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Multi-Scale Approach to Understanding Source-Sink Dynamics of Amphibians

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List of Acronyms

Aa	*Ambystoma annulatum*
AMAN	*Ambystoma annulatum*
Am	*Ambystoma maculatum*
AMMA	*Ambystoma maculatum*
Ao	*Ambustoma opacum*
AMOP	*Ambystoma opacum*
FLW	Fort Leonard Wood
HWE	Hardy-Weinberg equilibrium
IBD	Isolation by distance
LD	Linkage disequilibrium
NOVI	*Notophthalmus viridescens*
Nv	*Notophthalmus viridescens*
SVL	Snout vent length
TL	Total length

Keywords

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Preface

This project was initiated by Dr. Ray Semlitsch as Principal Investigator and Dr. Lori Eggert as co-Principal Investigator. Dr. Semlitsch brought a wealth of experience in herpetology and land use effects on amphibians and Dr. Eggert brought years of experience in population and ecological genetics. Between the initiation of the project in 2011 and the first quarter of 2015, both Dr. Semlitsch and Dr. Eggert contributed to the management of the project and the production of results. After the sudden and untimely passing of Dr. Semlitsch on June 10, 2015, Dr. Eggert became the PI for the project through its completion.

Abstract

Objective — The objective of this study was to improve our understanding of source-sink dynamics for subpopulations or populations of species of relevance to Department of Defense resource managers. Specifically, this project sought to better understand the impact of landscape heterogeneity on the source-sink dynamics of a species of conservation concern (the ringed salamander, *Ambystoma annulatum)* and a co-occurring species (the spotted salamander, *A. maculatum*) at Fort Leonard Wood, Missouri. Investigators sought to (1) define source habitat patches by identifying key environmental conditions associated with varying levels of reproductive success of *A. annulatum*, (2) determine whether species interactions influence source-sink dynamics, (3) identify habitat features associated with effective dispersal of *A. annulatum* among populations to define habitat connectivity, and (4) develop models to predict and manage population connectivity for salamanders in heterogeneous landscapes.

Technical approach — This study involved a combination of intensive ecological field studies, genetic analyses, and statistical modeling. Between 2011 and 2014, larvae were surveyed at each pond for three consecutive days within each sampling period. Field teams recorded the abundance of each species and that of invertebrate predators, other amphibians, and fish. During each year, visual encounter surveys and quadrat sampling for egg masses were also conducted. These surveys involved a single visit to each pond, during which data were recorded for the number of eggs observed and the number of metamorphs captured, respectively.

To study dispersal, investigators reared salamanders from hatchlings and manipulated larval densities to generate juveniles that differed in size. The activity level of juvenile salamanders was repeatedly measured to determine if movement behavior was repeatable, indicative of dispersers being intrinsically different from residents. Nine morphological traits were measured and regressed against distance moved. Whether habitat type or the interaction between body size and habitat affected initial movement distance was tested in a field study using replicated field enclosures to isolate the effects of natal and juvenile habitat on initial movement behavior. Investigators measured the surface area and maximum depth of each pond each time it was sampled. Pond attributes assumed to be constant over time (pond basin area, hydroperiod, canopy closure, emergent and submergent vegetation) were measured once during 2012.

Species-specific microsatellite loci were developed for *A. annulatum* and *A. maculatum*. After analysis of the 2012 data revealed limited genetic differentiation among ponds for both species, *A. opacum* (marbled salamander), a fall-breeding species, was added for comparison to the fall-breeding *A. annulatum.* After analysis of the 2013 data, the spring breeding *Notophthalmus viridescens* (eastern newt) was also added for comparison to the spring-breeding *A. maculatum*. Genotypes for all species were tested for genetic differentiation using traditional measures of genetic distance (F_{ST} and G'_{ST}) as well as Bayesian analyses to infer genetic partitions.

Landscape resistance was assessed using resistance surfaces generated in ArcGIS. The effects of distance and landscape resistance surfaces were assessed using CIRCUITSCAPE v4 and optimized using the "ResistanceGA" package for R. Further, pairwise G'_{ST} was used as the response variable and scaled effective resistance surfaces as the independent variable in a linear mixed effects model framework implemented in the "lme4" package for R. Model fits were assessed using AIC_C calculated from the linear mixed effects models.

Results — Habitat features, especially pond hydroperiod, forested habitat surrounding ponds including canopy cover, and the presence/absence of fish were found to be the strongest predictors of *A. annulatum* and *A. maculatum* distribution and population dynamics. Evidence was weak for species interactions being important factors in population dynamics in natural ponds, but strong evidence was observed for density-dependence and food web organization as critical components in experimental studies. Although the field data do not clearly support an important role for species interactions in source-sink dynamics in this system, the experimental data suggest that caution is warranted, and species interactions should not be disregarded as important contributors to source-sink dynamics.

The results indicate that metamorph size was strongly affected by intraspecific density and field observations indicated extensive interpond variability in metamorph size. Size affects dispersal in that intermediate sized metamorphs dispersed the farthest. A better understanding of the habitat and ecological factors affecting metamorph size in the field is an important next step to ensuring population viability and connectivity.

The absence of fish is a key characteristic of source ponds, and it is important that ponds be located within forested habitat. Field observations support a pattern of most ponds having few larvae. Spatial variation in larval density was four to ten times greater than temporal variation, suggesting that few source populations likely occur on the landscape, making their conservation priority even greater. Because growth in salamanders was affected by pond hydroperiod, it is recommended that ponds with a mosaic of hydroperiod regimes should be maintained.

Although genetic differentiation was limited, no support was found for land cover or topographic features to affect gene flow in any species, making such connectivity models irrelevant. Isolation by distance was evident and significant in *A. annulatum, A. maculatum,* and *A. opacum.* For both *A. annulatum* and *A. maculatum*, effective dispersal distances were estimated from the genetic data. The estimated dispersal distances were used to parameterize a demographic network model, which was used to assess the local importance of a population to maintaining connectivity, regional connectivity, and global contribution to gene flow of the entire network.

Benefits — This project's demographic modelling allowed for the simulation of the likely number of immigrants, emigrants, and philopatric individuals at each pond, determining average source-sink status, and assessing more integrative measures such as metapopulation capacity. Modeling results suggest that populations occur on a continuum of source to sink and that most populations fluctuate in their contributions.

Results from this model can ultimately be used to improve management concerning removal of anthropogenic stressors, mitigation of habitat loss, or facilitation of terrestrial and pond restoration for *A. annulatum* across FLW at the landscape level. The in-depth understanding of the source-sink dynamics of this representative species can be used to direct management at FLW to ensure species persistence, link management at this site to regional dynamics within the adjacent U.S. Forest Service Mark Twain National Forest, and can be applied to other ambystomatid salamanders of concern at other DOD facilities (e.g., *Ambystoma cingulatum* and *Ambystoma bishopi* at Fort Stewart, GA or Eglin Air Force Base, FL).

Objective

The technical objective of this project was to improve our understanding of source-sink dynamics for subpopulations and populations of species of relevance to Department of Defense resource managers (SISON-11-04). Specifically, this project sought to better understand the impact of landscape heterogeneity on the source-sink dynamics of a species of management concern (the ringed salamander, *Ambystoma annulatum)* and a co-occurring species (the spotted salamander, *A. maculatum*) at Fort Leonard Wood (FLW), Missouri. We used a multi-faceted approach in which we combined ecological, genetic, and modeling techniques to assess populations (i.e. ponds) at multiple spatial and temporal scales. This multi-scale approach allowed us to investigate the dynamic nature of source-sink populations. We sampled the abundance of *A. annulatum* and *A. maculatum* at local populations across seasons to understand the environmental features and species interactions associated with reproductive success or failure of ponds. Across a 7,140 hectare focal area within FLW, we used our data to investigate connectivity and occupancy in relation to dispersal habitat quality and landscape heterogeneity. Using polymorphic genetic markers, we inferred levels of gene flow among populations across the landscape over multiple generations to understand inter-pond dispersal and to identify which populations were providing emigrants.

We developed connectivity models based on occupancy, GIS habitat analysis, measures of molecular relatedness, and dispersal inferred from genetic analyses. By using a multi-scale approach to identify habitat features and species interactions associated with reproductive success at individual ponds, fine-scale genetic analyses, and the temporal dynamics of each of these processes, we investigated the contribution of individual populations to source-sink dynamics and species persistence at FLW.

This project had four specific aims: 1) to identify key environmental conditions associated with varying levels of reproductive success of *A. annulatum* to define source habitat patches, 2) to determine whether species interactions influence source-sink dynamics, 3) to identify habitat features associated with effective dispersal of *A. annulatum* among populations to define habitat connectivity, and 4) to develop models based on circuit and graph theory that can be used to predict and manage population connectivity for salamanders in heterogeneous landscapes.

The demographic modeling framework was used to determine the optimal locations on the landscape for future pond construction. The demographic network model has been made available in an R package called "ssmc" and is available with a fully documented user guide at https://github.com/wpeterman/ssmc. This approach has great potential to be beneficial and applicable to other systems in which the available population and life history parameters are uncertain, and where discrete habitat creation/restoration is an effective management strategy.

Background

A clear understanding of metapopulation dynamics and connectivity of habitat patches is essential for conservation and management as natural habitats become lost, altered, and fragmented due to changes in land use. This is absolutely required for on-the-ground management of at-risk species to ensure long-term persistence at a regional level. However, the spatial and temporal dynamics of metapopulations are not well understood for most species, especially for globally imperiled amphibians.

Two demographic processes are critical to metapopulation dynamics and species persistence. First, some local populations must exist in high-quality habitat patches (“sources”; Pulliam 1988) and produce surplus offspring frequently enough to balance losses in populations that occupy low-quality habitat ("sinks", Pulliam 1988). Second, connectivity among habitat patches on the landscape must allow successful dispersal and rescue of sink patches or colonization of new habitat patches. This second process is the result of species vagility, distance between ponds, and quality of the matrix between patches. Measurement of both these ecological processes in natural populations has seldom been achieved (but see Harrison et al. 1988, Hanski et al. 2000, Schtickzelle and Baguette 2004). Many studies focus on either reproduction alone or on dispersal and connectivity. However, processes such as source-sink population dynamics require understanding of both local population dynamics as well functional connectivity through dispersal. Ultimately, effective landscape-scale management of spatially structured metapopulations is greatly improved when factors affecting local population dynamics as well as dispersal and connectivity are considered.

Pond-breeding amphibians are distributed patchily across the landscape and are frequently assumed to exhibit metapopulation dynamics, especially in fragmented landscapes that may limit connectivity (Semlitsch 2008, Sinsch 2014). Metapopulations of pond breeding amphibians can be viewed from a “ponds-as-patches” perspective (Marsh and Trenham 2001, Richter-Boix et al. 2007). Patches include both the aquatic breeding habitat and the immediate surrounding terrestrial core habitat (95% core area usually within 300 meters) where the adult population is assumed to reside between breeding seasons (Semlitsch 1998, Semlitsch and Bodie 2003, Rittenhouse and Semlitsch 2007). Although a small number of juvenile individuals may disperse to non-natal ponds for breeding (Gamble et al. 2007), many amphibians have high site philopatry and small local population sizes, which can generate fine-scale population structuring (Beebee 2005). Genetic and statistical analyses are needed to effectively determine if pond-patches represent populations, subpopulations, or metapopulations.

Amphibians with a complex life cycle (both larval and adult stages) use aquatic habitats,(i.e., bogs, vernal pools, wildlife ponds) for mating, ovipositing, and larval growth. Aquatic habitats are highly dynamic, often filling and drying on an annual basis, and amphibians are specifically adapted to such processes (Wilbur 1980). The contribution of the aquatic habitat to the production of metamorphosing juveniles from ponds, a measure of local population recruitment and measure of individuals available for dispersal, are well understood for a number of species (e.g., Pechmann et al. 1989, Semlitsch et al. 1996, Berven 2009). Field studies have demonstrated that pond hydroperiod is a primary predictor of amphibian metamorphic success (Pechmann et al. 1989). In small, shallow, seasonal ponds or during drought years, ponds dry

early and few larvae reach a minimum body size to initiate metamorphosis and most die (e.g., in South Carolina ponds reproductive failure rates were 42-56% for 13 species over 16 years; Semlitsch et al. 1996). In large, deep, semi-permanent ponds or in wet years, ponds hold water longer and may produce large numbers of metamorphs of many species. However, ponds that persist too long can accumulate predators such as dragonfly larvae, crayfish, or fish that prey on amphibian larvae and drastically reduce survival of all but a few species, such as bullfrogs (*Lithobates catesbeianus*), that are resistant to predation (Shulse et al. 2010, 2012). Other pond features, such as the presence of emergent vegetation or shallow-slope banks, can ameliorate the effects of predators (Porej and Hetherington 2005, Shulse et al. 2010).

Salamanders (especially *Ambystoma*) have a life expectancy great enough (5-10 years) to experience a few "booms" in recruitment that compensate for adult attrition (Semlitsch et al. 1996). Modeling of amphibian populations indicates that long-lived species can "store" recruits in terrestrial habitats during these infrequent reproductive booms, whereas short-lived species cannot and depend more directly on larval success in aquatic habitats (Halpern et al. 2005, Harper et al. 2008). This pattern of "boom or bust" population dynamics contrasts greatly with the relatively constant level of annual reproductive success exhibited by most mammals and birds. This characteristic of amphibian populations means that multiple years of reproductive failure due to unfavorable aquatic conditions such as rapid drying during drought years or from anthropogenic factors like ditching or introducing fish can result in decline and eventual extinction of species at the local pond level (e.g., Semlitsch et al. 1996). Because breeding adults strongly prefer to return to ponds where they first breed (e.g., Oldham 1966, Breden 1987, Berven and Grudzien 1990), metamorphosing juveniles are likely the primary dispersal stage (sensu Gill 1978). A high rate of successful metamorphosis is critical to maintain local adult populations and produce dispersers to reestablish extirpated populations or found new populations. So, although ponds are especially critical for reproduction in short-lived species, terrestrial habitats are especially critical for adult populations in long-lived species, and the connections among pond populations are essential for regional persistence at the metapopulation level.

Though the existence of metapopulation dynamics is dependent on species dispersal ability, direct empirical evidence of dispersal rates between ponds (patches) is rare and inherently difficult to quantify (see results of a 7-year study by Gamble et al. 2007). Amphibians are generally thought to have low dispersal capabilities because of their small size and susceptibility to water loss; estimates of dispersal distances range from only 1-2 km (Berven and Grudzien 1990; Gamble et al. 2007). Behaviorally-based data, however, are constrained by measuring movement distances during rare dispersal events over a single lifetime whereas rescue or colonization processes are more likely to occur as the result of a series of movements over multiple stages and/or generations (Semlitsch 2008). However, using highly variable genetic markers, one can assess populations (ponds) that relate to each other (i.e., network topology) based on the level of connectedness, as well as pond importance (Dyer and Nason 2004, Garroway et al. 2008). For species with high dispersal ability, little to no differentiation will exist between populations (Sereno et al. 2006), whereas species with limited dispersal ability will be more severely impacted by fragmentation or habitat heterogeneity and patches may become completely isolated (Johnson et al. 2004, Richter and Broughton 2005). Major barriers to terrestrial dispersal (e.g., rivers, roads, row-crops) heavily impact gene flow (Noël et al. 2007,

Marsh et al. 2008), but effects of lesser barriers are not always obvious. For example, Marsh et al. (2005) found that even small forest roads limited the dispersal ability of red-backed salamanders (*Plethodon cinereus*) and pastures restricted movement of spotted salamanders (*Ambystoma maculatum*; Rittenhouse and Semlitsch 2006).

A conservation and management priority is the identification of high quality habitat and the factors that predict how populations respond to habitat loss or change. Such information is essential for habitat restoration and mitigation, and becomes critical when designating habitat patches as sources or sinks for prioritizing management efforts. Molecular approaches allow for powerful, indirect measures of movement among populations, and can identify populations that are net contributors “sources” or receivers “sinks” of dispersing individuals (Barson et al. 2009).

Materials and Methods

Study site — Fort Leonard Wood (Figure 1) is an active year-round military training facility located in the Ozark Highlands, Pulaski County, MO, USA. The facility encompasses 24,686 ha that is 80 % forested, and includes an extensive road system (both paved and gravel). Across the entire facility, there are more than 500 natural, constructed, and unintentional (i.e. deep tire ruts) bodies of water, primarily in the form of small (<0.04 ha) fishless, manmade wildlife ponds. We concentrated our efforts within a 7,140 ha focal area that contained both study species and all pertinent habitat types (Figure 1).

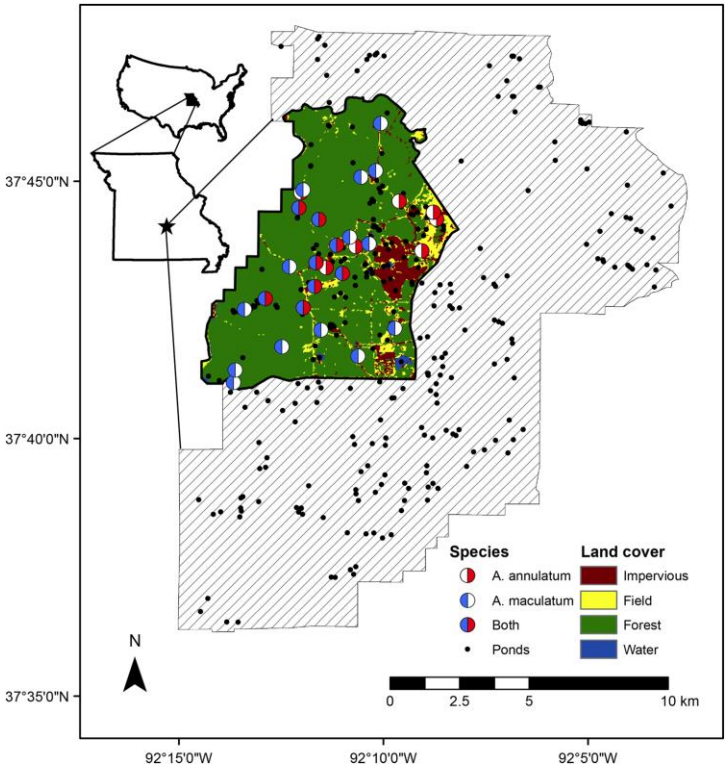

Figure 1. Map of Fort Leonard Wood, MO with land cover detail for the 7,140 ha focal area assessed in this study (From Peterman et al. 2015).

Occupancy, abundance and fitness of salamanders — During the first year of the study (2011-2012), we conducted initial surveys to determine the distribution of *Ambystoma annulatum* (ringed salamanders) and *A. maculatum* (spotted salamanders) and defined the focal area for sampling. Between 2012 and 2014, we surveyed 203 ponds within the focal area for larval *A. annulatum*, *A. maculatum*, and *A. opacum* (marbled salamanders). During the final year (2014-2015), we continued our surveys at 53 ponds that spanned a known gradient of abundance (as determined by the previous three years) for *A. annulatum* and *A. maculatum*, which would further our ability to differentiate high and low quality ponds for these species.

The majority of our survey efforts focused on sampling larvae, using a combination of dip net sweeps and mesh funnel traps. The number of dip nets and traps we deployed at each pond was scaled to each pond's surface area, such that different sized ponds received an equal survey effort (Shulse et al. 2010). We used funnel traps (2mm mesh size, 38 X 26 X 26 cm; Memphis Net and Twine) when the water was deep enough to cover trap openings and when we could access the site over the three days of the survey period. Traps were checked daily (maximum of 60 trap

checks per pond over three days). We split our sampling across two time periods within each year: late February to early April for *A. annulatum* and *A. opacum*, and late May and early June for *A. maculatum*. These sampling periods occurred at approximately 2/3 of the way through each species' larval period. We surveyed each pond for three consecutive days within each sampling period, and recorded the abundance of each species, as well as the abundance of all other captured organisms, including invertebrate predators, other amphibians, and fish.

During each year of the study, we also did visual encounter surveys and quadrat sampling for egg masses (October for *A. annulatum* and *A. opacum*; April for *A. maculatum*), and dip net surveys for metamorphosing individuals of both species (May for *A. annulatum* and *A. opacum*; June for *A. maculatum*). Each of these latter surveys involved a single visit to each pond, where we recorded the number of eggs observed and the number of metamorphs captured, respectively.

Dispersal studies — We measured the repeatability of movement behavior using lab and field assays. We reared salamanders from hatchlings in 1000 L pond mesocosms and manipulated larval densities to generate juveniles that differed in size. Animals metamorphosing from low density mesocosms (hereafter large; mean ± 1 SE: 41.0 mm ± 0.43) were larger than animals from high density mesocosms (hereafter small; 34.75 mm ± 0.34; ANOVA: $F_{1, 47} = 88.71$, $P < 0.001$). To determine if movement behavior was repeatable, indicative of dispersers being intrinsically different from residents, we repeatedly measured the activity level of 50 juvenile salamanders (large: N = 34; small: N = 16) during 10 repeated trials over a three month period (4 June – 9 September 2013). Activity was assayed in circular plastic arenas (8 cm deep x 30.48 cm diameter) with a moist paper towel substrate. After placing one juvenile in each arena, we allowed 10 minutes for acclimation and then video recorded behavior for 30 minutes using camera mounted above the arenas. We analyzed the videos to quantify the distance individuals moved and their latency to move. To further test intrinsic factors that may predict whether or not a salamander disperses, we tested the activity of 396 juvenile *A. annulatum* and 166 juvenile *A. maculatum* immediately following metamorphosis by measuring their latency to move and distance moved in a novel habitat. We then measured nine morphological traits (SVL, head width and length, tail length and width, humerus and femur length) and regressed the traits against distance moved.

We also tested whether habitat type or the interaction between body size and habitat affected initial movement distance in a field study. We used replicated field enclosures to isolate the effects of natal and juvenile habitat, respectively, on initial movement behavior. Each enclosure (N = 12; 2 m x 50 m) had plastic weave silt-fencing walls (90 cm tall) buried 15 cm into the ground. The walls were supported by wooden stakes. At each of the two sites, we constructed three replicate runways in both closed canopy deciduous forest (*i.e.* high quality habitat) and mixed grassland habitat (*i.e.* low quality habitat). We individually marked each salamander with a Passive Integrated Transponder (PIT) tag as in Ousterhout and Semlitsch (2014). When used in tandem with a portable antenna system, PIT tags allow otherwise prohibitively small bodied animals to be individual tracked from a distance, albeit a small one: 30 cm or less underground; 2499 cm^2 in the horizontal plane (the detection field is an ellipse, 24.3 cm in the direction parallel to the tag (long axis) and 10.6 cm in the direction perpendicular to the tag (short axis); (Connette and Semlitsch 2012, Ousterhout and Semlitsch 2014). We released cohorts of juvenile salamander on nights within 24 h of rain, and then tracked their movement daily for seven days

using a portable RFID system. We recorded the location of each recapture and calculated total distance moved.

Landscape studies — We measured pond attributes during sampling each year except in the case of static variables, which were measured once during the summer of 2012 (Table 1). We estimated pond area by measuring across the longest and shortest axes using an infrared range finder (Bushnell Yardage Pro) and multiplying the results. We treated hydroperiod as a continuous variable in all analyses, but divided it into four categories along a continuum: ephemeral (dries multiple times every year), seasonal (dries once a year during the summer), semi-permanent (dries during droughts), and permanent (never dries). Categories were assigned based on the results of observations made approximately every other month during 2012 and 2013 while conducting other studies, use of temperature loggers to detect drying date (Anderson et al. 2015), and historical data (K. Lohraff, FLW Wildlife Manager, pers. comm.). We estimated canopy closure using a spherical densitometer (Forestry Suppliers, Jackson, MS, USA) during full leaf-out at the four cardinal directions around each pond. We categorized within-pond habitat variables as emergent vegetation, submerged vegetation, cattails, coarse woody debris, duckweed, water shield, and lily pads. The percentage of each type was recorded for all ponds in summer 2012 using quadrat sampling. The slope of the pond basin was quantified by taking depth measurements at 1- and 2-m distances from the shoreline at multiple locations, calculating slope, and averaging values for each pond (methods from Semlitsch et al. 2015). Additional landscape data were gathered from digital orthophotomaps and land cover data layers available through University of Missouri's Spatial Data Information Center: http://msdis.missouri.edu.

Within-population genetic analyses — We developed species-specific microsatellite loci for *A. annulatum* and *A. maculatum* using a next-generation sequencing approach. During the first year of the study, we collected tissue samples for each species from FLW, extracted whole genomic DNA and prepared an Illumina paired-end shotgun library for each species. We analyzed the data for each species separately, using the program PAL_FINDER v0.02.03 (Castoe et al. 2012) to extract sequence reads containing microsatellites and developing primers in Primer3 (Rosen and Skaletsky 2000). We screened candidate loci for each species for amplification and polymorphism using a panel of seven *A. annulatum* and seven *A. maculatum*. From 591 candidate loci for *A. maculatum*, we screened 68 tetranucleotide loci and selected 18 for use in the study (Peterman et al. 2013a). From 749 candidate loci, we screened 150 tetra- and pentanucleotide loci for the *A. annulatum* and selected 22 for the study (Peterman et al. 2013b).

We collected samples during the field sampling phase of the study between 2012 and 2014. In March–April, 2012, we collected 12–53 *A. annulatum* from 20 ponds and 10–54 *A. maculatum* from 23 ponds within the focal area. We collected tail clips from late stage larvae of *A. annulatum*, and to minimize sampling of siblings, we sampled larvae systematically from the entire perimeter of the pond. For *A. maculatum*, we collected one late stage embryo from each individual egg mass. During spring 2013 for *A. maculatum* and fall 2013 for *A. annulatum*, we collected 332 *A. maculatum* late stage embryos from 17 ponds and 421 *A. annulatum* late stage embryos or larval tail clips from 19 ponds. All samples were collected during the breeding season to minimize the among year variation. We lumped all individuals sampled in ponds within 100 m of each other into one group for downstream analysis due to the likelihood of individuals within these ponds being closely related.

Table 1. Summary of landscape and pond parameters assessed in this study (from Peterman et al. 2014).

Parameter	Variable type	Data range	Rationale	Sources
Abundance Model				
Fish	Presence/absence	binary 1/0	Fish negatively influence amphibian reproduction, survival, abundance, and species richness	Sexton, Phillips & Routman, 1994; Porej, Micacchion & Heatherington, 2004; Werner *et al.*, 2007a; Shulse *et al*., 2010
Structure	Percentage of pond with vegetation	36 (0–100)	Vegetation provides breeding substrate and refuge from predators	Madison & Farrand, 1998; Shulse *et al.*, 2010; 2012
Hydroperiod	Categorical (ephemeral, seasonal, permanent)	categorical 1–3	Hydroperiod is a critical determinant of survival to metamorphosis and breeding population size	Semlitsch & Wilbur, 1988; Baldwin *et al.*, 2006
Canopy	Percentage of closed canopy above pond; pond average (linear and quadratic)	38 (0.00–96.00)	Larval survival and breeding population size is greater in closed canopy ponds	Baldwin *et al.*, 2006; Earl *et al.*, 2011
Predator Richness	Number of invertebrate predator species	5.45 (0.00–12.00)	Larval mortality increases with predators, and predator richness is correlated to abundance	Rannap *et al.*, 2009; Relyea, 2007
Ponds within 300 m	Count	8.5 (1–27)	Connectivity of ponds is a predictor of salamander occupancy	Cosentino, Schooley & Phillips, 2011

Forest within 300 m	Percent of buffer area	65 (1–100)	Salamanders abundance is strongly affected by the presence of forests in core terrestrial habitat	Shulse *et al.*, 2010; Porej, Micacchion & Heatherington, 2004; Semlitsch & Bodie, 2003
Forest within 1 km	Percent of buffer area	72 (15–100)	Habitat within 1 km is a better predictor of salamander occurrence	Charney, 2012
Distance to forest	Euclidean distance (m)	43 (0–260)	Juvenile and adult ambystomatid salamanders have higher survival in forested habitat	Rothermel & Semlitsch, 2006
Pond Age	Years	27 (4–75)	Ambystomatid salamanders are hypothesized to prefer mid- to late-succession ponds	Birx-Raybuck, Price & Dorcas, 2010
*Presence of *A. annulatum* and/or *A. opacum*	Presence/absence	Binary 1/0	*A. annulatum* and *A. opacum* may act as a fish-like predator that *A. maculatum* avoid	Urban, 2007
Slope	Change in depth cm / m	0.151 (0.00–0.42)	Shallow littoral zones are a predictor of amphibian richness, and may provide breeding sites and refuge from predators	Walls, 1995; Porej & Hetherington, 2005
Detection Model				
Date	Julian date	-	Larval activity patterns may change throughout the	personal observation

			sampling season	
Temperature	Degrees Celsius	8.35 (0.50–20.00)	Larval activity may be affected by water temperature	Almeida, 2010; Mott & Sparling, 2010
Method	Dip net or minnow trap	-	Efficacy of method may vary with larval vagility	Denton & Ricther, 2012
Percent cattail	Percent of pond with cattails	10 (0–95)	Dip net sweeps were potentially less effective in highly structured ponds	personal observation
Slope	Change in depth cm / m	0.151 (0.00–0.42)	Dip net sweeps were potentially less effective in steep ponds	personal observation

* Parameter only included in *A. maculatum* abundance model

After analysis of the 2012 data, we added a third species, the *A. opacum* (marbled salamander) for comparison to the fall-breeding *A. annulatum*. This allowed us to explicitly test the hypothesis that breeding pond availability and species life history strongly influences genetic structure in pond-breeding salamanders. We screened previously published microsatellite loci for Ambystomatids (Nunziata et al. 2011, Martin 2013, Peterman et al. 2013b) in *A. opacum* and selected 13 that were informative in the FLW populations. During the fall of 2013, we collected 129 larval tail clips from 12 ponds. After analysis of the 2013 data for the three ambystomatid species, we added a fourth species, *Notophthalmus viridescens* (Eastern newt) for comparison with the spring-breeding *A. maculatum*. We selected 10 microsatellite loci for this species after screening previously published loci for several newt species (Crowshaw and Glenn 2003, Jones et al. 2001, May 2011). During the spring of 2014 we collected 110 larval tail clips from 11 ponds.

Following genotyping of the study samples, we tested for full-siblings in our data sets with COLONY (Jones and Wang 2010) setting male and female mating to polygamous without inbreeding for a long run with full likelihood, high precision, and no sibship prior. For all samples that had greater than 95 % likelihood of being full siblings, we kept one haphazardly selected sample for downstream analysis.

We tested for Hardy-Weinberg Equilibrium (HWE) and linkage disequlibrium (LD) among pairs of loci with Genepop (Raymond and Rousset 1995, Rousset 2008). We conducted both tests using 1,000 burn-in steps and 100 batches with 1000 iterations per batch. Results of all tests were assessed for significance following a Bonferroni correction for the number of comparisons (Rice 1989). We used the "PopGenReport" package for R (Adamack and Gruber 2014) to test for the presence of null alleles and gstudio (Dyer 2014) to calculate allelic richness, observed and expected heterozygosity,

Among-population genetic analyses — For the 2012 data, we tested for genetic differentiation among populations using traditional measures of genetic distance (F_{ST} and G'_{ST}) in "PopGenReport" (Adamack and Gruber 2014). We assessed spatial population structure using the program Bayesian Analysis of Population Structure v6 (BAPS, Corander et al. 2003, 2008). In BAPS, we used a two-step analysis to assess the most likely number of genetic partitions for each species. The first step involved a spatial clustering of individuals to allocate each sample into its most likely genetic group. The second step refined the results by assessing admixture (Corander and Marttinen 2006). This approach uses the spatial coordinates of each sample location, Voronoi tessellation, and Markov Random fields to determine the maximum number of population clusters (K). For *A. annulatum* and *A. maculatum*, we tested for 2–15 and 2–22 clusters using ten replicates at each potential cluster number.

For the 2013 genetic data, we used the "gstudio" package for R (Dyer 2014) to assess genetic distances, but used the program STRUCTURE (Pritchard et al. 2000) to test for spatial population structure. After a 100,000 burnin period, we used 500,000 MCMC repetitions to test K=1-20 for *A. annulatum*, K=1-18 for *A. maculatum*, K=1-13 for *A. opacum*, and K=1-12 for *N. viridescens*. To infer the number of genetic clusters that were best supported by the data, we used the ΔK metric of Evanno et al. (2005).

Landscape resistance — The main objective of our landscape genetic analyses was to determine the features of the landscape that most affected dispersal, as measured by genetic differentiation between pairs of breeding ponds that were sampled in 2012. Additionally, we sought to determine rate and direction of gene flow through the use assignment tests or coalescent modeling. We generated landscape resistance surfaces using ArcGIS v10.3 (ESRI, Redlands, CA, USA) in order to test our hypothesis that the species differ in their response to landscape usage. Resistance surfaces were as follows: slope, aspect, land cover (forest, open water, field, and developed), topographic position index (Jenness 2006), topographic wetness index (Theobold 2007), and distance from ravine. With the exception of our land cover data that was downloaded from the U.S. Geological Survey (USGS, http://view.nationalmap.gov), all other surfaces were derived from 90 m resolution USGS elevation data following the methods outlined in Peterman and Semlitsch (2013). We iteratively tested the effects of distance and landscape resistance surfaces following the methods of Peterman et al. (2014b) within the "ResistanceGA" package for R (Peterman 2014, R Core Team 2015). We chose to assess landscape resistance using CIRCUITSCAPE v4.0 (McRae 2006) rather than least cost paths. CIRCUITSCAPE models random movement between populations across multiple pathways, while least cost path analysis assumes a single optimal pathway, the former better approximates dispersal and movement behavior of amphibians (Rittenhouse and Semlitsch 2006). Further, we used pairwise G'_{ST} as the response variable and centered and scaled effective resistance surfaces as the independent variable in a linear mixed effects model framework implement in the "lme4" package for R (Bates et al. 2015). We assessed model fits using AIC_C calculated from our linear mixed effects models.

Source-sink dynamics — Ultimately, source-sink dynamics within our system were assessed by combining pond-level abundance and size data with genetic-based dispersal data in a stochastic connectivity model. This model probabilistically distributes metamorphs produced from a pond across the landscape to adjacent ponds as a function of (1) survival, (2) probability of remaining philopatric to their natal pond, and (3) dispersal ability, which was modeled as a negative exponential decay. Metamorphs that survive and disperse in the model are distributed among adjacent ponds as a function of their probability of connectivity. From this model we were able to identify populations that were net producers (sources) and receivers (sinks) of salamanders. We were unable to rigorously assess population growth rates because of the longevity and life history of ambystomatid salamanders. Specifically, ambystomatid salamanders reach sexual maturity in 2–4 years, can live for up to 10 years, and breeding effort is largely tied to temperature and precipitation events. As such, assessment of local population growth rates would require longer-term monitoring and marking of individuals. However, we were able to identify some populations that were definitive sinks on our landscape. Specifically, when we found eggs deposited in ponds containing fish (predators of ambystomatid eggs and larvae) or in ephemeral ponds that dried before metamorphosis could be completed

Species interactions — We assessed interspecific interactions between *A. annulatum* and *A. maculatum* using a combination of outdoor mesocosm and indoor microcosm experiments. The outdoor mesocosm experiments were conducted in 1000 L cattle water tanks that were designed to mimic natural pond communities, as we controlled the initial inputs (e.g. leaf litter, planktonic organisms) but manipulated variables of interest (e.g. larval density). Microcosm experiments were conducted in aquaria indoors under controlled environmental conditions, which permitted

more mechanistic tests of different ecological processes. We analyzed body size and survival of each species to assess their effect on one another.

Results and Discussion

Occupancy, abundance and fitness of salamanders — We found that a combination of abiotic and biotic factors influenced larval occupancy and abundance of *A. annulatum*, *A. maculatum*, and *A. opacum* (Peterman et al. 2014, Ousterhout et al. 2015, Anderson et al. 2015). For all species, the presence of fish, including gamefish (Centrachidae), mosquitofish (*Gambusia* spp.) and other minnows (Cyprindae), severely reduced both the probability of occupancy of larval salamanders and their abundance (see Figure 2 in Anderson et al. 2015). In many cases, breeding efforts of all species ranged from absent to a single female in ponds with fish; in the few instances where breeding was attempted, we rarely encountered larvae, suggesting no recruitment from fish occupied ponds. Ponds with fish act either as unusable habitat or a sink habitat if salamanders deposit eggs. Therefore, absence of fish is a key characteristic of source ponds.

Because we repeatedly sampled ponds over three days, we were able to assess how detection probability varied among our focal species. For *A. annulatum*, we found that warmer water temperature (which was strongly correlated with sampling date) resulted in increased detection probability for occupancy analyses. Sampling for larvae of *A. annulatum* occurred during the late-winter and early-spring. During this time, the deeper regions of ponds maintain a more constant temperature than the shallower edges where we sampled, which more closely mirrored the air temperature. Detection of larvae may have been higher with warmer temperatures, because of a shift in habitat use from the more stable, deeper sections of the pond to the more variable edges of the pond that we sampled. We did not find any covariates that significantly influenced detection of *A. maculatum* in either an occupancy or abundance framework (Peterman et al. 2014, Anderson et al. 2015). In general, there was large variation in captures between days, indicating that multiple samples at a site will be needed to approximate the true abundance of animals. Our results indicate that consideration of seasonal effects on sampling procedures will likely improve detection of animals, leading to less bias in abundance or density estimates.

When comparing populations of *A. annulatum* over time, we found a strong correlation of consistently high densities of larvae between years for some ponds. In contrast, other ponds showed high stochasticity in larval densities, which is consistent with the "hot-spot" model of spatial demography (Gill 1978). Certain ponds are consistently sources, producing numerous recruits into the metapopulation, whereas others are either not contributing at all, or acting as sink habitats. Identification of each type of population is crucial in conservation planning so as to preserve regional connectivity and promote persistence through protection of the source habitats. We also observed a pattern of most ponds having few larvae, and only a few ponds have higher densities; spatial variation in larval density was four to ten times greater than temporal variation. This would suggest that the number of source habitats is minimal, making their conservation priority even greater.

Size at metamorphosis is a predictor of eventual fitness in pond breeding amphibians. Specifically, amphibians that metamorphose at smaller size have an increased risk of desiccation, depressed immune function, lower lipid levels and survival, later age of first reproduction, and lower fecundity (Scott 1994, Davis and Maerz 2009, Peterman et al. 2013c). We found a strong correlation between individual size during the mid-larval and late-larval stages. This indicates the size of larvae may be a predictor of a pond's contribution to the metapopulation, i.e. whether

it is a source or sink. While we found a strong negative relationship between population density and size at metamorphosis in mesocosm studies (Ousterhout and Semlitsch unpublished data, Anderson and Semlitsch 2014), in natural ponds intraspecific density had only a small effect on body size (Ousterhout et al 2015). From our sampling, fitness, as predicted by larval body size, was most strongly affected by the density of other *Ambystoma* salamanders. Both *A. annulatum* and *A. maculatum* were smaller in ponds that had a higher density of *A. opacum* (Ousterhout et al 2015). Further experiments with *A. opacum* are necessary to tease apart the mechanism of this relationship.

Dispersal — Despite the importance of dispersal to connecting source and sink habitats, there are significant gaps in our understanding of dispersal processes (Hanski 2004). We complemented the genetic approaches that sought to describe patterns of dispersal based on gene flow and the distribution of genotypes across the landscape with a series of laboratory and field experiments to examine factors affecting the propensity of an individual to disperse. Preliminary investigations suggest that dispersers differ from residents in a non-random manner. The movement behavior of individuals was consistent over a three-month period. In addition, we observed inter-individual variation in the distances moved, with larger bodied animals moving farther than small juveniles (Figure 2).

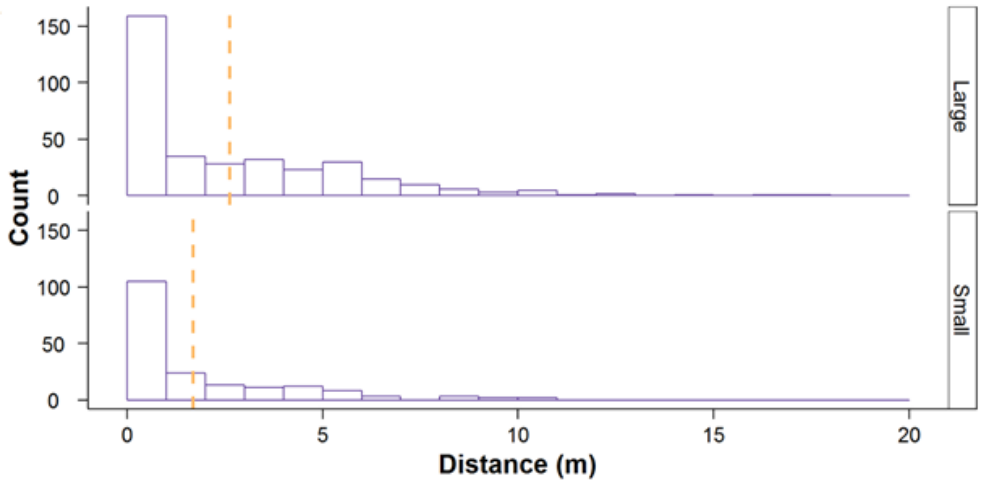

Figure 2. Observed frequency of distance moved (m) by juvenile body size in laboratory study. Dashed vertical line represents the mean from all trials.

These findings support the hypothesis that juvenile salamanders display non-random dispersal, with dispersal propensity being best predicted by morphological traits. However, these morphological traits differed between *A. annulatum* and *A. maculatum. Ambystoma annulatum* with intermediate body sizes and longer tails after a correction for body size moved farther (Figure 3). *Ambystoma maculatum* with shorter femurs for their body size moved farther. However, these were not strong relationships for either species (Ousterhout et al. unpublished data).

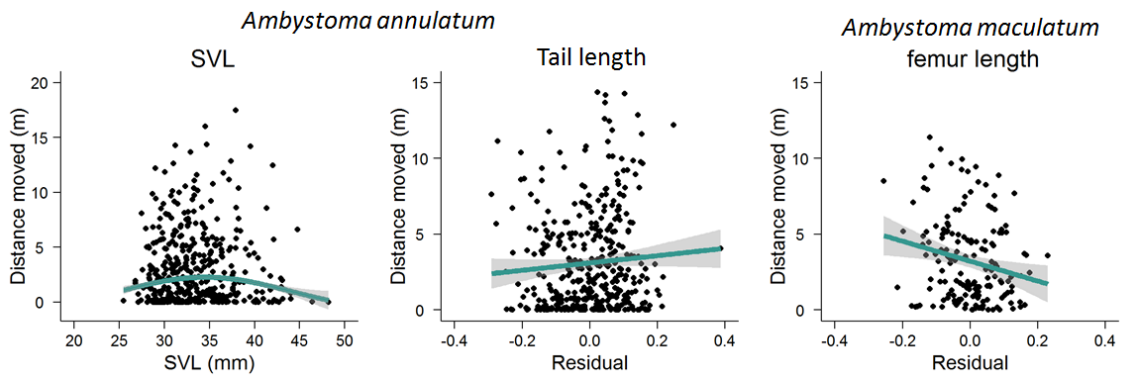

Figure 3. Activity in the lab (distance moved) of *A. annulatum* was predicted by body size (*left*) and the body size independent residual of tail length (*middle*). *A. maculatum* movement was predicted by femur length (*right*).

Although we found support for factors intrinsic to the individual predicting whether an individual is likely to disperse (e.g. body size), 67% of variation in movement behavior was not specific to the individual. This indicates that factors extrinsic to the individual (e.g. habitat quality, density dependence) play an important role in moderating movement behavior. Indeed, in a field assay of juvenile movement, we found that both juvenile body size and habitat quality affected how far animals moved over a week. *Ambystoma annulatum* of a medium body size moved the farthest, mirroring our findings in the activity assay. This finding suggests that ponds which produce intermediate sized individuals will contribute more individuals with a propensity to disperse within the metapopulation. Juveniles moved farther in forested habitats, indicating that high quality habitat may facilitate dispersal between pond populations. *Ambystoma annulatum* moved more frequently in grassland habitat, which is low quality for these forest dependent species (Ousterhout and Semlitsch unpublished data). We interpret this as individuals minimizing risk by moving quickly through unfavorable areas.

Landscape studies — For *A. annulatum*, we found that occupancy probability increased in ponds that were surrounded by greater percentage of forest within a 300 m buffer and in ponds that had a more permanent hydroperiod (Anderson et al. 2015). Similarly, *A. maculatum* occupancy probability increased in ponds surrounded by more forest, and also in ponds that had greater canopy closure (Anderson et al. 2015).

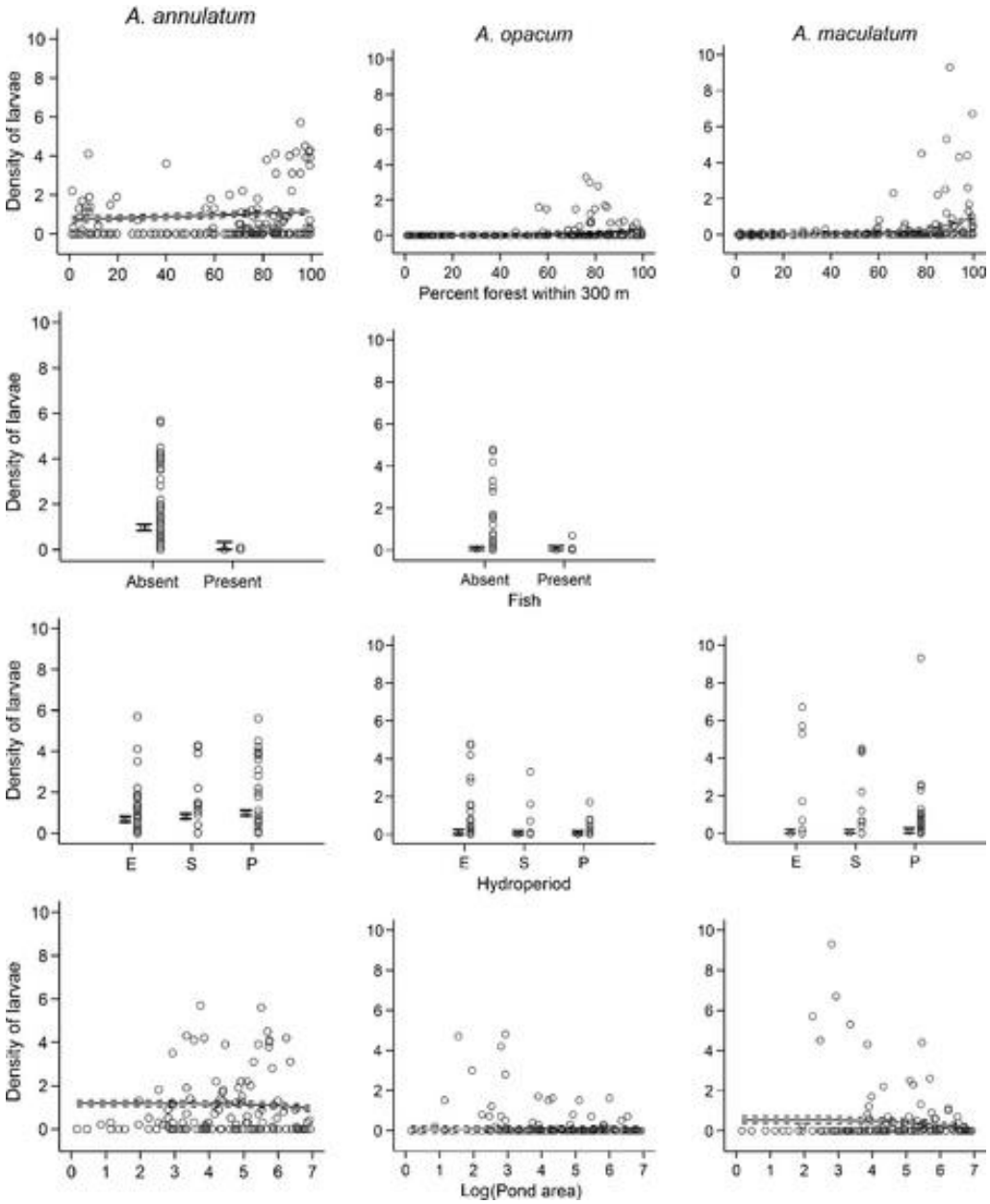

Figure 4. Estimated effects of percentage forest within 300 m, fish presence, hydroperiod, and pond area on the density of larvae of *A. annulatum*, *A. opacum*, and *A. maculatum* (larvae/m^2). Open grey circles represent observed values, open black circles or solid lines are predicted values, and error bars or dotted lines indicate ± 1 SE. Hydroperiod was evaluated under mean percentage of forest, fish absence, and mean pond area and classified as ephemeral (E), seasonal (S), and permanent (P) (from Ousterhout et al. 2015).

For abundance, both Peterman et al. (2014) and Ousterhout et al. (2015) found that increasing forest cover surrounding a pond resulted in higher larval densities for *A. annulatum* and *A. maculatum* (Figure 4). We also found that larger ponds had higher densities of *A. annulatum* compared with smaller ponds (Ousterhout et al. 2015), and that abundance increased when ponds were increasingly spatially clustered (i.e. a greater number of ponds within 300 m; Peterman et al. 2014). Similar to occupancy, larval abundance of *A. maculatum* increased in ponds with greater

canopy closure (Peterman et al. 2014). Maintaining such habitat features (forested habitat with high canopy closure) will likely benefit both our focal species, as well as other amphibians.
In addition to pond and landscape traits, climatic factors played a significant role in determining larval occupancy. Missouri experienced a severe drought that persisted from March to November 2012, and had significant but different consequences for *A. annulatum* and *A. maculatum* (Anderson et al. 2015). The onset of the drought coincided with the breeding period for *A. maculatum*; due to the drought, 34% of ponds where *A. maculatum* bred in 2012 dried prior to any larvae completing metamorphosis (i.e. complete reproductive failure, Table 2). Because *A. annulatum* requires more permanent ponds to complete their life cycle, such reproductive failure was not observed, as their ponds did not dry. However, the drought continued through the breeding period of *A. annulatum* (October), which resulted in a 15% reduction in the number of available ponds (i.e. ponds dried and did not refill). Identifying and incorporating such differential responses to environmental factors based on life history differences may be critical in conservation planning, particularly when evaluating the minimum necessary requirements of each species as well the factors that promote source versus sink habitat. Specifically, we recommend that ponds with a mosaic of hydroperiod regimes should be maintained to safeguard against regional population failure from climatic factors such as drought.

Table 2. Summary of ponds surveyed during egg, larval and metamorph sampling for the fall-breeding ringed (*Ambystoma annulatum*) and spring-breeding spotted (*A. maculatum*) between 2011 and 2013 at Fort Leonard Wood, Missouri, USA (from Anderson et al. 2015).

Species, year, and life stage	Number surveyed	Number occupied	Dry ponds during survey	Occupied but dried
Ambystoma annulatum				
2011				
Egg	144	63	6	2
2012				
Larvae	175	75	3	3
Metamorph	80	53	3	0
Egg	190	66	29	6
2013				
Larvae	198	82	2	0
Metamorph	81	76	0	0
A. maculatum				
2012				
Egg	83	101	15	32
Larvae	185	53	72	2
Metamorph	53	41	0	0
2013				
Egg	194	117	1	6
Larvae	197	99	21	4
Metamorph	98	78	4	0

Notes: "Number occupied" indicates the number of ponds where that life stage was detected. "Occupied but dried" indicates ponds that were occupied by that stage, but experienced drying prior to the next survey round, indicative of reproductive failure.

Within-population genetic analyses — Genotyping of our 2012 *A. annulatum* samples revealed that we had a high frequency of full siblings, despite our efforts in the field to avoid collecting from related individuals. We sampled 22 ponds for *A. annulatum* and collected 547 samples, but had to remove 44% of these samples due to high relatedness. After removal, only 15 of the 22 ponds had ≥10 samples remaining, and only these ponds were included in our analyses. For *A.*

maculatum, we removed 1.5% of the genotyped samples due to high relatedness, and only one of the 23 ponds from which we collected samples was removed from the analyses due to having insufficient sample size. We found no evidence of linkage disequilibrium for any pairs of loci in either species. For *A. annulatum*, four microsatellite loci (Aa_37, Aa_45, Aa_31, and Aa_4) deviated significantly from heterozygosity values expected under Hardy-Weinberg equilibrium (HWE). Further analyses suggested that these loci contained null alleles, thus we removed them and conducted our final analyses using 15 loci. For *A. maculatum*, no pond or locus deviated significantly from expectations under HWE, but one locus (Am_60) was monomorphic; we conducted our final analyses using 18 loci. We found moderate levels of genetic variability (Table 3) as defined by allelic richness and observed heterozygosity in both species. The inbreeding coefficient F'_{IS} values suggest that individual ponds do not contain inbred individuals.

For 2013-2014, we genotyped additional samples from *A. annulatum* and *A. maculatum*, as well as samples from *A. opacum* and *N. viridescens.* We found no evidence of linkage disequilibrium for any pairs of loci in any species. For *A. annulatum*, we found no evidence of null alleles or significant deviations from HWE and a low percentage of full siblings; we were able to include 19 microsatellite loci in our final analyses. For *A. maculatum*, we also found no evidence of null alleles or significant deviations from HWE and a very low percentage of full siblings. Our analyses for this species included 17 of the 18 loci used in the 2012 study. For *A. opacum*, we found that no locus deviated significantly from HWE, but that two of the 14 we optimized for this species were monomorphic. Analysis in COLONY suggested that two of the 129 individuals we genotyped were full siblings, thus our final analyses included 128 individuals from 10 ponds that were genotyped at 12 loci. For *N. viridescens*, we found no evidence of null alleles and no full siblings. Two of the 10 microsatellite loci were excluded from further analyses as one was monomorphic and the other deviated significantly from HWE. Our final analyses for this species included 110 individuals from 11 ponds that were genotyped at eight microsatellite loci.

Although allelic richness and F'_{IS} were slightly higher for *A. annulatum* and *A. maculatum* in 2013 than in 2012, the differences were not statistically significant (Table 3). Our analysis of *A. opacum* in 2013 revealed that this species has significantly lower heterozygosity and allelic diversity and higher F'_{IS} than either *A. maculatum* or *A. annulatum*, likely as a result of smaller population sizes at FLW. Our 2014 study of *N. viridescens* found higher allelic richness than any of the *Ambystoma* species but similar levels of heterozygosity. This species has a complex life history, as larvae develop in ponds and after 2-6 months, metamorphose into the terrestrial “eft” stage known to disperse for up to 7 years over long distances (Gill 1978). After a second metamorphosis, they become aquatic breeding adults. These life history differences make it difficult to interpret differences in allelic richness and F'_{IS}.

Table 3. Population genetic summary statistics for salamander species sampled at Fort Leonard Wood, MO.

2012 samples	*A. maculatum*	*A. annulatum*
N samples	642	306
N loci	15	18
N sampling sites	22	15
Observed heterozygosity	0.59	0.66
Expected heterozygosity	0.60	0.68
Allelic richness	3.08	3.44
F'_{IS}	0.013	0.038

2013 samples		*A. maculatum*	*A. annulatum*	*A. opacum*
N samples	332	421	129	
N loci	17	19	12	
N sampling sites	17	26	10	
Observed heterozygosity	0.62	0.69	0.45	
Expected heterozygosity	0.60	0.68	0.51	
Allelic richness	4.47	4.23	3.36	
F'_{IS}	0.047	0.063	0.210	
G'_{ST}	0.077	0.129	0.102	

2014 samples	*N. viridescens*
N samples	110
N loci	8
N sampling sites	11
Observed heterozygosity	0.60
Expected heterozygosity	0.71
Allelic richness	5.76
F'_{IS}	0.243
G'_{ST}	0.014

Among-populations genetic analyses — Our analysis of the effects of distance between sampling sites on genetic differentiation suggested that much of the genetic differentiation was explained by geographic distance alone (Figure 5).

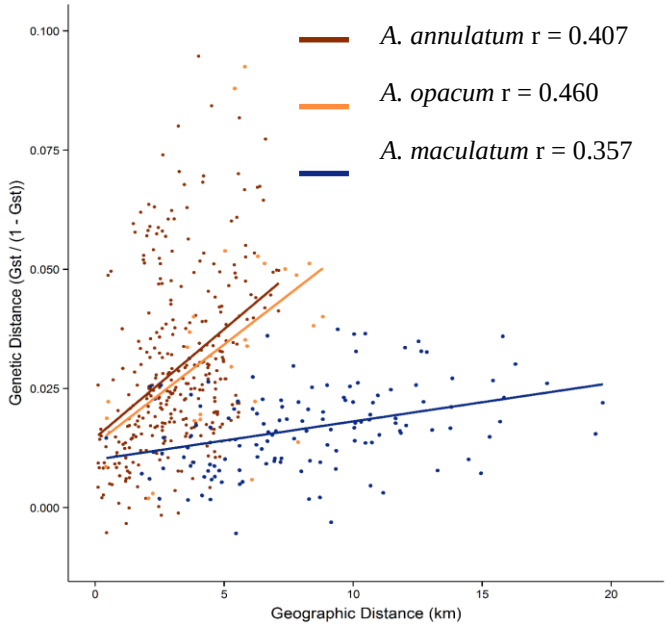

Figure 5. Scatter plot demonstrating significant isolation-by-distance (IBD) relationship for *Ambystoma annulatum*, *A. maculatum*, and *A. opacum*. Fit lines were determined from mixed effects models using a maximum likelihood population effects parameterization (from Peterman et al. 2015, Burkhart et al. unpublished data).

Overall levels of genetic differentiation across populations were measured for

each species using the metric G'_{ST} (Table 3). The results suggest higher levels of population structure in the fall-breeding species *A. annulatum* and *A. opacum* than in the spring breeding species *A. maculatum* and *N. viridescens*. To infer patterns of genetic clustering, the 2012 data for *A. annulatum* and *A. maculatum* were analyzed in the program Bayesian Analysis of Population Structure (BAPS), while the data from 2013 for the three Ambystoma species were analyzed in STRUCTURE. Because we obtained very similar results (Peterman et al. 2015, Burkhart et al. unpublished data) from the two methods of analysis, we present here the 2013 results (Figure 6). The two fall breeding species each consist of 2 genetic clusters (K=2) that are somewhat spatially segregated. The two clusters for *A. annulatum* are distributed in the northern and southern portions of the focal area with an area between them in which populations contain approximately equal proportions of each cluster (K=2, ΔK=134.35, Figure 6A). Because the distribution of *A. opacum* is more restricted at FLW than the other ambystomatid species, our sampling was not as continuous. Nevertheless, we recovered a pattern of north/south partitioning similar to that of *A. annulatum* (K=2, ΔK=50, Figure 6B). Clustering analyses revealed a single genetic cluster for *A. maculatum* not only within the focal area, but across a broader area within FLW (Figure 6C). Clustering analysis for *N. viridescens* for samples collected within the focal area suggest that populations of this species also comprise a single genetic cluster (Figure 6D).

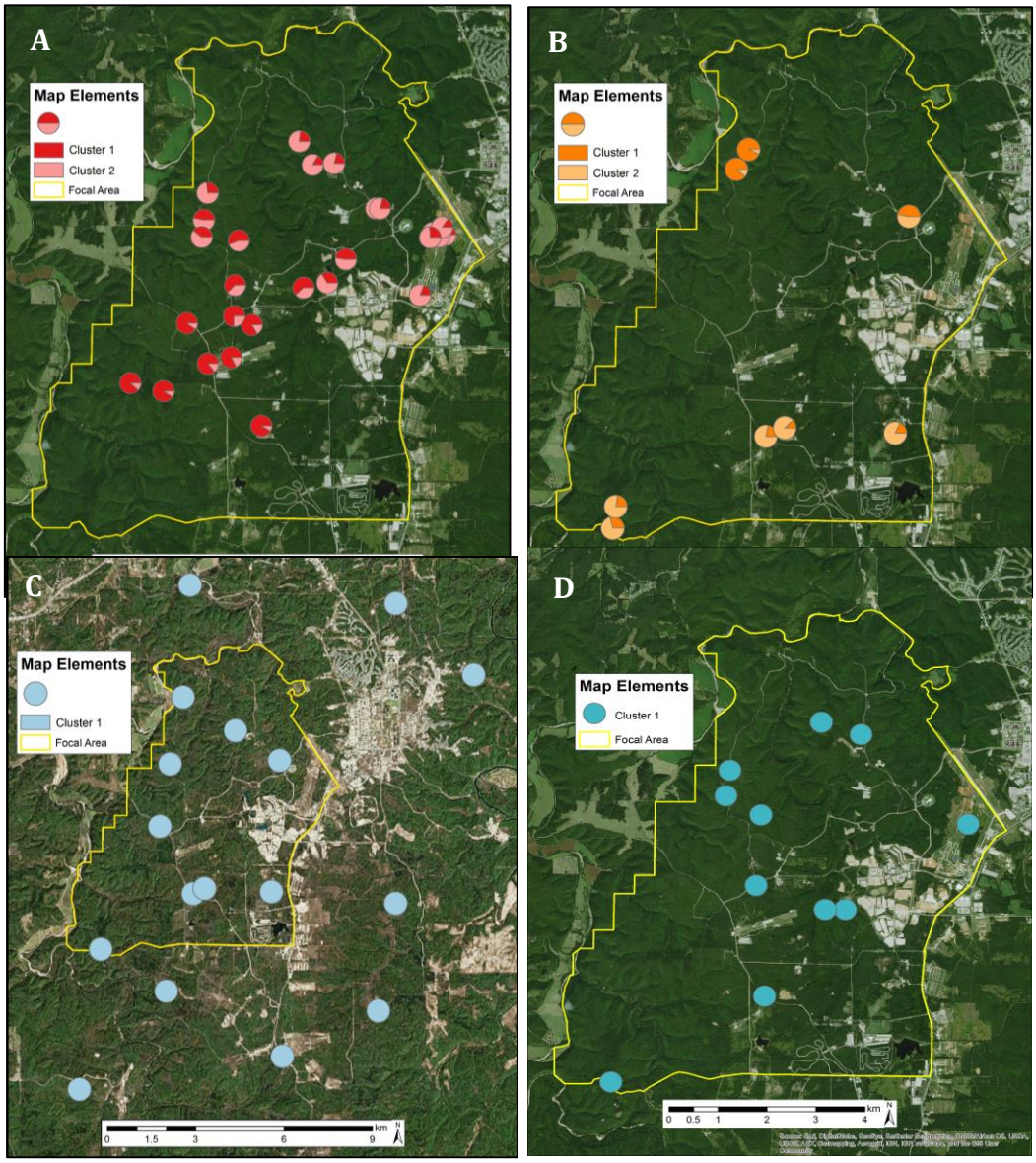

Figure 6. Results of genetic clustering analyses from program STRUCTURE for *A. annulatum* from fall 2013 (A), *A. opacum* from fall 2013 (B), *A. maculatum* from spring 2013 (C), and *N. viridescens* from summer 2014 (D).

Landscape genetics — The main objective of our landscape genetic analyses was to determine what features of the landscape most affected dispersal, as measured by genetic differentiation between pairs of breeding ponds that were sampled in 2012. Additionally, we sought to determine rate and direction of gene flow through the use assignment tests or coalescent modeling. Unexpectedly, we found limited genetic differentiation and no support for land cover or topographic features that affect gene flow in either *A. annulatum* or *A. maculatum* (Peterman et al. 2015). Rather, genetic differentiation was best explained by Euclidean distance (Figure 3, in Peterman et al. 2015); *A. annulatum* showed greater differentiation over shorter distances than

A. maculatum. We estimated the mean dispersal distance of *A. annulatum* to be 1693 m and the mean dispersal distance of *A. maculatum* to be 2050 m. The differences in genetic differentiation and dispersal ability also resulted in differences in spatial population structure. Specifically, *A. annulatum* showed substantial spatial structure, with three distinct clusters being identified by a Bayesian clustering algorithm in 2012 (Peterman et al. 2015) and two clusters being detected in 2013 (Burkhart et al. unpublished data). In contrast, no spatial structure was evident in *A. maculatum.* Because of the overall low genetic differentiation, we were unable to conduct analyses to determine rate and direction of gene flow. However, this comparative analysis emphasizes the importance of importance of considering variability in species dispersal ability when developing management criteria to better account for differences in dispersal ability.

We further explored the spatial structure and connectivity among *A. annulatum* breeding ponds using the more extensive genetic data set that was collected in 2013. Specifically, we created a genetic network of covariance, and assessed whether there was significant modularity in this network. Modularity arises in networks when nodes (habitat patches) form clusters or modules wherein nodes within a module interact extensively with each other, but rarely interact with nodes from different modules. Networks with a modular structure have been shown to be more resilient to disturbances as well as increase the persistence of metapopulations (Stouffer and Bascompte 2011, Fletcher et al. 2013). Our analysis of modularity revealed that *A. annulatum* breeding ponds at FLW do exhibit a significant modular structure consisting of four clusters or modules (Figure 7, in Peterman et al. *In press*). Our analysis of network modularity allowed us to assess the variable contributions of each pond to different aspects of the network, including participation coefficient, within-module strength, and patch strength.

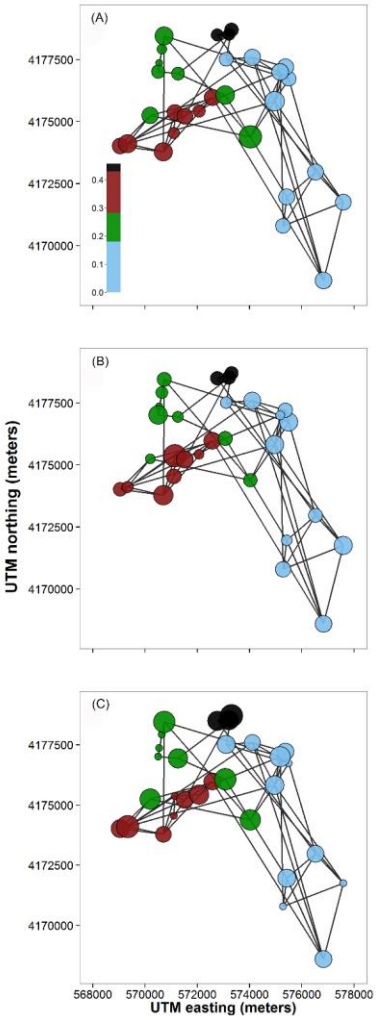

Figure 7. Network modularity of *A. annulatum* based on gene flow among ponds. Nodes (ponds) are color-coded to represent module membership. Node size represents contribution to total gene flow within the genetic network (patch strength) in (A), within module strength in (B), and participation coefficient in (C). Locations of nodes in plots reflect spatial locations of ponds on the FLW landscape. The scale bar indicates the cumulative contribution of each module to the total modularity (*Q*) of the network.

Participation coefficient measures a pond's contribution to connectivity across the landscape to other modules, within-module strength assesses the importance of a pond in maintaining connections within a module, and patch strength assesses a pond's overall contribution to gene flow within the network. Critically, we found an inverse relationship between participation coefficient and within-module strength (Figure 8), and more generally found that ponds were critical contributors to different aspects of network connectivity. Finally, we tried to determine the landscape, habitat, or connectivity features that affected a pond's role within the modular network. We found that an interaction between forest cover and connectivity had a negative effect on patch strength, or a pond's contribution to gene flow in the network. This surprising result could be indicative of a high rate of philopatry among individuals breeding and metamorphosing from ponds that are located in suitable forested habitat in close proximity to other ponds, resulting in limited long distance dispersal. Gene flow is likely high, however, among these clusters of highly connected ponds, and the genetic diversity within these populations is likely very similar. As such, more isolated populations harboring unique alleles may be greater contributors to the total genetic variability.

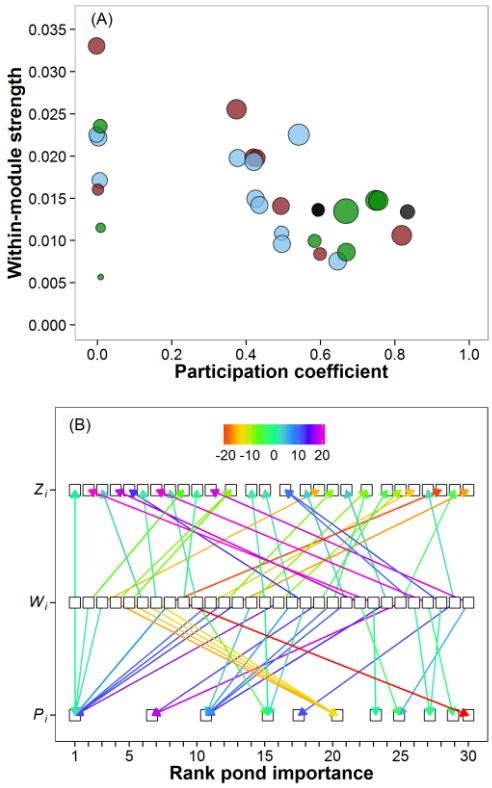

Figure 8. (A) Importance of ponds to gene flow in *Ambystoma annulatum* in relation to within-module strength (the importance of ponds for gene flow within modules) and between-module participation coefficient (extent to which gene flow occurs to all other modules from a pond). Size of points indicate the genetic contribution of each pond to the network (patch strength) and color represents module membership. (B) Rank importance of ponds changes depending upon whether patch strength (W_i), within-module strength (Z_i), or between-module participation coefficient (P_i) is the network metric used, indicating that populations are critical contributors to different aspects of connectivity within the network. Arrow color is scaled to the amount of rank change.

Assessment of source-sink status and pond importance - Demographic network model — Empirical data of dispersal in amphibians generally, and salamanders specifically, are extremely difficult and costly to collect (e.g., Berven and Grudzien 1990, Gamble et al. 2007). Since we were unable to reliably or rigorously assess directional movement among populations using genetic data, we relied on other methods to make inferences concerning the contribution and importance of each pond to the metapopulation, as well as source-sink status. Specifically, we

developed a demographic network model that uses Monte Carlo sampling to incorporate uncertainty in parameter estimates and to simulate thousands of model permutations. Briefly, the model incorporates empirical data collected in this study: abundance at each pond, mean size of larvae prior to metamorphosis, and dispersal distance estimated from genetic data. Additionally, the proportion of individuals that disperse to new breeding ponds was determined from the literature. Size of larvae was used to determine the probability of survival to reproduction, as described by Altwegg and Reyer (2003). For each of these parameters, a mean and standard deviation was used as an input into the model to create a normal distribution from which a random value was drawn at each iteration of the simulation. The likelihood of individuals successfully dispersing to adjacent ponds was a function of distance, and was justifiable because only distance had a significant effect on genetic differentiation. At the conclusion of each iteration, the number of philopatric individuals remaining at a breeding pond, as well as the number of immigrants and emigrants to and from each pond was determined. Additionally, at each iteration we sequentially removed each pond from the network and assessed the percent change in metapopulation capacity, which was determined by calculating the leading eigenvalue of the network connectivity matrix.

Because of the longevity of Ambystomatid salamanders (up to 10 years) and dependence upon rain and temperature cues to initiate breeding, assessment of local population dynamics is challenging. As such, it is impossible to determine whether a population, in the absence of immigration, is destined for local extinction (i.e. demographic sink). In lieu of this determination, we assessed the ratio of the local philopatric breeding population to the number of immigrants from surrounding populations. When the number of immigrants exceeded the number of philopatric individuals, a population was considered a sink, although we reiterate that such a population may have population growth rate less than one.

We assessed all three Ambystomatid species at FLW using our demographic network model. We found that 20, 8, and 5 ponds (9.7%, 3.9%, 2.4%) acted as sources ≥ 90% of the time for *A. annulatum*, *A. maculatum*, and *A. opacum*, respectively. Ponds that acted as sources for *A. maculatum* also tended to act as sources for *A. opacum*, but there was minimal relationship between source *A. annulatum* and *A. opacum* ponds. The majority of breeding ponds on the landscape serve as functional sinks most of the time for all three species, with only a limited number reliably and consistently acting as sources (Figure 9). Visually, sources and sinks appear to be interspersed across the landscape; however, assessment of spatial autocorrelation suggests that sources are significantly clustered in space for all species. Further research will be needed to better understand the basis for this clustering.

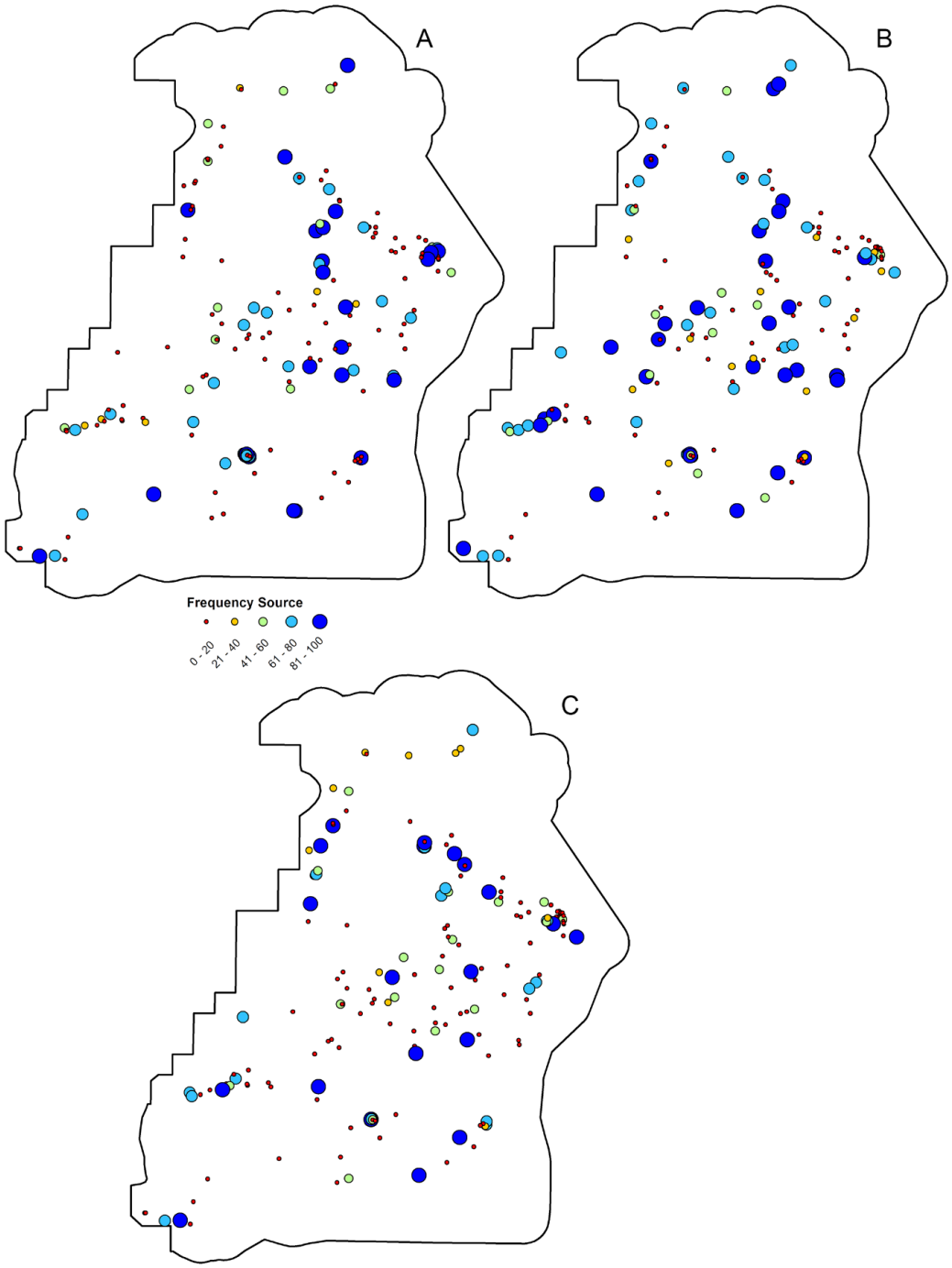

Figure 9. Maps of the FLW focal area showing the frequency that ponds act as sources, estimated from 10,000 iterations of the demographic network model for Ambystoma annulatum (A), A. maculatum (B), and A. opacum (C).

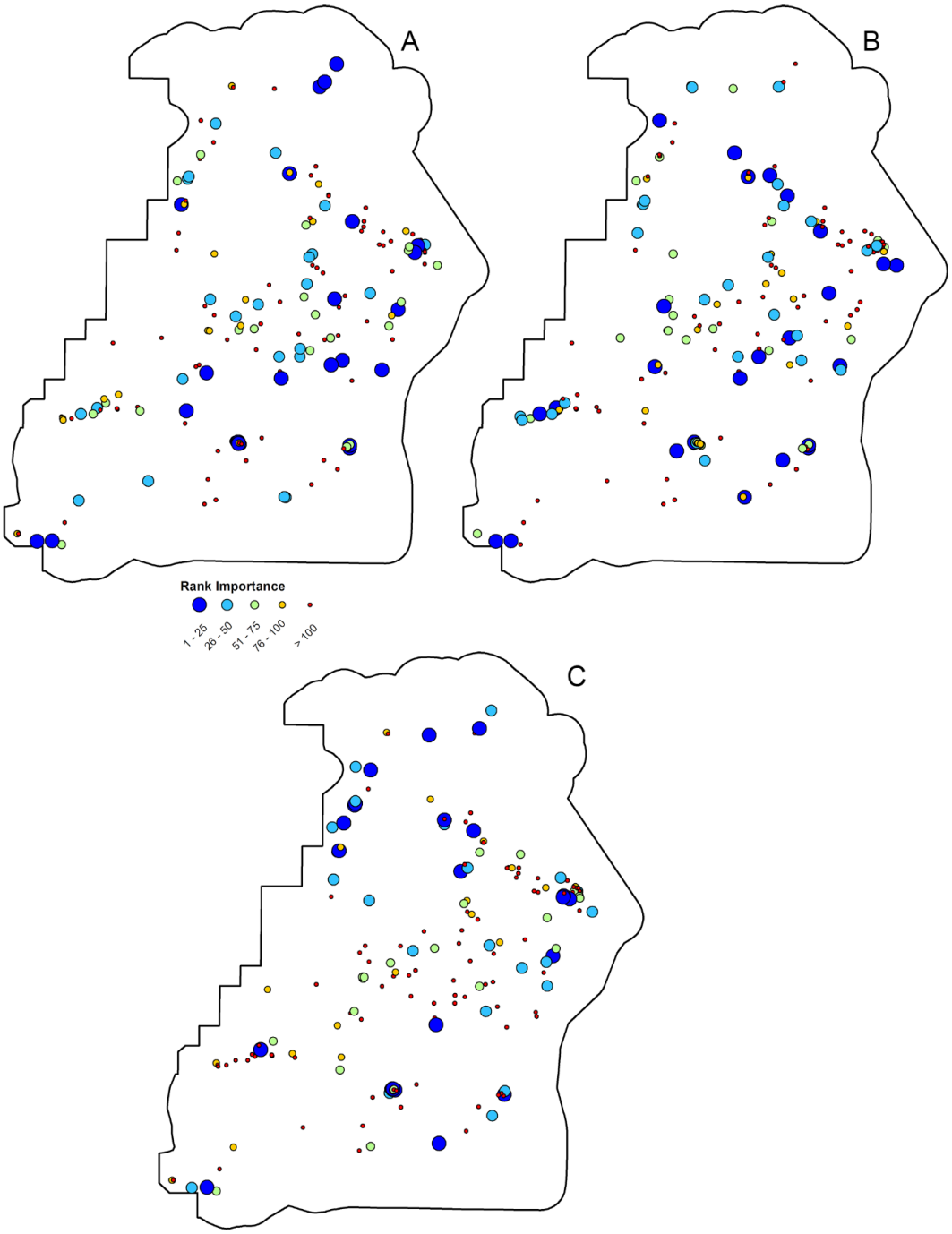

Figure 10. Maps of the focal area at FLW showing rank importance of ponds, determined by the percent change in metapopulation capacity when a pond was removed from the network and averaged over 10,000 iterations of the demographic network model for Ambyst.

In assessing the rank importance of each pond in contributing to the persistence of the metapopulation (i.e. metapopulation capacity), it is clear that different ponds on the landscape are most critical to different species (Figure 10). This finding corroborates previous analyses that found different pond features and habitat characteristics to be important predictors of larval abundance of *A. annulatum*, *A. maculatum*, and *A, opacum* and that overlap in use of breeding ponds by all three species is infrequent (Peterman et al. 2014).

In further assessing factors affecting the frequency that a pond acted as a source or sink, we found no indications that any species inhibited another. Specifically, the frequency that a pond was a source population in our demographic network simulations was not negatively affected by that pond acting as a source population for another species. In fact, ponds that were more frequently sources for *A. annulatum* also tended to be sources for *A. maculatum*, and vice versa. However, only source *A. maculatum* ponds tended to act as source ponds for *A. opacum*. For *A. annulatum*, increasing the canopy cover over a pond tended to increase the frequency a pond acted as a source, while increasing the hydroperiod (duration that a pond holds water) tended to have a negative effect. Canopy cover did not affect the frequency that *A. maculatum* populations were sources, but the amount of forest cover within 300 m of a pond and increasing hydroperiod both tended to decrease the source frequency. However, the effect of forest cover was three times less important than hydroperiod. Finally, only increasing hydroperiod had a negative effect on the source population frequency for *A. opacum.* Consistent among all three species is the fact that increasing hydroperiod tends to have a negative effect on the frequency at which a pond acts as a source. Hydroperiod has also been found to have significant effects on species richness, abundance, and invertebrate predator density (Peterman et al. 2014, Ousterhout et al. 2015, Semlitsch et al. 2015).

In addition to environmental and interspecific factors affecting the frequency a pond acts a source, we also assessed the effects of mean abundance and size of larvae. For each species we fit linear models with source frequency as the dependent variable and mean abundance and larval size as independent variables. In all three species, mean abundance and larval size were significant predictors of a pond acting as a source. However, abundance tended to be 3–5 times more important than size. For each species, these linear models explained >90% of the variance. These results indicate the single most important factor in a population acting as a source is the number of individuals that successfully metamorphose from a pond. In contrast, mean abundance, larval size, frequency of being source, nor any environmental variables were meaningful predictors of a ponds contribution to metapopulation capacity.

To determine the relative effect of each parameter in the demographic network model, we conducted a sensitivity analysis (Figure 11). This analysis highlighted that dispersal from the natal pond is critical to maintaining connectivity and increasing the likelihood of the metapopulation persisting. Additionally, a higher survival rate results in more salamanders available to disperse. In our model, survival probability is determined by the average size of larvae prior to metamorphosis, with larger individuals having a greater probability of surviving to maturity. Surprisingly, mean dispersal distance had a very minimal effect on metapopulation capacity. On the FLW landscape, these results may in part be a result of landscape that is densely covered with ponds and the landscape is relatively homogenous, making successful dispersal less dependent on the ability to traverse large distances through matrix habitat.

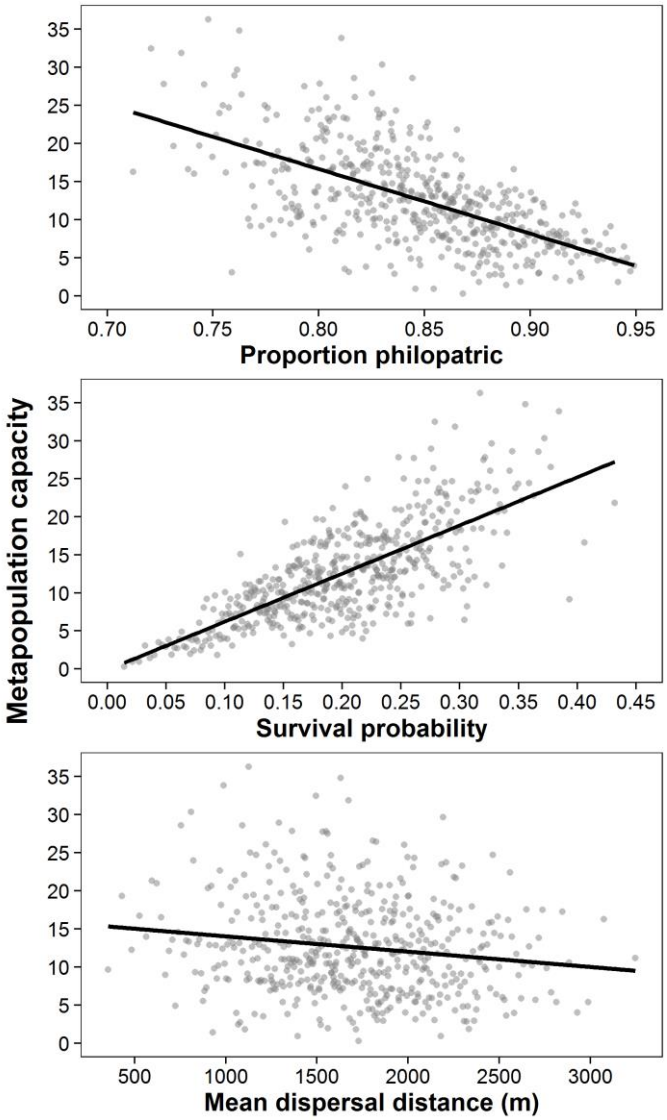

Figure 11. Sensitivity analysis of demographic network model parameters in affecting metapopulation capacity. As the proportion of individuals that remain philopatric to their natal pond increased, metapopulation capacity decreased, but as survival probability increased, so did metapopulation capacity. In general there was minimal effect of dispersal distance.

Identification of optimal sites for habitat creation — We extended the use of our demographic network model beyond assessment of existing ponds on the landscape and developed it into a tool for assessing the best location for pond creation to maximize the benefits to the metapopulation. To accomplish this, we first determined the critical landscape features necessary to support robust salamander populations as well as the logistical and geological limitations of where ponds can be created on the landscape. Our previous studies have clearly demonstrated the importance of forest cover for all three species (Peterman et al. 2014, Ousterhout et al. 2015). Because nearly all of the more than 500 ponds present at FLW are man-made, we used these to determine the physical and geological conditions most conducive to creating wetlands that retain water. Existing ponds on the FLW landscape generally occur on slopes with <17% grade, gravelly or silt-loam soils, and are < 675 m. from existing roads (necessary for equipment access to build ponds). Finally, we also imposed criteria that new ponds be at least 200 m from existing ponds to ensure that future resources were not excessively clumped. Within this suitable region, we generated random locations spaced 200 m apart, resulting in 608 potential locations to be evaluated. We then ran our demographic network model, and at each iteration, evaluated the

contribution of each potential pond location in turn. We set the abundance and size of larvae present at a potential pond to the mean of all existing ponds on the landscape in that iteration of the model (i.e. the newly created pond is an average pond). The change in metapopulation capacity of the network when each potential pond was added was then assessed, and the rank importance of each pond was determined as the average rank from 10000 iterations of the demographic model. In Figure 12, we denote the top 10 locations that increased metapopulation capacity the most for *A. annulatum*. It can be seen that these 10 sites are distributed across the focal area of FLW, indicating that there is no single region on the landscape in greatest need of management. Our current implementation of this approach only assesses the potential contribution of newly created ponds. Our approach could, however, be modified to assess existing ponds on the landscape for restoration, allowing resource managers to prioritize their efforts.

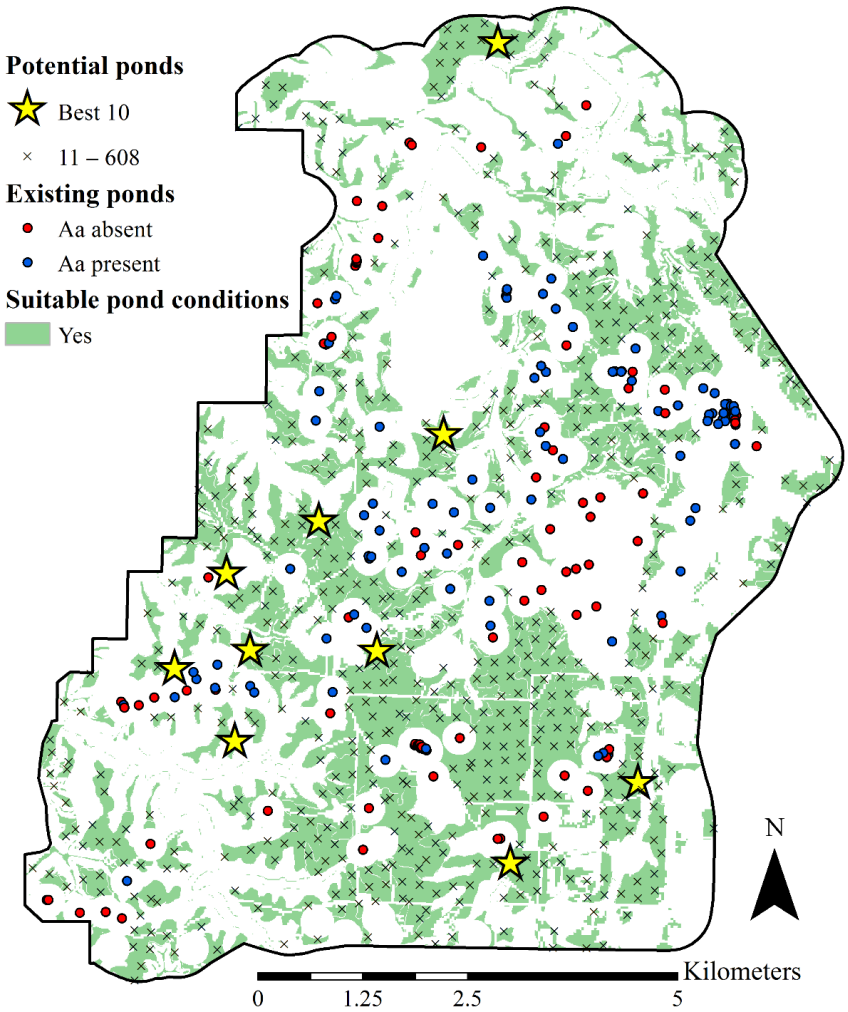

Figure 12. Map of the focal area of Fort Leonard Wood. Green shading indicates the region of the landscape suitable for the creation of *Ambystoma* breeding ponds. The yellow stars indicate the ten best locations for creating new ponds on the landscape that, if colonized and become average ponds on the landscape, will have the greatest contribution to the persistence of the metapopulation. Crosses indicate all of the 608 potential pond locations that were evaluated.

Species interactions: *Field observations* — In natural populations at FLW we found that larval abundances of *A. annulatum* and *A. maculatum* were positively correlated with one another (Ousterhout et al. 2015), which appears to be a general pattern among amphibians (Pechman et al. 1991, Semlitsch et al. 1996, Werner et al. 2007b). Positively correlated abundances, along with

the evidence that certain habitat traits are strong predictors of density and abundance of both species, indicate that features associated with a high quality pond for one species may be similarly good for other amphibians. This positive association among congeneric species also suggests that habitat features may be stronger predictors of density rather than interspecific associations (i.e. competition or non-fish predation) in regulating larval abundance. We have begun to investigate whether habitat traits have interactive effects with competitor and invertebrate predator densities to assess any context-dependent relationships, but as of yet have found no strong covariance among such predictors for either *A. maculatum* or *A. annulatum* (Anderson et al. unpublished data).

Species interactions: Mesocosm and microcosm experiments — In contrast to the patterns observed in natural populations, we observed strong interspecific interactions from several mesocosm experiments conducted on *A. annulatum* and *A. maculatum*. We observed no effects of intraspecific density on survival of either species (Anderson and Semlitsch 2014, Ousterhout and Semlitsch unpublished data), except in one experiment where severe winter temperatures in 2013-2014, in conjunction with high larval densities, resulted in low survival of *A. annulatum*. We did observe a strong negative relationship of *A. annulatum* density and *A. maculatum* survival, where high densities of *A. annulatum* resulted in few survivors of *A. maculatum* (Anderson and Semlitsch 2014; Anderson and Semlitsch unpublished data). However, the predation rate of *A. annulatum* on *A. maculatum* did not vary with increasing habitat complexity (Anderson and Semlitsch unpublished data), and the timing of breeding for each species was not as strong a factor in determining the outcome of interactions as was larval density (Anderson, unpublished). The presence of other top predators (e.g. dragonfly larvae, *N. viridescens*, or mosquitofish) altered the relationship of *A. maculatum* and *A. annulatum*, where the strength of their survival covariance differed depending on what other predators were present in the food web (Anderson and Semlitsch unpublished data). Of note, survival was lowest for both salamanders when they coexisted with multiple predators, which likely reflects a more realistic scenario and would match known survival estimates for larval salamanders (<1%, Semlitsch et al. 2014, Anderson et al. 2015b). We observed that invertebrate predator densities peaked in ponds with characteristics similar to those in which amphibian densities peaked, suggesting that larvae persist in the face of numerous predators. One potential mechanism that permits coexistence is non-consumptive effects among predators of salamanders. In a microcosm experiment, we found that larval dragonflies, voracious predators of larval amphibians, had reduced foraging rates in the presence of cannibalistic conspecifics, which resulted in increased survival of their prey (*A. annulatum*; TLA, unpublished data).

Using microcosms, we examined the functional response curve of different-sized larval *A. annulatum* larvae, and found that larger individuals foraged with a Type I functional response (a linear increase in consumption rate with increased prey densities) and smaller individuals a Type II response (an asymptotic increase in consumption rate where predators will reach constant consumption rate regardless of prey density; Anderson et al. unpublished data). This would indicate that the relative size of *A. annulatum* when *A. maculatum* hatches in the spring time could influence their effectiveness as a predator. Interestingly, we also found that if metamorphosis is delayed in *A. maculatum* such that they persist as larvae into the fall, they can be effective predators on hatchling *A. annulatum* (Anderson et al. unpublished data). Such a role reversal of intraguild predation based on life history differences has not been documented for ambystomatid salamanders.

Transition plan — The data collected to date inspired and supported the preparation of a conservation assessment and proposed management plan for *A. annulatum* beginning in the summer of 2014. This document is in preparation to be submitted for publication, and we have made a draft of the document available to regional natural resource managers.

The population viability analysis (PVA) we have completed for the *A. annulatum* will help to inform the conservation of closely related species, like the federally listed flatwoods salamanders (*A. bishopi* [Endangered] and *A. cingulatum* [Threatened]). This PVA is also in preparation to be submitted as a stand-alone manuscript, and the model predictions will be applied to flatwoods salamander recovery planning beginning in October of 2015.

Our findings and recommendations in both documents have already been shared with the team working to recover these two species through a series of Structured Decision Making Workshops held by the U.S. Fish and Wildlife Service. The student working on both the proposed management plan and the PVA has been asked to join both the Flatwoods Salamander Working Group and Federal Recovery Team, which will collaborate to facilitate the transition plan.

Conclusions and implications for future research/implementation

Our objective was to better understand the impact of landscape heterogeneity on the source-sink dynamics of a species of conservation concern (the ringed salamander, *A. annulatum)* and a co-occurring species (the spotted salamander, *A. maculatum*) at Fort Leonard Wood, Missouri through a combination of intensive ecological field studies, genetic analyses, and statistical modeling. Specifically, we sought to (1) identify key environmental conditions associated with varying levels of *A. annulatum* reproductive success to define source habitat patches, (2) determine whether species interactions influence source-sink dynamics, (3) identify habitat features associated with effective dispersal of *A. annulatum* among populations to define habitat connectivity, and (4) develop models to predict and manage population connectivity for salamanders in heterogeneous landscapes.

Overall, habitat features, especially pond hydroperiod, forested habitat surrounding ponds, canopy cover, and the presence or absence of fish appear to be the strongest predictors of *A. annulatum* and *A. maculatum* distribution and population dynamics. We found weak evidence for species interactions being important factors in population dynamics in natural ponds, but strong evidence of density-dependence and food web organization as critical components in experimental studies. The discrepancies between field and experimental studies may stem from imprecise field measures and/or failure of simplified experimental mesocosms to replicate the complex interactions found in natural systems. Therefore, despite lack of clear support from our field data, we would caution against disregarding species interactions as important contributors to source sink dynamics.

One of the most critical predictors of amphibian fitness is size at metamorphosis (e.g., Berven 1990, Scott 1994). In experimental mesocosms, we found that metamorph size was strongly affected by intraspecific density (Anderson and Semlitsch 2014, Ousterhout and Semlitsch unpublished data). We also observed extensive variability in metamorph size in field observations. However, the effects of intraspecific density were much less apparent (Ousterhout et al. 2015). Previous amphibian research has shown that size at metamorphosis can affect survival, age at maturity, and fecundity (Berven 1990, Scott 1994), and we found that size also affects dispersal (Ousterhout and Semlitsch unpublished data). Specifically, we found that intermediate sized metamorphs dispersed the farthest. A better understanding of the habitat and ecological factors affecting metamorph size in the field appears to be a critical next step to ensuring population viability and connectivity.

In this study, we went to great lengths to survey and study >200 ponds across a large landscape. The extent of these surveys necessitated some tradeoffs in survey intensity at each individual pond. Because of the brief period that adults are present in the breeding ponds and the challenges of effectively surveying egg masses, we invested our efforts in understanding environmental and ecological factors affecting larval salamanders. While the larval life stage is undoubtedly critical to future fitness and population persistence, it will likely be insightful for future research to more intensively monitor all life stages in populations at a smaller number of sites and to begin to understand how terrestrial habitat quality surrounding ponds affects survival and fecundity.

The clearest and most unsurprising result from our study was that ponds with fish act either as unusable habitat or a sink habitat if salamanders deposit eggs as fish are voracious predators of

amphibian embryos and larvae (Drake et al. 2014, Shulse 2012, 2013). Therefore, absence of fish is a key characteristic of source ponds. Further, we found that it is important for all of our studied ambystomatid species that ponds be located within forested habitat and that pond hydroperiod plays critically into the larval abundance and size. In general, we observed a pattern of most ponds having few larvae, and only a few ponds supporting large populations with higher densities; spatial variation in larval density was four to ten times greater than temporal variation. This suggests that there are likely few source populations on the landscape, making their conservation priority even greater. We also observed differential success and growth in salamanders that was affected by pond hydroperiod. Therefore, to gain the maximum benefit for the most species and to buffer against regional population failure from climatic factors such as drought, we recommend that ponds with a mosaic of hydroperiod regimes should be maintained.

One of our initial objectives was to develop connectivity models based on network circuit theory. Unfortunately, we found limited genetic differentiation and no support for land cover or topographic features that affected gene flow in *A. annulatum*, *A. maculatum*, *A. opacum*, or *N. viridescens* (Peterman et al. 2015, Burkhart et al. unpublished data), making such connectivity models irrelevant. The lack of genetic differentiation also precluded our use of coalescent models or assignment tests to effectively quantify directional dispersal among populations. However, our genetic data allowed us to assess effective dispersal distances for both *A. annulatum* and *A. maculatum*, which was essential to parameterizing our demographic network model.

Modularity analyses based on a network of genetic covariance for *A. annulatum* allowed us to assess the role and contribution of populations to different aspects of connectivity. Specifically, we assessed the local importance of a population to maintaining connectivity within modules, regional connectivity among modules, and global contribution to gene flow of the entire network. From these analyses, we found that, in general, no population was universally the most critical to all aspects of connectivity. While none of these measures are directly relatable to source or sink population designation, our findings suggest that management for a single type of population may not be the most beneficial to connectivity and gene flow across the landscape.

Ultimately, our most robust assessment of population source-sink status came from our demographic network model. This stochastic model incorporated dispersal distances derived from population genetic analyses conducted in this study as well as three years of population abundance and size estimates from field surveys. From this approach we were able to simulate the likely number of immigrants, emigrants, and philopatric individuals at each pond, determine average source-sink status, as well as assess more integrative measures such as metapopulation capacity. As an integrative measure assessing connectivity and amount of contribution, metapopulation capacity intuitively seems like a more holistic measure by which to evaluate a population's role and contribution. While creation and maintenance of sink populations on the landscape is certainly undesirable, our modeling suggests populations occur on a continuum of source to sink and that most populations fluctuate in their contributions. A singular focus on sources or sinks, at least in our pond breeding amphibian system, actually does very little to maintain connectivity and ensure metapopulation persistence. As such, we feel it is important to manage for large, robust population sizes, but also to critically evaluate the spatial location of populations on the landscape as well as local habitat features to maximize growth and survival.

Finally, we used our demographic modeling framework to determine the optimal locations on the landscape for future pond construction. We have found that abundance of larvae increases with

increasing forest cover and number of ponds within 300 m of a pond (Peterman et al. 2014). Additionally, hydroperiod and slope of the pond basin are important considerations in how ponds are constructed. Of greatest importance to successful amphibian breeding and recruitment is the exclusion of fish. Once fish are present, a pond is no longer suitable for amphibian reproduction. Therefore, future ponds at FLW should be constructed in forested habitat on slopes with <17% grade with gravelly or silt-loam soils. We provide no concrete recommendations regarding a target surface area or depth when constructing ponds, but emphasize that the maintenance of ponds of varying size and hydroperiod on the landscape is likely to have the greatest benefit for the greatest number of amphibian species, including *A. annulatum* (Semlitsch et al. 2015). Our demographic network model has been made available in an R package called "ssmc" and is available with a fully documented user guide at https://github.com/wpeterman/ssmc. We feel this approach has great potential to be beneficial and applicable to other systems where the available population and life history parameters are uncertain, and where discrete habitat creation/restoration is an effective management strategy.

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Appendix A: Scientific/Technical Publications and Presentations

Publications in preparation:

Burkhart, J. J, Peterman, W. E., Brocato, E., Romine, K. R., Willis, M. M., Anderson, T. L., Ousterhout, B. H., Drake, D. L., Semlitsch, R. D., and Eggert, L. S. Small-scale landscape genetic analysis of four co-occurring salamander species at multiple spatial scales. Landscape Ecology.

Burkhart, J. J., Shulse, C. D., and R.D. Semlitsch. Teasing apart the variation in the relationship of metamorph export and larval density estimates for pond breeding anurans. Copeia.

Heligman, Z.E., B.H. Ousterhout, C. Farmer, and R.D. Semlitsch. Behavior consistent with non-random dispersal in a juvenile salamander.

Messerman, A., and R. D. Semlitsch. A conservation assessment and proposed management plan for the ringed salamander (*Ambystoma annulatum*).

Messerman, A., and R. D. Semlitsch. A population viability analysis for the ringed salamander (*Ambystoma annulatum*).

Peterman, W.E., T.L. Anderson, D.L. Drake, B.H. Ousterhout, and R.D. Semlitsch. Using spatial demographic networks to identify optimal locations for wetland construction and restoration. Ecological Applications.

Walls, S. C., Ball, L. C., Enge, K M., Gorman, T. A., Palis, J. G., and R. D. Semlitsch. Overcoming roadblocks to recovery of declining amphibian populations in the United States. Bioscience.

Publications in review/revision:

Nussbaum, S. E., Ousterhout, B. H., and R. D. Semlitsch. Agonistic behaviour suggestive or territoriality among sympatric juvenile pond-breeding salamanders.

Ousterhout, B.H., and R. D. Semlitsch. Non-additive response of larval ringed salamanders to intraspecific density.

Semlitsch, R. D., Ousterhout, B. H., Anderson, T. L., Drake, D. L, Burkhart, J. J., Rowland, F. E., and W. E. Peterman. Are hot-spots consistent" Spatiotemporal variation and annual synchrony in larval abundance of pond-breeding salamanders.

Journal articles:

Anderson, T. L., B. H. Ousterhout, W. E. Peterman, D. L. Drake and R. D. Semlitsch. *In Press.* Life history differences influence the impacts of drought on two pond-breeding salamanders. Ecological Applications doi:10.1890/14-2096.1

Anderson, T.L., J. L. Heemeyer, W. E. Peterman, M. J. Everson, B. H. Ousterhout, D. L. Drake, and R. D. Semlitsch. 2015. Automated analysis of temperature dataloggers to determine inundation state of wetlands. Wetlands Ecology and Management. doi:10.1007/s11273-015-9439-x

Anderson, T. L., Hocking, D. J., Conner, C. A., Earl, J. E., Harper, E. B., Osbourn, M. S., Peterman, W. E., Rittenhouse, T. A. G., and R. D. Semlitsch. 2014. Abundance and phenology patterns of two pond-breeding salamanders determine species interactions in natural populations. Oecologia. doi:10.1007/s0042-014-3151-z.

Anderson, T. L. and R. D. Semlitsch. 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. Population Ecology 56: 265-273 **Editors Choice**

Drake, D. L., T. L. Anderson, L. M. Smith, K. L. Lohraff, and R. D. Semlitsch. 2014. Predation of eggs and hatchlings of the Ringed Salamander (*Ambystoma annulatum*) by native and non-native predators. Herpetologica 70: 378-387.

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Other publications:

Peterman, W. E. 2014. ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms.

Semlitsch, R. D, W. E. Peterman, L. S. Eggert. 2014. Understanding the complex spatial and temporal variation in source-sink dynamics of salamanders. Natural Selections: Department of Defense Natural Resources Programs, Fall 2014.

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Oral presentations:

Anderson, F. E. Rowland, and R. D. Semlitsch. 2015. Phenology and density influence intraguild predation between larval salamanders. Ecological Society of America Annual Meeting, Baltimore, MD. 2015.

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Anderson, T. L. and R. D. Semlitsch. Top predators and habitat complexity alter a larval salamander intraguild predation module. Ecological Society of America Annual Meeting, Sacramento, CA. 2014.

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Burkhart, J. J. Small scale landscape genetic analysis of three co-occurring salamander species at multiple spatial scales. Society for the Study of Amphibians and Reptiles. Lawrence, KS. 2015.

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Doll, S., B.H. Ousterhout, and R.D. Semlitsch. Marbled salamander (*Ambystoma opacum*) larvae behavioral response to ringed salamander larvae (*Ambystoma annulatum*) cues. MU Undergraduate Research & Creative Achievements Forum, Columbia, MO. 2014.

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Nussbaum, S.E., B.H. Ousterhout, and R.D. Semlitsch. Competition and aggression in juvenile *Ambystoma* salamanders. MU Undergraduate Research & Creative Achievements Forums, Columbia, MO. 2014.

Willis, M. M., Burkhart, J. J., Messerman, A. F., Semlitsch, R. D., and L. S. Eggert. Influence of life history on genetic diversity in salamanders. Summer Undergraduate Research and Creative Achievements Forum, University of Missouri, Columbia, 2015.

Appendix B: Datasets Used in the Genetic Analyses (online attachments)

From Burkhart et al. (unpublished data):

Aa_FLW_Fa2013* *A. annulatum* (embryos coll. Fall, 2013)

Am_FLW_Sp2013 *A. maculatum*

Ao_FLW_Fa2013 *A. opacum*

Nv_FLW_Su2014 *N. viridescens*

From Peterman et al. 2015

Aa_GenePop_2012 *A. annulatum*

Am_GenePop_2012 *A. maculatum*

From Peterman et al. (In Press, Ecosphere)

Aa_Genepop_2013 *A. annulatum* (embryos coll. Fall 2012, would metamorphose 2013)

*All genetic files are in GenePop format

Appendix C. User Guide Program 'ssmc' (online attachment)

ssmc: An R Package for assessing source-sink population status and contribution of populations to the metapopulation using demographic connectivity networks and Monte Carlo simulation