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Radio telemetry and welfare - experiences from a three-week study of released European green toads (*Bufotes viridis*) on the Baltic Island of Öland, Sweden

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Abstract

Amphibians are declining all over the world ultimately due to habitat loss, pollution, and climate change. Reintroduction of captive bred specimens is becoming a common conservation method, but difficult to monitor and its success is challenging to estimate. Radio telemetry (RT) has been shown to be an effective monitoring method to relocate released individuals but has rarely been evaluated for amphibians. Since amphibians have very delicate skin with many functions, the evaluation of the use of RT is both important from a conservation point of view but has also a welfare aspect.

In this study we tested the impact of RT waist belts on reintroduced European green toads (*Bufotes viridis*). To ensure appropriate fitting, the RT waist belts were fitted in laboratory before the reintroduction took place.

The results after three weeks in the field, showed a low number of skin abrasions on the toads and a low number of dropped RT waist belts. However, we discuss some improvements for the design and the fitting of the RT waist belts.

Introduction

Amphibians are in global decline due to factors such as habitat fragmentation, habitat destruction, pollution, the emergence of infectious diseases, and climate change (Luedtke et al., 2023). Reintroduction of captive-bred individuals is now being carried out as a promising conservation action (Backlin et al., 2015). However, the success of reintroduction projects is challenging to estimate, in part due to the cryptic behaviour and good camouflage of many amphibian species. Therefore, tracking techniques such as radio telemetry (RT) can be a useful method to follow individuals in the field (e.g. Feller & Kleeman, 2007; Holenweg & Reyer, 2000). In amphibians, invasive tracking techniques such as RT are more demanding in comparison with mammals. Amphibians are generally small, and most species weigh less than 100 g (Burow et al., 2012). They have delicate and permeable skin capable of osmotic regulation and, in many species, also capable of gas exchange (Wells, 2007). All amphibians frequently shed the outer layer of the epidermis, with some also ingesting the old skin (Wells, 2007). The function of this process is the need for renewal of the outer layer of skin cells, which is susceptible to mechanic abrasions and injuries (Burow et al., 2012; Wells, 2007). Additionally, in many terrestrial anurans, including the European green toad (*Bufotes viridis*; Laurenti, 1768; hereafter *B. viridis*), the ventral skin, particularly the pelvic patch, is thinner than the dorsal skin, highly vascularized, and is the primary area used for water uptake (Burow et al., 2012; Wells, 2007).

With the lack of a protective outer covering of the skin, any external attachment of devices poses the risk of friction injuries (Pough et al., 1998; Wells, 2007). A suggested method to reduce these problems, is to use a waist belt with a material that reduces friction (Bartelt & Peterson, 2000). It is recommended that the belt is produced of a material that will disintegrate and allow the waist belt to open after a certain time if the individual is lost (Burow et al., 2012).

A potential problem with waist belts is impaired agility and movement due to the added weight and design of the RT device, but so far, these negative effects have been found to be minimal (Bartelt & Peterson, 2000; Groff et al., 2015; Indermaur et al., 2008; Rathburn & Murphey, 1996). It is also recommended that the weight of the device is less than 10% of the bodyweight (White & Garrott, 1990).

B. viridis, is a widely distributed species associated with open sun-exposed landscapes, such as shore meadows and open pastures, often on a sandy substrate (Rogell et al., 2011). In the northern edge of its distribution, in southern Sweden, habitat changes (mainly bush encroachment and afforestation) and other factors have led to a critical situation with only a few isolated populations left (Wirén, 2010). Active translocations and re-introductions to localities where it has become locally extinct have been carried out since 1994 (Wirén, 2010). Using RT techniques on *B. viridis* is not common (Indermaur et al., 2008), and to our knowledge, never tested in Nordic conditions. Since the use of RT has the potential to improve the conservation of the *B. viridis*, but little is known about the effect of attached transmitters on the toad itself, our aim was to evaluate welfare-related aspects, especially skin condition and fitting of radio transmitter waist belts. Tests were performed both in field and in laboratory conditions.

Material & methods

Toads and transmitters

We equipped a group of 17 *B. viridis* with transmitter waist belts, 72 h before they were released into the wild (Fig. 1). The group consisted of nine males and eight females reared at Nordens Ark (58.4424°N, 11.4365°E) from eggs collected at ponds located in the Limhamn limestone quarry (55.5682°N, 12.9293°E) in 2017. We used adult toads with a bodyweight exceeding the weight of the

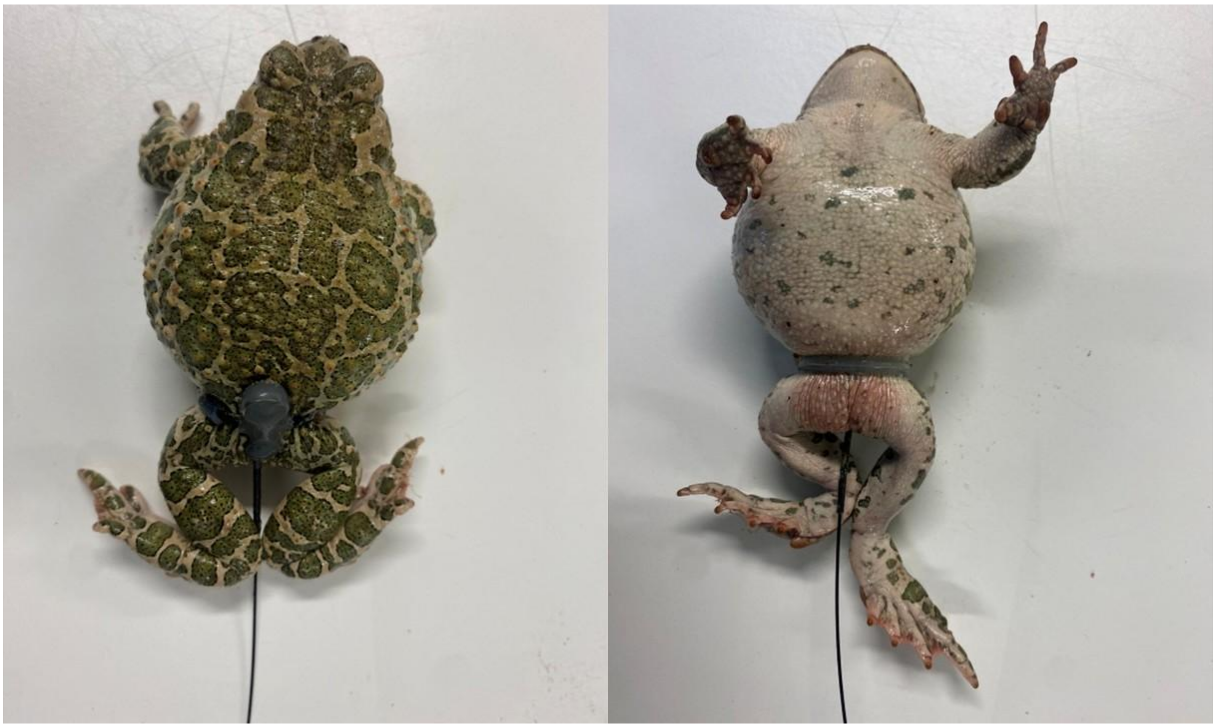

Figure 1. *Adult green toad equipped with transmitter with waist belt.*

transmitter belt at least ten times, as recommended by White & Garrott (1990). All individuals were three years old, and their weights ranged from 23.48 to 57.28 g, with an average of 41.95 g.

Transmitters were of the brand Holohil BD-2 (ranging from 0.5 to 2.0 km according to the manufacturer) with a weight of 1.8 g (Fig. 2). The waist belts were produced out of silicone tubing (Du-Bro silicone fuel tubing OD:3.6mm, ID:1.6mm), PVC tubing (DISTRELEC insulating hose PVC OD:1.8mm, ID:1.05mm P10518 BK001) and catgut (BRAUN F1144103 catgut plain) according to the suggested design of Burow et al. (2012) and weighed 0.5 g (Fig. 2). The belt was secured by the friction between the PVC and silicone tubes, which also made it possible for the belt to be released under excessive tension, such as entanglement in shrubs (Burow et al., 2012). The total weight of the waist belt and the transmitter was 2.3 g, representing on average 6% (4%-10%) of the adult toad bodyweight and the total length was 220 mm (Fig. 3).

The receiver used for the study was a TRX-48 in combination with a 3-element lightweight Yagi antenna, both from Wildlife Materials International Inc.

Location

The RT tracking and skin condition monitoring were carried out in the Högby hamn Natura 2000 area (57.1693°N, 17.0358°E) on the Baltic Island of Öland between the 1st and 19th of September 2020. Calling *B. viridis* males were last heard in the area in 1994, and by the year 2000, it was considered locally extinct. Since 2012, eggs, tadpoles, and toads in various age groups have been released with limited or unknown success. The area consists of a large open coastal meadow with restored ponds and wetlands, surrounded by farmland and forest. Cattle and sheep graze parts of the area and some areas are also maintained by mowing. The pastures are divided by stone walls and have scattered thickets of thorny bushes.

Figure 2. *Transmitter of the brand Holohil BD-2 (weight 1.8 g), attached to a waist belt produced out of silicone tubing, PVC tubing and catgut (weight 0.5 g), designed after Burow et al. (2012).*

Tracking and evaluation of welfare aspects

The toads were tracked with approximately 12 to 24-hour intervals over the study period (the ethical permit required a daily checking interval; *Dnr 01505-2020*). After successfully tracking an individual toad, we recorded the following: if the toad was alive or dead, skin condition under the belt, and the general activity and health status of the toad. As a part of the routine handling, we rinsed newly shed skin with water to reduce the accumulation of skin around the waist belt, which would otherwise further increase the risk of skin injuries. In cases where the toads were deeply burrowed in vegetation or soil, we chose not to dig them out on every occasion to reduce stress.

Waist belt fitting was tested in a small pilot study under lab conditions at Nordens Ark (15-21 December 2020). Five adult toads (three females, two males) had individual belts fitted on them. At first, a wet fitting method was used. With this method, both the toad and belt are wettened with water to reduce friction when sliding the belt over the hindlegs of the toad. After using the wet fitting method, a dry fitting method was tested. In this method, both the toad and belt are kept dry and free of debris (such as sand or other abrasive materials). Within the same trial, we tested if transmitter position influenced belt shedding. The transmitters had a flat side and a concave side. During the tests, either one of those sides could be in contact with the toad skin. Unless specified, the flat side of the transmitter was in contact with the skin when fitting the belts. Each day all toads were closely observed for 30 minutes. We looked at belt fit, shedding, and signs of discomforted behaviour (bloating and dragging hindlegs) during this period. Individuals that shed their belt during an initial 30-min observation period were refitted with an adjusted belt (tightened or loosened), or a different method was used (dry or wet fit), or both. Toads that shed their belt outside of this observation period had their belt refitted the next day.

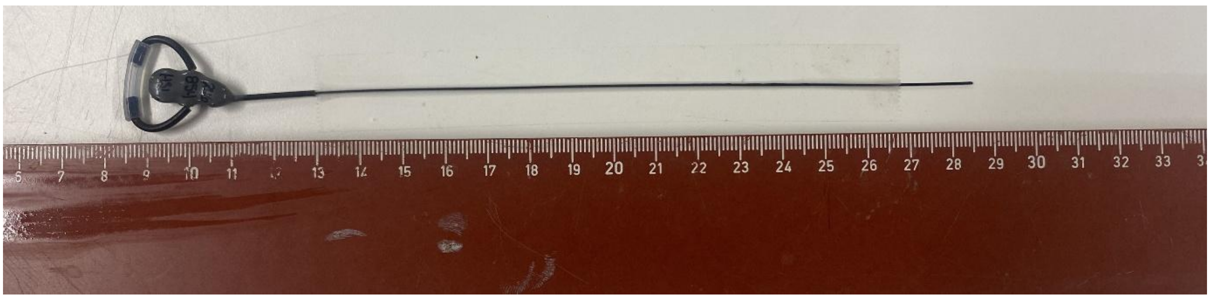

Figure 3. *Transmitter with waist belt used for tracking B. viridis. Total length 220 mm and total weight 2.3 g.*

Results

Mortality

We successfully recorded 264 positions during a three-week period. Of the 17 toads released into the wild, one was found dead 48 h after the release (Fig. 4). There were no visible signs of injury related to the RT waist belt on the dead toad and the skin had no discolouration. Starvation or dehydration are improbable causes after only two days since all toads were well fed before the release, and the area had plenty of water near the surface. The toad was found near a path where cows frequently passed, and therefore it is possible that cows may have trampled it and caused the death, but since there were no apparent signs of trauma, this is difficult to assess with certainty. Apart from that toad, no mortality was recorded in the group that retained their RT waist belts.

Waist belt related injuries

We observed skin lacerations at the hips associated with the RT waist belt on two toads (11.7%; Fig. 4). One individual developed laceration on the hips after 14 days when the belt was entangled in rush vegetation. Another toad developed lacerations after 19 days. Both toads were immediately removed from the experiment and both shed skin less than 24 h after removal of the belt. In the remaining observations of skin shedding, no lacerations were developed.

The toad found entangled in rush vegetation also showed signs of exhaustion (lethargic and unwilling to move) but recovered after two days of care in captivity.

Apart from this, we did not record any other signs of injury, impaired health, lowered activity or exhaustion in any of the remaining toads.

Waist belt fit and shedding

In the field study, we recorded shed RT waist belts in seven cases out of the 17 toads released (41.2%). In one case, the release mechanism had opened. In the remaining six cases, unopened, intact RT waist belts were recovered in sturdy woody vegetation such as inside or under thorny bushes (mostly *Prunus spinosa*). Shedding was unevenly distributed over time, with four toads that shed their belt within two days after release, and three toads that shed their belt gradually during the field test (Fig. 4).

There were 21 occasions where the transmitter had flipped or shifted from its original position on the back. In 11 out of these, the transmitter rotated around its attachment axis whit the antenna pointing toward the toads head, which was the cause of entanglement in the rush vegetation. In the remaining ten observations, the transmitter was still in its original position but was shifted to the side or to the belly of the toad due to a rotated waist belt. In one case, it was recorded that the transmitter had shifted to just behind the toad’s front legs.

In the laboratory test of belt fitting, two toads retained their belt at the first attempt (wet fitting; Fig. 5). The remaining three toads required two, three and seven attempts respectively, to retain their belts, including a shift from wet to dry fitting method. Out of these three cases, the transmitter was repositioned with the concave side in contact with the skin on two occasions. Tightening the belt reduced belt shedding in two cases. After 72 h no belts were shed.

All five toads in the laboratory test showed signs of discomfort when fitted with a belt, such as dragging their legs behind them or crawling, however this stopped within 48 h (Fig. 5).

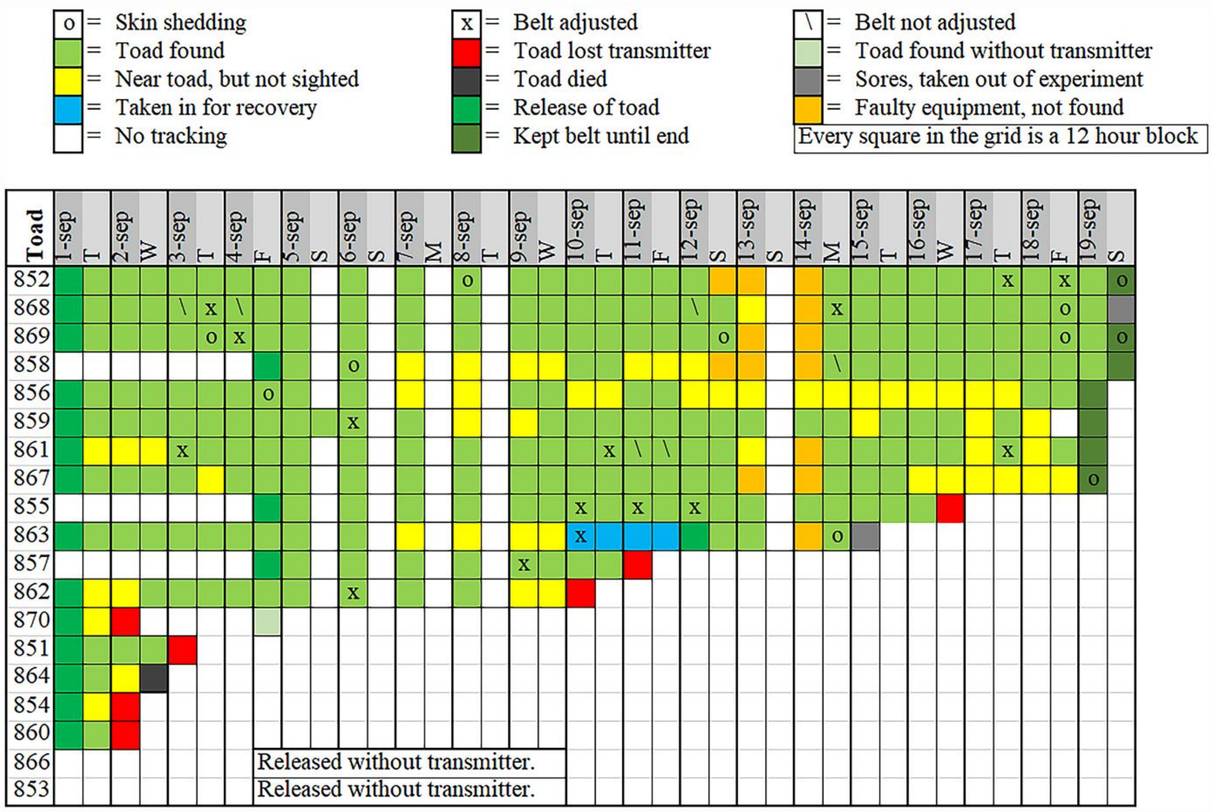

Figure 4. *Tracking record for 17 released green toads equipped with transmitter waist belts and two green toads without waist belts. The toads were tracked one or two times per day.*

Discussion

Tracking animals with radio telemetry is a suitable method when investigating activity, movement, and behaviour of animals, especially for animals such as the *B. viridis*, a night-active and well-camouflaged species. Our study shows that with the proper equipment, the RT technique can be considered low-invasive on *B. viridis*. Based on our results and the results from Indermaur et al. (2008), the only RT-studies on *B. viridis* that we are aware of, we suggest some insights and possible fields of development, both from an animal welfare and radio tracking point of view.

Mortality

Only one toad was found dead, early in the experiment, possibly from being trampled by cattle. It is unlikely that the waist belt directly contributed to its death. However, the waist belts may affect movement and agility and can thereby possibly increase mortality, either by decreasing the ability to escape predators *(Blomquist & Hunter, 2007; Sinsch, 1989)* or as shown in this study, by entanglement. Too long intervals between check-ups could therefore result in higher mortality from starvation or predation of entangled toads. However, this problem will most likely be small if using transmitters with an attachment point that would not allow the transmitter to flip to a forward position, since this causes the antenna to entangle.

Waist belt related injuries

We recorded two individuals with lacerations. This number is comparable with other studies (Bartelt & Peterson, 2000; Fellers & Kleeman, 2007; McAllister et al., 2004; Rathburn & Murphey, 1996). Some of these studies have a longer tracking period than our study, which might have led to a higher number of injuries. However, it is difficult to say if it was the frequent checks, waist belt design, water rinsing and removal of dead skin to reduce friction, or all in combination that contributed most to the low frequency of lacerations.

Due to difficulties in objectively recording belt tightness on every occasion, we did not register this. We aimed at keeping belts as loose as possible but still tight enough for the toads not to lose them. The high frequency of shed belts in the study could indicate however a too loose fit and a tighter fitting could have been used but on the other hand, this was very individual between the toads.

One of the two toads with skin injuries, was found entangled and exhausted. This is a strong indication that its efforts to break free caused the skin injuries. Our observations are thus in line with the observations of Bartelt & Peterson (2000) and Rathburn & Murphey (1996), who found that skin injuries developed when the waist belt was fitted too tight and that such injuries healed after the belt was loosened, as was also the case in our study.

Waist belt fit and shedding

Shedding of RT waist belts occurred in seven out of 17 toads, and it seems to be common also in other amphibian studies using RT. Our toads shed waist belts at a similar or higher rate (41.2%) compared to studies of other Anuran species (*Rana lessonae* and *Rana esculenta*, 8.3% (Holenweg & Reyer, 2000); *Rana draytonii*, 22% (Fellers & Kleeman, 2007); *Rana pretiosa*, 34% (McAllister et al., 2004); *Bufo boreas*, 36.8% (Bartelt & Peterson, 2000); *Rana draytonii*, 51.1% (Rathburn & Murphey, 1996); *Bufo b. spinosus* and *Bufotes viridis*, 41% and 56% (Indermaur et al., 2008)). It should be noted that the belt designs vary a lot (e.g. polyethene tubing, aluminium beaded chain, belt materials like copper, nylon, catgut, or cotton) between these studies. We know of no comparison between different belts, thus such a comparison seems like an important area for further study. As seen in the laboratory test it was also a great difference between different individuals in the fitting, hence a larger test group would be recommended.

The behaviour of the species also effects the fitting of the belts. For example, the burrowing habits of the *B. viridis* in sandy soils, probably makes it harder to fit the belt so it is not shed, compared to more aquatic or arboreal species.

Toad	15/12/2020	16/12/2020	17/12/2020	18/12/2020	19/12/2020	20/12/2020	21/12/2020	**Total shed**
female1	0d	0d	0	0	0	-	0	**0**
female2	2d	3/d	1*d	1*b	0	-	0	**7**
female3	0d	1d	1d	0	0	-	0	**2**
male1	0d	1d	1d	1*b	0	-	0f	**3**
male2	0d	0	0	0	0	-	0	**0**

	=	Wet fitting method
	=	Dry fitting method
#	=	Amount of belt sheddings
d	=	Bloating and/or dragging hind legs (discomforted behaviour)
/	=	Loosened belt
*	=	Tightened belt
f	=	Transmitter flipped forward
b	=	Belt fitted with bumpy side in contact with skin
-	=	No observation made

Figure 5. *Results of belt fitting test on five green toads under laboratory conditions.*

One suggested improvement of our RT waist belts would be to use a transmitter with an attachment point on the very tip to make it easier for it to flip back when entangled in vegetation, which would decrease the risk of getting stuck and thereby shedding the belt. Fitting belts in laboratory conditions so that adjustments to the tightness of the belts can be made before release in the field, also seems highly recommendable to reduce shedding. In our laboratory pilot study, waist belt fit seemed to be highly individual, where some toads kept their belt on the initial test period, and others had to be refitted multiple times. There is too little data about the wet versus dry-fitting method to draw any conclusions. However, it seems that the dry fitting method in combination with tightening the waist belt is favourable when toads repeatedly shed their belts. The results may be indicative of favourable fitting methods, but more testing is needed to confirm this.

If the waist belt technique is not markedly improved in these perspectives, it is highly advisable to start with a substantially higher number of belts than what is needed from a data collection point of view (Bartelt & Peterson, 2000; Groff et al., 2015; Rathburn & Murphey, 1996).

Like in the study of Langkilde & Alford (2002), our toads showed a change in behaviour up to 48 h after fitted with the waist belt. This implies a need to fit waist belts in controlled conditions well before releasing in the field, to minimize the risk of influencing the habituation of the toads to the new environment.

Conclusions

Our study shows that using radio telemetry waist belts to track and monitor released *B. viridis* is an appropriate and effective method, with respect to animal welfare, with low mortality and a low frequency of skin injuries. Our study gave valuable insight for further improvements to reduce belt shedding by testing other transmitter designs, new materials, and other ways of fitting the waist belts on the toads. In addition to this, belt tightness and material are essential to understand lacerations in amphibians. There is much potential for improvements in this field, both in finding appropriate belts for a given species and considering various behaviours expressed in different species.

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