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Evaluating the Contribution of Multiple Dispersal Pathways to the Genetic Population Structure of Northern Dusky Salamanders (*Desmognathus fuscus*)

by

William L. Miller

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Department of Biological Sciences

Towson University

8000 York Rd.

Towson, Maryland 21252

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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by William L. Miller entitled "Evaluating the Contribution of Multiple Dispersal Pathways to the Genetic Population Structure of Northern Dusky Salamanders (*Desmognathus fuscus*)" has been approved by his committee as satisfactory completion of the requirement for the degree of Master of Science.

Chair, Thesis Committee	Date 5-16-14
Committee Member	Date 5/16/2014
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ABSTRACT

Evaluating the Contribution of Multiple Dispersal Pathways to the Genetic Population Structure of Northern Dusky Salamanders (*Desmognathus fuscus*)

William L. Miller

Headwater species are organisms that are primarily constrained to the upstream terminus of river networks with limited capacities for both in-stream and overland dispersal. Movement along alternative dispersal pathways is suggested to contribute to gene flow and the overall stability of headwater populations. Six microsatellite markers were used to assess gene flow along in-network and out-of-network pathways in a species of headwater salamander, *Desmognathus fuscus,* over multiple spatial scales. Overall, genetic divergence was significant among all populations (F_{st} = 0.027 to 0.405) and at all hierarchical spatial scales. Genetic clustering analyses suggested limited gene flow within and among watersheds, indicating that both dispersal pathways are involved in maintaining gene flow among headwater populations. Increased genetic distance was associated with out-of-network distance and the degree of urbanization in upland habitat. These results suggest that significant dispersal occurred along terrestrial pathways, but dispersal resistance appears to be greatest along these pathways.

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INTRODUCTION

Dispersal among patches can be a key feature of the population dynamics of organisms that are distributed in spatially-explicit habitat patches. Dispersal facilitates population stability and genetic diversity, and is largely driven by the ability of an organism to move between habitat patches across a landscape matrix characterized by suboptimal habitat (Saunders et al., 1991; McKelvey et al., 1993; Turner, 1995; Fagan, 2002; Lowe and Allendorf, 2010). Most studies addressing patterns of dispersal have focused on two-dimensional landscape concepts with a planar geometry (Fagan, 2002). In this conceptual framework, ecological landscapes are represented by a network of discrete habitat patches that are all interconnected by a series of links, which function as corridors for the movement of both resources and organisms (Urban and Keitt, 2001).

In contrast, dendritic ecological networks are characterized by a bifurcating arrangement of sequentially occurring habitat patches (Fagan, 2002). True dendritic networks are self-similar, fractal-like landscapes with a branching hierarchical geometry (Grant et al., 2007). Dendritic networks are unique in the fact that the linear branches serve as both usable habitat and functional corridors, with confluences acting as both branch junctions and as unique habitat types (Grant et al., 2007). The spatial configuration of dendritic networks often restricts ecological processes to the reaches within linear systems, so that dispersal among populations is inherently tied to both the geometric configuration and topology of the network, with little influence of the surrounding landscape matrix (Fagan et al., 2010; Padgham and Webb, 2010).

Stream networks are hierarchically arranged, dendritic-like networks characterized by a high degree of spatial complexity. The ecological dynamics of stream networks are shaped by the highly variable hydrologic and geophysical characteristics of individual stream reaches. Consequently, patterns of resource availability, population distribution, and community composition may be highly structured throughout the network (Grant et al., 2007; Grant, 2011). The main channel of a stream network is created by a coalescence of smaller, heterogeneous stream branches, contrasting the self-similar, fractal-like nature of true dendritic networks (e.g., trees) where constituent branches are both physically and functionally similar to each other. While high degrees of internal ecological structure do distinguish stream networks from classical dendritic networks, patterns of dispersal among populations are expected to conform to the traditional framework of dendritic systems, driven largely by the linear nature of network topology.

Characteristics of Stream Networks

Traditionally, stream networks have been viewed as a physical element of the surrounding landscape mosaic (Wiens, 2002). This framework only addresses stream networks as internally homologous features of the surrounding terrestrial landscape and makes no distinction between stream reaches in regards to local structure and function. Other conceptual views address streams as important landscape features that act as ecological boundaries, which are connected to the surrounding terrestrial matrix by a series of flows and fluxes (Wiens et al., 1985; Wiens, 2002). While these concepts assign functionality in the context of the surrounding landscape mosaic, they do little to address the highly complex internal structure of stream networks. In contrast, more recent

conceptualizations focus on stream networks as complex, internally heterogeneous landscapes (often referred to as river- or streamscapes) with ecological processes driven primarily by internal hydrodynamic and geophysical properties, and further influenced by complex interactions with the surrounding floodplain (Wiens, 2002; Allan, 2004; Malard et al., 2006).

Water is the primary feature and an important limiting resource of aquatic habitats, where patterns of water availability shape the abundance and population condition of stream species (Resh, 1992; Golladay et al., 2002; Hakala and Hartman, 2004), as well as the spatial distribution, structure, and interactions of stream communities (Stanley et al., 1994; Closs and Lake, 1996; Lake, 2003). In stream systems water flow is generally unidirectional, with water travelling down elevation gradients (Muller, 1954). Vannote's River Continuum Concept (Vannote et al., 1980) emphasizes the influence of unidirectional stream flow on ecosystem dynamics by recognizing stream networks as a functional continuum characterized by a high degree of internal connectivity (rather than a summation of functionally distinct stream reaches). Flow facilitates the passive downstream transport, processing, and utilization of energy resources (Vannote et al., 1980). Flow also facilitates the passive movement of organisms from upper reaches to locations further down the river gradient (Anholt, 1995; Humphries and Ruxton, 2002).

While the River Continuum Concept represented a significant paradigm shift in understanding stream networks as a functionally connected system, it failed to address the total spatial and temporal complexity observed in stream systems. Later studies addressed the role that geophysical or artificial discontinuities (e.g. dams and waterfalls) and boundary dynamics (river-floodplain interactions) play in shaping the movement of

materials and organisms down the stream gradient (Ward and Stanford, 1983; Junk et al., 1989). What emerges from these conceptualizations is a system of connections and barriers to the movement of energy, materials, and organisms that is determined by the interaction between geomorphology and hydrology in stream networks. This complex interaction of spatial and hydrodynamic variance provides the foundation for the inherent heterogeneity observed in lotic ecosystems (Wiens, 2002).

Influence of Network Structure

Classifying stream reaches within a network is often difficult due to the innate complexity of stream systems. Traditionally, streams are classified by stream order (Strahler, 1957; Kuehne, 1962) with the goal of generalizing local ecological dynamics. When like stream orders combine, the new reach formed from the confluence increases in order (Strahler, 1957). Lotic systems can be broadly categorized into headwaters (stream order 1-3), mid-reaches (stream order 4-6), and lower reaches (stream order > 6; Strahler, 1957). While the stream order system is useful in generalizing biological function within a network, some have suggested that a system that incorporates the physical heterogeneity of stream networks is needed to extrapolate patterns among stream reaches (Perry and Schaeffer, 1987; Brussock and Brown, 1991). For example, adventitious tributaries often exhibit different physical and ecological characteristics when compared to true headwater streams (Gorman, 1986; Osborne and Wiley, 1992; Osborne et al., 1992), limiting the relevancy of using the stream order system when comparing biological patterns among low-order stream reaches from different parts of the stream network.

The spatial positioning of reaches within the stream network can influence community composition and population dynamics of aquatic organisms. The influence of network structure on community and population dynamics of many aquatic taxa, such as salamanders, has not been directly addressed; however several studies have addressed these issues for stream fish populations and communities. Generally, higher order reaches have increased species diversity and richness when compared with lower order reaches (Gorman and Karr, 1978). Adventitious tributaries will often exhibit greater species richness and diversity when compared to other low order tributaries, as they share a relatively direct connection with the main channel, allowing organisms to easily move between the main channel and adventitious reaches (Osborne and Wiley, 1992; Osborne et al., 1992; Thomas and Hayes, 2006). Additionally, the confluence formed between two connecting stream reaches may increase species diversity as a consequence of increased resource heterogeneity (Grant et al., 2007). Low order streams also exhibit more complex disturbance regimes (e.g. periods of seasonal drying), which may lead to increased mortality rates and decreased birth rates of sensitive species (Closs and Lake, 1996; Magoulick and Kobza, 2003). Mobile species may attempt to find refugia, such as permanent streams or pools, as intermittent streams dry, driving shifts in migratory patterns (Magoulick and Kobza, 2003; Perry and Bond, 2009).

The spatial configuration of reaches within the network may also determine patterns of demographic structuring. The complexity of a particular stream network can facilitate a wide variety of effects on population demographics and community structure. More complex stream networks feature a greater number of functionally diverse stream reaches and a ‘branchier’ network topology, when compared with functionally uniform,

'ladder-like' networks (Fagan et al., 2010; Grant, 2011). The spatial isolation imposed by stream network geometry may limit movements of organisms among stream reaches (Johnson et al., 1995). Topological complexity increases the number of potential links between stream reaches, decreasing the degree of spatial isolation occurring within the network. This leads to increased movement of individuals among populations, as well as higher species richness and diversity (Fagan et al., 2010). In contrast, stream reaches in less complex networks exhibit greater spatial isolation, which constrains dispersal for many species, and may lead to increased rates of local extirpation and decreased community diversity among reaches (Fagan et al., 2010).

Headwater Organisms

Movement patterns of organisms among populations are not only shaped by the physical structure of the landscape, but are also influenced by the life history characteristics and dispersal strategies of individual species. Life history traits and habitat requirements shape the spatial distribution of organisms across a landscape, while dispersal strategies influence how easily an organism can move through the landscape matrix. In stream networks, mobile species (such as large fish) that inhabit the main channel are expected to maintain a longitudinal pattern of movement, with migratory species being able to easily move throughout the network in the absence of physical barriers to dispersal (Branco et al., 2011). However, due to specific habitat requirements, obligate headwater species are often confined to low-order reaches, and are generally assumed to exhibit explicit genetic population structuring as a result of limited dispersal of individuals among low-order reaches (Finn et al., 2006; Finn and Adler, 2006; Finn et

al., 2007). Therefore, patterns of gene flow are determined by the ability of dispersers to navigate a matrix of suboptimal habitat.

Because headwater species require small streams occurring within the upstream terminus of stream networks to complete their life cycle (Meyer et al., 2007), they often have unique life cycle characteristics (e.g. multiphasic life cycles) that allow them to compensate for passive downstream drift and avoid seasonal disturbances. For example, directed dispersal by the adult stages of stream insects may offset passive downstream movement of larval stages (Hershey et al., 1993). Terrestrial or semi-aquatic adult stages may allow for dispersal among reaches via movement through terrestrial habitats (Finn et al., 2007; Grant et al., 2009), which may also be important for seasonal drought avoidance in ephemeral and intermittent streams (Robson et al., 2011).

Based on life history characteristics, several models of dispersal have been proposed to explain the development of spatially explicit genetic population structure among populations of headwater species (Fig. 1). Highly mobile and obligate aquatic species (e.g., brook trout; *Salvelinus fontinalis*; Kanno et al., 2011) may exhibit high degrees of movement within drainage basins due to their ability to navigate the main channel (stream hierarchy model; Meffe and Vrijenoek, 1988), with patterns of genetic population structure driven largely by isolation by stream distance (Hughes et al., 2009) and the presence of structural barriers to movement within the stream channel (Kanno et al., 2011). In contrast, headwater species with very restricted dispersal abilities (e.g., high-elevation black flies; *Metacnephia coloradensis*; Finn and Adler, 2006) may exhibit strong population structuring at the reach scale (Death valley model; Meffe and Vrijenoek, 1988). Genetic population structure of headwater species with the ability to

disperse terrestrially (e.g., giant water bugs; *Abedus herberti*; Finn et al., 2007) may be defined by clusters of closely associated headwater reaches, regardless of stream channel connections (headwater model; Finn et al., 2007). Finally, generalist species with strong propensities for both terrestrial and aquatic dispersal (e.g., European caddisflies; *Plectrocnemia conspersa*; Wilcock et al., 2003) may exhibit widespread gene flow, even over large spatial scales (Hughes et al., 2009).

Headwater species commonly utilize streams found in the upstream terminus of stream networks (true headwater streams), as well as small, low-order tributaries that flow directly into the main channel of the network (adventitious tributaries). While these stream classes are physically similar (Osborne and Wiley, 1992), adventitious and headwater streams may differ in habitat structure and may have different population dynamics and community composition. Although not definitively stated, patch size (stream length) and ecological connectivity are generally assumed to be greatest in the true headwaters due to the close association of low-order streams and the strong interface with upland habitat that often occurs in the upstream terminus (Freeman et al., 2007). In contrast, adventitious tributaries are generally assumed to represent smaller patch sizes and decreased landscape-scale connectivity. However, these streams are more closely associated with the main channel, allowing easier access for generalist species that will commonly utilize adventitious tributaries as thermal refugia and spawning sites (Thomas and Hayes, 2006).

Several studies have found that fish species richness was greater in adventitious tributaries when compared to true headwater streams (Osborne and Wiley, 1992; Thomas and Hayes, 2006). Thomas and Hayes (2006) postulate that this phenomenon is most

likely explained by the relative proximity of adventitious tributaries to the main channel and slight differences in local hydrologic and physical parameters. Their results indicate that warm-water species that were more common in mid- or high-order reaches were more commonly found in adventitious streams, which tended to have more stable hydrologic parameters and were slightly warmer than true headwater streams (Thomas and Hayes, 2006). Headwater species, such as brook trout and mottled sculpin (*Cottus bairdi*) were almost exclusively found in true headwater streams, suggesting that temperature regime is an important factor determining species assemblage composition (Thomas and Hayes, 2006).

In contrast to fish, species diversity for both stream invertebrate and amphibian communities seems to be greatest in the true headwaters. Insect diversity (Dieterich and Anderson, 2000) and production (Progar and Moldenke, 2002) is highest in true headwater streams, and is likely related to the limiting effects streambed slope has on the abundance of fish predators and competitors (Macneale et al., 2005). Likewise, other studies have found salamander abundance (Peterman et al., 2007) and nest site densities (Snodgrass et al., 2007) to be greatest in small, high-gradient streams, suggesting that stream amphibians also benefit from the absence of fish predators.

The persistence of populations within small, high-gradient stream reaches is often tied to the unique dispersal strategies employed by headwater species. Organisms inhabiting the headwaters are often prone to passive downstream drift associated with the unidirectional downstream flow of water (Anholt, 1995; Humphries and Ruxton, 2002). Alternate mechanisms of dispersal, such as intentional upstream movements or the use of multiple dispersal pathways, are essential for some taxa in maintaining population

persistence in headwater tributaries (Humphries and Ruxton, 2002; Grant et al., 2009). In stream networks, dispersal is either restricted to movements within the stream corridor (in-network) or between associated reaches across riparian or terrestrial habitat (out-of-network; Grant et al., 2007; Grant et al., 2010). Thus, differences in dispersal behavior, as well as spatial location within the stream network are considered factors determining genetic structure among populations (Grant et al., 2007; Grant et al., 2010). Organisms, such as fish and freshwater mollusks, are restricted to the water column and are thus constrained to in-network movements (Hughes et al., 2009). Patterns of gene flow among populations constrained to in-network movements are dictated largely by the influence of water flow (Hernandez-Martich and Smith, 1997), presence of predators (Gilliam and Fraser, 2001), distance along stream reaches (Primmer et al., 2004), and presence of in-network discontinuities (Wofford et al., 2005; Neville et al., 2006). Organisms, such as amphibians, insects, and crayfish, are capable of utilizing multiple dispersal strategies (both in and out-of-network dispersal). Thus, for these species directional dispersal biases (Lowe, 2003; Lowe et al., 2008), overland distances (Finn et al., 2007), and terrestrial fragmentation additionally influence gene flow (Wilcock et al., 2003; Watts et al., 2004). Hughes et al. (2009) predicts that movement of individuals between populations inhabiting headwater areas will be high due to the proximal relationship of streams within the headwater terminus and the ability of many headwater species to utilize multiple dispersal pathways. Populations inhabiting adventitious tributaries separated by the main channel may be relatively isolated because main channels may function as barriers to movement for headwater species (Hughes et al., 2009).

Urbanization and Fragmentation

Urbanization represents one of the most pervasive anthropogenic sources of habitat degradation and fragmentation affecting both terrestrial and aquatic habitats. Urbanization is an ambiguous term that has been defined in many ways. By its simplest definition, urbanization refers to the conversion of rural areas to modernized areas as a result of increased economic growth and development (Peng et al., 2000). Urbanization also involves the redistribution of humans from less-dense exurban localities to highly centralized, population-dense urban cores (Peng et al., 2000). In the United States, the urban population is expected to represent 90% of the total projected population by the year 2050 (~400 million individuals; UNPD, 2012). The effects of urbanization on natural landscapes are localized, but highly pervasive, strongly influencing local biodiversity and ecological dynamics. McDonald et al. (2008) implicate urbanization in the listing of approximately 8% of the total vertebrate species on the IUCN Red List, with a clear relationship between the proportion of the species range that is urbanized and the probability of being listed as near threatened or worse. While projected extinction risk due to urbanization may not be as great as other anthropogenic threats to biodiversity (e.g., global climate change; Thomas et al., 2004), the localized negative effects of urbanization are quite significant (McDonald et al., 2008) and disproportionately focused in coastal areas characterized by high rates of endemism (Ricketts et al., 2005).

In stream systems, urban development alters channel form, hydrology, and upland habitats (Paul and Meyer, 2001; Allan, 2004), and therefore may increase dispersal resistance along both in-network and out-of-network pathways. Human-made discontinuities, such as dams and culverts, increase in-network dispersal resistance by

disrupting stream flow and can serve to isolate headwater populations, most likely by limiting upstream dispersal (Morita and Yamamoto, 2002; Wofford et al., 2005; Blakely et al., 2006). Additionally, development within upland habitat increases out-of-network dispersal resistance, and can serve to further isolate semi-aquatic populations capable of out-of-network movements (Smith et al., 2009).

In addition to decreased dispersal of individuals among populations, urbanization can also lead to a reduction in habitat quality. Urbanization can subject stream systems to changes in flow regimes, hydrology, and water chemistry (Paul and Meyer, 2001). Increased surface impermeability and the reduction of riparian buffer zones can cause increased rates of surface water run-off (Arnold and Gibbons, 1996), leading to 'flashier' hydrographs during storm events when compared with non-urban watersheds (Lenat and Crawford, 1994). Additionally, reduced groundwater infiltration associated with impervious surfaces lowers water tables, creating a phenomenon that Groffman et al. (2003) refer to as "urban hydrologic drought." Hydrologic drought can lead to reductions in stream baseflow, which can disconnect a stream channel from the surrounding floodplain, limiting available riparian habitat for stream species and important ecosystem services. Greater surface run-off can also impact water chemistry by introducing contaminants into streams (Paul and Meyer, 2001). Changes in water chemistry can negatively impact community structure by decreasing species richness and diversity (Pratt et al., 1981), altering trophic patterns (Poff and Huryn, 1997; Moore and Palmer, 2005), and reducing population sizes (Medeiros et al., 1983), which in some cases can lead to local extirpation (Frissell, 1993).

PURPOSE

The overall purpose of this study was to understand: 1) the importance of out-of-network dispersal in promoting gene flow among headwater populations of the northern dusky salamander (*Desmognathus fuscus*); 2) the influence of urbanization on patterns of gene flow and genetic diversity in this species. Headwater species may be particularly sensitive to the effects of urbanization due to their patchy distribution throughout stream networks. Additionally, headwater species rely on alternative dispersal mechanisms, such as out-of-network movements or upstream dispersal biases, to maintain gene flow and promote population persistence in headwater tributaries (Grant et al., 2009; Humphries and Ruxton, 2002). Increasing dispersal resistance along in-network and out-of-network pathways may limit dispersal, increasing the chance of local-scale extirpation or loss of genetic diversity, or both.

Polymorphic microsatellite markers were used to infer dispersal of individuals among populations from patterns of genetic population structure among stream reaches. Microsatellites are sections of nuclear DNA in which two to six base pair sequences are repeated a variable number of times (Tautz, 1989). Microsatellites have relatively high rates of mutation and are capable of exhibiting fine-scale patterns of genetic divergence (Sunnucks, 2000), making them ideal markers for studying gene flow at smaller spatial scales.

Several studies have addressed the role that directed dispersal and out-of-network movements play in determining patterns of gene flow among headwater salamander populations. Wooten and Rissler (2011) found that patterns of genetic structure in black-

bellied salamanders (*Desmognathus quadramaculatus*) conformed largely to terrestrial ecoregions, implicating the importance of out-of-network gene flow among watersheds in these regions. In contrast, Lowe et al. (2006) suggested patterns of genetic structuring in the spring salamander (*Gyrinophilus porphyriticus*) were a function of the stream gradient suggesting the importance of within network dispersal for this species. These studies link dispersal behavior and genetic population structuring at specific scales, but do not address how patterns of gene flow may change across multiple spatial scales (Mullen et al., 2010).

Understanding the influence of urbanization in conjunction with the relative importance of multiple dispersal pathways has important management implications, and may provide additional insights into how anthropogenic disturbance influences gene flow in headwater networks. To date, only a single study exists that explores the influence of urbanization on genetic population structuring in Desmognathine salamanders. Munshi-South et al. (2013) investigated the effects of urbanization on genetic population structure and diversity of northern dusky salamander populations inhabiting severely impacted streams in New York City by comparing them to populations occurring outside of the city. The results of their study suggest that severe urbanization can result in increased genetic structuring (F_{st} = 0.213 – 0.514) and an overall reduction in heterozygosity (urban H_o = 0.229; suburban H_o = 0.425 – 0.890). However, the effect of landscape resistance along multiple dispersal pathways was not explored. Understanding the mechanisms influencing patterns of genetic population structure in urban settings may help shape management plans for salamanders, and other sensitive headwater taxa.

Objectives and Hypotheses

The specific goals of this study were to: (1) explore the role that both in-network and out-of-network dispersal play in maintaining patterns of gene flow among spatially distinct populations of headwater salamanders; (2) determine how urbanization may influence patterns of genetic population structuring and genetic diversity. Gene flow among streams was inferred using a hierarchical sampling regime, which compared streams paired within watersheds to those paired among neighboring watersheds, in an attempt to address the importance of multiple dispersal pathways. Mark-recapture data from a previous study (Grant et al., 2010) suggested that the probability of movement among relatively closely-spaced sampling localities (i.e., 300 to 500 m between sample locations) for northern dusky salamanders was similar along in-network corridors (within the same stream reach) when compared to sites paired along out-of-network corridors (among neighboring stream reaches). However, because of the sites were relatively close to each other, the Grant et al. study did not completely eliminate the possibility of within-network dispersal between neighboring sites. In this study, sites paired between watersheds (out-of-network) were separated by the Chesapeake Bay and therefore, movement between these paired sites within stream networks was highly unlikely. Additionally, sites within watershed were separated by greater distances (> 10 km) and stream channels of larger size that represented unsuitable habitat for dusky salamanders. Therefore, it was predicted that sites within watersheds would exhibit increased genetic population structuring when compared to sites paired across watershed divides (out-of-network). Urbanization was expected to increase genetic population structuring among closely associated streams. Additionally, urban development in close proximity to stream

reaches was predicted to decrease genetic diversity when compared to streams sampled in rural watersheds.

METHODS

Study Organism

The northern dusky salamander (*Desmognathus fuscus*) is a common streamside salamander distributed throughout eastern North America. These salamanders are important secondary consumers in headwater stream communities (Hairston, 1949). The northern dusky salamander is known to inhabit watersheds in the Baltimore Metropolitan area (Snodgrass et al., 2007) and is one of the more common species of streamside salamander in Maryland (D. C. Forester, pers. comm.). This species is a headwater species capable of utilizing multiple dispersal pathways, making it an ideal model for this study. Snodgrass et al. (2007) found that northern dusky salamander nest densities were highest in the uppermost reaches of stream networks with channel widths of < 2m, most likely due to the limiting effect that high stream gradient has on fish abundance. Fish are known to be capable of significantly reducing larval salamander densities where both co-occur (Sih et al., 1992).

Upon hatching, fully aquatic larval salamanders remain in the wetted channel for 9 to 12 months (Danstedt, 1975). Following metamorphosis, juvenile salamanders move to the stream bank and may disperse into the surrounding riparian area. Adult salamanders are most commonly associated with moist, wooded stream banks and groundwater seeps, where they can be found utilizing gravel, small rocks, and course woody debris as cover (Krysik, 1980). Using mark-recapture data, Grant et al. (2010) found strong upstream

dispersal biases (in-network) across all age groups (larvae, juvenile, and adult). Moderate rates of out-of-network dispersal were observed in juvenile salamanders, even over distances of as much as 500 m between streams.

Multiple studies have found that northern dusky salamander populations are negatively impacted by urbanization making it an ideal model for this question. Price et al. (2011) found that mean occupancy of larval-phase salamanders decreased from 1.0 to 0.57 of sampled streams four years after watershed urbanization. Several studies suggest that northern dusky salamander population size is negatively correlated to the degree of watershed urbanization (Price et al., 2006; Orser and Shure, 1972). Both Snodgrass et al. (2007) and Orser and Shure (1972) suggested that dusky salamanders are particularly sensitive to the effects of bank scouring and erosion, which often results from increased flow rates in urban streams.

Study Sites and Sampling Design

Salamander tissue samples were obtained from 16 streams occurring in the Piedmont region of central Maryland (Baltimore, Harford, and Montgomery counties; Figs. 2-3). Streams were evenly distributed between land-use classes (developed and rural; n = 8 for each). A nested, hierarchical study design was used in an attempt to quantify the relative importance of in-network and out-of-network dispersal routes (Fig. 4). At the scale of individual stream networks, two stream reaches were selected and were intended to represent one headwater stream and one adventitious tributary. Final study site locations within watersheds were limited by the presence of salamander populations. However, within watersheds, larger channels representative of suboptimal

habitat separated all sites. Watersheds were paired with closely associated reaches in adjacent watersheds between which out-of-network movements were expected. Paired watersheds were functionally isolated from one another, either by distance (> 15 km in-network distance) or by a functional barrier (e.g. Chesapeake Bay), in order to reduce the possibility of in-network movement between paired watersheds. A total of eight watersheds were sampled in this study (Fig. 3).

Urban streams were located in Owings Mills (Baltimore County) and Bel Air (Harford County), Maryland, both of which occur along major interstate corridors proximal to Baltimore City. Streams in Baltimore City were not used because salamander occupancy is limited due to the loss of suitable headwater habitat associated with stream burial. Elmore and Kaushal (2008) estimated that 66% of all streams occurring within Baltimore City have been buried with headwater streams accounting for the greatest proportion of all buried streams.

Rural and exurban streams were sampled in predominantly agricultural and residential areas of northern Baltimore County and Montgomery County, Maryland. Rural landscapes may not represent a true "control" due to the negative effects of both current and historic agricultural land-use. This study was limited to rural landscapes because the lack of availability of contiguous forested habitat in the Piedmont region of Maryland. To avoid introducing extraneous variables associated with differences in local geology and topology between ecoregions, streams from other geographic regions (e.g., Appalachian Range) were not included in this study.

Field Sampling

Tissue samples were collected from each stream from July through October 2012. Collection was confined to 100 to 300 m stream reaches, with no samples being collected below of the closest downstream stream confluence. Tissue samples from 20 to 30 northern dusky salamanders were collected in each stream reach, with one exception (Bynum Run 1; n = 11). Salamanders were located by overturning rocks and course woody debris along the stream margin and in associated groundwater seeps. Tissue samples were collected by aseptically removing 0.2 to 0.5 cm of the distal end of the tail from each salamander following standard animal care and handling protocol outlined by the Herpetological Animal Care and Use Committee (Beaupre et al., 2004). Samples were stored in 95% ethanol in the field and transferred to a -80°C freezer upon return to the laboratory.

Genetic Analysis

DNA was extracted from each tissue sample using the Promega Wizard® Genomic DNA purification kit animal tissue (mouse tail) DNA extraction protocol (Promega). DNA concentration (ng/µL) and purity (260/280) were verified using a NanoDrop 2000 (Thermo Scientific). Following DNA extraction and isolation, microsatellite primer pairs were used for polymerase chain reaction (PCR) amplification of microsatellite loci (Table 1). No previous microsatellite primers were developed in the northern dusky salamander, so a total of 21 di- and tetranucleotide microsatellite loci developed for related taxa were screened for use (*D. auriculatus*, Croshaw and Glenn, 2003; *D. ocoee*, Adams et al., 2005; *Plethodon cinereus*, Connors and Cabe, 2003*; Ensatina eschscholtzii*, Devitt et al.,

2009). All loci that failed to amplify, or that were monomorphic were excluded from this study. A subset of six tetranucleotide microsatellite loci from the southern dusky salamander (*D. auriculatus;* Croshaw and Glenn, 2003) and the Ocoee salamander (*D. ocoee*; Adams et al., 2005) proved to consistently amplify, exhibited heterozygosity, and were used in this study.

Microsatellite loci were amplified and optimized for use with RubyTaqTM PCR Master Mix (2X; USB Corporation) with unlabeled forward and reverse primers. Total reaction volume amounted to 25 µL (12.5 µL RubyTaqTM PCR Master Mix; 9.5 µL deionized H_2O; 1 µL of forward and reverse primer; 1 µL DNA template). Reaction conditions were: (1) Dau primers: denaturation at 94°C for two min; 35x (94°C for 30s, primer-specific annealing temperature for 30s, and 72°C for 45s); and a final extension of 72°C for five min; (2) Doc primers: denaturation at 95°C for five min; 35x (94°C for one min, primer-specific annealing temperature for one min, and 72°C for two min); and a final extension of 72°C for three min. Amplification success and fragment size were qualitatively screened using 1.5% agarose gel electrophoresis and a 100 base pair DNA ladder (Bionexus, Inc.). Optimized annealing temperatures ranged from 52°C to 56°C (Table 1).

PCR amplicon identity was verified for two microsatellite loci (Dau11 and Dau12) by cloning fragments into One Shot® Mach1™-$T1^R$ Chemically Competent *Escherichia coli* cells using the pCR®8/GW/TOPO® TA cloning protocol for sequencing (Invitrogen). Initial screening was carried out using ampicillin-selective media to test for the presence of the vector plasmid, and a blue-white screen was used to verify incorporation of the DNA fragment into the plasmid. Transformed colonies were

screened using PCR amplification of the M13 plasmid region (95°C for three min; 25x [94°C for 30s; 55°C for 30s; 72°C for 45s]; final extension of 72°C for five min). Successful amplification was confirmed using 1.5% agarose gel electrophoresis. Plasmid DNA was extracted using the PrepEase ™ MiniSpin Plasmid kit (USB) and then sent to Functional Bioscience, Inc. for sequencing. Due to difficulties in cloning, the sequences of the remaining four loci have yet to be verified.

Microsatellite analysis was conducted utilizing optimized PCR cycling conditions (Table 1) with fluorescently labeled forward primers (D2-PA, D3-PA, D4-PA) and unlabeled reverse primers. Amplicon volumes of 0.6 to 1.0 µL were added to 35 µL of Sample Loading Solution containing Hi-Di Formamide (Applied Biosystems) and a Genome Lab DNA Size Standard (400 kit; Beckman Coulter). Samples were placed in a 96-well sample tray and covered with mineral oil to prevent evaporation during analysis. Trays were analyzed using a Beckman Coulter Genetic Analyzer (CEQ 8000 platform). Raw data were analyzed using a Fragment Analysis Module that assigned fragment size (base pair length) based on the DNA size standard.

Allele identity was assigned via visual interpretation of microsatellite peaks. In those cases where a novel peak was observed, the reactions were repeated at least two times to verify. Fresh DNA samples were re-extracted and re-amplified in cases where PCR reactions failed to amplify for a particular locus after replication.

Data Analysis

Genetic Summary Statistics

Microsatellite genotypes were checked for the presence of null alleles, stutter products, or allelic dropout using MICRO-CHECKER (v.2.2; Van Oosterhout et al., 2004). Significant deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were tested using an exact test, with *P*-values estimated using a Markov chain method (Guo and Thompson, 1992; 10,000 dememorizations, 1,000 batches, 10,000 iterations per batch) implemented in Genepop (v4.2; Raymond and Rousset, 1995). Significance was assessed using an adjusted alpha value determined by a sequential Bonferroni correction to account for multiple comparisons (Holm, 1979). Additionally, tests for significant heterozygote deficiencies for all loci at each sample site were carried out using an exact test (Markov chain method; Guo and Thompson, 1992; 10,000 dememorizations, 1,000 batches, 10,000 iterations per batch) as implemented in Genepop (v4.2; Raymond and Rousset, 1995).

Genetic Diversity

Allele frequency data were used to estimate summary genetic diversity indices for each population. GenAlEx (v.6.5; Peakall and Smouse, 2006; Peakall and Smouse, 2012) was used to calculate the average number of alleles per locus (N_A), the effective number of alleles per locus (N_E), the number of private alleles, and observed heterozygosity (H_o). The inbreeding coefficient (F_{is}) was calculated using FSTAT (v2.9.3; Goudet, 1995). Allelic richness (AR) and unbiased expected heterozygosity (H_e) were calculated to compare variability among populations. Allelic richness is a measure of allelic diversity

that corrects for variance in sample size by rarefaction (Petit et al., 1998). Additionally, Nei and Roychoudhury's (1974) unbiased estimate of expected (Hardy-Weinberg) heterozygosity (H_e) provides a correction for small ($N < 50$) sample sizes, allowing for inter-population comparisons of average heterozygosity. Allelic richness and expected heterozygosity were calculated using the programs FSTAT (v2.9.3; Goudet, 1995) and GenAlEx (v.6.5; Peakall and Smouse, 2006; Peakall and Smouse, 2012), respectively.

Genetic Population Structure

Genetic differentiation among populations was estimated by calculating pairwise F_{st} values for all site pairs (Weir and Cockerham, 1984). Wier and Cockerham's F_{st} (1984) was calculated using the program FSTAT (v.2.9.3; Goudet, 1995). Other measures of genetic differentiation (Nei, 1973; Nei and Chesser, 1983; Jost, 2008), which correct for various sources of potential error associated with highly polymorphic loci, were calculated using the DEMEtics package (v.0.8-7; Gerlach et al., 2010) in program R (R Core Team, 2013). In the context of the current project, both Jost's D and Nei's G_{st} estimates were calculated to validate the degree of genetic divergence estimated from Wier and Cockerham's F_{st}. For all measures, *p*-values were generated using resampling (1000 bootstraps) and significance was assessed using an adjusted alpha determined by a sequential Bonferroni correction for multiple comparisons (Holm, 1979).

A hierarchical analysis of molecular variance (AMOVA) was performed using the package heirfstat (Goudet, 2005) in program R (R Core Team, 2013) in order to determine the partitioning of genetic structure related to the nested spatial scales represented by the study design. Hierarchical F-statistics were estimated at the following

four levels: (1) among individuals within reaches, (2) among reaches within watersheds, (3) among watersheds within paired watersheds, and (4) among watershed pairs. Significance was determined using likelihood-ratio statistics based on 1000 permutations.

The program STRUCTURE (v.2.3.4; Pritchard et al., 2000) was used to estimate the total number of genetic clusters (K) across all samples. Ten levels of K were tested with 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions after a burn-in of 200,000 steps, with 10 replicates for each level of K. The probability of each level of K was evaluated using the methods described by Pritchard et al. (2000) and Evanno et al. (2005). Both estimates of K were assessed using STRUCTURE HARVESTER (v. 0.6.93; Earl and vonHoldt, 2012). Bar plots were created using DISTRUCT (v.1.1; Rosenberg, 2004).

Population Parameters

The effective population size parameter, theta, and directional migration rates were estimated using a maximum-likelihood approach in the program LAMARC (v.2.0; Kuhner, 2006). Theta (Θ), which is an estimate of the effective population size (N_e) adjusted for the mutational rate of microsatellite loci (Kuhner, 2006), was estimated in order to investigate differences in N_e to assess what effect urbanization may have on population size. Effective migration rates (N_em), which represent the proportion of migrants from a source population that contribute to the gene pool of the recipient population (Beerli and Felsenstein, 2001), were calculated among streams within watershed pairs, in order to investigate the relative importance and probability of in-network and out-of-network gene flow. All estimates were carried out using 10 initial

MCMC chains of 10,000 trees and two final chains of 200,000 trees each. Each chain had a burn-in period of 1000 steps. All loci were assumed unlinked (i.e., no significant linkage disequilibrium).

Landscape Genetics Analyses

Locations of stream reaches were plotted in ARCGIS 10.1 (ESRI, 2012) using the National Hydrography Dataset (NHDplus, 2010). Two alternative gene flow models were tested in order to determine the relative importance of in-network and out-of-network gene flow in maintaining genetic population structure. All possible in-network distances were calculated using the Network Analyst extension in ARCGIS 10.1 (ESRI, 2012), which measured the shortest possible paired stream distance between all sites using the National Hydrography Dataset (NHDplus, 2010). All possible out-of-network distances were calculated using the RASTER package (v. 2.2-12; Hijmans and van Etten, 2012) in R (R Core Team, 2013), which generated a matrix of all pairwise geographic distances between sites. Mantel tests were implemented using the package ecodist (v.1.2.9; Goslee and Urban, 2007) in R (R Core Team, 2013) to test for correlations between genetic distance and both in-network and out-of-network distance.

The degree of urbanization was estimated using data from the National Land Cover Database (Fry et al., 2011) on percent impervious surface cover (%IPC) at a 30 m x 30 m cell resolution. The degree of urbanization was summarized by calculating %IPC within 500 m buffers placed around sampling sites using the Zonal Statistics extension in ARCGIS 10.1 (ESRI, 2012). The relationship between urbanization within stream drainages (%IPC within 500 m buffer around sampling localities) with both genetic

diversity (H_e and AR) and effective population size (Θ) was assessed using Spearman's rank correlations implemented in program R (R Core Team, 2013).

The relationship between dispersal resistance associated with urbanization and genetic population structuring was explored using a set of multiple regression models in R (R Core Team, 2013). Explanatory variables incorporated into these models included in-network distance (IN), out-of-network distance (OUT), and %IPC within a 500m buffer around out-of-network pathways among sites (estimated using Zonal Statistics extension). Models were ranked using Akaike's Information Criterion (AIC_c; Akaike, 1974; Hurvich and Tsai, 1989) in order to compare the explanatory power of each candidate model. Models incorporated all possible combinations of independent variables, as well as interaction terms.

RESULTS

Data Quality

A total of 313 DNA samples were successfully amplified across 6 tetranucleotide microsatellites, with sample sizes ranging from 11 to 22 individuals per stream (Table 2). Significant deviations from Hardy-Weinberg equilibrium were observed at 5.2% of all loci among stream site combinations. Possible null alleles were identified at two loci for five populations, with null allele frequencies ranging from 0.164 to 0.351 (Table 3). For most populations mean F_{is} was low (overall mean = 0.039). However, there was evidence for significant heterozygote deficiencies among individuals from Bynum Run 2 (p = 0.003; $\alpha = 0.0083$), and globally at the Dau8 locus ($p < 0.001$; $\alpha = 0.0031$). No significant linkage between loci was observed for any population.

Genetic Structure and Gene Flow

Overall, allelic diversity (mean N_A = 4.313; mean N_E = 2.856; mean AR = 3.937) and the number of private alleles (6 total) were low across all sampled streams (Table 2). The number of alleles per locus ranged from 2 (Dau6) to 14 (Dau12), with three populations exhibiting fixation at the Dau6 locus. Despite low allelic diversity, moderate levels of both observed (H_o = 0.367 - 0.692) and expected (H_e = 0.415 - 0.708) heterozygosity were found among streams (Table 2).

Spatial structuring was observed among sites, as all F_{st} comparisons were significantly different from zero. For paired population comparisons, F_{st} ranged from 0.027 to 0.405 (median = 0.137; Table 4). All other measures of genetic distance were consistent with the patterns of divergence observed with F_{st} (Tables 5-8); therefore, the following analyses use only F_{st} values. Comparisons made among paired streams were similar along both in-network (F_{st} = 0.035 - 0.156) and out-of-network pathways (F_{st} = 0.043-0.188; Fig. 5), despite the difference in mean distance along these pathways (in-network mean = 13.28 km, out-of-network mean = 2.08 km; Table 9).

The results of the AMOVA suggest significant genetic structuring at all hierarchical levels (Table 10). The greatest amount of genetic variation was accounted for among streams within watersheds (45.3%; F_{st} = 0.091). In contrast, genetic differentiation was lowest among watersheds nested within watershed pairs (F_{st} = 0.027). Genetic divergence among all watershed pairs was low (F_{st} = 0.045), with only 24.1% of genetic variance explained by differences among watershed pairs.

Results from STRUCTURE suggested a north to south gradient in spatial genetic population structure. The Pritchard et al. (2000) method suggested a K = 5, while a more conservative estimate (Evanno et al., 2005) supported a K = 2 with moderate sub-structuring (Figs. 6 and 7). The results of the STRUCTURE analysis and the concordant cluster analysis (Fig. 8), both indicated distinct genetic clustering among streams at the north (Winters Run and Bynum Run) and south (Seneca Creek and Patuxent River) end of the gradient (Fig. 7), and were generally grouped together as watershed pairs. In contrast, Jones, Gwynns, Gunpowder, and Little Gunpowder Falls watersheds exhibited a higher degree of genetic similarity, indicating higher gene flow across watershed boundaries among these watersheds.

Overall, migration rates were low (N_em = 0.105 to 0.512) among streams within each watershed pair (Tables 11-14). Migration rates were similar along in-network and out-of-network pathways. Migration was often asymmetrical between stream pairs, with higher immigration rates exceeding emigration rates (or visa versa) at many sites. Upstream migration rates were greater in five out of eight watersheds, but were greater in the downstream direction in the other four.

Mantel tests indicated weak relationships between distance along both dispersal pathways and genetic distance (F_{st}). Significant relationships were found between genetic distance and geographic distance (slope = 0.00134; 95% CI = 0.00084 – 0.00184; p = 0.001), as well as between genetic distance and stream distance (slope = 0.00018; 95% CI = 0.00010 – 0.00025; p = 0.019; Fig. 9). While the results were significant, neither geographic distance nor stream distance explained a large degree of genetic variance (Mantel r = 0.439 and 0.396, respectively).

Urbanization

Spearman's rank correlations indicated no significant relationship between %IPC within a 500 m buffer around sites and either H_e (slope = -0.002; r = -0.100; p = 0.713) or AR (slope = -0.014; r = -0.085; p = 0.754; Fig. 10). Theta values varied, ranging from 0.054 to 0.422 across all populations (Table 15). No significant correlation between Θ and the degree of urban development (%IPC) within a 500 km buffer of the stream was observed when a Spearman's rank correlation was applied (r = -0.006, p = 0.983).

Model selection indicated that the MLR model with the most support included out-of-network distance, %IPC along out-of-network dispersal paths, and their interaction (R^2 = 0.25; w_i = 0.44; Table 16). This model explains a greater degree of variance in F_{st} than either distance measure does alone. No model accounted for more than 25% of the total variance observed in pairwise genetic distances (Table 16), indicating other unmeasured parameters may be influencing genetic population structure.

DISCUSSION

Role of Multiple Dispersal Pathways

A nested, hierarchical study design was employed in order to assess how the use of multiple dispersal pathways may influence genetic population structure within and among watersheds. Significant degrees of genetic structuring were observed at multiple scales. Pairwise genetic distances varied widely, with some comparisons exhibiting strong genetic structuring. These results indicate that overall F_{st} values are greater for northern dusky salamanders when compared to other mobile salamanders capable of using out-of-network dispersal, such as *Dicamptodon tenebrosus* (Steele et al., 2009).

However, high pairwise F_{st} values are generally associated with specific populations (e.g. Seneca Falls 1) indicating that the context of the surrounding landscape may serve to functionally isolate specific populations.

Genetic population structuring was higher within watersheds than that observed among watersheds within watershed pairs, indicating higher gene flow among sites paired closely together, even along out-of-network corridors. Estimated migration rates were significant and did not differ between within-network and out-of-network comparisons, further signifying the presence of gene flow along both dispersal corridors. Likewise, STRUCTURE and cluster analyses suggest greater genetic similarity among closely associated watersheds, indicating that gene flow is occurring among proximal streams via terrestrial dispersal.

Low overall genetic divergence was observed at the landscape-scale ($F_{st} = 0.045$). For a species with limited dispersal capabilities, finding patterns of regional gene flow is somewhat surprising, and may suggest a widespread distribution of populations across headwater streams within the Piedmont region, acting as a conduit for gene flow across the landscape. Population structuring for organisms exhibiting low to moderate degrees of dispersal is expected to be shaped by distance, assuming a uniform distribution of populations across the landscape and a lack of dispersal resistance (Wright, 1943). The results of the Mantel tests largely conform to this model, and indicate a stronger relationship between overland distance and genetic distance when compared with the isolation by stream distance model, suggesting that patterns of gene flow are more associated with the ability of salamanders to move along out-of-network pathways.

Patterns of in-network gene flow generally conformed to previously established dispersal biases associated with this species and related stream salamanders. In five watersheds, net migration occurred in the upstream direction. If within-network dispersal was primarily driven by downstream drift (Muller, 1954; Muller, 1982), effective migration rates would be expected to occur uniformly in the downstream direction. However, several studies observed strong upstream dispersal biases in the adult phase of northern dusky salamanders (Grant et al., 2010), as well as several other related species (Lowe, 2003; Lowe et al., 2006; Lowe et al., 2008), presumably to avoid increased competition and predation in downstream habitats. This seems to be a phenomenon common among other unrelated headwater taxa, such as stream-dwelling macroinvertebrates (Fairbairn, 1985; Elliott, 2003; Macneale et al., 2005), indicating that the net movement of individuals in the upstream direction is an important characteristic, which may promote population stability when not impeded by habitat fragmentation (Smith et al., 2009).

Linking Gene Flow and Genetic Structure

High degrees of gene flow occurring among closely associated watersheds suggest that northern dusky salamander populations conform to the headwater model of gene flow proposed by Finn et al. (2007). While out-of-network gene flow was expected, given that previous studies established the use of terrestrial dispersal in Desmognathine salamanders (Grant et al., 2010; Wooten and Rissler, 2011), both the magnitude and relative importance in determining genetic population structure was surprising. Previous studies of genetic structuring in other stream-dwelling salamanders indicate stronger conformities to the stream hierarchy model (Meffe and Vrijenhoek, 1988), with genetic

population structure largely driven by gene flow within watersheds. However, this may be largely attributed to the specific life histories of the species studied, with a bias for large, charismatic species that are either facultative paedomorphs (*Dicamptodon copei*; Steele et al., 2009) or largely sedentary (*Cryptobranchus alleganiensis*; Unger et al., 2013). The above-mentioned characteristics restrict these species to the water column itself, unlike semi-aquatic salamanders, which are capable of moving into adjacent riparian habitat and may exhibit substantial capacities for out-of-network movement (e.g., *Dicamptodon tenebrosus*; Steele et al., 2009).

To date, only one previous study directly addressed the effect of out-of-network gene flow in Desmognathine salamanders. Wooten and Rissler (2011) observed evidence of out-of-network gene flow between black-bellied salamander (*D. quadramaculatus*) populations occupying separate stream networks, supporting the notion that terrestrial dispersal facilitates gene flow between populations. Wooten and Rissler (2011) found that patterns of phylogenetic structure conformed more to distinctions between terrestrial ecoregions and not to freshwater ecoregions, suggesting that out-of-network dispersal facilitates gene flow among associated streams. The patterns observed in the current study lend support for continued, contemporary out-of-network gene flow when compared to the broad, historic patterns observed by Wooten and Rissler (2011).

Observed patterns of genetic population structuring in northern dusky salamanders are more akin to the patterns observed in highly mobile stream insects. Wilcock et al. (2003) observed little genetic structuring between European caddisfly (*Plectrocnemia conspersa*) populations; even among streams separated by watershed divides. In both cases, widespread genetic exchange occurred across a large region with

habitat quality presumably driving considerable variation in the degree of genetic differentiation between streams. However, the magnitude of pairwise genetic divergence was greater in salamanders, with the difference in magnitude most likely representative of the relative ability of adult insects to disperse easily across long distances due to their ability to fly. While the results of the Mantel tests generally conformed to similar results observed in other taxa with limited terrestrial dispersal abilities (*Metacnephia coloradensis,* Finn et al., 2006; *Abedus herberti,* Finn et al., 2007; *Ephemerella invaria,* Alexander et al., 2011), geographic distance alone failed to explain a large proportion of the observed variance among pairwise F_{st} comparisons.

Urbanization

Factors which increase landscape resistance, such as anthropogenic disturbance, may account for a portion of the unexplained variance observed among pairwise F_{st} comparisons, particularly for comparisons that exhibit large degrees of genetic divergence over relatively short distances. Anthropogenic disturbance associated with urbanization in particular, has been shown to increase genetic structuring in Plethodontid salamanders (Noel and Lapointe, 2010; Munshi-South et al., 2013), as well as other stream-dwelling organisms (*Plectrocnemia conspersa*, Wilcock et al., 2003; *Coenagrion mercuriale*, Watts et al., 2004). The effects of urbanization would be expected to increase dispersal resistance, thereby leading to the isolation of populations that may otherwise share significant gene flow with closely associated streams. This loss of gene flow associated with watershed urbanization is a key conservation concern, as it may limit the ability of headwater organisms to offset the negative effects of downstream drift (Smith et al., 2009). Both increased genetic structuring associated with the loss of

dispersal among populations and decreased genetic diversity related to reductions in overall effective population sizes commonly associated with disturbed habitats (Watts et al., 2004; Ewers and Didham, 2006) may negatively impact the adaptive capacity of affected populations.

Moderate degrees of urbanization were observed within close proximity to several sampled stream reaches. No statistically significant relationship existed between genetic diversity and the degree of impervious surface coverage occurring within a watershed. However, both heterozygosity and allelic diversity were generally lower in streams experiencing the highest degrees of urbanization within closely associated upland habitat, although the loss of genetic diversity was less than predicted. This may indicate that populations may be resistant to the effects of moderate development. Munshi-South et al. (2013) observed significant and substantial losses of genetic diversity of northern dusky salamander populations found in severely urbanized streams (H_e = 0.151 to 0.293), which is not consistent with the relatively high degree of genetic diversity observed in the current study. While other studies implicate recent watershed urbanization with reductions in census population densities (Orser and Shure, 1972; Price et al., 2006; Price et al., 2011), the results of the current study would suggest that the effective number of breeders might not be significantly impacted by the degree of urbanization observed in this study. However, both time scale and the overall low degree of diversity observed across all sampled loci may obscure the relationship between the loss of genetic diversity and moderate degrees of landscape depreciation.

Overall, the results of the model selection process suggest that the model that best explained the variance in genetic distance incorporated the additive and interactive

effects of out-of-network distance and amount of impervious surface cover along out-of-network pathways. In this case, the degree of urbanization observed along out-of-network pathways appears to increase resistance to gene flow. This affect was most apparent among closely associated stream reaches. These findings reiterate the importance of out-of-network movements in facilitating gene flow and suggest that salamanders may be more sensitive to development at more local scales, where contemporary barriers may disrupt movements. These results are consistent with the findings of Munshi-South et al. (2013), who observed significant genetic isolation (F_{st} = 0.35 to 0.55) when comparing populations occurring within New York City to populations occurring in the surrounding rural/suburban landscape. Likewise, Wilcock et al. (2003) observed increased genetic structuring in urbanized settings for a highly mobile stream insect that otherwise showed high degrees of gene flow over large spatial scales.

While the model containing both distance and %IPC was more informative than isolation by distance models alone, it still failed to account for a large amount of the variance observed in pairwise genetic distance measures. This suggests that other unmeasured landscape parameters are exerting a significant degree of influence on dispersal patterns in northern dusky salamanders. For instance, Little Gunpowder 2 exhibited moderate degrees of isolation in the absence of any signature of urbanization in close proximity to the stream reach (0% IPC). Comparisons between rural and urban sites within the current study design may have been confounded by the presence of other anthropogenic activities, which may affect both genetic population structure and genetic diversity. The rural sites used in this study may have been disturbed due to the presence of agricultural activity in close proximity to study areas. Agricultural land use may affect

habitat quality and serve to functionally isolate populations along out-of-network pathways. Agricultural practices may lead to reductions in core riparian habitat, increased surface runoff, erosion, and the input of contaminants, all of which may negatively impact salamander populations (Willson and Dorcas, 2003).

Conservation Implications

While often assumed, the degree of gene flow associated with out-of-network movements may be under-represented in the literature for semi-aquatic, stream-dwelling organisms. Maintaining the capacity for dispersal is imperative when addressing the stability of headwater stream populations, especially since directed dispersal plays a role in offsetting passive downstream drift (Hershey et al., 1993), predator evasion (Lowe, 2003; Macneale et al., 2005), and avoidance of disturbance events (Perry and Bond, 2009; Robson et al., 2011). Headwater species significantly contribute to the overall biodiversity and ecosystem function of stream networks, even though their respective distributions are patchy and confined to low-order reaches (Meyer et al., 2007). Headwater species often fill a variety of roles that influence the habitat quality of downstream reaches, and serve as functional links in the trophic web of both terrestrial and stream systems (Meyer et al., 2007; Wipfli et al., 2007). The conservation, maintenance, and restoration of habitat within the upland matrix may therefore directly promote population resiliency in headwater networks, thereby supporting overall biodiversity and ecosystem function within stream networks and their associated riparian corridors.

These results provide support for the overall importance of both in-network and out-of-network dispersal in maintaining patterns of local and landscape-scale gene flow.

In particular, these results suggest that the conservation of both terrestrial and aquatic habitat in and around closely associated headwater streams is important for maintaining gene flow among these populations. Given the relationship between genetic structure and gene flow, further study is needed to determine how the topology and spatial arrangement of river networks may either facilitate or impede gene flow among streams. Likewise, understanding the effects of varying degrees of anthropogenic disturbance is important when addressing the conservation of organisms characterized by limited dispersal capacities and spatially discrete population structuring. Disturbance associated with deforestation, urbanization, and agricultural land-use are all expected to negatively impact stream habitat quality and population dynamics of headwater organisms (Allan, 2004; Lowe and Likens, 2006; Smith et al., 2009). Addressing questions regarding how natural and human-mediated processes shape patterns of gene flow in species utilizing headwater streams should be a key conservation concern when addressing the overall health of lotic landscapes, given the importance of these streams to the overall ecologic integrity of stream networks.

Table 1. Sequences of six tetranucleotide microsatellites, dye color, and optimal annealing temperature of PCR reaction used in analysis of *Desmognathus fuscus* genetic population structuring.

Locus	Primer Sequence	Dye Color	Annealing Temperature
Dau6*	F: GATCGCACGTTAAATAA	D4-PA (Blue)	53°
	R: GGCAGGAAAAGGTTAG		
Dau8*	F: GGAAACACCAGAAAAAGT	D3-PA (Green)	52°
	R: AAGCAGGATTAGGTGAATA		
Dau11*	F: GTCCCTCAGGCTTGATAAG	D4-PA (Blue)	56°
	R: TGTGCCTATCCAGTCATCTA		
Dau12*	F: CGACTTCTGAAACAACAAC	D4-PA (Blue)	56°
	R: CGGTTCTGAATTCCTTAC		
Doc1**	F: TGTGAAGGGTGTTCTCTTACTG	D2-PA (Black)	54°
	R: GCTGTTTGTGCTTTGACTTTAC		
Doc3**	F: CTCTCCCACTCTTCCTCAAGTA	D4-PA (Blue)	54°
	R: CTTCACCTTCGCTATGACTGT		

Primer Citations:
* Croshaw and Glenn, 2003
** Adams et al., 2005

Table 2. Sample size (N), number of private alleles (P), and mean diversity indices across 6 microsatellite loci from *Desmognathus fuscus* sampled at 16 stream locations in the Baltimore Metropolitan area. N_A–average number of alleles per locus; N_E–effective number of alleles per locus; H_o–observed heterozygosity; H_e–unbiased expected heterozygosity; AR–allelic richness; F_{is}–inbreeding coefficient.

Watershed	Reach	N	Private Alleles	N_A	N_E	H_o	H_e	AR	F_{is}
Bynum Run	Bynum 1	11	1	3.167	2.034	0.500	0.499	3.167	0.061
	Bynum 2	20	0	3.167	2.088	0.367	0.415	2.757	0.139*
Winters Run	Winter 1	20	0	3.500	2.400	0.508	0.479	3.310	-0.027
	Winter 2	20	1	4.500	2.707	0.525	0.565	4.043	0.072
Little Gunpowder Falls	Little 1	20	0	4.167	3.110	0.658	0.660	4.052	0.029
	Little 2	20	1	4.167	2.494	0.517	0.508	3.762	0.066
Gunpowder Falls	Gun 1	20	0	4.833	3.580	0.675	0.708	4.542	0.083
	Gun 2	20	0	5.333	3.169	0.692	0.638	4.634	0.035
Jones Falls	Jones 1	20	1	4.833	2.913	0.692	0.618	4.266	-0.110
	Jones 2	20	0	5.167	3.213	0.625	0.672	4.593	0.098
Gwynns Falls	Gwynn 1	22	1	5.167	3.481	0.720	0.671	4.594	-0.040
	Gwynn 2	20	0	4.500	3.433	0.617	0.675	4.326	0.102
Patuxent River	Patuxt 1	20	0	3.333	2.465	0.533	0.554	3.133	0.038
	Patuxt 2	20	1	4.833	2.838	0.500	0.513	4.205	0.026
Seneca Creek	Seneca 1	20	0	4.000	2.892	0.533	0.532	3.712	0.003
	Seneca 2	20	1	4.333	2.876	0.567	0.601	3.890	0.059

* Value is significantly different ($p = 0.003$; $\alpha = 0.0083$) from zero.

Table 3. Summary table of possible null alleles and their estimated degree of homozygote excess (F_{is}) for 6 microsatellite loci from 16 *Desmognathus fuscus* populations in the Baltimore Metropolitan area, with null allele frequencies estimated using the Van Oosterhout (2004) method.

Stream Reach	Locus	Null Allele Frequency	F_{is}
Winter 1	Dau8	0.2639	0.530
Jones 2	Dau8	0.3514	0.769
Gwynn 2	Dau8	0.3086	0.701
Patuxt 1	Doc1	0.3407	0.877
Patuxt 2	Dau8	0.1639	0.355

Table 4. Pairwise F_{st} values (below diagonal) and associated *p*-values (above diagonal) measured among *Desmognathus fuscus* populations sampled in the Baltimore Metropolitan area. Significance was based on 1,000 permutations.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
BYN2	0.135	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
WIN1	0.157	0.152	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
WIN2	0.144	0.135	0.156	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
LIT1	0.093	0.207	0.209	0.161	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
LIT2	0.212	0.263	0.274	0.185	0.097	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
GUN1	0.147	0.191	0.187	0.129	0.043	0.132	0	0.0004	0.0004	0.0004	0.0004	0.0017	0.0004	0.0004	0.0004	0.0004
GUN2	0.113	0.189	0.216	0.112	0.052	0.098	0.047	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
JON1	0.145	0.175	0.162	0.106	0.090	0.155	0.070	0.076	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
JON2	0.112	0.198	0.107	0.121	0.079	0.156	0.071	0.071	0.116	0	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
GWY1	0.095	0.166	0.183	0.129	0.040	0.140	0.027	0.056	0.088	0.092	0	0.0008	0.0004	0.0004	0.0004	0.0004
GWY2	0.097	0.134	0.137	0.068	0.061	0.140	0.029	0.032	0.046	0.043	0.035	0	0.0004	0.0004	0.0004	0.0004
PAT1	0.175	0.194	0.154	0.159	0.159	0.267	0.099	0.127	0.087	0.137	0.108	0.094	0	0.0004	0.0004	0.0004
PAT2	0.155	0.200	0.145	0.157	0.176	0.256	0.155	0.153	0.091	0.156	0.129	0.104	0.043	0	0.0004	0.0004
SEN1	0.311	0.357	0.344	0.300	0.212	0.405	0.161	0.272	0.231	0.237	0.179	0.197	0.188	0.287	0	0.0004
SEN2	0.164	0.133	0.167	0.146	0.110	0.252	0.062	0.136	0.113	0.128	0.075	0.071	0.079	0.137	0.121	0

Table 5. Pairwise D values (below diagonal) and associated *p*-values (above diagonal) measured among *Desmognathus fuscus* populations sampled in the Baltimore Metropolitan area. Significance was based on 1,000 permutations.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
BYN2	0.183	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
WIN1	0.235	0.208	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
WIN2	0.242	0.172	0.211	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LIT1	0.177	0.285	0.240	0.300	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LIT2	0.328	0.201	0.178	0.158	0.215	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
GUN1	0.320	0.336	0.335	0.310	0.266	0.269	0	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001
GUN2	0.237	0.271	0.331	0.385	0.335	0.345	0.296	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
JON1	0.281	0.234	0.217	0.320	0.379	0.342	0.290	0.337	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001
JON2	0.244	0.329	0.179	0.304	0.214	0.338	0.234	0.176	0.307	0	0.001	0.001	0.001	0.001	0.001	0.001
GWY1	0.198	0.254	0.333	0.244	0.161	0.272	0.145	0.168	0.225	0.262	0	0.002	0.001	0.001	0.001	0.001
GWY2	0.224	0.240	0.285	0.188	0.214	0.336	0.149	0.141	0.169	0.176	0.130	0	0.001	0.001	0.001	0.001
PAT1	0.295	0.300	0.234	0.314	0.311	0.446	0.216	0.265	0.181	0.287	0.255	0.240	0	0.004	0.001	0.001
PAT2	0.261	0.335	0.269	0.304	0.315	0.409	0.294	0.281	0.195	0.312	0.261	0.232	0.096	0	0.001	0.001
SEN1	0.470	0.448	0.470	0.411	0.387	0.655	0.330	0.456	0.350	0.389	0.359	0.305	0.272	0.405	0	0.001
SEN2	0.304	0.217	0.260	0.288	0.263	0.466	0.191	0.305	0.300	0.276	0.227	0.213	0.173	0.270	0.219	0

Table 6. Pairwise D_{EST} values (below diagonal) and associated *p*-values (above diagonal) measured among *Desmognathus fuscus* populations sampled in the Baltimore Metropolitan area. Significance was based on 1,000 permutations.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
BYN2	0.155	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
WIN1	0.203	0.212	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
WIN2	0.206	0.192	0.244	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LIT1	0.131	0.292	0.297	0.303	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LIT2	0.301	0.362	0.360	0.308	0.166	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
GUN1	0.280	0.316	0.300	0.272	0.129	0.250	0	0.001	0.001	0.001	0.002	0.003	0.001	0.001	0.001	0.001
GUN2	0.198	0.262	0.313	0.231	0.111	0.208	0.121	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
JON1	0.246	0.318	0.267	0.238	0.173	0.271	0.166	0.167	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001
JON2	0.203	0.301	0.140	0.271	0.168	0.310	0.185	0.125	0.270	0	0.001	0.001	0.001	0.001	0.001	0.001
GWY1	0.156	0.224	0.304	0.203	0.117	0.236	0.094	0.121	0.186	0.220	0	0.002	0.001	0.001	0.001	0.001
GWY2	0.182	0.209	0.254	0.146	0.170	0.307	0.093	0.092	0.125	0.126	0.077	0	0.001	0.001	0.001	0.001
PAT1	0.266	0.280	0.205	0.290	0.278	0.425	0.176	0.233	0.146	0.253	0.223	0.207	0	0.006	0.001	0.001
PAT2	0.231	0.316	0.242	0.277	0.280	0.385	0.254	0.247	0.161	0.278	0.224	0.194	0.058	0	0.001	0.001
SEN1	0.442	0.426	0.443	0.379	0.351	0.635	0.289	0.421	0.314	0.349	0.325	0.260	0.240	0.375	0	0.001
SEN2	0.272	0.192	0.228	0.258	0.225	0.445	0.145	0.270	0.272	0.235	0.191	0.173	0.139	0.238	0.181	0

Table 7. Pairwise G_{st} values (below diagonal) and associated *p*-values (above diagonal) measured among *Desmognathus fuscus* populations sampled in the Baltimore Metropolitan area. Significance was based on 1,000 permutations.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	NA	NA	NA	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001
BYN2	0.090	0	NA	NA	0.001	NA	0.001	0.001	NA	0.001	0.001	0.001	0.001	NA	0.001	0.001
WIN1	0.095	0.073	0	NA	0.001	NA	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	0.001	0.001
WIN2	0.100	NA	0.096	0	0.001	NA	0.001	0.001	NA	0.001	0.001	0.001	0.001	NA	0.001	0.001
LIT1	0.073	0.154	0.151	0.119	0	0.001	0.004	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001
LIT2	0.124	0.154	0.162	0.106	0.076	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
GUN1	0.099	0.136	0.133	0.100	0.034	0.099	0	0.005	0.001	0.001	0.031	0.012	0.001	0.001	0.001	0.001
GUN2	0.072	0.120	0.133	0.079	0.042	0.063	0.040	0	0.001	0.001	0.001	0.011	0.001	0.001	0.001	0.001
JON1	0.088	0.102	0.097	0.068	0.065	0.095	0.052	0.050	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001
JON2	0.078	0.136	0.080	0.087	0.054	0.101	0.051	0.049	0.070	0	0.001	0.003	0.001	0.001	0.001	0.001
GWY1	0.068	0.125	0.130	0.103	0.031	0.102	0.024	0.043	0.063	0.062	0	0.002	0.001	0.001	0.001	0.002
GWY2	0.066	0.097	0.093	0.059	0.045	0.091	0.029	0.028	0.033	0.033	0.033	0	0.001	0.001	0.001	0.001
PAT1	0.105	0.123	0.098	0.109	0.100	0.169	0.064	0.076	0.056	0.083	0.070	0.060	0	0.002	0.001	0.001
PAT2	0.089	0.099	0.070	0.104	0.125	0.152	0.107	0.091	0.059	0.101	0.094	0.072	0.041	0	0.001	0.001
SEN1	0.222	0.258	0.253	0.230	0.157	0.315	0.127	0.209	0.173	0.179	0.133	0.155	0.137	0.209	0	0.001
SEN2	0.102	0.098	0.116	0.110	0.074	0.177	0.044	0.091	0.071	0.084	0.050	0.052	0.052	0.092	0.097	0

Table 8. Pairwise $G_{st.EST}$ values (below diagonal) and associated *p*-values (above diagonal) measured among *Desmognathus fuscus* populations sampled in the Baltimore Metropolitan area. Significance was based on 1,000 permutations.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	NA	NA	NA	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	0.001	0.001
BYN2	0.072	0	NA	NA	0.001	NA	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	0.001	0.001
WIN1	0.077	0.060	0	NA	0.001	NA	0.001	0.001	NA	0.001	0.001	0.001	0.001	NA	0.001	0.001
WIN2	0.082	NA	0.083	0	0.001	NA	0.001	0.001	NA	0.001	0.001	0.001	0.001	NA	0.001	0.001
LIT1	0.055	0.142	0.139	0.107	0	0.001	0.005	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001
LIT2	0.106	0.142	0.150	0.094	0.064	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	0.001	0.001
GUN1	0.081	0.124	0.121	0.088	0.021	0.087	0	0.002	0.001	0.001	0.031	0.016	0.001	0.001	0.001	0.001
GUN2	0.054	0.107	0.121	0.067	0.029	0.050	0.027	0	0.001	0.001	0.001	0.013	0.001	0.001	0.001	0.001
JON1	0.071	0.090	0.084	0.055	0.052	0.083	0.040	0.037	0	0.001	0.001	0.002	0.001	0.001	0.001	0.001
JON2	0.060	0.123	0.067	0.074	0.041	0.088	0.038	0.037	0.057	0	0.001	0.004	0.001	0.001	0.001	0.001
GWY1	0.051	0.113	0.118	0.092	0.019	0.090	0.012	0.031	0.051	0.050	0	0.003	0.001	0.001	0.001	0.001
GWY2	0.048	0.085	0.080	0.046	0.032	0.078	0.017	0.016	0.021	0.020	0.021	0	0.001	0.001	0.001	0.001
PAT1	0.087	0.110	0.086	0.097	0.087	0.157	0.052	0.063	0.043	0.071	0.058	0.047	0	0.001	0.001	0.001
PAT2	0.071	0.087	0.058	0.091	0.112	0.140	0.094	0.078	0.047	0.089	0.082	0.059	0.029	0	0.001	0.001
SEN1	0.205	0.248	0.242	0.220	0.144	0.305	0.115	0.198	0.162	0.167	0.121	0.143	0.125	0.197	0	0.001
SEN2	0.084	0.085	0.103	0.097	0.061	0.165	0.032	0.078	0.058	0.071	0.038	0.040	0.039	0.079	0.085	0

Table 9. Pairwise in-network (below diagonal) and out-of-network distances (above diagonal) measured among sampling localities in the Baltimore Metropolitan area. All distances are expressed in kilometers (km). Bolded values indicate the distances between stream pairings established in the hierarchical study design.

Pop	BYN1	BYN2	WIN1	WIN2	LIT1	LIT2	GUN1	GUN2	JON1	JON2	GWY1	GWY2	PAT1	PAT2	SEN1	SEN2
BYN1	0	8.18	**0.86**	9.34	16.33	16.48	17.94	17.52	36.18	36.74	36.56	38.25	75.12	72.85	79.41	75.27
BYN2	**12.88**	0	8.68	**2.29**	22.84	20.74	24.11	21.98	38.51	38.04	39.08	39.36	76.98	74.13	81.16	76.75
WIN1	56.93	46.68	0	9.68	15.49	15.65	17.08	16.68	35.39	36.00	35.76	37.52	74.34	72.09	78.63	74.50
WIN2	42.92	32.67	**16.83**	0	22.70	19.90	23.81	21.17	36.98	36.31	37.59	37.58	75.25	72.29	79.40	74.95
LIT1	104.26	94.01	107.32	93.30	0	7.09	**1.96**	6.67	24.48	26.87	24.48	28.55	62.38	61.02	66.75	63.07
LIT2	98.73	88.48	101.79	87.78	**12.32**	0	6.48	**1.28**	20.09	21.50	20.35	23.14	58.94	57.00	63.27	59.27
GUN1	115.43	105.19	118.50	104.48	89.61	84.09	0	5.75	22.64	25.17	22.62	26.86	60.44	59.10	64.81	61.14
GUN2	111.21	100.96	114.27	100.25	85.38	79.86	**19.70**	0	19.37	21.04	19.58	22.69	58.12	56.28	62.46	58.52
JON1	136.23	125.98	139.30	125.28	144.95	139.42	156.12	151.90	0	4.82	**1.02**	5.99	38.95	36.91	43.25	39.19
JON2	132.61	122.37	135.68	121.66	141.33	135.80	152.51	148.28	**10.84**	0	5.77	**1.68**	38.95	36.21	43.11	38.75
GWY1	141.29	131.04	144.35	130.34	150.00	144.48	161.18	156.95	77.13	73.51	0	6.83	38.60	36.71	42.92	38.93
GWY2	131.39	121.14	134.45	120.43	140.10	134.57	151.28	147.05	67.23	63.61	**11.46**	0	37.69	34.80	41.81	37.39
PAT1	341.08	330.83	344.14	330.13	349.79	344.27	360.97	356.74	334.70	334.70	339.76	329.86	0	6.02	**4.38**	3.57
PAT2	334.43	324.19	337.50	323.48	343.15	337.62	354.33	350.10	328.06	324.44	333.11	323.21	**11.50**	0	8.15	**3.18**
SEN1	468.92	458.67	471.99	457.97	477.64	472.11	488.81	484.59	462.55	458.93	467.60	457.70	499.35	492.70	0	4.98
SEN2	468.53	458.28	471.60	457.58	477.25	471.72	488.42	484.20	462.16	458.54	467.21	457.31	498.94	492.31	**10.73**	0

Table 10. Results of the four-level AMOVA, highlighting the hierarchical partitioning of genetic variance among all streams, as related to the nested sampling scheme.

Source of Variation	*df*	Variance Components	Percentage of Variation	F statistics	*P*
Among all watershed pairs	3	0.187	24.1	0.045	0.006
Among watersheds within pairs	4	0.105	13.6	0.027	0.040
Among stream reaches within watersheds	4	0.351	45.3	0.091	0.001
Within stream reaches	614	0.132	17.0		

Table 11: Unidirectional effective migration rates (N_em) among streams within the Bynum Run and Winters Run watersheds, with the migrant source stream listed in the first column, and the recipient stream listed in the first row.

Pop	Bynum 1	Bynum 2	Winter 1	Winter 2
Bynum 1	-	0.192	0.312	0.105
Bynum 2	0.426	-	0.257	0.140
Winter 1	0.512	0.286	-	0.152
Winter 2	0.253	0.206	0.105	-

Table 12: Unidirectional effective migration rates (N_em) among streams within the Little Gunpowder Falls and Gunpowder Falls watersheds, with the migrant source stream listed in the first column, and the recipient stream listed in the first row.

Pop	Little 1	Little 2	Gun 1	Gun 2
Little 1	-	0.269	0.252	0.365
Little 2	0.416	-	0.273	0.269
Gun 1	0.340	0.256	-	0.350
Gun 2	0.303	0.195	0.457	-

Table 13: Unidirectional effective migration rates (N_em) among streams within the Jones Falls and Gwynns Falls watersheds, with the migrant source stream listed in the first column, and the recipient stream listed in the first row.

Pop	Jones 1	Jones 2	Gwynn 1	Gwynn 2
Jones 1	-	0.195	0.273	0.463
Jones 2	0.323	-	0.219	0.280
Gwynn 1	0.415	0.216	-	0.349
Gwynn 2	0.355	0.335	0.461	-

Table 14: Unidirectional effective migration rates (N_em) among streams within the Patuxent River and Seneca Creek watersheds, with the migrant source stream listed in the first column, and the recipient stream listed in the first row.

Pop	Patuxt 1	Patuxt 2	Seneca 1	Seneca 2
Patuxt 1	-	0.328	0.152	0.179
Patuxt 2	0.167	-	0.339	0.183
Seneca 1	0.120	0.351	-	0.386
Seneca 2	0.129	0.247	0.219	-

Table 15: Mutation-rate adjusted, effective population size estimates (Θ) summarized for all *Desmognathus fuscus* sampled in the Baltimore Metropolitan area.

Watershed	Locality	Θ	Support Interval
Bynum Run	Bynum 1	0.422	0.345 - 0.532
	Bynum 2	0.120	0.104 - 0.139
Winters Run	Winter 1	0.117	0.103 - 0.134
	Winter 2	0.054	0.047 - 0.064
Little Gunpowder Falls	Little 1	0.205	0.180 - 0.236
	Little2	0.100	0.087 - 0.115
Gunpowder Falls	Gun 1	0.165	0.144 - 0.191
	Gun 2	0.168	0.143 - 0.196
Jones Falls	Jones 1	0.235	0.207 - 0.271
	Jones 2	0.122	0.107 - 0.140
Gwynns Falls	Gwynn 1	0.123	0.108 - 0.141
	Gwynn 2	0.153	0.135 - 0.174
Patuxent River	Patuxt 1	0.080	0.070 - 0.095
	Patuxt 2	0.306	0.268 - 0.351
Seneca Creek	Seneca 1	0.143	0.125 - 0.163
	Seneca 2	0.146	0.128 - 0.169

Table 16. Results of multiple linear regression model selection based on Akaike's Information Criterion (AIC) for models explaining variation in genetic distance between *Desmognathus fuscus* populations as a function of distance along hypothetical dispersal pathways and landscape resistance represented as percent urbanization. Variables included in the models include OUT = geographic distance; IN = stream distance; IPC = % impervious surface cover. Models are arranged from most informative to least informative.

Model	**k**	**AIC_c**	**ΔAIC_c**	**w_i**	**R^2**
OUT + IPC + OUT:IPC	5	-656.86	0.00	0.44	0.25
IN + OUT + IPC + OUT:IPC	6	-654.75	2.11	0.15	0.25
IN + OUT + IPC + IN:OUT + OUT:IPC	7	-652.92	3.94	0.06	0.25
IN + OUT +IPC + IN:IPC + OUT:IPC	7	-652.58	4.29	0.05	0.25
IN + OUT	4	-652.38	4.48	0.05	0.06
OUT	3	-652.34	4.52	0.05	0.19
IN + OUT + IN:OUT	5	-652.22	4.64	0.04	0.22
OUT + IPC	4	-652.01	4.85	0.04	0.20
IN + OUT + IPC + IN:OUT	6	-651.19	5.67	0.03	0.23
IN + OUT + IPC	5	-651.07	5.79	0.02	0.21
IN + OUT + IPC + IN:IPC	6	-651.07	5.80	0.02	0.23
IN + OUT + IPC + IN:IPC + IN:OUT	7	-650.76	6.10	0.02	0.24
IN + OUT + IPC + IN:IPC + IN:OUT + OUT:IPC	8	-650.64	6.22	0.02	0.25
IN + OUT + IPC + IN:IPC + IN:OUT + OUT:IPC + IN:OUT:IPC	9	-648.32	8.54	0.01	0.25
IN	3	-647.12	9.75	0.00	0.16
IN + IPC	4	-645.32	11.54	0.00	0.16
IN + IPC + IN:IPC	5	-643.20	13.66	0.00	0.16
IPC	3	-630.55	26.31	0.00	0.03

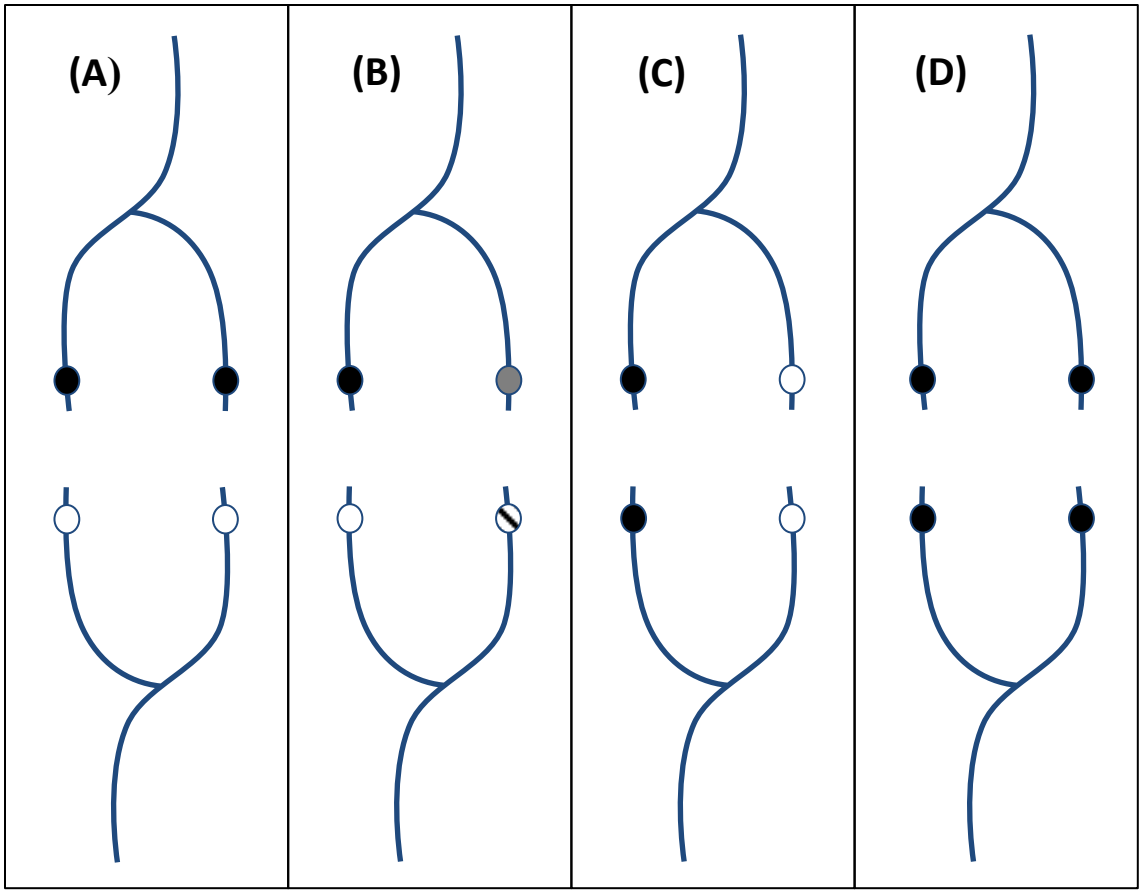

Figure 1: Four contrasting models of genetic population structuring across headwater networks. Each pane contains two adjacent watersheds with each colored circles representing distinct localities. (A) The stream hierarchy model predicts high degrees of gene flow within watersheds, but relative isolation between adjacent networks; (B) The Death-Valley model predicts that fragmentation and restricted dispersal capacities limits gene flow, leading to high degrees of genetic divergence between reaches; (C) The headwater model predicts that limited terrestrial dispersal will facilitate gene flow between closely associated headwater streams, with limited in-network gene flow due to headwater specialization; (D) The widespread gene flow model predicts that organisms with strong propensities for terrestrial and aquatic dispersal will exhibit widespread gene flow without regards to out-of-network boundaries. Figure adapted from Finn et al. (2007) and Hughes et al. (2009).

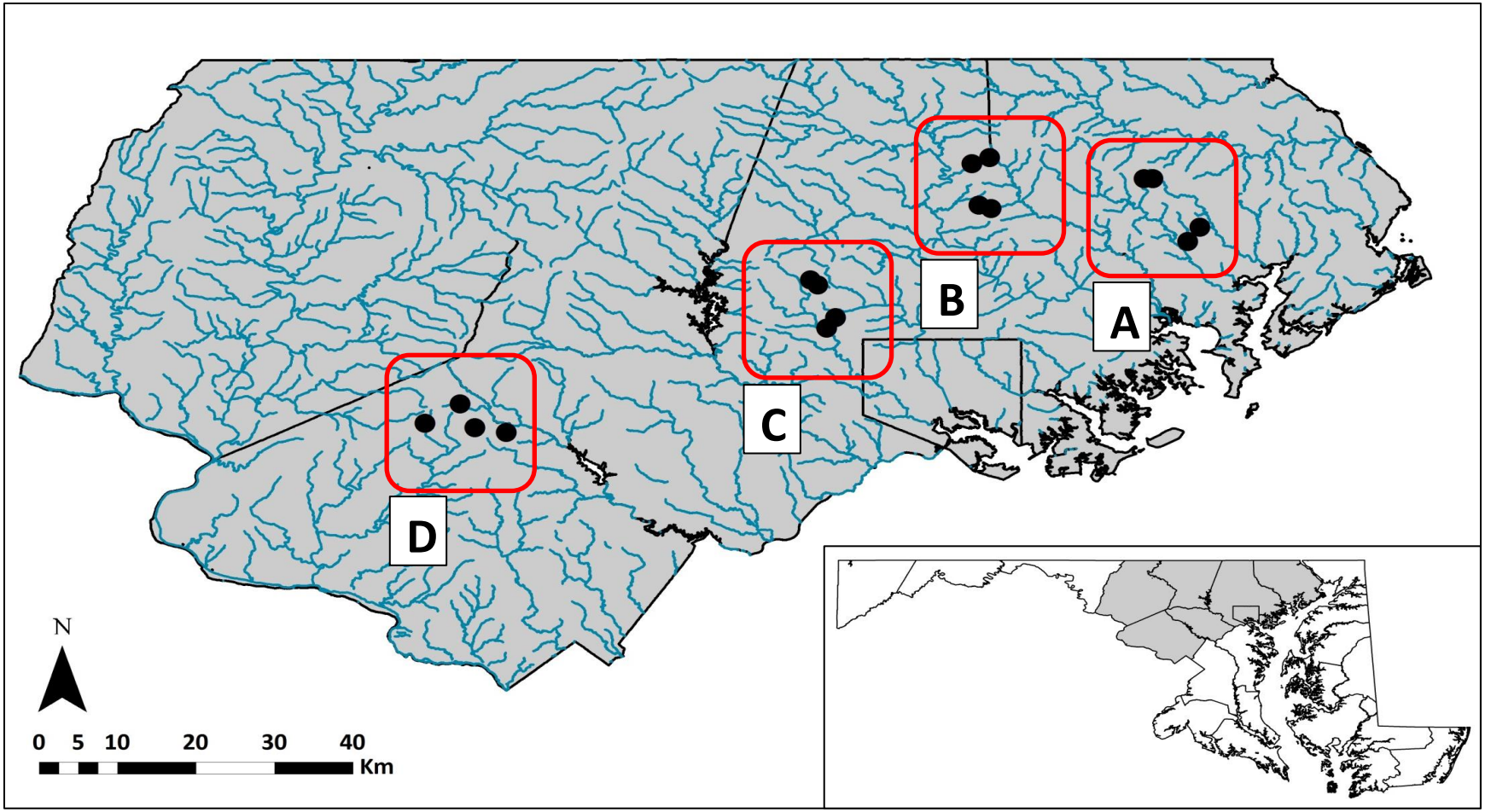

Figure 2. Map of sampled streams within piedmont counties in Maryland. Boxes indicate paired watersheds (A = Bynum Run–Winters Run; B = Little Gunpowder Falls–Gunpowder Falls; C = Jones Falls–Gwynns Falls; D = Patuxent River–Seneca Creek).

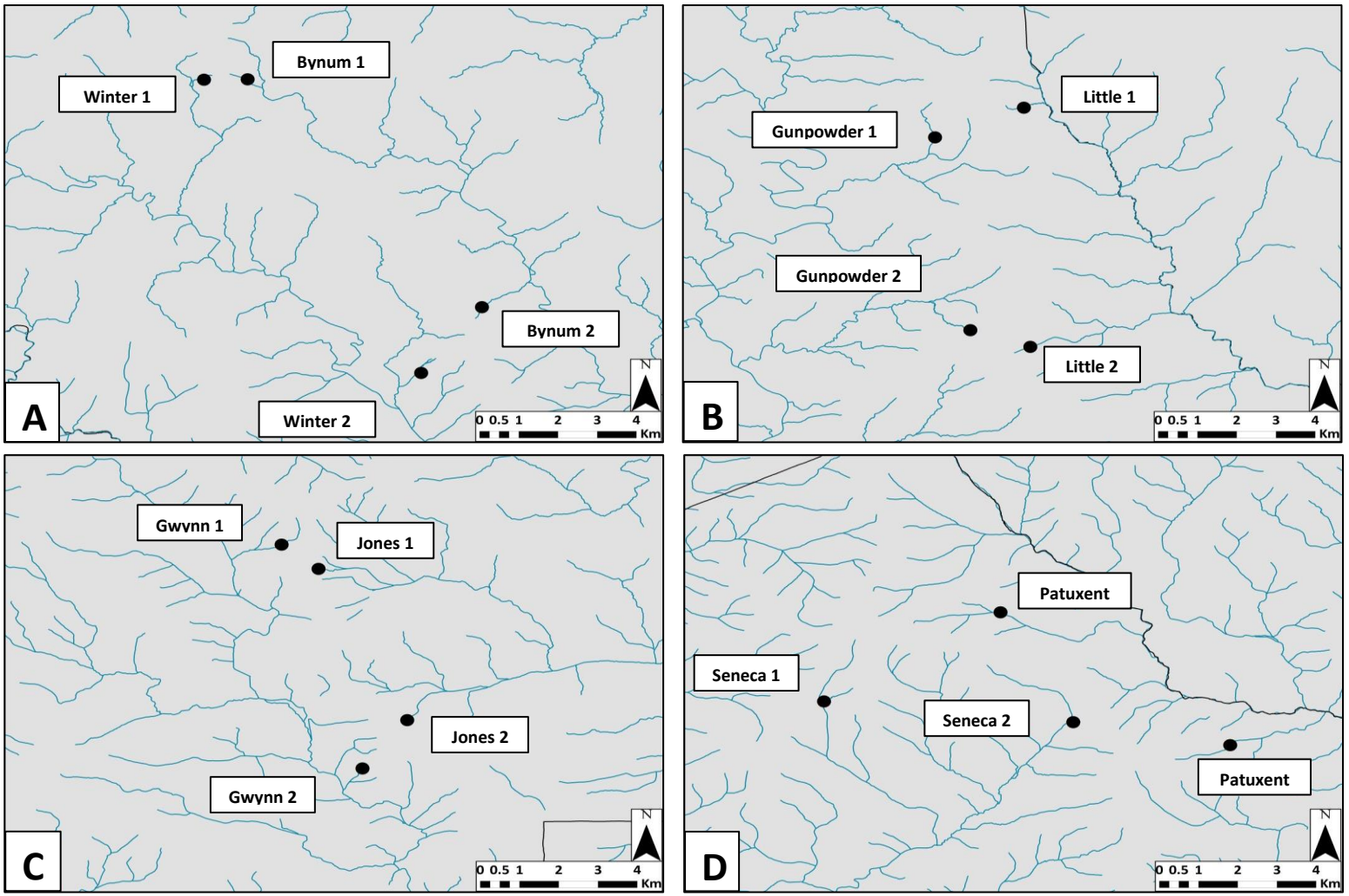

Figure 3. Map of sampled stream reach location within each pair of watersheds (A = Bynum Run–Winters Run, B = Little Gunpowder Falls–Gunpowder Falls, C = Jones Falls–Gwynns Falls, D = Patuxent River–Seneca Creek).

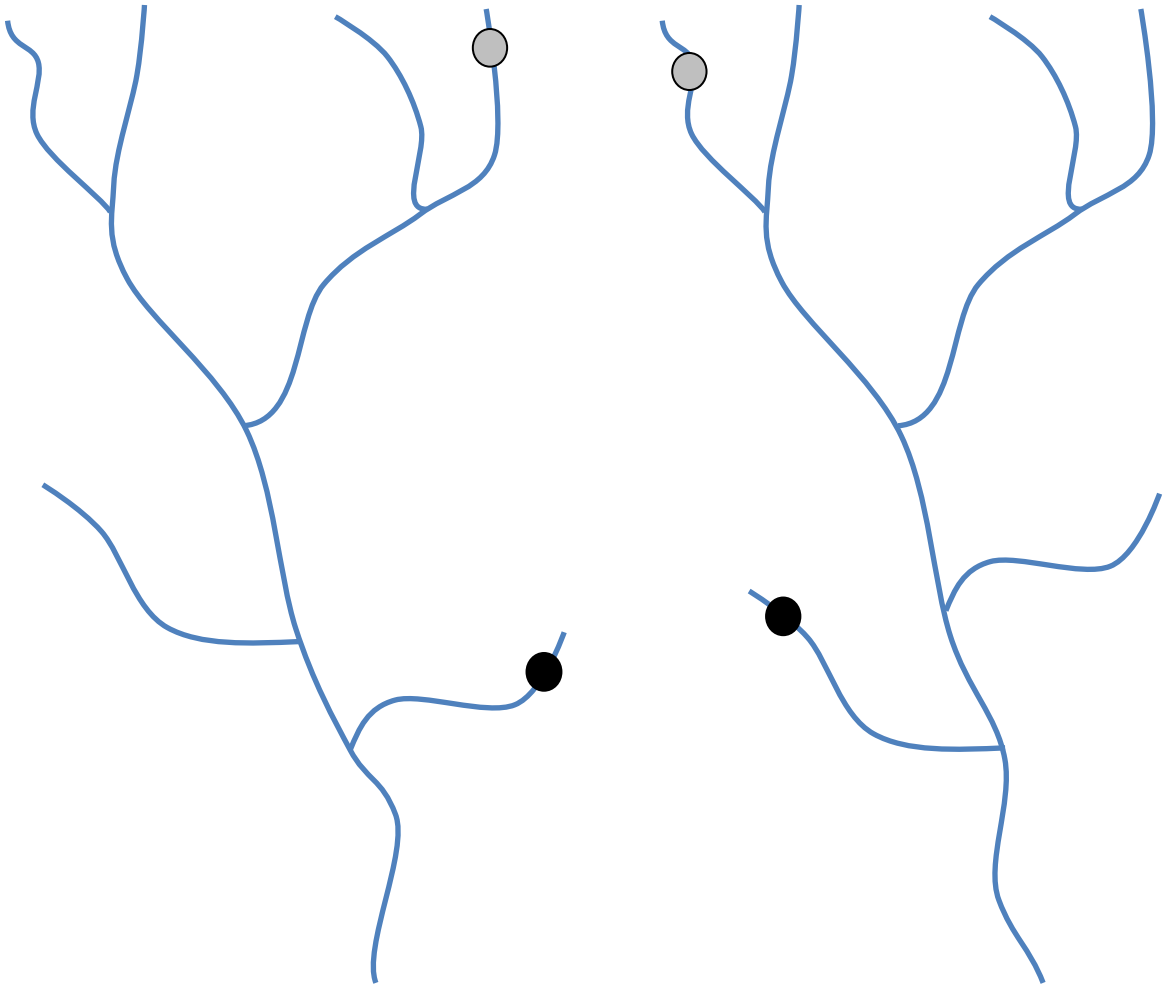

Figure 4: Graphical representation of the hierarchical sampling scheme employed in this study. Tissue samples were collected in two stream reaches within each watershed, intended to represent headwater streams (gray) and adventitious tributaries (black). Streams were paired with associated streams in a neighboring watershed. Streams paired within each watershed were separated by the main stream channel, which represents suboptimal habitat for headwater specialists. Neighboring watersheds were functionally isolated along in-network pathways by either distance (> 15 km) or a functional barrier to movement (e.g. the Chesapeake Bay).

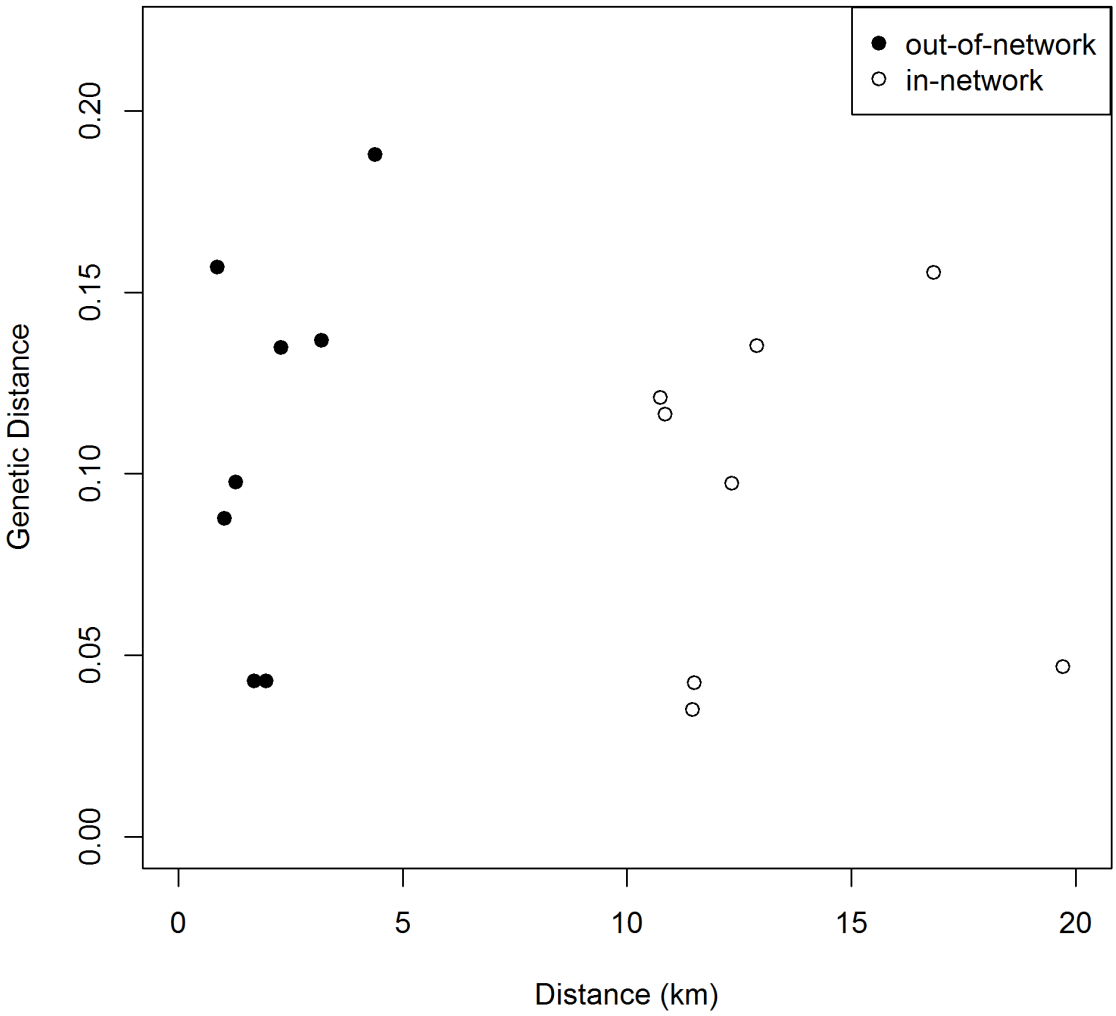

Figure 5: Plot comparing genetic distance (F_{st}) with distance along in-network and out-of-network distances between *Desmoganthus fuscus* populations. Distances are relative to the paired comparisons established in the nested study design.

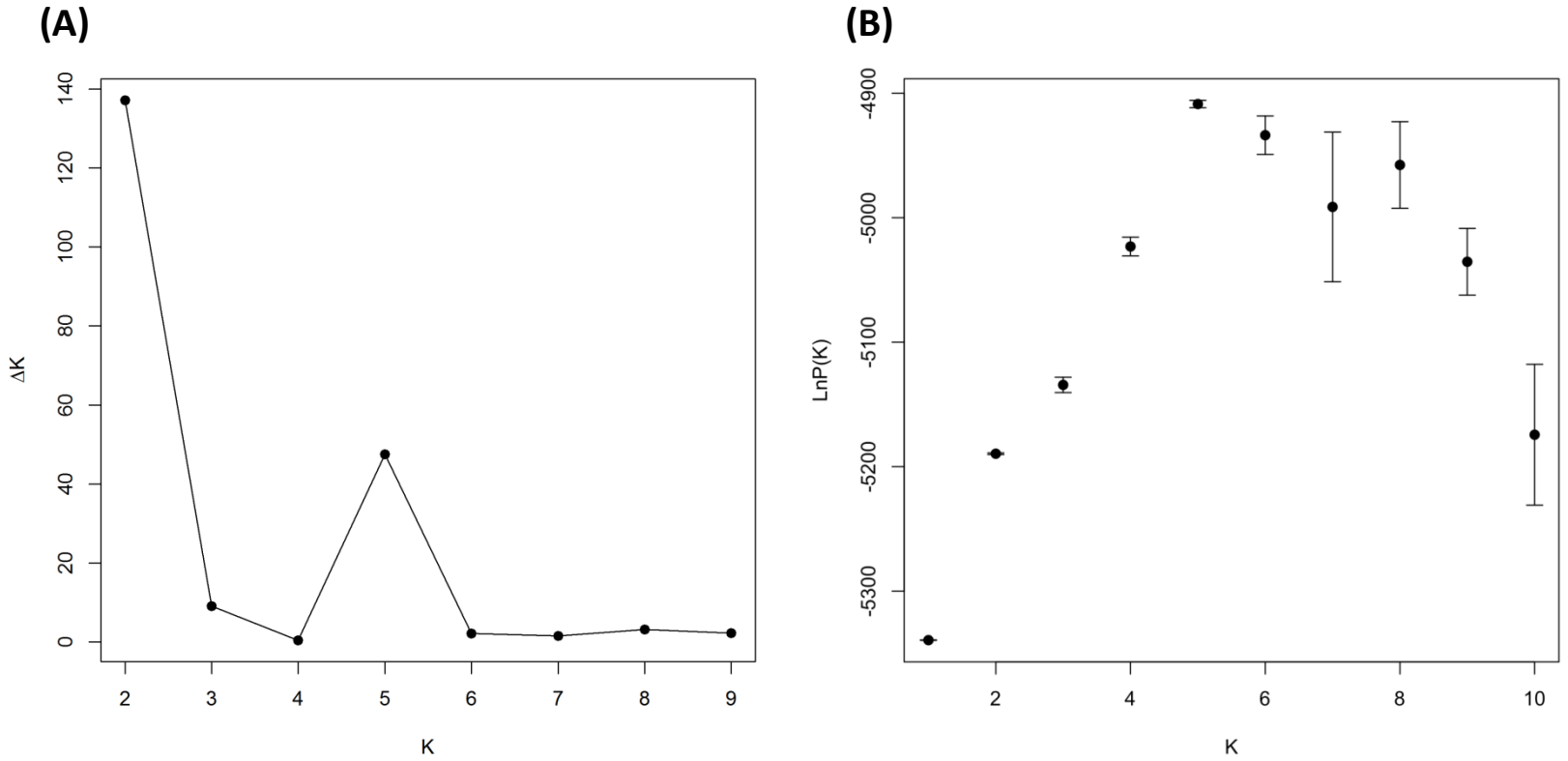

Figure 6: Estimations of the number of putative genetic clusters (K) for *Desmognathus fuscus* populations found in the Baltimore Metropolitan region. Two methods were used to estimate K: (A) the Evanno et al. (2005) method based on the ΔK statistic; and (B) the Pritchard et al. (2000) method based on the mean log probability of each level of K.

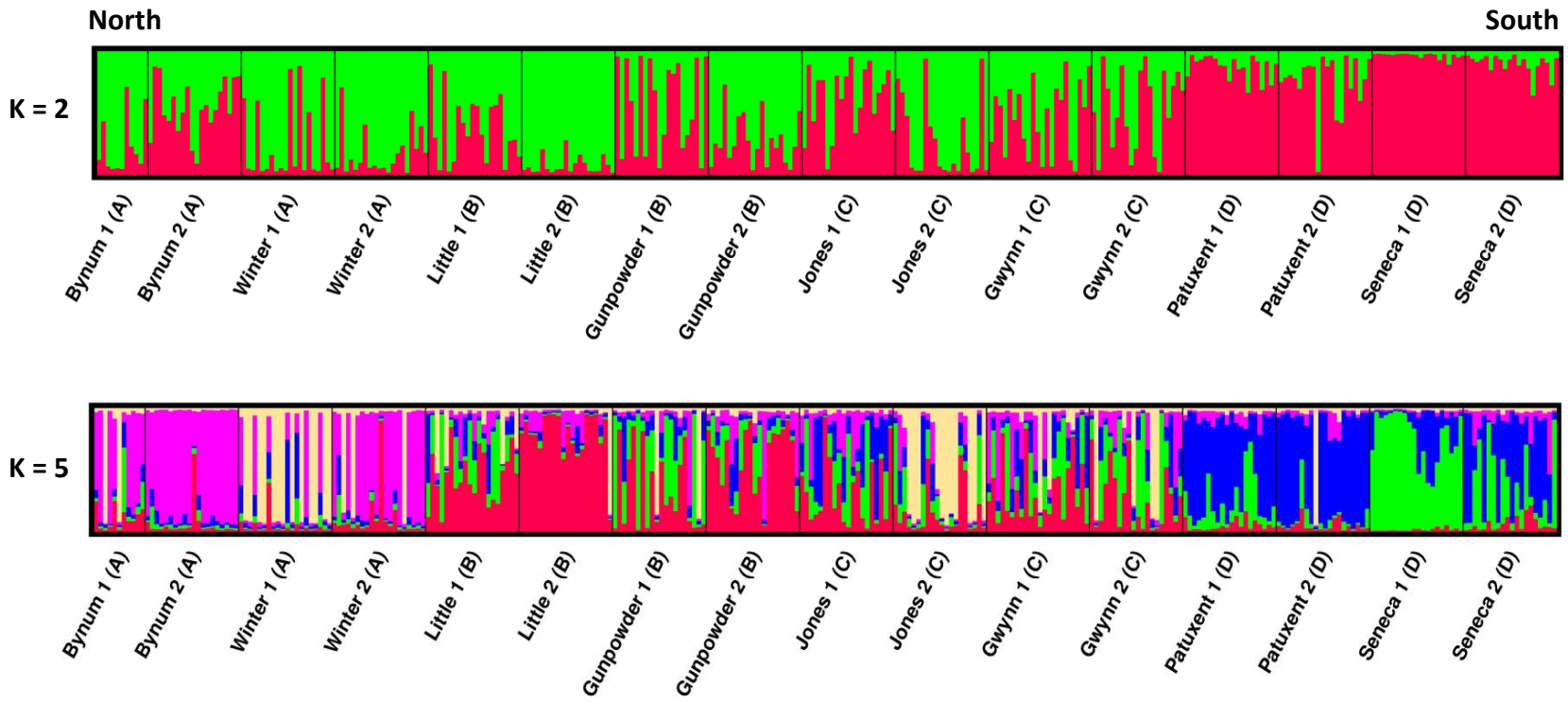

Figure 7. Bar plot representing the results from Bayesian assignment tests performed in STRUCTURE, for both a K = 2 and K = 5. Names of sampled streams are listed below each bar plot, with watershed pairs indicated in parenthesis after stream labels. Streams are arranged along a north–south gradient (from left to right). Colors on individual bars represent the probability of that individual being assigned to genetic units.

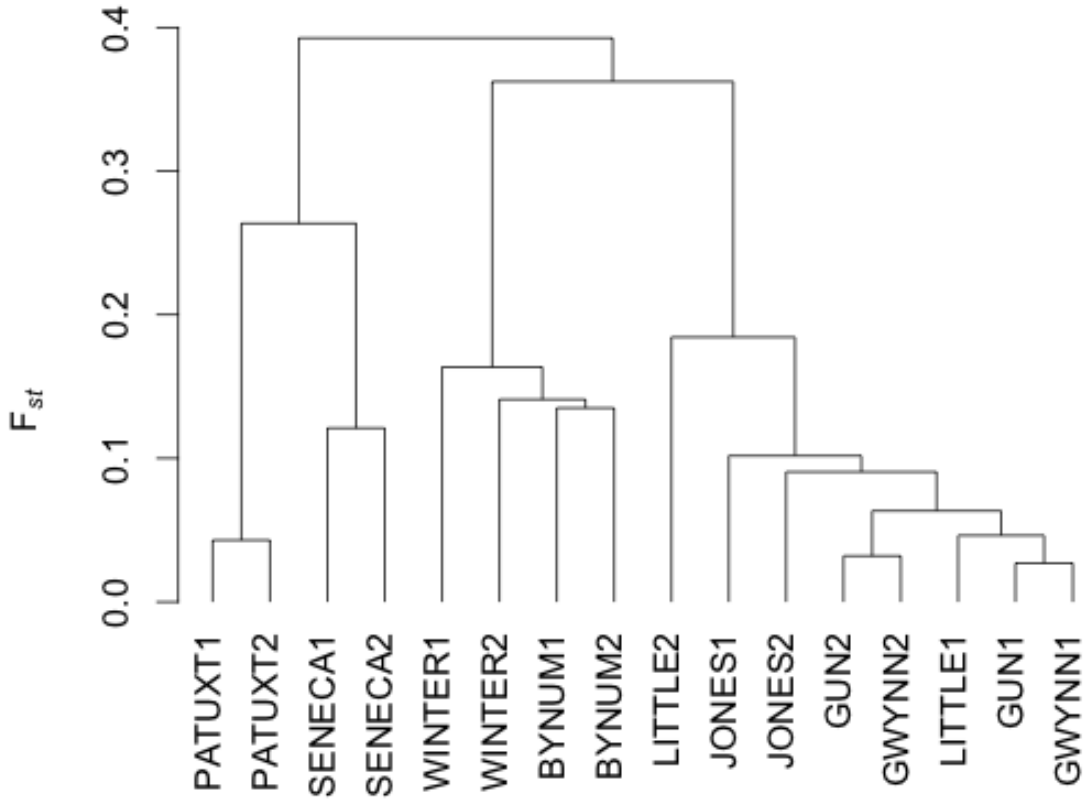

Figure 8. Cluster analysis results for populations of *Desmognathus fuscus* inhabiting the Baltimore Metropolitan area. Branch length represents the degree of genetic divergence (F_{st}) occurring between branches.

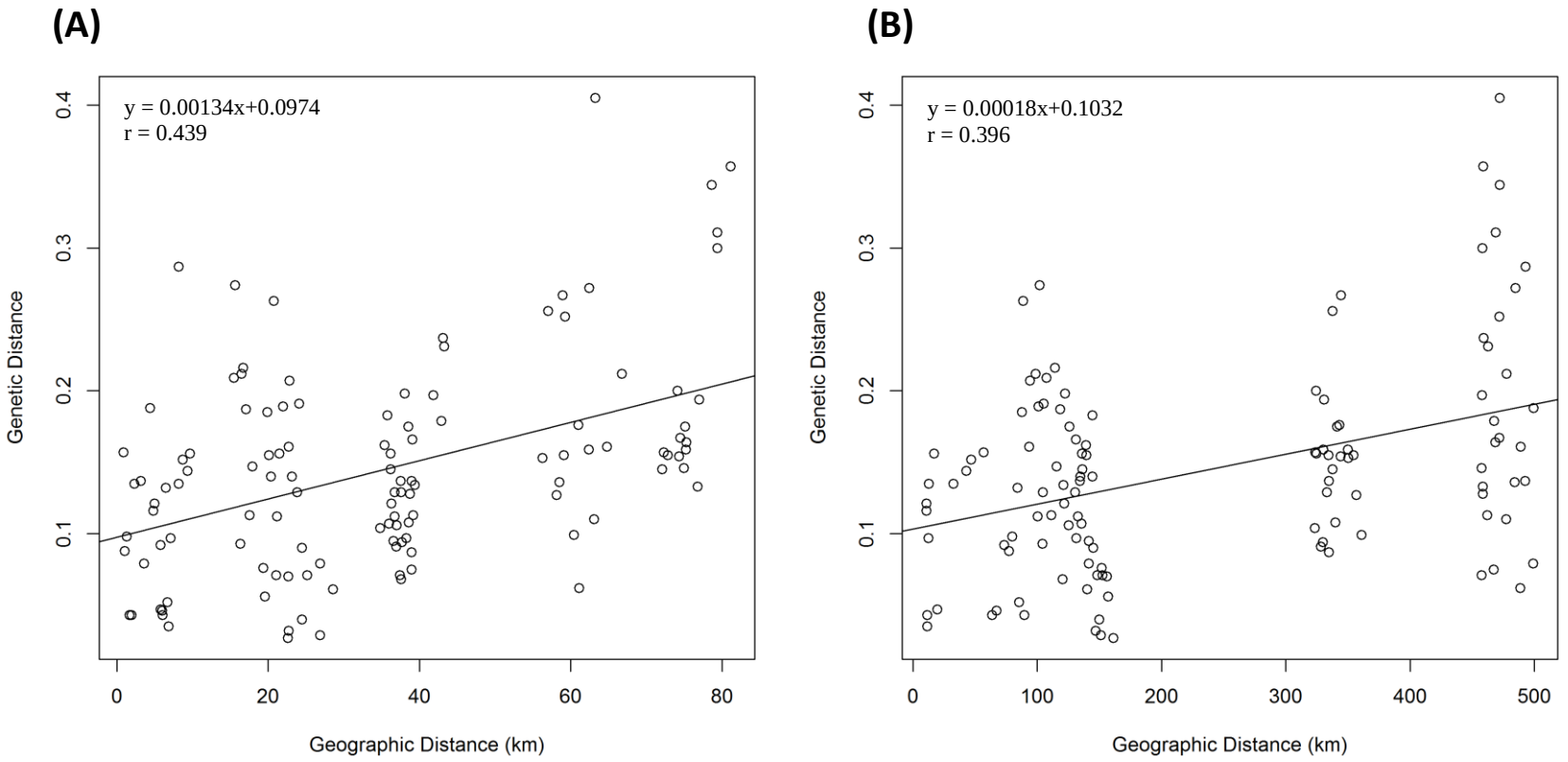

Figure 9. Isolation by distance plots representing the correlation between genetic distance (F_{st}) and geographic distance (km) along out-of-network dispersal pathways (geographic distance; A), and in-network dispersal pathways (stream distance; B).

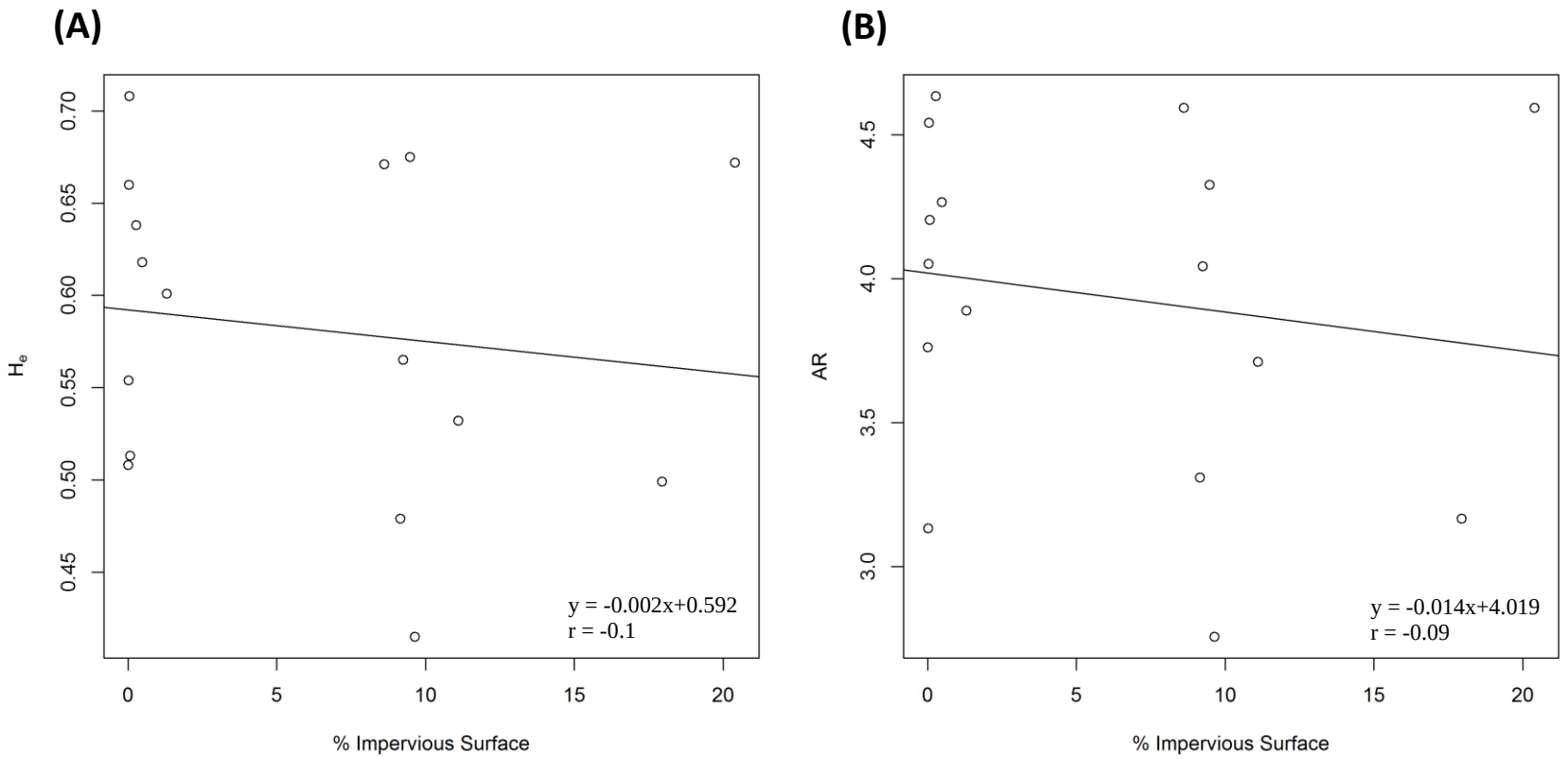

Figure 10. Relationship between watershed urbanization, estimated using %IPC within a 500m buffer around sampled stream, and standardized genetic diversity measures: (A) unbiased heterozygosity; (B) allelic richness.

APPENDIX A

IACUC Approval Notification

TOWSON UNIVERSITY

Department of Biological Sciences

Towson University
8000 York Road
Towson, MD 21252-0001

T. 410-704-3042
F. 410-704-2405

To: William Miller <wmille7@students.towson.edu>

From: Liliana Rao <LRao@vetmed.umd.edu>

Subject: IACUC Approval

I am writing to inform you of the approval of the protocol **IACUC # 071312 JS-03** in title "The effects of urbanization on gene population structure of a streamside Salamander (*Desmognathus fuscus*) over multiple spatial scales"

The approval for this protocol is, July 13, 2012. Your protocol is approved for a period of 3 years and Dr Louis DeTolla, Chair, IACUC TU will send an official letter of approval in near future.

Best Regards,

Liliana

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WILLIAM L. MILLER

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EDUCATION

Institutions Attended	Dates	Degree
Messiah College, Grantham, PA	2007-2011	B.S. Biology (Cum Laude)
Towson University, Towson, MD	2011-2014	M.S. Biology

PROFESSIONAL EXPERIENCE

Research Assistant (January 2014 – August 2014)
Towson University Department of Biological Sciences, Towson, MD
Teaching Assistant (September 2011 – December 2013)
Towson University Department of Biological Sciences, Towson, MD
Molecular Ecology Research Internship (May 2011 – August 2011)
Au Sable Institute of Environmental Studies, Mancelona, MI
Greenhouse and Animal Caretaker Assistant (Fall 2008 – May 2011)
Messiah College Department of Biological Sciences, Grantham, PA
Nature Interpreter and Kayak Guide (May 2010 – August 2010)
Whidbey Island Sea Kayaking Company, Langley, WA
Prairie Restoration Research Internship (May 2009 – August 2010)
Pacific Rim Institute for Environmental Stewardship, Coupeville, WA

PRESENTATIONS AND CONFERENCES

Miller, W., J. Snodgrass, and G. Gasparich. The Effects of Urbanization on Gene Flow and Genetic Population Structure in a Headwater Salamander, *Desmognathus fuscus*, Over Multiple Spatial Scales. Au Sable Institute of Environmental Studies Annual Research Symposium, Au Sable Institute of Environmental Studies, Mancelona, MI, August 2013. (Presentation)

Miller, W., G. Gasparich, and J. Snodgrass. The Effects of Urbanization on Gene Flow and the Genetic Structure of a Headwater Salamander, *Desmognathus fuscus*, Over Multiple Spatial Scales. 26th International Congress for Conservation Biology, Baltimore, MD, July 2013. (Poster)

Miller, W., J. Snodgrass, and G. Gasparich. The Effects of Urbanization on Gene Flow and Genetic Population Structure in a Headwater Salamander, *Desmognathus fuscus*, Over Multiple Spatial Scales. 2013 Joint Meeting of Ichthyologists and Herpetologists, Albuquerque, NM, July 2013. (Presentation)

Miller, W., J. Snodgrass, and G. Gasparich. The Effects of Urbanization on Genetic Differentiation and Gene Flow in a Headwater Salamander, *Desmognathus fuscus*, Over Multiple Spatial Scales. 14th Annual Student Research and Scholarship Expo, Towson University, Towson, MD, April 2013. (Poster)

Miller, W., and T. Evans. Effects of Habitat Fragmentation by Forest Roads on the Genetic Structure of Northern Red-backed Salamanders (*Plethodon cinereus*). Au Sable Institute of Environmental Studies Annual Research Symposium, Au Sable Institute of Environmental Studies, Mancelona, MI, August 2011. (Presentation)
Miller, W., and D. Foster. A Comparative Study of Fire Ecology and Small Mammal Effects on Vegetative Communities within a Puget Sound Shortgrass Prairie Remnant. Pennsylvania Academy of Science Annual Meeting, Pennsylvania State University Altoona Campus, Altoona, PA, April 2011. (Presentation)
M**iller, W**., and D. Foster. A Comparative Study of Fire Ecology and Small Mammal Effects on Vegetative Communities within a Puget Sound Shortgrass Prairie Remnant. Messiah College SOHNS Scholarship Symposium, Grantham, PA, December 2010. (Presentation and Poster)
Miller, W., and D. Foster. Initial Survey of Vegetation and Small Mammal Populations on a Puget Sound Shortgrass Prairie Remnant. Au Sable Institute Integrative Session, Pacific Rim Institute for Environmental Stewardship, Coupeville, WA, July 2010. (Presentation)
Miller, W., and D. Foster. Initial Survey of Vegetation and Small Mammal Populations on a Puget Sound Shortgrass Prairie Remnant. Messiah College SOHNS Scholarship Symposium, Messiah College, Grantham, PA, December 2009. (Presentation and Poster)
2008:
Kras, L., A. Derr, A. Stuckert, M. Thomas, and **W. Miller**. Unique Foraging Behavior of the Tricolored Heron. Messiah College SOHNS Scholarship Symposium, Messiah College, Grantham, PA, April 2008. (Poster)

PROFESSIONAL ASSOCIATIONS

Society for Conservation Biology
American Society of Ichthyologists and Herpetologists
Sigma Zeta (National Science and Mathematics Honors Society)
Beta Beta Beta (National Biological Honor Society)

HONORS/AWARDS

Field Naturalist Certification (Au Sable Institute)
Environmental Analyst Certification (Au Sable Institute)
Land Analyst Certification (Au Sable Institute)
Water Resource Analyst Certification (Au Sable Institute)
Provost Scholarship Recipient (Messiah College)
Pennsylvania Fish and Boat Commission: Conservation Award