Cover Page

Title: The Possibility of the Occurrence of Hay's and Kenk's Spring Amphipods Near the Purple Line Metro Route and Its Implications

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I certify that this is solely my statement. Affiliations are used for identification purposes only.

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I. Credentials and expertise

A graduate of Grinnell College (B.A.) and Yale University (Ph.D.), I have devoted most of my scientific career to the study of the biology of subterranean animals, especially in caves. I have written four books and over 100 scientific articles on the subject of subterranean life. My first book, *Cave Life,* published in 1982 by Harvard University Press, has been cited nearly 500 times in the scientific literature. My research over the past ten years has focused on shallow subterranean habitats, such as the seeps where Hay's and Kenk's spring amphipods are found. This work is summarized in the forthcoming book, written with Tanja Pipan, *Shallow Subterranean Habitats: Ecology, Evolution, and Conservation,* to be published by Oxford University Press in June, 2014. Since 2002, under a variety of contracts and cooperative agreements, I have been working on *Stygobromus* amphipods in national parks of the National Capitol region, including Rock Creek Park, George Washington Memorial Parkway, and others. This work culminated in the publication in *Northeastern Naturalist Monographs* in 2012 of "The Fauna of Seepage Springs and other Shallow Subterranean Habitats in the Mid-Atlantic Piedmont and Coastal Plain". I am a Fellow of the American Association for the Advancement of Science and a Visiting Fellow of the Karst Research Institute of the Slovenian Academy of Arts and Sciences, and am currently Professor of Environmental Science at American University.

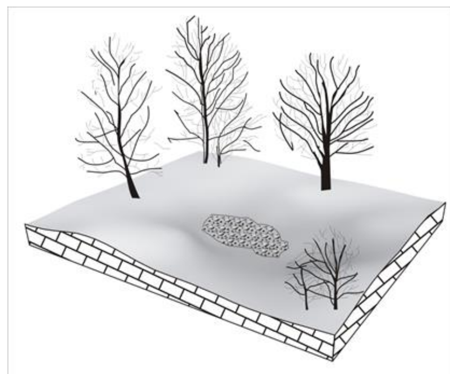
II. Introduction to the biology of *Stygobromus* amphipods in the Rock Creek drainage

There are a number of places along Rock Creek and its tributaries that harbor a remarkable and unique fauna of blind, eyeless invertebrates, especially *Stygobromus* amphipods. Ranging in size at maturity from 1/8 inch to ¾ inch, five different depigmented and eyeless *Stygobromus* species have been found in the Rock Creek basin [1]. Two of these species (*S. pizzinii* and *S. tenuis*) are relatively common both in terms of number of sites and geographic range, one is uncommon (*S. sextarius*), and two are rare, being limited to the Rock Creek basin (*S. hayi* and *S. kenki*). Aside from their size differences, these species (and the many *Stygobromus* species found in caves), share a common morphology of slender shape and elongated appendages [2]. Their lack of eyes makes them extremely vulnerable to predation by both invertebrates (such as dragonfly nymphs) and vertebrates (such as fish and salamanders) in any habitat with light. Their lack of pigment makes them vulnerable to *uv* light radiation damage in any habitat with light. On the other hand, these *Stygobromus* species, with their elongated appendages that increase ability to sense the environment around them, are clearly adapted to life in darkness, that is, life in groundwater.

While these species have no direct economic value, they are indicators of the health of the Rock Creek ecosystem. As elaborated below, their habitat, the shallow subsurface, makes them indicators of the overall health of the ecosystem, especially the forest riparian community. Successful management of these species, precariously positioned to suffer the impacts of many environmental insults to the Rock Creek basin, is a bellweather of the health of the ecosystem as a whole, including water quality.

In the Rock Creek drainage basin, there are four more or less distinct groundwater habitats, habitats that lack light, that could be habitat for *Stygobromus.*

The shallowest of these groundwater habitats, defining groundwater in the sense of water under the surface not exposed to light, can occur less than a foot beneath the ground. Meštrov [3] applied the term "hypotelminorheic" to these shallow groundwater habitats that are vertically isolated from the water table. They are miniature drainage basins, typically less than 100 yards in linear extent that are fed by subsurface water and underlain by either by clay or by some other impermeable material [4,5]. They create persistent wet spots where they emerge to the surface in seepage springs, which is where most of the amphipods are collected (by hand sampling). Few *Stygobromus*, including Hay's and Kenk's spring amphipods have been collected outside these habitats in the Rock Creek basin. The sketch and photograph below give a sense of the hypotelminorheic and seepage springs.



Left panel—typical seepage spring in Scotts Run Regional Park, Fairfax County. Right panel—sketch of seepage spring, showing its connection to nearby topography.

The second groundwater habitat in the Rock Creek basin is the underflow of tributary streams and Rock Creek itself. This aphotic habitat is called the hyporheic, and in many places harbors a rich fauna with numerous eyeless, depigmented, but very small sized species [6]. It is relatively easily sampled used a modified hand pump attached to a pipe with holes at the end that is driven into the stream bed. Rock Creek and tributaries were extensively sampled using the "Bou-Rouch" pump but almost no *Stygobromus* were found. There were less than one *Stygobromus* in 500 liters of hyporheic water [1], but one Hay's amphipod was collected in Rock Creek, near Rapid Bridge [1]. Based on this study, it seems likely that the hyporheic is not the primary habitat for either Hay's or Kenk's spring amphipods, but it may be a dispersal path. Except for the smallest *Stygobromus—S. sextarius,* all the other *Stygobromus* are much larger than the typical hyporheic species, most of which survive in the narrow spaces between sand and gravel

The third type of groundwater habitats (at least hypothetically—none have been sampled) in Rock Creek are shallow aquifers at a depth of about 10 to 15 feet. Historically, there have been a number of such shallow wells in the Washington, DC area. Most have been destroyed but one that was sampled in the 1970's occured off Edsall Road in Fairfax County, and harbored blind, white invertebrates, including an undescribed species of *Stygobromus* [7, 8]. No such wells in the Rock Creek basin are known to the author.   Water seeping out of deeply incised stream banks can be found at the same depth, and in Fort

Belvoir, harbors a unique species of *Stygobromus—S. phreaticus* [8,9]. Given its rarity in the hyporheic, it seems very unlikely but possible that Hay's and Kenk's spring amphipods occur in these deeper habitats.

Finally, there are deep aquifers capable of providing water to communities. An example is the fractured aquifer that supplies water to Poolesville, Maryland. The Poolesville wells are at depths of between 285 and 700 feet. These habitats have not been sampled for amphipods, but are unlikely to harbor any of the known *Stygobromus* species.

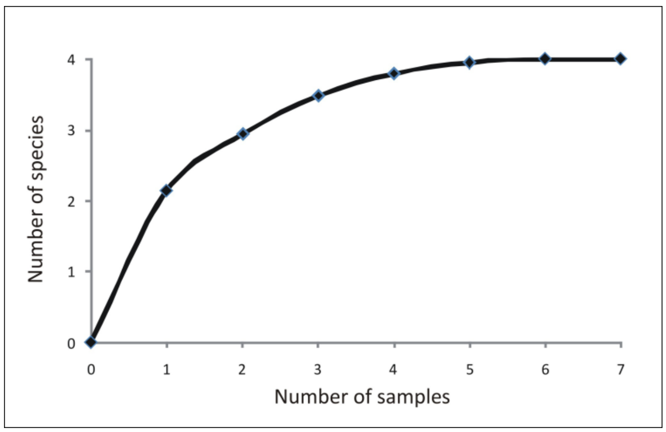
Given our present knowledge of *Stygobromus* species in the Rock Creek basin, the following generalities emerge:

1. The primary habitat of *Stygobromus* species is the very shallow "hypotelminorheic", situated directly below the leaf litter.
2. During seasons of lowered evapotranspiration (late fall to early spring), *Stygobromus* are relatively common in the exit of the water to the surface, that is, seepage springs. It seems likely that they emerge into twilight habitats to obtain food, which is in greater supply at the seepage spring than in the hypotelminorheic itself [10].
3. During periods of drought when seepage springs dry up, *Stygobromus* probably survives in the clay, which retains water [6]. Subterranean amphipods are known to burrow in clay [8].
4. The near absence of hypotelminorheic *Stygobromus* (including Hay's and Kenk's spring amphipods) from other types of groundwater suggests that dispersal and movement is likely through very shallow habitats, including leaf litter, but possibly also the hyporheic. Dispersal may occur at times of heavy surface flow, that is, sheet flow. Anecdotal evidence for dispersal through litter and very shallow sediments is the occurrence, albeit rare, of *Stygobromus tenuis,* a hypotelminorheic species, in ant mounds [8]! **As a result, threats to *Stygobromus* come more from surface disruptions and contamination rather than groundwater disruptions and contamination.**
5. Relative to *Stygobromus tenuis,* the most common *Stygobromus* in the Rock Creek basin, Hay's and Kenk's spring amphipods occur in smaller numbers of sites. This is likely the result of both competition with the larger *S. tenuis,* and the reduced dispersal and smaller ranges of smaller-sized *Stygobromus*, such as Hay's and Kenk's spring amphipods [6].

A proposed model of the habitat of Hay's and Kenk's spring amphipods is that of a series of small hypotelminorheic basins, typically 100 yards or so in linear extent and a couple of feet in depth, with water exiting at seepage springs during wetter times of the year (or times with reduced evapotranspiration). They are connected by dispersal pathways mostly likely both through the litter and possibly through the hyporheic of streams. In the language of conservation biology, *Stygobromus* species form metapopulations, with inter-connected sub-populations.

III. Ranges and potential ranges of Hay's and Kenk's spring amphipods

Range determination and determination of presence or absence of a given *Stygobromus* species is challenging because the collecting point (the seepage spring) is not the primary habitat for the species, and their appearance is seasonal and sporadic. In practice this means that repeated collecting at the same site often yields additional species records. For example, in our study of East Spring in Rock Creek Park, Kenk's spring amphipod was only found during one of five sampling periods [1]. Likewise, sampling nearby seeps also yields additional species. The graph below shows the "species accumulation curve" , created by counting the number of *Stygobromus* species in 1,2,3…seepage springs, with the particular seepage springs chosen at random (and this randomization repeated 100 times) [5].



In this example, four seepage springs are needed to adequately sample the fauna. However, Rock Creek Park and the area of Rock Creek around the proposed site of the purple line are well sampled [1, 11], the latter being extensively and intensively sampled by the Natural Heritage Program of the Maryland Department of Natural Resources. The following description of ranges is taken from the monograph of Culver, Holsinger, and Feller, published in 2011 [8].

Hay's amphipod is only known from five seepage springs in Rock Creek Park, one hyporheic site in Rock Creek (a single specimen), and a small spring at the south end of the National Zoological Park (the type locality). Kenk's spring amphipod is known from three seepage springs, also in Rock Creek Park. In addition, its range extends to the north into Montgomery County, Maryland, at Burnt Mills seepage spring near Northwest Branch and to a seepage spring on Coquelin Run, near the proposed site of the purple line. Both species likely occur in other seepage springs, but it seems likely that these will be in the Rock Creek basin. George Washington Memorial Parkway in Virginia has been extensively sampled, but no locations for either Hay's or Kenk's spring amphipod are known on the Virginia side of the river. It is probable that Kenk's spring amphipod occurs in sites in the northern part of Rock Creek Park, between the known Maryland and D.C. sites. Based on current ranges, it is more likely Kenk's spring amphipod will be found on the proposed site of the purple line, compared to Hay's spring amphipod.

It should be noted that many records for *Stygobromus tenuis* are known within the range of Hay's and Kenk's spring amphipods, and is known throughout the Rock Creek basin. In addition, the rare amphipod

Stygobromus sextarius, known from a total of six sites on both sides of the Potomac River, is found in a seepage spring near Rock Creek in Maryland, near the D.C. boundary line [12]. It may occur in other sites in the Rock Creek drainage as well.

IV. Threats to Hay's and Kenk's spring amphipods

The 1982 listing of the Hay's spring amphipod [12] cited two main threats to the Hay's Spring amphipod: (1) The increasing frequency of flooding of Rock Creek, which may remove individual amphipods and adversely affect habitat by removing leaves and sediment that form the species' spring habitat; and (2) construction activities affecting spring habitats. The five year review [13] confirmed that these threats remain and added increased recreational use of Rock Creek Park and changes in hydrology and water quality in the spring recharge areas as additional threats. The threat posed by flooding is one the species have faced for eons, but the severity of flooding has increased as a result of both climate change and by alteration of the drainage, especially the increase in impervious surfaces. The likely frequency of flooding varies, of course, with elevation of the hypotelminorheic habitat and associated seepage spring above the stream. Flooding can basically result in habitat removal. While this threat is real, I believe it is overemphasized in USFS reports [12,13], because the habitats of Hay's and Kenk's spring amphipod is riparian rather than the stream itself. The threat posed by construction activities, and to a certain extent recreational activities, are likely more important, especially those that have a direct impact on the recharge area (drainage basin) of seepage springs. Direct impacts include:

1. Compaction as a result of trail construction and use.
2. Tree removal within the recharge area, with resulting loss of habitat through reduction in the litter and hypotelminorheic zone.
3. Runoff into the recharge area of water from impermeable surfaces, which likely contains elevated levels of road salt and heavy metals as a result of tire wear [1].

The Chevy Chase Lake Sector Plan Appendix [15] lists lowering of regional water tables as a threat, but given the superficial nature of the hypotelminorheic habitat, this seems unlikely to be important. The purple line Environmental Impact Statement [16] is silent both on the potential occurrence of Hay's and Kenk's spring amphipods in the impact area and any possible threats.

Assessment of the threats to Hay's and Kenk's spring amphipods from purple line construction needs to be considered in the context of a metapopulation with patches of hypotelminorheic connected by leaf litter and the underflow of streams (hyporheic). The following activities within hypotelminorheic zones are incompatible with protection of the Hay's and Kenk's spring amphipods:

1. Track and ramp construction
2. Clearing of trees
3. Culvertization of streams
4. Construction of impermeable surfaces

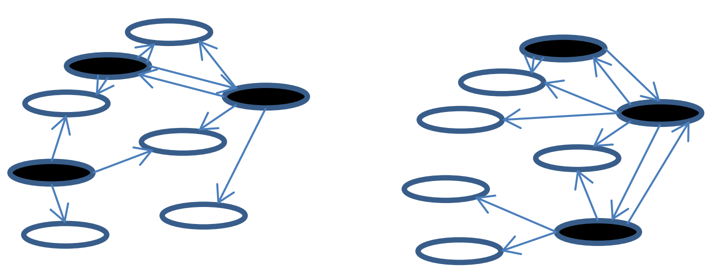
In the broader area of the hypoelminorheic (drainage basins) plus the area connecting them, the following activities represent threats to the species:

1. Any clearing of land that completely bisects the drainage basin, reducing the possibility of migration, and increasing the possibility of extinction
2. Any culvertization of tributaries that completely bisects the drainage basin, reducing the possibility of migration , and increasing the possibility of extinction
3. Construction of impermeable surfaces or culverts that increase the likelihood of toxic spills reaching hypotelminorheic habitats.

A key feature of any attempt to minimize the impact of the purple line on Hay's and Kenk's amphipods must begin with the identification of hypotelminorheic habitats. Given difficulties in finding *Stygobromus* in seepage springs, repeated collecting is in order. Even habitat identification is difficult because temporary rainwater pools and vernal pools unconnected with groundwater may be confused with seepage springs. Chemical measurements (especially conductivity, temperature, and pH [5]), but more dependable is the presence of seepage spring specialists. In Rock Creek, the isopod *Caecidotea kenki* is a very good and consistent indicator of shallow groundwater [8].

V. Towards a recovery plan for Hay's and Kenk's spring amphipods

The original listing [13] and a subsequent five-year review [14] suggests that because of the urbanized habitat and limited possibilities for protection, that no "no conservation benefits would ensue from a recovery plan" [14]. I believe that this is an overly pessimistic view, given what we now know about *Stygobromus* in these habitats. If hypotelminorheic habitats can be identified (see section IV) within an area, and these areas are protected (perhaps with a fence), and the integrity of the intervening surface habitat is maintained, protection would be enhanced. It would be important to include habitats that currently do not have either Hay's or Kenk's spring amphipods, because sub-populations of these species (and other *Stygobromus*) likely blink on and off in any particular site. Below is a hypothetical representation of an idealized network of protected sites. Dark ellipses have populations of either Kenk's or Hay's spring amphipods, and arrows indicate possible dispersal. All ellipses should have high levels of protection. The Kenk's spring amphipod candidate assessment [17] comes to a similar view about threats and protection strategies.



TIME 1 TIME 2

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