

Induced parental care in a poison frog: a tadpole cross-fostering experiment

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Summary statement

We triggered parental tadpole transport in males and females of a poison frog with asymmetric parental sex-roles by transferring unrelated tadpoles on the backs of adult frogs under natural conditions.

Abstract

Understanding the external stimuli and natural contexts that elicit complex behaviors, such as parental care, is key in linking behavioral mechanisms to their real-life function. Poison frogs provide obligate parental care by shuttling their tadpoles from terrestrial clutches to aquatic nurseries, but little is known about the proximate mechanisms that control these behaviors. In this study, we used *Allobates femoralis*, a poison frog with predominantly male parental care, to investigate whether tadpole transport can be induced in both sexes by transferring unrelated tadpoles to the backs of adults in the field. Specifically, we asked if the presence of tadpoles on an adult's back can override the decision-making rules preceding tadpole pick-up and induce the recall of spatial memory necessary for finding tadpole deposition sites. We used telemetry to facilitate accurate tracking of individual frogs and spatial analyses to compare movement trajectories. All tested individuals transported their foster-tadpoles to water pools outside their home area. Contrary to our expectation, we found no sex difference in the likelihood to transport nor in the spatial accuracy of finding tadpole deposition sites. We reveal that a stereotypical cascade of parental behaviors that naturally involves sex-specific offspring recognition strategies and the use of spatial memory can be manipulated by experimental placement of unrelated tadpoles on adult frogs. As individuals remained inside their home area when only the jelly from tadpole-containing clutches was brushed on the back, we speculate that tactile rather than chemical stimuli are triggering these parental behaviors.

Keywords: parental care, behavioral trigger, flexibility, spatial cognition, tadpole transport, telemetry

Introduction

Studying the external stimuli and contexts that induce and modulate complex behaviors, such as parental care, is key in linking the proximate mechanisms to the function of behavior (Brown, 1985; Taborsky et al., 2015). Understanding the constraints imposed by these proximate mechanisms on the behavioral flexibility can also provide insights into the evolution of traits associated with parental care. For example, the predisposition of many birds to respond to orange gape and loud begging calls with food provisioning explains, at least in part, the prevalence of brood parasites among altricial birds (Kilner et al., 1999; Redondo, 1993). Despite the diversity of vertebrate parental strategies (reviewed in Balshine, 2012), it has been suggested that many neuroendocrine mechanisms might be conserved across taxonomically distant groups (Brown, 1985; Dulac et al., 2014). Cross-fostering of young has been used to study neural and hormonal basis of parental care, offspring recognition, adaptive value of parental investment as well as other associated traits in many vertebrates (e.g., Buntin, 1996; Dewsbury, 1985; Francis et al., 1999). Amphibians show a variety of parental behaviors, including egg guarding, nest making, larval transport and provisioning (Crump, 1996; Wells, 2007), but the proximate mechanisms of amphibian parental care remain mostly unknown and standard methods such as cross-fostering are rarely applied (but see Ringler et al., 2016; Stynoski, 2009).

Poison frogs (Dendrobatidae) are a well-studied family of Neotropical frogs with complex life-histories that involve parental care, including egg attendance and larval transport from terrestrial clutches to aquatic deposition sites (Grant et al., 2006; Weygoldt, 1987). The adaptive value and the evolution of parental care in this group has attracted a considerable amount of research (reviewed in Summers and Tumulty, 2013), but the proximate mechanisms of these behaviors remain poorly understood (Roland and O'Connell, 2015). Several recent studies on the poison frog *Allobates femoralis* have revealed a surprising degree of flexibility in the parental care strategies of this species. In *A. femoralis* tadpole transport is primarily performed by males (Ringler et al., 2013). While males indiscriminately transport all tadpoles encountered inside their territory (Ringler et al., 2016), males become cannibalistic when establishing a new territory (Ringler et al., 2017), thus adjusting their parental responses towards unrelated clutches according to their territorial status. Females, in

turn, show compensatory parental care when the father disappears (Ringler et al., 2015a), and will only transport tadpoles from the exact location of their own clutch. Neither sex appears to use direct cues for offspring recognition (Ringler et al., 2016).

Tadpole transport in *A. femoralis* follows a sequence of stereotypical behaviors (Fig.1A, Movie S1). First, to pick up the tadpoles, the parent lowers the body posture and rotates in the clutch waiting for the tadpoles to wriggle on their back. Tadpole carrier will then transport to and sometimes distribute offspring between small terrestrial pools, tens to hundreds of meters away from the territory (Beck et al., in press; Erich et al., 2015; Ringler et al., 2013). Once at the pool, frogs appear to wait and inspect the site before finally submerging themselves in the water and allowing the tadpole to swim off (personal obs. by all authors). After the deposition, the parent returns to the home territory via direct path (Beck et al., in press). Recent tracking studies have revealed that *A. femoralis* relies on large scale spatial memory for finding the pools and homing (Beck et al., in press; Pašukonis et al., 2014; Pašukonis et al., 2016). Further, it has been proposed that some strategic planning of where to go and how many tadpoles to transport is involved (Ringler et al., 2013). Together, these findings suggest that the stereotypical action patterns involved in parental care are controlled by a rather flexible decision making process and extensive use of spatial memory. Understanding the stimuli that trigger and control such behavioral cascade would constitute a crucial step in understanding the proximate mechanisms involved. In turn, understanding the proximate mechanisms of parental care in poison frogs would provide key insights into the evolution of vertebrate parental care as well as amphibian cognition.

In this study, we used telemetry and an array of artificial deposition sites to test whether tadpole transport can be triggered in male and female *A. femoralis* by experimentally transferring tadpoles to the backs of unrelated adults in the field. Several decision-making rules involved in *A. femoralis* parental care have been demonstrated or hypothesized in recent years, such as the use of spatial cues for offspring recognition, context-dependent decision to transport, and context-dependent number of tadpoles transported (Erich et al., 2015; Ringler et al. 2013; Ringler et al., 2015a; Ringler et al., 2016a; Ringler et al., 2017). We were interested if the presence of the tadpoles on the back of an adult can override these decision-making rules naturally preceding the tadpole pick-up and induce the recall of spatial memory necessary for finding the tadpole deposition sites. Because females are more selective

in initiating parental care in this species, we expected females to be less likely to transport cross-fostered tadpoles. In addition, we expected males to be faster and more accurate in finding suitable tadpole deposition sites because of their predominant role in tadpole transport.

Material and methods

The study area and species

The study was carried out from January to March 2016 and 2017 in an experimental population established on a 5 ha river-island (Ringler et al., 2015b) near the Camp Pararé field site at the CNRS Nouragues Ecological Research Station in the Nature Reserve Les Nouragues, French Guiana (<http://www.nouragues.cnrs.fr>). The frogs in the study area rely primarily on an array of 13 artificial plastic pools (volume~12 l, inter-pool distance~20 m) for tadpole deposition.

Allobates femoralis is a small diurnal frog common throughout the Amazon basin and the Guiana Shield (Amezquita et al., 2009). During the rainy season males establish acoustically advertised territories on the forest floor where courtship and oviposition occur (Ringler et al., 2009). After 12-20 days of larval development, frogs transport 1-25 tadpoles and deposit them as far as 185 m from their territory in a variety of small terrestrial water bodies (Beck et al. in press; Ringler et al., 2013). *Allobates femoralis* mating system is polygynandrous, most mature individuals reproduce continuously throughout the rainy season, and males have been observed caring for up to five clutches simultaneously (Ursprung et al., 2011).

Frog sampling

At the onset of the experiment we performed a baseline sampling of all females and males in the study area. We caught the frogs with transparent plastic bags, photographed them for individual identification by their unique ventral coloration pattern (Ringler et al., 2015b), and marked their position on a detailed GIS background map (Ringler et al., 2016b) using a tablet (WinTab 8, Odys) with the mobile GIS software ArcPad 10.0 (ESRI). We sexed the individuals by the presence (male) or absence (female) of vocal sacs. Male territorial behavior (calling, aggression, courtship) and repeated recaptures allowed identifying male territories.

To obtain tadpoles for the experiments we searched through the leaf-litter around males' calling sites. In *A. femoralis*, clutches are typically laid on dry leaves within the male's territory. We marked the locations and followed the development of all detected clutches. To assure that tadpoles used for experiments were morphologically ready to be transported, we only used hatched tadpoles in the appropriate developmental stage (Gosner stage 24-25 (Gosner, 1960); usually 12-16 days after oviposition, Fig. S1). When not transported to the water, *A. femoralis* tadpoles can remain in the egg-jelly for multiple days after hatching (S. Weinlein unpublished data).

Experimental procedure

All experiments were started in the morning between 07:30 and 10:30, which corresponds to the daytime period when *A. femoralis* usually initiate tadpole transport (Ringler et al., 2013). We captured the frogs in their supposed home areas, fitted them with a transponder for tracking and subjected them to one of the two experimental treatments: (1) tadpole cross-fostering (tadpole group hereafter) or (2) egg-jelly swabbing (jelly group hereafter). The second treatment (jelly) was conducted in the second field season as an addition to the first treatment (tadpoles). Each experimental group included 10 males and 10 females and we did not repeatedly use the same individuals between the two field seasons.

Tadpole group: To test if tadpole transport can be induced, we forcibly placed tadpoles on frogs' backs. We first carefully removed the tadpoles from the jelly onto a moist leaf using a fine-tipped paintbrush. We then transferred the tadpoles one-by-one from the leaf to the back of an adult while holding the frog immobilized by the hind legs (Fig. 2; Movie S2). We transferred 8-12 tadpoles, which corresponds to the average number of tadpoles transported naturally. We waited for a few minutes until the tadpoles settled on the back and released the frogs as gently as possible to prevent the larvae from falling off. Occasionally, the frog lost a few tadpoles with a sudden escape movement during the release. We never observed the frog losing additional tadpoles after the initial release. We continuously observed the frog for 5-15 min immediately after the release during which time most frogs remained immobile. During this period, we often observed the tadpoles wiggling on the frog and sometimes repositioning themselves on the back. To minimize the chance of testing a male with his own tadpoles, we only used tadpoles collected outside

respective male's territory and its immediate neighborhood (at least 30 m from the tested male). To minimize the chance of testing a female with her own tadpoles, we used tadpoles collected at least 50 m away from the capture site of each tested female since females preferably mate with males within approximately 20 m radius (Ursprung et al., 2011).

Jelly group: To control for the effects of handling and tagging and to test if tadpole transport can be induced by chemical cues present in the egg-jelly, we did a follow-up experiment in 2017. We dipped the paintbrush into a clutch containing at least eight ready-for-transport tadpoles and gently brushed the egg-jelly on the back of the frog. The procedure was repeated eight times and the frogs were handled identically to the tadpole group.

Tracking

We used the harmonic direction-finding telemetry technique to track the individuals and followed the protocol that has been successfully used in *A. femoralis* in several contexts including the tadpole transport (Beck et al., in press; Pašukonis et al., 2014b; Pašukonis et al., 2014a). The system consists of a directional transceiver and a passive reflector (i.e. transponder) attached to the animal. The tag together with the attachment made up for less than 5% of frogs' body weight (frog weight ~ 2 g, tag weight < 0.1 g). We used a commercially available transceiver (R8, RECCO® Rescue System, Lidingö, Sweden) for tracking. Following the release, all frogs were located every 15-30 min during their daylight activity hours (~ 07:30 to 18:30). At each instance, we carefully approached the location of the signal and attempted to detect the frog visually. In cases of poor visibility or if an individual was hiding, we narrowed the signal to less than one meter. Every position fix was recorded on the background GIS map as described above. Whenever the frog was visible, we also recorded the number of tadpoles present and the current behavior (e.g., moving, hiding, depositing tadpoles). In 2016 (tadpole group), we followed the frogs until all tadpoles were deposited. Most frogs completed the tadpole transport and deposition within one day (mean duration=4.47 h, SD=2.48 h), but two individuals were tracked for two consecutive days (Table S1). All males were untagged immediately after tadpole deposition and were recaptured back in their territory over the following days. Since females do not show conspicuous behavior such as calling or territorial aggression, it was not always possible to confirm female homing by recapture. Thus,

we tracked three females that had moved the longest distance after the deposition to affirm that females also return to their home areas after tadpole transport. All three females tracked showed homing behavior and moved back to their original home areas after tadpole deposition. In 2017 (jelly group), the frogs were tracked for one day between 6.7 h and 9.4 h (mean duration = 7.92 h) and untagged in the evening between 17:30 and 18:45.

Movement analysis

Visualization and extraction of coordinates were done in the GIS software ArcGIS™ 10 (ESRI). To describe the spatio-temporal characteristics of frog movement to the pools, we quantified the latency to move, the speed of movement and the distance travelled to the first deposition site, and the total duration until all tadpoles were deposited. The latency to move was defined as the time taken to leave a five-meter radius area from the capture/release site. This cut-off was chosen based on the distance to the closest deposition site across all frogs and it allowed to separate various local movements around the release site from the directional movement towards the deposition site. Since *A. femoralis* do not move at night, for the two frogs that were tracked overnight, we subtracted 12 h for the calculations of movement speed. We compared the total duration of tadpole transport between males and females with and without subtracting the 12 h.

To estimate orientation accuracy, we calculated a straightness coefficient (SC) defined as the ratio between the straight-line distance and the actual path distance between the starting point and the first deposition site (such as SC = 1 would indicate a perfectly straight path). SC is a simple but robust estimator of the efficiency of a goal oriented path (Benhamou, 2004). For the frogs that did not visit a deposition site, we calculated the latency to move, the total path length, the distance to the farthest point moved from the capture sites, and the movement speed over the entire tracking period. We performed a Mann-Whitney U test for sex differences in temporal and spatial parameters. We also used Mann-Whitney U test to compare the speed of movement and SC of induced male tadpole transport with natural male tadpole transport (cf., Beck et al., in press). No tracking data on natural female tadpole carriers is currently available. For visual representation, we plotted pool direction-normalized trajectories derived from the linear interpolations of consecutive positions. All analyses were performed in GIS software ArcGIS™10 (ESRI) and R statistical

software (R Core Team, 2015).

Results

All 20 individuals from the tadpole group transported and deposited the cross-fostered larvae. Seventeen frogs deposited in artificial pools and three frogs used natural pools (Fig. 3A). Two frogs (males) distributed tadpoles between two pools and one frog (female) visited a dried-out deposition site before moving to an artificial pool. From the capture site to the first deposition site frogs moved on average 36.5 m (mean $\text{path}_{\text{female}}=35$ m, $\text{SD}=31$ m; mean $\text{path}_{\text{male}}=38$ m, $\text{SD}=20$ m) at an average speed of 10 m/h (mean $\text{speed}_{\text{female}}=8.5$ m/h, $\text{SD}=6$ m/h; mean $\text{speed}_{\text{male}}=11.7$ m/h, $\text{SD}=6.7$ m/h). The average latency to leave 5-m-radius area from the capture site was 1.92 h (mean $\text{latency}_{\text{female}}=2.02$ h, $\text{SD}=1.43$ h; mean $\text{latency}_{\text{male}}=1.83$ h, $\text{SD}=1.59$ h) and the total duration until deposition was on average 6.47 h (mean $\text{duration}_{\text{female}}=8$ h, $\text{SD}=9.05$ h; mean $\text{duration}_{\text{male}}=4.49$ h, $\text{SD}=2.26$ h). The average orientation accuracy was 0.87 (mean $\text{SC}_{\text{female}}=0.89$, $\text{SD}=0.14$; mean $\text{SC}_{\text{male}}=0.85$, $\text{SD}=0.11$). We did not find any significant differences between the sexes in any temporal and spatial parameters measured (Fig.3B, C; Mann-Whitney U test: $U_{\text{latency}}=44.5$, $p=0.7$; $U_{\text{full duration}}=48$; $p=0.9$; $U_{\text{daytime duration}}=54.5$; $p=0.8$; $U_{\text{speed}}=33$, $p=0.2$; $U_{\text{SC}}=65$, $p=0.3$). We also found no significant difference between movement speed and orientation accuracy of males during induced and natural tadpoles transport (Mann-Whitney U test: $U_{\text{speed}}=54$, $p=0.8$; $U_{\text{SC}}=44.5$, $p=0.7$).

Only one frog (male) out of 20 moved to a pool after being swabbed with jelly from tadpole-containing clutches. During the entire tracking period jelly group frogs moved on average 8.16 m ($\text{SD}=6.45$ m) at an average speed of 1.1 m/h ($\text{SD}=0.9$ m/h) and only three frogs (two males and one female) left 5-m-radius area from the capture sites.

Ethical statement

Our study was approved by the Animal Welfare Board of the University of Vienna (approval number: 2016-003) and by the scientific committee of the research station where fieldwork was conducted. All necessary permits were provided by the local authorities (DEAL: ARRETE n°2011-44/DEAL/SMNBSP/BSP). All sampling was conducted in strict accordance with current French and EU law and followed the

“Guidelines for use of live amphibians and reptiles in the field and laboratory research” by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists.

Discussion

We successfully triggered tadpole transport and deposition in all tested frogs by transferring unrelated tadpoles onto their backs. In contrast, all frogs except one subjected to the same handling procedure and brushed with jelly from tadpole-containing clutches remained in their home areas. Even though *A. femoralis* females rarely and selectively transport tadpoles, both sexes were equally likely to transport cross-fostered tadpoles. We also found no difference in accuracy or speed of finding deposition sites between males and females.

Our findings show that the decision-making processes and motor actions involved in tadpole pickup do not need to precede successful tadpole transport (Fig 1B). They also confirm a recent finding that *A. femoralis* do not use direct cues for offspring recognition (Ringler et al., 2016a), similar to results for egg-feeding behavior in strawberry-poison frog, *Oophaga pumilio* (Stynoski 2009). Our ability to experimentally bypass the tadpole pickup is especially surprising for females, which are very selective to the context in which they naturally pick up and transport tadpoles (Ringler et al., 2015a) and to the precise spatial cues for clutch recognition (Ringler et al., 2016a). It suggests that even though males and females show diverging offspring recognition strategies, the mechanisms underlying tadpole transport are likely to be shared between sexes. In addition, the similar spatio-temporal patterns of tadpole transport between the sexes suggest shared underlying spatio-cognitive capabilities. While parental care in poison frogs is typically classified based on species- and sex-specific parental roles, anecdotal observations and some experimental data suggest that both, the presumed species- and sex-specific parental roles may be more flexible than often assumed (Myers and Daly 1983, Ringler et al., 2015a; Souza et al., 2017; Tumulty et al., 2014; Vacher et al., 2017; Wells 2007, Table 11.3, p. 524–526). We speculate that the mechanisms underlying poison frog parental care are not species- nor sex-specific and therefore allow strong plasticity through ontogeny as well as flexibility in adults.

Our study provides limited information about the exact cues that trigger tadpole transport, but we speculate that tactile stimuli may play a predominant role. Dorsal swabbing with the jelly from clutches that contain ready-for-transport tadpoles did not trigger the parental behavior, suggesting that chemical cues passively released by tadpoles are not sufficient as a trigger. Tadpoles wriggling up and on the back of a parent could provide a variety of tactile cues to initiate the tadpole transport. Tadpoles of several tropical anurans, including dendrobatid frogs, are known to use tactile and vibrational cues for parent-offspring communication in the context of begging behavior (Jungfer, 1996; Jungfer and Weygoldt, 1999; Kam and Yang, 2002; Stynoski and Noble, 2012; Yoshioka et al., 2016). The tactile cues could work in combination with chemical cues actively released by the tadpoles once on the back of the parent, which might in part explain the delay to move after the tadpole transfer. The mechanisms by which tadpoles identify a suitable carrier and adhere to their back are not well understood, but are also likely to involve mechanical and chemical interactions (Grant et al., 2006; Lüddecke, 1999; Myers and Daly, 1980). Interestingly, different poison frog species vary in how strongly the tadpoles are attached to the back of the carrier (Myers and Daly, 1980; personal obs. by AP). Identifying these attachment mechanisms might provide crucial insights into the cues that trigger the tadpole transport in poison frogs.

Even though tadpole transport was triggered in all tested individuals, there was a marked and highly variable latency between the tadpole transfer and the initiation of the movement towards a deposition site. Several factors such as the nature of the trigger or the spatio-cognitive processes preceding long-distance movement may influence this latency, but we speculate that the motivational/hormonal state of the individual is likely to be the key element. Complex cycles of neuroendocrine parental care regulation in mammals, birds, and fish are often preceded by reproductive triggers such as mating (Brown, 1985; Dulac et al., 2014). In *A. femoralis*, females can produce clutches on average every eight days and males commonly have several clutches simultaneously inside their territory (Ursprung et al., 2011). As a result, mating and parenting generally overlap, but the exact reproductive state and thus the parental motivation most likely varies between individuals. Our results suggest that reproductively active adults are indeed in a general readiness state for tadpole transport and the variable latency to initiate transport might reflect the exact reproductive status of each individual. Interestingly,

male parental care has been shown to correlate negatively with territoriality and aggressiveness via steroid hormone levels in several vertebrates (Ros et al., 2004; Wingfield et al., 1990) including one frog species (Townsend and Moger, 1987). *Allobates femoralis* males appear to be an exception to this pattern as they maintain high aggression and territoriality levels while being in such parental readiness state. Neuroendocrine control of parental care in poison frogs has recently attracted some attention (Roland and O'Connell, 2015; Schulte and Summers, 2017), but to date the mechanisms of parental care in amphibians remain a virtually unexplored field.

To sum up, our results echo the findings of classical ethological studies on parental spatial behavior of digger wasps (*Ammophila spp.*, Baerends, 1941) and beeswolves (*Philanthus spp.*, Tinbergen, 1932) in revealing how a single trigger can induce a stereotypical behavioral cascade of fixed action patterns. Some key components of such sequence are usually controlled and modulated by memory. In *A. femoralis* the presence of tadpoles on the back induces a stereotypical sequence of orientation, fast directional movement, pool inspection, tadpole deposition, and homing: behaviors dependent on the recall of long-term spatial memory (Beck et al., in press; Pašukonis et al., 2014a; Pašukonis et al., 2016). The exact cues that trigger tadpole transport remain to be studied, but we speculate that tactile stimuli play a predominant role. We believe that our findings provide key behavioral data from the field and an experimental approach for future studies on the neuroendocrine basis of parental behavior and the associated cognitive processes.

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Data accessibility: All tracking data used and calculated variables have been uploaded as electronic supplementary material and deposited on the Dryad Digital Repository.

Competing interests: We have no competing interests.

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Figures

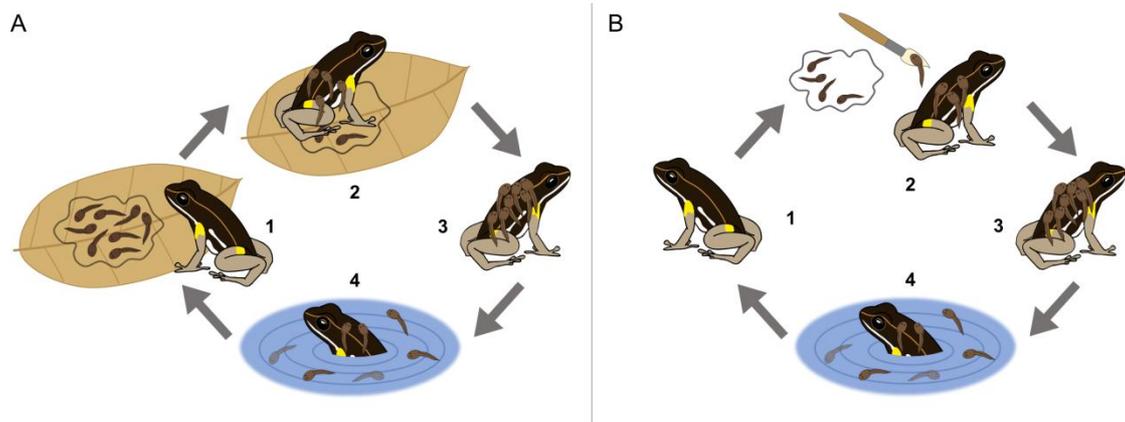


Figure 1. Schematic representations of the behavioral cascade involved in A) natural tadpole transport and B) induced tadpole transport. A) The numbered stages represent: 1) clutch recognition and homing; 2) tadpole pickup; 3) orientation and tadpole transport; 4) pool inspection and tadpole deposition. B) The numbered stages represent: 1) adult in home area and homing; 2) tadpole transfer from an unrelated clutch to the back of a frog; 3) orientation and tadpole transport; 4) pool inspection and tadpole deposition.

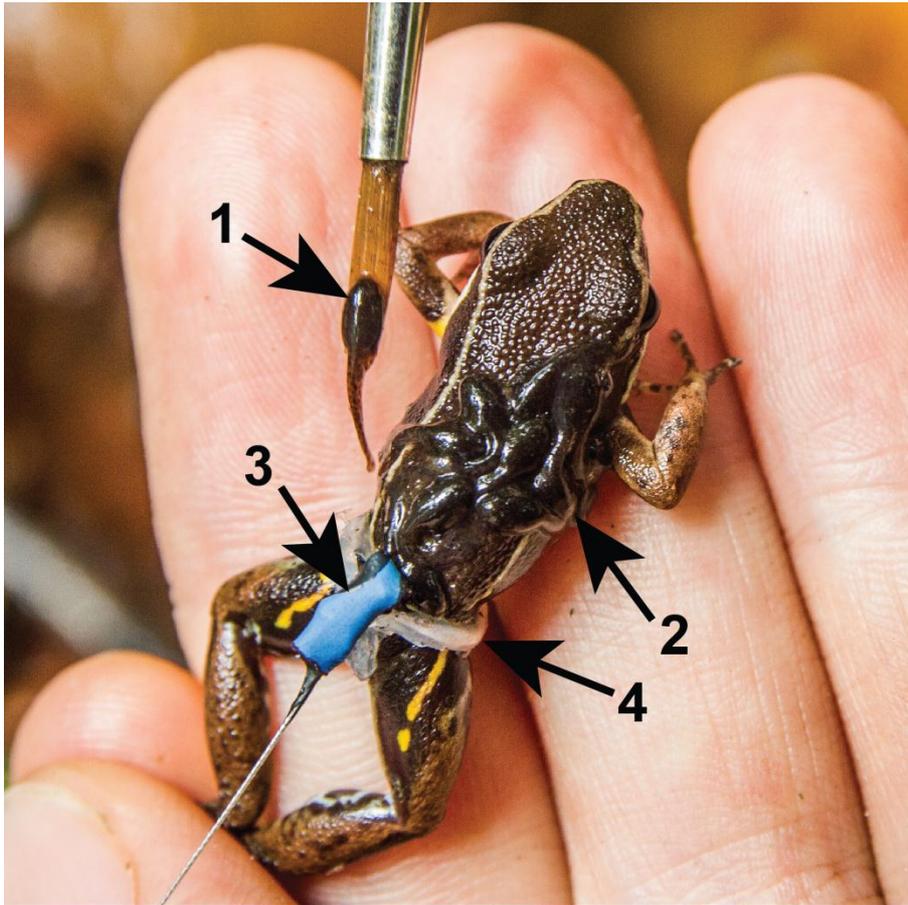


Figure 2. Photograph showing a captured frog wearing the tracking transponder during the tadpole transfer procedure. The numbers and arrows indicate: 1) a live tadpole being transferred with a fine-tip paintbrush; 2) tadpoles already transferred on the back of the frog; 3) a sealed diode with antenna used for tracking; 4) silicone waistband holding the tag.

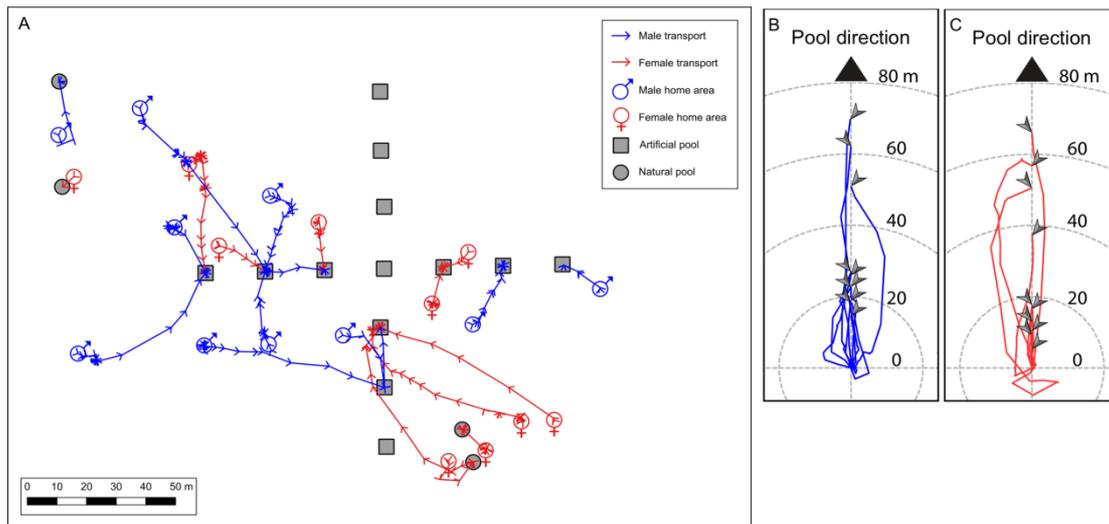


Figure 3. Study area map and frog trajectories and the showing induced movements. A) Schematic representation of the study area and the movement patterns of tested frogs during induced tadpole transport from their home areas to aquatic deposition sites. Grey squares represent artificial pool array, grey circles represent natural deposition sites visited by tested frogs, red Venus symbols and blue Mars symbols represent female and male home areas respectively. Red and blue lines represent interpolated female and male movement trajectories. Arrows on the lines mark every position recorded during tracking and indicate the direction of movement. B) C) Tadpole transport trajectories of B) males and C) females normalized for and shown until the first deposition site. Each line corresponds to a single individual. The grey arrows indicate the last point of each trajectory, i.e. the deposition site.

Table S1. All measured and calculated variables per individual for the tadpole group frogs. Each row corresponds to a different individual. Females indicated by “f”, males indicated by “m”. See materials and methods for detailed explanation.

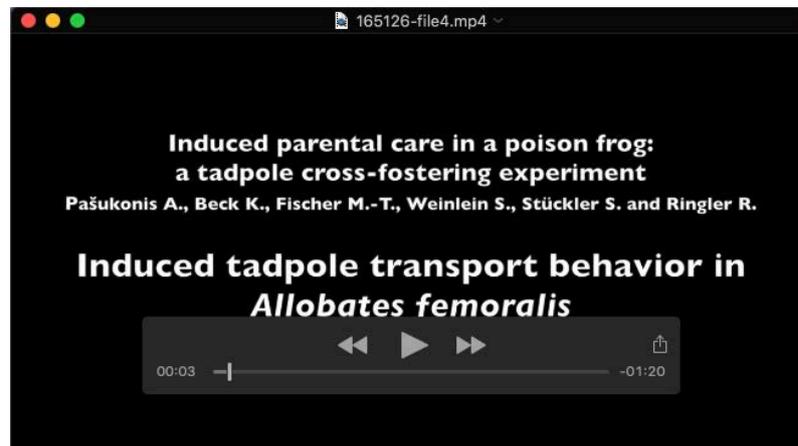
ID	Tadpoles added	Duration (h)	Speed (m/h)	Total path (m)	Path to 1st pool (m)	Straight distance (m)	SC	Latency to move (h)	Deposition site
f1	11	24.57	3.79	46.59	46.59	37.30	0.80	0.35	pool
f2	8	0.38	13.28	5.09	5.09	5.09	1.00	0.38	palm bract
f3	11	3.38	5.31	16.81	16.81	16.49	0.98	1.67	pool
f4	8	1.68	7.22	10.22	10.22	9.38	0.92	0.50	pool
f5	9	1.67	10.74	17.91	17.91	17.70	0.99	0.67	pool
f6	8	5.33	2.86	13.36	13.36	12.96	0.97	4.33	pool
f7	8	24.5	5.99	68.93	68.93	56.50	0.82	3.50	pool
f8	8	4.00	22.22	66.65	66.65	66.02	0.99	1.00	dry burrow then pool
f9	8	9.00	10.23	92.04	92.04	50.51	0.55	1.00	pool
f10	8	5.50	3.12	11.95	11.95	10.25	0.86	3.50	pool
m1	8	6.50	5.74	33.50	33.50	25.78	0.77	0.83	pool
m2	8	6.77	8.66	58.61	58.61	50.97	0.87	1.13	pool
m3	12	1.62	27.61	28.07	28.07	17.92	0.64	0.23	fallen tree
m4	8	4.17	9.79	24.47	24.47	22.32	0.91	1.00	pool
m5	8	6.75	8.64	49.11	28.80	26.92	0.93	0.83	2 pools
m6	11	1.67	11.18	14.91	14.91	14.66	0.98	0.67	pool
m7	12	5.50	5.74	25.84	25.84	18.62	0.72	4.50	pool
m8	8	6.70	11.17	72.24	72.24	69.47	0.96	0.35	pool
m9	8	7.25	9.34	67.70	67.70	62.11	0.92	4.35	pool
m10	10	2.50	18.88	47.19	26.75	22.45	0.84	0.75	2 pools



Figure S1. A photograph showing *A. femoralis* tadpoles hatched inside egg-jelly before being transported by an adult to the water. Tadpoles of similar developmental stage were used for inducing parental behavior.



Movie S1



Movie S2