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Improved procedure for implanting radiotransmitters in the coelomic cavity of snakes

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Abstract

Objective To investigate the expulsion of radiotransmitters in snakes and modify the surgical technique for coelomic implantation to prevent its occurrence.

Design To enable monitoring of snakes for an ecological study, radiotransmitters were implanted in 23 south-west carpet pythons (Morelia spilota imbricata) using the standard surgical technique. In a further 23 pythons we used a refinement of the technique, which anchored the tracking device, using non-dissolvable sutures, to the snake's rib-cage. We also
investigated the potential mechanisms for expelling the radiotransmitters in one snake that underwent an exploratory coeliotomy.

**Results** Of the initial group of snakes, 12 (52%) expelled the radiotransmitter between 4 days and 3 years post implantation. In the later group, which underwent the refined technique of implantation, none of the radiotransmitters was expelled and no adverse responses were observed.

**Conclusion** An appropriately sized radiotransmitter anchored to the rib-cage of the snake will prevent expulsion of the device and appears to be well tolerated. Non-attachment of the tracking device enables it to migrate along the length of the body, particularly during feeding and reproduction. Caudal positioning of the transmitter's antenna provides a possible pathogenesis for expulsion into the cloaca.

**Keywords:** coelomic cavity; pythons; radiotelemetry; radiotransmitter; surgical implantation

The use of telemetry has revolutionised the way in which many animal species, from fish through to elephants, can be studied.\(^1\) Radiotelemetry was first used in snakes in the late 1960s.\(^2\) In that study, 68 individuals of eight species of snake were force-fed a radiotransmitter, which lodged in their stomachs, and to prevent regurgitation, a nylon thread was tied around the body of the snake, anterior to the stomach, and sutured to the ventral scales.\(^2\) This methodology enabled telemetric research and led to increased interest in studies of snake ecology, including spatial and movement analysis, oviposition and winter hibernacula site selection, and thermoregulation.\(^2-9\) However, the limitations of force-feeding radiotransmitters to snakes became apparent during the early 1980s. Firstly, those
Radiotransmitters did not have antennas attached and therefore signal range was limited. Secondly, it was believed the presence of the radiotransmitter in the stomach caused changes in foraging behaviour and physiology. Thirdly, long-term studies were difficult to plan because of the uncertainty of the snake regurgitating the radiotransmitter at any stage. In recognition of this, Reinert and Cundall\textsuperscript{10} developed a method of implanting radiotransmitters equipped with a whip antenna to increase the range of the emitted signal. Snakes were anaesthetised with halothane gas and the radiotransmitters were surgically implanted in the coelomic cavity just anterior to the gonads, with the whip antenna extending through the musculature of the body wall and running subcutaneously in a cranial direction along the length of the snake. Subsequently, Weatherhead and Anderka described a subcutaneous implantation technique for both the radiotransmitter pack and antenna, but suggested this method was problematic for species or individuals smaller than black rat snakes (\textit{Elaphe obsoleta}) (i.e. < 375 g), as the subcutaneous bulge created could hinder the snake's movement and affect the size of shelter sites that could be selected.\textsuperscript{11}

Few radiotelemetry studies of snakes have outlined techniques to improve radiotransmitter implantation, although Anderson and Talcott advocated the need for increased reporting of outcomes of this type of procedure, in order to identify best-practice modern techniques and associated complications.\textsuperscript{12} Pearson and Shine\textsuperscript{13} reported a significant problem with surgically implanting radiotransmitters into the coelomic cavity of carpet pythons (\textit{Morelia spilota}). They implanted radiotransmitters with antennas in the coelomic cavity of 75 pythons (with the antenna directed posteriorly) and 14 (18.7\%) subsequently expelled it. The authors suggested that the pythons were able to absorb the radiotransmitter into the gastrointestinal tract and expel it with the faeces. A consequence of the loss of tracking devices is that animals are unable to be located in the field and are lost to the study, which is costly in terms
Materials and Methods

Study animals

Our study group comprised 46 south-west carpet pythons (*M. s. imbricata*) captured from several sites in southwest Western Australia: 25 females (average body mass 1535.5 ± 146.8 g, average snout–vent length 177.3 ± 9.0 cm) and 21 males (average body mass 658.0 ± 53.6 g, average snout–vent length 130.6 ± 3.5 cm). All pythons were captured between 2004 and 2008 and brought into a captive holding facility at the Department of Environment and Conservation, Dwellingup Research Centre, Dwellingup, Western Australia for surgical implantation of radiotransmitters. Pythons were generally held in captivity for 2 weeks to perform the surgery and allow a short recovery before release at the point of capture.

Radiotelemetry device

Five different sizes of radiotransmitter with a whip antenna (Holohill® models: 5 g SB-2, 11 g S1-2T, 13.5 g S1-2T, 16 g A1-2T, 25 g A1-2T; Holohil Systems Ltd, Canada) were used to ensure the radiotransmitter's mass constituted <5% of the body mass of each fasted python (i.e. no recent meal determined by palpation). The radiotransmitters ranged in operational life from 50 to 150 weeks, depending on battery size. The battery unit was covered in a
physiologically inert wax and the antenna (15 cm long) was coated with a fine, flexible, inert tube.

**Surgical procedure**

Each python was anaesthetised using isoflurane (Forthane, Abbott Pty Ltd) inhalational anaesthesia and maintained at preferred body temperature (PBT) of approximately 26°C by placing it on heat pads and conducting the procedure in a heated room. During induction of anaesthesia, each python was physically restrained and, while conscious, intubated with a lubricated uncuffed endotracheal (ET) tube of an appropriate size. (A small drop of local anaesthetic (e.g. lidocaine diluted to 1%) is recommended to desensitise the glottis.)

Once the animal was intubated, the ET tube was carefully secured around the jaw using Micropore® tape and the anaesthetic circuit was connected. Anaesthesia was induced using intermittent positive pressure ventilation (IPPV) at approximately 12 breaths/min, with 5% isoflurane gas in oxygen (at 1.5–2 L/min). Surgical anaesthesia was maintained with IPPV at approximately 2–4 breaths/min on 2–3% isoflurane gas in oxygen at a rate of 1.5–2 L/min. The python's heart rate was monitored during the procedure using a small vascular Doppler (Hadeco Echo Sounder, Doc Stock Medical Supplies, Sydney, NSW, Australia) at the position of the heart, in addition to monitoring superficial skin perception (i.e. level of response to gentle tactile stimulation), pain perception and righting reflexes. Pythons were recovered from anaesthesia by stopping the isoflurane and maintaining IPPV at 2–4 breaths/min with 1.5–2 L/min oxygen for 5 min, then using a paediatric resuscitation bag (Ambu bag™) to ventilate with room air until spontaneous breathing or voluntary movement.
was observed. At this point, the python was extubated and returned to a warm enclosure to complete the recovery process.

**Coeliotomy.**

Surgical implantation of the radiotransmitters was achieved via routine ventral coeliotomy.\textsuperscript{17–19} The surgical site was prepared with 3\% chlorhexidine in 70\% alcohol and an incision was made longitudinally at the junction between the first and second row of ventrolateral scales. The incision was no longer than 5 cm and located anterior to the cloaca by approximately 30\% of the snake's snout–vent length, ensuring it was caudal to the lung (Figures 1, 2). The incision was continued through the body wall to open the coelomic cavity to allow implantation of the transmitter.

It is generally considered ideal to sterilise the transmitters using ethylene oxide, zephiran chloride or gamma irradiation.\textsuperscript{20} However, for logistical reasons, in this study each radiotransmitter was soaked prior to implantation in a disinfectant solution of F10SC Veterinary Disinfectant at a dilution 1:125 for a minimum of 2 h and then rinsed with sterile saline solution (Baxter Healthcare Pty Ltd) immediately before insertion in the python's coelomic cavity.

In most cases the radiotransmitter was positioned with the antenna directed cranially, but in the first three surgical procedures the antenna was directed caudally, as in the previous study.\textsuperscript{13} After one of the pythons expelled the radiotransmitter, the procedure was modified and radiotransmitters were subsequently implanted with the antenna positioned cranially,
because it seemed likely that the caudal position of the antenna was associated with the device's expulsion.

Absorbable suture material (Vicryl 3/0©, Ethicon Inc.) was used to close the muscle layer in a continuous pattern, and the skin with horizontal mattress sutures in an everting pattern (polydioxanone 3/0©, Ethicon). An everting pattern is recommended for suturing reptilian skin, because of its strong tendency to invert and because the scales prevent true apposition.21,22 A waterproof transparent dressing with an absorbent pad (OpSite post-op®, Smith & Nephew, VIC, Australia) was used to cover the incision site and secured in place with an adhesive dressing (Elastoplast Sport Elastowrap, Beiersdorf Australia, North Ryde, NSW, Australia). As standard supportive postoperative therapy, each python was given a non-steroidal anti-inflammatory drug (NSAID) via intramuscular injection (meloxicam 0.2 mg/kg: Metacam®, Boehringer Ingelheim, North Ryde, NSW, Australia) in the paravertebral muscles for anti-inflammatory treatment and pain relief.16,21 Warmed fluids (normal saline and 5% glucose at a ratio of 1:1 at 20 mL/kg) were administered subcutaneously. Both injections were administered in the cranial third of the snake's body.

Radiotransmitters were placed as described in the coelomic cavity of 23 pythons captured early in the study. A further group of 23 pythons underwent a refinement of the technique. Prior to implanting the radiotransmitter, non-absorbable suture (Prolene 3/0©, Ethicon Inc.) was tightly wrapped twice around its base near the junction of the antenna (where there are two small ridges on the wax covering of the unit) and tightly knotted with two square knots. Without cutting the suture, another two square knots were made to create a small (∼0.2 mm) loop between the knots (Figure 3a). The radiotransmitter was then positioned in the coelomic
cavity as described and the uncut suture material was carefully looped around a rib by
passing the needle around the rib and associated muscle tissue from the caudal surface of the
rib through to the cranial surface (Figure 3b). The needle was then passed back to the
coelomic cavity and through the small loop between the square knots and the transmitter
secured using several square knots (Figure 3c). The suture material was cut and the surgical
incision site was closed as before.

**Radiotransmitter removal.**

The surgical technique for device removal followed that for implantation, with the suture
surrounding the ribcage being cut and the radiotransmitter and attached suture material being
carefully removed from the python's coelomic cavity. The site was sutured closed and the
snake recovered from anaesthesia as per the standard procedure.

**Recovery and monitoring**

After surgery the pythons were maintained in a warm (PBT ≈26°C), quiet environment for a
minimum of 5–7 days while being monitored for signs of postoperative complications. The
dressing remained on the wound for up to 5 days and was removed before the python was
released into the field. Pythons were then monitored by radiotelemetry (usually weekly) as
part of a broader research project carried out over 3 years.
All procedures were approved by Murdoch University Animal Ethics Committee W2028/07 and the Department of Environment and Conservation Animal Ethics Committee DEC AEC/55/2006 and DEC AEC 54/2006 permits.

Results

Initial radiotransmitter implantation technique

Of the 23 pythons with radiotransmitters implanted using the standard technique without ribcage-anchoring, 12 (52%) expelled the device between 4 days to more than 3 years (mean 259 ± 103 days) post surgery. Only 3 of the 12 expelled radiotransmitters were found to be associated with faecal material. The python that expelled the radiotransmitter after 4 days was still being held in captivity for postoperative recovery. It had been fed two small mice the day following surgery and the expelled radiotransmitter was contaminated with faeces. On examination of the surgical site, the incision was almost completely closed and healing well, demonstrating that the radiotransmitter had not migrated through the surgical wound. However, there was slight inflammation around the cloaca.

The phenomenon of radiotransmitter expulsion was investigated further during an exploratory surgical procedure in a large adult female python, which had carried a radiotransmitter for approximately 2 years. This animal was re-captured in the field because its radiotransmitter, which had been implanted without anchoring to a rib and with the antenna positioned caudally, had migrated towards the cloaca. When the python was captured the radiotransmitter was contained within a thin membranous sac, which contained urate-like granules, and was protruding from the cloacal opening. The veterinarian undertaking the
procedure noted that the thin membranous sac appeared to be mucosal tissue and identified it as cloacal tissue. It is likely that the radiotransmitter was about to be expelled through the membranous wall of the cloaca. There was also significant accumulation of faecal material anterior to the radiotransmitter, which was confirmed by radiography (Figure 4). Radiographs also indicated that the body of the radiotransmitter had moved caudally and the antenna was bent close to the base of the radiotransmitter, with its remaining length directed cranially (Figure 4).

**Anchored radiotransmitter implantation technique**

None of the pythons expelled radiotransmitters that had been surgically anchored to a rib (Table 1). When all implanted radiotransmitters were surgically removed during November 2008 upon completion of the research project, there was no evidence of inflammation within the coelomic cavity of these pythons and there were no adhesions surrounding any of the radiotransmitters. At the time of radiotransmitter removal all pythons appeared to be in good health, based on physical and clinical examinations.

**Statistical analysis**

Logistic multiple regression analysis confirmed that expelling the radiotransmitters was not associated with a python's sex, snout–vent length or body mass, but was strongly associated with the type of surgery performed ($t = -5.039$, $P \leq 0.001$, i.e. whether or not the radiotransmitter was anchored to a rib).
Discussion

The anchoring technique used by us when surgically implanting radiotransmitters in 23 carpet pythons proved to be 100% successful in preventing expulsion of the tracking device. We recommend that the suture securing the radiotransmitter to the ribcage should be positioned midway from the spinal column and should not be positioned on the distal aspect of the rib, in order to prevent tearing of the muscle tissue and resulting in the radiotransmitter being effectively unanchored.

Surgical implantation of radiotransmitters is a well-established technique used in studies of fish of the ectothermic taxa, but expulsion of the implanted tags is a widespread problem. Three ways of expelling implanted radiotransmitters have been reported in fish studies. All involve the development of proliferative fibrogranulation tissue containing myofibroblasts in response to the transmitter and the device is ultimately expelled along the path of least resistance. One route of expulsion is through necrotic muscle tissue at the incision site, as seen in approximately 7% of implanted African catfish (*Heterobranchus longifilis*) and 15% of laterally compressed bluegills panfish (*Lepomis macrochirus*) at higher temperature treatment conditions (20°C) and attributed to the incision wound not healing effectively. Because of this, in the present study each radiotransmitter and antenna was implanted intracoelomically, rather than positioning the antennae subcutaneously, to minimise the risk of inflammatory reaction and wound dehiscence as result of the antennae wire being near the incision site. Another route of expulsion is through necrotic muscle tissue that develops in an intact part of the body wall adjacent to the incision, which has been seen in Atlantic salmon (*Salmo salar*) but with no adverse effects or mortality of the study animals. The third route is through the intestine (transintestinal expulsion) and has been occasionally observed in
channel catfish (*Ictalurus punctatus*), African catfish (*Heterobranchus longifilis*) and rainbow trout. During necropsy procedures, implanted radiotransmitters have been found at various stages of encapsulation, including limited fibrous proliferation surrounding the transmitter, full encapsulation by fibrous connective tissue or following encapsulation when the transmitter has been located partly or fully inside the intestinal lumen. 

The cloacal region of snakes is a complex anatomical structure that has three chambers (the coprodeum, urodeum and proctodeum) with different functions, separated from each other by papillae and other structures. Pythons are constrictors and the controlled body wall muscle contractions and peristalsis associated with capturing and digesting their prey could easily move an unanchored radiotransmitter caudally through the coelomic cavity. Movement of the body wall musculature associated with general mobility may also result in the caudal movement of an unanchored transmitter. If the transmitter is pushed down to the most caudal point in the coelomic cavity, it will be lodged against the cloacal wall and could potentially perforate through and be expelled from the cloaca, with or without faeces (Figure 5). The likelihood of this occurring may be increased if the unanchored radiotransmitter is placed with the antenna directed caudally, which could create a sharp point where the antenna folds or bends, initiating or exacerbating pressure necrosis of the tissue.

Pearson and Shine suggested that expulsion of transmitters was a result of feeding events involving the consumption of whole prey, often large in size, following which the stomachs and intestines of pythons are quickly upregulated and increase substantially in size. This could result in pressure necrosis of the intestinal lumen wall adjacent to the transmitter, causing it to be encapsulated within the intestines and subsequently excreted with the faeces.
The minimum number of days taken for African catfish to expel an implant through the intestine was 8 days\textsuperscript{23} and a python in the present study expelled the implanted radiotransmitter within 4 days, which seems to rapid for encapsulation through the gastrointestinal wall.\textsuperscript{13} All of the fish that expelled implanted radiotransmitters through the intestine had a degree of intestinal inflammation and there was granulation tissue surrounding the radiotransmitter, but our exploratory coeliotomy of a carpet python that was captured while in the process of expelling a transmitter showed no evidence of inflammation at the implantation site or within the coelomic cavity.

Our investigations support the observation that a feeding event may allow an unanchored radiotransmitter to be pushed towards the cloaca as the digestion process progresses;\textsuperscript{13} however, only 3 of 12 expulsion events in our study were contaminated with faecal material, suggesting that a feeding event is not necessarily the initiating factor forcing the radiotransmitter out of the body.

Telemetry studies on fish have shown signs of irritation at the antenna exit wound and suggest that infection, inflammation or tissue necrosis may be problematic for implanted radiotransmitters that have antennas.\textsuperscript{26} Additionally, there was no evidence of an inflammatory response to the implanted transmitter. A previous study\textsuperscript{25} of channel catfish found that the prevalence of tissue reaction and radiotransmitters being expelled through the incision was greater with devices weighing 2.0% of the fish's body weight, compared with transmitters that were 0.5% of body weight. That study also found that radiotransmitters being expelled from the incision occurred more frequently in gravid females than in males or spent females (females that have produced eggs). We used an arbitrary rule of transmitter
weight being <5% of the python's body weight, but others have provided detailed
descriptions of quantifying and monitoring the choice of radiotrigger or device
attachment to animals in order to estimate the costs associated for the animal.33 When
researchers plan to attach a device to animals they must predict how it will affect the animal's
biomechanics, locomotion and survival, ranging from altered foraging behaviour, grooming
regimens, provisioning of offspring, movement patterns and exposure to predators, and also
provide animal ethics committees with a ‘discomfort index’ of the device.34 In the present
study we did not find evidence of adverse effects on survival or locomotion associated with
the anchored technique of radiotrigger implantation in any of the study pythons.

We recommend that in future studies the radiotriggers should be appropriate for the size
of the snake. A more elongated shape of radiotriggers would be beneficial, allowing
substantially more room for the snake's organs to change in size during digestion and
reproduction, particularly in gravid females.

There are no studies evaluating the efficacy of NSAIDs or local anaesthetics in reptiles and
doses are anecdotal or extrapolated from mammalian or avian studies.16 We recognised the
need to provide postoperative analgesia with meloxicam, but given the increased
understanding of the need for analgesia in reptiles, recommendations for analgesic regimens
associated with invasive surgical procedures, such as a coeliotomy, should involve the use of
both intra- and postoperative analgesic agents.15,16,21,33,35 However, the effects of pre-emptive
analgesia on anaesthetic induction or recovery times of reptiles following general anaesthesia
are still not fully understood.16,33,35
Acknowledgements

This project was funded by Murdoch University, the Department of Environment and Conservation and the Invasive Animals Cooperative Research Centre. We thank D. Pearson for help with the initial training and advice for implanting radiotransmitters and the numerous volunteers for their assistance in the surgeries, in particular J. Clarke, M. Connor, J. Bryant, A. Bryant, C. Bryant, J. Crow, S. Dundas and J. Hampton. R. Joy, M. Rouffignac and T. Messer helped with invaluable veterinary nursing knowledge.

References


Figure 1. Diagram of the position of an implanted radiotransmitter in relation to major organs within the coelomic cavity of a snake.
Figure 2. Radiograph of implanted radiotransmitter inside a male python's coelomic cavity. Note the antenna has moved within the python and is now more looped than originally positioned during surgery. The bulb on the radiotransmitter is additional temperature data loggers that were wax-embedded onto the radiotransmitter before implantation.
Figure 3. Diagram of the process of anchoring the radiotransmitter to a rib in the coelomic cavity of a snake to prevent it from migrating and potentially being expelled from the body. (a) Sutures looped twice tightly around the radiotransmitter and secured with square knots, then a further two square knots are made to create a loop between the knots. Note: the suture material is not cut. (b) The radiotransmitter is positioned in the coelomic cavity and the needle is pushed from the ventral surface of the ribcage (indicated by hatched lines) to the dorsal surface, over a rib and back to the ventral surface. (c) The needle is pushed through the loop created by the square knots attached to the radiotransmitter and secured completely with several more knots. The excess suture material is then cut.
Figure 4. Radiograph of a radiotransmitter and antenna that had been implanted in the coelomic cavity (without anchoring to the ribcage) of an adult female python (*Morelia spilota imbricata*), which was re-captured in the field (Leschenault Peninsula Conservation Park, Western Australia) to enable examination of the migrating radiotransmitter after the python had carried it for 2 years. The radiotransmitter had migrated from the incision site (approximately 10% of the snout–vent length, anterior to the cloaca) towards the cloacal opening (edge of the image). Note the significant build-up of faecal material anterior to the radiotransmitter, which may have resulted in the movement of the device towards the cloacal opening.
Figure 5. Schematic of the movement of an unanchored radiotransmitter towards the three chambers of the snake's cloaca. The lower panel shows how the radiotransmitter may be forced into one of the cloacal chambers and then expelled with or without faecal material.
Table 1. Summary of the two surgical techniques used to implant radiotransmitters in the coelomic cavity of southwest carpet pythons and the association with expelling of the device

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<th>Expelled (av. no. of days implanted ± SE)</th>
<th>Retained (av. no. of days implanted prior to removal ± SE)</th>
<th>Expelled (av. no. of days implanted prior to removal ± SE)</th>
<th>Total</th>
</tr>
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<tr>
<td>No. of pythons (av. body mass ± SE)</td>
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<td></td>
</tr>
<tr>
<td>Female (1420.5 ± 34.1 g)</td>
<td>6 (416 ± 186)</td>
<td>8 (688 ±112.3)</td>
<td>11 (248.4 ±112.3)</td>
<td>25</td>
</tr>
<tr>
<td>Male (693.9 ± 16.4 g)</td>
<td>6 (101 ± 48)</td>
<td>3 (420 ±126.4)</td>
<td>12 (308.3 ±21.3)</td>
<td>21</td>
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<tr>
<td>Subtotal</td>
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<td>11</td>
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