Practical considerations for the field study of American mink *Mustela vison* in lowland England

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Abstract. Because feral American mink are a successful alien carnivore worldwide, demands for field studies are likely to grow. Appropriate methods for trapping, use of anaesthesia, handling and radio-tracking are important from both academic and animal welfare view points. From accumulated field studies of mink over the last 30 years, including our own, we describe the problems that researchers will likely face in the field, and offer possible solutions for them. Surveys in the breeding and kit dispersal seasons likely inflate the estimated populations, and the best time to assess a local mink population is kit rearing season (May to July in the UK). The best trapping sites occur in the junction between a hedge/ditch and waterside cover. A mass of dry hay, with which the traps are wrapped, prevents mink from damaging their teeth. In the field mink is safely anaesthetised using a combination of a volatile agent (isoflurane), and injectable agents (medetomidine and ketamine mixture), which produces anaesthesia lasting for 60 minutes or more, and is reversible with another injectable agent (atipamezole). In lowland England triangulation is not necessary to locate animals by radio-tracking although obtaining radio signals is problematic when animals are under ground.

Key words: American mink, anaesthesia, England, introduced species, trapping.

In the UK, the arrival of the American mink, *Mustela vison*, has given rise to concern over its impact not only on game and fish industries, but also on native wildlife (Birks 1990; Macdonald 1995; Macdonald and Strachan 1999). These concerns were paramount when mink were found breeding in the countryside in the 1950s. Since then their potential negative impact (e.g. predation, spreading infectious diseases) has caused much debate among the general public and conservationists (Linn and Chanin 1978; Birks and Dunstone 1991; Clark 1991; Craik 1995; Macdonald 1995; Barreto et al. 1998a, 1998b; Strachan et al. 1998; Macdonald and Strachan 1999; Yamaguchi and Macdonald 2001). A number of studies and surveys on feral mink have been carried out in the UK (Chanin 1976; Birks 1981; Birks and Linn 1982; Ireland 1988; Dunstone 1993; Clode and Macdonald 1995; Halliwell and Macdonald 1996; Yamaguchi 2000). Considering that they are one of the most widespread introduced predatory species worldwide (Dunstone 1993; Macdonald and Strachan 1999), a continued demand for ecological studies and field surveys of

feral mink seems likely.

Although the field methodology for mink study has been developed through communications among field workers, written protocols and recommended techniques are not readily available. Furthermore, information about practical welfare of free-ranging animals, including mink, remains poor (M. Berdoy, personal communication) despite growing concern for the welfare of animals used in field research. A widely available guide to field methods would be beneficial for field workers, not only to obtain better study results, but also to improve practical animal welfare.

In this paper we describe methods for field study on free-ranging mink, suggest problems researchers may encounter in the field, and offer some solutions.

Study area and season

A number of factors should be taken into consideration when planning a study area. Possible effects of competition and predation caused by other species must

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Fig. 1. Seasonal changes in trapping success between May 1995 and August 1997.

be considered. Sympatric carnivores may influence ecology and behaviour of mink in various ways, especially their spacing patterns through resource competition (Lodé 1993; Clode and Macdonald 1995). In the UK, otters *Lutra lutra* and polecats *Mustela putorius* are considered the most important direct competitors to mink. A confounding problem for mink studies in the UK is a high human caused mortality. Researchers must try to establish good relationships with land owners and other local people and ask them not to kill mink before the start of field work.

The effects of the timing of a study on results should also be considered in planning. For example, surveying population during the breeding season (January to early April in the UK) or kit dispersal season (August to October in the UK) inflates the population size because of transient males (Birks 1981; Ireland 1988) or dispersing juveniles (Fig. 1). When a long term study is planned on the basis of resident mink, the best season to assess population size is during kit rearing season (May to July in the UK).

Trapping

In general, mink spend much of their time in the narrow strip of cover beside watercourses (Chanin 1976; Birks 1981). Traps set in this linear habitat corridor are likely to be encountered by mink (J. Birks, personal communication). The best sites include the following: the junction between a hedge/ditch and waterside cover, beside an obvious landmark such as a bridge or a big tree (especially a big hollowed willow tree near the river), a waterside parapet, or in a patch of cover on an otherwise

sparsely vegetated riverbank (J. Birks, personal communication). Traps close to water should be anchored to prevent them from rolling into the water. Also, the risk of traps being flooded by rising water after a heavy rain must be avoided.

For our studies, mink were trapped in commercially available, single-entry aluminium mink/rat cages of approximately $14 \times 14 \times 76$ cm (A. Fenn and Co., Redditch, Worcestershire, UK). The study area was divided into four stretches of river, each of which consisted of 3–8 km between two neighbouring locks. We set c. 20–30 traps per stretch — on average one every 200–300 m of riverbank although they were not set evenly because people, dogs or cattle had regular access to some areas. Also trapping was carried out during weekdays only because of too much public access during weekends. We were able to set or collect all traps within c. 7–8 hours, and check them within one hour, by using a boat. Trapping results gave us monthly demographic information for the entire study area, and this 4-week cycle of trapping was continued throughout the study period to monitor the population (Fig. 2).

After trying various baits including day-old chicks, rabbit flesh or dead laboratory rats, Ireland (1988) favoured day-old chicks as baits. Because it was easier to obtain than chicks, we baited traps with fresh herring which were easy to handle, did not need any preparation, and had quite a strong smell likely to attract mink (J. Birks, personal communication). After positioning a trap, baits (4 to 5 herring) were put into the trap, and herring fragments were smeared on objects close to the trap entrance. As slugs, shrews, mice etc. removed the bait, fresh ones were added to the traps at least every three

Fig. 2. Residency of mink between May 1995 and August 1997. Each bar represents the duration of residency, estimated from captures, radio-tracking and direct observations.

days. The traps were then wrapped with a loose mass of dry hay. The hay prevents trapped animals from biting the cage and damaging the teeth, because they tend to bite and pull the hay instead (J. Birks, personal communication). Eventually the animal will pull most of the covering of hay into the trap, making a hay-tunnel inside which it can keep warm and dry. Finally, the traps were

disguised using a variety of materials, such as grass and driftwood, so that the bright hay and trap were not obvious to passers-by. Traps were set in the afternoon and were checked early in the following morning to limit the duration for which a mink might be caught inside and reduce any capture-related stress to the animal.

Fig. 3. Anaesthetising box.

Anaesthesia

Good anaesthesia provides humane restraint, muscle relaxation to facilitate various procedures, and sufficient analgesia to prevent the animal from experiencing pain (Wolfensohn and Lloyd 1994). However, misuse of anaesthetic drugs can kill animals, and hence, it is necessary to develop skills for performing and monitoring anaesthesia accurately.

In our study, the trapped mink was transferred to a Perspex-sided, wooden floored, anaesthetising box ($15 \times 15 \times 48$ cm to fit the trap entrance) (Fig. 3) (Chanin 1976; Birks 1981) for the induction of anaesthesia by a volatile agent. A cotton wool ball, one third of the size of a fist, soaked with the agent of up to c. 5 ml, was put into a special compartment made of steel wire to induce anaesthesia (Fig. 3). Ireland (1988), as well as Chanin (1976) and Birks (1981), used ether for this purpose, but it has many problems including being an irritant to mucous membranes which makes induction unpleasant for the animal (Wolfensohn and Lloyd 1994). Halothane is very widely used, relatively safe for the animal and the cheapest of modern volatile agents (Wolfensohn and Lloyd 1994). It has been used on mustelids (polecat: Birks 1998; American mink: Bonesi 1996) and its average induction time was approximately 5 min on both species (J. Birks, L. Bonesi, personal communications). We initially used methoxyfluorane (Metofane: C-Vet Ltd., Bury St. Edmunds, Suffolk, UK) which produced a very slow induction (45.3 ± 6.5 min: mean $\pm$ standard error, $n = 15$) and hence was very safe for the animal. However, as we became familiar with the field anaesthetic procedure, we did not need this very safe and yet very slow agent — in low temperatures ($< 5^\circ\text{C}$) the induction took more than one hour. Later we substituted isoflurane

(IsoFlo: Schering-Plough Animal Health, Welwyn Garden City, Hertfordshire, UK) which was safer for both animals and humans than halothane (M. Lloyd, personal communication) and produced significantly quicker induction (10.7 ± 0.7 min, $n = 17$) than Metofane (Mann-Whitney U-test: $n = 15$ and 17 , $U = 0$, $P < 0.0001$).

If all handling procedures would be completed within c. 5 min, no more anaesthetic agents would be necessary, but otherwise, anaesthesia needs to be maintained throughout. In the field, unlike in the laboratory, maintaining anaesthesia by injecting anaesthetic agents is the most feasible option. There are many injectable agents, but only a few have been tested in the field. Bonesi (1996) administered to mink an intramuscular injection of ketamine hydrochloride (11 mg/kg of weight) and maintained 30 min of anaesthesia. But ketamine alone is generally insufficient for surgery, and no antagonist is available, necessitating careful monitoring until the animal recovers (M. Lloyd, personal communication). Hypothermia is a potential problem during this time, and also if other mink are captured on the same day researchers may lose precious time by staying with, and monitoring the recovery of, the first animal.

Medetomidine has been tested on polecat in the field: 0.1 ml per kg body weight of Domitor (1 mg/ml medetomidine hydrochloride: Pfizer Limited, Sandwich, Kent, UK) administered with intramuscular injection to the animal that had been already sedated with halothane provided good sedation for an additional 20 min or more (Birks 1998). The great advantage of medetomidine is that it can be reversed very rapidly with atipamezole. However, medetomidine alone does not induce general anaesthesia and is not suitable for surgery, and if skin or blood samples need to be collected, medetomidine alone may not be recommended from an animal welfare perspective (M. Lloyd, personal communication).

In a laboratory setting, a medetomidine-ketamine combination was reported to provide good anaesthesia for mink (Arnemo and Sølvi 1992). In the field, we used 0.1 ml to 0.4 ml of a Domitor and Vetalar (100 mg/ml ketamine hydrochloride: Pharmacia and Upjohn Limited Animal Health Business, Crawley, West Sussex, UK) mixture, which was mixed 3 : 2 in volume, and given by an intramuscular injection to the thigh. This mixture (0.2 ml of the mixture per kg body weight) produced complete immobilisation with good muscle relaxation lasting 60 min or more, which was very important for taking body measurements. While the animal was under anaesthesia, we covered it with a blanket to prevent

hypothermia. After handling, anaesthesia was reversed with an intramuscular injection to the thigh of Antisedan (5 mg/ml atipamezole hydrochloride: Pfizer Limited) of the same volume as Domitor. After receiving the Antisedan injection, the mink was placed back in the trap to recover — usually started to move within 5 min after injection. After complete recovery, which took 30 to 60 min, the mink was released at the site of capture, usually on the researcher's return from checking other traps.

Aging

In our study area, where all mink had dark fur (sepia to jet black), individuals were easily identified by unique ventral white spot patterns, and amongst 51 animals we caught during the study period, no two animals had the identical pattern. A mink was classified as a kit if it was observed or trapped with its mother before dispersal, at approximately 13 weeks old (early August in the UK). After that time it was classed as a juvenile until the onset of its first breeding season (January in the UK), at approximately eight months old, and after that as an adult. The age of a newly caught male was assessed by toothwear and baculum length, which was measured with a string held against the fur, and by toothwear and the presence of white hairs on the back of the neck for females. These hairs are derived from mating scars, indicating that the animal is older than c. 10 months (Ireland 1988). Adult males had significantly longer bacula (58 ± 1.0 mm: mean $\pm$ standard error) than age-known juvenile males (46 ± 1.0 mm) (Mann-Whitney U-test, $n = 25$ and 3, $U = 0.5$, $P = 0.02$). As Ireland (1988) suggested, toothwear may not always be a good indicator of the animals' age. We recorded several juveniles of known age whose toothwear was much worse than their mothers'. In general, however, if an animal has a perfect set of teeth without any noticeable wearing, it is likely a young individual less than one year old. The season of its appearance is also important to assess its age; most newly recorded individuals between late summer and early winter are dispersing juveniles (Fig. 2).

Blood sampling

To ensure a research animal's health and welfare, no more than 10% of total blood volume (equal to approximately 4.2 ml for a 600 gram female or 10.5 ml for a 1,500 g male) should be removed at any one time, and no more than 15% within a 3 months period (Wolfensohn

and Lloyd 1994). We took blood samples of approximately 1 ml for males and 3 ml for females from the jugular vein by using a 5 ml disposable plastic syringe with a 0.6×25 mm disposable needle (Terumo Europe N.V., Leuven, Belgium). Vacuum tubes (e.g. Vacutainers) may not be suitable to collect blood by venepuncture from an animal smaller than a cat because it will remove blood too quickly, causing the vein to collapse around the needle (S. Wolfensohn, personal communication). Drawing volumes were calculated for sex steroid hormone assays. To minimize the effects of daily fluctuations in hormone concentrations (Sundqvist et al. 1984; Tauson et al. 1988; Yamaguchi and Ishii 1991), blood samples were taken between 09-00 and 12-00 h.

To take blood samples, one must imagine a regular triangle whose base includes the anterior point of sternum and the ventral point of scapula, pointing towards animal's head. Then hairs need to be clipped from an area of c. 1 cm² around the third apex of the triangle, and a whirl of hair appears on the ventral side of the clipped area. By pressing gently on the part between the clipped area and the heart, the jugular vein will appear in the clipped area. The collected blood was transferred into a glass tube coated with ethylene diaminetetraacetic acid: EDTA (Becton Dickinson Vacutainer Systems Europe, B.P.No.37, 38241 Meylan Cedex, France), mixed with the anti-coagulant by gently tilting and rolling the glass tube, and cooled with ice in a polystyrene box in the field. Samples were later centrifuged at approximately $3,000 \times g$ for 5 min. The supernatant plasma and the blood cells were separated and stored at -70°C until analyses were conducted.

Mink necks became very thick in winter, making it difficult to find the jugular vein. For the collection of blood samples in the field, first attempts should be made in summer so that researchers can become familiar with the technique under more favourable conditions.

Tissue sampling

Although blood samples can be used for DNA analyses, mink-sized mammals cannot provide a large volume of blood. Furthermore, because mammalian red blood cells do not carry nuclei, collecting tissue samples for DNA analyses may be necessary. We took a skin sample from the ear for this purpose by using a 3 mm diameter biopsy punch (Stiefel Laboratories (UK) Ltd., Wooburn Green Bucks, UK), with which the skin was punched onto a small cork mat. The skin sample was stored in

BLB (250 mM EDTA, 5% SDS: sodium dodecyl sulphate, 50 mM Tris.Cl (pH 8.0): 2-amino-2-(hydroxymethyl)propane-1,3-diol, pH adjusted appropriately with HCl, autoclaved) in the field and later stored at -70°C . Ethanol (either 100% or 70%) can also be used to temporarily store tissue samples in the field. After taking the skin sample we applied an antibiotic spray, Duphacycline Aerosol (3.6% Oxytetracycline hydrochloride: Solvay Duphar Veterinary, Southampton, UK), to the wound to reduce the risk of topical infections.

Radio tags and radio-tracking

To study mink territoriality or movement patterns, radio-tracking is essential. Birks and Linn (1982) reported that a back-pack transmitter with harness attachment was not suitable to radio-track mink. We used commercially available waterproof radio tags (173–174 MHz, which are specifically allocated for use with wildlife radio tags in the UK) attached to collars (Biotrack Ltd, Wareham, Dorset, UK), and radio receivers (M57, Mariner Radar Ltd, Lowestoft, Suffolk, UK), with three-element Yagi antennas with either traditional rigid elements or flexible elements (Biotrack Ltd). Tags with “activity switches” proved to be more useful because we were able to know whether the animal was awake or not, even when its location did not change for several hours.

It has been suggested that the total weight of the radio-collar should be no more than 4–6% of body weight for animals above 50 g (Kenward 1987). Although the details are not available for earlier studies (e.g. Gerell 1969), this standard appears to have been achieved by the early 1980s. Radio-collars used by Ireland (1988) weighed between 20 and 30 g, and by Bonesi et al. (2000) c. 20 g., both of which were within c. 5% of the body weight of a female mink. Our radio-collars weighed approximately 15 g, less than 3% of the lightest adult female mink in our study area (c. 550 g). Researchers should also regularly monitor the effects of the collar on the animal's neck; in cases where serious chafing or other injuries are apparent, the collar should be removed. Gerell (1969) used collars made of copper which served as transmitter antenna loop, and reported skin chafing. Ireland (1988) also reported neck abrasion caused by loop antenna radio-collars. It is especially so in winter when mink's neck (especially in males) becomes very thick and there is virtually no difference in size between neck and head. The collar needs to be fitted tightly to en-

sure that the transmitter stays on. The abrasion may only wear hairs off around the neck, but may cause damages further into skin and soft tissues. To avoid this, Ireland (1988) developed leather collars into which a wire aerial was incorporated and reduced the abrasion dramatically. This method was further developed and the collar was glued to the guard-hairs of the neck (Ireland 1988). This is particularly useful for attaching collars to growing animals or individuals which may leave the study area. The collar is loosely adjusted so that it can be pulled off over the head by the mink once the adhesive has worn off.

We used 34 radio tags for up to 3 months in the field (no animal carried a collar for more than 3 months for welfare concerns). Although two transmitters failed while they were still on the animals, the others worked very well even in water. Signals could be detected from as far as 1.5 km away when the animal was above ground and tracked from a higher point. However, when mink were in rabbit warrens we frequently had to be within 50 m to detect a weak signal, as was reported in Ireland (1988).

To assess the accuracy of radio locations (Zimmerman and Powell 1995), we put the same radio collar in nine randomly selected sites in the study area, and tried to locate it with the same combination of a receiver and a rigid and a flexible Yagi antenna. The fixes were made approximately 100 m away from the collar using triangulation. Average fixing errors of $16 \text{ m} \pm 2.9 \text{ m}$ (mean $\pm$ standard error) were made with the rigid antenna, compared to $10 \text{ m} \pm 3.7 \text{ m}$ with the flexible antenna, but the difference was not significant (Kendall Rank Correlation, $n = 9$, $\tau = 0.036$, $P = 0.89$). These were comparable to the reported error of $9.67 \text{ m} \pm 7.17 \text{ m}$ ($n = 6$) by Bonesi et al. (2000). The flexible Yagi was much preferred for manoeuvring within the brush.

Triangulation was not necessary for mink radio-tracking in the study area where pasture was dominant, often extending to the water's edge. Areas with trees, shrubs and grasses were distributed in thin bands along the water courses, and places for mink to rest or to hunt were limited. As the result, we were always able to pinpoint exactly where the tracked animals were, without triangulation.

Following resident adult female mink was relatively easy in both summer and winter. However, following males was very demanding, particularly during the breeding season. Although Birks and Linn (1982) suggest that fixing locations twice a day may be sufficient to

assess the ranges of mink, we recorded the animals' locations every 15 min not only to investigate the range sizes, but also to obtain detailed data for their habitat use within the ranges.

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