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## TECHNICAL NOTE

### FIELD ANESTHESIA OF LEAST WEASELS (*MUSTELA NIVALIS NIVALIS*) WITH ISOFLURANE

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Nest Box;  
Portable Anesthesia Unit.

#### Abstract

The aggressive and agile nature of weasels makes the use of inhalant anesthetics preferable to injectable agents because inhalant anesthetics generally reduce handling time, cause less stress and present no risk of delivering the drug into an unintended area. Here we report on the use of the inhalant anesthetic isoflurane in free ranging least weasels. We used a battery powered portable anesthesia unit (Uninventor 400 Anesthesia Unit, AgnTho's AB, Sweden) to immobilize the animals in the field. The isoflurane liquid was filled in a syringe connected to a vaporizer where it mixed with air coming from an air pump. The animals inhaled the anesthetic first in a wooden chamber and then through a modified facial mask. Mean induction time in the anesthetic chamber was 4.8 minutes ( $\pm 0.3$  SE) with a setting of 4% isoflurane. The anesthesia was maintained with the facial mask and a setting of 2% isoflurane. Mean recovery time was 16.4 minutes ( $\pm 2.1$  SE) and mean recumbency time 40.7 minutes ( $\pm 2.9$  SE). We anesthetized 9 animals, had no fatalities during anesthesia and all animals recovered quickly. One animal was found dead 11 days post-capture of undetermined cause. Isoflurane is safe and easy to use with the tested setting. However we were unsuccessful in monitoring the animals following their release and we therefore recommend further evaluation of the anesthetic. In addition, we recommend the use of a wooden anesthetic chamber with bedding to avoid the risk of hypothermia.

## Introduction

Predation by stoats (*Mustela erminea*) and weasels (*M. nivalis*) may have a strong impact on the population dynamics of their prey [1, 2]. Habitat use and hunting behavior of these species have however, mostly been studied using capture-mark-recapture methods, hair-traps, tracking tunnels (two-openings tunnels with paper and ink on the bottom where weasels' tracks are left after the passage of the animals) or snow-tracking [3, 4]. Few studies have been based on radio-telemetry [but see for example 5], a research method that is often more effective [3] but which requires the anesthesia of captured individuals. A large number of anesthetics have been used in studies on small mustelids: e.g. ether [6, 7], phencyclidine hydrochloride and promazine [8] and ketamine [9]. Some of them are no longer in use or no longer produced (e.g. ether and phencyclidine). Others, such as ketamine, have the disadvantage of causing

muscle rigidity and should therefore be used together with sedatives, for example, xylazine or medetomidine [5, 10-12]. This however may prolong the handling time and increase the number of necessary agents. In addition, the use of injectable agents requires a great amount of handling and potential stress which can be reduced by using inhalant anesthetics instead [13]. In small and very agile animals the additional risk of injecting the drug into an unintended area, e.g. close to a nerve, is particularly high. Therefore, the use of inhalant anesthetic is recommended [3] and isoflurane in particular has been successfully used in small and medium sized mammals [e.g. 13, 14, 15, 16]. Kreeger *et al.* [17] found that black-footed ferrets (*Mustela nigripes*) were more “physiologically normal” when immobilized with isoflurane compared to ketamine-medetomidine. Indeed, isoflurane provides general anesthesia with the help of a vaporizer and supply gas with a minimal amount of metabolism within the animal’s body, thus contributing to the relatively high degree of safety [14, 18, 19]. Here we report on the use of isoflurane in the least weasel (*Mustela nivalis nivalis*) which, to the best of our knowledge, has not yet been tested in this species in the field [see reference 1 for use of isoflurane on captive least weasels]. We have used a light, battery powered, portable anesthesia unit which we found to be very practical and which has not been used in the field before on this species.

## Material & Methods

### Study area

The study area was located at Evenstad, Hedmark county, south-eastern Norway near latitude 61°25' N and longitude 11°06' E. The landscape consisted of patches of harvested forest stands, dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), interspersed among clear-cuts. Other common tree species were birch (*Betula* spp.), willow (*Salix* spp.) and rowan (*Sorbus aucuparia*).

The study area in a cool-moderate continental climatic zone, was semi-humid with an annual average temperature of 4°C (average temperature over 2001-2010 measured at Evenstad weather station, altitude 255m), and mean precipitation 789mm year<sup>-1</sup> (www.met.no). Snow cover normally lasts from November to April with large between year variation ranging from 20 to 120 cm.

### Methods

The trapping was conducted in 2007 and 2008 in June, July and August. We used wooden Edgar type trap boxes [20] provided with different types of bait (e.g., raw meat mixed with eggs, dry fish or dead voles, etc.). All Edgar traps were connected to a wooden nest box (ca. 36x25x25 cm) that we could use as an anesthetic chamber in the field. The box had a transparent hard-plastic cover on top which made it possible to observe the animal inside. On top of it, a removable steel cover created a dark environment designed to reduce the stress experienced by the captured individual [3]. Weasel trapping was part of a project on habitat use of small mustelids and their main prey, small rodents. Hence, the mustelid traps (Edgar type) were mostly located within or in the vicinity of two trapping grids (1.4 and 1.8 ha respectively) for small rodents containing a total of 291 “multi-capture” live-traps for voles (Ugglan Special N°3, Grahnab AB, Hillerstorp, Sweden) [see 21 for details on the trapping of small rodents]. Weasels could easily enter the vole traps, in which case they were led to the nest box by carefully opening the sliding bottom of the vole trap on top of the entrance to the nest box (the two openings were the same width). In 2007 trapping was preceded by snow-tracking and the use of 16 tracking tunnels to obtain presence indices of small mustelids within the study area. The vole traps were set for a total of 90 days between 2007 and 2009. This effort, added to the 60 trapping days with 20 wooden mustelid traps in 2007 and 2008, gave a total of 150 trapping days and 45150 trap-nights. All traps were checked twice a day. All necessary permits for

capture and handling animals were provided by the Norwegian Directorate for Nature Management and the Norwegian Animal Research Authority.

We used an Uninventor 400 Anesthesia Unit, size 23 x 13 x 10 cm, weight 1.8kg (AgnTho's AB, Sweden, <http://www.agnthos.se>) with a built-in air flow meter and a vaporizer calibrated for isoflurane. The concentration of isoflurane and volume of the air flow could be constantly adjusted as needed. By connecting the unit to a portable battery, it could be easily used in the field (Fig. 1).

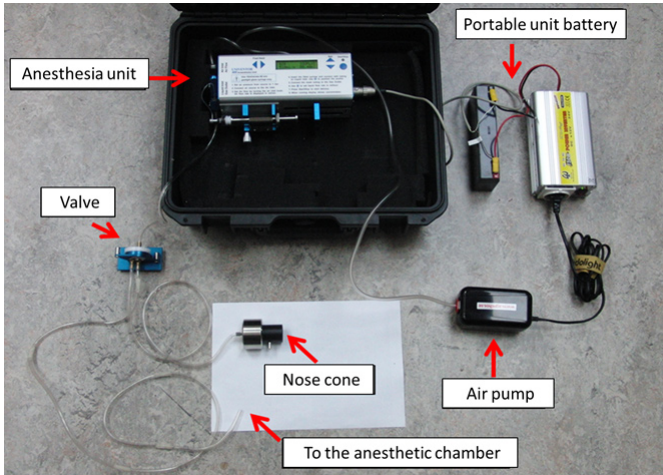


Fig. 1: Battery powered portable anesthesia unit for use in the field.

Isoflurane from a 10 ml gas-tight glass syringe was mixed with air in the vaporizer and was delivered in useful, safe concentrations for the anesthesia via the wooden chamber or a facial mask. The air came from an air pump connected to the unit (Fig. 1). From the vaporizer, isoflurane mixed with air flowed through a tube where an incorporated valve allowed the inhalant anesthetic to be directed either towards the anesthetic chamber or the facial mask. After induction in the anesthetic chamber, by turning a switch in the valve to reroute the anesthetic towards the facial mask placed around the animal's head, the anesthesia could be maintained outside the anesthetic chamber (Fig. 2).



Fig. 2: Least weasel receiving the anesthetic through the facial mask. The animal was ear-tagged and radio-collared.

In addition, the unit had a built-in alarm system in case the air-flow or the anesthesia was blocked. Induction dose in the nest box was set to 4% on the vaporizer which was reduced to 2%, and 200 ml/min air once the anesthetic was delivered through the facial mask. We chose these doses based on the manufacturer's recommended maintenance dose of 2.2% isoflurane at a flow of 200 ml of air per min for mice, which we were ready to adjust in accordance with the observed effect on the animal. The animal was placed on a hot water bottle covered with a thin blanket to keep it warm during anesthesia. Standard measurements (body weight and length) were taken and a radio-collar (Biotrack, collar cable tie, weight 1.2gr) and ear tag (Hauptner & Herberholz) were fitted. Radio-tracking of the collared individuals started shortly after release and was scheduled in three hours periods throughout day and night, with 15 minutes between each location. Once the collar was fit and the measurements taken, the animal was placed into the wooden nest box provided with bedding to avoid loss of body heat and the nest box placed open next to the original capture site. We waited until the animal was able to leave the box on its own. Induction time was calculated from the moment isoflurane was inhaled in the anesthetic chamber to the moment the animal was removed from the anesthetic chamber and placed on the hot water bottle covered with a blanket. Maintenance time was calculated from the moment the facial mask was placed over the animal's head until it was removed. Recumbency time was calculated from the moment the induction started until the animal was fully recovered. Recovery time was calculated from the moment the animal stopped receiving isoflurane until they were alert and effortlessly moving.

## Results

We captured a total of 9 least weasels in 2008 (Table 1). All were caught in small rodent traps. Weasels were transferred to the wooden nest box where they first inhaled the anesthetic and we did not observe any panic reaction. We fitted collars on 6 animals. For the remaining 3: one individual was considered too small (weighing less than 30 gr) for the collar and the two others were a by-catch in small rodent traps within an experimental enclosure connected to a different project and were thus not suitable for our study on the species' movements. The mean induction time with a setting of 4% isoflurane in the anesthetic chamber was 4.8 ( $\pm$  0.3 SE) minutes. The maintenance anesthesia time with a setting of 2% isoflurane, was 19.5 minutes ( $\pm$  1.9 SE). After removal of the facial mask it took 4.1 ( $\pm$  0.8 SE) minutes before we detected the first movements. The animals were alert and effortlessly moving after 16.4 ( $\pm$  2.1 SE) minutes. Recumbency time was 40.73 ( $\pm$  2.9 SE) minutes. We had no fatalities during the anesthesia but we found one dead individual 11 days after anesthesia. Based on our interpretation of the VHF radio-telemetry data it had died a week earlier. We were unable to successfully radio-track the other animals and recovered only two additional collars: one was recovered at a resting site three days after immobilization and one was found the day after

Table 1: Individual least weasels captured in 2008 in the vicinity of Evenstad (61°25' N 11°06' E), south-eastern Norway.

Date	Sex/ Age	Weight (gr)	Induction time (min.)	Maintenance time (min.)	Recovery time (min.)	Recumbency time (min.)	Respiration rate		
							10 min	15 min	25 min
19.7.2008	F/A	42	5.0	23.5	14.3	42.8	-	-	-
22.7.2008	F/A	40	4.8	21.2	15.0	41.0	-	-	-
22.7.2008	M/A	50	4.6	21.0	15.0	40.6	-	-	-
22.7.2008	M/*	40	3.0	22.5	25.0	50.5	-	-	-
24.7.2008	M/A	50	5.7	22.3	18.0	46.0	112	-	92
6.8.2008	F/A	40	4.5	22.5	18.0	45.0	92	100	68
6.8.2008	M/*	40	4.8	11.8	3.0	19.7	100	96	-
7.8.2008	F/J	36	6.5	7.5	24.0	38.0	68	-	-
2.9.2008	F/J	30	4.5	22.8	15.7	43.0	88	101	95

\* - Uncertain

immobilization. Due to the particular shape of weasels, fitting collars on them has a high risk of the animal losing them [3]. The remaining three collars were never found and we did not succeed in tracking the animals.

## Discussion

Despite our efforts in trapping least weasels, we were only able to catch nine. This was probably related to the extremely low density of voles in 2008 and 2009 [21], which most likely was accompanied by a crash in the least weasels population. In 2007, vole density was on the other hand extremely high (ca. 100 voles per hectare) and weasels may have been reluctant to enter a trap when prey was so readily available in most habitat types.

All animals recovered from the anesthesia, none had self-inflicted injuries and in spite of the mild pungency of isoflurane, none of them reacted or panicked when the isoflurane was introduced. We did not observe any emesis effect during anesthesia in accordance with a study by Horn *et al.* [22] showing no sign of isoflurane-induced emesis on a closely related species, i.e. ferrets (*Mustela putorius furo*). Weasels are known for biting at the trap when captured, to the point of breaking their teeth or skinning their noses [3, 23], we therefore believe that the dark environment and adequate bedding provided in the wooden nest box was particularly suitable as an anesthetic chamber for the species [3]. During anesthesia, the animals were monitored by counting the respiratory rate. This provided adequate safety for the animals considering the minimally invasive procedures that were performed and the short time the animals were receiving isoflurane. We prioritized keeping the animal under control continuously and maintaining the anesthesia as short as possible. We did not record systematically the respiratory rate except for 5 individuals (table 1) and we strongly recommend a follow-up study with monitoring of vital signs and blood gases to be carried out. An attempt to record the heart rate was made but due to pulse rates well over 200 it was considered less reliable than counting the respiratory rate. The small size of least weasels poses a challenge for pulse oximeter probe placement as pulse oximeters generally work well in animals weighing more than 200 g [14]. It was impossible to monitor eyelid reflexes as the facial mask covered most of the animals' head (Fig. 2).

The mean recovery time of 16 minutes was short compared to other studies on small mustelids using injectable anesthetics, such as the study from Gehring & Swihart [5] where the authors used ketamine-xylazine on long-tailed weasels (*Mustela frenata*) and recorded a mean recovery time of 26 minutes. In a study on the American marten (*Martes americana*) using isoflurane [16] recovery time was even shorter than in our case with a mean of 6 minutes.

The anesthesia unit was light and easy to operate in the field which allowed us to minimize the handling time. The isoflurane concentration and the air flow could be quickly adjusted and the unit could operate on air as well as on medical grade oxygen as recommended [24]. The anesthesia unit's calibration is such that whether using air or oxygen, the concentration of isoflurane shown on the display will be correct. According to the manufacturer the unit functions without any problems between 5°C and 35°C and our study was conducted at temperatures well within this range.

Unfortunately we did not succeed in monitoring the animals after the handling and this limits our interpretation of the safety of this technique. We therefore recommend further evaluation of the isoflurane on least weasels with consistent adequate telemetry monitoring of the animals after immobilization to assess post capture behavior and survival.

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Five “key references”, selected by the authors, are marked below (Three recommended (●) and two highly recommended (●●) papers).

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