Managing Endangered Species: Charting the Course of the Illinois Cave Amphipod with Non-lethal Censusing

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Abstract

In 1940 the Illinois Cave Amphipod, *Gammarus acherondytes*, was described as a new species. The only obligate subterranean amphipod of the genus *Gammarus* in North America, this unique crustacean was described from two caves in southwestern Illinois. By 1988, cave bioinventories had revealed *Gammarus acherondytes* in a total of six caves just southeast of metropolitan Saint Louis. Over time, groundwater quality deteriorated in the area as land use changed. In 1995 *Gammarus acherondytes* could not be found in two previous sites and was barely present in two others. The amphipod was listed as a federally endangered species in 1998. In 1999 bioinventory by The Nature Conservancy revealed six additional cave populations, two in groundwater basins where the amphipod was previously unknown. As an endangered species, *Gammarus acherondytes* presented a censusing dilemma. There was no way known to monitor the 12 cave populations of *Gammarus acherondytes* without killing the amphipods to count them. In 2000 a project was initiated to see if it would be possible to measure the population sizes without killing the tiny endangered animal. Experimental census transects were established in several caves. To eliminate sampling prejudice, quadrats were randomly placed within the transects. Using a hand-held 15X microscope it was possible to separate *Gammarus acherondytes* from three other species of co-occurring cave amphipods. All animals were identified, measured, and released immediately back into the stream. The method was painstaking and labor intensive, but successful. Full-scale censusing of the endangered species commenced in 2001.

Introduction

The subterranean amphipod, *Gammarus acherondytes,* was described by Hubricht and Mackin (1940) from specimens collected by Leslie Hubricht from Morrisons Cave (Illinois Caverns) and Stemler Cave in the karst of southwestern Illinois. Bousfield (1958) redescribed the species but added no new localities. Based on collections in the mid-1960s, Peck and Lewis (1978) added Fogelpole, Krueger/Dry Run, and Pautler Caves to the list of localities from which this amphipod was known. In 1976, Lewis visited Illinois Caverns and Stemler Cave to evaluate the sites for the Illinois Natural Areas Inventory. The cave communities were inspected and appeared intact at that time, but no collections were made. However, over the next 20 years the land use of the area began to change from primarily agricultural and second growth forest into a region with an increasing suburban component. Webb (1995) reported that *G. acherondytes* could no longer be found in Stemler Cave and only small numbers of the amphipods were present in the other sites (Pautler Cave was reportedly closed by the owner). Fueled by the growing interest in *G. acherondytes*, The Nature Conservancy conducted a bioinventory of caves in Monroe and Saint Clair Counties (Lewis, Moss, and Tecic, 1999). This project resulted in the report of six additional caves with populations of *G. Acherondytes*. During that same year, *Gammarus acherondytes* was added to the U.S. Endangered Species List.

During the bioinventory by The Nature Conservancy it became apparent that little had been done to provide a basis by which the populations of *Gammarus acherondytes* could be measured. Webb (1995) had collected amphipods in various parts of several caves using a biased sampling technique that considered only the amphipod subset of the total community. Lewis, *et al*. (1999) collected samples in a similar manner to produce results that could be compared to what had already been done. These samplings of the populations provided little data that could be duplicated to determine the ongoing situation with *G. acherondytes*, while killing the endangered animals that were purportedly being "saved."

Thus was born the *raison d'etre* for developing a non-lethal method for estimating populations: to provide a yardstick by which the status of *Gammarus acherondytes* could be measured in the future to see if the situation was getting better, getting worse, or staying the same. The best method for censusing anything is to count the entire population. This is obviously not possible with a cavernicolous invertebrate, therefore leading to the alternative of examining a subset of the population. Many methods are known for preparing population estimates. We have chosen to use one that was suggested to Julian J. Lewis by cave ecologist Thomas L. Poulson for population biology studies in the aquatic communities of the Flint-Mammoth Cave System of central Kentucky.

Population Estimate Methodology

In general, the method consists of counting and measuring all species present (not just a target organism of interest) in multiple, randomly-selected quadrats along a series of transects. Analyzing the entire community, rather than merely a population within it, provides a much more complete picture of what is happening in the ecosystem. Concerning measurement of the animals, many stygobitic organisms have populations that are skewed toward older (larger) individuals with fewer juveniles (smaller) or ovigerous females. Although it might be impossible to glean the exact size of an amphipod, an estimate of six millimeters for an amphipod places the animal in a subadult cohort that obviously differs from a two-millimeter brood release or an 18-millimeter adult. This provides important information when the entire community is measured.

The fauna

Aquatic cave communities are usually relatively simple, comprising a handful of species that can frequently be identified easily, even in the field by the naked eye. Unfortunately this is not the case in western Illinois cave streams in which there are four species of amphipods that are of approximately the same size and shape. Non-lethal identification of the amphipods was the most challenging part of the project.

Cave stream communities in the western Illinois karst of Monroe and Saint Clair Counties typically comprise an assemblage of species: the flatworm *Sphalloplana hubrichti* (stygobite); snails *Fontigens antroecetes* (stygobite); *Physella* sp. (stygobite or stygophile); isopods *Caecidotea packardi* (stygobite); *Caecidotea brevicauda* (stygophile); and amphipods *Gammarus acherondytes* (stygobite), *Gammarus troglophilus* (stygophile), *Bactrurus brachycaudus* (stygobite), *Crangonyx forbesi* (stygophile). Detailed analysis of the identification of these animals was presented by Lewis (2000).

Census transects

The first priority in the establishment of transects was the presence of a landmark felt to be of an enduring nature, such that a researcher desiring to repeat the census a century from now would have an excellent chance of finding the same spot again. For each riffle transect, when facing upstream the census start point was the point at which the riffle ended and pool habitat started on the right-hand side of the riffle. A square foot (30 by 30 centimeters) Surber sampler was used to collect samples. Randomization of the sample sites was done by selecting each sample site with a number taken from a random numbers chart (available in most statistics books). The starting spot in the random numbers chart was selected first by random selection on the chart. From the point selected, the numbers were read down the column and the first two digits used to select the sample spot. A flexible plastic tape measure was stretched down the right hand side (facing upstream) of the riffle. Using the random number, the first digit was used to select the number of feet up the tape for the first quadrat. The second digit was the percentage across the stream from the right hand bank. For example, if the first number was 4268, and the stream was 10 feet wide, the first quadrat would be placed four feet up the riffle and 20% (two feet) across the stream from the right bank. After the Surber sampler was placed, a ruler was placed in the shallowest and deepest part of the quadrat and the depth recorded. If the water depth was less than about 2.5 centimeters the animals present were censused *in situ*. If the water was deeper than 2.5 centimeters the gravel was dislodged and animals allowed to wash into the sampler. All rocks were visually inspected for animals clinging on them.

Several large plastic beverage cups were carried into the cave and used to wash any animals or other material clinging onto the net of the sampler into the plastic container on the bottom of the sampler. Usually 8 to 12 washings were adequate. The contents of the sampler were released at streamside into a plastic bowl. On the first day of censusing a four-inch square bowl was used and was immediately recognized as inadequate in size. That evening an 8- by 12-inch Rubbermaid plastic bowl was purchased and was found to be an ideal size for carrying into the cave as well as containing the samples. All animals except amphipods were identified immediately visually, measured with a millimeter grid placed in the bottom of the bowl, and released back to the stream. Amphipods were placed in a dish with a millimeter grid prepared by photocopying graph paper (five grids per centimeter) onto 8½ by 11 inch 3M Transparency Film. Initially an 8X Loop was utilized for identification of the amphipods, but the 15X magnification provided by a Waltex hand microscope was found to give better viewing of animals less than six millimeters in length. A 2.5X Optivisor was found to be ideal for identification of amphipods greater than ten millimeters and the other aquatic invertebrates present in the samples. Immediately after identification all invertebrates were released back into the stream.

As-noted habitat was characterized by measuring the water depth in centimeters and giving an approximate description of the composition. Small particle size was characterized as clay if it was smooth when rubbed between the thumb and forefinger, and sand if it was gritty to the touch. Gravel was anything larger than sand up to three centimeters in size, cobbles were larger than three centimeters. Pieces of breakdown present were measured and noted.

It was noted that some animals, particularly flatworms and snails, occurred mostly under larger pieces of rock. Thus, in each transect it was decided to use a timed census rock count. This method consists of picking up larger rocks, identifying all of the fauna present on them, sight-estimating the size, then returning the rock and animals immediately to the stream. It was decided to do five-minute timed counts and to lift rocks larger than about ten centimeters throughout the transect. The number of rocks surveyed and an estimate of the size of the rocks were included in the census data.

Results

The raw data was recorded in the cave and then transcribed into a standardized spreadsheet format. On this datasheet is contained the name of the site, the location within the cave of the census area, date and personnel conducting the census, random numbers used to generate the quadrats, a description of the quadrat microhabitat, and the lengths of all animals found.

Population size of the Illinois Cave Amphipod can be estimated by extrapolating the area sampled to encompass the total area of the transect. Alternately, the relative proportions of the populations in different caves (or different parts of the same cave) can be compared by analyzing the mean number of amphipods per quadrat (square foot), which requires no extrapolation. For example, areas censused in Fogelpole Cave ranged from 0 amphipods per quadrat in the nature preserve entrance area to 1.7 amphipods/quadrat in the upstream part of the caves. The largest populations were found in Pautler Cave (up to 1.3 amphipods per quadrat), Fogelpole Cave (1.7 amphipods per quadrat) and Frog Cave (up to 3.3 amphipods per quadrat).

The data can be analyzed in a variety of other ways. For example, microhabitat preference of *Gammarus acherondytes* was examined as a function of substrate versus water depth, with the data indicating that the amphipod strongly prefers gravel/cobble substrates in shallow water.

These are just a few examples of results. Complete data was presented by Lewis (2000, 2001).

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