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ORIGINAL PAPER

Field techniques for handling, anaesthetising and fitting radio-transmitters to Eurasian otters (*Lutra lutra*)

Lughaidh Ó Néill · Peter Wilson · Addy de Jongh · Tjibbe de Jong · John Rochford

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Abstract Practical issues for radio-tracking studies of otters (*Lutra* lutra) include their sensitivity to stress, their sensitivity to certain anaesthetic regimes and their unsuitability for standard collar mounted radio-transmitters. We examined the practicability of various field techniques for overcoming these issues in east and south east Ireland from April 2005 to July 2006. Thirty-four highly-stressed otters were restrained with ketamine and midazolam within minutes of capture, to fit externally-mounted transmitters or to transfer them to transport boxes. Eleven otters were fitted with harness mounted radio-transmitters, 2 were fitted with glued-on radio-transmitters and 15 were surgically implanted with intra-abdominal radio-transmitters in the field. The intra-abdominal transmitters were implanted under isoflurane anaesthesia within an hour of initial sedation. We experienced no complications with this anaesthetic regime. The abdominal cavity was accessed by a lateral approach. All surgeries were successful and we recorded no serious post-operative complications. The implantation procedure lasted less than 3 hours from capture to release such that almost all animals stayed within their territories, and pups were not abandoned. However, following their release, animals were sensitive to directed disturbance and could easily be forced to disperse.

Keywords Field-surgery · Harness · Implant · Sedative · Telemetry

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L. Ó Néill (✉) · P. Wilson · J. Rochford
Department of Zoology, School of Natural Sciences,
Trinity College Dublin,
Dublin 2, Ireland
e-mail: luoneill@tcd.ie

P. Wilson
e-mail: pwilson@eircom.net

J. Rochford
e-mail: rchfordj@tcd.ie

A. de Jongh · T. de Jong
Dutch Otterstation Foundatinon,
Spanjaardslaan 136,
8917 AX Leeuwarden, Netherlands

A. de Jongh
e-mail: addydej@xs4all.nl

T. de Jong
e-mail: addydej@xs4all.nl

Introduction

The near-threatened status of Eurasian otters (*Lutra lutra*) focused attention on research protocol (Council of Europe 1979; EU Habitats directive 92/43/EEC, CITES 1979; IUCN 2006) such that our understanding of certain aspects of their socio-biology remains meager because we could not readily capture and mark otters humanely (Chanin 2003; Kruuk 2006). Newly captured animals suffer short-term traumatic stress associated with capture, and long-term fatiguing stress associated with confinement and transportation (Nielson 1999). We have recently described a highly efficient trapping protocol involving trap alarms that minimises short-term stress by allowing us to capture, mark and release animals within a matter of minutes (Ó Néill et al. 2007). However, the similar circumferences of the otter neck and head make it unsuited to standard collar mounted transmitters that can be attached rapidly in the field (Melquist and Hornocker 1979; Kruuk 1995; Zschille et al. 2008). Consequently, the major methodological

issue for tracking studies of otters is identifying a transmitter attachment procedure that minimises long-term stress and population perturbation.

Several studies of native populations of the North-American river otter (*Lontra canadensis*) support implanting otters with radio-transmitters in the field without any period of retention in captivity (e.g. Melquist and Hornocker 1979; Blundell et al. 2002; Bowyer et al. 2003; Gorman et al. 2006). Nevertheless, we and other European researchers have found it difficult to receive the necessary permissions to follow this promising approach on the highly protected Eurasian otter (Hans Kruuk, Institute of Terrestrial Ecology Banchory, personal communication). We therefore tested two alternative methods of external attachment: harness mounted radio-transmitters (Mitchell Jones et al. 1984), and glued-on radio-transmitters as used for other semi-aquatic mammals (e.g. seal spp. [Fedak et al. 1983], platypus *Ornithorhynchus anatinus* [Gardner and Serena 1995]). Based on our findings, we received permission to implant otters with radio-transmitters in the field following the North American approach. We report on our experiences with anaesthetising exceptionally stressed Eurasian otters just after their capture, and our attempts at limiting long-term stress and population perturbation associated with attaching radio-transmitters.

Materials and methods

Study site and capture

The most recent Irish national otter survey indicated a widespread distribution (Bailey and Rochford 2006). The current study was conducted from April 2005 to July 2006 on the Liffey, King's and Boyne rivers in the east and south east of Ireland. These rivers were lowland limestone systems dominated by brown trout (*Salmo trutta*), salmon (*Salmo salar*) and crayfish (*Austropotamobius pallipes*). They also included stocks of pike (*Esox lucius*), perch (*Perca fluviatilis*), eels (*Anguilla anguilla*), stickleback (*Gasterosteus aculeatus*) and minnow (*Phoxinus phoxinus*). All three systems had otter densities of roughly 0.3–0.5 otters/km of river (L. Ó Néill, unpublished data). We captured otters with padded leghold traps fitted with trap-transmitters that alerted us as soon as an animal was caught and we arrived on site in a mean time of 22 min (standard deviation=14). The trapping technique is described and evaluated elsewhere (Ó Néill et al. 2007). The Bio-Resources Unit of Trinity College Dublin (201005) provided ethical approval for the research program and the Department of Health and Children (B100/3735) licensed us to implant otters with intra-abdominal radio-transmitters. Further trapping and tagging licenses were issued by the National Parks and Wildlife Service (1/2005, 3/2005, 8/2005, 9/2005, C15/2005, C68/2005, C69/2005).

Sedation and handling

We needed a chemical restraint that was safe to use on otters that had stepped on traps just minutes earlier and were clearly highly stressed. Ketamine with medetomidine, an intra-muscular anaesthetic combination used for sedating wild-caught otters (Fernández-Morán et al. 2001), was lethal for such highly stressed otters (T. de Jong and A. de Jongh, Dutch Otterstation Foundation, unpublished data), and so we used ketamine with midazolam (Spelman et al. 1993). For sufficient immobilisation to attach an external transmitter, we administered this drug combination at a dosage of 10 mg/kg and 0.25 mg/kg respectively (Spelman et al. 1993). When an animal was to be operated upon, we administered a lower dosage of 3 mg/kg and 0.08 mg/kg, respectively, to facilitate its transferral from the trap to the transport-box. Later in the study we adopted the lower dose even for animals being fitted with external transmitters because of the potential to administer multiple injections without exceeding guide levels.

To administer the intra-muscular injection we physically restrained the otter with a handling tongs (Fig. 1). These wooden tongs were 1-m long and the closed jaws encompassed a 12-cm diameter opening that was lined with soft rubber. We placed the tongs around the otter's thorax and injected the sedative to the gluteal mass when under control. We measured body temperature rectally as soon as possible, and relieved hyperthermia (>40°C) by partially immersing the otter and massaging its fur to aid water penetration. This is of critical importance as all otters struggled vigorously in the traps. We then fitted the animal

Fig. 1 Otter handling tongs. The otter is clamped around the thorax just behind the forelegs. When under control, a second researcher administers an intramuscular chemical restraining agent to the gluteal mass

with an externally mounted radio-transmitter or transferred it to a sturdy clear plastic 'transport' box (0.8×0.3×0.3 m). To induce light surgical anaesthesia we introduced 4% isoflurane to the transport box at a rate of 10 l/min 100% oxygen for 6 min using a portable anaesthetic machine with a precision vaporiser (O'Neill Medicalia Ltd., Liverpool, UK). When the otter lost its ability to right itself, we administered 1.5% isoflurane at a rate of 2 l/min through a facemask. We monitored muscle response to surgical manipulation throughout the surgery and varied the concentration of isoflurane as necessary to maintain light surgical anaesthesia.

External attachment of radio-transmitters

The external radio-transmitters measured approximately 3.5×2.5×1.5 cm with a 5-cm whip antenna and weighed just 15 g, or 0.3% of the weight of the smallest otter caught (4 kg) (TW-52 high power twin 10–28 cells, Biotrack Ltd., Peterborough, UK). The radio-transmitters were designed to have a lifetime of 10 weeks and a line-of-sight range of approximately 5 km. Owing to the rolling nature of the countryside in our study area, we were able to detect the tags from up to 2 km away under optimal conditions and up to 1 km under normal conditions. We fitted radio-transmitters externally to 11 otters with harnesses following the approach of Mitchell-Jones et al. (1984). Our harnesses used leather at the lighter end of the range suggested by Mitchell-Jones et al. (1984). The combined wet weight of harness and tag together was approximately 130 g, or 3.25% of the weight of the smallest otter caught. We also attempted to glue radio-transmitters directly to the fur of two otters using a thick flexible cyano-acrylate (Loctite Contact® 4860, Radionics, Dublin, Ireland). We coated the transmitter in glue and stuck it to the fur between the otter's shoulder blades with the aerial oriented towards the tail. By parting the fur we buried the transmitter as deeply as possible and then wrapped the surrounding fur over the radio-transmitter.

Surgical implantation of radio-transmitters

The surgically implanted radio-transmitters were encased in cylindrical, round-ended, silicone and polycarbonate tubes measuring 8.5×2 cm and weighing 28 g, or 0.7% of the weight of the smallest otter caught (TW-5FT high power twin cell with 1/2 AA, Biotrack Ltd. Peterborough, UK). Owing to the helical antenna, its intra-abdominal location and the increased life-time of these radio-transmitters (approximately 1 year), the normal detection range was just 300 m in the field. All surgeries were performed by L. Ó Néill under the supervision of P. Wilson (MRCVS). Our preferred surgical theatre consisted of a clean room within 30 min of the trap site, but when necessary we used a van fitted with a dedicated surgical table as a mobile theatre. We prepared otters for surgery outside the theatre. We shaved a 6×6-cm patch on the upper flank midway between the last floating rib and the hip bone, and clipped guard hairs from the surrounding 2–3 cm. We scrubbed the surgical site with 98% alcohol and disinfected it with sterile disinfectant swabs. We placed the otter upon the surgical table in lateral recumbence and covered it with a cloth drape followed by a sterile disposable surgical drape with a slightly narrower aperture (6 cm diameter) to prevent contamination by wet fur. All instruments and gloves and gown were sterile. We cleaned the tubes thoroughly and steeped them in 98% alcohol for at least 72 h prior to implantation.

We made a 3.5-cm dorso-ventral incision through the skin in the centre of the surgical site and separated the underlying tissue layers by splitting muscle fibres along their axes and by tearing fat layers dorso-ventrally (Melquist and Hornocker 1979; Hernandez-Divers et al. 2001). We pierced the peritoneum with a fine forceps and opened a 3.5-cm tear. We placed a stay suture in through the transverses abdominis and the peritoneum as the introduction of the transmitter often forced the innermost muscle layer and the peritoneum down and made it difficult to appose the peritoneal edges. We closed the muscle layers separately with three or four interrupted sutures using 3-0 absorbable monofilament suture (Monocryl, Ethicon, Johnson and Johnson, Belgium). We closed the skin layer for the first two otters with a continuous horizontal mattress, while for the remaining 13 otters we used four or five sub-cuticular interrupted 3-0 absorbable sub-cuticular sutures (Polysorb, USSC Auto Suture Company, France). We released the animal from the transport box when it began moving incessantly and scratching in a determined manner. We then prevented the animal from accessing the water manually by calmly placing ourselves in its path. When the animal was sufficiently coordinated to pass us in spite of our efforts we considered it sufficiently coordinated to be released safely. The animals did not appear unduly stressed by this.

Results

Sedation and anaesthesia

Values are given±standard deviations. Exertion associated with attempting to escape the trap and avoid the tongs meant that 11 of 36 animals were hyperthermic with temperatures ranging from 40–43°C. Early on in the study one hyperthermic (≥41°C) animal died under sedation due to inefficient monitoring of rectal temperature and a second died when its airway was partly blocked by ingested vegetation and its neck extension was not properly

preserved. The mean induction time for the high dose of ketamine and midazolam was 4±2 min (n=10). We apparently failed to administer the first injection properly on two occasions and had to deliver a second full dose. Animals given the high dose of ketamine and midazolam remained unconscious for 39±17 min and had become coordinated enough to be released 91±19 min after recovery (n=8). The lower dose was designed to allow for brief handling of conscious but uncoordinated and non-aggressive animals. The level of sedation was subjectively and conservatively assessed and the recorded induction times varied considerably (10±7 min) with seven animals losing consciousness (n=26). No complications ensued for any animal sedated with the lower dose including those animals that required two (n=5) or three (n=2) additional injections at the same dosage. In general, we concluded that the booster injections were necessary where the initial injection was improperly administered because of the animal's struggles.

Only those otters that had been given the low doses of ketamine and midazolam were anaesthetised with isoflurane. The induction time for the sedation with isoflurane in the transport box was 6±2 min (n=15). Induction proceeded smoothly and without complication in all cases. The isoflurane afforded us excellent control of the degree of anaesthesia. In one case we resolved the onset of irregular breathing by stopping administration of isoflurane and allowing the otter to breathe 100% oxygen for 1 min. Otters had regained consciousness 7±3 min after the flow of isoflurane was stopped. The otters were sufficiently coordinated to be safely released 37±9 min following recovery of consciousness.

External attachment of radio-transmitters

Harness-mounted radio-transmitters were retained by animals for 20±16 days (n=11) (Table 1). Five harnesses were retrieved from submerged snags upon which they had become securely entangled. Each of the five otters had escaped. The carcass of an animal (SAF5) that was recovered as a road traffic victim after 45 days, revealed an open sore on the inside of one foreleg caused by abrasion with the

Table 1 Summary details for otters tracked with intra-abdominally implanted, harness mounted, or glued-on radio-transmitters on the Boyne, King's and Liffey rivers in the Republic of Ireland in 2005–2006

Transmitter	Name	Sex	Weight (kg)	Tracking period (days)	Fate
Implant	AM5	♂	8.5	167	Alive Oct 2006
Implant	AM6	♂	8	164	Death (illegal snare)
Implant	AM1	♂	8	150	Alive Oct 2006
Implant	YAF1	♀	5.4	150	Alive Oct 2006
Implant	SAF3	♀	5	150	Alive Oct 2006
Implant	AF2	♀	5.8	90	Alive Oct 2006
Implant	SAF2	♀	4	90	Alive Oct 2006
Implant	SAM1	♂	5.3	90	Alive Oct 2006
Implant	YAM1	♂	7	80	Contact lost
Implant	AF3	♀	7	62	Contact lost
Implant	SAF4	♀	4.8	60	Contact lost
Implant	AF9	♀	6.5	10	Dispersed[a]
Implant	AF10	♀	6	13	Dispersed[a]
Implant	AM8	♂	8.5	12	Death (road traffic collision)
Implant	SAM3	♂	4.5	0	Contact lost
Harness	SAF5	♀	5.1	52	Tag retrieved
Harness	AF4	♀	6	40	Tag failure
Harness	AM7	♂	8.7	30	Tag retrieved
Harness	AF8	♀	7	24	Tag failure
Harness	SAM8	♂	6.9	22	Tag failure
Harness	AF5	♀	5.8	14	Tag retrieved
Harness	AM2	♂	9	14	Tag retrieved
Harness	SAF1	♀	5.1	9	Tag retrieved
Harness	SAM3	♂	4.8	6	Tag retrieved
Harness	AM4	♂	8	4	Tag retrieved
Harness	YAM3	♂	7.2	2	Tag retrieved
Glue	AF1	♀	6.8	17	Tag retrieved
Glue	YAM2	♂	5.4	16	Tag retrieved

[a] Dispersal in direct response to anthropogenic disturbance

leather strap of the harness. The harness leather was substantially weakened after 4 weeks and only two harnesses remained attached beyond this period (40 and 52 days). Therefore the harnesses could not remain attached much longer. Glued-on radio-transmitters were fitted to two animals and remained attached for 15 and 17 days. As far as we could determine, all animals fitted with external transmitters remained in home ranges surrounding the trap site.

Intra-abdominal radio-transmitter implantation

All surgery was successfully performed without serious complication. From time of primary incision to wound closure, surgery lasted 55 and 60 min for the first two otters. Placing the stay suture through the transverses abdominis and peritoneum prior to the insertion of the radio-transmitter reduced operating time to 41±6 min (n= 13). Slight bleeding occurred twice when small blood vessels in fat layers were ruptured, but we observed no other significant bleeding.

No animal exhibited post-operative complications that we could detect (Table 1). The first otter we operated on was killed in a road traffic accident 12 days later. A post-mortem found that the cuticular edges of part of the surgical wound were slightly apart owing to failure of the continuous horizontal mattress subcuticular suture. Some of the suture material was missing, indicating that the otter had bitten it off and that the otter may have caused the failure of the suture. Consequently, we adopted a more secure discontinuous suture pattern for the other 14 animals. In spite of the failure of the sutures, the wound was sealed, secure, showed no clinical evidence of infection and was healing normally. The skin layer displayed satisfactory progression in secondary healing. There were no adhesions between the transmitter and the omentum or the peritoneum. Professor P. Wilson (MRCVS) concluded from the necropsy that the animal was uninfected and that its death was unrelated to the surgery. We recaptured a second otter (AF10) after 13 days. This otter showed no clinical evidence of infection and the skin layer appeared fully healed.

Following the recapture of AF10 we lost contact with this otter. The signal displayed no abnormal fluctuation in either strength or frequency prior to loss of contact, suggesting that the animal dispersed rather than that the tag failed. We lost contact with two other individuals within the first fortnight. They were caught in the same trap and were very probably mother and cub. We lost contact with the juvenile (SAM3) immediately even though we were still in contact with the adult female (AF9). We noticed that although it worked, the juvenile's radio-transmitter rattled slightly. Another radio-transmitter from the same batch also rattled and failed several days after activation while it was still in our possession. We then lost contact with the adult female 9 days later following disturbance as we tried to investigate apparent inactivity. We could detect no abnormality in the radio-transmitter and concluded that the loss of contact was due to dispersal. We searched the region intensively (radius of 20 km from trap site) for several weeks without detecting either animal. We walked all streams from the bank and detected no signal though we were easily capable of detecting signals for nearby otters diving to depths of 2 m. We detected otters with implanted transmitters on 94.7%±4.6% of occasions (217 fixes for 14 otters) prior to loss of contact and we attributed most failures to receiver faults. This indicates that otters were detectable in the vast majority of holts. Therefore, we are confident that we would have detected any mortality. Excluding the three cases described above, all other individuals remained within the vicinity of the trap site and adults occupied exclusive territories with stable borders. Overall, three sets of instrumented lactating females and dependent offspring returned to each other following release and one case remains inconclusive (AF9 and SAM3 above).

Discussion

We experienced no significant complications associated with the surgical implantation procedure though it clearly entailed greater complexity and immediate risk than external attachment. The harnesses we used remained attached for shorter and more variable periods than those achieved by Mitchell-Jones et al. (1984) who developed the method. This probably reflects their having practiced on captive animals. Indeed, the retention of harnesses improved somewhat as the study progressed, though not significantly so. The harnesses could cause considerable discomfort to the animal, as has been observed for collars and harnesses with other mustelids (Fournier et al. 2001; Zschille et al. 2008). Furthermore, we often retrieved harnesses that had become securely entangled on underwater snags. This may present a risk of drowning, although all our animals successfully escaped from the harness. The glued-on radio-transmitters involved negligible risk of injury to the animal but the retention time was too short. We agree with the general consensus that surgical implantation is the best approach for tracking studies of otters (see e.g. Kruuk 2006).

Instrumented otters were released within 3 h of entering our traps. The short duration of the procedures meant that almost all animals occupied ranges including the trap site for several weeks following capture and dependent offspring and their mothers returned to each other. Three

instrumented lactating females successfully raised at least a single and two pairs of instrumented cubs respectively. It appears therefore, that the procedures we followed could be safely used on all classes of individuals, apart from heavily pregnant females or extremely juvenile individuals, without perturbing the population structure. However, otters were sensitive to directed disturbance during the weeks following capture.

By releasing the otters immediately we lost the ability to intervene where post-surgical complications such as wound failure occurred. Behaviourally induced wound failure caused the death of a female otter 3 days post-release (Hertweck et al. 1998) and a male mink 24 h post-release (Zschille et al. 2008). Periods of captivity for semi-aquatic mammals (e.g. a minimum of 72 h) can help deal with such complications (Hernández-Divers et al. 2001; Fernández-Morán et al. 2002, 2004; Zschille et al. 2008). Consequently, studies of native populations of Eurasian otters have kept animals in captivity for several days following surgery (e.g. Durbin 1996; Kruuk et al. 1993; König and König 1998; Kruuk 2006, but see also Arnemo 1991) as have studies of reintroduced Eurasian otters where the animals had to spend time in captivity in any event (Sjöåsen 1997; Saavedra 2002; Fernández-Morán et al. 2002; Niewold et al. 2003). However, prolonged retention may be counter-productive for species that are highly susceptible to stress or population-perturbation if there is a low risk of surgical complications. Behavioural and hormonal signals indicated that captive wild otters were highly stressed or approaching that state for 2–5 days following capture (Fernández-Morán et al. 2004). Territories of dead or removed otters are usurped rapidly (Erlinge 1967; L. Ó Néill, unpublished data) and prolonged captivity must increase the likelihood of dependent offspring being abandoned.

We observed no mortality associated with the surgical procedure. Similarly, Fernández-Morán et al. (2002) observed no surgical complications, yet 9% of otters died due to captive management. Evisceration is a likely consequence of wound failure where the abdomen is accessed by an incision through the linea-alba (e.g. Zschille et al. 2008; Melquist and Hornocker 1979). This risk is decreased by accessing the abdomen through a para-lumbar incision and muscle-split techniques (Melquist and Hornocker 1979; Hernandez-Divers et al. 2001; Bowyer et al. 2003; Blundell et al. 2002). This approach reduces the risk of behaviourally induced suture failure (Melquist and Hornocker 1979) and makes evisceration a less likely consequence because the muscle layers slide over each other and misalign the openings so that the sutures of the inner muscle layers and peritoneum cannot be accessed. Following this approach combined with a relatively small cuticular incision (3.5 cm) we observed satisfactory secondary healing where the cuticular sutures had opened, reassuring us greatly of the safety of the method. We therefore find that the risk of evisceration is not sufficient to necessitate the stress, mortality and population perturbation caused by keeping otters in captivity.

In conclusion,

1. Ketamine and midazolam should be used to sedate highly stressed otters.
2. Isoflurane provided great control of anaesthesia and was safe to use on otters restrained 20–30 min earlier with ketamine and midazolam.
3. Implanted radio-transmitters were superior to external transmitters both for the data they allowed us to gather and for the comfort and safety of the otters.
4. A lateral or para-lumbar approach resulted in a secure wound that did not require the otter to be kept under observation.
5. Capturing, marking and releasing the animals within 3 h avoided population perturbation.

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