FINAL REPORT

Hydroecology of Intermittent and Ephemeral Streams: Will Landscape Connectivity Sustain Aquatic Organisms in a Changing Climate?

SERDP Project RC-1724

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14. ABSTRACT

This project aims to understand how Southwest intermittent and ephemeral (dryland) streams provide critical habitat and population connectivity for obligatory aquatic species. The project examined how hydrology, hydrologic connectivity, and other riverine characteristics influence the community structure and population genetics (e.g. gene flow, structure, diversity) of amphibian and aquatic insect species across a gradient of flow permanence within Fort Huachuca and the surrounding Sky Island mountain ranges.

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Amphibians, aquatic invertebrates, climate change, frog, genetics, groundwater withdrawals, hydrology, hydrologic connectivity, intermittent rivers, landscape ecology, metapopulations, toad

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Abstract

Objective: This project aims to understand how Southwest intermittent and ephemeral (dryland) streams provide critical habitat and population connectivity for obligatory aquatic species. The project examined how hydrology, hydrologic connectivity, and other riverine characteristics influence the community structure and population genetics (e.g. gene flow, structure, diversity) of amphibian and aquatic insect species across a gradient of flow permanence within Fort Huachuca and the surrounding Sky Island mountain ranges. Specifically, the project addressed four main objectives that aim to provide both the science and management tools needed to ensure the conservation of aquatic species on DoD military lands in a rapidly changing environment. First, stream flow was measured to quantify flow permanence and hydrologic connectivity at multiple spatial scales. Second, the distribution and abundance of aquatic insects were characterized and modeled in relation to hydrology, riparian vegetation, and geomorphology. Third, population structure (gene flow) of insect and amphibian species was evaluated with contrasting life histories along a gradient of flow permanence and hydrologic connectivity.

Technical Approach: Quantifying the extreme spatiotemporal variability in streamflow of dryland rivers remains an ongoing challenge. This project addressed this knowledge gap by deployed electrical resistance sensors (novel approach to quantify streamflow occurrence at fine temporal intervals) at 40+ locations across Fort Huachuca to quantify network-scale longitudinal hydrological connectivity. Next, patterns and drivers of aquatic invertebrate communities were accessed using empirical field collections and statistical modeling. Invertebrate diversity was examined from multiple streams in Fort Huachuca that span a flow permanence continuum from highly intermittent to perennial, and the relative roles of flow permanence, habitat size, season, and microhabitat in determining taxonomic and functional (trait) structure were quantified. Then, information was combined on local and regional habitat characteristics to explain spatial patterns of invertebrate diversity and tested whether these patterns were predictable based on species' dispersal abilities. Finally, population structure (gene flow) and landscape genetics of amphibian and aquatic invertebrate species with contrasting life histories were investigated. How species' ecological strategies affect the regional balance of gene flow was examined within three amphibians [the canyon treefrog (*Hyla arenicolor*), red-spotted toad (*Anaxyrus punctatus*), and Mexican spadefoot (*Spea multiplicata*)] and three aquatic insects [*Abedus herberti* (Hemiptera: Belostomatidae), *Mesocapnia arizonensis* (Plecoptera: Capniidae), and *Boreonectes aequinoctialis* (Coleoptera: Dytiscidae)]. These species characterize a range of ecological strategies, driven primarily by different water dependencies and dispersal abilities, enabling species survival in arid and semiarid environments. Finally, project research examined a suite of hypothesized relationships between genetic connectivity and landscape connectivity across all species.

Results: Substantial within and across canyon hydrologic variability was evident during the study period, which lead to differences in patterns of longitudinal connectivity. Increased flow permanence of streams in Fort Huachuca was associated with increased functional richness, functional evenness, and taxonomic richness of invertebrate communities. Conversely, drying events reduced functional diversity across all measured indices. A saturating relationship was identified between functional richness and taxonomic richness, indicating functional redundancy in species-rich communities, which may promote resilience of ecosystem function to

environmental variation. The results also suggest that both local and regional factors influenced the structure of invertebrate communities, and the importance of each factor depended on the dispersal capacities of the organisms. Local and weak dispersers were more affected by site-specific factors, intermediate dispersers by landscape-level factors, and strong dispersers showed no discernable pattern. Unlike most other studies of dendritic networks, the results suggest that overland pathways, using perennial refugia as stepping-stones, might be the main dispersal routes in fragmented stream networks.

A positive relationship existed between population differentiation and water dependency for amphibians: e.g., longer larval development periods and site fidelity for reliable water sources. Global genetic differentiation was highest for canyon treefrogs, intermediate for redspotted toads, and lowest for Mexician spadefoots. Strong hierarchical clustering was present for canyon treefrogs with spatial clustering by mountain range. Red-spotted toads had moderate hierarchical structure with complex spatial patterns of genetic connectivity. Mexican spadefoots had little hierarchical structure with diffuse spatial clustering. Aquatic connectivity exhibited importance for all amphibian species, particularly when considered with topography (slope). The effect of spatial scale differed by species, with canyon treefrogs and Mexican spadefoots characterized by relatively consistent results at different scales in contrast to the stark differences in results for red-spotted toads at different scales. Direction and strength of the relationships between genetic distances and geographic distances between sampling localities matched predictions of population genetic structure according to invertebrate species' dispersal abilities. Moderate-disperser *Mesocapnia arizonensis* has a strong isolation-by-distance pattern, suggesting migration-drift equilibrium, whereas population structure in the flightless *Abedus herberti* is influenced by genetic drift and gene flow is the dominant force in the strong-flying *Boreonectes aequinoctialis*. Analyses also identified a strong spatial scale-dependence, in which landscape genetic methods performed well only for species that were intermediate dispersers.

Benefits: Rapid environmental change and limited management resources necessitate efficient and effective conservation planning that promotes the persistence of aquatic species in dryland environments. The completed research highlights the key role of hydrology in determining aquatic invertebrate diversity in dryland streams of Fort Huachuca and surrounding Sky Island mountain ranges. These findings emphasize the need to manage river systems for organisms that span a wide variety of dispersal abilities and local ecological requirements, and they highlight the need to preserve perennial refugia in fragmented networks, as they may ensure the viability of aquatic communities by facilitating recolonization after disturbance.

Knowledge of population attributes such as structure, connectivity, and genetic integrity are a fundamental part of successful conservation. Here, landscape genetic approaches were applied to integrate population genetics with emerging spatial statistics to examine how hydrology and the terrestrial matrix affects population genetic structure, diversity, and differentiation of obligatory aquatic species. Research findings highlight the utility and potential of species' ecological traits, in this case water dependency for amphibians and dispersal for aquatic invertebrates, in characterizing relationships between genetic and structural (landscape) connectivity. Genetic diversity is often a missing component in conservation planning and resource allocation despite its recognized role in species persistence. With increasing human demand for aquatic resources in arid environments, environmental change and habitat alteration will likely outpace the

resources and time necessary for single-species population genetics studies for many species of conservation concern. Using ecological information to predict relationships between genetic and landscape connectivity is a promising approach for multi-taxa inference and may help inform conservation efforts in which single-species genetic studies are not possible.

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List of Acronyms

AIC, Akaike's information criterion
DDR, distance decay relationship
DEM, digital elevation model
DoD, Department of Defense
EPA, Environmental Protection Agency
ER, electrical resistance
FEve, functional evenness
FRic, functional richness
HWE, Hardy-Weinberg equilibrium
IPCC, Intergovernmental Panel on Climate Change
MDZFP, mean duration of zero flow period
MLPE, maximum likelihood population effects
MRDM, multiple regression on distance matrices
MSI, Madrean Sky Islands
NMDS, non-metric multidimensional scaling
USDA, United States Department of Agriculture
ZFP, zero flow period

Keywords

Amphibians, aquatic invertebrates, climate change, frog, genetics, groundwater withdrawals, hydrology, hydrologic connectivity, intermittent rivers, landscape ecology, metapopulations, toad.

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Plate

Research team pictured in 2010 at the beginning of the DoD SERDP RC-1724 project. Left to right: Kristin Jaeger, Julian Olden, Dave Lytle, Meryl Mims, Kate Boersma, and Martin Schlaepfer. Not pictured are essential personnel Michael Bogan, Tiffany Garcia, Emily Hartfield Kirk and Ivan Phillipsen.

Objective

Improvements in our fundamental understanding of the links between hydrological and ecological processes in arid and semi-arid environments are needed for the proactive conservation of imperiled species and their habitats on and around DoD installations. Our project contributes to this challenge by examining how southwestern U.S. intermittent and ephemeral streams provide critical habitat and population connectivity for obligatory aquatic species (insects, amphibians), and addresses whether these ecological functions will be maintained in a changing climate. By incorporating quantitative modeling, field studies, and molecular genetics our research project directly address the core requirements detailed in the RCSON-10-02. Our proposal focuses on Fort Huachuca Army Base for concept development and tests concept generality on the Sky Island Mountain Ranges that surround Fort Huachuca. Insight gained from this research directly informs current and proposed future management actions of on DoD military installations for conserving desert streams and their aquatic inhabitants.

Our project has four main objectives that aim to provide both the science and management tools needed to ensure the conservation of obligatory aquatic species on DoD military lands in a rapidly changing environment:

1. Model and measure stream flow to quantify flow permanence and hydrologic connectivity at multiple spatial scales;
2. Characterize and model the distribution and abundance of obligatory aquatic organisms in relation to hydrology, riparian vegetation, and geomorphology;
3. Evaluate population structure (gene flow) of insect and amphibian species with contrasting life-histories along a gradient of flow permanence and hydrologic connectivity;
4. Advance our understanding of how obligatory aquatic species may respond to climate-induced changes in flow permanence and hydrologic connectivity.

By achieving each of these objectives our project contributed directly to the two main interests of RCSON-10-02. First, our research improved our scientific understanding of the variation and ecological significance of intermittent and ephemeral stream systems within the major biotic regions of the Southwest. Second, we developed knowledge and tools that provided insight into potential consequences of climate-induced changes to hydrology.

1. Hydrological connectivity and continuity of Fort Huachuca

1.1 Introduction

Hydrological connectivity refers to the water-mediated transfer of matter, energy and/or organisms within or between elements of the hydrologic cycle (Pringle, 2003). Operating in longitudinal, lateral and vertical dimensions, connectivity is a fundamental property of aquatic ecosystems (Ward and Stanford, 1989; Tockner and Stanford, 2002; Ward et al., 2002; Freeman et al., 2007). In intermittent and ephemeral streams, hydrological connectivity exerts particular control because drying and wetting events shape the spatiotemporal patchwork and linkages of habitats over time (Bunn et al., 2006). Drought conditions can interrupt longitudinal connectivity (e.g. surface water connections in the upstream and downstream direction), resulting in periods of temporary habitat loss and limiting dispersal of obligatory aquatic species and downstream transport of matter and energy (Dodds et al., 2004; Sponseller and Fisher, 2006; Doering et al., 2007; Nadeau and Rains, 2007). Consequently, longitudinal connectivity, notably the shifting spatiotemporal character between the flow presence and absence, can define the ecological structure and function of lotic ecosystems (Nilsson et al., 1989; Larned et al., 2009; Arscott et al., 2010).

Artificial interruption of longitudinal connectivity from the burial of headwater streams (Freeman et al., 2007), physical barriers such as dams and diversions (Nilsson et al., 2005) or dewatering associated with groundwater pumping (Falke et al., 2011) can result in temporary or permanent fragmentation of aquatic habitats (Fullerton et al., 2010). For already naturally fragmented dryland streams in arid and semi-arid systems, there is concern of further decreases in connectivity, particularly as a result of more frequent and severe droughts associated with forecasted changes in climate (Seager et al., 2007; Cayan et al., 2010) and greater human appropriation of freshwater resources (Sabo et al., 2010). Increasing temperatures and changes in precipitation patterns could alter flow timing, potentially increasing stream-flow intermittency, with subsequent impacts to aquatic biota (Levick et al., 2008; Sheldon et al., 2010).

Quantifying the considerable spatial and temporal variability of streamflow patterns that determines connectivity remains an ongoing challenge despite its established importance on ecological processes in river systems (Fullerton et al., 2010; Larned et al., 2010). The need to develop metrics describing longitudinal connectivity at broad spatial scales has become more pressing with increased research that focuses on riverscape patterns and processes (Fausch et al., 2002). Currently, metrics are limited and hindered by the substantial effort and monetary cost required to implement monitoring programmes across a channel network. Some methods such as field mapping the contraction and expansion of surface water in streams can effectively quantify longitudinal connectivity (e.g. Hunter et al., 2005; Larned et al., 2010; Turner and Richter, 2011). However, their accuracy is limited by the frequency of field visits, which can be time consuming, requiring the work of many individuals or dedicated work of a few, depending on the spatial extent of the area of interest. Repeat satellite imagery is applicable to areas of large spatial extent such as floodplain rivers (e.g. Puckridge et al., 2000, 2010), but may not be useful in smaller streams as a result of spatial resolution constraints. Other methods, including thermograph interpretation of field-deployed temperature sensors throughout a channel network, may not have a temporal resolution sufficient to capture shorter duration (<24 h) streamflow events typical of intermittent and ephemeral stream reaches (Constantz et al., 2001; Blasch et al., 2004; Gungle, 2006). Consequently, current models (hydrologic, hydraulic and statistical) would benefit from near-continuous, fine-scale measurements of flow timing and longitudinal connectivity at riverscape scales.

A technique using electrical resistance (ER) sensors provides a novel opportunity for quantifying network-scale longitudinal connectivity that requires substantially less effort than field mapping while exceeding the spatial and temporal resolution of the thermograph interpretation and modelling methods. We demonstrate the ER sensors' utility to measure flow timing and hence, longitudinal connectivity across a riverine network in the Huachuca Mountains characterized by clear transitions between perennial, intermittent and ephemeral reaches.

1.2 Methods

1.2.1 Study site

Our study site is the US Army Garrison, Fort Huachuca and surrounding areas along the eastern flank of the Huachuca Mountain Range within the Upper San Pedro River Basin, south-eastern Arizona, USA (Figure 1.1). The Huachuca Mountains are part of the Madrean Sky Island Region, a term illustrating the region's distinct biogeography, which is supported by the complex topography of isolated mountain ranges (maximum elevations of 1830 to 3350 m) that are separated by arid valleys (Plate 1.1). The climate in the Huachuca Mountains is semi-arid; mean annual precipitation is approximately 40 cm with 50–60% occurring as high-intensity, local convective thunderstorms associated with the North American monsoon season. Winter precipitation typically resulting from North Pacific frontal storms accounts for 21–35% of the annual precipitation. Frontal storms tend to be of broader spatial extent producing rain events of longer duration and less intensity (Ely et al., 1993), which turns to snow at higher elevations.

The region's climate and topographic complexity sustains a sharp geomorphic and hydrologic gradient. The canyons that comprise the Huachuca Mountains are composed of granite, limestone and other sedimentary geologic units. Stream channel morphology is characterized by cascade and bedrock reaches in the upper canyons draining the mountain front, which give way to step-pool, plane bed and pool-riffle channel forms downstream. Channel bed substrates range from large cobble and boulder in the upper reaches fining downstream to primarily sand and gravel. Calcium carbonate deposition has armoured the channel bed or contributed to a travertine step-pool morphology in some reaches both within and downstream of where the streams flow through the lime- stone unit. Wetlands located along streams in some of the canyons contribute silt, clay and fine organic debris, which deposit in low-gradient reaches that are interspersed among steeper reaches. Extending beyond the mountain front onto the valley floor, channels are primarily sand-bedded and ephemeral.

Figure 1.1 The Huachuca Mountain range with US Army Garrison, Fort Huachuca and Sierra Vista (gray) in foreground.

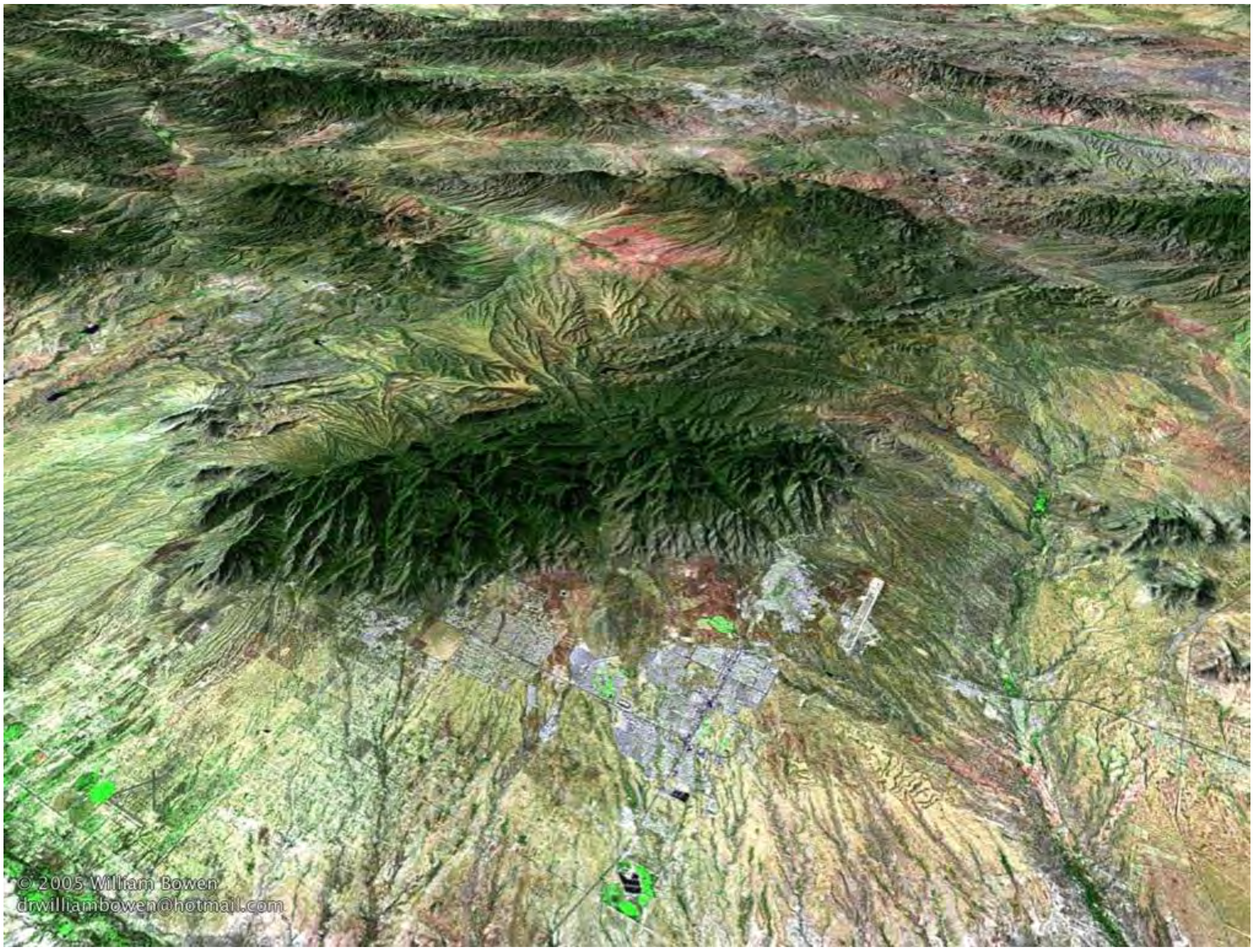


Plate 1.1 The Huachuca Mountain range as captured from the Mule Mountains. Photo credit: Ivan Phillipsen.

1.2.2 Approach

We installed 44 ER sensors at approximately 2-km intervals throughout eight canyons primarily on Fort Huachuca (Figure 1.2, Plate 1.2). The relatively systematic spatial array provides broad representation of perennial, intermittent and ephemeral reaches, which we define following Levick et al. (2008).

Perennial reaches are reaches with streamflow during all times of the year. Ephemeral reaches are characterized by short duration streamflow events occurring in direct response to local precipitation, most of which takes place during the late summer North American monsoon season. Intermittent reaches flow continuously for only certain times of the year and are supported by sources such as bedrock springs, melting snow or repeated monsoon events. These water sources locally recharge the water table to produce sustained streamflow with durations that extend beyond the ephemeral runoff response (Table 1.1).

Temperature loggers (TidBit v2, Onset Computer Corporation, Bourne, MA, USA) are customized to measure relative conductivity (the inverse to resistance) as a proxy for streamflow presence (Blasch et al., 2002; Goulsbra et al., 2009). The specific sensor modifications consist of replacing the thermistor with two polyvinyl chloride (PVC)-insulated copper wires soldered to the sensor circuit board and protruding from the encased water-proof datalogger (Figure 1.3). Insulation from approximately 4 mm of the end of each wire was stripped and the exposed wires were secured approximately 20 mm apart from each other at the top of the sensor. Following the methods described by Blasch et al. (2002) and Gungle (2006), the sensors were housed in a perforated ~5 by 15-cm PVC piece, shallowly buried (<10 cm) in the streambed and secured. The sensor housing was then leashed with wire cable to a nearby tree or rock in the event that it scoured during high streamflow conditions (Plate 1.3 and 1.4).

Electrical conductivity increases in wet sediments relative to dry sediments, and abrupt increases in relative conductivity values indicate the onset of streamflow. The changes in relative conductivity values are more marked and therefore easier to confidently interpret compared to temperature fluctuations in the thermograph-based approaches with no time delay between the signal of the surface water sensor and the true timing of streamflow (Figure 1.4).

Sensors were deployed in April 2010 at a 15-min logging interval and retrieved (for the purposes of this study) in August 2012 (Plate 1.3 and 1.4). We report on the major canyons on Fort Huachuca (Huachuca, Garden, Woodcutters and Blacktail Canyons) and Ramsey Canyon (immediate south of Fort Huachuca); collectively these locations provide a comprehensive investigation of hydrology and longitudinal habitat connectivity.

Figure 1.2 Site map of Fort Huachuca in the Huachuca Mountains, southeastern Arizona, USA.

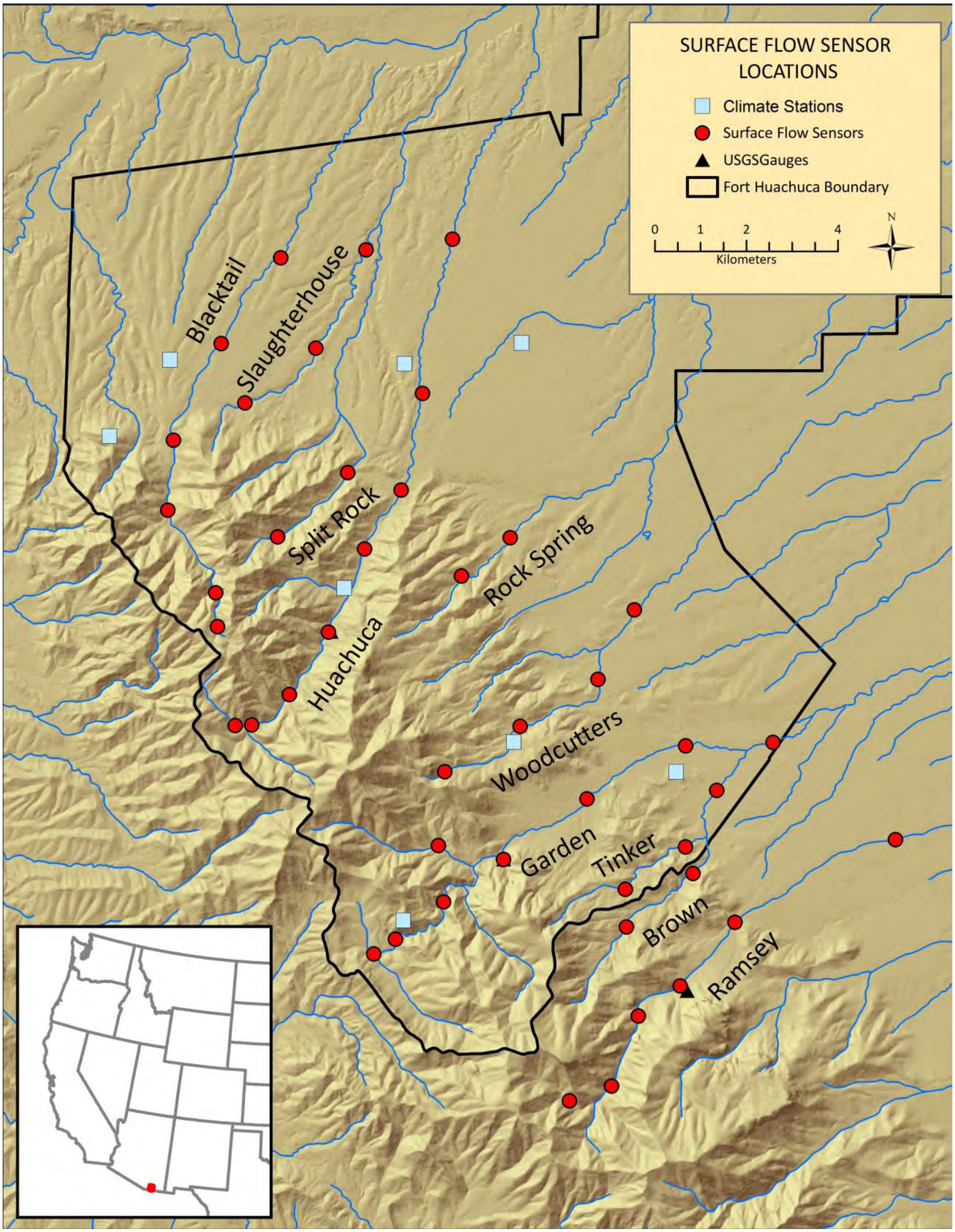


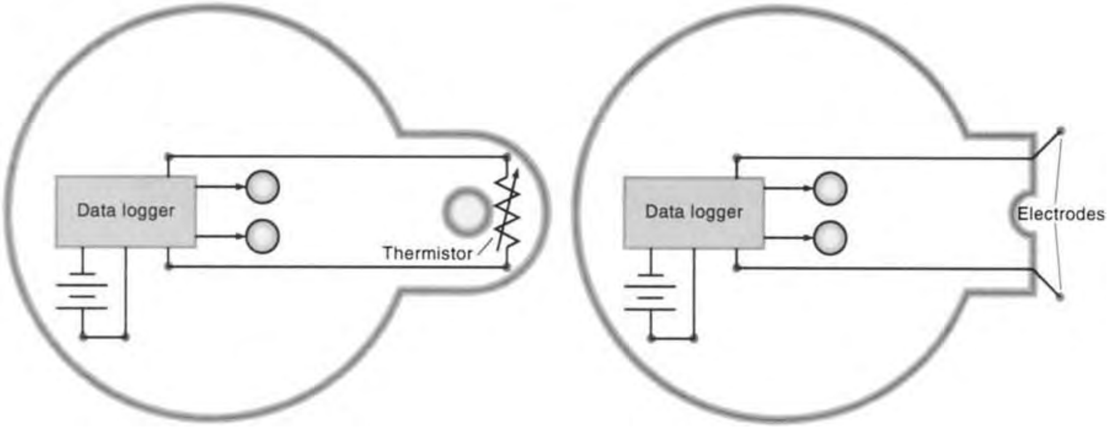
Plate 1.2. Illustration of electrical resistance sensor installation. Sensors are shallowly buried in the streambed along a gradient of hydrologic flow regimes (perennial, intermittent, ephemeral) within a canyon.

Table 1.1 Surface flow detection sensors installed in Huachuca Mountains, AZ. Flow type is represented by perennial (P), intermittent (I), and ephemeral (E) reaches. Channel distance is measured from source. NA indicates headwater reaches or instances where only sensor was installed and therefore channel distance is irrelevant.

Canyon	Sensor	Channel Distance (km)	Flow Type	Geology	Channel Morphology
Blacktail	BT1	NA	P	Sedimentary/Local Volcanics	shallow pool-riffle
	BT2	0.8	I	Limestone	colluvium/cascade
	BT3	2.3	I	Limestone	step-pool
	BT4	1.6	I	Granite	plane bed
	BT5	2.5	E	Alluvium	plane bed
	BT6	2.5	E	Alluvium	sand bed wash
Slaughterhouse	SL1		I	Granite	shallow step-pool
	SL2	2.2	E	Alluvium	plane bed
	SL3	3.0	E	Alluvium	sand bed wash
Split rock	SPR1		E	Granite	cascade
	SPR2	2.2	E	Granite	pool-riffle
Huachuca	H0	NA	P	Sedimentary/Local Volcanics	cascade
	H1	0.4	P	Sedimentary/Local Volcanics	shallow step-pool
	H2	1.2	E	Sedimentary/Local Volcanics	step-pool
	H3	1.7	P	Limestone	step-pool/travertine
	H4	2.0	I	Granite	step-pool/travertine
	H5	1.6	I	Granite	shallow step-pool
	H6	2.3	I	Alluvium	pool-riffle
	H7	3.8	E	Alluvium	plane bed
Rock Spring	RS1	NA	P	Granite	cascade
	RS2	1.5	I	Granite	pool-riffle
Woodcutters	W1	NA	E	Limestone	colluvium/cascade
	W2	2.2	I	Granite	pool-riffle
	W3	2.3	I	Granite	pool-riffle
	W4	1.9	E	Alluvium	sand bed wash
McClure	Mc1	NA	E	Limestone	step-pool

Garden	G1	NA	I	Volcanic	shallow step-pool
	G2	0.8	P	Quartzite	pool-riffle
	G3	1.8	P	Limestone	step-pool/travertine
	G4	1.2	P	Limestone	step-pool
	G5	3.6	I	Granite	pool-riffle
	G6	2.5	I	Alluvium	pool-riffle
	G7	2.1	I	Alluvium	sand bed wash
Tinker	T1	NA	I	Granite	step-pool
	T2	1.8	I	Granite	shallow step-pool
Brown	B1	NA	I	Granite	step-pool
	B2	2.0	I	Granite	shallow step-pool
	B3	2.3	E	Alluvium	sand bed wash
Ramsey	R1	NA	P	Granite	step-pool/cascade
	R2	1.2	P	Volcanic	step-pool
	R3	1.7	P	Limestone	step-pool
	R4	1.3	P	Granite	step-pool
	R5	1.9	I	Granite	pool-riffle
	R6	4.3	E	Alluvium	sand bed wash

Figure 1.3 Traditional temperature sensor (A) and developed electrical resistance sensors (B) used in the present study. TidBit v2, Onset Computer Corporation, Bourne, MA, USA.

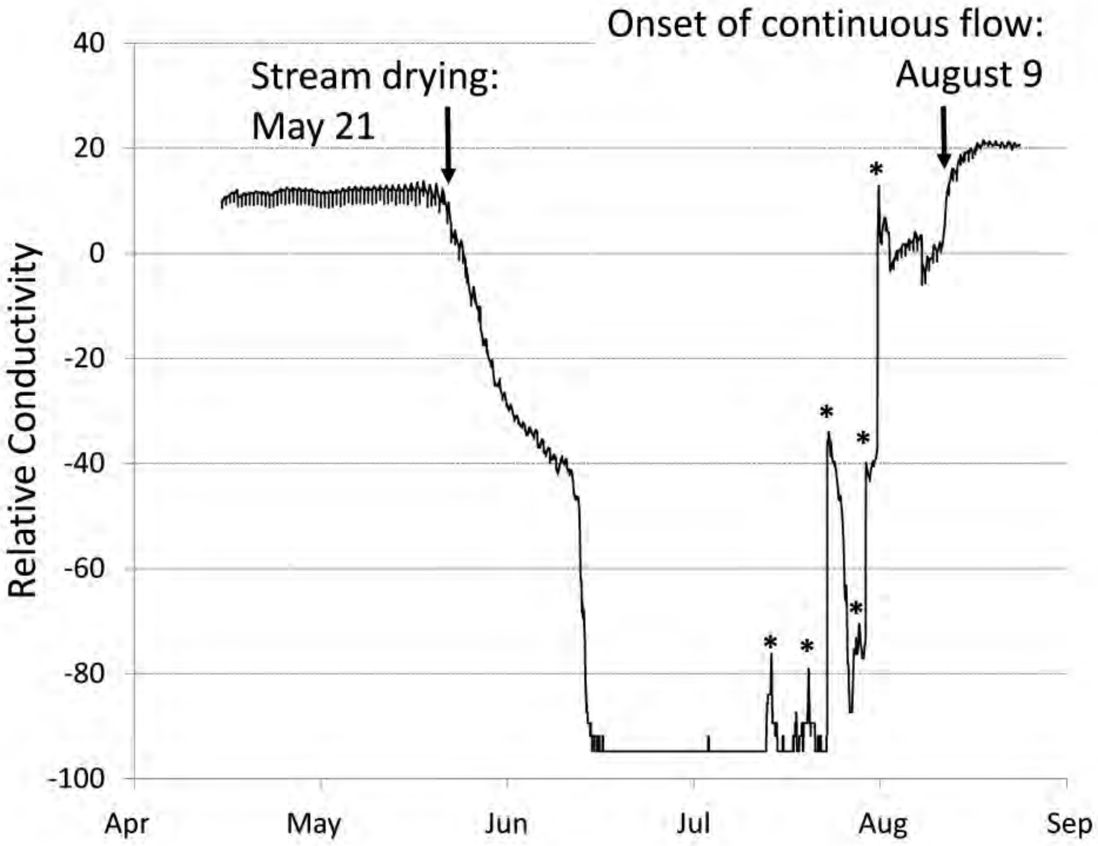

A. Temperature sensor

B. Electrical-resistance sensor

Plate 1.3 Post-doctoral researcher, Kristin Jaeger, deploying a electrical resistance sensor in Woodcutters Canyon, Huachuca Mountains. Photo credit: Meryl Mims.

Plate 1.4 PhD student, Meryl Mims, retrieving an electrical resistance sensor buried after a large flood. Photo credit: Jessie Hale.

Figure 1.4 ER sensor record in an intermittent reach in upper Huachuca Canyon (H2). Abrupt increases in relative conductivity values indicate onset of surface flow. Sustained declines in relative conductivity values indicate stream drying. * represent periods of short duration < 24 hrs streamflow from convective thunderstorm precipitation during the North American monsoon.



1.2.3 Streamflow continuity and connectivity

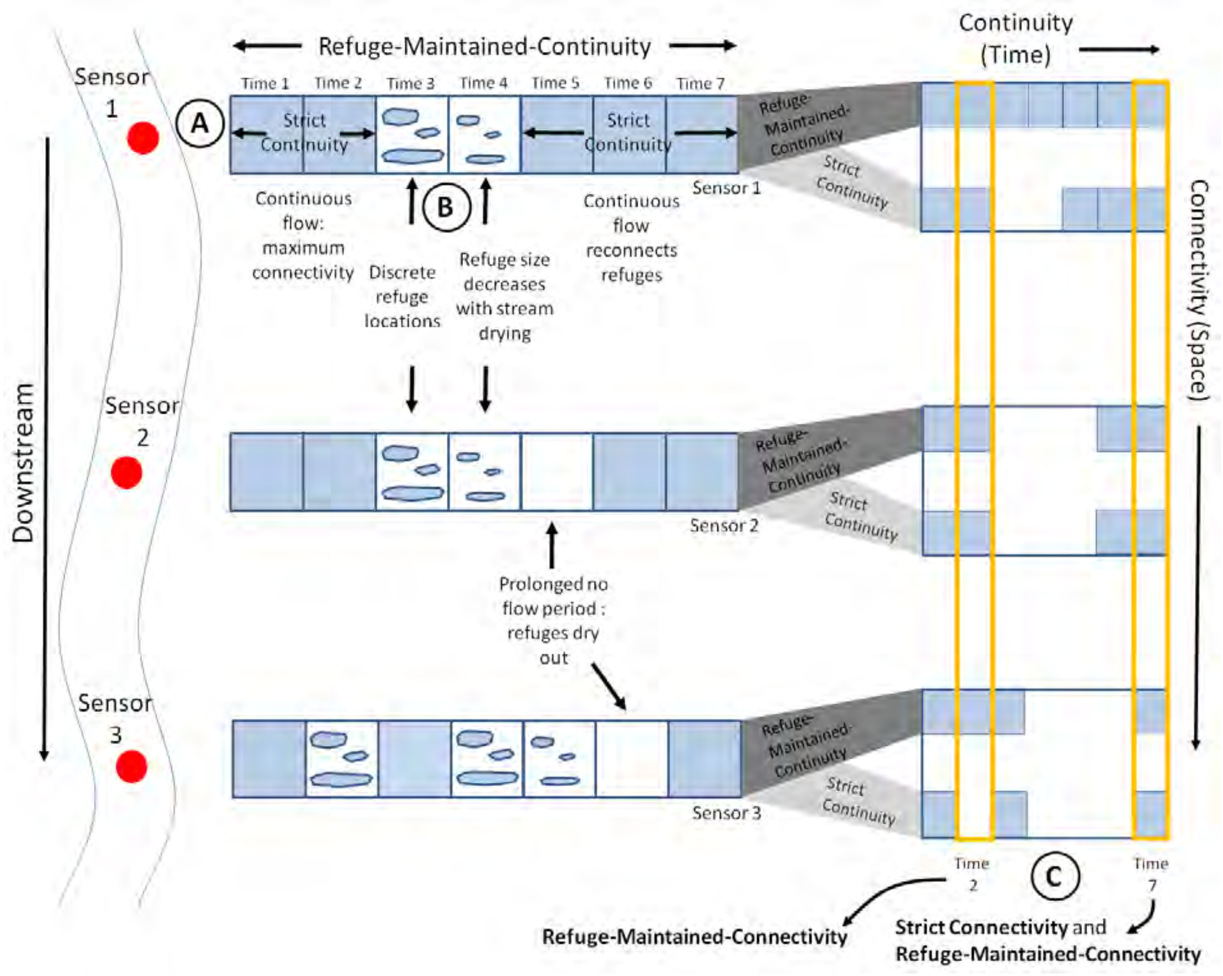
Longitudinal connectivity consists of both a structural component describing the shape, size and location of habitat patches and their physical linkage via hydrological corridors and a functional component describing the linkage of habitat patches by organism dispersal and gene flow. This distinction was originally made in the field of landscape ecology (Taylor et al., 1993). In the case of structural longitudinal connectivity (the focus of our paper and hereafter called connectivity), periods of stream drying and wetting events that link ephemeral, intermittent and perennial reaches create a patchwork of habitats at different locations and times during the year.

It is important to make the distinction between streamflow that is continuous in time at an individual sensor location (i.e. continuity) versus continuous in space across multiple sensor locations (i.e. connectivity). At an individual sensor location, the presence of surface water during consecutive time steps (15 min in our study) is considered to be continuous through time; a time period defined as 'strict continuity' (Figure 1.5). However, in the time following strict continuity when stream drying is occurring, contraction of surface water to discrete locations in the vicinity of the sensor can provide refuge for aquatic organisms (e.g. Labbe and Fausch, 2000; Magoulick and Kobza, 2003; Larned et al., 2009; Sheldon et al., 2010). If streamflow resumes before the remaining refuges dry out, the likelihood of local extirpation decreases. Thus, we consider this time period as 'refuge-maintained continuity', the criteria being that a sensor must have a signal of streamflow within a particular length of the last streamflow signal (Figure 3).

We recognize that this time period will vary according to the particular species or ecological process of interest and across river systems characterized by differing geomorphologies and climate conditions. For our purposes, we chose 48 h as a reasonable time period when water can be expected to remain in the channel following strictly continuous streamflow. This 48-h time period is based on field observations of stream drying dynamics in the Huachuca Mountains, notably the anecdotal identification of sizes and frequency of pools that serve as refuges in intermittent and ephemeral reaches.

Following with this line of reasoning, under the same strict interpretation, 'strict connectivity' is considered the condition when multiple sensors within a canyon simultaneously signal continuous surface flow. In this condition, we must assume continuous streamflow between sensors. During this time, aquatic species movement along a channel network is theoretically maximized (although this will vary across species, thus defining functional longitudinal connectivity). We quantify strict connectivity by summing time intervals of strict continuity that are simultaneous among sensors within a canyon. 'Refuge-maintained connectivity' consequently is composed of time periods that have streamflow under the 'refuge-maintained continuity' criteria (Figure 1.5). Connectivity is represented as the total number of days and the percent of the total monitoring period and the monsoon monitoring period (July-August). We recognize that continuity and thus connectivity values are dependent on sensor placement. Consequently, locations different from the sites we chose may result in different values of streamflow timing. However, we expect that the systematic spatial array of the sensors produces a reasonably accurate representation of spatiotemporal streamflow variation at the landscape scale.

Figure 1.5. Schematic of streamflow continuity (continuous in time) and connectivity (continuous in space) for three hypothetical sensors recording surface flow presence (blue) or absence (white) over seven time periods (boxes). Each box represents a 24-hour time period. Boxes with blue shapes represent time periods that support refuges. Consecutive time periods of flow at an individual sensor location is considered strict continuity (A). Refuge-maintained-continuity refers to time periods where continuous streamflow is interspersed with short time periods (<48 hours) of no flow conditions, which may support refuges (B). Strict connectivity refers to time periods when multiple sensor locations within a canyon simultaneously exhibit strict continuity (C, Time 7). Refuge-maintained-connectivity refers to periods when multiple sensor locations within a canyon simultaneously exhibit refuge-maintained-continuity (C, Time 2 and 7).



1.3 Results and Discussion

The ER sensors provide a record of streamflow continuity and longitudinal connectivity in hydrologically complex streams in the Huachuca Mountains, information relevant to both reach-scale and riverscape-scale aquatic investigations. Sustained declines in relative conductivity values for sensors in intermittent and some ephemeral reaches beginning in April and continuing through May indicate a distinct stream drying that corresponds to cessation of spring runoff. Intermittent and ephemeral reaches remained dry throughout June and the early part of July 2010. Short duration (<24 h) surface flow occurred in ephemeral and intermittent reaches in response to individual monsoonal rain events, which are recorded in 15-min time increments at Army Base-operated climate stations located throughout Fort Huachuca. Continuous flow in some intermittent reaches resumed in late July and early August as the monsoon season developed. Streamflow type (e.g. perennial, intermittent, ephemeral) only moderately corresponded to the underlying geologic unit, suggesting that the influence of geology on streamflow type may vary in relation to the particular positioning in the channel network (e.g. headwaters versus lower in the network).

Flow continuity varied substantially across the major canyons (Table 1.2, Figure 1.6); the highest was observed for Ramsey, followed by Garden, Huachuca, Woodcutters and Blacktail Canyon. Intermittent reaches, on average, experienced flow for 20% per year, whereas ephemeral reaches, on average, experienced 2% flow days (Table 1.2, Figure. 1.6). Flow at a particular sensor during an individual monsoon event had an average duration of 5.2 h in intermittent reaches and 3.5 h in ephemeral reaches.

Substantial within canyon hydrologic variability was also evident, leading to differences in patterns of longitudinal connectivity (Figure 1.7). Canyon-wide connectivity – referring to the full spatial extent of all ER sensors within an individual canyon from headwaters to downstream of the mountain front - was rare to non-existent (Figure 1.7). Despite the absence of total connectivity, however, extensive reaches within a canyon experienced longitudinal connectivity for substantial periods of time. Perennial and intermittent reaches along the mountain front in Garden and Ramsey Canyons remained connected for 97% and 39% of the monitoring period, respectively. By contrast, Woodcutters Canyon and Blacktail Canyon were never full connected across their longitudinal extent. Although we assume continuous flow between adjacent sensors that are simultaneously activated, hydrologic models can serve to interpolate the flow presence between sensor locations.

Refuge-maintained continuity calculations increased flow days in both intermittent and ephemeral reaches (Figure 1.8). The refuge-maintained criteria increased streamflow continuity values, but quantifying connectivity by the same criteria did not greatly change canyon-wide connectivity, and only increased connectivity by 1–2% in portions of some canyons. Regardless of the lack of influence in our study, refuge-maintained connectivity remains an important concept to longitudinal connectivity because of its applicability to a broad spectrum of time periods relevant to aquatic biota of choice (i.e. aspects that define functional connectivity) that varies widely according to the persistence of particular refuges in different hydro-climatic landscapes. Refuge persistence can range on the order of a few hours in dryland streams (Lytle et al., 2008), weeks to months in small streams in midwestern USA (Capone and Kushlan, 1991; Labbe and Fausch, 2000) or 1–2 years in larger floodplain river systems in Australia (Bunn et al., 2006; Sheldon et al., 2010).

Table 1.2 Summary statistics of flow permanence for major canyons in Fort Huachuca.

Sensor	Total Period (days)	Total Flow (days)	Total Dry (days)	Total % Flow Permanence	2010 % Flow Permanence	2011 % Flow Permanence	2012 % Flow Permanence
Garden Canyon							
G1	816	110	706	13.5	15.5	15.4	6.9
G2	816	816	0	100.0	100.0	100.0	100.2
G3	816	816	0	100.0	100.0	100.0	100.2
G4	816	788	28	96.6	100.0	100.0	53.6
G5	468	143	325	30.6	54.8	7.4	21.0
G6	816	43	773	5.3	15.2	0.4	1.0
G7	816	28	789	3.4	9.9	0.1	0.1
Huachuca Canyon							
H0	466	431	36	92.3	100.0	82.6	95.0
H1	644	215	429	33.4	63.5	11.2	21.7
H2	816	22	794	2.7	4.8	2.5	0.0
H3	816	816	0	100.0	100.0	100.0	100.0
H4	468	419	50	89.4	96.2	80.8	99.0
H5	120	48	71	40.5	40.5	29.0	35.0
H6	816	93	723	11.4	24.3	8.1	0.0
H7	816	2	813	0.3	0.2	0.4	0.0
Woodcutters Canyon							
W1	798	15	782	1.9	2.6	2.4	0.0
W2	816	68	695	8.3	22.1	2.8	0.1
W3	816	11	805	1.3	4.0	0.1	0.0
W4	816	16	800	2.0	5.8	0.2	0.0
Blacktail Canyon							
BT1	802	82	720	10.2	28.3	2.4	0.0
BT2	738	10	729	1.3	2.7	0.7	0.0
BT3	738	0	738	0.0	0.0	0.0	0.0
BT4	816	36	780	4.4	13.8	0.0	0.0
BT5	816	12	804	1.4	3.1	0.9	0.0
BT6	816	7	809	0.8	2.5	0.0	0.0
Ramsey Canyon							
R1	816	816	0	100.0	100.0	100.0	100.0
R2	816	321	503	39.4	100.0	16.5	0.3
R3	816	816	0	100.0	100.0	100.0	100.0
R4	816	658	157	80.7	100.0	78.0	59.3
R5	816	75	741	9.2	28.6	0.0	0.0
R6	816	5	811	0.6	1.9	0.1	0.1

Figure 1.6 Percent time of monitoring period (2010-2012) when surface flow was present at sensor locations (e.g., strict continuity). Inset table reports values for locations classified as intermittent and ephermal. Background colors and associated letters correspond to geologic units.

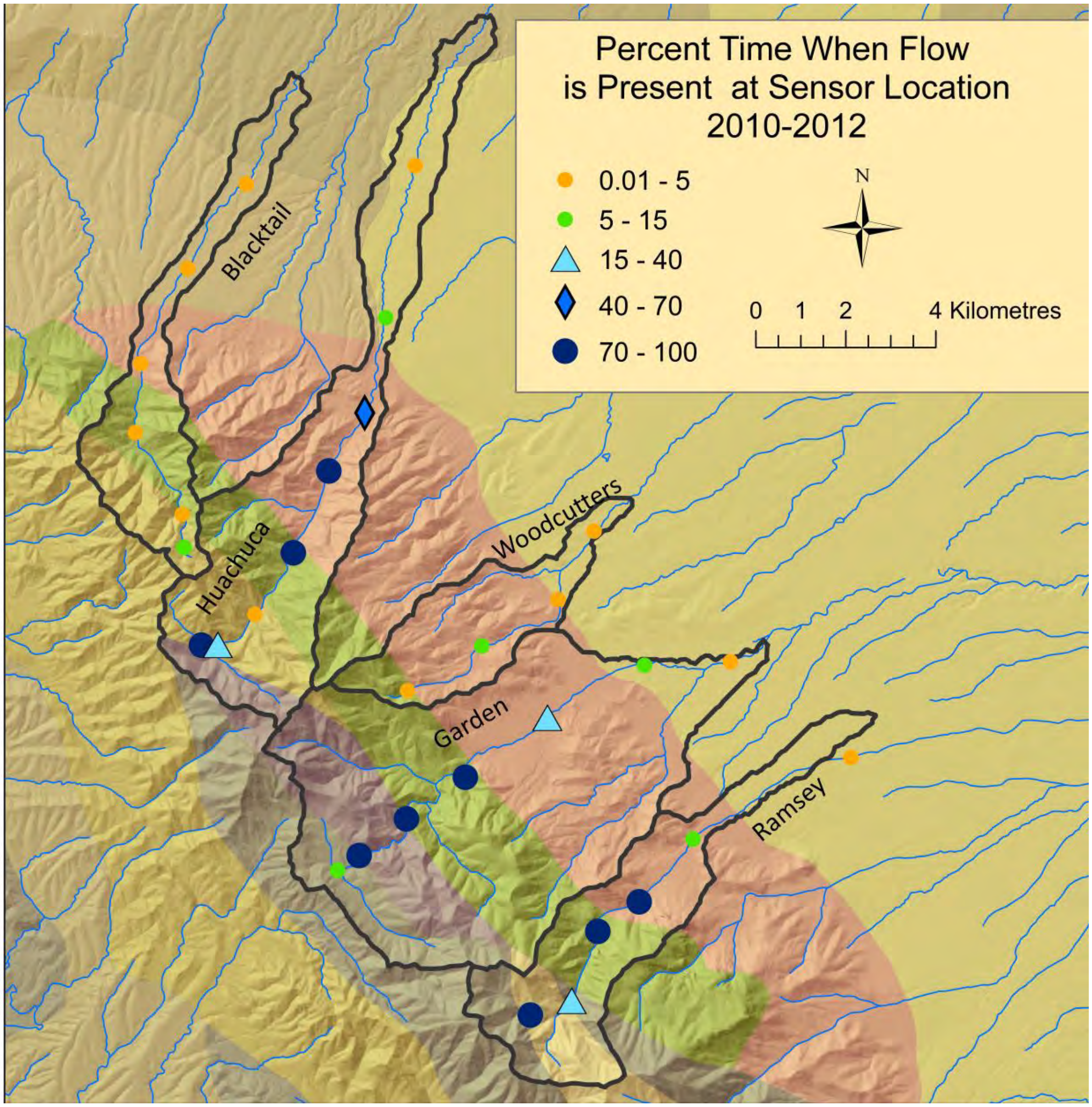


Figure 1.7 Percent time of continuity and connectivity by strict criteria during monitoring period (2010-2012). Percentages in black represent canyon-wide connectivity and percentages in blue represent upper-basin connectivity.

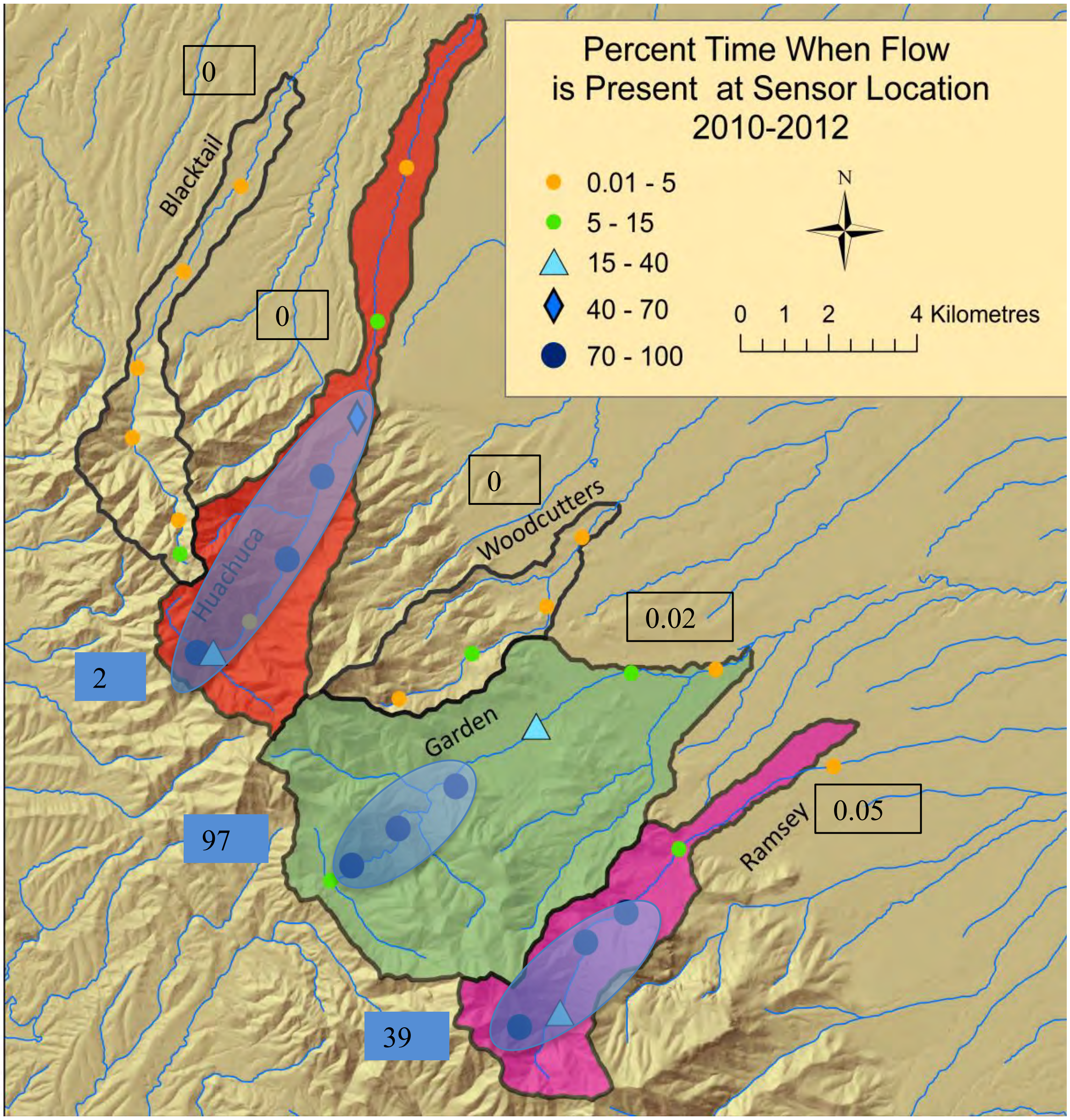
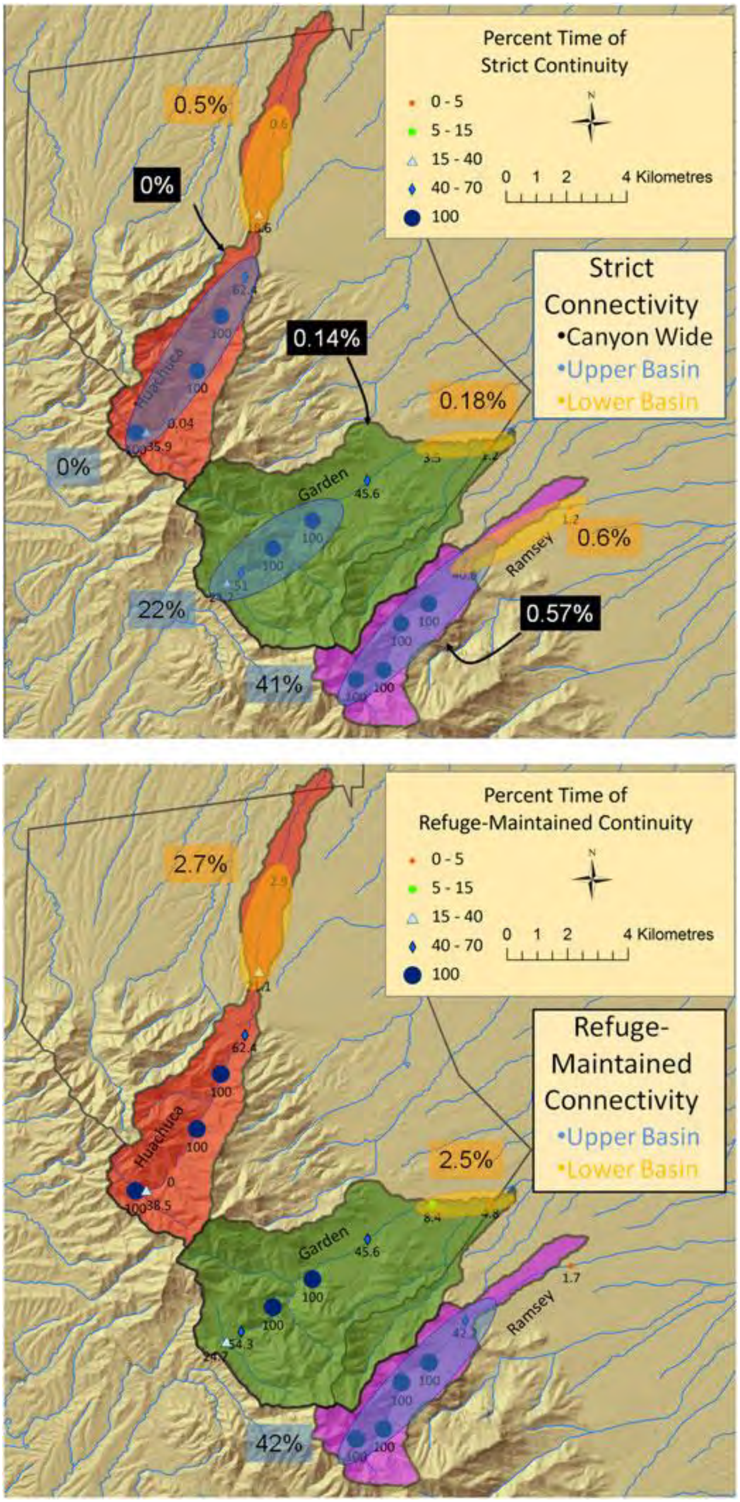


Figure 1.8 Comparison of strict (upper panel) vs. refuge-maintained continuity (lower panel) for Huachuca, Garden and Ramsey Canyons in 2010. Numbers adjacent to each individual sensor represent continuity values.



1.4 Conclusion

Using ER sensors, we have demonstrated a riverscape approach to quantify streamflow continuity through time and longitudinal connectivity through space. We accomplished this for several semi-arid watersheds in Fort Huachuca that are characterized by hydrologically complex flow patterns, but the proposed methodology is broadly applicable. A burgeoning area of research is focusing on the influence of longitudinal connectivity on population dynamics and ecological processes in dendritic river networks (e.g. Grant et al., 2007; Brown and Swan, 2010; Erös et al., 2011). Data generated from spatial arrays of surface flow sensors could contribute significantly to this effort by yielding information on streamflow timing and duration at a higher spatial and temporal resolution compared to previous methods and requiring substantially less effort and monetary cost compared to implementing field mapping programmes.

1.5 Acknowledgements

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2. Hydrology shapes taxonomic and functional structure of desert stream invertebrate communities

2.1 Introduction

Streams and rivers lie along a hydrologic continuum that ranges from highly intermittent flow, where surface water is present only occasionally, to perennial, where surface water occurs year-round (Poff et al. 1997). These differences in hydrology can shape patterns of species diversity (Wellborn et al. 1996, Williams 1996), affect the structure of riverine and riparian food webs (Sabo et al. 2010, McCluney and Sabo 2012), and drive evolutionary change in the organisms themselves (Lytle and Poff 2004, Lytle et al. 2008). The strong environmental gradients imposed by flow also impose a strong 'habitat filter' (sensu Southwood 1977, 1988) that directly controls the taxonomic and trait composition of communities (Williams 2006, Gallart et al. 2012, Mims and Olden 2012, Ledger et al. 2013b, Belmar et al. 2013). Strong habitat filtering may locally favor taxa adapted to one hydrological extreme or another, as well as generalists capable of persisting in a variety of habitats (Poff and Ward 1989, Williams 2006, Bonada et al. 2007), but how habitat filtering affects communities along gradients of hydrological variability is less clear.

Species richness and assemblage composition are commonly used to quantify aquatic communities and how they respond to perturbations (Bunn and Arthington 2002, Poff and Zimmerman 2010, Carlisle et al. 2011). However, trait-based measures of diversity, or functional diversity, may be more informative in describing ecological responses to environmental variability than taxonomy-based metrics alone (Walker 1992, Hoeinghaus et al. 2007, Cadotte et al. 2011). Functional diversity can be defined as the number, type and distribution of functions performed by organisms within an ecosystem (Díaz and Cabido 2001). A change in functional diversity may affect ecosystem processes, whereas a change in species diversity may elicit no ecosystem response at all because of functional redundancy (i.e., shared ecosystem-effect traits) among species. For example, Bonada et al. (2007) found no difference in macroinvertebrate species richness between permanent and intermittent rivers but did find significant among-site trait differences attributable to hydrology. Thus, a trait-based approach may identify consistent responses to disturbances or environmental gradients that are not revealed by taxonomic analyses alone. Functional diversity has important implications for the ability of communities to withstand and recover from disturbance and to respond to environmental change (Poff et al. 2006).

The form of the relationship between taxonomic and functional diversity determines the degree of functional redundancy in communities (Micheli and Halpern 2005). For instance, a positive linear relationship (slope = 1) indicates that species additions to a community result in new ecological functions, whereas a shallower, positive slope (<1) indicates low redundancy because some species share functional traits. Curvilinear relationships between taxonomic and functional diversity indicate communities rapidly acquire unique functions (associated with trait values) at low diversity levels and subsequently reach an asymptote at higher levels of diversity. More functionally diverse communities are thought to offer greater resilience because of greater ecological redundancy (Hooper et al. 2005), as has been shown for agricultural land use gradients (Fischer et al. 2007), forest-fire disturbances (Hidasi-Neto et al. 2012), and hydrologic-alteration gradients (Pool et al. 2010). Thus, the form of the relationship between taxonomic and functional diversity can reveal much about how communities might respond to ecological perturbations.

Plate 2.1 Fieldwork in Ramsey Canyon, Huachuca Mountains. Photo credit: Meryl Mims.

We sought to assess whether taxonomic and functional trait diversity differed across aquatic habitats that span a gradient of flow dynamics ranging from highly intermittent to perennial. We characterized the trait and taxonomic diversity of desert-stream invertebrate communities in a stream network in southeastern Arizona and then examined the relative roles of flow permanence, physical-habitat conditions, and season in determining the taxonomic and functional structure of the communities. We examined the taxonomic–functional richness relationship in these arid-land communities. We predicted that under more intermittent flow conditions, only specialized taxa would persist locally because of habitat filtering, which would lead to high similarity or compositional convergence among communities. Therefore, we expected both taxonomic and functional diversity to be positively related to increasing stream flow permanence and negatively related to the duration and number of stream drying events.

2.2 Methods

2.2.1 Study area and invertebrate collection

We collected aquatic invertebrates from 28 sites distributed across 7 arid-land streams in the Huachuca Mountains within the Upper San Pedro River Basin of southeastern Arizona, USA (Figure 2.1). The area receives ½ of its total yearly precipitation during the summer monsoon season (July–September) during short, intense thunderstorms and ½ during the winter season (November–April) from more protracted, milder frontal systems. Streams in the area consist of perennial headwaters that flow into intermittent sections as streams cross alluvial fans at canyon mouths. Further downstream, flows transition to ephemeral (Bogan et al. 2013). We distributed our sample sites among perennial, intermittent, and ephemeral reaches (classification follows (Levick et al. 2008), but we used a continuous-flow metric to quantify permanence (see below). Our sampling covered nearly all available perennial habitat in the eastern Huachuca Mountains, and we established additional sites to sample ephemeral and intermittent reaches when they became activated with surface flows. We sampled sites multiple times between 2009 and 2011 with a per-stream average of 21 sampling events. However, the number of sites and samples collected differed among streams because not all sites had flow or all microhabitats during each sampling event (Table 2.1) and were not sampled when dry. The period of our study spanned 5 dry seasons, 3 periods of ephemeral flows from summer monsoon rains, and 1 period of intermittent flows resulting from above-average winter precipitation. Most sampling occurred during autumn and winter (November and December, March and April, respectively) for a total of 144 site × sampling-event combinations (Table 2.1). More perennial (*n* = 113) than intermittent (*n* = 31) samples were collected because of the rarity of intermittent flow events.

Each site consisted of a 100-m-long stream reach in which all available microhabitats were sampled (primarily riffles and pools). For riffle samples (1–3/site), we disturbed 0.33 m^2 of stream substrate to a depth of 5 cm while capturing invertebrates immediately downstream with a D-net (500-μm mesh). We sampled pools (1–3/site) by sweeping the entire pool area including water column, surface, and pool benthos with a D-net at an effort of 10 s/m^2 pool habitat ((Bogan and Lytle 2007). We preserved samples in 95% ethanol and identified invertebrates in the laboratory to the finest taxonomic level possible, usually to genus or species for insects (including Chironomidae) and family or order for non-insects. We summed abundances from microhabitat samples collected from the same site during the same sampling event (e.g., 3 riffles) for each taxon and divided by the number of replicates to acquire relative abundances. Samples were taken from multiple locations in the same streams, so we tested for the possibility of nonindependence caused by spatial autocorrelation with Mantel tests in the *ade4* package (Dray and Dufour 2007) in R (version 2.15.2; R Project for Statistical Computing, Vienna, Austria). Based on these results, we accepted the null hypothesis that spatial location was not related to functional richness ($r = –0.0175$, $p = 0.6$) or species richness ($r = 0.0437$, $p = 0.16$). We also recorded relative habitat size of the wetted reach (<1 m^2, 1–10 m^2, 10–100 m^2, 100–1000 m^2, and >1000 m^2) and microhabitat type (river riffle, river pool, headwater stream riffle, headwater stream pool).

2.2.2 Hydrology and environmental variables

We measured in-stream flow variation by deploying 15 wet/dry electrical resistance (ER) sensors (Jaeger and Olden 2012) to quantify timing and continuity of stream flow near invertebrate sampling locations. The sensors logged relative conductivity at 15-min intervals from 15 April 2010 to 31 December 2011 as a proxy for the presence of surface water (see (Jaeger and Olden 2012)for in-depth description of sensor hardware, deployment, and data analysis). From these conductivity data, we calculated 4 stream-flow metrics for each sampling site using the nearest sensor: % flow permanence in year of sample, % flow permanence by season, mean duration (number of days) of zero flow periods (ZFP) each year, and number of ZFP each year. For the 2 flow-permanence metrics and duration of ZFP, we summed 15-min periods of both wet and dry conditions for the sampling period and individual zero flow periods, converting the time units to either days or years as appropriate for the final stream-flow metric. We used an average of 2010 and 2011 flow data to estimate flow conditions for the November 2009 invertebrate sampling period (16 samples), which occurred prior to deployment of sensors. The flow-permanence variables measured the percentage of time a reach had water in the sampling year and the proportion of days with flow in each season. Duration of ZFP indicates on average how long, in days, a stream has no surface water during drying events. A site that never dries will have a ZFP = 0. The number of ZFPs quantifies the number of drying events the site experienced in the year. These 4 metrics were calculated from the same flow-sensor records, but each was designed to characterize distinct components of the hydrologic regime that might influence macroinvertebrate occurrence.

Figure 2.1 Locations of study streams and sampling sites. Dots along streams mark the specific sampling site.

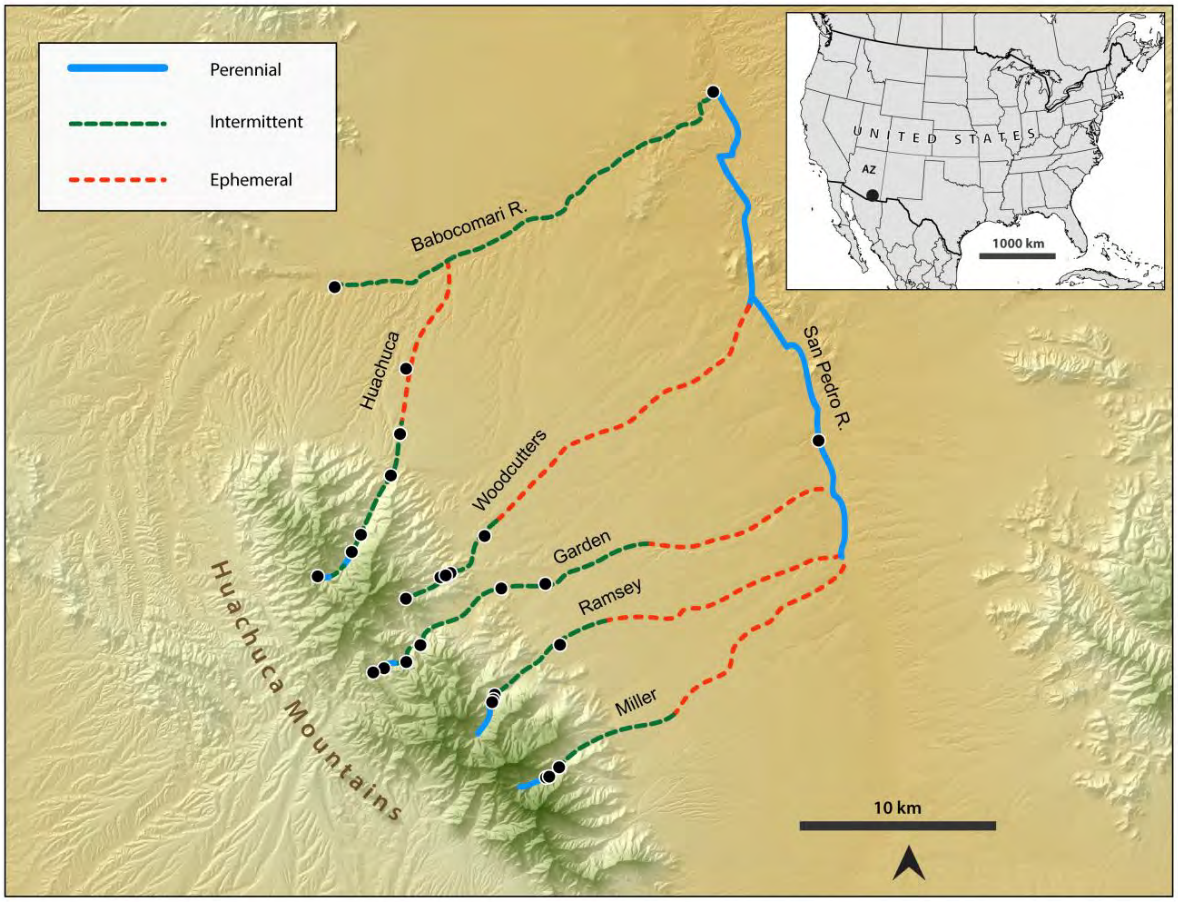


Table 2.1 Number of samples collected per year (after aggregation of replicate samples per microhabitat), season, and hydrological category from each of the 7 streams. The number of samples varied depending on whether intermittent sites had surface water during a given season. The total number of samples collected per stream is noted by *n*.

	Year			Season			Hydrological category		
Stream	2009	2010	2011	Fall	Monsoon	Winter	Intermittent	Perennial	*n*
Babocomari	0	3	3	3	0	3	2	4	6
Garden	7	16	16	18	7	14	6	33	39
Huachuca	4	14	14	12	9	11	8	24	32
Miller	6	0	0	6	0	0	0	6	6
Ramsey	6	13	14	18	4	11	2	31	33
San Pedro	2	6	7	6	4	5	0	15	15
Woodcutters	0	11	2	1	4	8	13	0	13

2.2.3 Functional trait data

We identified 234 freshwater macroinvertebrate taxa during our study. We developed a trait database based on information in >80 publications from primary literature, databases, and available specialist knowledge to define categorical trait states specific to the southwestern region of the USA (2.7 Appendix A). Each taxon was represented by a combination of traits, known as its functional trait niche (FTN) (Poff et al. 2006). Functional trait diversity was represented by a species × trait matrix for the 225 taxa for which we found complete trait information. We used 7 functional traits spanning a total of 30 modalities: body size, voltinism, respiration, functional feeding group (FFG), dispersal capability, diapause, and primary locomotion (Table 2.2).

We calculated taxonomic richness, Shannon diversity (H′), and evenness (Pielou index; (Pielou 1969); and functional richness (FRic), functional diversity, and functional evenness (FEve). FRic measures the volume of functional space occupied by a community (Cornwell et al. 2006, Villéger et al. 2008). FRic values are not constrained to the total number of trait modalities present in the species pool because they are calculated using the minimal convex hull that includes all species and quantifies the volume occupied by the community's traits (Villéger et al. 2008). We calculated functional diversity as H′ for each community (species traits × species abundance matrix) and for individual traits (species abundances within each trait state in each community sample). FEve describes the distribution of traits within a community (i.e., whether they are distributed evenly within occupied trait space) (Villéger et al. 2008). FEve ranges from 0 to 1. Low values represent unevenly distributed species traits within trait space (e.g., high density of species within a narrow range of trait space) and high values represent evenly distributed species traits throughout the functional trait space. FEve incorporates species abundances in calculation of the metric. FRic and FEve were calculated using the R-based *FD* package and the function *dbFD* (Laliberté and Legendre 2010, Laliberté and Shipley 2011).

2.2.4 Statistical analyses

All analyses were conducted in R, and significance was assigned at $p < 0.05$. We used simple linear regression to assess the relationships between taxonomic richness and diversity vs functional richness and diversity. We fitted a hyperbolic saturation curve of the form $y = ax/(b + x)$ to the nonlinear relationship between functional richness and taxonomic richness, where a is the asymptotic limit of the curve and b is the half-saturation constant. We interpreted a as the limit saturation point of trait states for a given community type and b as the rate at which trait saturation occurs. This type of curve arises when the number of species (x) is relatively large or unbounded, but the number of trait states (y) is finite. In our case, the total number of trait states was 30. We used linear regression for relationships involving taxonomic and functional trait diversity because these metrics incorporate relative abundance and richness.

Invertebrates often do not show a mean linear response to stream flow, but their responses may be strong at very high or low flows (Konrad et al. 2008). Therefore, traditional linear regression models could overestimate, underestimate, or fail to detect a relationship (Cade et al. 1999, Cade and Noon 2003) between diversity estimates and stream-flow metrics. To address this problem, we used quantile regression to estimate multiple rates of change (slopes) across the distribution rather than focusing solely on the change in the center of the distribution (Cade et al. 1999). In addition to detecting important relationships in different partitions of our data, quantile regression is appropriate for our analyses because: 1) variances are often heterogeneous across flow-permanence gradients; 2) flow permanence may be a limiting factor on invertebrate community composition, richness, and diversity; 3) the data might not always meet parametric assumptions, and 4) multiple interacting factors may contribute to invertebrate diversity. The upper quantiles (ceilings) identify the constraints imposed by stream flow that limit the maximum response. Cases where only the upper- or lower-most quantiles are significant indicate limiting relationships. The lowest quantile ($\tau = 0.05$) describes the minimum limit of response. We followed (Rogers 1992) recommendations to select the number of quantiles investigated, $n > 5/q$ and $n > 5/(1 - q)$, where q determines the limits of reliable extreme quantiles and n is sample size. Our analysis included 5 evenly distributed quantiles ($\tau = 0.05, 0.25, 0.5, 0.75$, and 0.95) to test the bivariate relationships among 4 diversity measures (FRic, FEve, taxonomic richness and diversity) and a set of 4 predictors (% flow permanence, % flow permanence by season, mean duration of ZFP, and number of ZFPs). The median quantile ($\tau = 0.5$) estimates the center of the distribution where 50% of the data lie above and 50% lie below the estimate (Cade et al. 1999) and provides an estimate of central tendency similar to a linear regression fit. For quantile regression tests we used the *rq* function in R, which uses the Barrodale and Robert algorithm to compute model fit in the *quantreg* package (version 4.98; (Koenker 2013).

We used variance partitioning analysis (*varpart* function in the *vegan* package version 2.0-6 in R) to isolate the variance explained by each set of abiotic variables and their combined effects: season (monsoon, autumn, and winter), flow (perennial, intermittent), microhabitat type (river riffle, river pool, stream riffle, stream pool, seep, and pond), and habitat size (<1 m^2, 1–10 m^2, 10–100 m^2, 100–1000 m^2, and >1000 m^2). The function uses adjusted R^2 to assess the partitions explained by the explanatory variables and their combinations (Peres-Neto et al. 2006). We ran permutation tests to test the significance of all constraints simultaneously (Oksanen et al. 2013).

Table 2.2 Description of the 7 traits assessed for 225 taxa of aquatic invertebrates collected from 7 streams in the Huachuca Mountains, Arizona.

Trait	Trait state
Body size	<9 mm, 9–16 mm, >16 mm
Voltinism	Semivoltine: <1 generation/y, univoltine: 1 generation/y, multivoltine: >1 generation/y
Dispersal	Aquatic passive, aquatic active, aerial passive, aerial active
Respiration	Integument, gill, plastron, spiracle, vesicle
Functional feeding group	Collector-gatherer, shredder, scraper/grazer, filter-feeder, piercer-plants, piercer-predator, engulfer-predator
Diapause	Presence of structures for diapause or known diapause, possible diapause or resistance (inferred in studies or found in closely related taxa), no diapause or resistance known
Locomotion or habit	Burrow, interstitial, sprawl (crawl), attached (clingers), full water swimmer, surface swimmer (skater), climber

Plate 2.2 *Abedus herberti* on Fort Huachuca. Photo credit: Michael Bogan.

2.3. Results

2.3.1 Invertebrate assemblage structure and diversity across sampling sites

A total of 124 taxa in 43 different families or taxonomic units were identified across all intermittent sites, whereas a total of 210 taxa (70 families) were found across all perennial sites. Diptera were the most diverse group regardless of hydrological category (intermittent: 12 families, 58 species; perennial: 13 families, 85 species). Ephemeroptera and Trichoptera were more diverse in perennial sites (6 families, 18 genera or species; 12 families, 12 genera or species, respectively) compared to intermittent sites (3 families, 6 species; 5 families, 6 genera or species). Seven major taxonomic groups of invertebrates were limited to perennial sites and were not found in intermittent sites (e.g., Amphipoda, *Procambarus* crayfish, the hemipteran *Abedus herberti*). Invertebrate abundances did not differ significantly between perennial headwaters and intermittent reaches (as seen by Bogan et al. 2013).

A saturation curve fit to the taxonomic–functional richness data estimated trait saturation as a = 25.75 and the rate at which trait saturation occurs as b = 33.79 ($R^2 = 0.64$, $p < 0.0001$; Figure 2.2A). When analyzed separately, intermittent and perennial sites showed significant saturating relationships between taxonomic richness and FRic (intermittent: $a = 25.66$, $b = 43.65$, $R^2 = 0.61$, $p < 0.0001$; perennial: $a = 21.73$, $b = 22.05$, $R^2 = 0.44$, $p < 0.0001$) indicating the addition of new taxa beyond the asymptote did not increase FRic and that trait saturation occurred at a slightly lower species richness in perennial (21.73) than at intermittent sites (25.66). The intermittent-only curve was potentially affected by a single site with high species richness. Reanalysis without this single point suggested a linear rather than a saturating form to the curve. FEve was weakly associated with both taxonomic richness ($R^2 = 0.05$, $p = 0.005$) and taxonomic diversity ($R^2 = 0.07$, $p = 0.001$) because the distribution of traits within communities (FEve) had more variation at species-poor than at species-rich sites (2.8 Appendix B).

We found a strong positive relationship between taxonomic diversity (H′) and functional diversity across all sites ($R^2 = 0.65$, $p < 0.0001$, slope = 0.31), indicating moderate levels of functional-trait redundancy among coexisting species (Figure 2.2B). Intermittent sites ($R^2 = 0.56$, $p < 0.0001$, slope = 0.34) and perennial sites ($R^2 = 0.58$, $p < 0.0001$, slope = 0.25) had similarly strong positive relationships between taxonomic and functional diversity. The pattern of functional redundancy was also robust for single functional traits: FFG ($R^2 = 0.67$, $p < 0.0001$, slope = 0.44), body size ($R^2 = 0.35$, $p < 0.0001$, slope = 0.25), and habit/locomotion ($R^2 = 0.45$, $p < 0.0001$, slope = 0.43) (2.10 Appendix D).

Taxonomic evenness was on average high (0.68), but varied across samples, indicating that some invertebrate communities were dominated by a few species. FEve and taxonomic evenness were not related ($R^2 = -0.01$, $p = 0.7$, $n = 142$).

2.3.2 Stream flow permanence as a driver of invertebrate diversity

As predicted, FRic was positively related to % flow permanence and negatively related to the duration and number of ZFPs (Figure 2.3A, B, 2.9 Appendix C, 2.11 Appendix E). All flow metrics showed strong relationships across multiple quantiles for FRic (95% of quantiles tested were significant). Maximum FRic occurred at perennial, continuously flowing stream sites (Figure 2.3A). FRic increased with % flow permanence and had uniform variance across the flow permanence gradient (lines parallel), but variances were heteroscedastic across flow increments (Figure 2.3A). FRic did not respond homogeneously to changes in the number of ZFP

(Figure 2.3B). The response of FRic was steep for the lower quantiles ($\tau = 0.05$ and 0.25, slope = –1.35, –1.38, respectively) and different from the rates of change for the upper quantiles (0.75 and 0.95, slope = –0.46, –0.31, respectively). This result indicates communities with low FRic respond more strongly to ZFPs than communities with high FRic. The median quantile was not significant and, thus, the relationship between number of ZFP and FRic would not have been detected using standard regression techniques (Figure 2.3B). FRic and mean duration of ZFP had similar patterns, where decreasing rates of change occurred at higher levels of FRic (2.11 Appendix E). However, FRic values were less certain at longer durations of ZFPs. Several quantiles were significant for the relationship between functional diversity and % flow permanence and mean duration of ZFP, which suggests these metrics are important predictors of functional diversity (Figure 2.3C, 2.11 Appendix E). The number of ZFPs and % flow permanence by season were weakly correlated with functional diversity (Fig 2.3D, 2.11 Appendix E).

Communities at the perennial (100% flow permanence) sites had less variable and more evenly distributed traits in niche space than sites that experienced drying. FEve exhibited a negative limiting relationship with the number of ZFPs and demonstrated a floor in evenness with confidence bounds of FEve 0.39 to 0.68 (2.12 Appendix F). The duration of ZFP was weakly negatively related to FEve for the lowest 2 quantiles (2.12 Appendix F). FEve declined with longer dry periods. Notably, one community had a nearly-even distribution of traits in a pool that experienced the longest observed dry period (231 d) between flow events.

As predicted, taxonomic richness increased with flow permanence and declined with increasing number of drying events and longer duration of ZPFs (Figure 2.3F). Taxonomic diversity increased significantly at a relatively constant rate with the 2 metrics of flow permanence (Figure 2.3G, 2.11 Appendix E). The median response was informative for estimating the relationship between the number of ZFPs and taxonomic diversity. However the relationship also was significant for the upper extreme quantile (0.95; Figure 2.3H), suggesting that the number of drying events a stream experiences places a limit on the maximum diversity a community attains. We also examined the relationships between flow metrics and Rao's Q and found similar positive relationships with flow permanence and negative relationships with zero flow metrics. Evidence of strong niche filtering along the flow-permanence gradient was shown by a high degree of functional dissimilarity at sites that never dry (100% flow permanence, mean duration of ZFP = 0, and number of ZFP = 0).

Sites had fairly low interannual variation in flow conditions (values across 16 sites, mean ± SD; % flow permanence in year of sampling: 92.10 ± 24.39, duration of ZFP: 9.67 ± 34.19). FRic was significantly lower in intermittent samples compared to perennial communities, with ~½ as much niche space occupied (Kruskal–Wallis $\chi^2 = 41.590$, $p < 0.0001$). Intermittent sites also had lower functional diversity (Kruskal–Wallis $\chi^2 = 27.957$, $p < 0.0001$). FRic and functional diversity typically were higher in perennial sites, but 1 intermittent site attained taxonomic and functional diversity values similar to those in perennial sites. During September 2011 this site was connected by flow to upstream perennial reaches, which possibly contributed to higher FRic values. Invertebrate taxonomic diversity (Kruskal–Wallis $\chi^2 = 24.419$, $p < 0.0001$) and taxonomic richness (Kruskal–Wallis $\chi^2 = 40.809$, $p < 0.0001$) were highest in perennial sites.

Figure 2.2 Macroinvertebrate functional richness (FRic) vs taxonomic richness ($y = 25.75x/[33.79 + x]$) (A) and functional diversity vs taxonomic diversity ($y = 0.31x + 2.04$) (B) based on $n = 144$ sampling events in the Huachuca Mountains, Arizona, USA. Sites are coded by whether they had flow during 100% of the sampling period (perennial) or not (intermittent).

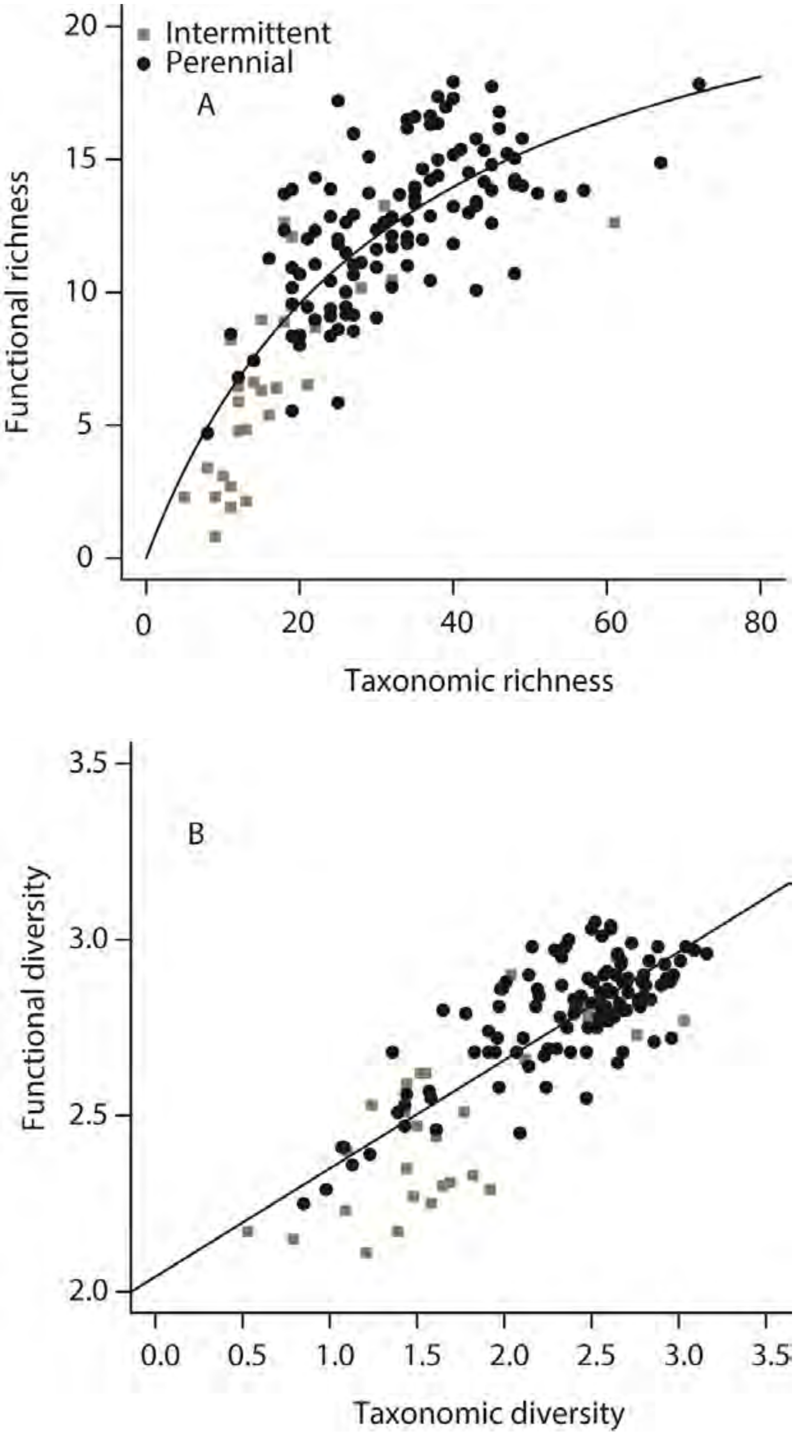
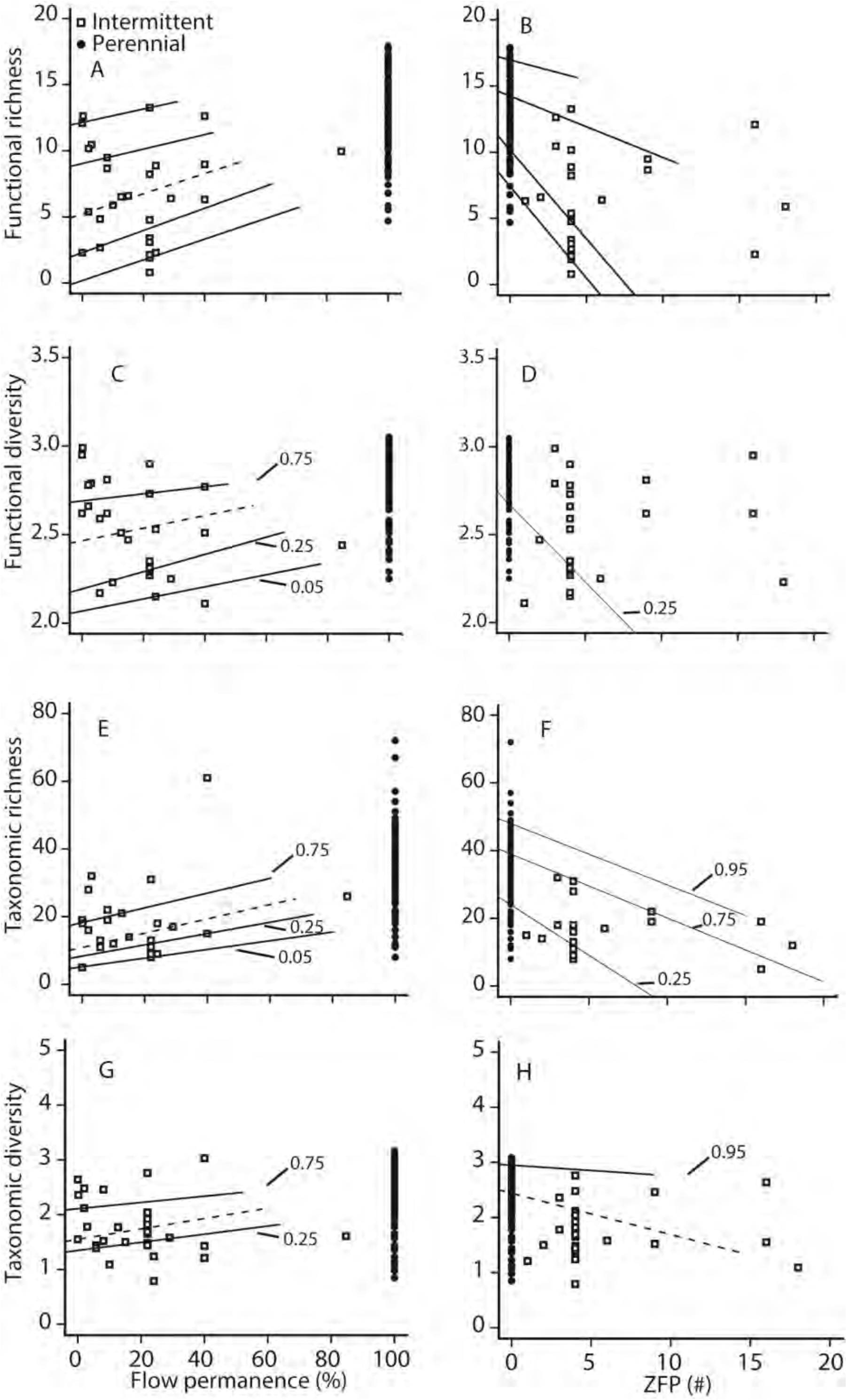


Figure 2.3 Quantile regressions of the relationships between functional richness (FRic) (A, B), functional diversity (C, D), Taxonomic richness (E, F), and taxonomic diversity (G, H) and % flow permanence (A, C, E, G) and number of zero flow periods (ZFP) (B, D, F, H). Only significant quantiles and the median quantile (dashed line) are shown. *n* = 144 for all plots.



2.3.3 Relative roles of habitat, hydrology, and season in shaping invertebrate communities

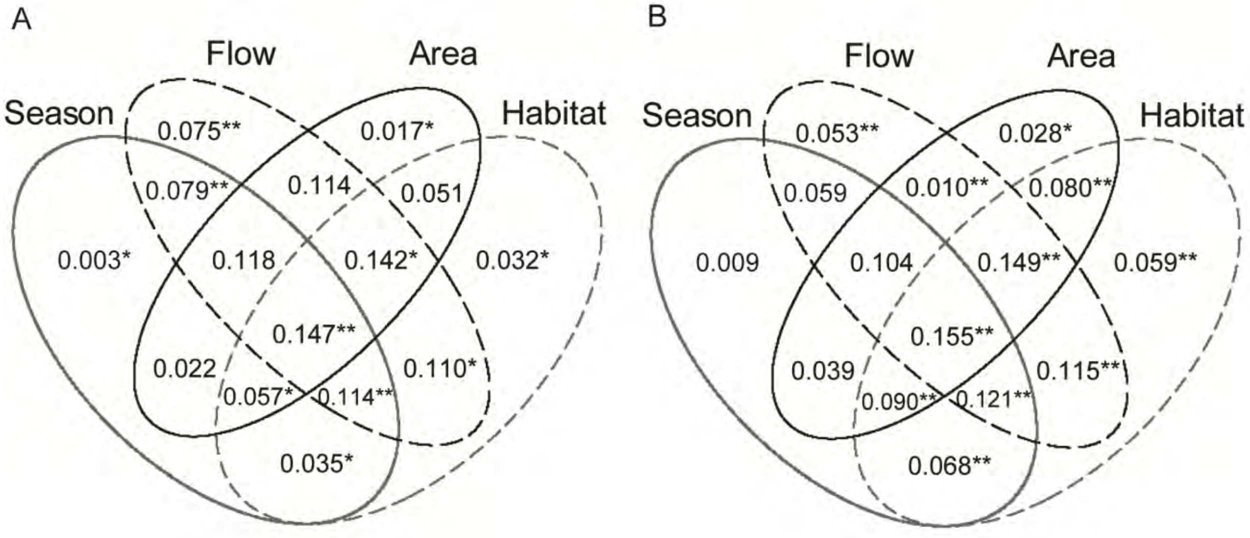
The main gradient in functional community composition was hydrology (flow: $R^2 = 0.075$; $F = 5.44$, $p = 0.005$) after controlling for the other environmental variables (Figure 2.4A). This result is congruent with the quantile regression analysis on flow metrics, indicating that functional composition and diversity indices respond strongly to hydrology. Microhabitat and total wetted area followed, explaining 3.2 and 1.7% of functional trait variation among sites, respectively. Season, although statistically significant, explained only 0.3% of the functional trait composition. The common variation explained in functional-trait composition contributed by all factors was 14.7% of the total variation explained (68.6%). On the other hand, the percentage of the variation in species composition among sites was nearly equal for site microhabitat (5.9%) and hydrology (5.3%) (Figure 2.4B). Habitat area explained a small portion of species composition (2.8%), and season was not a significant predictor ($p = 0.1$). These results show that microhabitat and hydrology explained a larger part of variation in species composition, whereas hydrology played a crucial role in explaining functional trait composition.

2.4 Discussion

We used continuous stream-flow data to examine the relationship between taxonomic and functional measures of both richness and diversity and to test for changes in diversity patterns in macroinvertebrate communities. Increased flow permanence was associated with increases in both taxonomic and functional richness of aquatic invertebrate communities. Diversity was lower in sites with more intermittent hydrological conditions, and hydrology explained more variation in trait and species composition than other environmental variables.

Functional richness was strongly related to taxonomic richness and diversity of stream invertebrates. The initial slope of this saturating relationship was steep, suggesting that at lower levels of taxonomic richness, communities have lower functional redundancy and less occupied niche space than at high taxonomic richness. Based on the saturating curve, functional richness would eventually peak at a higher taxonomic richness in intermittent (~51) than at perennial sites (~43), although intermittent sites was still generally lower than that of perennial sites. Moreover, the taxonomic and functional richness curve suggests that functional richness reached a saturation point (lower for intermittent sites) at which adding species to the community was not likely to add new trait combinations. Only 17% of intermittent sites had species richness greater than the saturation point (25) compared to 84% of perennial sites that had taxonomic richness >22 species. These results partially agree with those of (Beché and Statzner 2009) showing that trait saturation is rare in stream invertebrate communities. However, this was the case only at intermittent streams. Differences between their findings and ours could have arisen from the use of different traits (number of traits and trait states) and measures of functional richness (Petchey and Gaston 2006). Beché and Statzner (2009) used number of trait categories, whereas we calculated FRic using convex hull volume.

Figure 2.4 Variance partitioning (R^2) for trait composition (A) and taxonomic composition (B) of the matrix of environmental variables showing the respective contributions of the season, habitat area (Area), microhabitat type (Habitat), and the dominant hydrological flow category (Flow), their combinations, and combined effects. Fractions were tested on residuals (permutations = 9999). For significant combinations, * indicates $p = 0.01$, ** indicates $p = 0.001$.



We found a positive relationship between taxonomic and functional diversity. This relationship provides evidence for higher functional redundancy at higher levels of taxonomic diversity. Functional redundancy may offer resilience to environmental changes because of niche complementarity. A greater range of traits available could allow more efficient resource use (Loreau et al. 2001) and could provide insurance against changes in ecosystem function (Walker 1992). Lower functional diversity implies lower ecological redundancy, so if stream hydrology were to transition from perennial to intermittent, unique traits could be lost and that loss might lead to decreased productivity and disruption of ecosystem processes. For example, a single drying event could serve as a strong trait filter and cause the loss of drought-intolerant taxa (Poff 1997, Lamouroux et al. 2004, Cornwell et al. 2006) which might result in shorter food-chain length (Sabo et al. 2010). Thus, communities with lower functional richness and diversity may be susceptible to decreases in flow permanence because their communities have less redundancy and unused niche space. The communities that contained taxa with drought-adapted life histories showed levels of diversity similar to communities in perennial sites, thereby explaining the shallow slopes associated with diversity indices and flow metrics at the upper quantiles. Climate change is expected to increase drought conditions (Seager et al. 2007) by creating longer periods of low-flow conditions, resulting in intermittency of stream flow (Larned et al. 2010). We have shown that stream sites that experience more episodes and longer durations of no flow also have lower functional and taxonomic diversity.

If hydrology acts as a strong environmental filter, species should tend to occur at sites for which they are evolutionarily suited (Poff 1997, Lytle and Poff 2004), thereby resulting in lower functional richness and diversity in stream reaches with intermittent flow. Our results show that intermittent streams contained taxa with a higher degree of niche specialization (less trait volume occupied) than perennial streams (Figure 2.3), and that these taxa tended to be more functionally similar (as indicated by Rao's Q).

Only by evaluating multiple quantiles could we show that different aspects of stream hydrology differentially influenced multiple facets of functional and taxonomic diversity. Yearly flow permanence had a strong influence at low levels of diversity, richness, and evenness, whereas the number and duration of ZFPs constrained higher levels of diversity and richness. These results are congruent with those of other studies of intermittent streams, but they reveal new information about the relationship between stream intermittence and the functional response of communities. For instance, intermittent streams are characterized by low invertebrate richness (Williams 1996, Storey and Quinn 2008) and may support more specialist taxa and fewer predator taxa than perennial streams (Bogan et al. 2013). In general, invertebrate species diversity, abundance, and distribution are determined by flow (Statzner and Higler 1986, Statzner 2008, Oldmeadow et al. 2010, Arscott et al. 2010) and the length of the dry season (Williams and Hynes 1976), which is congruent with our observed pattern of lower FRic and FEve in intermittent sites. Bogan and Lytle (2011) found that altered stream flow (perennial to intermittent) after a severe drought changed species composition and extirpated top predators from stream reaches, but that species richness did not change. Our results provide evidence that species richness, FRic, and FEve are also reduced by declines in stream flow duration.

Functional evenness increased with flow permanence, signifying that traits became more regularly distributed in trait space as sites approach perenniality. Moreover, FRic was lower at intermittent than at perennial sites. Functional richness and FEve declined steeply across the less extreme numbers and durations of drying events (<10 events and 100 d dry, lower quantiles) followed by little change across the rest of the gradient. This relationship probably represents an ecological threshold (Groffman et al. 2006), where a few drying events influence all measures of functional diversity (signifying a strong environmental filter to those taxa without adaptations to cope with desiccation), but additional drying events have less effect on diversity measures. This result suggests that a single drying event would reduce species richness, functional richness, and the prevalence of drought intolerant taxa in streams that rarely experience drought. Moreover, this effect would be strongest for communities with lower levels of functional richness. We have shown that the duration of drying events and flow permanence are key extrinsic drivers of the responses of functional and taxonomic richness. Identifying ecological thresholds is imperative for making water-management decisions in dryland streams, especially where groundwater extraction contributes to the reduction in surface water flows.

Many authors separate aquatic habitats into discrete hydrological categories for convenience. However, our study shows that responses to hydrology fall along a continuum with high variance within hydrological groups. Thus, the responses of multiple measures of functional and taxonomic diversity to stream hydrology will vary depending on the region and length of the hydrologic continuum being examined. The upper and lower quantile regression lines for most diversity measures were farther apart at the more extreme end of the hydrologic continuum (intermittent flow, more ZFPs), and scatter between the upper and lower limit lines was considerable, indicating that other limiting factors influenced the diversity measures. The relationship between FRic and the 2 zero-flow metrics show that quantile regression lines started to converge in perennial sites, indicating that the functional niche space occupied was less heterogeneous (and thus, more predictable) in perennial habitats. These results concur with the habitat templet model prediction (Southwood 1977, Townsend and Hildrew 1994) that similar insect communities should occur in streams with similar environmental conditions, but contradict the prediction by (Poff et al. 2006) that infrequent low-flow disturbances should lead to high community similarity. Presumably, more extreme habitats have the harshest environmental filter and, therefore, one might predict extreme communities to be most similar. In support of this, we found strong limiting relationships between several flow permanence metrics and invertebrate diversity measures. However, intermittent sites tended to be more variable than permanent sites in their physical and chemical environment and to have high species turnover. This combination could lead to greater variation among and within intermittent communities, as was shown by empty niche space (lower FRic) and lower FEve at intermittent sites.

We showed that hydrology is a stronger gradient influencing functional composition of invertebrate communities than microhabitat, habitat size, and season (Figure 2.4A). However, in stream systems that exhibit less hydrologic variability, other factors, such as distance to perennial water, connectivity among habitats, or local variables, such as canopy cover, temperature, and water quality, may play an important role. Hydrology-influenced patterns of species diversity and community composition may scale up to important differences in ecosystem-level processes and foodweb dynamics. In a study on ponds, habitats with shorter hydroperiod had shorter food-chain length and fewer species at the intermediate-consumer and predator trophic levels compared to permanent ponds with longer hydroperiods (Schriever and Williams 2013). A similar study on streams in New Zealand demonstrated that streams with more variable temperature, hydrology, and geomorphology had shorter food-chain length (McHugh et al. 2010). In addition, in experimental stream mesocosms, induced drought conditions can cause loss of species and biomass that triggers restructuring of food webs (Ledger et al. 2013a). In light of projected climate change and increased human water use in coming decades (World Water Assessment Programme 2009, Marshall et al. 2010) and the importance of flow permanence for shaping the structure and function of invertebrate communities, it is imperative that arid and semi-arid streams receive additional research and management attention.

2.5 Acknowledgements

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2.6 Appendix A

References for trait state determination.

Order	Family	Taxon	Body size	Voltinism	Dispersal	Respiration	FFG	Diapause	Locomotion	Source
Amphipoda	Crustacea	*Hyalella*	3	3	1	2	2	2	3	1, 2
Annelida	Hirudinea	*Hirudinea*	2	2	3	3	6	2	5	3
Anostraca	Anostraca	*Anostraca*	2	3	3	2	4	1	5	1, 3
Basommatophora	Ancylidae	*Ferrissia*	1	2	1	1	3	3	4	1
	Ancylidae	Ancylidae	1	2	1	1	3	3	4	3, 4
Class:Arachnida	subclass:Acari	*Acari*	1	2	3	1	5	1	3	5,6
Coleoptera	Dryopidae	*Helichus lithophilus*	1	2	4	3	3	3	4	1,7, 8
		Helichus suturalis	1	2	4	3	3	3	4	1 ,7, 9, 8
		Helichus triangularis	1	2	4	3	3	3	4	1 ,7, 9,8
		Postelichus confluentus	1	2	4	3	3	3	4	7,9,10, 8
		Postelichus immsi	1	2	4	3	3	3	4	7,9, 10,8
	Dytiscidae	*Agabus*	1	2	4	3	6	1	5	1,11
		Desmopachria portmanni	1	3	4	3	6	3	5	1,7,10,11,12
		Hydroporinae	1	3	4	3	6	3	5	1 ,7,9, 11
		Hygrotus patruelis	1	3	4	3	6	3	5	1,11
		Hygrotus wardi	1	3	4	3	6	3	5	1,11
		Laccophilus fasciatus	1	2	4	3	6	3	5	1, 11,13
		Laccophilus maculosus	1	3	4	3	6	3	5	1, 11,13
		Laccophilus mexicanus	1	2	4	3	6	3	5	1, 11,13
		Laccophilus oscillator	1	2	4	3	6	3	5	1, 11,13
		Laccophilus pictus	1	2	4	3	6	3	5	1, 11,13
		Liodessus obscurellus	1	3	4	3	6	3	5	1 ,7,11
		Neoclypeodytes cinctellus	1	3	4	3	7	3	5	14,15,1,10,11
		Neoclypeodytes fryi	1	3	4	3	7	3	5	14,15,1,10,11

	Neoporus	1	3	4	3	7	3	5	14,15,1,10,11
	Rhantus atricolor	2	2	4	3	6	3	5	1,16
	Rhantus gutticollis gutticollis	2	2	4	3	6	3	5	1,16
	Sanfilippodytes	1	3	4	3	6	3	5	1,7,9,10,11
	Stictotarsus aequinoctialis	1	3	4	3	6	3	5	1,16
	Stictotarsus corvinus	1	3	4	3	6	3	5	1,16
	Stictotarsus roffi	1	3	4	3	6	3	5	1,16
	Stictotarsus striatellus	1	3	4	3	6	3	5	1,16
	Thermonectus marmoratus	2	2	4	3	6	3	5	15,14,1,17
	Thermonectus nigrofasciatus	2	2	4	3	6	3	5	15,14,1,17
Elmidae	*Heterelmis*	1	1	4	3	1	3	4	8,7,1
	Microcylloepus pusillus	1	2	4	3	1	3	4	18,1,8,7, 9
	Optioservus	1	1	4	3	1	3	4	18,15,7,1 ,19
	Zaitzevia	1	2	4	3	1	3	4	18,1,7,15,1, 20
Gyrinidae	*Dineutus sublineatus*	2	2	4	2	7	3	5	14,15,21
	Gyrinus plicifer	1	2	4	2	7	3	5	1 ,9,21, 22
Haliplidae	*Peltodytes callosus*	1	3	4	3	5	3	5	1,7,9,21
	Peltodytes dispersus	1	3	4	3	5	3	5	1 ,7,9,21
Hydraenidae	*Gymnochthebius*	1	2	4	3	3	3	4	14,10,1 ,21
	Hydraena	1	2	4	3	3	3	4	1,10,21
Hydrophilidae	*Anacaena (signaticollis)*	1	2	4	3	2	3	1	1,9
	Berosus miles	1	2	4	3	7	3	5	1,10,21,23
	Berosus punctatissimus	1	2	4	3	7	3	5	1,10,21,23
	Berosus rugulosus	1	2	4	3	7	3	5	1,10,21,23
	Berosus salvini	1	2	4	3	7	3	5	1,10,21,23
	Chaetarthria	1	2	4	3	2	3	5	1,10,21
	Cymbiodyta	1	2	4	3	2	3	5	1,24
	Enochrus aridus	2	2	4	3	2	3	3	1,10,25,26
	Enochrus piceus glabrus	2	2	4	3	2	3	3	1,10,25,26
	Enochrus pygmaeus pectoralis	2	2	4	3	2	3	3	1,10,25,26

		Helophorus	1	2	4	3	3	3	5	1,27
		Laccobius hardyi	1	2	4	3	3	3	5	1,28
		Tropisternus affinus	2	2	4	3	3	3	5	9,1,29
		Tropisternus lateralis	2	2	4	3	3	3	5	1,9,29
Decapoda	Cambaridae	*Procambarus*	3	2	2	2	1	2	1	1,3
Diplostraca	Limnadiidae	*Eulimnadia*	1	2	1	3	4	1	5	30,3
Diptera	Ceratopogonidae	*Atrichopogon*	1	2	1	2	7	2	3	1,7
		Forcipomyia	1	2	4	2	1	2	3	1 ,7
		Ceratopogonidae	1	2	1	2	7	2	3	1,7
	Culicidae	*Aedes*	1	3	4	3	1	1	5	15,10
		Anopheles	1	2	4	3	7	1	5	1,10
		Culiseta	1	3	4	3	1	1	5	1,9,10
		Culex	1	3	4	3	1	1	5	1,9,10
	Dixidae	*Dixa*	1	2	1	3	4	1	5	1,10
		Dixella	1	2	1	3	1	1	5	1, 9
	Dolichopodidae	Dolichopodidae	1	2	4	3	5	3	1	1,10
	Empididae	*Empididae*	1	2	4	1	7	3	3	1,7
		Ephydridae	1	2	4	3	2	3	3	1,9,10
	Muscidae	*Muscidae*	1	3	2	3	6	3	3	1,15
	Psychodidae	*Maruina*	1	2	1	3	2	3	4	7
		Pericoma	1	3	1	3	2	3	1	1,7
	Ptychopteridae	*Ptychoptera*	3	2	1	3	1	1	1	1,10
	Simuliidae	*Prosimulium*	1	2	4	1	4	1	4	1,10,31
		Simulium	1	3	4	1	4	1	4	1
	Stratiomyidae	*Caloparyphus*	2	2	4	3	2	1	3	1,10
		Euparyphus	2	2	4	3	2	1	3	1,10
	Syrphidae	*Syrphidae*	2	2	4	3	1	2	1	1,9
	Tabanidae	*Tabanus*	3	2	4	3	6	2	1	1,10,9
	Thaumaleidae	Thaumaleidae	2	2	1	3	3	2	3	1,9
	Tipulidae	*Dicranota*	3	2	4	3	2	3	1	1,9
		Limnophila	3	2	4	3	2	3	1	1,9

	Limonia	3	2	4	3	2	3	1	1,9
	Pedicia	3	2	4	3	2	3	1	1,9
	Tipula	3	2	2	3	2	3	1	1,7, 9
Chironomidae	*Ablabesmyia*	1	3	4	1	7	3	3	32, 38, 40, 47, 53
	Acricotopus	1	3	4	1	1	3	3	32, 38, 40, 47, 53, 56
	Apedilum	1	3	4	1	1	2	3	32, 38, 40, 47, 51, 53
	Apsectroptanypus	1	3	4	1	7	3	1	32, 38, 40, 47, 53
	Brillia	1	3	4	1	2	3	1	32, 38, 40, 41, 47, 53
	Bryophaenocladius	1	3	4	1	1	3	3	32, 38, 40, 47, 53
	Chaetocladius	1	3	4	1	1	2	3	32, 35, 36, 38, 40, 47, 53
	Chironomus	1	3	4	1	1	1	1	32, 33, 38, 39, 40, 47, 49, 51, 53, 54, 56
	Corynoneura	1	3	4	1	1	2	3	32, 36, 38, 39, 40, 47, 50, 53
	Cricotopus/ Orthocladius	1	3	4	1	1	2	4	32, 36, 38, 40, 46,47, 53, 56
	Cryptochironomus	1	3	4	1	6	3	2	32, 38, 40, 47, 53
	Demicryptochironomus	1	3	4	1	1	2	1	32, 38, 40, 46, 47, 53
	Diamesa	1	3	4	1	1	2	3	32, 38, 40, 46, 47, 50, 53
	Dicrotendipes	1	3	4	1	1	2	1	32, 38, 40, 41, 47, 49, 53
	Eukiefferiella rectangularis grp.	1	3	4	1	1	2	3	32, 38, 40, 41, 47, 50, 53, 56
	Eukiefferiella brehmi grp.	1	3	4	1	1	2	3	32, 38, 40, 41, 47, 50, 53, 56
	Eukiefferiella claripennis grp.	1	3	4	1	1	2	3	32, 36, 38, 40, 47, 48, 50, 53, 56
	Eukiefferiella coerulescense grp.	1	3	4	1	1	2	3	32, 38, 40, 46, 47, 50, 53, 56
	Eukiefferiella devonica grp.	1	3	4	1	1	2	3	32, 38, 40, 41, 47, 50, 53, 56
	Eukiefferiella gracei grp.	1	3	4	1	1	2	3	32, 38, 40, 41, 47, 50, 53, 56
	Heleniella	1	3	4	1	1	3	3	32, 38, 40, 41, 47, 53
	Heterotrissocladius	1	3	4	1	1	3	3	32, 38, 40, 47, 53
	Hydrobaenus	1	3	4	1	3	1	3	32, 34, 36, 37, 38, 39, 40, 45, 47, 52, 53, 56
	Krenosmittia	1	3	4	1	1	2	3	32, 38, 40, 46, 47, 53
	Labrundinia	1	3	4	1	7	2	3	32, 38, 40, 42, 47, 53
	Larsia	1	3	4	1	7	2	3	32, 36, 38, 40, 47, 53
	Limnophyes	1	3	4	1	1	2	4	32, 34, 36, 38, 39, 40, 46, 47, 53, 56
	Lopescladius	1	3	4	1	1	3	3	32, 38, 40, 47, 53
	Mesosmittia	1	3	4	1	1	3	3	32, 38, 40, 47, 53
	Micropsectra	1	3	4	1	1	1	7	32, 36, 38, 40, 47, 50, 53, 54,

								55
Microtendipes pedellus grp.	1	3	4	1	4	3	4	32, 38, 40, 47, 53
Nanocladius	1	3	4	1	1	3	3	32, 38, 40, 47, 53
Nilotanypus	1	3	4	1	7	3	3	32, 38, 40, 47, 53
Orthocladius (Symposiocladius)	1	2	4	1	1	3	1	32, 36, 38, 40, 47, 53
Parachaetocladius	1	3	4	1	1	3	3	32, 36, 38, 40, 47, 53
Paracladopelma	1	3	4	1	1	3	3	32, 38, 40, 47, 53
Paracricotopus	1	3	4	1	1	3	3	32, 38, 40, 47, 53
Parakiefferella	1	3	4	1	1	2	3	32, 36, 38, 40, 47, 53
Paramerina	1	3	4	1	7	3	3	32, 38, 40, 47, 53
Parametriocnemus	1	3	4	1	1	2	4	32, 38, 40, 42, 46, 47, 50, 53
Parasmittia	1	3	4	1	1	3	3	32, 38, 40, 47, 53
Paratanytarsus	1	3	4	1	1	2	4	32, 34, 38, 40, 43, 47, 49, 53, 56
Paratendipes	1	3	4	1	1	2	1	32, 36, 38, 40, 47, 49, 53
Parochlus	1	3	4	1	1	3	3	32, 38, 40, 47, 53
Pentaneura	1	3	4	1	7	3	3	32, 38, 40, 47, 53
Phaenopsectra	1	3	4	1	3	2	4	32, 38, 39, 40, 43, 46, 47, 53
Polypedilum	1	3	4	1	1	2	4	32, 38, 40, 42, 44, 47, 51, 52, 53, 56
Procladius	1	3	4	1	7	3	3	32, 38, 40, 47, 53
Psectrocladius	1	3	4	1	1	3	4	32, 38, 40, 47, 53, 56
Pseudochironomus	1	3	4	1	1	3	1	32, 38, 40, 47, 53
Pseudosmittia	1	3	4	1	1	2	3	32, 36, 38, 40, 47, 53
Rheocricotopus	1	3	4	1	1	3	4	32, 38, 40, 47, 53
Rheotanytarsus	1	3	4	1	4	2	4	32, 38, 40, 47, 49, 53
Saetheria	1	3	4	1	1	3	1	32, 38, 40, 47, 53
Smittia	1	3	4	1	1	2	1	32, 38, 40, 47, 53, 56
Stempellinella	1	3	4	1	1	3	1	32, 38, 40, 47, 53
Stenochironomus	1	3	4	1	2	2	1	32, 38, 40, 47, 49, 53
Stictochironomus	1	3	4	1	1	2	1	32, 36, 38, 40, 47, 49, 53
Tanytarsus	1	3	4	1	1	1	4	32, 38, 40, 43, 46, 47, 52, 53, 56
Thienemanniella xena	1	3	4	1	1	1	3	32, 38, 40, 46, 47, 52, 53
Thienemanniella fusca	1	3	4	1	1	1	3	32, 38, 40, 46, 47, 52, 53
Thienemannimyia grp.	1	3	4	1	7	2	3	32, 38, 39, 40, 47, 50, 53
Tvetenia bavarica grp.	1	3	4	1	1	3	1	32, 38, 40, 47, 50, 53

		Virgatanytarsus	1	3	4	1	1	3	7	32, 38, 40, 47, 53
Ephemeroptera	Ameletidae	*Ameletus*	2	2	4	2	3	2	5	1
	Baetidae	*Acentrella*	1	2	4	2	3	2	3	1
		Baetis	1	3	4	2	3	2	3	1
		Callibaetis	1	3	4	2	3	2	5	7,10
		Camelobaetidius maidu	1	3	4	2	3	2	5	7
		Fallceon	1	3	4	2	3	2	5	1,7
	Caenidae	*Caenis*	1	3	1	2	1	2	3	1
	Heptageniidae	*Ecdyonourus*	1	2	4	2	3	2	4	1,7
	Leptohyphidae	*Homoleptohyphes*	1	2	4	2	1	1	4	7,9
		Tricorythodes	1	2	2	2	1	2	3	1,7
	Leptophlebiidae	*Choroterpes*	1	2	1	2	1	2	3	1
		Farrodes	1	2	1	2	1	2	4	15,7
Gastropoda	Lymnaeidae	*Lymnaeidae*	2	2	3	2	3	2	3	3
	Hydrobiidae	*Pyrgulopsis*	1	2	1	2	3	3	4	57, 3,1,10,58
	Physidae	*Physidae*	2	2	2	1	3	1	7	10
	Planorbidae	*Planorbidae*	1	2	1	2	3	3	7	1,3
Hemiptera	Belostomatidae	*Abedus herberti*	3	2	2	3	6	3	7	7
		Lethocerus medius	3	2	4	3	6	3	7	7,10,59
	Corixidae	*Graptocorixa abdominalis*	2	3	4	3	6	3	5	15,7,60,61
		Graptocorixa gerhardi	1	3	4	3	6	3	5	15,7,61
		Graptocorixa serrulata	1	3	4	3	6	3	5	1,7,61
		Hesperocorixa	2	3	4	3	6	3	5	1,7,61
		Rhamphocorixa acuminata	1	3	4	3	6	3	5	15,10,61
		Trichocorixa uhleri	1	3	4	3	6	3	5	7,15,61
	Gerridae	*Aquarius remigis*	2	2	4	3	6	3	6	1,10,21
	Hebridae	*Hebrus*	1	2	4	3	6	3	6	15,21
	Nepidae	*Curicta pronotata*	3	2	4	3	6	3	7	15,1,7,10,62,63
		Ranatra quadridentata	3	2	4	3	6	3	4	1,21,63
	Notonectidae	*Buenoa arida*	1	3	4	3	6	3	5	1,10,64
		Buenoa arizonis	1	3	4	3	6	3	5	1,10,64
		Notonecta hoffmanni	2	2	4	3	6	3	5	1,10,81
		Notonecta lobata	2	2	4	3	6	3	5	1,10,81

	Veliidae	*Microvelia*	1	3	4	3	6	1	6	1,10,65
		Rhagovelia	1	3	2	3	6	2	6	1,7,66
Lepidoptera	Crambiidae	*Petrophila*	1	2	4	2	2	3	4	15
Megaloptera	Corydalidae	*Corydalus texanus*	3	1	4	2	7	2	4	7,10,67
		Neohermes filicornis	3	1	4	2	7	1	4	1,7,10,68
Odonata	Aeshnidae	*Oplonaeschna armata*	3	1	4	2	7	3	4	1,7,69
	Calopterygidae	*Hetaerina*	2	2	4	2	7	2	7	7,70
	Coenagrionidae	*Argia*	2	2	4	2	7	3	4	7,70
		Coenagrion / Enallagma	3	2	4	2	7	3	3	1
	Cordulegastridae	*Cordulegaster diadema*	3	1	4	2	7	3	1	1,10
	Gomphidae	*Erpetogomphus*	3	1	4	2	7	2	1	7,1,10,69
	Lestidae	*Archilestes grandis*	3	2	4	1	7	3	7	7,10,70
	Libellulidae	*Brechmorhoga*	3	1	4	2	7	3	3	15,69
		Libellula saturata	3	1	4	2	7	3	3	1,69
		Paltothemis lineatipes	3	1	4	2	7	3	3	1,7,69
		Pantala hymenaea	3	1	4	2	7	3	3	7,15,1, 69
Plecoptera	Capniidae	Capniidae	1	2	4	1	2	1	3	7,71,72
		Mesocapnia	1	2	4	1	2	1	3	7,39
	Chloroperlidae	Chloroperlidae	2	2	4	1	7	3	3	1,15, 7
		Sweltsa	2	1	4	1	7	3	3	7,73
	Nemouridae	*Malenka/Amphinemura*	1	2	4	1	2	1	3	1,7,73
Podocopida	Ostracoda	Ostracoda	1	2	3	3	4	1	5	74, 3,75
Trichoptera	Brachycentridae	*Micrasema*	1	2	4	1	2	3	4	1,76
	Calamoceratidae	*Phylloicus mexicanus*	2	2	4	2	2	3	3	1,7,77
	Helicopsychidae	*Helicopsyche*	1	2	4	1	1	2	4	1,7,76,78
	Hydrobiosidae	*Atopsyche*	2	2	4	2	7	3	4	1,7,15,76
	Hydropsychidae	*Cheumatopsyche*	1	2	4	2	4	3	4	1,10,76,78
		Hydropsyche/Ceratopsyche	2	2	4	2	4	3	4	1,7,76,78
	Hydroptilidae	*Culoptila*	1	3	4	1	3	3	4	1,7,76
		Hydroptila	1	2	4	2	5	3	4	1,10,76
		Metrichia	1	3	4	1	5	3	4	1,76

		Ochrotrichia	1	3	4	1	3	2	4	1,76
		Oxyethira	1	3	4	1	5	3	4	1,64
	Lepidostomatidae	*Lepidostoma - sp. A*	1	2	4	2	2	3	3	1,64
		Lepidostoma acarolum	2	2	4	2	2	3	3	1,7,79
	Leptoceridae	*Nectopsyche*	2	2	4	2	1	3	7	1,15,7,76
		Oecetis	1	2	4	2	2	3	3	1,7,76
	Limnephilidae	*Hesperophylax magnus*	3	2	4	2	2	3	3	1,7
	Philopotamidae	*Wormaldia*	1	1	4	1	4	3	4	1,10,7,78
	Polycentropidae	*Polycentropus*	2	2	4	1	7	3	4	1,7
	Psychomyiidae	*Tinodes*	1	3	4	1	3	3	4	1,76
Tricladida	Platyhelmenthies	Platyhelmenthies	2	2	1	3	6	2	7	3
Veneroida	Sphaeriidae	*Pisidium*	1	2	1	2	4	1	1	1,80,10

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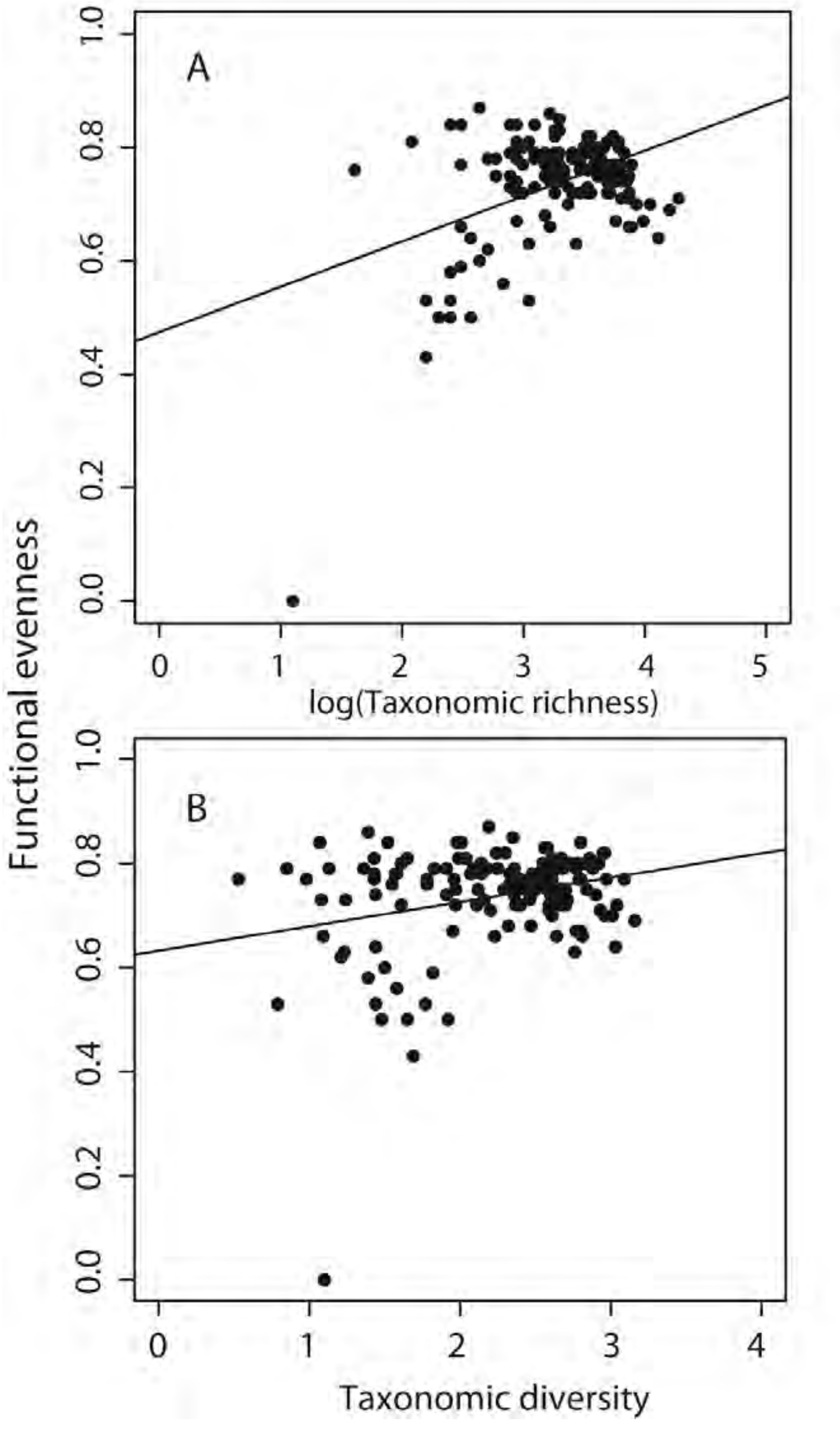
2.7 Appendix B

Functional evenness (FEve) vs taxonomic richness ($y = 0.08\log[x] + 0.474$) (A) and taxonomic diversity ($y = 0.05x + 0.6325$) (B).



2.8 Appendix C

Quantile regression models results. The 4 stream flow metrics are: % flow permanence in year of sample (flowyr), % flow permanence by season (flowsea), mean duration of zero flow periods each year (MDZFP), and number of ZFP each year (numZFP). NS refers to non-significance.

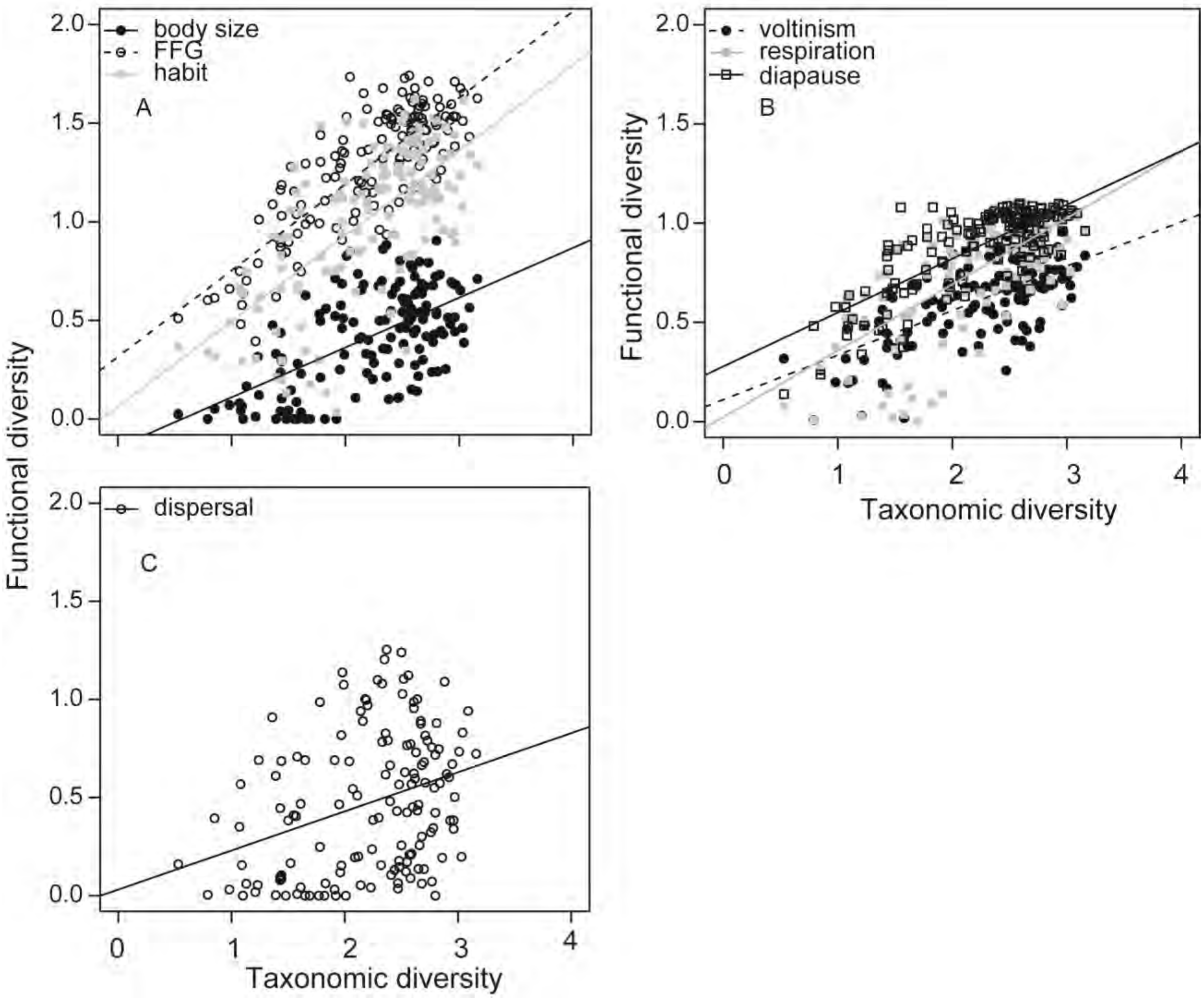
Relationship	Model equation	Regression quantile	*p* value
Functional richness (FRic)			
flowyr	0.078(flowyr) + 0.189	0.05	<0.0001
	0.084(flowyr) + 2.30	0.25	<0.0001
	0.076(flowyr) + 5.230	0.5	<0.0001
	0.055(flowyr) + 9.038	0.75	0.006
	0.051(flowyr) + 12.149	0.95	<0.0001
flowsea	0.083(flowsea) – 0.240	0.05	<0.0001
	0.082(flowsea) + 2.22	0.25	<0.0001
	0.070(flowsea) + 5.876	0.50	<0.0001
	0.048(flowsea) + 9.742	0.75	0.04
	0.044(flowsea) + 12.586	0.95	<0.0001
MDZFP	–0.118(MDZFP) + 7.430	0.05	0.00003
	–0.127(MDZFP) + 10.420	0.25	0.0008
	–0.085(MDZFP) + 12.630	0.50	0.04
	–0.045(MDZFP) + 14.310	0.75	0.05
	–0.045(MDZFP) + 16.970	0.95	0.002
numZFP	–1.38(numZFP) + 7.430	0.05	0.0002
	–1.348(numZFP) + 10.170	0.25	0.02
	–0.644(numZFP) + 12.60	0.50	NS
	–0.464(numZFP) + 14.240	0.75	0.00003
	–0.305(numZFP) + 16.970	0.95	<0.0001
Functional evenness (FEve)			
flowyr	0.002(flowyr) + 0.452	0.05	0.00001
	0.002(flowyr) + 0.501	0.25	<0.0001
	0.001(flowyr) + 0.632	0.50	0.014
	0.0003(flowyr) + 0.759	0.75	0.030
	0.000(flowyr) + 0.802	0.95	NS
flowsea	0.002(flowsea) + 0.449	0.05	0.00001
	0.0024(flowsea) + 0.486	0.25	0.00001
	0.0016(flowsea) + 0.615	0.50	0.017
	0.000(flowsea) + 0.7460	0.75	NS
	0.001(flowsea) + 0.789	0.95	NS

numZFP	–0.043(numZFP) + 0.67	0.05	<0.0001
	–0.033(numZFP) + 0.720	0.25	NS
	–0.006(numZFP) + 0.770	0.50	NS
	–0.004(numZFP) + 0.800	0.75	NS
	0.000(numZFP) + 0.840	0.95	NS
MDZFP	–0.004(MDZFP) + 0.67	0.05	<0.0001
	–0.003(MDZFP) + 0.73	0.25	0.022
	–0.001(MDZFP) + 0.770	0.50	NS
	–0.000(MDZFP) + 0.790	0.75	NS
	–0.001(MDZFP) + 0.840	0.95	NS
Taxonomic richness			
flowyr	0.128(flowyr) + 5.179	0.05	<0.0001
	0.167(flowyr) + 8.333	0.25	<0.0001
	0.211(flowyr) + 10.873	0.50	<0.0001
	0.218(flowyr) + 18.236	0.75	<0.0001
	0.175 (flowyr) + 31.474	0.95	NS
flowsea	0.131(flowsea) + 4.869	0.05	<0.0001
	0.182(flowsea) + 6.818	0.25	<0.0001
	0.200(flowsea) + 11.956	0.5	<0.0001
	0.212(flowsea) + 18.788	0.75	<0.0001
	0.170(flowsea) + 32.000	0.95	NS
MDZFP	–0.157(MDZFP) + 16.000	0.05	NS
	–0.235(MDZFP) + 24.000	0.25	<0.0001
	–0.179(MDZFP) + 31.000	0.5	0.003
	–0.129(MDZFP) + 39.000	0.75	NS
	–0.121(MDZFP) + 49.000	0.95	<0.0001
numZFP	–1.750(numZFP) + 16.000	0.05	NS
	–3.000(numZFP) + 24.000	0.25	<0.0001
	–1.625(numZFP) + 31.000	0.50	NS
	–1.889(numZFP) + 39.000	0.75	<0.0001
	–1.813(numZFP) + 48.000	0.95	<0.0001
Functional diversity			
Flowyr	0.003(flowyr) + 2.068	0.05	0.003
	0.005(flowyr) + 2.193	0.25	<0.0001
	0.004(flowyr) + 2.465	0.5	0.007
	0.002(flowyr) + 2.690	0.75	0.037
	0.0005(flowyr) + 2.950	0.95	NS
flowsea	0.003(flowsea) + 2.093	0.05	NS

	0.005(flowsea) + 2.199	0.25	<0.0001
	0.003(flowsea) + 2.49	0.5	0.013
	0.002(flowsea) + 2.73	0.75	NS
	0.0004(flowsea) + 2.95	0.95	NS
MDZFP	–0.005(MDZFP) + 2.41	0.05	0.028
	–0.007(MDZFP) + 2.68	0.25	0.0001
	–0.003(MDZFP) + 2.81	0.5	NS
	–0.002(MDZFP) + 2.89	0.75	<0.0001
	0.000(MDZFP) + 2.99	0.95	NS
numZFP	–0.060(numZFP) + 2.41	0.05	NS
	–0.083(numZFP) + 2.68	0.25	0.003
	–0.032(numZFP) + 2.81	0.5	0.057
	–0.016(numZFP) + 2.88	0.75	NS
	–0.003(numZFP) + 2.99	0.95	NS
Taxonomic diversity (H′)			
flowyr	0.002(flowyr) + 1.074	0.05	NS
	0.007(flowyr) + 1.347	0.25	<0.0001
	0.010(flowyr) + 1.550	0.5	<0.0001
	0.006(flowyr) + 2.109	0.75	0.031
	0.003(flowyr) + 2.704	0.95	NS
flowsea	0.001(flowsea) + 1.090	0.05	NS
	0.008(flowsea) + 1.304	0.25	<0.0001
	0.010(flowsea) + 1.540	0.5	<0.0001
	0.006(flowsea) + 2.065	0.75	0.024
	0.002(flowsea) + 2.760	0.95	NS
numZFP	–0.007(numZFP) +1.217	0.05	NS
	–0.073(numZFP) + 1.980	0.25	NS
	–0.075(numZFP) + 2.440	0.50	0.014
	–0.070(numZFP) + 2.67	0.75	NS
	–0.019(numZFP) + 2.950	0.95	<0.0001
MDZFP	0.002(MDZFP) + 1.130	0.05	NS
	–0.010(MDZFP) + 2.01	0.25	<0.0001
	–0.009(MDZFP) + 2.480	0.50	0.043
	–0.004(MDZFP) + 2.680	0.75	0.021
	–0.005(MDZFP) + 2.960	0.95	0.006

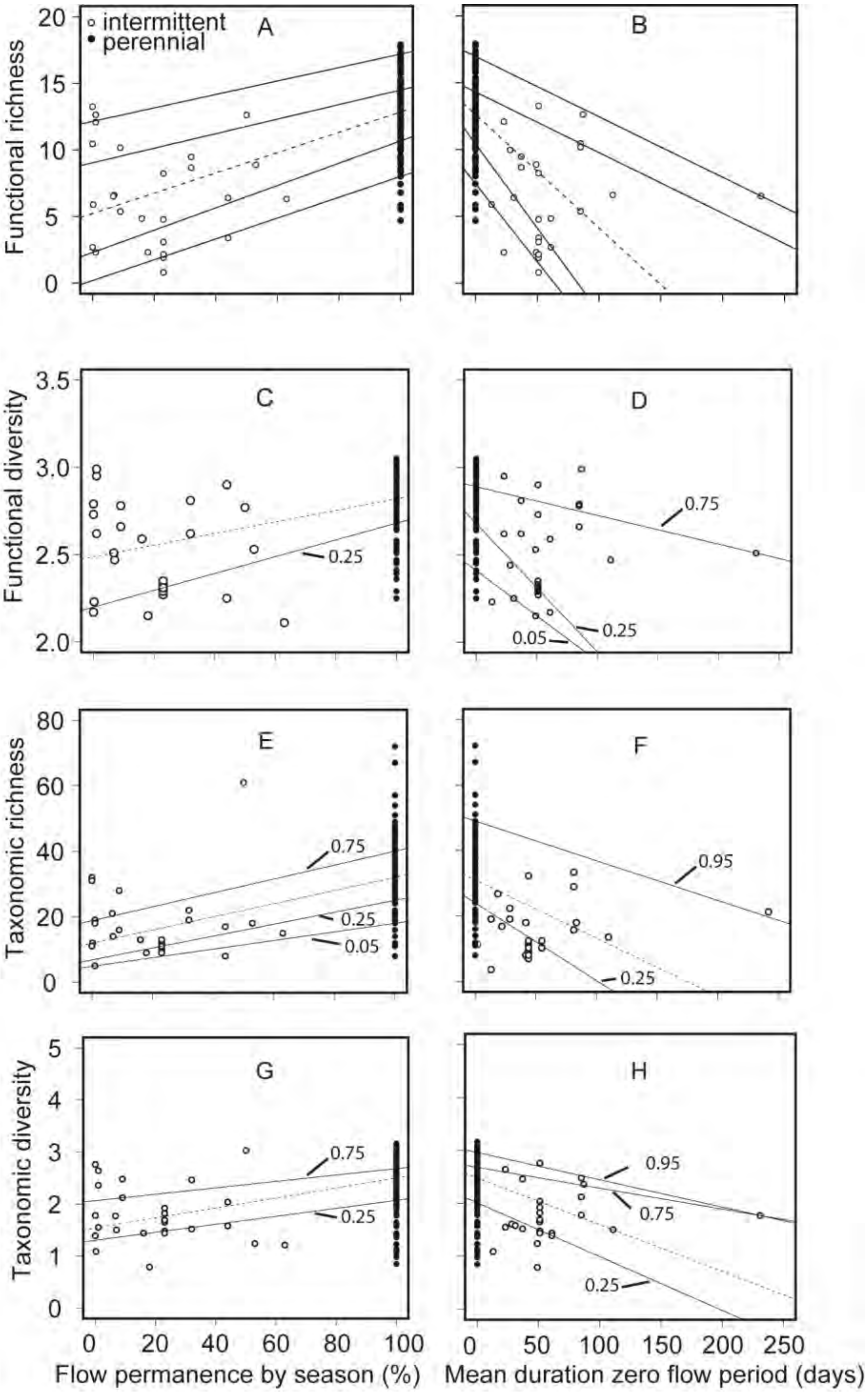
2.9 Appendix D

Relationship between responses of functional and taxonomic diversity for individual functional traits. A.—Functional feeding groups (*n* = 7 states), body size (*n* = 3), and habit/locomotion (*n* = 7). B.—Voltinism (*n* = 3, slope = 0.22, $R^2 = 0.43$, $p < 0.0001$), respiration (*n* = 3, slope = 0.33, $R^2 = 0.47$, $p < 0.0001$), and diapause (*n* = 3, slope = 0.27, $R^2 = 0.61$, $p < 0.0001$). C.—The relationship with dispersal was significant, but weak (*n* = 4, slope = 0.20, $R^2 = 0.10$, $p < 0.0001$).



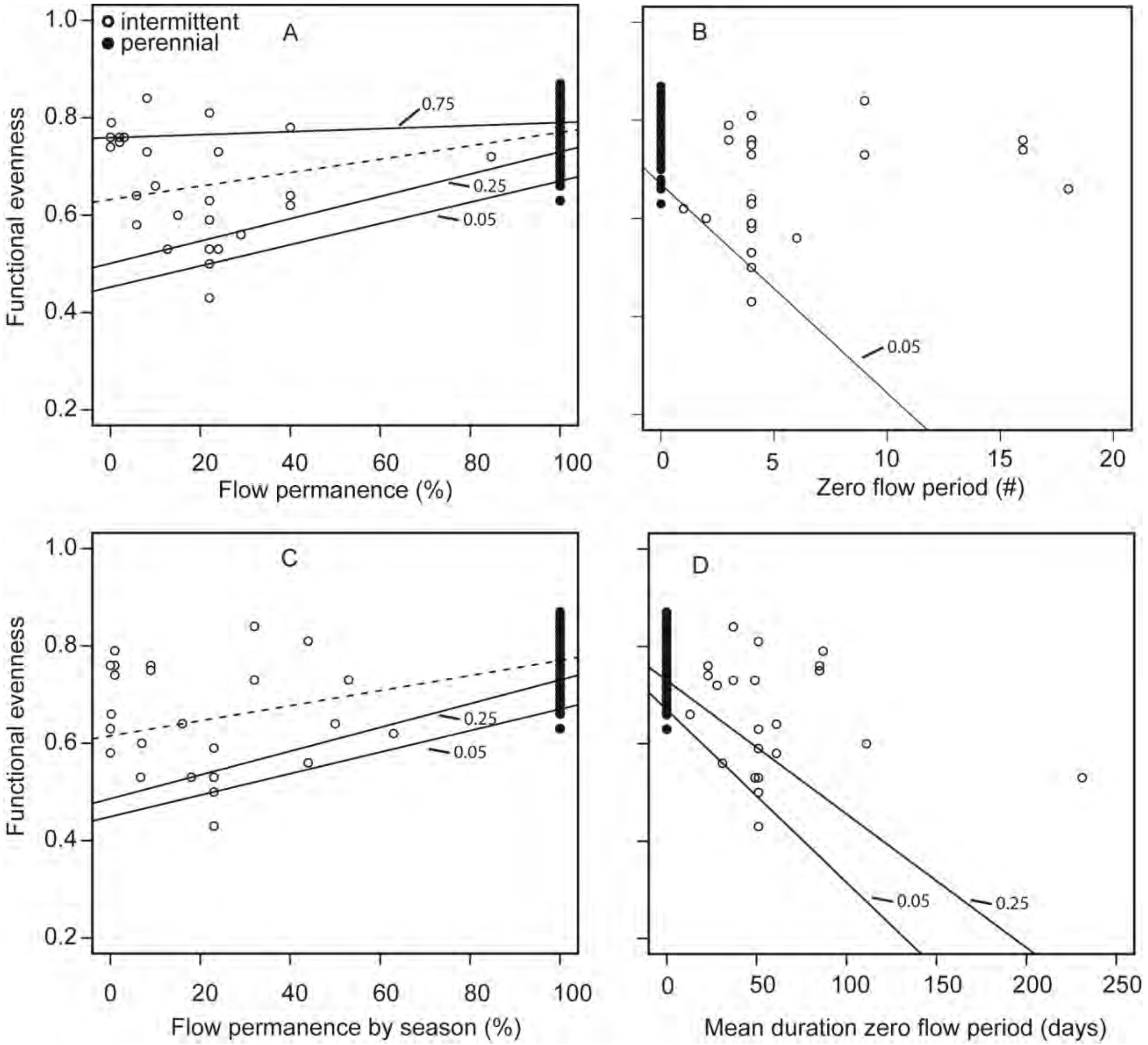
2.10 Appendix E

Quantile regression plots for the relationships between flow metrics % flow permanence per season and mean duration of zero flow periods and functional richness (FRic) (A, B), functional diversity (C, D), taxonomic richness (E, F), and taxonomic diversity (G, H). Only the significant quantiles are plotted and the dashed line is the median quantile.



2.10 Appendix F

Quantile regression for relationships between % yearly flow permanence (A), number of zero flow periods (B), % flow permanence by season (C), mean duration of zero flow period (D) and functional evenness (FEve). Only the significant quantiles are plotted, and the median quantile is the dashed line.



3. Dispersal strength determines meta-communities in a dendritic riverine network

3.1 Introduction

Studying patterns of biological diversity has been the foundation of numerous ecological pursuits over the past two centuries. Distance decay relationships (DDRs) – which describe the biogeographical phenomenon where taxonomic similarity between localities decreases or decays as the distance between them increases – have received considerable interest among ecologists (Nekola & White, 1999). Indeed, this ecological pattern is encapsulated in Tobler's first law of geography, which states that 'everything is related to everything else, but near things are more related than distant things' (Tobler, 1970, p. 236). DDRs have now been studied across a wide range of organisms and environments (reviewed in Soininen *et al*., 2007), but are still relatively understudied in riverine ecosystems (Thompson & Townsend, 2006; Leprieur *et al*., 2009; Brown & Swan, 2010; Bonada *et al*., 2012; Warfe *et al*., 2013). This is largely because streams and rivers are organized as complex dendritic networks rather than simple linear systems (Benda *et al*., 2004; Campbell Grant *et al*., 2007; Erős *et al*., 2012), thus necessitating the incorporation of network connectivity to explore the interactions among communities that are linked by dispersal (Fausch *et al*., 2002).

The environmental phenomena that drive any particular DDR can be decomposed into local and regional factors. Local factors include site-specific environmental attributes (e.g. substrate composition, channel depth, water temperature and chemistry) and biotic interactions (e.g., predator, competition, parasitism) that serve as filters, excluding some taxa and favouring others (Poff, 1997; Townsend *et al.*, 1997). Regional factors include landscape-level features that facilitate or impede the movement of organisms across landscapes. These features may include the dendritic structure of stream networks (Fausch *et al.*, 2002; Benda *et al.*, 2004), the spatial arrangement of suitable habitat patches across the landscape (Campbell Grant *et al.*, 2007; Erős *et al.*, 2012; Phillipsen & Lytle, 2013), and the simple Euclidean distance between sites. Therefore, a full understanding of the ecological processes underlying DDR patterns in stream networks must account at least for three main factors: environmental filters, dispersal of organisms and network topology.

Environmental harshness, both in terms of hydrologic regimes and physical conditions, can influence the role of local and regional forces shaping biodiversity patterns (Brown *et al.*, 2011; Heino, 2011). For example, unstable environments (e.g. aquatic habitats that experience severe, recurrent droughts) show a high degree of niche filtering, allowing only those species adapted to the local conditions to persist (Poff, 1997; Chase, 2007). In these circumstances, environmental forcing plays a much greater role than biotic interactions, such as predation or competition, in shaping patterns of species occurrence and community composition (Jackson et al. 2001). Therefore, harsh environmental conditions may cause meta-communities to be structured by local factors (Urban, 2004) and DDRs may not meet the expectation of decreasing community similarity with increasing distance.

Dispersal of aquatic-obligate riverine organisms is highly constrained by flow connectivity (Fausch *et al.*, 2002; Hughes, 2007; Schick & Lindley, 2007). In contrast, aquatic organisms that can disperse overland, such as flying forms of adult aquatic insects, can move both along drainages and across drainage divides (Bilton *et al.*, 2001; Petersen *et al.*, 2004). Therefore, variability in dispersal mode and ability will determine the extent to which local and regional factors structure assemblages of organisms (Bohonak & Jenkins, 2003; Cottenie & De Meester, 2004) and may be reflected in the shape of the DDR. For example, studies on aquatic invertebrate meta-communities in stream networks have reported that increasing dispersal strength results in a weakening of DDRs due to relaxation of dispersal limitation (Thompson & Townsend, 2006; Brown *et al.*, 2011). Very high dispersal rates could cause the homogenization of communities, because organisms can disperse to all available habitats and only the strongest competitors will survive (Kneitel & Miller, 2003; Leibold *et al.*, 2004).

The network topology, or spatial structure of the river network, also has important implications for dispersal and resulting meta-community structure (Muneepeerakul *et al.*, 2008; Auerbach & Poff, 2011). The use of within-network and overland dispersal pathways by aquatic organisms largely depends on the connectivity between the habitat branches, with the loss of connectivity constraining within-network dispersal (Fagan, 2002). Although critical for understanding the potential mechanisms shaping DDRs (Brown *et al.*, 2011), landscape resistance to the dispersal of organisms has been largely neglected in meta-community analyses (Moritz *et al.*, 2013). Landscape resistance quantifies ‘distances’ between communities that may yield more biologically informative DDRs than straight-line Euclidean distance, such as those associated with barriers to dispersal (e.g. high mountains or cliffs). To the present date, only Euclidean and network distance (i.e. the distance between sites along the riverine dendritic network) have been applied to stream networks, which fails to consider more realistic landscape variables in DDR analyses (McRae *et al.*, 2008).

In this study, we present a novel application of a landscape resistance modelling approach, originally developed for landscape genetic studies, to understand local and regional drivers of community structure. Dryland streams were used as a model system to test how environmental stability, dispersal capacity and network topology interact to structure aquatic meta-communities in dendritic networks. These streams experience frequent droughts and floods, which lead to strong niche filtering of stream organisms (Lytle, 2002; Lytle & Poff, 2004), with perennial habitats serving as refugia for species that need water during their entire life cycle to survive (Bogan & Lytle, 2011; Phillipsen & Lytle, 2013). Therefore, the high temporal and spatial variation in environmental conditions may disrupt expected longitudinal patterns of species’ replacement along the network (Bogan *et al.*, 2013). In these systems DDRs are expected to be weak or non-existent, with adjacent sites showing very different aquatic assemblages as a result of large among-site variation in environmental conditions (e.g. one site may flow year-round, while an adjacent site may flow only during rainy seasons). We focused on aquatic invertebrates because they possess a wide range of dispersal capacities (Bilton *et al.*, 2001; Bohonak & Jenkins, 2003) and are present over a wide range of environmental conditions (Rosenberg & Resh, 1993; Merritt *et al.*, 2008), as exemplified by the great diversity of biological traits that they exhibit (Statzner *et al.*, 2004; Poff *et al.*, 2006).

The aim of our study was to use DDRs to investigate the relationship between local and regional factors in explaining aquatic meta-community structure in fragmented dendritic networks. Here we define fragmentation as the loss of surface water connectivity along the river network. During low precipitation periods (typically in late spring and summer) some dryland streams experience very low flow, with sections becoming disconnected pools separated from one another by dry stream reaches. We used fine-scale local environmental variables and landscape resistance metrics to quantify the influence of local and regional drivers on DDRs for groups of species with different dispersal capacities. The following hypotheses were tested:

Plate 3.1 Invertebrate community sampling, Woodcutters Canyon, Huachuca Mountains. Photo credit: Tiffany Schriever.

Plate 3.2 Invertebrate community sampling, Garden Canyon, Huachuca Mountains. Photo credit: Tiffany Schriever.

H_1: DDRs should be weak for very strong and very weak dispersers (at the ends of the dispersal gradient) because meta-communities of weak dispersers show little spatial structure and meta-communities of strong dispersers are homogenized by competition. We predicted that DDR would be strongest in species with moderate dispersal strength.

H_2: Owing to high network fragmentation in dryland streams (i.e. longitudinal flow disruption during long dry seasons), no significant DDR should be found when using network distance. We predicted that network distance would have low explanatory power because of high spatial and temporal stream fragmentation, while our approach that considers landscape resistance to dispersal would provide greater explanatory power.

H_3: Owing to strong niche filtering (i.e. high environmental heterogeneity), DDRs associated with flow and environmental characteristics should be consistently stronger than DDRs associated with landscape resistance variables, regardless of the dispersal strength of the organisms. We predicted that DDRs associated with flow and environmental characteristics would be significant, regardless of species' dispersal abilities.

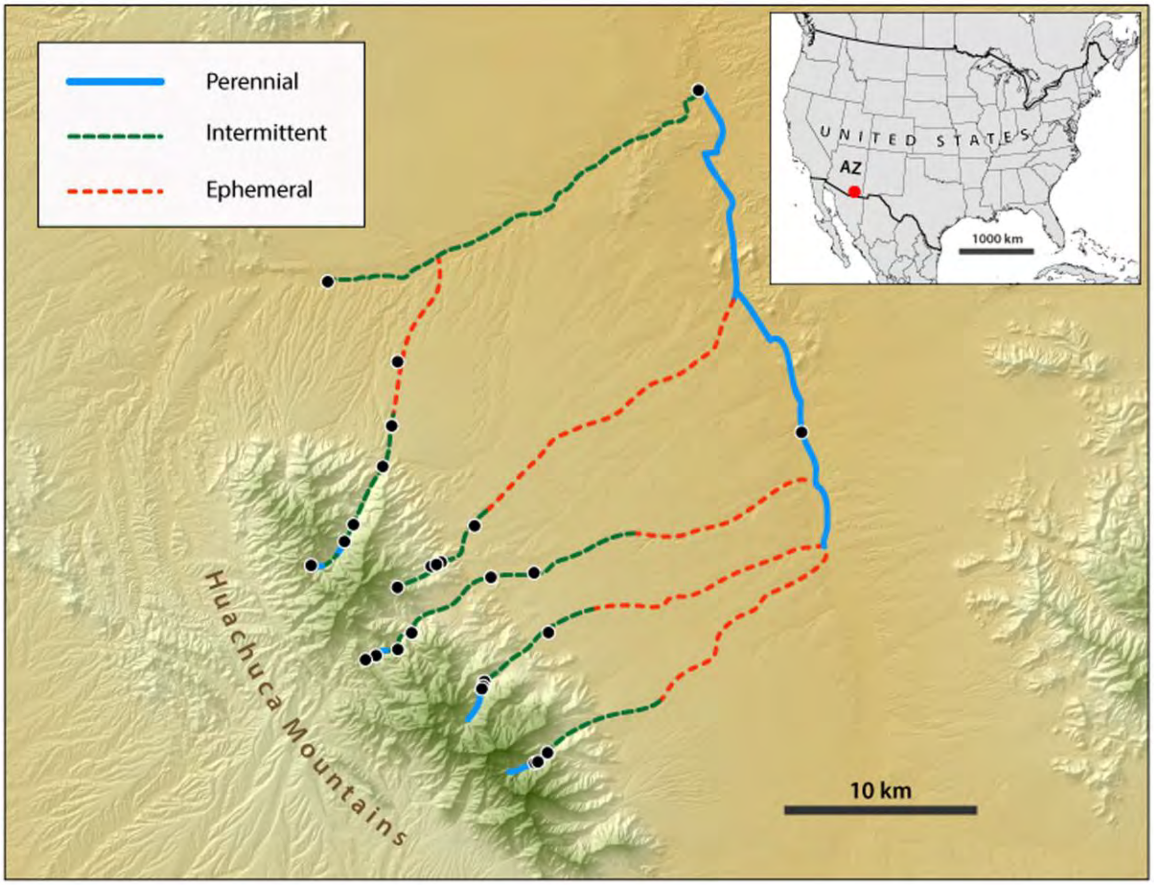
3.2 Materials and Methods

3.2.1 Study site and field sampling

We sampled aquatic invertebrates at 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA (Figure 3.1; Schriever *et al*., in press). Streams in the area generally have perennial flow in montane headwaters, intermittent flow in upper alluvial fan reaches, ephemeral flow lower on alluvial fans, and then alternating perennial and intermittent reaches in valley rivers (Bogan *et al*., 2013). We distributed our sample sites among perennial, intermittent and ephemeral reaches (classification follows Levick *et al*., 2008), but used a continuous flow metric to quantify permanence (see below). Sites were sampled three times a year (March/April, August/September, and November/December) between 2009 and 2011. The number of sites and samples collected differed among streams because not all sites had flow or all microhabitats during each sampling event. The study period spanned numerous dry seasons, several periods of ephemeral flows from summer monsoon rains, and one period of intermittent flows resulting from a wet winter. The majority of the sampling occurred during the fall (Nov/Dec) and winter (Mar/Apr) seasons for a total of 144 site × sampling event combinations.

Both riffle and pool microhabitats were sampled at each site, when present. For riffle samples we disturbed 0.33 m^2 of stream substrate to a depth of 5 cm while capturing invertebrates immediately downstream with a D-net (500-µm mesh). Pool samples consisted of sweeping the entire pool area including water column, surface, and pool benthos with a D-net at an effort of 10 s for every 1 m^2 of pool habitat (following Bogan & Lytle, 2007). Abundances from replicate microhabitat samples collected from the same site during the same sampling event (e.g. three riffles in November) were summed for each taxon and divided by the number of replicates to acquire relative abundances. Samples were preserved in 95% ethanol and invertebrates were identified in the laboratory to the finest taxonomic level practical, usually to genus or species for insects (including Chironomidae) and family or order for non-insects.

Figure 3.1 Map of sampling localities for stream invertebrates in south-eastern Arizona (USA). Inset map shows the location of the study area. Continuous blue line, perennial streams; short-dashed green line, intermittent streams; long-dashed red line, ephemeral streams. Black circles represent sampling localities. The map is based on a digital elevation model (DEM) at 10 m resolution.



During each visit, we measured water temperature, pH (Whatman pH indicators, Whatman International, Maidstone, UK) and conductivity (Milwaukee waterproof EC meter C65; Milwaukee Instruments, Rocky Mount, NC, USA), visually estimated canopy cover and benthic substrate on a percentage cover scale (0–100%; substrate categories: silt, sand, gravel, cobble and bedrock). We measured the timing and duration of streamflow through the deployment of 15 electrical resistance sensors (Jaeger & Olden, 2012), each representing the hydrological conditions at the nearest location of invertebrate sampling. The sensors logged the presence or absence of water in the stream channel at 15-min intervals from April 2010 to December 2011. From the sensor data, we calculated four hydrological metrics for each site: % flow permanence by year, mean % flow permanence by season (spring = May–June; monsoon = July–September; fall = October–November; winter = December–April), mean duration (number of days) of zero flow periods (ZFP) each year, and total number of ZFP each year. For the two flow permanence metrics and duration of ZFP, we summed 15-min time periods of both wet and dry conditions for the sampling period and for individual zero flow periods, converting the time unit to either days or years as appropriate for the final stream flow metric. We used an average of 2010 and 2011 flow data to estimate flow conditions for the November 2009 invertebrate sampling period (16 samples) that occurred prior to the deployment of sensors. Flow permanence is the percentage of time a given reach is wetted or flowing, while the duration of ZFP indicates how long (in days) a given reach is dry during each drying event. For example, a site with permanent stream flow would have a flow permanence of 100% and would receive a value of 0 for ZFP duration. While these metrics were all calculated from the flow sensor records, they were designed to characterize distinct components of the hydrological regime that may influence aquatic invertebrate occurrence and abundance.

3.2.2 Distance measures

We used four regional distance metrics (Table 3.1, Figure 3.2), two of which described to the physical distance between sites (geographical distance and network distance) and two of which described the resistance of the landscape to dispersal (topographic distance and perennial distance). *Geographical distance* is simply the straight-line Euclidean distance between two sites as determined from map coordinates. *Network distance* was generated via a least-cost path analysis in ARCGIS 9.3 (ESRI, Redlands, CA, USA). For this variable, only one pathway connects each pair of sites, and this pathway is restricted to the stream network. *Topographic distance* assumes that dispersal occurs along concave corridors such as streambeds, dry gullies, or low saddle points along mountain ridges. Flying and crawling insect adults are likely to follow these relatively cool and moist pathways to disperse from one wetted site to another (Bogan & Boersma, 2012; Phillipsen & Lytle, 2013). *Perennial distance* assumes that isolated perennial freshwater habitats act as stepping-stones for dispersal among communities in fragmented dendritic networks. For example, in arid landscapes perennial habitats are known to be critical for the survival of certain aquatic species when rivers cease to flow during droughts (Chester & Robson, 2011).

We generated the four regional distance measures from landscape data layers obtained from the Arizona State Land Department (https://land.az.gov/). Data layers used in our analyses included a digital elevation model (DEM; 10 m resolution), the stream network of the region (from the National Hydrology Dataset), and a map of perennial stream habitats. The latter was constructed using data for the San Pedro River watershed from the Nature Conservancy (http://www.azconservation.org/) combined with observations from field studies in the region (e.g. Bogan & Lytle, 2007; Bogan *et al*., 2013). We used ARCGIS 9.3 to generate new data layers and to calculate the distance (km) between all pairs of sites. The distances related to landscape resistance (topographic and perennial distances) were generated from the GIS data layers in the form of pixelated maps (i.e. rasters). Each raster map was used as input for the program CIRCUITSCAPE (McRae, 2006). CIRCUITSCAPE calculates the resistance of the landscape to dispersal between each pair of sites (analogous to electrical resistance in a circuit diagram), allowing for multiple pathways between sites. This pairwise resistance is a summation of the resistances of individual pixels in the input map. Pixels with high input values are hypothesized to offer high resistance to movement, and vice versa. Thus, pairwise resistances from CIRCUITSCAPE model the structural connectivity of communities, based on the landscape/habitat feature represented by the input map. We used the original values of the map pixels to assign resistance values to the raster maps. Using the original pixel values is more conservative than assigning relative costs of landscape features based on expert opinion (a practice that some have questioned; Spear *et al*., 2010). Before running the CIRCUITSCAPE analysis, we transformed the original values of the maps so that they were all on the same scale (1 for lowest resistance, 10 000 for highest resistance; results were qualitatively similar for different values of highest resistance). We performed a separate CIRCUITSCAPE analysis for both topographic and perennial distances, generating their two independent data sets of all pairwise resistance distances as output. See Phillipsen & Lytle (2013) for an example of CIRCUITSCAPE output in a population evolution context and how this relates to the underlying distance metrics.

In addition to the four regional distance measures, two local ecological distance measures were calculated: habitat distance and flow regime distance. *Habitat distance* was calculated as the dissimilarity between the multivariate centroids of each pair of sites based on their environmental characteristics, including canopy cover, conductivity, pH, and % of bedrock, cobble, gravel, sand and fines. *Flow regime distance* was calculated as the dissimilarity between the multivariate centroids of each pair of sites from a composite of flow metrics: % flow permanence in year of sample, % flow permanence by season, duration of zero flow periods each year (mean) and total number of zero flow periods each year. All variables were normalized (mean = 0; SD = 1) before analysis.

Figure 3.2 Hypothetical scenarios of species dispersal among sites in dryland streams based on each of the four regional distance metrics. In each scenario, the locations of three hypothetical communities are shown as white circles in a generic mountain landscape. Streams are depicted by dotted lines and thick black lines with arrowheads represent bi-directional species flow between pairs of communities. The paths of species dispersal in each scenario are determined by the hypothesized resistance to dispersal associated with the given landscape variable. In the topography scenario, for example, the underlying hypothesis is that dispersal is easiest in areas with strongly concave topography. Thus, gene flow is expected to be highest through areas with concave topography (shown as grey polygons in the figure). See the Table 3.1 for more detail on each of the regional distance metrics.

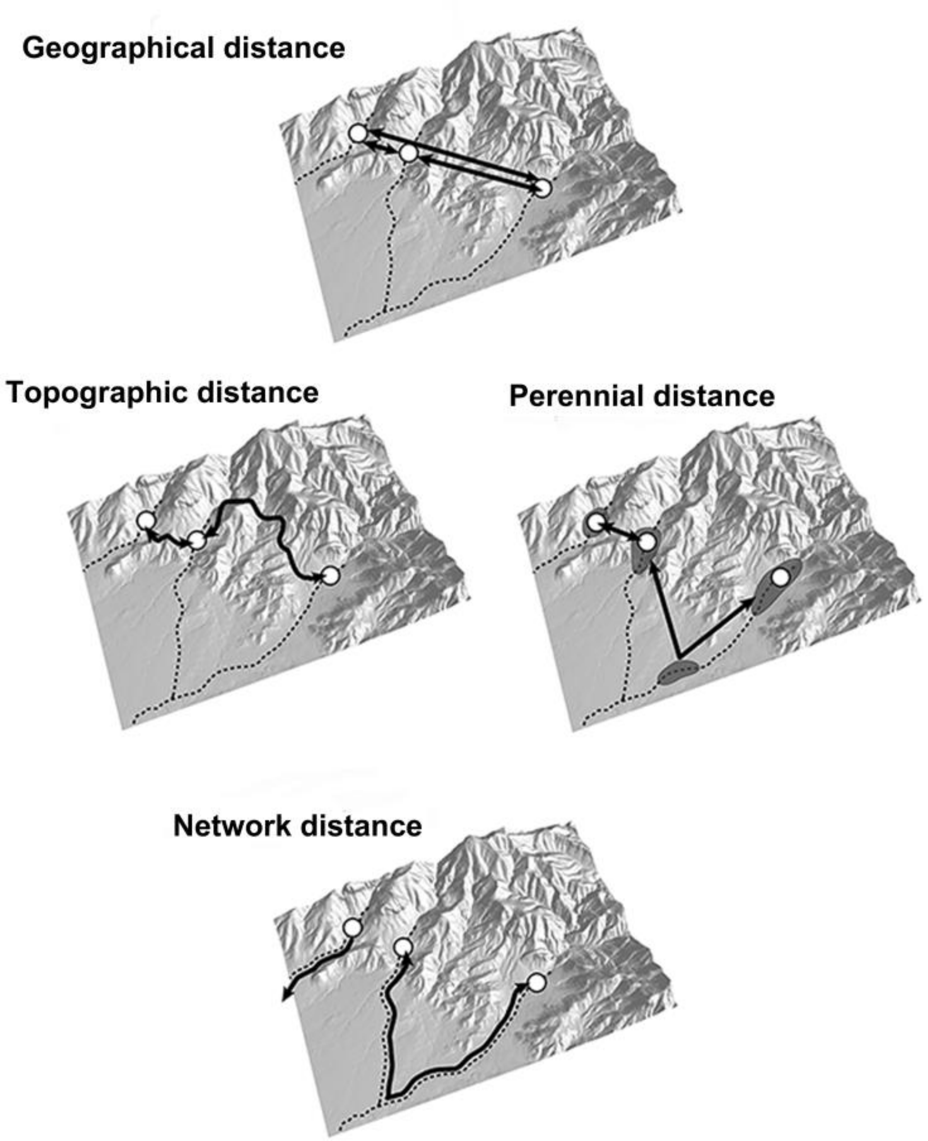


Table 3.1 Details of the four regional distance metrics used in this study.

Distance metric	Explanation	Hypothesized relationship to species flow
Geographical distance	Straight-line distance between sites in two-dimensional space.	Dispersal increases when the geographical distance between a pair of sites decreases.
Topographic distance	Pairwise resistances between sites based on low resistance of map pixels with concave topography and high resistance of pixels with convex topography.	Dispersal is highest in areas with strongly concave topography. Dispersal is lowest across areas with strongly convex topography.
Perennial distance	Pairwise resistances between sites based on low resistance of map pixels in patches of perennial freshwater habitats and high resistance of pixels in the matrix between these patches.	Dispersal increases in the presence of perennial freshwater habitats.
Network distance (stream network)	Pairwise least-cost paths between sites that strictly follow the stream/river network. Only one path exists between any pair of sites.	Dispersal occurs only within the stream/river network.

3.2.3 Statistical analyses

Prior to analyses, we placed each of the 225 aquatic invertebrate taxa into one of four categories: weak, local, moderate and strong dispersers (see 3.8 Appendix A). Weak dispersers (17 taxa) are aquatic obligates that spend nearly all of their life cycle within the stream (e.g. *Abedus herberti*). Local dispersers (142 taxa) have flying adult stages but can only travel short distances owing to their short life cycles and/or weak flying musculature (e.g. *Hydrobaenus* sp.). Moderate dispersers (64 taxa) have flying adult stages that can travel long distances but cannot cover the entire geographical range of our study (e.g. *Enochrus aridus*). Strong dispersers (10 taxa) are powerful fliers that can travel between any of the sites in our studied geographical range (e.g. *Lethocerus medius*). These categories were derived from a trait database specific to the study region built from over 80 publications from primary literature, existing databases and expert judgment (Schriever *et al.*, in press). Abundance data were log (x+1) transformed and then used to calculate the Chao dissimilarity index among all pairs of sites (using the function 'vegdist' in the R package VEGAN; Oksanen *et al.*, 2013). The Chao index was the most appropriate dissimilarity index to use because each dispersal ability group had a different number of taxa; it is intended to account for the effect of unseen shared species and thus reduce sample-size bias (Chao *et al.*, 2005). Habitat distance, flow regime distance and the four landscape distances (Table 3.1) were used as independent explanatory variables of Chao's index for each group of species (weak, local, moderate and strong dispersers). Spearman correlation tests were performed between all pairs of the explanatory variables. For those variables showing strong correlation (i.e. Spearman's $\rho > 0.5$ and P-value < 0.01), we used partial Mantel tests (mantel function in VEGAN package) to compare community data to the explanatory variable of interest while controlling for the correlated variable (Legendre & Legendre, 2012). The differences in the relationship between community dissimilarity and each of the distance metrics (geographical, network, topographic, perennial, flow regime and habitat distances) across dispersal classes was tested through an analysis of covariance (ANCOVA; Legendre & Legendre, 2012) with Chao dissimilarity as the dependent variable, each driver as a covariate, and the dispersal class as the grouping factor.

We fitted linear models to each distance metric, and performed F-tests to assess model performance. Models were tested for linearity using the diagnostic plots for generalized linear models (see 3.9 Appendix B). We used the glm.diag.plots function in the R package BOOT (Davison & Hinkley, 1997; Canty & Ripley, 2014), which makes a plot of jackknife deviance residuals against linear predictor, normal scores plots of standardized deviance residuals, plot of approximate Cook statistics against leverage/(1 − leverage), and case plot of Cook statistic. After validating the models we used an information-theoretic approach to compare the contribution of different explanatory variables that best described differences in invertebrate community composition. We derived the log-likelihood for each model and calculated Akaike's information criterion (AIC; Akaike, 1973; Burnham & Anderson, 2002) to rank the models from lowest to highest AIC. We only compared single variable models and the combination of local environmental distance metrics (flow regime and habitat) and landscape distance metrics (geographical, topographic, perennial and network distances), because our aim was to compare the importance of local versus regional filters for aquatic invertebrate meta-communities across a gradient of dispersal strength. Information for the rest of the models is shown in Appendix S3. Once the models were ranked, additional information-theoretic metrics were calculated. The difference between the AIC of a particular model and the AIC of the estimated best-fitting model (i.e. the model with the lowest AIC) is ΔAIC. We also calculated Akaike weights, the probability that the model is actually the best-fitting of the candidate models. The sum of Akaike weights across the models is 1.0. When the weight of the model with the lowest AIC is not close to 1.0, there is evidence for model selection uncertainty. We accounted for the non-independence of our data (represented by pairwise distances among sites) by using an R^2 approach for fixed effects in a linear mixed model to adjust for the inflation of sample size (Edwards *et al.*, 2008). As both the AIC and mixed model approaches yielded similar results, we only report the adjusted R^2 values for fixed effects (Table 3.2). The selection of AIC over adjusted R^2 values was based on the limited use of adjusted R^2 values in model building, owing to the lack of diagnostic and selection tools for linear mixed models (Edwards *et al.*, 2008). All analyses were conducted in R version 3.0.2 (R Core Team, 2013) and significance was assigned at $P < 0.05$. Bonferroni correction was used to adjust *P*-values for multiple comparisons.

3.3 Results

The six distance metrics displayed weak pairwise correlations. Only geographical, topographic and perennial distances were significantly correlated (Figure 3.3). Habitat and flow regime distances significantly explained community dissimilarity, regardless of the species' dispersal abilities (Table 3.2). Geographical, topographic and perennial distances significantly explained community dissimilarity for all dispersal groups except for the weak dispersers, and had a higher explanatory power for strong dispersers (Table 3.2). Network distance was not a significant predictor for any dispersal group. Associations between community dissimilarity and the explanatory variables varied considerably among the four dispersal groups (Figure 3.4). The relationship between community dissimilarity and the six distance metrics differed across the dispersal categories for all but one metric (network distance; Table 3.3).

Partial Mantel tests revealed that: (1) geographical distance was significantly correlated with the composition of strong dispersing taxa after controlling for topographic distance; (2) topographic distance was significantly correlated with the composition of moderate dispersers while accounting for geographical distance; and (3) topographic and perennial distances were correlated with the composition of moderate dispersers while controlling for each other (Table 3.4). Of all the models, local drivers (habitat distance plus flow regime distance) demonstrated the strongest relationships (i.e. the lowest AIC for single variable models) with the composition of weak, local and strong dispersers, while regional drivers (geographical, topographic, perennial and network distances) best-explained moderate dispersers (Table 3.5).

Table 3.2 Adjusted R^2 values, F statistic and P-value for each combination of aquatic invertebrate dispersal category (weak, local, moderate, and strong) and explanatory environmental (habitat distance and flow regime distance) and spatial (geographical, topographic, perennial and network distances) variables. Bonferroni correction was used to adjust P-values for multiple comparisons. Significant relationships ($P < 0.05$) are shown in bold. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

Explanatory variable	Weak			Local			Moderate			Strong		
	Adj R^2	F	P-value	Adj R^2	F	P-value	Adj R^2	F	P-value	Adj R^2	F	P-value
Habitat distance	0.13	51.1	< **0.01**	0.36	195.1	< **0.01**	0.08	33.3	< **0.01**	0.15	61.1	< **0.01**
Flow regime distance	0.09	37.2	< **0.01**	0.25	119.8	< **0.01**	0.08	29.6	< **0.01**	0.11	46.4	< **0.01**
Geographical distance	0	2.4	0.120	0.03	10.5	**0.001**	0.07	29.1	< **0.01**	0.09	37.7	< **0.01**
Topographic distance	0	0	0.964	0.06	25.3	< **0.01**	0.24	109.2	< **0.01**	0.02	9.6	**0.002**
Perennial distance	0.02	8	**0.005**	0.1	40.9	< **0.01**	0.27	129.3	< **0.01**	0.01	5.6	**0.019**
Network distance	0	1.7	0.195	0	0.6	0.457	0	0.1	0.788	0	0.5	0.481

Figure 3.3 Pairwise correlations among all local and landscape distance metrics calculated between 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA. HAB, habitat distance; GEO, geographical distance; TOP, topographic distance; FLO, flow regime distance; NTW, network distance; PRN, perennial distance. Blue indicates a positive correlation, while red indicates a negative correlation. The intensity of the colour indicates the strength of the correlation. Spearman's ρ values are shown inside each box. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

Figure 3.4 Distance decay relationships for each dispersal group of stream invertebrates sampled at 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA. WEAK, weak dispersers; LOCAL, local dispersers; MODERATE, moderate dispersers; STRONG, strong dispersers.

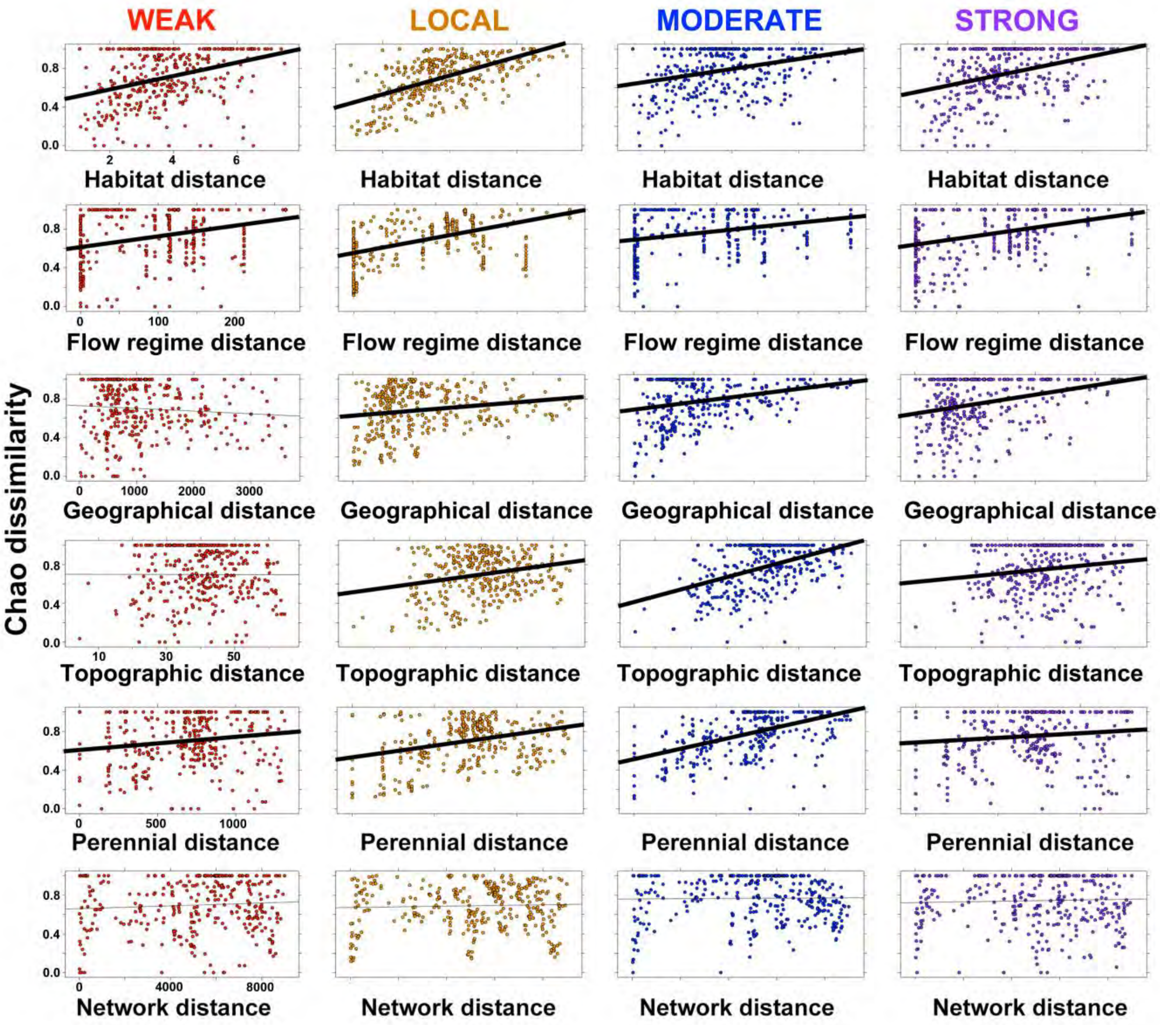


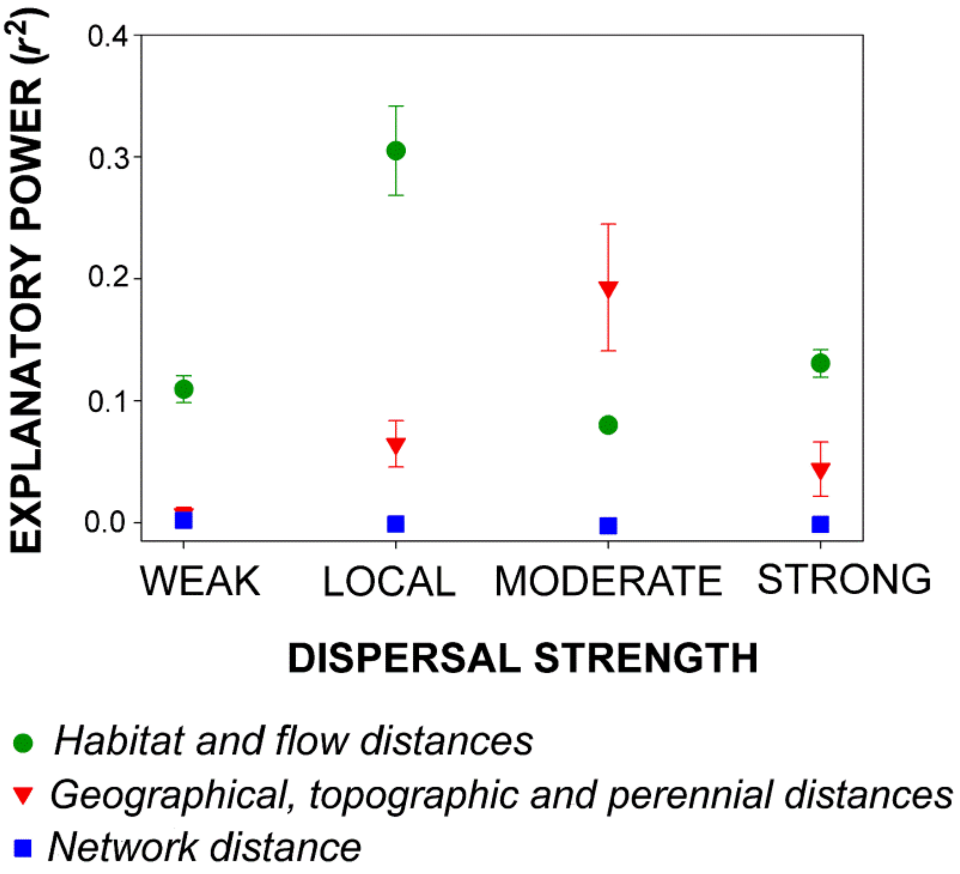
Table 3.3 Partial Mantel test results among those distance metrics that showed strong correlation between each other (Figure 3.3). GEO, geographical distance; TOP, topographic distance; PRN, perennial distance. * 0.01< $P < 0.05$; ** $P < 0.01$; n.s., not significant. Distances were calculated for 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

Comparison	Control matrix	*r*	*P*
Weak dispersers vs. GEO	TOP	−0.12	n.s.
Local dispersers vs. GEO	TOP	−0.02	n.s.
Moderate dispersers vs. GEO	TOP	−0.11	n.s.
Strong dispersers vs. GEO	TOP	0.28	**
Weak dispersers vs. TOP	GEO	0.08	n.s.
Local dispersers vs. TOP	GEO	0.2	n.s.
Moderate dispersers vs. TOP	GEO	0.43	**
Strong dispersers vs. TOP	GEO	−0.09	n.s.
Weak dispersers vs. PRN	TOP	0.19	n.s.
Local dispersers vs. PRN	TOP	0.22	n.s.
Moderate dispersers vs. PRN	TOP	0.33	**
Strong dispersers vs. PRN	TOP	0.04	n.s.
Weak dispersers vs. TOP	PRN	−0.11	n.s.
Local dispersers vs. TOP	PRN	0.09	n.s.
Moderate dispersers vs. TOP	PRN	0.26	*
Strong dispersers vs. TOP	PRN	0.11	n.s.

Table 3.4 ΔAIC (Akaike's information criterion) and weight for single variable models and the combinations (in italics) of habitat distance and flow regime distance (local distance metrics) and geographical, topographic, perennial and network distances (regional distance metrics). The lower the AIC, the higher the explanatory power of the model for each of the four invertebrate dispersal categories (weak, local, moderate and strong). Lowest AIC of single variables and combinations of variables for each dispersal category are marked in bold. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

Variable	Weak		Local		Moderate		Strong	
	ΔAIC	Weight	ΔAIC	Weight	ΔAIC	Weight	ΔAIC	Weight
Habitat distance	**15**	< 0.001	**60**	< 0.001	98	< 0.001	**20**	< 0.001
Flow regime distance	27	< 0.001	112	< 0.001	102	< 0.001	32	< 0.001
Geographical distance	60	< 0.001	205	< 0.001	102	< 0.001	40	< 0.001
Topographic distance	63	< 0.001	191	< 0.001	35	< 0.001	67	< 0.001
Perennial distance	55	< 0.001	177	< 0.001	**20**	< 0.001	71	< 0.001
Network distance	61	< 0.001	215	< 0.001	130	< 0.001	76	< 0.001
Local drivers	**0**	1	**0**	1	86	< 0.001	**0**	1
Regional drivers	53	< 0.001	179	< 0.001	**0**	1	37	< 0.001

Figure 3.5 Explanatory power of three different groups of distance metrics over stream invertebrates' community dissimilarity among 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA. Groups of distances: (a) habitat and flow distances; (b) geographical, topographic and perennial distances; and (c) network distance. Group (a) represents local environmental filters and group (b) represents regional landscape filters. The network distance is shown separately as having a very low power to predict community dissimilarity. The explanatory power is plotted against different categories of dispersal strength of stream invertebrates: weak, local, moderate and strong (see Materials and Methods section for a description of each category).



3.4 Discussion

We used distance decay relationships to examine the importance of local and regional drivers of aquatic invertebrate meta-community structure in dryland streams. DDRs have been used to examine the interaction of processes operating at local and regional scales for a wide range of organisms and ecosystems (Cottenie, 2005; Soininen *et al.*, 2007), including stream networks (Thompson & Townsend, 2006; Leprieur *et al.*, 2009; Brown & Swan, 2010; Warfe *et al.*, 2013). However, past studies have largely not explored the relative roles of local- and regional-scale landscape drivers within connected networks. The only published study that used DDRs to address the effect of hydrological connectivity on stream meta-communities showed, rather counter-intuitively, that the loss of connectivity enhanced DDR in a variety of organisms (Warfe *et al.*, 2013). However, environmental conditions in that study were not independent of geographical distance; therefore dispersal limitation and niche partitioning both played a role in shaping assemblage structure. Our results suggest that fragmentation impacts DDRs by altering the viable dispersal pathways, with organisms dispersing overland instead of using the stream network. As we hypothesized, meta-community structure was determined by three main factors: niche filtering due to local among-habitat differences, dispersal ability of the species, and landscape resistance (geographical distance, topography and availability of perennial refugia). Notably, we did not examine the influence of biotic interactions on invertebrate community composition for a number of reasons. First, our study streams do not contain predator fish species, and similarly predation effects from amphibians (tadpoles) and invertebrate beetles are minimal. Second, as supported by ecological theory and substantial empirical evidence, invertebrate communities in harsh intermittent streams such as those examined here are predominantly shaped by environmental factors (Bogan *et al*., 2013), whereas biological drivers play a more important role in environmentally-benign streams (Jackson *et al.,* 2001).

3.4.1 Niche filtering due to among-habitat differences

We hypothesized that high environmental heterogeneity would generate significant environmental DDRs for all dispersal categories. Indeed, we found that the local filters of habitat and flow regime had a stronger effect on community dissimilarity than regional filters across all dispersal classes, except for moderate dispersers. At intermediate levels of dispersal, organisms might be able to survive harsh environmental conditions such as floods and droughts by escaping and finding refugia (Velasco & Millan, 1998; Lytle, 1999), but at the same time they might not be able to reach all the available habitats, preventing meta-community homogenization via mass effects (Kneitel & Miller, 2003; Leibold *et al.*, 2004). The significant influence of local filters on meta-community patterns was not surprising given the high temporal and spatial variation in environmental conditions in the study region. Highly variable seasonal and interannual precipitation patterns and various geomorphic settings (e.g. bedrock canyons, alluvial fans) interact to create a patchy stream landscape. In these dryland streams, perennial reaches are adjacent to intermittent reaches, and intermittent reaches with seasonal flow in a wet year can be completely dry during the same period in a dry year (Jaeger & Olden, 2012; Bogan *et al*., 2013). The amount of water and how it is distributed within the year (i.e. frequency and timing of droughts and floods) have important consequences for water quality and habitat variables (e.g. canopy cover, river substratum). Accordingly, these local habitat filters should be extremely strong in dryland streams. Our results validate previous studies that linked these patchy environmental conditions to disruptions in the longitudinal patterns of stream invertebrate communities in the region (Bogan *et al.*, 2013). They are also in agreement with a recent study from north-western Australia, which found that flow and channel width best explained invertebrate meta-community patterns across a range of perennial and intermittent streams (Warfe *et al.*, 2013). Moreover, the importance of niche filtering in structuring meta-communities has been demonstrated for a variety of ecosystems (Soininen *et al.*, 2007), including ponds (Urban, 2004; Chase, 2007) and streams (Thompson & Townsend, 2006; Brown & Swan, 2010). As the loss of connectivity among stream reaches results from high flow heterogeneity (leading to high environmental heterogeneity), niche filtering can be expected to be strong in fragmented stream networks.

3.4.2 Dispersal ability

Aquatic invertebrates are known to have very different dispersal capacities, ranging from a few metres to thousands of kilometres (Kovats *et al.*, 1996; Bilton *et al.*, 2001; McCauley, 2006). Given that meta-community structure is highly dependent on geographical scale (Brown *et al.*, 2011; Maloney & Munguia, 2011; Nekola & McGill, 2014), differences in dispersal can be expected to affect DDRs (Nekola & White, 1999). Increasing dispersal ability is expected to enhance community similarity among sites and reduce beta diversity among habitat patches (Shurin *et al.*, 2009). Recent studies on invertebrate meta-communities in stream networks have provided evidence for this pattern, with DDR being weakened by increasing dispersal strength (Thompson & Townsend, 2006; Brown & Swan, 2010; Bonada *et al.*, 2012). However, we found a more complex unimodal pattern, with DDR peaking at intermediate dispersal strength for different measures of landscape resistance. This pattern might result from assemblages of weak dispersers showing no spatial structure as a result of dispersal limitation while assemblages of strong dispersers are more homogeneous across the landscape as a result of the absence of dispersal restrictions (Kneitel & Miller, 2003; Leibold *et al.*, 2004). Figure 3.5 illustrates this pattern by showing the explanatory power of local (habitat and flow distances) and landscape (geographical distance, topography and availability of perennial refugia) filters along the dispersal strength gradient.

Our results could be influenced by the lower flow connectivity and environmental stability in our dryland study system compared to more mesic stream systems. In low connectivity systems, weak dispersers are highly isolated, leading to species distributions ruled by ecological drift and niche filtering (Hu *et al.*, 2006). Therefore, in these fragmented systems, low connectivity coupled with differing environmental conditions can lead to adjacent sites having very different assemblages of weak dispersal species. Previous investigations on the flightless aquatic obligate *Abedus herberti* within our study area reported strong population genetic structure, with populations within the same stream drainage (less than 5 km apart) showing significant genetic differentiation (Finn *et al.*, 2007; Phillipsen & Lytle, 2013). This same pattern has been observed for the blackfly *Prosimulium neomacropyga* in isolated alpine headwater streams (Finn & Poff, 2011). On the other side of the spectrum, extremely strong dispersers can break down geographical barriers, occurring in all suitable habitats (Townsend *et al.*, 2003; McCauley, 2006; Thompson & Townsend, 2006; Brown & Swan, 2010; Bonada *et al.*, 2012). This would explain the decrease in the explanatory power of landscape variables over meta-community structure at the upper end of the dispersal strength gradient in the present study (Figure 3.5). We suggest that regional drivers should be important predictors of meta-community structure up to a certain dispersal distance threshold, beyond which dispersal is strong enough to break the limitation imposed by geographical barriers.

3.4.3 Distance among sites

Network distance did not significantly affect community dissimilarity for any of the four dispersal groups, as we hypothesized. This contradicts the general rule of aquatic invertebrates using the stream network as the main ‘highway’ for dispersal (Petersen *et al.*, 2004). While evidence supporting the ideas of the stream channel as the primary dispersal route and restricted overland dispersal between catchments continue to accumulate in the literature (Hughes, 2007; Brown & Swan, 2010; Rouquette *et al.*, 2013), previous investigations within our study area suggest that dryland streams might be exceptions to this rule. Many aquatic invertebrate species in our study region disperse laterally from stream corridors in search of other wetted habitats (Bogan & Boersma, 2012). Additionally, Bogan *et al*. (2013) reported an interruption of the river continuum, where invertebrate communities in distant headwater and lowland perennial streams were more similar to one another than to those in intervening intermittent reaches. Furthermore, Phillipsen & Lytle (2013) found no significant relationship between network distance and population genetic structure of *Abedus herberti.* Instead, they found that topography best explained genetic structure and suggested that overland dispersal resulted from flood-escape behaviour (Lytle, 1999; Lytle *et al.*, 2008), where individuals crawl from streams during floods and accidentally end up in adjacent drainages.

We found significant (but generally weak) DDRs for perennial habitat distance in all cases and for topographic distance in all cases except weak dispersers. This supports the hypothesis of overland dispersal (flight and crawling) being the main dispersal pathway for aquatic invertebrates in highly fragmented stream networks, such as those inhabiting dryland regions. Similarly, Campbell Grant *et al*. (2010) found evidence of high overland dispersal rates in newly metamorphosed juveniles of stream salamanders and suggested that the salamanders followed that dispersal strategy to increase population persistence across isolated headwater streams.

3.5 Conclusions

Our DDR analyses suggest that in highly heterogeneous stream networks, where environmental conditions vary greatly across space and time, local factors (i.e. niche filtering) may swamp regional influences (i.e. landscape filters) on aquatic invertebrate meta-community structure. However, this interaction between local and regional factors is dependent on species’ dispersal capacity, which determines their ability to colonize suitable habitats. Using a combination of landscape and local distance measures, we found evidence that local and weak dispersers were affected by local-scale factors, intermediate dispersers were affected by landscape-level factors, and strong dispersers showed no discernable pattern. This resulted in a hump-shaped relationship between dispersal ability and landscape-level factors, where only moderate dispersers showed significant DDRs. Stream corridors may not be a primary dispersal pathway in these networks, where frequent drought and flood disturbances generate habitat patches with low connectivity. Overland pathways, using perennial refugia as stepping-stones, might be the main dispersal route for aquatic invertebrates in these dryland stream networks (Phillipsen & Lytle, 2013).

Our DDR approach has the potential to generate timely management insights, such as the importance of preserving perennial habitat patches in fragmented river networks. Meta-communities of weak dispersal species are highly disconnected in dryland stream networks. As most of these species depend on perennial water sources for their survival (Bogan & Lytle, 2011; Hermoso *et al.*, 2013), intensifying droughts may have effects at both meta-population and meta-community levels. Our results suggest that perennial habitat patches may facilitate dispersal of aquatic invertebrates and thereby may ensure the long-term viability of populations. Conserving perennial habitats is of vital importance in dryland streams, and it will become increasingly important in basins experiencing flow reduction due to warmer temperatures and increased anthropogenic water use (Marshall *et al.*, 2010). Given the different responses of invertebrate meta-communities to our various geographical and environmental distance measures, we encourage future investigations to incorporate multiple regionally relevant measures of landscape

Plate 3.3 Woodcutters Canyon, Fort Huachuca, Arizona. Photo credit: Meryl Mims.

resistance into their studies. Further research is needed to better understand how environmental stability affects the balance between local and regional factors structuring meta-community patterns in dendritic networks, including work at different spatial scales and degrees of fragmentation.

3.6 Acknowledgements

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3.7 Appendix A

Aquatic invertebrate taxa list (sampled in 28 sites across seven dryland streams distributed within a 400 km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA) including their assignation into one of four dispersal categories: weak, local, moderate and strong dispersers.

Order	**Family**	**Genus/Species**	**Dispersal Category**
Acariformes	Acari	Acari	Weak
Amphipoda	Crustacea	Hyalella	Weak
Annelida	Hirudinea	Hirudinea	Weak
Anostraca	Anostraca	Anostraca	Weak
Basommatophora	Ancylidae	Ferrissia	Weak
Basommatophora	Ansylidae	Ancylidae	Weak
Decapoda	Cambaridae	Procambarus	Weak
Diplostraca	Limnadiidae	Eulimnadia	Weak
Gastropoda	Hydrobiidae	Pyrgulopsis	Weak
Gastropoda	Lymnaeidae	Lymnaeidae	Weak
Gastropoda	Physidae	Physidae	Weak
Gastropoda	Planorbidae	Planorbidae	Weak
Hemiptera	Belostomatidae	*Abedus herberti*	Weak
Hemiptera	Nepidae	*Curicta pronotata*	Weak
Platyhelmenthes	Platyhelmenthes	Platyhelmenthes	Weak
Podocopida	Ostracoda	Ostracoda	Weak
veneroida	Sphaeriidae	Pisidium	Weak
Coleoptera	Dryopidae	*Helichus lithophilus*	Local
Coleoptera	Dryopidae	*Helichus suturalis*	Local
Coleoptera	Dryopidae	*Helichus triangularis*	Local
Coleoptera	Dryopidae	*Postelichus confluentus*	Local

Coleoptera	Dryopidae	*Postelichus immsi*	Local
Coleoptera	Elmidae	Heterelmis	Local
Coleoptera	Elmidae	Microcylloepus	Local
Coleoptera	Elmidae	Optioservus	Local
Coleoptera	Elmidae	Zaitzevia	Local
Coleoptera	Hydraenidae	Gymnochthebius	Local
Coleoptera	Hydrophilidae	Chaetarthria	Local
Diptera	Ceratopogonidae	Atrichopogon	Local
Diptera	Ceratopogonidae	Ceratopogoninae	Local
Diptera	Ceratopogonidae	Forcipomyia	Local
Diptera	Chironomidae	Ablabesmyia	Local
Diptera	Chironomidae	Acricotopus	Local
Diptera	Chironomidae	Apedilum	Local
Diptera	Chironomidae	Apsectroptanypus	Local
Diptera	Chironomidae	Brillia	Local
Diptera	Chironomidae	Bryophaenocladius	Local
Diptera	Chironomidae	Chaetocladius	Local
Diptera	Chironomidae	Parachaetocladius	Local
Diptera	Chironomidae	Paracladopelma	Local
Diptera	Chironomidae	Corynoneura	Local
Diptera	Chironomidae	Paracricotopus	Local
Diptera	Chironomidae	Cricotopus-Orthocladius	Local
Diptera	Chironomidae	Cryptochironomus	Local
Diptera	Chironomidae	Demicryptochironomus	Local
Diptera	Chironomidae	Diamesa	Local
Diptera	Chironomidae	Dicrotendipes	Local
Diptera	Chironomidae	Heleniella	Local
Diptera	Chironomidae	Heterotrissocladius	Local
Diptera	Chironomidae	Hydrobaenus	Local
Diptera	Chironomidae	Parakiefferella	Local
Diptera	Chironomidae	Krenosmittia	Local
Diptera	Chironomidae	Labrundinia	Local
Diptera	Chironomidae	Larsia	Local
Diptera	Chironomidae	Limnophyes	Local
Diptera	Chironomidae	Lopescladius	Local
Diptera	Chironomidae	Paramerina	Local
Diptera	Chironomidae	Mesosmittia	Local
Diptera	Chironomidae	Parametriocnemus	Local
Diptera	Chironomidae	Micropsectra	Local
Diptera	Chironomidae	Nanocladius	Local
Diptera	Chironomidae	Nilotanypus	Local
Diptera	Chironomidae	Parochlus	Local
Diptera	Chironomidae	Pentaneura	Local

Diptera	Chironomidae	Phaenopsectra	Local
Diptera	Chironomidae	Polypedilum	Local
Diptera	Chironomidae	Procladius	Local
Diptera	Chironomidae	Psectrocladius	Local
Diptera	Chironomidae	Pseudosmittia	Local
Diptera	Chironomidae	Pseudochironomus	Local
Diptera	Chironomidae	Rheocricotopus	Local
Diptera	Chironomidae	Rheotanytarsus	Local
Diptera	Chironomidae	Chironomus	Local
Diptera	Chironomidae	Saetheria	Local
Diptera	Chironomidae	Parasmittia	Local
Diptera	Chironomidae	Smittia	Local
Diptera	Chironomidae	Stempellinella	Local
Diptera	Chironomidae	Stictochironomus	Local
Diptera	Chironomidae	Stenochironomus	Local
Diptera	Chironomidae	Tanytarsus	Local
Diptera	Chironomidae	Paratanytarsus	Local
Diptera	Chironomidae	Paratendipes	Local
Diptera	Chironomidae	Virgatanytarsus	Local
Diptera	Chironomidae	*Eukiefferiella brehmi*	Local
Diptera	Chironomidae	*Eukiefferiella claripennis*	Local
Diptera	Chironomidae	*Eukiefferiella coerulescens*	Local
Diptera	Chironomidae	*Eukiefferiella devonica*	Local
Diptera	Chironomidae	*Eukiefferiella gracei*	Local
Diptera	Chironomidae	*Eukiefferiella rectangularis*	Local
Diptera	Chironomidae	*Microtendipes pedellus* grp.	Local
Diptera	Chironomidae	*Thienemanniella fusca*	Local
Diptera	Chironomidae	Thienemannimyia grp.	Local
Diptera	Chironomidae	Thienemanniella xena	Local
Diptera	Chironomidae	*Tvetenia bavarica* grp.	Local
Diptera	Culicidae	Aedes	Local
Diptera	Culicidae	Anopheles	Local
Diptera	Culicidae	Culex	Local
Diptera	Culicidae	Culiseta	Local
Diptera	Culicidae	Psorophora	Local
Diptera	Dixidae	Dixa	Local
Diptera	Dixidae	Dixella	Local
Diptera	Dolichopodidae	Dolichopodidae	Local
Diptera	Empididae	Empididae	Local
Diptera	Ephydridae	Ephydridae	Local
Diptera	Muscidae	Muscidae	Local
Diptera	Psychodidae	Maruina	Local
Diptera	Psychodidae	Pericoma	Local

Diptera	Ptychopteridae	Ptychoptera	Local
Diptera	Simuliidae	Prosimulium	Local
Diptera	Simuliidae	Simulium	Local
Diptera	Stratiomyidae	Caloparyphus	Local
Diptera	Stratiomyidae	Euparyphus	Local
Diptera	Syrphidae	Syrphidae	Local
Diptera	Tabanidae	Tabanus	Local
Diptera	Thaumaleidae	Thaumaleidae	Local
Diptera	Tipulidae	Antocha	Local
Diptera	Tipulidae	Dicranota	Local
Diptera	Tipulidae	Limnophila	Local
Diptera	Tipulidae	Limonia	Local
Diptera	Tipulidae	Pedicia	Local
Diptera	Tipulidae	Tipula	Local
Ephemeroptera	Ameletidae	Ameletus	Local
Ephemeroptera	Baetidae	Acentrella	Local
Ephemeroptera	Baetidae	Baetis	Local
Ephemeroptera	Baetidae	Callibaetis	Local
Ephemeroptera	Baetidae	*Camelobaetidius maidu*	Local
Ephemeroptera	Baetidae	Fallceon	Local
Ephemeroptera	Caenidae	Caenis	Local
Ephemeroptera	Heptageniidae	Ecdyonourus	Local
Ephemeroptera	Leptohyphidae	Homoleptohyphes	Local
Ephemeroptera	Leptohyphidae	Tricorythodes	Local
Ephemeroptera	Leptophlebiidae	Choroterpes	Local
Ephemeroptera	Leptophlebiidae	Farrodes	Local
Hemiptera	Hebridae	Hebrus	Local
Lepidoptera	Crambidae	Petrophila	Local
Plecoptera	Capniidae	Capnia	Local
Plecoptera	Capniidae	Mesocapnia	Local
Plecoptera	Chloroperlidae	Chloroperlidae	Local
Plecoptera	Chloroperlidae	Sweltsa	Local
Plecoptera	Nemouridae	Malenka-Amphinemoura	Local
Trichoptera	Brachycentridae	Micrasema	Local
Trichoptera	Calamoceratidae	*Phylloicus mexicanus*	Local
Trichoptera	Helicopsychidae	Helicopsyche	Local
Trichoptera	Hydrobiosidae	Atopsyche	Local
Trichoptera	Hydropsychidae	Cheumatopsyche	Local
Trichoptera	Hydropsychidae	Hydropsyche	Local
Trichoptera	Hydroptilidae	Culoptila	Local
Trichoptera	Hydroptilidae	Metrichia	Local
Trichoptera	Hydroptilidae	Ochrotrichia	Local
Trichoptera	Hydroptilidae	Oxyethira	Local

Trichoptera	Hydroptilidae	Hydroptila	Local
Trichoptera	Lepidostomatidae	*Lepidostoma acarolum*	Local
Trichoptera	Lepidostomatidae	Lepidostoma sp.	Local
Trichoptera	Leptoceridae	Nectopsyche	Local
Trichoptera	Leptoceridae	Oecetis	Local
Trichoptera	Limnephilidae	Hesperophylax	Local
Trichoptera	Philopotamidae	Wormaldia	Local
Trichoptera	Polycentropodidae	Polycentropus	Local
Trichoptera	Psychomyiidae	Tinodes	Local
Coleoptera	Dytiscidae	*Desmopachria portmanni*	Moderate
Coleoptera	Dytiscidae	Hydroporinae	Moderate
Coleoptera	Dytiscidae	*Hygrotus patruelis*	Moderate
Coleoptera	Dytiscidae	*Hygrotus wardi*	Moderate
Coleoptera	Dytiscidae	*Laccophilus fasciatus*	Moderate
Coleoptera	Dytiscidae	*Laccophilus maculosus*	Moderate
Coleoptera	Dytiscidae	*Laccophilus mexicanus*	Moderate
Coleoptera	Dytiscidae	*Laccophilus oscillator*	Moderate
Coleoptera	Dytiscidae	*Laccophilus pictus*	Moderate
Coleoptera	Dytiscidae	*Liodessus obscurellus*	Moderate
Coleoptera	Dytiscidae	*Neoclypeodytes cinctellus*	Moderate
Coleoptera	Dytiscidae	*Neoclypeodytes fryi*	Moderate
Coleoptera	Dytiscidae	Neoporus	Moderate
Coleoptera	Dytiscidae	*Rhantus atricolor*	Moderate
Coleoptera	Dytiscidae	*Rhantus gutticollis*	Moderate
Coleoptera	Dytiscidae	Sanfilippodytes	Moderate
Coleoptera	Dytiscidae	*Stictotarsus corvinus*	Moderate
Coleoptera	Dytiscidae	*Stictotarsus roffi*	Moderate
Coleoptera	Dytiscidae	*Boreonectes striatellus*	Moderate
Coleoptera	Dytiscidae	*Thermonectus marmoratus*	Moderate
Coleoptera	Dytiscidae	*Thermonectus nigrofasciatus*	Moderate
Coleoptera	Gyrinidae	*Gyrinus plicifer*	Moderate
Coleoptera	Haliplidae	*Peltodytes dispersus*	Moderate
Coleoptera	Haliplidae	*Peltodytes callosus*	Moderate
Coleoptera	Hydraenidae	Hydraena	Moderate
Coleoptera	Hydrophilidae	*Anacaena signaticollis*	Moderate
Coleoptera	Hydrophilidae	*Berosus miles*	Moderate
Coleoptera	Hydrophilidae	*Berosus punctatissimus*	Moderate
Coleoptera	Hydrophilidae	*Berosus rugulosus*	Moderate
Coleoptera	Hydrophilidae	*Berosus salvini*	Moderate
Coleoptera	Hydrophilidae	*Berosus stylifer*	Moderate
Coleoptera	Hydrophilidae	Cymbiodyta	Moderate
Coleoptera	Hydrophilidae	*Enochrus aridus*	Moderate
Coleoptera	Hydrophilidae	*Enochrus piceus glabrus*	Moderate

Coleoptera	Hydrophilidae	*Enochrus pygmaeus pectoralis*	Moderate
Coleoptera	Hydrophilidae	Helophorus	Moderate
Coleoptera	Hydrophilidae	Hydrophilus	Moderate
Coleoptera	Hydrophilidae	*Laccobius hardyi*	Moderate
Coleoptera	Hydrophilidae	*Tropisternus lateralis*	Moderate
Hemiptera	Belostomatidae	*Belostoma flumineum*	Moderate
Hemiptera	Corixidae	*Graptocorixa abdominalis*	Moderate
Hemiptera	Corixidae	*Graptocorixa serrulata*	Moderate
Hemiptera	Corixidae	*Graptocorixa gerhardi*	Moderate
Hemiptera	Corixidae	Hesperocorixa	Moderate
Hemiptera	Corixidae	*Rhamphocorixa acuminata*	Moderate
Hemiptera	Corixidae	*Trichocorixa uhleri*	Moderate
Hemiptera	Gerridae	*Aquarius remigis*	Moderate
Hemiptera	Notonectidae	*Buenoa arida*	Moderate
Hemiptera	Notonectidae	*Buenoa arizonis*	Moderate
Hemiptera	Notonectidae	*Buenoa margaritacea*	Moderate
Hemiptera	Notonectidae	*Buenoa scimitra*	Moderate
Hemiptera	Notonectidae	*Notonecta hoffmanni*	Moderate
Hemiptera	Veliidae	Microvelia	Moderate
Megaloptera	Corydalidae	*Corydalus texanus*	Moderate
Megaloptera	Corydalidae	Neohermes	Moderate
Odonata	Aeshnidae	*Oplonaeschna armata*	Moderate
Odonata	Calopterygidae	Hetaerina	Moderate
Odonata	Coenagrionidae	Argia	Moderate
Odonata	Coenagrionidae	Coenagrion / Enallagma	Moderate
Odonata	Gomphidae	Erpetogomphus	Moderate
Odonata	Lestidae	*Archilestes grandis*	Moderate
Odonata	Libellulidae	Brechmorhoga	Moderate
Odonata	Libellulidae	*Pantala hymenaea*	Moderate
Odonata	Libellulidae	Sympetrum	Moderate
Coleoptera	Dytiscidae	Agabus	Strong
Coleoptera	Dytiscidae	*Boreonectes aequinoctialis*	Strong
Coleoptera	Gyrinidae	*Dineutus sublineatus*	Strong
Coleoptera	Hydrophilidae	*Tropisternus affinis*	Strong
Hemiptera	Belostomatidae	*Lethocerus medius*	Strong
Hemiptera	Nepidae	*Ranatra quadridentata*	Strong
Hemiptera	Notonectidae	*Notonecta lobata*	Strong
Odonata	Cordulegastridae	*Cordulegaster diadema*	Strong
Odonata	Libellulidae	*Libellula saturata*	Strong
Odonata	Libellulidae	*Paltothemis lineatipes*	Strong

3.8 Appendix B

Diagnostic plots for generalized linear models fitted to each combination of aquatic invertebrate dispersal category (weak, local, moderate, and strong) and explanatory environmental (habitat distance and flow regime distance) and spatial (geographical, topographic, perennial and network distance) variables.

Figure 3.8.1. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the flow regime distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

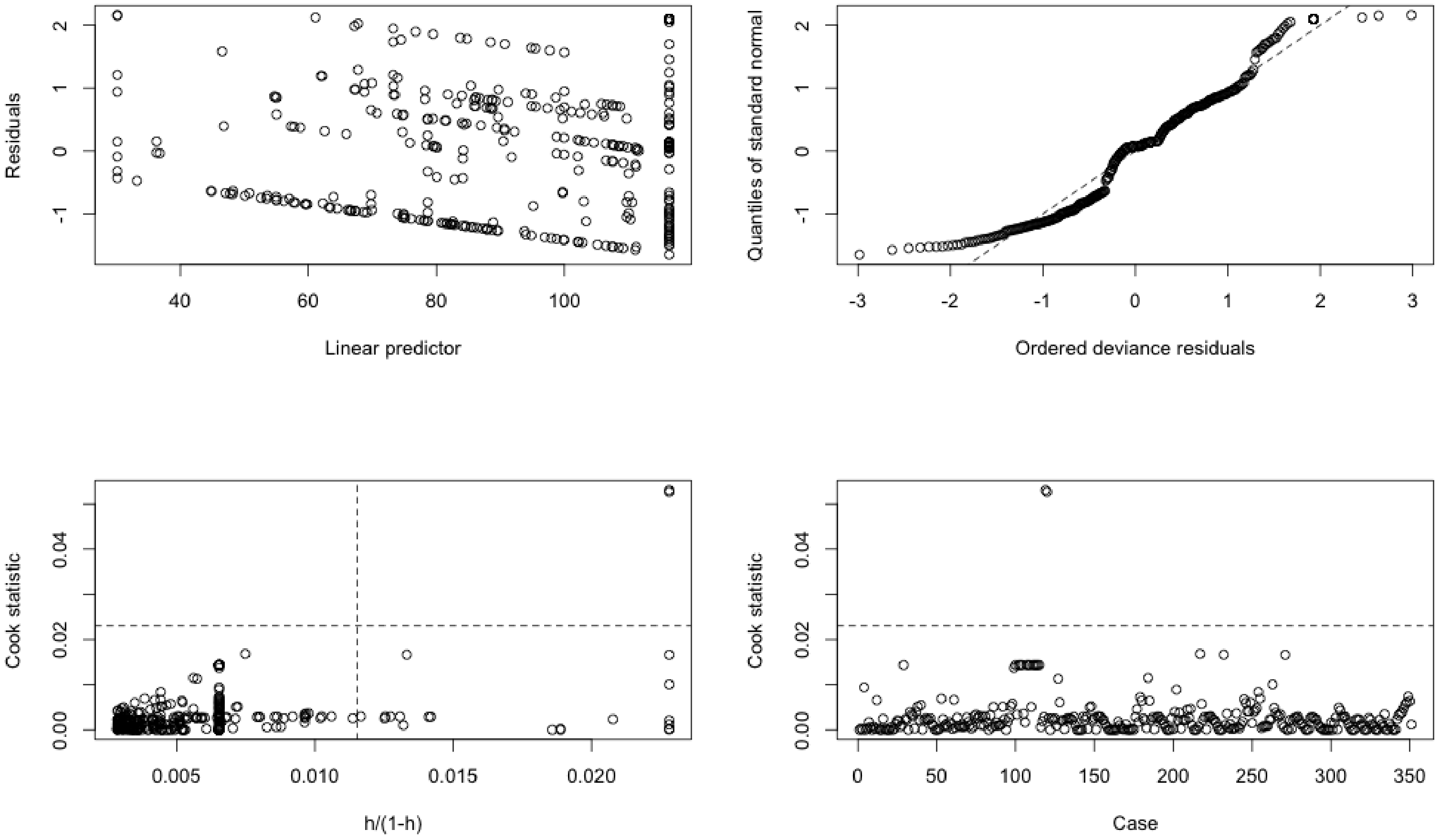


Figure 3.8.2. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the geographic distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

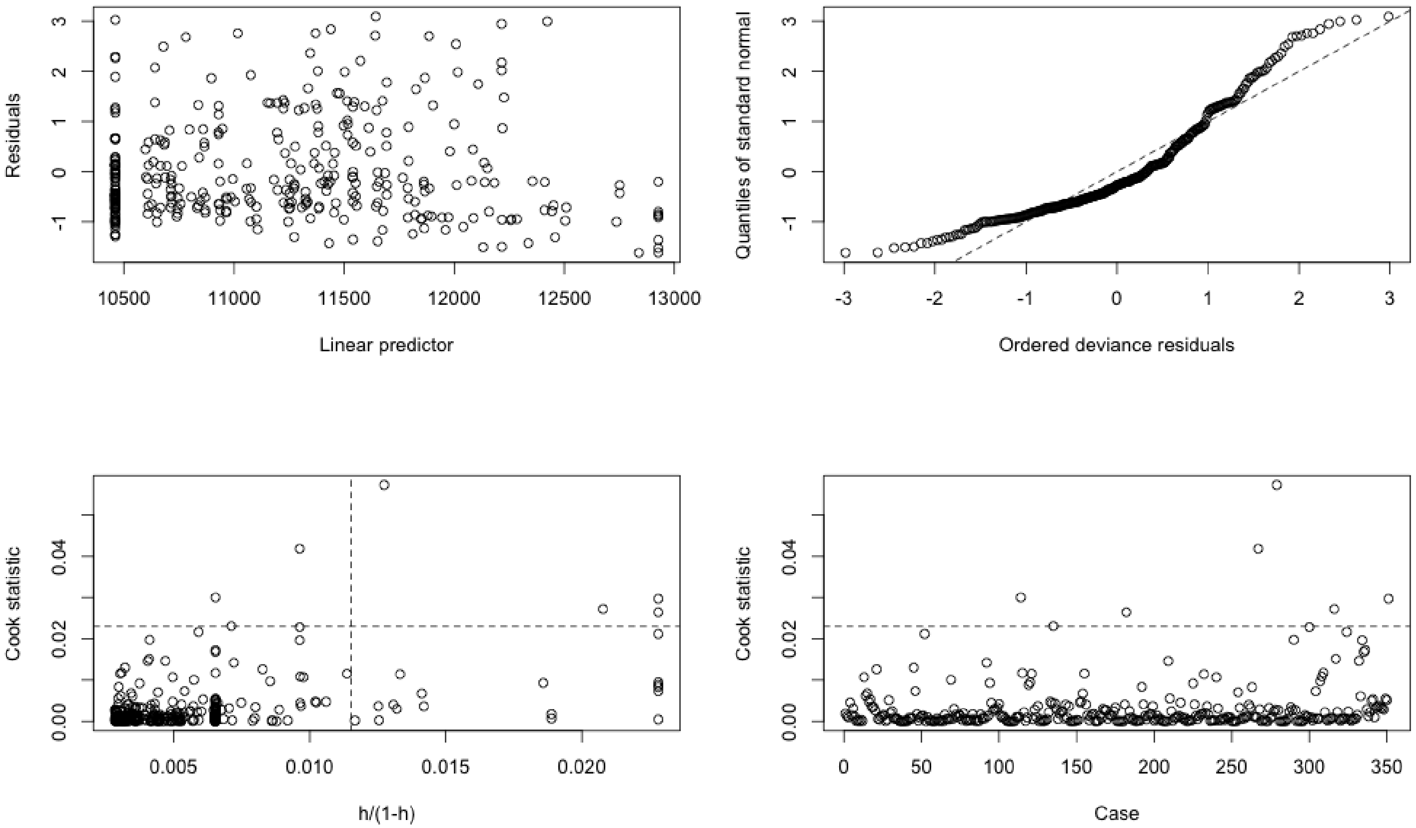


Figure 3.8.3. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the habitat distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

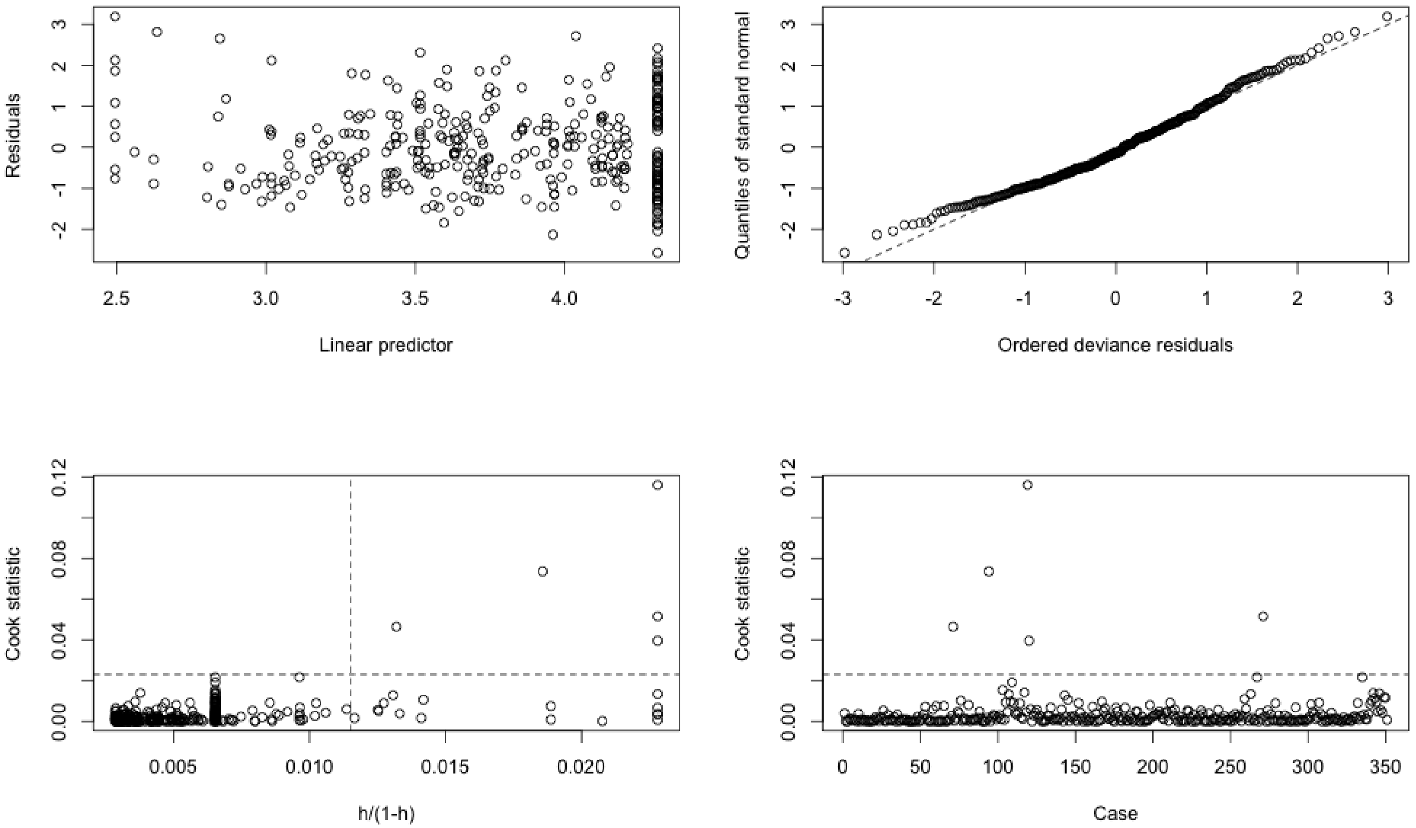


Figure 3.8.4. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the network distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

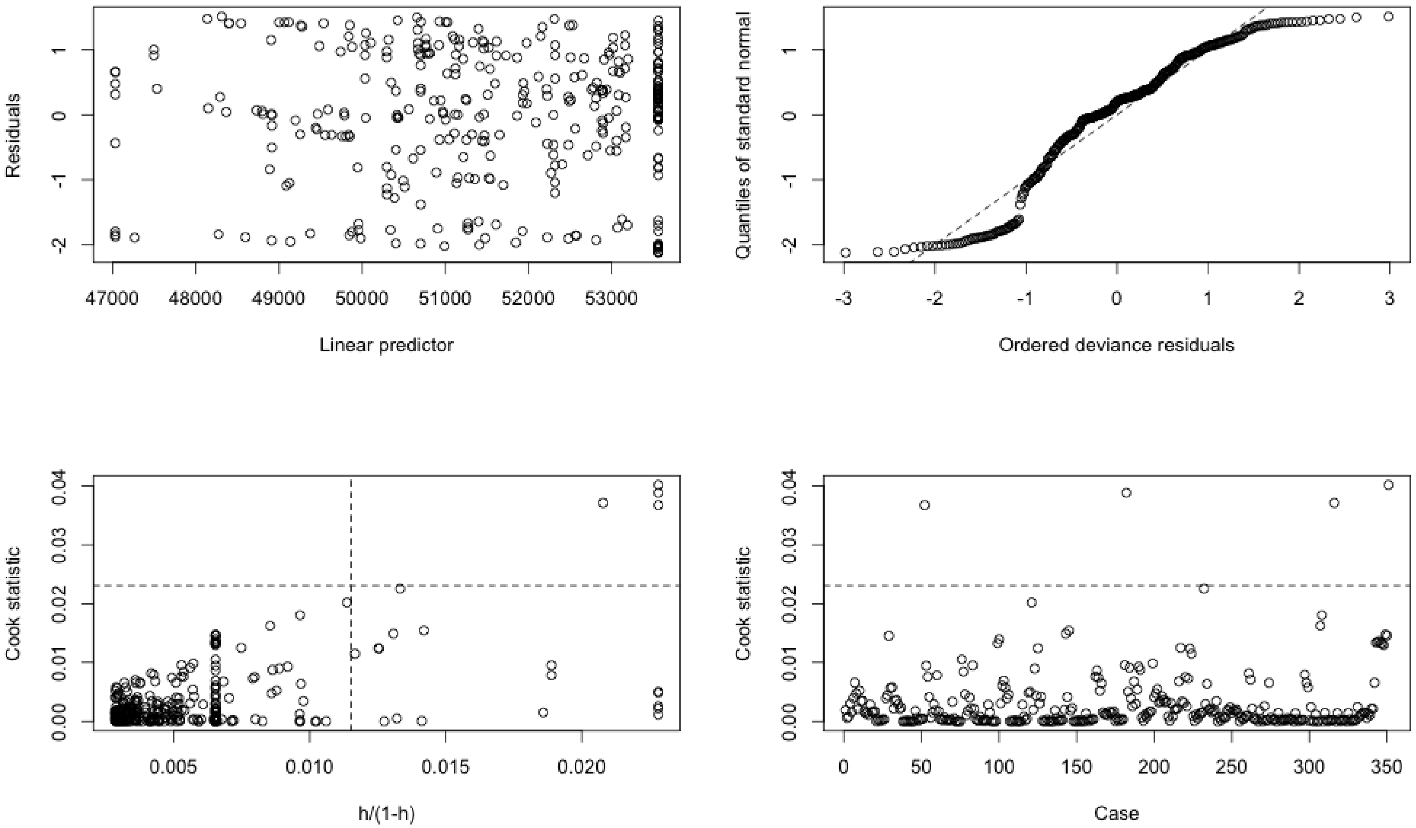


Figure 3.8.5. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the perennial habitats distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

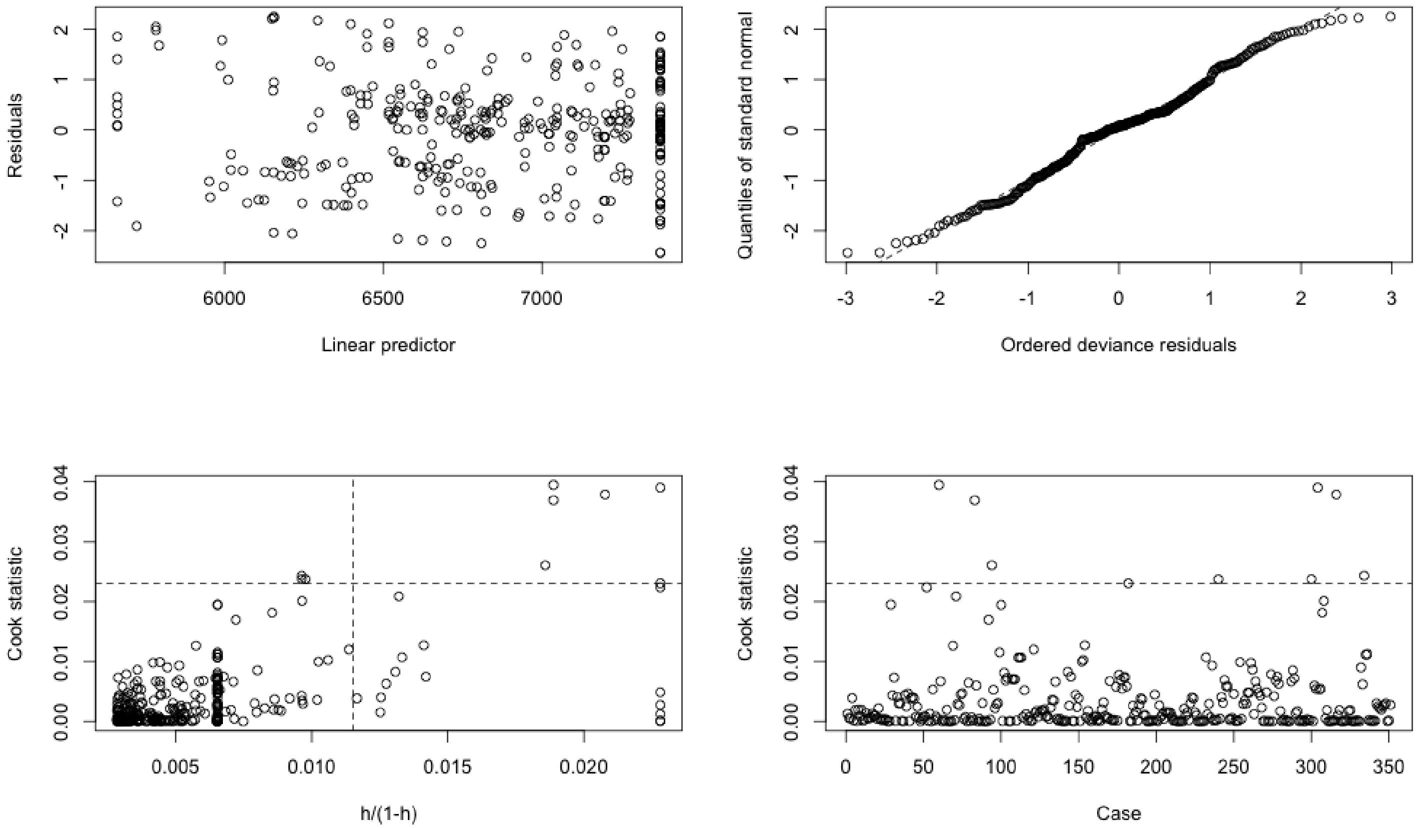


Figure 3.8.6. Diagnostic plots for generalized linear models with community dissimilarity of weak dispersers as the dependent variable and the topography distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

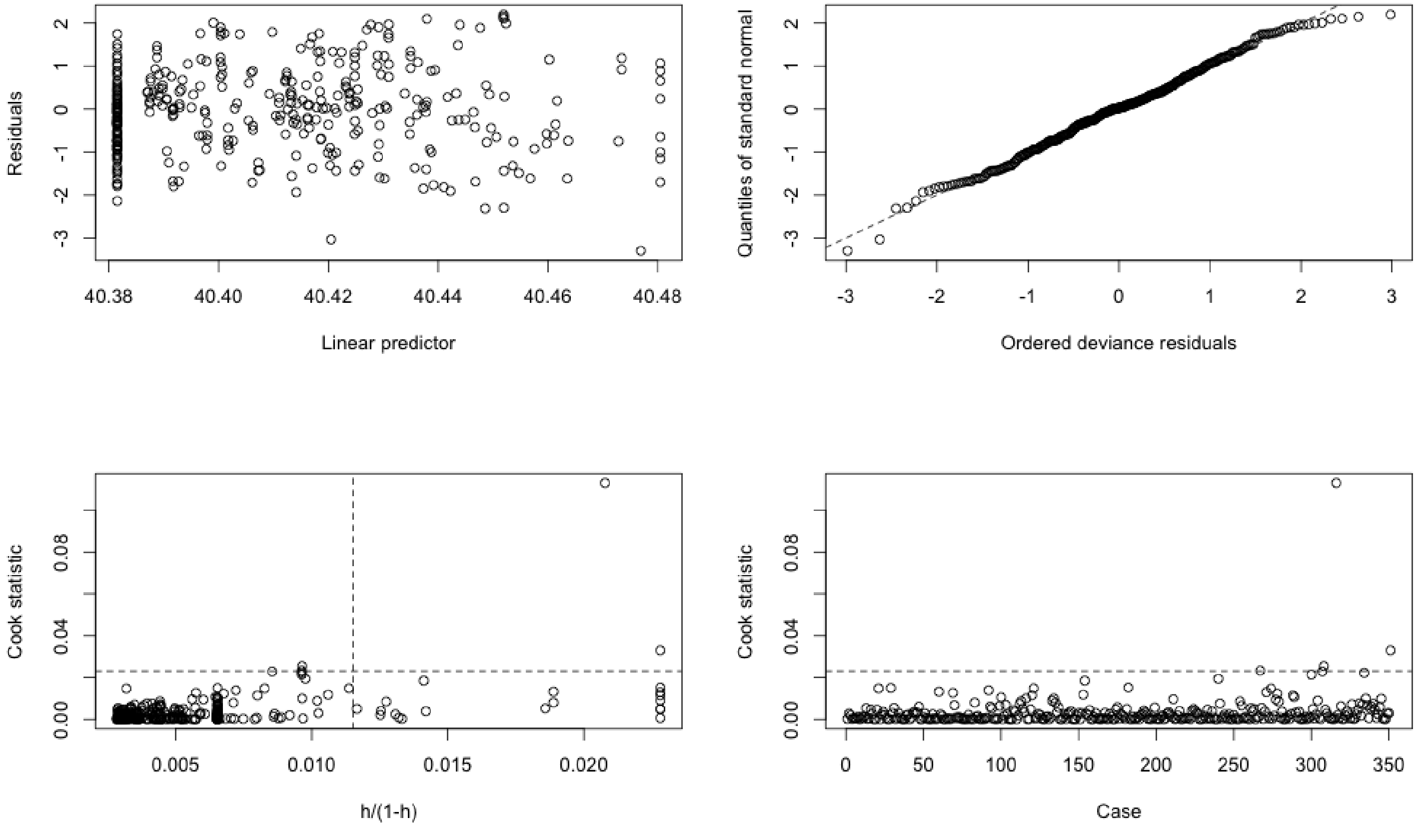


Figure 3.8.7. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the flow regime distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

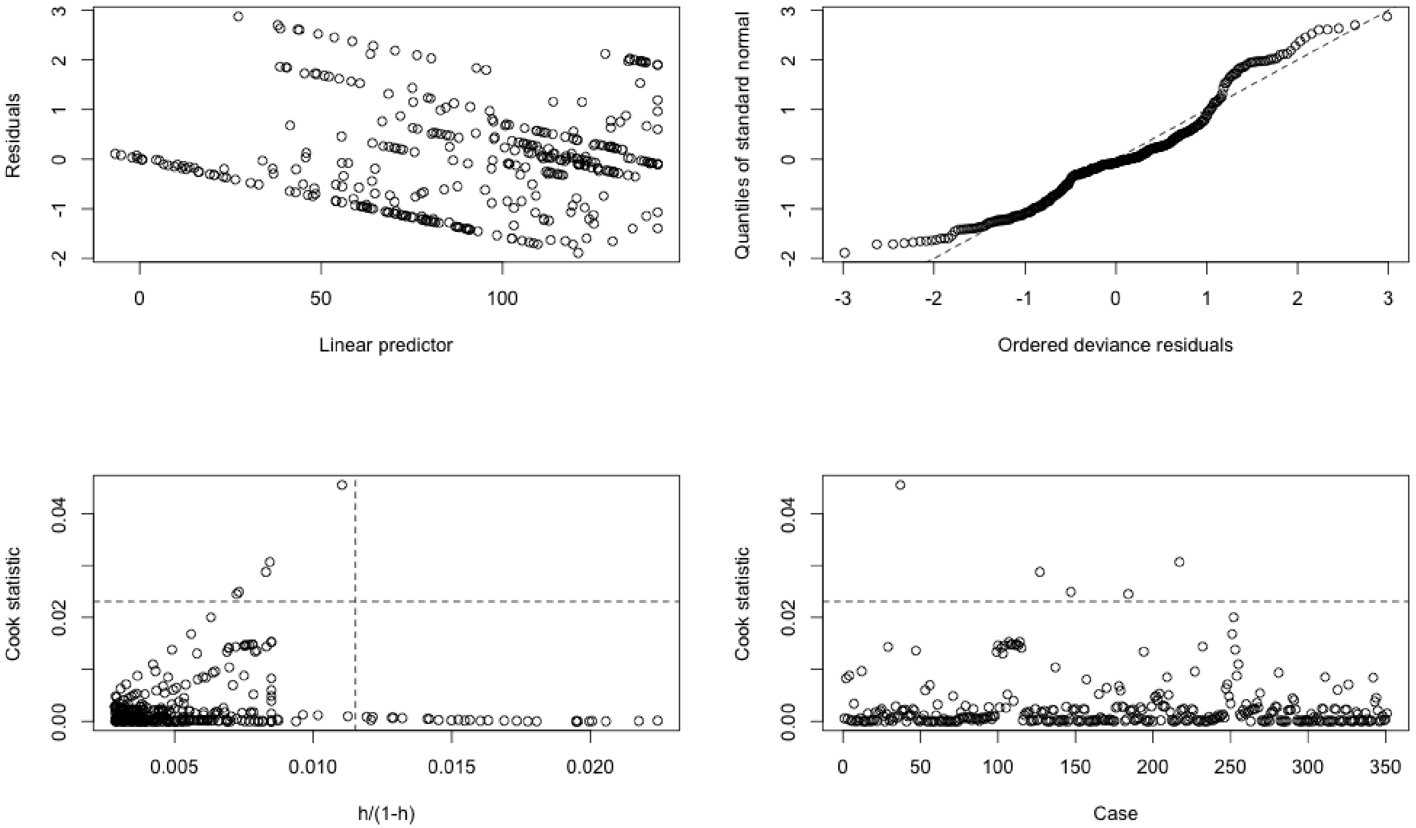


Figure 3.8.8. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the geographic distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

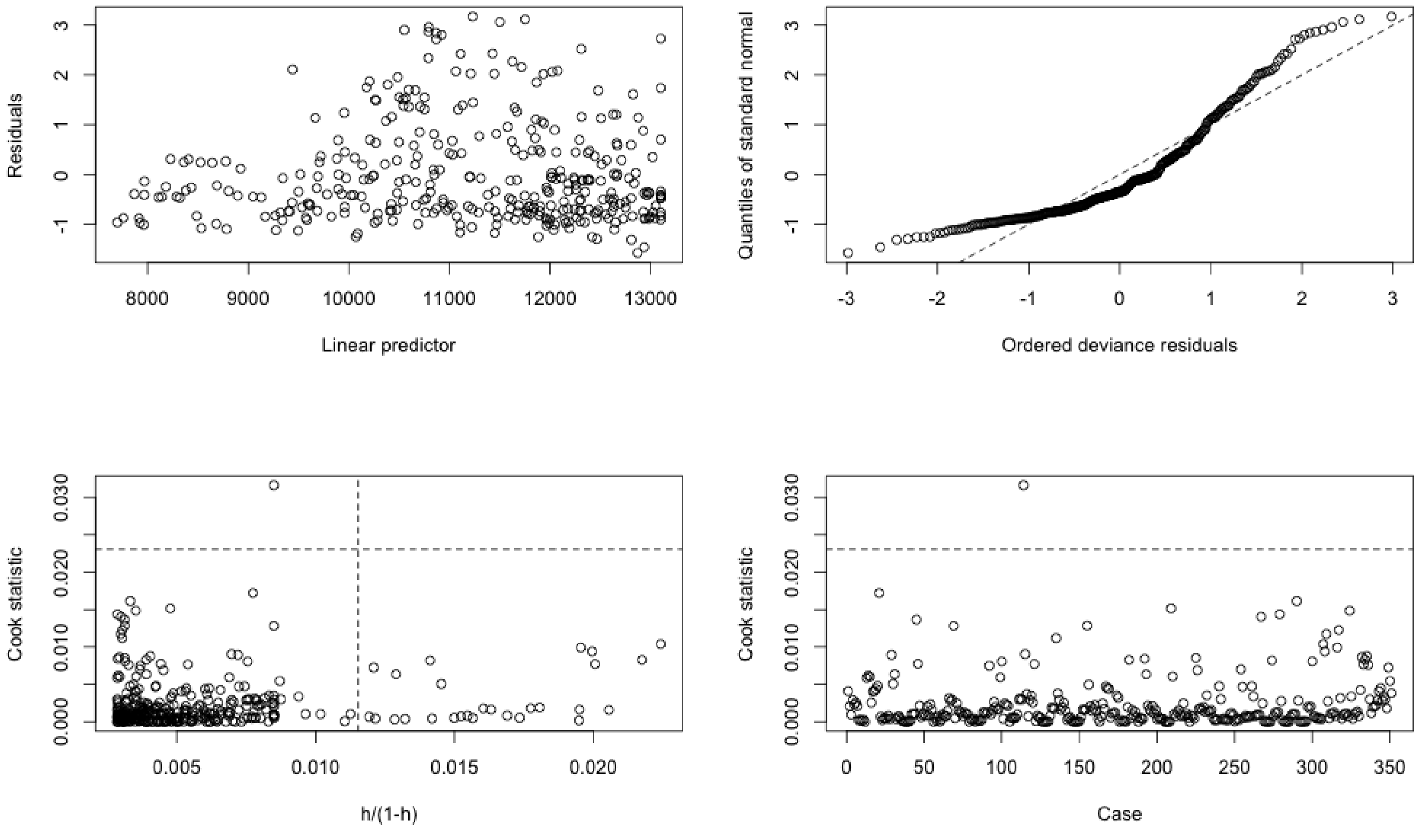


Figure 3.8.9. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the habitat distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

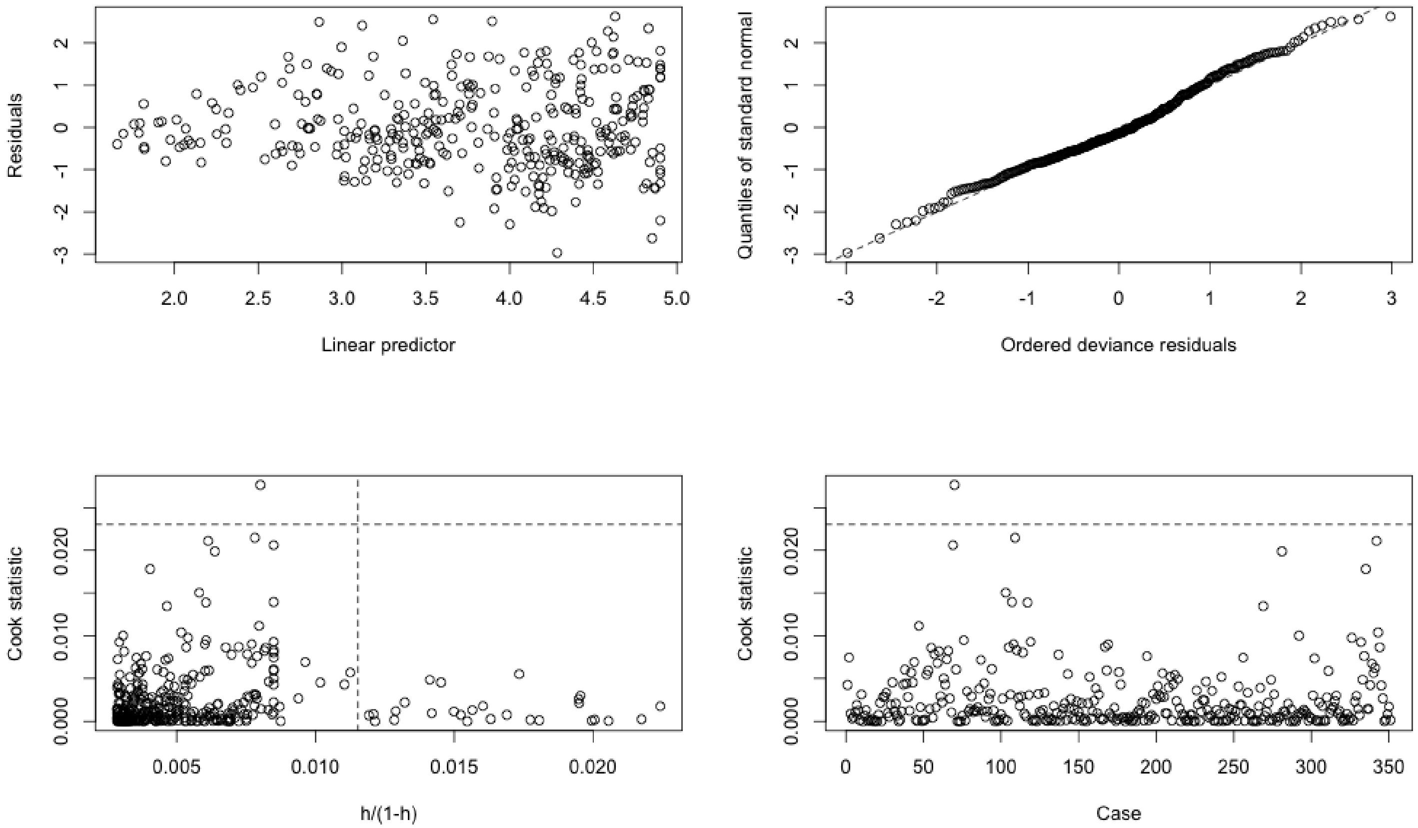


Figure 3.8.10. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the network distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

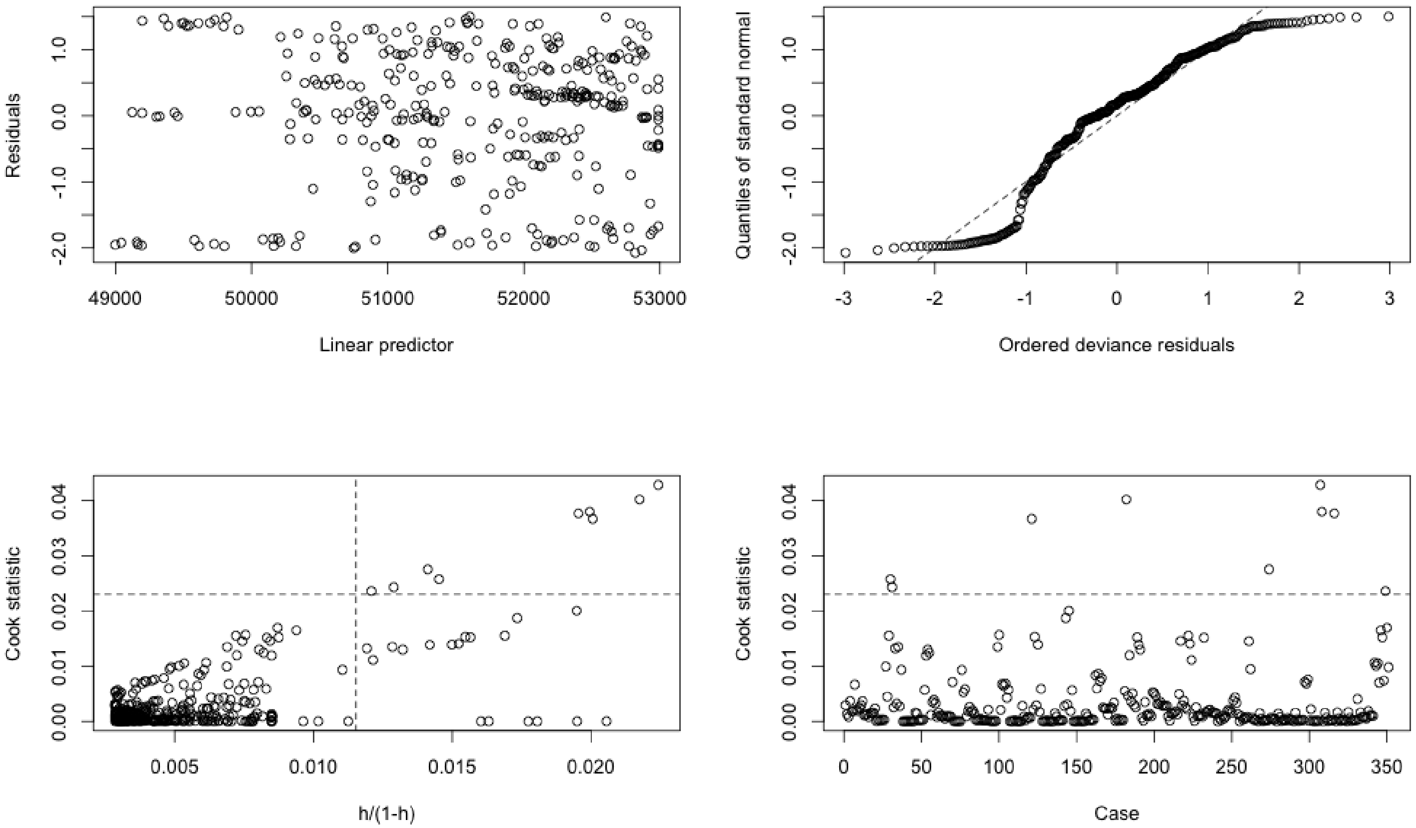


Figure 3.8.11. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the perennial habitats distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

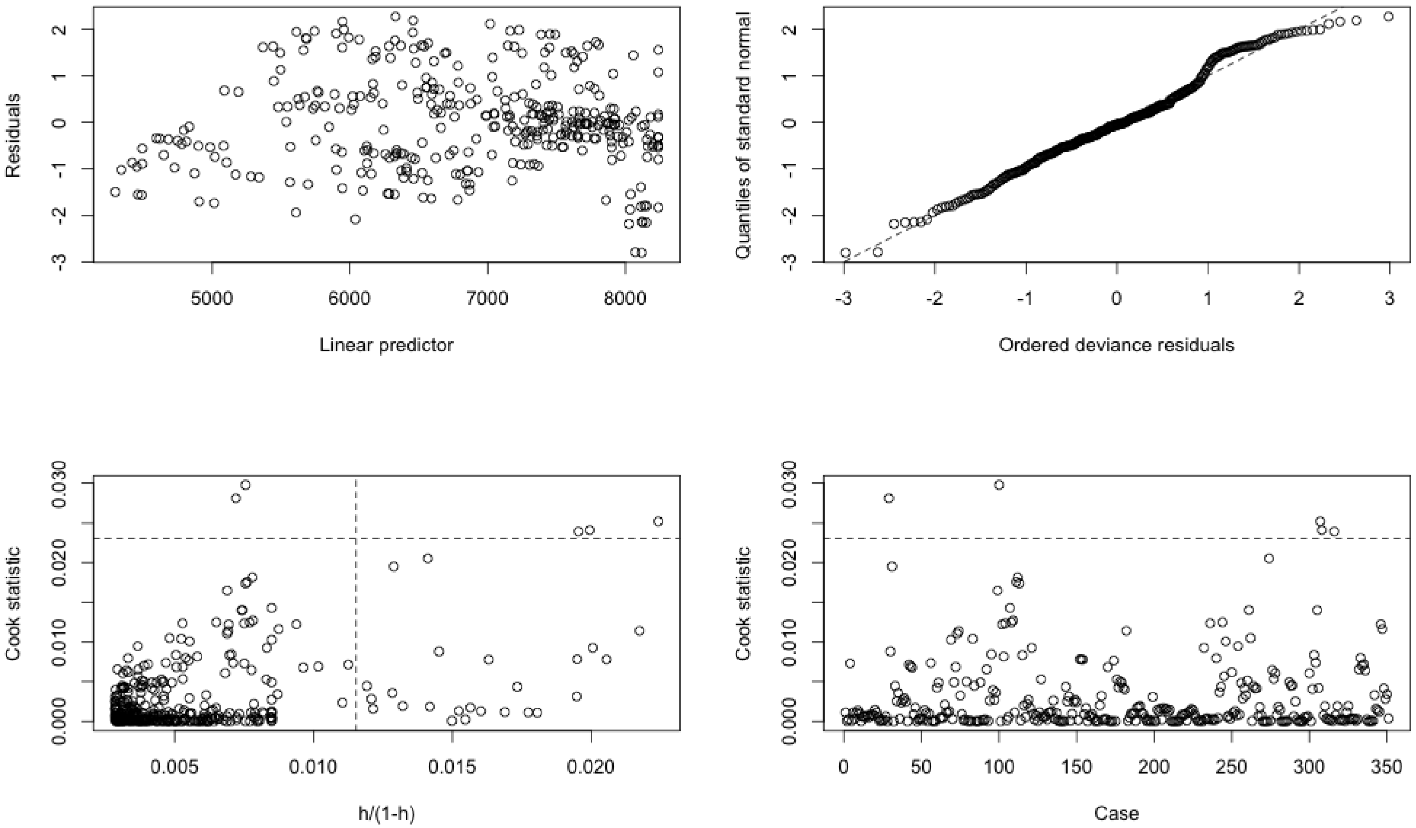


Figure 3.8.12. Diagnostic plots for generalized linear models with community dissimilarity of local dispersers as the dependent variable and the topography distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

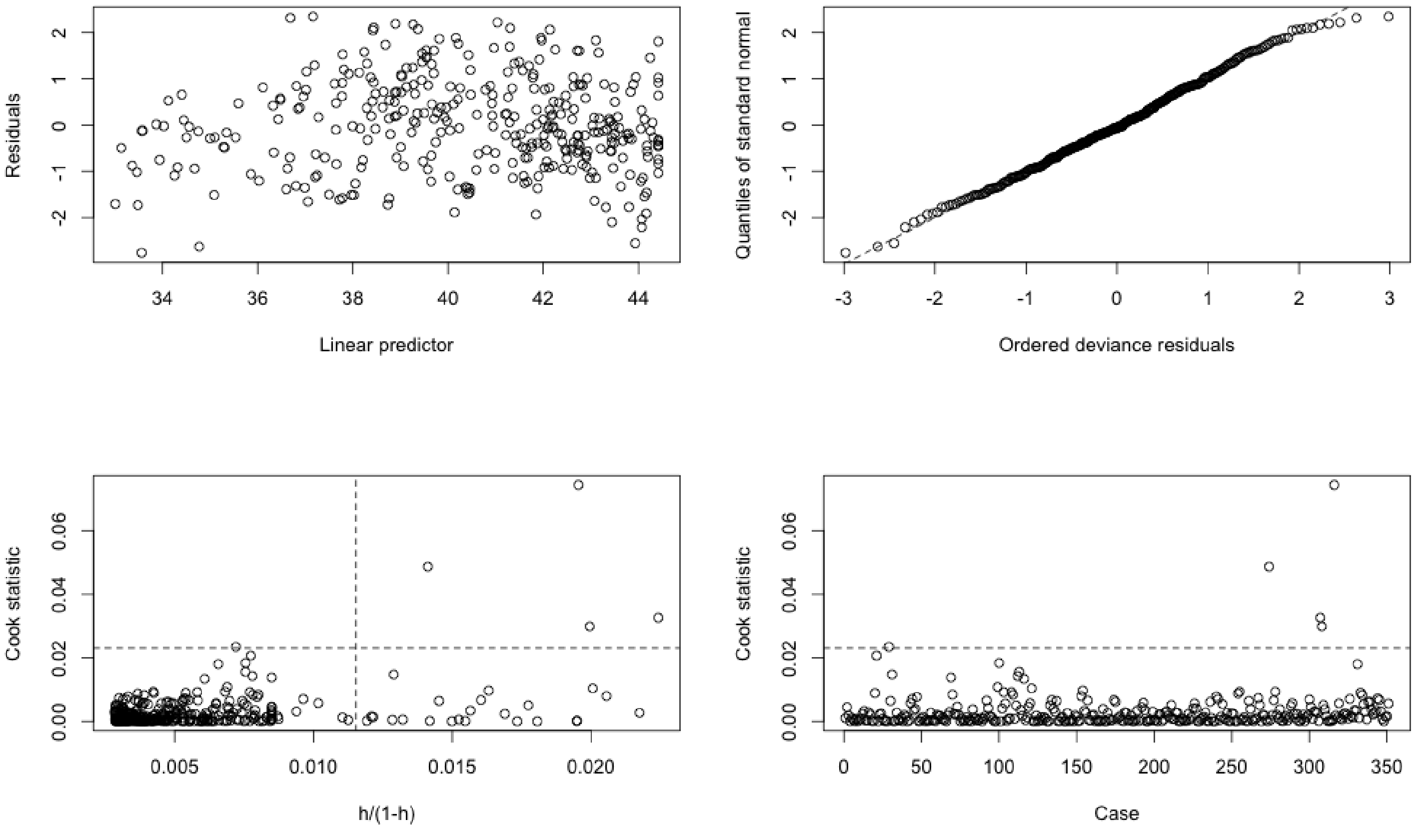


Figure 3.8.13. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the flow regime distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

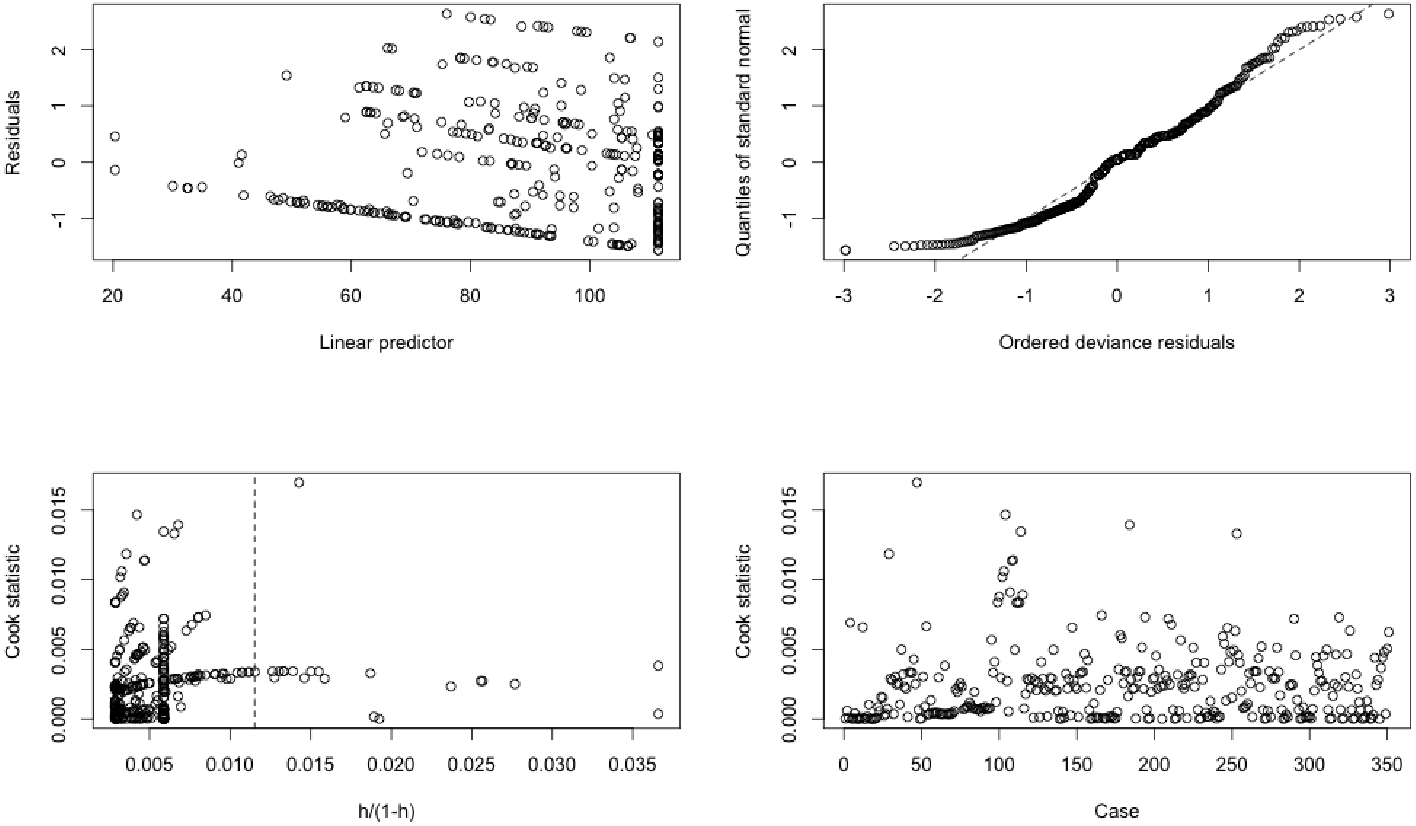


Figure 3.8.14. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the geographic distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

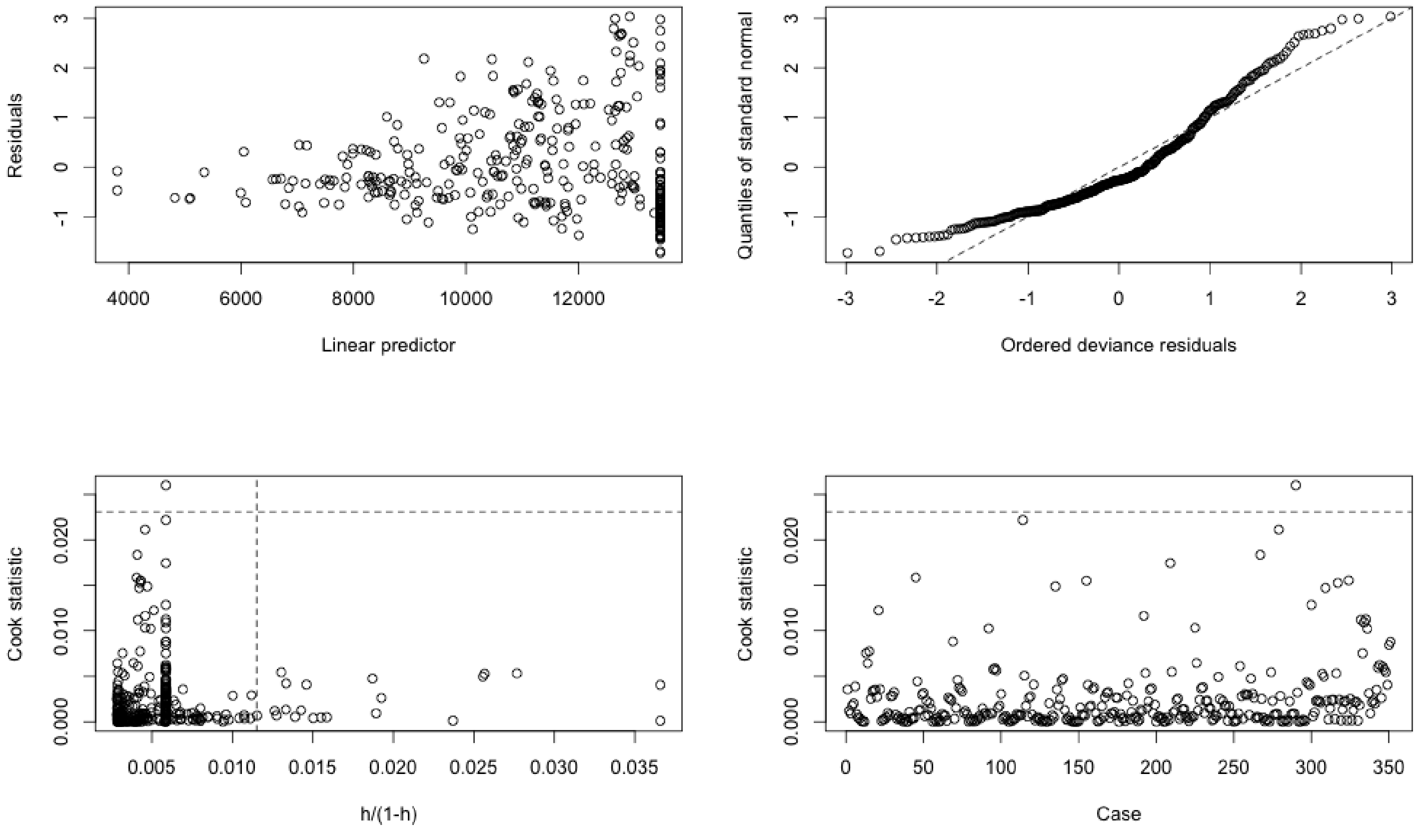


Figure 3.8.15. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the habitat distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

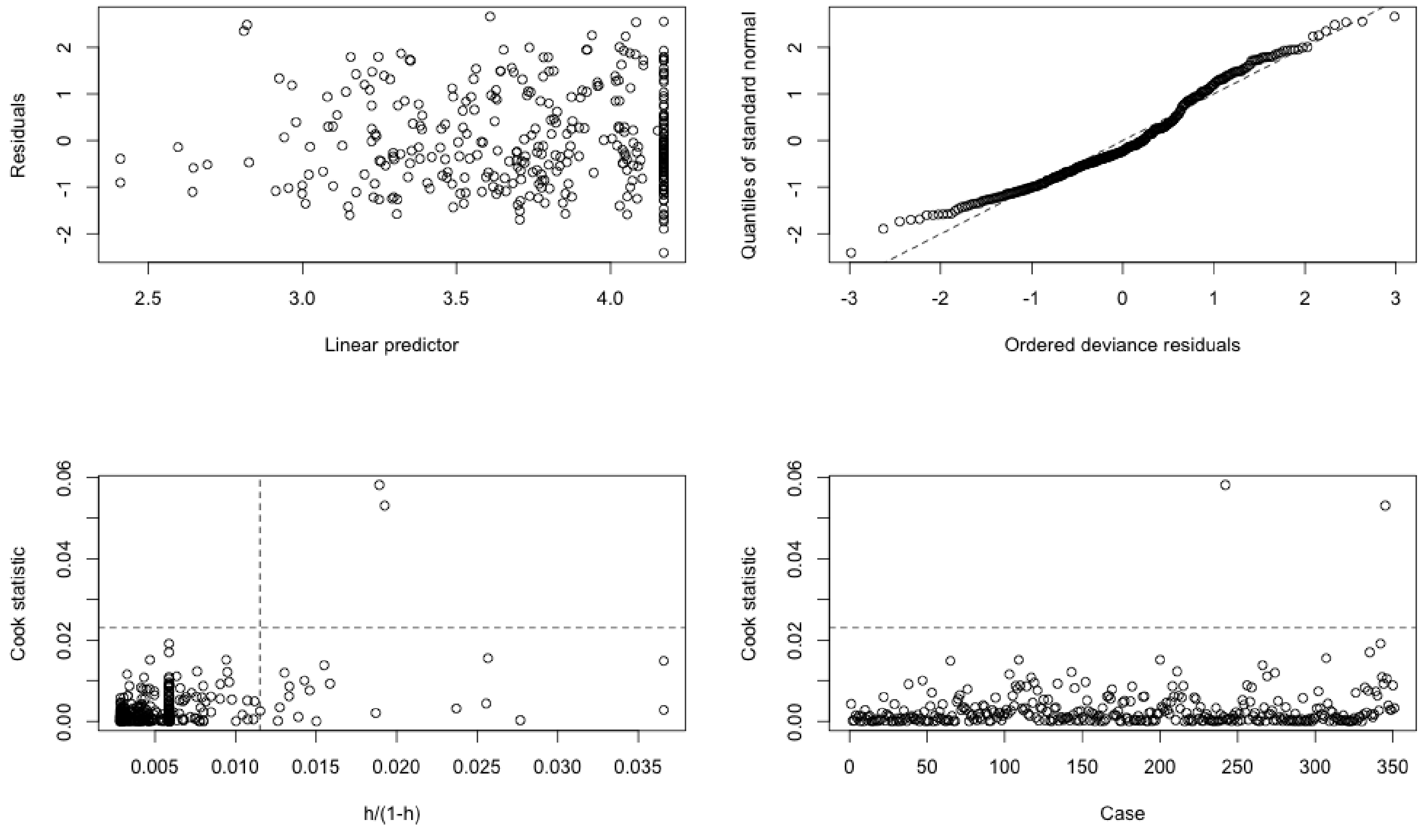


Figure 3.8.16. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the network distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

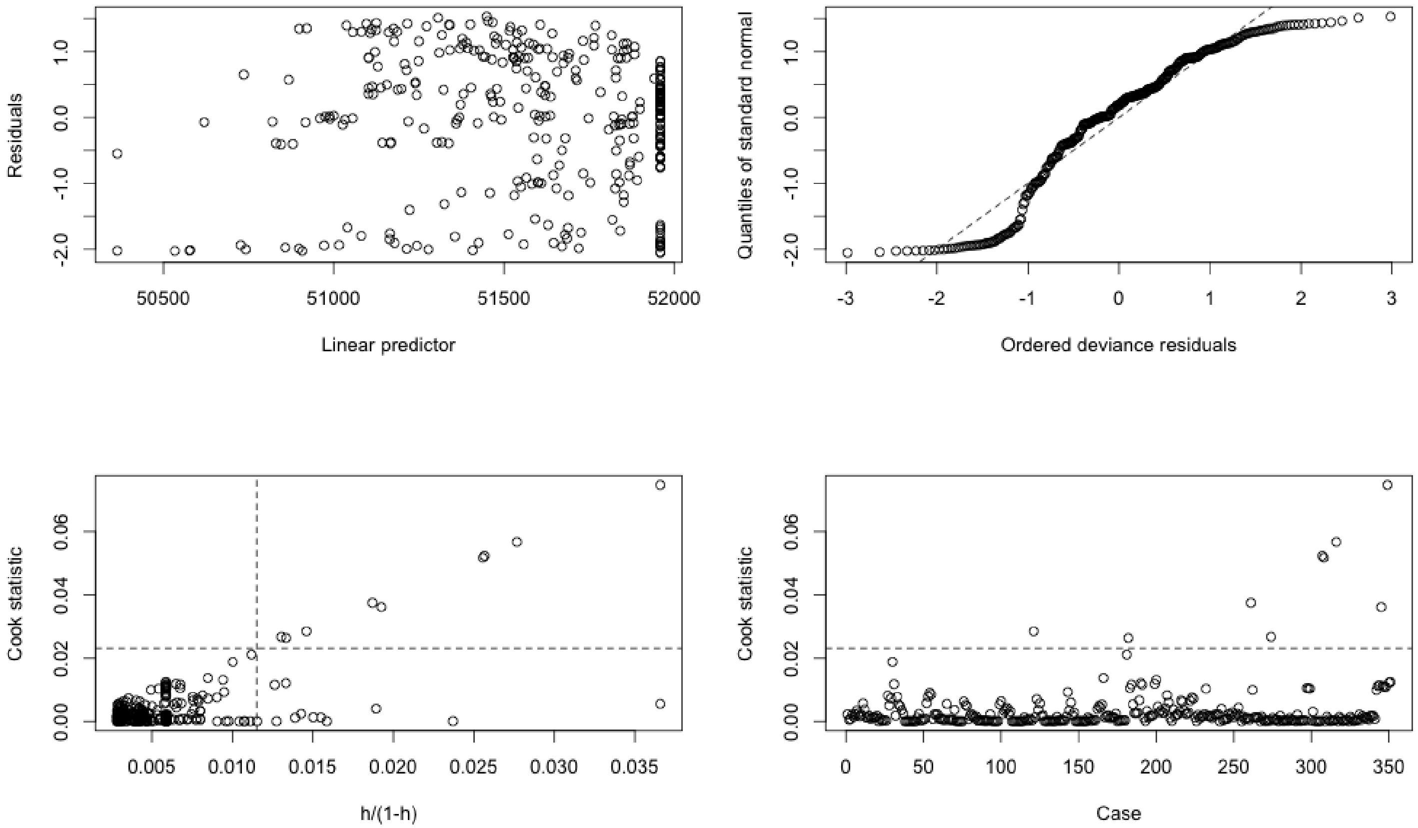


Figure 3.8.17. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the perennial habitats distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

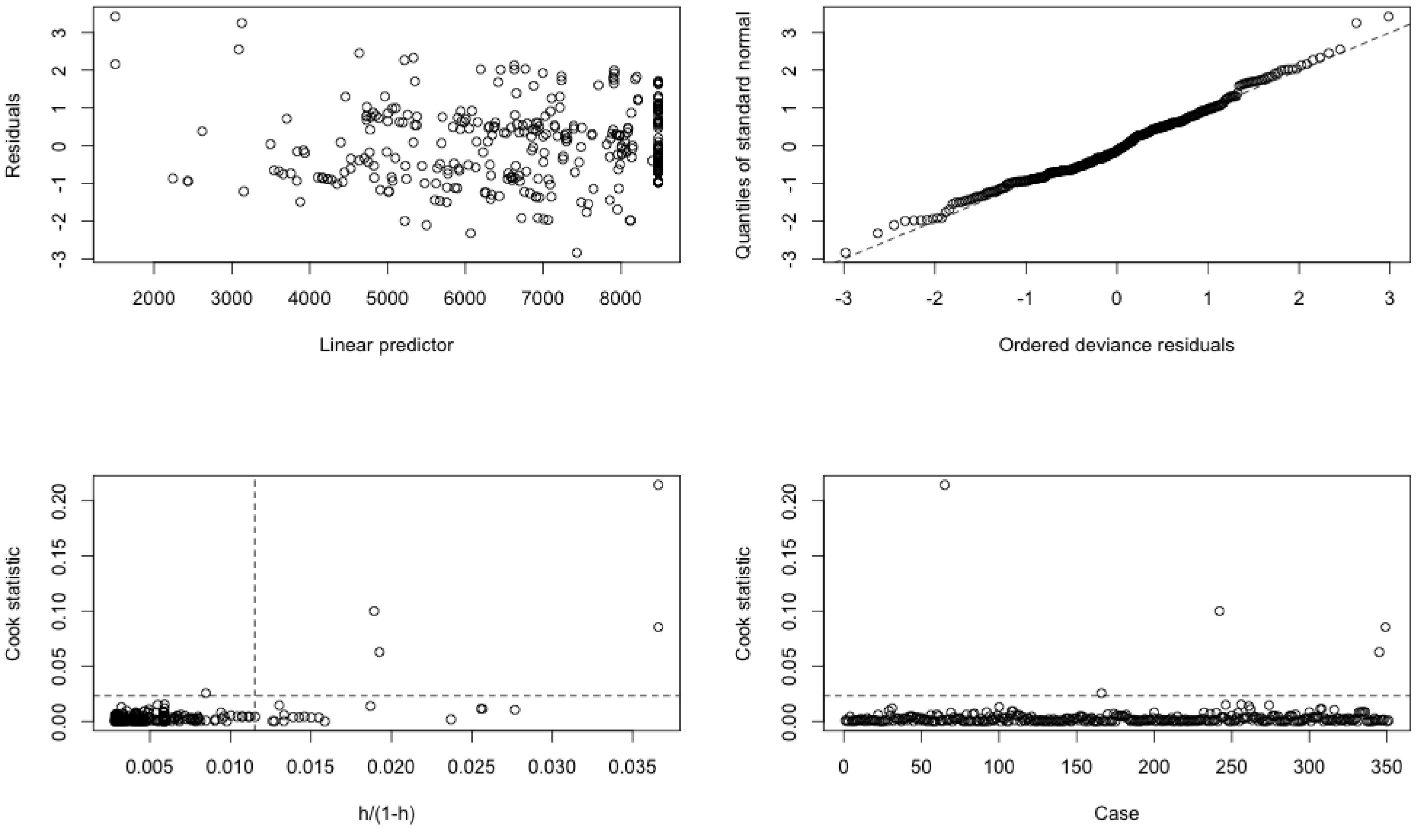


Figure 3.8.18. Diagnostic plots for generalized linear models with community dissimilarity of moderate dispersers as the dependent variable and the topography distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

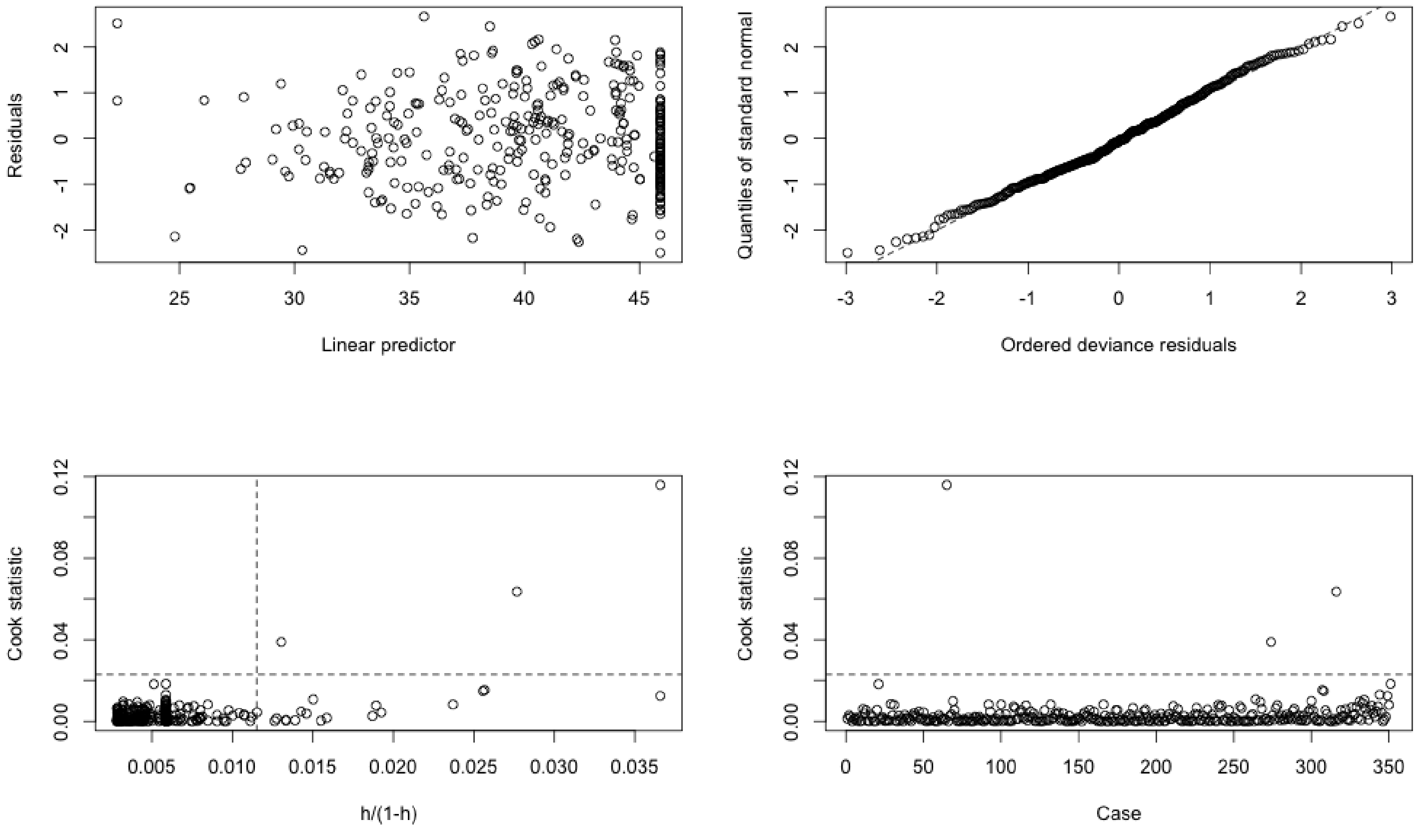


Figure 3.8.19. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the flow regime distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

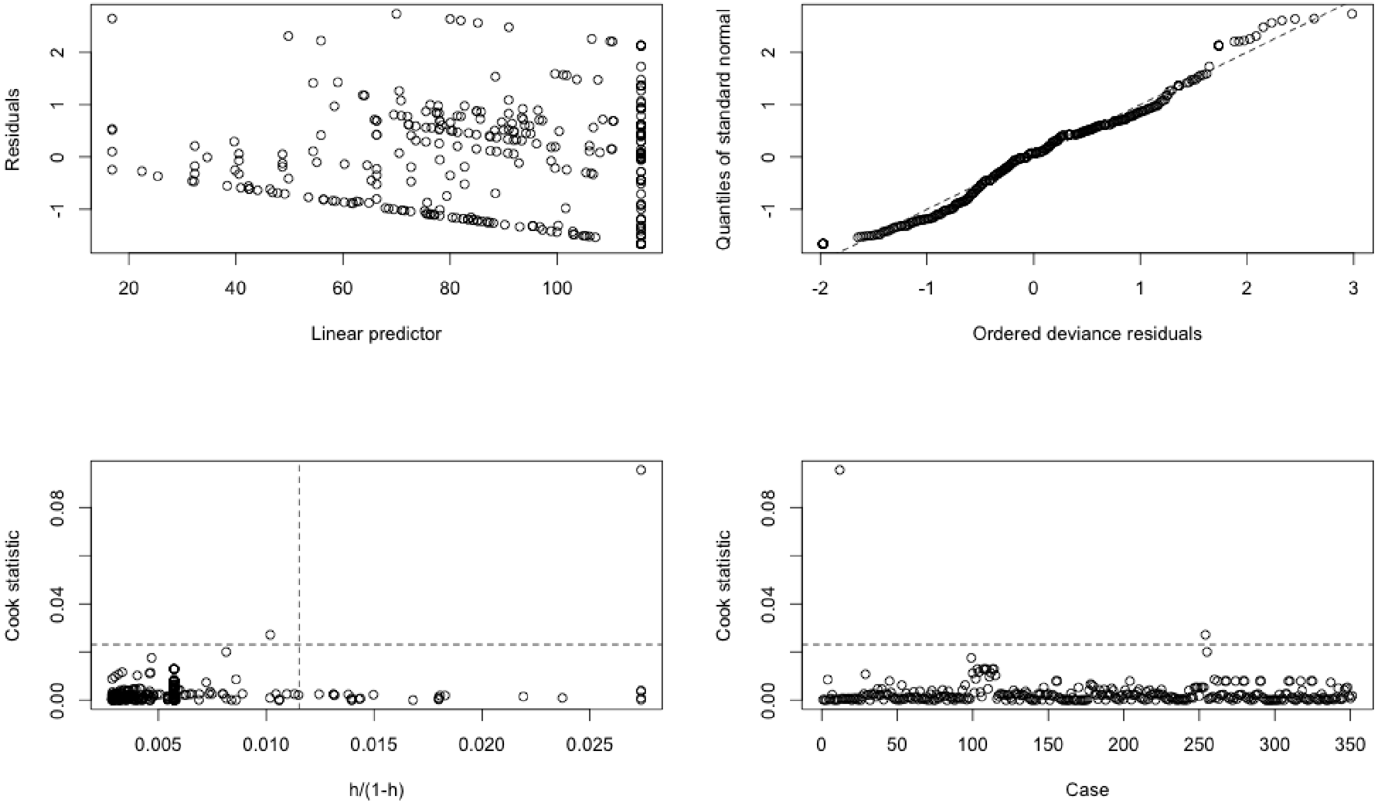


Figure 3.8.20. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the geographic distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

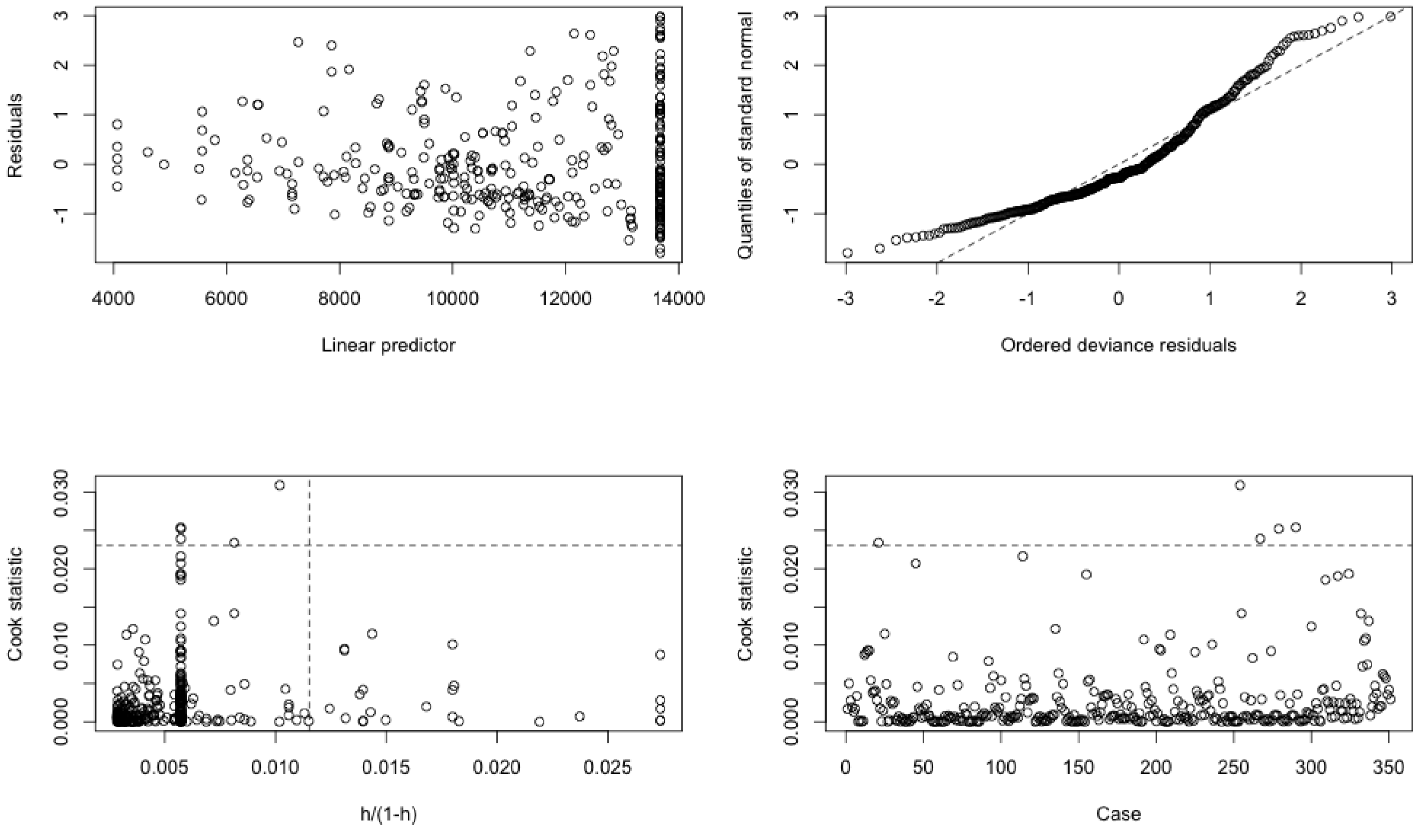


Figure 3.8.21. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the habitat distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

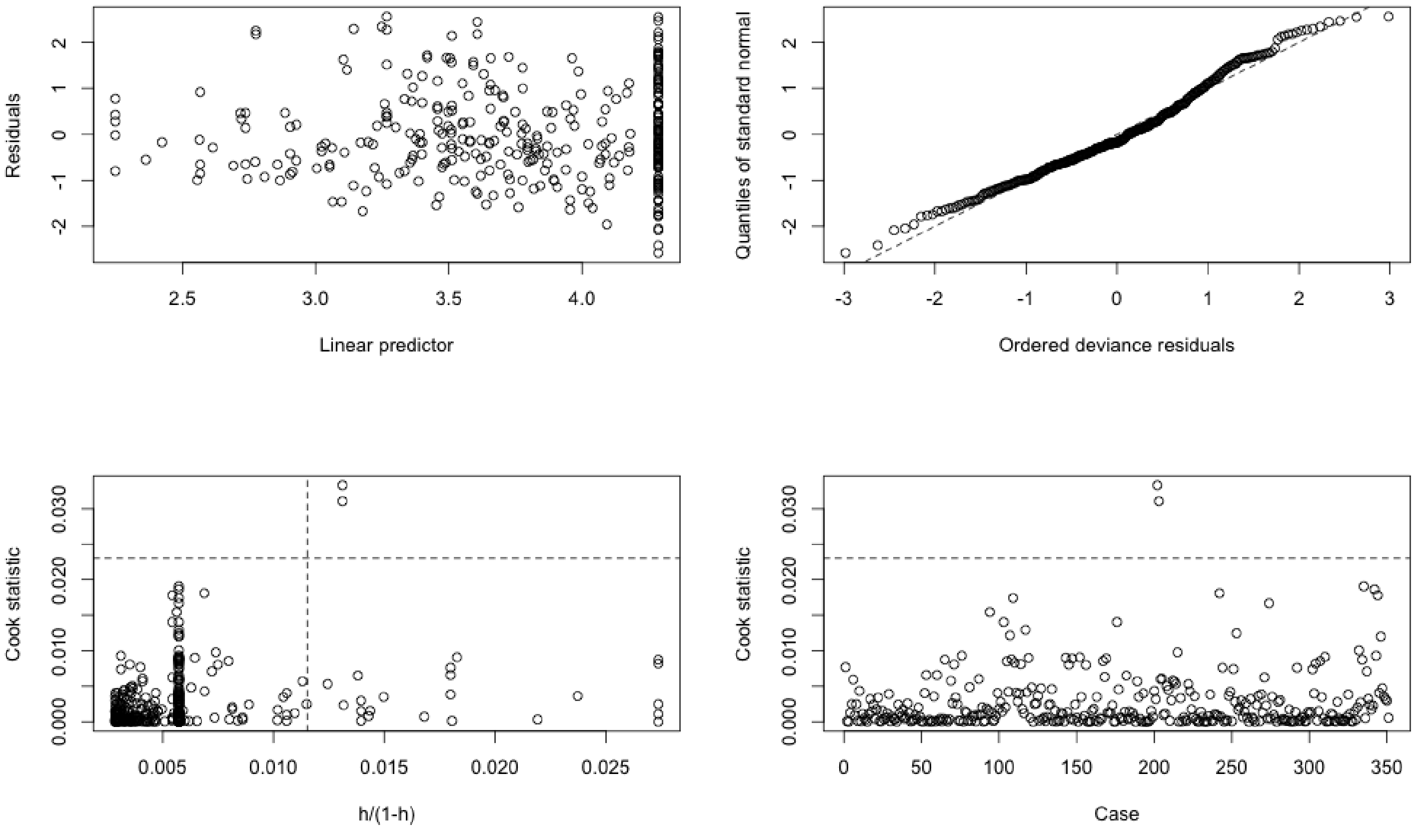


Figure 3.8.22. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the network distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

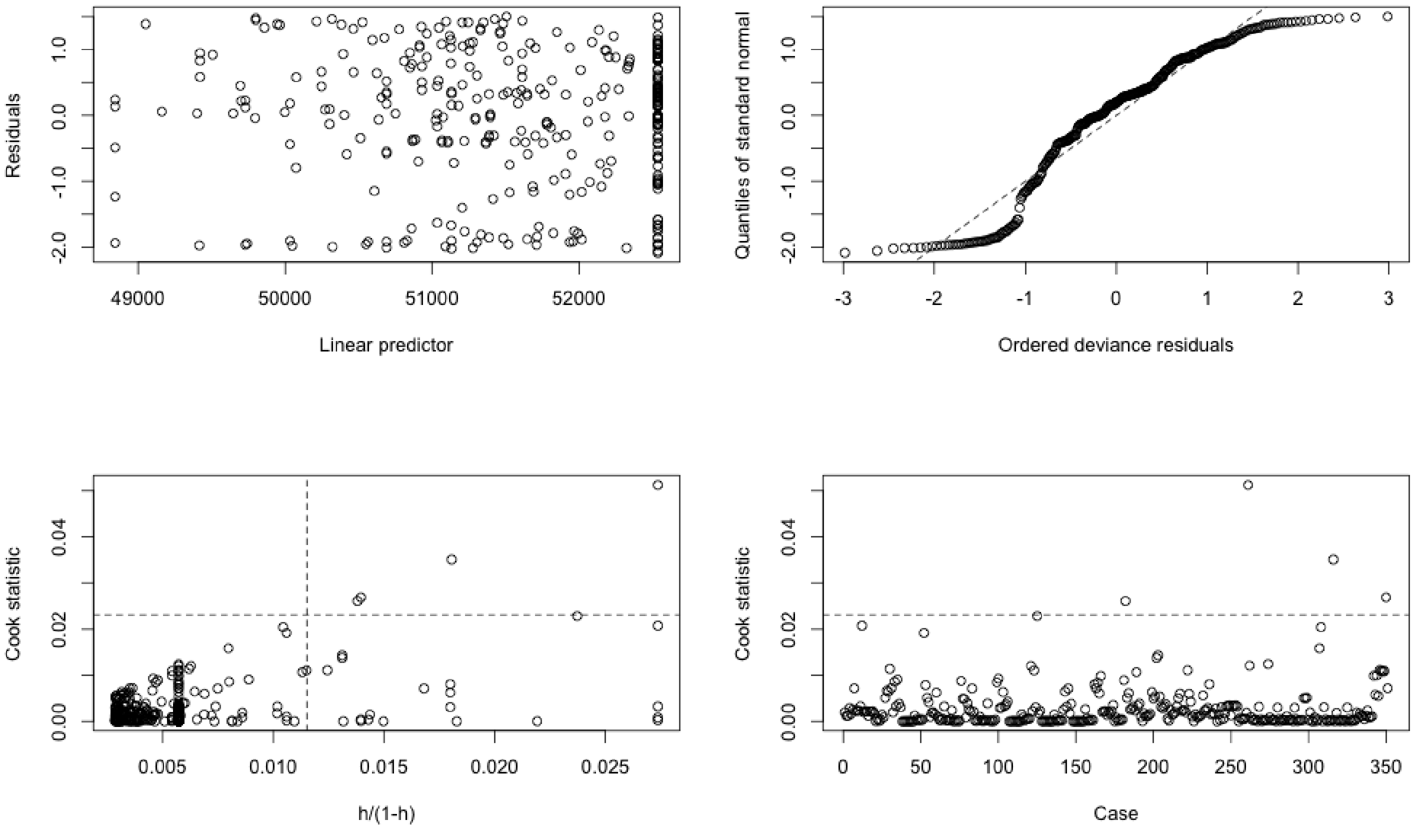


Figure 3.8.23. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the perennial habitats distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.

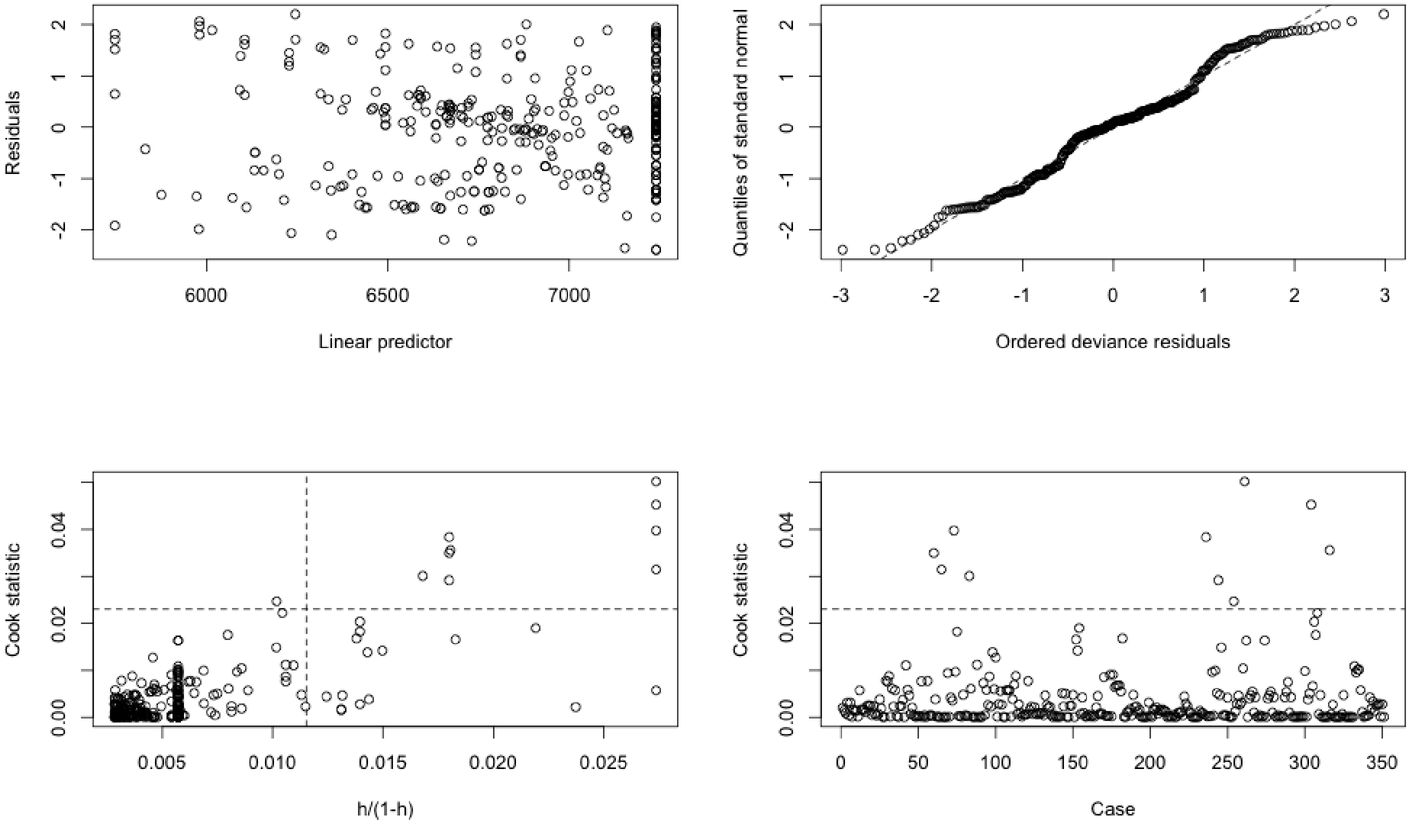
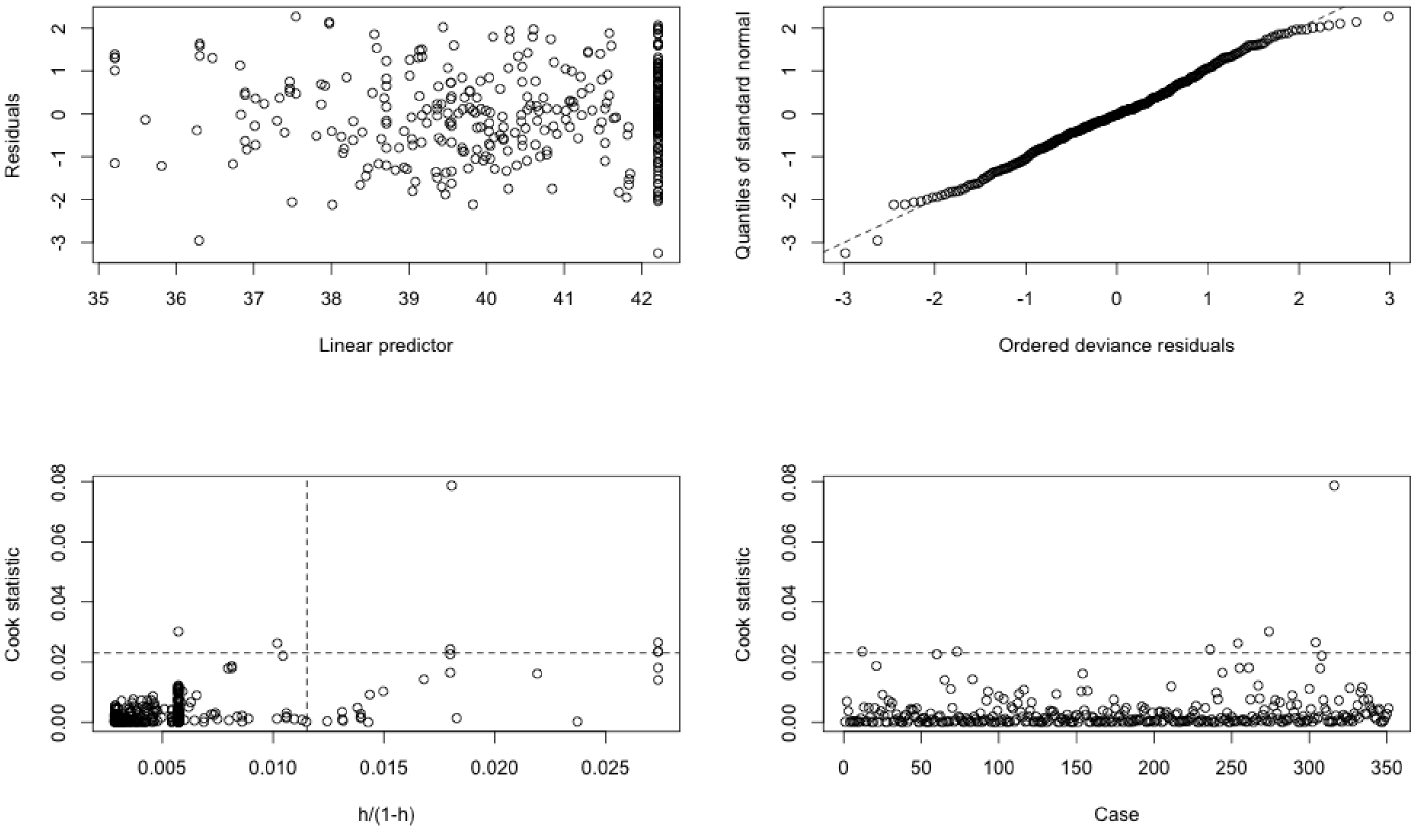


Figure 3.8.24. Diagnostic plots for generalized linear models with community dissimilarity of strong dispersers as the dependent variable and the topography distance between sites as the independent variable. Upper left: plot of jackknife deviance residuals against linear predictor; Upper right: normal scores plots of standardized deviance residuals; Lower left: plot of approximate Cook statistics against leverage/(1 − leverage); Lower right: case plot of Cook statistic. Aquatic invertebrates were sampled in 28 sites across seven dryland streams distributed within a 400-km^2 section of the Upper San Pedro River basin, south-eastern Arizona, USA.



4. Links between two interacting factors – novel habitats and non-native predators – and aquatic invertebrate communities in a dryland environment

4.1 Introduction

The Madrean Sky Islands (MSI) of the southwestern United States and northwest Mexico are characterized by high elevation mountain ranges separated by desert valleys, and the region's unique climate and geological history contribute to a patchwork of diverse habitats and resource availability (Gottfried et al. 2012). Aquatic habitats in the region punctuate the semi-arid landscape and include both naturally occurring aquatic habitat – perennial and intermittent streams, seasonal pools, wetlands, and springs – and novel habitat such as ponds, drainage ditches, and troughs. The diverse array of aquatic habitats is home to a unique regional assemblage of aquatic invertebrates with several hundred species in over 60 families, including Neotropical, Nearctic, and regionally-endemic taxa (Bogan, 2012). Determining how local habitat characteristics and co-occurring vertebrate species, including nonnative fishes and frogs, affect aquatic invertebrate communities is essential to conserving biodiversity in the region.

A diverse suite of behavioral, morphological, and life history traits enable aquatic invertebrates in the MSI to occupy a broad range of habitats with variable environmental characteristics. Water permanence is considered a key gradient along which invertebrate communities stratify (e.g. Della Bella & Chiarotti, 2005; Chapter 2). Lotic habitats in the MSI are characterized by dynamic seasonal drying and wetting cycles. They are connected by flowing water during the wet season but contract to a series of small perennial pools during the dry season (Bogan & Lytle, 2007). Additionally, MSI streams experience severe flash floods in the wet season (Lytle, 2000). These connectivity and disturbance events exert strong selective pressure on invertebrates occupying MSI stream pools (Lytle, 2003), resulting in a range of adaptations to flow events. For example, high dispersal ability allows individuals of many taxa to quickly recolonize habitats following flood or drought (Bogan & Boersma, 2012). Other taxa produce multiple broods each year (i.e., multivoltinism) and have short development times to minimize the time spent in a flood- or drought-prone habitat (Gray & Fisher, 1981). Seasonal floods and droughts in MSI streams can also affect the trophic dynamics of local communities. For example, lack of flow during the dry season leads to an increased concentration of resident predators, allowing predators to dominate these isolated pools numerically (>75% relative abundance of predators) and quickly consume any available prey (Bogan & Lytle, 2007).

The patchwork of natural aquatic habitat in the MSI region has undergone substantial changes due to human water use in the region over the last century. Water resource development for agricultural and domestic purposes has greatly reduced the amount of natural aquatic habitats in arid regions, and the reliability (i.e., permanence) of remaining surface waters is decreasing (Deacon et al. 2007; Larned et al. 2010). However, human water use has also resulted in novel perennial habitat in the form of man-made ponds or stock tanks (hereafter called "stock ponds"). Stock ponds are created to provide water for domesticated animals and are now a common feature of many dryland landscapes, including the MSI. As human activity threatens to dewater much of the natural aquatic habitat in these regions, stock ponds are becoming important surrogate habitat for native species (Peltzer et al., 2006). Previous research has identified the potential for man-made ponds to contribute significantly to the diversity of aquatic invertebrates in agricultural and urban landscapes (Céréghino et al. 2008; Legnouo et al. 2013).

Although stock ponds may function as important surrogate habitat for aquatic invertebrates, little is currently known about the similarities between invertebrate communities of naturally occurring perennial stream pools and man-made stock ponds. As both habitat types are perennial and, at least seasonally, have standing water, these habitats might be expected to support similar communities. However, stream pools experience severe flooding and scouring events to which stock ponds are not subjected. Moreover, the size of these habitats is often vastly different, with surface areas of stream pools measuring a few to tens of square meters whereas pond surface areas may be hundreds of square meters. To what extent do invertebrate communities in these two habitats overlap taxonomically? Can known differences in disturbance regime and other environmental characteristics such as habitat size affect the taxonomic and trait composition of these communities? Answering these questions is essential to determining whether stock ponds provide a surrogate habitat for aquatic invertebrate communities of threatened perennial stream pools.

Although stock ponds are important habitat for many native aquatic species in arid regions, they are also a vector for the invasion of non-native species. Novel freshwater habitats such as stock ponds are known to support, and even favor, non-native species (Johnson et al., 2008; Rahel & Olden, 2008; Letnic et al., 2014). The gains of surrogate habitat for native species may be offset by losses to competition with, and predation by, non-native species. In western North America, stock ponds provide perennial water essential to the survival and reproduction of non-native American bullfrogs (*Lithobates catesbeiana*) (Govindarajulu et al., 2006). The American bullfrog has been introduced widely outside its native range and is known to negatively affect many native species throughout North America (e.g. Snow & Witmer, 2010). Bullfrogs can reduce the overall abundance of aquatic invertebrates (e.g. Lawler et al., 1999), and diet analyses suggest that they target larger prey items such as crayfish, dragonfly nymphs, beetles and true bugs (Tyler & Hoestenbach, 1979; Werner et al., 1995; Balfour & Morey, 1999; Hirai, 2004). Previous research has also shown that native prey suffer heavy predation because they lack effective anti-predator responses to novel predators (Sih et al., 2010) like bullfrogs. In the MSI, both stock ponds and stream pools can also support other non-native vertebrates, including Western mosquitofish (*Gambusia affinis*). Mosquitofish, although less common than bullfrogs in stock ponds, are known to consume many small- and medium-sized invertebrate taxa, including mosquitos, amphipods, and true bugs (Linden & Cech, 1990; Peck & Walton, 2008).

Bullfrogs outside their native range are known to be detrimental to many vertebrate species (Snow & Witmer, 2010), including threatened amphibian species in the MSI (Rosen & Schwalbe, 1995; Jones & Timmons, 2010). Due to these potentially deleterious effects on native amphibian communities, wildlife agencies have engaged in an active bullfrog removal program in a portion of the MSI, in both novel and natural aquatic habitats. Though removal efforts have progressed for over a decade, little is currently known about the effect of non-native bullfrogs – or their removal – on the aquatic invertebrate communities in the MSI. Understanding how the presence of non-native predators affects aquatic invertebrate communities may also shed light on the indirect pathways through which these predators affect native amphibians, since aquatic invertebrates can serve as prey, competitors, and even predators of native amphibians (e.g., Werner & Anholt, 1996; Toledo, 2005).

Plate 4.1 *Lithobates catesbeiana* (American bullfrog) in the Huachuca Mountains. Photo credit: Meryl Mims.

Plate 4.2 American bullfrog removal (sniper) from a stock pond in the Huachuca Mountains. Photo credit: Julian Olden.

This study aims to explore links between two important factors – a novel perennial habitat and the presence of non-native predators – and the community composition of aquatic invertebrates in the MSI. First, we compare community composition (taxonomic and functional) of aquatic invertebrates in naturally occurring perennial stream pools with those from novel stock ponds. Although stream pools and stock ponds differ in disturbance regime and size, we hypothesized substantial overlap in community composition between these habitats due to the facts that both are perennial and support standing water habitat. However, we also expected to see some differentiation in trait composition. We hypothesized that traits such as multivoltinism and high dispersal ability may be favored in stream pool habitats as these traits are adaptations to more intense disturbance regimes. Second, we compared community composition, abundance, and richness of invertebrate communities in stock ponds with and without non-native vertebrate predators (bullfrogs and mosquitofish). Because bullfrogs and mosquitofish are known to predate on a variety of aquatic invertebrates, we expected to see reduced invertebrate abundance and richness associated with the presence of non-native predators. Finally, given the potential for selective predation by bullfrogs on larger taxa (e.g. Coleoptera and Hemiptera) and taxa without anti-predator behavior or morphology, we hypothesized that bullfrog presence may be correlated with a reduced abundance of larger taxa and taxa without predator defenses. By exploring patterns and links between these two interacting factors – a novel habitat and its associated non-native predators – and their correlations with aquatic invertebrate communities, we provide the first insight into the potential of stock ponds to serve as surrogate habitat for this diverse regional assemblage of aquatic invertebrates.

4.2 Methods

Our study sites were located in the Huachuca, Dragoon, and Galiuro Mountains of southeastern Arizona, USA (Figure 4.1) and included bedrock-bound stream pools in all three mountain ranges and stock ponds in the Huachuca Mountains. We sampled invertebrates in three stream pools: Garden Canyon (Huachuca Mountains), West Stronghold Canyon (Dragoon Mountains), and Ash Canyon (Galiuro Mountains) (Figure 4.1B). Stream pools occurred at similar elevations to one another and had similar habitat characteristics (e.g. bedrock substrate, open canopies) and similar surface areas (see 4.7 Appendix A). All study sites were perennial.

Stock pond sampling sites were located in the Huachuca Mountains and included three bullfrog removal ponds (Quote, Upper Mesa, and Campini Ponds, “removal ponds”), two ponds with bullfrogs present at time of sampling (Shell and Cholla Ponds, “BF ponds”), and one pond with bullfrogs and mosquitofish present at the time of sampling (Canelo Pond, “BF+M pond”) (Figure 4.1C). Criteria for pond selection included similar surface area and elevation (see 4.7 Appendix A). Spatial locations of removal ponds were restricted to within the 9.7 km bullfrog-removal radius from Parker Canyon Lake, and invaded ponds were located 2.5 - 3.1 km outside the radius (Figure 4.1C). Bullfrogs were collected from removal ponds by the Arizona Game and Fish Department in cooperation with the United States Forest Service between 2008 and 2012 and were bullfrog-free for 6 - 14 months prior to sampling (see 4.7 Appendix A). This post-removal time period was sufficient to assume adequate opportunities for adult insect recolonization of ponds, although differences among ponds may influence community structure.

Removal ponds were bullfrog-free during sampling with the exception of Quote Pond where a single male bullfrog was observed at the pond 14 days before sampling (J. Hale, personal observation). Quote Pond was previously bullfrog-free for a maximum of 6 months prior to sampling, depending on when the bullfrog migrated into the pond. Despite extensive efforts, the bullfrog was not confirmed killed or removed and was assumed present at the time of sampling (S. Kerr, personal communication). However, the intermittent presence of a single bullfrog over a short time period (weeks) is likely ecologically distinct from consistent bullfrog presence in greater numbers over multiple years, as were observed in invaded ponds. In addition, the abiotic and biotic characteristics of Quote Pond were more similar to other study sites than any alternatives; thus, we retained it as a removal pond sampling site. Upper Mesa Pond was bullfrog-free for approximately 14 months prior to sampling, and Campini Pond was bullfrog free for approximately 8 months prior to sampling. All stock ponds had salamanders (*Ambystoma* sp.) present at the time of sampling.

4.2.1 Field sampling and sample processing

Stream pools were sampled during the early summer prior to the wet season (June 2011) when streams had no flow and approximated lentic habitat. Streams were sampled early in the summer to ensure that sampling occurred before flash floods could reduce invertebrate abundance and richness. We vigorously swept a D-frame net with a 500 µm mesh through the water, above all substrates, and on the surface of the water for 10 s m^{-2} of stream pool surface area. This sampling effort was designed to maximize species detection without negatively impacting populations in isolated stream pools (see Bogan & Lytle, 2007). We measured water temperature (Traceable ® thermometer, Fisher Scientific Inc., U.S.A.), pH (indicator paper, Type CF Cat. No. 2614991, Whatman International Ltd.®, Maidstone, England, UK), and dissolved oxygen (CHEMets® Kit, Dissolved Oxygen K-7512, CHEMetrics Inc., Midland, VA, USA) before sampling each stream pool.

Stock ponds were sampled during the summer wet season (August 2012) when lentic invertebrate abundance and richness are thought to be highest. We conducted a visual search for bullfrogs prior to sampling using binoculars, visually estimated the number of bullfrog juveniles and adults, and noted the presence of egg masses and tadpoles. For invertebrates, we sampled a shoreline distance of 5 m in each quadrant for a total of 20 m per pond, using a habitat-targeted composite method to maximize aquatic invertebrate species detection. Using the same D-frame net as described above, we swept each 5 m stretch (approximately 1 m from the shore) for 60 seconds, starting with the top of the water column to capture fast swimming invertebrates and then sweeping towards the bottom (depth: 0.3 - 0.6 m). We pooled all quadrant samples for each stock pond. We measured temperature, pH, and dissolved oxygen levels at a depth of 0.3 m after sampling in each quadrant using the same equipment as noted above. Although stream pools and stock ponds were sampled in different years (2011 and 2012, respectively), annual variation in early summer lotic invertebrate communities is generally very low (Bogan, 2012). In addition, both temperature and precipitation were similar in 2011 and 2012 (annual mean maximum temperature for Sierra Vista, Arizona in 2011 and 2012 = 23.3 and 25.6 °C, respectively; annual total rainfall in 2011 and 2012 = 87.73 and 93.75 centimeters, respectively) (National Climatic Data Center). Thus, we predicted lotic invertebrate communities to be equivalent across years and therefore comparable to stock ponds that were sampled one year later. Given the density of bullfrog-free stock ponds in the study region, we assume a strong source pool.

Figure. 4.1 (A) Study region. (B) Nine study sites, including 3 natural stream pools (open squares: ASHC = Ash Canyon, WEST = Western Stronghold, GARD = Garden Canyon), 3 removal ponds (light grey inverted triangles: CAMP = Campini Pond, UPME = Upper Mesa Pond, QUOT = Quote Pond), and 3 invaded ponds (2 bullfrog ponds (BF), dark grey triangles: CHOL = Cholla Pond, SHEL = Shell Pond; 1 bullfrog and mosquitofish pond (BF+M), black triangle: CANE = Canelo Pond). (C) Ponds (n = 26) and stream segments (n = 5) that have been targeted for removal including 3 removal ponds (light grey triangles). Number of years each non-study pond was targeted for removal indicated by circle size and color: light grey, small circles = 1 or 2 years of removal (n = 12); medium grey, medium circles = 3 years of removal (n = 8); dark grey, large circles = 4 or 5 years of removal (n = 4). Dashed line indicates 9.7 km bullfrog-removal radius around Parker Canyon Lake.

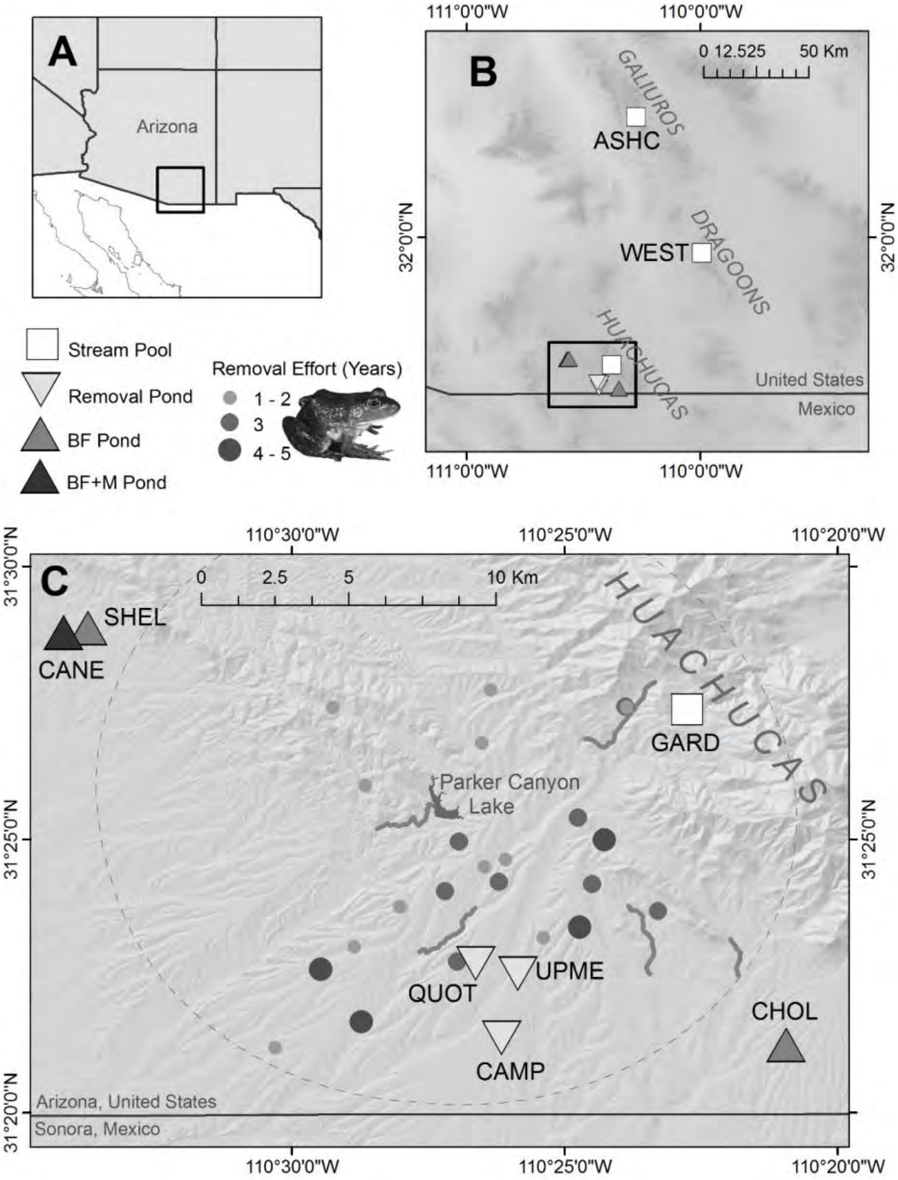


Plate 4.3 Invertebrate community sampling, Huachuca Mountains. Photo credit: Meryl Mims.

Plate 4.4 Invertebrate community sampling, Fort Huachuca. Photo credit: Meryl Mims.

All pond and stream pool samples were preserved in 95% ethanol and processed in accordance with U.S. EPA Benthic Macroinvertebrate Sampling Protocols (Barbour et al., 1999). We identified a subsample of at least 500 invertebrates from each stock pond sample. Campini Pond was used to create a collector's curve (Colwell & Coddington, 1994) and verify that *ca.* 500 individuals were sufficient to represent the invertebrate community. All individuals were identified in stream pool samples. Invertebrates were identified to the finest taxonomic unit practical (species, genus, or family) using Merritt, Cummins, and Berg (2008) for insects and Thorp and Covich (2001) for non-insects. Additionally, adult Hemiptera and Coleoptera were identified to species when possible using various regional and genus- or family-specific keys.

4.2.2 Trait characterization of invertebrate taxa

We collected trait data on most invertebrate taxa from peer-reviewed literature (e.g. Poff et al., 2006; Vieira, 2006; United States EPA GCRP, 2012) and used a regional trait database (see Schiever et al., in press) built from over 50 natural history publications for beetle and true bug species not currently listed in published trait databases (publications available from the authors upon request). We compared invertebrate functional composition using a database of five traits, including defense (anti-predator behavior and morphology), predatory habits, maximum body length, maximum dispersal distance, and voltinism. Maximum body length was assigned to taxa based on maximum adult body length unless data were available for larval stages. We assigned binary scores for physical or chemical defense (1 = present, 0 = absent) and predatory habits (1 = predator, 0 = not predator). We assigned ordinal scores for body length (1 = small, < 9 mm; 2 = medium, 9 - 16 mm; 3 = large, > 16 mm), maximum dispersal distance (1 = low, < 1 km; 2 = medium, 1 - 10 km; 3 = high, 10 - 100 km; 4 = very high, > 100 km), and voltinism (1 = univoltine, one brood per year; 2 = multivoltine, > one brood per year). We assigned trait values at the finest taxonomic level possible.

4.2.3 Statistical analyses

We used a Student's t-test to determine whether habitat variables (temperature, pH, dissolved oxygen, and surface area) in stock ponds co-varied with presence of non-native predators (Zar, 2010). When the assumption of normality was not met, we used a Mann-Whitney U test (Zar, 2010). We also used a Kruskal-Wallis test to determine if temperature, pH, dissolved oxygen, elevation, and surface area varied between invaded ponds, removal ponds, and stream pools (Zar, 2010). We used a Mann-Whitney U test to determine if invertebrate abundance and richness differed between removal and invaded ponds. As the abundance of non-native predators in stock ponds varied by several orders of magnitude, we also used a qualitative assessment to compare invertebrate abundance and richness in stock ponds in relation to the number of non-native predators (bullfrogs and mosquitofish) at each pond. Aquatic invertebrate taxon-specific analyses were determined by calculating the delta value defined as the difference in the log absolute abundance of each taxon between removal and invaded ponds. Positive delta values indicate higher abundance of invertebrate taxa in invaded ponds, and negative delta values indicate higher abundance in removal ponds. An index of confidence (described in Table 4.1) was calculated to reflect number of ponds and types of ponds in which species were or were not observed. Delta values of species present in only one pond were not calculated due to low confidence.

Multivariate statistics were used to determine correlations between habitat type or non-native predator presence and aquatic invertebrate community composition. Only presence-absence data were used for comparisons of stream pools and stock ponds, as these habitats were sampled differently and varied in size. Due to sample size limitations and the high number of taxa across sites, taxonomic community composition was measured at family-level resolution. Site-by-taxa and taxa-by-trait matrices were multiplied to create a site-by-trait matrix for functional trait composition analysis. Categorical traits were represented as presence-absence for a given trait state. Trait values for taxa present at a given site were then averaged to get a proportional value (0 - 1) for each trait state per site. We calculated assemblage dissimilarity using Bray-Curtis distance and applied nonmetric multidimensional scaling (NMDS) to visualize relationships among community composition, habitat type, and non-native predator presence in ordination space. We also calculated correlation coefficients between the axes of the NMDS ordinations with the respective families (taxonomic) or traits (functional) to determine which original variables had the highest contribution to the ordination. Multivariate analyses were performed using R version 2.15.0 (The R Foundation for Statistical Computing, 2013) with the 'vegan' package, version 2.0-3 (Oksanen et al., 2012). Only taxa and traits with correlation coefficients of $r \geq |0.3|$ are presented.

4.3 Results

We recorded a total of 74 invertebrate taxa during our study (41 identified to species, 23 identified to genus, and 10 identified to family), including 32 Coleoptera, 20 Hemiptera, 7 Odonata, 6 Diptera, 1 Ephemeroptera, and 8 non-insect taxa. Thirty-two taxa were exclusive to stock ponds (43% of all taxa), 28 taxa were exclusive to stream pools (38% of all taxa), and 14 taxa (19% of all taxa) were found in both habitats. Of the 32 taxa exclusive to stock ponds, 8 (25%) were exclusive to removal ponds, 6 (19%) were exclusive to invaded ponds, and 18 (56%) were present in both removal and invaded ponds.

Stream pools were significantly smaller than invaded (Kruskal-Wallis test, $\chi^2 = 3.86$, $P = 0.05$) and removal ponds (Kruskal-Wallis test, $\chi^2 = 3.86$, $P = 0.05$), with invaded pond surface area ranging from 593 - 1698 m^2 (average = 868 m^2), removal pond surface area ranging from 594 to 1698 m^2 (average = 1078 m^2), and stream pool surface area ranging from 2.25 to 5 m^2 (average = 3.42 m^2). Stream pools were significantly higher elevation than removal ponds (Kruskal-Wallis test, $\chi^2 = 3.86$, $P = 0.05$), but not invaded ponds (Kruskal-Wallis test, $\chi^2 = 2.33$, $P = 0.13$), with invaded ponds ranging in elevation from 1550 - 1666 m, removal ponds ranging in elevation from 1493 to 1616 m, and stream pools ranging in elevation from 1646 - 1890 m (see 4.7 Appendix A). We found no significant difference in temperature (Kruskal-Wallis test, $\chi^2 = 2.22$, $P = 0.33$), dissolved oxygen (Kruskal-Wallis test, $\chi^2 = 2.58$, $P = 0.28$), or pH (Kruskal-Wallis test, $\chi^2 = 0.11$, $P = 0.95$) between stream pools, removal ponds, and invaded ponds. There were also no significant differences in temperature (Student's t-test, $t_4 = 0.64$, $P = 0.56$), dissolved oxygen (Student's t-test, $t_4 = 1.56$, $P = 0.19$), pH (Mann Whitney U test, $z = -1.33$, $P = 0.13$), elevation (Mann Whitney U test, $z = -1.09$, $P = 0.28$), or pond surface area (Mann Whitney U test, $z = -0.22$, $P = 0.83$) between removal and invaded ponds.

There was no significant difference in invertebrate abundance (Mann Whitney U test, $z = -1.09$, $P = 0.28$) or richness (Mann Whitney U test, $z = -0.66$, $P = 0.51$) between removal and invaded ponds. However, a qualitative assessment revealed that invertebrate abundance and richness were lowest at Canelo Pond, which contained > 100,000 juvenile bullfrogs, bullfrog tadpoles, and mosquitofish (Figure 4.2). We observed a 25-fold decrease in total invertebrate abundance and a 2-fold decrease in taxonomic richness when comparing removal ponds to Canelo Pond.

Taxon-specific analyses revealed the strongest negative associations with non-native predator presence for the following taxa: *Tropisternus lateralis* (Coleoptera, exclusive to removal ponds), *Chironomus* sp. (Diptera, delta = -85.44), *Buenoa scimitra* (Hemiptera, delta = -63.89), and *Graptocorixa* sp. (Hemiptera, delta = -62.54) (largest negative deltas) (Table 4.1). The strongest positive associations between non-native predator presence and invertebrate taxa included the following: *Pantala* sp. (Odonata, exclusive to invaded ponds), *Libellula* sp. (Odonata, delta = 755.07), *Haliplus* sp. (Coleoptera, delta = 483.29), and *Anax* sp. (Odonata, delta = 329.77) (largest positive deltas) (Table 4.1).

NMDS ordination of taxonomic composition converged on a stable, 2-D solution (stress = 9.15%, $P<0.001$) and indicated moderate separation of ponds and stream pools along axis 1 and marginal separation of removal and invaded ponds along axis 2 (Figure 4.3). Veliidae ($r = 0.60$), Nepidae ($r = 0.60$), and Culicidae ($r = 0.78$) were positively correlated with axis 1, whereas Coenagrionidae ($r = -0.31$) and Hydracarina ($r = -0.30$) were negatively correlated with axis 1. Physidae ($r = 0.39$) was positively correlated with axis 2, and Haliplidae ($r = -0.44$) was negatively correlated with axis 2.

NMDS ordination of community trait composition converged on a stable, 2-D solution (stress = 3.54%, $P<0.001$) (Figure 4.4). The functional ordination revealed only marginal separation between ponds and stream pools (Figure 4.4) with a tendency for stream pools to contain more species with very high dispersal abilities. The only trait above the correlation cutoff of $r \geq |0.3|$ was very high dispersal which was positively correlated with axis 1 ($r = 0.37$) but also had a weak negative correlation with axis 2 ($r = -0.18$). Overall we found less separation in functional ordination space compared to taxonomic ordination space (Figure 4.3). We saw no evidence for differentiation between removal and invaded ponds in the functional ordination.

Table 4.1 Taxon-specific analyses. Positive deltas indicate a higher abundance in invaded ponds and negative deltas indicate a higher abundance in removal ponds. Confidence was determined based on the number of ponds in which each taxon was present. Pairs of numbers represent the number of ponds in each category (invaded & removal) in which each taxa was found (High: 2 & 3, 3 & 3, or 3 & 0; Medium: 3 & 1, 2 & 2, or 2 & 0; Low: 1 & 2, or 1 & 1). Taxa present in only one pond were removed from this analysis due to low confidence in their associations with the presence of bullfrogs.

Taxon	**Log Absolute Abundance Delta**	**Confidence**
Tropisternus lateralis	Only in removal ponds	Medium
Chironomus sp.	-85.44	High
Buenoa scimitar	-63.89	Medium
Graptocorixa sp.	-62.54	Low
Caloparyphus sp.	-58.52	High
Belostoma flumineum	-36.30	Low
Hygrotus nubilis	-34.33	Medium
Berosus infuscatus/fraternus	-27.15	High
Laccophilus fasciatus	-24.21	High
Hydracarina	-23.22	High
Berosus larvae	-17.21	High
Berosus stylifer	-15.31	Medium
Laccophilus sp. Larvae	-8.02	High
Glossiphoniidae	2.02	High
Callibaetis sp.	4.91	High
Ranatra quadridentata	7.21	Low
Corixidae sp. Larvae	26.61	Low
Lestes sp.	74.65	Medium
Coenagrion/Enallagma	76.98	High
Haliplus sp. 2	150.25	Low
Buenoa sp. Larvae	210.30	Low
Laccophilus maculosus	270.04	Low
Anax sp.	329.77	Low
Haliplus sp. 1	483.29	Low
Libellula sp.	755.07	Low
Pantala sp.	Only in invaded ponds	Medium

Figure 4.2 (A) Invertebrate abundance (log-scale) and (B) taxanomic richness in relation to the estimated density of non-native predators in each pond in three categories of pond: Removal, BF Pond (bullfrog), and BF+M Pond (bullfrog + mosquitofish). Ponds are listed from lowest to highest qualitative assessment of non-native predator density, with the following estimated abundance classes: 0 bullfrogs (removal ponds: Campini, Upper Mesa, and Quote Ponds), ~5 bullfrogs (Shell Pond), ~50 bullfrogs (Cholla Pond), and > ~100,000s bullfrog juveniles, tadpoles, and mosquitofish (Canelo Pond). Actual invertebrate abundance shown above bars in A ($\bar{x}$ = mean, x = count).

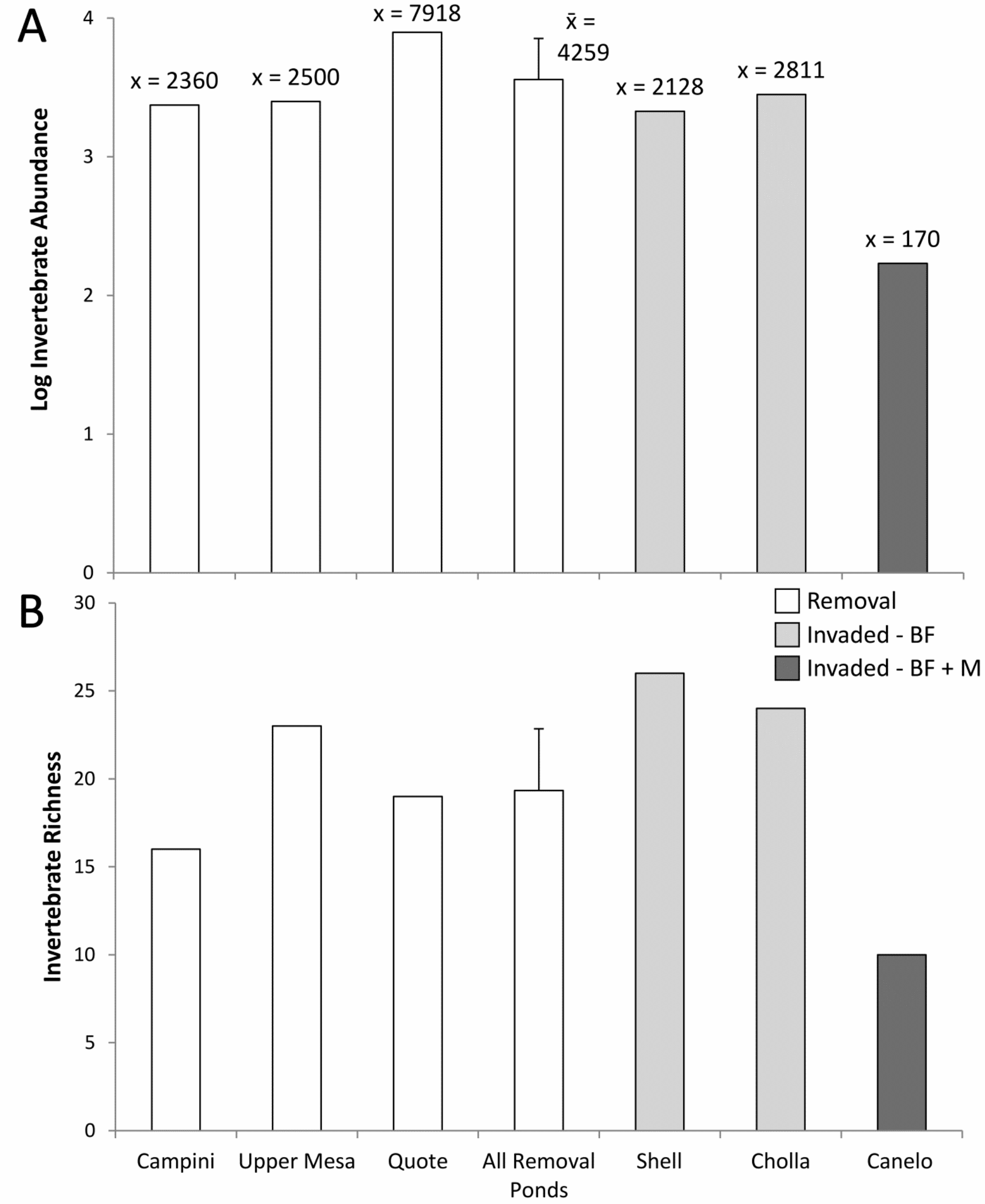


Figure 4.3 NMDS ordination of taxonomic community composition based on family presence-absence from 3 removal ponds (light grey inverted triangles), 3 invaded ponds (BF Pond: medium grey triangles; BF+M Pond: black triangle), and 3 natural stream pools (open squares). Lines form triangles to delineate hulls encompassing each habitat type. Pond abbreviations and locations included in Figure 4.1. Taxa contributing the most to axis 1 and 2 (r ≥ |0.3|) are denoted by text, and correlations are included in parentheses.

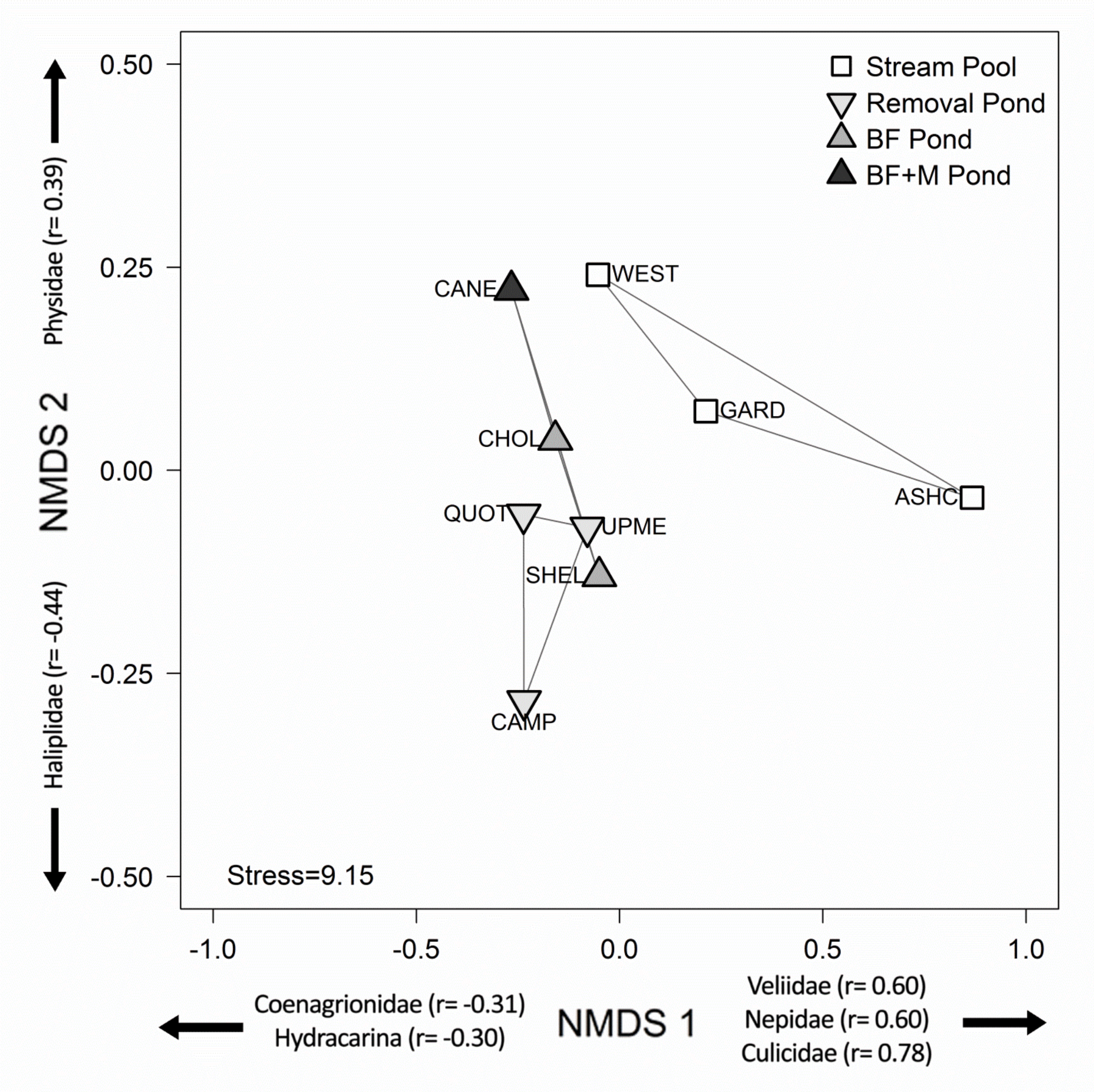
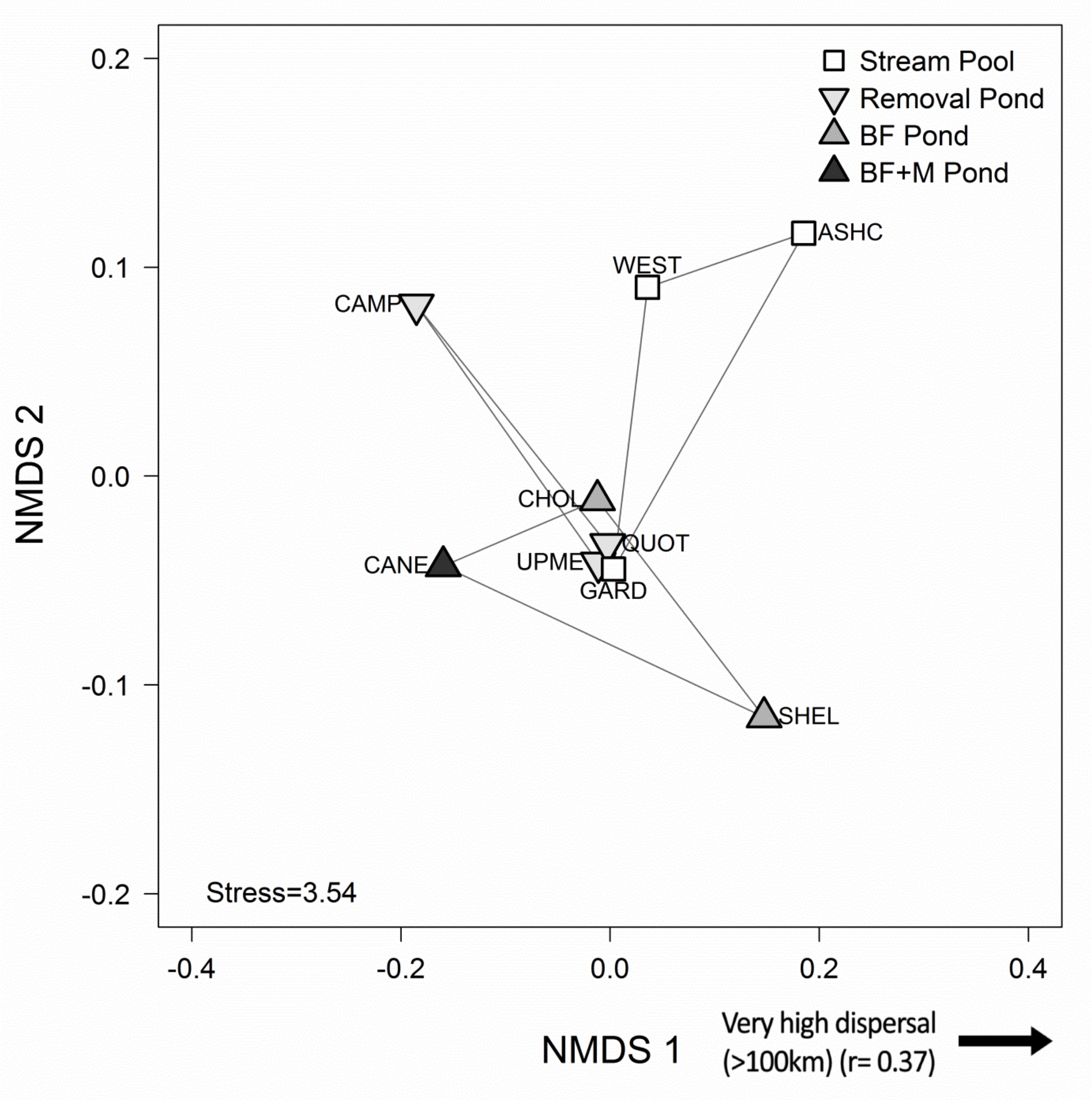


Figure 4.4 NMDS ordination of functional trait community composition based on family trait data from 3 removal ponds (light grey inverted triangles), 3 invaded ponds (BF Pond: medium grey triangles; BF+M Pond: black triangle), and 3 natural stream pools (open squares). Lines form triangles to delineate hulls encompassing each habitat type. Pond abbreviations and locations included in Figure 4.1. Traits contributing the most to axis 1 and 2 (r ≥ |0.3|) are denoted by text, and correlations are included in parentheses.



4.4 Discussion

Our study highlights the potential as well as the limitations of novel stock ponds to serve as surrogate habitat for aquatic invertebrates of the MSI. We found that when comparing invertebrate communities of stock ponds and stream pools – the two dominant perennial lentic habitat types in the region – some overlap in invertebrate community composition was observed. Functional (trait-based) similarity was considerable between stream pools and stock ponds. The only trait observed to differ markedly between these two habitats was very high dispersal ability (>100 km) which had higher representation in stream pools than stock ponds. Previous research has shown that dryland streams support species with traits that confer higher resilience to droughts and floods (e.g., Gray & Fisher, 1981; Bonada et al., 2007), including high dispersal ability. This may be because high dispersal ability is an important factor in the recolonization of stream pool habitats after high flow events. We found no evidence of an association between multivoltinism or predatory habits and habitat type as we predicted. This may be due to our limited ability to detect a relationship using presence-absence data with a small sample size.

Despite high functional similarity, taxonomic similarity between stream pools and stock ponds was lower. A large majority (81%) of invertebrate taxa were exclusive to either stock ponds or stream pools (43% exclusive to stock ponds, 38% exclusive to stream pools), indicating that stock ponds may support a sizable portion of the region's aquatic invertebrate diversity. This result is consistent with a previous study (Bogan et al., 2013), and may be explained by a number of potential mechanisms. First, the disturbance regimes of these two habitats differ markedly and may exclude poorly adapted taxa from one habitat type or the other. Second, the sizes of these habitats differ substantially. Habitat size is known to influence species richness in this region (Bogan et al., 2013; Section 2), with larger habitats supporting different and more rich communities. Third, stock ponds are visited by cattle and other livestock, which may contribute to increased nutrient levels and water turbidity. Previous research has shown that the composition of invertebrate assemblages in streams is correlated with environmental factors associated with disturbance caused by livestock grazing and trampling (Herbst et al. 2012). Given the narrow scope and correlative nature of our study, we cannot isolate particular drivers of taxonomic differentiation between habitat types or state with complete certainty what proportion of the region's invertebrate taxa are excluded from stock ponds. However, our observations suggest that although stock ponds may be an important surrogate habitat for some aquatic invertebrate taxa in the region and for a wide range of invertebrate functional diversity, these ponds may not be utilized by other taxa. Thus, relying on stock ponds alone as a surrogate habitat for perennial stream pools will not be a sufficient strategy to conserve all aquatic invertebrate taxa in the region.

Although stock ponds do serve as important surrogate habitat for many species in dryland environments (Peltzer et al 2006), novel aquatic habitats like stock ponds can also be home to many non-native species (Johnson et al. 2008; Letnic et al., 2014). Our examination of correlations between the presence of non-native vertebrate predators (bullfrogs and mosquitofish) and the richness, abundance, and community composition of aquatic invertebrate communities of stock ponds revealed some interesting patterns. First, we found that invertebrate richness and abundance did not differ markedly between two invaded ponds (with bullfrogs) and the three removal ponds (no bullfrogs or mosquitofish). Although we did not explicitly quantify bullfrog density, qualitative assessments indicated that bullfrog densities at the two ponds invaded by bullfrogs alone were likely relatively low (5-50 juveniles or adults; no tadpoles present). It is possible that with low densities of bullfrogs and with so few sample sites, evaluating the effect of bullfrogs on invertebrate richness and/or abundance is simply not possible through correlation only.

Despite this limitation, we did see substantially lower invertebrate abundance and richness at one pond with bullfrogs and mosquitofish (Canelo Pond) as compared to all other sites. Although this result should be interpreted with caution, we think is worth discussing for a few reasons. First, Canelo Pond did not differ markedly from other ponds with respect to abiotic and biotic characteristics other than the high abundances of bullfrogs and mosquitofish. Previous research has shown that large populations of invasive vertebrate predators can greatly reduce invertebrate abundance and richness (e.g. Lawler et al 1999; McCarthy et al., 2006; Fischer et al., 2013; Holitzki et al., 2013). Mosquitofish and bullfrogs in particular have been observed to affect abundance and richness of aquatic invertebrates in other systems, both individually and when present together (Lawler et al. 1999). Further studies, including experimental manipulations, are needed to be able to distinguish between the effect of bullfrogs and mosquitofish on aquatic invertebrates in the MSI. Identifying the density of bullfrogs and mosquitofish at which a detectible decline in the abundance or richness of aquatic invertebrates would be particularly useful for setting management objectives and thresholds for control of these non-native species.

Our observations of differences in the taxonomic composition of aquatic invertebrate communities in invaded ponds and removal ponds may be consistent with some selective predation by bullfrogs, and possibly mosquitofish. Bullfrogs are known to be effective predators of aquatic beetles (Balfour & Morey, 1999; Hirai, 2004), and this may explain why *Tropisternus lateralis* (Hydrophilidae), a conspicuous beetle species (medium size class, 9 - 16 mm), was absent from invaded ponds. Another conspicuous beetle species, *Berosus infuscatus/fraternus* (Hydrophilidae), was negatively associated with presence of non-native predators. However, small aquatic beetles (size class < 9 mm) were generally positively associated with presence of non-native predators, including *Haliplus* (Haliplidae) and *Laccophilus maculosus* (Dysticidae). We also found that the abundance of small midge larvae (Diptera: Chironomidae) was negatively associated with presence of non-native predators, with an average abundance that was orders of magnitude greater in removal ponds than in invaded ponds. Mosquitofish are capable of dramatically reducing the abundance of midge larvae (Linden & Cech, 1990); however, the negative relationship between non-native predator presence and midge abundance was consistent across all bullfrog-invaded ponds, not just the one pond with mosquitofish. Invaded ponds also supported lower abundances of small water mites (Hydracarina). It is possible that lower abundances of midges and mites in invaded ponds were the result of a bullfrog-induced trophic cascade in which bullfrog predation reduces the number of large invertebrate predators (e.g., water beetles) causing a release of mesopredators who, in turn, consume higher numbers of small prey items like midges and mites. However, the extent to which this relationship applies to other small invertebrate taxa is limited and possibly explains why we did not observe a general relationship between invertebrate size and non-native predator presence. Similarly, abundance of large invertebrate predators was only lower in invaded ponds for two of twelve large predator taxa. Further study is required to elucidate mechanisms driving taxon-specific effects of non-native predators.

We found little evidence that bullfrog presence caused changes in the functional composition of invertebrate communities. A qualitative assessment of invertebrate size class distributions across all ponds did not reveal any patterns of differential size class distribution, even in Canelo

Pond which had high abundances of predators known to have size-selective predation tendencies (4.9 Appendix C). This could be because our functional community composition analysis did not take into account ontogenetic shifts in size, where a species may fit into different size classes depending on its life stage (larvae, juvenile, or adult). For example, *Belostoma flumineum*, a species that was categorized as large (> 16 mm maximum body length), was found in our samples at smaller size classes as juveniles (J. Hale, personal observation), suggesting that assigning taxa to a size class, rather than measuring actual body length, may miss portions of the true size distribution of the invertebrate community. We also found little support for associations between non-native predator presence and anti-predatory behavior or morphology of aquatic invertebrates. One possible explanation for this result is that local species have no history of bullfrog or mosquitofish predation and hence not enough time has passed to allow for adaptation of anti-predator defenses. Previous research has shown that prey naivety and lack of anti-predator behavior may facilitate the establishment of non-native species and increase the impacts of non-native predators on native communities (e.g., Sih et al., 2010; Kuehne & Olden, 2012). It is also important to note that small sample sizes may have limited our ability to detect effects of bullfrog presence on the functional composition of aquatic invertebrate communities.

In conclusion, we found evidence that aquatic invertebrate communities in two perennial habitats – natural stream pools and manmade stock ponds – have considerable functional similarities despite modest taxonomic distinctiveness. This suggests that novel stock ponds may provide important surrogate habitat for at least some aquatic invertebrate taxa in the MSI, and that they also support a broad swath of the functional diversity found in natural stream pool habitats. As demand for water in the region grows, understanding the factors that influence whether novel habitats provide surrogate habitat for the native aquatic species will be an important part of conserving regional biodiversity. We also found that differences in richness and abundance of aquatic invertebrates in invaded versus removal ponds were minimal with the exception of one pond. However, data from that one pond suggests an association between relatively high densities of non-native vertebrate predators – bullfrogs and mosquitofish – and reduced richness and abundance of aquatic invertebrates. Our observations also suggest that ponds invaded by non-native predators may not suffer substantial reductions in invertebrate abundance and richness if non-native predators are kept at sufficiently low densities. However, more research is needed to determine which non-native vertebrate predators affect aquatic invertebrates and at what density those predators pose the greatest threat invertebrate biodiversity in the region. Continued efforts to understand and disentangle the effects of novel habitat and novel predators on native communities may help better direct management efforts in a region where tens of thousands of dollars are spent annually on the control of non-native species (S. Kerr, personal communication).

4.5 Acknowledgements

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4.6 Appendix A

Abiotic and biotic parameters in stock ponds (removal and invaded) and stream pools in the Madrean Sky Island region. Surface area, temperature, pH, and dissolved oxygen measurements for each stock pond are an average of measurements from each quadrant sampled. Years targeted indicates the years in which the Arizona Game and Fish Department attempted to remove bullfrogs, including years in which partial removal, complete removal (eradication), and no removal (no bullfrogs present) were achieved. Year of eradication indicates the year in which complete bullfrog eradication was completed and no bullfrogs have been observed at the site since, up until time of sampling, with the exception of Quote Pond that had a bullfrog present at time of sampling. Asterisk indicates presence of mosquitofish.

Sampling Site	Latitude	Longitude	Habitat Type	Surface Area (m^2)	Temp-erature (°C)	pH	Dissolved Oxygen (ppm)	Elevation (m)	Years targeted	Year of eradication
West Stronghold	31.935916	-109.994278	Stream Pool	3	29.0	7.0	2.0	1646	-	
Garden	31.456421	-110.379221	Stream Pool	5	14.0	7.3	6.0	1890	-	-
Ash Canyon	32.517008	-110.275438	Stream Pool	2	13.0	7.5	5.0	1798	-	-
Quote Pond	31.378760	-110.443785	Removal Pond	594	29.3	7.0	4.5	1493	2008, 2010, 2011	2011
Campini Pond	31.355943	-110.435582	Removal Pond	1698	28.6	7.0	8.0	1616	2012	2012
Upper Mesa Pond	31.375589	-110.430480	Removal Pond	962	27.8	8.5	11.0	1516	2008, 2011	2011
Cholla Pond	31.354079	-110.348949	Invaded Pond	855	29.2	8.0	12.0	1666	-	-
Canelo Pond*	31.479873	-110.569722	Invaded Pond	1698	25.8	7.0	6.0	1550	-	-
Shell Pond	31.481228	-110.562241	Invaded Pond	593	25.7	7.0	6.0	1563	-	-

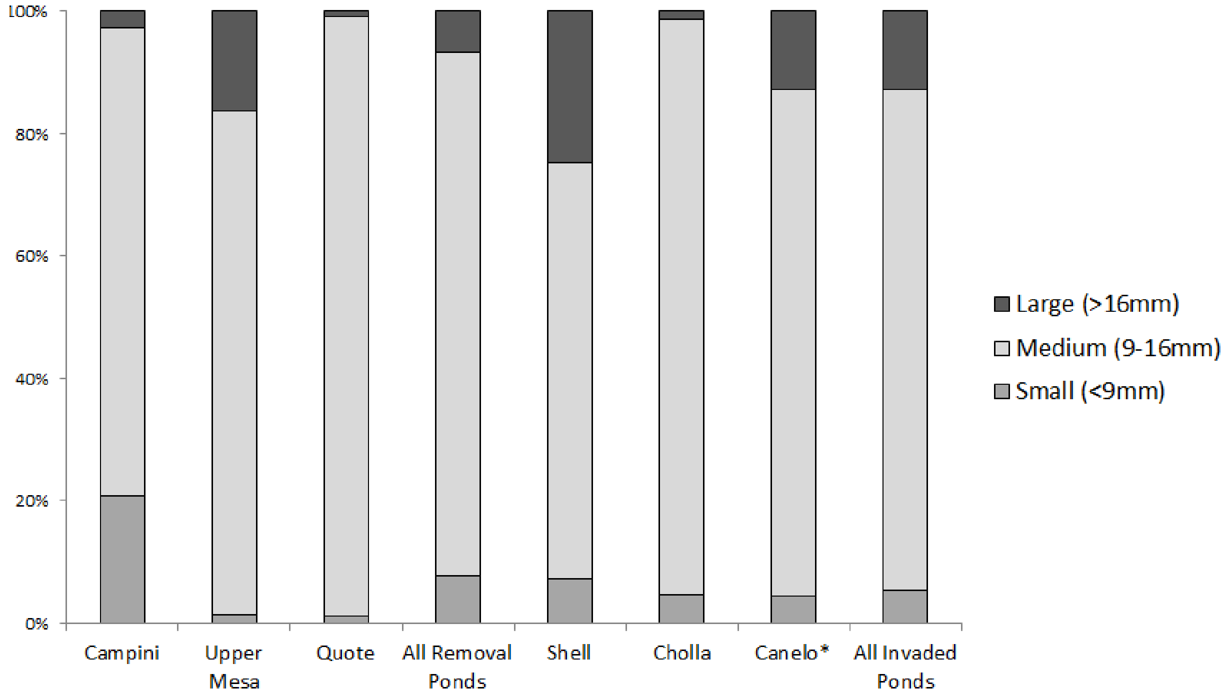
4.7 Appendix B

Invertebrate taxa identified from stock ponds and stream pools in the Madrean Sky Island region. Natural stream pool exclusive taxa were found solely in natural stream pools, man-made stock pond exclusive taxa were found solely in stock ponds, and both were found in both stock ponds and stream pools.

Natural Stream Pool Exclusive	Novel Stock Pond Exclusive	Both
Abedus herberti	*Anax* sp.	*Callibaetis* sp.
Agabus sp.	*Belostoma flumineum*	*Caloparyphus* sp.
Aquarius remigis	*Berosus infuscatus/fraternus*	Unidentified Chironomidae
Archilestes grandis	*Berosus* sp. larvae	Unidentified Culicidae
Berosus punctatissimus	*Berosus stylifer*	*Graptocorixa abdominalis*
Berosus rugulosus	Unidentified Bivalvia	*Haliplus* sp. 1
Berosus salvini	*Buenoa* sp. larvae	Unidentified Hydracarina
Buenoa arida	*Buenoa scimitra*	*Laccophilus fasciatus*
Buenoa arizonis	*Coenagrion/Enallagma*	*Microvelia* sp.
Ceratopogon sp.	*Corisella edulis*	Unidentified Oligochaeta
Cymbiodyta sp.	Corixidae larvae	Unidentified Ostrocoda
Desmopachria portmanni	Ephydridae larvae	Unidentified Physidae
Dineutus sublineatus	*Gerris* sp.	*Ranatra quadridentata*
Enochrus sp.	Unidentified Glossiphoniidae	*Rhantus gutticollis*
Gyrinus plicifer	*Graptocorixa* sp.	
Laccobius sp.	*Haliplus* sp. 2	
Laccophilus horni	*Hesperocorixa laevigata*	
Laccophilus pictus	*Hyalella* sp.	
Limonia sp.	Hydroporinae larvae	
Liodessus obscurellus	*Hygrotus* cf. *nubilis*	
Notonecta lobata	*Laccophilus* sp. larvae	
Paltothemis lineatipes	*Laccophilus maculosus*	
Rhantus atricolor	*Lestes* sp.	
Sanfillipodytes sp.	*Libellula* sp.	
Stictotarsus aequinoctialis	*Mesovelia mulsanti*	
Stictotarsus corvinus	*Morphocorixa lundbladi*	
Stictotarsus striatellus	*Notonecta indica*	
Thermonectus marmoratus	*Notonecta repanda*	
	Pantala sp.	
	Peltodytes callosus	
	Unidentified Planorbidae	
	Tropisternus lateralis	

4.8 Appendix C

Mesa, and Quote Ponds had bullfrogs removed by the Arizona Game and Fish Department. Shell, Cholla, and Canelo Ponds had bullfrogs present at time of sampling. Asterisk indicates presence of mosquitofish, *Gambusia affinis*, in Canelo Pond. Invertebrate taxa were categorized by maximum body size as either small (<9 mm), medium (9 - 16 mm), or large (> 16 mm).



5. Ecological strategies predict associations between aquatic and genetic connectivity for dryland amphibians

5.1 Introduction

Mounting concern for the survival of species in human-modified environments has strengthened interest in how species biology and landscape heterogeneity interact to structure populations. Population genetic structure is a fundamental consideration in applied conservation (Allendorf and Luikart 2007), which has led to greater research emphasis on integrating population genetics with emerging spatial methodologies to elucidate the landscape genetics of species (Manel et al. 2003). Substantial progress has been made in the last decade toward quantifying associations between landscape features and genetic connectivity of single species, and interest in explicitly testing for generalizations across multiple species is increasing (Manel and Holderegger 2013). Multispecies inference may be a promising way to identify emerging patterns of landscape influence on population connectivity across taxa and to reveal transferable relationships according to biological attributes such as dispersal ability (Richardson 2012) and life history (Bradbury et al. 2008, Hughes et al. 2013). Ecological strategies – the life history, biology, and behavior of a species – may provide a means to generalize associations between genetic connectivity and structural connectivity (i.e., the physical linkages between habitat patches via a landscape feature such as riparian networks or ridgelines: Taylor et al. 1993), or what is commonly considered “landscape connectivity”. The potential for generalized, multi-taxa relationships between genetic and landscape connectivity is important in light of limited management resources or logistical complications that render single species studies unfeasible for many species of conservation concern.

Aquatic habitat supports a range of obligate aquatic species; some species require perennial water for the duration of their life cycle, whereas others require water for only a short period to fulfill a portion of their life cycle. The water requirements, dispersal abilities, and other traits of aquatic species coupled with the distribution (spatial and temporal) of aquatic habitat may be major factors that influence the population structure of a diverse array of species including plants (Imbert and Lefèvre 2003, Nilsson et al. 2010), insects (Hughes et al. 2013, Robertson et al. 2014), fishes (Hughes et al. 2013), amphibians (Richardson 2012), and birds (Kingsford et al. 2004, Cadena et al. 2011). In arid and semi-arid (hereafter “dryland”) environments characterized by sparse, sometimes unpredictable, and often temporary aquatic habitat, the link between aquatic species ecology and aquatic habitat on the landscape in driving population structure may be more pronounced due to the arid terrestrial matrix between aquatic habitats (Finn et al. 2007, Phillipsen and Lytle 2013). Conversely, the need to reach temporally and spatially variable aquatic habitat may decouple the link between the aquatic habitat and population genetic structure for aquatic species with high dispersal ability (Chan and Zamudio 2009, Cadena et al. 2011).

Quantifying genetic connectivity among populations of aquatic animals – and determining how this is influenced by landscape factors such as hydrology – is critical for conservation planning now and in the future. Worldwide threats to water security for society and freshwater diversity require efficient and effective conservation planning (Vörösmarty et al. 2010). In recent decades, amphibians have declined globally as a result of major threats including habitat loss, disease, and non-native species (Stuart et al. 2004, Sodhi et al. 2008). These ubiquitous challenges for amphibians worldwide are also true of anurans (frogs and toads) in dryland environments. In the American Southwest, habitat loss is an ongoing challenge as limited perennial water is often appropriated for human use, and groundwater pumping results in continued reduction in surface water availability (Marshall et al. 2010, Jaeger et al. 2014). Current demand for and conflict over these resources foreshadows increasing demand and decreasing availability as climate change increases aridity in the region (Seager et al. 2007).

Anurans native to this region utilize a variety of life history and behavioral strategies to survive in a harsh, arid landscape. Some species are specialists that live in either perennial or ephemeral freshwater habitats, and others occupy niches intermediate to these two extremes. If these anurans have dispersal limitations that correspond with their habitat requirements, the patterns of population structure among these species may be as diverse as their ecological strategies related to water use.

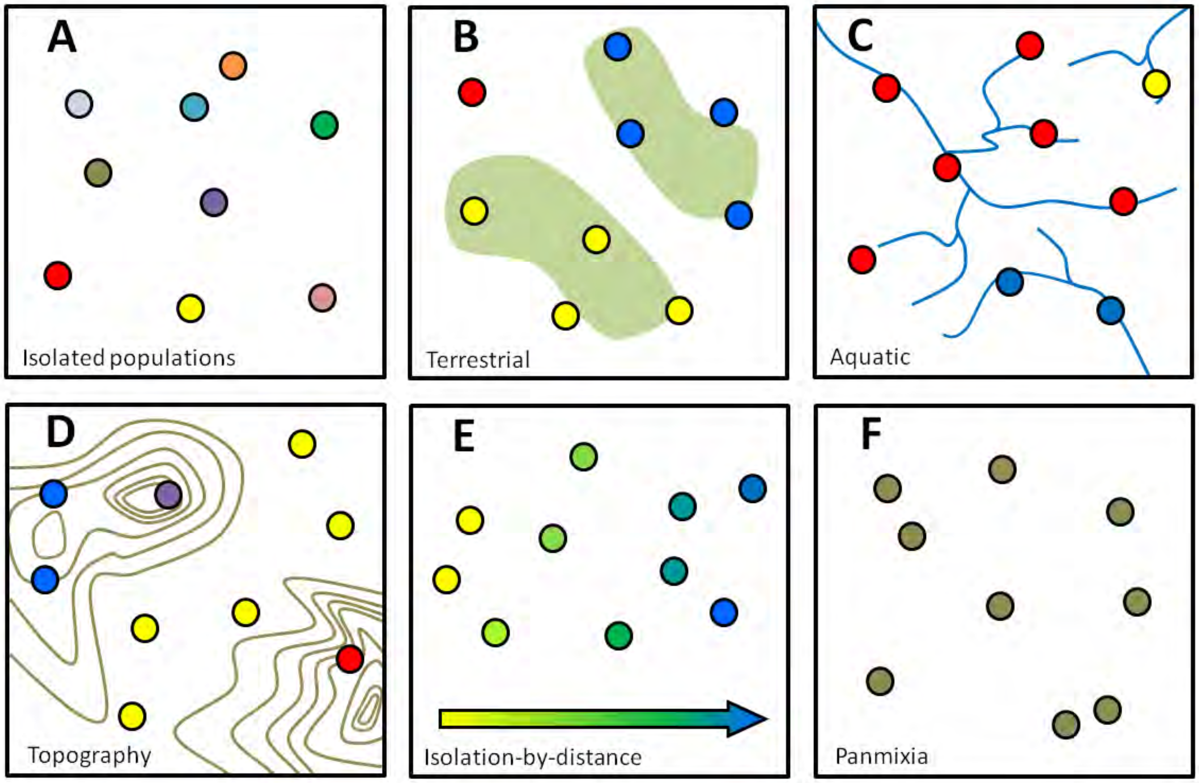
In this study, we examine the relationship between genetic and structural connectivity for three dryland anurans common to the American Southwest: the canyon treefrog (*Hyla arenicolor*), the red-spotted toad (*Anaxyrus punctatus*), and the Mexican spadefoot (*Spea multiplicata*). These species represent a range of water dependencies typical of dryland anurans and thus provide a unique opportunity to investigate whether ecological strategies – in this case defined by water requirements – can be used to generalize the associations between genetic and structural connectivity. Although canyon treefrogs have unique behavioral adaptations thought to minimize evaporative water loss, adults require frequent (possibly daily) access to water (Snyder and Hammerson 1993). The larval period is estimated to range between 6 and 11 weeks (Zweifel 1961, Stebbins 2003). Red-spotted toads are common near intermittent and ephemeral water sources in the Mojave and Sonoran deserts. They show evidence of some site fidelity, returning to similar stream sections and even burrows when displaced (Weintraub 1974). The larval period of red-spotted toads is estimated to last between 4 and 8 weeks (Tevis 1966, Brennan and Holycross 2006). Mexican spadefoots are ephemeral specialists that breed in temporary pools and ponds filled by summer monsoon thunderstorms. Vibrations from rainfall and/or thunder are thought to signal emergence of spadefoot toads that aestivate in burrows deep underground and emerge to breed in ephemeral pools (Dimmitt and Ruibal 1980). The larval period of Mexican spadefoots ranges between 2 to 4 weeks (Buchholz and Hayes 2000).

Our primary objective was to test whether water dependency – a defining element of aquatic species' ecological strategies – provides a means to generalize the likely mechanisms, and hence patterns, of anuran population structure. First, we hypothesized that genetic population connectivity was inversely related to water requirements, the primary driver of species ecology in an environment in which desiccation risk is a considerable threat for aquatic species (Table 5.1). We also expected that water availability explained a greater degree of population genetic structure (Figure 5.1C) as species-specific water requirements increased. Land cover, topography, and geographic distance were expected to have heterogeneous effects across species. We predicted that terrestrial resistance (canopy cover and urban land use) would be most important for canyon treefrogs (Figure 5.1B), the species with the highest desiccation risk for which permeability of the matrix between breeding sites may drive genetic connectivity.

Table 5.1 Genetic and landscape connectivity hypotheses. Hypothesized genetic connectivity models for each species (CT = canyon treefrog, RT = red-spotted toad, and MS = Mexican spadefoot) are indicated by "**X**". Hypotheses are visualized in Figure 5.1.

Hypotheses	**Species predictions**			**Description**	**Population genetics patterns**	**Landscape drivers and genetic connectivity correlation (+/-)**
	CT	RT	MS			
Isolated populations	**X**			High differentiation; no relationship with distance.	All populations distinct from one another; high population differentiation.	None: local processes (e.g., genetic drift) dominate.
Terrestrial	**X**			Canopy cover promotes genetic connectivity; development reduces connectivity.	Populations cluster within areas of high canopy cover; distinct populations in developed areas.	Canopy cover (+) and urban land use (-).
Aquatic	**X**	**X**		Connectivity correlated with hydrologic network (streams, ponds), precipitation.	Populations cluster along riparian networks and in areas with high precipitation.	Hydrologic network: streams and ponds (+); precipitation (+).
Topography		**X**	**X**	Slope (rise over run) negatively correlated with genetic connectivity.	Isolated populations in areas with steep terrain; high connectivity along flat ground.	Topography: slope (-).
Isolation-by-distance			**X**	Differentiation correlated with distance.	Population differentiation increases proportionally with distance.	Distance (+).
Panmixia			**X**	Low differentiation; no relationship with distance.	Little/no population differentiation.	None: migration/connectivity dominate.

Figure 5.1 Genetic connectivity hypotheses (see Table 5.1). Populations (circles) of the same color are genetically similar. Panels illustrate: isolated populations (A) with no landscape drivers of population differentiation; rather, local processes such as genetic drift drive genetic differentiation of populations. Terrestrial (B) describes genetic connectivity associated with low resistance land cover, such as areas with high canopy cover (shown in green). Aquatic (C) describes connectivity within riparian networks (shown as blue lines) or in areas with high precipitation. Topography (D) is characterized by high connectivity along flat ground (low slope) and high differentiation of populations separated by high slope, as shown with brown contour lines. Isolation-by-distance (E) describes an increase in genetic differentiation between populations as distance increases or along a cline (arrow). Panmixia (F) is characterized by genetically similar populations across the sampling range with no relationship to landscape.



We also expected that topographic resistance (slope) would limit gene flow for red-spotted toads and Mexican spadefoots, whereas canyon treefrogs are adept climbers and may disperse more easily across complex topography (Figure 5.1D). Alternatively, canyon treefrog populations may be so isolated and gene flow so low that genetic drift results in genetically isolated populations (Figure 5.1A). Finally, we predicted an isolation-by-distance pattern (Figure 5.1E) or panmixia pattern (Figure 5.1F) for Mexican spadefoots where gene flow is likely to be diffuse and relatively unimpeded by landscape factors.

5.2 Methods

The study region is the Madrean Sky Islands of southeastern Arizona (USA), characterized by many high mountain ranges separated by arid valley scrubland. The region's landscape is remarkably heterogeneous with large gradients in elevation, water permanence, precipitation, vegetation, and temperature. Our study focused on the Huachuca Mountains and surrounding mountain ranges and valleys, including the Santa Rita, Whetstone, Dragoon, and Mule Mountains. Summer monsoon rains and flash floods in our study region are seasonally predictable but spatially variable. Some areas may receive rain early in the season and stay wet during the entire monsoon while others may receive no rain all season. For these reasons, we used an opportunistic but spatially stratified sampling approach in order to maximize the chance of finding amphibians while attempting to balance the extent of our study, the density of sample locations, and the evenness of sampling locations on the landscape. Adult and larval amphibians were sampled during the spring and summer monsoon seasons of 2010, 2011, and 2012. A single toe clip (adult) or tail clip (larvae) was taken from each individual for DNA extraction and genotyping. Where possible, sampling sites were visited across multiple years in order to maximize the chance of sampling multiple families. Other sites consisted of multiple isolated pools within 1 km of each other to maximize the chance of sampling multiple families. Additional sampling details and effects of sampling strategy are reported in 5.7 Appendix A.

5.2.1 Genetic methods

Microsatellite marker information, genotyping details, marker screening procedures, and sibling removal procedures for larval samples are provided in 5.7 Appendix A. Briefly, population genetic diversity estimates of expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness (AR) were estimated with the program MSA 4.05 (Dieringer and Schlötterer 2003). We estimated effective population size (N_e) using the linkage disequilibrium method of Waples and Do (2008), as implemented in NeEstimator V2 (Do et al. 2014). Global genetic differentiation for each species was estimated using G'_{ST}, a standardized measure of genetic differentiation appropriate for multiple species comparisons (Hedrick 2005). Pairwise genetic distance (between each pair of sample sites) was calculated using D_{ps}, a method of measuring genetic differentiation based on proportion of shared alleles (Bowcock et al. 1994). Both G'_{ST} and D_{ps} were calculated with MSA 4.05. Individual-based hierarchical population structure was analyzed using the Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al. 2000). The most likely number of genetic clusters (K) for each species was determined using the delta-K method (Evanno et al. 2005). Genetic clustering methods are further described in 5.7 Appendix A, and genotype data are available through figshare (http://dx.doi.org/10.6084/m9.figshare.1205533).

Plate 5.1 A. Thunderstorm over Fort Huachuca, August 2010. B. *Spea multiplicata* (Mexican spadefoot). C. *Anaxyrus punctatus* (red-spotted toad). D. *Hyla arenicolor* (canyon treefrog). Photo credits: Meryl Mims

5.2.2 Landscape Genetics

Hypothesized landscape connectivity surfaces were built using CIRCUITSCAPE (McRae 2006). CIRCUITSCAPE uses circuit theory to simulate gene flow (i.e., "current") through a resistance surface in which landscape features hypothesized to promote gene flow are assigned low resistances, and landscape features hypothesized to inhibit gene flow are assigned high resistances. CIRCUITSCAPE allows gene flow across multiple pathways and reports pairwise summations of resistance between sampling locations. Modeling multiple pathways is appropriate for dryland anurans with high dispersal ability (Chan and Zamudio 2009). A geographic information system (ArcGIS 10.1, Environmental Systems Research Institute) was used to catalog and manipulate landscape data, and landscape resistance models, data, and sources are described in detail in 5.8 Appendix B. Nine landscape resistance surfaces in four broad structural connectivity categories were examined (Table 5.3). The first category was terrestrial and included three resistance surfaces: Canopy (resistance decreased with canopy cover), Urban (resistance increased with development), and LandCov (combination Canopy and Urban for which resistance was lowest with high canopy cover and highest for high development). The second category was aquatic and included three resistance surfaces: Stream (resistance was lowest for perennial/intermittent streams and ponds, moderate for ephemeral streams, and highest for areas with no aquatic habitat), PrecipET (resistance decreased as summer precipitation-evapotranspiration increased), and AvgWat (combination Stream and PrecipET for which resistance was lowest where both precipitation was high and aquatic habitat was available, and for which resistance was highest for dry areas with no aquatic habitat). The third category was topography and included Slope (resistance increased with slope). The fourth category was isolation-by-distance and included pairwise Euclidean distance between sampling locations (Euclidean) and one uniform, non-zero resistance surface (Null).

We evaluated relationships between pairwise genetic distance and pairwise landscape resistances using a mixed-effects modeling approach (van Strien et al. 2012). Through mixed-effects modeling, explanatory variables (pairwise landscape resistances) are treated as fixed effects, and sampling locations are treated as random effects to account for non-independent values in distance matrices (Yang 2004). Model fit was evaluated with the R^2_β statistic (Edwards et al. 2008) that compares a model with fixed and random effects (pairwise landscape distance or resistance and sampling location) to a null model with only the random effect (sampling location) and an intercept. Comparison of model performance metrics, such as p-values, allows for evaluating significant differences between models; however, no formal method of comparison has been developed or agreed upon for evaluating R^2_β values of different models. Therefore, we compared R^2_β values to one another directly as is common to-date in studies using this approach (van Strien et al. 2012). We also used multiple regression with distance matrices (MRDM) (Holzhauer et al. 2006, Balkenhol et al. 2009) as a complementary method to evaluate relationships between genetic and structural connectivity. MRDM methods are described in 5.8 Appendix B. All analyses were performed in R version 2.14.0 (R Development Core Team, 2012), using a modified version of lme4 (Bates et al. 2012) for mixed-effects modeling, PBKRTEST (Halekoh and Højsgaard 2012) for R^2_β calculation as described in van Strien et al. (2012), and ecodist (Goslee and Urban 2007) for MRDM.

Plate 5.2 Sampling amphibian larvae, Huachuca Mountains. Photo credit: Meryl Mims.

5.3 Results

We found support for the hypothesis that population structure (genetic differentiation) was correlated with species' water requirements. Global genetic differentiation was highest for canyon treefrogs ($G'_{\mathrm{ST}} = 0.57$), intermediate for red-spotted toads ($G'_{\mathrm{ST}} = 0.31$), and lowest for spadefoots ($G'_{\mathrm{ST}} = 0.09$). We found strong hierarchical clustering for canyon treefrogs (Figure 5.2A) with spatial clustering by mountain range (Figure 5.2D). Red-spotted toads had moderate hierarchical structure (Figure 5.2B) with complex spatial patterns of genetic connectivity (Figure 5.2E). Mexican spadefoots had little hierarchical structure (Figure 5.2C) with diffuse spatial clustering (Figure 5.2F). Delta-*K* results for all species and genetic clusters are included in 5.7 Appendix A. Sampling information and population genetic metrics are summarized in Table 5.2 and described in full in 5.7 Appendix A.

Landscape resistances and distances in three of four categories (terrestrial, aquatic and isolation-by-distance) were moderately to highly correlated for each species (5.8 Appendix B, Table 5.8.3). We evaluated correlated landscape resistances independently in a mixed-effects modeling framework to insure that collinear variables were not included in the same model. Mixed-effects models revealed a large difference in the strength of correlation between genetic and structural connectivity across species. R^2_β values for canyon treefrogs were more than double those for red-spotted toads and Mexican spadefoots (Table 5.3). Within species, R^2_β values for canyon treefrogs were highest for models in the terrestrial and aquatic categories, with AvgWater having the highest correlation across models ($R^2_\beta = 0.70$). For red-spotted toads, Urban had the strongest correlation with genetic connectivity ($R^2_\beta = 0.33$). However, R^2_β for other terrestrial resistances (Canopy and LandCov) were lower than overall aquatic resistances, and Urban was highly correlated ($r > 0.9$) with Null (isolation-by-distance category). Thus, the R^2_β for urban resistance may reflect the effects of null resistance rather than a true terrestrial effect. Finally, Mexican spadefoot genetic connectivity was most closely related to Euclidean distance in the isolation-by-distance category ($R^2_\beta = 0.31$).

We also examined relationships between genetic and structural connectivity within major genetic clusters for each species. For canyon treefrogs, we examined two nested clusters: the western cluster (CT-W) and the Huachuca Mountains cluster (CT-H) (Figure 5.2D). Results for the two nested clusters did not differ greatly from results for all canyon treefrog sites, though the support for isolation-by-distance increased marginally at finer scales (5.8 Appendix B, Table 5.8.4). The same was true for the only major genetic cluster of Mexican spadefoots (MS-E, Figure 5.2F) in which results differed only slightly by scale (5.8 Appendix B, Table 5.8.4). However, the two major genetic clusters for red-spotted toads were spatially complex, with one spatially clustered group (the Huachuca Mountains group, RT-H) and a larger, spatially diffuse group (the northern group, RT-N) (Figure 5.2E). Hierarchical structure was not found in RT-H and was moderate for RT-N. We found strong support for a relationship between genetic structure and topographic resistance for RT-N. Conversely, genetic structure of populations in the RT-H group had no relationship with landscape structure, indicating panmictic population structure (Table 5.3).

Figure 5.2 Spatial and individual hierarchical population structure for each species. Individual-based STRUCTURE results are shown in Panels A, B, and C. Each vertical bar represents one individual. Colors indicate the most likely genetic cluster assignments. Black vertical bars denote individuals from the same sampling locations. Each cluster was hierarchically analyzed for nested structure; nested structure results are shown directly below the original cluster. Hierarchical analyses were repeated until terminal clusters (K = 1) were reached. Panels D, E, and F: nested outlines group sampling locations into genetic clusters shown in panels A, B, and C, with study extent shown in black box of inset map (Panel E). Outline color corresponds to population clusters. Red to yellow shading in Panel F represents the transition between two clusters shown in Panel C (Mexican spadefoots). Major genetic clusters include: canyon treefrog-west (CT-W) and canyon treefrog - Huachuca Mountains (CT-H) (Panel D); red-spotted toad-north (RT-N) and red-spotted toad - Huachuca Mountains (RT-H) (Panel E); Mexican spadefoot - east (MS-E) (Panel F). Figure on next page.

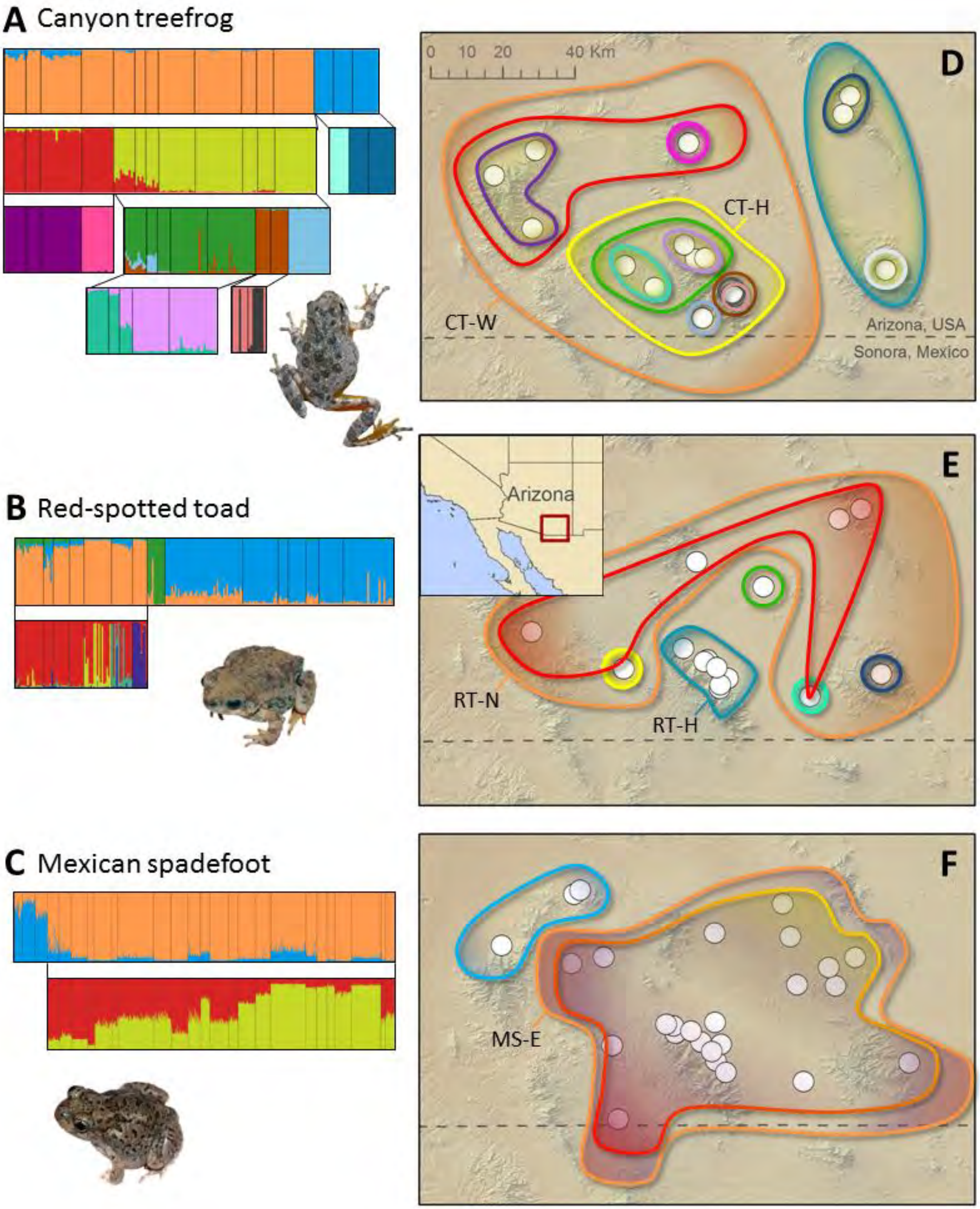

A Canyon treefrog
B Red-spotted toad
C Mexican spadefoot
D
0 10 20 40 Km
CT-H
CT-W
Arizona, USA
Sonora, Mexico
E
Arizona
RT-N
RT-H
F
MS-E

Table 5.2 Sample size, microsatellite information, and population genetics metrics for each species. Sample sizes are given as number of sampling locations (*N*), total individuals (*n*), *n* with all but one full sibling (sib) from each family removed, *n* with reconstructed parents included (described in 5.7 Appendix A), and average *n* ($\bar{n}$) per sampling location with all siblings, with only one full sibling, and with reconstructed parents. Microsatellite information includes loci count and average allelic richness (AR, averaged across populations, and adjusted for smallest sample size). Population genetic metrics include expected heterozygosity (H_E) and observed heterozygosity (H_O) calculated as averages of all populations, with reconstructed parents; median effective population size across populations ($\overline{N_e}$); and overall population differentiation (G'_{ST}). More information, including population genetic metrics by population and locus, is included in 5.7 Appendix A.

Species	**Sample size**				**Average *n***			**Loci**		**Population genetics**			
	N	*n*	*n*, no sibs	*n,* with parents	$\bar{n}$	$\bar{n}$, no sibs	$\bar{n}$, with parents	Count	AR	H_E	H_O	$\overline{N_e}$	G'_{ST}
Canyon treefrog	15	575	175	202	42.2	11.7	13.5	12	5.09	0.73	0.74	30.7	0.57
Red-spotted toad	15	660	233	252	41.1	15.9	16.8	14	5.29	0.76	0.74	83.0	0.31
Mexican spadefoot	26	1163	781	784	38.3	30.0	30.2	8	5.26	0.67	0.67	5951.7	0.09

Table 5.3 Mixed-effects modeling results for each species (CT = canyon treefrog, RT = red-spotted toad, MS = Mexican spadefoot) and major genetic clusters of red-spotted toads (RT-N = north, RT-H = Huachuca Mountains; Figure 5.2). Spatial data are described in full in 5.8 Appendix B. Single explanatory variable models are included above the line, and couplet models are below. Top R^2_β values are highlighted in bold font. Isolated populations and panmixia hypothesize no landscape effect, indicated by poor model performance across all other models. A dash indicates no support for isolated populations and panmixia. All R^2_β correlation coefficients are positive except those underlined. Slope is negatively correlated with D_{PS} for CT in all couplet models, and Euclidean is negatively correlated with D_{PS} for RT-N in the IBT + T couplet model.

Hypotheses	**Resistance layers/ distance**	**R^2_β, mixed-effects models**				
Species		**CT**	**RT**	**MS**	**RT-N**	**RT-H**
Isolated populations	*N/A*	-	-	-	-	-
Terrestrial (TE)	Canopy	0.68	0.26	0.28	0.06	0.02
	Urban	0.68	**0.33**	0.27	0.06	<0.01
	LandCov	0.69	0.27	0.28	0.07	<0.01
Aquatic (A)	Stream	0.67	0.32	0.28	0.10	<0.01
	PrecipET	0.68	0.30	0.28	0.06	<0.01
	AvgWater	**0.70**	0.31	0.28	0.09	<0.01
Topography (T)	Slope	0.35	0.27	0.29	**0.48**	0.01
Isolation-by-distance (IBD)	Euclidean	0.57	0.14	**0.31**	0.02	<0.01
	Null	0.67	0.30	0.28	0.06	<0.01
Panmixia	*N/A*	-	-	-	-	**Yes**
TE + T	Best of TE + Slope	0.78	0.58	0.49	0.62	0.04
A + T	Best of A + Slope	**0.85**	**0.61**	**0.50**	**0.66**	0.03
IBD + T	Euclidean + Slope	0.76	0.32	0.39	0.37	0.02
	Null + Slope	**0.85**	0.56	0.48	0.36	0.03

Combining topographic resistance with other landscape resistances provides a context for interpreting how the physical template (topography) and landscape features (e.g., streams, canopy cover, and urbanization) interact and affect genetic connectivity. Topographic resistance (slope) was not correlated with other landscape resistance surfaces, and for that reason we evaluated couplet models combining topography with landscape resistances in other categories (terrestrial, aquatic, and isolation-by-distance). R^2_β values for couplet models were higher than single resistance models across all species, and R^2_β values increased by the widest margin for red-spotted toads and Mexican spadefoots (Table 5.3). Perhaps most notably, the best couplet model for each species and within the northern red-spotted toad (RT-N) genetic cluster included both topographic and aquatic resistances. Canyon treefrogs had equivalent and high R^2_β values (0.85) for the couplet models combining topography + aquatic resistance and topography + isolation-by-distance. However, rather than the expected negative correlation with genetic connectivity, topographic resistance was positively correlated with genetic connectivity for canyon treefrogs. This was also true for the two finer-scale genetic clusters examined for canyon treefrogs (Appendix B, Table 5.8.4). This may indicate gene flow across high slope areas, such as mountain ranges and ridgelines, rather than across flat landscapes. Urbanization resistances were highly correlated with uniform resistance values for both genetic clusters of canyon treefrogs due to very low urban development within the spatial extent of those clusters.

Finally, MRDM results for each species and their genetic clusters largely corroborated mixed-effects modeling results, although there were some differences. Canyon treefrog genetic distances were most highly correlated with uniform landscape resistance, with the highest support for uniform landscape resistance (isolation-by-distance hypothesis) model. When accounting for high collinearity between the uniform and aquatic resistances within genetic clusters of canyon treefrogs (*vif* > or near to 10), conservative interpretation of the MRDM results also supports the uniform resistance-only model for both genetic clusters. For red-spotted toads (both overall and for the northern group), the best model included low resistance along river networks and ponds, topographic resistance, and uniform resistance. However, the correlation with uniform resistance was negative, indicating no support for the isolation-by-distance hypothesis. Finally, we found support for the aquatic hypothesis for Mexican spadefoots at multiple spatial scales. Results are summarized in 5.8 Appendix B.

5.4 Discussion

Characterizing the influences of species ecology and the landscape on genetic connectivity among populations contributes to fundamental ecological and evolutionary knowledge and is an important part of successful conservation of species (Allendorf and Luikart 2007, Manel and Holderegger 2013). Our findings highlight the utility and potential of species' ecological traits – in our case water dependency – in characterizing relationships between genetic and structural (landscape) connectivity across taxa. We found a positive relationship between population differentiation and increasing water requirements across three aquatic species. When considered independently, landscape drivers of genetic connectivity were largely predicted by hypothesized models built upon knowledge of water requirements, a defining characteristic of species ecology for desert amphibians. Aquatic connectivity had the strongest relationship with genetic connectivity across species when landscape drivers were combined with topography. This supports the notion that in arid environments, water and aquatic habitat are major factors in gene flow and landscape permeability for all aquatic species – from perennial to ephemeral specialists.

Desert anurans utilize a range of ecological strategies to survive in areas characterized by a spatial mosaic of perennial, intermittent, and ephemeral waters embedded in an arid landscape. Larval development periods restrict species' breeding habitats, and desiccation risk is a known driver of amphibian movements in arid environments (Tingley and Shine 2011). High mobility is one possible strategy for capitalizing on unpredictable availability of water and may drive high genetic connectivity observed in some desert anurans in the American Southwest (Mexican spadefoots in this study; Chan and Zamudio 2009). The high mobility of Mexican spadefoots and other ephemeral specialists in the region (e.g., Couch's spadefoot, *Scaphiopus couchii*, and the Great Plains toad, *Bufo cognatus*) may provide greater resiliency to temporal or spatial changes in habitat availability, and high genetic connectivity between populations may buffer the genetic consequences of some habitat loss.

High mobility, however, is only one end of a spectrum of potential strategies for aquatic desert life; other species may instead exhibit site fidelity to isolated pools with perennial or longer-term intermittent water. Both canyon treefrogs and red-spotted toads exhibit some degree of site fidelity and had greater population structure than Mexican spadefoots. Proximity to water is the hypothesized mechanism by which canyon treefrogs meet necessary water requirements for thermoregulation (Snyder and Hammerson 1993). Red-spotted toads also exhibit non-random occupancy of suitable breeding habitat (Dayton and Fitzgerald 2001) and fidelity for particular sites (Turner 1959, Weintraub 1974). These findings support our hypothesis of greater population structure with increasing water requirements. Aquatic habitat in the Sky Islands region is threatened by human water use (Marshall et al. 2010), a warming and drying climate (Seager et al. 2007), and an increased risk of catastrophic disturbances such as extreme fires (Brown et al. 2004). A drier landscape and loss of an already limited number of breeding sites may result in a greater risk of loss of genetic diversity for amphibians such as red-spotted toads, canyon treefrogs and others with small population sizes and/or high water requirements (e.g., Chiricahua leopard frogs, *Lithobates chiricahuensis*; Arizona treefrog, *Hyla wrightorum*; and Sonoran tiger salamander, *Ambystoma mavortium stebbinsi*).

Considered independently, the importance of different connectivity models varied across species. As predicted, terrestrial and aquatic connectivity were highly correlated with genetic connectivity for canyon treefrogs in mixed-effects models. But, the null resistance model (isolation-by-distance) performed similarly, and MRDM models provided strong support for an isolation-by-distance-only model. This indicates that distance alone may be an important driver of population genetic structure in this species. This may be particularly true at the spatial scale of a single mountain range where high elevation, lower temperature, and greater precipitation provide a high density and availability of permeable landscape. This is supported by the marginal increase in support for isolation-by-distance at a finer scale for canyon treefrogs. Two things may explain this result. First, distance may in fact be the primary driver of genetic connectivity between populations, particularly within a mountain range. Second, if landscape attributes such as aquatic connectivity are important for dispersal, the high permeability of the landscape may make it difficult to detect the importance of such features using a landscape genetics approach (Cushman et al. 2013).

For red-spotted toads, all mixed-effects models performed similarly with the exception of poorly supported Euclidean distance-only model. However, when major genetic clusters were considered independently, topographic resistance had the highest R^2_β for the northern red-spotted toad group (RT-N) as predicted, and the Huachuca group (RT-H) was panmictic, highlighting the variability of important landscape drivers of genetic connectivity introduced by spatial scale and extent of a study. Finally, mixed-effects models supported distance alone (Euclidean) as the best explanatory factor in Mexican spadefoot genetic connectivity.

Topography is the physical template upon which organisms interact with other landscape factors, and high slope is known to reduce genetic connectivity in some amphibians (Lowe et al. 2006). When topographic resistance was considered alongside other landscape factors, aquatic connectivity emerged as a dominant driver of genetic connectivity for all species. However, for canyon treefrogs, the correlation between topographic resistance and genetic connectivity was opposite the expected relationship, with high slope correlated with high genetic connectivity. Though canyon treefrogs have the highest water requirements of the three species we examined they are relatively well-adapted to dry conditions (Snyder and Hammerson 1993). Complex topography and high elevation ridges may not completely inhibit canyon treefrog gene flow, and our results suggest some genetic resilience to temporarily dewatered or disturbed habitat (e.g., from fires or a dry year) at finer spatial scales where individuals may be capable of recolonizing breeding habitat. Furthermore, mountain ranges may provide more refugia and less stressful environmental conditions for dispersing individuals due to wetter monsoon conditions, higher canopy cover, and lower temperatures than valley regions. Similar patterns of isolation-by-mountain range are described for sympatric insects described as headwater specialists (Finn et al. 2007, Phillipsen and Lytle 2013).

The relationship between genetic and aquatic connectivity for red-spotted toads may be driven by the species' affinity for bedrock pools. In ephemeral areas, adults might disperse along riparian networks in which refugia and the likelihood of finding suitable breeding habitat are greatest, and they may be deterred by the high slope of canyons, incised channels, or valley walls. Longitudinal connectivity may also be the result of rare but important downstream dispersal when larvae in these shallow bedrock reaches are displaced and washed downstream by flash floods.

Finally, the relationship between genetic and aquatic connectivity in a topographic context for Mexican spadefoots supports the idea that aquatic connectivity may be important for aquatic species with even the most ephemeral water requirements. Mexican spadefoots have the lowest hydrologic requirements of the three species in this study, but aquatic habitat remains a critical part of their life cycle. Furthermore, high slope topography may represent a barrier, may not provide adequate breeding habitat or underground refugia, or may be too energetically costly to navigate.

These findings highlight the potential predictive power of species biology and ecology in understanding population connectivity. Still, there are inherent limitations with this methodology. Gene flow estimates derived from microsatellite data reflect gene flow over the last few decades to centuries and do not always reflect present-day demographic or genetic processes (Waples and Gaggiotti 2006). Secondary contact after removal of a historical barrier may manifest as a cryptic genetic signal unrelated to current landscape processes (Landguth et al. 2010). Our study extent may have encompassed two major lineages of red-spotted toads that diverged during the late Pleistocene (Bryson et al. 2012). However, studies suggest similar or even increased landscape permeability for at least the last 1000 generations for this species (Holmgren et al. 2003, Pigati et al. 2009). Moreover, global genetic differentiation of red-spotted toads in this study was moderate to low, and with many populations in this study estimated to have modest N_e it is unlikely that the signal from secondary contact is detectable in this study (Whitlock and McCauley 1999).

Challenges stemming from collinearity, resistance surface parameterization, and spatial scale can also complicate detection and interpretation of relationships between genetic and structural connectivity. This is not unique to our study as collinearity among landscape resistance values is a persistent challenge in landscape genetics (Balkenhol et al. 2009). Our ability to account for collinearity in resistance values is limited because formal comparisons of R^2_β have yet to be developed. Thus, comparisons of close R^2_β values can make interpretation – and determining the "true" landscape driver – difficult. Therefore, though interpreting results in an ecological context does provide valuable insight (Cushman and Landguth 2010), R^2_β values must be interpreted with these correlations in mind. Defining landscape resistance values is a recognized challenge in landscape genetics (Spear et al. 2010, Cushman et al. 2013). It is likely that true landscape resistances are more complex than the simple resistance values used in our study, and non-linear relationships may exist between genetic and landscape connectivity (e.g., Peterman et al. 2014). However, our goal was to compare hypothesized relationships between genetic structure of populations and a suite of landscape factors, and we created simple landscape resistance layers that reflect our hypotheses and that were easily compared across species.

Our study's spatial extent captured meaningful genetic structure across all three species, but landscape effects may change at different spatial scales (Anderson et al. 2010, Murphy et al. 2010). Spatial patterns of genetic diversity indicated clean hierarchical structure for canyon treefrogs and clear isolation-by-distance patterns for spadefoots; however, red-spotted toads displayed spatially complex structure, and genetic connectivity within genetic clusters varied in its relationship to the landscape. Though we do not know the underlying cause of that spatial variability in red-spotted toads, complex effects of spatial scale highlight the need to carefully interpret patterns observed in one portion of a species' range when drawing management-relevant conclusions outside the study area. The landscape is often heterogeneous across a species' range, and edge verses central populations may have different population dynamics and undergo different selective pressures (Hardie and Hutchings 2010, Trumbo et al. 2013). The ranges of the three study species extend beyond the area of this study and encompass areas with drier climate, greater urbanization, and lower canopy cover than the geographic extent of this study. The variability observed for red-spotted toads emphasizes that relationships between landscape variables and genetic connectivity are not necessarily consistent across a range or at different spatial scales, and relationships may be fundamentally different in areas where the matrix between populations is drier, harsher, or offers fewer refugia.

In conclusion, we found that patterns of population structure, connectivity, and their landscape drivers are predicted by the water dependencies of anurans in dryland ecosystems. Our work supports recent studies highlighting the utility of multi-species inference and ecologically derived hypotheses (e.g., Hughes et al. 2013). Genetic diversity is often a missing component in conservation planning and resource allocation despite its recognized role in species persistence (Laikre et al. 2010). With increasing human demand for aquatic resources in arid environments, environmental change and habitat alteration will likely outpace the resources and time necessary for single-species population genetics studies for many species of conservation concern. When single-species studies are not feasible, the use of species' ecological information to predict relationships between genetic and structural connectivity may provide a promising alternative.

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Plate 5.3 Fort Huachuca canyon treefrog survey volunteers. Photo credit: Meryl Mims.

5.6 Appendix A, Sampling and genetic methods

5.6.1 Microsatellite amplification and screening

Whole genomic DNA extractions were performed using DNeasy 96 Blood & Tissue Kit (QIAGEN), and extractions were performed at the Molecular Ecology Research Lab at the University of Washington's School of Aquatic and Fishery Sciences. Mexican spadefoot loci were compiled from previously published microsatellite markers (Rice et al. 2008, Van Den Bussche et al. 2009). Canyon treefrog and red-spotted toad marker sets were developed by the Evolutionary Genetics Core Facility at Cornell University and are described in Table 5.7.2. Polymerase chain reaction (PCR) was used to amplify DNA for multiplexed loci using Multiplex PCR kits (QIAGEN). PCR products were genotyped using an ABI 3730 sequencer (Applied Biosystems) at the Oregon State University's Center for Genome Research and Biocomputing (Corvallis, OR). Genotypes were analyzed using the software GENEMAPPER 4.1 (Applied Biosystems), and alleles were binned using the program TANDEM (Matschiner and Salzburger, 2009). Loci were screened for the presence of linkage disequilibrium using the log likelihood ratio statistic for each pair of loci in each population was found (GenePop, Raymond and Rousset 1995). No evidence of consistent linkage between loci was found. Any significant pairwise linkage results occurred in < 13% of all populations for a given species; results not shown but available upon request. Loci were also screened for deviations from Hardy-Weinberg equilibrium (HWE) using the exact p-test (results presented in Table 5.7.3) as implemented in GenePop (Raymond and Rousset 1995), and the presence of null alleles was evaluated with Micro-Checker (Van Oosterhout et al. 2004) using adults only (spadefoots) or larval samples from which all but one full sibling were removed. With a Bonferroni correction applied, no significant deviations from HWE were observed for any locus in a given population for canyon treefrogs or Mexican spadefoots. Three significant deviations from HWE were observed for red-spotted toads (Table 5.7.3); however, because these deviations did not represent a considerable proportion of tests for any given population or locus, all markers and populations were retained in our analyses. Summary statistics for all retained loci (all species) are included in Table 5.7.4, including *F*-statistics calculated using MSA 4.05 (Dieringer and Schlötterer 2003).

Larval samples can bias population genetics findings by artificially inflating genetic differentiation due to family structure (Goldberg and Waits 2010); therefore, we screened all larval samples for full siblings using the program COLONY (Wang 2009). One sibling was retained from each family with fewer than six siblings. For families with six or more full siblings, there is a 98.4% chance of detecting both parental alleles for each locus, and we manually reconstructed two parental genotypes for use in population-based analyses for samples < 25 in order to achieve the maximum sample size. For individual-based analyses, only a single sibling was retained. This was confirmed by re-genotyping rare alleles (in < 3 individuals). We estimated statistical power of our final marker sets using POWSIM, a simulation-based computer program that estimates power (and α error) for chi-square and Fisher's exact tests when evaluating the hypothesis of genetic homogeneity (Ryman and Palm 2006). Sample sizes, number of samples, loci and allelic information, and number of generations can be combined under various scenarios to produce a hypothetical degree of genetic differentiation (measured as F_{ST}). We ran simulations for each species and used our actual sample size and number of samples (conservatively calculated without reconstructed parents), our median estimated N_e, numbers of loci and alleles, and allele frequency for simulations. Number of generations was then adjusted to approximate observed F_{ST}. Each species was simulated at a range of numbers of generations from 10 to 500 to calculate power at a range of F_{ST} output values, including one that approximated the observed $F_{ST.}$ Proportion of significant differences observed (200 runs) was 1.0 for all species in almost all scenarios. The 10-generation spadefoot simulations were the only scenario with a proportion of significant differences observed that fell below 1.0. However, the estimated F_{ST} for that run was roughly 1/10 the observed F_{ST}, indicating that a 10-generation simulation is not sufficiently long to reflect actual observed genetic differentiation for this species. These results indicate satisfactory statistical power given the loci and sample sizes in our dataset.

5.6.2 Hierarchical population structure and clustering

Individual-based hierarchical population structure was analyzed using the Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al. 2000). Each sampling site was treated as an independent putative population with a total of *n* putative populations for each species. Ten iterations of each *K* from 1 to *n* + 1 for each species were run for 1,000,000 cycles with a burn-in of 200,000 cycles. We used the locprior model with admixture and correlated allele frequencies. The most likely *K* was determined using the delta-*K* method (Evanno et al. 2005) in which the most likely value of *K* is assessed by the second-order rate of change in the log-likelihood. A delta-*K* value cannot be calculated for *K* = 1; thus, for cases in which *K* = 1 has the greatest log-likelihood, 1 is assumed to be the most likely *K* (Spear et al. 2012). This analysis was repeated for genetic clusters in which both K > 1 and *n* > 1 to identify hierarchical population structure until terminal clusters were described (Phillipsen et al. 2013). All STRUCTURE output and delta-*K* calculations are included in Table 5.7.5. STRUCTURE output was visualized using the program DISTRUCT 1.1 (Rosenberg 2004).

5.6.3 Assessment of potential sampling bias from variable sampling methods

Despite accounting for larval family structure, it is possible that variable sampling methods may introduce biases into genetic inference of population structure and connectivity. For example, COLONY accounts for full-sibling groups, but it is possible that half-siblings or other distant family structure inflates genetic differentiation between larval samples relative to adult samples. Also, Mexican spadefoot adults were collected along roadways in addition to breeding sites. It is not well known whether adults of this species exhibit breeding site fidelity or homing behavior; if they do, particularly for their larval pond, it is reasonable to expect that adults collected at breeding sites may be more genetically similar than those collected along roads. To examine whether we see evidence of large bias from these variable sampling methods, we paired larval samples with nearby adult samples for red-spotted toads and spadefoots. Canyon treefrogs were not included because we did not have enough adult samples to generate a sufficient number of adult-larval paired samples. We then examined genetic diversity and overall genetic differentiation within each group of samples collected by a given method (larval samples, adult samples, breeding site adults, and roadside adults).

Allelic richness and heterozygosity were similar across sampling methods within species. We found some evidence for higher differentiation among larval samples than adult samples for red-spotted toads, where G'_{ST} increased by 62% for larval samples compared to adult samples (Table 5.7.6). N_e was also lower for larval samples than adults. However, for spadefoots, there was modest evidence of lower genetic differentiation among larval samples than for adult samples, with G'_{ST} reduced by 34% for larval samples compared to adult samples. N_e was also lower for adult samples than for larvae for which the median N_e value was 10,000 (the estimate for infinite population sizes). Genetic differentiation between spadefoot adult sampling methods was minimal. However, for both species, not all "pairs" of larval and adult samples were spatially congruent. Some adult and larval red-spotted toad pairs were selected based on similar sample sizes and similar spatial locations, but with local processes identified as important for this species (for example, low N_e in some populations), the pattern of higher G'_ST may be spurious and requires further exploration.

We also explored the effects of multiple sampling replicates ("Reps") in space and time on genetic diversity indices. To do this, we used a paired sample approach for populations of all three species with two or more replicates (from which at least 10 % of the sampled individuals represented an additional replicate). These included 3 populations of canyon treefrogs, 6 populations of red-spotted toads, and 12 populations of spadefoots. Due to low sample sizes for two of the three species, we did not explore effects of sampling replicates on genetic differentiation between populations. Genetic diversity metrics (H_O, H_E, AR, N_e and the upper confidence limit of N_e (N_e high) calculated using a jackknifing approach and estimated as 10,000 for infinite values) were calculated with only one replicate and with multiple replicates. To control for sample size, multiple replicate samples were reduced to match sample sizes of one replicate only. Where possible, equal numbers of individuals were included from each replicate in the reduced sample. A paired t-test was then used to compare differences in H_O, H_E and AR, and a Wilcoxon signed-rank test was used to compare differences in N_e and the upper confidence interval of N_e given the non-normal distribution of differences for these values. Local processes such as drift due to low N_e or low migration rates as well as possible metapopulation dynamics were identified as playing a greater role in population genetic structure of canyon treefrogs and red-spotted toads, and the effect of multiple sampling replicates may be more apparent in species such as these with low N_e and greater family structure among larval samples. For that reason, the effect of replicates on these genetic diversity metrics was calculated for all species as well as for canyon treefrogs and red-spotted toads combined.

We found little evidence that sample replicates biased the results of this study (Table 5.7.7). We found no significant differences in genetic diversity measures between single and replicate samples for all species, and we found only one significant difference for canyon treefrog and red-spotted toads alone (H_E, p-value = 0.024). If a Bonferroni correction for multiple comparisons is applied, the result for H_E is not significant.

In summary, although we saw differences in estimates of genetic differentiation between larval and adult samples for both species, the range of G'_ST values within species were comparatively low. These potential biases did not result in overlap of G'_ST between species, and thus for this study we suspect that the potential bias did not affect the outcome of this study. We also found little support for the effect of number of spatial or temporal sampling replicates on the genetic diversity metrics of this study. However, our results are limited to our study species in a subsection of their ranges, and bias due to different sampling methods may be more substantial for other species or regions. Future consideration of biases from sampling methods both in empirical and simulation studies may be particularly important for development of predictive models in which small differences in connectivity estimates may have implications for resistance surface parameterization or management actions.

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Figure 5.6.1 Canyon treefrog sampling map.

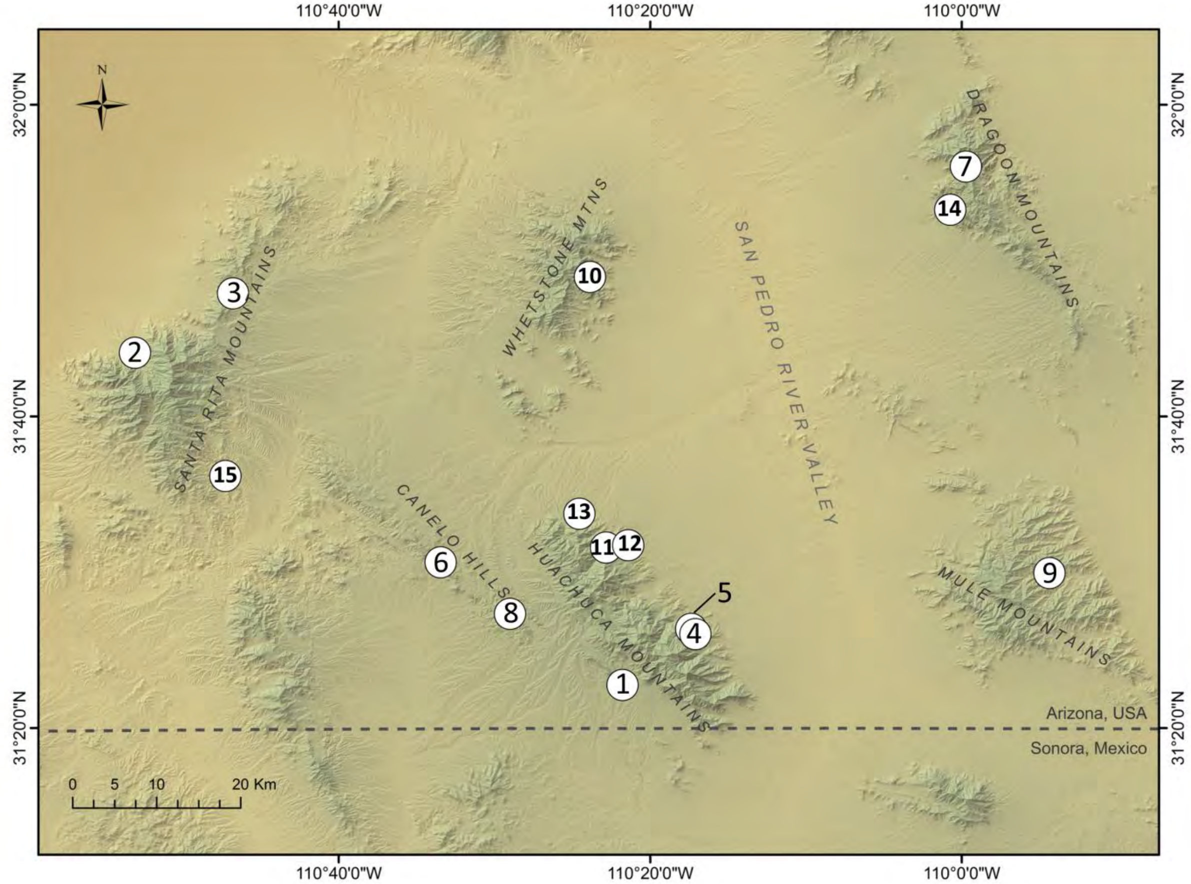


Figure 5.6.2 Red-spotted toad sampling map.

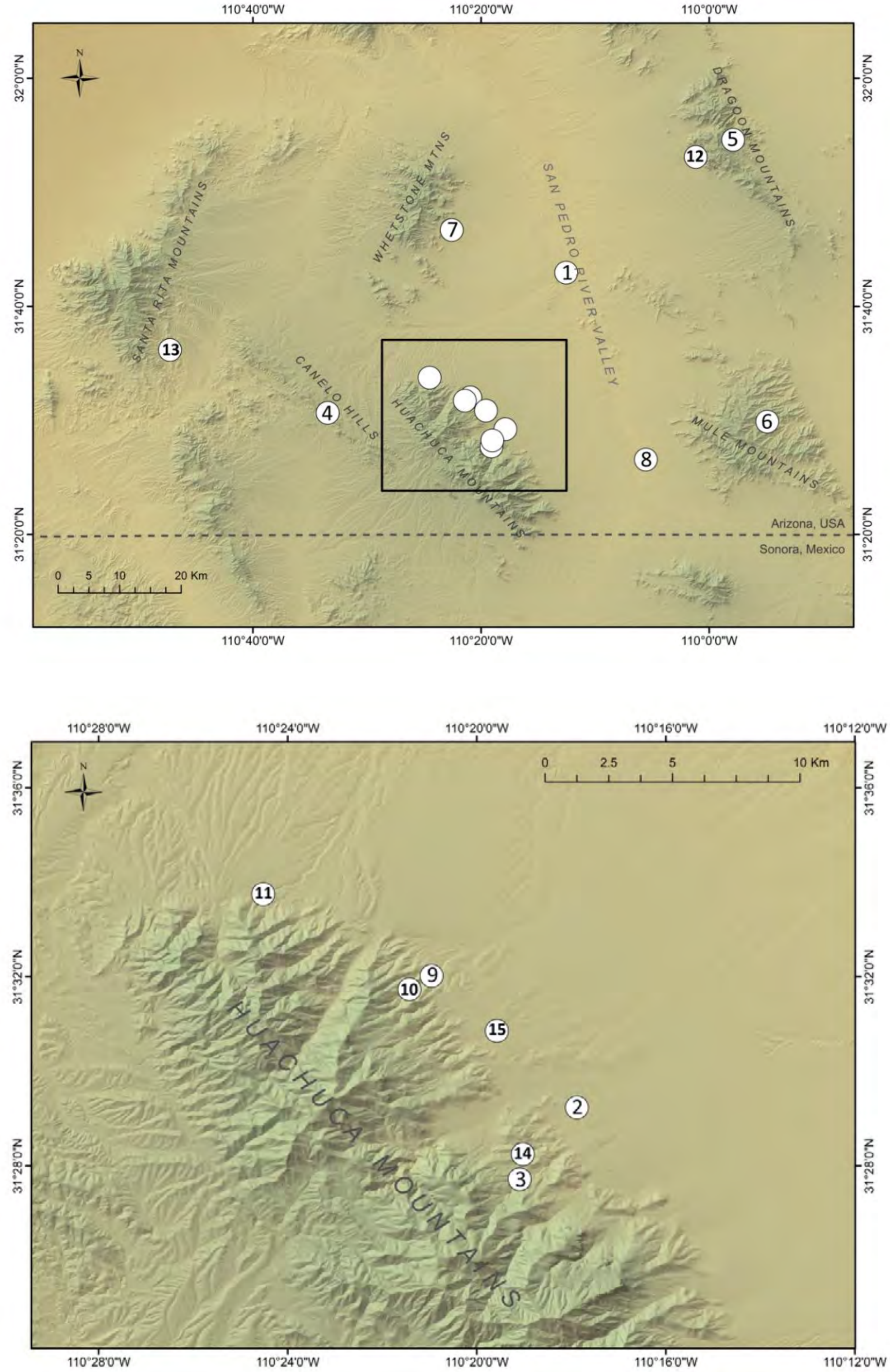


Figure 5.6.3 Mexican spadefoot sampling map.

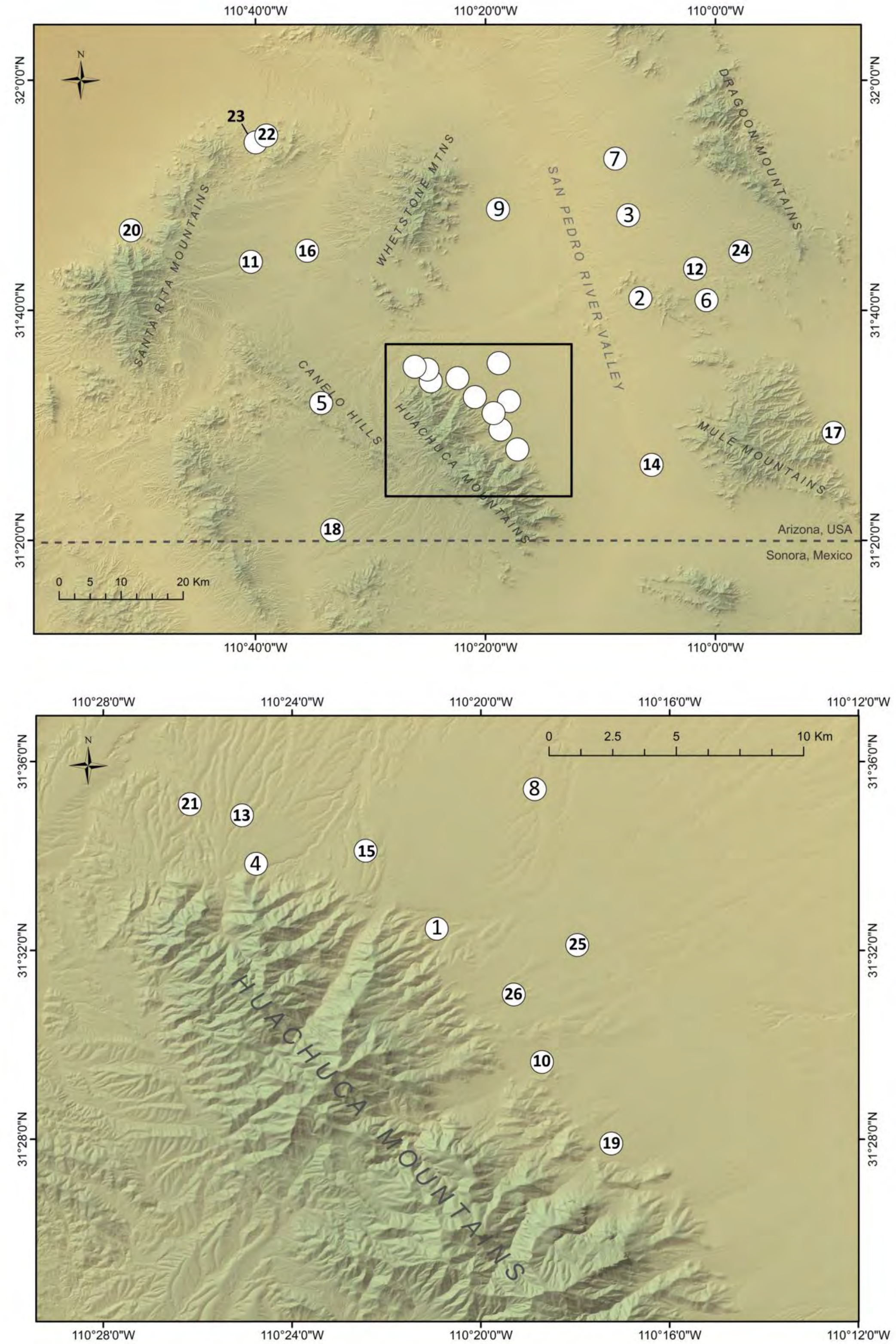


Table 5.6.1 Sample number and locations (UTM Zone 12); sample size with siblings removed (N All), with reconstructed parents (N with P), and with number and percent of adults; replicates (temporal or spatial); allelic richness (based on minimum sample size); expected and observed heterozygosity; effective population size (N_e) and the 95% confidence intervals for N_e calculated via a jackknifing method where "*Inf*" represents infinity; and F_{IS} values averaged over all loci. Results from Hardy Weinberg exact tests are included in Table 5.7.3. Additional information by locus and/or population are available from M.C. Mims upon request.

Canyon treefrog

Site	UTM Northing	UTM Easting	N All	N with P	N Adults	Percent Adults	Reps	AR	H_{E}	H_{O}	N_e	C.I.s for N_e		F_{IS}
1	560558	3471841	19	19	15	79	1	4.96	0.71	0.75	17.9	12.7	27.4	-0.07
2	510906	3511050	10	11	2	20	1	6.45	0.81	0.84	*Inf*	52.2	*Inf*	-0.06
3	520834	3518105	7	8	2	29	2	5.26	0.78	0.81	13.5	8.7	24.4	-0.09
4	567504	3478529	7	11	0	0	1	4.58	0.71	0.73	7.5	3.1	16.2	-0.05
5	567930	3477919	8	10	0	0	1	4.66	0.74	0.78	7.4	3.7	13.4	-0.09
6	541988	3486290	5	7	0	0	1	5.50	0.72	0.68	*Inf*	26.2	*Inf*	0.08
7	594936	3533556	12	12	4	33	1	4.53	0.63	0.58	17.6	8.7	64.2	0.09
8	549066	3480211	10	12	3	30	2	5.65	0.79	0.81	33.1	15.5	348	-0.05
9	603435	3485393	9	13	1	11	2	3.06	0.54	0.54	2.7	1.8	6.7	-0.02
10	556976	3520186	15	16	0	0	1	4.33	0.70	0.74	*Inf*	75.7	*Inf*	-0.08
11	558781	3488127	17	17	17	100	1	5.54	0.80	0.81	30.7	18.7	67.5	-0.02
12	561038	3488403	22	23	0	0	2	5.62	0.79	0.75	323.1	72.3	*Inf*	0.04
13	556064	3492110	6	9	0	0	1	5.79	0.79	0.84	75.1	25.9	*Inf*	-0.11
14	593398	3528429	9	12	0	0	1	4.35	0.64	0.60	19.7	9.6	83.5	0.04
15	520105	3496453	19	22	0	0	1	6.05	0.80	0.83	94.4	35.1	*Inf*	-0.05

Table 5.6.1, continued.

Red-spotted toad

Site	UTM Northing	UTM Easting	N All	N with P	N Adults	Percent Adults	Reps	AR	H_E	H_O	N_e	C.I.s for N_e		F_{IS}
1	574944	3509235	11	12	0	0	2	3.88	0.65	0.69	6.9	3.3	11.7	-0.09
2	566670	3483820	8	8	0	0	1	5.81	0.83	0.78	*Inf*	287.2	*Inf*	0.03
3	564771	3481006	15	15	0	0	1	5.55	0.79	0.75	302.1	49.1	*Inf*	0.05
4	542015	3486332	17	18	0	0	3	5.05	0.76	0.70	20	14.4	30.1	0.07
5	597845	3530951	9	11	0	0	1	5.99	0.80	0.82	*Inf*	49.9	*Inf*	-0.05
6	603007	3485338	9	11	0	0	2	4.36	0.67	0.68	2.9	2.3	4.9	-0.03
7	559054	3516045	6	9	0	0	1	5.28	0.77	0.73	*Inf*	180.6	*Inf*	0.02
8	586216	3479036	13	13	13	100	1	5.08	0.75	0.76	7.8	5	11.8	-0.04
9	561774	3488938	22	24	17	77	3	4.87	0.72	0.67	37.6	25.2	65.8	0.06
10	561034	3488404	6	6	0	0	1	5.93	0.79	0.72	*Inf*	*Inf*	*Inf*	0.05
11	556119	3492102	48	48	25	52	5	5.61	0.80	0.82	46.4	37.7	58.5	-0.04
12	592703	3528123	10	15	0	0	1	5.52	0.76	0.74	83	37.2	*Inf*	0.00
13	520105	3496453	17	18	2	12	1	5.75	0.78	0.72	144.6	55.6	*Inf*	0.05
14	564872	3481987	31	33	27	87	3	5.54	0.78	0.80	81.5	50.8	176.4	-0.04
15	563972	3486799	11	11	2	18	2	5.22	0.76	0.76	641.9	34.8	*Inf*	-0.02

Table 5.6.1, continued.

Mexican spadefoot

Site	UTM Northing	UTM Easting	N All	N with P	N Adults	Percent Adults	Reps	AR	H_E	H_O	N_e	C.I.s for N_e		F_{IS}
1	561802	3489747	36	36	17	47	3	5.26	0.67	0.64	1903.3	87.1	*Inf*	0.03
2	584463	3505799	21	21	21	100	1	4.86	0.68	0.67	12.2	4.6	41.2	0.00
3	582676	3519097	23	23	0	0	1	5.31	0.69	0.72	*Inf*	63.7	*Inf*	-0.06
4	555732	3492251	12	12	12	100	1	5.56	0.67	0.75	*Inf*	48	*Inf*	-0.14
5	540672	3488698	33	33	0	0	2	5.40	0.65	0.66	*Inf*	314.2	*Inf*	-0.01
6	593537	3505547	34	34	0	0	1	5.05	0.65	0.62	*Inf*	10400	*Inf*	0.04
7	580834	3528180	72	72	0	0	3	5.21	0.67	0.67	1157.8	132.2	*Inf*	-0.01
8	565055	3495225	36	36	0	0	2	5.41	0.69	0.67	377.7	78.6	*Inf*	0.07
9	564804	3519926	31	31	0	0	1	5.16	0.67	0.65	1845	64.3	*Inf*	0.02
10	565362	3484554	39	39	18	46	2	5.35	0.68	0.69	*Inf*	105	*Inf*	-0.01
11	530935	3511341	24	24	0	0	1	4.92	0.65	0.64	*Inf*	44.8	*Inf*	0.01
12	591925	3510598	16	16	16	100	2	5.08	0.67	0.70	*Inf*	32.7	*Inf*	-0.06
13	555249	3494145	36	36	0	0	1	5.47	0.68	0.65	*Inf*	67.5	*Inf*	0.03
14	586216	3479036	9	9	9	100	1	5.00	0.68	0.65	*Inf*	24.7	*Inf*	0.01
15	559400	3492783	34	34	20	59	3	5.16	0.65	0.65	82.5	32.7	*Inf*	-0.02
16	538646	3513134	25	25	25	100	1	5.30	0.64	0.60	618.9	50.5	*Inf*	0.05
17	611229	3484426	19	20	0	0	1	5.15	0.67	0.69	63.9	24.2	*Inf*	-0.05
18	542257	3468315	14	16	0	0	1	5.00	0.61	0.66	49.8	17.3	*Inf*	-0.09
19	567709	3481377	19	19	19	100	3	5.30	0.66	0.69	74.7	26.4	*Inf*	-0.06
20	514446	3516357	17	17	0	0	1	5.32	0.72	0.70	58.4	23.4	*Inf*	0.03
21	553502	3494577	74	74	9	12	3	5.34	0.66	0.65	*Inf*	147.3	*Inf*	0.01
22	531529	3530515	26	26	0	0	2	5.65	0.73	0.71	83.5	36.5	*Inf*	0.04
23	532978	3531631	25	25	0	0	1	5.26	0.69	0.68	*Inf*	160.3	*Inf*	0.00
24	598126	3513429	61	61	0	0	2	5.25	0.69	0.66	*Inf*	77.7	*Inf*	0.08
25	566518	3489137	19	19	4	21	1	5.52	0.69	0.68	79.1	28.9	*Inf*	0.01
26	564400	3487189	26	26	9	35	3	5.39	0.68	0.70	*Inf*	72.8	*Inf*	-0.03

Table 5.6.2 Locus, repeat length (di-, tri- or tetranucleotide), and primer sequences for the final microsatellite loci for red-spotted toads (*Anaxyrus punctatus*) and canyon treefrogs (*Hyla arenicolor*), developed by the Evolutionary Genetics Core Facility at Cornell University.

Canyon treefrog

Locus	Repeat	Forward primer (5' - 3')	Reverse primer (5' - 3')
ha40	tet	ACAACTCCCAGCATATATCTCTC	GTTCACTGTACTCAAATGGCCTC
ha280	tri	TCCTTCACACTCTAAGGTTGCTC	CGCACTTTATGAACAGATTTGCC
ha311	tet	ATAATTACAGTGATGCCGCCTTG	CAAGCAACCATCAACATATGTAGG
ha357	tet	TTGTATCACTTGTGCTATTGGGC	TAGTGCTGCATTTATGTGGAAGG
ha479	tet	GCATTGTTCAAGTATTACCAGGC	TGTTCTCACTTTGCAGTTGAAGG
ha568	tet	GAGGCAGATTAATAGGTGAACGG	CATCCAAACACATACATCAGGGC
ha664	tet	AATGCCACATGTAACTGAGTGTG	TCCATTACTAAAGTACACCAGCC
ha703	tet	AGGTAGGTAGGTGTGCTTACATG	ACACTTGTGTCTTGAGTCATTGAC
ha705	tet	ACAGAAGCTACACCTAACACCTC	AAATATTAACCACCGGAGTACCC
ha1435	tet	ACTAGGTCATTCATTAGATGTGGG	TGAAAGGCTTAACTCTTCCAAGC
ha1997	tet	TTCTAACAAAGCCTGAGACATCC	TTGGACCCTTTATGACTTGCTTG
ha2144	tet	TGGCCGGTGAGTGTATATCTATC	TTGGATACCTACCTCACAGTCTG

Red-spotted toad

Locus	Repeat	Forward primer (5' - 3')	Reverse primer (5' - 3')
ap71	tet	AACCCTTTGTGACAGAATGGTTC	TTTGGTTGTTCACATCTCTCTGG
ap213	tet	ATCTCATTTCCCTCAAACTGTGC	GAAACAGTGAGCCAAACATTCCC
ap360	tet	TGCTCAACACTACTGAAGACATC	AGGATCTGTCAGGAGCAGTTATC
ap1904	tri	CACGATGTGTCCCTCTTTGTTTG	GGAGTAGCAGAAGGAATGTTGTG
ap2524	di	CCAGAAGTCATATGATCAGCGTG	ATTCCACTGTTGTTACCACTGAC
ap3396	tet	GGCAAATGTCCACAAATGTACAG	TGAGTCAGATAAGCTAGATGTGGG
ap3587	tri	GACGGATGAGACCAACATAGAAC	GATTGAACAAGACAAGCCCAAAC
ap3591	tet	CCACATTAAATACTGGCGCCTAG	GACCGATTCTGCCATATCTGC
ap4565	tet	TGCATGCCACTGTAGATAATAGG	TAGAGATAGCACTTACACCTGGG
ap5418	tet	ACAAGTGGGTAGAAAGATATGGG	CAGGAGCTGCTGGAGAGTATTC
ap5818	tet	ACCTTGAATTCTTTGTCATGTTCC	CCAGGGAGCCATTATTTCAGATG
ap6204	tet	CTGCTGCAACTGACACTG	AAACATACAAGGCTGACTATGGG
ap9886	tri	TGCGTGTTTCCATGTACCATATG	CAGTACAGTGTGGATGTGAAAGG
ap10273	tri	ACCAATATCTATCCTCCGACGTC	ATGTGAGAATAGGTTAGCGTTCC

Table 5.6.3 *P*-values for Hardy Weinberg exact test for each population (rows) and locus (columns). Significant *p*-values with a Bonferroni correction applied are shown in bold. No data (*na*) indicates that only one allele was present for a given locus in a given population, or two alleles were detected but one was represented by only one copy.

Canyon treefrog

	280	357	40	664	1435	2144	311	705	1997	479	568	703
1	0.353	0.223	0.291	0.490	0.344	0.642	0.937	0.338	0.841	0.515	0.739	0.064
2	0.483	0.501	0.208	0.779	0.910	0.624	0.783	0.255	1.000	0.823	0.576	1.000
3	0.404	0.745	0.424	0.291	0.106	0.617	1.000	0.991	0.819	0.739	1.000	0.867
4	0.764	0.782	0.182	0.345	0.159	0.266	0.142	0.127	0.021	0.273	0.391	0.263
5	0.409	0.786	0.496	0.667	0.654	0.367	0.219	0.810	0.198	0.900	0.113	0.944
6	0.007	0.011	0.106	0.232	0.305	0.440	0.849	1.000	0.433	0.805	0.559	0.661
7	*na*	1.000	0.039	0.140	0.071	0.255	0.343	0.160	0.802	0.429	0.928	0.555
8	0.769	0.482	0.619	0.314	0.881	0.608	1.000	0.178	0.989	0.321	0.008	0.812
9	1.000	1.000	0.028	0.938	0.263	0.036	0.566	0.457	0.015	0.089	0.026	0.119
10	0.889	0.821	0.261	1.000	0.479	0.400	0.372	0.886	0.353	0.872	0.823	0.972
11	0.912	0.756	0.126	0.834	0.024	0.957	0.368	0.580	0.189	0.849	0.687	0.399
12	0.550	0.228	0.630	0.443	0.026	0.330	0.016	0.857	0.421	0.183	0.115	0.037
13	0.251	0.026	0.741	0.152	0.591	0.683	0.245	0.442	0.766	0.315	0.101	0.016
14	*na*	0.595	0.080	0.341	1.000	0.404	0.909	0.158	0.035	0.725	0.133	0.823
15	0.780	0.057	0.521	0.892	0.588	0.078	0.355	0.688	0.713	0.377	0.653	0.862

Table 5.6.3, continued.

Red-spotted toad

	10273	2524	3396	5818	3591	360	6204	71	213	3587	1904	4565	5418	9886
1	0.304	0.227	0.400	0.119	0.653	0.219	*na*	0.722	0.471	0.174	1.000	0.774	0.258	0.530
2	0.089	0.378	0.759	0.696	0.866	0.887	0.434	1.000	0.223	0.191	0.069	0.855	0.189	0.766
3	0.384	0.032	0.622	**0.001**	0.971	0.996	0.047	0.749	0.188	0.071	1.000	0.672	0.250	0.117
4	0.853	0.032	0.934	0.047	0.375	0.139	1.000	0.965	0.584	0.317	0.116	0.256	0.620	0.260
5	0.983	0.795	0.775	0.271	0.715	0.516	1.000	0.980	0.680	0.053	0.932	0.679	1.000	0.754
6	0.511	0.098	0.140	0.076	0.182	0.568	1.000	0.739	0.669	0.160	1.000	0.011	0.084	0.654
7	0.742	0.665	0.627	0.878	0.688	0.652	1.000	0.345	0.527	1.000	1.000	0.339	0.463	0.648
8	0.982	0.063	0.552	0.454	1.000	0.027	0.602	0.110	0.037	0.057	0.743	0.215	0.173	0.468
9	0.322	0.385	0.607	0.127	0.095	0.275	1.000	0.148	0.537	0.025	0.173	0.052	0.218	0.693
10	0.148	0.655	1.000	**0.003**	0.430	0.709	1.000	0.760	0.163	0.379	1.000	0.213	1.000	0.585
11	**0.002**	0.077	0.284	0.804	0.933	0.053	0.867	0.854	0.267	0.046	0.057	0.076	0.362	0.224
12	0.438	1.000	0.116	0.193	0.209	0.115	0.729	0.516	0.366	0.388	1.000	0.283	0.644	0.896
13	0.008	0.033	0.941	0.008	0.274	0.688	1.000	0.112	0.411	0.053	1.000	0.605	0.830	0.335
14	0.310	0.128	0.150	0.826	0.740	0.987	0.091	0.834	0.281	0.483	0.549	0.013	0.260	1.000
15	0.881	0.461	0.163	0.857	0.719	0.076	0.538	0.364	0.125	0.342	0.554	0.760	0.897	0.188

Table 5.6.3, continued.

Mexican spadefoot

	C7	D125	H115	D103	D111	D7	H129	20
1	0.942	0.081	0.563	0.057	0.804	1.000	0.884	0.564
2	0.072	0.443	0.026	0.409	0.400	1.000	0.234	0.228
3	0.906	0.337	0.828	0.133	0.918	1.000	0.496	0.504
4	0.012	0.874	0.259	0.260	0.724	*na*	0.293	0.387
5	0.809	0.072	0.883	0.534	0.747	*na*	0.348	0.448
6	0.300	0.073	0.888	0.106	0.588	*na*	0.408	0.766
7	0.698	0.016	0.340	0.547	0.100	1.000	0.309	0.487
8	0.363	0.046	0.281	0.419	0.710	0.084	0.522	0.351
9	0.275	0.521	0.135	0.829	0.980	*na*	0.188	0.740
10	0.717	0.811	0.898	0.166	0.763	*na*	0.157	0.941
11	0.129	0.687	0.350	0.002	0.997	*na*	0.094	0.893
12	0.660	0.840	0.465	0.492	0.499	*na*	0.232	0.591
13	0.415	0.652	0.382	0.039	0.312	*na*	0.496	0.378
14	0.842	0.650	0.421	0.099	0.879	*na*	0.302	0.976
15	0.423	0.339	0.015	0.210	0.457	*na*	1.000	0.268
16	0.529	0.323	0.664	0.255	0.167	*na*	0.018	0.462
17	0.585	0.086	0.127	0.965	0.828	*na*	0.442	0.642
18	0.411	0.519	0.305	0.465	0.340	*na*	1.000	0.083
19	0.831	0.666	0.129	0.916	0.401	*na*	0.327	0.361
20	0.818	0.181	0.071	0.902	0.955	0.431	0.725	0.745
21	0.589	0.576	0.252	0.526	0.807	*na*	0.613	0.468
22	0.902	0.332	0.217	0.197	0.607	0.297	0.462	0.991
23	0.832	0.237	0.284	0.355	0.554	1.000	0.574	0.000
24	0.967	0.907	0.300	0.160	0.902	0.002	0.147	0.732
25	0.879	0.574	0.831	0.657	0.831	*na*	0.239	0.286
26	0.963	0.835	0.208	0.897	0.710	*na*	0.865	0.322

Table 5.6.4 Characteristics of final microsatellite loci datasets for each species. Expected heterozygosity, observed heterozygosity, variability in PCR product size (Var), variability in PCR repeat number (VarRepN), allelic characteristics, and *F*-statistics are shown. Additional information available upon request from M.C. Mims.

Canyon treefrog

Locus	H_E	H_O	Var	VarRepN	Allele				F_{IS}	F_{ST}	F_{IT}
					Min	Mean	Max	Richness			
40	0.79	0.77	151.5	9.47	305	383.88	413	7.75	0.03	0.11	0.14
280	0.52	0.48	39.02	4.34	261	269.16	279	3.82	0.06	0.12	0.17
311	0.75	0.79	69.76	4.36	199	210.7	235	6.6	-0.05	0.13	0.08
357	0.66	0.71	21.85	1.37	204	214.81	232	4.62	-0.08	0.10	0.03
479	0.78	0.77	159.47	9.97	275	300.22	335	8.11	0.00	0.15	0.15
568	0.79	0.8	383.77	23.99	332	384.07	432	9.2	0.00	0.15	0.15
664	0.68	0.72	201.42	12.59	291	315.27	369	7.16	-0.06	0.19	0.14
703	0.84	0.83	644.4	40.28	305	397.43	499	9.19	0.00	0.09	0.10
705	0.77	0.79	394.52	24.66	320	360.16	416	7.33	-0.03	0.11	0.09
1435	0.77	0.77	298.06	18.63	178	307.3	375	7.32	0.02	0.13	0.14
1997	0.78	0.84	459.77	28.74	243	271.88	327	7.74	-0.09	0.14	0.07
2144	0.62	0.62	21.26	1.33	174	180.43	198	4.46	0.01	0.16	0.17

Red-spotted toad

Locus	H_E	H_O	Var	VarRepN	Allele				F_{IS}	F_{ST}	F_{IT}
					Min	Mean	Max	Richness			
71	0.84	0.85	123.98	7.75	233	335.74	365	6.58	-0.01	0.04	0.03
213	0.87	0.83	2266.66	141.67	258	367.51	430	7.27	0.02	0.04	0.06
360	0.83	0.85	86.57	5.41	376	396.41	416	6.55	-0.01	0.05	0.04
1904	0.56	0.58	15.53	1.73	280	291.49	298	3.33	-0.06	0.04	-0.02
2524	0.72	0.69	17.44	4.36	365	382.32	391	5.05	0.05	0.05	0.10
3396	0.86	0.9	154.33	9.65	242	266.35	310	7.33	-0.04	0.04	0.00
3587	0.72	0.67	21.67	2.41	132	143.51	159	4.85	0.09	0.08	0.16
3591	0.72	0.75	155.3	9.71	316	332.05	438	5.51	-0.05	0.03	-0.02
4565	0.87	0.85	344.37	21.52	324	361.4	424	7.7	0.02	0.04	0.06
5418	0.91	0.89	1864.16	116.51	286	359.4	490	9.28	0.01	0.03	0.05
5818	0.86	0.73	135.15	8.45	408	435.82	496	6.01	0.10	0.02	0.12
6204	0.44	0.42	75.94	4.75	159	184.95	191	2.57	-0.02	0.07	0.05
9886	0.62	0.59	71.82	7.98	203	217.94	233	3.73	0.02	0.04	0.07
10273	0.83	0.78	156.83	17.43	325	355.76	376	5.87	0.06	0.04	0.10

Table 5.6.4, continued.

Mexican Spadefoot											
Locus	H_E	H_O	Var	VarRepN	Allele				F_{IS}	F_{ST}	F_{IT}
					Min	Mean	Max	Richness			
20	0.86	0.86	82.69	5.17	150	178.23	202	7.86	0.00	0.01	0.02
C7	0.8	0.81	89.23	5.58	232	243.26	268	6.3	-0.01	0.01	0.00
D103	0.69	0.68	77.36	4.84	133	140.55	169	5.24	0.03	0.00	0.03
D111	0.84	0.86	144.76	9.05	84	103.07	136	7.18	-0.01	0.02	0.00
D125	0.79	0.77	77.11	4.82	198	210.66	242	6.42	0.03	0.01	0.04
D7	0.07	0.06	4.77	0.3	212	212.4	232	1.56	0.14	0.02	0.16
H115	0.7	0.71	16.11	1.01	84	95.06	112	4.61	-0.01	0.01	0.00
H129	0.64	0.62	59.25	3.7	186	195.91	218	4.49	0.04	0.01	0.05

Table 5.6.5 STRUCTURE results and delta-*K* calculations for each species. Results shown for each genetic cluster with best delta-*K* in bold, and clusters are shown in Figure 5.2.

CANYON TREEFROG

Canyon treefrog - all populations

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln"(*K*)|	Delta *K*
1	-9093.82	0.18	NA	NA	NA
2	**-8537.00**	**1.04**	**556.82**	**167.31**	**160.66**
3	-8147.49	2.23	389.51	181.21	81.12
4	-7939.19	14.73	208.30	7.21	0.49
5	-7738.10	15.62	201.09	46.76	2.99
6	-7583.77	25.84	154.33	116.84	4.52
7	-7546.28	108.98	37.49	101.37	0.93
8	-7610.16	741.54	-63.88	190.59	0.26
9	-7483.45	523.98	126.71	10.71	0.02
10	-7367.45	95.81	116.00	187.83	1.96
11	-7439.28	134.99	-71.83	16.60	0.12
12	-7494.51	138.59	-55.23	22.23	0.16
13	-7571.97	91.26	-77.46	35.08	0.38
14	-7684.51	270.39	-112.54	66.86	0.25
15	-7730.19	186.78	-45.68	14.96	0.08
16	-7760.91	300.21	-30.72	NA	NA

Canyon treefrog - western group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln"(*K*)|	Delta *K*
1	-7356.15	0.39	NA	NA	NA
2	**-6973.11**	**0.63**	**383.04**	**167.45**	**264.43**
3	-6757.52	12.86	215.59	18.68	1.45
4	-6560.61	17.87	196.91	39.79	2.23
5	-6403.49	6.76	157.12	123.87	18.33
6	-6370.24	18.98	33.25	22.25	1.17
7	-6314.74	33.30	55.50	87.91	2.64
8	-6347.15	25.78	-32.41	77.86	3.02
9	-6457.42	124.04	-110.27	88.56	0.71
10	-6479.13	85.90	-21.71	8.46	0.10
11	-6509.30	70.28	-30.17	31.15	0.44
12	-6570.62	102.93	-61.32	56.82	0.55
13	-6688.76	189.85	-118.14	NA	NA

Canyon treefrog - northwestern group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln"(*K*)|	Delta *K*
1	-2462.50	0.57	NA	NA	NA
2	**-2248.47**	**0.94**	**214.03**	**253.09**	**268.76**
3	-2287.53	17.77	-39.06	23.63	1.33
4	-2302.96	14.28	-15.43	16.94	1.19
5	-2301.45	13.78	1.51	NA	NA

Canyon treefrog - Santa Rita group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln"(*K*)|	Delta *K*
1	**-1690.58**	**0.38**	**NA**	**NA**	**NA**

Table 5.6.5, continued.

2	-1693.03	3.14	-2.45	0.12	0.04
3	-1695.36	4.32	-2.33	5.49	1.27
4	-1692.20	1.00	3.16	NA	NA
Canyon treefrog - southwestern group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-4457.82	0.24	NA	NA	NA
2	-4292.70	33.15	165.12	4.37	0.13
3	**-4131.95**	**11.36**	**160.75**	**105.96**	**9.33**
4	-4077.16	17.13	54.79	87.08	5.08
5	-4109.45	160.49	-32.29	84.61	0.53
6	-4057.13	24.78	52.32	99.66	4.02
7	-4104.47	56.91	-47.34	4.32	0.08
8	-4156.13	63.66	-51.66	44.54	0.70
9	-4252.33	121.29	-96.20	NA	NA
Canyon treefrog - Huachuca and Canelo group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-2770.95	0.54	NA	NA	NA
2	**-2754.07**	**5.33**	**16.88**	**53.81**	**10.09**
3	-2791.00	30.92	-36.93	10.50	0.34
4	-2817.43	36.99	-26.43	45.92	1.24
5	-2889.78	57.80	-72.35	110.59	1.91
6	-2851.54	29.00	38.24	NA	NA
Canyon treefrog - Canelo group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	**-660.86**	**0.43**	**NA**	**NA**	**NA**
2	-662.53	2.25	-1.67	2.25	1.00
3	-661.95	1.16	0.58	NA	NA
Canyon treefrog - northern Huachuca group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-2001.53	0.46	NA	NA	NA
2	-2030.85	38.31	-29.32	22.21	0.58
3	-2037.96	43.77	-7.11	25.50	0.58
4	-2019.57	28.44	18.39	NA	NA
Canyon treefrog - Carr Canyon group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-546.61	0.40	NA	NA	NA
2	**-490.71**	**3.44**	**55.90**	**69.73**	**20.24**
3	-504.54	13.51	-13.83	NA	NA
Canyon treefrog - eastern group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-1125.51	0.46	NA	NA	NA
2	**-938.96**	**0.60**	**186.55**	**223.24**	**369.57**
3	-975.65	20.74	-36.69	4.19	0.20
4	-1008.15	23.66	-32.50	NA	NA

Table 5.6.5, continued.

Canyon treefrog - Dragoons group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	**-687.94**	**0.69**	**NA**	**NA**	**NA**
2	-689.36	1.76	-1.42	1.81	1.03
3	-688.97	1.51	0.39	0.18	0.12
4	-688.76	1.94	0.21	NA	NA

RED-SPOTTED TOAD

Red-spotted toad - all populations

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-13215.02	0.46	NA	NA	NA
2	-12971.37	13.98	243.65	102.01	7.30
3	**-12829.73**	**8.44**	**141.64**	**63.70**	**7.55**
4	-12751.79	27.19	77.94	36.58	1.35
5	-12710.43	29.91	41.36	0.06	0.00
6	-12669.01	71.29	41.42	49.47	0.69
7	-12677.06	43.01	-8.05	30.23	0.70
8	-12654.88	64.53	22.18	116.90	1.81
9	-12749.60	178.93	-94.72	177.69	0.99
10	-13022.01	407.90	-272.41	298.99	0.73
11	-12995.43	403.13	26.58	325.88	0.81
12	-13294.73	468.77	-299.30	300.26	0.64
13	-13894.29	867.01	-599.56	929.46	1.07
14	-13564.39	505.81	329.90	753.24	1.49
15	-13987.73	953.76	-423.34	113.54	0.12
16	-14297.53	757.84	-309.80	NA	NA

Red-spotted toad - Huachuca group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	**-7728.50**	**0.40**	**NA**	**NA**	**NA**
2	-7739.63	9.16	-11.13	5.23	0.57
3	-7745.53	76.76	-5.90	104.14	1.36
4	-7855.57	181.07	-110.04	125.11	0.69
5	-7840.50	283.73	15.07	293.10	1.03
6	-8118.53	159.93	-278.03	11.19	0.07
7	-8407.75	350.29	-289.22	442.65	1.26
8	-8254.32	234.76	153.43	NA	NA

Red-spotted toad - Northern group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-4553.32	0.32	NA	NA	NA
2	-4449.05	8.94	104.27	18.66	2.09
3	-4363.44	29.85	85.61	20.58	0.69
4	**-4298.41**	**5.18**	**65.03**	**72.03**	**13.90**
5	-4305.41	16.50	-7.00	26.40	1.60
6	-4338.81	52.72	-33.40	3.73	0.07
7	-4375.94	36.93	-37.13	59.32	1.61

Table 5.6.5, continued.

8	-4472.39	58.42	-96.45	9.56	0.16
9	-4559.28	174.10	-86.89	NA	NA
Red-spotted toad - Northern sub-group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	**-2372.74**	**0.48**	**NA**	**NA**	**NA**
2	-2430.49	33.76	-57.75	35.43	1.05
3	-2452.81	87.30	-22.32	79.44	0.91
4	-2395.69	26.53	57.12	52.34	1.97
5	-2390.91	33.63	4.78	NA	NA
MEXICAN SPADEFOOT					
Mexican spadefoot - all populations					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-19503.12	0.18	NA	NA	NA
2	**-19351.07**	**6.00**	**152.05**	**68.00**	**11.33**
3	-19267.02	11.99	84.05	104.55	8.72
4	-19287.52	41.90	-20.50	110.89	2.65
5	-19418.91	160.98	-131.39	59.02	0.37
6	-19491.28	197.55	-72.37	1.44	0.01
7	-19565.09	289.89	-73.81	51.89	0.18
8	-19587.01	270.93	-21.92	9.04	0.03
9	-19617.97	181.86	-30.96	130.94	0.72
10	-19779.87	355.34	-161.90	284.66	0.80
11	-19657.11	235.36	122.76	277.71	1.18
12	-19812.06	273.94	-154.95	139.42	0.51
13	-19827.59	271.40	-15.53	212.04	0.78
14	-19631.08	210.34	196.51	250.04	1.19
15	-19684.61	217.28	-53.53	36.77	0.17
16	-19774.91	149.92	-90.30	158.59	1.06
17	-19706.62	130.22	68.29	258.44	1.98
18	-19896.77	365.91	-190.15	310.33	0.85
19	-19776.59	168.08	120.18	17.39	0.10
20	-19673.80	185.09	102.79	136.42	0.74
21	-19707.43	107.20	-33.63	8.41	0.08
22	-19732.65	220.54	-25.22	100.37	0.46
23	-19657.50	80.49	75.15	90.43	1.12
24	-19672.78	127.48	-15.28	59.26	0.46
25	-19747.32	242.03	-74.54	9.78	0.04
26	-19812.08	334.43	-64.76	NA	NA
Mexican spadefoot - Santa Rita group					
K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	**-1758.44**	**0.53**	**NA**	**NA**	**NA**
2	-1797.04	29.55	-38.60	14.24	0.48
3	-1821.40	37.96	-24.36	1.36	0.04
4	-1847.12	29.46	-25.72	NA	NA

Table 5.6.5, continued.

Mexican spadefoot - Huachuca group

K	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	-17565.59	0.07	NA	NA	NA
2	**-17497.87**	**10.61**	**67.72**	**129.18**	**12.17**
3	-17559.33	32.91	-61.46	24.50	0.74
4	-17596.29	83.92	-36.96	128.06	1.53
5	-17761.31	154.30	-165.02	0.93	0.01
6	-17927.26	178.78	-165.95	141.40	0.79
7	-17951.81	204.93	-24.55	95.89	0.47
8	-18072.25	302.79	-120.44	260.18	0.86
9	-17932.51	263.72	139.74	245.68	0.93
10	-18038.45	226.66	-105.94	96.13	0.42
11	-18048.26	243.41	-9.81	84.39	0.35
12	-17973.68	275.75	74.58	120.49	0.44
13	-18019.59	335.17	-45.91	109.97	0.33
14	-17955.53	137.98	64.06	69.75	0.51
15	-17961.22	154.04	-5.69	100.90	0.66
16	-18067.81	303.65	-106.59	266.85	0.88
17	-17907.55	147.00	160.26	261.14	1.78
18	-18008.43	248.44	-100.88	31.08	0.13
19	-18140.39	380.27	-131.96	285.94	0.75
20	-17986.41	220.01	153.98	87.47	0.40
21	-17919.90	158.72	66.51	41.97	0.26
22	-17895.36	169.39	24.54	52.57	0.31
23	-17923.39	144.17	-28.03	74.31	0.52
24	-17877.11	110.27	46.28	NA	NA

Table 5.6.6 Comparison of allelic richness, observed and expected heterozygosity, N_e (median estimate and the count of upper confidence intervals that include infinite population sizes), and genetic differentiation (G'_{ST}) between sampling methods for red-spotted toads and spadefoots.

Red-spotted toad	Allelic richness	H_O	H_E	N_e	N_e infinite C. I.	G'_{ST}
Adults	5.781	0.776	0.764	81.8	2 of 4	0.235
Larvae	5.673	0.749	0.772	35.0	1 of 4	0.380

Mexican spadefoot	Allelic richness	H_O	H_E	N_e	N_e infinite C. I.	G'_{ST}
Adults	4.985	0.667	0.664	618.90	10 of 11	0.103
Larvae	5.020	0.654	0.673	*Infinite*	10 of 11	0.077
Breeding Adults	4.937	0.656	0.667	732.10	6 of 6	0.102
Roadside Adults	5.024	0.677	0.661	197.60	4 of 5	0.099

Table 5.6.7 Spatial and temporal sampling replicates and genetic diversity (expected and observed heterozygosity, allelic richness, N_e, and the upper confidence interval for N_e estimate calculated via a jackknifing method). Significant results shown in bold font.

All species		
Paired t-test	*t*	*p-val*
H_E	1.723	0.100
H_O	-1.128	0.273
AR	1.191	0.248
Wilcoxon signed-rank test	*V*	*p-val*
N_e	101	0.5136
N_e *(upper C.I.)*	22	1
Canyon treefrog and red-spotted toads		
Paired t-test	*t*	*p-val*
H_E	**2.777**	**0.024**
H_O	-0.378	0.715
AR	1.707	0.126
Wilcoxon signed-rank test	*V*	*p-val*
N_e	19	0.944
N_e *(upper C.I.)*	9	0.447

5.7 Appendix B, *landscape resistance methods and additional landscape genetics results*

5.7.1 Landscape resistance and distance details and construction

We hypothesized relationships between genetic and structural connectivity along a gradient of species water requirements. Final hypotheses were organized into six categories. Two categories imply no correlation with landscape factors: isolated populations (high genetic differentiation between sampling locations driven primarily by low migration, genetic drift, and/or small populations) and panmixia (low genetic differentiation due to high gene flow between all sampling locations and/or large populations). The other four categories imply a specific relationship between genetic connectivity and some landscape factor. These categories are summarized in Table 5.8.1. Each category – terrestrial, aquatic, topography, and isolation-by-distance – was evaluated using one or multiple landscape resistance surfaces built with spatial data. These resistance surfaces and data are summarized in Table 5.8.1, and details for data layers and sources are included in Table 5.8.2.

Hypothesized resistances of structural connectivity between sampling locations were built using CIRCUITSCAPE (McRae 2006), a program utilizing circuit theory to simulate gene flow through a resistance surface. CIRCUITSCAPE allows for gene flow (i.e., "current") to travel across multiple pathways, reporting pairwise summations of resistance between sampling locations. To generate these pairwise data, we built raster maps of resistance (low to high) using data in Table 5.8.2. A geographic information system (ArcGIS 10.1, Environmental Systems Research Institute) was used to catalog and manipulate landscape data and generate resistance raster maps. Each resistance map was scaled for hypothesized landscape resistance to gene flow from 1 - 100 where 1 indicates low resistance and 100 indicates high resistance. The scale of resistance values was arbitrary and was designed to reflect hypothesized relationships between landscape features and genetic connectivity. We examined two additional scales of resistance (1 - 1000 and 1 - 10,000), but resistances and the relationships with genetic connectivity were highly correlated ($r > 0.99$). Thus only results for resistances from 1 – 100 are included in this manuscript.

To balance demand for computational resources with maintaining enough detail to realistically examine structural connectivity, all resistance maps were scaled to 60 m resolution, which involved resampling of some data layers. The spatial extent of resistance rasters ensured a buffer of at least 30 km from the edge of the raster to a given sample site. With this grain and extent, we were able to perform all CIRCUITSCAPE analyses in the pairwise source/ground modeling mode and using a cell connection scheme of eight neighbors, allowing maximum freedom of current flow. Due to the location of one Mexican spadefoot sampling site near the US-Mexico border, the 30 km buffer required use of spatial data from the US and Mexico. Data are generally more widely available and higher resolution for the US, and in some cases in our study Mexico's data are courser resolution (Table 5.8.2). However, it is unlikely that the resolution of data for Mexico would skew structural connectivity estimates between populations of any species, particularly with only one sample site very near the border.

Some landscape resistance layers had moderate to strong correlations with one another. These correlations are summarized in Table 5.8.3 and were taken into account in all analyses of genetic and structural connectivity relationships.

Mixed-effects modeling results for canyon treefrog and Mexican spadefoot genetic clusters
Mixed-effects modeling results (individual factors and couplet models) for canyon treefrog and Mexican spadefoot genetic clusters are presented in Table 5.8.4. Methods and results are presented in the main text.

5.7.2 Multiple regression with distance matrices (MRDM) methods and results

MRDM evaluates relationships between one or many explanatory distance matrices and a response distance matrix and uses permutation to determine statistical significance of the overall model. MRDM also informs significance of each explanatory variable in a model, and those significance values can help elucidate drivers among correlated structural connectivity hypotheses. We included one distance matrix (with the highest R^2_β value as calculated by mixed-effects modeling) from each structural connectivity category to build global MRDM models. For cases in which multiple variables were included in the supported model, we evaluated the variance inflation factor of each predictor variable. A variance inflation factor > 10 is considered a threshold at which collinearity of variables is problematic for interpreting model results (Kutner et al. 2004). Variance inflation factor was evaluated using the linear form of each model with the R package "car" (Fox and Weisberg 2011). Pairwise landscape resistances were then dropped according to significance (least significant dropped first) until only one resistance layer remained. We inferred strength of model fit by evaluating overall R^2 and the significance of explanatory variables (pairwise landscape resistances) included in the model. MRDM results are summarized in the main text of the manuscript and are presented in Table 5.8.5 of this appendix.

5.7.3 References

Fox, J., and S. Weisberg. 2011. An R Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Kutner, M., C. Nachsheim, and J. Netter. 2004. Applied Linear Regression Models. McGraw-Hill, New York, NY.

McRae, B. H. 2006. Isolation by resistance. Evolution **60**:1551-1561.

Table 5.7.1 Descriptions and data used to construct resistance layers for each connectivity hypothesis (data described in Table 5.8.2, and note that data used often include different sources for Arizona and Mexico). Isolated population and panmixia hypotheses presented in text are not included here because they are not associated with effects of any particular landscape driver. Additional details available upon request from M.C. Mims.

Resistance layers	Description	Data used	Type
Terrestrial			
Canopy	Resistance decreases with increased canopy cover.	Canopy Cover - Arizona; Canopy Cover - Mexico	Categorical
Urban	Resistance increases with development.	Urbanization - Arizona; Urbanization - Mexico	Categorical
LandCov	Resistance is lowest with high canopy cover and highest for high development.	Combination of Canopy and Urbanization resistance layers (reclassified)	Categorical
Aquatic			
Stream	Resistance is lowest for streams and ponds, moderate for ephemeral streams, and highest for areas with no aquatic habitat.	Streams - Arizona; Streams - Mexico; Ponds and lakes - Arizona; Geology - Arizona; Geology - North America; Streamflow permanence - geological inference; Streamflow permanence - known perennial reaches	Categorical
PrecipET	Resistance decreases as summer precipitation-evapotranspiration increases.	Precipitation - Arizona; Precipitation - Mexico; Evapotranspiration	Continuous
AvgWater	Resistance is lowest where precipitation is highest and aquatic habitat is available and is highest in dry areas with no aquatic habitat.	Average Stream and PrecipET resistance layers	Categorical + Continuous
Topography			
Slope	Resistance increases with slope.	Slope from digital elevation model (DEM)	Continuous
Isolation-by-Distance			
Euclidean	Pairwise Euclidean distance between sampling locations.	Euclidean distance	Continuous
Null	Uniform landscape resistance.	Null model	Uniform

Table 5.7.2 Data, details, resolution, and source information for spatial and environmental data used to create resistance layers.

Data	Details	Resolution	Source
Canopy cover - Arizona	2001 NLCD canopy density dataset.	30m	http://www.mrlc.gov/nlcd01_data.php
Canopy cover - Mexico	USGS Land Cover Institute.	250m	http://landcover.usgs.gov/nalcms.php
Urbanization - Arizona	2006 NLCD land cover dataset.	30m	http://www.mrlc.gov/nlcd2006.php
Urbanization - Mexico	USGS Land Cover Institute.	250m	http://landcover.usgs.gov/nalcms.php
Streams - Arizona	NHDPlus Version 2, downloaded from National Map Viewer (USGS).	*shapefile*	http://viewer.nationalmap.gov/viewer/
Streams - Mexico	Stream network supplied by Dale Turner, The Nature Conservancy.	*shapefile*	Dale Turner, The Nature Conservancy, personal request.
Ponds and lakes - Arizona	NHDPlus Version 2, downloaded from National Map Viewer (USGS).	*shapefile*	http://viewer.nationalmap.gov/viewer/
Geology - Arizona	Surface deposits identified for characterization of ephemeral streams.	*shapefile*	http://mrdata.usgs.gov/geology/state/state.php?state=AZ
Geology - North America	Surface deposits identified for characterization of ephemeral streams.	*shapefile*	http://ngmdb.usgs.gov/gmna/
Streamflow permanence - geological inference	Steamflow permanence records (Huachuca Mountains) as a function of geology.	*N/A*	Kristin Jaeger and Julian Olden, unpublished data.
Streamflow permanence - known perennial reaches	The Nature Conservancy, US Fish and Wildlife Service: documented and well-known perennial stream reaches.	*N/A*	http://azconservation.org/map_gallery/san_pedro_river_surface_water http://www.fws.gov/southwest/federal_assistance/pdfs/chapter%2010%20santa%20cruz%20river%20watershed.pdf
Precipitation - Arizona	PRISM: 30-year precipitation averages for months of June-Oct, 1981-2010.	800m	http://prism.nacse.org/normals/
Precipitation - Mexico	Climate Wizard: 50+ year precipitation averages, June-Oct, 1951-2002.	50km	http://www.climatewizard.org/

Table 5.7.2, continued.

Data	Details	Resolution	Source
Evapotranspiration	MODIS Global Terrestrial Evapotranspiration Data Set, June-Oct, 2000-2010.	5km	http://www.ntsg.umt.edu/project/mod16
Slope	Calculated with 9-m Digital Elevation Model from National Elevation Dataset.	9m	http://ned.usgs.gov/downloads.asp
Euclidean distance	Pairwise distances calculated using UTM location data in PASSaGE 2.	*distance*	http://www.passagesoftware.net/
Null model	Uniform resistance layer.	*flexible*	

Table 5.7.3 Pearson correlation coefficients between pairwise resistance/distance values for each species and their major genetic clusters, identified in Figure 5.2 of the main text.

Canyon treefrog

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.811							
Urban	0.967	0.745						
LandCov	0.859	0.990	0.794					
PrecipET	0.946	0.853	0.906	0.897				
Slope	0.167	-0.066	0.265	-0.026	0.024			
Stream	0.955	0.707	0.920	0.756	0.811	0.278		
Null	0.971	0.748	0.998	0.798	0.913	0.245	0.922	
Eucl	0.965	0.744	0.995	0.794	0.904	0.239	0.921	0.997

Red-spotted toad

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.842							
Urban	0.894	0.800						
LandCov	0.898	0.988	0.873					
PrecipET	0.945	0.876	0.857	0.913				
Slope	0.394	0.047	0.357	0.159	0.311			
Stream	0.966	0.758	0.845	0.820	0.830	0.412		
Null	0.946	0.826	0.951	0.894	0.925	0.446	0.883	
Eucl	0.906	0.797	0.894	0.861	0.882	0.457	0.848	0.949

Mexican spadefoot

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.842							
Urban	0.894	0.800						
LandCov	0.898	0.988	0.873					
PrecipET	0.945	0.876	0.857	0.913				
Slope	0.394	0.047	0.357	0.159	0.311			
Stream	0.966	0.758	0.845	0.820	0.830	0.412		
Null	0.946	0.826	0.951	0.894	0.925	0.446	0.883	
Eucl	0.906	0.797	0.894	0.861	0.882	0.457	0.848	0.949

Canyon treefrog - west

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.724							
Urban	0.955	0.648						
LandCov	0.786	0.991	0.707					
PrecipET	0.911	0.795	0.859	0.849				
Slope	0.080	-0.237	0.234	-0.189	-0.176			
Stream	0.950	0.595	0.900	0.658	0.738	0.247		
Null	0.960	0.649	0.998	0.710	0.871	0.209	0.899	
Eucl	0.872	0.591	0.922	0.639	0.751	0.306	0.846	0.923

Table 5.7.3 continued.

Canyon treefrog - Huachuca Mountains

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.523							
Urban	0.948	0.412						
LandCov	0.637	0.981	0.513					
PrecipET	0.933	0.700	0.836	0.798				
Slope	-0.284	-0.588	-0.106	-0.542	-0.497			
Stream	0.935	0.285	0.914	0.406	0.747	-0.039		
Null	0.956	0.401	0.998	0.506	0.850	-0.133	0.918	
Eucl	0.846	0.348	0.886	0.431	0.756	-0.204	0.804	0.894

Red-spotted toad - north

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.877							
Urban	0.894	0.754						
LandCov	0.926	0.984	0.851					
PrecipET	0.956	0.798	0.908	0.851				
Slope	0.093	-0.225	0.194	-0.091	0.086			
Stream	0.966	0.887	0.802	0.923	0.849	0.067		
Null	0.892	0.752	1.000	0.849	0.907	0.199	0.800	
Eucl	0.819	0.642	0.968	0.751	0.868	0.256	0.700	0.966

Red-sotted toad - Huachuca Mountains

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.585							
Urban	0.927	0.363						
LandCov	0.687	0.985	0.494					
PrecipET	0.972	0.548	0.956	0.665				
Slope	-0.271	-0.427	0.036	-0.397	-0.204			
Stream	0.983	0.603	0.865	0.688	0.914	-0.324		
Null	0.915	0.324	0.998	0.457	0.943	0.064	0.855	
Eucl	0.786	0.182	0.913	0.311	0.864	0.090	0.689	0.918

Mexican spadefoot - east

	AvgWat	Canopy	Urban	LandCov	PrecipET	Slope	Stream	Null
Canopy	0.821							
Urban	0.881	0.779						
LandCov	0.881	0.988	0.856					
PrecipET	0.941	0.858	0.846	0.900				
Slope	0.265	-0.139	0.197	-0.028	0.207			
Stream	0.961	0.728	0.827	0.795	0.814	0.278		
Null	0.940	0.805	0.941	0.878	0.923	0.299	0.867	
Eucl	0.904	0.782	0.879	0.848	0.883	0.291	0.837	0.948

Table 5.7.4 Mixed-effects modeling results for major genetic clusters for canyon treefrogs (CT-W: canyon treefrog – west; CT-H canyon treefrog - Huachucas) and Mexican spadefoot (MS-E: Mexican spadefoot - east). Genetic clusters are identified in Figure 2 of the main text. Top R^2_β values for single-resistance (top) and couplet-resistance (below) models are highlighted in bold font. IP and P hypothesize no landscape effect, indicated by poor model performance across all other models. A dash indicates no support for IP or P. All R^2_β correlation coefficients are positive with the exception of a negative relationship with Slope in models denoted with underlined text.

Hypotheses	Resistance layers/distance	R^2_β, mixed-effects models		
Species		CT-W	CT-H	MS-E
Isolated populations (IP)	*N/A*	-	-	-
Terrestrial (TE)	Canopy	0.64	0.06	0.22
	Urban	0.73	**0.80**	0.21
	LandCov	0.68	0.51	0.23
Aquatic (A)	Stream	0.71	0.79	0.23
	PrecipET	**0.74**	0.76	0.22
	AvgWater	0.73	0.79	0.23
Topography (T)	Slope	0.46	0.61	0.16
Isolation-by-distance (IBD)	Eucl	0.61	0.58	**0.29**
	Null	0.72	**0.80**	0.22
Panmixia (P)	*N/A*	-	-	-
TE + T	Best of TE + Slope	**0.86**	**0.85**	0.44
A + T	Best of A + Slope	0.85	0.84	0.22
IBD + T	Eucl + Slope	0.73	0.67	0.39
	Null + Slope	**0.86**	**0.85**	**0.46**

Table 5.7.5 MRDM results by species. Correlation between genetic distance and landscape resistance were consistent within a species or group and are shown as positive (+) or negative (-). R^2 and p-values generated from permutation tests are shown for the overall model, and p-values for each factor are included. Best models shown in bold font, and for best models with > 2 factors, variance inflation factors (*vif*) are included in italicized text just below each factor in the model. *For Mexican spadefoots, the Stream-only and Euclidean-only models performed similarly. A partial mantel test was used to evaluate correlation between genetic distance and either Stream or Euclidean while controlling for the other. The partial mantel statistic was more significant for Stream (p = 0.030) than for Euclidean (p = 0.093). †*vif* > 10 indicates high collinearity among predictor variables. Note that for both canyon treefrog clusters, *vif* values suggest interpreting results with caution.

Canyon treefrog						
	Null	AvgWat	Slope	LandCov	R^2	p-val
correlation	+	+	-	-		
1	0.201	0.980	0.945	0.915	0.54	0.001
2	0.002		0.954	0.915	0.54	0.001
3	0.002			0.928	0.54	0.001
4	**0.001**				**0.54**	**0.001**
Red-spotted toad						
	Null	Stream	Slope	Urban	R^2	p-val
correlation	-	+	+	+		
1	0.097	0.001	0.019	0.335	0.62	0.002
2	**0.009**	**0.001**	**0.023**		**0.61**	**0.001**
vif	*6.0*	*5.58*	*1.39*			
3	0.121	0.009			0.47	0.003
4		0.001			0.40	0.001
Mexican spadefoot						
	Eucl	Stream	Slope	LandCov	R^2	p-val
correlation	+	+	+	+		
1	0.256	0.179	0.668	0.956	0.44	0.001
2	0.234	0.129	0.51		0.44	0.001
3	0.138	0.095			0.43	0.001
4*		**0.001**			**0.40**	**0.001**
5	**0.001**				**0.38**	**0.001**
Red-spotted toad - north						
	Null	Stream	Slope	Lan	R^2	p-val
correlation	-	+	+	+		
1	0.068	0.259	0.034	0.928	0.73	0.027
2	**0.026**	**0.017**	**0.025**		**0.73**	**0.007**
vif	*2.95*	*2.85*	*1.07*			
3		0.15	0.059		0.48	0.046
4			0.061		0.40	0.061

Table 5.7.5, continued.

Red-spotted toad - Huachuca Mountains

	Null	Stream	Slope	Lan	R^2	p-val
correlation	-	+	+	+		
1	0.303	0.383	0.237	0.552	0.42	0.582
2	0.179	0.171	0.302		0.36	0.384
3	0.527	0.593			0.08	0.737
4	0.731				0.01	0.731

Canyon treefrog - west

	Null	PrecipET	Slope	Urban	R^2	p-val
correlation	+	-	-	-		
1	0.109	0.001	0.003	0.465	0.72	0.001
2	**0.001**	**0.001**	**0.002**		**0.71**	**0.001**
vif	*9.50*	*9.37*	*2.36*			
3	0.001	0.173			0.51	0.001
4	0.001				0.45	0.001

Canyon treefrog - Huachuca Mountains

	Null	AvgWat	Slope	Urban	R^2	p-val
correlation	+	-	-	-		
1	0.006	0.006	0.256	0.047	0.78	0.001
2	**0.005**	**0.006**		**0.019**	**0.74**	**0.001**
vif[†]	*15.39*	*16.44*		*1.44*		
3	0.014	0.071			0.55	0.012
vif[†]	*11.65*	*11.65*				
4	0.001				0.34	0.001

Mexican spadefoot - east

	Eucl	AvgWat	Slope	LandCov	R^2	p-val
correlation	+	+	-	-		
1	0.594	0.048	0.167	0.305	0.36	0.006
2		0.028	0.186	0.36	0.36	0.006
3		0.001	0.345		0.34	0.001
4		**0.001**			**0.31**	**0.001**

6. Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects

6.1 Introduction

The relationship between physical landscape structure and the population dynamics of individual species is often complex. Organisms that occupy nearly-identical geographic ranges may exhibit radically different population structures, and these differences will depend on the biological attributes of each species: their dispersal abilities, habitat requirements, life histories, and other factors. In particular, dispersal ability and habitat requirements are expected to have strong influences on gene flow, genetic drift, and other population-level process. Dispersal (which facilitates gene flow) between populations can be limited for species with strict habitat requirements, especially when that habitat is fragmented or rare in the landscape (Bonte et al. 2003), but such limitations can be overcome by species with strong dispersal abilities. Landscape features representing critical habitat requirements can interact with dispersal ability to either inhibit or facilitate the movement of individuals between habitat patches (Manel 2003). Similarly, the mode of dispersal may determine how different species respond to the same landscape features (Goldberg and Waits 2010).

For the long-term conservation management of species in the wild, it is essential to understand how processes such as gene flow and genetic drift affect genetic diversity within each species of concern (Toro and Caballero 2005; Allendorf and Luikart 2012), yet it is intractable to collect population-level data on every species in the landscape. Thus, an important goal of population genetics is to find general relationships between organisms with particular biological attributes (dispersal ability, habitat requirements, life history) and their population genetic structures across the landscape. Such information is especially needed in the context of climate change, where shifting habitat distributions are likely to affect rates of gene flow among populations as well as individual population sizes (Rice and Emery 2003).

Differences in dispersal ability and habitat requirements can lead to demographic processes that favor strong isolation of populations, step-wise connectivity across the landscape, or panmixia. Genetic differentiation between a pair of populations or among a set of populations reaches an equilibrium level when the homogenizing effect of gene flow is balanced by the differentiation that occurs due to genetic drift (for neutral loci when mutation is negligible). The stochastic evolutionary force of genetic drift occurs within each population and is a function of effective population size (N_e; Nei and Tajima 1981). Hutchison and Templeton (1999), building on Slatkin (1993), presented a framework to describe the relationship between pairwise genetic distance and geographic (Euclidean) distance that would arise under conditions of regional equilibrium as well as several forms of disequilibrium. Under equilibrium conditions, the line-of-best-fit will have an intercept near zero and a significant, positive slope (Type B in Figure 6.1). Variance in genetic distance will increase with geographic distance in this case, such that there is more scatter in the y-axis at greater geographic distances. This is the pattern produced at equilibrium under an isolation-by-distance (IBD) process, when gene flow follows a stepping-stone model (Malécot 1955, Kimura and Weiss 1964). This IBD pattern will not be observed when either gene flow or genetic drift is more influential. In the situation where one of these forces overwhelms the other we expect a flat line-of-best-fit (i.e. a non-significant slope). When gene flow is minimal and populations are diverging randomly due to drift, genetic distances are expected to be large at all spatial scales and the intercept should be well above zero (Type A in Figure 6.1). Variance in this case is expected to be high at all geographic distances. Populations are evolving mostly independently since they are isolated from each other by minimal gene flow. When gene flow is high across the region, genetic distances should be small and variance will be low at all geographic distances (Type C in Figure 6.1).

This is essentially panmixia, where there is only one homogeneous genetic group in the region.The patterns described above assume that geographic distances are calculated as Euclidean distance; however, many other intervening landscape features can affect rates of gene flow between pairs of populations. In this case a significant linear relationship between genetic and geographic distance may be detectable if the calculated geographic distance captures the effect of the landscape feature (Manel et al. 2003). For example, if dry land is a barrier to dispersal in an aquatic species, such that gene flow occurs exclusively along streams connected in a network, it may be that an association will only be revealed when pairwise genetic distances are regressed on pairwise stream network distances (Funk et al. 2005). Numerous methods in the field of landscape genetics aim to detect such relationships among multiple alternative landscape models (Balkenhol et al. 2009). As with Euclidean distance, significant relationships using landscape distances should be detected only when there is a regional equilibrium between gene flow and genetic drift.

In this study, we tested for linear relationships between genetic and geographic distances to determine how dispersal ability and habitat structure interact to affect the regional equilibrium of gene flow and drift within three aquatic insect species: a flightless giant water bug, *Abedus herberti* (Hemiptera: Belostomatidae); a moderate-disperser stonefly, *Mesocapnia arizonensis* (Plecoptera: Capniidae); and a strong-disperser diving beetle, *Boreonectes* (= *Stictotarsus*) *aequinoctialis* (Coleoptera: Dytiscidae). The three species represent the large range of dispersal abilities (low, medium, high, respectively) and habitat requirements (perennial water specialist, intermittent water specialist, generalist, respectively) that are observed in desert stream insect communities (Schriever et al. accepted). The freshwater habitats of these species are distributed across the Madrean Sky Island region of southwestern North America. This region is generally arid and its freshwater habitats are often fragmented into isolated segments with little or no direct hydrologic connectivity (Brown and Lowe 1980). Habitat fragmentation will likely be more severe in the coming decades, as climate models predict that conditions in the southwest will become increasingly arid (Seager et al. 2007). Most streams in the Madrean Sky Islands are restricted to the higher elevations of small, disjunct mountain ranges, which are isolated from each another by a matrix of lowland desert. The three insects live in many of the same stream drainages but typically occupy different microhabitats within those drainages.

We hypothesized that each of the three insect species exhibits one of the three population genetic structure patterns described above (Type A-C in Figure 6.1, Plate 6.1). *A. herberti* is a large-bodied (24-50 mm total length), predatory water bug that requires perennial freshwater habitats at all life stages. These habitats include flowing streams, wetlands, and in the driest months, stagnant pools. Such habitats are relatively rare in the study region and have a fragmented distribution. *A. herberti* is flightless and can only disperse between aquatic habitats by crawling short distances over land (Lytle 1999; Boersma and Lytle 2014), a distribution that has led to among-population behavioral differentiation in the species (Lytle et al. 2008). Two previous genetic studies (Finn et al. 2007; Phillipsen and Lytle 2013) also found evidence of strong genetic differentiation among populations of *A. herberti*. Based on these studies and the biology of *A. herberti*, we predicted that genetic drift will overshadow gene flow in this species and that no strong, significant IBD relationship would be detectable (Type A in Figure 6.1).

The stonefly *M. arizonensis* specializes on temporally-intermittent stream reaches, where there is no above-ground flow for some part of the year, or even for years at a time. Individuals diapause as larvae in subsurface hyporheic zones and then emerge to complete their life cycle when surface flows resume following rains. Males are brachypterous; their wings are small and ineffective for flight, whereas females have larger, functional wings (Jacobi & Cary 1996). The presumed flightlessness of the males and weak flight capability of the females of this species suggest that dispersal may be restricted to short-to-moderate distances between patches of intermittent stream habitat. We predicted a pattern of significant isolation-by-distance (Type B in Figure 6.1) for *M. arizonensis*, given its moderate dispersal ability and the relative continuity of its intermittent stream habitat in the region.

The diving beetle *B. aequinoctialis* is a strong flier and presumably has a strong capacity for long-distance dispersal. Relative to the other two insects in our study, *B. aequinoctialis* is a habitat generalist. It is commonly found in most perennial and intermittent stream habitats in the study region, including ephemeral reaches that may hold water for only a few days. Because *B. aequinoctialis* is likely to exhibit strong dispersal and has few habitat limitations, we predicted that high rates of gene flow across the region would minimize genetic distances for this species and that we would not detect a significant IBD pattern (Type C in Figure 6.1).

For each species, we tested for linear relationships between genetic distances and geographic distances using Euclidean distance for the latter as well as landscape-based distances that reflect alternative hypotheses describing the permeability of the landscape for each of the insect species. We developed and evaluated landscape genetics models to determine which, if any, of the landscape features have an important influence on population structure. We were particularly interested in any effects of hydrological connectivity on genetic distance, since these species occupy stream habitats in an arid environment.

Figure 6.1 Predicted relationships between genetic distances and geographic (Euclidean) distances between pairs of populations. The black line is the regression line and the shaded area shows the spread (i.e. variance) in the pairwise genetic distances across the geographic distances. When genetic drift is more influential than gene flow (Type A), as is predicted for species with low dispersal, the slope of the line should not differ significantly from zero, the intercept will be high, and the variance will be high across all geographic distances. For species with moderate dispersal, a positive slope is predicted (Type B). The intercept should be near zero, and variance should increase with increasing geographic distance. Gene flow should be more influential than drift for species with high dispersal (Type C). The regression line in this case should not significantly deviate from zero, the intercept should be small, and variance is low across all geographic distances.

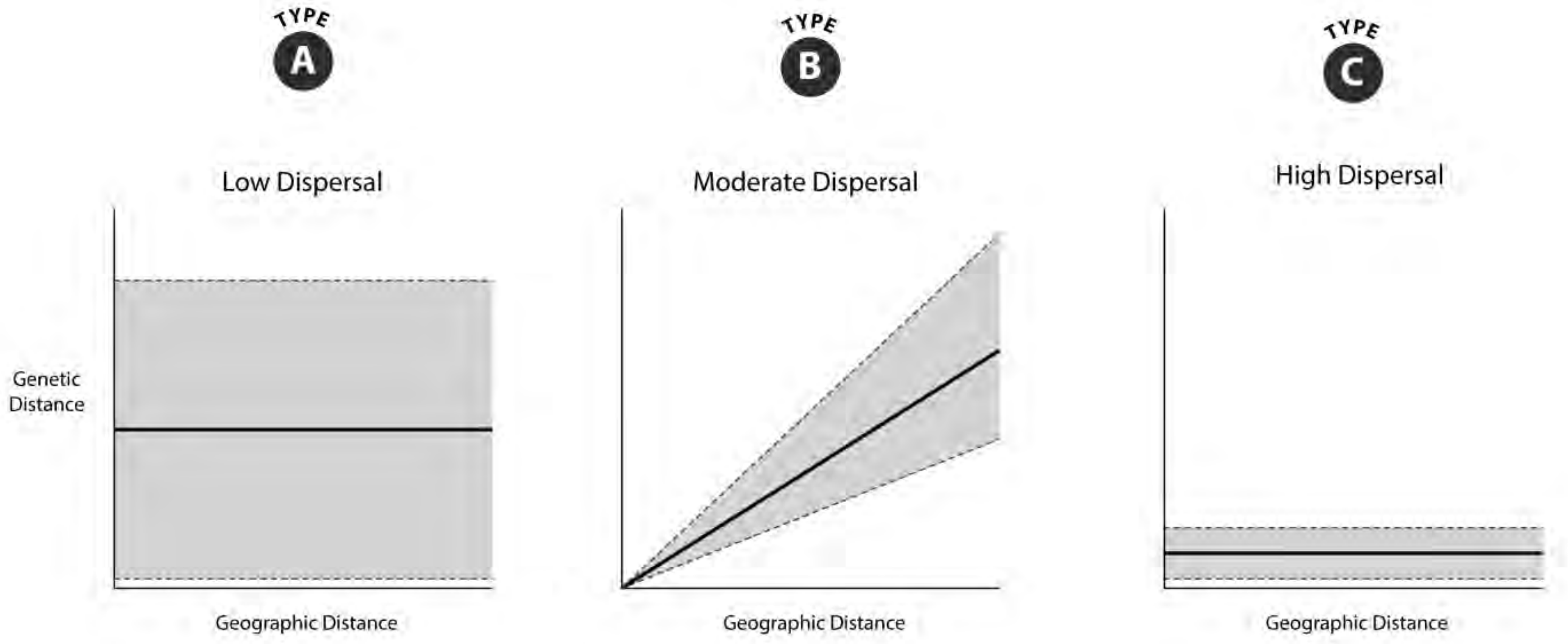


Plate 6.1 Three aquatic invertebrates of this study: *A. herberti* (top), *M. arizonensis* (lower left), and *B. aequinoctialis* (lower right). Photo credits: Mike Bogan.

6.2 Methods

6.2.1 Data Collection

Insect samples were collected in southeastern Arizona (USA) between 2010 and 2012 (Figure 6.2; Table 6.1). Most of the *A. herberti* samples were collected earlier, between 2002 and 2010 (Phillipsen and Lytle 2013). Whole adult specimens (and some larval specimens for *A. herberti* and *M. arizonensis* in a few localities) were sampled and preserved in 95% ethanol. Sampling for the three species was conducted at the same approximate spatial extent within the 25,000 km^2 study region (Figure 6.2, Plate 6.2). Sample sizes ranged from 9-94 (median=28) individuals per population for *A. herberti*, 10-42 (median=30) for *M. arizonensis*, and 9-55 (median=30) for *B. aequinoctialis* (Table 6.1).

Genomic DNA for *B. aequinoctialis, M. arizonensis,* and some *A. herberti* populations was extracted from tissue of each specimen using DNeasy Blood and Tissue Kits (QIAGEN). Multilocus microsatellite genotypes were generated for each individual using the protocols in Phillipsen & Lytle (2013), which was also the source of most of the *A. herberti* genotype data. Most of the data for *A. herberti* used in the present study was previously analysed by Phillipsen and Lytle (2013). A pilot analysis was used to identify useful microsatellite loci from a candidate set of >100 loci, based on sufficient among-population variability and conformity to Hardy-Weinberg equilibrium. Final numbers of microsatellite loci used per species were: 10 for *A. herberti* (Daly-Engel et al. 2012), 11 for *M. arizonensis*, and 8 for *B. aequinoctialis*. Information on the loci for the latter two species is provided in Appendix A. Loci were multiplexed for amplification via polymerase chain reaction (PCR) using Multiplex PCR kits (QIAGEN). Amplified PCR products were run on an ABI 3730 sequencer and individuals were genotyped using the GENEMAPPER 4.1 software (Applied Biosystems).

6.2.2 Population Genetics Analysis

We tested each sample of *n* individuals from a collection locality for deviation from HWE at each microsatellite locus using the program FSTAT (Goudet 2001). We generated two measures of genetic diversity for each sample: the average (across loci) expected heterozygosity of a sample (H_e) and average allelic richness (*AR*). For each species, the allelic richness of a sample was rarefied by the smallest number of complete genotypes among all the samples collected for that species.

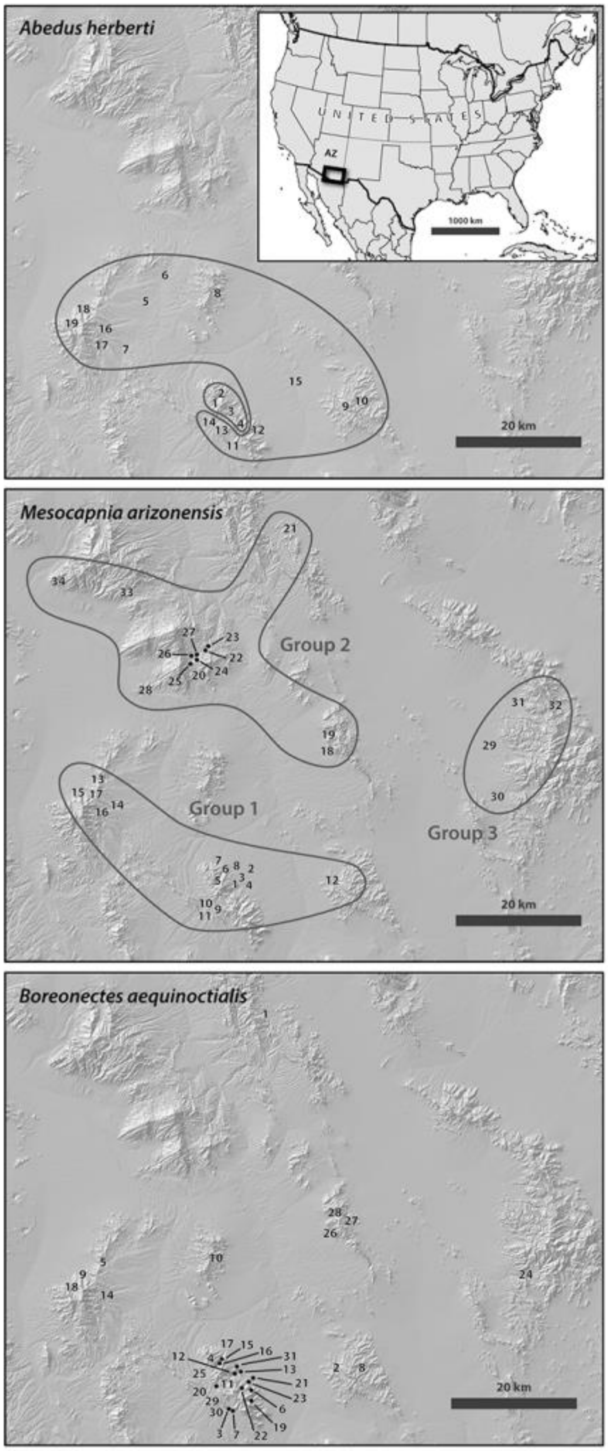
For the microsatellite loci used in our analyses, linkage disequilibrium was present for a few loci in some localities—however, disequilibrium was not consistent across any locus pairs in multiple sampling localities and there were no loci that deviated from HWE in more than a few localities. Genetic diversity also did not differ greatly among the three insects (Table 6.1). Although many of the samples were out of HWE in multilocus tests, as indicated by significant, positive F_{IS} values (Table 6.1), these deviations were influenced by only one or two loci in each population. This suggests that the cause of HWE deviations is not biological in nature, but technical (e.g. due to null alleles).

Table 6.1 Population sampling localities, sample sizes (*n*), geographic coordinates (UTM E and UTM N), and population metrics for *Abedus herberti*, *Mesocapnia arizonensis*, and *Boreonectes aequinoctialis* in southeastern Arizona, USA. Significant F_{IS} values are marked with an asterisk. In some cases, metrics were not calculated for a population due to small sample size.

Species	Locality	*n*	lat	lon	*AR*	H_o	H_e	F_{IS}	N_e
A. herberti	1	13	31.4996	-110.4094	3.56	0.52	0.55	0.07	-530 (15 - ∞)
	2	31	31.5187	-110.3875	4.52	0.57	0.68	0.17*	-1854 (87 - ∞)
	3	94	31.4732	-110.3511	4.38	0.59	0.68	0.14*	96 (51 - 334)
	4	63	31.4410	-110.3180	4.13	0.52	0.62	0.15*	669 (142 - ∞)
	5	38	31.7882	-110.6376	3.64	0.46	0.62	0.26*	3 (1 - ∞)
	6	25	31.8630	-110.5727	4.67	0.54	0.71	0.24*	20 (11 - 61)
	7	20	31.6518	-110.7045	3.64	0.48	0.56	0.14	25 (16 - 50)
	8	18	31.8104	-110.3943	3.89	0.46	0.64	0.30*	19 (11 - 41)
	9	24	31.4862	-109.9749	3.15	0.49	0.55	0.09	101 (53 - 480)
	10	28	31.4971	-109.9256	3.96	0.53	0.63	0.16*	68 (17 - ∞)
	11	30	31.3754	-110.3488	3.92	0.50	0.62	0.19*	684 (168 - ∞)
	12	9	31.4358	-110.2849	1.99	0.46	0.32	NA	74 (23 - ∞)
	13	21	31.4391	-110.3808	4.54	0.50	0.68	0.26*	110 (42 - ∞)
	14	31	31.4471	-110.4015	4.46	0.54	0.66	0.18*	9 (3 - 21)
	15	14	31.5563	-110.1401	4.14	0.46	0.66	0.31*	37 (24 - 65)
	16	50	31.7109	-110.7671	4.84	0.57	0.69	0.17*	150 (70 - 2999)
	17	11	31.6626	-110.7800	4.8	0.53	0.72	0.26*	-229 (24 - ∞)
	18	45	31.7596	-110.8441	4.23	0.57	0.63	0.10*	41 (14 - ∞)
	19	49	31.7278	-110.8805	4.06	0.52	0.64	0.18*	92 (32 - ∞)
M. arizonensis	1	34	31.5005	-110.3396	3.35	0.53	0.67	0.21*	-335 (360 - ∞)
	2	30	31.5222	-110.3169	3.37	0.51	0.69	0.26*	649 (79 - ∞)
	3	30	31.5176	-110.3209	3.29	0.56	0.67	0.17	-668 (96 - ∞)
	4	42	31.4953	-110.2891	3.55	0.57	0.72	0.21*	2552 (192 - ∞)
	5	30	31.5098	-110.3937	3.34	0.59	0.67	0.13	422 (92 - ∞)
	6	24	31.5453	-110.3715	3.3	0.49	0.67	0.27	-2340 (81 - ∞)
	7	11	31.5673	-110.3957	3.47	0.56	0.67	0.18*	-28 (-63 - ∞)
	8	30	31.5431	-110.3385	3.55	0.65	0.73	0.10	-185 (307 - ∞)
	9	30	31.4261	-110.4116	3.36	0.55	0.68	0.19*	-3082 (91 - ∞)
	10	36	31.4448	-110.4466	3.31	0.60	0.69	0.13	105 (55 - 457)
	11	30	31.4190	-110.4289	3.5	0.60	0.72	0.17*	-171 (55599 - ∞)
	12	10	31.5110	-110.0171	3.46	0.61	0.69	0.13	-36 (-113 - ∞)
	13	30	31.7993	-110.7975	3.59	0.57	0.72	0.21*	-180 (302 - ∞)
	14	23	31.7187	-110.7585	3.61	0.61	0.73	0.17	-528 (123 - ∞)
	15	30	31.7631	-110.8457	3.48	0.59	0.72	0.18	-133 (331 - ∞)
	16	30	31.7090	-110.7733	3.8	0.56	0.76	0.27*	-1863 (134 - ∞)
	17	30	31.7669	-110.8330	3.53	0.57	0.71	0.20*	-75 (-324 - ∞)
	18	30	31.8771	-110.0283	3.29	0.53	0.68	0.22*	-93 (212 - ∞)
	19	30	31.9198	-110.0292	3.3	0.50	0.7	0.30*	-101 (177 - ∞)
	20	30	32.0954	-110.4628	3.11	0.43	0.6	0.28*	-34 (-76 - ∞)
	21	30	32.5142	-110.1476	3.63	0.62	0.72	0.14	-330 (173 - ∞)
	22	30	32.1646	-110.4345	3.07	0.43	0.6	0.29*	-64 (332 - ∞
	23	31	32.1751	-110.4276	3.23	0.46	0.64	0.29*	-143 (69 - ∞)
	24	24	32.1419	-110.4622	3.26	0.50	0.64	0.23	-22 (-43 - ∞)
	25	14	32.1263	-110.4842	2.88	0.39	0.57	0.32*	-10 (-14 - ∞)
	26	14	32.1510	-110.4803	3.01	0.45	0.61	0.28	-7 (-9 - ∞)
	27	12	32.1509	-110.4621	3.25	0.51	0.65	0.21	-116 (52 - ∞)
	28	30	32.0503	-110.6392	3.21	0.51	0.65	0.21*	-64 (275 - ∞)
	29	18	31.8867	-109.4914	2.62	0.42	0.54	0.24	-39 (110 - ∞)

	30	30	31.7401	-109.4656	2.68	0.46	0.56	0.19	-101 (167 - ∞)
	31	30	32.0081	-109.3934	2.94	0.47	0.61	0.24*	-195 (108 - ∞)
	32	30	31.9997	-109.2718	2.83	0.46	0.59	0.23*	-86 (164 - ∞)
	33	14	32.3271	-110.6995	3.09	0.44	0.61	0.29*	-117 (42 - ∞)
	34	14	32.3608	-110.9281	3.33	0.53	0.66	0.21*	-26 (-66 - ∞)
B. aequinoctialis	1	30	32.5067	-110.2364	3.79	0.35	0.54	0.35*	-154 (94 - ∞)
	2	29	31.5008	-110.0046	3.39	0.36	0.47	0.25*	-57 (54 - ∞)
	3	16	31.3800	-110.3622	4.15	0.34	0.53	0.37*	349 (21.6 - ∞)
	4	37	31.5255	-110.4163	3.86	0.41	0.51	0.19	955 (43.9 - ∞)
	5	30	31.7982	-110.7806	3.64	0.37	0.47	0.23*	66 (23.1 - ∞)
	6	28	31.4379	-110.2855	3.62	0.36	0.52	0.31*	200 (22.4 - ∞)
	7	30	31.3744	-110.3495	3.93	0.36	0.52	0.31*	111 (26.3 - ∞)
	8	29	31.4971	-109.9255	3.89	0.39	0.49	0.22	-181 (42.3 - ∞)
	9	28	31.7629	-110.8456	3.73	0.39	0.48	0.19	-36 (-375.4 - ∞)
	10	20	31.8123	-110.3977	3.99	0.49	0.56	0.14	46 (17.1 - ∞)
	11	30	31.4542	-110.3758	3.89	0.41	0.52	0.21*	-41 (78.8 - ∞)
	12	36	31.4791	-110.3381	3.81	0.43	0.51	0.16	-53 (462 - ∞)
	13	30	31.4892	-110.3231	3.7	0.43	0.51	0.18	486 (28.1 - ∞)
	14	30	31.7109	-110.7671	3.94	0.33	0.53	0.38*	191 (35.2 - ∞)
	15	55	31.5187	-110.3875	3.45	0.40	0.46	0.13	-50 (-481.2 - ∞)
	16	23	31.5107	-110.3924	3.76	0.43	0.48	0.11	-81 (52 - ∞)
	17	16	31.5689	-110.3661	3.56	0.36	0.48	0.27*	-22 (258.6 - ∞)
	18	30	31.7293	-110.8815	3.94	0.39	0.54	0.28*	-45 (119.2 - ∞)
	19	29	31.4065	-110.2878	3.61	0.38	0.49	0.23*	-101 (65.2 - ∞)
	20	20	31.4313	-110.4454	3.33	0.40	0.46	0.13	40 (9.7 - ∞)
	21	16	31.4674	-110.2810	4	0.35	0.53	0.35*	-596 (21.8 - ∞)
	22	34	31.4410	-110.3180	3.64	0.40	0.49	0.19	551 (43 - ∞
	23	40	31.4591	-110.2957	3.64	0.37	0.49	0.25*	-93 (170.4 - ∞)
	24	30	31.7571	-109.3702	3.92	0.34	0.53	0.36*	-139 (55.7 - ∞)
	25	29	31.4471	-110.4015	3.83	0.38	0.51	0.26*	3115 (41.6 - ∞)
	26	30	31.8866	-110.0177	3.8	0.35	0.53	0.34*	-177 (48 - ∞)
	27	9	31.9118	-109.9560	3.9	0.39	0.55	NA	-26 (13.1 - ∞)
	28	29	31.9365	-109.9940	3.99	0.38	0.53	0.30*	264 (48 - ∞)
	29	33	31.4103	-110.4214	3.35	0.33	0.45	0.27*	646 (14.6 - ∞)
	30	30	31.3768	-110.3913	3.9	0.39	0.51	0.25*	592 (34.8 - ∞)
	31	31	31.5036	-110.3339	3.41	0.36	0.48	0.26*	-45 (163.1 - ∞)

Figure 6.2 Map of the study region in southeastern Arizona, USA, showing the distribution of sampling localities for the three insect species. The inset in the top panel shows location of the study region. The three major genetic groups identified in the STRUCTURE analysis for *M. arizonensis* are shown in the middle panel. Two major groups were identified for *A. herberti* (top panel) in a previous study (Phillipsen and Lytle, 2013). Our samples of *B. aequinoctialis* (bottom panel) were found to belong to a single group in the STRUCTURE analysis.



We quantified pairwise genetic differentiation between sampling localities using F_{ST} and its standardized analog, G'_{ST} (Hedrick 2005). We use F_{ST} in our basic isolation-by-distance analyses, for ease of qualitative comparison with the work of Hutchison and Templeton (1999) and other previous studies. Because it is standardized, G'_{ST} is appropriate for comparing landscape genetics models across species (see below). Both metrics were calculated using GenAlEx (Peakall and Smouse, 2006). We used a Mantel test (Mantel 1967) with 10,000 permutations in GenAlEx to test for an isolation-by-distance (IBD) relationship among the populations. Genetic distances and Euclidean landscape distances were used in the Mantel tests as both raw and transformed ($F_{ST}/(1- F_{ST})$ and log of Euclidean distance, respectively), as per the suggestion of Rousset (1997). The outcomes based on raw and transformed input distances were qualitatively very similar and we present only the former in our results. Given the possibility for null alleles in the datasets, we used the program FreeNA (Chapuis and Estoup 2007) to estimate null allele frequencies and to generate corrected F_{ST} estimates. Uncorrected and corrected F_{ST} values were similar (results not shown), which suggests that any effects of null alleles on the results is likely minimal.

We assessed the strength of genetic drift by estimating N_e for each population using the linkage-disequilibrium method (Hill 1981) implemented in the program LDNe (Waples and Do 2008). We ran the program under the random-mating model and report N_e estimates based on calculations that excluded rare alleles with frequencies less than 0.02 when sample size (S) was greater than 25. When $S \leq 25$, we adjusted the critical allele frequency (P_{crit}) to $1/2S < P_{\text{crit}} < 1/S$, as recommended by Waples and Do (2009). Negative values for $\widehat{N}_e$ from the LD method were interpreted as infinity (Waples and Do 2009).

We assessed the large-scale population genetic structure of the three insects using the program STRUCTURE (Pritchard et al. 2000). Where we found evidence for genetically distinct population groups, we tested for IBD within each group independently, to avoid confounding IBD patterns with the effects of large-scale, possibly historical, barriers to gene flow. STRUCTURE applies a Bayesian clustering algorithm to the multilocus genotypes of all the individuals in the analysis, sorting them into groups that best conform to Hardy-Weinberg and linkage equilibrium. For both species, we performed 10 independent runs of STRUCTURE (under the correlated allele frequencies model allowing admixture) for each value of K (from 1 to 20), which is the hypothesized number of distinct genetic groups in the dataset. Each run had 2×10^6 iterations with a burn-in of 1×10^5 iterations. We applied the ΔK method of Evanno et al. (2005) to identify the most likely number of major genetic groups, given the variation in results across the 10 replicate runs for each K value.

6.2.3 Landscape Genetics Analysis

We analyzed relationships between genetic and spatial data in order to investigate the influences of several landscape features (Table 6.2) on the population structures of the three insect species. Spatial data layers were analyzed in ArcGIS 9.3 software (Environmental Systems Research Inst.) using data provided by the Arizona State Land Department (www.land.state.az.us).

We calculated topographically-adjusted Euclidean distances between all pairs of populations using a digital elevation model (DEM; 10 m resolution). Using the same DEM, we generated the 'curvature' and 'elevation' landscape variables. 'Curvature' is a metric that describes whether local topography (within a 50 m moving window) is convex or concave. Portions of the landscape with convex topography should be drier and more exposed than those with concave topography, such as stream drainages, gullies, and saddles points on ridges. We hypothesize that dispersing aquatic insects are more successful at moving through concave portions of the landscape. ’Elevation’ was derived by determining the mean elevation of the sampling sites for a species and assigned this elevation as the part of the landscape that is the least resistant to gene flow for that species. The underlying hypothesis is that an optimal elevation exists through which dispersal is most frequent or successful, and that the mean elevation of our samples approximates that optimal value. Our samples were collected from across the range of elevations in which each species is found. Elevations higher or lower than the mean were assigned greater resistances, reaching a maximum resistance at ± 1 SD away from the hypothesized optimum. ‘Canopy’ was calculated from a vegetation canopy cover layer, such that the lowest resistance was at 100% canopy cover and highest resistance was at 0% cover.

The remaining four landscape variables were all related to hydrology. ‘Perennial’ and ‘intermittent’ both capture the distribution of freshwater habitat in the study area, for *A. herberti* and *M. arizonensis*, respectively. The locations and extents of perennial habitat patches in the study region were determined using data for the San Pedro River watershed from the Nature Conservancy (www.azconservation.org) combined with observations from field studies in the region (e.g., Bogan and Lytle 2007; Bogan et al. 2013). Intermittent habitat was mapped by selecting sections of stream channels in the study area that are within 2 km up and downstream from the interface between bedrock/mountain and alluvial/valley geologic areas, since these areas are where intermittent stream habitat most commonly occurs (Bogan et al 2013; Jaeger and Olden 2012). Geologic data was obtained from an ‘estimated depth to bedrock’ map (Digital Geologic Map 52) produced by the Arizona Geological Survey (www.azgs.az.gov). For the ‘intermittent’ and ‘perennial’ variables, the lowest resistance values were assigned to patches of intermittent and perennial freshwater habitats, respectively, while the intervening landscape matrix (dry streambeds and terrestrial habitat) was assigned the maximum resistance value. A data layer of the stream network in the study region (from the National Hydrology Dataset) was used to generate the ‘stream-resistance’ and ‘stream-strict’ variables. For ‘stream-resistance,’ resistance was low wherever there was a stream channel and resistance increased away from the stream up to a distance of 1 km, where it reached a maximum value. This variable allows for some overland movement between watersheds, even though most gene flow should occur along stream channels (i.e. within the stream network). By contrast, ‘stream-strict’ only allowed gene flow to occur within the branching stream network.

Plate 6.2 Garden Canyon, Fort Huachuca. Photo credit: Mike Bogan.

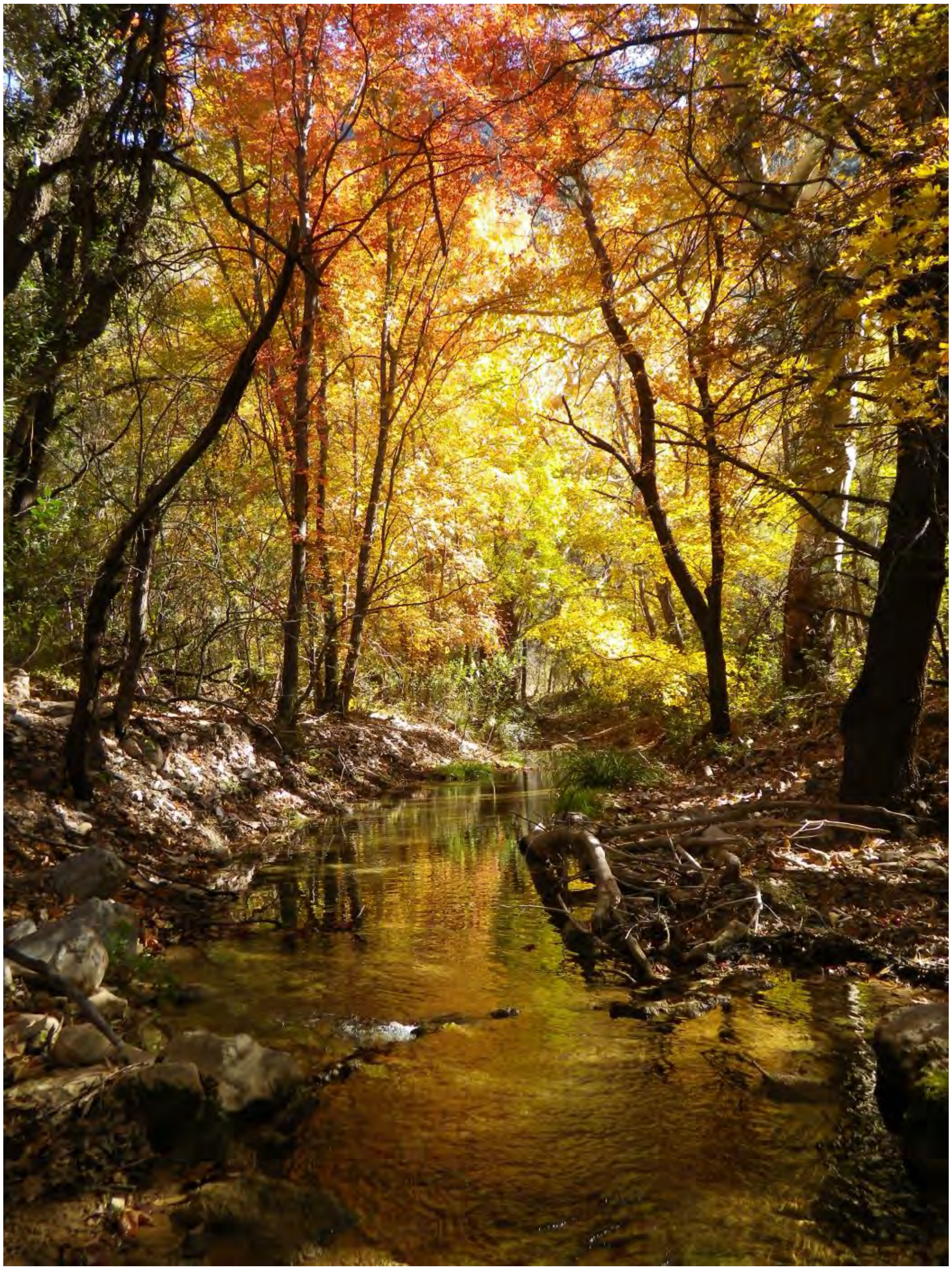


Table 6.2 Details of the landscape variables included in maximum likelihood population effects (MLPE) models.

Variable/Model Name	Hypothesized relationship to gene flow	Explanation	GIS source data
Euclidean	Gene flow follows a stepping-stone model, where dispersal is highest between neighboring populations and decreases as the pairwise Euclidean distance increases between populations.	Pairwise Euclidean distance, adjusted for topography.	NA
canopy	Dense canopy cover from trees and shrubs provides relatively cool and moist microenvironments that increase the chance of survival for dispersers.	Pairwise resistances between populations based on low resistance of map pixels with high percent cover and high resistance of pixels with low percent cover.	a
curvature	Dispersal is highest in areas with strongly concave topography. These areas tend to be canyons, gullies, and low saddle points between drainages. They may be relatively cool and moist and are often the places where water flows. Dispersal is lowest across areas with strongly convex topography. Ridgelines that separate drainages tend to have convex topography.	Pairwise resistances between populations based on low resistance of map pixels with concave topography and high resistance of pixels with convex topography.	b
elevation	Each of the three insect species has an optimum elevation zone, where dispersal is most likely to be successful.	Pairwise resistances between populations based on low resistance at an optimum elevation for each species (calculated as the mean elevation of sampled populations), with increasing resistance at higher and lower elevations.	B

perennial	*A. herberti* only. Perennial freshwater habitats, which exist as fragmented patches in the study region, act as stepping stones for dispersal among populations. This variable was only included in the analysis for *A. herberti*, since this species requires perennial habitat at all life stages.	Pairwise resistances between populations based on low resistance of map pixels in patches of perennial freshwater habitats and high resistance of pixels in the matrix between these patches.	C
intermittent	*M. arizonensis* only. Intermittent freshwater habitats provide stepping stones for dispersing *M. arizonensis*. This species specializes in intermittent habitats, which are fragmented across the study area.	Pairwise resistances between populations based on low resistance of map pixels in patches of intermittent freshwater habitats and high resistance of pixels in the matrix between these patches.	c, d
stream - resistance	Dispersal is easiest within the stream/river network, but can also occur over land. However, resistance to dispersal is relatively high over land due to decreased chance of survival for dispersers.	Pairwise resistances between populations based on low resistance of map pixels in the stream/river network and high resistance of pixels out of the network.	C
stream- strict	Dispersal occurs only within the stream/river network.	Pairwise least-cost paths between populations that strictly follow the stream/river network. Only one path exists between any pair of populations.	C

a. National Land Cover Database 2001 – Canopy (30 m resolution)
b. National Elevation Dataset (10 m resolution)
c. National Hydrography Dataset (NHD)
d. Arizona Geology from Arizona State Land Department

Each raster-based map was used as input for the program CIRCUITSCAPE (McRae 2006), which uses circuit theory to model gene flow among populations in a given landscape. The program quantifies the resistance of the landscape to gene flow between each pair of populations (analogous to electrical resistance in a circuit diagram), allowing for multiple pathways to connect the pair of populations. This pairwise resistance is a summation of the resistances of individual pixels in the input map; pixels with low resistance values offer the least resistance to movement, and vice versa. The minimum to maximum resistance ratio was 1:10,000. The matrix of pairwise resistances output from CIRCUITSCAPE models the structural connectivity of populations, based on the landscape/habitat feature represented by the input map (McRae et al. 2008). Our input rasters for CIRCUITSCAPE had a cell size of 50m. We used the options to connect raster cells via average resistance and to connect each cell to its eight neighboring cells.

Next, we developed statistical models of the relationship between genetic distance (G'_{ST}, as the response variable) and each of the landscape resistances/distances (as the explanatory variables) for each species. The goal was to generate a set of models—one for each of the explanatory variables—and select the best-fitting model from among them, in order to identify which landscape variables demonstrated the strongest association with genetic differentiation. The pairwise genetic and landscape distances within their respective matrices could not be analyzed by traditional regression methods because they violate the assumption of independence. Furthermore, it remains unclear how to appropriately account for non-independent pairwise data when using Akaike's information criterion (AIC; Akaike 1973) for model selection (Burnham and Anderson 2002). To correct for any bias in our data introduced by dependency among our pairwise data points, we used the maximum likelihood population effects (MLPE) method of Clark et al. (2002), which was recently applied to landscape genetics by Van Strien et al (2012). In this method, a linear mixed effects model is used as an alternative to traditional linear regression. A linear mixed effects model includes both fixed and random effects, where fixed effects are represented by explanatory variables included in the model and the random effects represent the dependency in the data (Oberg and Mahoney 2007). The covariate structure of the MLPE model incorporates a parameter (ρ) for the proportion of the total variance that is due to correlation between distances that involve the same sampling site (maximum possible value for ρ is 0.5). Model output can be used to estimate ρ, which provides a measure of the dependency in the data. Residual maximum likelihood (REML; Clark et al 2002) was used to obtain unbiased estimates of MLPE model parameters. For each model we generated, we calculated the R^2_β statistic as described by Edwards et al (2008) as a measure of model fit. We chose to analyze only univariate models because the interpretation of larger, multivariate models would be difficult due to the presence of multicollinearity among landscape variables. Because each of our models included only one landscape variable, ranking models by R^2_β allowed us to identify the variables most strongly associated with genetic structure in each of the three insect species. All MLPE analyses were performed using the R statistical package (R Development Team 2009).

6.3 Results

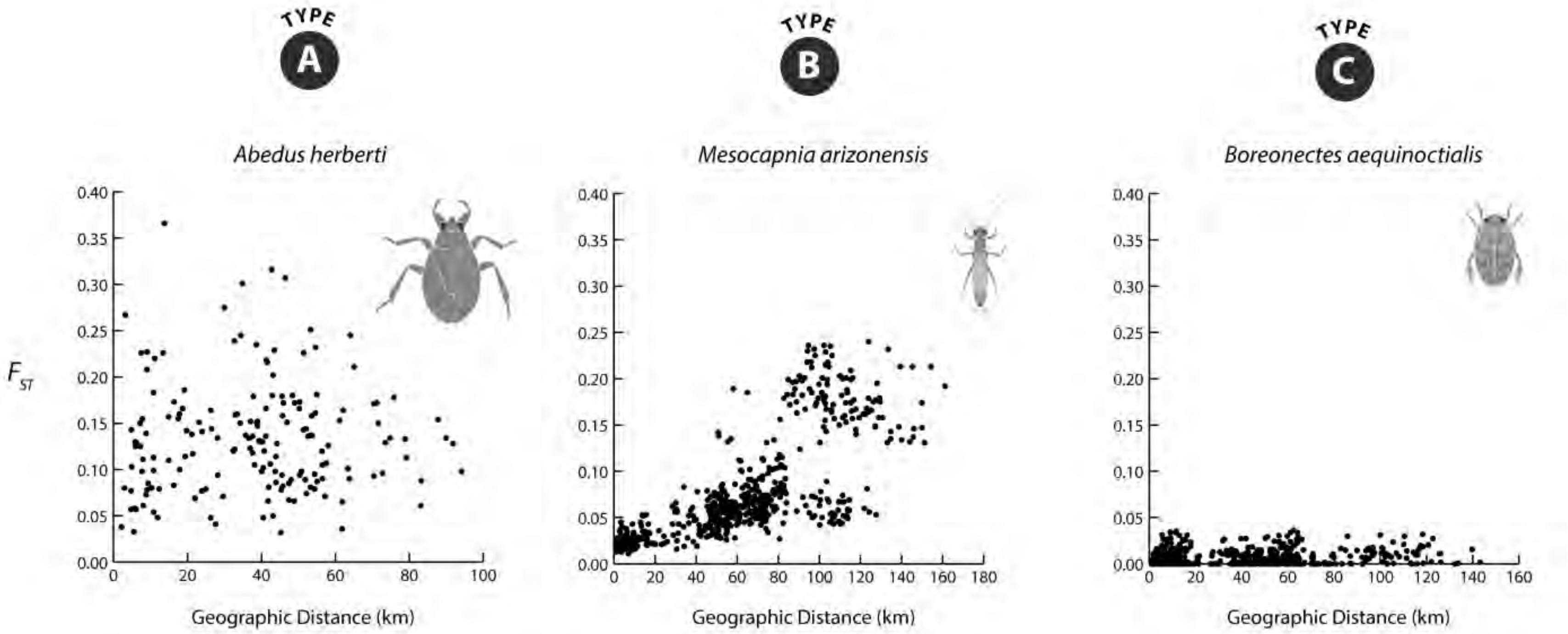
6.3.1 Population Genetics

Direction and strength of the relationships between genetic distances (F_{ST}) and geographic distances (Euclidean distance) between sampling localities matched our predictions of population genetic structure according to species' dispersal abilities (Figure 6.3). There was no significant IBD relationship for *A. herberti* and variance in F_{ST} was high (Mantel $r = 0.03$; P = 0.40). Pairwise F_{ST} values tended to be highest in this species. A strong pattern of IBD was found for *M. arizonensis* and variance in F_{ST} increased with geographic distance (Mantel $r = 0.72$; $P = 0.01$). In *B. aequinoctialis*, no significant IBD relationship was found between F_{ST} and Euclidean distance (Mantel $r = 0.07$; $P = 0.22$). F_{ST} in *B. aequinoctialis* was lower in magnitude and variance at all geographic distances as compared to the other two species. Global F_{ST} values were 0.12 for both *A. herberti* and *M. arizonensis*, and 0.006 for *B. aequinoctialis*.

Estimates of N_e were infinity (i.e. had negative estimates) for 3 (out of 19) population samples for *A. herberti*, 30 (out of 34) population samples for *M. arizonensis*, and 17 (out of 31) population samples for *B. aequinoctialis* (Table 6.1). Upper confidence limits were infinity for all but 8 populations in *A. herberti* and 1 population in *M. arizonensis*. Estimates of infinity are returned when the signal in the genetic data can be attributed entirely to sampling error, rather than genetic drift, which is the case for a very large population or when the population sample contains too little information (Waples and Do 2009). Considering only the non-infinity estimates, *A. herberti* had a median estimated N_e of 71 (n =16), *M. arizonensis* had a median estimated N_e of 536 (n = 4), and *B. aequinoctialis* had a median estimated N_e of 307 (n = 14). The linkage-disequilibrium method of N_e estimation is most reliable when true N_e is small (<50) (Waples and Do 2009), which seems most likely for *A. herberti*.

Results of the STRUCTURE analysis indicate that *A. herberti* and *M. arizonensis* in the study area are divided into two and three major genetic groups, respectively. In *A. herberti*, sampling localities 1-4 represent a group distinct from the remaining sampling localities (more detailed STRUCTURE results for this species are presented in Phillipsen and Lytle, 2013). Three distinct groups were found for *M. arizonensis*: Group 1 (sites 1-17), Group 2 (sites 18-28 and 33-34), and Group 3 (sites 29-32). The geographic distributions of the major genetic groups in *M. arizonensis* are shown in Figure 6.2 and the proportions of these groups represented in each sampling site are shown in Figure 6.4. We treated these groups as independent data sets in the landscape genetics analyses and present their results separately. Group 3 was not analyzed further due to the small number of pairwise comparisons among its four sampling sites. No evidence of genetic structuring was found in *B. aequinoctialis*. Our samples for this species appear to represent a single, panmictic population in the study area.

Figure 6.3 Empirical relationships between genetic distances (F_{ST}) and geographic (Euclidean) distances between pairs of populations. The patterns found for *A. herberti*, *M. arizonensis* and *B. aequinoctialis* closely matched the predictions for low, moderate, and high dispersal, respectively (see Figure 6.1).



6.3.2 Landscape Genetics

Model fit based on R^2_β revealed that several landscape factors are important for explaining landscape-level patterns for *M. arizonensis* (Table 6.3 and Figure 6.5). The best model for Group 1 was the Euclidean distance model, which had $R^2_\beta = 0.381$. Euclidean distance was also the best model for Group 2, with a strong fit of $R^2_\beta = 0.925$. The second and third best fitting models were 'curvature' and 'intermittent' in both groups. However, these variables were both strongly correlated with Euclidean distance (Table 6.4), making it difficult to determine which of the three variables are displaying causal linkages with genetic structure in *M. arizonensis*.

Values of ρ from the MLPE models (the proportion of the variance due to correlation between genetic distances that involve the same sampling site) for *B. aequinoctialis* were smaller than those of *A. herberti* and *M. arizonensis* (Table 6.3). The latter two species had similar ρ values.

No candidate models demonstrated a good fit for *A. herberti* or *B. aequinoctialis*, with R^2_β values less than 0.03 for all models (Table 6.3 and Figure 6.5). Given such poor-fitting models, we can conclude little regarding which landscape variables are the most important for driving population structure for these two species. This result is corroborated by the Mantel tests, which did not reveal significant IBD patterns for *A. herberti* or *B. aequinoctialis*.

The perennial aquatic habitat required for *A. herberti* has a patchy, disjunct distribution in the study area. Perennial stream reaches are limited to isolated, spring-fed reaches of mountain streams that may be separated by dozens of kilometers (Bogan & Lytle 2011; Jaeger & Olden 2012). The habitat distribution and weak dispersal ability (due to flightlessness) of this species have apparently resulted in a high level of genetic isolation among populations. Our data for *A. herberti* fit the Type A pattern (Figure 6.1), where gene flow is weak and genetic drift is high at all geographic distances. Additional evidence that genetic drift is strong in *A. herberti* came from the small estimated N_e values in this species.

The opposite pattern, Type C, was found for *B. aequinoctialis*. Gene flow appears to be high for this strong-flying beetle at all geographic distances and genetic drift is minimal. *B. aequinoctialis* also occupies a variety of freshwater habitat types, so it probably has more potential dispersal pathways than do the other two species. High gene flow in this species was also supported by our STRUCTURE analysis, which found that individuals from the 31 sampling localities most likely belong to a single genetic group.

Figure 6.4 STRUCTURE results for *M. arizonensis*. The most likely number of genetic groups for this species is three. For each sampling site, a bar is shown that depicts the proportional membership of that site for each of the major genetic groups. All but one of the sites (34) could be unambiguously assigned to one of the major groups.

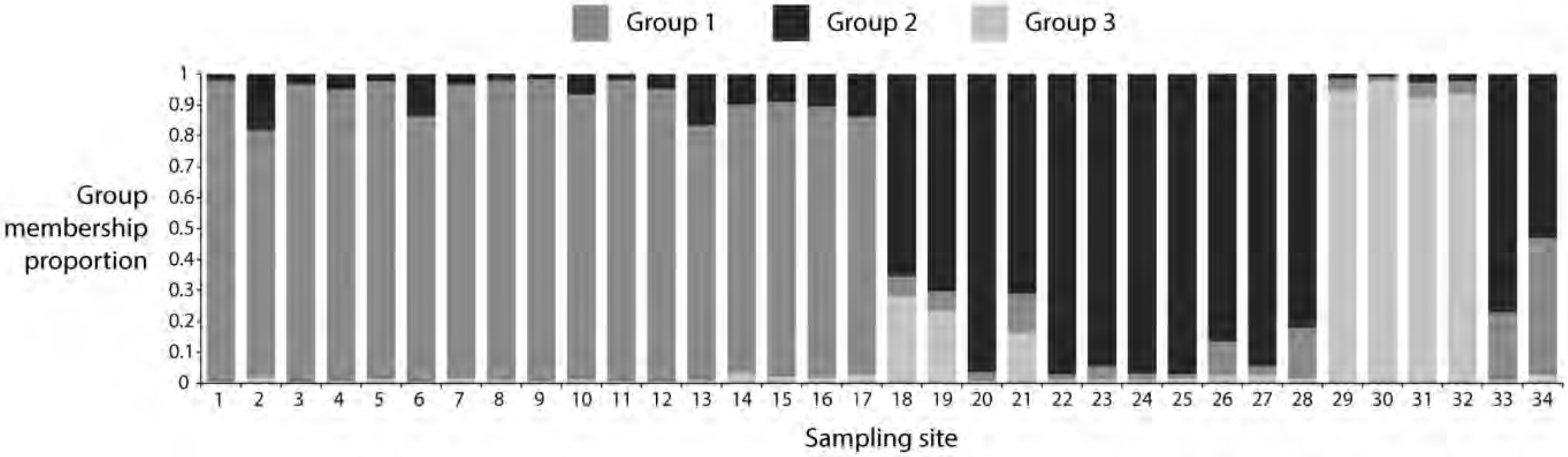


Figure 6.5 Graphical representation of maximim likelihood population effects (MLPE) model results. The y-axis is the model fit (R^2_β) of the MLPE model; models for each of the three insect species are listed on the x-axis. Model fit was very high for the best-fitting models in *M. arizonensis* Group 2. Fit was generally weaker in *M. arizonensis* Group 1. R^2_β was very low for all models in *A. herberti* and *S. aequinoctialis*.

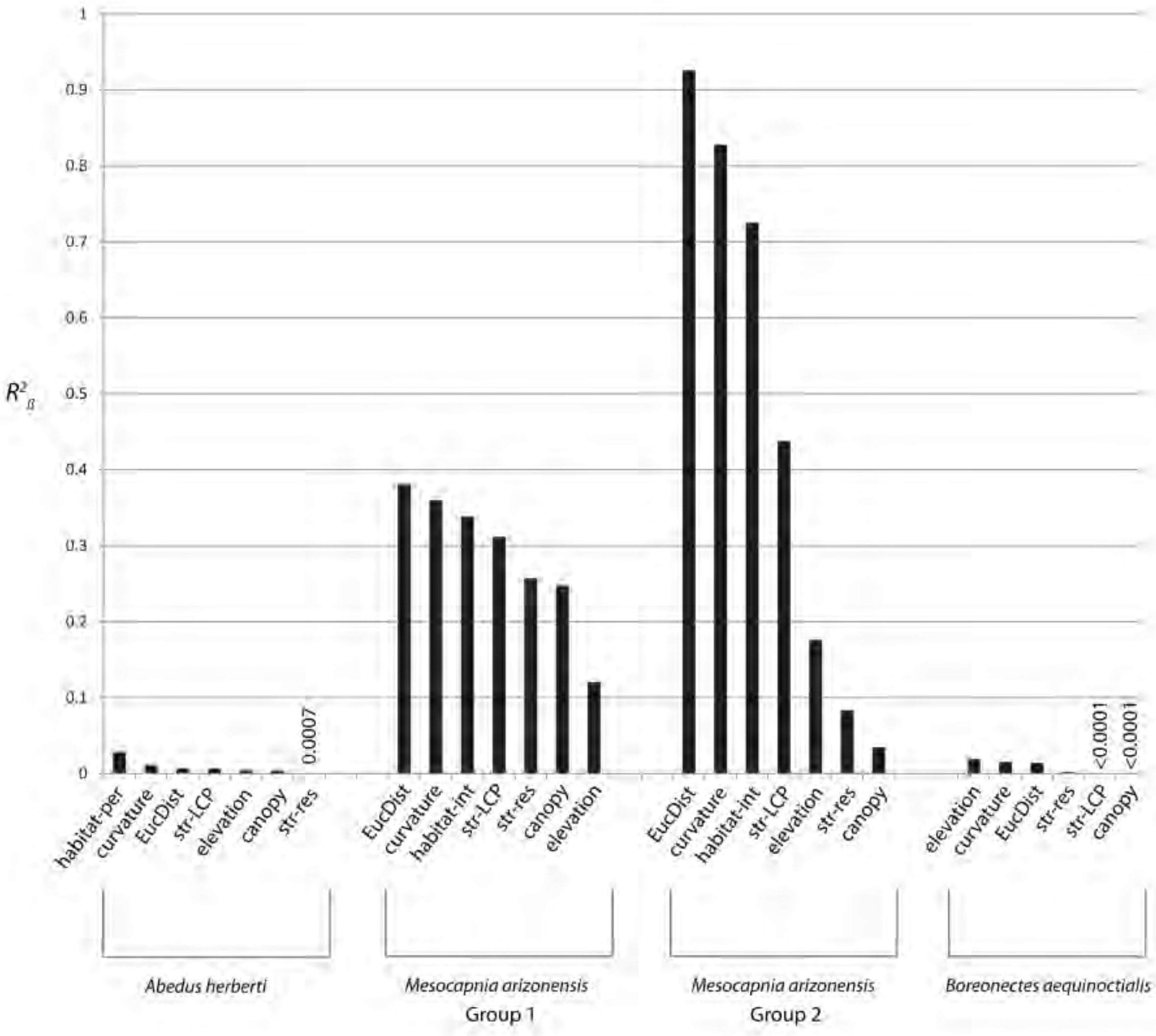


Table 6.3 Maximum likelihood population effects (MLPE) modeling results. Within each of the three insect species, there was model for each of several landscape variables. Models for a species are ranked from highest to lowest $\boldsymbol{R^2_\beta}$, a measure of model fit. The degree of dependency among distances included in the model that is due to correlation between values involving the same sampling site is given by $\boldsymbol{\rho}$ (maximum value of 0.5).

Species	Model	R^2_β	ρ
A. herberti	Perennial	0.0285	0.39
	Curvature	0.0113	0.39
	Euclidean	0.0070	0.38
	stream-LCP	0.0066	0.40
	Elevation	0.0049	0.39
	Canopy	0.0043	0.41
	stream-Res	0.0007	0.33
M. arizonensis – Group 1	Euclidean	0.3813	0.35
	Curvature	0.3596	0.35
	Intermittent	0.3383	0.35
	stream-LCP	0.3119	0.33
	stream-Res	0.2573	0.42
	Canopy	0.2479	0.45
	Elevation	0.1204	0.45
M. arizonensis – Group 2	Euclidean	0.9256	0.27
	Curvature	0.8274	0.33
	Intermittent	0.7252	0.28
	stream-LCP	0.4376	0.38
	Elevation	0.1761	0.48
	stream-Res	0.0833	0.43
	Canopy	0.0347	0.47
B. aequinoctialis	Elevation	0.0189	0.24
	Curvature	0.0152	0.24
	Euclidean	0.0142	0.24
	stream-Res	0.0021	0.25
	stream-LCP	0.0001	0.25
	Canopy	0.0000	0.25

6.4 Discussion

6.4.1 Landscape genetics of the three species

Genetic structure of *M. arizonensis* was best explained by geographic (Euclidean) distance between localities. The 'curvature' and 'intermittent' models also exhibited strong fits, but these models contain landscape variables that were both highly correlated with Euclidean distance. This evidence suggests that dispersal in *M. arizonensis* occurs in a stepping-stone pattern among patches of intermittent stream habitat, and that dispersal may be only minimally influenced by landscape curvature or that it occurs along intermittent stream corridors.

Genetic structure of both *A. herberti* and *B. aequinoctialis* was not explained by distance measures describing geographic proximity or landscape resistance to dispersal among localities. The fact that the model including geographic (Euclidean) distance had a poor fit for these species was expected given our previous IBD results; however it contrasts Phillipsen and Lytle (2013) which found that curvature had a small effect independent of Euclidean distance for *A. herberti*. In the latter study, however, conditional genetic distance (sensu Dyer et al. 2010) was used, not G'_{ST} that was calculated in the present study because it is standardized and appropriate for comparisons across species (Hedrick 2005). Rather than being a limitation of our data or analyses, our finding that none of the MPLE models had a good fit for *A. herberti* and *B. aequinoctialis* likely reflects the biological characteristics of these insects. If, as we suspect, gene flow is minimal in *A. herberti* and genetic drift is relatively strong, we would not expect to find a significant relationship between any landscape variable and genetic distance in this species. The pattern of high genetic differentiation introduced by genetic drift would overwhelm any signal of gene flow. For *B. aequinoctialis* on the other hand, high levels of gene flow indicate that no single landscape variable has a measurable effect on genetic distance. The relatively small values of ρ for this species (Table 6.3) may be another indicator of the interconnectedness of its populations that would result from high gene flow.

It is also interesting that gene flow among populations of these aquatic insects does not appear to be strongly influenced by direct hydrologic connectivity. The 'stream-resistance' and 'stream-strict' landscape variables were two representations of hydrological connectivity and neither was important for any of the species. Dispersal in these insects may not closely follow stream drainages—as might have been the case for *M. arizonensis* or *B. aequinoctialis* if dispersal occurred exclusively via movements of aquatic larvae—and may instead occur over watershed boundaries (Finn et al. 2007). Thus, overland movement of the terrestrial adult stage is likely to be more important for population connectivity than within-stream migration of aquatic juvenile stages (e.g., Lytle 1999, Boersma et al. 2014). The distribution of habitat patches does seem to be important, however, at least for *A. herberti* and *M. arizonensis*. The genetic patterns we revealed were likely influenced in part by the isolation of *A. herberti*'s perennial stream habitat and the relative continuity of *M. arizonensis*' intermittent habitat.

6.4.2 The effects of dispersal ability and spatial scale on the utility of landscape genetics methods

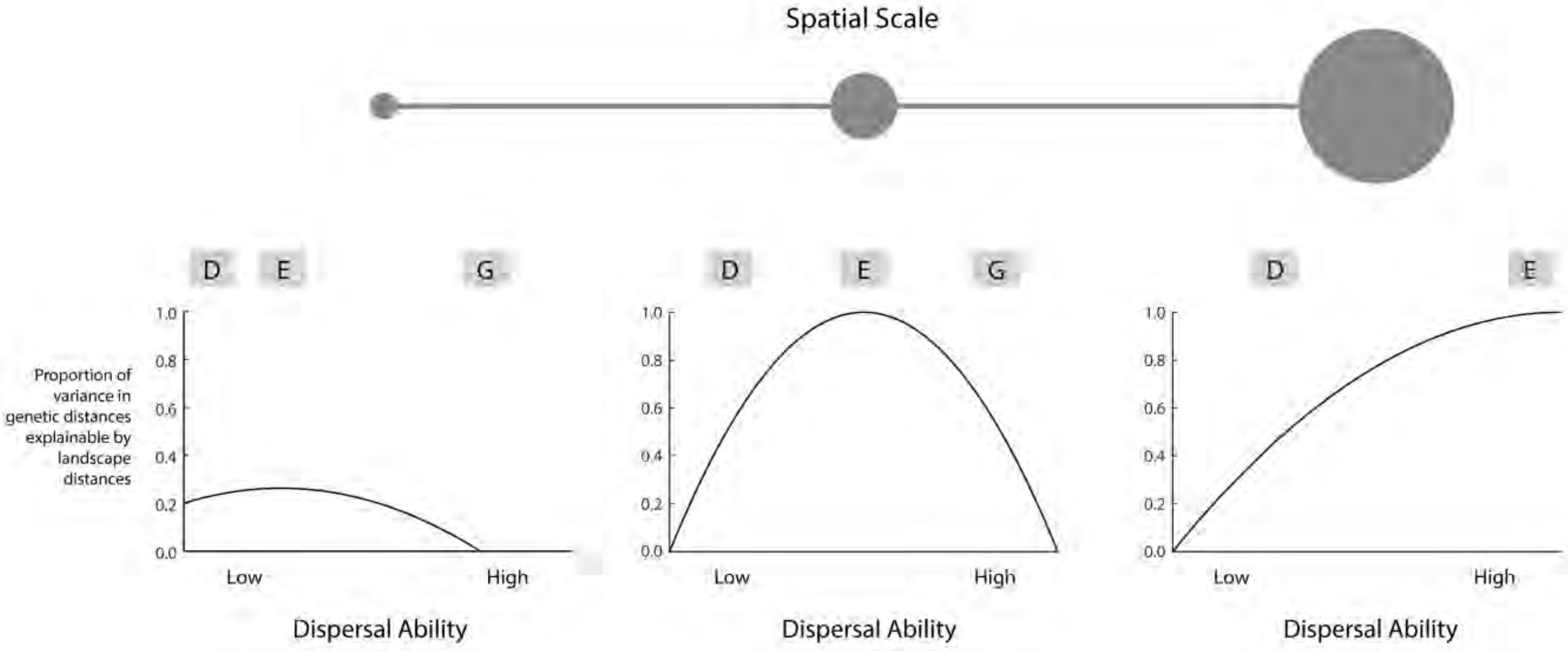
The interaction of gene flow and genetic drift drives the spatial signature of genetic similarity among populations, for neutral loci and when mutation can be ignored. Many landscape genetics analysis methods rely on these two evolutionary forces being in equilibrium across the set of populations being studied, such that a significant, monotonic relationship exists between genetic distance and geographic/landscape distance between pairs of populations (Balkenhol et al. 2009). Our results corroborate the finding of Slatkin (1993) that when either gene flow or genetic drift overwhelms the other force, no detectable relationship between genetic and geographic distances is expected. In these cases landscape genetics methods may not be very useful.

However, spatial scale (the spatial arrangement of sampling localities and total extent of the sample area) and the dispersal ability (i.e. distance) of the study organism can also be important determinants of whether there are spatial-genetic relationships that can be investigated with landscape genetics methods (Anderson et al. 2010). The relative strengths of gene flow and drift can vary across spatial scales, and dispersal is often relative to how spatial scale interacts with a species' perceptual range (Olden et al. 2004). For example, a 'small' spatial scale for a species with strong dispersal ability may be a 'large' spatial scale to a species with weak dispersal. The absolute scale at which landscape genetics will have the most explanatory power—when gene flow and genetic drift are in equilibrium—should vary with dispersal ability.

We can conceptualize how interactions between spatial scale and dispersal ability influence the proportion of the variance in pairwise genetic distances that can be explained by geographic/landscape distances (Figure 6.6). At the smallest spatial scale, gene flow-drift equilibrium will only exist for species with very low dispersal ability. Landscape genetics methods will have limited utility at this scale for moderate and strong dispersers, and perhaps even for weak dispersers. At intermediate spatial scales, landscape genetics methods will be most effective for moderate dispersers and relatively ineffective for both weak and strong dispersers. Finally, at the largest spatial scale, gene flow-drift equilibrium will only exist for strong dispersers and thus landscape genetics methods will be useful only for such species.

For the three insect species that we studied in the Madrean Sky Islands of southeastern Arizona, only the stonefly *M. arizonensis* exhibited an IBD pattern suggesting regional equilibrium between gene flow and genetic drift. We believe that genetic drift overwhelms gene flow in *A. herberti*, preventing any detectable IBD for this species at any spatial scale. Rampant gene flow in *B. aequinoctialis* is likely the reason we found no evidence for IBD in that species. If we were to sample *B. aequinoctialis* at a much larger spatial extent, we predict that we might find IBD for this species.

Figure 6.6 Conceptual model of how interactions between spatial scale and dispersal ability influence the proportion of the variance in pairwise genetic distances that can be explained by geographic/landscape distances. Drift (D) or gene flow (G) dominates under certain conditions, or these two forces can be at equilibrium (E). At the smallest spatial scale, equilibrium will only exist for species with very low dispersal ability. At intermediate spatial scales, equilibrium will exist for moderate dispersers but not for weak or strong dispersers. At large spatial scales, gene flow-drift equilibrium will only exist for strong dispersers.



6.4.3 Implications for conservation

Our findings are valuable for the conservation of freshwater habitats of the Madrean Sky Islands under the threat of increasing aridity. Predictions for southwestern North America in general (Seager et al. 2007) and the Madrean Sky Islands in particular (Coe et al. 2012) suggest increases in temperature and decreases in precipitation in the coming decades. Increased fragmentation and reduction of freshwater habitats are likely to occur, and transitions of perennial stream habitat to intermittent habitat will result in local extinctions of perennial specialists such as *A. herberti*. Indeed, this phenomenon has already been documented (Bogan & Lytle 2011), and it is clear that removal of a predator species has implications for the structure and function of the entire aquatic community (Boersma et al. 2014). Local extinctions in species with Type A population structure will potentially result in a loss of unique genetic variation. Although Type A species will be the most vulnerable to losses of genetic diversity, they may be less affected by reduced habitat connectivity in the future because their dispersal is already highly restricted.

An increase in intermittent habitat might be beneficial for species such as *M. arizonensis*, allowing populations to expand into new areas. However, increasing aridity will also result in concurrent losses of intermittent habitats as these transition to ephemeral flow patterns. If this happens, gene flow in *M. arizonensis* and other species with Type B population structure may be reduced such that genetic drift becomes dominant (as in Type A) and local extinctions become more likely. High gene flow species such as *B. aequinoctialis* with Type C population structure should be the least vulnerable to changes in population structure and losses of genetic diversity due to climate change.

The multi-species approach of this study allowed us to contrast the population structures of insects that represent three ecological syndromes, each defined by a combination of habitat requirements, life history, and dispersal ability. Thus, these species are model representatives of a diverse, co-distributed aquatic invertebrate fauna in the study region (Schriever et al., in review). An understanding of how the biological traits of these insects influence their population structures gives us insight into how gene flow and drift interact at the regional scale, not only for these species but for the multitude of aquatic invertebrates that share their habitats and dispersal abilities.

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7. Traits as a mechanistic, multi-taxa, and conservation framework for contemporary genetic inference

7.1 Introduction

Rapid environmental change and limited conservation resources necessitate efficient and effective conservation planning that promotes the persistence of native species (Williams *et al.* 2008; Glick *et al.* 2011). Knowledge of population attributes such as structure, connectivity, and genetic integrity are a fundamental part of successful conservation. For decades, population genetic approaches have been used to directly identify genetic scenarios that may compromise the health, resilience, or persistence of a species and its populations, such as inbreeding and outbreeding depression, or accumulation of deleterious alleles (Amos and Balmford 2001). Population genetics has also informed population distinctiveness and designation of management units (Fraser and Bernatchez 2001; Palsbøll *et al.* 2007), particularly for species that are difficult to monitor via traditional means (Schwartz *et al.* 2007). In recent years, the field of landscape genetics has integrated population genetics with emerging spatial statistics to examine how the environment affects population genetic structure, diversity, and differentiation (Manel *et al.* 2003). New technologies and increasing focus on adaptive genetic variance continue to expand the contribution of population and landscape genetics to conservation biology (Segelbacher *et al.* 2010; Bolliger *et al.* 2014).

Environmental change is outpacing the rate at which vulnerability to decline or extinction is assessed for most taxa, and increasing emphasis has been placed on using species' attributes – or traits – to identify at-risk species when single-species approaches are not feasible (Williams *et al.* 2008). Traits are defined as attributes measurable at the individual level that are comparable across species, and they include morphological, life history, trophic, behavioral, and other attributes. The utility of traits-based approaches is well recognized in community ecology, where traits are often used to investigate patterns across both taxa and ecosystems (McGill *et al.* 2006). Opportunities for multispecies, trait-based studies in landscape genetics continues to increase as analytical approaches converge (Bolliger *et al.* 2014) and open communication, data sharing, and collaboration are increasingly prioritized (Balkenhol *et al.* 2009; Storfer *et al.* 2010). Concurrently, calls for hypothesis-driven inquiry (Segelbacher *et al.* 2010) and multispecies inference (Manel and Holderegger 2013) underscore the need for strategic approaches that promote generalized frameworks in population and landscape genetics. Here, we propose guiding principles for the formal development, testing, and generalization of traits-based frameworks to advance the utility, efficiency, and effectiveness of genetic inference in contemporary ecology, evolution, and conservation.

7.2 Principles of Application

7.2.1 Species, population genetics, and the environment are connected mechanistically by traits

A traits-based framework for the generalization of population and landscape genetics builds upon long-recognized mechanistic connections between traits and population genetics. Population size and migration rates were among the first factors identified as fundamental to population genetic structure (Wright 1943), and both are primarily governed by traits such as fecundity, longevity, generation time, and dispersal ability. Recent evidence suggests that genetic diversity is strongly related to life history attributes across many animal species, where lower overall genetic diversity exists in longer lived and lower fecundity species compared to short-lived, highly fecund species (Romiguier *et al.* 2014). The same traits affecting population genetic structure are also associated with the vulnerability of species to environmental change (Williams *et al.* 2008). The underlying mechanistic links between traits, genetic structure, and the environment help provide the foundation for hypothesis-driven inquiry to avoid spurious correlations associated with strictly correlative studies (Cushman and Landguth 2010).

Because of the direct linkages between key traits and ecological processes, traits may better explain patterns of population and landscape genetics than implicit indicators such as predicted abundance (Peterman *et al.* 2014). Dispersal ability is perhaps one of the most important and widely recognized drivers of landscape genetic patterns across a range of taxa, including amphibians in the northeastern United States (Richardson 2012), freshwater fishes in the southeastern United States (Fluker *et al.* 2014), aquatic insects in western Switzerland (Alp *et al.* 2012), and woodland birds in Australia (Amos *et al.* 2014). The traits of breeding site fidelity and generation time explained differences in population genetic differentiation, landscape associations, effective population size, and sensitivity to land-use change for two otherwise ecologically similar salamander species in eastern United States (Whiteley *et al.* 2014). Territorial social organization, a behavioral trait, was associated strongly with population genetic diversity of two woodland lizard species following fire disturbance in Australia (Smith *et al.* 2014). Increasingly, combinations of traits that include dispersal ability, behavior and other ecologically limiting factors have proven useful in explaining differential landscape genetic patterns between species (e.g., Goldberg and Waits 2010; Kelly and Palumbi 2010). Statistical approaches such as multivariate analyses may ultimately help evaluate the performance of many traits simultaneously.

7.2.2 Traits provide currency for multispecies inference in population and landscape genetics

The extent to which patterns and processes are generalizable across space and species is a unifying research theme in ecology and evolution. Understanding the generality and uncertainty of patterns and processes across species is also of critical importance to managers seeking to build comprehensive multispecies or community-level conservation plans with relatively limited information. Recent multispecies genetic studies highlight the utility of considering many species at once in attributing patterns of population genetic structure to environmental gradients (Manel *et al.* 2012) or particular geographic "hot spots" of genetic diversity (Thomassen *et al.* 2011). We propose trait-based inference as a formal organizing framework for multi-taxa inference in population and landscape genetics. Traits have been applied successfully as the basis for multispecies generalization frameworks of population genetic structure for a range of freshwater organisms. For example, dispersal and life history traits predicted population genetic structure in almost three-quarters of >100 fish and aquatic macroinvertebrate species in Australia (Hughes *et al.* 2013). Population genetic structure for a diverse suite of aquatic taxa in the southwestern United States was also closely linked to dispersal and water dependency (Chapter 5 and 6, Panel

7.1), thus providing new opportunities to generalize these associations to other aquatic taxa in the region (Figure 7.1).

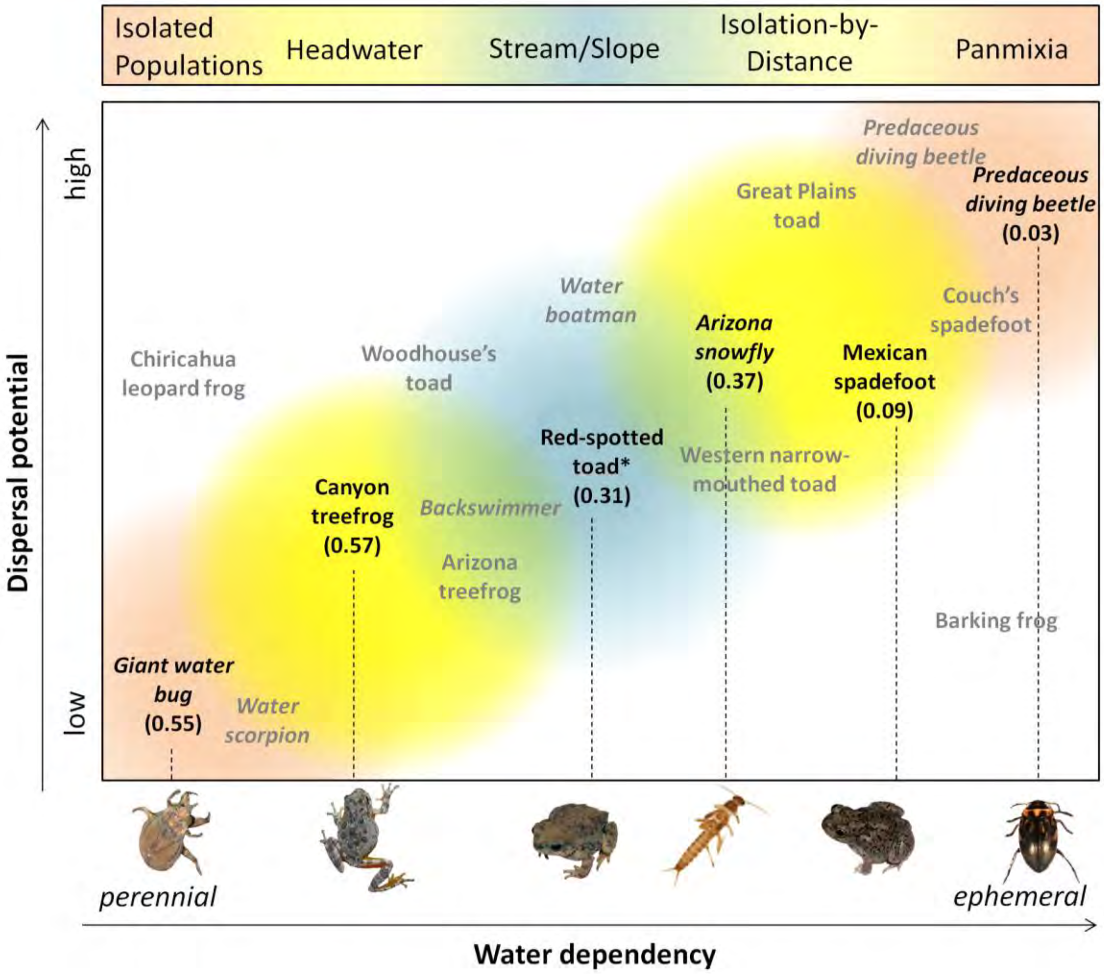
A framework for multispecies population and landscape genetic inference provides an important tool for managers and conservation practitioners tasked with applying the best available data to managing many species at once. For example, the two studies highlighted in Panel 1 provide the foundation for conservation prioritization of regional aquatic fauna. Dewatering of perennial springs and streams due to climate change and human water use in the southwestern United States (Marshall *et al.* 2010; Seager *et al.* 2012) may result in local extinction of genetically distinct populations of the canyon treefrog (*Hyla arenicolor*) and the giant water bug (*Abedus herberti*) with little chance of recolonization (Bogan and Lytle 2011). Alternatively, predaceous diving beetles (*Boreonectes aequinoctialis*) and Mexican spadefoots (*Spea multiplicata*) are well adapted to capitalize on ephemeral aquatic habitats that are temporally and spatially variable in availability, and population genetic analysis revealed high gene flow for both species within the range of these studies. These ephemeral-adapted species and others like them may be less vulnerable to changes in aquatic habitat or landscape features.

As traits-based frameworks are developed and tested, efforts toward multispecies inference must consider challenges inherent to population and landscape genetics. For example, the spatial scale (Balkenhol *et al.* 2009; Storfer *et al.* 2010), grain size (Galpern *et al.* 2012), and study extent (Moore *et al.* 2011; Trumbo *et al.* 2013) can have profound effects on the outcomes of population and landscape genetic studies within a species. Landscape genetics must also be interpreted in the context of temporal scale, considering both the imprinting of former landscape processes and the lag time to detect the effects of contemporary landscapes (Landguth *et al.* 2010). These challenges should be carefully and explicitly considered (Balkenhol *et al.* 2009), particularly when employing multispecies inference or developing predictive frameworks. At the same time, traits and multi-taxa inference may ultimately help identify scenarios in which species or populations are particularly sensitive to the spatial or temporal elements of a study design. We may find that the severity of spatial and temporal complications differs, and is possibly predicted, by species traits such as dispersal ability or generation time. For example, the temporal lag of landscape genetic signals is directly affected by population size and generation time (Whitlock and McCauley 1999; Landguth *et al.* 2010). Thus, traits may help reduce uncertainty due to the spatial and temporal challenges of genetic approaches.

7.2.3 Traits focus the utility of genetics studies for management and conservation goals

Traits may help determine *a priori* whether genetic approaches are the appropriate tool for the management or conservation goal at hand (Figure 7.2). Population and landscape genetics are employed to address a wide range of management goals (DeSalle and Amato 2004; Bolliger *et al.* 2014), and the relevance of genetic information to achieve these objectives is filtered by the degree and nature of population structure (Table 1). Traits may ultimately help elucidate the opportunities and limitations of genetic approaches to inform management actions. Technological developments in the field (Allendorf *et al.* 2010), increasing focus on adaptive genetic variance (Manel and Holderegger 2013), and careful study design (Segelbacher *et al.* 2010) may effectively expand or shift the management goals informed under different trait scenarios (Figure 7.2). But species dispersal ability, life histories, environmental tolerances, and phenology are increasingly linked to population genetic structure – and the degree to which this structure is influenced by the landscape. Traits can highlight cases in which interactions between population genetic structure and the landscape are likely, identifying valuable and informative pursuits in landscape genetics such as estimating landscape resistance to gene flow or identifying dispersal corridors or barriers (Figure 7.2, central oval). Alternatively, if the target species' traits suggest that population genetic structure will not be closely tied to the landscape (for example, panmixia or completely isolated populations, Figure 7.2, outer-most oval), then genetic inference may have limited utility in landscape management but could inform other management goals such as identifying genetically unique populations or estimating population risk due to inbreeding depression.

Figure 7.1 Population and landscape genetic structure along gradients of water dependency (x-axis) and dispersal potential (y-axis). In this case, dispersal potential is a summary index comprising known dispersal distance, body size, and desiccation tolerance. Hypothesized models of population structure are shown as shaded circles with labels shown above the plot. Empirical results for six aquatic species – three anurans and three aquatic insects – are shown in black text within the plot and correspond to photos along the x-axis. Global G'ST, a standardized value of genetic differentiation appropriate for comparison across species in which larger values indicate greater genetic differentiation (Hedrick 2005), is shown in parentheses below each study species. Additional aquatic species with distributions overlapping the six target taxa are shown in grey text according to their dispersal potential and water requirements. Common names are show for all taxa with macroinvertebrates shown in italics. Red-spotted toads marked with an asterisk (*) were also found to be panmictic in a subset of the study range; those results are not shown in this summary figure.



Panel 7.1 The landscape genetics of aquatic species in a dryland environment

Two recent studies evaluated the population and landscape genetics of aquatic taxa that span a range of water dependency and dispersal abilities in the Madrean Sky Islands of Arizona, USA (Chapter 5 and 6). Water availability in this dryland region ranges from isolated perennial springs to ephemeral washes that contain water for only hours or days out of the year. Aquatic species in the region have adapted to this gradient of water availability with a diverse range of ecological strategies, and water dependency ranges from requiring water for only a short portion of the life cycle to water dependency for the entire life cycle. Both studies found strong support for relationships between water dependency and dispersal potential and population genetic structure across taxa (Figure 7.1). Furthermore, species with intermediate dispersal and water dependency, like *Mesocapnia, etc,* had the strongest relationship with spatial (distance) and landscape factors (aquatic habitat and topography).

The taxa in these studies encompass a range of aquatic taxa spanning a gradient of both dispersal and water requirements. Trait databases characterizing the diverse assemblage of aquatic invertebrates in the Sky Islands region (e.g., Chapter 2) may help infer population genetic structure for species with similar or intermediate trait values to those in this study. For example, the water scorpion (*Curicta pronotata*), water boatman (*Trichocorixa uhleri*), and predaceous diving beetle (*Rhantus atricolor*) are well-represented by the dispersal and water dependency traits of the three focal invertebrates in Chapter 6 (Figure 7.1). Amphibian taxa such as the Great Plains toad (*Incilius alvarius*), Couch's spadefoot (*Scaphiopus couchii*), and Arizona treefrog (*Hyla wrightorum*) also fall along the same ecological strategies represented by amphibians in Chapter 5, and the inferred population genetic structure for the Great Plains toad and Couch's spadefoot (Figure 7.1) are supported by previous research (Chan and Zamudio 2009).

Both studies provide conservation and management-relevant information. Dewatering of perennial springs and streams due to climate change and human water use in the region (Marshall *et al.* 2010; Seager *et al.* 2012) may result in local extinction of genetically distinct populations of the giant water bug (*Abedus herberti*) and canyon treefrogs (*Hyla arenicolor*) with little chance of recolonization (Bogan and Lytle 2011). Alternatively, predaceous diving beetles (*Boreonectes aequinoctialis*) and Mexican spadefoots (*Spea multiplicata*) are well adapted to capitalize on ephemeral aquatic habitats that are temporally and spatially variable in availability, and population genetic analysis revealed high gene flow for both species within the range of these studies. These ephemeral-adapted species may be less vulnerable to changes in aquatic habitat or landscape features.

These two studies support the potential of a traits-based framework for multi-taxa inference and highlight important future directions to improve this framework. Considering additional traits may help characterize species that fall outside the general pattern of this bivariate trait analysis. For example, the barking frog (*Craugastor augusti*) has no aquatic larval life stage but also has low dispersal tendencies (Goldberg and Schwalbe 2004) and is thus not well-represented by the focal taxa (Figure 7.1). In addition, true dispersal potential – and its variability – are poorly understood for many aquatic organisms, and plasticity in larval development, desiccation tolerance, and behavior produce intraspecific variability in water dependency. Dispersal potential and water dependency are considered as relative metrics (amphibians, Chapter 5) or categorical variables (invertebrates, Chapter 2). Thus, even with diverse taxa representing a range of trait values as presented in these studies, there remains considerable uncertainty in the relationships between traits, the environment, and population genetic structure. Future efforts may reduce this uncertainty with more robust empirical measures of traits values coupled with simulation-based approaches to evaluate the role of inter- and intra-specific trait variability.

Figure 7.2 Population and landscape genetic structure along two focal trait axes. Models of population structure include, from lower left to upper right: isolated populations in which genetic drift dominates population structure ("Isolated populations" - red shading), genetically distinct groups of populations but local isolation-by-distance patterns ("Local IBD" - yellow shading), populations in which a landscape or environmental factor(s) drives population genetic structure ("Landscape effects" - blue shading), populations with isolation-by-distance structure throughout a region or species' range ("Regional IBD" - yellow shading), and panmictic populations ("Panmixia" - red shading). Regional and local isolation-by-distance patterns illustrate how the spatial scale of a study may reveal different population genetic patterns and processes. Nested ovals depict management-relevant information provided by population and landscape genetic approaches. For example, the outside oval encompasses all scenarios providing any baseline information on population structure and genetic diversity. The middle oval represents scenarios in which management units or dispersal ability may be informed. Finally, the central oval represents the core objectives of most landscape genetic studies – for example, to inform corridors and barriers to gene flow or estimate landscape resistance to dispersal.

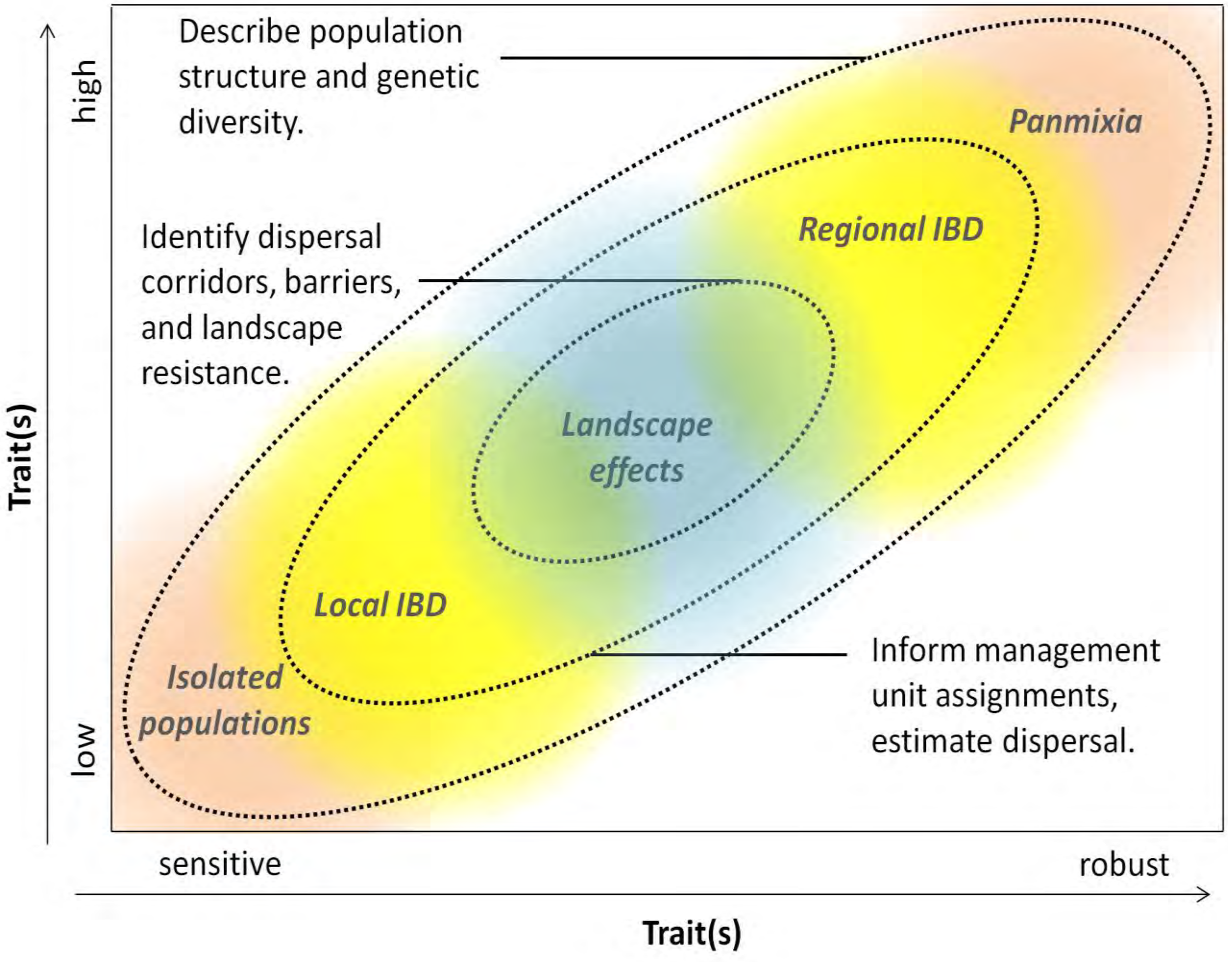


Table 7.1 Examples of management goals and considerations most relevant for different population genetic scenarios (isolated populations, isolation-by-distance, and panmixia).

Management goals	Knowledge gaps and considerations
Isolated populations	
- Identify unique and derivative populations - Identify and prioritize conservation of populations with high genetic diversity - Identify populations at direct risk from low genetic diversity and its associated threats (e.g. inbreeding depression, mutational meltdown)	- Genetic drift obscures potential importance of landscape and local factors in population genetic attributes and connectivity - Distinguishing each population as an independent management unit may not be feasible or efficient
Isolation-by-Distance	
- Implement metapopulation management strategies - Identify source populations and prioritize their conservation - Identify, maintain, and restore landscape corridors for population connectivity	- Genetic structure may not capture important contemporary landscape processes (e.g. landuse change) due to temporal lag - Identifying "true" drivers among collinear landscape variables can be difficult
Panmixia	
- Species of least concern - Carefully consider whether management of individual units within panmictic group is necessary or effective in achieving goals - Manage for overall abundance on landscape	- High dispersal may obsecure genetic signal of important local and landscape influences on demography - Panmictic neutral genetic variability may miss important structure in adaptive genetic variance

7.3 Future Directions

In order to implement a traits-based framework for the identification of linkages between and across species, genes, and environments, more rigorous and systematic evaluations of the geographic and taxonomic generalizations of population and landscape genetics will be required. Here we identify three focal areas fundamental to furthering and refining the use of traits as a framework for testing ecological and evolutionary processes across taxa and geography and for the rapid, efficient, and generalizable incorporation of landscape genetics into management and conservation practice.

7.3.1 Selecting informative traits: which ones and how many?

With many traits to choose from, a useful strategy may be to first select "focal" response traits that determine species resilience and resistance to environmental change (McGill et al. 2006). In some cases, one or two focal traits may be intuitive drivers of population genetic patterns; in other cases, multivariate analyses will facilitate the simultaneous consideration and comparison among a suite of focal traits. Life history traits (e.g., fecundity, generation time, and longevity) influence the growth rate of populations, can affect overall genetic diversity, and may ultimately shape population structure and species response to environmental change (Romiguier et al. 2014). Traits that characterize some ecological or physiological limitation, such as dispersal potential, thermal tolerance, or physicochemical requirements may also predict associations between genetic structure and the landscape. Several ecologically limiting traits have been related to population genetic structure, including invertebrate position in the intertidal zone (Kelly and Palumbi 2010), rate of water loss in salamanders (Peterman et al. 2014), and water dependency of aquatic organisms in an arid environment (Chapter 5 and 6). Ecologically limiting traits also provide the framework for inference of landscape genetic structure for other taxa facing similar environmental challenges (Figure 7.1).

Empirical examples of traits-based inference in landscape genetics often report trait values as categorical or relative measures, leaving considerable uncertainty about the strength of such relationships. Coupling technological advances in genetic resolution with more rigorous traits-based analyses will be necessary to realize the predictive potential of a traits framework. The opportunities for comparisons among many species are increasing, allowing for more formal evaluation of traits-based models to complement the largely qualitative evaluations to-date (but see Hughes et al. 2013). Predicting population structure via a uni- or bivariate trait framework already shows promise, and multivariate, multi-trait approaches will likely continue to refine and improve such models, capturing species that fall outside relationships built upon one or two focal traits. For example, though the two studies featured in Panel 7.1 support the potential of a traits-based framework for multi-taxa inference, they also highlight important future directions to improve this framework. Considering additional traits may help characterize species that fall outside the general pattern of a bivariate trait analysis. For example, the barking frog (Craugastor augusti) has no aquatic larval life stage but also has low dispersal tendencies (Goldberg and Schwalbe 2004) and is thus not well-represented by the bivariate trait framework (Figure 7.1). Additional traits such as generation time, habitat specificity, or breeding seasonality may better describe the population genetics of species poorly predicted by the focal traits.

7.3.2 Uncertainty and the importance of intraspecific trait variability

Intraspecific trait variability can have profound effects in community ecology (Bolnick et al. 2011) and may be particularly important in linking traits to population genetic structure, as extreme trait values (versus the mean) may have a disproportionately high effect on gene flow and population genetic structure. Yet traits-based inference often relies on a single trait value for a given species – typically a mean or maximum observed value. In many cases, this may be sufficient to distinguish species from one another (e.g., Blanck and Lamouroux 2006). However, trait variability is not well understood or quantified for many species. For example, a recent meta-analysis found quantitative dispersal estimates for only 62 stream fish species globally (Radinger and Wolter 2014), suggesting knowledge gaps of important traits such as dispersal ability even for species-rich groups. In the studies highlighted in Panel 7.1, dispersal potential and water dependency were considered as relative metrics (amphibians, Chapter 5) or categorical variables (invertebrates, Chapter 2). Availability of trait data may be limited for many taxa, and quantifying intraspecific trait variability for many species may not be feasible. True dispersal potential – and its variability – is poorly understood for many aquatic organisms, and plasticity in larval development, desiccation tolerance, and behavior produce intraspecific variability in water dependency. Simulation-based approaches (Epperson et al. 2010) and consideration of well-studied taxa may provide valuable opportunities to determine whether, and if so how, intraspecific trait variability affects the ability to relationship between traits, population genetic structure, and the landscape.

7.3.3 Traits as a unifying theme across fields

Traits offer a powerful currency for pressing conservation and management applications, are widely employed in community ecology, and are increasingly applied to address contemporary challenges such as multispecies vulnerability to climate change. Ultimately, traits provide a common currency to further integrate landscape genetics with the broader fields of ecology, conservation, and restoration which increasingly rely upon traits-based inference to inform multispecies management and conservation planning. Traits already show promise as a multispecies framework in population and landscape genetics (Kelly and Palumbi 2010; Hughes et al. 2013), and formal exploration of the strengths, limitations, and uncertainty of such a framework may promote rapid, efficient, and generalizable incorporation of landscape genetics results in management and policy.

7.4 Conclusions

Traits provide a promising framework for geographic transferability, cross-taxa comparison, and broader testing of ecological theories in landscape genetics. Here we propose leveraging available and ongoing landscape genetics research to formally explore, develop, and test traits as a common currency across taxa, geography, and disciplines. Such research would focus and unify emerging support of traits as important drivers of population and landscape genetic patterns. Moving forward, more rigorous, systematic evaluations of traits-based frameworks for population and landscape genetics will help evaluate their potential in addressing fundamental ecological, evolutionary, and conservation goals.

8. Conclusions and implications for future research/implementation

From the standpoint of biodiversity conservation in the arid Southwest, it is economically and strategically prudent to understand where and how to manage for conservation purposes well before species and ecosystems become endangered. Aquatic species depend not only on available water, but also on regular dispersal events between habitat patches as a source of genetic variation or to rescue locally extirpated populations. Thus, the key to protecting aquatic species on and around DoD installations will be to understand how climate change and water management affect the critical habitat elements upon which these species depend. This understanding will increase the likelihood of long-term persistence of both rare and common species, and avoid the costly compliance with formal listings. Our project directly addressed this challenge by advancing our understanding of how stream hydrology influences the landscape genetics of aquatic insect and amphibian populations on Fort Huachuca and surrounding mountain ranges. By doing so, we provide important insight into fundamental biological processes in intermittent and ephemeral streams. Furthermore, by using species with marked differences in their life-history requirements for permanent water we were able to make generalized predictions of how functionally-similar insect and amphibian species will respond to changes in natural or human-induced changes in hydrology.

We demonstrated a novel riverscape approach to quantifying streamflow continuity through time and longitudinal connectivity through space using a network of surface flow sensors. This was accomplished for several watersheds in Fort Huachuca that are characterized by hydrologically complex flow patterns; however the proposed methodology is broadly applicable elsewhere. Data generated from spatial arrays of surface flow sensors may yield critical information on streamflow timing and duration at a higher spatial and temporal resolution compared to previous methods, while at the same time require substantially less effort and monetary cost compared to implementing field mapping programs.

According to our research, the loss of water from perennial springs and streams on Fort Huachuca (caused by climate change, human water use, or forest fires) may result in local extinctions of genetically distinct populations of the canyon treefrog (*Hyla arenicolor*) and the giant water bug (*Abedus herberti*) with little chance of recolonization. These species are perennial specialists and require permanent water for their persistence, and local extinction events will result in a loss of unique genetic variation. Alternatively, predaceous diving beetles (*Boreonectes aequinoctialis*) and Mexican spadefoots (*Spea multiplicata*) are well adapted to capitalize on ephemeral aquatic habitats that are temporally and spatially variable in availability, and population genetic analysis revealed high gene flow for both species within the range of these studies. These ephemeral-adapted species and others like them may be less vulnerable to changes in aquatic habitat or landscape features. Finally, an increase in intermittent habitat might be beneficial for species such as the red-spotted toad (*Anaxyrus punctatus*) and the stonefly (*Mesocapnia arizonensis)*, allowing populations to expand into new areas. However, increasing aridity will also result in concurrent losses of intermittent habitats as these areas transition to ephemeral flow patterns. If this happens, gene flow in these and other intermittent species may be reduced such that genetic drift becomes dominant and local extinctions become more likely.

Desert anurans utilize a range of ecological strategies to survive in areas characterized by a spatial mosaic of perennial, intermittent, and ephemeral waters embedded in an arid landscape. Larval development periods restrict species' breeding habitats, and desiccation risk is a known driver of amphibian movements in arid environments. High mobility is one possible strategy for capitalizing on unpredictable availability of water and may drive high genetic connectivity observed in some desert anurans in the American Southwest. The high mobility of Mexican spadefoots and other ephemeral specialists in the region (e.g., Couch's spadefoot, *Scaphiopus couchii*, and the Great Plains toad, *Bufo cognatus*) may provide greater resiliency to temporal or spatial changes in habitat availability, and high genetic connectivity between populations may buffer the genetic consequences of some habitat loss.

High mobility, however, is only one end of a spectrum of potential strategies for aquatic desert life; other species may instead exhibit site fidelity to isolated pools with perennial or longer-term intermittent water. Both canyon treefrogs and red-spotted toads exhibit some degree of site fidelity and had greater population structure than Mexican spadefoots. Proximity to water is the hypothesized mechanism by which canyon treefrogs meet necessary water requirements for thermoregulation. These findings suggest that a drier landscape and loss of an already limited number of breeding sites may result in a greater risk of loss of genetic diversity for amphibians such as red-spotted toads, canyon treefrogs and others with small population sizes and/or high water requirements (e.g., Chiricahua leopard frogs, *Lithobates chiricahuensis*; Arizona treefrog, *Hyla wrightorum*; and Sonoran tiger salamander, *Ambystoma mavortium stebbinsi*).

Our work supports recent studies highlighting the utility of multi-species inference and ecologically derived hypotheses. Genetic diversity is often a missing component in conservation planning and resource allocation despite its recognized role in species persistence. With increasing human demand for aquatic resources in arid environments, environmental change and habitat alteration will likely outpace the resources and time necessary for single-species population genetics studies for many species of conservation concern. When single-species studies are not feasible, the use of species' ecological information to predict relationships between genetic and structural connectivity may provide a promising alternative. Our framework for multispecies population and landscape genetic inference provides an important tool for managers and conservation practitioners tasked with applying the best available data to managing many species at once. Species' traits provide a promising avenue for geographic transferability, cross-taxa comparison, and broader testing of ecological theories in landscape genetics. We urge researchers to leverage available and ongoing landscape genetics research to formally explore, develop, and test traits as a common currency across taxa, geography, and disciplines. Such research would focus and unify emerging support of traits as important drivers of population and landscape genetic patterns. Moving forward, more rigorous, systematic evaluations of traits-based frameworks for population and landscape genetics will help evaluate their potential in addressing fundamental ecological, evolutionary, and conservation goals.

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10. Appendices

Supporting Data

Olden, Julian; Mims, Meryl (2014): Ecological strategies predict associations between aquatic and genetic connectivity for dryland amphibians. Figshare: http://dx.doi.org/10.6084/m9.figshare.1205533

Phillipsen IC, Kirk EH, Bogan MT, Mims MC, Olden JD, Lytle DA (2014) Data from: Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.hb558

List of Scientific/Technical Publications

Cañedo-Argüelles, M., Boersma, K.S., Bogan, M.T., Olden, J.D., Phillipsen, I., Schriever, T.A. and D.A. Lytle. 2015. Dispersal strength determines meta-community structure in a dendritic riverine network. *Journal of Biogeography* 42: 778-790.

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