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Telemetry tagging methods for some freshwater reptiles

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Abstract. Reptiles are often ignored or under-studied in freshwater systems. An understanding of their biology and thus their role in aquatic communities can be greatly advanced by studies using radio telemetry. In turn, the value of radio telemetry for research depends on the availability of suitable and reliable methods of attaching or implanting radio transmitters. The present study describes transmitter attachment and implantation techniques for selected freshwater reptiles, including the eastern and northern long-necked turtles (*Chelodina longicollis* and *Chelodina rugosa*, respectively), the pig-nosed turtle (*Carettochelys insculpta*), Mertens' water monitor (*Varanus mertensi*) and the water dragon (*Physignathus lesueurii*). The effectiveness of the methods for each species is described and the potential pitfalls and challenges of each method are discussed. The literature abounds with techniques for attachment and implantation techniques, and the methods used in the present study are not wholly novel. The aim, however, is to provide detailed summaries, in one paper, of effective methods for attachment and implantation of radio-tags for freshwater reptiles with a diversity of sizes, shapes and attachment surfaces. Despite the focus on Australian freshwater reptiles, these methods are applicable to aquatic reptiles worldwide.

Additional keywords: *Carettochelys insculpta*, *Chelodina longicollis*, *Chelodina rugosa*, *Physignathus lesueurii*, radio telemetry, *Varanus mertensi*.

Introduction

Reptiles are often ignored or under-studied in freshwater systems (Gibbons 2003). This oversight is significant given that aquatic reptiles can achieve high densities and biomass (Iverson 1982; Shine 1991) and can occupy upper trophic levels in their communities (e.g. crocodiles, monitor lizards and pythons). As a result, the success of reptiles would be expected to induce cascading effects in their respective food webs (Pace *et al*. 1999). In addition, many species of freshwater reptiles are imperilled (i.e. threatened or endangered); however, the mechanisms causing the declines and ways to effectively manage for their recovery remain elusive as a result of our limited knowledge of their biology (Gibbons *et al*. 2000; Klemens 2000).

An understanding of the biology of freshwater reptiles and their role in aquatic communities could be further advanced by studies using radio telemetry. Radio telemetry remains the most popular method for repeatedly locating animals (White and Garrott 1990). The technique allows researchers to maintain intimate contact with their study animals, providing the opportunity to answer a range of questions regarding animal biology and conservation. Traditionally, radio telemetry has been critical for determining a species' spatial ecology, movements and habitat use, and useful in studies of feeding, reproduction, energetics, thermoregulation and refuge use, among other behaviours. The longevity of radio telemetry, however, also results from its usefulness in combination with other techniques (e.g. Eiler 1995; Broseth and Pederson 2000).

Reptiles make good subjects for radio telemetry for several reasons. First, the adults of most species are large enough to carry transmitters with sufficient battery life for several months of tracking. Second, the hard surfaces of some reptiles, such as turtles and crocodiles, facilitate easy radio-tag attachment and the long bodies of snakes and goannas are well suited for implanting radio-tags and associated aerials. Third, reptiles are secretive and many remain inactive for much of the day or season, contributing to our ignorance of their basic biology (Zug *et al*. 2001; Pough *et al.* 2003). In many cases, an understanding of their biology may only be revealed through intensive radio tracking (e.g. case studies herein).

The value of radio telemetry for research depends on the availability of suitable methods of attaching or implanting radio-tags (Pietz *et al*. 1995). To be effective, attachments must not only be secured for the duration of the study, but they must also only minimally influence the natural behaviours and health of the individual. We summarise the radio-tag attachment and implantation techniques that we have developed or modified for selected Australian freshwater reptiles: the eastern and northern long-necked turtles (*Chelodina longicollis* Shaw and *Chelodina rugosa* Fry, respectively), the pig-nosed turtle (*Carettochelys insculpta* Ramsay), Mertens' water monitor (*Varanus mertensi* Glauert) and the water dragon (*Physignathus lesueurii* Gray). Each of these species presents some unique challenges for successful radio-tag attachment and implantation. We do not include crocodilians because these are covered in detail elsewhere

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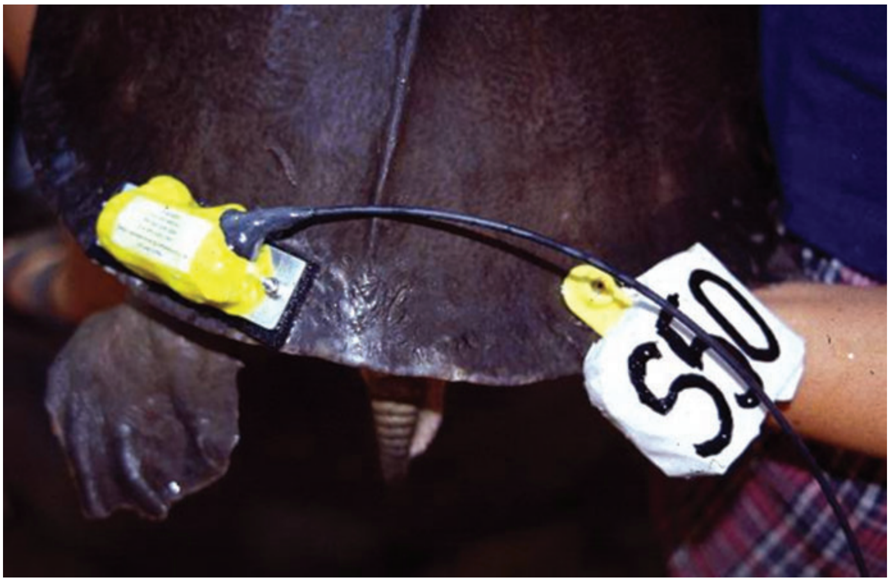

Fig. 1. Adult pig-nosed turtle (*Carettochelys insculpta*) showing a radio transmitter attached to the rear carapacial edge (left). The transmitter is ~6 cm long.

(Franklin *et al.* 2009) or snakes because their implant technique is similar to that for water monitors (Weatherhead and Anderka 1984). We recognise that these methods have been used in numerous studies worldwide that would include various modifications, but it is not our purpose to review these (see Boarman *et al*. 1998 for a review on attachment techniques for turtles). Rather, our aim is to provide a summary, in one paper, of some effective attachment and implantation methods for ecologists interested in using radio telemetry to study reptiles in freshwater systems.

External attachment in turtles

Long-necked turtles are common, freshwater species inhabiting dams, billabongs, swamps, rivers and creeks across much of Australia (Cogger 2000). The eastern long-necked turtle (*Chelodina longicollis*) occurs over much of temperate eastern Australian and the northern long-necked turtle (*Chelodina rugosa*) occurs in tropical New Guinea and in Australia from Cape York through to the Kimberly (Cogger 2000). The pig-nosed turtle (*Carettochelys insculpta*) is a freshwater species inhabiting rivers in New Guinea and the Northern Territory, Australia (Cogger 2000).

Compared with other reptiles, attaching radio-tags to turtles is rather straightforward because the carapace provides a site to securely fasten a tracking device. However, transmitters can fall off if not properly attached, and several researchers have reported adverse effects associated with the attachment process or the burden of carrying a transmitter (reviewed in Boarman *et al*. 1998). Many potential problems can be minimised if care is taken with the attachment method and location of the transmitter on the shell. We outline different modifications of a technique that we have used successfully on these turtles. Horny scutes cover the bones of the carapace in long-necked turtles, whereas only a thin skin covers the carapacial bone in the pig-nosed turtle.

We have attached two different styles of transmitters to turtles, both of which require attachment via bolts or other fasteners through holes drilled into the carapace. We have used Holohil Systems (Carp, Ontario, Canada), models SB-2F, SI-2F and AI-2F (6–30 g, 19–46 mm length (L), 9.5–17.0 mm diameter (D)), in which the battery and transmitting unit are hermetically sealed in a cylindrical brass case mounted onto two bendable end tabs with holes for attachment. We have also used Sirtrack (Havelock North, New Zealand; http://www.sirtrack.com) VHF transmitters (12–25 g, 25–50 mm L, 20–25 mm width (W)), where the battery and transmitting unit are sealed in waterproof epoxy and mounted onto aluminium plates with holes at either end for attachment. Each of the models is fitted with an ~20–25-cm whip antenna. Only slight modifications are required to accommodate the different transmitter designs and turtle shells.

Our preferred site of attachment is on top of the posterior marginal scutes and underlying the peripheral bones of the carapace, typically in a region above one of the hind limbs (Fig. 1). This region provides a broad platform for attachment and here the radio-tag is less likely to create drag or impede movement through substrate or vegetation. Placement of the radio-tag on the most posterior scutes (i.e. directly above the tail) may interfere with mating behaviour when males mount females.

We start by clearing the attachment area of any debris or algae and then drying it. Next, we pre-place the transmitter on the shell (with the aerial trailing behind) such that the holes in the end tabs or plates are aligned as closely as possible to the middle of two scutes in the long-necked turtles. Holes drilled too close to the outside edge can easily open from wear, creating a gap through which the fastener may slip. Holes drilled too close to the scute seams often result in excessive bleeding because this area is actually aligned with the centre of the underlying marginal bone (i.e. the scutes and underlying bones are offset), where an artery feeds the marginal bone. Drilling on or near the artery can cause necrosis of the marginal bone, leading to loss of the marginal bone and the radio-tag. Because pig-nosed turtles have no scutes, it is critical to ensure that the holes are drilled on or near the seams between the marginal bones, which are clearly visible beneath the skin, in this species. All other Australian turtles have hard scutes covering the marginal bones except for leatherback sea turtles, *Dermochelys coriacea* (Cogger 2000).

Pre-placement of the radio-tag serves two purposes. First, we mark the spots to be drilled, and second, we assess how the transmitter fits in relation to the curvature of the shell. The margins of the carapace are variably curved on each individual, presenting either a convex, concave or flat surface. For units mounted on flat metal plates (e.g. Sirtrack models), the attachment surface needs to be reshaped because any bending of the plate tends to crack the epoxy that fastens the transmitter to the plate. We use a putty epoxy (Shelleys Aqua Knead-it, Padstow, NSW, Australia), which is available in most hardware stores, to flatten the attachment surface. By applying the epoxy to the desired areas of the transmitter plate and pressing the transmitter and epoxy firmly onto the shell, a flat surface for attachment results. The epoxy sets in ~20 min (even in water), effectively filling any gaps between the plate and the curved shell. Transmitters with flexible end tabs (e.g. Holohil models) can more easily fit different degrees of curvature – the tabs can simply be bent to accommodate the different surfaces. In pig-nosed turtles, instead of using the epoxy, we cut strips of neoprene to the size of the transmitter (Fig. 1). The neoprene serves to soften the pressure of the radio-tag against the skin.

After the holes are drilled into the carapace, the radio-tags can now be attached using stainless steel bolts, locking nuts threaded with nylon to prevent loosening, and corrosion-resistant metal backing plates. The use of non-stainless steel bolts results in rust, which can cause infection. The backing plates function as washers, spreading the force of the bolt head over a larger area. Backing plates are only needed on the bottom, as the mounting plates or end tabs serve that purpose on the top. A number of manufacturers provide backing plates (e.g. Sirtrack), but we often find that modifications are necessary. For instance, Sirtrack provides a plate identical to the one that the transmitters are mounted onto, but we find it difficult to make the pre-drilled holes of the two plates align. We find that it is much easier to use two separate plates. Thin sheets of aluminium are cut to size, approximately equal to that of the scute in which the hole is drilled, and then bent to match the curvature of the scute. When cutting metal, it is important to ensure that any sharp or rough edges are sanded smooth to prevent injury to the turtle. When fastening the radio-tag to the shell, the nut should be on the top (transmitter side) to allow the turtle full leg movement (Fig. 1). We have observed abrasions on turtle legs when the nut or bolt extends too far below the carapace. Similarly, the threaded bolt should not pass much above the nut on top because excessive protrusions can more easily become entangled in vegetation or debris. Having several bolts of different lengths on hand allows an appropriate match to be made. Bolts can be cut to size using an angle grinder or hacksaw.

Once the radio-tags are securely bolted to the turtle, a few minor modifications may be necessary to ensure that the radio-tag is securely attached and safe for the turtle. We fill any gaps between the radio-tag and the shell with putty epoxy to prevent vegetation, debris or rubbish (e.g. fishing line, plastic bags) from becoming entangled. We have only observed three instances of radio-tags becoming entangled. In the first, the aerial became wrapped around thick vegetation. In the other two, the nuts became entangled in the thin nylon mesh of turtle traps. To prevent this latter problem from happening, we now fill all gaps around the edges where the nuts and bolt heads contact the plates with putty epoxy. This method of attachment routinely takes <15 min per turtle in the field and the unit can be removed just as quickly.

We have used this method to attach 191 Sirtrack models to 132 *C. longicollis* and 17 *C. rugosa* and 24 Holohil models to 24 *C. longicollis*. No radio-tags became detached and individuals were tracked for up to 2 years (Rennie 2002; Roe and Georges 2008). We have also used this method to attach Sirtrack models to 63 *C. insculpta* for up to 3 years (Doody *et al*. 2003; Davies 2005). Initially with *C. insculpta*, several of the units had fallen off (with necrotic marginal bones) by the end of the first year of the study because of the problems described above in the placement of the drill holes away from the bone seams. However, proper positioning of the units thereafter has yielded >95% success. In the nearly 300 attachments, only three radio-tags have become entangled, and the problem in two of these instances was corrected by filling the gaps with epoxy to streamline the attachment surface. All of the turtles that we tracked have been adults, so we cannot comment on the effectiveness of this attachment method in juveniles (the faster-growing life-history stage).

Surgical implantation into monitor lizards

Varanus mertensi is a semiaquatic goanna inhabiting many watercourses, billabongs, springs and soaks in tropical Australia (Cogger 2000). These goannas also inhabit anthropogenic water bodies such as dams and irrigation areas. Not surprisingly, large numbers of *V. mertensi* can be found in the various lakes and channels of the Ord River Irrigation Scheme in the East Kimberley of Western Australia, where the present methods were used.

Captured individuals were anaesthetised using inhaled isoflurane ether (Nichloas Piramol India, Mumbai, India). The head of a restrained animal was placed in a sealed plastic bag containing several cotton balls soaked in ~5 mL of isoflurane. The anaesthetic took ~5–15 min to take effect, although in some cases where individuals initially held their breath it took up to 20 min. Animals were deemed fully anaesthetised when pulmonary and cardiac activity ceased and their eye lids remained closed. A pinch test was applied to the tail of the animals to assess loss of touch sensation before continuing with the surgical procedures. Animals were not ventilated during surgery to avoid exhalation of the isoflurane inhalant anaesthetic.

An area ~10 cm × 10 cm on the lateral side of the abdomen, 2–3 cm forward of the hind limbs was cleaned with 70% chlorhexidine solution (Pfizer, Bentley, WA, Australia). All surgical instruments and the operating area were also sterilised with chlorhexidine, as were the radio transmitters. A small incision, 2–3 cm, was made longitudinally in the area treated with chlorhexidine using surgical scissors. After separation of the skin and intact muscle layers with forceps, access was gained to the abdominal cavity. Transmitters (3 g, 2.5 cm × 0.6 cm) with an external whip antenna (length ~10 cm) were inserted into the abdomen (Holohil Systems, Model 2I-T). The transmitters represented a range of 0.003–0.001% of the weight of the animals; the animals ranged in body mass from 1000 to 3000 g. The transmitters were positioned longitudinally within the abdominal cavity. A thin-walled brass tube with a 1-mm diameter and a length of 20 cm was threaded between the skin and muscle layers from the anterior point of incision along the lateral fold of the animal to just behind the head. The external antenna was positioned inside the tube and the tube was removed through a small incision made just behind the head, leaving the antenna in place under the skin along the lateral fold. This technique ensured that the antenna was straight and provided maximum signal strength and hence detection range. The small incision behind the head, made for the extraction of the brass antennae leader, was sealed using Vetbond (3M Veterinary Tissue Adhesive, St Paul, MN, USA). The larger incision in the ventral surface was closed with dissolvable sutures (Mono-dox 3/0 19 mm suture needle; Medcare, Cleveland, OH, USA). Suturing began 1–2 mm behind the cut surface of the skin and the two surfaces were held neatly together and sutured. Sutures were covered with Vetbond to assist in the adhesion of the closed incision and to prevent the sutures being exposed to the environment until the Vetbond had abraded away.

Animals were revived from the anaesthetic by inserting a small flexible plastic tube (surgical-grade Silastic), with a diameter of 2 mm, through the glottis to a depth of ~5 cm. Animals were artificially aspirated, via this tube, until most of the isoflurane within the lungs was removed. In general, two or three breaths into the lungs were sufficient for this task. Following removal of the isoflurane, the animals usually showed signs of increased heart rate within minutes. Most animals had full pulmonary function within 5–10 min. Recovering animals were assessed continually for both increased cardiac and pulmonary activity. In some instances, animals did not immediately regain strong cardiac activity. On these occasions, gentle cardiac massage was used until strong cardiac activity was noted. After establishing strong cardiac activity, pulmonary activity normally followed. After cardiac and pulmonary functions were re-established, the individuals usually woke within 10–15 min. Recovering individuals were held overnight to fully recover before being released at their point of capture the following day.

Surgical implantation of radio-tags using this technique revealed minimal long-term effects on observed individuals over the 2-year study period. During the study, 50 adults were surgically implanted with radio-tags in watercourses of the Kimberley region. Of these 50 individuals, 37 continued to be successfully located >16 days after implantation (Mayes 2007*a*), with four individuals tracked successfully for 2 years following implantation (Mayes 2007*a*). The ultimate fate of the remaining 13 (26%) individuals not located >16 days after surgery is not known.

The results of the study show that meaningful movement data, ranging up to 2 years after transmitter implantation, was obtained from 74% of *V. mertensi* surgically implanted with radio-tags (Mayes 2007*a*). The survival rate of *V. mertensi* following surgical implantation cannot be determined confidently, but may be higher than 74% given the unknown fate of the remaining *V. mertensi* not relocated >16 days after surgery. Our findings suggest that *V. mertensi* make seasonal movements between distant waterbodies, possibly in search of greater prey resources (Mayes 2007*a*, 2007*b*). Thus, it is likely that these individuals represent widely dispersing individuals following radio-tag implantation.

External attachment in water dragons

The water dragon (*Physignathus lesueurii*) is a medium-sized (up to 1 m in total length), semiaquatic lizard inhabiting riparian areas of rivers, streams, lakes and well-vegetated ponds and swamps in eastern Australia (Cogger 2000). Although generally terrestrial, water dragons often sleep in water (Thompson 1993; Turner 1999), and commonly dive into water when they feel threatened (Barnett 1931; Retallick and Hero 1994).

The basic design of the radio-tag attachment was a backpack or harness (Fisher and Muth 1995; Richmond 1998), but the details closely follow the design used by Warner *et al*. (2007) for the congeneric jacky dragon (*Amphibolurus muricatus*, a smaller, non-aquatic species; Cogger 2000). The harness was cut from a sheet of nylon mesh or flyscreen in the shape of a square with two extending straps (see Fig. 1 in Warner *et al*. 2007). The size of the square and its straps depended on the size of the animal, which varies markedly between the sexes in *P. lesueurii* (Cogger 2000). If the harness is too large it may get caught on vegetation, and if it is too small it may cause discomfort or abrasion in the lizard. However, correct sizing was easily accomplished with some trial and error (in hand). The straps, which can be 10 cm long, are trimmed to size once the harness is attached. The width of the straps should be ≥4 mm to provide adequate strength.

Once the harness is cut to the appropriate size, the radio transmitter can be glued to the harness using superglue (UHU Australia; Bolton Group, Buhl, Germany), before attaching the harness to the animal. Other glues can be used, but the glue must be waterproof and strong. The transmitter is positioned in the centre of the square with the antenna protruding in the opposite direction to the straps. Although the glue is fast-drying, better results were achieved by waiting 1 h for the glue to dry.

Once the glue has set, the harness can be fitted and attached. Attaching the harness generally requires two people (Warner *et al*. 2007). After placing the harness in the centre and holding the square part of the harness firmly on the lizard's back above the forearms, the straps are then pulled over the shoulders, crossed on the chest, and pulled up under the lizard's forearms. After pulling the straps snug, they are superglued to the square, and to one another where they cross on the chest (Fig. 2). The mesh should be held together at the glue points with forceps or another object with a non-stick surface. During this time, the lizard should be carefully restrained. We inserted duct tape under the glue points to prevent the harness from being glued to the lizard's skin (Fig. 2). However, Warner *et al*. (2007) purposely glued the harness to the body (both dorsally and on the chest) to prevent the harness from shifting during their study. They did not remove the mesh that was glued to the skin after the study, choosing instead to cut the rest of the harness off, leaving a small bit of mesh to be sloughed off within 1 month of removal. Our harnesses were easily removed by cutting the straps with scissors.

This technique was utilised on 11 water dragons for up to two months without any harness losses or any noticeable problems for the lizards. One strap of one harness was nearly torn off, possibly due to the harness snagging on vegetation. Warner *et al*.

Fig. 2. Sub-adult water dragon (*Physignathus lesueurii*) fitted with a radio-transmitter harness.

(2007) also experienced a similar problem with the harness of one individual, and some of their animals became entangled in vegetation. Warner *et al*. (2007) outlined the advantages of using nylon mesh over materials used previously (Fisher and Muth 1995; Richmond 1998), and our results indicate that the method is also useful for the semiaquatic *P. lesueurii*. In particular, the technique was robust to potential problems created by water, at least in the short term. The mesh material does not get waterlogged nor does it appear to break down, at least over a 2-month period. One lizard was found dead of unknown causes, but there was no evidence of problems created by the harness. We made several observations of animals with harnesses running, jumping, climbing and hiding without any obvious effects of the harness. Therefore, we reiterate the assertion of Warner *et al*. (2007) that the harness is an effective means of attaching radio-tags to lizards, and extend this recommendation to aquatic lizards. However, we recommend further assessment of the method. For example, our study was short term (e.g. weeks); the effectiveness of these harnesses over several months should be evaluated, both in captivity and in the field.

Summary

Reptiles are important components of aquatic communities, yet our understanding of their biology is limited (Gibbons *et al*. 2000; Klemens 2000). Radio telemetry can be an effective method for studying many aspects of field-active reptiles, but only if the device attachment and implantation techniques are reliable and safe for the animal. The decision of whether or not to attach or implant should be based on careful consideration of the attachment surfaces, body size and battery life/size, behaviour and habitats. In addition, some animals may not recover well from surgery. There are numerous published works outlining attachment/implantation techniques for reptiles, including freshwater species. However, our methods provide a good starting point for those interested in using radio telemetry to study freshwater reptiles. Aquatic animals often require additional considerations compared with terrestrial animals, and it is our hope that the methods outlined here will help pave the way for more research on these under-studied components of aquatic ecosystems.

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