Tracking of recently released European green toads using radio telemetry

A closer look at the radio telemetry tracking method and the welfare of Bufo viridis in regard to externally attached transmitter belts.

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Colophon

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NORDENS ARK

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SUMMARY

Nordens Ark is a private non-profit foundation that aims to conserve endangered animals by breeding, research and training. Since the early 2000's Nordens Ark has released hundreds of animals that were born at the zoo, one of the species that is released is the European green toad (*Bufo viridis*) which is listed as vulnerable in Sweden on the European red list. For the release and tracking of the toads a bird sanctuary near Löttorp, named Högby hamn Natura 2000 in Sweden, was chosen. This location was determined to be the release site because before the European green toad was listed as locally extinct in Sweden in the '90s it was the last location where this toad species naturally occurred in this province. The toad still occurs in circa 5 small and scattered population in the provinces of Skåne and Blekinge.

The goal of the project was to determine the feasibility of radio telemetry localization to monitor released green toads and evaluate the method as such in terms of health and welfare impact on the toad. To follow the toads, they were fitted with transmitter belts. The transmitter belts were designed and fitted in lab conditions to monitor the behaviour of the toads in reaction to the belt. After fitting the toads with the belts, they were tracked in the bird sanctuary at Högby Hamn for three weeks to see the effects of the belts in terms of health impact and to evaluate the method in field conditions. Afterwards, range experiments were performed to see how terrain and weather conditions influence the range of the transmitters.

The best fit of the belt seemed to be a "wiggle" fit, where there is enough room to wiggle the belt but not enough to pull it off the toad. Despite that, more toads shed their belts compared to other studies, as 44,4% of our toads retained their belts compared to 63,2% and 48.9% in other studies. The range of the signal is deemed sufficient for this experiment, but extra caution is needed when tracking in suboptimal weather conditions so that toads do not get out of range. This is because changes in air humidity, temperature and air pressure influence the refractivity of the air and thus influencing the signal range by scattering the radio wave.

Ultimately, toads were not negatively affected by the fitting of the belts and sore developments were rare. Due to the centre belt-hole placement in the transmitters there was an increased risk for toads to get stuck in sturdy vegetation and roots if the transmitter flipped to the front of the toad. One deceased toad was recorded (48 hours after release), however no obvious cause of death could be found or related to the fitting of the belt. Cow trampling might be a possible cause of its death.

More research about the design of the belt is needed to reduce shifting of transmitter and shedding of belt. Also, when preparing for field tracking, we found that, based on our experiences, fitting the belt on the toads in controlled lab conditions at minimum 3 days in advance is advantageous, so that they can get familiar with their belts and adjustment can be made if the belt is shed. The transmitter should have an anterior tube at the front of the transmitter instead of a centre tube in the middle of the transmitter to reduce the chance of a toad getting stuck due to the flipping of the transmitter. With a front attachment the chance of getting stuck is reduced.

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1 INTRODUCTION

1.1 GENERAL BACKGROUND

Nordens Ark

Nordens Ark is a private non-profit foundation that aims to conserve endangered animals by breeding, research and training. Nordens Ark releases endangered animals into the wild and helps to improve their habitats. Since the early 2000's Nordens Ark has released hundreds of animals that were born at the zoo, one of the species that is released is the green toad (*Bufo viridis*) which is listed as vulnerable in Sweden on the European red list.

The green toad

The green toad can be found in most of Europe to Central Asia and North Africa. Southern Sweden, Denmark, the Baltic states and Russia are the northernmost locations where the green toad can be found (1) (2) (3). In Sweden the green toad prefers to live in habitats that consist of shoreline meadows and shallow bodies of water that are surrounded by shrubs and grasses that are easily warmed up in early spring and summer (4).

Nowadays, both the land and the water habitats of the green toad are threatened, the former is important as the primary living habitat and the latter is important as breeding grounds for the green toad. The habitats of the green toad are reduced by anthropogenic and natural activities such as land-use change, agriculture, lowering of water levels, predation and disease (4).

Since 2009 efforts of restoring the habitat of the green toads have increased and more coastal habitats have since been restored by the County Administrative Board of Kalmar on the island of Öland. Öland is chosen as the primary release location since the green toad, before it was listed as locally extinct in this part of Sweden, was last found on the North-Eastern part of the island. The county board aims to expand the amount of suitable habitats on Öland in the future along the eastern shoreline by adding suitable hibernation sites and creating shallow ponds (4) (3).

Radio telemetry

Radio telemetry has been used to track animals since the 1960's. The technique consists of a three-part system; the receiver, an antenna and a transmitter. The animal is equipped with the transmitter that sends radio signals that can be picked up by the receiver and antenna. If the receiver finds the signal it will produce a beeping sound that will get louder when the receiver approaches the transmitter (5). Using radio telemetry one can track an animal's movements and which habitat it chooses to use (6).

1.2 PROJECT GOAL

The goal of the project is to determine the feasibility of radio telemetry localization (hereafter "RT)" to monitor released green toads and evaluate the method as such for *Bufo viridis*. in terms of health impact on the toad.

The sub-goals of the project are;

1. To design and fit the toads with transmitter belts in lab conditions and monitor the behaviour of the toad in reaction to the belt.

2. Perform range experiments with the transmitters to see how terrain and weather conditions influence the range of the transmitters.
3. Track the European green toads in the field from the 1st of September until the 19th of September to monitor the effects of the transmitter belts on the toads in terms of health, and to evaluate the method in field conditions.

1.3 Boundaries

The project starts at the 17th of August 2020 and ends at 22nd of January 2021 with a total of 22 weeks. The field data will be gathered from the 1st of September 2020 until the 19th of September. To monitor the health of the green toad, while it is wearing a belt attached transmitter, only the skin condition of the toad will be monitored, there will be no elaborate assessments on the toad to get a detailed result of the health of the toad and if the behaviour of the green toad is impaired by RT localization as far as we can assess within this project.

For the range of the transmitters no elaborate calculations will be made in regard to the various parameters like air humidity, temperature and air pressure. The only assessment about these parameters is whether the transmitter range is sufficient or not while tracking in different weather conditions.

1.4 Reading guide

In the introduction the project and its goals are explained as well as the project's boundaries. Following the introduction is the theoretical background where all necessary literature and technical information that is needed to get an understanding of the used methods, such as RT and weather conditions, for example. Subsequently, the methods for the fitting of the transmitter belts, the range experiments and the field tracking are explained in detail in the methods chapter. The results of the fitting of the transmitter belts, range experiments and field tracking are listed in the results chapter. In the next chapter the results of this study are compared to results of other studies, which is then followed by the conclusions of this study. Finally, the recommendations and advice about the effects of the belt on the toads and the RT method are given in last chapter.

2 THEORETICAL BACKGROUND

2.1 METHODS OF RADIO TELEMETRY

The transmitter can be found using two different methods, one more precise than the other, the homing method and the triangulation method. The *homing* method requires visual confirmation of the tracked animal in order to decide its geographical location. With this method the individual that perform the tracking has one receiver and one antenna trying to find the signal. When the signal is found a beeping sound will be produced that gets louder when the receiver gets closer to the transmitter (5). If the receiver is really close to the transmitter it will seem as if the signal is coming equally from all directions. If that happens the tracker can switch on the attenuator as it weakens the signal so that the transmitter signal again comes in from one direction. For even more accuracy, in dense vegetation or rock piles for example, the tracker can build a small antenna from a COAX cable that is split at the end exposing the metal wires of the cable, these wires can then be bent into a small trident shape. The whole converted cable can be placed in a sturdy pipe, like PVC, so that the makeshift antenna can be prodded into places where the animal with the transmitter might be located and the tracker cannot reach or see (7).

The other way of locating a transmitter uses triangulation. It involves three trackers all with their own receivers and antennas. The benefit of triangulation over homing is that direct sight of the tracked animal is not needed, because it can be calculated from the received signal of the three receivers to get the exact location of the transmitter. This is especially useful for areas where the tracker cannot go due to, for example, private property or while tracking fast moving easily disturbed animals. Using a map, the trackers can draw lines to pinpoint the location of the transmitter (5).

2.2 RADIO TELEMETRY TRANSMITTERS

The size of the transmitters is limited by the size of the animal, or rather the ability to carry a certain weight. This is especially true for toads and frogs, of which the transmitters and belt combination is recommended to be less than 10% of their body mass (8). When the transmitter and belt combination is 14% to 17% of the body mass the movement speed of the animal has been shown to be reduced by a third (9). Carrying a transmitter may also impair movement due to entanglement or increase visibility and thus increase the risk of predation. In studies where frogs that were equipped with transmitters, they showed different antipredator behaviour when exposed to simulated attacks of snakes and birds, but ultimately the transmitters did not significantly affect such behaviour. Northern Leopard Frogs (*Rana pipiens*) altered their method of escape when confronted with a simulated attack, while Wood frogs (*Rana sylvatica*) were not affected by the simulations but jumped more and moved in straighter lines. Frogs equipped with transmitter might be negatively affected in their survival (10). Thus, the results of the different studies show that the frogs can be either negatively or not affected by the transmitter.

2.3 EFFECTS OF WEATHER ON RADIO TELEMETRY TRANSMITTER RANGE

Radio waves can be influenced by air temperature, atmospheric pressure and air humidity. The strength of a radio signal is reduced when one or more of these parameters increases (11). Increased precipitation influences the strength of the signal of radio waves in a negative manner, while clear sky conditions do not have a negative effect (12). This is confirmed by another study that examined the effects of precipitation in combination with wind. The more precipitation and higher wind speeds are, the more the signal strength of radio waves was reduced (13). When temperature increases, the amount of light particles also increases. The result of this on the signal strength is that the light particles collide with the radio waves, reducing its strength and thus also reducing its range (14). All of these parameters have one thing in common. They influence the refractive index of air to vary in different places (11). Higher refractivity makes the radio wave bend more, weakening its signal and thus its range. It is not expected that weather will have major impact on the tracking of toads, but in certain situations tracking can be harder compared to optimal weather conditions.

2.4 Radio telemetry transmitter belt, design and welfare

In the past several attempts have been made with attaching a radio transmitter to frogs and toads. The biggest concern was the effect that the transmitter belt would have on the animals; would it be able to move as it normally would, would the animal be restricted in its behaviour, is the animal able to feed properly, would the belt negatively affect the skin of the animals, etc. (15) (6). Different types of belts are used for the tracking of frogs and toads, a belt that consists of beads that can be removed and added to tighten or loosen the belt (15), one that is made from stretch bead cord (6) and a belt that is made of an adjustable plastic tubing (16). Some belts are designed in such a way that over time it will degrade so that the transmitter will no longer be attached to the animal. This is usually provided by for example, catgut. Catgut is a surgical wire used to stitch wounds and dissolves after a few weeks.

A study which resulted in usable data from 26 western toads (*Anaxyrus boreas*) showed that 7 toads developed skin conditions and 6 toads shed their belt according to the belt design. The toads in the study were equipped with a plastic tube belt between 1993 and 1995. Developed skin conditions varied from mild excoriation to open wounds on one or both of their hips. The conditions were treated with vitamin E oil while the belt was moved from the wounds but not removed from the individual, resulting in healed wounds within a week. Body weight that was lost by the toads due to the conditions was gained back after treatment. Toads that developed these skin conditions were fitted with a belt that was too tight (16).

Another study with a sample group of 89 frogs (*Rana draytonii*), equipped with belts of beads that can be removed or added to loosen or tighten the belt, showed that out of these 89 toads only 6 developed small skin sores. A frog that developed skin sores was captured and isolated which allowed the skin to heal within two days. When frogs were captured that developed skin sores the belt was loosened resulting in the healing of the skin in less than 14 days. The study also showed that frogs equipped with a transmitter were not significantly affected in their weight (15).

3 METHODOLOGY

3.1 BELT DESIGN AND FITTING

The belts were designed using PVC tubing, silicon tubing, catgut thread, superglue and the transmitter (HOLOHIL Systems Ltd. Model: BD-2, centre tube). First the catgut was put through the hole in the transmitter so that both sides sticking out of the transmitter were equal in length. Catgut is surgical wire used to suture wounds that will dissolve after a few weeks. After this, two pieces of PVC tubing (ID: 1.07mm, OD: 1.48mm) of approximately 2 centimetres was put over the catgut on both sides. Catgut that stuck out of the PVC tubing was cut off so that the end of the catgut met up with the end of the PVC tubing. A small dot of superglue was put on the end of the PVC tubing so that it wouldn't slide off the catgut. To complete the construction of the transmitter belt, a piece of approximately 1,5-centimetre silicon tubing with a diameter of 2 millimetre was put over both ends of the PVC and catgut belt parts.

Figure 1, the constructed transmitter belt with silicon tubing, PVC tubing and catgut.

Before fitting the belt (figure 1) to a toad, we looked at the shape of the transmitter which had a bump on one side and was flat on the other. The flat part of the transmitter should be touching the toad since the bumpy side made the fitting of the belt substantially harder. To fit the belt, one of the hind legs of the toads was held so that the belt could be slid over that leg. After this the other leg was also held and the belt was slid over that leg. If the toads retracted both legs it would often help to slide the belt over one knee which would trigger a stretching movement of the toad, making it much easier to fit the belt on the toad. The transmitter part of the belt was slid past the ischium on the hind part of the toad. To make it easier to slide the belt over the toad skin, we used a small amount of water for lubrication.

The transmitter belts were fitted 96 hours before release in controlled lab conditions so that the toads could get familiar with the belts and behaviour could be monitored. After all the toads were fitted with their transmitter belts a check was done every subsequent 24 hours to adjust and refit the belts to the toads. If the toad was successful in getting the transmitter belt off, the belt was adjusted by sliding the silicon tubing further over the PVC tubing, making the belt tighter. Unfortunately, due to time constraints detailed observations or notes were not taken at an individual basis about the fitting of the belts in the period before release.

3.2 RANGE TESTS

Again, due to a lack of time, the range tests were conducted after the field test. A consequence of this might be that the overall range of the transmitters was impacted by the shortened battery life of the transmitters (HOLOHIL systems. Ltd guarantees a battery life of 10 to 20 weeks, with a standard battery life of 14 weeks). For the testing of the range of the transmitters, 15 transmitters (HOLOHIL Systems Ltd. Model: BD-2, centre tube), a receiver (Wildlife Materials International Inc., model: TRX-

48) and antenna (Wildlife Materials International Inc., model: 3-element lightweight folding antenna) were used. To monitor humidity, air pressure and temperature a weather monitoring sensor (Ruuvi weatherproof Bluetooth sensor) was used. The SMHI (Sweden's meteorological and hydrological institute) application was installed on an android phone to monitor the wind speed, wind direction and precipitation in millimetres per hour for the area in general. Also, the GPS coordinates application (smartphone, android) was installed for the monitoring of the GPS coordinates in longitude and latitude.

The transmitter range was tested at three locations, laying on the ground;

- Field location: 58.454033, 11.436822 (latitude, longitude) and,
- Road location: 58.436980, 11.422630 (latitude, longitude) and,
- Ekopark location: 58.448843, 11.409292 (latitude, longitude).

After the transmitters were placed on the ground, the air humidity (%), temperature (degrees Celsius), air pressure (hPa), wind speed (m/s) and direction, cloudiness (0,1,2,3 with 0 representing a clear sky and 3 completely overcast), precipitation (mm/h), and the direction in which we linearly walked to lose signal. After writing this down we moved linearly away until the point where no signal was received by the receiver. From this point on we moved closer in approximately 20 meter sections until a signal of one or more of the transmitters was received. If a signal was received, we slowly walked backwards until the signal was lost and those coordinates were noted. When all the maximum ranges of the transmitters were found all the parameters (humidity, temperature, etc.) were noted again and the transmitters were collected from their location.

When conducting the test at the road location a lot of interference was picked up by the receiver and passing traffic made it hard to hear the very faint beeping tone of the receiver. Therefore, the road location tests were moved to the Ekopark location.

Two smaller tests were also conducted to see the effects of soil cover and antenna length. One where the transmitters were placed at the coordinates of the field location but placed in a box and covered by 10 centimetres of soil. This was done to simulate a burrowed toad and what the effects of the layer of soil is on the range of the transmitter signal.

The other small test was to see how the transmitter antenna length influences the range. First a test was performed with the full length of the antenna of 16 cm. Then, the antenna was cut to a length of 6.7 and finally to a length of 2.7 cm.

3.3 Field release and tracking experiment

For the release and tracking of the toads a bird sanctuary near Löttorp, named Högby hamn Natura 2000 in Sweden, was chosen (57.168374, 17.032104, latitude, longitude). At this location 19 toads were released, of which 17 were fitted with a transmitter belt. Two of the toads released, at the release site, without a transmitter belt because they could not be fitted regardless of the tightness of the belt. Initially the intention was to release 20 toads in total but unfortunately one of the toads lost its transmitter before departure and we could not find it anymore since it had not been activated yet. The release was done at two occasions (1st of September 2020 and 4th of September 2020) and we used two methods. The first group was released with both a hard ("direct") release and a soft release (via an enclosure at the release site) (1st of September 2020). The hard release group, consisting of 7 toads, was released from the traveling box in which the toads came. The soft release, also consisting of 7 toads, was done so that the toads could get accustomed to the environment and to evaluate later what the difference in survival was between the two methods. The soft release toads were placed in a tent that was set-up at the release site 24 hours in advance. Within the tent we placed sand, branches and rocks to simulate the environment outside of the tent. In the second release (4th of September 2020), 5 transmitter fitted toads were released 4 days after the initial release. The five belts were kept in the case wild toads would be found that could be fitted with the belts instead. However, no wild toads were found during these 4 days and the captive toads were therefore released.

The tracking of the toads was done two times per day, one round (lasting ca. 2,5 hours) starting at 11:00 in the morning and one round starting at 20:00 in the evening. Upon locating a toad data about location, behaviour and condition was recorded in a form, (appendix A). For the tracking of the toads the same receiver and antenna combination as used with the range tests were used. In addition to this, the GPS coordinates application and Ruuvi sensor were used for the monitoring of the location of the toads and the outdoor temperature. An IR-temperature gauge (Nortec, model: testo 830-T4) was used to measure the temperature of the toads, which was done on the back of the toad between the eyes and hind legs of the toad. A flashlight was used to find toads if the tracking was done at night. Beforehand, we had decided to take out any toads that developed sores to heal and to be relocated.

When the signal of the receiver came equally from all directions, meaning that the transmitter/toad is nearby, we turned on the attenuator function on the back of the receiver to weaken the signal and receive it again from one direction. Usually this resulted in finding the toad in a short amount of time, as switching on the attenuator would bring is within 0.3 to 0.5 meters of the transmitter/toad. However, if the toad was located in, for example, a rock wall, it would be harder to find the toad and we often would benefit by detaching the cable of the antenna and use it as a makeshift prodding device in holes and crevices. In many cases when a toad was deeply buried in the ground or in a rock wall, we decided not to dig them out to decrease the risk of injuring them in the process and to reduce stress.

4 RESULTS

4.1 LAB TEST OF BEHAVIOUR AND BELT FITTING CONDITIONS

On Friday the 28th of august 20 toads (10 females and 10 males) were fitted, in the lab at Nordens Ark, with belts, four days before their release the following Tuesday. On the 29th of august, 24 hours after the first fitting, 9 toads had lost their belts. On the 30th of August, 24 hours after the second fitting, 8 toads had lost their belts. Males seemed more successful in getting their belt off compared to females, with 6 being male, 1 female and one unknown sex for the second fitting. All fittings were done in lab conditions. The toads resided in a 2*2m enclosure outside and were brought inside to be fitted with a transmitter belt. After fitting, the toads were placed inside in a 1,5*1,5m open box where they could get familiar with their belts.

Figure 2, a bloated toad dragging its hind legs in an attempt to get the belt off.

When fitting the toads with the belts there was varying behaviour observed. Some of the toads accepted the belt in a short amount of time, within 15 minutes after fitting, without exhibiting stressed behaviour. However, many of the toads exhibited stressed behaviour where the they would bloat themselves and drag their hind legs in an attempt to get the belt off (figure 2). This behaviour could continue up to 1,5 hours after fitting, but they also would eventually accept their belt. In addition to this, a few male individuals gave distress calls. Some toads also used their hind legs to "claw" the belt off by pushing one of their hind legs between the belt and skin. Two female toads were unable to be fitted with a belt regardless of how the belt was adjusted. It is speculated that this might be due to hip structure.

Transmitters that were fitted with a bit of wiggle room, but not too loose, were more susceptible to flip towards the front of the toad if the toad burrowed itself or crawled under shelter (figure 3).

Figure 3, burrowed toad with a half-flipped transmitter.

Belt weights varied in relation to body mass from 4-10% of the body weight (table 1). One of the toads was fitted with a belt that was 10% of its body mass (table 1), but ultimately none of the toads seemed to be negatively affected by the fitted belts and its weight in lab conditions.

Table 1, belt weights and belt percentage of total body mass of the toad.

Belt weight	g	Belt weight percentage of toad weight	%
Average	2.33	Average	6%
Minimum	2.21	Minimum	4%

Maximum	2.51	**Maximum**	10%

4.2 Range tests

Table 2, Signal range.

	Meters
Mean signal range	771
Median of signal range	806
Minimum signal range	383
Maximum signal range	991

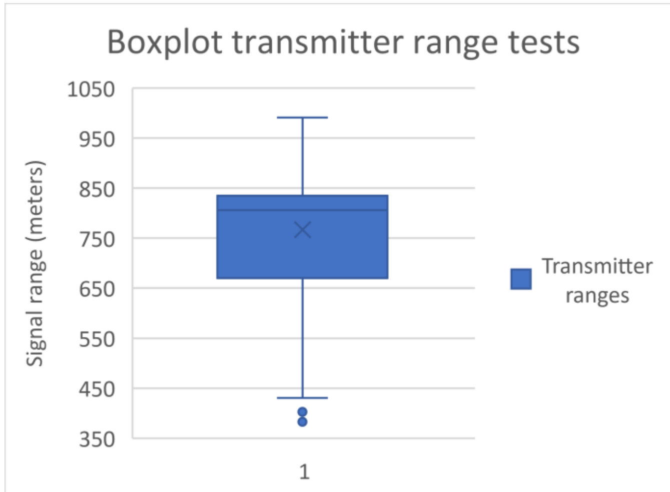

Figure 4, boxplot of transmitter signal ranges.

Range of signal was 771 meters at average, median 806 meters, at minimum 383 and at maximum 991 meters. There are 3 outliers in these tests; 383 meters, 403 meters and 403 meters. Half of all measurements are between 670 and 834.5 meters (figure 4). The average range is 771 meters (table 2), the minimum signal range without outliers is 431 meters. The median of all the range data is 806 meters (figure 4, figure 5).

In the Ekopark location signal was lost due to a large boulder and picked up again after approximately 300 meters. Vegetation did not seem to have influence on the signal range.

It was found that weather influenced the signal. Higher humidity shortened the signal (figure 6). Low air pressure shortened the signal (figure 7). Higher temperature shortened the signal (figure 8).

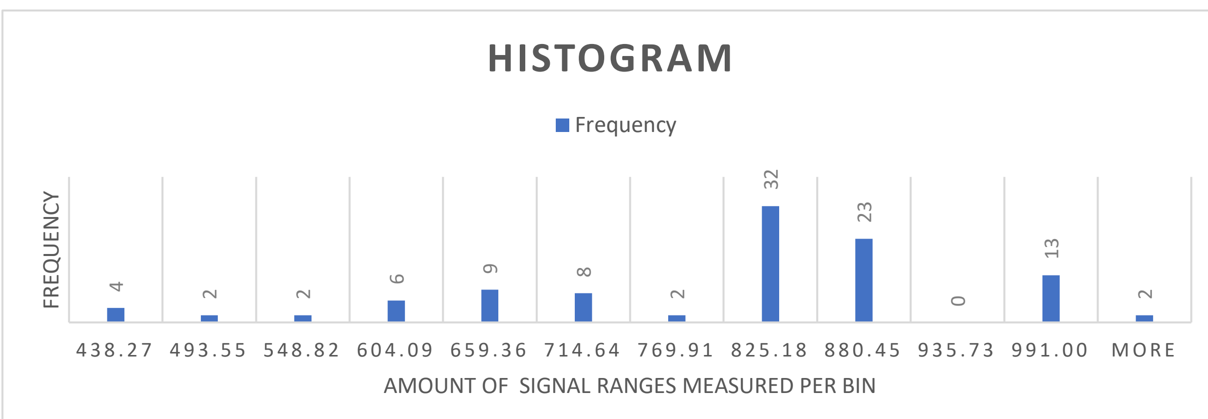

Figure 5, histogram of all signal ranges.

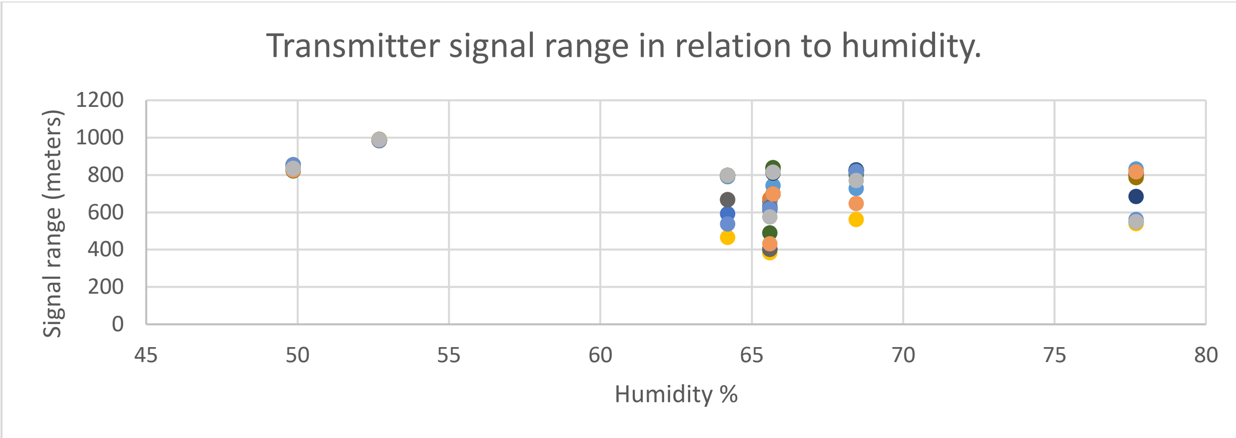

Figure 6, all transmitter signal ranges in relation to humidity.

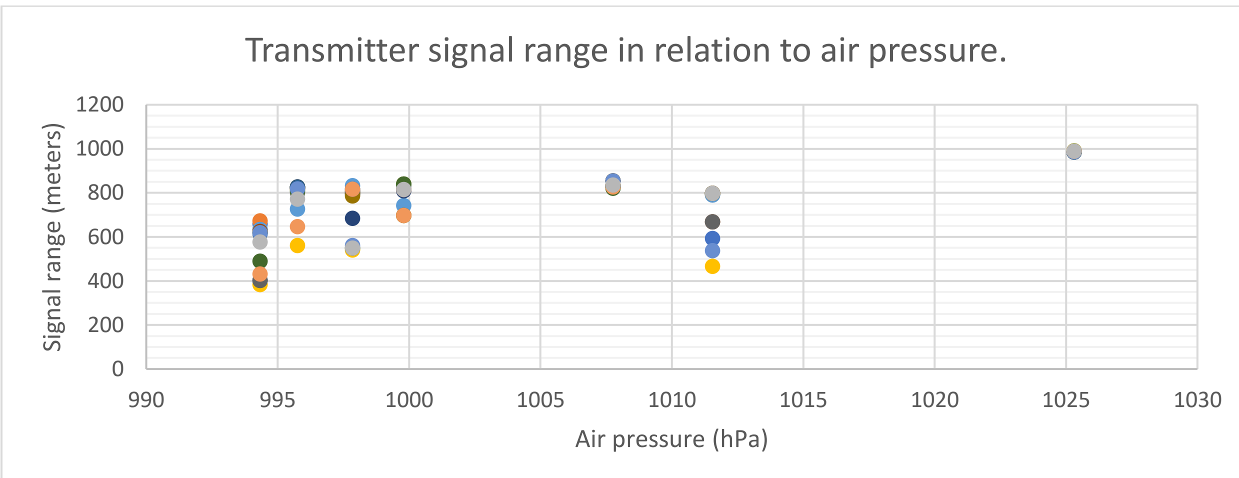

Figure 7, all transmitter signal ranges in relation to air pressure.

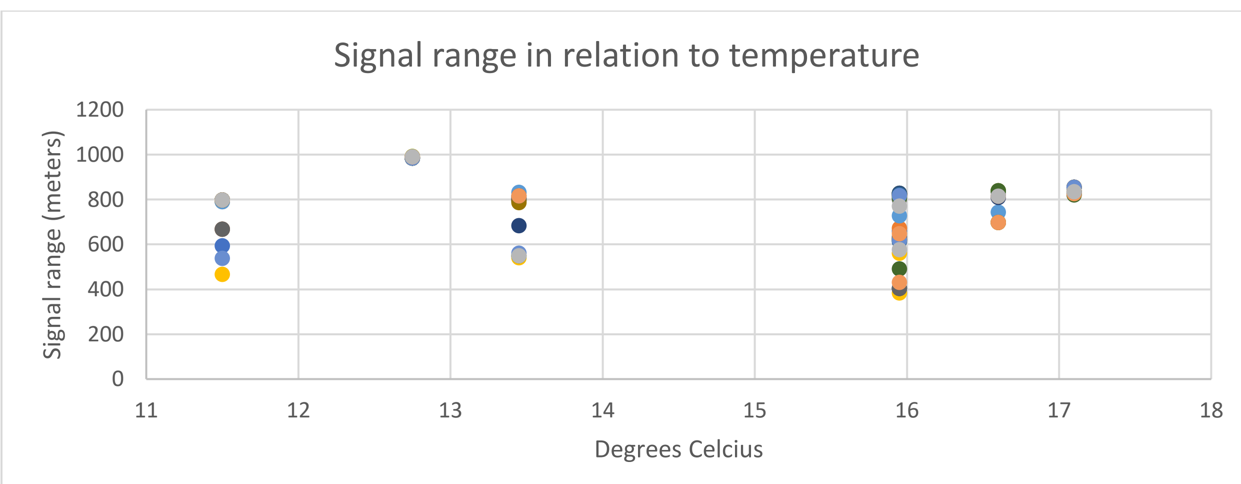

Figure 8, all transmitter signal ranges in relation to temperature.

4.3 TRACKING IN FIELD AND WELFARE

Fitting of belt.

A total of 243 observations of 17 toads were made between the 1st of September 2020 and the 19th of September 2020 (table 3). Two toads were released without transmitter as they could not be fitted regardless of how tight the belt was. Eight Toads kept their transmitter until the end of the tracking period on the 19th of September. In the first week four toads shed their belts and one was found dead with no obvious cause of its death (figure 9). In the second week of tracking two more toads lost their transmitters. In the third week one toad shed its transmitter and one was taken out of the experiment due to the development of sores (appendix B). In total seven toads lost or shed their transmitter, excluding the two individuals that could not be fitted with a transmitter. The shed transmitters were found in sturdy vegetation, there was one occasion where the release mechanism of the belt was activated. In 52 cases it was not possible to make a direct observation of a toad, primarily because the toad was in a location where it was not accessible, for example under a rock wall or in thick bushes.

Table 3, observations of the released toads based on Appendix B.

Observation:	No. of cases:
Number of releases of toads with transmitters	17
Toad found and observation made	243
Near toad, but not sighted	52
Belt adjusted	15
Faulty equipment, toad not found	13
Skin shedding	11
Toad lost transmitter	7
Toad kept belt until end of tracking period	8
Belt not adjusted	6
Toad taken in for recovery	4
Sores, toad taken out of experiment	2
Toad died	1
Toad, lost belt, later found again	1

Figure 9, top; belly of dead toad. Bottom: back of dead toad.

Welfare and sores.

In general, it seemed that the toads were not negatively influenced by the belts since they still would move up to 600 meters per day and were also found in tight burrows and shrew holes. However, no elaborate assessments or observations were made about this because comparative studies with toads having no transmitter belt is almost impossible to design under field conditions. We noticed that the transmitter could flip towards the front and/or shift to the belly of the toad, this increased the risk of a toad getting stuck due to twigs and roots getting stuck under the transmitter. One particular example of this is a toad that was stuck in a waterfilled ditch due to the flipping to the front and shifting to the belly of the transmitter. This resulted in the toad being stuck for three days in a location that was very hard to reach (deep water around). Later the toad was freed from its location and taken in for recovery for two days. After recovery the toad was released again, however after four days it developed sores on its hips and was taken out of the experiment. One other toad had developed sores on the very last day of tracking. It was noticed that before the sores developed, the skin of the toads was discoloured to a slightly brighter hue (figure 9, bottom). For 11 observations, shed toad skin had accumulated around the belt and was removed with the help of some water.

In total there were 21 occasions where the transmitter had flipped or shifted from its position. In 15 cases we adjusted the belt back to its original position, in the remaining six cases this was judged to be unnecessary. In one case it was recorded that the transmitter had shifted to behind the toad's front legs.

Range and localisation of toads

There were no problems recorded of toads getting out of range as the radio signal of the toad was always found when tracking started from a toads previously known location. It was noticed that the signal was negatively influenced in suboptimal weather conditions like rain and storms. Only when the equipment was faulty problems with range and finding toads occurred. Due to a temporarily broken antenna cable the range of the antenna was limited to 20 meters, which resulted in 13 missed observations.

The average time spend on finding toads after switching the attenuator function on is 2:35 minutes, with a minimum of 00:04 minutes, a maximum of 10:31 minutes and a median of 01:37 minutes.

5 DISCUSSION

5.1 BELT SHEDDING

Despite the limited time to prepare for the design and fitting of the belt similar results are reported by Bartelt and Peterson (16) in regards to time needed by the toads to get familiar with their belts. However, the design we used for our belts differs slightly compared to their study, the general design is the same, but the materials are different. This alteration in the design might have caused the increased shedding of belts as we finished the field experiment with 44.4% of the released toads, while Bartelt and Peterson were left with 63.2% of their western toads (*Bufo Boreas*). The study of Rathburn and Murphey (15) on ranid frogs (*Rana aurora draytonii*), ended with a similar percentage as our study, 48.9%. However, the belt design used by Rathburn and Murphey is different compared to ours and consisted out of a bead-chain belt.

5.2 WELFARE, HEALTH AND SORES

In terms of sore development our belt design seems to be performing well, in comparison with other studies, since only 2 out of 19 toads (10.5%) developed sores compared to 7 out of 38 (18.4%) by Bartelt and Peterson on the western toad (16). Rathburn and Murphey using their bead-chain belts on ranid frogs report sores in 6 out of 47 frogs (12.8%). Thus, out of three studies in total, our study on the green toad show the lowest incidence of sores. While tracking, our green toads did not seem to be negatively affected by the weight of the transmitter. Concerning the transmitter weight, our study design did not allow for a good evaluation if transmitter weight or design restricted natural behaviour or movement. As suggested by White and Garrot (8), the weight of the transmitters did not exceed 10% of their body weight. Blomquist and Hunter (10) researched the effects of external attachment of transmitters to wood frogs (*Rana sylvatica*) in reaction to simulated predation and vagility of northern leopard frogs (*Rana pipiens*), and concluded that there is no significant change in behaviour. While we did not make any elaborate assessment about the movement of our green toads, none of them seemed to exhibit altered movement due to the transmitters, compared to our previous studies of working with green toads for several years in field and in lab. Furthermore, none of our green toads were predated while having a transmitter belt attached.

5.3 SIGNAL RANGE

In optimum weather conditions with high air pressure, low humidity and low temperature our equipment reached radio signal ranges of up to 991m in the range tests. While tracking in the bird sanctuary we would still hear the signal after the toad had moved 600m from its previous location. These results are 65.1% higher compared to the results of Bartelt and Peterson ,who reported a signal range of 600m under optimal weather conditions (16), Under the conditions we experienced we found the range sufficient to track green toads in the wild. However, the range was not far from the upper limit. It is not unlikely that, studying more released toads under other conditions, the movement of the toads may exceed the chosen antenna range as the range from a transmitter is a function of its power and size. Rathburn and Murphey reported a signal range of up to 100m on land, which is much lower although since then technology has improved and better transmitter performance are possible with same sized transmitters. The drop in signal range by weather conditions is as expected sinceUkhurebor and Umukoro (11), Olasoli and Kalawole (12), Meng, Yee and Chong (13) and Amajama (14) wrote that an increase or decrease in humidity, temperature and air pressure changes the refractivity of air and thus influences the signal strength. This can be important to keep in mind under field studies, especially when animal movement is on the limit of antenna range.

6 Conclusion

On the 1st of September 2020 19 toads were released in Högby hamn Nature 2000 bird sanctuary on Öland, Sweden. The toads were fitted with transmitter belts and tracked using radio telemetry until the 19th of September 2020. The goal of the project was to determine the feasibility of RT localization to monitor released green toads and evaluate the method as such for Bufo v. in terms of health impact on the toad.

6.1 Range of transmitters and fitting of belt

The best fit of the belt seemed to be a "wiggle" fit, where there is enough room to wiggle the belt but not enough to pull it off the toad. Despite that, more toads shed their belts compared to other studies. The range of the signal is deemed sufficient for this experiment, but extra caution is needed when tracking in suboptimal weather conditions so that toads do not get out of range.

6.2 Welfare

Ultimately, toads were not negatively affected by the fitting of the belts and sore developments were rare. Due to the centre belt-hole placement in the transmitters there was an increased risk for toads to get stuck in sturdy vegetation and roots if the transmitter flipped to the front of the toad. One toad was found dead with no obvious cause for its death.

7 RECOMMENDATION AND ADVICE

Further research is recommended on the conditions that cause a toad to develop sores, conditions like, environmental factors and what belt tightness is optimal for the fitting of the belt and wellbeing of the toad. Furthermore, additional information is needed on what belt design is least likely to shift the transmitter from its position on the back of the toad. Especially important is to find a belt design that is less likely to be shed by the toad while maintaining a low risk of injury or developing sores In addition to the belt design, it is recommended to find more information about the hip structure of the toads and to evaluate if this information could be contributing to further reduction of belt shedding.

In preparation of field tracking, it is recommended to fit toads at minimum 3 days in advance with their belts, this amount of time is needed for the toads to get familiar with the belts and to make adjustments to the belts if toads are successful in shedding it. To reduce the risk of toads getting stuck in roots and sturdy vegetation due to the transmitter flipping to the front it is recommended to use transmitters with a frontal anterior tube (figure 10) instead of transmitters with a center tube. When tracking in suboptimal weather conditions consider increasing the amount of tracking per day to reduce the risk of a toad getting out of range and possibly being unfindable.

Figure 10, two designs of a BD-2 transmitter of Holohil Systems Ltd., One with a frontal anterior tube (top) and one with a center tube (bottom) (17).

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APPENDIX A

Form used to note observations and information about the toads, weather, location, date and time.

Toad ID				
Date/time				
Coordinates	Lat: Long:	Lat: Long:	Lat: Long:	Lat: Long:
Atm. temp.				
Toad temp.				
Habitat	☐ Grasses ☐ Shrubs ☐ Reeds ☐ Water ☐ Open	☐ Grasses ☐ Shrubs ☐ Reeds ☐ Water ☐ Open	☐ Grasses ☐ Shrubs ☐ Reeds ☐ Water ☐ Open	☐ Grasses ☐ Shrubs ☐ Reeds ☐ Water ☐ Open
Weight (g)				
Skin condition under belt	☐ No effect ☐ Sores on skin ☐ Badly damaged	☐ No effect ☐ Sores on skin ☐ Badly damaged	☐ No effect ☐ Sores on skin ☐ Badly damaged	☐ No effect ☐ Sores on skin ☐ Badly damaged
Toad condition	☐ Alive ☐ Dead ☐ Uncertain	☐ Alive ☐ Dead ☐ Uncertain	☐ Alive ☐ Dead ☐ Uncertain	☐ Alive ☐ Dead ☐ Uncertain
Activity of toad	☐ Hiding ☐ Resting in open ☐ Walking ☐ Hunting ☐ Uncertain	☐ Hiding ☐ Resting in open ☐ Walking ☐ Hunting ☐ Uncertain	☐ Hiding ☐ Resting in open ☐ Walking ☐ Hunting ☐ Uncertain	☐ Hiding ☐ Resting in open ☐ Walking ☐ Hunting ☐ Uncertain

APPENDIX B

Timeline of all toad observations, including effects of the belt on toad and adjustments to the belt.