Standard Operating Protocols to Support Conservation, Health, Welfare & Prosecution of Wildlife Crimes

Part II: Live Wildlife Crime Scene Investigation

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Actions taken at the outset of an investigation at a wildlife crime scene can play a pivotal role in the resolution of a case and successful prosecutions of offenders. Careful, thorough investigation is key to ensure that potential physical evidence is not tainted, lost or destroyed, nor potential witnesses overlooked. Many agencies have limited experience in combatting the illegal wildlife trade and crime scene investigation to support prosecution of wildlife crimes.

Prosecution of alleged perpetrators of wildlife crimes is often unsuccessful or flawed due to multiple challenges including:

- Varying legislations, standardisations and responsibilities
- Lack of knowledge of legal authorities regarding essential biological aspects
- Prosecutors unaware of the legal aspects of wildlife crimes, and the threats they pose to conservation, human and livestock health, international trade, economies and security
- Improper crime scene investigation, handling, storage and transport of samples
- Lack of systematic and comprehensive approaches to CSI, collection, handling, storage, transport of samples and chain of custody
- − Investigators, prosecutors, and laboratories not familiar with wildlife crime, and methods for investigation
- Ancillary investigation techniques not available
- Vague testimony due to limited trained in wildlife crimes for prosecutors and lack of supporting evidence

Acquisition of evidence to support prosecution of wildlife crimes and strengthening of sentencing guidelines requires standardized methods and procedures and a deeper understanding of the multiple threats posed by wildlife crimes. These Standard Operating Protocols have been developed to help agencies investigate and prosecute wildlife crimes.

These Standard Operating Protocols are one method of promoting quality crime scene investigation. The type and scope of a crime scene investigation will vary from case to case. Jurisdictions will want to carefully consider the procedures in this guide and their applicability to local agencies and circumstances.

This guide follows the recommendations articulated similarly as the *Crime Scene Investigation, A Guide for Law Enforcement* (Department of Justice 2000) developed by US representatives of law enforcement, the prosecution, the defence, and forensic scientists. This guide has been adapted to be used during wildlife crime investigation and tailored to frontline officers.

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Introduction

Wildlife trade is defined as the selling or exchange of wild animals or products made from them around the globe. It is legal to trade specific animals domestically within a country and internationally with the appropriate licenses and permits (Department of Agriculture, Water and the Environment 2021; CITES 2019). The illegal wildlife trade (IWT) is defined as all unlawful activities associated with the commercial exploitation and trade of wildlife specimens (living organisms or harvested parts thereof) (t'Sas Rolfes et al 2019).

Illegal wildlife trade (IWT) is the world's fourth largest organised crime industry trailing closely behind narcotics, human and drug trafficking. Due to its illegitimate nature, the significance and revenue generated from the IWT remains poorly understood, however it is estimated at up to USD 23 billion annually. Global illegal wildlife trade poses a major threat to environmental biodiversity, biosecurity and One Health, and directly threatens many animal species. If the IWT remains on its current trajectory then the overexploitation and unsustainable harvesting of wildlife will continue to push extinction of global biodiversity (Tow, Symes & Carrasco 2021).

This guide is intended for use by law enforcement and other responders who have responsibility for detecting illegal wildlife trade and managing crime scenes, preserving physical evidence, and collecting and submitting the evidence for scientific examination. Physical evidence has the potential to play a critical role in the overall investigation and resolution of a suspected illegal and/or criminal act. Realization of this potential depends on actions taken early in the investigation at the scene. Developments in technology and improvements in the analysis and interpretation of physical evidence recovered from crime scenes will place even greater importance on properly documented and preserved evidence. An important factor influencing the ultimate legal significance of this scientific evidence is that investigators follow an objective, thorough, and thoughtful approach. The goal of the investigator is to recognize and preserve physical evidence that will yield reliable information to aid in the investigation.

Investigators should approach the crime scene investigation as if it will be their only opportunity to preserve and recover these physical clues. They should consider other case information or statements from witnesses or suspects carefully in their objective assessment of the scene. Investigations may change course a number of times during such an inquiry and physical clues, initially thought irrelevant, may become crucial to a successful resolution of the case.

Forensic science is quickly becoming a steadfast ally in the fight against IWT worldwide. However, investigations into IWT continue to be unsuccessful due to a lack of understanding, training, support, funding, and improper handling of evidence that is crucial to the conviction of perpetrators. When managed and organised appropriately, forensic science will provide the key to building strong cases that can resist scrutiny in the court of law and bring culprits to justice. As stated by Locard's Exchange Principle, *"Every contact leaves a trace"*; so it is up to us to record, collect and analyse the evidence appropriately (Sanctuary Asia 2021).

References

CITES 2019, 'The CITES species', *CITES*, viewed 14 June 2022 <https://cites.org/eng/disc/species.php>

Department of Agriculture, Water and the Environment. 2021. 'Wildlife trade and the law', *The Australian Government*, viewed 18 June 2022, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8457769/

Sanctuary Asia. 2021. 'Forensic vs. wildlife crime: A battle that must be won', *Sanctuary Nature Foundation*, viewed 14 June 2022, <https://sanctuarynaturefoundation.org/article/forensics-vs.-wildlife-crime%3A-a-battle-that-must-be-won>.

Tow, J., Symes, W., Carrasco, L. 2021. 'Economic value of illegal wildlife trade entering the USA', PLoS ONE, vol. 16, no. 10.

't Sas-Rolfes, M., Challender, D.W., Hinsley, A., Veríssimo, D. and Milner-Gulland, E.J., 2019. Illegal wildlife trade: Scale, processes, and governance. *Annual Review of Environment and Resources*, *44*, pp.201-228.

I. How to Secure and Record Evidence during Wildlife Crime/Trade Investigations?

The aim of this chapter is to outline in detail the chronological steps that must be undertaken to build a strong, legitimate and comprehensive case against a person or an illegal wildlife trade enterprise. This includes the measures required before arriving at a crime scene, how to approach the crime scene and how to accurately record and secure all evidence.

1. Before arriving at the crime scene

1.1 Intelligence gathering

Where possible, the first step of a wildlife crime investigation takes place long before arrival at the scene. Gathering intelligence is vital to determine how the search will take place. Information such as the location, animal species and the approximate number of animals aids in planning and organising resources and equipment required. The majority of successful wildlife crime operations depend on tip offs (approximately 70%) (Anagnostou et al. 2020). It has been shown that intelligence-led investigations are more effective than investigations that are reactive, though in some cases an immediate reactive response by enforcement officers is of course necessary and the only option.

1.1.1 Types of intelligence

The types of intelligence that should be gathered can be simplified into six broad categories known as the 5WH framework: who, what, where, when, why and how (Wildlife Justice Commission 2022). Further details can be found in Box A.

Box A: The 5WH framework of intelligence.

Who? Wildlife crime organisations are made up of a complex network of individuals, including suppliers, couriers, organisers, packers, processors, poachers, farmers, and financiers. However, it is only the poachers and couriers that are most commonly arrested for wildlife trade crimes, as they are the individuals most exposed to law enforcement. The removal of these individuals from the operation has little impact on the functioning of the criminal network, as they can be easily replaced. Ideally the most powerful individuals should be targeted, for example the organisers and financiers, as removal of them will cause the network to collapse (Wildlife Justice Commission 2022).

	Identifying common patterns between different shipments can allow them to be linked to particular crime networks. In some circumstances, controlled deliveries can be used, in which a shipment that is known or suspected to contain illegal wildlife is allowed to be transported under controlled conditions in order to identify the people connected with the shipment and gather evidence against them (UNODC 2019).
What?	Identification of the species that is being traded is important for various reasons, including confirming that the trade of that particular species is in fact illegal.
Where?	Determining where the illegal trade activity is occurring is essential. Analysis of common trade routes should be performed and examine why they have been chosen. Typically a route will be selected not for speed or efficiency, but for evasion of law enforcement efforts. Checkpoints along main transport routes and at ports, airports and key entry points to national parks can aid in prevention of wildlife movement, and provide further information (Wildlife Justice Commission 2022).
Why?	It is clear that a large demand exists for traded wildlife, and therefore there is potential to yield substantial profit, as wildlife trade is the fourth most profitable transboundary crime worldwide (Tow, Symes & Carrasco 2021). Wildlife trade is perceived as a low risk high reward crime, as law enforcement efforts have historically had fewer resources for this form of crime, and consequently, apprehension of high level individuals within wildlife crime networks is rare (Wildlife Justice Commission 2022).
How?	The method of transport, how the wildlife was concealed and what it was packaged with can provide important information about where it has originated and potentially who has been involved (Wildlife Justice Commission 2022).
When?	It is important to gain intelligence about the timing of the illegal activity. When is it predicted to happen, or has it happened already? The timeliness of collection and dissemination of intelligence is vital to the success of the investigation. It is important to find a balance between taking time to gather as much intelligence as possible before commencing a search, whilst still acting quickly to avoid losing evidence or missing the illegal activity altogether. Up to date and timely information is important to inform decisions and maintain momentum in an investigation (Wildlife Justice Commission 2022; Cooper & Cooper 2013).

1.1.2 Sources of intelligence

Intelligence can be obtained from a variety of different sources. A key source of information comes from collaboration with non-government organisations (NGOs). Consultation with local rangers and community members can also be valuable in gaining information. Studies have

found that local rangers who are able to build trust with communities are more likely to be able to access information, as criminal networks often hire local people or require accommodation in the area. Local people may be more likely to report illegal activity if the perpetrator is not a member of their community due to fear of retaliation from their peers, or if they feel their access to their local resources is threatened (Anagnostou et al. 2020). Experts can be consulted, including biologists, naturalists, zoologists and veterinarians, who can provide valuable expertise. Following paper trails, such as correspondence or other documents is another source of useful intelligence (Cooper & Cooper 2013). Covert or undercover operations can be used, but generally require extensive legal authorization and most regions have strict requirements and conditions (UNODC 2019). But they can be useful in cases where other forms of intelligence are limited.

The international criminal police organisation (INTERPOL) is available to provide collaboration, technical support, operational support, and intelligence. They have a central reporting system called Ecomessage, where reports of wildlife crime from national police agencies can be submitted (Cooper & Cooper 2013).

1.1.3 Intelligence analysis and use

Depending on the source, intelligence varies in reliability, so it is necessary to verify then analyse it. Intelligence analysis software exists for this reason. Computer software and machine learning systems are increasingly being used to investigate and analyse illegal wildlife trade online. Intelligence analysis aims to understand the functioning of organised wildlife crime networks (Cooper & Cooper 2013; Di Minin et al. 2019).

The intelligence obtained then needs to be transferred to you in order to plan and execute searches. Various tools exist to facilitate this exchange, including INTERPOL, or the European Union Trade in Wildlife Information Exchange (EU-TWIX), which is a database that assists detection, analysis and monitoring of illegal wildlife activities (UNODC 2019).

Once ready to use the intelligence to begin planning a search, it is vital to first obtain a warrant that is valid in the region. Generally, in order to receive a warrant, there must be reasonable suspicion that illegal activity is occurring, which is demonstrated using intelligence (Nijman et al. 2019; UNODC 2019).

1.2 Legal considerations at crime scenes

Governments must implement institutional arrangements to hold all persons accountable for their actions. This includes those that engage in illegal trafficking of animals, as well as those organisations attempting to seize and secure illegally traded animals (UNODC 2020; OECD 2022).

Legislation is implemented at international, regional, national and local levels. It is largely covered by international treaties and conventions that hold countries accountable to efforts to track live trading across the globe. There are a number of international treaties and conventions that make up the international legal framework for wildlife crime. This includes the:

- Convention on International Trade in Endangered Species (CITES)
- 2000 UN Convention on Transnational Organised Crime (UNTOC)
- 2003 UN Convention Against Corruption (UNCAC). (CITES 2022)

Local levels then have smaller entities such as district and city councils that establish laws, customs and traditions in a particular locality (OECD 2022).

Animals are nevertheless still falling through the cracks. International conventions such as CITES, UNTOC and UNCAC are legally binding, meaning countries that sign must adopt its regulations into its national legal framework. This does not, however, guarantee that the crimes' sanctions are effectively enforced. Each case needs to be caught, investigated and prosecuted for criminals to be charged and for deterrence to be effective. Another issue faced by law enforcement is the issue of bribery and corruption of officers to allow trafficking to occur (Thailand NACC 2018). All this makes illegal wildlife trafficking across regions and international borders a complicated issue to police (OECD 2022).

In order to begin a seizing operation, NGOs work with government departments to gather evidence and build a case against one farm/live trade group, specific shipping routes and containers. A permit is then obtained to allow the right to seize and investigate a known farm/shipment (Cooper & Cooper 2013).

1.3 Preparation

The fundamentals of securing and performing an evidentially successful crime scene investigation relies greatly on proper preparation and rapid preservation of the location (Cooper et al. 2009; FWG 2014). The goal is to maximise the amount of viable evidence collected while disturbing the crime scene as little as possible. Preparation not only involves ensuring you have all the required equipment for securing and sampling evidence but also the right personnel, time of day and information on the potential of the crime (UNODC 2012). Intelligence gathering is a fundamental aspect of preparation prior to arriving at the crime scene to ensure you understand the crime scene you are investigating (Cooper & Cooper 2013). This aspect of preparation has been described previously in this report. Briefing of personnel on the site location, parameters, GPS coordinates and weather should be completed before you arrive on site. This will ensure you are prepared with appropriate equipment for the specific crime scene.

Recruitment of sufficient and qualified personnel that have been thoroughly briefed on the crime is essential for a complete investigation to be carried out. The team should consist of a minimum

of eight people, consisting of a primary investigator, two photographers, two to four searchers and two recorders and measurers. The exact tasks that each officer is responsible for should be defined prior to arriving on scene (Box B) and all team members must report to the primary investigator (Cooper & Cooper 2013).

Box B: A summary of the responsibilities of the crime scene investigation on-site team.

1. Primary Investigator
    a. Delegate roles to team members
    b. Ensure thorough and accurate records are taken
    c. Liaise with media or legal prosecution to provide case updates
2. Photographers
    a. Document the scene as found and contribute to evidence collection
    b. Ensure all photographs are labelled and recorded (further detail below)
3. Searchers
    a. Trace the scene in a specific pattern (discussed below)
    b. Alert photographers, and recorders to potential evidence
4. Recorders and measurers
    a. Create a scene drawing
    b. Record, bag and tag all evidence collected

2. Arrival at the Crime Scene

2.1 Boundaries

Crime scenes in which investigations may occur can vary greatly, depending on the stage of wildlife trade you are intercepting, this poses a challenge to preserving the crime scene (UNODC 2012). Investigation principles should be adapted to the specific environment you are in and a systematic approach to the crime scene investigation should be undertaken regardless of size and location of the scene.

The initial boundaries of the crime scene are established based on the intelligence received on the potential crime taking place. It is advantageous if the crime scene is located indoors as the scene is then contained within physical barriers, which therefore defines the area for your investigation. Outdoor crime scenes pose more difficult challenges, as boundaries of the crime scene must be formed based on your perception of the crime (Cooper 2009). There is always the

possibility to reduce the size of your primary crime scene, however placing boundaries too close to the primary evidence may cause disturbance of critical evidence further out.

Examining a crime scene requires use of the scientific model to perform a systematic evaluation of the scene, collect and preserve physical evidence which may reconstruct the events and identify, and link a suspect to the animals and crime scene for the purpose of solving the crime. This scientific method is based on formulating a hypothesis, testing the hypothesis and then validating or rejecting the hypothesis through deductive and inductive reasoning (Crispino 2008; Bevel 2001). To begin your wildlife crime scene investigation a few questions can be posed by the primary investigator which have been described in Box C.

Box C: Scientific method to follow for wildlife crime scene investigation

1. Has a criminal act been committed here?
2. Is the animal of a protected species?
3. Is there physical evidence present suggestive of potentially illegal activity related to wildlife?
4. Develop a hypothesis based on initial field observations
5. Look for evidence that supports or invalidates the hypothesis
6. Collect and document the evidence for further analysis and for documentation required during a court's judicial proceedings

2.2 Safety precautions

Safety for yourself and your team is of utmost importance in all crime scene investigations but even more so for wildlife as the evidence can be live and dangerous and is biological, sometimes including associated pathogens or chemicals. Safety can sometimes be overlooked when focus is on the investigation itself, increasing team members' susceptibility to potential hazards, so it is important to take time to consider and implement safety precautions. The adverse effects of hazards may not be initially apparent (e.g. delayed onset of symptoms of an infection or toxicity); if they occur, seek medical advice immediately. Hazards can also pertain to the environment and the weather you experience, with extreme weather conditions or rough terrain causing slip and trip hazards (Archer 2003). It is also important to beware of any traps set by the alleged criminals.

The collection and storage of biological evidence must be completed in a safe and methodological way and allow for safe transportation for laboratory personnel and delivery personnel (See sections 2.6.2 and 2.6.3 on labelling and transportation). The correct methods and appropriate Personal Protective Equipment (PPE) for specific hazards are highlighted in the

SOP: *"Live Wildlife Handling and Management for Frontline Law Enforcement Officers to Support Conservation, Health, Welfare and Successful Prosecution of Wildlife Crimes"* and in the UNODC document: *The potential of pathogen exposure from wildlife seizures - Guidance for evaluating and reducing the risks of transmission to frontline enforcement officers* (see Appendix II).

2.3 Securing and protecting the scene

The initial boundary of the crime scene is established based on a preliminary, and thus imperfect, assessment of the potential crime and its setting. The purpose of the boundary is to provide a visual guide to the most important areas to minimise the loss of potential evidence (UNODC 2012). It is important that if the crime scene is in a public area that appropriate boundaries are put in place (barriers and car diversions) to limit onlookers and vehicular traffic for safety reasons, to preserve evidence, and to maintain confidentiality for the investigation (Cooper 2013). A recording of the details of each person wanting to enter the scene should be made by the officer in charge, including of supervisors, management personnel, witnesses and individuals from their own and other agencies.

2.4 Initial walk through and scene search

The scene search can be divided into <u>two</u> main stages: the *initial walk through* and the *formal and systematic search* for evidence.

The initial walk through is designed to further assess the crime scene and evaluate what equipment and personnel will be needed for the thorough search, however no evidence is to be collected at this time apart from initial photographs (Bevel 2001). There are several precautions to consider when completing the initial walk through, which are listed in Box D. Once evaluated, the equipment necessary can be gathered and the borders of the crime scene can be re-established if required.

Box D: Precautions for investigators conducting the initial walk through

1. Take the most direct route, however not the route taken by the perpetrator
2. Enter and exit the crime scene using the same path
3. Take care to not disturb small pieces of evidence on initial walk through
    a. i.e. blood drops, shed hair, scales or feathers, foot or tyre prints
4. Evidence flags can be carried to mark small items for systematic search
5. Do not collect evidence; this is purely for scene evaluation

The formal and systematic scene search is a thorough investigation of the crime scene, where the evidence is recorded and sampled. The primary investigator will lead the search in a methodical fashion, choosing one of the documented search patterns commonly used in crime scene investigations (Box E). It is essential that the scene is not contaminated as this can lead to false hypotheses, unnecessary laboratory work and misleading questions in court (Bevel 2001; Cooper & Cooper 2013).

Box E: Search patterns for crime scene investigations

1. Standard search pattern
    a. Searchers will start in a straight-line side-by-side and walk through the area. They are required to be close enough (within arm's reach) to ensure all areas are searched.
2. Grid search pattern
    a. The crime scene itself is zoned into a grid formation and searchers are allocated each section of the grid to examine.
    b. Allows for better mapping and documentation of the crime scene.
3. Spiral search pattern
    a. Starting from the main evidence (animals) and working outwards or starting from the perimeter and searching inwards.

Time of day is important as natural light allows best visualisation of evidence. Therefore, it is recommended that wildlife crime investigations begin early in the morning if possible, to allow processing and movement of live animals. The needs of the animals must be of top priority when processing the scene to ensure their welfare is upheld. A veterinarian should be on site or on call nearby to attend to any clinical or welfare needs of live animals involved. If weather is an issue, try and examine fragile evidence or place protective shelter until the search can be continued. Re-searching the area after the sun has changed direction can allow investigators to have a different perspective and identify further evidence. In many cases, a good search will produce more items than are needed to prosecute the case (Cooper & Cooper 2013).

Items that are potentially relevant evidence to the case should be collected and recorded. The relevance of an item, if disputed or unsure, should be determined by the primary investigator and if an item is disregarded, this should be recorded with reasoning. It is important to remember that evidence not collected at this time is potentially evidence lost (Blom-Cooper 2006).

2.5 Recording the evidence

Recording of evidence in the field should follow a pre-planned set of standard operating procedures (SOPs) to ensure all information is recorded correctly. The steps to be taken after arrival at a wildlife crime scene to ensure accurate and durable records are collected are described in Box F.

Box F: Steps to photograph and record evidence (Cooper & Cooper 2013)

1. Take note of the date, time, name of the officer and relevant weather, and take a photo of this. This marks the start of the photography series for this location.
2. Photo series 1: complete series of images of the scene as first perceived.
3. Stop and wait for lead investigator to process the scene eg., set evidence flags and placards
4. Photo series 2: photographs of the actual evidence. This includes 2 photographs for every piece of evidence; one close-up including the numbered placard, and a second close-up of the evidence itself alone.
5. Images are then to be filed and archived, so that the files cannot be overwritten. DO NOT DELETE ANY IMAGES, because the numbering system must have continuity for court proceedings.
6. Create a duplicate of all photos where any and all alterations to the image can be made. The original of each image cannot be altered.
7. If possible, mapping of the crime scene should be done to provide a guide as to where everything was found. This can include any and all of the following: panoramic image of the entire scene, sketch or diagram of the crime scene.

2.5.1 Note taking

Taking notes is a crucial component to building a case during a crime scene investigation. Notes should be taken from the moment you step foot on the crime scene and can be handwritten or typed on a mobile device, tablet or computer. If live animals are involved it is of particular importance to pay attention to the five freedoms of animal welfare, listed in Box G. Notebooks for written information are essential. These can be supplemented with a voice recorder for dictated notes or sounds of interest.

Box G: The five freedoms of animal welfare (Cooper & Cooper 2013)

1. Freedom from hunger and thirst
2. Freedom from discomfort
3. Freedom from pain, injury or disease
4. Freedom to express normal behaviour
5. Freedom from fear and distress

Specific software that are accessible on tablet and smartphone can facilitate data capturing, an example being Epicollect. One advantage of collecting data digitally is that it significantly increases the sensitivity, specificity, timeliness, and representativeness of the data collected and facilitates organization, use and analysis. It allows for data collected on a previous day to be transferred and stored on a larger device, and recalled with ease when needed. Cloud technology has enabled data to also be accessed on multiple devices or by multiple people at a time. The challenges to consider are the reliance of this type of data collection on network availability and battery time of each device; however this can be aided by the use of portable generators and internet toggles (Cooper & Cooper 2013).

2.5.2 Photography

Photographing evidence is another important aspect of crime scene investigation for documentation of evidence to assist with reconstruction and explanation of events. It is imperative that photographs be taken before any interaction with the crime scene area. Photography is best taken in digital form and run through software such as Adobe Photoshop or GIMP to ensure adequate quality. These software have the ability to, for example, adjust contrast and colour on an image in the instance of poor visibility/sunlight at the scene (Cooper & Cooper 2013). Not only is photography a vital component of recording evidence, it is also readily accessible and feasible for teams who suffer financial constraints or restrictions on the number of personnel. As a supplement to photography and observation note taking, video recording is another valuable tool for collecting information at a crime scene. Guidelines for wildlife crime scene photography are detailed in Box H below.

Box H: Wildlife crime scene photography guidelines (Cooper & Cooper 2013).

1. All photographs should be taken by someone trained in forensic photography or someone competent with photographic techniques and camera handling
2. The camera is able to have flash activated or deactivated when needed
3. All photographs are stamped with the accurate date and time

4. Make sure that the photographer has access to a Crime Scene Photography Kit, complete with normal lens, wide-angle lens, close-up lens, filters, tripod, extra recording media, extra batteries, rulers of different colours including an 18% grey ruler for post-processing colour collection, flashlights, location placards, and evidence flags to be placed in the field of view
5. Covering of evidence or camera lens in the case of extreme heat, sunlight, rain or water.

2.5.3 Database

Every individual piece of evidence must be identified at a crime scene via label, and recorded into a database. Records should include:

- a number for identification
- where the evidence came from (i.e. country/region/farm)
- its location at the crime scene
- where it is being sent and when (i.e. live animals are going to be moved, so where they are being moved to and when must be recorded; samples (e.g., blood, swab, scales, feather etc.) may go to a laboratory for analysis or to a police station for storage so where and when they are moved must be tracked)
- any future processing the sample requires (e.g. diagnostic lab, off site examination centre, post-mortem, etc.).
-

Furthermore, each piece of evidence must be clearly labelled with any storage instructions that are crucial to maintaining its integrity (i.e. temperature range, no sunlight exposure, etc.) (Cooper & Cooper 2013).

2.6 Gathering the Evidence

2.6.1 Health and safety precautions

Accurate species identification can **only** be achieved through DNA testing of samples from confiscated animals. During wildlife crime investigations, a variety of biological samples including from live animals, dead animals and the environment should be secured for use as evidence (see Section 3 on collecting samples for DNA evidence and Appendix 1 for collection of additional samples for pathogen analyses). Before collecting any samples, health and safety precautions must be considered to prevent contamination of evidence and to protect personnel from hazards (see Box I).

Box I: Health and safety precautions to follow during sample collection to minimise hazards and sample contamination (Cooper & Cooper 2013; FWG 2014).

1. Always wear appropriate Personal Protective Equipment (e.g. disposable gloves and facemask) when handling samples. One person should handle the samples and remain "clean" (i.e. not touch non-sterilised equipment) while another person labels and records the evidence.
2. Do not eat, smoke, drink or touch your face when handling samples, or until your hands have been washed after handling samples.
3. Use disposable single-use items and equipment when possible for sample collection.
4. For non-disposable equipment, ensure it has been sterilised prior to use. Equipment used to collect DNA must be cleaned with bleach prior to sample collection to remove any prior trace of DNA.
5. Handle and store sharp items carefully, and dispose of any single-use sharps in a biohazard sharps container.
6. Store individual samples in separate containers, even if they have been taken from the same source.

2.6.2 Identification and labelling

Each piece of evidence should be appropriately identified and recorded for tracking.

Identification of live animals can be achieved by tagging ears, flippers or wings, banding birds or implanting microchips in any species. Microchipping is the preferred method for permanent and safe identification. The microchip is a radio-frequency identification device (RFID) with a unique 15-digit identification number (Cooper & Cooper 2013). The microchip is implanted under the skin using a wide gauge hypodermic needle and read with a hand-held reader (Cooper & Cooper 2013). Passive Integrated Transponder (PIT) tags can be used similarly, having have an internal microchip that is activated when it passes close to a special antenna.

Correct labelling of evidentiary samples, including samples collected from live or dead animals and the crime scene is of crucial importance. In order to identify the contents, A unique identifier must be inscribed at the packaging at a prominent place with a permanent marker. Avoid, e.g., placing the identifier solely on the lid of a container since once the lid is opened, the contents of the container is not identifiable.

Labels should include the following (Palmbach 2016):

- investigation or case number
- Date and time of seizure/collection
- Location
- Description of sample
- Identification number (i.e. microchip number) of animal sampled
- Seizing officer's initials/signature

Each sample should be placed in its own collection device (described in Table 2, on page 47), and then placed in a transparent plastic bag. A label should be included within the bag that can be easily viewed when the bag is sealed. The plastic bag must be sealed (with tamper-proof ties or tape) and it is recommended that the collector write initials, date and time over the seal. "Tamper-indicating" tape is also available which tears or shreds when removal is attempted. The outside of the bag should be labelled with the same identification details (Cooper & Cooper 2007). Labels must not be easily removed, or removal should show obvious damage in order to prevent tampering of evidence. However, labels should not damage the evidence itself. Waterproof, unerasable ink should be used on commercially available freezer-proof labels. It is recommended to photograph the final package with seal and labelling visible. If the package has to be reopened, avoid, whenever possible, destroying the original seal and open the package from another location. Upon resealing, the seal should be labelled in the same manner as mentioned above.

2.6.3 Transportation and storage

Whenever possible, transport should be done by the authorities. Private persons (e.g. members of NGOs) should never be assigned to transporting or storing any evidence since this may lead to concerns regarding the chain of custody (see below) and hence the admissibility of the respective item in court.

After collection, biological evidence samples should be promptly sent to a laboratory for forensic investigations. Fresh samples should be transported refrigerated at 4^{o}C and can be refrigerated for up to 7 days; thereafter frozen. Samples intended to be used for DNA analysis should be fixed in ethanol, not formalin (Cooper & Cooper 2013). If the collecting team is in doubt about appropriate methods for sample collection, transport or storage for specific testing, they should consult with the respective laboratory.

3. Chapter References

Anagnostou, M, Mwedde, G, Roe, D, Smith, R.J, Travers, H & Baker, J. 2020. 'Ranger perceptions of the role of local communities in providing actionable information on wildlife crime', *Conservation Science and Practice*, vol. 2, no. 6, pp. 202.

Archer, M.S. 2004. 'Rainfall and temperature effects on the decomposition rate of exposed neonatal remains', *Science & Justice,* vol. 44, pp. 35–41.

Bevel, T. 2001. 'Applying the scientific method to crime scene reconstruction', *Journal of Forensic Identification,* vol. 51, no. 2, pp. 150–162.

Bezerra-Santos, M.A, Mendoza-Roldan, J.A, Thompson, A, Dantas-Torres, F & Otranto, D 2021, 'Illegal wildlife trade: A gateway to zoonotic infectious diseases', *Trends in Parasitology*, vol. 37, no. 3, pp. 181-184.

Blom-Cooper, L. 2006. 'Experts in the Civil Courts', *Expert Witness Institute*, Oxford University Press, Oxford, UK.

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). 2022. 'How CITES works'*, CITES*, viewed 16 June 2022, <https://cites.org/eng/disc/how.php>.

Cooper, J.E., Cooper, M.E. 2007. 'Introduction to Veterinary and Comparative Forensic Medicine', Blackwell Publishing

Cooper, J.E., Cooper, M.E., Budgen, P. 2009. 'Wildlife crime scene investigation: Techniques, tools and technology', *Endangered Species Research,* vol. 9, pp. 229–238.

Cooper, J.E., Cooper, M.E. 2013. 'Wildlife forensic investigation: principles and practice, *CRC press*.

Crispino, F. 2008. 'Nature and place of crime scene management within forensic sciences', *Science & Justice,* vol. 48, pp. 24–28.

Di Minin, E., Fink, C., Hiippala, T., Tenkanen, H. 2019. 'A framework for investigating illegal wildlife trade on social media with machine learning', *Conservation Biology*, vol. 33, no. 1, pp. 210.

Forensic Working Group (FWG). 2014. 'Wildlife DNA sampling guide', *Partnership for Action against Wildlife Crime* (PAW), viewed 14 June 2022, <https://www.tracenetwork.org/wp-content/uploads/2012/08/Wildlife_DNA_Sampling_Guide_web.pdf>.

Nijman, V., Morcatty, T., Smith, J.H., Atoussi, S., Shepherd, C.R., Siriwat, P., Nekaris, K.A.I., Bergin, D. 2019. 'Illegal wildlife trade–surveying open animal markets and online platforms to understand the poaching of wild cats', *Biodiversity*, vol. 20, no. 1, pp. 58-61.

Organisation for Economic Cooperation and Development (OECD). 2022. 'Legal frameworks to deter and combat the illegal wildlife trade in Southeast Asia', *OECD*, viewed 16 June 2022, <https://www.oecd-ilibrary.org/sites/bb3eae76-en/index.html?itemId=/content/component/bb3eae76-en>.

Palmbach, T.M. 2016. 'Crime scene investigation and examination: Chain of evidence', in J Payne-James & RW Byard, *Encyclopaedia of Forensic and Legal Medicine*, Elsevier, pp. 679-685.

Thailand NACC. 2018. 'Interview with Thailand National Anti-Corruption Commission', Bangkok, Thailand.

Tow, J., Symes, W., Carrasco, L. 2021. 'Economic value of illegal wildlife trade entering the USA', *PLoS ONE*, vol. 16, no. 10.

United Nations Office on Drugs and Crime (UNODC). 2012. United Nations Publication.

United Nations Office on Drugs and Crime (UNODC) 2020. 'World wildlife crime report: Trafficking in protected species', *United Nations*, viewed 15 June 2022, <https://www.unodc.org/documents/data-and-analysis/wildlife/2020/World_Wildlife_Report_2020_9July.pdf>.

United Nations Office on Drugs and Crime (UNODC). 2019. 'Investigation measures and detection methods', *Criminal Justice Responses to Wildlife Trafficking*, viewed 15 June 2022, <https://www.unodc.org/e4j/en/wildlife-crime/module-3/key-issues/investigation-measures-and-detection-methods.html>.

Wildlife Justice Commission. 2022. 'Intelligence analysis', *Wildlife Justice Commission*, viewed 14 June 2022, <https://wildlifejustice.org/intelligence-analysis/>.

II. How to identify species during wildlife crime/trade investigations?

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international agreement between governments, aiming to protect wild species, limiting and monitoring their international trade. More than 37,000 species of wild animals and plants are listed and protected against trade by CITES (CITES 2019). Animals confiscated from illegal trade are often mis-identified. Recording the correct taxa traded is of vital importance for accurate trade data and for criminal prosecution efforts. Identification of the species is the first step to regulating trade of species in accordance with CITES. Identifying species in trade is even more difficult than in the wild, due to the often unknown origin location, associated habitat, and behaviour cues usually apparent in healthy, wild species (Linarce 2021). Adequate training of management authorities and law enforcement officers in identification is thus essential. Little has been written on identification of species in the illegal wildlife trade, with limited protocols in place for inspectors to follow. A standardized methodology to facilitate identification is needed to improve consistency and accuracy (Department of Agriculture, Water and the Environment 2021).

This chapter suggests chronological steps to be taken to help identify live species in wildlife crime/trade investigations; obtaining shipping details/history, differentiating animals by class (fish, amphibians, reptiles, bird and mammals), differentiating species within each class. These steps use a combination of a dichotomous key, visual identification, and DNA analysis to differentiate animal species. Methods of DNA testing will be compared and evaluated, accreditation requirements for 'experts' will be detailed and recommendations for the wildlife crime investigation protocols will be made.

1. Movement details/history

Movement details have to be looked at closely. Knowing the point of origin can help narrow down the list of species to those that are native to the original country or continent. There are often records which show types of species that are more commonly trafficked from certain countries or continents. The destination can also be just as important as the point of origin, in helping trace trade routes. Correlations can often be made between a wildlife species country of origin and destination countries where there is a high demand for that animal.

For shipments, the bill of lading is a legal document which shows the place of origin for a shipment, its route and its destination as well as the name of the sender (Zavagli 2021). Unfortunately, due to the illegal nature of wildlife trafficking, documents such as the bill of lading and certificates of origin are often forged or tampered with (Zavagli 2021), making it difficult to determine the shipping route and place of origin. In cases where the point of origin of a shipment

is known, there can still be discrepancies regarding the animals' source location due to limited wildlife trade enforcement and poor border security in lower-resource settings. Smuggling and illegal trade often occur across multiple international borders. Uganda is an example of a significant international exporter of live wildlife which often originate from other countries (Runhovde 2017). Thus, while shipping details accompanying trafficked wildlife may provide useful information in some situations and to investigate crimes, they cannot be wholly relied upon to assist with species identification.

2. Differentiation between Taxa

Correctly identifying species is crucial when investigating wildlife trafficking and determining the severity of the crime. This can prove to be a difficult task given the many species that exist worldwide. When beginning the process of identification, it helps to be aware of taxonomic classifications. All animals are ranked based on their genetic features in a hierarchical system, with the ranks being kingdom, phylum, class, order, family, genus and species (Dewey 2016). We suggest first determining which class the animal is in as this will narrow down the species options and direct the investigation towards the correct path.

There are 5 important classes of vertebrates which we discuss in this report: fish, birds, amphibians, reptiles and mammals. Each of these classes have their own specific physical features to record in order to identify the exact species. Choosing which class an animal is part of is a crucial first step. Sometimes this might seem challenging e.g., at first glance, a salamander from the amphibian class may be confused with a lizard from the reptile class. However, if prompts are provided for closer inspection, the class can be easily identified e.g., reptiles have scales with dry skin while amphibians have moist skin without scales (Zug 2013).

The use of a dichotomous key can assist inspectors in this initial process as they are easy to understand and follow. An example of a dichotomous key that would be useful in this situation is provided in Figure 1. Once the animal species has been narrowed down to a class, the inspector can follow the guidelines provided in this report below for that specific class to ensure they have recorded every defining feature.

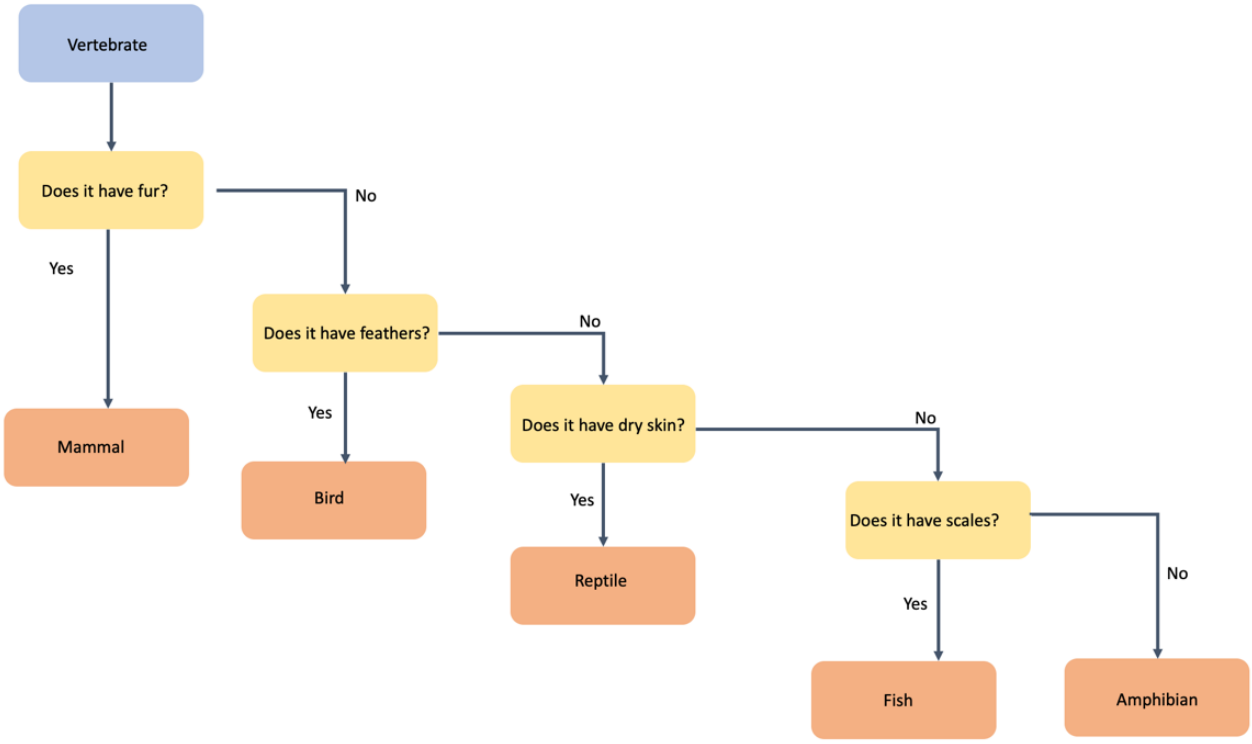


Figure 1. *A dichotomous key for differentiating between animal classes.*

NB: that some exceptions do occur, e.g. the pangolin is a mammal with scales.

3. Fish identification

When inspecting fish for import it is important to accurately determine which species are present. There are over 28,000 species of fish, 154 of which are protected by CITES and therefore subject to regulations under the Wildlife Protection (Regulation of Exports and Imports) Act 1982 (Weitzman et al. 2021; CITES 2019; Halstead 1994). Identification based on gross morphological features is the routine method for classification of different fish species (Department of Agriculture, Fisheries and Forestry 2012; Panprommin et al 2019; Shan et al 2021; Steinke et al 2009). The instructional steps below outline how to correctly photograph and record details of live specimens for identification.

Step 1: Records

- Record import permit, sender and recipient details.

- Record parcel ID and/or barcode.

- Record the current date, weight of parcel and any other identifying features.

- Inspect animals in 3 10x10cm windows. Note number of live fish, deceased fish and any other specimens. Use these numbers to predict number of animals in the entire tank.

- This information will accompany any evidence collected and is essential for traceability.

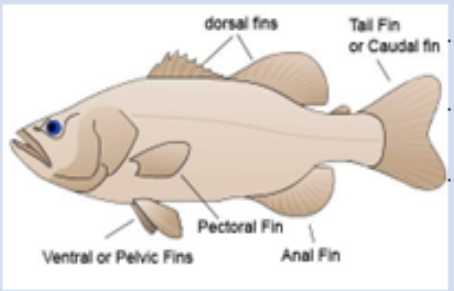
Step 2: Body

- Photograph fish from the lateral, dorsal and ventral aspect.

- Ensure the entire fish from snout to tail, including fins is in the frame.

- The fish should be straight and parallel to a ruler to indicate scale.

Step 3:



- Record number and location of fins.
- Photograph each set of fins.
- Ensure shape, fin spines, fin rays and pelvic fin claspers are apparent.

- Make note of presence or absence of adipose fin (this will be located behind the dorsal fins and there will be no exoskeleton, dermal skeleton or musculature).

Step 4:

- Take a photograph of gills.
- Gills are usually located on the lateral surface, however for some rays and sawfish may be on the ventral surface.
- If the gills are covered by skin or operculum gently fold this back.
- If not obvious from the photo record the number of gill slits or arches.

Step 5:

- Photograph the head of the fish ensuring the eyes are included.
- Photograph the dentition of the fish by opening the mouth.
- Photographs should be taken of the side and front profile.
- A ruler may be included f the lenght of the head and snout cannot be determined from the photographs of the whole fish.

(Bray 2015 ; Weitzman et al 2021 ; Branson et al 1967 ; Stewart et al 2014)

Despite wide use, studies have found that visual identification of fish species has low accuracy. Similarities between species of fish and variable morphology at different life stages can make correct identification difficult even for expert taxonomists (Department of Agriculture, Fisheries and Forestry 2012; Panprommin et al. 2019; Shan et al. 2021; Steinke et al. 2009). As such, a secondary method of species identification should be used to support the morphological classification.

Fish release DNA into their environment through tissues and secretions, this is called eDNA. Ideally, a sample of water should be taken to isolate eDNA to confirm the classification of species. eDNA sampling has high accuracy, can be standardised and is particularly useful when there are many fish in the one container of water, or when juvenile life cycle stages are involved (Panprommin et al. 2019; Shan et al. 2021; Steinke et al. 2009). Detailed steps for collecting a sample of eDNA in water can be found below.

Caution must be taken when preserving the eDNA sample for transport to prevent degradation. Addition of buffers followed by freezing is the most commonly used method for eDNA storage, however one study showed that samples preserved with lysis buffers can last for 2 weeks at room temperature (Renshaw et al. 2015).

Step 1: Water sample

- All PPE and equiptment must be clean and care must be taken to prevent contamination of the sample. The sterile gloves and specimen jars provided must be used for all eDNA sampling.

- Wearing gloves, scoop between 20mls and 2L into the sterile specimen jar.

- Larger volumes of water allow for a higher yeild of DNA, but fish welfare must not be compromised.

Step 2: Filtration

- Water should be passed directly from the sterile specimen jar, through the filtration device, into a sterile transport jar.

- The filter typically used is a glass fibre mesh with 0.45um gaps.

- In cases of high density housing, the glass fibre mesh should be swapped for a mixed cellulose acetate and nitrate filter.

- Care needs to be taken to prevent contamination of the sample at all times.

Step 3: Transport

- The lysis buffers CTAB and Longmire's must be immediately added to the filtered sample.

- The sample can then be placed into the liquid nitrogen transport container to freeze.

- The container should be labeled, classified as time sensitive, and sent off within the day.

- If a liquid nitrogen transport container is not available, the sample may be placed on ice, but must be classified as urgent.

(Artamonova et al 2018; Caraguel 2021; Dutrudi et al 2019; Hoboken 2015; Shan et al 2021)

Another method for the identification of fish species is DNA barcoding (Dutrudi et al. 2019; Steinke et al. 2009). This method may be employed in cases of uncertainty, or when more evidence may be required for prosecution (Halstead 1994; Steinke et al. 2009). Sample recovery for DNA barcoding is invasive and requires anaesthetisation of the fish. As such, it must normally be performed by a veterinarian who is licenced to practice in the relevant country. The veterinarian or trained professional performing the sampling should be competent in fish anaesthesia and fin clip sampling (Hoboken 2015).

4. Amphibian Identification

The Class Amphibia consists of over 6000 species, with the largest order Anura (frogs and toads) comprising over 5000 species. The two other orders are Caudata (salamanders, newts, sirens) and Gymnophiona (caecilians). They each vary greatly in body shape, presence of legs, colouring and pupil shape. Caecilians have no limbs and are more worm-like in appearance whereas salamanders closely resemble lizards in their shape except without scales. High quality photographs are key in identifying the many species of amphibians and helpful instructions to achieve this are outlined in Step 1 below. A checklist has also been provided to help the inspector include specific identifying features as well as key measurements.

Appropriate personal protective equipment (PPE) including face masks, nitrile gloves and safety glasses should be worn when handling amphibians as some species are poisonous. Gloved hands should be moistened with water prior to handling.

DNA samples are ideal for officially identifying a species. The historical method of toe clipping is not recommended as it is painful and incredibly stressful for the animal. A less invasive approach equally sufficient in DNA yield, is a buccal or skin swab. Buccal swabs are best processed when fresh, however similar DNA yields can be obtained when samples are frozen for storage and transportation (Pidancier et al. 2003). Recent studies have shown that skin swabs from both adult and larval life stages provide ample DNA for species identification (Pichlmüller, Straub & Helfer 2013). This approach is less invasive and requires less handling of the animals.

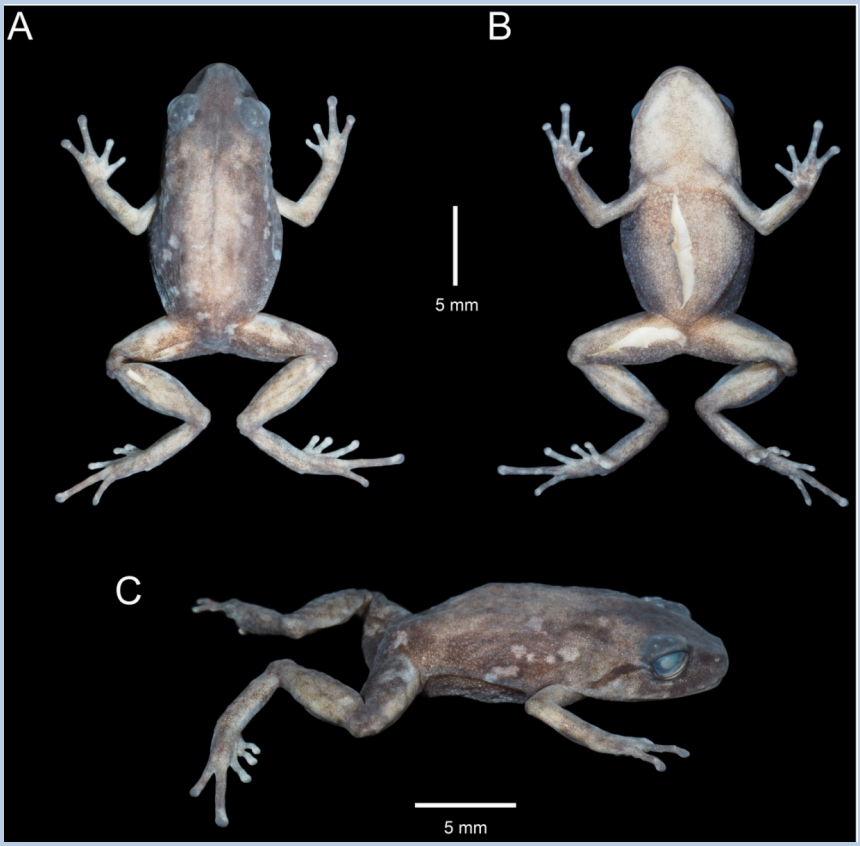
Step 1: Photographs

Take the following photographs of the animal. Include a ruler in the images where possible as well as the identification number for the animal.

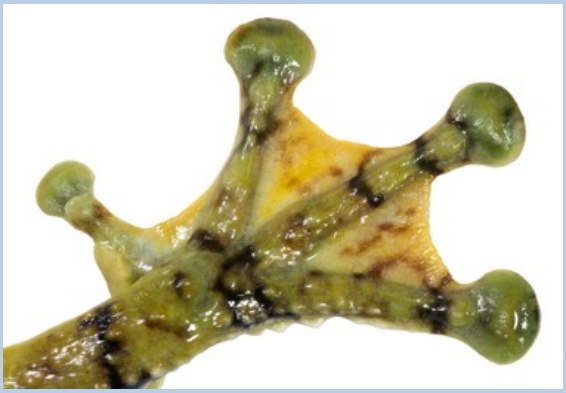
-Lateral aspect of head 

Step 1: Photographs continued

-Dorsal (A), ventral (B) and lateral (C) aspects



- Front and back feet



Step 2: Fill out the following form

Note: Some features are not present in all amphibian species

Pads on digits
☐Absent
☐Present
☐Wider than digits

Parotid glands
☐Absent/indistinct
☐Present

Metatarsal tubercle
☐Absent
☐Present
☐Pigmented
☐Unpigmented

Webbed Toes
☐No/Only slightly
☐Yes

Pupil Shape
☐Horizontal
☐Vertical
☐Cross-shaped

Tympanum (ear)
☐Distinct
☐Not distinct

Dorso-lateral fold
☐Yes
☐No

Back
☐Warty
☐Smooth

Snout-Vent length
___

Toe count
___ (Forelimb)
___ (Hindlimb)

(Michael, D *et al*, 2010, Amphibian Research Centre, 2013)

Step 3: Take buccal and skin swabs for DNA analysis

5. Reptile Identification

The reptilian class comprises over 8000 species in the orders Squamata (lizards, snakes), Testudines (turtles), Crocodilia (crocodiles, alligators) and Tuataras (lizard-like reptiles endemic to New Zealand). Characteristic features such as pupil shape, tongue colour and scale shape, colour and pattern can aid in distinguishing one species from another. High quality photographs are helpful for capturing different colours and body shapes, as outlined in Step 1. An example of a systematic checklist has also been provided in Step 2 for an inspector to use which involves scale counts and body descriptions. Figure 2 will be a useful addition to the checklist to assist inspectors with reptilian anatomical terms and Figure 3 is helpful for dorsal scale counts.

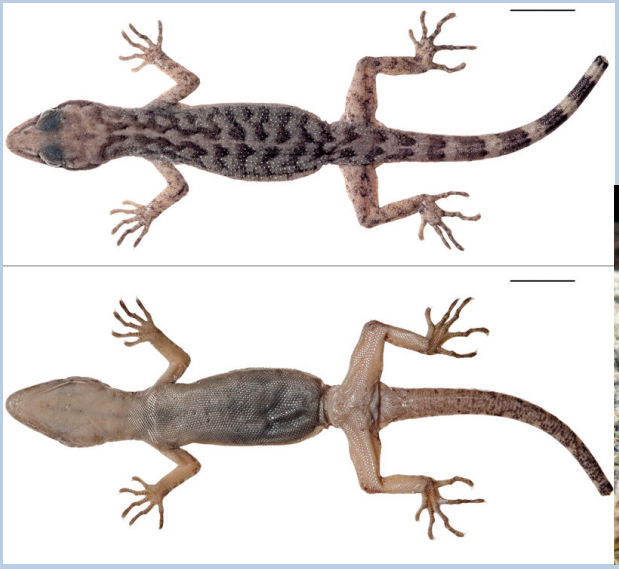
It is important to note that some reptiles may be aggressive and/or venomous and pose a threat to the safety of staff. Appropriate personal protective equipment (PPE) including face masks, nitrile or gauntlet gloves and safety glasses should be worn. A credited snake handler should be responsible for handling of venomous snakes.

DNA analysis is crucial for confirming species identification. DNA samples can be taken by swabbing the buccal cavity and cloaca. Previously tissue samples were taken by severing the tip of a toe or tail, however buccal and cloacal swabs are far less invasive and stressful for the animal while providing a more than satisfactory DNA yield (Miller 2006; Schulte, Ulrich et al. 2011). Any faeces or shed skin from the animal will also harbour DNA that can be used to identify the species (Horreo et al. 2015; Pearson et al. 2015).

Step 1: Photographs

Take the following photographs of the animal. Include a ruler in the images where possible as well as the identification number for the animal.

-Dorsal, ventral and lateral aspects

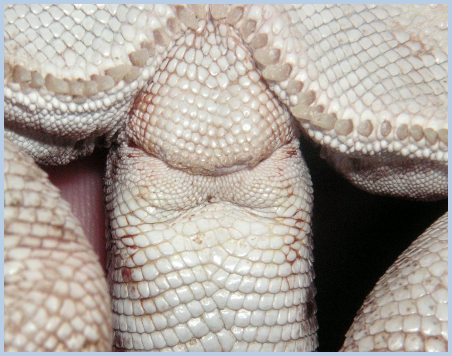


- Head-on view with mouth closed (left image) and with mouth open showing tongue and mouth lining (right image)

Step 1 (continued)

-Underside of hindfoot (if legs present).

-Close-up of vent



Step 2: Fill out the following form

Note: Some features are not present in all reptilian species

Head shape
☐Rounded
☐Wedge
☐Square
☐Short
☐Elongate
☐Pointed
☐Beak-like

Neck
☐Not distinct
☐Somewhat distinct
☐Distinct

Tail Shape
☐Short
☐Medium
☐Long
☐Blunt tip
☐Pointed tip
☐Slender
☐Tapered
☐Rounded

Pupil Shape
☐Round
☐Vertically elliptical
☐Horizontally elliptical

Head Scales:

General character:
☐Large, plate-like scales
☐Small scales, undifferentiated

Rostral Scale Shape:
☐Rounded
☐Large, shield-like
☐Small
☐Large
☐Pointed, projecting
☐Protruding
☐'Leaf' shaped
☐Flattened, shovel-like
☐Elongated, pointing downwards

Nasal Scale
☐Single
☐Semi-divided
☐Divided

Internasal Scales
☐Absent
☐Paired
☐Single

Prefrontal Scales
☐Absent
☐Paired
☐Single

Chin Shields
☐Absent
☐ 1x2
☐ 2x2

Submandibular Groove
☐ Not pronounced/absent
☐ Somewhat pronounced/shallow
☐ Pronounced

Head Scale Counts
___ Preoculars
___ Supraocular
___ Postoculars
___ Suboculars
___ Perioculars
___ Interoculars
___ Anterior Temporals
___ Upper Labials
___ Lower Labials
___ Rows between upper labials and eye

Dorsal Scale Count (mid-body)
___

Cloacal Scale/Anal Plate
☐Single
☐Divided

Ventral Scale Count
___

Snout-Vent Length
___

Shell-Length
___

Fourth Toe Lamellae (Hindfoot)
___

(Macdonald, S, 2019, Hsu, ER et al, 2017)

Step 3: Take buccal and cloacal swabs for DNA analysis

Figure 2. *The variety of head scales present on a reptilian head.*

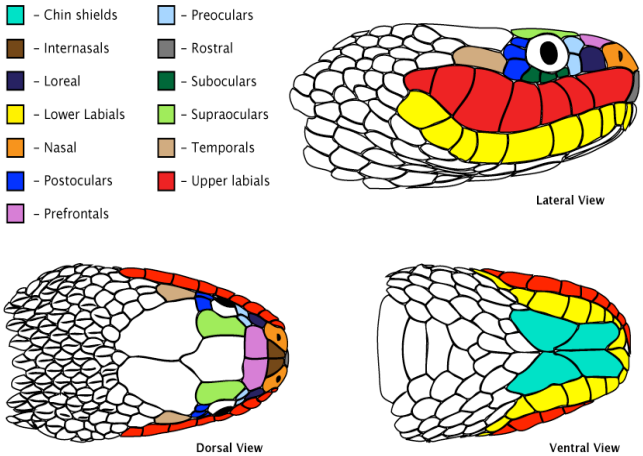
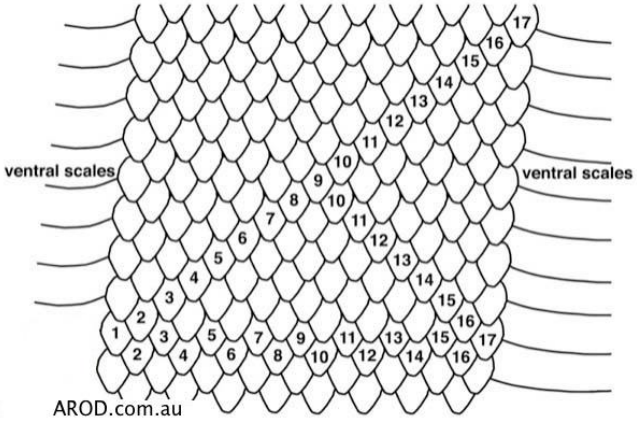


Figure 3. *How to count dorsal scales across the mid-body.*



6. Bird Identification

Over 11,000 different bird species have been identified in the world, with over 1,400 of those threatened with extinction (Del Hoyo, Collar & Bird Life International 2016). Currently, a total of 1,461 bird species are protected against CITES against international trade (CITES 2019). Bird species are best distinguished based on their unique morphological features.

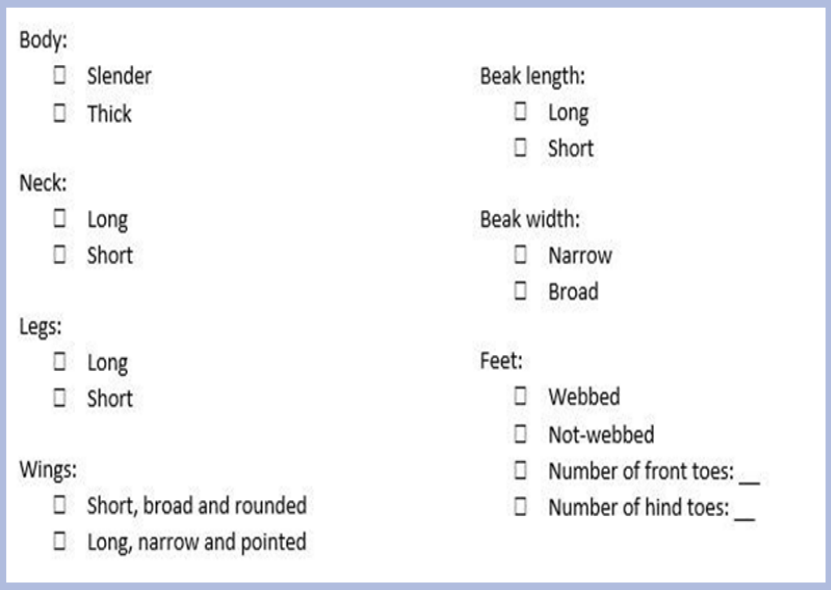
Useful features for experts to identify bird species include colour of plumage, size and shape of the bird, and call or song (Australian Museum 2022; Menkhorst et al. 2017). Most species have distinct plumage or colour pattern; however, it is important to note that lighting conditions or weather can influence appearance of plumage. Body proportions (body: neck: legs: wings) are crucial in narrowing down the identity of a bird. Beak shape and size correlates with feeding habits, and foot structure may help indicate a bird's natural habitat (Menkhorst et al. 2017). Some bird calls are distinctive and instantly recognisable, whilst others sound very similar to the call of other species and thus are less useful for identification (Australian Museum 2022). Outlined below are instructional steps for information to be collected and sent to bird experts to correctly identify the species.

Step 1: Photographs

Photograph the bird in full profile from the side, front and back.

Step 2: Body proportion details

Answers the following questions by ticking the most appropriate box or filling in the blank.



Body:
- ☐ Slender
- ☐ Thick

Neck:
- ☐ Long
- ☐ Short

Legs:
- ☐ Long
- ☐ Short

Wings:
- ☐ Short, broad and rounded
- ☐ Long, narrow and pointed

Beak length:
- ☐ Long
- ☐ Short

Beak width:
- ☐ Narrow
- ☐ Broad

Feet:
- ☐ Webbed
- ☐ Not-webbed
- ☐ Number of front toes: __
- ☐ Number of hind toes: __

Step 3: Call or song

Take an audio recording of the bird's call or song.

(Todd 2010; Gillete 2007; Wear 2006)

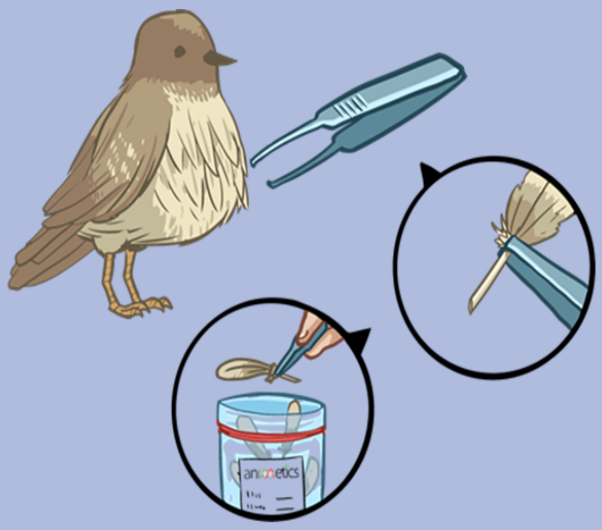
Bird species identification can be difficult, even for experienced bird watchers, therefore a feather sample should be taken for DNA testing. Feathers for DNA testing should be freshly plucked. DNA is contained at the base of the feather, originating just below the skins' surface. Moulted feathers are not recommended because they carry much less usable DNA and the origin of the feather is uncertain (Animal Genetics 2022; Linarce & Tobe 2013). Preferred feather collection sites on the bird are the chest, vent or rump. It is quite easy to pluck feathers from these areas and does not create a pinching sensation on the bird's skin (Animal Genetics 2022; Avian DNA Lab 2020). Feather samples should be taken by a veterinarian or other similarly trained personnel, to ensure safety of the bird and handler. Before feather collection, and between when collecting samples from multiple birds, the handlers' hands should be washed, (International Biosciences [n.d.]). Outlined below are the steps to properly collect a feather sample for DNA testing.

Step 4: Feather sample collection

1. Individually pluck 4 feathers from the bird's chest by pulling towards the beak using sterile tweezers. Pluck close to the skin to prevent feather breakage.

2. Place feathers in a sealed envelope.

3. Label envelope with the bird ID and relevant shipping details.



(Animal genetic services [n.d.])

The feather will be processed in a laboratory for species identification using mtDNA extraction, PCR amplification and DNA sequencing (Linarce & Tobe 2013; Speller, Nicholas et al. 2011). Mitochondrial DNA (mtDNA) can be extracted from the calamus, the blood clot located in the superior umbilicus (Linarce & Tobe 2013) or from the barbs of a feather (Speller, Nicholas & Yang 2011) (see Figure 4). This method requires destruction of 5-10mm of the feather-shaft terminus (Linarce & Tobe 2013). When feather barbs are removed from the distal portion of the feather, complete destruction of the feather is required (Speller et al. 2011). A whole feather should be plucked from the bird to maximise the diagnostic success of laboratory testing.

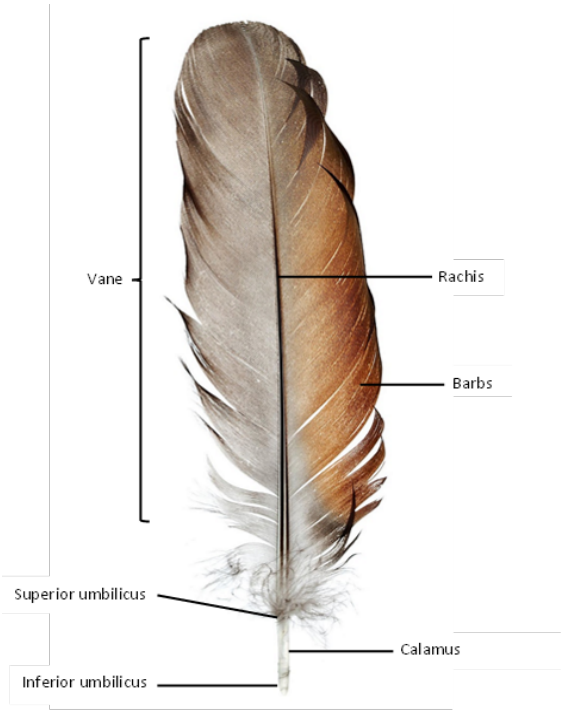


Figure 4. *A feather labelled to show the mtDNA extraction sites; the calamus, the blood clot located in the superior umbilicus and the barbs.* (*Klappenbach 2019)*

7. Mammal Identification

More than 6,496 mammals have been identified on earth (Burgin 2018) many of which are found in the illegal wildlife trade. Due to a vast diversity in mammals; the class can be further broken down into the following groups: ungulates (odd- and even-toed), carnivores, bats, cetacean, primates, elephants, marsupials and monotremes. The most commonly traded mammal is the Pangolin. The pangolin is most popular for the use of its scales in Chinese medicine and also one of the most frequently miss-classified mammals. In order to accurately identify mammal species, the identification process must be done in a systematic and repeatable manner.

Taking appropriate photographs is vital for visual species identification. Images of unique features such as; wingspan and wing shape for bats; head shape; tail length; fur pattern and length etc. are required. All photographs must contain the unique animal ID created for them and preferably a form of scale.

Step 1: Equipment

1. Pen
2. Paper
3. Camera
4. Tweezers
5. Dry sample collection pots
6. Scale
7. Gloves
8. Swabs
9. Purified water

Step 2: Record information

1. Date, time, location
2. Estimated size and weight of animal.
3. Form of containment
4. Create unique animal ID
5. Record animal taxa; use of dicotomous key recommended to avoid errors
6. Note any other details: the more the better

Step 3: Photographs of Mammals

Photograph the mammal in full profile from both sides (left and right), front and back.

Each image must contain the unique ID number created for each case a form of measure.

NOTE: Also photograph close-up of identifying features e.g. scales, claw, tail/horn length, wings



Sample collection from live mammals can be difficult, but is important for accurate identification of species by DNA analysis. Ideal samples for DNA are blood, cheek swabs, hair, faeces, or soft tissue. The least invasive are outlined below. In the case of the pangolin, scales can be used. Samples must be sent to the appropriate laboratory for DNA sequencing.

Step 4: Sample collection for Mammal DNA Analysis

1. Wear gloves
2. Use tweezers to pluck 20 hair with follicles intact. Ensure to handle hair by tip not root.
3. Collect dry blood:
   - Wearing gloves, moisten swab with purified water and rub across dried blood
   - Place in specimen jar, seal and label
4. Collect fresh blood: same as above, however no need to moisten swab
5. Faecal collection: wear gloves, scrape the surface layer (outside of faeces) into specimen jar with a swab.

8. DNA testing: Brief Note

Accurate species identification can only be ensured through DNA testing. Depending on the taxa in question, certain types of DNA testing are preferred. For example, in Diprotodontia, ND2 mitochondrial DNA marker testing yielded better results than Cytochrome B mitochondrial marker or Cytochrome Oxidase I (COI) barcoding marker (Wilson-Wilde 2010). Often during wildlife forensic investigations, DNA samples may not be of the best quality or of sufficient quantity; hence Primer modification techniques, such as LNAs, are an invaluable addition to the process to help amplify the DNA (Wilson-Wilde 2010). If there is an organism for which no information is recorded in current DNA databases e.g. GenBank, Next Generation Sequencing (NGS) can be used to characterize the organism. Ultimately, the DNA testing process used will be very case dependent.

9. Recommendations

Illegal wildlife trade is accelerating extinction of many exotic species and is detrimental to wildlife conservation. Currently, most wildlife seizures are not prosecuted. This can be partially due to dismissal or acquittal as a result of insufficient evidence (UNODC 2020). Species identification plays a critical role in criminal prosecution for wildlife trafficking. However, currently many animals are mis-identified. Identification and classification of species can be a challenge even for qualified experts and staff are often not sufficiently trained in this area (DAFF 2012; Shan et al. 2021; Steinke et al. 2009). A set of strict protocols for law enforcement officers to follow should be formulated and implemented to facilitate easier and consistent identification of species illegally trafficked between countries. Useful tools to aid in species identification, include dichotomous trees, visual identification and DNA testing. Training of staff will further enhance the identification process. If live specimens can be accurately and consistently identified, we are a step closer to the shutting down criminal wildlife trade activities and protecting endangered wildlife species.

Whilst border forces and customs officers are often responsible for illegal wildlife trade surveillance and enforcement, there are often no official related qualifications required. One recommendation is that all frontline staff complete a course in CITES species and ID (Zavagli 2021). Examples of agency-level training include that provided by the Department of Environment and Energy (DoEE) staff in Australia to staff responsible for surveillance of illegal animal imports. The training covers relevant legislation, key skills such as species identification and includes examples of illegal animal trade (DAFF 2012). Frontline staff should have access to experts in the animal field to ensure accurate species identification. This may include veterinarians, taxonomists, ecologists and scientists trained in DNA testing techniques.

DNA testing is the gold standard for species identification. It has significantly lower occurrence of errors than morphological methods (Animal Genetics 2022; Linarce & Tobe 2013). The routine use of DNA testing can assist border control officers in improving the integrity of the live animal trade supply chain and strengthen prosecution of wildlife crime cases (UNODC 2020). Ensuring capacity for collecting quality DNA specimens and securing resources and laboratory capacity to do bar coding is a vital component of wildlife crime law enforcement.

Live imported animals may also pose a risk to personnel through bites, scratches, envenomation and zoonotic or exotic diseases. Frontline staff should have at minimum training to provide an understanding of such hazards and have access to accredited handlers for snakes and other dangerous animals. A list of such experts should be included in the SOPs for enforcement officers and customs / border control (UNODC 2020; Zavagli 2021). See recommendations in associated Standard Operating Protocol: “Live Wildlife Handling and Management for Frontline Law Enforcement Officers to Support Conservation, Health, Welfare and Successful Prosecution of Wildlife Crimes” (2022).

10. Chapter References

Amphibian Research Centre. 2013. Accessed: Jun 18, 2022. >frogs.org.au/frogs<Animal Genetics 2022, Avian DNA Sexing (Feather), viewed 16 June 2022, <https://www.animalgenetics.us/Avian/DNA_Sexing/DNA-Sexing-Feather.asp>.

Animal genetic services [n.d.]. Avian DNA sexing, viewed 17 June 2022, <https://www.animetics.com.au/avian-dna-sexing/#collecting>.

Artamonova, V.S., Kolmakova, O.V., Kirillova, E.A., Makhrov, A.A. 2018. 'Phylogeny of Salmonoid Fishes (Salmonoidei) Based on mtDNA COI Gene Sequences (Barcoding)', Contemporary Problems of Ecology, vol. 11, no. 3, pp. 271–285.

Australian Museum. 2021. 'What bird is that?', viewed June 16 2022, < https://australian.museum/learn/species-identification/ask-an-expert/what-bird-is-that/>

Avian DNA Lab. 2020. 'How to pluck feathers from your bird for DNA Testing purpose?', September 5 2020, available at: https://www.facebook.com/watch/?v=2660573214208653

Bray, D.J. 2015. 'Identifying Fishes, in Bray', D.J. & Gomon, M.F. (eds) Fishes of Australia. Museums Victoria and OzFishNet, accessed 15 June 2022, http://fishesofaustralia.net.au/

Branson, B.A., Ulrikson, G.U. 1967. 'Morphology and histology of the branchial apparatus in percid fishes of the genera Percina, Etheostoma, and Ammocrypta (Percidae: Percinae: Etheostomatini)'. Transactions of the American Microscopical Society, pp.371-389.

Burgin, C.J., Colella, J.P., Kahn, P.L. and Upham, N.S., 2018. How many species of mammals are there?. Journal of Mammalogy, 99(1), pp.1-14.

Caraguel, C. 2021. 'Clinical training in ornamental fish of veterinary students in the school of animal and veterinary sciences', practical notes distributed in the topic 4110 Wildlife and Conservation Practice, University of Adelaide.

CITES 2019, 'The CITES species', CITES, viewed 14 June 2022 <https://cites.org/eng/disc/species.php>

Del Hoyo, J., Collar, N.J. 2016, 'HBW and BirdLife International illustrated checklist of the birds of the world', Bird Life International, Lynx Edicions, Spain.

Department of Agriculture, Fisheries and Forestry. 2012. 'Audit report: an examination of the effectiveness of department of agriculture, fisheries and forestry controls to manage biosecurity risk in the importation of freshwater and marine ornamental fish', Australian Government, no. 2012-13/03

Department of Agriculture, Water and the Environment. 2021. 'Wildlife trade and the law', The Australian Government, viewed 18 June 2022, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8457769/>

Dewey, T 2016, 'Animalia', Biology: A-D, pp. 43–45.

Dutrudi Panprommin, Kanyanat Soontornprasit, Siriluck Tuncharoen, Santiwat Pithakpol, Julatat Keereelang 2019. 'DNA barcodes for the identification of species diversity in fish from Kwan Phayao, Thailand', Journal of Asia-Pacific Biodiversity, vol. 12, no. 3, pp. 382-389

Gillete, H. 2007. 'Anodorhynchus hyacinthinus Disney', image, Wikimedia, viewed 17 June 2022, <https://commons.wikimedia.org/wiki/File:Anodorhynchus_hyacinthinus_-Disney_-Florida-8.jpg>.

Halstead, B. 1994. 'Wildlife legislation in Australia: Trafficking provisions', Australian Institution of Criminology, viewed 15 June 2022, <https://www.aic.gov.au/sites/default/files/2020-05/wildlife-legislation-in-australia.pdf>

Hoboken, N.J. 2015. '25 Wet-Mount Sampling Techniques in Fish' Exotic Animal Hematology and Cytology, John Wiley & Sons, Inc, USA, pp. 373–376.

Horreo, J.L., Peláez, M.L. and Fitze, P.S., 2015. Skin sheds as a useful DNA source for lizard conservation. Phyllomedusa, 14(1), pp.73-77.

International Biosciences [n.d.], 'Avian DNA Testing; Feather Collection Procedure', viewed 16 June 2022, <https://www.ibdna.com/downloads/FeatherAvianForm.pdf>

Klappenbach, L. 2019. 'Feather Anatomy and Function', image, ThoughtCo, viewed 17 June 2022, <https://www.thoughtco.com/feather-anatomy-and-function-129577>.

Linacre, A., Tobe S.S. 2013. 'Wildlife DNA analysis applications in forensic science'. Chichester, West Sussex, U.K: John Wiley & Sons Inc.; 2013.

Linacre, A. (2021). 'Wildlife crime in Australia'. Emerging topics in life sciences, 5(3), 487–494.

Menkhorst, P., Rogers, D., Clarke, R., Davies, J., Marsack, P., & Franklin, K. 2017. 'The Australian Bird Guide'. CSIRO Publishing. https://www.publish.csiro.au/book/6520/

Michael, D., Lindenmayer, D. 2010. 'Reptiles of the NSW Murray Catchment: A Guide to Their Identification, Ecology and Conservation'. Victoria: CSIRO Publishing.

Miller, H.C., 2006. Cloacal and buccal swabs are a reliable source of DNA for microsatellite genotyping of reptiles. Conservation Genetics, 7(6), pp.1001-1003.Nakagawa, 2018.

Panprommin, D., Soontornprasit, K., Tuncharoen, S., Pithakpol, S., Keereelang, J. 2019. 'DNA barcodes for the identification of species diversity in fish from Kwan Phayao, Thailand'. Journal of Asia-Pacific Biodiversity, 12(3), pp.382-389.

Pearson, S.K., Tobe, S.S., Fusco, D.A., Bull, C.M. and Gardner, M., 2015. Piles of scats for piles of DNA: deriving DNA of lizards from their faeces. Australian Journal of Zoology, 62(6), pp.507-514.

Pichlmüller, F., Straub, C. and Helfer, V., 2013. Skin swabbing of amphibian larvae yields sufficient DNA for efficient sequencing and reliable microsatellite genotyping. Amphibia-Reptilia, 34(4), pp.517-523.

Pidancier, N., Miquel, C. and Miaud, C., 2003. Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. Herpetological Journal, 13(4), pp.175-178.

Renshaw, M.A., Olds, B.P., Jerde, C.L., McVeigh, M.M. and Lodge, D.M., 2015. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. Molecular ecology resources, 15(1), pp.168-176.

Runhovde, S.R. 2017. 'Merely a transit country? Examining the role of Uganda in the transnational illegal ivory trade'. Trends in organized crime. 2017;21(3):215–34.

Schulte, U., Gebhard, F., Heinz, L., Veith, M. and Hochkirch, A., 2011. Buccal swabs as a reliable non-invasive tissue sampling method for DNA analysis in the lacertid lizard Podarcis muralis. North-Western Journal of Zoology, 7(2), pp.325-328.

Shan, B., Liu, Y., Yang, C., Zhao, Y., Zhang, G., Wu, Q., Sun, D. 2021. 'DNA Barcoding of Fish in Mischief Reef—Fish Diversity of a Reef Fish Community From Nansha Islands'. Frontiers in Marine Science, 7, p.618954.

Speller, C.F., Nicholas, G.P., Yang, D.Y. 'Feather barbs as a good source of mtDNA for bird species identification in forensic wildlife investigations'. Investig Genet 2**,** 16 (2011).

Steinke, D., Zemlak, T.S., Hebert, P.D.N. 2009. 'Barcoding nemo: DNA-based identifications for the ornamental fish trade', PloS One, vol. 4, no. 7, pp. e6300–e6300.

Stewart, T.A., Smith, W.L., Coates, M.I. 2014. 'The origins of adipose fins: an analysis of homoplasy and the serial homology of vertebrate appendages'. Proceedings of the Royal Society B: Biological Sciences, 281(1781), p.20133120.

Todd, M 2010, Hyacinth Macaw, image, eBird, viewed 17 June 2022, https://ebird.org/species/hyamac1

United Nations Office on Drugs and Crime (UNODC) 2020. 'World wildlife crime report: Trafficking in protected species', United Nations, viewed 15 June 2022, <https://www.unodc.org/documents/data-and-analysis/wildlife/2020/World_Wildlife_Report_2020_9July.pdf>.

Wear, P [n.d.]. Blue Macaw Puzzle, image, fineartamerica, viewed 17 June 2020, < https://fineartamerica.com/featured/blue-macaw-paul-wear.html?product=puzzle&puzzleType=puzzle-20-28>.

Weitzman, S.H., Parenti, L.R. 2021. 'Fish: classification', Britannica, viewed 14 June 2022, < https://www.britannica.com/animal/fish/Actinopterygii-ray-finned-fishes#ref63655>

Wilson-Wilde, L., 2010. Wildlife crime: a global problem. Forensic science, medicine, and pathology, 6(3), pp.221-222.

Zavagli, M. 2021. 'Red flag indicators for wildlife and timber trafficking in containerized sea cargo: a compendium and guidance for the maritime shipping sector', TRAFFIC and WWF.

Zug G.R. 2013. 'Reptiles and Amphibians of the Pacific Islands: A Comprehensive Guide'. Berkeley: University of California Press.

III. Sample collection for DNA Analysis for Species Identification

Analysis of DNA (short for deoxyribonucleic acid), the molecules that carry genetic information in an organism, is essential for accurate species identification. It has been used in human forensics for decades to help identify individuals. Biological DNA evidence collected from confiscated live animals can replace the need for their long-term maintenance in captivity during court cases and, combined with photographic and video evidence and records from the crime scene, negates the need for presentation of wild animals in court. DNA analysis can be used to investigate whether seized animals are farmed or wild caught, their geographical origin and familial relatedness.

Information that can be gained through genetic (DNA) analysis to support wildlife crime investigations:

- *Species Identification*
- *Region/Population of origin of an individual:* Genetic analyses may reveal the origin of animal or animal parts, respectively. A reference database is used to compare the DNA profile of a sample with the known distinct DNA profile of animals in distinct regions and/or populations. Publicly-accessible genetic reference databases exist for most species and subspecies (e.g. GenBank, Barcode of Life).
- *Distinct individual identification*: if reference DNA profiles of the specific individual are needed.
- *Sex determination*: Since male and female individuals carry distinct DNA sequences (for example the XX chromosomes in female mammals versus XY chromosomes in male mammals),
- *Parentage/Relatedness*: If samples or a DNA profile of all individuals in question are available, parentage and/or relatedness of the individuals can be determined by genetic analysis (e.g., to determine parentage of wild animal claimed to be farm-bred)

In almost any cell of an animal, two types of DNA are found: nuclear DNA and mitochondrial DNA. For forensic purposes, mitochondrial DNA is often better suited since nuclear DNA is more susceptible to degradation in decomposing tissue and a single cell may contain hundreds of copies of mitochondrial DNA compared to only 2 copies of the nuclear DNA. In any forensic case however, the respective genetic lab will decide which DNA to use and sampling approaches remain the same.

D-loop or hyper variable control region of mtDNA is used for analysis based on haplotypes. This has been successfully applied in Chinese sika deer and seahorses (Sahajpal, Mishra and Bhandari 2021). Similarly, spatial smoothing assignment has been used in the past to investigate illegal sale and trafficking of African elephant ivory and Luxembourg red deer (Alacs et al. 2009). This is invented under a theory that when populations become isolated, it creates a discrete variation

in genetic material as there is no exchange with other populations. This test can relate a sampled animal back to its original population, identity poaching hotspots, and differentiate between captive-bred and wild-caught animals (Cooper 2013). According to Alacs et al. (2009), 50% of geographic-specific alleles (16 STC loci) can be identified within 500km of their origin. However, this cannot be applied to all animals, depending on the information available on the DNA database. To identify familial relatedness in seized animals, specific markers in DNA can be identified and used to validate parent-offspring relationship in seized animals. Parentage can be assessed using a suite of hypervariable micro satellite markers (Sahajpal, Mishra and Bhandari 2021).

Laboratory submission

Laboratory accreditation to the ISO17025 standard is gold standard in wildlife forensic testing. However, accreditation to this standard is time consuming, expensive and requires a substantial level of staffing and infrastructure to achieve, which is not realistic for many wildlife forensic laboratories. The Society for Wildlife Forensic Science (SWFS) has established a set of Standards and Guidelines specifically for several disciplines within wildlife forensics (SWFS Standards and Guidelines 2018), please refer to SWFS for further details. Accredited labs have submissions evaluated by a UNODC wildlife forensic expert and by an independent panel of experts drawn from the Technical Working Group of the Society for Wildlife Forensic Science. Accredited laboratories (Table 1) can be contacted for guidance on collection, submission and transportation of samples.

Table 1: Accredited laboratories listed by CITES that may provide assistance and recommendations on sample collection and submission.

Laboratory name and location	Country, CITES region	QA standard	Sample types analysed	Contact name / email
Australian Centre for Wildlife Genomics, Sydney	Australia, Oceana	ISO17025	Terrestrial animal, Aquatic animal, Rhinoceros horn, Elephant ivory	Greta Frankham Greta.Frankham@austmus.gov.au
Criminalistic Service, Guardia Civil, Madrid	Spain, Europe	ISO17025	Terrestrial animal, Aquatic animal, Plant, Microorganisms	David Parra Pecharromán crimquimica@guardiacivil.org
Genomia Limited, Plzeň	Czech Republic, Europe	ISO17025	Terrestrial animal	Markéta Dajbychová marketa.dajbychova@genomia.cz
Institute of Forensic Medicine, Zurich	Switzerland, Europe	ISO17025	Terrestrial animal, Aquatic animal, Elephant ivory	Morf Nadja Nadja.Morf@irm.uzh.ch
James Hutton Institute, Aberdeen	United Kingdom, Europe	ISO 9001	Plants, Diatoms, Soil	Lorna Dawson Lorna.Dawson@hutton.ac.uk
Netherlands Forensic Institute, the Hague	The Netherlands, Europe	ISO17025	Terrestrial & Aquatic animal, Plant, Timber, Rhino horn, Elephant ivory, Pangolin	Irene Kuiper i.kuiper@nfi.minvenj.nl
Science and Advice for Scottish Agriculture, Edinburgh	United Kingdom, Europe	ISO17025	Terrestrial animal, Aquatic animal, Rhinoceros horn, Elephant ivory	Lucy Webster Lucy.Webster@sasa.gsi.gov.uk
US Fish and Wildlife Service, National Forensic Laboratory, Ashland	United States of America, North America	ISO17025	Terrestrial animal, Aquatic animal, Timber, Rhino horn, Elephant ivory, Pangolin	Ed Espinoza ed_espinoza@fws.gov
University of California, Davis	United States of America, North America	ISO17025	Terrestrial animal, Rhinoceros horn	Christina D Lindquist cdlindquist@ucdavis.edu

1. Types of biological evidence for DNA analyses for species identification

A range of samples can be collected from wildlife for DNA analysis. In general, sampling should be done by qualified and trained personnel. Personal Protective Equipment (PPE) must be worn, and gloves should be changed between sampling of different materials and/or samples to avoid cross-contamination. A wide array of commercial kits is available for sampling of genetic material. Using these kits in wildlife crime cases is highly recommended, as this reduces the risk of improper collection and handling of samples. All instructions of the respective manufacturer regarding the usage, storage and mode of shipment must be followed.

Biological evidence for DNA analyses from live animals include skin biopsies and scrapings, blood, urine, faeces, saliva, and hair/feathers/scales (Cooper & Cooper 2013). Non-invasive methods such as urine and faecal collection are preferred for live animals to minimise handling and associated stress to animals (Cooper & Cooper 2013). However, tissue biopsies and blood samples which are more invasive are preferable for DNA analysis (FWG 2014). The process of collection and securing different sample types can be found in Table 2.

Environmental evidence that can be collected from the scene to determine the recent presence of wildlife include faeces, regurgitated pellets from birds of prey, dropped hairs or feathers, scales from reptiles, and dried blood or other bodily fluids (Cooper & Cooper 2013). Samples should be collected promptly, as DNA evidence is easily contaminated and degrades quickly in the environment (FWG 2014). Wet samples and swabs should be allowed to air-dry prior to packaging to prevent condensation and spoilage that will adversely affect laboratory testing (Palmbach 2016).

Table 2: Appropriate collection methods for different sample types used in DNA analyses

Sample Type	**Collection**	**Storage/ preservation***
Tissue (skin from live animal)	Skin biopsy: via a biopsy punch should be performed by a veterinarian or other experienced, trained individual. Fresh sample: place tissue into a collection tube using tweezers and place tube in sealed plastic bag. Skin scraping: scrape multiple areas of skin with the back (blunt side) of a scalpel blade and smear onto a microscope slide. Place microscope slides in a slide mailer. Small pieces of skin can be placed into a collection tube.	Refrigerate fresh sample at 4^{o}C for up to 7 days. Thereafter freeze below -20^{o}C or keep in fixative. Fix in ethanol for DNA studies, otherwise fix in formalin.

Tissue (from dead animal)	For a fresh sample: cut a 1cm^3 piece of tissue (ideally muscle) using a scalpel and place into a collection tube using tweezers. Place tube in sealed plastic bag.	As above for tissue from live animal
Blood	At least 2 swabs are needed for each sample, as well as one control swab to test for contaminant DNA. Control swab: moisten the swab head in sterile water. Air dry, place swab back into collection tube, seal and label. Fresh blood: soak 1-2 drops of blood onto the swab head. Air dry, place swab back into tube, seal and label. Dried blood: 2 swabs are needed. 1st swab: moisten the swab head in sterile water then rub the swab across the dried blood. Air dry, place swab back into tube, seal and label. 2nd swab: on the area sampled with the 1st swab, use a fresh, dry swab to rub the area and soak up remaining moisture. Air dry, replace the swab into tube, seal and label. Place all sealed swabs into an evidence bag and seal.	Freeze below -20^{o}C.
Saliva (swabbed from mouth)	For live animals, a swab should be taken by a trained individual. Swabs should be placed in collection tubes.	Freeze below -20^{o}C.
Saliva (swabbed from surface)	Follow instructions as for blood. Swabs should be placed in collection tubes.	Freeze below -20^{o}C.
Hair	Use sterile tweezers, preferably plastic for more delicate handling. Pluck ~20 hairs with the root of the hair remaining attached. Handle hairs by the tip, not the root. Place plucked hairs in a universal container filled with saline. Shed hairs in the environment can be placed in a paper envelope.	Store dry or freeze below -20^{o}C.
Feathers	Use sterile tweezers, preferably plastic for more delicate	Store in transport

	handling. Pluck several young, growing feathers, ensure careful handling. Place feathers in a sealable plastic bag. For DNA studies, place feathers in a paper envelope and then in a sealable plastic bag. The same guidelines apply for moulted feathers.	medium, refrigerate at 4°C or freeze below -20°C.
Faeces	Place faeces into a universal container with a screw-cap using a gloved hand or sterile forceps for small droppings.	Freeze below -20°C.
Regurgitated pellets (birds of prey)	Place pellets into a universal container with a screw-cap using a gloved hand or sterile forceps.	Freeze below -20°C.

References: Cooper & Cooper 2013; FWG 2014

2. Stable isotopes

In wildlife crime investigation, stable isotope analysis is used as a method to discover what animals have consumed/eaten. The stable isotopes of carbon and nitrogen (δ13C and δ15N) ratios from animal tissue can provide information on the long-term composition and quality of their diets (Vedel 2022). This can also provide information on geographic origin. Mass spectrometry is a sensitive method to identify wildlife samples. Tissues, fluids, teeth or bones can be collected with different preparations such as drying, acidifying or packing (capsules of tin). Preferred techniques of sample preparation and collection can be provided from laboratories in your area. Stable isotopes provide evidence of diet changes that are frequent when animals move from the wild to a captive environment.

3. Faecal analysis

Faecal analysis is a non-invasive method of obtaining genetic material for DNA analysis. Faeces can also be analysed for ingested materials and provide an indication of an animal's diet over the past few days. Undigested materials can be separated from a binocular microscope for taxonomic analysis (Napolitano et al 2008). DNA from faecal materials can be extracted and genes of interest can be amplified for identification of dietary items. These can reflect their habitats, such as altitude, latitude and local tree compositions (Shutt et al. 2020). By comparing feeding characterisation and geographical distribution, we can conclude the origin of seized animals and determine whether they were recently taken from the wild or have been held in captivity for at least a few days (depending on gastro-intestinal transit time) (Napolitano et al. 2008).

4. Chapter References

Alacs, E.A., Georges, A., FitzSimmons, N.N., Robertson, J. 2009. 'DNA detective: a review of molecular approaches to wildlife forensics', *Forensic Science, Medicine, and Pathology*, vol. 6, no. 3, pp. 180–194.

CITES website < https://cites.org/eng>.

Cooper, J.E., Cooper, M.E. 2013. 'Wildlife forensic investigation: principles and practice', *CRC press*.

Forensic Working Group (FWG). 2014. 'Wildlife DNA sampling guide', *Partnership for Action against Wildlife Crime* (PAW), viewed 14 June 2022, <https://www.tracenetwork.org/wp-content/uploads/2012/08/Wildlife_DNA_Sampling_Guide_web.pdf>.

Napoliano, C., Bennett, M., Johnson, W., O'Brien, S., Marquet, P., Barria, I., Poulin, E., Iriarte, A. 2008. 'Ecological and biogeographical inferences on two sympatric and enigmatic Andean cat species using genetic identification of faecal samples', *Molecular Ecology*, vol. 17, no. 2, pp. 678–690.

Palmbach, T.M. 2016. 'Crime scene investigation and examination: Chain of evidence', in J Payne-James & RW Byard, *Encyclopaedia of Forensic and Legal Medicine*, Elsevier, pp. 679-685.

Sahajpal, V., Mishra, S., Bhandari, D. 2021. 'Forensic Analysis in Wildlife Crime Cases: Microscopy, DNA Profiling and Isotope Analysis', *Forensic Analysis Scientific and Medical Techniques and Evidence Under the Microscope,* IntechOpen, London.

Shutt, J.D., Nicholls, J.A., Trivedi, U.H., Burgess, M.D., Stone, G.N., Hadfield, J.D., Phillimore, A.B. 2020. 'Gradients in richness and turnover of a forest passerine's diet prior to breeding: A mixed model approach applied to faecal metabarcoding data', *Molecular Ecology*, vol. 29, no. 6, pp. 1199–1213.

SWFS Technical Working Group. 2018. 'Standards and Guidelines for Wildlife Forensic Analysis, Version 3'. Ed. Lucy M.I. Webster, *The Society for Wildlife Forensic Science*, 19th November 2018, pp.21.

Vedel, G., de la Peña, E., Moreno-Rojas, J.M., Gómez, J.C.M., Carranza, J. 2022, 'Stable carbon and nitrogen isotope values in hair reveal management differences and hidden practices in wild boar populations', *The Science of the Total Environment*, vol. 823, pp. 154071–154071.

IV. Are the animals farmed or collected from the wild?

Monitoring trade of wildlife sometimes requires assessment of whether animals are bred in captivity as claimed or were in fact collected from the wild and trafficked through a wildlife farm. This chapter focuses on the investigation of wildlife farms or breeding facilities that may use their facilities to sell or house animals illegally collected from the wild.

1. Seller Assessment

1.1. Facilities & equipment

On arrival at the site, record the stall holders/farm layout and the equipment they have. Take note of the cage construction, sizes, and the number of animals in each cage. It is important to note if the cages have appropriate husbandry requirements according to the species being sold. For example, heating lamps for reptiles/amphibians or water filters for aquatic species. Successful husbandry and species knowledge is very important for a legitimate, successful breeding program. Some of these same principles can be applied to inspection of wildlife intercepted at airports or other transit hubs by considering whether species-specific needs for transport have been provided as one would expect for legitimate breeders.

Considerations:

- Do the cages look well-constructed and maintained? Think about the materials they have been made from.
- Are the cages clean and appropriately stocked with bedding for the animals?
- Are the cages appropriate size for the number and size of animals contained?
- Are the animals caged in groups or individually?
- Are the animals caged all the same species or a range of species?
- Is the cage/container appropriately prepared for the intended travel time and method?

It is important to note ANY welfare considerations, regardless if you suspect the animals are farmed or wild caught.

Potential Red flags:

- *Poorly constructed cages with sharp corners/materials that could cause harm to the animals*
- *Cages unsuitable in size or design for the animals being housed*
- *Cages that do not provide adequate husbandry according to species*
- *Mismatch/range of different species housed together*
- *Dirty cages with uncomfortable animals*
- *Severely overcrowded cages*
- *Presence of injured or sick animals receiving no medical attention/alteration in care*

1.2. Seller information

When having a closer look at the stall or seller and associated postal address, consider the platform the seller is using and how they are advertising their sales. Take note of the location of their breeding facility as this information can be useful when considering the species they are breeding and selling. Consider if the animal is native to that area or if breeding would be the only viable option to have the species in the area. Also consider the distance they would have travelled with the animals from their breeding facility and if this seems viable.

Considerations when investigating wildlife breeders and sellers:

- Are the breeders able to discuss information about their farm including location, care, feeding and animal holding facilities?
- Are the animals being sold native to the location of the breeding facility?
- Are the breeders able to share their CITES export permit or government accreditation when asked?
- How long have they been breeding for and how many species do they breed?
- Do they have records and information on their breeding stock?
- What are their breeding protocols? How many breeding pairs do they have and how old are they?
- Do they have records related to veterinary visits or care?
- Are they able to provide information on family trees for the animals they are selling?
- Are the cages/containers appropriately labelled according to the animals inside?
-

Potential Red flags:

- *Animals being sold are native to the area of the 'breeding facility'*
- *Supposed breeders lack knowledge on the husbandry (feeding, veterinary care, breeding practices etc.) of the animals*
- *Unwillingness to share any documentation or information on the animals being sold*
- *Elevated price of animals*
- *Purposely mislabelled or fraudulent consignment details*
- *Seller known to authorities for crimes unrelated or related to animals, particularly trafficking offences (wildlife crime is often linked to other illicit trade activities)*

2. Animal examination

2.1. Species

Identification of species is one of the first and most important steps in these investigations. Please refer to the previous chapter for species identification recommendations. Once the species has been identified, it is important to consider the likelihood of whether this species has been bred in captivity, i.e. is it logistically viable for these animals to have been bred?

Information gathering on the animal

- What is the animal's life span?
- Does the species have any specific and demanding husbandry requirements?
- How easy and successful are captive breeding programs for this species?
- Example: Cheetahs are very difficult to breed in captivity. If large numbers of young cheetah are being sold, consider that they may have been taken from the wild.
- How rare is this species?
- Species listed as threatened, endangered or critically endangered by IUCN and/ or CITES should be investigated in-depth as there is incentive for criminals to sell these animals for high profit.
- How many animals of a species are being transported/sold/bred?
- Numbers and consistency of animals can be a clue in determining whether animals are being farmed or caught.
- Sexual maturity of the species
- Species that are late to sexually mature and have long gestation/rearing periods with low numbers of offspring are less productive, and thus unlikely to be being sold/transported in large numbers by legitimate breeders, especially large numbers of mature animals. Low hatching rates and poor neonate survival are barriers to breeding that should be considered for certain species, such as chameleons and other reptiles.
- When assessing a breeding facility, take note of the number of animals housed and the composition of the animals to determine if there are enough male and female animals to realistically produce the number of offspring being reported.

Red flags:

- *Overcrowded cages with inappropriate husbandry, poor animal comfort and welfare*
- *Large numbers of threatened or endangered species or those which have low production rates or don't breed well in captivity*
- *Mixed species housed together in the same cage or sellers selling a wide range of species*
- *Many breeders focus on one or two specific species and mixing may indicate they are wild caught species*

2.2. Age

Determining the exact age of live animals can be very difficult, especially with so many variables relating to the biology and history of the animal.

Evaluating age

- Categorise animals into an age group
  - Mammals: consider deciduous teeth and size to categorise as juvenile or adult
  - Birds: consider analysis of feathers, eyes or feet and categorise accordingly as juvenile, sub-adult or adult

2.3. Gender

The ability to determine gender varies greatly between species, with some animals only able to be sexed using blood tests. Where possible gender should be determined and recorded.

2.4. Markings/colouring

The use of a generic datasheet (e.g. the body maps below for dogs and cats) that bears the outline of an animal can be useful to record any specific features and markings. Depending on species, breeders often breed animals with the goal of certain desirable traits such as colourings (e.g. albino animals) and sizes. These sought-after phenotypic traits can lead to the indication that animals have been bred in captivity.

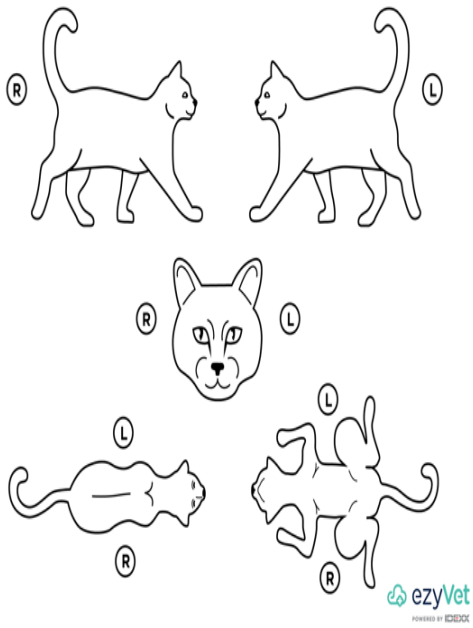


Figure*5:* An example of a body map for a feline

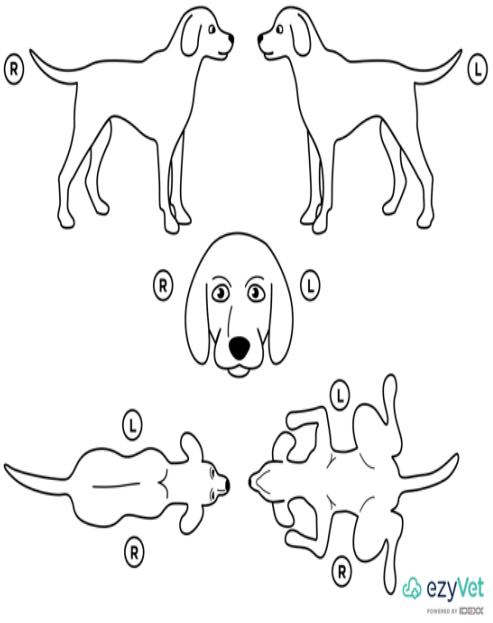


Figure 6: An example of a body map for a canine

2.5. Behaviour

Variable behaviours can be noted in different species to try and determine if they are captive bred. For example, birds bred in captivity tend to be calmer and not as easily frightened by noise or human presence, whereas those from the wild tend to show more excitable behaviour and exhibit damaged and soiled feathers and feet.

2.6. Body condition

All live animals should be examined where possible, providing first responder teams have a member(s) appropriately trained to do so, or an expert to call upon to assist. Wounds should be noted and photographed, and it should be considered whether these could be wounds related to trauma from capture or other welfare concerns.

Recognising body condition abnormalities

- Birds of prey caught in the wild can have shotgun damage, trap injuries, or abrasions caused by leather bands
- Changes in body composition due to altered diets and artificial selection for certain body shapes and sizes

  - Species specialists should be consulted to determine alterations in body composition as they can be variable and specific. E.g.:

    A study on domestic minks (Mustela vison) found that expanded skull size was a sign of farmed minks when compared to wild. Femur bones can also be measured during necropsy, with a study conducted by Zhou et al (2014) suggesting that captive bred minks tend to have larger, heavier and longer femur bones compared to wild-caught, due to high energy and nutritious diets provided by breeding facilities.

    In Dybowski's frogs, a theory has been suggested by Yang et al (2011) and Xia et al (2011) that wild frogs tend to develop larger and denser femur bones due to increased activity levels and more balanced nutrition when compared to captive bred.

- Animals poached from the wild and kept in captivity can suffer from wounds and lesions from capture or due to inadequate conditions in captivity.

2.7. Animal identification in breeding facilities

Identification methods will vary according to species, with the main methods of identification of exotic animals being microchipping and leg bands. It is important to cross-reference identification with breeder documentation. Tampering of identification methods should also be assessed, for example note any changes in shape/design of bird leg tags which could easily be tampered with to permit removal and replacement.

Examples of methods of identification

- Leg rings/bands for birds and some mammals
- Tags, drawings or photographs of individual markings
- Tattooing, branding or microchipping

Image 1: A leg ring for identification of a bird

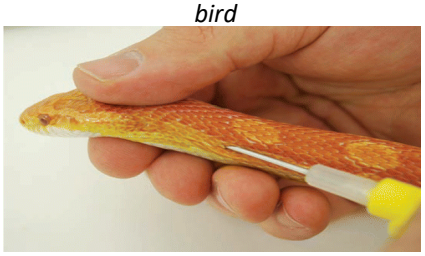


Image 12: The placement of a microchip in a reptile

3. Chapter References

CITES website < https://cites.org/eng>.

Xia R, Huang XM., Yang SH., Xu YC, Ying L., Dahmer TD. 2011, 'Morphometric discrimination of wild from farmed Dybowski's frog (*Rana dybowskii*) based on hindlimb length', *Journal of Forestry Research,* vol. 22, pp. 269 – 274.

Yang SH., Huang XM., Xia R, Xu YC. & Dahmer TD. 2011, 'Use of femur bone density to segregate wild from farmed Dybowski's frog (*Rana dybowskii*)', *Forensic Science International,* vol. 207, pp. 61-65.

Zhou, XL., Xu, YC., Yang, SH., Hua, Y & Stott, P 2015, 'Effectiveness of Femur Bone Indexes to Segregate Wild from Captive Minks, Mustela vison, and Forensic Implications for Small Mammals', *Journal of Forensic Sciences*, vol. 60, no. 1, pp. 72–75.

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V. Appendices

Appendix I

Additional evidentiary sample collection from confiscated wild animals

The illegal trade and transport of wildlife poses a significant public health risk. Unregulated movement of animals has the potential to spread animal diseases around the world and zoonotic diseases to people, with implications for public health, international trade, economies and security, food availability, ecosystem health and biodiversity. Collection of other samples from confiscated wildlife in addition to those collected for DNA testing can support identification of potential health concerns for animals and people, e.g., establishing cause of death if dead animals are found alongside live animals during a seizure; diagnostic testing to identify cause of illness in symptomatic animals; and/ or screening for known and novel pathogens (such as SARS-coronaviruses or other emerging viruses) in symptomatic or asymptomatic individuals. These analyses provide further supporting evidence for presentation in court cases to highlight the threats of illegal wildlife trade to wildlife, livestock and human health and support strengthening of penalties and sentencing guidelines.

Identifying cause of death should include recording of observations at the crime scene and a necropsy conducted in an appropriate facility by a veterinarian or other trained expert. Sampling and necropsies should only be conducted by trained personnel. It cannot be overemphasized that, when collecting biological samples, all handlers must use appropriate PPE (see Appendix II) to avoid any health hazards that might ensue in a suspected wildlife crime, e.g. poisons and pathogens (especially zoonotic agents). Those most likely to be exposed to zoonoses include individuals directly involved with the trade of animals including wildlife, such as hunters/poachers, farmers, butchers, couriers and sellers (Bezerra-Santos et al. 2021). Once on site at a wildlife crime scene this health risk extends to enforcement officers, which is why it is of vital importance to follow personal safety procedures outlined in Appendix 2 to protect both your health and safety, and broader public health.

All samples must be collected and stored in a manner that prevents destruction, degradation or cross contamination. Regarding the latter, multiple changing of the gloves is recommended, for

example in between handling different animals. Samples can be collected from healthy, sick or freshly dead wildlife to look for evidence of pathogens (of conservation or public health concern) such as viruses, bacteria and parasites, and to assess the overall health status of animals. Such samples, their storage and usage are noted in Table 3. If the country is a member of the World Organization of Animal Health (WOAH), identification of certain pathogens or diseases is required to be reported by laboratories to the relevant authority/ wildlife health focal point if the disease is classified as notifiable by WOAH.

If the collecting team is in doubt about appropriate methods for sample collection, transport or storage for specific testing, they should consult with the respective laboratory.

Laboratories and natural history museums may have facilities for storage or archiving of samples, and this should be established prior to sample collection. To prevent any confusion during transport of samples and subsequent investigations, correct labelling of samples is of crucial importance, as for any evidence collected from the crime scene (see labelling recommendations in section on collecting samples for DNA analysis).

Table 3: Additional samples to collect for pathogen and toxicology testing

SAMPLE TYPE	STORAGE	USES
Saliva (oropharyngeal swab[1])	- CRYOVIAL[2] with lysis buffer[3] (use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the swab shaft of the swab above the tip) - Refrigerate for up to 5 days or ideally freeze in dry shipper or dewar with liquid nitrogen. Transfer to -80°C freezer when possible	Pathogen PCR screening
Urine (free catch method or urogenital swab)	- Urine swab in CRYOVIAL[4] with lysis buffer (use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the swab shaft of	Pathogen PCR screening

[1] Swab refers to: terile,polyester-tipped swabs with either an aluminum or plastic shaft

[2] 'Cryovial" refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended.

[3] E.g. tris-EDTA; RNALater; others available inc ountry/ preferred by partner laboratories

[4] 'Cryovial" refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended.

	the swab above the tip) - Urine sample in cryovial with lysis buffer at approximate ratio of 1 part urine: 3 parts lysis buffer. - Refrigerate cryovial for up to 5 days or ideally freeze in dry shipper or dewar with liquid nitrogen. Transfer to -80°C freezer when possible	
Blood (serum; rbc/wbc pellet; thin blood smear (fixed)) (Use a nonheparinized syringe to collect blood (not to exceed 1% of the total body weight)	- 2 thin smears on glass microscope slides, fix with methanol or ethanol, and store in slide box. - Place rest of blood into a serum vacutainer (red-top) tube containing serum-clotting factor. After allowing blood to clot, spin tube in a centrifuge or allow to stand vertically on ice overnight. Use a sterile pipette tip and pipette gun to draw off serum and place into cryovial with lysis buffer. - Refrigerate for up to 5 days or ideally freeze in dry shipper or dewar with liquid nitrogen. Transfer to -80°C freezer when possible	Pathogen PCR screening; microscopy; serology
Faeces (fresh faecal sample or rectal swab)	- Pea size piece of fresh faeces in a 1.0 ml empty cryovial. - If faeces not available, rectal swab in CRYOVIAL[5] with lysis buffer (use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the swab shaft of the swab above the tip) - Refrigerate for up to 5 days or ideally freeze in dry shipper or dewar with liquid nitrogen. Transfer to -80°C freezer when possible	Pathogen PCR screening
Ectoparasites	In cryovial with 95% ethanol. Store at room temperature.	Entomology and pathogen PCR screening

[5] 'Cryovial" refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended.

Crop, stomach, intestinal contents		Toxicology screening
Tissue (from dead animal)	- Cut a pea sized sample of as many of: muscle; large intestine, small intestine, liver, lung, kidney, spleen, and brain; half of each placed in an empty cryovial and half (duplicate sample) in a cryovial with lysis buffer - Refrigerate for up to 5 days or ideally freeze in dry shipper or dewar with liquid nitrogen. Transfer to -80°C freezer when possible	Pathogen PCR screening; histology
Swabs from external lesions or wounds	- Fresh, discharging wound/lesion: soak swab head in wound discharge. Air dry, place swab into cryovial, seal and label. Freeze below -20C. - Dry wound/lesion: 2 swabs: 1st swab: moisten the swab head in sterile water then rub the swab across the wound/ lesion. Air dry, place swab into cryovial, seal and label. 2nd swab: on the area sampled with the 1st swab, use a fresh, dry swab to rub the area and soak up remaining moisture. Air dry, place swab into cryovial, seal and label. Freeze below -20oC. If multiple wounds/lesions, place swabs from separate lesions into separate cryovials and separate evidence bags.	Microbiology, virology

Note: The preferred option for disposal of infectious field sampling materials and necropsy waste of infected carcasses it to contain the waste and deliver it to a health facility that maintains a safe disposal system.

Chapter References

Bezerra-Santos, M.A, Mendoza-Roldan, J.A, Thompson, A, Dantas-Torres, F & Otranto, D. 2021. 'Illegal wildlife trade: A gateway to zoonotic infectious diseases', *Trends in Parasitology*, vol. 37, no. 3, pp. 181-184.

Appendix II

VI. Acknowledgement

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