# 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 ($17 \times 12 \times 9$ 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 $\pm$ SD of $11 \pm 3.2$ days and $14 \pm 6.8$ 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; $\pm1$ mm) and wet mass ($\pm0.001$ 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 $\times$ 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 ($\sim$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