DIPLOMARBEIT

Titel der Diplomarbeit

Displacement experiments to investigate the orientation and navigation abilities of the common toad *Bufo bufo*

verfasst von

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Contents

Abstract

The common toad *Bufo bufo* is mostly known for its migration behaviour but in contrast to its popularity, little is known how it navigates to its home pond. Current understanding of true navigation is mostly known from birds but recent studies have shown that amphibians, such as the red spotted newt, possess the ability to navigate home from, to them, completely unknown locations. To investigate if the common toad is capable of true navigation 120 toads were tested during their migration period. The toads were collected at a pond in Vienna during their migration and displaced approximately 3 km from the testing site. The toads were tested in an arena to see whether they were able to orientate towards their home pond. To eliminate chance orientations, two identical experiments at different locations were carried out. Each toad was tested again after four days to observe if the orientation behaviour changed. In the arena with only magnetic and celestial cues available the toads failed to orientate homeward and only a weak tendency to keep their former migration direction could be observed.

Keywords

True navigation, magnetic orientation, amphibians, magnetic map hypothesis, arena.

Introduction

History of research on magnetoreception

The ability of birds to navigate home over very long distances has always fascinated people and through research into how birds manage to do this, much information about magnetoreception has been collected during recent years (Wiltschko & Wiltschko 2005, Mouritzen & Ritz 2005, Rodgers & Hore 2008). Before 1970 experiments to train animals for magnetic discrimination failed or the results could not be reproduced (Kirschvink et al. 2001). Since it was proven about forty years ago that birds use magnetoreception for navigation, this phenomenon has triggered a lot of research for similar senses in other species (Rodgers & Hore 2009). Early experiments with birds were all displacement experiments to find out if birds can locate their position and navigate to their home. When it became clear that birds can use compass direction, experiments were made whereby the magnetic field around them was manipulated (Åkesson 1994). After this a large number of experiments with animals with migratory behaviour were carried out. To date magnetoreception has been found in up to fifty species (Maeda et al. 2008). There is evidence for magnetoreception from turtles to insects, amphibians and even micro-organism and fungi. (Fischer et al. 2001, Lohmann et al. 2001, Pazur et al. 2007, Wajnberg et al. 2010).

The earth's magnetic field

The earth has its own magnetic field which is similar to that of a regular bar magnet. It can provide compass information used for orientation but there are also other geomagnetic parameters which can be used for position finding, such as field intensity and inclination (Johnsen & Lohmann 2005). Two parameters which are gradual and non parallel such as the inclination and the field intensity of the earth´s magnetic field can be used for position fixing. This process, called bicoordinate position fixing, is required for true navigation (Griffin 1952). According to the magnetic map hypothesis the orientation is separated into two steps, first the map step where the individual uses parameters to locate itself on an internal map, followed by a second step, the compass step, where the desired direction to the goal is determined now that the current position is known (Munro et al. 1997).
Freake described true navigation as the ability of an organism, displaced to an unfamiliar location, to orient homeward without reference to familiar landmarks, goal-emanating cues, or directional information obtained during the displacement (Freake et al 2006). It is mostly known from birds but there is evidence that animals such as the sea turtle (*Caretta caretta)* and the eastern red-spotted

newt (*Notophthalmus viridescens)* can locate their position and are capable of true navigation (Fischer et al. 2001, Lohmann 2001).

The magnetoreceptor

It is very difficult to detect the magnetoreceptor because the magnetic field passes freely through an animal´s body, hence the receptor could be located anywhere in the animal (Johnsen & Lohmann 2005). The best example for this is the research on birds whereby the receptor was assumed to be in the upper beak of the birds and indeed evidence was found for magnetite particles but newer studies locate another receptor in the right eye of the birds (Wiltschko & Wiltschko 2005, Solov'yov & Greiner 2007, Lau et al. 2012). For most other animals capable of magnetoreception it is unknown where the receptor is located. In newts most of the extraocular photoreceptors are located in the pineal organ or in nearby brain areas (Adler 1976, Foster et al. 1994).

Up to date there are two fundamentally different magnetoreception mechanisms. The first one is the magnetite based mechanism which uses magnetite particles to interfere with the magnetic field and produce mechanic signals (Kirschvink et al. 2001). The second one is the radical pair mechanism in which cryptochrome proteins are activated by light and the magnetic field influences the duration of the activation (Lau et al. 2012). In experiments where newts were exposed only to long-wavelength (>500nm) light a shift of 90° in orientation could be observed compared to the full-spectrum or short-wavelength (<450nm) light (Phillips & Borland 1992). Covering the pineal organ of newts with a filter that blocks long-wavelength light caused them to shift their orientation about 90° leading to the assumption that the receptor is located in the pineal organ or somewhere nearby (Deutschlander et al. 1999). The receptor must be capable of distinguishing very small changes in the inclination and field intensity because compared to birds amphibians move only very small distances.

Magnetoreception in *Bufo bufo*

The common toads are widely known for its migration behaviour but still it is poorly understood which cues they use for orientation. It is known that they use landmarks, olfactory, visual cues but these cues only work for short distance orientation. They possibly use magnetic and celestial parameters but how exactly they navigate over long distances is still unknown. In amphibians most experiments have been carried out with newts or frogs and only few with toads (Sinsch 1987, Freake & Phillips 2005, Schlegel 2008). Until now, the only proof for true navigation in amphibians

is found in the eastern red-spotted newt *Notophthalmus viridescens* (Fischer et al. 2001).
The first evidence that the common toads uses the magnetic field as a cue for orientation was found about thirty years ago (Sinsch 1987). These experiments were made to find out what cues the toad used for orientation and distinguished between olfactory, visually and magnetic cues. There was evidence that a magnet taped on the head of the toad changed its orientation behaviour significantly compared to the control group with a similar non magnetic object. Heusser used displacement experiments to investigate the orientation abilities of the common toad (Heusser 1958)
Previous works at the University of Vienna confirmed the assumption that *Bufo bufo* uses magnetoreception for orientation. Landler showed in his thesis that the orientation behaviour of toads changed if the magnetic field around the arena were reversed (Landler & Gollmann 2011). In these experiments the toads aligned their movement after displacement along an axis similar to their former migration direction. Similar results were found in displacement experiments with the Californian newt *Taricha torosa* (Endler 1970). If toads have a light dependent compass, as is assumed in other amphibians, it is possible that they require sunlight for recalibration and are unable to preform the map step which they need for orientation without light. If the compass is light dependent or they preform the map step only during a specific time at the day they need time to get used to the new location. To avoid this orientation problem in experiments the individuals usually have a day or a week for orientation at the new location after displacement (Phillips et al. 1995, Sinsch 2007). In this work the toads were given four days at the new location, more may have been better but the experiments ran the whole breeding season and the motivation of the individuals to get to the breeding pond may decrease over time. To avoid a bias caused by variable migration motivation over the breeding period the experiments altered between the two testing sites every two days.

The experiments

This work tried to find out more details about the orientation of the common toad *Bufo bufo* and if it is capable of true navigation. For this purpose the toads were moved approximately 3 km away from their home pond to the testing site where they were tested if they can find the direction to their home pond. To avoid that the toads follow a non homing cue which leads them into the same direction as their home pond they were tested at two different locations to ensure that if they show a preferred direction towards home it is not by chance. The locations for the experiments were selected so that the direction to the home pond were clearly different from their former migration direction at the pond. Another reason to make two identical experiments at two different locations

was that if some environmental conditions at the testing site disturb the magnetic field and influence the orientation ability of the toads the second location functions as a backup.
The normal migratory range of the common toad is at its maximum about 3 km so it is very unlikely that the toads knew one of the testing sites or the magnetic conditions in this area. If they have never been to the place before and are still able to orient themselves to their home pond they may be capable of true navigation.
It is widely assumed that toads also could use celestial cues for orientation and therefore the cloudiness, the temperature and the phase of the moon at the testing site were recorded (Sinsch 1987, Heusser 1958).
In Austria the common toad is endangered and therefore only male toads were used for the experiments because *Bufo bufo* is an explosive breeder and so there are enough males available at the pond and the reproduction will not be disturbed (Heusser 1968). During the two weeks of migration more than 1500 toads migrating to the pond could be observed.

Methods

Setup and material

The toads were collected from a permanent pond at Bisamberg, a hill at the border of Vienna with the coordinates latitude: 48.3129416 and longitude: 16.3847351. Only male toads were collected and used for experiments. The testing sites were located south-east and south-west from the home pond of the toads. The toads migrate from all directions to the pond but only the ones heading west were caught for testing.

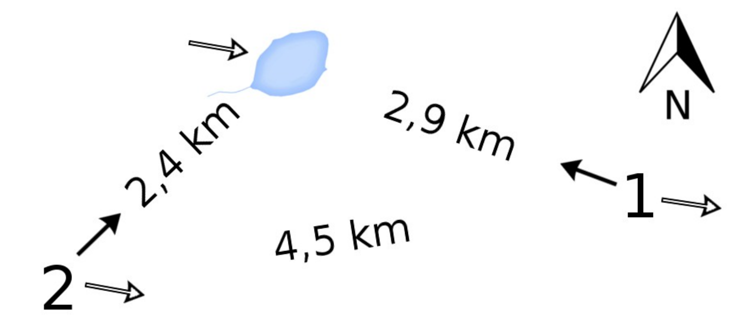

Figure 1: The pond and the two testing sites 1 and 2. The white arrow shows the migration directions of the toads at the pond and at the testing sites if they keep it the same. The black arrows are the direction from the testing sites to the pond.

The direction the toads were heading and the place where they were caught were recorded to determine later if they kept their migration direction. To determine the direction the toad was heading, a wooden stick was placed along the axis of the toad and with a compass the direction was measured with a five degree accuracy. The toads were placed into plastic buckets and transported by car to the testing site where the experiments took place.

Immediately after the toads had arrived at the testing site the experiments started with the first toad collected and ended with the last toad collected.

It was as quiet as possible during the experiments and buckets with the toads were placed 10 to 20 meters away from the experiments so they were not influenced by noises and lights.

The arena in which the toads were tested had a diameter of 121 cm, and the walls were 60 cm high. The complete arena was visually symmetrical on the inside. From inside of the arena the toads were able to see the sky but not the horizon. The ground of the arena was made of wood and the walls were made of plastic. The inside of the arena was covered with black opaque plastic foil so it was easy to clean.

A framework was built around the arena which was needed to hold the infra red spotlight and the camera. At the start of each experiment the toads were sat in the middle of the arena under a clay pot to calm down. A string was attached to the middle of the framework to lift the pod vertically from outside of the arena.

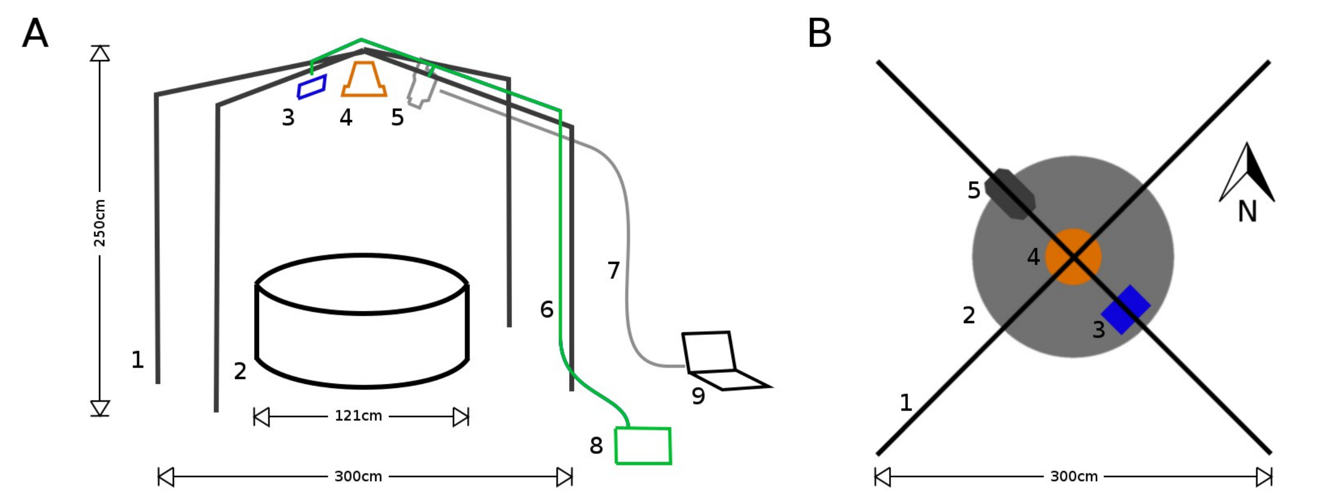

Figure 2: Experimental setup. A side view B top view; 1)Framework, 2) Arena, 3) Infrared spotlight, 4)Clay pot, 5) Infrared camera, 6)Power cable, 7) Video cable, 8)Battery, 9) Laptop.

For video documentation of the experiments a Panasonic NV-DS28EG camcorder with infrared-mode was used. The videos were analysed to determine the toad´s direction shortly before they reached the wall of the arena. During the experiments the camera was connected to a laptop where a live view of the video was used to determine the current position of the toad and the videos were recorded. The time stamp function of the camera was used to document the time of the experiments.

With this construction it was possible to see the movement of the toads during the experiments without disturbing them.
At the testing sites temperature, cloudiness and phase of the moon were recorded at the home pond where the toads where captured only the temperature was measured.

Procedure

The centre of the arena was marked with black tape and the toads were placed in the middle of the arena and covered with a clay pot of 20 cm diameter. Then they were given four minutes to calm down from the handling. When the four minutes had passed the pot was lifted up to the centre of the framework with a string from outside the arena and the toad had ten minutes to reach the wall of the arena. If a toad did not reach the wall within time it was excluded from further analysis.
After each trial the floor of the arena was wiped with a damp sponge to eliminate olfactory cues of the toads of previous experiments (Fischer et al. 2001). During the experiments the toads were filmed with the infra red camera and lit with an infra red spotlight.
The experiments took place between dawn and short after midnight. The toads were tested the same evening they were caught and four days later again at the same testing site. Each toad had four complete days to get used to the new location and recalibrate its internal compass. During these four days the toads stayed at the new location in a plastic bucket and kept wet so they do not wither.
Every two days the experiments switched to the other testing site.

Locations

Two locations near the home pond were chosen for testing and the experiments alternated between the two places every two days.
The first location where the experiments took place was a yard in Floridsdorf at the border of Vienna (latitude: 48.3045747, longitude: 16.4217351). The distance from the testing site to the pond was about 3 km. At the location the toads were stored in plastic buckets in an attic with the windows on the north side of the roof. The second location was at the Seeschlacht in Langenzersdorf (latitude: 48.2983406, longitude: 16.3614712). The distance to the pond was about 2,3 km. The toads were kept in plastic buckets in a small house with windows north-west and south-east but with very less light in the room itself. The distance between the two testing sites is about 4,5 km.

Data collection

Screen-shots of the recorded Videos were taken using the VLC media player 2.0.3 and then the image manipulating software GIMP 2.8 was used to determine the exact direction the toads were moving. For each toad a screen-shot of the video were taken when they were about 9 cm away from the wall which was 85% of the arena-radius.

Statistics

The data were analysed with standard circular statistics (Fisher 1995). The mean vectors of each sample were estimated and the Rayleigh-Test was used to proof significance for a uniform distribution. Rayleigh values were calculated with Oriana 4.02.

Results

Experimental animals

In total 116 toads were collected for the experiments; 20 of them were excluded from further analysis because they did not fulfil the requirements of the experiments or due to technical problems. In total 96 toads were successfully tested and reached the wall of the arena within 10 minutes.

The average weight of a toad was 37 g (minimum 24 g; maximum 57 g) and the average length 7,1 cm (minimum 6,3 cm; maximum 8,1 cm).

Experimental conditions

The migration started at the 9th of April and experiments took place from 10th to 23rd April 2013.

This year new moon was at the 10th of April and full moon at the 25th. The migration started about new moon and ended a few days before full moon. The average temperature during the experiments was 10° C with a maximum of 17° C and a minimum of 3° C. The experiments started at dawn and usually ended short after midnight.

Distributions

If possible only toads heading towards the pond were collected for analysis. The mean vector of the initial orientation of the collected toads was 107,1°.

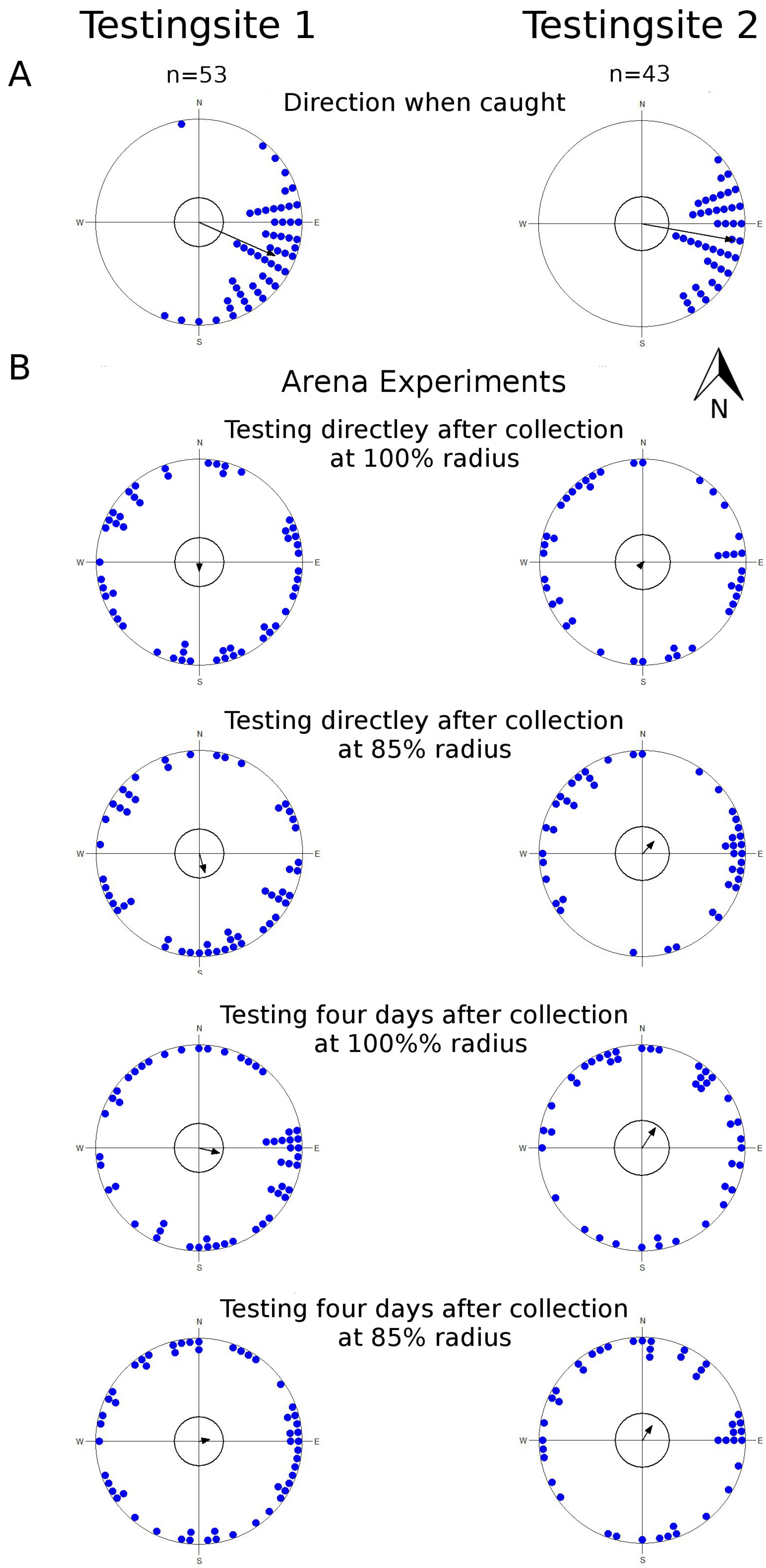

Figure 3: A Initial orientation of the collected toads at the home pond, B experiment results of testing site 1 and testing site 2. In total 96 toads were tested.

Table 1: Rayleigh test p values.

	Day 1 radius 85%	Day 1 radius 100%	Day 5 radius 85%	Day 5 radius 100%
Testing site 1 (n=53)	0,145	0,687	0,562	0,112
Testing site 2 (n=43)	0,363	0,687	0,224	0,095

Discussion

Against the expectations the toads did not orient themselves towards the home pond. No preferred direction could be determined and the results were not significantly distinguishable from an uniform distribution. This leads to the assumption that under the experimental conditions they were unable to orient themselves or are not capable of true navigation.

The arena was previously tested in other experiments and is therefore not a new method but may be too small to get significant results but due to technical difficulties the arena diameter was kept the same as in previous experiments that were made with the common toad. In the arena the toads had enough room for orientation but Landler showed with his experiments that the orientation became clearer the closer the toads came to the border (Landler & Gollmann 2011). An arena diameter of 121 cm may be not enough for significant results. A behaviour that could be interpreted as orientation was observed during the experiments so it is possible that the toads tried to orientate themselves but failed. According to Heusser the toads should have a big enough desire to return to their home pond but it may changed over time during the migration (Heusser 1958). One problem encountered was that the migration of the common toad usually starts at new moon and continued for two weeks till it was nearly full moon (Vignoli & Luiselli 2013). With more and more moonlight every day the toads started to see the wall of the arena as they were coming closer and then stopped shortly before they reached the wall and then often changed the direction to evade the obstacle and so the effective size of the arena got smaller. To exclude this bias from the analysis the direction they were heading was measured shortly before they reached the wall.

The infra red light did not seem to have influenced the toads during the experiments in any way.

The results show that the toads have a tendency to keep their migration direction the same day they were caught and even after four days the direction of the mean vector did not change much. It is possible that four days were not enough time for the toads to recalibrate there internal compass and so they still kept the same direction they were heading when caught.

Over the years different experiments were carried out to investigate the orientation ability of the common toad and still it is only barely understood how it works and what cues the toads use for orientation. The desire to return to the home pond during the migration period is strong but the arena experiments are difficult to interpret because the toads seem to have too little input for a clear navigation.

The research on navigation abilities of *Bufo bufo* probably should be bottom up not top down meaning more studies in the field to investigate how toads navigate in their natural habitat. If it is clear what cues they use for orientation and if they are capable of true navigation in the field it should be tested if only a few cues are still enough to navigate towards home because older displacement experiments looked quite promising (Sinsch 1987).

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Zusammenfassung

Seit etwa 40 Jahren ist bekannt, dass Tiere das Erdmagnetfeld als Orientierungshilfe benutzen um sich über längere Distanzen zu orientieren. Die ersten Versuche wurden mit Vögeln gemacht und obwohl Magnetorezeption auch in anderen Tiergruppen von Amphibien, Reptilien bis zu Mikroorganismen und Pilzen gefunden wurde, sind die meisten Daten lediglich zu Vögeln bekannt. Zum Orientierungsverhalten von Amphibien gibt es bereits seit längerem Studien doch ist im Vergleich zu Vögeln nur sehr wenig über deren Magnetsinn bekannt. Zu vielen Arten wie z.B. der Erdkröte gibt es fast keine Studien dabei ist die Erdkröte (*Bufo bufo)* aber gerade wegen ihres auffälligen Wanderverhaltens bekannt. Da das Erdmagnetfeld zwei nicht parallele graduelle Komponenten, die Feldstärke und die Inklination, aufweist, ist echte Navigation nur mit Hilfe des Magnetfeldes möglich. Verschiedenste Studien konnten zeigen, dass Veränderungen des Magnetfeldes einen Einfluss auf das Wanderverhalten der Erdkröte haben und daher ist anzunehmen, dass sie ähnlich den Zugvögeln das Magnetfeld der Erde als Orientierungshilfe benutzen.

In dieser Arbeit wurde mittels eines Arena-Versuches getestet ob Erdkröten sich nach einer Verfrachtung von rund drei Kilometern nur mit Hilfe des Magnetfeldes und freier Sicht auf den Himmel wieder in Richtung ihres Heimatgewässers orientieren können. Dazu wurden Kröten in einer optisch symmetrischen Arena mit 121 cm Durchmesser und 60 cm Wandhöhe getestet. Die Kröten wurden in die Mitte der Arena unter einem Tontopf gesetzt und nach vier Minuten wurde dieser in die Höhe gezogen. Die Tiere hatten daraufhin 10 Minuten Zeit sich zu orientieren und die Wand der Arena zu erreichen. Während des Versuches wurden sie mittels einer Infrarot Kamera gefilmt und die Videos später ausgewertet. Da die Vermutung besteht, dass der Magnetrezeptor bei Amphibien Lichtabhängig ist, brauchen sie nach der Verfrachtung möglicherweise einige Zeit um sich auf ihrer inneren Landkarte neu zu orientieren. Daher wurden die Kröten nach den Versuchen vier Tage an den jeweiligen Versuchsstandorten gehalten. Nach vier Tagen wurden sie ein zweites mal getestet ob sie sich in Richtung des Heimatgewässers orientieren können. Die Test wurden abwechselnd an zwei verschiedenen Standorten jeweils ca. drei Kilometer entfernt vom Laichgewässer durchgeführt wobei immer alle zwei Tage zwischen den Standorten gewechselt wurde. Durch den Wechsel der Standorte konnte sichergestellt werden, dass die Wandermotivation in beiden Versuchsgruppen die selbe ist und dass, wenn sie eine Orientierung in Richtung Heimatgewässer aufweisen diese nicht zufällig zustande gekommen ist.

Die Kröten waren nicht fähig sich in der Arena zu orientieren und die Verteilung konnte nicht

signifikant von einer zufälligen unterschieden werden weder am selben Tag an dem sie gefangen wurden noch vier Tage später. Es konnte eine Tendenz zur Beibehaltung der Wanderrichtung beobachtet werden, diese war jedoch nicht signifikant.

Summary

Since about 40 years it is known that animals use the earth´s magnetic field for long distance orientation. The first experiments were made with birds and although magnetoreception was found in other animal groups such as amphibians, reptiles and even micro-organism and mushrooms most of the data is only from birds.

There are already studies to the orientation behaviour of amphibians but compared to the research on birds only little is known. To many species such as the common toad (*Bufo bufo*) only a few studies exist although it is mostly known for its migration behaviour. The earth´s magnetic field provides two non parallel components, the inclination and the field intensity, true navigation with only the help of the magnetic field is possible. Studies showed that changes in the magnetic field influenced the toads orientation behaviour and therefore it is assumed that toads, similar to birds, use the magnetic field as clue for orientation.

In this work arena experiments were carried out to investigate if toads were able to orient homewards after a displaced up to three kilometres and with only the magnetic field and celestial clues available. The toads were tested in a visually symmetrical arena with a diameter of 121 cm and the walls were 60 cm high. The toads were placed in the middle of the arena under a clay pot which was lifted four minutes later. The toads had ten minutes for orientation and to reach the wall of the arena. During the experiments they were filmed with an infra-red camera and the videos were later evaluated.

The amphibian magnetoreceptor may be light depended and so they might need time for recalibration after displacement. Therefore the toads were kept four days at the testing site. After four days they were tested again if they are able to orient homewards. The experiments were carried out at two different locations approximately three kilometres away from their home pond. The experiments alternated every two days between the two testing sites to ensure that the migration motivation in both groups were about the same. The two testing sites ensured that the orientation homewards is not by chance.

The toad were unable to orient homewards under the experimental conditions and the distribution could not be distinguished from an uniformly distribution neither the day they were caught nor four days later. There was a weak but not significant tendency to keep the migration direction.

Lebenslauf

Persönliche Daten

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