

Use of Olfactory Cues by Newly Metamorphosed Wood Frogs (*Lithobates sylvaticus*) during Emigration

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Juvenile amphibians are capable of long-distance upland movements, yet cues used for orientation during upland movements are poorly understood. We used newly metamorphosed Wood Frogs (*Lithobates sylvaticus*) to investigate: (1) the existence of innate (i.e., inherited) directionality, and (2) the use of olfactory cues, specifically forested wetland and natal pond cues during emigration. In a circular arena experiment, animals with assumed innate directionality did not orient in the expected direction (suggested by previous studies) when deprived of visual and olfactory cues. This suggests that juvenile Wood Frogs most likely rely on proximate cues for orientation. Animals reared in semi-natural conditions (1500 l cattle tanks) showed a strong avoidance of forested wetland cues in two different experimental settings, although they had not been previously exposed to such cues. This finding is contrary to known habitat use by adult Wood Frogs during summer. Juvenile Wood Frogs were indifferent to the chemical signature of natal pond (cattle tank) water. Our findings suggest that management strategies for forest amphibians should consider key habitat features that potentially influence the orientation of juveniles during emigration movements, as well as adult behavior.

UNDERSTANDING the movements of migrants and dispersers and identifying habitats selected during the movements to and from breeding ponds is critical for identifying conservation strategies for pond-breeding amphibians (Semlitsch, 2008). Orientation and navigation play important roles in guiding amphibian movements in both the aquatic and terrestrial environments (Sinsch, 2006). Amphibians rely on a multisensory orientation system (Ferguson, 1971) that uses a wide variety of mechanisms, including path integration, beaconing, pilotage, compass orientation, and true navigation (Sinsch, 2006). Orientation during the transition between the aquatic and terrestrial stages (i.e., immediately post-metamorphosis) raises particularly interesting questions because of the change in locomotion, the availability of cues, as well as the potential carryover of sources of information between the larval and juvenile life stages (Hepper and Waldman, 1992). For example, orientation information acquired during the larval stage was found to influence American Bullfrog (*Lithobates catesbeianus*) movements during the onset of emigration in the terrestrial environment (Goodyear and Altig, 1971).

Migratory movements of adult amphibians toward breeding ponds and overwintering sites are well understood and have been shown to rely on a multitude of cues that are used in a hierarchical manner depending on the relative availability of cues (Dall'antonia and Sinsch, 2001). Olfaction plays an important part in adult orientation, with odors being used as directional cues for both short-distance orientation toward mates and long-distance homeward orientation (Tracy and Dole, 1969; McGregor and Teska, 1989). Adult breeding migrations were found to be guided by the chemical signature of the breeding ponds in both anurans (Forester and Wisnieski, 1991; Sanuy and Joly, 2009) and urodeles (Hershey and Forester, 1980; Joly and Miaud, 1993).

In contrast to the adult life stage, juvenile movements and orientation remain largely unstudied, despite their long-distance emigration and dispersal abilities (Cushman, 2006). Migrating juveniles are hypothesized to have a narrow perception range and consequently rely on proximate cues for orientation in the upland habitat (Rothermel and Semlitsch, 2002; Rothermel, 2004; Popescu and Hunter, 2011). Because natal experience is linked to habitat selection at later life stages (Davis and Stamps, 2004), the ability to recognize the chemical signature of the natal pond is thought to play a prominent role for juvenile orientation during emigration movements (Hepper and Waldman, 1992). The behavioral responses to natal pond odors are species-specific (i.e., reflect the species' affinity to aquatic or terrestrial environments), change during ontogeny, and exhibit high individual variability (Shakhparonov and Ogurtsov, 2003; Arhipova et al., 2005). The imprinting of specific odors in anurans can occur during the embryonic stage, as well as during two distinct stages of larval development (Gosner 19–21 and 31–41), and the discrimination between imprinted and novel odors during the aquatic stage is maintained during the early terrestrial stage (Ogurtsov and Bastakov, 2001).

Despite the strong evidence for the use of breeding/natal pond odors for orientation, little is known about the use of other olfactory cues during movements in the terrestrial environment. For the juvenile stage of our study organism, the vernal pool-breeding Wood Frog (*Lithobates sylvaticus*), previous research conducted in Maine, USA found evidence that forested wetlands attracted a substantial segment of the local juvenile cohort, and that directionality during emigration movements might be genetically inherited (Vasconcelos and Calhoun, 2004; Patrick et al., 2007). The attraction to forested wetlands has conservation implications because forested wetlands are a scarce resource in some landscapes. Also, anthropogenic changes in land use have the potential

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to affect populations in which directionality of emigration movements is inherited by attracting migrants into ecological traps (*sensu* Schlaepfer et al., 2002). However, in other landscapes, the emigration of juvenile Wood Frogs was either completely random (Popescu et al., 2012), or random across seasons and sites, but with evidence of local directionality that shifted on a seasonal basis (Timm et al., 2007; Homan et al., 2010). Upon exiting natal ponds, orientation does not necessarily reflect directionality farther in upland habitat (Jenkins et al., 2006; Roznik and Johnson, 2009); that is, it might be wrong to assume that habitat selected during emigration movements from the natal pond into upland is consistent with orientation at the pond edge.

Given the conflicting body of evidence on the orientation of post-metamorphic Wood Frogs and the need to better understand the orientation behaviors during the transition between the aquatic and terrestrial environments, the objectives of this study were to investigate whether or not newly metamorphosed *L. sylvaticus* possess innate directionality, and to evaluate the use of olfactory cues during emigration movements. For the first objective, we hypothesized that if animals possess innate directionality, unrelated to the landscape context, then eliminating potential cues, such as visual (i.e., celestial, landmarks), and chemical cues (i.e., olfactory), would not affect their preferred direction of movement. For the second objective, we examined the role of forested wetland and natal pond odors. Based on current literature, our predictions were: juveniles would be attracted to forested wetland olfactory cues, and juveniles would be able to recognize their natal pond odor.

MATERIALS AND METHODS

Amphibian collection.—We collected egg masses from roadside ditches and other small, ephemeral pools located on the University of Maine forests in central Maine, USA (44°54'16"N, 68°41'55"W). Wood Frog larvae were hatched in plastic wading pools (1 m diameter and 25 cm tall) filled halfway with water and covered with shade cloth to prevent predation on egg masses (two egg masses per pool). Upon hatching, larvae from wading pools were moved to 1500-liter self-sustaining mesocosms (cattle tanks), stocked with 1 kg of dried leaf litter and plankton from nearby vernal pools, where they were reared to metamorphosis. We stocked cattle tanks at a density of 80 larvae per tank; the larvae hatched in wading pools that were not transferred to cattle tanks were taken back to the collection sites and released. The tanks were covered with shade cloth to prevent colonization by other aquatic organisms and placed under closed canopy. Wading pools and cattle tanks were initially filled with water from a nearby well, and water level was maintained naturally by precipitation throughout the rearing period (Popescu and Hunter, 2011). For the experiment pertaining to the second objective, we also collected early stage tadpoles (Gosner 25–30; Gosner, 1960) from two artificial pools on Sears Island, Maine (44°26'36"N, 68°53'20"W; Vasconcelos and Calhoun, 2004), where innate directionality upon emergence was found to occur in juvenile Wood Frogs (Patrick et al., 2007). Tadpoles were also reared to metamorphosis in cattle tanks. When the animals reached the final stage of metamorphosis (Gosner 46), they were transferred individually to plastic containers (40 × 20 × 20 cm) with moist paper towels. Individuals were tested two days after removal from cattle tanks (they were given enough time to completely reabsorb the tail). The

third day (i.e., after testing), animals were returned to the sites where the egg masses ("local" animals) or tadpoles (Sears Island animals) were collected and released.

Circular arena design.—A circular arena was used to investigate whether or not juveniles possess innate directionality, as well as to obtain preliminary data on the use of olfactory cues during emigration. The experimental site was located on the University of Maine forests, in a 100 × 50 m clearing, where we erected a large canvas tent (6 × 4 m and 2.8 m in height). All sides of the tent were opened during the day to ensure air flow. We performed the experiment at night (starting at 2130 h EST) between 14 July and 3 August 2009. The tent eliminated the potential use of visual cues, such as celestial (Diego-Rasilla and Luengo, 2002), and landmarks (i.e., forest edges; Gibbs, 1998; Rothermel, 2004; Popescu and Hunter, 2011), and prevented the inflow of external airborne chemical cues. The test arena was a circular plastic container 1.8 m in diameter and 20 cm tall. Inside the arena, we marked a 1.5 m diameter circle at 10° intervals, which we used as the threshold for recording the direction of movement. We placed each animal under an opaque plastic container in the middle of the arena; we lifted the container using a pulley system (following a two-minute recovery period) and recorded the compass bearing (departure from magnetic North) at which each test animal crossed the 1.5 m circle inscribed on the floor of the test arena. Because we conducted the experiment in complete darkness and did not want to influence frog behavior, we used night-vision binoculars (Rigel Optics, DeWitt, Iowa) to observe animals from approximately 3 m outside the arena (Popescu and Hunter, 2011). The floor of the arena was wiped with paper towels and distilled water and allowed to dry between each trial in order to eliminate interference from odor trails left by previous animals (Adler, 1980; Diego-Rasilla and Luengo, 2002).

Circular arena experimental releases.—First, we tested whether or not newly metamorphosed Wood Frogs show innate directionality at emergence. We contrasted animals with assumed innate directionality from restored ponds on Sears Island (Patrick et al., 2007) and animals from University of Maine forests where they are known to be exiting natal wetlands without evidence of directionality (Popescu et al., 2012). The animals with innate directionality were hypothesized to orient toward the northeast (approximately 41–71° departure from magnetic North), in the direction of a forested wetland. Second, we conducted a preliminary test of the use of olfactory cues by juvenile Wood Frogs, specifically forested wetland cues, using animals from the second set of pools only (i.e., with no assumed innate directionality). Previous research suggested that juvenile amphibians are attracted to forested wetlands (Vasconcelos and Calhoun, 2004; Patrick et al., 2007), so the chemical signature would act as an attractant. The protocol was similar to the previous experiment, but we added a small tub (40 × 25 × 20 cm) filled with forested wetland organic soil adjacent to the circular arena, at a random bearing (220°). We then repeated the experiment with the tub located at 130° and 40° (a 90° and 180° shift from the initial location). The forested wetland organic soil was collected from a forested wetland located >10 km from the location of the larvae-rearing mesocosms, thus ensuring that animals would not recognize the soil as natal.

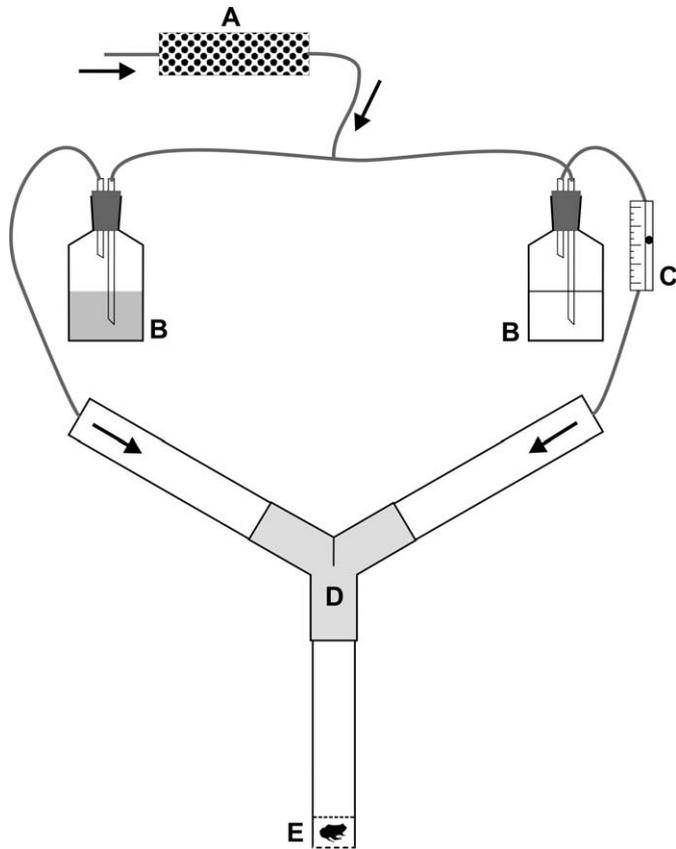


Fig. 1. Design of the Y-maze used to test the use of olfactory cues by juvenile Wood Frogs (*L. sylvaticus*): (A) activated carbon filter, (B) bubbling flasks containing the olfactory cues to be tested, (C) flowmeter, (D) PVC connector, and (E) wire mesh cage at the end of the arm where animals were released (used for acclimating animals for two minutes prior to release). Arrows indicate the direction of airflow; drawing not to scale.

Y-maze experimental design.—Following the preliminary investigation of the use of olfactory cues, we performed an olfactory dichotomous choice experiment. We built a Y-maze from clear acrylic tubing (5.7 cm in diameter), and a 3-way PVC connector (120° between each connection), with each arm 30 cm long (Fig. 1). To avoid mixing of the cues before the main (releasing) arm of the olfactometer, we inserted a 5 cm long separating wall inside the connector (D in Fig. 1) between the two arms receiving the cues. We used two olfactory cues: (1) forested wetland organic soil, and (2) natal pool (cattle tank) water. We tested these cues against distilled water, a control neutral cue. We pumped ambient air into 150 ml glass bubble flasks which contained either 100 ml of testing cue or distilled water. The forested wetland cue was prepared by mixing 60 ml of saturated organic soil with 40 ml of distilled water to bring it to a consistency that allowed bubbling. The natal pool cue was cattle tank water collected one hour prior to the beginning of the experiment. Because we used ambient air, we attached an activated carbon filter (clear PVC pipe, 25 cm in length and 5 cm in diameter filled with 4–6 mm activated carbon pellets) to remove impurities and eliminate ambient odors (Fig. 1). Air flow was maintained constant at 4 l/minute, measured with a Dwyer VFA-22 flowmeter (Dwyer Instruments, Inc., Michigan City, IN). The Y-maze was rinsed with distilled water and dried between the trials to eliminate both odors from previous animals and traces of cue (Forester and

Wisniewski, 1991). We randomly switched the treatment cue and the control between the left and right arms of the Y-maze to eliminate potential directional bias induced by the experimental setup itself. We also replaced the cues for every other trial in order to maintain a fresh source of odors.

Y-maze experimental releases.—We conducted the experiment in low, diffuse overhead red light oriented away from the setup, during 21–26 July 2010, between 0800 and 1200 h EST. After a two-minute acclimation period, we lifted a wire mesh gate (E in Fig. 1), and animals were allowed to move freely. An animal was considered to exhibit a preference once it moved more than halfway into one of the cue arms (Forester and Wisniewski, 1991). Based on our experience with the circular arena experiment, animals that did not reach the end of one of the arms in <10 minutes ($n = 6$) were not counted as successful trials and were discarded from the analysis. We conducted the experiment at constant temperature (22°C) and relative humidity (80%). For all experiments, each animal was used only once.

Statistical analyses.—For the circular arena trials, we used circular statistics (Batschelet, 1981) to investigate whether or not juveniles exhibit innate directionality. We used Oriana 3.13 (Kovach Computing Services, Anglesey, Wales, U.K.) to run separate Rayleigh tests for uniformity for animals with and without hypothesized innate directionality to test whether or not there is significant departure from randomness. We examined the circular variance for each treatment as a measure of spread of individual bearings. The circular variance takes a value between 0 (clustered around a mean angle) and 1 (dispersed with respect to the mean angle; Batschelet, 1981). After plotting the raw data, we also performed a *post-hoc* test for axial orientation for the Sears Island animals, which seemingly showed a bidirectional pattern. We transformed the raw data to axial by doubling the values, reducing any value >360° using modulo arithmetic, and converting the data back to a 0–180° range (Fisher, 1993).

For the preliminary investigation of the use of olfactory cues in the circular arena, we chose not to use circular statistics, because two of the possible outcomes of the experiment—attraction and avoidance—are not symmetrical processes (Haila et al., 1996). Attraction, revealed by concentration of animals around the cue, is a strongly deterministic process (Haila et al., 1996) for which the use of circular statistics would be warranted. However, avoidance is likely to lead to a random distribution (i.e., not necessarily concentrated in the opposite direction of the cue) because avoidance is not a deterministic process and leaves a large margin of variation (Haila et al., 1996). Therefore, we used the bearing departure from the location of the cue (clockwise and counterclockwise), which ranged between 0° (exact location of cue) and 180° (opposite location of cue) to assess the concentration of animals within ± 30 , 45, 60, and 90° from the cue location. We then used a G-test for goodness of fit to test whether or not the number of individuals observed within 60, 90, 120, and 180° arcs centered on the cue, respectively, was significantly different than expected under the assumption of randomness. We pooled the bearing-departure-from-cue data for all three cue locations.

For the Y-maze experiment, we used a G-test of goodness of fit, under the assumption of equal preference of treatment

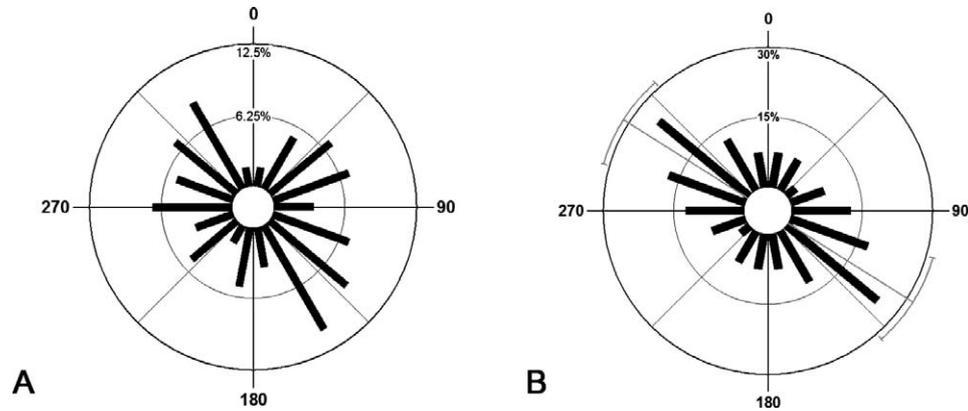


Fig. 2. Lack of orientation of juvenile Wood Frogs (*Lithobates sylvaticus*) (A) without assumed innate directionality, and (B) axial orientation of juveniles with assumed innate directionality (gray lines and arcs are mean axis and 95% confidence intervals, respectively). For (B), assumed directionality was toward northeast (41–71°; Patrick et al., 2007).

cues and control. We ran separate tests for forested wetland organic soil cue vs. distilled water, and natal pool vs. distilled water. For all tests, we used a significance level of $\alpha = 0.05$.

RESULTS

Circular arena experiments.—In the innate directionality experiment, juvenile Wood Frogs from the control, non-directional pools oriented at random (Fig. 2A), and did not show a departure from circular uniformity ($n = 57$, Rayleigh test; $Z = 0.267$, $P = 0.766$). Animals that originated from the pools where innate directionality was assumed (Sears Island) showed a significant axial (bidirectional) orientation ($n = 40$, Rayleigh test; $Z = 5.455$, $P = 0.004$), with a direction of $122.4^\circ \pm 40.4$ (Fig. 2B). Because the two groups of animals differed in their orientation, we were not able to test whether or not they shared a mean direction. The spread of bearings with regard to the mean direction was large for both groups, as expressed by the high values of the circular variance (0.809 for animals with assumed innate directionality; 0.932 for control animals).

In the same experimental setup, the forested wetland olfactory cues acted as a deterrent, rather than as an attractant for juvenile Wood Frogs. For all three locations of the wetland cue, 220° ($n = 29$), 130° ($n = 24$), and 40° ($n = 28$), animals tended to move away from the forested wetland cue (Fig. 3). When all three locations were pooled, significantly fewer individuals than expected reached the edge of the arena within 60° ($n = 5$; $G = 4.056$, $df = 1$, $P = 0.044$), 90° ($n = 7$; $G = 6.724$, $df = 1$, $P = 0.009$), and 120° arcs ($n = 11$; $G = 6.952$, $df = 1$, $P = 0.008$) centered on the cue (Fig. 4). A smaller than expected number of animals also reached the edge of the orientation arena in the half-circle centered on the cue (180° arc), but the results were not significant at $\alpha = 0.05$ ($n = 26$; $G = 3.187$, $df = 1$, $P = 0.074$). In addition, we observed 12 individuals out of 81 releases (14.8%) that initially started moving in the general direction of the forested wetland cue, but switched direction when they were approximately 50 cm from the cue, thus appearing to show active avoidance.

Y-maze experiment.—The Y-maze experiment also yielded evidence for avoidance of forested wetland cues. In 38 successful tests, 73.7% of the animals ($n = 28$) chose the distilled water over the forested wetland cue ($G = 8.878$, $df =$

1, $P = 0.003$). Frogs did not exhibit preference or avoidance of natal pool water; in 37 successful tests 40.5% ($n = 15$) chose the distilled water ($G = 1.332$, $df = 1$, $P = 0.248$).

DISCUSSION

Innate directionality during emigration movements.—We did not find evidence for innate directionality in juvenile Wood Frogs. The control animals oriented randomly when visual and olfactory cues were removed (Fig. 2A). Animals with assumed inherited directionality showed an axial orientation, but the general observed direction (122° , Fig. 2B) did not match the expected northeastern ($41\text{--}71^\circ$) direction suggested by Patrick et al. (2007). However, this finding does not exclude the possibility that even if directionality *per se* is not inherited, information on the strongest cues used for orientation might be transferred between successive generations. Moreover, most previous experimental evidence points at proximate cues as the main source of orientation immediately post-metamorphosis. For example, directionality of emergence in American Bullfrogs was found to be established during the late stages of metamorphosis, and it was influenced by the variation in the surrounding environment (Goodyear and Altig, 1971), both aquatic (e.g., shallow water) and terrestrial (e.g., presence of shade from adjacent trees). This line of evidence might explain the results of Vasconcelos and Calhoun (2004) and Walston and Mullin (2008), who found that juvenile emergence was directed toward tracts of closed-canopy forest. These studies were conducted in landscape settings where contiguous tracts of forests were concentrated in specific directions, which coincided with the direction of emigration movements. For Wood Frogs, researchers have shown high phenotypic plasticity and rapid evolutionary changes in the larval life-history stages (Skelly et al., 2002; Skelly, 2004), so it is not improbable that inherited directionality based on some prominent cue can be easily acquired, but also lost, if the local conditions change. This hypothesis is also supported by Miaud et al. (2003) who found that upon emergence, newly metamorphosed Common Frogs (*Rana temporaria*) from two populations located on the edge of a large lake showed directionality toward patches of suitable forest habitat. They concluded that the observed local adaptation for directionality of migrations in Common Frogs probably occurred in <100 frog generations, a period coinciding with major land cover changes.

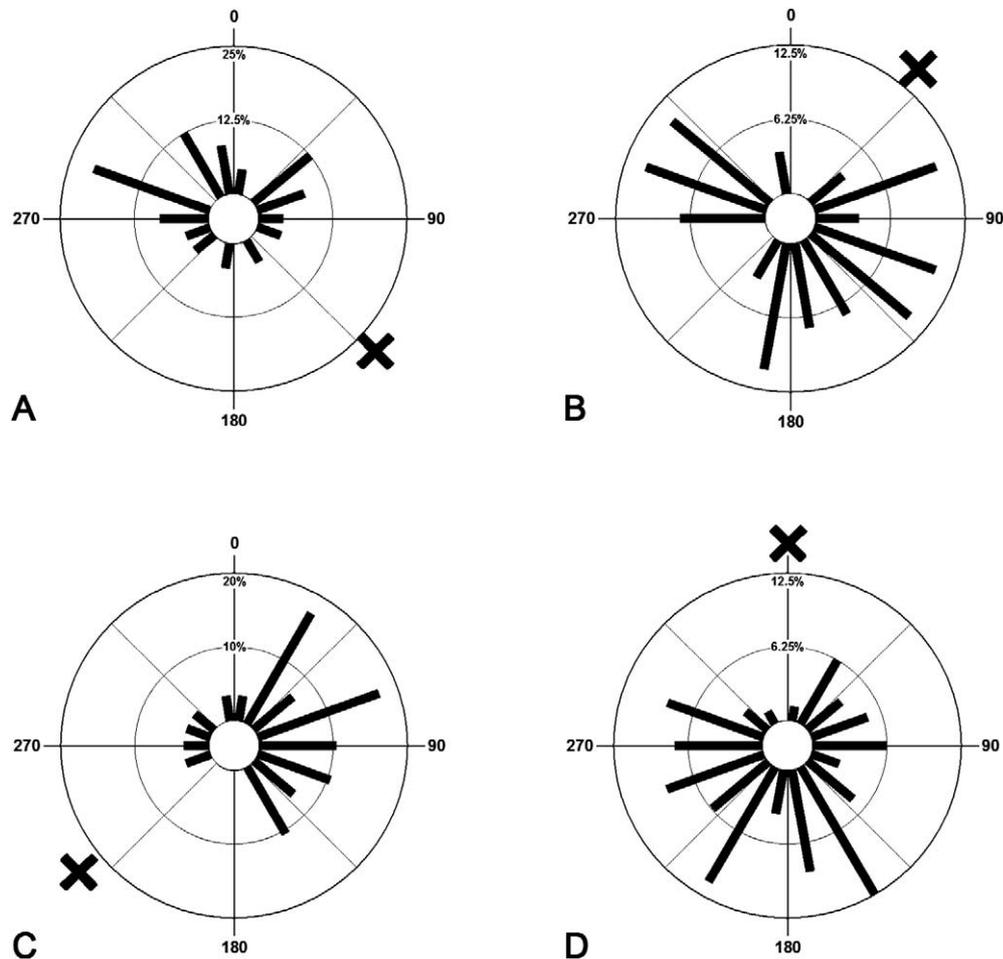


Fig. 3. Orientation of juvenile Wood Frogs (*Lithobates sylvaticus*) exposed to forested wetland olfactory cues in three circular arena trials with different locations of the cue source: (A) 130°, (B) 40°, (C) 220°, and (D) all trials combined and standardized to cue = 0° bearing. (X = location of cue).

The possibility of innate directionality also comes into play when thinking about wetland mitigation. Building artificial isolated wetlands (i.e., breeding pools mimicking the hydroperiod of natural pools) has been used as a mitigation strategy in the northeastern United States (Korfel

et al., 2010). If consistent directionality due to some prominent habitat feature unavailable in all directions occurs across seasons and is inherited across generations, then populations from older pools should show consistent directionality compared to populations from newly created pools. However, evidence from field studies in forested landscapes with both new artificial (Patrick et al., 2006) and natural breeding pools (Jenkins et al., 2006; Timm et al., 2007; Homan et al., 2010) showed that juvenile vernal pool amphibians exhibit non-directional orientation at emergence across many seasons, but directional orientation might be the norm in a given year in some settings (Jenkins et al., 2006; Homan et al., 2010). A switch in directionality across years for the same breeding pool refutes the role of innate directionality toward a stable patch of upland habitat. Instead it suggests that temporally and spatially variable environmental cues influence the orientation upon emergence, and questions the idea that juveniles might disperse into ecological traps (*sensu* Patrick et al., 2007; Homan et al., 2010).

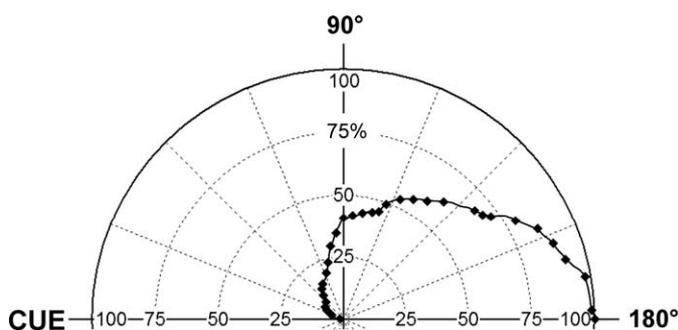


Fig. 4. Cumulative percentage of juvenile Wood Frogs (*Lithobates sylvaticus*) orienting with respect to forested wetland cues in a circular arena during three trials ($n = 81$). The circular axis shows the departure from cue location (angles 0–180°, where 180° represents the opposite side of cue location), and the linear axes show the cumulative percentage of animals (0–100%; i.e., approx. 10% of animals were within 45° of cue; 40% within 90°, etc.). Perfect attraction would be represented by a horizontal line from origin to cue; perfect avoidance would be represented by a horizontal line from origin to 180°.

Response to forested wetland cues.—Our research suggests that olfactory cues are used by juvenile Wood Frogs during emigration from the natal pool into the upland. Contrary to our hypothesis, based on existing literature (notably Vasconcelos and Calhoun, 2004; Patrick et al., 2007), juveniles tended to avoid forested wetland cues, and they

did so in two different experimental setups. Although attraction, rather than avoidance of forested wetlands, was expected due to juveniles' low tolerance to desiccation (Schmid, 1965), our research suggests that factors not driven by physiological requirements may play a more prominent role in habitat selection post-metamorphosis. The avoidance of forested wetland olfactory cues by animals raised in cattle tanks and not previously exposed to such odors prior to the experiment, and the discordance between our findings and previous studies raises two main questions: (1) what is the relative importance of cues used for orientation during migrations, and (2) what are the mechanisms inducing the avoidance of forested wetland cues?

For the first question, the attraction of newly metamorphosed Wood Frogs to forested wetlands suggested by Vasconcelos and Calhoun (2004) might have occurred as a result of non-olfactory cues (i.e., lack of canopy cover in the opposite direction of the forested wetland), overriding less strong cues, such as chemical signature of forested wetlands. Moreover, the relative importance of cues used for orientation may differ among individuals from the same population or cohort (Dall'antonia and Sinsch, 2001; Shakhparonov and Ogurtsov, 2003). Given such variability in individual behavior and the narrow perception range of juvenile amphibians (Rothermel and Semlitsch, 2002; Rothermel, 2004; Popescu and Hunter, 2011), it is likely that olfaction comes into play only when chemical signatures used for orientation override other cues.

For the second question, one explanation for the avoidance of forested wetlands could be the affinity of Wood Frogs for upland terrestrial habitat immediately post-metamorphosis. Newly metamorphosed Wood Frogs quickly emigrate and settle in upland habitat up to 150 m from the natal pool (Patrick et al., 2008). Because juveniles feed on invertebrates within the leaf litter layer, forested uplands might provide a better food resource for these animals compared to forested wetlands. Another explanation for the avoidance of forested wetland olfactory cues is that juveniles may associate this odor with that of the natal pool, given that in our landscape, vernal pools often occur in forested wetland complexes (Calhoun and deMaynadier, 2007). Thus, avoidance of forested wetlands could be equivalent to avoiding the proximity of natal pools where juveniles might experience higher competition for food with individuals in the same cohort. In addition, avoidance could be related to higher predation risk; for example, American Bullfrogs, which use seasonal pools during summer (Gahl et al., 2009), are known to predate on other anurans (Stewart and Sandison, 1972).

Response to natal pool odors.—We did not find avoidance or attraction behaviors in newly metamorphosed Wood Frogs with respect to the odor of natal pool (i.e., cattle tank water). The indifference to natal pond odors adds to the evidence that their natal ponds do not elicit a behavioral response for juveniles of terrestrial and semi-terrestrial anurans after the onset of emigration movements. For example, juveniles of the semi-aquatic Green Toad (*Bufo viridis*) and terrestrial Common Frog were attracted by the natal pool odor before the onset of emigration, whereas during emigration movements, they either avoided or were indifferent to the same cues (Shakhparonov and Ogurtsov, 2003; Arhipova et al., 2005), indicating a plastic response to natal pond cues.

The affinity to either terrestrial or aquatic environments post-metamorphosis affects the response to the natal pool odor and offers further support to our findings. Bastakov (1992) found that juveniles of the highly aquatic Pool Frog (*Rana lessonae*) were attracted by the odor of their natal pool, while Common Toad juveniles (*Bufo bufo*), a semi-aquatic species, tended to avoid the odor of their natal pool. In addition, Arhipova et al. (2005) found that the response of juvenile Green Toad and Common Frog to natal pool odors was dependent on whether animals were held in laboratory setting (i.e., 9–12 days) or were collected from the wild prior to the experiment. That is, natal pool cues elicited an attraction behavior from animals held in laboratory conditions, while animals caught in natural conditions were indifferent to such cues. In this context, experiments that minimize laboratory holding period (such as used in our experiment) and use animals raised in natural or semi-natural conditions (e.g., mesocosms) are more likely to identify real-world behaviors (Šamajová and Gvoždík, 2010).

Summary.—Orientation behaviors are deterministic responses to a hierarchy of environmental cues ranging from strongest to weakest. In our experiment, animals collected from a landscape that was assumed to induce innate orientation behaviors upon emergence failed to exhibit inherited directionality when tested in controlled conditions. Newly metamorphosed Wood Frogs were indifferent to natal pool odors, but exhibited strong avoidance of forested wetland chemical signature that potentially reflects upland habitat selection during emigration movements. Using two different experimental setups—circular arena and Y-maze—we also showed that relying on circular statistics to test for directionality fails to correctly identify behaviors when the ecological process is avoidance, rather than attraction to a particular cue.

However, we are still far from understanding the relative use of direct cues for orientation during juvenile upland movements and the importance of inherited directionality during the transition between the aquatic and terrestrial stages in anurans. Research aiming at differentiating between genetic variation and phenotypic variation (i.e., environmentally driven) in orientation behavior is needed (Via and Lande, 1985). Such research might be accomplished by accounting for sources of spatial and temporal variability and availability of cues, testing a hierarchy of potential cues, as well as using transplants and common garden experiments and genetic manipulations (i.e., hybrids from populations with different observed directional traits; Mousseau et al., 2000). This would potentially lead to a better understanding of habitat selection by juvenile amphibians, a critical and data-deficient life stage.

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