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Breeding Ecology and Habitat Use of Unisexual Salamanders and their Sperm-Hosts, Blue-Spotted Salamanders (Ambystoma laterale)

Kristine Hoffmann
UMaine, Kristine.hoffmann@maine.edu

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BREEDING ECOLOGY AND HABITAT USE OF UNISEXUAL SALAMANDERS AND THEIR SPERM-HOSTS, BLUE-SPOTTED SALAMANDERS (AMBYSTOMA LATERALE)

By

Kristine Elizabeth Hoffmann

B.S. University of Massachusetts, 2005

M.S. University of Florida, 2007

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Advisory Committee:

Aram J. K. Calhoun, Professor of Wetlands Ecology, Co-Advisor

Malcolm L. Hunter, Jr., Professor of Wildlife Ecology, Co-Advisor

James P. Bogart, Professor Emeritus

Daniel J. Harrison, Professor of Wildlife Ecology

Michael Kinnison, Professor of Evolutionary Applications

BREEDING ECOLOGY AND HABITAT USE OF UNISEXUAL SALAMANDERS AND THEIR SPERM-HOSTS, BLUE-SPOTTED SALAMANDERS (AMBYSTOMA LATERALE)

By Kristine Elizabeth Hoffmann

Dissertation Co-Advisors: Dr. Aram J. K. Calhoun, Dr. Malcolm L. Hunter, Jr.

An Abstract of the Dissertation Presented
in Partial Fulfillment of the Requirements for the
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Unsexual Salamanders within the Blue-Spotted Salamander Complex carry combinations of ambystomatid genomes (those of Blue-Spotted Salamanders, *Ambystoma laterale,* and Jefferson Salamanders, *A. jeffersonianum* in Maine). They are nearly all female, breed in wetlands, and use sperm of related species to reproduce. Little is known about their ecology to guide the conservation of this unique lineage. I examined breeding site occupancy, demographics, orientation, and terrestrial habitat selection of Unisexual Salamanders in comparison to Blue-Spotted Salamanders and other amphibians. I compared statistical tests of orientation to determine which was most appropriate for pitfall data.

Unisexual Salamander occupancy at breeding sites was positively related to counts of captured Blue-Spotted Salamanders, hydric soil, and vegetation characteristics. Blue-Spotted Salamander occupancy was related to the same vegetation characteristics, but Spotted Salamander occupancy was related to other characteristics.

I examined demographics and orientation of Unisexual Salamanders, Blue-Spotted Salamanders, Spotted Salamanders, and Wood Frogs (*Lithobates sylvaticus*) at four vernal pools.

The ratio of juveniles per female was not significantly different among taxa, though only 1% of my Blue-Spotted Complex Salamander populations were males. I used simulated and field data to examine the effects of common scenarios on statistical tests, and found the Rayleigh test to be most suitable as a test of uniformity and the Kruskal-Wallace test the most suitable test of homogeneity. Orientation of Blue-Spotted Complex Salamanders was generally like that of other taxa.

Telemetered Unisexual Salamanders migrated distances similar to those of parent species, and used sites with high numbers of burrows, lower temperatures, and low cover by forest floor vegetation. Ninety percent of Unisexual Salamanders stayed within the forest matrix, but some migrated to disturbed areas such as backyards.

These findings relate Unisexual and Blue-Spotted Salamanders to their community and habitat. These two salamanders are similar in their use of both breeding sites and non-breeding habitat, but the former may range beyond the reach of this sperm-host. Additionally, managers who conserve terrestrial habitat near the pool for other species may also be aiding in the movements of sympatric Blue-Spotted Complex Salamanders, and pools with few males may still support viable populations of the complex.

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Malcolm Hunter and Aram Calhoun were coauthors on all chapters. Chapter one was also coauthored by James Bogart and Erik Blomberg, and chapters three and four were coauthored by James Bogart.

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Upward Bound Math Science created prototype funnel traps. Kelly Ilseman and I co-authored a pedagogy article on this collaboration which was published in The Science Teacher in 2016. Appendix B gives instructions on trap construction, and was published in Herpetological Review in 2016. The University of Maine Student Chapter of The Wildlife Society assisted greatly in building these traps.

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CHAPTER 1

BREEDING HABITAT OCCUPANCY BY CRYPTIC POOL-BREEDING SALAMANDERS

Chapter Abstract

Efforts to conserve amphibian communities may be confounded when some amphibians are far more cryptic than others. For example, Blue-Spotted Salamanders (*Ambystoma laterale*) and Unisexual Salamanders (*A. laterale - jeffersonianum*) are more challenging to detect than Spotted Salamanders (*A. maculatum*). Therefore, information on breeding habitat use and co-occurrence is a critical requirement for conservation of these amphibians. Our objectives were to determine which environmental factors affect breeding site use by Blue-Spotted Salamanders, Unisexual Salamanders, and Spotted Salamanders and to examine co-occurrence. We used aquatic funnel traps to survey breeding salamanders at wetlands and modeled site occupancy while accounting for imperfect detection. Detection decreased with days post immigration. Blue-Spotted Salamander occupancy was predicted by vegetation characteristics related to low emergent vegetation cover, but this effect was based on relatively low captures. Unisexual Salamander occupancy was positively related to counts of captured Blue-Spotted Salamanders, hydric soil within 200 m, and vegetation characteristics related to low emergent cover. Spotted Salamander occupancy was positively related to forest cover within 200 m of the wetland, smaller wetland areas, and vegetation characteristics related to canopy closure. These differing relationships imply that management based solely on easily observed species may not conserve more cryptic salamanders.

Introduction

Researchers and managers often survey amphibians that congregate to breed in wetlands by documenting evidence of reproduction, such as courting adults, egg masses, or larvae (reviewed in Heyer et al., 1994; Dodd, 2010). These methods are integral to studying amphibian communities because adults are difficult to monitor throughout the remainder of the year, but detectability varies among species. Conservation of the whole suite of pool-breeding amphibians requires that research and management target even the most cryptic of species. Information on habitat use and co-occurrence could inform managers where rare and difficult to detect animals are likely to occur and if a more easily observed species may act as a management surrogate (Hunter et al., 2016).

Ambystomatid salamanders are of management concern because of habitat alteration and loss, with 45% of species listed as threatened on the IUCN Red List. Many taxa are state-listed throughout much of their range, and some states regulate breeding sites (Hunter et al., 1999; Maheny and Klemens, 2008; IUCN, 2016). Spotted Salamanders (*Ambystoma maculatum*) and Blue-Spotted Salamanders (*A. laterale*, abbreviated as LL) are diploid, bisexual species (with males and females), while Unisexual Salamanders are almost all female, usually polyploid, and reproduce through 'kleptogenesis' by using the sperm from certain sympatric species (e.g., *A.* (2)*laterale - jeffersonianum* use Blue-Spotted Salamander sperm and are abbreviated as LLJ; Uzzell, 1969; Bogart et al., 2009). Unisexual Salamanders and the species whose sperm they use are collectivly refered to as the Blue-Spotted Salamander Complex. Spotted Salamanders are common, while Unisexual Salamanders and Blue-Spotted Salamanders are both rarer (e.g., listed as Species of Special Concern or Endangered in many Northeastern and Midwestern states) and harder to document at breeding wetlands because their eggs are laid singly or in small, loose, transparent masses while Spotted Salamanders deposit relatively large, conspicuous egg masses

(Stille, 1954; Petranka, 1998). Over time, however, Unisexual Salamander egg masses may become more obvious as unviable eggs support fungal growth (Piersol, 1910).

Spotted Salamander breeding habitat is well documented as large, deep, seasonal wetlands with long hydroperiods and low salinity in forested areas (Rowe and Dunson, 1993; Calhoun et al., 2003; Skidds et al., 2007), but little is known about the breeding habitat selection of the Blue-Spotted Salamanders and their kleptogens (LLJ and LLLJ). Managers typically consider Blue-Spotted Salamanders to be vernal pool breeding amphibians, but this specialization is not reflected in the primary literature. Descriptions of the breeding habitat of Blue-Spotted and their kleptogens are generally brief and vary by hydroperiod (permanent to ephemeral), water depth, vegetation (barren to choked), degree of alteration (including roadside ditches and gravel pits), association with floodplains, water flow (stagnant to moderate), and surrounding land cover (Piersol, 1910; Bleakney, 1957; Anderson and Giacosie, 1967; Nyman et al., 1988; Downs, 1989; Van Buskirk and Smith, 1991; Klemens, 1993; Homan et al., 2007; Ryan and Calhoun, 2014). Other ambystomatids vary in the degrees to which they partition habitat with their respective kleptogen, with Small-mouth Salamanders (*Ambystoma texanum*, abbreviated TT) and LTT being more similar in climate niche than Jefferson Salamanders (*A. jeffersonianum*, abbreviated JJ) and LJJ (Greenwald et al., 2016).

Our objectives were to determine which environmental factors affect breeding site use by Blue-Spotted Salamanders, sympatric Unisexual Salamanders (primarily LLJ and LLLJ), and Spotted Salamanders, and to evaluate co-occurrence. If the Blue-Spotted Salamander Complex selects similar breeding habitat as Spotted Salamanders, then the latter may act as an indicator of Blue-Spotted Complex Salamander presence. Otherwise, knowing which sites are more likely to host these cryptic salamanders would allow managers and surveyors to tailor conservation strategies.

We expected that within-wetland variables would be more important for breeding habitat selection than landscape-scale factors for all three taxa (Calhoun et al., 2003); for example, we hypothesized that breeding occupancy would be higher in temporary and fish-less wetlands with relatively long hydroperiods. We hypothesized that the Blue-Spotted Salamander Complex and Spotted Salamanders would differ in their response to human-altered landscapes and forest cover (Klemens, 1993; Regosin et al., 2005; Windmiller et al., 2008). We expected that Unisexuals would have a high co-occurrence and similar habitat relationships with Blue-Spotted Salamanders due to their chromosomal overlap, but not necessarily with Spotted Salamanders (Greenwald et al. 2016).

Materials and Methods

Study Sites

We conducted surveys in 43 wetlands located within 13 km of Bangor, Maine. Land use ranged from managed forest to suburban, with some agriculture and developed open space (such as cemeteries). Coniferous and mixed forest were dominant and included White Pine (*Pinus strobes*), Balsam Fir (*Abies balsamea*), Eastern Hemlock (*Tsuga Canadensis*), Red Maple (*Acer rubrum*), and Red Oak (*Quercus rubra*). The Penobscot River, the Stillwater River, and Pushaw Stream flowed through the area, creating floodplains. We chose sites with a spring high water depth ≥ 10 cm.

In 2014 we set traps in 20 wetlands within 1 km of two focal vernal pools for reasons unrelated to the current work. In 2015 we expanded the geographic extent of the study and sampled 35 sites (23 new wetlands and 12 that were trapped in 2014) using a stratified sample scheme with four categories: (1) wide, slow moving, or stagnant sections of ephemeral streams, (2) classic vernal pools (small isolated depressions similar to those found in southern New

England), (3) floodplain wetlands (e.g., depressional wetlands or small streams embedded in floodplain), and (4) wetlands with long hydroperiods (i.e., permanent ponds or those that dried only in drought years). These categories were not mutually exclusive; e.g., a stream in a floodplain could fit categories 1 and 3. Eleven of these 43 wetlands were known to host the Blue-Spotted Complex prior to our study, but approximated our stratification (3 ephemeral streams, 4 classic vernal pools, 1 floodplain wetland, and 3 long hydroperiod wetlands) and we did not know which lineage of salamander occurred in each wetland.

Trapping

We trapped breeding adult salamanders at wetlands from 24 April until 9 May in 2014 and from 20 April to 8 May in 2015 using tall aquatic funnel traps constructed from fiberglass screening covering tomato cages (Hoffmann et al., 2016). The first migrant salamanders reached the wetlands the nights of 22 April 2014 and 18 April 2015. We trapped each wetland with 10 traps for three consecutive nights, and then rotated traps systematically among wetlands until we no longer caught salamanders in sites with egg masses (indicating adults had emigrated). We counted Spotted Salamanders, but took no further measurements. We weighed, individually marked (using VIE; Northwest Marine Technologies, Inc), and tail-clipped all Blue-Spotted Complex Salamanders under 7 g (we did not clip heavier individuals because our unpublished data indicates that salamanders ≥ 7 g in our area are exclusively Unisexuals). We cut a 0.5 by 0.3 cm tissue sample from each tail with surgical scissors (Nöel et al., 2011). We stored tail clips in 70% ethanol and mailed them to the University of Guelph for genetic testing to determine genomotype using microsatellite DNA analyses at six loci (AjeD75, AjeD94, AjeD283, AjeD346, AjeD378, and AjeD422), four of which can be used to differentiate between the genomes of Blue-Spotted and Jefferson Salamanders (Julian et al., 2003). The microsatellite DNA methods are described in detail elsewhere (Bogart et al., 2007; 2009). LLLJ are locally rare (detected in only 2 sites), so we pooled all Unisexual Salamanders (LLJ and LLLJ).

Environmental variables

We measured water depth at each site in April and returned in August or September to record data on hydrology, substrate, and vegetation at the within-site spatial scale. We used a handheld GPS (etrex 10, Garmin International, Inc.) to map and calculate wetland size by walking the high-water mark or the edge of the suitable breeding area (i.e. lentic, homogenous, continuous) in cases of floodplains and ephemeral streams.

We used ArcGIS 10.3 (ERSI, 2014) to delineate a 200-m wide buffer around each breeding site (henceforth “200-m landscape”). We chose 200-m based on the median emigration distance of local Unisexual Salamanders (Hoffmann, unpublished data); emigration distances of Blue-Spotted Salamanders and Spotted Salamanders are reported to be shorter (Semlitsch, 1998; Ryan and Calhoun, 2014). We created Boolean rasters from layers of impervious surfaces (MELCD 2004 IMPERV from the Maine Office of Geographic Information Systems), land cover (MELCD 2004 from the Maine Office of Geographic Information Systems), and soils (Natural Resources Conservation Service) and used Geospatial Modelling Environment (sic; Spatial Ecology LLC, 2014) to calculate the percent of each 200-m landscape that was covered by impervious surface (as an index of urbanization), forest, and hydric soils.

We reduced the number of variables in our models due to the relatively small number of wetlands we were able to survey. We used the ‘Vegan’ library (Oksanen et al., 2015) in Program R to calculate the principle components (i.e. linear combinations of correlated variables) of our vegetation data (% coniferous leaf litter, % deciduous leaf litter, canopy density, and % cover of open water, shrubs, emergent vegetation, submergent vegetation, floating vegetation, and downed woody debris) and of our numerical hydrology variables (spring max depth, summer max depth, % full in summer). The first vegetation principle component (VegPC1) accounted for 31.8% of the variance alone and with the second (VegPC2) accounted for 54.8% of the variance (Table 1). VegPC1 was positively related to canopy closure and deciduous leaf litter, and VegPC2 was positively related to emergent vegetation and coniferous leaf litter. Our depth principle component (DepthPC1) explained 77.5% of the variation, and reflected shallower depths.

We used the ‘Hmisc’ library in R (Harrell and Dupont, 2015) to create a correlation matrix and reduced our remaining number of covariates when two or more variables had $|r| \geq 0.5$. Specifically, we removed: 1) impervious surface cover in the 200-m landscape because it was correlated with VegPC1 ($r = 0.55$); 2) presence of inlets because it was related to presence of fish ($r = 0.55$); 3) shrub cover because it was related to streams ($r = 0.65$); and 4) soil at the edge of the wetland because it was related to soil at the center ($r = 0.54$). Although VegPC1 was correlated with forest cover within 200 m ($r = 0.60$), we kept both for interpretation at different scales, but did not include these covariates in the same model. We then z-standardized each continuous variable (Table 1).

Table 1. Predictor variables used to evaluate occupancy and detection probability of Unisexual Salamanders (LLJ and LLLJ), Blue-Spotted Salamanders, and Spotted Salamanders. We trapped salamanders during the spring of 2014 and 2015 for three consecutive nights in wetlands in central Maine, USA. Asterisks (*) indicate the contribution of variables from principal component analysis. Within a principle component, (–) indicates a negative relationship, and (+) indicates a positive relationship. Double asterisks (**) indicate counts of captures of a taxon used as a variable for another taxa, and were sampled with replacement (such that one individual Spotted Salamander caught on 2 days would count as 2 captures).

Parameter	Variable	Description	Mean, (min, max)
Detection	Date	Days between first migration and each trap night, used as a linear survey covariate	10.9 (2, 20)
Detection	Spring depth	Maximum wetland depth in cm	41 (14, 300)
Detection/ Occupancy	Wetland Area	Size of the sampled area in square meters	1,753 (46, 9978)
Detection/ Occupancy	VegPC1 * "Canopy Closure"	Deciduous litter cover (+, r = 0.75), canopy density (+, r = 0.82), floating vegetation cover (-, r = 0.82), and submergent vegetation cover (-, r = 0.81)	0 (-6.23, 2.15)
Occupancy	VegPC2 * "Emergent vegetation"	Coniferous litter cover (-, r = 0.65), emergent vegetation cover (+, r = 0.75), woody debris cover (-, r = 0.55), and open water (-, r = 0.73)	0 (-4.64, 2.52)

Table 1, continued

Parameter	Variable	Description	Mean, (min, max)
Occupancy	Fish	Fish bycatch	Binary, Fish at 28% of sites
Occupancy	DepthPC1* “Shallow depths”	Spring depth (-, r = 0.91), summer depth (-, r = 0.97), and percent dry in the summer (+, r = 0.75)	0 (-7.82, 1.37)
Occupancy	Forest	Percent of the 200-m landscape covered by forest	0.75 (0%, 100%)
Occupancy	Hydric soils	Percent of the 200-m landscape covered by hydric soils	0.36 (0%, 92%)
Occupancy	Soil center	Presence of muck or mineral soil at the center of the wetland	Binary, Muck at 70% of sites
Occupancy	Stream	Wetland present in slow moving section of a stream	Binary, stream at 28% of sites
Occupancy	Uni	Counts of Unisexuals**	3.45 (0, 53)
Occupancy	BSS	Counts of Blue-Spotted Salamanders**	0.19 (0, 3)
Occupancy	SS	Counts of Spotted Salamanders**	8.67 (0, 83)

Occupancy models

We fit the single season occupancy model (Mackenzie et al., 2006) using the library 'unmarked' in Program R (Fiske and Chandler, 2011) to model the relationships between environmental variables and salamander occupancy (the proportion of sites where the taxa are present, Mackenzie et al., 2006) while accounting for sites where animals may have been present without being captured (imperfect detection). We first attempted to model salamander occupancy under the multi-species occupancy formulation (Mackenzie et al., 2004) using program PRESENCE 9.8 (Hines, 2015), however we found that species interaction terms failed to converge, and so we instead employed a single-species approach for each taxon. We used the count of individuals for each taxon (sampled with replacement) as predictor variables in occupancy models of the other two taxa, which allowed us to explore relative interspecific effects on occupancy without directly modeling their co-occurrence (Werner et al., 2014). We also modeled the occupancy of the subset of wetlands that were not known breeding sites.

We identified four *a priori* detection models based on the biology of the salamanders and the constraints associated with our trapping scheme and modeled eight environmental variables that may influence the occupancy of each salamander. We expected detection to decrease with thicker vegetation (VegPC1), deeper springtime water, larger wetlands, and towards the end of the breeding season. We expected occupancy might vary with vegetation (VegPC1 or VegPC2), the percent of the 200-m landscape containing forest or hydric soil, counts of congeners (BSS, SS, or Uni), wetland area, and the presence of fish.

We fit the global model (which includes the additive effects of each covariate) for Unisexual Salamanders, but the global model for Blue-Spotted and Spotted Salamanders failed to converge. We used the most inclusive models for these two salamanders and the Unisexual global model to test for overdispersion using Chi-square goodness-of-fit tests on 1,000 bootstrap permutations (MacKenzie and Bailey 2004). The global model for Unisexual Salamanders and the most inclusive model for Blue-Spotted Salamanders adequately fit the data (χ^2_{Uni} = 90.540, p = 0.173, $\hat{c}_{Unisexual}$ = 1.22 and χ^2_{BSS} = 38.147, p=0.192, $\hat{c}_{Blue\text{-}Spotted}$ = 1.22). The Spotted Salamander data displayed characteristics of moderate overdispersion (χ^2_{SS} = 162.938, p <0.001. $\hat{c}_{Spotted}$=2.37). We adjusted ĉ (the ratio of the observed test statistic to that of the average of test statistics obtained from the bootstrap) as needed and ranked the Unisexual and Blue-Spotted Salamander models by AICc (Akaike's Information Criterion, adjusted for small sample size) and the Spotted Salamander models by QAICc (Quasi-likelihood AICc; MacKenzie and Bailey, 2004). We considered any model that ranked above the null (intercept only) model to have some support. We examined the 90% confidence intervals of each variable related to occupancy in models that ranked above the null model to determine if each variable was a significant predictor of salamander detection or occupancy.

Due to the overlapping 200-m landscape around some of our sampling sites, we examined the residuals of the most-inclusive models for spatial autocorrelation. We averaged the residuals across trap nights and used library 'ncf' in Program R (Bjornstad, 2015) to calculate the spline-correlog of each model. We consider models to be spatially autocorrelated at distances where the confidence intervals did not include zero. Spline correlograms indicated that the most-inclusive models of our three salamanders were not spatially autocorrelated therefore we did not include a spatial autocovariate in our models.

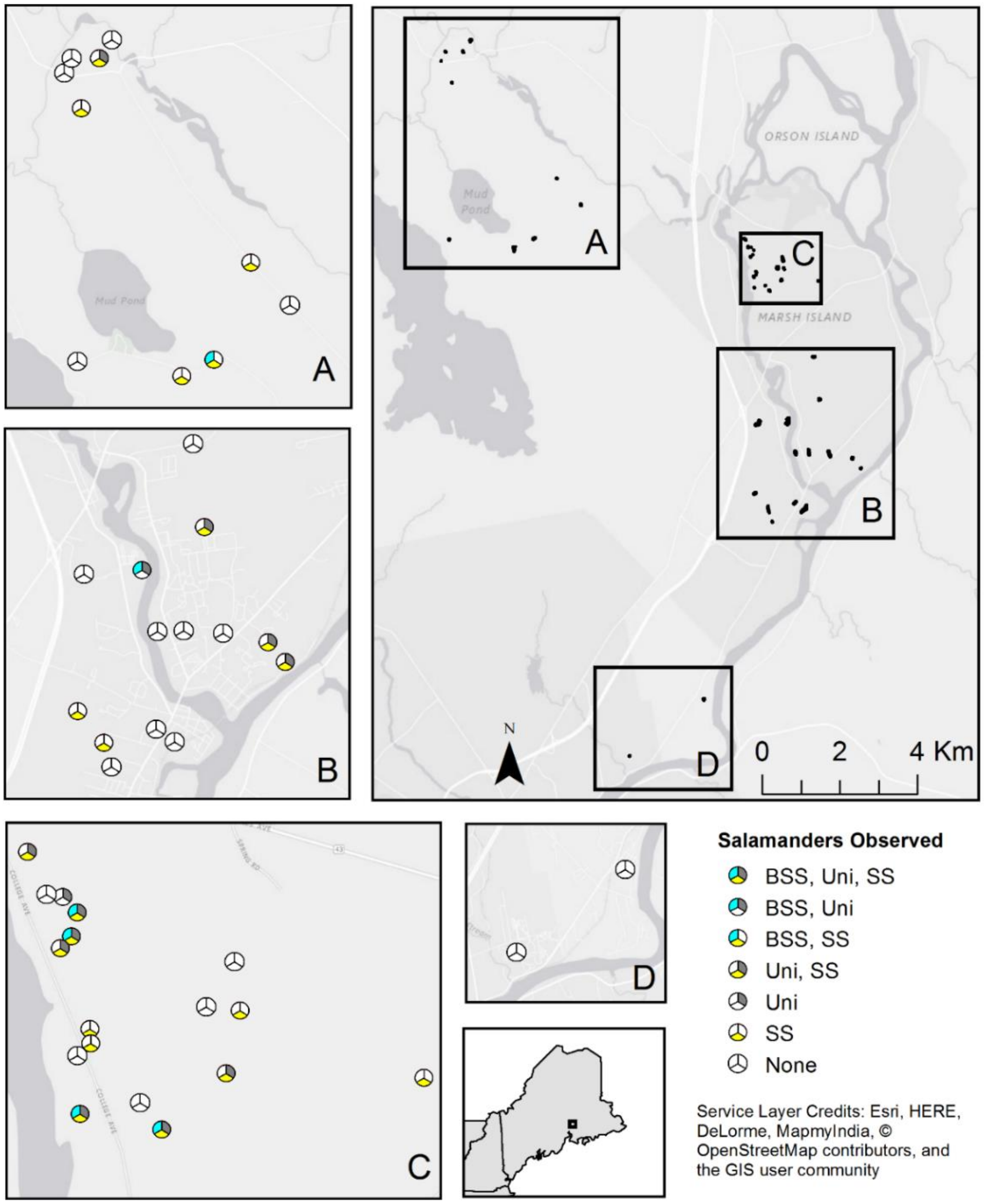

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Figure 1. Extent of the study area in central Maine, USA. Wetland locations are indicated in black on the main map (wetlands are scaled relative to each other but enlarged to be visible on the map). Inserts A through D indicate the salamanders caught in funnel traps at each site during three consecutive trap nights in the spring of 2014 and/or 2015 (BSS = Blue-Spotted Salamanders, Uni = Unisexual Salamanders [LLJ and LLLJ combined], and SS = Spotted Salamanders).

Results

We trapped 43 wetlands and captured Unisexual Salamanders (n = 149) in 13 of these, Blue-Spotted Salamanders (n = 8) in 6, and Spotted Salamanders (n=373) in 21 (Figure 1). The naïve occupancies (the number of sites where salamanders were captured divided by the total number of sites, S_D/S), which do not account for imperfect detection, were 0.30, 0.14, and 0.49, respectively. Of the 14 sites where the Blue-Spotted Salamander Complex was captured, there was only one site where we did not detect Unisexuals.

Detection of all species decreased during the trapping period, with date as a linear effect (Table 2). Detection probability of Unisexual Salamanders dropped from 0.91 (0.067 SE) on the day following immigration to 0.50 on the 11th day. Blue-Spotted salamander detection probability dropped from 0.635 (0.395 SE) to 0.50 on the third day, and Spotted Salamander detection probability dropped from 0.951 (0.035 SE) to 0.50 on the 11th day (Figure 2). For Blue-Spotted Salamanders, a model that contained a VegPC1 effect on detection ranked highly (AICc = 59.01), however estimates of the slope coefficient failed to converge for this model. There were generally low capture rates for this species, and captures only occurred in wetlands with high VegPC1 values. Given that detection probability is contingent on presence, lack of variation in VegPC1 among occupied sites likely produced the failed convergence, and we removed this model from consideration. For all taxa we retained date as a covariate effect on detection probability while evaluating occupancy.

Table 2. Ranking of single-season occupancy models, each with a single detection covariates. Salamanders were trapped during three consecutive nights during the spring of 2014 and 2015 in wetlands in central Maine, USA.

Unisexual Salamanders	K	AICc	ΔAICc	*w*
ψ(.)p(Date)	3	119.57	0	0.99
ψ(.)p(Spring depth)	3	129.34	9.77	0.01
ψ(.)p(.)	2	132.92	13.35	0
ψ(.)p(VegPC1)	3	134.75	15.18	0
ψ(.)p(Wetland area)	3	135.17	15.60	0

Blue-Spotted Salamanders	K	AICc	ΔAICc	*w*
ψ(.)p(Date)	3	58.99	0.00	0.73
ψ(.)p(.)	2	62.04	3.05	0.16
ψ(.)p(Wetland area)	3	64.18	5.19	0.05
ψ(.)p(Spring depth)	3	64.27	5.28	0.05

Spotted Salamanders	K	QAICc	ΔQAICc	*w*
ψ(.)p(Date)	4	84.68	0	0.99
ψ(.)p(Wetland area)	4	96.44	11.77	0
ψ(.)p(.)	3	96.79	12.11	0
ψ(.)p(Spring depth)	4	98.35	13.67	0
ψ(.)p(VegPC1)	4	98.55	13.87	0

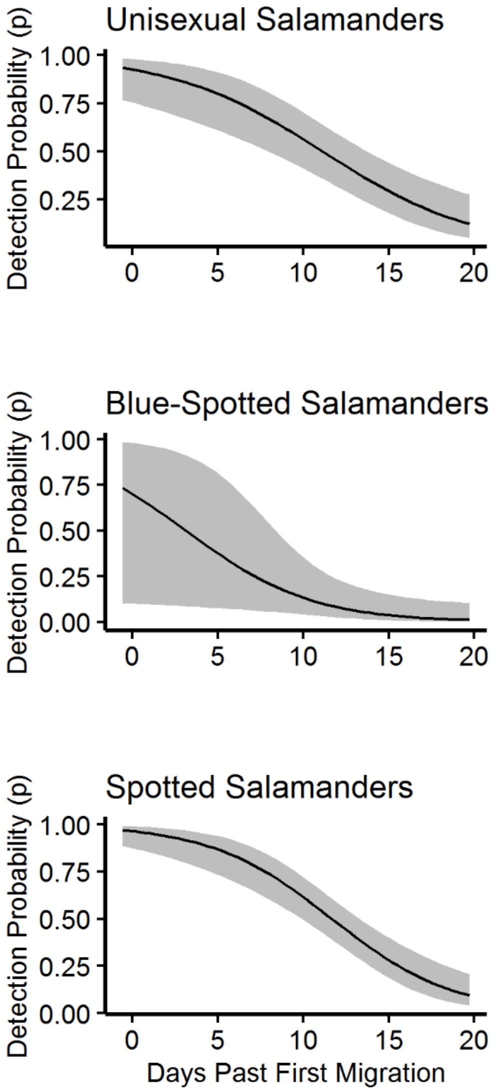

Figure 2. Relationship between detection probability and the number of days following initial immigration to breeding pools for three salamanders in central Maine, USA. Date was modelled as a covariate effect in single-season occupancy models based on salamander capture data collected during 2014 and 2015. Shaded areas represent 90% confidence intervals.

Models including covariates that described effects of congeners, landscape characteristics, and vegetation on occupancy tended to rank among the best models (Table 3, Table 4). Unisexual Salamander occurrence was positively related to the number of captured Blue-Spotted Salamanders, and this top model had over three times the weight of the next ranked model. Unisexual Salamander occurrence was also positively related to the proportion of the landscape within 200 m that contained hydric soil, and negatively related to the second vegetation principle component (which indicates occupancy increased with more coniferous leaf litter, woody debris, and open water, and decreased with more floating vegetation cover, Figure 3). The same vegetation relationship (VegPC2) was the only significant predictor of Blue-Spotted Salamanders, and had an effect on Blue-Spotted Salamander occupancy almost three times as strong as on Unisexual Salamander occupancy (Figure 4; Table 4). Although count of Unisexual Salamanders had a positive effect on occupancy by Blue-Spotted Salamanders ($\beta_{Uni} = 2.120$, SE = 1.467), the 90% confidence interval of β_{Uni} included zero (-0.293 to 4.534) so we cannot conclude that the effect is supported by our data. Spotted Salamander occupancy increased with the proportion of the landscape that was forested, and this top model had a weight about twice that of the next ranked model. Spotted Salamander occupancy also increased with the first vegetative principle component (which increased with deciduous litter and canopy density but decreased with floating and submergent vegetation cover), and decreased with the area of the wetland (Figure 5).

Table 3. Ranking of single-season occupancy models with scaled environmental covariates. Salamanders were trapped during three consecutive nights during the spring of 2014 and 2015 in wetlands in central Maine, USA. Only models with β estimates greater in absolute value than their standard errors are listed here. Each model contains a single covariate for occupancy and the number of days since the start of immigration as a covariate for detection.

Unisexual Salamanders	K	AICc	ΔAICc	w
ψ(BSS)p(Date)	4	114.25	0	0.6
ψ(Hydric soil)p(Date)	4	116.77	2.51	0.17
ψ(VegPC2)p(Date)	4	117.4	3.15	0.12
ψ(.)p(Date)	3	119.57	5.32	0.04
ψ(SS)p(Date)	4	120.73	6.48	0.02
ψ(Forest)p(Date)	4	120.85	6.59	0.02
ψ(Fish)p(Date)	4	120.86	6.6	0.02
ψ(.)p(.)	2	132.92	18.67	0

Blue-Spotted Salamander	K	AICc	ΔAICc	w
ψ(VegPC2)p(Date)	4	53.36	0	0.57
ψ(Uni)p(Date)	4	54.12	0.76	0.39
ψ(.)p(Date)	3	58.99	5.63	0.03
ψ(.)p(.)	2	62.01	8.68	0.01

Table 3, continued

Spotted Salamander	K	QAICc	ΔQAICc	w
ψ(Forest)p(Date)	5	82.16	0	0.35
ψ(Uni)p(Date)	5	83.74	1.57	0.16
ψ(Wetland area)p(Date)	5	84.03	1.87	0.14
ψ(VegPC1)p(Date)	5	84.65	2.49	0.1
ψ(.)p(Date)	4	84.68	2.51	0.1
ψ(Hydric soil)p(Date)	5	86.04	3.87	0.05
ψ(BSS)p(Date)	5	86.06	3.9	0.05
ψ(VegPC2)p(Date)	5	86.57	4.41	0.04
ψ(.)p(.)	3	96.79	14.63	0

Table 4. Estimates, standard errors, and 90% confidence intervals of environmental covariates of adult salamander occurrence. Salamanders were trapped in wetlands in central Maine, USA, during the spring of 2014 and 2015. Variables are listed in order of AICc or QAICc, and only those with confidence intervals that do not include zero are shown here. These statistics are based on a single covariate for occupancy and days past immigration as a covariate for detection.

Unisexual Salamander	β estimate	SE	Lower CI	Upper CI
BSS	1.15	0.835	0.132	2.879
Hydric soil	0.933	0.477	0.148	1.717
VegPC2	-0.811	0.418	-1.499	-0.122

Blue-Spotted Salamander				
VegPC2	-2.37	1.15	-4.253	-0.482

Spotted Salamander				
Forest	1.525	0.65	0.455	2.594
Wetland area	-1.211	0.66	-2.296	-0.126
VegPC1	0.993	0.506	0.161	1.825

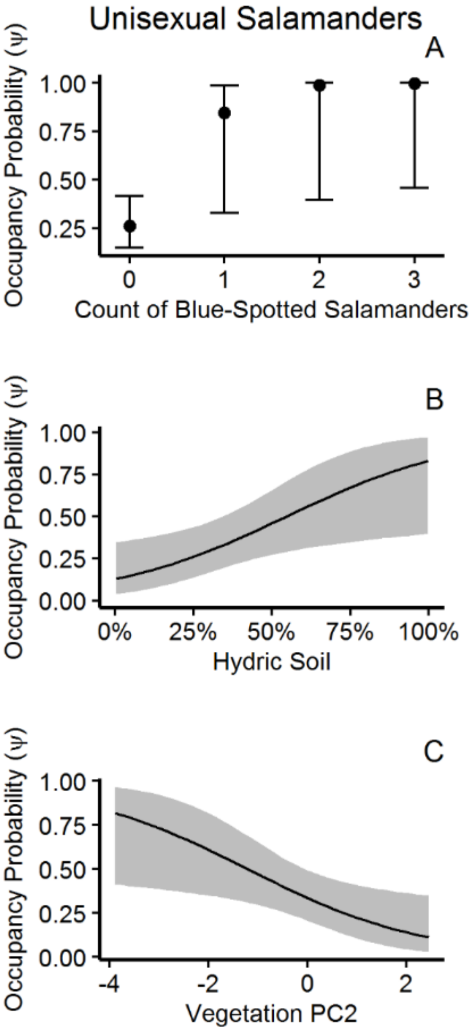

Figure 3. Relationships between occupancy probability of breeding Unisexual Salamanders in wetlands and occupancy covariates. Occupancy was related to A) the counts of captured Blue-Spotted Salamanders sampled with replacement, B) the proportion of the surrounding 200 m landscape that contains hydric soil, and C) a vegetation principle component that is positively related to emergent vegetation and negatively related to deciduous leaf litter, woody debris, and open water. Bars and shaded areas represent 90% confidence intervals.

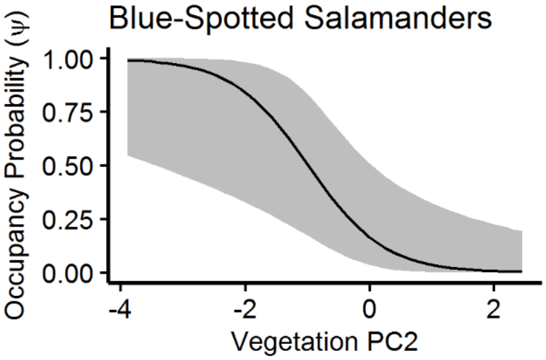

Figure 4. Relationships between occupancy probability of breeding Blue-Spotted Salamanders in wetlands and a vegetation principle component. This component is positively related to emergent vegetation and negatively related to deciduous leaf litter, woody debris, and open water. Shaded areas represent 90% confidence intervals.

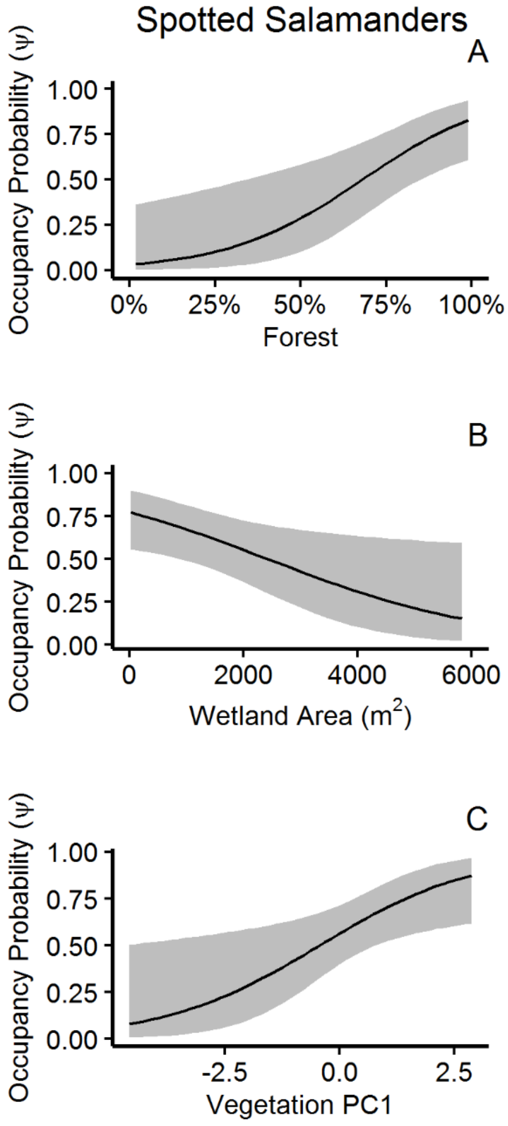

Figure 5. Relationships between occupancy probability of breeding Spotted Salamanders. Occupancy was related to A) the proportion of the surrounding 200 m landscape that is forested, B) the wetlands' area, and C) a vegetation principle component that is positively related to deciduous leaf litter and canopy density and negatively related to floating and submergent cover. Shaded areas represent 90% confidence intervals.

Predicted occupancy for Blue-Spotted Salamanders, based on an intercept-only occupancy structure and a date effect on detection probability, was 2.5 times higher (Ψ= 0.36 +/- 0.20 SE) than the naïve estimate (0.14, which estimated occupancy as the number of sites where the taxon was captured divided by the total number of sites). For Spotted Salamanders predicted occupancy (Ψ= 0.60 +/- 0.10 SE) was somewhat higher than the naïve estimate (0.49), and for Unisexual Salamanders predicted occupancy (Ψ= 0.36 +/- 0.09 SE) and naïve estimates (0.30) were more similar. When we removed wetlands that were known to host the Blue-Spotted Salamander Complex prior to our study, predicted occupancy of Blue-Spotted salamanders fell to 0.191 (SE = 0.13), that of Spotted Salamanders fell to 0.520 (SE = 0.11), and that of Unisexual Salamanders fell to 0.22 (SE = 0.08).

Discussion

Our study yielded insights into rates of wetland occupancy by ambystomatids that would not have been possible without incorporating repeated sampling and occupancy analysis. Although we captured Unisexuals at over twice as many wetlands compared to Blue-Spotted Salamanders, after accounting for the low detection of Blue-Spotted Salamanders their predicted occupancies were similar. Occupancies of both salamanders in the Blue-Spotted Salamander Complex were just over half that of Spotted Salamanders. However, our strength of inference varied across the three salamanders due to difference in capture rates.

The detection probability of all three salamanders decreased as the breeding season progressed and animals emigrated. Detection of Unisexual and Spotted Salamanders was initially high (90% and 95% on the day after migration began, respectively) and remained >50% for a week and a half. Blue-Spotted Salamanders had lower detection, starting at 63% the day after migration, and their detection declined more steeply compared to the other salamanders (Figure

2). We recommend that managers using funnel traps to survey ambystomatids begin trapping as soon as possible after immigration, and those surveying specifically for Blue-Spotted Salamanders should use many traps.

Blue-Spotted Salamander and Unisexual Salamander occupancy were associated with the same vegetation characteristics while Spotted Salamander occupancy was best-predicted by a different set. The most apparent difference is that the Blue-Spotted and Unisexual Salamanders selected wetlands with coniferous leaf litter while Spotted Salamanders selected wetlands with deciduous leaf litter. The remaining vegetation variables sorted onto two different principle components but both appear to describe wetlands with sparse vegetation. More specifically, Blue-Spotted and Unisexual Salamanders selected breeding habitat with low cover by emergent vegetation and high amounts of open water and woody debris (opposite of VegPC2 in Table 1), while Spotted Salamanders selected breeding habitat with high canopy density and low floating vegetation and submergent vegetation (VegPC1 in Table 1).

Spotted Salamanders used small wetlands, but wetland area was not supported as a predictor of either lineage in the Blue-Spotted Salamander Complex. Other studies have found that Spotted Salamanders were positively associated with wetland area (Rowe and Dunson, 1993; Calhoun et al., 2003; Skidds et al., 2007). This discrepancy is most likely due to scale. Our study included flood plain wetlands, permanent ponds, and forested wetlands that tend to be far larger than classic temporary pools. Our findings fit with the paradigm that Spotted Salamanders select vernal pools, which tend to be small, fishless, and temporary by definition (Calhoun et al., 2003; Grant, 2005).

At the 200-m landscape scale, Unisexual Salamander occupancy was predicted by high proportion of hydric soil, while Spotted Salamander occupancy was predicted by high forest cover. Ambystomatids are fossorial and it is possible that Unisexuals prefer hydric soil for their terrestrial non-breeding habitat. Ryan and Calhoun (2014) observed radio-implanted Blue-Spotted Salamanders summering in a Red Maple (*Acer rubrum*) swamp and a wet meadow, and Hoffmann (unpublished data) observed some Unisexual Salamanders residing in Alder (*Alnus incana*) swamps after emigration. The use of forests by Spotted Salamanders is well-established (deMaynadier and Hunter, 1998; Skidds et al., 2007; Pittman and Semlitsch, 2013), while the Blue-Spotted Complex Salamanders may be less forest dependent (Regosin et al., 2005).

It is not surprising that Blue-Spotted Salamander captures were the strongest predictor of Unisexual Salamander occupancy, as Unisexuals in Maine are dependent upon Blue-Spotted Salamanders to contribute sperm. Unisexual Salamanders often outnumber sperm-host species (Downs, 1978; Lowcock et al., 1991; Homan et al., 2007), which may account for their higher detection rate.

Our results indicate that Spotted Salamanders and the Blue-Spotted Salamander Complex may need to be managed separately, as they respond to different breeding habitat characteristics and the presence of Spotted Salamanders does not significantly predict the presence of the more cryptic salamanders. Oscarson and Calhoun (2007) recommend increased survey effort to target these species, and we further recommend increased survey effort in landscapes with large proportions of hydric soil and in wetlands with coniferous leaf litter and sparse vegetation. In surveys where Blue-Spotted Salamanders are of interest we recommend increased trap effort in wetlands where Unisexuals have been observed. Future studies may refine relationship between occupancy and vegetation or determine the applicability of our findings to other regions.

CHAPTER 2

PITFALLS OF ANALYZING PITFALL DATA ON A CIRCULAR SCALE

Chapter Abstract

Information on the construction and use of pitfall arrays at amphibian breeding pools is widely available, but there is little guidance regarding the statistical analysis of orientation data from these sites. We used simulated and field data to examine the effects of binning, pool shape, concentration, sample size, and multimodality on the outcomes of statistical tests. We examined the efficacy of three tests of the null hypothesis that orientation is random rather than directional. We found the Rayleigh test to be most suitable and caution against the use of Rao's spacing test on pitfall data as it produced high Type I and Type II error rates. We do not recommend the use of chi-squared goodness of fit for small samples or when the drift fence is not strictly circular. We also examined four tests of the null hypothesis that two samples have the same distribution. We recommend the Kruskal-Wallace test but not the Watson-Williams test as it resulted in high Type I error rates at concentrations similar to what we observed in the field. We advise against using chi-squared test of homogeneity due to problems with the expected values needed for this test, such as division by zero being undefined. Multiresponse Permutation Procedure should not be used for small samples due to high Type II error rates. Expert opinion indicated that some observations that were statistically significantly different were probably not ecologically different. We encourage future researchers to use conservative tests and to visually examine their results to ensure that their conclusions make sense ecologically.

Introduction

Drift fences with pitfall traps are commonly used to study amphibians, especially their movements at breeding sites. However, interpretation of pitfall data has many potential problems. First, sampling bias is likely because catchability may vary by species, season, body size or condition, sex, and site microtopography (reviewed in Dodd and Scott, 1994). Second, pseudoreplication readily occurs when an unmarked individual moves to and from the wetland more often than expected within a season, or when multiple seasons are combined with repeating breeders counted more than once (but see Santos et al., 2010). Challenges also exist in statistical analysis of pitfall data, including navigating the assumptions of statistical tests on directional data. Detailed guidance for planning and implementing drift fence studies exists (Dodd and Scott, 1994; Wilson and Gibbons, 2010) but information on analyses of directional data based on pitfall traps are lacking.

The fundamental feature of directional data from pitfalls surrounding breeding pools is circularity, with data distributed on a circular scale (i.e. degrees or radians) rather than a linear scale and the location of zero arbitrary. For example, 90° is the opposite of 270°, 300° is not “larger” than 3°, and 360° is the same as 0°. The mean direction of travel is represented by a mean vector found through trigonometry rather than the arithmetic mean, and the length of this vector indicates how concentrated (clustered together as opposed to spread out evenly around the circle) the observations are. Most parametric statistics cannot handle directional data, so researchers use circular statistics and non-parametric statistics. Circular data can either be continuous or binned into counts within arcs of the circle. Distributions restrict the choices of statistical tests and include (but are not limited to): the uniform distribution, where amphibians show no directionality (which can be considered random orientation); the von Mises distribution, the circular equivalent of a normal distribution; and multimodal distributions, which can be problematic as the mean vector may point in a direction avoided by amphibians but located between two modes.

Problems in analysis may also stem from the assumption that amphibians are migrating to or from the center of a circular wetland. Shoop (1968) speculated that an amphibian immigrating to a breeding site needs only to intercept the border rather than orient to its center, and that an animal emigrating eastward may exit at anywhere between 0° and 180°. Additionally, even in a round wetland some areas may be preferentially used. For example, a deep section in the north end of a vernal pool may dry last and skew the pitfall data to indicate that juvenile frogs tend to emigrate northwards. Natural wetlands may be oblong, linear, or highly irregular in shape and this may distort the distribution of amphibian orientation from what would be expected at a circular wetland. While authors typically acknowledge that their wetlands are not strictly circular and this could influence interpretation, we are unaware of any quantification of the influence of pool shape.

The most common null hypothesis in amphibian orientation is that the sampled population is uniformly distributed (e.g., that salamanders are leaving at random angles), and this hypothesis is assessed using a variety of test of uniformity (Table 5). These are often followed by a test of homogeneity to assess the null hypothesis that two samples come from the same population (e.g., the orientation of toads one year is not significantly different from a second year). The tests used by authors of pitfall studies on amphibian orientation vary in their limitations. For example, some authors treat animals as individuals located along a continuous circle (Dodd and Cade, 1998; Marty et al., 2005; Santos et al., 2010), while others bin animals into ordinal or nominal categories (Rothermel, 2004; Jenkins et al., 2006; Homan et al., 2010). This distinction may be critical, as it reflects a basic assumption of the statistical tests. To our knowledge, no one has directly compared tests that view drift fence data as continuous or binned counts to determine if results may be affected by the inherent binning by the fence.

In addition, there are problems in interpretation of results. Many of these tests may detect subtle differences in orientation across years or age classes, but statistical significance may not be ecologically relevant (Johnson, 1999). In fact, it has long been recognized that the perceived precision of drift fence data may be higher than is biologically significant (Shoop, 1968).

Table 5. Comparison of circular statistical tests (Batschelet 1981, Mardia and Jupp 2000, Zar 2010, Pewsey et al. 2013).

Test of uniformity	Accommodates multimodal data	Accommodates count data	Acknowledges circular scale	Other limitations
Rayleigh test	No	With correction	Yes	
Rao's spacing test	Yes	No	Yes	
Chi-squared GOF	Yes	Yes	No	Large sample size
Tests of homogeneity				
Chi-squared TOH	Yes	Yes	No	Large sample size
MRPP	Yes	Yes	Yes	Computationally intense
Kruskal-Wallis test	Yes	Yes	No	Uses ranks
Watson-Williams test	No	If groups are ≤ 10°	Yes	Equal and large parameters of concentration

In this chapter, we examine issues with statistical analysis of pitfall data to study amphibian orientation into and out of a breeding sites. We use simulated data and field data from vernal pools to: (1) contrast the results of various tests with emphasis on Type I and Type II errors; (2) examine how binning of data, multimodality, concentration, sample size, and fence shape might influence the results of these tests; and (3) evaluate expert opinion on when the difference between two samples is ecologically important rather than just statistically different. We break some assumptions and examine the consequences. Our goals are to alert other researchers to these pitfalls of pitfall trap analysis and to provide suggestions on selecting appropriate statistical methods.

Materials and Methods

Background on Tests of Uniformity

Researchers use tests of uniformity to determine if animals are orienting randomly or directionally. The Rayleigh test uses the length of the mean vector to determine if the data are too concentrated to be from a uniform distribution and assumes the von Mises distribution as an alternative (Batschelet, 1981; Zar, 2010; Pewsey et al., 2013). The Rayleigh test is intended for continuous data but can be used when bins are numerous or corrected for use with few bins (Mardia and Jupp, 2000; Rothermel, 2004; Vasconcelos and Calhoun, 2004). The Rayleigh test is not robust against multimodal distributions as a peak of individuals orienting northward could cancel out a peak of individuals orienting southward though doubling the angles will correct for this specific scenario (Batschelet, 1981; Bergin, 1991; Pewsey et al., 2013). Rao's spacing test is based on the sum of the lengths of arcs between data points compared to the sum of lengths of uniform arcs (i.e. lengths of 360° divided by the number of points) with a large difference being less likely under the null hypothesis (Batschelet, 1981; Mardia and Jupp, 2000). This test is relatively powerful with both unimodal and bimodal data and robust at small sample size but cannot be corrected for use of count data (Bergin, 1991; Dodd and Cade, 1998; Marty et al., 2005). Another alternative is the chi-squared goodness of fit test (chi-squared GOF) which compares observed frequencies to expected frequencies for binned data (Jenkins et al., 2006; Timm et al., 2007; Homan et al., 2010). Chi-squared GOF assumes an expected frequency of at least four individuals per bin (Batschelet, 1981). Expected frequencies are often found by dividing the total number of individuals by the number of bins (*n* / *k*). This test allows the researcher to decide how to bin the data, such that the number of bins can be reduced when the minimum number of points per bin is not met. Chi-squared GOF does not account for the proximity of bins, but treats these ordinal categories as unorganized, which can result in lower power (Pewsey et al., 2013). While each of these tests has flaws, there is little in the literature to help a researcher determine which flaws are acceptable and which should exclude a test form consideration.

Background on Tests of Homogeneity

Researchers have used chi-squared tests of homogeneity (chi-squared TOH), multiresponse permutation procedures (MRPP), Watson-Williams tests, and Kruskal-Wallis tests to compare two or more samples of pitfall data (such as different years, ages, and species) to determine if orientation across samples differs (Dodd and Cade, 1998; Vasconcelos and Calhoun, 2004; Patrick et al., 2007; Timm et al., 2007; Homan et al., 2010). The chi-squared TOH compares the distribution of two samples using a 2 x *c* contingency table, where *c* is the number of bins (Timm et al., 2007). As in the use of the chi-squared GOF test above, bins are arbitrary and the proximity of bins to one another is not taken into account. Minimum sizes still apply and Batschelet (1981) advises dropping bins with expected values of zero which some software applications do automatically. MRPP measures the Euclidean distances (arc lengths) between points of two or more samples and compares the mean within sample distance to the distances found in permutations of the data sorted into randomly assigned samples (Talbert et al., 2013). The Watson-Williams test examines differences in two or more samples by combining the mean vectors and comparing the length of the resultant vector to the length of the sum of the mean vectors (Batschelet, 1981). This test assumes the concentrations (κ) of the two samples are equal and large ($\geq$2) and that the samples are taken from a von Mises distribution. It is not intended to handle multimodal data or binning with arcs longer than 10° (Batschelet, 1981; Patrick et al., 2007). The Kruskal-Wallis test is a non-parametric test that ranks bins to determine if the sampled populations have the same distribution (Homan et al., 2010; Zar, 2010). This test assumes the samples have equal variance and does not take the circular scale into account when it ranks bins of data (Zar, 2010).

Simulations

We simulated animals *in silico* emigrating from the center of wetlands surrounded by drift fences with equally spaced pitfall traps (e.g., pitfalls every 5 meters along the fence). We determined the azimuths of 24 pitfall traps from both the center of a circle and the center of an ellipse with a major diameter twice as long as the minor diameter using SolidWorks 2015 (Dassault Systems) and calculated the range of angles that would lead an animal to each trap. Our hypothetical circular pool had pitfall traps located evenly around its perimeter at every 15°. For our ellipse pool to also have pitfalls spaced evenly around the perimeter, the arcs of the ellipse that represent the distance served by each trap varied from 11.05° to 22.40°.

We used Program R to simulate data and examined how binning into pitfalls, pool shape, sample size, concentration, and multimodality affect the efficacy of statistical techniques. We used “base R” to create uniform distributions (Figure 6), and the “circular” package (Lund and Agostinelli, 2015) to produce Von Mises distributions and bimodal distributions. For each distribution, we created 1,000 replicates of 30, 90, and 200 angles to represent animals emigrating from a wetland (e.g., 30 frogs emigrating per year for 1,000 years). Our directional distributions had a mean vector of north (0°) towards the wide side of the ellipse or east (90°) towards the narrow end of the ellipse. Our bimodal distribution had a mode at both north and east. For our test of uniformity, we used κ (concentration parameter) of 1, which gave the sample some clustering around the mode but enough dispersion for a realistic number of animals to emigrate in other directions (Figure 7) and approximated the median κ observed in our field data (see below). This level of concentration is significant under the Rayleigh test at $n = 30$ and $\alpha = 0.05$. For our tests of

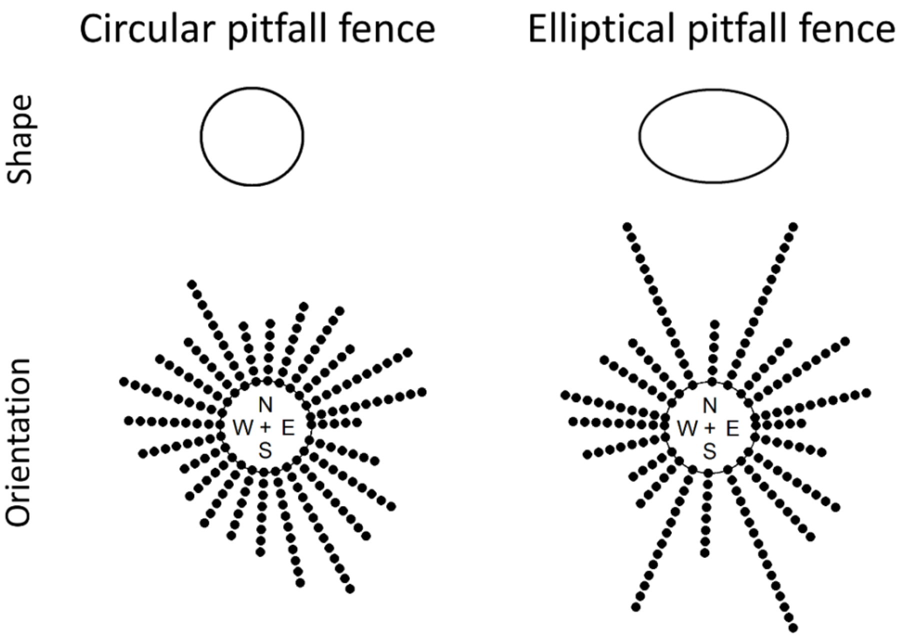

Figure 6. Effects of drift fence shape on orientation. Both plots show the same 200 simulated amphibians (i.e. random angles) leaving in a uniform distribution from the center of a pool but differ in how the animals were binned into 24 pitfalls traps. When the simulated animals where binned into pitfalls at an elliptical pool the distribution was distorted to show more animals exiting towards the long sides (N and S) of the pool.

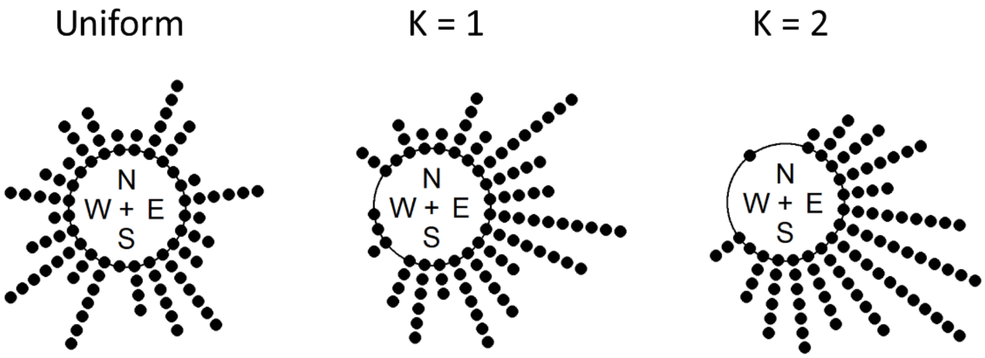

Figure 7. Effects of concentration on orientation. All three plots have a sample size of 90 simulated amphibians (random or semi-random angles), but the left plot shows a uniform distribution while the middle and right plots show eastward directional distributions of different concentration (κ). Note the increase in both white space to the northwest and kurtosis ("peakedness") of the mode.

homogeneity, we used both $\kappa = 1$ and $\kappa = 2$ so that we could examine the effects of varying κ (a parameter important for the Watson-Williams test). We used $\alpha = 0.05$ for all tests, and considered error rates below 6% to be acceptable.

We conducted Rayleigh tests, Rao's spacing tests, and a series of chi-squared GOF tests using the "circular" package and "base R." We did not correct for binning for the Rayleigh tests because we had more than 12 bins (Batschelet, 1981; Pewsey et al., 2013). We tested each distribution and then doubled the angles of the elliptical pitfall data to adjust for axial bimodal distribution; then we conducted another Rayleigh test and Rao's spacing test on these data. We used eight bins for our chi-squared GOF test with expected values of 12.5% for each bin (representing 45°/360° degrees). We ran further chi-squared tests to examine the effects of various expected values.

We then examined the efficacy of chi-Squared TOH, the Watson-Williams test, MRPP, and the Kruskal-Wallace test by comparing replicates of varying direction, pool shape (circular and elliptical), concentration ($\kappa = 1$ and 2), and sample size ($n = 30$, 90, and 200) using the "circular" and "Blossom" (Talbert et al., 2013) packages.

Field data

We completely encircled two vernal pools in Orono, Maine, and two in Old Town, Maine, USA, with drift fence arrays of silt fence and aluminum cans sunk into the ground every 5 m along both sides of the fence (Shoop, 1968; Dodd and Scott, 1994). Each fence had from 13 to 30 pairs of pitfall traps representing a variety of angles. We installed the fence within 2 m of the high-water mark of Pools 1, 2, and 4, and between 1 and 15 m of the high-water mark of Pool 3 owing to the study design of a related project. Pools were embedded in mixed forest, some with nearby development (Pools 3 and 4), and one was 5 m from a dirt road (Pool 1). We checked the fences daily throughout the spring and summer and on alternate days in the fall of 2013 and 2014. We covered pitfalls and laid down sections of fence for winter. Captured amphibians were released on the opposite side of the fence. Here we focus on emigrating salamanders in the Blue-Spotted Complex (*Ambystoma laterale* and *A. laterale* – dependent unisexuals), Spotted Salamanders (*A. maculatum*), and Wood Frogs (*Lithobates sylvaticus*).

We used a GPS (eTrex 10, Garmin International, Inc) to determine the coordinates of each pair of pitfall traps. We determined the azimuth from each pair of traps to the center of the pool using ArcMap 10.2 (ERSI). The drift fence at most pools was roughly elliptical in shape, though the ratio of major to minor diameter varied (1.50, 2.09, and 1.88 for Pools 1, 2, and 4). The fence of pool 3 was roughly heart-shaped with the major axis 1.55 longer than the longest minor axis.

We divided the amphibians at the four pools by three species (or complex), two age classes, and two years, creating 48 samples. We plotted the distribution of the samples and noted those that appeared bimodal. We conducted tests of uniformity and homogeneity by year and age class on samples with ten or more individuals (Jenkins et al., 2006). We expected most comparisons of years to be similar and expected that orientation of adults may differ from that of juveniles.

Expert opinion

Preliminary analysis revealed several sets of field data that looked visually similar but were highly significantly different using chi-squared TOH and MRPP (with $p < 1E\text{-}12$). We questioned if these statistical differences were ecologically relevant and therefore we explored the opinions of experts who have published studies on amphibian orientation. We plotted samples in Oriana 4.0 (Kovach Computing Services) and used Qualtrics Survey Software (Qualtrics Labs Inc. 2015) to create an online questionnaire where we presented 12 pairs of circular histograms that were all statistically different and asked each expert if they deemed the differences between the graphs ecologically relevant by answering yes, no, or unsure. We included three pairs that we assessed as ecologically significant, seven pairs that we deemed similar, and two for which we were uncertain. Experts were told to assume that each graph represented movement of 200 individuals out of a wetland and into a homogeneous upland.

Results

Test of Uniformity Simulations

The tests of uniformity had varying performance when using simulated samples with various binning, sample sizes, and distributions (Table 6). The Rayleigh test was robust when binning individuals into both pitfalls at a circular fence and pitfalls at an elliptical fence, but had marginally high Type II errors (7.4% to 8.6%) at low sample sizes. The test was robust against the distortion caused by the ellipse, and performed well for orientation towards both the long and short end of the ellipse. This test even performed well for the bimodal distribution.

Rao's spacing test was unable to handle binned data and produced very high Type I errors (100% in many cases) when examining uniform distributions of pitfall data. Rao's spacing test also had high Type II error at small sample sizes and at medium sample sizes with low concentration. Doubling the angles in the ellipse to adjust for possible axial bimodal distortion did not improve the performance of Rao's spacing test.

Chi-squared GOF was robust against binning medium and large samples of data at a circular pitfall fence but had high Type I errors (10.8% to 54.5%) when the data were binned at an elliptical pitfall fence. When we altered the expected values so that our eight bins represented three pitfall traps rather than 45° or split up pitfalls individually into 24 bins, chi-squared still produced high Type I errors. Only when we adjusted the expected values so that they varied by pitfall trap according to the length of the arc funneled into each pitfall did chi-squared GOF produce close to 5% Type I error for medium and large samples at $\alpha = 0.05$. The expected values for chi-squared GOF at low sample size were below the minimum values to meet the test's assumptions, leading to high Type II error rates (above 25% for directional simulations) and causing R to print error messages warning that the chi-squared approximation may be incorrect.

Test of Homogeneity Simulations

Once researchers have determined that two or more samples are not uniform, they often compare the samples to determine if they have significantly different distributions using a test of homogeneity. When comparing directional distributions, the Kruskal-Wallace test had lower Type I (east versus east) and Type II (north versus east) error rates than the Watson-Williams test, MRPP, and chi-squared TOH (Table 7). None of the tests of homogeneity was influenced by binning, though concentration and sample size affected some tests. The Watson-Williams test had marginally high Type I error rates (6.2% to 9.9%) at low concentration (similar to those concentrations observed in the field data). This test printed a warning that concentration was below its assumed minimum even when our simulations had $\kappa = 2$. MRPP had marginally high Type I error rates (6.0 to 6.9%) at high sample size and very high Type II error rates (33.7 to 41.8%) at low sample size. Chi-squared TOH had high Type II error rates (13.6 to 34.5%) at low sample size, but almost all simulations violated the assumption of minimum expected values in bins located away from the mean. This test often failed to produce a test statistic for east versus east comparisons (up to 90.6% of comparisons depending on sample size and concentration), due to bins with expected values of zero (as division by zero is undefined) located away from the mode.

Table 6. Proportion of Type I and Type II errors (α = 0.05) resulting from simulations of uniform and directional distributions of departures from a wetland. The paths of animals are represented as continuous angles, pitfall traps in a circular drift fence (PF), and pitfall traps in an elliptical drift fence. For directional distributions, κ = 1. Note that chi-squared GOF with 30 individuals breaks the assumption of expected values of at least four per bin (here expected values are 30 / 8 = 3.75). Error rates over 6% are in bold.

	Rayleigh test			Rao's spacing test			Chi-squared GOF (8 bins)		
N	30	90	200	30	90	200	30	90	200
Uniform distribution - Type I error rate									
Continuous	0.048	0.046	0.042	0.038	0.048	0.047	0.047	0.057	0.054
Circular PF	0.053	0.044	0.043	**0.295**	**1.000**	**1.000**	0.057	0.050	0.052
Elliptical PF	0.048	0.046	0.047	**0.623**	**1.000**	**1.000**	**0.108**	**0.243**	**0.545**
North distribution - Type II error rates									
Continuous	**0.079**	0	0	**0.484**	**0.131**	0.012	**0.289**	0	0
Circular PF	**0.074**	0	0	**0.175**	0	0	**0.280**	0	0
Elliptical PF	**0.084**	0	0	0.056	0	0	**0.266**	0	0
East distribution - Type II error rates									
Continuous	**0.084**	0	0	**0.522**	**0.143**	0.008	**0.300**	0.002	0
Circular PF	**0.081**	0	0	**0.207**	0	0	**0.288**	0.003	0
Elliptical PF	**0.086**	0	0	**0.061**	0	0	**0.257**	0.002	0
Bimodal distribution - Type II error rates									
Continuous	0.005	0	0	0.031	0	0	0.010	0	0
Circular PF	0.008	0	0	0.002	0	0	0.019	0	0
Elliptical PF	0.012	0	0	0	0	0	0.006	0	0

Table 7. Comparisons of tests of homogeneity. Program R printed error messages due to low expected values in Chi-squared TOH and due to low κ or unequal κ for the Watson-Williams test. (See supplemental material for a more detailed table.)

	Chi-squared TOH	Watson-Williams test	MRPP	Kruskal-Wallace test
East vs East Type I error	Low error	High error at low κ	High error at high sample size with low κ	Low error
North vs East Type II error	High error at low n	Low error	High error at low n and low κ	Low error

Table 8. Counts of emigrating amphibians at four vernal pools in Penobscot County, Maine, USA.

	Wood Frogs				Spotted Salamanders				Blue-Spotted Complex			
	Juveniles		Adults		Juveniles		Adults		Juveniles		Adults	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Pool 1	428	233	42	90	51	104	14	6	109	125	13	12
Pool 2	2	1	117	76	5	23	50	37	0	53	16	17
Pool 3	212	5	85	81	226	164	238	301	190	126	122	135
Pool 4	6359	8939	102	374	118	499	94	89	36	65	66	74

Field data

We captured Wood Frogs, Spotted Salamanders, and Blue-Spotted Complex Salamanders emigrating from all four breeding pools in both 2013 and 2014 (Table 8). Of the 48 combinations of pool, age class, and year, 42 had ten or more individuals. We will refer to these 42 as "samples." The median number of individuals in a sample was 87. The maximum likelihood estimate of the concentration parameter, κ, ranged from 0.075 to 1.845, with a median of 0.768. We had no *a priori* reason to expect a unimodal distribution, and visually estimated that nine of these samples were bimodal.

The Rayleigh test led us to accept the null hypothesis more frequently than did Rao's spacing test and chi-squared GOF. The Rayleigh test initially suggested that eight of the samples were uniform, including two samples we visually determined to be bimodal. Doubling the angles in these two sets reduced the p-value to the extent that we then concluded both were directional. Rao's spacing test and chi-squared GOF suggested that none of the samples were uniform. Only 36 samples had chi-squared GOF expected values large enough to fulfill the test's assumptions.

Orientation tended not to differ by year, and there were no striking differences between the results of the tests of homology, though chi-squared TOH and the Kruskal-Wallace test were more likely to accept the null hypothesis than the Watson-Williams test. Of 24 possible pairs of samples, 19 had over ten individuals caught in both years. Using MRPP, we found five pairs of samples to differ by year. When we tried to use this method to compare juvenile wood frogs at Pond 4 where sample sizes were above 6,000 using a laptop computer (Intel Duo Dual-Core CPU at 1.8 GHz and 3GB of RAM, Intel Corporation) the program produced no results after 24 hours. We then ran the code on a specialized system (AMD Vishera 8-Core CPU at 4.0 GHz and 16 GB of RAM, Advanced Micro Devices, Inc) and together with the comparisons of age classes the codes took approximately seven hours to run. The Kruskal-Wallace test suggested a different four pairs were different by year. The Watson-Williams test suggested we reject the null of homology for seven pairs of samples. When we did not drop bins with low expected values, chi-squared TOH indicated that four pairs differed by year, but when we dropped bins with low expected values and reduced our degrees of freedom (which occurred for six pairs) the test suggested we reject the null for seven comparisons.

Age classes were more variable in orientation, with Kruskal-Wallace most frequently accepting the null. Of the 24 pairs of samples, 18 pairs had more than 10 individuals for both ages. We rejected the null of homogeny for nine pairs using MRPP. We again had issues using this test on extremely large samples on our laptop. The Watson-Williams test suggested 13 pairs differed. The Kruskal-Wallace test lead use to conclude only four pairs differed by ages class. Chi-squared TOH found 13 pairs to differ when we did not drop bins with low expected values and was unable to calculate p-values on four comparisons (due to division by zero). When we dropped bins with low expected values, 14 comparisons differed.

Expert opinion

Seven amphibian ecology experts provided their opinions and they agreed that not all of the plots represented ecologically significant differences although they were all statistically significantly. The experts considered a minimum of five of the 12 comparisons to be not meaningfully different. Collectively they considered 17% ± 2.5% (s.d.) pairs of graphs to be ecologically different, 66.7% ± 2.8% pairs to be ecologically similar, and were unsure about 15.5% ± 1.2%.

Discussion

One of the first decisions to make when conducting analyses of pitfall data is which test can best address the data. We found that binning, shape, sample size, and concentration of simulated data had mixed effects on the efficacy of the statistical tests. We acknowledge that none of our tests was perfect, but clearly some were better than others. We make suggestions that favor tests with low error rates (even if they have violated assumptions) over tests with high error rates (but whose assumptions may have been better met).

We found the Rayleigh test to be most suitable for testing the null hypothesis that animals caught in a drift fence orient uniformly. The Rayleigh test is considered the most powerful test to determine if a distribution is unimodal (Pewsey et al., 2013) and is widely used in amphibian orientation analysis (e.g. Walston and Mullin, 2008; Roznik and Johnson, 2009; De Lisle and Grayson, 2011). The Rayleigh test had the lowest error rates for binned, elliptical data in our simulations. We were surprised that error rates were low for our bimodal distributions, though this test did require doubling of angles for bimodal field data. We caution that researchers should still be wary of using the Rayleigh test for bimodal data, as Bergin (1991) observed high errors in simulated data and De Lisle and Grayson (2011) reported a bimodal distribution that the Rayleigh test had suggested was uniform.

We caution against the use of Rao's spacing test on pitfall data, as the test produced high errors in our simulations and failed to indicate that any of our field samples were uniformly distributed. Pewsey et al. (2013) warned this test is inappropriate for binned data because it is based on distance between points, but amphibian researchers have paired this test with MRPP (Dodd and Cade, 1998; Marty et al., 2005; Santos et al., 2010) which also uses distances but was designed to handle binning (Talbert et al., 2013).

We do not recommend the use of chi-squared GOF for small samples or when the drift fence is not strictly circular. Chi-squared GOF does not take the order of bins into account, resulting in lower power (Pewsey et al., 2013). Jenkins (2006) addressed problems with low expected values by reducing the number of bins, which we speculate may also reduce power due to loss of precision. We recommend that researchers who need an alternative to the Rayleigh test with elliptical or irregularly shaped fences should adjust expected values to accommodate the variation in the angles represented by each pitfall.

For comparing samples, we recommend the Kruskal-Wallace test, although it does not recognize the circular scale and uses ranks (Zar, 2010). This test had the lowest error rates in our simulations, and most frequently led us to accept the null hypothesis for our field data. Researchers should avoid using the Watson-Williams test on pitfall data as the test requires observations to be more concentrated than may be reasonable for field data, and resulted in high error rates at concentrations similar to what we observed in the field. Patrick et al. (2007) used this method with concentrations close to or higher than we observed, and reported that orientation at one out of five pools differed statistically but not ecologically. We advise against using chi-squared TOH, as even with high sample sizes we had problems with insufficient expected values needed for this test. Although bins with low or zero expected values can be dropped or combined into larger bins, as done in the past by amphibian researchers (Jenkins et al., 2006; De Lisle and Grayson, 2011), doing so reduces the information available for interpretation (i.e., if animals are avoiding a section of the fence, we would be hesitant to remove that information). We recommend against using MRPP for small samples due to high observed error rates and because extremely large samples are unwieldy from a computational perspective.

Our experts agreed that not all comparisons that are deemed significantly different by tests of homogeneity may be ecologically relevant. The precision we were able to achieve in measuring directionality may be higher than what is biologically meaningful (Shoop, 1968). Both Jenkins (2006) and Patrick (2007) have reported significant differences that they deemed were not ecologically different using chi-squared TOH and the Watson-Williams test, respectively. We therefore advise that when researchers select a test they should consider how likely the test is to reject the null hypothesis (i.e. how conservative and powerful it is), and how this test might fit into their study design. Based on our expert opinions and our desire to find only differences that are ecologically meaningful, we recommend tests that are less likely to reject the null.

Statistics are a tool that we use to objectively model and infer the behavior of animals. Researchers must be sure they use an adequate tool that fits the data and scenario at hand, and should visually inspect their data and assure that the statistics corroborate common sense as to what is likely to be ecologically relevant. Plots can be produced easily in R and Oriana, and statisticians agree that visualization of data before conducting tests provides valuable insight (Mardia and Jupp, 2000; Pewsey et al., 2013). Field data are open to interpretation, with no clear right or wrong answers about their uniformity or similarity between samples, but choosing the most appropriate statistical test can help researchers work through the shades of grey.

CHAPTER 3

BREEDING ECOLOGY OF THE BLUE-SPOTTED SALAMANDER COMPLEX

Chapter Abstract

Knowledge of life history and demographics of animals that breed in vernal pools can inform management of these wetlands, yet little is known about the ecology of the Blue-Spotted Salamander Complex (*Ambystoma laterale* and *A. laterale - sp.).* We used drift fence arrays and radio telemetry to examine demographics and orientation of Blue-Spotted Salamanders and Unisexual Salamanders (differentiated with microsatellites) and compared the resulting metrics to two better-known species (Spotted Salamanders, *A. maculatum*, and Wood Frogs, *Lithobates sylvaticus*) at four vernal pools in central Maine. The ratio of dispersing juveniles per female of the Blue-Spotted Salamander Complex was not significantly different from that of other species, though sex ratio and genomotype ratio suggested 92% of our populations were triploid females (LLJ) and only 1% were diploid males (LL males). We are uncertain how Blue-Spotted Salamander Complex populations maintain recruitment with so few males. Orientation of Blue-Spotted Complex Salamanders exiting the pool was generally similar to other taxa for both age classes. The distribution of adult radio-implanted Blue-Spotted Complex Salamanders after emigration (up to 463 m from the pools) did not significantly differ from their orientation at the drift fence (at 2 m). Our work suggests managers who conserve terrestrial habitat near the pool for other species may also be aiding in the movements of sympatric Blue-Spotted Complex Salamanders, and pools with few males may still support viable populations of the complex.

Introduction

Vernal pools and other ephemeral wetlands are of conservation concern partly because some species of amphibians and invertebrates breed nearly exclusively in these sites and they are increasingly degraded or lost to land use changes (Semlitsch and Skelly, 2008; Calhoun et al., in press.). Managers typically identify vernal pools through specific indicator species, such as Fairy Shrimp (*Eubranchipus sp*.), Wood Frogs (*Lithobates sylvaticus*), and ambystomatid salamanders, and need information on the ecology of pool breeding fauna to better manage nearby terrestrial areas. For example, forest managers and urban developers can minimize their impact on vernal pool amphibians by maintaining a life zone of intact forest surrounding the pool within a specific radius or polygon based on the species migration distances and habitat selection (Semlitsch, 1998; Baldwin et al., 2006; Rittenhouse and Semlitsch, 2007). Knowledge of fine-scale movements, dispersal, and patch permeability allows a manager to maintain landscape connectivity (Rittenhouse and Semlitsch, 2006; Gamble et al., 2007; Pittman and Semlitsch, 2013; Osbourn et al., 2014; Cline and Hunter, 2014). Management is context dependent, such that species vary in their needs and may not behave consistently across a geographic gradient (Hocking et al., 2008; Baldwin et al., 2006; Rittenhouse and Semlitsch, 2007; Groff et al., in press).

Best management practices should be based on best available science and address diverse stakeholder needs (Calhoun and Klemens, 2002; Calhoun and deMaynadier, 2004; Levesque et al., 2017), but scientific knowledge may be incomplete and evolve constantly. For example, our knowledge of Blue-Spotted Salamander (*Ambystoma laterale*) habitat preferences is limited because of their rarity and is complicated by the presence of Unisexual Salamanders (*A. laterale - sp.*), an almost entirely female lineage of hybrid origin that is visually similar to Blue-Spotted Salamanders. Ecological studies often do not differentiate between Blue-Spotted and Unisexual Salamanders (Brodman and Jaskula, 2002; Regosin et al., 2005; Homan et al., 2007; Belasen et al., 2013; Windmiller et al., 2008), or may combine all ambystomatids so that inferences cannot be made specifically about these taxa (deMaynadier and Hunter, 1998; but see Lowcock, 1994). The clear majority of studies on this complex have focused on phylogeny, reproductive mechanisms, or geographic distribution (i.e. Ramsden, 2008; Bogart et al., 2009; and Charney, 2013) rather than migration, recruitment, or habitat relations. Conservation of Unisexual Salamanders and Blue-Spotted Salamanders is hindered by lack of knowledge.

Unisexual Salamanders are a monophyletic lineage which is usually polyploid and, in New England, contain the genomes of multiple species, and reproduce in New England using the sperm of Blue-Spotted Salamanders and Jefferson Salamanders (*A. jeffersonianum*, Uzzell Jr. 1964; Bogart and Klemens, 1997; Bogart and Klemens, 2008). The sperm is usually not incorporated into the offspring making them clones of the female parent (Bogart et al., 1989; Lowcock, 1994; Ramsden, 2008). These salamanders and other sperm-hosts are collectively referred to as the Blue-Spotted Salamander Complex (hereafter, the BSS Complex), and genetic testing is needed to discriminate between Unisexual Salamanders and the other taxa (Downs, 1978). We use the tradition of abbreviating the genetic composition (genomotype) of individuals by how many replicates of the genome of each species they contain (e.g.,LL for *A. laerale*, LLJ fo *A. (2) laterale - jeffersonianum*, and LLLJ for *A. (3) laterale - jeffersonianum*, Lowcock et al. 1987).

Breeding populations of the BSS Complex are usually dominated by Unisexual Salamanders with little genetic variation and the ratio of sperm-hosts to Unisexual Salamanders has long been speculated to affect the demographics of both (Clanton, 1932; Bogart and Klemens, 1997; Homan et al., 2007). Ploidy ratios vary geographically with higher proportions of Blue-Spotted Salamanders near the edge of the BSS Complex's range and populations of Blue-Spotted Salamanders without Unisexual Salamander being rare (Lowcock et al., 1991; Bogart and Klemens, 1997; Nöel et al., 2011; Charney et al., 2013). Relatively few studies have examined large enough samples to precisely determine the relative abundance of Blue-Spotted to Unisexual

Salamanders (i.e., genomotype ratio) and factors that contribute to the breeding ecology such as recruitment and orientation during migration into and out of breeding pools (but see Bogart and Klemens, 1997; Homan et al., 2007). Males are a limiting resource in the BSS Complex (Lowcock et al., 1991) and low viability of Unisexual Salamander eggs seems to be a product of competition for sperm and high mortality during embryonic development (Licht, 1989). Homan (2007) saw a decline in the salamanders in the BSS Complex during a five-year study and hypothesized that it was caused by loss of males and the resulting changes to sex-ratio.

While the BSS Complex uses different characteristics to select pools at the landscape and within-pool scales than the more-studied Spotted Salamanders (*Ambystoma maculatum*; chapter 1), similarities in breeding ecology may help inform management where the amphibians are sympatric. Our objective was to quantify the breeding ecology of the BSS Complex and compare their ecology to other pool-breeding species. Specifically, we examined genomotype ratios (for the Blue-Spotted Salamander Complex), sex ratios, an index of recruitment, and initial orientation. We expected ploidy ratios for the BSS Complex to be biased towards triploid Unisexual Salamanders (Bogart and Klemens, 1997; Nöel et al., 2011). We predicted that sex ratios would be male biased for bisexual species and that initial recruitment of Spotted Salamanders and Wood Frogs would be higher than that of the BSS Complex (Phillips and Sexton, 1989; Berven, 1990; Berven and Grudzienogical, 1990; Homan et al., 2007). We expected the BSS Complex to differ in orientation from Wood Frogs, but not necessarily from Spotted Salamanders (Regosin et al., 2005).

Materials and Methods

Study sites

We selected four vernal pools known to host large breeding populations of the BSS Complex, Spotted Salamanders, and Wood Frogs, in forests dominated by White Pine (*Pinus strobus*), Balsam Fir (*Abies balsamea*), Red maple (*Acer rubrum*), Red Oak (*Quercus rubra*), and Red Pine (*Pinus resinosa*). Pools 1 and 2 were on a relatively undisturbed parcel managed for forestry and abutting a large river (210 m wide, 73 and 81 m from the pools) in Old Town, Maine, USA. Both pools were ephemeral and dried in August or September. Pools 3 and 4 were near rural development (102 m and 121 m to impervious surfaces) in Orono, Maine, USA, and did not dry completely during the study.

Drift fence

We installed two concentric drift fences surrounding three of the pools and one fence at the fourth. The outer fences were about ten meters from the high-water mark to avoid springtime flooding of pitfalls during adult amphibian immigration. The inner fences were within two meters of the high-water marks to increase capture rates of dispersing juvenile amphibians. The fence at Pool 3 varied from two to ten meters from the high-water mark to avoid crossing a hiking path that abutted the pool (within two meters). Due to the varying radius of this fence and frequent trail use, we did not construct a second fence at this pool.

The drift fences were constructed from silt fencing buried 20 cm into the ground with aluminum cans (#10) as pitfalls (Shoop, 1965). Each pitfall trap contained a moist sponge, a funnel, and a stick to allow escape of small mammals (Perkins and Hunter, 2002). Pairs of pitfalls were spaced at every five meters along the fence, with one can each on both the inside and outside of the fence. We used a GPS (GPSmap 62stc, Garmin International, Inc) and ArcMap

10.2 (ERSI) to determine the azimuths from the pool centers to the traps (Walston and Mullin, 2008). We checked the fence daily in 2013 and 2014 from April to September, and every two days in October and November. We filled and covered pitfalls and laid down sections of fence each winter (Regosin et al., 2005).

We determined the sex of adult amphibians using secondary sexual characteristics and recorded counts of amphibians by pitfall and date. In 2013 we tail clipped and PIT tagged emigrating adult BSS Complex salamanders. In 2014 we PIT scanned all BSS Complex adults (both immigrating and emigrating) and tail clipped new captures. We did not PIT tag animals in 2014. We tail clipped one of every five BSS Complex juveniles captured at the inner fences in both years. PIT tagging occurred in the field without anesthetic by injecting the PIT tag (HPT12, 134.2kHz ISO FDXB tag; Biomark) into the body cavity just anterior to the left hind leg using a modified syringe (Perret and Joly, 2002; Cucherousset et al., 2008). We remove a 0.5 by 0.3 cm tail tissue sample (Nöel et al., 2011), which was analyzed using microsatellite DNA at 6 loci (AjeD75, AjeD94, AjeD283, AjeD346, AjeD378, and AjeD422; Julian et al., 2003) at the University of Guelph to determine genomotype. Salamanders with the same multi-locus genotype (MLG) for AjeD75, AjeD94, and AjeD283 were considered members of the same clone. Our microsatellite DNA methods are described in detail elsewhere (Bogart et al., 2007; Bogart et al., 2009).

Telemetry

We implanted radio transmitters into 44 Unisexual Salamanders to track their post-breeding movements. We selected large animals (>7 g) captured at the drift fence (which we refer to as candidates) and brought them to the lab where we selected the heaviest for surgery (which we refer to as tracked). We used tricaine methane sulfonate (MS-222) to anesthetize salamanders, and surgically inserted ATS A2415 transmitters (0.33 g, Advanced Telemetry Systems) into their body cavities (Faccio, 2003; Ryan and Calhoun, 2014). We re-located salamanders daily in 2013 and every three days in 2014. Our surgical and tracking methods are described in detail elsewhere (chapter 4). We used ArcGIS to measure the azimuth from the center of each salamander's breeding pool to its last known location (Roznik et al., 2009).

Demographics

Our counts of immigrating and emigrating adult females differed, presumably due to trespass (e.g. climbing over the fence) and within-pool mortality, so we used the higher of these counts for each species per pool. We used the ratio of emigrating juveniles per adult female as an index of initial recruitment so that we could directly compare our results to those of Homan et al. (2007). We compared the recruitment index of Wood Frogs and Spotted Salamanders to BSS Complex Salamanders using a Kruskal-Wallace test with years pooled. We estimated sex ratios by dividing counts of females by counts of males at the four pools for each year. We compared counts of genomotypes of tail-clipped adult and juvenile BSS Complex Salamanders across vernal pools using chi-squared goodness of fit.

Orientation

We compared the orientation of the BSS Complex to that of Wood Frogs and Spotted Salamanders as determined through the pitfall arrays. We also compared the distribution of azimuths from the pool to the last known location of telemetered Unisexual Salamanders to the orientation of these salamanders at the drift fence (i.e. overall orientation versus initial orientation). We plotted each sample with more than 10 individuals to inspect for multimodality

(Pewsey et al. 2013), conducted Kruskal-Wallace tests, and used the Bonferroni correction to adjust our criteria for significance due to the large number of tests of each null hypothesis.

We tested for uniformity using the Rayleigh test in the "circular" package for program R, with correction for any axial bimodality (Batschelet, 1981; Pewsey et al., 2013). This test had lower error rates than alternative tests in simulations of data from irregularly shaped drift fences and low sample sizes (Hoffmann et al, in prep.). We used counts at the inner fence for juveniles but combined fences for adults, as we did not release adults between fences so these were not recaptures.

We used the radio telemetry data to compare the orientation of adults at the drift fence to their post-migration movements from pools where at least 10 animals were tracked (Pools 3 and 4). As these comparisons were not part of our original objectives, we did not record which individual was captured in each pitfall, so we used a Kruskal-Wallace test and visually inspected the distributions.

Results

Demographics

Triploid Unisexual Salamanders dominated the BSS Complex populations at our sites. Of the 652 salamanders that were genomotyped, 92.18% were LLJ, 3.68% were LLLJ, and only 4.14% were Blue-Spotted Salamanders (LL; Table 9). Proportions of each genotype did not vary by age (χ^2=0.865, df = 2, P = 0.649). Most (86%) Unisexual Salamanders were clones of other salamanders in our sample, with only 96 unique individuals of 669 salamanders. Three clones were particularly common (n = 182, 140, and 58 salamanders). We found the most common clone at all four pools and the second and third most common clone at three sites. Other clones contained between 2 and 21 salamanders, and we observed these clones at either three (n = 5 clones) or two (n = 13 clones) sites.

Blue-Spotted Salamander Complex females greatly outnumbered males (78:1 with pools combined). Our genomotyped samples of the BSS Complex Salamanders included few Blue-Spotted Salamanders (LL), and they had sex ratios of 4:1 females to males in Pool 1, 6:1 in Pool 2, 1:1 in Pool 3, and 2:1 in Pool 4 but we caution that these are very small samples. In contrast, Wood Frog and Spotted Salamander breeding populations tended to be dominated by males (0.7:1 and 0.8:1 females per males with pools combined, respectively) (Table 10).

Blue-Spotted Salamander Complex indexes of recruitment were comparable to other taxa (Table 10). Although Wood Frog recruitment was higher than other taxa at three pools and Spotted Salamander recruitment was higher than the BSS Complex at three pools, none of these differences was significant (H = 1.192, df = 2, $P = 0.551$).

Orientation

Orientation of both life stages of all amphibians was non-random ($P > 0.0125$) at all pools with three exceptions in which distributions had low or many modes (see legend in Figure 8). Both juvenile and adult orientation tended not to differ between 2013 and 2014 with three exceptions.

Orientation of BSS Complex Salamanders was similar to that of Spotted Salamanders; adult Spotted Salamanders at Pool 3 were the exception (H = 8.8048, $P = 0.003$, Figure 8). Adult Wood Frog orientation differed from the BSS Complex at two ponds (Pool 3, H = 52.9531, $P << 0.05$ and Pool 4, H = 8.2956, $P = 0.004$), though the modes at Pool 4 were in the same general direction. Orientation by juvenile BSS Complex salamanders visually differed from juvenile Spotted Salamander orientation at Pools 2 and 3, but these difference were not significant ($P =$

0.039 at Pool 2 and $P = 0.051$ at Pool 3 with $P_{critical} = 0.0125$). Juvenile BSS Complex salamanders orientation and Wood Frog orientation differed significantly at Pool 3 ($H = 39.2411$, $P << 0.0042$) but not at Pool 4 or Pool 1 ($P \geq 0.1164$). Sample size was too low for comparison at Pool 2.

At Pools 3 and 4 we were able to compare the distribution of salamanders at the drift fence to the distribution of the final locations of tracked animals (n = 17 animals at the fence and 12 tracked animals from Pool 3, n = 21 animals at the fence and 16 tracked animals from Pool 4). All animals in these samples were LLJ. Neither of these comparisons were statistically significant ($P > 0.60$, Figure 9).

Table 9. Counts of genomotypes of Blue-Spotted Complex Salamanders at four vernal pools in Maine, USA. We sampled all emigrating adult salamanders in 2013 and unmarked immigrating and emigrating adults in 2014. We tail clipped one out of every five dispersing juveniles in both years. Samples were identified to genomotype using microsatellites.

Pool	**Age**	**Year**	**LL**	**LLJ**	**LLLJ**
Pool 1	Adult	2013	2	56	1
		2014	3	80	3
	Juvenile	2013	0	5	0
		2014	0	21	1
Pool 2	Adult	2013	5	15	0
		2014	2	40	2
	Juvenile	2013	0	25	2
		2014	5	40	1
Pool 3	Adult	2013	1	28	2
		2014	1	46	1
	Juvenile	2013	0	0	0
		2014	3	21	1
Pool 4	Adult	2013	1	65	1
		2014	2	125	8
	Juvenile	2013	1	32	1
		2014	1	42	0

Table 10. Counts of amphibians and associated demographics at four vernal pools in Maine, USA. Adult numbers are based on the higher count of emigrating or immigrating individuals and juvenile counts are based on emigrating individuals. We use the number of juveniles per adult female as an index of recruitment. Note that this table includes all Blue-Spotted Complex Salamanders, including those that were not genomotyped and so do not appear in Table 9.

Spotted Salamanders

Pool	Year	Female Adults	Male Adults	Juveniles	Sex Ratio F/M	Index of Recruitment
Pool 1	2013	22	19	31	1.2	1.4
	2014	13	12	104	1.1	8.0
Pool2	2013	47	61	5	0.8	0.1
	2014	36	33	23	1.1	0.6
Pool 3	2013	106	161	202	0.7	1.9
	2014	117	184	164	0.6	1.4
Pool 4	2013	90	136	110	0.7	1.2
	2014	90	88	499	1.0	5.5

Table 10, continued

Wood Frogs

Pool	Year	Female Adults	Male Adults	Juveniles	Sex Ratio F/M	Index of Recruitment
Pool 1	2013	57	60	409	0.9	7.2
	2014	75	84	233	0.9	3.1
Pool 2	2013	105	103	2	1.0	0.0
	2014	55	68	1	0.8	0.0
Pool 3	2013	70	139	208	0.5	3.0
	2014	42	71	5	0.6	0.1
Pool 4	2013	81	258	6166	0.3	76.1
	2014	211	263	8940	0.8	42.4

Blue-Spotted Salamander Complex

Pool	Year	Female Adults	Male Adults	Juveniles	Sex Ratio F/M	Index of Recruitment	(LLJ + LLLJ)/LL
Pool 1	2013	26	1	95	26.0	3.7	3.0
	2014	34	1	125	34.0	3.7	21.0
Pool 2	2013	33	2	0	16.5	0	30.0
	2014	39	1	53	39.0	1.4	47.0
Pool 3	2013	123	1	188	123.0	1.5	66.0
	2014	135	1	126	135.0	0.9	66.5
Pool 4	2013	225	2	36	112.5	0.2	28.5
	2014	143	0	65	---	0.5	27.7

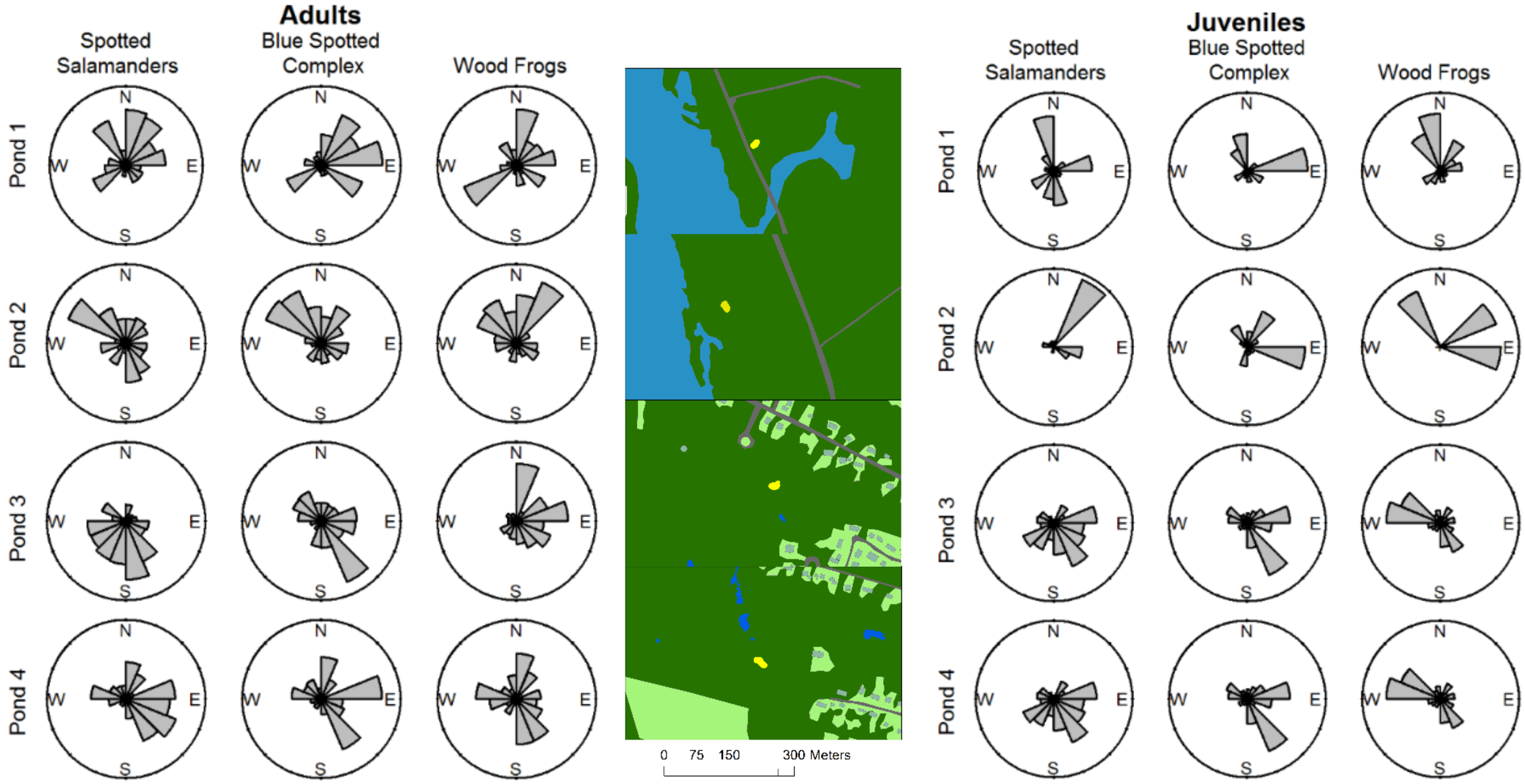

Figure 8. Orientation of Spotted Salamanders, Blue-Spotted Complex Salamanders, and Wood Frogs at four vernal pools in Maine, USA, using drift fence arrays in 2013 and 2014. Rose diagrams show directionality of animals emigrating from the ponds with years and sex pooled. The length of each bar indicates the proportion of amphibians with bearings within the bin. Cover types include forest (dark green), lawns and fields (light green), roads (grey), wetlands and river (blue), and vernal pool of interest (yellow, and enlarged). All distributions were non-random except for juvenile Spotted Salamanders at Pool 1, adult Spotted Salamanders at Pool 2, and adult Wood Frogs at Pool 4. Only adult Spotted Salamanders at Pool 4, juvenile Wood Frogs at Pool 4, and juveniles of the BSS Complex at Pool 3 differed significantly between 2013 and 2014

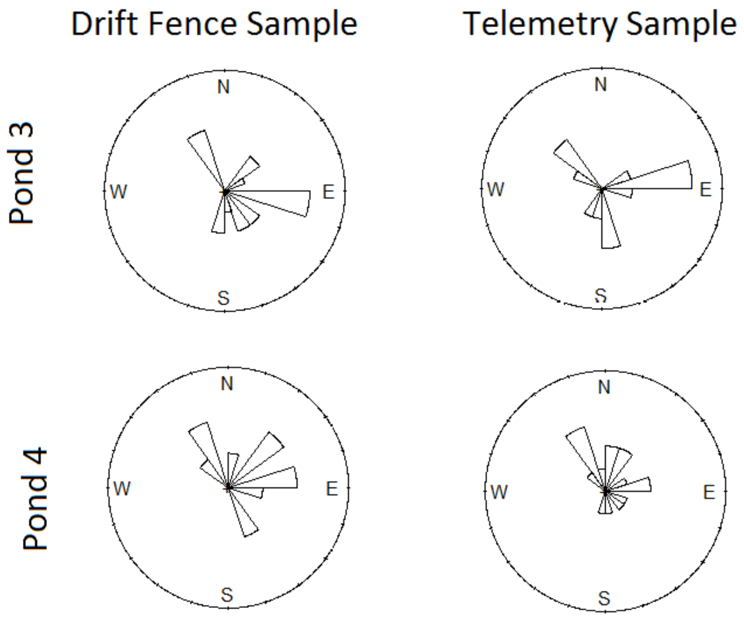

Figure 9. Comparison of the orientation of Unisexual Salamanders chosen as candidates for radio implant surgery to the final location of radio tracked animals at two pools in Maine, USA. Length of wedges represents the proportion of animals within each bin.

Discussion

We found that although Unisexual Salamanders have very different breeding strategies and sex ratios compared to Spotted Salamanders and Wood Frogs, they had similar indexes of recruitment and orientation patterns. These similarities suggest current management (based on better-studied species) may incidentally benefit the BSS Complex.

Our sample of BSS Complex salamanders is female dominated as a result of the high abundance of Unisexual Salamanders. Most Unisexual Salamanders at our vernal pools were triploids and clones. Our pools are mostly iced over during the breeding season so this result fits the hypothesis that sperm is less frequently incorporated into the offspring at cooler temperatures

(Bogart et al., 1989). Our sex ratios and ratio of polyploids to diploids ranged greatly. Our Pools 3 and 4 were somewhat more female biased than published studies, with reported ratios in the literature as high as 77 females per male and 93% Unisexual Salamanders (Clanton, 1932; Lowcock et al., 1991; Lowcock, 1994; Homan et al., 2007; Nöel et al., 2011)

Although Blue-Spotted Salamanders (LL) were rare, initial recruitment of the BSS Complex was not significantly lower than that of other amphibians. Our results contrast with that of Homan et al. (2007) who observed that Spotted Salamanders had about six times higher recruitment than the BSS Complex in a vernal pool over a five-year study. Our extremely female-biased sex ratio raises questions as to how so many juveniles were produced with so few males. It has long been hypothesized that as Unisexual Salamander populations outgrow that of their sperm donors they will eventually decline because low availability of sperm would limit reproduction, and then lower Unisexual Salamander abundance would allow the parent species' population to recover (Uzzell, 1969). Unisexuals have about half the fecundity of similar sized LL females and male Blue- Spotted Salamanders deposit fewer spermatophores for LLJ than for LL female (Uzzell, 1969). The number of females with which a male can mate is limited as, according to Uzzell (1969), each male Blue-Spotted Salamanders is capable of producing an average of 35 spermatophores and a minimum of 11 spermatophores are needed for successful fertilization of an LLJ egg mass. Clanton (1932) observed lower numbers, with a maximum of eight spermatophores per males. Therefore, as LL are rare in our populations, we should expect low rates of juveniles per BBS Complex females. It is possible that males trespass drift fences more readily than females (DeLisle and Grayson, 2011). Nöel et al. (2011) also questioned how Unisexual Salamanders may reproduce with low numbers of males (and potential absence of males) at sites where only diploid Unisexual Salamanders (LJ) were observed. Our findings suggest the BSS Complex has overcome these barriers to reproduction and continue to be productive with few sperm donors, but further work will be needed to determine how.

Our Wood Frog and Spotted Salamander sex ratios tended to be male biased and similar to those observed elsewhere (Howard, 1980; Berven and Grudzienogical, 1990; Hocking et al., 2008). The expected male bias in sex ratio has been attributed by authors to differences in age at maturity and frequency of breeding (Howard, 1980; Stenhouse, 1985; Phillips and Sexton, 1989). Male Wood Frogs, Blue-Spotted Salamanders, and Spotted Salamanders enter the breeding population a year before females (Homan et al., 2007). Roughly twice as many males from each clutch of Wood Frogs survives to breeding age due to the extra year of pre-breeding mortality for females (Berven, 1990). Our Blue-Spotted Salamander (LL) sex ratio was surprisingly female biased even though this species is bisexual. We speculate the ratios may be driven by environmental variables and competition with Unisexual Salamanders, or are simply an artifact of low sample size.

Both age classes of amphibians showed non-random directionality even though all ponds were surrounded by forest (Regosin et al., 2005; Jenkins et al., 2006; Timm et al., 2007; Walston and Mullin, 2008). The distributions of orientation at two drift fences were comparable to the distributions of last-known locations of telemetered adult Unisexual Salamanders, suggesting that drift fence data may provide some evidence of basic terrestrial habitat relations (Roznik and Johnson, 2009). Orientation of adult BSS Complex salamanders was similar to that of Spotted Salamanders and Wood Frogs in most pools corroborating the results of Regosin et al. (2005). Orientation is usually context dependent, and may change with habitat preferences, barriers, and microtopography (Jenkins et al., 2006; Roznik, et al. 2009). It is possible the river (Pools 1 and 2) and anthropogenic disturbance (Pools 3 and 4) affected orientation of all taxa similarly, but our plots do not provide strong evidence suggesting avoidance of these areas. The overlap of the BSS Complex orientation with that of both Wood Frogs and Spotted Salamanders is surprising, given their differences in life history. Wood Frogs may seek aquatic resources (such as seeps, streams, or forested wetlands) or upland forest while fossorial Spotted Salamanders prefer better drained soils with small abundant mammal burrows (Faccio, 2003; Baldwin et al., 2006; Rittenhouse and Semlitsch, 2007; Groff et al., in review). Our previous work (Hoffmann et al., in prep.) suggests that Unisexual Salamanders use breeding wetlands in landscapes with hydric soils while Blue-Spotted and Unisexuals Salamanders have been observed in both upland forest and forested wetlands (Regosin, et al. 2005; Belasen et al., 2013; Ryan and Calhoun, 2014). We suggest the movements and terrestrial habitat of the BSS Complex in New England might be similar to or intermediate of the movements and different habitats selected by Wood Frogs and Spotted Salamanders and therefore the BSS Complex may benefit from conservation efforts directed at these more-studied amphibians, but further work in different regions is needed.

Our work suggests that in some contexts managers who conserve forests adjacent to vernal pools for other pool-breeding species will likely be supporting movements of the sympatric BSS Complex. Further work with telemetry or with drift fences at pools in areas characterized by more heterogenous forests and without river barriers or in other geographic regions would strengthen this hypothesis. Our work also suggests that where few male Blue-Spotted Salamanders occur, populations of the Blue-Spotted Salamander Complex may still be viable. The overwhelmingly biased genomotype ratio and sex ratio of the BSS Complex in our pools raises questions about how these salamanders persist and what ecological mechanism drive these ratios, and we suggest that further work should address these questions.

CHAPTER 4

DO THE POST-BREEDING MIGRATION AND HABITAT USE OF UNISEXUAL SALAMNDERS DIFFER FROM THAT OF THEIR PARENT SPECIES?

Chapter Abstract

The behavioral phenotypes of hybrids are known to vary in similarity to their parent species. Unisexual Salamanders (*Ambystoma laterale – sp.*), which are the results of ancient hybridization, contain nuclear DNA of multiple parent species some of which they rely on as sperm-hosts, but the habitat preferences of these sperm-hosts differ from each other. We radio-tracked Unisexual Salamanders from four vernal pools to quantify their migration distances and post-breeding habitat selection and compared these to published accounts for Blue-Spotted Salamanders (*A. laterale*) and Jefferson Salamanders (*A. jeffersonianum*). Unisexual Salamanders migrated distances within the range reported for these sperm-hosts. We modeled microhabitat selection and found Unisexual Salamanders used sites with higher numbers of small mammal burrows, lower substrate temperatures, and lower cover by forest floor vegetation (up to 1 m above ground) than random sites, similar to the sperm-hosts. While 90% of Unisexual Salamanders remained in the forest matrix, we observed others under outbuildings or near forest-lawn edges and roads. We emphasize that migration distances are context specific, and we caution resource managers to be careful when designating management zones.

Introduction

The behavioral phenotypes of hybrids can vary from similar to a parent species to profoundly different due to the new combinations of alleles causing potentially wide-cross heterotic effects (Doherty and Gerhardt, 1983; Smith and Riechert, 1984; Page et al., 2001; Panov and Pavlova, 2010). These genetic variations may result in differential habitat selection (Jaenike and Holt, 1991). For example, some hybrids are less selective than either parent species, easing their fit into hybrid zones between allopatric parent populations, while others may remain sympatric and compete with, or even replace, parent species (Jaenike and Holt, 1991; Saino, 1992; Wood et al., 2016).

All unisexual teleost fish, unisexual amphibians, and many unisexual lizards are the result of past hybridizations between two or more bisexual species (here defined as species with separate males and females; Neaves and Baumann, 2011). Unisexual taxa are almost entirely female and either reproduce wihout sperm or with sperm contributed by males of bisexual species (Dawley, 1989). Unisexuals may use a wide variety of niches due to their hybrid origins, allowing some to thrive in different habitats, persist in changing environments, and reduce competition with parent species (Bullini, 1994; Mee and Rowe, 2010; Tarkhnishvili et al., 2010; Greenwald et al., 2016).

The Blue-Spotted Salamander Complex is the result of a 5-million-year-old hybridization event creating a lineage of modern salamanders carrying combinations of the genomes of Blue-Spotted Salamanders (*Ambystoma laterale*), Jefferson Salamanders (*A. jeffersonianum*), Tiger Salamanders (*A. tigrinum*), Small-Mouthed Salamanders (*A. texanum*), and, rarely, Streamside Salamanders (*A. barbouri;* Uzzell, 1964; Morris and Brandon, 1984; Kraus and Miyamoto, 1990; Spolsky et al., 1992; Bogart et al., 2009; Bi and Bogart, 2010). Unisexual Salamanders have nuclear DNA from two or more of these species and are almost always polyploid (Lowcock and Murphy, 1991; Bogart and Klemens, 1997, 2008). We use the tradition of abbreviating the genetic composition (genomotype) of individuals by how many replicates of the genome of each species they contain (e.g., LL for *A. laterale*, LLJ for *A. (2) laterale - jeffersonianum*, and LLLJ for *A. (3) laterale - jeffersonianum,* Lowcock et al. 1987). Because they usually lack males but are not capable of parthenogenesis, Unisexual Salamanders require the sperm of their bisexual parent species to stimulate egg development (Petranka, 1998; Bogart et al., 2009). They are outwardly similar in their appearance to their sperm-hosts and genetic methods are often needed for identification. Hereafter, we refer collectively to Unisexual Salamanders and their sperm-hosts as “the BSS Complex.”

Salamanders in the BSS Complex are known to congregate in seasonal wetlands to breed and travel post-breeding to terrestrial forest where they occupy burrows as "sit and wait" predators (Petranka, 1998). However, other aspects of habitat use may vary among the taxa within the BSS Complex. For example, Jefferson Salamanders and Blue-Spotted Salamanders partition habitat by altitude, with the former typically in well-drained uplands and the latter in lowlands (Nyman et al., 1988; Downs, 1989; Klemens, 1993). In addition, Jefferson Salamander are larger and capable of migrating farther than the smaller Blue-Spotted Salamanders (Williams, 1973; Douglas and Monroe, 1981; Ryan and Calhoun, 2014). Jefferson Salamanders use forested landscapes with low disturbance (Porej et al., 2004; Rubbo and Kiesecker, 2005; Greenwald et al., 2016), while some researchers have documented Blue-Spotted Salamanders in more open habitat that may have greater anthropogenic disturbance (Weller et al., 1978; Downs, 1989; Klemens, 1993; Regosin et al., 2005; Windmiller et al., 2008).

Unisexual Salamander habitat studies have been limited to establishing their geographic and climatic niche (Greenwald et al., 2016), examining the habitat relations of these salamanders to three tree species in the sub-canopy (Belasen et al., 2013), and breeding site characteristics (Hoffmann et al, in review). The post-breeding habitat selection and migration distances (with known start and end points) of Unisexual Salamanders are critical to informing management decisions but have not been quantified in the peer-reviewed literature.

Unisexual Salamanders bearing the DNA of Jefferson and Blue-Spotted Salamanders may have terrestrial habitat preferences and migration distances that are intermediate, similar to, or different from these parent species. Our goal was to understand the post-breeding habitat selection of Unisexual Salamanders in comparison to the sperm-host species. Specifically, we (1) quantified the emigration distances of LLJ and LLLJ, (2) examined their micro-habitat selection in late-spring and summer, and (3) compared these behaviors to published telemetry studies of Jefferson and Blue-Spotted Salamanders (Williams, 1973; Faccio, 2003; Ryan and Calhoun, 2014).

Materials and Methods

Study Sites

We captured emigrating salamanders at four vernal pools and followed them through landscapes with mixed and coniferous forest dominated by Red Pine (*Pinus resinosa*), White Pine (*P. stobus*), Eastern Hemlock (*Tsuga Canadensis*), Balsam Fir (*Abies balsamea*), Red Maple (*Acer rubrum*), and Red Oak (*Quercus rubra*). The two pools in Old Town, Maine, were located on a parcel managed for forestry and abutting a wide river (>200 m across). The forest matrix of the two pools in Orono, Maine, was penetrated by residential neighborhoods and fields. We had prior knowledge that large numbers of Unisexual Salamanders bred in these pools.

Capture, genetic testing, and surgery

We encircled the vernal pools with drift fences for a related study (chapter 3) and selected emigrating Unisexual Salamanders (>7g) to implant with radio transmitters following the methods of Madison et al. (2010). We only monitored large Unisexual Salamanders because previous research on Blue-Spotted Salamanders (LL) discouraged use of radio telemetry for this species due to its smaller size (Ryan and Calhoun 2014) and we are outside the geographic range of Jefferson Salamanders.

We anesthetized Unisexual Salamanders by submergence in 3.1 mM tricaine methane sulfonate (MS-222) neutralized to pH 7.0 with aqueous NaOH until loss of pain response (toe pinching). We used surgical scissors to remove a 0.5 by 0.3 cm tissue sample (Nöel et al., 2011) which we stored in 70% ethanol and shipped to the University of Guelph to determine genomotype using microsatellite DNA analyses at six loci (AjeD75, AjeD94, AjeD283, AjeD346, AjeD378, and AjeD422), four of which can be used to differentiate between the genomes of Blue-Spotted and Jefferson Salamanders (Julian et al., 2003). Our microsatellite DNA methods are described in detail elsewhere (Bogart et al., 2007, 2009).

We inserted ATS A2415 transmitters (0.33 g, Advanced Telemetry Systems) with the antennas removed and a PIT tag (0.09 g, HPT12, 134.2kHz ISO FDXB tag; Biomark) into salamanders' coelomic cavities using 10 mm longitudinal incisions in the left ventrolateral abdominal walls (Ryan and Calhoun, 2014). We closed the wounds with absorbable sutures (Model PDS Plus, RB-1 taper, Size 5-0, Ethicon Inc) and bathed the salamanders in distilled water until they were able to right themselves (Faccio, 2003; Madison et al., 2010; Ryan and Calhoun, 2014). The salamanders recovered overnight and were released under wet leaves outside the drift fence in 2013 and into the vernal pools in 2014. In 2013, we carefully excavated salamanders every two weeks when possible to examine incisions and measure weight.

We extended our telemetry season from 42 days (battery life of one transmitter) to 92 days (cumulative battery life of three transmitters) by replacing transmitters in six animals on 3 July 2013 and 8 August 2013 (after McDonough and Paton, 2007; Titus et al., 2014). We noted that transmitters did not seem to irritate internal organs. However, the skin was weak at the site of the original incision, and two animals were found with an open incision 7 and 12 days after the second re-implant surgery. In 2014, we tracked each salamander for the life of one transmitter (42 days), and made no attempt to regularly weigh animals. Ambystomatids may make large movements in fall (Faccio, 2003; Regosin et al., 2005; McDonough and Paton, 2007) but we did not attempt to track salamanders to their hibernacula due to welfare concerns associated with additional surgeries to replace expiring transmitters, therefore our results are not inclusive of all non-breeding habitat selection.

Telemetry

We re-located the radio-implanted salamanders daily in 2013 and every three days in 2014 using a receiver (Model R-1000, Advanced Telemetry Systems) and VHF antennae (Model RA-2AK, Telonics, Inc) for direct overhead localization (10 cm accuracy). At the end of the 2014 season when a transmitter's battery expired, we scanned the previous location and surrounding area for PIT tags using a Destron-Fearing transceiver (Model FS 2001A-ISO; Digital Angel Co., St. Paul, Minnesota, USA) mounted in a backpack with a battery and connected to a custom-built antenna (60-cm diameter circle of 20-gauge wire wrapped several times through a PVC frame on a 1.5 m handle; Blomquist et al. 2008). We scanned for up to 30 minutes (covering approximately a 20-m radius) before dismissing a salamander as lost. This technique extended tracking for 11 salamanders for an average of 15 days (range: 3-36 days). We assumed lost salamanders had either moved beyond the search area or were too deep underground to be detected (≥ 20 cm).

We recorded locations with a handheld GPS (GPSMAP 62stc and eTrex 10, Garmin International, Inc.) and marked them with a pin flag. We considered an animal not to have moved if it was estimated to be within 3 m of the flag on subsequent visits in 2013, and within 0.5 m of the flag in 2014.

Habitat Variables

We measured microhabitat variables at paired used and random plots in succession such that meteorological conditions and vegetation phenology were comparable within pairs. We measured environmental variables on 3-m radius plots for each used plot (Faccio, 2003). In 2014 we added 0.5-m radius plots to examine finer scale selection (when salamanders moved within the 3-m plot) based on new information about Blue-Spotted Salamanders (Ryan and Calhoun, 2014). Three random plots evenly surrounded each used plot (i.e., 120 degrees apart, with the first random plot located along a random bearing) to sample available habitat a salamander might have either passed through without settling or could have reached had it not chosen to stop where it did (Figure 10). Distances between used and random plots at the larger scale were based on the median step distance (distance between sequential locations) of all individuals combined during the previous two weeks in 2013, except for during the initial two weeks when we used the same weeks' median distance. The first three weeks' plots were 56 m apart, by the fifth week the distance decreased to 35 m, and from the seventh week on the plots were 6 m apart (the minimum to allow no overlap of plots). We used the distances measured in 2013 to space plots in 2014. Random points for the smaller movement scale were always located 6 m from the used plot.

We measured 22 environmental variables within each random and used plot, chosen based on previous habitat studies of Jefferson Salamanders, Blue-Spotted Salamanders, and Wood Frogs (Faccio, 2003; Rittenhouse and Semlitsch, 2007; Ryan and Calhoun, 2014; Groff et al., 2016). We recorded the land use within each plot as forest, yard/field, or wetland. We visually estimated the percent cover of bare soil, all leaf litter, coniferous leaf litter, water, grass, moss, rock, vegetation < 1 m tall, vegetation between 1 and 3 m tall, and vegetation > 3 m tall but < 10 cm DBH (diameter at breast height). We counted the number of stumps. We used a spherical convex densitometer (Model-A, Forestry Suppliers, Inc., Jackson, Mississippi, USA) to quantify canopy density and measured leaf litter depth to nearest 0.5 cm, soil moisture (soil moisture probe, FieldScout TDR200, Spectrum Technologies, Inc. Aurora, Illinois, USA), and soil temperature at ºC (digital thermometer, Model 9841, Taylor Precision Products, Las Cruces, New Mexico, USA) near the center of each plot. We measured the diameter and length of coarse woody debris (> 10 cm) to calculate the total area covered (cm^2) and recorded the maximum decay stage for 3-m plots only (Monti, 1997). Once a salamander had vacated the plot or its transmitter expired, we brushed away the leaf litter and recorded the number of horizontal and vertical small mammal burrow openings within 1 m^2 inside 3-m plots and within the plot for 0.5-

m plots (after Faccio, 2003, with horizontal burrows generally in the organic layer and vertical burrows, such as chipmunk holes, steeply penetrating the soil).

Analysis

We plotted salamander locations in ArcGIS 10.3 (ERSI) and used the “adehabitatLT” package (Calenge, 2006) in Program R to determine the length of each step, cumulative distance, and maximum straight line distance from the vernal pool for each salamander. We used a Kruskal-Wallace test to determine if maximum straight-line distances varied by pool and Spearnman’s rank correlation to determine if these distances were correlated with salamander weight (Jehle and Arntzen, 2000).

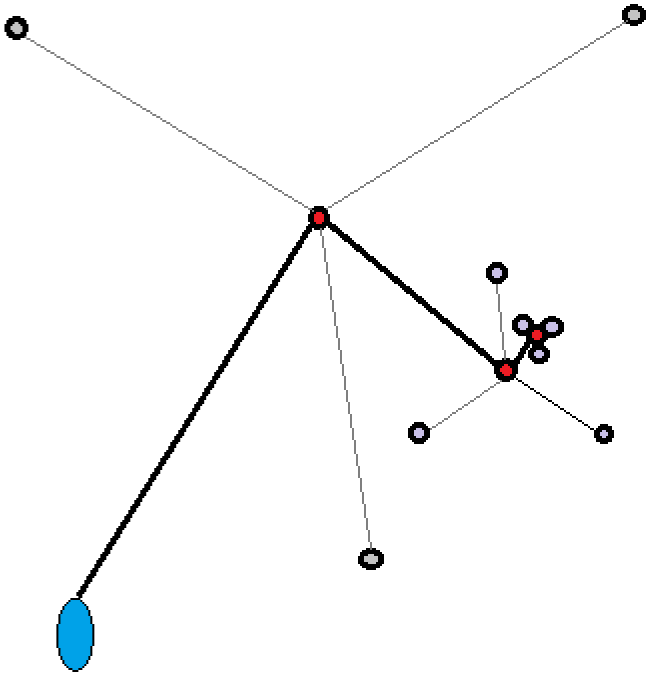

Figure 10. Schematic of used and random points. The path (thick black line) of a hypothetical Unisexual Salamander as it moved from a vernal pool (blue oval). Three random points (grey circles) were spaced 120º apart around each used location (red circles). The median distance moved by all salamanders in the previous two weeks was used to determine the distance of random plots from used plots (thin grey lines), such that random plots better represented the scale on which salamanders were making decisions than a constant distance.

We compared maximum straight-line distances for Unisexual Salamanders to published values for their parent species' mean post-breeding migration distances and 95% life zones (based on Williams, 1973; Faccio, 2003; Ryan and Calhoun, 2014). Faccio (2003) tracked six Jefferson Salamanders from two pools and followed Semlitsch (1998) in using a 95% confidence interval to determine the radius of a life zone that would include 95% of a study population's maximum distances from their breeding site. However, confidence intervals are intended to give precision of estimation of the mean, so we can interpret this distance as the area we can be 95% sure will include the mean of maximum distances moved by the salamanders, not the area that includes 95% of the salamanders. Ryan (2014) sorted the distances traveled by Blue-Spotted Salamanders in ascending order and determined which distance included 95%. We recalculated life zones for these published data sets, the radio-isotope tracked Jefferson Salamander of Williams (1973), and our own observations using t-scores to calculate the 95% quantile of the population (which we believe was Semlitch's intention, as we simply divide by the standard deviation rather than the standard error). Neither of these Jefferson Salamander populations have been genomotyped. However, we assume Williams' animals were JJ due to their location outside the geographic range of Unisexual Salamanders (Petranka, 1998; Charney, 2011) and that Faccio's salamanders were mostly JJ based on sex ratios of the breeding population (S. Faccio, personal communication). Also, half of Faccio's telemetered salamanders were males and therefore almost certainly JJ. The Blue-Spotted Salamander population was known to contain no Unisexual Salamanders (Ryan and Calhoun, 2014). Although in some cases female salamanders migrate further than males (Regosin et al., 2005; McDonough and Paton, 2007), we were not able to separate the published data by sex to directly compare migration distances between bisexual females and unisexuals.

We determined if Unisexual Salamanders maintained an initial bearing using individuals with more than one re-location. We found the difference between the initial bearing (the azimuth from the pool to the first re-location) and the bearing of each subsequent re-location, such that a small difference would indicate an animal continued in a straight line. We used the "circular" package (Lund and Agostinelli, 2015) in Program R to conduct a modified Rayleigh test to determine if the differences (pooled across all individuals) were significant (i.e. formed a uniform distribution or one with a peak at zero; Durand and Greenwood, 1958; Harrison, 1992).

We examined the orientation of salamanders around each pool. We used the last known location of each salamander in Rayleigh tests to determine if salamanders were distributed uniformly or directionally.

We paired use by individuals to their own availability at a given time rather than assume that all areas were equally available to every salamander without seasonal change (i.e., study design IV; Erickson et al. 2001). Additionally, we accounted for unequal numbers of re-locations and days tracked by weighing each plot by the proportion of days the salamander spent there, such that the experimental units were animals rather than re-locations (Aebischer et al., 1993; Thomas and Taylor, 2006).

We z-standardized all continuous variables. We inspected the data for collinearity to ensure no Pearson correlation coefficient was ≥ 0.7. We created 26 *a priori* models (Table 11) based on hypotheses about factors influencing salamander habitat selection including land use, microclimate, shelter, vegetation, and ground cover. We also included models of sperm-host habitat selection from the literature (Ryan and Calhoun, 2014). We used the "survival' package (Therneau, 2015) in program R to conduct conditional logistic regression to examine selection of microhabitat features at the 3-m and 0.5-m scales. This method is used for animals of limited mobility, as pairing a single used location with specific random location(s) reduces the standard error of the estimates (Compton et al., 2002; Gorman et al., 2013; Popescu et al., 2013). We used Akaike Information Criterion adjusted for small sample size (AICc) to rank models separately for each scale with package “AICcmodavg” (Mazerolle, 2016) and considered a model to be well supported with ΔAICc < 2 and to have some support with ΔAICc < 7 (Burnham and Anderson, 2002). We used the “support.Ces” package (Aizaki, 2012) to determine McFadden's R^2 (ρ), which measures the fit of the full model compared to that of the model with no covariates adjusted for the number of covariates. We found the model averaged estimates of covariates in models with a cumulative model weight of ≤ 0.9, and considered these covariates important if the confidence intervals of their odds ratios did not include 1.

Results

Of the 42 Unisexual Salamander sampled in this study, 39 were LLJ, two were LLLJ, and one was unidentified but greater than the maximum observed weight of local Blue-Spotted Salamanders (LL; Hoffmann et al., unpublished data). Seven clones were represented in this sample and included 21 salamanders (Appendix C). Weights did not vary by pool (Kruskal-Wallace Chi-square = 2.91, df = 3, *P* = 0.406) and ranged from 7.74 - 13.38 g, such that the transmitters represented ≤ 4.26% of body weight.

Post-operative recovery and fates

In 2013, all 15 salamanders showed signs of recovery (able to right themselves and walk) within one hour of surgery and were released the next morning, except for one salamander who escaped her container and was found desiccated. Another seven of these salamanders died during the study due to complications (n = 4), unrelated causes (n = 1), or unknown causes (n = 2). One transmitter failed prematurely. The other salamanders were tracked until their transmitters’ batteries were exhausted. Salamander weight did not decrease following surgeries.

Table 11. Models used to compare 3-m and 0.5-m plots used by Unisexual Salamanders to three paired random plots in central Maine, USA. The variables CWD (coarse woody debris) area, CWD decay stage, and forest were not included in models for 0.5-m plots due to lack of field measurements and lack of convergence caused by low variation.

	Model	Variables
	Land use	
1	LU global	Forest + Yard + Wetland
2	Natural	Forest + Wetland
3	Forest	Forest
4	Yard	Yard
	Shelter	
5	Sh global	Horizontal burrows + Vertical burrows + Leaf litter cover + Leaf litter depth + Stumps + Rock + CWD decay stage + CWD area
6	All tunnels	Horizontal burrows + Vertical burrows + Stumps
7	Mammal burrows	Horizontal burrows + Vertical burrows
8	Cover objects	Stumps + Rock + CWD area
9	Rotten wood	CWD decay stage + CWD area + Stumps
	Ground cover	
10	GC global	Leaf litter cover + Leaf litter depth + Rock + Moss + Water + Bare soil + Coniferous leaf litter
11	Leaves	Leaf litter cover + Leaf litter depth
12	Bare ground	Rock + Bare soil
13	Moist areas	Moss + Water

Table 11, continued

	Model	Variables
14	Needles	Coniferous leaf litter
	Microclimate	
15	MC global	Soil moisture + Soil temperature
16	Soil Moisture	Soil moisture
17	Soil Temp	Soil temperature
	Vegetation	
19	Veg global	Veg < 1m + Veg 1 to 3m + Veg > 3m + Canopy density + Grass
20	Understory	Veg < 1m + Veg 1 to 3m + Veg > 3m
21	Canopy	Canopy density
22	Low Veg	Veg < 1m + Grass
23	Shrubs	Veg 1 to 3m
	Literature	
24	Lit global	Soil temperature + Leaf litter depth + Soil moisture + Grass + Canopy density
25	Ryan 1 m best	Soil temperature + Leaf litter depth + Soil moisture
26	Ryan 10 m best	Grass + Canopy density

In 2014, we observed similar recovery following anesthesia and no mortalities during the study. We were unable to locate two salamanders after release due to either the high level of radio interference in some areas or because they moved beyond the area searched (approximately 800 m radiating from the pool).

Distances

We tracked Unisexual Salamanders from 5 to 94 days (mean = 51 days), during which they moved an average straight-line distance of 172 m (range = 6 - 403 m) from the wetland ("Pool Dist" in Table 11). Distance to the pool generally reached an asymptote within a week, as salamanders made large initial migrations with few short subsequent movements (Figure 11). Individuals ranged from 1 to 13 steps (movements between locations, mean = 3.95, sd = 2.31), and we found no evidence of maintaining the initial bearing for 37 individuals with two or more steps (121 subsequent steps; V = 0.026, *P* = 0.347, indicating subsequent steps were in random directions rather than peaking in distribution around the initial bearings). Salamanders were distributed uniformly (i.e. in random angles) around all pools, although orientation at Pool 4 was nearly-significantly directional (r = 0.413, *P* = 0.063).

In 2013, the mean step length was 41 m (sd = 51, max = 194 m) and represented the distances moved by salamanders in one night, with a mean cumulative distance of 191 m (sd = 76, range = 6 to 410 m). In 2014, the mean step length was 57 m (sd = 81, max = 355 m), but because we tracked less frequently this represents the distance moved in 3 nights. The mean cumulative distance in 2014 was 209 m (sd = 140, range = 47 - 463 m).

Maximum straight-line distance from the pool varied by pool (Kruskal-Wallis χ^2= 18.45, df = 3, *P* = 0.004), but was not related to the weight of the salamander (r = 0.090, *P* = 0.581). Salamanders in Old Town remained closer to the pools (mean = 112 m and 36 m) than salamanders in Orono (mean = 244 and 214 m). Distances at all pools were normally distributed ($p > 0.3$).

Ninety-five percent life zones for Unisexual Salamanders in our study also varied by pool (195 m, 74 m, 383 m, 415 m for Pools 1, 2, 3, and 4) and extended 362 m with all pools combined (Figure 12). The 95% quantile for distance traveled by Jefferson Salamanders in Indiana, USA, was 478 m (mean = 252 m, sd = 136 m, n = 86, based on Williams, 1973) while the zone for Jefferson/Unisexual Salamanders in Vermont, USA, was 143 m (mean = 92, sd = 25, n = 6, based on Faccio, 2003). The life zone for Blue-Spotted Salamanders was 149 m (mean = 64.9, sd = 50.1, n = 43, based on Ryan and Calhoun, 2014).

Macrohabitat

Thirty-Six Unisexual Salamanders remained within the forest matrix for the entire study, although 12 of these emigrated to post-breeding home ranges that were within about 20 m of forest-lawn or forest-hay field edges (Figure 13). Seven salamanders occupied swamps dominated by Alder (*Alnus incana*) and Highbush Blueberry (*Vaccinium corymbosum*). No salamanders were observed in a lawn or field, though the remaining four of salamanders had to have crossed lawns during emigration, and they spent the majority of the season underneath buildings (two salamanders under separate sheds with wooden floors and two salamanders under the same garage on a concrete slab). Only five salamanders crossed roads (two crossed a dirt road, one crossed a logging road, and two crossed paved roads), and two salamanders used plots within 10 m of roads. Salamanders also did not enter pine plantations east of Pool 2 and west of Pool 4.

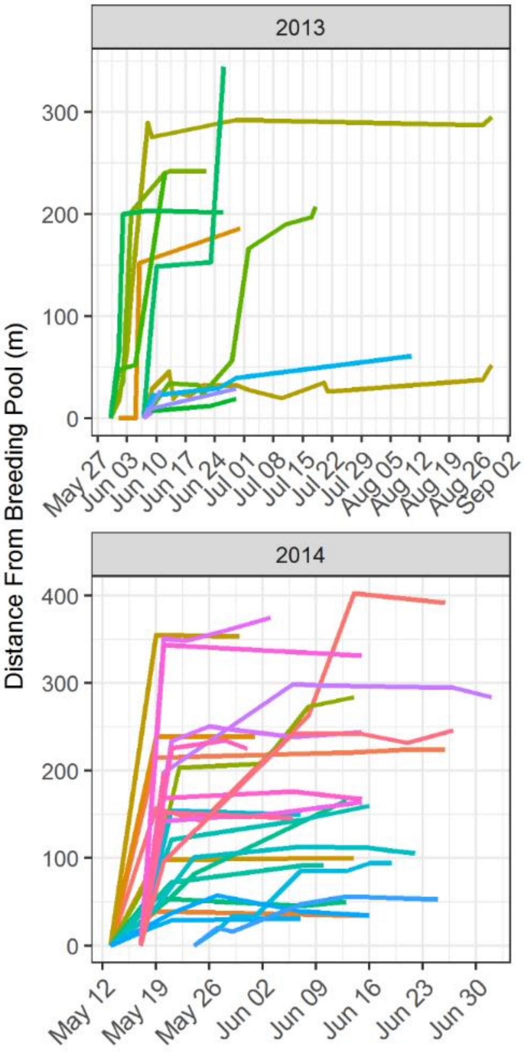

Figure 11. Distance from the breeding wetland for emigrating Unisexual Salamanders quickly reached an asymptote in central Maine, USA.

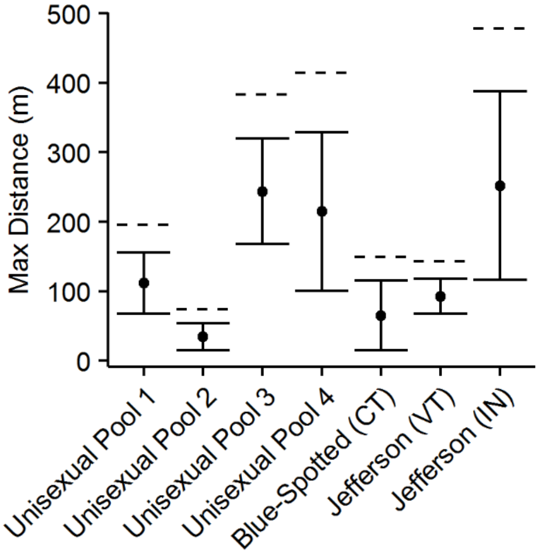

Figure 12. Mean distances of Unisexual Salamanders from four breeding pools in central Maine, USA, plus the mean distances observed for sperm-hosts in other tracking studies (based on Williams, 1973; Faccio, 2003; and Ryan and Calhoun, 2014). Error bars indicate the mean ± one standard deviation, and dashed lines represent the 95% quantile.

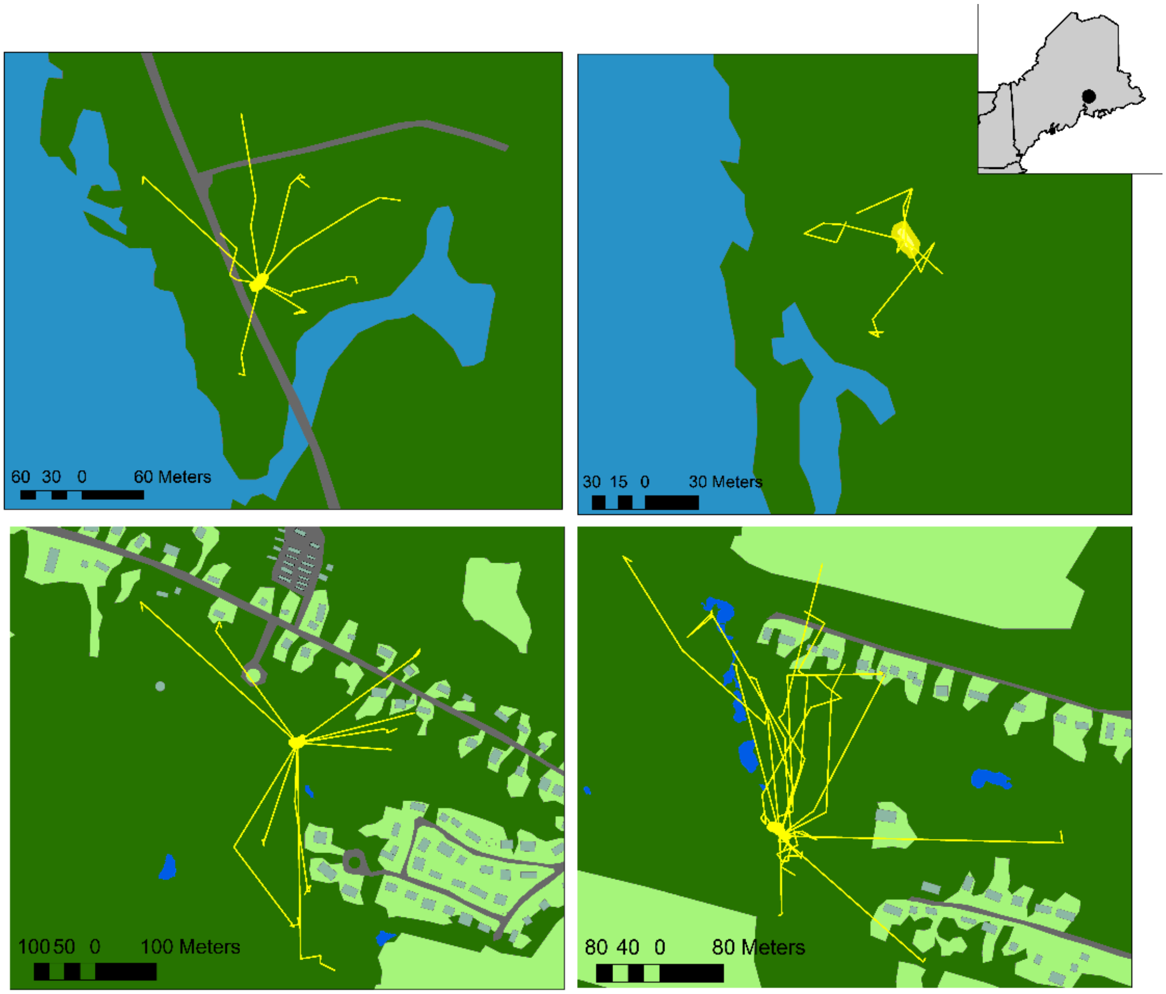

Figure 13. Emigration of Unisexual Salamanders from breeding pools. Pools in Old Town, Maine, USA, are on top and pools in Orono, Maine, USA are on bottom. Paths of radio tracked salamanders and their breeding sites are shown in yellow. Lawn and hay fields are light green, forest is dark green, roads and buildings are grey, and water is blue. Note that scales vary. Study site location is shown as the black dot in the insert map.

Microhabitat selection

The top ranked models of Unisexual Salamander habitat selection at the 3-m plot scale and 0.5-m plot scale overlapped. At the larger movement scale ($\geq$ 3 m), only the All tunnels model had substantial support (ΔAICc < 2, Table 12). Seven other models related to shelter, vegetation, and microclimate, including important models for Blue-Spotted Salamanders based in Ryan and Calhoun (2014) had some support (ΔAICc < 7), though McFadden's adjusted pseudo R^2 was low for the five models that did not include mammal burrows as a covariate. Selection of these models appears to be driven by three important covariates (Table 13), with Unisexual Salamanders more likely to use plots with more horizontal burrows, lower substrate temperatures, and less vegetation under a meter tall than random plots. At the smaller movement scale (< 3 m), both the All tunnels model and Mammal burrows model had substantial support, and the global shelter and soil moisture models had some support, though all models had low McFadden's adjusted pseudo R^2. The only covariate with a model averaged estimate odds ratio whose confidence interval did not overlap one at the small movement scale was the count of horizontal burrows.

Table 12. Top ranked Unisexual Salamander paired logistic regression models for used and random locations in central Maine, USA. Only models with ΔAICc < 7 are shown. K is the number of parameters, adjusted ρ2 is McFadden's adjusted pseudo R^2, and LL is the log likelihood.

Model	K	AICc	ΔAICc	w_i	Cum. *w*	Adjusted ρ^2	*LL*
3 m plot scale							
All tunnels	3	35.476	0.000	0.444	0.444	0.174	-14.711
Soil temp	1	37.639	2.163	0.150	0.594	0.136	-17.815
Mammal burrows	2	37.742	2.266	0.143	0.737	0.176	-16.858
Veg global	5	39.328	3.852	0.065	0.802	0.096	-14.596
MC global	2	39.541	4.065	0.058	0.860	0.080	-17.757
Ryan et al 1	3	39.651	4.175	0.055	0.915	0.043	-16.798
Lit global	5	40.457	4.981	0.037	0.952	-0.005	-15.158
Low veg	2	42.087	6.611	0.016	0.968	0.109	-19.031
0.5 m plot scale							
All tunnels	3	82.99085	0	0.443486	0.443486	0.037	-38.4276
Mammal burrows	2	83.44444	0.453587	0.353497	0.796983	0.034	-39.6906
Sh global	6	85.09519	2.10434	0.154856	0.951839	0.016	-36.3062
Soil moisture	1	89.74988	6.75903	0.015107	0.966946	0.057	-43.865

Table 13. Important model averaged parameter estimates (β), standard errors, odds ratios with confidence intervals and descriptive statistics for Unisexual Salamander paired logistic regression models for plots of used and random locations in central Maine, USA. Only covariates from models included in 90% of the cumulative model weight and with 95% confidence intervals or odds ratios that did not include one are shown.

Covariate	β Estimate of scaled data	SE of scaled data	Odds ratio of scaled data	95% CI lower	95% CI upper	Used mean	Used SD	Used Min	Used Max	Rand mean	Rand SD	Rand Min	Rand Max
3-m plot scale													
Horizontal burrows	0.588	0.246	1.801	1.112	2.917	1.269	1.351	0	6	0.687	1.13	0	7
Soil temp	-0.879	0.425	0.415	0.181	0.955	13.854	2.78	8	27	14.623	3.182	7	31
Veg < 1m	-0.681	0.332	0.506	0.264	0.971	17.234	19.523	0	83	25.875	26.366	0	100
0.5-m plot scale													
Horizontal burrows	0.702	0.297	2.018	1.128	3.609	1.45	1.431	0	6	0.843	1.278	0	6

Discussion

Unisexual Salamander post-breeding movement patterns were similar to other ambystomatids, characterized by long movements during emigration over a few nights followed by infrequent and shorter movements in their post-breeding home range (Figure 11; Williams, 1973; Madison, 1997; Titus et al., 2014). Unisexual Salamanders moved as far as 463 m from the pool. While individuals moved about five times on average during the study, five salamanders (of those tracked over 20 days) moved only once (from the pool to the summer location) and remained within the same 3-m plot for the season. This stationary behavior has been reported for other ambystomatids and also directly observed in Wood Frogs (*Lithobates sylvaticus*; Douglas and Monroe, 1981; Rittenhouse and Semlitsch, 2007). This behavior is presumed to be a reflection of their "sit-and-wait" predatory strategy.

Mean, median, and 95th percentiles of amphibian migration distances are used to justify the conservation of terrestrial habitat through regulatory or management zones (Semlitsch, 1998; Semlitsch 2000). However, these distances have not been widely quantified across and within species, which may be problematic for managers. For example, distances of Unisexual Salamanders from Pools 1 and 2 were less than half those recorded from Pools 3 and 4, perhaps due to variation in these landscapes, the river acting as a barrier, or competition for burrows in the larger populations (Regosin et al., 2004). Making management decisions for the latter pools based solely on the former would be ill-advised.

Unisexual Salamanders in our study generally migrated within the variation of distances observed for Blue-Spotted Salamanders and Jefferson Salamanders (Figure 12). Mean distances from the pool and 95% life zones for Unisexual Salamanders at 3 of our 4 pools were larger than those of Blue-Spotted Salamanders in Connecticut (Ryan and Calhoun, 2014) and Jefferson Salamanders in Vermont (Faccio, 2003), but Jefferson Salamanders in Indiana had a larger mean and life zone distances (Williams, 1973). Other references also list mean distances of hand captured Unisexual Salamanders (presumably LLJ) in Michigan, USA, as intermediate (110 m) and radioisotope tagged Jefferson Salamanders in Kentucky, USA, as farther (250 m; Douglas and Monroe, 1981; Belasen et al., 2013). This comparison does not consider variation due to geographic location, and we recommend future work to directly compare taxa at the same site.

Ninety percent of tracked Unisexual Salamanders stayed in the forest matrix, but we were surprised to find 13 of 22 Unisexual Salamanders from our Orono pools had post-breeding home ranges near or within residential neighborhoods (i.e. within about 20 m of lawns or fields). We are unsure if salamanders settled in these areas because they interpreted the neighborhoods as barriers, if they were responded to unmeasured variables, or if they sought out these locations. These scenarios have drastically different management implications. Ambystomatids are known to cross open areas, however they also avoid forest edges (deMaynadier and Hunter, 1998; Gibbs, 1998; Regosin et al., 2005; McDonough and Paton, 2007; Pittman and Semlitsch, 2013). Forest edges are associated with reduced soil moisture, canopy density, and coarse woody debris and increased forest floor disruption, predation, and temperature (reviewed in Lindenmayer and Fischer, 2006). Pesticide and herbicides may contaminate lawns, but these areas also have high primary production and may have high plant and invertebrate diversity (Falk, 1976; Frankie and Ehler, 1978; McKinney, 2008). Buildings may act as large cover objects to reduce fluctuation in temperature and moisture compared to surrounding areas. We cautiously suggest further study to determine 1) which scenario is occurring, 2) if salamanders in more urban areas behave similarly, and 3) if salamanders residing near lawns have lower survival than those in forest interior.

Our top Unisexual Salamander microhabitat selection models included those based on shelter, vegetation, and microclimate, as well as one based on microhabitat selection of Blue-Spotted Salamanders (from Ryan and Calhoun, 2014). Ground cover and land use covariates were not supported, presumably due to the homogeneity of the landscape in our study area. Only three covariates were important: horizontal burrows, forest floor vegetation (herbaceous and woody plants within a meter of the ground), and soil temperature.

The most important feature for predicting use by Unisexual Salamanders was also important in previous studies of microhabitat selection by their parent species and other ambystomatids. Horizontal small mammal burrows were selected by Unisexual Salamanders both during large movements (such as during immediate post-breeding movements) and during shorter movements within their post-breeding home range. The association of ambystomatids with burrows is well documented (Williams, 1973; Douglas and Monroe, 1981; Madison, 1997; Regosin et al., 2004; Osbourn et al., 2014). Horizontal burrows are particularly important and are selected over vertical by both *A. jeffersonianum* and *A. maculatum* in the summer in Vermont (Faccio, 2003).

Minimal forest floor vegetation and low temperatures may be the results of shaded areas that remain moist, and therefore indicate conditions conducive to thermoregulation and osmoregulation. Jefferson Salamanders likewise select areas shaded by shrubs (Faccio, 2003). Salamanders in general are thought to behaviorally thermoregulate by selecting cool refugia, but temperature relations are rarely observed in the field where other needs (such as food resources) may outweigh the benefits of optimal temperature (Feder and Pough, 1975; Stebbin and Cohen, 1995; Welsh and Lind, 1995). Stebbin and Cohen (1995) also suggest that selection of low temperatures may aid in recovery from high metabolic demands, such as migration and breeding.

With the exception of the riparian area and neighborhoods, our study sites were relatively homogenous and we cannot rule out the possibility that Unisexual Salamanders also select habitat based on other variables important to the sperm-hosts. Factors such as leaf litter, shrubs, logs, soil moisture, and canopy may be important (Faccio, 2003; Ryan and Calhoun, 2014), but escaped our attention due to consistently high availability in our relatively forested study area. Other studies have found associations between Unisexual Salamanders and Red Maple (*Acer rubrum*) and between this taxon pooled with Spotted Salamanders and canopy cover, root density, litter depth, non-vascular plants, ambient light intensity, woody cover, and mid-story canopy (deMaynadier and Hunter, 1998; Belasen et al., 2013).

Unisexual Salamanders select microhabitat and travel distances within the known parameters of behavior of their sperm-hosts. Blue-Spotted Salamanders and Unisexuals in Maine also select breeding sites based on the same vegetative characteristics (Hoffmann et al, in review). The overlap in habitat features important to Unisexual Salamanders with those of their parent species may allow the former to colonize landscapes wherever sperm-hosts are present, although, in the case of less vagile sperm hosts, Unisexuals Salamander may require larger forest patches (Mee and Rowe 2010). We suggest future work to track sperm-hosts and Unisexual Salamanders from the same wetlands to directly compare habitat selection for differences that might allow coexistence of the taxa.

Unisexual Salamanders are generally more abundant than their sympatric sperm host, but are unusual among vertebrates in their reproductive system and therefore warrant conservation. We recommend maintaining small mammal populations to provide burrows, avoiding use of lawn chemicals since some salamanders resided near lawns, and further studies to examine the use of rural and suburban/exurban neighborhoods by ambystomatids. We emphasis that migration distances are context specific, and we caution resource managers to be conservative in designating management zones.

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APPENDIX A. ERROR RATES OF TEST OF HOMOGENEITY

Table 14. Error rates in tests of homogeneity of departures. Amphibian paths are represented as continuous angles, pitfall traps (PF) in a circular drift fence, and pitfall traps in an elliptical drift fence with α = 0.05. Error rates over 6% are in bold.

	Chi-squared TOH					
	n = 30		n = 90		n = 200	
	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)
East vs. East - Type I Error Rate						
Continuous	0.028	0.000	0.047	0.020	0.059	0.036
Circular PF	0.014	0.002	0.044	0.022	0.046	0.042
Elliptical PF	0.022	0.001	0.045	0.018	0.057	0.041
East vs. East - Type I Error Rate						
Continuous	**0.137**	**0.345**	0.000	0.016	0.000	0.000
Circular PF	**0.148**	**0.335**	0.000	0.015	0.000	0.000
Elliptical PF	**0.136**	**0.262**	0.000	0.007	0.000	0.000

Table 14, continued

Watson-Williams test

	n=30		n=90		n=200	
	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)
East vs. East - Type I Error Rate						
Continuous	**0.075**	0.050	**0.066**	0.041	**0.097**	0.051
Circular PF	**0.073**	0.054	**0.069**	0.042	**0.091**	0.050
Elliptical PF	**0.074**	0.052	**0.062**	0.039	**0.099**	0.047
East vs. East - Type I Error Rate						
Continuous	0.001	0.000	0.000	0.000	0.000	0.000
Circular PF	0.001	0.000	0.000	0.000	0.000	0.000
Elliptical PF	0.001	0.000	0.000	0.000	0.000	0.000

MRPP

	n=30		n=90		n=200	
	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)	(κ =1)	(κ = 2)
East vs. East - Type I Error Rate						
Continuous	0.039	0.057	0.030	0.055	0.060	**0.061**
Circular PF	0.037	0.054	0.043	0.053	**0.063**	0.050
Elliptical PF	0.037	0.057	0.044	0.041	**0.069**	0.050
North vs. East - Type II Error Rate						
Continuous	**0.418**	0.002	0.002	0.000	0.000	0.000
Circular PF	**0.337**	0.002	0.002	0.000	0.000	0.000
Elliptical PF	**0.412**	0.002	0.002	0.000	0.000	0.000

Table 14, continued

	Kruskal-Wallace					
	n = 30		n = 90		n = 200	
	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)	(κ = 1)	(κ = 2)
East vs. East - Type I Error Rate						
Continuous	0.051	0.055	0.058	0.041	0.050	0.044
Circular PF	0.052	0.050	**0.061**	0.033	0.052	0.041
Elliptical PF	0.057	0.049	0.048	0.039	0.058	0.041
North vs. East - Type II Error Rate						
Continuous	0.026	0.000	0.000	0.000	0.000	0.000
Circular PF	0.030	0.000	0.000	0.000	0.000	0.000
Elliptical PF	0.036	0.000	0.000	0.000	0.000	0.000

APPENDIX B. AN INEXPENSIVE DEEP-WATER FUNNEL TRAP

Researchers studying Ambystomatid salamanders often capture adults with unbaited minnow traps at aquatic breeding sites, but traps that are fully submerged do not allow the enclosed salamanders to reach the water surface to breathe. This animal welfare concern can be addressed by limiting minnow traps to shallow areas, by checking the traps frequently enough to release animals before drowning, or by floating the traps (Wilson and Dorcas, 2004). Our research required us to trap Ambystomatids in large, deep wetlands where we believed our sampling would be inadequate if limited to the shallow edges, and time constraints did not allow us to visit sites multiple times each day. We were also unsure if floating traps, even with an aquatic drift fence, would effectively capture adult Ambystomatids. We sought a surrogate to minnow traps.

Some alternative traps have a vertical chamber that allows access to the surface, but have other drawbacks. For example, one funnel trap (Mushet et al,. 1997) extends above the water surface, but requires welding and costs approximately $45 each (versus $11 for a collapsible mesh minnow trap; Willson & Dorcas, 2004). Commercially available crayfish traps (Johnson and Barichivich, 2004) are bulky, making them difficult to store and transport in large numbers. Traps made from trashcans are stackable but have not been reported to capture terrestrial species (Luhring and Jennison, 2008), which may be capable of climbing the sides and slipping out under the lids.

We describe a modified trap (Figure 14) developed through collaboration with high school students in the Upward Bound Math Science Program at the University of Maine (described in Ilseman and Hoffmann, 2016). We used a 35 by 107 cm (about 14 by 42 inches) galvanized tomato cage as a frame, and enclosed the inverted cage in a fiberglass screening sack (Figure 15, Figure 16). We used a bolt cutter to remove the tines, and bent them in half to use as stakes. We cut a 40-cm diameter circle of screen for the floor and used a paint pen to trace the bottom of the frame (36-cm diameter) where we would later sew a seam. We cut a 90 by 122 cm rectangle for the walls; folded it in half and sewed along the edge to form a tube about 36 cm in diameter. We cut three 10 by 20 cm rectangles to make straps for staking the trap to the substrate; folding and sewing these along their long axes produced straps stronger than one layer of screen. We cut four 40 cm diameter half circles, folded these in half, and sewed them to create funnels with a narrow end about 3 cm in diameter and a wide end of about 18 cm. We sewed the funnels onto the wall high enough to allow room for the seam along the floor. We sewed the bottom and straps on simultaneously. We then inverted the sack, and cut entrance holes for the funnels. While we did not include aquatic drift fences, silt fence or screening could be sewn directly to the walls of the trap for this purpose.

We assembled the traps in the field by inserting the frames into the sacks, closing the excess material at the top using hair elastics, and staking them into place. Frames can be stacked, and sacks and stakes can be carried to the site easily inside a sack. We constructed 90 of these traps. Each trap used under $7.00 of material.

We tested this design using 10 traps for 5 nights at a site with a large number of breeding spotted (*Ambystoma maculatum*), blue-spotted (*A. laterale*), and unisexual (*A. laterale* and *A. laterale - jeffersonianum*) salamanders. Between 5 and 8 traps captured salamanders each night, with no trap empty for more than 3 nights. The average number of salamanders per trap per night was 3.9 and the maximum was 15 spotted salamanders and 35 blue-spotted salamanders. We observed no mortality or injuries. Unfortunately, our research objectives did not include comparing the efficacy of our traps with other trap designs.

By-catch at a variety of wetlands included eastern newts (*Notophthalmus viridescens*), four-toed salamanders (*Hemidactylium sctutatum*), northern redbelly dace (*Clinostomus eos*), central mudminnows (*Umbra limi*), sticklebacks (Gasterosteidae), leeches (Hirundinae), predaceous diving beetle larvae (Dytiscidae), caddisfly larvae (Trichoptera), dragonfly larvae (Anisoptera), mosquito larvae (Culicidae), and other invertebrates**.** Wood frogs (*Lithobtes sylvaticus*), pickerel frogs (*L. palustris*), green frogs (*L. clamitans*), and spring peepers (*Pseudacris crucifer*) were captured in shallow sites where the tops of funnels were at the water surface, and we expect anurans could be targeted by attaching the funnels higher.

Our tomato cage traps were effective and affordable. They are light, cheap, easy to transport and store, and allow animals to reach the surface.

Figure 14. Assembly of traps in the field. (A) The components of the trap: altered tomato cage, fiberglass screening sack, stakes, and hair elastic equipped with flagging tape and a tag. (B) Inserting the frame into the sack is made easier by pulling the funnels inside-out so they do not get caught on the bars of the cage. (C) A view looking down into the trap after inverting the funnels. (D) The top of the sack is gathered and secured with the hair elastic, and the trap is stacked to the substrate through straps at the bottom of the sack.

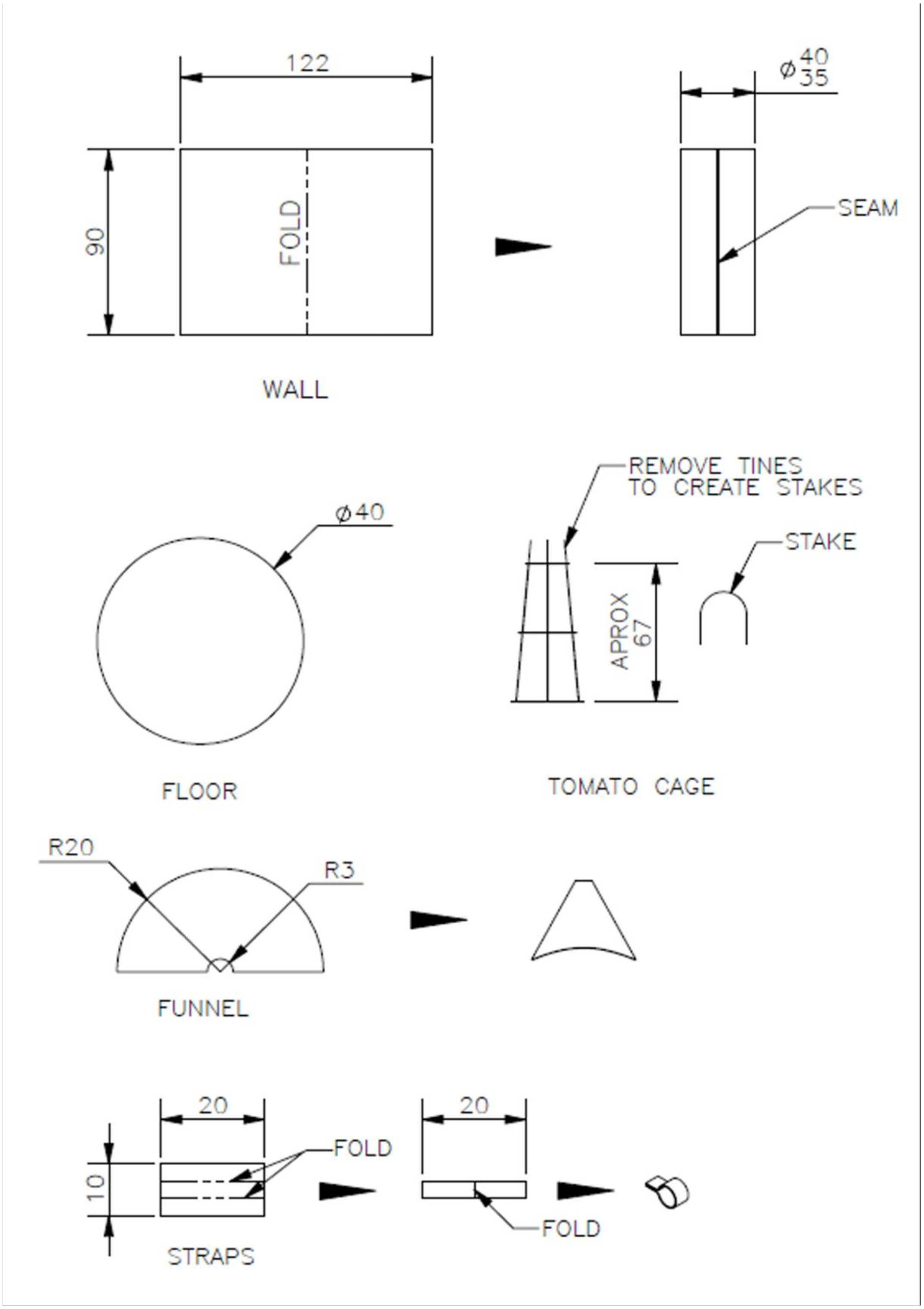

Figure 15. Components of the trap. Measurements in cm. Walls, floors, funnels, and straps are made of fiberglass screening, and the frame and stacks are created from a tomato cage.

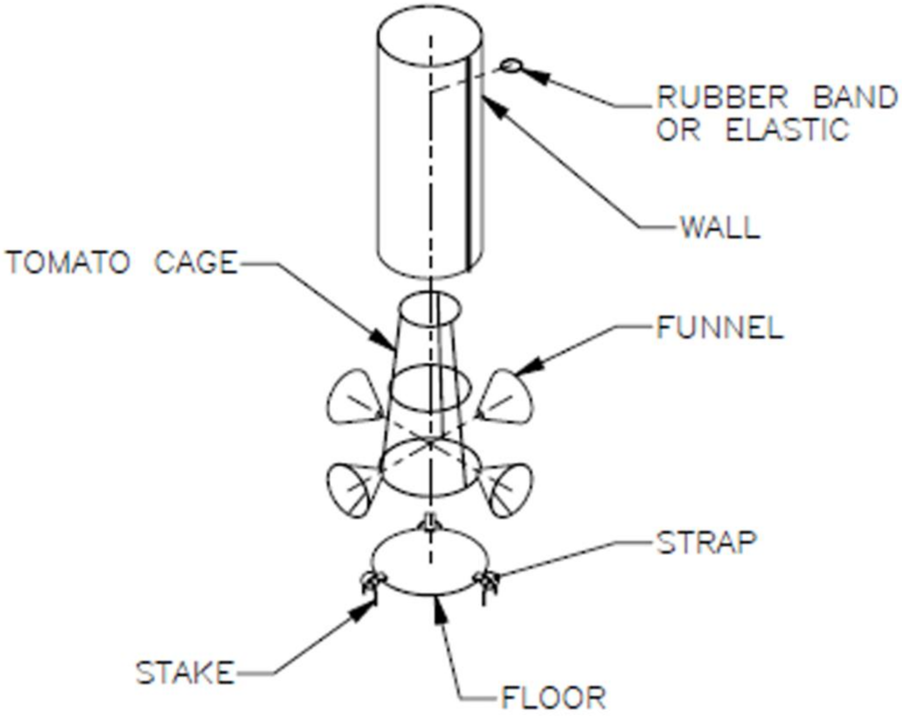

Figure 16. Exploded view of the trap.

APPENDIX C. SUMMARY DATA FOR 42 UNISEXUAL SALAMANDERS RADIO TRACKED FROM FOUR VERNAL POOLS IN MAINE

Table 15. Summary data for 42 Unisexual Salamanders radio tracked from 4 vernal pools. ID includes the pool of origin followed by the identification number of each animal. Genomotype indicates both ploidy and how many Blue-Spotted and Jefferson Salamander genomes each individual contains. Clone indicates which animals were identical at 3 loci. Weight and SVL (snout to vent length) were measured under anesthesia prior to transmitter implant surgery. The number of 3-m plots represents the amount of movements > 6 m for which habitat data was recorded, while 0.5-m plots represent 1-6 m movements in 2014. Max step indicates the maximum distance moved between successive relocations, which occurred daily in 2013 and every 3 days in 2014. Pool Dist is the maximum Euclidean distance each salamander traveled from the breeding site. The fates of each salamander include mortality events related to the implanted transmitters (MT), mortality events that were unrelated to the transmitters (MU), premature transmitter failure (TF), and battery expiration (BE).

ID	Genomotype	Clone	Weight (g)	SVL (mm)	Release Day	Days tracked	# 3m plots	# 0.5m plots	Max Step	Cumulative Dist	Pool Dist	Fate
P1.763	LLJ		9.5	72	6/7/2013	69	6	0	27	79	61	BE
P1.1	LLLJ		8.2	76	5/13/2014	34	2	3	54	76	54	BE
P1.2	LLJ		8.5	74	5/13/2014	40	3	0	84	167	166	BE
P1.3	LLLJ		9.8	78	5/13/2014	40	2	2	72	98	91	BE
P1.4	LLJ	E	7.8	73	5/13/2014	40	4	1	74	166	160	BE

Table 15, continued

ID	Genomotype	Clone	Weight (g)	SVL (mm)	Release Day	Days tracked	# 3m plots	# 0.5m plots	Max Step	Cumulative Dist	Pool Dist	Fate
P1.5	LLJ	F	8.9	74	5/13/2014	82	3	5	68	141	113	BE
P1.6	LLJ	F	10.4	77	5/17/2014	43	1	1	154	161	154	BE
P1.7	LLJ	E	12.2	80	5/24/2014	29	3	3	54	110	95	BE
P2.608	LLJ		11.7	76	6/7/2013	5	0	0	28	28	28	TF
P2.670	LLJ		9.4	73	6/8/2013	23	2	0	37	37	37	MT
P2.692	LLJ		9.9	80	6/7/2013	14	1	0	6	6	6	MU?
P2.1	LLJ		9.0	76	5/13/2014	37	3	1	33	63	31	BE
P2.2	LLJ		10.3	72	5/13/2014	47	5	4	37	91	57	BE
P2.3	LLJ		10.6	71	5/24/2014	71	2	4	55	116	56	BE
P3.1	LLJ	D	10.3	76	5/17/2014	75	2	3	198	392	299	BE
P3.10	LLJ	G	9.5	81	5/17/2014	48	3	4	234	274	251	BE
P3.11	LLJ	D	9.1	78	5/17/2014	48	3	1	351	422	375	BE

Table 15, continued

ID	Genomotype	Clone	Weight (g)	SVL (mm)	Release Day	Days tracked	# 3m plots	# 0.5m plots	Max Step	Cumulative Dist	Pool Dist	Fate
P3.12	LLJ		9.6	78	5/17/2014	35	3	2	142	169	164	BE
P3.3	LLJ	G	8.7	77	5/17/2014	75	2	1	343	359	343	BE
P3.5	LLJ		9.8	81	5/17/2014	32	3	2	169	185	176	BE
P3.6	LLJ	D	10.1	80	5/17/2014	41	1	1	225	251	234	BE
P3.7	LLJ	D	9.5	74	5/17/2014	38	1	0	196	196	196	BE
P3.8	LLJ		9.7	81	5/17/2014	35	1	1	152	160	152	BE
P3.9	LLJ	G	10.0	80	5/17/2014	75	4	2	148	274	246	BE
P4.401	Unknown		11.5	83	6/1/2013	45	5	0	152	183	186	BE
P4.629	LLJ	A	10.1	80	6/7/2013	86	14	0	30	229	53	BE
P4.662	LLJ		8.3	70	5/30/2013	91	7	0	178	394	295	BE
P4.718	LLJ	B	12.6	81	5/30/2013	48	4	0	171	342	242	BE
P4.811	LLJ		8.8	71	6/7/2013	64	8	0	126	298	208	MT

Table 15, continued

ID	Genomotype	Clone	Weight (g)	SVL (mm)	Release Day	Days tracked	# 3m plots	# 0.5m plots	Max Step	Cumulative Dist	Pool Dist	Fate
P4.871	LLJ		13.4	77	5/30/2013	48	4	0	193	246	241	BE
P4.872	LLJ	A	10.6	82	6/7/2013	53	3	0	15	34	19	MT
P4.899	LLJ	B	12.9	78	5/30/2013	94	4	0	144	218	203	BE
P4.930	LLJ		11.9	73	6/7/2013	87	3	0	194	364	345	MU
P4.1	LLJ		9.6	72	5/13/2014	79	3	1	157	463	403	BE
P4.2	LLJ		9.5	71	5/13/2014	52	2	3	215	225	224	BE
P4.3	LLJ	C	8.6	76	5/13/2014	35	2	2	39	47	39	BE
P4.4	LLJ	C	10.8	83	5/13/2014	50	2	1	240	244	240	BE
P4.5	LLJ	D	7.7	67	5/13/2014	41	2	2	98	102	100	BE
P4.6	LLJ	D	11.2	81	5/13/2014	41	1	1	355	369	355	BE
P4.7	LLJ		8.7	75	5/7/2014	41	4	1	203	404	196	BE

BIOGRAPHY OF THE AUTHOR

Kristine “Kris” Hoffmann grew up with a vernal pool in her back yard in Massachusetts. She volunteered in high school for the Massachusetts Division of Fisheries and Wildlife and the Town of Sterling Conservation Commission, and graduated from Wachusett Regional High School. She earned her Bachelor’s degree in Biology with a minor in Wildlife and Fisheries Conservation from the University of Massachusetts. She held internships at the University of Massachusetts, Brookhaven National Laboratory, Massachusetts Audubon, and the Michigan State University studying a variety of reptiles and amphibians, as well as sea lampreys. She earned her Master of Science degree at the University of Florida with a thesis focused on the effects of an exotic treefrog on the native frog community. She then became a teaching fellow at the University of Virginia’s College at Wise before returning to school to earn her doctorate at the University of Maine. After years in the south, returning to New England to study vernal pools felt like going home. Kris is a candidate for the Doctor of Philosophy degree in Wildlife Ecology from the University of Maine in May 2017.