

Final Report

**Early Natural History and Larval Habitat Utilization of the Spring Cavefish,
Forbesichthyes agassizi, in Southern Illinois**

Submitted to:

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28 June 2000

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Introduction:

Family Amblyopsidae, cavefishes and swampfishes, is comprised of four genera and six species found in the eastern United States (Woods & Inger 1957, Lee et al. 1980, Berra 1981, Page & Burr 1991). Since their discovery by W. T. Craigie 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 & Inger 1957, Poulson 1963). Cavefishes are restricted to the southeastern/central United States, from Missouri and Oklahoma to the Carolinas and Virginia, and from southern Indiana to northern Georgia and Alabama (Woods & Inger 1957, Page & Burr 1991). Although the family is widespread within this area, the species have restricted ranges. Specific habitat requirements and limited range of cavefish have 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 & Boston 1995).

Spring Cavefish (*Forbesichthys agassizi*) is a nocturnal species ranging in distribution from southeastern Missouri to south-central Tennessee (Pflieger 1997, Page & Burr 1991). Spring Cavefish are characterized by diminutive eyes (functional only for light detection), enhanced sensory (i.e., lateral-line) system, absent pelvic fins, and small body size (maximum total length 90 mm) (Woods & Inger 1957). 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 & Welch 1978). As judged from measurements of morphology, physiology, and life history, Poulson (1963) supported the idea of cave colonization by this species.

Spring Cavefish are locally abundant but appear to be restricted to clear springs with associated groundwater streams (Burr et al. 1996). The largest known, and well studied, population of Spring Cavefish, located at Rich's Pond, Kentucky, has been extirpated. In Illinois, Spring Cavefish are known from 11 localities representing 5 counties (Alexander, Hardin, Jackson, Johnson, and Union) (Figure 1). Spring cavefish do not currently have a conservation status in Illinois, probably because of a lack of biological information (Scott Ballard, pers. comm.). The species was listed as of special concern in 1969 and as endangered in the state of Illinois in 1973 (Lopinot & Smith 1973). Missouri harbors only one known population of Spring Cavefish (which has been listed as state endangered), and collectively, these populations (Missouri and Illinois) represent the northwestern extent of the species range.

Previous Studies of Spring Cavefish in Illinois

Spring Cavefish are dependent on clear, cool springs and appear to be locally abundant in springs along bluffs at LaRue-Pine Hills Ecological Area in Union County, Illinois. Forbes (1882) described specimens from Pine Hills, 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..."

Spring Cavefish abundance and size distribution measurements have been somewhat sketchy in previous studies. Layne and Thompson (1952) conducted searches at two of the springs at Pine Hills in August, 1950, but provided limited biological information. Weise (1957) qualitatively assessed variation within a spring in relation to habitat; more Spring Cavefish were found in the "rocky" upstream area of the spring/stream than downstream. He attributed this to increased cover and decreased predation at the upstream end. However, water quality parameters such as temperature, pH, dissolved oxygen, and turbidity were not measured on a regular basis, limiting the conclusions Weise could make about factors affecting distribution of cavefish within a spring. Smith and Welch (1978) conducted a mark/recapture study at LaRue-Pine Hills to estimate population sizes and examine connectivity of springs. Estimates of population size for springs in Union County ranged from 18 to 302 individuals. Marked individuals were never recaptured at another spring location, suggesting each spring is isolated. Many of the studies conducted to date have been anecdotal and based on qualitative data only. Additionally, most studies have been limited to factors that affect only adults within the population and did not consider habitat requirements of young or larvae.

Since Weise (1957) and Smith and Welch (1978), no research has been conducted on Spring Cavefish in Illinois. Eigenmann (1909), Poulson (1963), and Hill (1968, 1969a, 1969b, 1971) provide the only other known biological information for this species. Comparing current data to data collected during Smith and Welch's (1978) study would provide pertinent information on changes in the populations over the last 22 years. There is also a need to understand the reproductive cycle and habitat use of young fish, a question that has remained unanswered in the past.

Early Natural History

Studies have been conducted on distribution, oxygen preference, and food habits of Spring Cavefish, but very little information is available on their reproductive biology (Hill 1968, 1969a). Weise (1957) found gravid females at LaRue-Pine Hills during almost every month of the year. 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. Some authors (Weise 1957, Hill 1969a) have suggested Spring Cavefish are dependent on underground spawning habitats to complete their life cycle. Reduced numbers of adults on the surface during late winter, the time corresponding to reproduction, has led to the conclusion that spawning occurs only underground. Adults that remained on the surface during winter tended to resorb eggs (Weise 1957).

Timing and mode of reproduction has long been a mystery for this species. Eigenmann (1909) and subsequent authors (Weise 1957, Poulson 1963, Smith & Welch 1978) hypothesized Spring Cavefish to be gill brooders, based on the discovery by Eigenmann and later Poulson, of eggs and young in the gill cavity of *A. spelaea*. Gill brooding, unique among teleost fishes, has never been confirmed for Spring Cavefish. Judging from the jugular-positioned anus found in all members of the family, several authors theorized that this unusual anatomical feature facilitated gill brooding behavior.

Objectives:

The goals of this project were to conduct field studies of Spring Cavefish in Union County, Illinois, in order to 1) document timing and occurrence of reproduction, 2) describe local abundance and population demography of both adults and larvae, 3) describe habitat utilization (water quality, substrate, temperature, water velocity) of the larvae and adults.

Methods:

Three populations were monitored during winter and spring 2000 at LaRue-Pine Hills Ecological Area (Figure 2). They include Elm (or Maple) Spring, McCann Spring, and Otter Pond Spring at LaRue-Pine Hills. Big Creek, Cache River drainage, was monitored during early spring for evidence of reproduction.

All three springs studied at Pine Hills exit the base of limestone bluffs through basin-shaped structures of either tile, concrete, or metal piping and flow via a surface spring run into the bordering swamp. Elm Spring is located just inside the Forest Road 345 gate at the southern entrance (Figure 3). The spring is approximately 100 yards in length between the spring exit and the entrance into the swamp. The upper "rocky" area is about 25 yards, with mud/silt comprising the other 75 yards (Weise 1957). A culvert beneath the gravel road separates the rocky, upland reach from the lower, bottomland reach. McCann Spring is located just north of Levee road and has a morphology similar to Elm Spring (Figure 4). Major differences, however, are the overall steeper gradient of the stream run, the mud/silt reach of the stream continues for a greater distance before entering the swamp, and the presence of a smaller tributary spring seep. Otter Pond Spring is located on the unimproved road near the Forest Road 345 gate at the southern entrance leading to Otter Pond. This small spring has less output at base flow and is directly impacted by traffic on the gravel road due to absence of a culvert (Figure 5). Following rain events, Otter Pond Spring experiences greater flows relative to the other two springs at LaRue-Pine Hills due to output from Twilight Cave. The lower reaches of both Elm Spring and Otter Pond Spring are directly influenced by water-level changes in the adjacent swamp. Additional descriptions of the LaRue-Pine Hills springs can be found in Gunning and Lewis (1955), Weise (1957), and Smith and Welch (1978). Big Creek was monitored just downstream of a large unnamed spring near Nash Road, south of Anna. Big Creek represents a new locality for this species and was the site where larvae were first observed in 1999. The spring at Big Creek has a much larger and turbid discharge than the springs at LaRue-Pine Hills.

Reproduction

Beginning in January 2000, study sites at LaRue-Pine Hills were monitored for evidence of reproduction by the presence of young-of-the-year (YOY) in the surface stream. Initial monitoring was accomplished by conducting timed searches along the spring run. After young were discovered, the springs were sampled through 31 March with drift nets, light traps, and timed searches. When possible, total lengths (TL) were obtained from 10 young each sampling date; these data were used to estimate growth rates. Care was taken to minimize stress on young cavefish. We feel our methods were not detrimental since we were able to capture young, obtain lengths, and release them with no apparent harm.

Drift nets (small-meshed dipnets) were used to determine if YOY were drifting in the current and to ascertain the source of YOY found in the spring run. Two nets were placed simultaneously in the stream (one at the spring head and one just downstream of the culvert) and checked at either 30-minute or 1-hour intervals between late afternoon (~ 3 p.m.) and dark (~ 8 p.m.). Drift nets were utilized twice at Elm Spring (18 February and 19 February) and once at McCann Spring (26 February) following locally heavy rain events to increase the likelihood of capturing young in the spring discharge.

The early life stages of many fish species are known to be attracted to light, and light traps have been successfully utilized to passively study habitat affinities of threatened species such as White Sands Pupfish (S. Adams, personal observation). Light traps used in this study were modifications of the Plexiglas design described by Killgore (1994). Three narrow slots in a two-liter plastic bottle allowed for entry of small fish but prevented them from exiting. A chemical light stick (Cyalume Yellow) was placed in the center of the trap to provide a light source. Although Weise (1957) found adult Spring Cavefish to be photonegative, nothing was known about the phototactic response of early life stages. Light traps were utilized as an exploratory procedure to determine if young cavefish could be captured with a light trap. Light traps were set at the LaRue-Pine Hills springs on five separate dates.

Fish Abundance and Size Distribution

To determine habitat use and fish abundance, each spring run was divided into three stations based on distance from the spring head and habitat type (e.g., Figure 3). Three plots, 1.5 meters long, were established within each station (Figure 6). A wax pencil was used to mark rocks along the edge of the spring to assure sampling was conducted in the same plot each season. Sampling was accomplished starting at the most downstream station. Prior to sampling fish within a given plot, it was blocked off at the upstream and downstream border with nets having a mesh size of 1.6 mm. Fish were removed from the plot using a D-frame dipnet and placed directly into a bucket filled with ambient spring water. As each plot was completed, the most upstream net of the plot remained in place until the downstream net was positioned in the next plot; this prevented error due to movement of fish into or out of the plot before sampling. Wet weight and total length were measured on every fish in order to construct length/frequency histograms and to calculate condition. Once all cavefish were measured and debris placed back into the spring, cavefish were returned to the spring. Abundance of other vertebrates (i.e., amphibians, reptiles, and other fish) was also documented within each plot. Sampling was conducted at LaRue-Pine Hills springs during 12-16 January and 14-28 May, representing the winter and spring seasons, respectively.

Habitat Use

Habitat data corresponded with fish abundance sampling during 12-16 January and 14-28 May. Water quality (temperature, conductivity, dissolved oxygen concentration, and pH) was measured once in each station per sampling trip. Substrate and cover type was quantified within each plot using a method modified from Vadas & Orth (1998). A 20-cm square was placed in a given plot three times along a diagonal to quantify habitat (Figure 6). Substrate and cover type (Table 1) were recorded at each corner of the square and in the center. This method of quantifying habitat allowed us to integrate 15 measurements of each variable per plot. Types of aquatic plants, percent canopy cover, stream width, stream depth, and water velocity were also

measured at each plot. Longitudinal position within the stream, water quality, and microhabitat were recorded during timed searches for YOY cavefish.

Results and Discussion:

Reproduction

Gravid females were not observed during this study. Weise (1957) reported gravid females in the field from almost every month of the year but stated there was evidence to suggest most fish ready for spawning disappeared in late winter. We found fewer adults (>45 mm) on the surface during our winter sampling trip compared to spring, and cavefish on the surface in winter had lower condition values compared to similar-sized individuals in the spring (Figure 7). Lower condition of cavefish in winter was not likely due to decreased food availability since amphipods, the primary food source, were qualitatively abundant during our winter sampling. Based on a laboratory study, Weise (1957) hypothesized breeding adults that could not move underground to spawn would resorb eggs. Our winter sampling occurred in mid-January, and it is possible adults collected during this time were either not part of the reproductive pool or were unable to move underground; consequently, these fish may have been forced to resorb eggs, accounting for the reduction in condition. The seasonal difference in condition of cavefish in winter and spring requires further data collection.

Larval Habitat Use

The presence of cavefish larvae indicated that reproduction occurred in late winter at all three springs at LaRue-Pine Hills. Larvae (<8 mm) were first observed in two springs (Elm Spring and McCann Spring) at LaRue-Pine Hills on 16 February 2000, but were not observed in Otter Pond Spring until 27 February. No young were found at Big Creek during sampling of the spring effluent on 6 February and 4 March. Young initially found at LaRue-Pine Hills were larvae and represent only the second known reports of this life stage for Spring Cavefish throughout its range and the first report of this life stage for the LaRue-Pine Hills populations. In fact, this is only the second species of the family Amblyopsidae in which larvae have been

seen. Larvae were found by visually searching the stream and were subsequently monitored by using drift nets and light traps.

A total of 96 cavefish larvae, 26 cavefish adults, and 732 larval salamanders (Eurycea lucifuga and E. longicauda) were captured during drift sampling at Elm Spring on 18 February, 19 February, and 27 February. On 26 February, 11 cavefish larvae, 4 cavefish adults, and 241 larval salamanders were captured during drift sampling at McCann Spring. At both springs, relatively equal numbers of cavefish larvae and adults were captured at the spring head and culvert sets. No distinctive diel pattern existed in the occurrence of larval cavefish in the drift; however, adult cavefish and larval salamanders were more abundant at dusk. Amphipods were also abundant in drift samples.

Based on drift net sampling, cavefish at the larval stage (fin folds were present) appeared to be drifting out of the spring head into the surface environment. Previous authors (Weise 1957, Hill 1971) suggested spawning occurs only underground and that young remain underground until they reach a larger size (post-larval). We concur with the hypothesis of an underground spawning since no gravid females or eggs could be found in the surface stream. However, our results suggest young may exit underground soon after hatching and that this species may not be a gill brooder as previously suggested (Weise 1957, Poulson 1963). Gill brooding in amblyopsids has been observed in only one species, and young were not released from the gills until they were beyond the larval stage. A major advantage of drifting from the subterranean environment to the surface may be the lack of suitable food items for YOY in hypogean habitats and avoidance of cannibalism by adults (Hill 1969b).

Light trap sampling was highest at Elm Spring because of the abundance of larvae in drift samples. Light traps were successful in capturing both larval and juvenile cavefish (Table 2), but tended to capture more fish at the larval stage. On two occasions when both traps with and without light sticks were set, 16 fish were collected in traps containing light sticks and 5 in traps without light sticks (Table 2). Further experiments are required to determine whether young fish are attracted to the light or if traps are simply acting as "activity traps" and incidentally catching fish when they become more active at night. Light traps did allow us to determine longitudinal

distribution of young cavefish at Elm Spring. Visual searching for larval fish was not feasible once the spring run entered the swamp, and light traps provided a method to document presence/absence of young in these areas. Of the 27 young captured in light traps from the lowland reach of Elm Spring, 10 were found in the stream beyond where the stream entered the swamp. It appears the lowland reach, including the swamp, are important nursery habitat for young cavefish at Elm Spring.

Higher numbers of young fish were observed at Elm Spring, and sufficient data to determine growth rates could only be obtained for Elm Spring. Growth rates collected during this study were higher compared to values reported by Hill (1971) for surface young at Rich's Pond, Kentucky (Figure 8). This variation may be explained by the difference in food availability between the two sites. Young fish at Rich's Pond fed primarily on Chironomidae, whereas Weise (1957) reported that the main food item for fish at LaRue-Pine Hills was an amphipod (Gammarus troglophilus). It is possible that the amphipod community at Pine Hills provides a more nutritional food base for the fish.

Fish Abundance and Size Distribution

Weise (1957) reported seasonal variation in abundance for Spring Cavefish at LaRue-Pine Hills. At Elm Spring he found 25 cavefish on 23 January and 104 on 14 April, but he concluded the size composition of age classes ruled out the increase in numbers being related to reproduction. During our study, we observed a similar trend at both Elm Spring and Otter Pond Spring. At Elm Spring we found 56 fish on 14 January and a total of 276 fish on 16 May (Figure 9). Of the 276 fish collected in May, 227 were YOY (25.5 - 36 mm TL) based on calculated growth rates (Figure 8). At Otter Pond Spring we found 25 fish on 16 January and 124 fish on 15 May (Figure 10). As judged from growth data in our study it is possible that Spring Cavefish collected during Weise's 14 April sampling trip were YOY. Few fish were captured at the McCann Spring site. In winter, a total of only 15 fish were found and only 11 were observed during spring sampling. As opposed to the other two springs, YOY comprised less than half of the total fish in the spring

sample. Poorer recruitment at McCann Spring may be due, in part, to its higher gradient and lack of an imminent relationship with the swamp relative to Elm and Otter Pond springs.

Habitat Use

Previous researchers of the cavefish at LaRue-Pine Hills focused their efforts on the headwater portion of the springs where fish appeared most abundant and were easily captured underneath cover objects (Gunning & Lewis 1955, Weise 1957, Smith & Welch 1978); however, Gunning & Lewis (1955) also found cavefish to be relatively abundant in the adjacent swamp. We designated three sampling stations at each spring (Above Road, Below Road, and Lowland) to encompass the longitudinal change in habitat from the spring head to the confluence with the swamp. All three springs have a distinct headwater reach (Above Road), characterized by a rocky substrate and relatively constant water quality, and a lowland reach (Lowland) that is characterized by soft substrates and seasonally variable water quality (Table 4); the middle reach (Below Road) generally had habitat characteristics intermediate between the headwater and lowland reaches (Tables 3,5,6).

In both seasons sampled, cavefish abundance tended to be higher in the headwater reach of all three springs (although exceptions did occur). Fish could be found underneath and within the interstices of pebble-sized rocks at all three sites. Cavefish were particularly abundant underneath large boulders present in the headwater reach of Elm Spring. During the spring sample at Elm Spring (273 fish), many cavefish were found exiting deep crayfish burrows (*Cambarus diogenes*) located underneath the large boulders and pebbles. It is difficult to ascertain specifically whether the habitat characteristics of the upstream reaches are being selected for or, if the abundance of cavefish is simply due to proximity to the spring seep. Previous researchers have noted a tendency for movement between surface and subterranean habitats dependent on time-of-day (Gunning & Lewis 1955, Weise 1957). Additionally, amphipods, reportedly the major cavefish food item, were abundant in the headwater reach of all three springs. Our sampling revealed that overall conditions in headwater reaches, especially the presence of large cover items, were hospitable to and provided daytime refuge for cavefish.

Although cavefish were generally most abundant in the headwater reaches, they were also numerous at times in the Lowland and Below Road stations. Twenty-one cavefish were captured in the Lowland station from Elm Spring during winter; fish were hiding underneath leaf litter, the dominant cover type, that was often embedded in the soft substrate (Table 3). Cavefish abundance was high (102 fish) at the Below Road station of Otter Pond Spring in the spring sample (Table 6). Refuge was being provided by a fallen log that had created a small pool within the stream run. The input of leaf litter and woody debris, particularly larger branches and logs, were important cover items for cavefish in the Below Road and Lowland stations and demonstrates a direct value of the forested area bordering the streams.

Drift sampling, light traps, and timed-searches indicated that YOY cavefish were abundant in the mid and lowland reaches. The dispersal of YOY cavefish from the spring head towards the swamp is probably due to drifting at the larval stage. Larval and early juvenile cavefish could be found associated with leaf litter, woody debris, and macrophytes present in the mid and lowland reaches. Hill (1969b) found that dipteran larvae were the primary food item of YOY cavefish. Although we did not specifically sample invertebrates, dipteran larvae have been found in the soft substrates of the lowland reach of Elm spring (Weise 1957). Predation pressure is probably exerted on YOY cavefish in the lowland reaches from swamp-inhabiting fish (e.g., Gambusia affinis, Umbra limi, and Fundulus dispar) (Gunning & Lewis 1955), but the abundance of adult cavefish in the headwater reaches may also be a threat to YOY survival (sensu Hill 1969b). The presence of YOY cavefish in the mid and lowland reaches of all three springs indicates the importance of the entire spring length, from the headwaters to the swamp, in the maintenance of surface population size.

Conservation and Management:

Relatively little is known about the basic biology of Spring Cavefish throughout its range, particularly reproductive biology and early natural history. We have established that reproduction occurred in all three springs studied at LaRue-Pine Hills, and substantial recruitment of YOY Spring Cavefish into the population (based on abundance of YOY in the spring sample)

occurred at Elm Spring and Otter Pond Spring. Abundance data indicate adults in spawning condition are underground during late winter and young drift to the surface soon after hatching.

Lowland, swamp portions of the springs appear to be important nursery areas for YOY Spring Cavefish. Previous researchers have indicated Spring Cavefish are rarely found below the spring run and have often dismissed the potential significance of the swamp habitat (Layne & Thompson 1952, Weise 1957). We monitored growth of larval cavefish into sub-adults in the lowland reach of Elm Spring, demonstrating that YOY cavefish successfully recruited within this habitat type. Further studies will help elucidate the relationship between the swamp habitat and early natural history of cavefish. Identification of critical habitat needs of this critical life stage will help managers evaluate proposals such as water-level manipulations within the swamp and may have range-wide implications for habitat protection.

At La-Rue Pine Hills, Spring Cavefish potentially face a threat from the location of the gravel road. At two of our sites, a culvert runs underneath the road and provides a conduit for cavefish to move from the spring head to downstream habitats. No culvert exists at Otter Pond Spring which is directly impacted by the road. From our observations, culverts appear to provide habitat for adult cavefish, with some moving into the culvert during the daytime and exiting at night to feed (much the same as if it were the spring head). Road construction or replacement of the culverts can potentially impact the population if precautionary measures are not taken. Knowledge of the seasonal abundance and reproductive timing of Spring Cavefish should be taken into consideration when conducting road construction.

Low population size and restricted range of Spring Cavefish in Illinois prompted Smith & Welch (1978) to suggest retention of the protected status afforded Spring Cavefish in 1969. Smith & Welch (1978) recommended increased monitoring and protection of Spring Cavefish in the Ecological Area due to limited habitat and ease of collection. This species is currently listed as endangered in Missouri because only one population is known to exist. It appears that the Elm Spring population of Spring Cavefish is currently stable when compared with population data provided by Weise (1957) and Smith & Welch (1978). We do not, however, have historic

data for the other two Pine Hills springs sampled and are unable to assess historic changes in population size.

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Table 1. Types of substrate and instream cover measured for springs at LaRue-Pine Hills Ecological Area.

Substrate Types	Instream Habitat
Gravel	Overhanging Vegetation
Sand	Aquatic Macrophytes
Silt	Leaf Litter
Mud	Undercut Banks
Detritus	Woody Debris
	Boulder (> 60.4 cm)
	Cobble (25.4-60.4 cm)
	Pebble (7.6-25.4 cm)
	Gravel (.02-7.6 cm)

Table 2. Dates and localities light traps were set for springs at LaRue-Pine Hills Ecological Area. Traps set with lights are those that had a Cylume light stick in the center, no Cylume stick was placed in traps without lights.

Spring	Date	Number of Traps Set with Lights	Number of Fish Caught in Traps with Lights	Number of Traps Set without Lights	Number of Fish Caught in Traps without Lights	Total Number of Fish Caught
Elm	18 February	3	2	0	0	3
Elm	19 February	5	3	0	0	5
McCann	19 February	1	5	0	0	5
Elm	26 February	16	0	0	0	0
Elm	27 February	2	0	0	0	0
McCann	27 February	4	0	0	0	0
Otter Pond	27 February	2	2	1	0	2
Elm	4 March	7	14	7	5	19
Otter Pond	4 March	2	1	0	0	1

Table 3. Substrate, cover, and Spring Cavefish Abundance at Elm Spring, LaRue-Pine Hills. Substrate and cover values were taken at 15 points within each plot. Substrate values are reported in Table 1. Cover was categorized and then converted to percentages. Large cover items indicate whether there was a presence or absence of large cover (boulders, large woody debris, etc.). Cavefish abundance is the total number of fish captured within the station for each season.

Station	Depth of Water (cm)	Width of Plot (m)	Substrate	Cover	Large Cover Items	Cavefish Abundance (Winter)	Cavefish Abundance (Spring)
Lowland	3.3	2.1	Mud/Detritus	48% Leaf Litter 32% No Cover 20% Woody Debris	Present	21	3
Below Culvert	1.9	1.6	Silt/Mud	69% No Cover 27% Leaf Litter 4% Woody Debris	Absent	0	0
Above Culvert	1.8	1.1	Gravel	28% No Cover 27% Boulder 16% Woody Debris 18% Leaf Litter 11% Pebble	Present	35	273

Table 4. Water quality parameters for springs at LaRue-Pine Hills. Mean values are reported for each station and data are listed as winter/spring for each parameter.

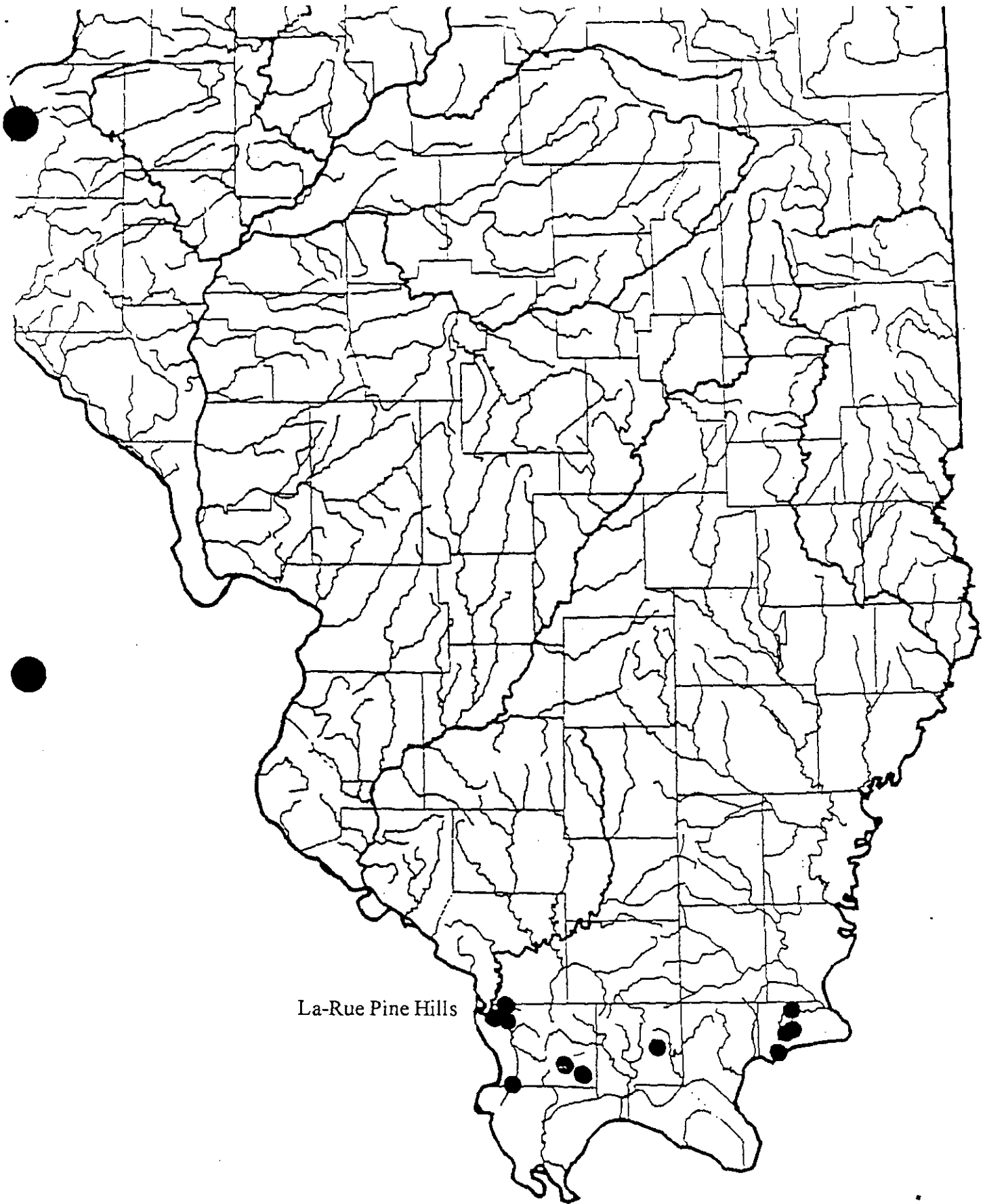
Spring	Station	pH	Temperature (° C)	Dissolved Oxygen (mg O ₂ /L)	Conductivity (µmhos)
Elm	Lowland	7.7/8.2	7.0/16.2	10.50/9.78	120/182
	Below Road	7.7/8.2	10.4/14.7	10.16/11.28	135/164
	Above Road	7.6/8.0	12.0/13.9	9.60/11.68	95/159
Otter Pond	Lowland	7.8/8.1	11.0/14.6	9.62/8.25	165/185
	Below Road	7.8/8.0	11.5/15.4	9.65/9.05	170/192
	Above Road	7.6/7.7	12.4/13.6	9.13/8.18	170/182
Class	Lowland	7.8/8.1	8.8/14.2	10.78/8.21	165/220
	Below Road	7.8/7.7	9.6/13.6	11.05/8.88	185/220
	Above Road	7.5/7.9	11.8/13.1	10.77/8.63	200/180

Table 5. Substrate, cover, and Spring Cavefish Abundance at McCann Spring, LaRue-Pine Hills. Substrate and cover values were taken at 15 points within each plot. Substrate values are reported in Table 1. Cover was categorized and then converted to percentages. Large cover items indicate whether there was a presence or absence of large cover (boulders, large woody debris, etc.). Cavefish abundance is the total number of fish captured within the station for each season.

Station	Depth of Water (cm)	Width of Plot (m)	Substrate	Cover	Large Cover Items	Cavefish Abundance (Winter)	Cavefish Abundance (Spring)
Lowland	3.1	1.5	Silt/Mud	36% No Cover 31% Macrophyte 24% Woody Debris 9% Leaf Litter	Absent	1	3
Below Culvert	2.0	1.7	Silt/Gravel	29% Leaf Litter 27% Macrophyte 24% No Cover 18% Woody Debris 2% Gravel	Present	3	0
Above Culvert	3.0	1.5	Silt/Gravel	42% Gravel 29% Macrophyte 11% Woody Debris 6% Leaf Litter 9% Pebble 3% No Cover	Present	11	6

Table 6. Substrate, cover, and Spring Cavefish Abundance at Otter Pond Spring, LaRue-Pine Hills. Substrate and cover values were taken at 15 points within each plot. Substrate values are reported in Table 1. Cover was categorized and then converted to percentages. Large cover items indicate whether there was a presence or absence of large cover (boulders, large woody debris, etc.). Cavefish abundance is the total number of fish captured within the station for each season.

Station	Depth of Water (cm)	Width of Plot (m)	Substrate	Cover	Large Cover Items	Cavefish Abundance (Winter)	Cavefish Abundance (Spring)
Lowland	2.6	1.4	Silt/Mud	56% Leaf Litter 33% No Cover 11% Woody Debris	Present	3	1
Below Culvert	3.9	0.8	Silt/Gravel	47% No Cover 33% Leaf Litter 11% Woody Debris 9% Gravel	Present	6	102
Above Culvert	0.8	0.8	Gravel	38% Leaf Litter 26% No Cover 18% Gravel 18% Woody Debris	Present	16	21



La-Rue Pine Hills

Figure 1. Known localities of Spring Cavefish based on data from Illinois Natural History Survey and Southern Illinois University, Carbondale.

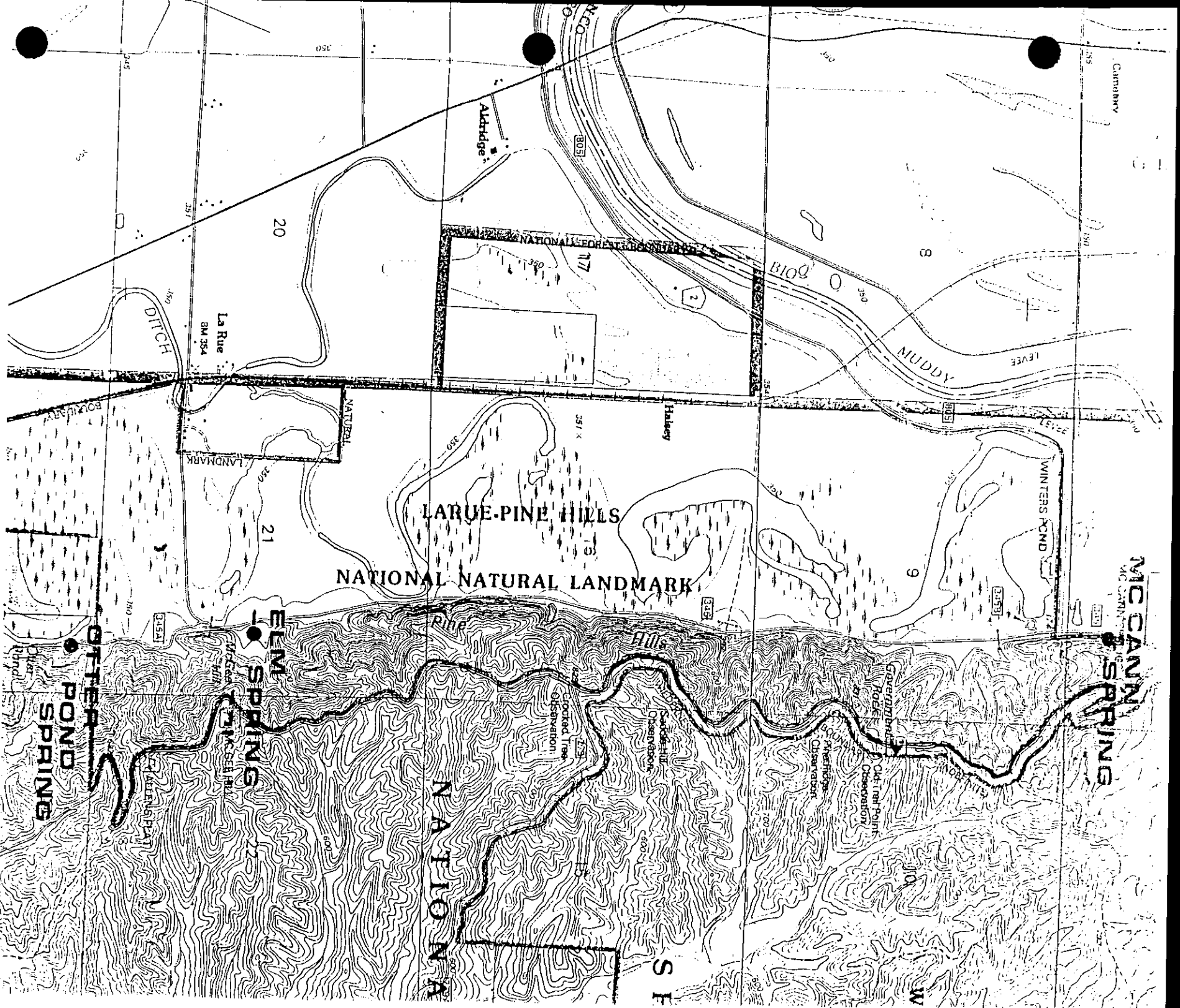


Figure 2. Topographic map depicting locations of the three springs in the Larue-Pine Hills Ecological Area.

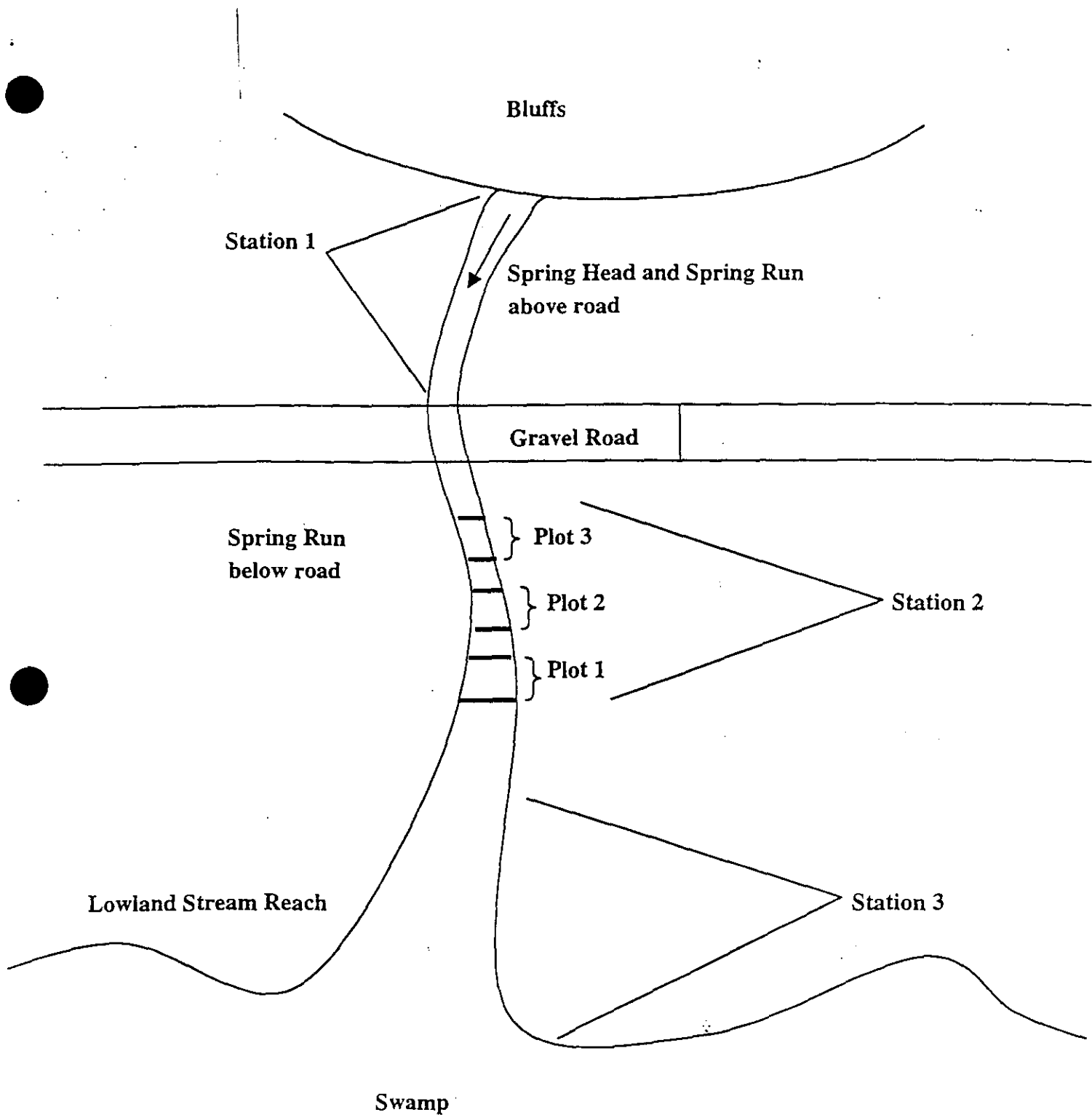


Figure 3. Diagram of Elm Spring at the south end of LaRue-Pine Hills Ecological Area. Three stations were established within the spring representing the spring run above the road, the spring run below the road and the lowland portion of the stream. Three plots were sampled within each station.

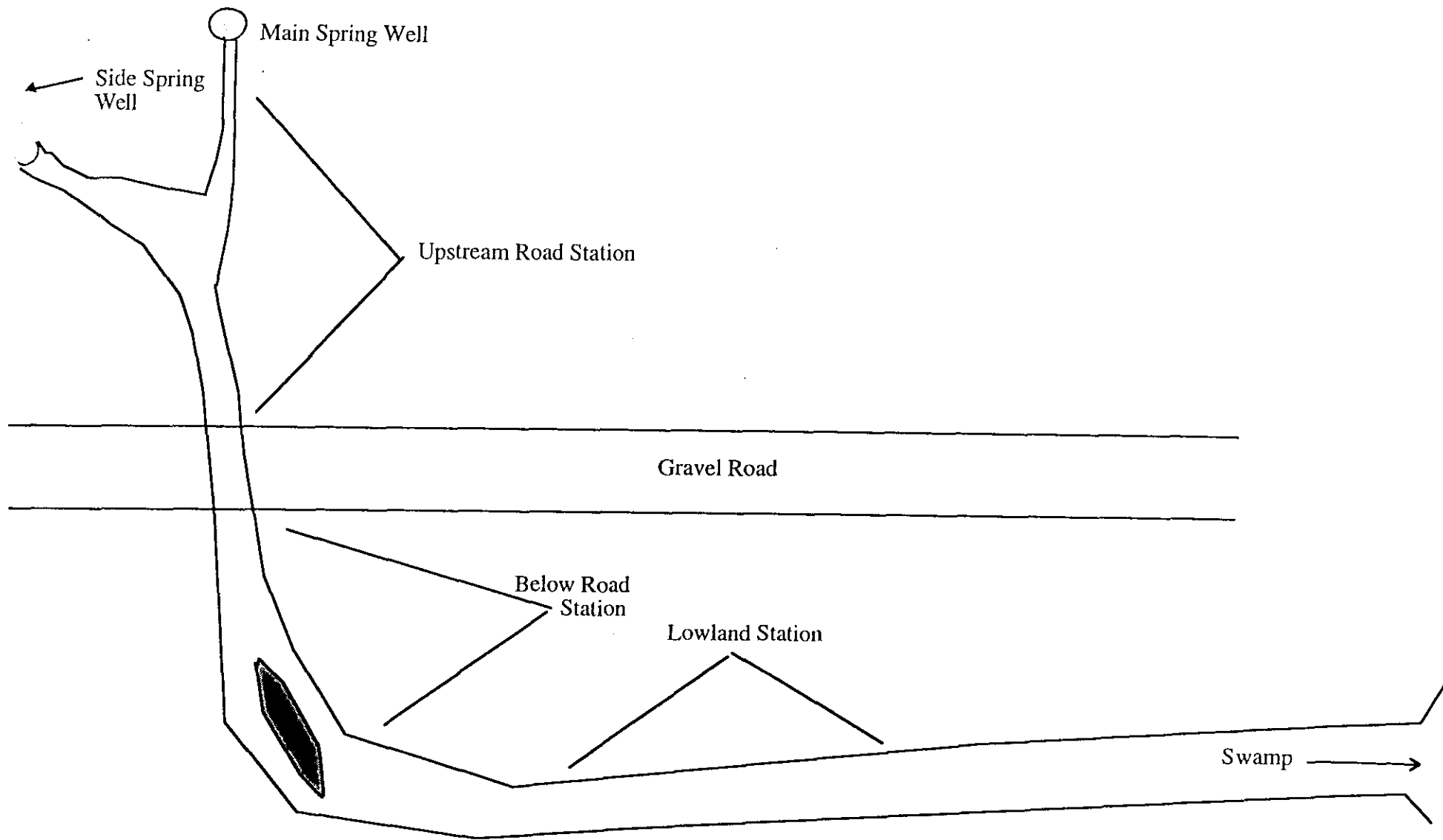


Figure 4. Diagram of McCann Spring, north of Levee Road, LaRue-Pine Hills Ecological Area. The three stations along the stream are labeled. Three plots were established within each station for sampling of fish abundance and habitat.

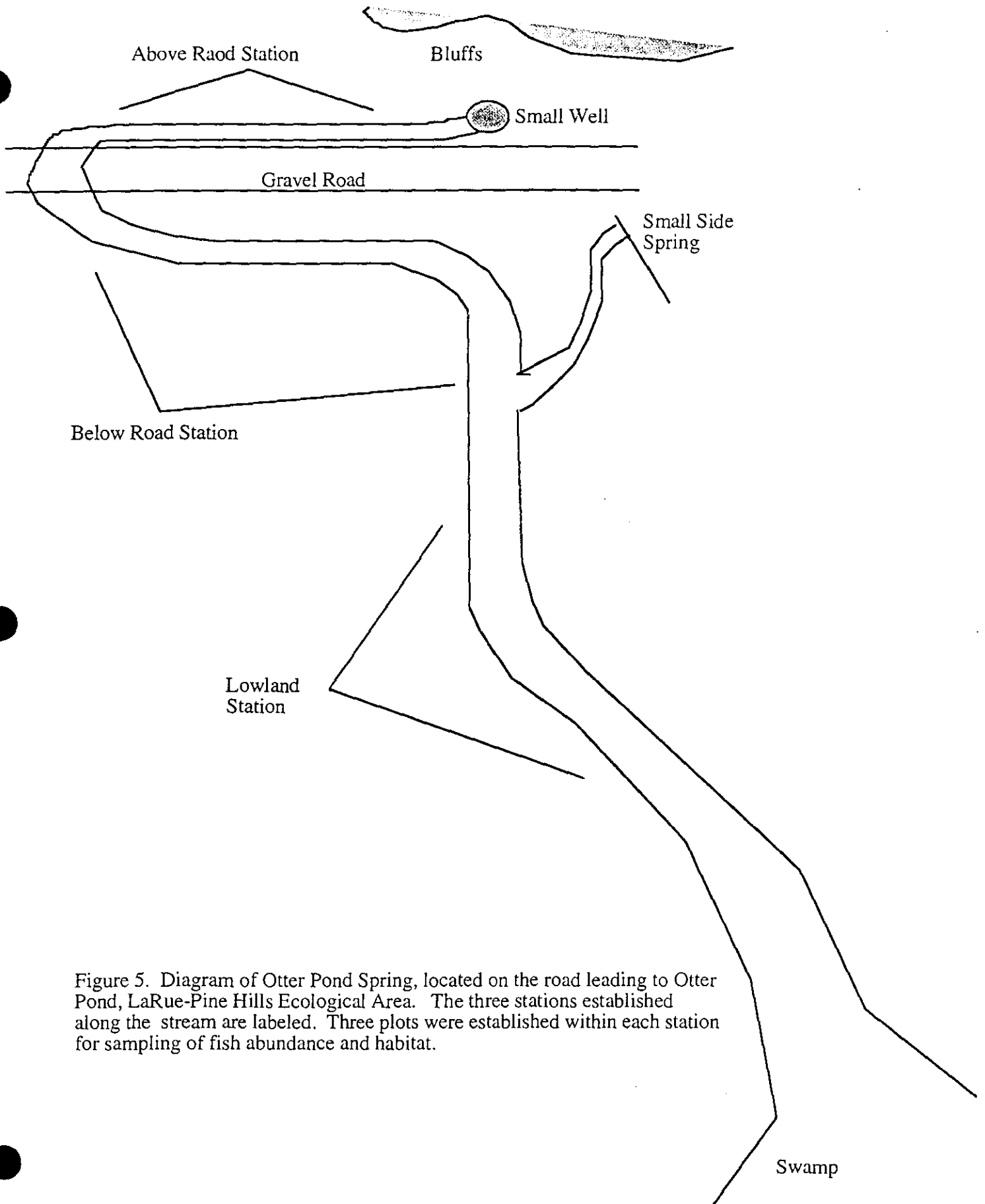


Figure 5. Diagram of Otter Pond Spring, located on the road leading to Otter Pond, LaRue-Pine Hills Ecological Area. The three stations established along the stream are labeled. Three plots were established within each station for sampling of fish abundance and habitat.

Sampling technique for each plot.

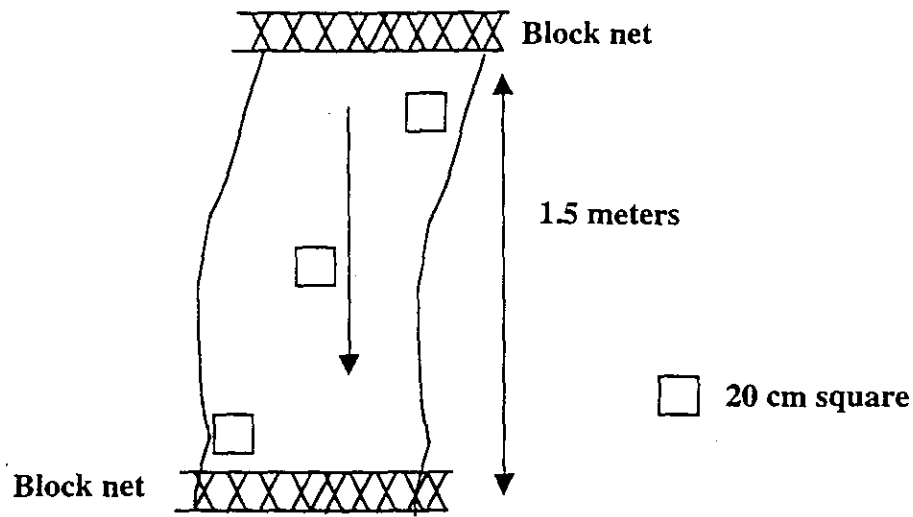


Figure 6. Diagram of the sampling procedure for each plot. Block nets were placed at both the upstream and downstream edge of the plot. A 20 cm square was positioned in the stream three times along a diagonal. At each square, cover and substrate were determined for the edges and center of the square to give a total of 15 measurements.

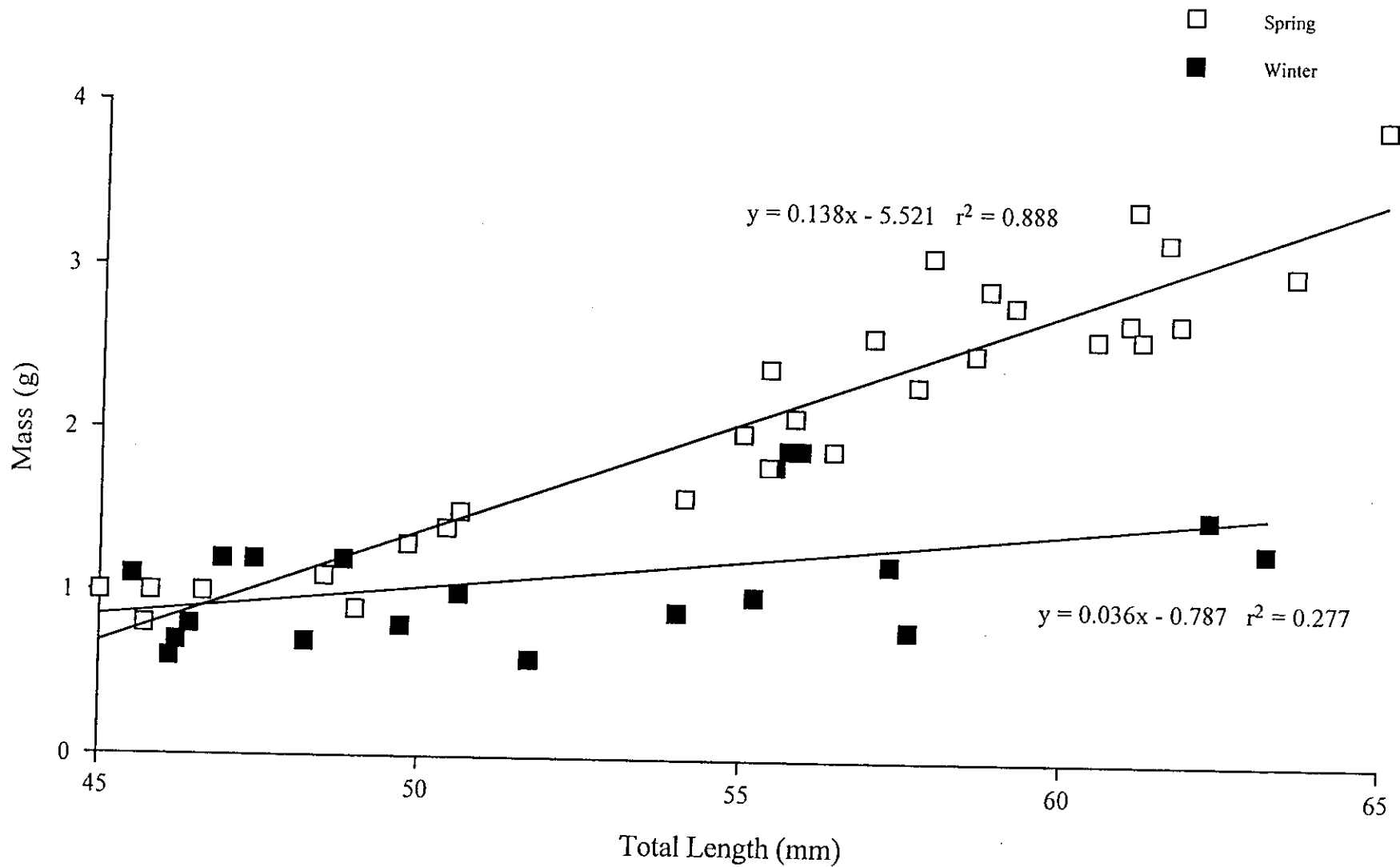


Figure 7. Condition analysis for Spring Cavefish at Elm Spring, LaRue-Pine Hills. Only individuals 45 mm total length and larger were used in the analysis. Open squares represent data collected from Cavefish during spring sampling, closed squares represent data collected during winter.

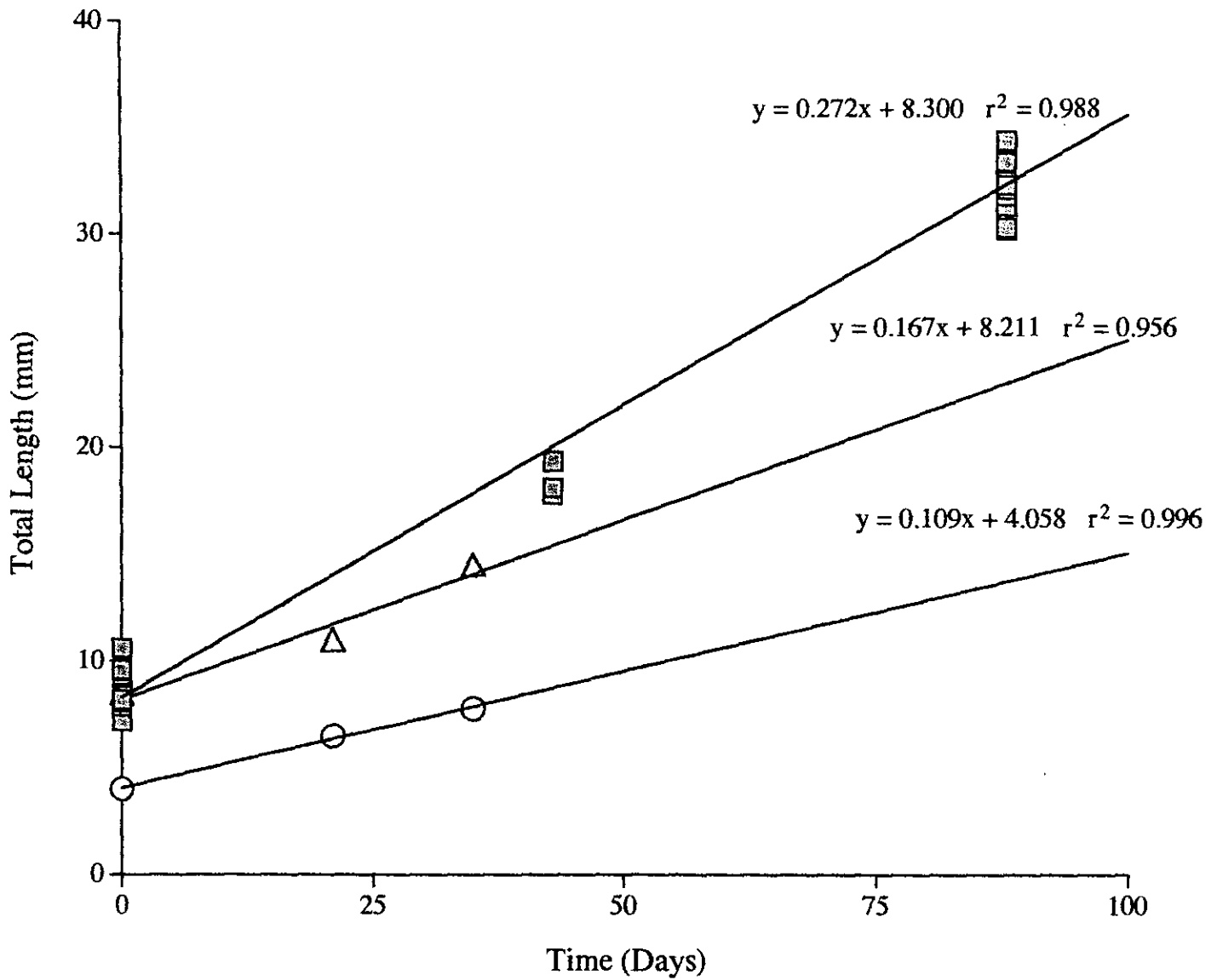


Figure 8. Growth rates for Spring Cavefish. Squares represent data collected during the present study at Elm Spring, LaRue-Pine Hills. Triangles represent data collected from surface Spring Cavefish and circles represent data collected from hypogean (cave) Spring Cavefish in a study at Rich's Pond by Hill (1971). Time 0 does not represent hatching, rather it represents first observation of cavefish by the respective author.

Elm Spring

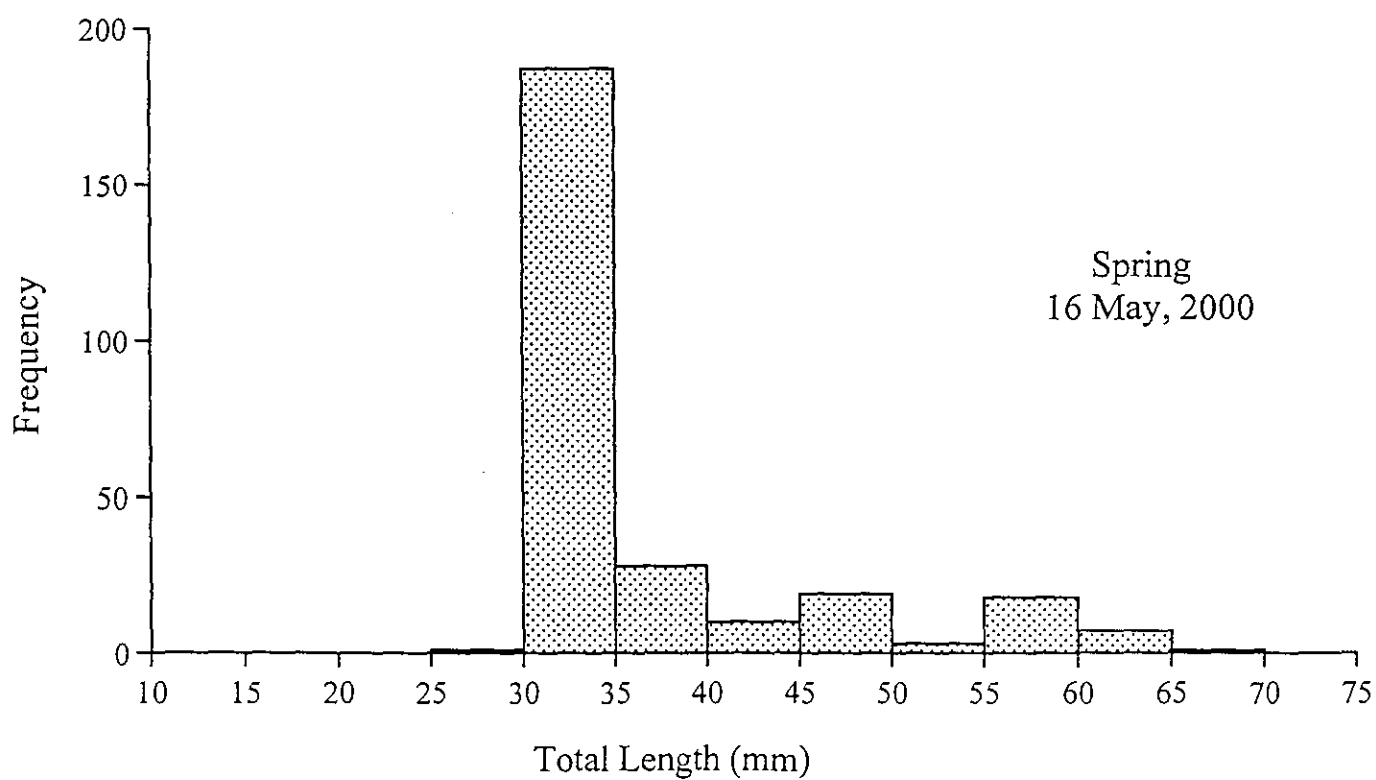
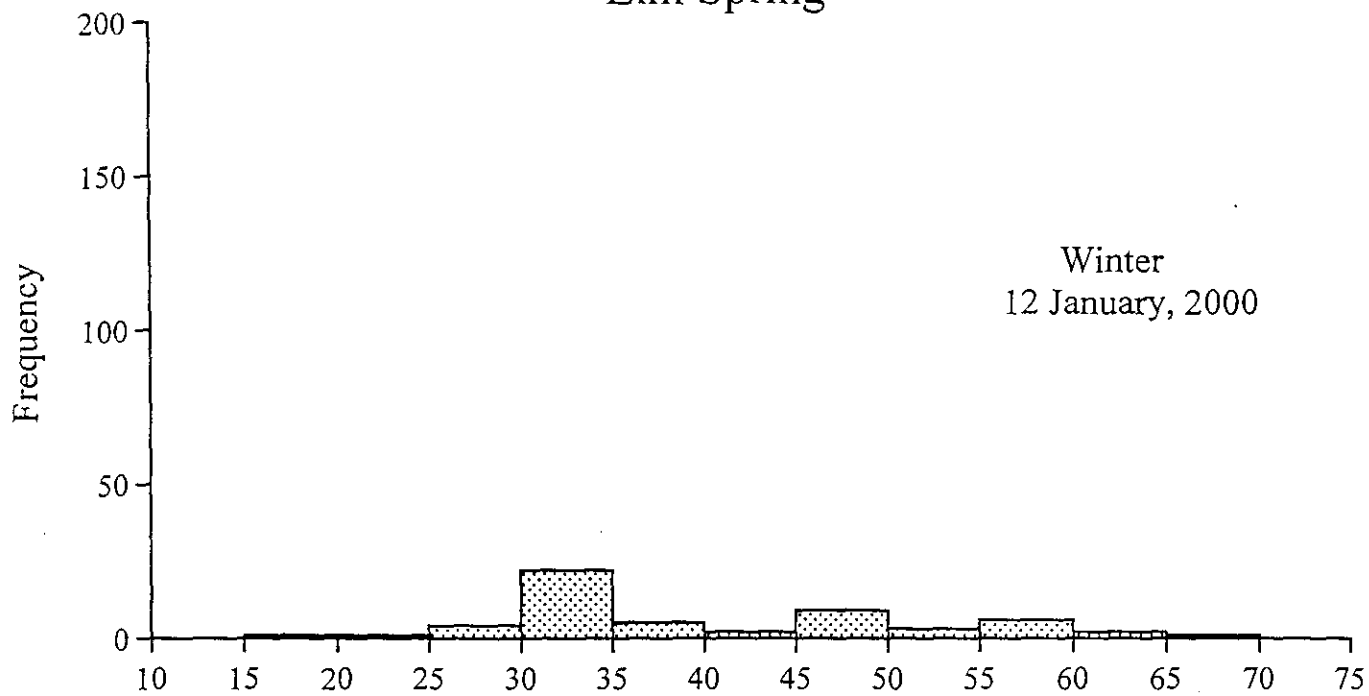


Figure 9. Size frequency histogram for Spring Cavefish at Elm Spring, LaRue-Pine Hills. Total number of fish caught in the winter was 56 and total caught in the spring was 276.

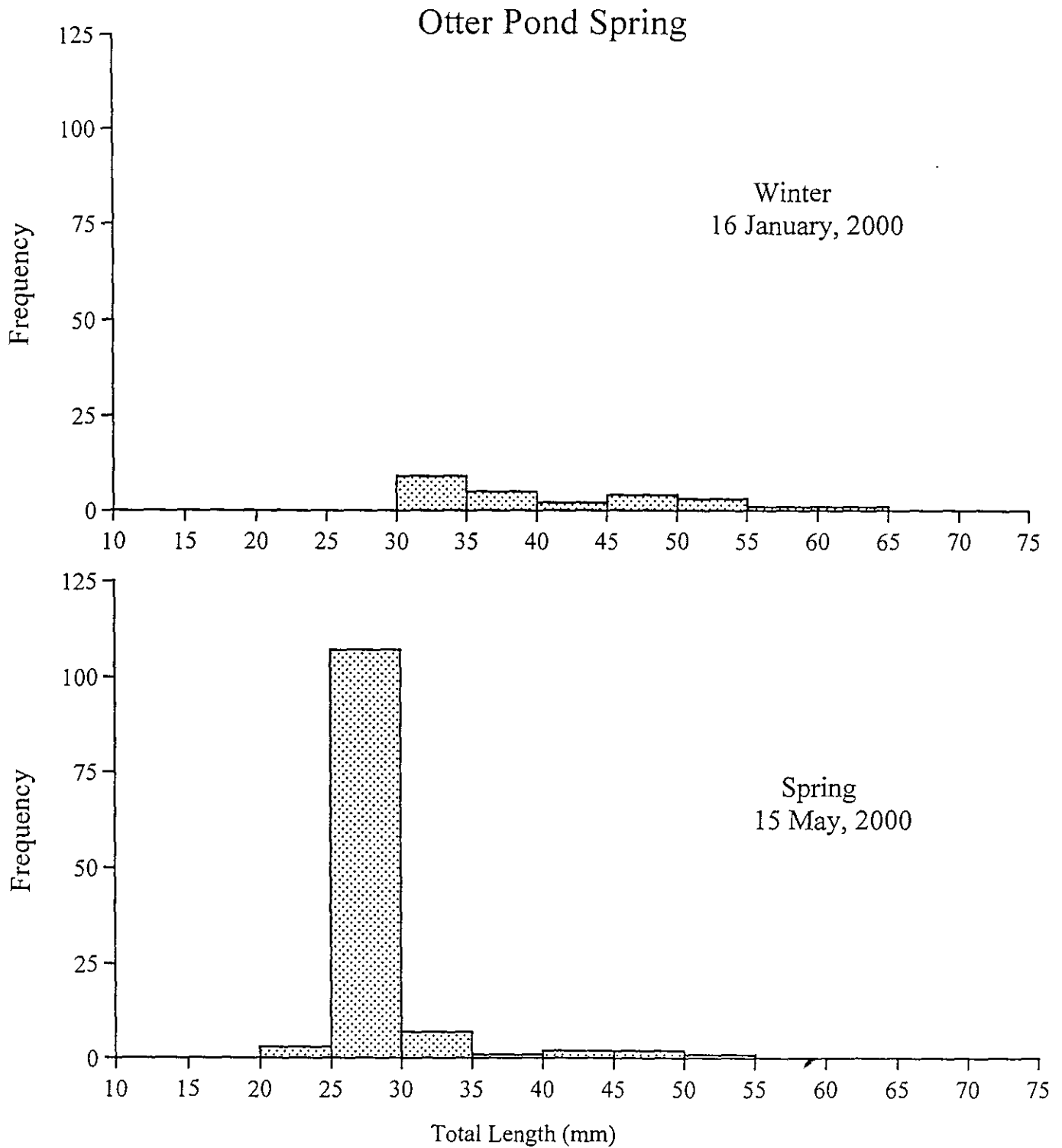


Figure 10. Size frequency histogram for Spring Cavefish at Otter Pond Spring, LaRue-Pine Hills. Total number of fish caught in the winter was 25 and total caught in the spring was 123.

McCann Spring

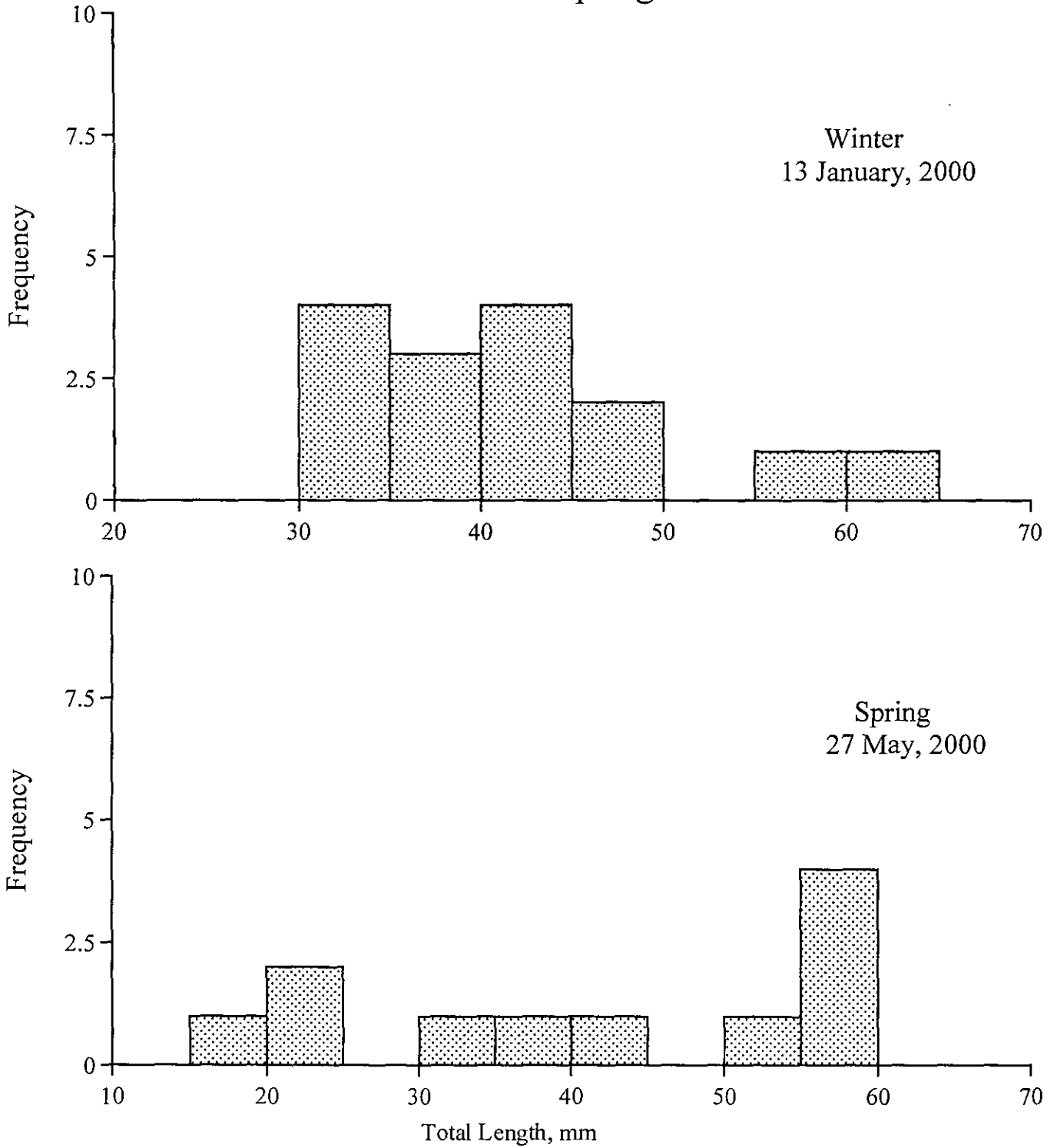


Figure 11. Size frequency histogram for Spring Cavefish at McCann Spring, LaRue-Pine Hills. Total number of fish caught in the winter was 15 and total caught in the spring was 11.