The Use of Forensic Gellifters to Collect Human DNA Off Trafficked Animal Specimens

Abstract

Overexploitation is a leading threat to species worldwide, with 72% of threatened and non-threatened species suffering from its pressures. The illegal wildlife trade (IWT) is worth millions of dollars fuelled by demand across international and domestic markets in traditional medicines, the exotic pet trade, consumption and luxury goods.

Up until now use of forensics in tackling the IWT has been focused on the animals, seeking to identify the species and geographical origins of seized goods. This study has identified a gap in the research where collection and analysis of human based evidence in IWT cases is being overlooked.

Using black gellifters, a common and inexpensive forensic tool for fingerprint lifting, this study attempted to collect and analyse trace levels of human DNA from fingerprints deposited on three commonly trafficked animal specimens, ivory, a feather and crocodile skin. Comparisons were made between specimens and analysis was carried out to see if the scanning process involved in imaging fingerprints from gellifters impacted DNA recovery.

Results showed failure to recover DNA in quantities needed for successful profiling in any samples, with average DNA concentrations of <1pg/µl. Fingerprints collected from ivory had the highest average DNA concentrations at 0.241 pg/µl fingerprints from feather and crocodile skin had averages of 0.218 pg/µl and 0.209 pg/µl respectively. Human DNA concentrations retrieved between specimens were not found to be significantly different. Scanning of the gels resulted in significantly lower average DNA concentrations compared to not scanning (p-value = 0.00198).

Removal and replacement, after fingerprint collection, of the gellifter's protective acetate layer and possibly gellifter composition were discussed as likely contributors to overall low DNA concentrations. The wavelengths of light used, and supplementary removal and replacement of

the acetate required, during the scanning process were the two factors concluded as contributing to the significantly lower DNA concentrations retrieved from scanned samples.

This study shows in theory that collection of human trace DNA from fingerprints left on ivory, feathers and crocodile skin is possible using black gellifters, but the protocol used is not efficient and further research is needed to improve upon it.

Acronyms:

AFWFN	Africa Wildlife Forensic Network
ASEAN-WEN	Association of Southeast Asian Nations Wildlife Enforcement Network
CaribWEN	Caribbean Wildlife Enforcement Network
CEN	Customs Enforcement Network
CITES	Convention on International Trade in Endangered Species of Wild Flora & Fauna
CODIS	Combined DNA Index System
CRS	Cambridge Reference Sequence
DEFRA	Department for Environment, Food & Rural Affairs
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
HA-WEN	Horn of Africa WEN
HTA	Human Tissue Act
ICCWC	International Consortium on Combating Wildlife Crime
INTERPOL	International Criminal Police Organization
IWT	Illegal Wildlife Trade
LEMIS	Law Enforcement Management Information Systems
mtDNA	mitochondrial DNA
NAWEG	North America Wildlife Enforcement Group
NDNAD	National DNA Database
NGO	Non-governmental organisation
PCR	Polymerase Chain Reaction
rCRS	Revised Cambridge Reference Sequence
ROAVIS	Red de Observancia y Aplicación de la Normativa de Vida Silvestre de Centroamérica y la República Dominicana
SAWEN	South Asia Wildlife Enforcement Network
SD	Standard Deviation
SRMs	Standard Reference Materials
STR	Short Tandem Repeat
SudWEN	South America Wildlife Enforcement Network
TCM	Traditional Chinese Medicine
TM	Traditional Medicine
TWIX	Trade in Wildlife Information Exchange
UNEP	United National Environmental Programme
UNEP	United National Environmental Programmes
UNODC	United Nations Office on Drugs & Crime
USFWS	United States Fish and Wildlife Service
UV	Ultraviolet
WCO	World Customs Organisation
WEN	Wildlife Enforcement Network
WEN-SA	Southern African Wildlife Enforcement Network
WFN	Wildlife Forensic Network
WWF	World Wildlife Fund
ZSL	Zoological Society London

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Figure 11. Average DNA concentrations of collated data from scanned and non-scanned samples for each item. n=36 samples were analysed. There is no significant difference between DNA concentrations of fingerprints lifted from each item p=0.441 (Friedman test).

Chapter 1 - Introduction

1.1 Animal as Products

1.1.1 Legal Wildlife Trade

Many animals exist amongst the human population as commodities, domesticated over thousands of years to serve purposes including but not limited to, companionship, sustenance and physical labour. These types of animal groups, pets, food animals and working animals, are within reason, bred, traded and slaughtered within the realms of legal and moral frameworks. In addition to domesticated animals' humans have also found uses for non-domestic animals or "wildlife" (Oldfield 2012). Traded wildlife is sourced both directly from the wild and from captive breeding facilities and can include protected and endangered species under certain circumstances (CITES 2019). Like domestic animals' certain species of wildlife are desired for the pet trade and plenty of vertebrate species, fish, birds, reptiles and mammals are legally traded domestically and internationally to meet demand (Bush et al. 2014). Parrots and reptiles cover some of the most common groups desired in the exotic pet trade but demand extends to small mammals such as sugar gliders which the US imports from Indonesia (Auliya et al. 2016; Souviron-Priego et al. 2018; Campbell et al. 2019). Commercial fishing is a large-scale example of legal wildlife trading, the Food and Agriculture Organisation of the United Nations (FAO) most recent statistics placed the world marine catch, fish taken from the wild for human use, at 79.3 million tonnes (FAO 2018). Other legal wildlife trades include more controversial products such as tiger bone harvested from captive breeding populations and sent from South Africa to East and Southeast Asia (Williams et al. 2017). On the African continent, 23 countries, allow trophy hunting of charismatic megafauna such as lion (*Panthera leo*) and giraffe (*Giraffa sp*.), although more recent country figures may differ (Lindsey et al. 2007). Assuming the individual has the correct permits, trophies can be taken back to their home countries for personal use and display. Trophy hunting is argued to be an important source of income for local economy, with permits

costing hunters up to $350,000 (Di Minin et al. 2016) and the industry employing people from the surrounding community with money also being fed back into conservation efforts (Lindsey et al. 2006; Angula et al. 2018). The topic is hotly debated by conservationists with some arguing trophy hunting maintains biodiversity and others that it is detrimental (Di Minin et al. 2016; Macdonald et al. 2017; Batavia et al. 2019). In Europe moose hunting managed by landowners and co-operatives is a popular form of game hunting for consumption, domestic trade in moose accounts for 2% of all meat consumption in Sweden and Norway (Naevdal et al. 2012) . The middle east has a long history of importing camels as working animals from pastoral facilities in Somalia and Sudan, at USD $1000 per adult animal the trade is a significant contributor to pastoral communities (Younan et al. 2016). Exotic leathers and furs including crocodile, ostrich and mink are regularly traded for use in the luxury goods market and organisation regularly voice concerns over the ethics and welfare of the animals harvested or farmed for this industry (Belleau et al. 2004; Muthu 2017).

As seen above the legal trade in wildlife is a thriving and diverse business, estimated at upwards of $86 billion (Van Uhm 2016). With such opportunity for wealth available in the trade of wildlife it stands within reason that people will take advantage of the high profit margins available outside the restrictions of the law.

1.1.2. Wildlife Crime

At the very basic level a “wildlife crime” is any illegal activity involving or aimed at a wild animal. This can range from persecution of a species, theft of wild bird eggs, hunting/poaching, trade in protected species or the knowing introduction of an invasive species (Nurse 2011) .Defining what constitutes a wildlife crime from a legal perspective differs from country to country, It is up to each individual country to define and assess not only what they deem a wildlife crime but also the severity of the crime and the subsequent punishment. This may be influenced by, amongst other things, cultural or religious beliefs, country priorities or available resources.

1.1.3. Illegal Wildlife Trade

1.1.3.1. Defining the Issue

Of all wildlife crimes, the illegal wildlife trade (IWT) is the most prominent of our times (Wilson-Wilde 2010) and often touted as a key contributor to the current supposed sixth mass extinction event (Ceballos et al. 2015; McCallum 2015). As with legal trade, IWT includes the sale and exchange of live animals and animal products but carried out without permits, permissions or outside of legal frameworks. The method of collection i.e. is it taken from the wild, the number of animals or the type of animal and whether it is protected can all influence the legality of the transaction (Phelps et al. 2016).

1.2. The Scale of IWT

1.2.1. Financial

Estimates of the worth of the illegal trade in animals range from US$5 billion to US$23 billion (May 2017) calculated using figures reported between 2011 – 2016 from over a dozen different sources including specialist non-governmental organisations (NGOs) in wildlife trade such as TRAFFIC, governmental institutes such as International Criminal Police Organization (INTERPOL) and United Nations Environmental Programme (UNEP) and more generalised wildlife conservation NGOs such as Wild Wildlife Fund (WWF). These figures place the IWT alongside illegal drugs and arms dealing as one of the top three illegal financial operations globally (Broad et al. 2003; D'Cruze & Macdonald 2016; Milner-Gulland 2018).

1.2.2. Quantities of Animal Products Traded

Quantifying the number of individual animals and species that are traded, and the global reach of those trades, to any degree of accuracy relies on meticulous record keeping and consistent communication between nations. At present there are a multiple databases and organisations which hold data on details of both legal and illegal trade, including: TRAFFIC; the European Union

(EU) and Africa Trade in Wildlife Information Exchanges (TWIX); the World Wildlife Seizure database (World Wise); US Fish and Wildlife Service, Law Enforcement Management Information System (USFWS LEMIS) and The World Customs Organization's (WCO) Customs Enforcement Network (CEN). The Convention on International Trade in Endangered Species of Wild Flora & Fauna (CITES), an international agreement put in place to help regulate the trade in wildlife, is one of the best known publicly available resources for tracking legal trade and as of 2017 CITES began compiling data on illegal seizures. Table 1 goes into these databases in further detail, the listed databases are some of the most common cited in the literature and should not be taken as a complete representation of all possible databases available.

Seizures provide some of the only data available for understanding the scope of the IWT, but a seizure represents a failed transaction. For the IWT to thrive as it is, there must be regular successful trades happening and therefore any figures available on the scope of the IWT are only really extrapolations of these failures and therefore not wholly reliable. A report produced in 2016 by the United Nations Office on Drugs & Crime (UNODC), based on analysis of data collated by the World Wise database, reported 162,000 recorded seizures from roughly 1999 up to 2015, made up of 7000 species (United Nations 2016). There is no standardised method for recording seizures across nations, some will record by weight, some by individual animal and some may include multiple species in a single seizure record. Therefore, the report does not detail how many individual animals were included in those 162,000 seizures. To put into context how many total animals this could account for, a single seizure of pangolins in 2016, weighed in at seven tonnes, potentially representing up to 14,000 individual pangolins (Environmental Investigation Agency 2019). Based on 162,000 seizures, each seizure would only need to contain 6 individual animals to account for a million animals illegally traded during that time.

Database	Description	Who has access	References
CITES Trade Database	Records of legal trade as reported by CITES parties (governments agreed to be bound by the convention)	• Legal trade – Public • Illegal seizures – Members of the International Consortium on Combating Wildlife Crime (ICCWC)	(CITES 2019)
TRAFFIC	Monitoring and collation of wildlife trade from resources including CITES and media articles	• Free public access to TRAFFIC publications and bulletins	(TRAFFIC 2019b)
EU TWIX	Reported wildlife seizures and offences from all 28 EU member states	• Member law enforcement authorities	(EU-TWIX 2005)
Africa TWIX	Reported wildlife seizures and from five Central Africa member countries (Cameroon, Congo, Gabon, Democratic Republic of the Congo, and Central African Republic)	• Member law enforcement authorities	(TRAFFIC 2018c)
WCO CEN	Database of seizures and offences related to all trafficked items not just wildlife related from WCO member countries	• WCO member custom officers	(WCO 2019)
USFWS LEMIS	Legal and illegal wildlife imports to the USA	• Raw data held by USFWS. • Map of seizures between 2003 – 2013 publicly available via Wildlife Trade Tracker online platform	(TRAFFIC & WWF 2013; Smith et al. 2017)
World Wise	Amalgamation of seizure data from multiple databases	• Raw data held by United Nations Office on Drugs & Crime (UNODC). • Results of data analysis presented in the publicly available "World Wildlife Crime Report"	(United Nations 2016)

Table 1. Most commonly cited databases holding information on global legal and illegal wildlife trade data and information on who has access to the information held by them.

The reality is that most seizures contain significantly more than this and therefore reports that tens of millions of animals are being traded are far from exaggerated but rather prudent. In the same UNODC report, 120 countries, both low- and high-income nations, were registered as having made seizures between 1999-2015, either imports or exports, that contained wildlife. These figures mean just under 62% of the existing 195 recognised countries have been touched in some capacity by the IWT. Figure 1 shows 51 countries with reported seizures and prosecutions related to the IWT, covering 12 months between October of 2017 and 2018,

reported in the two most recent TRAFFIC Bulletins at time of writing (TRAFFIC 2018a; TRAFFIC 2018b) demonstrating the IWT is persisting at a global scale.

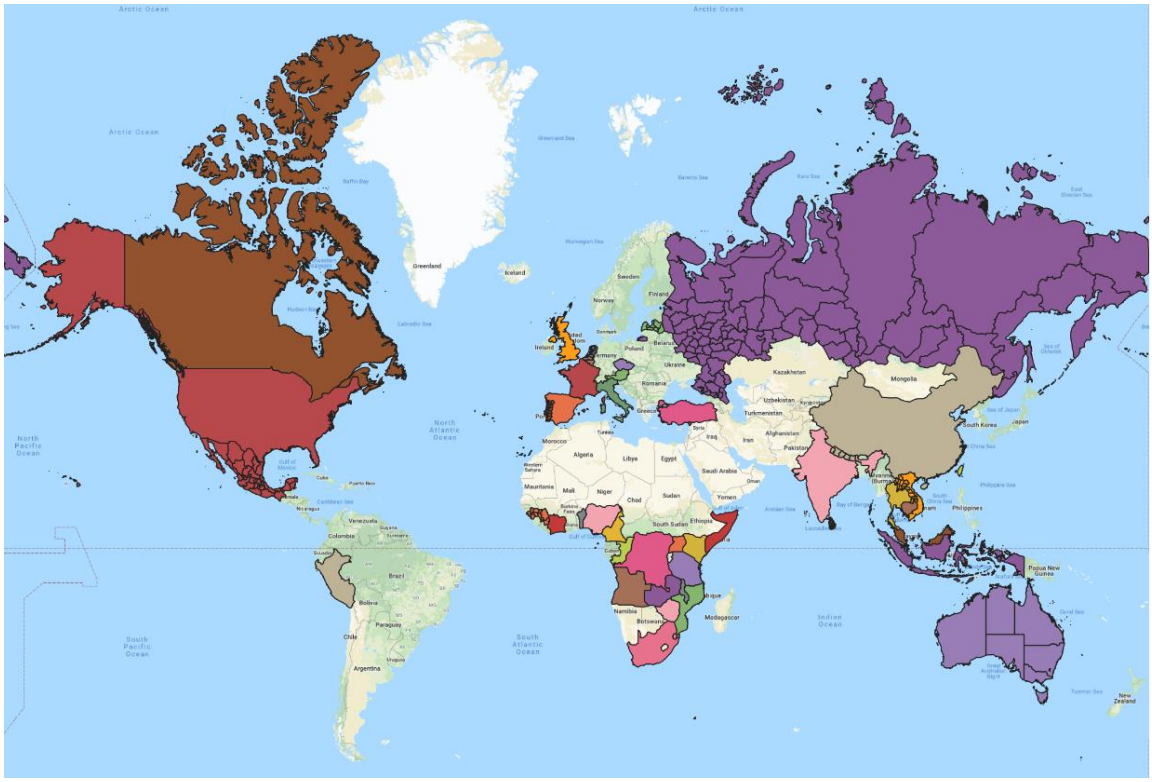

Figure 1. Map showing 51 countries reporting seizures or prosecutions related to the illegal wildlife trade between October 2017 and 2018. Map created by author in QGIS 3.4 using data sourced from April 2018 and October 2018 TRAFFIC Bulletins (TRAFFIC 2018a; TRAFFIC 2018b).

Many countries, mainly key exporters in animal goods with high biodiversity and coveted endemic species also deal in domestic trade (Van Uhm 2016). A survey of live animal markets in South China, outside of major cities, where enforcement is lacking, found multiple native species for sale, their status' ranging from critically endangered to vulnerable by IUCN, or held under categories I or II of CITES (Chow et al. 2014). This single study found over 7000 animals being traded across 7 different cities in South China, the majority destined for consumption (Zhang et al. 2008). Other significant domestic markets include multiple African nations, including the Congo basin, Tanzania, Benin and savannah areas, which are dominated by sustenance hunting (bushmeat) and traditional medicine (Wilkie & Carpenter 1999; Fischer et al. 2014; TRAFFIC 2018b), South East Asia for consumption, souvenirs and traditional medicine (Van Song 2008)

and South America, including Peru and other nations surrounding the amazon basin who's markets deal in the exotic pet trade and selling wildlife for biomedical research (Leberatto 2016).

1.2.3. Drivers and Enablers of IWT

Like any trade the IWT involves a supply chain, producers, consumers and middlemen all involved in a single transaction. Motivations of individuals in the supply chain may be driven by factors seemingly external to the initial consumer demand. In source nations of traded wildlife which are classified as low income, it is recognised that the poverty experienced in these nations is a key driver behind the IWT (Duffy et al. 2016). At a domestic level, wildlife markets selling bushmeat are fuelled by a full range of people, both higher and lower income families who eat bushmeat out of personal preference (McNamara et al. 2016) and individuals seeking sustenance they are unable to afford elsewhere, in some cases it may be there main source of protein (Pooley et al. 2015; Fa et al. 2015). Limited earning opportunities in poverty-stricken nations will also push individuals towards either opportunistic hunting, i.e. killing wildlife in the hopes of finding a buyer or alternatively being drawn into more organised crime syndicates for a more reliable income (Cooney et al. 2017). Whilst top down is the demand for the wildlife from the bottom up the recruitment of hunters and traders is potentially driven by the lack of alternative reliable income sources. A study by Harrison et al. (2015) showed that poverty alleviation, ensuring the basic needs of the population are addressed, is effective in curbing the illegal wildlife trade, strengthening this concept.

With poverty being a driver on one end of the scale, the increasing wealth of consumers is also driving the trade in wildlife (Challender 2011). Both the desire and the ability to demonstrate wealth fuels luxury goods markets and exotic pets and foods as status symbols; the harder it is to get, the more it costs and therefore the more impressive it is to possess it (Courchamp et al. 2006). Resources such as ivory, rhino horn, exotic animal skins, big cats and rare reptiles as pets,

consumption of endangered species such as sturgeon eggs, are all considered tangible demonstrations of wealth by those that seek them.

Cultural practices are a significant driver of the illegal wildlife trade. Traditional medicines (TM) often associated with the IWT are used all over the world although they do not always include the use of animal products. An estimated 80% of the population of Africa use TMs (Boakye et al. 2015) and over 100 million Europeans are currently using TMs (World Health Organisation 2013). When used for medicinal purposes consumers believe certain animal parts and products, when consumed or applied topically will cure any number of ailments, from AIDs to impotence (Byard 2016).

The population of China are some of the biggest consumers of wildlife parts in the world through their use in cultural medical practices, widely known as traditional Chinese medicine (TCM) (Wyatt & Kushner 2014; Zhang & Yin 2014). It has been reported that TCM accounted for three quarters of all the wildlife seizures in Hong Kong in the past five years (Zhang et al. 2008; Nijman 2010; McNamara et al. 2016). Hong Kong and China's international borders are one of the biggest hubs for movement of wildlife contraband between Asia and the rest of the world (the Hong Kong Wildlife Trade Working Group (HKWTWG) et al. 2018; Yi-Ming et al. 2000).

Large economic gains from the illegal wildlife trade have been touted in recent years as a potential funding source for terrorist organisations (Scientific American 2013; McNeish 2014). There is suggestion this claim has been overstated and there appears to be little tangible evidence supporting it, instead there is a call for governments to accept more accountability (Felbab-Brown 2018). Political links to the illegal trade are widely recognised (Smith & Walpole 2005), corruption by politicians, officials and law enforcement through exploitation of their positions to help facilitate access of species and movement of goods consistently hampers both national and international efforts to tackle the trade (Smith et al. 2003; Smith & Walpole 2005; Van Uhm & Moreto 2018).

1.2.4. Most Commonly Trafficked Animals and their Uses

Media coverage of wildlife crime often seem to be focused on charismatic megafauna; elephant and rhino poaching for ivory and horn and tigers and other big cats for bones and skins. The actual scope of species targeted for illegal trade is far more encompassing, as noted above 7000 species were recorded in seizures between 1999-2015 many of which are unlikely even known to the general public. Mammals, birds and reptiles are the three most trafficked groups of animals, targeted for the pet trade, TCM and as trophies (Symes et al. 2018) but fish, molluscs, sharks/rays and amphibians are also targeted and traded regularly. The size and intended use to the buyer will influence both the state an animal will be trafficked in, either in parts or whole, live or dead and the number of represented animals which can be trafficked. African Grey Parrots (*Psittacus erithacus*) are the one of the most trafficked bird species, destined for the exotic pet trade they must be transported live, limiting the numbers and methods they can be moved around by (Martin et al. 2018; Eniang et al. 2011).Pangolins, by comparison, as the "most trafficked animal in the world", are sought after for their scales and less likely to need to be trafficked whole or alive, meaning significantly more units can be traded or smuggled at a time (Heinrich et al. 2016). Additionally, some animals are rarer, elusive or need significant resources to obtain, so numbers traded may be low but perceived value by buyers high: rhino horn for example is now thirteen times the price per kilogram it was a decade ago as the number of animals sharply declines (Biggs et al. 2013; Rivalan et al. 2007). Bearing this in mind, quantity may not always be representative of demand (Aisher 2016).

Table 2 gives examples of animals targeted for the IWT, the resources they are targeted for and the market destination for end products. This list is by no means exhaustive but rather shows the variety of species targeted and colour coding has been used to demonstrate the overlap in uses across described species.

Group	Species	Classification	Resource	Market Destination				References
				Exotic pet trade	Traditional medicines	Luxury goods markets	Consumption	
Mammals	Tiger (*Panthera tigris*)	Endangered	Skins, penis, bones, claws, whole (live or dead)					(Moyle 2009)
	Chinese pangolin (*Manis pentadactyla*)	Critically endangered	Scales, whole (live or dead)					(Heinrich et al. 2016)
	Sloth Bears (*Melursus ursinus*)	Vulnerable	Bile					(Dutton et al. 2011)
	Greater slow loris (*Nycticebus coucang)*	Vulnerable	Whole (live)					(Shepherd 2010)
Reptiles	Boa constrictor (*Boa orophias*)	Endangered	Skins, whole (live/dead)					(Da Nóbrega Alves et al. 2008)
	Green sea turtle (*Chelonia mydas*)	Endangered	Shell, whole (live/dead), eggs					
Fish	Russian Sturgeon (*Acipenser gueldenstaedtii*)	Critically Endangered	Eggs (caviar)					(van Uhm & Siegel 2016)
	Blue Shark *(Prionace glauca)*	Near threatened	Fins					(Clarke et al. 2006)
Birds	African Grey Parrot (*Psittacus erithacus*)	Endangered	Whole (live)					(Eniang et al. 2011)
	Helmeted Hornbill (*Rhinoplax vigil*)	Critically endangered	Ivory casque					(Beastall et al. 2016)

Table 2. Selection of illegal traded species representing the four of the main trafficked animal groups. Colour blocking define known markets for relevant end products, greyed areas denotes where there is no demand in this market. Classification of species sourced from IUCN Red List database (IUCN 2019).

1.3. Enforcement of Wildlife Laws

1.3.1. Introduction

In many nations, even high income ones, resources put aside to tackle IWT are limited, existing legislation contain loopholes which can be exploited and enforcement and funding is often lacking (Bennett 2015; Gokkon 2018). Tackling the IWT requires training or means to identify species being seized, freedom from corruption and harsh punishments to discourage other traffickers. Evidence suggests these requirements are lacking for enforcement agencies across the world and have been for many years (Rowcliffe et al. 2004; Wellsmith 2011; Cochran et al. 2018). Severity of their penalties for the same crime can vary between neighbouring countries and this lack of consistency is being exploited by criminals (UNEP 2019). Traffickers may poach in one country, prep the goods in another and ship from a third knowing each act would be treated differently within different nations (TRAFFIC 2012; Gristwood 2019). In efforts to improve on this lack of cohesion regional and international frameworks and networks have been established to pool resources and knowledge bases, these are discussed in further detail below.

1.3.2. Wildlife Enforcement and Information Exchange Networks

The IWT has no respect for borders, trade happens within and between continents and regions. Information, resource and skill sharing are important tools in building investigations and creating understanding of how networks operate and interact. To this effect wildlife enforcement networks (WENs) have sprung up intent on addressing these needs (Table 3). Attempts to aid in facilitating information by providing training for concerned nations and trying to standardise their level and style of investigations most notably come via the Wildlife and Forest Crime Analytic Toolkit produced by the ICCWC available to all government wishing to work towards tackling wildlife crime (UNODC 2012).

Name	Acronym	Countries Included	Year of conception	References
South Asia Wildlife Enforcement Network	SAWEN	Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, Sri Lanka	2015	(SAWEN 2015)
Caribbean Wildlife Enforcement Network	CaribWEN	TBC	Development stages	(Weissgold 2016)
Central America WEN	ROAVIS	Belize, Costa Rica, El Salvador Guatemala, Honduras Nicaragua, Panama, Dominican Republic	2010	(ROAVIS 2010)
Horn of Africa WEN	HA-WEN	Djibouti, Ethiopia , Kenya, Somalia , South Sudan, Sudan, Uganda	2016	(Bekele 2016)
North America Wildlife Enforcement Group	NAWEG	Canada, United States, Mexico	1995	(Commission for Environmental Cooperation 1995)
South America Wildlife Enforcement Network	SudWEN	Argentina, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Venezuela. In the process of engaging Uruguay, Bolivia, Suriname, Guyana and French Guyana.	2014	(CITES 2016a)
Association of Southeast Asian Nations Wildlife Enforcement Network	ASEAN-WEN	Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Vietnam and Thailand	2005	(ASEAN Cooperation on Environment 2005)
Southern African Wildlife Enforcement Network	WEN-SA	TBC	Development stages	(CITES 2016b)

Table 3. Established and developmental Wildlife Enforcement Networks (WENs) created with the intention of facilitating information and skill sharing amongst enforcement agencies to improve on IWT investigations.

Other prominent networks include the previously mentioned EU and Africa TWIX's (TRAFFIC 2019a). The TWIX platforms can be accessed by enforcement officials and hold information on seizures and have mailing lists available to encourage and enable communications between country officials to allow actions to be implemented in real time. The initiatives have proved successful with the platform directly leading to arrests and collaborative investigations between Nigeria, Hong Kong and Cameroon (TRAFFIC 2018c).

1.3.3. Global Frameworks Supporting Wildlife Enforcement

The global reach of the IWT has been discussed in length, it is logical that frameworks which span all concerned nations are required to help provide some level of standardisation in the approach to tackling it. Though not solely dedicated to the IWT the United Nations Convention against Transnational Organised Crime and Convention against Corruption are both existing frameworks which are applicable to the problem (Price 2017). However, the ICCWC was established in 2010 as a targeted solution (UNODC 2019). The ICCWC is an alliance of five major inter-governmental organisations, CITES, INTERPOL, UNODC, WCO and the World Bank hoping to provide support to wildlife enforcement agencies through training, guidelines and toolkits utilising the expertise of all five collaborative organisations. Of the five CITES is the most pertinent to the trade in wildlife; established in 1975 it provides a framework where countries (referred to as parties) can voluntarily join and agree to be bound by the rules of the convention. Parties must adopt a licencing system which controls the trade (import and export) of CITES listed species which are organised into three Appendices (I, II and III) dependent on their threatened status. Appendix I includes species at risk of extinction and all trade is prohibited except in exceptional circumstances. Member parties are required to provide annual reports on their trade (legal and illegal) of CITES species. If done correctly the use of CITES should promote sustainable legal wildlife trade (Challender et al. 2015).

Although legally binding CITES does not have the power to prosecute, this responsibility still lies on the enforcement agencies of the member parties to implement their own national legislation. In recent years CITES and been scrutinised and concerns raised regarding its efficiency (Maher & Sollund 2016). It is argued the framework is open to corruption leading to forgery of licences (Wyatt 2009) and it leaves thousands of non-CITES listed species open to exploitation (Lavorgna et al. 2018). Finally, though parties are legally bound by the agreement there is no global enforcement agency in place that can force compliance so it is reliant on the dependability of parties (Reeve 2014).

With even global frameworks ultimately relying on enforcement at a national level, it is important those enforcement agencies are equipped with resources and skillsets which are obtainable, manageable and sustainable in the long-term.

1.3.4. Prosecutions in the IWT

In order to successfully prosecute in cases of IWT a survey of enforcement agencies identified four focal areas they believe are pivotal to their work; species identification methods, detection methods, intelligence gathering and investigative resources which are defined in Table 4 (World Bank Group 2018).

Focal Area	Definition	Resources/Methods
Species Identification Methods	Positively identifying a species to confirm it is an illegal good.	Identification manuals, wildlife forensics, mobile apps
Detection Methods	Finding concealed goods/evidence in cargo, on a person or at a crime scene	Sniffer dogs, search warrants, training in body language, metal detectors
Intelligence gathering	Tracing movements of suspects, their interactions and sources of income	Drones, social media monitoring, security camera surveillance
Investigative resources	Basic tools to carry out day to day work	GPS, patrol vehicles, manpower, computers

Table 4. Focal areas deemed pivotal to the work of enforcement agencies investigating IWT. Table created using data from survey conducted and reported by World Bank Group (2018).

A review by TRAFFIC of IWT cases between 1997 – 2018 describes many examples where these focus areas, both independently and collaborative have contributed to enforcement agencies carrying out seizures and prosecutions (TRAFFIC 2019c). Cases in the report within the last 15 years have seen social media (*intelligence gathering*) and sniffer dogs (*detection*) more commonly being utilised as tools. However, the bulk of seizures and prosecutions listed since 1997 appear to be due to discovery, interception or confiscation of items at airports by customs officials or at border check points (*investigate resources*), either with or without an accompanying trafficker. For enforcement agencies if somebody is found carrying a specimen which is proven to be illegal (*species identification*) it provides the basis of a strong case against that individual. For unaccompanied items, found in abandoned luggage for example, other methods need to be deployed to connect an individual to the seizure. This may be done through, intelligence gathering such following paper trails associated with accompanying paperwork or reviewing CCTV to identify who was travelling with the case, which as long as handled correctly can be admissible in court (World Bank Group 2018). An additional method to link an individual to a crime is forensic evidence, such as DNA or fingerprints, this type of evidence has been shown to play a pivotal role in how criminal cases are processed (Peterson et al. 2013). Within 450 pages of the collated examples from the report by TRAFFIC, human evidence is only reported as influencing the outcome of two cases, one to help prosecute and one to clear of involvement (BBC 2012; TRAFFIC 2019c)

Although the TRAFFIC report described cannot be taken as a complete picture of prosecutions in the IWT it is comprehensive. The lack of use of human related forensic evidence described is notable and appears to be an unutilised approach in investigation of IWT cases particularly when knowledge bases and resources surrounding human evidence are so well established as this paper will go on to detail further.

This paper intends to investigate the use of a common-place forensic technique that will address this gap in collecting human biological and physical evidence in IWT cases. Potentially providing a standardised, cost-effective method that can be implemented and used by multiple enforcement agencies both within WENs and globally. This study hopes to produce examples of data which could be used as reliable evidence in court and used to compare the involvement of individuals in multiple cases helping to ascertain the roles played by individual suspects and evidence of wider criminal networks.

1.4 Aims and Objectives

1.4.1. Aims

This study intends to investigate the use of an existing forensic technique to address the apparent gap in analysing human DNA evidence in relation to IWT cases. The use of gellifters as an affordable and viable tool in the fight against the illegal wildlife trade will be investigated. By encompassing multiple aspects of human trace DNA collection and analysis from commonly trafficked animal specimens, this study will build upon existing research where gellifters have been used to lift fingerprints off pangolin scales.

1.4.2. Objectives

To achieve the aforementioned aims the following four objectives are the cornerstones of this study:

1. Establish whether human DNA can be collected from animal parts using gellifters
2. Determine which, of the trialled specimens, are best suited towards the use of gellifters in forensic work.
3. Determine if the process involved in scanning gellifters to produce images of collected fingerprints affects attempts at dual recovery.

4. Assess the reality and practicalities of using gellifters in the investigation of wildlife crime

1.5 Thesis Outline

Chapter 2 – Research Design

A description and history of the existing forensic work carried out in both human and wildlife crime cases, concluding with a summary of the methodology based on the chapter findings.

Chapter 3 – Methodology

A full outline of the steps taken in this study to produce the final datasets, from fingerprint deposition through to DNA quantification and statistical analysis.

Chapter 4 – Results

Presentation of the results obtained from statistical analysis of the attained datasets.

Chapter 5 – Discussion

A critical analysis of the methodology used and discussion of possible causal factors behind the results. Applications of the study findings in a field setting are also scrutinised

Chapter 6 – Conclusion

A final summary of the overall findings of the study, recommendations for improvements on current methodology and exploration of alternative directions to pursue in this field of research.

Chapter 2 - Research design

2.1 Use of forensics in Non-Wildlife Crime

2.1.1 Background

Forensics, the application of scientific methods in relation to criminal investigation or matters of law, reportedly dates back thousands of years to ancient China. This is based on the discovery of a seal on a document dating back to third century BC, with a clear and intentional impression of a thumb print assumed to be placed there so the document could be traced back to the specific owner/author. This has led some to believe the individual held some understanding of the individuality of fingerprints (Berry & Stoney 2001). Putting aside this single speculative piece of evidence, fingerprinting remains one the first applications of human identification in modern-day forensics. The recognition that each person has unique ridge detailing on their fingertips and the birth of modern-day fingerprinting came about in the mid to late 19th century through individual's such as Sir William Herschel, Dr Henry Faulds and Sir Edward Henry (Berry & Stoney 2001). Sir Edward Henry eventually designed the "Henry Classification System" breaking fingerprints down in eight classes made up of arches, loops and whorls, describing the shapes of the ridges we see on our fingertips and the impressions made from them, this was the official system taken on by Scotland Yard at the time (Chang & Fan 2002). The first well-known instance of a fingerprint being a key piece of evidence in the outcome of a criminal case occurred in 1905 in the United Kingdom. The Stratton brothers were convicted of murder by a jury after one of the brothers thumbprints was shown to match a print found on an emptied safe at the scene of the crime (Cole 1999).

Even before fingerprinting, forensic techniques were used to identify the use of poisons such as arsenic and match bullet casings to weapons as science edged its way into the world of crime investigation. Fast forward to the 1980s and the discovery of the "genetic fingerprint" (also referred to as DNA fingerprinting, DNA profiling, DNA typing) where an individual's unique DNA

characteristics can be identified, isolated and a profile produced from biological samples such as semen or spit opened up a whole new world for forensics (Jeffreys et al. 1985). The basics of DNA profiling take advantage of repetitive sequences of highly variable DNA found in genes, which do not attribute to gene function but are unique to the individual, these are known as minisatellites. Over time the protocol for producing DNA profiles has developed and the newest and most common approaches use PCRs (polymerase chain reactions). PCRs amplify fragments of DNA referred to as microsatellites (smaller units than mini satellites) or STRs (short tandem repeats) from even small or degraded samples to produce multiple copies so there is more genetic material to work with. Analysis of the genetic material allow forensic investigators to identify repetition at specific locations (loci) on a DNA strand, different enforcement agencies have a set number of loci they use as standard identification points. If a sample taken from a crime scene and a sample from a suspect are found to have matching repetitions at the same loci points the likelihood of the samples not being from the same person (identical twins aside) is 1 in a billion (Weir 2007).

DNA evidence was first used in a criminal case in 1986 by creating a DNA profile from semen samples taken from murder victims and matched against samples provided by residents of the local town (Cobain 2016). Since then DNA databases have been established which hold DNA profiles on record for comparison against DNA evidence, examples of these databases include the UKs National DNA Database (NDNAD) and the USA's Combined DNA Index System (CODIS), the second largest and the largest in the world respectively which hold millions of profiles between them (Butler 2012).

2.1.2 DNA from Fingerprints

Traditionally samples such as semen, spit or blood were used to create DNA profiles given the large amounts of DNA they contain. However, protocols quickly became increasingly sophisticated and within a decade of discovering genetic fingerprinting researchers found you

could collect, and profile even small trace samples of DNA left behind after an individual had handled or touched a surface for even a short amount of time. This is referred to as “trace” or “touch” DNA (van Oorschot & Jones 1997; Daly et al. 2012). The amount of touch DNA deposited during handling is highly variable ranging between 0 nanograms (ng) to 170 ng dependent on individuals and surfaces (Burrill et al. 2019). Identification of an individual using DNA fingerprinting can be carried out using <1.0 ng of DNA, making touch DNA samples a valuable piece of evidence if successfully collected and analysed (Life Technologies Corporation 2012; Williams et al. 2013).

When handling or touching surfaces, if not wearing gloves, a fingerprint may be left behind by the individual. As well as providing identification through fingerprint comparison the material or “touch DNA” left behind in a fingerprint has been found to be sufficient to produce a DNA profile for the individual who deposited it (van Oorschot & Jones 1997; Schulz & Reichert 2002; Williams et al. 2013). Therefore, fingerprints have the potential to provide two viable forms of identification to link an individual to a handled item.

Fingerprints are collected in a myriad of ways; powders which adhere to sweat residue, help the fingerprint to be visualised this is then followed by a lifting or imaging technique to preserve and record the fingerprints. Lifting techniques include adhesive lifts (commonly referred to as mini-tapes), casts and gellifters (Subhani et al. 2019), powdered prints can also be photographed. Collecting touch DNA from fingerprints can be carried out either prior to lifting by swabbing the fingerprint whilst still on the original surface or post lifting by processing any biological material present on the lifting material. Ideally investigators will want dual recovery from a single sample, the fingerprint image and the DNA sample (Sinelnikov & Reich 2017). Swabbing a fingerprint directly before lifting statistically produces better recovery rates of DNA but can destroy the ridge detailing of the print itself (Fieldhouse et al. 2016). With lifting, not all biological material will be collected from the target site, but the fingerprint will be preserved. For lifting techniques

where the lifting material can be extracted from directly, such as mini-tapes, the risk of loss of DNA is minimised. Any technique which requires the DNA from the lifter to be transferred to another material before extraction can take place will further increase the potential loss of DNA.

2.1.3 Introduction to Gellifters

Gellifters are a method of evidence collection used in forensic science, designed to collect unpowdered and powdered fingerprints, shoe prints and micro traces. They are cheap to purchase, up to £10 for a pack of ten gels and there are three types of gellifters currently on the market, black, white and transparent/clear all made using bovine gelatine. Which colour gel is used is dependent on the evidence being collected and whether it has been treated, for example BVDA International a forensic product manufacturer, recommends using the transparent gel for fingerprints which have been powdered and black gels for untreated fingerprints (BVDA International 2019). The flexibility of gellifters allows evidence collection from irregular surfaces as they can be rolled or pressed onto the collection site making contact with as much of the area as possible. All gel types consist of three layers, a protective transparent polyester film, the gelatine layer itself and either a layer of linen rubber or a second clear polyester film, Figure 2 demonstrates the composition of the black gellifters which are the type being used in this study. To collect evidence the protective polyester film is removed, and the exposed gelatine layer placed over the desired target site. Once the evidence has been collected gellifters can be photographed or scanned to produce a digitally enhanced image of any fingerprint or shoeprint impressions, or micro traces like hair can be removed from the low tack surface of the gel using tweezers.

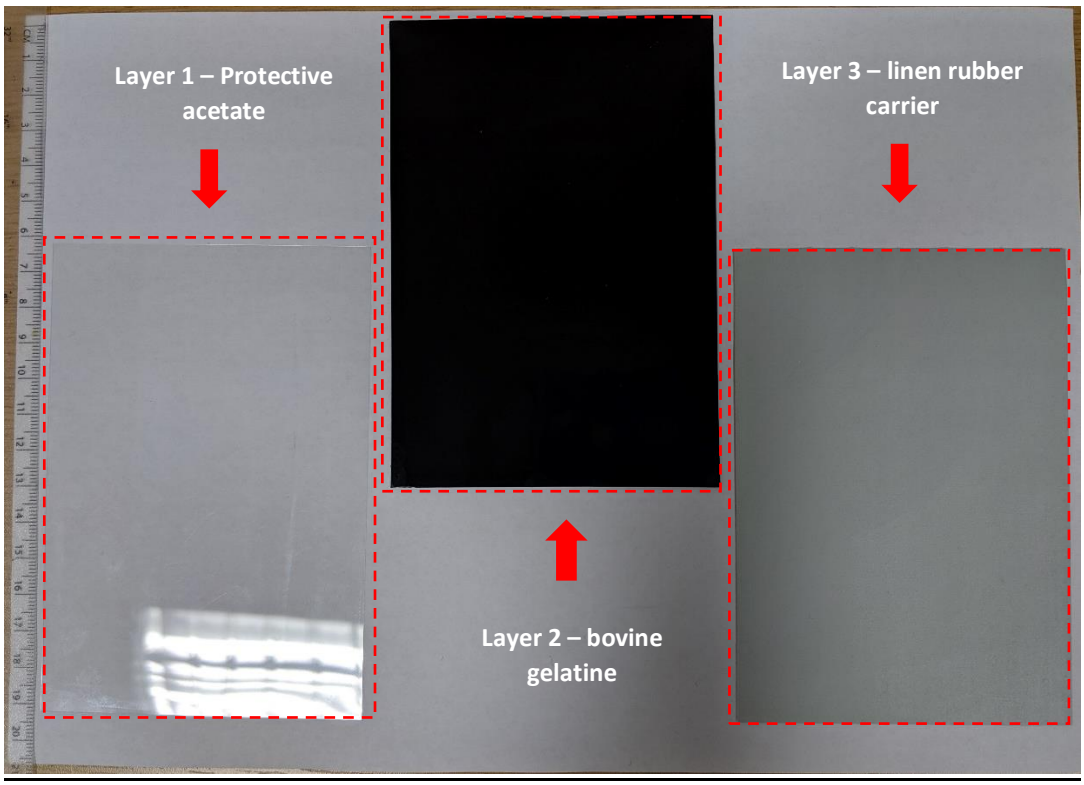

Figure 2. Diagram of deconstructed gelatine lifter with individual layers outlined in red. Layer 1 protective acetate. Layer 2 bovine gelatine. Layer 3 linen rubber carrier layer, Image: Authors own.

2.1.3.1 DNA Extraction from Gellifters

There have been minimal attempts to extract DNA from fingerprints lifted using gels and those that have focused on fingerprints enhanced using powders and then lifted with transparent gellifters (Parsons et al. 2011; Zieger et al. 2019; Subhani et al. 2019). The few attempts made have been successful in not only extracting DNA but in quantities required for DNA profiling, Zieger et al. (2019) found that more than 80% of DNA material is transferred from fingerprint to gelatine lift during collection. Current tested methods for extraction from gels include direct extraction, through proteolytic digestion and standard spin columns and indirect approaches whereby the gels are swabbed, and extractions carried out on the swabs. Problems described during direct extraction protocols on transparent gels include the gel melting during the incubation period and subsequently blocking spin column filters requiring fiddly attempts to clear them without damaging their integrity (Parsons et al. 2011). Where the direct proteolytic digestion method was used on gels Zieger et al. (2019) concluded that despite the method being

more effective in terms of recovery they would still recommend swabbing the gels and extracting from these due to the elaborate protocol required carrying out proteolytic digestion. Other studies using the swabbing technique confirm it as a successful method for extracting DNA from enhanced fingerprints collected using gellifters (Subhani et al. 2019). There appears to be no attempt recorded in the published literature to extract DNA from any kind of evidence, fingerprint of otherwise, collected using black gellifters.

2.2 Use of forensics in Wildlife Crime

2.2.1 Introduction to Wildlife Forensics

The Society for Wildlife Forensic Science, describes the term wildlife forensics as "*the application of a range of scientific disciplines to legal cases involving non-human biological evidence*" (Society for Wildlife Forensics 2019). Using this definition wildlife forensics is applicable to cases of crimes against both humans and wildlife, for example forensic entomology, using insects in criminal investigation to assess time and even cause of death of a cadaver, is commonly used in murder investigations and would fall under the above description of wildlife forensics. Despite the potential dual application of the term, a literature search for "wildlife forensics" tends to bring up papers where focus lays on the use of forensic science in cases of crimes against wildlife rather than against humans, particularly in relevance to the illegal wildlife trade (Ogden et al. 2009; Cooper & Cooper 2013; Richards et al. 2014; Sudhir & Dixit 2016). Being able to identify with certainty the animal or plant product which has been seized, the population it has come from or in rare cases the individual identity of the product will help draw up more robust cases when tackling wildlife crime. With solid evidence there can be no ambiguity as to whether a crime has been committed if it can be shown the product is indeed a species that falls under a trade ban (Peppin et al. 2008). Identification can be carried out through studying the morphology of fur, teeth, claws and other body parts and can prove a quick and inexpensive method where applicable to expose not only illegal items but instances where individuals are

attempting to pass on substitutes as the real thing (Bell 2011; Sharma & Sharma 2016; Tridico et al. 2014). Wildlife DNA forensics is one branch of forensic science which has captured the imagination of researchers (Wolinsky 2012). With an overall aim to analyse DNA from seized wildlife in an attempt to identify the species, population or individual identity of the sample (Peppin et al. 2008) it is a useful tool when morphologically features of a sample have been lost (Shivji et al. 2002) or when there is uncertainty as to whether the trade or possession of the item is covered by national legislation (Gupta 2018). Toxicology analysis of hair, fur, feathers or skin is another approach of wildlife forensics which can help establish whether wildlife has been exposed to harmful chemicals in their environment (Richards et al. 2014). Veterinary practitioners can play a role in wildlife forensics by carrying out post mortems on wildlife carcasses to establish cause of death or assessing live animals to better understand the conditions they may have been kept or transported in (Stroud 1998). Where legislation allows trade in animal products from a certain time period forensic techniques can help age samples to confirm their authenticity and avoid traders trying to pass off modern items as older to circumnavigate these types of laws (BBC 2010).

2.2.2. Wildlife Forensic Networks

In addition to the previously mentioned WENs, wildlife forensic networks (WFNs) have also been established with aims to provide training, information and advice and development of new forensic techniques in supporting wildlife crime investigations. The most prominent of the WFNs is TRACE which works closely with TRAFFIC, the Society of Wildlife Forensic Sciences and PAW UK Forensic Working group (TRACE 2019a) . TRACE and TRAFFIC have collaborated to bring focus to the African & Asian continents and two offshoots have been established the ASEAN Wildlife Forensic Network (ASEAN WFN) (WFN 2019) and the African Wildlife Forensic Network (AWFN) (Pietsch 2018).

2.2.3 Limitations in the Field of Wildlife Forensics

Forensic techniques using human biological evidence are well established with detailed protocols in place for the retrieval and processing of samples. In 2003 the Human Genome Project successfully completed the sequencing of all the genes in human DNA (Collins et al. 2003). The precursor to the human genome project was the Cambridge Reference Sequence (CRS) for human mitochondrial DNA, the DNA found in cell mitochondria and passed down maternally, which was first published in 1981 with a corrected version rCRS (revised Cambridge Reference Sequence) being released in 1999 (Andrews et al. 1999). These breakthroughs allowed human mtDNA Standard Reference Materials (SRMs) to be produced which are used in forensics as quality control to positively confirm a DNA sample is human in origin. The benefit of forensic work on human biological material is that this reference system exists and is readily available, a sample can quickly be identified as human and if enough material is present it can then be profiled and compared against known profiles held on databases or collected using warrants.

Whilst there is only one species of human on this planet there are thousands of species of animals and plants. For wildlife DNA forensics to be utilised to its full capacity equivalent mtDNA SRMs and databases would need to be produced for all species, particularly focal species in wildlife crime. Currently there is no such reliable publicly available resource and researchers must rely on non-validated sequences shared by other researchers on platforms such as GenBank which do not require any sort of process of quality control from those uploading their data (Dawnay et al. 2007). There are calls for mtDNA sequencing of species targeted by the IWT such as the proposed 'ForCyt' wildlife DNA database (Ahlers et al. 2017), but it is recognised the process would be multi-tiered. First a positive identification of taxa will be required from an expert and any other supporting evidence possible to confirm the species as well as strict processing of samples to ensure any potential future positive comparisons cannot be bought

into question (Ahlers et al. 2017). Though the work is underway, this size and type of database will require significant commitment and time to build up.

Forensic analysis is also a costly endeavour (TRACE 2019b), whilst there is much positive in the WFNs mentioned above it should be noted that TRACE and TRAFFIC are both UK based organisations and as a high-income nation the UK has more resources available to put into this kind of research. Funding and support for the AWFN also came from UK sources, the People's Postcode Lottery and Department for Environment, Food & Rural Affairs (DEFRA) and officers travelled to and from the UK to receive and provide training (DEFRA 2018). Without continued overseas funding or financial support from their home nations it is unlikely this kind of training could be sustainable for enforcement agencies from low-income nations.

Wildlife crime cases do not however only rely on non-human biological evidence, where an item has been handled there is always potential for human DNA to be present. If an individual can be proven to have handled illegal goods, there is opportunity for a case to be built against them. Most enforcement agencies will likely have whole teams or dedicated individuals well versed in the analysis of human forensic evidence and existing databases in place containing DNA profiles and fingerprints. In contrast to wildlife forensics there would be no need for financial input into training or development of new methods saving both time and money and utilising existing resources.

Bearing this in mind and the knowledge that human DNA analysis is a common and robust forensic practice, more work is needed focusing on obtaining human orientated evidence, such as fingerprints and DNA in cases related to the IWT.

2.3 Gellifters, Fingerprints and the IWT

Resources targeted by traffickers for the IWT can focus on whole animals or specific parts, for elephants it is their ivory for pangolins their scales. This must be considered when looking into

the application of forensic techniques towards fighting the IWT, it is important that techniques can be shown to work on these targeted resources which are more likely to be seized and available as evidence. Surface type can significantly affect the quality of fingerprint recovery, the porosity of an item is a key factor; non-porous surfaces have poor absorption and therefore the fingerprint residue sits on the surface putting it at higher risk of environmental damage (Yamashita et al. 2010; Madkour et al. 2017). Much of the research into surface suitability has been carried out on man-made materials but studies have been done on materials such as leather and human skin (Färber et al. 2010; DownHam et al. 2015). Other than leather there is a lack of research into fingerprint recovery from animal products and much of it within the last five years, the few that have been attempted are discussed here. In a 2016 paper it was demonstrated that human fingerprints could be enhanced and lifted off elephant ivory up to a month after deposition using small scale fingerprint powdering materials (Weston-Ford et al. 2016). The same method was found to be applicable to hippo and sperm whale teeth. Another team, found success in enhancing and photographing fingerprints on feather and eggs from birds of prey commonly targeted in wildlife crimes, using a range of powders and cyanoacrylate fuming (McMorris et al. 2015). More recently researchers successfully lifted fingerprints from pangolin scales using black gellifters (Figure 3).

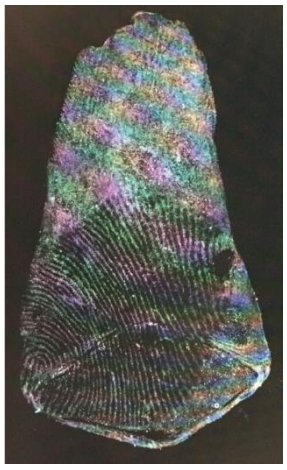

Figure 3. Image of fingerprint on pangolin scale collected using black gelatine lifter – Image: University of Portsmouth (Mills 2018)

The images of fingerprints taken from pangolin scales showed clear ridge detailing and identifiable prints without the need to treat the surface with powder prior to lifting (Mills 2018). Gellifters have also been tested under various conditions in South Africa to assess their usability and durability in less controlled environments which more realistically mimic field conditions which investigator may face at a wildlife crime scene (Mayer 2019). The study did not attempt collection from animal parts but did demonstrate that gellifters could be used in multiple environments and transported over long distances without detriment to the quality of the print.

These discoveries are all promising steps forward in the application of forensic techniques to tackling the IWT. Both techniques, powdering and gel lifting require training for wildlife crime investigators to consistently be able to produce viable fingerprint evidence. Even with training there is no guarantee a fingerprint will have clear enough ridge detailing for individual identification. As discussed above dual recovery of fingerprints and touch DNA will provide better opportunities for comparisons with suspects should either of the identification methods fail. If dual recovery can be successfully carried out on commonly trafficked animal parts it will provide more opportunities for investigators to profile suspects, this is what this research hopes to achieve.

2.4. Developing an Extraction Protocol for Black Gellifters

Given the lack of examples on DNA extraction from black gellifters trials were required to establish which protocol would be most suitable given the time and resources available for this project. Direct extraction was attempted first following the success of (Parsons et al. 2011) method using the QIAGEN DNA Investigation Kit protocols. Like the transparent gels, the black gels melted during the incubation period and were found to both stain and clog the min-elute columns during centrifugation at every stage of the protocol. The final elution was dark purple/black in appearance. A second trial attempted to overcome the melting by “steeping” the gels in proteinase K & Buffer ATL for ten minutes then separating the lysate from the gel into

a new microcentrifuge tube. The intention was to give the proteinase K & buffer ATL time to lyse any cells present then continue with incubation and the remainder of the protocol without the gelatin lift and avoid the melted gelatin clogging the columns (Elkins 2013). However even in the short time the gel was steeping it began to leech a black colour into the buffer mixture and although it did not clog the column the end-product was still purple/black in appearance. To assess if DNA was present in the samples a nano-drop was run. Nano-drops determine quantities of nucleic acid by passing UV light through 1µl of a sample suspended between two optical fibers by surface tension. Nucleic acid absorbs UV light so by assessing the amount of UV which passed through the sample the quantity of DNA present can be ascertained. Results from the nano-drop were inconclusive and it is likely the black colour interfered with the ability of the UV light to pass through the sample. The resources were unavailable to attempt any further methods to confirm the presence of DNA from the direct extraction samples. Without reliable evidence the direct extraction method was suitable and based on the findings of similar papers it was decided to use a wet-dry swabbing technique and subsequent extraction on the swabs for the final protocol (Pang & Cheung 2007; Zieger et al. 2019).

2.5 Research Design Summary

An eight-step methodology for this study was established based on a combination of the above research and the resources available, a summary of the methodology is presented in Figure 4.

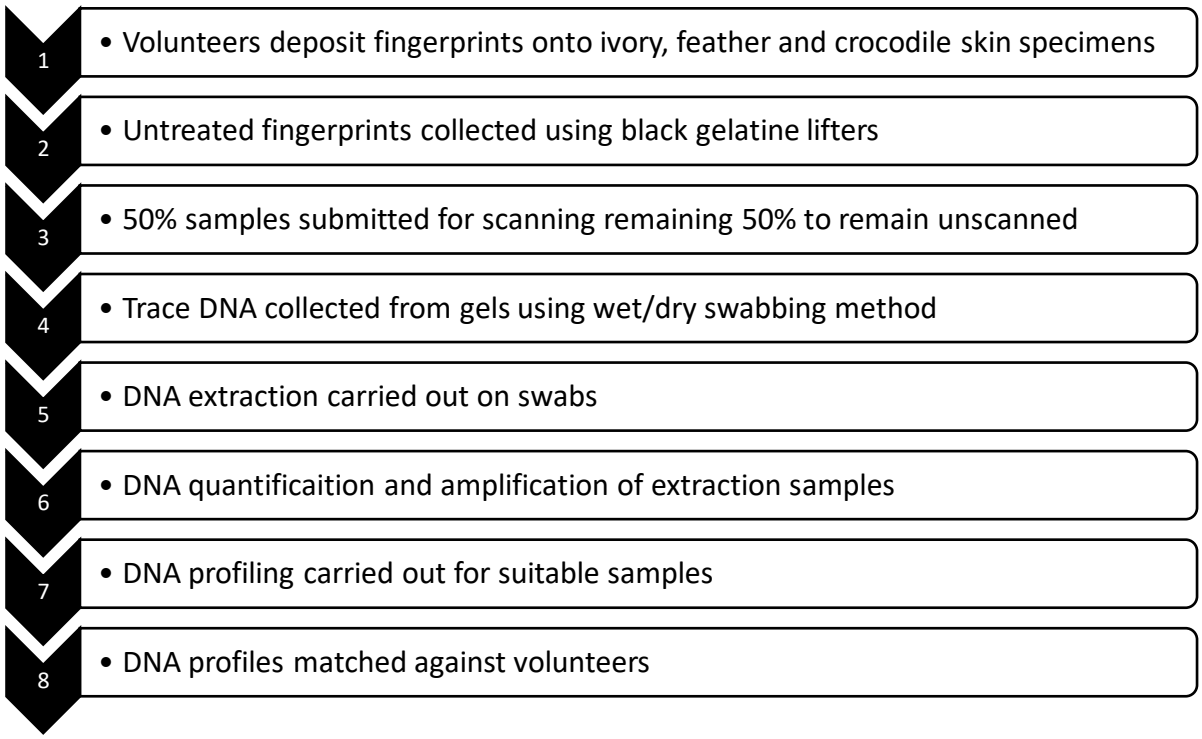

Figure 4. Summary flowchart of methodology decided upon for the current study based on resources available to the author and research discussed in Chapter 2.

Chapter 3 – Methodology

3.1. Selection Process

3.1.1. Animal Specimens

Specimens from the Zoological Society of London (ZSL) archives were made available for this project, items were chosen to represent the three most commonly trafficked animal groups, mammals, birds and reptiles. The final items chosen had two key similarities, they had a flat surface wide enough to accommodate at least one thumb print and they were in a condition akin to something that may be found in a real-life scenario related to the IWT. This was intended to help standardise the fingerprinting and collection technique as much as possible and ensure results could be applicable to field work or investigations. Final specimens chosen were one ivory comb, a feather from unknown species and a crocodile skin bag, also unknown species. The ivory comb and crocodile skin bag were genuine customs seizures handed over to ZSL for storage, the origin of the feather is unknown but likely from a species being held in the zoological collection at ZSL.

3.1.2. Volunteers and Ethics

An email request to participate in this project was sent out to ZSL staff members and students, from the replies twenty volunteers were selected on a first come first serve basis. The samples volunteers were asked to provide, cheek swabs and fingerprints, fall under the definition of "human tissue" as described by the Human Tissue Act (HTA) 2004 as "*material that has come from a human body and consists of, or includes, human cells*" under the act is it a crime to possess human tissue samples with intent of analysing the DNA without the consent of the person who provided the sample. To conform with ethical and legal requirements as laid out in the HTA (Human Tissue Act 2004) a consent form was drawn up (Appendix I) which volunteers had to read and sign before participating in the project. The consent form informs volunteers

their DNA will be collected and profiled from their cheek swab and fingerprints and that the results will only be used for comparisons within this study not run through any external databases. Volunteer identity is kept anonymous through a coding system whereby each volunteer is assigned a letter and numbers 1-6 assigned to each of the lifts being carried out, so for each sample there is an individual code full coding system available to view in Appendix II. Gellifters from ivory were assigned numbers 1 & 2, from the feather 3 & 4 and from the crocodile skin 5 & 6. Odd numbered lifts (1,3,5) were scanned and even numbered lifts were not (2,4,6). The document detailing assignation of letters to volunteers and thus the identity of the volunteers, was password protected and held on a secure server only accessible by the author.

3.2. Preparation

3.2.1. Specimens

All specimens were cleaned using distel high level laboratory disinfectant between each thumbprint and each volunteer. For standardisation the same 3x3cm area of each specimen was used by each volunteer to deposit their thumbprint, Figure 5 shows each specimen and their marked area for volunteers to place their thumbs.

3.2.2 Gellifters

Gel lifters were provided in sealed pouches, prior to the fingerprinting each gel was marked out into six 3cm x 3cm sections with a border surrounding them (Figure 6). The borders were the only areas of the gels handled, either with gloved hands or sterile forceps. Two sides of the gel remained borderless; these sides were used as a guide for placing the sampling area of the gel on the sampling area of the item by lining up the corner of the free edges with the corner

of the 3x3cm square on the item. Sterile scissors were used to cut up the gels into six individual gelatine squares.

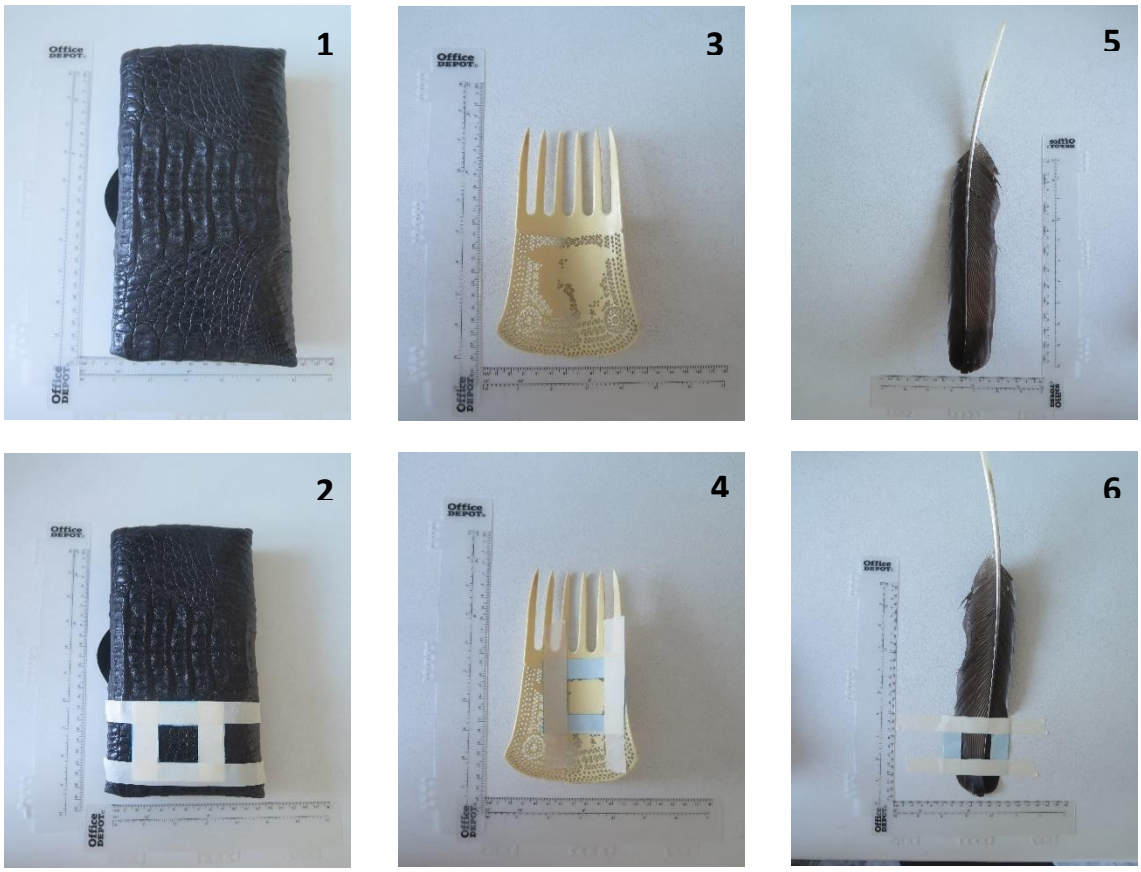

Figure 5 – Images 1 & 2 - Crocodile skin bag with and without 3x3cm marked sampling area; 3 & 4 - Ivory comb with and without 3x3cm marked sampling area; 5&6 - Feather with and without 3x3cm marked sampling area.

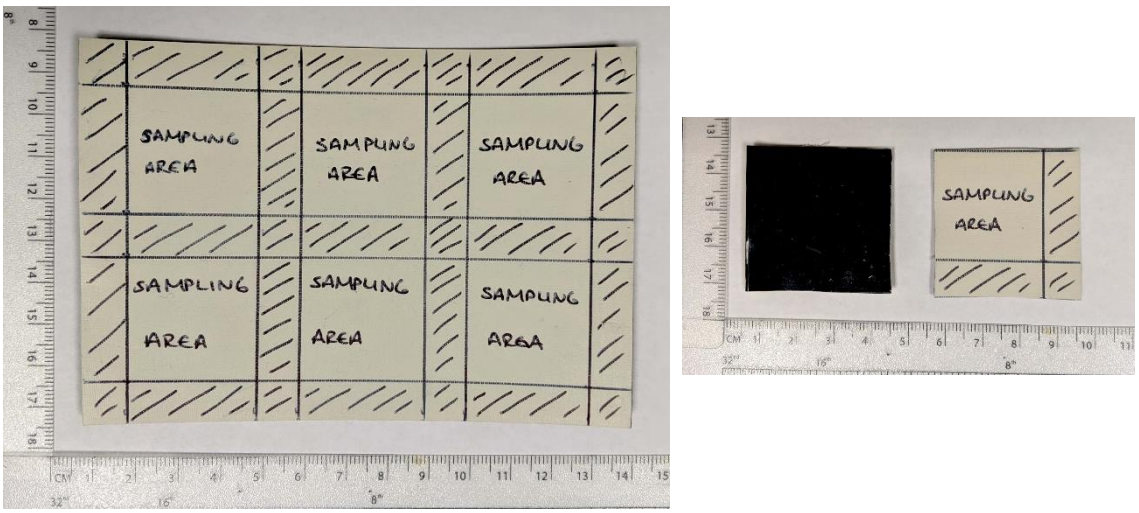

Figure 6. Image of whole gellifter marked for sampling, and front and back images of individual section of gellifter used to lift one fingerprint. Hatched areas represent border handling areas. Image: Authors own.

3.3 Fingerprinting

3.3.1 Deposition

Each volunteer was asked to not eat or wash their hands for 30 minutes before partaking in the project, this was allow natural oils to build up on the skin for more effective fingerprinting and to avoid food traces contaminating the cheek swabs and gels. Volunteers were asked to complete the following steps; 1) rub hands together for 5 seconds 2) place right thumb onto the pre-marked area of the specimen and apply mild pressure for 5 seconds 3) wait for the gel lift to be applied and the area to then be cleaned 4) Repeat step 2 with the left thumb. The rubbing of the hands resulted in "groomed fingerprints" to help standardise DNA deposition between volunteers, it has also been shown to results in higher DNA yields (Subhani et al. 2019). Volunteers completed these same four steps for every specimen resulting in six fingerprints in total per volunteer, two per specimen. Volunteers also provided a cheek swab sample, using a plain cotton swab they rubbed the inside of each cheek for five seconds either side. Swab tips were immediately removed using a sterile scalpel and placed into a labelled 1.5ml tube and stored in the freezer at -20^{o}C.

3.3.2. Collection

Once a print had been deposited the acetate layer was removed from the gel using sterile forceps only touching the bordered handling area. The 3x3cm sampling area of the gel was then placed on specimen and gone over with a roller lengthways, widthways and diagonally to encourage as much contact between the gel and item as possible. The gel was then removed, and the acetate layer replaced using sterile forceps. The gel was then rolled over to remove any air bubbles and placed into a sealed labelled plastic bag.

3.3.3 Scanning

Half of the gels were transported to University of Portsmouth in cool bags to recover fingerprints using the BVDA GelScan gelatine lifter scanner. The scanner works by illuminating black gellifters with three separate light sources and taking a high resolution image, equivalent to 1046 dpi or an image taken by a 155 mega pixel camera (BVDA 2019). Prior to scanning the machine was wiped down inside and out using a damp cloth to remove any dust. Gels were taken from their sealed bags, again only being handled by their bordered edge and the acetate layer removed. The BVDA GelScan can scan gels up to the size of 7.2 x 14.4 inch at any one time. This capability allowed all three gels for each volunteer (one for each specimen) to be scanned at the same time. The gels are vacuumed onto the scanner plate to guarantee a flat surface and avoid any reflections which may interfere with the final image. The exposed gelatine layer did not come into contact with any part of the GelScan machine. Once the scan was complete the acetate layer was replaced, and the gels placed back into their sealed bags. This process was repeated for each volunteer's gels.

3.4 DNA

3.4.1 Gel Swabbing

Gels were swabbed with individually wrapped sterile cotton swabs using a wet dry method. One side of the border was removed from the gel using sterile scissors and a small incision made alongside the upper border to provide a visual guideline on the 3x3cm area with the volunteer's fingerprint. Distilled water was dropped onto one swab using a pipette the wet swab was then drawn over the gel from left to right and top to bottom with a rolling motion. A dry swab was run over in the same process immediately after. Swab tips were removed with sterile scalpel and both tips placed into the same 1.5ml tube. Scalpels, scissors and gloves were changed

between every volunteer's set of gels. Swabs were stored in the freezer at -20°C the acetate was replaced on the gels and the gels stored in the fridge at 4°C.

3.4.2 DNA Extraction

Extractions were carried out on the gel swabs using the QIAGEN DNA investigator Kit and following standard operation procedure for surface and buccal. To begin 20 µl proteinase K and 600 µl Buffer ATL were added to the sample, pulse vortexed for 10 seconds then incubated at 56°C for one hour. During incubation samples were removed from the incubator every 15 minutes and pulse vortexed for 10 seconds then replaced. After one-hour samples were briefly centrifuged, 600µl Buffer AL was added to the samples and they were then pulse-vortexed for 15 seconds. Following this samples were incubated for ten minutes at 70°C, after five minutes the timer was paused, samples were removed and pulse-vortexed for 10 seconds then replaced in the incubator and the timer restarted. After ten minutes samples were briefly centrifuged, 300 µl of ethanol was added to each sample and samples were pulse-vortexed for 15 seconds. Sample tubes were again briefly centrifuged to remove any liquid from the inside of the lids. QIAamp MinElute columns were removed from the fridge and placed in collection tubes provided with the kit. From the samples 700 µl of lysate was carefully added to the MinElute columns, lids closed and then centrifuged at 6000g for one minute, the flow through was then discarded from the collection tube and the column replaced. Remaining lysate from the sample was then transferred to the same column and centrifuged again, flow through was once again discarded and column replaced in the collection tube. Each column then had 500 µl of buffer AW1 added and was centrifuged at 6000g for one minute, flow through was discarded and column replaced; 700 µl buffer AW2 was then added and columns centrifuged at 6000g for one minute, flow through discarded and column replaced; 700µl ethanol was then added and centrifugation at 6000 for one minute was repeated, flow through was discarded. Columns were then centrifuged at full speed (16000g) for four minutes to remove any final lysate and flow

through and collection tube discarded. Columns were placed into a labelled 1.5ml microcentrifuge tube, column lids were opened and incubated at room temperature for 10 minutes. Finally, 20µl Buffer ATE was carefully added to the column directly above the central membrane; column lids were closed and incubated for a further one minute at room temperature then centrifuged at full speed for one minute. The 1.5ml microcentrifuge tube was checked to ensure final product was present, if satisfied columns were then discarded and extraction samples immediately transferred to the freezer for storage at -20^{o}C.

3.4.3 DNA Quantification

The Investigator® Quantiplex®Pro RGQ Kit was used for quantification of extraction samples following the manufacture protocol for using manual setup and template files in Q-Rex and Rotor- Gene Q. The kit uses quantitative real-time polymerase chain reaction (qPCR) to establish if sufficient DNA is present for further DNA profiling and to establish if a sample if male or female by amplification of two specific target regions of the human genome.

Reaction mixtures were made up using a master mix containing 9 µl each of Quanitplex Pro RGQ Reaction Mix and Quantiplex Pro RGQ Primer Mix Total and 2 µl of either our experimental sample or template DNA sample, a total volume of 20 µl per reaction. Template DNA samples are known concentrations of DNA, referred to as DNA standards, four DNA templates were used in this study, each template was repeated, to bring a total of eight DNA standards included in each run alongside the experiment samples from the gellifters. Non-Template Controls (NTCs) were included to detect contamination, these are reactions excluding template or experimental DNA and instead 2 µl QuantiTect Nucleic Acid Dilution Buffer was added to bring the reaction solution up to the required 20 µl. All reactions were added to the Rotor-Gene Cycler, the Q-Rex software was calibrated according to the kit protocol, then the programme was run. The Q-Rex Absolute Quantification HID analysis method was used for assessing concentrations. This method established absolute concentrations for the experimental samples in this study by

comparing their amplification curves against curves of the standards. Concentration results for samples are provided as pg/µl.

3.5 Statistical Analysis

To assess the normality of the datasets in the current study a Shapiro-Wilk Test was used. This test is better suited to smaller datasets of <50 which was true for most of our datasets, but it is effective up to 2000 samples therefore was also applied to our pooled scanned and not scanned samples of 60 (Royston 1992). Results with p value of <0.05 suggest evidence of non-normality in the dataset. All datasets tested resulted in a significance value of $p<0.05$ which confirmed they did not fit the normal distribution model. Histograms of the datasets further corroborated this. The failure of the data to show normal distribution meant non-parametric tests were chosen for analysis, specifically the Wilcoxon-Signed Rank test for analysing differences between two datasets (Woolson 2008) and the Friedman Test for comparisons between three datasets (Cleophas & Zwinderman 2016). Both tests are non-parametric and appropriate for repeated measures data, where data comes from a single group which has had multiple measurements applied to it. Our dependent sample for all tests was the DNA concentration and independent samples were; whether the gel was scanned or not scanned and then the items, ivory, feather and crocodile skin. All tests were carried out at a significance level of $p<0.05$ using R Studio statistics package.

CHAPTER 4 – RESULTS

4.1. Incidental Results

4.1.1. Cheek Swabs

Cheek swab samples were collected to produce reliable profiles for each volunteer for comparisons should DNA collected off items be suitable for STR profiling. As detailed below none of the gel extractions produced DNA concentrations robust enough to carry this step out therefore the cheek swab data were not be used for this purpose. On average 7384 pg/µl of DNA was extracted from the cheek swabs. Four of the seventeen female volunteer samples showed contamination with male DNA, but all concentration levels were insignificant at <1 pg/µl. The cheek swab quantification results show that the extraction method used on the cotton swabs can detect significant levels of DNA and therefore the use of the QIAGEN DNA Investigator Kit and its protocol were justified for their use in this study.

4.1.2 Fingerprints

Figure 7 shows that finger-marks were successfully retrieved from each item using the black gellifters. This study did not attempt to review the quality of the fingerprints retrieved from the items, but the images will be kept on record for future analysis. The scanned images also demonstrated that the inclusion of a designated handling area was an important factor in helping to reduce contamination of the sampling area.

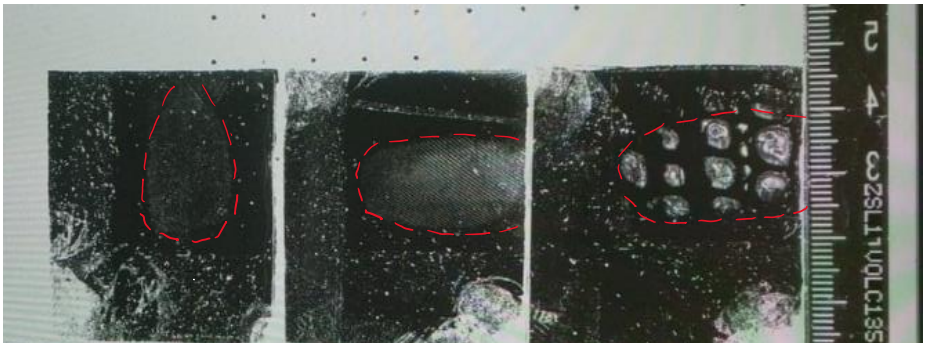

Figure 7. GelScan image of three gellifters in from a single volunteer in this study. Finger-marks outlined in red. From L-R, Ivory, Feather, Crocodile Skin

4.2 Contamination, Outliers and Negatives

4.2.1. Negative Results

A total of 120 fingerprints were taken from twenty volunteers across the three items, ivory, feather and crocodile skin. Table 5 details the number and corresponding percentage of failures, where DNA concentration values were zero, for each set of samples. Failures were generally spread across volunteers but for volunteers D, M & S analysis failed to detect any human DNA from their gelatine lifts for two out of the three items lifted from. Across 120 gellifts taken (40 ivory, 40 feather, 40 crocodile skin), 46% had no DNA recovered at all. All gel lifts applied to clean non-spiked items were free of DNA, indicative of a reliable cleaning method between volunteers and that gels used were DNA free.

	Ivory		**Feather**		**Crocodile**	
	Scanned	Not scanned	Scanned	Not scanned	Scanned	Not scanned
No. Negative results n=20	10 (50%)	8 (40%)	13 (65%)	5 (25%)	11 (55%)	8 (40%)
Total per item n=40	18 (45%)		18 (45%)		19 (48%)	
Total – All n=120	55 (46%)					

Table 5. Number and percentage of fingerprint samples with zero DNA concentration values across individual items. (Full details of all results can be found in Appendix III).

4.2.2. Outliers

Two gellifters, VD4 and VL1, both from male volunteers, displayed problematic quantification results which were considered outliers in the full dataset. Details of the DNA concentrations found are displayed in Table 6, as shown male DNA concentrations were high compared to the rest of the samples which averaged at <1pg/µl. Additionally no human DNA was detected in these samples, for male samples Male:Human DNA concentration should have a ratio of 1:1,

reasons behind the lack of human DNA are currently unknown. As paired non-parametric tests were being used for further analysis equal sized datasets were required. Based on these two outliers and the requirements of the statistical tests being used the decision was made to remove all results from volunteers D & L from further statistical analysis. After removal of volunteers D & L data, Shapiro-Wilks tests were repeated on the updated datasets to ensure non-normal distribution was maintained and non-parametric tests were still appropriate. Evidence of non-normality was found in all updated datasets and analysis went ahead with Wilcoxon Paired Rank and Friedman tests.

Sample Code	Male DNA (pg/µl)	Human DNA (pg/µl)
VD4	12.358	0
VL1	14.755	0

Table 6. Details of outlier datapoints taken from all samples showing high Male DNA concentrations compared to the rest of the datasets and Male:Human DNA concentrations differing from the expected 1:1 ratio. ID codes corresponding to volunteer D, gelatine lift from feather not scanned (VD4) and volunteer L gelatine lift from Ivory scanned (VL1).

4.2.3. Contamination

One NTC control reaction included in the quantification of gellifters from ivory showed the presence of human DNA at a concentration of 0.241 pg/µl. As controls NTCs reactions should have been free of DNA at the end of quantification cycles. DNA extractions were carried out in five batches (excluding cheek swabs, see Appendix IV for breakdown of batch contents), a negative was included for each extraction batch. Male and human DNA was found to contaminate two of the five batch extraction negatives (negatives NEB1 and NEB4) with an average contamination level of 0.56 pg/µl (±SD 0.21, n=2) for the two contaminated negatives.

Results showed male contamination in 18 of the 78 female extractions (23% contamination rate), the average level of male contamination was found to be 0.071 pg/µl (± SD 0.149, n=78) across all female volunteer gel lifts (Figure 8).

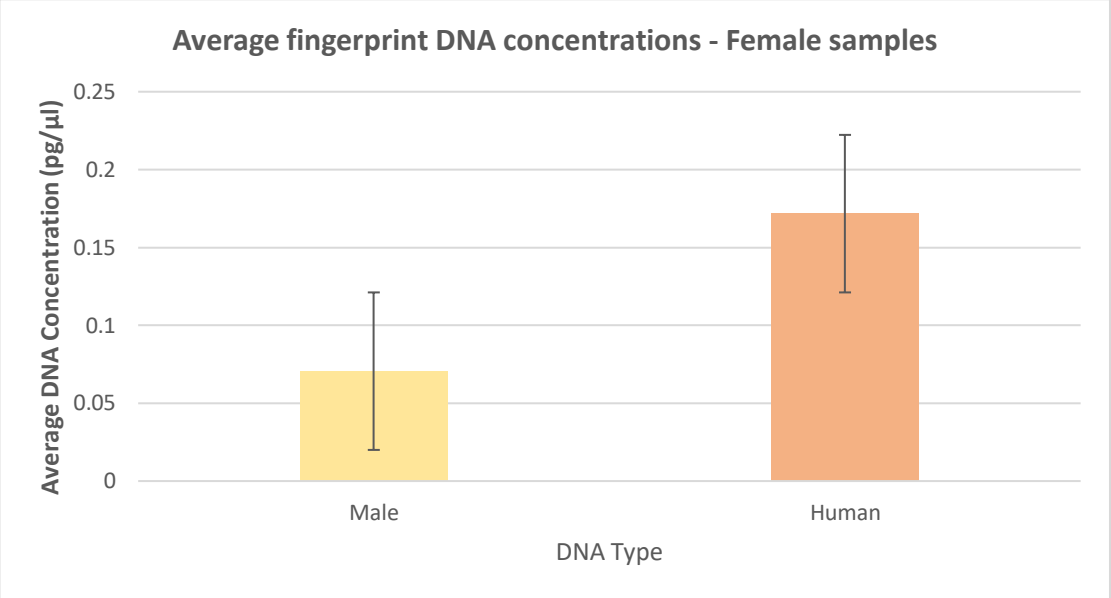

Figure 8. Average DNA concentration of male DNA contamination and human DNA in all female volunteer samples, n=78, male=0.071 pg/µl; human=0.172 pg/µl. Human DNA concentrations were found to be significantly higher than male concentrations p=0.0013 (Wilcoxon Sign Rank Test).

Not all contaminated female samples fell within the previously mentioned contaminated batches and extractions and quantification were carried out at different sites suggesting contamination may have occurred at both stages. A Wilcoxon Signed Rank Test of the quantification data for female fingerprint lifts, indicated that male DNA concentrations were significantly lower (n=13, $p<0.0013$) than overall DNA concentrations. Based on this we believe the dataset from female volunteers to be reliable for further analysis as the male contamination should not impact any comparisons.

Non-contaminated male samples would be represented by a 1:1, Male:Human DNA ratio in the results. After outlier data was removed average male (0.20 pg/µl) and human (0.36 pg/µl) DNA concentrations for male samples had a Male:Human ratio of 5:9 (Figure 9) suggesting female contamination within the male samples. A Wilcoxon Signed Rank Test indicated no statistical

significance between the male and human DNA concentrations for male samples (n=30, p=0.05) and the decision was made to include male samples in further analysis.

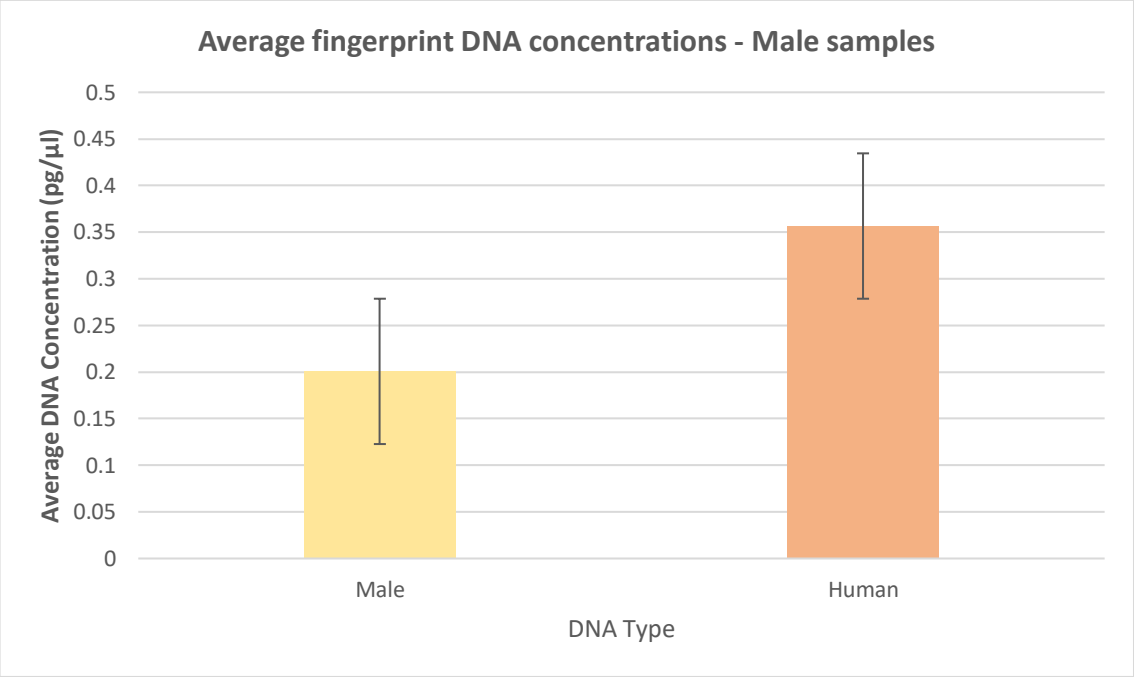

Figure 9. Average DNA concentrations for male volunteer samples, n=30, male = 0.20pg/µl; human=0.36 pg/µl. Human DNA concentrations were found to not be significantly higher than male concentrations p=0.05 (Wilcoxon Signed Rank Test).

4.3. Specimens

4.3.1. Ivory

Collation of scanned and non-scanned gelatine lift DNA concentrations for trace DNA samples lifted from Ivory resulted in an average of 0.241 pg/µl (± SD 0.270, n=36) of DNA being salvaged from each gelatine lift. Non-scanned ivory lifts had a higher average DNA concentration compared to scanned, 0.318 pg/µl (± SD 0.320, n=18) vs 0.165 pg/µl (± SD 0.178, n=18) respectively. A Wilcoxon Signed Rank test showed this difference was not significant (n=18, p-value = 0.149).

Scanned	Not- Scanned	Collated*	Scanned vs Non-Scanned**
Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Significance level p<0.05
0.165 ± 0.178	0.318 ± 0.320	0.241 ± 0.270	p=0.149

*Table 7. Average DNA concentrations and results from Wilcoxon Signed Rank Test, from fingerprints deposited on ivory. n=18 samples were analysed for scanned, non-scanned and Wilcoxon Signed Rank test, n=36 samples were analysed for collated samples. *Collated column represents analysis on combined scanned and non-scanned results. **Statistical analysis between scanned and non-scanned samples using Wilcoxon Signed Rank Test.*

4.3.2. Feather

DNA concentrations from fingerprints deposited on the feather averaged at 0.218 pg/µl (± SD 0.344, n=36), similar to ivory. A significant difference was found between scanned (0.131 pg/µl) and non-scanned (0.306 pg/µl) DNA concentrations from fingerprints retrieved from the feather. Non-scanned gels displayed a significantly higher rate of retrieval based on a Wilcoxon Signed Rank test (n=18, p=0.010).

Scanned	Not- Scanned	Collated*	Scanned vs Non-Scanned**
Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Significance level p<0.05
0.131 ± 0.217	0.306 ± 0.418	0.218 ± 0.344	p=0.010

*Table 8. Average DNA concentrations and results from Wilcoxon Signed Rank Test, from fingerprints deposited on a feather. n=18 samples were analysed for scanned, non-scanned and Wilcoxon Signed Rank test, n=36 samples were analysed for collated samples. *Collated column represents analysis on combined scanned and non-scanned results. **Statistical analysis between scanned and non-scanned samples using Wilcoxon Signed Rank Test.*

4.3.3. Crocodile

The average DNA concentration for fingerprints collected from crocodile skin was 0.209 pg/µl (± SD 0.267, n=36), close to both results from ivory and feather fingerprints. Non-scanned gels showed a higher average DNA concentration of 0.276 pg/µl (± SD 0.327, n=18) compared to the

scanned average of 0.143 pg/µl (± SD 0.163, n=18). A Wilcoxon Signed Rank test found no statistical significance (n=18, p=0.164) between DNA concentrations retrieved from scanned vs non-scanned gels for the crocodile skin bag.

Scanned	Not- Scanned	Collated*	Scanned vs Non-Scanned**
Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Average DNA ± SD (pg/µl)	Significance level p<0.05
0.143 ± 0.163	0.276 ± 0.327	0.209± 0.267	p=0.164

*Table 9. Average DNA concentrations and results from Wilcoxon Signed Rank Test, from fingerprints deposited on crocodile skin. n=18 samples were analysed for scanned, non-scanned and Wilcoxon Signed Rank test, n=36 samples were analysed for collated samples. *Collated column represents analysis on combined scanned and non-scanned results.**Statistical analysis between scanned and non-scanned samples using Wilcoxon Signed Rank Test.*

4.4. Comparisons

4.4.1. Scanned vs Non-Scanned

Non-scanned gels showed greater average DNA concentrations across the board (Figure 10) compared to scanned gels. Despite all items showing this result the difference was only found to be significant for the feather. The scanned and non-scanned data was collated for all items to obtain a better consensus on whether the process involved in scanning the gels negatively impacted the amount of DNA which could be collected from them. A Wilcoxon signed rank test was performed on the collated data and non-scanned gels were found to have significantly higher concentrations of DNA comparable to scanned (n=54, p-value = 0.00198). Possible reasons behind this difference are discussed below.

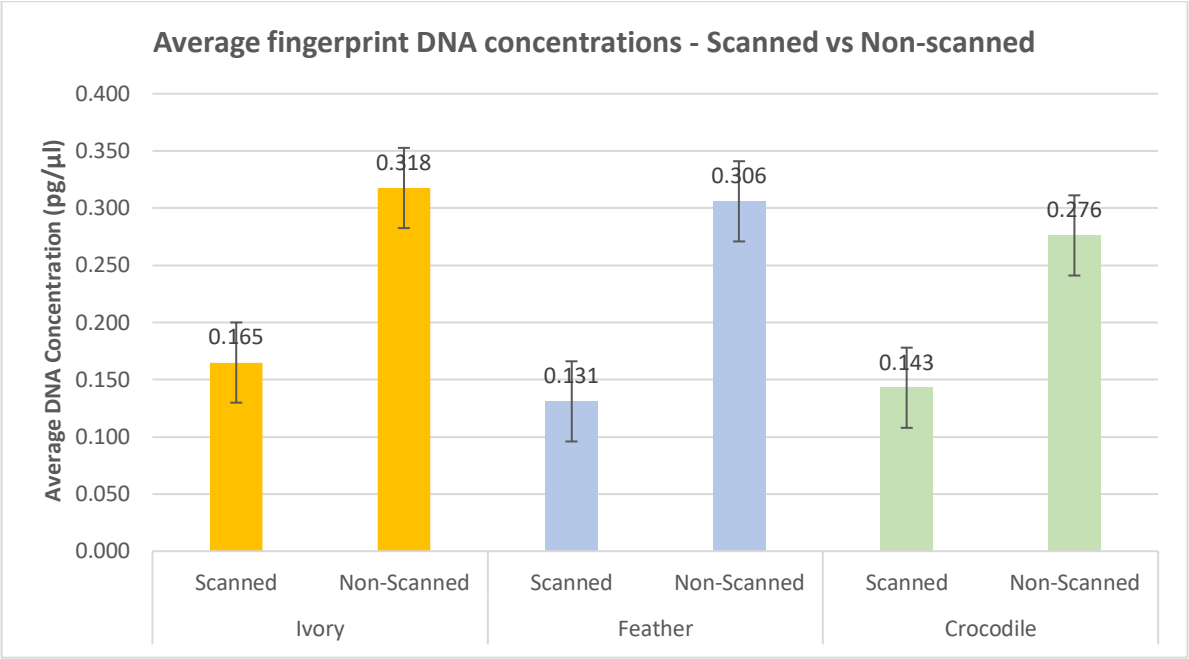

Figure 10. Average DNA concentrations for scanned and non-scanned fingerprints taken from ivory, feather and crocodile skin collected using gelatine lifts. n=18 for all scanned and non-scanned samples. Scanned and non-scanned data was collated, and analysed, average DNA concentrations were significantly higher for non-scanned samples, n=54, p=0.00198 (Wilcoxon Signed Rank Test).

4.4.2. Specimen vs Specimen

Non-scanned average DNA concentrations were similar for gelatine lifts from all three items at 0.318 (± SD 0.320, n=18), 0.306 (± SD 0.418, n=18) and 0.276 pg/µl (± SD 0.327, n=18) for ivory, feather and crocodile respectively. Scanned samples also showed little variation between items with ivory samples having the highest average concentration at 0.165 pg/µl (± SD 0.178, n=18) compared to 0.131 pg/µl (± SD 0.217, n=18) for the feather and 0.143 pg/µl (± SD 0.163, n=18) for crocodile skin. Out of all the items the ivory had the best retrieval rate with a total average of 0.241 pg/µl (± SD 0.270, n=18) of DNA for fingerprints taken from ivory. Analysis was carried out to see if there was any significant difference to DNA concentrations collected from the gels dependent on the item they had been in contact with. Scanned and non-scanned data was collated for each item (Figure 11), and then compared using a Friedman test, no significant difference was found between the items (n=36, p= 0.441).

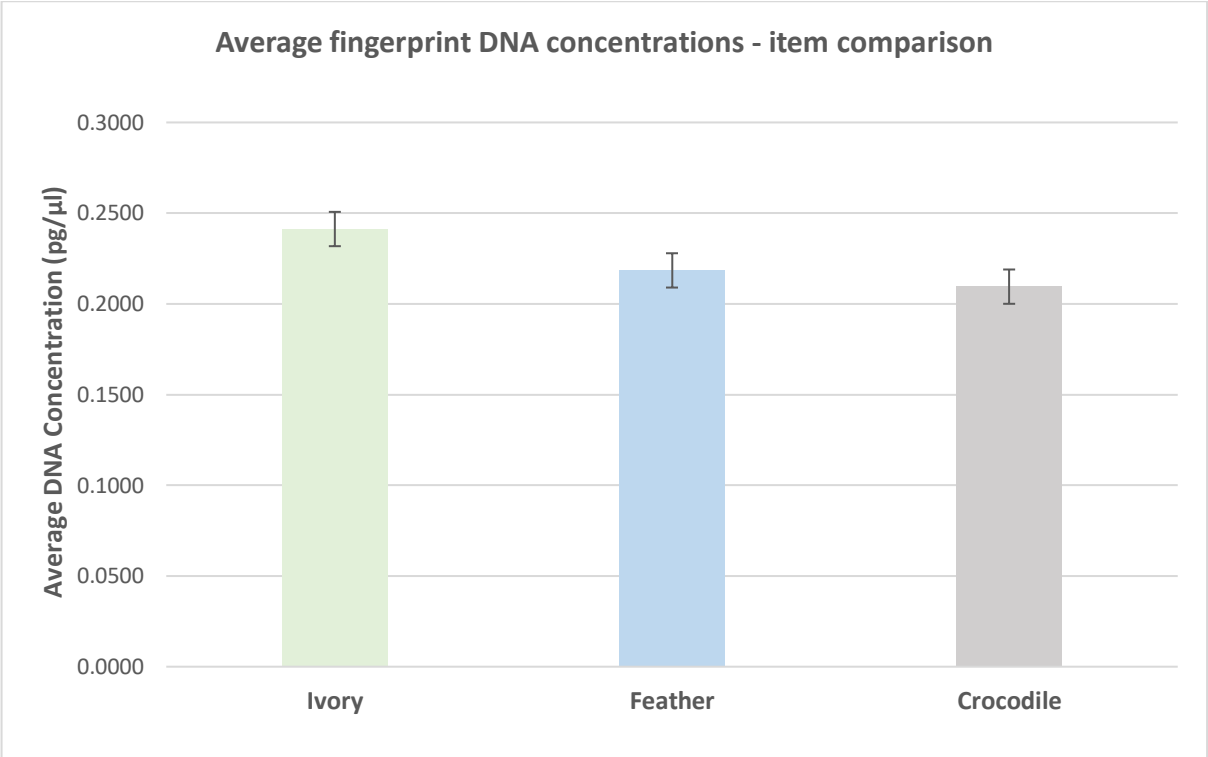

Figure 11. Average DNA concentrations of collated data from scanned and non-scanned samples for each item. n=36 samples were analysed. There is no significant difference between DNA concentrations of fingerprints lifted from each item p=0.441 (Friedman test).

Further Friedman tests were run comparing only the scanned data for all three items and then non-scanned data. Neither test identified a statistically significant difference between DNA concentrations across the three items, (n=18, Scanned, p= 0.752； Non-scanned, p=0.3162).

Comparisons were also made using a Wilcoxon signed rank test, comparing two items at a time with scanned and non-scanned data collated. No pairings (ivory/feather, ivory/crocodile, feather/crocodile) were found to have significantly different DNA. From this we can assume for this study the type of item being collected from does not influence the amount of DNA recovered from the gels.

Chapter 5 - Discussion

5.1 Dataset Reliability

5.1.1. DNA Quantities

DNA concentrations for this study averaged at the incredibly low levels of <1 pg/µl for each item, both scanned and non-scanned datasets. The average amount of DNA, referred to as the C-value, found in a single human haploid nucleus (like a gamete) is ~3.5 pg. Diploid somatic cells (any generic biological cell making up an organism) contain double this meaning a single cell from a human should contain around 7pg of DNA (Gregory 2015). At < 1pg/µl samples were found to contain ~ $1/7^{th}$ of a single cell's worth of DNA per microlitre. Our extractions were eluted in 20 µl of buffer putting the maximum DNA yield for our samples at 20 pg or just under 3 cells worth of DNA, but it is likely lower as not all buffer would pass through the column. Typically the minimum amount of DNA required for successful profiling is between 0.2 – 0.5 ng (Williams et al. 2013), this is equivalent to 200-500 pg putting into perspective how negligible the levels of DNA found in this study were.

5.1.2 Stochastic Effects

Analysis of low levels of DNA is not unheard of, successful STR profiling was carried out using DNA from a single cell over twenty years ago (Findlay et al. 1997). The process is referred to in the literature as Low Copy Number (LCN) testing (McCartney 2008). LCN testing typically works with <100pg of total DNA sample, significantly more than the maximum 20pg available in this current study, run at a higher number of amplification cycles, 34 vs 28 (Buckleton 2009). Stochastic effects are a recognised issue in the testing of low-level DNA, these are random sampling effects where the PCR reaction may sample an imbalance of the available alleles in a heterozygous locus or sample no alleles in a homozygous locus (Timken et al. 2014). If a sample has a low concentration of DNA the transfer of a true representation of its contents into a

reaction is increasingly unreliable. Depending on what has found its way into the reaction from the total sample primers may be unable to find the specific region they are targeting for amplification and quantification results would record it as absent or alternatively any contamination DNA may be amplified instead of the target DNA giving “false” results, referred to as allelic drop out and drop in respectively or locus dropout where both alleles are missed (Gill et al. 2009; Aditya et al. 2011).

These stochastic effects are a potential explanation behind many of the results seen in this study. Locus drop-out would produce the zero results found for several volunteers across the items and allele drop in would provide explanation for the sporadic male contamination seen in multiple female samples. The extremely low levels of DNA found and the increasing impact of stochastic effects on smaller samples does mean the reliability of the results in this study can be called into question.

5.1.3. Contamination

It is accepted that during this study whilst every effort was made to ensure working conditions were sterile, neither sets of labs used for the extraction and quantification/amplification were completely sterile environments and the GelScan device was also not sterile. Contamination may have occurred at almost any point of the procedure and as discussed above given the low levels of DNA already being worked with even trace amounts of contaminating DNA have influence on the results. An additional explanation for the contamination of female samples is from DNA transfer prior to volunteers depositing fingerprints. Indirect transfer of male DNA to items during female volunteer’s deposition is possible if the volunteer had shaken hands with a man in the recent past (Meakin & Jamieson 2013; van Oorschot et al. 2019) . As a form of “trace DNA” it may not have affected the results if the original fingerprint had contained higher levels of DNA as stochastic effects would not have been so prominent. The level of male contamination in this study was found to be insignificant but it is impossible to tell what levels of third-party female

contamination may have been present in female samples. Human DNA levels may well be the result of amplification of drop-in contamination through DNA transfer. Although the decision was made to go ahead with further statistical analysis on the data as discussed above it is appreciated the reliability of the data sets is low.

5.2. Causal Factors Behind Low DNA Yields

5.2.1. Background

Multiple other studies have had success in sequencing trace DNA from fingerprints, not only directly from swabbing the fingerprint but also after transfer to a lifting material and then again after further transfer to a swab (Plaza et al. 2016; Sinelnikov & Reich 2017; Templeton et al. 2017) . Key differences between this current study and the aforementioned studies are 1) black gellifters were used rather than transparent or white 2) fingerprints were not enhanced using powders or other methods prior to lifting 3) ivory, feathers and crocodile skin are novel items for an attempt of this kind of study. The potential implications for each of these difference on DNA yields and other considerations are discussed below.

5.2.2. Non-Enhancement and Black Gellifters

This study is a continuation of the successful lifting of fingerprints from pangolin scales using black gellifters (Mills 2018). A key reason for the use of black gellifters is that enhancement of the fingerprint through powdering isn't required and the perceived belief being able to miss out this step would be beneficial to the application of their use in field conditions.

The chief aim of powdering is to visualise latent prints so that investigators can effectively lift or photograph as much of the fingerprint as possible. Multiple studies have been done into whether powders have a detrimental effect on ability to generate STR profile from touch DNA and none have been found (Gino & Omedei 2011; Alem et al. 2017; Templeton et al. 2017).

Therefore, not powdering the fingerprints in this study should have had no negative effect on DNA recovery.

Without a better understanding of the composition of the black gellifters compared to transparent or white lifters it is difficult to say whether the black gels themselves influence DNA recovery rates. It is recommended that this study be replicated with powdered fingerprints using black and transparent/clear gellifters as independent variables to further investigate the role black gellifters play in influencing DNA recovery.

5.2.3 Specimen Type

5.2.3.1 Crocodile Skin

Crocodile skin is made up of highly keratinised scales and is waterproof in nature (Milinkovitch et al. 2013). Scales are adjoining and create an uneven surface, making fingerprint collection difficult. As seen in Fig 5 the gellifters were unable to efficiently make contact with the full sampling area even with rolling. Fingerprints already contain low levels of DNA so inability to lift a full fingerprint as seen with the crocodile skin, would prove significant. In contrast it has been argued that rough or uneven surfaces encourage more cells to be dislodged due to abrasion and therefore should have higher levels of DNA available for recovery (Wickenheiser 2002). Fingerprint images for crocodile skin samples (Figure 7) showed much more distinct sites of deposition comparable to ivory and feather, but less distinct ridge edges. This could support the abrasion theory, if the images are depicting actual cells rather than ridge detailing through secretions.

5.2.3.2. Feather

Under a microscope the natural weave of feathers is comparable to some fabrics so similar fingerprint enhancement techniques have been trialled and found to be successfully specifically using powders (McMorris et al. 2015). The scanned lifts in this study (Figure 7) clearly showed

deposited finger-marks on the feather, suggesting residue had been collected by the gel. Ridge detailing did not appear to be present, but the striations of the feather were visible; notably these striations were not picked up in the areas outside of the point of deposition again suggesting the visible residue was exclusively from the volunteer and not any contamination on the feather. From a cursory look at the fingerprint scans and knowing that touch DNA can successfully be used for STR profiling it is surprising not a single sample came back with suitable levels of DNA. One explanation may be that the weave on the feathers was not tight enough and residue, ergo DNA, was able to "leak" between gaps. Looking more closely at the feather scans we can see thin black lines within the finger mark where the gel has not picked up any residue which supports this theory.

5.2.3.3. Ivory

Like most teeth ivory is made up of a dentine material with a protective enamel layer (Vollrath et al. 2018). It is a relatively non-porous material which places it in the more difficult category for fingerprint collection (Madkour et al. 2017). The ivory comb used in this study was polished ivory which has been found to lend itself better to fingerprint enhancement compared to unpolished ivory (Weston-Ford et al. 2016). It stands to reason that the polished ivory allowed the fingerprint residue to sit on the surface with minimal if any absorption. This could explain why ivory had the highest level of DNA retrieval compared to the crocodile skin and feather which had more uneven surfaces.

5.2.3.4. Comments on Specimen Type

In this study fingerprints were immediately collected after deposition and therefore were not subject to the types of environmental factors which normally affect fingerprints on non-porous surfaces. Whilst the low DNA recovery from crocodile skin and feathers have potential explanations it is surprising that none of the ivory samples achieved higher DNA yields. This increasingly points to the idea that the black gellifters have an influence on DNA recovery.

5.2.4. Observation of Cellular Material

Trace DNA is recognised as being a low-level source of DNA comparable to other forms of evidence such as blood or other bodily fluids (Wickenheiser 2002). At low levels efforts should be made wherever possible to reduce the chances of DNA transfer and subsequent loss and different steps in the protocol. For the current study the main points of potential loss of DNA were at the lifting stage if not all the DNA was collected from the item using the lifts, at the swabbing stage if not all DNA material was collected from the surface of the gel and at the extraction stage. It has been found that around 80% of DNA from enhanced fingerprints can be transferred to transparent gellifters and 67% of DNA can be recovered from swabbing of the lifters (Zieger et al. 2019). Assuming black gellifters have the same transfer rate it would place DNA recovery at a maximum of ~54% of total DNA available from the fingerprints in a best-case scenario. In reality cotton swabs are shown to have poor collection and retention rates for untreated touch DNA samples, and that anywhere between 20-76% of DNA collected by swabs is lost during the extraction phase (Verdon et al. 2014; Bruijns et al. 2018).

No attempt was made to check whether all or significant amounts of cellular material had been transferred at each stage. Viewing of the fingerprint under a microscope after deposition and then again after the gel lift had been applied as well as the gel before and after swabbing would have help establish the level of DNA transfer which had occurred and if further action was needed to improve on the technique.

5.2.5 Storage Methods and Periods

Out of necessity the period between fingerprint deposition and scanning was six weeks, it was a further four weeks before extractions and quantification were carried out. This places a ten-week period between collection and extraction with gels being stored at 4^{o}C and then subsequent extractions at -20^{o}C.

Successful DNA recovery from trace samples and fingerprint quality decreases with increasing time lapsed when exposed to environmental factors, the impact is lessened when samples are stored under controlled conditions (Yao et al. 2004; Raymond et al. 2009).

Gellifters are normally stored at room temperature or 20°C but there are no detrimental effects to storing in a fridge as done in this study (BVDA International 2019). Storage at 4°C also allowed the samples to be held under more controlled conditions which as discussed is preferable for touch DNA samples. QIAGEN DNA Investigator kit protocol advises that extractions can be used immediately for amplification or stored at -20°C (QIAGEN 2012). The storage methods used in this study were therefore in keeping with professional recommendations and should not have had a significant detrimental effect on DNA yield.

5.3. Explanations for Statistically Significant Results

5.3.1. Summary

The only significant result found in this study were the differences in average DNA concentrations for scanned and non-scanned samples. The stochastic effects from such low DNA concentrations take away any true validity of this finding but as scanned samples were subject to multiple instances of interference the results are still of interest and possible explanations are provided below.

5.3.2. Transportation

During transport to the scanning site and when not being scanned gels were stored in cool bags. Repeated opening and closing of the bag to remove and replace samples and then sitting under the scanner for up to two minutes meant gels were subject to fluctuating temperatures. The maximum temperature fluctuation would have been from the storage temperature of 4°C to room temperature of 20-25°C and back to 4°C with several hours between each temperature stage. Freezing and thawing of DNA samples has been shown to cause DNA degradation but this

occurs after multiple cycles and when DNA is frozen at low temperatures such as -20°C (Brunstein 2015; Alketbi 2018). Fingerprints appear to be hardy to temperature fluctuations, with retrieval found to be possible from paper and plastic, porous and non-porous materials respectively, when subjected to temperatures ranging from -20°C to 150°C (Iten 2012). Fingerprints have even been recovered after exposure to extreme temperatures of 300°C in simulated arson crime scenes (Gardner et al. 2016). It is unlikely the samples in this study were affected by the minimal and short-term temperature changes experienced during scanning.

5.3.3. Acetate Layer

In two of the three studies detailing successful retrieval of trace DNA from gellifters the handling of the protective acetate layer differed from this study. One study did not replace the acetate layer and instead immediately transferred gels into tubes in preparation for extraction (Parsons et al. 2011). The second study carried out extractions on both the gels and its corresponding acetate layer (Subhani et al. 2019), the third study did not provide any detail on the fate of the acetate layers. In the current study the acetate layers were seen only as a protective layer for storage and no attempts were made to retrieve any DNA from them. Product information for the gellifters used indicate no detriment to collected material with the removal and replacement of the non-adhesive acetate layer (BVDA International 2019). The lifters were not originally designed for trace DNA retrieval but are used for collecting micro traces such as hair. The low-tack nature of the gellifters does put material on them at risk of being dislodged. With already low levels of DNA present in trace evidence, even negligible loss of DNA from the removal and replacement of the acetate in this study could have been significant.

For the scanned samples the acetate layers had to be removed before being scanned and then replaced for transportation, a total of two removals for the entire process from collection to extraction. If material is lost during the acetate removal/replacement process this extra removal compared to non-scanned samples may have dislodged further biological material from the gels.

This acetate theory would provide a simple and elegant explanation not only for the overall low concentrations found but also the significant difference between scanned and non-scanned datasets.

5.3.4. Ultraviolet (UV) Light

The GelScan machine used for visualizing fingerprints on the gels uses three separate light sources for illumination, the wavelengths of light used are undisclosed by the manufacturers. Fingerprint enhancement using UV light is a tool commonly used by forensic scientists. There is a lot of evidence that short term exposure to UV radiation can have a detrimental impact on DNA recovery and that distance from the light source is a contributing factor (Frégeau et al. 2000; Nicholson et al. 2005; Ballari & Martin 2013; Gršković et al. 2013; Kumar et al. 2015). Andersen & Bramble (1997) found that exposure of bloody fingerprints to short-wave UV light for 15 minutes had a significant enough effect on DNA quantities that amplification and profiling was not possible. This finding is notable in relation to the current study as blood has much higher DNA concentrations comparable to trace DNA samples. Scanning of the gels in this study took anywhere up to two minutes. If a UV wavelength is present as one of the three sources of illumination used by the GelScan machine, the closeness of the light sources to the gels and the two-minute scan time may be a strong contributing factor to the significant difference between scanned and non-scanned samples.

In this study gel lifters were stored in cool bags which did not subject them to exposure to light sources during storage and the non-scanned samples were not removed until the point of extraction. Degradation caused by UV light may explain the results from scanned samples, but it does not explain the overall low levels seen throughout the results.

5.4. Application of Gellifters in a Field Environment

A contributing factor behind the design of this study was, in the event of success, that black gellifters could have been a cheap and easy to use tool for investigators in the field. The lack of powder enhancement required, the ease of storage and relative ease of use all lend to its inclusion in a field kit.

Although unsuccessful the discussion in this study has raised many considerations about the reality of working with trace DNA evidence in tackling the IWT. As discussed, a variety of environmental factors can negatively impact the quantity and quality of trace DNA. Smuggling methods used by wildlife traffickers can be extreme; live animals hidden on a person or drugged and crammed into suitcases and illegal items disguised as everyday objects are all methods used by traffickers (Hoyland 2011; Ingber 2013; Reuter & O'Regan 2017; Dasgupta 2018) . Land, sea and air are all utilised potentially putting trafficked animals and products under a variety of weather and temperature pressures (Ferrier 2010). Dependent on where in the supply chain an animal or animal product has been seized there is a strong chance any viable trace DNA material has been damaged or degraded beyond use and serious consideration should be given to using resources to try and collect DNA evidence, particularly if the investigating force has no DNA database in place for comparisons.

A second consideration is the number of individuals who may have handled an item. IWT supply chains are notoriously difficult to pick apart, some transactions may involve a simple two person poacher and buyer handover, others may involve complicated criminal networks with multiple individuals and the goods could have gone through many hands before reaching the point of retrieval (Ayling 2013; Reuter & O'Regan 2017). Mixed DNA samples, where two or more individuals information is present, are difficult to interpret, small samples in trace DNA even harder as stochastic effects are amplified and allelic drop in and drop out could cause an entire suspect to be missed in analysis (Hu et al. 2014).

In this study under controlled conditions contamination of samples occurred, in field conditions contamination risks are intensified and investigators would have to be diligent in their collection techniques. Field conditions can be dangerous for those tackling the illegal wildlife trade with murder of anti-poaching units and activists not unheard of, to benefit all involved a simple approach as possible to evidence collection would be preferable (BBC News 2018; Burke 2018). What we know of the use of black gellifters from this study and the recent pangolin study (Mills 2018) points to them currently being better focused as a tool for fingerprint collection.

Chapter 6. Conclusion

6.1. Study Findings

As far as it can be found in the published literature this study is the first attempt to extract trace DNA from fingerprints lifted with black gellifters. It is also the first attempt in the published literature to analyse human DNA deposited on trafficked animal specimens of any kind. This current study has shown it is possible in theory to collect and quantify trace human DNA samples from ivory, feathers and crocodile skin using black gellifters. The method used however did not obtain DNA in the quantities required for successful DNA typing and therefore use in criminal investigations. Analysis further demonstrated scanned samples had significantly lower DNA concentrations compared to non-scanned samples. All results in this study should be approached with caution due to the stochastic effects prompted by the very low average DNA concentrations (<1pg) found in this study.

Item surface type, collection and extractions using cotton swabs, and the black gellifters were discussed as potential contributing factors to the low DNA yields in this study. UV light, transportation and failure to analyse acetate cover layers were further discussed in relation to the significantly lower DNA concentrations seen in scanned samples.

All factors considered bought forward the conclusion that black gellifters and removal/replacement of the acetate layer as the two most likely causal factors in the overall low DNA concentration seen in this study. A combination of an additional round of removal/replacement of the acetate layer and possible use of UV light during the scanning process were deemed to play a role in the significantly lower DNA concentrations from scanned samples.

However, better understanding of the composition of black gellifters and which wavelengths of light are used to illuminate gels during scanning are required before they are can reliably be considered as contributing factors.

6.2 Future Recommendations

This work would benefit from repeat studies comparing other forms of DNA retrieval and extraction from black gellifters including direct extraction, tape lifts and DNA wipes to see if they have better success in retrieving DNA quantities at levels needed for profiling.

It is also recommended that the acetate layer be included in any future analysis, either through observations under a microscope to identify the presence of cellular material, inclusion in extractions or if possible, the omission of the layer completely after fingerprint collection.

The use of non-invasive fingerprint imaging should also be explored on the same item types used in this study, whereby a fingerprint image can still be recorded but without the need for lifting. This would allow for direct DNA recovery from fingerprints and may result in higher yields and a better understanding of which kinds of animal products are suited to this kind of forensic work.

The scanned fingerprint images from this study should be further analysed to establish whether viable prints have been obtained from ivory, feather and crocodile skin. If identifiable prints are found this would be a first for all these animal products using black gellifters and a significant discovery within itself. It would further support the findings of work done with black gellifters on pangolin scales and in turn the use of these gels in fingerprint evidence collection in cases pertaining to the IWT where these animal products are involved.

This study has proven much work needs to be done before fingerprints collected using black gellifters can be a reliable source of DNA evidence in tackling the illegal wildlife trade. These results suggest black gellifters are currently better suited for fingerprint evidence collection in IWT cases and DNA evidence should be collected using more robust methods.

The use of forensic techniques in the IWT to collect biological human evidence is still in its infancy but the potential for future work is great. We would encourage further studies using the

black gellifters but also with other emerging techniques and expand the number of animal products that are investigated.

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APPENDIX I –ETHICAL CONSENT FORM

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1. Purpose of the Project

We would like to invite you to participate in an MSc research project provisionally titled "Can human DNA be extracted from fingerprint lifts collected using gelatine lifters off commonly trafficked animal parts".

The illegal wildlife trade (IWT) is currently one of the biggest threats to species globally (Van Uhm 2016), responsible for the demise of large charismatic megafauna including rhino and elephant (Ayling 2013; Underwood et al. 2013), parrots, the most threatened bird species in the world (Pires 2012) as well as more elusive species such as the pangolin (Owen 2014). Fuelled by demands for parts used in traditional medicines (Zhang et al. 2008) and as trophies for the elite the IWT has even been linked to organised crime (Biggs et al. 2013; Biggs et al. 2017) and estimated to be worth up to $10 billion a year (Milner-Gulland 2018) making it a lucrative endeavour. It is apparent this is now a transnational issue requiring the collaboration of multiple world leaders and governments (Rosen & Smith 2010).

The use of forensics in solving human related crimes is constantly evolving and has been a staple in investigations for some time (Kayser & De Knijff 2011) with every increasing sensitivity in methods of collection and profiling of trace materials helping to build cases for or against concerned parties. Now there is a surge research targeted at wildlife forensics, with particular focus on analysis of DNA samples to identify species, populations and more regarding the wildlife sample (Ahlers et al. 2017; Ogden et al. 2009). There are significant limitations with wildlife forensics however, much of the time it requires existing profiles to match against to confirm a species. At the moment the most commonly used resource for this is GenBank and as it is unregulated its reliability is questionable, proposals have been put forward for regulated databases but as yet none exist (Ahlers et al. 2017). In comparison multiple well established human DNA banks exist containing millions of profiles for comparison (National DNA database 2013). Given the urgency surrounding IWT there is a need to see which existing methods in forensic investigation can be applied to wildlife crime cases. It has already been shown that gelatine lifters are effective tools in lifting trace evidence of various surfaces and that DNA can be successfully extracted from the gels (Plaza et al. 2016; Ricci et al. 2007), however so far there is no apparent attempt to use the lifts on animal parts. The amalgamation of attempting to obtain human trace evidence off of animal parts using gelatine lifters seems a natural bridge between current forensic capabilities and progression towards prosecutions related to IWT.

2. Description of the Research Collection of Samples and Medical Information

We will take the following samples from you for this project:

1. A cheek swab to collect cells which can be used to produce your DNA profile
2. Fingerprints and associated fingerprint residue deposited on multiple different animal specimens
3. Fingerprints and associated fingerprint residue deposited directly onto a gelatine lifter for control purposes

All samples will be labelled with a code. Only Alexandra Thomas will have access to the safeguarded database that identifies which volunteer applies to each code. It is necessary to retain this information in the event you need to be invited back for repeat sampling.

3. Financial Compensation/Costs

You will not be paid to participate in this project. Your DNA profile and fingerprints will be used only for research purposes and will not be sold.

4. Potential Benefits of Participating in the Project

You should not expect to personally benefit from this research. The main reason you may want to participate is help the project manager Alexandra Thomas with their MSc thesis.

5. Confidentiality

We will make every attempt to protect your confidentiality and to make sure that your personal identity does not become known. This signed consent form will be stored in a locked file that will be accessible only to the project manager (Alexandra Thomas). The DNA samples and fingerprints you provide will be labelled with a coded system stored in a safe guarded database that only the project manager will have access to. Only your name and email will be stored in this database. Your DNA profiles will not be used for anything other than comparison against profiles created from the fingerprinting touch samples, they will not be entered into any public database or sold to a third party.

6. Project Results

If you are interested in the results you can request a copy of the final report by selecting the option below. If research from this project is published in professional journals, there will be no traditionally used identifying information, such as your name, address or telephone number included in the publications.

7. Alternatives to Participating in the Project

The alternative option is not to participate.

8. Voluntary Participation

The choice to participate in this research by providing fingerprints and a cheek swab is completely up to you.

9. Withdrawal from the Project

If you would like to withdraw from this project you can contact Alexandra Thomas and she will destroy any samples of yours that have been obtained for the study.

If you have any questions about the project, about your rights as a research participant, or about any research-related injury, please contact Alexandra Thomas s080156@my.ed.ac.uk .
By agreeing to participate in this research, you must agree to ALL of the following statements:

- I voluntarily agree to donate a cheek swab DNA sample from which a profile will be created to be used for this research project.
- I voluntarily agree to provide fingerprints on multiple animal specimens with the understanding they will be lifted using gelatine lifters and an attempt to extract DNA from them will go ahead

- I voluntarily agree to allow my DNA profile to be used for comparison against samples extracted from the aforementioned fingerprints

Please sign your name here if you agree with the above statements.

Your signature: _____

Date: _____

☐ Please tick here if you would like to receive a copy of the final report

APPENDIX II – ANONYMISED SAMPLE CODING SYSTEM

Volunteer	Cheek swab	Ivory comb Scanned	Ivory comb not scanned	Feather scanned	Feather not scanned	Crocodile skin bag scanned	Crocodile skin bag not scanned
A	VA0	VA1	VA2	VA3	VA4	VA5	VA6
B	VB0	VB1	VB2	VB3	VB4	VB5	VB6
C	VC0	VC1	VC2	VC3	VC4	VC5	VC6
D	VD0	VD1	VD2	VD3	VD4	VD5	VD6
E	VE0	VE1	VE2	VE3	VE4	VE5	VE6
F	VF0	VF1	VF2	VF3	VF4	VF5	VF6
G	VG0	VG1	VG2	VG3	VG4	VG5	VG6
H	VH0	VH1	VH2	VH3	VH4	VH5	VH6
I	VI0	VI1	VI2	VI3	VI4	VI5	VI6
J	VJ0	VJ1	VJ2	VJ3	VJ4	VJ5	VJ6
K	VK0	VK1	VK2	VK3	VK4	VK5	VK6
L	VL0	VL1	VL2	VL3	VL4	VL5	VL6
M	VM0	VM1	VM2	VM3	VM4	VM5	VM6
N	VN0	VN1	VN2	VN3	VN4	VN5	VN6
O	VO0	VO1	VO2	VO3	VO4	VO5	VO6
P	VP0	VP1	VP2	VP3	VP4	VP5	VP6
Q	VQ0	VQ1	VQ2	VQ3	VQ4	VQ5	VQ6
R	VR0	VR1	VR2	VR3	VR4	VR5	VR6
S	VS0	VS1	VS2	VS3	VS4	VS5	VS6
T	VT0	VT1	VT2	VT3	VT4	VT5	VT6
CONTROLS	VZ0	VZ1	VZ2	VZ3	VZ4	VZ5	VZ6
AUTHOR DNA SAMPLE	CO						

VOLUNTEER CHEEK SWABS

		Cheek Swabs	
Volunteer	**M/F**	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl
A	F	0	6126.182141
B	M	8512.516757	10636.33623
C	F	0	10033.02986
D	M	13220.66141	13681.5805
E	F	0	5330.330932
F	F	0	4789.782772
G	F	0	6714.926441
H	F	0	4247.531461
I	F	0.548245029	6723.57892
J	M	5403.093727	4675.950786
K	F	0.222436403	9967.818927
L	M	4114.531556	4170.786633
M	F	0.360529409	10272.00403
N	F	0.509238099	3485.906724
O	F	0	6117.74545
P	M	22549.27655	18845.33499
Q	F	0	4803.058986
R	M	8609.203469	11688.43627
S	F	0	2853.523886
T	M	2455.556991	2518.741107

VOLUNTEER FINGERPRINTS COLLECTED FROM IVORY

		Ivory Comb Scanned		Ivory Comb Not Scanned	
Volunteer	**M/F**	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl
A	F	0	0	0	0.584318068
B	M	0	0	0.883281415	1.028831953
C	F	0	0.285589923	0	0
D	M	0.253310888	0.61477439	0	0.121220853
E	F	0	0	0.292371211	0
F	F	0	0.291718976	0	0.464713837
G	F	0	0.294162651	0.18781699	0.23430552
H	F	0.1325519	0.336931168	0	0.819482026
I	F	0	0.299338503	0	0.212791886
J	M	0	0	0.445065468	0.469852108
K	F	0.194597673	0.489492545	0	0
L	M	14.75518873	0	0	0
M	F	0	0	0	0
N	F	0	0	0.283055249	0.319269429
O	F	0	0.238971231	0	0.281504427
P	M	0	0	0	0.547425737
Q	F	0	0	0.22634287	0
R	M	0	0.228774699	0.369595606	0.753609307
S	F	0	0	0	0
T	M	0	0.503994803	0.268597463	0

VOLUNTEER FINGERPRINTS COLLECTED FROM FEATHER

		Feather Scanned		Feather Not Scanned	
Volunteer	**M/F**	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl
A	F	0	0.153688191	0.445398912	0.1667068
B	M	0	0.859413357	0.377678904	1.916054512
C	F	0	0	0	0.154675925
D	M	0.782895158	0	12.3583997	0
E	F	0	0	0	0.38075526
F	F	0	0	0.347308911	0
G	F	0	0	0	0
H	F	0	0	0.690388272	0.359954798
I	F	0.153373248	0	0.387438753	0
J	M	0.461932156	0.382636224	0	0
K	F	0.121131349	0.173838019	0	0.348092612
L	M	0	0	1.232466731	0.339118827
M	F	0	0	0.279088321	0.148696709
N	F	0	0	0	0.103564958
O	F	0	0	0.457325279	0.335958457
P	M	0	0.25771779	0.197228657	0.337951036
Q	F	0	0.189308327	0	0.405745874
R	M	0.573278007	0.341298299	0.442163962	0.41118056
S	F	0	0	0	0.308960299
T	M	0	0	0.979607785	0.127882799

VOLUNTEER FINGERPRINTS COLLECTED FROM CROCODILE SKIN

		Crocodile Skin Bag Scanned		Crocodile Skin Bag Not Scanned	
Volunteer	**M/F**	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl	Male DNA Concentration pg/µl	Human DNA Concentration pg/µl
A	F	0.212664838	0	0	0
B	M	0	0	0	0.606157792
C	F	0	0.398159293	0	0
D	M	0	0	0	0
E	F	0	0.150336145	0	1.100351924
F	F	0.608282095	0	0.271763548	0.459352128
G	F	0	0.15739767	0	0
H	F	0	0.284223516	0	0.816107121
I	F	0	0.272642788	0	0
J	M	0	0	0.220038236	0.198667275
K	F	0	0.273818578	0.213471749	0.246478355
L	M	0	0	0	0.88690919
M	F	0	0	0	0
N	F	0	0.302740471	0	0.105788122
O	F	0	0	0	0.256211637
P	M	0	0.21140804	0.283055714	0.759987688
Q	F	0	0	0	0.187230718
R	M	0	0	0	0.23284549
S	F	0	0	0	0
T	M	0	0.521653337	0.518832129	0

APPENDIX IV – BATCH DETAILS

DNA extractions from samples were carried out in batches and as standard practice a negative was included in each batch, the following table shows which negative is applicable to which batch and the samples included in each of this batches.

Batch	Samples	Negative Sample Code
1	VA0	**NEB0**
	VB0	
	VC0	
	VD0	
	VE0	
	VF0	
	VG0	
	VH0	
	VI0	
	VJ0	
	VK0	
	VL0	
	VM0	
	VN0	
	VO0	
	VP0	
	VQ0	
	VR0	
	VS0	
	VT0	
	Z0	
	CO	
2	VA(1-6)	**NEB1**
	VB(1-6)	
3	VC(1-6)	**NEB2**
	VD(1-6)	
	VE(1-6)	
	VF(1-6)	
4	VG(1-6)	**NEB3**
	VH(1-6)	
	VI(1-6)	
	VJ(1-6)	
5	VK(1-6)	**NBE4**
	VL(1-6)	
	VM(1-6)	
	VN(1-6)	
	VO(1-6)	
	VP(1-6)	
6	VQ(1-6)	**NEB5**
	VR(1-6)	
	VS(1-6)	
	VT(1-6)	
	Z(1-6)	