



# Movement of amphibians through agricultural landscapes: The role of habitat on edge permeability



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## ABSTRACT

Long-term population persistence depends on successful dispersal and colonization. Within agricultural landscapes, dispersing individuals encounter a variety of edge types. How individuals respond to edges can dictate whether they are permeable to dispersal or act as barriers, with consequences for population connectivity. Using two amphibian species, bullfrogs (*Lithobates catesbeianus*) and Blanchard's cricket frogs (*Acris blanchardi*), we conducted two studies to address (1) how abiotic factors influence habitat choice and (2) how habitat choice and movement behavior change based on edge type (edges were between grass–corn, grass–forest, and forest–corn habitats). In the first experiment we found that both species preferred high soil moisture environments during both the day and night; and at night neither species showed a preference for ground or canopy cover. However, during the day bullfrogs had a preference for both ground and canopy cover, while cricket frogs showed no preference. In the second experiment, we found that bullfrogs had no overall preference for one habitat type over another; they were observed traveling along the edge of two habitats. Cricket frogs, on the other hand, showed a strong aversion to forest habitat. We concluded that behavioral responses to different habitats may explain current population trends; movement along edge habitat may allow bullfrogs to readily disperse through altered landscapes whereas cricket frog declines may occur in areas experiencing reforestation due to increased isolation. Conservation practices in agricultural land will likely improve landscape connectivity for these species, especially for declining cricket frog populations.

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## 1. Introduction

Maintaining functional connectivity in dynamic landscapes is imperative for population persistence and depends on the dispersal capabilities and movement behaviors of a species as well as the spatial configuration of the landscape (Brown and Kodric-Brown, 1977; Fahrig, 2003; Baguette and Van Dyck, 2007). Behavioral responses to edge may be particularly important in fragmented landscapes, which are characterized by increased area of edge. Edge permeability, which depends on an organism's willingness to cross from one habitat type into another, can play a large role in population connectivity and explain distribution patterns (Stamps et al., 1987; Haynes and Cronin, 2006; Stasek et al., 2008). Edges with high permeability will enhance connectivity between patches, whereas impermeable edges will serve as barriers to dispersal (Stamps et al., 1987). Edge permeability is a function of an individual's habitat preferences resulting from the interaction of physiological

constraints (thermal and desiccation stress), resource requirements, and risk of predation (e.g. Russell et al., 2007; Rykken et al., 2011; Janin et al., 2012). Understanding species responses to edge is critical for predicting connectivity between populations and long-term population persistence; it is of particular interest for species with low mobility, like amphibians.

Studies of habitat or edge permeability have largely focused on the effects of forest fragmentation (e.g. Gobeil and Villard, 2002; Rykken et al., 2011). Population connectivity is particularly important for species like amphibians that experience high turnover rates and exist as metapopulations or patchy populations (Smith and Green, 2005). Studies of amphibian movement at forest edges have found that forests have higher permeability than other habitat types (Vasconcelos and Calhoun, 2004; Rittenhouse and Semlitsch, 2006; Nowakowski et al., 2013; but see Graeter et al., 2008), which has been attributed to desiccation risk. Open canopy habitats tend to have higher temperatures and lower humidity, resulting in greater water loss (Rothermel and Semlitsch, 2002; Cosentino et al. 2011; Nowakowski et al. 2013). Other studies of edge and habitat permeability have emphasized the importance of vegetation structure and ease of movement (e.g. Lopez-Barrera

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et al., 2007; Cortes-Delgado and Perez-Torres, 2011), physiological stress (Janin et al. 2012), and presence of refugia (Graeter et al., 2008; Roznik and Johnson, 2009).

Permeability of habitat may differ between species depending on whether it is a habitat generalist or specialist. In general, forest associated species have a preference for forest and open canopy associated species have a preference for open canopy habitat (Haddad, 1999; Ries and Debinski, 2001; Rittenhouse and Semlitsch, 2006; Roznik and Johnson, 2009). For these species with narrower habitat tolerances, functional connectivity of a landscape may be reduced by land-use changes and fragmentation. However, for generalist species there is evidence to suggest that functional connectivity in fragmented landscapes remains high (Haddad, 1999; Gobeil and Villard, 2002). For example, grassland specialist butterflies are less likely to leave grassland habitat than habitat generalists (Haddad, 1999; Ries and Debinski, 2001). Habitat generalists use a wider diversity of resources and may have a greater range of environmental tolerances than specialists, which could result in a decreased sensitivity to habitat edges. However, studies comparing differences in responses of generalist and specialist species to fragmentation and edge habitat are limited (Gobeil and Villard, 2002) and the generalization of observed patterns remains to be tested across taxa.

We conducted two studies to determine the relative importance of (1) abiotic variables and (2) habitat types on choice and movement behavior of juvenile amphibians using outdoor enclosures and natural edges, where marked amphibians could be tracked. Our specific objective was to assess how responses to habitat edges within an agricultural landscape differed between two species with different sensitivities to terrestrial land-use. We used bullfrogs (*Lithobates catesbeianus*) and Blanchard's cricket frogs (*Acris blanchardi*) to address our objective. We predicted that both species would prefer conditions that reduce desiccation risk (presence of ground cover, canopy cover, and high soil moisture) during the day, but not at night. We predicted that habitat edges would be equally permeable to bullfrogs (no preference between forest, corn, and grass habitats) because they are habitat generalists and that cricket frogs would prefer open canopy habitat to forested habitat because they are open canopy specialists.

Elucidating how these species respond to habitat edges may help explain why bullfrogs are able to maintain stable or expanding ranges within their native and non-native ranges and why cricket frogs are experiencing declines across the northern, eastern, and western edges of their range (Gray and Brown, 2005). Our study has direct implications for dispersal and population connectivity (and thus population persistence) in fragmented landscapes and provides valuable information on the differences in permeability between edge habitats and between species that differ in life histories.

## 2. Methods

### 2.1. Brief natural history of species used in this study

Both bullfrogs and cricket frogs are considered colonizers with maximum recorded dispersal distances of 7 km and 1.6 km, respectively (Casper and Hendricks, 2005; Gray et al., 2005). Bullfrogs are found in a range of habitats and are invasive in the western United States and other countries worldwide (Casper and Hendricks, 2005); cricket frogs, however, are associated with open canopy habitats (Lehtinen and Skinner, 2006; Trumbo et al., 2012) and are experiencing declines in parts of their range (Gray et al., 2005). Both species are semi-aquatic and can be found at the edge of breeding habitats throughout the spring and summer. Bullfrogs overwinter in permanent aquatic habitats and cricket frogs

overwinter terrestrially, most likely close to their natal pond (Irwin et al., 1999). Furthermore, cricket frogs are active during the day and have been observed traveling overland during the day (Gray et al., 2005, Youngquist personal observations).

### 2.2. Animal collection and rearing

All studies were conducted using juveniles because they are likely the dispersing stage (Semlitsch, 2008). All animals for this study were reared in outdoor mesocosms at Miami University's Ecology Research Center (ERC), Oxford, Butler Co., OH. We set up mesocosms by adding 1000 L water, 1 kg leaf litter, and algal inoculates from a local fishless pond two weeks prior to tadpole addition. To obtain cricket frog metamorphs, we collected 10 amplexed pairs from a pond at the ERC in May 2011. We maintained egg masses at Miami University's Animal Care Facility at 12:12 light dark cycle at 21 °C until hatching, at which time they were added to mesocosms on 20 June 2011. Cricket frog tadpoles were used in an experiment testing the effects of nitrate concentration on larval development and metamorphosis. Metamorphs used for this study were reared at a density of 30 tadpoles per mesocosm and exposed to 0 or 1 mg/L nitrate; nitrate concentration at this level are within natural ranges (<2 mg/L; Rowe et al., 2004) and had no effect on survival or time to or size at metamorphosis (Youngquist, unpublished data). Because bullfrogs typically have larval periods exceeding one year, we collected overwintered bullfrog tadpoles from two private ponds in Oxford, OH in May and June 2011; we immediately added tadpoles to mesocosms. The movement of late stage tadpoles for terrestrial studies is a common technique and likely does not impact movement behavior of juveniles (e.g. Rothermel and Semlitsch, 2002). Bullfrogs were between Gosner stages 27–36 (Gosner, 1960). Bullfrogs from each location were reared separately at a density of 15 tadpoles per mesocosm.

Bullfrogs began to metamorphose on 24 June 2011 and cricket frogs on 22 July 2011. After metamorphosis, we maintained metamorphs at the Animal Care Facility on a 12:12 light dark cycle at 21 °C. We fed metamorphs crickets *ad libitum* until initiation of the experiments. Bullfrogs were held until enough individuals had metamorphosed to conduct an experiment, up to three weeks. For Experiment 1 (described below) cricket frogs were also held for up to three weeks. However, for Experiment 2 (described below) we held cricket frogs for up to six weeks to allow individuals to reach a size of 0.5 g because preliminary studies found newly metamorphosed individuals (generally less than 0.3 g) were too small to leave a discernable trail of fluorescent powder. Due to bullfrogs metamorphosing one month earlier than cricket frogs, we tested species separately in both Experiments 1 and 2. All research protocols used in this study were approved by Miami University (IACUC 827).

### 2.3. Experiment 1: preferences for habitat based on environmental variables

We tested three environmental variables: ground cover, canopy cover, and soil moisture. These variables can be used to mimic conditions of forested versus unforested habitats. Each treatment and species was tested separately. We constructed eight 3.6 × 3.6 m enclosures using 1 m silt fence buried 15 cm in a grassy field at the ERC. Grass within enclosures was kept short (≤10 cm) throughout the study to prevent potential confounding effects caused by tall grass. We split each enclosure in half, such that one half was on the north side and the other half was on the south side, with a removable silt fence divider. To control for potential orientation bias due to external cues, we randomly assigned each half as “with” or “without” ground cover, canopy cover, or high soil moisture treatment.

We manipulated ground cover by haphazardly placing eight 30 × 30 cm plywood cover boards into half of each enclosure. Plywood was used to mimic downed woody debris; we used plywood because it enabled us to precisely control for the amount of cover placed in each enclosure. We mimicked canopy cover by stretching 90% shade cloth over half of each enclosure. We increased soil moisture by adding 180 L of water to half of the enclosure (~26.9 L/m<sup>2</sup>). This quantity was determined from preliminary trials and was the maximum amount that could be added before soil saturation caused runoff into the other half of the enclosure. All treatments were set up the same day as the nighttime trial.

To determine nighttime choice, we released eight animals into each of the eight enclosures at sunset and added the barrier after 6 h, at which time we collected all individuals and recorded the side of the enclosure frogs were found. We then returned individuals to their original enclosure for an additional 3 h to determine daytime choice. The initial plan for a 6 h choice period during the day was reduced to 3 h after high bullfrog mortality during the first experiment (likely due to heat and desiccation stress). We weighed all individuals before and after each trial. When an individual was found, it was placed in a plastic container with a damp paper towel and transferred to the lab to be weighed; on average, 2 h passed between the end of a trial and being weighed. The same individual was used for both day and night trials of a single experimental treatment. Different individuals were used to test each variable. We used unique toe-clips for identification of individuals; no more than two toes per foot were clipped and no more than three toes per individual.

Bullfrog experiments were conducted on 17–18, 25–26, and 27–28 July 2011 for ground cover, canopy cover, and soil moisture, respectively. Cricket frog experiments were conducted on 3–4, 9–10, and 11–12 August 2011 for ground cover, canopy cover, and soil moisture, respectively. Over all treatments, the average starting bullfrog mass was 10.8 ± 2.9 g and the average cricket frog mass was 0.35 ± 0.09 g.

For the ground cover experiment, we determined choice based on the side of the enclosure individuals were found on and whether or not individuals were under the cover boards. To ensure that our presence did not influence an individual's final location, we visually assessed the number of frogs not under cover boards before entering an enclosure. We collected these individuals first before methodologically searching the enclosure from one side to the other for additional individuals. While our methods may have overestimated use of cover boards, no frogs were seen moving under or out of cover boards during the search. For the ground cover experiment with bullfrogs, temperatures exceeded 38 °C in the afternoon and some bullfrogs suffered mortality. We analyzed our data using two datasets, one including all animals where they were found and one including only survivors. Choice for the canopy cover and soil moisture treatments were based on the side of the enclosure individuals were found. We measured relative humidity, air and ground irradiance temperature (Extech RH101), and percent soil moisture (30 cm depth; Moisture Point MP-917, Environmental Sensors Inc.) twice in each side of each enclosure at the end of a trial, as appropriate for a given experiment (Table A1). Due to equipment malfunctions, we were unable to obtain measures for each environmental variable for every trial. However, between the bullfrog and cricket frog experiments, a complementary set of data was collected.

For all analyses we treated each enclosure as the replicate. All response variables, proportion of individuals choosing a particular habitat and abiotic factors (temperature, relative humidity, soil moisture), were analyzed using Hotelling's  $T^2$  tests, which account for an individual's choice of one side precluding choice of the other side. All proportion data were arcsine-square-root transformed. We calculated percent body weight gain or loss for each trial with

95% confidence intervals. We excluded enclosures in which we recovered 1 or 0 individuals: two enclosures from the daytime ground cover experiment with bullfrogs (survivor-only dataset) and one enclosure from the daytime ground cover experiment testing cricket frogs. Bullfrog enclosures were excluded to due mortality; for the cricket frog enclosure we were unable to determine the cause of frog loss. We also excluded individuals that were found more than one day after the end of a trial (inclusion did not change statistical results).

#### 2.4. Experiment 2: movement behavior at habitat edges

We characterized movement behavior and habitat choice by releasing individuals at the edge between two habitats and tracking their movement with fluorescent pigment powder. Edge habitats in this study were forest–grass, forest–corn, and grass–corn. All release sites were located at the ERC. We replicated each edge-type twice and released a minimum of 24 individuals per edge type (released two or three individuals per site per night). A total of 72 bullfrogs (12.3 ± 0.5 g) and 75 cricket frogs (0.56 ± 0.01 g) were released. All corn edges had a buffer region (less than 3 m width) between the first row of corn and either tall grass or forest understory that was a mix of mown grass, weedy vegetation, and bare dirt. At one corn–grass edge replicate, there was a narrow gravel road (less than 3 m width) between the edge of the corn and the continuous grassy field. Roads of this nature are common in agricultural landscapes and are part of the edge habitat. The forest–grass edges were abrupt with high vegetative contrast; at one location the edge was a small drainage ditch covered in a thin layer of gravel overgrown with grasses.

At each edge, three individuals were released at least 10 m apart. Bullfrogs were released on the nights of 21, 25, 27, 28, 31 August and 1 September 2011. Cricket frogs were released on the nights of 11, 13, 15, 20, and 21 September 2011. Temperature was above 15 °C at all release times except one night when it was 7.2 °C; 1/6 bullfrog releases and 4/5 cricket frog releases occurred on nights where it had rained earlier in the day or the day before.

To track individual frog movement, we used fluorescent powder (DayGlo Color Corporation). This method has been used successfully to study movement in a variety of taxa (e.g. Graeter et al., 2008; McShea and Gilles, 1992; Furman et al., 2011). Animals were weighed and released at edge habitats an hour after sunset; we chose a nighttime release because the majority of amphibians disperse during the night. We covered animals in fluorescent powder and placed them individually under a bucket with a string stretched over a shepherd's hook. After a 5-min acclimation period, we raised the bucket, allowing the individual to move freely. Based on preliminary releases, we allowed bullfrogs a 3 h window in which to move; cricket frogs were given a 6-h period to move. Cricket frogs were given a longer period because of short dispersal distances (less than one meter) observed after three hours in preliminary studies, compared to >10 m for bullfrogs. Using a limited window of time enhanced our ability to recapture individuals before they ran out of powder, while allowing enough time for individuals to make a choice. It was important to be able to recapture individuals to maximize accuracy in determining final habitat choice at the end of the time period.

At the end of the allotted time period, we used a UV flashlight to follow and mark the paths with flags at each change in direction. We measured distance and direction between flags, which allowed us to calculate path length, path displacement (net distance traveled), path straightness (displacement/total distance), orientation (net direction traveled), and habitat preference. Path characteristics were calculated using standard trigonometric functions. Habitat preference was determined by where the individual was found

or where the trail ended. Edge habitat was defined as 2 m on each side of the release location; individuals or paths that ended in this corridor were scored as having chosen “edge” habitat. A 2 m definition for edge encompassed the maximum buffer region between habitats (see above) and ensured that the path ended within of one habitat or another.

Path displacement and straightness were analyzed using multivariate analysis of co-variance (MANCOVA) (SAS 9.3) with edge-type as the treatment. We used mass as a covariate and implemented a spatial and time block (release day) to account for variation in location and time released. However, spatial block was not significant and was dropped from the model; release day was significant and was included. We normalized our data by log transforming distance data and arcsine-square-root transforming the straightness data. To analyze orientation, we standardized the data by orienting the habitat edge lines along the north–south axis. This allowed us to combine data from edge replicates. In reality, both grass–corn edges were split along the north–south axis with grass on the west side; in the case of forest edges, two forest habitats were on the north side (one replicate for forest–corn and one for forest–grass), one the west side (forest–grass), and one on the east side (forest–corn). Orientation was analyzed using non-parametric circular statistics; we opted to use non-parametric analyses based on small sample sizes and lack of normality. We tested for similarity between sites using Watson two-sample test of homogeneity. Given no difference between replicates, we combined data sets and tested for circular uniformity using Watson’s one-sample test for circular uniform distribution. All circular statistics were run using the Circular package in R (R v2.12.1; Agostinelli and Lund, 2011). We analyzed habitat choice using Chi-square test (R v2.12.1), with separate tests for each edge type. We analyzed each edge type separately because not all habitat options were available at each edge habitat. We combined edge replicates for all Chi-square tests because there were no site differences. For all analyses, all paths less than 1 m in total distance were removed from analysis (final sample sizes: bullfrog  $N = 68$ ; cricket frog  $N = 59$ ).

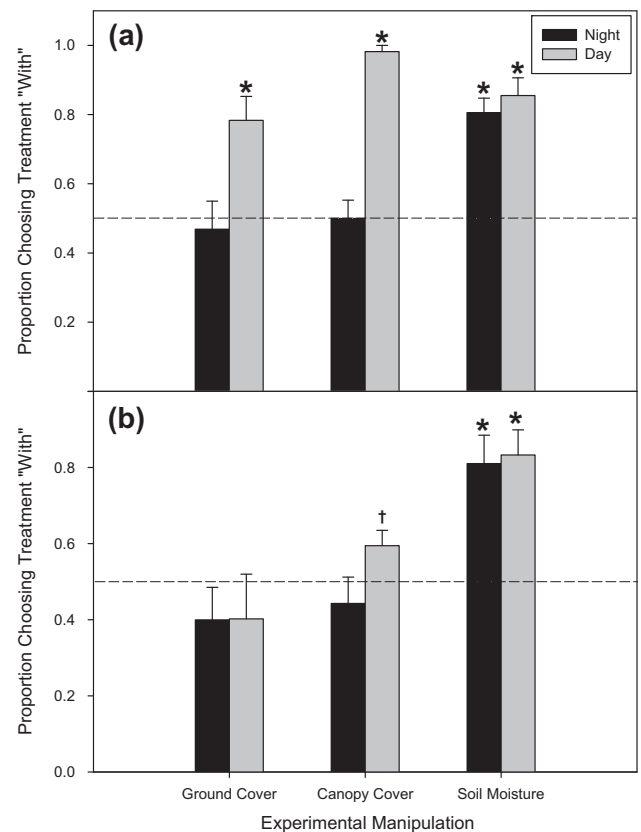
### 3. Results

#### 3.1. Experiment 1: preferences for habitat based on environmental variables

##### 3.1.1. Ground cover

Bullfrogs did not show a preference for the presence of ground cover during the night ( $F_{1,7} = 0.17$ ,  $p = 0.69$ ; Fig. 1a); when bullfrogs were found on sides with cover boards, they did not use cover boards ( $F_{1,7} = 75.67$ ,  $p < 0.0001$ ). For the daytime trial, 29/62 animals survived 6 h. Looking at all individuals (alive and dead-where-found), there was preference for the side with cover available ( $F_{1,7} = 11.97$ ,  $p = 0.01$ ; Fig. 1a). However, we found there was no preference for cover use ( $F_{1,7} = 0.63$ ,  $p = 0.45$ ). When considering only those that were alive, the majority were found under cover (24/29), but use of cover boards was not significant ( $F_{1,5} = 3.11$ ,  $p = 0.14$ ). During the nighttime trial, bullfrogs had no change in mass; during the day, bullfrogs found alive under cover boards had no change in mass but those that were not under cover and alive experienced a 9% loss in mass (Table A2).

Cricket frogs showed no preference between habitats with or without cover board during the nighttime trial ( $F_{1,7} = 1.24$ ,  $p = 0.30$ ) and they did not utilize the cover boards ( $F_{1,7} = 268.54$ ,  $p < 0.0001$ ). Similarly, during the day we found no preference for the presence of cover boards ( $F_{1,6} = 1.05$ ,  $p = 0.34$ ) and cricket frogs did not use cover boards ( $F_{1,6} = 12.1$ ,  $p = 0.01$ ; Fig. 1b). During the night cricket frogs gained an average of 4% mass but during the day individuals lost an average of 5% mass (Table A2).



**Fig. 1.** Choice for ground cover, shade, or moisture, by (a) bullfrogs and (b) cricket frogs. “Proportion ‘with’” indicates the proportion of individuals (mean  $\pm$  SE) found on the side of the enclosure with ground cover, with canopy cover, or with high moisture. For bullfrog daytime ground cover choice, the value is for all individuals (both alive and perished). ‘\*’ Indicates a significant choice, and ‘†’ indicates a marginally significant choice ( $0.06 < p > 0.05$ ); the dotted line indicates the no choice threshold.

##### 3.1.2. Canopy cover

Bullfrogs showed no preference for canopy cover during the night ( $F_{1,7} = 0.0$ ,  $p = 0.98$ ), but there was strong preference for canopy cover during the day ( $F_{1,7} = 231.36$ ,  $p < 0.0001$ ) with only one animal choosing not to use canopy cover (Fig. 1a). During the night bullfrogs showed no change in mass; however, during the day they lost up to 7% of body mass (Table A2).

Cricket frogs showed no preference for canopy cover during the night ( $F_{1,7} = 0.76$ ,  $p = 0.41$ ) but there was a marginal preference during the day ( $F_{1,7} = 5.50$ ,  $p = 0.052$ ; Fig. 1b). Cricket frogs had no change in mass during the night, but experienced, on average, a 5% loss in mass during the day with no difference between individuals found under canopy cover or in the open (Table A2).

##### 3.1.3. Soil moisture

Bullfrogs preferred the high moisture environment both during the night ( $F_{1,7} = 25.99$ ,  $p = 0.0014$ ) and during the day ( $F_{1,7} = 24.22$ ,  $p = 0.0017$ ; Fig. 1a). Most individuals were found buried under grass or in crevices along the fence. Overall, bullfrogs experienced no change in mass during the day or night; there was no apparent difference in mass change between individuals found in high or low moisture environments (Table A2). However, only 12 individuals were found in the low moisture half during the night and 9 during the day.

Cricket frogs had preference for high soil moisture both during the night ( $F_{1,7} = 14.13$ ,  $p = 0.0071$ ) and during the day ( $F_{1,7} = 16.41$ ,  $p = 0.0049$ ; Fig. 1b). Cricket frogs had no change in mass over the night but did experience up to an average 6% decrease in mass during the day. It appeared as though cricket frogs found in the high



moisture side of the enclosures experienced greater loss in mass (Table A2); however, there were only eight individuals found in the low moisture environment during the day.

### 3.1.4. Environmental variables

Over all three days of trials, the average temperature ( $\pm$ SE) at the end of the nighttime trials was  $21.5 \pm 1.0$  °C for bullfrogs and  $19.1 \pm 2.0$  °C for cricket frogs; average nighttime percent humidity ( $\pm$ SE) was  $86.3 \pm 5.1$  for bullfrogs and  $67.9 \pm 5.2$  for cricket frogs. During the day, average temperature ( $\pm$ SE) at the end of trials was  $36.5 \pm 1.8$  °C for bullfrogs and  $32.0 \pm 0.5$  °C for cricket frogs; daytime average percent humidity ( $\pm$ SE) was  $46.2 \pm 0.6\%$  for bullfrogs and  $39.7 \pm 5.2\%$  for cricket frogs. Cover boards caused a slight decrease in ground irradiance in the afternoon ( $F_{1,7} = 5.82$ ,  $p = 0.0466$ ); canopy cover resulted in cooler ground irradiance ( $F_{1,7} = 29.78$ ,  $p = 0.0009$ ), warmer air temperature ( $F_{1,7} = 19.34$ ,  $p = 0.0032$ ), and higher relative humidity in the afternoon ( $F_{1,7} = 5.13$ ,  $p = 0.0578$ ); high moisture treatments had significantly higher levels of soil moisture after both the nighttime ( $F_{1,7} = 87.44$ ,  $p < 0.0001$ ) and daytime trials ( $F_{1,3} = 257.83$ ,  $p = 0.0005$ ; Table A1).

## 3.2. Experiment 2: movement behavior at habitat edges

### 3.2.1. Bullfrogs

A total of 72 bullfrogs were released and 68 had paths greater than 1 m in length; 67% were recovered at the end of the fluorescent trail. On average, bullfrog path distance was  $15.2 \pm 2.1$  m (range 1.11–108.8 m). Bullfrogs showed circular uniform orientation (random) at all edge types (Fig. 2a) and, overall, no clear preference for one habitat type over another. However, there was preference for edge habitat at corn–forest edges ( $X^2 = 7.92$ ,  $df = 2$ ,  $p = 0.019$ ). For the multivariate response of path straightness and displacement, there was an effect of edge that was strongly driven by path straightness (MANOVA  $F_{4,114} = 3.03$ ,  $p = 0.0203$ ; ANOVA straightness  $F_{2,58} = 4.16$ ,  $p = 0.0205$ ; displacement  $F_{2,58} = 1.04$ ,

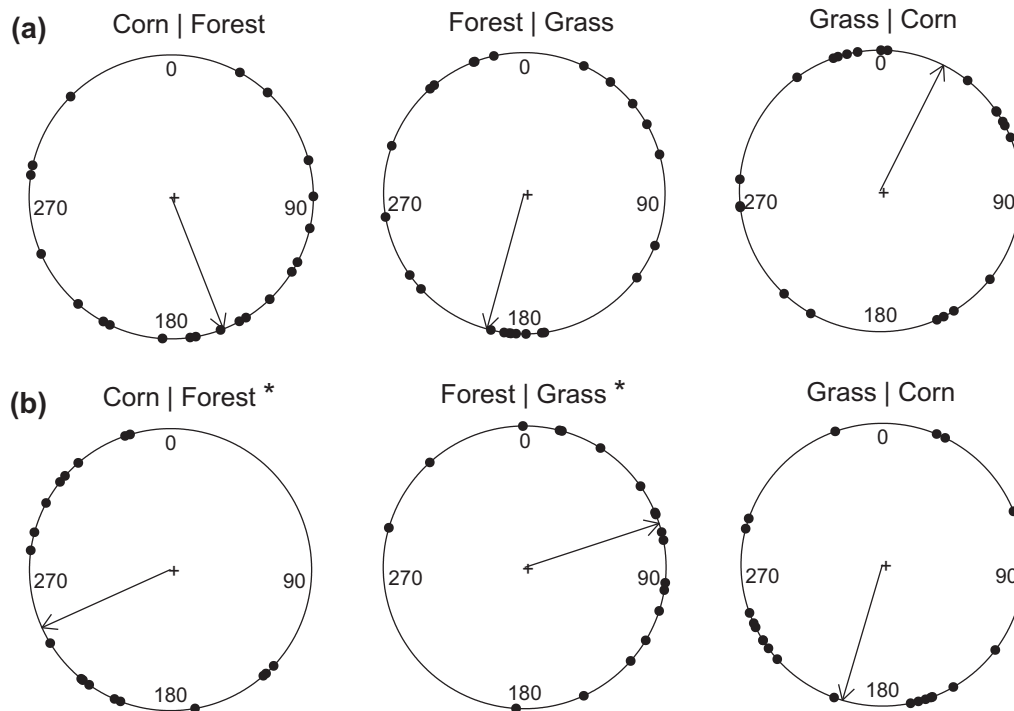
$p = 0.3596$ ; Fig. 3a). Bullfrogs had straighter paths in forest–grass edge habitats and least straight paths in grass–corn. Path displacement was greatest in grass–corn and corn–forest edge habitats.

### 3.2.2. Cricket frogs

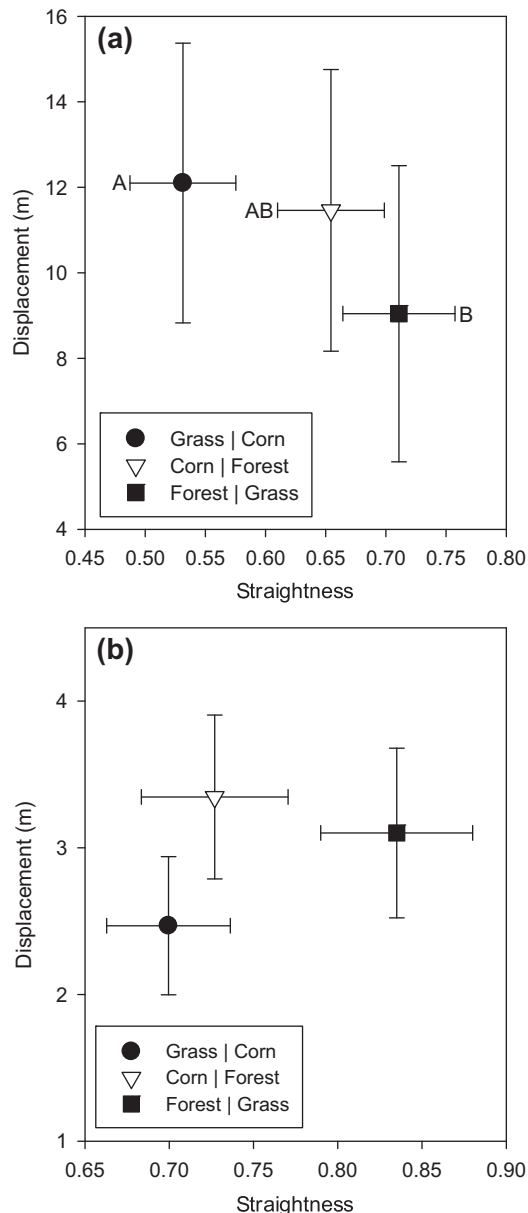
A total of 75 cricket frogs were released and 59 had paths greater than 1 m in length; 37.3% were recovered at the end of the fluorescent trail. On average, cricket frog path distance was  $4.0 \pm 0.4$  m (range 1.01–20.4 m). Cricket frogs had non-uniform circular orientation in forest–corn ( $U^2 = 0.1992$ ,  $p < 0.05$ ) and forest–grass ( $U^2 = 0.1697$ ,  $p < 0.01$ ) edge habitats; in both cases cricket frogs oriented away from forest habitat (Fig. 2b). Orientation was random at grass–corn edges. These trends were reflected in the choice results: cricket frogs preferred non-forest habitat (grass–forest  $X^2 = 10.33$ ,  $df = 2$ ,  $p = 0.0057$ ; corn–forest  $X^2 = 12$ ,  $df = 2$ ,  $p = 0.0025$ ) and preferred edge at grass–corn edges (grass–corn  $X^2 = 13.65$ ,  $df = 2$ ,  $p = 0.0011$ ). For the path straightness and displacement analysis, there was no edge effect on path displacement or straightness, although cricket frogs did show a trend to travel straighter in grass–forest edge treatments ( $F_{2,51} = 2.55$ ,  $p = 0.0877$ ; Fig. 3b).

## 4. Discussion

Maintaining population connectivity in fragmented landscapes is vital for the stability of regional populations. Of important consideration is how dispersing individuals respond to edge habitats. Permeable edges may enhance connectivity and allow for the rescue and recolonization of populations while impermeable edges can lead to population isolation (Stamps et al., 1987). Edge permeability is determined by the willingness of an individual to cross between two distinct habitat types and is a function of innate habitat preferences and the need to minimize physiological stress (e.g. desiccation). This study demonstrated that while species with different life history strategies both make choices to minimize physiological stress in confined enclosures, response to edges indicates



**Fig. 2.** Mean orientation direction traveled by (a) bullfrogs and (b) cricket frogs. Dots indicate individual frogs; arrows indicate mean direction traveled at each edge; each half of a circle (partitioned along 0–180 axis) corresponds to habitat type as indicated by above heading (e.g. 0–180 corresponds to Forest, while 180–360 corresponds to corn at the corn–forest edge). \*' Indicates significant orientation in direction of mean angle.



**Fig. 3.** Multivariate response of path displacement (mean  $\pm$  SE) and straightness (mean  $\pm$  SE) to edge type of (a) bullfrogs and (b) cricket frogs. Letters indicate significant difference between edge-types.

that other factors influence habitat choice; furthermore, species responses to edges may be indicative of regional population trends. Our study indicates that bullfrog distribution may be less hindered by changes in land-use between forest, grassland, and agriculture, while cricket frogs may be at risk of isolation by changes in habitat that increases forest cover.

#### 4.1. Role of abiotic factors in habitat choice and movement decisions

Understanding which environmental factors are critical to habitat choice may be key to predicting how species will respond to different habitat types. For example, a species' innate response to enter or avoid open canopy habitat may affect the likelihood of crossing roads (Andrews and Gibbons, 2005; Aresco, 2005). Our study clearly demonstrates that microhabitats that reduce desiccation are important determinants for habitat choice in amphibians; this is consistent with other studies demonstrating a preference for amphibians to travel through habitats that minimize desiccation (e.g.

Rothermel and Semlitsch, 2002; Cosentino et al., 2011; Nowakowski et al., 2013). In Experiment 1, individuals chose habitat with lower ground temperature and higher humidity (shade) and higher soil moisture. While we did not measure environmental variables in the field for Experiment 2, other studies found amphibians experienced less desiccation in forest than in field or corn crop, likely due to lower temperatures and higher humidity, and not due to differences in soil moisture (e.g. Rothermel and Semlitsch, 2002; Rothermel and Semlitsch, 2006; Nowakowski et al., 2013). Therefore, we may have predicted a preference for forest. Instead, bullfrogs showed no overall preference of any habitat while cricket frogs avoided forest. Field habitats often have higher levels of soil moisture than forest (Rothermel and Semlitsch, 2002; Rothermel and Semlitsch, 2006) and this could have been a factor in observed habitat choices. Soil moisture may have played a larger role in cricket frog habitat choice because there was a greater amount of rainfall during Experiment 2, which could have resulted in higher topsoil moisture in the open canopy habitats. Most amphibians travel during rainy nights; therefore, even if the decision by cricket frogs to avoid forest was based on increased soil moisture after rain and not avoidance of canopy cover, the implications of our study remain unchanged as the conditions of the experiment match natural conditions for travel. With regards to bullfrogs, the lack of rain during Experiment 2 may have impacted movement decisions and the differences in weather between bullfrog and cricket frog trials limits our ability to draw direct comparisons. However, bullfrogs showed no preference for any one habitat during dry periods when they would be under greater desiccation stress and we may infer that there would be no preference during wet periods as well (Popescu and Hunter, 2011).

The movement patterns at the edges were in agreement with the nighttime habitat preferences in the enclosures; neither bullfrogs nor cricket frogs showed preferences for habitats with ground or canopy cover. However, during the day, bullfrogs changed behavior in preference for covered habitats. Daily and seasonal changes in habitat preference is ubiquitous in nature and may make predicting habitat use more difficult because species may rely on or utilize a wider range of habitats than generally thought. Intimate knowledge of all habitats used by a species throughout its life cycle is necessary for proper species management. Furthermore, our results indicate that individuals choosing to travel through open canopy habitat at night may be at risk during the day if they are not able to reach suitable protection from abiotic or biotic stressors. Specifically, bullfrogs in Experiment 1 had no preference for canopy or ground cover at night, but did during the day and suffered high mortality without ground cover. Therefore, bullfrogs dispersing through agricultural fields at night may risk desiccation and mortality during the day. Lack of habitat preference at night may render bullfrogs more vulnerable to land-use changes that increase bare ground. The lack of a large shift in night-versus-day use of cover board or shade in cricket frogs may be a result of less stressful weather conditions experienced by cricket frog in Experiment 1 and direct comparisons of microhabitat use between species should be made with caution. However, the different weather conditions experienced by each species in both experiments reflect natural differences due to bullfrogs metamorphosing earlier than cricket frogs. Species with higher mobility may suffer higher rates of extinction within fragmented landscapes due to increased time in inhospitable environments (Gibbs, 1998; Thomas, 2000). The ability to discern favorable microhabitats, higher moisture in the case of amphibians, may reduce risks associated with traveling through open habitats.

#### 4.2. Response to habitat may predict regional distributions

The observed response of bullfrogs and cricket frogs to microhabitat and edges matched our predictions based on natural

history and may help explain regional population trends. As with many species that have become invasive, bullfrogs are human-associated and thrive in anthropogenic landscapes (Adams et al., 2003; D'Amore et al., 2010). One reason for the bullfrogs' predilection to anthropogenically disturbed sites is the increased availability of permanent aquatic habitats, due to changes in hydrology of natural water bodies or the creation of new ponds and lakes (D'Amore et al., 2010). Our study indicated that bullfrogs had a tendency to move along the edge of agricultural fields and traveled farthest when released at corn edges; these structurally simple edges would allow for unimpeded travel. While the presence of the buffer region along corn edges could have affected the behavior of individuals compared to the abrupt grass-forest edges, these buffer regions are common in agricultural habitats and our study sites reflect actual habitat encountered by individuals. Habitat fragmentation and edge habitats are often believed to enhance the spread of non-native species (e.g. With, 2002; Holway, 2005). Our results indicate that the increase in edge availability associated with agricultural landscapes may enhance bullfrog dispersal and increase chances of reaching new breeding sites. Thus, we would predict bullfrogs to rapidly expand their ranges in agricultural landscapes (Ficetola et al., 2010).

In contrast to bullfrogs, cricket frogs are experiencing enigmatic declines in parts of their range. One hypothesis for their declines is the natural succession of grasslands and agricultural lands to forest in the eastern United States; some areas of cricket frog declines coincide with increased forest cover, while regions with high cricket frog density are managed for agriculture (Lehtinen, 2002; Lehtinen and Skinner, 2006). Our results indicate that cricket frogs prefer to travel through grassy areas and avoid forest, rendering grasslands, hay fields, and pasture land as potential dispersal corridors and forested habitats as potential behavioral barriers. Increases in forest cover could cause increased isolation of extant populations. Furthermore, isolation may be more strongly felt by cricket frogs because they are functionally an annual species with only a very small percentage of a population surviving more than one year (Lehtinen and MacDonald, 2011). Thus, in the absence of immigrants, a single year of recruitment failure may lead to local extirpation and regional declines. There is some evidence with plants to indicate that short-lived (annual) species may be more susceptible to extinction in fragmented habitats due to greater population fluctuations (Fischer and Stocklin, 1997; Stehlik et al., 2007). Therefore, reforestation may cause cricket frog declines both by reducing availability of preferred breeding habitats in grassland and increasing isolation of breeding populations; forest associated range contractions may reflect this species' historic range before widespread deforestation due to agriculture.

#### 4.3. Land management in agricultural landscapes

It is important to note that for both of these species corn crop did not appear to be a behavioral barrier to dispersal, despite the lack of cover in agricultural fields which could render agricultural fields as leaky barriers or evolutionary traps for dispersal (Schlaepfer et al., 2002). The simple structure of row crops may appeal to dispersing individuals as easily traversable ground and in some instances cropland may enhance connectivity (Goldberg and Waits, 2010). The ability to readily disperse through agricultural lands may explain why both species are locally abundant in agricultural landscapes and frequently breed in agricultural ponds. Agricultural lands, with proper management, may provide good quality habitat, allow for easy dispersal, and play an important role in species conservation (e.g. Rustigian et al., 2003; Cereghino et al., 2008). Increasing or changing the type of cover available may improve the habitat quality of agricultural fields for dispersal. For example, salamanders were found to experience less desiccation

stress and have increased movement in soybean crop over corn due to increased canopy cover of the soy bean plants (Cosentino et al., 2011). For species that utilize ground cover, such as bullfrogs in this study, farming practices like conservation tillage that leave vegetative debris on the fields (Mannerling and Fenster, 1983) may provide cover for dispersing individuals. The implementation of conservation programs such as the Conservation Reserve Program (CRP), which removes land from agricultural production and creates grassland habitats, has benefited a wide diversity of wildlife (Farrand and Ryan, 2005). Encouraging farmers to enroll in conservation programs increases the amount of quality habitat and may also increase connectivity by creating grassland corridors within agricultural landscapes. For grassland associated amphibians, such as cricket frogs, CRP land may greatly benefit local and regional populations; this is particularly pertinent in the western part of the cricket frog range that was not historically forested. Over the long term, populations in agricultural landscapes may experience temporal differences in connectivity due to crop rotations, changes in tillage regimes, and changes in CRP land coverage.

#### 4.4. Conclusions

As humans continue to modify the landscape, species will be continuously confronted with novel habitat types. Therefore, it is imperative that we understand how different land-uses affect movement behavior for at-risk species if we are to create effective species management plans and conserve regional landscape connectivity. Conservation strategies such as no-till and CRP may enhance landscape connectivity for generalist and grassland species within agricultural landscapes by reducing the costs of dispersal. Movement behavior studies offer mechanistic understanding of dispersal patterns that are necessary for accurate modeling of population dynamics (Hawkes, 2009; Stevens et al., 2006) and for distinguishing which land management strategies would be most effective for species conservation.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2014.04.028>.

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