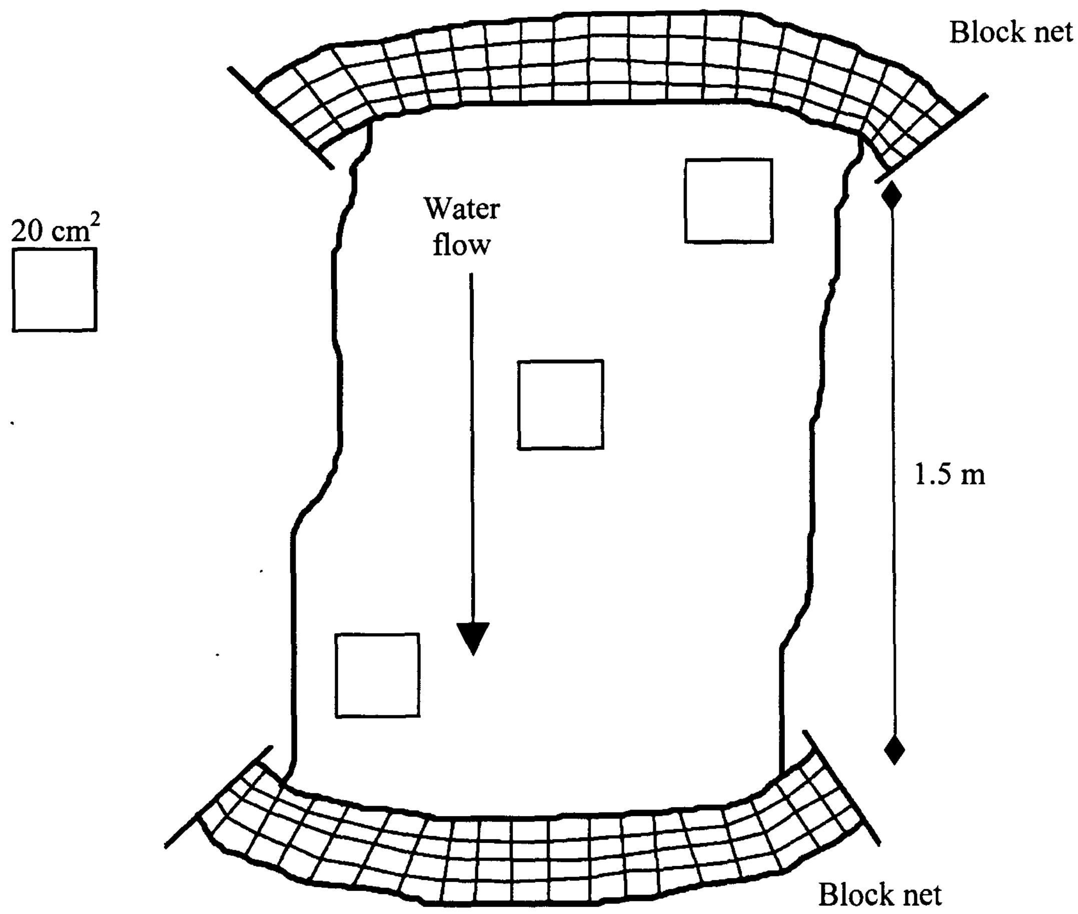

Figure 6. Diagram of the seasonal sampling procedure for each plot within three streams at LaRue-Pine Hills Ecological Area, Union County, Illinois. Block nets were placed at both the upstream and downstream edge of the plot. A 20 cm square was positioned in the stream three times along a diagonal. At each square, cover and substrate were determined for the edges and center of the square to give a total of 15 measurements.

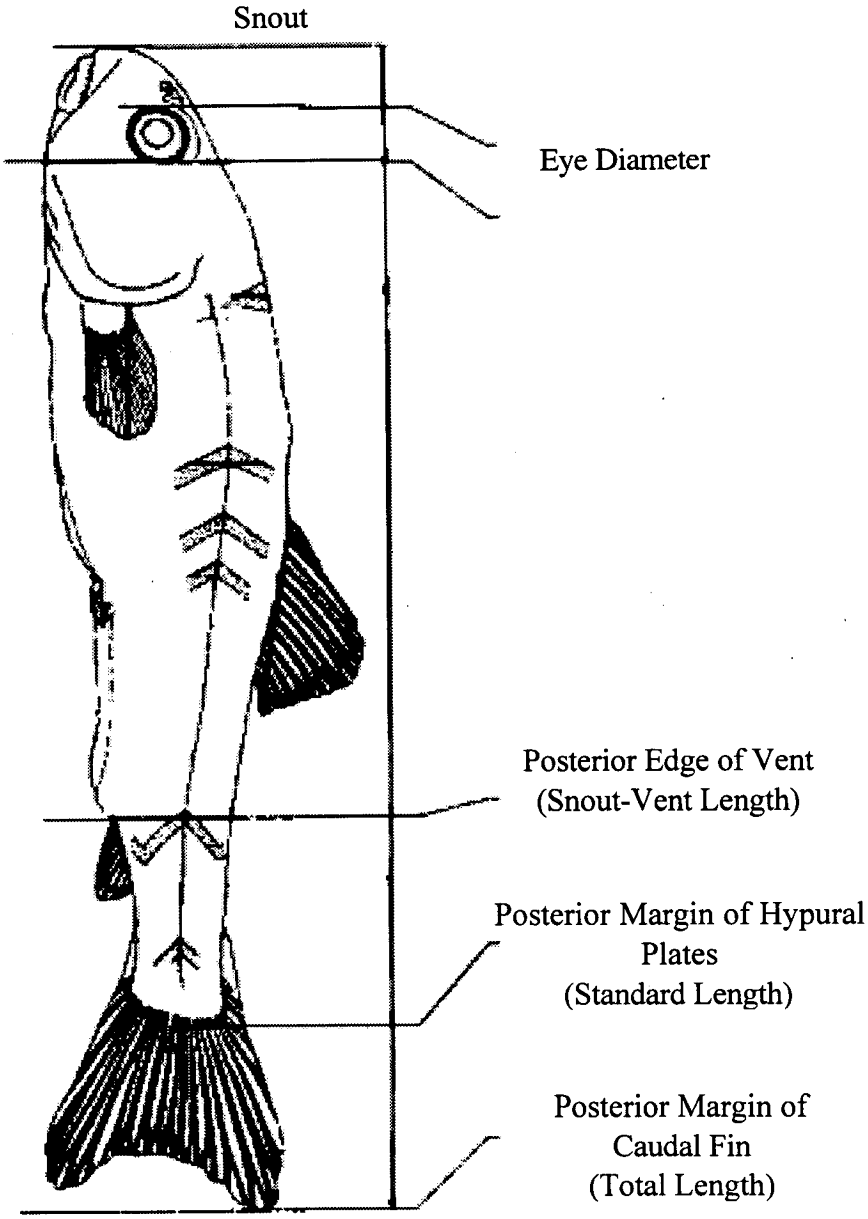

Figure 7. Morphometric measurements made on young of year and adult cavefish from LaRue-Pine Hills RNA, Union County, Illinois. (Modified from Snyder and Muth 1990).

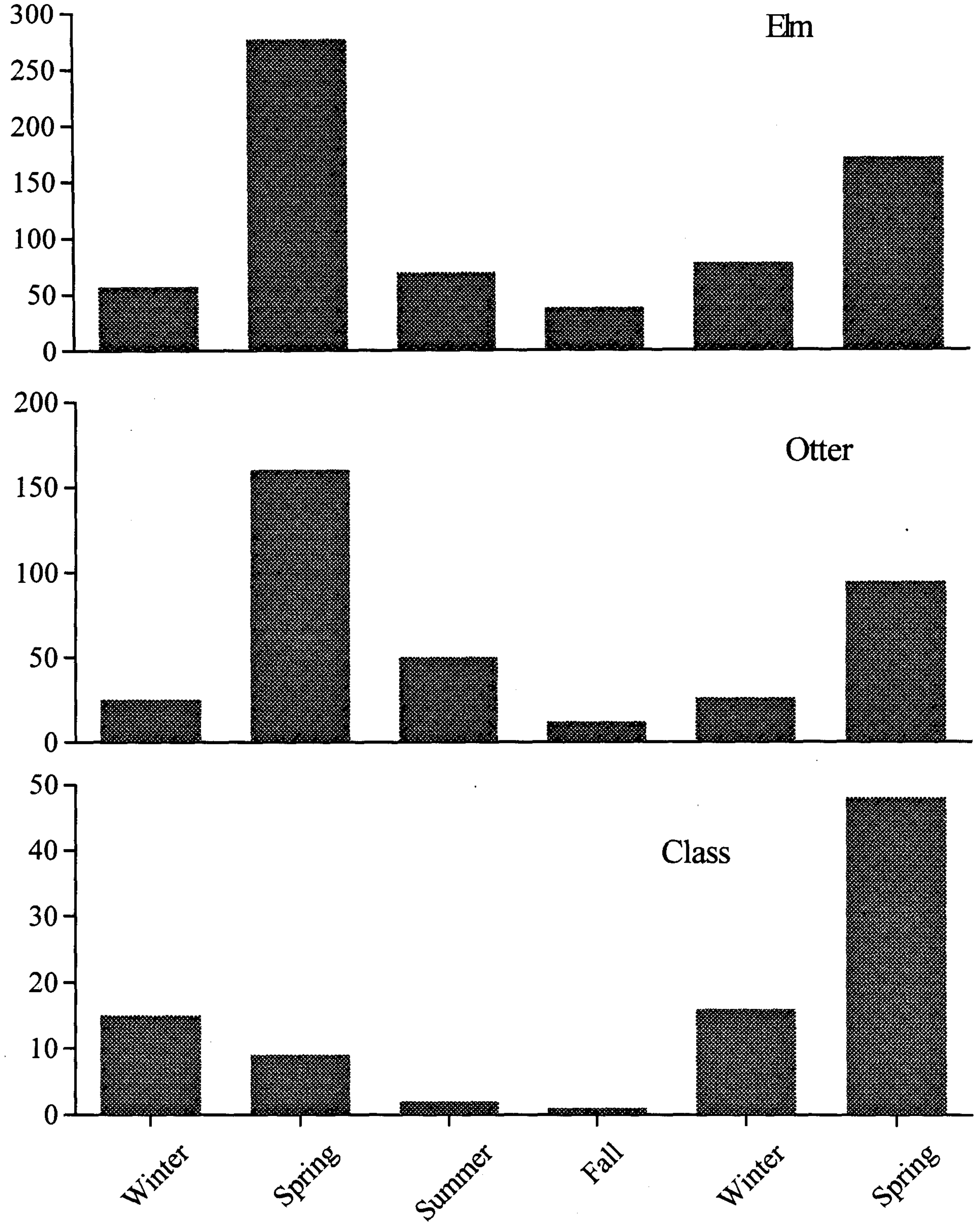

Figure 8. Seasonal changes in spring cavefish abundance for three springs at LaRue-Pine Hills RNA, Union County, Illinois.

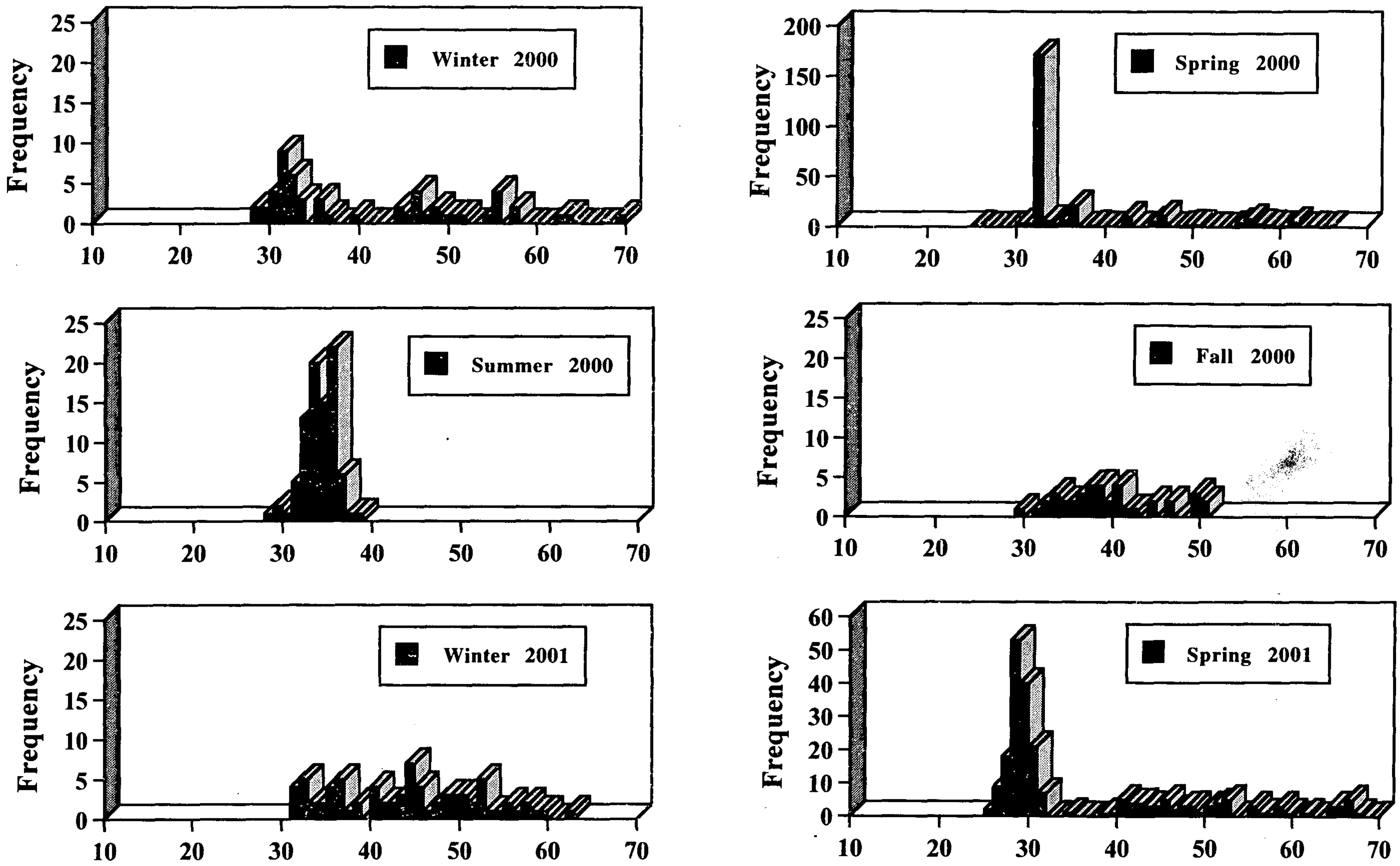

Figure 9. Length frequency histograms for spring cavefish at Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois, by season.

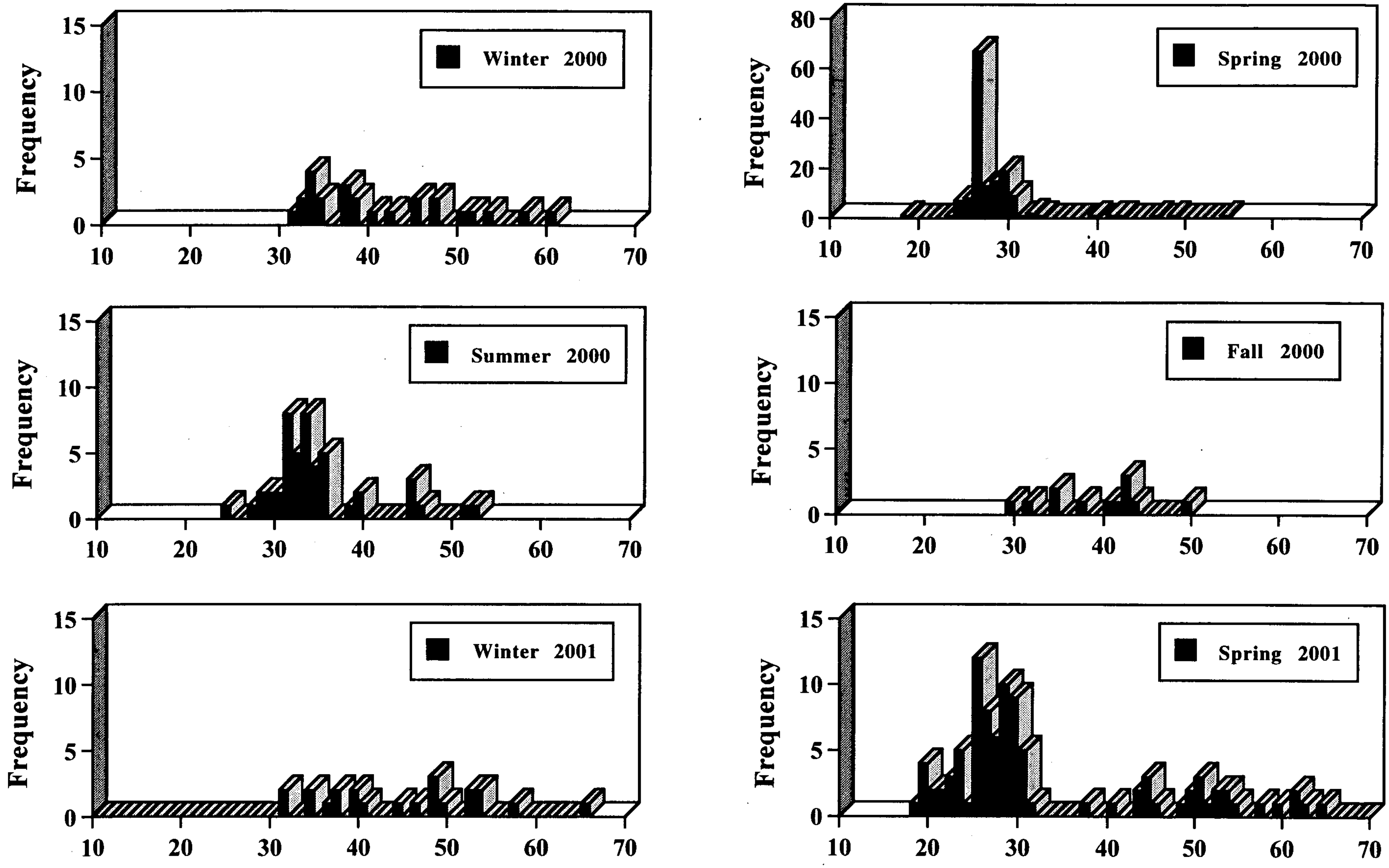

Figure 10. Length frequency histograms for spring cavefish at Otter Pond Spring at LaRue-Pine Hills RNA, Union County, Illinois, by season.

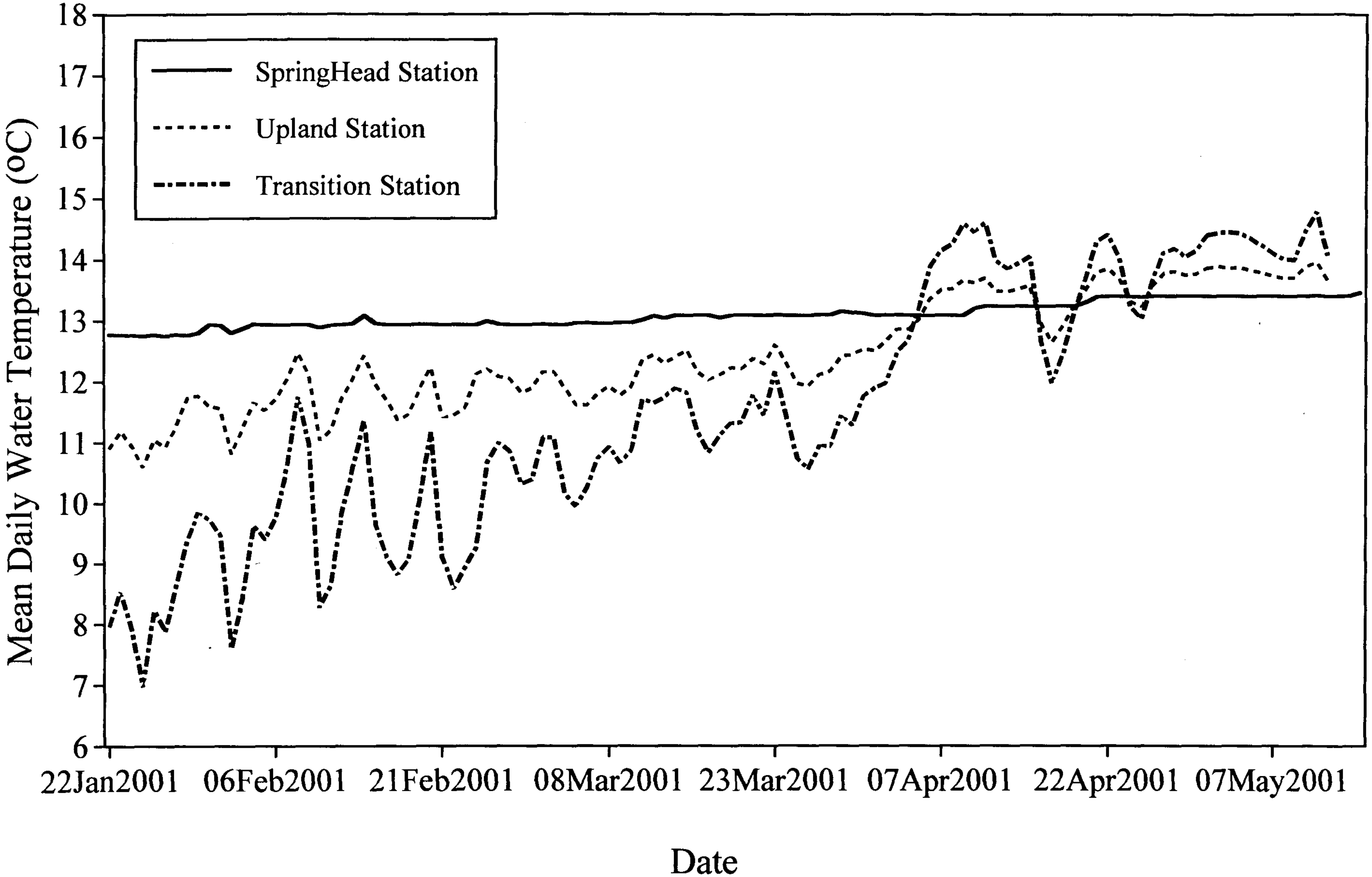

Figure 11. Mean daily water temperature at three stations in Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois.

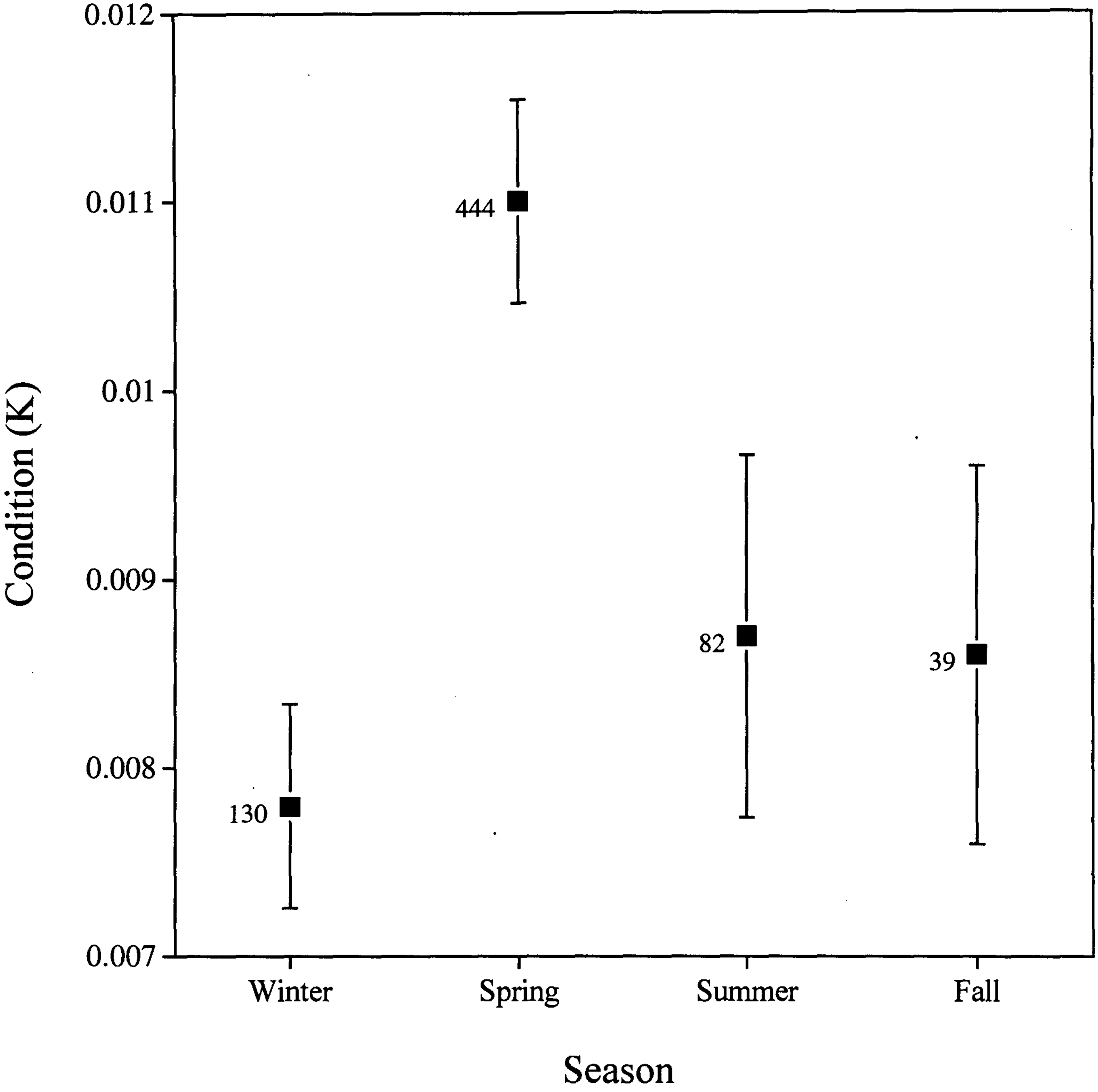

Figure 12. Mean condition for spring cavefish at Elm Spring at LaRue-Pine Hills RNA, Union County, Illinois. Error bars represent ± 2 standard errors.

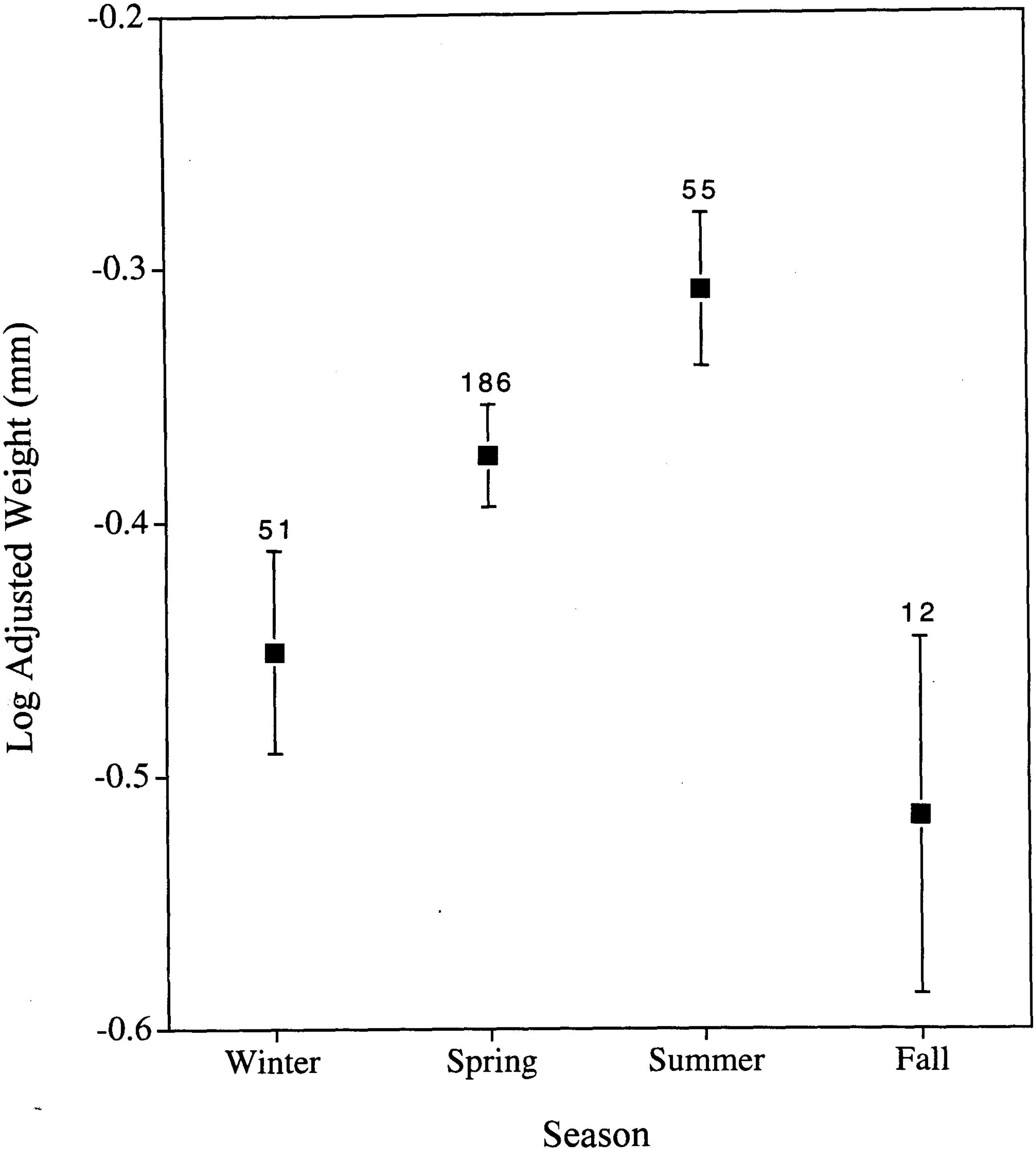

Figure 13. Mean condition for spring cavefish at Otter Pond Spring at LaRue-Pine Hills RNA, Union County, Illinois. Error bars represent ± 2 standard errors.

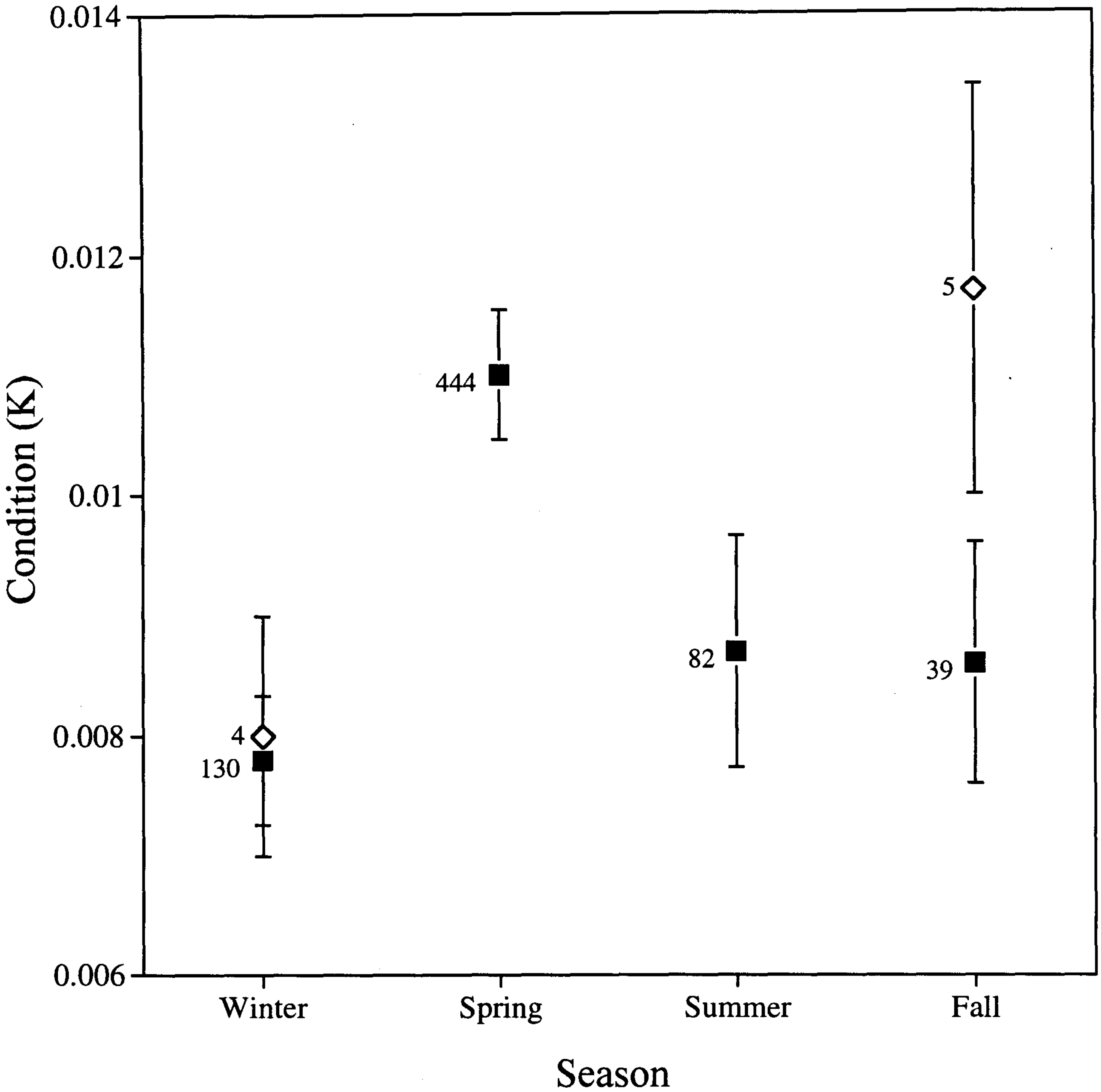

Figure 14. Mean condition for spring cavefish at Elm Spring at LaRue-Pine Hills RNA, Union County, Illinois. Squares represent fish in seasonal sampling plots and diamonds represent reproductively mature individuals found outside the seasonal plots. Error bars represent $\pm$ 2 standard errors.

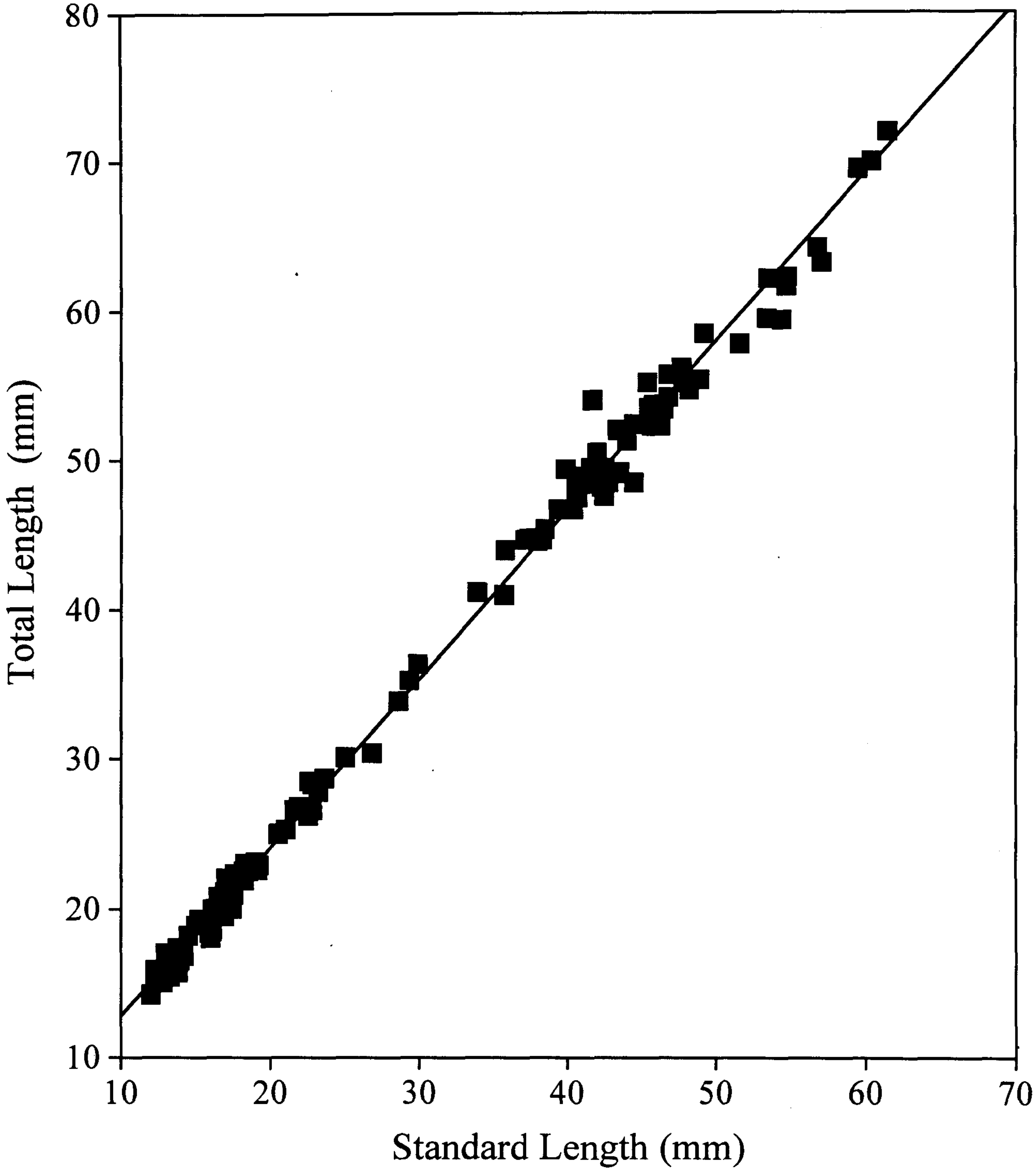

Figure 15. Regression line of standard length and total length for spring cavefish at LaRue-Pine Hills RNA, Union County, Illinois.

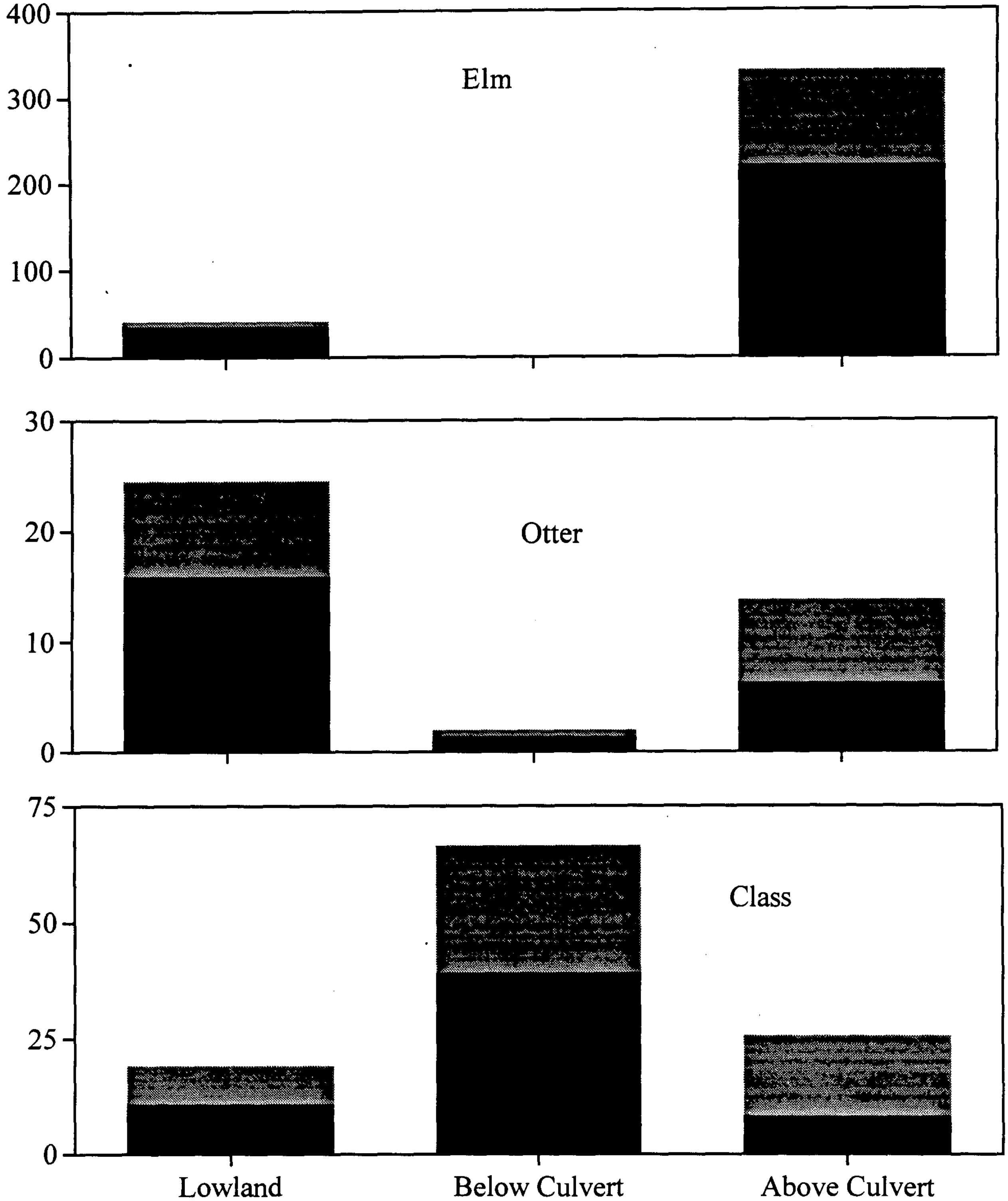

Figure 16. Abundance of spring cavefish in stations at three springs at LaRue-Pine Hills RNA, Union County, Illinois. Dark bars represent spring cavefish < 36 mm and light bars represent spring cavefish > 36 mm.

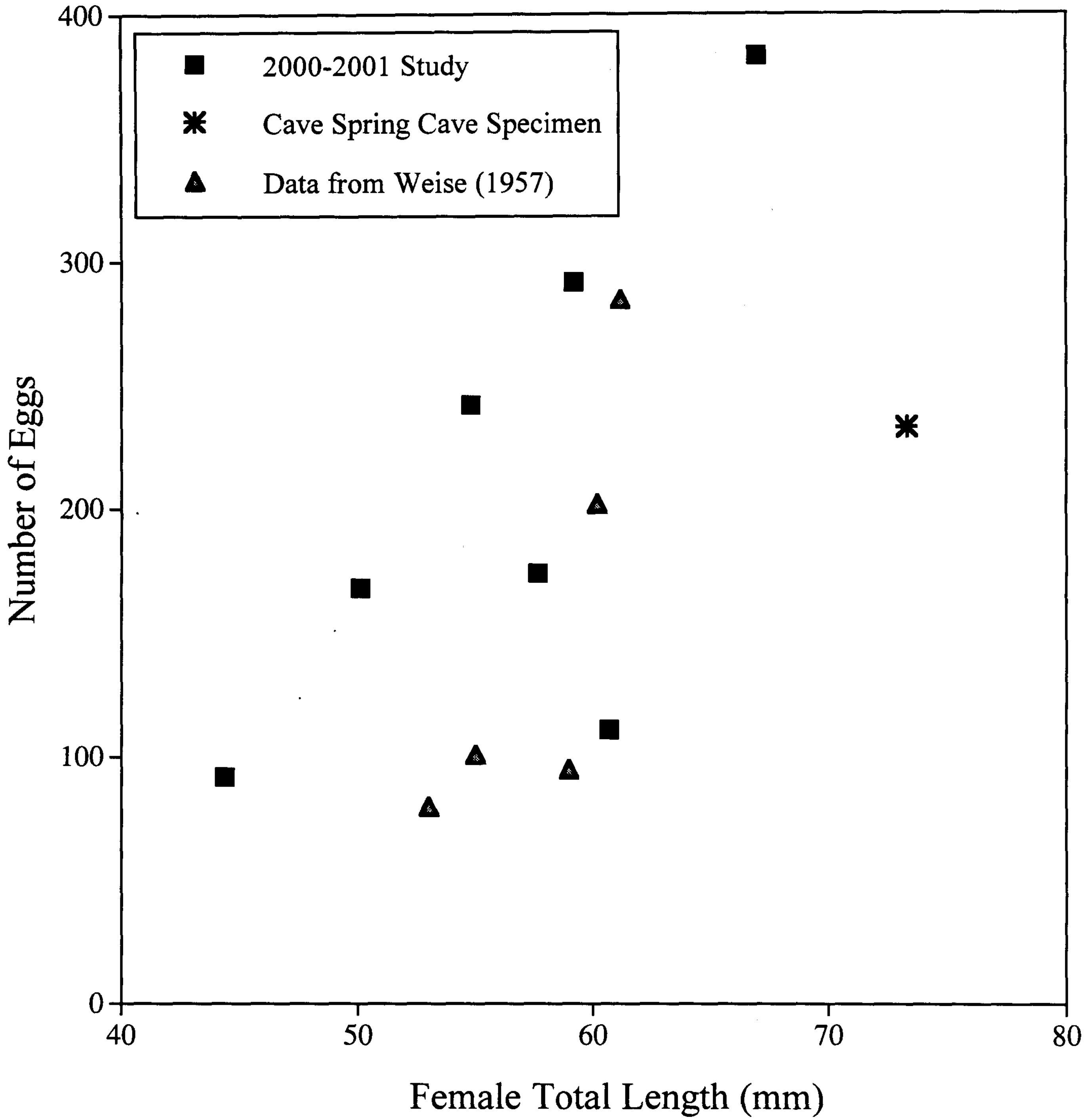

Figure 17. Number of eggs plotted against total length for females collected at Elm and Class Springs at LaRue-Pine Hills RNA, Union County, Illinois, and Cave Spring Cave, Union County, Illinois.

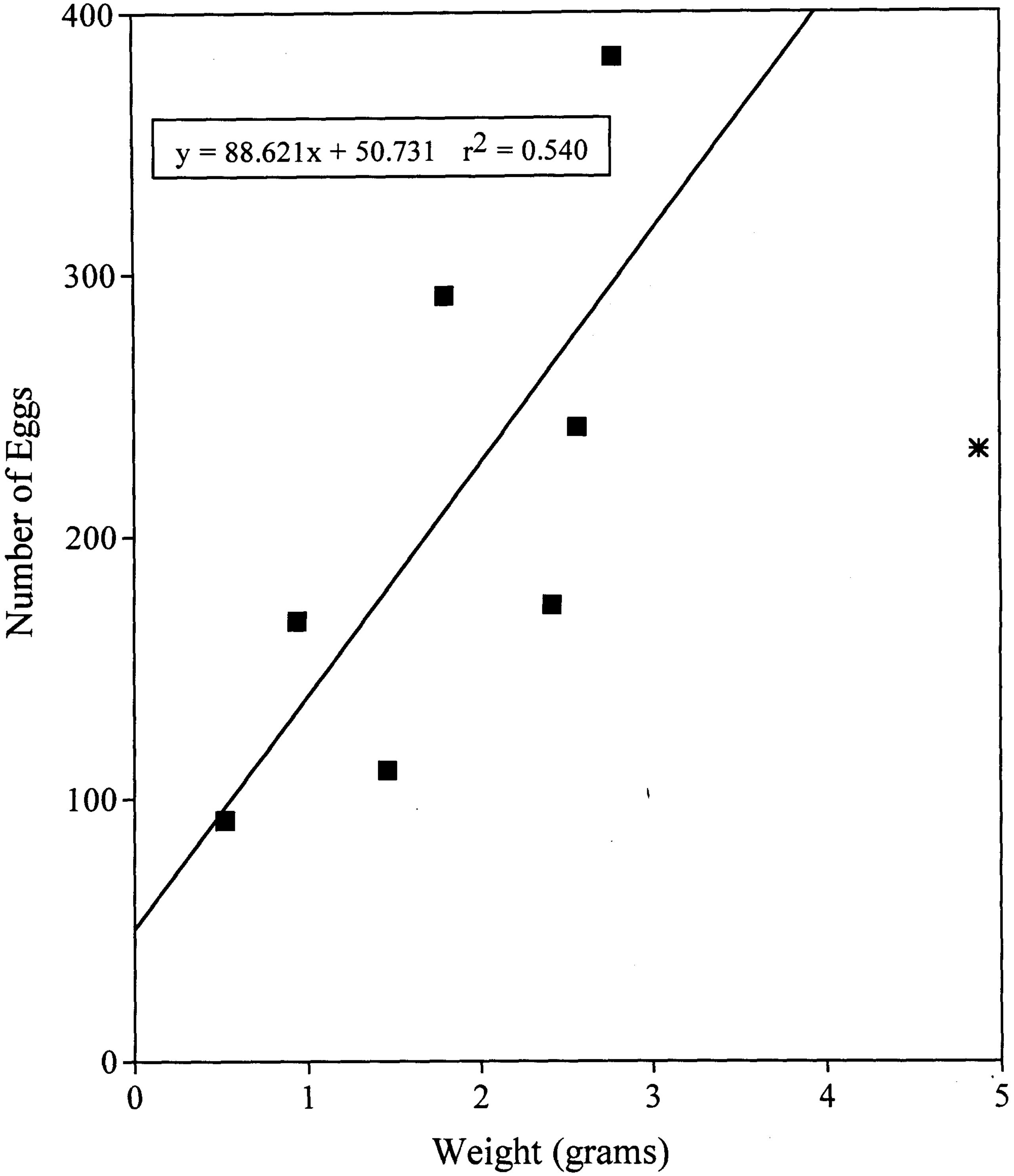

Figure 18. Relationship between female weight (mm) and total number of eggs for spring cavefish at Elm and Class Springs at LaRue-Pine Hills RNA, Union County, Illinois.
* denotes the single specimen from Cave Spring Cave (the only cave specimen)

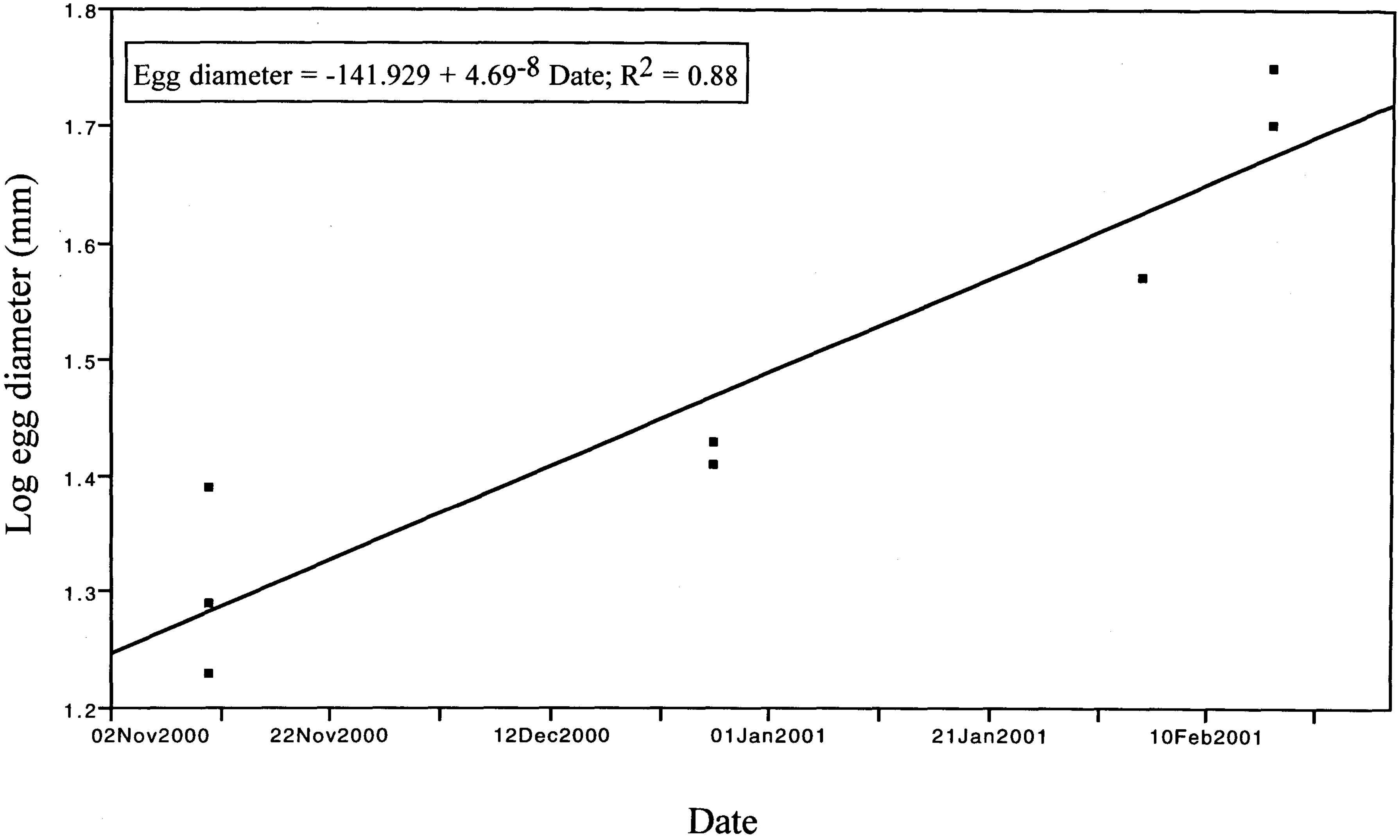

Figure 19. Log egg diameter plotted against date for spring cavefish collected at Class Spring and Elm Spring at LaRue-Pine Hills RNA and Cave Spring Cave in Union County, Illinois.

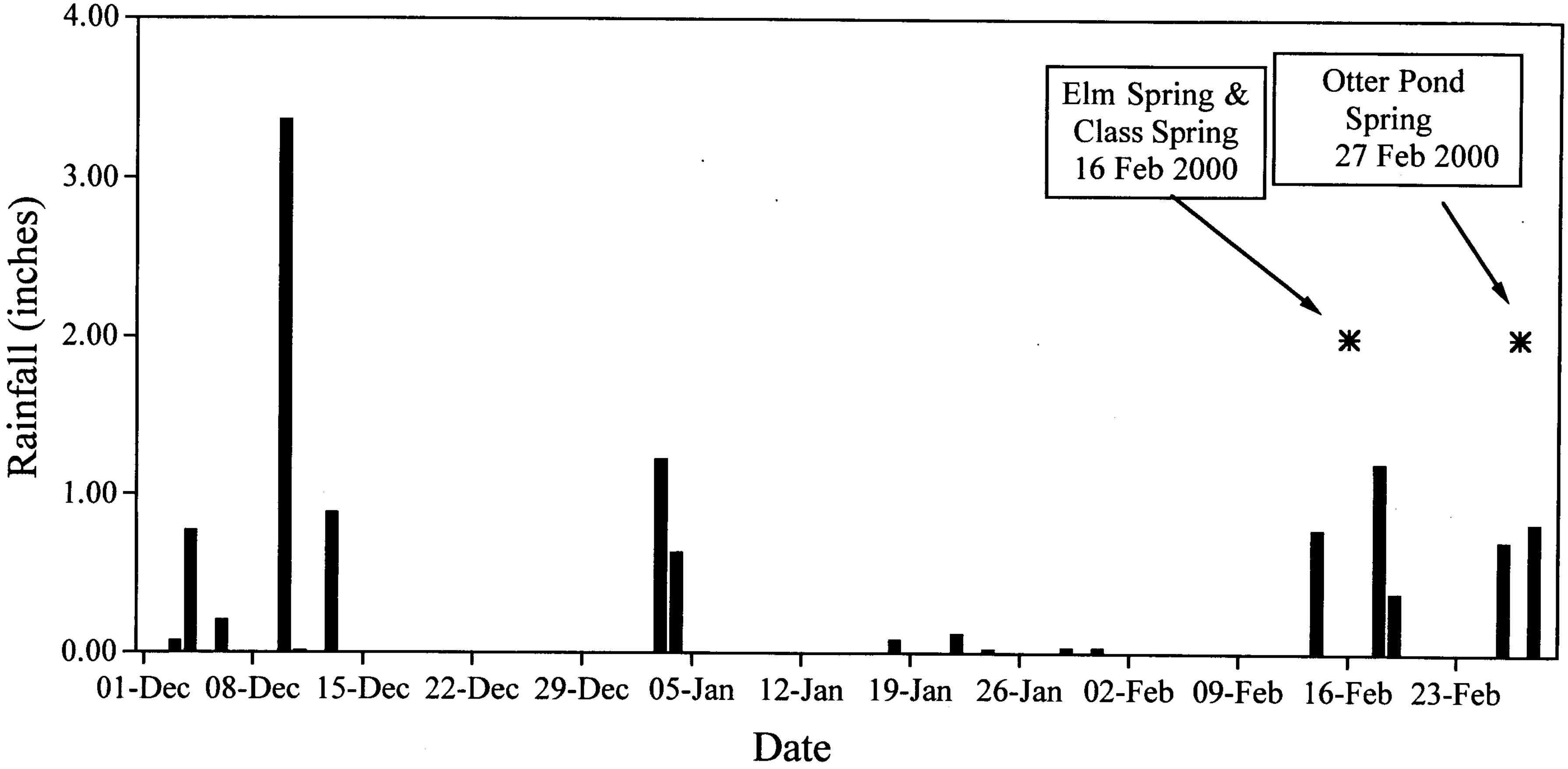

Figure 20. First appearance of larval spring cavefish at LaRue-Pine Hills RNA, Union County, Illinois, and rainfall data taken from a gauging station from December 1999 through February 2000 at Grand Tower, Illinois.

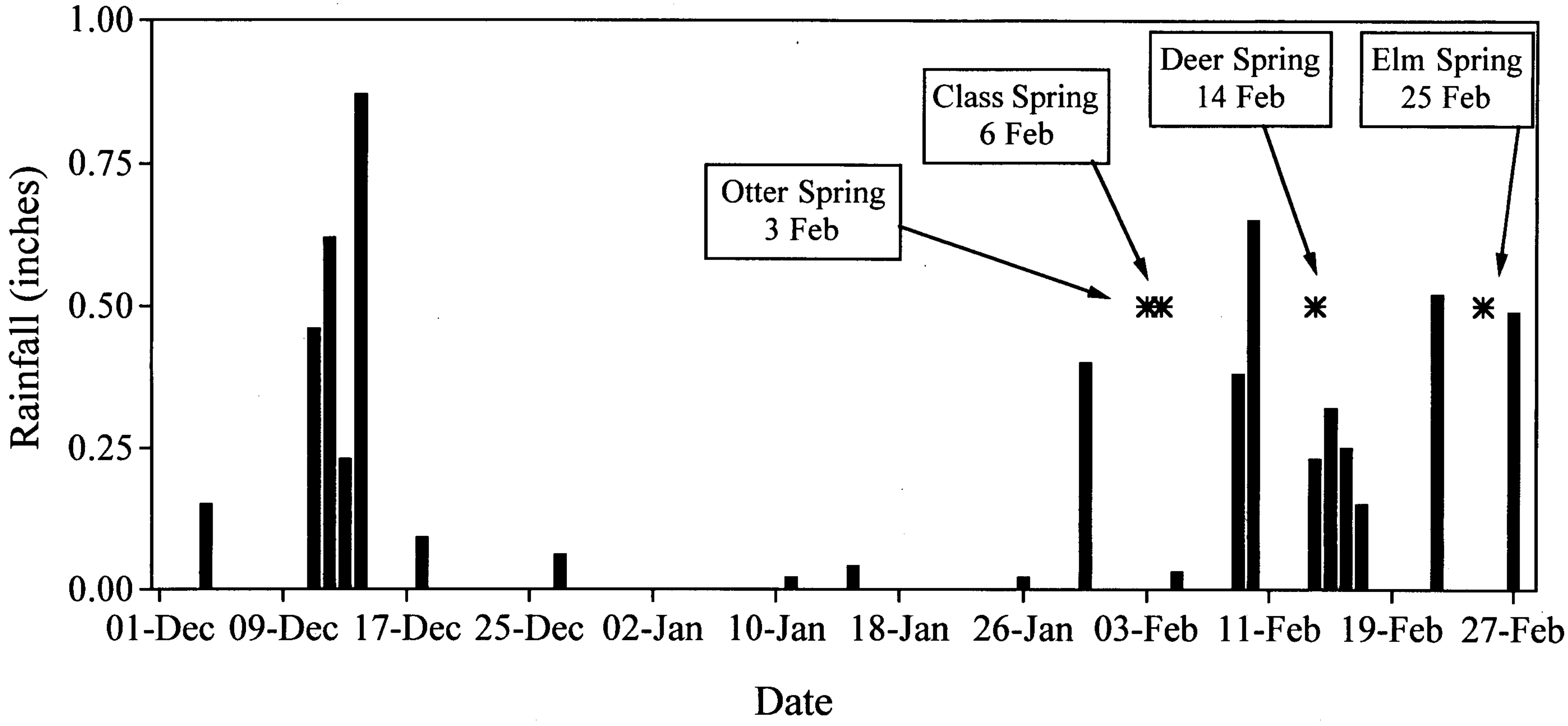

Figure 21. First appearance of larval spring cavefish at LaRue-Pine Hills RNA, Union County, Illinois, and rainfall data taken from a gauging station from December 2000 through February 2001 at Grand Tower, Illinois.

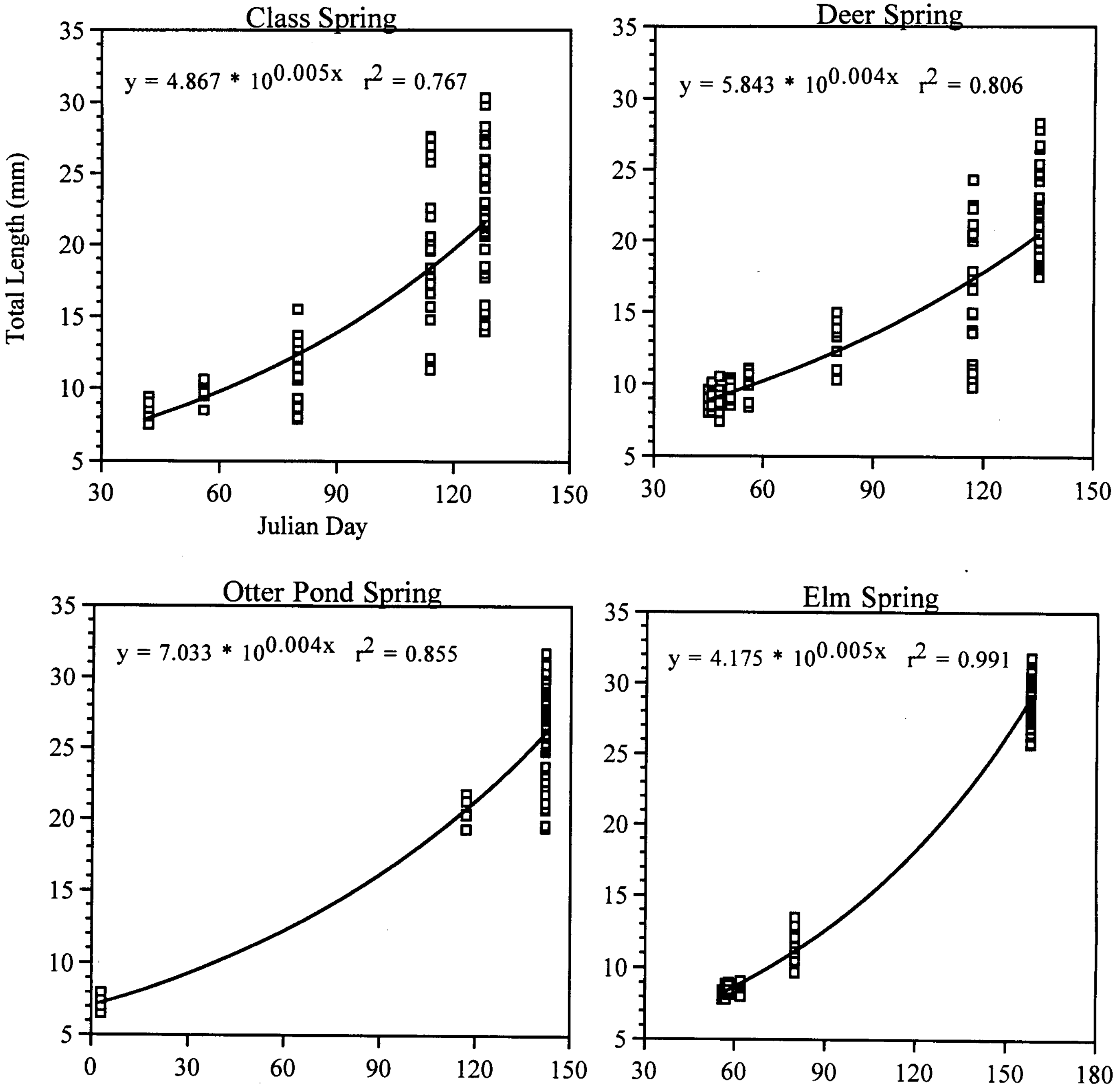

Figure 22. Growth of young-of-the-year spring cavefish in four springs at La-Rue Pine Hills RNA, Union County, Illinois, in 2001. Exponential regression curves provided the best fit to the data.

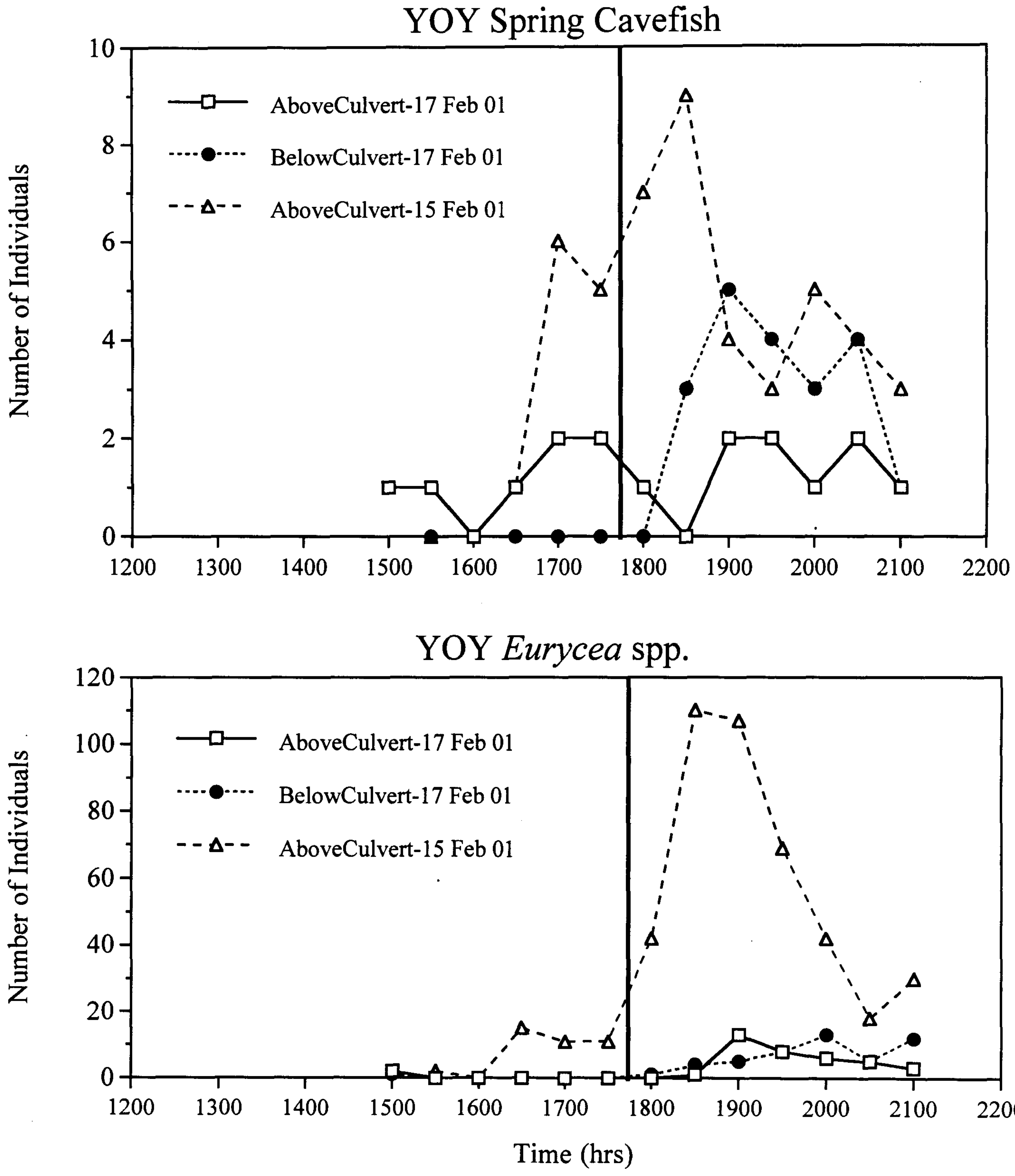

Figure 23. Diel drift patterns of young-of-the-year (YOY) spring cavefish and *Eurycea* at Deer Spring, LaRue-Pine Hills RNA, Union County, Illinois. A vertical line distinguishes day and night samples.

to avoidance of thermal fluctuations. This strategy would result in decreased stress and metabolic rate, allowing more effective use of lipid stores.

Condition was examined among seasons using analysis of covariance (ANCOVA) with length as the covariate and weight as the dependent variable for cavefish in Elm and Otter Pond Springs. Class Spring was omitted because of low sample sizes. Condition of individuals in Elm Spring could not be compared using ANCOVA because the assumption of parallelism of lines was violated (Test of Parallelism: $P < 0.02$). This implies the length-weight relationship was not congruent among all seasons. Variation in slopes may have been influenced by the inconsistency of size classes present in the sample (lack of cavefish > 36 mm in the summer sample). Therefore, data were converted to condition values ($K = \text{weight}/\text{length}^3 \times 1000$) and compared using analysis of variance (Figure 12). Using K values, condition was significantly higher in spring compared to all other seasons. All assumptions were met for analysis of covariance for condition at Otter Pond Spring (Test of Parallelism: $P = 0.69$). There was a significant covariate effect (total length) on weight ($P < 0.001$) and a significant effect of season ($P < 0.001$). Summer condition was significantly higher than all other seasons and spring was significantly higher than fall and winter. Fall and winter condition was not significantly different (Figure 13).

Low condition values in fall and winter may be due to several possibilities. During fall sampling, we observed gravid females in the most upstream portion of Elm Spring, adjacent to the spring head. This area did not correspond to one of our standardized plots; therefore, the individuals were not included in the seasonal condition analysis. No gravid females were found in our standard plots at either Elm or Otter Pond springs, and it is possible fish sampled during fall represent individuals not in the "reproductive pool." When mean condition for reproductively mature individuals was overlaid onto the seasonal condition graph, we see these individuals have increased condition compared to those sampled in our plots during fall (Figure 14). In fact, reproductively mature individuals exhibit mean condition values similar to individuals in the spring. Low condition in winter may be a direct result of post-spawning condition. By spring and summer, high lipid content was present in cavefish (the lipid deposits being visible through the body wall). Increased condition in the summer at Otter Pond Spring relative to Elm Spring may be a result of the lack of adults observed at Elm Spring during our summer sample.

We generated a regression model based on 118 fish for total length on standard length in order to provide a method of conversion for data among papers and studies. The regression equation: Total Length = 1.637 + 1.125(Standard Length) was supported by a R^2 value = 0.995 and $P < 0.0001$ (Figure 15).

Longitudinal distribution and habitat selection - Summaries of water quality measurements are provided for each spring and station in Tables 9, 10, and 11. Station had a significant overall effect on the distribution of cavefish at LaRue-Pine Hills RNA (Figure 16). Gravel or silt/gravel substrate, large cover in the form of pebbles and boulders, and a moderate gradient typically characterized the Upland Stations. The Transition Stations usually exhibited a decrease in stream gradient, a change from gravel to silt substrate and little to no cover. Lowland Stations had primarily a mud/detritus substrate and cover in the form of leaf litter and woody debris. The Upland Stations had a significantly higher mean number of cavefish (mean = 15.07; $\pm$ 2.6 SE) compared to the Transition (mean = 3.91; $\pm$ 2.8 SE) and Lowland Stations (mean = 1.38; $\pm$ 2.9 SE) for all springs combined. No significant differences were found between the Transition and Lowland Stations. There was a significant interaction between location and station in the overall model ($P < 0.0001$). Class and Elm Springs exhibited a similar trend, with highest mean abundance in the Upland Station compared to the Transition and Lowland Stations. Otter Pond Spring did not demonstrate a significant difference in cavefish abundance between Upland and Transition Stations; however, Upland and Lowland Stations were significantly higher than Lowland and Swamp stations. There was also a significant interaction between location, season, and station for the overall model ($P < 0.023$). As a result of the significant interactions observed in the model, many of the subsequent habitat analyses will be broken down either by location or both by location and season.

We attempted to provide a habitat model for each spring during each season using stepwise regression. Cavefish greater than 36 mm (assumed to be 1+) were analyzed separately from cavefish less than 36 mm (assumed to be YOY). Results of these analyses are reported in Tables 12 and 13.

At Elm Spring, habitat models for adult cavefish were generated for every season except summer, which did not contain adult fish. High R^2 values were indicative of the models for this spring (ranging from 0.64 to 0.99). The variable that accounted for a large amount of variation in every season was boulder cover. Boulders were characteristic of the habitat in the Upland Station and are where a majority of fish were collected. Pebble cover and small gravel substrate also explained some of the variation in cavefish abundance.

For Otter Pond Spring models were generated for every season. However, they were not as congruent among seasons as Elm Spring in variables that explained variation in cavefish abundance. In winter, gravel substrate and pebble cover explained 82% of the variation in abundance. During this season, adults were found primarily in the most upstream plots within the spring. In spring, amphipod density, conductivity and silt/gravel substrate explained 87% of the variation. Cavefish were negatively correlated to amphipod density and this is likely a result of amphipod depletion where large numbers of cavefish were present. Conductivity generally varied longitudinally throughout the spring and a positive correlation with this variable indicated cavefish were more abundant in the upstream stations (Upland and Transition) as opposed to the downstream stations (Lowland and Swamp). The importance of silt/gravel substrate during spring is explained largely by the presence of adults in the Transition Station. During summer, amphipod abundance was the only significant variable, accounting for 52% of the variation. Adults were negatively correlated to amphipod abundance, similar to the spring season. It is interesting to note spring and summer are also the two seasons in which condition is highest for cavefish at Otter Pond Spring and there may be a direct relationship between depletion of amphipods and increase in cavefish condition during this time. In fall, three variables explained 67% of the variation in cavefish abundance. Cavefish were negatively correlated to width and lack of cover and positively related to depth. During fall, cavefish were mainly found in narrow plots that contained a cover item. In one instance, the cover item (a log) was found just below a riffle; this resulted in a scouring effect, which created a deeper pool.

We were only able to provide a seasonal habitat model for adults at Class Spring during winter. Spring did not produce a significant model and there were only two adult cavefish in the summer sample and one in the fall. In winter, cavefish were positively correlated with dissolved oxygen and gravel cover and negatively correlated with conductivity ($R^2 = 0.94$). In other words, they were found principally at the Upland Station (which has a lower conductivity, higher dissolved oxygen, and gravel cover).

Habitat models were constructed for YOY cavefish in spring, summer, and fall at Elm Spring, winter at Otter Pond Spring, and spring at Class Spring. Other seasons either did not contain young-of-the-year or did not produce a significant model. At Elm Spring, the primary variables accounting for variation in fish abundance were very similar to habitat models for adults. Overall, gravel substrate and boulder cover were the two primary variables related to YOY abundance. Young cavefish at Otter Pond Spring in the winter were negatively correlated to plot width and silt/gravel substrate. These individuals were found in soft mud substrate in plots that were relatively narrow. At Class Spring, silt substrate explained 37% of young cavefish abundance. Young at Class Spring had an affinity for Lowland Plot 3, which was primarily silt substrate.

Spearman Correlation was used to examine relationships between abundance of cavefish and other aquatic organisms by location. There was no difference in the variables that were correlated to cavefish abundance for the two size classes (<36 mm and >36 mm TL) so all cavefish were combined. For Elm Spring, cavefish abundance was positively correlated to presence of larval *Eurycea* spp. (Spearman $r_s = 0.2928$; $P < 0.03$). At Otter Pond Spring cavefish abundance was negatively correlated to presence of slough darters (Spearman $r_s = 0.323$; $P = 0.005$), and positively correlated to larval salamanders (Spearman $r_s = 0.507$; $P < 0.0001$). At Class Spring only abundance of larval salamanders was significantly correlated with spring cavefish (Spearman $r_s = 0.327$; $P = 0.018$). Other species collected but not showing significant correlations with abundance of cavefish include: western mosquitofish (*Gambusia affinis*), northern starhead topminnow (*Fundulus dispar*), central mudminnow (*Umbra limi*), pirate perch (*Aphredoderus sayanus*), grass pickerel (*Esox americanus*), banded pygmy sunfish (*Elassoma *zonatum*), bluegill (*Lepomis macrochirus*), slough darter (*Etheostoma gracile*), central newt (*Notophthalmus viridescens*), devils crayfish (*Cambarus diogenes*), and water scorpions (*Nepa apiculata*). Central mudminnow and adult spring cavefish have been reported to prey on young cavefish (Gunning and Lewis 1955) and pirate perch are known to feed on swampfish (*Chologaster cornuta*) (Shepherd and Huish 1978), implying a high probability they would also consume spring cavefish. Other species listed above have not been documented preying on spring cavefish, but it is likely that grass pickerel occasionally eat them. The negative correlation of cavefish to slough darters at Otter Pond Spring may just be a spurious relationship resulting from seasonal partitioning of the habitat by these two species. Spring cavefish were found in the Swamp Station predominantly in the spring season, whereas slough darters utilized the same habitat in the winter.

Positive correlation of spring cavefish and *Eurycea* spp. during each season is likely related to the similarities in life history traits exhibited by these species. Adult salamanders are thought to move into underground habitat in winter/early spring to lay eggs. After the eggs hatch, the young salamanders drift downstream and settle in the surface habitat (presumably due to increased food quality). We were unable to detect species level identifications between larval *Eurycea lucifuga* and *E. longicauda* in the field, so they were grouped for the analyses. Although only preliminary analyses have been conducted on habitat models for larval *Eurycea* in this study, it appears that similar habitat characteristics are important in their distribution relative to spring cavefish.

Devil's crayfish were often found in association with spring cavefish, particularly at the Upland Station at Elm Spring. Devil's crayfish are known to build burrows which extend a few meters underground and it is possible spring cavefish are utilizing these burrows as a pathway underground or as a refuge during low water conditions. When conducting seasonal sampling we would often find burrows in the Upland Station under large boulders. Cavefish could be seen darting into the burrows and could be stirred out by gently prodding a stick into the hole.

Reproduction and Early Life History

Reproduction- Gonadal development - Gravid females were observed between 11 November 2000 and 27 February 2001, at Elm Spring, Class Spring, and Cave Spring Cave. No gravid females were found at Otter Pond Spring during this study. A total of 39 gravid females were observed: 6 at Elm Spring, 8 at Class Spring, and 29 at Cave Spring Cave (Table 14). Mature males were not detected until later than females, and were found between 6 January and 12 February 2001, at Elm Spring and Cave Spring Cave. A total of 9 mature males were observed: 3 at Elm Spring and 7 at Cave Spring Cave (Table 14).

At Elm Spring we found gravid females between 11 November and 6 January (Table 14). Gravid females were easily distinguished during this time period because eggs were visible through the body wall. During our winter seasonal sampling, on 7 February, we observed in the field what we believed to be gravid females. These individuals had a deep yellow coloration in the opercular and abdominal region and a large yellowish-cream body, which appeared to be the ovary. However, two specimens were collected and brought back to the laboratory and found to have large lipid deposits, but no eggs. These fish may have been females since they had the yellowish coloration that is characteristic of other female amblyopsids (pers. comm., Brown and Johnson) and of female spring cavefish in particular (personal observation). Weise (1957) reported gravid females in the field from almost every month of the year but stated there is evidence to suggest most fish ready for spawning disappear in late winter. He only provided complete fecundity data on 5 females that were held in the laboratory from September through March; he did not collect what he assessed to be gravid females from any other time of the year. It is possible Weise (1957) mistook individuals with yellow coloration and presence of large fat deposits as gravid females during spring and summer. Other than a single small gravid female collected in June by Smith and Welch (1978), no other researchers have observed gravid females at any time other than fall through mid-winter.

After initially finding mature females at Elm Spring, we began an intensive monitoring regime at Cave Spring Cave. We observed a total of 154 cavefish over 9 sampling trips into the cave and found 36 reproductively mature individuals, from which length and weight measurements were taken on 14 individuals (Table 14). The average number of cavefish counted in the cave was 22, with a maximum of 40 counted on the first trip. Diffuse white lights and/or red lights (Petzl headlamp with red lens) were used to make observations and did not seem to negatively affect the cavefish. Gravid females were obvious from above due to a distended abdominal region. However, once males matured, it was often difficult to distinguish whether individuals were male or female without actually examining the gonads with a strong light. Reproductively mature individuals in the cave ranged in size from 49.5 to 74.5 mm total length. Condition was similar between reproductively mature individuals, although female condition (mean = 0.011 $\pm$ 0.001 SE; N = 9) tended to be higher than that of the male (mean = 0.009 $\pm$ 0.002 SE; N = 5).

We hoped to elucidate details of spring cavefish reproduction by monitoring Cave Spring Cave. Other studies have suggested spring cavefish are unable to reproduce unless they are underground. On each trip to the cave, we searched for eggs (both in the gill cavity and on substrate) and YOY. At the time of our last trip (27 February 2001) into the cave we were unable to find adult cavefish, primarily due to high water from snowmelt. High flow resulted in increased turbidity in the pools, further limiting our ability to make observations. On the previous trip (12 February 2001) we found females still carrying eggs, indicating reproduction may not have occurred at that point. This is noteworthy because we were already finding YOY on the surface at Class, Otter Pond, and Deer Springs.

On the surface at Elm and Class Springs, gravid female cavefish were only found at the most upstream stations. At Elm Spring, gravid females were found under a large boulder adjacent to the spring head and could not be collected at any of our permanent seasonal plots. At Class Spring gravid females were found both at the tile at the spring head and just downstream of the confluence of North Branch (corresponding to Upland Plot 2). The cavefish observed at the tile were swimming openly in the water column whereas those in Upland Plot 2 were found under a cobble size rock. Cavefish near the spring head are likely at an advantage because of the close proximity of the underground environment. In the cave environment at Cave Spring Cave, we rarely found spring cavefish under cover items and then only during high flow. Cavefish often swam free of the bottom, which was bedrock overlaid with silt, and appeared to move in a haphazard pattern, often coming into contact with other objects and fish in the pool. This is similar to behaviors exhibited by other cave amblyopsids (Poulson 1963).

Data for fecundity measures taken in the laboratory on 8 females from Elm Spring, Class Spring, and Cave Spring Cave are summarized in Table 15. Total number of eggs was counted for each female and compared to data collected by Weise (1957). Egg number was significantly correlated with female total length and this relationship varied in a similar fashion to data collected by Weise (1957) (Figure 17). The one exception being a single individual collected during this study from Cave Spring Cave, that had fewer eggs compared to smaller-sized individuals from the surface. It is interesting that the one cave specimen had fewer eggs, as would be predicted in the cave environment because of decreased food availability. Further investigation is needed to determine if this individual represents a unique trend in the cave environment or if it was simply an anomaly. When number of eggs is plotted against female wet weight cavefish collected in surface waters exhibit a higher number of eggs/female body weight compared to a larger individual in the cave (Figure 18).

Relationship between egg diameter and number of eggs ($P > 0.05$) or between egg diameter and female total length ($P > 0.05$) was not significant. We found a significant increase in log egg diameter over time ($P < 0.001$; $R^2 = 0.88$) (Figure 19). Egg diameter increased from approximately 1.25 mm in mid-November to 1.70 in mid-February. Weise (1957) reported reproductively mature females with egg diameters between 1.5 and 2.0 mm, indicating females collected in mid-February during this study were close to spawning. Within a single female there was little variation in egg diameter suggesting female spring cavefish do not produce multiple clutches.

Although we were unsuccessful in illuminating details of spawning for this species, the information provided herein increases accuracy for data on timing of reproduction and changes in fecundity parameters prior to reproduction. Cave Spring Cave is an excellent location for pursuing details of spring cavefish reproduction and it is likely this location will continue to provide scientific information because of the environmental ethics of the current landowners.

The last issue that should be addressed pertaining to spring cavefish reproduction is the hypothesis of gill brooding in this species. Many authors (Eigenmann 1909, Weise 1957, Poulson 1963) have suggested that spring cavefish are gill brooders based on the observation of gill brooding in *Amblyopsis spelaea* (Eigenmann 1909, Poulson 1963). At this time we feel it is presumptuous to assume this species is a gill brooder. *Amblyopsis spelaea* is unique among the amblyopsids in exhibiting a very distended opercular cavity relative to head size. Spring cavefish on the other hand, have a relatively small head and it is hard to imagine even a small number of eggs and larvae being held in the gill cavity. It is possible this species conforms to the reproductive behaviors exhibited by the closely related pirate perch which also has a jugular vent but is a nest builder.

<u>Initial appearance of young cavefish</u> - Based on presence of larval cavefish in winter of 2001, reproduction occurred in all study springs, including Deer Spring. Young-of-the-year (YOY) spring cavefish first appeared on the surface at LaRue-Pine Hills RNA in Otter Pond Spring on 3 February 2001 (Table 16). Four individuals (6.5-8.0 mm TL) were captured in the Lowland Station in winter quarterly population sampling. Low numbers of YOY despite extensive sampling of the spring indicated this was not a major "pulse" of young at Otter Pond Spring. Larvae appeared in the other springs in early to late February 2001 which is similar to time of first appearance in 2000 (Figure 20). In both 2000 and 2001, timing of larval appearance on the surface corresponded with increased rainfall following a period of low rainfall in January (Figures 20 and 21). This correlation suggests appearance of larval cavefish in the surface springs was assisted by increased discharge due to local rainfall patterns.

Larval cavefish were next found at Class Spring on 4 February 2001 during quarterly sampling. Twelve larvae (7.5-8.1 mm TL) were captured in Upland Plot 1 and one larva (7.8 mm TL) was found in Upland Plot 4 located in the North Branch. We further investigated appearance of larvae on 6 February. During a one-hour set, 2 larvae were captured in a drift net placed directly below the road culvert, and 6 larvae were captured in a net placed below the main spring head in Plot 1. Fish ranged from 8.4 - 9.2 mm total length (TL). Also, 1 larval cavefish was observed at the spring head of Watercress Spring. Locations of larval cavefish indicated larvae emerged from the resurgences of all three primary springs comprising Class Spring. On 10 February 2001 at 1800 hr, 34 larval spring cavefish were observed in a 1-m stretch of shoreline just downstream of Transition Plot 1 and just upstream of Lowland Plot 3. These fish were stationary on the bottom (3 cm deep) and often in groups of 2-3 fish. They were in the open (i.e., not under cover) oriented upstream where flow was approximately < 5 cm/s. When an individual was disturbed by the observer or by an amphipod swimming near it, the fish would swim up to the surface in a quick burst and then immediately swim back to settle on the bottom, not far from its original position. A few larval cavefish were observed in close proximity (in one instance, very near the mouth) of western mosquitofish *Gambusia affinis*, but the mosquitofish did not appear to feed on the cavefish. A concentration of YOY spring cavefish continued to be observed in this reach of Class Spring through April, and were found there during our quarterly population sampling in May.

We found 1 YOY spring cavefish at Otter Pond Spring after 30 min of searching on 10 February 2001. The individual was underneath a boulder located in Lowland Plot 2. The spring was searched on 14 February, but no YOY fish were found. On 26 February, one YOY and one adult cavefish was observed in Side Spring. Otter Pond Spring was searched again on 2 March and 29 YOY were found distributed from the SpringHead Station to the Swamp Station, including the Side Spring. Both primary springs comprising Otter Pond Spring were important in dispersing larval cavefish from subterranean habitats.

On 14 February 2001, we observed four adults and two YOY near the SpringHead Station at Deer Spring. The spring had significant flow and we positioned drift nets at the SpringHead Station and farther downstream, just before the confluence with North Branch (AboveCulvert Station). From 1615-2030 hrs, two larval cavefish (8.4 mm TL) and 32 larval salamanders (*Eurycea*) were captured at the SpringHead Station. From 1710-2030 hrs, 40 larval cavefish (8.0-9.6 mm TL) and 71 larval salamanders were collected in the drift at the AboveCulvert Station. No larval or adult cavefish were ever observed in North Branch.

Elm Spring was intensively monitored for the presence of young cavefish starting on 27 December 2000. We first observed larvae (7.9-8.5 mm TL) on 25 February 2001. Many fish were found distributed from SpringHead Station to where the stream enters the swamp; numerous salamander larvae were also present. Young cavefish were stationary on the stream bottom and oriented upstream in flow of 2-3 cm/s.

Larval cavefish were remarkably similar in size (6.5-10 mm TL) when they initially appeared in each spring at LaRue-Pine Hills in both 2000 and 2001, suggesting a change in life stage, behavior, physiological requirement, etc. occurred that increased the likelihood of larvae being washed to the surface. Eigenmann (1909) reported larval *Amblyopsis spelaea* hatch at 5 mm and leave the gill cavity around 10 mm once most of the yolk has been absorbed. Larval spring cavefish captured on the surface did not have yolk (or had very little) and could conceivably fit a gill brooding scenario as described by Eigenmann (1909) for *A. spelaea.* However, Hill (1971) reported larval spring cavefish from a Kentucky cave (apparently free-swimming and not in the gill cavity) that were 4-5 mm TL; he did not comment on the status of the yolk. Circumstantial evidence based on larval lengths indicates spring cavefish probably hatch at around 4-5 mm TL and become vulnerable to drifting to the surface at around 6.5-10 mm TL once the yolk has been consumed. If spring cavefish gill brood, retention time of larvae in the gills is probably less than for *A. spelaea.*

Growth and development - Young-of-the-year spring cavefish grew at an exponential rate following appearance in the surface springs. On average, fish obtained total lengths of 20-23 mm by mid to late April in all springs, with some individuals reaching 30 mm by early June (Figure 22). Relatively rapid growth by young was also found by Poulson (1963) who reported YOY were 15-32 mm standard length (SL) in approximately June-July and 25-36 mm SL by late fall.

Substantial variation in size distribution was found in later samples, particularly at Class Spring, Deer Spring, and Otter Pond Spring. This was partly due to the periodic appearance of smaller spring cavefish near 10 mm TL in these springs, indicating multiple YOY cohorts existed. For example, on approximately day 80 at Class Spring, a second group of fish less than 10 mm was distinctly present (Figure 22). Also, a smaller cohort distinct from the other fish was present in Deer Spring on day 120 (Figure 22). In general, each spring was defined by the presence of one strong cohort of young cavefish with the "leaking" of young hatched at a later date through April. In contrast, the size distribution at Elm Spring indicated only one cohort was present in this spring (Figure 22).

Young collected at first appearance possessed little or no yolk and had already begun exogenous feeding. Exogenous feeding was determined by the presence of unidentified food items within the digestive tract as seen through the gut wall. Presence of finfolds and well-developed pectoral fins (ray elements developed) indicated the cavefish were metalarvae (Snyder and Muth 1990) at first appearance in the springs. The smallest specimen preserved from the surface was 6.34 mm (SL) and 8.03 mm (TL) collected on 16 February 2001 from Elm Spring. The largest specimen collected that still possessed finfolds was 9.09 mm (SL) and 10.78 (TL) collected on 27 February 2000 from Elm Spring (11 days after first appearance).

During development of the spring cavefish, the vent undergoes a migration from a typical position anterior to the anal fin, as in most other fishes, to the jugular position seen in adults. The closely related pirate perch has a jugular vent similar to the cavefishes (Etnier and Starnes 1993). The vent position in pirate perch has been used to determine their age (Jordan 1878, Mansueti 1963). This seems applicable to the cavefish as well. The percentage of the standard length that the snout-vent length accounted for decreased from 59.77% in larvae to 20.29% in adults (Table 17).

Loss of the finfolds and position of the vent (not in the jugular position) were used to designate young spring cavefish as juveniles. Juveniles (24.10-24.97 mm SL) had a vent that was posterior to the pectoral fin. Poulson (1963) reported that vent migration was completed at 25 mm (SL) with a range of 15 – 32 mm. We preserved a specimen on 8 May 2001 that was 15.95 mm (SL) and 18.90 (TL) that had no finfolds. This specimen appeared to have a vent in an intermediate position between larvae (collected in February 2000 and 2001) and juveniles (collected in June 2001). Its length and eye diameter as a percentage of standard length fit midway between the larval and juvenile stages (Tables 17 and 18). However, the specimen's snout-vent length as percentage of standard length fell within the range exhibited by larvae (Table 17). Because of these differences, the specimen has been classified as an early juvenile in Tables 17 and 18.

Adults exhibited smaller eye diameter and snout-vent lengths as percentage of standard length than the earlier life history stages (Table 17). All adults measured had jugular vents. The smallest adult, 25.70 (SL) and 31.70 (TL), was collected 6 December 2000 and is of great interest. This specimen is not much longer than the juveniles that were collected in June 2001 (Table 18). However, the vent was jugular in position (had completely migrated) and eye diameter as a percentage of standard length was the same as the other adults. This specimen was probably spawned the previous winter and further indicates that position of the vent can be used as an indicator of age in small individuals.

Downstream dispersal - We captured larval cavefish and larval salamanders (*Eurycea* spp.) in drift samples from Class Spring and Deer Spring in February 2001 (Table 19). Both *Eurycea longicauda* and *E. lucifuga* were present in samples, but individuals could not be consistently and reliably distinguished in the field. At Deer Spring, 130 cavefish and 762 salamanders were captured at four stations ranging from the resurgence (SpringHead Station) to 55 m downstream (Pool Station). Fish ranged in size from 7.4 to 10.5 mm TL. The majority of fish were collected at two stations (AboveCulvert and BelowCulvert) located in run/riffle habitats where maximum water velocity was 11-21 cm/s. The most downstream station (Pool Station) was located in a pool/run that had a maximum water velocity of 3 cm/s. Although the pool/run contained a very high concentration of YOY cavefish, relatively few were captured in the drift directly downstream (Table 19). During diel sampling at Deer Spring, drift density of YOY cavefish ($P = 0.049$; $N = 3$) and salamanders ($P = 0.049$; $N = 3$) was significantly higher at night (Table 20). Both taxa followed a similar pattern of increased drift density around sunset with a gradual decrease over the next 2- 3 hrs (Figure 23). At Class Spring, fish were collected in the drift directly at the SpringHead Station and in the Transition Station. No fish were captured in a drift net (Watercress Station) positioned in a run (maximum water velocity = 13 cm/s) directly downstream of a high concentration (35 over two meters of shoreline) of YOY cavefish.

Previous researchers who have found young cavefish on the surface in late winter/early spring have concluded the fish hatched underground and migrated to the epigean environment (Weise 1957, Poulson 1963, Hill 1969a). Our collection of larval spring cavefish in drift nets positioned at the heads (resurgence) of springs at LaRue-Pine Hills also suggests underground spawning is occurring. The appearance of larvae on the surface was correlated with increased local rainfall and increased discharge in the springs. Therefore, the mechanism for dispersal of larval cavefish from underground to the surface was through entrainment in currents and subsequent downstream drift in flowing water. Given the observation by Hill (1969a) that only 1 of 1,920 larvae collected underground had food in the stomach and high rates of cannibalism by adults, it appears adaptive for cavefish larvae to disperse to epigean environments.

Once on the surface, we documented further downstream dispersal by drifting, the extent of which depended on local conditions (spring morphology and velocity) in a particular spring and time-of-day. Larval drift was high at Deer Spring from the spring head to the gravel road, and settling occurred in a run/pool which had low water velocities (0-3 cm/s). At Class Spring, larvae drifted from the spring head and settled in the Lowland Station. It is intriguing that cavefish larvae on the surface drifted more at night, a phenomenon observed in larvae of many other fishes (Brown and Armstrong 1985). Explanations for larval fish drift range from foraging behavior to nocturnal disorientation (reviewed in Armstrong and Brown 1983). It is unclear at this time the proximate reasons for larval cavefish drift; however, it does appear to be an important mechanism of dispersal for this species, both between subterranean and epigean environments and within epigean spring/stream systems.

<u>Habitat</u> - A total of 183 young spring cavefish were observed during 3-min visual searches at four springs. In general, young were present from the spring head to our most downstream sampling station. At Elm, Otter Pond, and Class Springs, Station had a significant effect on YOY abundance (two-way ANOVA; $F_{2,4} = 11.55$; $P = 0.022$). Abundance was highest in the Lowland Station (Tukey HSD test), accounting for 83% of fish observed. Lowland Stations were usually 1-2 ^{0}C cooler than the spring head and characterized by slow flow and soft substrates. Mean number of larvae was not different between Elm, Otter Pond, and Class Springs (two-way ANOVA; $F_{2,4} = 3.87$; $P = 0.1157$).

Typically, YOY spring cavefish were concentrated in one location at a given spring, in part due to the dynamics of downstream drifting. At Class Spring, fish were most abundant in

Lowland Plots 2 and 3 (Table 21 and Figure 2). Lowland Plot 2 was covered by a bed of watercress that was providing cover. Lowland Plot 3 marked the abrupt transition from gravel to soft substrates, where fish were conspicuous on the stream edge in slow currents. Seventy-two percent of YOY observed at Otter Pond Spring was located in Swamp Plot 4 and Lowland Plot 1 (Table 22 and Figure 4); fish were stationary on the soft bottom where flow was 1-5 cm/s. At Elm Spring, YOY were only observed in Lowland Plots 3 and 4 (Table 23 and Figure 3) and were located on the stream edge in leaf litter. The physical habitat was different at Deer Spring from all other springs in that the higher stream gradient resulted in a run-riffle-pool complex. Young cavefish were most abundant in pools or slow flowing runs (Table 24). A vast majority of the young were located in Plot 7, a slow flowing run containing large amounts of leaf litter (Table 24 and Figure 5).

Analysis of microhabitat data collected from the Lowland Station of Class Spring corroborated general observations that water velocity was an important determinant of habitat use by young spring cavefish. Sixty-one YOY spring cavefish were captured during point-abundance sampling of 16 locations (Table 25). Only one variable, flow, emerged from the stepwise procedure to be a significant predictor of fish abundance ($P = 0.038$; $r^2 = 0.27$). Fish were negatively associated with higher flows (standardized regression coefficient = - 0.52). Areas of spring/stream systems with flows less than 5 cm/s appear to be suitable habitat for larval/early juvenile spring cavefish.

Longitudinal abundance patterns, coupled with the drift data, indicate the entire surface portion of the spring/stream systems are utilized by young spring cavefish. The lowland reaches, in particular, are important nursery areas for young cavefish. High density of organisms in a habitat is not always the best indicator of habitat quality (Van Horne 1983). Hill (1969a) found that young cavefish on the surface primarily consumed oligochaetes, chironomids, and copepods. These taxa are presumably abundant in the low velocity/soft substrate areas in pools and lowland reaches of springs at Pine Hills. The fact that we documented significant growth of YOY spring cavefish through the first 4-5 months from pools and lowland reaches of three springs indicates these areas were providing quality nursery habitat. A detailed study of the invertebrate fauna of the spring/stream systems is needed to better evaluate food resources available to spring cavefish.

Quarterly population sampling in the spring of 2001 was 4-5 months following initial occupation of the habitats by YOY spring cavefish, and highest density of YOY cavefish continued to be in the lowland reaches at Otter Pond Spring and Class Spring. However, YOY cavefish abundance drastically decreased in the Lowland Station of Elm Spring. During the time period YOY spring cavefish were in the Lowland Station of Elm Spring, water levels were relatively high in the swamp and the Lowland Station was completely contiguous with the swamp. Many other species of lowland fishes inhabit the Pine Hills swamp, some being piscivorous (Gunning and Lewis 1955, Weise 1957).

On the evening of 24 April 2001 we observed adult spring cavefish (3-4), pirate perch *Aphredoderus sayanus* (2), central mudminnow *Umbra limi* (1) and YOY grass pickerel *Esox americanus* (15-20; 30-50 mm) in association with YOY spring cavefish in the lowland reach of Elm Spring. Mudminnows will eat spring cavefish (Gunning and Lewis 1955), and grass pickerel generally begin to consume fish by 15 mm (Becker 1983). Adult spring cavefish on the surface are known to be cannibalistic (Hill 1969a). Low numbers of YOY cavefish in the lowland reach of Elm Spring on 7 June 2001 could have been due to migration, but circumstantial evidence indicates predation upon YOY cavefish may have been substantial. Lowland reaches of spring/stream systems appear to provide suitable nursery habitat for young spring cavefish, but they must contend with predation threats from a variety of species. How this predation pressure compares with that in subterranean habitats and upland reaches of springs (which is also probably high because of the abundance of adult cavefish) is not known.

ototactic behavior - The phototactic response of spring cavefish was examined on 7 sampling s from 26 February to 27 April 2001 at Elm Spring and Otter Pond Spring (Table 26 and 27). ficantly more YOY spring cavefish (N = 7; P = 0.006) were captured in traps containing a light stick (overall mean = 1.6 fish/trap) than in traps with no light stick (overall mean = /trap). Selection for baited light traps was demonstrated during 6 of 7 replicate trials; on h an equal number was collected in each treatment (Table 26 and 27). Selection for traps baited with yellow light sticks was also found in the water scorpion *Nepa apiculata* (N = 7; P = 0.034), amphipods of the genus *Gammarus* (N = 7; P = 0.002), Coleoptera (N = 7; P = 0.024), Trichoptera (N = 7; P = 0.025) and young crayfish (N = 7; P = 0.032) (Table 26 and 27). Young spring cavefish were also captured in traps baited with green (1.8 fish/trap) and red (1.5 fish/trap) light sticks. No adult spring cavefish were captured in light traps.

The photoresponse of fish is known to change with development (Romero 1985, Masuda and Tsukamoto 1996). Adult spring cavefish are photonegative (Eigenmann 1909, Weise 1957), and typically only at night can they be seen in springs without overturning cover items. The adults will dart frantically when exposed to a white light at night. Conversely, we observed that larval and early juvenile spring cavefish do not respond when exposed to a white light. It was not uncommon to observe young cavefish on the edges of spring runs independent of cover in mid afternoon.

The light trap experiment indicates young spring cavefish, similar to many other young fishes, have a photopositive response and are attracted to light traps. A close relative of the spring cavefish, the pirate perch, is secretive and nocturnal as an adult, but larvae are highly vulnerable to capture by light traps (Killgore and Baker 1996). It could be argued that young cavefish were not attracted to the light, but were perhaps attracted to the concentration of prey in or around the traps. Adult spring cavefish do not use vision to detect food but rely heavily on thigmotaxis, they only consume food items that come into contact with some part of the body (Eigenmann 1909, Weise 1957, Hill 1969a). The feeding behavior of larval spring cavefish is not as well known, but food items retrieved from traps were typically too large for consumption by larval/early juvenile cavefish. Also, the concentration of prey did not seem to attract adult cavefish. Food availability cannot be completely ruled out, but, in our opinion, the selection of traps baited with light sticks demonstrates a positive response by young spring cavefish to yellow light.

The most cave-adapted cavefishes (e.g., *Amblyopsis*) have very reduced eyes but still can detect light and exhibit a photonegative response (Eigenmann 1909). Some have suggested this ability to detect light enables cavefishes to remain in subterranean habitats, but others have concluded the ability to detect light is a relict characteristic not under selective pressure in the cave environment (Green and Romero 1997). Unlike other cavefishes, the spring cavefish inhabits both cave and epigean environments. Our data and the diet study by Hill (1969a) indicate it may be adaptive for young cavefish to disperse to epigean habitats. Further research is needed to determine the significance of a photopositive response by young spring cavefish.

Conservation and Management

Throughout its entire range, status of the spring cavefish has recently been described as "currently stable" (Warren et al. 2000). The state of Missouri lists the spring cavefish as "endangered" since it is known from only two springs at one locality (Benton Hills Swamp); the Missouri population is unique in that it is the only documented locality west of the Mississippi River. In Illinois, spring cavefish are known from 5 counties, including approximately 16 different springs and caves in the Shawnee Hills (Smith and Welch 1978). Eight of these localities are within the LaRue-Pine Hills Research Natural Area and afforded protection by the U.S. Forest Service. The population in Cave Spring Cave is protected through the Illinois Department of Natural Resource Natural Heritage program.

From our research on four of the large populations of spring cavefish at LaRue-Pine Hills RNA and comparisons with Smith and Welch (1978), we conclude these populations, as a whole, are stable. It is encouraging that, in general, our fish abundance estimates were comparable to Smith and Welch (1978) and we found evidence of reproduction in all study springs. Based on the criteria for listing outlined by the Illinois Endangered Species Protection Board, we found no evidence to warrant legal protection to the species under the Illinois Endangered Species Act. However, due to the sensitive nature of their habitat (caves, springs, and spring runs) and relatively small population size at a given location, we recommend the species be added to the Watch List to insure future monitoring. A comprehensive survey of caves and springs in southern Illinois should be conducted to better document the distribution of spring cavefish in the state.

It is important spring cavefish populations at LaRue-Pine Hills RNA, the largest known populations in Illinois, continue to be protected and monitored. Of some concern is the substantial decrease in abundance observed at Class Spring since the survey by Smith and Welch (1978). A change in physical habitat in the epigean portion of the spring does not appear to be the culprit. Smith and Welch (1978) reported that two of the springs at Pine Hills had elevated levels of ammonia and phosphate from runoff associated with agriculture; we only measured basic water quality parameters during our study and cannot discern if pollution has played a role. Presence of relatively large numbers of YOY in late winter/early spring of 2001 is a positive indicator; however, in late spring/early summer, the entire spring was consumed by back-flooding of the Big Muddy River. At the time of this report, we do not know how the flooding impacted recruitment.

Preserving important habitat features of the spring/streams is necessary (e.g., the large boulders at Elm Spring), but maintaining the integrity of the spring resurgences and allowing movement between surface and subterranean environments, is essential. Observed changes in seasonal abundance and size distribution suggested spring cavefish were moving between the surface and subterranean environments. Presence of gravid females near the spring heads and larvae drifting from the resurgences indicate access to underground habitats is necessary for reproduction. Spring heads at Class, Elm, and Otter Pond Springs have been modified (since at least the 1950's) by the installation of tiles and stone basins. To what extent, if any, these structures have effected movement of spring cavefish is not known.

Managers and future investigators should consider the impact of roads and associated culverts on the movements of spring cavefish at LaRue-Pine Hills RNA. Our data, coupled with the findings of other researchers, suggest the following cycle: larval drift to epigean habitats and lowland reaches of spring/streams - growth and recruitment in the lowland reaches - migration back to the spring resurgence as subadults or adults - movement underground for reproduction. All four study springs have a road crossing within approximately 15 - 35 m of the spring resurgence and three of the springs have a culvert at the road crossing. At all four study springs, we documented the dispersal of larvae well beyond (downstream) the road crossing. Increased velocities (> 40 cm/s) associated with culverts are known to be barriers to upstream movement by small fishes (Warren and Pardew 1998). At base flow, water velocities associated with the culverts at Elm Spring and Class Spring were approximately 5 - 10 cm/s and 10 - 15 cm/s, respectively. Although the swimming performance of spring cavefish is not known, these values probably do not exceed the maximum swimming abilities of adult cavefish; therefore water velocity is probably not a concern in culverts at these springs.

Of greater concern, is blockage of culverts by debris or the creation of plunge pools directly downstream. A very high number of adult and YOY spring cavefish (> 181) were found downstream of the road at Deer Spring; they were probably washed downstream during spates. These fish appear trapped downstream and are unable to migrate to the spring head to reproduce because of the elevation difference between the culvert and the pool. In its current physical state, habitat downstream of the road at Deer Spring is an "ecological sink" for spring cavefish. This is based on current knowledge that spring cavefish only reproduce underground. Perhaps position of the culverts could be modified to allow upstream movement of fish. Another consideration is to redirect surface storm runoff that currently flows into Deer Spring during spates (Figure 5). The decrease in discharge during spates would reduce scouring in Deer Spring.

Otter Pond Spring historically flowed in a culvert under the road leading to Otter Pond. Welch (1973) reported that cavefish were trapped in a plunge pool directly below the culvert. Since Welch (1973), the culvert has been removed and the spring flows directly over the road. Motorized vehicles drive down the road on a regular basis. At night, we observed spring cavefish in the ruts produced by vehicles at the stream crossing. Additional modification of the spring at the road crossing could result in the formation of deep pools directly in the road. Our data on larval drift indicate pools are likely locations for YOY spring cavefish and salamanders to settle in high numbers. Vehicle traffic on the road during presence of YOY could be very detrimental. Ballard (1994) recommended the road to Otter Pond be closed to motorized traffic. We encourage the U.S. Forest Service to further contemplate this issue.

Spring cavefish is the only cavefish species in Illinois and is a unique natural resource of the state. Fortunately, a number of the springs/spring streams in which they are known to occur are relatively protected within the LaRue-Pine Hills Research Natural Area. Our study builds upon previous research conducted on the status of these populations by Smith and Welch (1978). The spring cavefish at Pine Hills should be monitored on a regular basis, particularly given the aforementioned concerns. Our intention was to employ sampling procedures that could be repeated by future investigators to maximize compatibility of data sets and information learned concerning status of the populations.

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Table 1. Collection records for the spring cavefish in Illinois from the Southern Illinois University at Carbondale (SIUC) Fluid Vertebrate Collection and Illinois Natural History Survey (INHS) Fish Collection.

Catalogue #	Stream	Drainage	County	Year
SIUC9783	Clear Spring	Big Muddy/Mississippi River	Jackson	1984
SIUC10643	Clear Creek	Mississippi River	Union	1953
SIUC10642	Pine Hills Spring	Mississippi River	Union	1954
SIUC10641	Pine Hills Swamp	Mississippi River	Union	1954
SIUC10639	Pine Hills Spring	Mississippi River	Union	1968
SIUC10638	Pine Hills Spring	Mississippi River	Union	1981
SIUC8952	Pine Hills Spring	Mississippi River	Union	1983
SIUC24816	Cypress Creek	Cache/Ohio River	Union	1995
SIUC30770	Bear Track Hollow	Ohio River	Pope	1998
SIUC31026	Bear Track Hollow	Ohio River	Pope	1998
SIUC34457	Big Creek	Ohio River	Hardin	1999
SIUC35612	Big Creek	Cache/Ohio River	Union	1999
SIUC37913	Elm Spring	Mississippi River	Union	2000
INHS17543	Pine Hills Swamp	Mississippi River	Union	1958
INHS17556	Pine Hills Swamp	Mississippi River	Union	1967
INHS17563	Pine Hills Swamp	Mississippi River	Union	1963
INHS17584	Pine Hills Swamp	Mississippi River	Union	1971
INHS18098	Cave Spring Cave	Mississippi River	Union	1970
INHS18100	Cave Spring Cave	Mississippi River	Union	1968
INHS18101	Clear Creek	Mississippi River	Union	1972
INHS26675	Hogthief Creek	Big Creek/Ohio River	Hardin	1975
INHS26844	Max Creek	Bay Creek/Ohio River	Johnson	1976
INHS26857	Big Creek	Ohio River	Hardin	1976
INHS26858	Max Creek	Bay Creek/Ohio River	Johnson	1976
INHS26883	spring	Mississippi River	Union	1948
INHS26901	Cave Spring Cave	Mississippi River	Union	1976
INHS26953	spring	Mississippi River	Union	1881
INHS36200	Cave Spring Cave	Mississippi River	Union	1995
INHS37654	Cave Spring Cave	Mississippi River	Union	1996
INHS43197	Pine Hills Swamp	Mississippi River	Union	1997
INHS68216	Layoff Cave	Ohio River	Hardin	1985
INHS86467	spring	Mississippi River	Union	1878
INHS86468	spring	Mississippi River	Union	1882
INHS86469	spring	Mississippi River	Union	1882
INHS86470	spring	Mississippi River	Union	1896
INHS86471	spring	Mississippi River	Union	1900
INHS87625	Hogthief Creek	Big Creek/Ohio River	Hardin	1982
INHS88481	Decker Spring	Big Creek/Ohio River	Hardin	1984
INHS88505	Pine Hills Swamp	Mississippi River	Union	1953
INHS88506	Pine Hills Swamp	Mississippi River	Union	1948

Table 2. Latitude and longitude for study springs located in the LaRue-Pine Hills RNA, Union County, Illinois. Coordinates are from a Garmin® 12XL GPS unit. Numerical classification of each spring in Welch (1973) is provided.

Spring	Latitude	Longitude	Welch (1973)
Class Spring*	N 37^0 35.305	W 89^0 26.415	4 and 5
Elm Spring	N 37^0 32.746	W 89^0 26.350	2
Otter Pond Spring	N 37^0 32.536	W 89^0 26.302	1
Deer Spring	N 37^0 33.778	W 89^0 26.446	7 and 12**

* We referred to Class Spring as "McCann Spring" in a previous report (Adams et al. 2000) to IDNR.

** Data presented in Welch (1973) does not accurately match data in this report on Deer Spring; however, the two springs listed are the most similar in location.

Table 3. Substrate and instream cover types used to classify habitat in plots during seasonal sampling at three springs at LaRue-Pine Hills RNA, Union County, Illinois.

<u>Substrate</u>	
Mud	Soft mud with a thick layer of detritus
Silt/Mud	Some mud present but mixed with silt (< 0.074 mm), firmer substrate and little to no detritus layer
Silt/Gravel	A gravel bottom (0.2 – 4.0 cm) with a moderate layer of silt
Gravel	Gravel that is too small to be considered cover (0.2 – 4.0 cm).
<u>Instream Cover</u>	
No cover	
Leaf Litter	Leaves that have fallen in the stream and are not embedded in the substrate
Woody Debris	Logs or sticks in the stream large enough to provide cover
Macrophyte	Instream aquatic plants that may provide cover
Gravel	Gravel too large to be considered substrate and which has been seen to provide cover for cavefish (4.0 – 7.6 mm)
Pebble/Cobble	Rocks between 7.6 and 60.4 cm
Boulder	Rocks greater than 60.4 cm

Table 4. Three-way analysis of variance table depicting the effect of location, season, and station on total spring cavefish abundance (mean cavefish/m^2) for three springs at LaRue-Pine Hills RNA, Union County, Illinois. Data were collected between winter 2000 and spring 2001 (a total of six seasons).

Source	Npram	DF	Sums of Square	F-ratio	P-value
Model		35	34.778	9.484	< 0.0001
Error		124	12.991	1.910^{-21}	
C. Total		159	47.769		
Location	2	2	3.592	17.143	< 0.0001
Station	2	2	11.097	52.960	< 0.0001
Location*Station	4	4	10.199	24.337	< 0.0001
Season	3	3	2.071	6.589	0.0003
Location*Season	6	6	0.451	0.717	0.6359
Station*Season	6	6	0.981	1.562	0.1637
Location*Station*Season	12	12	2.539	2.020	0.0276

Table 5. Observations of spring cavefish in the ceramic tile structure at the spring head of Class Spring, LaRue-Pine Hills RNA, Union County, Illinois. Data collected between January 2000 and May 2001.

Date	Time (hr)	Number of Spring Cavefish
15 Jan 00	0945	5
18 Feb 00	2130	9
19 Feb 00	2230	11
25 Feb 00	2030	7
12 Jan 01	1500	5
30 Jan 01	2000	11
6 Feb 01	1920	15
8 Feb 01	2300	14
10 Feb 01	2120	4
11 Feb 01	1730	4
	2200	7
14 Feb 01	2000	3
	2100	5
17 Feb 01	1400	0
	2230	1
20 Feb 01	2300	2
25 Feb 01	1630	0
27 Feb 01	1330	0
21 March 01	1850	3
24 April 01	2030	5
15 May 01	1500	0

Table 6. Total number of spring cavefish in each plot during the six sampling seasons for Class Spring, LaRue-Pine Hills RNA, Union County, Illinois.

		2000				2001	
		15 Jan	**28 May**	**2 Aug**	**12 Nov**	**4 Feb**	**8 May**
Station	**Plot**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**
Upland	**1**	3	2	0	0	13	4
	2	1	3	0	1	4	7
	3	7	1	0	0	4	0
	4	1	0	0	0	2	0
Transition	**1**	0	0	0	0	*	0
	2	1	0	0	0	*	0
	3	2	2	0	0	*	0
Lowland	**1**	0	3	0	0	*	*
	2	1	0	0	0	*	*
	3	0	0	2	0	*	37
Total		**16**	**11**	**2**	**1**	**23**	**48**

* denotes plots that were not sampled

Table 7. Total number of spring cavefish in each plot during the six sampling seasons for Elm Spring, LaRue-Pine Hills RNA, Union County, Illinois.

		2000				2001	
		12 Jan	**16 May**	**1 Aug**	**11 Nov**	**7 Feb**	**7 June**
Station	**Plot**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**
Upland	**1**	15	195	7	3	14	99
	2	13	52	30	23	44	34
	3	9	25	45	13	14	34
Transition	**1**	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
Lowland	**1**	13	0	0	0	4	1
	2	6	0	0	0	0	0
	3	1	3	0	0	1	0
Total		**57**	**276**	**82**	**39**	**73**	**168**

Table 8. Total number of spring cavefish in each plot during the six sampling seasons for Otter Pond Spring, LaRue-Pine Hills RNA, Union County, Illinois.

		2000				2001	
		16 Jan	14 May	31 July	19 Nov	3 Feb	22 May
Station	**Plot**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**
Upland	1	1	7	3	0	2	4
	2	1	10	0	1	4	1
	3	14	4	7	6	14	25
Transition	1	3	78	22	5	0	0
	2	1	4	18	0	0	5
	3	2	19	1	0	1	4
Lowland	1	1	0	0	0	1	4
	2	0	1	0	0	2	2
	3	2	0	0	0	2	1
Swamp	1	*	4	0	0	0	32
	2	*	1	0	0	0	5
	3	*	26	0	0	0	0
	4	*	5	0	0	0	16
Total		**25**	**160**	**55**	**12**	**26**	**94**

* denotes plots that were not sampled.

Upland Station, we obtained a range of abundance between 48 and 278 cavefish, with higher fish abundance in the spring sample. This is not incongruent with Smith and Welch (1978), assuming our sampling plots in the Upland Station represent a majority of the fish present. If anything, their estimates may be conservative for this site. Population abundance was also estimated by Weise (1957) who estimated winter and spring densities to be 25 and 104 fish, respectively. Assuming no differences exist in sampling efficiency, our data suggest an increase in spring cavefish abundance since Weise conducted his surveys in 1954. Photos in Weise (1953) suggest major changes in cover type in the Upland Station of Elm Spring. Large boulders, now in place, were not present in his picture. This is potentially critical because the boulders appear to provide quality habitat, as evidenced by the high concentrations of cavefish in plots with large boulders. Welch (1973) also makes note of the boulders and their importance in cavefish distribution within Elm Spring.

At Class Spring, our numbers (between 7 and 23) are drastically lower from Smith and Welch (1978) who designated Class Spring (referred to as Spring 3) as the largest and healthiest population of cavefish at Pine Hills with an estimate of 302 ± 201. A total of 46 fish were tagged at this site, which is at least double the number of fish we observed in the Upland Station in either the winter or spring during our study. This decrease is noteworthy since our sampling approach was probably more intense than previous studies. They also stated that typically 30-40 cavefish could be found swimming in the tile at the spring head on any given night. We made counts of cavefish when possible during this study in the tile and these numbers are reported in Table 5. Our count data at the spring head (0-15) were much lower than numbers reported by Welch (1973). We are unaware of any data that describe temporal habitat changes that might have occurred in Class Spring to elicit this decrease in cavefish abundance. Welch's study in 1973 was the last to examine aquatic populations of organisms at Class Spring and it is possible major habitat changes have occurred over the 28-year period. However, photographs of Class Spring in the early-1950's appear similar to the present day habitat, thus major changes in physical habitat are not a likely explanation for the decrease. Other possible explanations include changes in water chemistry, overcollecting, or natural population fluctuation. At the time of this