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

Part I: Handling and Management of Confiscated Live Wildlife

Developed by Lucy Ogg Keatts*, Wayne Boardman** and Anne-Lise Chaber**, through the generous support of the U.S. Department of State Bureau of International Narcotics and Law Enforcement Affairs (INL)-funded project: “Confiscation and Management of Live Wildlife as Evidence: Promoting Conservation, Health, Welfare and Successful Prosecution.” * Wildlife Conservation Society (WCS), ** University of Adelaide

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TABLE OF CONTENTS

Introduction

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) (1). Trade can include all activities relating to the human harvesting, transportation, commercial exchange (involving money or barter), and end use of wildlife and harvested wildlife products, both at local levels and across legal jurisdictions. IWT has an estimated financial value of USD 23 billion annually, is widespread in many countries, and is a concern for wildlife conservation being responsible for substantial losses in biodiversity due to uncontrolled capture and trade of endangered species, and detrimental impacts from introduction of invasive species and wildlife diseases (1,2,3). Wildlife trade is also a concern for public health, livelihoods, security and economies due to the potential for spread of zoonotic agents, introduction of pathogens that threaten agricultural production and trade, and links to organized crime and criminal networks (2,3,4,5).

As a wildlife product moves from the point of harvest to the consumer it typically changes hands several times between multiple actors. Suppliers introduce products into the trade chain, other actors facilitate the trade in some way (intermediaries), or purchase the final product (consumers) (1).

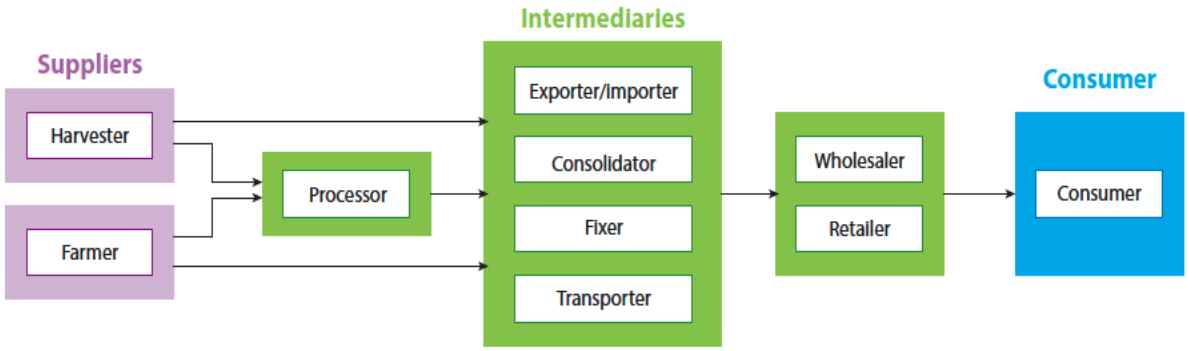


Figure 1. Illegal wildlife trade chain (1)

The overarching aim of this document is to develop best practice standard operating protocols to ensure:

- Frontline officers understand how to maintain high-welfare standards for live wildlife ('living evidence') confiscated from IWT
- Frontline officers can implement safe and appropriate handling for live wildlife ('living evidence') during confiscations from IWT
- Best-practice processes are in place to facilitate appropriate disposition of confiscated wildlife after initial holding period
- Best-practices are implemented for collation and storage of evidence to promote successful prosecution of criminal actors whilst maximizing future benefits to the conservation of wild populations.

Why do we need standard operating protocols for confiscation and management of live wildlife as evidence?

Management of confiscated live wildlife is complex, often involving multiple species and taxa, and associated with potential hazards including from bites, other trauma or disease transmission (from animals to humans and vice-versa). Managing the needs of these varied and often stressed and injured animals requires trained expertise, and adequate investment in personnel and financial resources, from confiscation through immediate and longer-term care, and management or re-release. In many cases, these are inadequate and the survival rate and/or welfare status of confiscated wild animals falls below desired levels. Poor identification of species, and inadequate collection and management of evidence also contribute to poor success in prosecution of illegal wildlife trafficking cases.

Standard operating protocols will provide personnel on the frontlines of illegal wildlife trade enforcement with guidelines on how to prepare, organise and activate an illegal wildlife trade unit from the perspective of safe and effective handling and management of live wildlife during and immediately after a confiscation, to promote health, animal welfare and successful prosecution of criminal cases, and to support decision-making for the final disposition of confiscated wildlife: i) repatriation, ii) conservation translocation (including reintroduction), iii) long term captive management, iv) euthanasia.

The ability of enforcement staff to achieve the standards and follow protocols outlined in this document may at the current time be limited by the lack of resources e.g., access to personal protective equipment, wildlife handling equipment or appropriate training. This document can help identify needs for additional equipment and training, and provide a starting point for achieving best-practices for protecting the health and welfare of people and wildlife during confiscation events.

These guidelines are for frontline officers and are not intended to cover husbandry during long-term housing of animals. If an animal is being held for more than 48 hours then enforcement officials should contact rescue centres or zoos for advice on feeding and husbandry.

These Standard operating protocols for the confiscation and management of live wildlife include the following:

1. **Preparedness for Confiscation and Management of Life Wildlife as Evidence**
2. **Pre-seizure Planning and Preparation**
3. **Protocols Required During and After the Confiscation Process**
4. **Decision-making Tree for Disposition of Confiscated Wildlife**

The protocols cover practices along various stages of the live wildlife evidence chain, including:

i) Preparedness planning

ii) Essential steps and communications when planning a confiscation event

iii) Key assessment of the seizure environment

iv) Minimising zoonotic and anthropozoonotic disease risk through correct use of personal protective equipment (PPE) and good standards of biosecurity

v) Species Identification

vi) Unique Animal Marking/ ID and record taking

vii) The safe and ethical handling, restraint and control of live wild animals at the point of confiscation to avoid injury to humans and animals

viii) Collecting and securing critical evidence at confiscation to facilitate identification of animals and support prosecution efforts - i.e. wildlife forensics (e.g., microchipping, sample collection for DNA barcoding, real-time PCR, photography and videography)

ix) Preparing and selecting appropriate temporary holding and transport containers and conditions for specific species/taxa and the safe movement of animals into those containers, ensuring appropriate welfare considerations for specific species/taxa

x) Developing country-specific networks and facilities for specialist animal handling and longer-term disposition

xi) Considering the benefits and risks of the three ultimate disposition options for confiscated live wildlife:

   a. Maintaining the animals in captivity for the remainder of their natural lives
   b. Returning the animals to the wild
   c. Humane Euthanasia

xii) Managing and presenting evidence from live wildlife in trafficking cases to achieve successful criminal prosecution without requiring live animals to appear in court.

Indicators of success

Indicators of Success include the following:

1. A well-organized illegal wildlife trade unit with clear jurisdiction and a clear and effective management structure, whose participants have clearly aligned job descriptions and roles, a high level of skills and knowledge on handling and management of a range of live wildlife taxa, are appropriately equipped to ensure the safety of animals and humans;

and are motivated and passionate advocates to safely and effectively restrict illegal wildlife trade

2. Frontline officers understand the importance of evidence collection at the point of confiscation, and the chain of custody for evidence is documented and maintained to support successful prosecution
3. Confiscated animals are handled and managed to the highest standards of welfare and health and rate of survival increases
4. The fate of confiscated animals aligns with the highest standards of conservation, welfare, health and management of the taxa involved
5. Ultimately, the illegal wildlife trade is minimised thus improving conservation and welfare outcomes.

I. Preparedness for the Confiscation and Management of Life Wildlife as Evidence

Introduction

Effective and successful confiscation of wildlife and subsequent prosecution of illegal wildlife traders necessitates considerable investment in organization, preparation and training. It requires the establishment of a detailed organisational structure, with clearly defined job descriptions, roles and responsibilities and authorities, well trained staff in those roles, adequate funding and a suite of operating procedures and protocols

Training needs to be done well before any seizure event and repeatedly updated on a regular basis so that when a confiscation event occurs the seizure process runs smoothly and safely, and everyone knows what, how and when actions are required and who is responsible for which aspects. Training can take the form of role plays and simulation exercises of a seizure event, and a planned programme of such events should be organised. As part of an iterative training process, the team involved in a seizure event should always debrief in a standardised way as soon as possible after an event (whether simulated or real) to ensure learnings from the exercise are adapted into future preparation and standard operating procedures.

In the field, personnel need to have guidelines for many eventualities given the unpredictable nature and hazards associated with some wildlife confiscations, and need to follow due process to ensure the chain of custody for evidence (live and dead wildlife and samples collected from them) is maintained. Confiscated species need to be identified, assessed, provided with identification, provided with high levels of husbandry that follows the Five Freedoms of animal welfare[1]. In order to be able to provide suitable level of care, taxa management and husbandry guidelines need to be developed ahead of any confiscation event. Personnel need to know how to handle animals and be able to transport them safely and appropriately to holding sites from the seizure site. Thus, other essential preparedness training includes: the handling and management of animals, species identification, disease prevention and correct use of PPE, and a systematic, organised, detailed training on the collection of evidence and securing of a crime scene during a seizure event to support subsequent prosecution.

A systematic, detailed calendarized training program should exist and be organised for any new recruits, as well as current employees as best practices change and improve. Designation of a training programme development officer within the Illegal Wildlife Trade (IWT) team, whose

[1] The Five Freedoms are globally recognized as the gold standard in animal welfare, encompassing both the mental and physical well-being of animals; they include: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury, and disease; freedom to express normal and natural behavior; and freedom from fear and distress.

responsibility is to ensure the training programme is adhered to, will increase the likelihood of sustained, effective training and maintenance of high-skill sets important for the teams' success.

1. Establishment of a successfully functioning confiscation management unit

The structure of the organisation needs to be defined within each jurisdiction, and a unit might comprise Ministry of Environment personnel, field rangers, customs and border officers, biosecurity officers, logisticians, drivers, animal handlers and carers, and/ or veterinarians. An effective management and communication structure is needed to work effectively with a strong team culture.

All roles and responsibilities within a confiscation unit must be defined, with job descriptions clearly outlined and a clearly publicised organogram that notes established authorities within the unit. Awareness of appropriate roles and responsibilities in agencies/ organizations to whom the unit must report or with whom the unit should coordinate is also essential for effective preparedness. In particular, there should be a close link between the decisions that confiscating authorities and their agents need to make and those that CITES Management Authorities need to make. There is the opportunity for close collaboration between the two bodies (if separate) that should be utilised.

Many factors often need to be considered before determining how a confiscated live animal should be managed. Consequently many different areas of expertise are required to ensure sufficient information for optimal decision-making. (6) Confiscating authorities and their agents are therefore encouraged to develop local, national, regional and international contacts and form a Confiscation Advisory Network (6) with specialists including:

- Taxonomic expertise to enable rapid and accurate identification to species/subspecies level
- Expertise on animal handling, particularly of venomous, large or aggressive species
- Medical and veterinary expertise on human and animal health, including quarantine, animal handling, anaesthesia, diagnostic/screening procedures, and vaccination requirements
- Wildlife rescue, husbandry and behavioural expertise
- Appropriate legal expertise
- Logistical expertise to advise on holding and transport.

To enable this, confiscating authorities are encouraged to proactively establish points of contact with:

- Local, regional, and international wildlife rescue/rehabilitation centres, zoo authorities and associations, and sanctuaries which may be able to provide expert advice and in some cases short- or long-term holding
- In-country World Organisation for Animal Health (OIE) focal points and government/university veterinary and wildlife departments, which may be able to advise on animal health, husbandry and welfare issues
- In-country CITES Management and Scientific Authorities, and the CITES Secretariat
- In-country wildlife crime enforcement and border authorities
- Occupational health and safety departments and public health agencies
- Other in-country wild animal and plant health and animal welfare agencies, organizations and advisory bodies as appropriate

In the context of confiscations, if a confiscating authority is in a country that is bounded by CITES regulations, then the CITES protocol for managing CITES-listed species should be adhered to, carried out under the 'Management Authority'.

2. Important Procedures For Routine Update

- Keeping current all contact lists e.g., for expert animal handlers or identification specialists, forensics teams, veterinarians, CITES, and rescue centers (examples of tables to collect contact details of experts to assist with animal confiscations can be found in Appendix 4)
- Maintaining all equipment, materials and consumables (including PPE) that might be used in a confiscation event
- Assessing inventory of equipment, materials and consumables and planning for the purchase of new items in a timely manner as required.
- Management of all information collected from seizure events into a centralised database (e.g., source, date, location, species, quantity, intended destination and purpose; where animals are being held and ultimate disposition; samples collected and where they are sent and results of any analyses) (7). This information can be used to analyse the effectiveness of the processes and procedures implemented. In addition, this type of data can also be recorded in a number of regional [e.g. the European Union's Trade in Wildlife Information Exchange (EUTWIX; www.eutwix.org)] and international databases (e.g., World Customs Organisations Customs Enforcement Network and World Wildlife Seizure Database (World wise) (8).

3. Staff Health

There is always a possibility of disease transmission between animals and staff. In order to minimize these, the following precautions should be taken for any team member who might be in the proximity of wild animals (i.e. at a confiscation):

Pre employment:

1. All new staff should have a thorough pre-employment health check for baseline information gathered by a certified and accepted doctor before commencing work. This could include the following:
    a. Full clinical examination
    b. TB test i.e. Mantoux test with follow up radiographs if required by the doctor
    c. Faecal parasite check and treatment as required
    d. Blood group and allergy assessment
    e. Evaluation of up to date vaccination status and vaccination as necessary i.e., tetanus, polio, measles, hepatitis B, rabies
    f. Other tests as deemed necessary by the doctor
2. Staff with compromised immune systems will be at higher risk of catching diseases and should not be involved in confiscations.

During Work:

1. All staff have a responsibility to ensure they minimize the chance of disease transmission to and from animals and avoiding activities that might result in injury. All staff should exercise personal hygiene at all times e.g. wash hands with soap and water after handling potentially contaminated objects and animals. Team managers must ensure materials are available to do so.
2. All care giving staff should wear suitable PPE when required, know where to access the PPE and know how to correctly put on, remove and dispose of PPE
3. All staff should receive a health check by a certified and accepted doctor once a year. This should follow the format of the pre-employment health check. The vaccination status should be updated.

4. Resourcing

Resources include funds, expertise, holding and quarantine capacity, and personnel. One of the most important factors that influences decision-making is the level of resources available to the confiscating management authority. Resources may vary depending on conservation and other national priorities and are finite, so authorities will need to consider what actions are possible and feasible. Outreach to external experts can provide support when needed if personnel resources are limited within a unit. These expert lists should be compiled ahead of time and updated regularly. Management of confiscated individuals may be transferred to responsible third parties managed under an agreement or MOU.

Resource considerations when deciding potential disposition of confiscated wildlife include:

- Are there sufficient resources to repatriate the animals to the country or area of origin to the standards needed for high health, safety and welfare?
- Are there sufficient resources available to implement a reintroduction/ translocation/ reinforcement program particularly for species classified as threatened?
- Are sufficient resources available to ensure long-term captive management of the individual to an appropriate welfare level?
- Does the proposed allocation of resources reflect the conservation status of the animals? If not, can we collate supporting documentation to lobby appropriate decision-makers to release more resources

Resources to improve Illegal Wildlife Trade detection:

Every effort should be directed to improving intelligence, including close scanning of all levels of social media, relationships with informants, and developing novel methods for the detection of the illegal wildlife trade and prosecution of cases. Where possible, the unit should develop and advertise hotlines so that people who suspect illegal wildlife trade can contact someone in authority and report it as soon as possible. Well trained and managed wildlife detection dogs can be an invaluable method to detect the illegal wildlife trade. This method of detection has been effectively applied in several jurisdictions, but requires the establishment of a specific unit aimed at resourcing and managing suitable dogs. Collation of robust intelligence helps to strengthen prosecution, and the more rapidly IWT is detected and wild animals are rescued, the better their chance of survival and positive conservation outcomes.

5. Staff training and Operational Protocols

Protocols, training manuals and training exercises should be established and implemented (Includes simulation exercises and role-play to ensure optimum confiscation occurs) for all people who work in the confiscation management unit on:

- Species identification
- Collection, storage and tracking of samples for DNA analysis
- Animal welfare following the Five Freedoms/Domains
- Safe and welfare-appropriate animal handling for highly trafficked taxa/ species
- Safe and appropriate short-term management and husbandry for key relevant taxa/ species
- Safe and appropriate transportation of key relevant taxa/ species
- Health and Safety
  - Principles of risk management
  - Methods of disease transmission
  - Personal hygiene and infection prevention and control
  - Use of PPE
- Securing a crime scene, collection of evidence and samples, and chain of custody maintenance
- Important contacts for expert support, coordination and reporting
- Deciding on disposition of confiscated wildlife

Protocols, training manuals and training exercises should be established and implemented for all people who work with wildlife during and after confiscation along the chain of custody in the following areas:

- Health and Safety
  - Principles of risk management
  - Methods of disease transmission
  - Principles of biosecurity
  - Personal hygiene and infection prevention and control
  - Use of PPE
  - Cleaning and disinfection procedures, both personal and for equipment

  - Zoonotic disease risk management including: – vaccinations required to work safely with wildlife taxa and which workers need to take special care (e.g. pregnant, immunocompromised, very young, elderly)
  - Recognising and reporting accidental exposures and signs of disease and when to seek medical advice.
- Recognising and recording animal care events such as management, feed, treatment, any signs of disease
- High standards of welfare and appropriate handling and husbandry for key relevant taxa/ species
- Important contacts for expert support
- Quarantine and isolation practices for at-risk wildlife
- Responding to sick or injured wildlife
- Assessing and managing biosecurity risks prior to the movement of wildlife
- Deciding on disposition of wildlife

The following are additional important protocols to have in place. These documents support the best practices for management of all species. The development of these manuals will allow for planned management of confiscated animals rather than an ad hoc approach. These documents should be updated as new systems, equipment, holding centres and science become available to improve on them. They should be available in electronic and written forms (to be carried on field operations) and should be updated following seizure events as needed:

- **Protocols for effective cross- country and cross- border counter illegal wildlife tracking collaborations**
  It is important to build relationships and agreements with all aspects of the confiscation unit including third parties. It is important to have ready access to a network of specialist consultants that can act as an advisory panel on species-specific needs and issues. An up to date contact list needs to be maintained. Temporary housing agreements can be pre made with local sanctuaries, rescue/rehabilitation centres or zoo authorities. (6). A register should be maintained and a program of inspection of the facilities needs to be conducted to ensure they comply with the standards expected.

- **Protocols and Manuals for Management and Husbandry of Commonly Confiscated Taxa**
  Within each jurisdiction, resources should be applied using local expertise and the network advisory group, to develop and keep updated a Management and Husbandry Manual for Commonly Confiscated Taxa which can be used when the identification of the confiscated species is confirmed. See appendix for template.

- **Protocols for Storage and Management of Equipment**

All equipment required for the confiscation of wildlife should be obtained, managed and stored appropriately including vehicles, animal transport containers, animal handling equipment, communication equipment, computers, cameras, PPE etc. An inventory and register are maintained and specific personnel are designated to this task.

- **Protocols for Storage of Samples**

  All samples collected as part of the chain of custody are to be stored appropriately so as to support a prosecution and increase likelihood of a conviction. This will require specific rooms and buildings, or laboratories or police units, specific freezers etc. A standard labelling system for samples should be agreed upon and understood across the unit. A computerised database should be developed on all confiscation events and include details on case no, date, GPS location, species, quantity, disposition and a computerised register of samples related to a specific confiscation event case should be maintained and allocated to a specific person and updated when samples are moved during any testing or court cases.

II. Pre-seizure Planning and Preparation

Pre-planning and preparation for a seizure is the most important step in the confiscation process and requires a well-rehearsed and informed team[11]. Both individuals involved in this industry and the animals being illegally traded pose a considerable threat to the teams involved in the seizure, through potential physical injury and/ or exposure to potentially zoonotic pathogens. Furthermore, planning is critical in ensuring that adequate evidence is collected during the seizure to obtain a later conviction, should presentation in court be required. For this reason, it is also vital that detailed, real-time records regarding all stages of the process be taken during the seizure.

1. Health Risks to Humans

Health status of staff carrying out operations

Pathogens can pass from confiscated wildlife to humans, but also from humans to wildlife. The following guidelines reduce health risks for both:

- Staff carrying out planned live animal confiscations should be vaccinated for a minimum of rabies and tetanus
- Any staff participating in primate confiscations must be free of tuberculosis (TB). Staff participating in primate confiscations should be tested for TB every 6 months, typically by an intradermal tuberculin skin test.
- No persons with any current or recent (within a few days) clinical signs of illness (coughing, sneezing, fever, diarrhea, rash, cold sores, etc.) should participate in the confiscation
- Staff with compromised immune systems will be at higher risk of catching diseases and should not be involved in confiscations.

The following is excerpted from Guidelines for the Safe Handling of Wildlife and Wildlife products during Counter-wildlife Trafficking Enforcement Operations (WCS 2021)(9):

The types of operations where law enforcement officers may come into contact with wildlife vary widely, from being in the same room as wildlife during an operation (e.g. during inspection of a wildlife trading facility, or at customs); to a search of a market or suspected wildlife criminal's property where it is anticipated wildlife products or live animals will be detected and seized. For each situation the level of health risk varies and precautions that need to be taken change accordingly. The key factors influencing health risk that need to be considered prior to the operation are (i) the type of operation, (ii) the environment in which the wildlife is present and (iii) the species of wildlife present:

1.1 Type of operation

If an operation involves handling or close contact with live wildlife, level of risk is high due to the risk of injury and disease transmission through bites, scratches, splashes to the face with urine, faeces or saliva and aerosols (e.g., exhaled air or coughs/sneezes from animal)**.** When dead, fresh wildlife is handled, there is still a disease risk, however, risk of injury and disease transmission is lower. With dry wildlife products (no longer containing tissues or secretions) e.g., ivory, rhino horn, bones, pangolin scales etc., the health risk is low.

1.2 The environment in which the wildlife is present

Indoor spaces, especially those with limited air flow (e.g., trader's house, restaurant, airports, warehouse) are environments that are high risk for transmission of airborne pathogens. Outdoor environments are usually lower risk for transmission of airborne pathogens if there is good air flow (e.g., border crossings, road check points). If an outdoor area is crowded and staff are likely to frequently come within 2 meters of animals or people, the risk increases (e.g., crowded markets).

1.3 The species of wildlife likely present

Certain species pose a higher risk for either causing injury or carrying serious diseases that can infect humans. Enforcement staff must assess when a situation poses or represents a higher risk by understanding which species are most likely to cause injury or carry serious zoonotic diseases, and how the diseases are transmitted.

Risk of injury: All wild animals have the potential to cause injury to people who handle and/or have close contact with them. Large (> 15kg) or venomous wildlife species are most likely to cause injury. This includes venomous snakes, medium to large felids and bears. All primates and carnivores > 3kg are considered high risk due to their speed, dexterity or potential aggressive nature. Expert assistance from a rescue center or zoo is a requirement for confiscation of these species, to allow experienced handling and potentially chemical immobilization. If the species of snake cannot be identified, treat it as venomous.

Risk of disease: All mammals and birds pose a risk of carrying serious diseases that can infect humans (See Appendix 2). Primates, bats, rodents and carnivores are mammals of particular concern. Although reptiles (turtles, lizards, snakes) can also carry zoonotic pathogens, human infections can usually be treated and the diseases are rarely fatal.

2. Risks to Live Wildlife

Animals are likely to become stressed and possibly struggle when captured or handled. This raises the risk of the animal overheating, especially for mammals in a hot climate. Conducting confiscations at cooler times of day is advisable, as is having cold water ready to cool the animal if they start to overheat. If mammals cannot be caught quickly, the confiscation may need to be postponed to avoid hyperthermia. To reduce risks of injury to personnel and/ or animals, it is essential that the correct PPE, handling, restraint and holding equipment is available to confiscation units, and that they have been trained in its use. PPE, equipment and appropriate usage are summarized in Chapter 3 of these guidelines.

3. Establishment of Operational Protocols

A protocol for preparation including an **operational checklist** should be developed and implemented prior to any confiscation event to facilitate a smooth and safe confiscation and collection of adequate evidence and records for potential subsequent prosecution. The checklist below is an example:

1. What information needs to be shared with the team or local partners– have you done a briefing?
2. Do all personnel know their roles in the seizure?
3. Is the key contact list updated and do you have the contact details of all expert personnel who might be needed?
4. Who is in charge of the seizure event?
5. Is the intelligence on the illegal trade valid and accurate?
6. What species are involved and estimated numbers?
7. What's the condition and circumstances of species involved?
8. Where will the confiscation/seizure take place?
9. When will the confiscation/seizure take place?
10. Who will be involved?
    a. Law enforcement officers
    b. Police
    c. Wildlife handlers
    d. Logistical planner (transport, housing)
    e. Veterinarians and veterinary technicians
    f. Taxonomy specialists
    g. Species-specific wildlife expert
    h. Regional quarantine representative
    i. CITES liaison (6)

11. Who needs to know what? Has everyone that needs to know been alerted?
12. Do you have the means to effectively communicate before, during and after the seizure?
13. Do you have means of recording events and ensuring an effective crime scene including cameras, videos, note pads, voice recorder, species identification, numbers, animal identification?
14. Who will be assigned to undertake the photography and note taking of the crime scene?
15. Do you have all the equipment necessary for the initial seizure? Is this equipment clean and disinfected, in working order and ready to use?
16. Health and Safety
    a. What health and safety needs to be considered for the animals involved
    b. Are the suppliers/intermediaries potentially dangerous – are you prepared for all eventualities?
    c. Do you have sufficient PPE to protect against zoonotic diseases and dangerous animals?
17. Have you organised where the animals are going to be moved and how they are going to be transported?
18. Do you have all the personnel and resources to move animals to the holding facilities?
19. How will the holding facilities be managed?

III. Protocols Required During and After Confiscation

At the confiscation scene, law enforcement personnel and animal carer personnel and veterinarians should work closely following pre-determined protocols as best as possible to make an assessment of the situation.

1. Initial Assessment Protocol

Designated team members should make an initial observational assessment of the scene. This will include the species (where known; if unknown include genus or family), quantity of animals, observational health status and housing of specimens; assessment of risks; list of key evidential samples to collect.

For guidance on securing the crime scene and the collection of key photographic, video, note and sample evidence to be collected during a confiscation event, please refer to: Standard Operating Protocols to Support Conservation, Health, Welfare & Prosecution of Wildlife Crimes Part II: Live Wildlife Crime Scene Investigation.

Once an overall assessment has been made, and taking into consideration chain of custody evidence, the next steps include ensuring high standards of biosecurity, safe and welfare-appropriate handling, examination and treatment of animals, species identification, specimen and record collection.

Chain of custody

This term refers to the continuous documentation of the custody, transport, transfer, analysis and final deposition of any item of evidence. Failure to adhere to this process may lead to evidence not being accepted in court. Each item of evidence should have its own chain of custody form that is sent along during transfer of the item. The form should include the following information:

- Description of item
- Date/Time/Names/Signature of individual and agency transferring and receiving item
- Purpose for transfer
- Analysis performed
- Any alteration of the item

2. Species Identification Protocols

Animals confiscated from illegal trade are often mis-identified. Identifying a specimen in the hand is harder than identifying a live specimen in the wild due to the often unknown origin location, associated habitat, and behaviour cues usually apparent in healthy, wild species (10,11). Accurate identification of individuals can be extremely challenging, particularly when the number of animals to be identified is large and they are similar in appearance; however, decisions based around conservation status, health and origin for example, require precise identification and consideration of each individual animal. Without knowing the exact species, decisions on animal care, disposition and sometimes prosecution are severely limited. Accuracy is more important than immediacy because the success of a court case may depend on the correct identification. Identification of the species contributes to accurate trade data and is the first step to regulating trade of species in accordance with CITES. Adequate training of management authorities and law enforcement officers in identification is thus essential.

There are many references, online image databases, online and stand-alone computerised keys, guides, posters etc on species identification but a systematic approach needs to be taken to ensure the species or taxa can be identified, and a standardized methodology to facilitate identification will improve consistency and accuracy.

Appendix 5 of this document 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. Personnel need to have been trained in the method for identification selected by the confiscation unit, have the correct equipment and manuals, understand basic taxonomy, look for obvious physical characteristics.

In order to identify species, manuals and guides can be used, but identification keys (e.g. Figure 2), including dichotomous keys protocols need to be developed and available with the help of local taxonomic experts e.g., from a natural history museum or university. Taxonomic experts for highly-trafficked animal families need to be available at a confiscation event or for rapid consultation to support accurate identification. Sometimes identifying species is not possible e.g. young animals or eggs or hatchlings in which case supporting of the animals until they reach an age where they can be specifically identified might be the only option.

Once the identification of the species is confirmed, an action plan for the individual can be developed. Management decisions should be made based on the population of origin, not necessarily the country of origin.

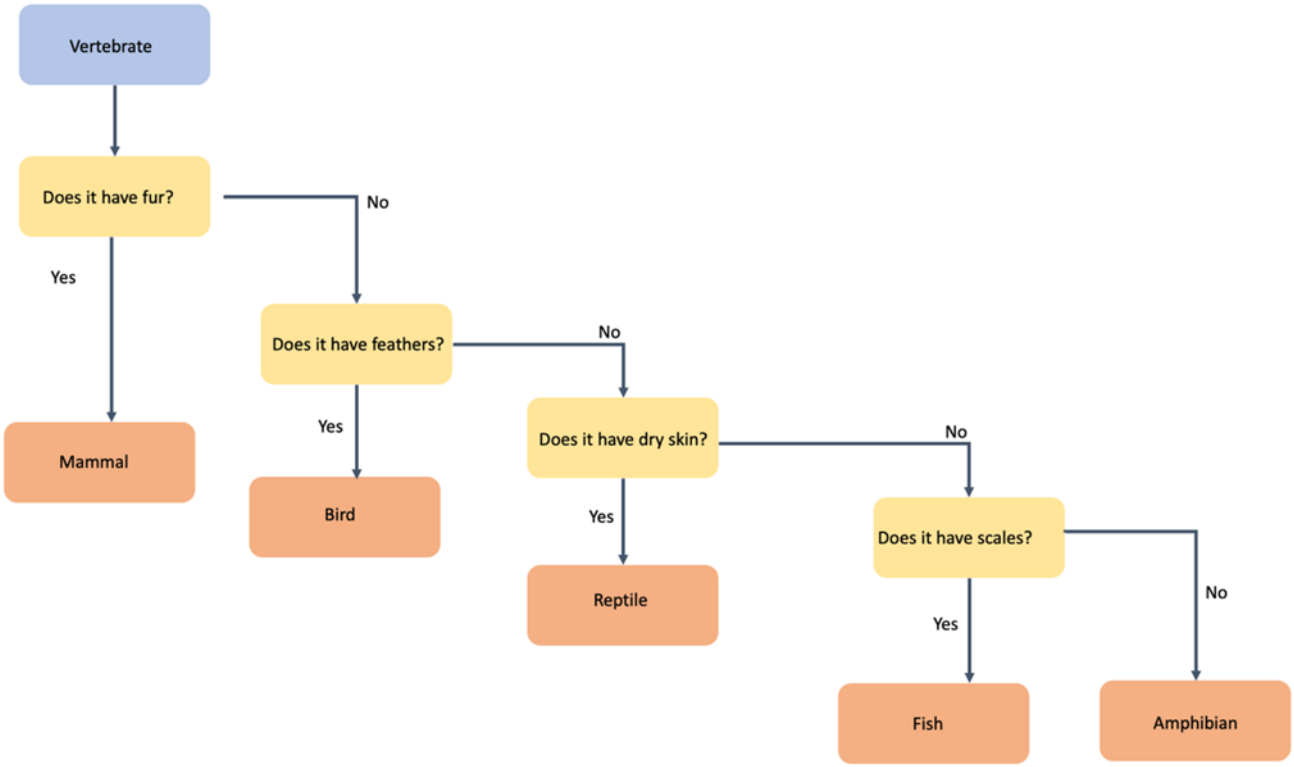
NB A confiscated live specimen whose species identity is not known (even if suspected) should never be released into the wild as this poses an unacceptable risk to global biodiversity. Unless there are strongly supported mitigating circumstances, individuals in general should only be considered for release in their native range, and, if known, within their population of origin.

These restrictions are necessary in order to avoid introducing alien invasive species, novel pathogens, causing genetic pollution, and altering the genetic structure of the species.

Examples of Species Identification guides:

CITES Wiki Identification Manuals
CITES reference manuals
Birds
Dragons
Tortoises and freshwater turtle
Key to the main groups of lizards and snakes

Figure 2**.** A dichotomous key for differentiating between animal classes.



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

3. Unique Animal Identification/ Marking and Information Collection

Keep good records for confiscated animals. The minimum information should include species (or genus/ family if unsure), date and location of where animal was confiscated, condition and any trauma or health concerns and if they were mixed with other species. Ideally each animal should be given a unique ID for record keeping so the animal can be tracked.

Identification of live animals can be achieved by tagging or tattooing ears, flippers, or wings, banding birds or implanting microchips or PIT tags. These should only be performed by well-trained professionals. Combining these forms of identification along with a labelled, recorded and tracked photographic collection of the animal helps to ensure traceability when animals are moved and to ensure chain of custody evidence is maintained. 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 (12). The microchip is implanted under the skin using a wide gauge hypodermic needle and read with a hand-held reader (12). 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.

Confiscation units should have documented protocols for:

- Identification (or marking) of individual animals e.g. tattoo, non-toxic paint, pit tags/ microchip/ leg band/ other method
- Recording relevant written information on individual and group animal cases (including location, health information, samples collected, treatments
- Maintaining and storing records
- Reporting suspicious signs of disease and unexpected or unexplained deaths in wildlife to appropriate authorities
- Contact list for local laboratories who could provide advice on sample collection, storage and diagnostics if confiscated wildlife showing symptoms of disease.

4. Health and Safety Protocols

Biosecurity and the use of PPE are very important to reduce sharing of pathogens across different species and taxa and to reduce injury in confiscation personnel. Good biosecurity includes ensuring diseases are not transmitted from humans to animals, as well as from animals to humans.

Understanding and managing risk is key for good biosecurity. The best way to manage infectious disease risks is to understand both the hazard (pest or disease) and the available options for minimising risks to a safe or acceptable level. A knowledge of potential pathogens that are associated with animal species and environments allows workers to: i) identify hazards ii) assess the risk associated with each hazard iii) implement appropriate infection control measures to

ensure both animal and human health risks are properly managed iv) monitor and evaluate the control measures regularly. Risk management guidelines have been developed by IUCN (13) which should be considered at all times during the confiscated animal seizure-holding-quarantine- disposition continuum.

4.1 Managing biosecurity risk associated with confiscated wildlife

Management of confiscated wildlife is considered to pose a high biosecurity risk. In addition to the physical risks associated with the necessary human intervention, the biosecurity risks are amplified because:

- The disease status of each animal is unknown
- individuals may have an unknown and untraceable history and provenance
- there is a high likelihood of previous inappropriate or substandard husbandry, housing, grouping and poor biosecurity
- individuals may have been illegally transported, including across state or international borders
- individuals may have been illegally collected from the wild
- individuals may have been "abnormally" mixed with other individuals or species of similar or greater risk. Assessing and managing the biosecurity risks associated with confiscated wildlife is challenging due to the high number of unknowns.

Standard practice is to apply the precautionary principle and assume a high biosecurity risk, unless concrete information is available to indicate a different approach. In some cases this will involve euthanasia of the animals and appropriate disposal of the carcass to destroy any infectious agents that may be involved. In some circumstances (e.g. for some endangered species or for legal reasons), it may be necessary or desirable to hold the live animals in an appropriately biosecure facility. It should be kept in mind that confiscated animals may be infected with multiple pathogens, including novel pathogens or those for which there is no known testing regime. For these reasons, it is recommended that confiscated animals should be maintained in the strictest isolation and receive full veterinary inspection and investigation.

Confiscation authorities should have documented protocols for:

- Personal hygiene, including hand hygiene (see below)
- Biosecurity management of clothing, boots and other personal equipment
- Selection and use of Personal Protective Equipment (Example below)
- Equipment hygiene
- Facility hygiene
- Waste management
- Managing biosecurity risks associated with sick wildlife, carcasses and biological samples.

Confiscation units should have documented protocols for:

- Zoonotic disease risk management
- Worker training programs around zoonotic disease risk management
- Bite/scratch protocol and staff health monitoring after an operation, including reporting and notifying suspected zoonotic disease of wildlife origin in a wildlife worker
- Worker disease screening and/or vaccination

Appropriate hand washing is the simplest proven method for reducing risk of contracting a disease. Plan for washing in the field—bring supplies to confiscation events (water, soap, bucket, paper towels and or sanitizing gels or wipes). Below guidance is from the USAID PREDICT Safety Guide for Biosafety and PPE Use (14)

Always wash your hands before:

- Handling animals
- Preparing food
- Eating
- Treating wounds or administering medications
- Contact with a sick or injured person or animal
- Inserting or removing contact lenses

Always wash your hands after:

- Touching an animal, samples, waste, products or animal equipment
- Collecting and handling diagnostic samples
- Visiting a wet market or farm
- Working with hunters and handlers of animals or animal products
- Preparing foods, especially raw meat or poultry
- Using a toilet
- Blowing your nose, coughing or sneezing into your hands
- Treating wounds
- Touching a sick or injured person
- Touching garbage or other contaminated materials

Hand Washing Technique:

- Wet hands with water (turn on water source with paper towel or clean handle during washing process)
- Use liquid, bar or powder soap
- Work up a lather for 15-20 seconds
- Wash lower arms, wrists, and under and around nails
- Rinse hands
- Turn off water source with paper towel or clean handle
- Dry hands with a fresh paper towel
- Do not take the paper towel with you (dispose of onsite/ collect in trash bag).

4.2 Protocols for the use of Personal Protective Equipment (PPE)

(from: Guidelines for the Safe Handling of Wildlife and Wildlife products during Counter-wildlife Trafficking Enforcement Operations (WCS 2021)(9))

4.2.1 Why use PPE?

If employed correctly, PPE forms a barrier between you and the animal that you are confiscating. Zoonotic viruses or bacteria can be transmitted from animals to humans in the air an animal breathes out, in splashes of saliva, urine or faeces, and via bites or scratches. Therefore, it is important that the air you breathe is filtered, that your skin, eyes and mouth are covered to protect from splashes, that you have a layer of clothing that can be removed at the operation site once the operation is complete, and shoes that can be washed. Figure 3 shows examples of full and partial PPE.

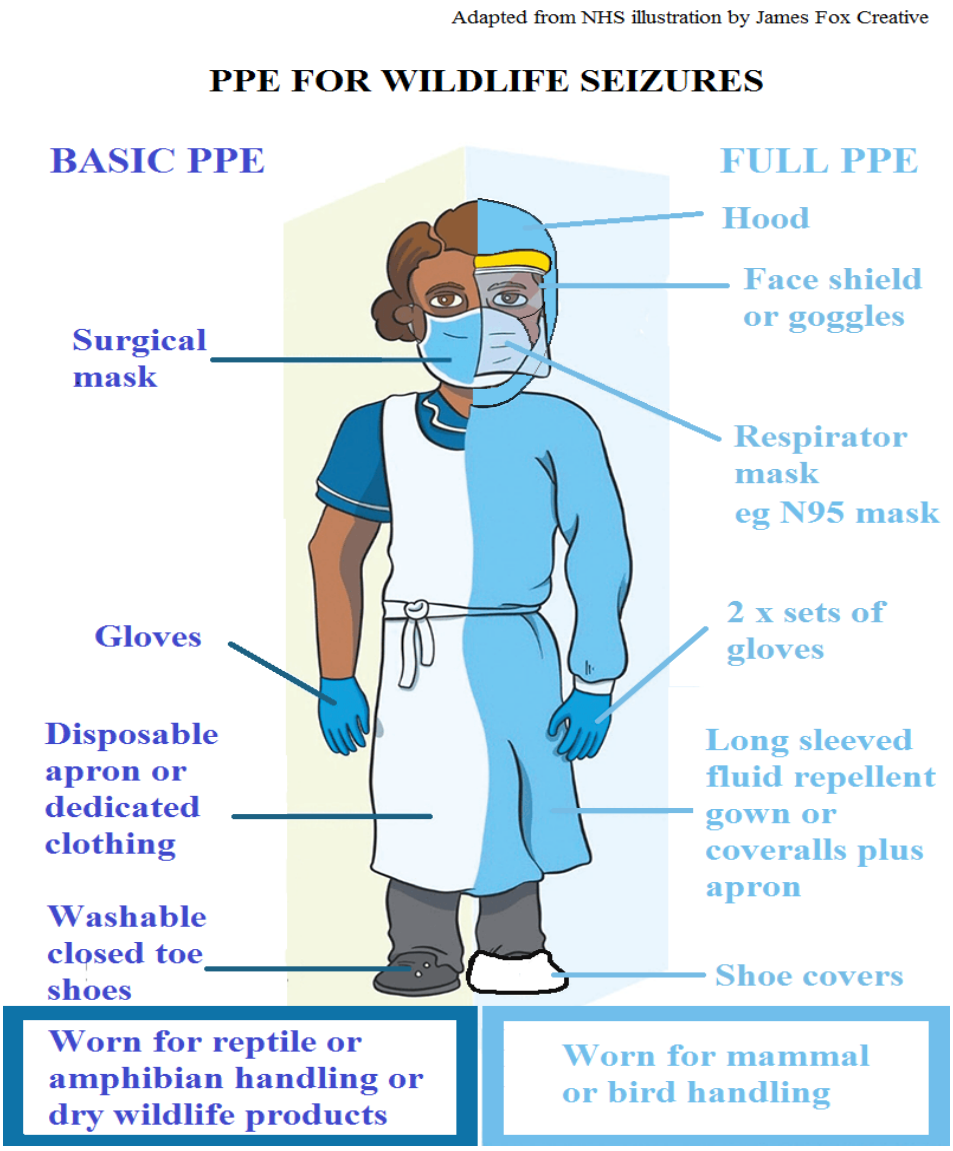


Figure 3. Full and basic PPE to be used in wildlife seizures. Basic PPE is used for live or fresh dead reptile or amphibian handling or dry wildlife products. Full PPE is used for live or fresh dead mammals or birds.

It is vital officers put on and remove PPE correctly to ensure they are properly protected. All law enforcement officers working with wildlife should have attended training on the correct use of PPE prior to any operation. The following video shows how to put on and take off PPE safely, including hand hygiene and important viewing. However, this is not a replacement for training:

https://www.cdc.gov/vhf/ebola/hcp/ppe-training/n95Respirator_Coveralls/donning_01.html

PPE such as gloves, steel toe capped shoes and long trouser legs and sleeves can also offer some protection from bites and scratches but should be combined with careful and correct wildlife handling to avoid injury. Table 1 lists recommended PPE and equipment selection depending on the wildlife species being confiscated. Further information on equipment is given in Section 2.7.

4.2.2 Choosing the correct level of PPE for an operation

In order to assess risks, it is preferable to find out as much information about the species of wildlife, environment and type of activity before an operation begins. However, this will not always be possible. Having masks, nitrile gloves, washable shoes and 80% + alcohol gel ready for any operation will allow an operation site to be entered and for further risk assessment to be made. Both surgical masks and respirator masks should be carried.

- Surgical masks should comply with the standards of the region from which they are purchased (European standard EN14683, US standard ASTM, Chinese standard YY 0469).
- Respirator masks are graded according to their filtration performance. The following grades of mask would be suitable for enforcement operations as they all significantly reduce the risk of inhaling an infectious aerosol (by >93%). The masks should comply with the standards of the region from which they are purchased:
    - N95 (US standard NIOSH 42CFR84)
    - FFP2 (European standard EN149)
    - KN95 (Chinese standard GB2626)
    - Korea 1ST Class (Korean standard KMOEL-2017-64)
    - DS2 (Japanese standard JMHLW-2000)
    - P2 (Australian standard AS/NZS1716)

For indoor sites (e.g., traders house, restaurant, airports, warehouse), a respirator mask must be worn. For crowded outdoor sites where enforcement staff are likely to frequently come within 2 meters of animals or people (e.g., crowded markets), a respirator mask must be worn.

For outdoor sites with good airflow (e.g., border crossings, road check points), a surgical mask can be worn.

Table 1: Summary of PPE and wildlife handling equipment to be used by wildlife taxa

Form of wildlife	Class	Sub-group	Sub-group	Example species	PPE to use	Wildlife handling equipment needed if live animals
Live or fresh dead	Mammals	Primates	Large >15kg	Large macaques, orangutan	Full PPE	Seek expert help
			Medium 3-15kg	Some macaque species, gibbons, langurs	Full PPE	If handling necessary, seek expert help
			Small 0-3kg	Lorises, Tarsiers	Full PPE	Soft nets, gauntlet gloves, transport crate
		Carnivores	Large >15kg	Bears, large felids	Full PPE	Seek expert help
			Medium 3-15kg	Leopard cat, civets, otters	Full PPE	If handling necessary, seek expert help
			Small 0-3kg	Martens, weasels, mongoose	Full PPE	Nets, gauntlet gloves, shield, cage door barrier, transport crate
		Rodents		Squirrels, rats	Full PPE	Soft nets, gauntlet gloves, shield, cloth bags, transport crate
		Bats			Full PPE	Gauntlet gloves, towel, fine net, torch, cardboard transport box
		Pangolins			Full PPE	Smooth sided transport box, towel to cover box, gauntlet gloves
	Birds	Poultry/ waterfowl			Full PPE	Soft non-knotted mesh net, towel, transport crate
		Raptors		Owls	Full PPE	Gauntlet gloves, towel, transport crate
		Psittaciformes		Parrots	Full PPE	Soft non-knotted mesh net, towel, transport crate
	Reptiles	Snakes	Venomous	Cobra, viper	Basic PPE	Use snake specialist – seek expert help
			Non-venomous	Python, boa	Basic PPE	Snake hook, clear plastic tubes, plastic shield, snake tongs, hessian bags/pillow cases, transport box
		Turtles/ tortoises			Basic PPE	Gauntlet gloves, transport crate
		Lizards		Monitor lizards, water dragons	Basic PPE	Net, gauntlet gloves, transport crate

For SEIZURE of mammals or birds (live or fresh dead), full PPE should be worn by the personnel handling the wildlife or personnel coming within 2 meters of the wildlife. Full PPE includes a respirator mask, face shield or goggles, long sleeved fluid repellent gown or coveralls with hood, nitrile gloves, washable closed toe shoes and shoe covers (see Figure 3).

For SEIZURE of reptiles or amphibians (live or fresh dead) or dry wildlife products (those no longer containing tissues or secretions) basic PPE can be worn by the personnel handling the wildlife or personnel coming within 2 meters of the wildlife. This consists of a surgical mask, nitrile gloves, disposable apron or dedicated clothing (that can be removed at the end of the confiscation) and washable closed toe shoes (see Figure 3).

4.2.3 Practical PPE solutions

If law enforcement officers cannot access the PPE outlined above, there are some alternatives:

- Where gowns/coveralls are not available, a plastic rain poncho that covers body, arms, legs and head can be used and disposed of at the end of the operation. Care must be taken not to contaminate the wearer when the poncho is pulled over the head at the end of the operation.
- If dedicated clothing/cotton coveralls are not available, any normal clothing with long trouser legs can be worn. At the end of the operation, the clothes must be removed in the PPE removal area and washed in a work washing machine. Care must be taken not to wear the clothes away from the enforcement operation site.
- If visors are not available, sunglasses can be used to protect eyes from splashes.
- If aprons are not available, plastic bin bags can have a hole cut out for head and arms and worn over dedicated clothing. It must be disposed of at the end of the operation.

4.2.4 Reusing masks

Reusing respirator masks is possible but should only be done as a last resort in crisis situations where there is a shortage of available PPE. Care should be taken to check that after cleaning the mask still fits the users face well. The following 3 protocols for reusing masks have been trialled for COVID-19 however their effectiveness against novel pathogens is unknown;

- Store the used mask for 3 days at room temperature (21–23°C) and 40% humidity. All SARS-CoV-2 viruses on the mask will be dead in 3 days (15)
- Heat mask for 60 min at 70°C by hanging the mask in an oven using plastic or wooden clips. Masks need to be > 6” from the walls of the heater to prevent mask degradation (16)

- Boil masks for 5 minutes and then air-dry. The elastic band should not be immersed in boiling water. Do not stir while boiling to avoid disturbing the physical structure of the mask (16).

4.3 Bite/scratch protocol

If a member of staff gets bitten or scratched, the following should be done:

- Injured person notifies other staff and work stops
- The bite or scratch should be washed for 5 minutes with soap and running water. In the event of a macaque bite or scratch, wash for 15 minutes with povidone-iodine.
- Apply an antiseptic with anti-viral properties to the wound e.g., iodine-based disinfectant such as povidone-iodine
- If mucous membranes become contaminated with a splash of animal urine/faeces/saliva then use eyewash/saline to do a 5 minute continuous flush. If the splash is from a macaque, then do a 15-minute continuous flush of any exposed mucous membranes
- If the injury/bite is from a bat or carnivore then get post-exposure rabies vaccination as soon as possible (within 24 hours)
- Consult a doctor if a bite or scratch has penetrated the skin barrier as antibiotics will likely be prescribed
- 24 hours after the bite/scratch occurs, ensure staff member is examined by doctor to assess for swelling, pain, heat, fever
- If the bite or scratch is from a macaque or from a primate that has been housed with macaques then B Virus emergency protocol must be triggered immediately.

After an enforcement operation involving wildlife, if any enforcement officer or those involved in the confiscation event feel sick they should seek medical attention immediately and inform the doctor that there has been contact with wildlife. If a breach of PPE occurred during the operation and known exposure to wildlife occurred, staff should seek medical advice immediately

5. Handling and Restraint of Animals

(from: Guidelines for the Safe Handling of Wildlife and Wildlife products during Counter-wildlife Trafficking Enforcement Operations (WCS 2021))

Live imported animals 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. Wildlife capture and handling has the potential to result in serious injury to both the handler and the animal. It should only be undertaken if absolutely necessary. In all operations, handling of live wildlife should be minimized by doing the following:

- Confiscate the animal without handling it directly e.g., if it is already in a cage that can be moved, use this rather than moving the animal into another transport cage
- Enlist external expertise to assist e.g., zoo/rescue center personnel or other experienced personnel familiar with handling the species (e.g., pet shop owner, wildlife trader or wildlife farm staff)
- Frontline staff should have access to accredited handlers for venomous snakes and other high-risk animals. A list of such experts should be included in the SOPs for enforcement officers and customs/ border control (17,18).
- If external expertise is not available on the day of the operation, maintain the animal on site (i.e., at the point of seizure) until assistance is available from experienced personnel
- Avoid physical capture of animals by encouraging an animal to enter a transport crate using patience or food as enticement

If enforcement personnel feel that there is no option but to handle the wildlife, the following criteria must be met, <u>before the operation begins.</u> The officers who will handle the wildlife should:

- Understand the potential disease risk posed by the environment in which the operation is occurring
- Understand the potential disease and injury risks from and to the wildlife on site
- Have the appropriate training and experience to handle the species of wildlife present, and have access to appropriate wildlife handling equipment
- Have access to, and understand how to use appropriate Personal Protective Equipment (PPE)
- Understand how to manage bites/scratches from wildlife
- Feel confident that the health risks of the operation can be mitigated

If the above criteria are not met, then the operation involving wildlife should not proceed. If enforcement staff are not properly trained, live animal handling should not be attempted.

Under no circumstances should confiscations of wildlife > 15kg (medium to large felids, bears) knowingly be undertaken without expert assistance, to allow for experienced handling and chemical immobilization if needed. If primates or carnivores 3-15kg cannot be confiscated without handling (e.g. by encouraging an animal to enter a transport crate using patience or food as enticement), expert assistance for handling should be sought. Under no circumstances should confiscations of venomous snakes knowingly occur without the assistance of trained, expert snake handlers.

Physical handling of animals can often be avoided by encouraging an animal to enter a transport crate using patience or food as enticement. This is always the preferred option if possible, as it minimizes risk to both handler and animal. Animals are likely to become stressed and possibly struggle when captured or handled and cold water should be ready to cool the animal if they start to overheat. If mammals cannot be caught quickly, the confiscation may need to be postponed to avoid hyperthermia. Here we provide example guidelines for handling commonly confiscated taxa.

5.1 Mammals

Small mammals **(<3kg):** e.g., loris, mustelids, squirrels, rats.

- Gauntlet gloves and a secure neck grip are often sufficient for this group of mammals.
- If a net is used, fine mesh is needed to ensure claws don't get entangled.
- Once in the net, grasp the animal securely through the net around the head and neck and carefully remove the net.
- Small mammals have sharp teeth – handling must be done quickly to prevent the animal from turning its head and biting.
- Take care, if wearing thick gloves, not to exert too much pressure which can restrict the animal's breathing.

Bats:

NB: Flying foxes and other bats can carry lyssaviruses (including Rabies). Transmission is via bites and scratches from infected animals, or if saliva from infected animals contacts broken skin, the eyes, mouth, or nose.

- If in small enclosure throw towel over bat to restrict flight, then grasp with gauntlet gloved hands.
- If in a big enclosure where flight is possible, shine a bright light on a perched bat to daze it then grasp with gauntlet gloved hands.
- If nets are used the hoop must be big enough to allow open wings to pass through easily and the mesh should be fine.
- Bats can be transported in cardboard boxes.

Safety consideration for bat handlers-

- Suitable gloves are essential to protect hands
- Protective eyewear and face coverings should be worn
- When selecting gloves, size of the bats, dexterity, and puncture resistance need considered
- Split-leather cowhide work gloves are the most puncture-resistance of the gloves and are suited to handling bats weighing 40 up to and including 100g. These gloves are low dexterity.
- Deerskin work gloves have both good puncture-resistance and dexterity for handling small to mid-sized bats weighing 4 up to and including 40g.
- A puncture-resistant gauntlet or sleeve protector will also protect the forearms and the back of hands from bites and scratches
- Handlers should be up to date with Rabies Vaccination.
- Handlers should be properly protected by donning the correct Personal Protective Equipment (PPE).
- Cover all wounds and cuts
- Careful hand washing after handling the animals
- Long sleeved shirts and long pants to protect the arms and legs
- Enclosed shoes

Medium mammals (3-15kg):

Medium sized primates eg gibbons, langurs, some macaques

- Primates should be enticed into a transport cage or self-closing cage using food.
- Handling with nets and gloves should only be a last resort and must only be attempted by very experienced handlers as the risk of injury to both handler and animal is high. Contact experts for assistance if handling is necessary.
- Chemical immobilization using a blow pipe or jab stick is preferable to using nets and gloves but should only be attempted by experienced personnel from a rescue center or zoo.
- Medium sized carnivores eg leopard cats, civets, otters
- Carnivores should be enticed into a transport cage or self-closing cage using food.
- Handling with nets and gloves should only be a last resort and must only be attempted by very experienced handlers as the risk of injury to both handler and animal is high. Contact experts for assistance if handling is necessary.

Pangolins

- Pangolins are shy and likely to curl into a defensive ball when handled – don't let the animal curl around your arm as it can be painful.
- Pangolins can be easily moved when curled up. If they uncurl, hold by the tail with one hand, the other hand supporting under the body.

- They have sharp claws that can cause cuts and scratches so leather gloves should be used.
- Pangolins get stressed very easily and stress can lead to fatalities. It is very important to keep stress to a minimum by keeping people away from the animals, keeping quiet, and covering the box with a towel to reduce visualization of handlers.

Large mammals (>15kg): e.g., bears, large primates, large carnivores.

Expert help should always be brought in. No human contact during confiscation, chemical immobilization often needed.

5.2 Birds

The primary concern when handling captured birds should be their health and wellbeing, and all efforts should be made to avoid stress and injury. Birds can be captured using netting techniques, or a towel if cornered, being mindful of the number of attempts and distress induced. Use approved handling techniques such as the ringer's hold to control the head, legs and wings, whilst maintaining a quiet and calm environment.

- Stress in birds can be severe so keep handling time to a minimum. Stressful handling can cause a significant increase in temperature and respiratory rate. Many of these animals may already be ill or malnourished and a stress response could potentially compromise their immune system further, placing the animals more at risk of developing disease
- Birds do not have diaphragms and so if squeezed too tightly they cannot breathe. Excessive restraint should be avoided to eliminate potential for respiratory stress from thoracic compression. Take care when using gloves and towels not to restrict breathing.
- For tame or domesticated birds, allow bird to step up onto your hand by placing your hand or finger under their chest. Restrain by gently holding toe between the thumb and finger (19)
- As most illegally traded wild birds are not tame, capture with a towel or net is appropriate (20).
- If in a small cage use gloved hands and a towel.
- If in a large cage or aviary, use a soft, non-knotted hoop net to catch bird.
- Birds are very fragile and a hoop net can easily fracture a bird's leg or wing if not used gently.
- Once in the net, hold birds head or beak while carefully removing the net.
- If handling larger species with long beaks such as egrets, wear eye protection (see below for raptors).
- Secondary personnel may be required for larger, more aggressive/active animals.
- Birds may be lightly rolled in a towel or calico bag for additional restraint
- For towelling: select an appropriate sized towel for the bird, e.g., budgies can be held with a face cloth; cockatiels with hand towels; macaws with bath towels (21). Place towel quickly

and gently on top of the bird to minimise chance of escape and duration of handling (20). Once covered, the bird can be gently restrained through the towel and transported to a cage/aviary (22).

- Note that catching a bird from above or behind is considered stressful as it mimics a predatory attack, but it can be difficult to catch wild birds without using this technique (23).
- Avoid damage to feathers: birds use feathers for maintaining body heat as well as flying.
- Place birds in a dark box or towel-covered holding cage for calming
- Line box/crate with a towel or newspaper to allow the bird to grip and prevent further slippage during transport.

Raptors/ Birds of Prey

NB: Birds of prey have strong, sharp beaks and talons that pose significant risk to humans. If raptors are being confiscated, wear gauntlets and make sure the legs and talons are controlled.

- Thick gloves/ gauntlets and a towel are required (24).
- Grasp legs first and then the head.
- The initial grasp to restrain the legs should be above the stifles, close to the body to prevent leg fracture (especially important in long limbed species). Placing the index finger between the legs provides a secure grip that is comfortable for the bird (24).
- Proper restraint for examination requires 5 points of contact, two wings, two feet and control of the head (24)
- A towel can be used to cover the bird's head and wrap the wings.

Parrots

All personnel involved should be aware of the risk of zoonotic diseases which can be contracted either directly from parrots or indirectly from contaminated materials and air (25). In any facility holding parrots, personal and environmental hygiene measures should be in place. In addition, any personnel with impaired immunity should avoid any direct or indirect contact with birds (25).

5.3 Amphibians

Amphibians are highly susceptible to stress, so handling should be kept to a minimum. Where it cannot be avoided, handling must be as short, gentle, and quiet as possible. If the animal is, at any time, showing signs of acute stress, such as tonic immobility or overheating, the patient should be temporarily released.

- Disposable, non-powdered gloves should be worn when handling adult amphibians, and ideally changed between handling successive animals to prevent pathogen transfer, both between animals and to the handler.
- Gloves also double as a protective measure for the handler against toxic skin secretions produced by some amphibian species (26).
- When handling tadpoles, gloves should not be worn due to an unknown toxicity. Studies have observed up to 100% mortality in tadpoles following only 90 seconds of direct contact with both nitrile and latex gloves (27).
- Dechlorinated water should be used to wet the gloves prior to touching the skin to prevent irritation and support the mucous layer which serves as a protection against pathogens and to facilitate gas exchange (28).
- In situations where gloves are not available, hands should be washed between animals with 70% ethanol and allowed to dry, and then thoroughly rinsed with clean water, to prevent alcohol contacting the animal.
- The animal should be caught with a soft-shell net and immediately placed into a clear container or plastic bag sufficient for a visual examination and movement to holding facilities.
- For sample collection or medicine administration, physical restraint is often needed. Extreme care should be taken to not traumatize the animal (29).
- Frogs and toads should be physically restrained by grasping the waist and extending the hindlimbs to prevent kicking. The handler's other hand may be placed just caudal to the forelimbs on the pectoral girdle for extra support.
- Smaller animals weighing less than 5 grams should be restrained by placing the animal's dorsum between the middle and ring finger, with the thumb immobilizing the ventrum and hindlimbs.
- Salamanders should be restrained by grasping the dorsal aspect of the both the pectoral and pelvic girdles with separate hands (29). The handler can then restrain the limbs with their fingers, taking care not to grasp the tail or handle too roughly since tail autonomy can occur.

5.4 Reptiles

Non-venomous snakes:

- Remember, if a species of snake cannot be identified, treat it as venomous and call an expert.
- A snake hook can be used to pick up a non-venomous snake and place it in front of a clear plastic tube (hold the plastic tube with snake tongs) so the snake chooses to slide into the tube. The tube should be just wider than the head of the snake. As the snake slides into the tube, both the tube and the snake are grabbed quickly with the head and upper part of the body within the tube. Alternatively a hessian sack or pillow case can be used.
- If the snake will not enter the tube or sack, use the snake hook to gently pin the head of the snake to the ground to allow the handler to grasp the head.
- The head is held behind the occiput using the thumb and middle finger, while the index finger is placed on top of the head. Care must be taken not to put too much pressure on the joint at the back of the head (atlanto-occipital joint) as it may snap.
- When holding a snake, the body must be supported. If unsupported, the snake may feel insecure and thrash about.
- Large snakes should never be handled by one person alone; for every 3 feet of snake, there should be an extra person to assist with handling.
- If a snake appears to be dead, never pick it up with bare hands as snakes can play dead and then bite.

Lizards:

- Catch monitor lizards with a net and gauntlet gloves.
- They should then be grasped through the net over the shoulders, the net removed then hold one hand on the shoulders, one hand over the pelvis and the tail secured under the arm.
- Monitor lizards have very strong jaws and care should be taken to avoid bites.

Turtles/tortoises:

- Tortoises tend to be shy and easy to handle.
- Some turtles, such as snapping turtles and soft-shell turtles, may deliver a serious bite and should be handled using gauntlet gloves.
- Avoid turning turtles/tortoises on their backs.

6. Wildlife Handling Equipment

6.1 Minimum equipment list for live animal or fresh dead seizures

(from: Guidelines for the Safe Handling of Wildlife and Wildlife products during Counter-wildlife Trafficking Enforcement Operations (WCS 2021)(9))

On ALL operations, masks (both surgical and respirator e.g., N95), nitrile gloves, washable shoes and alcohol gel should be carried to allow an operation site to be entered and for further risk to be assessed.

For a live or fresh dead animal seizure/confiscation, additional equipment should be brought to the site:

- PPE. See Table 1 for level of PPE needed by wildlife taxa. Make sure extra PPE is taken in case items become torn or damaged.
- Wildlife handling equipment and transport crates. See Table 1 for equipment by wildlife taxa.
- Container of water for handwashing, and antibacterial soap
- Hand alcohol gel that contains at least 80% alcohol
- Disinfectant for cleaning equipment after use: bleach and Virkon. If Virkon unavailable, information on other disinfectants is available here: **http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf**
- Scrubbing brush
- Large waste bags; one for infectious waste disposal (such as disposable PPE) and one for equipment that will be disinfected for re-use (such as plastic goggles, face shields and rubber boots)
- Disinfectant wipes or bottle of disinfectant spray for disinfecting outside of waste bags before they go in the vehicle
- First aid kit including povidone-iodine for washing any scratches or bites, eyewash, dressing to cover any scratches or bites

If performing confiscations of macaques, due to the risk of B virus, you should also carry:

- 1 L of saline eye wash
- Freshly prepared 1:20 dilution of household bleach for initial wash of skin if it becomes exposed (NOT to be used on mucous membranes)

6.1.1 Gloves

Gauntlet gloves provide protection from smaller animal bites and scratches. They will not necessarily protect from medium sized carnivore or primate bites. Hexarmor Hercules 400R6E gloves are good. Leather welders gloves can suffice if custom gloves are not available. Gauntlets should be slightly loose on the hand so if an animal bites, the finger can slip sideways and be missed. Care is needed not to harm the animal when wearing thick gloves as the ability to feel how tightly the animal is being held is reduced.

A B

Figure 4. Gauntlet gloves for animal handling. A Hexarmor Hercules 400R6E gloves, B leather welders gloves

6.1.2 Equipment for snakes

Snake hooks (Figure 5A) are very useful for lifting snakes from containers, directing their movement and gently pinning heads to the floor. Clear plastic tubes (Figure 5B) are useful to allow snakes to slide into and allow containment of the head. The tubes can be easily made from any clear plastic pipes (e.g. used for building/manufacturing) that is then cut down to size and a cap fitted. Plastic shields (Figure 5C) can be used to capture slightly aggressive non-poisonous snakes by trapping the head against the floor with gentle pressure to allow the handler to move in and grasp behind the head. Grasping snake tongs (Figure 5D) are not suitable for direct handling of snakes as they can easily cause injury if not used correctly. However they are useful for removing dishes, feeding or holding plastic tubes. Hessian bags or pillow cases with enough room to tie a knot in the top can be used to transport snakes inside a transport box.

Figure 5. Snake handling equipment. A. snake hook, B. clear plastic tubes, C. plastic shield, D. grasping snake tongs. (Photos Fowler 2008).

6.1.3. Nets

Hoop nets are essential pieces of equipment. They have a long, strong pole fixed to a metal or fiberglass hoop supporting the net, allowing the handler to keep a distance from the animal. The hoop of the net should be large enough that it can be placed over the animal with enough room to avoid the hoop edge injuring the animal. The holes in the net should be small enough so the animal cannot put its head through the holes and risk strangulation and cannot stick arms or legs through the holes. The net should be deep enough to allow the net to be folded over or twisted to trap the animal inside (see Figure 6B). The thickness and type of mesh used will depend on the species. Soft mesh should be used for small animals, non-knotted mesh for birds and strong/thick mesh for larger animals. A variety of sizes and mesh strengths should be purchased to allow capture of a wide range of species.

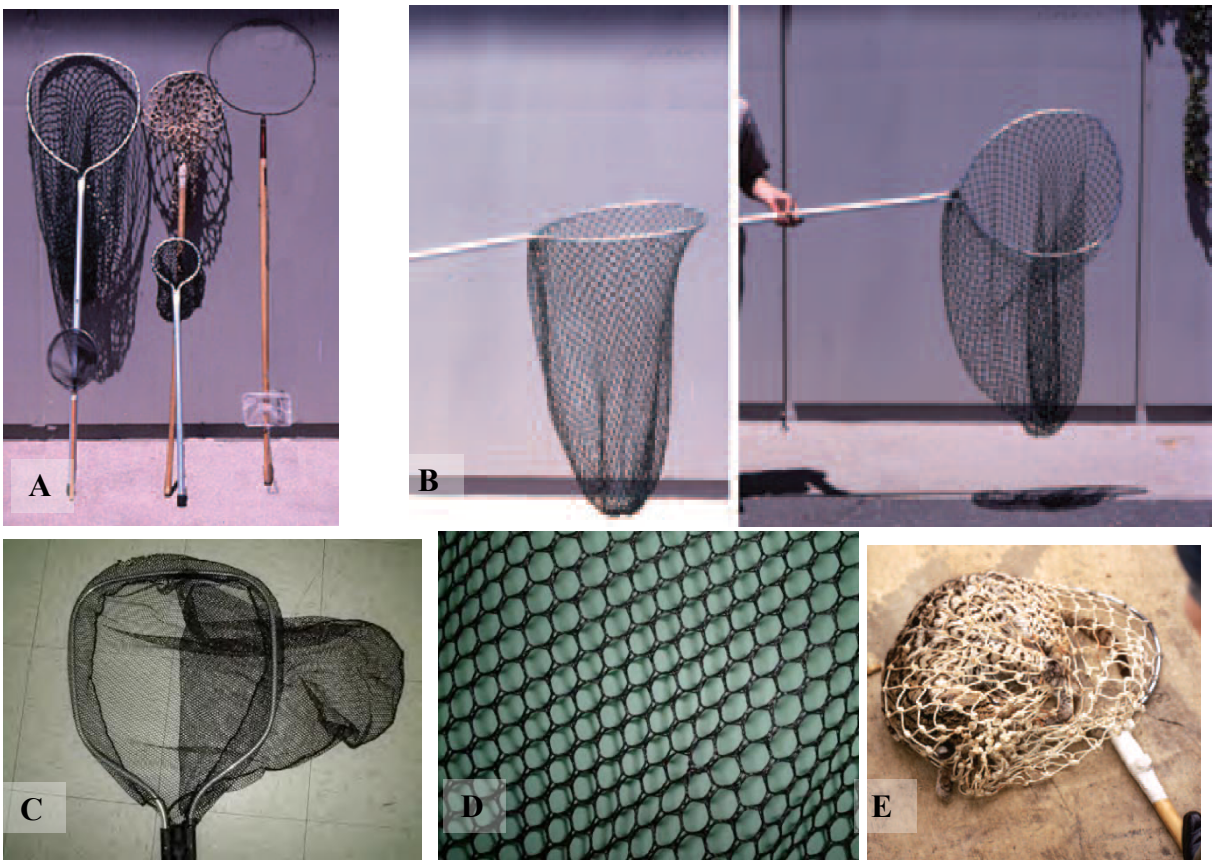


Figure 6. Hoop nets. A.Having a range of hoop nets allows capture of different species. B. Hoop nets must be deep enough to allow folding or twisting to trap animal in bottom of net. C. Nets for birds must be soft and not-knotted. D. Close up of non-knotted mesh for birds. E Strong rope nets are used for larger animals but the holes are still small enough to stop the animal poking a leg or head through a hole. Photos Fowler 2008 and KFBG 2009

7. DNA Sample Collection from Confiscated Wildlife

From: Standard Operating Protocols to Support Conservation, Health, Welfare & Prosecution of Wildlife Crimes Part II: Live Wildlife Crime Scene Investigation

Analysis of DNA (short for deoxyribonucleic acid), the molecules that carry genetic information in an organism, has been used in human forensics for several decades to help identify individuals. Accurate wildlife species identification can also be ensured through DNA testing.

DNA testing of biological evidence collected from 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.

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.

Sampling:

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 testing includes skin biopsies and scrapings, blood, urine, faeces, saliva, and hair/feathers/scales (12). Non-invasive methods such as urine and faecal collection are preferred for live animals to minimise handling and associated stress to animals (12). However, tissue biopsies and blood samples which are more invasive are preferable for DNA analysis (30). The process of collection and securing different sample types can be found in Table 2. Further details on specific sample collection and types of analyses for DNA testing (including reference labs) can be found in Appendix 6.

When sampling biological material for genetic analysis in a wildlife crime case, standardized procedures and protocols must be obeyed regarding chain of custody, labelling, transport and storage:

- During sampling, all collection material and tools (swabs, containers, knives, scissors etc.) must be free of "external" DNA from the collector or other possible sources of contamination. Use of sealed and disposable tools is recommended.
- Appropriate storage and transport are crucial to avoid degradation or contamination of the material; different materials require distinct conditions. If in doubt, it is always best to contact the laboratory performing the genetic analysis for the relevant information.

Labelling:

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 :

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

Each sample should be placed in its own collection device (as described in Table 2), 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. Labels must not be easily removed, or removal should show obvious damage in order to prevent tampering of evidence.

Table 2: Collection methods for different sample types and their use for laboratory tests.

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. Fixed sample: place tissue into a universal screw-cap container with fixative. Fresh samples and fixed samples must be placed in separate sealed plastic bags. 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°C for up to 7 days. Thereafter freeze below -20°C or keep in fixative. Fix in ethanol for DNA studies
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. For a fixed sample: cut a $1cm^3$ piece of tissue (ideally muscle) using a scalpel and place into a universal screw-cap container with fixative. Fresh samples and fixed samples must be placed in separate sealed plastic bags.	Refrigerate fresh sample at 4°C for up to 7 days. Thereafter freeze below -20°C or keep in fixative. Fix in ethanol for DNA studies, otherwise fix in formalin.
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.	Freeze below -20°C.

	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.	
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°C.
Saliva (swabbed from surface)	Follow instructions as for blood. Swabs should be placed in collection tubes.	Freeze below -20°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°C.
Feathers	Use sterile tweezers, preferably plastic for more delicate 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. The same guidelines apply for moulted feathers.	Store in transport 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.

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. Open the package from another location. Upon resealing, the seal should be labelled in the same manner as mentioned above.

8. Protocols for overnight/ short-term holding

Once confiscated, animals should be taken directly to rescue centers. If this is not possible and they have to be held overnight, animals need to be provided for while minimizing handling and continuing biosecurity precautions. If an animal will be held for more than 48 hours then enforcement officials should contact rescue centres or zoos for advice on feeding and husbandry. These protocols are not intended to cover longer-term housing for confiscated wildlife. Appendix 1 recommends components to include in Management and Husbandry Manuals for Commonly Confiscated Taxa. Wildlife care facilities should ensure welfare, species-specific husbandry and biosecurity needs are met, and practical considerations are listed in Appendix 3.

General principles for short-term care of confiscated live wildlife

- Specific staff should be designated to care for the confiscated animals to minimize exposure risk to other members of the team.
- Keep animals in a quiet and dark place with minimum disturbance.
- If the transport crate is of sufficient size (large enough for an animal to stand up and turn around) and the animal is only going to be kept for 24-48hrs before transfer to a rescue center or release site, then keep the animal in the transport crate overnight. This minimizes risk from further handling of the animal.
- Provide water – enough so the animal can drink but not submerge itself as it may be weak and at risk of drowning. If an inbuilt water container is present in the transport crate, water can be provided without having to open the crate. Otherwise, a low, wide, heavy and damage resistant water dish can be used and a cage divider can be used to keep the animal at the back of the crate while the door is opened and water or food put in, without risk of escape.
- Provide food. The only animals which should not have food provided in the first 24 hrs after confiscation are snakes. Ensure the food provided is appropriate for the species of animal (seek advice from zoos/ rescue centers if unsure).
- Ensure the animals don’t overheat – ensure the animal is in a shaded and cool area.
- Ideally keep animals in a well-ventilated area to reduce the risk of airborne pathogens.
- Ideally keep animals in an isolated area where staff are not walking through.
- Don’t mix wildlife species. Aim to keep a minimum of 1m between different species.

- Practice good biosecurity when going near animals. Remember disease can travel both ways, from humans to animals and from animals to humans. If there is a possibility that the animal will be reintroduced to the wild, it's important to make sure they don't pick up a disease while being held in captivity. Staff need to wash their hands and put on basic PPE before going in to feed/check animals and follow PPE removal protocol when leaving the room where the animal is being kept. To make following these protocols easier, it helps to have alcohol gel, boots and respirator masks (e.g., N95 masks) on a table and a trash can set up at the entry to the room.
- Place a tray or plastic sheeting under the cage to catch urine or faeces for easy disposal.
- If large confiscations of multiple animals occur, sick and healthy animals should be separated
- Once the animals have been transferred to a rescue center or released, disinfect/mop with 1:10 diluted bleach and ventilate the area for 30 minutes before personnel enter without masks.

9. Protocols for Wildlife Transportation

Transporting wild animals subjects them to additional stress. Thus, moving them over the minimal distance possible and as few times as possible is ideal, whilst also ensuring an appropriate final disposition. Local movement over a short distance may necessitate less rigorous adherence to normal standards of transportation, however IATA guidelines (https://www.iata.org/en/programs/cargo/live-animals/) should be followed wherever possible. Requirements vary for different species but the following considerations are always important:

9.1 Duration of travel

- Consider how long the journey will be and if any contingencies need to be made to ensure the safe transportation
- An Emergency Response Protocol should be developed at all levels for escaped animal and/ or accident or human injury

9.2 Animals Needs

- Allow for acclimatisation where possible; accompaniment by familiar staff where appropriate – especially social/head-reared animals
- Consider the conditions en route including temperature, humidity and ventilation, altitude that the animal is to be transported which may have an effect on the animals physiology, the conditions of the road
- Food and water provision as required during journey
- Vehicle availability

- Tranquilizers/sedatives can be used under expert guidance in certain situations

9.3 Vehicles/Trucks/Trailers

These need to be in good safe working order; non-slip flooring/loading ramp; adsorbent bedding; experienced driver. Crate >60kg will need forklift capability

9.4 Transport Crate Selection

- The size of the crate/container depends on the species. It should be large enough for an animal to stand up and turn around whilst avoiding excessive movement and injury (IATA)
- 25% of the vertical surfaces of crates should be ventilated with holes.
- Labelled with 'Live Animal', 'Wild Animal', 'venomous', 'Dangerous animal', 'this way up'... etc as appropriate
- Construction must be suitable to:
    - prevent escape or animal reaching out;
    - prevent accidental or easy opening;
    - allow provision of food/water as appropriate;
    - provide adequate ventilation;
    - ensure non-slip flooring;
    - allow cleaning and disinfection between use
    - provide adsorbent/grilled flooring for urine/faeces; ± internal padding; If crate slightly raised off the floor it allows drainage of urine. If the animal is being held overnight, a tray or plastic sheet can be slid underneath the crate to collect urine and minimize soiling of the animal.

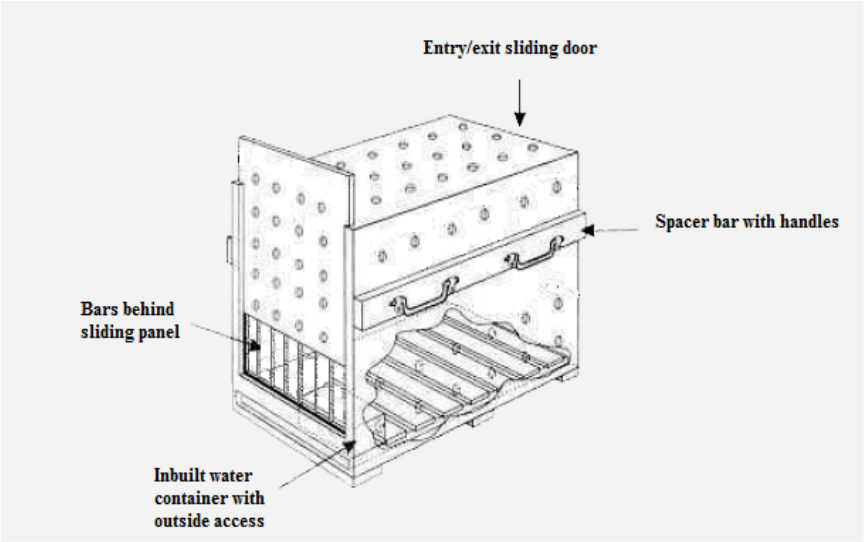


Figure 7. An example transport crate. Advice on specific requirements of crates for various wildlife taxa can be gained from speaking to in country rescue centers dealing with specific taxa.

9.5 Further information on transport crates and associated equipment

Having a selection of strong wooden or metal transport crates of varying sizes is advisable. It is essential to have a sliding door on crates to allow more controlled access to animals while minimizing risk of escape. This allows the crate to be put directly up against a cage for transfer of animals. Having one end covered in mesh or bars with a sliding metal or wooden panel over the top (Fig. 7) allows visualization of the animal and access to inbuilt water containers without having to open doors. It is often necessary to hold animals overnight before they are taken to a rescue center or released so having in-built water and food containers with outside access is important. Specific requirements of crates for various wildlife taxa can be gained from speaking to rescue centers experienced in dealing with specific taxa.

Figure 8. Transport crates for particular wildlife taxa. A Crate for Pangolin: a smooth sided wooden box minimizes the risk of self-trauma. Photo: WCS Vietnam B: Plastic box for non-venomous snakes with holes for ventilation

9.5.1 Shields and cage door barriers

A shield (Figure 9A) can be used to encourage small animals to move into a transport crate. A cage door barrier, such as a strong piece of plywood (Figure 9B) can be used if an animal needs to be transferred from a cage with a swing door to a transport crate. The barrier can be used to cover the opening of the swing door until the transport box can be put flush against the cage. This technique only works for animals that are not strong enough to push the barrier away.

A

B

Figure 9. A: Shield for encouraging animals to move into transport cage; B: a cage door barrier to stop escape of an animal while opening a swing door and putting transport crate against cage. Photos: Fowler 2008

9.5.2 Self-closing cages

Self-closing cages, such as Tomahawk traps, can be used if an animal is in a large enclosure and handling is deemed too dangerous. The trap is baited with food and the animal enters the cage to get the food, triggering the door to close. The disadvantage of these traps is that it can be time consuming waiting for the animal to go into the cage of their own accord.

Figure 10. A tomahawk trap can be used for small to medium sized animals. Photo: PREDICT Tanzania Team

9.6 Taxa-Specific Transport Guidelines

Fish

- Plastic bags: 1/3 water, 2/3 air; in Styrofoam-lined, rigid, leak-proof box; transport under low light
- Larger species may include filters and/or oxygen bubbled through water during transport
- Fasting before shipment to ↓N waste; addition of salt can reduce osmotic stress on freshwater fish.
- Water at cooler end of preferred temp range reduces metabolic requirements and N waste

Snakes/lizards

- Normally cloth bags in Styrofoam lined wooden box, ventilation
- Double bag venomous snakes, appropriate label on box
- For non-venomous snakes, a clear plastic box (to allow visualization of the inside of the box) with a lid and ventilation holes is adequate If a box with a sliding lid (ideally made of clear plastic) can be made, this is even better, allowing gradual opening of the box and so better control of the snake. Ideally the snake should be inside a sack inside the box

Amphibians

- Often in plastic containers, appropriate air holes, within wooden box; ± sphagnum moss etc
- Aquatic species in water double bagged.
- Attention to temperature during shipment – provision of heat packs as appropriate

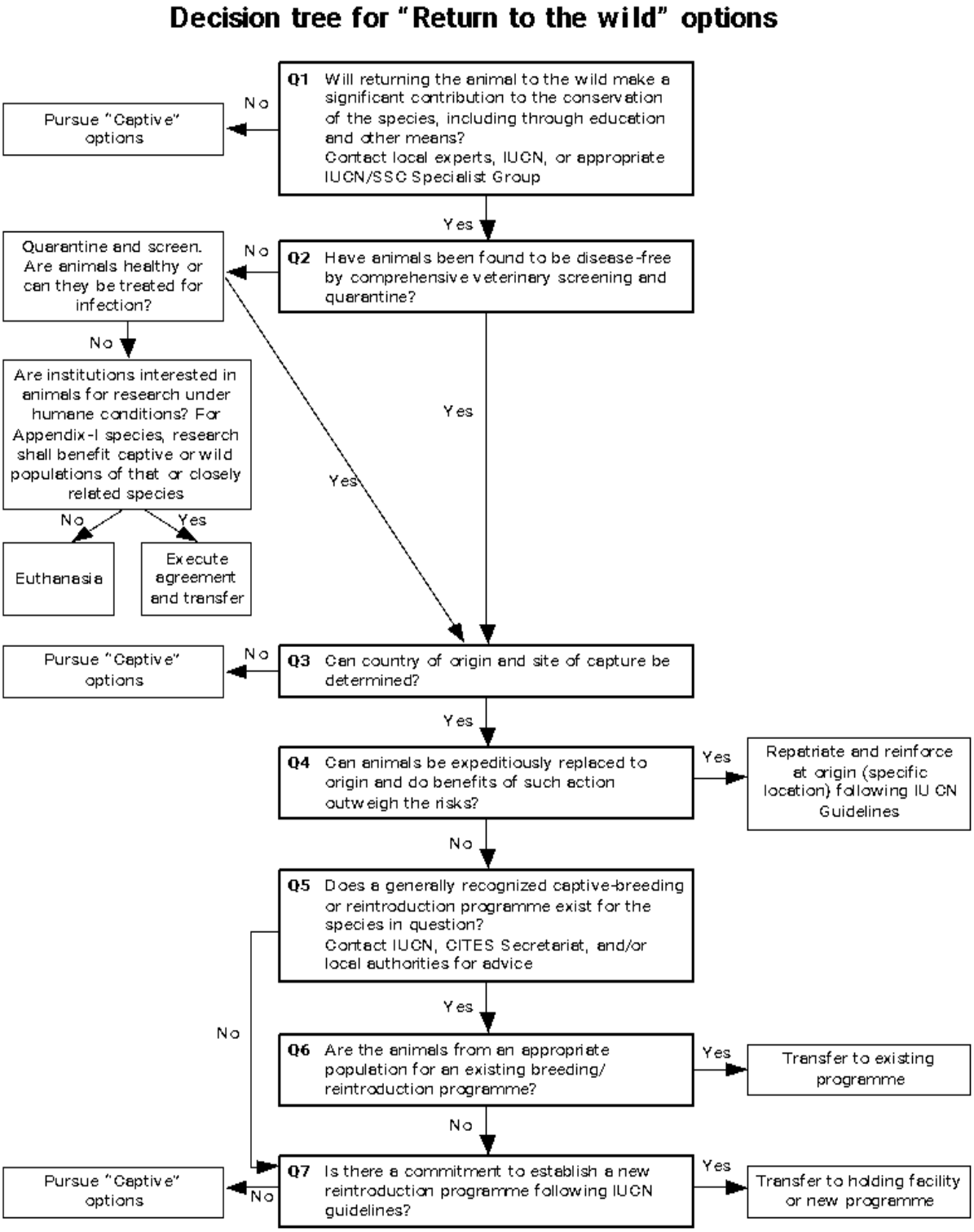
Birds

- Ideally individual compartments with adequate food/water (preventing spillage and faecal contamination)
- Should be able to perch with head upright and tail off floor.
- Cargo lights left on on long flight to permit feeding
- For birds, the crate should be a minimum size of roughly double the height of the bird in all three dimensions but not be too large or the bird will flap and damage itself. Wire mesh crates should be avoided for birds as they can easily damage feathers

Mammals

- Social groups may be shipped in the same container as deemed appropriate
- Attention to temperature – heat for small mammals; ice for sea otters etc
- Adequate/ongoing provision of nutrition for small mammals with high metabolic rates
- Pangolins are very susceptible to self-injury – they can tear off claws on metal cages. They are also very easily stressed, so wooden transport crates with solid smooth sides that minimize visual contact and potential for self-injury are best for transportation

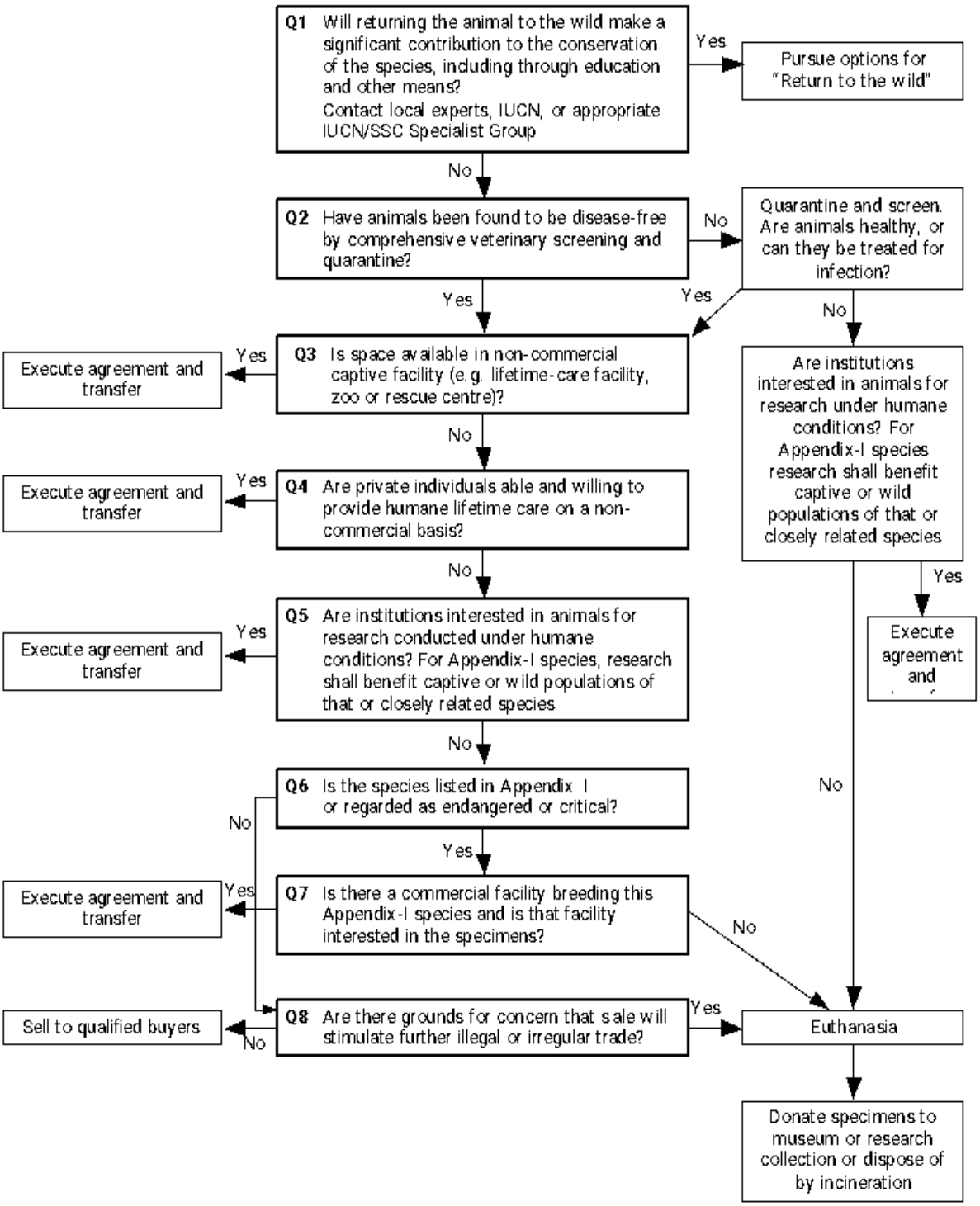
IV. Decision-Making Tree for Disposition of Confiscated Wildlife



Disposal of confiscated live specimens: https://cites.org/eng/res/10/10-07R15.php

IUCN Guidelines for Reintroduction: https://portals.iucn.org/library/efiles/documents/2013-009.pdf

Decision tree for "Captive" options



Disposal of confiscated live specimens: https://cites.org/eng/res/10/10-07R15.php

Appendix I

Key Components to Include in a Management and Husbandry Manual for Commonly Confiscated Taxa

Taxonomy – species or taxonomic groups

Conservation Status

Natural History

- Weights and measurements
- Age determination
- Distinguishing features
- Distribution range and habitat
- Social structure
- Diet in the wild

Captive Husbandry Guidelines

Housing/Environment/Feeding

- Spatial requirements
- Suitable type of holding and Size of holding area
- Substrate
- Diets and supplements
- Presentation of food
- Materials for housing
- Shelter/screening
- Water
- Furnishings, including suitable vegetation
- Humidity/temperature/thermoregulation
- Cleaning routine
- Record keeping
- Routine checks
- Normal Behaviours
- Abnormal behaviours

Health Requirements

- Health checks
- Environmental hygiene

- Chemical restraint
- Physical Examination
- Known health problems
- Quarantine procedures

Welfare friendly transportation Guidelines

- Container design
- Furnishings
- Timing of transportation
- Animals per container
- Water and food

Rehabilitation and Release Procedures

- Triage, health assessment, suitability for release
- Rehabilitation facilities and husbandry– see above
- Disease risk analysis
- Optimum timing of release
- Release location assessment
- Soft release vs hard release
- Post release monitoring

Appendix II

Examples of zoonotic diseases that can be found in wildlife species encountered in enforcement operations.

Examples of Zoonotic Diseases associated with Bushmeat and Live Animals (66)

Pathogen	Wildlife involved	Wildlife products	Trade type	Public health issues	Refs
Viruses					
Simian foamy virus (retrovirus)	Non-human primates	Bushmeat	International	Increase in pathogenicity following cross-species transmission	[11]
Cytomegalovirus (herpesvirus)	Non-human primates	Bushmeat	International	Concern for immunocompromised people	[11]
Lymphocryptovirus (herpesvirus)	Non-human primates	Bushmeat	International	B-cell tumors in immunocompromised individuals	[11]
Bacteria					
Escherichia coli	Birds, duiker	Live animals, bushmeat	National, international	Urinary-tract infection, meningitis, septicemia	[4,12]
Klebsiella pneumoniae	Birds	Live animals	National	Pneumonia, urinary-tract infection, septicemia	[4]
Salmonella enterica serovar Typhimurium	Birds	Live animals	National	Gastrointestinal infection	[4]
Listeria monocytogenes	Pangolin, red hog	Bushmeat	International	Meningitis, septicemia, and abortion in immunocompromised people	[12]
Staphylococcus aureus	Pangolin, duiker, red hog, fish	Smoked fish, bushmeat	International	Osteomyelitis, endocarditis, pneumonia, bacteremia, toxic shock syndrome	[12]
Parasites					
Baylisascaris procyonis (nematode)	Raccoons	Live animals	International	Neurological signs, visceral larva migrans	[7]
Toxocara sp. (nematode)	Raccoons	Live animals	National, international	Neurological signs, visceral larva migrans	[7]
Trichinella spp. (nematode)	Black bear, grizzly bear	Meat products	International	Intestinal, muscle, and neurological clinical signs	[1]
Cryptosporidium spp. (protozoan)	Non-human primates	Live animals	National	Intestinal clinical signs	[15]
Hyalomma aegyptium (tick)	Turtles	Live animals	International	Potential vector of zoonoses (e.g., *Borrelia turcica*; Crimean–Congo hemorrhagic fever virus)	[3]

Serious zoonotic diseases that can be found in confiscated wildlife These diseases can be fatal in humans and transmitted through bites, scratches, facial splashes, or inhalation of aerosols(9)

Disease	**Common wildlife hosts**	**Route of transmission**
Cercopithecine herpes-1 (B virus)	Macaques or other primates housed with macaques	Bites, scratches, facial splash with saliva, urine or faeces
Rabies	All mammals but particularly bats and carnivores	Bites, scratches, facial splash with saliva or urine
Hantaan virus	Rodents	Inhalation of aerosols, less commonly through rodent bites
Highly Pathogenic Avian Influenza (H5N1)	Birds	Contact with saliva, nasal secretions or blood
Coronaviruses responsible for Severe Acute Respiratory Syndrome SARS-CoV-1 and SARS-CoV-2	Bats*, mustelids, pangolins	Bites, scratches, facial splash with saliva, urine or faeces

* The origins of SARS-CoV-2, the causative agent of COVID19, remain unknown, but its genome indicates a strong likelihood that the reservoir species is a bat (Zhang et al. 2020). Other wildlife species may act as intermediate hosts as civets did with SARS-CoV-1

Appendix III

Holding facility construction and management

Ideally, every wildlife care facility should be designed, constructed and maintained with biosecurity risks in mind. In practical terms, this involves the following considerations:

- Easy and effective cleaning (and if necessary, disinfection) of enclosures, furnishings and equipment
- Ease of work flow management
- Appropriate management of animal food, biological and water wastes
- Good drainage (without cross-contamination), with no accumulation of water or waste products
- Security from operational disruption by flood, bushfire, or other events
- Appropriate areas for isolation of individuals or groups
- Species-appropriate facilities to minimise physiological stress
- Ease of capture, transfer and movement of animals
- Appropriate airflow and air exchange (to minimise pathogen load)
- Appropriate ambient temperature and humidity (to minimise individuals' physiological stress)
- Isolation from domestic animals, free-ranging wildlife, feral animals, pest species and invertebrate pests
- Facilities that allow for safe and hygienic animal handling, examination, treatment and postmortem processes
- Animal food preparation and storage areas
- Hand washing and showering and change areas for workers
- Wash bay for vehicles and larger equipment
- Visitor hygiene facilities (at a minimum hand washing facilities)
- Appropriate segregation of different groups of wildlife in care, so that abnormal species mixing does not occur, and to ensure social species are housed in appropriate age and health cohorts
- Facilities for worker food preparation, storage and meals, separate to those used for animals.
- Wildlife holding facilities should be secure, preferably with a well-defined and secure perimeter that prevents both escape of animals and entry by animals outside of the perimeter.

Appendix IV

Example of tables to collect contact details of experts to assist with animal confiscations

As these guidelines are localized, the tables below should be expanded to relevant wildlife species/ taxa and populated with names and contact details of personnel and facilities who can provide expert assistance and longer-term housing of animals. Contacts should be checked and validated every few months.

Wildlife type	**Expert contact details (Name, affiliation, specialization, phone, email)**	**Date Contact Updated**
Venomous snakes or amphibians		
Large mammals (>15kg) that need chemical immobilization (large primates, large carnivores, bears)		
Other species (primates, small carnivores)		

Wildlife type	**Rescue Centre Name and Contact Details (Name, specialization, address, phone, email)**	**Date Contact Updated**
Reptiles and Amphibians (turtles, snakes, lizards, frogs, salamanders etc)		
Birds		
Primates		
Large carnivores (E.g. bears, tigers)		
Other species (e.g., small carnivores, pangolins etc)		

Appendix V

How to identify species during wildlife crime/trade investigations?

From: Standard Operating Protocols to Support Conservation, Health, Welfare & Prosecution of Wildlife Crimes Part II: Live Wildlife Crime Scene Investigation

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 (18). 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 (18), 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 (63). 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.

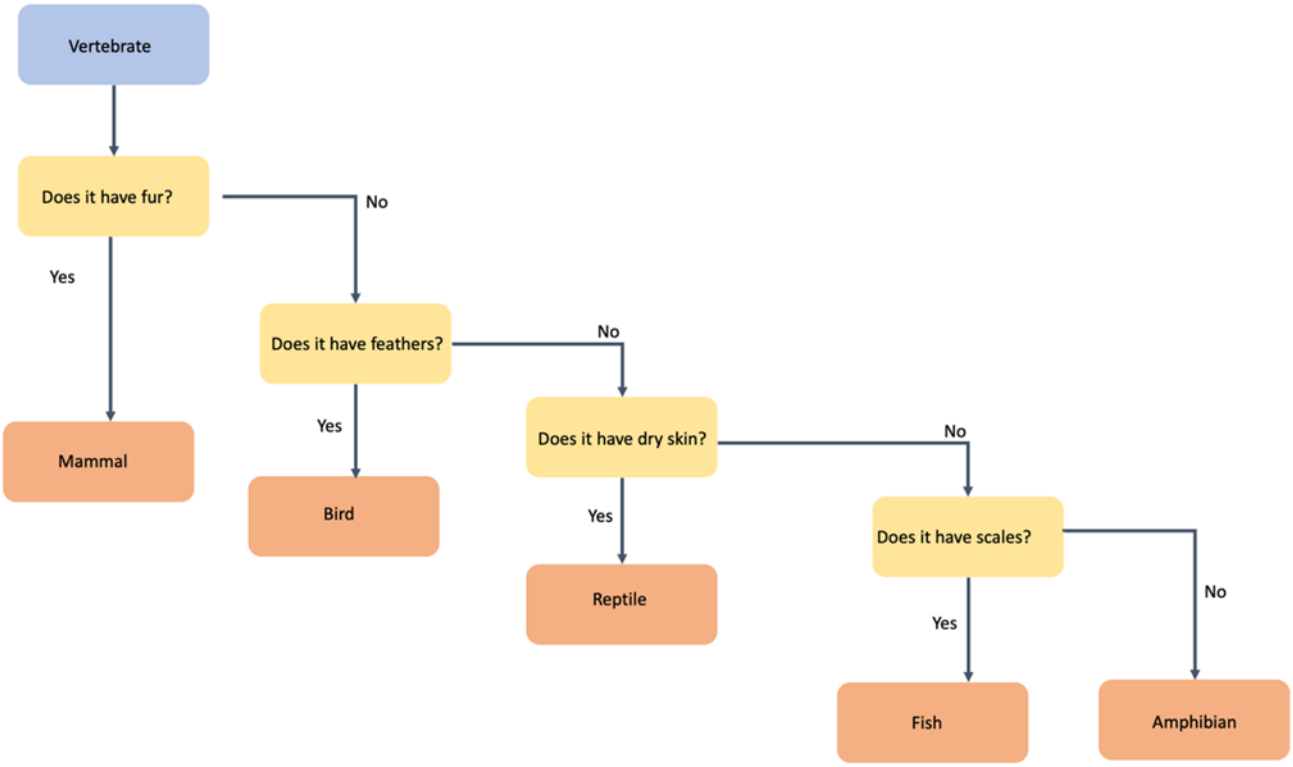
2. Differentiation between Taxa

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 (64). 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 (65). 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 Section 2 of these guidelines in Figure 2 (also shown below). 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 2 from Section 2 of Guidelines**.** A dichotomous key for differentiating between animal classes



NB: 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. Identification based on gross morphological features is the routine method for classification of different fish species (33, 34, 35). The instructional steps below outline how to correctly photograph and record details of live specimens for identification.

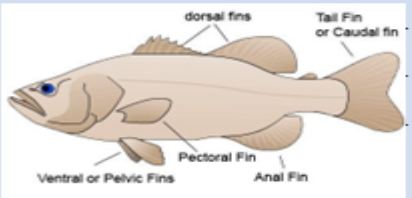
Step 1: Records

- 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 (33; 34; 35, 39). 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 (33; 34; 35). 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 (Xing 2022; 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.

(40,41,42)

Another method for the identification of fish species is DNA barcoding (33,35). This method may be employed in cases of uncertainty, or when more evidence may be required for prosecution (32,35). 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.

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. 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 (45). Recent studies have shown that skin swabs from both adult and larval life stages provide ample DNA for species identification (46). This approach is attractive due to being less invasive and requiring less handling of the animals.

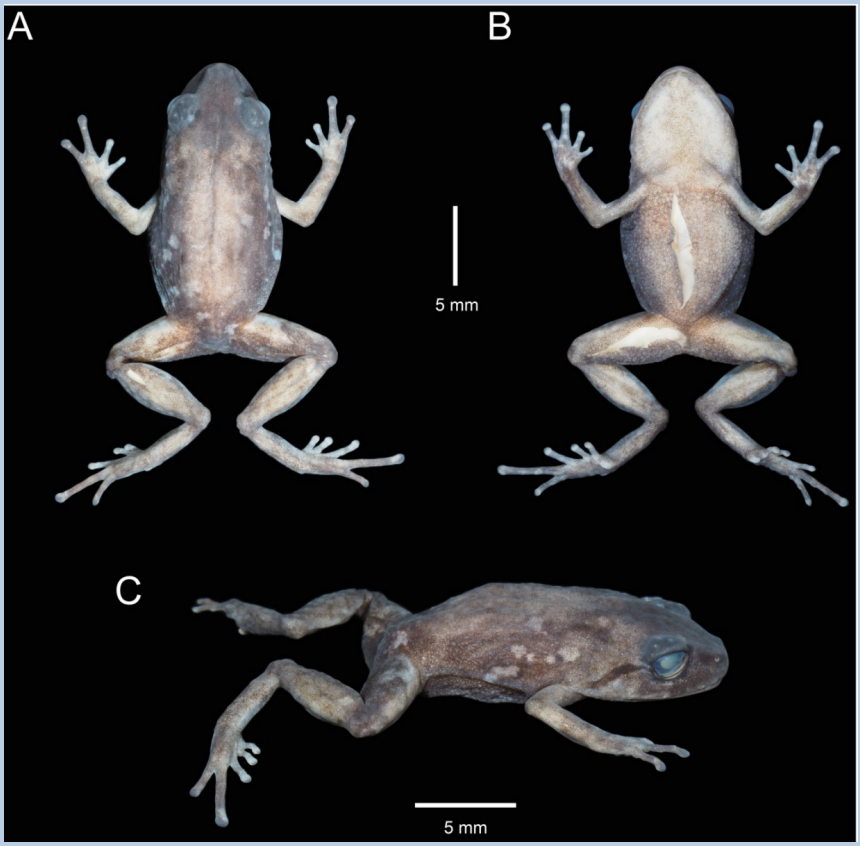
Step 1: Photographs

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 11 will be a useful addition to the checklist to assist inspectors with reptilian anatomical terms and Figure 12 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. An accredited 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 (47). Any faeces or shed skin from the animal will also harbour DNA that can be used to identify the species (48,49).
et al. 2015).

Figure 11. The variety of head scales present on a reptilian head.

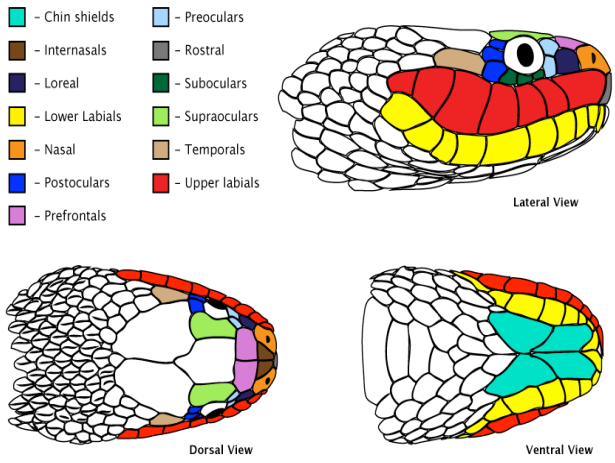
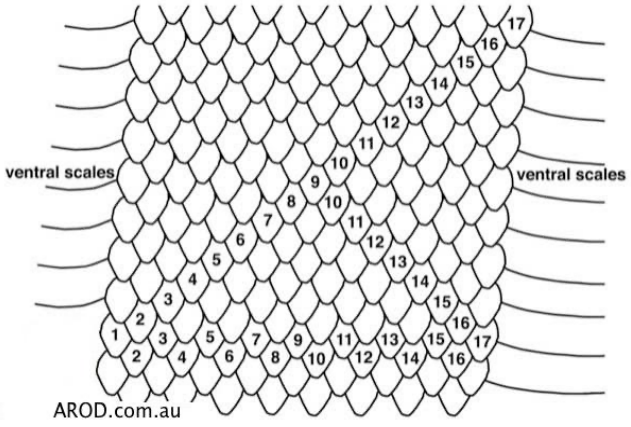


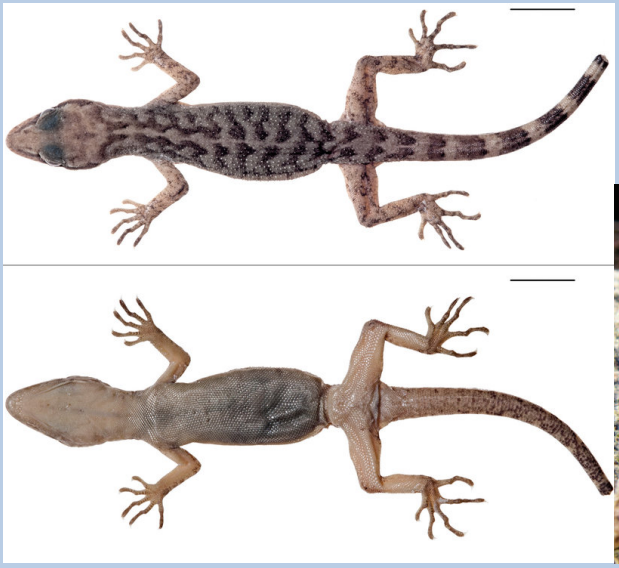
Figure 12. How to count dorsal scales across the mid-body.



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

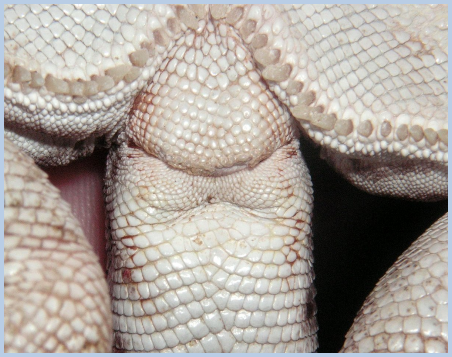


- Head-on view with mouth closed (left image) and with mouth open showing tongue and mouth lining (right image)

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
___

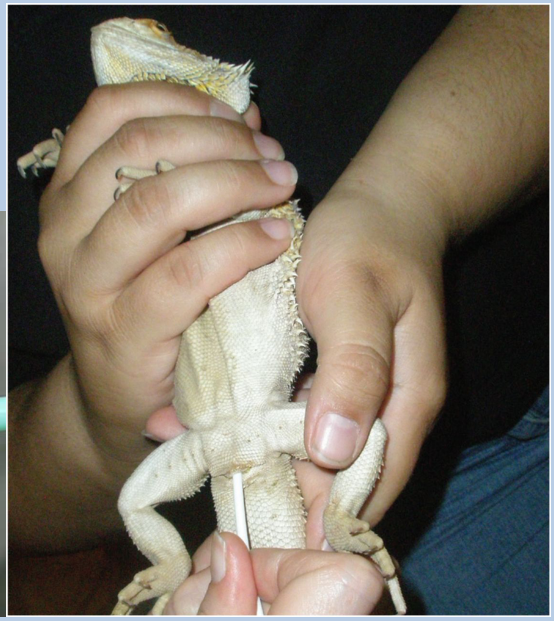
Snout-Vent Length
___

Shell-Length
___

Fourth Toe Lamellae (Hindfoot)
___

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

Step 3: Take buccal and cloacal swabs for DNA analysis



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)

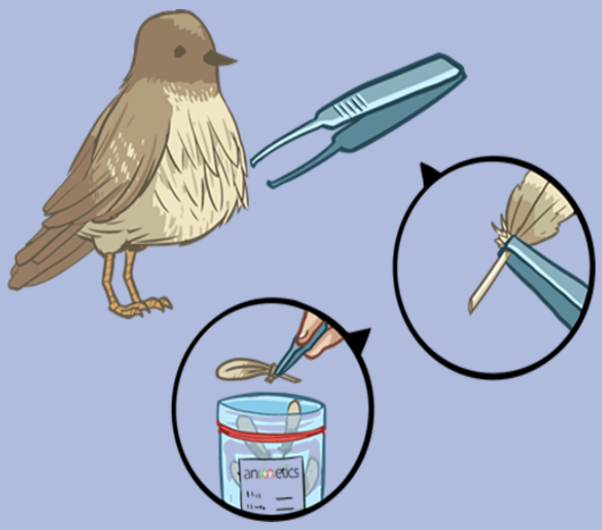
Bird species identification can be difficult, even for experienced bird watchers, therefore a feather sample should be taken for DNA testing. Feathers for DNA testing should be freshly plucked. DNA is contained at the base of the feather, originating just below the skins' surface. Moulted feathers are 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 13). 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.

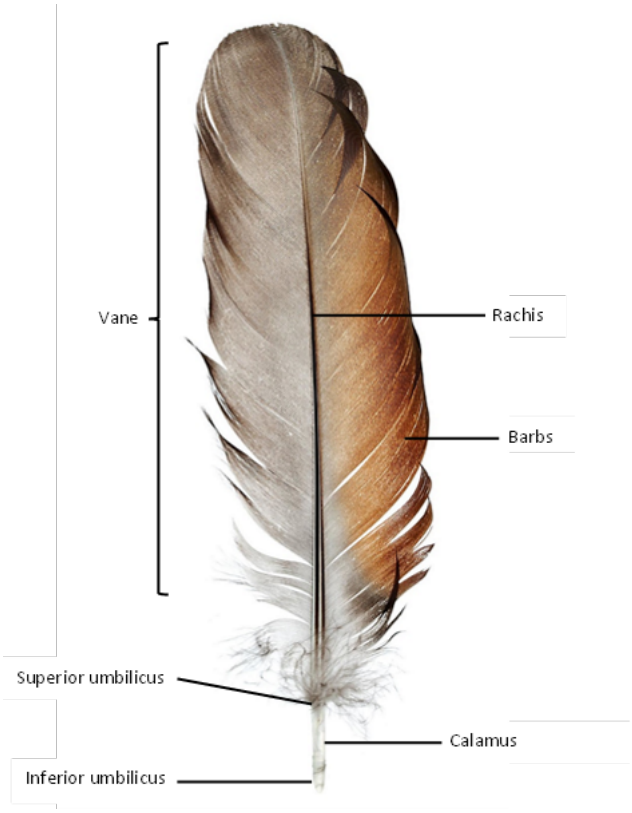


Figure 13. A feather labelled to show the mtDNA extraction sites; the calamus, the blood clot located in the superior umbilicus and the barbs. (Klappenbach 2019)

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.

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

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 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 (56). 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 (56). 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 (17). 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 (34, 35, 67). 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 (18). Examples of agency-level training include that provided by the Department of Environment and Energy 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 (67). 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 (10, 42, 52). 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 (17). 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 (17, 18).

APPENDIX VI

Sample Collection for DNA

From: Standard Operating Protocols to Support Conservation, Health, Welfare & Prosecution of Wildlife Crimes Part II: Live Wildlife Crime Scene Investigation

DNA analysis

In order to carry out DNA sequencing, a range of body tissues can be collected and analysed. These can be undertaken by investigators using the following techniques, however if a direct blood sample is indicated, this must be undertaken under veterinary or other trained expert supervision. Refer to the previous appendix section on Species ID for full DNA collection techniques.

DNA can be used to gain a variety of information including geographical origin and evidence of familial relatedness. Mitochondrial DNA (mtDNA) is commonly used in DNA analysis, which can be extracted from cooked/dried meats, claws, tanned hides, dried shark fins and feathers. Geographical origins can be identified from mtDNA, which is crucial information to differentiate between captive-bred and wild-caught animals.

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 (57). Similarly, spatial smoothing assignment has been used in the past to investigate illegal sale and trafficking of African elephant ivory and Luxembourg red deer (58). 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 (12). 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 (57).

Sample collection for DNA analysis

Deceased animals: muscle, teeth, bone

Live animals/environmental samples: saliva, hair, faeces, vomit

Blood collection

Blood from live animals can be obtained by swabbing superficial wounds or direct blood sampling (under veterinary/ expert supervision)

3 swabs are required for blood sampling

Swab 1: purified water sampled with swab 1 to test for contaminants

Swab 2: soak up fresh blood or moistened dry blood

Swab 3: sample remaining moisture from the area

Each swab needs to be placed into an individual sterile tube, sealed and labelled

Considerations

Direct blood sampling gives the most accurate results, however veterinary/ expert supervision is required

All samples must be collected while wearing gloves and masks to reduce potential contamination and disease risk

All samples should be placed into the appropriate evidence bag, sealed and labelled with the animal's signalment, date and location as detailed in prior section

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 (59), 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 3) can be contacted for guidance on collection, submission and transportation of samples.

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 (60). 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.

Table 3: 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

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 (61). 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 (62). 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) (61).

Collecting a faecal sample

- Ensure to wear gloves while collecting a faecal sample
- Collect approx. 1tsp of dried or fresh faeces from the cage or animal
- Place sample into a sterile container and label (animal signalment, date, time, freshness of sample)
- Store sample in a fridge or cooled box until able to send to the lab
-Appropriate sampling protocols should be discussed with the laboratory being used for specific requirements needed including storage and logistical limitations

APPENDIX VII

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 section 3.4.2) 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 (2). 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 section 2.1 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 Table4. 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 4: Additional samples to collect for pathogen and toxicology testing

SAMPLE TYPE	STORAGE	USES
Saliva (oropharyngeal swab[2])	- CRYOVIAL[3] with lysis buffer[4] (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[5] with lysis buffer (use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the swab shaft of 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	Pathogen PCR screening
Blood (serum; rbc/wbc pellet;	- 2 thin smears on glass	Pathogen PCR screening;

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

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

[4] E.g. tris-EDTA; RNALater; others available incountry/ preferred by partner laboratories

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

thin blood smear (fixed)) (Use a nonheparinized syringe to collect blood (not to exceed 1% of the total body weight)	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	microscopy; serology
Faeces (fresh fecal 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[6] 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
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,	Pathogen PCR screening; histology

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

	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	
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.

APPENDIX VIII

Considerations for the Chemical Restraint of Confiscated Wildlife

When might chemical restraint be necessary during the confiscation process?
Expertise, careful planning, appropriate facilities, equipment and drugs are crucial for the safe chemical restraint of confiscated wildlife to ensure the optimal animals welfare and human safety outcomes. Safer and more effective chemical restraint options and remote drug delivery systems will reduce the need for physical restraint in many instances. Many animals are capable of inflicting significant injury through bites, scratches, blows or envenomation. The risk to the animal is equally significant with potential to run into obstacles resulting in wounds, fractures, concussion or death. Excessive physical restraint can result in suffocation, contusions or fractures. Young maybe affected and injured or killed and capture myopathy can be a serious outcome.

Many simple procedures in suitable animals can be accomplished quickly and safely using appropriate physical restraint methods and equipment. However, on most occasions chemical restraint will be required. The method of capture, chemical restraint, drugs and drug delivery must be based on sound principals, experience, knowledge and the current published and available literature (71). All chemical restraint procedures should be undertaken by an experienced wildlife veterinarian with experienced technical help, following legal and health, safety at work requirements and following best practice procedures.

There are many occasions when chemical restraint is required during the confiscation process. Detailed planning and preparation is essential for all anaesthetic procedures to ensure successful outcome. All local legal requirements should be exercised during the deployment of anaesthetic and sedative agents for the chemical restraint of confiscated animals and expertise in the use of and handling of anaesthetic agents, human and animal first aid and CPR techniques. A manual of chemical restraint procedures and protocols for commonly seen confiscated animals should be developed locally. A list of equipment and drugs should be maintained and stored safely and appropriately

The reasons for using chemical restraint may facilitate the following

I. Clinical examination
II. Precise and safe sample collection for species identification purposes
III. Diagnostic samples in the case of sick animals
IV. Treatment of sick and injured confiscated animals
V. Identification of the individual ie PIT tags, bands
VI. Relocation

Methods of Chemical Restraint

The clinical pharmacology of chemical restraint drugs, drug delivery systems and principals of wild animal chemical restraint are discussed elsewhere (70, 72). It is beyond the scope of this document to describe in detail chemical restraint procedures for all taxa but information is available and clinical experience is necessary. Inhalation anaesthesia using primarily isoflurane in oxygen and a suitable circuit is a commonly used method of chemical restraint for small mammals, birds and some reptiles that are often restrained by hand or in capture net, a towel, blanket, hessian sac or calico bag. Alternatively, isoflurane in oxygen can be run into a induction box when restraint is difficult to achieve or through the hessian sac/calico bag. In water administration of agents such as MS222 or clove oil can be provided for fish or amphibians should chemical restraint be required. Alternatively, chemical restraint drugs can be administered by intramuscular injection either into a large muscle mass by direct injection through a capture net, hessian sac or calico bag in a restrained animal. Knowledge of the important anatomy is essential to ensure correct injection placement. Similarly, when animals are large and dangerous, chemical restraint can be provided intramuscularly by remote delivery of drugs using a pole syringe, blow pipe, dart pistol or rifle. Again, knowledge of and experience with this equipment are essential as is the understanding and safe monitoring of the animal under anaesthesia and correct and safe recovery procedures (70,72).

What are the risks and complications associated with chemical restraint

All anaesthetic procedures should be carried out by an experienced wildlife veterinarian with expert technical assistance and follow evidence based best practice. However, there are risks associated with any anaesthetic procedure for both animals and humans if the correct procedures are not followed and there is limited experience.

Risks and Complications for the Animal

Inaccurate weight estimation can lead to animals being underdosed or overdosed. Underdosing can lead to continued stress for the animal as induction can take time. Overdosing can lead to respiratory distress and cardiac failure and death. Every effort should be made to obtain accurate weight estimates to facilitate safe anaesthesia.

Stress prior to and during the chemical restraint procedure can lead to physiological changes that result in respiratory depression, acidosis, hypoxaemia (lack of oxygen in circulation), hyperthermia (overheating) and myopathy. To minimise these effects, chemical restraint should take place in a quiet environment and undertaken as quickly as possible. Hypothermia can also be an issue leading to complications – all animals should be kept warm during the anaesthetic process as normal thermoregulation can be compromised when under anaesthesia.

Bloat, vomiting and regurgitation can also be a major concern especially for ruminants and correct positioning in sternal recumbency with the nose being held lower than larynx is important. Following all anaesthetic procedures, it is prudent to debrief to ensure improved safety procedures and protocols can be used in the future. All anaesthetics should be monitored and recorded by trained personnel.

Risks and Complications for People

All anaesthetic drugs if not safely managed can be a source of danger for veterinarians and other staff. Standard operating procedures must be developed for safe use of all anaesthetics and all drugs should be stored safely and accounted for. Sufficient time should be allowed for the anaesthetic to work to ensure it is safe to approach. Stimulating large dangerous animals using a stick from a distance can allow the veterinarian to gauge if the animal can be approached. Maintaining an acceptable depth of anaesthesia by regularly monitoring including top ups as required will ensure safety of all staff is maintained

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