The significance of spatial memory for water finding in a tadpole-transporting frog

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Abstract

The ability to associate environmental cues with valuable resources strongly increases the chances of finding them again, and thus memory often guides animal movement. For example, many temperate region amphibians show strong breeding site fidelity and will return to the same areas even after the ponds have been destroyed. In contrast, many tropical amphibians depend on exploitation of small, scattered and fluctuating resources such as ephemeral pools for reproduction. It remains unknown whether tropical amphibians rely on spatial memory for effective exploitation of their reproductive resources. Poison frogs (Dendrobatidae) routinely shuttle their tadpoles from terrestrial clutches to dispersed aquatic deposition sites. We investigated the role of spatial memory for relocating previously discovered deposition sites in an experimental population of the brilliant-thighed poison frog, *Allobates femoralis*, a species with predominantly male tadpole transport. We temporarily removed an array of artificial pools that served as the principal tadpole deposition resource for the population. In parallel, we set up an array of sham sites and sites containing conspecific tadpole odour cues. We then quantified the movement patterns and site preferences of tadpole-transporting males by intensive sampling of the area and tracking individual frogs. We found that tadpole-carrier movements were concentrated around the exact locations of removed pools and most individuals visited several removed pool sites. In addition, we found that tadpole-transporting frogs were attracted to novel sites that contained high concentrations of conspecific olfactory tadpole cues. Our results suggest that *A. femoralis* males rely heavily on spatial memory for efficient exploitation of multiple, widely dispersed deposition sites once they are discovered. Additionally, olfactory cues may facilitate the initial discovery of the new sites.

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Valuable resources are scattered in complex environments and the ability to learn about cues associated with these resources in space strongly increases the chances of relocating them. Understanding how memory guides the movement of free-ranging animals remains among the top challenges in behavioural biology (Fagan et al., 2013; Mueller & Fagan, 2008). Factors such as concentration and stability of resources, the scale of animal movement and animals' cognitive abilities may either favour or constrain memory-based spatial strategies. For example, learning about scattered, small and fluctuating resources is cognitively more demanding and less reliable than learning about localized, large and stable resources (Mueller & Fagan, 2008). A number of insects (e.g. honeybees; Gould, 1986), birds (e.g. hummingbirds; Cole, Hainsworth, Kamil, Mercier, & Wolf, 1982) and mammals (e.g. primates; Janmaat, Ban, & Boesch, 2013) have been shown to rely on flexible spatial-learning strategies when foraging for scattered ephemeral resources. It remains unknown how widespread such spatial-learning strategies are among other taxa and in contexts other than foraging.

Amphibians are a particularly interesting group for studying the evolution of spatial strategies, given that most amphibians show regular movements between terrestrial and aquatic sites for breeding. More importantly, amphibians show some of the most diverse reproductive behaviours among terrestrial vertebrates. Their breeding behaviour ranges from explosive breeding where thousands of individuals gather for a few days in large permanent ponds to prolonged breeding in small scattered ephemeral pools with intensive parental care (Crump, 2015; Duellman & Trueb, 1994; Wells, 1977). The associated spatial and learning strategies must be just as diverse, but as yet they remain largely unstudied.

To date, research on amphibian learning and movements has focused almost exclusively on nocturnal, pond-breeding amphibians of temperate regions. Laboratory experiments have successfully identified several cues learned and used by some anuran amphibians, especially toads (i.e. Bufonidae), for orienting in small arena settings (e.g. Adler, 1980; Dall'antonia & Sinsch, 2001; Daneri, Casanave, & Muzio, 2015; Grubb, 1976; Sotelo, Bingman, & Muzio. 2014). In the field, amphibian spatial orientation experiments have focused on sensory modalities (e.g. olfaction, vision, magnetoreception, reviewed in Ferguson, 1971; Sinsch, 1990, 2006) and have rarely addressed the cognitive mechanisms, such as path integration, beaconing or spatial learning, underlying the movements (but see Fischer, Freake, Borland, & Phillips, 2001; Ishii, Kubokawa, Kikuchi, & Nishio, 1995; Joly & Miaud, 1993). Both laboratory experiments and observations of orientation behaviour in the field suggest that many amphibians are able to use learned cues to orient in their local area (Dole, 1968; Grubb, 1976; Sinsch, 2007; Sinsch & Kirst, 2015). However, very few studies have purposely investigated how experience guides amphibian movements under natural conditions (but see Pašukonis, Warrington, Ringler, & Hödl, 2014).

Many temperate region amphibians show strong natal philopatry to stable ponds or streams and they will return to them after experimental translocations even when a closer suitable breeding habitat is available (e.g. *Bufo bufo*, Heusser, 1960; Schlupp & Podloucky, 1994; *Triturus alpestris*, Joly & Miaud, 1989; *Taricha rivularis*, Twitty, 1959). Further, some amphibians continue migrating to their breeding areas long after the aquatic sites have been destroyed (Heusser, 1960; Pechmann, Estes, Scott, & Gibbons, 2001). These observations can be explained by natal site imprinting guided by route following (e.g. Ishii et al., 1995; Shoop, 1965) and/or an ability to perceive long-distance cues originating from the target (i.e. beacons), such as the natal pond odour (e.g. Grubb, 1973; Joly & Miaud, 1993). However, such mechanisms are insufficient to explain the ability to relocate small and scattered reproductive resources in complex habitats, such as tropical rain forests. In contrast to temperate species, many tropical frogs depend on small and fluctuating resources such as ephemeral forest pools for reproduction. On the one hand, learning about where such ephemeral pools are and/or are likely to occur in the future would increase the efficiency of resource exploitation. On the other hand, the fluctuation in resource location and availability makes the learning harder and learned information less reliable. It remains unknown how tropical frogs respond to such trade-offs and whether they rely on spatial memory for effective exploitation of scattered fluctuating resources.

Poison frogs (Dendrobatidae) are a group of small diurnal frogs from the Neotropics and are ideal to address these questions. They display some of the most complex spatial behaviours known among amphibians, such as long-term territoriality (Pröhl, 2005) and tadpole transport (Weygoldt, 1987). Males and/or females of most species shuttle their tadpoles from terrestrial oviposition sites to multiple, widely dispersed, ephemeral aquatic sites such as flooded areas, cavities in fallen or standing trees, or water-filled leaf axils for tadpole rearing (for reviews of dendrobatid parental behaviour see Summers & Tumulty, 2013; Wells, 2007; Weygoldt, 1987). In a recent study, Liu, Day, Summers, and Burmeister (2016) showed that a poison frog, *Dendrobates auratus*, could use flexible learning strategies in a serial spatial reversal task. The authors speculated that this flexibility has evolved in response to the dynamic landscape of their reproductive resources, but it remains unknown whether dendrobatids use spatial memory to find suitable aquatic sites. Alternatively, individuals could adopt uninformed search strategies and scan for environmental cues such as local odour to find the deposition sites every time they transport tadpoles. However, tadpole transport is associated with significant costs, such as energetic expenditure, increased exposure to predators and loss of mating opportunities. Given these associated costs to the transporting parent we hypothesized that poison frog movements during tadpole transport are guided by spatial memory of previously discovered deposition sites.

We investigated the significance of spatial memory for relocating the deposition sites in an experimental island population of the well-studied species *Allobates femoralis*, a small dendrobatid frog with predominantly paternal long-distance tadpole transport (E. Ringler, Pašukonis, Hödl, & Ringler, 2013). Males of this species are known to use multiple, widely dispersed terrestrial pools for tadpole deposition (Erich, Ringler, Hödl, & Ringler, 2015). In addition, when translocated within their local area, these territorial frogs show very accurate homing, which suggests some use of spatial learning (Pašukonis et al., 2013, 2014). To test whether *A. femoralis* males rely on spatial memory to find known deposition sites, we

temporarily removed an array of artificial pools that had served as the principal tadpole deposition resource for the population over the previous few years and quantified the movement patterns of tadpole-transporting males by intensive sampling of the area and by following individual frogs. We predicted that if tadpole-transporting males rely on spatial memory, they would concentrate their movement around those removed pool sites. Further, we expected the frogs to search at multiple pool sites as an indication of flexible memory use in an environment with scattered resources.

Methods

Study Species

Allobates femoralis is a small (snout–urostyle length approximately 25 mm) diurnal leaf litter frog common throughout the Amazon basin and the Guiana Shield (Amézquita et al., 2009). At the onset of the rainy season, males establish and vocally advertise territories, which they aggressively defend for up to several months (M. Ringler, Ringler, Magaña Mendoza, & Hödl, 2011; M. Ringler, Ursprung, & Hödl, 2009). Courtship takes place within the male's territory where a clutch of approximately 20 eggs is laid in leaf litter (Montanarin, Kaefer, & Lima, 2011; E. Ringler, Ringler, Jehle, & Hödl, 2012; Roithmair, 1992). Both males and females are highly polygamous and iteroparous, and males have been observed to attend up to five clutches simultaneously (Ursprung, Ringler, Jehle, & Hödl, 2011). Tadpole transport takes place 15–20 days after oviposition and is mainly performed by males after heavy rainfall (Aichinger, 1991; E. Ringler et al., 2013). Tadpoles are deposited in a variety of terrestrial water bodies, such as floodplains, peccary wallows, palm fronds and holes in fallen trees (see M. Ringler, Hödl, & Ringler, 2015 and references therein). Adult frogs can be sexed by the presence (male) or absence (female) of the vocal sacs. Individual frogs can be unambiguously recognized by their unique ventral coloration patterns (E. Ringler, Mangione, & Ringler, 2014).

Study Area

The study was carried out at the beginning of the rainy season January–April 2014 on a 5 ha river island near the 'Camp Pararé field site at the CNRS 'Nouragues Ecological Research Station' in the Nature Reserve 'Les Nouragues', French Guiana (3°59′ N, 52°35′W; M. Ringler, Mangione, Pašukonis, et al., 2014). The island was not inhabited by *A. femoralis* before March 2012 when 1800 tadpoles were experimentally introduced (E. Ringler et al., 2014). The tadpoles were sampled from a nearby autochthonous population and released in a linear array of 20 artificial pools (volume ca. 10 litres, interpool distance 10 m) on the island. In September 2012, we removed every second pool, leaving an array of 10 pools 20 m apart (Fig. 1). By March 2013, 36 adult males and 31 females had successfully established and were reproducing, using the remaining pools for tadpole deposition (E. Ringler et al., 2014).

Territory Sampling

To determine male territories, two to six people conducted extensive visual and acoustic surveys from 22 January to 24 February across the entire island. All detected frogs were captured with transparent plastic bags, sexed, photographed for identification and their

behaviour was noted. We considered males to be territorial when they were calling, in courtship, or when they showed aggressive reactions to a playback simulating an intruder (M. Ringler et al., 2011). All spatial locations of frogs were recorded on pocket computers (MobileMapper 10; Ashtech/Spectra Precision) in the GIS software ArcPAD 10 (ESRI, Redlands, CA, U.S.A.) using a detailed background map (M. Ringler, Mangione, Pašukonis, et al., 2014).

Experimental Set-up and Tadpole-carrier Sampling

Prior to the experimental manipulations, we extensively surveyed the area for natural tadpole deposition sites. We found one water-filled palm frond, one temporary puddle on a trail, a waterfilled burrow and one larger pool at the base of a fallen tree that contained *A. femoralis* tadpoles. Additional palm fronds that held only water but could have been used for tadpole deposition were removed. Artificial pools had 19–106 (mean = 49.5, SD = 28.5) tadpoles per pool on the day of pool removal.

To test whether males remember the tadpole deposition locations, all artificial pools were removed simultaneously on 6 February. All removed pool sites ('pool sites' hereafter) were filled with soil and covered with leaf litter, but we left in place the yellow labelling stakes that had previously marked the pool sites (Supplementary Fig. S1). All tadpoles were collected in two 6-litre buckets (approximately 250 tadpoles per bucket). To allow the tadpoles to remain on the island, and to emerge near their original deposition sites, we suspended the buckets in the proximity of the pool array axis and approximately 0.5 m above the ground, one in the northern (bucket N) and one in the southern (bucket S) half of the island (Fig. 1). This was sufficient to prevent *A. femoralis* males from using the buckets as deposition sites.

The intensive monitoring of the pool area started on 7 February, the day after pool removal. The final experimental array was established on 9 February, after an initial observation period of 2 days. As expected, during the first 2 days after pool removal we caught tadpole-carriers arriving at the pool sites marked by the yellow stakes. Unexpectedly, we also observed some males arriving beneath the suspended tadpole-containing buckets. In response to these observations, we extended our experimental set-up to control for (1) a potential sampling bias, (2) frog attraction towards the yellow stakes and (3) a potential odour effect at the pool sites. To control for (1) and (2) we established 10 control sites, each 5 m from a pool site, and marked them with an identical yellow stake ('control sites' hereafter; Fig. 1). Likewise, we established 10 sites to control for (3) the effects of residual tadpole odour at pool sites ('odour sites' hereafter). Every day (with an exception of a few rainless days) during the course of the experiment, we collected all water and sediments from both tadpole-containing buckets (approximately 10 litres of 'tadpole water'). For each of the 10 odour sites, we diluted 1 litre of tadpole water with 9 litres of river water and spilled it at the respective locations. These sites were also marked with identical yellow stakes. The 10 odour sites were evenly distributed in between the pool sites and offset by 5 or 20 m to the left or the right from the pool array (Fig. 1).

During the monitoring from 7 to 24 February, we evenly sampled all sites, including the suspended buckets, by slowly walking between the consecutive sampling sites and visually

scanning the area. Two to four people visually inspected the area of the previous pool array every 1–2 h between 0800 and 1800 hours for 7 days and every 2–4 h for another 10 days, aiming to capture as many tadpole-transporting males as possible. Before release, we recorded tadpole-carrier capture location, photographed them for identification, and counted the tadpoles on their back. The tadpoles were counted as accurately as possible without manipulating them. We did not observe any change in movement patterns between the initial 2-day observation period after pool removal and the rest of the sampling period (Supplementary Table S1 and Fig. S2). However, for the site preference overview we only report the directly comparable numbers of frogs captured at different sites during the period when the full experimental array was in place (9–24 February).

Focal Tracking

The visual tracking of tadpole-carriers was performed from 7 to 16 February. A subset of the tadpole-transporting males captured during the array sampling was visually followed after release. Specific males were selected arbitrarily, based on observer availability. Each observer was assigned to track a single frog as long as possible, until the frog was eventually lost from sight for approximately 30 min, or until nightfall. Thereby, we were able to follow one to four frogs simultaneously. The observer kept a distance that allowed good visibility but did not appear to disturb the moving frog (usually 1–2 m). Occasional disturbances by the observer, other fieldworkers in the area or natural causes, such as falling branches or passing animals, caused the frogs to hide in the leaf litter before moving again. As a result, the calculated average frog movement speeds are minimum estimates. While following a frog, the observer marked its trajectory with a string and additional time stamp markings (Supplementary Fig. S1). Time was marked irregularly on the string every 1–15 min depending on the speed of the frog and the risk of losing it from sight while marking. Occasional longer gaps occurred when a frog was lost from sight but found again. The string trajectories were later digitized using a detailed GIS background map of the area (M. Ringler, Mangione, Pašukonis, et al., 2014). In addition, we collected movement data of several tadpole-carriers during a different tracking study from 6 to 10 April. These data are not directly comparable because of changes in tracking methods and experimental set-up but provided important observations of removed pool site visits after a period of approximately 2 months.

Frog Sampling Analysis

All spatial data were analysed in the GIS software ArcGIS 10 (ESRI). To infer the most likely origins of the specific tadpole transport events, we calculated the territory centres of all males that showed territorial behaviour during the entire sampling period from 22 January to 24 February (Fig. 1). Territory centres were calculated as the centroids ('Mean Center'-function in ArcGIS 10) of all encounter points that were associated with territorial behaviour, excluding tadpole transport points and locations where a male was apparently homing back to its territory after tadpole transport.

Because of intensive sampling by several people, tadpolecarriers were sometimes recaptured at multiple locations during the same tadpole transport event. We considered recaptures of the same individual as separate transport events only if there was at least a 1-day gap

between recaptures or a male was recaptured with more tadpoles than on the previous capture. Distances from the territory centres were only calculated for the first capture location of every transport event. A previous study had found that tadpole-carrying *A. femoralis* males tend to transport more tadpoles when travelling further from the territory (E. Ringler et al., 2013). To re-evaluate these findings, we fitted a linear mixed-effects model to the data using tadpole number as dependent variable, distance from the territory centre as the predictor and frog ID as a random effect. We log-transformed the transport distances because the number of tadpoles transported is limited by clutch size and cannot increase indefinitely with transport distances. The analysis was done using the 'lme4' (Bates, Mächler, Bolker, & Walker, 2014) package for the 'R' environment for statistical computing (R Core Team, 2015).

To evaluate the site preference based on capture–recapture data, we only considered individuals captured during 9–24 February, when all test and control sites were in place. Frogs were considered at a specific site if they were found within a 1 m radius (measured on the GIS background map) from the centre of the site. Multiple captures of the same individual at different site types (i.e. pool site, odour site and bucket) were included. Recaptures of the same individual at the replicates of the same site type (e.g. pool site 1 and pool site 2) were excluded.

Movement Trajectory Analysis

Visualization, extraction of coordinates and distance measurements were done in ArcGIS 10. To quantify the space use patterns of tracked frogs, we estimated their utilization distributions (UD) with a biased random bridge approach using a movement-based kernel density estimator (MKDE; Benhamou, 2011) as implemented in the 'adehabitat' package v1.8 (Calenge, 2006) for 'R'. MKDE can incorporate both time and distance between the consecutive relocations (i.e. step length and duration) into the estimation of a UD, and thus this method is particularly suitable for our tracking data at high frequency but with irregular intervals. As model parameters we used a maximum step duration (T_{max}) of 60 min, an interpolation time (τ) of 1 min, a minimum step length (L_{min}) of 1 m, and a minimum smoothing parameter (h_{min}) of 1 m. The diffusion coefficient (D) was estimated using the maximum likelihood function (BRB.likD) implemented in the package 'adehabitat'. The resulting probability density distribution was evaluated at a raster cell size of 1×1 m. To show the overall movement patterns during tadpole transport, the % volume raster cell values of all individual MKDEs were summed and divided by the number of individuals for averaging. The resulting combined UD was re-evaluated by inversely mapping the averaged % volume values linearly to the range 1–100% and then using the 'percent volume contour' tool of 'Hawth's Analysis Tools for ArcGIS' (Beyer, 2004) for establishing combined % volume contours. For visualizing the combined space use pattern, we used a colour gradient to represent the cumulative volume under the aggregated UD, which is indicative of the encounter probability of frogs. Two of 15 tracked individuals were excluded from this analysis because they showed very little movement during the tracking period.

To describe the directionality of movement between pool sites along the array axis, we used all trajectory parts within a 10 m radius from each pool site, starting from the point where a

frog first reached the pool. All trajectory parts were extracted, shifted to a common starting point and rotated with the pool array axis running from 0° to 180°. We measured the bearing from the centre of the pool site to the location closest to crossing the 10 m radius circle. Because the expected orientation to other pool sites is diametrically bimodal (0° or 180°), we transformed the observed angles (a_i) to a unimodal distribution using the angle doubling procedure (Batschelet, 1981). The transformed angle (b_i) is equal to $2a_i$ if $2a_i < 360°$. If $2a_i > 360°$ then $b_i = 2a_i–360°$. This procedure results in a unimodal distribution around 0°. Directionality of movement between the pool sites was tested with Rayleigh's test for unimodal distribution using the circular statistics program Oriana 4.02 (Kovach Computing Services, Pentraeth, U.K.).

Ethical Note

Our study was approved by the scientific committee of the 'Nouragues Ecological Research Station' where fieldwork was conducted (http://www.nouragues.cnrs.fr). All necessary permissions were provided by the 'Centre National de la Recherche Scientifique' (CNRS) and by the 'Direction Régionale de l'Environment, de l'Amenagement et du Logement' (DEAL: ARRETE n°2011-44/DEAL/SMNBSP/BSP). All experiments were conducted in strict accordance with current French and EU law. We adhere to the 'Guidelines for use of live amphibians and reptiles in field and laboratory research' by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. Experiments were conducted in a free-ranging frog population dependent on artificial reproductive resources (artificial pools). Our temporary experimental resource manipulation did not have a lasting effect on the population, which was monitored again in the following year (winter 2015). Tadpoles collected from the removed artificial pools have successfully emerged and remained in the local population. For the purpose of this study, we captured the frogs using transparent plastic bags and identified them with photographs of their unique ventral coloration patterns. Animals were handled briefly (up to a few minutes) before release on the first encounter and usually identified through the bag without handling on the consecutive recaptures. The movement pattern data reported in this study were observational and did not involve tagging the animals.

Results

Territories and Tadpole Transport

During the entire sampling period, we recorded a total of 56 males at a total of 331 capture points. Of these, 220 points were associated with territorial behaviour. During the experimental sampling period, we recorded 49 independent tadpole transport events by 34 different individuals. All of the tadpole transport events were from territorial males with known territory locations (Fig. 1). Tadpole-transporting males were first captured 2–144 m (mean = 54.5 m, SD = 30.6 m) away from their respective territory centre. They were transporting 2–19 tadpoles (mean = 8.7, SD = 4.4). We found a strong and significant positive relation between the distance from the territory centre and the number of tadpoles transported (effect size (r) = 0.48, $P < 0.001$, $N = 47$). Two transport events were excluded from this analysis because the tadpole counts were not available.

Site Preference

A total of 29 tadpole-transporting males were captured during the period when the experimental array was fully in place. Of these 29 individuals, 13 were captured at least once within 1 m of a pool site (mean visits per site = 1.3, range 0–3). Three individuals were found at experimental odour sites and 10 frogs were captured beneath one of the two suspended buckets, holding large quantities of tadpoles. No frogs were found at the marked control sites.

Movement Patterns

We followed 15 different males during 20 different tadpole transport events resulting in an accumulated distance of 1503 m during a total of 7627 min of focal tracking. Each male was followed on average for 508 min (minimum = 44 min, maximum = 1109 min, SD = 334 min). During this time, each individual moved on average 100 m (minimum = 3 m, maximum = 302 m, SD = 84 m), resulting in an average speed of 0.56 m/min (SD = 1.03 m/min). In general, tadpole-carriers performed short bouts of rapid movements with longer motionless pauses. The maximum speed measured over a short bout was 10.47 m/min.

Of 15 tadpole-carriers tracked, 13 visited experimental sites and most of the individuals visited multiple sites (Figs. 2 and 3, Supplementary Table S2). Tracked frogs visited all except one (pool 10) of the removed pool sites and single individuals visited up to six different pool sites (mean = 2.6, SD = 1.6). Pool sites were visited by up to six different males (mean = 3.8, SD = 1.9) and frogs often revisited the same pool sites on several occasions. In addition, seven of 15 males visited one of the suspended tadpole buckets. Finally, during the additional short tracking period 2 months after pool removal, five of 11 tracked frogs were observed visiting five different pool sites. Two of these five individuals were previously tracked males revisiting removed pool sites that they had already visited 2 months earlier.

Males that visited removed pool sites spent on average 18% (maximum = 81%, SD = 23%) of their total tracking time within 2 m of one of the sites. In addition, males that visited one of the tadpolecontaining buckets spent on average 22% (maximum = 48%, SD = 19%) of the tracking time within 2 m of these sites. The UD analysis revealed that highly used areas of tracked individuals included nine of 10 pool sites and both tadpole-containing buckets (Fig. 3).

In general, frogs showed a corridor-like movement between the pool sites, pausing at one site before moving in a highly directed manner on to the next site (Figs. 2 and 4). The orientation when moving from one pool site to the next showed a highly significant directionality (expected bearing = 0°, mean bearing = 347.3°, 95% CI = 325°–9.7°, Rayleigh test: $P < 0.001$, $N = 34$). Tadpolecontaining buckets apparently attracted frogs during their movement from pool to pool (Fig. 2b). Four males showed an abrupt change in movement direction from pool 14 to the bucket approximately 10 m away (Figs. 2b and 4). Finally, two males showed a change in movement direction at pool 18 and left the linear pool site array for a natural deposition site 36 m away.

Discussion

In line with the spatial memory hypothesis, the initial capture locations and tracked movements of tadpole-transporting male *A. femoralis* were highly concentrated around and directed towards the exact locations of the removed pools. Further, single frogs visited up to six different sites distributed over 100 m linear distance. This constitutes strong evidence that spatial memory plays a predominant role in the poison frog's ability to relocate multiple, small, widely distributed water bodies.

Most of the observed movement was highly directional and strongly oriented towards one of the removed pool sites. However, the suspended buckets containing large numbers of tadpoles also attracted tadpole-carrying males from at least 10 m away. This effect is most likely explained by orientation towards water containing high concentrations of tadpole odour cues. *Allobates femoralis* only uses terrestrial deposition sites and frogs could not identify the suspended buckets as potential deposition sites by visual cues. The use of olfactory cues for orientation is widespread in nocturnal temperate region anurans (e.g. Ferguson, 1971; Oldham, 1967; Sinsch, 1987). Recent research has also demonstrated that the dendrobatid frog *Ranitomeya variabilis* uses olfactory cues to avoid depositing offspring with cannibalistic conspecific tadpoles (Schulte et al., 2011). Further studies are needed to identify which components and what amount of conspecific tadpole cues attract the tadpole-transporting males of *A. femoralis*.

Crucially, olfactory beaconing can be excluded as the principal mechanism for locating removed pool sites in our study. The experimental odour sites explicitly placed close to the removed pool array did not have a clear effect on frog movement. Further, the frequent heavy rainfall during our study period would have diluted and eventually eliminated residual olfactory cues at removed pool sites. In contrast, similar olfactory cues (water previously containing tadpoles) were restored on a daily basis at the experimental odour sites but still failed to attract the frogs. The contrasting results between the buckets and the experimental odour sites are probably due to strong quantitative and/or qualitative differences in olfactory cues between the sites where large numbers of tadpoles were always present (buckets) and where diluted water with presumed odour cues was spilled (experimental odour sites). Olfactory cues are particularly hard to quantify and this was not in the scope of our field study, but the olfactory effects should be further investigated under more controlled conditions.

In concordance with our predictions, frogs knew several different deposition sites in the area. This rules out natal imprinting as an exclusive learning mechanism underlying the observed movement patterns and suggests a more flexible learning process. Interestingly, 2 months after pool removal, some tadpole-carrying males continued to visit the old pool sites. Two of these individuals were observed revisiting removed pool sites that they had already visited 2 months earlier. Such persistence of spatial memory might be favoured by the ephemeral nature of the natural deposition sites. Many natural pools might dry out for periods of time during the reproductive season but refill again later. Further, certain areas continuously provide natural temporary water bodies, such as fallen palm fronds, which have a continuous overturn as old ones decay and become unsuitable but new ones become

available. Revisiting such areas might lead to discovering new deposition sites. It remains unclear how males discover such ephemeral pools in the first place, but conspecific tadpole odour detection might be one of the mechanisms used to find sites already used by other males.

This study replicates and supports a recent finding that male *A. femoralis* seem to anticipate the distance to the deposition sites (E. Ringler et al., 2013). The strong correlation between the transport distances and the number of tadpoles transported suggests that males prefer to transport large numbers of tadpoles when travelling to distant pools, but they might transport smaller batches of tadpoles and distribute them across pools when the chosen pools are closer. This also provides additional albeit indirect evidence for the males' spatial knowledge of the area. Spreading of tadpoles across several pools by male *A. femoralis* was recently demonstrated and described as a bet-hedging strategy, using parentage assignments of tadpoles in pools (Erich et al., 2015). Our study provides the first behavioural evidence that males know multiple deposition sites over a large area, which would allow males to make such informed decisions about tadpole distribution. Our findings could be generalized and provide insights into the spatial cognition of other dendrobatid species. For example, female strawberry poison frogs, *Oophaga pumilio*, return to multiple deposition sites to provision their offspring with unfertilized eggs (Brust, 1993). Females appear to use fine-scale spatial cues for offspring recognition (Stynoski, 2009), but how they find their way around and keep track of multiple simultaneous tadpole-rearing sites remains unknown. Several poison frog species, including *A. femoralis*, have been shown to avoid or to prefer deposition sites of a specific size, containing specific prey or predator types (Brown, Morales, & Summers, 2008; McKeon & Summers, 2013; Rojas, 2014). Integrating such information about resource quality with spatial memory could allow poison frogs to develop complex and efficient resource exploitation strategies.

An ability to integrate directional and positional cues to create novel routes as seen in some mammals, birds and insects has been proposed to form the basis of the presumed cognitive maps (Jacobs & Menzel, 2014; Jacobs & Schenk, 2003). Given the relatively distant and small goal, we believe that general directional cues ('bearing map' sensu Jacobs & Schenk, 2003) from the territory alone are not sufficient to explain such accurate orientation to the removed pool sites. Frogs often spent a large proportion of their total travel time stationary at the locations of the missing pools, which suggests that males recognize some positional cues near the deposition sites ('sketch map' sensu Jacobs & Schenk, 2003). Translocation studies of territorial *A. femoralis* males have also revealed that these frogs rely on experience with the area to find their way back to the territory (Pašukonis et al., 2013, 2014). They take direct homing paths from arbitrary release points, which is often considered a hallmark of map-like orientation in other animals (Jacobs & Menzel, 2014). In addition, a recent laboratory study showed that one poison frog species has the cognitive abilities to use visual cues and flexible learning strategies in a serial spatial reversal task (Liu et al., 2016). Taken together these findings suggest that poison frogs may form and use flexible spatial map-like representations of their surroundings. Such spatiocognitive capabilities have so far been demonstrated only in some mammal, bird and social insect species.

Further experimental work is needed to test the flexibility of poison frogs' spatial memory for taking completely novel direct routes to the deposition sites. This ability is likely to have adaptive significance in the dynamic landscape of shifting male territories and scattered ephemeral reproductive resources. Tadpole transport over long distances involves energetic costs and the risk of losing mating opportunities or the territory altogether. Further, travelling males are probably more exposed to predation. Indeed, a snake predated one of the tracked males in this study and a second frog received injuries, probably from a bird. Considering all the associated costs of tadpole transport, the selective pressure for flexible spatial memory use allowing efficient way finding between the territory and the deposition sites must be high.

Overall, our study demonstrates that *A. femoralis* rely heavily on spatial memory for efficient use of small and scattered reproductive resources. We cannot fully explain the observed spatial behaviour by uninformed search, beaconing or natal imprinting and more complex and flexible learning strategies probably underlie poison frog movements. Given similar behaviour and ecological requirements, our findings probably generalize to other dendrobatid frogs and corroborate the emerging view that spatial-learning abilities are ubiquitous among, and potentially ancestral to, all vertebrates. However, little is known about amphibian spatial cognition and their movements in general, especially in the tropics where taxonomic and behavioural amphibian diversity is highest. The diversity of amphibian spatial behaviours provides a unique system for understanding how environmental factors such as the stability and distribution of reproductive resources might shape spatiocognitive abilities in animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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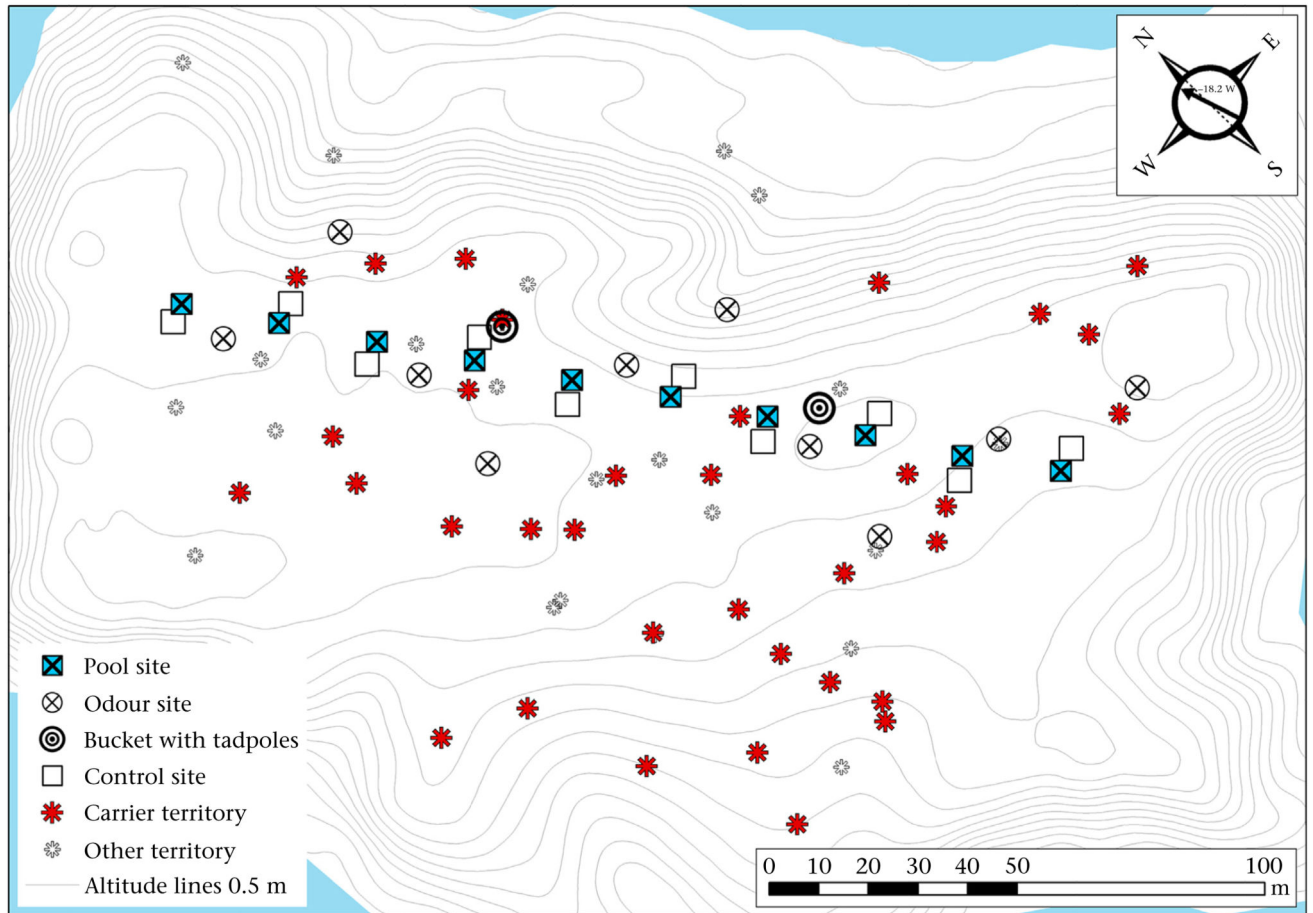
Figure 1.
Map of the study area showing the experimental set-up and the distribution of male territories. Crossed squares represent the linear array of 10 artificial tadpole deposition sites that were removed during our experiment; crossed circles represent 10 experimental sites where water with tadpole odour was spilled; concentric circles represent the two suspended buckets containing large numbers of tadpoles; empty squares represent 10 control sites. Stars indicate territory centres of 56 sampled males. Red stars highlight the territories of 34 males that were sampled during tadpole transport. Contour lines (0.5 m) are drawn in light grey and the Arataye river in blue.
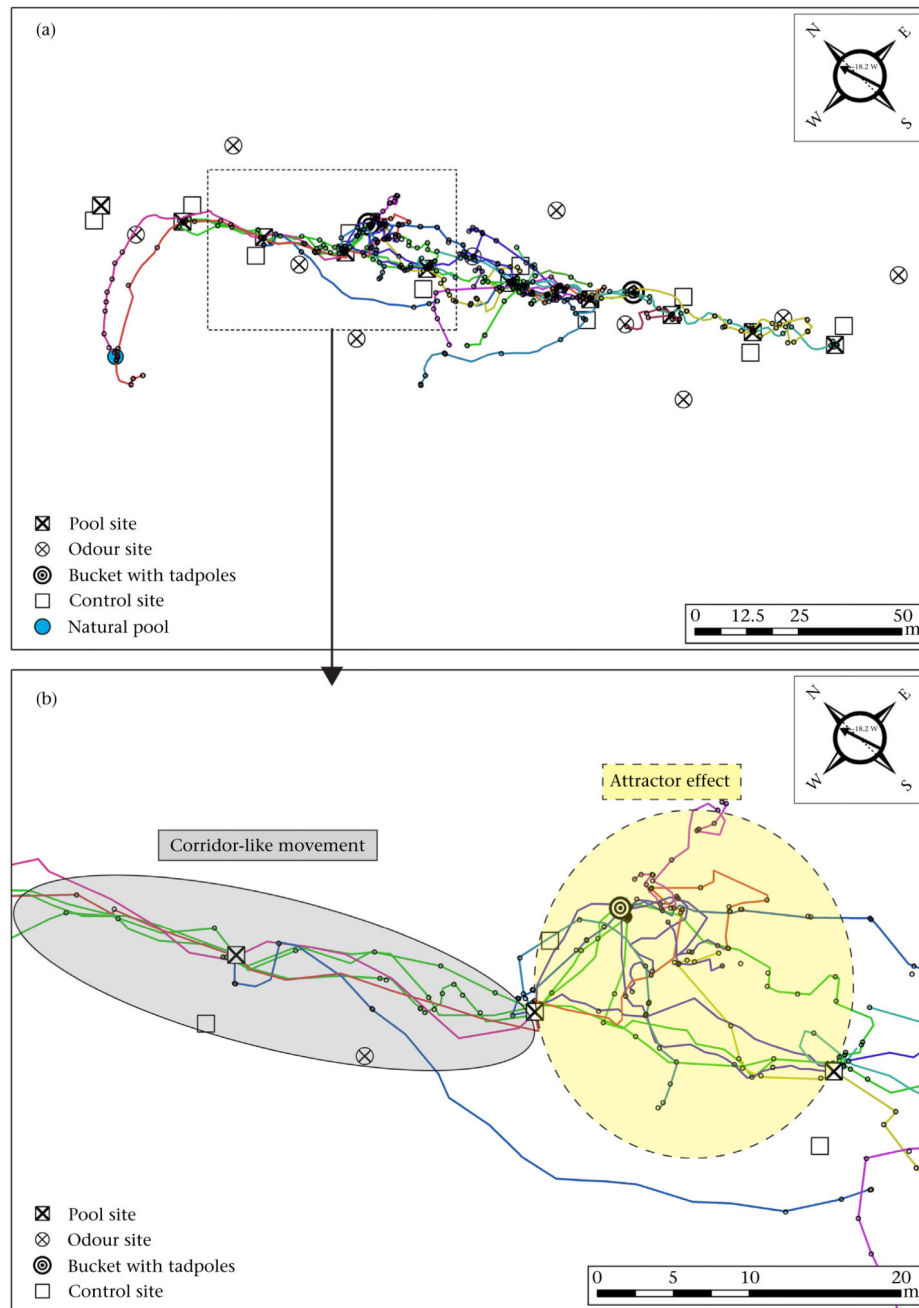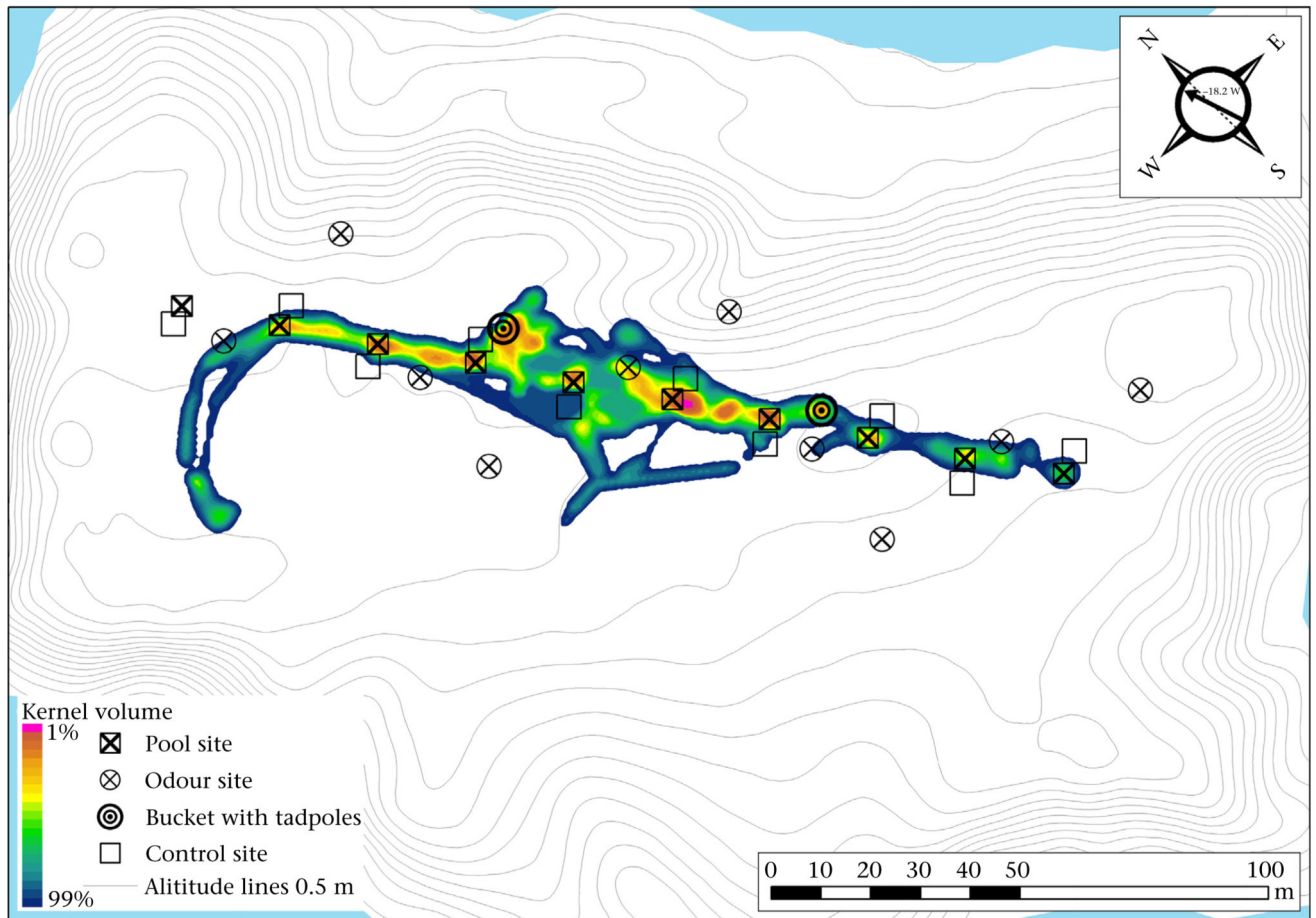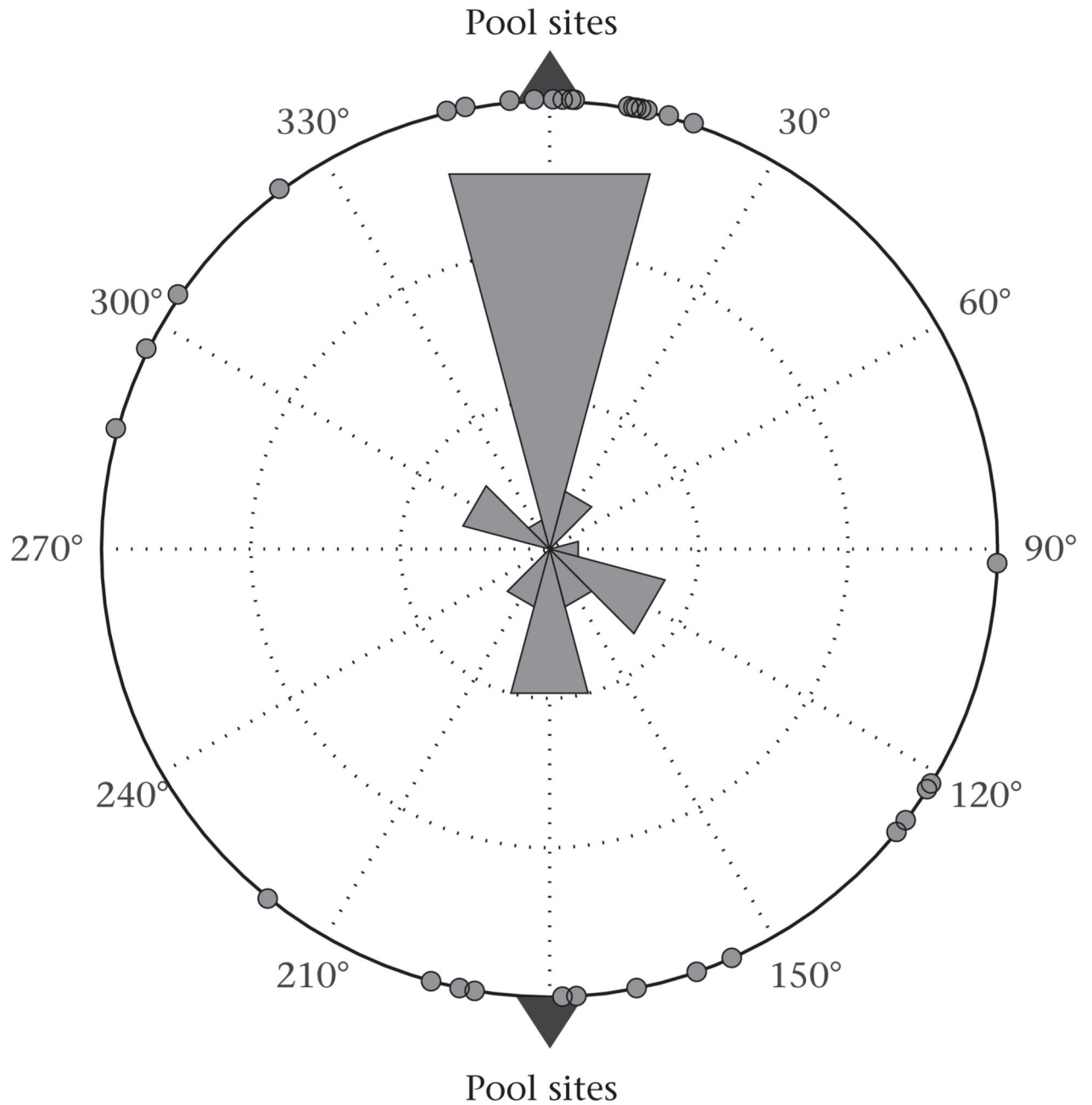
Figure 2.

Trajectory map showing movement patterns of tadpole-transporting males during the experiment. Crossed squares represent the linear array of 10 artificial tadpole deposition sites that were removed during our experiment; crossed circles represent 10 experimental sites where water with tadpole odour was spilled; concentric circles represent the two suspended buckets containing large numbers of tadpoles; empty squares represent 10 control sites; the blue circle represents a natural deposition site that was used by frogs during the experiment. Different colour lines represent trajectories of different individuals. (a) The

whole area covered by tracked tadpole-carriers. Overall, frogs showed a corridor-like movement along the array of pool sites. (b) A close-up of pool sites 6, 7, 8 and the N-bucket. An example of a corridor-like movement between pool sites is shaded in grey. The catchment area of the tadpole bucket to which frogs diverted when moving between the pool sites is outlined with a dashed line and shaded in yellow.

Figure 3.
Aggregated utilization distribution (UD) from the bridged-kernel analysis showing the overall pattern of space use of 13 tadpole-transporting males along the removed artificial tadpole deposition sites. The heat map shows the % volume under the combined UD, represented by the colour gradient where low % values correspond to areas of highest use. Crossed squares represent the linear array of 10 artificial tadpole deposition sites that were removed during our experiment; crossed circles represent 10 experimental sites where water with tadpole odour was spilled; concentric circles represent the two suspended buckets containing large numbers of tadpoles; empty squares represent 10 control sites. Contour lines (0.5 m) are drawn in light grey and the Arataye river in blue.

Figure 4.
Rose diagram showing the directionality of movement from the removed pool sites. The plot centre represents the position of nine pool sites visited during frog following. Black arrows at 0° and 180° indicate the axis of the pool site array. Each point represents a bearing of an individual that moved away from the pool site. Grey bars show the grouping of these bearings for every 30° where the bar length represents the number of bearings. The same

individuals were sampled multiple times if they visited a different site or revisited the same site on different occasions.