AQUATIC INVERTEBRATE FAUNA SURVEY AT HUECO SPRINGS CREEK, COMAL COUNTY, TEXAS

Prepared for Paul Price Associates, Inc.

By Jean Krejca and Peter Sprouse
Zara Environmental LLC
USFWS endangered species permit no. TE028625-0
708 Elliott Ranch Road, Buda, Texas 78610
www.zaraenvironmental.com
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Abstract

Zara Environmental conducted field surveys for the endangered cave-adapted amphipod *Stygobromus pecki* (Peck's Cave Amphipod), Comal Springs Dryopid Beetle (*Stygoparnus comalensis*), and Comal Springs Riffle Beetle (*Heterelmis comalensis)* along the stream emanating from Hueco Springs, Comal County, Texas, under contract for Paul Price Associates, Inc. Jean Krejca and Peter Sprouse conducted this work on 22, 27, and 28 August 2003, with assistance during part of that time from Scott Harden and Andrew Clamann. Sampling techniques were chosen to increase the likelihood of finding cave or groundwater adapted organisms. Although the sampling techniques were successful enough to find other subterranean species in the interstitial stream habitat, a single endangered amphipod was found only at the spring orifice where it has been collected in the past. None of the ten sampling stations downstream of the springhead contained *Stygobromus pecki*, *Stygoparnus comalensis*, or (*Heterelmis comalensis*. These results are congruent with past studies that have found only the amphipod at the spring head.

Background

The endangered amphipod, *Stygobromus pecki*, occurs at only two sites, Comal Springs and Hueco Springs. The species is considered exclusively subterranean due to its morphology (lacking eyes and pigment) and localities where it is known from (springheads and a well). Most of the specimens that have ever been collected are from Comal Springs, but a single specimen was collected from Hueco Springs by Barr (1993). A more recent study (2003, unpublished) yielded multiple individuals from Hueco Springs and many others from Comal Springs (Randy Gibson and Scott Harden, personal communication, September 2003). All of the collections at Hueco Springs were taken from drift nets placed over spring orifices (and a well-house pipe) located at the upstream most points along the spring run. The recent collections of this species from Comal Springs were also all from upwellings in course gravels or spring heads. Other habitats away from these upwellings were sampled and found to have *Stygobromus russelli*,

which appears to be more tolerant of alluvial environments away from spring heads (Randy Gibson and Scott Harden, personal communication, September 2003). According to Arsuffi (1993), at Comal Springs this species has been taken from the spring run outside the orifices even though this is not the primary habitat. It should be understood, however, that the spring runs downstream of the spring heads at Comal have numerous tiny openings and upwellings, so therefore the spring run habitat is not actually a downstream interstitial zone, it actually consists of many small springs. Barr (1993) also found *Stygobromus pecki*, near known spring orifices or in spring runs that had many smaller outlets within it. For example, her sites in Comal 2 had outlets evident for about 36.6 m downstream of the primary outlet.

The endangered Comal Springs Dryopid Beetle (*Stygoparnus comalensis*) is also a subterranean species, and known only from Comal Springs and Fern Bank Springs (the latter in Hays County). Collections of this species have been made during the months of April through August (USFWS 1997), suggesting that the time we were sampling was appropriate. Past studies by Barr (1993) using drift nets, kick netting and hand searching have attempted to find this species at Hueco Springs to no avail.

The endangered Comal Springs Riffle Beetle (*Heterelmis comalensis)* is known only from Comal Springs and San Marcos Springs (the latter of which is in Hays County). This species lives in the gravel substrate and have high population densities between February and April (Bosse et al. 1988), suggesting that our sampling time was not ideal. The species was not found at Hueco Springs by Barr (1993).

The objective of this study is to determine precisely how far along the spring run, or what distance from the spring orifice itself, at Hueco Springs the endangered amphipod, *Stygobromus pecki*, may occur. Another objective is to sample the stream for the two endangered beetles, the Comal Springs Dryopid Beetle (*Stygoparnus comalensis*), and Comal Springs Riffle Beetle (*Heterelmis comalensis)*. The beetles are not known to occur at this site, but since they occur at nearby springs (one of which also harbors the amphipod), we felt it important to also sample for beetles. Prior studies with the objective of finding the amphipods have focused collecting efforts in the most ideal habitat, the spring orifice. In order to sample less ideal habitat downstream of the spring orifices, a variety of techniques beyond those employed in past studies were used. Primarily these additional techniques involved sampling the gravel interstitial zone in the stream bed that may have tiny openings to more extensive subterranean voids, or may simply provide additional habitat.

Materials and Methods

Ten sampling sites were set up at 50-meter intervals along the stream below the outlet of Hueco Springs on the west side of River Road (also called Hueco I or

Hueco A by Barr 1993) beginning at the site of a breached concrete dam just west of River Road. The intervals were marked and determined using a hand-held GPS unit, and are therefore approximate. At nine out of ten of the sampling sites the first three methods were used to find amphipods, and at four other selected sites the two other methods were used:

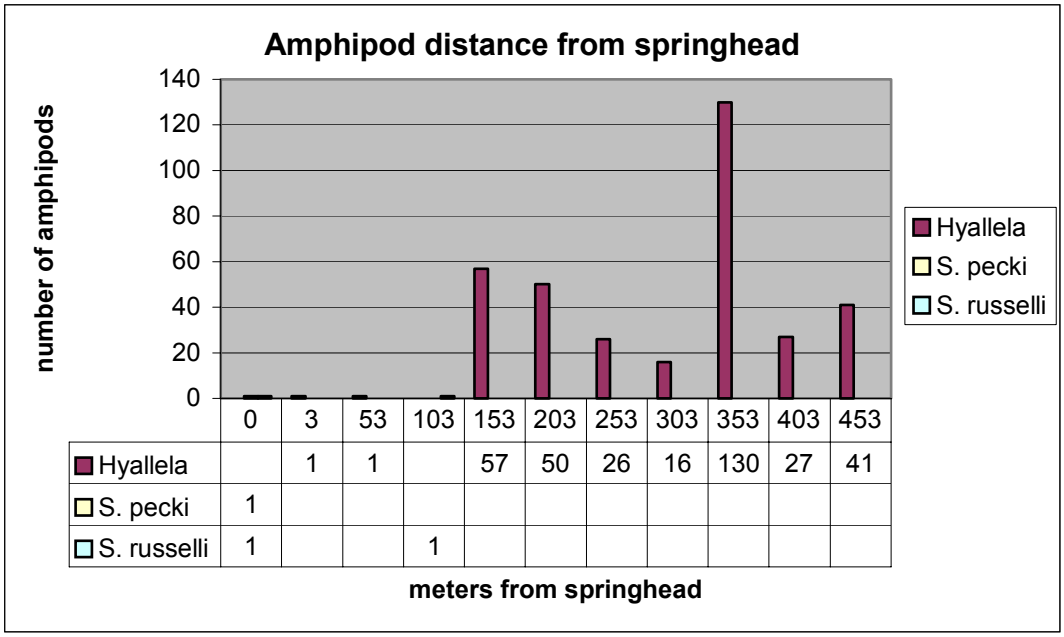
1. A transect was made across the stream at each site which involved one person digging up the streambed to a depth of up to 10 cm for approximately 5 m of stream length with a shovel and substrate kicking while the second person held open a drift net to catch detritus and organisms that were released from the stream gravels (see Figure 1). This procedure was repeated 5-10 times to cross the width of the stream run. The net contents were carefully inspected for fauna. Sampling always progressed upstream (eg. First sample HS1, then HS2, etc.) throughout the day to avoid contamination of downstream fauna from organisms that were accidentally released at an upstream location.
2. At each site gravels were shoveled from the stream bottom into a five-gallon pickle bucket while the drift net was held open immediately downstream. To inspect those gravels for fauna, first the larger gravels were washed and removed with a series of ½ inch and ¼ inch mesh screens. Then the remaining pebbles and wash water were inspected in a white enamel pan and run through a 500-micron mesh net (see Figure 2).
3. Baited inverted bottle traps were made and placed at each site and later retrieved and inspected for fauna (see Figure 3). To make the traps, the top part of a bottle was cut off and inverted, creating a cone into which fauna could enter seeking bait. These were weighted with gravel and placed in calm areas of the stream at each transect.
4. At three selected sites, pits (up to 0.6 m deep, 1 m wide) were dug into the stream gravels to sample the difficult-to-reach deeper interstitial habitats (see Figure 4). In these pits fine mesh dipnets were used to strain the subsurface water, the surface of the water was also carefully monitored for displaced fauna that was caught on the surface tension, and the deepest gravels were shoveled into a five gallon bucket for careful inspection. Baited bottle traps were also buried in two out of three of these pits and retrieved and inspected 24 hours later (see Figures 5, 6 and 7).
5. At the spring orifice, a 500-micron mesh drift net was anchored to the bottom of the streambed and left in place for 3 hours in order to verify that our team could find and identify the endangered species from where it is already known to exist (see Figure 8).

Field identifications of all amphipods were made. The common epigean (surface-dwelling) spring species, *Hyallela azteca* were preserved in 70% ethanol (Figure 9). Any cave-dwelling species of the genus *Stygobromus* were carefully and immediately carried in stream water to the truck where they were identified live by Jean Krejca and Scott Harden using a dissecting microscope and identification keys (Holsinger 1967), photographed, then released in compliance

with permit protocols (Figures 10 and 11). Identifications of insects, including aquatic beetles, were made by Andrew Clamann.

Results

Transect sampling resulted in the collection of large numbers of the common stream amphipod *Hyallela azteca*. Digging in streambed gravels turned up one specimen of the cave-adapted amphipod *Stygobromus russelli* at transect HS8. Only one specimen of *Stygobromus pecki* was encountered, from a drift net anchored over the spring aperture. Table 1 summarizes the amphipod distribution from the springhead to the site of the old dam, 453 m downstream. Table 2 details the sampling effort and other taxa that were collected at each site, and appendix A includes images of each transect. Figure 12 shows a map of the transect localities.



	0	3	53	103	153	203	253	303	353	403	453
Hyallela		1	1		57	50	26	16	130	27	41
S. pecki	1										
S. russelli	1			1							

Table 1. Species of amphipods (including *Hyallela azteca, Stygobromus pecki* and *Stygobromus russelli*) found at various distances from the springhead. The site at 0 m distance is the spring orifice itself, the site 3 m downstream is site HS10, at 53 m downstream is site HS9, etc.

Site HS1: This is the site of the old dam. It is about 7 m wide here, up to 0.5 m deep, and a stream riffle. On 22 August 2003, Jean Krejca, Peter Sprouse and Andrew Clamann sampled for a total of 4.25 person hours. Five *Hyallela azteca* amphipods were found in four gallons of shoveled gravel, the remaining 34 were from the drift net and hand picking. One beetle (Elmidae: *Microcylloepus*), 1 true bug (Naucoridae: *Ambrysus*) were also found at this site. The baited inverse bottle trap was set for 5 days, and contained two *Hyallela azteca* amphipods.

Site HS2: It is about 5 m wide here, up to 0.5 m deep, and a stream riffle. On 22 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 4 person hours. No *Hyallela azteca* amphipods were found in 2.5 gallons of shoveled gravel, the remaining 27 were from the drift net. Two beetles (Elmidae: *Microcylloepus*) were also collected from this site. The baited inverse bottle trap was set for 5 days, and found to contain no amphipods.

Site HS3: It is about 10 m wide here, a stream pool, and up to 1.5 m deep. On 22 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 3.5 person hours. Ten *Hyallela azteca* amphipods were found in 2.5 gallons of shoveled gravel and stream sediments, the remaining estimated 120 were from the drift net. This site had a lot of aquatic vegetation that contained large numbers of *Hyallela azteca* amphipods. The baited inverse bottle trap was set for 5 days, and found to contain no amphipods.

Site HS4: It is about 6 m wide here, up to 1 m deep, and a stream pool. On 28 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 3 person hours. A single *Hyallela azteca* amphipod was found in 3.3 gallons of shoveled gravel, the remaining 15 were from the drift net. One snail was also collected from this site. The baited inverse bottle trap was set for 7 hours, and contained no fauna.

Site HS5: It is about 7 m wide here, up to 0.5 m deep, and a stream riffle. On 28 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 2 person hours. No gravel was shoveled because the habitat was too full of clay, therefore lacking the interstitial spaces needed for amphipods to exist in the subsurface. Twenty-one *Hyallela azteca* amphipods were collected from the drift net and five others were counted but not collected. One insect larva was also collected from this site. The baited inverse bottle trap was set for 7 hours, and contained no fauna.

Site HS6: It is about 10 m wide here, up to 1 m deep, and a combination of stream pool and stream riffle. On 28 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 3 person hours. Two *Hyallela azteca* amphipods were found in 1.3 gallons of shoveled gravel, the remaining 37 were from the drift net and 11 more were counted but not collected. Three beetles (2 Elmidae: *Microcylloepus*, 1 Elmid exuvia) and 1 mayfly larva (Tricorythidae: *Tricorythodes*) were also collected from this site. The baited inverse bottle trap was set for 7 hours, and contained no fauna.

Site HS7: It is about 11 m wide here, up to 0.75 m deep, and a stream pool. On 28 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 2 person hours. Ten *Hyallela azteca* amphipods were found in 0.6 gallons of shoveled gravel, the remaining 47 were from the drift net. There was very little gravel available at this site for shoveling. Three insect larvae and one ant were also

collected at this site. The baited inverse bottle trap was set for 7 hours, and contained no fauna.

Site HS8: It is about 14 m wide here, up to 1.2 m deep, and contains both stream riffle and stream pool habitats. Because of our heavy sampling in the pit dug within this transect (Pit 2) and in the nearby Pit 1, we chose not to perform additional gravel digging or kick netting. The baited inverse bottle trap was set on 27 August 2003 for 1 day and contained three terrestrial surface isopods and a water penny.

Site HS9: It is about 7 m wide here, up to 0.5 m deep, and a stream raceway. On 27 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 3 person hours. A single *Hyallela azteca* amphipod was found in 1.6 gallons of shoveled gravel and none were found from the drift net. This site is very swift, had little loose gravel and vegetation and therefore very few amphipods. One terrestrial isopod, 7 beetles (Elmidae: *Microcylloepus*) and 1 mayfly larva (Baetidae: *Plauditus*) were also collected at this site. The baited inverse bottle trap was set for 1 day, and contained 8 flatworms.

Site HS10: It is about 6 m wide here, up to 0.5 m deep, and a stream raceway. On 27 August 2003, Jean Krejca and Peter Sprouse sampled for a total of 3 person hours. No amphipods were found in 1.6 gallons of shoveled gravel, though a single *Hyallela azteca* amphipod was found in the drift net along with a troglobitic isopod, probably *Cirolanides texensis*. One beetle (Dytiscidae: not *Haideoporus*) and 2 snails were also collected at this site. The baited inverse bottle trap was set for 1 day and contained 12 flatworms.

Spring head: This is the central spring orifice, located only 3 m upstream of site HS10. It is about 6 m wide here, up to 0.75 m deep, and a stream raceway. While this site is already known to contain the endangered *Stygobromus pecki*, we felt it was necessary to sample this site simply to demonstrate that our methodology was sufficient to capture and identify the endangered species should we find it at a downstream site. On 27 August 2003, Jean Krejca, Peter Sprouse, and Scott Harden anchored a drift net to the central spring orifice and left this net on for three hours. No kick netting, gravel digging or bottle trapping was done in order to avoid unnecessarily damaging the endangered species or its known habitat. The net was found to contain about 5 cave adapted *Lirceolus* isopods, one cave-adapted *Stygobromus russelli* amphipod, an unidentified snail, and one cave-adapted *Stygobromus pecki* amphipod. The two *Stygobromus* amphipods were identified live, photographed, and the *Stygobromus pecki* was released live at the spot were it was found.

Pit 1: This site is located between HS8 and HS9 in a dry gravel bank that sits in approximately the middle of the streambed. The pit was dug about 0.5 m from the edge of the stream on 27 August 2003. Jean Krejca, Peter Sprouse and Scott Harden sampled here for a total of 3 person hours. Four springtails, 5

beetles, 1 insect, 22 snails and three possible symphylans were found in 1.6 gallons of gravel shoveled from the bottom of the pit. A baited bottle trap was set for 24 hours and found to contain no fauna.

Pit 2: This site is located within transect HS8 in a shallow (0.1 m) submerged gravel bank that is surrounded by deeper water. The pit was dug on 27 August 2003. Jean Krejca, Peter Sprouse and Scott Harden sampled here for a total of 2.25 person hours. A single stygobitic amphipod was found in 2.5 gallons of gravel that were sorted from the bottom of the pit, and no amphipods were found in the drift net that was placed downstream during digging. An additional 2 person hours were spent examining and photographing the single specimen of *Stygobromus russelli* that was found at this site. A baited bottle trap was set for 24 hours and contained about 20 *Physa* snails, 5 aquatic oligochaetes, 15 flatworms and one water penny.

Pit 3: This site is located in transect HS2 in a dry gravel bank that sits on the river right side of the streambed. The pit was dug about 0.5 m from the edge of the stream on 28 August 2003. Jean Krejca and Peter Sprouse sampled here for a total of 1.5 person hours. No fauna was found in 5 gallons of gravel shoveled from the bottom of the pit. One eyeless springtail was collected from the surface tension of the water, and about four others were seen but not collected. There was no baited bottle trap set at this site.

Site	person hours	width (m)	Max depth (m)	stream flow	gravel sorted (gallons)	*Hyallela azteca*	*Stygobromus russelli*	*Stygobromus pecki*	*Cirolanides texensis*	*Lirceolus*	Beetles and Other Collections
HS1, dam	4.25	7	0.5	riffle	4	41					1 beetle (Elmidae: *Microcylloepus*), 1 true bug (Naucoridae: *Ambrysus*)
HS2	4	5	0.5	riffle	2.5	27					2 beetles(Elmidae: *Microcylloepus*)
Pit 3 (at HS2)	1.5	n/a	n/a	n/a	5						1 eyeless springtail
HS3	3.5	10	1.5	pool	2.5	130					
HS4	3	6	1	pool	3.3	16					1 snail
HS5	2	7	0.5	riffle	0	26					1 insect larva
HS6	3	10	1	pool/riffle	1.3	50					3 beetles (2 Elmidae: *Microcylloepus*, 1 Elmid exuvia), 1 mayfly larva (Tricorythidae: *Tricorythodes*)
HS7	2	11	0.75	pool	0.6	57					3 insect larvae
HS8	0	14	1.2	pool/riffle	0						3 surface isopods, 1 water penny
Pit 2 (at HS8)	2.25	n/a	n/a	n/a	2.5		1				
Pit 1 (between HS8 and HS9)	3	n/a	n/a	n/a	1.6						Four eyeless springtails, 5 beetles, 1 insect, 22 snails and three possible symphylans
HS9	3	7	0.5	raceway	1.6	1					1 terrestrial isopod, 7 beetles (Elmidae: *Microcylloepus*), 1 mayfly larva (Baetidae: *Plauditus*)
HS10	3	6	0.5	raceway	1.6	1			1		1 beetle (Dytiscidae: not *Haideoporus*), 2 snails
Spring head	3*	6**	0.75	raceway	0		1	1		5	
* = unmanned net was left over spring for 3 hours											
** = entire width was not sampled at spring orifice, see results for details											

Table 2. Details of sampling effort and findings at each site. The sites are 50 m apart, with site HS1 is 453 m downstream of the springhead at the old dam, site HS2 is 403 m downstream of the springhead, etc.

Conclusions

Organisms showing subterranean adaptations were found at several localities, demonstrating that our techniques were sufficiently thorough for detection of cave fauna. Examples include eyeless springtails at Pit 3 and Pit 1, eyeless isopods, genera *Lirceolus* and *Cirolanides*, at the springhead and at HS10, and eyeless amphipods, genus *Stygobromus*, at the springhead and at Pit 2. The collection of *Stygobromus russelli* at a site downstream of the springhead demonstrates that this species is capable of dwelling in alluvial habitats, a thesis supported by recent collecting efforts in Comal Springs (Randy Gibson and Scott Harden, personal communication, September 2003). However, no individuals of the endangered amphipod, *Stygobromus pecki*, were found beyond the spring orifice itself where they are known to occur. There is also no evidence in the literature that there is seasonality to the range of this species, though certainly the biology of the species is not very well studied. The historic collecting information summarized in the background section, combined with recent studies and this study, indicate that the Peck's Cave Amphipod is unlikely to occur beyond the springhead, or that the population levels beyond the springhead are so low that the extensive sampling effort in this study was inadequate to detect them.

Although multiple beetles of the family Elmidae were collected, no Comal Springs Dryopid Beetles (*Stygoparnus comalensis*) or Comal Springs Riffle Beetles (*Heterelmis comalensis)* were found. Our collection period (August) for the Comal Springs Dryopid Beetle corresponds to times when they have been found at other sites, but the collection times for the Comal Springs Riffle Beetle was not ideal, since the populations peak from February to April. The historic collecting information summarized in the background section combined with our sampling effort indicates that the range of these two species does not extend to this site, or that their populations at this time of year are too low to detect them.

Personnel

Jean Krejca: The principal biologist for this project and subterranean species specialist. Jean has a Bachelor's degree in Zoology, and is a Ph.D. Candidate in Evolution, Ecology and Biology at the University of Texas. Her dissertation work is on cave adapted aquatic fauna, biogeography and hydrology of Texas and North Mexico. Since 1991 she has worked as a cave biologist and her experience in that area spans across the United States (California, Texas, Nevada, Illinois, Missouri, Indiana, Tennessee, North and South Carolina) as well as Mexico, Belize, Thailand and Malaysia. Texas cave biology experience started in 1997 and includes extensive collections of aquatic cave fauna for research, monitoring for endangered species, and working as a Karst Invertebrate Specialist for the U.S. Fish and Wildlife Service. In addition she has

been involved with a variety of public outreach efforts such as public talks, field trips, and cave biology photography.

Peter Sprouse: Peter has been exploring and studying caves since 1970, having led the exploration of Sistema Purificación in Mexico, one of the longest and deepest caves in the world. He began collecting cave fauna for study by taxonomists in 1977, and has six species named in his honor. He attended the University of Texas at Austin in 1974 as a geology major, and since 1991 he has worked professionally in the fields of cave biology, management, and cartography. He holds US Fish and Wildlife Service endangered species permit number TE014168-0. He currently manages over 50 caves in central Texas, conducting fire ant control and building cave gates. His experience in conducting karst surveys for invertebrate cave fauna habitat is very extensive. The National Speleological Society has given him the prestigious Lew Bicking Award, named him an NSS Fellow, and he was the medal winner in the 1980 and 1986 NSS Cartographic Salons.

Scott Harden: A consulting biologist for this project, with a specialty in crustacean taxonomy. Scott received his B.A. in Geology at the University of Texas at Austin in 1981. Involved in organized caving (survey, exploration) since 1970, he began working with subterranean fauna in 1981. In the mid 1980's he did some pioneer work sampling non-cave stream sediments to discover new localities and new species of subterranean aquatic fauna in central Texas, and thus has a number of these species named in his honor. Recent work (2001 to present) includes taxonomic assistance with a Ph.D. thesis and contracting for a USFWS and Texas State University project, collecting and identifying fauna from springs.

Andrew Clamann: Andrew is a biologist with Paul Price Associates, Inc. (PPAI). His specialty is in benthic macroinvertebrate identification. He has conducted numerous biological evaluations as well as a variety of environmental assessments adhering to various local, state, and federal guidance. Andrew has six years of experience identifying benthic macroinvertebrates including over 25 rapid bioassessment and receiving water assessment samples from across Texas and phytoplankton samples from south central Texas and Lavaca Bay. Prior to PPAI, Andrew's laboratory experience includes two years of identification, curation and establishment of a database for the Odonata collection for the University of Texas Brackenridge Field Laboratory under Dr. C. Riley Nelson.

Figures

Figure 1. Sampling with drift net at transect HS5. Bottom gravels were churned up using a shovel, and resulting debris was collected in the drift net to sample for invertebrate fauna.

Figure 2. A ½ inch mesh screen was placed on top of a ¼ inch mesh screen for washing and inspecting gravel from stream bottoms and pits.

Figure 3. Inverted bottle trap. Top part of a bottle was cut off and inverted, creating a cone into which fauna could enter seeking bait. These were weighted with gravel and placed in calm areas of the stream at each transect.

Figure 4. Sampling at Pit 1 in stream bank gravels near HS8. Gravel shoveled from the submerged bottom of the pit was washed using screens before inspection.

Figure 5. Bottle trap, pre-baiting, prepared for burial in streamside gravels.

Figure 6. Bottle trap submerged in bottom of pit dug in stream bank gravels.

Figure 7. Bottle trap re-covered with stream bank gravel to original grade, to be recovered later.

Figure 8. Drift net was anchored over the highest volume rising in Hueco Springs and left in place to collect fauna.

Figure 9. Common *Hyallela azteca* found at nearly all of the stream transects.

Figure 10. *Stygobromus pecki* showing lack of distal posterior lobe at the basis of pereiopod 7 (only pereiopod six is clearly visible in this photograph due to the movement of the animal being photographed, but the morphology of these two legs was the same). *Stygobromus* amphipods were identified live in the field using dissecting scopes and keys.

Figure 11. Field identification and photography of amphipods.

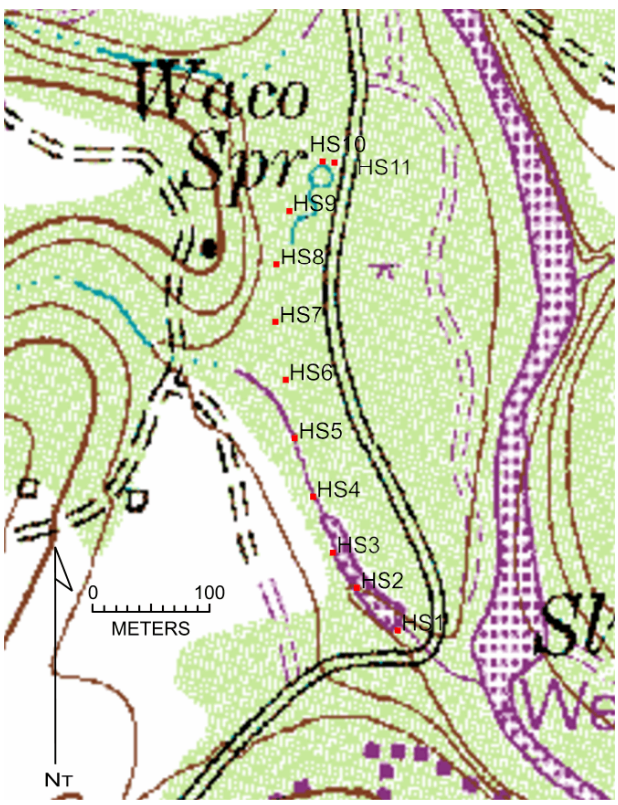


Figure 12. Location of sampling sites

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Appendix A Photos of all sampling localities.

Transect HS1 is the site of the destroyed dam, and is the lowermost site sampled. No sampling was done east of River Road.

Transect HS2, digging Pit 3 in stream bank gravel.

Transect HS3

Transect HS4

Transect HS5

Transect HS6

Transect HS7

Transect HS8, buried bottle trap.

Transect HS9

Transect HS10