



ALPBIONET2030

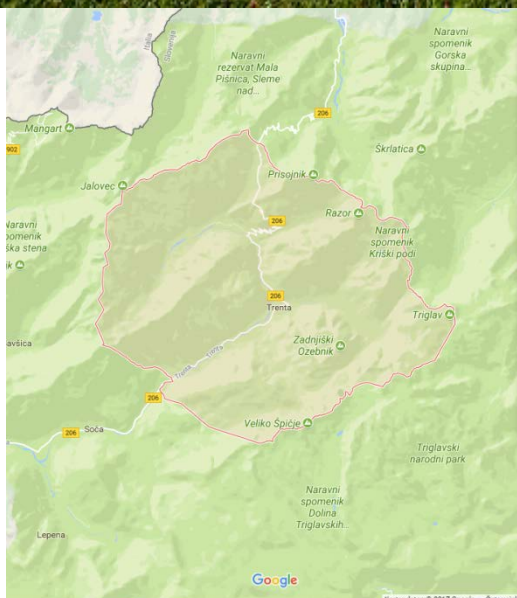
Integrative Alpine wildlife and habitat management for the next generation

REPORT

Transnational workshop on
standardisation of wildlife forensic
methods, Carnia, Venzone, Italy

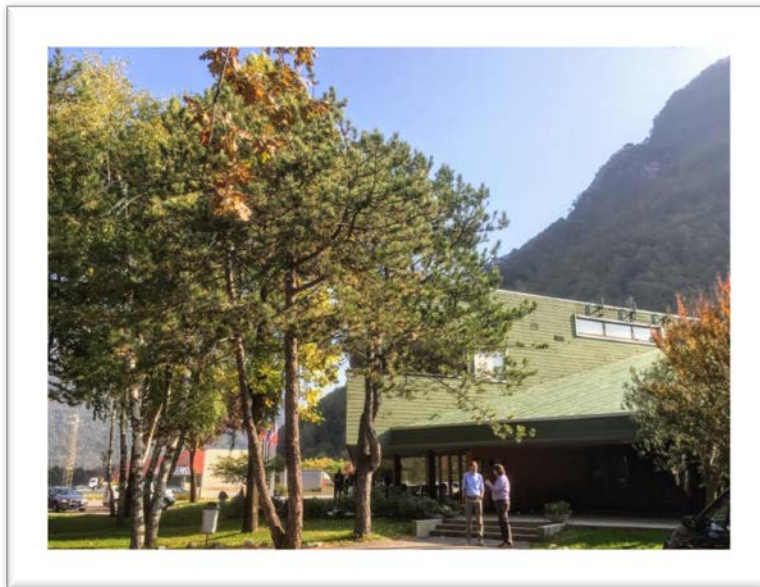
12 October 2017, Hotel Carnia, Venzone

(Alpbionet2030 – Work Package 2)



1. Introduction

Participants from Italy, Slovenia and Austria, from authorities responsible for dealing with wildlife-related crime and interested experts, met in Carnia to work on discussing standard operating procedures (SOPs) on wildlife forensics.



2. WORKSHOP PART I

To further the goals of ecological connectivity, ALPBIONET2030 aims to enhance the capacity of local wildlife crime teams by presenting and discussing state-of-the-art methods and procedures. The forensic expert team from the Research Institute of Wildlife Ecology (FIWI) of the Vetmeduni Vienna gave an overview of the basics of wildlife forensic science presented such methods and discussed with participants the situation in the Alpine regions of Slovenia and Italy (Triglav National Park and Prealpi Giulie National Park).

Prevention and prosecution of wildlife crimes (illegal killing, damaging health of wildlife, keeping of wildlife and wildlife products, and - although this is not the focus of this project – illegal wildlife trade) on a broader transnational scale is generally hampered by varying legislation, different standards, different levels of knowledge, focus and responsibility.

Wildlife may be the victim of human illegal actions, but may also cause damage to human interests.



In forensic investigations we may encounter clinical cases (animals still alive, but usually only flagship species such as large carnivores or raptors are represented), and cases of animals found dead.

Dead animals may not necessarily have been the victim of crime, there are a lot of deaths due to natural causes (especially diseases)

– these have to be ruled out in forensic investigations.

There are also human-induced “legal” causes of death (vehicle collisions, other accidents with human infrastructure, fireworks, legal hunting) – these also have to be ruled out.

Illegal causes may include hunting/trapping, including hunting by prohibited methods of otherwise legally huntable game species.

Forensic tasks usually involve

- Visiting crime scene and collating site information and data (e.g. photos), sampling, transportation of samples
- Necropsy, lab tests, storage
- Report filing

This needs the collaboration of many experts and institutions, and it has to be cost-effective. Authorities sometimes do not ask clear questions that may facilitate and focus lab work.

Forensic cases do not just involve experts, there are also other stakeholders (hunters, farmers, game wardens, national parks, NGOs), but often also the media and the public (social media!) because of high interest in wildlife crime. Passing information has to be done with care, a strict chain of the communication hierarchy has to be respected. Leaking information may hamper persecution and conviction.

Pathological examinations include a macroscopic and a microscopic examination to determine the cause of death. Bacteriological, virological and parasitological investigations, as well as toxicological (poisons, pesticides, heavy metals, drugs...) investigations are part of this. Various chemical analyses exist – you have to check with the labs beforehand what kind of



sample they need and the costs of analysis. Botanical investigations concerning plant parts within an animal's stomach may also be necessary – but specialists in botanical investigation are very rare. Radiography can be used to detect bullet fragments, fractures, bone density. It should be done before necropsy, especially in all suspicious deaths. Digital x-ray has an accredited system so that the name and number of the file cannot be changed – it is very important in forensic investigations to keep the original image unaltered (same for photos from the original location of the body). Other imaging techniques include MRI – but if you suspect illegal shooting, never put it into an MRI, as the bullets will be extracted from the body by the machine and damage it. Analysis of blood cells, enzymes, electrolytes, etc. can give information on the immunological or physiological status, but is not possible for all samples.

Another quite recent method is stable isotope analysis to determine feeding ecology, physiological status, water use, movement patterns, origins. Stable isotopes give you location signatures (where the animal came from). This requires specialized labs, but holds huge potential for future investigations. It can be very useful in legal questions.

Entomological investigations (e.g. stage of larvae of blowflies) may help in calculating the minimum post-mortem interval. Sampling has to be done by the first responding person, because these larval stages develop further.

There are some minimum requirements for the first responding forensic lab.

It should be a fully established veterinary pathology lab with ability for necropsy and histology, radiography, sometimes parasitology, and an existing network of collaboration with other labs. Experts in **wildlife** pathology should be at hand to evaluate the necessity of further investigations – sometimes to argue with a prosecutor who may want to save on costs. Appropriate containers have to be used – different samples require different packaging. Storage and treatment methods of samples vary (chilling, freezing, formaline, ethanol/methanol) and have different pros and cons.

All this has to be taken into consideration when working on wildlife crimes.

The **potential applications of DNA** analysis in wildlife crime were explained.

These include Sample ID (species, region, population, individual), matching samples (antlers – skull/jawbone; carcass – blood on clothing/car), sex determination, parentage/relatedness.

The source material for sampling can include almost any biological material (saliva, faeces, hair, blood, urine, tissue, bone fragments, antlers, eggshells, museum samples). The critical factor is the quality of the sample. Which DNA marker/method can be used depends on the question and the source material.



Currently the “next generation” sequencing is very expensive (3-5000 €/run), which can be a limiting factor if only a few samples are included per run.

There are some differences in the results in different labs, which can be a problem for comparing results. Labs can share reference samples with each other so that sample analysis results become comparable. E.g. In Slovenia they may have good references for Slovenian populations, which may not be available in the Austrian or Italian database. To check whether an animal is from a particular population you need to have this type of reference.

What trace DNA cannot tell us:

- Trace DNA cannot really tell us about the age of an individual, because there is too much variation between individuals.
- You can also not tell whether a sample came from a living or dead animal.
- Size, colour or markings of an individual.

A real problem is **sample contamination**. E.g. sometimes a hunting dog might lick your dead animal, and you may get dog DNA on the sample, even though bite marks may indicate predation by e.g. a fox. DNA degradation complicates the analysis and

interpretation. In addition, analysis is expensive and takes time (weeks). → DNA alone is not sufficient to determine cause of death, you need the other necropsy tools combined.

Comments and questions from participants:

- Protocols are important, but often more important is the direct and quick contact and fast procedures to solve the crime. A lot of the time the limit is language – interpreting the English/German/Italian may lead to misunderstandings. Italy has a collaboration with the Austrian and Slovenian police force, and they can use a central phone number for wildlife crimes that are transnational. The logistic part is important.
- How important is it to call an expert to make a first analysis on the site you find a dead animal, when the animal is difficult to transport (e.g. bear)? Depends on the situation- time of year, time it takes to organize transport/time it takes for expert to arrive. The first responder has to be able to judge (usually carabinieri). Refrigeration as soon as possible.
- What happens if you find out that an animal has died from disease related to sick domestic animals? Is this a case of illegal death? - No, in this case it is not an illegal death. In European countries it is a crime if wildlife is killed through purposeful introduction of disease, but not if domestic animals are sick and pass on their parasites or pathogens. There is often contact between domestic animals and wildlife – sometimes also animals walking across country borders. On the other hand, if livestock is e.g. grazing where it is not supposed to be (e.g. in the core zone of a national park), then it is illegal. But these cases would lead to long legal fights...
- Regarding bone fragments, the quality of the result depends on the time of getting the sample. But with bone, does it matter? Bone has very little DNA, but some can be accessed – nevertheless over time the DNA fragments become smaller, and the smaller they are the less useful. The type of bone matters too – skull bone does not have much DNA left, but a leg bone with some marrow is a very good source. The types of markers that can be used also depend on the quality.
- On fresh tracks in snow there is supposed to be a technique for retrieving cells – what is the technique? - Theoretically it is possible, but it may be difficult to collect material that way. You could potentially filter down snow samples, and with newer sequencing technologies it would be possible to do an analysis, but the failure rate would be quite high at this stage. In a court case they would probably not accept it because of the possibility of contamination. A better strategy would be to follow the tracks until you find some feces.

- In Slovenia, when they do a census of wolf and bear populations, they collect feces from both species. The success rate of DNA analysis for bears is much higher (90%) than that for wolves (50-60% success)– why? - This could have to do with the diet of the animal, as well as with different types of digestion. It could also have to do with the age of the sample, degree of degradation. Winter samples are generally much better than summer samples due to better preservation in the winter. This may also mean that you need to gather many more wolf samples than bear samples.
- Do the insects provide hints of different locations of the animal? - Some experts are able to determine where the animal came from based on the type of larvae. Also, some larvae may have entered the animal if it was still alive. It has to be recorded on which body part the sample comes from.
- Using isotope analysis – what if a bird or mammal moves from Italy to Slovenia? - There is a certain amount of time it takes for the signature to change, it depends on the hair growth rate, so you have to know that.
- The use of Diclofenac and its effect on wildlife should also be mentioned.

3. WORKSHOP PART II

Presentation of some wildlife forensic cases

Some concrete cases encountered over the last several years in Austria were presented. These included cases of:

- Poisoning by the pesticide *Carbofuran*, which is banned as a pesticide, but which still appears to be available and leads to a protracted and painful death. So all cases of the use of this are illegal killings.
- Poisoning by rodenticides, which cause death by internal bleeding. Used to kill rats, it causes secondary poisonings in raptors/scavengers. This may be used for intentional killing, but may also cause unintentional poisoning of non-target species (which is illegal, even if unintended!).
- Lead intoxication in birds, mostly non-lethal gunshot, ingestion of lead shot directly or indirectly.
- A project about bird crime in Austria, Czech Republic, Hungary, Serbia and Slovakia - the PannonEagle project, an EU Life Project - was presented. This is a project run by Birdlife Hungary in cooperation with several international partners.. The main objective of the project is to increase the population of

the Eastern imperial eagle in the pannonian region through significantly decreasing the non-natural mortality caused by persecution incidents.

- The case of an illegal killing of a lynx in Austria was presented. Forensic investigations through DNA showed that the dead lynx, of whom a pelt was found in a bag, came from the Kalkalpen region and was related to two known lynx. The fur pattern was also compared with photos of the live animal, which allowed to confirm the identity of the dead animal. After the analysis the hunter admitted the illegal shooting. She was convicted to a payment of 240 daily rates because of endangering an animal population, lost the hunting license for 2,5 years. The taxidermist was sentenced to a payment of 360 daily rates because of a false witness report and illegal commercial use of a CITES annex A species.
- Another case presented was that of a dead wildcat. The necropsy showed lacerations and broken ribs. It was determined to be a car accident. The forensic team then checked if this was really a wildcat using morphological criteria. It was determined that this could be a wildcat, but genetic analysis showed it was a hybrid with a wildcat mother and a domestic cat father.
- The use of DNA analysis to determine insurance questions: roe deer collisions – in Austria insurance covers collisions only for game species, but what if there is no carcass? In one investigated case, some hairs were found on the front fender, but suspiciously placed. The hair samples were examined and swabs taken from the blood traces. DNA extracted and sequencing showed that the hair was clearly from roe deer, and the blood matched as well – but there were two different sequences, indicating two (unrelated) individuals, while the hunter had claimed the accident was with one deer only. It could have been a very unlikely case of heteroplasmy. A second method could not confirm a second individual.
- A second case reported was the accurate predator identification from game and livestock kills. Compensation is paid to farmers if the killer is a wild predator. Often the DNA results are clear and show what predator it was, but there are some cases where there are doubts between wolf and dog. Signatures of domestication include an adaptation of the dog to a starch-rich diet – they have evolved multiple copies of genes for digesting starch, of which wolves have only an ancestral two copies. New lab tests can measure the gene copy number and compare samples this way. Further work is needed to optimize low quality samples.

Questions and comments:

- Do you find poisoned birds in Austria quite often? - Every third week.

- In Italy, collisions for other species may also be covered. It is case-dependent.
- Although no cases were presented, there may also be poisonings by drugs used by humans in bait and poisoning by artificial sweeteners used by humans – these are quite toxic to carnivores.

4. WORKSHOP PART III

Discussion with and feedback from stakeholders centered on the preparation of a SOP Handbook, as foreseen in the Albionet2030 project. This will be produced in the different Alpine country languages. The handbook may be web-linked (maybe with a QR code).

Q: What would you need to be included in the handbook for your daily work?

1. Lists of contacts, labs in the different countries.

There are different structures in the different countries. It would be good to indicate what possible links there are in border areas to authorities and labs in the other countries.

(In Italy one of the problems is that it is not really clear if a dead animal is found, who is supposed to handle this, what the legal requirements are.

In Slovenia, like in Austria, the hunters are the first instance responsible for this.)

The **handbook** should include information on whom to inform, an organization chart of the different institutions handling forensics and their main tasks. It was suggested that the handbook should include **the complete process**, from what happens when a dead animal is found to what happens during a prosecution in court.

2. Protocol

- Sampling
- Storage of samples
- What methods to use in what situation/for what purpose
- A special box on what not to do in the first instance!

3. Case flow chart – if this, then do that...

4. Examples of cases that went wrong due to wrong procedures

(An output of this project should be to inform all police units of the proper procedures to follow. A training workshop for police units is foreseen in the LifeLynx project – Alpbionet-produced materials can be used!)

Implementation → Inform administration, police (BK)

Presentations will be made available to participants as downloadable PDF.

This report and presentations can be downloaded at this link:
<https://tinyurl.com/forensicworkshop2017>