



# No evidence of natal habitat preference induction in juveniles with complex life histories



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Individual variation in physiology and behaviour can have strong effects on ecological and evolutionary processes. Natal habitat, one source of individual variation, can influence individual phenotype, behaviour and fitness through effects on eventual habitat selection. Natal habitat preference induction occurs when individuals match stimuli in their selected habitat to those of their natal habitat. Natal habitat can also affect habitat selection through its influence on body condition (silver spoon effect). We tested for natal habitat preference induction and body-condition-dependent habitat selection in two species with complex life histories, the spotted salamander, *Ambystoma maculatum*, and the small-mouthed salamander, *Ambystoma texanum*. We reared salamanders from hatchlings in mesocosms with leaf, grass or control substrate, and tested juvenile habitat selection through two behavioural assays. We found weak evidence of larger salamanders having decreased latency and sampling more habitats, lending support to the body-condition-dependent habitat selection hypothesis in these species. Juveniles preferred grass litter cues regardless of the substrate in their natal mesocosm, suggesting natal habitat preference induction may not occur in species with complex life histories. We propose that species with complex life histories use simple movement rules, such as moving along habitat gradients, to select postnatal habitat when moving through a novel environment.

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Individual variation in behaviour can have strong effects on ecological and evolutionary processes (Bolnick et al., 2003; Davis, 2008; Sih, Bell, & Johnson, 2004). One source of variation is natal habitat, which influences individual phenotype (Monaghan, 2008), behaviour (Davis & Stamps, 2004) and fitness (Morris, 2011; Stamps & Davis, 2006). Conditions experienced during early developmental stages can have long-term consequences for individual development and fitness, including growth rate, metabolism, immune function and fecundity (Lindström, 1999; Metcalfe & Monaghan, 2001; Monaghan, 2008; Scott, 1994).

Natal habitat can affect fitness and individual phenotype beyond natal ontogeny through subsequent effects on habitat preference and selection. Selected habitat ultimately determines the ecological interactions and selective pressures that an individual experiences and affects many broader processes, including metapopulation dynamics, local adaptation and sympatric speciation (Clobert, Le Galliard, Cote, Meylan, & Massot, 2009). Several hypotheses

suggest that individual experience can have strong effects on the habitat selection of individuals within a landscape. One hypothesis, natal habitat preference induction (NHPI), posits that exposure to stimuli in the natal habitat increases the probability that an animal will prefer and settle in habitats with similar stimuli (Davis & Stamps, 2004). This hypothesis assumes that individuals are equally able to sample all habitats. However, individuals vary in their physiology such that some are more limited in the number of habitat patches they are able to search or are less able to compete for high-quality patches (Davis, 2007; Morris, 2003). Thus, habitat selection may be determined by an interaction between individual body condition and preference, or silver spoon effects (Davis, 2007; Stamps, 2006). Other factors affecting habitat preference include innate habitat preference via genetics and fixed action patterns that are condition dependent. Natal habitat-related hypotheses of future habitat preference and selection have been primarily tested in species with similar habitat requirements throughout ontogeny (Davis & Stamps, 2004). To our knowledge, the relationship between natal habitat and later habitat preference has yet to be systematically tested in groups that experience an abrupt change in niche over the course of development, such as the change from aquatic to terrestrial habitat experienced by metamorphosed pond-breeding amphibians.

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To test whether NHPI or body-condition-dependent habitat selection occurs in species with complex life-history strategies, we conducted a set of experiments with two widely distributed pond-breeding salamanders: the spotted salamander, *Ambystoma maculatum*, and the small-mouthed salamander, *Ambystoma texanum*. Although these species are sympatric over much of their ranges, *A. maculatum* is thought to be a forest specialist and *A. texanum* is hypothesized to be a habitat generalist (Petranka, 1998). We reared salamanders from hatchlings in experimental mesocosms with different natal substrates to simulate different habitat types, and conducted two assays of the quality of recently metamorphosed juveniles and their habitat preferences through habitat choice tests. We predicted that juveniles would display NHPI and show an increased probability of selecting terrestrial habitat cues similar to the substrate of their natal pond. Alternatively, if juveniles have innate or condition-dependent preferences, we expected *A. maculatum* to select forest habitat cues preferentially, and *A. texanum* to have no preference.

In the second assay, we tested the response of juveniles to water-borne cues that would indicate proximity to their natal wetland. We hypothesized that individuals would preferentially select habitat different from their natal wetland given the high density of conspecifics and predators immediately surrounding wetlands (Patrick, Harper, Hunter, & Calhoun, 2008; Pittman, Osbourn, Drake, & Semlitsch, 2013; Rittenhouse & Semlitsch, 2007). As such, we predicted that individuals would distance themselves from natal pond water-borne cues and prefer cues dissimilar to their natal pond. In both assays, we also predicted that juveniles with better body condition would sample more habitat types (silver spoon effect).

## METHODS

### Experimental Design

#### Mesocosms

We initiated 60 1000-litre cattle tank mesocosms in late February 2009 in a fenced outdoor research facility at the University of Missouri, Columbia, MO, U.S.A. in the same manner as similar studies (e.g. Earl, Luhring, Williams, & Semlitsch, 2011). We filled mesocosms with tap water, allowed the water to stand and dechlorinate over 14 days, and added 1 kg of a randomly assigned litter type ( $N = 20$  of each litter treatment). We simulated two types of pond substrate corresponding to commonly encountered pond types within a landscape: deciduous oak (*Quercus* spp.) litter (forest ponds) and grass litter (old-field ponds). We used a synthetic cloth substrate (8 cm squares of high-density polyethylene PAK knit shade cloth with 1 mm gauge mesh; Hummert International, St Louis, MO) to create a third mesocosm type in which substrate was present but lacked cues associated with leaf or grass litter. We inoculated mesocosms with a 300 ml concentrated aliquot of plankton from natural ponds to establish natural plankton and periphyton communities. Mesocosms were left uncovered to allow colonization by flying insects, such as dipterans, as an additional food source for larvae. Water levels were maintained at approximately 50 cm during the experiment.

We collected litter, egg masses and adult breeding pairs from natural ponds in the Thomas Baskett Wildlife Research Area, Boone County, Missouri. Eggs were collected within 24 h of oviposition (*A. maculatum*). We also captured pairs of adult salamanders (*A. texanum* and *A. maculatum*) by hand and allowed them to breed in 18.9-litre buckets filled with rain and well water. Following breeding, adults were returned to their point of capture. We stored eggs in a mix of rain and well water in 18.9-litre buckets until they hatched. Within 24 h of hatching, we placed 25

larvae from each clutch into each of the three mesocosm treatments (75 larvae total per clutch; 7 *A. texanum* clutches, 13 *A. maculatum* clutches). We randomly assigned mesocosm substrate type, salamander species and clutch to each tank. We checked mesocosms nightly for metamorphosing salamanders (gills largely reduced and all four legs well developed) beginning 1 June 2009 and removed metamorphosed individuals. We stored metamorphosed salamanders individually in plastic containers (17 × 12 × 9 cm) in a secure facility at the University of Missouri (25–28 °C; 12:12 h light:dark cycle). The plastic containers contained damp sphagnum moss that had been soaked in deionized water and wrung out. The juveniles were tested a mean ± SD of 11 ± 3.2 days and 14 ± 6.8 days after metamorphosis in the litter cue and water-borne cue test, respectively. Because all animals were housed indoors in individual containers and in the absence of the cues tested, it is unlikely that housing affected habitat preference. To minimize handling of animals prior to behavioural experiments, snout–vent length (SVL; ±1 mm) and wet mass (±0.001 g, Mettler AT-100 electronic balance, Mettler Toledo, Columbus, OH, U.S.A.) were not measured until after all behavioural experiments for an individual were completed. To prevent salamanders from imprinting on cues in their housing, we did not feed individuals during the course of the experiment. Following assays, animals were used in a separate experiment. Animals were collected under Missouri Department of Conservation Wildlife Collector's Permit 8908 and maintained under University of Missouri – Columbia Institutional Animal Care and Use Committee protocol 3368.

#### Choice tests

We constructed choice test chambers from transparent round plastic containers (8 cm deep × 26 cm in diameter) and placed a thin line of clear caulk through the centre to keep cues in their respective halves. A baffle of clear tape lined the top edge of the chambers to prevent salamanders from climbing out. We arranged 16 chambers into four rows and columns, and randomized whether the centre line of each chamber was oriented north–south or east–west. The orientation of each chamber remained constant for all experiments. Before each trial, we randomized the half to which each treatment was assigned.

Two types of cues were used in behavioural assays: litter and water-borne. We used litter cues to assess habitat preference of recently metamorphosed salamanders entering the terrestrial environment. Litter cues were obtained by grinding either grass or leaf litter with deionized water into a fine paste to limit differences in structure and moisture gradients that may otherwise provide shelter or influence choices. We homogenized batches of each litter type (leaf and grass) on the night of trials from extra litter that was collected at the same time and place as the litter used for mesocosm initiation. Litter cues were lightly drained (moist but not dripping) prior to placing a thin (~5 mm) layer on assigned sides of the choice test chambers. We used water-borne cues to test whether recently metamorphosed salamanders preferred habitat different from their natal wetland. Water-borne cues were obtained by saturating WypAll cloths (Kimberly-Clark, Dallas, TX, U.S.A.) with water collected from mesocosms with either leaf or grass litter for at least 24 h. We lightly wrung out cloths so that they were saturated but not dripping prior to placing them in the test chambers. We selected water from mesocosms that contained the same substrate and species as the focal animal, but did not have siblings of that individual.

All behavioural trials were conducted at night to correspond with natural activity periods of recently metamorphosed salamanders (Semlitsch & Pechmann, 1985). Litter trials were conducted 20–24 June 2009 and water-borne cue assays were

conducted 27 June–21 September 2009. The laboratory was dark during trials, and we used green light-emitting diode (LED) headlights to make observations. We placed one individual under an opaque cup in the centre of each arena, and allowed the juveniles to acclimate for 5 min after the last animal was placed in the arena. We recorded latency, or the time until first habitat selection, and subsequent positions of juveniles every 3 min for 1 h. Salamanders were determined to use one cue only if the entirety of their body was on one half of the chamber. We also recorded at each interval the location of animals that had parts of their body on both halves of the chamber or that climbed on the sides of the choice chamber, but we excluded these data from the analyses.

## Analysis

### Effects of natal habitat

To determine the effects of natal habitat and species on juvenile morphology and ontogeny, we conducted three three-way ANOVAs using the Anova function in package 'car' (Fox & Weisberg, 2011). Specifically, we tested whether the size, survival or length of larval period of salamanders was predicted by natal substrate type (i.e. cloth, leaves or grass), species, or a two-way interaction. Treatments were applied at the mesocosm level, so we used mesocosm means of all dependent variables. Juvenile size is a strong predictor of lifetime fecundity, and length of larval period can have large implications for survival in natural ponds if individuals are unable to metamorphosis (Scott, 1994). Because preliminary analysis demonstrated that the relationship between SVL and mass was different for each species, we included both as metrics of size.

### Behavioural assays

We tested factors that affected individual habitat preference behaviour by building generalized linear mixed models (GLMM) using 'glmer' in package 'lme4' (Bates, Maechler, & Bolker, 2012). We analysed the litter and water-borne cue experiments separately. The responses of interest for each assay were latency, number switches and proportion of time spent in grass habitat (water cue test) or proportion of time spent on natal habitat type for the litter cues as no animals reared in cloth substrate mesocosms were tested. To account for the split-plot design, we included a random intercept with an interaction between mesocosm and behavioural trial in all models. Although we assayed some salamanders in the water-borne cue assay after testing that individual in the litter cue assay ( $N = 39$ ), preliminary analysis indicated that previous testing and its interaction with species or substrate was nonsignificant in any of the water-borne cue models. We interpreted this as evidence that carryover effects were absent, and we included these animals in both analyses and removed whether an individual had been previously tested from the models. We fitted latency and number of switches to a Poisson error distribution, and time spent in habitat type of interest was modelled using a binomial error distribution. All model residuals met assumptions of normality and homoscedasticity with these error distributions.

We developed four models a priori to test specific hypotheses concerning habitat preference. We hypothesized that habitat selection could be driven by differences in habitat specialization (species), by natal substrate type (NHPI), or their interaction. Our model set included one single factor model for each hypothesis, a random intercept model, and a global model with the fixed factors species and natal mesocosm substrate type, and their interaction.

We also developed eight models a priori to test for factors affecting latency and switches. We hypothesized that there may be differences in activity levels between species or due to body condition (size and natal substrate type). Our model set included one

single factor model for each fixed effect, all possible models with one two-way interaction, a global model with all possible interactions, and a random intercept model. The global model included species, natal substrate type, SVL, all two- and three-way interactions, and a random intercept term. Preliminary analyses indicated that SVL was highly correlated with days since metamorphosis ( $r^2 > 0.90$ ), so we elected to use SVL as it allowed for tests of body-condition-dependent habitat selection.

We ranked the models according to their Akaike Information Criterion ( $AIC_c$ ) values, corrected for small sample size. All models with a  $\Delta AIC_c$  of less than 2 had substantial support, models with a  $\Delta AIC_c$  of less than 5 received some support and models with a  $\Delta AIC_c$  of greater than 5 had limited support. We present all models, and we determined those in the 95% confidence set according to Akaike weights (Burnham & Anderson, 2002). If species or natal substrate was included in a well-supported model, we conducted planned a priori contrasts (grass versus leaf mesocosm; cloth versus grass and leaf mesocosms) using 'glht' in package 'multcomp' (Hothorn, Bretz, & Peter, 2008). All statistical analyses were performed using R 2.15.1 (R Development Core Team, 2012).

## RESULTS

### Effects of Natal Habitat on Juvenile Morphology and Ontogeny

Salamanders from mesocosms with leaves had the greatest mass at metamorphosis ( $N = 85$ ; mean  $\pm$  SD:  $0.71 \pm 0.11$  g), followed by salamanders from grass mesocosms ( $N = 95$ ;  $0.52 \pm 0.12$  g) and cloth mesocosms ( $N = 30$ ;  $0.37 \pm 0.13$  g) ( $F_{1,41} = 27.15$ ,  $P < 0.001$ ). Substrate affected SVL similarly to mass, with larger salamanders metamorphosing from mesocosms with leaves ( $28.8 \pm 2.3$  mm), followed by grass ( $25.9 \pm 2.6$  mm) and cloth ( $22.4 \pm 2.5$  mm) ( $F_{2,41} = 29.00$ ,  $P < 0.001$ ). *Ambystoma maculatum* individuals were smaller at metamorphosis ( $N = 129$ :  $N_{\text{Cloth}} = 22$ ,  $N_{\text{Leaves}} = 49$ ,  $N_{\text{Grass}} = 58$ ; SVL:  $24.7 \pm 2.9$  mm) than *A. texanum* individuals ( $N = 81$ :  $N_{\text{Cloth}} = 8$ ,  $N_{\text{Leaves}} = 36$ ,  $N_{\text{Grass}} = 37$ ; SVL:  $28.5 \pm 3.4$  mm;  $F_{1,41} = 23.06$ ,  $P < 0.001$ ) and had a shorter mean larval period (*A. maculatum*:  $183 \pm 17$  days; *A. texanum*:  $197 \pm 20$  days;  $F_{1,41} = 5.90$ ,  $P = 0.02$ ). There were no interactions between species and substrate for any morphological (SVL, mass) or ontogenetic (length of larval period) response variable.

### Behavioural Response to Litter Cues

We assayed the response of 48 recently metamorphosed salamanders ( $11 \pm 3$  days postmetamorphosis) to grass and leaf litter cues ( $N = 12$  for each species and natal substrate combination). Latency, the mean  $\pm$  SD time before an individual selected their first habitat, was  $1.6 \pm 1.7$  min. The random intercept model was best supported by the latency data, suggesting none of the variables included in the model strongly affected time until first habitat selection (Table 1). The number of habitat switches made was best supported by the species model; *A. texanum* switched habitats ( $3.0 \pm 2.2$  switches) more often than *A. maculatum* ( $1.2 \pm 1.3$  switches) (Tukey test:  $Z = 0.96$ ,  $P < 0.001$ ). There was also some support for the species\*SVL model (Table 1, Fig. 1), although these differences appeared to be driven by species-level processes. As SVL increased, *A. maculatum* made fewer switches, while *A. texanum* switching behaviour did not change with SVL ( $\chi^2_1 = 1.95$ ,  $P = 0.16$ ; Fig. 1). Time spent on natal habitat type was best described by the species\*substrate model. Salamanders spent more time on grass ( $76.1 \pm 29\%$ ) than on leaves ( $23.9 \pm 29\%$ ) (Tukey test:  $Z = 5.10$ ,  $P < 0.001$ ). Juveniles raised in grass mesocosms spent more time on their natal litter than did individuals raised with a leaf substrate (leaves:  $23 \pm 30\%$ ; grass:  $75 \pm 29\%$ ;  $Z = -4.94$ ,  $P < 0.001$ ). In

**Table 1**  
AIC<sub>c</sub> model comparison for litter cue behavioural assays

Model	AIC <sub>c</sub>	K	ΔAIC <sub>c</sub>	w <sub>i</sub>
<i>Latency</i>				
<b>Random intercept</b>	<b>41.1</b>	<b>2</b>	<b>0.0</b>	<b>0.467</b>
<b>SVL</b>	<b>43.0</b>	<b>3</b>	<b>1.9</b>	<b>0.179</b>
<b>Species</b>	<b>43.3</b>	<b>3</b>	<b>2.2</b>	<b>0.152</b>
<b>Substrate</b>	<b>43.3</b>	<b>3</b>	<b>2.2</b>	<b>0.148</b>
<b>Species * SVL</b>	<b>46.9</b>	<b>5</b>	<b>5.8</b>	<b>0.025</b>
Substrate * SVL	47.8	5	6.7	0.016
Species * Substrate	48.2	5	7.1	0.013
Global	55.9	9	14.8	<0.001
<i>Switches</i>				
<b>Species</b>	<b>85.4</b>	<b>3</b>	<b>0.0</b>	<b>0.680</b>
<b>Species * SVL</b>	<b>87.9</b>	<b>5</b>	<b>2.5</b>	<b>0.196</b>
<b>Species * Substrate</b>	<b>89.1</b>	<b>5</b>	<b>3.7</b>	<b>0.111</b>
Global	95.3	9	9.9	0.005
SVL	95.6	3	10.2	0.005
Random	96.4	2	11.0	0.003
Substrate	98.6	3	13.2	<0.001
Substrate * SVL	100.2	5	14.8	<0.001
<i>Habitat preference</i>				
<b>Species * Substrate</b>	<b>273.4</b>	<b>5</b>	<b>0.0</b>	<b>0.980</b>
Substrate	281.2	3	7.8	0.020
Species	297.6	3	24.2	<0.001
Random	299.0	2	25.6	<0.001

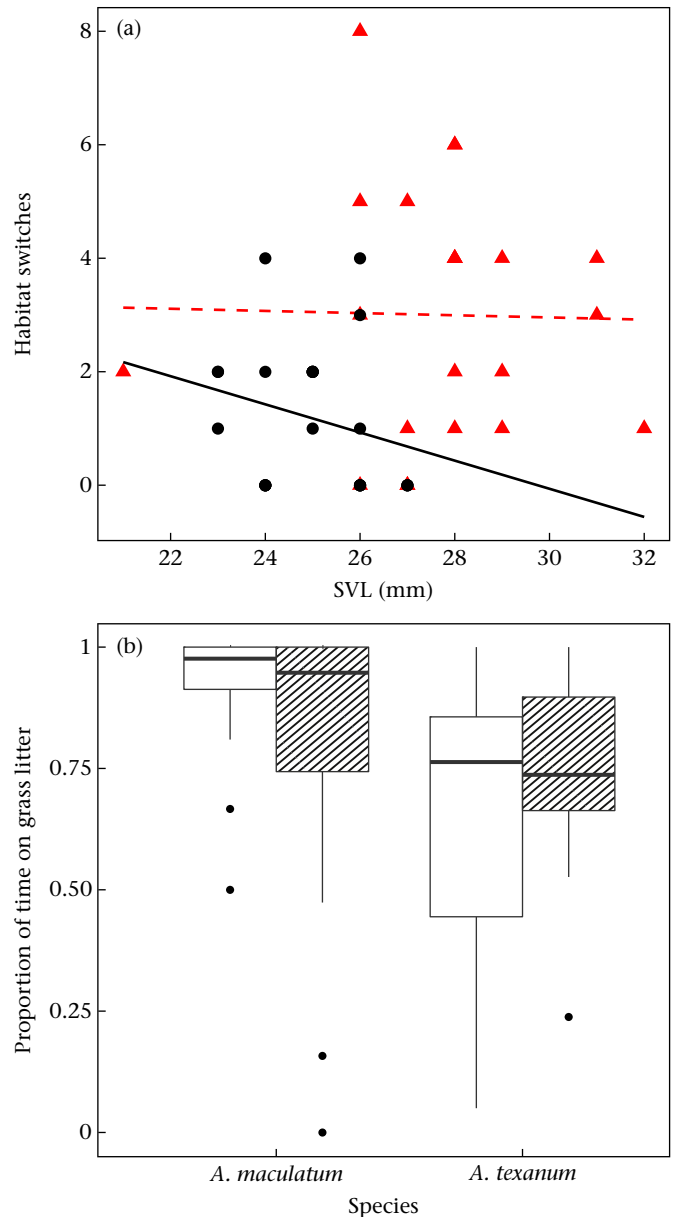
AIC<sub>c</sub>: Akaike Information Criterion values, corrected for small sample size; K is the number of parameters estimated in the model; w<sub>i</sub>: Akaike weight; SVL: snout–vent length. Akaike weight can be interpreted as the probability that a model is the best approximating model in the set. Bold-faced models were included in the 95% confidence set according to Akaike weight.

contrast to our expectations of species behaviour, *A. texanum*, the presumed habitat generalist, spent a greater proportion of time on leaves ( $36.2 \pm 35.2\%$ ) than did the presumed forest specialist, *A. maculatum* ( $9.8 \pm 16.2\%$ ) ( $Z = 4.81$ ,  $P < 0.001$ ).

#### Behavioural Response to Water-borne Cues

We tested for preferences of water-borne cues on 180 recently metamorphosed salamanders (*A. maculatum*:  $N = 106$ ,  $N_{\text{Cloth}} = 21$ ,  $N_{\text{Leaves}} = 44$ ,  $N_{\text{Grass}} = 41$ ; *A. texanum*:  $N = 74$ ,  $N_{\text{Cloth}} = 8$ ,  $N_{\text{Leaves}} = 33$ ,  $N_{\text{Grass}} = 33$ ;  $14 \pm 7$  days postmetamorphosis). Latency was best described by the global model (Table 2). Overall, *A. maculatum* had greater latency ( $5.80 \pm 9.77$  min) than *A. texanum* ( $2.84 \pm 6.90$  min) (Tukey test:  $Z = 2.60$ ,  $P < 0.01$ ; Fig. 2). While the latency of *A. maculatum* was similar across natal substrates (cloth:  $5.86 \pm 9.88$  min; natural:  $5.79 \pm 9.81$  min), *A. texanum* had shorter latencies when reared on cloth than when reared on natural substrates (cloth:  $0.38 \pm 1.06$  min; natural:  $3.14 \pm 7.24$  min; Fig. 2). This result may be driven by the small sample size of *A. texanum* reared in cloth mesocosms ( $N = 8$ ) relative to individuals reared in natural substrate mesocosms ( $N = 66$ ). As the size of juveniles increased, *A. maculatum* became more latent, while *A. texanum* was less latent (Fig. 2).

The number of habitat switches an individual made was best described by the global model with the species\*substrate, substrate and SVL models also being strongly supported (Table 2). Larger juveniles tended to make more switches than smaller juveniles, but this difference was not significant. Individuals reared in grass tanks made more switches than animals reared in mesocosms with leaves (grass versus leaves:  $Z = 2.77$ ,  $P < 0.01$ ; grass:  $1.65 \pm 1.76$  switches,  $N = 74$ ; leaves:  $1.36 \pm 2.10$  switches,  $N = 77$ ); however, individuals that metamorphosed from cloth substrate tanks did not differ in switching from the other two treatments (cloth versus leaves and grass:  $Z = 0.04$ ,  $P = 0.97$ ;  $0.93 \pm 1.31$  switches,  $N = 29$ ). There was a nonsignificant tendency for *A. texanum* to make more



**Figure 1.** (a) Switching behaviour of *Ambystoma maculatum* (circles, solid line) and *A. texanum* (triangles, dashed line) in relation to snout–vent length (SVL) in the litter test. (b) Proportion of time spent on grass litter by juveniles of both species from each natal substrate type (open bars: leaves; hatched bars: grass). Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles).

switches ( $1.51 \pm 2.01$  switches,  $N = 74$ ) than *A. maculatum* ( $1.34 \pm 1.75$  switches,  $N = 106$ ) ( $Z = 1.54$ ,  $P = 0.12$ ).

Habitat selection in the water-borne cue trials was best described by the substrate model (Table 2). We found a nonsignificant tendency for individuals reared in cloth substrate mesocosms to spend less time on grass tank cues (parameter estimate  $\pm$  SE:  $19.8 \pm 35.5\%$ ) than salamanders reared in grass substrate mesocosms ( $43.4 \pm 32.5\%$ ) or leaf substrate mesocosms ( $59.0 \pm 32.6\%$ ) (Tukey test: cloth versus grass and leaves:  $Z = -1.44$ ,  $P = 0.15$ ). Juveniles reared in mesocosms with grass and leaf substrate did not differ in their habitat preference in this assay ( $Z = 0.59$ ,  $P = 0.56$ ). There was also strong support for the random intercept model, and all models were included in the 95% confidence set.



**Table 2**  
AIC<sub>c</sub> model comparison for water-borne cue behavioural assays

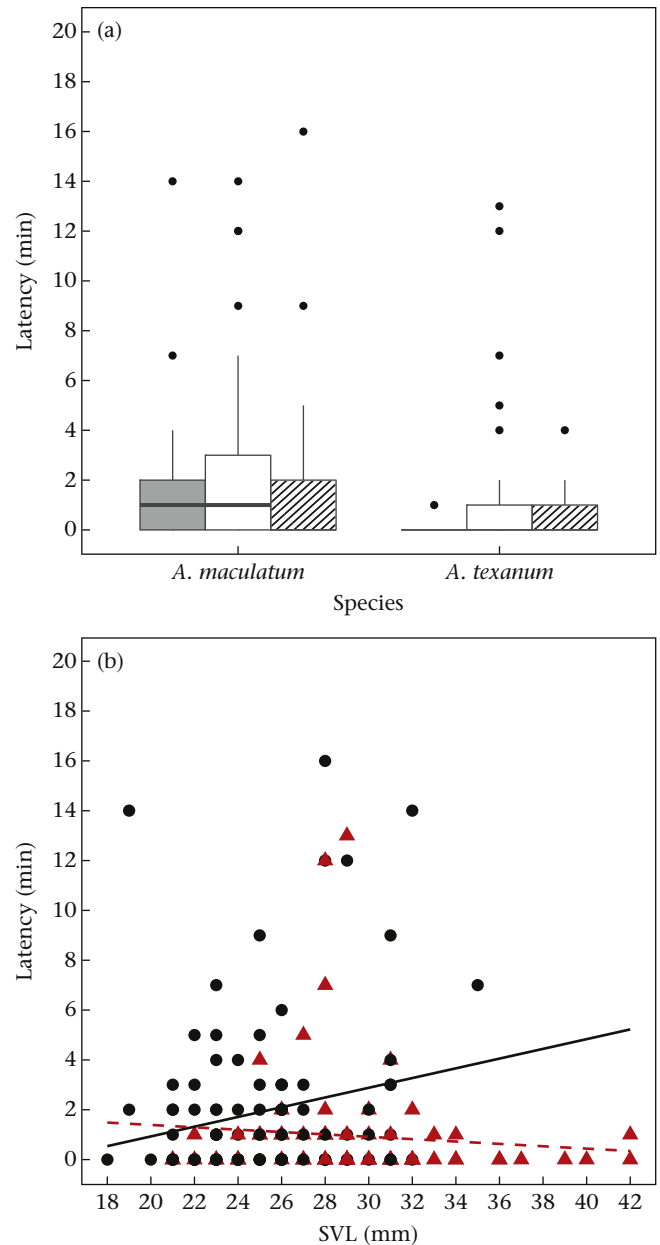
Model	AIC <sub>c</sub>	K	ΔAIC <sub>c</sub>	w <sub>i</sub>
<i>Latency</i>				
<b>Global</b>	<b>430.8</b>	<b>13</b>	<b>0.0</b>	<b>0.990</b>
Species * SVL	440.9	5	10.1	0.010
Species * Substrate	452.2	7	21.4	<0.001
SVL	452.8	3	22.0	<0.001
Species	452.9	3	22.1	<0.001
Random	454.3	2	23.5	<0.001
Substrate * SVL	454.3	7	23.5	<0.001
Substrate	455.9	4	25.1	<0.001
<i>Switches</i>				
<b>Global</b>	<b>358.8</b>	<b>13</b>	<b>0.0</b>	<b>0.295</b>
<b>Species * Substrate</b>	<b>359.1</b>	<b>7</b>	<b>0.3</b>	<b>0.253</b>
<b>Substrate</b>	<b>360.5</b>	<b>4</b>	<b>1.7</b>	<b>0.130</b>
<b>SVL</b>	<b>360.6</b>	<b>3</b>	<b>1.8</b>	<b>0.126</b>
<b>Random</b>	<b>362.4</b>	<b>2</b>	<b>3.6</b>	<b>0.120</b>
<b>Species</b>	<b>362.7</b>	<b>3</b>	<b>3.9</b>	<b>0.043</b>
Species * SVL	362.7	5	3.9	0.018
Substrate * SVL	362.9	7	4.1	0.017
<i>Habitat preference</i>				
<b>Substrate</b>	<b>1522.8</b>	<b>4</b>	<b>0.0</b>	<b>0.521</b>
<b>Random</b>	<b>1524.3</b>	<b>2</b>	<b>1.5</b>	<b>0.248</b>
<b>Species</b>	<b>1525.7</b>	<b>3</b>	<b>2.9</b>	<b>0.123</b>
<b>Species * Substrate</b>	<b>1526.0</b>	<b>7</b>	<b>3.2</b>	<b>0.107</b>

AIC<sub>c</sub>: Akaike Information Criterion values, corrected for small sample size; K is the number of parameters estimated in the model; w<sub>i</sub>: Akaike weight; SVL: snout–vent length. Akaike weight can be interpreted as the probability that a model is the best approximating model in the set. Bold-faced models were included in the 95% confidence set according to Akaike weight.

## DISCUSSION

Our study does not support the NHPI hypothesis (litter assay) or the hypothesis that juveniles will move away from their natal pond (water-borne cue assay) in animals with complex life histories. In the litter test, juveniles preferred grass regardless of their natal substrate. This finding could support an alternate hypothesis, and indicate that individuals may have a fixed habitat preference rather than one that is plastic to stimuli received during the natal period. In animals with a simple life-history strategy, postnatal habitat selection can be informed by natal habitat conditions (Massot & Clobert, 2000, and references therein). Additional information regarding habitat quality is gathered by juveniles in species that forage with their parent(s) before leaving their natal range (e.g. Mannan, Mannan, Schmidt, Estes-Zumpf, & Boal, 2007). These two sources of information available to individuals before the postnatal stage can lead to habitat matching, and thus NHPI. In contrast to this, animals with complex life histories leave a familiar natal habitat and enter a novel habitat in which they have no prior knowledge. For these species, NHPI would not be a useful strategy for habitat selection during initial dispersion. Rather, simple movement rules, such as moving along a moisture or topography gradient, may maximize fitness.

Under laboratory conditions, larger juvenile salamanders, namely *A. texanum* and individuals with a greater SVL, had decreased latency and sampled more habitats. We speculate that this behaviour could be driven by two nonmutually exclusive factors. First, larger juveniles may be physiologically capable of sampling more habitats (Pittman, 2013). Indeed, field and physiological studies indicate that larger juvenile salamanders have lower desiccation rates and greater lipid stores than smaller individuals (Peterman, Locke, & Semlitsch, 2013; Scott, Casey, Donovan, & Lynch, 2007; Spotila, 1972). Individuals with greater energy stores and/or lower desiccation rates can spend more time searching habitats, thus reducing the risk of habitat prospecting behaviour of larger individuals. Alternatively, larger salamanders may be more



**Figure 2.** (a) Latency to first habitat selection by *Ambystoma maculatum* and *A. texanum* from each natal substrate type (open bars: leaves; hatched bars: grass; solid bars: cloth) in the water-borne cue assay. (b) Relation between latency to first habitat selection and snout–vent length (SVL) for *A. maculatum* (circles, solid line) and *A. texanum* (triangles, dashed line). Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles).

motivated to find better habitat (Pittman, Osbourn, & Semlitsch, 2014; Nathan et al., 2008) and to escape high rates of density-dependent mortality closer to the natal pond (Patrick et al., 2008). If size is a predictor of terrestrial competitive ability, then larger juveniles may sample more habitats to find an optimal patch. Smaller juveniles would be less able to compete for prime habitat, and thus may settle sooner, sampling fewer habitats. Size in terrestrial salamanders is a strong indicator of competitive ability and territory quality (Mathis, 1990, 1991), but the effects of size on habitat selection have not been tested in a pond-breeding amphibian. Additional experiments are necessary to determine whether either of these mechanisms is driving the increased

switching behaviour and/or decreased latency that we observed in larger juveniles.

Habitat preference in our study was similarly fixed for both species. Contrary to the natural history of *A. maculatum*, which is thought to be a forest specialist, but less surprising for *A. texanum*, a habitat generalist (Petranka, 1998), both species had a strong preference for grass litter cues. This does not support our hypothesis of habitat specialization, which predicted that *A. maculatum* would prefer leaf substrates and that *A. texanum* would show no preference. Our study is not the first to find a forest specialist salamander preferring grass habitat (Pittman & Semlitsch, 2013). Semlitsch et al. (2012) found that graycheek salamanders, *Plethodon metcalfi*, moved slowly and had a tortuous path when travelling through mowed grass, similar to their movement through leaf litter and soil, but unlike their rapid, straight-line movement through asphalt and gravel. Similar findings were reported in a field experiment with a pond-breeding amphibian. Juveniles of *A. maculatum* metamorphosing from ponds in Missouri were more likely to enter herbaceous habitats than they were a second-growth forest or recently burned habitat (Osborn, 2012). This selection of herbaceous habitats by salamanders could be driven by moisture content. Juvenile salamanders may be attracted to the higher moisture content in grass fields and old clear-cuts than in forests (Rothermel & Luhring, 2005; Rothermel & Semlitsch, 2002). However, amphibians also experience higher mortality in field habitats, likely due to higher maximum temperatures (Rothermel & Luhring, 2005; Rothermel & Semlitsch, 2002). More direct data on the movement and survival of individuals across time are required to test this hypothesis.

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## References

- Bates, D. M., Maechler, M., & Bolker, B. M. (2012). *lme4: Linear mixed-effects models using Eigen and Eigen*. Vienna, Austria: R Foundation for Statistical Computing.
- Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulseley, C. D., et al. (2003). The ecology of individuals: incidence and implications of individual specialization. *American Naturalist*, *161*, 1–28.
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multi-model inference: A practical information-theoretic approach* (2nd ed.). New York, NY: Springer.
- Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, *12*, 197–209.
- Davis, J. M. (2007). Preference or desperation? Distinguishing between the natal habitat's effects on habitat choice. *Animal Behaviour*, *74*, 111–119.
- Davis, J. M. (2008). Patterns of influence of natal experience on habitat choice. *Quarterly Review of Biology*, *83*, 363–380.
- Davis, J. M., & Stamps, J. A. (2004). The effect of natal experience on habitat preferences. *Trends in Ecology & Evolution*, *19*, 411–416.
- Earl, J. E., Luhring, T. M., Williams, B. K., & Semlitsch, R. D. (2011). Biomass export of salamanders and anurans from ponds is affected differentially by changes in canopy cover. *Freshwater Biology*, *56*, 2473–2482.
- Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Thousand Oaks, CA: Sage.
- Hothorn, T., Bretz, F., & Peter, W. (2008). Simultaneous inference in general parametric models. *Biometrics*, *50*, 346–363.
- Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, *14*, 343–348.
- Mannan, R. W., Mannan, R. N., Schmidt, C. A., Estes-Zumpf, W. A., & Boal, C. W. (2007). Influence of natal experience on nest-site selection by urban-nesting Cooper's hawks. *Journal of Wildlife Management*, *71*, 64–68.
- Massot, M., & Clobert, J. (2000). Processes at the origin of similarities in dispersal behaviour among siblings. *Journal of Evolutionary Biology*, *13*, 707–719.
- Mathis, A. (1990). Territoriality in a terrestrial salamander: the influence of resource quality and body size. *Behaviour*, *112*, 162–175.
- Mathis, A. (1991). Territories of male and female terrestrial salamanders: costs, benefits, and intersexual spatial associations. *Oecologia*, *86*, 433–440.
- Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, *16*, 254–260.
- Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*, 1635–1645.
- Morris, D. W. (2003). Toward an ecological synthesis: a case for habitat selection. *Oecologia*, *136*, 1–13.
- Morris, D. W. (2011). Adaptation and habitat selection in the eco-evolutionary process. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 2401–2411.
- Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., et al. (2008). A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 19052–19059.
- Osborn, M. S. (2012). *Initial juvenile movement of pond-breeding amphibians in an altered forest habitat* (Unpublished doctoral thesis). Columbia, MO: University of Missouri.
- Patrick, D. A., Harper, E. B., Hunter, M. L., & Calhoun, A. J. K. (2008). Terrestrial habitat selection and strong density-dependent mortality in recently metamorphosed amphibians. *Ecology*, *89*, 2563–2574.
- Peterman, W. E., Locke, J. L., & Semlitsch, R. D. (2013). Spatial and temporal patterns of water loss in heterogeneous landscapes: using plaster models as amphibian analogues. *Canadian Journal of Zoology*, *140*, 135–140.
- Petranka, J. W. (1998). *Salamanders of the United States and Canada*. Washington, D.C.: Smithsonian Institution Press.
- Pittman, S. E., Osborn, M. S., Drake, D. L., & Semlitsch, R. D. (2013). Predation of juvenile ringed salamanders (*Ambystoma annulatum*) during initial movement out of ponds. *Herpetological Conservation and Biology*, *8*, 681–687.
- Pittman, S. E., Osborn, M. S., & Semlitsch, R. D. (2014). Movement ecology of amphibians: a missing component for understanding population declines. *Biological Conservation*, *169*, 44–53.
- Pittman, S. E., & Semlitsch, R. D. (2013). Habitat type and distance to edge affect movement behavior of juvenile pond-breeding salamanders. *Journal of Zoology*, *291*, 154–162.
- Pittman, S. E. (2013). *Movement ecology of juvenile pond-breeding salamanders: Implications for the management and conservation of amphibian populations* (Unpublished doctoral thesis). Columbia, MO: University of Missouri.
- R Development Core Team. (2012). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rittenhouse, T. A. G., & Semlitsch, R. D. (2007). Distribution of amphibians in terrestrial habitat surrounding wetlands. *Wetlands*, *27*, 153–161.
- Rothermel, B. B., & Luhring, T. M. (2005). Burrow availability and desiccation risk of mole salamanders (*Ambystoma talpoideum*) in harvested versus unharvested forest stands. *Journal of Herpetology*, *39*, 619–626.
- Rothermel, B. B., & Semlitsch, R. D. (2002). An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology*, *16*, 1324–1332.
- Scott, D. E. (1994). The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*, *75*, 1383–1396.
- Scott, D. E., Casey, E. D., Donovan, M. F., & Lynch, T. K. (2007). Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. *Oecologia*, *153*, 521–532.
- Semlitsch, R. D., Ecrement, S., Fuller, A., Hammer, K., Howard, J., Krager, C., et al. (2012). Natural and anthropogenic substrates affect movement behavior of the southern graycheek salamander (*Plethodon metcalfi*). *Canadian Journal of Zoology*, *90*, 1128–1135.
- Semlitsch, R. D., & Pechmann, J. H. K. (1985). Diel pattern of migratory activity for several species of pond-breeding salamanders. *Copeia*, *1985*, 86–91.
- Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*, 372–378.
- Spotila, J. R. (1972). Role of temperature and water in the ecology of lungless salamanders. *Ecological Monographs*, *42*, 95–125.
- Stamps, J. A. (2006). The silver spoon effect and habitat selection by natal dispersers. *Ecology Letters*, *9*, 1179–1185.
- Stamps, J. A., & Davis, J. M. (2006). Adaptive effects of natal experience on habitat selection by dispersers. *Animal Behaviour*, *72*, 1279–1289.