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**MONITORING THE USE OF ARTIFICIAL LOG PILE OTTER HOLTS
USING HAIR ANALYSIS FROM BEDDING**

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Abstract: Nineteen, approximately ten year old log pile holts (LPH) in mid-Wales, UK, were dismantled prior to reconstruction and any bedding from couches removed for analysis. The plant material making up the bedding was identified and animal guard hairs contained within it were determined to species. The bedding material simply consisted of opportunistically available material with little evident selection. Hairs of cow and sheep were clearly brought in with the bedding but it is suggested that hairs of otter, badger, fox, dog, cat, American mink and polecat indicate use of the LPH by the species concerned. A possible pine marten record is also considered.

INTRODUCTION

Artificial log pile holts (LPH) for use by otters, have been in use in Wales for well over a decade and, in consequence, some of the first ones to be erected are now in need of refurbishment. It is usually the forest brush, often used to cover them, which decays first and needs replacing. After removing the old covering the inner chambers of the LPH are exposed and Geoff Liles and Louise Midgley of "Otters in Wales" and their co-workers took the opportunity to remove all bedding from couches in the chambers of 19 refurbished holts. This material was taken to Cardiff University's Field Centre at Llysdynam in mid-Wales where both the botanical make up of the bedding and, in particular, the identity of any mammal hair incorporated into the bedding were determined. Although clear from the evidence of spraints which LPHs had been entered by otters, identification of hair in bedding would confirm if otters would lie up there and which other species may have made use of the holts.

MATERIALS AND METHODS

The bedding from each chamber of each holt was collected separately, and, where the bedding was apparently layered, each layer was separately bagged. Each sample was loosely sealed into a polythene bag and appropriately labelled. The samples were then stored in a dry place until required for analysis. Prior to analysis the samples were stored for a few days in a deep freeze in order to kill any insects within the bedding.

Using a powerful desk lamp, each bag of bedding was searched thoroughly for mammal guard hairs which were usually easier to see when the light source came from behind. The guard hairs from each bag were put into a wide necked screw topped jar to await identification and the bedding material re-bagged for botanical analysis. The finer underhairs were not collected as they could not be identified. The bedding material from each sample was separated into its component parts by species and plant part (e.g. *Quercus robur* and *Salix cinerea*, leaves and twigs). Each component was then weighed.

Analysis of the hairs is a far more exacting process than that of sorting the plant material in the bedding. Conventional methodologies generally require that observation of the three layers of the hair, the cuticle, the cortex and the medulla, each need separate and often intricate preparation (HAUSMAN,

1930, 1932; MATHIAK, 1938; WILLIAMSON, 1951; WILDMAN, 1954; FORD and SIMMENS, 1959; SHORT, 1978; TERRINK, 1991).

The key to the hair of West European Mammals by TERRINK (1991) was useful but techniques in several keys advocated cutting up to ten cross section slides and ten cuticular slides per hair and this was unnecessary for most hairs considered and impractical in terms of time, equipment and the quantity of material for identification. Two of the present authors devised a specific hair key for this project (COWELL and THOMAS, 1999) and fast and simple histological techniques for mounting and identifying hairs, based on a reference collection of hair compiled by Cardiff University and The National Museum of Wales.

After trials of a range of techniques two were developed which proved adequate for the purpose providing sufficient information to identify the range of species we were likely to find in otter holts in Wales.

- a) Cellulose acetate cast technique (permanent fix - 3 mins to carry out).

The best techniques for observing the cuticle of hairs are generally based on making a cast in a suitable medium (e.g. gelatin or nail varnish) (WILDMAN, 1954; WILLIAMSON, 1951; TERRINK, 1991). Our technique involved the use of cellulose acetate sheet, which was used for casts but initially used to observe the medulla. A 5mm wide strip of cellulose acetate sheet was cut to a length slightly shorter than a microscope slide. Using a pipette, acetone was thoroughly coated along the length of the slide and the cellulose acetate strip immediately laid in the liquid lowering it on from one end of the slide. Superfluous acetone was poured or blotted off. The acetate strip was then attached to the slide. The hair was then laid lengthways on the acetate strip securing the tip with a drop of acetone. The hair is then stretched using forceps and the base fixed with acetone, as at the tip, then the entire length of the hair is flooded with acetone. Another cellulose acetate strip of approximately the same dimensions is placed on top of the hair. The hair is then sandwiched between two fused strips of acetate allowing the medullary structure to be seen. The technique can be used with several hairs at a time and takes about three minutes to carry out. To get a cast of the hair do not use the second acetate strip but after fixing the hair with acetone wait two minutes, gently peel the hair away avoiding damage to the distal end, then refix the hair alongside the cast for future reference.

- b) The "Sellotape" method - semi-permanent. Time taken - 30 seconds.

A simple and fast technique for making semi-permanent mounts involved the use of clear adhesive tape (Sellotape). The hairs were arranged along the length of a glass slide and a length of Sellotape the same length as the slide was held at each end and very rapidly put on to the slide to avoid hair movement due to static electricity. The tape was pressed and rubbed firmly down over the hairs to avoid air bubbles. For most species cuticular and medullary structures can be seen. Using TERRINK (1991) and our own key (COWELL and THOMAS, 1999) the hair samples were individually identified.

RESULTS

- a) **Bedding materials:**

For each chamber of each holt, and, where appropriate, each layer of each couch of bedding, a species list of plant material present was constructed and quantified by weight. Detailed results would be inappropriate and repetitive but several general observations can be made. Many couches consisted of fine twigs and leaves of the brush species used to cover the LPH. These were probably collected within the holt. As the covering brush is usually of local origin some of the material such as *Salix*, *Alnus* and *Quercus* leaves could have been brought in from outside the holt.

Other bedding material reflected the location of the holt. Where wetland with *Phragmites* or *Phalaris* was nearby these two grasses frequently predominated in the bedding. Where the surroundings were grassland *Agrostis*, *Holcus* and the rush *Juncus effusus* were frequent. In some cases the mosses *Rhytidiadelphus squarrosus* and *Thuidium tamariscinum* typical of deciduous woodland floors in mid-Wales were abundant. Occasionally other debris was found in the bedding including black polythene silage wrapping, clear polythene sheet, red polystyrene baler twine and

expanded polystyrene cup fragments. In many cases the lower layers of the couch were well rotted with fresher material above indicating fairly long continued use of that site.

b) **Hairs:**

The types of hairs found in the 19 holts examined are listed in Table 1. A number of holts were unused by otters but all contained some hairs although the dog (*Canis*) hairs probably came from the animal owned by one of the authors, which was generally present when holts were refurbished. Similarly human (*Homo*) hair is probably contamination during holt demolition and sample collection process.

Table 1. Hair types found in the 19 holts examined

Holt No.	Hair present of
1	<i>Lutra, Vulpes, Bos, Canis</i>
2	<i>Lutra, Bos</i>
3	<i>Lutra, Bos, Canis, Muridae, Homo, feathers</i>
4	<i>Meles, Bos, Canis, Muridae, Ovis, Oryctolagus</i>
5	<i>Lutra, Bos, Felis, Oryctolagus</i>
6	<i>Bos, minimal bedding</i>
7	<i>Lutra, Meles</i>
8	<i>Lutra, Bos, Oryctolagus</i>
9	<i>Lutra, Bos, Meles, feathers</i>
10	<i>Felis, Canis, Mustela putorius, Vulpes</i>
11	<i>Lutra, Bos, Canis, Felis, Homo, Mustela vison</i>
12	<i>Lutra, Bos, Mustela vison, Canis</i>
13	<i>Felis, Bos</i>
14	<i>Lutra, Bos, Canis, Muridae, Ovis</i>
15	<i>Lutra, Bos, Ovis</i>
16	<i>Ovis, Canis, Oryctolagus, Martes martes</i>
17	<i>Canis</i>
18	<i>Lutra</i>
19	<i>Lutra, Bos</i>

DISCUSSION

The provision of LPHs for otters has almost certainly been an aid to their recovery in England and in Wales (ANDREWS et al., 1993; STRACHAN and JEFFERIES, 1996) providing refuge particularly in places where natural holts are scarce. Although several of the authors are familiar with otter couches in open cover e.g. *Phragmites* beds, little is reported of their couches within holts in general and LPHs in particular. Our results suggest that the otter might first utilise the plant fragments which originate from the brush used to cover the LPH and which subsequently fall inside accounting for the frequent use of coniferous leaves and fine twigs where these are LPH construction materials. Because the bases of some couches are well rotted with fresher material above, it would suggest that these couches are renewed as necessary. Clearly much of the material comes opportunistically from the immediate environs of the holt as evidenced by the presence of cattle (*Bos*) and sheep (*Ovis*) fibres. The source of the dog hairs has been suggested as belonging to the LPH reconstruction crew. Cat (*Felis*) hair probably represents the fact that cats are the commonest larger mammal to be seen in the countryside by both day and night even at distances of over a kilometre from the nearest house (SLATER, 1994) and might therefore be expected to explore most areas in the course of time.

Otters clearly used most LPHs and if dog, cattle and sheep hair are excluded as coming from external sources, several holts were used exclusively by otters. Although the bedding was generally free of spraints, the occasional occurrence of broken amphibian bones might suggest occasional faecal contamination and might account for the small quantities of "mouse" (*Muridae*) and rabbit (*Oryctolagus*) hair and unidentified "feathers." Having accounted for the presence a range of possibly non-resident species, the presence of fox (*Vulpes*), badger (*Meles*), mink and polecat (*Mustela* sp.) suggested that LPHs offer sanctuary to most of the larger carnivorous mammals found in Wales. The record of pine marten (*Martes martes*), one of the rarest mammals in Wales, is one which needs reconfirmation. It was recorded from a part of Wales where the species has been recorded in recent

times and, if the record proved to be correct, then the use of LPHs might be targeted into other non-riparian habitats for the benefit of other vulnerable species.

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Résumé : Étudier l'Utilisation des Caliches Artificielles en Rondins par l'Examen des Poils de Litière

Au Pays de Galles, 19 caliches artificielles en empilements de rondins (LPH), construites environ 10 ans auparavant, ont été démantelées avant reconstruction, et à cette occasion la totalité des litières a été prélevée pour analyse. Le matériel constitutif de ces litières a été identifié, y compris les poils de mammifères pour diagnose spécifique. Le matériel de base semble être amassé de façon opportuniste par l'occupant, sans sélection nette. Des poils de bovins ou de moutons sont distinctement apportés et nous suggérons que la présence, dans la litière, de poils de loutre, blaireau, renard, chien, chat, vison américain ou putois est un indice d'utilisation du gîte concerné par l'espèce en question. Un cas possible de martre a été aussi envisagé.

Resumen: Monitoreo del Uso de Madrigueras Artificiales De Nutrias Utilizando Análisis de Pelos del Lecho

En Gales UK, 19 madrigueras artificial de madera (LPH) de aproximadamente 10 años de edad fueron desmanteladas antes de ser reconstruidas y todo lecho en las madrigueras fue recogido para ser analizado. Se identificó el material vegetal constituyente de los lechos y los pelos guardianes de animales contenidos dentro de este fue determinado hasta el nivel de especie. El material de los lechos consistió simplemente en el material disponible oportunamente, con poca evidencia de selección. Los pelos de vacas y ovejas fueron claramente acarreados con el lecho, pero se sugiere que los pelos de nutrias, tejones, perros, zorros, gatos visones americanos y turones indican el uso de los LPH por estas especies. Un posible registro de marta también es considerado.