

EFFECTS OF PHENOTYPE- AND CONDITION-DEPENDENT FACTORS
ON JUVENILE DISPERSAL OF THE
RINGED SALAMANDER (*AMBYSTOMA ANNULATUM*)

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by
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EFFECTS OF PHENOTYPE- AND CONDITION-DEPENDENT FACTORS
ON JUVENILE DISPERSAL OF THE
RINGED SALAMANDER (*AMBYSTOMA ANNULATUM*)

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EFFECTS OF PHENOTYPE- AND CONDITION-DEPENDENT FACTORS ON JUVENILE DISPERSAL
OF THE RINGED SALAMANDER (*AMBYSTOMA ANNULATUM*)

ABSTRACT

Dispersal is the movement of individuals from their natal population to a different breeding population. Long distance dispersal movements are rare, with most individuals staying within their natal population (hereafter residents). The frequency with which individuals disperse to new population or settle near natal sites has strong consequences for individual survival and fitness, as well as ecological and evolutionary processes. Despite the importance of dispersal, there have been limited empirical tests of the factors affect whether an individual disperses or is philopatric.

This is particularly true for amphibians of the family Ambystomatidae. These pond-breeding salamanders have complex life cycles, with aquatic larvae and terrestrial juveniles and adults. Despite abundant research on the breeding migrations and larval life stage of these salamanders, factors affecting juvenile dispersal remain poorly resolved. This is particularly true for phenotype-dependent factors, such as body size and body condition, which are generally controlled for rather than manipulated. An understanding of factors driving dispersal in Ambystomatidae is critically needed. One-third of all amphibians are currently threatened with extinction due to habitat loss and fragmentation, and climate change. By understanding the factors that affect dispersal in this family of salamanders, we can manage at the individual, population, and metapopulation level.

My dissertation research used laboratory and field experiments to measure effects of phenotype- and condition-dependent factors on movement in a mole salamander

(*Ambystoma annulatum*). The primary objectives of my study were to i) describe the effect of natal density on juvenile phenotype, and ii) determine the effect of individual variation in phenotype-dependent factors (i.e., behavior, morphology, and body condition) and condition-dependent factors (i.e., natal population density and juvenile habitat quality) on dispersal.

To identify the effects of natal population density on juvenile phenotype, I conducted a replicated pond mesocosm experiment. I manipulated the density of ringed salamanders (*Ambystoma annulatum*) over 10 levels and compared the model fit of four functional forms of body size, body condition, date of metamorphosis survival, and percent lipids. In general, I found support for non-additive density dependence, with the negative impact of each additional individual decreasing in magnitude as density increased. After measuring the effect of natal conditions on juvenile phenotype, I conducted a series of experiments to describe the effect of phenotype- and condition dependent factors. I reared salamanders from larvae under different natal densities, resulting in juveniles that differed in the conditions they experienced (i.e., natal density), as well as their body size, condition, and morphology. I also measured the exploration behavior of individuals. Using PIT telemetry, I tracked the initial movement of juvenile salamanders in two different habitat conditions (forest and field habitat). I found that both phenotype- and condition-dependent factors affect juvenile dispersal. I also found an interaction between these two types of factors, suggesting that redundant cues over ontogeny affect dispersal. Collectively, these studies indicate that both aquatic natal habitat and its effects on juvenile phenotype, as well as juvenile terrestrial habitats should be considered when managing amphibian populations and metapopulations.

CHAPTER 1

INTRODUCTION

Dispersal is the movement of individuals from their natal population to a different breeding population (Semlitsch 2008). Long distance dispersal movements are rare, with most individuals staying within their natal population (hereafter residents). The frequency with which individuals disperse to new population or settle near natal sites has strong consequences for individual survival and fitness, as well as ecological and evolutionary processes (Clobert et al. 2001; Hanski and Gaggiotti 2004; Clobert et al. 2012). Despite the importance of dispersal, there have been limited empirical tests of the factors that affect whether an individual disperses or remains a resident (Ronce 2007; Lowe and McPeck 2012; Pittman et al. 2014).

Evolutionary models and empirical studies indicate that dispersers differ from residents in their internal state, or phenotype-dependent factors, and in their response to external cues, or condition-dependent factors (Bowler and Benton 2005; Clobert et al. 2009; Edelaar and Bolnick 2012; Lowe and McPeck 2014). For example, dispersers might be more sensitive to deteriorating habitat conditions or high population density, and thus less likely to remain philopatric. However, the relationship between phenotype-dependent factors and dispersal remains poorly understood, especially in taxa that have continuous morphological variation. Whereas larger individuals in a cohort may be expected to be more likely to disperse because they can move more efficiently (e.g. longer limbs, greater energy stores) (Harrison 1980; Denno et al. 1991), smaller individuals in a cohort are less able to win competitions and may need to move farther from their natal population to obtain resources (Cheptou et al. 2008). Because the

mechanisms underlying differences between dispersers and residents is unknown in most taxa, it is often implicitly ignored despite its importance for developing ecological and evolutionary theories, understanding the demographic consequences of dispersal to population dynamics, and determining the flow of nutrients between ecosystems.

This is particularly true for amphibians of the family Ambystomatidae. These pond-breeding salamanders have complex life cycles, with aquatic larvae and terrestrial juveniles and adults. Although numerous studies have investigated breeding migrations and the larval life stage of these salamanders, factors affecting juvenile dispersal remain poorly resolved. Amphibians are generally philopatric, with 78 – 95% of juveniles in a cohort returning to their natal pond to breed (Berven and Grudzien 1990; Sinsch 1997; Trenham et al. 2001; Gamble et al. 2007), however, the mechanisms leading to dispersal remain unknown. This is especially the case phenotype-dependent factors, such as body size and body condition, which are generally controlled for rather than manipulated.

The primary objectives of my dissertation was to i) describe the effects of natal density on juvenile phenotype, and ii) determine the effects of individual variation in phenotype-dependent factors (i.e., behavior, morphology, and body condition) and condition-dependent factors (i.e., natal population density and juvenile habitat quality) on dispersal in a pond-breeding salamander. In Chapter 2, I describe a mesocosm experiment that identified the effects of natal population density on juvenile phenotype. In Chapters 3 and 4, I present the results of field experiment where I determine the effects of phenotype-dependent factors (body size, body condition, morphology, exploration behavior), condition-dependent factors (natal population density and habitat type), and their interaction on dispersal.

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CHAPTER 2

NON-ADDITIVE RESPONSE OF LARVAL RINGED SALAMANDERS (*AMBYSTOMA ANNULATUM*) TO INTRASPECIFIC DENSITY

Abstract

Conditions experienced in early developmental stages can have long-term consequences for individual fitness. High intraspecific density during the natal period can affect juvenile and eventually adult growth rate, metabolism, immune function, survival, and fecundity. Despite the important ecological and evolutionary effects of early developmental density, the form of the relationship between natal density and resulting juvenile phenotypes is poorly understood. To test competing hypotheses explaining responses to intraspecific density, we experimentally manipulated the initial larval density of ringed salamanders (*Ambystoma annulatum*), a pond-breeding amphibian, over 11 densities. We modeled the functional form of the relationship between natal density and juvenile traits, and compared the relative support for the various hypotheses based on their goodness of fit. These functional form models were then used to parameterize a simple simulation model of population growth. Our data support non-additive density dependence and presents an alternate hypothesis to additive density dependence, self-thinning and Allee effects in larval amphibians. We posit that ringed salamander larvae may be under selective pressure for tolerance to high density and increased efficiency in resource utilization. Additionally, we demonstrate that models of population dynamics are sensitive to assumptions of functional form of density dependence.

Introduction

A central goal of ecology is to determine the factors influencing population dynamics (Cole 1954). The size of a population is determined by both non-feedback mechanisms, such as external perturbations (e.g. disease, catastrophes, and boom years), and by density dependent factors, which can stabilize population sizes around a mean. Negative density dependence regulates populations through intra- and interspecific competition, whereby per capita resources are reduced, resulting in decreased growth, fecundity, or survival of individuals, and ultimately lower population growth (reviewed in Herrando-Perez et al. 2012a). Population regulation through negative density dependence is broadly observed in natural and experimental systems across a range of organisms. In a meta-analysis of 1198 species, Brook and Bradshaw (2006) found that density dependence is a pervasive feature of population dynamics. Density dependence is central not only to theories of population regulation, but also to community structure and species interactions (Wright 2002).

When experienced during early developmental stages (i.e. before sexual maturation), conditions such as high intraspecific density can have immediate and long-term consequences for individual development and fitness. Effects can include altered growth rate, metabolism, immune function, and decreased fecundity and there is substantial evidence for these patterns in many taxa (Lindström 1999; Monaghan 2008; Herrando-Perez et al. 2012a). For example, zebra finches (*Taeniopygia guttata*) reared in experimentally larger broods had greater mortality before and after fledging, weighed less, had higher metabolism, poorer immune responses, and bred later than those raised in experimentally smaller broods (De Kogel 1997; Monaghan 2008). Similar patterns occur

in explosive breeders, such as salmonids. Fry from stream reaches of high intraspecific density have lower survival, depressed growth and thus delayed maturity, poorer immune-response, select different habitat, and are more likely to disperse (Post et al. 1999; Bult et al. 1999; Einum et al. 2006).

The effects of increased intraspecific density have also been broadly documented in pond-breeding amphibians. Amphibian larvae reared at high densities have lower survival to metamorphosis, slower growth, later metamorphosis, and emerge from ponds as smaller juveniles (Wilbur 1976; Scott 1994; Loman 2004). While amphibian populations are thought to be regulated during terrestrial life-history stages (Wilbur 1980; Pechmann 1995; Biek et al. 2002), the phenotype resulting from larval environments have fitness implications that carry over into later life-history stages. Smaller juveniles have an increased risk of desiccation, depressed immune function, lower lipid levels, decreased survival, later age of first reproduction, and lower fecundity (Scott 1994; Davis and Maerz 2009; Peterman et al. 2013).

Although the relationship between natal intraspecific density and adult phenotype is broadly understood in many taxonomic groups, there is not a consensus concerning the form of functional responses because most studies are limited to test five or fewer densities (Wilbur 1977, Semlitsch and Caldwell 1982, Vonesh 2005, Anderson and Semlitsch 2013). We sought to test four competing hypotheses concerning the relationship between density and phenotype: 1) that density dependence is additive (e.g. Anderson and Semlitsch 2013), 2) that density dependence is non-additive (e.g. Morin 1988), 3) that self-thinning occurs (Semlitsch and Caldwell 1982), and 4) that Allee effects occur (Wilbur 1977; Smith-Gill and Gill 1978). Additive density dependence

occurs when there is a linear response to increased density with each additional individual, thus resulting in an equal change in the response of interest (Figure 1). Additive effects would be expected if each additional individual resulted in a proportional decrease in resources. Alternatively, if the response is less than expected by an additive model, non-additive density dependence may be occurring (Figure 1). Self-thinning occurs when competition is reduced as individuals metamorphose, allowing the remaining larvae to develop in lower densities. This results in individuals at high densities performing similarly or better than individuals at medium densities (Figure 1). Allee effects occur when populations with a density smaller than a critical mass perform more poorly than those with a density slightly greater than the critical mass (Figure 1). Such positive density dependence can result from difficulty in finding mates, or modification of the environment (e.g. toad larvae stirring sediment to release food; Wilbur 1977).

To test these contrasting hypotheses, we experimentally manipulated the initial larval density of a pond-breeding amphibian, the ringed salamander (*Ambystoma annulatum*), and compared the functional form of eventual juvenile phenotype to previously proposed models. To better fit density dependence, we tested 11 densities ranging from 0.001 to 0.036 larvae/ L which encompasses the range of larval salamander densities found in natural ponds (Semlitsch 1987, Scott 1990; Ousterhout et al. in review). As individuals metamorphosed, we measured size, body condition, and length of larval period. We also assayed energy stores and survival. To test if models of population growth are sensitive to the functional form of density dependence, we built a series of simple population growth models differing only in the shape of density dependence. Here

we report evidence of non-additive density dependence. Additionally, we demonstrate that population models are sensitive to the functional form of density dependent size, highlighting the importance of our findings for modeling population dynamics.

Materials and Methods

Study System

The ringed salamander is a pond-breeding amphibian endemic to the Ozark Plateau (southern Missouri, western Arkansas, and eastern Oklahoma; Petranka 1998). Fossorial adults migrate to breeding ponds late August – October (Hocking et al. 2008). Following oviposition, eggs hatch, and larvae overwinter in ponds. While most surviving offspring metamorphose in May, individuals emerge from ponds as late as July (Hocking et al. 2008).

Mesocosm Experiment

To test potential mechanisms of conspecific density on larval ringed salamanders, we conducted a pond mesocosm experiment. We tested the following densities (larvae/1000L mesocosm): 9, 12, 16, 21, 24, 27, 30, and 33 (N = 4); 6 and 18 (N = 9); and 36 (N = 7). We increased the replication of three treatments (6, 18 and 36 larvae/mesocosm) as those animals were later used for another experiment. We established experimental pond mesocosms (1000 L volume, 1.52 m diameter polyethylene cattle tanks) in the same manner as similar studies (Earl et al. 2011). We arranged mesocosms in a rectangular array in a fenced research facility at the University of Missouri, Columbia, Missouri. On 05-Sept-2011 each mesocosm was filled with tap water, and left to dechlorinate for 14 d.

We added 2 kg air dried leaves (primarily *Quercus* spp. and *Acer* spp.) to each mesocosm on 19-Sept-2011 to create a nutrient base. We inoculated each mesocosm with aliquots of zooplankton (4L added in total; 22-Sept to 11-Oct-2011) to establish natural plankton and periphyton communities. Mesocosms were left uncovered to allow colonization by flying insects (e.g. dipterans) as an additional food source for larvae. Water levels were maintained at a constant depth (approximately 50 cm) during the experiment.

We collected early stage salamander embryos at similar developmental stages on 28-Sept-2011 from four ponds at Fort Leonard Wood, Missouri, USA. Egg masses were stored in the laboratory at 10° C, approximately the water temperature of natural ponds (Anderson et al *unpublished data*), until they hatched. Following hatching but prior to feeding, we combined all salamander larvae from the four ponds, and then randomly assigned each larva to an initial density treatment (hereafter, treatment) following a randomized block design (larvae added 15-, 16-, and 21-Oct-2011). Each treatment was then randomly assigned to a mesocosm. As larvae were assigned to treatments, we inspected them for damage and irregularities, and replaced individuals if one of those conditions existed.

Beginning in Apr-2012, we searched for metamorphosed salamanders by checking mesocosms after sunset with a light at least every other night. Individuals were considered to have metamorphosed if their gills were < 1 mm and their tailfin was reabsorbed (Mott and Maret 2011; Ousterhout et al. 2014). Mesocosms were searched until 01-June-2012, when we drained all mesocosms and thoroughly searched the leaf litter for remaining salamanders to calculate survival. At metamorphosis, we recorded date of metamorphosis, wet mass (± 0.001 g, Mettler AT-100 electronic balance, Mettler

Toledo, Columbus, Ohio, USA) and snout-vent length (SVL; ± 1 mm) Animals that failed to metamorphose prior to the end of the experiment were only included in the survival modeling..

To assay energy stores, we immediately euthanized animals by immersion in tricaine methanesulfonate (MS-222) from eight treatments levels (all except 6, 18, and 36 individuals/mesocosm). Individuals were stored at -20° C until the conclusion of the mesocosm experiment. To test for effects of density on energy stores, we homogenized samples by mesocosm and then determined the amount of crude lipids using a modified Folch extraction followed by gravimetric lipid determination (Fisheries and Illinois Aquaculture Center, Southern Illinois University).

Statistical Analysis

We were interested in six responses to natal density: length of larval period, body size (SVL), body condition, percent crude lipids, and survival to metamorphosis. Length of larval period was defined as the elapsed time from the addition of hatchlings to a mesocosm to an individual's metamorphosis. We defined body condition as the size independent mass. We calculated body condition by mean scaling the mass to decouple variance from measurement scale and means. We then regressed mass against SVL, and used the residuals as the response in the body condition models (Berner 2011).

We conducted nonlinear regressions to compare our data to five possible mechanisms underlying density dependence (Figure 1; Harper and Semlitsch 2007, Rollinson and Hutchings 2013). Nonlinear least-squares models relax the assumption of linearity, but still require independence, equal variance, and normally distributed errors

(Bates and Watts 1988). If additive density dependence was occurring such that each additional individual decreased the per capita resources equally, a linear model with a negative slope would be best supported (Anderson and Semlitsch 2014). If responses are less than predicted by the additive model, non-additive density dependence may be occurring, a model of a decelerating curve would be best supported. A convex curve would be best supported by the data if self-thinning was occurring (Semlitsch and Caldwell 1982). Finally, if an Allee effect was occurring, a concave curve would best fit the data (Wilbur 1977). In all cases, the strength of density dependence would increase with steeper slopes.

We compared our data to these proposed mechanisms using four functional equations models. We tested for an additive response using a linear model ($y = ax + b$). We used a Shepherd model $y = ax/(b+x^c)$ and an exponential function ($y = ae^{-bx}$) to test for non-additive density dependence. Finally, we used a Ricker equation ($y = axe^{-bx}$) to test for Allee effects and self-thinning, depending on the parameter space (Figure 1). Because nonlinear regression does not use least-squares, non-nested models cannot be compared using Akaike Information Criteria. We instead compared candidate nonlinear regression models for each response variable by calculating an adjusted r^2 as a measure of goodness of fit (Crawley 2013). Models that account for the same amount of variation as the null intercept model had an adjusted r^2 of zero. For all analyses, mesocosm was the experimental unit, and as such, mesocosm means were used. Preliminary analysis revealed block accounted for very little variation ($P > 0.60$), so we did not consider it in our models. All analyses were performed using *nls* in R 3.0.2 (R Core Team 2015).

Results

Length of Larval Period

Salamanders began metamorphosing 14 April, two weeks after we began checking mesocosms. Ninety-nine percent of all individuals surviving to metamorphosis had left the mesocosms by 1 June, the day we concluded the experiment. Initial larval density affected length of larval period (ANOVA, $F_{10,44} = 13.87$, $P < 0.0001$). The mean length of the larval period was 18 d longer for individuals from the highest density treatment (mean \pm 1 SD: 205 days \pm 8.7) than those from the lowest density treatment (mean 187 days \pm 6.4 SD). Two non-additive models best fit our data, and the linear model also received support (non-additive: adjusted $r^2 = 0.72$ and 0.69 , linear: adjusted $r^2 = 0.69$; Table 1, Figure 2a).

Body Size

Body size was negatively affected by initial density (ANOVA: $F_{10,44} = 25.35$, $P < 0.0001$). Juveniles metamorphosing from high density mesocosms were 22% smaller than animals from low density mesocosms (low: 45.9 mm \pm 2.5, high: 35.8 mm \pm 3.0). A non-additive model best described the relationship between initial density and SVL (adjusted $r^2 = 0.83$; Table 1, Figure 2b).

Body Condition

Body condition did not vary with initial larval density (ANOVA: $F_{10,44} = 1.47$, $P = 0.18$) and no model received appreciably more support than the null model (adjusted $r^2 = 0.11$; Table 1, Figure 2c). Body condition was 0 ± 0.08 (minimum: -0.14, maximum: 0.35),

where a body condition of zero indicated an animal's mass corresponded to its SVL, a negative body condition indicated animals weighed less than their SVL would predict, and a positive body condition signaled that animals weighed more than their SVL would indicate.

Survival

There was no relationship between initial larval density and survival to metamorphosis (ANOVA: $F_{10,44} = 1.5$, $P = 0.17$). Survival was $91.3 \% \pm 17$ (minimum: 42.9%; maximum 100%). No model received substantially more support than the null model. The best fitting model explained little of the variance (adjusted $r^2 = 0.07$; Table 1, Figure 2d).

Energy Stores

As density increased, percent crude lipid decreased nonlinearly (ANOVA: $F_{7,23} = 29.927$, $P < 0.0001$). Individuals reared in the highest density treatments had 34% lower lipid content than those reared in the lowest density treatment (low: 12.90% crude lipids ± 0.65 ; high: 8.52% crude lipids ± 0.80). The relationship between lipids and initial density of larvae was best described by the non-additive model (adjusted $r^2 = 0.63$; Table 1, Figure 2e).

Discussion

Here we show that intraspecific natal density exerts clear non-additive effects on juvenile phenotype. The non-additive model accounted for at least 8% more variation than the next most supported hypothesis for all responses affected by density, except for length of

larval period (3%) and survival (0%). Individuals metamorphosing from low density ponds were larger and metamorphosed earlier than juveniles from higher density treatments, but the differences between treatments diminished as density increased. We did not detect an effect of density on survival. As most amphibians occur at high larval densities in natural ponds, the diminishing difference between treatments at high densities may help account for the limited explanatory power of intraspecific density in field studies (Van Buskirk 2005; Ousterhout et al. 2015).

Two non-exclusive mechanisms could result in the non-additive density dependence observed in this study: resource limitation and increased stress hormones. The lipid assay in this study indicates that food resources limited energy assimilation. Percent crude lipids decreased nonlinearly; the percent crude lipid of animals in the lowest density mesocosms was 51% greater than animals reared in the highest density pond. If resource limitation was the mechanism resulting in non-additive density dependence, we would expect there to be lower quantities of zooplankton in ponds with a higher density of larvae. This would result in non-additive density dependence if beyond a threshold density of larvae zooplankton density was uniformly low. However, with one exception (Scott 1990), previous studies have found no effect of the density of salamander larvae on the density, biomass or composition of zooplankton (Figiel and Semlitsch 1990; Van Buskirk and Smith 1991; Davis 2012), including one study with ringed salamanders (Anderson et al *unpublished data*). We suggest that while resource limitation is certainly occurring, further experiments that frequently sample zooplankton will be required to capture the highly cyclic dynamics of food resources and test the resource limitation hypothesis.

Alternatively, the non-additive density dependence observed in this study may be caused by increased stress hormones as a result of more frequent encounters with conspecifics in higher density ponds. Elevated stress in response to conspecifics has been well documented in amphibian larvae (Glennemeier and Denver 2002; Crespi and Denver 2004; Rot-Nikcevic et al. 2005; Davis and Maerz 2009; Davis 2012), and is directly linked to reduced foraging (Crespi and Denver 2004) and size at metamorphosis (Davis and Maerz 2009). Davis (2012) observed a strong effect of intraspecific density on the stress hormones of larval spotted salamanders (*A. maculatum*). However, there was no effect of food additions on stress hormones, indicating in that study that stress rather than resource limitation affected metamorph traits.

Additionally, it seems unlikely that aggressive interactions alone could account for the patterns we observed. While larvae of *Ambystoma* can be agonistic in their interactions, levels of aggression vary greatly between species (Walls and Jaeger 1987; Mott and Maret 2011). Cannibalism does occur among larvae of ringed salamanders (Nyman et al. 1993). If cannibalism happened in our experiment, the high survival we observed indicates that it occurred infrequently. Furthermore, we did not observe any limb, body or tail injuries suggestive of overt aggression when salamanders were recaptured at metamorphosis. However, previous studies have found that the perception of density, rather than direct aggression, may be sufficient to alter behavior. When high intraspecific density was mimicked through exposure to clay models of wood frog (*Rana sylvatica*) larvae, Rot-Nikcevic et al. (2005) found individuals were smaller and had elevated stress hormones at metamorphosis. In low density treatments, encounters between larvae by chance should occur less frequently than in high density mesocosms.

This would correspond to fewer stress responses interrupting activities such as foraging. Following an encounter with another larva, stress hormones do not immediately decrease, but rather do so over time. In medium and high density treatments subsequent encounters may occur before normal behavior is resumed, resulting in the diminishing difference between treatments as density increases. Experiments quantifying encounter rates in different densities and stress hormones corresponding to these encounters are required to test this hypothesis.

We found no effect of initial larval density on survival to metamorphosis. This supports our speculation that resource levels were not limiting, at least not to degree of causing starvation. Additional experiments with greater densities would be required to test this. Alternatively, the uniformly high survival to metamorphosis may indicate non-sequential density dependence, whereby the effects of high larval density are not experienced immediately (i.e. mortality), but carried over as costs into the juvenile stage through smaller body size at metamorphosis. Such non-sequential density dependence through decreased fitness in later life history stages is characteristic of species that breed in variable environments (Berven and Gill 1983; Smith 1987; Semlitsch et al. 1988).

The non-additive response of larval ringed salamanders to initial intraspecific density presents evidence for an alternate hypothesis to additive density dependence, self-thinning (Semlitsch and Caldwell 1982), or an Allee effect in larval amphibians (Wilbur 1977; Smith-Gill and Gill 1978). As suggested by Semlitsch and Caldwell (1982), the different phenotypic patterns observed in response to high natal density may be due to alternative evolutionary pathways: dispersal ability and tolerance (Gill 1978). Gill (1978) suggested that in situations where the environment is deteriorating and unlikely to

improve, there will be selection for rapid growth and a short natal period (i.e. dispersal ability). Semlitsch and Caldwell (1982) observed this strategy in spadefoot toads (*Scaphiopus holbrooki*). Spadefoot toads are explosive breeders that utilize ephemeral wetlands. As the habitat this species uses is unlikely to improve (e.g. longer hydroperiod), selection favors rapid larval growth. While species with short larval periods, such as spadefoot toads, are predicted to undergo selection for dispersal ability, Gill (1978) predicted species with longer larval periods may evolve to be more tolerant of higher densities, and thus selection would increase efficiency of resources utilization. We would expect ringed salamander to fall under the tolerance model. Ringed salamanders breed in the fall and over winter in relatively permanent ponds before metamorphosing in the late spring – early summer. Cold temperatures during the early larval period would prevent individuals from attaining a minimum size or surviving dispersal if they metamorphosed early (i.e. low selection for dispersal ability). Thus, we expect selection to favor improved resource utilization efficiency (i.e. tolerance) in ringed salamanders. This evolutionary pathway would account for the diminishing effect of increased natal density; while individuals at low densities grow faster than those at high densities, the effect of increased density may be dampened by selection for efficiency of resource use, resulting in non-additive density dependence.

The effects of early developmental environment on juvenile and adult phenotype have far reaching individual survival and fecundity consequences. By elucidating the non-additive relationship between initial intraspecific density and individual traits, this study may aid future models in using empirical data rather than expert opinion (Cosentino et al. 2011; Richardson 2012). The equations presented in this study can aid in

simulating the characteristics of salamanders metamorphosing from ponds which differ in intraspecific density. Resulting phenotypic differences could be used to select survival probabilities, movement behavior, age of first reproduction, or fecundity (Scott 1994; Chelgren et al. 2006). Additionally, models of discrete population dynamics are sensitive to the functional form of density dependence. For example, population models with an exponential decline in survival as a function of density have larger regions of stability than models based on linear declines (May and Oster 1976; Bellows 1981). To demonstrate the sensitivity of population models to the functional form of density dependence, we simulated population dynamics using different equations for the shape of density dependence. In this model, body size was a function of the population size and the probability of reproduction and fecundity were a function of body size. The functional form of the density dependent size model was parameterized with a linear, exponential, Ricker, or Shephard equation (from Table 1; for more details see Appendix A). Both the stable population size and amplitude of population oscillations were sensitive to functional form of density dependence (Figure 3). While this was a very simple modeling exercise, it highlights the importance of accounting for nonlinearities in density dependence.

In conclusion, our study suggests that non-additive density dependence can occur among larval amphibians. By testing four-times more densities than previous studies, we were able to better fit nonlinearities in the response of salamanders to density dependence. We suggest the proximate mechanism for nonlinearities are stress-mediated behaviors and the ultimate mechanism is tolerance to high density conditions through selection on resource utilization. Experiments are required to test these proposed

mechanisms. Intraspecific density affected juvenile phenotype in a nonlinear manner, with the strength of effects decreasing as initial density increased. By modeling the effects of density on phenotype, our experiment contributes to a larger body of work focused on how organisms adapt to negative density dependence. Such studies are important for developing population models that can inform theory and ultimately predict dynamics in the face of anthropogenic change.

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Table 1. Models with parameter estimates (± 1 SE) and adjusted r^2 values. Models are ordered by their goodness of fit. Parameters (a , b , c) were used in equations as defined in the methods. Parameters for the Ricker models suggested Allee effects, rather than self-thinning. However, in all cases, these models fit the data poorly.

Hypothesis	Model	a	b	c	Adjusted r^2
<i>Length of larval period (days)</i>					
Non-additive	Shepherd	161.554 ± 12.621	-0.132 ± 0.229	0.933 ± 0.021	0.72
Additive	Linear	184.803 ± 1.313	0.634 ± 0.058		0.69
Non-additive	Exponential	185.213 ± 1.268	-0.003 ± 0.0003		0.69
Allee effects	Ricker	31.662 ± 1.561	0.052 ± 0.002		0
<i>Body size (SVL) at metamorphosis</i>					
Non-additive	Shepherd	52.395 ± 6.002	-0.390 ± 0.407	1.105 ± 0.032	0.83
Non-additive	Exponential	46.503 ± 0.587	0.008 ± 0.001		0.77
Additive	Linear	45.973 ± 0.546	-0.305 ± 0.024		0.75
Allee effects	Ricker	8.523 ± 0.502	0.067 ± 0.003		< 0
<i>Body condition at metamorphosis</i>					
Non-additive	Shepherd	0.006 ± 0.062	-6.562 ± 39.750	1.000 ± 3.155	0.11
Non-additive	Exponential	8.188 ± 117.996	0.811 ± 2.397		0.09
Allee effects	Ricker	-0.001 ± 0.005	0.051 ± 0.231		0.004
Additive	Linear	0.009 ± 0.025	0.000 ± 0.001		0.003
<i>Survival to metamorphosis</i>					
Additive	Linear	0.973 ± 0.050	-0.003 ± 0.002		0.07
Non-additive	Shepherd	0.545 ± 0.334	-1.603 ± 0.681	0.880 ± 0.160	0.07
Non-additive	Exponential	0.975 ± 0.052	0.003 ± 0.002		0.03
Allee effects	Ricker	0.170 ± 0.015	0.060 ± 0.0034		< 0
<i>Percent crude lipids at metamorphosis</i>					
Non-additive	Shepherd	8.805 ± 6.053	-3.399 ± 0.628	1.027 ± 0.181	0.63
Non-additive	Exponential	13.805 ± 0.702	0.015 ± 0.002		0.55
Additive	Linear	13.219 ± 0.571	-0.149 ± 0.025		0.53
Allee effects	Ricker	2.220 ± 0.176	0.070 ± 0.004		0.04

Figure 1. The functional form, models, and parameter space that would support each hypothesis. Responses include survival, size at metamorphosis, body condition, or percent crude lipids. For the response of length of larval period, we would expect the each curve to be reflected over the x-axis (e.g. additive density dependence would exhibit a positive slope and non-additive would be supported by an increasing function at a decelerating rate rather than a decreasing function). A linear model is used to test for no density dependence and additive density dependence. A non-additive response to natal density is tested with a Shepherd function and exponential model. A Ricker equation is used to test for self-thinning and Allee effects.

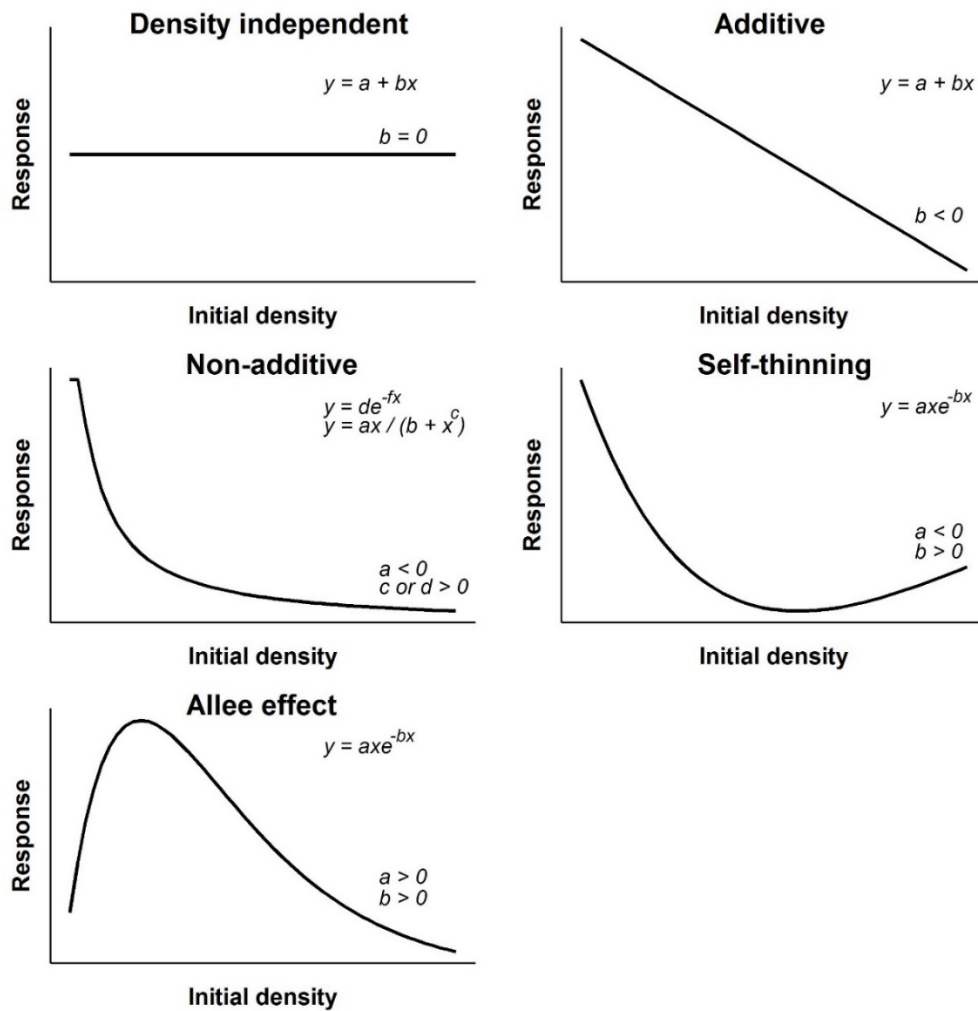


Figure 2. Predicted model values (solid black line) with 95% prediction intervals (dashed lines) for initial larval density and (a) days to metamorphosis (N = 55), (b) body size (SVL; N = 55), (c) body condition (N = 55), (d) survival to metamorphosis (N = 55), and (e) percent crude fat (N = 30) as a function of initial larval density. A Shepherd equation was used to fit all responses except for survival, which was fit with a linear model. Dots represent mesocosm means

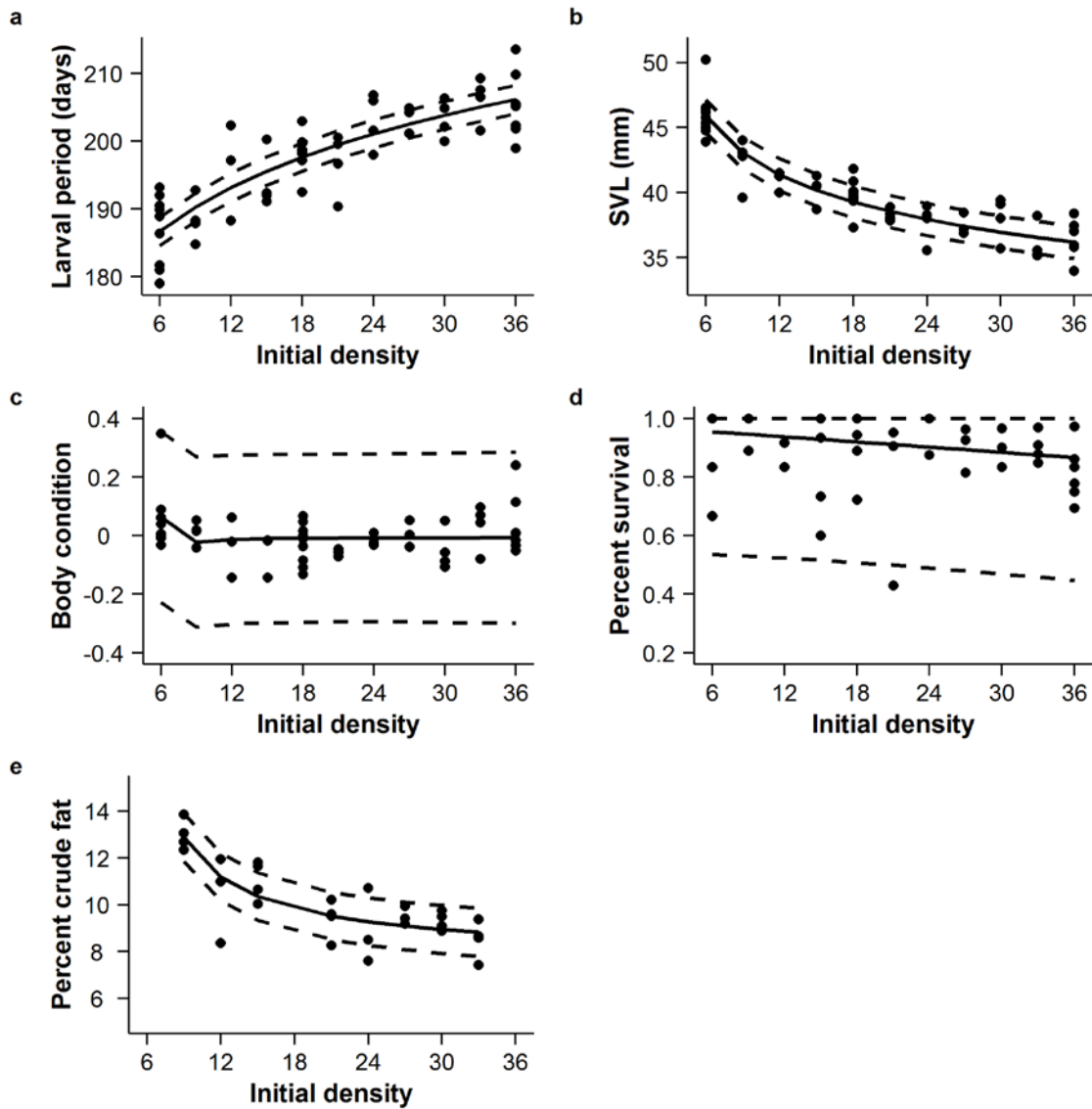
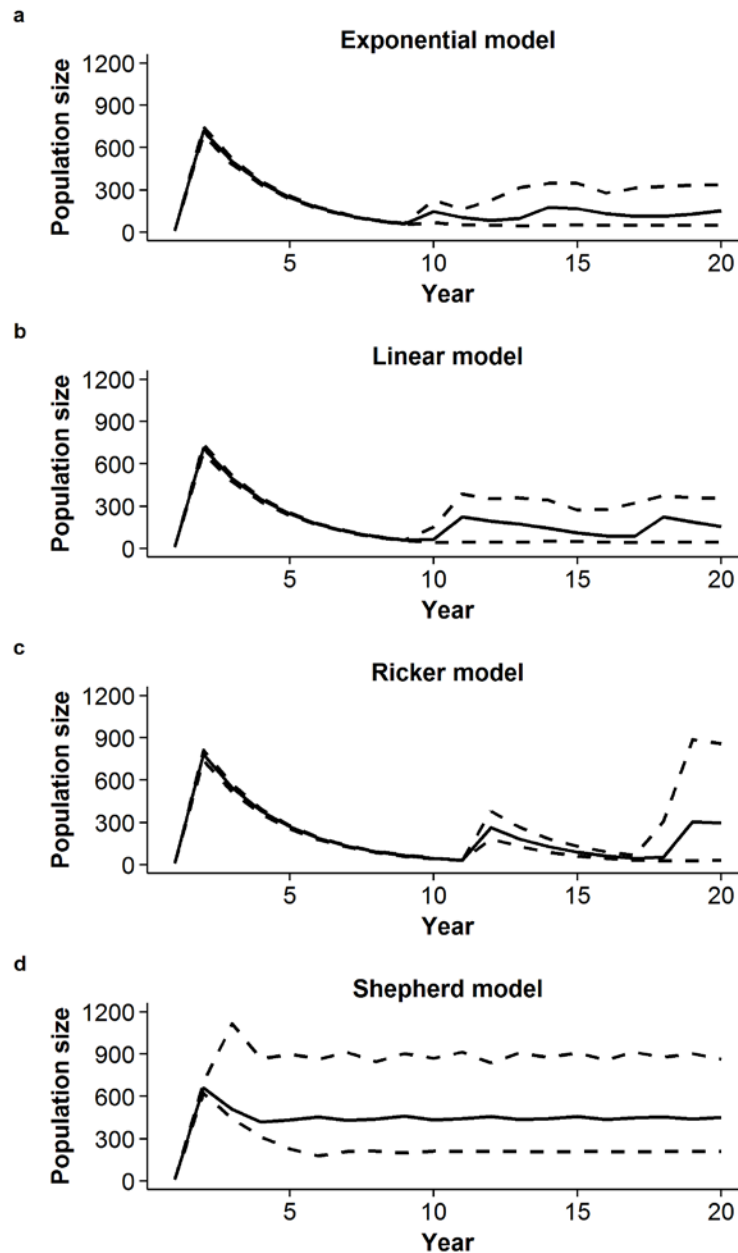


Figure 3. Predicted dynamics of a population over 20 years differed based on assumptions of the functional form of density dependent size. Solid lines represents a mean population estimate from the bootstrap analyses parameterized with an (a) exponential, (b) linear, (c) Ricker, or (d) Shepherd equation. The 95% confidence interval for each parameterization is represented with a dashed line. Population estimates are based on 10,000 bootstrapped simulations



CHAPTER 3

ASYMMETRIC EFFECTS OF PHENOTYPE- AND CONDITION-DEPENDENT FACTORS ON DISPERSAL IN A POND-BREEDING SALAMANDER

Abstract

Long distance dispersal movements are rare in nature, yet those few individuals that successfully arrive at a new population have a disproportionately large effect on ecological and evolutionary processes. Individuals that disperse to a new population can differ from non-dispersing residents behaviorally, morphologically, and in their response to external conditions such as population density. However, the effects of such phenotype- and condition-dependent factors on dispersal remain poorly tested in amphibians, a taxon that exerts a strong effect on the flow of biomass between aquatic and forest ecosystems. We conducted a field experiment to assess whether three phenotype-dependent factors (exploration, body size/condition, and morphology) or a condition-dependent factor (natal population density) affected dispersal in a pond-breeding salamander (ringed salamander, *Ambystoma annulatum*). We manipulated conditions experienced by juveniles by altering the larval density in pond mesocosms. After individuals metamorphosed, we assayed their exploration in a novel field test in the laboratory and measured three body components that could affect dispersal (limbs, trunk and tail). We also assessed the repeatability of exploration behavior over the three-month period when juvenile salamanders make initial dispersal movements. We then individually marked salamanders and tracked their movement in field enclosures as a proxy for dispersal. We found that exploration was repeatable over the three-month

period when juvenile salamanders make initial dispersal movements. Morphology and natal population density, but not exploration, had an effect on the distance moved by individuals in the field. These findings support the hypothesis that amphibian dispersal occurs over a series of sustained movements rather than in a single burst. This study also indicates that both phenotype- (morphology) and condition-dependent factors (natal population density) play an important role in modulating movement behavior, and ultimately, dispersal.

Introduction

Natal dispersal (hereafter dispersal) is the permanent movement by an individual from its natal population to a new breeding population (Semlitsch 2008). Dispersal is rare, with most individuals staying within their natal site (hereafter residents). The frequency with which individuals disperse to a new population or settle near natal sites has strong consequences for individual survival and fitness, population demography and persistence, metapopulation dynamics, and the distribution and abundance of species (Hanski 1999; Clobert et al. 2001; Bowler and Benton 2005; Templeton et al. 2011). Ultimately, dispersal drives gene flow, and can impede or facilitate adaptation and speciation (Clobert et al. 2001; Bowler and Benton 2005). Despite the importance of dispersal to ecology and evolution, there are significant gaps in the understanding of dispersal processes (e.g., Ronce 2007; Lowe and McPeck 2012; Pittman et al. 2014). While genetic approaches have provided descriptions of the broad patterns of dispersal based on gene flow and the distribution of genotypes across the landscape, there has been less study of underlying mechanisms of movement and factors determining whether an individual

disperses or remains a resident, as well as the associated fitness consequences (Clobert et al. 2009).

Evolutionary models and empirical studies indicate that dispersal does not involve a random sample of individuals (Clobert et al. 2009; Edelaar and Bolnick 2012; Lowe and McPeck 2014). Rather, individuals that disperse differ from residents in phenotype- and condition-dependent factors. Phenotype-dependent factors are determined by the individual's internal state, and can include physiology, morphology, behavior, and life-history traits, whereas condition-dependent factors are external cues such as conspecific density and habitat quality (Figure 1; Bowler and Benton 2005; Clobert et al. 2009; Edelaar and Bolnick 2012). However, these differences between dispersers and residents are often implicitly ignored despite their importance for developing ecological and evolutionary theories, understanding the demographic consequences of dispersal to population dynamics, and determining the flow of nutrients between ecosystems.

In this study, we assessed whether phenotype- or condition dependent factors affected movement in a pond-breeding salamander. Specifically, we tested for effects of three phenotype-dependent factors (morphology, exploration behavior, and body size/condition) and one condition dependent factor (natal intraspecific density) on movement (Figure 1). Although these questions have been examined extensively in invertebrates, the generality of these findings remain unknown, as the relative contributions of phenotype- and condition-dependent factors have received little attention in vertebrates, and particularly those with complex life histories. Additionally, pond-breeding amphibians represent a large export of nutrients and energy from aquatic to terrestrial ecosystems (Gibbons et al. 2006; Capps et al. 2015). By determining how far

amphibians disperse and what factors affect dispersal distance, we can better model where these nutrients are ultimately deposited.

External morphology is a readily observed phenotype-dependent factor that can contribute to differences in dispersal between individuals. For example, in many insects, individuals with a high propensity for long-distance dispersal will have longer wings, whereas non-dispersers will have absent or small wings (Harrison 1980). Some plants exploit similar dispersal polymorphisms, producing both light seeds with pappus-structures for long-distance wind-dispersal and heavy seeds for short-distance dispersal (Cheptou et al. 2008). Although distinct dispersal morphs can be found in plants and invertebrates, such distinct polymorphisms for dispersal do not occur among vertebrates. However, continuously varying morphology can have an effect on dispersal (Lowe and McPeck 2012 but also see Dingemanse et al. 2003; Videlier et al. 2014).

Exploration is an individual's behavioral response to a novel situation (Reale et al. 2007), and it is predicted to be positively correlated to dispersal (Reale et al. 2007 but see Selonen and Hanski 2006). Animals that move more in a new habitat (i.e., explore more) are expected to be more likely to move to a new breeding site, rather than remaining within familiar habitat and settling adjacent to their natal site. Because exploration also exposes individuals to predation and potentially hostile environments, it is likely under strong selection (Smith and Blumstein 2008). While this positive relationship between exploration and dispersal has been observed in natural populations of Great tits (*Parus major*, Dingemanse et al. 2003), Trinidad killifish (*Rivulus hartii*, Fraser et al. 2001), to our knowledge, a relationship between exploration and movement in the field has not been tested in amphibians.

Whereas morphology and exploration are both phenotype-dependent factors, population density can affect dispersal as a phenotype- or condition-dependent factor. Individuals from high density habitats are more likely to have a reduced body size and body condition (Herrando-Perez et al. 2012; Ousterhout and Semlitsch 2016), and in many systems, these smaller individuals are competitively inferior. Thus, when competition for resources is high, small individuals may be more likely to disperse farther, to reach patches with lower density where they can acquire resources. However, there is also empirical evidence for dispersal distance to correspond to large body size (Bowler and Benton 2005; Clobert et al. 2009). In addition to having a competitive advantage, large individuals also consistently perform better in locomotor studies, moving farther, faster, and having greater stamina than smaller individuals (Goater et al. 1993; Beck and Congdon 2000). For example, naked mole rats (*Heterocephalus glaber*) that dispersed had greater energy reserves, and therefore greater body condition, than residents (O’Riain et al. 1996). Population density can also be a condition-dependent factor. There is widespread evidence among invertebrates, birds, mammals, and reptiles that departure from natal sites increases with intraspecific density (Lambin et al. 2001; Bowler and Benton 2005; Ronce and Clobert 2012). In wing-dimorphic insects, wing size, and thereby dispersal propensity, is most strongly affected by population density (Denno et al. 1991).

We tested the effects of phenotype- and condition-dependent factors on salamander dispersal using movement in a field experiment as a proxy. We reared juvenile salamanders from hatchlings in pond mesocosms that differed in intraspecific density to generate animals that differed in body size/condition (Ousterhout and

Semlitsch 2016) and a condition-dependent factor. After individuals metamorphosed, we assayed their exploration in a novel field test in the laboratory and measured three body components that could affect dispersal (limbs, trunk and tail). We also assessed the repeatability of exploration behavior over the three-month period when juvenile salamanders make initial dispersal movements. We then individually marked salamanders with Passive Integrated Transponder (PIT) tags and tracked their movement in field enclosures for one week. We predicted that there would be a positive relationship between initial dispersal distance and body size/condition, natal density, exploration in the laboratory, and limb length.

Materials and Methods

Study Species

We conducted all assays with a pond-breeding amphibian, the ringed salamander (*Ambystoma annulatum* Cope 1886). The ringed salamander is endemic to the Ozark Plateau and Ouachita Mountains of Missouri, Arkansas, and Oklahoma. Adults lay eggs in ponds during the fall (late August – early October), aquatic larvae overwinter in ponds, and then metamorphose in the late spring and early summer (early May – early July; Hocking et al. 2008). As is generally the case for pond-breeding amphibians, the aquatic larval stage is well studied, but little is known regarding individual behavior from the time juveniles enter the terrestrial environment until they return as adults during breeding migrations 2-3 years later (Semlitsch 2008; Pittman et al. 2014). During this interval, juveniles will encounter novel, unfamiliar habitat and must seek out suitable refuges, namely small mammal burrows in hardwood and mixed hardwood-pine forests (Osborn

et al. 2014). The frequency of dispersal remains poorly resolved for *Ambystoma*, but it is estimated that 9-22% of juveniles in a cohort will disperse to a new breeding site (Trenham et al. 2001; Gamble et al. 2007). Genetic dispersal distance is estimated to be 1693 m for ringed salamanders (Peterman et al. 2015).

Animal Rearing

We reared salamanders from hatchlings in experimental pond mesocosms (1000 L volume, 1.52 m diameter polyethylene cattle tanks) in a fenced research facility at the University of Missouri, Columbia, Missouri. We established mesocosms as in similar studies (Ousterhout and Semlitsch 2016). We filled each mesocosm with tap water to a depth of 50 cm (see Table 1 for dates). After allowing the mesocosms to dechlorinate for at least 5 d, we added 1 kg of air-dried leaves (primarily *Quercus* spp. and *Acer* spp.; Table 1) to each mesocosm. We inoculated each mesocosm with aliquots of zooplankton to establish natural plankton and periphyton communities (Table 1). We left mesocosms uncovered throughout the duration of the larval period (September 2012 – July 2013) to allow colonization by flying insects (e.g., dipterans) as an additional food source for larvae.

We collected embryos at similar developmental stages from several partial egg masses in two ponds at Fort Leonard Wood, Pulaski County, Missouri. After embryos hatched, we mixed hatchlings from different masses and randomly assigned animals to mesocosms (Table 1). To generate juvenile salamanders with different body sizes, we randomly assigned a natal density treatment to each mesocosm (low: 6 larvae/mesocosm [N = 32 mesocosms]; medium-low: 12 larvae/mesocosm [N = 14]; medium: 18

larvae/mesocosm [N = 12]; high: 36 larvae/mesocosm [N = 14]). We increased replication of low-density mesocosms to generate sufficient juveniles for all experiments. During the last third of the larval period, we searched mesocosms for metamorphosed animals every other night after sunset (Table 1). We considered an individual to have metamorphosed if its gills were less than 2 mm in length and tailfin was reabsorbed (Mott and Maret 2011; Ousterhout et al. 2014). At metamorphosis, we recorded date of metamorphosis, wet mass (± 0.01 g, Mettler AT-100 electronic balance, Mettler Toledo, Columbus, Ohio, USA) and snout-vent length (SVL; ± 1 mm). We housed salamanders individually in plastic containers with moist sphagnum moss substrate and fed them small mealworms (*Tenebrio molitor*) and fruit flies (*Drosophila melanogaster*) *ad libitum*. The animal care facility was maintained at 25 - 28 °C and a 12:12 h light:dark schedule.

Repeatability of Exploration Behavior

To test if exploration was consistent, we measured the exploration behavior of 50 juvenile ringed salamanders during 10 repeated trials over a three-month period (4 June – 9 September 2013; Figure 2). For this experiment we used ringed salamanders from the 12 larvae/mesocosm (N = 32 juveniles) and 36 larvae/mesocosm natal density treatments (N = 16 juveniles). We conducted all trials at night (2000 – 2400 h), the active period for juvenile salamanders, in a dark laboratory to encourage natural behavior. Exploration was assayed in circular plastic arenas (8 cm deep x 30.48 cm diameter) with a moist paper towel substrate. A baffle of clear tape lined the top edge of each arena to prevent salamanders from climbing out. We placed blinds between arenas so that salamanders could not visually interact with other animals. After placing one juvenile in each arena,

we allowed 10 min for acclimation and then video recorded behavior for 30 min using an infrared camera (IRB420 Infrared Bullet Camera, Optiview, Jacksonville, Florida, USA) mounted above the arenas. Observers used red light to watch salamanders and prevented any escape attempts.

Statistical Analysis—We calculated total distance moved, time spent moving, and latency to move from the video recordings using Ethovision XT9 (Noldus Infomation Technology 2012). We sampled the video at a rate of 3 sec⁻¹ and considered an animal to have moved if the distance between samples was > 1 cm (distance moved) or velocity exceeded 10⁻⁷ m sec⁻¹ (time moving and latency to move). While we were initially interested in all three responses, time spent moving and distance moved were strongly positively correlated (Spearman's Rank Correlation: $r_s = 0.93$, $P < 0.001$). We analyzed total distance moved as it is most closely related to dispersal distance, and did not consider time spent moving.

To determine if repeatability was dependent on the temporal scale of the experiment, we built different models to examine each response over three time scales (Figure 2): short-term (N = 2 measurements), medium-term (N = 4), and long term (N = 10). We independently tested two responses, distance moved and latency to move (time until first movement), over each of these three time scales by constructing six generalized linear mixed models (GLMM). We only analyzed ringed salamanders that had complete time series (short term: N = 50; medium term: N = 49; long term: N = 42), so as to not systematically underestimate repeatability (Bell et al. 2009). We built a null intercept model with a random effect of individual ID for each response by time period combination. Additionally, we built a full GLMM that contained as covariates date of

trial (continuous), natal density (medium-low or high density), body condition, treatment by date of trial interaction and treatment by body condition interaction for each response by time period combination. We calculated body condition as size-independent mass. We regressed mean scaled mass against SVL, and used the residuals to represent relative body condition. We did not include SVL in the full GLMM, because it was collinear with natal density (Pearson's correlation coefficient $r = -0.81$) and prevented model convergence. We scaled and mean-centered date of trial and body condition prior to analyses. To account for non-independence of individuals due to multiple tests over time, we also included a random effect of individual identity.

We constructed the GLMMs using JAGS (Plummer 2003) through R (package *R2jags*, R version 3.1.3; R Core Team 2012; Su & Yajima 2014), because the Bayesian framework is more tractable for the analysis of models with complex error structures and unbalanced datasets (Hilbe 2007). We assumed uninformative (flat) priors for all parameters using a uniform distribution. Posterior summaries were based on three Markov chains run for 300,000 iterations and thinned at a rate of 100 iterations after a 50,000 iteration burn-in, resulting in approximately 2,500 samples of the posterior distribution for each estimated parameter. We verified convergence with a Brooks-Gelman-Rubin statistic ($\hat{R} < 1.1$ indicates satisfactory convergence) and performed posterior predictive checks (Bayesian P -value) to assess the adequacy of model fit (0.51 - 0.56; Kery 2010). To determine if individual behavior was repeatable over multiple trials, we derived the Intraclass Correlation Coefficient (ICC) from the posterior distribution. We calculated ICC as the proportion of the total variation that was attributable to between individual differences (Nakagawa and Schielzeth 2010). We

report ICC without covariates as a conservative estimate of repeatability (intercept model in Table 2), but will focus our results and discussion on the models that included covariates. We used the standard deviation as a measure of dispersion unless otherwise indicated, and estimates are presented as the posterior mean with its 2.5 and 97.5 % credible interval (CI).

Exploration Measurements

We measured the exploration of juvenile salamanders ($N = 182$) from 22 May – 12 June 2013. For this experiment we used juveniles from the low ($N = 71$), medium ($N = 73$), and high ($N = 38$) natal density treatments. Assays were conducted as in the repeatability assay, with the exception that we recorded each salamander on one night, as exploration behavior was repeatable.

Morphological Measurements

We took dorsal photographs of all juveniles when they were under anesthesia (see marking methods below). Animals were placed on their ventral side with arms and legs extending out from the body and bending at a 45° angle and the elbow/knee joint. We measured three body components that could affect dispersal: trunk, limbs, and tail (Figure 3). We expected trunk shape to affect undulatory walking performance (Barclay 1946). We defined the trunk as the area from the anterior insertion of the forelimbs to the posterior insertion of the hind limbs. We expected limb length to affect stride length, and thus walking performance (Bennett et al. 1989). We measured limbs as the length of the humerus and radius/ulna or femur and tibia/fibula, respectively, from the insertion point

of the limb to the second joint. We analyzed tail shape as a predictor of energy reserves (Wells 2007). We defined the tail as the posterior insertion of the hind limbs to the posterior tip of the tail. We used ImageJ (Rasband 2013) to measure trunk length, maximum trunk width, tail length, maximum tail width, forelimb length, and hind limb length. We measured SVL as the distance from the anterior end of the head to the posterior insertion of the hind limbs.

We quantified morphological variation in trunk, limbs, and tail by using Principal Component Analysis (PCA) to generate size-adjusted shape variables (Adams and Beachy 2001; Lowe and McPeck 2012). Each covariance matrix included log-transformed SVL, and two measurements specific to a body component (trunk length and maximum width, forelimb and hind limb length, and tail length and maximum width). We extracted three principal components from the three covariance matrices representing the three body components (trunk, limbs, and tail). The first principal components (PC1) were expected to represent body size variation, because SVL was correlated with all morphological measurements. The second and third principal components (PC2 and PC3) are body-size independent shape variation, and these were used in future analyses (Table 3 and Table 4). This analysis used the ‘prcomp’ function in R version 3.1.3 (R Core Team 2015).

Movement Assays

We individually marked juveniles using Passive Integrated Transponder (PIT) tags as in Ousterhout and Semlitsch (2014). PIT tags allow otherwise prohibitively small animals to be tracked over sustained periods of time from a limited distance (< 30 cm underground;

Connette and Semlitsch 2012; Ousterhout and Semlitsch 2014). Briefly, we anesthetized each juvenile by immersing it in a buffered 1% solution of tricaine methanesulfonate (MS-222) until it failed to right itself or respond to toe pinching. After rinsing the salamander in spring water, we assigned it a PIT tag size based on weight and trunk length: 12 mm PIT tag (12 mm x 2.12 mm, 0.115 g, full duplex, HPT12; Biomark, Boise, ID, USA): 3.48 g – 1.49 g; 9 mm tag (9x2.12 mm, 0.08 g, full duplex, HPT9, Biomark): 2.65 g – 1.01 g; 8 mm tag (8.5x1.4 mm, 0.033 g, full duplex, HPT8, Biomark): < 1.00 g.

We released marked salamanders into replicated experimental enclosures at Fort Leonard Wood, Pulaski County, Missouri. Previous studies have effectively used long, rectangular enclosures to assay amphibian movement (Rosenberg et al. 1998; Rothermel and Semlitsch 2002; Osbourn et al. 2014). Each enclosure (50 m x 2 m) was made from 90 cm tall silt fence wall that extended 15 cm underground. We created a baffle on the top of the fence by folding 10 cm of fabric down, and suspending it at a 90° angle. We constructed six enclosures at each of two sites. At each site, we built three enclosures in a closed-canopy forested habitat and three enclosures in a mixed grassland. The selected sites were level and within 100 m of known ringed salamander breeding ponds, which is well within the known utilization space of pond-breeding salamanders (Rittenhouse and Semlitsch 2007).

We released cohorts of eight to ten juvenile ringed salamanders (mean \pm 1 SD: 9.5 ± 1.5 salamanders) into the northern side of the enclosure on nights within 24 h of rain. For each cohort, we randomly assigned individuals from the same natal density treatment to an enclosure in each habitat type. Whenever possible, we concurrently released cohorts of salamanders from each density treatment into each habitat type at

both sites. After sunset (2000 h – 2215 h) all juveniles assigned to a runway were placed under a plastic shoebox at the end of an enclosure and allowed to acclimate for 30 min, after which the box was removed. We also placed a small piece of sphagnum moss from each salamander's individual container into the shoebox to provide an olfactory reference point for animals to move away from. We released 28 cohorts from 25 May– 24 June 2013 (N = 268). We searched each runway daily for 7 d post-release using a portable RFID system (FS-2001F-ISO reader and BP portable antenna, Biomark). When salamanders were detected, we marked their location along the length of the enclosure. Because the shape of the enclosure limited lateral movement, we only quantified movement in one dimension parallel to the long axis of an enclosure. After the seventh day of tracking, we attempted to recapture and remove all released animals. We recorded the location of every detected salamander, and measured the distance between subsequent detections.

Statistical Analysis

We assessed if movement distributions had fat-tailed distributions, characteristic of distributions of dispersal distances using 'kurtosis' in package *ar agricolae* (de Mendiburu 2014). We then built two generalized linear models (GLM) to test if exploration, natal density, or body condition had an effect on movement rate (m day^{-1}) or the proportion of times an individual moved ($\text{count of movements times detected}^{-1}$), respectively. The models included the distance moved during the exploration assay, natal density, and body condition as predictors. We calculated size-independent body condition as in the repeatability analysis. We used the covariates of enclosure habitat and release cohort to

account for differences in environmental conditions over space and time. We did not include SVL in the analysis, because it was collinear with natal density treatment ($r = -0.81$). We fit movement rate using a negative binomial error structure ('glm.nb' in package *MASS*; Venables and Ripley 2002), and proportion of times moved was fitted with a binomial error distribution ('glm'). We then constructed two additional GLMs to test whether movement rate (m day^{-1}) or the proportion of times an individual moved (count of movements times detected $^{-1}$) were affected by any of the size-adjusted morphology axes describing trunk, limb, or tail arm morphology (PC2 and PC3). These morphological models also included the covariates of enclosure habitat and release cohort. Initial plotting suggested a possible quadratic relationship between morphology and distance moved, so we also included quadratic terms for each PC2 and PC3. All analyses were done in R version 3.1.3 (R Core Team 2015).

Results

Within a trial, ringed salamanders moved 2.34 ± 2.92 m (mean \pm 1 SD; $N = 420$). The latency, or time until first movement, of juvenile ringed salamanders to move was 5.83 ± 4.86 min ($N = 420$). We observed considerable variation between individuals and trials. In 15% of repeatability trials an individual animal did not move ($N=64$); however, all juveniles were observed moving in at least one trial over the course of this assay. Long movements were less common; ringed salamanders moved farther than 10 m in 3% of trials ($N = 11$; maximum: 17.49 m). We similarly observed considerable variation in latency to move. Animals moved instantaneously in 4% of trials ($N = 17$). In support of our prediction that movement behavior was repeatable, the distance moved by individuals

was repeatable over all three time periods (Figure 4, Table 2, ICC: 0.25 – 0.31). Distance moved decreased with each trial over the short- and medium-term, but there was no effect of date on the distance moved over the long-term (Table 2). Latency to move was also repeatable over time, however, to a lesser degree than distance moved (Table 2, ICC: 0.18- 0.26). None of the covariates tested had an effect on latency to move (Table 2).

Ringed salamanders had a fat-tailed distribution of movement rates in field enclosures (kurtosis: 7.19; kurtosis of a normal distribution is 0). Salamanders moved at a rate of 11.37 ± 9.67 m day⁻¹ and had a $68 \pm 23\%$ probability of moving between detections (Figure 5a, b). When ringed salamanders were tracked in field enclosures, exploration was not a predictor of movement rate ($F_{1,171} = 2.37$, $P = 0.13$) or the proportion of times an individual moved ($F_{1,171} = 1.14$, $P = 0.29$). During the exploration assays, salamanders moved 1.91 ± 2.69 m ($N = 182$). Likewise, body condition did not affect movement in field enclosures (movement rate: $F_{1,171} = 2.52$, $P = 0.11$; proportion of times moved: $F_{1,171} = 1.08$, $P = 0.30$). However, we did observe a relationship between morphology and movement. Individuals with relatively longer forelimbs and shorter hind limbs moved at a greater rate (Figure 5c, limb PC2: $F_{1,167} = 6.10$, $P = 0.015$). Morphology did affect proportion of times moved. There was a marginally significant trend for natal density to affect juvenile movement rate (Figure 5d, $F_{2,171} = 2.74$, $P = 0.07$), but there was no effect on the proportion of times moved ($F_{2,171} = 0.0001$, $P = 0.99$). Salamanders reared in medium- and high-density mesocosms moved farther than those reared in the low-density tank.

Discussion

In this study, we tested whether phenotype- or condition-dependent factors affected dispersal in a juvenile pond-breeding salamander. Despite the importance of dispersal to ecological and evolutionary processes, the mechanisms of these often long-distance movements and their fitness consequences remain poorly understood for many taxa, including amphibians. We found repeatable exploration behavior during the three-month period of initial juvenile dispersal. Additionally, we saw asymmetric effects of phenotype-dependent factors (morphology) and condition-dependent factors (natal population density) on movement in the field (Figure 6).

Exploration Behavior

This study provides, to our knowledge, the first evidence of repeatable exploration in a salamander. Twenty-four to thirty-three percent of the variation among individuals in distance moved and 19-29% of variation in latency to move was attributable to individual differences. This is consistent with other studies that have examined the repeatability of behaviors. In a meta-analysis of behavioral repeatability, approximately 35% of variation among individuals in a behavior could be attributed to individual differences, and the repeatability for exploratory behavior was marginally greater than that observed in our study (approximately 40%, Bell et al. 2009). The other two studies that have tested for repeatable behavior in juvenile amphibians have also found consistent behavior. Marsh frogs (*Rana ridibunda*) had appreciably higher repeatability than that observed in this study (activity: 91%; latency: 96 %; Wilson & Krause, 2012). However, behavior was measured over 24 h, and as such the repeatability may not be a strong test of a behavior

like dispersal, which occurs over longer periods of time (Bell et al. 2009). Similarly, juvenile natterjack toads (*Bufo calamita*) had repeatable exploration over 6 week (30-63%), and there was a trend for the distance toads moved to be repeatable after one year (Maes et al. 2013). These findings mirror those in our study, and (Maes et al. 2013)(Maes et al. 2013)support the hypothesis that juvenile amphibians disperse in a series of discrete movements until they reach sexual maturity (1-3 years of age, Semlitsch 2008; Semlitsch et al. 2014).

Morphology

Limb shape affected the distance moved by salamanders. Salamanders with long forelimbs and short hind limbs move farthest. This may reflect the trade-off between terrestrial walking performance and aquatic swimming performance. Elongated limbs increase stride length, and thus walking performance (Bennett et al. 1989). However, long hind limbs increase drag during undulatory swimming, decreasing aquatic performance of near-metamorphosis larvae and breeding adults. As noted by Lowe and McPeck (2012), who observed a similar pattern between limb morphology and dispersal in the stream salamander (*Gyrinophilus porphyriticus*), elongation of only the forelimbs in recently metamorphosed juveniles may allow individuals to walk with greater efficiency without appreciable reducing swimming performance.

No Effect of Behavior

We predicted exploration to be positively correlated to dispersal (Reale et al. 2007 but see Selonen and Hanski 2006), because animals that moved more in a new habitat (i.e.,

explored more) would also be more likely to move farther from their natal site, rather than settling adjacent to their place of birth. However, we did not observe an effect of exploratory behavior in the laboratory on movement rate or proportion of times moved in field enclosures. Exploration behavior is expected to be under strong selection, because it also exposes individuals to predation and potentially hostile environments (Smith and Blumstein 2008). This is particularly true for juvenile amphibians, which experience intense predation pressure during their first night in terrestrial habitat (Pittman et al. 2013). As a result, exploration may not be a strong predictor of dispersal. Future experiments should consider using a composite score, which combines several behavioral metrics (e.g., exploration, activity, and boldness; Dingemanse et al. 2003). It is also possible that the lack of a relationship was due to the use of field enclosures, which constrain movement and bias detections towards animals that remain within enclosures. However, given the available technology for tracking small bodied (Ousterhout and Semlitsch 2014), there currently is not a better solution for collecting direct individual movement data over multiple days from juvenile pond-breeding amphibians. Because this is the first study to test for an effect of exploration behavior on movement under natural conditions in an amphibian, future research should determine whether our non-significant result is the general pattern for amphibians.

Natal Density

There was a trend for natal density to affect the movement rate of salamanders, with salamanders from higher density mesocosms moving farthest. Natal density can have a strong effect on both phenotype- and condition-dependent factors that may influence

movement behavior. For many taxa, population density has a strong effect on the number of individuals initiating dispersal (i.e. emigrating). Emigration from natal sites increased with intraspecific density in invertebrates, birds, mammals, and reptiles (Lambin et al. 2001; Bowler and Benton 2005; Ronce and Clobert 2012). We observed a similar pattern in this study, with individuals from high-density natal populations moving farther than those from low-density populations. However, because the effect of natal density was non-significant, our finding could alternatively indicate that amphibians do not use natal density as a cue to disperse. Pond-breeding salamanders are explosive breeders, and high-density conditions in the aquatic larval environment may be the norm. Density experienced immediately following metamorphosis may provide a more reliable cue for dispersal decisions. Alternatively, salamanders may use multiple redundant cues across ontogeny to make dispersal decision, as is the case for the common lizard (*Lacerta vivipara*). Dispersal in the common lizard was affected by sequential environmental conditions in the maternal habitat, prenatal habitat, and postnatal habitat (Massot et al. 2002). Future experiments that manipulate larval and juvenile density will be required to test these hypotheses.

Natal density could also influence dispersal through its effect on body size and condition. Individuals from high density habitats are more likely to have reduced body size and body condition (Herrando-Perez et al. 2012; Ousterhout and Semlitsch 2016), as was the case in this study (post-hoc ANOVA: $F_{2,179} = 100.3$, $P < 0.001$). However, the relationship between body size and dispersal is less consistent than that between density and dispersal, with larger individuals dispersing in some systems and smaller ones in others (O’Riain et al. 1996; Bowler and Benton 2005; Clobert et al. 2009). In this study,

smaller animals (i.e., reared in a medium- or high-density mesocosm) moved farther than larger animals. This may indicate that motivation to disperse has a stronger effect on individuals than ability to disperse. Larger amphibians have greater energy stores (Ousterhout and Semlitsch 2016), and this may allow them to move longer distances without foraging. Amphibians with larger bodies are also able to move farther, faster, and have higher stamina than smaller individuals (Goater et al. 1993; Beck and Congdon 2000). These larger salamanders may also be able to move under drier conditions. Water-loss is a critical issue for terrestrial amphibian survival, and smaller animals lose water at a greater rate than larger ones due to a larger surface-area-to-volume ratio (Peterman et al. 2013). In this manner, a larger body size may allow an individual to move under environmental conditions under which smaller salamanders would have to take refuge in moist microhabitat. Thus, smaller bodied amphibians are less physiologically able to disperse. However, if natal population density is an honest indicator of terrestrial population density, animals from high-density natal habitats may be more motivated to disperse than those from low-density natal populations. Alternatively, smaller animals may move farther to find unoccupied burrows. In a system where size structures competition, smaller sized salamanders may have to move farther than large sized salamanders to compete for resources.

Conclusions

We found support for repeatable exploration in a pond-breeding salamander. By examining exploration behavior over three-months, our study provided biologically-relevant support for the hypothesis that dispersal occurs in a series of movements, rather

than in a single bout immediately following emergence from the natal pond. Additionally, we found support for phenotype- and, to a lesser degree, condition-dependent factors affecting movement in ringed salamanders. Animals that moved farther differed morphologically, but not behaviorally or in body condition. Additionally, there was a trend for natal density to have a positive relationship with movement. These findings support the hypothesis that both phenotype- (morphology) and condition-dependent factors (natal population density) play an important role in moderating movement behavior and ultimately, dispersal. However, further experiments will be required to determine if natal density alone informs dispersal, or if redundant cues across ontogeny such as juvenile density affect dispersal distance

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Table 1. Experimental design for larval mesocosms. Ringed salamander mesocosms were established in two groups in the fall of 2012, because of an extended breeding season. Juveniles metamorphosed in 2013. Preliminary analyses indicated no effect of group, so it was not included in analyses (N = number of mesocosms, Inoculated = dates over which inoculum added, and Amount = total volume of inoculum added to each mesocosm, Metamorph searches = nights metamorphs were searched for).

Group	N	Filled	Leaves added	Inoculated	Amount	Larvae added	Metamorph searches
1	52	8-9 Sep	14 Sep	16 Sep - 24 Oct	2.25 L	30 Oct	15 Apr – 15 Jul
2	20	1-4 Oct	26 Oct	30 Oct - 1 Nov	4.02 L	4 Nov	15 Apr – 15 Jul

Table 2. Parameter estimates for full models of juvenile distance moved and latency to move. The 95% credible intervals of bolded values and the 90% credible intervals of italicized parameters did not overlap 0. Density = natal density, Cond = body condition, * = interaction, ICC = interclass correlation coefficient, and SD = standard deviation.

		Short term				Medium term				Long term			
		Mean	SD	2.50%	97.50%	Mean	SD	2.50%	97.50%	Mean	SD	2.50%	97.50%
Full model													
Distance	Intercept	6.71	0.48	5.68	7.67	6.14	0.37	5.41	6.85	5.93	0.25	5.38	6.39
	Density	-0.53	0.37	-1.25	0.27	-0.33	0.27	-0.83	0.20	-0.32	0.18	-0.63	0.08
	Date	<i>-0.77</i>	<i>0.47</i>	<i>-1.71</i>	<i>0.15</i>	<i>-0.44</i>	<i>0.26</i>	<i>-0.97</i>	<i>0.08</i>	0.07	0.17	-0.26	0.44
	Cond	-0.17	0.56	-1.33	0.89	0.15	0.34	-0.52	0.81	-0.07	0.30	-0.76	0.38
	Density*Cond	0.14	0.47	-0.71	1.12	-0.06	0.29	-0.63	0.51	0.15	0.34	-0.20	0.71
	Density*Date	0.38	0.35	-0.27	1.06	0.14	0.19	-0.26	0.52	-0.13	0.13	-0.39	0.11
	ICC	0.25	0.12	0.09	0.55	0.26	0.10	0.12	0.51	0.31	0.12	0.17	0.59
Latency	Intercept	5.47	0.24	4.99	5.92	5.54	0.17	5.20	5.87	5.63	0.12	5.40	5.86
	Density	0.13	0.17	-0.17	0.47	0.14	0.12	-0.08	0.38	<i>0.15</i>	<i>0.09</i>	<i>-0.03</i>	<i>0.32</i>
	Date	-0.05	0.22	-0.47	0.38	0.08	0.14	-0.19	0.36	-0.04	0.07	-0.19	0.10
	Cond	-0.18	0.26	-0.70	0.36	-0.10	0.18	-0.78	0.26	0.12	0.13	-0.13	0.42
	Density*Cond	0.18	0.21	-0.27	0.61	0.13	0.15	-0.17	0.44	-0.04	0.11	-0.29	0.15
	Density*Date	0.10	0.16	-0.22	0.41	-0.02	0.10	-0.21	0.17	0.09	0.05	-0.01	0.19
	ICC	0.18	0.11	0.05	0.48	0.21	0.09	0.07	0.42	0.26	0.07	0.14	0.40
Intercept model													
Distance	Intercept	5.99	0.15	5.70	6.29	5.74	0.11	5.52	5.96	5.52	0.10	5.33	5.71
	ICC	0.24	0.11	0.09	0.53	0.25	0.10	0.11	0.51	0.39	0.16	0.16	0.72
Latency	Intercept	5.64	0.10	5.45	5.83	5.70	0.07	5.56	5.82	5.84	0.05	5.74	5.93
	ICC	0.20	0.12	0.05	0.49	0.23	0.10	0.08	0.46	0.30	0.08	0.16	0.46

Table 3. Percent of total variance explained and eigenvectors (factor loadings) for principle components derived from morphological measurements of ringed salamanders used in the exploration assays.

Body component	Variance explained (%)	Eigenvectors		
Head		SVL	Head width	
PC1	94	-0.71	-0.71	
PC2	6	-0.71	0.71	
Trunk		SVL	Trunk length	Trunk width
PC1	88	-0.60	-0.60	-0.53
PC2	12	0.37	0.38	-0.85
PC3	>1	-0.71	0.70	0.01
Tail		SVL	Tail length	Tail width
PC1	86	-0.58	-0.60	-0.55
PC2	11	0.49	0.28	-0.83
PC3	3	-0.65	0.75	-0.13
Arm		SVL	Humerus length	Ulna/radius length
PC1	76	-0.62	-0.54	-0.57
PC2	17	-0.11	0.78	-0.62
PC3	6	0.78	-0.32	-0.54
Leg		SVL	Femur	Tibia/fibula length
PC1	76	-0.62	-0.55	-0.57
PC2	17	-0.08	0.76	-0.65
PC3	7	-0.78	0.36	0.51

Table 4. Percent of total variance explained and eigenvectors (factor loadings) for principle components derived from morphological measurements of ringed salamanders released into the field enclosures.

Body component	Variance explained (%)	Eigenvectors		
Head		SVL	Head width	
PC1	94	0.71	0.71	
PC2	6	-0.71	0.71	
Trunk		SVL	Trunk length	Trunk width
PC1	94	0.59	0.58	0.56
PC2	5	0.34	0.45	-0.82
PC3	<1	0.73	-0.68	-0.07
Tail		SVL	Tail length	Tail width
PC1	92	0.58	0.58	0.57
PC2	5	-0.44	-0.37	0.82
PC3	3	0.69	-0.73	0.04
Arm		SVL	Humerus length	Ulna/radius length
PC1	61	0.66	0.41	0.63
PC2	28	0.19	-0.90	0.38
PC3	11	-0.73	0.13	0.67
Leg		SVL	Femur	Tibia/fibula length
PC1	58	0.70	0.42	0.58
PC2	33	0.01	-0.82	0.58
PC3	9	-0.71	0.40	0.58

Figure 1. Conceptual diagram of (a) the relationship between phenotype- and condition-dependent factors and (b) traits representing these factors in our study. Lines represent one trait having an effect on another.

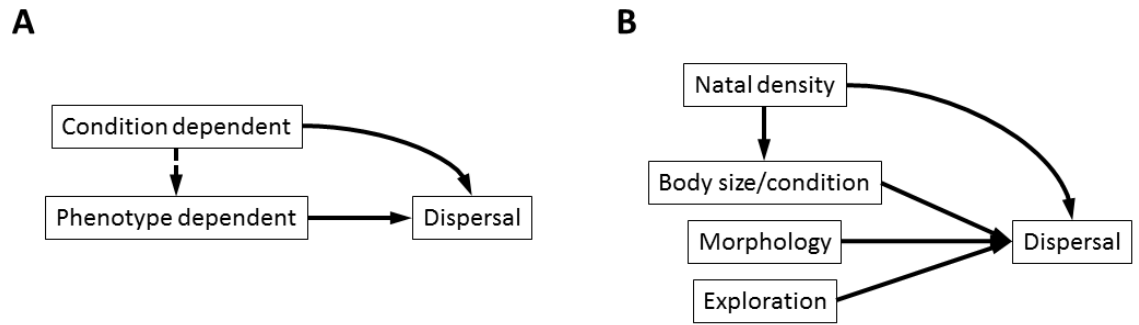


Figure 2. Timeline of behavioral observations. Numbers below the bar refer to dates.

Brackets above the timeline contain all trials included in the indicated time scale.

Timeline is not to scale.

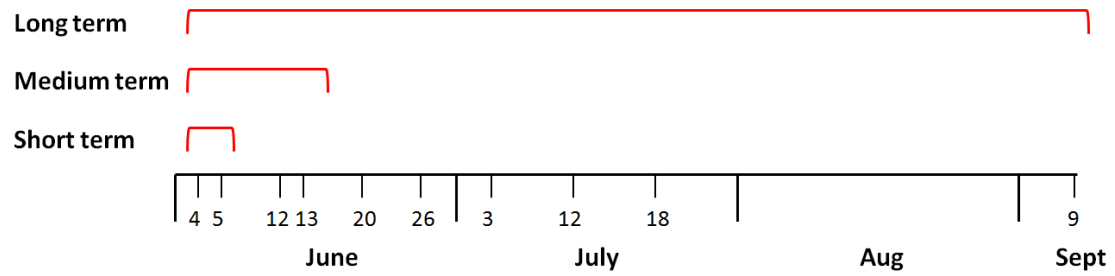


Figure 3. Diagram of trunk, limb, and tail measurements.

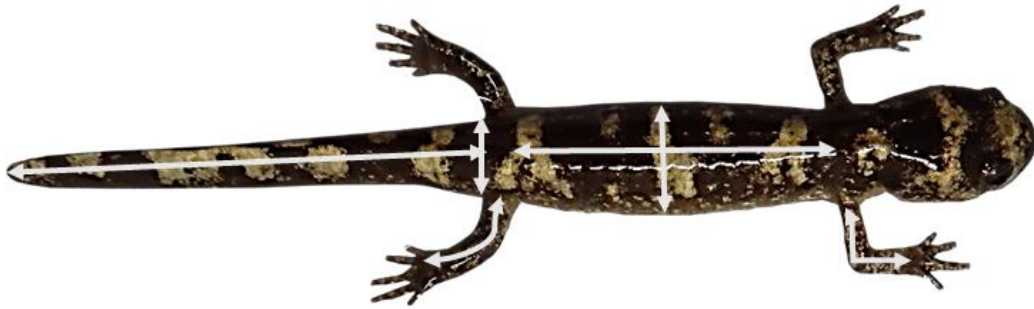


Figure 4. Distance moved by ringed salamanders from the (a) low density natal mesocosms and (b) high density natal mesocosms. If behavior was perfectly repeatable (ICC = 1), all lines would be horizontal. Dots represent individuals and lines connect the observations for an individual. X-axis are not to scale and y-axes differ between species.

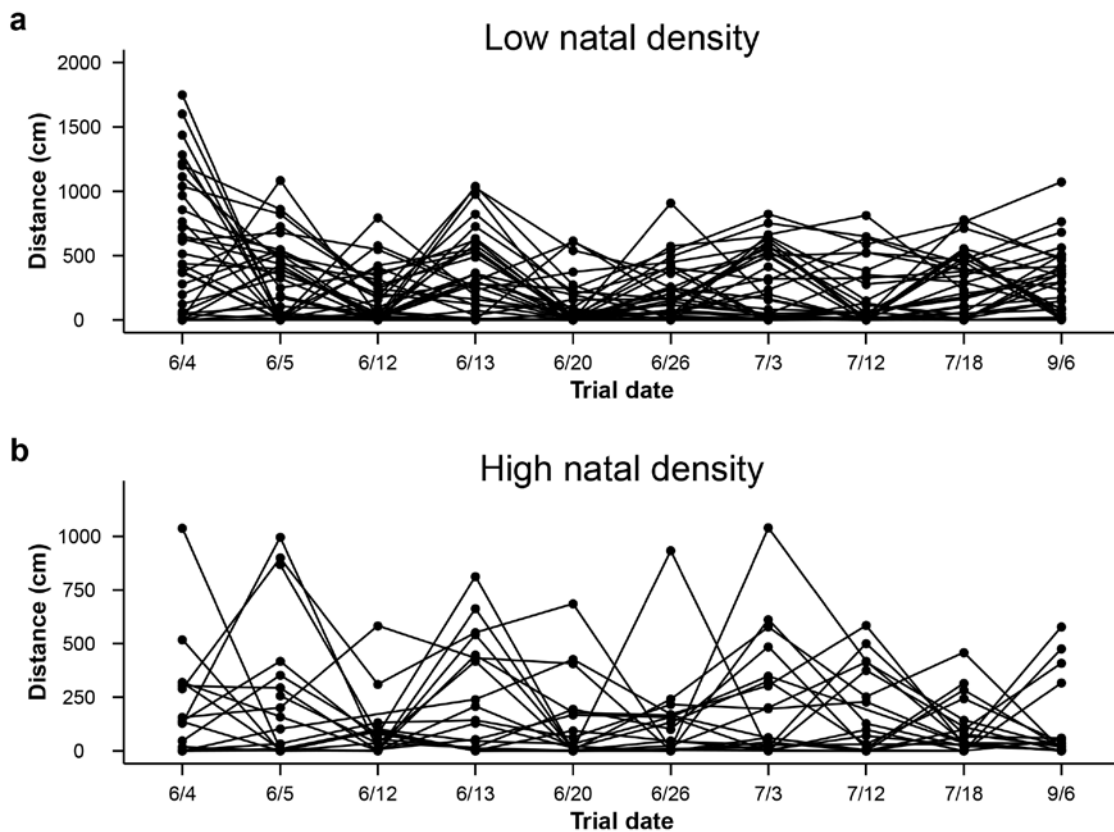


Figure 5. Histogram of the (a) movement rate and (b) probability of moving between detections (vertical line represents median) and estimated effects of (c) limb PC2 and (d) natal density treatment on movement rate ($m \cdot day^{-1}$). The effect of natal density treatment was evaluated under mean body condition and exploration, forest habitat, and cohort 1. Each dot (c) or open circle (d) represents one individual and these observations are jittered horizontally. Solid lines and filled circles are the predicted values and dashed lines and error bars are 95% confidence intervals for the effect of limb PC2 and natal density, respectively.

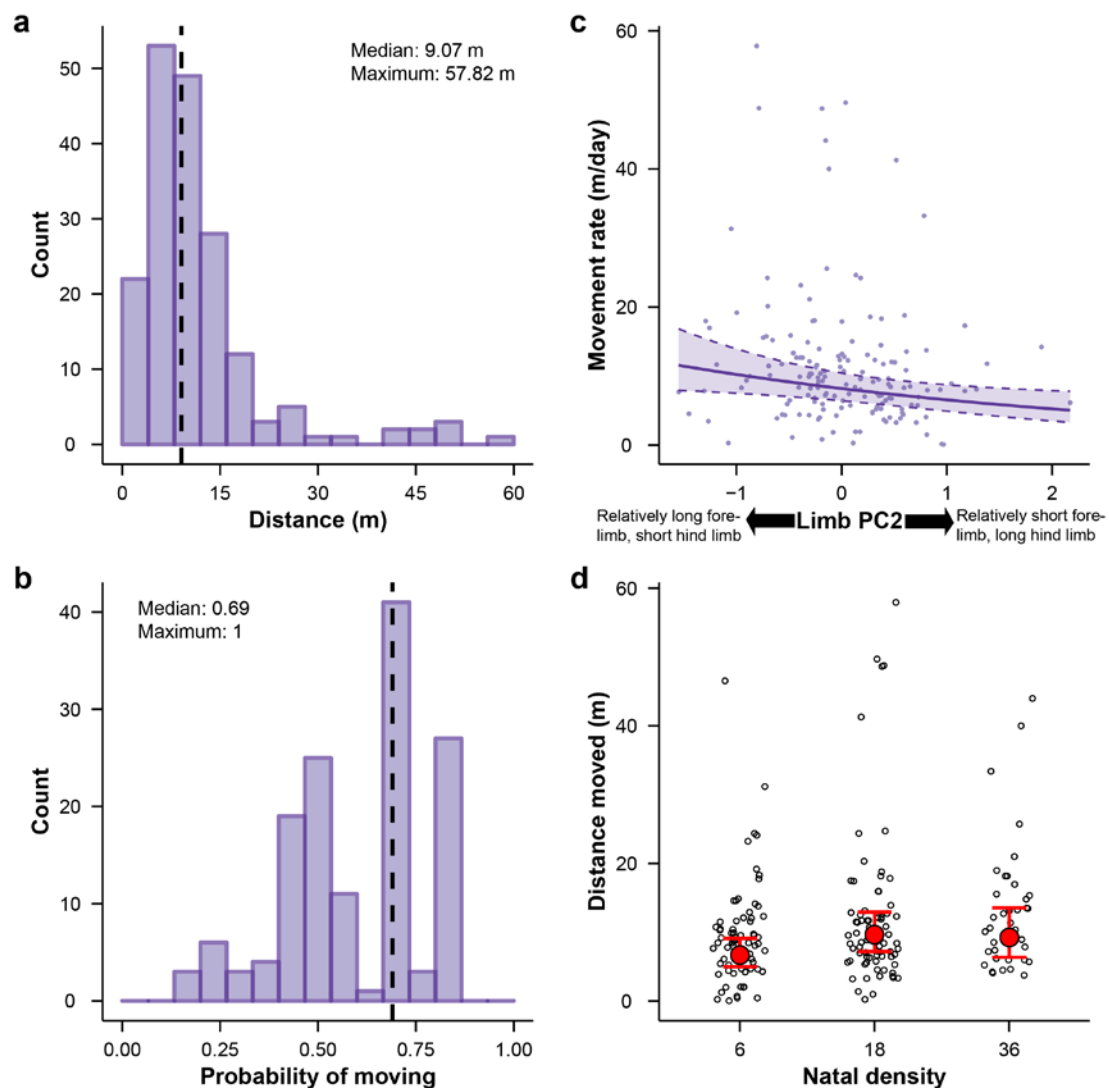
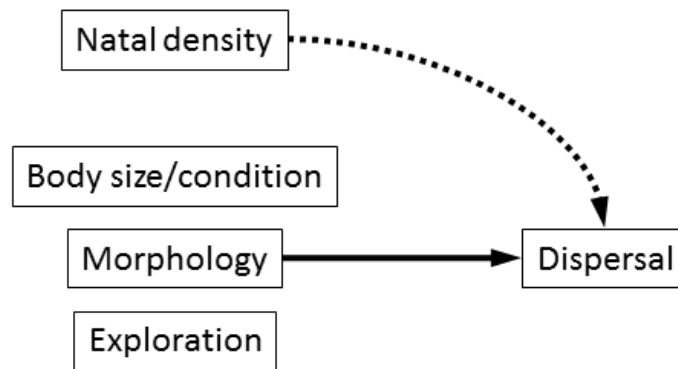


Figure 6. Conceptual diagram of findings, with solid lines representing $P < 0.05$ and dotted lines representing $P < 0.10$.



CHAPTER 4

NATAL POPULATION DENSITY AND HABITAT AFFECT DISPERSAL IN A POND-BREEDING AMPHIBIAN, THE RINGED SALAMANDER (*AMBYSTOMA ANNULATUM*)

Abstract

Dispersal is a pervasive behavior that has a large impact on many ecological and evolutionary processes. Theoretical studies of dispersal suggest that both phenotype- and condition-dependent factors should affect movement phases, including departure and transience. However, empirical tests have been largely limited to molecular approaches. Although such ultimate measures of dispersal capture general patterns, experiments are required to resolve the mechanistic underpinnings of dispersal. This lack of direct tests of factors affecting dispersal is particularly pronounced for pond-breeding amphibians. In this study, we tested whether phenotype- or condition-dependent factors affected the probability of dispersal or the distance moved during dispersal in a juvenile pond-breeding amphibian, the ringed salamander (*Ambystoma annulatum*). We reared salamanders at different natal densities to generate juveniles that differed in body size and in the population density cues that they received. After salamanders metamorphosed from ponds, we individually marked and tracked the movement of each animal in field enclosures in forest or grassland habitat. We tracked 324 juveniles and analyzed two phases of dispersal: departure, the probability of an individual leaving its natal site, and transience, the linear distance moved by an individual before selecting a habitat and settling. We examined the effects of two phenotype-dependent factors, body size and body condition, and two condition-dependent factors, natal density and habitat type, on

each dispersal phase. We found that body size had an effect on departure, whereas an interaction between natal density and habitat affected the distance moved by juveniles during transience. Our study suggests that habitat conditions, rather than ability as conferred by body size, affects departure. Additionally, the findings we present here indicate that juvenile salamanders use redundant cues over ontogeny that affect the probability of an individual dispersing. Although our findings support theoretical frameworks that indicate dispersal is influenced by both phenotype- and condition-dependent factors, this study also indicates asymmetries in the effects of these factors on different phases of dispersal.

Introduction

Dispersal, or the permanent movement by an individual from its natal population to a new breeding population (Semlitsch 2008), is a ubiquitous behavior that allows the exploitation of ephemeral resources generated by local disturbances and bet-hedging in temporally variable environments (Ronce 2007). The distance and frequency of dispersal impacts the scale at which species are affected by stochastic events such as disturbances, and thereby is a key determinant of many ecological processes, including population dynamics and persistence, connectivity within metapopulations, and the abundance and distribution of species (Clobert et al. 2001). Although the broad effects of dispersal are widely recognized, its underlying causes at the individual level remain poorly understood (Matthysen 2012).

This lack of an understanding of the mechanistic underpinnings of dispersal can largely be attributed to a paucity of empirical work (Ronce 2007; Holyoak et al. 2008).

Several authors have attempted to stimulate research efforts by proposing conceptual frameworks which decompose dispersal into distinct movement phases: departure, transience, and settlement (Bowler and Benton 2005; Clobert et al. 2009; Pittman et al. 2014). Departure, or when an individual leaves its natal site, is followed by transience, which continues until an individual selects a home range and settles (*i.e.*, settlement; Clobert et al. 2009). The timing and duration of each of these movement phases are likely key determinants of whether individuals settle within their natal population (philopatry) or continue moving until they reach a new breeding population (dispersal).

When individuals depart from their natal site and how far they move before settling are mediated by 1) individual variation in phenotype-dependent factors and 2) population level variation in condition-dependent factors. Phenotype-dependent factors are determined by the individual's internal state (e.g., physiology, morphology, behavior, and life-history traits) whereas condition-dependent factors are external cues such as conspecific density and resource availability which indicate inbreeding risk, kin competition, or habitat quality (Bowler and Benton 2005). Condition-dependent cues can be received at the time of dispersal or during previous life stages through carry-over effects (Beckerman et al. 2002; Pechenik 2006; Benard and McCauley 2008). In common lizards (*Lacerta vivipara*), pre- and post-natal intraspecific population density affects the likelihood of juvenile dispersal (Massot et al. 2002). Carry-over effects can also affect phenotype-dependent factors. For example, conditions in the natal habitat such as competition or predation can induce phenotypic plasticity in behavior or morphology. In wing dimorphic insects, natal population density has a strong carry-over effect on

whether individuals develop into a long-winged dispersal morph during later life stages (Harrison 1980; Denno et al. 1991).

Pond-breeding amphibians are an excellent system for studying the causal mechanisms of transition between initial dispersal movement phases (Pittman et al. 2014). These amphibians have a complex life cycle, and conditions in the aquatic larval stage, such as high intraspecific density, have a strong effect on juvenile phenotype (Scott 1990; Ousterhout and Semlitsch 2016), as well as the growth and survival of juveniles and adults (Semlitsch et al. 1988; Scott 1994; Relyea 2001; Altwegg and Reyer 2003; Van Allen et al. 2010). Amphibians have strong philopatry, with 78 – 95% of juveniles in a cohort returning to their natal pond to breed (Berven and Grudzien 1990; Sinsch 1997; Trenham et al. 2001; Perret et al. 2003; Gamble et al. 2007). Juvenile amphibians provide a source of subjects for experimental studies of movement behavior at smaller spatial scales than would be possible for most other vertebrates. Juvenile dispersal among *Ambystoma* is generally 200 – 500 m (Trenham et al. 2001; Gamble et al. 2007), whereas anuran dispersal is typically 1000 – 1500 m (Berven and Grudzien 1990). Intraspecific variation in vital rates and dispersal parameters can be equal to or greater than those between species of amphibians (Stevens et al. 2010), suggesting that external conditions experienced prior to (*i.e.*, carry-over effects) and during dispersal (*i.e.*, condition-dependent factors) may have strong effects on dispersal. Despite abundant research on breeding migrations, factors affecting amphibian dispersal remain poorly resolved. This is particularly true for phenotype-dependent factors, such as body size and condition, which are rarely considered (Lowe and McPeck 2012). Although effects of habitat type on dispersal have been tested (Rothermel and Semlitsch 2002; Pittman 2013; Osbourn et

al. 2014), the effects of other condition-dependent factors, such as natal or terrestrial population density remain unknown. Additionally, whether there are carry-over effects of larval density on juvenile dispersal remains untested.

In this study, we tested if body size or condition, habitat quality, or carry-over effects of natal population density influenced on the departure or transience of ringed salamanders (*Ambystoma annulatum* Cope 1886). We reared animals under different natal conditions, generating animals that differed in body size and condition. We tracked their initial movement in two habitat types: closed canopy forest and a mixed grassland. We hypothesized that some individuals would be more capable of long-distance dispersal because of larger body size. Larger amphibians have greater lipid stores (Scott et al. 2007; Ousterhout and Semlitsch 2016), lower rate of water loss (Peterman et al. 2013), and longer strides (Bennett et al. 1989). Alternatively, smaller individuals may be more likely to disperse, because they receive a cue from their natal habitat (high intraspecific density) that terrestrial habitat quality may be low, as is the case in many invertebrates (Harrison 1980). We predicted that juveniles would be less likely to settle in high-risk habitat (grassland; Pittman 2013) and that movement strategies of salamanders across the two habitat types would differ by natal density treatment due to the different cost/benefit structure imposed by carry-over effects. Understanding the effects of the interaction of an individual's internal state with the external environment (e.g., habitat quality or terrestrial density) on movement is the first step to understanding key features of dispersal. Such mechanistic understanding is critical to theories of the evolution of dispersal, its demographic consequences to populations, and developing real solutions to mitigate anthropogenic change.

Materials and Methods

Study Site and Species

A field experiment was conducted at two sites within Fort Leonard Wood, Missouri (37.92°N, 92.17°W). Fort Leonard Wood is an active military training facility encompassing 24,852 ha in the northern Ozark Highland. Eighty percent of Fort Leonard Wood is forested, characterized by oak-hickory forests (*Quercus* spp., *Q. stellata*, *Carya* spp., and *C. texana* canopy; *Rhus aromatic* and *Cornus florida* understory) or short-leaf pine plantations (*Pinus echinata*), and 9% is grassland.

The ringed salamander is endemic to the Ozark plateau and the Ouachita Mountains of Missouri, Arkansas, and Oklahoma (Petranka 1998). Adults migrate to ponds August – October and oviposit on submerged substrates. Ringed salamander larvae overwinter in ponds and metamorphose late April – early July (Hocking et al. 2008; Semlitsch et al. 2014). Of the ponds occupied by ringed salamanders at Fort Leonard Wood, 74% of the habitat within 200 m is forest and 15% is grassland. When juveniles of *Ambystoma* emigrate from their natal ponds, they respond to high risk environments with increased movement (Pittman 2013) and are more likely to settle in closed canopy forest habitat (Osbourn et al. 2014). When not moving on the surface, juveniles are fossorial, utilizing insect and small mammal burrows (Semlitsch 1981; Trenham 2001).

Larval Mesocosms

We established pond mesocosms (1000 L volume, 1.52 m diameter, polyethylene cattle tanks) on 5 September 2011 and 9 September 2012 with dechlorinated tap water, leaves,

and zooplankton in a fenced research facility at the University of Missouri, Columbia, Missouri (Ousterhout and Semlitsch 2016). We collected ringed salamander eggs at similar developmental stages from multiple clutches at several ponds (2011: N = 3; 2012: N = 2) at Fort Leonard Wood. On 15-21 October 2011 and 14 September 2012 we randomly assigned hatchlings to mesocosms and mesocosms to an intraspecific density treatment: low density (6 larvae/mesocosm, 2011: N = 9 mesocosms, 2012: N = 32), medium density (18 larvae/mesocosm, 2011: N = 9, 2012: N = 12), or high density (36 larvae/mesocosm, 2011: N = 7, 2012: N = 8) treatment. We increased replication of low-density mesocosms to generate an equal number of juveniles from all natal density treatments. Beginning in April of each year, we searched the mesocosms after sunset with a light for metamorphosed salamanders at least every other night. We considered animals to have metamorphosed if their gills were less than 2 mm in length (Mott and Maret 2011; Ousterhout et al. 2014). We measured snout-vent length (SVL; ± 1 mm) and wet mass (± 0.001 g, Mettler AT-100 electronic balance, Mettler Toledo, Columbus, Ohio, USA). We individually housed juveniles in plastic containers with wet sphagnum moss and fed animals fruit flies (*Drosophila melanogaster*) and small mealworms (*Tenebrio molitor*) weekly.

Experimental Enclosures

We used replicated experimental enclosures (N = 12) to isolate the effects of natal and juvenile habitat, respectively, on initial movement behavior. We constructed enclosures at one site in 2012, and we added a replicate site in 2013. We selected sites that were level, and, to increase the likelihood that habitats in our experiment reflected those

encountered by wild juvenile ringed salamanders, all enclosures were within 100 m of ringed salamander breeding ponds. At each site, we constructed long, rectangular enclosures (2 m x 50 m) in closed canopy forest (N = 3 per site) and mixed grassland habitat (N = 3 per site). Previous studies have effectively used similarly shaped enclosures to assay amphibian movement (Rosenberg et al. 1998; Rothermel and Semlitsch 2002; Osbourn et al. 2014). In the forest enclosures we controlled for the amount of coarse woody debris ($5.00 \text{ m}^3 - 6.05 \text{ m}^3$; conic-paraboloid method; Fraver et al. 2007). Each enclosure was constructed from plastic weave silt-fencing walls (90 cm tall) buried 15 cm into the ground and supported by wooden stakes. To prevent individuals from climbing out of enclosures, we folded the top 10 cm of fencing inwards and secured it to form a 90° angle.

Experimental Procedure

We marked each salamander with a Passive Integrated Transponder (PIT) tag as in Ousterhout and Semlitsch (2014). When used in tandem with a portable antenna system, PIT tags allow otherwise prohibitively small bodied animals to be individually tracked from a distance, albeit a small one (30 cm or less underground; Connette and Semlitsch 2012, Ousterhout and Semlitsch 2014). To mark animals, we anesthetized each juvenile by immersing it in a 1% solution of tricaine methanesulfonate (MS-222) until it failed to right itself or respond to toe pinching. After rinsing the animal in spring water, we weighed the juvenile (Mettler Toledo, LLC, Columbus, OH, USA; $\pm 0.01 \text{ g}$). We assigned juveniles a PIT tag size based on their weight: 12 mm PIT tag (12 mm x 2.12 mm, 0.115 g, full duplex, HPT12; Biomark, Boise, ID, USA): 3.51-1.38 g; 9 mm tag

(9×2.12 mm, 0.08 g, full duplex, HPT9, Biomark): 0.91 g – 2.65 g; 8 mm tag (8.5×1.4 mm, 0.033 g, full duplex, HPT8, Biomark): 0.52 g – 1.87 g. If half the torso length of a salamander was equal to or less than that of the length of the PIT tag assigned by mass, we implanted that individual with the next smallest PIT tag size. To implant the tag, we made a 3 mm incision to the muscle and skin anterior to the left hind limb, and inserted the tag into the body cavity. Following implantation with the PIT tag, we monitored salamanders until they recovered from anesthesia and righted themselves. We held all juveniles individually in plastic containers until their release in the field.

We released cohorts of eight to ten juvenile salamanders (mean \pm SD: 9.5 \pm 1.5 salamanders) into the northern side of enclosures within 24 h of rain. For each cohort, we randomly assigned individuals of the same natal density treatments to a site, habitat, and enclosure. Whenever possible, we concurrently released cohorts from each density treatment into runways in each habitat type and, in 2013, at each site to ensure temporal replication. However, this was not always possible because animals from low-density mesocosms metamorphosed 5 to 10 d before juveniles from high natal density mesocosms (2012: low-density: 21 April \pm 4.9 d, high-density: 30 April \pm 6.9 d; 2013: low-density: 25 May \pm 3.8 d, high-density: 30 May \pm 3.1 d). After sunset (2015 h – 2215 h), we placed all juveniles assigned to a runway under a plastic box at the end of an enclosure. We also placed a small piece of sphagnum moss from each salamander's individual container into the box as a negative cue for salamanders to move away from. After a 30 min acclimation period, we removed the plastic box. We released 18 cohorts in 2012 (30 April – 6 June; N = 175) and 28 cohorts in 2013 (25 May– 24 June; N = 268).

We searched each runway every 24 h for 7 d post release by passing a portable RFID antenna (FS-2001F-ISO reader and BP portable antenna, Biomark) twice over the entirety of each enclosure. During the second pass the antenna was rotated 90° around its vertical axis to maximize the detection field of the antenna (Ousterhout and Semlitsch 2014). The length of the antenna handle allowed us to remain outside of the enclosure during daily searches, minimizing soil compaction (Osbourn et al. 2014). When salamanders were detected, we marked their location along the length of the enclosure. We quantified movement in one dimension, because the rectangular shape of the enclosure limited lateral movement. After tracking on day 7, we attempted to recapture all detected salamanders to confirm survival and maintain a constant density of animals in the enclosures. When individuals occupied burrows, we limited our excavations to 0.063 m² to balance recapturing animals while minimizing the effects on enclosures.

Environmental Measures

We measured surface soil temperatures using data loggers (Thermochron iButtons, Maxim Integrated Products, San Jose, CA, USA). We weatherized the data loggers by dipping them three times in plastic tool dip (Plasti Dip, Plasti Dip International, Blaine, MN, USA). We then placed them in mesh wire cages, and positioned them against the soil substrate under the leaf litter (forest enclosures) or grass (grassland enclosures). We deployed two data loggers in one enclosure of each habitat type at each site. In 2013, we measured air temperature and relative humidity (RH) using HOBO data loggers (U10-003, Onset Computer Corporation, Bourne, MA, USA). Two data loggers were deployed at each habitat type at each site at a height of approximately 0.5 m. We used precipitation

data from a weather station (Waynesville Regional Airport at Fournery Field, Weather Underground, <http://www.wunderground.com>) located 4 km from our sites. When site and habitat specific measures were unavailable (e.g., RH in 2012 or logger failed), we interpolated missing values by regressing observations of the variable of interest against the observations at the weather station, site, and habitat.

Statistical Analysis

We used ANOVA to assess the effects of natal density and its interaction with release year on juvenile salamander body size, body condition and date of metamorphosis. We calculated body condition as size-independent mass by regressing the residuals of mean-scaled mass against SVL. We used generalized linear mixed models (GLMMs) to test for effects of natal density, body condition, body size, and/or habitat on 1) departure or 2) transience. In the departure analysis, we assumed a Bernoulli error distribution. We considered an animal to have departed if it moved more than 5 m over the 7 d of tracking, because the home range of *Ambystoma* is approximately 10 m² (Semlitsch 1981; Kleeberger and Werner 1983). For transience, we used the number of times an animal moved and the total distance moved as dependent variables, modeling assuming a Poisson and a negative binomial distribution, respectively. We considered an animal to have moved if consecutive locations differed by more than 0.5 m. For all three GLMMs, we tested for effects of habitat (forest or grassland), natal density (low, medium, or high), and body condition. In addition, we treated site as a covariate and runway crossed with cohort (*i.e.*, animals released at the same time into the same runway) as a random effect to account for non-independence of individuals. Because the number of detections was

positively correlated with the number of moves a juvenile made (Spearman's rank correlation: $\rho = 0.47$, $P < 0.001$) and the total distance moved ($\rho = 0.22$, $P < 0.001$), we included an offset of the number of times an animal was recaptured to adjust for individual differences in detection. Preliminary analyses indicated that cohort size did not affect departure or transience, so we did not include it as a covariate in models. We were missing body condition for 40 individuals; to maximize our sample size, we removed condition from the analysis after verifying it did not have an effect on any of our response variables.

We also examined the effects of weather on the probability of an individual moving and how far it moved between recaptures. We constructed GLMMs with the interaction between density and habitat, and the covariate site to control for phenotype- and condition-dependent factors. We included the independent variables soil temperature, air temperature, relative humidity, and whether measurable precipitation occurred. The models had a random effect of individual crossed with runway and cohort to account for multiple observations of each individual and non-independence of animals in the same enclosure at the same time. We assumed a zero-inflated Bernoulli distribution for the binary response of whether an individual moved, and a negative binomial distribution for the response of distance moved. In addition to assessing the weather cues that salamanders use to make movement decisions, we also tested the window of time over which they respond to cues: during the previous night during which we expected movement to occur (2200 h previous day – 0400 h day of tracking), or the entire previous 24 h (0400 h previous day – 0400 h day of tracking). We compared models for each weather window using AIC. All continuous variables in models were centered and scaled

and models met assumptions of normality, homoscedacity, and collinearity. For each model, we report the goodness of fit with a marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by the full model; Nakagawa and Schielzeth 2013). In a meta-analyses, the mean amount of variance (R^2) explained by ecological and evolutionary studies was 2.51-5.42% (95% CI: 1.99 – 7.05%; Moller and Jennions 2002). Analyses were performed in Program R (R Core Team 2015). We constructed GLMMs in the glmmADMB package with ‘glmmadmb’ (Fournier et al. 2012; Skaug et al. 2016). We tested for the significance of each term using the “Anova” function in the car package (Fox and Weisberg 2011). Confidence intervals were calculated using “confint”.

Results

Effects of Density on Juveniles

Salamanders metamorphosing from higher density mesocosms had a smaller body size (Figure 1; $F_{2,318} = 298.02$, $P < 0.001$) than those metamorphosing from lower density mesocosms. Juveniles reared at a low natal population density were 14% larger than those reared at the medium density and 26% larger than those reared at the high density. Likewise, there was a negative association between natal density and juvenile body condition (Figure 1; $F_{2,279} = 17.15$, $P < 0.001$). The body condition of salamanders reared in low density mesocosms was three times greater than that of salamanders reared in medium or high-density mesocosms. Natal density also affected date of metamorphosis, with juveniles from higher density mesocosms metamorphosing later than those reared in

low density mesocosms ($F_{2,318} = 52.95$, $P < 0.001$). In 2012, animals metamorphosed a month earlier than in 2013.

Departure

Departure was affected by SVL ($\chi^2 = 5.17$, $P = 0.023$). Smaller juveniles were more likely to depart (*i.e.*, move more than 5 m from their release site over 7 d) than larger juveniles (Figure 2). For every 1 mm increase in SVL, the log odds of departing decreased by a factor of -0.95 (Table 1). There was no effect of habitat type, natal density, or the interaction between habitat type and density on departure (Table 1). Our model accounted for 16% of the variance (marginal $R^2 = 0.02$, conditional $R^2 = 0.16$).

Transience

We assessed transience as the number of movements and distance that salamanders moved over one week. Salamanders changed locations 2.13 ± 1.33 times (mean \pm SD) and moved 45.27 ± 29.14 m (range: 0-147.89 m) over the week that we tracked them. Salamanders that moved more frequently also moved farther ($r = 0.55$, $P < 0.001$); however, there was substantial variation in this relationship (Figure 3). There was a trend for a negative relationship between SVL and the number of times an individual moved (Table 2 and Figure 3). There was also a trend for the number of times a salamander moved to be affected by an interaction between natal density and habitat (Table 2 and Figure 3). When salamanders were released into grassland enclosures, juveniles from higher density natal mesocosms moved more times than juveniles from lower density mesocosms. However, salamanders in the forest showed the opposite patterns, with

juveniles from higher density natal mesocosms moving fewer times than juveniles from lower density mesocosms. This model accounted for 17% of the variance (marginal $R^2 = 0.03$, conditional $R^2 = 0.17$).

The distance moved by juveniles was affected by natal density. Juveniles from higher density natal populations moved farther than those from lower density natal populations (Table 2 and Figure 3). There was also an interaction between natal density and habitat (Table 2 and Figure 3). In the forest habitat, there was a weak effect of natal density. Salamanders in forest enclosures moved 48.79 ± 29.90 m over 7 d and 9.14 ± 6.59 m per relocation. Although there was not a strong effect of density in the forest, in the grassland, there was a negative relationship between natal density and distance moved. In the grassland habitat, juveniles moved less than those in the forest enclosures (39.27 ± 26.88 m), but farther between observations (13.97 ± 14.20 m relocation⁻¹). In the grassland habitat, there was also a greater difference in the distance moved per relocation between individuals reared at different natal densities. Juveniles reared in high-density mesocosms moved twice as far as those reared in low-density mesocosms. There was no effect of SVL on distance moved (Table 2). The model of distance moved captured 65% of the variance (marginal $R^2 = 0.03$, conditional $R^2 = 0.65$).

Effects of Weather

The probability of a salamander moving, or how far it moved was best predicted by a 24 h window (probability of moving $\Delta AIC = 42.74$; distance moved $\Delta AIC = 86$). There was a strong effect of all weather variables on movement probability and distance (Table 3). Juveniles were most likely to move, and moved the farthest, when there was

precipitation, higher relative humidity and soil temperature, and lower air temperature (Table 3). The weather models fit the movement data better than the models with only phenotype- and condition-dependent factors (probability of moving: marginal $R^2 = 0.31$, conditional $R^2 = 0.42$; distance moved: marginal $R^2 = 0.15$, conditional $R^2 = 0.77$).

Discussion

Although theoretical studies of dispersal suggest that both phenotype- and condition-dependent factors should affect movement phases, including departure and transience, empirical tests of theory have been largely limited to molecular approaches. While such ultimate measures of dispersal capture general patterns over evolutionary time, they do little to test the underlying processes. This paucity of empirical tests is particularly true for pond-breeding amphibians. In this study, we tracked 324 juvenile ringed salamanders to determine the relative effects of phenotype- and condition-dependent factors on two phases of movement: departure and transience. We found that departure was affected by body size, whereas transience was affected by an interaction between natal population density and habitat. Additionally, we observed effects of weather conditions within 24 h of tracking on the probability of moving and distance moved.

Population density experienced during the natal period can have complex effects on behaviors, including dispersal. One manner in which natal density can influence dispersal is through its effect on juvenile phenotype. Individuals from high-density populations are generally smaller and have a reduced body condition relative to conspecifics from lower density populations (Herrando-Perez et al. 2012; Ousterhout and Semlitsch 2016), as was the case in this study. Although body size often has an effect on

dispersal, the direction of the relationship varies between taxa, with smaller individuals dispersing in some systems whereas larger individuals disperse in others (O’Riain et al. 1996; Bowler and Benton 2005; Clobert et al. 2009). In amphibians, the effects of body size on juvenile dispersal has rarely been tested. This is particularly true for pond-breeding salamanders, with only two other studies to our knowledge having tested for effects of body size (Rothermel 2004; Osbourn et al. 2014).

Here, smaller juveniles were more likely depart than larger animals. There was also a trend for smaller animals to be more transient, moving more frequently. This negative relationship between body size and dispersal suggests that dispersal ability may not have a strong effect on departure, and thus dispersal propensity. Larger amphibians have longer limbs, allowing them to move farther and faster than smaller individuals (Goater et al. 1993; Beck and Congdon 2000). These larger amphibians also have greater energy stores (Ousterhout and Semlitsch 2016) and desiccate less rapidly (Rothermel and Semlitsch 2002; Peterman et al. 2013). In these ways, larger amphibians are more capable of movement, and thus may be expected to have a greater probability of departing. Despite these factors, smaller animals were more likely to depart than larger individuals in our experiment. Osbourn et al. (2014) observed a similar pattern; when ringed and spotted salamander (*A. maculatum*) juveniles were released in enclosures, smaller individuals were more likely to depart. Similarly, when spotted salamanders were released in an old pasture, body size had no effect on their probability of being recaptured at a forest edge (Rothermel 2004). These findings, in addition to the lack of an effect of body condition on departure or transience in this study, suggest that ability to disperse, at

least as discussed here, does not have a strong effect on initial movement in pond-breeding salamanders.

In addition to affecting individual phenotype, natal density can also affect dispersal through carry-over effects of a condition-dependent factor. After body size was controlled for, we observed effects of natal density on the distance moved by salamanders during transience, as well as a trend for natal density to affect the number of times and individual moved. Population density has a strong effect on departure and transience in many taxa, with more individuals leaving their natal population as population density increases (Lambin et al. 2001; Bowler and Benton 2005; Ronce and Clobert 2012). For species with complex life histories, natal density may directly affect dispersal if it is an honest indicator of resource availability in juvenile or adult habitat. Because juveniles and adults of *Ambystoma* are fossorial, and rarely available for surface sampling outside of breeding migrations, it is unknown whether there is a relationship between natal density and surrounding terrestrial population density, and thus if it functions as a reliable cue for salamanders. However, future observational studies could seek to address this question. Alternatively, natal density may be indirectly affecting transience through phenotype-dependent factors other than body size not measured in this study such as stress hormones (Bellure et al. 2004) or immune function (Snoeijs et al. 2004).

We also observed an effect of habitat on ringed salamander dispersal. The terrestrial life stages of many species of *Ambystoma* are thought to prefer forested habitats (Petranka 1998). However, the effects of this preference on movement have rarely been tested in field experiments, particularly in juveniles. In this study, juveniles moved farther in the forest, but the distance moved between recaptures was greater in the

grassland. Other studies in amphibians have made similar observations. Rothermel and Semlitsch (2002) found no difference in the total distance moved by spotted salamanders when released into a grassland or forest habitat. However, there was a nonsignificant trend for the movement rate of to be greater in the field. Similarly, Pittman and Semlitsch (2013) observed longer steps (*i.e.*, fewer turns) when tracking spotted salamanders in a grassland relative to the forest. These findings may indicate that the forest is easier for juveniles to move in, and thus they make more frequent, smaller movements, whereas the in grassland juveniles movement is higher risk, and thus salamanders move long distances when weather conditions are appropriate.

While we have largely considered condition-dependent factors as a cue received at one point in ontogeny, there was support in our study for multiple cues over the course of development affecting transience. We observed no effect of natal density on transience in the forest habitat, which is thought to be preferred by ringed salamanders (Petranka 1998). However, there was a strong effect of density on transience in the grassland, with individuals from high-density natal populations moving farthest. This suggests that redundant cues of habitat quality over ontogeny affect juvenile salamander dispersal. When salamanders experienced either a natal or juvenile habitat with high resource availability (*i.e.*, low natal density or forest habitat), there was no effect on movement. However, when individuals encountered both a natal and a terrestrial habitat with low resource availability, they moved more frequently and farther. The effects of sequential environmental conditions across ontogeny affecting dispersal have also been observed in the common lizard (*Lacerta vivipara*, Massot et al. 2002). However, to our knowledge, this is the first evidence for a similar effect in an amphibian.

Conclusions

By manipulating natal and juvenile habitat and tracking juveniles in field experiment, our study tested the effects of phenotype- and condition-dependent factors on two movement phases. Whereas body size, a phenotype-dependent factor, affected the probability of departure, transience was affected by an interaction between natal density and habitat type. Our study suggests that 1) motivation rather than ability has a larger effect on departure and 2) redundant cues over ontogeny affect the probability of an individual dispersing. Although our findings support theoretical frameworks that indicate dispersal is influenced by several processes, they also indicate that not all processes contribute equally to all phases of dispersal.

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Table 1. Predicted effects of habitat type (Habitat = forest or mixed grassland), natal population density (Density = low, medium, or high), body size (SVL), and site on departure probability in ringed salamanders (*Ambystoma annulatum*). Estimate are the restricted maximum likelihood estimates (95% confidence intervals) and χ^2 are the Wald chi-square values.

Predictor		Estimate	χ^2	DF	P
<i>Intercepts</i>					
Intercept	Grassland, Low density	3.36 (0.83, 5.90)			
Habitat	Forest	0.52 (-1.07, 2.12)	0.53	1	0.465
Density	Medium	0.02 (-1.98, 2.02)	0.001	2	0.999
	High	-0.17 (-2.65, 2.31)			
Habitat*Density	Forest, Medium	-0.01 (-2.68, 2.66)	0.32	2	0.851
	Forest, High	-0.78 (-3.51, 1.94)			
<i>Slopes</i>					
SVL		-0.99 (-1.84, -0.14)	5.17	1	0.023
Site		-2.18 (-4.41, 0.06)	3.65	1	0.056

Table 2. Relationship between transience in ringed salamanders and (forest or mixed grassland), natal population density (Density = low, medium, or high), body size (SVL), and site. Estimates are the restricted maximum likelihood estimates (95% confidence intervals) and χ^2 are the Wald chi-square values.

Predictor		Estimate	χ^2	DF	P
Number of moves					
<i>Intercepts</i>					
Intercept	Grassland, Low density	-0.89 (-1.25, -0.53)			
Habitat			1.12	1	0.289
	Forest	0.11 (-0.17, 0.40)			
Density			0.87	2	0.646
	Medium	0.19 (-0.18, 0.56)			
	High	0.23 (-0.26, 0.72)			
Habitat*Density			5.82	2	0.054
	Forest, Medium	-0.26 (-0.66, 0.14)			
	Forest, High	-0.49 (-0.99, 0.002)			
<i>Slopes</i>					
SVL		-0.13 (-0.26, 0.01)	3.38	1	0.066
Site		-0.12 (-0.32, 0.08)	1.38	1	0.241
Distance moved					
<i>Intercepts</i>					
Intercept	Grassland, Low density	1.98 (1.59, 2.36)			
Habitat			0.71	1	0.400
	Forest	0.17 (-0.14, 0.49)			
Density			11.49	2	0.003
	Medium	0.37 (-0.02, 0.76)			
	High	0.69 (0.22, 1.16)			
Habitat*Density			14.69	2	< 0.001
	Forest, Medium	-0.37 (-0.80, 0.06)			
	Forest, High	-0.89 (-1.39, -0.39)			
<i>Slopes</i>					
SVL		-0.11 (-0.23, 0.02)	2.74	1	0.098
Site		-0.04 (-0.27, 0.19)	0.12	1	0.730

Table 3. Comparison of weather models predicting probability of juveniles moving (Moved) and distance moved (Distance). Time represents whether weather variables were averaged just over the night before tracking (Night only, 2200 – 0400 h) or for the 24 h before tracking (24 h, 0400 – 0400 h).

Response	Time	AIC	Δ AIC
Moved	24 h	2179.2	-
	Night only	2226.7	47.5
Distance	24 h	7798.1	-
	Night only	7818.1	20

Table 4. Parameter estimates for the probability of ringed salamanders moving and distance moved. In addition to the weather variables, the models included the covariates density, their interaction, and site to control for phenotype- and condition-dependent factors. RH is relative humidity and precipitation is a binary response of whether measurable precipitation occurred. All predictors were mean values from the 24 h model (0400 – 0400 h). Estimate is the restricted maximum likelihood estimates (95% confidence intervals) and χ^2 are the Wald chi-square values.

Predictor	Estimate	Probability of moving			Estimate	Distance moved		
		χ^2	DF	P		χ^2	DF	P
Soil temperature	0.36 (0.20, 0.51)	20.86	1	< 0.001	0.17 (0.10, 0.25)	21.35	1	< 0.001
Air temperature	-0.40 (-0.61, -0.20)	14.75	1	< 0.001	-0.21 (-0.30, -0.12)	21.36	1	< 0.001
Precipitation	1.95 (1.68, 2.22)	202.51	1	< 0.001	0.67 (0.53, 0.82)	80.72	1	< 0.001
RH	0.59 (0.45, 0.73)	65.19	1	< 0.001	0.11 (0.04, 0.19)	8.58	1	0.003

Figure 1. The effects of natal population density on juvenile ringed salamander a) body size (SVL), b) size independent body condition, and c) date of metamorphosis by year.

Each box spans the 25th percentile to the 75th percentile. The midline represents the median, whiskers extend to the highest value that is within 1 standard deviation of the median, and dots represent outliers.

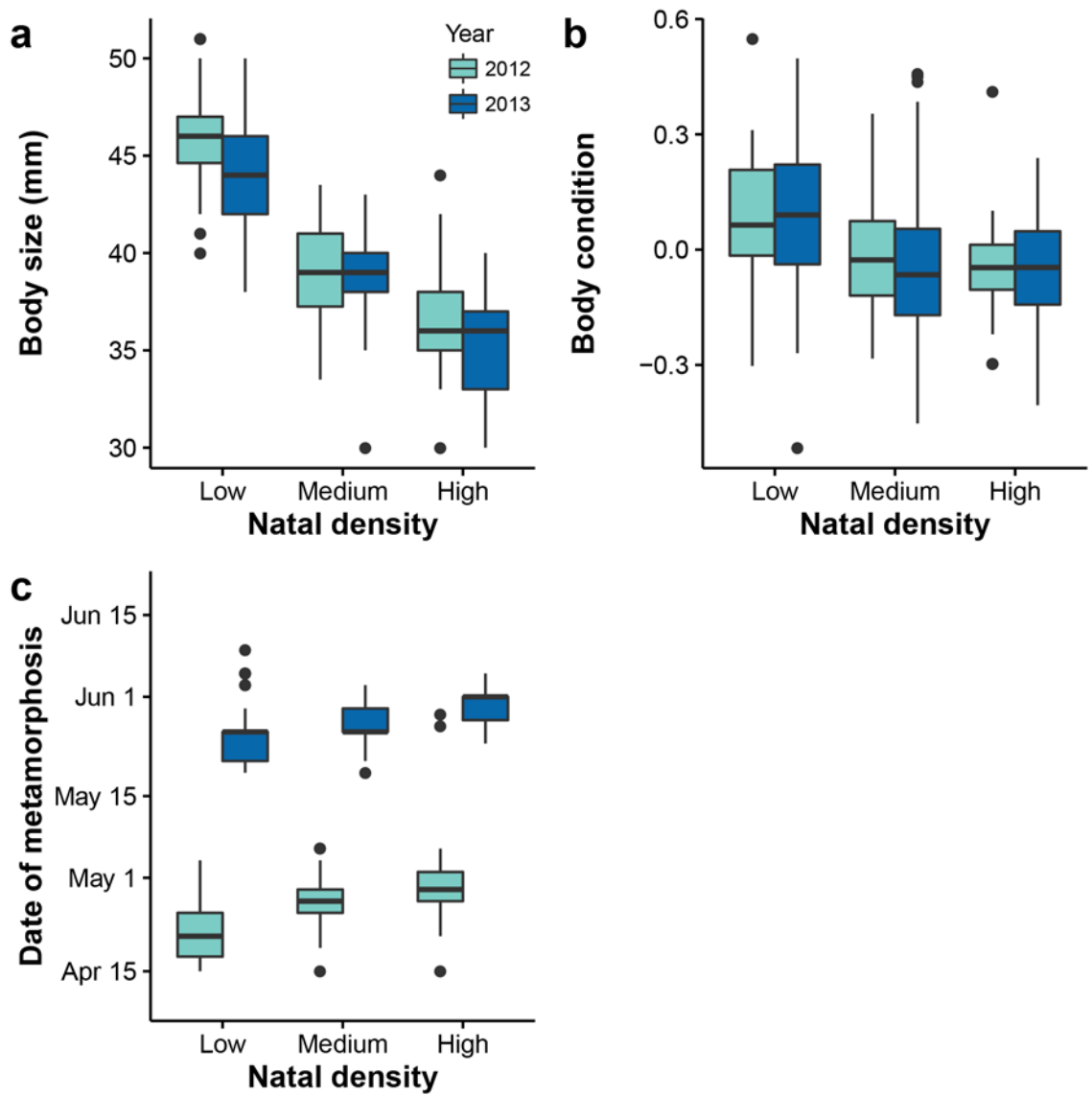


Figure 2. The effects of body size (SVL) on departure in ringed salamanders. Dots represent observations of individual salamanders, the line is the predicted values, and shaded area represents the 95% confidence intervals.

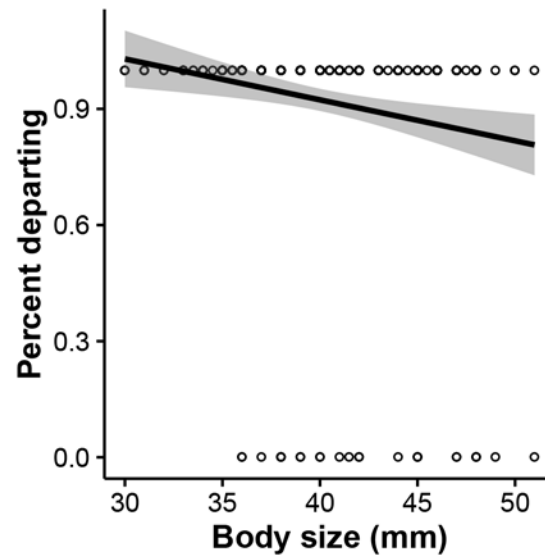
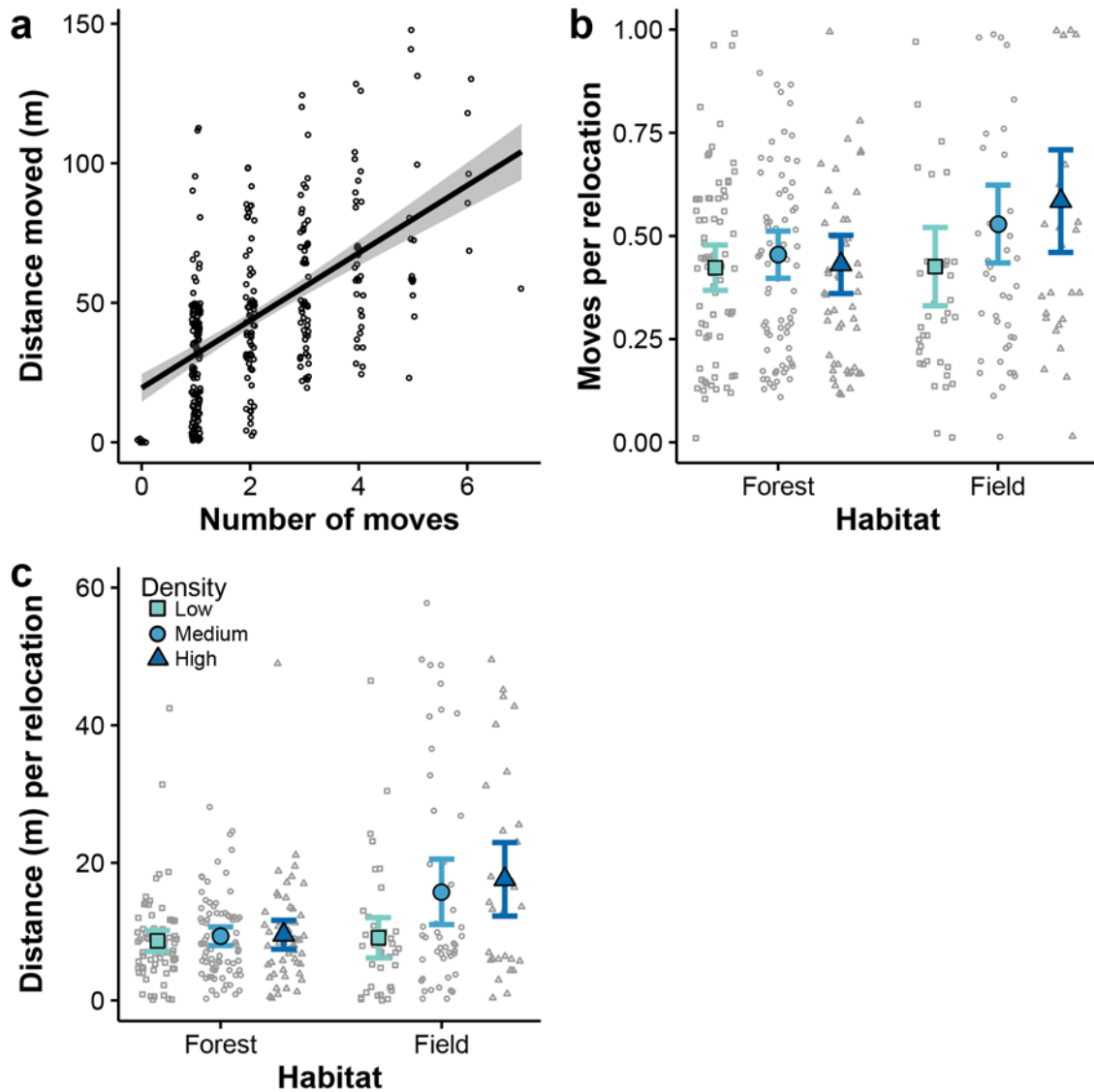


Figure 3. Transience in juvenile ringed salamanders. a) Relationship between two measures of transience, with the shaded area representing the 95% confidence interval and open circles representing individual observations. Effect of habitat and natal density on b) moves per relocation and c) distance per relocation. Open shapes represent observations, filled shapes represent means, and error bars are 95 % confidence intervals.



CHAPTER 5

CONCLUSIONS

In these experiments, I tested the relative effects of phenotype- and condition-dependent factors on dispersal in a juvenile pond-breeding salamander. Although dispersal is an important driver of ecological and evolutionary processes, the mechanisms of these often long distance movements and their fitness consequences remain poorly understood for many taxa, including amphibians. My dissertation indicates that both phenotype-dependent (body size and morphology) and condition-dependent (natal population density and habitat) factors play an important role in moderating movement behavior, and ultimately, dispersal. In addition to supporting theoretical frameworks that indicate dispersal is influenced by both phenotype- and condition-dependent factors, these studies also indicate asymmetries in the effects of these factors on different phases of dispersal.

Summary

Chapter 2 – Non-additive response of larval ringed salamanders (Ambystoma annulatum) to intraspecific density

- There was no effect of larval density on survival or body condition
- Body size, length of larval period, and lipid stores of juveniles had a non-additive response to natal population density, with the additional individuals having less of an affect at high densities than at low densities.
- This suggests that ringed salamander larvae are under selective pressure for tolerance to high density and increased efficiency in resource utilization.

- Predictions of population models are sensitive to assumptions of the functional form of density dependence.

Chapter 3 – Asymmetric effects of phenotype- and condition-dependent factors on dispersal in a pond-breeding salamander

- Exploration was repeatable over three months, with individual accounting for 24-33% of variation. This supports the hypothesis that amphibian dispersal occurs over a series of sustained movements rather than in a single bout.
- Distance moved in the field had a fat-tailed distribution, characteristic of dispersal distributions.
- There was no effect of exploration behavior on distance moved.
- The distance moved by salamanders was affected by morphology. Individuals with long forelimbs and short hind limb moved farthest. This may reflect a trade-off between terrestrial walking performance and aquatic swimming performance.
- Salamanders from high-density natal populations moved the farthest.

Chapter 4 – Natal population density and habitat affect dispersal in a pond-breeding amphibian, the ringed salamander (Ambystoma annulatum)

- Departure was affected by body size. Smaller bodied animals were more likely to depart than those with larger bodies were.
- Transience was affected by an interaction between natal density and habitat. Juveniles from high-density natal populations and in low quality juvenile habitat

moved the farthest. This suggests that juvenile salamanders use redundant cues over ontogeny affect the probability of an individual dispersing.

APPENDIX A

SIMULATION OF POPULATION DYNAMICS

Materials and Methods

We constructed a model of discrete population growth for a female only population with overlapping generations. We assumed that all individuals in the population were the same (*i.e.* no age structure) and that survival was density independent, as was the case in our experiment. At year 0 the population had 10 females and the model ran for 20 years, with each time step constituting a year. Population growth was dependent on 1) the population size in the previous time step, 2) body size as a function of population size, 3) body size dependent per capita probability of reproduction, 4) per capita fecundity as a function of body size, and 5) a constant per capita mortality (30%). We bootstrapped our experimental data and ran the population model 10,000 times for each functional form tested with our experimental data. We present the mean values from these simulations in the manuscript. All simulations were conducted in R.

Body size as a function of population size

We modeled the relationship between body size and population size using the same approach as with our experimental data. For each run of the model we randomly drew 1004 body size measurements from our mesocosm experiment, with replacement. We used these data to model the functional form of density dependence as described in the Methods using a linear, exponential, Shepherd, and Ricker equation in turn. The

coefficients from the functional form model were used to parameterize the body size as a function of total population size in the population model.

Reproduction

The probability of an individual reproducing in a year increased logarithmically as a function body size (Fig. 1). Following Taylor and Scott (1997) we modeled this relationship as

$$b(t) = 0.2 * \arctan(0.45 * s(t) - 18.25) + 0.47$$

where $b(t)$ is the per capita probability of reproducing a year and $s(t)$ is body size as a function of population size. Fecundity in the model increased linearly with body size.

Individuals below a minimum body size threshold (28 mm) did not reproduce.

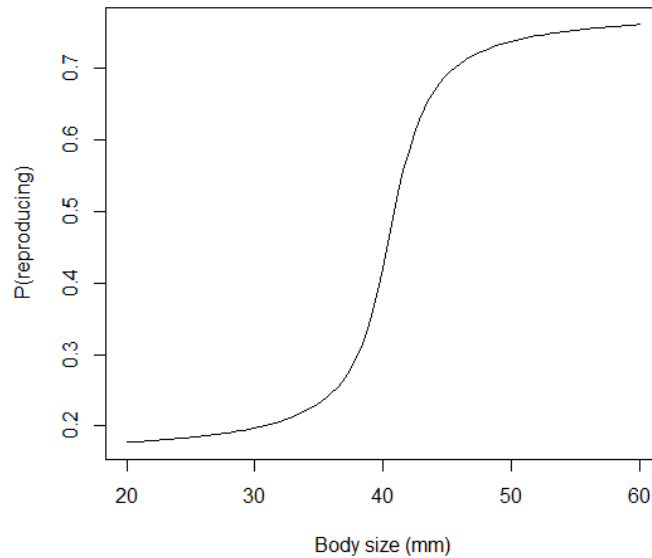


Figure A1. Per capita probability of reproducing as a function of body size

Example R Code for Shepherd Equation:

```
out <- data.frame(ITER = numeric(), YEAR = numeric(), N = numeric())

run <- data.frame(ITER = numeric(nt), YEAR = numeric(nt), N =
numeric(nt))

system.time(
for (i in 1:rep){
  size<-data[sample(1:nS,nS,replace=TRUE),]

  df<-aggregate(cbind(size$Treatment, size$SVL) ~
size$Tank,FUN="mean",na.rm=T)

  names(df) <- c("Tank","Treatment","SVL")

  svl <-nls(SVL ~ beta1*Treatment/(beta2+Treatment^beta3), data=df,
    start = list(beta1=50, beta2=-1, beta3=1), trace=F, control=a)

# svl<-nls(SVL ~ beta1*Treatment/(beta2+Treatment^beta3), data=df,
#          start = list(beta1=50, beta2=-1, beta3=1), trace=F)

  for ( t in 1:(nt-1)){
    s[t] <- (coef(svl)["beta1"]*N[t])/((coef(svl)["beta2"] +
N[t]^coef(svl)["beta3"]))

    b[t] <- 0.2*atan(0.45*s[t] - 18.25) + 0.47 #probability of
reproducing

    c[t] <- 7.45*s[t] - 208.6 #fecundity, if SVL
less than 28 don't reproduce

    c[t] <- c[t]*(c[t]>0)

    N[t+1] <- N[t] + N[t]*(b[t]*c[t]) - d*N[t]

  }

  run$ITER <- rep(i,nt)
  run$YEAR <- seq(1,nt)
  run$N <- N
  out <- rbind(out,run)
})
```

Results

Stable population size and the amplitude of population oscillations depended on the functional form of the relationship between body size and population size. Oscillations increased in amplitude as the fit of the functional form to the data decreased.

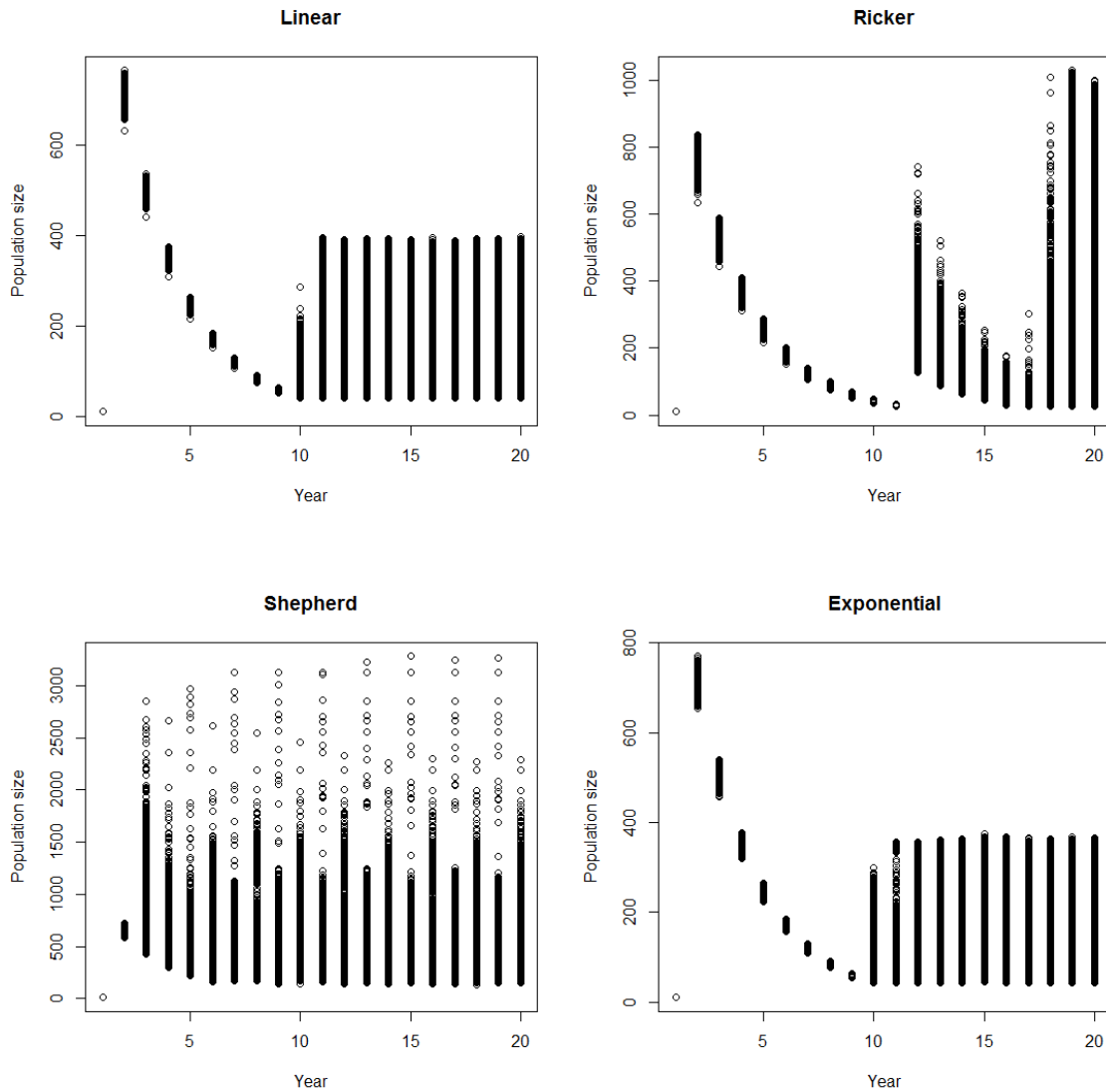


Figure A2. Population model results for 10,000 bootstrapped simulations for each functional form of density dependent size

Literature Cited

Taylor BE, Scott DE (1997) Effects of larval density dependence on population dynamics of *Ambystoma opacum*. *Herpetologica* 53:132–145.

VITA

Brittany Hunter Ousterhout was born 26 March 1986 in Lake Forest, Illinois to Cindy and Andy Ousterhout. She graduated from Lake Forest High School in 2004 and went on to earn a B.A. in Environmental Sciences from Dartmouth College in 2008. After working as a field technician, bagel maker, produce stacker, and barista, Brittany began her Ph.D. at the University of Missouri with an emphasis in Ecology and Evolution and College Science Teaching in May 2011. Her dissertation research was conducted at Fort Leonard Wood, Missouri and investigated the effects of individual variation and habitat on juvenile dispersal. Brittany will begin a post-doctoral appointment at the University of Arkansas in May 2016 where she will study the coexistence in damselfly communities.