Status Survey for the Sequatchie Caddisfly
*Glyphopsyche sequatchie* Etnier and Hix
(Trichoptera: Limnephilidae) in Alabama and Tennessee

Final Report

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Introduction

The Sequatchie caddisfly, *Glyphopsyche sequatchie* Etnier and Hix, is a rare, limnephilid caddisfly whose known range is limited to three spring sites in Marion County Tennessee. The species was discovered in March 1994 in Owen Spring Branch, approximately 25 air km west of Chattanooga (Etnier and Hix 1999). A second population was discovered four years later in Martin Spring, a tributary to Battle Creek, approximately 19 air km west-northwest from the type locality (Etnier and Hix 1999). The third known population was discovered in 2010, when a single individual was discovered at Clear Spring, approximately 2.1 km south-southwest of the type locality. Owen Spring Branch emerges from Sequatchie Cave within Sequatchie Cave State Natural Area, a 4-hectare (10-acre) preserve and park that was designated by the State of Tennessee in 2001. Martin Spring and Clear Spring are located on private property. Due to the species’ reduced range and perceived threats, the U. S. Fish and Wildlife Service recognized the Sequatchie caddisfly as a Candidate for Federal Listing in 1999 (USFWS 1999).

Most monitoring efforts for *G. sequatchie* have consisted of periodic, informal site visits by species experts at known localities. Searches at new sites have been limited. Etnier (1997) completed surveys at five new locations in September 1995, but no individuals of *G. sequatchie* were observed. Etnier (1997) identified seven additional survey sites as potential habitats, but these sites were not visited in subsequent years.

A single population estimate has been made since the species was described. Etnier and Hix (1999) surveyed Owen Spring Branch from the cave opening downstream to the Valley View Highway bridge crossing, arriving at a population estimate of 500-5,000 individuals. Since 2001, less than 10 individuals have been observed at the Martin Spring site, so it appears that this population is even smaller.
Based on the low number of surveys completed across the species' range and the lack of recent population estimates for Owen Spring Branch and Martin Spring, the U. S. Fish and Wildlife Service recognized the need for a comprehensive, range-wide survey effort and revised population estimate. Working with the Cookeville, Tennessee Ecological Services Field Office, we initiated a study to address several research goals: (1) completion of a range-wide survey effort to locate new populations; (2) completion of new population estimates at all known sites; and (3) an evaluation of the species' genetic diversity and gene flow between populations.

Methods

Field Surveys. Field surveys for immatures and adults were completed September 13-16, 2010 (throughout the Sequatchie Valley and Battle Creek system, Marion and Sequatchie counties, Tennessee; one additional site in the Crow Creek system, Franklin County); April 13-15, 2011 (Tennessee River Valley of northeastern Alabama); October 10-11, 2011 (type locality and Clear Spring), and October 22, 2011 (type locality). A total of 40 sites were visited in Alabama and Tennessee (Table 1; Appendix A, Figures 2-3). Survey efforts focused on cave springs or spring runs - habitats similar to those observed at the species' three known occurrences. Survey methods consisted of visual searches (e.g., hand-picking of larvae/pupae from rocks and woody debris), benthic sweeps for larvae using D-frame dip-nets, aerial sweeps for adults, and ultraviolet light trapping for adults.

Population Estimate. Population estimates for Owens Spring and Martin Spring were attempted in September 2010. We used the number of larvae captured by hand within a given stream reach, our assumed sampling efficiency, and the amount of suitable habitat present at a locality to generate a population estimate. At Owen Spring Branch, the large pool near the cave opening was the primary survey area (an approximate 60-m reach), but subsequent searches were made downstream to the Valley View Highway bridge crossing. At Martin Spring, searches were completed within an approximate 100-m reach below the cave opening. At Owen Spring Branch, we wanted to employ a more rigorous, quantitative sampling method (e.g., transects or sampling grids), but we ultimately decided on a more qualitative approach to avoid impacts to *G. sequatchie* larvae and pupae. We concluded
Table 1. Summary of cave/spring sites visited in Alabama and Tennessee (2010-2011)*

<table>
<thead>
<tr>
<th>#</th>
<th>Cave / Spring</th>
<th>County</th>
<th>Location</th>
<th>Comment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Tennessee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Owen Spring Branch</td>
<td>Marion</td>
<td>35.12061 / -85.59397</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>2</td>
<td>Martin Spring</td>
<td>Marion</td>
<td>35.16645 / -85.78878</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>3</td>
<td>Clear Spring</td>
<td>Marion</td>
<td>35.10372 / -85.60358</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>4</td>
<td>Dancing Fern Cave</td>
<td>Marion</td>
<td>35.13292 / -85.59045</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>5</td>
<td>Tate Cave Spring</td>
<td>Marion</td>
<td>35.17683 / -85.80733</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>6</td>
<td>Bible Spring</td>
<td>Marion</td>
<td>35.11242 / -85.74690</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>7</td>
<td>Sherwood Spring</td>
<td>Franklin</td>
<td>35.06525 / -85.91620</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>8</td>
<td>Sweden Cove Spring</td>
<td>Marion</td>
<td>35.07625 / -85.79543</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>9</td>
<td>Fish Trap Spring</td>
<td>Marion</td>
<td>35.07076 / -85.74393</td>
<td>Floodplain of Battle Creek</td>
</tr>
<tr>
<td>10</td>
<td>King Spring</td>
<td>Marion</td>
<td>35.04068 / -84.70329</td>
<td>Dry and within residential area</td>
</tr>
<tr>
<td></td>
<td>Hightower Spring</td>
<td>Marion</td>
<td>35.03997 / -85.69899</td>
<td>Dry and within residential area</td>
</tr>
<tr>
<td>11</td>
<td>Druin Spring</td>
<td>Marion</td>
<td>35.06845 / -85.64521</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>12</td>
<td>Blue Spring</td>
<td>Marion</td>
<td>35.08913 / -85.63351</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>13</td>
<td>Pryor Cave Spring</td>
<td>Marion</td>
<td>35.09493 / -85.62090</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>14</td>
<td>Francis Spring</td>
<td>Marion</td>
<td>35.09640 / -85.57277</td>
<td>Small and in floodplain of Sequatchie River</td>
</tr>
<tr>
<td>15</td>
<td>River Cave Spring</td>
<td>Marion</td>
<td>35.15253 / -85.60398</td>
<td>Dry</td>
</tr>
<tr>
<td>16</td>
<td>Brown Spring</td>
<td>Marion</td>
<td>35.15126 / -85.56151</td>
<td>No flow, poor habitat</td>
</tr>
<tr>
<td>17</td>
<td>Cowan Spring</td>
<td>Marion</td>
<td>35.16223 / -85.55487</td>
<td>Small spring run, no access</td>
</tr>
<tr>
<td>18</td>
<td>Daniel Cave</td>
<td>Marion</td>
<td>35.26083 / -85.48442</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>19</td>
<td>Cedar Spring Cave</td>
<td>Marion</td>
<td>35.26487 / -85.48018</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>20</td>
<td>Cookston Cave</td>
<td>Sequatchie</td>
<td>35.28985 / -85.46185</td>
<td>No access but currently being used as trout farm</td>
</tr>
<tr>
<td>21</td>
<td>Boynton Spring</td>
<td>Sequatchie</td>
<td>35.31325 / -85.44377</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>22</td>
<td>Stone Cave Spring</td>
<td>Sequatchie</td>
<td>35.31135 / -85.42800</td>
<td>See Appendix A</td>
</tr>
<tr>
<td>23</td>
<td>Stone Cave</td>
<td>Sequatchie</td>
<td>35.33513 / -85.42944</td>
<td>Short spring run, next to roadway</td>
</tr>
<tr>
<td>24</td>
<td>Minton Spring</td>
<td>Sequatchie</td>
<td>35.44133 / -85.35892</td>
<td>Small seep, poor habitat</td>
</tr>
<tr>
<td>25</td>
<td>Byrd Spring</td>
<td>Sequatchie</td>
<td>35.50455 / -85.31002</td>
<td>Small seep, poor habitat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alabama</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Houston Spring</td>
<td>Jackson</td>
<td>34.81121 / -85.95806</td>
<td>No access</td>
</tr>
<tr>
<td>27</td>
<td>McCary Spring</td>
<td>Jackson</td>
<td>34.87867 / -86.00578</td>
<td>No access</td>
</tr>
<tr>
<td>28</td>
<td>Flag Spring</td>
<td>Jackson</td>
<td>34.78477 / -86.03333</td>
<td>No access</td>
</tr>
<tr>
<td>29</td>
<td>Selby Spring</td>
<td>Jackson</td>
<td>34.74117 / -86.14130</td>
<td>Good habitat; Agapetus, Irontoquia, Pycnopsyche observed</td>
</tr>
<tr>
<td>30</td>
<td>Isbell Spring</td>
<td>Jackson</td>
<td>34.68813 / -86.18774</td>
<td>High volume flow; no caddisflies</td>
</tr>
<tr>
<td>31</td>
<td>Sauta Cave</td>
<td>Jackson</td>
<td>34.61650 / -86.13134</td>
<td>Bedrock substrate, with some cobble/boulder; Rhyncophila, Agapetus observed</td>
</tr>
<tr>
<td>32</td>
<td>Daniel #1 Spring</td>
<td>Jackson</td>
<td>34.73645 / -86.30911</td>
<td>Poor habitat or no access; no caddisflies observed</td>
</tr>
<tr>
<td>33</td>
<td>McGehee Spring</td>
<td>Marshall</td>
<td>34.50291 / -86.37554</td>
<td></td>
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<tr>
<td>34</td>
<td>Baker Spring</td>
<td>Madison</td>
<td>34.60204 / -86.42472</td>
<td></td>
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<tr>
<td>35</td>
<td>Beason Spring</td>
<td>Madison</td>
<td>34.73843 / -86.37580</td>
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<tr>
<td>36</td>
<td>Berry Spring</td>
<td>Madison</td>
<td>34.81645 / -86.43777</td>
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<tr>
<td>37</td>
<td>Cold Spring</td>
<td>Madison</td>
<td>34.74624 / -86.53030</td>
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<tr>
<td>38</td>
<td>Prater Spring</td>
<td>Madison</td>
<td>34.96597 / -86.40000</td>
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<tr>
<td>39</td>
<td>Valhermosso Spring</td>
<td>Morgan</td>
<td>34.50926 / -86.68024</td>
<td></td>
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<tr>
<td>40</td>
<td>Cave Spring</td>
<td>Morgan</td>
<td>34.54617 / -86.85113</td>
<td></td>
</tr>
</tbody>
</table>

*Withers (2003) provides additional descriptions of some Tennessee sites.
that a more quantitative, intensive sampling technique would cause significant habitat disturbance within the spring run and could potentially harm (crush) *G. sequatchie* larvae and pupae. We believed a more qualitative approach would cause fewer direct and indirect impacts on the species.

**Genetic Divergence/Estimation of Gene Flow.** Examination of genetic diversity and degree of gene flow between the Owen Spring Branch and Martin Spring Run populations (and any others found) were objectives of the project; however, due to the extremely low numbers of individuals observed at the Martin Spring site, we considered it imprudent to sacrifice any Martin Spring individuals. Ten individuals from Owen Spring Branch were preserved in absolute ethanol and stored in a -20°C freezer to allow future comparisons if/when the Martin Spring population rebounds or another thriving population is discovered. To insure the integrity of preserved tissues, DNA was isolated from five individuals from Owen Spring Branch using tissue excised from a single abdominal segment and amplified via PCR. DNA was extracted using a GeneJet DNA Extraction Kit (Thermo Fisher Scientific, Waltham, MA) per the manufacturer's suggested protocol. A portion of CAD (CAD Piece 1; Moulton & Wiegmann 2004) was amplified using the author's suggested protocol. The resulting amplicons were gel-purified, cleaned using a Qiagen Gel Extraction Kit (Qiagen Corp, Germantown, MD) per the manufacturer's suggested protocol, and cycle sequenced using big dye terminators (Applied Biosystems, Foster City, CA). Cycle sequencing reactions were cleaned using Centri-Sep columns (Princeton Separations, Adelphia, NJ) and analyzed using an ABI 3730 Genetic Analyzer.

**Results**

**Field Surveys.** Surveys throughout the Sequatchie Valley, Battle Creek, and Crow Creek systems of Tennessee and other spring habitats in Alabama yielded no new occurrences for the species (Appendix B). We were unable to receive landowner permission to access about half of the Alabama sites, so these sites were not surveyed (Table 1). Of the remaining sites surveyed, Selby Spring (Site #29) contained the most suitable habitat conditions for the species. We observed other caddisfly genera at Selby Spring (e.g., *Agapetus, Pycnopsyche*) but no individuals of *G. sequatchie*. 
Three Tennessee sites – Dancing Fern Cave, Sweden Cove Spring, and Boynton Spring (Sites #4, 8, and 23) – appeared to have adequate water quality and habitat conditions to support the species and could serve as potential reintroduction sites in the future. Of the three, Dancing Fern Cave/Spring appears to have the best habitat (e.g., higher volume flow, good canopy cover, less silt) and is more inaccessible than the other two sites. Another site, Stone Cave (Site #23, Walling property), is most similar in general appearance to Owen Spring Branch and Martin Spring. It emerges from a large cave and has a downstream channel with similar dimensions; however, the site has been greatly modified by an impoundment and appears to be receiving high sediment loads from areas upstream in the watershed. A significant restoration effort would be necessary before the species could be reintroduced at the site.

The remaining spring sites in Tennessee were dry or they had been modified physically, as evidenced by a lack of canopy cover, moderate to heavy siltation, eroded banks, and the presence of impoundments. One notable observation during our surveys was the presence of coal fines at nearly all Sequatchie Valley sites where the species was absent; coal fines were not observed at sites known to support the species.

The species continues to be common and stable at the type locality, Owens Cave, in Marion County, but it was rare at the remaining two sites – Martin Spring and Clear Spring. At Martin Spring, the species was observed near the cave opening but only downstream of the constructed stone wall. On September 13, 2010, only 6 individuals were observed during a one-hour search by four persons. Two individuals were found among leaves at the stream margin, two individuals were found on root mats of a large sycamore (Platanus occidentalis), and the remaining individuals were observed on wood at the stream bottom. At the time of our survey (September 2010), Martin Spring was clear and cold (12.8°C). The substrate was composed of a mixture of clean cobble, gravel, and sand, with no coal fines. Siltation was negligible, but the substrate was covered by strands of filamentous algae beginning approximately 30 m downstream of the stone wall (see Appendix B, Site #2). Canopy cover was low (< 50%). The left riparian zone consisted of a steep wooded slope, but natural vegetation was limited within the right riparian zone, which was narrow (<5 m wide) and partially maintained as an open field. We did not observe non-native rainbow trout (Oncorhynchus mykiss) within our search area, but trout were observed about 300 m
downstream near the home of Mr. Kelly. Some of these individuals were large (> 45 cm [18 inches] long).

Owen Spring Branch was slightly warmer than Martin Spring (13.5°C vs. 12.8°C), but its conductivity value was lower (212µS/cm vs. 269 µS/cm). Habitat conditions were similar at both sites, but Owen Spring Branch lacked the filamentous algae observed within Martin Spring. Substrates were composed of boulder, cobble, and gravel, with lesser amounts of sand and silt. Silt deposits were limited primarily to areas near shore. Pieces of trash such as bottles, rusted metal, aluminum cans, and other unknown items were noticeable throughout the area near the parking lot and pedestrian access. In fact, the amount of broken glass observed along the stream bottom was significant and could represent a hazard to anyone wading in the spring run. Two areas along the right descending bank lacked vegetation and were likely contributing fine sediment (silt/sand) during periods of high flow. These areas included the pedestrian access (near the wooden bench and large boulder) and the sloped bank near the parking area. An eroded, non-vegetated path led from the parking area down to the spring run, forming a small alluvial deposit at the bottom of the slope.

Our September 2010 survey (a four man-hour effort) at Owen Spring Branch demonstrated that *G. sequatchie* is still common at the site. We located about 50 larvae within an approximate 60-m sampling reach at the head of the spring run. Larvae were attached to wood and rocks, while others were observed crawling or resting on the stream bottom. We also observed larvae as far downstream as the Valley View Highway bridge crossing, just above its confluence with the "lagoon." Water temperatures in this area (about 14.2°C) were slightly higher than that observed in upstream reaches.

During October 2011 collections at Owen Spring Branch, we determined that mature larvae seek out quiet depositional areas containing branches or logs for pupation. One log (0.15 m wide by 0.46 m long) located approximately 70 m below the cave opening contained about 30 pupae. Numbers started to drop about 300 m upstream of the Valley View Highway bridge crossing, and no pupae were observed within sight of the road, which is well upstream of the lagoon. No larvae or pupae were observed in the canal. Larvae could
have migrated upstream from the lower reaches because we did observe larvae at the road crossing in 2010.

Adults were present at Owen Spring Branch in late October and were observed flying in the canopy above Owen Spring Branch. In general, adults were absent from the area immediately below the cave opening (an approximate 50 meter reach), but they were common farther downstream where there was adequate canopy cover. Lack of suitable cover near the cave opening likely explains why no adults were observed in that area. Adults were difficult to capture by net, and light trapping proved futile because night-time temperatures were lower than optimal (< 60°C at dusk). It is likely that adults of this species are strictly diurnal.

We did not observe the species at Clear Spring, despite a two-man, one-hour search of the entire spring run from Valley View Highway upstream to the source (approximately 145 m). The spring run of Clear Spring was much smaller (only 2-3 m wide) than the other two known localities, and there was a considerable amount of silt on the stream bottom. The left riparian zone was extensive (wooded slope), but the right riparian zone was narrow (1-2 m wide), consisting primarily of a well-maintained lawn. Based on our field observations, habitat conditions were poor at this site, and we considered the population to be very small or extirpated.

**Population Estimate.** Based on the low numbers observed at Martin Spring, and our inability to locate individuals at Clear Spring, we were unable to arrive at population estimates for those sites. The species may be extirpated from Clear Spring, and it appears that the species is rare at the Martin Spring site and likely consists of fewer than 100 individuals.

For Owen Spring Branch, we estimate a population size of 1,500 – 3,000 individuals – a range comparable to that reported by Etnier and Hix (1999). We base our estimate on the number of larvae (50) observed during our September 2010 sampling effort (4 man-hours spent in 60 m of habitat), our assumed sampling efficiency (a range of 25-50%), and the amount of suitable habitat present between the cave opening and the Valley View Highway bridge crossing (about 900 m):

- 50% sampling efficiency: \( (50 \text{ inds}) \times (2) \times \left( \frac{900\text{m}}{60\text{m}} \right) = 1,500 \text{ inds} \)
• 25% sampling efficiency: (50 inds)*(4)*(900m/60m) = 3,000 inds

**Genetic Divergence/Estimation of Gene Flow.** Although the scarcity of *G. sequatchie* at Martin Spring did not allow us to complete genetic analyses, high-quality DNA was verified from extractions performed on tissue excised from 5 larvae from Owen Spring Branch, thus validating both our preservation and DNA extraction methodologies. These individuals were sequenced for a small portion of the CAD gene (Figure 1) and were identical; based on these results, Owen Spring Branch appears to support a virtually clonal population. Our extracted templates from Owen Spring Branch are stored in a -20°C freezer in the Moulton laboratory at The University of Tennessee and will remain viable for decades.

![Figure 1](image.png) A portion (649 base pairs) of the CAD gene obtained from 5 *Glyphopsyche sequatchie* specimens from Owen Spring Branch. All five specimens were invariant.

**Discussion**

Our survey efforts provide further evidence that *G. sequatchie* is a narrow endemic that is extant at only 2-3 sites in Tennessee. The species appears to be stable at the type locality (Owen Spring Branch) but is likely declining or in low numbers at Martin Spring and Clear Spring. The species’ isolated status, low abundance, annual life cycle, potentially low genetic diversity, and limited range contribute to its vulnerability to extirpation.

The species continues to face a variety of threats as outlined in USFWS (2011). The three known populations are threatened by habitat destruction or modification - siltation, point and nonpoint source discharges from municipal and industrial activities, and...
introduction of contaminants during episodic events. In the past, the species was likely extirpated from spring habitats within the Sequatchie Valley due to impacts from coal extraction activities taking place within recharge areas on the Cumberland Plateau. Many streams in the valley continue to have coal fines dispersed within their substrates. Interestingly, coal fines were not observed at the three known localities. Besides its extremely limited range, several other threats to this species have been noted (USFWS 2011), including beaver activity, siltation, agricultural, municipal, and industrial chemical runoff (both direct and from subsurface flows), vandalism, pollution from trash thrown into the springs, and predation from non-native fish (e.g., rainbow trout). Specific threats for each of the three known localities are outlined below.

**Owen Spring Branch.** Threats at this site include (1) siltation, originating from sediment runoff in upstream areas (recharge zone) or from on-site erosion of non-vegetated areas along the right bank; (2) lack of woody substrate; (3) crushing of individuals by pedestrians who walk or wade in the spring run; (4) trash inputs, which cover up suitable habitat areas (pools) and may contribute contaminants to the site; (5) inundation of the site by beavers, which may cause stagnation or aggradation of pool habitats; and (6) inputs of warm water, sediment, or other pollutants from the “lagoon”, which creates unfavorable habitat conditions for the species downstream of Valley View Highway at all times of the year.

**Martin Spring.** Threats at this site include (1) siltation, originating from sediment runoff in upstream areas (recharge zone) or from stormwater erosion of non-vegetated areas; (2) lack of woody substrate; (3) excessive algal growth in downstream reaches (beginning approximately 30 m below the cave opening); (4) predation by non-native species (rainbow trout); and (5) lack of shade (high water temperatures).

**Clear Spring.** Threats at this site include (1) siltation, originating from sediment runoff in upstream areas (recharge zone); (2) lack of wood; and (3) contaminated stormwater runoff (lawn fertilizer, pesticides).
Conservation Recommendations. We believe that there are multiple conservation measures that the U.S. Fish and Wildlife Service and its partners can implement to protect and improve the conservation status of *G. sequatchie*. Our recommendations and comments are provided below:

1. Limit professional surveys and public access at known locations (Owen Spring Branch and Martin Spring) to reduce habitat disturbance and prevent unnecessary and excessive “takeings” of *G. sequatchie*. Professional collections at Martin Spring should be stopped; collections at Owen Spring Branch should be limited. Public access is already limited (controlled) at Martin Spring, but Owen Spring Branch receives a significant amount of public visitation. Limiting access to riparian areas and the spring run itself would help protect bank and instream habitats and prevent crushing of *G. sequatchie* individuals.

2. Acquire property within the drainage basins (recharge area) of Owen Spring Branch and Martin Spring. This would provide added protection against sedimentation, potential contamination of groundwater, and other indirect impacts from stormwater runoff on the Cumberland Plateau.

3. Revegetate and protect riparian habitats surrounding both springs, especially those areas near the parking lot and pedestrian access of Sequatchie Cave State Natural Area. Increased bank protection at Owen Spring Branch would reduce sedimentation originating on-site, and increased canopy cover would help reduce the excessive algal growths observed at Martin Spring.

4. Control beaver activity within Owen Spring Branch through periodic surveys and trapping. In the past, portions of Owen Spring Branch have been inundated by beaver dams, causing degraded habitat conditions - reduced flow, siltation, and stagnation. Dam removal could cause significant physical disturbance of Owen Spring Branch, including scouring of substrates and displacement of *G. sequatchie*.

5. Augment habitats in both spring runs with large woody material.

6. Remove trash from Owen Spring Branch (e.g., bottles, tires, etc.).

7. Redirect or limit the flow of water coming from the lagoon. A significant portion of Owen Spring Branch, roughly from midway between the cave entrance and the
Valley View Highway crossing (and downstream), would likely be more suitable for the species (lower temperatures, lower sediment inputs) if flows from the lagoon could be redirected to the Little Sequatchie River. Water quality characterization of the lagoon would be helpful to quantify what effects it is currently having on downstream reaches of Owen Spring Branch.

8. Increase the number of known habitats in the Sequatchie Valley through captive propagation, relocation, and augmentation activities.

9. Investigate the impact of rainbow trout on the Martin Spring population of *G. sequatchie*. Gut analyses could be performed to determine if trout are feeding on *G. sequatchie* larvae or pupae; however, the low abundance of *G. sequatchie* may limit the success of these analyses. If it can be shown that trout are causing a significant decrease in *G. sequatchie* abundance, existing trout should be removed and trout stockings in downstream reaches of Battle Creek should be discontinued.

10. Search for additional populations of *G. sequatchie* in Tennessee and Alabama. Other suitable habitats have not been searched; previously searched sites (e.g., Dancing Fern Cave) could be revisited.

11. Conduct water chemistry characterizations of known and potential sites to identify the species' preferred habitat conditions and evaluate the suitability of potential reintroduction sites.

12. Acquire or establish cooperative agreements with property owners at potential reintroduction sites; improve habitat conditions at these sites in anticipation of reintroduction efforts.

**Acknowledgements**

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Martin Cave, Clear Spring, Boynton Spring, and Stone Cave, respectively. Mapping assistance was provided by Mr. Greg Abernathy (Kentucky State Nature Preserves Commission).

References


Appendix A

Figure 2 – Study Area in AL and TN (2010-2011)
Figure 3 – Tennessee Study Area (2010-2011)
Appendix B

Site Descriptions, Maps, and Photographs
Sequatchie Cave / Owen Spring Branch, Marion County (Type Locality)

Site Number: 1

Location: off Valley View Highway, approx. 0.3 mi (0.5 km) NNW of Sequatchie

Coordinates: 35.12061 / -85.59397

Property Owner: State of Tennessee

Field Notes: Type locality. September 13, 2010: Temp: 13.5°C; Dissolved Oxygen: 8.17 mg/L; pH: 8.16 S.U.; Conductivity: 212 μS/cm. Larvae observed from cave opening to Valley View Highway bridge (September 2010); pupae observed within 300 m of Valley View Road bridge in October 2011; approximately 30 pupae observed on one log. Caddisfly genera (larvae) observed in September 2010: Cheumatopsyche, Goera, Micrasema, Pycnopsyche, Psilotreta (abundant). Photographs on following page.
Sequatchie Cave / Owen Spring Branch, Marion County (Site 1 - Type Locality)

Photographs: A, cave opening; B, pedestrian access at cave opening; C, pool below cave opening; D, pool below cave opening (downstream view); E, small dam below pool; F, channel just above Valley View Highway bridge crossing (upstream view).
Martin Spring, Marion County
Site Number: 2
Location: off Martin Springs Road, approx. 6.7 mi (10.8 km) NW of Smithtown
Coordinates: 35.16645 / -85.78878
Property Owner: n/a
Martin Spring, Marion County (Site 2)
Photographs: A, cave opening; B, concrete wall at cave opening; C, spring run below cave opening (downstream view); D, filamentous algae in channel.
Clear Spring, Marion County

Site Number: 3

Location: off Valley Road, approx. 1 mi (1.6 km) SSW of Sequatchie

Coordinates: 35.10372 / -85.60358

Property Owner: Ms. Inez Ireland

Field Notes: Site forested on left descending bank, right riparian zone with a narrow vegetated strip (1-2 m) but primarily consisting of a maintained lawn. Cave opening on hillside above spring run, with spring cascading downhill. Habitat conditions quite different from Owen Spring Branch and Martin Spring. Substrates dominated by sand/silt, with a few small patches of cobble; some root mats and woody debris. Several Pycnopsyche cases observed on wood and roots. Ultraviolet light trap collection (bucket trap) made on October 11, 2011: Molanna tryphena Betten, Pycnopsyche luculenta (Betten), Ironoquia punctatissima (Walker), Hydroptila gunda Milne, Hydroptila armata Ross, and Oecetis inconspicua (Walker). Photographs: A, cave opening; B, spring run (downstream view).
Dancing Fern Cave/Spring, Marion County

Site Number: 4
Location: off TN 28, approx. 1 mi (1.6 km) N of Sequatchie
Coordinates: 35.13292 / -85.59045

Property Owner: Dennis Elliott

Field Notes: Site forested, with good canopy cover (approaching 100%) — most intact riparian zone of any site in Sequatchie Valley; good water clarity, with almost no siltation/sedimentation; coal fines present. In September 2010: Temp: 14.5°C; Dissolved Oxygen: 9.09 mg/L; pH: 8.03 S.U.; Conductivity: 276 μS/cm. Good potential as reintroduction site. Immatures observed: Pycnopsyche, Glossosoma, Goera. Pleurocerid snails (abundant). Ultraviolet light trap collection (bucket trap) made on September 15, 2010 — total of 10 spp: Molanna musetta Betten, Triaenodes marginatus Sibley, Mystacides sepulchralis (Walker), Goera sp., Oecetis inconspicua (Walker), Micrasema scotti Ross, Psychomyia flavida Hagen, Pycnopsyche antica (Walker), Hydroptila decia Etnier & Way, and Hydroptila sandersoni Mathis & Bowles (new state record).
Tate Cave Spring, Marion County
Site Number: 5
Location: off Ladds Cove Road, approx. 1.2 mi (1.9 km) NW of Martin Spring
Coordinates: 35.17683 / -85.80733
Property Owner: n/a
Field Notes: Site modified, with most of flow directed to pond adjacent to cave opening. Little flow coming from cave opening, much less than other sites. Site completely open, no canopy cover. No caddisflies observed. Photographs: A, cave opening with pond; B, pond overflow below cave opening.
Bible Spring, Marion County
Site Number: 6
Location: off Battle Creek Road, approx. 2.2 mi (3.5 km) NW of Smithtown
Coordinates: 35.11242 / -85.74690
Property Owner: n/a
Field Notes: Spring emerges from cave and forms large pool/pond. Boulders at cave opening, otherwise silt substrates with abundant aquatic vegetation (max. depth – 0.8 m). Sunfish observed in pond. Temp: 14.0°C. Photographs: A, cave opening; B, pond below cave opening.
Sherwood Spring, Franklin County

Site Number: 7

Location: off Sherwood Road (TN 56), approx. 10 mi (16.1 km) SW of Martin Spring.

Coordinates: 35.06525 / -85.91620

Property Owner: n/a

Field Notes: Spring emerges from rock cliff line, forming small pool (0.25 m maximum depth). Water clear. Substrate composed of gravel/sand with scattered cobble. Abundant water cress and other emergent plants. Larval cases of *Pycnopsyche* and *Goera* observed on cobble.
Sweden Cove Spring, Marion County

Site Number: 8

Location: off Sweetens Cove Road, approx. 6.4 mi (10.3 km) WNW of Kimball

Coordinates: 35.07625 / -85.79543

Property Owner: n/a

Field Notes: Spring located in roadside park, emerging from rock cliff line and forming spring run with abundant water cress. Little or no sedimentation; no coal fines observed. Riparian zone generally devoid of natural vegetation due to park setting. In September 2010 – Temp: 13.6°C; Dissolved Oxygen: 5.66 mg/L; pH: 7.58 S.U.; Conductivity: 319 µS/cm. Photographs: A, spring emergence; B and C, spring run.
Druin Spring, Marion County

Site Number: 11

Location: off Summertown Road and US 64, approx. 1.2 mi (1.9 km) W of Jasper.

Coordinates: 35.06845 / -85.64521

Property Owner: Robert McVey

Field Notes: Spring surrounded by cow pasture but protected by fencing that surrounds spring source and run. Spring used as water source, as evidenced by concrete spring house and water lines. Substrate composed of cobble/gravel/sand with moderate siltation. Canopy cover 75-100%. Larval cases of Glossosoma and Psilotreta observed on cobble.
Blue Spring, Marion County
Site Number: 12
Location: off Cumberland Avenue, approx. 0.6 mi (1 km) NW of Jasper
Coordinates: 35.08913 / -85.63351
Property Owner: City of Jasper
Field Notes: Spring emerges as long canal-like pool at base of cliff in large depression. Emerges again to the west, with no canopy cover and excessive filamentous algae. No flow observed at either location. At emergence, substrate very rocky, with boulder and cobble substrates and some silt (max. depth – 1 m); western emergence with silt/sand substrates. Sunfish abundant in forested portion (*Lepomis* and *Micropterus*). Temp: 18.6°C; Dissolved Oxygen: 3.14 mg/L; pH: 7.81 S.U.; Conductivity: 260 μS/cm. Photographs on following page.
Blue Spring, Marion County (Site 12)
Photographs: A-C, cave opening, showing canal-like pool and forested canopy; D, second area of emergence, showing open canopy and algae.
Pryor Cave Spring, Marion County
Site Number: 13
Location: off Valley View Highway, approx. 1.4 mi (2.3 km) N of Jasper.
Coordinates: 35.09493 / -85.62090
Property Owner: Joe Hogan
Field Notes: Spring modified into an impoundment, with silt/sand substrate, emergent vegetation, and algae.
Daniel Cave, Marion County
Site Number: 18
Location: off Old Dunlap Road, approx. 4.5 mi (7.2 km) NNE of Whitwell.
Coordinates: 35.26083 / -85.48442
Property Owner: n/a
Field Notes: Cave opening closed by collapse; spring run with excessive sand/silt deposits and coal fines. Spring source forested; no caddisflies observed.
Cedar Cave Spring, Marion County
Site Number: 19
Location: off Old Dunlap Road, approx. 4.9 mi (7.9 km) NNE of Whitwell.
Coordinates: 35.26487 / -85.48018
Property Owner: n/a
Field Notes: Spring emerges from rock cliff and bordered by man-made rock wall and brick structure (pumphouse?). No flow visible; canopy cover 100%. Substrate consisting of large cobble/boulder. No caddisflies observed; pleurocerid snails abundant. On September 15, 2010: Temp: 13.5°C; Dissolved Oxygen: 9.32 mg/L; pH: 7.79 S.U.; Conductivity: 191 μS/cm.
Boynton Spring, Sequatchie County
Site Number: 21
Location: off Boynton Road and Old Whitwell Highway, approx. 5 mi (8 km) SW of Dunlap.
Coordinates: 35.31325 / -85.44377
Property Owner: Boynton family
Field Notes: Spring emerges from hillside beneath several large boulders. Used as water supply by Boynton family. Man-made dam downstream creates pool at spring source. Substrates primarily cobble/gravel with some coal fines. Few caddisflies observed (Pycnopsyche and Goera); pleurocerid snails abundant. On September 15, 2010: Temp: 13.8°C; Dissolved Oxygen: 9.21 mg/L; pH: 8.02 S.U.; Conductivity: 243 µS/cm.
Stone Cave, Sequatchie County
Site Number: 23
Location: off Old Whitwell Highway, approx. 3.4 mi (5.5 km) SE of Dunlap.
Coordinates: 35.33513/ -85.42944
Property Owner: Walling family
Field Notes: Spring emerges from large cave opening and enters man-made impoundment (according to landowner, pond over 30 years old). Substrates primarily silt, with recent deposition. According to landowner, pond siltation increased significantly with onset of new development on the ridge (plateau) above. No caddisflies observed. On September 15, 2010: Temp: 14.0°C; Dissolved Oxygen: 7.89 mg/L; pH: 7.95 S.U.; Conductivity: 338.9 µS/cm. Pond outflow much warmer – 21.0°C.
Selby Spring, Jackson County, Alabama
Site Number: 29
Location: off County Route 145 and AL 79, approx. 5 mi (8 km) NW of Scottsboro.
Coordinates: 34.74117 / -86.14130
Property Owner: n/a
Field Notes: Spring emerges from large cave opening and drains to unnamed tributary of Dry Creek. Good habitat; substrates primarily gravel with some cobble and woody debris. Site with about 100% canopy cover. Caddisfly genera observed: Agapetus, Ironoquia, Pycnopsyche.