DISSERTATION

„The role of learning in the spatial behavior of Neotropical poison frogs (Dendrobatidae)“

Verfasser

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Pats nuoširdžiausias ačių visiems mano artimiesiams už jūsų palikymą!
On the other hand, I am pained to confess that my many observations and experiments with the several species of true frogs found here, conducted without an intermission for four months, have yielded but little evidence that these creatures possess a particle of intelligence. It almost proved, indeed, to be labor lost,—

‘To perch upon a slippery log,
And sit in judgment on a frog.’

Charles Conrad Abbott “The Intelligence of Batrachians”, Science (1884).
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Summary

Spatial learning has been studied in a variety of mammal, bird and insect species and has become one of the broadest comparative topics in animal cognition. However, ectothermic vertebrates, especially amphibians, have been grossly neglected in this research area, at least in part because they were not considered to have advanced learning abilities.

At the same time, directed long-distance movements, such as mass spring migration to breeding ponds in temperate-region amphibians, have drawn attention to amphibian orientation mechanisms. Several authors have suggested that amphibians might use learned local cues to find their way around but to date empirical evidence for such learning is lacking. Furthermore, orientation and navigation have been studied almost exclusively in temperate regions, while some of the most complex spatial behaviors (e.g., territoriality and tadpole transport) are found in tropical amphibians, such as the poison frogs (Dendrobatidae). My aim is to bridge this knowledge gap by studying the role of learning in the spatial behavior of poison frogs.

I investigated movement patterns and orientation mechanisms of two territorial dendrobatid frogs with paternal tadpole transport. I experimentally displaced males from their territories and manipulated artificial tadpole deposition sites in the field. Movement patterns were quantified by telemetry and extensive field observations. I found that males of Allobates femoralis return to their home territories by a nearly straight line from several hundred meters but only from areas that individuals have potentially explored during their lifetimes. I also found that tadpole-transporting A. femoralis males return to the exact locations of the tadpole deposition sites even after the artificial pools had been removed. Finally, I demonstrate that male Ameerega trivittata can home from distances twice as long as A. femoralis, an ability most likely linked to their longer natural movements during tadpole transport.

Together, the results of this thesis document surprising way-finding abilities of Neotropical poison frogs. They reveal that learning plays a major role in their spatial behavior. I suggest that poison frogs form a large-scale spatial map that they use for flexible navigation, but the exact cues that they use still remain unknown. These findings corroborate the emerging view that spatial learning ability is ubiquitous among and potentially ancestral to all vertebrates. Amphibians are a key taxon in understanding vertebrate evolution but little is known about amphibian spatial cognition and the neural mechanisms behind it. Future research on the cognitive mechanisms behind amphibian orientation could give key insights into the evolution of spatial cognition.
Zusammenfassung

Räumliches Lernen wurde bereits bei vielen verschiedenen Säugetieren, Vögeln und Insekten untersucht und stellt ein breites Feld der vergleichenden Verhaltensforschung dar. Wechselwarme Wirbeltiere, insbesondere Amphibien, wurden dabei jedoch weitestgehend ignoriert, unter anderem deswegen, weil ihnen bisher höher entwickelte Lernfähigkeit generell abgesprochen wurde.

Gleichzeitig führten aber die bemerkenswerten Langstreckenbewegungen von Amphibien in temperaten Regionen, wie etwa die frühjährlichen Laichwanderungen zu Fortpflanzungsgewässern, zu einem verstärkten Interesse an den Orientierungsmechanismen. Als eine Orientierungsmöglichkeit wurden im Gedächtnis gespeicherte lokale Reize in Betracht gezogen, was jedoch bisher nicht nachgewiesen werden konnte. Außerdem wurden Orientierung und Navigationsvermögen von Amphibien beinahe ausschließlich bei temperaten Arten studiert, wohingegeben die komplexesten räumlichen Verhaltensweisen (z.B. Territorialität und Kaulquappentransport) bei tropischen Amphibien, wie etwa Pfeilgiftfröschen (Dendrobatidae), zu finden sind. Ich habe mir zum Ziel gesetzt, die Bedeutung des Lernens im räumlichen Verhalten von Pfeilgiftfröschen zu untersuchen, um diese Wissenslücke zu schließen.


Insgesamt zeigt sich in der vorliegenden Dissertation eine erstaunliche Orientierungsfähigkeit neotropischer Pfeilgiftfrösche, in welcher Lernen eine wichtige Rolle spielt. Es ist anzunehmen, dass Pfeilgiftfrösche sich eine umfangreiche räumliche Karte ihrer Umgebung aneignen können, welche ihnen die flexible Orientierung ermöglicht. Die konkreten hierzu genutzten sensorischen Informationen sind allerdings weiterhin unbekannt. Meine Ergebnisse bestätigen die zunehmende Ansicht, dass räumliches Lernen eine Fähigkeit
General introduction

Towards an integration of spatial cognition and ecology

Most animal movements are not random and animals use a multitude of biotic and abiotic cues to direct their behavior. An ability to associate cues with specific resources or locations can strongly increase animals’ chances of survival, and thus learning strategies are ubiquitous across the animal kingdom. However, the amount and the complexity of learned information used range from simple modulation of chemotaxis to large-scale spatial map representations (Jacobs 2012). The learning mechanisms behind the myriad spatial strategies used by animals are the topic of the broad comparative field of spatial cognition. Until recently, the majority of research in this area was done under controlled laboratory conditions (reviewed in Jacobs & Menzel 2014). How learning guides the behavior of free-ranging animals was rarely addressed or quantified (but see Tinbergen 1972; Cartwright & Collett 1982). Meanwhile, researchers in ecology have traditionally had a strong focus on the scale and patterns of animal movements (Levin 1992; Edwards et al. 2007). The two fields are becoming integrated and are producing some outstanding research (Fagan et al. 2013) driven by fast-paced technological developments in animal tracking techniques and analytical tools (Wikelski et al. 2007; Bridge et al. 2011; Kays et al. 2015), as well as integrative conceptual frameworks (Jacobs & Schenk 2003; Nathan et al. 2008; Jacobs & Menzel 2014). Nonetheless, although spatial cognition and movement ecology are already among the most comparative topics in the study of behavior, some taxonomic groups and habitats have remained neglected to date.

Amphibian spatial cognition and orientation

Spatial learning is being studied in an ever-increasing variety of mammals, birds, and invertebrates. However, ectothermic vertebrates, especially amphibians, have been grossly neglected in this research area, at least in part because they were not considered to have advanced learning abilities. Traditional conditioning methods of experimental psychology have been used with a few anuran amphibians (i.e., frogs and toads) and have produced a rather limited and equivocal picture of these species’ ability to form even simple associations (e.g., McGill 1960; Boice 1970; Thompson & Boice 1975). These “attempts to train frogs” (Thompson & Boice 1975) are riddled with methodological issues and with difficulties interpreting amphibian behavior (reviewed in Thompson & Boice 1975; Suboski 1992). As with many other taxa, more naturalistic conditions in the laboratory (e.g., using heat as a negative stimulus and water as a reward) have resulted in much higher and faster learning rates in amphibians (e.g., Elepfandt 1985; Brattstrom 1990; Daneri et al. 2007), but the
number of studies remains limited.

Meanwhile, directed long-distance movements, such as mass spring migration to breeding ponds in temperate-region amphibians, have drawn attention to amphibian orientation mechanisms (Boulenger 1912; Cummings 1912; Ferguson 1971; Sinsch 1990). Laboratory experiments have been successfully used to investigate which cues some amphibian species can use to orient on a small scale. At least toads (i.e., Bufonidae) have been able to learn visual, olfactory, body orientation, and even geomagnetic cues (Williams 1967; Grubb 1976; Janes & Falkenberg 1980; Dall'antonia & Sinsch 2001). Furthermore, different individuals under the same experimental conditions learn different cues and single individuals sometimes learn several redundant cues (Adler 1971; 1980; Dall'antonia & Sinsch 2001). Recent experiments revealed that toads of at least one species (Rhinella arenarum) can learn to use both geometrical features and directional visual cues in an arena experiment (Sotelo et al. 2014; Daneri et al. in press).

In the field, spatial orientation experiments have focused almost exclusively on sensory modalities (e.g., olfaction, vision, magnetoreception) rather than on the cognitive mechanisms (e.g., path integration, beaconing, spatial learning). Translocations from breeding and home sites combined with sensory ablation have been commonly used to evaluate the significance of different amphibian sensory modalities for homing (Oldham 1967; Twitty et al. 1967a; Dole 1968; Grant et al. 1968; Sinsch 1987). Several sensory modalities have been implicated in this ability, most commonly olfaction (Oldham 1967; Grubb 1975; Sinsch 1987; Ishii et al. 1995; Nakazawa & Ishii 2000), magneto-reception (Fischer et al. 2001; Diego-Rasilla et al. 2005; Landler & Gollmann 2011), and a celestial compass (Ferguson et al. 1965; Ferguson & Landreth 1966; Landreth & Ferguson 1967). The use of breeding chorus sounds as a beacon during spring migration has been often suggested as a possible cue but the empirical evidence is equivocal (Gerhardt & Klump 1988; Murphy 2003; Bee 2007; Bee 2007). Some amphibians seem to integrate and compensate different sensory modalities depending on cue availability (for reviews see Ferguson 1971; Sinsch 2006), however, what role specific cues play in the spatial orientation process remains unknown for most species (but see Ishii et al. 1995; Fischer et al. 2001). Orientation accuracy has usually been quantified using initial orientation in outdoors arenas (Oldham 1967; Sinsch et al. 2006; Landler & Gollmann 2011; Sinsch & Kirst in press), drift fences with pit-fall traps (Twitty et al. 1967b; Grant et al. 1968) or rudimentary tracking techniques such as thread bobbins releasing thread as the animal moves (Dole 1968; Tracy & Dole 1969; Sinsch 1987; Ishii et al. 1995). The full movement trajectories have rarely been quantified (but see Matthews 2003), thus it remains unknown how exactly these cues guide behavior. While the use of global directional cues, for example with celestial and magnetic compasses, has been identified in amphibians (Ferguson & Landreth 1966; Landreth & Ferguson 1967;
Deutschlander et al. 1999; Diego-Rasilla et al. 2005), it remains unknown how most amphibians choose the correct homing direction after experimental translocations. Magnetic map sense has been identified in one newt species (Phillips et al. 1995; Fischer et al. 2001) but has not been found in several others (Sinsch 2006; 2007; Sinsch & Kirst in press). Overall, magnetic map sense based on the earth’s magnetic field can be considered irrelevant for accurate orientation over the relatively small scale of most amphibian movements (Phillips 1996; Sinsch 2006).

Both laboratory experiments and some observations of orientation behavior in the field suggest that many amphibians use learned local cues to find their way around (Dole 1968; Grubb 1976; Sinsch 2007; Sinsch & Kirst 2015), but to date direct empirical evidence for such learning is lacking. Furthermore, the potential learning mechanisms have rarely been suggested or described. Orientation towards large breeding ponds in temperate-region amphibians could be explained by natal imprinting and an ability to perceive long-distance cues originating from the goal (i.e. beacons), such as the natal pond odor (Grubb 1973; Joly & Miaud 1993; Sinsch 2007). However, such mechanisms are insufficient to explain the ability to relocate terrestrial home areas or small reproductive resources in complex habitats, and more complex spatial learning strategies are likely to be involved in such behaviors. Finally, fully understanding amphibian movement strategies and spatial cognition will require detailed knowledge of their movement patterns, but such data are currently lacking (Sinsch 2014).

**Why track tropical amphibians?**

Amphibians show some of the most diverse reproductive behaviors among vertebrates. Their breeding behavior ranges from explosive breeding where thousands of individuals gather in ponds and leave the eggs unattended, to prolonged breeding with biparental care and pair-bonding (Wells 1977; Duellman & Trueb 1994; Crump 2015). It follows that the associated spatial behaviors and navigational abilities must be just as diverse, but as yet they remain largely unstudied.

The miniaturization of radio transmitters has strongly expanded the range of species suitable for telemetry, including many amphibians (van Nuland & Claus 1981; Stouffer et al. 1983; Jehle & Arntzen 2000; Sztatecsny & Schabetsberger 2005). In parallel, harmonic direction-finding techniques (Mascancioni & Wallin 1986) have been used to track even smaller amphibians (Leskovar & Sinsch 2005). Unfortunately, limited detection range and issues concerning the external attachment of the devices to amphibians have resulted in predominantly methodological studies (e.g., Langkilde & Alford 2002; Rowley & Alford 2007; Gourret et al. 2011; but see Leskovar & Sinsch 2005; Pellet et al. 2006).

Surprisingly, telemetry techniques have rarely been used to monitor and gain new insights into amphibian spatial orientation. More generally, movements of amphibians and
reptiles (with the notable exception of sea turtles) have been studied much less than other vertebrates. Currently, none of the hundreds of movement studies listed on the international database Movebank (www.movebank.org) involve amphibians. Holyoak et al. (2008) analyzed one thousand randomly selected publications on organism movements (including plants, fungi, algae, bacteria and protozoa) published between 1997 and 2006, of which 48.5% were on vertebrates. Among vertebrate studies, only 2.9% were on amphibians as compared to 4.9% on reptiles, 24.7% on mammals, 28.9% on fishes, and 39.2% on birds (Holyoak et al. 2008). Only a fraction of these studies actually quantified the movement patterns of individuals by telemetry. Further, these tracking studies have almost exclusively focused on nocturnal, pond breeding amphibians of temperate regions, especially in Bufonidae (e.g., Miaud et al. 2000) and Ranidae (e.g., Matthews & Pope 1999). The vast majority of these studies represent only a few of amphibian reproductive modes, such as explosive and prolonged pond breeders. A variety of complex reproductive behaviors, such as territoriality, terrestrial breeding, parental care, and vertical migrations are much more common in the tropical regions.

Tracking movement patterns of tropical anurans remains very challenging because many of them are small and move in highly complex three-dimensional habitats. However, compared to other small vertebrates such as small birds or mammals, they are a much more accessible system because of their relatively slow and limited movements. Therefore, tropical amphibians are also a highly promising system for understanding how tropical vertebrates exploit resources and find their way around such complex environments. Finally, there has been a major global decline in amphibian diversity, mainly caused by habitat loss, pollution, and global spread of infectious fungal diseases (Stuart et al. 2004). Tropical regions where the amphibian diversity is highest are particularly affected. A good understanding of amphibian movement patterns and orientation mechanisms will be critical for implementing effective conservation measures (Pittman et al. 2014).

The study system: poison frogs (Dendrobatidae)

Poison frogs (Dendrobatidae) are a group of small diurnal Neotropical frogs characterized by territoriality and parental care (Weygoldt 1987). They display some of the most complex social and spatial behaviors known among amphibians. In most species males and/or females are highly territorial and perform obligatory tadpole transport from terrestrial clutches to aquatic deposition sites. There is great variation in parental investment and tadpole deposition strategies between different species. While some species have uni-parental care and transport relatively large clutches to single deposition sites, others show flexible or bi-parental care and different levels of brood-partitioning behavior (Wells 1980; Weygoldt 1980; 1987; Aichinger 1991; Caldwell 1997; Erich et al. 2015; Ringler et al. in press). Many
Dendrobatid frogs rely on multiple widely dispersed ephemeral deposition sites such as flooded areas, fallen tree-holes or bromeliad leaf axils for tadpole rearing (e.g., Rojas 2014; Ringler et al. 2015). Topics such as territoriality, evolution of parental care, aposematism, and mimicry have been studied extensively in poison frogs (Weygoldt 1987; Summers & Clough 2001; Pröhl 2005; Summers et al. 2006; Brown 2013), but their complex spatial movement patterns and way-finding abilities have not yet been quantified.

While a variety of reproductive and social behaviors can be observed in dendrobatid frogs, the predominant system (e.g., most species in the large genera of Allobates and Ameerega) is male territoriality and male tadpole transport with no further parental care. Male clutch attendance and tadpole transport is considered to be the ancestral form of parental care in dendrobatids (Weygoldt 1987). A diverse set of derived behaviors can be found within this general type of reproductive system. For example, in some species males stop calling while attending to a single clutch while in others, males can have many clutches simultaneously and might not show any other parental care besides regular tadpole shuttling. For the majority of species, fine details of the reproductive behavior remain unknown.

During my thesis I studied three dendrobatid species representative of two large and abundant genera: Allobates and Ameerega. Most of the species in these two groups show overall similar reproductive behavior (as described above) but the two genera belong to two separate root clades of the dendrobatid phylogenetic tree (Grant et al. 2006). Most of the research reported in this thesis was done with one of the best-studied tropical frogs, Allobates femoralis (Fig. 1). Allobates femoralis is a small cryptic leaf-litter dendrobatid whose reproductive behavior, communication system, population genetics, ecology, and biogeography are well-studied (Hödl 1987; Roithmair 1992; Narins et al. 2003; Amézquita et al. 2009; Ringler et al. 2012; in press). At the onset of the rainy season, males establish and vocally advertise territories, which they aggressively defend against rival males for up to several months (Roithmair 1992). Females show non-aggressive site fidelity and these “listening sites” are usually situated outside males’ territories (Ringler et al. 2009; illustrated in Fig. 1). Courtship takes place inside the male’s territory where a small clutch of approximately 20 eggs is laid in the leaf-litter and abandoned by the female. Both males and females are highly polygamous and iteroparous: females will mate with multiple males and males attend to several clutches at the same time, five being the highest number observed so far (Ursprung et al. 2011). Tadpole transport takes place 15-20 days after oviposition and is mainly performed by males after heavy rainfall (Aichinger 1991; 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. While in the majority of cases males transport the tadpoles, recent research has shown that females can flexibly take over the tadpole transport when males disappear (Ringler et al. in press).
The representative of the other genus, *Amereega trivittata*, is also a relatively well-studied species (Roithmair 1994b; a; Rojas et al. 2006; Acioli & Neckel-Oliveira 2014; Luiz & Contrera 2015). It is one of the largest and most widespread dendrobatid frogs and it co-occurs with *A. femoralis* over large parts of their distribution range (Amézquita et al. 2006). As in *A. femoralis* males defend territories and perform tadpole transport. Up to 40 tadpoles (Roithmair 1994a and chapter 6) are transported at once to standing water bodies such as flooded forest areas or pools formed in partially dry creek-beds (personal observation). No female tadpole transport and no multiple clutches have been reported for this species but more detailed studies are needed.

Figure 1. Overview of reproductive behaviors of the Neotropical poison frog *Allobates femoralis*. (1) Males defend and vocally advertise terrestrial territories. (2) Females show site fidelity to their listening sites from which they visit territorial males (3). Courtship and oviposition occurs in the leaf-litter inside male’s territory. Males may have several clutches from different females at different developmental stages. (4) After two to three weeks of development, males transport tadpoles to extraterritorial deposition sites and (5) return back to their territory. (6) Juveniles disperse in their terrestrial habitat.

The aims of the thesis

Over the course of my studies, I investigated the role of learning in the spatial behavior of poison frogs by quantifying and analyzing their movement patterns and orientation mechanisms in the field. The aim of my project was threefold: (1) to develop
suitable methods for quantifying movement patterns of small tropical amphibians in the field, (2) to gain insights into spatial movements and orientation ability of dendrobatid frogs, and (3) to understand whether learning plays an important role in these movement patterns. In chapter 1 we revealed and quantified the high homing performance of territorial male *A. femoralis*. In chapter 2 we applied, for the first time, telemetry with a harmonic direction finder to quantify the short-distance homing trajectories of *A. femoralis*. In addition, we tested an arena assay to quantify the initial orientation of translocated territorial males. In chapter 3 we used the telemetry technique developed in chapter 2 to obtain long-distance homing trajectories and to test whether male *A. femoralis* rely on experience with the area for successful homing. In chapter 4 we manipulated artificial tadpole deposition sites in the field to test if the male *A. femoralis* learn the exact locations of these deposition sites. In chapter 5 we quantified the homing performance after experimental translocations in a closely related frog, *Allobates talamancae*. In the final chapter 6 we used radio telemetry to track *A. trivittata* in the field and quantified distances traveled by males during tadpole transport and their homing trajectories after long-distance translocations. Throughout these chapters I document the movement patterns and way-finding abilities of Neotropical poison frogs during both natural and experimentally manipulated movements. I apply and evaluate several methods suitable for quantifying small amphibian movements in the field. I interpret our findings in the light of poison frog natural histories and the potential underlying cognitive mechanisms. Overall, I argue that many poison frog movements are guided by memory.

References


the Linnean Society, 98, 826–838.


Chapter 1

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Reference:
The Homing Frog: High Homing Performance in a Territorial Dendrobatid Frog *Allobates femoralis* (Dendrobatidae)

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Abstract
Dendrobatidae (dart-poison frogs) exhibit some of the most complex spatial behaviors among amphibians, such as territoriality and tadpole transport from terrestrial clutches to widely distributed deposition sites. In species that exhibit long-term territoriality, high homing performance after tadpole transport can be assumed, but experimental evidence is lacking, and the underlying orientation mechanisms are unknown. We conducted a field translocation experiment to test whether male *Allobates femoralis*, a dendrobatid frog with paternal extra-territorial tadpole transport, are capable of homing after experimental removal, as well as to quantify homing success and speed. Translocated individuals showed a very high homing success for distances up to 200 m and successfully returned from up to 400 m. We discuss the potential orientation mechanisms involved and selective forces that could have shaped this strong homing ability.

Introduction
Amphibians are among the most sedentary vertebrates with daily movements often reduced to just a few tens of meters (for a review, see Russell et al. 2005; Wells 2007). Despite their lethargic nature, many amphibians occasionally show movements, such as spring migration, over longer distances, ranging a few hundred meters or more. This behavior requires a set of specialized orientation skills. Urodeles, for example, have served as a major model in unraveling vertebrate navigation based on magnetic map sense (Phillips 1996), while field studies on bufonid anurans have revealed multisensory orientation systems that can rely on different sensory modalities depending on cue availability (Ferguson 1971; Sinsch 1987).

Homing performance after experimental translocations from home or breeding sites has been widely used to study animal orientation (Müller & Wehner 1988; Walcott 1996). Translocation experiments not only provide information on a species’ ability and motivation to home back but also can suggest potential orientation mechanisms. In amphibians, translocation experiments indicate the use of spatial maps at least by some species, but the nature of these maps remains unclear (Sinsch 2006). On the one hand, some newt species show remarkable homing from unfamiliar release sites several kilometers away (Twitty et al. 1964), an ability most likely based on magnetic map sense (Phillips et al. 1995). On the other hand, the orientation of other amphibian species is restricted to potentially familiar areas where some sort of landmark learning must occur (Sinsch 1987, 2007).

In anurans, research on homing ability has focused almost exclusively on nocturnal species of the temperate regions, especially bufonids (Bogert 1947; Dole 1972; Sinsch 1987) (but see Gonser & Woolbright 1995; Nowakowski et al. 2012 for work on other groups). Regarding methodology, Wells (2007) pointed out that a control group, quantifying the recapture rate without displacement, is often missing in anuran translocation experiments, which makes it hard to estimate the return success, as a high
Materials and Methods

Quantify their homing speed and success. Capable of homing after being displaced as well as to migration experiment to test whether male Ringler et al. 2009). We conducted a field translocation experiment to test whether male A. femoralis is only found in the drier ‘terra firme’ forest. The Homing Frog

Study Animals and Area

Allobates femoralis (Dendrobatidae) is a dendrobatid leaf litter frog common throughout the Amazon basin and the Guiana shield (Amézquita et al. 2009). Males occupy long-term territories that are vocally advertised and used for oviposition by females (Ringler et al. 2012). Tadpoles are later transported by males to aquatic deposition sites outside their home territory (A. Pašukonis, M. Ringler, H. B. Brandl, R. Mangione, E. Ringler & W. Hödl, pers. obs.; Roithmair 1992; Ringler et al. 2009). We conducted a field translocation experiment to test whether male A. femoralis are capable of homing after being displaced as well as to quantify their homing speed and success.

Materials and Methods

Study Animals and Area

Allobates femoralis is a small (snout-urostyle length approximately 25 mm) territorial dendrobatid frog. At the onset of the rainy season, males establish multipurpose territories, which are vocally advertised and defended for up to several months (Roithmair 1992; Ringler et al. 2009). Playback of an advertisement call of a simulated intruder reliably elicits antiphonal calling or direct phonotactic approach by the resident male (Hödl 1987). Individual frogs can be identified and recognized by their unique ventral coloration patterns (Ursprung et al. 2011b).

The study was carried out within one reproductive season of A. femoralis between Feb. 25, 2012 and April 4, 2012. Frogs were sampled from a single local population in an area of approximately 15 000 m² near the field camp ‘Saut Pararé’ (4°02’N, 52°41’W, WGS84) in the nature reserve ‘Les Nouragues’, French Guiana. The study area consists mainly of primary lowland rainforest bordering the ‘Arataye’ river to the south. Typically for the rainforest of the Guiana shield, the area has a complex relief (30–140 m asl) composed of small hills and ridges covered in ‘terra firme’ forest and partitioned by numerous small creeks in the lower parts. Flat portions of the creeks form wet areas that are overgrown by açai palms (Euterpe sp.) (Fig. 1). Allobates femoralis is only found in the drier ‘terra firme’ forest.

Translocation Experiment

Translocations took place from Feb. 25, 2012 to Mar. 14, 2012. During this period, 50 territorial males were captured and translocated at equal numbers (10 per translocation distance) to five distances (50, 100, 200, 400, and 800 m) in all cardinal directions (north, east, south, and west). Additionally, 10 control individuals were captured and released without translocation (0 m). All frogs were identified by comparing digital images of their unique ventral coloration patterns.

Calling males were detected and identified as territorial if they showed stereotypical territorial defense behavior (calling and phonotactic approach), which was elicited by broadcasting conspecific advertisement calls, simulating an intruder. Frogs were captured with transparent airtight plastic bags and placed in an opaque container until release. Precise capture positions were recorded with mobile GIS software ArcPad™ 10.0 (ESRI, Redlands, CA, USA) on pocket computers (MobileMapper™ 6, SpectraPrecision, Westminster, CO, USA) using a detailed background map based on a grid of reference points and natural structures. Upon capture, translocation distances and cardinal directions were randomly assigned to the individual frogs. Release points were marked and located using the same detailed background map for distances up to 200 m. More distant release points were located using the GPS of the pocket computers. Translocations southwards were restricted to a single 400-m and to no 800-m release points, due to the proximity of the river on the southern edge of the study area (Fig. 1). All frogs were released within 2 h.
after their capture. Control frogs were released 10 min–30 min after their capture.

Capture sites of all translocated frogs, as well as of the 10 control individuals, were inspected daily during the peak calling time of *A. femoralis* from 15:00 to 18:00 h (A. Pašukonis, pers. obs.; Kaefer et al. 2013) for at least 14 d or until recapture. Original capture locations of all frogs were first inspected acoustically for calling males in approximately 5 m radius. If no calling individuals were present, a call of a simulated intruder was broadcast from the capture site. All frogs detected during the scanning were captured and identified. Post-14 d, the territories of non-recaptured males were inspected every few days for an additional 6–20 d. Finally, all territories of non-recaptured males were inspected on the 3 and 4 of April.

Five independent variables were measured as potential predictors of return success and speed: translocation distance, direction, water obstacle density, relief complexity, and the frogs’ age. *Translocation distance* was measured as the linear distance from capture to release site. *Translocation direction* corresponded to one of the four cardinal directions (north, east, south, west). *Water obstacle density* was estimated as number of creeks per meter. This measure was taken because *A. femoralis* rather seems to avoid crossing running water (A. Pašukonis, pers. obs.). *Relief complexity* was measured as the ratio between surface (3D) and linear (2D) distances. *Age* was estimated as a binary variable approximating an individual’s age from long-term capture–recapture data of the population (Ursprung et al. 2011a). Frogs were identified by their unique ventral coloration (Ursprung et al. 2011b) and classified as ‘new’ (newly captured frogs in 2012, N = 37) or ‘old’ (recaptures from 2011 and 2010, N = 13). The possibility that some males classified as ‘new’ were older unknown individuals could not be excluded.
Data Analysis

Return success was statistically analyzed using a multiple logistic regression model with the five independent variables outlined above. Return time was analyzed using a multiple linear regression model with the same independent variables. One frog that was recaptured post-14 d was not considered in the return time analysis because the exact return time was unknown. Surface distances were calculated in ArcGIS™ 9.3 (ESRI), using a topographic map of the area. All statistical analyses were performed with SPSS™ 19.0 (IBM, Armonk, NY, USA).

Results

Fifty-eight percent of the translocated frogs returned to their home territories within 22 d after the translocation event (Table 1). All control frogs were recaptured inside their home territories within 3 d. While return success was high for translocation distances up to 200 m (87%), it strongly decreased at 400 m (30%), and no frogs returned from 800 m. Logistic regression revealed that translocation distance was the only factor significantly predicting homing success (Table 2).

Table 1: Male Allobates femoralis homing success after experimental translocation

<table>
<thead>
<tr>
<th>Translocation distance (m)</th>
<th>N</th>
<th>Homing success (% recaptured)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>800</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>Control (0)</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Output table of the multiple logistic regression model showing a significant correlation between translocation distance and homing success, *p < 0.05

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Estimate (β)</th>
<th>Standard error</th>
<th>Wald</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance</td>
<td>-0.012</td>
<td>0.004</td>
<td>10.12</td>
<td>0.001*</td>
</tr>
<tr>
<td>Direction</td>
<td>-0.004</td>
<td>0.004</td>
<td>0.811</td>
<td>0.368</td>
</tr>
<tr>
<td>Relief</td>
<td>-5.9</td>
<td>12.91</td>
<td>0.209</td>
<td>0.648</td>
</tr>
<tr>
<td>Water</td>
<td>-98.61</td>
<td>78.21</td>
<td>1.59</td>
<td>0.207</td>
</tr>
<tr>
<td>Age</td>
<td>1.82</td>
<td>1.27</td>
<td>2.04</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Model statistics: $R^2 = 0.52$ (Cox & Snell), 0.7 (Nagelkerke); Model $\chi^2(1) = 36.74, p < 0.001$.

Return time for translocated frogs varied from 1 to 14 d ($\bar{x} = 3.86, SD = 3$), while all control frogs were recaptured within 3 d ($\bar{x} = 1.4, SD = 0.84$). Only one frog was recaptured after the daily territory inspection period of 2 wks and was not considered in return time analysis. Minimum return time was 1 d for 50 and 100 m, 2 d for 200 m, and 3 d for 400 m. Maximum return time was 6, 4, 11, and 14 d for 50, 100, 200, and 400 m, respectively. The multiple linear regression model revealed that only translocation distance significantly predicted return time (Table 3; Fig. 2). Translocation direction, water obstacle density, relief complexity, and frogs’ age did not have a significant effect on return time.

Table 3: Output table of the multiple linear regression model showing a significant correlation between translocation distance and recapture time, *p < 0.05

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Estimate (β)</th>
<th>Standard error</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance</td>
<td>0.527</td>
<td>0.005</td>
<td>3.05</td>
<td>0.006*</td>
</tr>
<tr>
<td>Direction</td>
<td>0.108</td>
<td>0.005</td>
<td>0.607</td>
<td>0.550</td>
</tr>
<tr>
<td>Relief</td>
<td>-0.006</td>
<td>45.93</td>
<td>-0.032</td>
<td>0.975</td>
</tr>
<tr>
<td>Water</td>
<td>0.024</td>
<td>84.54</td>
<td>0.137</td>
<td>0.892</td>
</tr>
<tr>
<td>Age</td>
<td>-0.307</td>
<td>1.09</td>
<td>-1.74</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Model statistics: $R^2$ Linear = 0.372, $F(5, 27) = 2.61, p = 0.054$.

Fig. 2: Scatter plot showing the correlation between translocation distance (m) and time until recapture in the home territory (days). Circle size represents the number of individuals, while numbers above each column represent the number of individuals per distance. The regression line with mean confidence intervals is plotted. A single outlier point outside the 14-d daily territory check period is marked by x.
Discussion
We found that translocated male *A. femoralis* did return to their home territories from up to 400 m within just a few days. Homing performance was high for up to 200 m but steeply decreased for longer distances. Translocation distance was the only factor significantly predicting return time and success. The results of our study not only provide experimental evidence of high homing performance in *A. femoralis*, but they also underline the species’ suitability for studying orientation in dendrobatid frogs. Strong site fidelity, loud advertisement calls, and stereotypic territorial defense behavior make it possible to reliably detect the presence of individuals and thus allow for good estimations of homing success and speed.

It is important to consider the homing distances in relation to the territory size of *A. femoralis* because males spend the vast majority of their time within this area (Ringler et al. 2009). When approximated by a circle, the average area defended by males in our study population is 13.9 m in diameter (cf. Ringler et al. 2011), which is 29 times smaller than the maximum homing distance. Longer homing distances have been reported for some anuran species. Bogert (1947) and Jameson (1957) observed individuals returning from over 1 km in *Anaxyrus (= Bufo) terrestris* and *Pseudacris (= Hyla) regilla*, respectively. However, even though the maximal return distance has interesting implications regarding potential orientation mechanisms, a more relevant measure of homing ability is the return success expressed as a percentage of individuals returning from a given distance. To the best of our knowledge, we report the highest homing success of any amphibian for comparable translocation distances.

Failure to return to the home territory can be attributed to orientation failure, loss of motivation, or direct costs such as fatigue and predation. In our opinion, direct costs are unlikely to fully explain the effect of distance on homing success. Predation pressure is relatively low as the majority of the territorial males can be observed conspicuously advertising their presence for several weeks or months from a single location without being predated upon (A. Pašukonis, pers. obs.; Kaefer et al. 2013; Roithmair 1994). Further, male *A. femoralis* routinely transport tadpole loads equivalent to 20% of their body mass to widely dispersed deposition sites (A. Pašukonis, pers. obs.; E. Ringler, A. Pašukonis, W. Hödl & M. Ringler, in prep), a task that is potentially physically more demanding than the return journey alone. It is possible that some frogs returned but were not able to reestablish their territories. However, this effect is probably small as the majority of the territories (16 of 21) of non-returning frogs remained vacant even after 20 d. As the high homing performance across shorter distances indicates a strong general motivation to return, we consider that low homing success for distances above 200 m is best explained by orientation failure.

There are at least five basic orientation mechanisms described in amphibians: path integration, beaoning, piloting, compass orientation, and true navigation (Sinsch 2006). In the present study, all frogs were translocated in opaque airtight bags, and their orientation was changed multiple times during handling and transport. Path integration, which relies on the animal having followed the full outwards path by itself, is disrupted by such experimental translocation. Beaconing is based on a direct sensory contact to the goal, and it could explain homing from shorter but is unlikely for longer distances where direct sensory contact to the home territory is limited. One crucial question in disentangling different orientation mechanisms is whether or not homing is dependent on previous experience with the area. If homing ability relies on experience, a piloting mechanism based on spatial learning of landmarks is implied. If not, this would suggest a true navigation based on map sense, that is, ability to extrapolate long-range directional gradients to position yourself in an unfamiliar area (Phillips 1996).

Our results suggest a homing ability threshold for *A. femoralis* between 200 and 400 m. The ability to home back from such relatively long distances, together with a clear upper limit, is consistent with piloting between landmarks or some sort of local area map. Interestingly, males have been found transporting tadpoles up to 185 m (\(x = 38.6, \ SD = 34.5, \ N = 129\)) away from their territory (E. Ringler, A. Pašukonis, W. Hödl & M. Ringler, in prep), which would allow them to explore and learn landmarks in a correspondingly large area. The fact that the distances at which homing success was high are within the range of the longest distances covered by tadpole-transporting adults further supports the idea of experienced based orientation. However, this apparent fit might also suggest that tadpole-transporting distances are limited by orientation ability. Further research should explore the effects of familiarity with an area on the homing ability of dendrobatid frogs in greater detail.

Despite the well-known spatial complexity of dendrobatid behavior, homing has only been investigated in one species (Strawberry poison frog, *Oophaga pumilio*, McVey et al. 1981; Nowakowski et al. 2012).
McVey et al. (1981) performed short distance (<20 m) translocations and found a very high homing performance (88% recapture from 12 m, N = 11 and 20 m, N = 6). Contrastingly, Nowakowski et al. (2012) found that only 67% of translocated *O. pumilio* returned from 20 m (N = 30) and 57% from 30 m (N = 30), which is a rather poor performance when compared to *A. femoralis* (87% recapture from 50, 100, and 200 m; N = 30). However, there are several differences in the spatial ecology of *A. femoralis* and *O. pumilio*, most importantly larger territory size and longer tadpole transport distances in the case of *A. femoralis* (Pröhl & Berke 2001; Stynoski 2009; Ringler et al. 2011). Further, in *O. pumilio* females perform the tadpole transport and in addition return to the tadpole deposition sites to feed their offspring with unfertilized eggs (Weygoldt 1980; Brust 1993). Consequently, in *O. pumilio* females can be expected to have better orientation ability. Unfortunately, the study of McVey et al. (1981) does not provide a sufficient sample size to draw conclusions in this respect, while the study of Nowakowski et al. (2012) does not specify the sex of translocated individuals.

Attending multiple clutches could have played a major role in selecting for a high homing performance in *A. femoralis* males, which were found to have up to five clutches at the same time (Ursprung et al. 2011a). In such cases, failure to return to the territory after tadpole transport would result in the loss of the other clutches and thus in a severely reduced reproductive output. If extra-territorial tadpole transport and attending multiple clutches were a major selective force in shaping orientation mechanisms, we should find that dendrobatid species lacking these traits would not perform as well in a homing task. The relatively low homing performance of *O. pumilio*, a dendrobatid frog with shorter tadpole transport distances, observed in the study by Nowakowski et al. (2012) is consistent with this hypothesis, but more rigorous comparative work is necessary.

**Acknowledgements**

Our study was approved by the scientific committee of the research station where fieldwork was conducted (http://www.nouragues.cnrs.fr/F-conseil.html). All necessary permissions were provided by the ‘Centre National de la Recherche Scientifique’ (CNRS) and by the ‘Direction Régionale de l’Environnement de Guyane’ (DIREN). All sampling was conducted in strict accordance with current French and EU law and followed the ASAB guidelines for the treatment of animals in behavioral research and teaching. We are grateful to the staff of CNRS Guyane for the logistic support during the fieldwork. This work was supported by the Austrian Science Fund (FWF): projects W1234-G17 (PI: Thomas Bugnyar) and P24788-B22 (PI: Eva Ringler).

**Literature cited**


resistance to movement of the poison frog, *Oophaga pumilio*, in the lowlands of northeastern Costa Rica.


Chapter 2

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Reference:
Homing trajectories and initial orientation in a Neotropical territorial frog, *Allobates femoralis* (Dendrobatidae)

Andrius Pašukonis1*, Matthias-Claudio Loretto1, Lukas Landler2, Max Ringler3,4 and Walter Hödl3

**Abstract**

**Introduction:** The ability to relocate home or breeding sites after experimental removal has been observed in several amphibians and the sensory basis of this behavior has been studied in some temperate-region species. However, the actual return trajectories have rarely been quantified in these studies and it remains unknown how different cues guide the homing behavior. Dendrobatidae (dart-poison frogs) exhibit some of the most complex spatial behaviors among amphibians, such as territoriality and tadpole transport. Recent data showed that *Allobates femoralis*, a frog with paternal tadpole transport, successfully returns to the home territories after experimental translocations of up to 400 m. In the present study, we used harmonic direction finding to obtain homing trajectories. Additionally, we quantified the initial orientation of individuals, translocated 10 m to 105 m, in an arena assay.

**Results:** Tracking experiments revealed that homing trajectories are characterized by long periods of immobility (up to several days) and short periods (several hours) of rapid movement, closely fitting a straight line towards the home territory. In the arena assay, the frogs showed significant homeward orientation for translocation distances of 35 m to 70 m but not for longer and shorter distances.

**Conclusions:** Our results describe a very accurate homing behavior in male *A. femoralis*. The straightness of trajectories and initial homeward orientation suggest integration of learned landmarks providing a map position for translocated individuals. Future research should focus on the role of learning in homing behavior and the exact nature of cues being used.

**Keywords:** Homing, Orientation, Telemetry, Dendrobatidae, *Allobates femoralis*

**Introduction**

The ability to quantify individual movement patterns using modern telemetry techniques has been crucial in understanding animal orientation. Homing ability and trajectories following translocations from home or breeding sites have been widely used to study the orientation mechanisms involved (e.g., homing pigeons [1], desert ants [2]).

Directed, relatively long distance movements, such as mass spring migration to breeding ponds in temperate-region amphibians, has historically drawn much attention to amphibian orientation ([3,4], reviewed in [5,6]). A tendency to move towards and to relocate home or breeding sites after experimental removal has been observed in many amphibians (e.g., *Anaxyrus terrestris* (Bufonidae) [7], *Lithobates pipiens* (Ranidae) [8], *Pseudacris regilla* (Hylidae) [9], *Allobates femoralis* (Dendrobatidae) [10], *Taricha rivularis* (Salamandridae) [11]). Several sensory modalities have been implicated in this ability, most commonly olfaction [12-14], magneto-reception [15,16], and a celestial compass [5,17]. Often, integration and compensation of different modalities depending on cue availability have been suggested (reviewed in [6,18]).

Even though the importance of certain sensory modalities has been revealed for some temperate-region amphibians, the exact homing trajectories have not been quantified (but see [3,4,18,19]). Thus it remains unknown how exactly these cues guide the behavior.

Traditionally, orientation mechanisms in amphibians have been studied by observing initial orientation over a few meters in arena setups or over longer distances...
using drift fences and pit-fall traps. In an attempt to track individuals, several authors have attached trailing devices to the backs of larger anurans, i.e. a thread bobbin, which releases a thread as the animal moves [8,13,20,21]. However, this tracking method hinders animal movements, often results in injuries or a complete inhibition of homing [8,22], and is limited to large species. The miniaturization of radio transmitters has strongly expanded the range of species suitable for telemetry, including many amphibians [23,24]. In parallel, harmonic radar [25] and harmonic direction-finding techniques [26] were developed, allowing to track even some of the smallest amphibians. Unfortunately, limited detection range and problematic external attachment on amphibians have resulted in predominantly methodological studies (e.g., [27-29], but see [30,31]). Telemetry techniques have rarely been used to gain new insights into amphibian orientation.

Orientation has mostly been studied in nocturnal temperate-region anurans, especially bufonids, but it is in tropical amphibians that we find some of the most complex spatial behaviors. Dart-poison frogs (Dendrobatidae) are a group of diurnal Neotropical frogs characterized by territoriality and parental care, which includes tadpole transport [32]. Despite the fact that dendrobatid parental care has been investigated in great detail [32-34], very little is known about the related spatial behaviors. *Allobates femoralis* is a small dendrobatid frog common throughout the Amazon basin and the Guiana Shield [35]. At the onset of the rainy season, males establish territories, which are vocally advertised and defended for up to several months [36,37]. Courtship, mating and oviposition take place in the leaf-litter within the male’s territory. Tadpoles are later transported to widely dispersed aquatic deposition sites, such as temporary pools in flooded areas or holes in fallen trees, as far as 185 m away from the territory [38]. Recent data have shown that experimentally translocated territorial males can return from up to 400 m with increased homing success rates under 200 m [10].

In the present study we further describe the homing behavior of male *A. femoralis* with a specific focus on the actual movement patterns during return to their home territory. We used telemetry with miniature passive transponders to quantify the homing trajectories of territorial males, displaced over 50 m. Additionally, we translocated males from their home territories into a circular arena within their natural habitat and quantified the initial movements after release.

**Results**

**Telemetry experiment**

Fifteen out of 17 male frogs equipped with transponders continued to show territorial behavior. Out of 15 individuals translocated for 50 m, 12 successfully returned to their home territories. Three individuals did not move from their release sites for three to four days, after which we removed their tags and returned them to their home territory to avoid any long-term effect on individuals’ health and behavior. Two individuals lost the reflector antenna and could not be located en route but were recovered back in their home territories. Ten individuals were successfully tracked en route. On average the trajectory straightness coefficient was very high (Mean$_{SC}$ = 0.93, SD = 0.075) and the inferred homing trajectories were closely fitting a straight homewards line (Figure 1). Translocated individuals showed a significant initial homeward orientation (Mean = 344.5°, 95% CI = 304.7° – 243.3°, Rayleigh-test p = 0.01, n = 8).

Total return time ranged from 4 h to 102 h (Mean = 37.82 h, SD = 29.49). During their diurnal activity period, individuals spent significantly more time immobile than moving (median$_{moving} = 4.88 \, h$, Q1–Q3$_{moving} = 4–8.06 \, h$, median$_{stationary} = 14.88 \, h$, Q1–Q3$_{stationary} = 4.38–23.13 \, h$, $Z = -1.97$, Wilcoxon p = 0.05, n = 10). As seen from the quartile values, there was relatively little variation in the active movement time necessary for frogs to return to their home territory but a much greater variation in the time they spent immobile.

**Arena assay**

A second order circular tests for unimodal distribution revealed a significant homeward orientation for the
individuals from the mid-range (n = 13, Mean vector = 345.1°, 95% CI = 275.7° – 49.1°, Hotelling’s F = 5.49, p = 0.02) but not for the close-range (n = 14, Hotelling’s p > 0.1) and far-range samples (n = 11, Hotelling’s p > 0.1) (Figure 2). Confirming the between-group difference, there was a significant difference in distribution between the mid-range and the close-range (Hotelling’s two sample F = 4.06, p = 0.03) as well as mid-range and far-range samples (Hotelling’s two sample F = 4.64, p = 0.02) but not between the close and far-range samples (Hotelling’s p > 0.1). Overall, we could not unambiguously confirm a successful homing after the trial for six individuals (three for the close-range and three for the far range).

**Discussion**

Our tracking experiment revealed that homing trajectories of translocated male *A. femoralis* are characterized by rapid movements, closely fitting a straight line towards the home territory, with occasional long periods of immobility lasting up to several days (Figure 3). We did not observe any search patterns and the initial movements over several meters after release were already oriented homewards. Detailed analysis of the very first movements within one meter in an arena setup revealed significant orientation for translocation distances of 35 m to 70 m but not for longer and shorter distances.

The most intriguing finding is the accuracy of the observed homing trajectories. The straightness of trajectories over similar distances is more comparable with path integration based homing in desert ants (e.g., [2]) or direct guidance in social insects (e.g., [39]) rather than any homing trajectories described in amphibians [8,40]. However, experimental translocation from territories excludes the possibility of path integration in our study. Direct guidance (also termed beaconing) requires direct sensory contact to the goal. We can exclude direct visual contact with the goal in the dense rainforest. The role of olfaction in orientation has been demonstrated for several anuran species, but in these cases animals were orienting in an open landscape to a large goal such as a breeding pond [12-14,20]. It is difficult to imagine how olfactory guidance would explain such an accurate homing to small terrestrial territories in a forest understory with hardly any stable winds. Alternatively, it has been suggested that some animals could form ‘olfactory maps,’ which allow them to navigate an area, but the empirical evidence is equivocal [41]. Magnetic compass and magnetic maps have been implicated in long distance homing of several species [42], including urodele amphibians.

![Figure 2 Arena orientation](image-url)
but the shallow magnetic gradients of the earth’s magnetic field could not account for the observed homing precision at these relatively short distances [43]. Acoustic orientation has been well documented for male A. femoralis in a territorial defense context [44–46] and females most likely use males’ advertisement calls as beacons to find territory owners over several tens of meters [47]. We speculate that translocated individuals could use other territorial males as acoustic beacons or even integrate their calling positions and identities into a full acoustic map of the area. To the best of our knowledge, the potential existence of such orientation mechanism has not yet been investigated. Broad acoustic gradients such as provided by a nearby river could be used in addition. The function of broad acoustic gradients in homing has been often discussed in anuran orientation, usually in the context of a breeding chorus (e.g., [7,14,48]), but there is little evidence of acoustics being a primary cue in anuran homing.

Other types of landmark-based orientation are also possible. However, the lack of any observable search patterns and strong initial orientation suggest, that if spatial learning is involved, landmarks may be integrated on an internal map and not simply used as intermediate beacons. Translocation distances of 50 m are well inside the potential range explored by most individuals during tadpole transport [38] or juvenile dispersal (M. Ringler, R. Mangione, A. Pašukonis, E. Ringler, unpublished data) and spatial information could be acquired during these movements. Homing success in A. femoralis is very high up to 200 m [10], which corresponds to the maximum distances covered by tadpole transporting adults, and further suggests the role of familiarity with the area in homing behavior. More generally, many dendrobatid frogs are likely to rely on spatial learning for finding small and widely dispersed aquatic tadpole deposition sites in complex environment but research in this area is lacking.

The long immobility periods shown by most individuals before moving could be related to the time necessary to accumulate or to perceive temporally varying orientation cues, such as conspecific vocalizations. We did not observe any clear relation between frogs’ activity during homing and the time of the day or the amount of precipitation. We do not think that catching or tag attachment induced stress caused these latencies. Our experience from a long-term recapture study of A. femoralis has shown that most males resume their previous activities such as calling or courtship after being caught or even toe-clipped (M. Ringler, R. Mangione, A. Pašukonis, E. Ringler, unpublished data) and personal obs. by A. Pašukonis). Further, the average return time from 50 m for non-tagged animals (2 days, [10]) is similar to the one observed for tagged individuals in this study (38 h). Of course, it is possible that the removal from a familiar location results in stress induced passiveness and immobility. However, we regularly observed translocated frogs waiting for hours on exposed, slightly elevated structures at the release site, which is an indication of an alert state rather than passiveness.

Initial movements of frogs translocated into the arena for distances comparable to the tracking experiment (35–70 m) support the assumption that A. femoralis perceives some cues directly at the release site. However, the overall results are equivocal, as the individuals with territories closer than 35 m and further than 70 m to the arena did not show significant homeward orientation. A
strong motivation to escape from the arena might over- 
ride the immediate homing motivation. More specif- 
ically, frogs displaced over very short distances might have 
had less motivation to orient towards their territory be- 
fore leaving the arena. On the other hand, individuals 
coming from further than 70 m might have not been 
able to perceive the orientation cue within the given trial 
time. In the arena, most individuals moved within the 
first 90 min and the remaining few were excluded from 
the trial, while after the release at natural sites in the 
tracking experiment, some frogs remained stationary for 
more than 24 h before initiating their movements. This 
indicates, that if the orientation cues are only temporally 
available or need to be accumulated for correct orienta-
tion, the arena setup was not suitable to test for this 
ability. It is important to note, that even the males that 
were displaced to the arena from longer distance 
returned to their home territories. Additional tracking of 
individuals after testing them in the arena would be ne-
cessary to understand how the direction choice in the 
arena relates to the overall homing motivation and 
trajectories.

To the best of our knowledge, this is the first study quan-
tyfying how territorial frogs return after experimental 
removals. It is also one of few studies addressing amphib-
ian orientation by quantifying full homing trajectories. 
Even though the HDF method has its clear limitations 
such as short detection range (10–30 m) and no possibility 
to identify individuals based on the signal, we demon-
strate that it can be a useful tool for understanding movement 
patterns of small animals in complex environments, such 
as the tropical rainforest. Several authors have reported 
problems, such as altered behavior and injuries, from ex-
ternal attachment methods on anurans [28,31,50]. Our at-
traction method was generally suitable for a short-term 
(up to five days) tracking but could be problematic for 
longer-term studies. Frogs exhibited all their natural re-
productive behaviors such as calling, territorial defense, 
courtship, and on one occasion even tadpole transport 
while having a reflector attached. However, on some occa-
sions, we observed skin abrasions around the waist after 
removing the tag. Observed injuries healed in a few days 
and at least in some cases we know that males continued 
to defend territories for weeks and months following the 
tracking.

Conclusions
Overall, our results underline the importance of quanti-
yfying the individual trajectories after experimental dis-
placements in understanding amphibian orientation. 
Taken together with measures of initial homeward orien-
tation, they reveal a very accurate homing behavior in A. 
femoralis. The straightness of trajectories and initial 
home orientation suggest an integration of learned 
landmarks providing a map position for translocated indi-
viduals. We suggest that spatial knowledge acquired 
during juvenile dispersal or tadpole transport mediates 
this behavior but the actual cues being used remain un-
known. Future research should focus on the importance 
of familiarity with an area for successful homing and the 
nature of sensory cues being used.

Materials and methods

Study animals and area
Allobates femoralis is a small (snout-urostyle length ap-
proximately 25–30 mm) territorial dendrobatid frog. 
Playback of an advertisement call of a simulated intruder 
reliably elicits antiphonal calling or direct phonotactic 
approach by the resident male [44]. Individual frogs can 
be identified and recognized by their unique ventral col-
oration patterns [49].

The study was carried out within one reproductive 
season of A. femoralis between 19 January 2013 and 30 
March 2013. Frogs were sampled from a single popula-
tion in an area of approximately 3 ha near the field camp 
‘Saut Pararé’ (4°02’ N, 52°41’ W, WGS84) in the nature 
reserve ‘Les Nouragues’, French Guiana. The study area 
mainly consists of primary lowland rainforest bordering 
the ‘Arataye’ river to the south.

Telemetry experiment

We used the harmonic direction-finding (HDF hereafter) 
telemetry technique to obtain homing trajectories of ex-
perimentally translocated territorial males. The HDF sys-
tem consists of a directional transceiver and a passive 
reflector. The transceiver emits and recaptures a radio 
signal which gets reflected from the tag attached to an 
animal, thereby providing directional information (for 
more details see [26,29]). The miniature size of the re-

flector tags allows this technique to be used on smaller 
animals than permitted by conventional active radio 
tracking. We used a commercially available transceiver 
(R8, RECCO Rescue System, Lidingö, Sweden) with re-

flector tags consisting of a Schottky diode soldered bet-
tween two antennas. Antennas were made of 40 μm 
steel strands forming a 2 cm by 10 cm T-shaped dipole 
with the braze point sealed in a heat-shrink tubing. We 
attached the reflectors to the frogs using a waistband 
made of 1 mm diameter silicon tubing similarly to 
Gourret and Schwarzkopf [27]. The short part of the T-
shaped antenna was secured inside of the tube and the 
waistband was fixed with a cotton thread going through 
the inside of the tubing. The tag together with the at-
tachment made up for less than 5% of frogs’ body weight 
(range of frog weight = 1.4 – 2.3 g, max of tag weight = 0.07 g).

The telemetry experiment took place from 21 January 
2013 to 15 March 2013. During this period, 18 territorial 
males were captured and equipped with reflector tags.
Calling males were detected and identified as territorial if they showed stereotypical territorial defense behavior (calling and phonotactic approach), which was elicited by broadcasting conspecific advertisement calls, simulating an intruder. Frogs were captured with transparent airtight plastic bags, photographed for identification, and their precise capture positions were recorded with the mobile GIS software ArcPad™ 10.0 (ESRI, Redlands, CA, USA) on pocket computers (MobileMapper™ 10, Spectra-Precision, Westminster, CO, USA) using a detailed background map based on a grid of reference points and natural structures. Each frog was equipped with a reflector and immediately released at its initial capture position, where it was observed for a 24 h period to confirm normal territorial behavior with the reflector. Each frog was located at least once during this period and confirmed to behave territorially if it showed any of the following behaviors: calling, phonotactic approach of a simulated intruder, courtship. Two males were excluded at this stage and their tags were removed because no territorial behavior was observed, and another individual lost the waistband and could not be located again.

When normal territorial behavior with the tag was confirmed, each frog was captured with an airtight plastic bag, placed in an opaque container and translocated 50 m away from the home territory (n = 15). Each animal was designated to one of five displacement directions (N, E, S, W and NW), avoiding terrain and vegetation where tracking might have been impossible. The release points were located and marked using the same detailed background map on a pocket computer. Allobates femoralis, like most dendrobatid frogs, is diurnal and does not move during the dark hours, which was confirmed by preliminary observations and during this study. Therefore, individuals were tracked during the daylight hours (07 00 h to 19 00 h) until they returned to their territories. Locations were visited approximately every hour with occasional shorter intervals when the frog was found actively moving. Longer intervals were sometimes forced by bad weather conditions and/or additional time taken to relocate the frog.

To locate the frogs, we followed the increasing amplitude of the reflected signal until visually spotting an individual. In cases of poor visibility or if an individual was hiding in the leaf litter, we narrowed the signal to less than one meter. If an individual remained stationary and hidden for longer periods, we carefully uncovered the frog at least once a day to make sure that the tag had not fallen off and that the individual had no injuries. Occasionally, the tag had twisted to the side or underneath the frog, in which case the frog was carefully manipulated by pulling the antenna to reposition the tag. These manipulations never took more than a minute and frogs never moved more than a meter as a result of them. Because the harmonic signal does not carry an individual signature and handling would be necessary for identification, we never translocated more than one frog at a time in the same area.

Trajectory analysis
Initial visualization, extraction of coordinates and distance measurements were done in the GIS software ArcGIS™ 10 (ESRI, Redlands, CA, USA). The geographic coordinates of all locations were projected (UTM, zone 22 N, WGS84) and extracted as X- and Y-coordinates in meters. We grouped points spaced less than two meters apart to a single position, as they might have fallen within the measurement error of the exact position in the field.

We calculated a straightness coefficient (SC) for the path between the release site and the home territory as the ratio between the straight-line distance and the actual path distance, with a ratio of one indicating a perfectly straight path. To test for initial homeward orientation, we considered the bearing between the release point and the first position further than two meters away from the release point (n = 8, mean\(\bar{d}_{\text{distance to first position}} = 6.6\) m, SD = 4.2). Significant homeward orientation was tested using Rayleigh’s test for unimodal distribution. Two individuals were excluded from this analysis because the first recovery positions were too far (> 20 m) from the release point to be seen representative for initial orientation. The Rayleigh test was performed with a circular statistics program Oriana 4.02 (Kovach Computing Services, Pentraeth, Wales, UK).

Return time analysis
To analyze return times, we considered diurnal activity hours from 7 00 h to 19 00 h. We further split the total return time of each individual into time actively moving and time stationary. Any time interval where an individual moved further than two meters between two consecutive relocalizations was considered as time active. We compared the two activity modes using the Wilcoxon Signed-Rank test for comparison of paired samples using the R Stats package 3.0.1 [51].

Arena assay
We further assessed the initial orientation ability of male A. femoralis in an experimental arena setup. A circular arena with a diameter of 240 cm and 40 cm height was set up at an arbitrarily chosen location in the natural habitat. The walls of the arena where made out of blue plastic tarpaulin and supported by fixed poles, while the floor was covered with an off-white, thick, plastic sheet, marked with a grid as a reference for position measurements.

To test for initial orientation after experimental translocation, territorial males were caught in their territories and released in the arena, following the same catching
and translocation protocol as in the telemetry trials. Single individuals were placed under a release device in the center of the arena and left to habituate for 15 min.

The release device consisted of two upturned plastic flowerpots, stacked one over the other. After the outer part was lifted with a string, the frog could leave the inner part to all directions through cutout exit holes. This release design was necessary because some individuals showed immediate escape behavior when left exposed with no cover. Trials were filmed for 90 min without experimenter presence using a wide angle video camera (GoPro™ HD HERO2, Woodman Labs Inc., San Mateo, CA, USA) on a tripod. After leaving the release device and reaching the edge of the arena, frogs usually circled and eventually climbed over the wall. Individuals were left to home back to their territories. Frogs that did not exit the release device within 90 min were returned to their home territories and excluded from the analysis.

Fifty-six arena trials were conducted from 19 January 2013 to 30 March 2013. Nine trials were excluded for technical problems (n = 3) or because frogs did not leave the release device within 90 min (n = 6). Comparisons of individual photographs revealed that nine individuals where tested twice and in such cases only the first trial was included in the analysis.

Initially, we translocated frogs that had their territories within 30–70 m around the arena to enable a direct comparison with the homing trajectories of the frogs that were used in the tracking experiment where they were translocated over distances of 50 m. Subsequently, we included frogs closer (5–30 m) and further away (70–105 m) from the arena to assess the effects of translocation distance on the accuracy of initial orientation.

**Initial orientation analysis**

In total, we analyzed 38 valid arena trials. We used a custom script written by the first author for MATLAB 7.11.0.584 (The MathWorks Inc., Natick, MA, USA) to code and extract the trajectory of each frog in the arena. A graphical representation of the arena with the reference grid was simultaneously displayed with the video recording of each trial and each hop position of the frog was coded with a mouse click at the corresponding location in the graphical representation. Each position was exported as the corresponding X- and Y-coordinates with the origin at the center of the arena. Arena trajectories were analyzed between an inner circle with 30 cm radius and an outer circle with 100 cm radius, in order to avoid the wall influenced movements close to the release device and close to the arena wall (Figure 4).

We used the coordinates to calculate the orientation bearing of each individual at 100 cm away from the center and the SC of their trajectory from 30 cm to 100 cm away from the center. Similar to the telemetry trials, the SC was calculated as the ratio between the straight line and the actual path distance in the arena. The expected homeward orientation bearings were calculated from the coordinates of each capture position as recoded on the digital map and the center of the arena.

To test for significant homeward orientation in different distance groups, we used the second order Hotelling’s circular test for significant unimodal distribution of each sample. In addition, we looked for significant differences between distributions obtained from displacements over different distances, by using the Hotelling’s two sample test. For significant mean directions a 95% confidence interval was calculated. Statistical analyses were performed with Oriana 4.02.

Our study was approved by the scientific committee of the research station where fieldwork was conducted. All necessary permissions were provided by the ‘Centre National de la Recherche Scientifique’ (CNRS) and by the ‘Direction Régionale de l’Environnement de Guyane’ (DIREN). All sampling was conducted in strict accordance with current French and EU law and followed the ASAB guidelines for the treatment of animals in behavioral research and teaching.

**Competing interests**

The authors declare that they have no competing interests.
Authors’ contributions
AP designed the study, collected, analyzed, and interpreted the data and drafted the manuscript. MCL participated in the design of the study, data collection, analysis, and interpretation. LL participated in the statistical analysis and interpretation of the data. MR and WH reviewed and provided valuable comments on the design of the study and advised on the GIS data processing. WH participated in the design of the study. MCL, LL, MR and WH reviewed and provided valuable comments on the manuscript. All authors read and approved the final manuscript.

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Chapter 3

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Reference:
Poison frogs rely on experience to find the way home in the rainforest

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Among vertebrates, comparable spatial learning abilities have been found in birds, mammals, turtles and fishes, but virtually nothing is known about such abilities in amphibians. Overall, amphibians are the most sedentary vertebrates, but poison frogs (Dendrobatidae) routinely shuttle tadpoles from terrestrial territories to dispersed aquatic deposition sites. We hypothesize that dendrobatid frogs rely on learning for flexible navigation. We tested the role of experience with the local cues for poison frog way-finding by (i) experimentally displacing territorial males of Allobates femoralis over several hundred metres, (ii) using a harmonic direction finder with miniature transponders to track these small frogs, and (iii) using a natural river barrier to separate the translocated frogs from any familiar landmarks. We found that homeward orientation was disrupted by the translocation to the unfamiliar area but frogs translocated over similar distances in their local area showed significant homeward orientation and returned to their territories via a direct path. We suggest that poison frogs rely on spatial learning for way-finding in their local area.

1. Introduction

Repeated attempts have been made to formulate a conceptual and evolutionary framework for animal navigation, integrating the findings from birds, mammals and insects [1,2]. Among vertebrates, comparable spatial learning abilities have been found in birds and mammals, as well as some turtles and teleost fish. Furthermore, potentially homologous pallial regions responsible for spatial learning have been identified in all of these taxa [3]. Meanwhile, very little is known about spatial learning and navigation in amphibians—the root branch of all tetrapods.

Spatial memory and its flexibility are expected to vary with a species’ mode of locomotion, home-range size, habitat complexity and the variability of the resources being exploited. Overall, amphibians are the most sedentary vertebrates (for a review, see [4,5]), but some of them face challenging navigational tasks. Poison frogs (Dendrobatidae) are restricted to the highly structured Neotropical habitats where they show some of the most complex spatial behaviours among amphibians. These small territorial frogs with terrestrial clutches rely on water for tadpole development. Consequently, poison frogs routinely shuttle tadpoles on their back from the terrestrial clutches to suitable deposition sites, many of which are temporary and widely dispersed [6].

Homing after experimental translocations has been observed in many amphibians (for a review, see [7]), but to date the research on amphibian orientation has largely focused on the sensory basis (e.g. [8,9]) and not the cognitive mechanisms. Some amphibians use large-scale directional cues, but such cues cannot account for accurate way-finding within a few hundred metres [10,11]. Several authors have suggested that learned local cues might be important for amphibian homing performance (e.g. [7,12]), but empirical evidence is lacking. We hypothesize that
dendrobatid frogs rely on experience with the local area for flexible way-finding in their complex habitat.

There are three main challenges to testing the dependence on experience for navigation in the field: (i) finding an animal strongly motivated to orient towards a goal, (ii) tracking the movements, and (iii) being able to quantify or exclude the familiarity with a given area. We overcame them by (i) experimentally displacing highly territorial males of a poison frog, *Allobates femoralis*, (ii) using a harmonic direction finder with miniature transponders to track these small frogs in their natural habitat [13], and (iii) using a natural river barrier to separate the translocated frogs from any learned local cues.

2. Material and methods

*Allobates femoralis* is a small, territorial, leaf-litter frog common throughout the Amazon basin and the Guiana Shield [14]. Translocated males return to their territories from up to 400 m [19]. To test whether experience with the local cues affects *A. femoralis* way-finding, we fitted 46 territorial males with miniature transponders and translocated them between 187 and 365 m away from their territories. To exclude the possibility of en-route path integration via visual, olfactory or magnetic cues, we transported frogs along an indirect path in an opaque, airtight container with a freely rotating rod magnet. Twenty-five frogs were released in the area occupied by the same population (i.e. mainland; mean translocation distance $\mu_{\text{mainland}} = 254$ m, s.d. $= 58.8$ m), whereas 21 frogs were released at equivalent distances in the centre of an approximately 5 ha river island (i.e. island; mean translocation distance $\mu_{\text{island}} = 301$ m, s.d. $= 51.2$ m) with an isolated population of *A. femoralis* (electronic supplementary material, figure S1). Both release areas provided comparable and suitable habitats for *A. femoralis*, but the frogs do not naturally cross the river barrier. Frogs released on the island had an area with a radius of about 100 m to move in before reaching the waterfront (see the electronic supplementary materials and methods for more details).

Figure 1. Polar plots comparing homeward orientation of frogs translocated to a familiar area, i.e. (a,c) mainland and an unfamiliar area, i.e. (b,d) island. (a,b) Full trajectories derived from linear interpolations of consecutive positions within 100 m from the release point on the mainland and the island, respectively. Each line represents a different individual. (c,d) Vector plots for individuals that moved at least 25 m from the release point ($n_{\text{island}} = 12$, $n_{\text{mainland}} = 14$). Vector direction corresponds to the home direction normalized bearing at approximately 25 m from the release point. Vector length corresponds to the path straightness until that point. Each white arrow represents a different individual, while the filled arrows show the mean orientation. Significance levels from the second-order Hostelling’s test and a two-sample Hostelling’s test are shown.
After the release, we tracked the individual movement patterns for about 7 days or until the frog returned to the home territory. We localized each frog every 15–60 min during their daylight activity period using a commercially available harmonic direction finder (RECCO R8, Recco AB). The position of each frog was recorded on a pocket computer (MobileMapper 10, Spectra-Precision) in ArcPAD (ESRI) using a detailed background map (see the electronic supplementary materials for the full tracking procedure). Experiments were carried out between 1 February 2014 and 22 April 2014 near the field camp ‘Saut Pararé’ in the nature reserve ‘Les Nouragues’, French Guiana.

For each frog, we analysed the X- and Y-coordinates of each position in ArcGIS 10 (ESRI), after averaging points that were spaced less than 1 m apart (estimated mapping error in the field). We normalized all movement vectors towards the home territory of each frog, so that 0° was the common home bearing. We further described trajectories by a straightness coefficient (SC), defined as the ratio of straight path length to total path length. To test for significance of homeward orientation, we considered only those frogs that moved at least 25 m away from their release point \( (n_{\text{island}} = 12, n_{\text{mainland}} = 14) \) and used a vector length weighted, second-order Hotelling’s test (Oriana 4, Kovach Computing Services). The vector length corresponds to the SC until the point where the bearing was measured. The island and mainland bearing distributions were compared with Hotelling’s two-sample test. For visual representation, we plotted home direction normalized trajectories derived from the linear interpolations of consecutive positions (see the electronic supplementary materials and methods for more details).

3. Results

While on the mainland, frogs that moved at least 25 m away from the release point showed significant homeward orientation \( (n = 14, \text{mean vector direction} = 354°, 95\% \text{ CI} = 303.5°–30°, \text{Hotelling’s } F = 14.6, p < 0.001; \text{figure 1a,c}) \), the frogs on the island showed no significantly preferred direction \( (n = 12, \text{Hotelling’s } p > 0.1; \text{figure 1b,d}) \). At 25 m, there was a significant overall difference in bearing distribution of frogs on the mainland from those on the island (Hotelling’s two sample \( F = 3.78, p = 0.038 \)). On the mainland, seven frogs returned to within a few metres of their capture location and another three moved at least 100 m homewards. All 10 frogs that moved at least 100 m away from the mainland release sites were strongly homeward oriented \( (n = 10, \text{mean vector direction} = 7°, 95\% \text{ CI} = 356°–17°, \text{Hotelling’s } F = 62.8, p < 0.001) \) and showed direct homing trajectories \( (\text{Mean}_{\text{SC}} = 0.77, \text{s.d.} = 0.18; \text{figure 2}) \).

4. Discussion

The fact that frogs choose a direct path home when released in their local area but are disoriented in an unfamiliar area demonstrates that experience with local cues is necessary for successful homing. Our findings cannot be solely explained by the use of large-scale gradients or goal-associated cue guidance (e.g. distant visual, olfactory or acoustic beacons). They suggest a role of spatial learning.

True spatial learning requires processing and storing information about distances and directions. It allows novel routes to be created in the familiar area \[2\]. There are several ways to use learned cues that do not require spatial information processing. The most common non-spatial solutions include using goal-associated cues as beacons, using a sequence of intermediate beacons, or using cues as triggers for associated motor routines that bring the animal to the goal. All of these mechanisms allow an animal to orient along specific learned routes or when in direct sensory contact with the goal. Strong initial orientation and straight homing trajectories of homebound frogs are inconsistent with route-based orientation. We cannot claim with certainty that the performed homing paths were completely novel. However, we find it very unlikely that all homebound individuals had previously taken these short-cuts to their territories from the arbitrarily chosen release sites. Even though some males maintain stable territories for up to several months, the overall territorial system is dynamic and regular re-shuffling of positions occurs \[16,17\]. Inflexible, route-based navigation would require a rapid learning of multiple new routes over a large area whenever a territorial shift occurs. The type of homing observed in translocated \textit{A. femoralis} is suggestive of a learned spatial map, but more experiments will be needed to test this hypothesis.

\textit{Allobates femoralis} relies on the exploitation of multiple, widely dispersed, temporary aquatic deposition sites for successful reproduction. In a recent study, \textit{A. femoralis} males were found transporting tadpoles up to 180 m away from their territory. In addition, males transported more tadpoles at once when going to more distant deposition sites, which suggests some spatial knowledge of the area \[18\]. Tadpole transport takes several hours or even days. This comes at a direct cost in the form of lost reproductive opportunities and a risk of losing the territory altogether. Under such circumstances, we expect a direct selection for a strong spatial memory and the ability to use it flexibly to find the optimal route to deposition sites and back home.

Qualitatively comparable spatial learning abilities have been shown in a broad range of vertebrates \[1,3\]. Amphibians are a key taxon in understanding vertebrate evolution but little is known about amphibian spatial cognition and the neural mechanisms behind it. Our findings suggest that among amphibians at least dendrobatid frogs rely on learning for successful orientation. Amphibians display an extreme diversity of reproductive behaviours \[19\] resulting in an extremely diverse spatial ecology. Thus, understanding the cognitive mechanisms behind amphibian orientation could give key insights into the evolution of spatial cognition.
Data accessibility. All positional data used for analysis have been uploaded as the electronic supplementary material.

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References

Chapter 3 - supplementary materials and methods

Telemetry equipment

We used the harmonic direction-finding (HDF hereafter) telemetry technique to obtain homing trajectories of experimentally translocated, territorial Allobates femoralis males. The HDF system consists of a portable transceiver and a passive reflector (i.e. transponder). The transceiver emits a microwave signal and recaptures a harmonic frequency reflected from the tag attached to an animal, thereby providing directional information (for more details see [1,2]). The small size of the reflector tags allows this technique to be used on smaller animals than would be possible with conventional radio tracking (i.e. VHF telemetry). We used a commercially available transceiver (RECCO® R8, Recco AB). The transponder consisted of a Schottky diode soldered between two antennas. Antennas were made of 40 µm steel strands forming a 3 by 12 cm T-shaped dipole with the braze point sealed in heat-shrink tubing and silicone. We attached the transponder to the frog using a waistband made of 1 mm diameter silicon tubing. The short part of the T-shaped antenna was secured inside the tube and the waistband was fixed with a cotton thread going through the tubing. The tag together with the attachment amounted to less than 5% of the frogs’ body weight (frog weight ~ 2 g, tag weight < 0.1 g).

Experimental areas

Frogs were sampled near the field camp ‘Saut Pararé’ (4°02’ N, 52°41’ W, WGS84) in the nature reserve ‘Les Nouragues’, French Guiana. The study area consists mainly of primary lowland rainforest bordering the ‘Arataye’ river to the south. The two experimental areas were the riverside surrounding the field camp (i.e., mainland) and a river island of approximately 5 ha in front of the field camp (i.e., island) (Fig. S1). The mainland sampling area sustains a population of at least 300 calling males distributed in patches. The area can be characterized as ‘terra firme’ forest with a complex terrain of small hills and ridges (~ 30 – 50 m a. s. l.) partitioned by swamps and creeks in lower areas. The island is mostly covered in ‘terra firme’ forest with less complex terrain (~ 30 – 40 m a. s. l.) and lacks any persisting creeks. It was previously uninhabited by any dendrobatid frog species, including A. femoralis. However, an experimental population of A. femoralis was been introduced in 2012 and has successfully established on the island. At the time of the study, the population had about 60 calling males, approaching the density of the mainland sampling area. The Arataye river forms a strict physical barrier between the two populations, as width and current strength do not allow for any intentional crossings of A. femoralis individuals. For this study, all frogs were sampled from the mainland population.
**Choice of individuals and translocation procedure**

Calling males were detected and identified as territorial if they showed stereotypical territorial defence behaviour (calling and phonotactic approach), which was elicted by broadcasting conspecific advertisement calls, simulating an intruder [3,4]. Individuals could be recognized and identified by their unique ventral coloration pattern [5]. Frogs were captured with a transparent airtight plastic bag, weighed and photographed ventrally for identification. Their precise capture locations were recorded with the mobile GIS software ArcPad™ 10.0 (ESRI) on a pocket computer (MobileMapper™ 10, SpectraPrecision) using a detailed background map, based on a network of reference points and natural structures [6]. Each territorial male was equipped with a transponder and immediately released at its initial capture location. We revisited the territories of the tagged males over the next 36 h to confirm their territorial behaviour after tag attachment. Males were only used for translocation if they were observed calling, reacting aggressively to a simulated intruder, or in courtship with a female during at least one of the visits. Over the whole study period, we tagged 72 territorial males, of which 46 were used for the translocations.

Selected males were collected from their territories in the morning hours, translocated and subsequently released in the afternoon. Each frog was placed in an individual, clean, airtight plastic bag. All bags were placed in an opaque waterproof barrel equipped with a suspended, freely rotating neodymium rod magnet (Ø = 15 mm, length = 100 mm, residual magnetism = 1.17-1.21 T). The barrel was rotated multiple times after each frog was added, during transport and before the release. Between one and five frogs were transported at the same time. All frogs were transported to the field camp (Fig. S1) before being released at their corresponding release sites on the island or the mainland. The average time between capture and release was 210 min (SD = 33 min) for the mainland translocations and 194 min (SD = 62 min) for the island. The average translocation distance was 254 m (min = 187 m, max = 365 m) on the mainland and 301 m (min = 193 m, max = 364 m) on the island. Frogs released on the mainland were translocated away from the river (northeast, north or northwest) or towards the river (southeast, south or southwest), depending on the location of their territory (Fig. S1). Individuals were not translocated towards east and west to avoid their release across a water barrier such as a creek or a swamp. To achieve comparable translocation distances for frogs released on the island, we selected frogs with territories close to the river, resulting in translocations towards southeast, south, or southwest. Frogs translocated to the island were released approximately in the centre of the island, spaced out by at least 10 m when multiple frogs were translocated at once. All frogs were released at the base of understory palms (*Astrocaryum* sp.), which are often used as calling perches by territorial males.
Tracking procedure

Following the release, all frogs were continuously tracked during their daylight activity hours (~ 7 h 30 to 18 h 30). *Allobates femoralis* is exclusively diurnal and we never observed a frog move between 19 h 00 and 7 h 00. During the tracking hours, each frog was relocated every 15–60 min. Longer intervals occasionally occurred due to bad weather conditions and/or additional time needed to relocate a frog.

To locate the frogs, we followed the increasing amplitude of the reflected signal until visually spotting an individual. In cases of poor visibility or if an individual was hiding, we homed in to the signal within less than one meter. If an individual remained stationary and hidden for longer periods, we carefully uncovered the frog at least once a day to make sure that the tag had not fallen off and that the individual showed no injuries. Because the harmonic signal does not carry an individual signature, we colour coded the transponders. A frog’s identity was always visually confirmed if there was any ambiguity in the spatial separation of simultaneously tracked frogs. Occasionally, we handled the frogs to fix or replace a broken or miss fitting waistband or transponder. These manipulations never took more than a few minutes.

Every position fix was recorded on a background GIS map as mentioned above. Occasionally, frogs moved out of the mapped area, in which case we mapped the positions by measuring the distance and compass direction from the nearest mapped reference point. Distance was measured with a laser rangefinder (DLE 50; Bosch) and compass direction was measured with a precision compass (Suunto Tandem 360PC/360R DG, magnetic inclination zone 2; Suunto).

Initially, we planned to track all frogs for seven full days or until their return to the home territory. However, several factors determined shorter or longer tracking periods. On the island, shorter periods were forced by predation events (2 frogs after 3 and 6 days); injury (1 frog after 2 days) and lost transponders (1 frog after 3 days). On the mainland, 7 frogs returned to their territories in 3 to 5 days. In addition, the tracking period on the mainland was extended (8 to 14 days) for 8 frogs because of an extended dry period, which resulted in very low mobility of frogs. Frogs that moved homewards after the first heavy rain were tracked until they returned to their home territory. All frogs were returned to their territories at the end of the tracking period and many of them were subsequently observed showing territorial behaviour.

Data analysis

Initial visualization, extraction of coordinates, and distance measurements were done in the GIS software ArcGIS™ 10 (ESRI). The geographic coordinates of all locations were
projected (UTM, zone 22N, WGS84) and extracted as X- and Y-coordinates in metres. We averaged consecutive coordinates of an individual when they were within less than one metre, which we considered to be our measurement error. We normalized all trajectories by shift and rotation to a relative zero release point and a single home bearing at 0°. We calculated a straightness coefficient (SC) as the ratio between the straight-line distance and the actual path distance, with a ratio of one indicating a perfectly straight path.

To test for initial homeward orientation, we considered only those frogs that moved at least 25 m away from their release point. We measured the bearing between the release point and the position closest to the 25 m-radius circle crossing. The SC was measured between the same two points. We used second-order Hotelling’s circular statistics test for a significant unimodal distribution of each sample, i.e. island and mainland. The test weights each angular measure by the vector length, which corresponds to the SC value in our data set (Oriana 4.02, Kovach Computing Services, Pentraeth, Wales, UK). In addition, we used the same procedure to test the homeward orientation of the mainland frogs that moved at least 100 m from the release point. The SC for the total homing path of these frogs was also calculated.

**Ethical statement**

Our study was approved by the scientific committee of the research station where the fieldwork was conducted. All necessary permissions were provided by the ‘Centre National de la Recherche Scientifique’ (CNRS) and by the ‘Direction Régionale de l’Environnement de Guyane’ (DIREN). Permit numbers: ARRETE n°2011-44/DEAL/SMNBSB/BSP du 19/07/2011 and ARRETE n°2013-04/DEAL du 14/01/2013. All sampling was conducted in strict accordance with current French and EU law and followed the ASAB guidelines for the treatment of animals in behavioural research and teaching.

**References**


Supplementary figure

Figure S1. Map of the study area showing home territories of translocated frogs (coloured circles) and release sites (arrowheads) connect with lines. Contour lines (1 m) are in light grey; creeks and Arataye river in dark grey; swamps marked as tussock on the white background; field camp marked with a camping symbol. All frogs were transported to the field camp before being released at their corresponding release sites on the island or the mainland.
Chapter 4

This chapter consists of a late-stage manuscript in preparation for a peer-reviewed journal.
Do poison frogs rely on learning for finding tadpole deposition sites?

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Abstract

An ability to associate cues with specific resources or locations can strongly increase animals’ chances of survival, and thus learning often guides the movement. Spatial learning is being studied in an ever-increasing variety of species, but amphibians have been grossly neglected in this research area. Many tropical amphibians depend on small and fluctuating resources such as ephemeral forest pools for reproduction, but it remains unknown if they rely on learning for effective exploitation of these resources. Neotropical poison frogs routinely shuttle their tadpoles from terrestrial clutches to widely dispersed aquatic deposition sites. We investigated if a poison frog Allobates femoralis relies on learning for relocating artificial deposition sites in the rain forest. For this purpose, we temporarily removed an array of artificial pool previously used for tadpole deposition by the frogs in the area and set up an array of control sites. We quantified the individual movements and site preference of tadpole-transporting adults by intensive sampling and observations in the field. We found that A. femoralis return to the exact locations of the tadpole deposition sites even after the artificial pools had been removed. This demonstrates that A. femoralis remembers the locations of multiple widely dispersed deposition sites. Further, we found evidence that tadpole-transporting adults are attracted to the olfactory cues of conspecific tadpoles. Future research will have to determine what exact cues are being learned and how they are used for efficient way-finding in this complex environment.

Keywords: amphibian learning, spatial cognition, tadpole transport, Dendrobatidae
Introduction

All animals use environmental cues to direct their movement. An ability to associate cues with specific resources or locations can strongly increase animals’ chances of survival, and thus learning strategies are ubiquitous across the animal kingdom. However, the complexity and amount of learned information used ranges from simple modulation of chemotaxis to large-scale spatial map representations (Jacobs 2012). Until recently, the majority of research on spatial learning has been done under controlled laboratory conditions (reviewed in Jacobs & Menzel 2014). How learning guides the behavior of free-ranging animals was rarely addressed or quantified (but see Tinbergen 1972; Cartwright & Collett 1982). As a result of recent technological developments in telemetry techniques and analytical tools (reviewed in Kays et al. 2015), as well as new integrative conceptual frameworks (Jacobs & Schenk 2003; Nathan et al. 2008; Jacobs & Menzel 2014), these questions have emerged at the forefront of animal behavior research (Fagan et al. 2013). While spatial cognition and movement ecology are already among the most comparative topics in the study of behavior, some taxonomic groups and habitats, such as tropical amphibians, have remained neglected to date.

Spatial learning is being studied in an ever-increasing variety of mammals, birds, and invertebrates. However, ectothermic vertebrates, especially amphibians, have been grossly neglected in this research area. Meanwhile, directed long-distance movements, such as mass spring migration to breeding ponds in temperate-region amphibians, have drawn attention to amphibian orientation mechanisms (reviewed in Sinsch 1990; 2006). Some laboratory experiments have been successful in revealing what cues a few anuran amphibians, especially toads, use to orient on a small scale (e.g., Grubb 1976; Adler 1980; Dall'antonia & Sinsch 2001; Sotelo et al. 2014; Daneri et al. in press). In the field, spatial orientation experiments have focused almost exclusively on sensory modalities (e.g., olfaction, vision, magnetoreception) (reviewed in Ferguson 1971; Sinsch 2006) rather than on the cognitive mechanisms (e.g., path integration, beaconing, spatial learning) (but see, Ishii et al. 1995; Fischer et al. 2001).

Both laboratory experiments and observations of orientation behavior in the field suggest that many amphibians use learned cues to find their way around (Dole 1968; Grubb 1976; Sinsch 2007; Sinsch & Kirst in press), but to date direct empirical evidence for such learning is lacking (but see Pašukonis et al. 2014). Further, the range of natural movements of most amphibians remains unknown and probably underestimated (Sinsch 2014). Orientation towards large breeding ponds in temperate-region amphibians could be explained by natal imprinting and an ability to perceive long-distance cues originating from the goal (i.e., beacons), such as the natal pond odor (Grubb 1973; Joly & Miaud 1993; Sinsch et al. 2006).
However, such mechanisms are insufficient to explain the ability to relocate terrestrial home areas or small reproductive resources in complex habitats, and more complex spatial learning strategies are likely to be involved in such behaviors. To date, research on learning and spatial orientation has focused almost exclusively on nocturnal, pond-breeding anurans of temperate regions. A variety of complex reproductive behaviors, such as territoriality, terrestrial breeding, and tadpole transport are found mostly in tropical regions. Unlike in temperate regions, many tropical anurans depend on small and fluctuating resources such as ephemeral forest pools for reproduction. It remains unknown whether tropical frogs rely on learning for effective exploitation of these resources.

Poison frogs (Dendrobatidae) are a group of small diurnal Neotropical frogs characterized by territoriality and parental care (Weygoldt 1987). They display some of the most complex spatial behaviors known among amphibians, such as long-term territoriality and tadpole transport. 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, fallen tree-holes or water-filled leaf axils for tadpole rearing (e.g., Brown et al. 2008; Rojas 2014; Ringler et al. 2015a). While many aspects of dendrobatid frog behavior have been well documented (Amézquita et al. 2011; Brown 2013; Summers & Tumulty 2013; Weygoldt 1987) it remains completely unknown, how these frogs find the tadpole deposition sites. In fact, even the tadpole transport distances have not been reported for all but one species (Allobates femoralis, Ringler et al. 2013). We hypothesize that learning plays a major role in the ability to relocate the deposition sites, once they are discovered. Alternatively, poison frogs could search for deposition sites using environmental cues such as local odor every time they transport tadpoles. We tested this hypothesis in a well-studied species Allobates femoralis: a small dendrobatid frog with paternal long-distance tadpole transport (Ringler et al. 2013), which uses multiple deposition sites widely dispersed in the forest (Erich et al. 2015). To test if males remember the tadpole deposition locations, we manipulated artificial deposition sites in the field and quantified individual movements by intensive sampling and observations.

Materials and 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 (Ringler et al. 2009; 2011). Courtship takes place inside the male’s territory where a small clutch of approximately 20
eggs is laid in the leaf-litter (Roithmair 1992; Montanarin et al. 2011; Ringler et al. 2012). Both males and females are highly polygamous and iteroparous, and males have been observed to attend up to five clutches simultaneously (Ursprung et al. 2011a). Tadpole transport takes place 15-20 days after oviposition and is mainly performed by males after heavy rainfall (Aichinger 1991; 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 (cf., Ringler et al. 2015a and references herein). 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 (Ursprung et al. 2011b).

**Study area**

The study was carried out at the beginning of the rainy season January – April 2014 on a 5-ha river island in close vicinity of “Camp Pararé” at the CNRS “Nouragues Ecological Research Station” in the Nature Reserve “Les Nouragues”, French Guiana (3°59´N, 52°35´W; Ringler et al., 2014). The island was not inhabited by *A. femoralis* before March 2012 when approximately 1800 tadpoles where introduced in a controlled way (Ringler et al. 2014). The tadpoles were sampled from a nearby autochthonous population and released in a linear array of 20 artificial pools (volume ~10 L, inter-pool distance ~10 m) on the island. In September 2012, we removed every second pool, leaving an array of 10 pools spaced by 20 m. By March 2013, 36 adult males and 31 females had successfully established and were reproducing, using the remaining pools for tadpole deposition (Ringler et al. 2014).

**Territory sampling**

To determine male territories, two to six people conducted extensive visual and acoustic surveys from 22 January - 24 February across the entire island. All detected frogs were captured with transparent plastic bags, sexed, photographed for identification, and their behavior was noted. We considered males to be territorial when they were calling or in courtship, or when they showed aggressive reactions to a playback simulating an intruder (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) using a highly detailed background map (Ringler et al. 2014).

**Experimental setup and tadpole carrier sampling**

We extensively surveyed the area for natural tadpole deposition sites before the experiment. As natural deposition sites containing tadpoles, we only found one palm frond that held water, one temporary puddle on a trail, a water filled borrow, and one larger pool at
the base of a fallen tree. Several palm fronds that held water and could have been used for tadpole deposition were removed. All artificial pools were well used for tadpole deposition.

To test if males remember the tadpole deposition locations, all remaining artificial pools were removed simultaneously on 6 February. We counted on average 49.5 (SD = 28.5) tadpoles per pool on the day of pool removal. All removed pool sites (“pool sites” hereafter) were filled with soil and covered with leaf litter while we left the yellow labeling stakes that had previously marked the pool sites. All collected tadpoles were distributed in two 6 L buckets (approximately 250 tadpoles per bucket). We suspended the buckets 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 allowed the tadpoles to develop and emerge but prevented *A. femoralis* males from using the buckets as deposition sites.

During experimental monitoring from 7 – 24 February, we aimed to capture a maximum number of tadpole-transporting males. Two to four people visually inspected the area of the previous pool array every 1 - 2 hours between 8:00 and 18:00 hours for 7 days and every 2 – 4 hours for another 10 days. We attempted to capture all frogs sighted. Before release, we recorded their location, photographed them for identification, and counted the number of tadpoles. The tadpoles were counted as exactly as possible without manipulating them. Occasionally, some tadpoles fell off in the capture bag. These tadpoles were gently returned on the back of the male. Our previous observations have shown that males continue transporting and depositing tadpoles even when the tadpoles are removed and replaced on the frog.

During the first two experimental sampling days, we noted a considerable number of tadpole-carriers right next to the yellow stakes marking the pool sites and occasional males sitting exactly beneath the suspended tadpole-containing buckets. In response to these observations, we adjusted our experimental setup to control for a potential sampling bias and frog attraction towards the yellow stakes and a potential odor effect at the pool sites. We established 10 control sites, each 5 m away from a pool site and marked with an identical yellow stake (“control sites” hereafter; Fig. 1). Likewise, we established 10 sites to control for effects of residual tadpole odor at pool sites (“odor 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 L “tadpole water”). For each of the 10 odor sites, we diluted 1 L of tadpole water with 9L of river water and spilled it at the respective locations. These sites were also marked with identical yellow stakes. The 10 odor sites were evenly distributed in-between the pool sites and offset by 5 or 20 meters to the left or the right from the pool array (Fig. 1).

For the remainder of the experimental sampling period (9 – 24 February), we sampled all sites, including the suspended buckets evenly, by slowly walking between the consecutive
sampling sites and visually scanning the area. Only the frogs captured during this period were included in the site preference analysis.

Focal tracking

The visual tracking of tadpole carriers was performed from 7 – 14 February. Some of the tadpole-transporting males captured during the array sampling were visually followed after release. Specific males were selected arbitrarily, based on observer availability and the time when tadpole carriers were encountered during the day. One to four frogs were followed simultaneously. Each observer was assigned to track a single frog as long as possible, until the frog was eventually lost, out of sight for approximately 30 min or until nightfall. 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, passing animals, etc., caused the frogs to hide in the leaf-litter before moving again. As a result, the calculated average frog movement speeds are lower estimates. While following the frogs, the observers marked the trajectories with a strings and additional time-stamp markings. Time-stamps were marked irregularly every 1 – 15 minutes depending on the speed of frog movement and the risk of losing the frog out of sight while marking. Occasional longer gaps occurred when the frogs were lost out of sight but refound later. The string-trajectories were later digitized using a detailed GIS background map of the area (Ringler et al. 2014). In addition, we noted the pool sites visited by tadpole carriers during an unrelated tracking study from 6 – 10 April.

Frog sampling analysis

All spatial data were analyzed in the GIS software ArcGIS 10 (ESRI). To determine the alleged origins of transported tadpoles, we analyzed the capture locations of all encountered males and calculated the territory centers of all males that showed territorial behavior during the entire sampling period from 22 January – 24 February (Fig. 1). Territory centers were calculated as the centroids (“Mean Center”-function in ArcGIS 10) of all points that were associated with territorial behavior, excluding tadpole transport points and encounters where a male was apparently homing back to its territory after tadpole transport.

Because of intensive sampling by several people, tadpole carriers 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 (i) there was at least a one-day gap between recaptures or (ii) a male was recaptured with more tadpoles than on the previous capture. Distances from the territory centers 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 away from the
To re-evaluate these findings, we fitted a linear mixed-effect model to the data using tadpole number as dependent variable, distance from the territory center 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” package for the “R” environment for statistical computing (R Development Core Team).

To compare the number of captures at different experimental sites, we only considered individuals captured during the full experimental array period from 9 – 24 February. Multiple captures of the same individual at different site types (i.e., pool site, odor site, and bucket) were included. Recaptures of the same individual at the replicates of the same site type were excluded to avoid pseudoreplication. Frogs were considered at a specific site if they were found within a one-meter radius (measured on the GIS background map) from the center of the site.

**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 biased random bridge approach using movement-based kernel density estimator (MKDE) (Benhamou 2011) as implemented in the “adehabitat” package v1.8 (Calenge 2006) for the “R”. MKDE can incorporate both time and distance between the consecutive relocations (i.e., step length and duration) into estimates of UD, and thus this method is particularly suitable for our high frequency but irregular interval tracking data. As model parameters we used maximum step duration (Tmax) of 60 min, interpolation time (tau) of 1 min, the minimum step length (Lmin) of 1 m, and the minimum smoothing parameter (hmin) of 1 m. For visualizing the space use patterns, we used the volume under UD function and a color gradient representing the contours of different UD cumulative frequency levels. 10 out of 15 tracked individuals had sufficient data to perform the analysis.

To describe the directionally 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 site. For analysis and graphical representation, all trajectory parts were extracted and normalized angularly and to a common starting point with the array axis running from 0° – 180°. We measured the bearing from the center of the pool site to the location closest to crossing 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. 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°. Significant orientation was tested with Rayleigh’s test for
unimodal distribution using the circular statistics program Oriana 4.02 (Kovach Computing Services, Pentraeth, Wales, UK).

Research permits and ethical statement

Our study was approved by the scientific committee of the research station where fieldwork was conducted (http://www.nouragues.cnrs.fr/F-conseil.html). All necessary permissions were provided by the ‘Centre National de la Recherche Scientifique’ (CNRS) and by the ‘Direction Régionale de l’Environnement de Guyane’ (DIREN). All experiments were conducted in strict accordance with current French and EU law.

Results

Territories and tadpole transport

During the entire sampling period, we recorded a total of 331 male capture points from 56 different individuals. Out of these, 220 points were associated with territorial behavior. 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 between 2 m and 144 m (mean = 54.5 m, SD = 30.6 m) away from their respective territory center. They were transporting from 2 to 19 tadpoles (mean = 8.7, SD = 4.4). We found a strong and significant positive relation between the distance from the territory center 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 individuals were captured during the main sampling period with a full experimental array. Out of these 29 individuals, 13 were captured at least once within one-meter from one of the pool sites (mean per site = 1.3, SD = 0.82). Three individuals were found at experimental odor 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 tadpole carriers over an accumulated distance of 1503 m during a total of 7627 min of focal tracking. This represents 20 different tadpole transport events of 15 different individuals. Each individual was followed on average for 508 min (min = 44 min,
max = 1109 min, SD = 334 min). During this time, each individual on average moved 100 m (min = 3 m, max = 302 m, SD = 84 m), resulting in an average speed of 0.56 m/min (SD = 1.03 m/min). In general, the movement of tadpole carriers consisted of short bouts of rapid movements and longer motionless pauses. The maximum speed measured over a short bout of one min was 10.47 m/min.

Thirteen out of 15 tadpole carriers tracked visited experimental sites and most of the individuals visited multiple sites (Table 1, Fig. 2 and 3). 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 individuals (mean = 3.8, SD = 1.9) and individuals often revisited the same pool sites on several occasions. In addition, 7 out of 15 males visited one of the suspended tadpole buckets and some of them revisited these buckets on multiple occasions. Finally, during a short tracking period two months after pool removal, five frogs were observed revisiting five different pool sites.

Males that visited removed pool sites, spent on average 18% (max = 81%, SD = 23%) of their total tracking time within two meters from one of the sites. In addition, males that visited one of the tadpole-containing buckets spent on average 22% (max = 48%, SD = 19%) of the tracking time within two meters of these sites. Utilization distribution was calculated for 10 individuals with sufficient data. Highly used areas of these 10 individuals (25% cumulative UD frequency) included 9 out 10 pool sites and 1 tadpole-containing bucket (Fig. 3).

In general, frogs moved in a corridor-like fashion between the pool sites, pausing at one site before moving on in a very directed manner to the next site (Fig. 2, 3 and 4). The orientation when moving from one pool site to the next one showed a highly significant directionality (expected bearing = 0°, Mean vector = 347.3°, 95% CI = 325° – 9.7°, Rayleigh-test p < 0.001, n = 34). Tadpole-containing 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 (Fig. 2b and 4). Finally, two males showed a change in movement direction and left the linear pool site array at pool 18 toward a natural deposition site 36 m away.

Discussion

Our study clearly shows that memory plays an important role in poison frog ability to relocate small widely distributed deposition sites. Both initial capture locations and movement patterns were highly concentrated around the exact locations of the removed pools. During our relatively short study period, we observed 37.5% all recorded territorial males in
the population (21 out 56) visit at least one of the pool sites while transporting tadpoles. Further, frogs knew up to six different sites distributed over 100 m linear distance.

Even though the additional control sites for odor cues were added ad hoc after our initial observations, the results are telling. High concentration of conspecific tadpole odor seems to attract tadpole-carrying males at least from ten meters away. The suspended buckets containing large amounts of tadpoles had a clear attractor effect. A number of individuals were found sitting for several hours directly underneath the buckets. This effect is mostly likely explained by continuously overflowing water containing high concentrations of residual tadpole odor cues. *Allobates femoralis* only uses terrestrial deposition sites so the suspended buckets did not represent a potential deposition sites for the males. Neither the containers themselves, nor the specific locations could have been previously associated with potential deposition sites either. In contrast, too few individuals visited any experimental odor sites to attribute any important effect. These contrasting results are probably due to strong quantitative and/or qualitative differences in olfactory cues between the sites where large amounts of tadpoles were always present (buckets) and where diluted water with potential odor cues was spilled (experimental odor sites). The olfactory cues are notoriously hard to quantify and this was not in the scope of our field study. The use of olfactory cues is widespread in temperate region anurans (Oldham 1967; Ferguson 1971; Sinsch 1987). Recent research has also demonstrated that a dendrobatid frog *Ranitomeya variablis* uses olfactory cues to avoid depositing their offspring with cannibalistic conspecific tadpoles (Schulte et al. 2012). Further studies will be needed to identify what type and amount of conspecific tadpole cues attract the tadpole transporting males of *A. femoralis*.

Considering our observations on the potential attractor effects of tadpole odor, one could argue that residual odor cues can explain the high rate of removed pool site visitation. However, this potential effect would have been diluted by frequent rainfall during our study period. In contrast, similar olfactory cues (residual water previously containing tadpoles) were restored on a daily basis at experimental odor sites but it did not have a clear effect on frog movements. Further, even two months after pool removal, five tadpole-carrying males were observed revisiting the sites of the missing pools. This persistence of memory might be surprising considering that frogs could have learned the absence of the deposition sites and that new deposition sites were already available at that time. However, many natural pools are ephemeral and might dry out for periods of time even during the reproductive season. Further, certain natural areas continuously provide temporary resources, such as fallen palm fronds, which become unsuitable as the material decays but the new ones will become occasionally available. It remains unknown, how males discover such ephemeral pools in the first place. Conspecific tadpole odor recognition might be one of the cues used to detect 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 (Ringler et al. 2013). Strong positive correlation between the transport distances and the number of tadpole transported suggests that males prefer to transport large amounts of tadpoles when traveling to distant pools, but they might distribute smaller amounts of tadpoles when the chosen pools are closer. Such bet-hedging strategy of male *A. femoralis* was recently supported by genetic data (Erich et al. 2015). Our study provides behavioral evidence that males know multiple deposition sites over a large area. It remains unknown what cues are learned and how they are used to find these precise locations in such complex habitat. While simple directional cues from the territory could be sufficient, our data rather suggest that males recognize the exact positions of the deposition sites. Males often spent a large proportion of their total travel time stationary at the location of the missing pools. If the males were using directional cues, they would have to be very accurate and combined with at least some local landmarks that allow the recognition of the sites of interest. Recent translocation experiments of territorial *A. femoralis* males have revealed that these frogs rely on experience with the area to find their way back to the territory (Pašukonis et al. 2013; 2014). Further, the direct homing paths are suggestive of a map-like behavior similar to mammals, birds and honeybees (Jacobs & Menzel 2014). Our findings support the idea that poison frogs learn a spatial map of the area, which they can use flexibly to identify the direct routes to the sites of interest. Tadpole transport over long distances probably involves high energetic costs, a cost of loosing mating opportunities, and a risk of loosing the territory altogether. Further, traveling males are probably more vulnerably to predation. Indeed, a snake predated one of the tracked males in this study and a second frog was injured probably by a bird. Considering all the associated costs of tadpole transport, the selective pressure for cognitive mechanisms allowing efficient way-finding between the territory and the deposition sites must be high.

Overall, our study demonstrates that at least some tropical amphibians rely heavily on learning for efficient use of reproductive resources. These findings corroborate the emerging view that spatial learning ability is ubiquitous among and potentially ancestral to all vertebrates. Amphibians are a key taxon in understanding vertebrate evolution but little is known about amphibian spatial cognition and their movements in general. Future research on the cognitive mechanisms behind amphibian orientation could give key insights into the evolution of spatial cognition.

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Author’s contributions

AP designed the study, collected, analyzed, and interpreted the data and drafted the manuscript. KT participated in the design of the study. KT, RM, JS, IW, and WH collected the data. MR and ER participated in data analysis and provided valuable comments on the manuscript. All authors read and approved the final manuscript.

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Figure 1. Map of the study area showing the experimental setup and the distribution of male territories. Crossed squares represent the linear array of ten artificial tadpole deposition sites that were removed during our experiment; crossed circles represent ten experimental sites where water with tadpole odor was spilled; target symbols represent the two suspended buckets containing large amounts of tadpoles; empty squares represent ten control sites. Star symbols indicate territory centers of 56 sampled males. Red-filled starts highlight the territories of 34 males that were sampled during tadpole transport. Contour lines (1 m) are drawn in light gray and the Arataye River in blue.
Table 1. Summarized results of tadpole carrier tracking. Each row represents a different individual. Columns show the visitation of different pool sites (1 to 10) and suspended buckets containing tadpoles (N or S). Total distance traveled, total duration of following, and time stationary near pool sites or near buckets are summarized.

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Table 1. Summarized results of tadpole carrier tracking. Each row represents a different individual. Columns show the visitation of different pool sites (1 to 10) and suspended buckets containing tadpoles (N or S). Total distance traveled, total duration of following, and time stationary near pool sites or near buckets are summarized.
Figure 2. Trajectory map showing movement patterns of tadpole-transporting males during the experiment. Crossed squares represent the linear array of ten artificial tadpole deposition sites that were removed during our experiment; crossed circles represent ten experimental sites where water with tadpole odor was spilled; target symbols represent the two suspended buckets containing large amounts of tadpoles; empty squares represent ten control sites; the blue circle represents one natural deposition site that was used by frogs during the experiment. Different color lines represent trajectories of different individuals. A) Shows the whole area covered by tracked tadpole carriers. Overall, frogs showed a corridor-like movement along the array of pools sites. B) Shows 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 where frogs diverted when moving between the pool sites is outlined with a dashed-line and shaded in yellow.
Figure 3. Heat-map showing overlaid patterns of space use by 10 tadpole-transporting males. The heat-map grid size is 1 m. The contours were calculated with volumes under UD function. The UD volume contours are drawn up to 90% cumulative frequencies for each individual and represented by the color gradient. The 25% contours are outlined in black. Crossed blue squares represent the linear array of ten artificial tadpole deposition sites that were removed during our experiment; blue star symbols represent the two suspended buckets containing large amounts of tadpoles. 9 out of 10 pool sites and 1 out of 2 buckets fall in the highly used area of 25% cumulative frequency.
Figure 4. Polar plot showing the directionality of movement within 10 m from the removed pool sites. The plot center represents the normalized position of nine pool sites visited during frog following. Black arrows at 0° and 180° indicate the normalized direction towards other pool sites. In addition, arrow at 120° indicates the direction of the nearest bucket from the pool site 14. Different colors represent different individuals. Same individuals were sampled multiple times if they visited a different site or revisited same site on different occasions.
Chapter 5

This chapter consists of a manuscript formatted and ready for submission to a peer-reviewed journal.
Homing performance in a territorial dendrobatid frog, *Allobates talamancae*

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Abstract

The ability to return home or to breeding sites after experimental translocations has been observed in many amphibians but the exact homing success has rarely been quantified. Poison frogs (Dendrobatidae) are characterized by territoriality and tadpole transport. Their complex spatial behaviour and strong territoriality makes them particularly interesting and suitable for quantifying homing success. Recent studies have shown that males of a dendrobatid frog *Allobates femoralis* show one of the highest homing performances among amphibians. In this study, we quantified the homing performance after experimental translocations in a small poison frog *Allobates talamancae*. We translocated 27 males for 20 m or 200 m from their territories. Seventy seven percent of them returned successfully within an average of 11 days. The homing success was 83.3 percent for short-distance (20 m) and 70 percent for long-distance (200 m) translocations. We also tested their ability to return from across a creek as an obstacle. One out of five translocated individuals returned. Our study confirms that high homing performance is characteristic of the genus *Allobates*. More comparative studies are needed to reveal how different spatial ecologies influence homing ability in the dendrobatid frogs and amphibians in general.

Key words: amphibians, behaviour, site fidelity, spatial orientation, translocation
Introduction

Many amphibians show high site fidelity (Sinsch 2014) and are able to find their way back after experimental translocations (reviewed in Sinsch 1990). Such homing ability has mostly been studied in nocturnal temperate-region amphibians (e.g., *Anxyurus terrestris* (Bufonidae) (Bogert & Station 1947), *Lithobates pipiens* (Ranidae) (Dole, 1968), *Pseudacris regilla* (Hylidae) (Jameson 1957), *Taricha rivularis* (Salamandridae) (Twitty et al. 1964)), where individuals seasonally move between fixed breeding and hibernation sites. While it is well established that many amphibians are capable of homing, comparative data of homing performance in different species is scarce. Estimations of recapture potential without translocation or the use of tracking devices are essential for reliable estimates of homing time and success. However, most homing studies in amphibians lack appropriate control group to estimate recapture potential and did not track animal after translocation. As a result, reliable measures of homing performance are lacking for most amphibians.

Tropical amphibians show extremely diverse reproductive and spatial behaviors, such as long-term territoriality and long-distance movements between fluctuating breeding sites. However, homing ability has rarely been studied in tropical species (but see Gonser & Woolbright 1995, Nowakowski et al. 2013, Pašukonis et al. 2013, 2014a, 2014b). Poison frogs (*Dendrobatidae*) are a group of small diurnal Neotropical frogs that show some of the most complex spatial behaviors among amphibians. Dendrobatid frogs are characterized by territoriality and tadpole transport from terrestrial clutches to widely distributed aquatic deposition sites (Weygoldt 1987). Many poison frogs rely on homing as the water bodies for tadpole development are often situated outside their territory (e.g., Roithmair 1994, Ringler et al. 2009). Homing ability after experimental translocations has been studied only in strawberry poison frog, *Oophaga pumilio* (McVey et al. 1981, Nowakowski et al. 2013) and in *Allobates femoralis* (Pašukonis et al. 2013, 2014a, 2014b). Some males of *A. femoralis* return from translocation distances up to 400 m (Pašukonis et al. 2013). Furthermore, tracking experiments revealed that *A. femoralis* relies on experience with an area for homing and barriers like rivers disrupt the initial homeward orientation (Pašukonis et al. 2014b). Nowakowski et al. (2013) found that only 67 percent of translocated *O. pumilio* return from 20 m (N = 30) and 57 percent from 30 m (N = 30), suggesting a smaller homing radius in this species. Contrary to most *Allobates* species, female *O. pumilio* deposit their tadpoles in phytotelmata within or close to their defended territories. Differences in life history, such as smaller territories and shorter tadpole transport distances in the case of *O. pumilio* (Pröhл &
Berke 2001) might explain lower homing performance. More comparative studies are needed to understand if such natural history traits determine the homing performance in dendrobatid frogs.

*Allobates talamancae* is a small dendrobatid frog common in Central America. Males defend territories in the leaf litter and transport tadpoles to aquatic deposition sites in the forest floor outside their territory (Summers 2000 & personal observation). If homing ability is related to the spatial behavior for the species, such as extraterritorial tadpole transport, we might find that high homing performance is characteristic of the genus *Allobates*. In this study, we quantified the homing success and speed of male *A. talamancae* after experimental translocations.

**Materials and Methods**

**Study species and area**

*Allobates talamancae*, Aromobatidae (Grant et al. 2006), is a small dendrobatid frog (average 20.5 mm SVL cf. Cope 1875) from Central America. Males advertise and defend small territories (mean size of 13.1 m², SD = 4.4 m²; Summers 2000). Mating and clutch deposition takes place in the leaf litter in the male’s territory throughout the year and peaks during the rainy seasons (Savage 2002). Males transport eight to 29 tadpoles on their back to small water bodies on the ground (Duellman 1994, Savage 2002, Summers 2000). Females have also been observed carrying tadpoles (pers. comm. in Summers 2000). Most of the deposition sites are situated outside of the males’ territories (personal observation). Males mainly call in low light periods, in the morning and late afternoon sitting on the leaves or on elevated structures, like tree stumps and branches. Calling usually peaks in the afternoon after rain (Savage 2002). Males start calling or show phonotatic approach towards a playback of an advertisement call of a simulated territorial intruder (Lechelt et al. 2014). The sexes are distinguishable in field by their color: males have a dark colored throat and chest, whereas females and juveniles have a white throat and belly (Savage 2002, Summers 2000). All observed frogs in the area were caught, photographed, identified and recognized by their unique coloration patterns on the body side.

The study area encompasses a fallow cacao plantation within secondary lowland rainforest adjacent to the tropical field station La Gamba (N 8°42’03.7”, W 83°12’06.1”) and the national park “Piedras Blancas”, Costa Rica. The selected plot was a flat low area bordering a creek on one side and a steep slope on the other.
Translocation procedure

The present data was collected over two field experiment periods during the beginning of the rainy season 2012 and 2013. Long-distance translocations were carried out from 2 – 31 August 2013. We located the territorial frogs by their advertisement calls and captured a total of 35 males. Calling was often stimulated by playing a recording of a male advertisement call close to supposed territories. Capture locations were recorded with a GPS device (MobileMapper™ 6, SpectraPrecision, Westminster, CO, USA) using a mobile GIS software ArcPad™ 10.0 (ESRI, Redlands, CA, USA) and marked with a flagging tape. To confirm male territoriality, we attempted to recapture them within the next few days at the marked initial capture sites. Confirmed territorial males were captured in an airtight plastic bag, placed in an opaque container and rotated. Two groups of five individuals each were translocated 200 m away from their territories into two different directions. One group was released along the creek bordering the study area, the other one up the hill and away from the creek. Translocations were done on 3 and 4 August. The release sites were marked and recorded on a GPS device. To quantify the recapture potential without translocation, we rotated and released eleven territorial males in their territory (0 m) after the first recapture. Additionally, five of the control group frogs were translocated across the creek (40 m to 70 m away from the territory) to test if creeks constitute an obstacle for homing. A closely related A. femoralis typically avoids crossing running water and will make detours to find a terrestrial crossing point even over small and shallow creeks (A. Pašukonis, unpublished data). The creek in our experimental area was three to five meters wide and it did not provide any terrestrial crossing possibilities.

To quantify the homing ability of translocated frogs and the recapture success within the control group, we attempted to recapture the translocated and control males back in their territory. The territories of all manipulated frogs were checked several times a day for ten days or until recapture. The side coloration pattern of each captured frog was compared to the digital images. Sampling was paused from 12 – 17 August and continued until 31 August 2013 by checking the territories once a day between 9:30 and 10:00.

Data for short-distance translocations (20 m) was obtained from a previous study by L. Kopeinig (unpublished data). Her observations took place from 21 July to 16 August 2012. No GPS data was recorded. Twelve frogs were translocated to 20 m, then four of them to 50 m, and one of them to 100 m. However, there are some important experimental procedure differences between the two datasets. In 2012, same individuals were repeatedly translocated to each cardinal direction for each distance starting from 20 m towards West. Only the frogs that returned four times from 20 m (once from each cardinal direction) were consequently translocated to 50 m and so forth. Individuals were not rotated before translocation. For the
best possible comparison, only the first translocation to 20 m for each individual was used to compare with the dataset from 2013.

Data analysis

Returnees which were recorded after the sampling break were considered as outliers (3 individuals) because their return time estimates were not accurate. We included them in the analysis, but to point out the difference, we displayed results including and excluding the frogs recaptured after the regular sampling period of 10 days. All statistical analyses were performed in “R” (Team R.D.C., 2008).

Results

Seventy seven percent of the translocated frogs returned to their home territories within 21 days after the translocation (Table 1). All of the recaptured control frogs were recorded inside their home territories within a maximum of seven days. Seven out of ten frogs translocated over 200 m returned to their home territories. Out of these, three returned back from the hillside and four along the creek. Ten out of twelve frogs translocated for 20 m returned to their territories. Return time for 200 m translocated frogs varied from 3 to 21 d ($\bar{x} = 11.14$, SD = 7.82). Control frogs were recaptured within 7 d ($\bar{x} = 4$, SD = 1.9). Return time for 20 m translocated frogs varied from 1 to 12 d ($\bar{x} = 4.2$, SD = 3.3) (Table 1 and Fig. 1). There was no observable difference between the recapture time of control group and frogs translocated for 20 m (Wilcoxon rank sum test: $W = 47.5$, $p = 0.87$). The sample size of frogs returned from 200 m was too small for a statistical comparison.

The recapture success of all groups was similar (Table 2). We recaptured 81.8 % of control frogs and the total homing success was 77.3 %. There was no detectable difference between recapture success of control group and either of translocated frog groups (Fisher’s exact test: $p = 0.76$). Out of five frogs translocated over the creek, one returned during our sampling period. Overall, we found returnees from all three directions, which were included in the experiment.

Discussion

Our results show a high homing performance in *A. talamancae* males translocated for 20 m and 200 m. Taken together with similar findings in *A. femoralis*, it demonstrates that high homing performance is characteristic of the genus *Allbobates*. There was no significant
difference between the recapture time of the control group and the frogs translocated for 20 m suggesting that frogs returned as fast as we could possibly detect given our overall recapture potential. Further, the mean return time did not vary strongly between the translocation distances; however, there was a much bigger variation in the return time from far away. Finally, one translocated frog overcame the creek demonstrating the ability to cross such obstacles.

The recapture success of the control group (81.8%) was comparable to the homing success of the translocated frogs (77.3%) indicating that more individuals might have returned but were not sampled. Further, the similarity to the recapture rate of control frogs shows that they returned at the highest rate we could detect with our sampling methods. Alternatively, some non-territorial males were included in the sample or shifted their territories during the sampling period. Overall homing success is comparable to that found in a larger dendrobatid frog, *A. femoralis* (Pašukonis et al. 2013). Smaller body size of *A. talamancae* (mean SVL *A. talamancae* = 20.5 mm cf., Cope, 1875; mean SVL *A. femoralis* = 26.8 mm cf. Roithmair, 1992) does not appear to be a limiting factor for the long-distance homing ability. Interestingly, the recapture potential of the control group was lower in *A. talamancae* (81.8% recaptured, mean recapture time 4 days) than in *A. femoralis* (100% recapture, mean recapture time 1.4 days) even though *A. femoralis* territories were sampled only once a day. This difference might reflect a higher site fidelity and stronger territoriality in *A. femoralis*, especially as territorial males were usually detected by stimulating with a playback. We may have failed to detect the presence of a male due to a lack of reaction to the playback. Since males returned from 200 m almost as successfully as from 20 m, the maximum homing distance for *A. talamancae* remains unknown. Homing success of *A. femoralis* males decreases steeply from distances longer than 200 m (Pašukonis et al. 2013), which corresponds to the maximum tadpole transport distances in this species (Ringler et al. 2013). Tadpole transport distances for *A. talamancae*, as for most other dendrobatid frogs, remain unknown.

There was also a strong variation of recapture time between individuals translocated over the same distance with a large overlap between the distances. Some individuals took as long as 11 days to return from 20 m while others returned in three days from 200 m. Similar findings were reported for *A. femoralis* (Pašukonis et al. 2014a). Tracking experiments in *A. femoralis* showed that homing consisted of up to several days of immobility and shorter periods of rapid movements (Pašukonis et al. 2014a). Translocations with *A. femoralis* were done over longer periods and part of the variation could be explained by changing weather conditions. We translocated all males simultaneously for 200 m so the weather conditions cannot explain the observed variation. These results indicate individual differences in homing
behaviour. The large variation in return times could be due to different physical conditions, experience and motivational states of individuals. Weaker individuals might travel slower and pause more often for foraging. In addition, males having clutches to attend should be more motivated and move faster. If *A. talamancae* rely on experience for orientation, different experience of individuals with a given area might also explain the variation in return time. Furthermore, terrain structure and the specific path chosen could affect return time and ability. While highly structured areas could increase the duration, areas that fit better to the male’s needs could also interrupt or even stop homing behaviour.

A recent study revealed that *A. femoralis* relies on experience for successful homing (Pašukonis et al. 2014a). Movements during tadpole transport or juvenile dispersal could lead to a learned spatial map used for orientation. In other amphibian species, translocation experiments also suggest the role of familiarity with an area on homing ability (reviewed in Sinsch, 2006). Tadpole transport potentially requires a good knowledge of water bodies to carry offspring to. Pašukonis et al. (2014a) reported that *A. femoralis* shows the highest homing success in amphibians. Our results suggest that high homing performance is characteristic of genus *Allobates*. This ability could be linked to regular tadpole shuttling between home territories and extraterritorial deposition sites, which is common in this genus. More comparative translocation studies of dendrobatid frogs may reveal the link between such natural history traits and the homing performance.

**Acknowledgements**

We want to thank Stefanie Jernej, Daniel Philippi, Dennis Kollarits, Laura Terzic, and Christian Wappl for their help in the field. Special thanks goes to the whole team of the tropical field station La Gamba, Costa Rica. The data in 2013 was collected during a field course supported by the University of Vienna and we are grateful to Max Ringler and Walter Hödl for making it possible. The work was also supported by Austrian Science Fund (FWF) project W1234-G17. The experiments were conducted in accordance with the guidelines of the Association for the Study of Animal Behaviour (ASAB) for the treatment of animals in behavioural research and teaching.

**Authors’ contributions**

AP designed the experiment. CP, SW, LK, and AP collected the data in the field. CP, SW, SJ analysed the data. CP, SW and AP wrote the manuscript.
References


Figures and tables

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Table 1: Summary statistics for recapture time (days) of translocated territorial *A. talmancae*.

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Table 2: Male *A. talmancae* homing success after experimental translocation.
Figure 1: Recapture time in days of frogs translocated for 0 m (control), 20 m, and 200 m from their territories. Results for 200 m are shown excluding and including three outlier points (17, 20 and 21 days). Black boxplot bars indicate the median and x-symbols indicate the mean.
Chapter 6

This chapter consists of an early-stage manuscript in preparation for a peer-reviewed journal.
Long-distance parental movements and spatial orientation in a Neotropical territorial frog *Ameerega trivittata* (Dendrobatidae)

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Abstract

Understanding amphibian movement patterns and orientation mechanisms has been among the top challenges in herpetology. These questions have mainly been studied in nocturnal amphibians of temperate regions, especially in Salamandridae and Bufonidae, but some of the most complex spatial behaviors are found in tropical frogs. Poison frogs (Dendrobatidae) are a group of diurnal Neotropical frogs characterized by territoriality and tadpole transport. We recently showed that *Allobates femoralis*, a small dendrobatid frog with paternal tadpole transport, successfully returns to the home territories after experimental translocation of up to 400 m. Tracking of translocated *A. femoralis* revealed a remarkable precision during homing. Further, these frogs rely on experience with the area to find their way back. There is some evidence that this ability is present in other territorial dendrobatids but limited to shorter distances.

*Ameerega trivittata* is one of the largest dendrobatids with similar reproductive behavior but with larger territories than *A. femoralis*. The larger body size of this species provides a unique opportunity to use radio transmitters for quantifying homing trajectories and study movement patterns over long distances. We used radio-telemetry to track tadpole-transporting male *A. trivittata* and carried out translocation experiments with territorial individuals. We hypothesize that homing ability of male *A. trivittata* is related to the distances covered naturally during tadpole transport. Tracked males traveled several hundred meters during tadpole transport (maximum of 364 m one-way straight-line distance). Supporting our initial hypothesis, translocated males were able to orient and return to their territory from as far as 792 m. We suggest that dendrobatid frogs use their experience acquired during exploratory behavior necessary to find the deposition sites to flexibly find the way back home after translocations.
Introduction

How seemingly sedentary amphibians accomplish long-distance directed movements, such as mass spring migration in temperate regions has been subject to scientific debate for over a hundred years (Abbott 1884; Boulenger 1912; Cummings 1912), but our knowledge is still rather limited. A tendency to move towards and to relocate home or breeding sites after experimental removal has been observed in many amphibians (Bogert 1947; Jameson 1957; Twitty et al. 1964; Dole 1968; e.g., van Nuland & Claus 1981; Stouffer et al. 1983). Even though the importance of certain sensory modalities has been revealed for some temperate-region amphibians (Ferguson 1971; Ishii et al. 1995; Fischer et al. 2001; Sinsch 2006), the exact homing trajectories have rarely been quantified (but see Matthews 2003), and thus it remains unknown how these cues guide the behavior. Results of several translocation studies point toward the importance of familiarity with an area for homing ability (Oldham 1967; Sinsch 2007; e.g., Sinsch & Kirst in press), but direct evidence is very scarce (but see Pašukonis et al. 2014b). Further, the extent of natural movements of most amphibians remains unknown and largely underestimated (Pittman et al. 2014; Sinsch 2014). The miniaturization of radio transmitters has strongly expanded the range of species suitable for telemetry, including many amphibians (van Nuland & Claus 1981; Stouffer et al. 1983; Rowley & Alford 2007). Surprisingly, telemetry techniques have rarely been used to gain new insights into amphibian spatial orientation.

Research on homing behavior, as well as natural movement patterns has focused almost exclusively on nocturnal, pond breeding amphibians of temperate regions, especially in the families of Salamandridae (e.g., Grant et al. 1968; Schabetsberger et al. 2004), Bufonidae (e.g., Sinsch 1987; Miaud et al. 2000) and Ranidae (e.g., Oldham 1967; Matthews & Pope 1999). The vast majority of these studies represent only a few of amphibian reproductive modes, such as explosive and prolonged pond breeders. A variety of complex reproductive behaviors, such as territoriality, terrestrial breeding, and parental care are much more common in the tropical regions. It follows that the associated spatial behaviors must be as diverse but they remain largely unstudied (but see Seebacher & Alford 1999; Schwarzkopf & Alford 2002). While most temperate-region amphibians exploit relatively stable breeding resources, such as large ponds, tropical amphibians usually depend on fluctuating ephemeral resources such as temporary forest pools.

Poison frogs (Dendrobatidae) are a group of diurnal Neotropical frogs characterized by territoriality and parental care, such as tadpole transport (Weygoldt 1987; Ringler et al. 2013). Despite the fact that dendrobatid parental care has been extensively studied (Weygoldt 1987; Summers et al. 2006; Brown 2013), very little is known about the related spatial
behaviors. We recently showed that *Allobates femoralis*, a small dendrobatid frog with paternal tadpole transport, successfully returns to the home territories after experimental translocations of up to 400 m (Pašukonis et al. 2013). Tracking experiments revealed remarkable precision during homing and initial orientation after translocations (Pašukonis et al. 2014a). Further, we could show that *A. femoralis* relies on experience with an area for successful orientation (Pašukonis et al. 2014b).

The return success of tranlocated male *A. femoralis* is very high for distances up to 200 m but it steeply drops off for longer distances. Interestingly, 200 m correspond to the maximum distances covered by tadpole transporting adults (Ringler et al. 2013) indicating the ecological relevance of such homing ability. Further, the experience required for accurate orientation might be gathered during the tadpole transport or exploratory movements while looking for deposition sites. Homing success in a closely related and ecologically very similar dendrobatid *Allobates talamancae* revealed comparatively high homing success from up to 200 m (chapter 5). However, a more distantly related dendrobatid *Oophaga pumilio*, unlike *A. femoralis* and *A. talamancae* do not perform as well after translocations (McVey et al. 1981; Nowakowski et al. 2012). Even though comparable data are lacking, *Oophaga pumilio* seems to have smaller territories and shorter tadpole transport distances (Pröhl & Berke 2001) in comparison to *Allobates* species. These findings suggest that the homing ability is widespread in dendrobatids, but the distances from which the frogs are able to orient depend on natural history traits such as the territory size and the tadpole transport distances. We hypothesize that orientation is dependent on previous experience with an area acquired during dispersal, territory establishment and tadpole transport. Species with larger territories and longer tadpole transport distances have a possibility to explore and learn landmarks in a larger area and will orient better after translocations of longer distances.

*Ameerega trivittata* is a large dendrobatid frog with similar reproductive behavior but not closely related to *A. femoralis* within the dendrobatid family (Grant et al. 2006). Males have larger territory sizes than *A. femoralis*, and transport larger amounts of tadpoles to terrestrial deposition sites (Roithmair 1994a). The transport distances and the associated movement patterns are unknown. Larger body size of this species provides a unique opportunity to use radio transmitters for obtaining homing trajectories and to study movement patterns over long distances. We tracked tadpole-transporting male *A. trivittata* and carried out translocation experiments with territorial individuals. We quantified the homing success and movement trajectories using radio-telemetry. We hypothesize that homing ability of male *A. trivittata* is related to the distances covered naturally during the tadpole transport.
Materials and Methods

Study species
We studied movement and orientation behavior of male *A. trivittata*, one of the largest (mean male SVL = 39 mm) and most widely distributed dendrobatid frogs (Amézquita *et al.* 2006). Males vocally advertise and defend large territories on the forest floor (residency time up to 116 days, territory size up to 156 m², cf. Roithmair 1994b), where courtship and oviposition takes place. Males have been observed to attend to only one clutch at a time but more data is needed (Roithmair 1994a). After approximately 18 days of larval development, males transport up to 41 tadpoles to temporary aquatic depositions sites outside their territory (Roithmair 1994b; a; Acioli & Neckel-Oliveira 2014; Luiz & Contrera 2015). In our study area, tadpoles were usually deposited in large to medium terrestrial pools such as rain flooded depressions and partially dried creek beds (personal observation). Like the majority of dendrobatid frogs, *A. trivittata* is diurnal, however, most calling activity takes place early in the morning between 04 40 h and 09 00 h (Roithmair 1994b). Individuals can be recognized by their distinct coloration patterns (personal observation).

Study site
The data were collected around the onset of the rainy season from 15 October - 18 November 2014, at the Panguana biological field station on the lower Rio Llullapichis, Amazonian Peru (9°35’S, 74°48’W). The study area consists of lowland rainforest with a moderate relief, intersected by several trails and creeks, and bordering a pastureland on one side. This area was chosen for the high density of calling *A. trivittata* as well as for accessibility necessary to follow the moving frogs. The undergrowth varied from thick liana forest to relatively open mature closed-canopy forest. We mapped the creeks, trails, and the forest edge of our study area by averaging multiple GPS readings (device used: MobileMapper 10; Ashtech/Spectra Precision) taken on a cloudless days.

Tracking equipment
We used miniature radio transmitters (BD-2X from Holohil Systems Ltd. and NanoTag series from Lotek Wireless Inc.). Transmitters were attached externally, using thin medical quality silicon tubing. The transmitter was positioned dorsally and the waistband was secured at the thinnest part of the waist. An additional strand of silicone tubing between the legs prevented the waistband from rotating (Fig. 1). Initially, a few frogs developed skin abrasion from the waistbands (which were immediately removed). The size of the waistbands was adjusted accordingly and no injuries were observed after. The radio transmitters together
with the attachment weighed between 0.38 and 0.5 g. In our sample, territorial males weighted on average 5.6 g (range: 4.5 – 6.8 g) and the tags were chosen so that they never accounted for more than 10 % of the body weight. *Ameerega trivittata* males routinely carry tadpole loads above 10 % of their body weight, and thus we assume they are adapted to withstand such weights. We used a portable radio tracking receiver (Sika, Biotrack Ldt.), a Yagi-antenna and a hand-held machete to follow and locate the frogs in the field.

**Tadpole transport**

During the study period, we opportunistically captured nine tadpole-transporting males. Before release, we counted the number of tadpoles, weighted, photographed on a millimeter paper, and equipped the frog with a radio transmitter. Captured frogs were kept in a net-cage at the capture site between 15 min and 2 h 45 min, before being tagged and released. After release, we relocated the frog three to seven times a day during daylight hours (5:30 to 18:30). The exact number of relocations per day depended on the accessibility of the location and the number of frogs being tracked simultaneously. We attempted to visually spot each individual, which was not always possible when the frog was hiding or in very dense vegetation. Alternatively, we attempted to triangulate the location as closely as possible. We visually spotted the males at least once a day. When possible, we recorded the frog’s behavior and the approximate number of tadpoles remaining. Movements longer than approximately 15 m were recorded by averaging 30 GPS readings. For smaller scale movements that would not be well represented via GPS signal, we measured the distance and the direction from the previous location. All data were recorded and stored in the GIS software ArcPAD 10 (ESRI) using a handheld computer/GPS device (MobileMapper 10; Ashtech/Spectra Precision). We attempted to track the frogs until tadpole deposition and back to the territory (total tracking time 1 - 11 days). After tadpole deposition, we assumed the frog to be back at its territory if it showed no directional movement for three days (n = 6). One individual was predated by a snake after tadpole deposition. Tracking was also terminated if any signs of skin abrasions were noticed (n = 2).

**Translocations from territories**

We scanned the area for calling males during the peak calling time. Males were identified as territorial if they were observed calling in the same area on at least two different days. Twenty-four selected males were captured and equipped with a short range tracking device (harmonic-direction finder, for details see Mascanzoni & Wallin 1986; Pašukonis *et al.* 2014a) and released for an observation period of 1 - 7 days. During this period, we checked the fit of the waistband, observed territorial behavior and local movements. Tags were
removed if any potential signs of wear or distress behavior (e.g., extensive hiding periods) were noticed. Between 25 October and 6 November, 12 of the previously tagged individuals were equipped with radio transmitters and translocated 203 m - 792 m (mean = 476 m, SD = 198 m) away from their territory northwards or southwards (Fig. 2). Single individuals were placed in an airtight opaque container. The container was rotated multiple times and brought to the release site by an indirect path. Translocation direction and the exact release sites were chosen based on the relative accessibility of area for tracking and avoiding releasing the frogs very close to the forest edge. After release, the frogs were relocated 4 to 6 times a day following the same procedure as for tadpole-carrier tracking. We attempted to track the frogs until the return to immediate vicinity of the capture point (i.e., home territory). The tracking of two individuals was terminated by a malfunction and loss of transmitter after 7 and 12 days respectively. One frog was still near the release site after 7 days. We untagged and returned this individual back to its territory.

Data analysis

Visualization of trajectories, extraction of coordinates and distance measurements were done in the GIS software ArcGIS 10 (ESRI). For the estimation of maximum tadpole transport distances, we measured the straight-line distance between the two most distant points within one tadpole transport event. One point usually represented the estimated home territory or the capture location, if the tracking was terminated prematurely. The second point represented the furthest tadpole deposition site recorded. One frog was excluded because it did not show any movements before we noticed an abrasion caused by a bad fitting of the waistband. Another individual was excluded because the tadpoles disappeared without any potential deposition site in the vicinity. For better visualization of homing trajectories, we normalized all trajectories by shift and rotation to a common release point and a single homeward bearing at 0°. To test for initial homeward orientation, we measured the bearing between the release point and two points early in the movement trajectory. The two points were the closest positions available from the release site in 5 - 20 m and 20 - 50 m range. Individuals that did not have a recorded position within these distance ranges were excluded from the respective analysis. Significant homeward orientation was tested using Rayleigh’s test for unimodal distribution of angles. The Rayleigh test was performed with a circular statistics program Oriana 4.02 (Kovach Computing Services, Pentraeth, Wales, UK).

Permits and ethical statement

The study was approved within the projects of the Panguana research station granted to Dr. Juliane Diller. The experiments were conducted in strict accordance to the European
and Peruvian law and following the guidelines of the Association for the Study of Animal Behaviour (ASAB) for the treatment of animals in behavioral research and teaching.

Results

Tadpole transport

We tracked 9 individuals on 10 tadpole transport events. Frogs were transporting between 15 and 32 (mean = 22.1, SD = 6.2) tadpoles. Some males distributed tadpoles over several deposition sites. The maximum straight-line distances that the frogs traveled during tracking were between 131 m and 364 m (mean = 223 m, SD = 96 m).

Homing

Ten out of 12 translocated males returned to their home territory during our observation period. Males took between 3 and 12 days (mean = 7.3 days, SD = 2.8 days) and returned from all translocation distances. The maximum return speed was 3 days from 702 m. In general, males spent up to several days near the release sites and then returned in a direct manner (Fig. 3). Frogs showed a tendency to move homewards at 5 - 20 m (expected bearing = 0°, Mean vector = 300.5°, 95% CI = 255° – 346°, Rayleigh-test p = 0.05, n = 10) and a strong homewards orientation at 20 - 50 m (expected bearing = 0°, Mean vector = 349°, 95% CI = 317° – 22°, Rayleigh-test p < 0.003, n = 7) from the release site.

Discussion

Our study revealed surprisingly long distances traveled by tadpole-transporting male *A. trivittata*. Supporting our initial hypothesis, translocated males were able to orient and return to their territory from longer distances with higher accuracy than most amphibians studied so far. Territorial males returned from the longest translocation distances (792 m) so the maximum homing distances for some individuals are likely to be even higher. To the best of our knowledge, it is the first study that uses telemetry to quantify tadpole transport distances in dendrobatid frogs. It is also one of very few studies to quantify movement patterns of tropical amphibians with telemetry in general (also see Schwarzkopf & Alford 2002; Brown et al. 2006).

While many amphibians move within a range of several hundred meters during their lifetime, most of such movements reported so far consist of seasonal movements, dispersal and rare events by single individuals (Sinsch 2014). In contrast, *A. trivittata*, like most dendrobatid frogs, routinely shuttle the tadpoles from their territory to widely distributed
deposition sites. *Allobates femoralis* is the only other dendrobatid species where tadpole transport distance have been quantified albeit by capture-mark-recapture (Ringler et al. 2013) or by visual tracking in experimental population (chapter 4) and not under completely natural conditions. The tadpole transport distances we observed 364 m (mean = 223 m, max = 364 m, SD = 96 m) are approximate two times longer than the maximum (185 m cf. Ringler et al. 2013; 144 m cf. chapter 4) and four times longer than the average (28 m cf. Ringler et al. 2013; 55 m cf. chapter 4) distances reported for *A. femoralis*. Concordantly, the ability to orient and home back after experimental translocations extends to distances at least two times longer than in *A. femoralis*. The link between orientation ability and tadpole transport distances suggests the ecological relevance of such highly developed homing ability of some dendrobatid species. In both *A. femoralis* and *A. trivittata* the maximum tadpole transport distances seem to be far below the distances from which the frogs are able to home. However, our sample size for *A. trivittata* is small (9 individuals) and the maximum distances observed are likely to increase with larger samples size. Furthermore, we tracked individuals for no more than two weeks of their lifetime. The sum of movements during dispersal, within- and between-season territorial shifts, and all tadpole transport and exploration will likely include much larger areas. We suggest that dendrobatid frogs use their experience acquired during exploratory behavior necessary to find the deposition sites to flexibly find the way back home after translocations.

Learned cues can be used as simple beacons or integrated in spatial representations of varying levels of complexity (Jacobs 2012). The flexible ability to choose the nearly shortest path home from arbitrary release points in both *A. trivittata* (this study) and *A. femoralis* (chapters 2 and 3) is suggestive of map-like understanding of the area. Beaconing is based on a direct sensory contact with cues originating from the goal. It has been suggested as a potential orientation mechanism for several amphibians migrating to and away from large breeding ponds in temperate regions (Grubb 1973; Joly & Miaud 1993; Sinsch 2007). Direct visual, olfactory, or acoustic beaconing is very unlikely over hundreds of meters in dense rain forest with complex relief. Map-like behavior under natural conditions has been mostly studied in open spaces, such as birds and honeybees in flight or sea turtles in open water (Jacobs & Schenk 2003; Lohmann et al. 2007; Cheeseman et al. 2014), where large visual, olfactory, or magnetic gradients are available. Little is known about how animals find their way in the understory of the rain forest where many cues, such as distant landmarks are limited. Magnetic gradients cannot explain accurate orientation on such relatively small scale (Phillips 1996). Visibility can be very limited for the frogs at their release site (Fig. 4), however most individuals are able to choose correct initial direction with very little searching movement. Both acoustic and olfactory cues are abundant in complex habitats and anuran
amphibians rely heavily on both sensory domains at different stages of their life (Sinsch 1987; Gerhardt & Huber 2002; Byrne & Keogh 2007; Schulte et al. 2011). We hypothesize that dendrobatid frogs are able to integrate such extended acoustic and/or olfactory landmarks in a flexible spatial map.

Our study provides limited but important insights into the complexity of movement patterns and orientation mechanisms of tropical amphibians. It also demonstrates the suitability of miniature radio-transmitters for quantifying the movement patterns of small amphibians in complex habitats, such as the rain forest. We believe that tropical anurans with their diversity of spatial and social behaviors are an extremely promising group for studying the evolution of spatial strategies and the associated cognitive mechanisms. Finally, in the current context of dramatic amphibian decline, the knowledge of natural movement scales and patterns is of primary importance for effective conservation measures and planning.

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Authors’ contributions

AP designed the experiment, collected, analyzed, and interpreted the data and drafted the manuscript. ML and WH collected the data and participated in the experimental design. All authors read and approved the final manuscript.

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Figures

Figure 1. Photograph of a male *A. trivittata* transporting 30 tadpoles while wearing a miniature radio transmitter attached with a waistband made out of fine silicone tubing.
Figure 2. Map of the study area showing the territories (colored circles) and the release sites (arrowheads) of 12 translocated males. Area filled with “tussock” represents the pastureland. Blue lines represent the creeks. Camp symbol represent the field station.
Figure 3. Polar plot showing homing trajectories over 800 m of the nine translocated frogs for which full homing trajectories were recorded. All trajectories are release site (center of the plot) and home direction (0°) normalized. Each line corresponds to a different individual.
Figure 4. Photographic panorama-view from one of the successful homing trajectory points showing the complexity and the density of the habitat structure. A) Photograph taken from higher-end perching height of a calling *A. trivittata* (approximately 1 m). B) Photograph taken from a usual perching height of a moving *A. trivittata* (approximately 0.3 m).
General discussion

In this thesis I document the outstanding way-finding abilities of the Neotropical poison frogs in their complex natural environment and point towards the potential cognitive mechanisms underlying this behavior. I found that learning guides the natural movements of the dendrobatid frogs during their parental duties (i.e. tadpole transport). Furthermore, they can use their experience to find the direct way back to their territory after experimental translocations. Both natural distances covered during the tadpole transport and the range of their homing ability are far more expansive than previously reported specifically for dendrobatid frogs as well as for most amphibians in general. Our studies highlight the suitability of small tropical frogs for studying and understanding the complex movements and cognitive abilities underlying animal spatial strategies in complex habitats.

In his paper titled “The intelligence of the batrachians” Abbott (1884) remarks: “Frogs, as a class, are not migratory. They frequent a given pond or stream; and, sustained by the insect-life that comes to them but is not sought, they pass an eventless life, trusting, as it were, to luck. Such an existence requires no intellectual exertion, and none is made.” While taken from a different era and slightly out of context (Abbott acknowledged the “superior intelligence” of toads), this quote illustrates both the evolutionary approach to cognition and the misconceptions about amphibian behavior that still prevail today. Amphibians show some of the most diverse reproductive behaviors among vertebrates and many aspects of their social and spatial behavior are likely to rely on or be fine-tuned by experience. However, learning experiments with amphibians are still scarce and the spatial behaviors of most species still remain to be quantified. As has been pointed out in the experimental psychology literature (Thompson & Boice 1975; Suboski 1992), it can be particularly difficult to find a motivating reward, appropriate naturalistic context, and easily interpretable behaviors suitable for designing experiments with amphibians. Spatial behavior is particularly suitable for naturalistic experiments on amphibian cognition as well as for understanding the role of cognition in the wild. For example, tadpole transport behavior of dendrobatid frogs can be easily replicated under controlled experimental conditions as well as observed and manipulated in the field (Ringler et al. in press). Recent empirical and conceptual advances in research on animal movement and spatial cognition are providing increasingly powerful frameworks for understanding the proximate and ultimate causes (Tinbergen 1963) of spatial behavior (Nathan et al. 2008; Jacobs & Menzel 2014).

While the findings presented in this thesis clearly demonstrate the importance of experience for poison frog way-finding, it remains completely unknown which sensory modalities are necessary and/or sufficient for poison frog orientation. The scale and the accuracy of poison frog spatial orientation in their complex environment challenge the current
knowledge of the sensory basis of amphibian orientation. Magnetic map sense has been implicated in long-distance homing of several species (Lohmann et al. 2007), including one newt species (*Notophthalmus viridescens*, Fischer et al. 2001), but the shallow magnetic gradients of the earth’s magnetic field could not account for the observed orientation precision of most amphibians under relatively short distances (Phillips 1996; Sinsch 2006).

Dendrobatid frogs are unique among amphibians in their exclusively diurnal activity. This would suggest a strongly developed visual system but the dense rainforest limits the visibility of distant landmarks, especially at ground level (see chapter 6, Fig. 4). I maintain that visual landmarks could play part in small-scale movements and recognition of specific locations (e.g., home territory), but that it does not explain the long-distance homing abilities. In many cases, translocated frogs spend a considerable amount of time alert (up to several days) but immobile near the release site. I have observed translocated *A. femoralis* perched on higher structures (approximately 1 m) than is usual for this terrestrial species, but in general they showed very little searching behavior, which would be expected if they depended on visual landmarks. To me, the long waiting times and exposed perches are suggestive of extended cue perception, such as odor or sound. The long waiting times may indicate that animals might need to identify cues that are only temporarily available (e.g., certain time of the day, weather conditions) or accumulate and integrate the necessary cues for orientation. Olfactory cues have been shown to play a role in the orientation of several nocturnal temperate-region anurans (e.g., Grubb 1973; Sinsch 1987; Ishii et al. 1995). Olfaction has also been suggested as the ancestral sensory orientation modality for mammals and birds (Jacobs 2012). Results of our tadpole deposition site manipulations (chapter 4) and recent research on antipredatory strategies have shown that dendrobatid frogs are sensitive to olfactory cues at least over short distances (Schulte et al. 2011; Schulte & Lötters 2013). It remains unclear whether perceivable stable environmental odors exist and can be used as extended landmarks at the understory of the rainforest. Sound is the primary domain of communication in anurans. Some species use the sound of breeding chorus as an orientation beacon at long ranges (e.g., Gerhardt & Klump 1988; Bee 2007; but see Murphy 2003; Buxton et al. 2015). In close range, both females and males across species use conspecific calls as acoustic beacons to home in on for mating or aggressive interactions (Wells 1977; Ursprung et al. 2009). Females often navigate noisy breeding choruses by resolving spectral and temporal acoustic patterns to localize the best mate with great spatial accuracy (Bee 2012). Simple beaconing mechanisms, however, are unlikely to explain the flexibility and the scale of poison frog spatial orientation. In theory, a navigator could use a sound source not only for direct guidance but also as an extended spatial landmark. Multiple sound sources could then form an acoustic mosaic map, similar to the one proposed for local odor navigation.
in birds (Papi et al. 1972). I hypothesize that poison frogs integrate acoustics and/or olfactory landmarks into map-like representations of the area. In addition, local visual landmarks could be used for place recognition. Further research is needed to understand the sensory basis and the flexibility of poison frog spatial representations.

The results and interpretations presented in this thesis are based exclusively on experiments and observations of male poison frogs. This raises the question whether the abilities of male poison frogs are unique or, instead, typical of all dendrobatids and maybe of amphibians more generally. I chose to study only male spatial behavior because males are predominantly the ones performing tadpole transport and displaying territoriality in our study species. Strong male territoriality allowed us to have a better control of individuals’ motivation for homing during translocation experiments and provided a reference for the origin of movements during tadpole transport. However, females show territoriality and/or tadpole transport in several dendrobatid species (e.g., Oophaga pumilio, Weygoldt 1980; Stynoski 2009). Additionally, recent findings suggest that parental duties of poison frogs might be much more flexible than previously thought (Ringler et al. in press): the females of our main study species, A. femoralis, take over the tadpole transport when males disappear. We have also observed tadpole-transporting females using the artificial deposition sites and visiting removed pool sites during the experiments. These anecdotal observations suggest that females know about the deposition sites available even though they use them much less frequently than males. Females mate with multiple males in an area with an approximately 20 m-radius (Ursprung et al. 2011), and they most likely use males’ advertisement calls as beacons to find territory owners. Males will also mate with multiple females and take care of several clutches at the same time. It remains unknown how females keep track of their various clutches with different mating partners and recognize when one of the males is missing, but spatial and other complex learning processes are likely to play a role. Similarities and differences of male and female spatial cognition in dendrobatid frogs is a very promising topic for future research.

It is tempting to conclude that dendrobatid frogs show unique cognitive abilities that have been selected by their social environment, complex habitat, and dispersed fluctuating reproductive resources. However, jumping to such conclusions is premature because the cognitive abilities and spatial behavior of most amphibians, especially in the tropics, remain unknown. Many other tropical (e.g., genus Engystomops, Marsh et al. 1999), as well as some temperate-region (e.g., Bombina variegata) amphibians rely on widely dispersed ephemeral resources for reproductions. These species constantly face a trade-off between learning and re-using known resources close by vs. exploring for potentially better ones. The ephemeral nature of small water bodies should promote a high level of behavioral flexibility and
complex spatial strategies. Future comparative research between species relying on stable predictable resources, such as large temperate-region ponds, and small fluctuating resources, will provide insights into how resource distribution or even geographical regions can shape animal cognition.

The ability to quantify individual animal movements is essential for our understanding of animal spatial strategies and cognitive mechanisms behind them. However, tracking most small animals in complex habitats, such as amphibians in the tropical rain forest, remains difficult. Major challenges for tracking tropical amphibians include their small size, sensitive skin, secretive (often nocturnal) habits and their highly structured, difficult to access habitat. Many amphibian-tracking studies fell short of their goals simply because of inappropriate tracking and external device attachment methods that inhibit animal movements and/or cause injuries (e.g., Grubb 1970; Eggert 2002; McAllister et al. 2004; e.g., Pellet et al. 2006). To avoid the difficulty associated with attaching tags to amphibians, several researchers have used implanted transmitters, but such manipulations are risky in the field and are only suitable for certain large amphibians (e.g., Stouffer et al. 1983; Spieler & Linsenmair 1998; Jehle & Arntzen 2000; Miaud et al. 2000). I have gradually developed safe tag attachment methods using waistbands and harnesses made from lightweight, skin-safe silicone-based materials, which could potentially be used for many small anuran species. By now, I have successfully used this tag attachment method on three Neotropical poison frog species and the animals tagged in this way were observed to engage in all usual behaviors such as moving, feeding, climbing trees, calling, mating, uptaking and transporting tadpoles (Fig. 1).

Figure 1. Photographs of three dendrobatid species illustrating the wide range of natural behaviors during tracking (chapters 3, 4, 6, and unpublished data). From left to right, Allobates femoralis male equipped with a harmonic transponder transporting tadpoles. Dendrobates tinctorius male equipped with miniature radio transmitter in courtship with a female. The courtship resulted in a fertilized clutch. Photographs of A. femoralis and D. tinctorius taken at the Nouragues nature reserve, French Guiana. Territorial male Ameerega trivittata equipped with miniature radio transmitter calling on a perch (Panguana field station, Amazonian Peru). Photographs by A. Pašukonis.
We have successfully used simple visual following, harmonic direction finder, and radio-telemetry to quantify the movement patterns of poison frogs in their natural habitat. However, the scope of our tracking projects was limited by the labor-intensive manual data collection in the field. Even after long periods of extensive data collections in the field, we gained only rather limited knowledge on the natural movements of poison frogs. At the moment, only miniature radio transmitters and transponders used for harmonic direction finding provide a possibility to track local movements of small amphibians for longer periods at a fine spatiotemporal resolution, albeit in a relatively small area. Harmonic direction finding system uses smaller tags without battery life limitations but they offer a very limited detection range, on the order of tens of meters, as opposed to hundreds of meters for radio tags (chapers 2, 3, and 6; Mascanzoni & Wallin 1986; Rowley & Alford 2007). The major limitation of both systems is the manual signal detection process. In order to record the position of an animal, it has to be found in the field using the directionality and the strength of the received signal. Subsequently, the position of an individual can be recorded on a map or using a GPS device. This further introduces a positional error dependent on the GPS signal or the local map accuracy. Therefore, the spatiotemporal resolution of animal movements tracked using these techniques is directly limited by manpower, accessibility of the area, mobility, and activity hours of the species in question. Recent developments in GPS logging techniques are revolutionising the study of larger mobile animals (Kays et al. 2015). However, the GPS loggers small enough for most amphibian species are not likely to be available anywhere in the near future. Presently, automatization of radio tracking techniques is essential for collecting data on local movements from a large number of individuals from a variety of small species in diverse environments. To the best of my knowledge, very few automated modular radio-tracking systems have been developed so far and all of them focus on low spatial resolution movements of large species (Cochran et al. 1965; Kays et al. 2011; Ward et al. 2013). The reported positional error derived from triangulation is usually on the order of tens to a hundred meters, which is not sufficient to quantify the fine scale movements of most terrestrial small animals. The goal for studying fine scale movement patterns of small animals would be to develop a modular automated radio tracking system functioning over a smaller area but with a higher spatial resolution and by using the lightest radio transmitters possible (Fig. 2).
Figure 2. Schematic representation of a hypothetical modular automated tracking system based on a wireless sensor network tracking two poison frogs in the forest. The network is composed of a data collector node and at least two tracking receiver nodes that function by triangulation. Optional receiver nodes (represented by dashed circles) can be added to increase the positional accuracy and to expand the detection range.

Overall, I reached all three main goals outlined for this thesis. I developed suitable tracking techniques for quantifying the movement patterns of small terrestrial amphibians in the field. Using these techniques, I gained insights into the range, the patterns, and orientation accuracy of natural as well as experimentally manipulated movements. Finally, I was able to demonstrate that learning plays a key role in guiding the spatial behaviour of dendrobatid frogs. As an extension of these goals, I suggest three main directions for future research in this area: (1) advancing automated tracking techniques to make them suitable for quantifying movement patterns of small animals at a high spatiotemporal resolution; (2) developing an empirically grounded comparative framework for understanding the diversity of amphibian spatial strategies; (3) designing hypothesis-driven laboratory and field experiments and using neurobiological techniques to understand the cognitive abilities underlying amphibian spatial movements.
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Publication overview and contributions

Thesis chapters

I am the first author of the chapters 1-4, 6. I was responsible for the experimental design, data collection, analysis, interpretation, and writing in these studies. I am the last author of the chapter 5. I was responsible for the experimental design and the supervision of the data collection, analysis, and writing in this study. For the specific contributions of co-authors, see the corresponding manuscripts.

Chapter 1: peer-reviewed and published article.

Chapter 2: peer-reviewed and published article.

Chapter 3: peer-reviewed and published article.

Chapter 4: article in preparation for submission
**Pašukonis, A.**, Trenkwalder, K., Mangione, R., Ringler, M., Ringler, E., Steininger, J., Warrington, I. & Hödl (in prep.). Do poison frogs rely on learning for finding tadpole deposition sites?

Chapter 5: article ready for submission

Chapter 6: article in preparation for submission
Additional co-authored work

In the following studies I was involved either in study design, data collection, data analysis, or have substantially contributed to the data interpretation and writing. For the specific contributions, see the corresponding manuscripts.


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Personal data
Born 27.05.1986 in Vilnius, Lithuania.
Lithuanian citizenship.

Research Interests
(1) Animal movements and spatial cognition
(2) Bioacoustics
(3) Tropical biology

Education
2006 - 2008 Bachelor in Biology, Université Joseph Fourier (Grenoble 1), Grenoble, France
2008 - 2009 Bachelor in Biology, visiting student, University of Washington, Seattle, USA
2009 - 2010 Master’s in Evolution and Ecology, Ecole Normale Supérieure de Paris, Paris, France. Research project “Budgerigar (Melopsittacus undulatus) contact call plasticity: application of sound analysis methods”, supervised by Dr. Timothy F. Wright, New Mexico State University, USA.
2010 - 2011 Master’s in Comparative Ethology (graduation with distinction), Université Paris 13, Paris, France. Research project “Environmental influences on the vocalization of New Zealand’s endemic parrot, the kea (Nestor notabilis)”, supervised by Dr. Ximena Nelson, University of Canterbury, New Zealand.
2011 - 2015 PhD candidate; DK Programme Cognition and Communication, University Vienna, Austria (scheduled graduation 14th September 2015)

Academic activities
2008 - 2009 Undergraduate research project on Permian tetrapod classification supervised by Dr. Christian Sidor, Burke Museum, Seattle, USA
from 2013 Teaching at the University of Vienna: lectures in "Biology of tropical amphibians", field course assistant in Costa Rica 2013 "Tropical biology", field course lecturer in French Guiana 2014, 2015, “GIS field methods, behavioral ecology and diversity of Neotropical amphibians”.

Grants and awards
2009- 2011 French national scholarship for academic achievements
2013 “Nouragues travel grant” from French National Research Agency (CNRS), PI – The role of spatial learning in the orientation and reproductive behavior of a territorial Neotropical frog, Allobates femoralis. French Guiana (January – April 2014)
2014 “Nouragues travel grant” from French National Research Agency (CNRS), collaborator on a joint project with Dr. Mark Bee, University of Minnesota - An experimental test of individual voice recognition in a dart- poison frog, Allobates femoralis. French Guiana (February-March 2015).
Conference contributions

2012
World Herpetology Congress, 08/12 Vancouver, Canada. Poster: The homing frog – high homing performance in a territorial dendrobatid frog Allobates femoralis (Aromobatidae)

2013

2013

2014
European Conference on Behavioural Biology 07/14, Prague, Czech Republic. Oral presentation: Experimental evidence of learned spatial maps in a poison frog, Allobates femoralis.

Publications


Science communication
Illustrated article “Kea fascination”, November 2011, PsittaScene – Magazine of the World Parrot Trust