Expert Opinion on Current Threats to
Hay's Spring Amphipod (*Stygobromus hayi* ((Hubricht and Mackin, 1940))
and Kenk's Amphipod (*Stygobromus kenki* Holsinger 1978)

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Purpose

The purpose of this affidavit is to provide an expert opinion that will assist in the conservation of endangered and candidate amphipods and their habitats.

Credentials

I received a B.S. in Biology from the University of Notre Dame in 1982. In 1984, I earned an M.S. in Zoology from Northwestern State University. My Ph.D. in Zoology was awarded by Ohio State University in 1991. Both my M.S. thesis and doctoral dissertations involved the study of freshwater organisms and ecosystems. From 1991 to 1992, I was a postdoctoral associate at Ohio State University and from 1992 to 1993, I was a postdoctoral scholar at Miami University in Ohio. Both of these positions were focused on research and conservation of freshwater invertebrates.

Since 1993, I have been on the faculty of the Department of Zoology (Biology as of July, 2013) at Miami University. Originally hired as an assistant professor, I was promoted to associate professor in 1999 and to professor in 2004. Faculty promotions at Miami are based on demonstrated accomplishments in both research and teaching. My research interests center on ecology, evolution, and conservation of freshwater invertebrates. I teach undergraduate and graduate courses in ecology, conservation biology, and invertebrate zoology.

One focus of my research is on the evolution and conservation of endangered amphipods found in springs of the Chihuahuan Desert of southeastern New Mexico and west Texas. Since 2001, my students and I have conducted extensive research on amphipods of the *Gammarus pecos* species complex. Members of this group are limited to isolated springhead and spring brook habitats. The complex consists of three described species, all of which are federally listed as endangered, and additional undescribed species. Two of the three described species were listed in the past year; research from my laboratory was instrumental in these successful listings. My research has identified several undescribed species that are new to science and which, after we complete descriptions, are likely to meet criteria for listing under the U.S. Endangered Species Act. This research has been funded by the National Science Foundation, the U.S. Fish and Wildlife Service, the New Mexico Department of Game & Fish, and the World Wildlife Fund. Two M.S. theses (Gervasio 2002; Haan 2012) and one Ph.D. dissertation (Seidel 2009) have been produced, along with a number of papers in peer-reviewed journals (Gervasio et al. 2004, Sei et al. 2009, Seidel et al. 2009, 2010). I regularly consult with management agencies (New Mexico Department of Game & Fish, U.S. Fish and Wildlife Service) and nongovernmental organizations (primarily The Nature Conservancy) on questions regarding conservation of freshwater invertebrates, including amphipods.

Because each desert *Gammarus* species is limited to a single spring system, their population biology and the conservation issues facing them are very similar to those of the Hay's spring amphipod and Kenk's amphipod. In each case, issues of hydrology and groundwater flow, and threats to water quality and quantity comprise the greatest risks of extinction. Thus, I am qualified to provide this expert opinion.

Biology of Amphipods

It is widely accepted that we are in a biodiversity crisis. While large numbers of species worldwide are threatened with extinction, aquatic ecosystems face some of the greatest threats. Freshwater organisms form a disproportionate share of the world's biodiversity, but also make up a disproportionate share of species that are at risk of extinction. While charismatic species such as elephants and tigers are widely known to be imperiled, many additional species that are small and occupy isolated habitats are also at great risk of extinction. Such is the case for a number of freshwater amphipods in North America. Included among these are six species listed as endangered, and one species that is a candidate for listing.

Freshwater amphipods are small, shrimplike crustaceans that occupy lake, stream and spring habitats. Several hundred species are described from North America; there are likely many more species that are undescribed. These species occupy benthic (bottom) habitats and cling to plants. A large number of species are limited to caves, seeps, and other underground habitats. Because amphipods are sensitive to pollution, their presence often indicates clean water; at the same time, declines in population abundance may be indicative of declining water quality. These species may be herbivorous or carnivorous; many of them feed on decaying leaves and other organic material that sinks to the bottom of aquatic ecosystems. Thus, they are vital links in the movement of energy and cycling of nutrients through ecosystems.

Distribution of Hay's spring amphipod and Kenk's amphipod

The amphipod genus *Stygobromus* is composed of a large number of subterranean-dwelling species distributed in North America and Europe. Seepage springs and other shallow subterranean habitats in the coastal plain and piedmont of the mid-Atlantic contain a number of endemic species (species found nowhere else) within the group, along with other endemic taxa (Culver et al. 2012). Many of these amphipods have highly restricted ranges and are often found in relatively few seepage springs. Included among these are Hay's spring amphipod (*Stygobromus hayi*) and Kenk's amphipod (*Stygobromus kenki*). Both of these species are found in tributary watersheds of the Potomac River in the District of Columbia and Montgomery County, Maryland.

Hay's spring amphipod is small (less than 10 mm in length), nonpigmented, and eyeless. It is listed as Endangered by the U.S. Fish and Wildlife Service and the International Union for the Conservation of Nature (IUCN), and Critically Imperiled by NatureServe. The range of Hay's spring amphipod is limited to seepage springs associated with the floodplain of Rock Creek as it flows through Rock Creek Park in the District of Columbia. Four of these springs have confirmed populations, while amphipods believed to be this species have been collected from three additional sites but have not been confirmed by species experts (USFWS 2013a). All seven sites are located along a three-mile reach of the floodplain, and therefore, likely are not spatially independent in terms of environmental conditions. Because these discrete seepage springs are separated by habitats that do not have records for this amphipod, it is likely that either populations of Hay's spring amphipod are isolated from one another or there are undiscovered populations between known sites.

Kenk's amphipod is smaller (less than 6 mm in length) than Hay's spring amphipod, but it is also nonpigmented and eyeless. It is a candidate for listing by the U.S. Fish and Wildlife Service, has not been assessed yet by IUCN, and is considered Imperiled by NatureServe. The known range of Kenk's amphipod includes three seepage springs in the floodplain of Rock Creek in Rock Creek Park (DC), a seepage spring on private land along Coquelin Run in the Rock Creek watershed of Montgomery County (MD), and a seepage spring in the drainage of the Northwest Branch of the Anacostia River in Montgomery County (U.S. Fish and Wildlife Service 2013b). A record of this species from Fairfax County (VA) was later determined to be wrong. Kenk's amphipod and Hay's spring amphipod are known to co-occur at one site (Culver et al. 2012). Because these discrete seepage springs are separated by habitats that do not have records for this amphipod, it is likely that either populations of Kenk's amphipod are isolated from one another or there are undiscovered populations between known sites.

Seepage spring habitats

The habitat occupied by these amphipods consists of moist, leaf-covered areas with minimal flow. In effect, water "seeps" to the surface (versus springheads with significant flow). Culver et al. (2012) characterized these as "wet spots in the woods." As such, flow varies across seasons, with low flow and drying occurring during hot periods with low rainfall. Major features of these habitats include persistent moisture due to groundwater above a shallow (less than 50 cm) layer of clay or other impermeable material, relatively large amounts of organic matter such as leaves, and typically less than 1 hectare of surface area (summarized in Culver et al. 2012). These habitats differ from streams and hyporheic (water that "leaks" beneath a stream) areas in water chemistry and temperature. The fact that they occur in woodlands slows evaporation and limits temperature in summer time. Conversely, winter temperatures are warmer than surrounding habitats because the seepage springs are fed by groundwater (Culver et al. 2012). In sum, these habitats are small, spatially discrete areas with greater thermal stability than other aquatic habitats due to both groundwater flow and their location within deciduous woodlands.

Because of their small size and locations in floodplains, seepage springs are easily overlooked when biological surveys are conducted. Furthermore, because Hay's spring amphipod and Kenk's amphipod are small and often found under leaves and rocks in these systems, they may be undetected during any particular survey. Thus, it is likely that they occupy additional seepage springs within the floodplains of Rock Creek and Coquelin Run that would not be uncovered without more comprehensive sampling. This would particularly be the case for areas that are currently wooded, such as Rock Creek Park, and wooded land along Coquelin Run and its tributaries.

Threats to amphipods

The U.S. Endangered Species Act lists five factors that may lead to a determination that a species is endangered or threatened. The U.S. Fish and Wildlife Service has determined that the primary factor affecting both amphipod species is "present or threatened destruction, modification, or curtailment of habitat or range." This is undoubtedly the most important category of threat to these organisms for a number of reasons. Because these species are found in small, spatially discrete habitats, each habitat patch is vulnerable to factors extrinsic to the patch itself. Thus, beyond just destruction of the actual site, activities in surrounding areas are also likely to impact amphipods occupying each of these seepage springs. Because each species is known from only a small number of patches, loss of any one represents a significant portion of the total range of the species. Finally, the number of individuals of each species occupying each patch appears to be low such that the likelihood that all individuals may be extirpated due to a single event is high. Because the springs are isolated from one another, the likelihood that individuals from one spring are able to re-colonize a spring where the population has been extirpated is very low. Once extirpated, a site is not likely to regain amphipods without human intervention. Given all of these considerations, each species constitutes a single nonequilibrium metapopulation, a term used by ecologists to describe a group of small, discrete populations that are isolated from one another. We know from modeling and experimental work that such nonequilibrium metapopulations are doomed to extinction as individual populations are lost (i.e., a population cannot reappear once it is lost if there is currently little or no dispersal from other populations).

A number of human activities have been identified as threats to survival of these amphipod populations (U.S. Fish and Wildlife Service 2013a, b). These include degradation of water quality due to pollution with toxic substances, sewage infiltration, and sediment run-off from construction sites; habitat destruction due to deforestation and other land use changes; and lowered groundwater levels due to decreased recharge of aquifers. The limited spatial extant of each species places it at risk of harm from any one or combination of these threats.

Pollution

Increased mortality of amphipods due to pollution of Coquelin Run and Rock Creek and their tributaries and floodplains is a likely consequence of activities associated with the construction of the Purple Line and attendant construction and development of additional buildings, parking, and the like on both sides of the Run and its watershed. Furthermore, because such development may also occur in recharge zones for the seepage springs inhabited by amphipods, even development that appears to be distant from the streams and springs may have adverse effects on the amphipods.

In the Environmental Impact Statement for the Purple Line, MTA identified numerous sites with high potential for concern about hazardous materials. This includes at least three high-potential sites that are located less than 300 feet from Coquelin Run in the vicinity of Connecticut Avenue (see Figure 4-29; MTA 2013). Two additional medium/high-potential and five other sites with insufficient data are also located within less than 500 feet of Coquelin Run. All of these sites are within the Chevy Chase Lake Sector Plan area, an area that includes the private land site for Kenk's amphipod. All of these sites also lie within, upstream of, or close to a Sensitive Species Project Review Area that is presumed to indicate the general locality of Kenk's amphipod in Coquelin Run (determined using Maryland Department of Natural Resources' MERLIN Online web site). Construction activities associated with the proposed station at Connecticut Avenue are likely to introduce toxic substances or hazardous materials from these sites via either contamination of water or sediments that run off of the site into Coquelin Run. Because these high impact sites likely include petrochemicals and dry cleaning chemicals that are persistent in the environment, such pollution has the potential to affect not only Coquelin Run but also Rock Creek. Contamination of the underlying aquifer by such pollutants could result in increased mortality of amphipods inhabiting subterranean and surface habitats on both sides of these creeks, due to subsurface flow of water. This would put all populations of both Kenk's amphipod and Hay's spring amphipod at risk. Blasting and assorted other construction activities also run the risk of disrupting sewage pipes with the potential for release of sewage into Coquelin Run and Rock Creek. Increased urbanization associated with building of the Purple Line and its stations, such as the one at Connecticut Avenue in particular, also increases the possibility of pollution due to run-off of fertilizers from suburban lawns that replace woodlots and introduction of pollutants associated with increased automobile traffic of both residents who are drawn to live near the station, and those driving to the station from further away. Fertilizers lead to increases in biochemical oxygen demand (BOD), leading to loss of sensitive species such as amphipods. Automobile pollution may include both petrochemical exhaust and some metals. Overall, it seems likely that without stringent pollution prevention measures designed to prevent toxic levels of BOD and pollution, construction of the Purple Line and associated development will greatly increase the chances for contamination of Coquelin Run, its tributaries and floodplain. It will also increase the chances for contamination of Rock Creek, its tributaries, and floodplains. These will put both species of amphipods at greater risk of extinction.

Before the Purple Line can be built, hazardous materials from identified sites must be isolated and removed, with minimal release of these into surface or ground waters. If the Purple Line is constructed, grading and landscaping must control stormwater runoff and groundwater infiltration so as to minimize the potential for contamination of the streams, their floodplains, and the underlying aquifer. Stormwater runoff should be managed within the vicinity of the streams and their floodplains, while protection of groundwater needs to occur throughout the region overlying the aquifers that feed seepage springs occupied by amphipods.

Habitat destruction

This threat is likely to take several forms, including deforestation and conversion to lawns, increased impervious surfaces leading to greater flashiness of streams, and alteration of stream beds and channels. Building of the Purple Line will entail removal of significant numbers of trees in the floodplain of Coquelin Run and in the Rock Creek floodplain. These trees serve several important functions relating to survival of amphipods: 1) they shade seepage springs, limiting increases in water temperature and evaporation of water during the summer (thus maintaining more stable habitat); 2) they provide leaves which serve as food and cover for amphipods; 3) they serve as buffer areas that stabilize the flow of run-off into streams. Thus, loss of woodlots associated with construction of the Purple Line may lead to increased water temperatures in seepage springs, decreased food items available for amphipods and other invertebrates, and increased variability in stream flow, with much higher peak flows of shorter duration. These higher peak flows may act to "flood out" the seepage springs, causing loss of amphipods downstream.

Construction of the Purple Line and the attendant economic development (i.e., construction of businesses, parking lots, and residences) that will occur in the vicinity of Purple Line stations will significantly alter the flow of Coquelin Run and increase the total impervious surface in the watershed. Much of this will be associated with the terminal station in Bethesda and the proposed Connecticut Avenue station, both of which are located less than 1000 feet from Coquelin Run; the latter is less than 500 feet from the stream and adjacent to the Sensitive Species Project Review Area that is presumed to contain Kenk's amphipod. Over 5100 hundred feet of culvert will be laid in stream beds over the course of the Purple Line, decreasing infiltration of stream water and increasing flow rates during storm events. Construction and increased impervious surface associated with crossing of Rock Creek (approximately 1400 feet upstream of the mouth of Coquelin Run) and greater development in the vicinity of Lyttonsville also has the potential to affect amphipods in Rock Creek; known sites for both species are less than 10 miles downstream. Increases in impervious surfaces lead to several effects on streams that are associated with precipitation events. These effects are a function of how storm water is handled. In the absence of proper storm water management, water that strikes roofs and parking lots moves rapidly across impervious surfaces until it reaches a stream. These streams become very "flashy," meaning that water levels rise and fall rapidly while maximum flow increases. Under these circumstances, streams rise out of their banks and flood events become more frequent. The increased height of streams and spillage into floodplains can pose threats to seepage springs, because a spring may become inundated by the adjacent stream. This creates the potential for increased amphipod mortality as bottom habitat is disrupted and high flow washes amphipods downstream where they may find unsuitable habitat or be subject to fish predation. If stormwater is managed via storm sewers, precipitation is diverted to storm drain outfalls or put into retention ponds. These measures limit groundwater recharge (see section below).

Seepage springs that contain amphipod populations, or have the potential to contain them, must be protected. If the Purple Line is to be built, construction activities must be designed to have minimal impacts on the streams and their floodplains. Such minimal impacts include maintaining as much riparian forest as possible to act as buffers, limiting the installation of culverts, minimizing the operation of heavy equipment in the floodplains, and maintaining natural stream channels. The sites of spring seeps that contain, or have the potential to contain, amphipod populations must be protected from trespass by individuals attracted to the neighborhoods by the Purple Line.

Groundwater recharge
Seepage springs represent locations where groundwater emerges to the surface. Thus, the flow of water at a spring is determined by the water table. The rate at which aquifers are recharged is a function of precipitation and infiltration of surface water into the aquifer. Thus, groundwater, surface water, and precipitation are linked. Any activity that decreases infiltration of surface water into the ground or that changes the flow of groundwater will affect the rate at which groundwater flows from any given spring. These effects are perhaps best known from springs in the western USA. Irrigation in the arid west is accomplished in two ways: interception of precipitation (typically as snowmelt) with storage in reservoirs, and mining of groundwater. Not surprisingly, these activities lead to decreased flow from natural springs and as a result, many springs have dried up in places such as west Texas (reviewed in Brune 1981). These same processes may occur with construction of the Purple Line and attendant suburban development.

Creation of impervious surfaces due to construction will require stormwater management. In most instances, such management is designed to either move storm water rapidly into storm sewers and larger streams or to direct storm water to retention basins where it is allowed to evaporate or infiltrate. In both of these cases, natural recharge of the aquifer is inhibited. Furthermore, blasting that might occur during construction of the Purple Line (see Chapter 5 of the EIS) creates the possibility that flow of groundwater may be altered. This is especially true just east of Jones Mill Road, which lies in the floodplain of the confluence of Coquelin Run and Rock Creek. If groundwater flow to existing seepage springs is altered or disrupted, the effect will be to decrease amphipod habitat. Groundwater recharge to a spring is often a complex process: it occurs both in the immediate vicinity of the spring seep and also elsewhere in the aquifer. In a sense, it can involve changes *anywhere* in the aquifer. Thus, such effects may occur both in the immediate vicinity of the spring, in the immediate vicinity of construction, and elsewhere in the aquifer, which is likely to include both sides of the stream. Because the primary habitat of these amphipods is subterranean, it is critical that patterns of groundwater recharge be understood and that this knowledge is used to protect critical zones of recharge within the aquifer.

Recommendations
After review of pertinent documents and relying on my experience and expertise, I conclude that **construction of the Purple Line preferred alternative has the potential to cause harm to Hay's spring amphipod and Kenk's amphipod**. Thus, the project Environmental Impact Statement conclusion that no endangered species are affected by the preferred alternative is not supported by available information. Because Hay's spring amphipod is listed as Endangered under the Endangered Species Act, **a Section 7 consultation with the U.S. Fish and Wildlife Service is necessary**. Furthermore, consideration of effects on both amphipod species should be included in the EIS as mandated by federal and state laws.

The Endangered Species Act seeks protection and recovery of listed species. Because the implementation of the act is to be based on the "best science," it requires both research and active management to achieve these objectives. The eventual goal of the act is recovery and delisting of species.

Research needs

- Extensive surveys of Rock Creek, Coquelin Run, their tributaries, and floodplains are needed in order to determine whether these species of amphipods are found at additional sites. Such surveys need to be both spatially and temporally extensive (i.e., they need to cover many sites and multiple times of the year). Further surveys should also be conducted in suitable habitat of adjacent watersheds in order to learn whether the range of these species extends beyond the Rock Creek basin.

- Genetic analyses should be performed to aid in identification of these species. At the present time, identification is based on expert analysis of morphological features. Description of DNA "barcodes" (short gene sequences unique to each species) would allow identification of these species using DNA sequencing. Once the barcodes are described and verified, this approach would not require the same reliance on a small number of taxonomic experts in order to place a species name on a specimen.

- Further genetic analyses should be undertaken to determine whether dispersal and gene flow occurs among sites for each species. This is critical in order to determine whether individual sites were historically isolated from one another and whether they are currently isolated from each other. If they are isolated, they will function independently and will face higher risk of extinction because once a population is extirpated, it will not be restored by migration from other populations.

- Mapping of groundwater aquifers and recharge zones under current land use conditions is necessary in order to understand potential impacts of future development.

Management needs

- Creation of a "refuge" population of each species should be undertaken, perhaps in conjunction with the National Zoological Park. Such a population would be protected from environmental degradation associated with suburban development in Montgomery County and the District of Columbia, while also serving as a source population for future recovery of the species if either is extirpated from sites it currently occupies. Development of such a population by an institution such as a zoo also provides opportunities for public outreach and education concerning the plight of endangered species, especially the small, unknown species that make up the bulk of the world's imperiled biodiversity.

- Establishment, via translocation and introduction, of additional populations of each species at sites within the current range that are deemed suitable. By increasing the number of individual populations, the potential for extirpation will decline. Habitat connecting extant and new populations should be protected to encourage dispersal and gene flow among sites.

- Extant sites, and newly established sites, should be protected from pollution and excessive surface water discharge through the creation and maintenance of forested buffer zones along Coquelin Run, Rock Creek, and other localities.

- Increases in impervious surface should be minimized and maintenance of forest should be encouraged, especially in aquifer recharge zones.

Recovery outline
These species should be deemed recovered when

- they have sufficient populations (sites) to ensure survival even if individual populations are extirpated;

- each individual population is large enough that effects of inbreeding and genetic drift are minimal;

- connectivity (via dispersal and gene flow) among populations is restored to natural levels.

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