WILDLIFE

Scanning electron microscopy and energy-dispersive x-ray spectroscopy (SEM-EDX) confirms shooting of a hen harrier (*Circus cyaneus*)

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SUMMARY

The hen harrier (*Circus cyaneus*) remains severely restricted as a breeding species in England despite sufficient habitat for over 300 breeding pairs. Human persecution is the main limiting factor and in the UK, there have been 45 confirmed incidents of shooting of hen harriers since records began (Royal Society for the Protection of Birds unpublished data). The authors report the pathological examination of a hen harrier, the detection of suspected ballistic fragments by radiograph and explain how scanning electron microscopy and energy-dispersive x-ray spectroscopy (SEM-EDX) was used to confirm (i) the composition of one ballistic remnant and (ii) that the remnant had been projected into and had damaged the bone. The authors report the use of post-analysis software to discriminate apparent anomalies produced by the proprietary SEM-EDX software package and discuss broader uses of SEM-EDX for wildlife crime investigation.

BACKGROUND

The hen harrier (*Circus cyaneus*) remains severely restricted as a breeding species in England despite sufficient habitat for over 300 breeding pairs (Fielding and others 2011). Human persecution is the main limiting factor and in the UK, there have been 45 confirmed incidents of shooting of hen harriers since records began (Royal Society for the Protection of Birds unpublished data). Since 2002, Natural England's Hen Harrier Recovery Project has monitored the numbers of breeding birds in England and used radio and satellite tags to track their movements (Natural England 2009). As a component of this project, hen harriers found dead are examined postmortem to investigate the health of the population. In the past, shooting of birds has been diagnosed using forensic examination including imaging, dissection and histopathology (Cooper and Cooper 2007, Munro and Munro 2008).

Scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDX) is a procedure for investigating the structure and elemental composition of a sample. A beam of electrons is scanned across a sample; the resulting secondary electrons are converted into a digital signal, which generates a microscopic image of the sample (Goldstein and others 2003). The electron beam is then focused onto a chosen target and the resulting emitted x-ray signal is detected by an energy dispersive detector. This result is presented to the user as a spectrum in which the intensity of characteristic x-rays is shown as a function of their energy.

The authors report the pathological examination of a hen harrier, the detection of suspected ballistic fragments by radiograph and explain how SEM-EDX was used to confirm (i) the composition

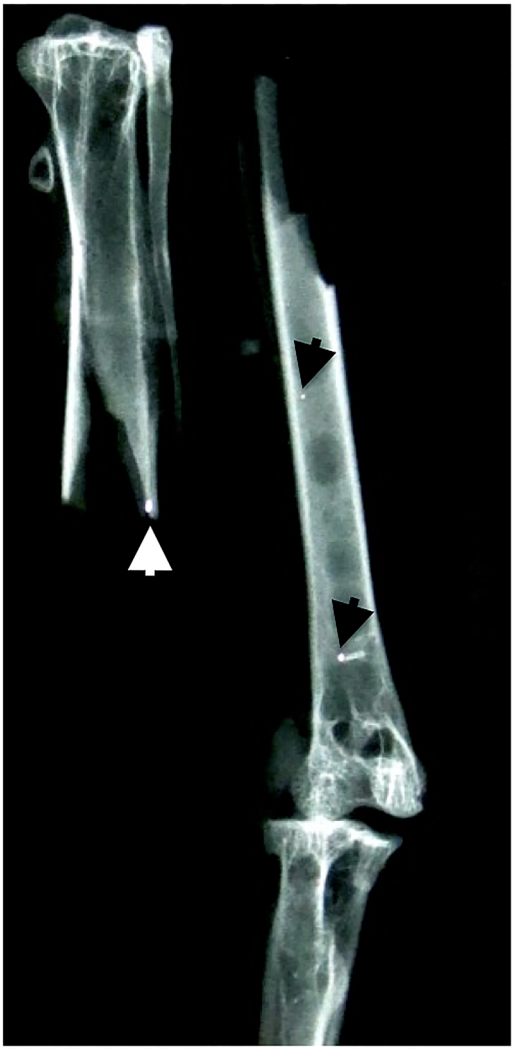

FIG 1: Plain craniocaudal radiograph of the left tibiotarsus and tarsometatarsus showing three radiodense objects (white and black arrowheads)

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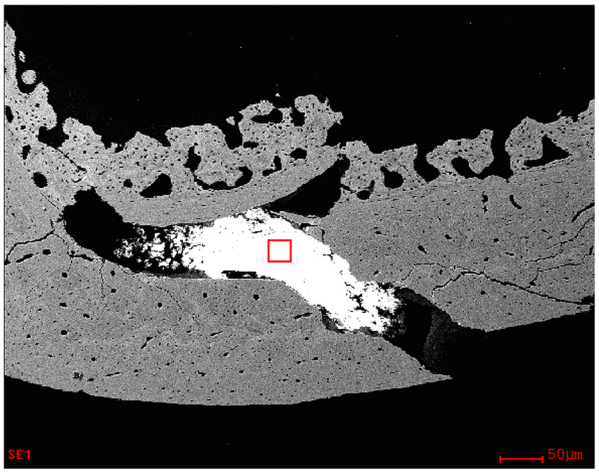

FIG 2: Scanning electron micrograph of the foreign object (white) embedded in the proximal tibiotarsal bone fragment of the hen harrier (grey). The red square is the target for energy dispersive x-ray spectroscopy

of one ballistic remnant and (ii) that the remnant had been projected into and had damaged the bone.

CASE PRESENTATION

A juvenile female hen harrier was fitted with a satellite transmitter at 32 days of age. Thirteen months later (and seven days after the bird's last movement was detected), the carcase was located on land managed for grouse shooting in Yorkshire. The carcase was in an advanced state of decomposition. There was a complete, simple, oblique fracture of the diaphysis of the left tibiotarsus. Six tail feathers had complete transverse fractures of the rachides.

INVESTIGATIONS

Radiography after postmortem examination

Lateral and craniocaudal radiographs of the left and right legs (Ultralight 300; 50 Kv, 60 mA, 0.03 s using mammography film) were taken (Fig 1). Three objects with metallic radiodensity were seen within or adjacent the fracture fragments. These objects were consistently located on both lateral and craniocaudal views and using different radiographic cassettes.

Scanning electron microscopy and energy dispersive x-ray spectroscopy (SEM-EDX)

The proximal fragment of the left tibiotarsus was coated with a layer of gold-palladium to improve conductivity and reduce charge accumulation during scanning. Scanning electron microscopy (JEOL JSM-5500LV) of the largest radio-opaque object showed a deformed area of foreign material embedded in, and damaging, the bone (Fig 2). Selected areas were chosen for energy dispersive x-ray spectroscopy. The spectrum of energies emitted from the object initially revealed a high proportion of lead (Pb) and a smaller proportion of niobium (Nb).

The original scan x-ray spectrum data were analysed using DTSA II software (Desktop Spectrum Analyser, National Institute for Standards and Technology – Ritchie 2012) to further discriminate the spectral peaks. Fig 3a shows the postanalysis spectrum including gold (Au) and palladium (Pd). Fig 3b is a zoomed-in portion of the sample spectrum showing most of the main peaks. Minor amounts of antimony (Sb) and arsenic (As) are visible.

DISCUSSION

The presence of the fracture of the left tibiotarsus and three foreign objects of a similar radiodensity to metal within the left tibiotarsus suggested this bird had been hit by a metal projectile and that the fracture was associated with the projectile's kinetic energy. The tail feather damage was consistent with acute trauma, such as shooting, fighting or a collision (pers. comm.

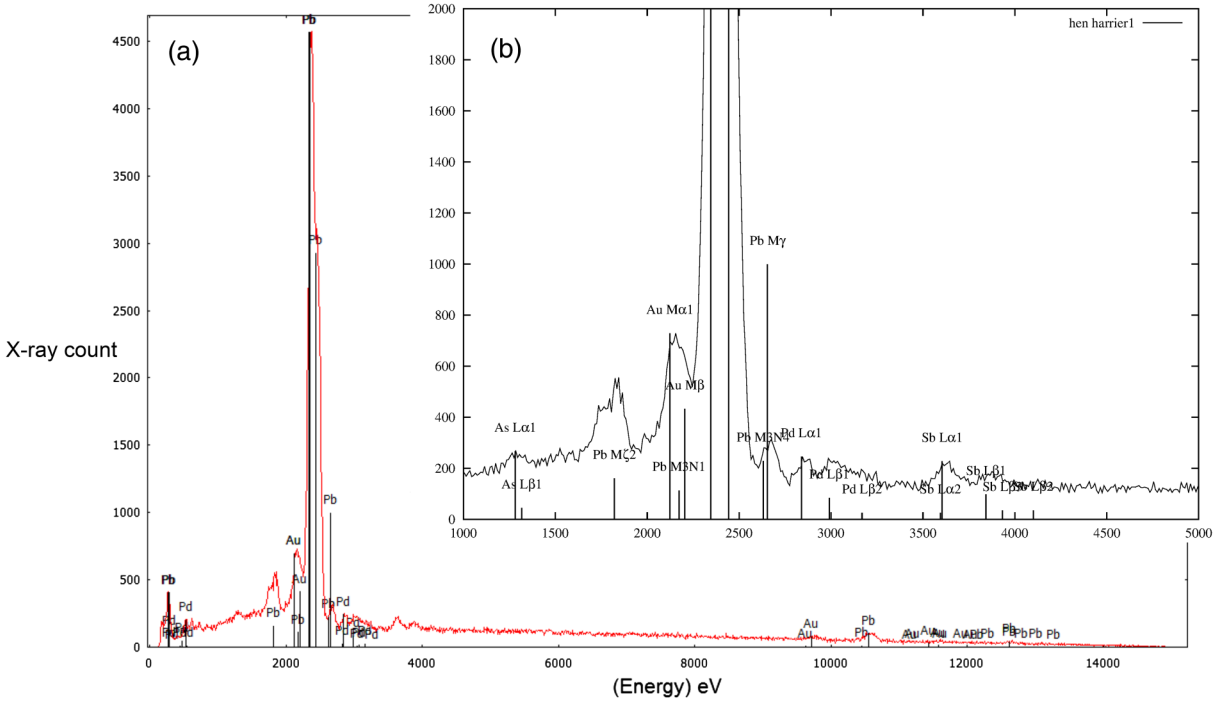

FIG 3: Foreign object energy-dispersive x-ray spectroscopy (EDX) spectra postanalysis with DTSA II software: (a) A predominance of lead (Pb) and the presence of gold (Au) and palladium (Pd). (b) Additional trace elements (arsenic (As) and antimony (Sb)) with identified Siegbahn x-ray notations

Brian Etheridge, July 18, 2013). SEM-EDX of the radiodense object embedding in the bone, and the associated damage, provided convincing evidence that one or more high-energy, predominantly lead projectiles caused the fracture. Alternative hypothetical causes of this fracture and the origin of the foreign objects (e.g. blunt trauma, predation and/or scavenging behaviour) were thus rejected and the authors concluded the bird was shot. As there was no significant bone resorption or remodelling around the metal or adjacent bone, the authors inferred that the injury likely led to the rapid death of this hen harrier.

Analysis with DTSA II (Fig 3) discriminated the gold-palladium coating as the cause of the apparent niobium and demonstrated the failure of the original autoidentification software within the proprietary package. DTSA II also revealed the presence of small amounts of antimony and arsenic, both commonly used to render additional characteristics to lead ammunition (Wallace 1990, Dufosse and Touron 1998).

SEM-EDX has also been used to detect *microscopic* gunshot residue (GSR) in the wounds of human beings (Biro and others 2010). This technique will be of particular interest in the investigation of suspected persecution of wild animals wherein a ballistic object is lacking or the carcase is markedly decomposed. Under ideal circumstances SEM-EDX of GSR from a wound may also determine the firing distance and ammunition type used (Chang and others 2013). On a 'case-by-case' basis, SEM-EDX of GSR on a suspect can confirm that the person recently fired a weapon (Saverio Romolo and Margot 2001, Dalby and others 2010). Used in concert, these techniques may contribute to the evidence of association between a suspect and an incident and allow detailed reconstruction of a crime. This evidential value could significantly deter potential persecutors and poachers, directly aiding the conservation of threatened species.

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Competing interests None declared.

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