ASSEMBLAGES OF SALAMANDERS (FAMILY PLETHODONTIDAE) IN CWA 305(b) FULLY-SUPPORTING, PARTIALLY-SUPPORTING, AND NON-SUPPORTING STREAMS ON WALDEN'S RIDGE, TENNESSEE

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To the Graduate Council:

I am submitting a thesis written by Jason M. Houck entitled "Assemblages of Salamanders (Family Plethodontidae) in CWA 305(b) Fully-Supporting, Partially-Supporting, and Non-Supporting Streams on Walden's Ridge, Tennessee". I have examined the final copy of this thesis and recommend that is be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Environmental Science

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Abstract

Surface mining throughout the southeastern United States leading to acid mine drainage (AMD) has caused significant declines in plethodontid salamander communities in AMD affected streams. The Clean Water Act (CWA) requires that each navigable body of water in the United States be assigned a use designation and a support status based upon the stream's use designation and how it is affected by local pollution to be published in the CWA 305(b) report. Three streams on the Cumberland Escarpment, outside of Chattanooga, Tennessee, representing CWA 305(b) fully-supporting, partially-supporting, and non-supporting status were surveyed for aquatic plethodontid salamanders. Plethodontid salamanders were collected in a non-destructive manner using coverboards and visual encounter surveys from April 2002 to October 2002. Wilcoxon's Signed Ranks Tests indicated that there was a significant difference in the abundance of plethodontid salamanders in the fully-supporting stream when compared to both the partially-supporting and non-supporting streams, while there was no significant difference between the partially-supporting and non-supporting streams. Aquatic salamanders were observed to be affected by AMD in the streams surveyed. These results suggest that a biotic survey coupled with the established abiotic testing may be necessary to adequately assess CWA 305(b) support status.

Introduction

Anthropogenic acidification of streams in the southeastern United States has been a widespread problem since the early twentieth century. The southern Appalachian Mountains of eastern Tennessee and western North Carolina, along with the Cumberland Plateau of central Kentucky, Tennessee, and Alabama were the subject of a relentless search for coal during the late nineteenth and early twentieth centuries. Because large deposits of coal in these areas were close to the surface, surface mining became an extremely profitable business. Years after the mining ended, there continue to be residual effects of these activities on the surrounding habitat (National Research Council 1992).

Anthropogenic movement of large quantities of soil and rock during the process of surface mining can lead to a large sediment load in the surrounding streams. When this process exposes soil and rock strata containing pyrite, oxidation takes place in the presence of oxygen and water, leading to acid mine drainage (Wieder 1993).

The mechanism for the acidification of streams generally involves two reactions: Pyrite (FeS_2) is oxidized in an aqueous environment to produce aqueous sulfuric acid (H_2SO_4); and Iron III (Fe^{3+}) in an aqueous environment reacts to form various iron oxides resulting in a high concentration of hydrogen ions (H^+) and a low pH (USGS 1995). These reactions occur in a similar fashion where surface mining has occurred throughout the United States. With the enactment of the Surface Mining Control and Reclamation Act of 1977 (SMCRA), regulations required mining agencies to ensure that water discharges from mining operations was buffered to ensure a pH range of 6.0-9.0. Uncontrolled discharges prior to SMCRA had seriously affected over 12,000 km of streams and over 12,000 hectares of land in the Appalachian coal mining region alone (Wieder 1993).

The principal federal statute for maintaining water quality in the United States is the Clean Water Act of 1977. Among other things, the Act requires that each state assign every navigable body of water a designated use. These uses include recreation, public water supply, propagation of fish and wildlife, agriculture, and industry (33 U.S.C.A. § 1313(c)(2)(A)). A key goal for the Act is for navigable waters in the United States to be considered safe for swimming and fishing (recreation). The "swimmable/fishable" designation is the second most stringent water quality designation behind public water supplies. Once these designations are in place, they are evaluated every three years to determine whether the waters are supporting their use designation. The three primary ratings given to water systems under this designation scheme are 1) fully-supporting, 2) partially-supporting, and 3) non-supporting. The evaluated system's support status is based upon the criteria set by its use designation, and how many of those criteria it meets (Moya and Fono 2001). For example, Mullens Creek in Prentice Cooper State Forest outside of Chattanooga, Tennessee, is a fully supporting stream based on its designation as suitable for the propagation of fish and wildlife. For this reason, the Tennessee Department of Environment and Conservation uses it as a reference stream upon which to base support status of other streams in the area (Denton *et al.*2000). Conversely, South Suck Creek, on the border between Hamilton and Marion Counties just outside Chattanooga, Tennessee, is a non-supporting stream due to low pH, siltation, heavy metal contamination, and high iron levels (Denton *et al.* 2000).

Because of their water permeable skin and their dependence on streams and seeps for food, habitat and reproduction, amphibians, especially salamanders, make excellent indicators of water quality. Salamanders are more taxonomically diverse in the southeastern United States, with seven families, nineteen genera and seventy-five+ species, than in any other region of the world (Petranka 1998). The Plethodontidae is the largest family of salamanders and contains the most common, diverse, and abundant salamanders in the southeastern United States (Petranka 1998).

The presence or absence of salamanders in a stream ecosystem can be an excellent "yardstick" with which to determine that ecosystem's overall health (Dunson *et al.* 1992; Heyer *et al.* 1994). This dependence on water quality does not apply only to salamanders whose life cycle includes a water-dependant larval stage, but also to the direct developing species (Dunson *et al.* 1992). In the Chattanooga, Tennessee area, the direct developing species are all members of the genus *Plethodon* (Family Plethodontidae). This genus has no aquatic larval stage (Petranka 1998). The larval stages occur entirely inside of the egg, with the newly hatched salamanders being anatomically and morphologically identical to the adults except for size (Petranka 1998).

However, species of salamanders that breed aquatically are especially susceptible to the effects of acidification of streams. For example, species of the plethodontid genera *Desmognathus* and *Gyrinophilus* have obligate aquatic larval stages and cannot escape from the stresses associated with low pH. In studies throughout the United States and Canada it has been observed that, in acidified streams, plethodontid salamander eggs are effected in such a way that hatching does not occur (Dunson and Connell 1982, Cook 1983,Gore 1983, Clark 1986, Roudebush 1988, Freda *et al* 1991;

Corn and Vertucci 1992). Freda *et al.*(1991) determined that after hatching, resistance to acidic conditions increases as the animal matures. This resistance is especially pronounced in *Desmognathus monticola* and *Desmognathus quadramaculatus* (Freda *et al.* 1991). Although adult *D. monticola* and *D. quadramaculatus* have been observed in acidified streams, there is no indication that these individuals were born there, or were successfully reproducing. Freda *et al* (1991) determined that, in general, desmognathine species were acid insensitive over a short term. In contrast, long-term exposure to low pH can cause subtle behavioral changes, including a reduction of feeding and reproductive behaviors (Roudebush 1988). Researchers begin to see significant loss in both species richness and overall abundance of individuals at a pH below 5.0 in studies conducted through the United States and Canada (Matthews and Morgan 1982; Gore 1983; Clark 1986; Roudebush 1988; Kucken *et al.* 1994).

All members of the family Plethodontidae are lungless. Lungless salamanders must maintain equilibrium with their environment with respect to hydration. These species must take up water through their skin in order to survive dehydration associated with normal activity or extended periods of moisture stress (Spotila 1971). However, when the water used is acidic, hydration either does not take place, or is detrimental to the health of the animal (Spotila 1971). Metal salts forced into solution by the lowering of pH, a result of acid mine drainage, can affect the salamander's ability to absorb water by changing the concentration gradient across the cell membranes (Sadinski 1992). In some cases this attempt by the animal to hydrate can even cause further dehydration (Sadinski 1992).

The purpose of this study was to use plethodontid salamanders as an indicator species for water quality in the Tennessee River Gorge. The Tennessee River Gorge (TRG) consists of 26,000 acres of the Cumberland Mountains carved out by the Tennessee River and represents the fourth largest river canyon east of the Mississippi River (Tennessee River Gorge Trust 2002). The streams selected for investigation were Mullens Creek, North Suck Creek, and South Suck Creek (Fig. 1), all of which drain into the TRG. All of these stream systems are designated for propagation of fish and wildlife and received a support status of fully-supporting, partially-supporting, and non-supporting, respectively, based upon the extent of AMD and other detrimental effects on the waterways. Plethodontid salamanders, being reliable indicators of water quality and typically occurring abundantly within the study area, were chosen to evaluate whether the support status designations established for use in compiling the CWA 305(b) report were reflected in the stream biota. The CWA 305(b) report testing is currently based strictly upon abiotic criteria. Specifically, the hypothesis was made that the plethodontid salamander fauna in the fully-supporting stream will exhibit higher relative abundances than in the partially-supporting stream, which will in turn have higher relative abundances than in the non-supporting stream.

Materials and Methods

This study involved the use of coverboards as well as visual encounter surveys. The coverboards were made from corrugated roofing material purchased from a local hardware store and measured 42 x 48 inches. This material was chosen for its light weight, ease of emplacement, and its resistance to degradation. This resistance to degradation ensured that it would not harm the environment, even when exposed for long periods of time to sunlight or water (Heyer *et al.* 1994; Minahan *et al.* 2002).

The coverboards were placed along the bank of each stream with approximately one third of the board in or over the water. This gave animals the opportunity to remain hidden and still enter the water. Since the target species are facultatively aquatic organisms, this method should have ensured the greatest success (Grant *et al.* 1992).

During sampling the date, time, approximate temperature, and humidity were recorded at each site. The sampling then began with the coverboards being turned over and the substrate examined. This exposed any salamanders present underneath the boards. Whenever possible, the animals were collected and placed into a plastic cooler to be identified. The animals were subsequently released at the same site where they were collected.

Upon completion of the coverboard surveys, a one-hour visual encounter survey was made at each site following the methodology outlined in Heyer *et al.* (1994). After noting the start time, the remainder of the stream and surrounding riparian habitat below the high water mark was surveyed 50 meters upsteam and downstream from each coverboard site. This effort consisted of turning over rocks, rolling logs, and examining some of the many seeps feeding the stream. Special consideration was given during each survey to ensure that all natural cover was returned as close to its original position as possible. This was done to ensure that the disturbance of the microhabitat underneath each cover object was minimized (Heyer *et al.* 1994).

The salamanders collected via coverboards and visual encounter surveys were photographed using a Kodak DC 290 digital camera, their life stage was noted, and the species location in or around the stream were recorded using a Garmin III+ GPS receiver. Once each specimen was recorded, they were returned to the approximate location at which they were captured. This process was repeated for each sampling site.

STUDY AREAS

The study occurred from April 2002 through October 2002. The sites surveyed (Fig. 1) were chosen based upon the sampling regime used by the Tennessee Department of Environment and Conservation (TDEC). The three sites were identified by TDEC and used as water sampling locations for incorporation into the 305(b) report on water quality that is submitted to the U.S. Environmental Protection Agency (EPA). The mines affecting the partially-supporting and non-supporting streams are no longer active and are located at the headwaters of both streams along the northwestern rim of the Cumberland Escarpment. Values for data collected by TDEC are listed in Table 1 (TDEC 2000).

1) **Mullens Creek**-Fully Supporting (Fig. 2)

Mullens Creek is located within Prentice Cooper State Forest and Wildlife Management Area in Marion County, Tennessee and is used as a reference stream by TDEC to establish "baseline" water quality data for incorporation in the CWA 305(b) report. The site surveyed was located at 35.12436^{0}N 85.44306^{0}W. This location was accessed by a 4x4 road approximately 2.5 miles from the entrance to Prentice Cooper.

2) **North Suck Creek**-Partially Supporting (Fig. 3)

North Suck Creek is the border between Hamilton and Marion Counties. The site surveyed was located approximately 20 meters upstream of the confluence of South Suck

Creek at 35.14594^{0}N 85.38878^{0}W. The site was accessed from US Highway 27 leading up onto Walden's Ridge approximately 2 miles south of the entrance to Prentice Cooper.

3) **South Suck Creek**-Non-Supporting (Fig. 4).

South Suck Creek flows from Walden's Ridge to its confluence with North Suck Creek at US Highway 27. The site surveyed was approximately 20 meters upstream of the confluence of North Suck Creek at 35.14575^{0}N 85.38961^{0}W. The site was accessed from US Highway 27 approximately 2 miles south of the entrance to Prentice Cooper.

SAMPLING INTERVALS

Three coverboards were placed at each sampling location. The sampling schedule was based upon precipitation. Within 48 hours of a rain event, a sample was taken on each of the three sites. Due to the drought in the Tennessee Valley over the course of late Spring though the early Fall 2002, the sampling regime adopted remained fluid and was based upon rainfall events rather than calendar dates

COMPARISON REASONING

In comparing the different sampling localities, it was assumed: 1) other than the water quality within the stream, the available salamander habitat was homogeneous, and 2) that the criteria set by TDEC and EPA for the assessment of water quality was used uniformly when the data was collected for the compilation of the 305(b) report. With these assumptions in mind, the following hypothesis was made:

Hypothesis:

Salamander populations inhabiting the fully supporting stream will exhibit significantly higher abundance when compared to the partially supporting stream, which will in turn exhibit significantly higher abundance when compared to the non-supporting stream.

H_A=Fully supporting stream population > Partially supporting stream population > Non-supporting stream population.

Testing

The comparison testing performed was a Wilcoxon Signed-Ranks Test. This test is a non-parametric version of the standard t-test, and can be used in place of a t-test when a normal parametric test cannot be completed (Ferguson and Takane 1989). Three tests were performed comparing Mullens Creek to South Suck Creek, Mullens Creek to North Suck Creek, and South Suck Creek to North Suck Creek, (α=0.05). Individual observations included the total number of salamanders collected on a given date at each survey site.

Results

A significant difference was observed in the salamander abundances between Mullens Creek and South Suck Creek and between Mullens Creek and North Suck Creek. In both cases, Mullens Creek showed significantly greater salamander abundances. There was no significant difference in salamander abundances between South Suck Creek and North Suck Creek (Table 2) although two salamanders were observed in North Suck Creek while it was dry. Both salamanders were observed to be *P. glutinosus*. The only aquatic salamanders observed throughout the course of the study were observed in Mullens Creek.

The salamanders collected and the collection dates are listed in Table 3. The results obtained from post hoc statistical testing indicate that there is a significant difference in the abundance of plethodontid salamanders in a fully-supporting stream (Mullens Creek) when compared to a partially-supporting stream (North Suck Creek) and a non-supporting stream (South Suck Creek).

Discussion

During the course of this study the southeast United States suffered from a drought. The drought occurred from the late summer through the early fall and was likely the reason that no salamanders were collected at any of the three survey sites from July 25th through August 15th, 2002. Upon the return of consistent rain, in October of 2002, the sampling regime once again yielded positive results in only one of the streams.

The salamanders collected from both Mullens Creek and North Suck Creek included two slimy salamanders (*Plethodon glutinosus*). These animals were observed both in Mullens Creek and North Suck Creek when both creeks were dry. This species represents the only direct developing species collected during the study. Direct developing species do not have an aquatic larval stage as part of their life cycle life cycle (Petranka 1998). The *P. gluntinosus* individuals collected in this study were collected in the riparian area within the high water line of the surveyed streams. It is probable that these individuals entered the streambed from the surrounding forest in order to forage.

All of the *P. glutinosus* collected were adults. There was no evidence that these salamanders were members of a persistent population.

The desmognathine species collected represented various life stages of species in the genera *Desmognathus* and *Gyrinophilus*. Larval forms and adults of *Gyrinophilus porphyriticus, Desmognathus monticola*, and *D. fuscus* were collected in Mullens Creek. This indicated resident populations.

The parameters utilized by TDEC while compiling the data used to designate support status of streams for the CWA 305(b) report include only abiotic factors. These parameters include things such as pH, dissolved oxygen (DO), heavy metal content, and siltation (Denton *et al* 2000). The data collected in this study supports the idea that, with regard to plethodontid salamanders, the CWA 305(b) support status designation for the three streams reflects some biological reality, although a partially-supporting designation is not partially-supporting for salamanders It could be inferred that within the parameters of the study conducted, the partially supporting support status designation is enough to eliminate aquatic plethodontid salamanders from the ecosystem. Therefore, there would be no aquatic salamanders left in the ecosystem when and if the stream was further affected and was dropped to a non-supporting status. The evidence suggested that plethodontid salamanders are affected by AMD, although terrestrial salamanders may be able to utilize partially supporting habitat. The dramatic difference observed in the differing pH values of the streams surveyed (Table 1) suggests that AMD is affecting the aquatic salamander populations.

The results of this study mirrored results seen throughout the southeastern United States. A study conducted in the North Chickamauga Creek watershed, approximately five miles from the survey sites of this study, yielded similar results (Combs 1997). It was found that significantly smaller populations of salamanders existed in AMD affected streams than in pristine reference streams (Combs 1997).

Clark (1986) found that the best predictor of hatching success in spotted salamanders, *Ambystoma maculatum*, was pH. Reduced hatching success, increased susceptibility to fungal infection, and physiological stresses on the eggs and embryos causing developmental deformities were observed in breeding ponds exposed to a pH of 4.51 (Clark 1986). The hydrogen ion toxicity in the larval and egg stages of these salamanders would reduce the persistence of these populations if exposure to low pH continued (Clark 1986).

In the Great Smoky Mountains National Park (GSMNP), exposed pyritic and Anakeesta rock formations caused pH effects similar to those observed in AMD affected streams (Kucken *et* al. 1994). In one such study it was observed that low stream pH significantly reduced the density and diversity of aquatic and streamside salamander communities (Kucken *et* al. 1994). This reduction in the aquatic salamander populations due to low pH indirectly altered terrestrial salamander communities (Kucken *et* al. 1994). It was hypothesized that this may have occurred in conjunction with a reduction or elimination of species with aquatic larval stages (Kucken *et al* 1994).

Anakeesta rock formations, containing large amounts of pyrite, are found throughout the southeastern United States (Mathews and Morgan 1982). Evidence has suggested that the low pH values associated with this rock formation being exposed to oxidation are lethal to salamanders as well as many macroinvertebrates and fish in the GSMNP (Mathews and Morgan 1982). Therefore, similar problems with regard to salamanders can be expected to occur in the Appalachian Mountain regions with pyrite containing geology (Mathews and Morgan 1982). In most cases, these natural phenomenon have been observed to "buffer" themselves over time through a change to more acid tolerant flora and fauna (Mathews and Morgan 1982). In contrast, surface mining creates a shock effect on the aquatic system from which it does not have sufficient time to adapt (Mathews and Morgan 1982).

The data collected in this study are consistent with observed AMD effects on plethodontid salamander communities in other areas of the United States and Canada where surface mining has occurred. However, the evidence does not conclusively establish that AMD is the only factor involved in the decline of these populations. Other explanations my include edge effects from forest cutting or road building.

Road building has been shown to adversely effect aquatic salamander communities. Increased runoff and erosion in disturbed areas has been observed to cause increased siltation in nearby streams. In addition, potentially toxic petroleum products released from vehicle traffic may enter nearby streams (Orser and Shure 1972). Orser and Shure (1972) determined that there is an inverse relationship between salamander density and the degree of environmental disturbance.

Forestry has been shown to effect both aquatic and terrestrial salamanders alike. The most likely explanation for the disappearance of terrestrial and facultatively aquatic salamanders from clearcuts in the southern Blue Ridge is that leaf litter standing crop is significantly reduced and what is left contains less moisture (Ash 1995). Reduced forest cover over streams as a result of logging practices increases water temperature, increases siltation and bank erosion, and reduces dissolved oxygen (Ash 1995).

Moreover, it is unclear which of the cumulative effects of AMD, including increased heavy metal contamination and reduced DO, may play a role in the reduction of aquatic salamander communities. The effect these factors have on the stream health individually is not well documented, as one usually is compounded by another (Charles 1991). Nevertheless, the results of this study are consistent with the hypothesis that surface mining has an impact on aquatic salamander communities.

Further analysis would be crucial in determining if this holds true for other partially supporting and non-supporting streams with the same use designation in Tennessee. The observed similarity between the abiotic support status criteria and salamander abundance supports the hypothesis that plethodontid salamanders are good indicators of water quality. The results of this study suggest the importance of biotic surveys in determining stream support status.

Figures and Tables

Figure 1

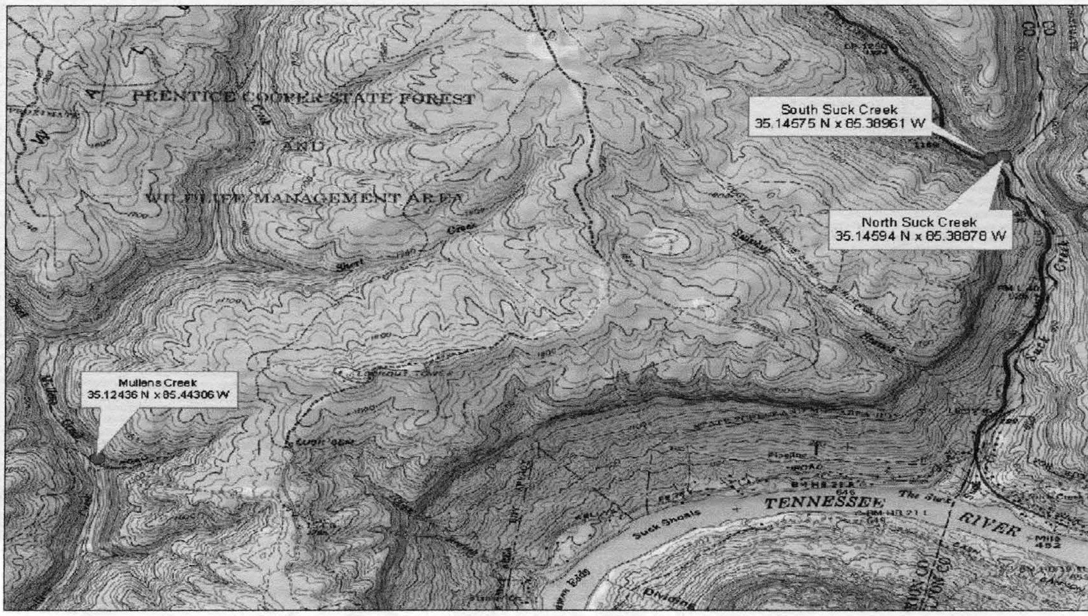

Figure 1. This figure displays the location of the three stream sites used in this study. Sites include Mullens Creek, South Suck Creek, and North Suck Creek located in Marion County, TN. Sampling for North and South Suck Creeks was done approximately twenty yards upstream respectively from the confluence.

Figure 2

Figure 2. Mullens Creek study site pictured on August 15, 2002. At the time this picture was taken the creek was dry allowing the seasonal high water mark to be viewed on either side of the creek channel.

Figure 3

Figure 3. North Suck Creek pictured on August 15, 2002. No flow was observed in the creek channel. This high water mark and riparian vegetation used in this area were observed slightly above the rocks.

Figure 4

Figure 4. South Suck Creek survey site pictured on October 1, 2002. At this point the creek had just begun normal flow following the late summer drought.

Table 1-Raw Data Obtained by TDEC During 305(b) Sampling

Location	Support Status	Stream Order	pH When Tested	Forest Cover	Elevation (ft)	Date Sampled (TDEC)
Mullens Creek	Fully-Supporting	1	7.62	60%	1420	7-Mar-2000
North Suck Creek	Partially-Supporting	1	5.49	40%	1060	7-Mar-2000
South Suck Creek	Non-Supporting	1	4.66	30%	1060	7-Mar-2000

Table 1. TDEC surveyed Mullens Creek, North Suck Creek, and South Suck Creek in March, 2000. Support status for incorporation into the CWA 305(b) report was determined based upon this data.

Table 2-Results of Wilcoxon's Signed Ranks Test

Streams Tested (α=0.05)	Wilcoxon's W Statistic	Critcal Value	2-tailed p
Mullens Creek(F) vs. South Suck Creek(N)	21	9	0.0313
Mullens Creek(F) vs. North Suck Creek(P)	21	9	0.0313
South Suck Creek(N) vs. North Suck Creek(P)	0	9	1.0000

Table 2. A total of nine surveys were used in the sampling (n=9). The stream's support status is designated by F, P, and N where F=fully-Supporting, P=Partially-Supporting, and N=Non-Supporting. Wilcoxon's W Statistic and P-values were calculated by the statistical software package Analyze-It in MS Excel.

Table 3-Individual Survey Results

		Loaction			Notes
Date	Species	Mullens Creek (F)	S. Suck Creek (N)	N. Suck Creek (P)	
30-Apr-02	*D. monticola*	3	0	0	
15-May-02	*D. monticola*	4	0	0	1 Leopard Frog @ S.Suck
	G. porphyriticus	1	0	0	
8-Jun-02	*D. fuscus*	1	0	0	Several crayfish, 2 leopard frogs
	D. monticola	3	0	0	
	P. glutinosus	1	0	0	
8-Jul-02	*D. monticola*	4	0	0	No flow in N. Suck
	D. fuscus	1	0	0	
	P. glutinosus	1	0	2	
25-Jul-02	Salamanders	0	0	0	Very Dry, no salamanders seen
1-Aug-02	Salamanders	0	0	0	Very Dry, no salamanders seen
15-Aug-02	Salamanders	0	0	0	Very Dry, No flow in all three streams
1-Oct-02	*D. monticola*	9	0	0	
	Unknown sp.	2	0	0	
22-Oct-02	*D. monticola*	7	0	0	2 Individuals brought back for comparison
	Unknwn sp.	2	0	0	1 Specimen will be sent to S. Tilley

Table 3. This table lists the survey dates and the salamanders collected along with notes pertaining to the sites. The stream's support status is designated by F, P, and N where F=Fully-Supporting, P=Partially-Supporting, and N=Non-Supporting.

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