Strengthening the wildlife forensic toolkit through the adoption of human specific approaches to identification

by

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List of Abbreviations

ACE-V – Analysis, Comparison, Evaluation, Verification

AFIS – Automated Fingerprint Identification System

BMP – Black Magnetic powder

BWCO – Borough Wildlife Crime Officers

CAST – Centre for Applied Science Technology

CITES – Convention of International Trade in Endangered Species

CODIS – Combined DNA Index System

CSE – Crime Scene Examiners

DFO – 1, 8 – Diazalfluoren-9

EU – European Union

FIT – Footprint Identification Technique

IFRG – International Fingerprint Research Group

IOZ – Institute of Zoology

IQR – Interquartile Range

ISFG – International Society for Forensic Genetics

IWT – Illegal Wildlife Trade

LCN – Low Copy Number

LOD – Limit of Detection

LR – Likelihood Ratio

MCAR – Missing Completely at Random

MI – Multiple Imputation

MICE – Multiple Imputation by Chained Equations

MNOC – Minimum Number of Contributors

NDIS – National DNA Index System

NGO – Non-Governmental Organisation

NWCU – National Wildlife Crime Unit

PAW – Partnership for Action Against Wildlife Crime

PCR – Polymerase Chain Reaction

PD – Physical Developer

PI – Principal Investigator

POI – Person of Interest

QS – Quality Sensors

RFU – Relative Fluorescent Units

RSPCA – Royal Society for Prevention of Cruelty to Animals

SMP – Supranano™ Magnetic Powder

SPR – Small Particle Reagent

STR – Short Tandem Repeat

SWFS – Society for Wildlife Forensic Science

TPPR – Transfer, Persistence, Prevalence and Recovery

UKAS – United Kingdom Accreditation Service

UNODC – United Nations Office on Drugs and Crime

UV – Ultraviolet

VMD – Vacuum Metal Deposition

WCO – Wildlife Crime Officers

WCU – Wildlife Crime Unit

ZSL – Zoological Society of London

Abstract

Wildlife crimes, including poaching and illegal trade, pose serious threats to both wildlife and human populations. A history of apathy and ineffective enforcement has allowed offenders to view these crimes as “low-risk, high-reward.” As a result, they persist, target a growing range of species, and there is a pressing need to rethink investigative approaches.

This thesis identifies a major gap in wildlife crime investigations: the underuse of traditional human forensic techniques, including DNA profiling and fingerprinting. Whilst wildlife preservation is the driver, this research centres people, both perpetrators and practitioners. It explores the largely untested potential of wildlife derivatives (including elephant ivory, tiger claw, sawfish rostrum, tortoise shell, deer antler, and gorilla skull) as surfaces for recovering fingerprints and human touch DNA and assesses law enforcement capacity to carry out this work.

Recognising the novelty of wildlife derivatives, and constraints on handwashing and face-touching (grooming) behaviour during COVID-19, I first examined how handling technique and grooming affect fingermark and touch DNA deposition. Results demonstrated grooming significantly impacted deposition, while handling technique did not.

These findings informed the methodology for comparative tests of four fingerprint enhancement techniques (including monochromatic, and fluorescent fingerprint powders, and gelatin lifters) and four touch DNA recovery techniques (cotton, flocked, and foam swabs, and mini-tapes) on multiple wildlife derivatives. Reduced-scale red fluorescent magnetic powder excited using a 365nm light source proved most effective for fingerprint enhancement across derivatives, providing high contrast on their patterned surfaces. Foam swabs, rarely used for touch DNA, yielded the highest average DNA recovery, significantly outperforming mini-tapes on several derivatives, likely due to their surface area, malleability, and retention properties. PCR inhibitors were suspected of affecting DNA sample analysis from multiple derivatives, particularly from the surface of ivory.

Surveys of UK wildlife crime officers (WCO) and crime scene examiners (CSE) confirmed a limited use of human forensics, inconsistent evidence recovery by WCO’s, and minimal training for CSEs in wildlife crime investigations. In-person training improved both capacity and likelihood of future CSE engagement.

This thesis calls for greater forensic parity in wildlife crime and recommends integrating CSEs and adopting effective tools, such as reduced scale red fluorescent magnetic powders and foam swabs, to improve investigative outcomes and enforcement efforts.

Author's Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Introduction

The problem of wildlife crime

Wildlife crime consists of a broad spectrum of activities, geographic ranges, and species of interest. The illegal wildlife trade (IWT) is one of the most recognisable iterations of wildlife crime and stands as a global crisis, with over 160 countries having reported incidences of either illegal import, export or transit of at least 4000 species in recent years (UNODC, 2024). These figures are already cause for alarm, but, due to the inherently covert nature of the act, they are likely to still be underestimations of the true scale and complexities of the IWT (Nijman and Shepherd, 2021). Other well documented examples of wildlife crimes include poaching, for both trophies and consumption, (National Wildlife Crime Unit, 2020), animal persecution, (RSPB, 2019; Goodall, 2021), and nest/roost destruction (Voigt and Kingston, 2016). The resulting impacts of unchecked wildlife crime include unsustainable levels of biodiversity loss (Wittemyer et al., 2014) and its subsequent cascading ecological impacts, increased zoonoses risks and pandemic like events (Bezerra-Santos et al., 2021), and undermining of law enforcement, strengthening the risk of social and economic instability within a nation (Marijnen, 2017). Despite the long-term risks posed by failing to address wildlife crime, this form of criminal activity endures due to a systemic legacy of ineffective interventions (Wilson and Boratto, 2020).

Enforcement efforts in wildlife crime

There have been both proactive and reactive approaches to tackling wildlife crime. Proactive approaches focus on deterrence tactics aimed at preventing wildlife crimes from being carried out in the first instance. Examples include, attempts to change perceptions and drivers towards engaging with wildlife crime through educational programmes,

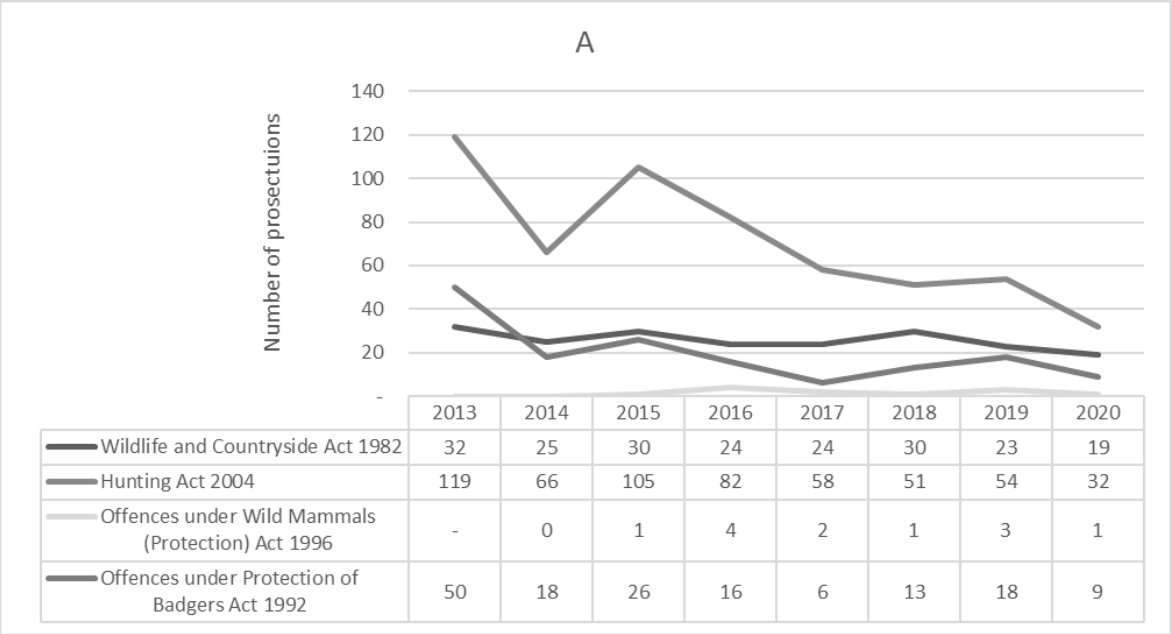
community engagement, alternative livelihoods, and implementation of policies and legislation (Travers et al., 2019; Lemaître and Hervé-Fournereau, 2020; Didarali et al., 2022). Deterrence is in part achieved through instilling a mixture of greater understanding of the impacts of wildlife crime to both animals and communities, and a fear of apprehension and subsequent repercussions (Lam et al., 2023). Reactive approaches occur after the fact and include investigating crimes which have already been committed, enforcing the extensive national legislation and international agreements that exist, and gathering intelligence which can feed back into more proactive work (Anagnostou et al., 2020).

Enforcement success in wildlife crime has been varied; high profile arrests such as that of the “Ivory Queen” (BBC News, 2019) and Lin-Zhang gang (Environmental Investigation Agency, 2021) suggest promising developments in targeting principal players in trafficking rings. However pre-pandemic, seizure rates remained consistent or increased for a range of species (C4ADS, 2024), indicating such arrests were not acting as sufficient deterrents. Post pandemic, as the world began reopening its borders, there has seen a return to large scale seizures and an increase in exploitation of maritime trade routes (Environmental Investigation Agency, 2024). This indicates, despite COVID-19 raising public awareness of the potential role wildlife trade plays in the spread of zoonotic (Booth et al., 2021) diseases, demand for illegal wildlife goods is still present and criminal organisations are adapting their approaches to movements of goods. Source nations with targeted high risk species continue to struggle with making significant progress (Sherman et al., 2022) while lenient sentencing and chronically low prosecution and conviction rates is a repeated concern (Salum et al., 2017a; Omifolaji et al., 2022; Sosnowski et al., 2022). Suggested underlying factors that impede both proactive and reactive approaches include; 1) limited resources, 2) overwhelming scale, 3) corruption, 4) apathy, and 5) ineffective deterrents (Wellsmith,

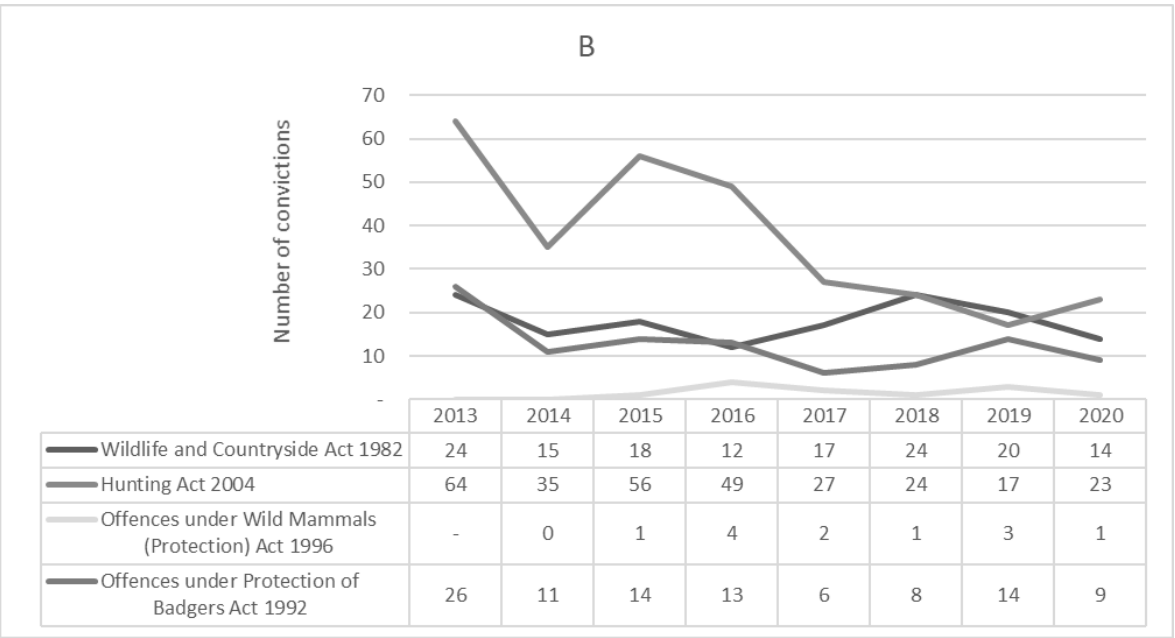
2011). These challenges lead to an inference that wildlife crime is a low risk, high reward activity worthy of criminals time (Salum et al., 2017a; Sollund and Runhovde, 2020).

A tunnel vision approach to forensic interventions

Significant amounts of wildlife crime discussions centre highly biodiverse low-income nations as key exporters of wildlife goods, and highly endangered charismatic species, such as pangolin, as flagship representatives of the issue. However, high income nations which play a large role in imports and transit also experience failure in the enforcement arena (Sosnowski et al., 2022) and species designated as least concern by the International Union for Conservation of Nature (IUCN) are still the target of illegal activities (Enari, 2021) . The UK, for example, is well placed to support wildlife crime investigations; it has a government funded National Wildlife Crime Unit (NWCU) (a UK police intelligence unit which provides operational support to law enforcement carrying out wildlife crime investigations), stakeholder involvement through the Partnership for Action Against Wildlife Crime (PAW) collaboration, as well as clear policy describing their priority areas. Though lauded for their contribution to international efforts to tackle wildlife crime, such as the IWT challenge fund, a recent United Nations report recommend the UK strengthen their domestic policies and efforts (UNODC, 2021). Advice underscored by the increased number of reports of crimes against badgers and bats, two priority species, (Wildlife and Countryside Link, 2020) but decline in prosecutions and convictions under key wildlife legislation (Figure 1.1).



	2013	2014	2015	2016	2017	2018	2019	2020
Wildlife and Countryside Act 1982	32	25	30	24	24	30	23	19
Hunting Act 2004	119	66	105	82	58	51	54	32
Offences under Wild Mammals (Protection) Act 1996	-	0	1	4	2	1	3	1
Offences under Protection of Badgers Act 1992	50	18	26	16	6	13	18	9



	2013	2014	2015	2016	2017	2018	2019	2020
Wildlife and Countryside Act 1982	24	15	18	12	17	24	20	14
Hunting Act 2004	64	35	56	49	27	24	17	23
Offences under Wild Mammals (Protection) Act 1996	-	0	1	4	2	1	3	1
Offences under Protection of Badgers Act 1992	26	11	14	13	6	8	14	9

Figure 1.1: *Number of prosecutions (A) and convictions (B) under four key pieces of UK wildlife legislation between 2013-2020.*

Across all nations and crimes, during an investigation law enforcement seeks to confirm, firstly, whether a crime as taken place and secondly to positively identify the human criminal(s) responsible. This is achieved in part through the production of robust evidence to inform and direct criminal investigations. Evidence types are vast but one consistent evidence type utilised in criminal investigation is forensic evidence (Ling et al., 2021). Whilst

veterinary forensic pathology has been regularly implemented to ascertain cause of death in wildlife crime cases (Millins et al., 2014; Cooper, 2021), it is species identification, through the use of DNA barcoding, that been the main focal area for forensics (Gouda et al., 2020). This body of work addresses the need to positively identify wildlife and their derivatives particularly in the absence of morphological characteristics. Globally different species are afforded different levels of legislative protection depending on context. Therefore, positive identification is vital in establishing whether a crime has taken place, and which punishment should subsequently be afforded. The discipline has demonstrated its value by contributing to several wildlife crime investigations (Caniglia et al., 2010; Ghosh et al., 2019). A natural progression from species identification has been the need for individual identification or parentage analysis. This has been used to help link individual wildlife parts to crime scenes (Moore and Frazier, 2019), to link shipments (Wasser et al., 2018), to identify individual animals from private collections (Gupta et al., 2011) or to camera trap records (Hiby et al., 2009), and to establish the number of individual animals that are victims in a crime (Moore and Frazier, 2019). Species identification and individual identification in wildlife crimes commonly employ mitochondrial DNA (mtDNA), and Short Tandem Repeat (STR) profiles of nuclear DNA (nDNA) respectively (Gouda et al., 2020). STR typing uses the same concepts as human DNA profiling and the sequencing of the human genome has allowed for human DNA profiling to become standard practice in criminal investigations, and multiple commercial kits are available for use. To recreate such a commonplace use of species or individual level identification for wildlife is a daunting prospect considering the thousands of species that fall victim of wildlife crimes (Johnson et al., 2014; Cardinali et al., 2023) and due to the extensive resources required progression in this area is significantly slower (Moore and Frazier, 2019). Additionally there are high standards to be met before it can be taken seriously within the wider forensic and law enforcement community (Ogden, 2010).

ISO/IEC 17025 and 17020 accreditation is the internationally recognised standard, and often legal requirement, for forensic laboratories and practitioners to prove their competency to collect process forensic evidence (Rankin and Welsh, 2013; UNODC, 2016a). A 2016 CITES and United Nations Office on Drugs and Crime (UNODC) commissioned survey of 110 wildlife forensic associated laboratories found just 22 were externally audited under these standards (UNODC, 2016a).

A more traditional use of forensics in criminal investigation is the application of human identity testing, often presented as fingermark or DNA evidence (Ling et al., 2021). Global infrastructure for human identity testing, including accredited laboratories, is constantly growing (McAndrew et al., 2023) and a wealth of research, knowledge, techniques and tools exist for utilisation by law enforcement. Despite ongoing contributions to solving human-on-human crime the literature suggests its application and development, is low in wildlife crime contexts (Figure 1.2). This is interesting given the theory and concepts behind both fingerprints and human DNA profiles have both been contextually applied to identification of wildlife. Possible reasons for a lack of application and research in this area include i) the observed separation between practitioners of human and wildlife forensics, ii) a lack of awareness/interest by researchers as to the cross-applicability of the methods, iii) unpalatable costs associated with human forensic methods when investigations only lead to small penalties or iv) the methods are not applicable in most wildlife crime cases.

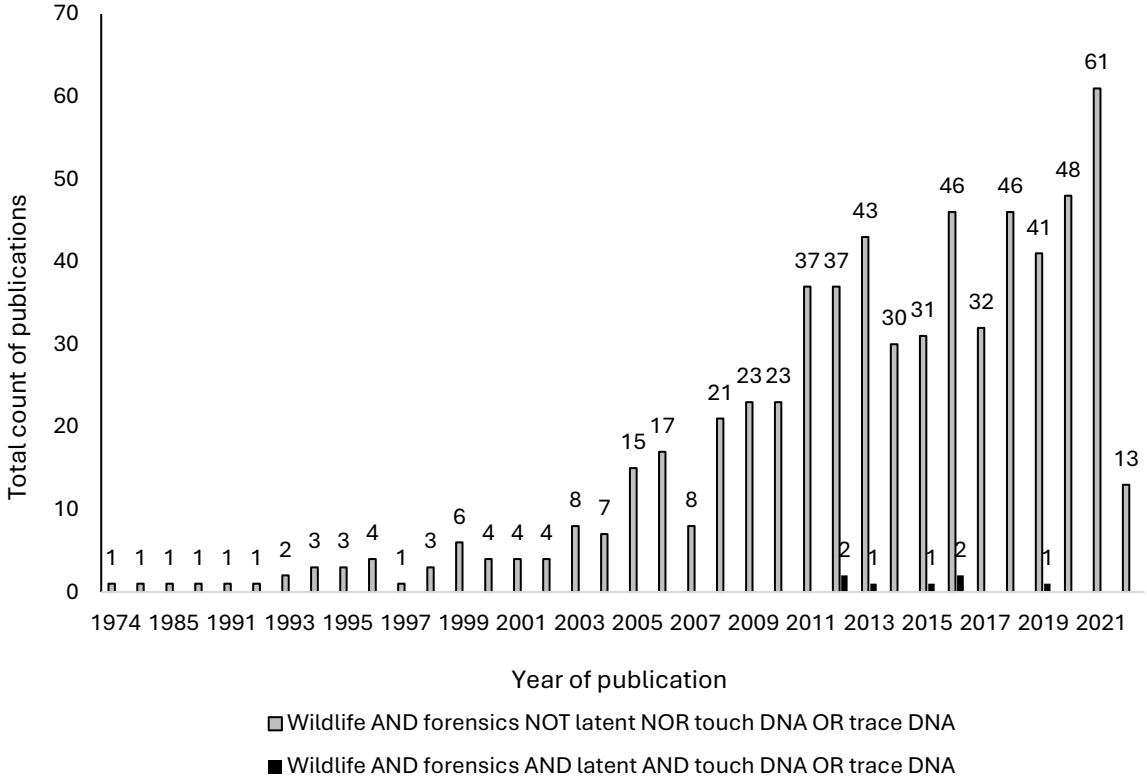


Figure 1.2: *Number of hits from a literature search using the Boolean terms "Wildlife" AND" forensics" NOT" latent" NOR" touch DNA" OR 'trace DNA' and a literature search using the Boolean terms "wildlife AND forensics" AND including "latent" AND "touch DNA" OR "trace DNA".*

Regardless of the reason, the main aim of any criminal investigation is to identify a suspect and establish a link between the suspect and the illegal activity under investigation. Species or individual identification of wildlife can establish if a crime has been committed and as such is of value in seizures where a suspect is claiming goods are of legal origin. However, where no suspect is yet identified it is limited in its evidential value to find one. Two UK cases of raptor and badger persecution demonstrate this phenomenon. In both cases, carcasses, of white tailed eagles and a badger respectively, were reported to law enforcement with the condition of the carcasses rendering morphological assessment possible for species identification (BBC News, 2021, 2023). Both incidents occurred in rural areas with no immediate suspects and with the carcasses themselves appearing to be the only tangible evidence available. The position of the carcasses strongly indicated human

involvement or handling therefore by PAW's own guidelines present as possible surfaces for consideration for recovery of human trace evidence (PAW Forensic Working Group, 2014). To the best of available knowledge human trace evidence was not carried out at either scene, and both cases remain unsolved. These types of cases, where species identity is not in question and a lack of direct evidence, such as witness testimony, exists suggest a logical need to re-evaluate approaches to wildlife crime investigations and the role human identity testing could play. Through human trace evidence recovery additional opportunity should present itself to increase the amount of evidence linking an individual to an associated crime beyond reliance on direct evidence. This in turn can strengthen or create new leads in cases where insufficient evidence is presented to identify a suspect or garner a conviction. Unlike species identification, human based identification may also unearth links to other crimes, including those non-wildlife related, shedding light on the suspected crossovers in organised criminal networks (Wyatt et al., 2020). It appears that the forensic community is overlooking the constant in all wildlife crimes: the human perpetrator.

Research gaps, aims, and objectives

There is currently stunted approach to the application of forensics in wildlife crime investigations, with little consideration given to the potential of leveraging human identity forensics techniques and infrastructure. At the time of writing this thesis there are just 13 peer reviewed publications on the subject of human trace evidence recovery from the surface of wildlife derivatives, including ten publications on fingermark recovery and three on touch DNA recovery. Chapter one of this thesis provides a full literature review covering these publications. However, to summarise the key takeaways that influenced this thesis' research questions; firstly, each of these publications focuses on either a single species or scenario type and typically investigates a limited number of techniques. This is despite forensic wildlife crime interventions needing to function on a global scale, across a diverse

set of circumstances and evidence types. Secondly, both within these studies and the wider literature, there is a lack of effort to assess the existing approaches and capacity of law enforcement to recover human forensic evidence in wildlife crime investigations. As a result, there is insufficient understanding of how tested techniques can be realistically implemented into real-world contexts. Thirdly there is a general lack of recognition regarding the novelty of wildlife derivatives as surface types for trace evidence recovery. Consequently, little scrutiny has been given to whether existing methodological design guidelines, developed around more commonly encountered man-made objects, are suitable or require adaptation. Although not an original focus of this thesis, due to the events of the COVID-19 pandemic, this third point became increasingly relevant as ethical and behavioural constraints relevant to the work, such as increased hand-washing and reduced face-touching, altered how forensic research could be conducted safely.

1. This thesis aims addresses these gaps by answering the following research questions: How do variations in touch DNA and fingerprint deposition techniques affect the reliability and methodological design of forensic research in novel investigative contexts e.g. human trace evidence recovery from the surface of wildlife derivatives?
2. How effective are existing fingerprint and touch DNA recovery techniques in handling the variability of wildlife-derived materials encountered in forensic contexts?
3. In what ways is forensic science currently used in wildlife crime investigations by a UK urban police force, and can targeted training improve its application by law enforcement?

These questions are explored and answered within a literature review and five empirical chapters, the aims of which are presented in Table 1.1. The thesis concludes with a general discussion on their findings, a critique of the work that was carried out, and suggestions of future work to further develop the themes investigated in this thesis.

Table 1.1. Individual empirical chapter aims of this thesis and the associated research questions they endeavour to answer: Strengthening the wildlife forensic toolkit through the adoption of human specific approaches to identification.

Research question	Chapter	Aim
1	2	Investigate the impacts of deposition and preparation methodologies on fingermark grade outcomes.
1	3	Investigate the impacts of deposition and preparation methodologies on quantities of deposited touch DNA.
2	4	Compare the efficacy of low-cost field deployable enhancement techniques for fingermark recovery from the surfaces of wildlife derivatives.
2	5	Compare the efficacy of low-cost field deployable human touch DNA recovery techniques from the surfaces of wildlife derivatives.
3	6	Explore the current approaches and attitudes towards the use of forensics in wildlife crime investigations within UK wildlife crime officers.
3	6	Explore and build capacity in current and future involvement, perceptions, and knowledge of wildlife crime investigations within metropolitan based crime scene examiners.

Chapter 1: Human identity forensics in wildlife crime

Preface

Preface: A version of this chapter has been published in: A. Thomas, L. Gibson, S. McColl, R. Rae, R. Ogden, N. Dawnay. What is it vs Who did it? A review of the lack of human focused forensic evidence in the context of wildlife crime, *Forensic Science International: Animals and Environments,* 4 (2023) 100073. https://doi.org/10.1016/j.fsiae.2023.100073 (Appendix I).

1.1 Introduction

This chapter reviews the existing research into the application of two main forms of evidence used in human identity testing, fingermarks, and DNA evidence in wildlife crime forensic investigation. It further highlights the limited number of instances where they have been applied in wildlife crime research and investigations. This review was carried out in a traditional approach combining several evidence gathering methods including the identification of relevant stakeholders in the field; a review of UK government and policing related policy and guidance documents; identification through United Kingdom Accreditation Service (UKAS) of common forensic methods used in human identification; and a trawl of the existing scientific literature of the most common methods.

1.2 Human Identity Forensics: Fingermarks

1.2.1 Background and composition.

On almost all individuals fingertips there exist friction ridges made up of a finite number of characteristics which present themselves in patterns, unique to everyone known as their fingerprint. The modern-day human populace has exploited this uniqueness for identification and security purposes and fingerprints are now used to gain access to phones, personal banking, places of home and work and as a means of border security (Smith and

Miller, 2021). Their most recognisable use however is by law enforcement to link a suspect to a crime by way of their location, enhancement, and preservation at a scene of crime or on evidence. Once enhanced or recovered from evidence or crime scenes unidentified fingerprints, referred to operationally as “fingermarks”, of sufficient quality can be compared against fingerprints of known individuals or against other unidentified fingermarks to establish a match. Though their composition involves a complexity of factors which is a topic of much debate, superficially they are composed of an amalgamation of secretions from the palms of hands (eccrine), dermis (sebaceous), and in adults the axillary and pubic regions (apocrine), coupled with skin or environmental contaminants (e.g. beauty products, food grease, pollen, dust) (Sears et al., 2012). Their composition changes almost immediately upon deposition, with time, environmental exposure and the substrate type (porous vs non-porous) all influencing longevity (Girod et al., 2012; Cadd et al., 2015; Bouzin et al., 2020). The immediate change to fingermarks occurs with the evaporation or absorption (dependent on surface type) of water and volatile lipids. Water loss results in a “waxier” fingermark as the remaining organic and inorganic compounds become concentrated. Salts will also crystallise and become vulnerable to physical erosion and ultraviolet (UV) exposure (Girod et al., 2012). Over the course of around thirty days most lipid components of sebaceous secretions will reduce significantly; squalene and unsaturated fatty acids are lost first with saturated fatty acids and non-volatile lipids including wax esters and triglycerides being more durable (Seah et al., 2005; Weyermann et al., 2011; Frick et al., 2020). As well as water, temperature, humidity, UV exposure and other forms of radiation contribute to the longevity of latent fingermark constituents (Seah et al., 2005; Mountfort et al., 2007; De Paoli et al., 2010; Paine et al., 2011). Despite this volatility fingermarks have been recovered decades after deposition (Tapps et al., 2019;

Bouzin et al., 2020) and after days or weeks of environmental exposure (Hagan and Green, 2018; Colella et al., 2020).

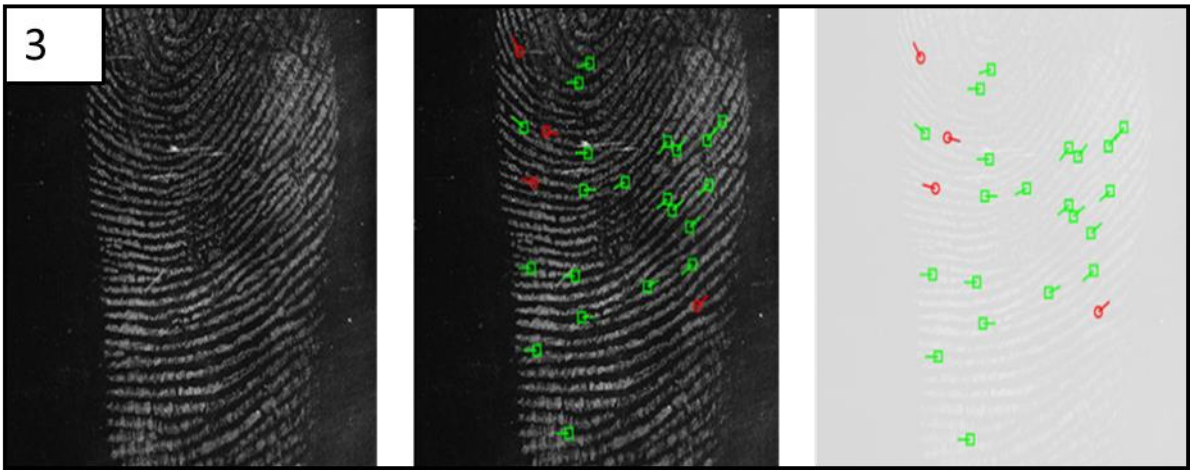


Figure 1.3. *(left) Latent fingermark with no mark-up in original state; (middle) Highlighted bifurcations and ridge endings as would be placed by an AFIS; (right) A "map" of minutiae that would be searched against. Images generated using Fingerprint Minutiae Viewer (FpMV) software* (National Institute of Standards and Technology, 2014).

For processing of fingermarks the Analysis, Comparison, Evaluation, Verification (ACE-V) approach is widely adopted (Needham et al., 2022). Historically each phase was carried out by hand however increasingly countries are utilising biometric Automated Fingerprint Identification Systems (AFIS) in their workflows (Drozdowski et al., 2019). A traditional AFIS functions via algorithms focused on identifying and tagging fingerprint minutiae, specifically bifurcations and ridge endings (Figure 1.3), creating a "map" for comparison (Moses, Kenneth R et al., 2011). Three countries hosting large biometric databases, China, the USA, and the UK are notable players within wildlife crime either as import, export, or transit countries (Wong, 2019; Tow et al., 2021) or as vocal advocates for improved international efforts (UK Government, 2019b). The transnational nature of wildlife crimes is well documented and in this vein INTERPOL hosts an international AFIS accessible to member nations (INTERPOL, 2021b).

Table 1.2: *Common fingermark enhancement methods presented in order of their recommended sequential application. Adapted from the Fingermark Visualisation Manual* (Home Office, 2022).

Method	Theory	Practical limitations	Porosity	Process
Fluorescent examination	Exploitation of fluorescing constituents either within fingermarks or substrates to provide contrasting illumination.	Requires a dark environment which can be difficult to achieve outside laboratory conditions.	Any	Physical
Powder	Applied directly to a substrate with the design of having a stronger affinity to fingermark constituents comparable to the surface they have been deposited on	Poor application technique can result in damage to the fingermark.	Non-Porous, Semi-Porous	Physical
Powder suspensions	Fine powder incorporated through a solution of detergent and water believed to interact with eccrine and sebaceous components of fingermarks.	Requires a water wash step after application which can be messy and impractical to contain at a scene.	Non-Porous, Semi-Porous	Physico-chemical
Vacuum metal deposition	Functions via the sequential evaporation of metals (gold, zinc) onto a surface within a vacuum. Fatty acids within fingermarks inhibit the layering process so that they become visible within the coated substrate.	An expensive process requiring specialist equipment and training. Irregular shaped objects can be difficult to process if areas are shielded from direct line of evaporation vessels	Non-Porous, Semi-Porous	Chemical
Ninhydrin	Targets the amine group within amino acids triggering a colour change reaction known as Ruhemann's purple.	Optimal process conditions are difficult to achieve at scene and humidity oven size in laboratory limits the size of items which can be processed.	Semi-Porous, Porous	Chemical
Basic Violet 3	A staining process which targets sebaceous sweat constitutes, shed skin cells and other contaminants to produce a purple mark.	The rinsing step can make containment of the hazardous dye difficult at scenes	Non-Porous	Chemical
Cyanoacrylate (superglue) fuming	Polymerisation of ethyl cyanoacrylate (superglue) triggered by water within eccrine sweat results in the accumulation of a "noodle-like" structure presenting as a white residue onto a fingermark	Optimal process conditions are difficult to achieve at scene and cabinet size in laboratory limits the size of items which can be processed.	Non-Porous, Semi-Porous	Chemical
Indandione/DFO	Reacts with amino acids within a fingermark resulting in a pink product which is best viewed using fluorescent examination	The reaction is initiated through heating making it problematic to carry out at scene.	Semi-Porous, Porous	Chemical
Physical developer	Fingermark constituents trigger a disturbance within a stable silver-based solution resulting in deposition of silver at the disturbance site.	Highly impractical to implement at scene. Cannot be followed up by subsequent enhancement techniques.	Semi-Porous, Porous	Chemical

1.3. Fingermarks: Application of methods in wildlife crime

Much of existing fingermark recovery research has been focused on “traditional” crime scenes and evidence types; these include vehicles, weapons, clothing and household goods or infrastructure such as doors and window frames. This focus has spilled over into the wildlife crime context with fingermark recovery attempted on similar substrates in environments associated with wildlife crime activity (Mayer, 2019). A less traditional evidence type, but one of vital importance in wildlife crime, are animals and their derivatives. Comparative to “traditional” evidence types there has been minimal research of fingermark recovery in this area. The research that does exist can be loosely grouped into surface type and are as follows; leathers and skins, inclusive of mammalian and reptile species, ivory, horn, antlers, feathers, eggs, fur, and pangolin scales (Table 1.3).

1.3.1 Fingermarks: Leather and skins

Leather and animal skins are animal products commonly encountered in ever day circumstance, most often seen in the guise of accessories such as wallets or belts and upholstery such as car seats. As such they are regularly encountered in non-wildlife case work and enhancement of fingermarks from these items are among some of the earliest associated work in this hybrid area. Leather is considered a problematic substrate due to its texture, porosity, and the multiple process stages it may be encountered in (Home Office, 2022). Despite the regularity in which leather items are encountered in criminal investigations success in fingermark retrieval is lacking (Downham et al., 2015). Vacuum metal deposition (VMD), superglue fuming, iron oxide powder suspension, a combination of superglue and iodine fuming and the development of a novel fingerprint development membrane with a ninhydrin developing agent have all proved successful (Yang and Lian, 2014; Downham et al., 2015; Zheng et al., 2017) at developing identifiable marks on a range of leather types. However, results are often inconsistent, and many marks enhanced of poor

quality. Due to the intensive processes involved in its creation, including tanning and dyeing, the properties of leather differ from the raw original skins from which it is derived. The only work carried out retrieving fingermarks from raw animal skins is through the substitution of domestic pig skin for human skin in associated research (Beaudoin, 2012; Siah, 2020). Black magnetic powder and cyanoacrylate fuming have both proved successful in recovering fingermarks off pig skin, even after environmental exposure but the onset of putrefaction quickly deteriorates marks (Baran, 2009). Although there are few similar "hairless" mammals that these methods could be trialled on the ones that do exist, including hippo (*Hippopotamus amphibius)* and elephant *(Elephantidae sp.),* are high value targets within IWT (Underwood et al., 2013; Andersson and Gibson, 2018).

Reptiles represent one of the most trafficked wildlife groups, entering both legal and illegal markets as live specimens destined for the exotic pet trade and coveted reptile skins/leathers for high-end fashion markets (Brazaitis, 1986; UNODC, 2016b; Sosnowski and Petrossian, 2020). Studies indicate that despite appearances reptile skin has some degree of permeability to contaminants and water (Stokes and Dunson, 1982; Weir et al., 2016) and likely fall under the "semi-porous" category. As a surface type for retrieving fingermarks there is additional complexity with background patterning and scale structure risking interrupting ridge lines, however marks have been successfully enhanced on both snake and lizard species (Eveleigh, 2009). Notably this work was conducted on both live and deceased specimens, making it applicable to both live seizures and worked goods. On live specimens Lightning White Fingerprint Powder® showed the most success, likely due to the contrast it produced against the patterned scale coloration of many species tested. Lightning Black Fingerprint Powder® successfully enhanced marks on more uniform light reptile skins such as the ventral side of alligator (*Alligator mississippiensis*). Cyanoacrylate fuming coupled with rhodamine fluorescing stain excited using 530nm wavelength viewed

through a 590nm barrier filter was effective at enhancing marks on multiple deceased species specimens. These same species also had marks successfully enhanced using the tested fingerprint powders. In keeping with existing knowledge of surface type influence on fingermark retrieval it was reported the smaller and rougher the scales the more limited the enhancement success. In this research the movement of live specimens either led to the destruction of powdered marks or problems with image capture. Within the IWT trade transport conditions of live reptiles is often poor (Wyatt, 2013). When subjected to transport conditions it has been found reptiles can demonstrate periods of prolonged inactivity (Mancera et al., 2014). Though improving the welfare of the animal would be of an immediate priority, this temporary period of inactivity could prove useful for fingerprint powdering in cases of seized reptiles. The issue of movement could also be overcome by use of newly developed techniques such as gelatin lifters which could recover enhanced marks from the body of the animal in a non-invasive manner.

Table 1.3. Summary of findings in the peer-reviewed literature investigating fingerprint detection methods from the surface of wildlife derivatives. The table outlines wildlife type, deposition and enhancement techniques, variable conditions considered (e.g., time, environmental exposure) and the specificity and quality of fingermark recovery achieved.

Group	Substrate type	Deposition method	Deposition type	Enhancement/Recovery	Variables	Specificity	Maximum grade achieved	Reference
Avian	Feather	Undirected	Natural	Mason Vectron Quasar 2000/30 connected to an Integrated Rapid Imaging System (IRIS), Black magnetic powder (BMP), magneta flake, red and green magnetic fluorescent, aluminium flake and magnetic bi-chromatic powders and cyanoacrylate fuming	Time	Positive enhancements obtained using red and green magnetic fluorescent up to 21 days after deposition.	4/4	(McMorris et al., 2015)
Avian	Egg	Undirected	Natural	Mason Vectron Quasar 2000/30 connected to an Integrated Rapid Imaging System (IRIS), BMP, magneta flake, red and green magnetic fluorescent, aluminium flake and magnetic bi-chromatic powders and cyanoacrylate fuming	Time	Usable prints obtained using black magnetic + magnetic bichromatic up to 14 days after deposition	4/4	(McMorris et al., 2015)
Avian	Egg	Consistent pressure 10 seconds	Natural	Variable light sources, Mason Vactron Quaser 40 MH, Cyanoacrylate fuming + Basic yellow 40 dye	None	Usable prints obtained, with an increase in grade achieved through the use of viewing filters.	3/4	(Darby et al., 2015)
Avian	Feather	Consistent pressure 2 seconds	Natural + Groomed	Blue Crime-Lite 82S (10% bandwidth 420-470nm with a 445nm peak) + yellow long pass filter (1% cut-on point – 476nm), Green magnetic fluorescent powder	Time + Environmental exposure	Usable prints obtained up to 60 or 14 days after deposition when stored indoors or outdoors respectively	4/4	(McMorris et al., 2019)

Mammal	Ivory	Undirected	Natural	BMP, Small particle reagent, cyanoacrylate fuming, BMP plus VMD	Time	Usable prints obtained after two weeks using cyanoacrylate fuming	Not described	(Azoury et al., 2001)
Mammal	Ivory	Medium pressure 1-2 or 10 seconds	Natural, sebaceous, and amino acid pads	Supranano Black Magnetic and Black Powder, Jet Black magnetic powder and cyanoacrylate fuming, 532 nm laser	Time + Sensitivity	Powders with particle sizes <40µm performed best, with usable prints recovered up to 1 week after deposition and positive enhancement achieved ridge up to 28 days post deposition	6/6	(Weston-Ford et al., 2016)
Mammal	Antler/Horn	Undescribed	Deposited in blood	Vapour phase cyanoacrylate + R.A.M stain, leucocrystal violet	None	Positive enhancement achieved using both described techniques	Not described	(Otis and Downing, 1994)
Mammal	Antler	Undescribed	Undescribed	Cyanoacrylate fuming + Volcano Black granular fingerprint powder, iodine fuming, ninhydrin, silver nitrate, magnetic fingerprint powders	Moisture + Temperate + Time	Usable prints obtained using magnetic fingerprint powders up to 16 hours after deposition	Not described	(Czarnecki, 2002)
Mammal	Scale (pangolin)	Undirected 5 seconds	Natural	Gelatin Lifters + GelScan	Time	Usable prints obtained up to four months after deposition	4/4	(Moorat et al., 2020)
Reptile	Scale	Undescribed	Sebaceous	Polilight w/ 590m barrier filter, Cyanoacrylate fuming + rhodamine stain, white or black fingerprint powder	None	Usable prints obtained using both methods on a range of species	Not described	(Eveleigh, 2009)

1.3.2 Fingermarks: Feathers

Globally it is suspected that avian trafficking is underreported and that a significant proportion of animals are trafficked live for the pet trade (Heinrich et al., 2020). Other species, particularly raptors, are persecuted for their perceived threat to livestock or game species such as grouse (Madden et al., 2019). Feathers are a unique structure found only amongst birds and their interlocking barbs and barbules have been compared to fabric weave, which at a macro level renders them as a porous material. Feathers are at a high risk of disturbance from handling or environmental exposure with barbules readily separated. Coupled with the often-flamboyant colours and patterns on feathers which hinder the ability to render strong contrasts between mark and background, it makes them a difficult surface type for fingermark retrieval. There have been just two complimentary pieces of research looking into fingermark retrieval from feathers (McMorris et al., 2015, 2019). For fabrics, VMD and cyanoacrylate fuming are the recommended approaches for fingermark retrieval with VMD the favoured approach on natural materials; powders of any kind are suggested as ineffective (Home Office, 2022). VMD has not been attempted on feathers but cyanoacrylate fuming has, and been found to be one of the least effective approaches (McMorris et al., 2015). It was postulated this was due to the hydrophobic nature of feathers but as cyanoacrylate is regularly used on non-porous and inherently hydrophobic surfaces it is more likely the porosity of the feathers was a contributing factor as superglue fuming is not recommended on porous materials. Fluorescent magnetic powders, specifically red and green were found to be the most consistently successful enhancement technique under controlled conditions.

The species trialled in these studies, kestrel, sparrowhawk, buzzard, red kite, and golden and white-tailed eagles have similar colour plumage, and as fabric comparisons were the underlying theory of approach plumage weave count rather than colour was a key focus.

However, if fluorescent powder enhancement is to be a continued line of research, plumage colour may be an important future consideration. Birds light sensitivity range sits between 300 – 700nm, this is inclusive of the UVA (320-400nm) end of the UV spectrum (100 – 400nm) (Rajchard, 2018). Feathers of several bird species, including heavily trafficked brightly coloured parrots and songbirds, have been found to fluoresce under UV light (Hausmann et al., 2003; Burns and Shultz, 2012). This may impact the ability of a fluorescing mark to stand out against a fluorescing background and considerations should be taken when considering which colour powders and subsequently wavelengths to use during enhancement and photography.

The second piece of research looking at fingermark recovery from feathers focused on environmental effects over time on green magnetic fingerprint powder development (McMorris et al., 2019). Marks were recovered up to 21 days after deposition with the location of the feathers, semi-protected or not from the elements, and precipitation having a significant effect on the success rates of recovery. Some relationship was also seen between both soil and air temperature and successful mark recovery. Marks recovered from control feathers left indoors were recovered up to 60 days after deposition. As noted by McMorris *et al*. (2019), happening upon a singular feather, as used in this study, is an unlikely scenario in case work. A whole, or part, carcass is commonly seen in raptor persecution cases. These are at risk of scavenging and the likelihood of feathers and thus marks being disturbed. Even in these instances knowledge that identifiable marks can be recovered after such long periods is beneficial; even if minutiae detail has been disturbed there is still opportunity to identify handling sites for subsequent swabbing for DNA recovery. For live trafficking, the nature in which birds are often packaged, stuffed in tubes or bottles (Australian Federal Police, 2015), and the inevitable movement of the birds themselves mean chances of mark recovery from feathers will be greatly diminished and

there are greater opportunities for mark recovery from the packaging. It is important to consider these types of contexts when deciding which types of wildlife specimens to trial forensic techniques on.

1.3.3 Fingermarks: Eggs

Egg theft and egg smuggling is a separate vein of avian associated wildlife crimes (Formentão et al., 2021). Eggs are easily concealed and have been known to be worn on a person's body for transport purposes (Rosen and Smith, 2010). Therefore sophisticated trade routes are not always a requirement and individual criminals can have devastating impacts (Walker, 2011; BBC News, 2018; Hammer, 2021). Egg shells are widely diverse in size, shell thickness, and surface pattern, and importantly to fingermark enhancement shells are porous. This porosity, which varies inter and intra species (Jaeckle et al., 2012; Bowers et al., 2015) allows the exchange of oxygen and carbon dioxide and is an important consideration for potential enhancement treatments if dealing with live eggs. Research on fingermark recovery from eggs as a food item initially found limited success with small particle reagent (SPR), a type of powder suspension (Ferguson et al., 2013). A later study concluded cyanoacrylate fuming followed by rhodamine 6G treatment was the most effective treatment but found best results when the egg had been refrigerated for fifteen minutes prior (Hong et al., 2019). Both these studies require potential life-threatening interference with the egg, submersion, refrigeration, and exposure to toxic substances and as such not suitable for application in many wild egg theft crimes.

Research in this area with a focus on wildlife crime found black magnetic powder had a 96% success rate at positively developing fingermarks on bird of prey eggs with enhancement possible up to 14 days after deposition (McMorris et al., 2015). In the McMorris *et al*. (2015) study eggs were described as a non-porous material. However, with the knowledge of the inherent porosity of bird's eggs, a semi-porous designation is also appropriate. Given this,

powder suspensions become a viable option for attempts at enhancement however the involvement of surfactants and need to wash the object makes their application to live trade limited. The only other study investigating fingermarks on non-domestic avian eggs also utilised cyanoacrylate fuming but with a subsequent Basic Yellow 40 dye treatment (Darby et al., 2015). Different wavelengths were used to excite fluorescent components within the fingermarks but resulted in maximum grades of just one and two (on a scale of zero – four). When viewing filters were applied marks increased in quality up to grade three overcoming the patterned background of lapwing and grey partridge eggs. Despite their light uniform coloration, the same results were not achieved on Canada goose and White-tailed eagle eggs. These species possess more notably porous egg surfaces, and the failure was attributed to the potential for the eggs to absorb the Basic Yellow 40 dye across its whole surface obscuring latent prints. In these studies, no effort was made to lift the fingermarks despite the smooth uniform surface of eggs being an ideal candidate for attempts with gelatin lifters. If the quality of the fingermark can be retained during the lifting process, analysis may be significantly easier as the problematic patterned background factor would be removed without the need for cycling through various wavelengths.

1.3.4 Fingermarks: Ivory, horn, and antler

Ivory, horn, and antler are commonly associated with a wide variety of wildlife crime activities, with deer poaching being one of the UKs priority areas. Some of the earliest studies focusing on contextual fingermark retrieval from wildlife parts were on deer antlers related to poaching cases (Otis and Downing, 1994). Mature antlers are exposed, regenerative, porous, rough bone which exist in different developmental states including a velveteen stage. On mature antlers, black magnetic fingerprint powder was found to be the superior method for consistent fingermark retrieval compared with cyanoacrylate fuming,

ninhydrin or granular powders (Otis and Downing, 1994). Over several days fingermarks became increasingly more difficult to enhance, presumed to be due to the porosity of the antlers causing absorption of constituents. Work on latent print enhancement on human bone drew similar conclusions also finding black magnetic powder the favoured technique (Steadman and Andersen, 2003). Chemical enhancement was hindered due to the reactions with organic material within the antler, with ninhydrin turning the entire surface area of the antler purple rendering any contrast to surface and ridge detail minimal. A similar phenomenon was seen with leather (Yang and Lian, 2014) demonstrating a theme with the application of chemical enhancement methods on organic materials. Further work expanded to include enhancement of bloody fingerprints on both antler and horn, a keratin based substance (Czarnecki, 2002). The study concluded cyanoacrylate fuming followed by fluorescent dye stains to be a viable technique for latent fingermark enhancement differing from the conclusions drawn in the first study. It should be noted no attempt at comparisons with other enhancement techniques were attempted and no description of the maturity of the antlers given. The porosity of antlers decreases over time making their growth stage of vital importance to viable fingermark enhancement techniques (Brockstedt-Rasmussen et al., 1987).

A perceived issue of fingermark enhancement for many animal products is their rough surface, as generally the smoother the surface the easier it becomes. Of all high risk trafficked animal products the smooth surface of polished ivory appears an appropriate case study to trial techniques. Whilst the term ivory is most commonly attributed to elephant tusks the term itself is applicable to several commercially traded mammalian teeth or tusks including elephant, walrus, narwhal, some toothed whales, hippo, and warthog (Baker et al., 2020). Several of these have recently been included in the UK's Ivory Act 2018 (UK Government, 2023). Ivory is porous, comprised almost entirely of dentine with a thin

layer of cementum, and in both elephants and walrus tusk tips are coated in enamel but this is eventually worn away and absent in older animals (Baker et al., 2020). Hippo ivory is sourced from both their upper and lower canines, and their enamel layer is more permanent covering about two-thirds of the tooth. To date there are two published studies investigating latent fingermark enhancement on ivory, both elephant, conducted 15 years apart (Azoury et al., 2001; Weston-Ford et al., 2016). Both these studies found Black Magnetic Powder (BMP) (standard and reduce scale powder respectively) suitable enhancement techniques including in a field setting. The main development seen between studies was increased success rate for longer intervals between deposition and enhancement, with the reduced scale (Supranano™) powder successfully enhancing prints up to 28 days after deposition. As an indicator of the continued focus on megafauna, this research has spawned the largest uptake in interest in application of fingerprinting techniques in wildlife crime cases and demonstration of its value. Fingerprinting kits have been produced and distributed both domestically and overseas with NGO support, with reports that use of these techniques have directly led to arrests (Foreign, Commonwealth and Development Office, 2018).

1.3.5 Fingermarks: Pangolin scales

Pangolin scales have recently become a high profile evidential item in IWT, in response several countries have carried out actions specific to the pangolin species (Moorhouse et al., 2021). Despite this, historical and continued demand has resulted in seizures containing tens of thousands of individual scales, representing thousands of individual pangolins (Ullmann et al., 2019). Though the number of seizures continues to increase these are not synonymous with conviction and arrest rates (Challender and Waterman, 2017; Omifolaji et al., 2022). Pangolin scales are keratin based, overlapping to form a protective layer on

the dorsal side. The surface presents as a smooth material with shallow grooves running vertically from the tip to the base. Under scanning electron microscope they have been revealed to be non-porous, opening up the number of enhancement methods available to them (Moorat et al., 2020).

At the time of this review just one attempt has been made to retrieve latent prints from pangolin scales using gelatin lifters (Moorat et al., 2020). Gelatin lifters are used to recover both treated and untreated latent marks, then subsequently scanned or photographed and enhanced using software such as Photoshop™ (Bleay et al., 2011). Latent marks on pangolin scales were retrieved up to four months post deposition and whilst the mean grade failed to reach over two point five for any periods, over 28% of all grades were three or above, and as such considered of forensic interest. There is sound logic behind the proposed use of gelatin lifters as a tool for use in wildlife investigations; they are affordable, portable, durable, and pliable, allowing them to be applied to uneven surfaces and used in field settings where chemical or traditional powdering techniques are unsuited and in nations with minimal resources. Limitations for this method start to creep in surrounding documentation of the latent prints. Optimum photography is carried out using specialised GLScan equipment, a large stationary scanning machine. As it currently stands to achieve best results practitioners would be required to collect marks in-situ and transport to the nearest lab with a GLScan machine which could be a significant distance or even located in a different jurisdiction. The research proposed the use of smart phones as an alternative, a method which is increasingly being investigated (Warren, 2013; Haertel et al., 2021). A second limitation is the fact that individual scales, such as those used in this study, are usually recovered in large quantities. With minimal resources available to wildlife crime case workers, analysis of hundreds or thousands of individual scales is impractical. Live or whole pangolins are traded on a smaller scale (Challender et al., 2020) and present a more

practical example of case work where gelatin lifters could be applied. However, due to the overlapping scales on whole specimens there is higher opportunity for latent marks to bridge multiple scales or be destroyed from friction of rubbing scales. Application of gelatin lifters also relies on an informed idea of the existence and positioning of a latent mark, without this a gel may be applied in a manner which cuts through a mark. As such this work would benefit from a preliminary step of investigating techniques for visualising latent marks, through oblique lighting, forensic light sources, or powdering.

1.4 Human Identity Forensics; Trace DNA

1.4.1 Background and Composition

Like fingermarks, DNA profiles are used in forensic investigation to identify an individual and can be full or partial in nature (Jobling and Gill, 2004). The laboratory pipeline for the processing of human DNA evidence is well established with validated methods and instrumentation available. The aim of forensic DNA analysis is to generate a STR profile amplified from a series of known loci, each displaying a maximum of two alleles in a single source profile (Figure 1.4). The data is reduced into a string of allele repeat numbers that can be compared to a reference sample or searched against a national or international DNA databases. During criminal investigations, DNA may be sampled from sources including blood, hair, saliva, and semen left behind at crime scenes, often because of physical or sexual abuse. However, where these evidence types are not readily available, “trace” or “touch” DNA, that which is transferred from person to object via physical contact, may be recovered (van Oorschot et al., 2010; Tozzo et al., 2022). The factors that affect the presence and retrieval of trace DNA include pre-factors such as the donor, handling time, surface type and post-factors like time since deposition and environmental exposure (Raymond et al., 2009; Alketbi and Goodwin, 2019c; Burrill et al., 2019). This is not to imply that as evidence

types they are one of the same; although DNA can be recovered from fingermarks (Subhani et al., 2019), fingermarks can exist without detectible DNA, and trace DNA can exist independent of fingermarks. Current understanding of the cellular contents and origins of trace DNA is limited with many possible origins noted including cell free DNA (Quinones and Daniel, 2012), anucleate corneocytes (Burrill et al., 2021b), nucleated epithelial cells from hands (Burrill et al., 2019) and fragmentary cells (Alessandrini et al., 2003). More recently, it has been proposed that trace DNA originates from various locations or bodily fluids, specifically shed keratinocytes from the outer layers of an individual's hand, nucleated epithelial cells from fluids (e.g. eyes, saliva, nasal fluids) or body parts in contact with hands and cell free DNA either endogenous to the hands (e.g. sweat) or exogeneous (e.g. transferred onto the hands from an external source) (Burrill et al., 2019).

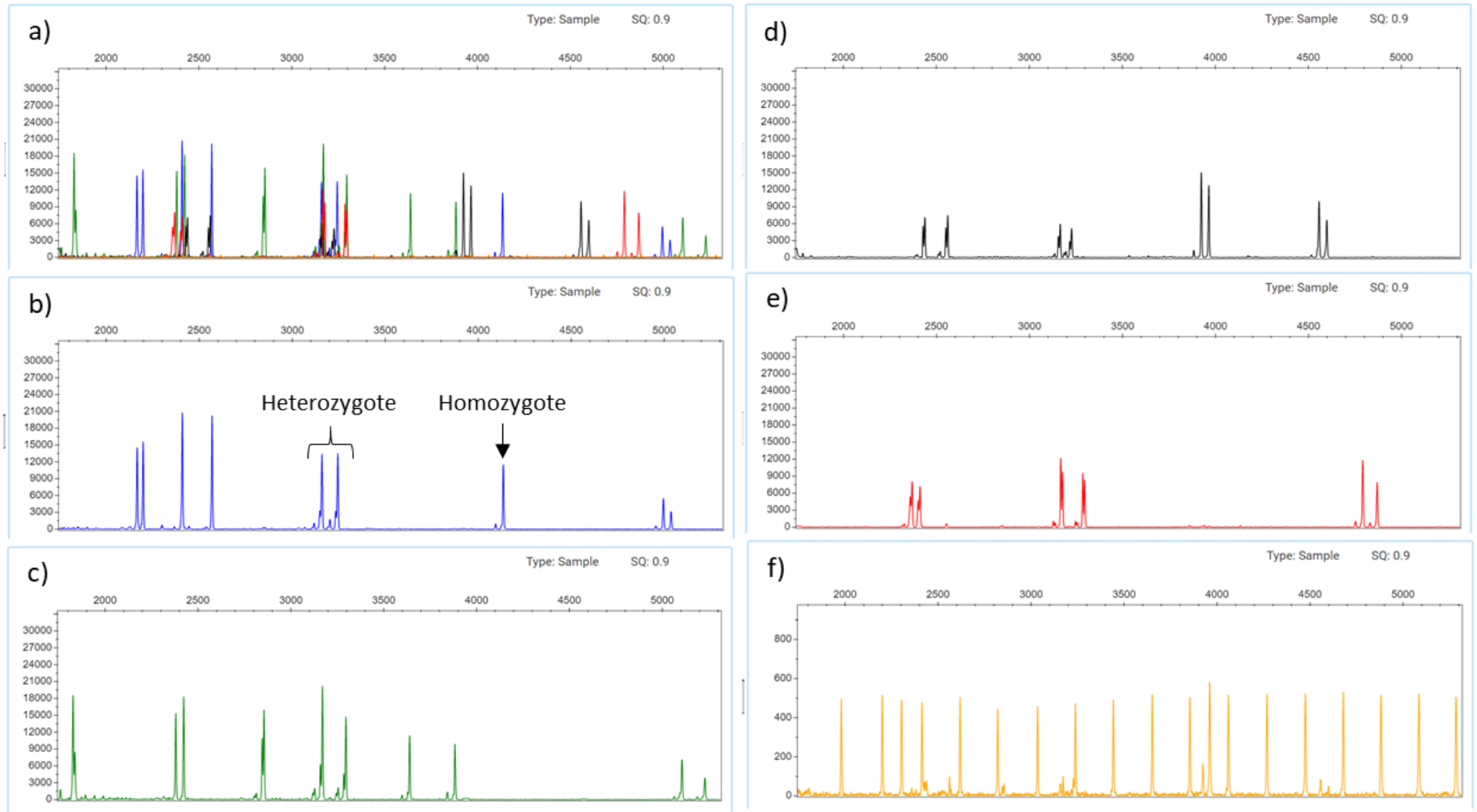


Figure 1.4. *A single source human STR profile viewed on GeneMapper™ software with Relative Fluorescent Units (RFU) on the Y-axis and fragment size (base pairs) on the x-axis. (5a) Overlay of five channel spectra showing all full STR profile. (5b) Five STR loci amplified in blue channel showing example heterozygote and homozygote alleles at loci. (5c) Five STR loci and Amelogenin amplified in green channel. (5d) Four STRloci amplified in yellow channel. (5e) Three STR loci amplified in red channel. (5f) Size standard used to identify fragment length of STR alleles presented in orange channel.*

1.5 Trace DNA: Application of methods in wildlife crime

1.5.1 Trace DNA: Deer

Like fingermark research some of the earliest attempts at human trace DNA retrieval in the context of wildlife crime was conducted in response to deer poaching. Minitapes, a common tool used for trace DNA retrieval from clothing (Verdon et al., 2014b), were tested for use on limbs of deer handled by hunters (Tobe et al., 2011). The method was successful but due to the low levels of DNA recovered the researchers were forced to use a modified protocol adapted for low copy number (LCN) samples during amplification. In a second iteration of the study the LCN approach was overcome through pooling of samples (Tobe et al., 2013). However, the experimental design of their study meant Tobe *et al*. (2013) had the luxury of knowing their combined samples originated from the same "perpetrator". Whilst a single individual handling a carcass may be true for small scale crimes such as deer poaching, in reality the supply chains of many wildlife crimes are complex and several individuals may be involved either along the whole chain or within just one of the links (Cerling et al., 2016; UNODC, 2016b). Mixed source DNA is considered complex and combined with the already problematic low levels of DNA in trace samples future studies should include several donors to better emulate real life cases. However with over a decade of development in the area of trace DNA recovery techniques such as direct PCR make processing of challenging samples more accessible (Cavanaugh and Bathrick, 2018).

1.5.2 Trace DNA: Bird of prey, corvid, and rabbits

In many cases of wildlife crime, the carcass, either whole or in parts, is a commodity therefore encountering a carcass as evidence at a crime scene which has been exposed to the elements, may be less common than encountering it in transit or on a person. In contrast, carcasses of species which are targeted for persecution have no value to the

offender and may be left or concealed at the scene of the crime. This is common in crimes against birds of prey whose carcasses are regularly found outside having been exposed to the elements for undetermined amounts of time (RSPCA, 2019). The impact of prolonged elemental exposure on trace DNA recovery has been investigated and evidence shows temperature and humidity both impact the persistence of DNA however whether this is positively or negatively is concurrent with the type of surface the DNA has been deposited on (Alketbi and Goodwin, 2019c). In one study, minitapes were chosen to remove human DNA from rabbit (a common bait), corvid and bird of prey carcasses in both controlled and exposed conditions (Mcleish et al., 2018). Profiles were obtainable form corvid and rabbit after two days of elemental exposure including heavy rainfall and up to ten days on carcasses kept in controlled indoor conditions with the rapid decomposition of the carcasses cited as a contributing factor to the decreasing ability to recover DNA. Bird of prey carcasses had only one day of exposure with rainy conditions but found significant difference in success depending on the species. Other external factors such as scavengers or invertebrates associated with decomposition may also contribute to the decline in available DNA. This was suspected to be true in a study of trace DNA recovery from pig skin submerged in water (Meixner et al., 2020). Both this study and that detailed in (Mcleish et al., 2018) managed to produce full DNA profiles from carcasses after being exposed to water. Once conclusion was that trace DNA persisted longer in cold, standing water but a full profile was still retrievable after one day of immersion in running water which is in keeping with (Mcleish et al., 2018) who retrieved reportable DNA samples from corvid carcasses exposed to rain after two days. Effects of rain exposure on trace DNA retrieval in wildlife cases deserves more research, given poaching incidents in certain countries peak during rainy seasons, as poachers attempt to capitalise on rangers inability to navigate flooded protected areas and the lack of tourists (Kyando et al., 2017).

1.6 Summary

Several key themes flow through human identification in wildlife crime. To begin with the literature shows it is possible to recover human evidence from wildlife derivatives using standard techniques, without the need to deviate from the general recommended procedures. Wildlife derivatives are seemingly subject to the same rules of porosity, texture, and environmental exposure that must always be considered by crime scene examiners when selecting a technique. Colourful, patterned, skins and coats of animals can be a challenging factor in producing a good contrast between substrate background and fingermark. For species destined for the pet trade or as ornamental display pieces these flamboyant features are a driving factor behind their demand, therefore overcoming this problem is imperative. Very few of the studies reviewed here attempted to compare enhanced mark quality on the substrate to lifted marks, despite lifting being a standard procedure by forensic investigators. Chemical enhancements often failed potentially because raw animal products are organic material which react in conjunction with fingermark residue rendering any contrasts that do occur of minimal quality. The techniques that do work, powders, and gelatin lifts, can be cost effective, field deployable and in the case of powders do not require expensive laboratory infrastructure for analysis. This makes them ideal candidates for take up in by those investigating wildlife crime who cite a lack of resources as an obstacle to enforcement. Notably researchers have placed no consideration the downstream impacts of fingermark enhancement techniques on potential DNA recovery, human or animal. Dual trace evidence recovery from fingermarks is an increasing consideration by practitioners for both fresh and archived fingermarks (Kumar et al., 2015; Solomon et al., 2018; Subhani et al., 2019) and the techniques employed can have significant impact on ability to recover DNA profiles.

Despite decades of successful proofs of concepts on several species there has been only one recorded instance of findings being translated into applied casework. One explanation behind this is that there has been no real need for recovery of such evidence types. Wildlife crime investigations can often begin from a “caught red handed” scenario, whereby an individual is found in possession of wildlife products, commonly seen during seizures at borders. As such the need to link an individual to the crime is superfluous. This is a weakness in the continued chronicling of making IWT synonymous with all wildlife crime and thus focusing efforts on highly trafficked species. By assuming this narrative and failing to establish robust methods of linking individuals to wildlife crimes a whole subset of cases is being ignored. It does injustice to the equally pressing matter of domestic, non-trade related, wildlife crimes such as seen in the USA and the UK who, as discussed, have a poor track record in wildlife crime conviction rates (Wildlife and Countryside Link, 2020; Sosnowski et al., 2022). Persecution and human-wildlife conflict cases in these countries may rarely see an individual caught in possession of a wildlife product as the wildlife product itself is not a target for commercial gain. The small-scale nature of these crimes, the comparably high resources available, including accredited laboratories and well-established databases, place such nations in prime position to lead in human evidence recovery in wildlife crimes. Ignoring human evidence also fails to consider the additional intelligence it can bring to investigations. For example, DNA barcoding with ivory has resulted in linking shipments and thus identifying supply chains and trafficking routes. This could also be achieved through the presence of repeated instances of the same human DNA profile or fingermark on multiple shipments identifying a repeat offender or common link in supply chains.

It is evident from increasing rates and simultaneous decrease in convictions that current attempts to tackle wildlife crime are fraught with problems. Along the way forensic

solutions posed have focused on the wildlife rather than the perpetrator. This work, specifically individual identification of wildlife has important applications, but they are limited by resources, lack of accreditation, need on a large scale and the sheer volume of wildlife species involved. By contrast human identity testing in forensic applications is a globally established industry, with recognised and well-rehearsed best practice methods. Human identity testing benefits from existing databases and infrastructure, particularly in the global north, but with more and more global south stakeholder countries developing in this area, such as India's new National AFIS (National Crimes Record Bureau, 2021) and Kenya's new forensic laboratory (Siele, Martin, 2022).

Any prosecution team will benefit from having an much evidence as possible at their disposal. Recovery and presentation of human trace evidence in wildlife crime cases provides clear links of perpetrators to wildlife products that other types cannot provide. As such it is recommended that more research is conducted looking into human trace evidence recovery from common substrates encountered in wildlife crime cases. Whilst this review has focused on wildlife products and their derivatives there is also opportunity to consider traps, snares, weapons, transportation boxes and vehicles. For several of these evidence and material types there will be existing research or guidance on best practice methods, but work is needed to contextualise them into the world of wildlife crime. Considerations should be made dependent on the seizure type or crime scene location. For example, seizures from shipping containers will have undergone different environmental exposure and periods since deposition comparative to air cargo, similarly crime scenes in an arid desert environment will have had significantly less moisture exposure than those in tropical humid environments affecting recommended recovery methods.

Fingermark work should look beyond just enhancement on substrates and investigate effective methods of mark retrieval to overcome problems in establishing contrast on patterned backgrounds. Trace DNA work in this area is very much in its infancy but will benefit from including mixed profile scenarios, more modern processing techniques and interactions with fingermark recovery techniques. It is important that such research is completed in appropriate contexts. To do this, researchers must work closely with law enforcement to understand their resource limitations, what types of evidence they most commonly encounter at wildlife crime scenes, what national priorities are, and the practicality of applying developed techniques.

Finally, there needs to be recognition of the complimentary nature of species identification and human identification forensic work. What species identification lacks in terms of accreditation and recognition within the wider forensic community, human identification possesses in abundance. Species identification benefits from ample examples of proof-of-concept work as well as media, funding, and research interest whereas in these areas human identity work is in its infancy. Encouraging these veins to work together could result in robust forensic investigation in wildlife crimes, with the recovery and analysis of several streams of forensic evidence being possible. The idea of paired wildlife and human forensic labs who agree to take on relevant evidence processing from wildlife crime cases at their respective crime scenes could be considered. As well as utilising each institutions unique skill set it will strengthen the relationship between the wildlife and human forensic community potentially increasing knowledge sharing opportunities and more cohesive and streamlined case work. A challenge will be the need for human forensic laboratories to find the time and resources to process wildlife crime related evidence. Efforts to access these resources will be strengthened by demonstration of the impacts of wildlife crimes on the economy, communities, and biodiversity. Better recording of wildlife crimes should be a first

step in this area, as is being called for in the UK within campaigns to make wildlife crimes notifiable (Wildlife and Countryside Link, 2022a) and recommendations for centralised wildlife databases within the European Union (EU) for better monitoring (Engel, 2023). It is evident that the presence of wildlife crime attendees that, singularly or collectively, possess a holistic set of forensic skills capable of processing and collecting both wildlife and human focused evidence is missing within wildlife crime investigations. Even if resources do not allow immediate processing of evidence, its correct and effective collection opens avenues for utilisation of archival evidence when circumstances allow in the future. This has the potential to improve prosecution and conviction rates and act as a serious deterrent to wildlife criminals, providing in a part a solution to the ongoing crisis of wildlife crime.

Chapter 2: Investigating impacts of handling manner and grooming preparation on fingermark quality: Identifying a reliable method of deposition.

2.1 Introduction

Since their recognition as an useful identification tool in criminal investigation during the late 19th and early 20th century (Cole, 2001) fingermarks have become one of the most recognisable types of forensic trace evidence recovered at crime scenes (Kaplan et al., 2020; Ling et al., 2021). The discovery of latent fingermarks (Lee and Pagliaro, 2013), those invisible to the naked eye, now sees fingermarks as one of the most significant types of forensic evidence that contribute to arrests, prosecutions and convictions, particularly when coupled with other forms of evidence (Peterson et al., 2013; Steele, 2020). In the UK alone, between 2022 and 2023 law enforcement carried out over 400,000 searches of latent fingermarks against ten prints stored in the national AFIS database, IDENT 1, resulting in just over 14,000 matches (UK Home Office, 2024). Therefore, there is a vested interest by the forensic community to continue optimisation and innovation into fingermark recovery techniques. An ongoing discussion in this field of work is how to design effective experiments investigating and comparing the efficacy of enhancement methods. The process is significantly complicated by the fact the dependent variable, in this case fingermarks, are highly variable both between and within individual donors even before the introduction of any influencing variables (Sears et al., 2012; Steiner et al., 2019). Without confidence in the similarity of quality of fingermarks undergoing testing, there could be ambiguity as to whether poor experimental results are the product of technique efficacy, methodological limitations, or due to a failure of deposition at the outset (Chadwick et al., 2018). This phenomenon of variability is colloquially referred to as “the donor effect” and attempts to control it can be broadly grouped into two categories, the physical way in which

a fingerprint is deposited, the “deposition technique” and what constituents that fingerprint consists of, the “composition”. Existing attempts to control deposition technique and composition are described below.

2.1.1 Controlling the deposition technique

The pressure, angle, and period of contact a finger has with a surface have all been shown to subsequently impact the quality of an enhanced fingermark (Fieldhouse, 2015; Hefetz et al., 2019). Increasing pressure has been shown to correlate with an increase in width and length of a fingermark leading to distortion, reducing the distance between ridges and risking the loss of detail through merging of ridges or bifurcations (Mil’shtein and Doshi, 2004; Jasuja et al., 2009). A simplified approach to minimising excessive use of pressure include descriptive instructions, such as applying contact “as naturally as possible” (Harush-Brosh et al., 2020) or “gently touching” a surface (Hong et al., 2019). However instructions such as these are open to subjective interpretation, and it has been shown that too light a mark can also misrepresent ridge detailing (Hefetz et al., 2019) and most donors are incapable of repeated depositions at the same pressure when left to their own volition (Mil’shtein and Doshi, 2004). Some of the most comprehensive work in attempting to manage deposition pressure has involved the use of mechanical aids to accurately measure the amount of pressure being applied. The most rudimentary of these approaches measures exerted pressure using a top pan balance, having donors release their fingers when a designated weight is met (Jasuja et al., 2009). However, it has been shown this method is not capable of reproducible or consistent results and as donors take varying amounts of time to reach the desired pressure it further introduces an unintended but known influencing variable, the amount of time in contact with the substrate (Steiner et al., 2019). More sophisticated attempts to control pressure, including development of

mechanical devices which remove any control of the deposition process from the donor and relegate it to either a human operator (Fieldhouse, 2015) or through machine automation (Reed et al., 2016). Both devices demonstrated a greater level in reproducibility of deposited marks comparative to other methods but have not been adopted by the wider research community. This may be due to their intricacies making mass production difficult, and their designs limiting the types of surfaces that be deposited on, making their use on unusual surfaces, such as wildlife specimens, impractical.

2.1.2 Controlling fingermark composition

As outlined in section 1.4.1 the constituents of a fingermark are a vital factor of consideration in choice of enhancement method. As such artificial manipulation of depositions can significantly impact results by causing over- or under-representation of certain constituents, favouring particular methods. Where novel enhancement techniques are being proposed representation of certain constituents may be desired to establish which are involved in the mechanism of the technique. Methods to achieve this include artificial secretions presented as printed test strips (Kupferschmid et al., 2010), chemical pads (Steiner et al., 2020) or pipetting or stamping formulated residues (Sisco et al., 2015). Artificial secretions are contentious as they deviate so significantly from the complexity of natural fingermark residue mixtures and have been shown to have unreliable reactions with some enhancement types (Zadnik et al., 2013; Steiner et al., 2020). As such their use is never encouraged to draw conclusions about the suitability of techniques for operational use. Alternative “natural” approaches to producing target constituents include inducing eccrine secretions by wearing plastic gloves (Fagert, 2023), or loading on sebaceous secretions by rubbing their fingers on their nose or cheeks (Kim et al., 2019). The fingerprint visualisation manual, produced by the UK Home Office, is one of the most comprehension

resources available for effective operational decision making in choice of fingermark enhancement technique (Home Office, 2022). In recognition of the ongoing challenges of standardisation Sears et al (Sears et al., 2012) published a paper outlining the methodology used to generate the data that underpins the guidelines presented in the Home Office manual. A point stressed throughout the paper and similar guidelines published by the International Fingerprint Research Group (IFRG) (Almog et al., 2014) is that the use of "groomed" fingermarks, whereby donors purposefully touch their face, particularly the t-zone, prior to deposition, is strongly discouraged. The reasoning given is that "naturally deposited" marks are better representative of latent marks encountered operationally due to the grooming practice significantly increasing the amount of some fatty acids and lipids, particularly squalene in a deposited mark (Croxton et al., 2010; Moraleda Merlo et al., 2023). Interestingly, despite this assumption being repeated throughout the associated literature, I can find no published research which directly compares experimental and casework fingermark composition. At least one recent study has stated that despite groomed marks resulting in a higher lipid content, this assumption of natural fingermarks being a better representation of operation marks may not hold entirely true (Moraleda Merlo et al., 2023). Another study has highlighted that latent marks possess significantly less water content than previously thought and most contain at least some additional sebaceous constituents (Kent, 2016). In addition, marks "naturally" deposited under laboratory conditions fail to take into consideration the contextual factors, such as stress, excitement, and fear which may be experienced by a criminal, or victim, during a crime, and subsequently influence their actions, such as face touching, or rate of sweating.

Though drawing attention to the fact fingermarks are not compromised of solely eccrine secretions, the recommendations of Sears et al (Sears et al., 2012) for generating "natural fingermarks" include no handwashing within 30 minutes prior to deposition, and avoidance

of activities including makeup application and deliberate touching of the face. Arguably these controls push these natural fingermarks into a territory of over representation of eccrine secretions, as the only glands found on the palms, as there has been an active effort to avoid the introduction of contaminants or sebaceous secretions. By comparison, the IFRG guidelines recommend caution in the use of a hand-washing step and provide no specific guidance on any other controls in generating a "natural" fingermark. Throughout the literature researchers employ a range of methodological interpretations of both of these sets of guidelines ranging from absolutely no attempt to control variables (Dawkins et al., 2020), to avoiding hand-washing within a certain time-frame (McMorris et al., 2015) to opting for the use of groomed prints without specific justification (Lohar et al., 2022).

2.1.3 Other methods of control

Other reported contributing factors affecting latent fingerprints deposition include, donor age and sex (Tozzo et al., 2022), lifestyle associated contaminants present on hands (Bleay et al., 2021), substrate type (Bacon, 2012) and time elapsed between deposition and collection (Colella et al., 2020). As already discussed, the hand-washing step is often introduced to control for contaminants and it is recommended to recruit of a range of donors of mixed sex, age, and suitability, to deposit fingermarks (Almog et al., 2014). The latter step of establishing "suitability", may be superfluous given the inter-variability of a single donor depositions within and between days. At later stages of research, where surface area size allows, techniques such as depletion series use the same finger to deposit a diminishing quantity of constituents. This technique allows for the assessment of sensitivity of enhancement methods. Split fingerprints are another common approach whereby a deposited mark is halved, and different techniques applied to each respective half, (Sears et al., 2012; Almog et al., 2014). By using the same mark for comparisons,

variables such as pressure, constituents and donor intra-variability are better controlled. However, the method functions best on uniform flat surfaces which can be physically separated. On other surface types, methods to circumnavigate the inability to physically separate, include placing two of the same surface type together and depositing so that the seam of connection is placed along the medial line. This method is not conducive to physical processes which fair best on a continuous surface, such as powdering (Sears et al., 2012) however and almost impossible on any irregular or curved surfaces. As such experimental design for research into fingermark enhancement on irregular and novel surfaces, such as wildlife specimens, is challenging.

In Chapter 4 of this study, I will be comparing the efficacy of reduced scale magnetic fingerprint powders and gelatin lifters on a range of wildlife specimens. Wildlife specimens present as organic surface types; unless purposefully altered to the contrary, they are traditionally non-uniform across their surface, due to natural growth patterns and weathering (Figure 2.1).

Figure 2.1. An example of an elephant ivory tusk unsuitable for deposition control methods, such as depletion series, due to the variation in texture and colour seen along its length.

As such it is impractical in many cases to use the recommended methods of split fingermarks and depletion series. In addition, whilst traditional surface types, such as paper, glass, plastic, or metal are in abundance, access to wildlife specimens is a greater challenge.

This may be particularly true if the specimens represent high-value or rare species and therefore may be required for future preservation work or vital for the species future preservation, and unavailable for potentially destructive applications such as chemical fingermark enhancement techniques. In general, it should be expected that there will be a finite number of wildlife specimens available for research. Therefore, there is an increased desirability to confidently deposit fingermarks during proof-of-concept as repetitions cannot be guaranteed. The techniques being investigated in Chapter 4, powders and gelatin lifts, both fall under “physical processes” (Home Office, 2022) capable of adhering and interacting to both the water and lipid constituents in eccrine and sebaceous secretions respectively (Gaw and Ramotowski, 2012), a factor which makes powders such a versatile and highly utilised enhancement technique. Given this, the risks of introducing an abundance of favourable constituents, which favour the method, by using “groomed” fingerprints may be limited, comparable to say a lipid dye such as Basic-Violet 3. Taking these factors into consideration this chapter will assess the impact of deposition method and pre-deposition grooming on the quality of powder enhanced fingermarks on porous and non-porous substrates, with the aim to identify a methodology that can be taken forward for use in Chapter 4 that results in the highest chance of deposition without significant deviation from operational latent fingermarks.

2.2 Materials and methods

2.2.1 Substrate choice and volunteer recruitment

In an effort to standardise interpreted handling technique between substrates, and consideration that later chapters will involve handling wildlife specimens which are 3D in nature, cylindrical versions of two porous (pine dowel and cardboard) and two non-porous (black polypropylene and reflective stainless steel) substrates were chosen for this study

(Figure 2.2). Each cylinder was divided into two areas, the smaller for fingermark deposition and the larger designated for DNA deposition for use in a trace DNA iteration of this study outlined in Chapter 3. Prior to use, to remove any fingermark residue, all substrates were wiped down using DNAaway™ (Thermo Scientific™) and left for at least one hour to dry before being handled by participants. In total eight participants were ethically recruited with an equal number of males and females assigned at birth represented (Liverpool John Moores University ethical approval reference 21/PBS/004). Individual shedder status was not determined as part of recruitment.

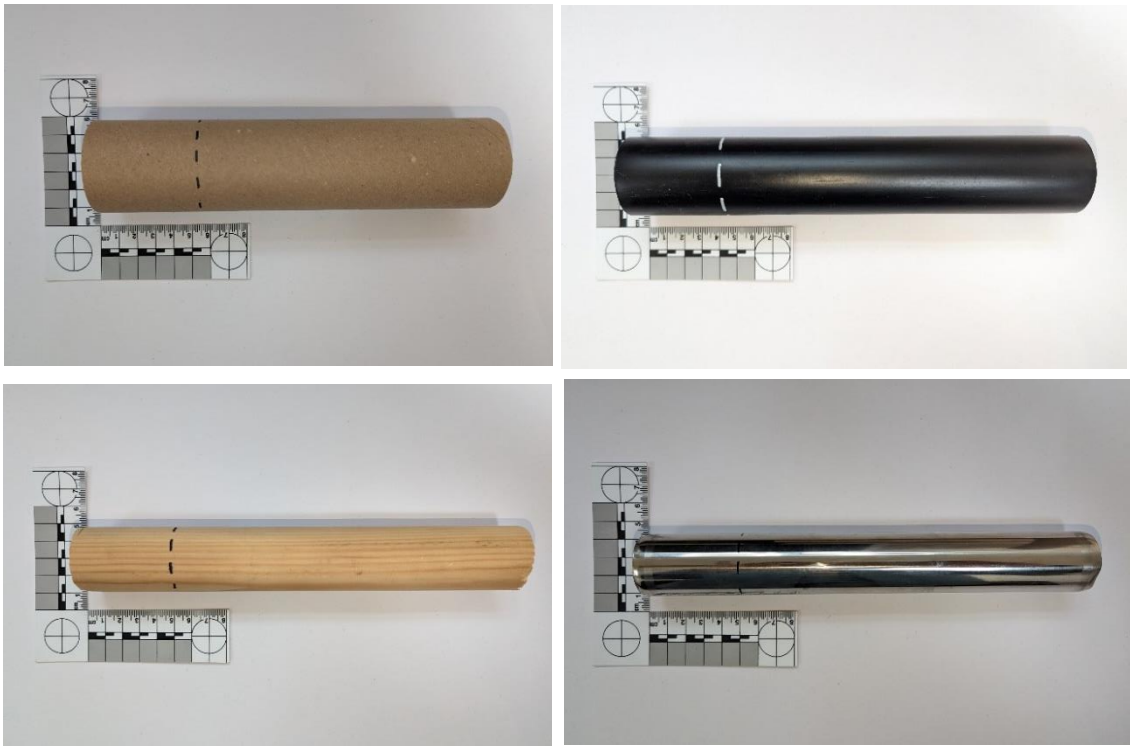


Figure 2.2: Images of the four substrates deposited on during this study. Clockwise from top left, cardboard cylinder (porous), polypropylene cylinder (non-porous), stainless steel cylinder (non-porous) and pine dowel (porous).

2.2.2 Fingermark Deposition

As a requirement of ethical approval, in relation to COVID-19 risk assessments, prior to each deposition session all participants were asked to wash their hands. For natural fingermarks participants refrained from touching themselves or objects between handwashing and deposition, a period of around 15 minutes to allow time for eccrine secretions to replenish.

Whilst eccrine glands are concentrated on the hands and soles of the feet they also exist in abundance on the forehead (Haskell, 2010). As such the area is not recommended for inclusion in studies requiring "grooming" to load fingerprints with sebaceous secretions (Sears et al., 2012). However, to more closely simulate a "natural" fingermark of mixed constituents, for groomed preparations in this study participants were asked to rub their fingers along their forehead and, bridge of nose. All participants were asked to rub their fingertips together to evenly distribute residues present.

Four deposition methods were tested, three methods included a set two seconds of contact time between the surface and fingertip, but instructions left pressure of deposition open to subjective interpretation by the participant (methods 1, 2, 4) the final method attempted to control pressure through use of a top pan balance but with variable contact time depending on participant (Table 2.1). Digit used (forefinger, thumb, ring) and handedness (left, right) were randomised across samples. All participants (N = 8) contributed equally to each study treatment and substrate, resulting in a total N = 128 individual fingermarks, N = 32 fingermarks per deposition method and N = 64 fingermarks per preparation type. After deposition fingermarks were aged for 30 minutes prior to application of enhancement techniques.

Table 2.1: Deposition methods employed in this study, comprising of three, light touch, heavy touch, mechanical, drawn from existing literature and one, undirected, novel devised by study investigators.

No.	Deposition method	Fingermark protocol	Reference
1	Light touch	'Lightly' press assigned fingertip onto designated area of substrate for two seconds.	(Richmond, 2004)
2	Heavy touch	'Heavily' press assigned fingertip onto designated area of substrate for two seconds.	(Richmond, 2004)
3	Mechanical	Press assigned fingertip onto designated area of substrate, which sits on scales, until a consistent force of 500g is read on the scale.	(Jasuja et al., 2009)
4	Undirected	Press fingertip onto designated area of substrate for two seconds. Interpretation of method approach is left up to participant.	Novel to his study

2.2.3 Fingermark enhancement and photography

In July of 2021, The City of London Police (CoLP) forensic services department provided in-person training, inclusive of crime scene and laboratory-based techniques, in fingermark enhancement and photography. The training included simulated crime scenes, during which qualified forensic personnel evaluated my practical techniques. SceneSafe™ Supranano™ Black and White Magnetic Powders, applied using a sterile magnetic wand, were used for cardboard and pine (black), and polypropylene (white) respectively. SceneSafe™ Bronze latent fingerprint powder was used for stainless steel and applied using a sterile disposable fibreglass zephyr brush.

Treated fingermarks were photographed with a reference scale, using a tripod mounted Sony DSLR A850 with Sony 100mm f2.8 macro lens attachment, positioned parallel to the fingermark, with a ten second delay for enhanced stability. Fingermarks were lit using ambient light and images taken using multiple apertures in both RAW and JPEG format. Fingermark images were exported into Photoshop© and the following edits applied to RAW images: i. scaled to 1:1 ii. converted to grayscale iii. sharpened. The first two edits were to bring images in line with standard requirements for uploading to an AFIS and the state in which fingerprint experts would most commonly carry out assessment. If images required further improvement to facilitate grading, then colour inversion, brightness, and contrast editing tools were also utilised. Post photography all fingermarks were lifted using SceneSafe Crystal-Tabs™ as per manufacturer recommendations.

2.2.4 Fingermark Grading

Fingerprint experts within the CoLP forensic service department provided training in correct methodology in fingermark grading. Training was inclusive of all steps of the ACE-V system and involved assessment of correct identification and comparison of fingerprint patterns,

and minutiae both manually and via AFIS. Fingermarks were graded using the Home Office 0 – 4 Centre for Applied Science Technology (CAST) grading scheme , (0 = no evidence of fingermarks; 1 = weak development; 2 = limited development; 3 = strong development; 4 = very strong development) (Sears et al., 2012). Enhanced fingermarks were first photographed in-situ on the substrate and then on the SceneSafe Crystal-Tabs^{TM} lifts post lifting. For each deposition both versions of the photographed fingermarks were graded and the highest scoring of the two taken as the final grade. This approach was taken due to the difficulties in photographing enhanced fingermarks on the more reflective substrate surfaces and subsequently having high enough quality images for analysis. The CAST grading scheme differs to casework where a qualitative assessment is made using the ACE-V framework (Needham et al., 2022). In the UK, this process results in a final verdict of insufficient (lacking sufficient detail for drawing comparisons), comparable (sufficient detail to compare against a known suspect's ten print) or searchable (sufficient detail to upload and search against latent and ten prints stored in an AFIS database). Although CAST grades were used as the main dependent variable in this study to support the validity of their grading, fingerprint experts from the City of London Police were also asked to grade the fingermarks using their ACE-V system.

2.2.5 Statistical analysis

Unless otherwise stated all statistical analysis in chapters 2, 3, 4 and 5 were performed using R statistical software and functions within the pre-loaded core "stats" package (R Core Team, 2024). Graphs, unless otherwise stated, were created in R statistical software using the "ggplot2" package (Wickham, 2016). The CAST grading scheme is intended for interpretation as ranked ordinal data and therefore non-parametric tests, such as Kruskal-Wallis, Mann-Whitney U and Chi-squared are recommended (Hockey et al., 2021). For

analysis of this data set the Kruskal-Wallis was used when assessing results across the full CAST grading scheme (0 – 4). Chi-squared, or, when conditions were not met, Fishers exact, was used for assessing results when CAST grades had been collapsed into new factors, for example pooling of CAST grades 0 -2 into “marks not of forensic interest” and CAST grades 3 – 4 into “marks of forensic interest”.

2.3 Results

2.3.1 Fingermark enhancement

Across all samples 75% (*N* = 96) of fingermarks were graded $\geq$1 using the CAST grading system, showing at least some evidence of contact. Fingermarks graded three or above are considered suitable for identification and subsequently of forensic interest in the context of a criminal investigation, 43% of all fingermarks (*N* = 55) were graded $\geq$ 3 (Figure 2.3).

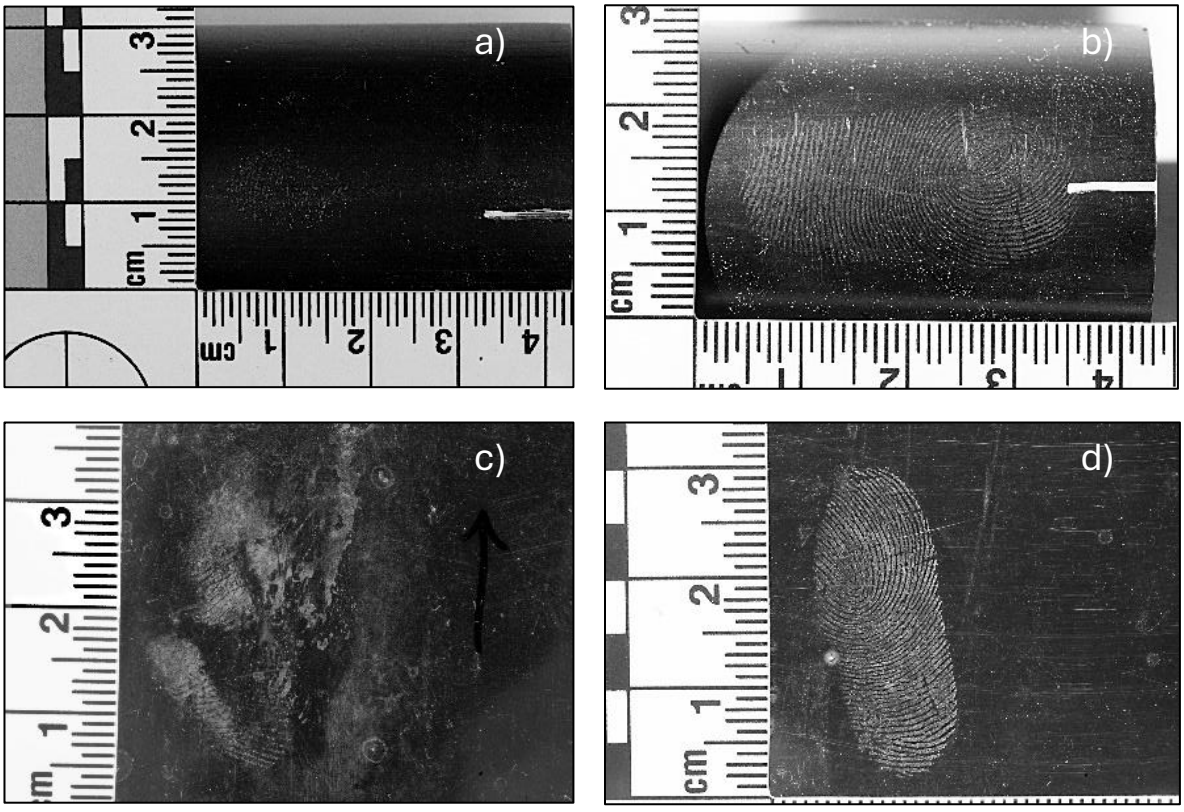


Figure 2.3: Examples of fingermarks enhanced during this study which have been converted to grayscale using Photoshop© software. Fingermarks depicted in a and c are examples of fingermarks classified as not of forensic interest and those in b and d as of forensic interest in terms of quality.

Comparison of CAST and CoLP assessments showed 100% of fingermarks with a 0 – 1, 2 or 4 CAST grade awarded by the PI were assigned into a singular assessed category by the CoLP. Marks awarded CAST grades of 0 and 1 were designated as insufficient, marks awarded CAST grade 2 were designated as comparable and CAST grades of 4 were designated as searchable. For fingermarks given a CAST grade of 3, 16% were designated as comparable by CoLP and the remaining 84% as searchable (Figure 2.4).

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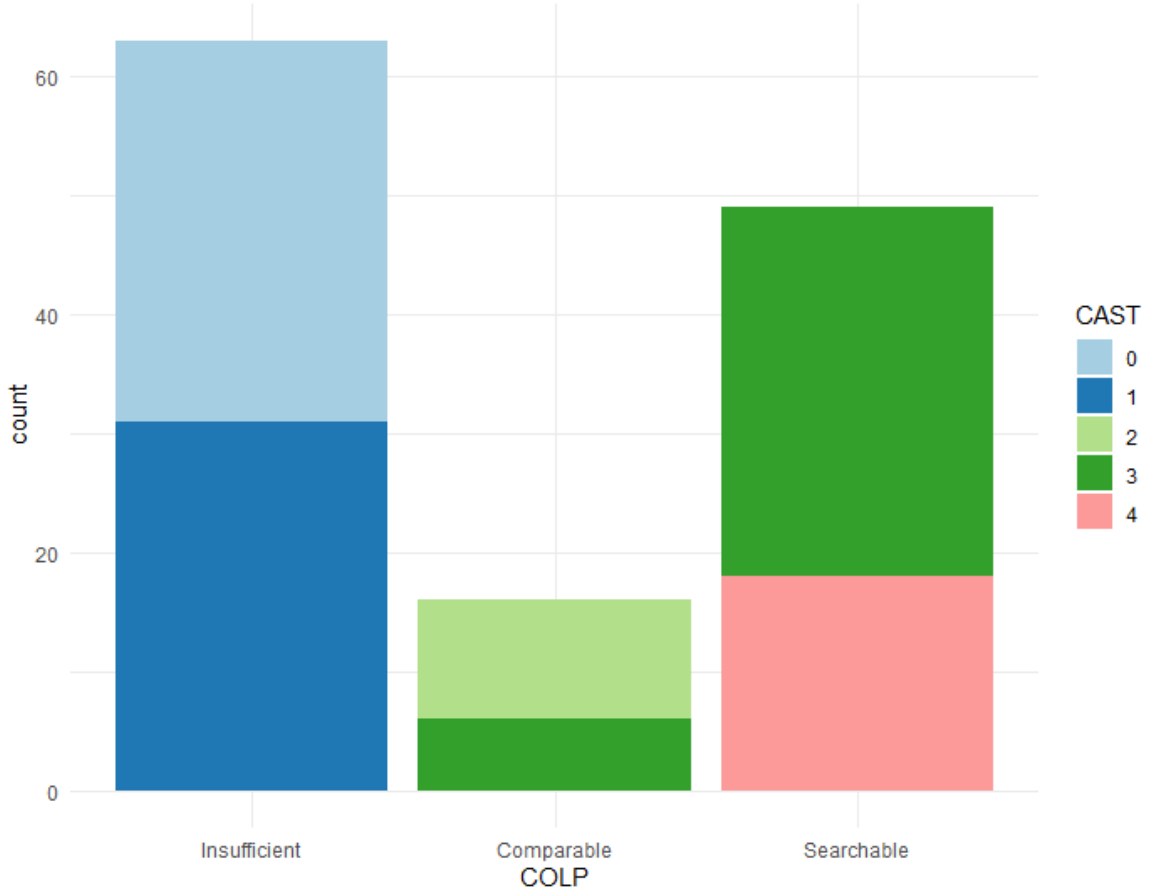

Figure 2.4. Stacked bar plot showing the distribution of CAST grades (0 – 4) within each CoLP designation (Insufficient, Comparable, Searchable). Each bar represents the total frequency of fingermarks awarded for each CoLP designation with segments indicating the proportion of each CAST grade within them.

2.3.2 Deposition technique

Within pooled samples the mechanical method produced the most fingermarks graded ≥3 (53%, *N* = 17) the undirected method the least (38%, *N* = 12) and the light and heavy touch

methods an equal quantity (41%, *N* = 13). A Kruskal Wallis test showed no significant association between deposition method and CAST grade, (x^2 = 2.02, *df* = 3, *p-value* = 0.57) for pooled samples. (Figure 2.5). No significant association was found when results were separated into porous and non-porous substrates and whether they had been deposited naturally or with a grooming step. Individual participants were found to be capable of producing a range of grades across all substrate types.

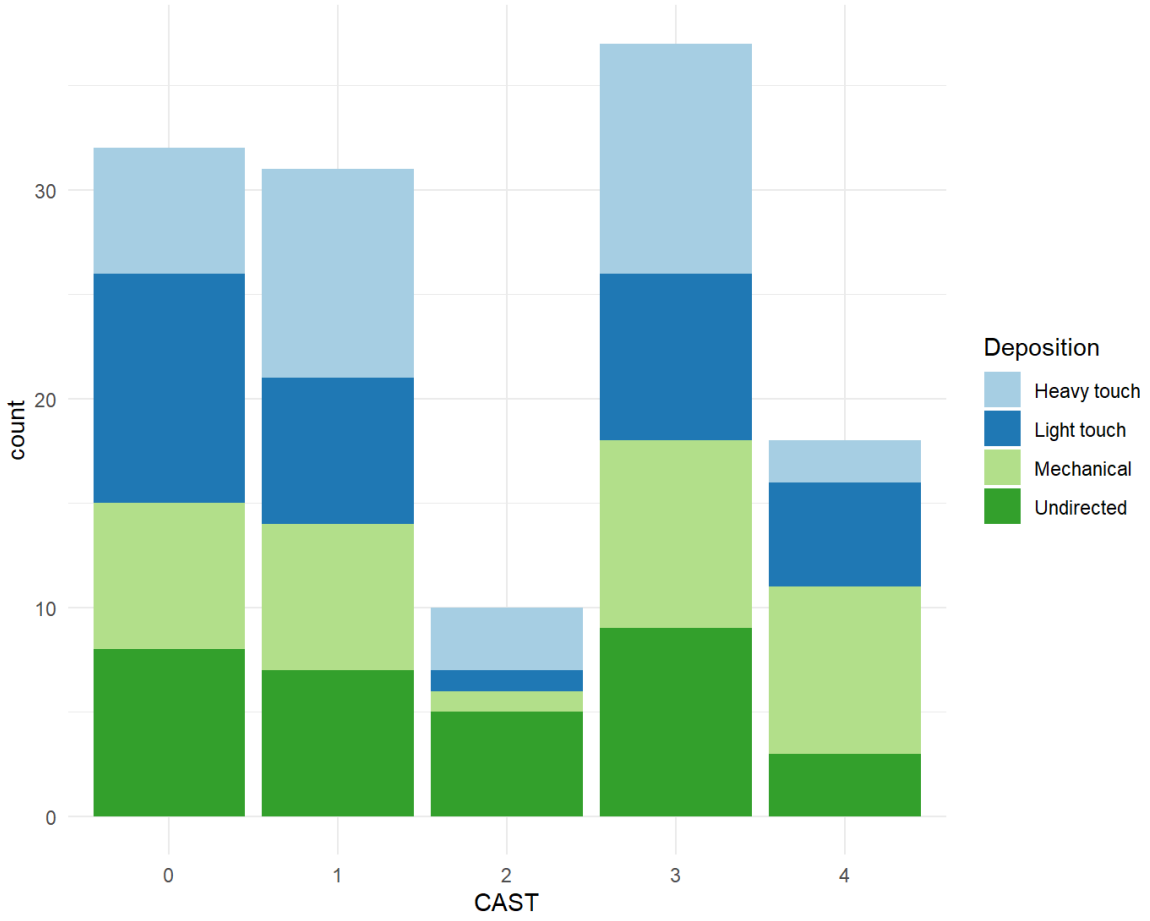

Figure 2.5. Stacked bar plot showing the distribution of deposition methods within each CAST grade. Each bar represents the total frequency of fingermarks awarded for each CAST grade with segments indicating the proportion deposited by each method.

2.3.3 Preparation

A total of *N* = 18 grade >3 marks were recovered from natural depositions and *N* = 37 from groomed. A Kruskal Wallis test found significant association between preparation prior to deposition (groomed vs natural) and awarded CAST grade (x^2 = 18.30, *df* = 1, $p < 0.001$, with

a higher number of CAST grades >1 deposited when using a groomed preparation technique. When grouping substrates by porosity this significance was only applicable to the porous items, wood, and cardboard (x^2 = 29.117, *df* = 1, p < 0.001) (Figure 2.6).

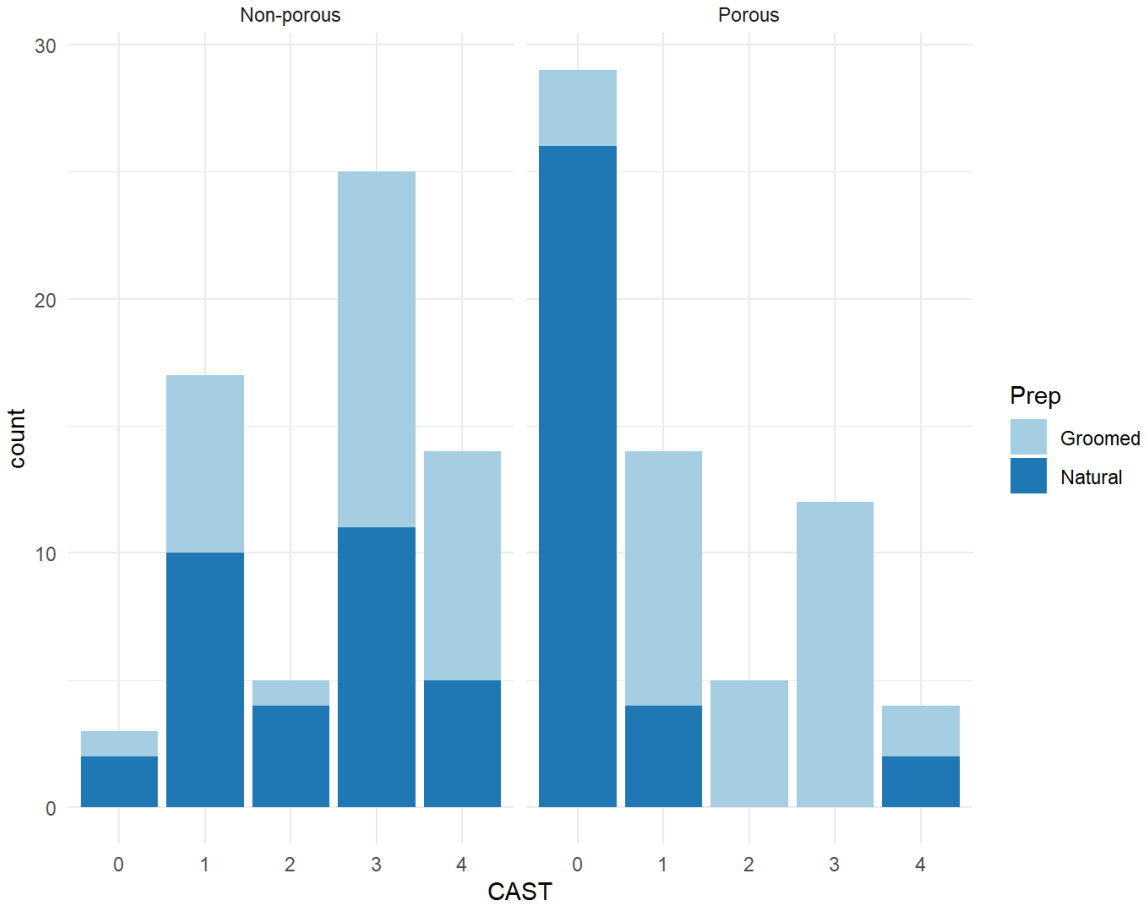

Figure 2.6. Stacked bar plot showing the distribution of preparation (groomed and natural) within each CAST grade for both non-porous and porous substrates. Each bar represents the total frequency of fingermarks awarded for each CAST grade with segments indicating the proportion deposited by each preparation method.

2.3.4 Marks of forensic interest

Fingermark grades were reassigned to produce a binomial dependent variable with 0 representing fingermarks not of forensic interest (grades 0-2) and 1 representing fingermarks of forensic interest (grades 3-4). A Chi-square test of independence found no significant association between deposition method and type of mark, forensic interest or not, recovered. A significant association (x^2 = *10.329, df = 1, p < 0.01*), was found between preparation method, groomed or natural, and type of mark recovered. A binary logistic

regression was carried out with deposition method, porosity and preparation included as independent variables. The first iteration of the model indicated deposition method was not a significant variable in predicting whether a fingermark was of forensic interest or not. The final best fit model was run with porosity and preparation as independent variables. From this model the odds of a fingermark of forensic interest being enhanced on a non-porous surface was not found to be significantly greater than the odds of enhancing one on a porous surface . The odds of a fingermark of forensic interest being enhanced using a groomed digit was found to be 2.61 times the odds for natural digits. (Table 2.2).

*Table 2.2. Results of binary logistic regression analysis on collapsed grade categorisations with preparation and porosity as independent variables including coefficient (Coef), p-value, and odds ratio (OR). Groomed and non-porous were used as reference categories for preparation and porosity, respectively. Significance values of < 0.01 and < 0.001 denoted by * and ** respectively.*

	Preparation [Groomed]			**Porosity [Non-porous]**		
Grade categorisation	*Coef*	*p-value*	*OR*	*Coef*	*p-value*	*OR*
Forensic interest All Mark CAST 0 [0 – 2] 1 [3 – 4]	0.96	0.03*	2.61	--- 18.240	0.99	0.00
Forensic interest “True Mark” CAST 0 [1 -2] 1 [3 – 4]	0.56	0.23	01.75	0.80	0.09	2.22
Positive deposition “True Mark” CAST 0 [0] 1 [1 – 4]	3.3	1.16^{e}-06**	26.45	3.61	7.54e-07**	36.90

Zero graded “fingermarks” by definition “show no evidence of a fingermark” and are therefore distinct from true latent fingermarks (those graded 1 - 4). Zero grades accounted for 25% of all samples (*N* = 32) in this study, and in order to analyse the results for “true fingermarks” were subsequently removed from the dataset. Within the sample set of “true fingermarks”, groomed fingermark deposits had a higher rate of CAST grade 3 marks comparable to natural marks (Figure 2.7), but a chi-square test of independence found no

significant relationship between preparation and CAST grades within “true” fingermarks. True fingermarks were reassigned for a second time based on whether they were of forensic value (CAST grade 3 – 4) or not (CAST grade 1-2). In this iteration of the results a chi square test of independence found no significant association between preparation method and the forensic value of a deposited mark. A binary logistic regression analysis was repeated on this new dataset and none of the influencing variables were found to have significance in relation to whether a fingermark of forensic value would be enhanced.

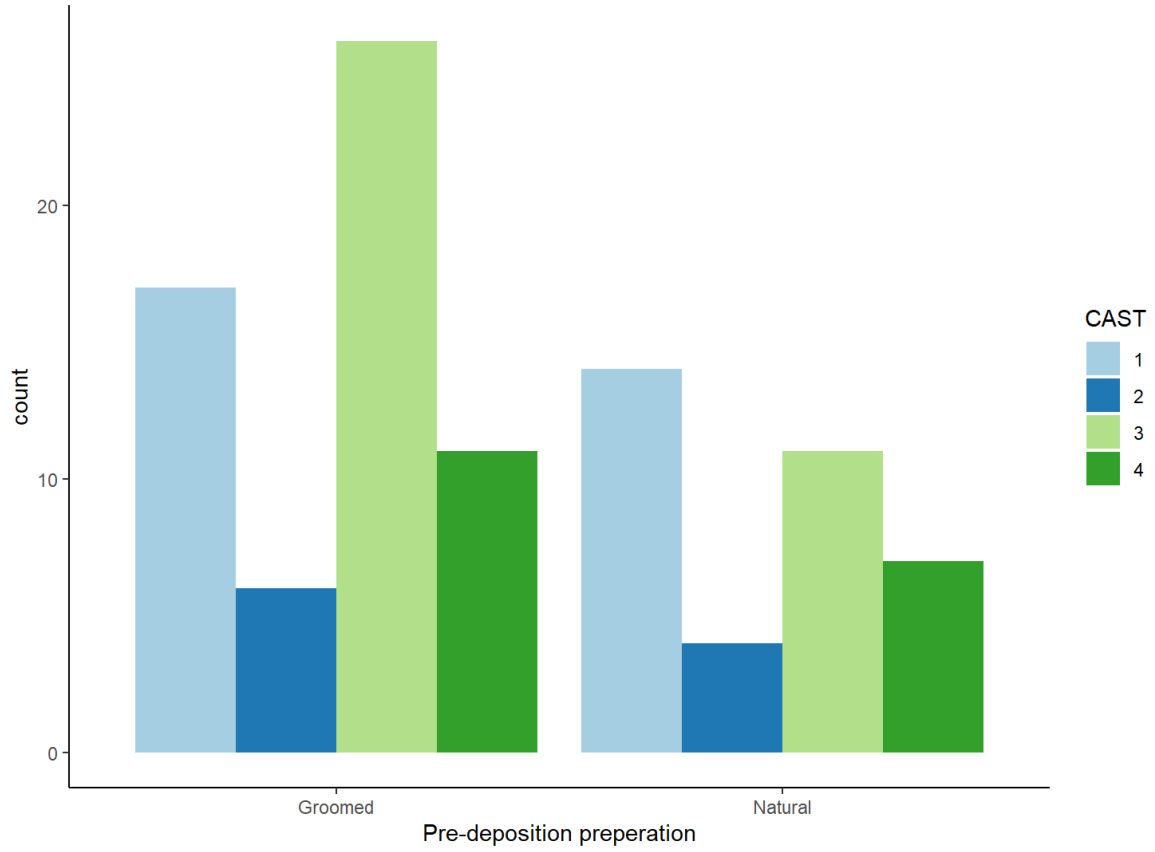

Figure 2.7. Bar plot of frequency of awarded CAST grades 1 – 4 dependent on whether volunteers deposited fingermarks using groomed or natural preparations. Results shown are taken from the “true fingermark” dataset which excludes 0 CAST graded fingermarks. A chi-square independence found no significant relationships between CAST grades award within the groomed and natural fingermark depositions in the “true fingermark” dataset.

2.3.5 Successful vs Unsuccessful Depositions

In response to the above findings one final iteration of the data was explored looking at how deposition method, material porosity and preparation relate to whether a fingermark of any quality is enhanced. Fingermarks were reassigned a third and final time as to whether

they were successfully deposited (CAST grades 1 - 4) or not (CAST grade 0). A Chi square test of independence found a significant association between preparation and whether a mark was successfully deposited or not ($x^2 = 22.04$, df = 1, $p < 0.001$) (Table 2.3).

*Table 2.3. Outcomes of tested associations between preparation and deposition methods and awarded CAST grades, covering full and collapsed grade categorisations. † and ∞ denotes associations tested with Kruskal Wallis and chi-square tests of independence, respectively. Significance values of < 0.01 and < 0.001 denoted by * and ** respectively.*

	Preparation			Deposition method		
Grade categorisation	*x^2*	*df*	*p-value*	*x^2*	*df*	*p-value*
Full scope CAST† [0 – 4]	18.30	1	1.892e-05**	2.02	3	0.57
Forensic interest All Mark CAST∞ 0 [0 – 2] 1 [3 – 4]	10.33	1	0.001*	1.88	3	0.60
Forensic interest "True Mark" CAST∞ 0 [1 -2] 1 [3 – 4]	0.82	1	0.37	2.44	3	0.49
Positive deposition "True Mark" CAST∞ 0 [0] 1 [1 – 4]	22.04	1	2.67e-06**	2.44	3	0.49

No significant association was found between deposition method and absence or presence of a mark. A binary logistic regression using preparation and porosity as indicator variables was again found to be best fit for the data. Outputs showed both porosity and preparation had a significant relationship with whether a fingermark was enhanced or not (*$p < 0.001$* and *$p < 0.001$* respectively). The odds of a groomed digit resulting in a successful deposition were 26.45 times that of a natural digit and the odds of a fingermark being successfully enhanced on a non-porous surface were 36.9 times that of a porous surface (Table 2.2).

2.4 Discussion

2.4.1 CAST grading quality assessment

In forensic research it is crucial that any drawn conclusions intended to guide law enforcement strategies are supported by high quality data. As such in later research stages, the IFRG stresses the importance of involving fingerprint experts to validate assessments made by researchers with minimal experience in fingermark interpretation. Although this thesis presents early-stage proof of concept, to ensure confidence in the results, the decision was made to validate the grading conclusions drawn, through consultation by CoLP fingerprint experts. Comparison of grades in this study with classifications by CoLP showed that grades 0, 1, 2, and 4 fell into distinct categories: insufficient (0–1), comparable (2), and searchable (4) whilst grade 3 marks bridged two classifications, comparable and searchable (Figure 2.4). It has been shown that professional fingerprint examiners tend to agree on assessments for the highest and lowest quality marks but may deviate outside of these extreme parameters (Ulery et al., 2012; Hicklin et al., 2020). Although the CAST and ACE-V grading schemes are not directly comparable, the results show that marks at the extremes of the CAST scale correspond to single classifications by CoLP, supporting this phenomenon. Within the CAST scheme grades 3 and 4 are considered of forensic interest due to their identifying capacity. Both the comparable and searchable classifications of CoLP's system offer as equivalent quality indicator. As such it is unsurprising that grade 3 marks bridged these two classifications due to the higher expertise of CoLP examiners being able to make more discernible interpretations of the useability of a grade 3 mark from a case work perspective. The classification of all grade 2 marks as "comparable" with no overflow into neighbouring classifications, comes at a surprise, as it indicates they hold a distinct level of quality and use to fingerprint experts. This value is contrary to their designation within the CAST system whereby they are grouped with 0 and 1 grades and seen as non-identifying in

quality (Hockey et al., 2021). Although other grading schemes exist, the Home Office CAST scheme remains widely used in research. These results challenge conclusions drawn from method rejections due to a lack of grade 3 or 4 marks, despite a high frequency of grade 2. Given the strength in quality assessment overlap with the CoLP assessments the CAST grading conclusions in this study are taken with confidence. However, I have highlighted there is a disconnect between the interpreted value of the CAST scale grades and the value of the same fingermark in actual casework scenarios.

2.4.2 Deposition method

When conducting research into fingermark enhancement techniques, investigators face the challenge of attempting to measure impacts on a dependent variable that is characterised by its inherent variation, even before the introduction of any independent variables under consideration. Fieldhouse's 2011 study on fingermark reproducibility through managing deposition pressure (Fieldhouse, 2011), highlighted that enhancement methods can be wrongly deemed ineffective, when in reality, poor results may be a product of the deposition method itself. As such the underlying theory of many attempts to control deposition pressure has been, if a method can produce "good quality" fingermarks, under a range of conditions, then a poorly graded fingermark deposited using that same method can be more easily attributed to the enhancement technique under investigation. The results of this study found that using a top pan balance to control deposition pressure resulted in the highest proportion of grade 3 and 4 marks. It is suspected that the method, whilst rudimentary, goes some way to removing subjective interpretation of pressure possibly leading to more confidence in the deposition and limiting opportunities for distortion caused by hesitant movements. As shown with the high number of absent fingermarks within the "light touch" depositions in this study, when left to their own interpretation donors may err too far on the side of caution to detrimental effect. Despite

some variance seen withing grade frequencies there was no significant relationship found between deposition method and CAST grade in any of the breakdowns conducted as part of this study. Therefore I concur with others in the literature (Steiner et al., 2019) that whilst attempts to control deposition pressure may provide some improvement in quality most methods are not sophisticated enough to overcome the natural inter and intra variability of donor depositions. These results also highlight the strength of results seen from purposefully manufactured pressure control deposition instruments (Fieldhouse, 2011; Reed et al., 2016). However, until such tools can be mass produced and adapted for work on novel surfaces, such as the wildlife specimens that will be featured in Chapter 4, the variability of deposition pressures may have to be considered an inevitable factor of consideration in research. To this end if high quality fingermarks cannot be consistently produced then an experimental design that includes equitable distribution of grade frequencies coupled with randomised application of enhancement methods may be alternative approach. In research guidelines, control of deposition pressure is recommended in early stages but as research progresses into pseudo and full operational trials it is encouraged to instead simulate how objects would be handled in a real case scenarios (Sears et al., 2012; Almog et al., 2014). Within this studies' experimental design, the "undirected" method most closely aligns with this guidance. The results demonstrate such a method does successfully represent intra-variability within donor depositions as well as producing the lowest range within grade frequencies.

2.4.3 Preparation

The decision to use groomed or natural fingermarks, holds significant influence over the communities interpretation of experimental outcomes. Results from existing research consistently demonstrate the act of grooming influences the composition of fingermarks

(Croxton et al., 2010; Kim et al., 2019; Moraleda Merlo et al., 2023). One consistent is that squalene is found to be a major component of both natural and groomed marks, degrading significantly over the space of a few days (Pleik et al., 2016). Another study found that changes in the proportions of certain lipid groups were correlated suggesting they originate from the same endo/exogenous sources (Moraleda Merlo et al., 2023). Notably composition differences do not extend to amino acids, an important component in chemical enhancement techniques including ninhydrin and indandione (Croxton et al., 2010). In support of this at least one recent paper found no significant variation in ninhydrin enhanced marks regardless of the preparation activity involved (Lange and Carlysle-Davies, 2024). Despite the strength of conviction regarding groomed and natural fingermark differences a systematic review of studies assessing fingermark composition failed to identify any conclusions which could be applied to the wider population or scenarios outside of the experimental setting (Robson et al., 2022). This was in part due to the significant variability in experimental design amongst papers. The findings related to change in lipid quantities within groomed marks appears to be the crux of the argument that "natural" fingermarks are inherently closer to ones encountered operationally. Fingerprint powders were the main choice of enhancement method in this thesis, due to their cost effectiveness and high rate of deployment by forensic practitioners in operational settings. Their functionality is tied to adhesion with both eccrine and sebaceous secretions, including water and fatty acids, in fingermarks. The loss of water in eccrine secretions through absorption or evaporation has been said to reduce the effectiveness of some powders (Bleay et al., 2021). The role of water is seen as important enough that in cases of older marks, a historical practice of "huffing" has been used to re-humidify prints and restore their water content to help powder adhesion processes. Conversely it has been suggested they work best on fresh, sebum rich deposits (Bécue and Champod, 2023) and as such an

increase in sebaceous constituents, including lipid quantity and composition has the potential to increase their effectiveness. With fingerprint powders being one of the most widely used enhancement methods on a global scale (Bleay et al., 2018) and one of the tested mediums of Chapter 4 of this thesis , there is a responsibility to carefully consider these different interactions and how best to imitate the complexity of latent fingermarks. As a general observation of the literature there is little to no evidence to support “natural” depositions being better representations of operational fingermarks. Indeed, there are several studies which demonstrate that face touching (i.e. grooming in an experimental setting) increases in correlation with events that induce increased arousal, emotion, stress, and cognitive load (Spille et al., 2021; Ralph et al., 2022) and it is reasonable to assume that a criminal activity of any kind could be conducive to any of these responses. As such it calls into question that a “natural” fingermark deposited under experimental, and therefore non-stressful, conditions can truly be considered representative of a fingermark deposited at a crime scene. Particularly when following guidelines such as Sear et al (Sears et al., 2012) which discourage the deliberate touching of faces, artificially manipulating donor actions in favour of eccrine secretions. In the context of this research the consideration of a “natural” fingermark was further complicated by the extenuating circumstances of COVID-19. During the period of experimentation recommendations to undertake diligent hand-washing, and minimising face touching were parts of public service announcements to control the spread of COVID-19. As such individuals were already deviating from normal activities which help build the complex constituents seen within fingermarks. In addition to this, as a requirement of ethical approval, participants were required to wash their hands before handling any objects, without being able to partake in a minimum 30-minute period of “normal activity” to accrue national secretions on their fingertips. Deposits from freshly washed hands have been found to contain a higher water content, less material, and be

significantly less durable than “dirty” (i.e. natural) deposits (Keisar et al., 2019; Czech et al., 2020) .

Given the factors introduced by COVID-19 protocols, along with stringent guidelines and concerns in the literature about the negative implications of using groomed marks, a significant deviation in quality between groomed and natural marks was expected across all depositions in this study. However, this expectation was not reflected in the results, which consistently demonstrated that grooming had no significant relationship with the quality of a deposited mark but did have a significant relationship with the likelihood of a positive deposition. One theory to explain these results is that the powders used in this study possess an equitable affinity for adhesion to both eccrine and sebaceous constituents but the act of grooming increases quantities available for adhesion. The proportional similarities in grade qualities for positive depositions (Figure 2.7) hold credence to this theory as it suggests that the additional quantity of fingermarks seen in groomed marks are equitably distributed throughout grades. This implies not that grooming adds more of a “good thing” but rather more of “everything” that is relevant to enhancement using magnetic and brass flake fingerprint powders. For research purposes this holds an important distinction as it suggests that grooming can beneficially support research in ensuring fingermarks *are* deposited whilst simultaneously producing a range of grades. Traditionally depletion series are used to produce the same effect to assess the sensitivity of enhancement techniques on both poor and high quality marks (Almog et al., 2014). This study demonstrates that this same variation can be achieved using groomed marks across several participants. As it is known grooming does fundamentally change the composition of a fingermark this phenomenon may not be replicated with other enhancement techniques that target different constituents to fingerprint powders. However given it is mostly lipid composition that changes and only three of the most common fingermark

enhancement processes target these constituents (Home Office, 2022) these results may in fact be applicable for several enhancement techniques. A factor not considered in this study but that would be required to investigate this phenomenon further is time elapsed since deposition. Fingermarks were left to age for 30 minutes and as such would be considered "fresh" with minimal time elapsed to allow for evaporation of water or degradation of lipid constituents. In this study over 50% of positive fingermarks for both natural and groomed depositions were considered of useable quality. By comparison in 2013 on average 10 % of marks recovered at scene were of sufficient quality to be taken forward as evidence in court (Brown et al., 2013). Though this quantity may have increased in the decade since there is still a disparity in the number of useable marks recovered under controlled conditions, both within this study and in studies with an ageing variable and the use of natural and groomed marks (McMorris et al., 2019). Other studies that closely replicate real crime scene conditions, with no preparatory activity and substrates aged as they would be in operational settings, show results that more closely align with the expected 10% recovery rate when using the most common enhancement techniques including powders and ninhydrin (Dawkins et al., 2020). The similar quality distributions and high recovery rate of both natural and groomed marks in this study suggest that contextual simulation of fingermark deposition may be a more important control in experimental design than the preparation or deposition technique employed.

2.4.4 Porosity

While deposition and preparation methods are widely discussed in fingermark research, forensic practitioners are unlikely to have access to such detailed contextual information about a suspect's actions. Consequently substrate porosity becomes the key guiding factor for enhancement method choice (Home Office, 2022) and is why both porous and non-porous substrates were included in this study. Fingermarks deposited on non-porous

substates hold the benefit of retaining constituents at a surface level. As such physical enhancement techniques such as powders can readily adhere to the exposed secretions and offer opportunity to enhance high quality marks with continuous ridge detailing and pore level detail (see section 3.1 for detailed descriptions of fingerprint detail levels) (Champod et al., 2016). However, due to their positioning they are vulnerable to damage through accidental or purposeful, physical, or environmental processes such as wiping with a cloth or hand or being washed away with running water. Even without interference over time fingermark components, likely fatty acids, can migrate across non-porous surfaces potentially impacting the effectiveness of chemical enhancement techniques that target such constituents, such as VMD (Popov et al., 2017). Water is also readily lost through evaporation, occurring within minutes if maintained at body temperature (37^{o}C) for the majority of eccrine depositions, and at a slower, but still rapid, rate for mixed depositions (Keisar et al., 2019). By contrast when fingermarks are deposited on porous substrates constituents are absorbed into the surface pores. Eccrine deposits are absorbed within seconds of deposition, with the depth of absorption correlated to porosity and relative humidity (Champod et al., 2016). Through rendering eccrine constituents inaccessible at surface level, they are better protected from physical damage though are still vulnerable to being removed with water. This preservation is best seen in cellulose-based products such as paper, cardboard and wood, where the water-soluble deposits, specifically, amino acids securely bind to the static cellulose compounds (Jelly et al., 2009). Assuming sufficient amino acids are present in the deposition, this binding factor prevents the dispersal of amino acids through the surface and effectively and securely retains an impression of the fingermark for a significant, but not indefinite, period (Champod et al., 2016). By comparison sebaceous secretions may remain on the surface of a porous surface for hours to days, and some superficial quantities may remain for years. Temperature has a significant

effect on rate of sebaceous secretion diffusion, increasing significantly above 35°C (Champod et al., 2016). Semi-porous substrates are categorised by their slower rate of diffusion of both eccrine and sebaceous constituents comparable to truly porous substrates, taking minutes to hours (eccrine) to days to weeks (sebaceous) for full diffusion to occur (Champod et al., 2016). Although depositions were aged for a maximum of 30 minutes in this study, based on the above factors, it is assumed that most water-soluble eccrine secretions would have diffused into the porous surfaces by time of enhancement, leaving primarily sebaceous secretions which have had minimal time to degrade. Similarly, within 30 minutes a large quantity of the water content from marks on non-porous surfaces is likely to have evaporated. Fingerprint powders are generally not recommended for use on porous substrates however this is not due to absolute ineffectiveness but rather because of the superior performance of chemical methods such as ninhydrin and indandione (Holder et al., 2011). The results support this assessment with a range of grades recoverable on porous substrates but with significantly fewer overall positive depositions (CAST grades 1 – 4).

However as with preparation the lack of significant relationship between porosity and CAST grades within positive enhancements comes at a surprise. One suggestion is that a minimum quantity of constituents, of either eccrine, sebaceous, or mixed origin, is required on a surface for powders to adhere successfully, and once this threshold is reached, their effectiveness stabilises, regardless of the surface type. Quality then becomes a greater matter of well-established factors, such as pressure, angle and the nuances of individual donors (Chadwick et al., 2018) which provides the range of grades seen. The sensitivity of a powder will affect the threshold and this has been proven in part through the use of depletion series in studies where in some cases depletion has no effect on grade outcome (La Rocca et al., 2024) and in others increasing depletions are correlated with increased

number of unsuccessful deposits though not necessarily decreasing quality (Chadwick et al., 2021). This theory would also explain why preparation only held a significant relationship with CAST grades (inclusive of 0 graded marks) on porous items. Natural (eccrine) depositions are known to contain lower quantities of materials overall and be quickly diffused into porous surfaces, therefore eccrine depositions on porous surfaces are less likely to readily reach this proposed "threshold" rendering powders ineffective. Grooming by comparison introduces a higher quantity of constituents allowing the threshold for powder adhesion to be met. Interestingly, on low porosity paper where natural fingermark constituents have minimal penetration it has been shown that enough residue is present that techniques, such as powdering, normally favoured for non-porous surfaces may be suitable for use (Almog et al., 2004). This supports the suggestion that in the case of powdering, grooming simply introduces more, but not necessarily favourable, constituents to adhere to as high quantities of available eccrine secretions could produce the same suitable environments. As non-porous surfaces do not absorb constituents, the "threshold" figure is more easily reached regardless of preparation type and constituent make-up. This is theoretically even with immediate water loss from eccrine heavy marks, as it has now been proven this accounts for a smaller proportion of fingermarks total composition than previously believed (Kent, 2016).

2.5 Summary

The aim of this chapter was to assess the impacts of deposition method and preparation on recovered fingermark CAST grades and subsequently identify a protocol to take forward for Chapter 4 of this thesis. It was found that deposition method had no significant relationship with the quality of a deposited mark, regardless of substrate type. Preparation prior to deposition, through the act of loading fingerprints by "grooming", did have a significant relationship with the enhanced fingermark but this was limited to whether a fingermark

was deposited or not and did not extend to a relationship with the quality of the mark. Therefore whilst ”grooming” is more likely to result in a deposited fingermark at a binary level the preparatory action retains a variability of grades across depositions when enhancing using fingermark powder. A similar relationship was found between substrate porosity and successful fingermark depositions.

As a result of this work, it is suggested that the use of grooming in fingermark research may be less contentious than previously discussed, at least regarding studies focused on fingerprint powders. Its use in experimental design may simply increase the likelihood of the deposition, without introducing a bias within enhancement and recovery testing outcomes. In certain contexts, such as the extenuating factors introduced by COVID-19 in this study or early stages of experimental research, this introduction of additional, but not overtly favourable constituents, may be a sought-after characteristic.

Though the number of participants used in this study was more than suggested numbers for early-stage research, the overall dataset and number of fingermarks remains small, and a larger scale study would be required to achieve more reliable statistical interpretation. In addition, both the logistical and circumstantial factors bought on by COVID-19, including inability to allow “natural” depositions to be accrued over a set period, and excessive handwashing procedures both within the experimental requirements and general cultural behaviours of the time, may have caused deviation of both groomed and natural deposition constituents from what would have been seen outside of these circumstances. Fingermarks were also not aged, a decision made to replicate the conditions that will be seen in Chapter 3, whereby the logistical limitations of having singular examples of each wildlife specimen require same day depositions and enhancements. However, ageing for a full 24 hours is likely to influence the results due to degradation and diffusion of constituents.

In general, these findings highlight the complexities of controlling fingermark depositions in research but raise evidence to call into question the significant aversion to grooming practices and whether “natural” depositions are better representative of latent fingermarks encountered operationally. Despite the limitations discussed these results provided enough confidence to pursue the use of groomed fingermark deposits and an “undirected” deposition technique for comparison of enhancement techniques on wildlife specimens in Chapter 4.

Chapter 3: Investigating impacts of handling manner and grooming preparation on direct trace DNA transfer: Identifying a reliable method of deposition.

3.1 Introduction

In the 1920's Edmond Locard postulated his famous theory, "Every contact leaves a trace" which would later become better known as Locard's exchange principle (Mistek et al., 2019). The theory conceptualises the notion that when two objects make contact there is an exchange in materials and this exchange may be of use in a forensic context. Whilst Locard's work helped develop the world of forensic science I see today, particularly with regards to fingerprinting technology, it wouldn't be until 1997 that his exchange principle would be applied to the area of DNA profiling, with the introduction of the phenomenon of DNA recovery from touched objects (van Oorschot and Jones, 1997). Within the decades since it's discovery, trace DNA has become a fundamental aspect of forensic investigation and the communities understanding of its origins, limitations, and affecting factors continues to develop (Alketbi, 2018; Burrill et al., 2019; Tozzo et al., 2022). Public awareness of its evidentiary role in both incrimination and exoneration has also increased dramatically in the advent of a boom in true crime media (Rickard, 2023). Therefore, as with fingermark evidence, there is an appetite to further research and understanding of the subject matter. Researchers concerned with maximising the applications of trace DNA for forensic investigation focus on investigating its transfer, persistence, prevalence, and recovery (TPPR). To design effective experiments to investigate these factors it is necessary to understand the mechanisms which facilitate deposition of DNA onto the sampling surface, i.e. DNA transfer, so that they can be exploited for experimental contexts. However, unlike with fingermark research, there appears to be little attempt to understand the complexities of DNA transfer with the specific intention of using the knowledge to standardise or inform

experimental design. This is evidenced by the wide range of approaches to trace DNA deposition seen within the literature including absence and presence of handwashing (Stoop et al., 2017; Alketbi and Goodwin, 2019c), grooming ((Subhani et al., 2019; Alketbi, 2022a) and shedder status assessments (Johannessen et al., 2021; Jansson et al., 2022) with little context as to reasoning behind design choices. Some studies seek to simulate real world scenarios and implement no controls, sampling from everyday objects that exist and are interacted with by donors in their natural environment (Boyko et al., 2020) whereas others have donors interact with objects in their intended manner but within an experimental setting (Pfeifer and Wiegand, 2017). Reviews of the literature have raised this issue of inconsistent or unreproducible study design (Gosch and Courts, 2019; Meakin et al., 2021) and whilst the ISFG provide some guidance this largely focused on replicating operational workflows at the recovery and analysis stage rather than model of deposition;

"*the experimental design used to collect the data need to be comparable to the methods used in the case"* (Gill et al., 2020).

As DNA profiling techniques have become more sensitive and reliable, forensic investigators are more readily able to answer the question of who the recovered DNA belongs to (Kokshoorn et al., 2017). As a result the boom in DNA transfer research has been spurred by a desire to answer the question of how the recovered DNA ended up on the sampled surface, commonly referred to as the "activity level" (Gill et al., 2020). Van Oorschot describes eight different routes for the transfer of DNA onto a final sampled surface, of which two distinct categorisations exist, "direct" and "indirect" transfer (van Oorschot et al., 2019). Direct transfer is the most simplistic of activity levels, where the original source e.g. a hand, deposits onto the sampled surface, e.g. a handle; indirect transfer occurs when the deposited DNA is transferred again, meaning the original source of the recovered DNA

may not ever make contact with the sampled surface. The discovery of indirect transfer has important implications for the development and analysis of activity level propositions (van Oorschot et al., 2021; Buckleton et al., 2022), those that sit at level II in the hierarchy of propositions (Cook et al., 1998). Below I discuss some of the known factors that play a role in direct and indirect DNA transfer and how they may influence deposition protocols.

3.1.1 Shedder status

The tendency of an individual to deposit DNA onto surfaces is referred to as their "shedder status". As with fingermarks there has been a long-held perception that certain individuals have a greater proclivity for depositing useful forensic material than others (Lowe et al., 2002). This theory has been supported by a range of studies indicating the existent of "good" and "poor shedders" who are capable of consistently depositing higher or lower quantities of DNA comparable to others (Goray et al., 2016; Johannessen et al., 2021). The commonality within the general public of these two classes of "shedders" is still unknown but at least one study suggests the majority of individuals do not fall into these extremities of shedder classification but rather sit within the media (Kanokwongnuwut et al., 2018). An individual's shedder status will have implications as to how much DNA is expected to be recovered from an object they have touched, and their contributions to mixture profiles. The second factor is important for casework considerations as shedder status has potential to feed into Bayesian networks assessing the probability of alleles being present as a result of either direct or indirect transfer (Fonneløp et al., 2017). In terms of consistencies within the populations it has been suggested that men and younger people, are more likely to be categorised as "good" shedders (Ceballos et al., 2015; Goray et al., 2016; Manoli et al., 2016; Fonneløp et al., 2017) though whether this is the result of endo or exogenous variables has not been explored.

In the opposite vein there have also been multiple studies which challenge the concept of shedder status and demonstrate a lack of reproducibility in status categorisation across both short (days) and long (years) periods of time (Manoli et al., 2016; Tan et al., 2019; Lee et al., 2023). This lack of reproducibility can have implications if selecting donors based on shedder status for inclusion in research studies, as an assessment made outside of the experimental period may be irrelevant on the day of deposition. Recent studies have recommended to effectively incorporate the nuances of "shedder status" into evaluations an individual's quantity of DNA that is transferred should be considered as a distribution and this whole distribution should be factored into probability assessments (Samie et al., 2020). As it stands selecting an individual for inclusion in experimental work based on a single assessment of shedder status is unlikely to produce a reliable narrative and may be a waste of resources. However increasingly it is being shown that extrinsic and as such controllable variables, such as actions prior to donation, may play a large role in quantities of DNA deposited (Miller et al., 2021) and these will be discussed further below.

3.1.2 Pre-deposition activities

As the understanding of trace DNA origins develops there has been interest in the role bodily secretions, such as sweat and sebum, may play in the transfer of target forensic materials for DNA transfer. A study looking at the impacts of handwashing and contact with sebaceous and non-sebaceous areas of the body, on direct and indirect transfer demonstrated that post handwashing, profiles could not be obtained from fingerprints deposited by direct transfer. By contrast a profile could be obtained as a result of indirect transfer from touching a sebaceous area of a second individual after hand-washing. The same result could not be achieved when touching a non-sebaceous area (Zoppis et al., 2014) demonstrating that sebum may be playing a significant role in DNA transfer. Another

study supporting this theory demonstrated that lower quantities of DNA were recovered from “inactive” hands that were restricted from touching objects and self-comparable to “active” and recovered DNA quantities were correlated with DNA quantities found on the face, a sebaceous area (Jansson et al., 2022). Other studies have shown DNA profiles can be recovered from depositions where contact has not been made with sebaceous areas (Oleiwi et al., 2015; Subhani et al., 2019) though touching of sebaceous areas can increase the percentage of alleles recovered (Subhani et al., 2019). These studies suggest both eccrine sweat and sebaceous secretions may act as vectors for DNA transfer which may have important implications in experimental design, especially when deciding to limit self-touch (i.e. grooming). Assuming trace DNA depositions are facilitated to some degree by such secretions the implications of handwashing become more apparent. Early on it was demonstrated that controlling for hand-washing impacted the number of alleles recovered from substrates, with a general tendency of increasing numbers of alleles observed with greater periods of time post-handwashing (Lowe et al., 2002; Phipps and Petricevic, 2007). These results suggest DNA undergoes an initial removal process through hand-washing followed by a natural re-accumulation. Again however, like shedder status, there has been demonstrations in the literature to the contrary, with handwashing resulting in no significant difference in trace DNA depositions (Goray et al., 2016; Szkuta et al., 2017). In these studies however there was at least a 5 minute period between hand-washing and deposition and it has been shown that accumulation of DNA occurs within 15 minutes of hand-washing, though notably this is from exogenous sources rather than the hands themselves (Burrill et al., 2021a). Whilst most studies have investigated handwashing through the use of standard soap and water at least one study has looked at the impacts of hand sanitizer on trace DNA deposition, an important variable of consideration within the events of COVID-19 (Bini et al., 2023). Here it was found that whilst hand-sanitiser does

decrease the quantity of DNA recovered enough alleles can be identified to warrant a sample of being of forensic interest. It is evident that hand-washing steps in experimental designs for trace DNA depositions introduce potentially complex impacts on DNA quantities. Whilst it may potentially remove any non-self-DNA from an individual's hands, if denied the ability to perform natural activities post-handwashing it may result in failure to accumulate quantities of self-DNA reminiscent of real-world scenarios due to the importance of exogenous sources. As such a period of accumulation followed by the expectation of non-source alleles introduced through indirect transfer during natural activity should be standard consideration in research studies.

3.1.3 Handling time, manner, and substrates

It has been suggested that a large proportion of DNA transfer takes place at the initial point of contact (van Oorschot and Jones, 1997; Meakin et al., 2021) and therefore prolonged handling will not result in higher levels of deposition. Indeed it has been found that a period of just two seconds of contact can provide sufficient quantities of DNA to produce full profiles and handling time has no significant impact on quantities recovered (Sessa et al., 2019). It has also been shown that sampling of habitually used items will result in the main user being a major donor a large proportion of the time, however, DNA of another individual can be detected after a single use of the same item (Atkinson et al., 2022). This supports the theory that a notable amount of DNA is deposited with minimal handling but can accumulate to significant levels through separate handling events. The same study demonstrated a negative correlation between first and second user contributions with increased handling time by the second user. This observation feeds into another phenomenon of a second user being capable of removing a first users DNA through the handling process and important consideration when including multi-person depositions

into experimental designs. The theory of handling by a second user resulting in removal is supported by other studies having shown that separate repeated handling events do not result in a “saturation” point where no more DNA can be deposited or recovered and that the repeated handling has an accumulative effect of unknown end point (Jansson et al., 2024). Although degradation will play a role, without a removal element by the second user it is less likely to achieve the significant differences in DNA that have been observed between first and second users over time.

As such research indicates that rather than length of time the friction and pressure applied during handling have a greater influence on DNA transfer (van Oorschot et al., 2019; Sessa et al., 2023). Studies have shown that a combination of increasing pressure and friction results in a higher rate of DNA transfer comparable to other passive combinations (Goray et al., 2010) and increasing pressure significantly increases quantities of deposited DNA and subsequently number of alleles (Tobias et al., 2017; Hefetz et al., 2019). When giving verbal instructions regarding the handling process for trace DNA depositions this will need to be taking into consideration as guidance that encourages handling practices at either end of the scale (i.e. very gently to very heavy handed) could influence results. In most cases friction will be introduced through the substrate type being deposited on and it has been found that greater quantities of DNA tend to be recovered from rough, porous surfaces which are likely to introduce a greater level of friction between hand and surface (Goray et al., 2010; Daly et al., 2012; Burrill et al., 2019).

Though not the focus of this study whilst initially friction and pressure seem logical variables for influencing trace DNA transfer it does raise further questions as to the origins of trace DNA. If significant quantities of DNA are deposited during single short-term contact these may feasibly originate from exogeneous sources, present on the surface of the skin, as laid

out in 3.1.2. In the event of any increase in deposited quantities correlated with increased friction and pressure this may subsequently be attributed to loosening and release of DNA from the surface of the epidermis. However reviews highlight there is conflicting data and ambiguity regarding the role corneocytes from the epidermis play in contributing to trace DNA deposits (Burrill et al., 2019). It is likely trace DNA deposits are an amalgamation of endogenous and exogenous sources on the skin and as such the active suppression or introduction of specific actions need to be carefully considered for their possible impacts on deposition quality.

3.1.4 Artificial secretions

One of the few attempts to standardise the quantity of trace DNA depositions has borrowed from fingermark research and manufactured a synthetic proxy (Arsenault et al., 2023). The work is influenced by the incoming knowledge of sebum's role in DNA transfer which has not been factored into other studies that had used suspension fluids unrepresentative of vectors for trace DNA deposits, such as bodily fluids or buffer solutions. The research looks promising as it goes to efforts to consider the deposit medium, and inclusion of both cell free and cellular trace DNA, however, is still in the early stages and unlikely to become readily available to the wider research community for some time.

Although there seems to be less concern within the DNA community surrounding deposition protocols comparable to those discussed with fingermarks in 2.1 the realities of controlling variability in trace DNA are still present. There appears to be no consistent or recommended approach within the literature that can be adopted. To add to this complexity in Chapter 5 of this study donors will be asked to handle wildlife objects which are likely to be novel to them and therefore they will have no frame of reference on their weight, texture, or robustness. Without such context the way individuals will handle the

items could be removed from their natural handling tendencies and influence outcomes. For example, too tentative a handling could result in lower quantities of DNA being deposited due to minimisation of friction and pressure.

To this end the objective of this chapter is to investigate the impacts of substrate handling technique and grooming activities on quantity and quality of trace DNA deposits on porous and non-porous substrates. Results are to be interpreted the intention of identifying a methodology of reliable deposition that can be taken forward for use on wildlife specimens.

3.2 Materials and method

3.2.1 Substrate preparation

As described in 2.2.1 two porous and two non-porous cylindrical substrates were used for this study (Figure 2.2) with an area representing ¾ of each substrate designated for trace DNA deposition. DNAaway™ (Thermo Scientific™) was used to clean the handling area and left to dry for a minimum of one hour. Control background swabs were taken for each substrate, using a cotton wet/dry swabbing method, to ascertain the effectiveness of the cleaning process. Samples were deposited by the same eight participants, from Chapter 2 and deposited trace DNA samples during the same session as their fingerprint depositions. All participants provided a buccal sample at time of deposition to be used for DNA profile comparison against amplified trace DNA samples.

3.2.2 Trace DNA deposition

The same procedure for creating groomed and natural depositions in 2.2.2 were deployed for trace DNA depositions which took place directly after fingerprint depositions without any additional loading or handwashing steps. Four handling methods were employed, as described in Table 3.1. For all handling methods participants used both hands in the

process, excluding the “Mechanical” method, where logistics dictated the use of a single hand; in these instances, participants were given the choice of which hand to use. Within 30 minutes of deposition fingerprint powders, the same used to enhance fingerprints in Chapter 2, were applied to the area. This approach was taken to pseudo mimic a standard forensic investigator approach whereby fingerprint powdering has been prioritised over swabbing. Negative control swabs of unhandled, powdered substrates, (*N* = 16) were included. Participants contributed equally to each study treatment and substrate resulting in a total of *N* = 128 trace DNA samples.

Table 3.1: Trace DNA deposition methods employed in this study, comprising of three, light touch, heavy touch, mechanical, drawn from existing literature and one, undirected, novel devised by study investigators.

No.	Deposition method	Verbal instructions for deposition protocol	Reference
1	Light touch	‘Gently’ handle substrate using both hands for ten seconds.	(Richmond, 2004)
2	Heavy touch	‘Firmly’ handle substrate using both hands for ten seconds.	(Richmond, 2004)
3	Mechanical	Grip the substrate allowing enough pliability for the principal investigator (PI) to manipulate the substrate for ten seconds.	(Jasuja et al., 2009)
4	Undirected	Handle for ten seconds. Interpretation of method approach left to participant.	Novel to his study

3.2.3 Swabbing

A wet/dry swabbing protocol described by Hedman et al (Hedman et al., 2021a) was used however to more closely represent UK collection protocols, NaCl, the Swedish standard for moistening, was substituted with pure distilled water at middle limit recommendations for appropriate surface type assignation. Following these protocols for the pine dowel and cardboard tubing (porous substrates) trace DNA was recovered using a cotton head moistened with 100 µl distilled water positioned at 60^{o} to the surface wiped over the surface four times whilst rotating and applying medium to hard pressure. This method was repeated for the polyethylene and stainless tubes (non-porous substrates) but using 50 µl

of distilled water. Focus was given to swabbing areas where fingerprint powdering had highlighted clear evidence of handling.

3.2.4 Extraction and quantification

Wet and dry swabs from the same sample were pooled in a single 2ml microcentrifuge tube. Samples underwent DNA extraction using the QIAamp DNA investigator kit (Qiagen) following the manufacturers recommended protocol for isolation of DNA from surface and buccal samples with low numbers of target molecules and the higher recommended volumes of Buffer AL, ATL, AW1 and AW2 and ethanol used to ensure full immersion of both swabs. As per manufacturer recommendations carrier RNA and QIAshredder spin columns were both used during the extraction process and final trace DNA samples were eluted to 20µl. A negative extraction control was included in each extraction batch. Buccal swabs from participants were extracted using the same kit following the manufacturers recommended protocol without modifications for low DNA quantities. All samples underwent DNA quantification using the Qiagen Investigator Quantiplex Pro RGQ Kit (Qiagen) (10µl reaction volume) following manufacturers recommended protocol using a Rotor-Gene® Q 5-Plex HRM (Qiagen). This qPCR-based assay targets both small and large autosomal and Y-chromosomal loci to produce quantification results for both total human and male DNA. DNA degradation is assessed via the human and male degradation indices, calculated via the ratio of small to large amplicons. The kit includes an internal control to detect potential PCR inhibition. The Rotor-Gene® Q is validated for use with reaction volumes between 10 µl - 25 µl and the decision was made to go with the lowest volume threshold due to limited reagent availability and a desire for a high-throughput setup. Samples were randomly distributed between plates and across runs to ensure any resulting

observations could be reliably interpreted. results were analysed with Qiagen Q-Rex software.

3.2.5 STR profiling

STR amplification was carried out at as per manufacturer recommended guidelines using the Qiagen Investigator 24plex QS Kit (25µl reaction volumes, 30 cycles) on a SeqStudio™ Genetic Analyzer Instrument (applied BioSystems) with default fragment analysis and injection settings. The investigator 24plex QS kit amplifies 22 autosomal STR markers including the 20 core CODIS loci and two additional forensic markers, SE33 and DYS391, and Amelogenin for sex typing. The kit further includes two internal quality sensors (QS) that can be used to detect inhibition and degradation in each reaction. Amplification was carried out on all samples that had a concentration greater than the laboratory defined limit of detection (LOD) (200pg total input) which equates to ~13.33pg/µl when 15µl DNA was added to the STR reaction (N = 6). Where samples did not meet these criteria the next (N = 6) highest concentrations were chosen to ensure an equitable representation of groomed and natural depositions (see Table 3.4).

Profiles were analysed using GeneMapper ID-X and EuroForMix 4.0 (Bleka et al., 2016) software. A 30 RFU threshold was applied for allele calling and 15% for stutter detection. Minimum number of contributors (MNOC) was estimated by the maximum number of alleles at any given locus. Likelihood ratios (LRs) for each person of interest (POI) were calculated in EuroForMix using parameters set by the in-built “Optimal quantitative LR” (automatic model search) function. LRs were converted to verbal qualifiers for ease of reporting using the scale produced by the European Network of Forensic Science Institutes (Willis et al., 2015) Propositions followed recommendations by Buckleton et al. (Buckleton et al., 2014) and are outlined in Table 3.2.

Table 3.2. Propositions taken into consideration in LR calculations, representing the positions of the prosecution (H_p) and defence (H_d).

Hypothesis	Description
H_p (Prosecution)	The DNA originated from the POI (original depositing volunteer) and $N-1$ unknown contributors.
H_d (Defence)	The DNA originated from N unknown contributors.

3.2.6 Statistical analysis

All samples (*N* = 128) were successfully quantified, a Shapiro-Wilk test was performed and showed that the distribution of DNA concentrations departed significantly from normality (W = 0.5145, p-value < 0.001). Based on this median and interquartile range (IQR) were used to summarise variables and non-parametric tests were used to assess relationships between variables. Kruskal Wallis tests were used to compare DNA concentrations against the following categorical variables, deposition method, volunteer, and substrate. Wilcoxon signed rank tests were used to compare DNA concentrations recovered dependent on pre-deposition activities (i.e. presence or absence of grooming).

3.3 Results

3.3.1 Exploratory results

Across all samples, a median DNA concentration value of 0.63pg/µl and IQR of 1.87 pg/µl was recovered (Table 3.3). Visualisation of the data showed a high variance across DNA concentrations deposited by each volunteer (Figure 3.1). A Kruskal Wallis test identified a significant relationship between volunteer and deposited DNA concentration (H (7) = 32.77, *p-value* = 2.92*e* – 05). A follow up post hoc analysis identified volunteer 1 as depositing a significantly different DNA concentration comparable to volunteers 3 ($p < 0.05$), 4 ($p < 0.01$), 5 ($p < 0.01$) and 7 ($p < 0.01$). All standard curves for quantification target regions (male, human, male degradation, and human degradation) showed a good linear relationship ($R^2 > 0.99$) between quantification cycle (cq) values and concentrations. PCR efficiency consistently sat within the acceptable 90% – 110% range.

Table 3.3. Mean and standard deviation ($\bar{x}$), median (M), and interquartile range (IQR) of DNA concentration (pg/µl) recovered across various influencing variables, including deposition method (N = 32 per method type), preparation (N = 64 per preparation type), porosity (N = 64 per porosity type), substrate type (N = 32 per substrate type) and all samples (N = 128).

Influencing variable	**DNA concentration pg/µl**		
	$\bar{x}$	**M**	**IQR**
Deposition method			
Heavy touch	3.22 ± 5.5	1.61	2.53
Light touch	1.60 ± 4.4	0.56	0.93
Mechanical	2.12 ± 3.4	0.78	1.77
Undirected	2.10 ± 4.3	0.52	0.68
Preparation			
Groomed	2.63 ± 4.6	0.82	2.37
Natural	1.89 ± 4.4	0.51	1.48
Porosity			
Porous	3.15 ± 5.5	1.04	2.31
Non-porous	1.37± 2.8	0.44	0.92
Substrate			
Wood	3.80 ± 6.2	1.99	2.58
Cardboard	2.50 ± 4.8	0.78	1.74
Plastic	1.26 ± 2.5	0.31	0.92
Metal	1.50 ± 3.2	0.50	1.02
All samples	2.26 ± 4.6	0.63	1.87

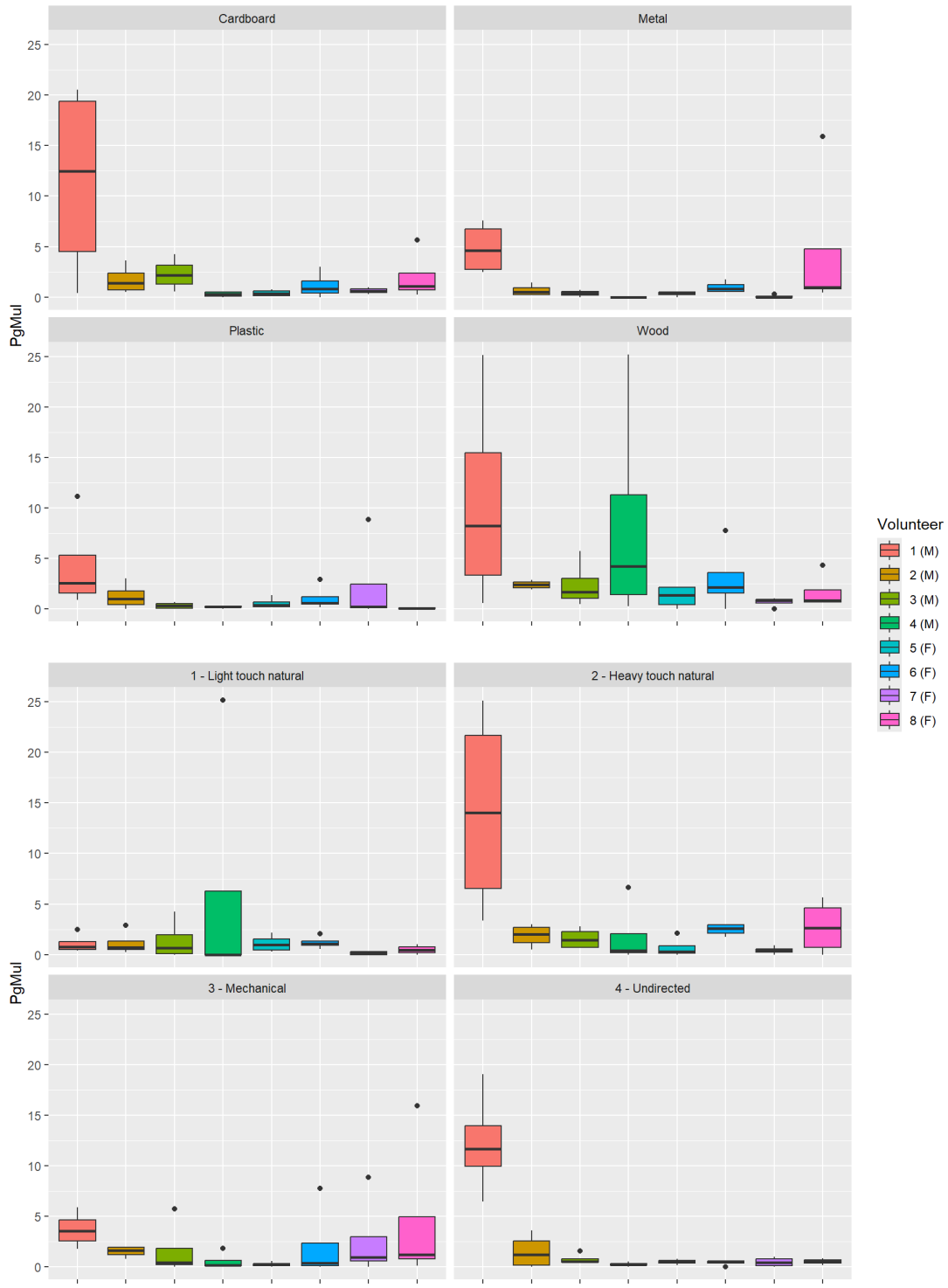

Figure 3.1. Boxplots showing distribution of DNA concentrations (pg/µl) recovered from depositions forall eight participants under different depositing conditions. . (M) = assigned male at birth, (F) = assigned female at birth. Outliers are shown as individual points.

3.3.1 Deposition method

The heavy touch method of deposition resulted in the highest median quantity of trace DNA recovered across substrates at 1.61 pg/µl, as well as the highest variability with an IQR of 2.53 pg/µl. This was followed in descending order by Mechanical, Undirected, and Light touch (Table 3.3). The results of Kruskal Wallis tests revealed no significant differences in the rank totals of deposition methods (H (3) = 5.6073, p-*value* = 0.13) (Figure 3.2). Repeated tests on porous and non-porous sample sets presented the same order of method efficacy in relation to quantity to of DNA recovered, but to no significant degree of difference.

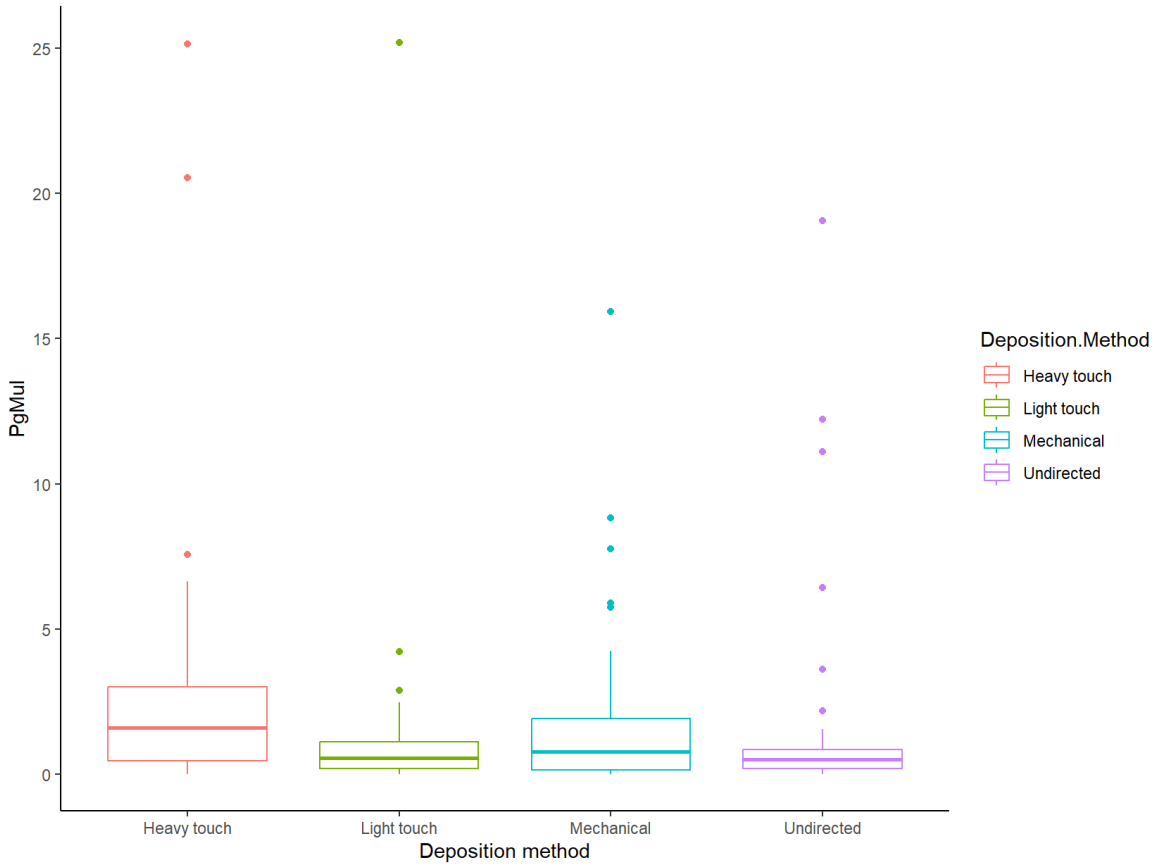

Figure 3.2. Boxplot showing distribution of DNA concentration (pg/µl) for each deposition method (heavy touch, light touch, mechanical and undirected). Outliers are shown as individual data points.

3.3.2 Preparation

Grooming prior to deposition resulted in a higher median concentration of DNA depositions 0.82 pg/µl comparable to non-preparation (natural) depositions 0.51pg/µl. A Wilcox Signed

Rank test showed a significant relationship between recovered DNA concentrations and preparation activity (W = 2534, z = -2.1, p < 0.05) (Figure 3.3).

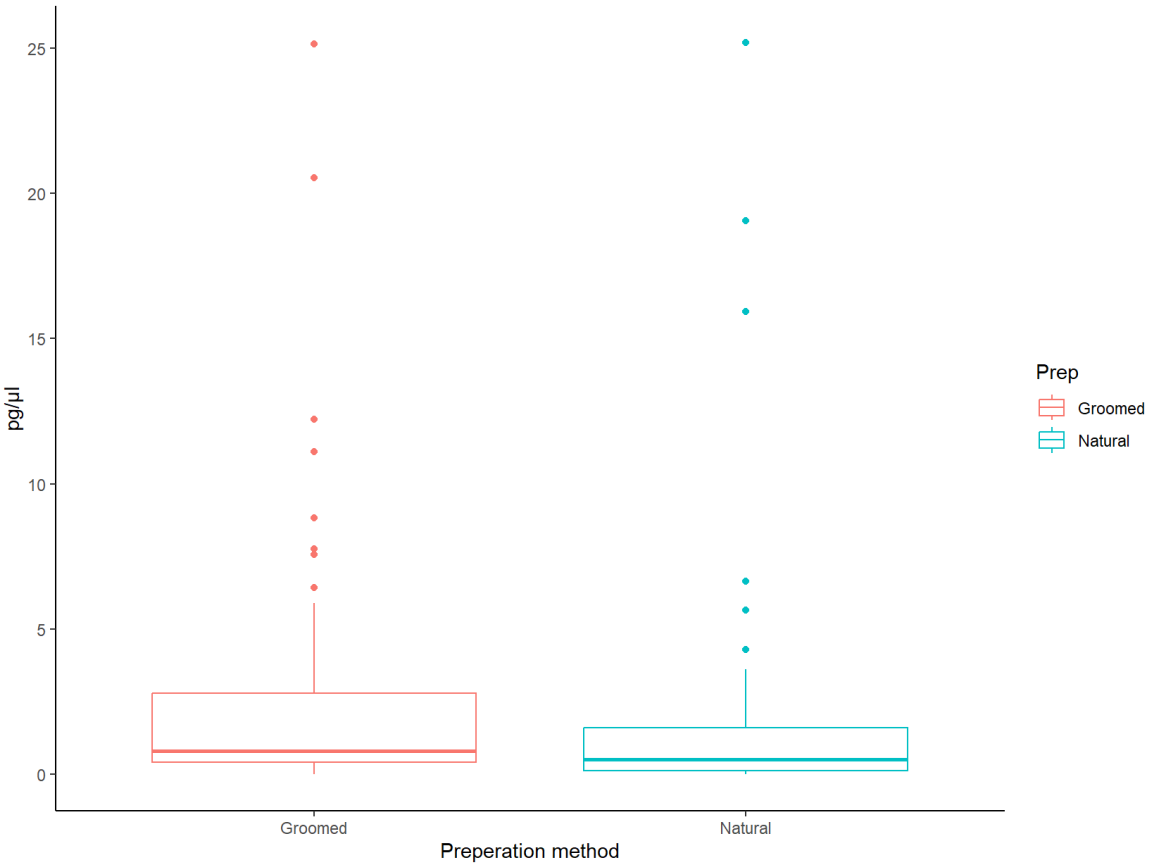

Figure 3.3. Boxplot showing distribution of DNA concentration (pg/µl) for each preparation method (groomed and natural). Outliers are shown as individual data points.

3.3.3 Substrate

The highest median DNA concentration was recovered from wood (1.99pg/µl), followed in descending order by cardboard (0.78 pg/µl), metal, (0.50pg/µl) and plastic 0.31pg/µl (Figure 3.4). A Kruskal Wallis test found a significant association between substrate and recovered DNA concentration (H (3) = 16.43, *p-value* = 0.001). A post hoc analysis indicated significantly higher concentrations of DNA were recovered from wood comparable to plastic ($p < 0.01$) and metal ($p < 0.01$). A Wilcox signed rank test indicated porosity also had a significant relationship (W = 1271, z = -3.7, $p < 0.001$) with DNA concentration pg/µl. The median recovered DNA concentration from porous items was 1.04 pg/µl and from non-porous 0.44pg/µl.

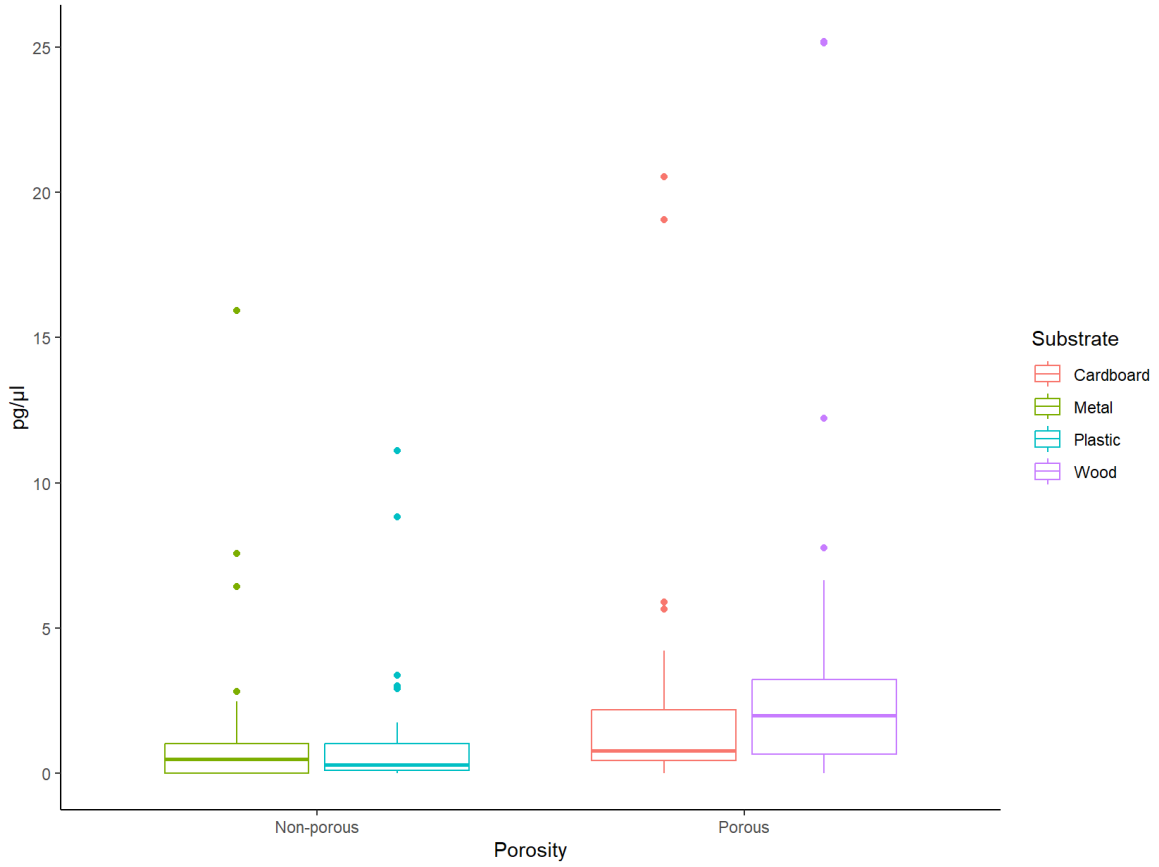

Figure 3.4. Boxplot showing distribution of DNA concentration (pg/µl) for non-porous (metal and plastic) and porous (cardboard and wood) surface types. Outliers are shown as individual data points.

3.3.4 Interactions

No combination of deposition method and preparation activity was found to deposit significantly higher quantities of DNA compared to others (H (7) = 13.458, *p-value* = 0.06) within the collated samples.

3.3.5 PCR Amplification

Out of ($N = 12$) samples taken forward for profiling (N = *4*), resulted in failed PCR's with an average DNA concentration of 3.63 $\pm$ 1.52 pg/µl. The average DNA concentration of successfully amplified samples was 16.27 $\pm$ 8.09 pg/µl. Analysis of peak height ratios of the internal PCR controls quality sensors (QS) indicated no occurrence of PCR inhibition within amplified samples (Figure 3.5).

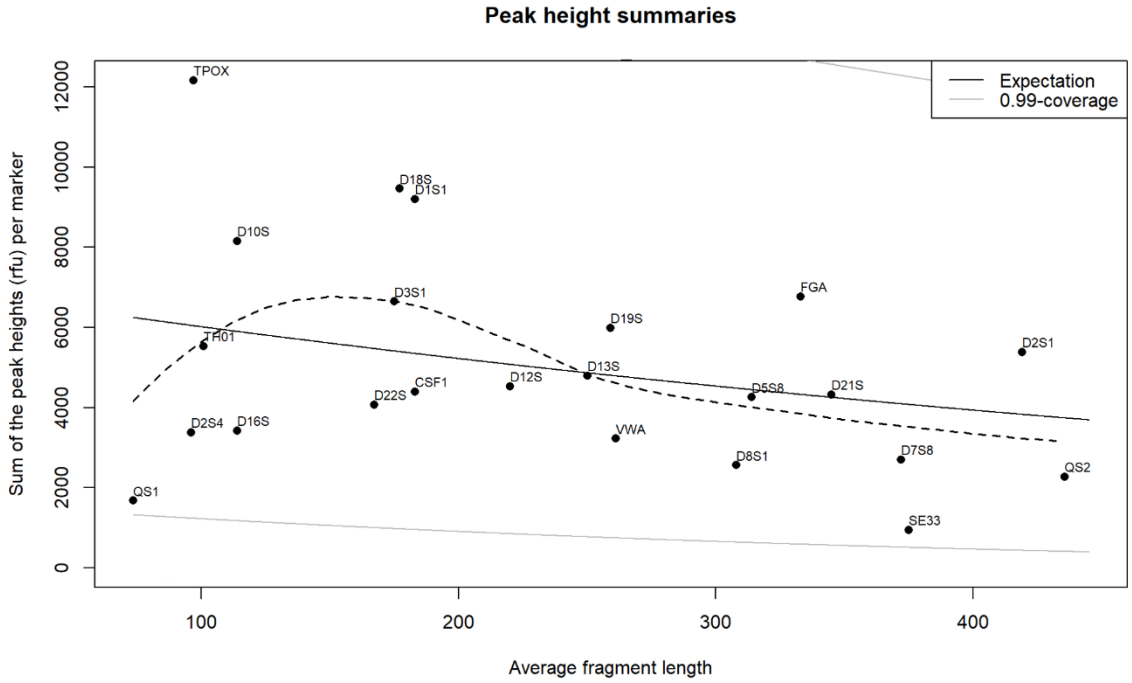

Figure 3.5. Scatter graph of sum of peak heights produced at each marker in relative fluorescence units (RFU) plotted against the average fragment length of marker. The presence and height balance of QS1 and QS2 markers suggest the absence of inhibitors during PCR. The downward trend in peak heights with increasing fragment length indicates the presence of degradation within the sample.

Using the automatic model search function in EuroForMix four of the eight amplified samples were identified as requiring degradation to be factored into the best fit models. Presence of degradation was further confirmed via the presence of a downward trend in peak heights with increasing allele fragment length (Figure 3.5). Calculations of LR by EuroForMix produced five samples with an LR equivalent to a qualifying statement of "Very strong evidence" in support of Hp (Figure 3.6). Of these four were produced using groomed preparations (Table 3.4). Match rates were calculated against all other volunteers for samples where an "Uninformative" LR was achieved against their expected volunteer match. Volunteers with the highest match rate were subbed in as POI to the H_p hypothesis to identify any instances of cross contamination.

The same process was repeated subbing in the PI's profile as POI. Within sample 89 replacing the expected volunteer (7) with volunteer 8 resulted in a qualifier of "Limited

support" (log10(LR) = 1.236) for the H_p hypothesis. Within sample 131 replacing the expected volunteer (8) with the PI's profile resulted in a qualified of "Very strong evidence" (log10(LR) = 22.9) to support the H_p hypothesis. Taking into consideration sample 131 possessed a minimum number of contributors (MNOC) equivalent to two individuals a further H_p hypothesis was run with both PI and Volunteer 8 as POI's and no unknown contributors. A resulting qualifier of "Very strong evidence" (log10(LR) = 21.43) was produced in this iteration of the model indicating contamination by the PI within the sample at some point during the experimental workflow (Figure 3.7). Sample 79 recovered the largest overall quantity of DNA across all samples but only produced a 0.27 match rate with the relevant participant and an "Uninformative" LR qualifier. Analysis was run individually subbing in all eight participant profiles and the PI's profile. None were found to produce a LR above an "Uninformative" qualifier.

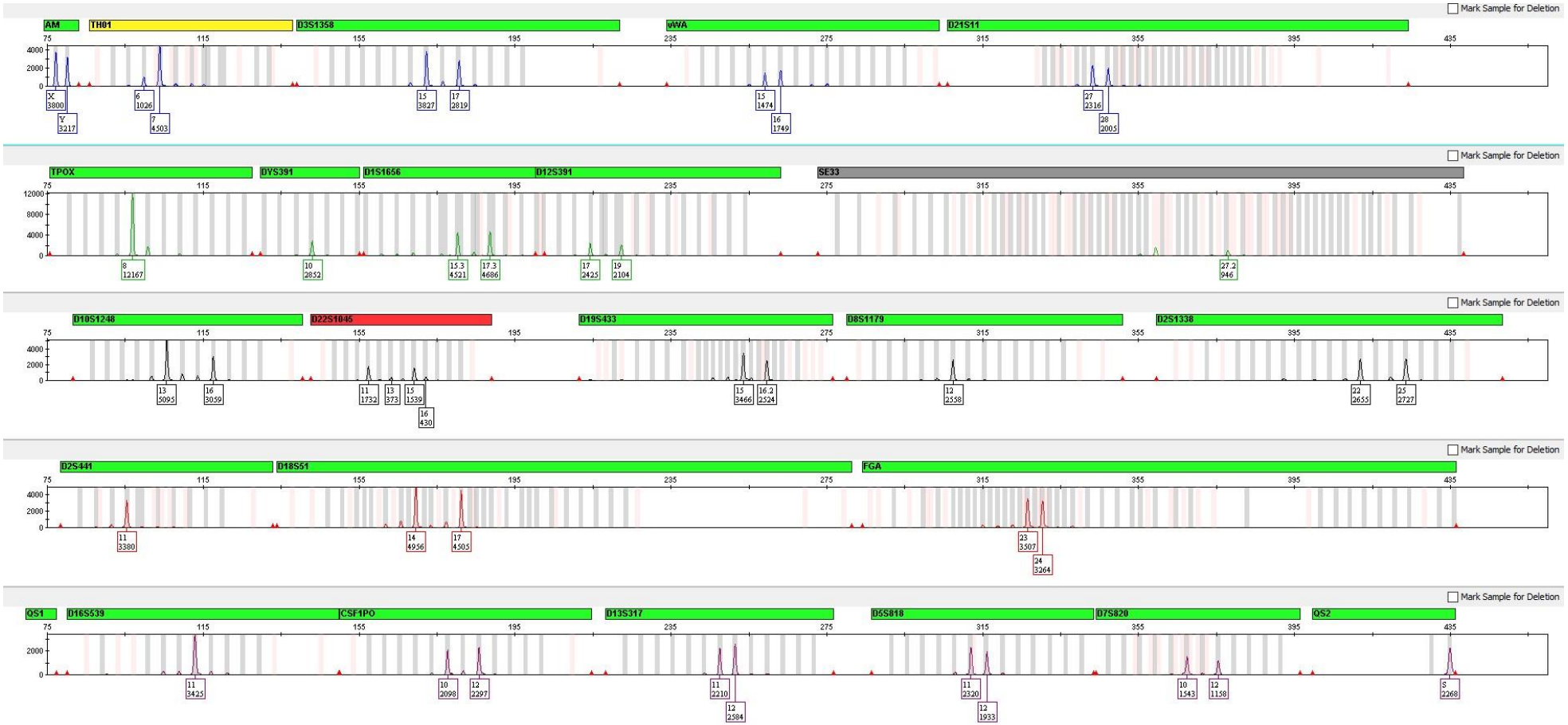

Figure 3.6. Electropherogram of sample 84 which produced a 0.98 match rate and descriptive LR qualifier of "very strong evidence" in support of the H_p proposition: The DNA originated from the POI (original depositing volunteer) and $N-1$ unknown contributors via analysis through EuroForMix software. Yellow highlighted marker headers are indicative of a genotype quality that sits within the "passing" and "low quality" range, red highlighted headers indicate a genotype quality within the low range. Grey marker headers indicate manual editing; in the instance of the presented sample this is a result of removing an off-ladder peak from the dataset.

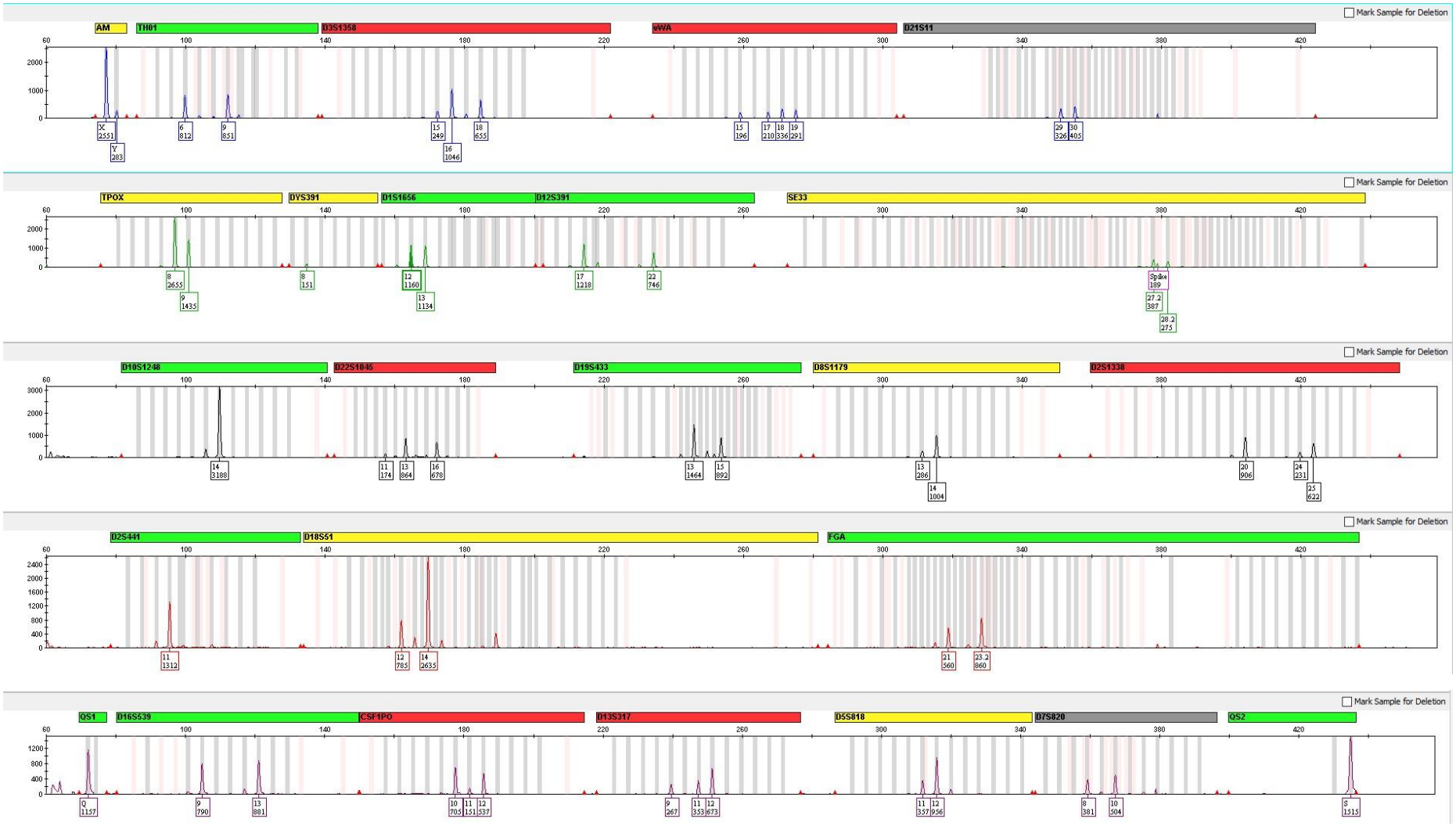

Figure 3.7. Electropherogram of sample 131 demonstrating a mixed profile as a result of contamination by the PI. Analysis through EuroForMix software produced a 0.48 match rate and descriptive LR qualifier of "Uninformative" in support of the H_p proposition: The DNA originated from the POI (original depositing volunteer) and $N-1$ unknown contributors via analysis through EuroForMix software. Yellow highlighted marker headers are indicative of a genotype quality that sits within the "passing" and "low quality" range, red highlighted headers indicate a genotype quality within the low range. Grey marker headers indicate manual editing; in the instance of the presented sample this is a result of removing off-ladder peaks.

Table 3.4. Characteristics of amplified samples analysed using EuroForMix software including minimum number of contributors (MNOC), match rates and likelihood ratios (Log10(LR) for original depositing volunteers. Descriptive evidence qualifiers adapted from (Buckleton et al., 2020).

							Known contributor as POI H_p		
Volunteer	**Preparation**	**Handling**	**Substrate**	**DNA concentration pg/µl**	**Allele count**	**MNOC**	**Match rate**	**Log10(LR)**	**Evidence qualifier**
7 (89)	Natural	Mechanical	Wood	1.04	17	2	0.08	-13.19	Uninformative
5 (74)	Natural	Light touch	Wood	2.17	Fail	Fail	NA	NA	NA
6 (9)	Groomed	Heavy touch	Cardboard	3.00	Fail	Fail	NA	NA	NA
2 (25)	Natural	Undirected	Cardboard	3.61	Fail	Fail	NA	NA	NA
3 (92)	Groomed	Mechanical	Wood	5.74	Fail	Fail	NA	NA	NA
1 (63)	Groomed	Undirected	Plastic	11.12	40	1	0.98	20.03	Very strong
1 (97)	Groomed	Undirected	Wood	12.23	69	2	1.0	16.99	Very strong
8 (131)	Natural	Mechanical	Metal	15.92	60	2	0.48	-1.633	Uninformative
1 (27)	Natural	Undirected	Cardboard	19.04	40	1	1.0	25.96	Very strong
1 (13)	Groomed	Heavy touch	Cardboard	20.52	29	1	0.94	15.76	Very strong
1 (84)	Groomed	Heavy touch	Wood	25.13	42	2	0.98	20.99	Very strong
4 (79)	Natural	Light-touch	Wood	25.18	39	1	0.27	-76.34	Uninformative

3.4 Discussion

Recovery and matching of DNA profiles, deposited by a POI via direct transfer, is an important part of a forensic practitioners investigative arsenal. Understanding factors influencing DNA-TPPR aids in the development of research studies looking to identify best practice recovery methods, particularly when introducing novel surfaces or evidence types. In this chapter the influence that handling technique, pre-deposition grooming activities, and surface type have on quantity and quality of DNA depositions was investigated.

As addressed in the introduction to this chapter studies will often assess an individual's shedder status prior to their inclusion in a study. This may be done to ensure a range of individuals are included, in an attempt to guarantee sufficient quantities of DNA are deposited or to provide additional information for DNA transfer probability models (Fonneløp et al., 2017; Tan et al., 2019). The results presented in this chapter indicate that certain individuals can transfer significantly higher amounts of DNA to surfaces under experimental conditions. I have also shown that this higher rate of deposition transcends influencing variables (Figure 3.1) and is not the result of contamination (Table 3.4). However, in the case of these results, since the high performing participant, and to this point all other participants, deposited their individual samples within a single day, the findings only demonstrate that on the day of testing they experienced a set of intrinsic and/or extrinsic circumstances which rendered them as a "good shedder". Beyond this and without further work to see if the results are reproducible, I can at best suggest that there is evidence towards the fact that during experimental research, on the day of deposition one individual may significantly outperform other contributors and present as a "good shedder".

Although the heavy touch method resulted in the highest recovered DNA concentrations it did so with considerable variance. By contrast the light touch and undirected methods,

despite resulting in lower DNA concentrations, showed a lower variance across volunteers. Of further interest is that the light touch method bought participant one's (identified as a "good shedder" in this study), deposited DNA quantities more closely in line with the rest of the participants. This is despite results showing their persistence in high deposition rates across other factor combinations. A possible explanation for this comes from the knowledge that a significant proportion of DNA is deposited at initial point of contact (Sessa et al., 2019). Under an instruction of handling with a light touch it may be assumed that minimal friction and pressure is being exerted by participants during handling, factors found to have a significant influence on trace DNA transfer (Tobias et al., 2017; Hefetz et al., 2019). If in this instance the "good shedder" is by nature already "heavy handed" and prone to exerting higher pressure and as a result rates of friction, than their peers, then an instruction to handle "lightly" could have helped control for this behaviour. This theory is supported when looking at the undirected depositions, which can in some way be considered a control for handling technique. The "good shedder" produced significantly higher quantities of DNA using this technique and the ability to reduce this through instruction to handle "lightly" provides support to the theory it is as a result of a natural tendency to handle objects with greater force than their peers. This suggests that even subjective instructions may be beneficial in helping to standardise participant handling technique and as such deposition rates. In this study the mechanical method exerted control of the movement of the substrate in the participants hand to the PI. This required participants to hold the substrate with a loose enough grip to allow the PI to manually manipulate it in their hand, again leading to an assumption of a lower exertion of pressure by the participant. However, the mechanical method displayed the second highest level of variance amongst depositions and more importantly was the single incident of known contamination by the PI within profiled samples. Therefore, the mechanical method may

be introducing a higher risk of contamination due to the involved nature of the technique. Despite the observed differences, the lack of significant difference found between deposition methods in this study is encouraging in the context of experimental design for Chapter 5 of this thesis. In this study uniform shapes for each substrate were used to help standardise the application of technique interpretation by each volunteer. However, the wildlife items that will be presented for handling in Chapter 5 will differ significantly in shape, size, weight, and texture. As such it is expected there will be a degree of variability in the way they handled, even if provided with instruction, depending on an individual's familiarity with the item and growth in confidence as they handle items in turn.

An observation of greater concern are the low quantities of trace DNA recovered during this study, consistently achieving mean quantities lower than seen in similar studies which also employed hand-washing, short DNA reconstitution periods, and grooming steps (Alketbi and Goodwin, 2019b). Plotted qPCR amplification curves did not exhibit signs of PCR inhibition, and PCR efficiency percentages sat within acceptable thresholds, however the low reaction volume used may have contributed to the observed results. The low input quantity of extracted sample DNA would have introduced significant stochastic effects such as failure to introduce any target DNA into the reaction despite it being present in the extraction elution. This hypothesis could have been tested through technical replicates with larger reaction volumes however resource limitations did not allow for these steps. It is also possible that that low-yields were a result of interference at the extraction stage (Cornwell et al., 2020). Magnetic fingerprint powders, used on wood, cardboard, and plastic substrates, have been shown to severely impact DNA recovery which may provide explanation (Lin et al., 2017). There appears to be no work looking at the impacts of bronze latent fingerprint powders on trace DNA recovery, though aluminium latent fingerprint powder has been shown to impart the least impact (Lin et al., 2017). However recovery of

low quantities of trace DNA, similar to observed in this study, from stainless steel have been reported (Ramsey, 2021). An additional compounding issue may have arisen from the COVID-19 pandemic and as suggested in section 2.4.3 an un-naturally high rate of handwashing and use of hand-sanitiser carried out by participants even outside of the experimental parameters. Fingerprint powders were introduced as part of the trace DNA recovery process in this study to mimic the standard protocol of prioritising fingerprints over DNA recovery at a crime scene.

Although the results have shown that trace DNA is recoverable in quantities and of quality to make it of use for forensic casework, even after treatment with fingerprint powders, the low quantities recovered cannot be ignored. Therefore, going forward for Chapter 5 of this thesis fingerprint powders will not be introduced as part of the experimental design, and, resource allowing, the recommended reaction volume of 20µl will be used in the quantification protocol.

Results showed that swabs of groomed depositions collected significantly higher quantities of trace DNA comparable to natural depositions. As experimental design dictated participants could not self-touch between handwashing and natural depositions the absence of significant depletion of sebaceous secretions on the hands can be assumed. Therefore, these observations draw strength to the suggestions in the literature that sebaceous secretions may play a role in facilitating the transfer of trace DNA (Subhani et al., 2019; Jansson et al., 2022). This is further strengthened by the outcomes of DNA profile analysis in this study, whereby for samples originating from natural depositions that met the defined LOD, alleles were identified as coming from either contamination by the PI or unknown contributors, possibly because of secondary DNA transfer. The only natural deposition sample that showed exception was attributed to the “good shedder” identified

in this study, already known to deposit significantly higher quantities of DNA. By comparison alleles recovered from groomed depositions could routinely be analysed to produce LR's which supported the H_p proposition that the DNA sample originated from the POI (original depositing participant) suggesting there is an important role self-touching plays in direct DNA transfer. Therefore, groomed depositions may be a favourable approach in research looking at direct transfer or comparing recovery methods, to both guarantee higher quantities of DNA and likelihood of that DNA originating from the original intended source.

Surface type is considered an important factor when assessing whether and how trace DNA can be recovered in a forensic context (Alketbi, 2018). An in-depth study looking at impact of physicochemical surface properties on identification of biological traces found that rough, hydrophilic surfaces retained more DNA and subsequently yielded more complete profiles (Recipon et al., 2024). Other studies have supported this with greater quantities of useable genetic material being deposited on rough, porous substrates, (Daly et al., 2012; Burrill et al., 2019). The results of this study further support these findings with wooden substrates retaining significantly higher quantities of DNA comparable to metal and plastic surfaces though notably cardboard did not. The significance of wooden substrates lays with the surface type producing a greater degree of friction between hands and surface and subsequently dislodging of biological materials containing DNA. Though cardboard is porous, it is less textured than wood, suggesting that texture (or roughness) may exert a greater influence on trace DNA depositions comparable to porosity.

As shown in this study even under controlled conditions mixed DNA profiles can occur. The alleles that were observed in the highest quantified sample were not confidently associated with any suggested POI in tested propositions and therefore their origins can only be speculated. Control swabs taken post cleaning with DNA AWAY™ returned negative for the

presence of DNA indicating the cleaning process sufficiently removed any background DNA that may have been present. Therefore, as already ascertained through LRs, the PI was an unlikely contributor to the sample, the alleles were likely the result of indirect transfer (van Oorschot et al., 2019). In operational settings trace DNA sampling routinely results in mixed or partial profiles and therefore single source profiles, whilst holding a place in experimental settings, are not always representative of real-world scenarios.

3.5 Summary

The aim of this chapter was to assess the impacts of deposition method and preparation on direct trace DNA transfer, with the intention of taking forward a protocol for use in a later chapter.

Results showed that manner of handling had no significant impact on quantities of trace DNA recovered. However certain techniques showed higher variance in depositions between volunteers comparable to others, with both the light touch and undirected methods resulting in more consistent rates of depositions however in lower quantities. Although generally low DNA quantities were recovered across samples “grooming” or “loading” was found to have a significant impact on quantities of deposited DNA. This has provided further evidence to the literature suggesting sebaceous secretions may play a role in DNA transfer. The impact of substrate type on DNA recovery mirrored results seen elsewhere in the literature and successful extraction, amplification, and profiling were carried out on multiple samples indicating low recovered quantities are unlikely due to poor operator technique.

Based on the findings of this study it is decided to go ahead with the “undirected” deposition technique for trace DNA depositions in Chapter 5, allowing participants to handle the wildlife specimens in whatever manner they feel is appropriate for a set period

of time. This technique has been chosen due to its low variance between participants and as it presents as a closer representation of how a POI may handle the specimens during criminal activity. Although the introduction of a grooming step has been shown to produce higher quantities of deposited DNA it was a result of grooming before every new instance of handling. Logistics surrounding access to specimens are likely to require participants to handle all wildlife specimens in Chapter 5 in a single session. As such there are health and safety concerns surrounding the repeated touching of an individual's face after handling wildlife specimens, particularly where provenance is unknown. Therefore, to overcome potentially low quantities of deposition which may render results uninterpretable, multiple donors will be asked to deposit on each specimen in each session. It has been demonstrated that for a single wildlife seizure a complex supply chain may exist with multiple individuals participating at each point in their movement. Therefore, for the purposes of Chapter 5 of this study, the inclusion of multiple donors over singular donors using grooming will both help increase the likelihood of sufficient DNA quantities being present on the surface and more closely simulate operational encounters. This study has achieved its aim of investigating the impact of deposition method and preparation activity have on trace DNA deposits and identified a suitable protocol to take forward for Chapter 5.

Chapter 4: Recovery of fingermarks from wildlife items: A comparison of low cost, field deployable techniques

4.1 Introduction

With over a century of research behind them fingermarks are one of the most recognisable and repeatedly used pieces of forensic evidence to link a suspect to a crime (Bleay, 2014). Their value is demonstrated by their ongoing contributions to the outcomes of cases and detection of crime, both as an inclusionary and exclusionary tool, including cold cases (Federal Bureau of Investigation, 2024), serious crimes (Forsyth, 2020), volume crime (Bond, 2009) and wildlife crimes (Foreign, Commonwealth and Development Office, 2018). Juries draw more confidence in their verdicts when forensic evidence is included in the decision making process (Ling et al., 2021) and the public perceive fingerprints to be one of the most accurate evidence types (Kaplan et al., 2020). However as a forensic discipline it is not without controversy and has been embroiled in serious instances of miscarriage of justice (Lawson, 2003). A 2009 report by the US National Research Council of the National Academic of Sciences sent ripples through the forensic world when it raised concerns about the scientific rigour behind many non-DNA forensic disciplines, drawing particular criticism on the perceived heavily subjective processes surrounding fingerprinting (National Research Council, 2009). This subsequently led to reviews of the admissibility of fingerprint evidence, the language surrounding the reporting of it and how it should be conveyed in court on a global scale (Campbell, 2011; Champod, 2015). Since then regulations and frameworks such as the EU Council Framework Decision 2009/095/JHA (European Union, 2009) and UK Accreditation of Forensic Service Providers Regulations 2018 (UK Government, 2018) have been implemented requiring forensic providers to undergo accreditation at the ISO/IEC 17025 level to demand some level of standardisation in examination of DNA and dactyloscopic data. To carry out fingermark analysis examiners

exploit the finite number of characteristics all fingerprints are made up of. These have been categorised in three levels of detail (Figure 4.1); level one detail, pattern type and ridge flow, are superficial and repeat between individuals but can help narrow down a search or aid in quick exclusion. The four basic fingerprint patterns are arch, tent, loop, and whorl though sub-divisions of each pattern type exist. Level three detail, pores, and ridge edge shapes, require high resolution images to clearly observe and rely on deposited marks to be devoid of smudging or smearing and present a strong contrast between print and background. Given this are not routinely used in fingermark comparison.

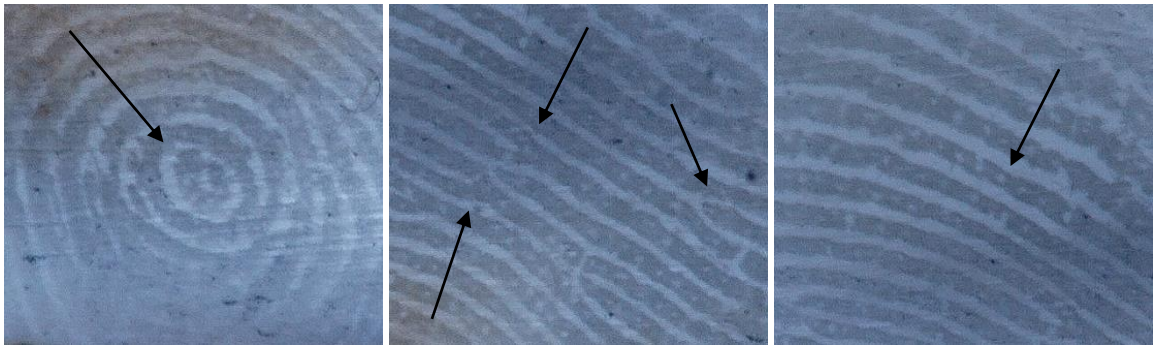

Figure 4.1: Fingermark enhanced using Supranano™ magnetic black powder on a hippo tusk, depicting left to right; level 1 detailing of a whorl pattern, level 2 detailing of bifurcations and ridge ending and level 3 detailing of pores.

It is level two detail, ridge characteristics known as minutiae, which is most exploited by law enforcement for identification purposes. Much like river systems fingermark ridges are not a single continuous flow, they can be interrupted, split apart, or be isolated. The six ridge characteristics observed at the level two detail in fingermarks are ridge endings, bifurcations, lakes, spurs, independent ridges, or crossovers. Their unique number, relative positions to each other, the core (centre) of the mark and the delta (a triangular shaped region of ridges demarking a change in direction) are what fingerprint examiners scrutinise and compare when analysing fingermark images.

Despite the ongoing concerns raised as to their reliability (Campbell, 2011; Bitzer et al., 2019), to date two identical fingerprints from different individuals have never been recorded. Efforts have been made to ascertain the reliability of these assumption, and recent research using the powerful pattern recognition capability of artificial intelligence has found it was not possible to predict intra-person relatedness using level 2 details, the main exploited by fingermark experts, (ridge endings and bifurcations) although it is possible to predict intra-person relatedness using level 1 (pattern type and ridge flow) detailing (Guo et al., 2024). These discoveries lend credence to and reinforce reliability of using level 2 detailing for individual identification purposes as its uniqueness extends to both intra and inter-person comparisons. Not only this the ability for intra-person relatedness using level one detailing broadens the possibilities of linking crime scenes through latent marks from different fingers of the same individual, previously an impossible task. All these factors suggest the routine inclusion of fingerprint evidence into casework is a worthwhile endeavour, yet it is not being applied as standard to wildlife crime investigations where I see chronically low prosecution and conviction rates (Thomas et al., 2023).

Latent marks, those invisible to the naked eye, are the most encountered fingermark evidence. To maximise their benefits as a tool of identification chosen enhancement methods should result in the greatest degree of contrast and clarity between the visualised ridge detailing and the background it has been deposited on. To achieve this enhancement techniques should ideally only target fingermarks constituents and not react with background substrates or vice versa. The chemical composition of fingermarks is complex differing within and between individuals at any given point in time depending on a host of both endogenous and exogenous factors. Three possible biological secretions are proven to contribute to the makeup of fingermark residue, these are, in order of abundance, eccrine,

sebaceous, and apocrine sweat (Bleay et al., 2021). Within these three secretions a range of organic and inorganic components, including water, amino acids, proteins, salts, sebum comprising of fatty acids, squalene and wax esters, have been identified and exploited by both physical and chemical fingermark enhancement processes (Girod et al., 2012).

In the UK current recommended workflows for appropriate choice fingermark enhancement methods are presented in the Fingermark Visualisation Manual, collated and published by the Home Office in conjunction with the Forensic Science Regulation Unit, the College of Policing, and the National Crime Agency, the most recent edition having been published in 2022 (Home Office, 2022). They use porosity as the leading influencing factor affecting process selection. As per their recommendations on all surface types (non-porous, semi-porous, porous) practitioners should first carry out optical non-invasive inspections using appropriately positioned light sources within the visible and UV spectrum in combination with filters where needed. Post this initial inspection process recommendations deviate dependent on the surface porosity. On non-porous substrates the most effective recommended sequence is VMD, powders, powder suspensions and finally lipid dyes. On semi-porous surfaces it is black magnetic powder, followed by either VMD, superglue fuming (plus enhancement) or powder suspensions, then indandione, ninhydrin, and finally PD (plus enhancement). The powder suspension workflow skips both indandione and ninhydrin phases due to it involving wetting the surface rendering them inoperable. Ninhydrin is globally a first choice enhancement method for marks on porous substrates (Zampa et al., 2020) and was the Home Office's recommended process until superseded by indandione in 2022, PD (plus enhancement) is recommended as the final step. Magnetic fingerprint powders are included in suggested porous workflows on untreated wood or wood treated with oils (Home Office, 2022). A host of other enhancement techniques not referenced in these workflows are also available including

SPR, single and multi-metal deposition, leuco crystal violet (LCV) and iodine fuming. Despite all the options that have become available to examiners over the years, fingerprint powders, ninhydrin and superglue fuming remain the three techniques most used in situ (Gomes et al., 2023).

In serious and volume crimes, exhibit types encountered vary but will include items or locations that are likely to have been handled in the context of the crime or circumstantially by suspects or victims. Examples include weapons, documents, entry and exit points, drinking glasses, or food and drink containers. In a wildlife crime exhibits may include traps, cages, poisons, weapons, and importantly the wildlife itself. Regardless of the surface in question examiners must strip back to the basic questions posed to all pieces of evidence encountered. At a crime scene level these may include; any known context of sequence of events, which exhibits are likely to provide the most information, which forensic evidence type to prioritise if certain techniques will compromise others, the perishable nature of the material, health and safety and the logistics of handling, transport and storage if laboratory techniques are to be utilised (European Network of Forensic Science Institutes, 2021). At an exhibit level considerations' will include: the porosity, texture, colour, and material of the item and precursory or imminent environmental exposure.

Wildlife specimens present as an oddity for application of fingermark enhancement techniques. This is not only due to the irregularity in which they will be encountered by the wider law enforcement work force but by the lack of comparable substrates where recommendations on best practice approaches to fingermark recovery are available. Leather is the only animal product consistently represented in the literature and considered a "low yield" surface type; minor success has been found enhancing marks on leather with superglue fuming, iodine fuming (Downham et al., 2015; Zheng et al., 2017), powder

suspensions (Fairley et al., 2012) and less traditional methods including marble slurry powder and plant derivatives (Vadivel et al., 2021) but these methods rarely achieve high rates of ridge detailing. Without comparable references a wildlife specimen exhibit must be systemically assessed as any other evidence type would be. Assuming a porous nature to the wildlife specimen lab based chemical processes indandione and ninhydrin would be recommended best practice. However, both methods work through reaction with amino acids present in fingermarks resulting in a coloured product that renders ridge detailing a pink/purple colour, known as Joullié's Pink (indandione) or Ruhemann's purple (ninhydrin). Amino acids are the building blocks of protein, and as such amino acid reagents are unreliable for use on organic materials where amino acids are abundant. This renders them unsuitable for use on wildlife derivatives and this was proven by Otis et al (Otis and Downing, 1994) who saw the entire surface of antlers reacting when treated within ninhydrin. This leaves examiners with two choices, either magnetic powders followed by PD (and enhancement) or PD alone depending on whether the exhibit holds similar characteristics to untreated wood or not. Secondly the wildlife specimen could be approached as a semi-porous surface (as is the case of leather). In this scenario the examiners first step, post optical processes, would be the use of magnetic powders. As discussed in Chapter one fingermark recovery from wildlife derivatives and carcasses has been proven using several of these methods (Table 1.3). Within their results two techniques repeatedly demonstrated high efficacy, these were magnetic powders (Azoury et al., 2001; Czarnecki, 2002; Eveleigh, 2009; Darby et al., 2015; McMorris et al., 2015, 2019; Weston-Ford et al., 2016) and the use of fluorescence (Otis and Downing, 1994; Eveleigh, 2009; Darby et al., 2015; McMorris et al., 2015, 2019). Given the representation of magnetic powders in iterations of both porous and semi-porous recommended workflows, in positive outcomes across related research streams and the proven effectiveness and inclusion in

existing wildlife forensic “toolkits” of Supranano™ black magnetic fingerprint powders on both cardboard and untreated pine in Chapter 2 of this thesis, fluorescent and non-fluorescent Supranano™ magnetic powders (SMP) have been chosen as techniques for comparison.

Where existing studies chose to focus on either a single species or derivatives from within a related group the aim of this study is to establish if any single technique demonstrates a degree of useability across multiple taxa rendering it a more cost effective and transferable method across a global stage. To this point gelatin lifters have also been chosen as a recovery/enhancement method for comparison given their proven success in recovering fingermarks from pangolins (Moorat et al., 2020) and arguments as to their potential suitability as a forensic tool in challenging conditions presented by international wildlife crime cases (Mayer, 2019).

4.2 Materials and Methods

4.2.1 Identification and preparation of specimens

Earlier studies in this area of work have focused on a specific taxon making their global applicability limited. Wildlife seizures often contain mixed shipments of various wildlife species as well as other types of illegal goods (FATF, 2020; van Uhm et al., 2021), with speculation that criminal syndicates involved in wildlife crime are diversifying their acquisitions, potentially, and ironically in response to their own activities limiting access to certain species. Trialling techniques on multiple taxa was a key step in this research as a key aim was identifying a technique that can be applicable to multiple streams of casework maximising its potential use. As well as variety, inclusion of taxa known to be targeted within the breadth of wildlife crime was attempted to offer greatest opportunity for translation of results into real world scenarios. As such the wildlife specimens used in this study were

sourced from collections held at the Institute of Zoology (IOZ), Zoological Society of London (ZSL) where confidence in their authenticity as genuine wildlife goods could be ascertained. In addition, several of the specimens held at IOZ originate from the Metropolitan Police Service wildlife crime unit storage facility, and as such represent examples of real evidence seized in wildlife crime investigations in London.

A total of *N = 12* specimens (Figure 4.2) were included in this study, comprising of six derivative types (tooth, skin, bone, claw, horn, and shell), originating from twelve different taxa (snake, tortoise, tiger, gorilla, elephant, deer, bovine, hippo, antelope, sawfish, and conch). As well as being grouped by derivative type (Table 4.1) specimens were assigned as either textured (snakeskin, elephant skin, ungulate skin, antler, conch shell and tortoise shell) or smooth (elephant ivory, hippo ivory, sawfish rostrum, tiger claw, bovine horn, and gorilla skull).

To remove any existing fingermarks each specimen was wiped down using mild detergent followed by a second dry tissue to soak up any excess liquid and then left to air dry for at least one hour. Specimens were then inspected using a handheld 365nm UV wavelength torch to confirm there were no existing fluorescent properties within the specimens themselves that may affect subsequent fingermark enhancement.

a)

b)

c)

d)

e)

f)

g)

h)

i)

j)

k)

l)

Figure 4.2. Photographs of whole specimens used in this study inlaid with macro images depicting surface texture: a) juvenile gorilla (Gorilla gorilla gorilla) skull, b) red deer antler (Cervus elepahus), c) bovine horn (sp. Unknown) d) mounted tiger (Panthera tigris) claw, e) tortoise (sp unknown) shell modified into guitar, f) snake (sp unknown) skin, g) sawfish (Pristidae sp) rostrum, h) hippo (Hippopotamus amphibius) tusk, i) elephant (sp unknown) tusk j) conch (Strombus sp) shell k) elephant (sp unknown) skin l) antelope (sp unknown) skin.

4.2.2 Deposition of fingermarks

Ethical approval was granted from LJMU's Research Ethics Committee (Approval reference [21/PBS/004]) prior to recruitment of any volunteer donors. Twenty donors of unknown

shedding status were recruited through invitation emails circulated to internal ZSL mailing lists. On arrival donors were given a brief reminder of the background of the research and the study design they would be taking part in that day. Donors deposited fingermarks using a combination of the “grooming” and “undirected” techniques devised in Chapter 2 of this paper and found to be a practical and effective method of depositing fingermarks on multiple surface texture types. Grooming was achieved by donors rubbing their fingertips over the bridge of their nose, forehead and back of their neck and then rubbing fingertips together to evenly disperse any collected secretions. Donors were then asked to press their fingertip onto the specimen for two seconds using either their forefinger, middle finger, or thumb on either their dominant or non-dominant hand.

The order in which donors deposited on specimens as well as the digit and hand used was randomised across all samples Each donor deposited one fingermark per enhancement method for each specimen resulting in a total of $N = 48$ depositions by each donor, a total of $N = 80$ depositions per specimen, and a collective total of $N = 960$ fingermark deposits in this study. To avoid risk of constituent depletion, donors deposited all 48 fingermarks within the course of a day with a maximum of 6 fingermarks, one per designated digit on each hand, deposited in a single session. Post deposition and prior to any enhancement the 365nm light source was used to inspect for the presence of fluorescent contaminants in deposited untreated fingermarks.

4.2.3 Enhancement and recovery method choice and application

Three enhancement methods were compared, SceneSafe™ red and yellow, fluorescent SMP (all specimens), SceneSafe™ black (elephant ivory, hippo tusk, antler, conch, claw, tortoise shell, skull, antelope skin, bovine horn) or white SMP (elephant skin, sawfish rostrum, snakeskin), and SceneSafe™ BVDA polyester backed black gelatin lifters (all specimens).

Black or white coloured fingerprint powder choice was dictated by highest contrast afforded compared to background surface colour and established in proof-of-concept experiments. All fingerprint powders were applied using a magnetic wand held perpendicular to the surface and moved in a circular motion. Once the mark was considered sufficiently enhanced the powder was returned to the pot and the empty magnetic wand used to collect any excess powder deposited on the surface. Gellifters were cut to a size of $3cm^2$ and applied in accordance with BVDA recommended lifting protocols for fingermarks (BVDA, 2024). All fingermarks were enhanced within 30 minutes of deposition.

4.2.4 Photography of fingermarks

Enhanced fingermarks were photographed in a dark room environment, using a tripod mounted Sony DSLR A850 with Sony 100mm f2.8 macro lens attachment. Specimens were placed on a table and the camera lens positioned parallel to the fingermark with a reference scale in place, a ten second delay was used during each shot to maximise opportunity for camera stabilisation. Marks enhanced using fluorescent fingerprint powders were photographed in four states, twice pre-lifting and twice post-lifting with gelatin lifter; pre lifting i) illuminated by white light ii) excited using a 365nm UV wavelength handheld torch, post-lifting iii) without acetate cover on gelatin lifter illuminated with white light iv) without acetate cover on gelatin lifter excited using a 365nm UV wavelength handheld torch. Fingermarks enhanced using black or white fingerprint powders were photographed in two states one pre-lifting and once post-lifting: pre lifting i) directly on the specimen illuminated by white light and post lifting ii) without acetate cover on the gelatin lifter using white light. Untreated fingermarks recovered using gelatin lifters were photographed in one state i) without acetate cover on the gelatin lifter illuminated by white light. In all photographing scenarios lighting (UV and white) was positioned at a 45^{o} angle or higher to the fingermark

with the aim of finding a point at which the fingermark was fully illuminated but reflections were minimised. Once powdered fingermarks had been photographed, they were lifted using gelatin lifters. All fingermarks were photographed within 1 hour of enhancement and or lifting.

4.2.5 Grading of fingermarks

All photography stages of enhanced/recovered fingermarks were graded, resulting in a maximum of four grades for fingermarks enhanced using magnetic fluorescent fingerprint powders, two grades for marks enhanced using magnetic black or white fingerprint powders and one grade for fingermarks recovered using gelatin lifters. All enhanced/recovered marks were graded using the same Home Office CAST grading system (Sears et al., 2012) within which grades of $\geq$3 are considered identifiable and therefore of forensic interest in the context of identification through fingermark quality. A multi-grading approach was taken to assess any change in perceived grade quality dependent on the stage of enhancement.

4.2.6 Statistical analysis

Repeating protocols outlined in 2.2.5 a Kruskal-Wallis test was used to compare CAST grades recovered by different enhancement methods and on different specimen types. when assessing results across the full CAST grading scheme (0 – 4). In addition, fingermarks grades at differing enhancement states were compared using a Wilcoxon signed rank test. Fishers exact tests were used to measure associations between enhancement methods and CAST grades for each independent and grouped specimen type and a chi-square test used for the same purpose for all pooled samples. Associated standardised residuals for grouped specimen types were subsequently represented through mosaic plots.

4.3 Results

4.3.1 Evaluation of enhancement techniques

4.3.1.1 Claw & Horn

Red fluorescent SMP and Yellow fluorescent SMP recovered the highest number of fingermarks ≥3 for claw (*N* = 7) and horn (*N* = 9) respectively and black gelatin lifters the lowest for both specimen types. For horn red fluorescent SMP was the only enhancement method which resulted in CAST grade 4 marks (Table 4.1). Across both individual and pooled specimen types in this group a Kruskal Walis test found significant association between enhancement method and recovered CAST grade (horn: $x^2 = 22.51$, df = 3, p-value = < 0.001; claw: $x^2 = 16.16$, df = 3, p-value = < 0.01; pooled: $x^2 = 35.60$, df = 3, p-value = < 0.001). Mosaic plots displaying Pearsons residuals show black gelatin lifters produced a higher-than-expected proportion of low CAST grades for both substrates independently and combined (Figure 4.4).

4.3.1.2 Bone

Fingermarks of CAST grade >3 were recovered from 44% of depositions on gorilla skull and 5% of depositions on antler. Red fluorescent SMP resulted in the highest number of >3 CAST grades for both bone specimens (Figure 4.3). Lowest CAST grades were most frequently recovered using black gelatin lifters for both bone specimen types (Table 4.1). A Kruskal Wallis test found a significant association between recovered CAST grade and enhancement method used for independent and pooled samples (antler: $x^2 = 9.21$, df = 3, p-value < 0.05; skull: $x^2 = 9.88$, df = 3, p-value < 0.05; pooled bone: $x^2 = 13.17$, df = 3, p < 0.01). The use of black gelatin lifters resulted in a higher expected rate of CAST grade 0 marks being recovered (Figure 4.4).

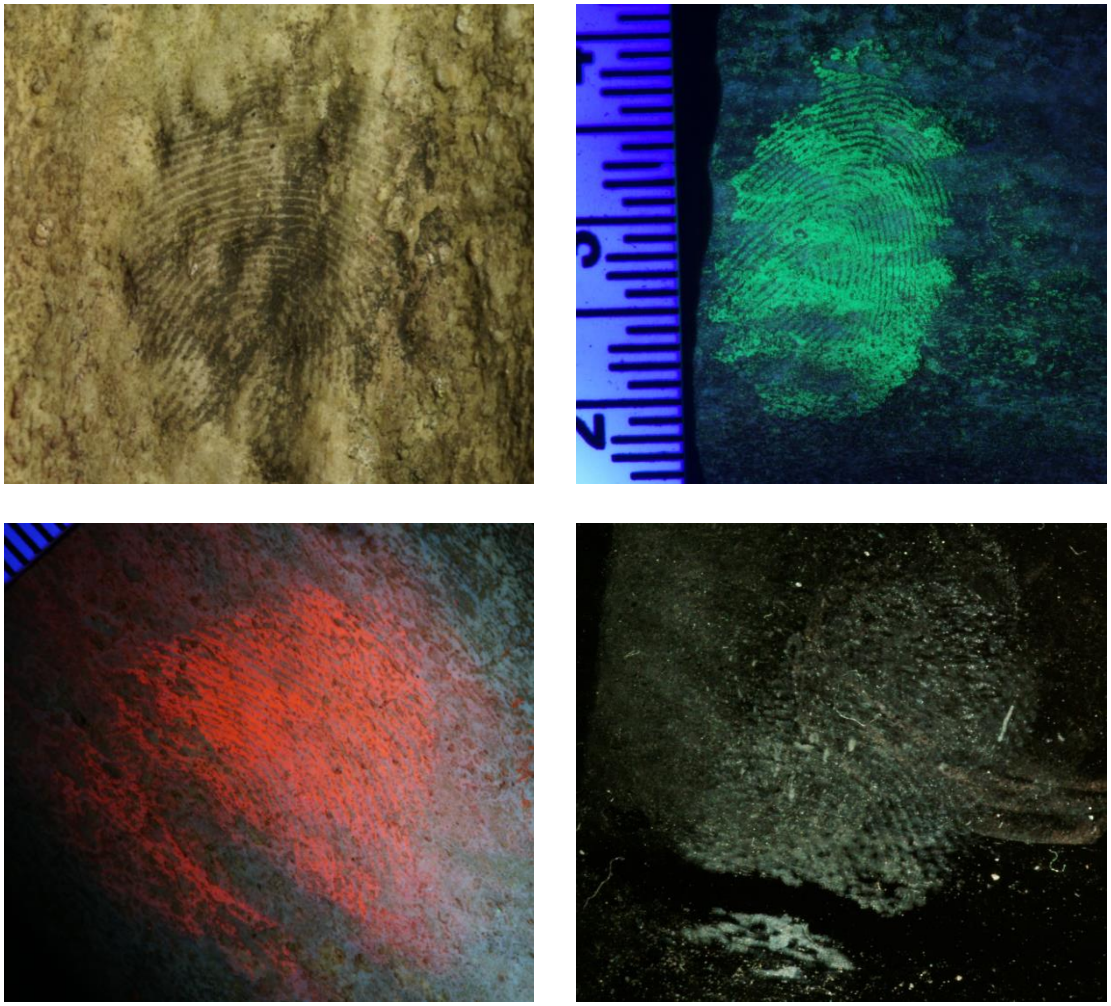

Figure 4.3. Clockwise from top left, examples of fingermarks recovered from antler using; Supranano™ black magnetic powder, Supranano™ yellow, fluorescent magnetic powder excited with 365nm UV torch, black gelatin lifter, Supranano™ red fluorescent magnetic powder excited with 365nm UV torch.

4.3.1.3 Ivory & Substitutes

CAST grades ≥3 were recovered at a rate of 61% (elephant), 45% (hippo) and 12.5% (conch) in ivory and ivory substitute samples. (Table 4.1). All tested recovery methods could produce CAST grades ≥3 on both elephant and hippo ivory (Figure 4.5). Red fluorescent and black SMP enhanced CAST grades >3 on conch. Black gelatin lifters resulted in the lowest number of marks of forensic interest across ivory and ivory substitute specimens. A Kruskal Wallis test found a significant association between grade quality recovered between enhancement methods within pooled ivory specimens, conch and hippo ivory but not elephant ivory (hippo ivory: $x^2 = 8.5$, df = 3, $p < 0.05$; conch: $x^2 = 24.36$, df = 3, $p < 0.001$;

pooled ivory: x^2 =26.20, df = 3, p < 0.001). Within pooled ivory, conch, hippo, and elephant ivory samples black gelatin lifters produced a higher-than-expected frequency of grade 0 and/or 1 marks (Figure 4.4).

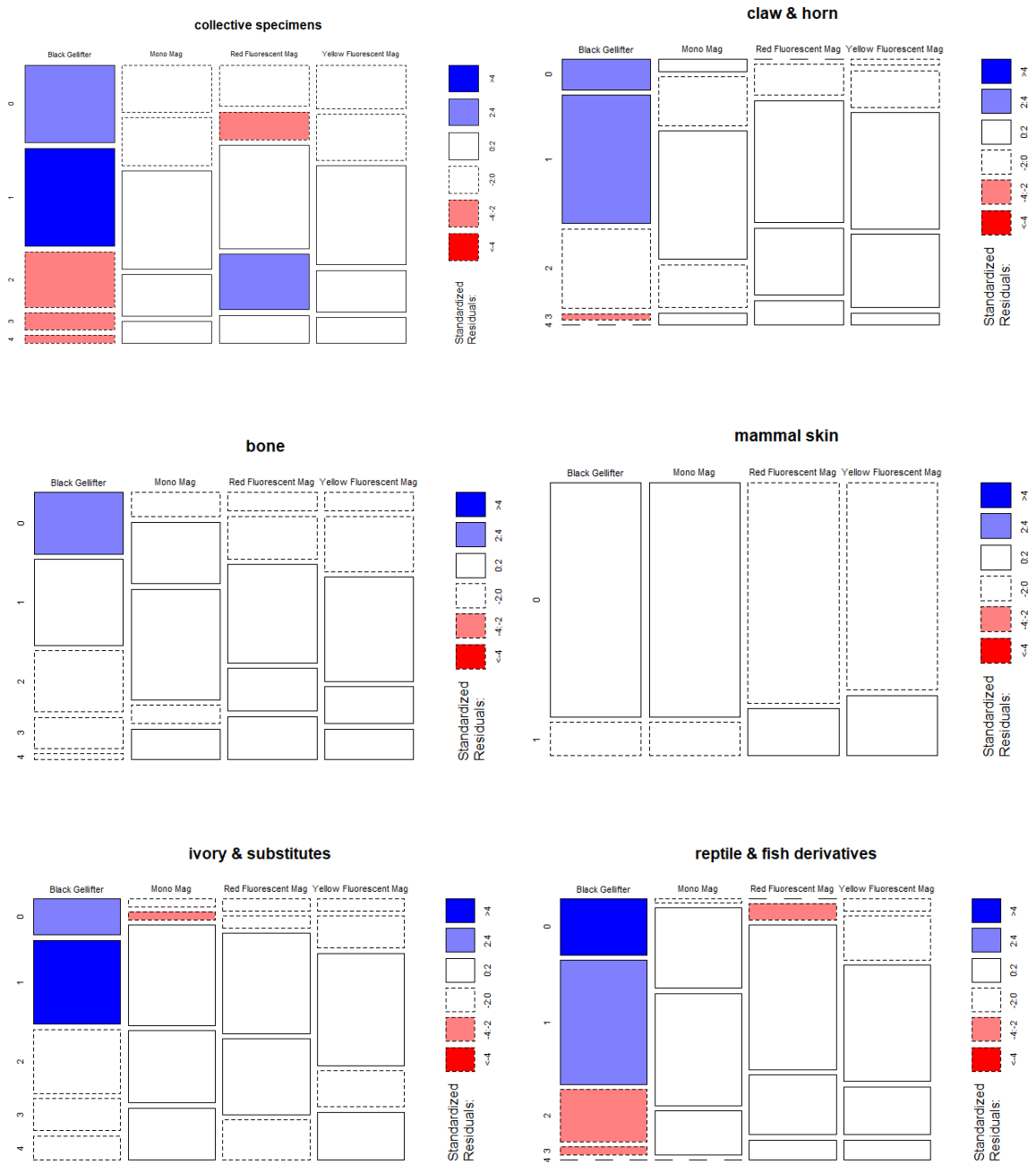

Figure 4.4: Mosaic plots visualising the frequency of CAST grade (y axis) against enhancement method (x axis), residuals. Box heights are proportional to % of cast grades seen within each enhancement category for each specimen group. Standardised residuals indicating significant deviations from the null models are represented by colour, the darker the shade the higher the significance. Blue represents a higher-than-expected number of observations (residuals >2) and red a lower-than-expected number of observations (residuals <-2). All plots bar mammal skin depicts significantly higher than expected observations of low cast grades recovered when using black gelatin lifters.

4.3.1.4 Mammal skin

All enhancement methods failed to recover grades >2 from either type of mammal skin (Table 4.1) demonstrating evidence of contact only (Figure 4.5). A fingermark of CAST grade 1 was enhanced from depositions on 21% of elephant skin and 11% of antelope skin samples. Yellow fluorescent SMP performed best for elephant skin and black gelatin lifters for ungulate skin (Table 4.1). No significant association was found between enhancement method and CAST grades recovered from mammal skins, pooled or independently.

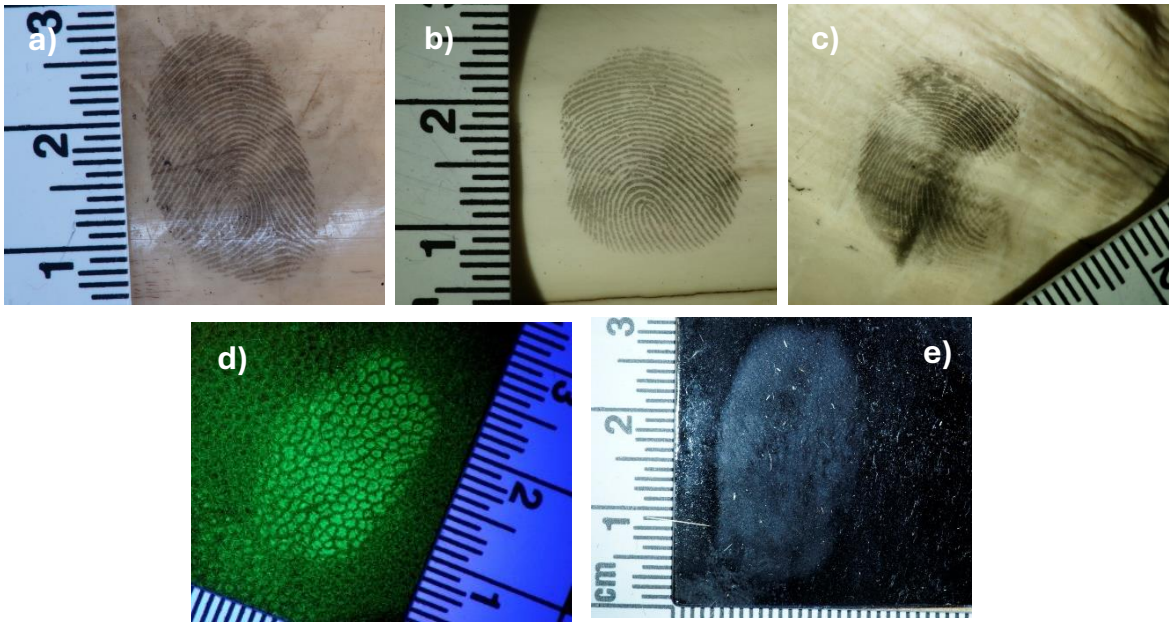

Figure 4.5: Examples of the highest grade fingermarks recovered from different specimen types using their best performing enhancement method a) grade 4 mark on elephant ivory enhanced using Supranano™ black magnetic powder, b) grade 4 mark on hippo ivory enhanced using Supranano™ black magnetic powder, c) grade 3 mark conch shell enhanced using Supranano™ black magnetic powder, d) grade 1 mark elephant skin enhanced using Supranano™ yellow fluorescent magnetic powder excited with a 365nm wavelength torch and e) untreated grade 1 mark ungulate skin recovered using black gelatin lifter.

4.3.1.5 Reptile & Fish

Red fluorescent SMP recovered the highest number of >3 CAST grades for sawfish rostrum (*N* = 8), and tortoise shell (*N* = 12) and black gelatin lifter the highest number for snake skin (*N* = 1) (Figure 4.6). Black gelatin lifters produced the lowest number of >3 CAST grades for both sawfish rostrum and tortoise shell (Table 4.1).

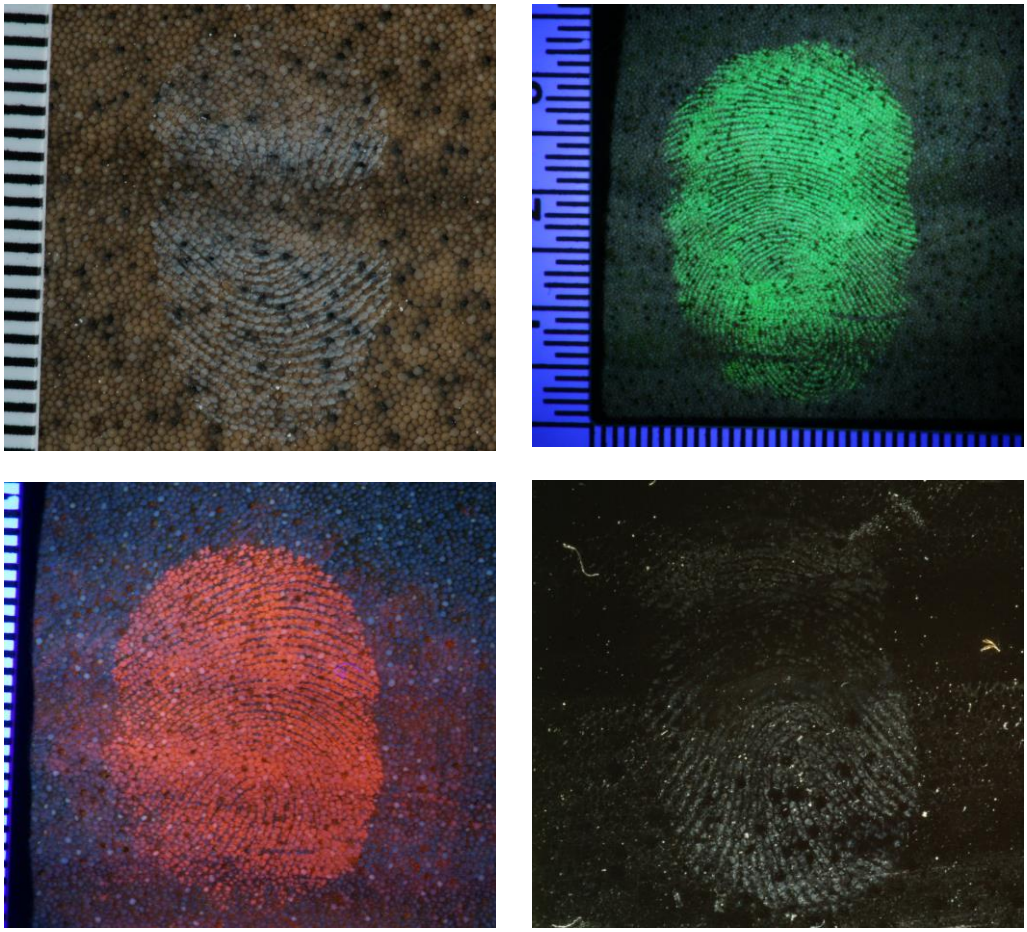

Figure 4.6: Examples of fingermarks enhanced on sawfish rostrum using all four tested enhancement methods. Clockwise from top left: Supranano™ white magnetic power, Supranano™ yellow, fluorescent magnetic powder excited with 365nm wavelength torch, black gelatin lifter, Supranano™ red fluorescent magnetic powder excited with 365nm wavelength torch.

Fingermarks graded >3 were recovered from 34% of depositions on tortoise shell, 28% of depositions on sawfish rostrum and 1% of depositions on snakeskin. A Kruskal Wallis test found a significant association between enhancement method and maximum CAST grade for pooled ($x^2 = 66.46$, df = 3, $p < 0.001$) and individual, sawfish ($x^2 = 16.63$, df = 3, $p < 0.001$), tortoise shell ($x^2 = 30.15$, df = 3, $p < 0.001$), snakeskin ($x^2 = 28.69$, df = 3, $p < 0.001$). A visualisation of standardised Pearson's residuals shows a higher-than-expected frequency of 0 – 1 level grades for black gelatin lifters and a lower-than-expected frequency of zero grades for red fluorescent SMP. This trend was seen within each of the individual reptile & fish specimens as well as collectively (Figure 4.4).

4.3.1.6 Pooled specimens

Across all pooled samples (*N* = 960), a Kruskal Wallis test found a significant association between enhancement method and recovered CAST grade (x^2 = 81.01, df = 3, p < 0.001). Visualisation of the standardised residuals (Figure 4.4) indicates that black gelatin lifters resulted in significantly more fingermarks graded ≤1 and significantly fewer CAST grades ≥2 than expected. The use of red fluorescent SMP resulted in significantly fewer CAST grades of one and significantly more CAST grades of three than expected.

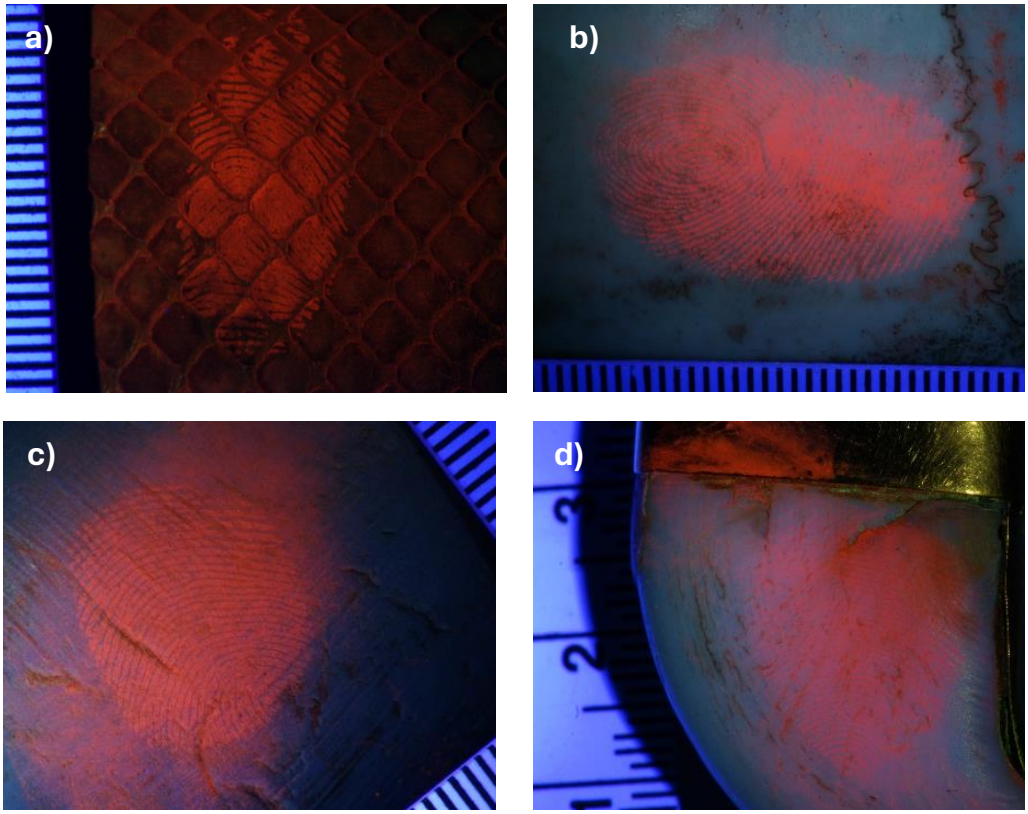

Figure 4.7: Examples of fingermarks enhanced using Supranano™ red fluorescent magnetic powder excited with 365nm wavelength light on a) snakeskin, b) gorilla skull, c) bovine horn, d) tiger claw.

4.3.1.7 Textured specimens

Using a Kruskal Wallis test a significant association (x^2 = 39.52, df = 3, p < 0.001) was found between enhancement method and maximum CAST grade achieved within textured specimens. Outputted standardised residuals of indicate red fluorescent SMP resulted in lower-than-expected rates of grade 1 marks. Black gelatin lifters resulted in lower-than-

expected rates of grade 2 and 3 marks and higher than expected rates of grades 0 and 1 marks as demonstrated by standardised residuals of <-2 and >2, respectively.

4.3.1.8 Smooth specimens

A significant association was found between enhancement method and maximum CAST grade achieved within smooth specimens using a Kruskal Wallis test ($x^2 = 59.78$, df = 3, $p < 0.001$). Black gelatin lifters resulted in lower-than-expected mark grades of 3 and 4 and higher than expected mark grades of 0 and 1, as indicated by standardised residuals of >2 and <-2, respectively. Standardised residues of <-2 were presented for grade 1 marks enhanced by red fluorescent SMP indicating lower than expected rates (Figure 4.8).

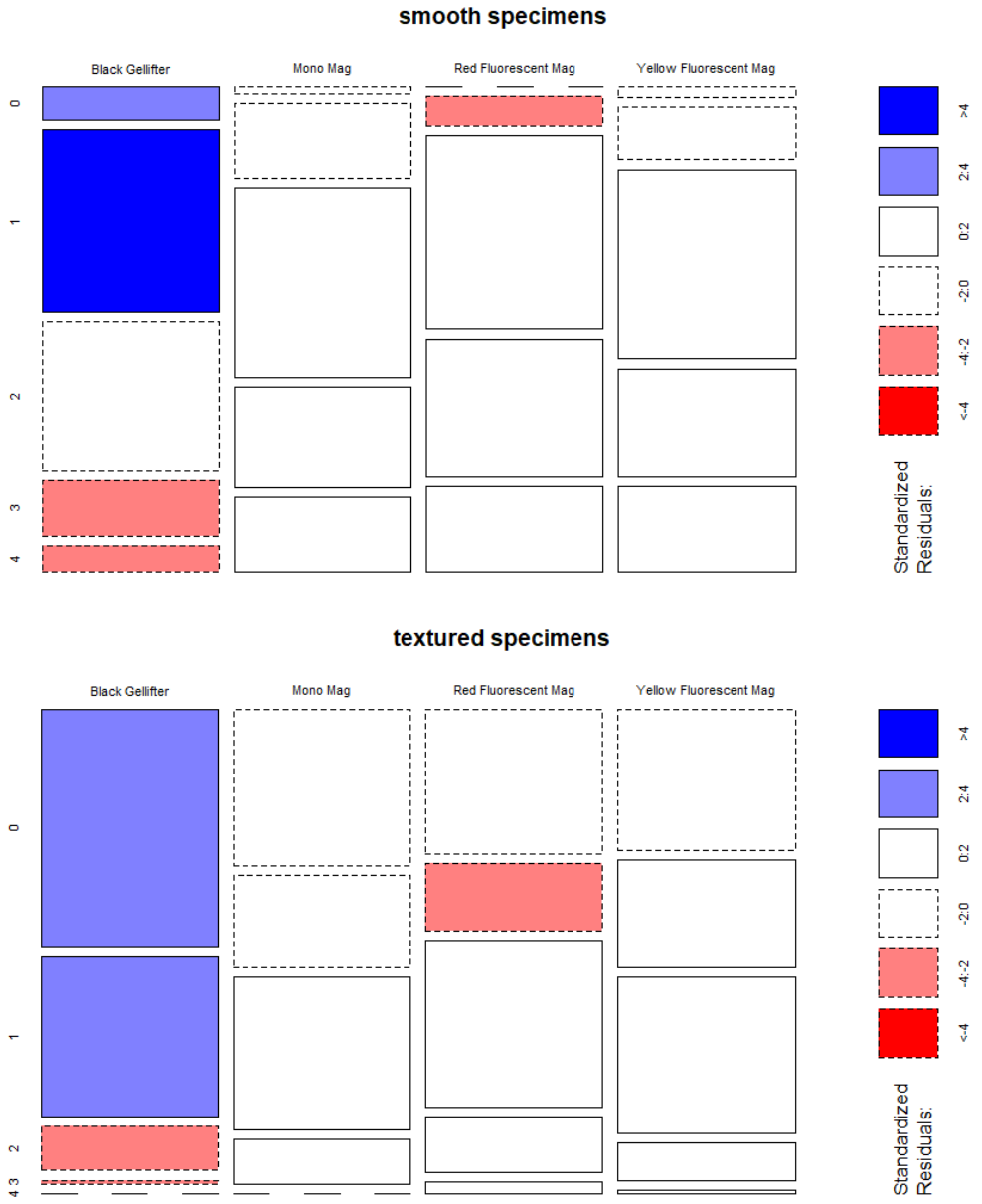

Figure 4.8. Mosaic plots visualising the frequency of CAST grade (y axis) against enhancement method (x axis), residuals for both smooth (top) and textured (bottom) specimen types. Box heights are proportional to % of cast grades seen within each enhancement category for each specimen group. Standardised residuals indicating significant deviations from the null models are represented by colour, the darker the shade the higher the significance. Blue represents a higher-than-expected number of observations (residuals >2) and red a lower-than-expected number of observations (residuals <-2). Significantly higher than expected observations of low CAST grades (0 – 1) and lower than expected observation of high CAST grades (3 – 4) were seen in fingermarks recovered using black gellifters. Significantly lower than expected observations of low CAST grades (0 – 1) were seen in fingermarks enhanced using Supranano red magnetic fluorescent powder excited using a 365nm wavelength torch.

Table 4.1: Frequency of CAST grades achieved for each combination of specimen type and enhancement method variables.

	Bone		**Claw & Horn**			**Ivory & Substitutes**		**Mammal skin**		**Reptile & Fish**		
CAST Grade	*Antler*	*Skull*	*Claw*	*Horn*	*Conch*	*Elephant Ivory*	*Hippo Ivory*	*Elephant skin*	*Ungulate skin*	*Sawfish rostrum*	*Snakeskin*	*Tortoise shell*
Red Fluorescent												
0	3	0	0	0	3	0	0	14	19	0	0	0
1	7	0	2	3	1	0	2	6	1	1	2	1
2	8	8	11	9	12	5	8	0	0	11	18	7
3	2	5	5	6	3	9	7	0	0	5	0	10
4	0	7	2	2	1	6	3	0	0	3	0	2
Yellow Fluorescent												
0	3	0	0	1	1	2	0	13	18	0	1	2
1	8	1	2	4	5	0	3	7	2	4	5	2
2	8	9	13	6	14	7	7	0	0	9	14	6
3	1	5	3	9	0	3	6	0	0	3	0	9
4	0	5	2	0	0	8	4	0	0	4	0	1
Mono magnetic												
0	4	0	2	0	2	0	0	17	18	0	1	0
1	7	3	4	4	1	0	1	3	2	8	9	3
2	8	10	12	9	11	6	8	0	0	6	10	12
3	1	2	2	5	6	6	6	0	0	6	0	5
4	0	5	0	2	0	8	5	0	0	0	0	0
Black gelatin lifter												
0	9	1	1	4	7	2	0	19	16	1	4	9
1	8	6	10	11	10	2	9	1	4	11	15	5
2	3	7	8	5	3	7	6	0	0	7	0	6
3	0	5	1	0	0	5	3	0	0	1	1	0
4	0	1	0	0	0	4	2	0	0	0	0	0

4.3.2 Impact of pre and post lifting enhancement state on mark quality

For comparison of grades between photographed states of enhancement, samples were split into two data sets, those treated with fluorescent powders (both red and yellow grouped) and those treated with mono magnetic powders (both black and white grouped). Fingermarks treated with mono-chromatic powders were compared in two states of enhancement, raw (powdered fingermark in-situ on the specimen) and lifted (powered fingermark on a gelatin lifter). A Wilcoxon signed rank test found no significant difference between grades awarded pre and post lifting using gelatin lifters for fingermarks enhanced using mono-chromatic powders ($V = 954$, *p-value* = 0.394). The mono-chromatic sample set was further broken down into textured and smooth specimens and Wilcoxon signed rank tests carried out on each separate dataset. No significant relationships were found in either scenario. Fingermarks treated with fluorescent powders were compared at four states of enhancement, raw (powdered fingermark with no UV excitation in-situ on the specimen), fluoresced (powdered fingermark with UV excitation in-situ on the specimen), lifted (powdered fingermark with no UV excitation on a gelatin lifter) and lifted and fluoresced (powdered fingermark with UV excitation on a gelatin lifter) (Figure 4.9). A Friedman rank sum test showed there was a significant difference between marks awarded in each of the different enhancement states ($X^2(3) = 184.62$, p-value = 2.2e-16). A post-hoc analysis established this significant difference was applicable to grades awarded in the raw and fluoresced states (p-value < 0 .001). The fluorescent enhanced sample set was further broken into textured and smooth samples and the Friedman test's repeated. A significant relationship was found to exist between grades at raw and fluoresced states for both textured ($p < 0.05$) and smooth ($p < 0.001$) specimen surface types.

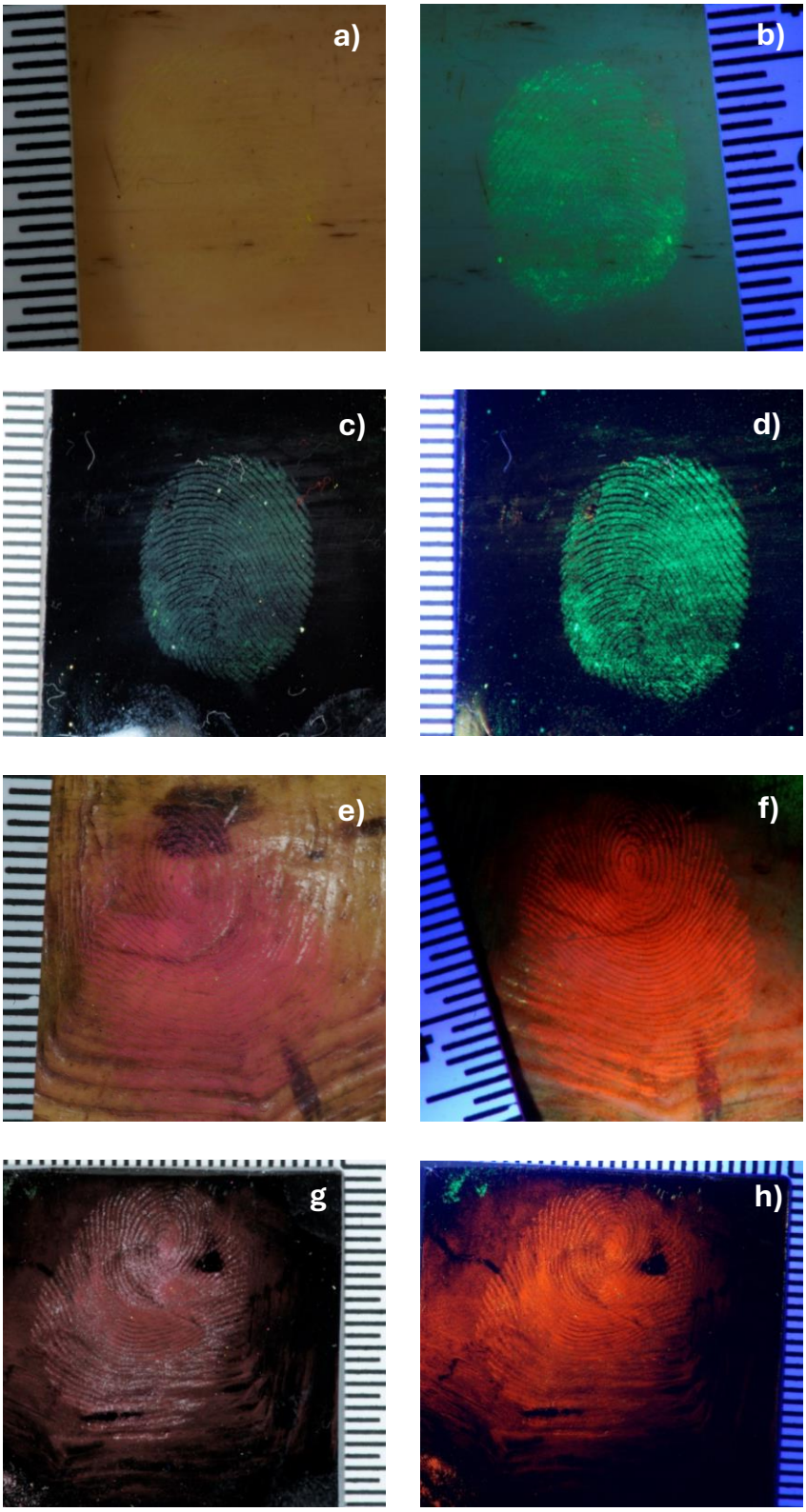

Figure 4.9. Images of Fingermarks enhanced using Supranano yellow (a-d) and red (e-h) magnetic fluorescent powders photographed in four unique recovery states on ivory (a-d) and tortoise shell (e-h): directly without fluorescence enhancement (a,e), directly with fluorescent enhancement (b, f), lifted without fluorescent enhancement (c,g) and lifted with fluorescent enhancement (d,h). The impact that surface colour and texture can have on choosing the best recovery state for grading is clearly demonstrated.

4.4 Discussion

4.4.1 Impact of texture, porosity, and colour group characteristics

When presented with a surface for potential fingermark recovery texture will be a priority assessment due to its major influence on method choice. The UK Forensic Science Regulator has defined texture as the "difference in height between the peaks and troughs of any surface features present" (Forensic Science Regulator, 2013). Based on these definitions, the visual topography and the feeling to touch in this study I assigned the following specimens as "textured" surfaces; elephant, snake, and antelope skin, tortoise shell, conch shell and antler and the following as "smooth"; hippo, and elephant ivory, sawfish rostrum, gorilla skull, claw, and horn. However, with no defined thresholds there will always be a level of subjectivity in assignment of this descriptor and particularly within the context of wildlife specimens where there can a wide-ranging presentation of surfaces within closely related groups. An in-depth assessment of porosity was not carried out however based on existing work in this area for each specimen, outside of the context of forensic applications, all substrates were assumed to have some level of porosity sitting within a porous to semi-porous designation.

Textured (rough) surfaces are routinely described as challenging for fingermark enhancement and recovery. This is in part due to interactions at the point of deposition whereby residue is only deposited on the highest peak points creating breaks in the ridge detailing where contact has failed to penetrate to lower levels creating a uniform complete print. I found this assessment to be true within the grouped specimen types with a higher frequency of CAST grades not considered of forensic interest recovered from "textured" specimens comparable to "smooth" specimens. Though textured surfaces often result in residue deposition at just the highest peaks there will be instances where increased deposition pressure allow for contact to be made at both high and low levels if the

topography is less exaggerated. It is suspected this phenomenon contributed to the recovered CAST grades from tortoise shell being more closely aligned with results from “smooth” specimens and images of enhanced marks clearly show full contact between finger pad and tortoise shell surface was regularly made.

Gelatin lifters have more traditionally been used for recovery of latent footwear marks or to lift powdered marks, their application for recovery of untreated latent marks, as used in this study is less common (Bleay et al., 2018). They have been suggested for fingermark recovery from textured surfaces as their malleable nature allows for manipulation across both low and high areas of surface topography (Maloney, 2017). However it has been shown that as texture (and porosity) of a surface increases the efficacy of gelatin lifters decreases and experimentally they perform best on smooth, non-porous surfaces (Bleay et al., 2011). Therefore, their overall inferior performance in this study is unsurprising, first due to all specimens being suspected porous or semi-porous in nature and secondly due to the highly textured nature of some of their surfaces. The limited malleability of gelatin lifters is evidence in lifts from the tortoiseshell where it has failed to penetrate the numerous ridges present despite other enhancement methods identifying ridge detail is likely to be present. The substrates in which gelatin lifters performed significantly worse on, these being tiger claw, bovine horn, conch, sawfish, tortoise shell and snakeskin, lend to the theory that texture was a significant influencing factor in the results produced by gelatin lifters. Though tiger claw, bovine horn and sawfish were placed into the “smooth” group of specimens all possessed superficial characteristics on their surfaces, such as scratches or very shallow divots or striations that marginally sets them apart from the smooth polished surface of elephant and hippo ivory.

An additional reason for the lower efficacy of gelatin lifters found is unrelated to the specimens but rather best practice methods of their use. In this study gelatin lifters were photographed using a digital camera and not a GLScan machine to visualise recovered prints. This equipment was used by Moorat et al. (Moorat et al., 2020) when successfully recovering latent fingermarks from pangolin scales, a textured surface, and is the gold standard method of visualising untreated marks recovered using gelatin lifters. In the case of footwear mark recovery guidance states that if GLScan or similar enclosed light equipment is not available then powdering may be a more appropriate approach however it will result in loss of fine detail (Bandey and Bleay, 2010). The cost and size of GLScan machines means access to the resource will be limited for most law enforcement. Photography of gelatin lifters using digital cameras and appropriate lighting has been shown to be an effective method of documentation (Tibben et al., 2023) and is also listed as a suitable method by manufacturers (BVDA, 2024). These findings show gelatin lifters on untreated fingermarks are not a universally appropriate fingermark recovery method from wildlife specimens and their success on pangolin scales is an exception rather than the rule. However, the results of this chapter have demonstrated their suitability to lift and preserve powdered marks, without any significant loss in quality, from a range of wildlife derivative surfaces. As such they may still be considered a useful tool in a wildlife crime scene first responders forensic arsenal.

Red fluorescent SMP recovered fingermarks graded as forensic interest, from nine of the twelve specimens included in this study. SceneSafe™ SMP are considered micro-scale powders with smooth spherical particles of an average size of $\leq$~40µm capable of adhering to a wide range of fingermark constituents (Weston-Ford et al., 2016). Despite the name they are not the same as nano-technology powders such as quantum dots, which are significantly smaller in size and carry higher health and safety risks (Gaw and Ramotowski,

2012). Powders as an enhancement process are fundamentally a mechanical (physical) process with the theory of their use underpinned by particles preferentially adhering to fingermark constituents comparable to the surface they have been deposited on (Bleay et al., 2021). Powders are generally not recommended for use on porous, textured surfaces due to a tendency for powder particles becoming “trapped” within the texture or pores of the surface obscuring any enhanced ridge detailing, this is particularly true of metal flake powders one of the most commonly used powder types globally (Home Office, 2022). However as techniques have developed magnetic powders overcome this problem by retaining particles on the applicator and depositing only on fingerprint residue, as well as being able to remove trapped excess powder, improving the clarity of enhanced ridge detailing (Sodhi and Kaur, 2001). Small scale powders provide a greater surface to volume ratio resulting in easier adhesion to fingermark constituents however as the number of fine particles increases it becomes inversely effective on increasingly porous surfaces (Gürbüz et al., 2015). This may provide reasoning as to why SMP has found such success in both this study and Weston-Ford et al (Weston-Ford et al., 2016) as the small (but larger than other reduced scale powders) particle size finds a balance between a high rate of adhesion whilst avoiding saturation of pores.

All three Supranano magnetic powders compared came from the same manufacturer (SceneSafe™) and to the best my knowledge will only vary in formulae by the choice of chemical(s) used to produce the desired colour and/or fluorescence. To view fine detail and minutiae in an enhanced mark a suitable level of contrast must be present between the mark and the background surface. The further apart two colours on a colour wheel the greater the contrast provided, the highest contrast being afforded to those opposite each other, for example blue/yellow, red/green. Black and white provide a high level of contrast and therefore it is unsurprising I saw Supranano black magnetic powder performing well on

ivory, and ivory substitutes. The rest of the specimens used in this study can be loosely grouped into three colour groups; grey (bovine horn, sawfish rostrum and elephant skin); brown (snakeskin, tortoise shell, antler); yellow/cream (claw, skull, antelope fur). As ivory ages it forms a yellow/brown patina, the elephant ivory used in this study appears to be undergoing this process and has a darker hue comparable to the whiteness of the conch and hippo tusk. This perceived transitional colour state is reflected in the fact that red fluorescent SMP outperformed black SMP in the frequency of forensic interest marks recovered. It is suspected that as ivory ages further and the patina deepens then the red powders may begin to outperform black powders to a higher degree. Though marketed as "red" the colour of the powder, once applied, leans subjectively closer to a pink/purple side of the spectrum. Simple colour theory provides some explanation for the success of red fluorescent SMP on yellow, cream, and brown surfaces, as the two colour groups sit far enough apart on the spectrum to produce a reasonable level of contrast. Grey is a mixture of white and black and the best high contrast colour available will vary depending whether it lands closer to black or white on a scale. I see this played out within this study's tested specimens, as both yellow and red fluorescent SMP performed similarly. Notably it was on elephant skin, the darkest and most uniform grey specimen, where the only instance of yellow, fluorescent SMP outperforming red fluorescent SMP occurred.

A second consideration for the success of red fluorescent SMP is that the wavelength of light used in this study (365nm) resulted in a stronger excitation and subsequent fluorescence strength of red vs yellow powder. However peak fluorescence of the powders used in this study occur with excitation using 415 - 450 nm and 515 – 535 nm wavelengths, for yellow and red respectively, making the 365nm wavelength used suboptimal on both counts. Additionally, there was a significant improvement in fingermark grade between powdered marks in their raw and fluoresced states, indicating that the excitation by this

wavelength was beneficial to the efficacy of both yellow and red fluorescent powders. Therefore, it is unlikely the choice of wavelength used is a significant factor in the overall greater success of the red fluorescent SMP.

In recent years it has been established that bio-fluorescence is surprisingly common across the animal kingdom, showcasing in mammals (Travouillon et al., 2023), reptiles (Paul and Mendyk, 2021), invertebrates (Ainsworth et al., 2008) and birds (Hausmann et al., 2003). Whilst it was not observed in this study, potential background fluorescence of animal specimens should be a consideration when deploying any fluorescent based enhancement methods as it may influence powder colour, filter, and wavelength choice. However, where this may present as a limitation this study shows that gelatin lifters may be suitable as a lifting tool to recovery the powdered fingermark and provide a stronger contrasting background.

4.4.2 Impact of morphological and composition characteristics

4.4.2.1 Mammal skin

Skin structure and hair/fur density varies widely amongst mammals influenced by their evolutionary adaptations (Lillywhite and Stein, 2009; Springer et al., 2021; Mohammed et al., 2022). Successful fingermark recovery from human skin has been carried out (Trapecar and Balazic, 2007) but the diversity of non-human mammalian skins means assumptions regarding porosity and texture, which significantly impact fingermark recovery, may not allow these findings to be translated. To become useable for commercial purposes animal skins must go through an intensive manufacturing process which can significantly alter their physical structure and porosity (Covington, 2009; Gil et al., 2013). Elephant skin presents itself as a challenging surface for fingerprint recovery due to it being heavily fissured providing ample opportunity for ridge detail to be interrupted (Lillywhite and Stein, 2009).

The lack of sweat and sebum glands render it a dry surface with its permeability reliant on manual wetting in its natural state and the skin structure resulting in greater spread of water meaning fingermark deposits may be quickly distributed(Martins et al., 2018). The suede like texture of the underside of antelope fur used in this study was soft to the touch but for the purposes of fingermark recovery would be considered a textured and porous surface (Bleay et al., 2018). As expected, given these properties this study struggled to enhance any high-quality fingermarks from mammal skins using any of the tested methods. However, given evidence of touch on multiple samples the potential for trace DNA recovery is present.

4.4.2.2 Bone

Skull and antler are structurally analogous bone with a high collagen content, albeit with significant textural differences. The literature states that highly textured surface types are more problematic for fingermark enhancement (Home Office, 2022). The results of this study mirror this with a greater frequency of higher CAST grades being recovered from the smooth gorilla skull comparable to the textured antler despite their similar composition. However, the difference in these results may have been artificially heightened as a visual and tactile inspection of the skull suggested it may have been treated with a consolidant. It has been shown bone treated in this manner is more amenable to fingermark enhancement comparable to non-treated bone however mark enhancement on untreated bone is possible (Steadman and Andersen, 2003) and I would recommend further work to include non-treated bone into this comparison study. As with the existing study on fingermark recovery from antler I hypothesise the magnetic quality of the powder overcame the problematic texture of the antler by allowing easy removal of excess powder within its

grooves. However further comparison work including a non-magnetic powder would be needed to determine this (Otis and Downing, 1994; Czarnecki, 2002).

4.4.2.3 Ivory & substitutes

Elephant ivory and hippo ivory are the upper incisors and upper and lower canines or lower incisors, respectively. They are considered semi-porous materials, both structured with a thin layer of cementum covering a dentine core. In both groups an enamel layer is also present but covering only the most heavily used portions of the teeth. Both the cementum and enamel layers may or may not be present in an ivory sample depending on what portion of ivory length is being targeted and whether it has been "worked" or not. Both specimens used in this study are examples of "raw" polished ivory, making them smooth, uniform, light, surfaces which, on initial inspection, lend themselves well to fingermark enhancement, was an assumption confirmed by the results seen in this chapter. Though conch shells can be used as ivory substitutes they are more often sought after as their own independent commodity (Pavitt et al., 2021). Their composition differs significantly from elephant and hippo ivory, compromised predominantly of calcium carbonate and considered a porous material (Li et al., 2023). The conch shell used in this study had a smooth but rippled surface texture, this coupled with its porosity, could provide explanation for the comparably lower frequency of fingermarks classified as of forensic interest seen as finger pads may be making contact with the high points of the surface rippling whilst failing to make contact with the lower topography areas.

4.4.2.4 Reptile and Fish derivatives

The outer shell and scales of the tortoise and snake skin specimens used in this study are composed of β-keratins and can be categorised as "semi-porous" in nature (Weir et al., 2016). On first inspection both specimens provided complex backgrounds for fingermark

enhancement: the tortoise shell had a ridged surface with a mottled patterning of dark and light browns. The snakeskin had similar background patterning of light and dark areas and whilst overall flatter topography than the tortoise shell, the overlap of each individual scale offered multiple opportunities for interruption of ridge detailing and minutiae.

An initial assumption regarding the low efficacy of gelatin lifters on tortoise shell is that due to the topography of the surfaces participants finger pads did not make contact with the lower areas of the surfaces. However, marks enhanced using powders indicate deposition itself is unlikely to be a factor as they clearly show contact made at both high and low points (Figure 4.9). An alternative reasoning is that although gelatin lifters are malleable the complexity of the surface area prevented the gelatin lifters from fully moulding to the shape. I saw evidence of this with the tortoise shell lift(s) whereby ridge detail failed to be recovered between the thinnest ridges of the shell. However this phenomenon was not seen in fingermark recovery using gelatin lifters from pangolin scales (Moorat et al., 2020) which present a similarly ridged surface type. Unlike tortoise shell the textural problems raised by snakeskin were present across all enhancement types with ridge and minutiae detail being lost at the intersection of scales (Figure 4.7). In reptilian species with larger individual scales this overlap may not present as such as significant problem as substantial portions of a fingermark may be deposited onto a single scale. Sawfish skin is comprised of dermal denticles, tooth like structures that in their natural state create a rough sandpaper type texture (Welten et al., 2015). The rostrum used in this study has undergone unidentified preservation techniques which had altered the expected texture, rendering it as a smooth, shiny, surface with a slight undulation caused by a natural depression running along the central length of the rostrum. This slight undulation may have contributed to the poor performance of gelatin lifters on this specimen if full contact were not being made but given the trivial depth of the depression this presents as an unlikely scenario.

4.4.2.4 Claw & Horn

Made up of hard alpha keratin, claw and horn of a range of species function as defensive, attack and display tools in their natural state and as such have evolved to withstand significant wear and tear (Li et al., 2010; Rothschild et al., 2013). Both bovine horn and cat claw grow as a keratinous sheath which covers a bony core resulting in a variation of density along their length, as they grow outer layers of keratin are shed, this can be an entire sheath as in feline claws or in flakes as with bovine horn (Homberger et al., 2009). It is known there is a level of porosity within horn (Li et al., 2010) and human nail, another keratinous substrate, and as such claw and horn could be classified as semi-porous surface types in the context of fingermark recovery work. In all but the cheetah (*Acinonyx jubatus*) species cats' claws are retractable, protected by skin folds when not being used (Homberger et al., 2009; Vipin et al., 2016), in contrast bovine horn is continuously exposed to the elements and is often polished to improve appearance for commercial uses so may be intercepted in a wider range of states than claw. In this study both the horn and claw had visible imperfections running horizontally and vertically though none of any comparable depth as seen on the antler and both could be considered smooth surfaces in comparison. The horn was light grey in colour with lighter mottling dispersed across the surface and the claw an off white more uniform colouring. Bovine horn can be used as a faux substitute f tiger claw and as such a technique for fingermark recovery that is successful on both derivative types would prove beneficial when species identification is not immediately available (Vipin et al., 2016). It should be noted that though the findings related to bovine horn found in this study may not be immediately transferable to rhino horn, one of the most heavily trafficked wildlife derivatives within wildlife crime, there are several similarities in their morphology

(Hieronymus et al., 2006). Additionally wild Bovidae suffer from their own pressures within the IWT and warrant inclusion in wildlife crime intervention research (Vipin et al., 2022).

4.4.3 Feasibility of real-world application

Transitioning proof of concepts into practical implementation is a key step in allowing research to move beyond theoretical concepts towards making a tangible impact in casework to the benefit of law enforcement (Weyermann et al., 2023). To make this transition, resource and infrastructure access, technique and applicability awareness, and appetite for take up should all be explored.

As discussed powders are the most used fingerprint enhancement tool amongst forensic practitioners (Gomes et al., 2023). In the UK alone it is estimated 50% of all fingerprint identifications per annum are from marks developed using powders (UK Home Office, 2022). Whilst reduced scale or fluorescent magnetic powders are not part of standard UK toolkits they are utilised elsewhere (Bleay et al., 2018) and their application method functions in the same way as standard magnetic powders. Their comparable functionality means forensic practitioners will not require specialist training and from a purely skill-based perspective they could theoretically be readily incorporated into workflows. A more apparent obstacle is validation, a necessary requirement by many regulators before a new technique can be deployed in the field. Aluminium, magneta flake, black magnetic and black granular powders manufactured and supplied by SceneSafe™ are referenced as holding similar specifications to those included in original powder validation studies suggesting the validation of their red fluorescent SMP used in this study is highly feasible (UK Home Office, 2022).

Ability to efficiently collect, store and analyse fingerprint evidence is a key step to seeing it adopted in wildlife crime cases. Whilst access to an AFIS streamlines the ability to search

and compare latent marks against a database they are not a requirement to successful use of fingerprint evidence (Moses, Kenneth R et al., 2011). This has been proven in India who regularly reported thousands of successful latent mark recoveries and matches prior to their recent full migration to a national AFIS (Bureau and Bureau, 2020). In Tanzania, a hub for wildlife crime, fingerprints are the leading forensic evidence type presented in court (Jilala and Lwoga, 2022). Of course there are exceptions such as Nigeria, who's judicial system is sceptical in its attitude towards fingerprint evidence in part due to their self-awareness of national systematic failures resulting high error rates in fingerprint analysis protocols (Ezegbogu and Omede, 2023). Like Nigeria, a lack of resources ranging from logistical accessibility to crime scenes, budgetary and sheer scale of the demands for forensic services in the global south (Jilala and Lwoga, 2022; Wamuyu et al., 2023) has pushed calls for a more sustainable and cost effective approach to forensic investigation coined "frugal forensics" (Bouzin et al., 2023). Reasons for fingerprint powders being so heavily deployed by CSEs include their low cost, ability for use at a crime scene and lack of detrimental impact on sequential processes. Crime scene examination limits the amount of time passed between deposition and enhancement, risk of mark destruction or disturbance through improper handling or transport, avoids logistical challenges of movement and storage of large and awkward shaped items, and importantly in the context of wildlife, is the most practical option if dealing with a live specimen. However, wildlife crime is not isolated to the global south and does not occurring within a vacuum of low-income nations with minimal resources. Participation by the global north as importers and transit routes (Engler et al., 2007; Olsen et al., 2021) of illegal wildlife goods is well documented and domestic wildlife crime within UK, the EU, and North America is an ever present threat. Unlike nations in the global south these players in the wildlife crime sphere possess AFIS, accredited techniques, personnel and laboratories and regularly include fingerprint

evidence in violent and volume crime casework. The FBI's AFIS, known as the Integrated Automated Fingerprint Identification System (IAFIS) is one of the largest in the world containing fingerprints of over 156 million individuals (both criminal and civil) as of 2021 (United States Government, 2021). The UK's AFIS is referred to as IDENT1 and holds 8.4 million individual profiles as of March 2020. Therefore the inclusion of fingermark evidence within wildlife crime context is not only realistic but arguably a minimum expectation for countries who have made statements surrounding their commitment to end the IWT (UK Government, 2014). An example case where attempts at fingerprint evidence recovery could have been beneficial include the 2024 discovery of seven Aldabra giant tortoises carcasses in an Exeter woodland (Grierson, 2024). As shown by this research fingermark recovery from tortoise shells is feasible and a successful mark enhancement could have been used at a later date to prove suspects handling of the tortoises. As well as national AFIS there is the international version managed by Interpol. Member nations can search against this database, containing over 220,000 records, if they suspect international involvement in the crime they are investigating (INTERPOL, 2021b). The Five Country Conference (FCC) Data Sharing Agreement is a similar arrangement involving international sharing of biometric data, in this case fingerprints, between the UK, Australia, Canada, New Zealand and the USA (UK Government, 2016). For cases of illegal trade where intelligence sharing is vital to progress investigations these types of international data sharing agreements or databases could prove valuable.

Appetite for the use of, and investment in, fingerprinting kits for use in wildlife crimes already exists. Kits inclusive of black SMP have already been deployed, and utilised worldwide based on the research conducted by Weston-Ford et al (Weston-Ford et al., 2016). The limiting factor seen in existing research in this area is that solutions are targeted at a singular species, often representations of charismatic megafauna. This dilutes the

usefulness of such toolkits as their perceived application is narrow and risk duplicating the efforts and investment into individual kits when a single more comprehensive one could achieve further impactful reach.

This research indicates that expanding guidance and toolkits to include UV light sources, red fluorescent SMP, and gelatin lifters (as a recovery tool for mark preservation), would significantly widen the pool of wildlife applicable for their use, rendering the kits of greater value for money and deployable in more countries and a greater number of case studies. Not only this, fingermark recovery and analysis transcend wildlife crimes making resource investment a more beneficial long-term investment for law enforcement agencies.

4.5 Summary

This study has found that fresh fingermarks of forensic interest quality (grades $\geq$3) can be recovered from elephant and hippo ivory, tortoise shell, bovine horn, primate skull and tortoise shell at a rate of >25% of cases. Similar quality fingermarks can also be recovered from deer antler, tiger claw, conch shells and snakeskin at a lower rate of success. Supranano™ red fluorescent magnetic powder excited using a 365nm wavelength torch and photographed in-situ on a specimen was found to be the most universally effective enhancement method. No significant loss of grade quality was found after lifting powdered fingermarks using gelatin lifters and therefore this tool presents as a viable collection method for preservation of treated fingermarks. Though fluorescent powders are not routinely used in the United Kingdom they are more globally (Bleay et al., 2018) and results from this study and others including fingermark enhancement on bird of prey eggs and feathers (Darby et al., 2015; McMorris et al., 2015) suggest fluorescent magnetic powders should be included in any kits developed specifically for use in wildlife crime investigations and mandated first responders trained in their use. Despite their reported success in fingermark recovery off pangolin scales (Moorat et al., 2020) black gelatin lifters used in

isolation presented the least effective fingermark recovery method used in this study and I would not recommend them as a primary recovery method of untreated fingermarks in wildlife crime case work. Identification of areas of contact using fingerprint powders is possible on all specimen types evaluated in this study presenting opportunities for investigation into alternative trace evidence recovery attempts such as trace DNA. This study has shown that collection of fingerprint evidence is possible from wildlife specimens using low cost, field deployable techniques. More work is required to assess the applicability of these techniques in aged fingermarks and those which have undergone environmental exposure.

It is not suggested that fingerprint evidence collected in wildlife crime cases will be the arbiter of the outcome of a prosecution or conviction. However, based on the results of this study, their practical application, proof of efficacy, positive contribution to case outcomes, and the need for “frugal forensics” in highly invested nations it is recommend this evidence type deserves consideration and inclusion in wildlife crime investigations.

Chapter 5: Human DNA recovery in the context of wildlife crime: Comparison of trace DNA collection methods from wildlife specimens.

5.1 Introduction

The use of forensics has opened a wealth of opportunities to better understand and investigate wildlife crimes with a particular focus on the IWT. Forensic disciplines including veterinary pathology (Brownlie and Munro, 2016), ballistics (Pankowski et al., 2018), biometrics (Hiby et al., 2009), DNA analysis (Garofalo, 2021), digital (Haas, 2023), and accounting (Viollaz et al., 2018) shed light on high risk species and regions, exploited trade and financial routes, and methods of trapping and killing. With weak or lack of evidence cited as a limiting factor in the progression of prosecution and convictions in wildlife crimes (Salum et al., 2017b), the use of forensic evidence has the potential to provide law enforcement a much needed lifeline to improve in this area. As discussed in section 1.1 by far the most applied forensic techniques is the use of DNA barcoding. The importance of wildlife forensics, and enthusiasm for its development within relevant stakeholder groups is underpinned by its continued contribution to positive outcomes in real world case work. As such, significant funding has been put into building relevant infrastructure, such as the recently (2023) built laboratory in Malawi (TRAFFIC, 2023), to help law enforcement take advantage of this burgeoning area of forensics. However wildlife DNA analysis is expensive and therefore despite these investments, as of July 2023 CITES has a directory of just 13 dedicated wildlife forensic labs existing globally (CITES, 2023), a limiting factor for an inherently global criminal activity such as the IWT. Positively, of these, 10 are accredited to ISO 17025 the international recognised quality assurance standard of competency for units undertaking forensic analysis in range of disciplines (Ross and Neuteboom, 2022). By comparison a search of UKAS ISO 17025 accredited labs capable of analysing human “DNA crime scene stains”, produces six results in the UK alone (UKAS, 2024) and over 70 countries

possess a national DNA database or report the use of human forensic DNA analysis in criminal investigations (Amankwaa and McCartney, 2021).

Several wildlife forensic disciplines mirror their human counterparts and have developed in parallel, with similar timelines existing for the exploration of using DNA fingerprinting to carry out individual identification in both humans and animals; the latter first published in relation to birds (Burke and Bruford, 1987) just two years after the introduction of the concept in human focused forensics. As forensic techniques have become more accurate and sensitive both subjects have delved into the recovery of increasingly smaller amounts of DNA, known as “touch” or “trace” DNA allowing for forensic identification of both humans and wildlife to take place in the absence of more traditional sample types such as blood or hair (van Oorschot and Jones, 1997; Chan et al., 2024). As the understanding of the nuances of wildlife crime have broadened it is clear its impact travels far beyond the ecological and environmental spheres it has historically been discussed within. Wildlife crime has reportedly been linked to zoonotic disease risk (Bezerra-Santos et al., 2021), drugs and, arms trafficking (van Uhm et al., 2021; Anagnostou and Doberstein, 2022), terrorism, and increased risk of violence (both by and towards perpetrators (Büscher, 2018)) and economic instability (Cardoso et al., 2021; Massé et al., 2021). Therefore, it has become increasingly important to identify the players driving and facilitating these crimes and provide strong evidentiary links between wildlife crimes and other organised criminal activity. In this vein the recovery of human forensic evidence becomes pertinent as human involvement is a factor that transcends all crime types, something that wildlife forensics cannot achieve in isolation. Similarly, wildlife and its derivatives are the common thread within wildlife casework presenting not only as the “victim” of the crime, but also as a piece of evidence, and a surface on which evidence can be collected from. Though wildlife crime is inherently linked to violent activity it is unlikely that evidence such as human blood, an ideal candidate

for DNA profiling, will be present on a specimen or at a crime scene. What can be assumed in many cases is that the wildlife has been handled, either by a singular or multiple individuals at varying stages of a criminal act and/or trade route, including but not limited to acquisition, transport, and delivery. Consequently “touch” DNA, DNA sources which are invisible to the naked eye and, potentially deposited during handling of an object, becomes an informed candidate for targeted recovery from wildlife. “Touch” DNA is normally recovered in very small quantities, impacted by degradation, inhibition, time and, environmental factors, and therefore presents as a challenging evidence type for analysis in forensic science (Burrill et al., 2019). Efficacy of recovery methods are influenced by surface type characteristics such as porosity and texture (Alketbi and Goodwin, 2019b) and recommendations on most appropriate techniques are often developed based on commonly encountered evidence types such as windowsills, weapons and, clothing (Barash et al., 2010; Dziak et al., 2018). Wildlife specimens present as surface types which the average law enforcement officer will have rarely encountered, particularly urban workforces, and as such they may struggle to apply existing recommendations into these more unique contexts.

This study aims to compare four DNA recovery methods to ascertain whether human trace DNA can be recovered from a range of wildlife specimens and if any of the tested methods present as superior candidates for inclusion in standard wildlife crime scene protocol training or forensic kits. If successful these findings will provide an additional and informed tool for evidence recovery in wildlife crime investigations, open up opportunities for identification of human perpetrators, and allow for greater collaboration between law enforcement agencies involved in investigations into organised criminal activity.

5.2 Materials & Methods

5.2.1 Substrate preparation

Six wildlife derivatives, deer antler, snake skin, elephant skin, conch shell, elephant ivory and antelope fur were sourced from the ZSL Biobank archive. Specimens were chosen as common representatives of wildlife goods seized as illegal trade commodities. Additionally results from Chapter 4 demonstrated that despite powdering failing to yield significant numbers of high-quality fingermarks on deer antler, snake skin, elephant skin, conch shell, and antelope fur, evidence of handling was consistent making them candidates for trace DNA recovery. Each specimen was divided into four sampling areas of equitable surface area size. The surface texture of the ventral of the elephant skin, snake skin, and antelope fur presented differently to the dorsal side (which represents the outward facing surface in a live or whole specimen) and as such only the dorsal side of these specimens was sampled in this study. In the case of snake and elephant skin this was the same side sampled during Chapter 4 (Figure 4f & 4i respectively) but an alternative side, fur presenting, for antelope fur. Prior to any deposition session each specimen was cleaned with the surface decontaminant DNA AWAY™, which Chapter 2 has shown to be effective at removing trace DNA, then left to air dry for at least one hour. This process was repeated post sampling session and then specimens stored in air tight boxes, also cleaned with DNA AWAY™ between sessions.

5.2.2 DNA deposition

Forty participants were ethically recruited for this study (Liverpool John Moores University ethical approval reference 21/PBS/004) and randomly divided into ten groups of four. Participants did not carry out any handwashing or other preparatory steps to emulate true trace DNA depositions. Wildlife specimens were displayed on a table and a group of four

participants were asked to stand in front of a specimen of their choice. For antler, ivory, and conch shells participants were asked to pick up the object in front of them and handle it in its entirety for one minute total with the only instruction to ensure they make contact with all four sampling areas. For snakeskin, elephant skin, and antelope fur, as only one side of the object was being sampled participants were asked to handle only the visibly presented dorsal surface. After one minute of handling participants were asked to move to the object on their immediate left until all participants had handled all objects once in the same session. This process was repeated for all ten groups of participants. The decision was made to have multiple handlers for each item to maximise the likelihood of sufficient quantities of trace DNA being deposited and subsequently recovered to allow for statistical comparison of recovery methods to take place as well as conduct mixed DNA analysis. As such buccal swabs for the purpose of participant DNA profiling were collected from each participant by having them rub an Whatman™ buccal omni swab on the inside of both cheeks. As per manufacturer design post swabbing, swab heads were immediately ejected from their handles directly into microcentrifuge tubes.

5.2.3 Recovery methods

Four recovery techniques were trialled; forensic grade MW104 cotton swab (SceneSafe™), 4520CS01 flocked swab (Copan™), sterile foam tipped applicator swab (Whatman™), and minitapes (SceneSafe™), (Figure 5.1). The sampling area for each method was randomised across groups and specimens. This was done to remove any potential bias caused by certain areas of a specimen being repetitively handled more excessively than others due to being more natural points of contact. The differing shapes of each of the three swabs meant slight variations on swabbing technique were required to ensure the full surface area of the swab was utilised.

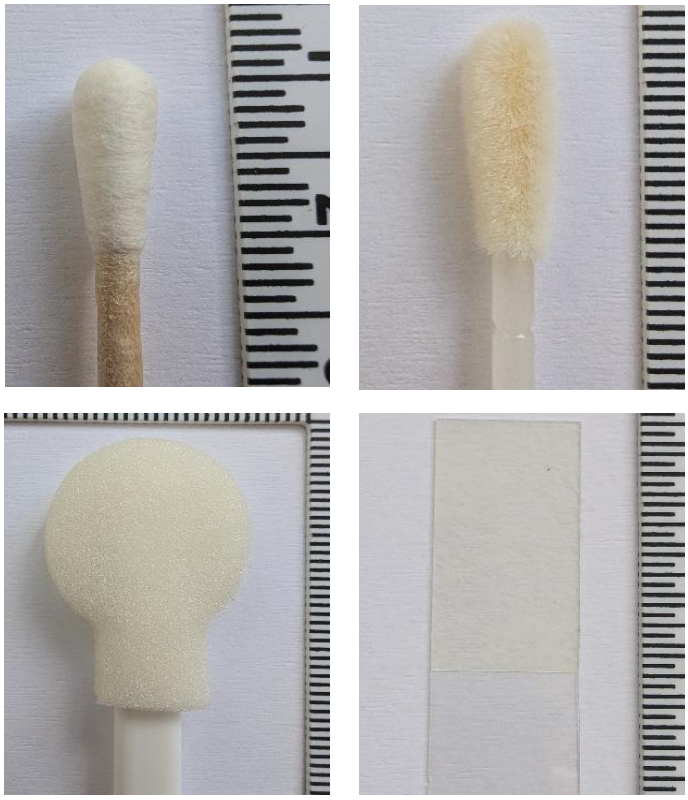

Figure 5.1: Images of each tested DNA recovery method, (L-R); SceneSafe™ forensic grade MW104 cotton, Copan 4520CS01 flocked, Whatman sterile foam tipped applicators, and SceneSafe™ minitapes.

A wet-dry approach was taken for cotton swabs, with sterile distilled water used to moisten the wet swab. This was then drawn across the sampling area using even pressure whilst rotating the swab then followed by a dry swab drawn in a direction perpendicular to the first. The flocked swabs had a flattened structure with two larger surfaces flanked by smaller sides, sterile distilled water was dropped onto one large side of the swab head and drawn across the sampling area in the same manner as the cotton swabs but without rotation. The swab head was then flipped and the process repeated perpendicular to the first swabbing direction. A similar approach was used for the foam swabs whereby one side was wetted and drawn across the area before flipping and repeating. Foam swabs demonstrated more water resistance than cotton or flocked and sterile distilled water had to be applied in smaller quantities spread across the surface area of the swab head and then allowed additional time to absorb into the swab material prior to sampling. Mini tapes were applied to the sample area working from the centre of the area outwards in a spiral pattern,

stopping once the entire sampling area was covered or the tape lost tack, which ever took place first. Flock swab tips were ejected into sterile microcentrifuge tubes immediately after swabbing, wet and dry cotton swab pairs were grouped and placed in their entirety including wooden handle into one sterile transport tube, foam swabs were placed in their entirety into sterile transport tubes and tapes were placed in their entirety into their original sterile containers. All samples were subsequently stored at -20^{o}C until extracted.

5.2.4 Extraction, Quantification, Amplification and STR profiling.

Foam, and cotton swab heads were separated from their handles using a sterile scalpel and placed into microcentrifuge tubes, paired wet/dry cotton swab heads were placed in a single tube. The sampling end of each minitape was separated from the non-tack handling end using a sterile scalpel and pushed to the bottom of a microcentrifuge tube with the sampling side facing inwards. Flocked and buccal swabs remained in the microcentrifuge tube they were placed in at the point of sampling. For all samples DNA was extracted using the QIAamp® DNA Investigator kit, as per manufacturer recommendations, QIAshredder spin columns were included as a step in the extraction process for cotton, foam, and flocked swabs and the upper quantity of recommended buffers and reagents used as a necessity to ensure full sample immersion. Quantification was carried out on all samples using the Qiagen Investigator Quantiplex Pro RGQ Kit (Qiagen) following manufacturers recommended protocol using a Rotor-Gene Q 5-Plex HRM (Qiagen) and quality analysis carried out using Qiagen Q-Rex software and Quantification Assay Data Handling and STR Setup Tool v 4.3. Out of *N* = 240 trace DNA samples *N= 11* resulted in failed reactions and did not produce any quantification data for the internal control, with the same results seen upon reruns of the samples. The concentrations of the remaining *N* = 229 samples were used in statistical analysis to compare the efficacy of DNA recovery methods under

investigation in this study. To confirm the presence of donor alleles, and absence of contamination, a sub-set of samples were taken forward for STR profiling, these represented the two highest DNA concentration samples for each substrate/method combination (*N* = 48). Of these *N* = 43 were successfully amplified. STR Amplification was carried out using the Qiagen Investigator 24plex QS Kit (25µl reaction volumes). The same protocol for STR profiling that was used in Chapter 3 and described in 3.2.5 was repeated for successfully amplified samples in this chapter. In addition to using EuroForMix to estimate MNOC a manual calculation was carried out by looking at the maximum number of alleles at any given loci in the profile. Propositions were repeated four times for each sample, subbing in each individual relevant group participant for the POI in each subsequent repeat (Table 5.1).

Table 5.1. Propositions taken into consideration in LR calculations, representing the positions of the prosecution (H_p) and defence (H_d).

Hypothesis	Description
H_p (Prosecution)	The DNA originated from the POI (1 of 4 possible depositing participants) and $N-1$ unknown contributors.
H_d (Defence)	The DNA originated from N unknown contributors.

5.2.5 Statistical analysis

A Shapiro-Wilk test was performed to assess normality within DNA concentrations of the assessed dataset (*N* = 229), results showed significant departure from normality (W = 0.48, p-value < 0.001). Mean, median and IQR were subsequently used as exploratory values and non-parametric tests used to analyse relationships between variables. A Kruskal-Wallis rank sum test followed by post hoc pair-wise comparisons was carried out to compare recovery methods for each individual specimen type as well as across pooled data. A second Shapiro-Wilk test was carried out on the dependent variable, number of alleles, for amplified samples (*N* = 43) and found data to be normally distributed (W = 0.97, p-value = 0.30). Mean

and standard deviations were used as summarising variables for the data and an ANOVA carried out to compare variances across influencing variables.

5.3 Results

5.3.1 Evaluation of DNA recovery by tested method types

Trace DNA collection using foam swabs resulted in the highest average yield of DNA across pooled samples followed, in descending order, by flocked swabs, cotton swabs, and minitapes (Figure 5.2). A Kruskal-Wallis test demonstrated there was a statistically significant difference in quantities of DNA recovered when comparing recovery methods (H(3) = 27.23, *p-value = 5.252e-06*). A post hoc pairwise comparison using Wilcoxon rank sum test with Bonferroni continuity correction found this significance was applicable to foam vs cotton (*p-value = 0.00026*), foam vs minitape (*p-value = 4.7e-06*) and flocked vs minitape (*p-value = 0.02*) relationships but not cotton vs minitape (*p-value = 0.15*), foam vs flocked (*p-value = 0.07*), or cotton vs flocked (*p-value = 0.16*). Out of *N = 25* samples which either failed or displayed possible inhibition 44% were collected using cotton swabs, 32% using foam swabs, 16% using flocked swabs and 8% using mini- tapes.

5.3.2 Evaluation of DNA recovery from different specimen types

A Kruskal Wallis test found no significant difference in average DNA concentrations recovered across specimens (*N* = 229, H(5) = 4.97 , *p-value = 0.42)*. Highest average DNA concentrations were recovered from ivory (*N* = 38, 150.40 pg/µl), conch (*N* = 39, 80.48 pg/µl) and antler (*N* = 40, 90.20 pg/µl) and lowest from elephant skin (*N* = 36, 34.84 pg/µl), antelope fur (*N* = 38, 25.67pg/µl) and snake skin (*N* = 38, 35.90 pg/µl) (Table 5.1). Of failed and possibly inhibited samples 32% were recovered from the surface of conch shell, 20% each from ivory and elephant skin, 12% from fur and 8% each from antler and snake skin.

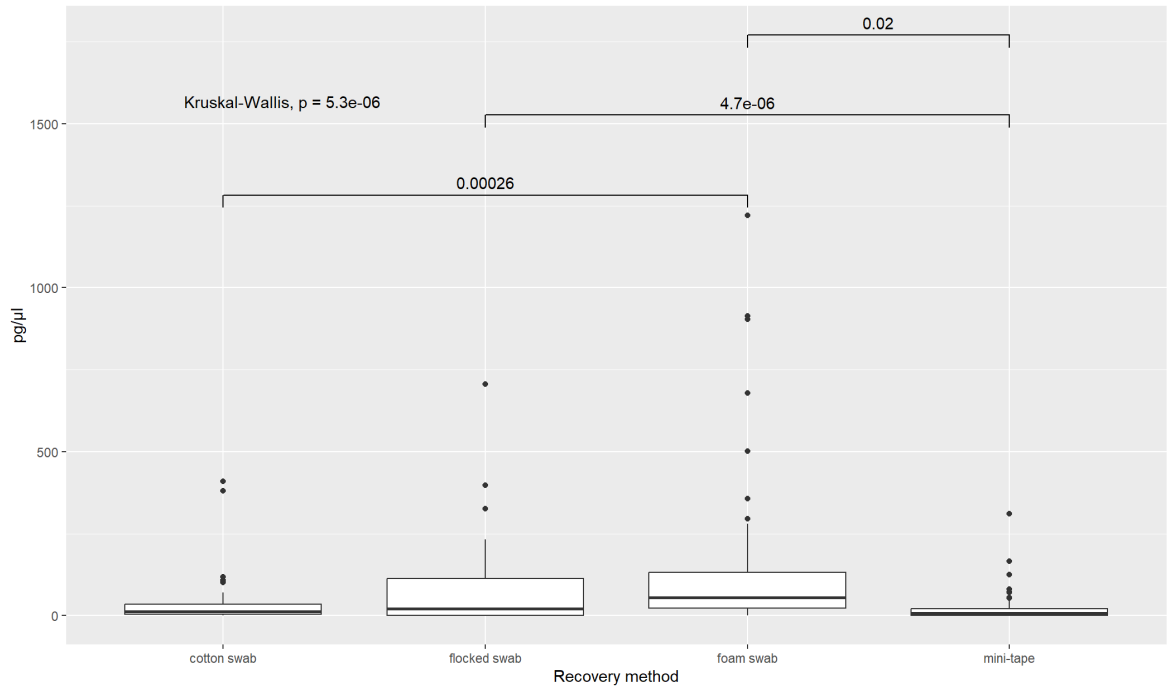

Figure 5.2. Boxplot showing distribution of DNA concentration (pg/µl) for each recovery method (cotton swab, flocked swab, foam swab and mini-tape). Outliers are shown as individual data points. Results of Kruskal Walis tests between recovery methods is displayed in the top left corner (p-value = 5.252e-06), along with corresponding statistically significant relationships, only, between recovery methods, identified through post-hoc testing. .

5.3.3 Evaluation of swab types within specimens

Foam swabs recovered the highest mean DNA concentration for both antler and conch, followed in descending order by flocked swabs, cotton swab and minitape for conch and minitape, flocked swabs and cotton swabs for antler. Both displayed extreme outliers (Figure 5.3), and a review of median values showed that when accounting for these a flocked swab was the best performing recovery type on both substrates (Table 5.2). A Kruskal Wallis test found no significant difference in DNA yield between recovery methods for samples from either antler (H(3) = 0.42, *p-value = 0.94*) or conch (H(3) = 6.5, *p-value = 0.09*). Results from elephant skin and snake skin revealed similar total average DNA concentrations recovered (Table 5.2) and method recovery efficacy presenting in the order of foam, flocked, minitape, and cotton swabs from highest to lowest recovery rate respectively for both specimens. Kruskal-Wallis rank sum tests found significant statistical

differences in DNA quantities dependent on method for elephant skin (H(3) = 8.86 , p-value < 0.05) but not snake skin (H(3) = 7.7, p-value = 0.05). Post hoc pairwise comparisons using Wilcoxon rank sum tests with Bonferroni continuity correction found this significance *applicable* to only foam vs cotton (p-value < 0.05) and foam vs minitape methods (p-value < 0.05).

Sampling from antelope fur resulted in the lowest average DNA yields across all specimen types with foam swabs recovering the highest average DNA quantities. A Kruskal-Wallis rank sum test found statistically significant differences between recovery methods (H(3) = 12.858, p-value < 0.01). A post hoc test pairwise comparison using Wilcoxon rank sum tests with Bonferroni continuity correction found this was only applicable to foam swabs vs minitapes (p-value = 0.0046) (Figure 5.3).

Table 5.2. Mean ($\bar{x}$), median (M), and interquartile range (IQR) of DNA concentrations(pg/µl) recovered for each combination of specimen type and tested recovery method.

	Recovery method											
	Cotton swab			*Flocked swab*			*Foam swab*			*Mini tape*		
Specimen	$\bar{x}$	M	IQR	$\bar{x}$	M	IQR	$\bar{x}$	M	IQR	$\bar{x}$	M	IQR
Antler	69.4	28.2	56.8	78.06	64.6	154.7	130.19	34.0	130.1	83.15	63.4	96.4
Conch	29.9	21.1	37.6	112.35	115.8	187.4	168.48	62.1	142.1	14.37	6.1	8.6
Elephant skin	6.8	3.1	11.7	50.63	24.0	86.9	60.84	50.7	60.0	15.26	14.4	18.8
Antelope fur	27.7	6.5	10.7	22.53	17.7	25.0	47.93	28.2	33.5	4.67	4.7	7.2
Ivory	60.3	14.1	27.6	157.25	67.9	170.6	396.00	236.2	384.0	3.60	3.2	3.1
Snake skin	16.7	9.6	12.4	38.22	20.7	64.9	68.32	76.9	61.6	21.70	18.3	30.1

Mean and median DNA quantity recovered from ivory was highest comparable to all other specimen types. Recovery from ivory using foam swabs also resulted in the highest average concentration for all recovery methods across all specimens. The use of minitapes on ivory

also presented the lowest average concentration for all recovery methods across all specimens. Within ivory samples a Kruskal-Wallis rank sum test found statistically significant differences between recovery methods ($H(3) = 8.96$, p-value = 0.03). A post hoc pairwise comparisons using Wilcoxon rank sum tests with Bonferroni continuity correction did not identify any one specific relationship between recovery methods to explain this.

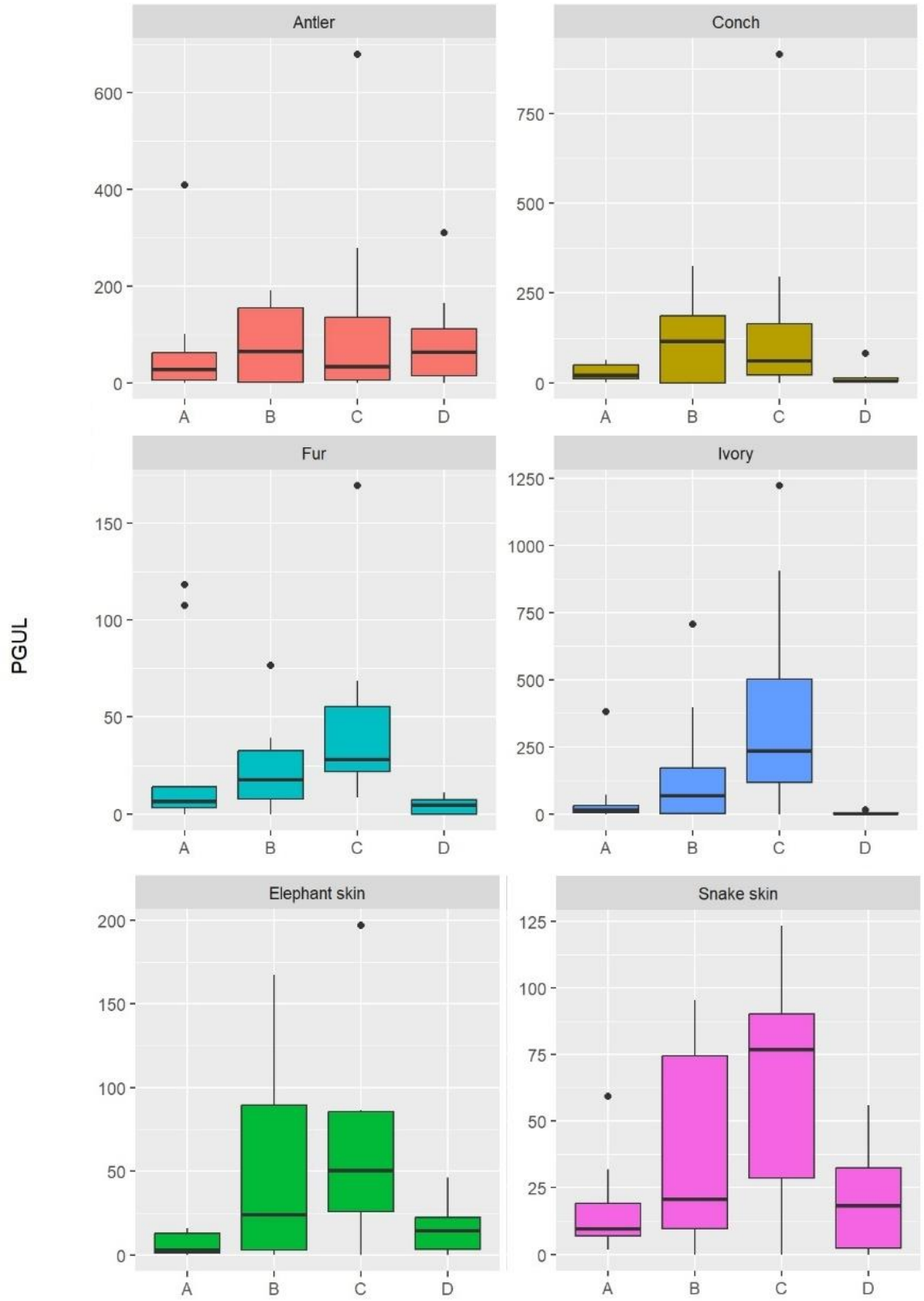

Figure 5.3. Box and whisker plots showing distribution of DNA concentrations (pg/µl) recovered by each recovery method (A - cotton swab, B – flocked swab, C – foam swab, D – minitape) for individual specimen types. Clockwise from top left: antler, conch, elephant skin, snake skin, ivory, antelope fur. Black data points are representative of potential outliers.

5.3.4 STR profiling

Out of *N = 48* samples taken forward for profiling *N = 5* resulted in failed PCR, a repeat run did not solve the problem. This resulted in only *N = 43* samples being taken forward for further analysis. Average number of amplified alleles were calculated for each recovery method. The highest average number of alleles were recovered in descending order of foam swab, cotton swab, flocked swab and minitapes (Table 5.3). An ANOVA found no significant difference in the average number of alleles recovered by method type.

Table 5.3: Mean ($\bar{x}$) and standard deviations (sd) for number of alleles recovered for each specimen and recovery type combination. Missing values indicate where standard deviations could not be calculated.

	Specimen												
Recovery	Antler		Conch		Elephant skin		Antelope fur		Ivory		Snake skin		Aggregate
method	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$
Cotton swab	71	23	74	6	53	NA	61	28	53	28	45	28	60
Flocked swab	85	25	49	33	55	28	88	11	19	4	63	26	60
Foam swab	60	-	61	12	68	36	74	13	-	-	89	4	69
Minitape	93	18	44	-	46	-	32	21	13	1	47	6	46
Aggregate	80	20	59	19	57	22	63	26	30	22	61	24	59

An ANOVA test found a significant difference in average number of alleles recovered from specimens ($F(5, 37) = 3.50$, *p-value = 0.011)). P*ost hoc testing via revealing a significantly lower number of alleles recovered from ivory, comparable to antler ($p < 0.001$) and antelope fur ($p < 0.05$) (Figure 5.4).

All samples presented evidence of degradation, and its presence was recommended for inclusion into best fit models identified by EuroForMix. Calculation and visualisation of peak height summaries further confirmed this assessment (Figure 5.5).

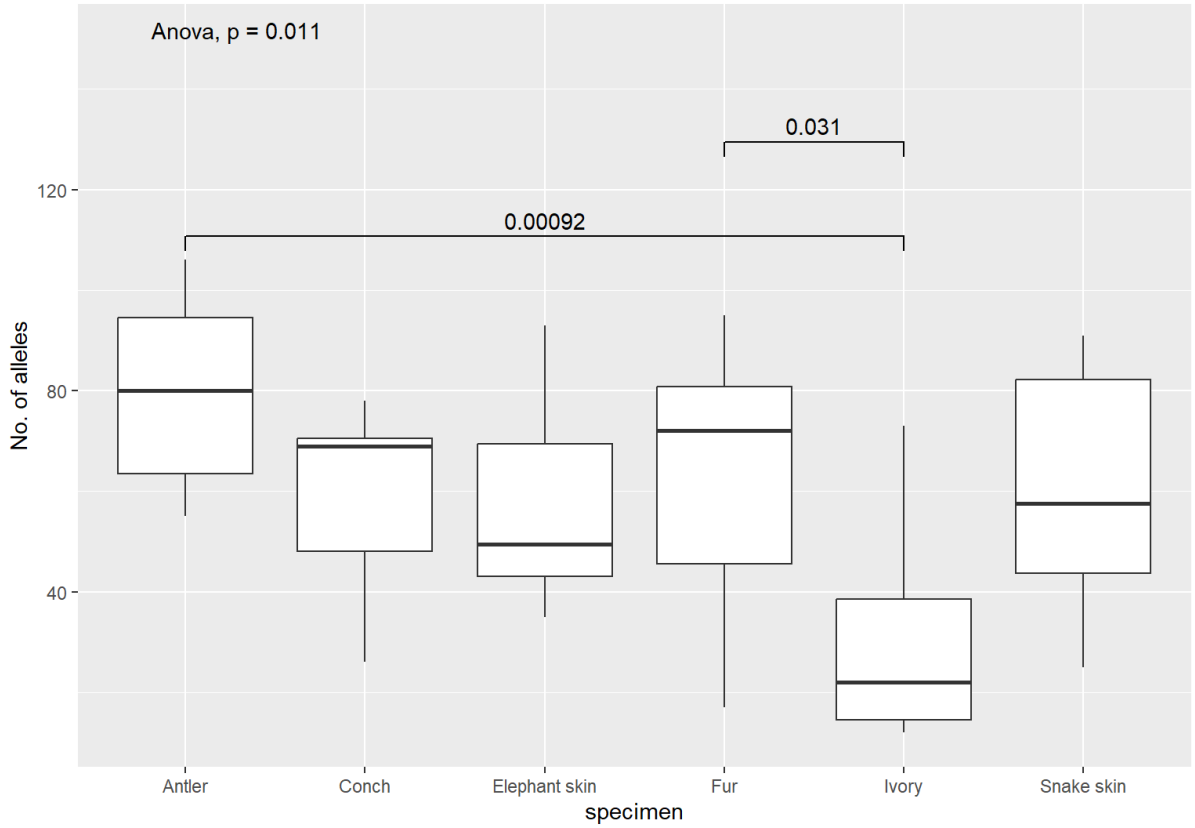

Figure 5.4. Box and whisker plots showing the distribution of allele counts recovered from individual specimen types. P-value of an ANOVA test between specimen types is displayed in the top left corner along with statistically significant differences, only, in allele counts recovered from specimen type, identified through posthoc testing using ivory as a control group.

Peak height ratios of internal PCR controls QS were calculated and from this analysis PCR inhibition was identified in *N = 14* profiled samples (Figure 5.6), the majority of which derived from swabs taken from ivory (Table 5.4).

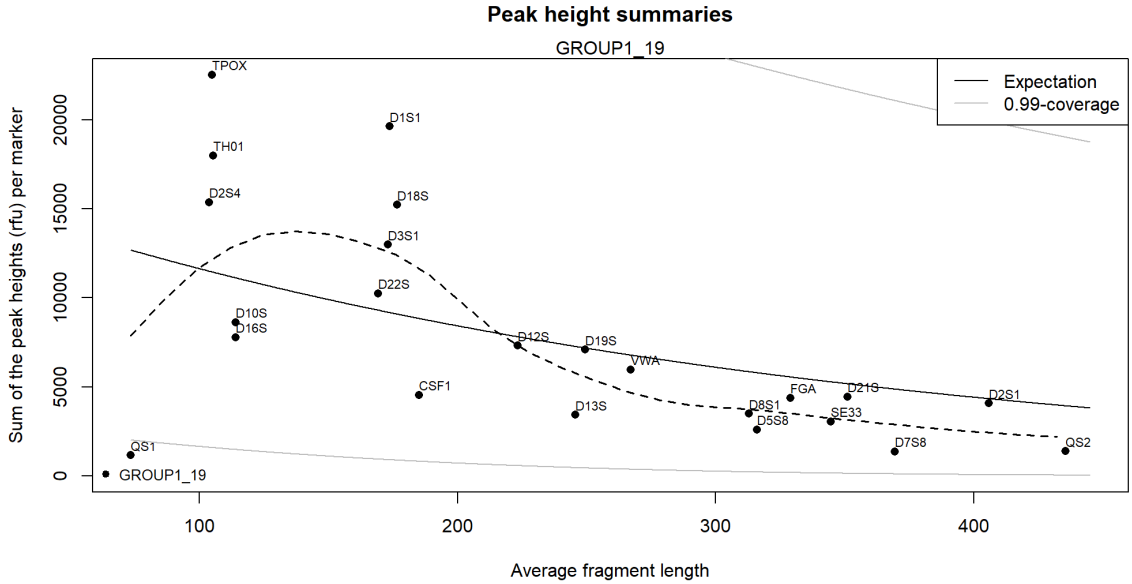

Figure 5.5. Scatter graph of sum of peak heights produced at each marker in relative fluorescence units (RFU) plotted against the average fragment length of marker for a random sample from the Chapter 5 dataset. The presence and height balance of QS1 and QS2 markers suggest the absence of inhibitors during PCR. The downward trend in peak heights with increasing fragment length indicates the presence of degradation within the sample.

MNOC were estimated for each sample by looking at the maximum number of alleles at any given loci. Two samples were estimated to have a minimum of *N* = 5 contributors (Figure 5.7). These were subsequently excluded from analysis using EuroForMix which has a suggested maximum capacity of four unknown contributors in a model due to excessive computing times. Match rates and LRs based on propositions outlined in 5.2.4 were calculated using EuroForMix. Of the *N* = 41 samples analysed *N* = 19 produced an LR equivalent to a qualifying statement of "Very strong evidence" for the H_p proposition outlined in Table 5.1, for at least one of the four participants who handled the item in question (Figure 5.8).

Table 5.4. Total DNA quantities (pg/µl), the maximum likelihood ratio (log10(LR) achieved by a single contributor and the descriptive equivalent for amplified samples which either failed or displayed PCR inhibition.

Specimen	Method	Total pg/µl	Max log10(LR)	Descriptive equivalent	PCR diagnosis
Conch	Foam swab	914.42	12.05	Very strong support	Inhibition
	Flocked swab	326.23	2.882	Moderate support	Inhibition
	Mini tape	16.69	N/A	Fail	Failed PCR
Elephant skin	Flocked swab	167.65	1.467	Limited support	Inhibition
	Cotton swab	13.41	3.441	Moderate support	Inhibition
	Foam swab	196.90	20	Very strong support	Inhibition
	Mini tape	25.21	N/A	Fail	Failed PCR
	Cotton swab	16.09	N/A	Fail	Failed PCR
Ivory	Cotton swab	381.37	3.393	Moderate support	Inhibition
	Mini tape	15.47	1.678	Limited support	Inhibition
	Flocked swab	127.89	0.8277	Limited support	Inhibition
	Flocked swab	706.78	8.648	Very strong support	Inhibition
	Mini tape	4.38	4.128	Strong support	Inhibition
	Foam swab	117.49	N/A	Fail	Failed PCR
Snake skin	Cotton swab	59.3	7.762	Very strong support	Inhibition
	Flocked swab	92.22	6.233	Very strong support	Inhibition
Antler	Cotton swab	102.32	12.32	Very strong support	Inhibition
	Foam swab	103.65	N/A	Fail	Failed PCR
Fur	Mini tape	9.87	0.1472	Uninformative	Inhibition

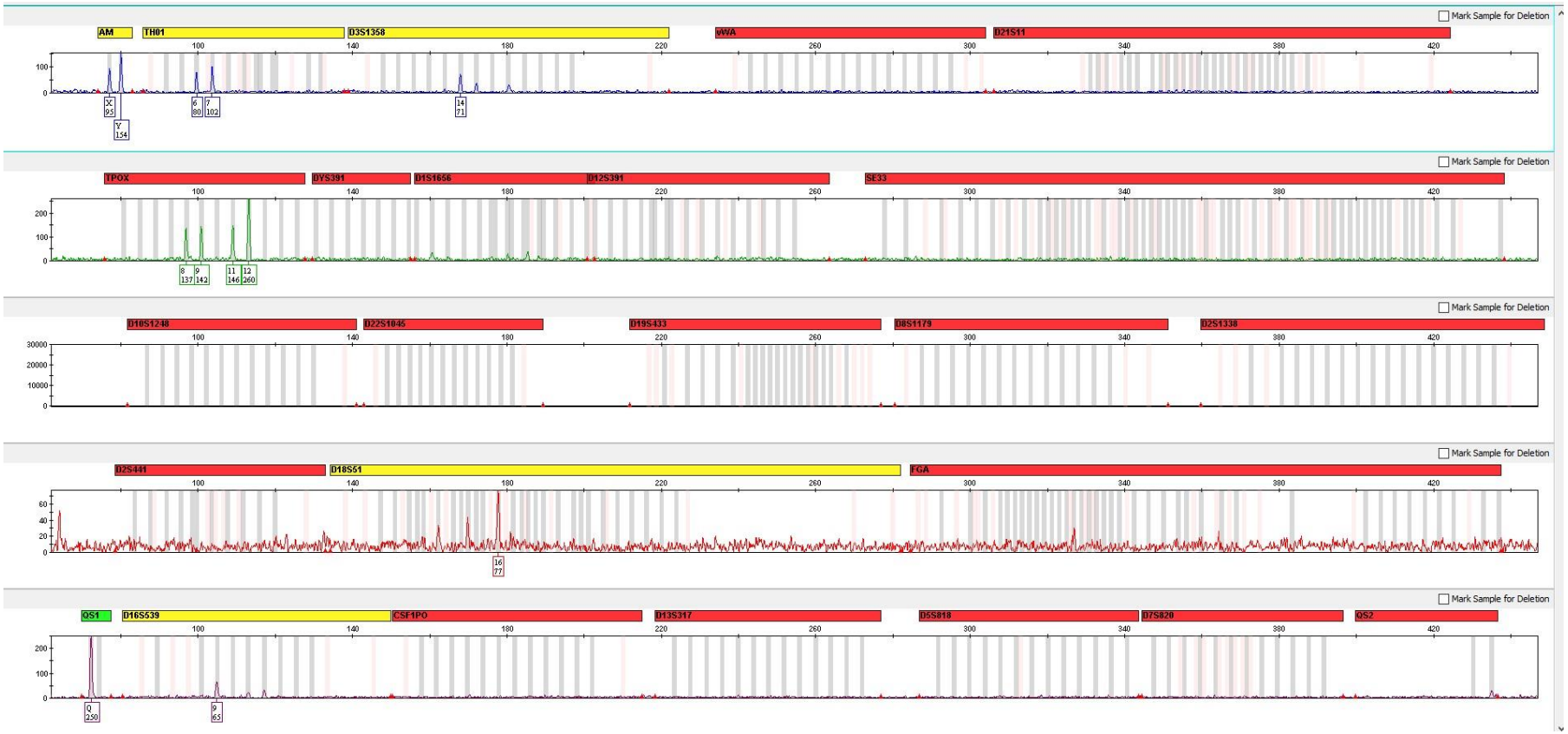

Figure 5.6. An electropherogram produced using GeneMapper™ software showcasing a mixed DNA profile recovered from ivory using minitape. Amplification of four alleles at the TPOX locus suggests places the MNOC at an estimated two donors. The presence of the quality sensory (QS) 1 peak but absence of the second QS peak indicate inhibition during PCR.

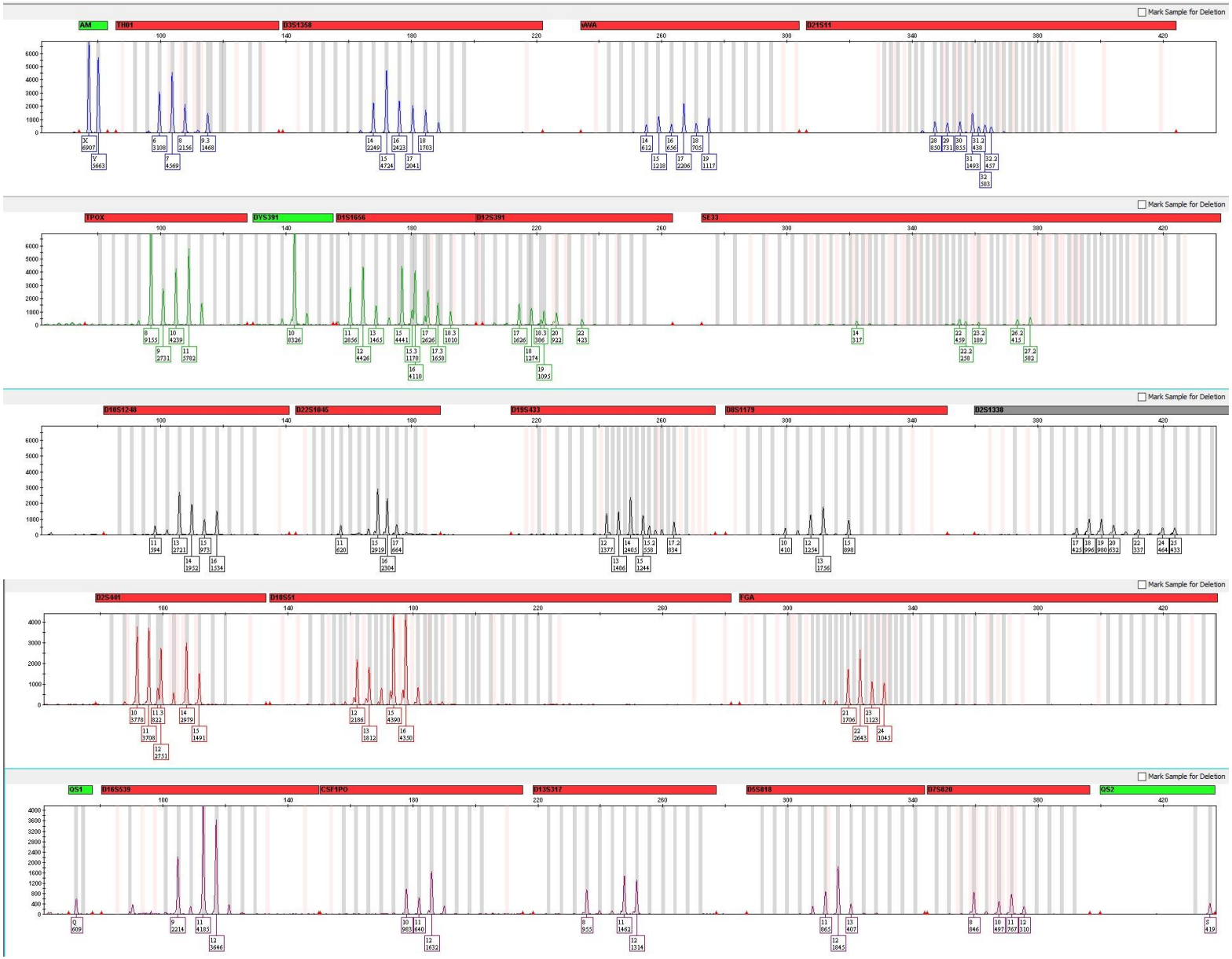

Figure 5.7. An electropherogram produced using GeneMapper™ software showcasing a mixed DNA profile recovered from an antler using minitape. Nine alleles are present at the D1S1656 locus leading to an estimation of a minimum of five contributors to the sample. Presence and balance of both QS peaks indicates a successful PCR without inhibition.

A linear model was fitted to predict LR with match rates and showed match rates had a statistically significant effect and explained a substantial proportion of variance ($R^2 = 0.28$, $F(1, 166) = 64.23$, $p < .001$ (Figure 5.8).

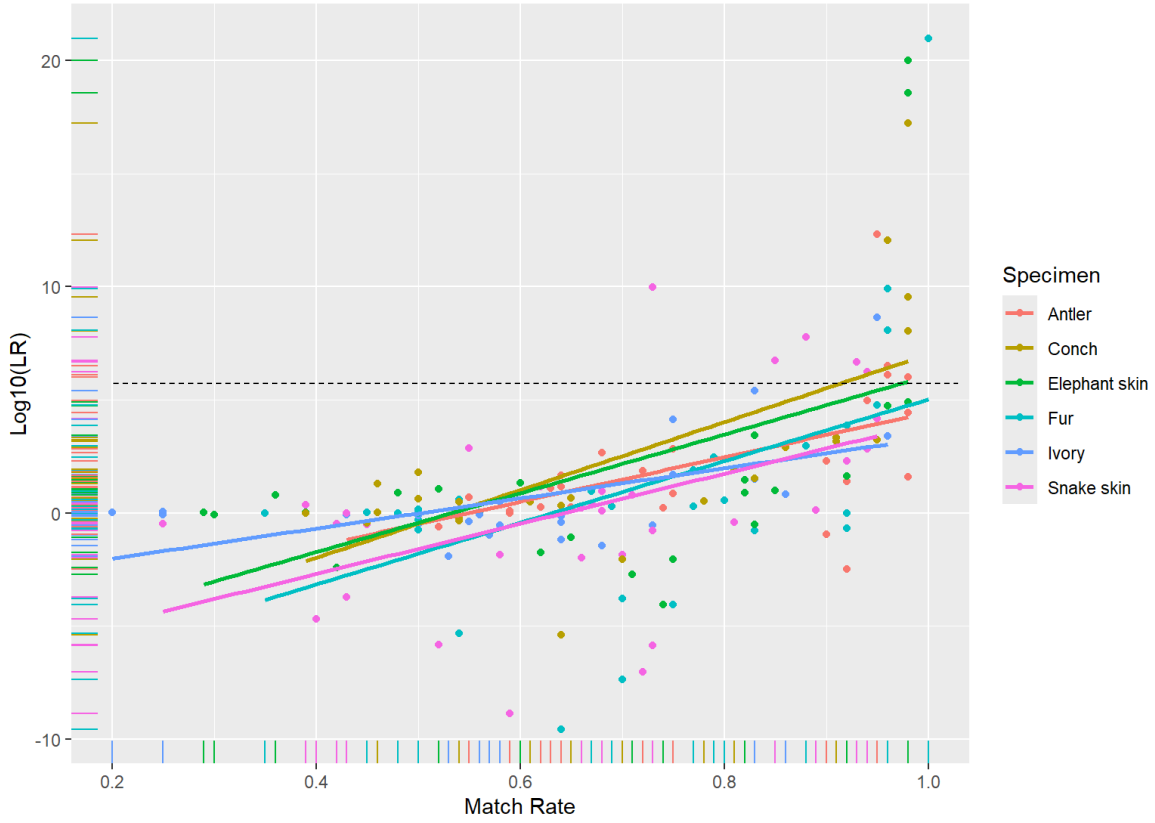

Figure 5.8. Scatter pot of match rate against likelihood ratio (Log10(LR)) including regression lines for each specimen type. The Log10(LR) value threshold (y = 6), above which a verbal qualifier of "Very strong support" is awarded is depicted via the dotted line.

5.4 Discussion

5.4.1 Trace DNA recovery from wildlife specimens

Trace DNA samples have historically been challenging to analyse however as processes advanced their adoption into routine criminal investigation has become common place (van Oorschot et al., 2021). When investigating the best method of DNA recovery the same considerations are taken regardless of the surface type being presented, these include but

are not limited to surface porosity and texture (Alketbi and Goodwin, 2019b). In fingermark research, an area with greater focus than trace DNA recovery in the context of wildlife crime (Thomas et al., 2023), substrate porosity assignation is three tiered, either porous, semi-porous, or non-porous (Home Office, 2022). Leather, the most common animal product encountered, is considered semi-porous (Home Office, 2022) and this term has also been used to describe other biological materials (Weston-Ford et al., 2016). Other studies indicate there is at least some level of porosity in ivory (Vollrath et al., 2018), conch (Hou et al., 2004), reptile skin (Weir et al., 2016) and antler (Evans et al., 2005). The semi-porous categorisation is rarely used in the research literature for trace DNA recovery techniques with substrate receiving either porous or non-porous assignations. Therefore, in the context of this study the tested specimens tested would fall into the "porous" category and further categorised as either smooth (ivory, conch, antelope fur) or rough (snake skin, elephant skin, antler). Jurisdictions across the globe have employed two key recovery techniques as their "go to" methods categorised in their application choice by the porosity of the items. These are the wet/dry cotton swab for non-porous materials, and minitapes for porous materials (Burmuzoska et al., 2022). In keeping with this minitapes have been the chosen recovery method for the few existing studies looking at trace human DNA recovery from wildlife specimens and have successfully been used to recover reportable DNA profiles from deer fur, corvid, buzzard and, hare carcasses (Tobe et al., 2013; Mcleish et al., 2018). However work on live domestic animal fur has employed the wet/dry swabbing method (Monkman et al., 2022, 2023). Whilst this study did successfully recover DNA using both minitapes and wet/dry swabbing techniques they were the worst two performing methods on average and recovered DNA at lower average concentrations comparable to other studies even with the multi contributor factor (Tobe et al., 2013; Monkman et al., 2022, 2023). These traditionally adopted methods outperformed foam or flocked swabs in only

two instances; minitapes outperformed both cotton and flocked swabs, but not foam, for recovery from antlers and wet dry swabbing with cotton swabs outperformed flocked swabs, but not foam, on fur.

A key distinction between this study and the existing studies is the origin of the wildlife derivatives being used; all other studies worked from either fresh carcasses or live animals whereas this study worked from items which had undergone chemical preservation (snake skin, antelope fur and, elephant skin) or had been long since removed from their living counterpart (elephant ivory, conch shell and, antler). Results from this study found that overall, significantly lower quantities of DNA were recovered from the three specimens that had undergone some kind of preservation technique comparable to ivory, conch and, antler which are assumed to have not. Chemical processes used in taxidermy or preservation have been shown to impede DNA analysis of wildlife specimens when attempting species identification (Hall et al., 1997; Hebenstreitova et al., 2024) and leather is known to contain PCR inhibitors derived from the tanning process that impact downstream analysis of human trace DNA (Bright and Petricevic, 2004). Evidence of inhibition was present in profiled samples for all three of the “preserved” specimen types but was most prolific within trace DNA samples recovered from elephant skin, an outcome echoed in quantification data for the full dataset. This analysis also found a significant difference between recovery technique efficacy for elephant skin, snake skin, and antelope fur, with minitapes and/or cotton swabs recovering significantly lower amounts of DNA than foam swabs, a result not seen with the “non preserved” specimens. The results for elephant and snake skin mirror other studies which show minitapes outperform the wet dry swabbing method on porous surfaces with the assumption they more effectively avoid collection of PCR inhibitors (Barash et al., 2010). Indeed, trace DNA samples recovered from the surface of elephant and snake skin using cotton swabs showed higher rates of inhibition comparable to

minitapes in both profiled and quantified datasets, although sample numbers were too low to draw meaningful statistical comparisons. Minitapes did not outperform wet dry swabbing on antelope fur and I hypothesise this may be due to the collection and subsequent introduction of inhibitors such as melanin known to be present in animal hair and fur (Kirkinen et al., 2022; Vajpayee et al., 2023). The only instance of PCR inhibition seen in successfully profiled trace DNA samples from fur was in a sample collected via minitape but was also observed for cotton swab and foam swab collections in the wider quantified dataset. The phenomenon of hair and debris collection by minitapes has been observed by Tobe et al (Tobe et al., 2013) during their deer leg sampling. Though they make no mention of their possible influence on the analysis process the presence of melanin and other inhibitors from collected dirt such as humic acid (Sutlovic et al., 2008) could provide explanation as to their need for a modified protocol. As such PCR inhibition provides a possible theoretical explanation as to why low DNA quantities were consistently recovered from fur, snake skin, and elephant skin, despite the snake and elephant skin being subjectively “rough” surfaces which have been shown to better surfaces for DNA retention and yield (Goray et al., 2010). However the inhibition and failure breakdown within the larger quantified dataset do not fully support this theory as inhibition was observed across the board and most commonly within touch DNA samples recovered from the surface of conch shells Significant further work is required to understand the role inhibition may or may not be playing in these results. Future work will benefit from including multiple examples of each wildlife derivative type to sample form the surface of to ascertain the reproducibility of these results.

A factor that may have been a greater contributor to lower recovered quantities of DNA on skins and fur may was the experimental design. For all items participants were instructed to “handle” the item and left to a subjective interpretation of what this should consist of,

using the “undirected” protocol outlined in Table 3.1. The only distinction given, was that participants were instructed to handle only the visibly presented surface (dorsal side) for these three items, but free to handle the conch, ivory and, antler in their entirety. No further description was given on what “handling” within these parameters should consist of. When observing participants interact with conch, ivory, and antler the items were routinely lifted from the table, moved from hand to hand and turned in hands during the handling period. By contrast participants were observed interacting with the furs and skins through stroking movements. Increased pressure and friction have been found to significantly influence trace DNA depositions (Tobias et al., 2017; Alketbi, 2018; van Oorschot et al., 2019). In this study it is assumed a stroking movement would place less pressure and friction then gripping and turning on a surface which would result in the discrepancies seen in these results. Although a heavy handling technique was not found to be result in a significantly higher quantity of DNA in Chapter 3 it did produce the highest average quantities in part supporting this theory. This interpretation is also in keeping with existing advice to forensic examiners whereby it is important to consider how an object may have been handled when carrying out targeted trace DNA swabbing (Gosch and Courts, 2019; van Oorschot et al., 2019). Leathers and fur are routinely used by the fashion industry and thus may be presented as bags, shoes or wallets, commodities in the IWT (Heinrich et al., 2019; Sosnowski and Petrossian, 2020). In these formats the objects will have been handled in a manner closer to the way ivory, conch, and antler were in this study and thus potentially yield more trace DNA evidence and as such these surface types should not be negated from trace DNA recovery consideration based on the outcome of this study.

An additional factor to consider is that the tanning process can impact leather porosity (Gil et al., 2013). At least one study looking at trace DNA recovery from leather steering wheels classified the surface as a rough, non-porous material (Comte et al., 2019). For these surface

types nylon flocked swabs have been shown to be one of the most effective method of DNA recovery (Alketbi and Goodwin, 2019a). Again this chapter's results mirror these with nylon flocked swabs outperforming both minitape and cotton swabs for elephant and snake skin and notably being outperformed by cotton swabs for fur which, assuming altered porosity, could be considered a smooth, non-porous surface (assuming participants did not go against the grain of the fur when handling) for which cotton swabs are often the recommended recovery type (Verdon et al., 2014a; Hartless et al., 2019). If non-porosity is assumed, I would expect to see cotton swabs outperform minitapes on elephant and snake skin however this discrepancy has already been explained by supposed more efficient collection of PCR inhibitors by cotton swabs.

Whilst minitapes are often recommended over cotton swabs for porous surfaces, it is almost always in the context of absorbent fabrics (Daly et al., 2012; Verdon et al., 2014b; Alketbi, 2022b; Währer et al., 2023). Wooden objects as a porous representative have also been considered and minitapes shown to be more effective than cotton swabs when the wood has been classified as both "rough, porous" (Alketbi and Goodwin, 2019b), and "smooth, porous"(Verdon et al., 2014a). Given the specimens tested in this study fall into both of these categories the averagely poor performance of minitapes is somewhat surprising particularly on ivory, conch, and fur, where they were outperformed by cotton swabs and in the case of ivory and fur low concentrations and allele numbers were observed. Possible explanations include practitioner skill set, inadequate extraction or substrate to tape size ratio, are all known factors affecting effective trace evidence recovery (Verdon et al., 2014b; Wood et al., 2017). However, minitapes recovered the second highest average DNA concentration for samples taken from antlers, at an average concentration higher than 79% of other averages across specimens and recovery methods. Mini tapes also recovered an average of 117 alleles from antler a number higher than 92% of other

specimens and recovery method combinations and demonstrated good allele recovery on substrates, including elephant skin and conch, despite low DNA concentrations. These results suggest experimental design nor operator to be a contributing factor to the results seen from minitapes as they have outperformed cotton swabs on three of the six items and a possible theory is available (PCR inhibition) for their low results for fur has been provided.

Foam swabs are an uncommon trace DNA recovery method in forensics and traditionally used in conjunction with FTA cards for buccal sample collection (Date Chong and Wallin, 2022). Nylon flocked swabs are more recognisable in day to day forensic work but due to their high price are often sidelined for cotton swabs due to budgetary constraints experienced by law enforcement (Budowle et al., 2022). However several studies have demonstrated that both foam and flocked swabs have been found to be equitable or even outperform a variety of swab and minitapes types in a range of different scenarios (Hansson et al., 2009; O'Brien, Robert and Figarelli, Debra, 2012; Verdon et al., 2014a; Hartless et al., 2019; Hedman et al., 2021b; Bruijns, 2024). This study found that, despite their unconventional application as a trace DNA recovery method, foam swabs were a superior recovery type across all specimens, both in terms of DNA quantity and allele average. Whilst this is keeping for the literature on smooth, porous surfaces it goes against existing studies which found foam swabs one of the least effective recovery method on rough, porous surfaces (Verdon et al., 2014a). Flocked swabs presented as a second-tier recovery method in all specimens bar fur for DNA concentrations and resulted in the second highest allele average. However flocked swabs showed a greater variance in allele recovery within specimen types comparable to foam swabs, with minitapes and/or cotton swab either matching or exceeding numbers of alleles recovered in four of the six specimens. By comparison foam swabs did not recover the highest number of alleles for only two of the

specimens, antler, and ivory, being outperformed by minitapes and cotton swabs, respectively.

It has been suggested that the flexibility afforded by the structure of foam swab heads allows greater penetration into porous substrates thus recovering higher quantities of material. Additionally the more open structure of foam swabs may facilitate better DNA release (Wood et al., 2017; Bruijns et al., 2018). The flexible structure may also allow for greater manipulation across uneven surface types, able to penetrate creases and grooves in textured surfaces, such as the elephant skin in this study. Though one existing study found foam swabs ineffective on rough, porous surfaces (Verdon et al., 2014a) brick was used as the representative substrate which can be argued presents a highly skewed degree of roughness and porosity comparable to the specimens included in this study. Conflicting results and guidance on best method recovery for different surface types is common in the literature, and as this is the first instance of comparison of trace DNA recovery methods on wildlife specimens, I do not find this discrepancy unusual.

Finally in this study the sampling area of the foam swab was the largest of all recovery types which may have resulted in a greater quantity of DNA being recoverable in the first instance due to the larger surface area. This theory is also suggested by Hedman et al (Hedman et al., 2021b) who found differences in recovery efficiency between large and small foam swabs. Foam swabs have also been found to perform better than other swab types on large sampling areas of both porous and non-porous surfaces for microbial sampling (Jansson et al., 2020). Whilst swabs and minitapes have been found to be equitable in effectiveness for sampling large areas of fabric (Alketbi, 2022a) to date studies looking into the effects of surface area on performance of recovery methods do not appear to have included foam swabs in their comparisons. Therefore, given their superiority over other swabs on large

sampling areas in other contexts, the sampling size of area may provide additional explanation as to my results. For all specimens bar conch, the area sizes sampled in this study would be considered “large” in the context of the study by Alketbi & Salem (2022). Whilst this does not appear to have been investigated in the literature, I postulate that foam swabs may also be better at isolated recovery of DNA, and avoiding the recovery of PCR inhibitors, comparable to cotton swabs, which is why they performed significantly better on the taxidermy specimens included in this study, an observation also seen in the larger quantified dataset.

Foam swabs have been found to perform significantly better at allele recovery from wooden surfaces (Hartless et al., 2019) however at least one study has found despite foam swabs recovering high amounts of DNA this did not always translate to alleles/profiles (Verdon et al., 2014a). By comparison, this study found the higher concentrations recovered by foam swabs tended to correlate to higher allele recovery. However, my results do report the same phenomenon in one instance, ivory, categorised the same as the wood used in the study, that is as a smooth, porous surface. Observations from ivory samples were the most unexpected of this study; whilst recovered samples had the highest average DNA concentrations across specimens, they had significantly lower number of alleles recovered compared to other specimens and profiled samples showed the highest rate of inhibition compared to all other specimens. None of the recovery methods tested on ivory resulted in the minimum number of alleles expected from a four person mixture (Perez et al., 2011). The reasons for this are unclear as the same outcome was not seen in the conch shell, a visually similar surface type which actually displayed a higher rate of possible inhibition across the quantified dataset. As the low allele count was seen across recovery techniques it suggests an interaction with DNA deposits taking place at substrate level. In species identification work using ivory as raw material calcium is seen as a risk factor for PCR

inhibition (Kitpipit et al., 2016) however this is unlikely to have been a contributing factor in the context of this study due to the non-destructive trace DNA recovery methods used. However, it should not be ignored that inhibition and reaction failure was most common amongst samples taken from the surface of two derivatives with high calcium levels, conch, and ivory. PCR inhibition by way of humic acid presents as a more likely scenario for the high rates of inhibition seen in both elephant derived products tested in this study. Humic acid is a known PCR inhibitor found in soil and plant materials and efforts are made to remove it or develop assays that can withstand its presence in the context of forensic casework (Coutu et al., 2016; Potoczniak et al., 2020). Elephants are routinely observed using their tusks to dig into the ground, seeking water, food sources and to loosen soil which they then apply to their skin for the proposed purpose of sun protection. In fact it is humic acid which may play a significant role in making this behaviour a viable method of UV protection (Kaiser et al., 2019). Therefore, the presence of humic acid is highly possible in raw, unwashed samples; in the context of this study humic acid may be present in the pores of ivory however its lack of water solubility (Klučáková and Pekař, 2005) raises the question as to whether the swabbing techniques used would reliably recover it from the pores. Further work is needed to identify the inhibitors at play in trace DNA samples recovered from ivory and compare these to other wildlife derivatives. The results seen in this study highlight the importance of looking beyond just quantification results when carrying out recovery method comparisons particularly on novel substrates.

5.4.2 Feasibility of real-world application

It is estimated there are over one hundred countries who either have DNA database for forensic investigation either already in place or in some stage of development (Machado and Granja, 2020). However, these databases contain fewer profiles comparable to AFIS.

Combined DNA Index System (CODIS) is the FBI's main software for management of DNA profiles; the National DNA Index System (NDIS) contains DNA profiles contributed by enforcement and is a facet of the larger CODIS program. In April 2021, the twenty millionth DNA profile was uploaded to the database, this is only ~13% of the number of fingerprint profiles available on IAFIS although it should be noted that profiles stored via NDIS are almost exclusively criminal offenders unlike IAFIS. The UK's system known as the National DNA Database (NDNAD) contained ~2.5 million profiles as of March 2020 (NPCC, 2020), around 30% of the number held in IDENT1. These lower numbers of searchable profiles suggest DNA retrieval from a wildlife crime without a POI to compare against may not have as high a chance as matching compared to fingerprint evidence. However Interpol's shared DNA database, which works in a similar capacity to their AIFS, contains around 247,000 profiles, over 20,000 more than their AFIS (INTERPOL, 2021a). Notably for the shared DNA database profiles contain no nominal data and it is at the discretion of the member country in ownership of the profile as to whether they wish to share. Though ethical concerns exist about the possession and sharing of biometric data (Mordini, 2017) there appears to be existing infrastructure that could lend well to the argument of putting in time and resources to retrieving human centric trace evidence from wildlife specimens to help solve associated crimes.

Whilst access to infrastructure and databases may not be barriers to the use of human DNA evidence in wildlife crimes financial resources afforded to investigations may be. Storage and analysis of DNA evidence is costly; operation of the UK's NDNAD is estimated to cost ~£2.5 million annually (Amankwaa and McCartney, 2019) and studies in North America found the average laboratory costs for analysis of DNA evidence to be ~$1000 – 1500 per sample (Davis and Wells, 2019; Wickenheiser, 2021). Enforcement institutes must carry out cost-benefit analysis within their decision to choice of investigative approach. These

assessments will go beyond a linear observation of inputted costs and successful conviction outcomes and encompass physical and emotional harm caused by the criminal activity as well as economic losses such as time off work (Heeks et al., 2018). The value of wildlife crime is routinely reported as being quantifiable to billions, or even trillions of dollars but at a wider scale its long-term impacts transcend monetary value (Wyatt, 2022). Whilst these impacts suggest the investigation and deterrence of wildlife crimes warrants investment for tools such as human DNA analysis, the reality is more complex.

In the UK, the NWCU does not receive permanent funding from the government. It's most recent round of funding bought it through to 2020 but there have been calls to parliament for permanent funding to be received (UK Government, 2020b). Funding specifically for forensic case work is available through PAW, FWG, 'Forensic Analysis Fund"(TRACE, 2021) and the 'Raptor Forensics Fund' to 'support forensic testing in wild raptor crime investigations' was launched by Wildlife Justice in 2020 (Wild Justice, 2020). However, this is currently earmarked and utilised for wildlife species identification not human trace evidence recovery or analysis, again highlighting a narrow vision within forensic interventions in this context. Despite the lack of permanent funding for the NWCU the UK is not averse to funding the protection of wildlife. In 2019 the prime minister announced a £220 million international biodiversity fund (UK Government, 2019a), the Illegal Wildlife Trade Challenge Fund has distributed £26 million between 85 projects since its inception (UK Government, 2020a) and the short-lived Wildlife Crime Tech Challenge fund (2014 – 2017) dedicated six out of ten of its spotlighted projects related to strengthening forensic evidence and data sharing to UK and USA based recipients. However forensic investment is haphazard, a review by SWFS and UNDOC on casework capacity implied a lack of funding and international coordination were major setbacks in the progression of wildlife forensics (UNODC, 2016a) though with almost a decade since this review the landscape may have

changed. In contrast the review of international funding for IWT by World Bank indicated the law enforcement category (which I have interpreted to include wildlife forensic) to be in receipt of the second highest cumulative commitment amount at $253 million (World Bank Group, 2016). However, this category still trailed behind protected areas management which received $609 million or almost half of all funding distributed between 2010-2016. At time of publication forensics was referred to just once as a tool in relation to work undertaken by UNODC within the whole review.

Beyond wildlife crime, a review by the UK Forensic Science Regulator of human centric forensics as a whole stated the lack of funding is putting "justice at risk" (Tully, 2021). Similar opinions have been voiced in more recent years about the threats to the future of forensic science due to the lack of funding (Gallop, 2020; Geddes, 2021), although in 2020 the UK Home Office announced £28.6 million of funding for forensics (Forensic Capability Network, 2021). The UK however has a distinct focus on funding research into digital and cyber projects comparable to more original approaches such as fingerprint and DNA work (Morgan and Levin, 2019). This may explain why wildlife forensic in all its factions is struggling from a perceived lack of funding as whilst innovative the approaches still fall under this "traditional" umbrella.

At a baseline level enforcement possess the knowledge, skills, and infrastructure to implement human trace DNA recovery at wildlife crime scenes and seamlessly integrate it into investigative workflows as with any other crime type. However, given the higher associated costs relative to fingermark recovery there will need to provide sufficient proof of its evidentiary value to encourage the funding required to carry it out. Diversification of the existing forensic funding pots dedicated to species identification would provide vital

support in this area and further encourage enforcement to consider all evidence types available to them.

5.5 Summary

The potential for human trace evidence recovery in wildlife crime cases has been overlooked as an investigative avenue. This study shows the recovery of fresh human trace DNA deposits from wildlife specimens is possible at rates of allele quantities expected in multiple person mixtures. These results indicate foam swabs present as a “catch-all” effective recovery method transcending a range of challenging textural diversity. Whilst foam swabs are not routinely included in comparison studies due to their lack of use in-situ by law enforcement and high costs (Comment et al., 2023) I have demonstrated their unique suitability for use in wildlife crime casework.

The nature of wildlife crimes mean that scenes and evidence may not be encountered until several days, or even weeks, after the initial deposition of DNA. Further work is needed to assess the efficacy of foam swabs in simulated real-world scenarios, inclusive of persistence studies and environmental impacts. Taxidermy introduces a potentially complex element to recovery method decision making and investigators may encounter failure when using traditional wet/dry swabbing or mini taping on taxidermized items despite previous success on non-taxidermized carcasses or live specimens. Additional work is recommended to investigate the role PCR inhibition may play in downstream DNA analysis for human trace evidence recovery from the surface of wildlife derivatives . Microscope analysis of surface porosity, of both taxidermized and raw wildlife derivatives, would ensure a fully informed view of influencing factors can be considered by forensic practitioners. Finally further work is required to investigate the effectiveness of foam swabs on non-taxidermized fresh wildlife

carcasses and derivatives to establish their suitability as a true “catch all” recovery method applicable to a wide range of wildlife crime case work.

Though wildlife crime is a prolific problem the potentially minimal applicability human DNA recovery from wildlife derivatives is recognised, however it is argued when it can be utilised the inclusion of human DNA forensic evidence in casework can be of significant benefit to the positive outcome of investigations. The combined factors of lack of evidence being a limiting factor to wildlife crime prosecutions and convictions, human trace DNA being a high-value evidence type and, the low contextual incident of application make a strong argument for the use of more expensive resources being utilised as long-term compound costs will remain comparatively low offset against potential beneficial outcomes.

The results of this chapter indicate further consideration should be given to the recovery of human trace DNA from wildlife items at wildlife crime scenes. Successful recovery and DNA profiling would present as a unique opportunity for robust links to suspects suspected of involvement, a form of intelligence which may not otherwise be present itself during the course of an investigation. Should resources allow, foam swabs should be the favoured recovery method in this line of work. However, if these are part of their standard kits forensic practitioners should not be discouraged from utilising either flocked swabs, minitapes or cotton swabs given their success in demonstrated ability to recover trace DNA in both this study and others.

Chapter 6: Investigating the approaches, perceptions, and awareness of UK urban police forces in relation to the use of forensics in wildlife crime investigations.

6.1 Introduction

A key theme mentioned throughout this thesis is the lack of resource investment acting as a barrier to development of effective wildlife crime interventions. However not all intervention types suffer equally, with research showing that available resources are consistently funnelled into enforcement based activities (Plowman, 2020). Enforcement approaches broadly cover the concept of end-to-end investigative steps, including intelligence and evidence gathering, arrest, prosecution, and conviction. Whilst most enforcement activities are carried out by law enforcement, it is common in a wildlife crime context for intelligence and evidence gathering steps to involve, or even rely on, external stakeholders (Nurse, 2020; INTERPOL, 2023). Non-law-enforcement agencies have long been engaged at multiple points in the investigative journey, either purposefully and collaboratively with law enforcement (Nurse and Harding, 2022), or without direct intent through data collection carried out in the context of conservation biology (Kurland et al., 2017). Their role in enforcement based activities is being continually scrutinised by stakeholders (White, 2013; Duggan and Newcomer, 2015; Nurse, 2016, 2020); as well as in self-reflection by organisations, as they decide to what degree they wish to participate in investigations and the costs and benefits of their involvement towards successful prosecutions. In 2021, The Royal Society for Prevention of Cruelty to Animals (RSPCA), which possess a historical precedent for bringing forward private prosecutions in wildlife crime cases, announced a desire to begin passing a larger portion of their prosecutor role to the crown prosecution service (RSPCA, 2021). This was presented as part of their "strategy to 2030", with the complexity of casework, and crossover with other criminal

enterprises outside of their expertise as an animal focused organisation, being cited as contributing factors. Involvement of wildlife or environmental focused non-law-enforcement agencies can be advantageous, as their objectives require a strong baseline knowledge of the commodities and victims involved in wildlife crimes as a pre-requisite. Despite their global prevalence, wildlife crimes remain a niche criminal activity for many law enforcement agencies, and therefore possession of specialised knowledge or training in the subject matter is unlikely. In-depth knowledge provided by external agencies can help contextualise decisions made during intelligence and evidence gathering activities improving the efficacy of investigations. As such with highly experienced and knowledgeable organisations such as the RSPCA stepping back from prosecutions, it is important to review the capacity of law enforcement to step in and fill these gaps.

In the UK wildlife related offences are non-notifiable, meaning they are excluded from crime statistics compiled by the Home Office. Without this data, understanding the extent of crimes, to what degree legislation is being enforced, and which methods of investigation are being used, remains challenging. Even when available, crime statistics are known to be insufficient in both representing the extent of criminal activity and the effectiveness of enforcement (Loveday, 2000; Brunton-Smith et al., 2023). At present the success of enforcement activities is routinely measured in seizures and prosecution outcomes, of which fines and nominal prison time are the most prolific method of consequence (Bamwine, 2019; Lynch et al., 2020; Hutchinson et al., 2023). Rarely are intervention methods assessed for their capacity building value, deterrent effects, or cascading impacts on other crime types, despite their known associations (Adhiasto et al., 2023). With little impact data available there is room to question the efficacy of many wildlife crime enforcement activities such as seizures. It is difficult to assess whether variability in seizure rates, or arrests of smugglers, is inherently linked to enforcement activity. Increased

seizures could be a result of a higher rate of trade taking place rather than more efficient detection; a decrease in number of seizures could be due to a change in trade routes, better concealment tactics or change in demand, rather than reduction in illegal activity. Within expansive supply chains, individuals tasked with moving items across borders may also fail to represent significant players facilitating and driving trade. As reactive based approaches with a punitive aim, on the spot seizures can consequently fail to address the underlying drivers of wildlife crime activities, including poverty, lack of alternative livelihoods, coercion, and considered cultural and social acceptability. Therefore the ethics and risks of exploitation related to enforcement and often associated militarisation involvement also require consideration (Duffy, 2022).

Despite these concerns research has shown the absence of enforcement approaches in non-regulatory interventions, such as grassroots community engagement, can be detrimental to overall success (Sherman et al., 2022). It is also recognised that community engagement level work alone will not solve the problem of wildlife crime and local communities may welcome law enforcement activities assuming it is carried out effectively (Roe and Booker, 2019; Travers et al., 2019). Even with successful enforcement campaigns, repercussions are often considered minor comparative to the impacts of wildlife crime and there are routine requests for harsher penalties. However high profile cases with lengthy prison sentences suggest there is traction in this area and that wildlife crimes are being taken more seriously (Environmental Investigation Agency, 2021). These cases are often the result of long-term intelligence led investigations afforded significant resources, international collaboration, and achieved through the collection of a range of intelligence and evidence types. Examples include “Operation Crash” an investigation focused on rhino horn theft and smuggling by organised criminal gangs, conducted by Irish, and US law enforcement (U.S. Department of Justice, 2017), and “Operation Dragon” a two year

investigation into turtle and tortoise trade involving law enforcement from India, and Malaysia, and the not for profit organisation, Wildlife Justice Commission (WJC) (Wildlife Justice Commission, 2018). The outcomes of these operations demonstrate the benefits to utilisation of multiple factions and resources within law enforcement agencies.

As the pool of stakeholders with a vested interested grows and the impacts of wildlife crime become more apparent, it is a reasonable assumption that there will be scrutiny on investigation quality and impact as a result. The pressure on law enforcement will be further exacerbated by the discussed events of external support loss, expectations of greater penalties and need to justify the favourable funding of enforcement activities over other intervention types. A peripheral stakeholder who holds interest in investigation quality includes suspect defence teams. Whereas historically defendants may have been willing to pay fines trivial comparable to the gains of trade, as prison time and high fines become more realistic outcomes of a guilty verdict, the low risk-high reward appeal that wildlife crime has become associated with diminishes. As such the defence may increasingly look for arguments towards case dismissal; this risks undermining law enforcement, wasting resources, and negates the perceived increased risk afforded to high fines and prison time, forcing a continuous seesaw of the risk, reward balance perception. These concerns are not unfounded, in at least one review of wildlife related citations in Canda, dismissals accounted for a third of decisions, though reasons for the high number were unclear (Lynch et al., 2020). Justification is further evidenced by a pattern of compromised cases related to poor investigative practices at administrative and procedurals levels including crime scene management, evidence handling and collection, and chain of custody reporting (Ceccato and Uittenbogaard, 2013; Salum et al., 2017b; UNODC, 2020; Wildlife and Countryside Link, 2022b). This phenomenon is not isolated to wildlife crimes with scrutiny of law enforcement activities at an all-time high (Walsh, 2023).

Considering these pressures a number of organisations have developed training programmes focused on capacity building for those engaged in wildlife crime investigations. TRACE (TRACE, 2024), the UNODC (UNODC, 2024a), the International Consortium on Combatting Wildlife Crime (UNODC, 2012) , PAW and NWCU (National Wildlife Crime Unit, 2023), ZSL (Global Wildlife Program, 2018), the International Fund for Animal Welfare (International Fund for Animal Welfare, 2021), and TRAFFIC (TRAFFIC, 2024b) represent just some of the organisations both independently, and collaboratively contributing to this endeavour. Topics covered are far ranging but one of the most prevalent avenues of interest is the application of forensic techniques and best practice approaches to crime scene investigation. As laid out in 1.2 the application of forensics in wildlife crime investigations has seen significant interest in recent decades but is skewed towards recovery and analysis of wildlife specimens, derivatives, and DNA concerning their use in species identification. Training programmes and resources can mirror this bias with a focus on helping parties detect incidences of wildlife crime through effective morphological identification (Baker et al., 2020; Murray et al., 2023) or collection, storage, and analysis of wildlife samples (Ogden et al., 2009; Partnership for Action Against Wildlife Crime Forensic Working Group, 2017; UNODC, 2024b). Others deliver more holistic packages, championing recipients ability to walk away with a comprehensive awareness of end-to-end crime scene management inclusive of considerations for both human and wildlife related forensic evidence (PAW Forensic Working Group, 2014; UNODC, 2019; Merwyn et al., 2020). Some such as the TRACE network goes as far as to partner training with infrastructure development, supporting the application of skills sets and newly acquired techniques in the long-term to ensure they remain a sustainable facet of wildlife crime investigations (TRAFFIC, 2023).

Targeted recipients of these training programmes are often what is collectively referred to as “wildlife crime scene first responders” (UNODC, 2019); individuals involved in wildlife

crime interventions who may be first to arrive at a scene. Dependent on an organisations interpretation of the role, recipients could encapsulate, rangers (IFAW, 2022), veterinarians (Smith-Blackmore, 2023) and border and customs officials (TRAFFIC, 2024a). Due to breadth of types of potential "first responders", training is often delivered with the perception that attendees existing knowledge of best practice forensics is either absent in its entirety or severely lacking (Potter and Underkoffler, 2021).

Several training packages include content on the correct method for handling and collecting forensic evidence. However, whether the first responders are mandated to do so must be considered. Both the UNDOC "Wildlife Crime Scene Guide for First Responders" (UNODC, 2019) and the PAWFG "Guide to the Use of Forensic and Specialist Techniques in the Investigation of Wildlife Crime" (PAW Forensic Working Group, 2014) stress upon the importance of only carrying out evidence collection where mandated otherwise deferring to those who are. Who is mandated to collect forensic evidence is dependent on individual jurisdictions and the forensic evidence in question. UK protocols dictate forensic evidence collection from a crime scene, inclusive of fingerprints and human DNA be deferred to qualified CSE in possession of ISO 17020 accreditation, and since 2013 National DNA regulatory guidance states that the recovery of human DNA should be carried out at the primary crime scene (PAW Forensic Working Group, 2014). If impossible it must be transported and opened in a laboratory with an ISO 17205 accreditation, lengthening an investigation and risking evidence degradation over time. The presence of a CSE may only be triggered through requests by police officers (Bitzer et al., 2022; Plombon et al., 2023). Therefore, an officer requires a suitable degree of understanding as to the potential forensic evidence that presents itself within the crime scene. Wildlife crime scene training for officers helps build their capacity to carry out an initial assessment as to the cost benefit analysis of a request for a CSE to attend making it an important and necessary endeavour

in the UK. However, as holders of, and authorities on applicable ISO accreditations CSE's remain the ultimate subject matter experts in the forensic element of an investigation and their presence at crime scenes provides valuable knowledge and skillsets that may help avoid case compromission.

As part of their role CSE's must also make an assessment as to whether their presence is warranted, or if they can provide guidance to the attending officer dependent on the facts and context presented to them by the officer. A long term study into the skillsets of CSE's found that the basis for professionalism and success come from a sound and well-rounded knowledge of their subject matter, bolstered through training and exposure (Kelty et al., 2011, 2017, 2023). Despite these factors CSE's rarely appear to be the focal recipients of training related wildlife crime investigations. Without inclusion in wildlife crime scene investigation training programmes CSE's lose the opportunity to experience contextual application of their knowledge and skills. This inherently limits the advice they can offer to officers attending crime scenes and their capacity to assess the value of their own contributions and/or presence. Therefore, forensic training of just "wildlife crime first responders", may be a necessary but stunted approach to associated capacity building programmes.

Through delivery of training programmes focused on forensic potential at wildlife crime scenes, and surveys of UK wildlife crime officers (WCO) and CSE's this study aims to achieve the following: map the current approaches and perceptions of forensic evidence collection in wildlife crime investigations, establish whether a gap exists that could benefit from the professional input of CSE's, and assess the value tailored face to face training programmes may hold for greater CSE input in wildlife crime investigations.

6.2 Method

6.2.1 Questionnaire composition and delivery

6.2.1.1 Wildlife crime scene officers

In May of 2023, a short presentation was given to a group of Metropolitan Police Borough Wildlife Crime Officers (BWCO); these are serving officers who have volunteered to be responsible for the investigation of wildlife crimes in their borough as part of their standard duties. The presentation included descriptions of existing research into the recovery of human trace evidence from wildlife specimens, inclusive of early proof of concept results found in Chapters 3 and 4 of this study. The objective of the presentation was to demonstrate to officers that there is justification for the attempt of human trace evidence recovery from wildlife specimens and encourage them to build its potential into their considerations during future wildlife crime investigations. Post in-person training, a member of the Metropolitan Police Wildlife Crime Unit (WCU) was recruited to act as gatekeeper and circulate an online questionnaire (Appendix II), hosted by Microsoft Office Forms™, to email mailing lists of all UK law enforcement officers involved in wildlife crime investigations. This included but was not limited to, all Metropolitan Police BWCO's and members of the NWCU. Completion of the survey was voluntary, and background information and purpose of the questionnaire was included in the email request as well as at the start of the questionnaire itself. Definitions were provided in-situ for jargon, such as "Direct evidence". A copy of the presentation was made available for those who did not attend the in-person training event. The questionnaires objectives were threefold: i. to gain an insight into officer's wildlife crime scene attendance rates and caseload types, ii. understand how the collection of forensic evidence at wildlife crime scenes is currently approached iii. garner officers opinions on perceived contributors to unsuccessful prosecutions and convictions in this area of work. Respondents were given the opportunity to provide additional information related to forensic evidence collection in wildlife crimes

not covered within structured questions through an open-ended question at the end of the questionnaire. Question types were a mixture of demographic, open, ranked, and multiple choice. Questionnaire design was aesthetically simple and comprised of sixteen questions total, with the intention of being able to be completed in less than ten minutes, as these factors have been shown to increase response rates in web-based surveys (Sammut et al., 2021). This dataset is referred to as the WCO dataset from this point forward.

6.2.1.2 Crime Scene Examiners

In collaboration with the Metropolitan Police WCU a two-hour training module was developed for in-person delivery to all Metropolitan Police CSE's. The module was delivered over the course of 10 repeated sessions spanning 10 weeks between January – March 2024 as part of a routine annual training cycle. Module content was divided into three components: an introduction to the activities undertaken by the Metropolitan Police WCU, including types of crimes, methods of investigation and the challenges they face, the second component outlined forensic opportunities and best practice methods for human trace evidence recovery from wildlife crime scenes based on existing literature, the findings of Chapters 4 and 5 of this thesis, and responses from the WCO surveys, the third section involved an interactive case study discussion where CSE's were invited to comment on how they would have engaged with real world case examples should they have been in attendance.

An accompanying questionnaire was developed as part of the training module (Appendix III) and designed to be completed as a pre and post training exercise. Section one, Questions 1-4, was completed prior to the delivery of any training content with two key objectives: i. to ascertain current rates of CSE engagement in wildlife crime, including training and case involvement, ii. establish CSE's existing perceptions around wildlife crime, including a

crossover question with the WCO questionnaire on contributing factors to unsuccessful prosecutions and convictions. Section two, Questions 5 – 15, was to be completed after receipt of training and designed with the objective of assessing whether the training resulted in any change in CSE's perceptions, knowledge, and apathy towards participation, in wildlife crime casework. Question types were a mixture of multiple choice, Likert-type, and Likert-scale. Likert scales were designed to assess two specific outcomes of the training, these being whether CSE's, had i. acquired knowledge related to wildlife crime, ii. were likely to participate in future activities related to wildlife crime. This dataset is referred to as the CSE dataset from this point forward. To encourage completion, hardcopies of questionnaires were disseminated at the beginning of each training session with a verbal explanation as to their design and purpose. As with WCO questionnaires designed to be aesthetically simple and capable of completion in ten minutes or under.

Both questionnaires received ethical approval for this study through the ZSL Human Ethics Committee (ZSLHEC-006) and were voluntary in their completion.

6.2.2 Analysis

6.2.2.1 Missing data

A total of (*N* = 46) questionnaires were returned from the WCO and (*N* = 206) from CSE. Collated questionnaires were reviewed for blank responses or anomalies, such as selecting more than the maximum number of options, in a multiple-choice answer. Within WCO responses (*N* = 22) instances of missing data were identified across (*N* = 10) questionnaires, representing 22% of responses (Figure 6.1). Within CSE responses (*N* = 15) instances of excessive choice were identified in multiple choice questions. These responses were subsequently considered null and void and treated as "missing" data. A further four

instances of missing data were identified from CSE responses totalling (*N* = 19) missing entries across (*N* = 19) questionnaires representing 9% of responses (Figure 6.2).

A Little's test to assess a missing completely at random (MCAR) assumption was carried out in R statistical software v. 4.1.1. using the "nanier" package (Tierney and Cook, 2023). Resulting *p* values led to accepting the null hypothesis that the data was MCAR for both WCO (test statistic = 38.6, *p-value = 0.136*) and CSE surveys (test statistic = 59, *p-value = 0.684*). Following Mirzaei et al (Mirzaei et al., 2022) recommendations for MCAR datasets multiple imputation (MI) was carried out on both datasets. MI for WCO data was performed in R statistical software v. 4.1.1 using the "class" package (Venables and Ripley, 2002) and the k-nearest neighbours (kNN) algorithm function as recommended for categorical variables (Memon et al., 2023).. A k value of k = 7 was used whereby $k = \sqrt{N}$, with N representing total number of samples. Due to presenting as a constant value of "Yes", MI was unable to be carried out on responses to the question "Would you be likely to submit wildlife items (whole or parts) for attempted human trace evidence recovery if it was proven to be achievable?". This question was subsequently omitted from MI and statistical analysis but still included in discussion. MI for CSE data was carried out using the Multiple Imputation by Chained Equations (MICE) algorithm function of the "mice" package in R statistical software v. 4.1.1 (van Buuren and Groothuis-Oudshoorn, 2011). All questions were included in the datasets and ten imputations carried out, resulting in 11 total versions of the dataset. The resulting pooled imputations used for subsequent statistical and descriptive analysis. Graphs were produced using a mixture of Microsoft excel and the "ggplot2" (Wickham, 2016) and "ggmice" (Oberman, 2023) packages in R.

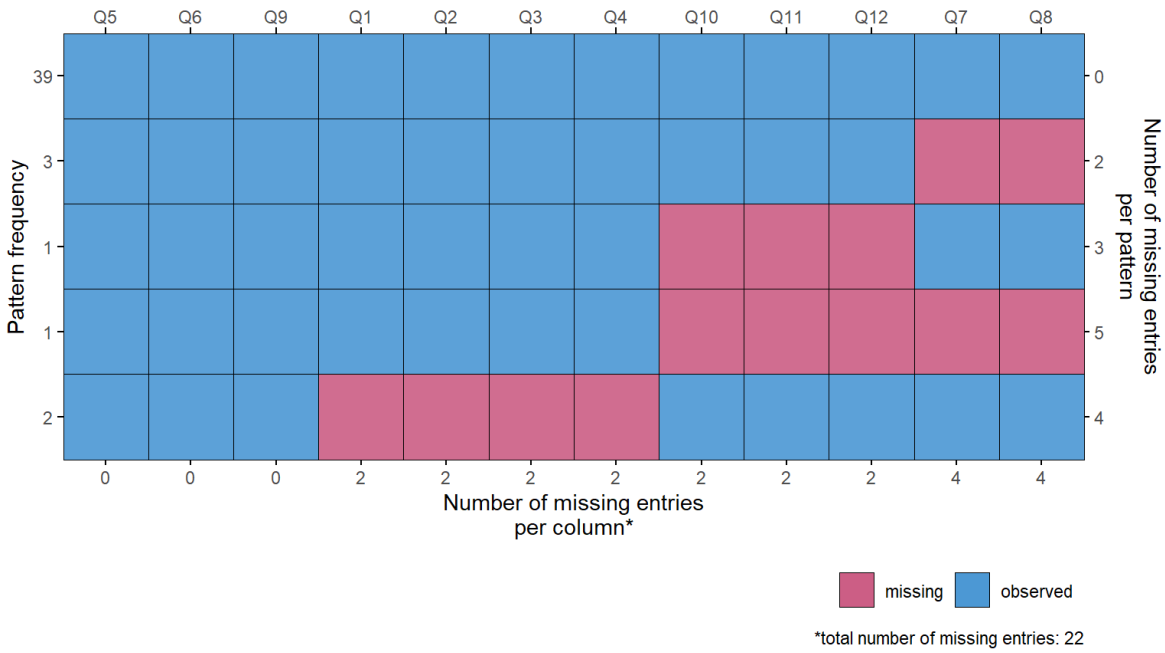

Figure 6.1. Frequency and pattern of missing data in wildlife crime officer questionnaire dataset.

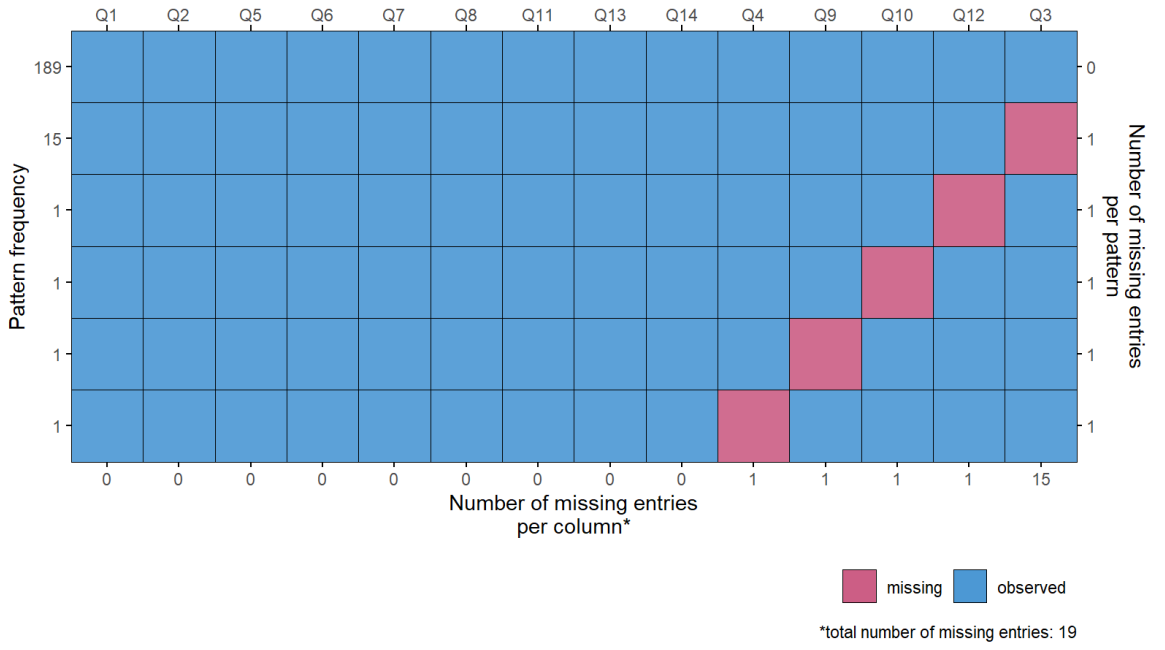

Figure 6.2. Frequency and pattern of missing data in crime scene examiner questionnaire dataset.

6.2.2.2 Statistical analysis

6.2.2.2.1 WCO datasets

Chi square tests of independence with a Monte Carlo procedure were used to assess associations between variables within the WCO datasets. Post hoc analyses were carried out for significant associations and standardised residuals used to ascertain what level associations were operating at.

6.2.2.2.2 CSE datasets

Multiple linear regression was carried out using the total sum of Likert items in the two Likert-scales, i. acquired knowledge related to wildlife crime, ii. likelihood to participate in future activities related to wildlife crime as dependent variables. Independent variables were selected by comparing AIC values of model variations. Presence of heteroscedasticity in selected models showing significance was tested using a studentized Breusch-Pagan test.

6.2.2.2.3 Open-ended responses

Open ended responses for all respondents (CSE and WCO) were analysed using a thematic analysis with an inductive coding scheme approach. The six-phase method outlined by Braun and Clarke (Braun and Clarke, 2006) was used to guide the thematic analysis methodology. Open comments were first transcribed from hard-copy questionnaires into soft copy format, during this process responses were read through several times to familiarise myself with the data. Responses were then read through line by line and coding carried out. Codes were then collated and collapsed into possible themes using rough thematic maps to sort the data until preliminary themes were identified that were then taken forward for a more in-depth review. Proposed themes were then thoroughly reviewed and broken into sub-themes where necessary, during this process themes were consistently referred back to the original dataset to ensure they appropriately represented the data and coding that had taken place. Once full confidence that themes and sub-themes

were a good representation of the dataset they were defined and results presented graphical following guidance by Rouder et al (Rouder et al., 2021).

6.3 Results

6.3.1 Wildlife Crime Officers

6.3.1.1 Demographics

Out of (*N* = 46) respondents, 52% (*N* = 24) identified as BWCO, 11% (*N* = 5) as part of the NWCU and 41% (*N* = 19) as neither. Two respondents (*N* = 2) self-identified as falling under both BWCO and NWCU categories. The majority of both BWCO (38%, *N* = 9) and NWCU (40%, *N* = 2), respondents had worked in these roles for more than five years.

6.3.1.2 Crime Scene Attendance Rates and Case Proportion

The majority of the respondents (*N* = 18) attended more than 15 wildlife crime scenes in person a year. The second most common attendance rate presented at the other end of the spectrum with *N* = 13 respondents attending 0 – 5 wildlife crime scenes in person a year (Figure 6.3). The majority of officers (52%, *N* = 24) listed poaching as their highest case load proportion, followed by badger persecution (20%, *N* = 9), CITES (9%, *N* = 4), raptor persecution (7%, *N* = 3), cyber enabled wildlife crime (4%, *N* = 2) and bat persecution (2%, *N* = 1). Freshwater pearl mussels were reported as the lowest proportion of caseload for 72% (*N* = 33) of respondents followed by CITES (11%, *N* = 5), cyber enabled wildlife crime (9%, *N* = 4) and poaching (2%, *N* = 1) (Figure 6.4).

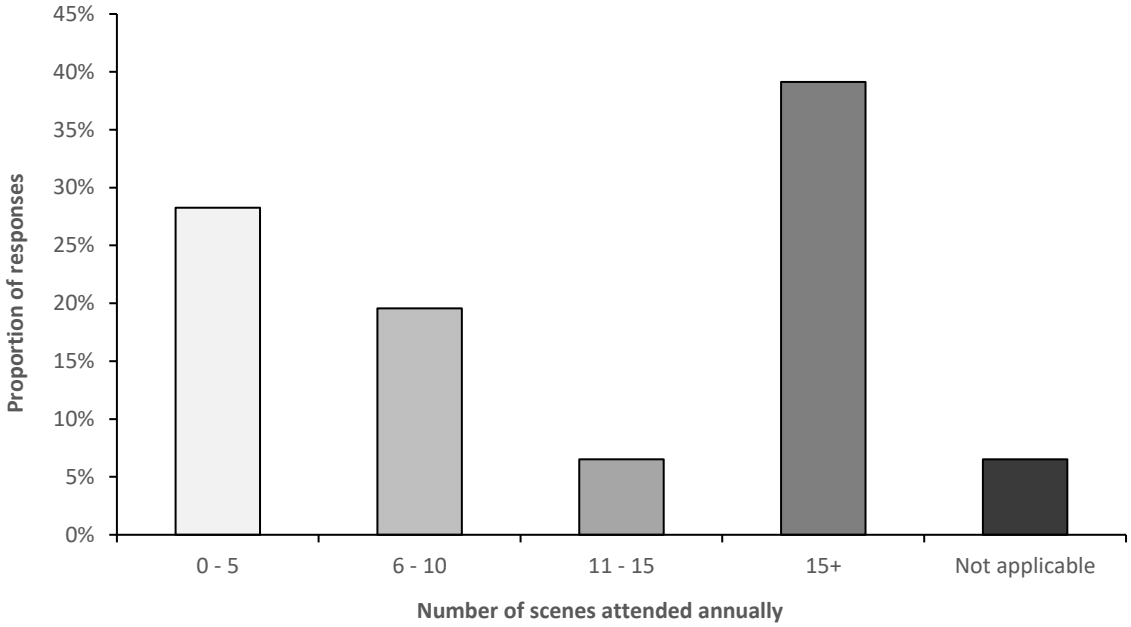

Figure 6.3. Frequency of number of wildlife crime scenes attended annually by WCOs.

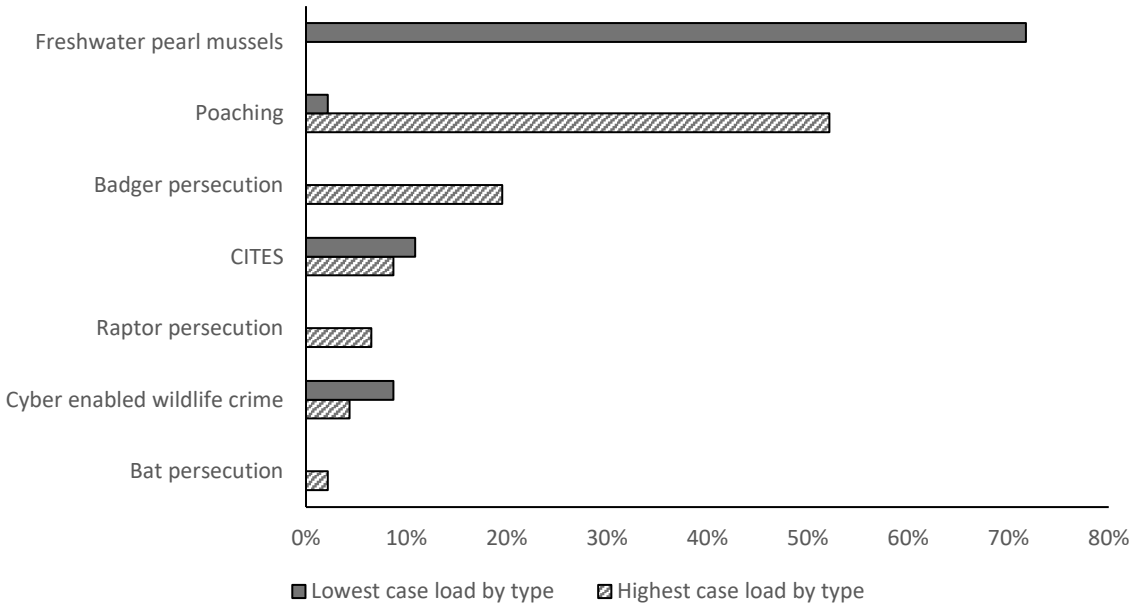

Figure 6.4. Percentage frequency of national wildlife crime priorities listed as either highest or lowest proportion of case types.

6.3.1.3 Unsuccessful prosecutions/convictions

Prioritisation of other crime types was placed as one of the biggest contributors to unsuccessful prosecutions/convictions by 59% (*N* = 27). A lack of evidence was the second most listed highest contributor at 48% (*N* = 22), followed by a lack of funding (37%, *N* = 17), manpower (30%, *N* = 14), infrastructure and other (13%, *N* = 6) (Table 6.1). Lack of

prioritisation paired with lack of evidence was the most common combination of contributing factors listed by respondents (*N* = 10) (Table 6.1).

Table 6.1. Frequency of paired variables perceived as highest contributors to failed prosecutions and convictions by WCOs.

Lack of…	*Evidence*	*Funding*	*Infrastructure*	*Manpower*	*Prioritisation*	*Other*	*Blank*
Evidence		5	1	5	10	1	0
Funding			2	1	8	1	0
Infrastructure				1	0	0	1
Manpower					5	0	1
Prioritisation						2	1
Other							1
Blank							

6.3.1.4 Successful evidence types

Out of (*N* = 46) respondents, 63% (*N* = 29) listed "direct" evidence as one of the types of evidence they believe most greatly contributes to successful prosecutions and/or convictions in wildlife crime cases. This was followed by "forensic" (52%, *N* = 24), expert, (39%, *N* = 18), "primary" (22%, *N* = 10), "circumstantial" (15%, *N* = 7) and "secondary" (7%, *N* = 3). The most cited combined response was "direct" and "forensic" evidence (24%, *N* = 11) (Table 6.2). Evidence type definitions can be found in Appendix II.

Table 6.2. Frequency of evidence types perceived as highest contributors to successful prosecutions and convictions by WCOs.

Evidence type	*Direct*	*Circumstantial*	*Primary*	*Secondary*	*Forensic*	*Expert*	*Blank*
Direct		6	3	1	11	8	0
Circumstantial			0	0	1	0	0
Primary				2	3	1	1
Secondary					0	0	0
Forensic						9	0
Expert							

6.3.1.5 Forensic evidence submissions

Wildlife carcasses (52%, *N* = 24) were the most common type of evidence submitted for forensic analysis by respondents (Figure 6.5). Some iteration of wildlife evidence (live, derivative or carcass) was listed as the highest proportion of submitted evidence types for 67% (*N* = 31) of respondents. Vehicles presented as both the second most (after wildlife grouping) and least common submitted evidence type at 13% (*N* = 6). The least commonly submitted evidence types were "Other" (48%, *N* = 22), items listed as examples included: tools, clothing, firearms, and documentation. There was a close majority split between respondents who had submitted an item for human trace evidence recovery (53%, *N* = 25) and those who had not (47%, *N* = 21). Within those who had the majority had submitted for both fingerprints and DNA (37%, *N* = 17).

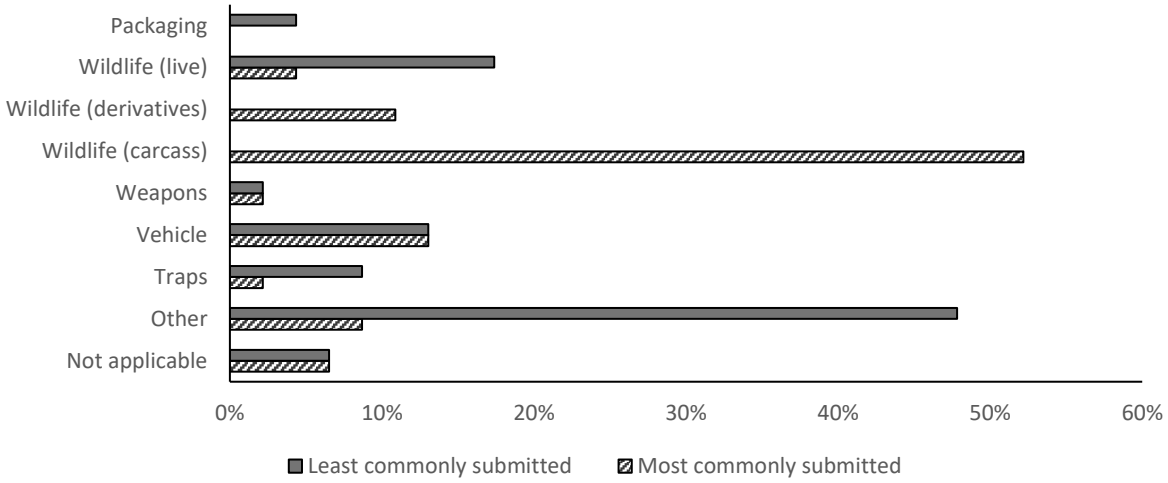

Figure 6.5. Frequency of most and least common submissions for forensic analysis by evidence type by WCOs.

6.3.1.6 Associations

Fifteen Chi square tests of independence were carried out using "length of service", "annual number of scenes attended" and "highest caseload proportion of by crime type" as dependent variables (Table 6.3). Of tested association nine variable combinations demonstrated statistically significant associations and these are presented in detail below.

*Table 6.3. Results of chi square tests of independence between questionnaire responses in WCO's questionnaires. Statistically significant associations denoted as * (p < 0 .05) or ** (p < 0.01).*

Independent variables	Length of service as WCO		Annual number of scenes attended		Highest caseload proportion by crime type	
	x^2	p - value	x^2	p - value	x^2	p - value
Largest proportion of evidence types submitted for forensic analysis	75.45	0.0025**	43.02	0.0025**	53.86	0.025*
Historical submission of evidence for human trace recovery	18.45	0.2304	22.67	0.03348*	62.41	0.0004998**
Largest contributing variables to successful case outcomes	15.21	0.957	14.15	0.8511	25.09	0.7331
Largest contributing variables to unsuccessful outcomes	20.06	0.7591	22.97	0.3013	48.68	0.02499*
Length of service as a WCO			47.05	0.001**	65.395	0.0015**
Annual number of scenes attended					120.9	0.0004998**

6.3.1.6.1 Caseload proportion

A significant relationship found between an officers highest case proportion type and: which factors they considered to be the greatest contributors to unsuccessful prosecutions and convictions $X^2(N = 46) = 53.86$, *p < 0.05*; whether they had historically submitted wildlife crime scene evidence for human evidence recovery $X^2(N = 46) =$, $p < 0.001$; the largest proportion of evidence types submitted for forensic analysis $X^2(N = 46) = p < 0.05$; length of service of as a wildlife crime officer $X^2(N = 46) = p < 0.01$); and annual number of scenes attended $X^2(N = 46) = p < 0.00$). Investigation and visualisation of standardised residuals demonstrate which levels of significance are operating at within variables (Figures 6.6 – 6.10). A higher-than-expected frequency of officers with cyber enabled wildlife crime as their highest caseload cited lack of infrastructure or "other" as the greatest contributing factors to unsuccessful prosecutions and convictions (Figure 6.6). Prioritisation of other crimes was cited at a higher-than-expected rate for officers with raptor persecution as their

highest caseload. A higher-than-expected frequency of traps are submitted for forensic analysis by officers whose largest case proportion is badger persecution (Figure 6.7).

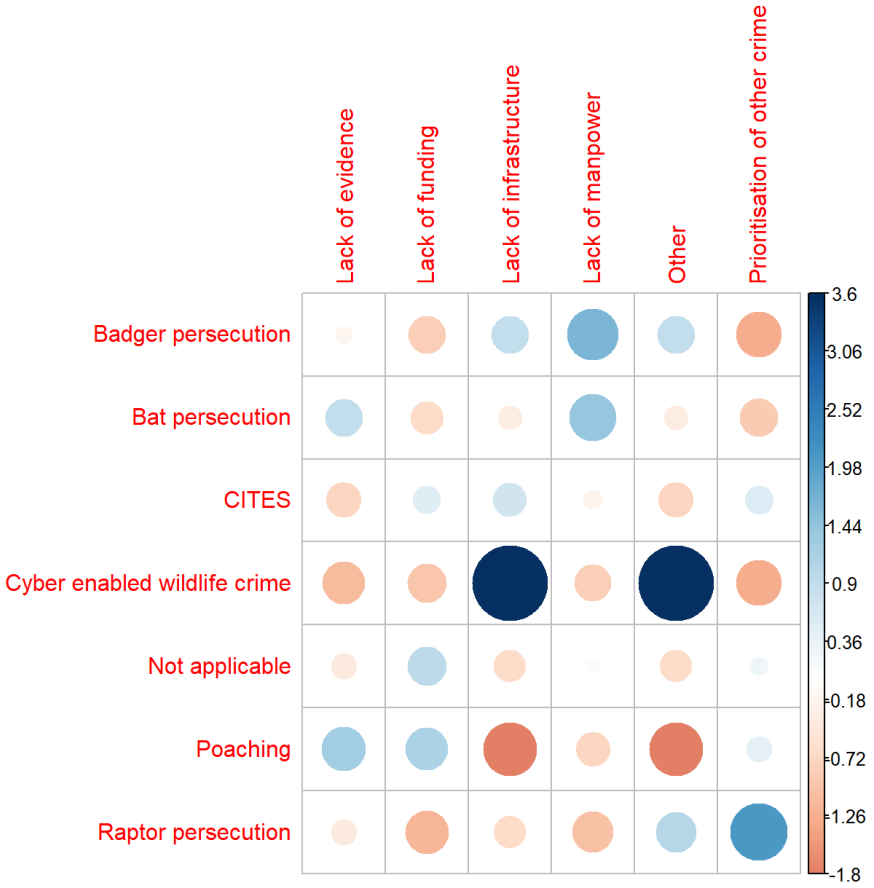

Figure 6.6. Correlation matrix of standardised residuals amongst factors of a WCO's highest proportion of caseloads and what they considered as significant contributing factors to failed prosecutions and convictions. Circle size and colour depth are representative of correlative strength and contribution level to the chi-square test for the tested association. Increasing size and opaqueness show increasing strength in positive (blue) and negative (red) correlation between variables.

A higher-than-expected frequency of wildlife (derivatives) are submitted for forensic analysis by officers with either CITES or cyber enabled wildlife crimes as their highest case proportion. Live wildlife submissions occur at a higher-than-expected rate amongst officers with CITES as their highest caseload.

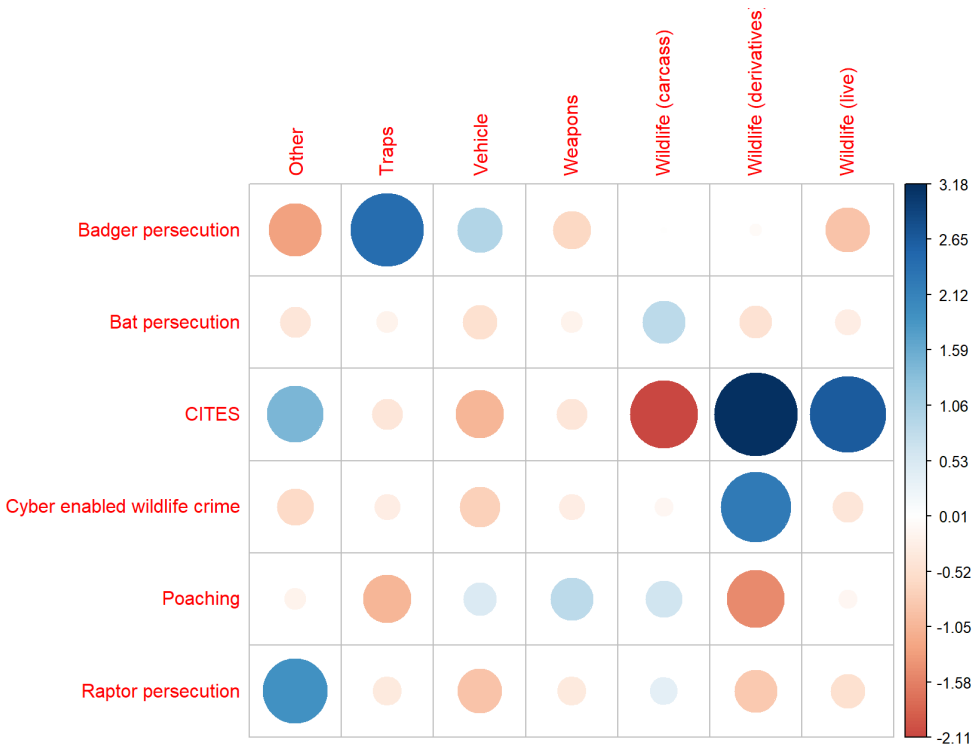

Figure 6.7. Correlation matrix showing standardised residuals amongst factors of a WCO's highest proportion of caseloads and highest proportion of evidence types submitted for forensic analysis of any kind.

A higher-than-expected frequency of officers with badger persecution as their highest caseload had submitted human DNA but not fingerprints for analysis from wildlife crime scenes and a higher-than-expected frequency of officers with CITES as their highest caseload proportion had submitted fingerprints but not human DNA for analysis from a crime scene (Figure 6.8). Officers in service for 1 – 2 had a higher-than-expected number of bat persecution cases as their most common case type, those in the role for 3-5 years a higher-than-expected rate of cyber enabled wildlife crime as their most common case type. Longest serving officers (5+ years) had a higher-than-expected proportion of CITES cases as their most common case type (6.9)

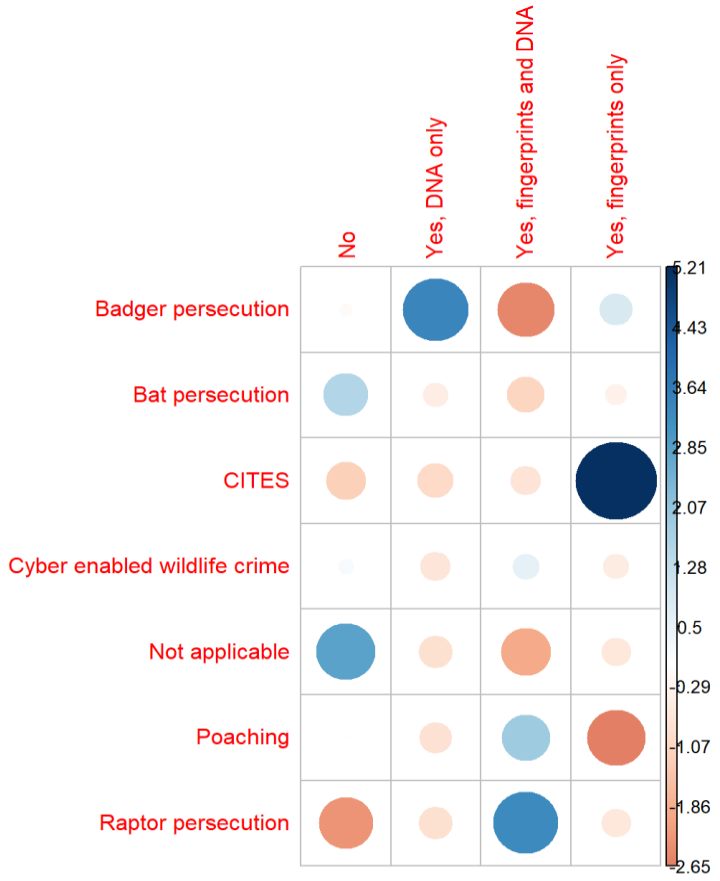

Figure 6.8. Correlation matrix showing standardised residuals amongst factors of a WCO's highest proportion of caseloads and whether they had ever submitted something for human evidence recovery from a wildlife crime scene.

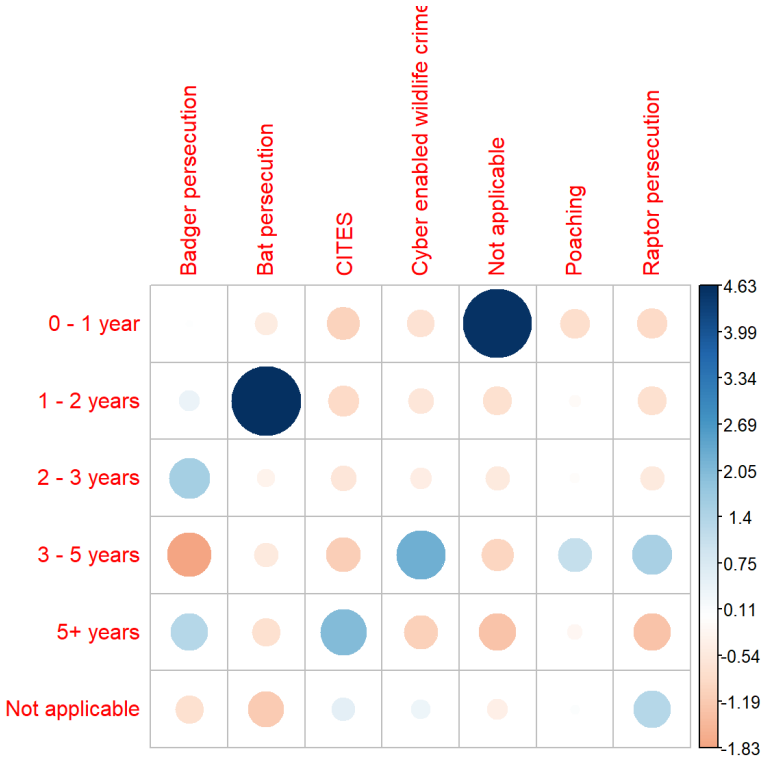

Figure 6.9. Correlation matrix showing standardised residuals amongst factors of highest caseload proportion and length of time in service as a WCO.

A higher-than-expected frequency of officers attending 11 – 15 crime scenes annually listed CITES as their highest caseload proportion (Figure 6.10). A higher-than-expected number of officers attending 0 – 5 scenes annually had bat persecution as their highest caseload proportion, the same officers had a lower-than-expected rate of raptor persecution as their highest caseload proportion.

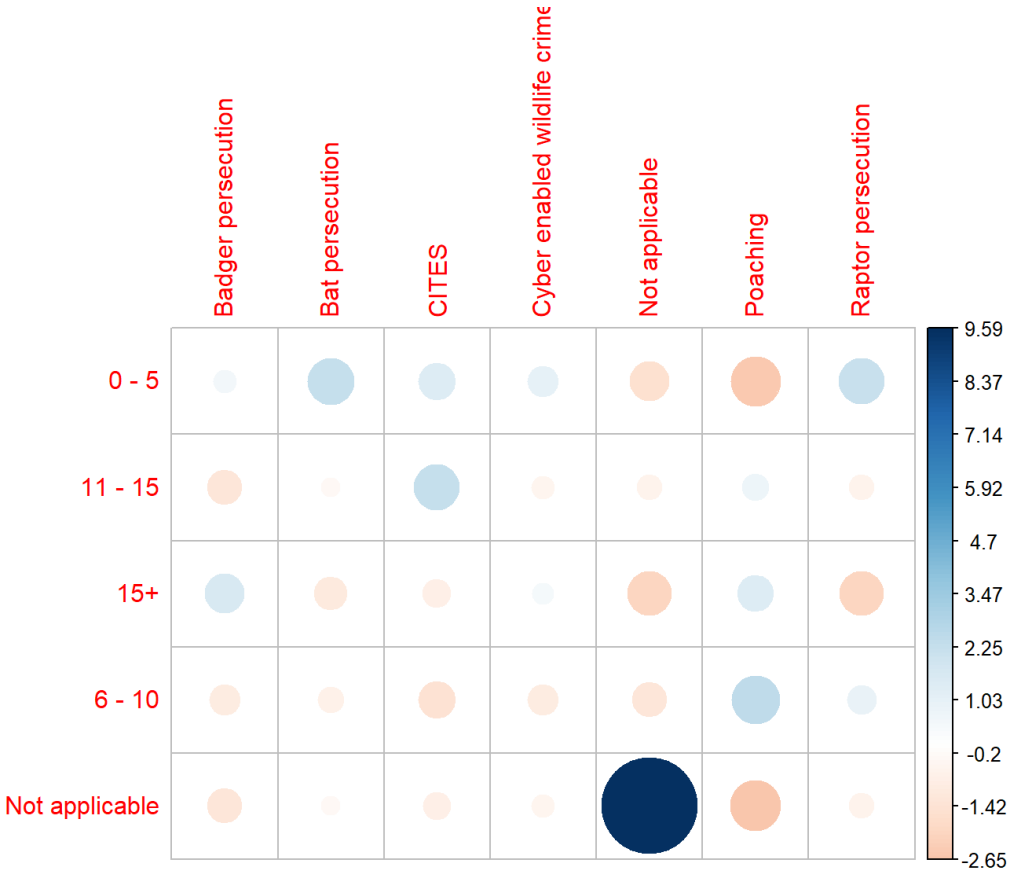

Figure 6.10. Correlation matrix showing standardised residuals amongst factors of highest caseload proportion and number of scenes attended annually by WCOs.

6.3.1.6.2 Annual frequency of scene attendance

A significant association was found between number of scenes attended annually and: most common evidence types submitted for forensic analysis $X^2(N = 46) = 43.02$, $p < 0.001$; whether they have historically submitted anything for human evidence recovery, $X^2 (N = 46) = 22.67$, $p < 0.05$; and their length of time in service in the role $X^2(N = 46) = 22.67$, $p < 0.01$). Standardised residuals indicate a higher-than-expected frequency of officers who attend 11

– 15 scenes a year having live wildlife submissions being their biggest proportion of evidence types submitted for forensic analysis (Figure 6.11) and having submitted evidence from wildlife crime scenes for fingerprint recovery only (Figure 6.12). Officers attending between 0 – 5 wildlife crime scenes a year also submitted a higher-than-expected frequency of “other” types of evidence for forensic analysis and were more likely to have been in their roles for 1 – 2 years. Those in the role 2 – 3 years, or 5 or more years, were more likely to attend over 15 crime scenes annually (Figure 6.13).

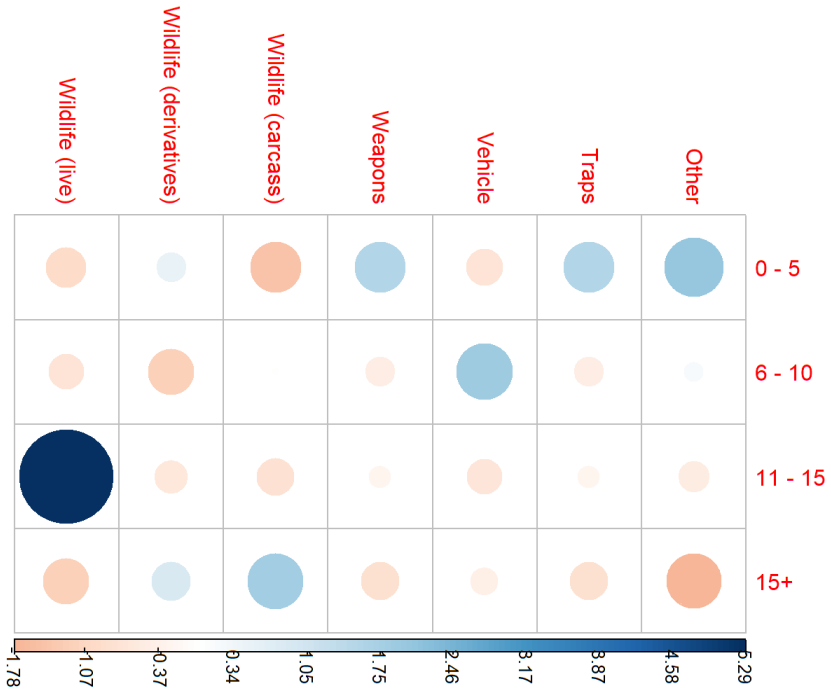

Figure 6.11. Correlation matrix showing standardised residuals between factors of the number of scenes attended annually by WCOs and their most commonly submitted evidence type for forensic analysis.

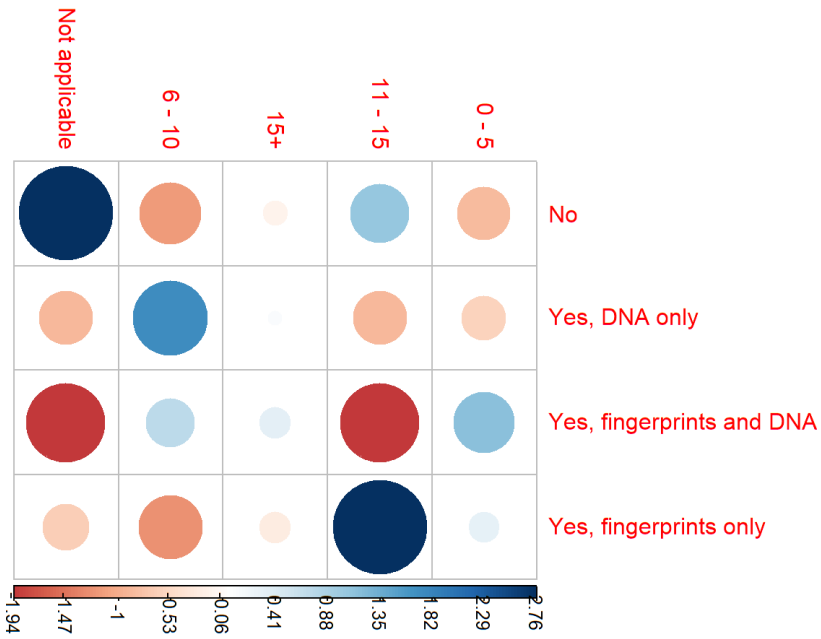

Figure 6.12. Correlation matrix showing standardised residuals between factors of the number of scenes attended annually by WCO's and whether they have ever submitted anything from a wildlife crime scene for human forensic analysis.

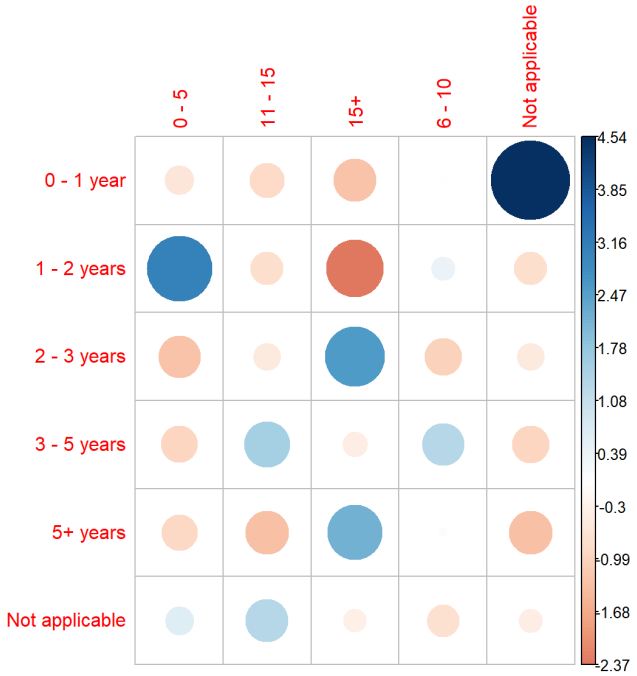

Figure 6.13. Correlation matrix showing standardised residuals between factors of the number of scenes attended annually by WCO's and their length of time in service in the role.

6.3.1.6.3 Length of service

Length of time somebody has been in a wildlife related role had a significant relationship with the highest proportion of evidence types being submitted for forensic analysis (Figure 6.14). Officers in their roles for 1 – 2 years submitted weapons at a higher-than-expected frequency than other evidence types. Vehicles were submitted at a higher-than-expected frequency by officers in their role for 2 – 3 years, wildlife (carcass) by those in their role 3 – 5 years and traps by those in their role 5+ years.

Figure 6.14. Correlation matrix demonstrating strength of association between a WCO's length of service in a wildlife related role and their largest proportion of evidence types submitted for forensic analysis.

6.3.2 Crime Scene Examiners

6.3.2.1 Descriptive statistics of pre-training questions

The majority of respondents had neither received any training related to wildlife crime (80.8%, *N* = 168) or a call to attend a wildlife crime scene (75.4%, *N* = 157). When asked to rate their existing knowledge on wildlife crime, CSE's gave an average response rate of 2 and the majority of responses were skewed towards the lowest end of the presented Likert type scale 1 (Non-existent) to 5 (Excellent), 25.3% (*N* = 55) rated their knowledge as one and 50.67 (*N* = 110) at two. Lack of prioritisation was the most cited factor contributing to unsuccessful prosecutions and convictions (67.4%, *N* = 231), followed by lack of manpower (37.61%, *N* = 129) and lack of evidence (34.1%, *N* = 117) (Table 6.4)

Table 6.4. Aggregate count data of pooled MI dataset of evidence types perceived as largest contributors to failed prosecutions and convictions

Lack of…	*Evidence*	*Funding*	*Infrastructure*	*Manpower*	*Prioritisation*	*Other*	*Blank*	Total
Evidence		43	0	135	447	67	121	813
Funding			0	54	235	0	33	322
Infrastructure				35	129	11	11	186
Manpower					422	45	83	550
Prioritisation						56	272	328
Other							74	74
Blank							15	15
Total	0	43	0	224	1233	179	609	2288

6.3.2.2 Descriptive statistics of post-training questions

6.3.2.2.1 Likert type

An average response rate of 3.0 was given for both Likert type questions. In response to the statement "I feel attending wildlife crime scenes may increase my workload to unsustainable levels." 46.6% (*N* = 99) skewed towards disagreement (Strongly Disagree or Disagree). The majority (41.07%, *N* = 88) overall disagreed (Strongly disagree or disagree), they did not have the resources available to effectively attend wildlife crime scenes.

6.3.2.2.2 Likert Scale

Out of a maximum composite score of 20 a calculated mean central tendency for Likert scale questions resulted in (16.50 ± 0.2) for confirmation of an increase in knowledge related to the subject of wildlife crime, and (15.0 ± 0.25) for an increased likelihood of participation in future wildlife crime related events, as a result of the delivered training. Table 6.5 indicates percentage responses for each individual question within the two presented Likert scales.

Table 6.5. Percentage of Likert scale and Likert type responses amongst MI CSE survey datasets. † denotes questions which contained missing entries in complete case dataset prior to MI.

Likert Scale	Response %				
	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Increased likelihood of future participation					
Q9. I consider participation in wildlife crime casework to be an increased priority. †	1.38	10.6	35.0	29.0	23.9
Q10. I am more likely to respond to a call to attend a wildlife crime scene. †	3.2	6.5	21.7	32.7	35.9
Q13. I am interested in receiving more training sessions focused on wildlife crime case work.	2.4	10.6	28.9	28.4	29.8
Q14. I am interested in supporting further research related to wildlife forensics.	1.9	14.4	24.0	24.0	35.6
Increased knowledge of subject matter					
Q5. I have a greater understanding of the types of wildlife crime casework encountered by the Metropolitan police.	1.4	1.9	13.5	34.6	48.6
Q6. I have a greater understanding surrounding the associated impacts of wildlife crimes	1.0	2.4	15.9	40.4	40.4
Q7. I feel more confident in identifying potential evidence at a wildlife crime scene.	1.0	2.9	23.6	42.8	29.8
Q8. I feel more confident in which human trace evidence recovery techniques can be used on wildlife carcasses and derivatives.	1.0	3.4	16.8	44.2	34.6
Likert type					
Q11. I feel attending wildlife crime scenes may increase my workload to unsustainable levels.	22.6	24	32.2	13.9	7.2
Q12. I do not feel I have the resources available to me to effectively attend wildlife crime scenes. †	11.58	29.49	31.33	18.43	9.22

6.3.2.3 Relationships

Model comparison found existing level of knowledge, knowledge acquisition, and choice of lack of funding (as a contributing factor to failed prosecutions and convictions), as best fit explanatory variables for Likert Scale responses on likelihood of future participation.

The model was accepted after a studentized Breusch-Pagan test of 11.19 with 6 degrees of freedom and p-value – 0.08, confirmed homoskedasticity.

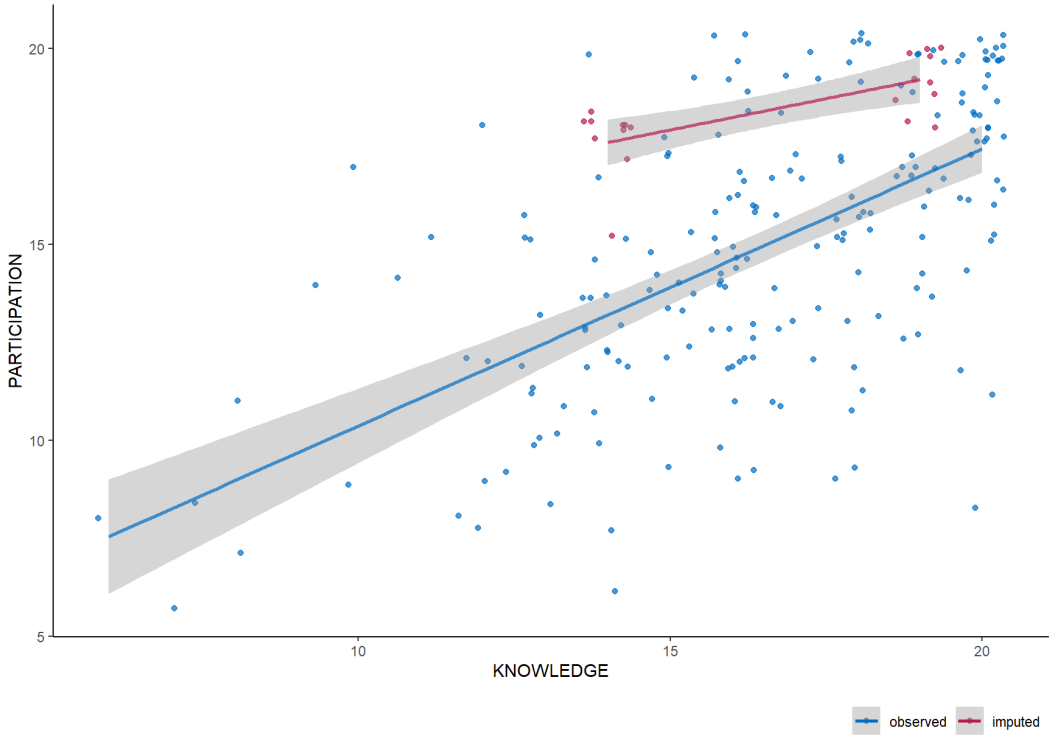

Figure 6.15. Scatter plot with regression line showing a statistically significant correlation between Likert Scale responses for knowledge acquisition and likelihood of future participation in wildlife crime related events.

For every incremental increase in composite knowledge acquisition score a statistically significant ($p < 0.001$), 0.69-point increase in composite score of likelihood of future participation was seen (Figure 6.15). Moving from a self-assessed knowledge rating of 1 to 2 gained a statistically significant (*p-value = 0.01*) 1.18-point increase in composite participation likelihood score and a movement of 1 to 3 gained a statistically significant (p-value = 0.002) 1.8-point increase in composite participation likelihood score (Figure 6.16). If lack of funding was chosen as a contributor to unsuccessful prosecutions and convictions this resulted in a statistically significant (*p-value = 0.01*) estimated 1.4-point increase in composite score of participation likelihood over non-choice (Figure 6.17). No significant

relationships were found between the Likert Scale of knowledge acquisition and any dependent variables.

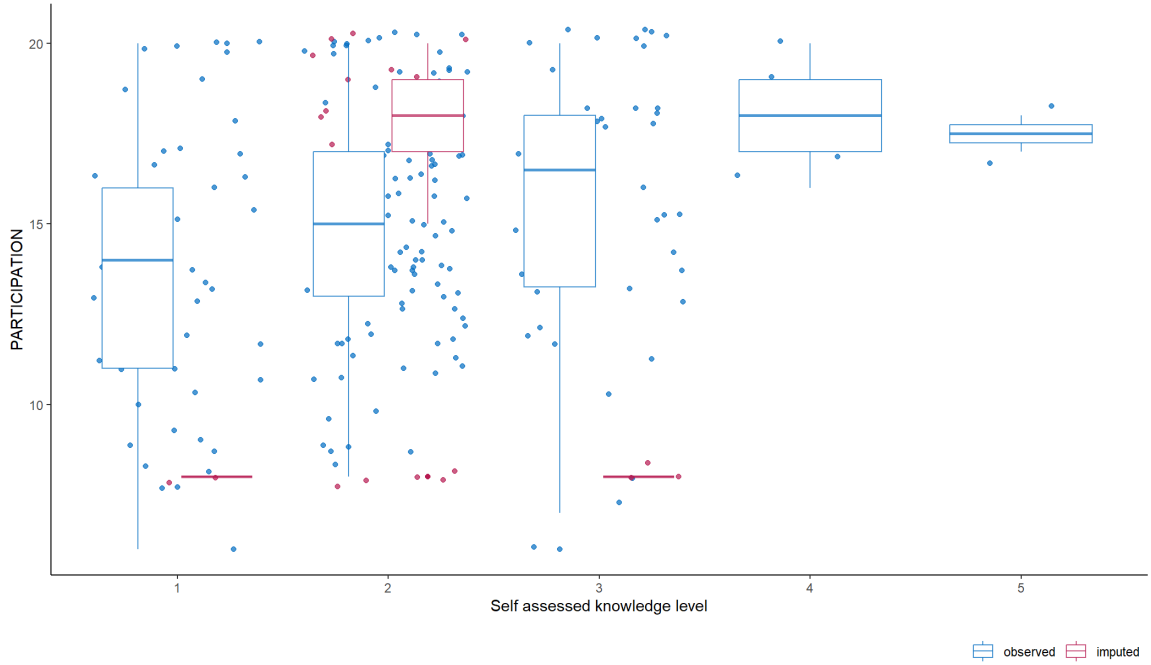

Figure 6.16. Boxplots showing distribution of summed Likert scores for likelihood of future participation for each self-assessed knowledge level among crime scene examiners for both observed and imputted data. The plot also shows individual data points to help visualise the spread of data. Each data point has been randomly offset using the geom_jitter function to mitigate overplotting.

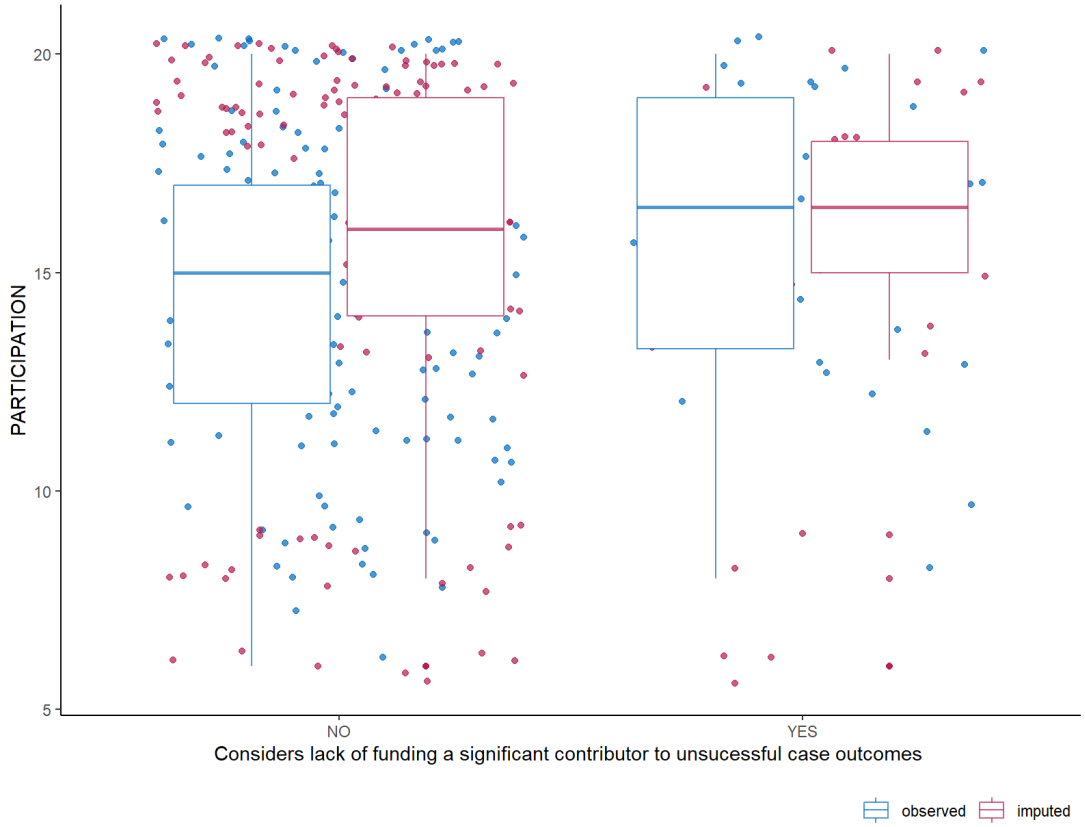

Figure 6.17. Boxplots showing distribution of summed Likert scores for likelihood of future participation whether or not crime scene examiner respondents consider lack of funding a significant contributor to unsuccessful case outcomes. The plot also shows individual data points to help visualise the spread of data. Each data point has been randomly offset using the geom_jitter function to mitigate overplotting. .

6.3.3. Open-ended responses

Two categories "personal capacity" and "external influences" were identified as themes within open-ended responses of both CSE and WCO (Figure 6.18 & 6.19). Further sub-themes of "Low staffing levels", "lack of training", "Workloads too high", "Lack of prioritisation", "Officer apathy" and "Failure to invite" were established. A third theme of "equipment" was identified within CSE responses only, with further sub-themes of "Methods not validated" and "Best equipment not available" (Figure 6.18).

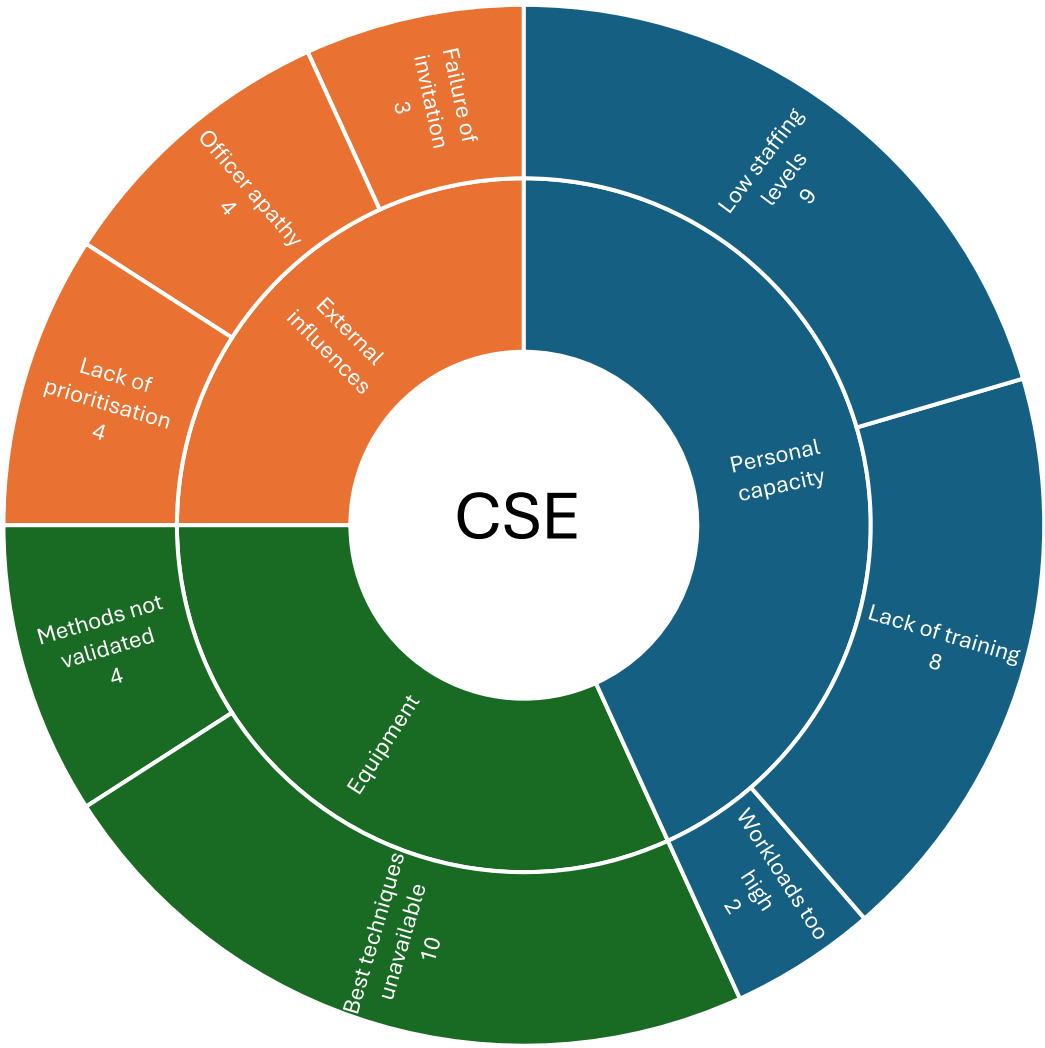

Figure 6.18. Sunburst diagram showing frequency of responses to themes and sub-themes established during thematic analysis of open-ended responses in CSE questionnaires related to organisational limitations to effective wildlife crime investigations. External influences reference personnel level actions outside of direct CSE involvement.

Figure 6.19. Sunburst diagram showing frequency of responses to themes and sub-themes established during thematic analysis of open-ended responses in WCO questionnaires related to organisational limitations to effective wildlife crime investigations. External influences reference personnel level actions

6.4 Discussion

Before encouraging the increased involvement of forensic practitioners in wildlife crimes it is important to first establish how and when forensics is currently being used. Understanding case load demographics and commonly encountered evidence types helps to visualise whether a gap exists for CSEs to fill. It is evident that WCO's place significant value on forensic evidence with it cited as a the second most common contributor to successful prosecutions and convictions. Despite its collectively agreed value this studies results paint an inconsistent approach to its use amongst officers, largely influenced by the types of case they proportionally encounter. Case studies included in guidance documents such as those provided by PAW, provide contextual insights as to possible reasons behind

these deviations (PAW Forensic Working Group, 2014). In incidents involving domestic UK species, species identity is not in question, therefore greater effort appears to be placed on collection of a larger diversity of evidence types, often focused on linking a human perpetrator to the crime. By comparison case studies involving CITES species are the result of seizures, where a suspect is already identified, and as such heavy focus is placed on carrying out species identification of the seized wildlife specimens. The results on common forensic submissions by case type strongly mirror the approaches seen in these case studies indicating tailored approaches to evidence collection by WCOs, potentially because of guidance literature. Despite forensic submissions both taking place and holding perceived importance, survey results reinforce the narrative that lack of evidence presents a challenge for police officers investigating wildlife crimes. As such there is an expectation that at least attempts will be made to capitalise on all potential avenues of forensic evidence whenever the situation presents itself. The results indicate this is not the case, and evidence of almost 50% of WCOs showing a failure to consider human trace evidence in their wildlife crime scenes seems a gross oversight. Inexperience fails to fully explain this phenomenon. No significant relationship was found between length of service and historical submission of human trace evidence, and low, high, and middling, rates of scene attendance showed variation as to types of human trace evidence submitted. I would hypothesis that the higher number of scenes attended annually would provide greater number of opportunities for both DNA and fingerprint evidence collection. However, WCO's attending 11 – 15 scenes a year showed significantly higher than expected rates of only fingerprint submissions and those attending 6 - 10 scenes a year a skew towards DNA collection only. The inconsistencies seen here are challenging to interpret. Again, experience fails to paint a full picture; tendency towards one evidence type through learned experience would still require trial and error of both DNA and fingerprint collection at some point in a WCO's wildlife case

experience, which these results do not present. It is possible that their encountered case types simply do not present opportunities for both types of human trace evidence recovery, but this is unlikely given the breadth of evidence types demonstrated in case studies (PAW Forensic Working Group, 2014). WCO's focused on fingermark recovery only may be deterred by the high costs associated with DNA evidence, particularly given reports of concerns around funding limitations (Figure 6.19). Although the aggregate value of DNA evidence across all crime types is up for debate its contribution to individual cases remains strong (Amankwaa and McCartney, 2021). As such, with comparatively low numbers of wildlife crimes comparable to volume crimes and lack of evidence a concern, complete dismissal based on funding concerns could still be considered a blinkered approach to investigations. One potential explanatory variable is the required involvement of CSE's. Officers attending 11 – 15 scenes a year had a higher-than-expected rate of CITES cases and submission of live wildlife specimens for forensic analysis. Any scene involving a live animal will be time sensitive, therefore it is reasonable to assume a WCO may decide to focus on prioritising evidence which can be bagged up for fingerprint enhancement attempts, over waiting on the arrival and attendance of a CSE for human DNA recovery at scene. This theory fails in my DNA focus example however, as officers attending 6 – 10 scenes a year had a higher-than-expected rate of poaching cases as their highest caseload, and vehicles as their most commonly submitted evidence type for forensic analysis. Vehicles present as large objects that benefit from on scene attendance by CSE's due to the logistics and challenges of moving them. As surface types they are suitable for recovery of both trace DNA and fingermarks without the need for lab based techniques (Kirgiz and Calloway, 2017; Giovanelli et al., 2022) making in-situ collection at crime scenes both possible and recommended per guidelines. Further work is needed to understand individual officers motivations behind focus on singular human trace evidence types in wildlife crime

investigations. Work should also be carried out to establish whether there is a pattern of significant deviation between their approaches to wildlife investigations and other crime types, such as volume crime, as alluded to by one officer in their open-ended comments (Appendix III).

Concerns raised within open comments further demonstrate the value of CSE's professional experience when considering forensic interventions in wildlife crime case work. Despite both WCO's and CSE's being presented with research results on best practice methods of human trace evidence recovery from wildlife specimens, established in Chapters 1, 4 and 5 of this study, only CSE's raised valid concerns related to their accessibility and validation (Figures 6.17 & 6.18). The ability to critically assess presented forensic interventions and question the legitimacy of training programmes is an important requirement of training recipients. These skillsets will help to reduce the risk of investment or implementation of ineffective or inadmissible methodologies so often cited as hampering investigations (Ceccato and Uittenbogaard, 2013; Salum et al., 2017b; UNODC, 2020; Wildlife and Countryside Link, 2022b). A significant relationship was found between a WCO's length of time in service and highest proportion of case types. Whilst there is insufficient information available to reliably organise case types by complexity, as alluded to throughout this thesis, cases related to the IWT are comparatively heavily represented in the media, and thus investigations are at risk of scrutiny and may be entrusted to more experienced officers. Though professional experience may provide benefits to investigations, it can also lead to officers being forced to take on larger, complex caseloads beyond the scope of new recruits (HM Inspectorate of Prisons, 2024). This in turn increases regular exposure to traumatic events leading to burnout and impacts to mental health (Craddock and Telesco, 2022). My results indicate experienced WCO's may be at risk of these same pressures, with those in the role for 5+ years having a larger proportion of CITES cases and numbers of scenes

attended annually. The UK College of Policing recommends a wildlife crime officer investigates wildlife crimes (College of Policing, 2022) and in England and Wales, it is recommended that police forces should have at least one designated wildlife crime officer (Nurse and Harding, 2022). Within the Metropolitan Police this recommendation is expanded to encompass each individual borough. This places a potential for a minimum of seventy-five wildlife crime officers (43 police forces and 32 London boroughs) spread across England and Wales. In 2022 the Wildlife and Countryside Link estimated 4457 reported incidents of wildlife crime in England and Wales (Wildlife and Countryside Link, 2023). Based on these figures individual officers would need to handle 60 wildlife cases a year, or 5 per month to cover all reports. Whilst the survey question in this study specifically refers to crime scene attendance, not general caseloads, with only 39% of officers attending an estimated 1.25 wildlife crime scenes a month, and 28% of officers attending a maximum of 0.4 scenes a month it suggests UK law enforcement may be under resourced in manpower to meaningfully deliver on investigations. Interestingly this assessment is not one shared by surveyed officers. A lack of manpower was only the fourth most cited reason for failed prosecutions and convictions, potentially negating any significant concerns of burnout.

By contrast CSE's placed lack of manpower as the second most assumed factor contributing to unsuccessful prosecutions and convictions. As with police officers high caseloads and backlog, repeated exposure to violent scenes and lack of resources are known contributors to stress and burnout amongst forensic practitioners (Almazrouei et al., 2021). Several open comments given by CSE's allude to these challenges, with staffing levels repeatedly cited as a limitation to participation (Figure 6.17), suggesting a heightened level of sensitivity to perceptions of increased workloads. For a significant proportion of CSEs this training module was their first exposure to the subject of wildlife crime and the casework experienced by the WCU and therefore the perception of a newly introduced crime type

would result in higher workloads is understandable. This perception was anticipated, and a point made during the training to provide CSE's with an expected rate of scene attendance at less than one per month. This figure was based on the number of CSEs employed by the Metropolitan Police, attending WCU detectives professional experiences, and data from officer surveys. Responses indicate inclusion of this guidance had a positive impact on CSEs perceptions; more than double the number of respondents disagreed involvement in wildlife crime would increase workloads to unsustainable levels comparative to those who agreed with the statement. The neutral response given by a third of responses could suggest CSEs already consider their workloads unsustainable, a concern reinforced by open comment response (Figure 6.17). Though significant case numbers may not be an issue, wildlife crimes can present as violent or disturbing scenes (BBC News, 2021). If increased forensic engagement in wildlife crimes is a desired outcome for law enforcement, serious consideration should be given to providing adequate resources to CSEs so they can handle what, for many, will be a novel situation which they will not have had the opportunity to develop coping mechanisms for (Cartwright and Roach, 2022).

It is clear that there is an absence of a standardised and complete approach to human trace evidence recovery in wildlife crimes. WCO's confidently and regularly submit wildlife specimens for forensic analysis but falter in their approach to decisions surrounding trace DNA and fingermark recovery. This chapter paints a telling picture of lack of communication and engagement between the CSE's and WCO's. Open comments in both sets of surveys considered apathy by their colleagues to be an influencing factor to lack of CSE involvement in casework (Figure 6.17). Despite this suggestion my results do not portray a systemic aversion to wildlife crime scene attendance by CSE's but rather a systematic failure to actively include them in both training and casework.

Lack of resources for wildlife crime investigations remains a named concern in England and Wales (Nurse and Harding, 2022). These findings suggest that perception of what these resources are can differ within organisation groups influenced by their individual roles. However, lack of prioritisation is seen by both groups as a leading contributor to failed outcomes. Between the completion of this training and writing of this chapter the Metropolitan police WCU was disbanded and detectives re-assigned within the organisation. This has left the Greater London area without a co-ordinated and expert input into wildlife crime investigations, putting casework prioritisation and outcomes at the mercy of individual detectives and officers. Due to the voluntary nature of WCO role it can be assumed there exists an above average interest in wildlife crimes amongst them. However, it cannot be assumed this extends en masse to elsewhere within the police force inclusive of CSEs. As individual contributions play such an important role in wildlife crime investigations in the UK (Nurse and Harding, 2022) if the disbandment of these dedicated units is not simultaneously delivered with awareness and skill training for the wider enforcement populace there presents a serious risk of collapse of wildlife crime investigations in the Greater London region. The findings of this chapter have demonstrated that a 2-hour dedicated training programme for CSE's can result in knowledge acquisition, and an increase in potential for future participation, inclusive of higher prioritisation of wildlife crime case work. Such training has the potential for high returns on quality of crime scene management and evidence handling in wildlife crimes for minimal investment of both time and resources.

6.5 Summary

My findings support those seen in other reports that forensic support is lacking in wildlife crimes risking ineffective and sporadic contributions to overall investigative outcomes (Frances Goodrum et al., 2023). Collection of wildlife specimens present as key focal area

for WCO's, indicating the success of current wildlife forensic training programmes, however there is an overall lack of standardisation in management of wildlife crime scenes in the UK. A significant proportion of officers fail to consider fingermarks or trace DNA in their investigations, indicating a strong potential for missed evidence. A portion of the training delivered in these programmes focused on drawing attention to the potential for human trace evidence recovery from wildlife specimens. There are encouraging reports from surveyed WCO's that attempts of this strategy have positively contributed to casework. Evidence of operational benefits coupled with findings of best practice methods from Chapters 4 & 5 in this thesis help build a foundation of justification for their considered application in wildlife crimes. However, due to existing protocols and mandates the lack of inclusion of CSE's in wildlife crime casework inherently limits possibilities for wider take up of these methods. Overall CSE's agreed that the training increased their knowledge on the subject of wildlife crime. The significant correlation between a CSE's knowledge on the subject of wildlife crime and their likelihood of participation in an associated case demonstrates the value of their inclusion in training exercises. Given CSE attendance at a scene must be triggered by officer invitation WCO's need to take on a more proactive role in communicating and advocating for their involvement in relevant training. WCO's, particularly those still new to their roles, should also consider the significant contributions CSE's can make beyond their scientific expertise such as investigative experience, community engagement, institutional understanding and engagement with other departments (Chowdhury, 2021).

Chapter 7: General discussion

7.1. Contributions to understanding current approaches to the use of forensics in wildlife crime investigations

The introduction and literature review of this thesis revealed that the focal aspect of forensic interventions in wildlife crime investigations is firmly set upon wildlife identification (Thomas et al., 2023). As such a significant proportion of research was found to have objectives that geared towards the development of robust methodologies in both morphological and molecular identification techniques. In turn associated capacity building endeavours for enforcement actors were shown to be skewed towards training in the identification, collection, handling, and preservation of wildlife specimens. I have discussed how this apparent tunnel vision in forensic interventions hinders progress by pooling limited resources into the development of new costly infrastructure, techniques, and training programs that serve a function with limited transferability outside of their immediate context. In recognition of a need to address this, and the continued narrative of low resourcing and prioritisation, the review drew attention to the chronic failure to capitalise on existing forensic infrastructure, theories, and techniques in wildlife crime investigation, namely the recovery and analysis of human trace evidence. A critical analysis of the minimal work that had been done in this area showed it presented with a narrow focus on singular species or scenarios. Chapter 1 therefore concluded there was not only an overarching lack of consideration of human trace evidence recovery in wildlife crime investigations but also a failure to consider that technique functionality must accommodate the diversity in which wildlife crimes present themselves.

The results from Chapter 6 of this study, of which one objective was to investigate the current use of forensics in wildlife crime investigations in a UK context, provided evidence

in support of the conclusions drawn in Chapter 1. Results from its surveys confirmed that lack of prioritisation and evidence are considered by UK law enforcement to be significant contributors to negative outcomes in wildlife crime investigations. In addition, they revealed that direct and forensic evidence is considered an important contributor to obtaining successful outcomes in investigations. These insights provide important guidance on direction for resource investment; these being efforts to increase the prioritisation of wildlife crimes and collecting larger quantities of evidence with focus on direct and forensic in the first instance. Importantly I have revealed that despite a consensus on the need for more forensic evidence, there is a lack of consistency in officers approach to its use in wildlife crime investigation and that there is a high rate of absence in attempts at human trace evidence recovery. This was evidenced by responses from both officer and crime scene examiner surveys with a lack of evidence submissions by the former and a lack of invitation to attend crime scenes for the latter. These results however demonstrated that a lack of contextual knowledge within CSEs, beneficial to ability to effectively contribute to investigations, can be overcome through face-to-face training. Results from the post-training assessment indicate that such focused training sessions can also help improve perceptions of priority and likelihood of participation in investigations.

7.2 Contributions to identification of human trace evidence recovery techniques viable for use on wildlife specimens.

Chapters 4 and 5 aimed to identify human trace evidence recovery techniques effective on multiple types of wildlife derivatives, recognizing that diversifying forensic evidence collection fits in strategically with enforcements opinions on successful wildlife crime interventions and that wildlife specimens are already key targets for forensic submissions. To this end this thesis has produced a body of evidence that the recovery of fresh

fingermarks and trace DNA depositions is a feasible endeavour from wildlife specimens of various colours, textures, and materials and that specific techniques significantly outperform others in their efficacy.

Within Chapter 4 it was demonstrated that the reduced scale Supranano™ powders that showed promise in research on fingermark enhancement on ivory (Weston-Ford et al., 2016) are also capable of enhancing fresh fingermarks of identifiable quality from antler, hippo ivory, conch shell, snake skin, sawfish rostrum, primate skull, *Panthera* claw, tortoise shell and bovine horn. I have shown however that mono-chromatic powders often fail to produce the necessary degree of contrast that is desired for easy interpretation and photography of ridge detailing for subsequent grading purposes. Instead, it was shown that a powder with fluorescent properties offers up a higher rate of success in providing contrast and this contrast can be further strengthened through excitation using a commercially available UV torch projecting a 365nm wavelength. Red fluorescent powders were found to provide a strong level of contrast on multiple specimens, and it was postulated this was due to colour theory placing red as further away on the colour wheel comparable to the major presenting colour of the specimens tested in this study.

In this thesis experimental outcomes demonstrated that despite their success on pangolin scales (Moorat et al., 2020), gelatin lifters generally perform poorly in untreated fingermark recovery from wildlife specimens. However, when used to lift powdered fingermarks they can retain ridge definition without any significant loss in grade quality, indicating their useability in an alternate capacity in wildlife crime investigations. This finding reinforces the concerns laid out in Chapter 1 regarding existing research efforts having too narrow a focus of singular species and as such missing full potential of tools that may render them of higher consideration for a wider uptake amongst the forensic community.

Chapter 1 revealed research into trace DNA recovery from wildlife specimens was even more scarce than that looking into fingermark recovery. Chapter five of this thesis has provided evidence that greater consideration should be given for its inclusion in both research and investigations. It was found that fresh trace DNA can recovered from ungulate fur, elephant skin, elephant ivory, conch shell, antler and snake skin. As with fingermarks one method of recovery, a wet/dry swabbing technique using foam swabs, presented itself as capable of performing successfully across a diverse range of specimen types. Not only did this method recovery high average quantities of DNA it was demonstrated this translated into numbers of alleles. This made mixed profile interpretation possible, to the degree that LR's equivalent of "very strong support" for the prosecution proposition could be produced for known contributors to the sample. Results from Chapter 5 also raise the point that certain recovery techniques introduce potential for inhibitory constituents found in wildlife specimens, particularly those that have been taxidermized, into the PCR workflow making them less suitable for use. The success of foam swabs was attributed to their larger surface area, looser weave, and malleability, allowing for greater ability for manipulation across the varying presented textures and the subsequent easier release of DNA during extraction.

As a result of this thesis, I have identified Supranano™ red fluorescent magnetic fingerprint powder and Whatman foam swabs as methods that demonstrate wide-ranging applicability for recovery of fresh trace evidence deposits from wildlife specimens.

7.3 Contributions to the wider forensic research community

Chapters 2 and 3 aimed to identify a deposition technique to be used in later stages of this thesis however their results additionally offered insights into factors influencing fingermark deposition and DNA transfer. On all counts handling technique was not found to hold any

significant relationship with depositions but the act of “grooming” or “loading” does. The results provided further evidence that sebum may play an important role in trace DNA transfer contributing important knowledge to the discussion on the origins of trace DNA and potential consequences of actively abstaining from face-touching as part of experimental designs. Our results on the role grooming plays in influencing fingermark depositions and subsequent enhancement using powders were more complex. However enough evidence was provided to justify the methods chosen for depositions in Chapter 4, and to open up conversations as to whether the existing strict guidelines regarding the inclusion of “grooming” activities are fully justified in being representative of operational scenarios.

7.4 Contributions to strengthening the wildlife forensic toolkit

This thesis proposed an original observation and subsequently a supporting body of evidence that forensic interventions in wildlife crime lack the comprehensive approach seen in other crime types, with a chronic failure to consider human trace evidence at related crime scenes. To address this gap novel research was carried out comparing trace evidence recovery techniques on a wide range of wildlife specimens. Resulting experiments identified a reduced scale magnetic fluorescent fingerprint powder and foam swab that would be capable of performing trace evidence recovery in a multitude of wildlife crime contexts. Therefore, this thesis has provided law enforcement and forensic communities with the potential to expand the quantity and diversity of evidence types collected during wildlife crime investigations. Outcomes of this thesis also established that, in the current UK context, even if these techniques are validated promptly, their immediate adoption is unlikely without significant efforts to integrate wildlife crime into CSE training and improve communication between officers and CSEs. However, it is shown that within a city based

CSE workforce an increase in awareness and prioritisation is achievable through short face to face training programmes.

7.5 Study limitations and future work

The experimental limitations of Chapter 2 – 5 in this research are recognised and namely include deviation from current suggested guidelines in conducting trace evidence research, such as those provide by the IFRG (Almog et al., 2014). For example, although existing guidelines are not prescriptive and Chapter 2 justified the use of groomed fingermarks in this thesis, especially due to the abnormal anthropogenic environments created by COVID-19, their use remains contentious within the forensic community. This makes the results of Chapter 4 subject to greater scrutiny than if "natural" fingermarks were used. Fortunately, the timing of experimentation for Chapter 5 allowed for a closer adherence to other research seen in the trace DNA literature. However, neither set of experiments included any significant period of ageing and whilst confident the experimental designs were sufficient in their aims of comparing recovery techniques, they lacked the ability to draw conclusions regarding the persistence of fingermarks and trace DNA on the tested specimens. This limitation presents with a natural progression for future work into persistent studies with experimental designs including time, exposure to environmental and simulated storage and transport scenarios conditions as variables. This will provide important contextual knowledge to enforcement as to when best to place appropriate forensic resources in an operational setting.

In the long-term to ensure adoption of these techniques by law enforcement and the wider forensic community it will be important to ensure research is in keeping with expectations put forward by relevant bodies and have the techniques validated. Within Chapter 4 the fingermark enhancement and recovery techniques chosen for comparison were based on their existing success in the literature but also due to the fact Supranano™ BMP and gelatin

lifters have both already been included in forensic recovery kits specifically developed and deployed for use in wildlife crime casework. It therefore seemed a logical and justifiable approach to test their efficacy on a wider variety of specimens so that organisations in possession of these kits could realise and employ their full potential. However, it is recognised that inclusion of a more common fingerprint powder, such as aluminium, magneta flake, or standard black magnetic would have strengthened the work by introducing a powdering technique for comparison that is already validated and utilised by the majority of CSEs. To further investigate the value of red fluorescent SMP as an enhancement technique work should be done to compare it against more standard issue powders and lab-based enhancement techniques. Finally, although this thesis demonstrated that within the UK there will be required steps before the identified techniques can be implemented it does not delve into their potential for implementation outside of a UK setting. This opens another line of needed research into investigating the current use of forensics in wildlife crime investigations on an international scale and their capacity for deployment of the techniques identified in both this study and others.

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Appendix I

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Review article

What is it vs Who did it? A review of the lack of human focused forensic evidence in the context of wildlife crime

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ABSTRACT

Wildlife crime suffers from low prosecution and conviction rates, with a lack of evidence and resources cited as hurdles to enforcement. Forensic evidence is used in human-on-human crimes to identify perpetrators and link individuals to criminal activity. Forensics approaches in the context of wildlife crime are heavily focused on non-human evidence using DNA barcoding to establish species and geographical origins. In human-on-human crime fingermarks and DNA profiling are two of the most recognisable forensic evidence types, both with significant global infrastructure, which contribute to prosecutions and convictions. Wildlife products can be the only physical evidence type available in a wildlife crime but attempts to recover human forensic evidence from them is a relatively unexplored area. The research that does exist demonstrates fingermark and touch DNA evidence can be collected in many contexts from several different species. Despite this there has been only one report of utilisation of this type human evidence recovery in wildlife case work. Failure to consider all potential evidence types has a negative impact on wildlife crime investigations. There is a need to experimentally assess the benefits and limitations associated with the collection of human evidence from wildlife items. This article introduces key factors that affect the recovery of human fingermarks and touch DNA evidence before focussing on the limited number of instances where these methods have been applied to wildlife forensic research and what considerations should be taken when developing further work in this field.

Introduction

Wildlife crime consists of a broad spectrum of activities, geographic ranges, and species of interest. The illegal wildlife trade (IWT) is one of the most recognisable iterations of wildlife crime and stands as a global crisis. Over 140 countries have reported incidences of either illegal import, export or transit of at least 6000 species [1]. Other well documented examples of wildlife crimes include, illegal poaching/-hunting/fishing [2], animal persecution [3,4] and nest/roost destruction [5]. Contributing to biodiversity loss [6], zoonoses risks [7], and violence [8], wildlife crime and its impacts are firmly on the radar of governments, NGO's and law enforcement agencies. However, despite its recognition, wildlife crime may still be underestimated or mis-reported in its scale, not wholly understood in its subtleties [9], and suffers from low prosecution/conviction rates [10,11], and failed interventions [12].

The UK, and elsewhere, tend to focus their interventions on critically endangered charismatic megafauna and the IWT [13,14]. Neither these species, nor the IWT, are fully representative of the diversity within wildlife crimes and evidence shows that species designated by IUCN as 'least concern' are still the target of illegal activities, such as human-wildlife conflict [15,16] or specimen collection [17]. Policies have often failed to make a positive impact on broader wildlife crime or at the domestic level, perhaps as a result of such hyperfocus on flagship species [13]. There are both proactive and reactive approaches to tackling wildlife crime. Proactive approaches focus on deterrence tactics; educational programmes, community engagement, alternative livelihoods, policies, and legislation aim to prevent and deter wildlife

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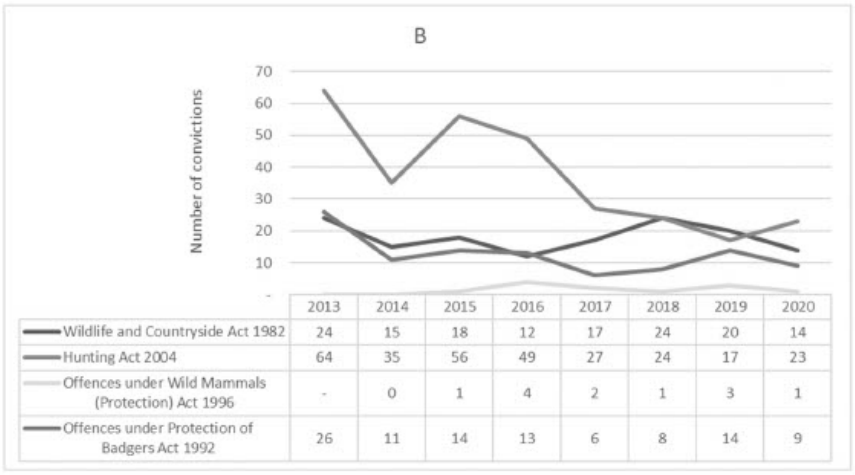
crimes from being carried out in the first instance. Reactive approaches include investigating crimes which have already been committed, enforcing the extensive national legislation and international agreements that exist and gathering intelligence which can feedback into more proactive work.

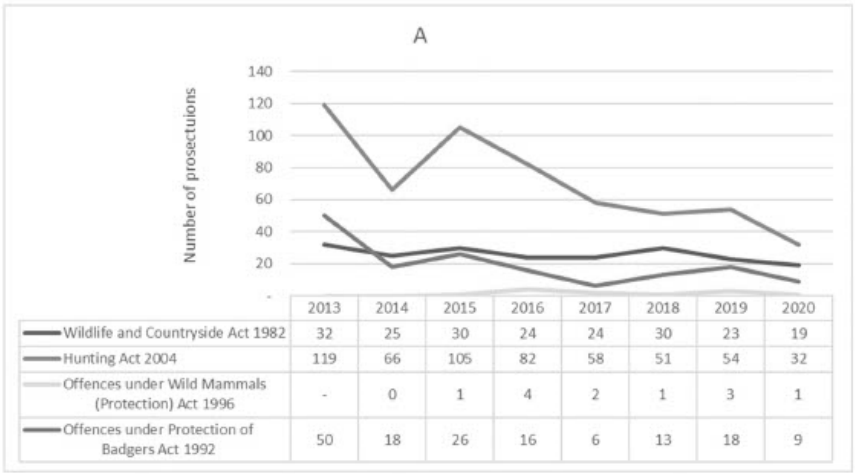
Enforcement success in wildlife crime has been varied; high profile arrests such as that of the "Ivory Queen" [18] suggest promising developments in targeting principal players in trafficking rings. However pre-pandemic seizure rates remained consistent or are increasing for a range of species [19], indicating such arrests are not acting as sufficient deterrents. Nations with high risk species have been found to struggle [20] while lenient sentencing is a repeated concern [11,21]. Suggested underlying factors that impede enforcement include; 1) limited resources, 2) overwhelming scale, 3) corruption, 4) apathy, and 5) ineffective deterrents [22]. These challenges lead to an inference that wildlife crime is a low risk, high reward activity [21,23].

A lot of wildlife crime discussion focuses on highly biodiverse low-income nations as key exporters of wildlife goods. However, enforcement problems are not isolated to these areas and high income nations which play a large role in imports also lack in this arena [11]. For example, the UK is well placed to support wildlife crime investigations; it has a government funded National Wildlife and Rural Crime Unit (NWRCU), stakeholder involvement through the Partnership for Action Against Wildlife Crime (PAW), as well as a clear policy describing their priority areas [6]. Though lauded for their contribution to international efforts to tackle wildlife crime, such as the IWT challenge fund, a recent UN report recommend the UK strengthen their domestic policies and efforts [24]. Advice underscored by the increased number of reports of crimes against badgers and bats, two priority species, [25] but decline in prosecutions and convictions under key wildlife legislation (Fig. 1a and b).

Across all nations and crimes, law enforcement seeks to achieve



	2013	2014	2015	2016	2017	2018	2019	2020
Wildlife and Countryside Act 1982	24	15	18	12	17	24	20	14
Hunting Act 2004	64	35	56	49	27	24	17	23
Offences under Wild Mammals (Protection) Act 1996	-	0	1	4	2	1	3	1
Offences under Protection of Badgers Act 1992	26	11	14	13	6	8	14	9



	2013	2014	2015	2016	2017	2018	2019	2020
Wildlife and Countryside Act 1982	32	25	30	24	24	30	23	19
Hunting Act 2004	119	66	105	82	58	51	54	32
Offences under Wild Mammals (Protection) Act 1996	-	0	1	4	2	1	3	1
Offences under Protection of Badgers Act 1992	50	18	26	16	6	13	18	9

Fig. 1. Number of prosecutions (A) and convictions (B) under four key pieces of UK wildlife legislation between 2013 and 2020.

positive and accurate identification of the human criminal(s) responsible, and the production of robust evidence to inform and direct criminal investigations. Evidence types are vast but one consistent evidence type utilised in criminal investigation for identification purposes is forensic evidence [26]. In the context of wildlife crime, forensics has seen significant investment in recent decades. Whilst veterinary forensic pathology has been regularly implemented to ascertain cause of death [27,28], species identification, through the use of DNA barcoding, has been the main focal area for identity forensics [29]. This work addresses the need to positively identify the species of wildlife and their derivatives for both case work and intelligence gathering, particularly in the absence of morphological characteristics. From it has stemmed the existence of databases containing animal barcode data, including the Barcode of Life Data Systems (BOLD) [30], GenBank [31], and ForCyt [32] as well as international and domestic collaborations including the African Wildlife Forensic Network [33] and PAW forensic working group [34]. The discipline has demonstrated its value by contributing to several wildlife crime investigations [16,35]. A natural progression from species identification has been the need for individual identification or parentage analysis. This has been used to help link individual wildlife parts to crime scenes [36], to link shipments [37], to identify individual animals from private collections [38] or to camera trap records [39], and to establish the number of individual animals that are victims in a crime [36]. Species identification and individual identification in wildlife crimes commonly employ mitochondrial DNA (mtDNA), and Short Tandem Repeat (STR) profiles of nuclear DNA (nDNA) respectively [29]. Within species identification universal primers have been developed for several mtDNA loci however the cytochrome *b* (cyt *b*) gene and the cytochrome oxidase 1 (COI) gene, are most commonly utilised [40]. STR typing uses the same concepts as human DNA profiling with multiple STR loci identified, in the case of humans 17–24 loci, and analysed to establish their size allowing a profile to be built. The sequencing of the human genome has allowed for human DNA profiling to become standard practice and multiple commercial kits be produced. To make STR typing as common in wildlife forensics it would require a similar rigorous approach to identifying suitable STR loci including a representative sample from the population; this is a daunting prospect for the thousands of species that fall victim of wildlife crimes many of which are critically endangered [41,42]. As such there are a minimal number of wildlife species STR typing has been developed for and due to the extensive resources required progression in this area is significantly slower [36]. One pressing limitation with the area is the need for high standards to be met, within both laboratories and practitioner communities, for wildlife forensic science to be taken seriously within the wider forensic and law enforcement community [43]. ISO/-IEC 17025 and 17020 accreditation is the internationally recognised standard, and often legal requirement, for forensic laboratories and practitioners to prove their competency to collect process forensic evidence [44,45]. A 2016 CITES and UNODC commissioned survey of 110 wildlife forensic associated laboratories found just 22 were externally audited under these standards [44]. Though a lack of accreditation does not equate to a lack of capability or skill, it may result in associated evidence collected or processed at/by these establishments/individuals being bought under scrutiny.

A more traditional use of forensics in criminal investigation is the application of human identity testing, often presented as fingermark or DNA evidence [26]. Global infrastructure for human identity testing, including accredited laboratories, is constantly growing [46] and a wealth of research, knowledge, techniques and tools exist for utilisation by law enforcement. Despite ongoing contributions to solving human-on-human crime the literature suggests its application and development, is low in wildlife crime contexts (Fig. 2). This is interesting given the theory and concepts behind both fingerprints and human DNA profiles have both been applied in wildlife crime contexts. Possible reasons for a lack of application and research in this area include i) the observed separation between practitioners of human and wildlife forensics, ii) a lack of awareness/interest by researchers as to the cross-applicability of the methods, iii) unpalatable costs associated with human forensic methods when investigations only lead to small penalties or iv) the methods are not applicable in most wildlife crime cases.

Regardless of the reason, the main aim of any criminal investigation is to identify a suspect and establish a link between the suspect and the illegal activity under investigation. Whilst species or individual identification of wildlife can establish if a crime has been committed and is of

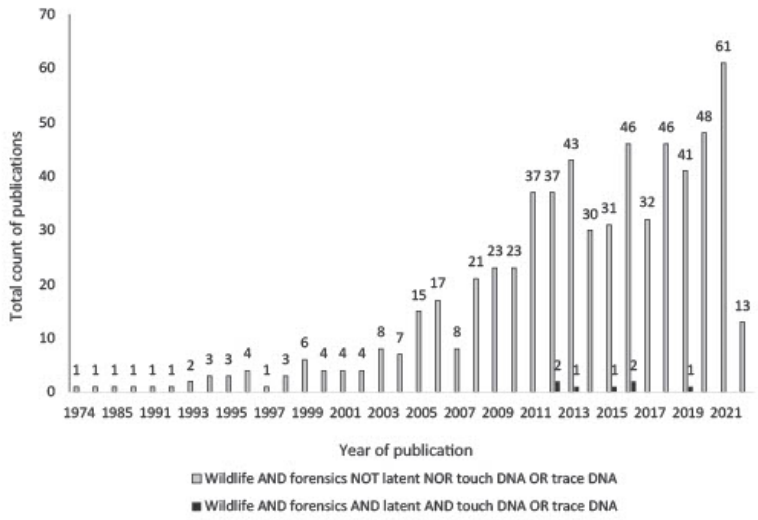


Fig. 2. Number of hits from a literature search using the Boolian terms "Wildlife" AND "forensics" NOT "latent" NOR "touch DNADNA" OR 'trace DNA' and a literature search using the Boolian terms "wildlife AND forensics" AND including "latent" OR "touch DNA" OR "trace DNA".

value in seizures where a suspect is claiming goods are of legal origin, it cannot always provide this link. Two recent UK cases of raptor and badger persecution demonstrate this phenomenon. In both cases carcasses, of white tailed eagles and a badger respectively, were reported to law enforcement with the condition of the carcasses rendering morphological assessment possible for species identification [47,48]. Both incidents occurred in rural areas with no immediate suspects and with the carcasses themselves appearing to be the only tangible evidence available. The position of the carcasses strongly indicated human involvement or handling. These types of cases suggest a logical need to increase the amount of human identity testing in wildlife crime investigation. Through this approach opportunity should present itself to increase the amount of evidence directly linking an individual to an associated crime, strengthening such cases where insufficient evidence is presented to identify a suspect or garner a conviction. Unlike species identification human based identification may also unearth links to other crimes, including those non-wildlife related, shedding light on the suspected crossovers in organised criminal networks [49].

This article reviews the two main forms of evidence used in human identity testing, fingermarks, and DNA evidence, and highlights the limited number of instances where they've been applied in wildlife crime research and investigations. This review was carried out in a traditional approach combining several evidence gathering methods including the identification of relevant stakeholders in the field; a review of UK government and policing related policy and guidance documents; identification through UKAS of common forensic methods used in human identification; and a trawl of the existing scientific literature of the most common methods.

Fingermarks: background and composition

All fingermarks are made up of a finite number of characteristics which present themselves as a unique pattern on an individual's fingertips. Once enhanced or recovered from evidence or crime scenes unidentified marks of sufficient quality can be compared against fingerprints of known individuals or against other unidentified marks to establish a match. Their composition changes almost immediately upon deposition, with time, environmental exposure and the substrate type (porous vs non-porous) all influencing longevity [50–52]. They are composed of an amalgamation of secretions (eccrine, sebaceous, and apocrine) coupled with skin or environmental contaminants (i.e. beauty products, food grease, pollen, dust) [53]. The immediate change to fingermarks occurs with the evaporation or absorption (dependent on surface type) of water and volatile lipids. Water loss results in a "waxier" fingermark as the remaining organic and inorganic compounds become concentrated. Salts will also crystallise and become vulnerable to physical erosion and UV exposure [52]. Over the course of around thirty days most lipid components of sebaceous secretions will reduce significantly; squalene and unsaturated fatty acids are lost first with saturated fatty acids and non-volatile lipids including wax esters and triglycerides being more durable [54–56]. As well as water, temperature, humidity, UV exposure and other forms of radiation contribute to the longevity of latent fingermark constituents [56–59]. Despite this volatility fingermarks have been recovered decades after deposition [50,60] and after days or weeks of environmental exposure [61,62].

For processing of unknown marks the Analysis, Comparison, Evaluation, Verification (ACE-V) approach is widely adopted [63]. Historically each phase was carried out by hand however increasingly countries are utilising biometric Automated Fingerprint Identification Systems (AFIS) in their workflows [64]. A traditional AFIS functions via algorithms focused on identifying and tagging fingerprint minutiae, specifically bifurcations and ridge endings (Fig. 3a), creating a "map" for comparison [65]. Three countries hosting large biometric databases, China, the USA, and the UK are notable players within wildlife crime either as import [66], export or transit countries [67] or as vocal advocates for improved international efforts [68]. The transnational nature of wildlife crimes is well documented and in this vein INTERPOL hosts an international AFIS accessible to member nations [69].

Fingermarks: crime scene and laboratory enhancement methods and photography

Latent fingermarks, those invisible to the naked eye, are the most common type of fingermark encountered at crime scenes with no reason to believe wildlife crimes would be an exception [71]. Initial detection of fingermarks allows for more targeted application of enhancement methods, conserving resources, and time. This is commonly achieved through multispectral forensic light sources or simple oblique lighting [72]. Once detected enhancement treatments, chemical, physical or a combination, allow for the visualisation of the fingermark. Treatments do not have to be used in isolation but due to the potential interactions between sequential treatments a strict order of approaches is followed [73]. A breakdown of the most common fingermark enhancement methods is provided in Table 1. For further analysis, and their utilisation and preservation as evidence, a record of an enhanced fingermark must be obtained, one of sufficient quality for repeated reference and identification. Photography using Digital Single Lens Reflex (DSLR) cameras is the most consistently used documentation approach, however with

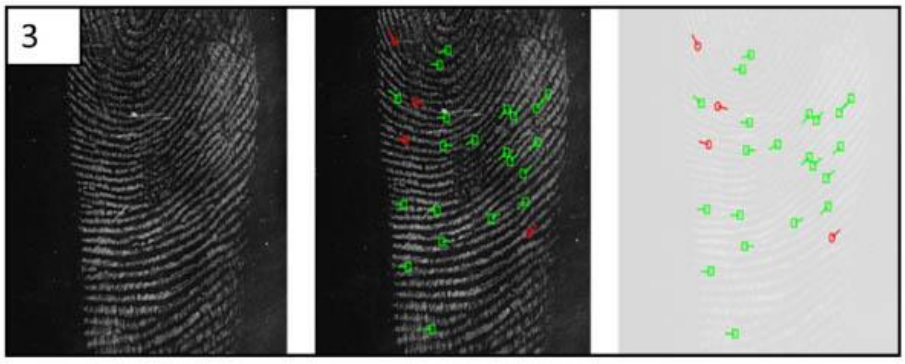


Fig. 3. (left) Latent fingermark with no mark-up in original state; (middle) Highlighted bifurcations and ridge endings as would be placed by an AFIS; (right) A "map" of minutiae that would be searched against. (2a). Images generated using Fingerprint Minutiae Viewer (FpMV) software [70].

Table 1
Common fingermark enhancement methods presented in order of their recommended sequential application.

Enhancement method	Theory	Practical Limitations	Porosity	Physical or Chemical
Fluorescent examination	Exploitation of fluorescing constituents either within fingermarks or substrates to provide contrasting illumination.	Requires a dark environment which can be difficult to achieve outside laboratory conditions.	Non-porous, semi-porous, porous	Physical
Powder	Applied directly to a substrate with the design of having a stronger affinity to fingermark constituents comparable to the surface they have been deposited on	Poor application technique can result in damage to the fingermark.	Non-porous, semi-porous	Physical
Powder suspensions	Fine powder incorporated through a solution of detergent and water believed to interact with eccrine and sebaceous components of fingermarks.	Requires a water wash step after application which can be messy and impractical to contain at a scene.	Non-porous, Semi-porous	Physico-chemical
Vacuum metal deposition (VMD)	Functions via the sequential evaporation of metals (gold, zinc) onto a surface within a vacuum. Fatty acids within fingermarks inhibit the layering process so that they become visible within the coated substrate.	An expensive process requiring specialist equipment and training. Irregular shaped objects can be difficult to process if areas are shielded from direct line of evaporation vessels	Non-porous, semi-porous	Chemical
Ninhydrin	Targets the amine group within amino acids and constituents within eccrine sweat triggering a colour change reaction known as Ruhemann's purple.	Optimal process conditions are difficult to achieve at scene and humidity oven size in laboratory limits the size of items which can be processed.	Semi-porous, porous	Chemical
Basic Violet 3	A staining process which targets sebaceous sweat constituents, shed skin cells and other contaminants to produce a purple mark.	The rinsing step can make containment of the hazardous dye difficult at scenes	Non-porous	Chemical
Cyanoacrylate (superglue) fuming	Polymerisation of ethylcyanoacrylate (superglue) triggered by water within eccrine sweat results in the accumulation of a "noodle-like" structure presenting as a white residue onto a fingermark	Optimal process conditions are difficult to achieve at scene and cabinet size in laboratory limits the size of items which can be processed.	Non-porous, semi-porous	Chemical
1,8 – Diazafluoren – 9 – one (DFO)	Reacts with amino acids within a fingermark resulting in a fluorescence which must be subsequently viewed using fluorescent examination	The reaction is initiated through heating making it problematic to carry out at scene.	Semi-porous, porous	Chemical
Physical developer	Fingermark constituents trigger a disturbance within a stable silver-based solution resulting in deposition of silver at the disturbance site.	Highly impractical to implement at scene. Cannot not be followed up by subsequent enhancement techniques.	Semi-porous, porous	Chemical

Source: Adapted from the Fingermark Visualisation Manual [73].

the ever increasing availability of affordable high quality smart-phone cameras research is being done surrounding their use as a tool in latent print photography [74]. Alternatively the use of physical tools, including tape, gellifters and silicone, allows for the removal of the fingermark from the substrate itself [73]. Successful fingermark lifting is of particular importance on curved, irregular, or highly reflective surfaces where photography can be problematic [75,76].

Fingermarks: application of methods in wildlife crime

Much of existing fingermark recovery research has been focused on "traditional" crime scenes and evidence types; these include vehicles, weapons, clothing and household goods or infrastructure such as doors and window frames. This focus has spilled over into the wildlife crime context with fingermark recovery attempted on similar substrates in environments associated with wildlife crime activity [77]. A less traditional evidence type but one of vital importance in wildlife crime are animals and their derivatives. Comparative to "traditional" evidence types there has been minimal research of fingermark recovery in this area. The research that does exist can be loosely grouped into surface type and are as follows; leathers and skins inclusive of mammalian and reptile species, ivory, horn, antlers, feathers, eggs, fur, and pangolin scales (Table 2).

Fingermarks: leather and skins

Leather and animal skins are commonly encountered animal products most often seen in the guise of accessories such as wallets or belts and upholstery such as car seats. As such they are regularly encountered in non-wildlife case work and enhancement of fingermarks from these items are among some of the earliest associated work in this hybrid area. Leather is considered a problematic substrate due to its texture, porosity, and the multiple process stages it may be encountered in [73]. Despite the regularity in which leather items are encountered in criminal investigations success in fingermark retrieval is lacking [87]. Vacuum metal deposition (VMD), superglue fuming, iron oxide powder suspension, a combination of superglue and iodine fuming and the development of a novel fingerprint development membrane (FDM) with a ninhydrin developing agent have all proved successful [77,87–89] at developing identifiable marks on a range of leather types. However, results are often inconsistent, and many marks enhanced of poor quality. Due to the intensive processes involved in its creation, including tanning and dyeing, the properties of leather differ from the raw original skins it is derived from. The only work carried out retrieving fingermarks from raw animal skins is through the substitution of domestic pig skin for human skin in associated research [90,91]. Black magnetic powder and cyanoacrylate fuming have both proved successful in recovering fingermarks off pig skin, even after environmental exposure but the onset of putrefaction quickly deteriorates marks [92]. Although there are few similar "hairless" mammals that these methods could be trialled on the ones that do exist, including hippo (*Hippopotamus amphibius)* and elephant *(Elephantidae sp.)*, are high value targets within IWT [93,94]. However, movement of such large species into laboratory environments for chemical enhancement techniques such as cyanoacrylate fuming is unfeasible.

Reptiles represent one of the most trafficked wildlife groups, entering both legal and illegal markets as live specimens destined for the exotic pet trade and coveted reptile skins/leathers for high-end fashion markets [95–97]. Studies indicate that despite appearances reptile skin has some degree of permeability to contaminants and water [98,99] and likely fall under the "semi-porous" category. As a surface type for retrieving fingermarks there is additional complexity with background patterning and scale structure risking interrupting ridge lines, however marks have been successfully enhanced on both snake and lizard species [100]. Notably this work was conducted on both live and deceased specimens, making it applicable to both live seizures and worked goods.

Table 2
Breakdown of existing studies investigating methods of fingermark recovery from wildlife specimens.

Group	Substrate type	Deposition method	Deposition type	Enhancement	Visualisation, Collection & Photography	Variables	Specificity	Maximum grade achieved	Reference
Avian	Feather	Undirected	Ungroomed	Black magnetic powder (BMP), Black magnetic powder (BMP), magneta flake, red and green magnetic fluorescent, aluminium flake and magnetic bi-chromatic powders and cyanoacrylate fuming	Nikon D5100 digital SLR camera with an 18–55 mm lens or a 60 mm micro-Nikon lens + Mason Vectron Quasar 2000/30 connected to an Integrated Rapid Imaging System (IRIS)	Time	Positive enhancements obtained using red and green magnetic fluorescent up to 21 days after deposition.	4/4	[78]
Avian	Egg	Undirected	Ungroomed	BMP, Black magnetic powder (BMP), magneta flake, red and green magnetic fluorescent, aluminium flake and magnetic bi-chromatic powders and cyanoacrylate fuming	Nikon D5100 digital SLR camera with an 18–55 mm lens or a 60 mm micro-Nikon lens + Mason Vectron Quasar 2000/30 connected to an IRIS	Time	Usable prints obtained using black magnetic + magnetic bi-chromatic up to 14 days after deposition	4/4	[78]
Avian	Egg	Consistent pressure 10 s	Ungroomed	Cyanoacrylate fuming + Basic yellow 40 dye	Variable light sources + DSLR camera + Mason Vactron Quaser 40 MH + Canon EOS 5D Mark II with a 100 mm f/2.8 L-series macro lens	None	Usable prints obtained, with an increase in grade achieved through the use of viewing filters.	3/4	[79]
Avian	Feather	Consistent pressure 2 s	Groomed + Ungroomed	Green magnetic fluorescent powder	Blue Crime-Lite 82 S (10 % bandwidth 420–470 nm with a 445 nm peak) + yellow long pass filter (1 % cut-on point – 476 nm) + Nikon D200 with 40 mm f2.8 Nikon micro lens	Time + Environmental exposure	Usable prints obtained up to 60 or 14 days after deposition when stored indoors or outdoors respectively	4/4	[80]
Mammal	Ivory	Undirected	Ungroomed	BMP, Small particle reagent (SPR), cyanoacrylate fuming, BMP and VMD	Undescribed	Time	Usable prints obtained after two weeks using cyanoacrylate fuming	Not described	[81]
Mammal	Ivory	Medium pressure 1–2 or 10 s	Ungroomed, sebaceous and amino acid pads	Supranano Black Magnetic and Black Powder, Jet Black magnetic powder and cyanoacrylate fuming	Adhesive tape and a Nikon D4 camera fitted with a 105 mm Macro Nikkor lens and a 532 nm laser	Time + Sensitivity	Powders with particle sizes < 40 μm performed best, with usable prints recovered up to 1 week after deposition and positive enhancement achieved ridge up to 28 days post deposition	6/6	[82]
Mammal	Antler/ Horn	Undescribed	Deposited in blood	Vapour phase cyanoacrylate + R. A.M stain, leucocrystal violet	Forensic light source + photography	None	Positive enhancement achieved using both described techniques	Not described	[83]
Mammal	Antler	Undescribed	Undescribed	Cyanoacrylnte fuming + Volcano Black granular fingerprint powder, iodine fuming, ninhydrin, silver	Copy stand lighting + 4 × 5 Crow Graphic Camera	Moisture + Temperate + Time	Usable prints obtained using magnetic fingerprint powders up to 16 h after deposition	Not described	[84]

(continued on next page)

Table 2 (*continued*)

Group	Substrate type	Deposition method	Deposition type	Enhancement	Visualisation, Collection & Photography	Variables	Specificity	Maximum grade achieved	Reference
				nitrate, magnetic fingerprint powders					
Mammal	Scale (pangolin)	Undirected 5 s	Ungroomed	None	Gelatin Lifters + GelScan + Photoshop	Time	Usable prints obtained up to four months after deposition	4/4	[85]
Reptile	Scale	Undescribed	Sebaceous	Cyanoacrylate fuming + rhodamine stain, white or black fingerprint powder	Polilight w/ 590 m barrier filter + photography	None	Usable prints obtained using both methods on a range of species	Not described	[86]

On live specimens Lightning White Fingerprint Powder® showed the most success, likely due to the contrast it produced against the patterned scale coloration of many species tested. Lightning Black Fingerprint Powder® successfully enhanced marks on more uniform light reptile skins such as the ventral side of alligator (*Alligator mississippiensis*). Cyanoacrylate fuming coupled with rhodamine fluorescing stain examined using 530 nm wavelength viewed through a 590 nm barrier filter was effective at enhancing marks on multiple deceased species specimens. These same species also had marks successfully enhanced by fingerprint powders. In keeping with existing knowledge of surface type influence on fingermark retrieval it was reported the smaller and rougher the scales the more limited the enhancement success. In this research the movement of live specimens either led to the destruction of powdered marks or problems with image capture. Within the IWT trade transport conditions of live reptiles is often poor [101]. When subjected to transport conditions it has been found reptiles can demonstrate periods of prolonged inactivity [102]. Though improving the welfare of the animal would be of an immediate priority, this temporary period of inactivity could prove useful for fingerprint powdering in cases of seized reptiles. The issue of movement could also be overcome by use of newly developed techniques such as gellifters which could recover enhanced marks from the body of the animal in a non-invasive manner.

Fingermarks: feathers

Globally it is suspected that avian trafficking is underreported and that a significant proportion of animals are trafficked live for the pet trade [103]. Other species, particularly raptors, are persecuted for their perceived threat to livestock or game species such as grouse [104]. Feathers are a unique structure amongst birds and with their interlocking barbs and barbules resembling fabric weave, which at a macro level renders them as a porous material. Unlike fabric, feathers are at a high risk of disturbance from handling or environmental exposure with barbules readily separated. Coupled with the often-flamboyant colours and patterns on feathers which hinder the ability to render strong contrasts between mark and background, it makes them a difficult surface type for fingermark retrieval. There have been just two complimentary pieces of research looking into fingermark retrieval from feathers [105, 106]. For fabrics, VMD and cyanoacrylate fuming are the recommended approaches for fingermark retrieval with VMD the favoured approach on natural materials; powders of any kind are suggested as ineffective [73]. VMD has not been attempted on feathers but cyanoacrylate fuming has, and been found to be one of the least effective approaches [105]. It was postulated this was due to the hydrophobic nature of feathers but as cyanoacrylate is regularly used on non-porous and inherently hydrophobic surfaces it is more likely the porosity of the feathers was a contributing factor as superglue fuming is not recommended on porous materials. Fluorescent magnetic powders, specifically red and green were found to be the most consistently successful enhancement technique under controlled conditions.

The species trialled in these studies, kestrel, sparrowhawk, buzzard, red kite, and golden and white-tailed eagles have similar colour plumage, and as fabric comparisons were the underlying theory of approach plumage weave count rather than colour was a key focus. However, if fluorescent powder enhancement is to be a continued line of research plumage colour may be an important future consideration. Birds light sensitivity range sits between 300 and 700 nm, this is inclusive of the UVA (320–400 nm) end of the UV spectrum (100–400 nm) [107]. Feathers of several bird species, including heavily trafficked brightly coloured parrots and songbirds, have been found to fluoresce under UV light [108,109]. This may impact the ability of a fluorescing mark to stand out against a fluorescing background and considerations should be taken when considering which colour powders and subsequently wavelengths to use during enhancement and photography.

The second piece of research looking at fingermark recovery from feathers focused on environmental effects over time on green magnetic fingerprint powder development [106]. Marks were recovered up to 21 days after deposition with the location of the feathers, semi-protected or not from the elements, and precipitation having a significant effect on the success rates of recovery. Some relationship was also seen between both soil and air temperature and successful mark recovery. Marks recovered from control feathers left indoors were recovered up to 60 days after deposition. As noted by the author happening upon a singular feather, as used in this study, is an unlikely scenario in case work. A whole, or part, carcass is commonly seen in raptor persecution cases. These are at risk of scavenging and the likelihood of feathers and thus marks being disturbed. Even in these instances knowledge that identifiable marks can be recovered after such long periods is beneficial; even if minutiae detail has been disturbed there is still opportunity to identify handling sites for subsequent swabbing for DNA recovery. For live trafficking, the nature in which birds are often packaged, stuffed in tubes or bottles [110], and the inevitable movement of the birds themselves mean chances of mark recovery from feathers will be greatly diminished and there are greater opportunities for mark recovery from the packaging. It is important to consider these types of contexts when deciding which types of wildlife specimens to trial forensic techniques on.

Fingermarks: eggs

Egg theft and egg smuggling is a separate vein of avian associated wildlife crimes [111]. Eggs are easily concealed and have been known to be worn on a person's body for transport purposes [112]. Therefore sophisticated trade routes are not always a requirement and individual criminals can have devastating impacts [113–115]. Egg shells are widely diverse in size, shell thickness, and surface pattern, and importantly to fingermark enhancement shells are porous. This porosity, which varies inter and intra species [116,117] allows the exchange of oxygen and carbon dioxide and is an important consideration for potential enhancement treatments if dealing with live eggs. Research on

fingermark recovery from eggs as a food item initially found limited success with small particle reagent (SPR), a type of powder suspension [118]. A later study concluded cyanoacrylate fuming followed by rhodamine 6 G treatment was the most effective treatment but found best results when the egg had been refrigerated for fifteen minutes prior [119]. Both these studies require potential life-threatening interference with the egg, submersion, refrigeration, and exposure to toxic substances and as such not suitable for application in many wild egg theft crimes.

Research in this area with a focus on wildlife crime found black magnetic powder had a 96 % success rate at positively developing fingermarks on bird of prey eggs with enhancement possible up to 14 days after deposition [105]. The authors considered eggs as a non-porous material but with the knowledge of the inherent porosity of bird's eggs, a semi-porous designation is also appropriate. Given this, powder suspensions become a viable option for attempts at enhancement however the involvement of surfactants and need to wash the object makes their application to live trade limited. The only other study investigating fingermarks on non-domestic avian eggs also utilised cyanoacrylate fuming but with a subsequent Basic Yellow 40 dye treatment [120]. Different wavelengths were used to excite fluorescent components within the fingermarks but resulted in maximum grades of just one and two (on a scale of zero – four). When viewing filters were applied marks increased in quality up to grade three overcoming the patterned background of lapwing and grey partridge eggs. Despite their light uniform coloration, the same results were not achieved on Canada goose and White-tailed eagle eggs. These species possesses more notably porous egg surfaces, and the failure was attributed to the potential for the eggs to absorb the Basic Yellow 40 dye across its whole surface obscuring latent prints. In these studies no effort was made to lift the fingermarks despite the smooth uniform surface of eggs being an ideal candidate for attempts with gellifters. If the quality of the fingermark can be retained during the lifting process, analysis may be significantly easier as the problematic patterned background factor would be removed without the need for cycling through various wavelengths.

Fingermarks: ivory, horn, and antler

Ivory, horn, and antler are commonly associated with a wide variety of wildlife crime activities, with deer poaching being one of the UKs priority areas. Some of the earliest studies focusing on contextual fingermark retrieval from wildlife parts were on deer antlers related to poaching cases [121]. Mature antlers are exposed, regenerative, porous, rough bone which exist in different developmental states including a velveteen stage. On mature antlers, black magnetic fingerprint powder was found to be the superior method for consistent fingermark retrieval compared with cyanoacrylate fuming, ninhydrin or granular powders [121]. Over several days fingermarks became increasingly more difficult to enhance, presumed to be due to the porosity of the antlers causing absorption of constituents. Work on latent print enhancement on human bone drew similar conclusions also finding black magnetic powder the favoured technique [122]. Chemical enhancement was hindered due to the reactions with organic material within the antler, with ninhydrin turning the entire surface area of the antler purple rendering any contrast to surface and ridge detail minimal. A similar phenomenon was seen with leather [88] demonstrating a theme with the application of chemical enhancement methods on organic materials. Further work expanded to include enhancement of bloody fingerprints on both antler and horn, a keratin based substance [123]. The study concluded cyanoacrylate fuming followed by fluorescent dye stains to be a viable technique for latent fingermark enhancement differing from the conclusions drawn in the first study. It should be noted no attempt at comparisons with other enhancement techniques were attempted and no description of the maturity of the antlers given. The porosity of antlers decreases over time making their growth stage of vital importance to viable fingermark enhancement techniques [124].

A perceived issue of fingermark enhancement for many animal products is their rough surface, as generally the smoother the surface the easier it becomes. Of all high risk trafficked animal products the smooth surface of polished ivory appears an appropriate case study to trial techniques. Whilst the term ivory is most commonly attributed to elephant tusks the term itself is applicable to several commercially traded mammalian teeth or tusks including elephant, walrus, narwhal, some toothed whales, hippo, and warthog [125]. Several of which have recently been included in the UK's Ivory Act 2018 [126]. Ivory is porous, comprised almost entirely of dentine with a thin layer of cementum, and in both elephants and walrus tusk tips are coated in enamel but this is eventually worn away and absent in older animals [125]. Hippo ivory is sourced from both their upper and lower canines and their enamel layer is more permanent covering about 2/3 of the tooth. To date there are two published studies investigating latent fingermark enhancement on ivory, both elephant, conducted 15 years apart [127,128]. Both studies found Black Magnetic Powder (BMP) (standard and reduce scale powder respectively) suitable enhancement techniques including in a field setting. The main development seen between studies was increased success rate for longer intervals between deposition and enhancement, with the reduced scale (Supranano™) powder successfully enhancing prints up to 28 days after deposition. As an indicator of the continued focus on megafauna, this research has spawned the largest uptake in interest in application of fingerprinting techniques in wildlife crime cases and demonstration of its value. Kits have been produced and distributed both domestically and overseas with NGO support, with reports that use of these techniques have directly led to arrests [129].

Fingermarks: pangolin scales

Pangolin scales have recently become a high profile evidential item in IWT, in response countries have carried out actions specific to the pangolin species [130]. Despite this and several other international interventions to curb it, historical and continued demand has resulted in seizures containing tens of thousands of individual scales, representing thousands of individual pangolins [131]. Though the number of seizures continues to increase these are not synonymous with conviction and arrest rates [10,132]. Pangolin scales are keratin based, overlapping to form a protective layer on the dorsal side. The surface presents as a smooth material with shallow grooves running vertically from the tip to the base. Under scanning electron microscope they have been revealed to be non-porous, opening up the number of enhancement methods available to them [133].

One attempt has been made to retrieve latent prints from pangolin scales using gelatin lifters [133]. Gelatin lifters are used to recover both treated and untreated latent marks, then subsequently scanned or photographed and enhanced using software such as Photoshop™ [134]. Latent marks on pangolin scales were retrieved up to four months post deposition and whilst the mean grade failed to reach over two point five for any time frames over 28 % of all grades were three or above, and as such considered of forensic interest. There is sound logic behind the proposed use of gelatin lifters as a tool for use in wildlife investigations; they are affordable, portable, durable, and pliable, allowing them to be applied to uneven surfaces and used in field settings where chemical or traditional powdering techniques are unsuited and in nations with minimal resources. Limitations for this method start to creep in surrounding documentation of the latent prints. Optimum photography is carried out using specialised GLScan equipment, a large stationary scanning machine. As it currently stands to achieve best results practitioners would be required to collect marks in-situ and transport to the nearest lab with a GLScan machine which could be a significant distance or even located in a different jurisdiction. The research proposed the use of smart phones as an alternative, a method which is increasingly being investigated [135,136]. A second limitation is the fact that individual scales, such as those used in this study, are usually recovered in large quantities. With minimal resources available to wildlife crime case

workers analysis of hundreds or thousands of individual scales is impractical. Live or whole pangolins are traded on a smaller scale [137] and present a more practical example of case work where gelatin lifters could be applied. However due to the overlapping scales on whole specimens there is higher opportunity for latent marks to bridge multiple scales or be destroyed from friction of rubbing scales. Application of gelatin lifters also relies on an informed idea of the existence and positioning of a latent mark, without this a gel may be applied in a manner which cuts through a mark. As such this work would benefit from a preliminary step of investigating techniques for visualising latent marks, through oblique lighting, forensic light sources, or powdering.

Touch DNA - background and composition

Like fingermarks, DNA profiles are used in forensic investigation to identify an individual and can be full or partial in nature [138]. The laboratory pipeline for the processing of human DNA evidence is well established with validated methods and instrumentation available. The aim of forensic DNA analysis is to generate a STR profile amplified from a series of known loci, each displaying a maximum of two alleles in a single source profile (Fig. 4). The data is reduced into a string of allele repeat numbers that can be compared to a reference sample or searched against a national or international DNA databases. During criminal investigations, DNA may be sampled from sources including blood, hair, saliva, and semen left behind at crime scenes. However, in non-violent crimes where injury or physical human-human abuse has not occurred, touch DNA, that which is transferred from person to object via physical contact, may be recovered [139,140]. Like fingermarks, the factors that affect the presence and retrieval of touch DNA include pre-factors such as the donor, handling time, surface type and post-factors like time since deposition and environmental exposure [141–143]. This is not to imply that as evidence types they are one of the same; although DNA can be recovered from fingermarks [144], fingermarks can exist without detectible DNA, and touch DNA can exist independent of fingermarks. Current understanding of the cellular contents and origins of touch DNA is limited with many possible origins noted including cell free DNA [145], anucleate corneocytes [146], nucleated epithelial cells from hands [142] and fragmentary cells [147]. More recently, it has been proposed that touch DNA originates from various locations or bodily fluids, specifically shed keratinocytes from the outer layers of an individual's hand, nucleated epithelial cells from fluids (e.g. eyes, saliva, nasal fluids) or body parts in contact with hands and cell free DNA either endogenous to the hands (e.g. sweat) or transferred onto the hands [142].

Touch DNA – crime scene recovery methods

To maximise the chance of obtaining a full DNA profile it is important to use a device that can provide an efficient and selective collection of traces, to preserve their integrity by limiting contamination and degradation and to allow an effective recovery of biological material. A large number of collection methods exist including, wet/dry single or double swabbing [148,149], taping [150,151], FTA paper [152], scraping [153], vacuum sampling [154] and cutting [155]. The efficacy of the methods varies based on the substrate and therefore, like fingermarks, become important factors to consider when collecting human touch DNA.

Swabbing

Swabbing is the most widely used method of collection due to its versatility and ability to sample in hard-to-reach areas. The number of swab types and manufacturers producing vastly different products and researchers with varying results conducted in controlled conditions raises questions about the suitability of swab types, whether they meet scientific criteria and are the best choice for specific sample type and

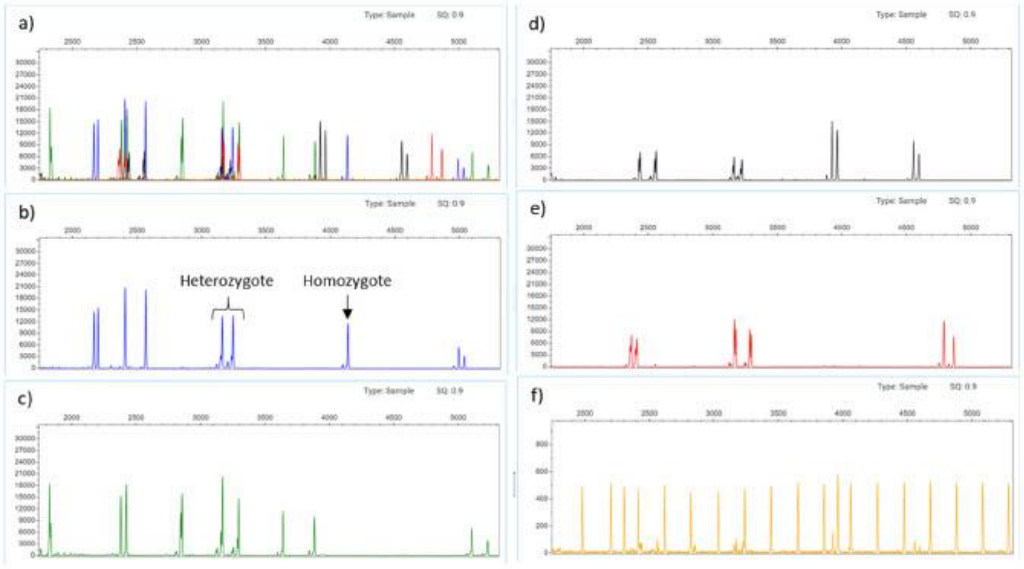


Fig. 4. A single source human STR profile viewed on Genemapper software with Relative Fluorescent Units (RFU) on the Y-axis and fragment size (base pairs) on the x-axis. (5a) Overlay of five channel spectra showing all full STR profile. (5b) Five STR loci amplified in blue channel showing example heterozygote and homozygote alleles at loci. (5c) Five STR loci and Amelogenin amplified in green channel. (5d) Four STRloci amplified in yellow channel. (5e) Three STR loci amplified in red channel. (5 f) Size standard used to identify fragment length of STR alleles presented in orange channel.

substrate. The effectiveness of a swab is influenced by several factors: the material, the thickness and length, how tightly the material is wound, the shape, design and whether the swab or transport tube preserves the DNA [156]. The double swabbing wet/dry method [157] has been suggested as the most effective swabbing technique with data showing that blood from a singular substrate (glass) can be collected using a range of swab types following this technique [158]. This method has also been identified as usable for touch DNA from both primary and secondary transfer [159,160]. Cotton swabs are most used due to their low cost, simplicity of use and ease of transportation for police and forensic departments although nylon flocked swabs and foam swabs are both well researched alternatives. Research looking into the most effective swab type assessed the Prionics cardboard evidence collection kit, COPAN 4N6FLOQSwabs, Puritan FAB-MINI-AP and Sarstedt Forensic Swab with data suggesting that the Copan flocked swabs presented the best overall performance [161]. The type of buffer solution used to wet the swab has been reported to affect the ability to dislodge and recover touch DNA bound to surfaces [162,163].

Adhesive lifting tapes

Lifting tapes are commonly in use today for the recovery of textiles fibres, hair, shoeprints, fingerprints, gunshot residues, cellular material and DNA as they are efficient non-destructive methods for obtaining concealed DNA [164] and can be used similarly to swabs for sampling specific locations on items or larger areas [165]. Taping for trace evidence consists of repeatedly pressing the sticky side against a material or surface and lifting for subsequent DNA extraction and comparison to swabs suggests Minitapes recover higher DNA concentrations except when used on non-porous surfaces [161]. This is also observed in other research which has shown that BVDA Gellifters and Scenesafe FAST tape outperform traditional cotton swabs when sampling fingerprints from 100 % cotton [149]. The conclusion being that BVDA Gellifters and Scenesafe FAST tape could be used as a substitute for cotton swab as they perform equally or better than cotton swabs particularly when collecting touch DNA samples. Further work has shown higher DNA recovery rates for mini-taping and scraping sampling methods independent of the substrates [148]. It can be seen from these studies that tape lifting is a suitable method of collection for both fabrics and non-porous surfaces such as plastic. Although it has been proven that stronger adhesion leads to higher yield of touch DNA, the tack complicates the extraction process leading sampling to be labour intensive.

For the processing of both swabs and lifting tapes DNA can be lost at both the extraction and quantification steps [166,167]. With the already low levels present in touch DNA it therefore becomes important to choose the most efficient workflow for processing samples. In this regard direct PCR amplification is increasingly being used for touch DNA samples so that potential loss of DNA can be circumnavigated by avoiding the extraction, purification, and quantification steps [168].

Touch DNA: application of methods in wildlife crime

Touch DNA: deer

Like fingermark research some of the earliest attempts at human touch DNA retrieval in the context of wildlife crime was conducted in response to deer poaching. Mini-tapes, a common tool used for touch DNA retrieval from clothing [150], were tested for use on limbs of deer handled by hunters [169]. The method was successful but due to the low levels of DNA recovered the researchers were forced to use a modified protocol adapted for low copy number (LCN) during amplification. In a second iteration of the study the LCN approach was overcome through pooling of samples [170]. However, the authors had the luxury of knowing their combined samples should have come from the same "perpetrator" as this was a controlled study. Whilst a single individual handling a carcass may be true for small scale crimes such as deer poaching, in reality the supply chains of many wildlife crimes are complex and several individuals may be involved either along the whole chain or within just one of the links [95,171]. Mixed source DNA is considered complex and combined with the already problematic low levels of DNA in trace samples future studies should include several donors to better emulate real life cases. However with over a decade of development in the area of touch DNA recovery techniques such as direct polymerase chain reaction (PCR) make processing of challenging samples more accessible [168]. As such this work is worthwhile repeating, with un-pooled samples, but using modern direct PCR techniques.

Touch DNA: bird of prey, corvid, and rabbits

In many cases of wildlife crime, the carcass, either whole or in parts, is a commodity therefore encountering a carcass as evidence at a crime scene which has been exposed to the elements, may be less common than encountering it in transit or on a person. In contrast, carcasses of species which are targeted for persecution have no value to the offender and may be left or concealed at the scene of the crime. This is common in crimes against birds of prey whose carcasses are regularly found outside having been exposed to the elements for undetermined amounts of time [172]. The impact of prolonged elemental exposure on touch DNA recovery has been investigated and evidence shows temperature and humidity both impact the persistence of DNA however whether this is positively or negatively is concurrent with the type of surface the DNA has been deposited on [141]. In one study, mini-tapes were chosen to remove human DNA from rabbit (a common bait), corvid and bird of prey carcasses in both controlled and exposed conditions [173]. Profiles were obtainable form corvid and rabbit after two days of elemental exposure including heavy rainfall and up to ten days on carcasses kept in controlled indoor conditions with the rapid decomposition of the carcasses cited as a contributing factor to the decreasing ability to recover DNA. Bird of prey carcasses had only one day of exposure with rainy conditions but found significant difference in success depending on the species. Other external factors such as scavengers or invertebrates associated with decomposition may also contribute to the decline in available DNA. This was suspected to be true in a study of touch DNA recovery from pig skin submerged in water [174]. Both this study and that detailed in [173] managed to produce full DNA profiles from carcasses after being exposed to water. Once conclusion was that touch DNA persisted longer in cold, standing water but a full profile was still retrievable after one day of immersion in running water which is in keeping with [173] who retrieved reportable DNA samples from corvid carcasses exposed to rain after two days. Effects of rain exposure on touch DNA retrieval in wildlife cases deserves more research, given poaching incidents in certain countries peak during rainy seasons, as poachers attempt to capitalise on rangers inability to navigate flooded protected areas and the lack of tourists [175].

Summary

Several key themes flow through human identification in wildlife crime. To begin with the data shows it is possible to recover human evidence with standard techniques without any need to deviate from the general recommended procedures. When considering the wildlife item as any other type of evidence encountered in a criminal investigation it is subject to the same rules of porosity, texture and environmental exposure that must always be considered. Colourful, patterned skins, and coats of animals can be a challenging factor in producing a good contrast between substrate background and fingermark. For species destined for the pet trade or as ornamental these flamboyant features are a driving factor behind their demand, therefore overcoming this problem is imperative. Very few of the studies reviewed here attempted a duality or comparison of enhanced mark quality on the substrate comparable to lifted marks, despite this simulating standard procedure by forensic investigators. Chemical enhancements often failed potentially because raw animal products are organic material which react in

conjunction with fingermark residue rendering any contrasts that do occur of minimal quality. The techniques that do work, powders and gelatin lifts particularly, can be cost effective, field deployable and in the case of powders do not require expensive laboratory infrastructure for analysis. This makes them ideal candidates for take up in by those investigating wildlife crime who cite a lack of resources as a stumbling block to enforcement. Notably researchers have placed no consideration the downstream impacts of fingermark enhancement techniques on potential DNA recovery, human or animal. Dual evidence recovery from fingermarks is an increasing consideration by practitioners for both fresh and archived marks [144,176,177] and the techniques employed can have significant impact on ability to recover DNA profiles. Magnetic powders, one of the most successful fingermark recovery techniques found in this review, have been found to have minimal impact on subsequent DNA recovery [176], making this work an ideal candidate for expansion into touch DNA recovery studies. This also feeds into the need for proper forensic training for wildlife crime scene first responders in the theory and practical application of general evidence handling including contamination minimisation and prioritisation of different evidence types. There are instances where media images of wildlife seizures show law enforcement handling goods without gloves suggesting even basic forensic practices are not being employed [178,179].

Despite decades of successful proofs of concepts on several species there has been only one recorded instance of translation of findings into applied work. One explanation behind this is that there has been no real need for recovery of such evidence types. Wildlife crime investigations can often begin from a "caught red handed" scenario, whereby an individual is found in possession of wildlife products, commonly seen during seizures at borders. As such the need to link an individual to the crime is superfluous. This is a weakness in the continued chronicling of making IWT synonymous with all wildlife crime and thus focusing efforts on highly trafficked species. By assuming this narrative and failing to establish robust methods of linking individuals to wildlife crimes a whole subset of cases is being ignored. It does injustice to the equally pressing matter of domestic, non-trade related, wildlife crimes such as seen in the USA and the UK who have a poor track record in wildlife crime conviction rates [11,25]. Persecution and human-wildlife conflict cases in these countries may rarely see an individual caught in possession of a wildlife product as the wildlife product itself is not a target for commercial gain. The small-scale nature of these crimes, the comparably high resources available, including accredited laboratories and well-established databases, place such nations in prime position to lead in human evidence recovery in wildlife crimes. Ignoring human evidence also fails to consider the additional intelligence it can bring to investigations. For example, DNA barcoding with ivory has resulted in linking shipments and thus identifying supply chains and trafficking routes. This could also be achieved through the presence of repeated instances of the same human DNA profile or fingermark on multiple shipments identifying a repeat offender or common link in supply chains.

It is evident from increasing rates and simultaneous decrease in convictions that current attempts to tackle wildlife crime are fraught with problems. Along the way forensic solutions posed have focused on the wildlife rather than the perpetrator. This work, specifically individual identification of wildlife has important applications, but they are limited by resources, lack of accreditation, need on a large scale and the sheer volume of wildlife species involved. By contrast human identity testing in forensic applications is a globally established industry, with recognised and well-rehearsed best practice methods. Human identity testing benefits from existing databases and infrastructure, particularly in the global north, but with more and more global south stakeholder countries developing in this area, such as India's new National AFIS [180] and Kenya's new forensic laboratory [181].

Any prosecution team will benefit from having an much evidence as possible at their disposal. Recovery and presentation of human trace evidence in wildlife crime cases provides clear links of perpetrators to wildlife products that other types cannot provide. As such it is recommended that more research is conducted looking into human trace evidence recovery from common substrates encountered in wildlife crime cases. Whilst this article has focused on wildlife products and their derivatives the work can be expanded to include traps, snares, weapons, transportation boxes and vehicles. For several of these evidence and material types there will be existing research or guidance on best practice methods but work is needed to contextualise them into the world of wildlife crime. Considerations should be made dependent on the seizure type or crime scene location. For example seizures from shipping containers will have undergone different environmental exposure and time frames since deposition comparative to air cargo, similarly crime scenes in an arid desert environment will have had significantly less moisture exposure than those in tropical humid environments affecting recommended recovery methods.

Fingermark work should look beyond just enhancement on substrates and investigate effective methods of mark retrieval to overcome problems in establishing contrast on patterned backgrounds. Touch DNA work in this area is very much in its infancy but will benefit from including mixed profile scenarios, more modern processing techniques and interactions with fingermark recovery techniques. It is important that such research is completed in appropriate contexts. To do this researchers must work closely with law enforcement to understand their resource limitations, what types of evidence they most commonly encounter at wildlife crime scenes, what national priorities are, and the practicality of applying developed techniques.

Finally, there needs to be recognition of the complimentary nature of species identification and human identification forensic work. What species identification lacks in terms of accreditation and recognition within the wider forensic community, human identification possesses in abundance. Species identification benefits from ample examples of proof-of-concept work as well as media, funding, and research interest whereas in these areas human identity work is in its infancy. Encouraging these veins to work together could result in robust forensic investigation in wildlife crimes, with the recovery and analysis of several streams of forensic evidence being possible. The idea of paired wildlife and human forensic labs who agree to take on relevant evidence processing from wildlife crime cases at their respective crime scenes could be considered. As well as utilising each institutions unique skill set it will strengthen the relationship between the wildlife and human forensic community potentially increasing knowledge sharing opportunities and more cohesive and streamlined case work. A challenge will be the need for human forensic laboratories to find the time and resources to process wildlife crime related evidence. Efforts to access these resources will be strengthened by demonstration of the impacts of wildlife crimes on the economy, communities and biodiversity. Better recording of wildlife crimes should be a first step in this area, as is being called for in the UK within campaigns to make wildlife crimes notifiable [182] and recommendations for centralised wildlife databases within the EU for better monitoring [183]. Ultimately it is recommended that wildlife crime scene first responders receive high quality training in forensic techniques and that subsequently wildlife crime scenes be processed the same as any other high priority crime. This includes the same considerations being taken surrounding evidence collection and handling and best practice forensics. Even if resources do not allow immediate processing of evidence it opens avenues for utilisation of archival evidence when circumstances allow in the future. This has the potential to improve prosecution and conviction rates and act as a serious deterrent to wildlife criminals, providing in a part a solution to the ongoing crisis of wildlife crime.

Ethical statement

The authors declare no ethical approval was necessary for the creation of this review manuscript. The data presented represents the authors understanding of the subject matter gathered through personal research and stakeholder discussions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review article.

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Appendix II

Understanding the collection and use of evidence in United Kingdom wildlife crime case work

As new methods and techniques for evidence recovery in wildlife crimes are developed it is imperative that their suitability for real world deployment is established. This questionnaire aims to understand the collection and use of evidence in wildlife crime cases, with a focus on forensic evidence and identify where, if any, gaps exist.

Collated data may be analysed and the results published. If you wish to withdraw any data collected or issue a complaint, email louise.gibson@ioz.ac.uk. By completing and submitting this questionnaire, you consent to this data being collected and processed for research

1

Are you part of the National Wildlife Crime Unit?

- ◯ Yes
- ◯ No

2

If yes, how long have you been part of the NWCU?

- ◯ 0 - 1 year
- ◯ 1 - 2 years
- ◯ 2 - 3 years
- ◯ 3 - 5 years
- ◯ 5+ years

3

Are you a Borough Wildlife Crime Officer (BWCO)

- ◯ Yes
- ◯ No

4

If yes, how long have you been a BWCO?

- ◯ 0 - 1 year
- ◯ 1 - 2 years
- ◯ 2 - 3 years
- ◯ 3 - 5 years
- ◯ 5+ years

5

Please describe your role

6

On average how many wildlife crime scenes do you attend in person a year

- ◯ 0 - 5
- ◯ 6 - 10
- ◯ 11 - 15
- ◯ 15+
- ◯ Not applicable

7

Please reorder the below UK priority areas from largest to smallest relative to the proportion of your cases they account for

- Cyber enabled wildlife crime
- CITES
- Raptor persectuion
- Freshwater pearl mussels
- Poaching
- Bat persecution
- Badger persecution

8

Please order the following evidence types from largest to smallest relative to the proportion you submit for forensic analysis of any kind.

- Wildlife (carcass)
- Traps
- Wildlife (live)
- Wildlife (derivatives)
- Vehicles
- Packaging
- Weapons
- Other

9

If other please describe

10

In your opinion which of the below evidence types most greatly contribute to successful prosecutions/convictions in wildlife crime cases. You may select up to two options.

Direct	evidence learned directly, by the witness presenting it e.g. witness saw or heard the crime
Circumstantial	evidence which is not drawn from direct observation of a fact or event e.g., an individual was seen with stolen items
Primary	evidence of the existence of an object e.g. a knife in an assault case
Secondary	evidence which has been reproduced from an original document, such as a photocopy of a document or a photograph that would have been considered primary evidence
Forensic	evidence from forensic tests
Expert	e.g. an expert in a particular field of science may be able to provide information to a jury

Please select at most 2 options.

- [ ] Direct
- [ ] Circumstantial
- [ ] Primary
- [ ] Secondary
- [ ] Forensic
- [ ] Expert

11

Have you ever submitted anything from a wildlife crime scene for attempted human trace evidence recovery

- ◯ Yes, fingerprints and DNA
- ◯ Yes, fingerprints only
- ◯ Yes, DNA only
- ◯ No

12

Would you be likely to submit wildlife items (whole or parts) for attempted human trace evidence recovery if it was proven to be achievable?

- ◯ Yes
- ◯ No

13

If no please elaborate on why

14

In your opionion which of the below options contribute the most to unsuccessful prosecutions/convictions. You may select up to two options.

Please select at most 2 options.

- ☐ Lack of funding
- ☐ Lack of manpower
- ☐ Lack of infrastructure
- ☐ Lack of evidence
- ☐ Priorisation of other crime types
- ☐ Other

15

If other please elaborate

16

Please add any additional information regarding evidence collection and processing in wildlife crime cases you feel may be of interest

Appendix III

Prior to today's session:

1. **Have you ever received training on the topic of wildlife crime?**

   A. Yes, in-person only
   B. Yes, eLearning only
   C. Yes, both in-person and eLearning
   D. No

2. **Have you ever had a call-out to a wildlife crime scene?**

   A. Yes and attended
   B. Yes but didn't attend
   C. No

3. **In your opinion which of the below options contributes the most to unsuccessful prosecutions/convictions. You may select up to two options.**

   A. Lack of funding
   B. Lack of manpower
   C. Lack of infrastructure
   D. Lack of evidence
   E. Prioritisation of other crimes
   F. Other

If other please elaborate (more space overleaf)

4. **How would you rate your general knowledge on the subject of wildlife crime?**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Non-existent Excellent

As a result of today's session:

5. **I have a greater understanding of the types of wildlife crime casework encountered by the Metropolitan police.**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Strongly disagree Strongly agree

6. **I have a greater understanding surrounding the associated impacts of wildlife crimes.**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Strongly disagree Strongly agree

7. **I feel more confident in identifying potential evidence at a wildlife crime scene.**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Strongly disagree Strongly agree

8. **I feel more confident in which human trace evidence recovery techniques can be used on wildlife carcasses and derivatives.**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Strongly disagree Strongly agree

9. **I consider participation in wildlife crime casework to be an increased priority.**

   ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

   Strongly disagree Strongly agree

10. I am more likely to respond to a call to attend a wildlife crime scene.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Strongly disagree Strongly agree

11. I feel attending wildlife crime scenes may increase my workload to unsustainable levels.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Strongly disagree Strongly agree

12. I do not feel I have the resources available to me to effectively attend wildlife crime scenes.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Strongly disagree Strongly agree

13. I am interested in receiving more training sessions focused on wildlife crime case work.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Strongly disagree Strongly agree

14. I am interested in supporting further research related to wildlife forensics.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Strongly disagree Strongly agree

Training Day Feedback Form:
Gaining insight into crime scene examiners views on wildlife crime casework

As new methods and techniques for evidence recovery in wildlife crimes are developed it is imperative that their suitability for real world deployment is established. This questionnaire aims to understand the collection and use of evidence in wildlife crime cases, with a focus on forensic evidence and identify where, if any, gaps exist.

Responses to this questionnaire are **anonymous** however collated data may be analysed, and the results published. By completing and submitting this questionnaire, you consent to this data being collected and processed for research. If you wish to issue a complaint or have further questions regarding this questionnaire, please email louise.gibson@ioz.ac.uk.

Please use this space to elaborate on question 3 or add any additional information regarding evidence collection and processing in wildlife crime cases you feel may be of interest.

Q3.

Other comments: