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Movin' & Groovin' Salamanders: Conservation Implications of Large Scales and Quirky Sex

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MOVIN' & GROOVIN' SALAMANDERS:
CONSERVATION IMPLICATIONS OF LARGE SCALES AND QUIRKY SEX

A Dissertation Presented

by

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DEDICATION

For all the vernal pool critters I met over the years.

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I am deeply indebted to my wife, Sydne, for the love and support she gave me throughout this endeavor. I would also not have been able to do any of this without the support of my mom, dad, sister and brother. And, of course, Annie.

I am grateful to have had the opportunity to spend three springs roaming from pond to pond. I thank the land itself, along with the plants, creatures, weather and all that graces its surface. I am thankful for the landowners who allowed us on their land, and I am thankful for all landowners who maintain open, unposted land for all to enjoy.

I am very thankful for the hard work of my three assistants in carrying out the physical chores of this dissertation. With the heroic efforts of Charley and Ethan, we completed 1151 pond surveys. Meanhwile, in the genetics lab, Andrea sacrificed an entire summer to the unisexual salamanders. A big piece of each of them is in this dissertation.

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Many other individuals aided along the journey, and I have done my best to acknowledge all of them in the appropriate chapters.

ABSTRACT

MOVIN' & GROOVIN' SALAMANDERS: CONSERVATION IMPLICATIONS OF LARGE SCALES AND QUIRKY SEX

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Mole salamanders (*Ambystoma*) and woodfrogs (*Lithobates sylvaticus*) are abundant in New England and depend on ephemeral wetlands for breeding. Their aquatic habitats have been well studied and are protected by several local and regional regulations. State endangered species laws also protect mabled salamanders (*A. opacum*), Jefferson salamanders (*A. jeffersonianum*), and blue-spotted salamanders (*A. laterale*). However, these amphbibians spend most of their adult lives in terrestrial habitats that remain poorly protected and elusive to researchers.

In chapter 1, I developed a novel technique using passive integrated transponders for tracking small animals. I used this technique to track marbled salamanders walking up to 200 m from their breeding pond during post-breeding migrations.

In Chapter 2, I examined the importance of multiple habitat variables for predicting the distributions of woodfrogs and spotted salamanders at 455 ponds in western Massachusetts. Based on a variable-comparison technique I developed, the best predictor for either species of amphibian was the amount of forest in the surrounding

landscape. Both species were found more frequently in upland forests where the ponds are least protected by state and federal wetland regulations.

In chapter 3, I used my data from chapter 2 and three other similar data sets to conduct an analysis of spatial scale and to parameterize a recently published resistant kernel model. The complex model parameterized by an expert panel did significantly worse than the null model. The distributions of both amphibians were best predicted by measuring the landscape at very large scales (over 1000 m). The most effective scales for conservation may be largest for organisms of intermediate dispersal capability.

In chapter 4, I explored the evolution and genetics of the Jefferson/blue-spotted/unisexual salamander complex. I framed research into the fascinating unisexual reproductive system with a model that relates nuclear genome replacement, positive selection on hybrids, and biogeography of the species complex. I parameterized this model using genetic data taken from salamanders spanning Massachusetts and an individual-based breeding simulation. If paternal genomes are transmitted to offspring with the frequencies reported from laboratory experiments, then my model suggests that there must be strong selection favoring unisexuals with hybrid nuclei.

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CHAPTER 1

TERRESTRIAL PASSIVE INTEGRATED TRANSPONDERS FOR TRACKING SMALL ANIMAL MOVEMENTS

1.1 Abstract

Measuring terrestrial movements of small animals poses a substantial technological challenge. I developed very long (up to 130 m) passive integrated transponder (PIT) detectors with which I tracked salamanders (Caudata) migrating from breeding ponds to their upland habitat >200 m away. In all 60 trials, salamanders were detected when released near the antennae. In a second test, I tracked 7 of 14 tagged marbled salamanders (*Ambystoma opacum*) migrating >65 m, well beyond the area protected by existing wetland buffer regulations in Massachusetts. The mean rate of movement for these salamanders (x = 0.9 m/min; SE = 0.1 m/min) was substantially higher than rates of movement reported for related salamanders with radio implants. These PIT antennae offer researchers a means to study small animal movements with less disruption of the animals' natural movement patterns than is caused by other available techniques.

1.2 Introduction

The pond breeding marbled salamander (*Ambystoma opacum*) is threatened in Massachusetts, and protecting its upland habitat requires knowing how far salamanders travel from breeding ponds to their terrestrial home territories (Semlitsch 1998). Due to challenges associated with tracking these small salamanders, few estimates of their migration distances are available (Williams 1973, Douglas and Monroe 1981, Gamble et al. 2006).

Techniques appropriate for large, abundant organisms are inappropriate for small, rare animals. With larger salamanders, radio-implants are possible, although surgery may impact the health and behavior of the study individuals (Windmiller 1996). Transmitter cost and limited battery life also constrain experimental designs (Madison 1997, Madison and Farrand III 1998, Montieth and Paton 2006, McDonough and Paton 2007). Techniques requiring recapture of animals (e.g. drift fencing; Enge et al. 1997) are labor intensive, capture non-target species, and interfere with regular movement patterns (Sheppe 1967). Radioactive tags have provided insight into movements of small salamanders, although health concerns and logistic constraints prevent the use of these techniques in many long term studies (Semlitsch 1981, Ashton 1994). Harmonic radar has recently proven to be a safe way to track very small organisms; however, the tags can be detected only from a short distance and do not allow for individual identification (Pellet et al. 2006).

Passive integrated transponders (PIT) present a promising approach for estimating movement rates of small animals. Tiny PIT tags (8 mm × 1 mm) with unique identification codes can be implanted into animals, and, because they have no batteries, may last for the life of the animals (Gibbons and Andrews 2004). When recaptured using traditional techniques, PIT tags allow researchers to identify individuals when they are recaptured (Germano and Williams 1993, Ott and Scott 1999, Perret and Joly 2002). Detectors placed at fixed locations along streams facilitate detailed studies of fish movements (Prentice et al. 1990 *a*, b; Castro-Santos et al. 1996, Burns et al. 1997, Zydlewski et al. 2006). On land, antennae at culverts, around tree bases, and in small mammal burrows have been used to track movements of desert tortoises (Boarman et al.

1998), lizards (Gruber 2004), and rodents (Harper and Batzli 1996), respectively. Most of these techniques have thus far required that study organisms be funneled into small areas for detection or capture.

I examined a technique for tracking individuals carrying PIT tags across a 2-dimensional surface (e.g. the ground) that does not require funneling through confined areas. My objective was to determine efficacy of using such antennae to track salamander movements.

1.3 Methods

1.3.1 Study Area

I tested half-duplex PIT systems at a seasonal pond surrounded by >1,000 ha of protected mixed-hardwood forest in the Holyoke Range in western Massachusetts. The closed-canopy forest was dominated by eastern hemlock (*Tsuga canadensis*), white pine (*Pinus strobus*), oaks (*Quercus* spp.), birches (*Betula* spp.), maples (*Acer* spp.) and hickories (*Carya* spp.) and had a sparse understory layer. This pond and 13 other nearby ponds supported approximately 1,000 to 1,500 adult marbled salamanders that were part of a long term meta-population study (Gamble et al. 2006, Jenkins et al. 2006, Gamble et al. 2007). Other species observed at the focal pond included spotted salamander (*Ambystoma maculatum*), red-spotted newt (*Notophthalmus viridescens*), four-toed salamander (*Hemidactylium scutatum*), and wood frog (*Rana sylvatica*). I placed antennae up to 300 m from the north of the pond (Fig. 1) because a large concentration of migrating adult marbled salamanders entered and exited the pond from that direction in previous years (Jenkins et al. 2006). The terrain sloped upwards heading away from the

pond, averaging 5° for the first 100 m, 25° for the second 100 m, and 40° for the final 100 m.

I tested full-duplex PIT systems on the grounds of the S. O. Conte Anadromous Fish Research Center in Turners Falls, Massachusetts. I placed antennae within the interior of a mixed-hardwood forest approximately 200 m southeast of the Connecticut River and 100 m northeast of a cleared field. The closed-canopy forest was dominated by northern red oak (*Quercus rubra*), eastern hemlock (*Tsuga canadensis*), white pine (*Pinus strobus*), and birches (*Betula* spp.) and had a sparse understory layer. Terrain was level. Amphibian species observed at this site included eastern red-backed salamander (*Plethodon cinereus*), American toad (*Bufo americanus*), and Fowler's toad (*B. fowleri*).

1.3.2 Antenna Design

I adapted rectangular antennae used in streams (Zydlewski et al. 2006) to lie across the ground and stretch >100 m. An antenna can detect a PIT tag crossing at any point over its length, though I cannot determine the precise crossing location along the antenna.

I designed antennae for 2 types of PIT transceivers: a Digital Angel (St. Paul, MN) FS1001A full duplex transceiver (FD) and a set of Texas Instruments (Dallas, TX) Series 2000 half duplex transceivers (HD). I powered both with 12-volt batteries. The PIT transceivers, batteries, switching circuits, and tuning boxes were all housed in separate weather-resistant plastic containers.

I used fundamental electrodynamics principles to develop the working rules I followed in designing my antennae (Griffiths 1999; Appendix A). In short, inductance (which depends upon antenna geometry) and capacitance (which depends in part on fixed

capacitors) must yield a natural resonant frequency that matches the output frequency of the PIT transceiver. Interested parties can contact the corresponding author for technical specifications.

In large antennae, capacitive coupling between the wire and the earth's surface may cause the antennae to de-tune during rain events, especially when low capacitance values are needed to tune the circuit. To avoid complications of weather-dependent tuning, the wire may be wrapped with a cylindrical insulator of sufficient diameter to make the external capacitance insignificant (Appendix B).

To construct the FD antennae, I placed a pair of 76-m plastic coated lamp wires parallel to each other 0.2 m apart (Fig. 2a) and wrapped them in closed cell polyethylene foam cylinders (o.d. = 0.03 m).The HD antennae consisted of a pair of lamp wires approximately 0.05 m apart and 130 m long (Fig. 2b). The HD system did not require foam insulation because its internal capacitance was much greater than the capacitance between the wire and the earth's surface. One side of the antenna loop lay on the ground and I propped up the other side on guide sticks. I left an additional 10 m at the ends of the HD antennae so that I could fine tune the inductance.

For coarse tuning in the FD antenna, I attached a set of fixed capacitors in series with the transceiver. I used a tuning box built into the FD transceiver for fine tuning (Texas Instruments sells separate tuning boxes for tuning the HD antenna). To tune, I first set inductance of the antenna by adjusting the length of the wire, then adjusted capacitance to maximize the read-range.

For both the FD and HD antennae, I raked leaf litter from a 0.5-m buffer on either side of the wires. I then gathered small sticks locally and laid them perpendicular to the

wire every 0.15 m, giving the appearance of miniature rail-road tracks (Fig. 2). The sticks guided salamanders so that the PIT tags they carried were optimally oriented for detection. Although the travel direction of a salamander was altered for a few centimeters, I did not funnel salamanders from a large space to a smaller space. The sticks also provided sufficient space for salamanders to pass freely under the HD foam insulation.

1.3.3 Antenna Testing

I tested detection rate for both the HD and FD systems and I separately tested the utility of the design for the HD system by tracking migrating marbled salamanders. To assess detection rate under varied weather conditions, I placed salamanders at randomly selected points adjacent to the antenna and allowed them to walk across. For the FD system, I used 12-mm × 1-mm PIT tags tied with dental floss to the backs of juvenile eastern spotted newts (*Notophthalmus viridescens)* with snout-vent-lengths from 3.5 cm to 3.8 cm. I set newts at 12 random points during a nighttime rainstorm. Without re-tuning the antennae, I then repeated this procedure at 18 random points during a sunny day. To measure detection rate of the HD array, I allowed marbled salamanders to cross at 30 locations during a clear day. I affixed a 12-mm wedge transponder to the tail of each marbled salamander using Krazy Glue® cyanoacrylate (Elmer's Products, Inc., Columbus, OH). Before application, I wrapped tags with strips of paper made from cotton and linen to aid in glue adhesion.

I tested the utility of antenna arrays for measuring length of postbreeding migrations of marbled salamanders. Using the HD system, I estimated the distance that marbled

salamanders migrated from their breeding pools to their upland territories. I placed antennae at 66 m, 130 m, 200 m, and 300 m from the high water mark of one

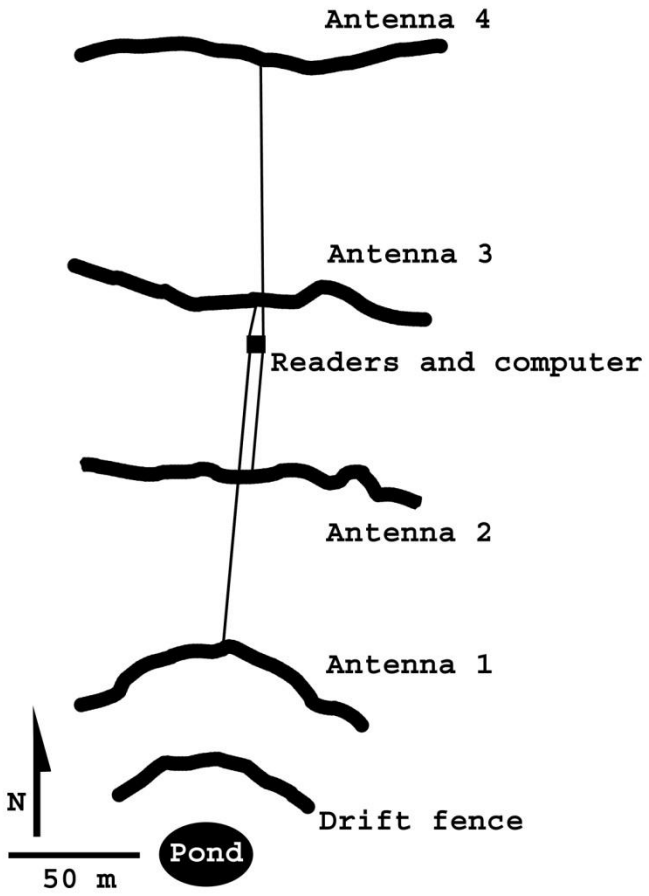

Figure 1.1. Diagram of the Holyoke Range field site and equipment used to track adult marbled salamanders during postbreeding migrations in Massachusetts, September and October 2007.

vernal pool (Fig. 1). These antennae bisected the path of any animal walking north from the pond. Twinaxial shielded cables connected each antenna to one central box containing a computer and transceivers that controlled the antennae.

At 13 m from the pond high water mark, a drift fence with pitfall traps caught migrating salamanders. I affixed tags (either HD 12-mm wedge transponder or HD 23.1-

mm glass transponder) to the tail of each salamander with glue as described above. I held 2 marbled salamanders and one spotted salamander (*A. maculatum*) overnight to demonstrate that tags stayed affixed for the sampling period. After tagging, I released salamanders on the upland side of the drift fence near where I captured them. To conserve battery power, I only turned on the antennae during nights that I released tagged salamanders (27 Sep, 9 Oct, 11 Oct, and 19 Oct 2007).

I used detection events and time stamps recorded by the computer to estimate distribution of distances between breeding pond and salamander home territories as well as salamanders' rates of travel. Because I focused on breeding adults, I expected >96% of salamanders to be migrating to upland habitat, not dispersing to another pond (Gamble et al. 2007). In this analysis, I included only 14 tagged salamanders released from 2 central pitfall traps on rainy nights when antennae were operating. I excluded salamanders released from peripheral traps (n = 2), released on nights when the forest floor remained dry (n = 6), or released towards non-operational antennae (n = 1). My methods were approved by the University of Massachusetts Institutional Animal Care and Use Committee (protocol 25-02-01).

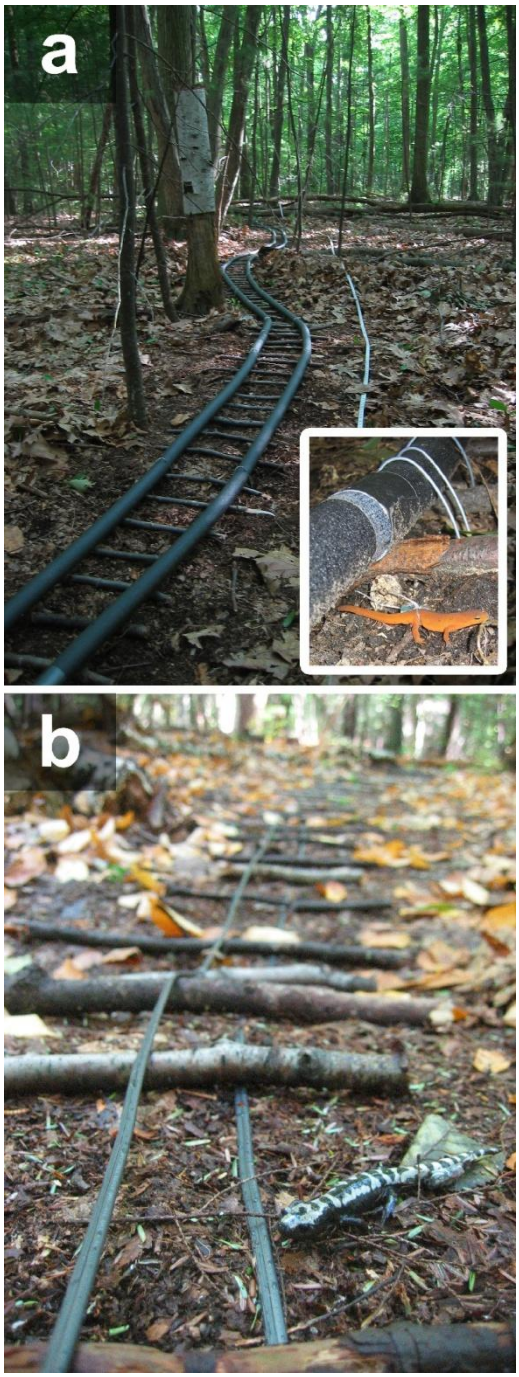

Figure 1.2. Examples of passive integrated transponder (PIT) antennae in the field. A tagged juvenile red-spotted newt crosses under the full duplex (FD) antenna at the S. O. Conte Anadromous Fish Research Center in Turners Falls, Massachusetts (a). A tagged adult marbled salamander approaches a half duplex (HD) antenna in the Holyoke Range in Massachusetts (b).

1.4 Results

The FD and HD transceivers detected salamanders in all 30 trials, which suggests that the system is likely to detect >95% of tagged salamanders that occur under similar conditions. Both the HD and FD antennae remained tuned despite changes in ambient temperature, humidity, and precipitation.

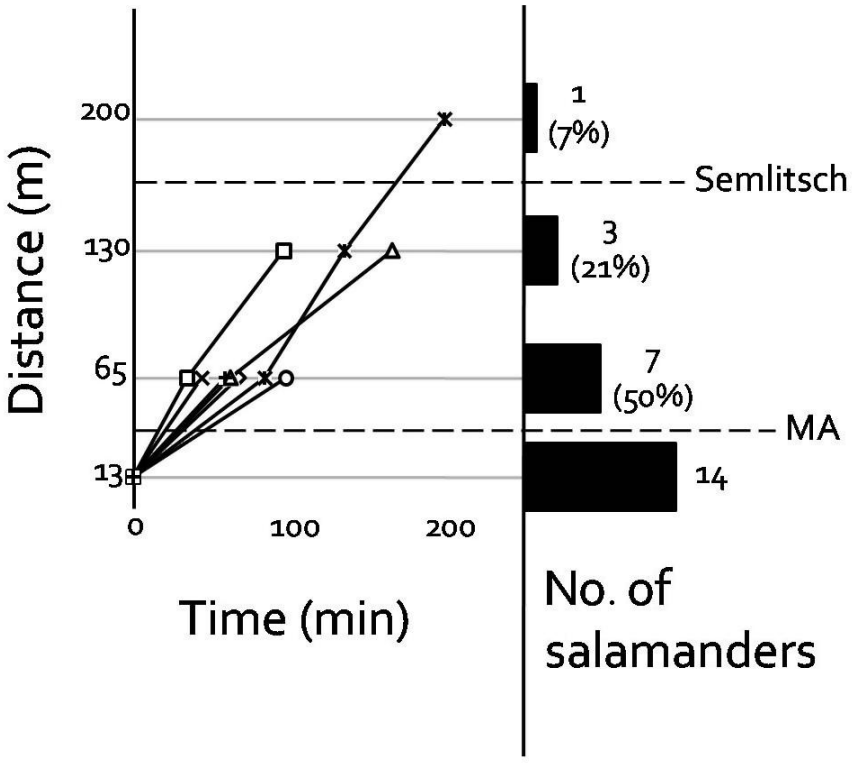

Figure 1.3. Movements of 14 adult marbled salamanders away from a pond during postbreeding migrations on 4 nights (27 Sep, 9 Oct, 11 Oct, and 19 Oct 2007) in the Holyoke Range in Massachusetts. At least half of the salamanders went farther than the Massachusetts 30-m Buffer zone (MA), whereas I detected only 1 salamander (7% of sample) beyond Semlitsch's (1998) proposed 164-m buffer zone (see text).

Of the 14 migrating marbled salamanders released on rainy nights from the central pitfall traps towards functioning antennae, I detected 7 at the 66-m antenna, 3 at the 130-m antenna, one at the 200-m antenna, and none at the 300-m antenna. Salamanders detected at the 130-m antenna were a subset of those detected at the 66-m antenna and included the salamander detected at the 200-m antenna (Fig. 3). Mean rate of movement for the 7 salamanders was 0.9 m/min (SD = 0.2; range = 0.5 – 1.2 m/min).

1.5 Discussion

I demonstrated that long PIT tag antennae may be used to estimate movement rates and extents for small animals. Movement rates of migrating marbled salamanders I documented are similar to movement rates of untagged spotted salamanders observed by Windmiller (1996). By contrast, a study of migrating spotted salamanders using radio tag implants reported much slower rates of movements (max. < 0.3 m/min; Madison 1997). It is possible that behavior of salamanders may be affected by implantation of radio transmitters, a phenomenon well documented in other taxa (Withey et al. 2001). Less invasive techniques like the one I developed may be necessary to obtain unbiased estimates of the movement ecology of small animals.

The 2 major advantages of these arrays over traditional drift fences are that animals can move freely across each antenna and that non-target species are not caught. With traditional drift fences, animal movements are stopped until a researcher releases them. Distance moved in a night may reflect frequency at which traps are checked more than it reflects natural movement patterns of study animals. Furthermore, drift fences

deflect animals from their natural movement trajectory and force them to walk until they reach a trap.

I estimated minimal distances that salamanders traveled to upland territories, yet even these low estimates place the home territories of half of my study animals more than twice as far from their breeding pool as the distance protected by current wetland buffer regulations (Fig. 3; Griffin 1989). Improving detection rate would yield higher estimates of salamander travel distances. Modified study designs could include extending antennae to detect salamanders that would have walked around the edges during this pilot study, tracking salamanders for several consecutive nights of their migration, and permanently implanting tags to avoid loss.

The cost of a multi-year study of upland salamander movements using the HD system is comparable to the cost of using aluminum drift fencing. The cost of using drift fencing increases substantially as traps are checked more frequently and study duration increases. Once installed, PIT arrays allow continuous long term monitoring with little added costs. The most labor intensive part of the PIT antenna array was laying the cross-sticks to guide salamanders, which took approximately 4 person-hours per 100 m, much less than the 15-20 person-hours needed to install 100 m of drift fence (Windmiller 1996). In future trials, I plan to preform antennae with guide sticks in the lab to expedite installation and removal at the field site. The PIT readers can be reused for many other experiments, whereas the costs of drift fence installation and monitoring are almost entirely non-recoverable. I borrowed the readers I used from ongoing fish research at no cost. Multiplexing systems under development (W. Leach, Oregon RFID, Portland,

Oregon, personal communication) may soon eliminate the need for separate transceivers, which will substantially reduce equipment costs further.

High detection rates likely depend upon good antenna maintenance and require that animals cross the antenna on the soil surface. My detections of salamanders during heavy rain in the FD trials and during heavy rain in the postbreeding migrations across the HD antennae demonstrated that antennae function during inclement weather. The FD antenna remained installed for a month without requiring re-tuning and functioned well during nighttime and daytime trials. However, leaves piling on the antennae, snow accumulation, or rodents chewing on the wires could make them ineffective. The PIT tags need to be oriented parallel to the magnetic field lines produced by the antennae (generally circles centered on each wire) and within about 5 cm of one of the wires to be detected. Marbled salamanders can be tracked effectively during migration (a critical portion of their life cycle; Semlitsch 1998) because they walk on the surface. As with most available techniques, long PIT antennae are not likely to detect salamanders during other parts of the year when they are underground. A tagged animal remaining stationary at an antenna could inhibit detection of other animals passing the same antenna, because PIT transceivers cannot detect >1 tag simultaneously at the same section of an antenna. However, in my field experiment with marbled salamanders, none of the 11 detection events lasted more than a few seconds, indicating that animals move quickly past antennae and are unlikely to interfere with other salamander detections. Removing leaf litter and other potential cover may deter animals from resting at the antennae and increase antenna effectiveness.

Maintaining a power supply at the field site is another consideration for employing PIT antenna arrays. I carried a lead acid battery to the site and only operated the antennae during narrow time windows. In locations where systems can be connected to fixed electrical lines, generators, or solar panels, these power sources may facilitate long term studies that require continuous monitoring (Boarman et al. 1998, Achord et al. 2004, Meynecke et al. 2008). Although solar power can be a reliable source of energy in remote locations, it requires an area with direct sunlight and could add a few thousand dollars to the initial cost.

Future arrays might be configured as grids of antennae to allow measurement of animal locations along 2 coordinate axes. Tagged animals residing within the area covered by the grid would be detected as they crossed antennae. Each detection could be treated as a recapture in a mark-recapture analysis. Researchers who are already using implanted PIT tags for long-term identification of individuals could address questions about within-territory movements and dispersal of their study animals by incorporating the system I described.

Table 1.1. Estimated cost (in US$) for a hypothetical study of salamander movements during breeding migrations using a half duplex (HD) passive integrated transponder PIT antennae system or a traditional drift fence based on data collected in the Holyoke Range, Massachusetts, October and September 2007. Detection rings (HD antennae or drift fence) would be placed at 60 m and 110 m from the centers of 10 ponds. Ponds would be monitored 20 nights a year for 3 years.

	Equipment	PIT Tags	Setup labor	Monitoring labor	Total
HD system	110,000	2,000	6,000	10,000	130,000
Drift fence	20,000		30,000	70,000	120,000

1.5.1 Management Implications

Most of the life cycle of most pond breeding amphibians is spent in upland habitat, yet protecting this habitat has proven difficult in part due to lack of knowledge of their

migration distances (Semlitsch 1998). My study suggests that, at my focal pond, the Massachusetts 30-m wetland buffer zone (Massachusetts Wetlands Protection Act. MGL c.131 s.40) would not provide effective protection of marbled salamander habitat (Fig. 3). Using PIT antennae with multiple taxa at many ponds, researchers might determine whether such regulations are adequate to conserve upland habitat. During spring migrations, researchers can deploy this system across a range of sites to estimate what percentage of animals move beyond proposed pond buffer distances.

1.6 Acknowledgments

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Figure 1.4. A marbled salamander (*Ambystoma opacum*) migrating with PIT tag affixed, captured on an automatically triggered camera installed in the uplands.

CHAPTER 2

A VARIABLE-COMPARISON APPROACH TO UNDERSTANDING AMPHIBIAN DISTRIBUTIONS

2.1 Abstract

Conserving pond-breeding amphibians requires us to know what habitat features are most important in controlling their distributions. While researchers are generally discouraged from publishing exploratory analyses, I argue for the importance of such broad studies that compare the importance many predictor variables. To handle the limitations of variable selection routines, I developed a variable comparison method that utilized multi-model inference, data partitioning, and univariate techniques. I fit a suite of habitat variables to observations of spotted salamander (*Ambystoma maculatum*) and woodfrog (*Lithobates sylvaticus*) occurrences at 455 ponds in Massachusetts. Important predictors for both species were water conductivity and percent forest cover in the nearby landscape. I found evidence that both species are more common in upland forests where the ponds are least protected by state and federal wetland regulations.

2.2 Introductions

Globally, conservation biologists are concerned about the survival of many amphibian taxa (Barinaga 1990, Blaustein et al. 1994, Stuart et al. 2004). An important approach to protecting amphibians such as spotted salamanders (*Ambystoma maculatum*) and woodfrogs (*Lithobates sylvaticus*) that breed in ephemeral wetlands ("vernal pools") is through wetland regulation laws that safeguard their breeding habitats (Semlitsch 2000, Zedler 2003). Regulations protecting vernal pools in New England exist at state and federal levels, and efforts are underway to strengthen these regulations (Calhoun et al.

2003, Burne & Griffin 2005, Department of the Army 2010, NHESP 2009). Only a subset of vernal pools receive protection under these laws, and it is not known whether the protected ponds are actually the ones that are best for breeding amphibians.

In developing wetland regulations so that they best protect amphibians, it is important to know what characteristics of the wetlands and surrounding uplands are most important for amphibians. This will help both in deciding which wetlands to protect and what types of land use activities should be allowed nearby. Here, I seek to understand what habitat variables are most important for supporting breeding populations of spotted salamanders and woodfrogs in Massachusetts. Previous studies have examined coarse scale landscape characteristics driving amphibian distributions, however few of these studies attempt to distinguish between different types of forest communities (*e.g.* Guerry & Hunter 2002, Homan et al. 2004, Herrmann et al. 2005, Clark et al. 2008).

Ecology is, at its core, concerned with discovering what factors influence the distribution of organisms. Often, as in the present case, many details are known about separate pieces of the organism's life cycle, but modeling their distributions remains elusive because large components of their life history remain poorly understood (Storfer 2003, Trenham & Shaffer 2005). Yet conservation demands timely answers as to what are the most important factors for the species persistence. Driven by the need to understand their study systems, ecologists regularly employ variable selection procedures such as stepwise selection and data-dredging, despite statisticians' warnings that these techniques result in biased estimates, overfit models, and arbitrary conclusions (Burnham & Anderson 2002, Whittingham et al. 2006). My goal in this study is to compare multiple predictor variables in order to better understand amphibian ecology and to guide

conservation policy. I am not seeking to rank different predictive models, but rather to understand the relative importance of the individual parameters in a multivariate framework.

There are likely to be many complex variables influencing species distributions. One way forward would be to embark on separate studies of small sets of pre-selected predictor variables. This strategy would avoid the pitfalls of model selection routines, yet without companion studies comparing the relative importance of all the variables in context, our ecological insights might be impoverished, progress would occur at a slower pace, and our collective efforts might reproduce some of the follies of variable selection within a single study. Researchers are often advised to use preliminary exploratory data sets to compare the importance of many variables in unpublished studies, but only publish follow up studies on a few choice parameters (Anderson et al. 2001). If we lean too far in this direction, the relative importance of the useful and useless variables would remain hidden in the unpublished preliminary studies. This may result in a situation akin to the "file drawer" problem that causes over-estimates of effect sizes in meta analyses (Rosenthal 1979). If particular experimental approaches tend to show significance for a focal variable, even if that variable seems unimportant with other experimental approaches, the literature will populate with studies from researchers who attempted the significance-yielding approach. Each lab's publications might separately claim significance for their focal variables and we would have little immediate guidance for policy makers. We would lose sight of the big picture. Is the focal variable still important when considered in the sea of other variables, or only in select experimental

designs? To understand the balance, some level of exploratory analysis ought to be cherished in journals.

In order to progress, ecologists need rigorous ways to compare many useful and useless variables at once and publish these findings. Anderson et al. (2001) suggest that we need to develop more *a priori* models to reduce the number of parameters. In the present case, there are in fact many variables with prior empirical and theoretical support and I identified 18 biotic and abioitic variables for inclusion in this study. Given that I expect all of these variables to have at least some influence on amphibian distributions, I aim to rigorously identify which are most important. To accomplish this, I developed a routine that seeks consensus from univariate hypothesis testing, multi-model inference within an information-theoretic framework, and data partitioning procedures (Anderson et al. 2000, Fielding & Bell 1997). With this approach, I can provide estimates of variable importance and coefficients along with estimates of uncertainty in these values. Combining multiple techniques allows me to filter out results that are peculiar to one particular technique. By presenting the results of all of these tests together, I allow readers to assess the relative influence and consistency of each variable examined. I apply this approach to a study of 455 ponds in western Massachusetts. I compare the performance of habitat variables in predicting amphibian presence, and draw new practical insights into amphibian ecology and conservation.

2.3 Methods

2.3.1 Study Area

I selected ponds within two focal areas in western Massachusetts centered on the Housatonic River watershed and the Connecticut River watershed. Each of the areas spans approximately 30 km from east to west and 60 km from north to south. Both areas contain a mix rural residences and urbanized town centers in a matrix of forest and agriculture. Forests are dominated by the following species, in decreasing order of abundance: red maple (*Acer rubrum*), white pine (*Pinus strobus*), white ash (*Fraxinus americana*), red oak (*Quercus rubra*), paper birch (*Betula papyrifera*), black cherry (*Prunus serotina*), sugar maple (*Acer saccharum*), eastern hemlock (*Tsuga canadensis*), quaking aspen (*Populus tremuloides*), yellow birch (*B. alleghaniensis*), black birch (*B. lenta*), American beech (*Fagus grandifolia*), white oak (*Q. alba*), and several other species at lesser abundances. In these areas, I observed the following amphibian species associated with spotted salamanders and woodfrogs during the study: red-spotted newts (*Notophthalmus viridescens*), salamanders in the Jefferson/blue-spotted complex (*Ambystoma jeffersonianum/laterale*), marbled salamanders (*Ambystoma opacum*), four-toed salamanders (*Hemidactylium scutatum*), spring peepers (*Pseudacris crucifer*), gray treefrogs (*Hyla versicolor*), green frogs (*Lithobates clamitans*), bullfrogs (*Lithobates catesbeianus*), pickerel frogs (*Lithobates palustris*), and American toads (*Anaxyrus americanus*).

2.3.2 Pond selection

For this study, I adopted a sampling approach that allowed inclusion of many more sites than in other similar published studies. In structuring data collection, there are two main strategies for dealing with observation error. Proponents of a multilevel model framework for pond sampling would advocate for visiting each site multiple times in order to better model observation error and decouple this source of error from the process error (Royle et al. 2005). Given limited funds and time, a multilevel modeling strategy that requires three visits per site effectively cuts in third the number sites. With sampling ponds for amphibians, there are a large number of extrinsic factors causing high levels of among-site variance that would be difficult to account for by repeated sampling, and which likely swamp out the effects of observation error for small sample sizes. These factors include land use history, hydrogeologic complexities, predation, disease outbreaks, and yearly demographic stochasticity (Marsh & Trenham 2001, Brooks 2005, Harp & Petranka 2006). I argue that to understand the effect of habitat, it is more efficient in this situation to maximize the number of sites surveyed by visiting each site only once. Large sample sizes are necessary to average across the large random inter-site noise. Large sample sizes are also especially important in this type of study where the goal is to compare a large number of predictor variables and maintaining an adequate ratio of observations to variables may be difficult. Observation error is dealt with by making every attempt to minimize bias in the sampling scheme, and drawing sober conclusions from the data that carefully consider which process variables might be expected to correlate with observation error. Sampling with this method allows the data to have the added advantage of being more useful in the short term to regulatory agencies

interested in mapping as many different locations of species occurrences as possible. The data from this study is currently being used by the Massachusetts Natural Heritage and Endangered Species Program to map and protect habitat.

I selected 455 ponds in the Connecticut and Housatonic River watershed areas using GIS with the Massachusetts potential vernal pool data layer (PVP; Burne 2001, www.massgis.gov). To understand the impacts of human land use on amphibians, I sought to include ponds with wide ranging levels of anthropogenic disturbance. Compton et al. (2007) used a resistant kernel model to score ponds according to connectivity and habitat quality at three spatial scales: local, neighborhood, and regional. A simple random draw from the available pools would not result in a data set that spans this connectivity space. To maximize the variance of landscape configurations in the sample, I selected a stratified set of ponds that spanned the range of local and neighborhood connectivity scores within the study region. To minimize bias due to spatial and temporal autocorrelation, pond survey dates were assigned such that sites visited within a local area within a few days of each other spanned the local and regional connectivity space.

Field technicians and I sampled sites in the Housatonic River watershed area in 2008 and 2009, and in the Connecticut River watershed area in 2009 only. To maximize the independence of the data sets from the two years in the Housatonic region, all ponds sampled in 2009 were a minimum of 1 km from ponds sampled in 2008.

I selected a suite of variables that I expect to correlate with habitat features important to amphibians, including pond characteristics, terrestrial forest characteristics and geospatial characteristics. Each of these variables is supported by a body of

literature, but to save space I include only a representative citation for each. At the ponds, field technicians and I recorded the surface area (Windmiller 1996), conductivity (Horne & Dunson 1994), pH (Rowe & Dunson 1993), observations of fish (Gunzburger & Travis 2005), emergent shrub vegetation (Eagon & Paton 2004), and tree canopy over the ponds (Eagon & Paton 2004). In the surrounding landscape, we measured the amount of forest cover (Homan et al. 2004), the density of downed logs (Faccio 2003), categories of human land use (Calhoun et al. 2005) and tree species. In addition, we calculated the amount of incoming solar radiation (Windmiller 1996) and the elevation (Vasconcelos & Calhoun 2004) at each pond.

2.3.3 Data collection

Field technicians and I performed diurnal visual surveys for spermatophores, egg masses, larvae, and adult amphibians during the 2008 and 2009 woodfrog and spotted salamander breeding season (April 2 to May 17). We used Garmin 76-CSx handheld GPS devices to navigate to PVP locations. We walked the entire perimeter of each pond at the water edge. At very large ponds, or ponds with extensive terrestrial obstructions, we stopped walking the pond perimeter after one hour. We used polarized sunglasses and dip nets when necessary to aid in detection. We sprayed equipment with 10% bleach between pond locations to reduce the spread of disruptive microorganisms.

Spermatophores produced by spotted salamanders cannot be distinguished from spermatophores produced by salamanders in the *A. jeffersonianum/laterale* complex (hereafter 'Jefferson salamanders'). Spermatophores detected in the absence of eggs

(n=30) were classified as spotted salamanders because Jefferson salamander eggs occurred at a much lower rate than spotted salamander eggs (0.11 compared to 0.45).

We measured the pond perimeter by pacing the entire shore. This was combined with a shape complexity index derived from a sketch of the pond outline to estimate the pond area. We recorded whether or not fish were observed during the survey and we estimated the percent tree canopy and the percent cover by emergent shrubs over each pond. We measured the water pH and conductivity using OAKTON Instruments (Vernon Hills, Illinois, U.S.A.) PTTestr35 meters. While use of these meters gives occasionally spurious pH readings, I found in a separate study that there is enough repeatability to use the relative trends in pH across many ponds (N. D. C. unpublished data).

At the four cardinal directions, we measured variables about the terrestrial habitat surrounding the pond. We visually estimated percent canopy cover by trees over 13 cm diameter at breast height within 30 m of the pond edge using cover classes which were later averaged across all four directions to calculate a mean percent coverage for each pond. We also recorded the dominant canopy species. Similar species that may be confused in the field, or that hybridize readily were lumped together in our data. Thus, *Quercus velutina* is included with *Q. rubra*, *Betula populifolia* is included with *B. papyrifera*, *Populus grandidentata* is included with *P. tremuloides*, *Fraxinus pennsylvanica* is included with *F. americana*, and we do not distinguish among species in the genera of *Salix*, *Carya*, *Picea*, *Prunus*, and *Ulmus*. When something other than forest covered the landscape, we recorded the type of cover as either agriculture, railroad, paved

road, dirt road, lawn, field, water bodies, powerline, or other human infrastructure (typically buildings or industrial).

Each canopy cover type or tree species was assigned a fractional score reflecting the number of other species recorded in that cardinal direction. These scores were then averaged across all directions for each pond. Only cover types that occurred in at least 30 plots were included in the statistical analyses. We also counted the number of downed logs over 10 cm diameter within 2.5 cm of the ground on a line transect going 30 m away from the pond. In 2008, these terrestrial measures were estimated from the pond edge, while in 2009, we walked a transect out 60 m, and recorded the number of logs crossed by the transect out to 60 m, along with dominant tree species at 60 m. The 2009 60-m and 30-m terrestrial habitat data were combined to match the 2008 data. The four cardinal directions were combined for each pond to give a single estimate for each terrestrial parameter.

I calculated elevation from the digital elevation model (DEM) available from Mass-GIS averaged within 30 m of each pond using the statistical software R (R Core Development Team 2009). I calculated the mean solar radiation within 30 m of each pond for April 15th by applying the solar radiation tool in ArcMap 9.2 to the DEM shapefile re-sampled to a 20-m pixel size. This tool takes into account slope, aspect, and shading from nearby topography. The percent forest canopy cover within 300 m of each pond was calculated from the National Land Cover Database (www.mrlc.gov) forest cover layer using R.

I included a few predictor variables in the models to deal with some of the likely sources of observation error. These variables were the watershed in which pools were

sampled (Connecticut River or Housatonic River), observer (N. D. C., C. S. Eiseman, or E. T. Plunkett), year (2008 or 2009), and date. I included latitude as a predictor variable to deal with spatial autocorrelation at the regional scale. Because the two main rivers run parallel to each other in two North-South valleys, both "watershed" and "elevation" are tightly correlated with longitude, and thus we did not include longitude in the model.

All predictor variables were scaled so that they ranged between 0 and 1. I chose this standardization because many of the variables were measured as percentages and this scaling allows for meaningful comparisons among variable coefficients. After dropping tree species that occurred in less than 30 plots, I re-standardized these variables so that the remaining tree species at each site summed to one. Pond area and conductivity were log-transformed before standardizing. I combined the observations of spermatophores, eggs, larvae and adults into simple detection/non-detection variables for spotted salamanders and woodfrogs. I then performed logistic regression analyses separately for the two species using the "glm" function in the R "stats" package.

2.3.4 Data analysis

I examined each predictor variable in the full model, in univariate models, in a multi-model averaging routine, and in several different sets of partitioned data. I sought consensus from these methods, considering the best variables to be only those that performed well in all of the techniques applied.

With multi-model averaging, I wish to have inference about each variable's performance in all possible models, although there are far too many possible models for practical analysis of them all. Given *n* parameters, the number of parameters in each

possible model varies from one, up to n in the full model. A simple random subset of all possible models would produce results that primarily reflect the performance of variables in models of intermediate lengths. Instead, I used a stratified random subset of all possible models, by selecting $n - 1$ models containing that variable from each model size between 2 and $n - 1$ parameters.

For each selected model, I calculated the focal variable importance as the change in the model AIC (ΔAIC) that results from adding the focal parameter. Across all models sampled for the focal variable, I calculated the mean and standard deviations of ΔAIC. For each parameter, I also reported the number of models for which ΔAIC is negative, and I calculated a separate mean and standard deviation of the parameter coefficient only using these models. To examine the stability of parameter performance across different subsets of samples, I used a three-fold cross validation procedure. I split the data into three random subsets and repeated the model averaging routine while holding out each of the thirds in turn. I made 33 such splits giving a total of 99 cross validation data subsets for each variable. Because the cross validation data sets are by definition smaller than the full data set, the AIC values are not comparable to the full data set AICs. I therefore compared cross-validation results to the full results by using variable ranks based on relative ΔAIC within each model.

I also examined the performance of each variable in the full model and in the model with no other predictor variables. For the full model, I calculated the ΔAIC for each variable. For the univariate models, I calculated what the p-value would be for each parameter in a hypothesis-testing framework. Univariate significance was determined using a Bonferonni family-wise adjusted error rate of 0.05 divided by the number of

parameters considered separately for each of the four model averaging routines. I also calculated the rates at which the survey outcomes (detection/non-detection) were correctly classified by the univariate and full models.

Tree species were treated separately from the other predictor variables by first performing the model-averaging routine on the tree species and then including the best tree species in the model averaging routine for the other predictor variables. Because I did not explicitly include the observation-error variables in the tree species models, I separately examined potential biases due to differences in observer, watershed, and year. To do this, I subset the data by each of these variables as in the cross-validation procedure and examined the stability of the variables across each split.

In most of the analyses, the response variables have two levels: no eggs detected and eggs detected. It is likely that detection error is correlated with the amphibian population size: the more eggs present in a pond, the more likely we are to detect them. Thus, the response variables may be a better proxy for population size than actual presence or absence of amphibians. To examine how the correlation between detection error and breeding effort influences the results, I ran another set of analyses in which the response variables were reclassified based on a ten-egg threshold. The two response categories in this analysis are: less than ten eggs detected and ten or more eggs detected.

2.4 Results

My field technicians and I detected spotted salamanders at 237 sites, and woodfrogs at 236 sites (158 of these contained both woodfrog and spotted salamanders). The mean pond area was 42,000 m^2 (SD = 100,000, range: 4.6 – 1,416,000), mean conductivity was

225 μS (SD = 287, range = 3 – 2690), mean pH was 7.1 (SD = 0.9, range = 4.6 – 9.8), mean cover of emergent vegetation was 20% (SD = 27), mean pond canopy cover was 27% (SD = 31), mean forest cover within 30 m was 58% (SD = 27) mean forest cover within 300 m was 67% (SD = 24), the mean log density was 0.76 logs per 30-m transect (SD = 0.75, range = 0 – 4.75), and the mean elevation was 300 m (SD = 150, range = 35 – 650). Land use categories that we encountered at more than 30 sites were fields (n = 72), lawns (n = 63), paved roads (n = 34), and other human infrastructure (n = 44).

From the tree species multi-model averaging, the top ranking species for spotted salamanders that were consistent across all data subsets were red oak (*Quercus rubra*), black birch (*Betula lenta*), and silver maple (*Acer saccharinum*; Figure 2.1, Table 2.1). Silver maple was negatively correlated with spotted salamander detection, while there was a positive correlation with black birch and red oak. These three were also the variables that would be considered significant in a univariate model. For woodfrogs, red oak is the only species that is consistent across all groups and is also significant in the univariate models (Table 2.1). Woodfrogs were positively correlated with red oak. When the top ranked tree species were combined with the other parameters for spotted salamanders, the predictor variables that performed consistently well across all tests were forest canopy within 300 m (positive correlation), conductivity (negative correlation), logs (positive correlation), black birch (positive correlation) and elevation (positive

Table 2.1. Relative performance of tree species in predicting spotted salamander (*Ambystoma maculatum*) presence.

Species[a]	Occurr.[b]	ΔAIC[c]	Select[d]	Coeff[e]	P[f]	% Corr[g]	Rand splits[h]	Categ splits[i]	+/-[j]
Quercus rubra	210	-11 (4)	306	2.6(0.5)	0.0003*	57	2.2(1.3)	3(2)	7/0
Betula lenta	86	-11 (2)	306	3.9(0.3)	0.0007*	55	2.3(1.3)	4(4)	7/0
Acer saccharinum	33	-9 (5)	301	-4.6(0.9)	0.003*	57	2.6(1.2)	3(3)	0/7
Pinus strobus	276	-4 (4)	264	1.6(0.6)	0.15	57	6(2)	11(6)	5/0
B. alleghaniensis	93	-3.7 (1.5)	306	1.9(0.2)	0.008	56	5(3)	10(6)	5/0
B. papyrifera	204	-1.5 (1.4)	261	1.6(0.3)	0.05	57	8(4)	12(6)	4/1
Populus deltoides	54	-1 (2)	185	-1.9(0.3)	0.018	55	8(3)	11(5)	0/5
A. saccharum	176	-1 (2)	149	1.2(0.3)	0.3	52	9(3)	10(4)	7/0
A. rubrum	315	-1 (2)	130	1.2(0.4)	0.3	59	9(3)	9(5)	5/2
Fagus grandifolia	83	0.4 (1)	104	0.95(0.1)	0.09	52	11(4)	9(5)	5/1
Salix spp.	72	1.2 (1)	34	1.5(0.3)	0.3	55	12(3)	14(1)	3/2
Prunus spp.	179	1.5 (0.7)	21	1.46(0.13)	0.9	52	14(3)	8(6)	4/2
Q. alba	70	1.5 (0.5)	0		0.3	52	15(4)	13(5)	3/1
Fraxinus americana	218	1.6 (0.7)	13	1.13(0.16)	0.5	54	13(3)	11(4)	3/2
Ulmus spp.	30	1.6 (0.5)	4	1.76(0.06)	0.5	53	14(3)	13(2)	3/2
Picea spp.	51	1.7 (0.5)	5	1.61(0.16)	0.7	52	15(3)	13(6)	2/3
Populus tremuloides	147	1.7 (0.4)	1		0.6	53	14(3)	12(5)	3/2
Tsuga canadensis	155	1.7 (0.4)	0		0.4	52	16(3)	9(4)	3/4
Carya spp.	30	1.7 (0.2)	0		0.5	52	15(4)	13(7)	2/1

[a] Species that performed consistently well are shaded

[b] Number of plots (out of 455) in which species were observed

[c] Mean (SD) change in AIC due to focal parameter in 306 models

[d] Number of models (out of 306) in which ΔAIC < 0

[e] Mean (SD) coefficient in logistic regression calculated only from models in which ΔAIC < 0

[f] Based on univariate logistic regression

[g] Percentage of points correctly classified by focal parameter. The full and null models correctly classified 63% and 52% of points, respectively.

[h] Mean (SD) variable rank in 99 subsets of 1/3 of the full data set

[i] Mean (SD) variable rank in 7 data subsets split by observer, river watershed, and year

[j] Number of times mean coefficient was positive / negative in data split by categories

Table 2.2. Relative performance of tree species in predicting woodfrog (*Lithobates sylvaticus*) presence.

Species[a]	Occurr.[b]	ΔAIC[c]	Select[d]	Coeff[e]	P[f]	% Corr[g]	Rand splits[h]	Categ.splits	+/-[i]
Quercus rubra	210	-19 (7)	302	3.9(0.8)	4E-06*	59	1.02(0.14)	1.4(1.1)	7/0
Acer saccharinum	33	-6 (4)	289	-2.8(0.9)	0.006	55	4(2)	5(2)	0/7
Betula lenta	86	-5 (2)	297	5.4(0.9)	0.007	53	5(3)	7(3)	7/0
Salix spp.	72	-4 (4)	258	-2.2(0.9)	0.007	55	5(3)	7(4)	1/6
Fagus grandifolia	83	-3 (2)	274	3.9(0.8)	0.006	55	7(4)	9(6)	7/0
Carya spp.	30	-1.7 (1.3)	291	-8.5(1.6)	0.17	53	9(5)	10(7)	1/4
B. alleghaniensis	93	-1.5 (1.8)	237	4.8(0.9)	0.018	54	9(4)	11(4)	6/0
Pinus strobus	276	-1 (3)	180	-1.3(1.1)	0.05	55	9(3)	9(4)	1/6
Populus deltoides	54	-1 (2)	179	-2.1(1)	0.06	54	10(4)	12(7)	2/4
B. papyrifera	204	-0.5 (1.3)	205	2(0.9)	0.07	56	11(4)	11(6)	5/2
Picea spp.	51	-0.5 (1.7)	177	-2.3(1)	0.1	53	11(5)	11(4)	0/7
Tsuga canadensis	155	0 (2)	127	-2.1(1.3)	0.6	52	11(4)	8(7)	2/5
Populus tremuloides	147	0.2 (1.5)	109	1.7(1.3)	0.3	52	12(4)	12(6)	6/1
Fraxinus americana	218	0.3 (1.7)	104	1.2(1.5)	0.5	52	13(3)	12(3)	4/3
A. rubrum	315	0 (3)	70	-1.4(1.8)	0.4	54	13(3)	12(4)	3/4
Prunus spp.	179	0.5 (1.2)	93	2.2(1.1)	0.3	52	13(4)	12(5)	6/1
A. saccharum	176	1.2 (1.4)	32	0(3)	0.5	52	15(2)	15(3)	5/2
Q. alba	70	1.4 (0.5)	6	-1(6)	0.4	52	16(3)	14(4)	4/3
Ulmus spp.	30	1.7 (0.5)	6	5(3)	0.6	53	16(3)	11(6)	4/3

[a] Species that performed consistently well is shaded.
[b] Number of plots (out of 455) in which species were observed
[c] Mean (SD) change in AIC due to focal parameter in 306 models
[d] Number of models (out of 306) in which ΔAIC < 0
[e] Mean (SD) coefficient in logistic regression calculated only from models in which ΔAIC < 0
[f] Based on univariate logistic regression
[g] Percentage of points correctly classified by focal parameter. The full and null models correctly classified 64% and 52% of points.
[h] Mean (SD) variable rank in 99 subsets of 1/3 of the full data set
[i] Number of times mean coefficient was positive / negative in data split by categories

Table 2.3. Relative performance of variables in predicting spotted salamander (*Ambystoma maculatum*) presence.

		1-egg threshold[a]						10-egg threshold[b]	
Variable[c]	ΔAIC[d]	Select[e]	Coeff[f]	Full ΔAIC[g]	*P*[h]	% Corr[i]	Rand splits[j]	Select	Coeff
Forest canopy (300 m)	-15 (12)	462	2.1(0.4)	-5.2	1.6E-10*	64	3(2)	462	2.5(0.4)
Conductivity	-14 (12)	462	-2.1(0.6)	-2.3	5E-8*	63	3(2)	462	-1.3(0.8)
Logs	-14 (8)	462	3(0.4)	-8.1	1.1E-6*	60	4(3)	437	1.7(0.4)
Pond canopy	-13 (8)	462	-0.2(0.2)	-5.4	0.7	53	4(2)	462	-1.1(0.3)
Emergent vegetation	-12 (7)	462	0.8(0.2)	-5.5	0.16	53	5(2)	462	-0.04(0.19)
pH	-10 (6)	462	0(1.1)	-4.5	6E-4*	58	6(2)	431	-0.3(0.8)
Betula lenta	-8 (3)	462	3.7(0.4)	-6.3	7E-4*	55	7(3)	462	2.5(0.4)
Elevation	-7 (8)	425	2(0.6)	-0.5	1.9E-6*	58	8(3)	435	2.2(0.7)
Date	-6 (2)	462	1(0.2)	-4.7	0.013	56	9(3)	462	1.97(0.19)
Acer saccharinum	-6 (5)	462	-3.8(0.9)	-1.8	0.003	57	9(3)	194	-3.8(0.6)
Pond area	-4 (2)	461	-1.1(0.3)	-1.7	0.07	53	11(3)	462	-1.4(0.2)
Forest canopy (30 m)	-4 (8)	233	1.5(0.4)	2	1.2E-6*	59	11(2)	139	1.3(0.3)
Observer	-2 (4)	267	-0.6(0.3)	1	0.18	55	15(4)	234	0.1(0.3)
Year	-1 (3)	230	-0.6(0.3)	0	0.03	55	14(2)	41	-0.3(0.5)
Quercus rubra	-1 (4)	168	1.9(0.5)	1.9	3E-4*	57	16(3)	250	1.6(0.3)
Human infrastructure	0 (2)	168	-2.6(0.5)	1.2	0.011	53	17(3)	36	-2.5(0.3)
River watershed	0 (2)	130	-0.3(0.8)	0	0.4	53	16(1)	107	-0.2(0.9)
Solar radiation	0 (3)	159	1.9(0.4)	2	0.003	56	17(3)	231	-2.3(0.5)
Fish	0.3 (0.8)	137	-0.5(0)	0.6	0.08	54	19(4)	313	-0.73(0.1)
Field species	1.1 (1.1)	59	-1.6(0.2)	1.1	0.05	53	20(2)	13	-1.8(0.2)
Lawn	1.2 (0.8)	31	-2.5(0.3)	0.8	0.07	54	19(3)	75	-4(0.6)
Latitude	1.5 (0.6)	18	0.7(0.1)	1.9	0.4	48	21(2)	48	-0.94(0.12)
Paved road	1.6 (0.4)	0		1.6	0.2	54	21(3)	321	3.1(0.6)

[a] Response variable is based on detection/non-detection of any egg masses. Eggs detected in 232 ponds.

[b] Response variable is based on detection/non-detection of 10 or more egg masses. Ten or more eggs detected in 108 ponds. All variables under here have same definitions as in the 1-egg threshold analyses.

[c] Variables that performed consistently well are shaded.

[d] Mean (SD) change in AIC due to focal parameter in 462 models

[e] Number of models (out of 462) in which ΔAIC < 0

[f] Mean (SD) coefficient in logistic regression calculated only from models in which ΔAIC < 0

[g] Change in AIC due to focal parameter in the full model.

[h] Based on univariate logistic regression

[i] Percentage of points correctly classified by focal parameter. The full and null models correctly classified 69% and 53% of points.

[j] Mean (SD) variable rank in 99 subsets of 1/3 of the full data set

Table 2.4. Relative performance of variables in predicting woodfrog (Lithobates sylvaticus) presence.

	1-egg threshold[a]							10-egg threshold[b]	
Variable[c]	ΔAIC[d]	Select[e]	Coeff[f]	Full ΔAIC[g]	*P*[h]	% Corr[i]	Rand splits[j]	Select	Coeff
Pond canopy	-19 (9)	380	0.9(0.2)	-8.4	0.0004*	57	1.9(0.9)	380	0.3(0.2)
Emergent vegetation	-19 (7)	380	1.32(0.18)	-10.2	0.003	56	2.2(1.3)	380	-0.5(0.2)
Quercus rubra	-13 (5)	380	3.2(0.4)	-10.1	2E-6*	59	4(2)	37	1.2(0.2)
Fish	-13 (6)	380	-1.28(0.18)	-6.8	2E-6*	59	4(2)	195	-0.8(0.1)
Conductivity	-11 (10)	380	-1.8(0.4)	-3.1	7E-6*	59	4.5(1.5)	380	-1.4(0.4)
pH	-10 (8)	380	-0.5(1)	-2.8	0.0002*	57	5(1.3)	380	-1(0.5)
Forest canopy (30 m)	-4 (6)	289	1.3(0.3)	0.9	1.4E-5*	59	9(2)	123	1(0.2)
Latitude	-4 (2)	375	1.2(0.3)	-6.1	0.11	55	9(3)	300	1.3(0.3)
Pond area	-4 (2)	379	-1(0.3)	-2.2	0.14	53	9(2)	380	-2.4(0.3)
Date	-1.9 (1)	370	0.52(0.15)	-2.3	0.4	56	12(4)	380	-1.7(0.2)
Logs	-2 (3)	293	0.6(0.5)	-0.1	0.02	55	11(3)	177	1.1(0.2)
River watershed	-1 (3)	170	0.9(0.4)	0	0.12	54	11.1(1.6)	154	1(0.4)
Elevation	-1 (3)	153	1.2(0.4)	1.8	0.0011*	57	13(2)	191	1.7(0.4)
Year	0 (2)	79	-0.8(0.5)	0	0.5	52	14.3(1.5)	185	0.7(0.4)
Human infrastructure	0 (1.4)	129	-2.2(0.3)	1	0.03	54	15(4)	80	-2.9(0.5)
Forest canopy (300 m)	0 (3)	99	1(0.2)	1.9	0.001*	58	15(2)	154	1.3(0.2)
Solar radiation	0.9 (1.5)	73	1.4(0.5)	1.3	0.02	53	17(2)	103	2.4(0.5)
Lawn	1.4 (0.5)	2	-1.97(0.15)	0.7	0.2	54	17(2)	0	
Paved road	1.7 (0.4)	0		1.8	0.3	53	19.1(1.8)	0	
Field species	1.7 (0.4)	3	-1.35(0.07)	2	0.3	52	19(2)	6	1.8(0.1)
Observer	2 (1.8)	40	1.3(0.3)	1.9	0.7	52	19(3)	231	1(0.2)

[a] Response variable is based on detection/non-detection of any egg masses. Eggs detected in 236 ponds.

[b] Response variable is based on detection/non-detection of 10 or more egg masses. Ten or more eggs detected in 107 ponds. All variables under here have same definitions as in the 1-egg threshold analyses.

[c] Variables that performed consistently well are shaded.

[d] Mean (SD) change in AIC due to focal parameter in 380 models

[e] Number of models (out of 380) in which ΔAIC < 0

[f] Mean (SD) coefficient in logistic regression calculated only from models in which ΔAIC < 0

[g] Change in AIC due to focal parameter in the full model.

[h] Based on univariate logistic regression.

[i] Percentage of points correctly classified by focal parameter. The full and null models correctly classified 69% and 52% of points.

[j] Mean (SD) variable rank in 99 subsets of 1/3 of the full data set.

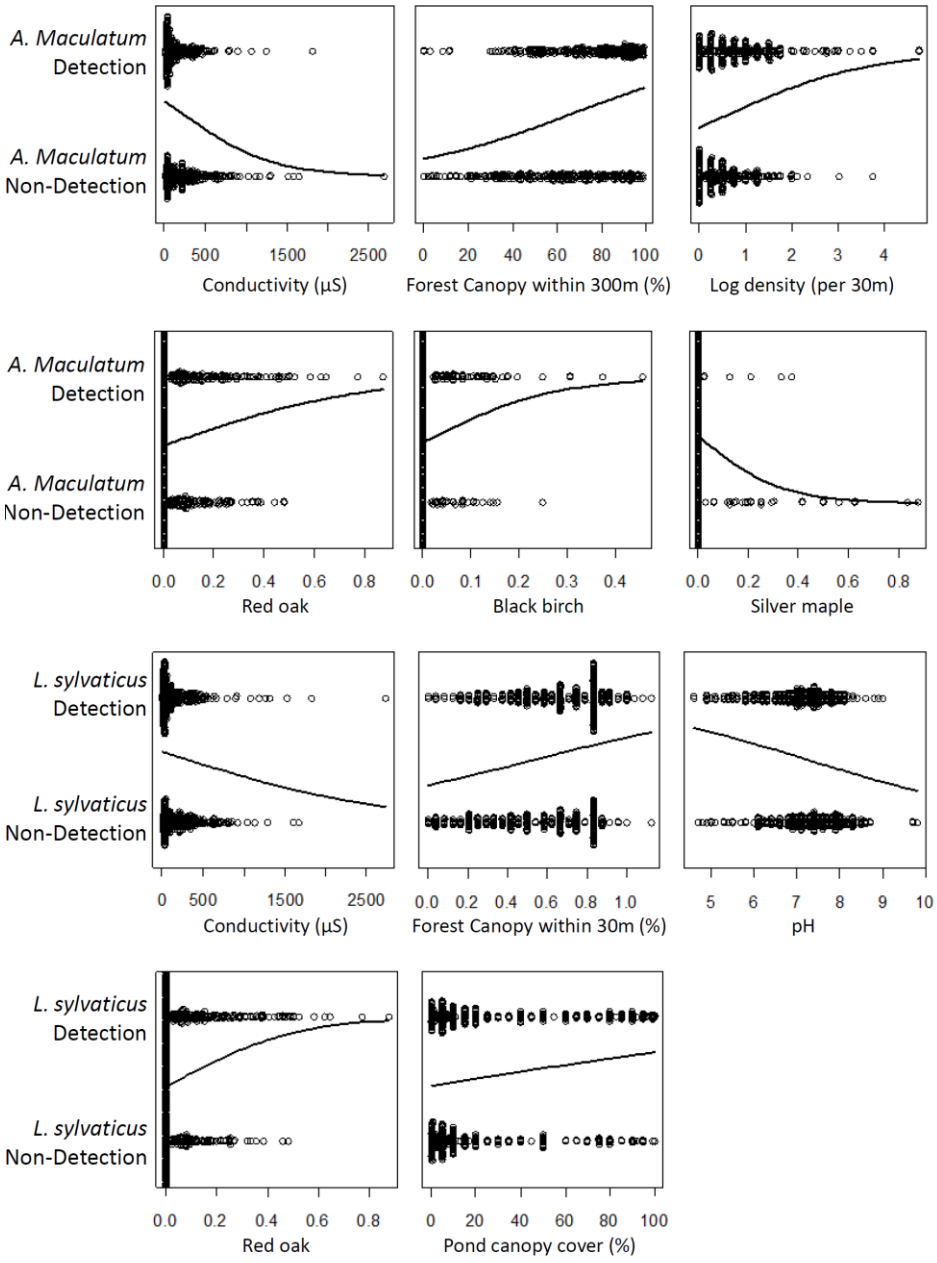

Figure 2.1. Spotted salamander (*Ambystoma maculatum*) and woodfrog (*Lithobates sylvaticus*) detection versus selected predictor variables at 455 sites surveyed in western Massachusetts between 2008 and 2009. All data are binary, points are spaced above and below the detection and non-detection levels for readability. Curves represent univariate best fits from logistic regression for each variable.

correlation; Table 1). For woodfrogs, the best predictor variables were pond canopy (positive correlation), red oak (positive correlation), fish (negative correlation), conductivity (negative correlation), pH (negative correlation), and forest canopy within 30 m (positive correlation; Table 2). Several ponds had unexpectedly extreme pH values, yet after discarding ponds where pH was more than two standard deviations from the mean, the strong correlations with pH remained. The full spotted salamander model with 23 parameters predicted 69% of the points correctly, while a null prediction of universal presence would predict 53% of points correctly. The univariate correct classification rates for spotted salamanders ranged from 48% to 64%. The full woodfrog model with 21 variables correctly classified 69% of points, while the null model had a correct classification rate of 52%. The univariate correct classification rate for woodfrogs ranged from 52% to 59%.

2.5 Discussion

The variable comparison process allows us to understand the most important variables driving amphibian distributions while minimizing some of the arbitrariness associated with model selection schemes. By seeking consensus from several different approaches, I am able to discard likely spurious peculiarities of a particular technique. The multi-model averaging routines yield measures of stability for each of the variables, and I consider the best performing variables to be those with the least variance in the parameter estimates among models. By including a univariate filter, and examining the consistency of the coefficients under different data partitions, I am able to discard variables such as emergent vegetation that may perform well in one multivariate selection routine but not

in other routines. My method sets a higher bar for acceptance of a variable as a robust predictor than if I were to use a single selection technique. Ultimately, this approach allows me to offer several novel insights into amphibian ecology.

As other researchers have found, the amount of terrestrial forest cover surrounding ponds appears to be important for both woodfrog and spotted salamander persistence (Guerry & Hunter 2002, Homan et al. 2004, Herrmann et al. 2005, Clark et al. 2008). My measurements of forest types only extended 30 m from the ponds, whereas the focal species likely use habitat much further away (Semlitsch 1998). However, in comparing ponds to each other, the relative composition of tree species within 30 m is likely representative of the relative composition of tree species at further distances. Evidence of this spatial autocorrelation is seen in the near and far plots from 2009. The abundance of each of the tree species at the pond edge was strongly positively correlated with the abundance of that species at 60 m away.

Red oak and black birch appear to indicate suitable habitat for both amphibians, while silver maple appears to indicate poor habitat. Red oak and birch are both associated with dry upland sites in the focal region, while silver maple occurs primarily in riparian areas (Reed 1988, Swain & Kearsley 2001). Other riparian species, such as cottonwood and willow also tended to be negatively correlated with amphibian detections. These trends are reflected in the positive correlation of both amphibian species with elevation. Potential causes of this correlation may be that riparian forest soil is too moist for overwintering habitat, that heightened levels of aquatic predators occur in riparian areas, or that human developments are concentrated in lowland areas near rivers.

Whatever the explanation for negative correlation of amphibian presence with lowland wet forests, the trend suggests that state and federal wetland regulations which focus on vernal pools near larger wetlands areas are not protecting the best breeding sites for these focal species. Based on the available statewide GIS data, 56 % of my ponds fell within areas that would likely fall under the Massachusetts Wetlands Protections Act (MGL c.131 s.40). This act has authority over wetlands, 100-year floodplains, and 61-m buffer strips around perennial streams. However, at the ponds outside of these wetland areas my technicians and I had higher rates of detection for spotted salamanders, woodfrogs and Jefferson salamanders (Figure 2.2). These data suggest a need for sensitivity to landscape context of wetlands if wetland regulations are intended in part to protect amphibian habitat.

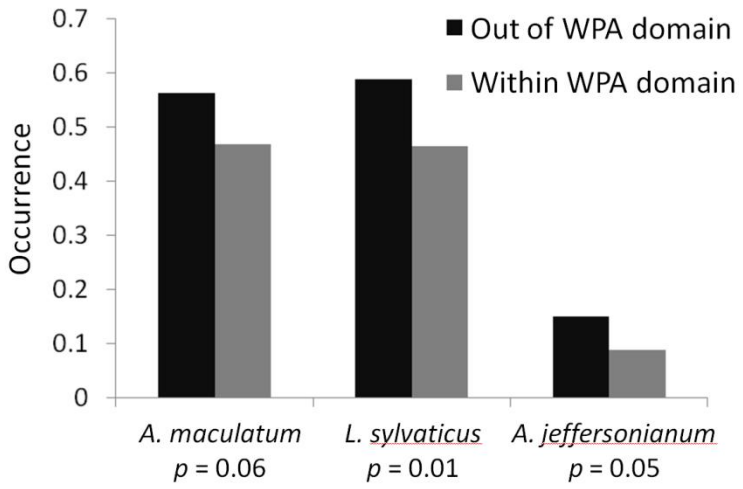

Figure 2.2. Detection rate of breeding spotted salamanders (*A. maculatum*), woodfrogs (*L. sylvaticus*) and Jefferson salamanders (*A. jeffersonianum*/*laterale*) at 254 sites estimated to be within the domain of the Massachusetts Wetlands Protection Act (WPA) and at 201 sites estimated to be outside of the WPA domain. The *p*-values reflect univariate chi-square tests.

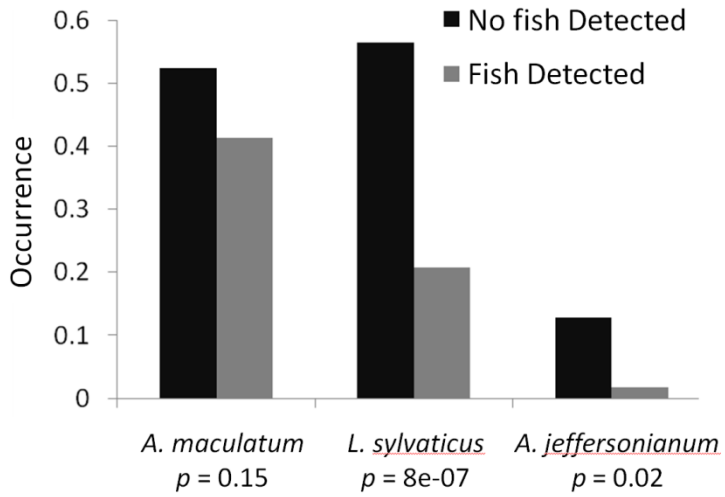

Figure 2.3. Detection rate of breeding spotted salamanders (*A. maculatum*), woodfrogs (*L. sylvaticus*) and Jefferson salamanders (*A. jeffersonianum*/laterale) at 397 sites where no fish were detected and at 58 sites where fish were detected. The *p*-values reflect univariate chi-square tests.

Another interesting trend I found was the difference between the prevalence of woodfrogs and spotted salamanders in ponds where fish were detected. Both species are considered to be obligate vernal pool breeders, and are used by state and federal regulatory agencies as indicators of wetlands largely free of established predatory fish populations which may predate eggs of vernal pool breeding amphibians (Gunzburger & Travis 2005, NHESP 2009). Although my technicians and I did not distinguish between predatory and non-predatory fish, or between established populations and transient individuals, we detected woodfrogs at much lower rates in ponds where we detected fish. However, this trend is much less pronounced for spotted salamanders (Figure 2.3), consistent with the findings of Egan and Paton (2004). This may reflect the fact that spotted salamanders, unlike woodfrogs, have a very firm outer membrane that protects their eggs. Interestingly, fish did have a large effect on the probability of detecting Jefferson salamanders in our study. Jefferson salamanders have a similar ecology to

spotted salamanders, however the outer membrane on their eggs is much thinner and less rigid, and likely more susceptible to predation (Kenney & Burne 2001).

Logs were positively correlated with both amphibian species, however it was a much better predictor for spotted salamanders. Other studies have suggested that logs create important salamander microhabitat, and this is consistent with the present findings (Faccio 2003, Montieth & Paton 2006). It is also possible that log density might be an indicator of forest age and disturbance history, to which the salamanders are responding. I do not see strong negative correlations between amphibian species and early-successional tree species that indicate recent disturbance, such as quaking aspen, black birch or white birch (Leak & Smith 1996, Sutherland et al. 2000). Together, these observations may suggest that Massachusetts amphibian populations can recover from forest disturbance within the first generation of pioneering tree species, as long as enough time has elapsed for coarse woody debris to accumulate.

While the statistical significance of the parameters is evident, the relatively low correct classification rates suggest that there are other important sources of variance. As described in the methods, I expected high rates of unexplained variance due to complex site-specific processes. The single-visit sampling scheme likely caused additional noise because of increased rates of false-negative survey results. Among the variables that I was unable to account for, hydroperiod exerts considerable influence on vernal pool amphibians (Karraker and Gibbs 2009). While my ponds cover a vast range of hydroperiods, the substantial effort required to measure this variable was beyond the scope of my study. If the main effect of the measured variables is consistent across the range of hydroperiods, then the inability to measure hydroperiod should not undermine

the conclusions of the study. If upland forests host better amphibian populations in all cases, then, in the long run, maintaining upland forest will be important for amphibians regardless of the unexplained variance in our short term data set. If there is an interaction between hydroperiod and my measured variables, or among any of the measured variables themselves, then this could be more problematic. As with all ecological studies, one of the biggest limitations in interpretation of the measured variables is that I cannot draw inferences to other regions or other years. My data cannot speak to whether or not, in situations with altogether different hydroperiods or ecological regimes, amphibians may instead be found most commonly in lowland forests.

Given that I do not separately model observation error, conclusions drawn within this study do need to be assessed critically. It seems unlikely that terrestrial log density, upland forest cover, or the presence of fish should be correlated with observation error. However, it is plausible that sites with dense pond canopy, high pH, high conductivity, or dark silver maple leaves could have water that is harder to see through, and thus drive correlations with egg mass detection. One might also expect higher error at high elevations because colder temperatures may have shifted the timing of egg laying to be later than our surveys began. However, my technicians and I actually detected eggs at higher rates at higher elevations, which is consistent with my other habitat findings and with our understanding of the system. The fact that the trend observed for silver maple is largely consistent with the relationships observed for willow, cottonwood, red oak, elevation, and the WPA wetland areas gives me confidence that this relationship is real. If these correlations are a result of observation error, one might expect these trends to be absent in the small ponds that are easiest to thoroughly survey. Yet, if I include only the

67 ponds that represent the smallest 15% of the ponds, all of these dominant trends remain.

My sampling scheme allowed me to achieve the high sample sizes necessary for a broad examination of many variables simultaneously. While I have sacrificed the in-depth precision that a single-variable study can provide, any variable that performed poorly in this study is likely not a major driver of the focal amphibian distributions. Though my methods lay the groundwork for more narrowly focused follow up studies, pending regulatory reform will not wait for all of the experimental studies to be completed, but must reflect the best current thinking.

2.5.1 Management Implications

By selecting parameters that are readily measured at many field sites, I have assessed variables that are likely to be employed by active managers and the broader public. My data suggests that if managers wish to use only one variable to quickly assess the potential of a vernal pool to support spotted salamanders and woodfrogs, they should measure the amount of forest cover in the surrounding landscape. These data ought to compel policy-makers to contemplate the larger scale landscape context of wetlands. Not all vernal pools are the same, and much of the difference in habitat quality is due to the composition and configuration of the surrounding landscape. Efforts should be made to protect isolated upland pools that do not currently enjoy full regulatory protection despite the fact that they likely make better breeding habitat for sensitive amphibians.

2.6 Acknowledgments

I thank the following individuals. C. Eiseman and E. Plunkett collected field data. M. Jones, L. Willey and S. Jackson provided ideas about wetland regulation and error models. P. Warren, K. McGarigal, T. Fuller, and A. Richmond provided manuscript input. Field work was supported by J. Russell, H. Russell and S. Record. Funding sources included a National Science Foundation Graduate Research Fellowship, a Massachusetts Natural Heritage and Endangered Species Program grant, and an Amherst College Lloyd I Rosenblum Memorial Fellowship.

Figure 2.4. Jefferson-type salamander (*Ambystoma jeffersonianum*) eggs detected during a pond survey.

CHAPTER 3

ON SPATIAL SCALE AND EXPERT OPINON: PARAMETERIZING AN AMPHIBIAN MODEL

3.1 Abstract

Spatial scale is a fundamental part of understanding ecology and crafting effective conservation policy. The choice of scale in designing experiments is a common problem facing amphibian researchers. Scale parameters and other parameters used in complex ecological models are often assigned based upon scant data or expert opinion. A recent resistant kernel model used to prioritize pond breeding habitat relies upon parameterizations of spatial scale and land cover resistance values. I optimize parameter values for both spatial scale and landscape resistance using 896 ponds from 5 studies in Massachusetts and Rhode Island. I find that models using resistance values assigned by an expert panel are significantly worse than the null model at predicting amphibian distributions. Using 30-m forest cover data, the best scale for predicting spotted salamander (*Ambystoma maculatum*) distributions was 1650 m (support interval: 1150 – 2150 m). For woodfrogs (*Lithobates sylvaticus*), the best scale was 1150m (support interval: 800 – 1900 m). When using 5-m resolution GIS data, I found a second peak in likelihood at scales under 200 m. The most effective scale for conservation may be largest for organisms of intermediate dispersal capability.

3.2 Introduction

Conserving sensitive species in the face of human development requires us to understand what types of land uses adversely impact the target species and at what spatial scale.

Identifying appropriate scales is a fundamental problem in both ecology and conservation biology (Levin 1992, Noss 1992). For example, knowledge of the distance that peripheral habitat disturbances penetrate into core habitat areas helps determine the minimum habitat area needed to maintain viable populations and metapopulations in reserves (Laurance 2000).

The role of metapopulations in structuring communities of amphibians and other organisms has been debated in the literature (Marsh & Trenham 2001, Freckleton and Watkinson 2002, Smith and Green 2005). Resolving this debate is important for conservation, because it informs the spatial scale at which action is needed. If networks of amphibian ponds function as classic metapopulations, then conservation efforts must focus on connecting large areas with interconnected ponds. If ponds do not act as classic metapopulations, then small scale, single-pond conservation efforts may be somewhat effective.

In a recent GIS-based model prioritizing pond-breeding salamander habitat in Massachusetts, Compton and colleagues (2007) segregated the effects at the population-level, metapopulation-level, and regional level. Measuring landscape features at multiple scales is a common approach used in studies to predict amphibian breeding distributions (Guerry and Hunter 2002, Homan et al. 2004, Herrmann et al. 2005, Baldwin et al. 2006 a, Cunningham et al 2007, Clark et al. 2008). Measurements are often made at a few discrete sizes that are selected *a-priori* based upon direct movement studies, of which there are very few. The local scale parameter in the resistant kernel model, for instance, was based upon one season of radio telemetry at a Rhode Island golf course and one season of radio telemetry in Vermont. It is not clear that the scale arrived at from such

direct movement studies is really the scale at which the model will best perform. Herrmann and colleagues (2005) conducted an analysis over 7 scales between 100 m and 2000 m, and found that the distributions of several amphibians are well predicted by scales up to 1000 m, much further than the expected seasonal migration distance. However, that study only incorporated 61 ponds, and thus had limited statistical power.

Besides a scale parameter, the resistant kernel model also relies upon resistance values assigned to 24 land cover types. However, little data exists to assign values to these parameters, therefore the researchers used the opinion of an expert panel for the land cover resistances. Yet, in the absence of data, expert opinion does not necessarily offer an improvement (Pearce and Cherry 2001). Few studies have rigorously tested the success of expert panels in assigning meaningful values. The ability to successfully assign resistance values to these land use types would not only improve the model, but would also offer guidance for policy makers seeking to regulate the types of activities permitted near wetlands.

In this study, I use breeding surveys for spotted salamanders (*Ambystoma maculatum*) and woodfrogs (*Lithobates sylvaticus*) at 896 ponds to conduct a statistically powerful analysis to determine the scale at which these amphibians respond to habitat fragmentation. I then use these data to optimize the resistance parameters of the resistant kernel model and evaluate the success of the expert panel parameterization.

3.3 Methods

3.3.1 Data sets

For this study, I aimed to include as many vernal pool studies as I could locate. The studies needed to include pond locations and detection/non-detection of spotted salamanders and woodfrogs. I identified and contacted 16 primary investigators of vernal pool research in the eastern United States, including authors of at least six data sets from published literature. Authors of two of the recently published papers were unable to locate their data. From these contacts, I was able to obtain seven separate donated data sets. After discarding data sets that did not have an effective sample size (defined as the smallest of the two outcomes, detection or non-detection) of at least 30 sites, and discarding data sets with spatial overlap, I was left with only three contributed data sets, to which I added two of my own. These included: one in Rhode Island with 151 ponds (S. Egan, unpublished data), one in suburban Boston with 105 ponds (Clark et al. 2008), one in the Quabbin Reservation in central Massachusetts with 171 ponds (D. Clark, Massachusetts Department of Conservation, unpublished data), one in the Connecticut River Watershed in central Massachusetts with 103 ponds (N. D. C., unpublished data), and one in the Housatonic River Watershed in western Massachusetts with 366 ponds (N. D. C., unpublished data). All of these areas except the Quabbin Reservation contain a mix of many land uses including residential, industrial, forests, and fields. The Quabbin Reservation is composed almost entirely of forests, timber cuts, and a large reservoir. Aspects of survey methodology such as timing, intensity, and frequency of visits, differed substantially between studies. All relied upon diurnal visual and auditory surveys for

some combination of egg masses, spermatophores, larvae, and adults of the target amphibians in the sampled ponds.

3.3.2 Simple Scale Analysis

For all of the data sets I conducted scale analyses using a simple model of percent forest cover within fixed radii circular buffers centered on the focal ponds. Forest cover serves as necessary overwintering habitat for both spotted salamanders and woodfrogs in the region (Regosin et al. 2005). I measured the percent canopy cover using the 2001 National Land Cover Data canopy density layer (www.epa.gov/mrlc). I conducted a single analysis for each species with all of the data sets combined, as well as separate analyses on each data set alone. In all models, amphibian breeding detection was used as the response variable, with forest cover as the predictor variable in a logistic regression. In the combined model, a categorical variable distinguishing the data sets was included as a covariate. When the data sets were analyzed individually, I included latitude, longitude, and the interaction term between latitude and longitude as covariates. I varied the buffer radius at 50-m intervals between 50 m and 20,000 m, calculating the logistic regression likelihood at each step. I generated a maximum likelihood estimate for the best scale parameter, and a support interval defined as the highest and lowest radii that produced models within two log-likelihood units of the maximum likelihood (Edwards 1992).

To see if I would arrive at smaller optimal scales with GIS data sampled at finer resolutions, I also performed the analysis on 5-m resolution land cover data using the ponds from the four Massachusetts datasets. Rhode Island was excluded from this

analysis because the data layer I used only covers Massachusetts. I generated 5-m resolution land cover data using the 0.5-m resolution forest cover layer from the Massachusetts Office of Geographic and Environmental Information (www.mass.gov/mgis). I performed the scale analysis with buffer radii ranging from 10 m to 5,000 m at 10-m intervals. The land cover pixel size and maximum buffer radii were determined by my computer processing limits.

I designed a null model to identify potential biases that could be introduced by a combination of noisy GIS data, pond location errors, and the fact that larger buffer circles sample from a greater number of cells. In this null model, I used the same GIS-based measures of forest cover as the predictor variable, but I used forest cover as measured on the ground during pond visits as the response. These data were available only for the Connecticut River watershed and Housatonic River watershed datasets. During field sampling in these regions, the percent forest canopy cover within 30 meters of the edge of each pond was recorded. I converted this local forest cover into a binary variable (greater or less than 50%) to fit my logistic regression model. The expectation is that GIS-based forest cover measured in the smallest radii buffers should best predict local forest cover at the pond, and models should get monotonically worse as buffers increase.

3.3.3 Resistant Kernel Optimization

For both woodfrogs and spotted salamanders, I optimized the resistance values and scale parameter for the local-connectivity resistant kernel model developed by Compton et al. (2007) using the three data sets containing urbanized areas in Massachusetts. This model assigns a resistance value for movement of amphibians through each land cover type,

ranging from one to infinity, with one being minimal resistance. For a focal pond, a Gaussian kernel is used to evaluate the connectedness of each cell in the surrounding landscape to that pond. The scale parameter sets the standard deviation of this kernel. The same sized kernel is then used to produce a weighted sum of these connectedness values, including only cells with suitable non-breeding habitat (forest) around each pond. In the original parameterization, the authors set the scale parameter to 124 m, and assembled an expert panel of seven researchers to assign land cover resistance values.

Land-cover maps were generated at 30-m resolution from 2005 aerial photographs using the methods described by Compton et al (2007). I fixed the resistance of cells containing forest at one, and varied the resistance of 23 other cover types used by Compton et al. between 1 and 40, with 15 steps evenly spaced along a log scale (Table 1). I also examined a null model where all resistances were fixed at 1. The null model is nearly identical to the circular buffer model used in the scale analysis, except that forest is weighted based on distance from the center according to the Gaussian envelope.

The resistant kernel model generates a habitat score for every pond, and I used these scores in a logistic regression to predict observations of breeding amphibians. The Housatonic River Watershed, Connecticut River Watershed, and Boston data sets were combined into a single statistical model by incorporating a categorical variable with three levels, one for each data set. Likelihood values were used to assess the fit of the model to the data, by iteratively optimizing one parameter at a time with R statistical software (R development core team 2009). While all 15 step sizes were tried for a focal parameter, the other parameters were held fixed. The focal parameter was then fixed to the maximum likelihood value, and the next parameter was run. The procedure continued to

rotate through all of the parameters repeatedly until the parameter values no longer changed. In tests, I found that for a given scale parameter, the starting parameters had no effect on the output of the procedure. However, if I allowed the scale parameter to vary along with the other parameters, then the starting values influenced the outcome. This is not surprising, given that the scale parameter only has meaning relative to the resistance values; if I double both the scale parameter and the resistance values, I will end up with the exact same resistant kernel output. Therefore, I optimized the resistance values separately for each of the scale steps, and then constructed a likelihood curve for the scale parameter from these parameterizations.

For each parameter, I calculated the influence as the difference in likelihood between the minimum and maximum likelihood estimates obtained by changing that variable. I also used the coefficient of variation in parameter estimates as a measure of parameter stability.

3.4 Results

3.4.1 Simple Scale Analysis

For predicting spotted salamander distributions from forest cover in concentric circles, the likelihood curve for the combined data sets peaked at 1650 m (support interval: 1150 m – 2150 m) for the 30-m resolution data. Using 5-m resolution data with the four combined Massachusetts data sets, the likelihood curve for spotted salamanders peaked at 2460 m (support interval: 1080 m – 2870 m). With woodfrogs as the response variable, the model using 30-m resolution data peaked at 1150 m (support interval: 800 m – 1900

m) and the model using 5-m resolution data peaked at 1670 m (support interval: 710 m – 5000 m).

Examining the data sets individually, clear likelihood peaks for spotted salamanders are seen between the 1000 m to 3000 m radii in the 30-m resolution models from the Connecticut River watershed, Housatonic River watershed, Quabbin Reservation and Boston area (Fig. 1). The Rhode Island data optimal radius was not reached until 9500 m. For woodfrogs using the 30-m resolution data, the likelihood peaked at scale parameters between 700 m and 1500 m for the three Massachusetts datasets, however it peaked at 250 m for the Rhode Island dataset. In addition to the peak in likelihood at larger scales for spotted salamanders, local maxima are also seen at much smaller radii with the 5-m resolution data in the Connecticut River watershed and the Quabbin Reservation. Woodfrogs showed small scale local maxima using the 5-m resolution land cover for all data sets except the Quabbin Reservation. For the null model predicting forest cover measured during pond visits, the likelihood peaked within the first 100 m and decreased rapidly and monotonically as the buffer radii increased for both land cover resolutions.

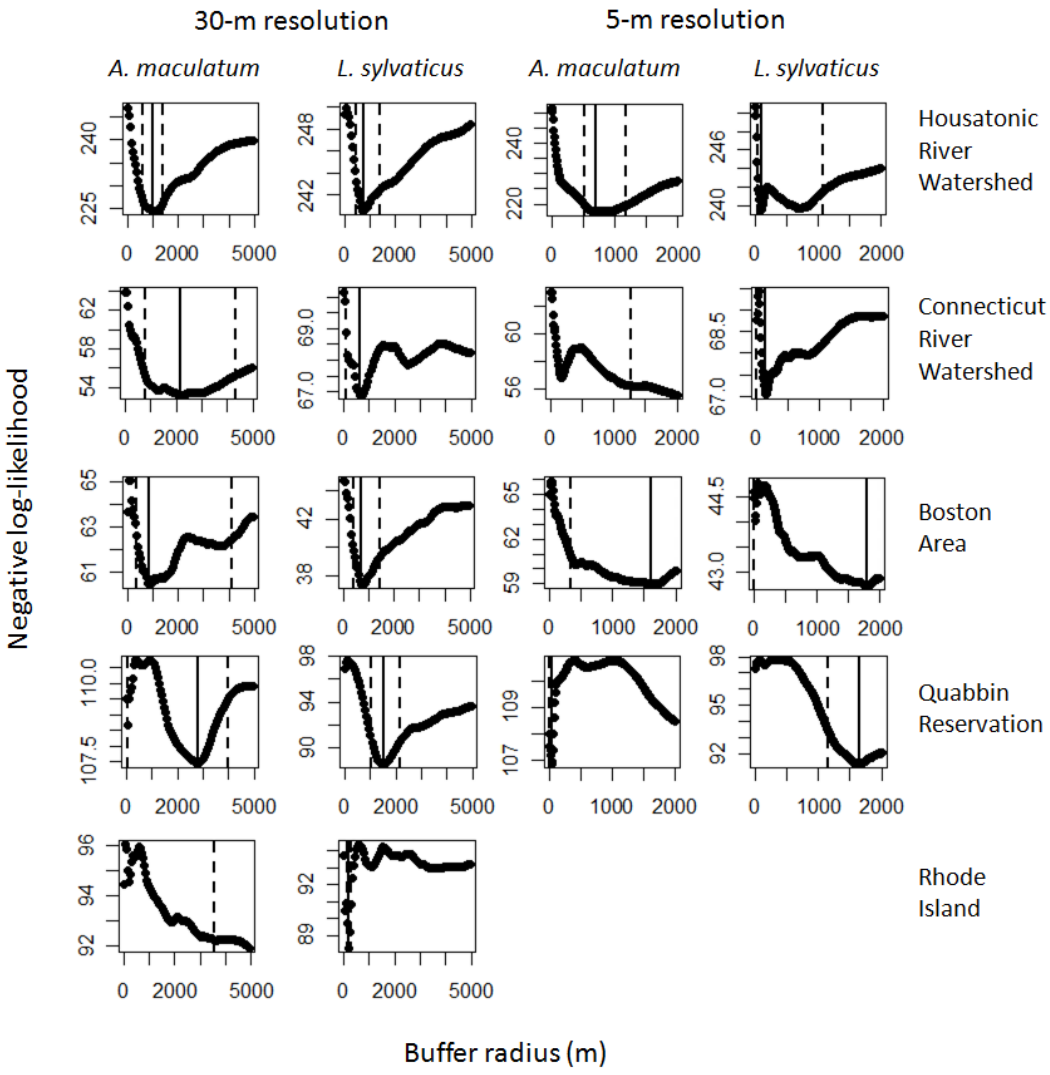

Figure 3.1. Likelihood curves for buffer radius used to measure percent forest cover surrounding ponds. Percent forest is used to predict detections of breeding spotted salamanders (Ambystoma maculatum) and woodfrogs (Lithobates sylvaticus) at focal ponds in five study regions. For the 30-m resolution data, I used the 2001 National Land Cover Data canopy density layer. The 5-m resolution data is resampled from 0.5-m resolution forest cover data from the Massachusetts Office of Geographic and Environmental Information. Solid vertical lines indicate the maximum likelihood estimate. Dashed vertical lines indicate the support interval within two log-likelihood units of the maximum likelihood. For ease of viewing, I do not display the full extent of radii used in the model, but only the portions in which most features are expressed in all of the plots. In the 30-m resolution Rhode Island data, the maximum likelihood for A. maculatum occurred above the maximum displayed scale, at 12,550 m. In the 5-m resolution Connecticut River watershed data, the maximum likelihood for A. maculatum occurred at 4,300 m.

3.4.2 Resistant Kernel Optimization

In the resistant kernel model, when land cover resistances were allowed to vary, the best fit to the spotted salamander data was achieved when the scale parameter was set to 3030 (support interval: 1380 m – 3030 m) (Fig. 2), with no other scales falling in the likelihood support interval. For woodfrogs, the best fit was achieved at 1060 m (support interval: 290 m - 3030 m). When land cover resistance values were set to the expert panel values the maximum likelihood of the scale parameter for spotted salamanders and woodfrogs were 2330 m (support interval: 1380 m – 3030 m) and 480 m (support interval: 370 m - 1060 m), respectively. In nearly all cases, the null model out-performed the expert panel model.

The land cover types that influenced the model fit the most were vernal pool, non-forested wetland, minor street or road, and unpaved road (Table 1). Optimized resistance values varied across scales with a mean coefficient of variance for all parameters' optimal resistance values of 0.8. There was very low correlation between mean optimized resistance values and the resistance values as judged by the expert panel (r^2 = 0.1; Figure 3.3).

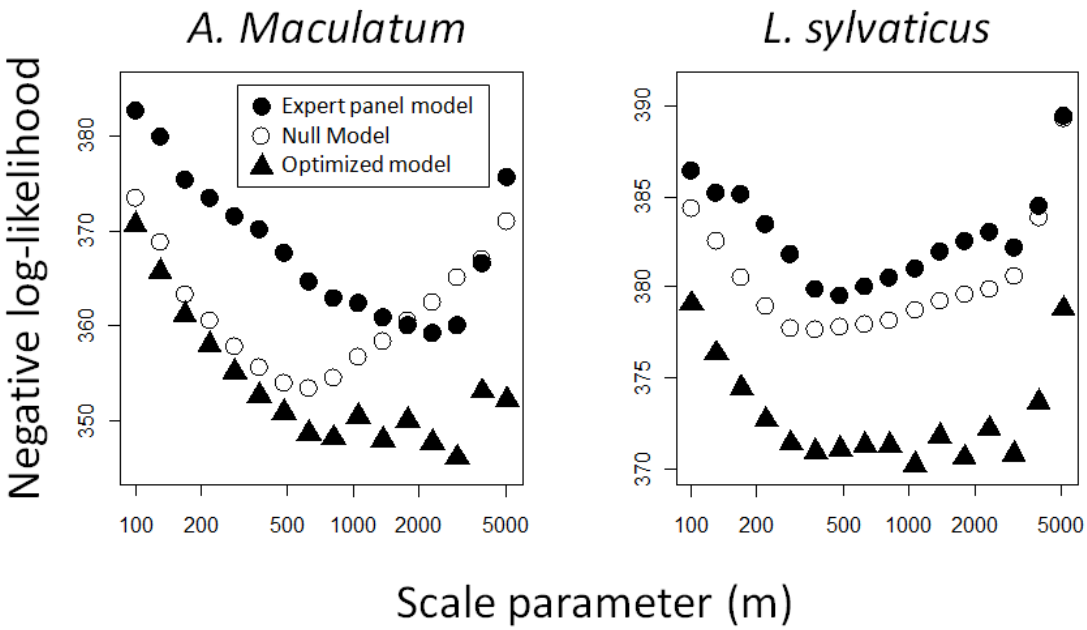

Figure 3.2. Likelihood for various parameterizations of resistant kernel model in predicting spotted salamander (*Ambystoma maculatum*) and woodfrog (*Lithobates sylvaticus*) distributions at vernal pools in Massachusetts. Landcover resistances are set to the optimized values, values determined by an expert panel, or set to unity in the null

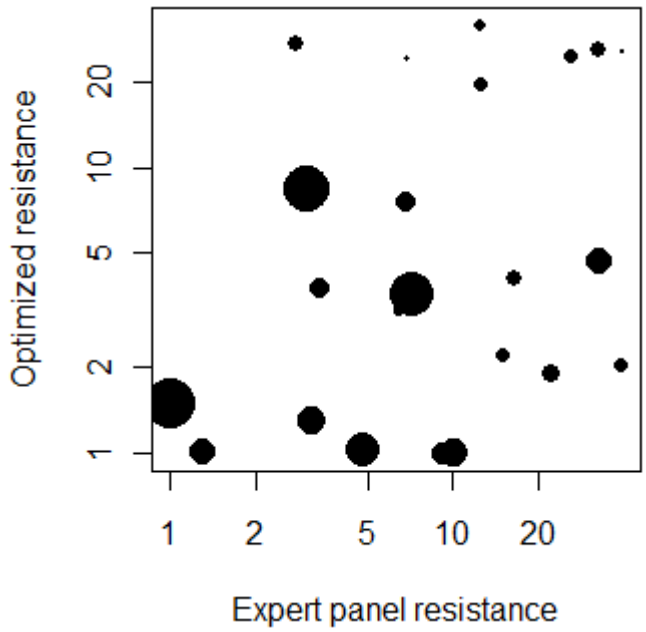

Figure 3.3. Relationship between 23 landscape resistance values for salamander dispersal as calculated by optimization procedure and as judged by an expert panel. The areas of the circles are proportionate to the mean influence of the variable in the optimization procedure.

Table 3.1. Resistance value ranks and influence of land cover types averaged across 15 different scales fit to salamander breeding survey data..

		Ambystoma maculatum		*Lithobates sylvaticus*	
Cover Type	Compton Rank[a]	Influence[b]	Optimized Rank[c]	Influence	Optimized Rank
Vernal pool	1	12 (11)	3.5 (4.6)	6(7)	9.2 (6.6)
Nonforested wetland	5	10 (4)	14.3 (6.7)	3.4(1.4)	15.6 (1)
Minor street or road	12	9 (3)	8.9 (6.4)	4.8(1.4)	4.1 (4.9)
Unpaved road	8	6 (3)	2.1 (3)	3.7(1.2)	1.8 (3.3)
Powerline	6	4 (2)	4.8 (5.1)	2.5(0.8)	1 (0)
Row crop	14	4 (3)	1 (0)	2.5(0.6)	3.5 (4.6)
Stream: 1st order	3	3 (1.3)	1.8 (3.3)	2(1.2)	6 (5.9)
Major highway	21	3 (1.5)	9.6 (7)	1.1(1.3)	14.9 (1.2)
Pasture	13	2.3 (1)	1 (0)	1.6(0.5)	4.1 (4.8)
Low-density residential	10	2 (1)	10 (8.2)	2.4(1)	1 (0)
Old field	7	2 (1.2)	4.1 (6)	1.4(0.3)	2.1 (3)
PondLake	19	1.5 (1)	5.4 (7.9)	1.5(0.9)	18.5 (2)
Major road	18	1.3 (0.6)	14.7 (1.8)	0.7(0.4)	14.7 (2.2)
Stream: 4th order	22	1.2 (1.1)	19.9 (5.3)	1.3(0.7)	18.7 (2.7)
Stream: 2nd order	4	1.2 (1)	19 (7.3)	0.7(0.6)	14.3 (7.5)
Railroad	17	1 (0.5)	3.4 (6.5)	1(0.4)	2.4 (3.8)
Expressway	23	0.9 (1.2)	5.3 (7.1)	0.5(0.5)	2.1 (2.9)
Stream: 3rd order	15	0.9 (0.5)	19.6 (2.1)	0.6(0.3)	14.9 (5.1)
Urban	20	0.8 (0.6)	20.8 (1.4)	1(1)	17.9 (2.7)
High-density residential	15	0.6 (0.3)	21.8 (0.8)	0.6(0.2)	18.6 (5.2)
Orchard	9	0.5 (0.5)	11.9 (5.6)	0.2(0.2)	10.3 (9.1)
Nursery	10	0.2 (0.1)	20.8 (2.6)	0.22(0.16)	17.8 (2.1)
Salt marsh	23	0.03 (0.02)	20.7 (1.9)	0.02(0.03)	17.3 (1.9)
Forest	1	0 (0)	1 (0)	0(0)	1 (0)
Missing data	1	0 (0)	1 (0)	0(0)	0 (0)

[a] Focal land cover resistance rank, relative to all land covers as assigned by expert panel in Compton et al. (2007).

[b] Maximum change in AIC exerted by focal land cover, averaged over all parameterizations, with standard deviations in parentheses.

[c] Focal land cover resistance rank, relative to all land covers in the best fitting parameterization optimized to our data.

3.5 Discussion

3.5.1 Simple Scale Analysis

The distributions of spotted salamanders in the datasets are best predicted by measuring land cover at distances between approximately 1000 m and 3000 m from breeding ponds. This radius is substantially larger than the scales of wetland protection laws, the "95% life zone," or the scale parameter originally used to parameterize the resistant kernel model (Griffin 1989, Semlitsch 1998, Compton et al 2007). One explanation for the difference is that the life zone scale reflects population-level processes, while this study may reflect larger scale metapopulation-level processes. Both the life zone concept and original resistant kernel parameterization are based on annual salamander migration distances. Habitat characteristics within the migration distance of breeding ponds should influence adult survival and thus predict population growth parameters. My models are not based on population size, but rather on the presence of detectable populations which depends in part on colonization rates. The scale at which landscape characteristics influence colonization ought to be determined by dispersal distance. Dispersal distances as calculated through individual movement studies and genetic analyses on pond breeding amphibians are in the range of the optimal scales found in my models (Semlitsch 2008).

In most cases, however, woodfrog detections were best predicted by measuring landcover at smaller radii than spotted salamanders. Dispersal studies do not suggest that woodfrog dispersal distances are any smaller than spotted salamander dispersal distances (Semlitsch 2008). In fact, because frogs are able to hop over obstacles and use con-specific vocalizations to locate ponds, I might expect woodfrogs to be better at colonizing

isolated ponds in a fragmented landscape than spotted salamanders (Smith and Green 2005).

Perhaps the high vagility of woodfrogs means that very few ponds in these landscapes are sufficiently isolated to prevent colonization. If ponds are so close to each other that they all receive many dispersing juveniles every year, then the distribution of breeding populations would not be explained by metapopulation processes (Marsh and Trenham 2001, Smith and Green 2005). Instead, the availability of upland habitat within the adult migration distance of ponds might be a better predictor of presence of a detectable breeding population. Indeed, the scales that worked best for woodfrogs are a bit closer to what we would expect their migration distance to be (Baldwin et al. 2006b). In addition, in the 5-m scale analysis woodfrogs showed a more substantial small-scale peak than spotted salamanders for three of the four data sets examined.

Paradoxically, organisms that are capable of dispersing great distances may be benefited more by small scale conservation than organisms with smaller dispersal distances. Conservation at the spatial scale of metapopulation processes might be most important for organisms in which the isolation distance between habitats is near the limit of their ability to disperse (Figure 3.4). For instance, birds that can easily fly great distances over fragmented areas would be able to make use of small isolated habitat fragments. Indeed, in a study with similar methods to this one, bird distributions were best predicted by smaller scales than most of the optimal scales arrived at for amphibians in this study (N. D C. and S. Lerman unpublished data). At the other end of the spectrum are organisms with very poor dispersal abilities compared to the isolation distance between habitats. For such organisms, large-scale conservation would be of little

benefit. Consider the extreme example of the filmy fern, *Trichomanes intricatum*, which lives in scattered moist caves and only reproduces vegetatively (Ebihara et al. 2008). Conservation of areas much beyond the individual caves it lives in would likely offer little help to the fern.

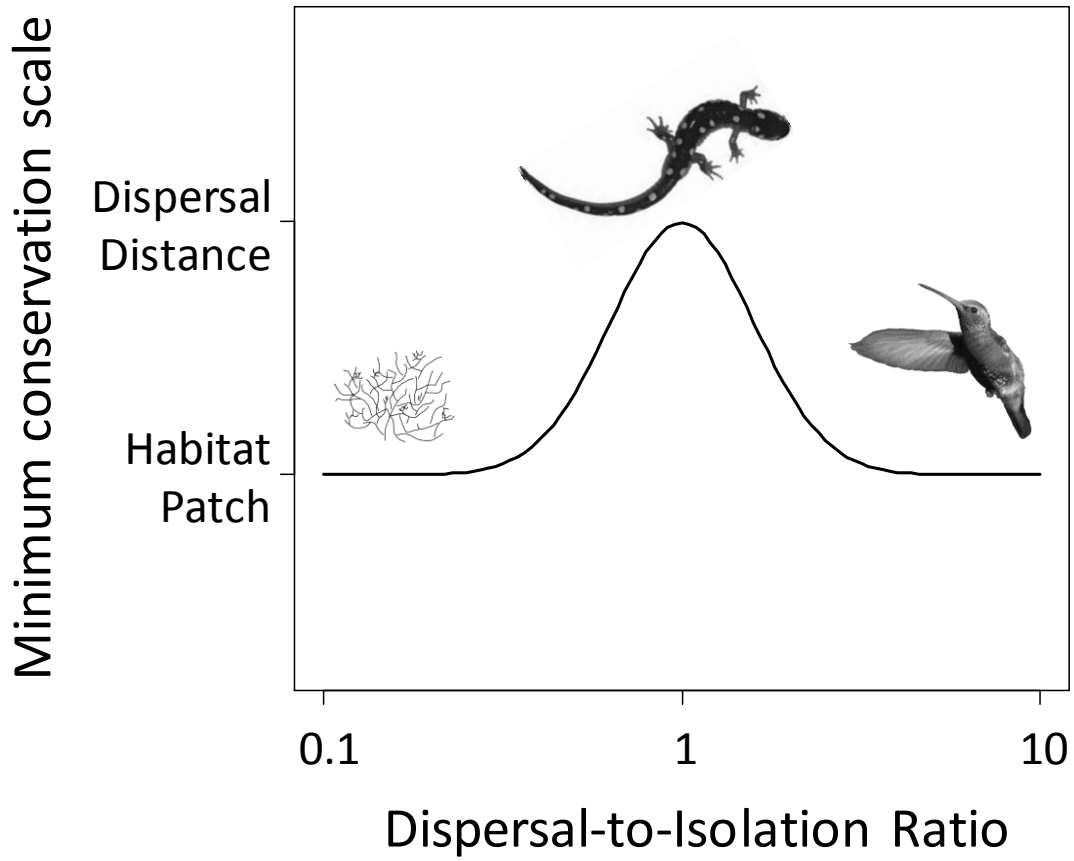

Figure 3.4. Conceptual relationship between the minimum effective conservation scale and an organisms' dispersal ability relative to the isolation distance between habitat patches. Organisms pictured from left to right are: filmy fern, spotted salamander, and humming bird.

3.5.2 Resistant Kernel Optimization

Despite the sophistication of the resistant kernel algorithm, when the expert panel resistances are used, the model is outperformed by my simple model that measures only percent forest area surrounding ponds. Compared to the resistant kernel model as originally parameterized, a higher likelihood is attained by the optimized simple circular buffer model in all three regions for both amphibian species. The likelihood of the null model parameterization was higher than the expert panel parameterization at almost all scales. There are four scales for spotted salamanders at which the expert panel likelihood is greater, and these are explained by the fact that peaks of the likelihood curves occur at different scales in the two models. A smaller scale peak is expected in the null model because the resistances are minimized and therefore the effective kernel volume is larger for a given scale. The fact that the optimized model is many log-likelihood units greater than the other models demonstrates that, if parameterized correctly, the resistant kernel model can offer a much better fit to the data than the simple model. The expert panel parameterization, however, apparently resulted in a worse model.

The optimization procedure produced parameter values substantially different from those of the expert panel. The relative optimized resistance values for land covers including row crops, pasture, and all types of roads were much lower than their expert panel values for both amphibian species. Other land covers, including non-forested wetlands, second order streams, and vernal pools had higher optimized resistances than expected.

The high resistance of vernal pools, particularly for woodfrogs, can likely be explained by its disproportionate influence. The influence of a land cover type is related

to its area and its proximity to the sampling locations. The land cover class designating vernal pools has an enormous influence even though it occupies very little area because it occurs at the center of the resistant kernel. The resistance of vernal pool cells likely functioned as a counterbalance scaling parameter by shrinking the resistant kernel volume for a given scale. As the scale parameter grew larger, so too did the resistance of vernal pool cells.

While the expert panel parameterization did not offer an improvement over the null model, I also have reason to distrust the parameter values obtained by the optimization procedure. Of particular concern are the high variances in the parameter values across scales, suggesting instability in the optimized values. Due to sample size limitations and processor constraints, I did not include a hold-out dataset to test the optimized model against. Perhaps my sample size is too low to appropriately optimize this model. Yet, in light of my efforts to track down all useable vernal pool data sets, I feel that a study of much greater magnitude is unlikely to occur soon. This is a large sample compared to other vernal pool studies. With 574 ponds, a presence/absence ratio of 0.99 and 0.98 and 23 land cover types, there are still more than 12 times as many samples as land cover types.

Inconsistencies in the land cover resistances may in part reflect the inability of a single parameter to capture the myriad types of direct and indirect impacts that land uses can have on the various amphibian life stages. A river near a breeding pond might serve as a source for predators of salamander eggs and larvae, a barrier to dispersing juvenile salamanders, yet have little impact on migrating adults. If migrating adults move more quickly across a parking lot than they do through a forest, then it might make sense for

parking lots to have a lower resistance than forest so that the kernel spreads out further. Yet, lowering the resistance of parking lots seems clearly at odds with such negative impacts as runoff and direct mortality from cars that are associated with parking lots.

3.5.3 Conclusions

Echoing the sentiment of Ockham's razor, in the absence of data, simple models with few parameters may be preferable to complex models parameterized by expert opinion. In the case of amphibians, accurately predicting the influence of many different land use types on populations may be prohibitively complex given our current resources. While I have parameterized the resistance values for the data in this study, perhaps other researchers would arrive at substantially different values in other areas, or using other response variables such as genetic distance. It is quite clear that in this region, my focal species need upland forest habitat. To identify target ponds for conservation I would recommend simple models based on this one known parameter, rather than opting for complexity. To be effective, any model and any long-term conservation initiative requires application at the appropriate spatial scales. The relative importance of population scale versus metapopulation scale influences may vary from species to species, and more work is needed to describe this balance. My study suggests that maintaining vernal pool assemblages is best done by coordinating conservation efforts over fairly large scales, up to 1 – 6 km diameter areas. Pursuing conservation of amphibians through small scale actions such as wetland buffer zones might protect populations in the short term, but may not allow for colonization events that are important for species such as spotted salamanders in the long term.

3.6 Acknowledgments

I thank E. Plunkett, B. Compton, and E. Ene for provding code to run the resistant kernel model. Amphibina survey data sets were provided by B. Windmiller, J. Regosin, P. Paton, R. Baldwin, S. Egan, D. Clark, M. Aliberti, and J. Cunningham and A. Calhoun. Additional feedback was provided by P. Warren, K. McGarigal, A. Richmond, and T. Fuller.

Figure 3.5. A spotted salamander (*Ambystoma maculatum*) migrating across a golf course.

Figure 3.6. Woodftogs (*Lithobates sylvaticus*) migrating across a road during a snow storm.

CHAPTER 4

RELATING HYBRID ADVANTAGE AND GENOME REPLACEMENT IN UNISEXUAL SALAMANDERS

4.1 Abstract

Unisexual salamanders of the *Ambystoma* genus have a complex and fascinating reproductive history. The frequency with which paternal genomes are incorporated into offspring has been debated by researchers and is a key parameter necessary to understand this system. Paternal genome incorporation allows unisexual salamanders to carry nuclear material from five distinct congeneric species. Hybrid nuclei might offer superior fitness over pure species in ecotones, or hybrid nuclei could represent a costly relict of the lineage history. I frame research into the unisexual reproductive system with a model that relates nuclear genome replacement, positive selection on hybrids, and biogeography of the species complex. To parameterize the model, I present microsatellite and mitochondrial sequence data from 15 ponds straddling the range boundary of *A. jeffersonianum* and *A. laterale* in Massachusetts. I also execute an individual-based simulation of the fate of hybrid genomotypes in contact with a single host species over time. I find that, if genome replacement occurs at a rate greater than 1/10,000, then there must be compensating positive selection of similar magnitude in order to maintain observed levels of hybrid nuclei. Future researchers may use the framework I developed as a guide to evaluating the hybrid superiority hypothesis.

4.2 Introduction

The study of bizarre biological systems offers both fascination and the hope that we will gain deeper insights into the standard pathways of evolution (Dawley 1989). Of particular interest to evolutionary theorists are vertebrates that appear to circumvent ordinary sexual reproduction, such as unisexual salamanders in the *Ambystoma* genus (Judson and Normark 1996, Schlupp 2005). These salamanders have a complex reproductive history that involves recurrent nuclear hybridization between five modern species, yet only one ancient monophyletic mitochondrial lineage (Hedges 1991, Robertson et al. 2006). The literature is replete with research and debate about two aspects of unisexual salamander biology: the geographic distribution of genomotypes, and the frequency with which nuclear genome replacement occurs (Clanton 1934, Uzzell 1964, Morris and Brandon 1984, Bogart 1989, Lowcock 1989, Elinson et al. 1992, Spolsky et al. 1992, Petranka 1998, Bogart 2003, Lanoo 2005, Bogart et al. 2007, Bi et al 2008, Bogart and Klemens 2008, Ramsden 2008). These two aspects of their biology ought to inform each other, however I am aware of no attempt to formally combine inquiries into genome replacement and biogeography into one framework. The present study is founded on the realization that observing unisexuals carrying nuclear genomes at great distances from the donor species allows one to calculate an upper limit for the rate of genome replacement in the absence of selection. In this paper, I elucidate my intuition by establishing a mathematical framework that relates genome replacement, selection, and observations of genomotype distributions in unisexual salamanders.

Unisexual salamanders reproduce primarily through gynogenesis, wherein females produce eggs that are clonal copies of themselves requiring sperm only for

activation of embryo development (Bogart et al. 2007, Bi et al. 2008). Paternal DNA is not typically incorporated into the offspring. Sometimes, however, eggs are reduced in ploidy number relative to the female prior to mating and sometimes the male genome is incorporated into the developing embryo, elevating the offspring ploidy (Bogart 1989). These incorporated sperm-donated genomes will be passed on to subsequent generations when offspring reproduce gynogenetically. If offspring never transmit paternally derived genomes (hybridogenesis), then gynogenesis would be lost from the system through a ratcheting effect that tightens every time a hybridogenetic offspring arises.

While occasional incorporation of paternal genomic material is known from other gynogenetic vertebrates (Nanda et al. 1995), *Ambystoma* are distinguished by the frequency with which such incorporation is thought to occur. There is ample evidence that both reduction of eggs and incorporation of sperm nuclei occur in unisexual salamanders. In the field, the nuclei of unisexual salamanders usually include a combination of genomes from one or more of: *A. laterale*, *A. jeffersonianum*, *A. tigrinum*, *A. texanum*, and *A. barbouri*. Which species' genomes unisexuals carry is influenced in part by what local host species are present (Bogart et al. 2009). Several different ploidy levels have been observed in adults, in eggs, and even in eggs produced by the same female (Bogart 1989, Bogart et al 1989, Elinson et al. 1992). Rarely, males occur in the lineage, which suggests that the genome containing the W sex chromosome can be lost during reproduction (Uzzell 1964, Bogart and Klemens 1997, Bogart 2003). In the lab, Bogart et al. (1989) found sperm nuclear incorporation at rates of 27% and 70% in water temperatures of 6°C and 15°C, respectively. Based largely on these observations suggesting high rates of genome replacement, Bogart et al. (2007) concluded that a new

term, "kleptogenesis" was warranted to describe the system. However, there has been extensive debate over the prevalence of genome replacement, and researchers continue to struggle to quantify the rate at which it occurs in nature (Spolsky et al. 1992, Bogart 2003, Ramsden 2008).

Beyond a semantic discussion of naming the reproductive mechanism, quantifying the rate at which male genomes replace unisexual genomes is key to untangling the peculiarities of this system. Genome replacement is an essential component of the lineage's evolutionary history, and likely the means through which unisexuals can reap the benefits of sex while potentially avoiding the costs of producing males (Maynard Smith 1978, Maynard Smith 1992). One of the striking features of unisexual salamanders is that they can be found deep in the heart of one host species' geographic range carrying nuclear genomes that are derived from distant species (Figure 4.1). Populations of LJJ (designating hybrid nucleus with one *A. laterale*, "L," genome and two *A. jeffersonianum*, "J," genomes; Lowcock et al. 1987) unisexuals are found in some areas where neither *A. laterale* nor *A. jeffersonianum,* occur, but only *A. texanum* occurs (Morris and Brandon 1984, Lowcock 1989). Unisexuals in northern Wisconsin, northern Maine and Nova Scotia maintain copies of the *A. jeffersonianum* genome even though they are 400 – 900 km from the nearest *A. jeffersonianum* populations (Petranka 1998, Bogart and Klemens 2008).

Figure 4.1. Ranges of *Ambystoma laterale*, *A. jeffersonianum*, and unisexuals containing hybrid nuclei of the two species. Adapted from Petranka (1998) and Bi et al. (2008).

One explanation for the success of unisexuals is that, by maintaining hybrid nuclei, they specialize in occupying ecotones where the niches of the two host species overlap (Moore 1977, Kraus 1985). Yet, the distribution of unisexuals is not easily explained by obvious ecotones. Consider the population of isolated LLJ unisexuals sympatric with *A. laterale* in northern Wisconsin (Figure 4.1). From North to South, beginning adjacent to this unisexual population, there is a 500 km portion of the *A. laterale* range where no unisexuals occur followed by a 200 km area in which both *A. laterale* and LLJ unisexuals occur and then the northwestern edge of the *A. jeffersonianum* range. On purely ecological grounds, it is difficult to explain why LLJ unisexuals are not continuous throughout this range (Uzzell 1964). Lowcock (1989) resolves such disjunct populations

as evidence that *A. jeffersonianum* had a more northerly distribution at the height of the climatic warm period that ended approximately 4,000 years ago (Viau et al. 2002). If these isolated unisexual populations are relicts from an historic climate, are the J genomes they carry adaptively advantageous today, or costly baggage from their past? Costs of carrying foreign nuclei may include environmental maladaptations, sexual selection by the host species, and accumulation of deleterious mutations in the absence of recombination (Muller 1964, Dawley and Dawley 1986, Lowcock et al. 1991).

Our ecological interpretation of unisexual salamanders is colored by what we think the rate of genome replacement is. If genome replacement happens very slowly, then the Wisconsin unisexuals may be on the path to replacing all of their J genomes with L genomes from the local males, but this process simply takes a long time. If genome replacement happens rapidly, however, then we must suspect that positive selection maintains the J genomes.

The goal of this study was to provide a formal framework to assess the adaptive advantage of hybridization in relation to the rate at which genome replacement occurs. To accomplish this, I collected data on genomotype distributions across a portion of the range boundary between the two host species. I also performed a stochastic simulation to understand the fate of neutral genomes in a hybrid unisexual lineage that only has contact with one host species. I then combined the field and simulation data in a model that uses the genome replacement rate as a basis to assess whether hybrid nuclei are maintained in-situ simply as a relic of the past, by contemporary dispersal, or by positive selection on hybrids.

Figure 4.2. (Top) Blue spotted salamander (*Ambystoma laterale*) and Jefferson-type salamander (*Ambystoma jeffersoninaum*). Both salamanders are in the hands of S.Record.

4.3 Methods

4.3.1 Study Region

Massachusetts is bisected by the northeastern range limit of *A. jeffersonianum* and is near the southern limit of *A. laterale* (Petranka 1998, Bogart and Klemens 2008). Unisexuals as well as both host species are protected under the state endangered species act. The Natural Heritage and Endangered Species Program (NHESP) of the Massachusetts Division of Fisheries and Wildlife maintains species occurrence records and has expressed a management need for more research assessing the status and distributions of populations within the state. For these reasons, I confined the study to Massachusetts, in an area approximately 190 km from East to West and 70 km from North to South. Consistent with Bogart and Klemens (1998, 2008), the NHESP considers the south flowing Connecticut River to be an approximate dividing line separating *A. jeffersonianum* to the west from *A. laterale* to the east, with unisexuals occurring throughout (NHESP, unpublished data).

4.3.2 Sample collection

A team of professional herpetologists coordinated by NHESP on a volunteer basis collected genetic material from 15 towns across Massachusetts: Richmond, Lenox, Lanesborough, Holyoke, Sunderland, Gill, Wilbraham, New Salem, Grafton, Northborough, Westborough, Boxborough, Westford, Newton and Easton. Town selection was based on an attempt to gain maximal geographic coverage while visiting sites in the NHESP database that were known to have productive breeding populations. In each town, visits were paid to a single known *Ambystoma laterale/jeffersonianum*

breeding site and salamanders were captured during the beginning of the breeding season, March 26 – April 4, 2009. The samples from Northborough were collected in 2003. Genetic material was collected from between 20 and 26 salamanders at all sites except for Lenox, Wilbraham, New Salem and Grafton, where the researchers were only able to obtain 7, 11, 4, and 2 samples, respectively. Salamanders were captured by hand while migrating to breeding ponds and using minnow traps placed in breeding ponds overnight. From each salamander, researchers collected one toe or tail tip and released the salamander. Samples were stored in 95% ethanol until extraction. Lab technicians and I extracted DNA following Fetzner (1999).

4.3.3 Nuclear Genomotypes

Lab technicians and I used two nuclear microsatellites (*Aje*D346 and *Aje*D94) to distinguish between *A. jeffersonianum* and *A. laterale* genomes (Julian et al. 2003, Ramsden et al. 2006). From the extracted DNA, we performed PCR with a 120 s initialization at 94°C, followed by 34 cycles of 45 s at 94°C, 45 s at 50°C and 90 s at 72°C. Samples were held at 72°C for a final elongation step lasting 600 s. We ran the PCR product on agarose gels and measured the allele sizes against a 100 bp ladder run in a parallel gel lane. We compared the allele sizes to the following sizes for known species from J. Bogart's unpublished data: 170-270 bp for *A. jeffersonianum Aje*D94, 134-198 bp for *A. laterale Aje*D94, 152-256 bp for *A. jeffersonianum Aje*D346, and 240-336 bp for *A. laterale Aje*D346 (J. Bogart, personal communication). These sizes provide slightly larger ranges than the available published data which were drawn from a more limited

geographic distribution (Julian et al. 2003). I used the results of the microsatellite analyses to construct a map of nuclear genomotypes in Massachusetts.

4.3.4 Mitochondrial Haplotypes

To map the distributions of *A. jeffersonianum, A. laterale,* and the unisexual mitochondrial haplotypes across the state, lab technicians and I sequenced a portion of the mitochondria D-loop (Shaffer & McKnight 1996, Bogart et al. 2007) from 85 salamanders. The goal was to sequence genetic material from individuals with both hybrid and pure nuclear genomotypes at each population. Using the results of the nuclear microsatellite data, we identified at least three pure and three hybrid salamanders for mitochondrial sequencing from each population where possible.

We used primers 007 and DL1 identified by Shaffer and McKnight (1992) to obtain sequences over a region approximately 485 bp long. The PCR protocol involved a 120 s initialization at 94°C, followed by 24 cycles of 60 s each at 94°C, 48°C, and 72°C. For the first five cycles, the transition from 48°C to 72°C was achieved by ramping up at 0.5°C/s. Subsequent cycles were not ramped. Samples were held at 72°C for a final elongation step lasting 600 s. Samples were cleaned using QIAquick PCR Purification kits (Qiagen, California) followed by Millipore Ultrafree Centrifugal Filters with a 10 kDa nominal molecular weight limit (Millipore Corporation, Massachusetts). We performed forward and reverse sequencing reactions using CEQ Dye-labelled Dideoxy-Terminator Cycle Sequencing kit (Beckman-Coulter, California). Sequences were prepared according to manufacturer instructions and analyzed using a CEQ 2000XL (Beckman-Coulter) automated sequencer. Sequences were aligned, edited, and compared

to reference sequences from the GenBank sequence database (http://www.ncbi.nlm.nih.gov/) using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI). I used the results of the mitochondrial sequencing to produce a map of mitochondrial haplotype distributions in Massachusetts.

4.3.5 Simulation

I constructed an individual-based simulation of unisexual reproduction to track changes in genome frequency that would be expected randomly under the case of no selection. I treated genome replacement as a phenomenon that consisted of the random loss and gain of entire genomes. It has been shown that recombination between L and J genomes can occur within unisexuals, such that genomes could also be replaced a small piece at a time (Bi and Bogart 2006, Bi et al. 2007). I did not attempt to account for these processes in the model, although I surmised that the rate of genome replacement would still have the same qualitative effect whether the genome was treated as one unit, divided into 14 chromosomes, or divided into 40 billion base pairs.

The model began with a uniform genomotype for all unisexuals in the population and assumed that population size, *N*, remained constant. In test simulations, I found that population size had no effect on the mean rate at which J genomes were lost from the population. As would be expected, population size did have a strong effect on the variance in simulation outcomes. For these simulations, however, I was interested in how the mean fate of hybrid nuclei is influenced by genome replacement. In simulations, I set *N* to be approximately the size that a breeding pond could support. This would represent the smallest population unit in the field, and thus model the largest variance. As I scale

inference up to the larger region, I would expect the actual trajectory of genomotypic change to more closely approach the model mean trajectory.

There were four steps in the model (Figure 4.3). In the first step, each salamander produced a set number of eggs with some probability of reduction in ploidy number (p_r). The probability that each egg's genomotype was identical to that of the mother was (1-p_r), while the probability that one of the mother's genomes was randomly discarded from the egg was p_r.

In the second step, a whole male genome was randomly incorporated into each egg with a probability of p_i. Incorporation in this model only counted if the genome would be passed on to subsequent generations. If the male genome would be incorporated only in the adult but selectively excluded prior to egg formation, this was not included in p_i.

In the third step, fitness coefficients were assigned to the embryos based on their genomotypes. For my parameterization, this consisted of setting the fitness to zero for all haploid embryos, all pentaploid embryos, and all embryos where no "L" occurred (Bogart 2003). All other embryos were assigned a fitness of one. Because I began the bulk of my simulations with LLJ salamanders, the selection against pure "J" salamanders was equivalent to selection against haploid salamanders.

In the final step, all of the embryos from all of the salamanders in the focal generation were pooled, and *N* of these were selected using a random binomial draw with probability of selection weighted by the fitness coefficients assigned in step three. These selected individuals were then used as the starting adult population for the next generation.

In parameterizing the model, I set N at 100 individuals, the number of eggs per individual to 200 (Petranka 1998), and I varied p_r and p_i on a log scale between 1 and 10^{-5} with six steps for each parameter. At every parameterization, I ran 24 simulations for 1000 generations each. I also ran 200 simulations at a parameterization of $p_r = 0.05$, and $p_i = 0.05$. For each run, I fit an exponential decay curve to the proportion of individuals with "J" genomes remaining as a function of the number of generations elapsed. I then fit a linear model to the logarithm of the mean values of the decay constant as a function of the logarithm of $p_r p_o$ for regions of the parameter space in which the simulation run time was sufficiently long to characterize the decay curves. All simulations were performed in R statistical software 2.10.0 (R Development Core Team 2009).

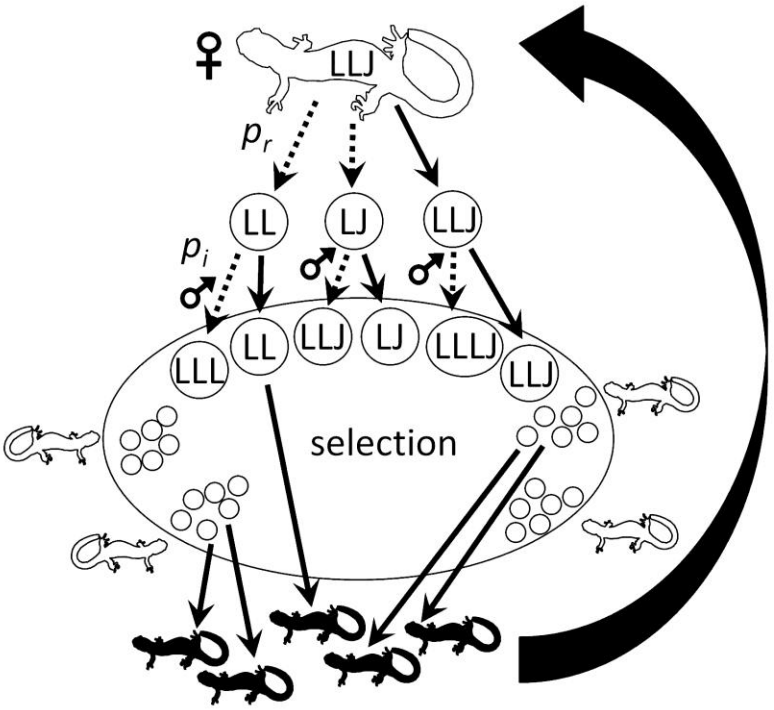

Figure 4.3. Schematic of individual-based simulation of unisexual reproduction. A unisexual generates eggs, each with a probability (p_r) of ploidy reduction. Male genomes are incorporated to elevate ploidy with a probability, p_i. Relative fitness coefficients are assigned to all embryos from all salamanders in the population. These fitness coefficients are used as weights in a binomial sampling process to select the next generation of adults.

4.3.6 Analytic Model

I constructed an analytic model to relate the rate of genome replacement to the question of whether there is selective pressure maintaining J genomes in areas far beyond the range limits of *A. jeffersonianum.* I then parameterized this model using the results of my genetic analyses and simulation. I also used a basic diffusion model to evaluate the potential for J genomes to be maintained in areas far outside of the *A. jeffersonianum* range via ongoing dispersal.

4.4 Results

4.4.1 Nuclear Microsatellites

I was able to assign nuclear genomotypes for both microsatellites *Aje*D94 and *Aje*D346 in 148 salamanders. I excluded from the analyses other salamanders for which only one of the microsatellites successfully amplified (n = 60), salamanders for which both microsatellites showed alleles in the overlap region between the two species (n = 2), and salamanders for which genomotypes assigned using the two microsatellites were inconsistent with each other (n = 8).

At four ponds in western Massachusetts, I found 31 salamanders carrying alleles only within the *A. jeffersonianum* (J) size range (Figure 4.4). At four ponds in eastern Massachusetts I found 13 salamanders carrying alleles only within the *A. laterale* (L) size range. I found 104 salamanders carrying hybrid nuclei distributed across the state. I did not find hybrid salamanders in the Easton population in southeastern Massachusetts. This is the same area in which Bogart and Klemens (2008) report the only populations lacking hybrids in the state. In central Massachusetts, I found one salamander at the Sunderland

pond that had a hybrid nucleus at *Aje*D346 but *Aje*D94 failed to amplify. At Grafton, *Aje*D346 amplified for only one of the two salamanders and displayed a hybrid nucleus, but *Aje*D94 did not amplify. Of the salamanders in the part of the state where both unisexuals and *A. laterale* are sympatric, seven out of 66 salamanders carried only L genomes, while the rest had a combination of L and J genomes. For five of these seven individuals I also sequenced their mitochondria.

4.4.2 Mitochondrial Haplotypes

I obtained mitochondrial sequences that matched known unisexual sequences from 47 salamanders representing all but the Easton population in southeastern Massachusetts (Figure 4.4). I obtained *A. jeffersonianum* sequences from 17 salamanders representing four ponds in the western portion of the state and I obtained *A. laterale* sequences from 21 salamanders at five ponds in the eastern portion of the state, including Easton. These distributions match those of Bogart and Klemens (1997, 2008). I did not find unisexual mitochondrial sequences in any of the salamanders for which I scored the nucleus as containing only L genomes. For all haplotype variants obtained, I deposited representative sequences in GenBank (accession numbers JF693886- JF693891).

4.4.3 Simulation

When I set the probabilities of reduction (p_r) and incorporation (p_i) greater than or equal to 0.001, J genomes were seen to decay appreciably out of the population within the 1000 generations included in the simulations. The mean rate of decay fit an exponential curve very well (Figure 4.5). Starting with a population of pure LLJ individuals, when both p_r and p_i are set to 0.05, J genomes would be expected to occur in half of the individuals

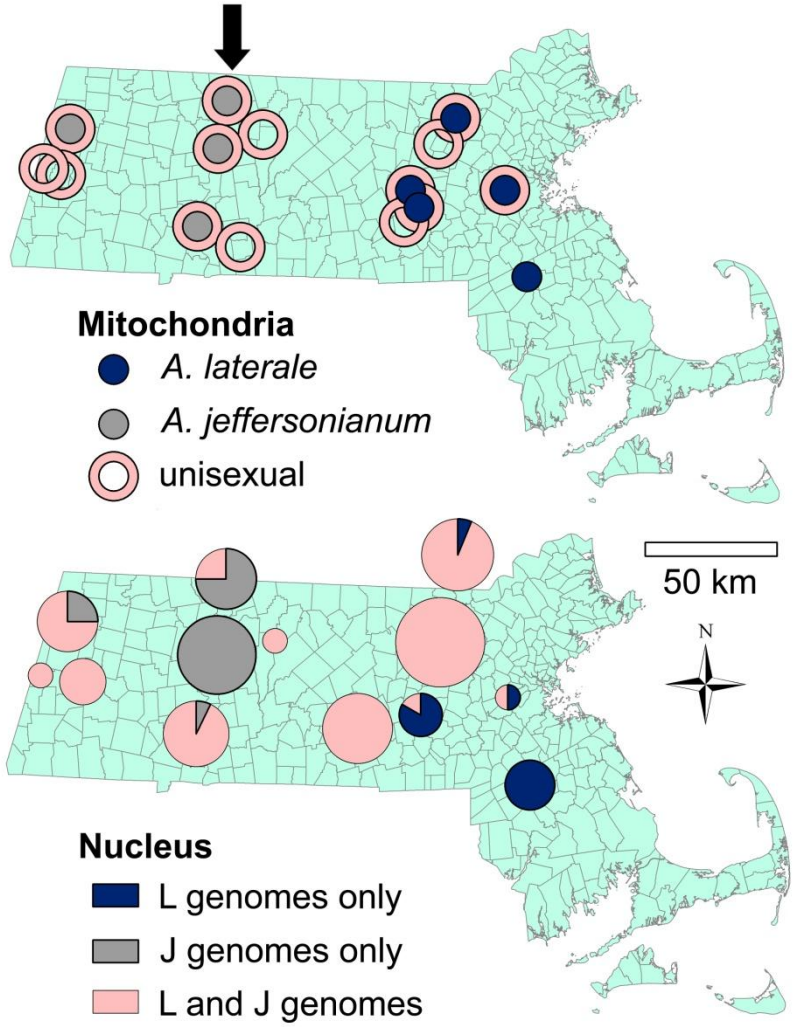

Figure 4.4. Distributions of mitochondrial and nuclear genotypes at 15 breeding ponds in Massachusetts. (Top) Distributions of *Ambystoma laterale*, *A. jeffersonianum* and unisexual salamander mitochondria. A portion of the mitochondrial D-loop was sequenced from 85 salamanders to determine haplotype presence at each pond. The arrow at the top of the map indicates the approximate position of the south-flowing Connecticut River, which is used in the state endangered species records as the approximate eastern edge of the *A. jeffersonianum* range. (Bottom) Distributions of nuclear genomotypes in 148 salamanders. Microsatellites were used to determine whether nuclei contained only *A. jeffersonianum* (J) genomes, only *A. laterale* (L) genomes, or both. Pie chart sizes represent the total number of salamanders scored. To avoid overlap, pie chart centers are displaced from actual breeding locations. The Grafton site, and the lone individual with a unisexual mitochondria in the Sunderland site are excluded from the bottom map because only one of the microsatellites successfully amplified.

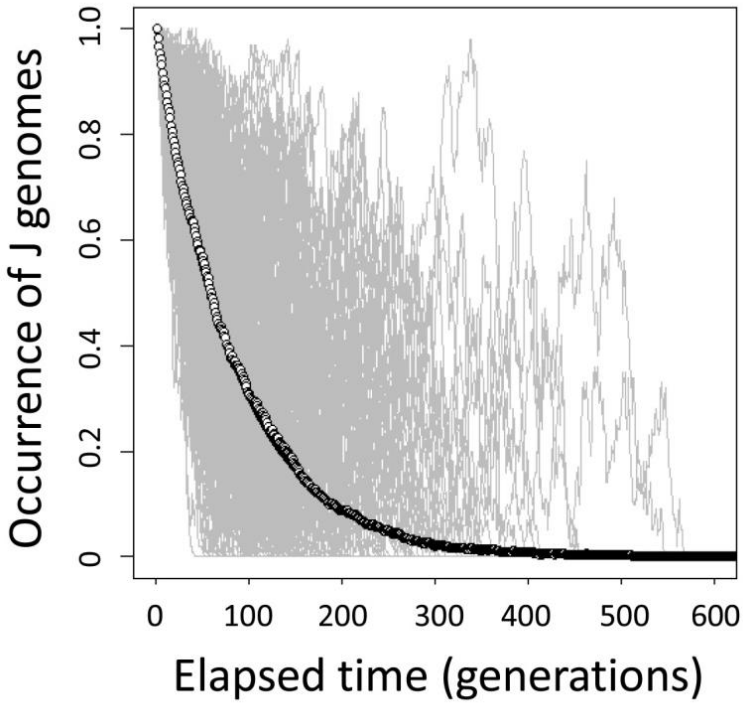

Figure 4.5. Occurrence of J genomes over time for simulated populations of unisexual salamanders breeding with *A. laterale* males. The probabilities of egg ploidy reduction and sperm incorporation were both set to 0.05. The model ran for 1000 generations in 200 iterations. Each iteration is plotted as a gray line. Points represent the mean occurrence of J genomes at each time step. An exponential curve with a characteristic decay time of 82 years is plotted as a dark line beneath and largely obscured by the mean points.

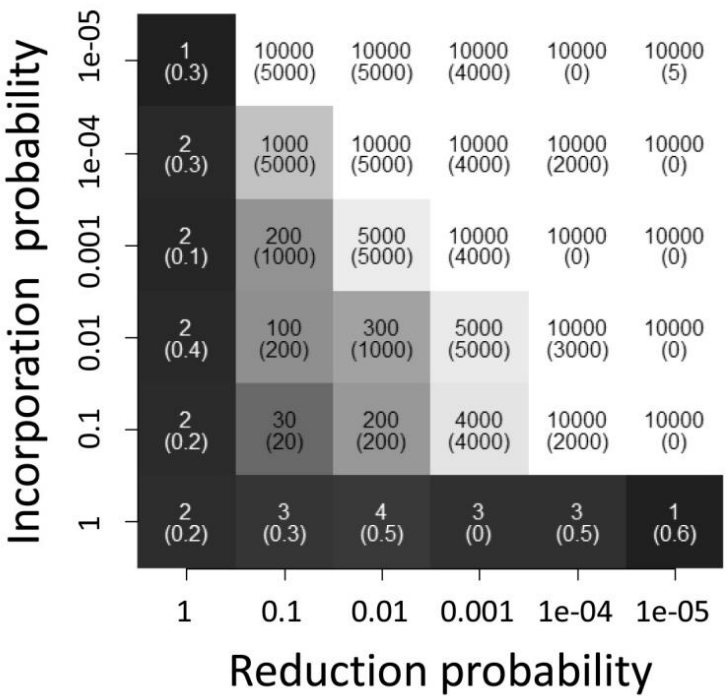

Figure 4.6. Half-life for the loss of J genomes from a population that began with pure LLJ genomotypes. "Incorporation probability" is the probability that sperm genomes will elevate ploidy, and "reduction probability" is the probability that eggs will have reduced ploidy relative to the mother. Every square represents 24 iterations of 1000-year simulations. Numbers represent median half lives of the best-fit exponential functions, measured in generations, with standard deviations in parentheses.

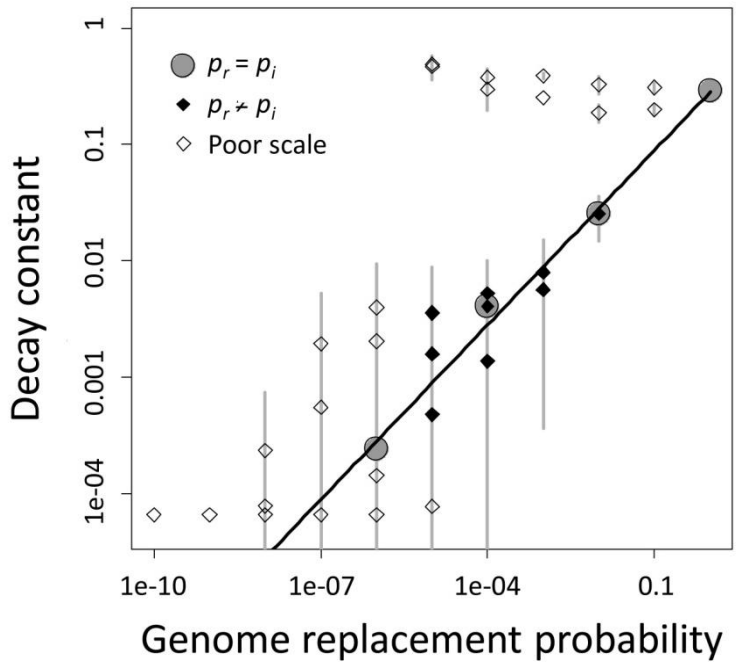

Figure 4.7**.** Mean decay constants calculated for the loss of J genomes from populations of pure LLJ genomotypes. The probability of genome replacement is calculated as the product of the probabilities of genome reduction (p_i) and sperm genome incorporation (p_r). The trend line was fitted only to the four parameterizations where p_i and p_r are equal and less than 0.0001, shown as gray circles. Empty diamonds represent points where the decay constant was either too large or too small for reliable fitting of the exponential model.

after 57 generations. When I started the populations with all LLJ individuals, the mean decay rate was twice that as when I began with a population of LJJ individuals. As the reduction probability and incorporation probability decreased, the length of time that J genomes remained in the population increased as a function of their joint probability (Figure 4.6). When p_r and p_i were both equal and between 1 and 0.001, the mean exponential decay constant, λ, was well described by:

$$\lambda = k\sqrt{p_r p_i}, \tag{4.1}$$

where k is a constant equal to 0.29 for my simulations (Figure 4.7). At lower values of p_r and p_i, the decay was rate was too low to be characterized within 1000 generations.

4.4.4 Analytic model

The simulation demonstrated that, if whole genome replacement occurs, a population of LLJ individuals breeding only with *A. laterale* males would lose all J genomes at an average rate described by an exponential decay function. We can greatly simplify the problem by ignoring the particulars of ploidy reduction and elevation and lumping p_r and p_i together into the single phenomenon of genome replacement that occurs with probability, $p_g = p_r p_i$. In reality, the functional relationship between these three probabilities may be more complicated. In particular, we might expect the probability of sperm nuclear incorporation to increase if an egg has lowered ploidy. In the derivation that follows, however, we are concerned with the minimum rate at which J genomes would be lost from the population. Relaxing the assumption that p_r and p_i are independent would allow J genomes to be lost from the population even more rapidly. It is also

reasonable to confine our analyses to the cases where $p_r = p_i$, otherwise we would expect the average ploidy of individuals in the population to be unstable.

Beginning with a population of LJJ individuals, the expected occurrence of J genomes in a population is governed by:

$$J(t) = J_0 e^{-\lambda t/2}, \tag{4.2}$$

where $J(t)$ is the proportion of individuals containing J genomes, J_0 is the initial occurrence of J genomes, t is the elapsed time (in generations), and λ is the decay constant. If the initial population consisted of all LLJ individuals, the "½" in the exponent would be removed.

So far, I have described the neutral case in which there is no selective advantage to J genomes in an *A. laterale* population. If there is positive selection, *s*, this will act to reduce the effect of λ. Equation (4.2) becomes:

$$J(t) = J_0 e^{-(\lambda - s)t/2}. \tag{4.3}$$

As I have defined it, *s* is related to and the same order as the familiar fitness coefficient, although the two are not identical. Here, *s*, describes the change in the exponential decay function. However, when fitness is added to the model, the proportion of J genomes is no longer well described by an exponential function. This is because relative fitness only matters when there is variance in the population. Due to the fact that my simulations begin with uniform populations, fitness has little influence when the model starts, but becomes more important as variance develops. Therefore, the familiar fitness coefficient would have to be slightly larger than *s* in order to compensate for this effect.

Combining equations (4.1), (4.2), and (4.3) yields:

$$s - k\sqrt{p_g} = \frac{2\ln\left(\frac{J(t)}{J_0}\right)}{t}. \tag{4.4}$$

We can now apply equation (4.4) to the experimental observations. Let us ignore contemporary dispersal for a moment, and consider the populations in eastern Massachusetts where unisexuals and *A. laterale* are sympatric. Assume that the populations once consisted of LJJ unisexuals hybridizing with *A. jeffersonianum.* At a time τ generations before the present, *A. jeffersonianum* had been completely replaced by *A. laterale.* Note that the math is equivalent if the sexual species have stayed in place but the unisexuals colonized the *A. laterale* ponds carrying LJJ nuclei. In the field data, I found J genomes in 59 out of 66 salamanders in the *A. laterale* – unisexual region. Five of the 7 with no J genomes had *A. laterale* mitochondria, and I do not know the origins of the other two mitochondria. We can say then that at least 97% of the unisexuals in the *A. laterale* region carry J genomes.

Estimating τ may be tricky for eastern Massachusetts, but we can place some extreme lower bounds on it. At the very least, the distribution of the species in Massachusetts reported by Uzzell in 1964 matches the current distribution. If we use 2.5 years as the average generation time between *A. laterale* and *A. jeffersonianum*, 1964 was approximately 18 generations ago (Petranka 1998).

Using the estimate of 0.29 for *k* from the simulation, a lower limit of 18 for τ, and a lower limit of 0.97 for $\frac{J(\tau)}{J_0}$, we can rearrange equation (4.4) into this inequality:

$$s > 0.29\left(\sqrt{p_g} - 0.01\right). \qquad (4.5)$$

The key implication to take away from equation (5) is that as long as the genome replacement probability is greater than 1/10,000, there must be compensating positive selection on J genomes to keep them at observed levels. Given our initial constraints that $p_g = p_r p_i$, and $p_r = p_i$, the limit for the rates of ploidy reduction and sperm nuclear

incorporation are each 0.01 assuming the neutral scenario. If we use the rate for nuclear incorporation observed by Bogart et al. (1989) in the lab of $p_i = 0.27$, we find that the positive selection term must be greater than 0.08.

The case for positive selection becomes clearer when we expand the scope of inquiry to include the entire unisexual range. If we suspect that LLJ populations in Nova Scotia have not been in contact with *A. jeffersonianum* populations for at least 1,600 years, since the end of the sub-Atlantic climatic cooling (Viau 2002), then the maximum rate of genome replacement if J genomes are selectively neutral would be approximately $1x10^{-7}$. Could the J genomes be maintained through dispersal from nearby populations of *A. jeffersonianum*? The distance between the eastern Massachusetts LLJ populations and the *A. jeffersonianum* range is more than 50 km. If we consider the system to be at equilibrium, we could treat the problem as a dispersal-dependent cline following the basic diffusion model (Fisher 1937, Slatkin 1973, Barton and Hewitt 1985). The source of dispersing J genomes would be the eastern edge of the range of *A. jeffersonianum* males. Individual unisexuals disperse with a standard deviation, σ, between generations. I treat the tendency of J genomes to be replaced by L genomes as mathematically equivalent to a form of negative selection acting against J genomes in all of the eastern ponds where only *A. laterale* males occur (Robertson 1960). Such a cline would have a characteristic spatial scale of $\sigma/\sqrt{\lambda}$, and a width of the same order (Barton and Hewitt 1985).

To estimate dispersal, we can use data from the closest species that has been sufficiently studied, *A. opacum* (Gamble et al 2007). Fittingly, the field site for that study was in central Massachusetts near the eastern extent of *A. jeffersonianum.* Setting σ to 0.17 km

from the Gamble et al. data, we find that the maximum negative selective pressure for a cline to be maintained over 50 km would be approximately 10^{-5}. Thus, from equation (4.1), we find that ongoing dispersal could only be important in maintaining J genomes in eastern Massachusetts if the rate of genome replacement is less than 10^{-9}.

There are two primary ways in which the equilibrium assumption in the dispersal model could be incorrect, neither of which undermine my argument. If *A. laterale* only arrived in eastern Massachusetts recently, then I have already addressed this problem above in calculating the decay time of J genomes. If the system is not in equilibrium because unisexuals only began dispersing recently, then the equilibrium assumption is quite conservative. This second case would yield a steeper cline and it would be even more difficult to explain the presence of J genomes in far eastern Massachusetts by dispersal.

4.5 Discussion

Past studies of unisexual *Ambystoma* have suggested high rates of genome replacement while describing hybrid nuclei distributed far beyond species' contact zones. Here, I have shown that these two ideas cannot be simultaneously true in the absence strong selection. As seen in the mitochondrial results, *Ambystoma jeffersonianum* and *A. laterale* have distinct distributions in Massachusetts. Yet, I found the *A. jeffersonianum* nuclear genomes in every unisexual salamander that I identified throughout the *A. laterale* range. With frequent genome replacement and no selection, my simulation predicted that populations of unisexuals breeding only with *A. laterale* would rapidly lose most of the J genomes. If genome replacement occurs at any appreciable rate, then positive selection must be acting to maintain hybrid nuclei.

What are the advantages to a hybrid nuclei far within the range limits of one species? On face value, it would seem that in northern populations where only *A. laterale* persists, L genomes would produce the phenotypes best adapted for the environment. Further, if male *A. laterale* preferentially mate with pure *A. laterale* females, either by choice or by phenology, then sexual selection should be against J genomes (Dawley and Dawley 1986, Lowcock et al. 1991). Compounding these adaptive disadvantages is the fact that the J portions of the unisexual genomes have no means for recombining with like genomes, and thus should be degrading under the force of Muller's ratchet (Muller 1964). Any repairs made to deleterious mutations must be made by replacement of J portions of the genome with L portions of the genome (Bi and Bogart 2006, Bi et al. 2007).

I can posit some sources of positive selection on the J genomes. Perhaps the distributions of unisexual salamanders do reflect intermediate ecotones between *A. laterale* and *A. jeffersonianum* habitat, where they enjoy hybrid superiority (Moore 1977). The peculiar feature of these ecotones is that they would not fully align with the current geographic border of the two species, but would include disjunct portions of the unisexual range far into the range of *A. laterale* (Lannoo 2001, Bi et al 2008). Another explanation for positive selection on J genomes could potentially be found in cytonuclear interactions (Fishman and Willis 2006). For instance, the unisexual mitochondria in these populations may have co-evolved with the J genome to the extent that functionality breaks down if the J genome is replaced by an L genome. This type of effect might explain the puzzling requirement for unisexuals across their entire range to maintain an L, with only one reported exception (Bogart and Licht 1986). However, in other parts of their range, unisexuals with no J genomes are commonly encountered (Petranka 1998). If cytonuclear

interactions explain the persistence of J genomes in Nova Scotia and northern Wisconsin, then these interactions must have arisen only in those branches of the lineage, and possibly independently. Perhaps, a cytonuclear requirement has developed in which at least one copy of any nuclear genome other than L be present for viability, although it is difficult to speculate on exactly what this mechanism would be. Furthermore, occasional unisexuals with only L genomes have been reported (Lowcock et al. 1991).

Imperfect knowledge of the distribution of unisexuals might also influence my conclusions. If, in fact, vast stretches of the unisexual range remains unstudied and populated largely by LLL individuals, then perhaps the LLJ populations really represent the last remnants of a stochastic decay. Sampling bias in the literature towards studying populations where J genomes persist is plausible (Lowcock et al 1991). The original identification of unisexuals was based upon observable phenotypic differences due to hybrid nuclear genomes (Clanton 1934). Populations of LLL unisexuals would presumably be phenotypically similar to *A. laterale* populations, except for the prevalence of females. That these populations could go undetected would not seem terribly surprising. Collecting the data to resolve this question is fairly straightforward.

Another possibility is that genome replacement truly does not occur very frequently in nature. Colder temperatures in northern climates might cause genome replacement rates to fall towards zero. Perhaps lab-specific conditions other than temperature caused the high genome replacements rate observed by Bogart et al (1989). If we accept that sperm incorporation does happen 27% of the time in nature, then the model implies that the positive selection term in favor of a hybrid nucleus must be greater than 0.08. This might

seem to be quite a substantial reduction in fitness of LLL unisexuals, especially knowing that *A. laterale* with pure nuclei must be surviving reasonably well in the same ponds. My framework provides a formal foundation for exploring the hypothesis of hybrid superiority as it relates to genome replacement rates. This framework offers direction on the future types of data that could be collected to test the model predictions. Specifically, field researchers could determine whether unisexuals produce reproductively viable offspring with pure nuclei, the distributions of pure-nuclei unisexuals, the isolation time of unisexual populations from their nuclear parental species, the rates of sperm incorporation in the field, and the rates of egg reduction in the field. Incorporating these data back into the simulation model will allow us to make more specific predictions and better understand one of the most fascinating, yet ecologically vulnerable, vertebrate systems.

4.6 Acknowledgments

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Figure 4.8 A newly metamorphosed Jefferson-type salamander, likely in the unisexual lineage.

APPENDIX A

ELECTROMAGNETIC THEORY GOVERNING PASSIVE INTEGRATED TRANSPONDER ANTENNAE

When in the presence of the correct frequency alternating magnetic field, a PIT tag is powered by the external field to return a signal carrying a unique code. Thus, a PIT tag needs no batteries of its own. Instead, detection depends upon proximity to an antenna producing the appropriate fields. The antenna will in turn receive the signal sent by the tag, and relay it to the transceiver which powers the antenna (Prentice et al. 1990b). In this section, I use fundamental electrodynamics principles to develop the working rules that I used to construct my antennae. For additional information on electrodynamic principles, see Griffiths (1999).

The basic circuit used to generate the magnetic fields consists of 1) a wire forming the antenna, 2) a set of capacitors, and 3) a transceiver supplied by the PIT manufacturer. The antenna functions as an inductor, with a self-inductance (L) that is entirely dependent upon the antenna geometry. Together, a capacitor and an inductor form a resonant oscillator, with a natural resonant frequency

$$f_0 = \frac{1}{2\pi\sqrt{LC}}, \tag{A.1}$$

where C is the capacitance in the circuit as measured in Farads, L has units of Henries, and f_0 has units of Hertz.

Tuning an antenna amounts to matching its resonant frequency to the output frequency of the PIT transceiver. Given that PIT tag systems operate at frequencies determined by the manufacturers (in our case, 134.2 KHz), the experimenter must adjust the inductance and capacitance in order to attain a circuit with the appropriate resonant frequency.

Capacitance is the easiest part of the circuit to adjust because fixed capacitors can be bought at low cost and can be combined following basic electronics rules. When stringing

capacitors in series, the total capacitance is the reciprocal of the sum of the reciprocals of the individual capacitances. When connecting capacitors in parallel, the total capacitance is the sum of the individual capacitances. Additional capacitance will be contributed by the cable connecting the antenna to the transceiver and by the antenna itself. The cable capacitance is directly proportional to the cable length. Because we use shielded cables, this value is largely independent of environmental factors. Cable capacitance can be determined easily by measuring the capacitance of a short length of cable and scaling up.

In large antennae, capacitive coupling between the wire and the earth's surface may be more troublesome, especially when low capacitance values are needed to tune the circuit. A capacitor may be thought of as 2 conductors (in this case, the wire and the earth below) separated by an insulator (such as the air). Capacitance increases as 1) the distance between the conductors decreases, 2) the surface area of the conductors increases, and 3) the dielectric constant (κ) of the insulator increases. On rainy days the earth's surface becomes a very good conductor and water raises the dielectric constant of the space around the wire, increasing the capacitance in the circuit. To avoid the complications of weather-dependant tuning, the wire may be wrapped with a cylindrical insulator of sufficient diameter to make the external capacitance insignificant. To be conservative, we assume a worst case scenario in which the outside of the insulator is soaked with salty water and forms a perfect conductor. For the sake of brevity, I will not fully derive this capacitance here, but using Gauss's law (Griffiths 1999), the capacitance between the wire and earth surface is found to be:

$$C = \frac{\kappa 2\pi\varepsilon_0 s}{\ln\left(\frac{a}{b}\right)}, \tag{A.2}$$

where κ is the dielectric constant of the insulating material, ε_0 is the permittivity of free space, *s* is the total length of wire, *a* is the diameter of the wire, and *b* is the outside diameter of the insulator.

Inductance may also be predicted from basic laws of electrodynamics. Here I derive the inductance of the antenna configuration I employed: a very long narrow loop of wire. The same general procedure may be followed to derive the inductance for any antenna configuration.

First, we calculate the magnetic flux through the loop (see Griffiths 1999). Modeling the loop as 2 infinitely long parallel wires carrying opposite currents, we find that the magnetic flux through the loop is twice the integral of the magnitude of the magnetic field from a single wire integrated over the area of the loop. The magnitude of the magnetic field at distance *s* from a single long wire carrying a current *I* is given by:

$$B = \frac{\mu_0 I}{2\pi s}, \tag{A.3}$$

where μ_0 is the permittivity constant ($1.6 * 10^{-6}$ H/m; Griffiths 1999). The total flux through a loop of length l, width d, and wire thickness α, is given by:

$$\Phi = 2\int_{\alpha}^{d} B da \tag{A.4}$$

$$\Phi = 2\frac{\mu_0 I}{2\pi} l \int_{\alpha}^{d} \frac{1}{s} ds \tag{A.5}$$

$$\Phi = \left(\frac{\mu_0 I}{\pi} l\right) \ln\left(\frac{d}{\alpha}\right). \tag{A.6}$$

This gives the flux for a single loop of wire. The self inductance for the coil when we consider the possibility of *N* loops is then given by:

$$L = \left(\frac{\mu_0 N^2}{\pi} l\right) \ln\left(\frac{d}{\alpha}\right). \tag{A.7}$$

We can efficiently tune the antenna by understanding that the inductance is linearly related to the length of the antenna, the square of the number of loops, and the natural logarithm of the ratio of the antenna width to wire thickness:

$$L \propto N^2 l \ln\left(\frac{d}{\alpha}\right). \tag{A.8}$$

APPENDIX B

DERIVATION OF CAPACITANCE BETWEEN WIRE AND GROUND

For a linear charge distribution, we construct a cylindrical Gaussian surface around the line.

$$\oint \mathbf{E} \bullet da = \frac{Q_{enc}}{\epsilon_0} \tag{B.1}$$

$$\oint E da = \frac{Q_{enc}}{\epsilon_0} \tag{B.2}$$

$$E \oint da = \frac{Q_{en\,c}}{\epsilon_0} \tag{B.3}$$

$$E(2\pi sd) = \frac{Q_{enc}}{\epsilon_0} \tag{B.4}$$

$$Q_{enc} = \lambda d \tag{B.5}$$

$$E(2\pi sd) = \frac{\lambda d}{\epsilon_0} \tag{B.6}$$

$$E = \frac{\lambda}{2\pi\epsilon_0 s} \tag{B.7}$$

$$\mathbf{E} = \frac{\lambda}{2\pi\epsilon_0 s}\hat{\mathbf{s}} \tag{B.8}$$

$$\Delta V = -\int_b^a \mathbf{E} \bullet ds \tag{B.9}$$

$$\Delta V = -\int_b^a \frac{\lambda}{2\pi\epsilon_0 s} ds \tag{B.10}$$

$$\Delta V = -\frac{\lambda \ln\left(\frac{a}{b}\right)}{2\pi\epsilon_0} \tag{B.11}$$

$$C = \frac{Q}{V} = \frac{\lambda d\, 2\pi\epsilon_0}{\lambda \ln\left(\frac{a}{b}\right)} = \frac{2\pi\epsilon_0 d}{\ln\left(\frac{a}{b}\right)} \tag{B.12}$$

APPENDIX C

INDIVIDUAL DATA ON SALAMANDERS IN THE AMYBSTOMA JEFFERSONIANUM/LATERALE COMPLEX FROM WHICH GENETIC MATERIAL WAS SAMPLED

Sex, Snout-vent-length (SVL), and mass were determined in the field. Nuclear genomotypes were determined by comparing fragment sizes of microsatellites AjeD94, and AjeD346 to known allele size ranges for A. laterale (L) and A. jeffersonianum (J). A segment of the cytochrome-b gene in the mitochondria was amplified using universal primers that should amplify fragments for all Ambystoma species (Univ) and primers that should only amplify fragments from unisexual salamanders (Hyb). Presence (1) or absence (0) of PCR product was examined for each individual whose genetic material was extracted. A portion of the mitochondrial D-loop was also sequenced and compared to known sequences from the two sexual species and that of unisexuals (U). Letters in parentheses indicate individuals for which a positive match was obtained using the Basic Local Alignment Search Tool on the GenBank sequence database website, however the samples were too noisy to obtain complete clean sequences using Sequencher, and thus are less robust results. Genotypes with the suffix "-SNP" indicate that individual contained a single nucleotide polymorphism relative to the consensus sequence for the lineage. Within each of the three lineages, all of the variant sequences contain the same SNP. "Fail" indicates unsuccessful attempts at sequencing.

					Nuclear		Mitochondria		
Town	Indiv.	Sex	SVL (mm)	Mass (g)	D94	D346	Hyb	Univ	D-loop
Boxborough	DB-1	F	72	9.7	LJ	LJ	1	1	U
Boxborough	DB-2	F	76	10.2	LJ	LJ	1	1	U
Boxborough	DB-3	F	75	10.4	LJ	LJ	1	1	
Boxborough	DB-4	F	84	11.2	LJ	LLJ	1	1	
Boxborough	DB-5	F	79	11.5	LJ	LLJ	1	1	
Boxborough	DB-6	F	81	12	LJ	LJ	1	1	
Boxborough	DB-7	F	76	10.3	LJ	LJ	1	1	
Boxborough	DB-8	F	85	16	LJ	LLJ	0	1	U
Boxborough	DB-9	F	69	6.3	LJ	LJ	1	0	
Boxborough	DB-10	F	75	8.3	LJ	LJ	1	1	
Boxborough	DB-11	F	79	9.8	LJ	LJ	1	1	
Boxborough	DB-12	F	85	14	LJ	LLJ	1	1	
Boxborough	DB-13	F	89	12.2	LLJ	LLJ	1	1	U
Boxborough	DB-14	F	77	11.1	LJ	LJ	1	1	
Boxborough	DB-15	F	75	11.2	LJ	LJ	1	1	
Boxborough	DB-16	F	79	6.7	LJ	LJ	1	1	
Boxborough	DB-17	F	75	10.2	LJ	LLJ	1	1	
Boxborough	DB-18	F	81	9.6	LJ	LJ	1	1	
Boxborough	DB-19	F	81	10.9	LJ	LJ	1	1	

Boxborough	DB-20	F	81	11.9	LJ	LJ	1	1	
Boxborough	DB-21	M	75	7.5	LJ	LLJ	1	1	
Boxborough	DB-22	F	73	8.7	LJ	LJ	1	0	
Boxborough	DB-23	F	78	8.5	LJ	LJ	1	1	
Boxborough	DB-24	F	80	9.7	LJ	LJ	1	1	
Boxborough	DB-25	F	76	9.2					
Boxborough	DB-26	F	83	11.3					
Boxborough	DB-27	F	76	7.3					
Boxborough	DB-28	F	79	10.9					
Boxborough	DB-29	F	78	10.7					
Boxborough	DB-30	F	80	8.4					
Boxborough	DB-31	F	77	11.4					
Boxborough	DB-32	M	85	10.4	LJ	LLJ			
Boxborough	DB-33	F	80	10.5					
Boxborough	DB-34	F	80	7.6			1	1	
Boxborough	DB-35	F	85	12.4					
Boxborough	DB-36	F	87	11.5					
Boxborough	DB-37	F	73	8.3					
Boxborough	DB-38	F	77	9.7					
Boxborough	DB-39	F	80	7.9					
Boxborough	DB-40	M	74	5.4	LJ	LLJ	1	1	
Boxborough	DB-41	F	81	12.6					
Boxborough	DB-42	F	78	8					
Boxborough	DB-43	F	75	8.6					
Boxborough	DB-44	F	59	4.1					
Easton	EA-1	F	58	4	L		0	1	(L)
Easton	EA-2	J	41	1.7	L	LL	0	1	L
Easton	EA-3	M	45	3.3	LL	LL?	0	1	L-SNP
Easton	EA-4	F	51	5.5	L?	L	0	1	(L)
Easton	EA-5	F	46	3.8	L?	L	0	1	(L)
Easton	EA-6	M	51	2.6	LL	??	0	1	L
Easton	EA-7	F	49	3.2	L?	?	0	1	(L)
Easton	EA-8	F	42	3.5	L		0	1	L
Easton	EA-9	J	44	2.2	L		0	1	(L)
Easton	EA-10	F	51	3.1	LJ		0	1	L
Easton	EA-11	M	48	3	L?	LL?	0	1	L
Easton	EA-12	M	51	3.2			0	1	(L)
Easton	EA-13	M	47	3.3		L?	0	1	(L)
Easton	EA-14	F	41	4		L	1	1	L
Easton	EA-15	F	47	5.2		L?	1	1	(L)
Easton	EA-16	M	42	3.6		LL	0	1	L-SNP
Easton	EA-17	F	53	5.1		LJ	1	1	L-SNP

Easton	EA-18	F	41	3.4		L?	1	1	(L)
Easton	EA-19	M	55	3.6	L		1	1	(L)
Easton	EA-20	F	51	4.1	LL	L	1	1	(L)
Easton	EA-21	J	38	2.3			1	1	(L)
Easton	EA-22	F	39	4.3			1	1	(L)
Easton	EA-23	M	47	3.1	LL		1	1	(L)
Easton	EA-24	F	58	4.9		LJ	1	1	L
Easton	EA-25	F	47	4.2					
Easton	EA-26	M	51	3.2					
Easton	EA-27	J	33	0.8					
Easton	EA-28	M	56	4.5					
Easton	EA-29	J	36	1.3					
Easton	EA-30	J	36	1					
Easton	EA-31	F	50	3.4					
Easton	EA-32	M	58	5					
Easton	EA-33	M	50	4.1					
Easton	EA-34	M	58	4.9					
Easton	EA-35	M	63	4.5					
Easton	EA-36	M	35	0.3					
Easton	EA-37	M	43	3.6					
Easton	EA-38	J	30	1.2					
Easton	EA-39	F	53	4.1					
Easton	EA-40	F	49	3.5					
Easton	EA-41	M	53	2.7					
Easton	EA-42	F	54	3.4					
Easton	EA-43	F	41	3.7					
Easton	EA-44	M	55	4.2					
Easton	EA-45	F	54	4					
Easton	EA-46	J	37	1.2					
Easton	EA-47	J	43	3					
Easton	EA-48	J	37	2					
Easton	EA-49	F	43	3.2					
Easton	EA-50	J	28	1.9					
Easton	EA-51	J	24	2.2					
Easton	EA-52	M	50	3.3					
Easton	EA-53	J	37	1.6					
Easton	EA-54	J	33	1.5					
Easton	EA-55	M	41	3.5					
Easton	EA-56	M	48	3.6					
Easton	EA-57	M	51	4.7					
Easton	EA-58	F	43	3.5					
Easton	EA-59	M	40	3.4					

Easton	EA-60	J	33	1.3					
Easton	EA-61	M	41	3.5					
Easton	EA-62	M	53	3.9					
Easton	EA-63	F	42	3.3					
Easton	EA-64	F	37	2.6					
Easton	EA-65	M	46	4.9					
Easton	EA-66	M	45	3.6					
Easton	EA-67	F	41	2.7					
Easton	EA-68	M	53	4.9					
Easton	EA-69	F	35	3.9					
Easton	EA-70	M	36	3					
Easton	EA-71	F	55	4.5					
Easton	EA-72	F	45	4.1					
Easton	EA-73	F	42	3					
Gill	BT-1	M	79	12.5			0	1	
Gill	BT-2	M	87	13.75		J	0	0	
Gill	BT-3	M	79	14	JJ	J	0	1	
Gill	BT-4	M	75	12.75	JJ	J	0	1	
Gill	BT-5	M	81	13	?JJ	JJ	0	1	(J)
Gill	BT-6	M	84	11.75	JJ	JJ	0	1	
Gill	BT-7	M	68	9.75	JJ	JJ	0	0	fail
Gill	BT-8	M	79	10.25	JJ	JJ	0	1	(J)
Gill	BT-9	F	72	12.75	LJJ	LLJJ	1	1	U
Gill	BT-10	M	81	10	JJJ	J	0	1	J
Gill	BT-11	F	70	9.75		LLJJ	0	1	U
Gill	BT-12	F	87	16.25	JJJ		0	1	J
Gill	BT-13	F	82	15	LJJ	LJ	1	1	U
Gill	BT-14	F	81	15.25	LJJ	LJ	1	0	
Gill	BT-15	M	78	11.25	JJ	J	1	1	J
Gill	BT-16	M	79	10	JJ		1	1	J
Gill	BT-17	M	88	14		J	1	1	
Gill	BT-18	M	76	13.75	JJ	J	1	1	
Gill	BT-19	M	77	11			1	1	
Gill	BT-20	M	84	11.25			1	1	
Grafton	JEK2-1	F	78.2	11.3		LJ	1	1	U
Grafton	JEK2-2	F	70.1	9.0			1	1	U
Holyoke	HOLY-1	F				LLJJ	1	1	
Holyoke	HOLY-2	M	82.3	8.4	JJ		0	1	
Holyoke	HOLY-3	F	72.9	6.4	LJJ	LLJJ	1	1	U
Holyoke	HOLY-4	F	77.1	10.6	LJJ	LLJJ	1	0	
Holyoke	HOLY-5	F	78.4	9.6			0	1	
Holyoke	HOLY-6	F	76	8.5	LJJ	LLJJ	1	1	

Holyoke	HOLY-7	F	83.3	11.2	LJJ	LLJJ	0	1	
Holyoke	HOLY-8	F	80.9	9.3	LJJ	LLJJ	0	1	
Holyoke	HOLY-9	F	76.5	10.6	LJJ	LLJJ	1	1	
Holyoke	HOLY-10	F	78.7	8.1	L	LLJJ	1?	1	U
Holyoke	HOLY-11	M	83.7	11.2	JJ		0	1	J-SNP
Holyoke	HOLY-12	F	82.7	12.7	LJJ	LLJJ	1	1	
Holyoke	HOLY-13	F	82.8	13	LJJ	LLJJ	0	1	U
Holyoke	HOLY-14	F	71	5.7	LJJ	LLJJ	1	1	
Holyoke	HOLY-15	F	75	11.1	LJJ	LLJJ	1	1	U
Holyoke	HOLY-16	F	67	4.9			1	1	
Holyoke	HOLY-17	F	78	8.3	LJJ		1	1	
Holyoke	HOLY-18	F	77	11.1			1	1	
Holyoke	HOLY-19	F	84	12.6	JJ	JJ	1?	1	J-SNP
Holyoke	HOLY-20	F	79	8.9	LJJ	LLJJ	1	1	
Holyoke	HOLY-21	F	96	14.4	LJJ	LLJJ	1	1	
Holyoke	HOLY-22	F	79	8.9	LJJ	LLJJ	1	1	
Holyoke	HOLY-23	F	74	6.7			1	1	
Holyoke	HOLY-24	F	73	6.5					
Holyoke	HOLY-25	F	76	10.8					
Holyoke	HOLY-26	F	79	9.8					
Holyoke	HOLY-27	F	81	10.4					
Holyoke	HOLY-28	F	76	8.3					
Holyoke	HOLY-29	F	83	12.3					
Holyoke	HOLY-30	F	74	8.3					
Holyoke	HOLY-31	M	79	9			1?	1	
Holyoke	HOLY-32	F	84	10.3					
Holyoke	HOLY-33	F	84	13.4					
Holyoke	HOLY-34	F		6.7					
Holyoke	HOLY-35	F		10.3					
Holyoke	HOLY-36	F		14.8					
Holyoke	HOLY-37	F		5.8					
Holyoke	HOLY-38	F		12.5					
Holyoke	HOLY-39	F		10.4					
Holyoke	HOLY-40	F		8.3					
Holyoke	HOLY-41	F		11.1					
Holyoke	HOLY-42	F		10.9					
Holyoke	HOLY-43	F		9.1					
Holyoke	HOLY-44	F		9.6					
Holyoke	HOLY-45	F		11.9					
Holyoke	HOLY-46	F		8					
Holyoke	HOLY-47	F		9					
Holyoke	HOLY-48	F		8.6					

Holyoke	HOLY-49	F		9.7					
Holyoke	HOLY-50	F		13.2					
Holyoke	HOLY-51	F	77	7.2					
Holyoke	HOLY-52	F	78	8.1					
Holyoke	HOLY-53	F	84	11.7					
Holyoke	HOLY-54	M	74	7.4					
Holyoke	HOLY-55	F	80.5	11.5					
Lanesborough	TTO-1	F	81	17.5	LJJ	LJJ	1	1	U
Lanesborough	TTO-2	F	81	16.7	LJJ	LJJ	1	1	U
Lanesborough	TTO-3	F	80	16.6			0	0	
Lanesborough	TTO-4	F	82	12.6	LJJ	LJJ	1	1	U
Lanesborough	TTO-5	F	78	15.4	JJ	LJJ	1	1	
Lanesborough	TTO-6	M	72	9.9	JJ	JJ	0	1	J
Lanesborough	TTO-7	F	85	17.3	JJ	JJ	0	1	J
Lanesborough	TTO-8	F	83	15.7	LJJ	LJJ	1	1	(U)
Lanesborough	TTO-9	F	81	12.4		JJ	0	1	
Lanesborough	TTO-10	F	86	15.1		LJ	1	1	fail
Lanesborough	TTO-11	F	91	17.6	JJ	JJ	0	1	J
Lanesborough	TTO-12	F	79	10.3	LJJ	LJJ	1	1	fail
Lanesborough	TTO-13	F	83	15.7	LJJ	LJJ	1	1	
Lanesborough	TTO-14	F	91.3	16.1	LJJ	LJJ	1	1	
Lanesborough	TTO-15	F	89.6	14.6	LJJ	LJJ	1	1	
Lanesborough	TTO-16	F	80.3	12.9	LJJ	LJJ	1	1	
Lanesborough	TTO-17	F	87.1	14.8		LJ	1?	?	
Lanesborough	TTO-18	F	85.3	14		LJ	1?	?	
Lanesborough	TTO-19	F	84.1	12.6			1?	?	
Lanesborough	TTO-20	F	93	15.6			1	?	
Lanesborough	TTO-21	F	76.6	10.6					
Lanesborough	TTO-22	M	87.3	12.1		JJ	1	1	
Lanesborough	TTO-23	F	77.4	12			?	1	
Lanesborough	TTO-24	F	92.6	20.5					
Lanesborough	TTO-25	F	92.1	16.8					
Lanesborough	TTO-26	F	82.3	10.3					
Lanesborough	TTO-27	F	77.3	12.4					
Lanesborough	TTO-28	F	92.9	17					
Lanesborough	TTO-29	F	87.4	13.8					
Lanesborough	TTO-30	F	88.5	16					
Lanesborough	TTO-31	F	92.7	12.2					
Lanesborough	TTO-32	M	75.9	8.8					
Lenox	TTX-1	F	72.5	8.5	LJJ	LJJ	1	1	U
Lenox	TTX-2	F	83.2	14.4	LJJ	LJJ	1	1	fail
Lenox	TTX-3	F	85.5	12.6	LJJ	LJJ	0	1	U

Lenox	TTX-4	F	89	15.3	LJ	LJJ	1	1	U
Lenox	TTX-5	F	82	10	LJJ	LJJ	1	1	U
Lenox	TTX-6	F	93	17.4	LJJ	LJJ	1	1	
Lenox	TTX-7	F	85	14.8	LJ	LJJ	1	1	U
New Salem	LM-1				LJ	?J			fail
New Salem	LM-2					?J			U
New Salem	LM-3				LJ	?J			fail
New Salem	LM-4								fail
Newton	JVR1-1	F	69.1	7.3			0	1	(L)
Newton	JVR1-2	F	84.3	11.9			1	1	(U)
Newton	JVR1-3	M	60.5	4.2			0	1	
Newton	JVR1-4	F	76.7	10.0			1	1	
Newton	JVR1-5	F	81.9	9.9			1	1	
Newton	JVR1-6	F		12.2			1	1	
Newton	JVR1-7	F		9.0			1	1	
Newton	JVR1-8	F		12.8			1	1	
Newton	JVR1-9	F		11.2			1	1	
Newton	JVR1-10	F		11.1			1	1	
Newton	JVR1-11	F		13.1			1	1	
Newton	JVR1-12	F		10.5			1	1	
Newton	JVR1-13	F	80.2	10.0			1	1	
Newton	JVR1-14	F		10.2			1	1	
Newton	JVR1-15	F		8.4			1	1	
Newton	JVR1-16	F	79.4	9.3			1	1	
Newton	JVR1-17	F	86.6	13.3			1	1	
Newton	JVR1-18	F	85.1	14.3	LJJ		1	1	
Newton	JVR1-19	F	80.0	10.3	LJJ		1	1	
Newton	JVR1-20	U/F	58.5	4.7	L?	L	0	1	L
Newton	JVR1-21	F	72.6	7.9	LJ	LJ	1	1	U
Newton	JVR1-22	F	78.3	7.5		L	1	1	U
Newton	JVR1-23	F	58.2	5.1	L		0	1	L
Newton	JVR1-24	F		9.1	LJ				
Newton	JVR1-25	F	81.9	11.9					
Newton	JVR1-26	F	75.8	7.8					
Newton	JVR1-27	F	71.9	8.2					
Newton	JVR1-28	M	62.4	4.5			0	1	
Newton	JVR1-29	F	74.4	8.6					
Newton	JVR1-30	F	69.8	7.9					
Northborough	WN-1	J					1	1	
Northborough	WN-2	F			LJ	LJ	1	1	
Northborough	WN-3	M			LJ	LJ	1	1	U
Northborough	WN-4	F			J		1	1	

Northborough	WN-5	M			LJ	LJJ	1	1	L
Northborough	WN-6	F			LJ	L	1	1	
Northborough	WN-7	M			LJ	LL	1	1	L
Northborough	WN-8	M			J	LL	1	1	L
Northborough	WN-9	F			LJ	LJ	1	1	
Northborough	WN-10	F			LJ	LJ	1	1	
Northborough	WN-11	F			LLJ	LJ	1	1	
Northborough	WN-12	F			L?J	LJ	1	1	U
Northborough	WN-13	F			L?J	LJ	1	1	U
Northborough	WN-14	F			L?J	LJ	1	1	
Northborough	WN-15	F			LJ	LJ	1	1	
Northborough	WN-16	F			L?J	LJ	1	1	
Northborough	WN-17	F			LJ	LJ	1	1	
Northborough	WN-18	F			LJ	LJ	0	1	(U)
Northborough	WN-19	F				LJ	0	1	U
Northborough	WN-20	F			LJ	LJ	1	1	
Northborough	WN-21	F			LJ	LJ	1	1	
Northborough	WN-22	F				LJ	0	1	
Northborough	WN-23				L?J	LJ	0	1	
Northborough	WN-24	F				LJ	1	1	
Northborough	WN-25	F				L?J	1	1	
Richmond	NDC-1	M	70		L?		1	1	
Richmond	NDC-2	F	70		L?	LJ	0	1	
Richmond	NDC-3	F	80			LJ	1	1	U
Richmond	NDC-4	F	70				1	1	
Richmond	NDC-5	F	80				1	1	
Richmond	NDC-6	F	80		L?		1	1	
Richmond	NDC-7	F	75				1	0	
Richmond	NDC-8	F	75		L?	J	0	1	(U)
Richmond	NDC-9	F	68			LJ	1	1	
Richmond	NDC-10	F	88		LJ	LJ	1	1	U
Richmond	NDC-11	F	70	9.5			1	1	
Richmond	NDC-12	F	75	11			1	1	
Richmond	NDC-13	F	85	15	L?J		1	?	U
Richmond	NDC-14	F	85	17		LJ	1	1	
Richmond	NDC-15	F	85	15			1	1	
Richmond	NDC-16	F	83	14.5		LJ	1	1	U
Richmond	NDC-17	F	80	13.5			1	1	
Richmond	NDC-18	F	80	15			1	1	
Richmond	NDC-19	M	75	10.5			1	1	U
Richmond	NDC-20	F	90	16			1	1	
Sunderland	ARS-1	F	90	15	?JJ	J	0	1	J

Sunderland	ARS-2	M	82	11.5	JJ	JJ	0	1	J
Sunderland	ARS-3	M	84	10.25	?J	JJ	0	1	
Sunderland	ARS-4	M	87	10.75	JJ	J	0	1	J
Sunderland	ARS-5	F	97	17.75	JJ	JJ	0	1	J
Sunderland	ARS-6	F	87	18.5	JJ	JJ	0	1	
Sunderland	ARS-7	M	93	13.25	JJ	JJJ	0	1	J
Sunderland	ARS-8	M	81	9	JJ	J	0	1	J
Sunderland	ARS-9	M	88	9.75		J	0	1	
Sunderland	ARS-10	F	96	19.5	JJ		0	1	
Sunderland	ARS-11	M	88	10.5	JJ	JJ	0	1	
Sunderland	ARS-12	F	98	21.5	JJ	JJ	0	1	
Sunderland	ARS-13	F	92	16	J	J	0	1	
Sunderland	ARS-14	F	95	17.25	J	J	0	1	J
Sunderland	ARS-15	F	90	17.25		LJ	1	1	U
Sunderland	ARS-16	M	86	13			1	1	J
Sunderland	ARS-17	M	86	10.5	J	JJ	0	1	
Sunderland	ARS-18	F	99	19.25	J	JJ	?	1	(J)
Sunderland	ARS-19	M	86	10.5	J	JJ	0	1	
Sunderland	ARS-20	M	87	13		JJ	0	1	
Sunderland	ARS-21	F	91	13.75			1	1	(J)
Sunderland	ARS-22	F	92	18	?J	JJ	0	1	
Sunderland	ARS-23	F	96	19.75	?J	J	0	1	
Sunderland	ARS-24	F?	92	14	J	JJ	?	1	
Sunderland	ARS-25	M	84	10.5					
Sunderland	ARS-26	M	86	12					
Sunderland	ARS-27	M	84	11.75					
Sunderland	ARS-28	F	96	15.75					
Sunderland	ARS-29	M	83	12.25					
Sunderland	ARS-30	M	89	9.25					
Westborough	JEK1-1	M							
Westborough	JEK1-2	M	59.2	3.9					
Westborough	JEK1-3	M	61.9	4.6					
Westborough	JEK1-4	M	59.4	3.6					
Westborough	JEK1-5	M	61.4	4.0					
Westborough	JEK1-6	M	60.5	3.5					
Westborough	JEK1-7	M	63.4	5.9					
Westborough	JEK1-8	M	61.1	4.7					
Westborough	JEK1-9	M		4.2					
Westborough	JEK1-10	M	57.9	4.9					
Westborough	JEK1-11	M	67.6	5.5					
Westborough	JEK1-12	M	63.2	5.1					
Westborough	JEK1-13	M	64.6	5.1					

Westborough	JEK1-14	M	61.9	4.2					
Westborough	JEK1-15	M	61.0	5.0					
Westborough	JEK1-16	M	59.1	4.3					
Westborough	JEK1-17	M	60.6	4.5					
Westborough	JEK1-18	F	77.4	10.5					
Westborough	JEK1-19	M	56.7	3.8					
Westborough	JEK1-20	M	62.0	3.5					
Westborough	JEK1-21	M		5.6					
Westborough	JEK1-22	F		7.2					
Westborough	JEK1-23	M		4.0					
Westborough	JEK1-24	M		4.0					
Westborough	JEK1-25	M		5.9					
Westborough	JEK1-26	M		4.8					
Westborough	JEK1-27	M	62.6	4.8					
Westborough	JEK1-28	F	80.1	10.7					
Westborough	JEK1-29	U/F	58.0	4.0					
Westborough	JEK1-30	F	71.1	7.9					
Westborough	JEK1-A	M	68.1	3.6			0	1	
Westborough	JEK1-B	F	80.2	9.6			1	1	U
Westborough	JEK1-C	F	78.1	8.0			1	1	U
Westborough	JEK1-D	F	70.7	7.7			1	1	
Westborough	JEK1-E	F	75.4	7.5			1	1	
Westborough	JEK1-F	F	74.7	8.2			1	1	
Westborough	JEK1-G	M	61.2	4.5			0	1	
Westborough	JEK1-H	F	67.2	5.4			0	1	
Westborough	JEK1-I	U/F	57.0	4.3			0	1	
Westborough	JEK1-J	M	55.2	4.0			0	1	
Westborough	JEK1-K	F	73.6	7.0			1	1	
Westborough	JEK1-L	F	72.0	7.6			1	1	U
Westborough	JEK1-M	M	61.5	3.9			0	1	
Westborough	JEK1-N	M	57.2	3.9			0	1	
Westborough	JEK1-O	F	79.0	7.6			1	1	
Westborough	JEK1-P	U	56.9	3.3			0	1	
Westborough	JEK1-Q	U	58.4	3.7		L	0	1	L
Westborough	JEK1-R	M	53.4	3.2	L	?	0	1	
Westborough	JEK1-S	U	61.1	5.1	L	L	0	1	
Westborough	JEK1-T	F	60.2	5.1	L		0	1	
Westborough	JEK1-U	M	63.6	5.2	L	L	0	1	L
Westborough	JEK1-V	M	55.6	3.7	L	L	0	1	L
Westborough	JEK1-W	F		8.0	LJ	LJ	0	1	U
Westborough	JEK1-X	U/F	57.8	4.2	L	L	1	1	L
Westborough	JEK1-Y	M		4.4					

Westborough	JEK1-Z	M	59.8	4.9					
Westborough	JEK1-AA	M	60.6	3.8					
Westborough	JEK1-BB	F	74.1	8.3					
Westborough	JEK1-CC	M	63.8	4.9					
Westborough	JEK1-DD	F	71.1	7.2					
Westford	ROB-1	F	79	8.6	LJ	LJ	1	1	
Westford	ROB-2	F	73	6.7	LJ	LJ	1	1	
Westford	ROB-3	F	74	7.6	LJ	LJ	1	1	
Westford	ROB-4	F	77	9.2	LJ	LJ	1	1	
Westford	ROB-5	M		4.2	??	LJ	0	1	
Westford	ROB-6	F	85	11.8	LJ	LJJ	1	1	
Westford	ROB-7	F	80	9.9	LJ	LJ	1	1	
Westford	ROB-8	F	81	10.5		LJ	1	1	
Westford	ROB-9	M	58	6	JJ	L	0	1	fail
Westford	ROB-10	F	76	9.1	LJ	LJ	1	1	
Westford	ROB-11	F	65	6.8	LJJ	LJJ	1	1	U
Westford	ROB-12	F	85	12.2	LJJ	LJJ	1	1	
Westford	ROB-13	M	55	4.1		L	0	1	
Westford	ROB-14	M	56	4.7	?	LL	0	1	L
Westford	ROB-15	F	65	5.8	LJJ	LJ	1	0	U
Westford	ROB-16	M	62	5		LL	0	1	L
Westford	ROB-17	F	62	7.1	LJJ	LJ	1	1	
Westford	ROB-18	F	85	11.3	LJ	LJJ	1	1	U
Westford	ROB-19	F	90	13.9	LJ	LJ	1	1	
Westford	ROB-20	F	71	9.9	LJJ	LJ	1	1	
Westford	ROB-21	F	57	5.6	LJJ	LJ	1	1	
Westford	ROB-22	F	80	8.2		LJ	1	1	
Westford	ROB-23	M	58	5.3	LJJ		1	1	
Westford	ROB-24	M	64	6.3	LJJ	L	1	1	
Westford	ROB-25	M	62	4.9			1	1	
Wilbraham	ARW-1	F	71	9.25	LJ	?J	1	1	U
Wilbraham	ARW-2	F	77.5	14.25	LJ	?J	1	1	U
Wilbraham	ARW-3	F	72	9.25	LJ	?J	1	1	(U)
Wilbraham	ARW-4	F?	89.5	16	LJ	?J	1	1	U-SNP
Wilbraham	ARW-5	F	62	5.5	LJ	?J	1	1	
Wilbraham	ARW-6	F	69.5	8.5	LJ	?J	?	1	U
Wilbraham	ARW-7	F	84	12	LJ	?JJ	1	1	fail
Wilbraham	ARW-8	F	71	9.75		J	1	1	U
Wilbraham	ARW-9	F	87	17.75		??J	1	1	
Wilbraham	ARW-10	F	73	10.25		??J	1	1	
Wilbraham	ARW-11	F	80.5	13	LJ	??J	1	1	U

APPENDIX D

SOURCE CODE FOR SIMULATING UNISEXUAL BREEDING

Functions for simulating genomotypic trajectory of a population of unisexual salamanders breeding only with Ambystoma laterale males, written for R Statistical Software.

```
############################
#
# simulation
#
# function to run repeated simulations for a given parameter space
# and return:
#       1) a matrix of J frequencies over time for each run
#       2) an array of adult genomotypes from each year
#
############################

simulation <- function(
        num.indiv,
        num.eggs, #per individual
        num.generations,
        end.ratio, #rule for truncate run once certain low % J is reached
        prob.reduction, #probability that a female will reduce an egg
        prob.incorporation, #probability that a male genome will get incorporated
        max.ploidy,
        selection, #this is the selective pressure AGAINST J alleles
        selection.pos=0,
        num.reps,
        initial.genomotype = c('J','J','L',NA,NA)
  ){

    adult.genomotypes <- array(dim=c(num.indiv,max.ploidy+1,num.generations))
      for(p in 1:(max.ploidy+1)){
        adult.genomotypes[,p,1] <- initial.genomotype[p]
      }

    egg.genomotypes <- array(dim=c(num.indiv,max.ploidy+1,num.eggs))
    embryo.genomotypes <- array(dim=c(num.indiv,max.ploidy+1,num.eggs))
    embryo.survival <- array( dim=c(num.indiv,num.eggs))
    J.occ.mat <- array(dim=c(num.reps,num.generations))


    plot(-1,-1, xlim=c(0,num.generations),ylim=c(0,1))

  for(r in 1:num.reps){
    J.occurrence <- vector('numeric',num.generations)
    J.occurrence[1] <- 1
    k <- 1
    stop.run <- FALSE
    num.survive <- num.indiv
    while(stop.run==FALSE){
```

```
#step one, make eggs with some probability of reduction
egg.genomotypes[] <- NA
embryo.survival[] <- NA
for(i in 1:(min(num.indiv,num.survive)) ){
  adult.ploidy <- sum(!is.na(adult.genomotypes[i,,k]))
  reduce <- rbinom(n=num.eggs,size=1,prob=prob.reduction) #vector choosing whether reduction
  happens
  for(e in 1:num.eggs){
    if(reduce[e] == 1){
      egg.genomotypes[i,1:(adult.ploidy-1),e] <- sort(
              sample(x = adult.genomotypes[i,1:adult.ploidy,k],
                size = adult.ploidy-1,
                replace = FALSE),
              )
    }else{
      egg.genomotypes[i,,e] <- adult.genomotypes[i,,k]
    }
  }



#step two, make embryos with some probability of incorporation

  incorporate <- rbinom(n=num.eggs,size=1,prob=prob.incorporation)
  for(e in 1:num.eggs){
    egg.ploidy <- sum(!is.na(egg.genomotypes[i,,e]))
    if(incorporate[e] == 1){
      embryo.genomotypes[i,1:(egg.ploidy+1),e] <- sort(c(egg.genomotypes[i,1:egg.ploidy,e],"L"))
        #always only "LL" males available
    }else{
      embryo.genomotypes[i,,e] <- egg.genomotypes[i,,e]
    }


#step three, apply selection to embryos-
    embryo.ploidy <- sum(!is.na(embryo.genomotypes[i,,e]))
    if( embryo.ploidy > max.ploidy | embryo.ploidy < 2){
      embryo.survival[i,e] <- 0
    }else{
      if(sum(embryo.genomotypes[i,,e]=="L",na.rm=TRUE)==0){
        embryo.survival[i,e] <- 0
      }else{
        ratio.J <-
  sum(embryo.genomotypes[i,,e]=="J",na.rm=TRUE)/sum(embryo.genomotypes[i,,e]=="L",na.rm=
  TRUE)
#         if(ratio.J>0){
            embryo.survival[i,e] <- max((1 - selection*ratio.J),0)
#         }else{
#           embryo.survival[i,e] <- 1
#         }
      }
    }

  }
```

```
        }


        embryo.survival[is.na(embryo.survival)] <- 0
        num.survive <- sum(embryo.survival>0,na.rm=TRUE)#just in case we get higher mortality than we
           need to sample in next row
        if(num.survive>0){
           winning.embryos <-
           sample(x=1:(num.eggs*num.indiv),size=min(num.indiv,num.survive),replace=FALSE,prob=embr
           yo.survival)
           cell.num <- winning.embryos
           num.rows <- dim(embryo.survival)[1]
           col.num <- ceiling(cell.num/num.rows)
           row.num <- cell.num - (col.num-1)*num.rows


           #finally, make this year's winning embryos next year's adults
           for(i in 1:num.indiv){
              adult.genomotypes[i,,k+1] <- embryo.genomotypes[row.num[i],,col.num[i]]
           }

           #get current generation proportion of "J"
           num.with.J <- 0
           for(i in 1:num.indiv){
              num.with.J <- num.with.J + min( sum(adult.genomotypes[i,,k] == "J",na.rm=TRUE),1)
           }
           J.occurrence[k] <- num.with.J / num.indiv

        }
           points(k,J.occurrence[k],pch=9)
        if( J.occurrence[k] <= (J.occurrence[1])*end.ratio | k == (num.generations-1)|num.survive==0){
           stop.run <- TRUE
        }
        k <- k+1

        J.occ.mat[r,] <- J.occurrence

     }#end while loop over number of generations
  }#end for loop over number of reps

  output <- list(J.occ.mat = J.occ.mat, adult.genomotypes = adult.genomotypes)

  output
}

################################
################################
################################
#
# stepper
#    steps over the space of incorporation rate and ploidy reduction rate
#
#
################################
```

```
stepper <- function(
        reduction.min,
        reduction.max,
        reduction.step,
        incorporation.min,
        incorporation.max,
        incorporation.step,
        log.scale = c(TRUE,TRUE),
        num.indiv,
        num.eggs, #per individual
        num.generations,
        end.ratio, #rule for truncate run once certain low % J is reached
        max.ploidy,
        selection, #this is the selective pressure AGAINST J alleles
        num.reps,
        path #path on hard drive to write outputs
        ){
          random.name <- sample(100000,1)
          params<- matrix(nrow=15,ncol=1,
            dimnames=list(c(
              'reduction.min',
              'reduction.max',
              'reduction.step',
              'incorporation.min',
              'incorporation.max',
              'incorporation.step',
              'log.scale-1',
              'log.scale-2',
              'num.indiv',
              'num.eggs',
              'num.generations',
              'end.ratio',
              'max.ploidy',
              'selection',
              'num.reps'),
            NULL))
          params[,1] <-c(
                reduction.min,
                reduction.max,
                reduction.step,
                incorporation.min,
                incorporation.max,
                incorporation.step,
                log.scale[1],
                log.scale[2],
                num.indiv,
                num.eggs,
                num.generations,
                end.ratio,
                max.ploidy,
                selection,
                num.reps)

          write.csv(params,file=paste(path,'stepParams',random.name,'.csv',sep=''))
          reduction.seq<-get.steps(reduction.min,reduction.max,reduction.step,log.scale=log.scale[1])
```

```
        incorporation.seq<-
        get.steps(incorporation.min,incorporation.max,incorporation.step,log.scale=log.scale[2])
        print(reduction.seq)
        print(incorporation.seq)

        output.array <-
        array(dim=c(length(reduction.seq),length(incorporation.seq),num.reps,num.generations),
                dimnames=list(reduction.seq,incorporation.seq,NULL,NULL)
              )
        for(red in 1:length(reduction.seq)){
          for(inc in 1:length(incorporation.seq)){

            sim.out<-simulation(
              num.indiv=num.indiv,
              num.eggs=num.eggs,
              num.generations=num.generations,
              end.ratio=end.ratio,
              prob.reduction=reduction.seq[red],
              prob.incorporation=incorporation.seq[inc],
              max.ploidy=max.ploidy,
              selection=selection,
              num.reps=num.reps)

            output.array[red,inc,,] <- sim.out$J.occ.mat
            save(output.array,
              file=paste(path,'stepper_output_array',random.name,'.R',sep='')
            )

            adult.genomotypes <- sim.out$adult.genomotypes
            save(adult.genomotypes,
              file=paste(path,'stepper_adult_gen_',red,'r_',inc,'i_',random.name,'.R',sep='')
            )

            fit.sim.out(sim.out$J.occ.mat)
            print(paste('reduction.rate= ',reduction.seq[red]))
            print(paste('incorporation.rate= ',incorporation.seq[inc]))
          }
        }
      }

##################################
##################################
##################################
#
# selection.stepper
#     step over a range of selections
#
##################################


selection.stepper <- function(
      select.min,
      select.max,
      select.step,
      num.indiv,
```

```
log.scale = TRUE,
num.eggs,
num.generations,
end.ratio,
prob.reduction,
prob.incorporation,
max.ploidy,
num.reps,
path){
  random.name <- sample(100000,1)
  params<- matrix(nrow=12,ncol=1,
    dimnames=list(c(
      'select.min',
      'select.max',
      'select.step',
      'num.indiv',
      'log.scale',
      'num.eggs',
      'num.generations',
      'end.ratio',
      'prob.reduction',
      'prob.incorporation',
      'max.ploidy',
      'num.reps'),
    NULL))
  params[,1] <-c(
      select.min,
      select.max,
      select.step,
      num.indiv,
      log.scale = TRUE,
      num.eggs,
      num.generations,
      end.ratio,
      prob.reduction,
      prob.incorporation,
      max.ploidy,
      num.reps)

  write.csv(params,file=paste(path,'selectstepParams',random.name,'.csv',sep=''))
  select.seq<-get.steps(select.min,select.max,select.step,log.scale=log.scale)
  print(select.seq)


  output.array <- array(dim=c(length(select.seq),num.reps,num.generations),
          dimnames=list(select.seq,NULL,NULL)
        )
  for(sel in 1:length(select.seq)){
    sim.out<-simulation(
        num.indiv=num.indiv,
        num.eggs=num.eggs,
        num.generations=num.generations,
        end.ratio=end.ratio,
        prob.reduction=prob.reduction,
        prob.incorporation=prob.incorporation,
```

```
                max.ploidy=max.ploidy,
                selection=select.seq[sel],
                num.reps=num.reps)

            output.array[sel,,] <- sim.out$J.occ.mat
            save(output.array,
              file=paste(path,'select_stepper_output_array',random.name,'.R',sep='')
            )

            fit.sim.out(sim.out$J.occ.mat)
            print(paste('select.size= ',select.seq[sel]))

        }
        output.array
      }


##################################
##################################
##################################
##################################
#
#  size.stepper
#     step over a range of sizes
#
##################################


size.stepper <- function(
      size.min,
      size.max,
      size.step,
      log.scale = TRUE,
      num.eggs,
      num.generations,
      end.ratio,
      prob.reduction,
      prob.incorporation,
      max.ploidy,
      selection,
      num.reps,
      path){
        random.name <- sample(100000,1)
        params<- matrix(nrow=12,ncol=1,
          dimnames=list(c(
            'size.min',
            'size.max',
            'size.step',
            'log.scale',
            'num.eggs',
            'num.generations',
            'end.ratio',
            'prob.reduction',
            'prob.incorporation',
            'max.ploidy',
```

```
            'selection',
            'num.reps'),
          NULL))
        params[,1] <-c(
            size.min,
            size.max,
            size.step,
            log.scale = TRUE,
            num.eggs,
            num.generations,
            end.ratio,
            prob.reduction,
            prob.incorporation,
            max.ploidy,
            selection,
            num.reps)

        write.csv(params,file=paste(path,'sizestepParams',random.name,'.csv',sep=''))
        size.seq<-get.steps(size.min,size.max,size.step,log.scale=log.scale)
        size.seq <- round(size.seq)
        print(size.seq)


        output.array <- array(dim=c(length(size.seq),num.reps,num.generations),
                  dimnames=list(size.seq,NULL,NULL)
                )
        for(siz in 1:length(size.seq)){
          sim.out<-simulation(
              num.indiv=size.seq[siz],
              num.eggs=num.eggs,
              num.generations=num.generations,
              end.ratio=end.ratio,
              prob.reduction=prob.reduction,
              prob.incorporation=prob.incorporation,
              max.ploidy=max.ploidy,
              selection=selection,
              num.reps=num.reps)

            output.array[siz,,] <- sim.out$J.occ.mat
            save(output.array,
              file=paste(path,'size_stepper_output_array',random.name,'.R',sep='')
            )

            fit.sim.out(sim.out$J.occ.mat)
            print(paste('pop.size= ',size.seq[siz]))

        }
      }
##################################
##################################
##################################
##################################
#
# fit.sim.out
#     fit an exponential function to the simulation output
```

```
#
###################################

fit.sim.out <- function(J.occ.mat,type='exponential')
  {
    if(is.matrix(J.occ.mat)){
      J.occ.mean <- apply(J.occ.mat,2,mean)
      J.occ.sd <- apply(J.occ.mat,2,sd)
      J.occ.extinction <- vector('numeric',dim(J.occ.mat)[1])
      for(r in 1:dim(J.occ.mat)[1]){
        J.occ.extinction[r] <- min((1:dim(J.occ.mat)[2])[J.occ.mat[r,]==0])
      }
      end.model<-min(J.occ.extinction)
    }else{
      J.occ.mean <- J.occ.mat
      J.occ.extinction <- min((1:length(J.occ.mat))[J.occ.mat==0])
      end.model <- J.occ.extinction
    }

    plot(1:length(J.occ.mean),J.occ.mean)
    data <- J.occ.mean[1:end.model]
    years <- 1:end.model

    if(type=='linear'){
      lm.out <- lm(data~years)
      intercept <- lm.out$coefficients[1]
      slope <- lm.out$coefficients[2]
      abline(intercept,slope)
      output <- list(lm.out,extinction.times=J.occ.extinction)

    }else{
      if(type=='exponential'){

        optim.target <- function(L,data){
          x <- 1:(length(data))
          residual <- data - exp(-L*x)
          sum.sq <- sum( residual^2 )
          sum.sq
        }


        optim.out<-optimize(interval=c(0,1),f=optim.target,data=data)
        L<-optim.out$minimum
        curve( exp(-L*x),add=TRUE)
        output <- list(optim.out,extinction.times=J.occ.extinction)

      }else{

        optim.target <- function(params,data){
          C <- params[1]
          p <- params[2]
          B <- params[3]
          x <- 1:(length(data))
            residual <- data^(1/p) - (C*x + B)
          sum.sq <- sum( residual^2 )
```

```
            sum.sq

          }

          optim.out<-optim(par=c(C=1,p=1,B=0),fn=optim.target,data=data)
          C<-optim.out$par['C']
          p<-optim.out$par['p']
          B<-optim.out$par['B']
          curve( (C*x+ B)^(p),add=TRUE)
          output <- list(optim.out,extinction.times=J.occ.extinction)
        }
      }
      print(output)
      output
    }

##################################
##################################
##################################
##################################
#
# get.steps
#     get a sequence of steps given a min/max step size
#     if log.scale==FALSE, then step size is multiplied by each level to get the next level
#     if log.scale==TRUE, then step size is added to each level to get the next
#
#
##################################
    get.steps<-function(value.min,value.max,value.step,log.scale=TRUE){
        if(log.scale){
          value.seq <- 10^seq(log10(value.min),log10(value.max),log10(value.step))
        }else{
          value.seq <- seq(value.min, value.max, value.step)
        }
        value.seq
    }

##################################
```

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