EVALUATION OF THE ADAPTIVE CAPACITY OF MOLE SALAMANDERS (AMBYSTOMATIDAE) TO A CHANGING CLIMATE IN WESTERN MASSACHUSETTS

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EVALUATION OF THE ADAPTIVE CAPACITY OF MOLE SALAMANDERS
(AMBYSTOMATIDAE) TO A CHANGING CLIMATE IN WESTERN MASSACHUSETTS

A Dissertation Presented

by

KRISTOPHER JONATHAN WINIARSKI

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Department of Environmental Conservation
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EVALUATION OF THE ADAPTIVE CAPACITY OF MOLE SALAMANDERS

(AMBYSOMATIDAE) TO A CHANGING CLIMATE IN WESTERN MASSACHUSETTS

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DEDICATION

I dedicate this dissertation to my late father Len.
ACKNOWLEDGEMENTS

Funding to support my time as a research associate at the University of Massachusetts Amherst was provided by a Department of Interior Northeast Climate Science Adaptation Center graduate fellowship. This dissertation would not have been possible without the support of my family, friends, colleagues and my committee. My committee included co-chairs Kevin McGarigal and Curt Griffin and outside member Liz Willey whom all provided supported throughout the dissertation process. I was fortunate to be a graduate student in the Department of Environmental Conservation which during my time as a graduate student fostered an environment that supported excellent graduate level courses and learning opportunities. I was also lucky to be a research fellow in the Northeast Climate Science Adaptation Center which wouldn’t be at the University of Massachusetts without the effort of the PIs including my committee co-chair Curt Griffin. Addie Rose Holland, Jeanne Brown, Toni Lyn Morrelli and Michelle Staudinger at the Northeast Climate Science Adaptation Center provided important fellows training, support for my project and I was lucky to have them as friends and officemates. Kevin McGarigal’s previous students devoted an incredible amount of effort and time into collecting the marbled salamander photograph capture-recapture data that I analyzed for this dissertation including: L. Gamble, B. Timm, E. Plunkett and M. Chesser along with many technicians and volunteers. I also appreciate the support Maximillian Matthe provided when I was using his software AmphIdent to match the thousands of marbled salamander photos. I also need to thank Andrew Whiteley and those that helped with the sample collection of a salamander
landscape genetics dataset that I also analyzed for this dissertation including: Brad Compton, Megan Chesser and Jason Estes. Much of my dissertation involved using and through computer simulation evaluating the R package ResistanceGA which was developed by Bill Peterman at Ohio State University. I couldn’t have completed those two chapters without Bill’s assistance and feedback and I have enjoyed working with him and been inspired by his scientific accomplishments. A number of folks also provided analytical support for my salamander survival analysis including Jeff Laake who assisted me with the use of his R package RMark and Erik Blomberg.

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I would have never pursued environment studies if not for the support of my mother Jayce, my father Lenny and my younger brother Jason. My father and his friends spent many hours taking my brother and I fishing along the coast of Narragansett Bay and introducing us to the natural world. They also strongly encouraged me to attend college and not pursue the kind of tough physical work they did day in and day out. Once my brother and I were older my mother
woke up countless times in the early morning hours to drive us to Narragansett Bay with our boat, outboard, friends and fishing poles without any complaints. Last but not least, I could have never accomplished this milestone without the support of my lovely wife Sarah. During the course of writing this dissertation we had three boys Fletcher, Silas and Emmet and enjoyed many hikes and adventures together as a young family with our bird dog Blue in the woods around our home in east Leverett.
ABSTRACT

EVALUATION OF THE ADAPTIVE CAPACITY OF MOLE SALAMANDERS 
(AMBYSTOMATIDAE) TO A CHANGING CLIMATE IN 
WESTERN MASSACHUSETTS

MAY 2019

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Directed by: Professor Kevin McGarigal
The primary goal of my dissertation was to investigate the adaptive capacity of mole salamanders in western Massachusetts, specifically marbled salamander (*Ambystoma opacum*), to future changes in climate. This involved the analysis of two existing datasets including i) a nearly decade-long photograph capture-recapture dataset (first described by Gamble et al. 2009) and ii) a landscape genetics dataset (first described by Whitely et al. 2014). My dissertation also included two chapters focused on computer simulations to better understand the behavior and inferences from the statistical models fit to the empirical datasets I modeled and the effects of error in the data on model parameter estimates.

In Chapter 1, I simulated multistate capture histories (CHs) by varying state survival (ϕ), detection (p) and transition (ψ), number of total capture occasions and releases per capture occasion and then modified these scenarios to mimic false rejection error (FRE), a common misidentification error, resulting from the failure to match photographs of the same individual in photograph capture recapture datasets. I then fit a multistate model and estimated accuracy, bias and precision of state-specific ϕ, p and ψ to better understand the effects of FRE on different simulation scenarios. The effect of FRE on bias was not consistent among parameters and differed by CH scenario. As expected, ϕ was negatively biased with increased FRE (except for the low ϕ low p CH scenario simulated with a low sample size), but I found that the magnitude of bias differed by scenario (high p CH scenarios were more negatively biased). State transition was relatively unbiased, except for the low p CH scenarios simulated with a low sample size, which were positively biased with FRE, and high p CH scenarios simulated with a low sample
size. My results demonstrate how FRE leads to relatively high bias in parameter estimates in a multistate model with the exception of $\psi$ when estimated using an adequate sample size.

In Chapter 2, I modeled landscape resistance surfaces to identify landscape characteristics that are highly resistant to dispersal and movement while also identifying areas in the landscape with high connectivity for *A. opacum* and spotted salamanders (*Ambystoma maculatum*). Here, I fit multi-scale/layer landscape resistance surfaces to estimate resistance to inferred gene flow. A resistance surface with forest land cover at a 500m Gaussian kernel bandwidth, and normalized vegetation index at a 100m Gaussian kernel bandwidth was the top optimized resistance surface for *A. maculatum*. A resistance surface with traffic rate and topographic curvature, both at a 500m Gaussian kernel bandwidth was the top optimized resistance surface for *A. opacum*. My findings highlight the success of using a novel analytical approach in a multi-scale framework with applications beyond amphibian conservation.

In Chapter 3, I assessed the performance of the R package ResistanceGA to correctly optimize resistance surfaces in relation to sample size, level of spatial autocorrelation in the true resistance surface, and level of variance in genetic distance data. ResistanceGA was able to reliably optimize resistance surfaces under a range of scenarios, resulting in optimized surfaces that were typically highly correlated with the true data-generating surface. Correlation between the true and optimized resistance surfaces remained high with increased variance in genetic distance, but only when sample size was moderate to high ($\geq 50$). Model selection error was also driven by sample size with low rates of type I error when simulations had moderate to high
sample sizes, even with moderate to high variance in pairwise genetic distances and spatially correlated alternative surfaces. Type I error was greater in multivariate simulations, as individual surfaces that were used to develop the true multivariate resistance surface were frequently identified in isolation as the top model due to the increased AIC penalty with multivariate models. Overall, my simulations highlight the accuracy of ResistanceGA for optimizing resistance surfaces with moderate to high sample sizes and highlight the success of a modified-bootstrap procedure towards more robust model selection.

In Chapter 4, I fit a multistate survival ($\phi$) model to the photograph capture recapture dataset to i) measure unbiased estimates of $\phi$ and breeding frequency, ii) explore whether climate variables relate to these measures and, if so, iii) investigate whether $A. opacum$ life history strategy could buffer population decline with future environmental change. I found a significant cost of breeding on $\phi$, especially on female $\phi$, with much lower $\phi$ for breeders who migrate to the vernal pool (VP) to breed compared with $\phi$ of non-breeders who “skipped” breeding and stayed in the upland forest habitat. I also found significant variability in $\phi$ of breeding individuals, especially females, as $\phi$ was a function of how long individuals were at VPs breeding, which depends on when dry VPs fill with water in the Fall. Annual $\phi$ was also driven by total summer precipitation, with lower $\phi$ of $A. opacum$ in years with higher summer precipitation. I found that females often “skipped” breeding, with $>60\%$ of females transitioning from breeders to non-breeders in a given year but found no evidence that climate variables drove transition rates from breeder to non-breeder or vice versa.
Results of my research provide new insights regarding the adaptive capacity of *A. opacum* populations to future environmental change. First, genetic differentiation of *A. opacum* was found to be driven by roads and landscape curvature in our study area which has resulted in population clustering (K=3; Whiteley, McGarigal, & Schwartz, 2014). This suggests that *A. opacum* may have difficulty tracking future climate change if movement, dispersal and ultimately gene flow are restricted due to both anthropogenic and natural topographic features of the landscape. Second, multi-state survival model estimates suggested that climate change could directly reduce *A. opacum* survival through increases in summer precipitation amounts and indirectly reduce *A. opacum* survival through increases in the length of the breeding interval due to increases in fall temperature and decreases in precipitation as mortality rates at the vernal pool. Lastly, no evidence was found that individual *A. opacum* could pick up on environmental cues that breeding conditions would be “riskier” in a given year due to a longer breeding interval and “skip” breeding at higher rates (particularly females), to buffer the population from such environmental conditions.
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CHAPTER 1

EFFECTS OF PHOTO AND GENOTYPE-BASED MISIDENTIFICATION ERROR ON ESTIMATES OF SURVIVAL, DETECTION AND STATE TRANSITION USING MULTISTATE SURVIVAL MODELS

Introduction

Knowledge of wildlife population dynamics is a crucial step towards species conservation and necessary if we wish to improve our understanding of the potential effects of climate change and future land use change. Accurate and precise parameter estimates of population vital rates (e.g., adult and juvenile survival rates, fecundity, etc.) are necessary for deciphering population dynamics and in forecasting future population projections. Survival ($\phi$) can be estimated using capture-mark-recapture (CMR) models, but accuracy of the estimates relies on meeting model assumptions, which often include: 1) individuals in a population all have an equal probability of being marked and recaptured; and 2) marks are permanent and they are observed, identified and recorded accurately at recaptures (Cormack, 1964; Jolly 1965.; Seber, 1965). Traditional CMR techniques depend on physical capture, tagging and subsequent recapture, resighting or recovery. Photo and genotype-based CMR are less invasive, not requiring physical capture, but, photo-based CMR depends on focal species having unique visual markings (Gamble, Ravela, & McGarigal, 2008; Morrison et al., 2011) and genotype-based CMR relies on the presence of highly polymorphic molecular markers (Taberlet et al., 1997; Lukacs & Burnham, 2005).
Photo and genotype-based CMR are now more feasible due to readily available software packages, which match large libraries of photo or genetic samples (Valière, 2002; Ayres & Overall, 2004; Gamble et al., 2008; Morrison et al., 2011). With photo-based CMR software, the matching process is typically not completely automated (although see Sherley et al., 2010), but relies on the user manually reviewing each photo with the most similar photos in the dataset to identify matches, with the specific measure of similarity being the major differences among software (Gamble et al., 2008; Bolger et al., 2012). Once photos are reviewed, individual capture histories (CHs) can be inferred from photo matches, which is required data input for CMR models. Unlike photo-based CMR, genotype-based CMR is not limited to species with distinct individual markings, as DNA samples can be collected without physical capture from hair samples or feces and with physical capture from saliva or tissues (Taberlet et al., 1997; Puechmaille & Petit, 2007; Latham et al., 2012). Individual identification is possible with polymorphic molecular markers, such as microsatellite loci or single nucleotide polymorphisms (Paetkau et al., 2004).

Advances with these techniques (e.g., digital photo quality for photo-based CMR and laboratory protocols for genotype-based CMR) have led to improvements in photo- and genotype-based CMR, but like the more traditional CMR approaches, they are not without error (Creel et al., 2003). One particularly important source of error in these non-invasive CMR methods is the misidentification of true matches; i.e., failure to match a new photo or genetic sample with an existing photo or genetic sample of the same individual, leading to incorrectly
concluding that it is a different individual. These "false rejections" are typically due to poor photo quality or image processing issues (e.g., significant photo glare) in the case of photo-based identification, or DNA degradation leading to false alleles or allelic dropout error in the case of genotype-based identification (Drechsler, Helling, & Steinfartz, 2015). False rejection error (FRE) is measured by estimating the percentage of known match photos or genetic samples (from the same individual) that are not identified as matches by the respective software. For photo-based CMR, FRE is a consequence of low similarity scores between matching and non-matching pairs of photos, and can differ depending on the number of top ranking photos reviewed per photo and the overall photo library size (Gamble et al., 2008; Morrison et al., 2011). For genotype-based CMR, FRE is driven by the proper selection and number of loci, PCR errors due to poor DNA quality and allele-shifting artifacts (Drechsler et al., 2015).

False rejection error can be as high as 25% for photo-based CMR, but is typically significantly lower for genotype-based CMR datasets (Drechsler et al., 2015). Unfortunately, FRE is not usually integrated into open CMR models under the assumption that if FRE is relatively low it will not significantly bias CMR model parameter estimates, even with a number of available statistical approaches recently developed (Lukacs & Burnham, 2005; Wright et al., 2009; Link et al., 2010; Yoshizaki et al., 2011; Bonner & Holmberg, 2013). This is in spite of simulation findings that even low FRE will bias estimates of \( \phi \) (Morrison et al., 2011). Negative bias occurs because false rejections cause erroneous CHs resulting in a capture history with a non-detection estimate instead of a detection estimate and the creation of an additional 'ghost
history’ comprised of a single detection. Capture histories of both types contribute to lower estimates of $\phi$ and detection ($p$). False rejection error has previously been found to bias estimates of $\phi$ and $p$ using simulated data but has not been evaluated within a multistate modeling framework. Multistate models allow for estimation of an additional state transition parameter, which estimates the probability of individuals transitioning among pre-defined “states”.

Here, we simulate the effects of FRE on parameter estimation in a multistate model framework by generating multistate CHs under a gradient of realistic FRE rates. We use different scenarios of high and low $\phi$ and $p$, different combinations of number of capture occasions and releases per capture occasion. Multistate models are an important class of CMR models and have been described as a unifying CMR modeling approach due to the fact that “states” can describe multiple aspects including age, geographic location, breeder or non-breeder, etc. making them applicable to a range of applications (Lebreton et al., 1992).

**Materials and methods**

To determine the effects of photo- and genotype-based CMR FRE on bias, precision and accuracy of estimates of $\phi$, $p$ and $\psi$, we simulated multistate (two states) CHs under four different CH scenarios (Table 1.1.) and numerous sample sizes (number of capture occasions and number of releases per capture occasion) using available R code (Kery & Schaub, 2012). All CH scenarios had constant and relatively low transition probabilities between states, but the transition probability from state A to B (0.3) was set slightly higher than the transition
probability from B to A (0.2) (Table 1.1.). We simulated CHs under a scenario of 3 total capture occasions (to represent a shorter-term research study) and under a scenario of 10 total capture occasions (to represent a longer-term research study) along with a varying number of releases per capture occasion (25, 50, 100, 500 or 1,000) for each unique CH scenario. For each simulated CH, each individual capture had a probability of being misidentified (i.e., falsely rejected) following a Bernoulli process (0.00, 0.01, 0.05, 0.10, 0.15, 0.20 or 0.25) with estimates spanning the range of values reported in empirical studies (Bolger et al., 2012). When an error occurred, the CH was modified to reflect the error and a ‘ghost’ history was created. For example, if we had a CH of AAAB000000, and the 2nd capture was deemed to be a “false rejection” based on the Bernoulli process, then the initial CH was modified to A0AB00000 and a new ‘ghost’ capture history was also created 0A00000000. It is important to note that our simulation assumed that an individual could only be captured once and that it was not possible for a ‘ghost’ to be recaptured.

For each unique simulation (4 combinations of $\phi$ and $p \times 7$ FRE rates $\times 5$ release per capture occasion $\times 2$ study durations $= 280$ unique simulations total; Table 1.1.), we ran 1,000 iterations. Each iteration, we fit a time invariant multi-state model ($\phi$, $p$, $\psi$) with an identity link function in program MARK (White & Burnham, 1999) using the RMark package (Laake, 2013) for model parameterization in R. Our simulation code discarded iterations where the Hessian was not positive singular or when program MARK gave a warning in respect to model convergence. We derived estimates of model parameters ($\phi_A$, $\phi_B$, $p_A$, $p_B$, $\psi_{AB}$, $\psi_{BA}$; $A =$ state A, $B =$ state B)
using maximum likelihood (White & Burnham, 1999). We then calculated root mean square error (RMSE), a common measure of accuracy, as,

\[
RMSE(\hat{\phi}) = \sqrt{\frac{\sum_{i=1}^{n} (\hat{\phi}_i - \phi)^2}{n-1}},
\]

where \(\hat{\phi}_i\) is a survival estimate from a single iteration, \(\phi\) represents the true \(\phi\) and \(n\) is the number of iterations. We calculated relative bias (hereafter, simply "bias") as

\[
Rbias(\hat{\phi}) = \frac{\sum (\hat{\phi}_i - \phi) / \phi}{n}.
\]

Standard error (a measure of precision) was calculated as,

\[
SE(\hat{\phi}) = \sqrt{\frac{\sum_{i=1}^{n} (\hat{\phi}_i - \hat{\phi})^2}{n-1}},
\]
where $\hat{\phi}$ is the mean of the $n$ survival estimates. We computed the RMSE, mean bias and mean standard error across the 1,000 iterations for each multistate model parameter ($\phi_A$, $\phi_B$, $p_A$, $p_B$, $\psi_{AB}$, $\psi_{BA}$) for each unique CH scenario (Table 1.1.).

**Results**

**Survival**

As expected, $\phi$ decreased in accuracy with increased FRE, with lower accuracy when CHs were simulated using only 3 capture occasions and a lower number of releases per capture occasion (Fig. 1.1.; left panels). Survival of CHs simulated with 10 capture occasions decreased in accuracy with increased FRE and was lowest in those scenarios simulated with high $p$ (Fig. 1.1.; left panels). Survival estimates were more negatively biased (with the exception of the low $\phi$ low $p$ scenario) with increased FRE, but we found that the magnitude of bias differed by the CH scenario simulated (e.g., high $p$ vs. low $p$) (Fig. 1.1.; center panels). Bias in $\phi$ was greatest with the high $p$ CH scenarios (>15% at 25% FRE) and linearly increased with FRE (Fig. 1.1.; center panels). Precision of $\phi$ decreased with increased FRE and was lowest with the low $\phi$ low $p$ CH scenario (Fig. 1.1.; right panels). Accuracy, bias, and precision were similar for estimates of $\phi_B$ (Appendix A).
Detection

Similar to ϕ, accuracy of p decreased with increased FRE and was lower with the CH scenarios simulated using only 3 capture occasions and a lower number of releases per capture occasion (Fig. 1.2.; left panels). Detection of CHs simulated with 10 capture occasions decreased in accuracy with increased FRE and was lowest in those scenarios simulated with high p (Fig. 1.2.; left panels). Bias of p was greatest with the low p CH scenarios with bias increasing with FRE (> -35% at a 25% FRE) (Fig. 1.2.; center panels). Bias in p was positive with the low φ low p scenario simulated with low overall sample size and did not show a strong relationship with FRE (Fig. 1.2.; center panels). Detection estimates were least precise with CH scenarios with low φ low p, with precision decreasing with increased FRE under all CH scenarios, except under the CH scenario of low φ high p, where precision increased with increased FRE (Fig. 1.2.; right panels). Accuracy, bias, and precision were similar for estimates of p<sub>B</sub> (Appendix B).

State-transition

Accuracy of ψ decreased with increased FRE and was lower with the CHs simulated using only 3 capture occasions and a lower number of releases per capture occasion (Fig. 1.3.; left panels). As predicted, state transition was relatively unbiased, except with simulations parameterized using a low overall sample size (Fig. 1.3.; center panels). Precision of ψ was low with the high φ CH scenarios in comparison to the low φ CH scenarios and decreased with FRE
(Fig. 1.3.; right panels). Accuracy, bias, and precision estimates were similar for estimates of \( \psi_{BA} \) (Appendix C).

**Discussion**

Our simulations confirmed that misidentification error, specifically, FRE, can lead to bias and reduced accuracy and precision in both state-specific \( \phi \) and \( p \) (confirming results found in past simulations). False rejection error did not bias estimates of \( \psi \) (only when simulations were performed using inadequate sample sizes). Overall, the magnitude of the effect of FRE depended on the absolute value of the parameter being estimated (i.e., \( \phi \), \( p \), or \( \psi \)), FRE rate, and the number of simulated capture occasions and number of releases per capture occasion (overall sample size). Effects of FRE and overall sample size on the accuracy, bias and precision of \( \phi \) and \( p \) are of particular concern given the implications for population modeling (see below). Fortunately, precision in \( \phi \) estimates do not appear to be overly sensitive to FRE, although is lower in those simulations representing a shorter-term study and a lower number of releases per capture occasions and may introduce additional uncertainty into subsequent population models (see below).

Estimates of \( \psi \) were unbiased and insensitive to FRE with the exception of scenarios simulated with a low number of capture occasions and number of releases per capture occasion (particularly those CH scenarios simulated with low \( p \)). Accuracy of \( \psi \) was also insensitive to FRE when simulated with an adequate sample size, with inaccuracy of estimates likely due to a
higher number of iterations estimating $\psi$ at the boundary and not a result of model convergence issues. To our surprise, precision of $p$ and $\psi$ estimates sometimes increased with FRE, which was counterintuitive to our initial predictions and needs to be further investigated. Our finding that $\psi$ estimates were unbiased and relatively robust to FRE was not surprising, as ‘ghosts’ could not be recaptured in our simulation framework (transition probabilities are conditioned on individuals being captured multiple times in defined “states”). In theory, recapturing of ‘ghosts’ is likely to be extremely rare as it would be a result of false acceptance error (FAE), which is the probability of samples (e.g., photos) from different individuals being falsely matched during manual review. False acceptance error rates have previously been found to be very low and this rate will be even lower with ‘ghosts’ (Bolger et al., 2012).

**Reduce false rejection error**

Our results highlight that unbiased, and more precise and accurate CMR parameters, particularly $\phi$, can be achieved if FRE is relatively low (<5%) or eliminated. For photo-based CMR, FRE can be reduced by increasing processing effort per image (e.g., more precise cropping to only include relevant pattern), reducing overall photo library size and comparing results between available photo recognition software which use different techniques and algorithms for photo matching (Gamble et al., 2008; Morrison et al., 2011; Matthé et al., 2017). Although, if the difference in similarity measure between matching photos and non-matching photos is relatively high, reducing library size may not significantly decrease FRE (and may not
be a practical alternative regardless) and an alternate approach may be to filter and remove low-quality photos (e.g., debris on pattern, heavy glare) to reduce overall FRE.

False rejection error with genotype-based CMR has been significantly reduced due to improvements in field protocols, laboratory procedures, and advancements with software (Paetkau et al., 2004; Lukacs & Burnham, 2005). Selecting the proper and adequate number of loci is also crucial for obtaining highly confident exclusion probabilities, to ensure individuals are correctly classified. Knowledge of these loci is species-specific and better understood for some species than others. If feasible, and if a high error rate is a concern, using multiple CMR techniques (photo and genotype-based CMR) instead of just a single CMR technique may be a feasible option to reduce FRE (Drechsler et al., 2015).

**Incorporating false rejection error into CMR models**

Recently, both ad-hoc and post-hoc approaches have been developed to deal with FRE. Ad-hoc approaches include the ‘conditioning approach’, which involves filtering and discarding initial captures of non-ghosts. This approach was found to produce better estimates (in terms of RMSE) compared with ‘unconditioned’ data when FRE was >5% (Morrison et al., 2011). Unfortunately, this leads to a loss of overall data, as it requires removing capture information and results in lower precision with parameter estimates.

Post-hoc solutions to the bias caused by ghost histories seem analogous to issues caused by transients (Yoshizaki et al., 2011), although transients and residents have independent $p$
probabilities, whereas ghosts and non-ghosts produced from misidentification do not (Gamble et al., 2008). Traditional CMR models for transients that assume data are drawn from multinomial distributions are inappropriate, preventing the derivation of a multinomial likelihood function (Morrison et al., 2011; Yoshizaki et al., 2011). Recently, statistical approaches have been developed to incorporate misidentification with Bayesian, unweighted least squares and chi-square statistical approaches that perform well under certain scenarios (e.g., those where capture probabilities are high). Although potentially flexible, many existing statistical approaches incorporating FRE focus on estimating population size, rather than $\phi$, with closed population models but are not yet incorporated into existing CMR software packages.

**Implications for population modeling**

Slight changes in survival rate (<5%), especially adult survival can significantly change estimates of population growth, particularly for species with high adult survival, late maturation and few offspring (Heppell, Caswell, & Crowder, 2000). Bias in $p$ can also have negative implications with estimating population size, which was not simulated in this study, but complements past studies looking at the effects of FRE on estimating population sizes in closed population models (Heppell et al., 2000; Lukacs & Burnham, 2005; Link et al., 2010; Yoshizaki et al., 2011). If ignored, bias in both $\phi$ and $p$ can potentially lead to management decisions and actions that are based on wrong estimates. The fact that bias in $\psi$ was relatively insensitive to
FRE (except in scenarios with inadequate sample sizes) suggests that $\psi$ may be more robust to FRE and adds to the overall broad applicability of this class of CMR models.

**Future directions**

Our simulation results are most relevant to those situations where only one sample (photo or genetic) per individual is collected per capture occasion. Multiple samples of individuals per capture occasion could theoretically lead to more accurate CHs depending on the matching protocols used. For example, allowing any new sample from the current capture occasion that matches an existing individual in the library to result in a "recapture" for that occasion (i.e., allowing multiple opportunities to confirm a match) could reduce overall FRE. Conversely, having multiple samples of the same individual that do not match individuals in the library due to poor sample characteristics could lead to higher numbers of ‘ghosts’ created per capture occasion. Exploring FRE in this context of having multiple samples from the same individual per capture occasion is important as it may lead to different levels of accuracy, bias and precision in parameter estimates and will require different statistical methods to incorporate into CMR models (although see Morrison et al., 2011 for a relevant example).

Improvements also need to be made in better understanding the mechanisms behind FRE. For photo-based CMR, FRE is currently based on the percentage of known pair matches (e.g., photos matched by “eye”) that are not found to be matches by the respective matching software. In reality, the photo-recognition software outputs a relational database with photo matches (e.g.,
photo A and photo B do not match, but photo A and photo B match photo C, thus photo A and B match). Thus, FRE may decrease with an increased overall number of photos of the same individual or an increased number of capture occasions, where misidentification error is not necessarily due to a “bad” photo that will not rank highly with any other photos of the same individual in the dataset (as our multistate CH code simulates), but is a photo that will rank highly with other photos of the same individual and thus eventually match with a photo that it does not directly match with due to the nature of the relational database. Testing known match photos that are not constrained to just being pairwise matches (e.g., multiple photos of the same individual) could provide insight into how this error changes with overall number of photos by individual. In theory, FRE could significantly decline if there are more than a couple of photos per individual.
Table 1.1 Summarized parameter values used for simulating capture histories used to evaluate effects of false rejection error on multistate model parameters.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>$\phi_A$</th>
<th>$\phi_B$</th>
<th>$p_A$</th>
<th>$p_B$</th>
<th>$\psi_{AB}$</th>
<th>$\psi_{BA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>high $\phi$ high $p$</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
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<td>0.90</td>
<td>0.40</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td>low $\phi$ high $p$</td>
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<td>0.90</td>
<td>0.40</td>
<td>0.90</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td>low $\phi$ low $p$</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.30</td>
<td>0.20</td>
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</tbody>
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Figures

Figure 1.1 Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of $\phi_A$ estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
Figure 1.2 Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of pA estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
Figure 1.3 Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of ψ_{AB} estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
CHAPTER 2

MULTI-SCALE RESISTANT KERNEL SURFACES DERIVED FROM INFERRED GENE FLOW: AN APPLICATION WITH VERNAL POOL BREEDING SALAMANDERS

Introduction

Foundational ecological theory on the importance of spatial scale in ecology (Wiens, 1989; Levin, 1992) has recently been validated by empirical studies evaluating environmental and anthropogenic predictors in a multi-scale context, which involves consideration of landscape variables not just at their original scale but at multiple spatial and/or temporal scales (McGarigal et al., 2016). This includes studies evaluating predictors at multiple scales with i) species-habitat relationships (Johnson et al., 2002; Grand et al., 2004; Chambers et al., 2016; Timm et al., 2016), ii) species abundance (Chandler & Hepinstall-Cymerman, 2016) and iii), and landscape resistance (Zeller et al., 2014; Cushman et al., 2016; Krishnamurthy et al., 2016; Zeller et al., 2017). These studies highlight that environmental and anthropogenic predictors measured at multiple scales (hence incorporating varying ecological neighborhood sizes; sensu Addicott et al., 1987) not only result in improved inferences and predictions, but importantly provide a more nuanced understanding of how species relate and utilize their environment (McGarigal et al., 2016). These studies highlight the potential of a multi-scale evaluation of environmental and anthropogenic predictors with a broad range of ecological disciplines and analyses.
The goal of landscape genetics is to understand gene flow and spatial genetic patterns within species in response to landscape composition and configuration with a reliance on conceptual ideas and tools from landscape ecology, population genetics and spatial statistics (Manel et al., 2003; Manel & Holderegger, 2013a). Understanding genetic connectivity and the spatial layers driving (or limiting) gene flow and resulting genetic spatial patterns is crucial if applied conservation goals include: i) management of the landscape to restore genetic connectivity to isolated populations to ensure long term viability (Greenwald, 2010; Whiteley et al., 2015), or ii) management of the landscape to allow for species movement or rapid adaptation to future landscape and environmental changes such as those driven by climate change (Velo-Antón et al., 2013).

Landscape genetics studies have previously been conducted at multiple spatial extents to compare differences in landscape predictors of gene flow at population and individual levels (Dudaniec et al., 2013; Keller, Holderegger, & Strien, 2013) and explored multiple scales in a more indirect manner (Murphy et al., 2010; Van Strien, Keller, & Holderegger, 2012; Coster et al., 2015), but only recently has a landscape genetics study estimated landscape resistance (LR) with environment and anthropogenic predictors at multi-scales within a single spatial level (Zeller et al., 2017). Advances have also been made with analytical approaches to measure landscape resistance (Richardson et al., 2016), including the optimization of landscape resistance surfaces (LRS) using genetic algorithms (Peterman, 2018) rather than pseudo optimization of resistance (Shirk et al., 2010) or the reliance on expert derived a priori assessments of LR, which
have been found to perform poorly (Charney, 2012). LRS optimization is one such ecological analysis that could improve by assessing the anthropogenic and environmental drivers of gene flow at multiple scales.

Here, we fit multi-scale LRS for two Abystomatid salamanders. We used genetic distance datasets of spotted salamanders (*Ambystoma maculatum*; AMMA) and marbled salamanders (*Ambystoma opacum*; AMOP) from a recent study by Whiteley, McGarigal, & Schwartz (2014), which found that although ecologically very similar, AMMA and AMOP had differing genetic structures and rates of inferred gene flow across the landscape. We hypothesized that for both species inferred gene flow would be highest through natural areas that facilitate movement and survival in our study area, such as forested habitats with high densities of vernal pools (VPs) with topography well-suited for the physiological conditions required for salamander migration and dispersal (e.g., specific range of topographic wetness), and that inferred gene flow would be more constrained in areas with high densities of roads and urban development or by natural topographic barriers on the landscape such as ridges or large rivers. We predicted that the differences found in the genetic patterns and inferred gene flow between AMMA and AMOP would potentially be explained by species-specific differences in the relationships between environmental, topographic and anthropogenic features with LR and differences in the species specific optimized spatial scales. We also sought to identify which VPs across our study area were most important for species-specific gene flow based on their landscape context and inferred connectivity at local, neighborhood and regional levels.
Methods

Study site

Our study was conducted in the Pioneer Valley of western Massachusetts (Fig. 2.1.). The Pioneer Valley is bisected by the Connecticut River and is characterized in the north by an agricultural valley interspersed with residential development and in the south-central by high density urban development. The Pioneer Valley also contains high elevation features (e.g., Holyoke Range) and transitions gradually to the west and east into areas of higher elevation dominated by forest interspersed with lower density residential development.

Population sampling

We collected larval AMMA and AMOP from 19 VPs (S1-S19) and 29 VPs (M1-M29), respectively, distributed across the Pioneer Valley (Fig. 2.1.). Larval AMMA were collected during the Summer of 2007 and 2008 and larval AMOP were collected during the Spring of 2010. We sampled VPs by visually scanning the VP perimeter after dusk with a headlamp. We collected approximately 30 larval salamanders from each sampled VP, ensuring that the complete perimeter of the VP was sampled. The specific number of individuals sampled varied by VP and depended on local population size and reproductive success prior to sampling (Whiteley et al., 2014). A tissue sample (tip of tail) was taken from each individual as a source
of genetic material and individuals were then released back into the VP. See Whiteley et al., (2014) for more detail regarding larval salamander sampling.

**Landscape genetics analysis**

We extracted DNA from each larval tail clip with a standard salt precipitation procedure. AMMA and AMOP were genotyped at eight micro satellite loci: *AmaD321, AmaD95, AmaD287, AmaD328, AmaC40, AjeD23, AmaD49, AmaD184* and *AMaD49, Aop36, AmaD95, AmaD184, AmaD42, AmaD328, AjeD23*, and *AmaD321*, respectively (Julian et al. 2003a, b; Croshaw et al. 2005). We used Qiagen multiplex buffer (Qiagen, Inc.) with the manufacturer recommending thermal cycler profile for micro satellite amplification. We used an Applied Biosystems 3130x1 capillary sequencer to determine the size of PCR fragments. We used Gene mapper and PeakScanner (Applied Biosystems) to score individual genotypes based on the ROX 500 size standard run with each individual. Whiteley et al., (2014) reported detailed population genetic analyses for this same set of populations for both species, including an analysis of the influence of full-sibling families on population genetic structure. The practice of removing full-siblings for some population genetic analyses has recently been called into question (Waples & Anderson, 2017). Based on the previous analysis of our focal populations, inclusion of full-sibling families in the analysis increased the signal of genetic differentiation (Whiteley et al., 2014). Therefore, we chose to limit the final dataset to a single randomly sampled full-sibling per family from each VP for each species.
We calculated chord distance ($D_c$) between local populations with GENODIVE version 2.0 (Meirmans & Tienderen, 2004). We discarded two AMMA local populations (Appendix D and Appendix E) and seven AMOP local populations (M4, M11, M14, M24, M26, M28 and M29) that contained 10 or fewer estimated individuals prior to the calculation of genetic distance. We fit an isolation by distance model for AMMA and AMOP with $D_c$ as the response variable and Euclidean distance (m) as the independent variable using a linear mixed-effects model (R package lme4; Bates et al., 2015) with a maximum-likelihood population effects (MLPE) parameterization to account for the non-independence of values within pairwise distance matrices (Clarke, Rothery, & Raybould, 2002).

**Multi-scale evaluation of spatial layers**

We evaluated 13 environmental and anthropogenic spatial layers that we hypothesized could predict the gene flow and genetic patterns of AMMA and AMOP across the study area (Table 2.1.). Spatial layers included: 1) topographic curvature, which we calculated using DEM Surface Tools (Jenness, 2013), 2) impervious surfaces (2005), 3-6) forest land cover at 4 temporal snapshots (1971, 1985, 1999 and 2005), 7) normalized difference vegetation index from July of 2012 (NDVI), 8) potential vernal pools (PVP), 9) slope, 10) topographic position index (TPI), 11) traffic rate, 12) topographic wetness index (TWI), and 13) water flow rate (Fig. 2.2.). We increased the cell resolution for all spatial layers from the original resolution to 60m using the Resample tool in ArcGIS to make our analysis computationally feasible. To evaluate
surfaces at multiple spatial scales, we smoothed all resulting 60m surfaces using a Gaussian kernel with 100m, 500m, 1000m, 1500m and 2000m bandwidths with the R package gridio (http://jamba.provost.ads.umass.edu/web/plunkett/gridio.html; Plunkett.)

**Multi-scale single layer landscape resistance surface optimization**

We used the R package ‘ResistanceGA’ (Peterman, 2018; https://github.com/wpeterman/ResistanceGA) to first optimize a LRS for each spatial layer independently to determine the best supported Gaussian kernel bandwidth for each spatial layer and then to fit multi-scale/layer LRSs. ResistanceGA uses a genetic algorithm (R package GA; Scrucca, 2013) to iteratively optimize the resistance of a layer using 8 different functional transformations (e.g., monomolecular or Ricker family transformations) and a shape and maximum resistance parameter. We limited the functional transformation in our analysis to monomolecular for all spatial layers except TWI, where we also allowed transformations in the Ricker family. We used pairwise genetic distances (chord distance; $D_C$) between local populations as the dependent variable and scaled and centered effective pairwise resistance distances between local populations as the independent variable, which were calculated using CIRCUITSCAPE version 4.0.3 (McRae et al., 2008a) in the model (based on the transformed resistance surface). We used an eight-neighbor connection scheme to measure average resistance distances between all sample pools. ResistanceGA fits a LMM (R package lme4; Bates et al., 2015) using the MLPE parametrization to account for the non-independence of values within
pairwise distance matrices (Clarke et al., 2002). We used AICc (Akaike’s information criterion corrected for small/finite sample size; Akaike, 1998) as our objective criterion during iterative genetic algorithm optimization.

**Multi-scale/layer landscape resistance optimization**

We optimized multi-scale/layer LRSs as follows. First, we restricted consideration to the best supported (delta AICc < 5) single layers at their best spatial scale. Next, we computed Pearson’s correlation coefficients for each pairwise combination of the retained univariate layers at their best spatial scale. Next, we fit pairwise combinations of the retained covariates at their best spatial scale, but restricted from consideration any combinations of covariates with correlations >0.7 to avoid multicollinearity (Appendix D and E). We limited multi-layer models to a maximum of two covariates due to difficulties in model convergence of more complex models. We found that the multi-scale/layer LRSs that we derived from this restricted set of single layer spatial scales did not outperform the best supported single-layer LRSs, based on AICc. We suspected that this might be due to the moderate correlations between layers at their optimized spatial scale (Appendix D and E), despite the restrictions we imposed to safeguard against multicollinearity. In addition, we suspected that this approach might be masking scale complementarity among covariates; e.g., a fine-scale covariate complementing a coarse-scale covariate. Consequently, we then selected our top two performing single-layer LRSs for AMOP and AMMA and fit all two-layer combinations of these two covariates at all spatial scales,
including the original scale (60 m) and smoothed layers at all bandwidths (100m, 500m, 1000m, 1500m and 2000m). Again, we restricted from consideration any pairwise combinations of the two covariates (at any scale) with correlations > 0.7.

**Multi-scale/layer landscape resistance surface prediction averaging**

To develop a single LRS, we model averaged predictions from our “All Combinations” multi-scale/layer LRSs for models with delta AICc < 10. We rescaled all multi-scale/layer LRSs from 1 to 100 prior to prediction averaging and rescaled the final prediction averaged multi-scale/layer LRS from 1 to 40 to match the LRS range used in Compton et al., (2007). We then measured the Spearman’s rank correlation coefficient between the prediction averaged AMMA and AMOP LRSs and the expert derived LRS in Compton et al., (2007). We used the function rasterCorrelation from the R package spatialEco (Evans, 2017) to highlight where in the study area the AMMA and AMOP LRSs showed agreement and disagreement in LR value. We also measured the Spearman’s rank correlation coefficient of the best supported AMMA and AMOP multi-scale/layer and the corresponding multi-layer LRSs at the original 60m scale.

**Scoring and identifying important vernal pools**

We identified high quality and highly connected VPs for AMMA and AMOP by scoring them at local, neighborhood and regional levels following Compton et al., (2007). Our approach
differed in that our resistant kernels (see below) were driven by an empirically optimized multi-scale/layer LRS versus an expert derived single-scale/layer LRS.

Local score.—VP scores at the local level were determined by the intensity of forest land cover in 2005 within the ecological neighborhood of each PVP defined by a Gaussian kernel. The Gaussian kernel bandwidth $b$ (the standard deviation of a bivariate normal curve) was 124m, which was based on the 66th percentile of maximum migratory distances from VPs for 28 individual spotted salamanders (Mcdonough & Paton, 2007), as in Compton et al., (2007). We calculated the local score for each PVP by summing the Gaussian weights of forested cells surrounding the pool (Appendix F).

Neighborhood score.—VP scores at the neighborhood level reflect how well a VP is connected to neighboring vernal pools. VP scores at the neighborhood level were based on a Gaussian resistant kernel (Compton et al., 2007). Briefly, a Gaussian resistant kernel uses a multi-directional least-cost path algorithm that measures the functional distance from a focal cell to every neighboring cell within a defined dispersal distance (Compton et al., 2007; Cushman, Lewis, & Landguth, 2014). Compton et al., (2007) used a bandwidth of approximately 400m, which was the standard deviation of a normal curve of dispersal distances from a study of AMOP dispersal distances among 14 vernal pools in our study area (Gamble, McGarigal, & Compton, 2007). We increased the Gaussian resistant kernel bandwidth $b$ to 800m because we found that a 400m bandwidth did not adequately discriminate neighborhood scores among VPs across our landscape (Appendix F). This was due to the LR values of our LRSs being higher on average
than the values in Compton et al., (2007), limiting the spread of our Gaussian resistant kernels. We calculated the neighborhood score for each VP by summing the resistant kernel value of neighboring VPs overlapping the focal VP.

Regional score.—VP scores at the regional level were determined by the total number of VPs within a VP “cluster” (Compton et al., 2007). Clusters consisted of discrete overlapping neighborhood kernels on the landscape and were identified using the function patch scan in the R package gridio. Briefly, we first applied a Gaussian resistant kernel similar to our neighborhood Gaussian resistant kernel but increased the bandwidth (2800m for AMMA and 2000m for AMOPs) to better capture gene flow over multiple generations at a broader spatial scale (Appendix G). A bandwidth >2000m for AMOPs resulted in very large clusters of VPs which didn’t allow us to distinguish regional scores amongst VPs in our landscape as all VPs received a high regional score. The same was not true for AMMAs and we felt that a higher Gaussian kernel bandwidth could be justified for AMMA due to a life history that potentially allows for higher regional connectivity (Burkhart et al., 2017). We calculated the regional score for each VP by counting the number of VPs in the "cluster" containing the focal VP.

To calculate a final species-specific score for each VP, we first rescaled each score (local, neighborhood and regional) from 1 to 10 and then computed the geometric mean. We also measured the Spearman’s rank correlation coefficient between the AMMA, AMOP and Compton et al., (2007) VP geometric mean scores.
Results

Genetic differentiation and gene flow

Genetic differentiation among local VP populations (measured by $D_c$) was stronger with AMOPs than with AMMAs across our study area (Fig. 2.3; Appendix F and G). Whiteley et al., (2014) identified three population-level clusters of VPs sampled for AMOPs, and only 1 population-level cluster for AMMAs, indicating landscape features were likely limiting gene flow more for AMOPs than for AMMAs. AMMA exhibited a weaker linear increase in genetic differentiation with increasing geographic distance (Fig. 2.3.). See Whiteley et al., (2014) for more detail regarding genetic differentiation among populations for AMOPs and AMMA.

Multi-scale/single-layer landscape resistance surfaces

The single-layer LRS that best described genetic pattern and inferred gene flow for AMMAs based on AICc was our most recent temporal representation of forest land cover (2005)(Fig. 2.4.). Forest land cover in 2005 was optimized with a reverse monomolecular transformation, 1.66 shape and 259 maximum resistance value, and with forest land cover smoothed using a 500m bandwidth Gaussian kernel. Resistance increased with decreasing forest land cover. Top surfaces based on AICc also included LRSs derived from NDVI (500m bandwidth) and impervious surfaces (500m bandwidth) spatial layers (Fig. 2.4). The single-layer LRS that best described genetic pattern and inferred gene flow for AMOPs based on AICc was
traffic rate (Fig 2.4.). Traffic rate was optimized with a monomolecular transformation, 0.08 shape and 180 maximum resistance value, and with traffic rate smoothed using a 500m bandwidth Gaussian kernel. Resistance increased with increasing traffic rate, although resistance asymptotically approached its maximum value at relatively low traffic rates. Topographic curvature (1000m bandwidth) and impervious surfaces (500m bandwidth) LRSs also performed well in describing the genetic pattern and differentiation of AMOP (Fig. 2.4.). Surprisingly, LRSs optimized using the PVP spatial layer performed poorly for both species at all spatial scales (Fig. 2.4.). More recent representations of forest land cover (2005) performed better than past representations of forest land cover for both AMMAs and AMOPs (Fig. 2.4.). LRSs modeled at the original 60m spatial scale performed relatively poorly compared to the smoothed layers for both species (Fig. 2.4.). For example, the “best” spatial layer for AMOP (traffic rate at a 500m bandwidth) was >9 AICc units better than traffic rate at the original 60 m spatial scale, and forest land cover (2005) at 500m bandwidth was >10 AICc units better than forest land cover at the 60m scale (Fig. 2.4.). TWI for AMOPs was the only LRSs which performed better at the original spatial scale based on AICc (Fig. 2.4.).

**Multi-scale/layer ecological resistance surfaces**

The multi-scale/layer LRSs derived by fitting spatial layers at their optimized spatial scale did not perform better than single-layer LRSs at their optimized spatial scale. For example, with AMMA, an LRS optimized from forest land cover (500m) had an AICc value 0.57 less than
a LRS optimized from both forest land cover (500m) and NDVI (500m). Similarly, with AMOP, an LRS optimized from traffic rate (500m) had an AICc value 0.32 less than a LRS optimized from both topographic curvature (1000m) and traffic rate (500m). However, LRSs derived from “All Scale Combinations” of the top two performing single-layer LRSs performed better than the single-layer LRSs and the multi-scale/layer pseudo-optimized bandwidth combinations. For AMMA, the top model included forest land cover (2005) smoothed using a 500m bandwidth Gaussian kernel and NDVI smoothed using a 1000m bandwidth Gaussian kernel (Appendix I). For this model, resistance decreased with increasing forest land cover, optimized with a reverse monomolecular transformation and 1.88 shape and 490 maximum resistance value, and decreased with increasing NDVI, optimized with a reverse monomolecular transformation and 1.54 shape and 470 maximum resistance value (Fig. 2.5.). For AMOP, the top model included traffic rate and topographic curvature, both smoothed using 500m bandwidth Gaussian kernels (Appendix J). For this model, resistance increased rapidly with increasing traffic rate, optimized with a monomolecular transformation and 0.21 shape and 237 maximum resistance value, and increased with topographic curvature, optimized with a monomolecular transformation and 1.08 shape and 250 maximum resistance value (Fig. 2.5.). The prediction averaged LRS from the multi-scale/two-layer “All Scale Combinations” LRS showed high resistance for AMMA in non-forested areas and high resistance for AMOP within the vicinity of roads and in areas on the landscape with high topographic curvature (Fig. 2.6.).
Correlation between the AMOP and AMMA LRSs was moderate (Spearman’s rank correlation coefficient = 0.70) with the different species-specific drivers (modeled at different spatial scales) of LR resulting in areas of agreement and disagreement in values across the study area (Appendix K). Correlation was higher between the AMMA LRS and the Compton et al., (2007) expert derived LRS (Spearman’s rank correlation coefficient = 0.70) than between the AMOP LRS and the Compton et al., (2007) expert derived LRS (Spearman’s rank correlation coefficient = 0.43)(Appendix K). Correlation between the multi-scale LRSs and the LRSs optimized at the original pixel size (60m) was moderate at best. The “best” AMMA LRS included forest land cover (500m) and NDVI (100m) and had a moderate correlation with the original forest land cover (60m) and NDVI (60m) optimized LRS (Spearman’s rank correlation coefficient = 0.70). The “best” AMOP LRS included topographic curvature (500m) and traffic rate (500m) and had very low correlation with the original topographic curvature (60m) optimized LRS (Spearman’s rank correlation coefficient = 0.11).

**Vernal pool scores**

Local scores were high for VPs outside of the Pioneer Valley where the land cover is dominated by forest (Fig. 2.7.). Neighborhood scores for AMMAs were highest in portions of the study area with low LR and high VP densities, which occurred in the western portion of the study area and in the Holyoke range (Fig. 2.8.). Neighborhood scores for AMOPs were also highest in portions of the study area with low LR and high VP densities, which occurred in the
portion of the study area east of the Connecticut River (Fig. 2.8.). Regional scores for AMMA were highest in a handful of VP clusters on both the west and east side of the Connecticut River, whereas regional scores for AMOPs were highest mainly on the east side of the Connecticut River (Fig. 2.8.). Overall scores (i.e., geometric mean of local, neighborhood and regional scores) for AMMAs were highest in multiple clusters of VPs in largely forested regions of our study area, which included the Holyoke range and areas west and east of the Pioneer Valley (Fig. 2.8.). Overall scores for AMOPs were highest in clusters of VPs in areas of low topographic curvature and away from roads in our study area, which occurred more in the eastern portion of the study area (Fig. 2.8.). In contrast to the moderate correlation patterns found between the AMMA and AMOP LRSs, correlation between the AMMA and AMOP VP scores was relatively low (Spearman’s rank correlation coefficient = 0.43)(Appendix L). Similar to the patterns observed for LRSs, correlation was higher between the AMMA and the Compton et al., (2007) VP scores (Spearman’s rank correlation coefficient = 0.70) than between the AMOP and the Compton et al., (2007) VP scores (Spearman’s rank correlation coefficient = 0.47)(Appendix L and M).

**Discussion**

Our research adds to the growing number of ecological disciplines and analyses successfully incorporating a multi-scale evaluation of relevant environmental and anthropogenic predictors (McGarigal et al. 2016) and highlights the benefits of such an approach when
optimizing LR. More specifically, our study resulted in the following major findings. Our multi-scale/layer LRSs empirically optimized using ResistanceGA performed better than LRSs derived from spatial layers at their original spatial scale and revealed different possible environmental and anthropogenic predictors of genetic pattern and inferred gene flow for these two ecologically similar species. Our focal species, VP breeding salamanders, also provided a study case in which genetic connectivity was constrained by the spatial configuration of the local populations across the landscape. Resistant kernels allowed us to i) model this connectivity between a VP and its surrounding uplands and among VPs at neighborhood and regional scales based on empirical dispersal data, and ii) score VPs based on this connectivity at multiple levels to better inform conservation of VPs in our study area.

Landscape resistance surfaces accounting for ecological neighborhood perform best

Multi-scale/layer LRSs empirically optimized using ResistanceGA performed better than single or multi-layer LRSs derived from spatial layers at their original spatial scale, demonstrating the importance of considering ecological neighborhood size (sensu Addicott et al., 1987) when deriving LRSs. Moreover, LRSs optimized with spatial layers smoothed at mid-scales (i.e., 100-500m bandwidth Gaussian kernels) performed best, and this is generally consistent with recent multi-scale explorations of species habitat use and movement (Grand et al., 2004; Zeller et al., 2017). For example, our “best” AMOP LRS based on environmental and anthropogenic surfaces at their original 60m spatial scale was topographic wetness index and our
“best” AMMA LRS was forest land cover (1985), based on AICc. Our multi-scale prediction averaged LRSs for AMMAs and AMOPs were much different than the multi-layer LRSs modeled at the original 60m spatial scale and would have resulted in different patterns and interpretation of species-specific LR and VP scores at the neighborhood and regional level.

Environmental and anthropogenic predictors of landscape resistance

Optimized multi-scale/layers LRSs revealed possible different environmental and anthropogenic predictors of genetic pattern and inferred gene flow for the two ecologically similar focal species. Environmental and anthropogenic predictors of LR and the species-specific differences were mostly in agreement with recent landscape genetics findings with VP-breeding amphibians and other taxa. Our AMMA multi-scale/layer LRS showed decreased resistance with increasing forest land cover (smoothed at 500m) and a finer scale measure of land cover type (as proxied by NDVI smoothed at 100m) and is consistent with several studies that have found reduced forest land cover and increased agriculture and residential development associated with increased population isolation and reduced gene flow with amphibians (Spear & Storfer, 2008; Greenwald, Gibbs, & Waite, 2009; Greenwald, Purrenhage, & Savage, 2009). Our AMOP multi-scale/layer LRS reflecting high resistance at relatively low traffic rates and increased resistance with topographic complexity (or curvature) is also consistent with other amphibian landscape genetics studies (Zellmer & Knowles, 2009; Richardson, 2012; Coster et al., 2015).
We were surprised with our optimized LRS for AMOP by the high resistance in the forested portions of our study area with high topographic curvature, as we expected that some of these areas were comprised of more intact habitat in our study area for VP-breeding salamanders. This suggests that AMOPs potentially cannot disperse well in rugged terrain despite high forest cover, which is in agreement with other studies showing that topography can influence genetic structure (Funk et al., 2005; Giordano, Ridenhour, & Storfer, 2007; Murphy et al., 2010). It is worth noting that VPs M16 to M21 did exhibit high levels of gene flow in what our LRS predicts as having high resistance due to high topographic curvature, but these pools were in relatively close proximity (mean pairwise Euclidean distance = 387m; Whiteley et al., 2014). Less surprising was the observed high resistance to inferred gene flow by roads for AMOP. High resistance to gene flow caused by roads has been well documented with many taxa (Balkenhol & Waits, 2009; Jackson & Fahrig, 2016) and has been previously documented in VP-breeding amphibians (Richardson, 2012; Coster et al., 2015). Roads have been found to cause population declines with VP-breeding amphibians due to direct mortality and VPs close to roads which are treated (e.g., salted) have been found to have reduced fecundity (Gibbs & Shriver, 2005; Karraker, Gibbs, & Vonesh, 2008). Surprisingly, we did not find a steady increase in LR with increasing traffic rate which has been documented in other taxa (Shirk et al., 2010). Instead, we observed more of an all or nothing response to roads (Fig. 2.5.). This could suggest that just the physical barrier of a narrow single lane road may be enough to impede gene flow of salamanders than the higher levels of direct mortality of adults and juvenile salamanders found with multi-lane roads (e.g., interstate highways) or that roads are correlated with an unknown spatial feature
of the landscape. In contrast to roads and our expectations, we found that the Connecticut River, a large river bisecting the study area, did not significantly reduce inferred gene flow, which contradicts findings that rivers and other large water bodies are important natural impediments to gene flow for VP-breeding amphibians (Richardson, 2012; Coster et al., 2015).

**Recent landscapes best describe gene flow**

Multi-decadal temporal snapshots of land cover imagery for our study area allowed us to explore which temporal snapshot of our landscape best described the present genetic patterns and inferred gene flow. Contrary to recent findings that landscapes can have legacy effects in which genetic patterns and gene flow are best described by landscape patterns 20+ years prior to genetic sampling (Spear & Storfer, 2008; Dudaniec et al., 2013), we found that the most recent forest land cover (2005) best described the genetic pattern and inferred gene flow of both AMMA and AMOPs. Indeed, for both species, forest land cover from 1971 performed poorest compared to forest land cover from 1985 and 1999. This suggests that the generational time of these two species may allow gene flow and genetic patterns to rapidly adjust to changes in landscape structure compared with longer-lived species with longer generational times.

**Population-level genetic clustering and gene flow with ecologically similar species**

We observed significant differences between AMMA and AMOP in the population-level clustering across the landscape and with the environmental and anthropogenic features best
predicting gene flow, which is consistent with previous studies comparing the landscape genetics of ecologically similar species (Steele, Baumsteiger, & Storfer, 2009; Goldberg & Waits, 2010; Richardson, 2012; Coster et al., 2015; Peterman et al., 2014; Burkhart et al., 2017). AMMA had no evidence of population-level clustering, a relatively weak pattern of isolation by distance, and little variation in family-level genetic structure (Whiteley et al., 2014), and this is consistent with past AMMA studies (Purrenhage, Niewiarowski, & Moore, 2009; Richardson, 2012; Coster et al., 2015; Peterman et al., 2014; Burkhart et al., 2017). In contrast, AMOP showed population-level clustering and stronger patterns of isolation by distance and variation in family genetic structure which is also consistent with past AMOP studies (Peterman et al., 2014; Burkhart et al., 2017). These findings are likely the result of differences in AMMA and AMOP life histories, phenology and morphology. For example, AMMA are larger bodied and able to disperse further on the landscape than AMOP, although on average they tend to disperse shorter distances than AMOP due to the fact they are able to breed in a wider range of VPs due to greater flexibility in hydroperiod requirements than AMOPs (Gamble et al., 2007; Peterman et al., 2014; Burkhart et al., 2017). Burkhart et al., (2017) hypothesized that differences in breeding phenology (AMOP breed in fall while AMMA breed in Spring) likely allow AMMA to breed in a wider range of VP hydroperiods than AMOP who gain advantages in Spring metamorph size by breeding in the prior Fall with the tradeoff of being able to breed in fewer VPs across the landscape. AMMA also have much larger effective population sizes ($N_b$) than AMOP (AMMA $N_b = 422 +/- 122$ SE, AMOP $N_b = 96 +/- 47$) (Whiteley et al., 2014), longer generation length (Petranka, 1998) and
potentially lower natal philopatry than AMOP due to their ability to breed in more VPs on the landscape (Petranka, 1998).

**Identifying important vernal pools on the landscape**

Our resistant kernel approach allowed us to identify highly connected VPs in our study area with high amounts of local forested habitat, although further effort will be needed to assess finer scale VP characteristics and species-specific occupancy and/or abundance. VP hydroperiod, chemistry (e.g., conductivity), micro habitat characteristics (e.g. logs, etc.) and tree species composition are known to be important drivers of species presence/abundance (Charney, 2011) and were not included in our VP scoring at local, neighborhood and regional levels. Recent evidence also suggests that VPs that have higher productivity have higher rates of gene flow, which would result in differences in scores at the neighborhood and regional level (Murphy et al., 2010; Coster et al., 2015).

Our empirically-based final VP scores differed somewhat from the expert-based scores of Compton et al., (2007), suggesting the importance of using empirical approaches when available, and this is consistent with other studies that have demonstrated superior performance of empirical approaches over expert-opinion approaches (Wasserman et al., 2010; Mateo-Sánchez et al., 2015; Shirk et al., 2015; Zeller et al., 2018). However, it is worth noting that our empirically-based VP scores were not directly comparable to Compton et al., (2007) expert-based scores, as the latter were not species-specific, Nevertheless, the Compton et al., (2007) VP
scores were more similar to our AMMA VP scores than our AMOP VP scores, perhaps reflecting greater familiarity with AMMA among the regional experts given that it is the more common and better studied species.

It is also worth noting that our spatial dataset of VPs is limited to potential VPs that have not been field verified. A comprehensive field verified dataset does exist, but the spatial distribution of certified VPs are biased to those areas on the landscape under consideration for residential/commercial development. Also, since potential VPs are based off of imagery, smaller VPs are likely missing from this spatial layer. This spatial layer also includes no information on VP quality which we know is driven by factors such as hydroperiod, with species preference often differing with hydroperiod (Peterman et al., 2014; Semlitsch et al., 2015). Upland forest composition and age may also be a factor in determining VP quality, but was not incorporated in scoring of VPs at the local level in our study, although a recent study found no effect of forestry practices on VP amphibian gene flow (Coster et al., 2015).

**Assumptions and limitations**

Our findings are subject to a couple of noteworthy assumptions and limitations. First, our multi-scale/layer LRS approach did not allow us to fully explore all spatial layer/bandwidth combinations due to the excessive computational demands of ResistanceGA’s genetic algorithm optimization. To successfully fit models at the spatial extent of our landscape, we were required to coarsen our original 30m rasters to 60m, thus sacrificing potentially important fine-scale
information about landscape patterns. Although, coarsening cell resolution has been found to
have minimal impact on inferences in this context (McRae et al., 2008; Cushman & Landguth,
2010) and given that the top performing models were composed of spatial layers smoothed at
100-500 m, the coarsening may not have been consequential in this case. In addition, to represent
ecological neighborhoods at varying spatial scales, we pseudo-optimized the neighborhood scale
for each layer by evaluating a predetermined and limited number of neighborhood sizes (i.e.,
Gaussian kernel bandwidths). This approach did not allow us to identify the very best
neighborhood scale on a continuum of possibilities but represented a reasonable tradeoff between
finding the best scale and computational feasibility. In addition, we were unable to fit complex
multi-layer models involving more than two or three layers due to computational deficiencies in
the data and the challenges of optimization in a higher dimensional parameter space. We deemed
this limitation acceptable and better than the null model alternative but recognize that
overcoming this partly technical limitation should be a focus of future work. Indeed, the inability
to fully optimize the neighborhood scale in complex multi-layer models is potentially a serious
shortcoming. Recall that our two-stage approach of pseudo-optimizing spatial layers at their
“best” spatial scale and then developing multi-layer LRSs performed poorer than our single-layer
LRSs, and we attributed this to the high correlation between spatial surfaces optimized at similar
kernel bandwidths (Appendix E and F). Our two-layer models evaluated across all combinations
of the predetermined and limited number of scales resulted in significantly better models,
highlighting the importance of a fully multi-scale/layer optimization. The most recent version of
ResistanceGA includes optimization of Gaussian kernel bandwidth (using the R package
spatstat) within the genetic algorithm, which makes possible full multi-scale/layer optimization. However, our preliminary examination of this capability suggests that there are still some major technical challenges to overcome for large datasets involving multiple spatial layers. Overcoming these technical challenges remains a priority of future work.

Second, we used Gaussian resistant kernels (Compton et al., 2007) to compute neighborhood and regional connectivity scores for VPs. The resistant kernel bandwidth we used was difficult to select because we did not know what the dispersal kernel would be on a completely non-resistant landscape. Our chosen 800m bandwidth at the neighborhood scale may have underestimated dispersal distance in a non-resistant landscape. Similarly, the selection of the bandwidth for the regional connectivity scoring was somewhat arbitrary because it reflected a temporal component regarding connectivity over multiple generations. We simply selected a bandwidth that gave us a distribution of VP scores that allowed us to discriminate amongst VPs at the study area level.

Conclusions

Our findings confirm that multi-scale approaches (in combination with multiple layers) are not only feasible but can result in improved models of species-environment relationships (McGarigal et al., 2016) and thus should be considered in all future studies optimizing landscape resistance. Our findings also confirm previous multi-species comparisons which have shown that we should not assume that ecologically similar species have comparable rates of gene flow and
genetic differentiation and that it is incorrect to assume that environmental and anthropogenic predictors of landscape resistance for those species are the same (Richardson, 2012; Burkhart et al., 2017). Thus, management practices geared towards the conservation of one species may not be beneficial for other assumed ecologically similar species. Species-specific resistant kernels derived from our multi-scale LRS allowed us to score VPs across our study area based on genetic connectivity and highlight an approach that we feel could be applicable to the conservation of wide range of taxa beyond VP breeding salamanders.
**Tables**

Table 2.1 Spatial layers used to model landscape resistance of marbled (*A. opacum*) and spotted (*A. maculatum*) salamanders and our justification for their inclusion in landscape resistance modeling process, derivation and supporting literature where a particular spatial layer was found to be an important driver of gene flow in a previous study.

<table>
<thead>
<tr>
<th>Spatial Layers</th>
<th>Justification</th>
<th>Source</th>
<th>Derivation</th>
<th>Supporting Literature</th>
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<tbody>
<tr>
<td>Topographic curvature</td>
<td>High values limit dispersal ability and gene flow</td>
<td>DEM Massachusetts GIS (<a href="http://www.mass.gov/anf/research-and-techno-serv-support/application-geographic-information-massgis/datalayers/">http://www.mass.gov/anf/research-and-techno-serv-support/application-geographic-information-massgis/datalayers/</a>)</td>
<td>Total Curvature (see Jenness 2013)</td>
<td>Funk et al. 2005; Murphy et al. 2010</td>
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<tr>
<td>Impervious surfaces</td>
<td>Impedes movement and cause direct mortality</td>
<td>Massachusetts Conservation Assessment and Prioritization System (<a href="http://www.umasscaps.org/data_maps/data.html">http://www.umasscaps.org/data_maps/data.html</a>)</td>
<td>Percent impervious</td>
<td>Murphy et al. 2010</td>
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<tr>
<td>Normalized difference vegetation index</td>
<td>High values enable movement and gene flow</td>
<td>Global Web Enabled Landsat Data website (<a href="http://globalweld.cr.usgs.gov/">http://globalweld.cr.usgs.gov/</a>)</td>
<td>Normalized Difference Vegetation Index (NDVI) value generated from Band3_TOA_REF and Band4_TOA_REF.</td>
<td>Spear and Storfer 2008</td>
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<tr>
<td>Potential vernal pools</td>
<td>High densities of pools enable movement and gene flow</td>
<td>Massachusetts GIS; Natural Heritage and Endangered Species Program (<a href="http://www.mass.gov/anf/research-and-tech/it-serv-and-">http://www.mass.gov/anf/research-and-tech/it-serv-and-</a></td>
<td>Photo interpretation of color infrared aerial photographs by the</td>
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<tr>
<td><strong>Topographic position index</strong></td>
<td>Different features may enable or impede movement and gene flow</td>
<td>DEM Massachusetts GIS (<a href="http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/">http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/</a>)</td>
<td>Massachusetts Natural Heritage and Endangered Species Program (Burne 2001)</td>
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<tr>
<td>Topographic wetness index</td>
<td>Movement and gene flow impeded by areas too &quot;wet&quot; or too &quot;dry&quot;</td>
<td>Designing Sustainable Landscapes (<a href="http://www.umass.edu/landeco/research/dsl/products/dsl_products.html">http://www.umass.edu/landeco/research/dsl/products/dsl_products.html</a>)</td>
<td>Richardson 2012</td>
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<tr>
<td>Water flow rate</td>
<td>High flow rates impede movement and gene flow</td>
<td>Massachusetts Conservation Assessment and Prioritization System (<a href="http://www.umasscaps.org/data_maps/data.html">http://www.umasscaps.org/data_maps/data.html</a>)</td>
<td>Log-scaled FP8 Flow accumulation from DEM</td>
<td>Coster et al. 2015</td>
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Figures

Figure 2.1 Study area in the Pioneer Valley in western Massachusetts. Nineteen vernal pools were sampled for larval spotted salamanders (*A. maculatum*) (S1-S19) and 29 vernal pools were sampled for larval marbled salamanders (*A. opacum*) (M1-M29). Larval *A. maculatum* were collected during the Summer of 2007 and 2008 and larval *A. opacum* were collected during the Spring of 2010.
Figure 2.2 Spatial surfaces used to model landscape resistance for spotted (*A. maculatum*) and marbled (*A. opacum*) salamanders including: topographic curvature, impervious surfaces (2005), forest land cover (1999), normalized difference vegetation index (July 2010), potential vernal pools, slope, topographic position index, traffic rate, topographic wetness index, and water flow rate. Forest land cover 1971, 1985 and 2005 were also included in the analysis (not shown here). Each spatial surface was evaluated at its original resolution (60m) and at multiple spatial scales with surfaces smoothed using a Gaussian kernel at 100m, 500m, 1000m, 1500m, and 2000m bandwidths.
Figure 2.3 Genetic distance (chord distance; $D_C$) versus geographic distance (km) for spotted ($A. maculatum$) and marbled ($A. opacum$) salamanders in the Pioneer Valley in western Massachusetts. $D_C$ values for both species are based on a subset of the data with one randomly sampled full-sibling per family from all vernal pools. Two $A. maculatum$ and seven $A. opacum$ vernal pools that contained 10 or fewer full-sib families were removed prior to the calculation of genetic distance. A linear regression and 95% CI were fit to $A. maculatum$ and $A. opacum$ genetic distance data and shown here.
Figure 2.4 Akaike's information criteria corrected for small sample sizes (AICc) for single-layer landscape resistance models which were optimized at the original pixel size (60m) and different Gaussian kernel bandwidths (100m, 500m, 1000m, 1500m and 2000m) for spotted (*A. maculatum*; top panel) and marbled (*A. opacum*; bottom panel) salamanders. Also included is the AICc of the “top” multi-layer resistance surface.
Figure 2.5 Response curves demonstrating the multi-scale/layer contribution and relationship of forest land cover 2005 (500m Gaussian kernel bandwidth) and normalized difference vegetation index (100m Gaussian kernel bandwidth) to landscape resistance for spotted salamanders (*A. maculatum*; left panels) and traffic rate (500m Gaussian kernel bandwidth) and topographic curvature (500m Gaussian kernel bandwidth) to landscape resistance for marbled salamanders (*A. opacum*; right panels).
Figure 2.6 Landscape resistances surfaces derived from the predicted averages of the top “All Scale Combinations” multi-scale/two-layer surfaces for spotted (*A. maculatum*; left panel) and marbled (*A. opacum*; right panel) salamanders.
Figure 2.7 Local vernal pool scores for spotted (*A. maculatum*) and marbled (*A. opacum*) salamanders were derived by summing the Gaussian kernel volume of forest land cover cells (2005) based on a Gaussian kernel (124m Gaussian kernel bandwidth) centered on each vernal pool in the study area.
Figure 2.8 Resistant kernels (800m Gaussian kernel bandwidth) were used to score vernal pools at the neighborhood level with each vernal pool’s neighborhood score derived by summing the volume of neighboring vernal pools resistant kernel volumes (top row) for spotted (*A. maculatum*) (left panels) and marbled (*A. opacum*) salamanders (right panels); resistant kernels (2800m and 2000m Gaussian kernel bandwidth for *A. maculatum* and *A. opacum*, respectively, were used to score vernal pools at the regional level with each vernal pool’s regional score derived by the sum of vernal pools within each resistant kernel “cluster” (middle row); and vernal pool scores based on the geometric mean of the local, neighborhood and regional scores (bottom row).
CHAPTER 3

EVALUATION OF THE R PACKAGE ‘RESISTANCEGA’: A PROMISING APPROACH TOWARDS THE ACCURATE OPTIMIZATION OF LANDSCAPE RESISTANCE SURFACES

Introduction

For just over a decade, the field of landscape genetics (LG) has made important contributions to conservation biology, epidemiology and molecular and evolutionary ecology (Segelbacher et al., 2010; Sork & Waits, 2010; Manel & Holderegger, 2013; Petren, 2013; Van Strien et al., 2014). LG early success and rapid development can be attributed to an active research community that has put a strong emphasis on identifying and addressing research needs and improving quantitative rigor (Manel et al., 2003; Balkenhol, Waits, & Dezzani, 2009; Epperson et al., 2010; Richardson et al., 2016). This has resulted in a number of simulation-based studies to address the many components of landscape genetics study design (e.g., spatial scale (Cushman & Landguth, 2010; Galpern, Manseau, & Wilson, 2012), contrast in landscape resistance (hereafter LR) (Cushman, Lewis, & Landguth, 2014; Shirk, Landguth, & Cushman, 2018), thematic resolution (Cushman & Landguth, 2010), landscape fragmentation (Cushman, Wasserman, Landguth, & Shirk, 2013), sampling regimes (Oyler-McCance et al., 2013), number of molecular markers and allelic richness (Landguth et al., 2012; Oyler-McCance et al., 2013), sample size (Landguth et al., 2012; Oyler-McCance et al., 2013), generational time (Landguth & Cushman, 2010; Oyler-McCance et al., 2013a) and genetic non-equilibrium (Zeller et al., 2016; Shirk et al., 2018), along with the development of a plethora of analytical approaches and their
subsequent evaluations (Cushman et al., 2006; Van Strien, Keller, & Holderegger, 2012; Graves, Beier, & Royle, 2013; Cushman et al., 2013; Gruber & Adamack, 2015; Zeller et al., 2016; Franckowiak et al., 2017; Row et al., 2017; Shirk et al., 2018; Peterman, 2018). Unfortunately, due to the nature of the genetic and spatial data, these analytical approaches (e.g., derivatives of the Mantel test), when rigorously evaluated, have tended to perform poorly (e.g., high type I error rates; Balkenhol et al., 2009; Legendre & Fortin, 2010; Graves et al., 2013; Manel & Holderegger, 2013; Cushman et al., 2013). These analytical issues have created a formidable challenge for landscape genetics, and arguably has resulted in a bottleneck limiting LG further development and success (Storfer et al., 2010; Richardson et al., 2016).

Recently, several analytical methods have been proposed to overcome previous limitations or shortcomings (Galpern et al., 2014; Rousset & Ferdy, 2014; Shirk et al., 2018; Peterman, 2018). ResistanceGA, an R package used to optimize landscape resistance surfaces (LRS), is one such approach that was developed to address shortcomings of existing analytical approaches (Peterman, 2018). ResistanceGA utilizes a genetic algorithm (R package GA; Scrucca, 2013) to optimize LRS for both univariate continuous or categorical surfaces and multivariate combinations (continuous and/or categorical) with pairwise genetic distance (GD) data. ResistanceGA’s approach is unique as LRSs do not need to be defined a priori (e.g., Compton et al., 2007) or pseudo-optimized (e.g., Shirk et al., 2010), as they are empirically optimized via the genetic algorithm. A detailed description of the algorithm is provided in the Materials and Methods.
Here, we simulated univariate and multivariate LRSs and evaluated ResistanceGA’s ability to recover the true LRS under a variety of scenarios, including: a) the true surface having different levels of spatial autocorrelation; b) the true surface having different functional transformations for the conversion to LR; c) varying sample size (i.e., number of sample point locations); and d) varying levels of variance in the measure of GD between sample points. In addition, we evaluated model selection performance of ResistanceGA when alternative, often correlated, surfaces are present. These alternative surfaces were either spatially correlated environmental predictors, the correct predictor measured at the wrong spatial scale, random surfaces uncorrelated with the true surface, or surfaces derived from both random and correlated surfaces.

**Methods**

We simulated both univariate and multivariate LRSs to evaluate the performance of the R package ‘ResistanceGA’, as outlined in Figure 3.1. and described below.

**Simulating landscape resistance surfaces**

We simulated continuous spatially autocorrelated surfaces (hereafter referred to as fractal surfaces) using the R package ‘RandomFields’ (Schlather et al., 2015). We created the fractal surfaces at two different extents (50² or 100² pixels) and two different levels of spatial autocorrelation (RMexp scale = 1 or 25 in the RMexp function) (Fig. 3.1.). The results based on
the performance metrics described below were similar between landscape extents; therefore, unless otherwise noted below we report only the results for the 50² pixel extent. For the coarse-scale fractal surface, we also generated correlated surfaces at varying levels of correlation (Pearson's r = 0.9, 0.7, 0.5, 0.3, and 0.1) using an analytical approach that retained the original level of spatial autocorrelation. In addition, for this scenario, we also smoothed the original fractal surface with a Gaussian kernel at varying bandwidths (4 and 7 pixels) using the R package ‘gridio’ (http://jamba.provost.ads.umass.edu/web/plunkett/gridio.html).

Next, to create a true LRS for the univariate simulations we transformed the original fractal surface into a LRS by specifying a functional transformation (Monomolecular or Inverse Ricker) and shape (always equal to 3) and maximum resistance (50 or 200) parameters using the Resistance.tran function in ResistanceGA (Fig. 3.1.; Table 3.1.; Peterman, 2018). To create a true LRS for the multivariate simulations we used ResistanceGA’s Combine Surfaces function. Briefly, two of the individual fractal surfaces were transformed using specified functional transformations, shape and maximum resistance parameters for each surface, as described above, and then and these individual surfaces were added together and divided by the minimum LR value to create the final LRS (Fig. 3.1.; Table 3.1.; Peterman, 2018).

Sample points

For each simulation, a varying number of sample points (10, 25, 50, 75 or 90) were probabilistically distributed across the true LRS with the likelihood of sampling a pixel being
inversely related to its LR value (Fig. 3.1.). We excluded sample points from being placed on the edge of the surface (10 pixels for $50^2$ pixel landscape and 20 pixels for the $100^2$ pixel landscape) due to potential issues in calculating landscape resistance distances (LRD, see below) from those pixels (Koen et al., 2014).

**Pairwise genetic distances**

For each simulation, we estimated LRD between all of the pairwise sample points on the true LRS using CIRCUITSCAPE (McRae et al., 2008b). We developed a surrogate for GD by adding random noise to pairwise LRD. We evaluated five levels of variance in genetic distance for our simulation (standard deviation = 0.001, 0.1, 0.25, 0.5 and 1.25). This procedure allowed us to emulate varying strengths (from extremely weak to very strong) in the relationship between LRD and GD (Fig. 3.2.; Appendix M).

**Landscape resistance surface optimization**

Optimization of the true LRS and alternative LRSs was performed using a genetic algorithm (R package GA; Scrucca, 2013) as implemented in the R package ResistanceGA (Peterman, 2018). In our application, the optimization of a LRS worked as follows:

1. The original fractal surface was rescaled from 0 to 10.
2. A "population" of "individuals" was generated (equal to 15x the number of parameters to be optimized), in which each "individual" was assigned random
values for the set of parameters needed to transform the rescaled fractal surface into a LRS. The parameters included: (a) the functional transformation (Inverse-Reverse Monomolecular, Inverse-Reverse Ricker, Monomolecular, Reverse Monomolecular, Inverse Monomolecular, Inverse Ricker, and Distance), (b) the shape of the transformation, and (c) the maximum resistance value of the transformation (maximum value = 2,500). For each "individual" or unique set of parameter values, the fractal surfaces was transformed into a LRS.

iii) For each "individual", pairwise LRDs between the sample point locations were calculated using CIRCUITSCAPE (McRae et al., 2008b).

iv) For each "individual", a linear mixed effects model with a maximum likelihood population effects parameterization (MLPE) was fit, in which our surrogate measure of GD was treated as the response and the scaled and centered LRD was treated as the predictor, and the pairwise combinations of point locations represented the observations. The MLPE parameterization was used to account for the non-independence among the pairwise data (Clarke et al., 2002) and has been shown to perform better than other regression-based approaches in this context (Shirk et al., 2018).

v) For each fitted model, Akaike information criterion adjusted for small sample size (AICc) was computed, with each model penalized by the overall complexity of the model (4 parameters for univariate models and 7 parameters for the multivariate two-layer models), and with sample size \( n \) equal to the number of
sampled points (not the number of pairwise observations used in the MLPE model).

vi) For the "population" of fitted models, the top 5% of "individuals" (i.e., unique parameterizations) based on AICc were retained for the next "generation" to form the "reproducing population" and the remaining "individuals" were discarded.

vii) For the selected "individuals", there was a probability of first a “mutation” (0.10) and then a “crossover” (0.85). Here, “mutation” involved the replacement of an “individual’s” single parameter value with a random value, while “crossover” simulated breeding with another “individual” and the creation of a new “individual” by averaging parameter values of the two breeding “individuals”.

viii) Steps 2-7 were repeated until there was no improvement in AICc (default = 25 iterations without improvement; maximum number of iterations = 1,000).

**Scenarios**

For the univariate simulations, we evaluated 8 different LRS scenarios representing unique combinations of spatial autocorrelation, functional transformation, and maximum LRD value (Table 3.1.). Thus, each scenario represented either a fine- or coarse-scaled fractal surface transformed into a unique LRS based on the specified parameters of the transformation. For each of these scenarios, we evaluated five different sample sizes and five different levels of variance in our surrogate measure of GD, as described above, for a total of 200 different scenarios. For
each scenario, we conducted 50 iterations to reflect the stochastic processes involved in generating the fractal surface, sampling point locations, and adding random "noise" to the LRDs to generate our surrogate measure of GD. For scenario #5 (Table 3.1.), we also optimized alternative spatially correlated surfaces (5 levels) or smoothed surfaces (2 levels) to evaluate model selection performance in realistic cases where the spatial surfaces hypothesized to drive gene flow and genetic differentiation are often highly correlated.

For the multivariate simulations, we evaluated the following combination of spatial autocorrelations, functional transformations, and maximum resistance values: Surface 1 was parameterized as a fine-scale fractal surface (RMexpscale = 1) transformed using a shape value of 3 and maximum resistance value of 100. Surface 2 was parameterized as a coarse-scale fractal surface (RMexpscale = 25) transformed using an Inverse Ricker transformation with a shape value of 3 and a maximum resistance value of 100. For this set of LRS parameters, we evaluated a single sample size of 75 across five levels of variance in our surrogate measure of GD, as described above, for a total of five different scenarios. For each scenario, we conducted 50 iterations to reflect the stochastic processes involved. For each iteration, we also optimized seven other alternative univariate and multivariate resistance surfaces (surface 1, surface 2, surface 1 and a random surface, surface 2 and a random surface, the two random surfaces, and the random surfaces individually) to evaluate model selection performance in realistic cases in which the model set under consideration is generated using an all-subsets approach.
**Performance metrics**

To evaluate ResistanceGA performance in the univariate simulations, we computed several metrics. First, for each iteration of the 200 scenarios, we computed the Pearson's correlation (r) between the true and optimized LRS and summarized the mean and standard error from the 50 iterations run for each scenario. Second, for each of the 25 scenarios (5 sample sizes by 5 levels of variance in genetic distance) associated with the LRS scenario 5 (Table 3.1.), we computed type I error rates, defined as the percentage of the iterations in which the true model was not selected as the top model when in competition with the corresponding correlated or smoothed alternative surfaces, and the percentage of the iterations in which each of the alternative surfaces was selected as the top model. Third, for each of the 200 scenarios, we computed the percentage of the iterations in which the correct functional transformation was selected by the optimization. Lastly, for those iterations in which the correct functional transformation was optimized, we also measured root mean square error (RMSE), bias and standard error (SE) of the optimized shape and maximum resistance parameters by scenario.

To evaluate ResistanceGA performance in the multivariate simulations, we computed a similar set of metrics. First, for each iteration of the five scenarios (levels of variance in GD), we computed the Pearson's correlation (r) between the true and optimized LRS and summarized the mean and standard error for the 50 iterations run for each scenario. Second, for each scenario, we computed the type I error rates, as defined above, and the percentage of the iterations in which the true model and each of the alternative all-subsets models was selected as the top model.
Lastly, for each scenario, we also used a modified-bootstrap procedure as an alternative and potentially more robust way to evaluate competing models (Peterman, 2018). Specifically, we used the Resist.boot function in ResistanceGA and defined a subsample of 75% of the pairwise response and LRD data from the optimized multi-surface model (without replacement) and ran 1,000 bootstrap iterations in which the function refit the MLPE model for each surface (based on the bootstrap sample). For each of the 50 iterations for each scenario, the Resist.boot function calculated the percentage of the 1,000 bootstrap iterations in which each model was selected as the top model and its average model weight and rank based on AICc; we combined results across the 50 iterations for each scenario and summarized the mean and standard error in these three statistics.

**Results**

**Correlation between true and optimized landscape resistance surfaces**

For the univariate simulations, patterns in the correlations between the true and optimized LRSs were generally consistent across all of the LRS scenarios (Appendix N) and spatial extents (S5) that we evaluated. Correlations were relatively high (>0.8) across all levels of variance in GD when the sample size was ≥50 (e.g., Fig. 3.3.). If the sample size was ≤25, the correlation between surfaces depended strongly on the level of variance in GD. Although the pattern of variation across sample size and level of variance was similar between the fine-scale fractal surfaces (LRS scenarios 1-4; Table 3.1.) and coarse-scale fractal surfaces (LRS scenarios
5-8; Table 3.1.), the correlations were consistently slightly higher with the coarse-scale surfaces (Appendix N). Similarly, the correlations were consistently higher with the Monomolecular versus the Inverse Ricker transformations (Appendix N). The maximum resistance value (50 vs. 200) used to develop the true LRS appeared to have no overall effects on correlation between the true and optimized LRSs (Appendix N).

For the multivariate simulations, correlations were very high (~0.95) between the true and optimized LRSs at all levels of variance in GD (Table 3.2.), although recall that all of these simulations were run with a relatively large sample size (75 sample points).

**Model selection error**

Type 1 error rates were relatively low (<25%) and AICc model weights for the true LRS correspondingly high (>0.75) across levels of variance in GD when the sample size was ≥50, except at the highest level of variance (sd = 1.25) when the relationship between LRD and GD was very weak (e.g., Figs. 3.4.-3.5.). Conversely, when sample size was ≤25, type I error rates were consistently relatively high (>25%) (Fig. 3.4.) and AICc model weights for the true LRS varied substantially (as low as 0.15) across levels variance in GD (Fig. 5). However, when the top model was not the true LRS (i.e., type I error), the top model was usually one of the LRSs highly correlated (r ≥ 0.7) with the true LRS or the moderately smoothed version of the true LRS, except at the highest levels of variance in GD when the sample size was small (e.g., Fig. 3.6.).
For the multivariate simulations, type I error rates were consistently 20-30% across levels of variance in GD for the only sample size (n=75) and single LRS scenario that we evaluated (Table 3.2.). Thus, the true multivariate LRS was selected as the top model more than 70 percent of the time regardless of the strength in the relationship between LRD and GD, and it received an AICc model weight of between 71-80% (Table 3.2). Even when the true multivariate LRS was not selected as the top model, the top model was most often one of the two component LRSs optimized in isolation and ranked first due to the decreased parameter count and reduced AICc penalty (Fig. 3.7.). The modified-bootstrap procedure produced more robust model selection results with the true LRS selected as the top model on average >95% of the time with an average model rank of ~1 and average model weight of >0.95 (Fig. 3.8.; Appendix P). Not surprisingly, model selection performance using the modified-bootstrap procedure decreased slightly (~5%) with increased variance in GD (Fig. 3.8.; Appendix P).

**Optimization of functional transformation and shape and maximum resistance parameters**

For the univariate simulations, patterns in the percentage of the iterations in which the correct functional transformation was selected by the optimization across sample sizes and levels of variance in GD were generally consistent across all of the LRS scenarios (Table 3.1.) that we evaluated (S3.5.). Simulations with moderate to high sample sizes (≥50) had a high percentage (>75%) of iterations optimized using the true transformation at all levels of variance in GD when the correct transformation was the Inverse Ricker, and at all but the highest levels of variance
when the correct transformation was the Monomolecular (e.g., Fig. 3.9). Generally, sensitivity to sample size and level of variance in GD was greater for Monomolecular versus Inverse Ricker transformations (e.g., Fig. 3.9). In particular, when the strength of the relationship between LRD and GD was very strong (i.e., low variance in GD) and the functional relationship was Monomolecular, the correct transformation was almost always selected by the optimization; however, when the strength of the relationship between LRD and GD was very weak (i.e., high variance in GD), the correct transformation was more often not selected. More specifically, when the true transformation was Monomolecular, and it was not selected by the optimization, the selection usually favored the Inverse Ricker or Inverse-Reverse Monomolecular (Appendix R). Similarly, when the true transformation was Inverse Ricker and it was not selected by the optimization, the selection usually favored the Inverse-Reverse Monomolecular (Appendix S).

The accuracy and precision of the optimized shape and maximum resistance parameters, as reflected by the RMSE, bias and SE of the estimates, decreased rapidly as sample size decreased and level of variance in GD increased across all scenarios (Table 3.3; Appendix T - Y). However, the magnitude and rate of deterioration in accuracy and precision in these two parameter estimates as sample size and the strength of the relationship between LRD and GD decreased varied considerably among scenarios, and much more so than with the other performance metrics. In particular, the accuracy and precision of the shape parameter was considerably greater for the scenarios based on the Inverse Ricker transformation than those based on the Monomolecular transformation (Appendix T, V and X). This pattern was true for
the maximum resistance parameter as well, but the differences between transformations were much less dramatic (Appendix U, W and Y). In addition, bias in the estimate of maximum resistance decreased markedly as the true maximum resistance value increased; indeed, bias was negligible across all scenarios when the true maximum resistance was 200 (Appendix W).

**Discussion**

In the field of LG, a true optimization of LR based on GD data has eluded researchers since the origin of the discipline. Instead, researchers have had to resort to constrained grid search approaches (but see Wang, Savage, & Shaffer, 2009; Graves et al., 2013; Peterman, 2018), in which a limited parameter space is explored, resulting in a finite number of alternative models being assessed (Shirk et al., 2010). Similarly, in multi-scale studies, typically the individual input surfaces are rescaled a priori at several discrete scales, usually by smoothing the surface with a user-defined kernel, and then confronted with the GD data to determine the strength of each fit in order to select the best scale for each surface, and then the univariate surfaces are combined in various combinations and the strength of fit is determined to select the best multi-scale, multivariate model (Zeller et al., 2016). The ResistanceGA R package was developed to offer a method for conducting true optimization of a LRS (including multi-scale optimization) when confronted with GD data (Ruiz-Lopez et al., 2016; Peterman, 2018). However, a rigorous examination of the algorithm's performance under widely varying but realistic scenarios had not been conducted until this study.
In this study, we examined a limited number of scenarios, but covering a broad range of parameter space, in which we simulated true LRSs encompassing both coarse- and fine-scale fractal surfaces at two different spatial extents and using two functional transformations (Inverse Ricker and Monomolecular) at two different maximum resistance values by which the fractal surfaces were converted into a LRS. Moreover, we simulated both univariate and multivariate surfaces as truth, and in scenarios where we competed the true surface against a set of closely related surfaces, we made our test of the algorithm's performance liberal (e.g., making it easy for the optimization to select an alternative LRS over the true LRS as the top model). For the univariate simulations, the alternative surfaces included surfaces spatially correlated with the true surface and the true surface smoothed at two different scales. These scenarios represent an environmental variable that is correlated with the true predictor or the inclusion of the true predictor measured at the wrong spatial scale. For the multivariate simulations, the alternative surfaces included the two component surfaces in isolation, representing realistic cases in which an all-subsets model selection approach is used. We also evaluate the algorithm's performance across a range of realistic sample sizes (10–90 sample point locations) and across the range of signal-to-noise ratios representing the strength of the relationship between LRD and GD.

Overall, across the broad and realistic range of scenarios we evaluated, we confirmed the ability of ResistanceGA to effectively optimize LRSs, even with the relatively low signal-to-noise ratios present in most empirical LG datasets, so long as the sample size is relatively large (generally ≥50). However, this conclusion comes with the following important caveats. First, the
performance of ResistanceGA appears to be quite robust to changes in the input surface characteristics, at least in terms of degree of spatial autocorrelation and spatial extent. We found very little differences in any of the performance metrics we examined between the coarse- and fine-scale fractal surfaces and the two spatial extents (50^2 or 100^2 pixels) we evaluated. In contrast to our findings, however, Cushman & Landguth (2010) found that both grain (pixel size) and spatial extent had small but statistically significant effects on estimates of inferred gene flow. Galpern et al., (2012) also found that spatial grain affected the results, with evidence of the landscape driving gene flow only when spatial layers were evaluated at spatial grains coarser than the original 200m grain size. These findings warrant additional simulations to evaluate the effect of spatial grain on the performance of ResistanceGA.

Second, and unsurprisingly, the performance of ResistanceGA was sensitive to the combination of sample size and strength of signal-to-noise in simulated GD datasets. Performance in most metrics deteriorated rapidly for sample sizes ≤25, and the deterioration was especially evident when the strength in the relationship between LRD and GD was relatively weak (e.g., SD ≥ 0.5 in our surrogate measure of GD; Fig. 3.2.). ResistanceGA performed well at sample sizes ≤25 only when LR was a relatively strong predictor of GD (e.g., SD ≤ 0.25 in our surrogate measure of GD; Fig. 3.2.). These findings generally agree with previous simulation studies that found model selection error to be low only at reasonably large sample sizes (Landguth et al., 2012; Oyler-McCance et al., 2013; Row et al., 2017; Shirk et al., 2018). It is worth noting that the sample sizes needed to accurately select the true LRS was much less in our
study using ResistanceGA (Fig. 3.3.) than reported in these previous simulation studies (>200 in Landguth et al., 2012; Oyler-McCance, Fedy, & Landguth, 2013), although differences in simulation approaches (e.g., sampling schemes) between studies make it difficult to make direct comparisons. Nevertheless, it is encouraging that ResistanceGA’s optimization may perform well with fewer observations previously used analytical approaches (Shirk et al., 2018). The relatively poor performance of LRSs optimized with low sample sizes in our study and these others is somewhat sobering given the plethora of LG studies using relatively low sample sizes.

Third, the functional relationship between the original continuous surface and the LRS is expressed through the parameterization of the selected transformation function, and we found that there was a tradeoff between selecting the correct transformation function and the accuracy and precision of the corresponding parameter estimates. Specifically, ResistanceGA was more successful in identifying the Inverse Ricker function as the correct transformation compared to the Monomolecular when the sample size was small and the relationship between LRD and GD was weak, but the accuracy and precision of the shape and maximum LRD parameters was less. In addition, ResistanceGA estimated the maximum resistance of the LRS with much less bias when the true LRS had a higher maximum resistance value (Appendix W), suggesting not surprisingly that the algorithm performs better when there is greater contrast in the LRS. Overall, it appears that the only way to ensure that the optimized transformation is an accurate and precise translation of the input layer is with a large sample size, and this becomes increasingly true as the strength of the relationship between LRD and GD weakens.
Lastly, our use of ResistanceGA's modified-bootstrap model selection procedure to evaluate alternative models with our multivariate simulations substantially reduced type I error (~20%). This approach showed great promise for model selection with spatially correlated alternative LRSs and strongly suggests that applying a more “traditional” model selection approach using AICc and the full dataset should be used with caution. The high type I error rate we observed with the traditional approach was primarily a result of one of the individual LRSs used to develop the true LRS often having the lowest AICc. Currently, LRSs are AIC penalized by the number of parameters being estimated (4 parameters for a univariate model and 7 parameters for two-surface multivariate models) and this is potentially over penalizing multivariate models. Unfortunately, a “true” bootstrap approach where models are repeatedly optimized with a subset of the data is not feasible here to prevent issues of model overfitting with optimized LRSs due to computational limitations.

In conclusion, our simulation study provides evidence that ResistanceGA is able to effectively optimize LRS under a wide range of realistic LG scenarios, making it a valuable and powerful tool for future LG analyses. Nonetheless, there are limitations, and our study serves as a reminder that advances in analytical methods are not a panacea for making inference from challenging data sets (e.g., small sample sizes, high variance in genetic data). The highest level of variance assessed in our simulation was intended to represent a worst-case scenario (i.e., extremely weak relationship between LRD and GD) and, as expected, type I error rates were quite high (25 to 90% depending on sample size) under these conditions. However, even under
these worst-case scenarios the correlation between the optimized LRS and the true LRS remained quite high (> 0.80) so long as the sample size was ≥ 50. This suggests that secondary analyses requiring a parameterized LRS (e.g., corridor mapping or reserve design) may not be too adversely affected by type I error if the sample size is relatively large. Nonetheless, caution is needed when interpreting the drivers of LR. Our study has shed light on how sample size, degree of spatial autocorrelation and model selection approaches with spatially correlated alternative surfaces affects ResistanceGA’s performance optimizing LRSs. There are numerous other aspects of population genetics and landscape features that can affect landscape genetics inferences, such as landscape composition, number of genetic markers, and sampling design that still need to be investigated through simulations with ResistanceGA. There are also unknowns regarding parameterization of the genetic algorithm used in ResistanceGA and whether changes in default parameters could potentially improve inference or decrease computational time (e.g., What is the best mutation rate, crossover rate and number of “individuals” in a population? How much of an improvement in AICc is justifiable for producing another generation?). The performance of ResistanceGA in relation to these and other features is unknown but is an area of important future research that will require a more direct simulation of genetic processes to assess. In addition, multivariate simulations should be expanded to more than two layers to be more realistic and to better assess the performance of ResistanceGA’s modified-bootstrap model selection procedure. Lastly, given increasing recognition of the importance of multi-scale relationships (Zeller et al., 2016), simulations are needed to evaluate ResistanceGA's scale optimization implementation.
Tables

Table 3.1 Univariate scenarios for simulating the true landscape resistance surface. Scenarios represent unique combinations of: i) RMexpscale, which controls the level of spatial autocorrelation in the simulated continuous fractal surface, ii) transformation for converting input surface values to landscape resistance values, iii) shape and iv) maximum resistance parameters associated with the functional transformation.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>RMexpscale</th>
<th>Transformation</th>
<th>Shape</th>
<th>Max Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Monomolecular</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Inverse Ricker</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Monomolecular</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Inverse Ricker</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>Monomolecular</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>Inverse Ricker</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>Monomolecular</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>Inverse Ricker</td>
<td>3</td>
<td>200</td>
</tr>
</tbody>
</table>
Table 3.2 Performance metrics for the multivariate simulations by level of variance (standard deviation) in genetic distance including: i) Pearson’s correlation (r), between the true and optimized resistance surfaces, ii) type I error rate (%), defined as the percentage of 50 iterations in which the true surface was not selected as the top model, and iii) Akaike’s Information Criteria adjusted for small sample size (AICc) weight of the true optimized surface. The mean and standard errors (SE) were derived from the 50 random iterations.

<table>
<thead>
<tr>
<th>Performance metric</th>
<th>0.001</th>
<th>0.1</th>
<th>0.25</th>
<th>0.5</th>
<th>1.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation (Mean/SE)</td>
<td>0.960 / (0.006)</td>
<td>0.95 / (0.01)</td>
<td>0.95 / (0.01)</td>
<td>0.95 / (0.01)</td>
<td>0.95 / (0.1)</td>
</tr>
<tr>
<td>Type I error rate (%)</td>
<td>28</td>
<td>20</td>
<td>30</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>True model AICc weight (Mean/SE)</td>
<td>0.72 / (0.06)</td>
<td>0.80 / (0.06)</td>
<td>0.71 / (0.06)</td>
<td>0.74 / (0.06)</td>
<td>0.78 / (0.05)</td>
</tr>
</tbody>
</table>
Table 3.3 Root mean square error, bias and standard error (SE) for the optimized shape and maximum resistance parameters for the univariate scenarios by level of variance (standard deviation) in genetic distance for the univariate scenarios #S1-S4 (Table 3.1; scenarios simulating a fine fractal surface) with a sample size of 50.

<table>
<thead>
<tr>
<th>Variance Level in Genetic Distance (Standard Deviation)</th>
<th>Shape</th>
<th>Max Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
<td>0.10</td>
</tr>
<tr>
<td>S1</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>S2</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>S3</td>
<td>0.00</td>
<td>0.12</td>
</tr>
<tr>
<td>S4</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

|                                                        | 0.01   | 0.04 | 0.22 | 0.36 | 0.40 | 1.22   | 3.79  | 4.04  | 3.56  |
| S1                                                     | 0.02   | 0.02 | 0.02 | 0.01 | 0.02 | 0.40   | 1.31  | 1.22  | 1.23  |
| S2                                                     | 0.00   | 0.07 | 0.29 | 0.68 | 0.42 | 0.32   | 0.14  | 0.11  | 0.40  |
| S3                                                     | 0.00   | 0.00 | 0.00 | 0.00 | 0.01 | 0.04   | 0.14  | 0.21  | 0.17  |
| S4                                                     | 0.01   | 0.10 | 0.30 | 0.52 | 0.97 | 11.55  | 23.27 | 29.32 | 30.42 |
|                                                        | 0.02   | 0.02 | 0.02 | 0.03 | 0.07 | 7.03   | 13.44 | 14.73 | 16.05 |
| S3                                                     | 0.00   | 0.11 | 0.32 | 0.70 | 1.07 | 12.43  | 22.86 | 25.70 | 36.63 |
| S4                                                     | 0.01   | 0.01 | 0.01 | 0.02 | 0.05 | 5.62   | 13.48 | 15.86 | 21.07 |
Figures

Figure 3.1 Flow chart depicting the univariate and multivariate surface simulation steps involved in evaluating the R package ResistanceGA. Steps included: i) simulating continuous fractal surfaces using the R package RandomFields and using ResistanceGA to transform these surfaces to landscape resistance surfaces; ii) placing sample points probabilistically across the landscape resistance surfaces and measuring pairwise resistance distances; iii) adding variance to these pairwise measures of resistance distances to create a surrogate measure of genetic distance in which the strength of the relationship between resistance distance and genetic distance decreases with increasing standard deviation; and iv) optimizing the original surfaces and alternative spatially correlated and kernel smoothed surfaces.

1) We simulated spatial surface(s) using the R package randomFields. We specified the RMesquite (1 = fine or 25 = coarse) argument to control the level of spatial autocorrelation in a given surface(s). 2) We transformed simulated surface(s) into a resistance surface using a specified functional transformation (Monomolecular or Inverse Flicker) and specifying a shape (3) and maximum resistance parameter (69 or 200). For each iteration, we placed a varying number of sample points (10, 25, 50, 75 or 90) probabilistically distributed across the true LRS with likelihood of sample point at a pixel being inversely related to its landscape resistance value.

2) We measured resistance distances between sample points using CIRCUITSCAPE. We then developed a surrogate for genetic distance by adding random noise to the pairwise resistance distance. We evaluated five levels of variance in our genetic distance data (standard deviation = 0.05, 0.1, 0.25, 0.5 and 1.25). We then used R package ResistanceGA to optimize the true surface(s) with our measure of genetic distance to evaluate ResistanceGA's ability to ability to recover the true resistance surface with varying levels of variance in genetic distance data. Spatially correlated alternative surfaces were also optimized to evaluate model selection performance.

Note: Multi and single surfaces were optimized in the multi-surface simulation.
Figure 3.2 Correlation between pairwise genetic distances (noise was added to pairwise resistances distances with varying standard deviations to develop a surrogate for genetic distance) and pairwise resistance distances, with the pairwise resistance distances derived from the true resistance surface, by level of variance in genetic distance and sample size.
Figure 3.3 Pearson's correlation ($r$) between the true and optimized resistance surfaces by sample size and level of variance (standard deviation) in genetic distance, shown here for a representative single-surface scenario (Scenario #5, Table 3.1.).
Figure 3.4 Type I error rates (% of 50 iterations) in which the true model was not selected as the top model among a model set of alternative spatially correlated and smoothed surfaces based on Akaike's Information Criteria adjusted for small sample size (AICc) (see text for details), by sample size and level of variance (standard deviation) in genetic distance, shown here for a representative univariate scenario (Scenario #5, Table 3.1).
Figure 3.5 Mean (of 50 iterations) model Akaike's Information Criteria adjusted for small sample size (AICc) weights and standard error of the optimized true surface (based on AICc) among a model set of alternative spatially correlated and smoothed surfaces (see text for details), by sample size and level of variance (standard deviation) in genetic distance, shown here for a representative univariate scenario (#5, Table 3.1.).
Figure 3.6 Percentage of 50 iterations in which each alternative model, including the true model and alternative correlated and smoothed surfaces (see text for details), was selected as the top model based on Akaike's Information Criteria adjusted by small sample size (AICc), by sample size (panels) and level of variance (standard deviation) in genetic distance, shown here for a representative univariate scenario (Scenario #5, Table 3.1.).
Figure 3.7 Percentage of 50 iterations in which the true multivariate resistance model and each of the alternative univariate and multivariate resistance models (see text for details) was selected as the top model based on Akaike's Information Criteria adjusted by small sample size (AICc), by level of variance (standard deviation) in genetic distance.
Figure 3.8 Results of the multivariate modified-bootstrap procedure. Top panel: Average model rank based on Akaike’s Information Criteria adjusted by small sample size (AICc) computed from 1,000 modified-bootstrap iterations for each of 50 iterations of a scenario that varied in level of variance (standard deviation) in genetic distance. Lower panel: Average of the average percent top model from the 1,000 modified-bootstrap iterations for each simulation iteration.
Figure 3.9 Percentage of 50 iterations in which the correct functional transformation was selected for the optimized true surface, by sample size and level of variance (standard deviation) in genetic distance for the Inverse Ricker (left panel) and Monomolecular (right panel) univariate scenarios #1 and #2 in Table 3.1.
CHAPTER 4

UNBIASED ESTIMATES OF SURVIVAL AND BREEDING FREQUENCY OF A VERNAL POOL BREEDING SALAMANDER AT THE NORTHERN EXTENT OF ITS RANGE

Introduction

Many wildlife species are at risk of future population declines due to habitat loss and fragmentation, land use change, and invasive species (Ceballos, Ehrlich, & Dirzo, 2017). Climate change has the potential to exacerbate these factors and limit a species ability to buffer such population declines (Cahill et al., 2012). A popular tool to better understand the effects of climate change on wildlife species is a bioclimatic envelope model (Jeschke & Strayer, 2008). A bioclimatic envelope model, often referred to as a niche model, relates a species current presence and absence (or only presence) to abiotic variables (e.g., temperature and precipitation), and then predicts species distribution in the future based on climate projections, often at a broad spatial scale (Jeschke & Strayer, 2008; Sohl, 2014). Such climate niche models can often be applied with already existing or less intensive monitoring, such as species occurrence datasets (e.g., citizen science datasets)(Sohl, 2014; Langham et al., 2015). While such an approach may provide important insight into the future distribution of a species, it lacks any mechanistic processes relating climate directly or indirectly to population dynamics. Also, these models fail to incorporate how species’ life history strategy, phenotypic plasticity, dispersal and colonization ability, and genetic diversity may buffer declines due to environmental change in the future.
(referred to as a species’ adaptive capacity; Beever et al., 2016). For most wildlife species, a lack of monitoring data (especially long-term datasets) leaves us with little information to investigate a species adaptive capacity to buffer the effects of climate change, which arguably limits or compromises the development of species priority lists, vulnerability assessments, or adaptation strategies (Beever et al., 2016).

Where long term individual-based data exists (e.g., tracking or capture-recapture data), demographic models can be used to estimate population vital rates (e.g., adult survival or reproductive rates) and estimate the relationship between vital rates to climate variables for a more mechanistic understanding of how climate change may affect future population dynamics (Jenouvrier et al., 2012). Estimating population vital rates can also shed light on a species life history strategy, which could either work to exacerbate population declines or buffer declines in the face of environmental change. For example, demographic models may reveal that when environmental conditions are poor for reproduction (e.g., low fecundity rates during a drought), individuals may choose not to breed (or fail breeding). If survival ($\phi$) is significantly higher for those non-breeding individuals, populations may be buffered from significant decline during periods of poor environmental conditions (Church et al., 2007; Blomberg et al., 2012).

Amphibians are of particular concern to state and federal wildlife agencies as they are rapidly declining globally, primarily as a result of land use development and disease, with such declines only exacerbated due to climate change (Houlahan et al., 2000; Adams et al., 2013). While climate change has not been found to be the primary driver of amphibian declines it could
become a larger contributor to population declines in the future (Miller et al., 2018). Studies of pond or vernal pool (VP) breeding amphibians have focused primarily on species occupancy or counts at the aquatic larval or metamorph stage with less focus on estimating population vital rates (although see Bailey et al., 2004; Church et al., 2007; Muths et al., 2010; Schmidt et al., 2014; Lau et al., 2017). This is mainly due to the logistical difficulties and the effort involved in tracking adults through time and analytical issues involved with fitting statistical models (although, previous modeling shortcomings have been overcome in recent decades; Bailey et al., 2004). The few studies and modeling applications that have explored population demographics and focused on vital rates have shown a disproportional importance of both adult and juvenile \( \phi \) on population trends (Biek et al., 2002; Vonesh & De la Cruz, 2002; Plunkett, 2009). This suggests that previous declines are likely a result of extrinsic factors (e.g., land use change) decreasing adult or juvenile \( \phi \) and implies that if climate change is going to be an important driver of population decline in the future it will likely have to directly or indirectly drive juvenile and/or adult mortality rates.

We fit a multistate survival model to: i) measure unbiased estimates of \( \phi \) and breeding frequency by controlling for imperfect detection (p) of a VP breeding salamander, ii) explore whether climate variables were important drivers of important population vital rates and, if so, iii) investigate whether the salamanders life history strategy could potentially buffer population decline based on these relationships and future environmental changes (e.g., climate change). The multistate survival model, referred to as a “modified robust design” (Bailey et al., 2004),
includes an observable/breeding state and an unobservable/non-breeding state, and was fit to an almost decade long photo capture-recapture dataset of a VP breeding salamander where females are known to frequently “skip” breeding (Gamble et al., 2009), but where little is known regarding the drivers of “skipping”. We had a number of hypotheses regarding the drivers of salamander φ and salamander breeding frequency, including: i) there would be a significant cost of breeding on φ, with individuals “skipping” breeding and staying in the forested upland habitat (non-breeding habitat) having significantly higher annual φ, ii) weather conditions, specifically, summer drought conditions, decreasing adult φ, iii) summer drought conditions acting as an environmental cue that φ or fecundity would be reduced in the subsequent breeding opportunity causing more individuals to “skip” breeding, or summer drought conditions increasing “skipping” if suboptimal conditions prevented individuals from being in condition to breed, and iv) lack of precipitation during the migration period increasing “skipping” if individuals could not migrate to VPs without optimal migratory conditions.

**Methods**

**Study species**

Our study focused on the marbled salamander (*Ambystoma opacum*), a VP breeding salamander, which has a unique breeding strategy compared with other similar VP breeding salamanders, in that they migrate from upland terrestrial habitat in late Summer to breed in the
dry VP basin. Female *A. opacum* will often “skip” breeding following a breeding attempt and stay in the upland terrestrial habitat through the breeding season while males are known to typically breed every year and not “skip” breeding (Gamble et al., 2009). Females deposit eggs in leaf litter and brood the eggs until the VP begins to fill, typically a few weeks or as long as a month or two, after egg laying. Once inundated the eggs develop and then hatch into aquatic larvae that overwinter in the VPs. Metamorphs develop and then emerge the next late Spring/early Summer and don’t return to the VPs to breed for three to five years (Houlahan et al., 2000; Adams et al., 2013). *A. opacum* can disperse several hundred meters to breed in a non-natal VP, although only about 4% of individuals disperse to breed in a VP that isn’t their natal VP (Gamble, McGarigal, & Compton, 2007). *A. opacum* range includes much of the eastern United States, with our study area being at the very northern extent of their range (Petranka, 1998).

**Study area**

Our study was conducted in the Holyoke Range in western Massachusetts, USA (Fig. 4.1). The study site is mostly mixed-deciduous hardwood forests but is bisected by a 30-m wide powerline corridor and contains a number of logging roads and walking trails. The fourteen VPs on the study site exist in two “clusters” with 10 VPs in the western section of the study area in close proximity (within a few hundred meters of each other) and four VPs in the eastern section of the study area spaced further apart (500 to 1500m) (Fig. 4.1). The VPs vary in hydroperiod
and water depth, with most VPs drying between June and September and others closer to permanent ponds during an average year. Differences in hydroperiod result in different structural vegetative communities at the VPs, including shrub-dominated, open water and shallow (open or vegetated) VPs. Populations of breeding female and male *A. opacum* at the VPs range from 0 to ca. 250 individuals and 0 to ca. 400 individuals, respectively. *A. opacum* have not been detected at any VPs within 1250m of the study site.

**Field methods**

We monitored populations of *A. opacum* at our study site from 2000 to 2009 (except 2007). Each VP had a drift fence around the perimeter with numerous pitfall traps spaced out along the perimeter of the drift fence (See Jenkins, McGarigal, & Timm, 2006 for more details). We checked traps daily from May through November of each year to capture metamorphs emerging from the VPs in the late Spring and early Summer and breeder adults in the early Fall when they migrate to the VPs to breed. Captured adults in the Fall were sexed (visual inspection of the cloacal region for swelling) and a digital photograph was taken of each individual’s unique dorsal pattern in a specially designed photograph box (beginning in 2002) to improve photograph quality and photograph consistency (Gamble, Ravela, & McGarigal, 2008). Captured metamorphs were toe clipped, with a pattern based on their natal VP so when they were later captured as adults their natal VP was known to estimate rates of juvenile dispersal (Gamble et
al., 2007). During the off-season, we closed all pitfall traps and created frequent openings along the drift fences to allow passage of animals.

**Salamander photograph processing**

To develop unique individual photograph capture histories, we matched our photographs using the image matching software AmphIdent (http://www.amphident.com), with a specialized AmphIdent module designed specifically for *A. opacum* and our specific photographs (Matthé et al., 2017). We performed several photograph preprocessing steps prior to matching images with AmphIdent’s matching algorithm. First, we straightened the salamander’s bodies in all photographs using the image editing software ImageJ (https://imagej.nih.gov/ij/). Briefly, this involved manually marking the spine of the individuals prior to an operation that warps the spine to a straight line by adjusting adjacent pixels to the spine accordingly and then cropping each photograph to a rectangle around each salamander. Then, using AmphIdent, we cropped all photos to a consistent portion of the dorsum of each salamander from the front legs to the rear legs (Appendix Z). After cropping, AmphIdent processed each photograph and created a binary red/black image (Appendix Z). We reviewed all 12,022 red/black images and discarded 1,793 images where it was apparent heavy glare from the camera flash on the original photograph had heavily distorted the red/black image.
Image matching to develop individual capture histories

AmphIdent (http://www.amphident.com) uses a pixel-based approach to measure a similarity score between all images in the database (Matthé et al., 2017). Here, each red/black image was scaled down by 25% per dimension, assigning to the resulting pixels the average of the original $4 \times 4$ pixels. The similarity score for all image pairs was based on the sum of the absolute differences of corresponding pixel values in both images. To improve robustness against translation, scaling and cropping differences, one image was scaled and translated by combinations of different scales and translations. The final similarity score was the maximum score calculated over all the investigated transformations. Once a similarity score was measured between all image pairs in the database, we reviewed each image in the database with the 7 highest ranking images based on the similarity score (Appendix AA). More specifically, we either i) selected one of the 7 images as a “match” if its pattern matched the focal image pattern or ii) concluded that none of the 7 images had patterns that matched the pattern of the focal image. Once we manually reviewed each image, AmphIdent created an individual capture history dataset that we used for our subsequent capture-recapture analysis. In a previous analysis, a 100 image pairs from the photo dataset were visually matched to evaluate error (false rejection error rate) with AmphIdent (Gamble et al., 2008; Matthé et al., 2017). Specifically, the 100 pairs of visually matched images were used to determine how many matching photos AmphIdent failed to rank in the top 10 based on the similarity score (FRR = 8.8%; Matthé et al., 2017).
Statistical analysis

Multi-state survival model design

Due to the nature of our capture scenario in which individual salamanders were only captured at the VP (immigrating to and then emigrating from the VP) when breeding, and the fact that we knew that females often “skip” breeding in any given year (Gamble et al., 2009), we fit a parameterization of a multi-state model referred to as a “modified open robust design” (Bailey et al., 2004; Gamble et al., 2009). This unique specification of a multi-state model has two states in which breeding individuals are in an “observable state” and non-breeding individuals are in an “unobservable” state. Since the detection probability (p) of individuals in the “unobservable” state is 0, \( \phi \) of individuals in the “unobservable” state must be a function of \( \phi \) of individuals in the “observable” state. Here, \( \phi \) of individuals in the “unobservable” state is a function of \( \phi \) of individuals in the “observable” state during the non-breeding interval when all individuals are in the upland habitat, with the assumption being that regardless of state, \( \phi \) in the upland habitat is the same (Bailey et al., 2004; Gamble et al., 2009). State transition (\( \Psi \)) is only allowed at the end of the non-breeding interval (i.e., individuals can \( \Psi \) if they survive the interval) and constrained to be zero during the breeding interval (fixed with \( \Psi \) observable to unobservable (\( \Psi_{\text{OU}} = 0 \)) and \( \Psi \) unobservable to observable (\( \Psi_{\text{UO}} = 0 \)). For males \( \Psi_{\text{UO}} \) was constrained to be 1 to improve model convergence. Our capture scenario also resulted in differing interval lengths as the length of the breeding interval (and the subsequent non-breeding
interval) is a function of the median date when individuals (by sex and VP) arrive at the VP and leave the VP. Females tend to stay at the VP for a longer duration as they will brood their eggs and leave when the VP begins to be inundated with water (Petranka, 1998). Males leave the VP earlier and tend to have longer non-breeding intervals in the upland habitat. Due to differences in VP hydrology, particular VPs tend to fill with water earlier than other VPs. To control for differences in interval lengths by sex and VP we measured the median immigration date and median emigration date to calculate overall interval lengths. Survival probabilities are the only non-instantaneous parameters and were estimated on a biweekly unit, with interval $\phi$ adjusted based on the realized time interval (Bailey et al., 2004; Gamble et al., 2009). A log link function was used to model $\phi$, as this link specification allows $\phi$ to = 1 when the time interval length equals 0. Monitoring did not take place in 2007 so $p$ was set to zero for those capture occasions (15 and 16). We modeled capture histories from the six VPs (2,3,4,5,6,12) where the average number of captures entering the VP to breed was > 20 and we removed any capture histories from the other monitored VPs (1,7,8,9,10,11,13,14) in the study area. We also removed any capture histories of individuals that had dispersed to breed in different VPs through time, however this was a small percentage of overall capture histories (<4%; Gamble et al., 2009).

**Evaluating explanatory variables**

$\phi$ – Covariates were modeled on two habitat terms (VP or upland) and included sex, year and a measure of total summer (June, July and August) precipitation, which was only modeled
on the upland term (individuals in the upland during the non-breeding interval). Total summer precipitation for our study area from 2001 to 2009 was downloaded from the PRISM climate data website (http://prism.oregonstate.edu/).

Ψ - Covariates modeled included sex, total summer precipitation and total migration precipitation (a measure of the total precipitation two weeks before and a week after the mean immigration date). Total migration precipitation for our study area from 2001 to 2008 was also downloaded from the PRISM climate data website (http://prism.oregonstate.edu/).

p – No covariates were modeled on detection (i.e., p(.)). Time varying covariates resulted in convergence issues with estimates of Ψ.

Goodness of fit and model testing

Goodness of fit was assessed using the software UCARE (Choquet et al., 2009). A variance inflation factor (c = χ²/DF) was estimated to perform model selection procedures using Quasi Akaike’s Information Criteria (QAIC)(Burnham & Anderson, 2002).

Model selection framework

Our approach to fitting models involved three iterative steps to find the “top” performing φ, Ψ and p model(s) based on QAIC, as an all model combination approach of biologically reasonable φ, Ψ, and p models was unfeasible due to the high number of possible models. First,
we fit all biologically reasonable $\Psi$ models, with detection constant ($p(.)$) and a $\phi$ model with the VP $\phi$ and upland term “saturated”. We then took the $\Psi$ model(s) within 2 delta QAIC and fit all biologically reasonable VP $\phi$ models with the upland term “saturated”. Lastly, we took $p(.)$, the top $\Psi$ model(s) (from step 1) and the top VP $\phi$ model(s) (from step 2) and fit all biologically reasonable upland $\phi$ models to obtain a final model set. All models were fit in Program MARK (White & Burnham, 1999) with models specified and executed using the R package RMARK (Laake & Rexstad 2008).

**Results**

After filtering distorted/poor quality images we had a total of 10,229 captures (3,424 female and 6,805 male captures/photographs) from our 16 capture occasions from 2000 to 2009 (no sampling in 2007; Table 4.1). This resulted in the capture of a total of 3,791 unique individuals from 6 VPs. The goodness-of-fit assessment in UCARE revealed slight overdispersion in the data ($\hat{c} = 1.63$). Detection was estimated to be 0.72 (0.01SE). Model selection results indicated heterogeneity in $\phi$ (Table 4.2). Survival during the breeding interval different by habitat, with probability of $\phi$ in upland habitat (individuals in the non-breeding state) higher than the probability of $\phi$ of individuals at the VP (breeding state), which also varied among years depending on the length of the sex and VP specific breeding interval length (Tables 4.2 & 4.3; Figs. 4.2 & 4.3). Breeding interval $\phi$ varied between $\sim$0.75 to $\sim$0.95 for males and female breeders at the VP and between $\sim$0.95 to $\sim$0.99 for male and female non-breeders.
depending on the length of the breeding interval (Figs. 4.2 & 4.3). Survival was lower for males than females in the VP habitat during the breeding interval, although, 95% confidence intervals of the beta estimates overlapped (Tables 4.2 & 4.3; Figs. 4.2 & 4.3). Survival during the non-breeding interval only included individuals in the upland habitat in the observable/breeding state and depended on the total amount of summer precipitation, with those non-breeding intervals with high summer precipitation having a lower probability of \( \phi \). Non-breeding interval \( \phi \) varied between \~0.50 to \~0.65 for males (Figs. 4.4 & 4.5). Similar to breeding interval survival, the probability of \( \phi \) during the non-breeding interval depended on the length of non-breeding interval (Figs. 4.4 & 4.6). Model selection results also indicated heterogeneity in \( \Psi \) (Table 4.2). Probability of \( \Psi_{(OU)} \) differed by sex, with female \( \Psi_{(OU)} \) \~0.40 and male \( \Psi_{(OU)} \) \~0.05 (Table 4.3). Probability of \( \Psi_{(UO)} \) for females was \~0.85 (Table 3). Probability of male \( \Psi_{(UO)} \) was constrained to be equal to 1 to reduce issues of model convergence (Table 4.3). Total migration precipitation was included in the 2\(^{nd}\) highest ranking model based on QAIC, but beta estimates overlapped with 0 (Table 4.3).

**Discussion**

**Survival**

There was a significant cost of breeding on survival for *A. opacum*, especially for females, with survival lower for breeders in the VP habitat compared with survival of non-
breeders who “skipped” breeding and stayed in the upland forest habitat. This finding is consistent with studies of studies evaluating the cost of reproduction (Creighton, Heflin, & Belk, 2009; Blomberg et al., 2013). There was also variability in annual survival of breeding individuals, especially females, as survival was a function of how long individuals were at VPs breeding, which could vary annually by weeks depending on when VPs began to fill with water. There was also annual variability in survival during the non-breeding interval and, contrary to our hypothesis and previous findings (Church et al., 2007; Cayuela et al., 2014; Cayuela et al., 2016; Weinbach et al., 2018), survival was lower in the two wettest summers of our study. It is possible that *A. opacum* movement rates are higher under these types of environmental conditions, exposing individuals to more predators and predation, or the fossorial burrows that these salamanders use to forage in the upland forest become flooded and individuals must migrate above ground exposing them to higher predation rates. Our estimates of annual survival for breeders (~0.45 to ~0.60) and non-breeders (~0.50 to ~0.65) are within estimates of annual survival for other VP breeding salamanders (Trenham et al., 2000; Taylor, Scott, & Gibbons, 2006; Church et al., 2007).

**Breeding frequency**

Confirming previous research on VP breeding salamanders (Bailey et al., 2004; Church et al., 2007; Kinkead & Otis, 2007; Cayuela et al., 2014) and results from a previous analysis of this dataset by Gamble et al. 2009 with fewer years of monitoring data, we found that a high
percentage of breeding females transitioned each year to non-breeding the following year ($\Psi_{(OU)} \approx 60\%$). Gamble et al. 2009 found some evidence that less females breed following a summer drought, although our analysis revealed no such patterns relating drought conditions to a decrease in females breeding. We also found no evidence that the amount of precipitation during the migration period to the VPs was an important driver of transition (S4.3). This suggests that *A. opacum* may not be able to pick up on environmental cues (or they are cues we did not model) that might predict lower breeding survival or breeding success which has been found with other VP breeding salamanders (Church et al., 2007; Kinkead & Otis, 2007). The fact that a high percentage of females transitioned from breeding to non-breeding each year ($\Psi_{(OU)}$) indicates that following breeding many females are not in condition to breed for two years.

**Implications of environmental change for a species at the northern extent of its range**

Total summer precipitation is predicted to increase in our study area based on regional downscaled climate models (Rawlins, Bradley, & Diaz, 2012), suggesting a future increase in *A. opacum* survival during the non-breeding interval. Although, future Summer precipitation increases are projected to be fairly modest with high interannual variability. This suggests that Summer drought conditions are highly likely in the future, especially during extremely hot summers (Dai, 2013; Cook et al., 2014). We found that annual survival was driven by the length of the breeding interval which is indirectly linked to climate, as the breeding interval length is a function of when VPs fill with water in the Fall. VPs have been predicted to fill later in the Fall as regional downscaled climate models for our study area predict drier and warmer Fall seasons.
(Brooks, 2009; Rawlins et al., 2012). However, these are predictions are under the assumption that leaf off date will shift later in the Fall in the future. A recent study suggests that earlier leaf on date actually results in earlier leaf off dates (Keenan & Richardson, 2015), which would change future predictions regarding VP hydrology as evapotranspiration rates would change and decrease earlier in the Fall season (Brooks, 2004). Contrary to our hypotheses, the climate variables we modeled had no relationship or a weak relationship to *A. opacum* transition. Total migration precipitation was in our 2nd best transition model, but the beta estimate overlapped with zero. This suggests that *A. opacum* are not able to predict breeding conditions (e.g., cost of breeding in a given year) based on prior Summer conditions and that migration conditions are not an important driver of transition.

**Assumptions and limitations**

Our findings are subject to a couple of noteworthy assumptions and limitations. First, we had to fix detection to be constant in all of our models to improve model convergence. If detection was not constant through time it could bias estimates of survival and transition. Also, our ability to understand the drivers of transition were hampered due to the fact that we had no information on those individuals not breeding as they did not visit the VP to breed. Captures of these individuals would have allowed us to estimate survival of non-breeders without constraining survival of non-breeders to be the same as survival of breeders during the non-breeding interval. In theory, estimates of non-breeding survival are biased low as it involves
estimates of survival from individuals migrating to and from the VPs. Captures of individuals in the non-breeding state would also allow us to measure body condition to evaluate whether body condition differs by state.

Next steps

Summer precipitation was an important driver of survival during the non-breeding interval. Future work needs to investigate whether this variable is correlated with other climate variables and should explore other climate variables that could be driving survival for this species at the northern extent of its range (e.g., winter severity).

Conclusions

Here, a multistate survival model, referred to as a “modified robust design”, fit to our long-term photograph capture-recapture dataset allowed us to: i) measure unbiased estimates, and ii) improve our understanding of the drivers of survival and breeding frequency of A. opacum. We found evidence that climate has both direct and indirect influence on survival of A. opacum, which suggests that climate change could reduce overall abundance of A. opacum even in those populations at the northern extent of A. opacum’s range. The flexibility of Program MARK allowed us to incorporate differing interval lengths by sex, VP and specific interval by using the Log link function to model survival as a function of the length of the interval and coding the analysis in R using the R package RMARK allows us to make our code available to
others modeling similar datasets in the future. Previous analyses to model similar datasets were completed using the software MSSURVIV, which is less accessible to potential users (Bailey et al., 2004; Gamble et al., 2009).
Tables

Table 4.1 Total Counts of unique captures of marbled salamander (*A. opacum*) immigrating to (I) and emigrating out (E) from six of the largest vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts between 2000 and 2009 by sex and vernal pool (no sampling in 2007). Total counts do not represent the total number of individuals immigrating and emigrating as some individuals may have had their photograph removed from the photograph dataset due to distortion of the dorsal pattern from glare on the photograph or “trespassed” the drift fence and not captured during a specific capture occasion.

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106
Table 4.2 Performance of multistate models estimating marbled salamander (*A. opacum*) survival ($\phi$) and transition ($\Psi$) for six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts, based on photograph capture-recapture data collected from 2000 to 2009. For $\phi$, terms were modeled by habitat with a vernal pool habitat (Pool) and upland habitat (Upland). Terms modeled on $\phi$ by habitat included sex and total summer precipitation (total_sum_ppt). All $\phi$ models included an interaction with biweekly interval length (Interval_biweek) to control for the length of the intervals which differed by vernal pool, sex and interval. The model included two states, unobservable and unobservable, which were synonymous with breeder and non-breeder. Terms modeled on $\Psi$ included state (stratum), sex, total migration precipitation (migr.precip.mm) and total summer precipitation (total_sum_ppt). All models included a stratum and sex interaction as females were found in a previous analysis of this dataset by Gamble et al. (2009) to “skip” breeding while males did not.

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<td>194.081738</td>
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Table 4.3 Beta estimates for our top performing model based on QAIC for marbled salamander (*A. opacum*) survival (\( \phi \)), detectability (\( p \)) and transition between states (\( \Psi \)) for six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts, based on photograph capture-recapture data collected from 2000 to 2009. Upland represents upland habitat and Pool represents vernal pool habitat. SexFemale and SexMale represents salamander sex and total_sum_ppt represents total summer precipitation (June, July and August). All \( \phi \) models included and interaction with biweekly interval length (Interval_biweek) to control for the length of the interval which differed by sex, vernal pool and interval. Transition models (\( \Psi \)) included two states (stratum 1 = observable/breeding and stratum 2 = unobservable/non-breeding). All models included a stratum and sex interaction as females were found in a previous analysis of this dataset by Gamble et al., (2009) to “skip” breeding while males did not. Beta estimates are represented in the table along with the the lower and upper 95% confidence interval (lcl and ucl).

<table>
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<th>Beta</th>
<th>estimate</th>
<th>lcl</th>
<th>ucl</th>
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</thead>
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<td>( \phi )(Upland:Interval_biweek)</td>
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<tr>
<td>( \phi )(Pool:SexFemale:Interval_biweek)</td>
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<td>-8.54E-02</td>
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<td>( \phi )(Pool:SexMale:Interval_biweek)</td>
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<td>( \phi )(Upland:total_sum_ppt:Interval_biweek)</td>
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<tr>
<td>( p )(Intercept)</td>
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<td>( \Psi )(Intercept)</td>
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Figures

Figure 4.1 Map of the study site in the Holyoke range in western Massachusetts, USA. A total of 14 vernal pools were sampled for marbled salamanders (*A. opacum*) from 2000 to 2009 (Gamble et al., 2009). The six vernal pools filled in black (2,3,4,5,6,12) had on average >20 individuals and were included in the capture recapture analysis.
Figure 4.2 Estimates of survival probability ($\phi$) by breeding interval for marbled salamander (*A. opacum*) for six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts from 2000 to 2009 in relation to vernal pool, sex and state (1 = observable/breeder and 2 = unobservable/non-breeder). Error bars represent standard errors.
Figure 4.3 Breeding interval length (two week intervals) for marbled salamanders (*A. opacum*) in six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) which was calculated based on the median Julian date of salamander immigration and emigration and separately by sex and vernal pool.
Figure 4.4 Estimates of survival probability ($\phi$) by non-breeding interval for marbled salamanders (*A. opacum*) in six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts from 2000 to 2009 in relation to vernal pool and sex. Note: estimates here include only those individuals in the observable/breeding state. Error bars represent standard errors.
Figure 4.5 Total summer (June, July and August) precipitation in our study area (Fig. 4.1) in Western Massachusetts between 2001-2009. Precipitation data were obtained from the PRISM website (http://prism.oregonstate.edu/).
Figure 4.6 Non-breeding interval length (biweekly count) of female (left panel) and male (right panel) marbled salamanders (*A. opacum*) for six vernal pool populations (Fig. 4.1; 2, 3, 4, 5, 6, and 12) in western Massachusetts by vernal pool. Interval length was calculated based on the median Julian date of salamander immigration and emigration which was calculated by sex and vernal pool.
APPENDIX A

ROOT MEAN SQUARE ERROR, RESIDUAL BIAS AND STANDARD ERROR OF $\Phi_A$ ESTIMATES

Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of $\Phi_A$ estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
APPENDIX B

ROOT MEAN SQUARE ERROR, RESIDUAL BIAS AND STANDARD ERROR OF PA ESTIMATES

Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of pA estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
APPENDIX C

ROOT MEAN SQUARE ERROR, RESIDUAL BIAS AND STANDARD ERROR OF $\Psi_{AB}$ ESTIMATES

Root mean square error (left panels), residual bias (center panels) and standard error (right panels) of $\Psi_{AB}$ estimates with the four different CH simulation scenarios. False rejection rate ranged from 0% to 25%. Lines represent mean values of the 1,000 simulated iterations. Line style represents number of releases per capture occasion and line color represents number of capture occasions simulated (3 or 10 capture occasions).
PEARSON CORRELATIONS FOR SPATIAL LAYERS AT THEIR OPTIMIZED GAUSSIAN KERNEL BANDWITH FOR A. MACULATUM

Pearson correlations for spatial layers at their optimized Gaussian kernel bandwidth for spotted salamanders (*A. maculatum*).
APPENDIX E

PEARSON CORRELATIONS FOR SPATIAL LAYERS AT THEIR OPTIMIZED GAUSSIAN KERNEL BANDWITH FOR *A. OPACUM*

Pearson correlations for spatial layers at their optimized Gaussian kernel bandwidth for marbled salamanders (*A. opacum*).
APPENDIX F

GENETIC DISTANCE AMONG 17 VERNAL POOL SAMPLES OF LARVAL A. MACULATUM

Genetic distance (chord distance; $D_C$) among 17 vernal pool samples of larval spotted salamanders (A. maculatum) in western Massachusetts. $D_C$ values are based on a subset of the data with one randomly sampled full-sibling per family from all vernal pools. Two A. maculatum vernal pools that contained 10 or fewer full-sib families were removed prior to the calculation of genetic distance.

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### APPENDIX G

#### GENETIC DISTANCE AMONG 22 VERNAL POOL SAMPLES OF LARVAL *A. OPACUM*

Genetic distance (chord distance; $D_c$) among 22 vernal pool samples of larval marbled salamanders (*A. opacum*) in western Massachusetts. $D_c$ values are based on a subset of the data with one randomly sampled full-sibling per family from all vernal pools. Seven *A. opacum* vernal pools that contained 10 or fewer full-sib families were removed prior to the calculation of genetic distance.

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APPENDIX H

AICc AND R²m FOR MULTI-LAYER/SCALE LANDSCAPE RESISTANCE SURFACES WHICH WERE “ALL COMBINATIONS” OF OUR BEST SINGLE-LAYER LRSSS FOR *A. MACULATUM*

Akaike's information criteria corrected adjusted for small sample sizes (AICc) and R²m for multi-layer/scale landscape resistance surfaces (LRSSs) which were “All Combinations” of our best single-layer LRSSs AICc for spotted salamanders (*A. maculatum*) sampled in vernal pools in the Pioneer Valley of western Massachusetts.

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APPENDIX I

AICc AND R²m FOR MULTI-LAYER/SCALE LANDSCAPE RESISTANCE SURFACES WHICH WERE “ALL COMBINATIONS” OF OUR BEST SINGLE-LAYER LRSS FOR *A. OPACUM*

Akaike’s information criteria corrected adjusted for small sample sizes (AICc) and R²m for multi-layer SCALE landscape resistance surfaces (LRSs) which were “All Combinations” of our best single-layer LRSs AICc for marbled salamanders (*A. opacum*) sampled in vernal pools in the Pioneer Valley of western Massachusetts.

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APPENDIX J

CORRELATION IN THE *A. MACULATUM* AND *A. OPACUM* LANDSCAPE RESISTANCE SURFACES OPTIMIZED USING THE R PACKAGE RESISTANCEGA
APPENDIX K

SPEARMAN’S RANK CORRELATION OF THE GEOMETRIC MEANS OF THE LOCAL, NEIGHBORHOOD AND REGIONAL VERNAL POOL SCORES FOR *A. MACULATUM* AND *A. OPACUM*

Spearman’s rank correlation of the geometric means of the local, neighborhood and regional vernal pool scores from the spotted (*A. maculatum*) and marbled (*A. opacum*) salamander scores derived from the landscape resistance surfaces and resistant kernels and the geometric vernal pool score derived from the Compton et al., (2007) expert derived landscape resistance surface and resistant kernels.
APPENDIX L

VERNAL POOL SCORES BASED ON THE GEOMETRIC MEAN OF THE LOCAL, NEIGHBORHOOD AND REGIONAL SCORES DERIVED FROM THE UNDERLYING EXPERT DERIVED LANDSCAPE RESISTANCE SURFACE

Vernal pool scores based on the geometric mean of the local, neighborhood and regional scores derived from the underlying expert derived Compton et al., (2007) landscape resistance surface and resistant kernels (not shown here).
APPENDIX M

PLOTS OF OUR SURROGATE MEASURE OF GENETIC DISTANCE WITH ORIGINAL RESISTANCE DISTANCE

Plots of our surrogate measure of genetic distance (i.e., “noise” added to resistance distances) against original resistance distance by level of variance (upper panel standard deviation of 0.001; middle panel standard deviation of 0.25; bottom panel standard deviation of 1.25) at a sample size of 75.
PEARSON'S CORRELATION (R) BETWEEN THE TRUE AND OPTIMIZED RESISTANCE SURFACES

Pearson's correlation (r) between the true and optimized resistance surfaces by: i) level of variance (standard deviation) in genetic distance and ii) sample size, shown here for all univariate scenarios (Table 3.1.).
PEARSON’S CORRELATION (R) BETWEEN THE TRUE AND OPTIMIZED RESISTANCE SURFACES BY SPATIAL EXTENT (50² VS. 100²)

Pearson’s correlation (r) between the true and optimized resistance surfaces by spatial extent (50² vs. 100²) for scenario #5 of our univariate simulations (Table 3.1.).
APPENDIX P

AVERAGE AICC WEIGHT OF THE AVERAGE BOOTSTRAP AICC OF THE TRUE AND ALTERNATIVE MODELS FROM THE MULTIVARIATE MODIFIED-BOOTSTRAP ANALYSIS

Average Akaike’s Information Criteria adjusted for small sample size (AICc) weight of the average bootstrap AICc weight from the 1,000 bootstrap iterations of the true and alternative models from the multivariate modified-bootstrap analysis by level of variance in genetic distance.
APPENDIX Q

PERCENTAGE OF 50 ITERATIONS IN WHICH THE CORRECT FUNCTIONAL TRANSFORMATION WAS SELECTED WITH THE RICKER TRANSFORMATION

Percentage of 50 iterations in which the correct functional transformation (Inverse Ricker in upper panels and Monomolecular in lower panels) was selected for the optimized true surface by: i) sample size and ii) level of variance (standard deviation) in genetic distance for all univariate scenarios (Table 3.1.).
APPENDIX R

FREQUENCY OUT OF 50 ITERATIONS IN WHICH EACH TRANSFORMATION WAS SELECTED DURING OPTIMIZATION WHEN MONOMOLECULAR WAS THE TRUE FUNCTIONAL TRANSFORMATION

Frequency out of 50 iterations in which each transformation was selected during optimization when Monomolecular was the true functional transformation by level of variance (standard deviation) in genetic distance for our univariate simulations. Panels are labeled by: i) sample size (10, 25, 50, 75 or 90), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX S

FREQUENCY OUT OF 50 ITERATIONS IN WHICH EACH TRANSFORMATION WAS SELECTED DURING OPTIMIZATION WHEN INVERSE RICKER WAS THE TRUE FUNCTIONAL TRANSFORMATION

Frequency out of 50 iterations in which each transformation was selected during optimization when Inverse Ricker was the true functional transformation by level of variance (standard deviation) in genetic distance for our univariate simulations. Panels are labeled by i) sample size (10, 25, 50, 75 or 90), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX T

ROOT MEAN SQUARE ERROR FOR THE OPTIMIZED SHAPE PARAMETER OF THE FUNCTIONAL TRANSFORMATION

Root mean square error for the optimized shape parameter of the functional transformation by: i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by: i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX U

ROOT MEAN SQUARE ERROR FOR THE OPTIMIZED MAXIMUM RESISTANCE VALUE OF THE FUNCTIONAL TRANSFORMATION

Root mean square error for the optimized maximum resistance value of the functional transformation by; i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX V

BIAS FOR THE OPTIMIZED SHAPE PARAMETER OF THE FUNCTIONAL TRANSFORMATION

Bias for the optimized shape parameter of the functional transformation by: i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by: i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
BIAS FOR THE OPTIMIZED MAXIMUM RESISTANCE PARAMETER OF THE FUNCTIONAL TRANSFORMATION

Bias for the optimized maximum resistance parameter of the functional transformation by: i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpScale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX X

STANDARD ERROR FOR THE OPTIMIZED SHAPE PARAMETER OF THE FUNCTIONAL TRANSFORMATION

Standard error for the optimized shape parameter of the functional transformation by: i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by: i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX Y

STANDARD ERROR FOR THE OPTIMIZED MAXIMUM RESISTANCE PARAMETER OF THE FUNCTIONAL TRANSFORMATION

Standard error for the optimized maximum resistance parameter of the functional transformation by: i) level of variance (standard deviation) in genetic distance, and ii) sample size for our univariate simulations. Panels are labeled by: i) simulated functional transformation (Inverse Ricker or Monomolecular), ii) level of spatial autocorrelation (RMExpscale = 1 or 25), and maximum resistance value (50 or 200).
APPENDIX Z

AMPHIDENT WAS USED TO CROP THE DORSUM PATTERN USING THE IMAGE MATCHING SOFTWARE AMPHIDENT

Amphident was used to crop the dorsum pattern using the image matching software AmphIdent (bottom panel). AmphIdent translates every cropped photograph into a red/black image which is used to measure a similarity score between all images in a dataset.
APPENDIX AA

REPRESENTATION OF THE MANUAL IMAGE MATCHING PROCESS WITH THE IMAGE MATCHING SOFTWARE AMPHIDENT

Representation of the manual image matching process with the image matching software AmphIdent. For each image in the database, 7 images with the highest similarity score are made available for review to the user. The user either i) selects a matching image or ii) selects no image if a matching image is not among the 7 images available for review. Shown here are 3 images where the user has to review the 7 images with the highest similarity score. In this scenario, the first image with the highest similarity score is the matching image of the focal image.
Biweekly female breeding interval length and total summer (June, July and August) precipitation from 2001 to 2009. Precipitation data for our study area was obtained from the PRISM website (http://prism.oregonstate.edu/).
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