The newsletter of the Society for Wildlife Forensic Science

Vol. 6 No. 2 | September 2021

SWFS NEWS

Inside this edition: Toxicological Tools, Detection Dog, Wildlife Veterinary, Pesticides, Rapid Detection PCR

How to build capacity for wildlife forensics in Africa during the Covid 19 pandemic?

Lessons from a hybrid course model for ivory identification training in Malawi

Author: Dr Stephanie Pietsch, Southern Africa Programme Coordinator of TRACE Wildlife Forensics Network (TRACE)

TRACE Wildlife Forensics Network (TRACE) supports capacity building for wildlife forensics and wildlife law enforcement in partnership with governments in several African countries. However, during the Covid 19 pandemic international and regional travel restrictions constitute major obstacles to various wildlife forensic capacity building activities in Africa. This also concerned a recent training course organised by TRACE on the morphological identification of different types of ivory and ivory substitutes. In 2020, TRACE was planning to sponsor the participation of several African wildlife law enforcement officers at the ivory identification training course offered by Dr. Ed Espinoza at the U.S. Fish & Wildlife Service (USFWS) National Forensics Laboratory in Ashland, Oregon, USA. Due to the Covid 19 pandemic, the training course in the US had to be postponed for an indefinite period of time. Another ivory ID course planned as part of the African Wildlife Forensics Network (AWFN) meeting in Kruger National Park, South Africa in July 2021, had to be cancelled as well.

The global ivory trade has been a key wildlife conservation issue for decades. Traditionally, the approach of ivory traffickers was to smuggle either complete or fragmented pieces of elephant tusks from Africa to Asia, where they would then be processed locally. However, recent investigations (for example by the NGO, TRAFFIC) have uncovered a trend in ivory being processed in Africa before being smuggled to end markets. There are additional concerns that the prohibition of domestic commercial trade in elephant ivory in some countries globally may lead to an increase in trade of hippo ivory as a substitute.

continued on page 4

Welcome from the SWFS President

Dear SWFS Members,

Welcome to the Eleventh edition of the SWFS Newsletter.

I hope this Autumn Newsletter finds everyone healthy and well. As our Society continues to navigate the new world, it will come to no surprise to find a shorter Newsletter. The SWFS board and committees have been operating as normal and we have a few notable updates.

Over the summer there were several changes to the SWFS board. The following board members completed their terms and have moved on to impact the Wildlife Forensic Community in different ways. I would like to recognize Rob Ogden, Kathy Moore and Rebecca Johnson for the dedication, involvement and expertise they brought to the SWFS board over the last several years in their role on the SWFS board. Their input and expertise will be missed. With three members terming out the board circulated a request for new board members in April 2021. I am pleased to announce that the board has voted in three Wildlife Forensic Professional who will be great additions to the team.

I am pleased to welcome back Dr. Rebecca Johnson, Associate Director for Science and Chief Scientist at the Smithsonian National Museum of Natural History as the President-Elect, Dr. Sherryn Ciavaglia, Wildlife DNA Forensic Unit at SASA as the Membership Director and Professor Gila Kahila Bar-Gal at the Koret School of Veterinary Medicine as the Director of Policy. Please take a moment and congratulate each of these SWFS members on their new Board positons.

Now on to Conference thoughts, as the 2021 year is more than half way gone the SWFS board and conference planning committee are starting to meet again to discuss how to move forward with a meeting in July of 2022. How this meeting will look as not been decided at this time, as there are many variables in play. Current communication on SWFS meetings have been

Officers 2019-2021
President: Tasha L. Bauman, M.S.
President-Elect: Dr. Rebecca Johnson
Treasurer: Dee Dee Hawk
Secretary: Nadja Morf

Board of Directors:
Audit/Assessment: Edgard Espinoza
Certification: Kim Frazier
Communications: Daniel Xu
Proficiency Program: Brian Hamlin
Membership: Dr. Sherryn Ciavaglia
Policy and Partnership: Gila Kahila Bar-Gal
Director: Frankie Sitam
Director: Antoinette Kotze

Editor
Tasha Bauman

www.wildlifeforensicscience.org

Welcome from the SWFS President

minimal at best, please know we are working to have something in place come July.

As always, I would like to extend my gratitude and thanks to all that have contributed to the SWFS Newsletter, as well as the production team that put this periodical together.

Best wishes

Tasha Bauman

IN THIS ISSUE

© SWFS 2021 - To reproduce content from this newsletter please contact Tasha Bauman: tasha.bauman@wyo.gov

How to build capacity for wildlife forensics in Africa during the Covid 19 pandemic? Lessons from a hybrid course model for ivory identification training in Malawi

continued from front page

This development is a challenge for wildlife enforcement officers in Africa given that smaller pieces of ivory and carved ivory are more difficult to detect and identify. Since ivory originates from a range of species whose protection status varies across the African continent, species identification is key to CITES enforcement efforts.

The time is ripe but the timing is not ideal

It is clear that there is an urgent need to build capacity within the wildlife law enforcement community for ivory identification. The director of the Department of National Parks and Wildlife (DNPW) in Malawi, Mr. Brighton Kumchedwa, approached TRACE with the request to provide training for ivory ID amongst his senior investigators. DNPW Malawi currently has only two wildlife officers proficient in ivory identification, which is not sufficient to keep up with the backlog of ivory cases awaiting prosecutions in the local courts of all over Malawi.

The poor prospects for an in-person ivory ID training in the near future led TRACE, Dr. Espinoza and DNPW Malawi to collaborate and explore options for an online ivory ID training. After initial doubts, the idea for a hybrid course concept was born: A combination of face-to-face practical sessions in Malawi with online learning under the guidance of an international instructor and leading ivory ID expert, Dr. Espinoza. Another important key player for this hybrid course concept was Mr. Fyson Suwedi - a senior investigation officer at DNPW Malawi. He had previously attended an in-person ivory ID training by Dr. Espinoza during the last SWFS meeting in Denver, Colorado, USA in June 2019. Since then he has successfully applied his skills in several ivory court cases in Malawi. Mr. Suwedi was immediately willing to accept the proposed role as local co-instructor to build capacity for ivory ID amongst his colleagues in Malawi. TRACE arranged all logistics for the ivory ID course including the necessary equipment, national travel of participants and conference hotel with funding from the People's Postcode Lottery Project (UK-PPL). Seven wildlife law enforcement officers from DNPW and one veterinary scientist of the Central Veterinary Laboratory (CVL) participated in the hybrid ivory ID training held over two half days in Lilongwe, Malawi from 10th to 11th August 2021 (Figure 1).

The ivory ID course programme was based on the "CITES Identification Guide for Ivory and Ivory Substitutes" (4th Edition, 2020) but customized to fit the needs of DNPW Malawi, especially with regards to those ivory-bearing species and ivory substitutes commonly found in trade in Southern Africa. The DNPW Malawi provided a reference collection of ivory for this training. The participants learnt how to identify ivory from different

Participants of the online ivory identification training held in Malawi during the COVID-19 pandemic.

continued on page 5

How to build capacity for wildlife forensics in Africa during the Covid 19 pandemic? Lessons from a hybrid course model for ivory identification training in Malawi

continued from page 4

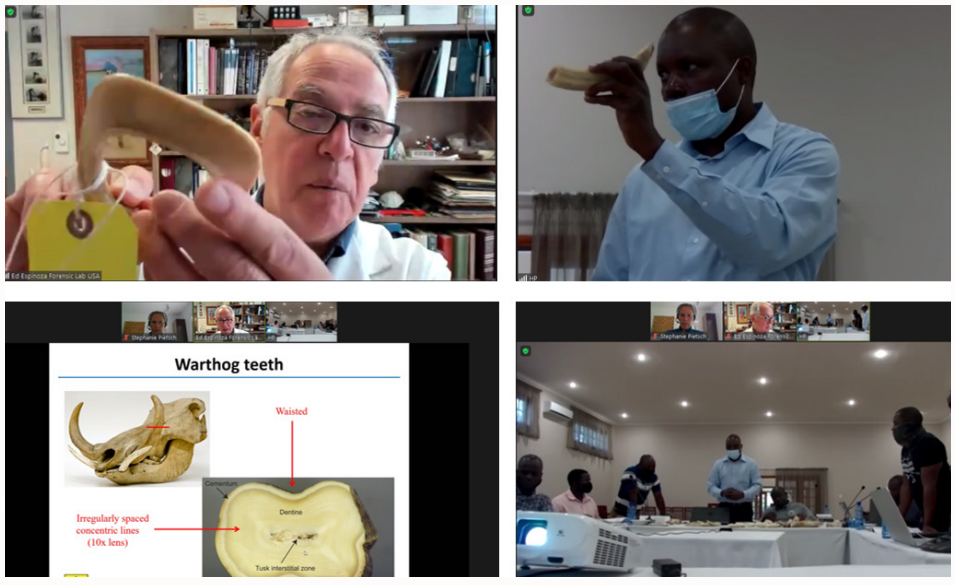
Participants of the online ivory identification training held in Malawi during the COVID-19 pandemic.

species including mammoth, elephants, hippopotamus, warthog and cetaceans.

They were also trained to differentiate between real ivory and ivory substitutes such as vegetable ivory, bone, hornbill and plastic. Online learning sessions given by Dr. Espinoza alternated with practical exercises led by Mr. Suwedi in Malawi. Co-instructor Suwedi also used the local language Chewa to impart knowledge and expertise in both theory and practice to his Malawian colleagues. The participants learnt how to use traditional tools for morphological ivory identification such as UV light, magnification lens, protractors and rulers for Schreger angle measurements. In addition, modern tools (e.g. clip-on smartphone microscope with LED UV lights) and different smartphone apps were also tested for their performance in the field. Despite the hybrid character of this ivory ID training, the participants soon became actively involved, very much focused and asked a multitude of excellent questions during the training course. After successful completion, all participants were awarded a certificate, which is important to demonstrate their qualification as expert witnesses for ivory cases in court. Feedback provided by the participants showed that this innovative training model was a real success story and a great collaboration project between TRACE, USFWS and DNPW Malawi. After this positive experience the organizers agreed to roll-out this course model to other African countries.

Lessons learnt: Online hybrid training courses can successfully bridge the gap. Well organized and qualified local co-instructors supporting the practical exercises on the ground and the on-site availability of an extensive ivory reference collection are both key to the training success. However, we are all looking forward to onsite training courses with personal interactions in Africa.

After successful completion, all participants were awarded certificates and congratulated with fist bumps.



Online learning session for ivory ID training delivered by Dr. Ed Espinoza via zoom.

Optimization of toxicological and forensic tools in the investigation of wildlife poisoning

Department of Socio-Sanitary Sciences, Area of Toxicology, Faculty of Veterinary, University of Murcia, Campus Espinardo, 30100 Murcia, Spain

Authors: Irene Valverdea, Silvia Espína & Antonio J. García-Fernándeza

This communication summarizes the PhD (to be defended in Oct-Nov 2021) developed by Irene Valverde Domínguez and supervised by Antonio J. García Fernández and Silvia Espín Luján at University of Murcia (Spain).

Wildlife is exposed to many threats worldwide which may cause important population declines, including e.g., deforestation, illegal hunting, illegal trade and poisoning. In order to improve the fight against wildlife poisoning, this thesis aims to provide additional toxicological and forensic tools. This requires increasing the knowledge on standardisation and protocolisation of methods for classifying animal carcass decomposition, assessing the degradation of compounds in carcasses, and collecting available information on analytical techniques.

Post-mortem examination and toxicological analysis are essential for a proper diagnosis of the cause of death by poisoning. However, investigators often require an estimate of the time of death, which is best determined by identifying the stage of carcass decomposition. For this purpose, a scoring method was proposed to classify the stages of carcass decomposition and thus provide an estimate of the time of death in small-size raptors. Twelve carcasses of Common kestrel (Falco tinnunculus) were exposed to external weather conditions (in the period 4-19 July 2019) in Murcia, South-eastern Spain. The ambient temperature and relative humidity, body core temperatures and carcass weights were measured at intervals over the study period. Necropsies were performed (2 birds at each interval) at 1-2 hours, 24 hours, 72 hours, 96 hours, 7 days and 15 days after death. The necropsy of a previously frozen bird was performed to act as a comparison with non-frozen fresh individuals. Six stages of the post-mortem autolytic process were selected: fresh carcass, moderate decomposition, advanced decomposition, very advanced decomposition, initial skeletal reduction and complete skeletal reduction. To classify the carcasses according to these categories, a scoring method is proposed considering 5 parameters: state of the eyeballs, tongue/oral cavity, pectoral muscle, internal organs and other features. Several parameters affecting the process of the decomposition are discussed.[1]

In a second step, a study to evaluate the persistence of bromadiolone (a second- generation anticoagulant rodenticide, SGAR, frequently involved in secondary poisoning in rodent predators worldwide) in poisoned carcasses was carried out. The bromadiolone was analysed in liver of experimentally-dosed Common kestrels at different stages of carcass decomposition to understand the possibility of detecting bromadiolone in cases of wildlife poisoning and the potential risk of tertiary poisoning. Twelve non-releasable individuals (destined to be euthanized) were divided into the bromadiolone-dose group (dosed with 55 mg/kg b.w.) and the control group. Hepatic bromadiolone concentrations found in each stage of decomposition were: 3000, 2891, 4804, 4245, 8848, and 756 ng/g d.w. at 1–2 h (fresh carcass), 24 h (moderate decomposition), 72 h, 96 h (advanced decomposition), seven days (very advanced decomposition), and 15 days (initial skeletal reduction) after death, respectively. Liver bromadiolone concentrations in carcasses remained relatively stable over the first four days and raised on day 7 of decomposition under the specific conditions of this experiment, presenting a risk of causing tertiary poisoning. However, at the initial skeletal reduction stage, liver bromadiolone concentration declined, which should be considered to interpret toxicological analyses and for proper diagnosis. This experimental study provides for the first time some light to better understand the degradation of SGARs in carcasses in the wild. [2]

Publications describing different analytical methods for anticoagulant rodenticides (AR) analysis in biological samples are growing. A compilation of the overall picture has been carried out to standardise methodologies and compare the analytical procedures applied for AR determination in the literature. Using

continued on page 7

Optimization of toxicological and forensic tools in the investigation of wildlife poisoning

Continued from page 6

this information, a scoring system was developed for those techniques using liver and blood as matrices, and the techniques were ranked considering different criteria (i.e., sample amount required, recoveries, LOQ, number of AR analysed, points of the calibration curve and multi-class methods). This review shows an overview of the main methods used for AR analysis in forensic toxicology and will help to elucidate future directions to improve multi-residue techniques to detect the AR involved in wildlife lethal poisoning.[3]

Several heterogenic techniques using diverse matrices, extractants and analytical instruments at different conditions have been described in the literature for different compounds. Therefore, it is essential to monitor the performance of different laboratories applying a variety of methodologies in the determination of toxic compounds involved in wildlife poisoning worldwide, to ensure comparable results. For this reason, we carried out a comparative study to make a first approach in assessing the individual performance characteristics of the analytical procedures applied to detect toxic compounds involved in wildlife poisoning between different laboratories. Four laboratories of reference in veterinary forensic toxicology in Spain have participated. Chicken liver samples were spiked with 11 strategically selected substances, including bromadiolone, brodifacoum, difenacoum, warfarin, chlorophacinone, carbofuran, aldicarb, methiocarb, diazinon, chlorpyrifos and parathion. Each laboratory carried out the analyses with their routine techniques and reported their results (mean concentrations in the spiked liver samples for each substance, repeatability, recoveries, and limits of detection/quantification of their techniques). In this interlaboratory comparison, the laboratory performance was expressed in terms of z-score in accordance with ISO13528:2015. Despite their different extraction procedures, instrumentation, and chromatographic conditions, in general all the participant laboratories have accurate and comparable results for all the compounds evaluated. However, some techniques stand out because they are more economic and environmentally respectful.[4]

Toxicovigilance and risk assessment studies are essential to reinforce the knowledge of the number of illegal poisoning cases and the substances involved in these crimes. In a final chapter, we started a European network, focused on veterinary forensic toxicology laboratories, in order to improve communication between laboratories in the fight against wildlife poisoning, specially focused on raptors. For this purpose, a questionnaire was designed and sent by email to 118 laboratories. It had 39 questions on different topics (e.g., laboratory activities, analytical information). A total of 28 replies were received. The different analytical techniques and data collection should be harmonized, and a sufficient communication between laboratories is needed to create an effective network. This study detected strengths and pitfalls that will help to harmonize methodologies and increase pan-European capacities.[5]

[1] Valverde I, Espín S, María-Mojica P, García-Fernández AJ. 2020. Protocol to classify the stages of carcass decomposition and estimate the time of death in small-size raptors. European Journal of Wildlife Research, 66:1–13.

[2] Valverde I, Espín S, Gómez-Ramírez P, Navas I, Sánchez-Virosta P, Torres-Chaparro MY, Jiménez P, María-Mojica P, García-Fernández AJ. 2020. Temporal persistence of bromadiolone in decomposing bodies of Common Kestrel (Falco tinnunculus). Toxics, 8:98.

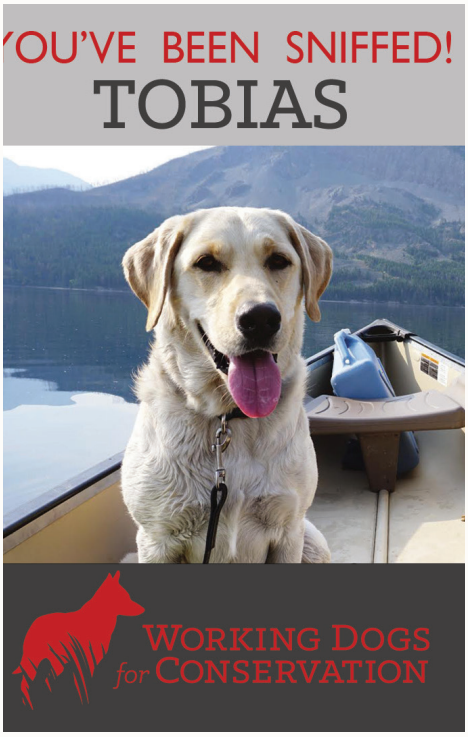
[3] Valverde I, Espín S, Gómez-Ramírez P, Navas I, Sánchez-Virosta P, Torres-Chaparro MY, Jiménez P, María-Mojica P, García-Fernández AJ. 2021. Wildlife poisoning: a novel scoring system and review of analytical methods for anticoagulant rodenticide determination. Ecotoxicology, 30(5): 767-782.

[4] Valverde I, Espín S, Luzardo O, Mateo R, Soler-Rodríguez F, Gómez-Ramírez P, Navas I, Sánchez-Virosta P, Torres-Chaparro MY, Jiménez P, María-Mojica P, García-Fernández AJ. Interlaboratory comparison to determine toxic compounds involved in wildlife poisoning. In preparation.

[5] Valverde I, Espín S, Gómez-Ramírez P, Sánchez-Virosta P, García-Fernández AJ, Berny P. Developing a European network of analytical laboratories and government institutions to fight against raptor poisoning. *Under review.*

New textbook chapter highlights ways that conservation detection dog teams and ecological monitoring can be harnessed to support

Author: Ngaio Richards, Forensics & Field Specialist for Working Dogs for Conservation, Instructor and Coordinator, Department of Wildlife Forensic Sciences and Conservation, University of Florida.



Partnered with capable handlers, specially trained dogs are an asset to ecological and environmental monitoring efforts, and in various forensic and enforcement endeavors. However, dog-handler team efficacy can be significantly undermined if the strengths and limitations of this tool, relative to the information or action sought, is not fully understood from the outset.

A chapter titled 'The Role of Conservation Dog Detection and Ecological Monitoring in Supporting Environmental Forensics and Enforcement Initiatives' in the recently published textbook 'Wildlife Biodiversity Conservation – Multidisciplinary and Forensic Approaches' sets out to address this and related concerns. The textbook was edited by Susan Underkoffler and Dr. Hayley Adams in the University of Florida's newly created Department of Wildlife Forensic Sciences and Conservation. The chapter itself was written by a team of canine handlers with detection backgrounds spanning conservation and ecological monitoring, law enforcement and search and rescue. Committed to upholding and strengthening the integrity of this field wherever possible, these authors sought to underscore the wealth of olfactory science accumulated to date, while acknowledging and legitimizing the artistry that comes from gathering data with dogs - extraordinary animals whose successful deployment requires a different approach to that used with conventional sampling equipment.

The enhancement of ecological monitoring applications at the interface of environmental forensics and enforcement is discussed around further incorporation of dog-handler teams. The notion of proactive monitoring, to facilitate sustainable preservation of organisms and ecosystems, and to lessen the strain on enforcement resources, is a prevailing theme. Also covered are the types of information that can best be gathered with the help of dog teams, and how to contextualize the importance of seemingly low find rates and sample sizes, for example in the case of rare species or in verifying the success of non-native species eradication efforts.

In short, the chapter seeks to clearly delineate the strengths and limitations of conservation dog-handler detection by addressing some of the misconceptions we commonly encounter in this profession. To gain a further sense of the relative strengths and limitations of the dog team method, a similar discussion can be found in Liczner et al. (2021) 'Training and usage of detection


© Working Dogs for Conservation

continued on page 9

A Matter of Interpretation: Wildlife Law Enforcement and the U.S. Migratory Bird Treaty Act

Continued from page 8

dogs to better understand bumble bee nesting habitat: Challenges and opportunities', particularly the discussion appended in SI: 'Additional considerations for using detection dogs to find bumble bee nests and in related conservation efforts'. Co-authored by WD4C trainer-handlers, this paper and the supplementary information candidly discusses the challenges, from training and fielding perspectives, of using dog teams to find bumble bee nests. It also offers some (perhaps surprising) information-gathering opportunities made possible merely by the participation of dogs. Topics discussed include: the types of dogs used in conservation detection applications, sample procurement relative to training considerations, suggested field sites for deploying detection dogs to locate bumble bee nests and proxy targets for bumble bee nests.

Meet the Board: Brian C. Hamlin

What's your current position?
Since 1999 I have been working as a forensic scientist in the genetics section of the United States Fish & Wildlife Service (USFWS) Office of Law Enforcement (OLE) National Fish & Wildlife Forensics Laboratory (NFWFL), located in the beautiful mountains of southern Oregon, USA.

Can you give me a brief overview of what it is you do in your work?
The big picture involves providing support to our law enforcement user groups in the form of genetic analyses and subsequent case reports, and if needed, attesting to those finding in the court of law. On a day-to-day basis, this typically involves mtDNA sequencing for species identification, and the application of autosomal STRs for individual identification and/or geographic assignment, including determination of sex origin. This work encompasses a wide range of species from across the globe, however, I have a special interest in cervids. I also enjoy participating on various task groups for the OSAC Wildlife Forensic Subcommittee and my current role on the SWFS Board as Proficiency Director.

How did you first get involved in Wildlife Forensics?
By accident! While in graduate school I was able to spend a year at the USFWS OLE NFWFL volunteering in the genetics section working on a project for individual identification of N.A. Elk. That initial exposure opened my eyes to the incredible work being doing and the challenges/opportunities that lie ahead in the field. I was able to join the genetics team not long after that and have been rolling along in my current role ever since.

What has surprised you most about working in Wildlife Forensics?
The casework. It never stops and even when you think you've seen or heard of just about everything… Here comes something else to give you pause! Then you get busy working

continued on page 10

Meet the Board: Brian C. Hamlin

Continued from page 9

the case or conducting research, validation, and application. Rinse and repeat.

What do you find most challenging about Wildlife Forensics?
Hmm. Staying on top of accreditation demands, keeping track of best practices, including what you "shall" vs. what you "should" be practicing, and all that entails. The lack of standardized and commercially available kits for use in Wildlife Forensics has been a challenge since day one. Those in the wildlife field understand this challenge. Those working in the Human Forensics arena, not so much.

What would you say most motivates you to do what you do?
Working in this profession has a direct impact on the well-being of our worlds flora and fauna. That is motivating in itself. Plus, I really enjoy the process of letting the evidence tell its story through genetics. The moment the "what is it" question is answered always brings some degree of satisfaction.

What were you doing before you began your current position?
Working as a wildlife biologist and attending graduate school. Much of that time was spent working in the field with a variety of fish and mammal species found in the Midwestern portion of the USA.

Tell me about someone who has influenced your decision to work in Wildlife Forensics?
It wasn't a single person, but just about everyone I met during the year I spent volunteering at the USFWS OLE NFWFL. They were an inspiring bunch!

Where did you grow up?
Missouri. In the heartland of the United States. I grew up playing sports like baseball and soccer, while spending as much time as I could in Ozark streams canoeing and fishing or exploring the hardwoods and prairies across the state.

What might someone be surprised to know about you?
I grew up in a family that loved raising and training bird dogs. I currently have three English Setters and hope to add a couple more to my pack as soon as can afford them (LOL).

What's next for you in your work? What are you looking forward to?
Let's see. I've got a constant flow of cases in various stages from newly assigned to ready for reports to be written. I also have Proficiency Director duties to attend to (Please see our Proficiency Testing Update in this newsletter). Then some research! Like casework, the research never stops and that always gives me something to look forward to. OdoPlex - a validated method involving species ID, sex identification, and individual identification of N.A. White-tailed deer and Mule deer will soon be available in the upcoming FSIAE SWFS Wildlife Forensics special edition. On the distant horizon is a similar project called WapitiPlex, which involves validation and standardization of a method for individual identification of N.A. Elk.

What would you tell someone who is thinking about starting a career in Wildlife Forensics?
Be open, think outside the box in terms of your education and life experiences and how they might be applied in Wildlife Forensics. Experience gained by volunteering or an internship could open your eyes to the possibilities that exist and temper expectations (this is not your televisions CSI show).

What do you do when you aren't working?
I have a seekers soul. I spend free time casting about looking for morel mushrooms, recreationally prospecting for gold, and I absolutely love spending time training my dogs and looking for Galliformes.

Wildlife Veterinary Forensics: A New Opportunity for Veterinary Technicians in Conservation

Author: Katrina Mishel, MA Conservation Biology, Miami University Ohio

Veterinary technicians should be the optimal choice in assisting wildlife veterinarians and conservationists in wildlife forensics. Veterinary technicians are vital components to the growth of wildlife veterinary forensics that can be used to help convict poachers, determine causes of death, monitor ecosystem health, and much more. It is strongly advised to become a certified veterinary technician and have a certificate or degree in veterinary forensics in order to be successful in the field. There are continuing education opportunities and degrees available around the globe.

Veterinary forensics is increasingly being utilized in wildlife and conservation cases.

Wildlife veterinarians are assessing critical health factor impacts on wildlife population dynamics, developing new technologies, collecting biological data, managing emerging diseases, and more. This increase of veterinary medicine in conservation focuses on the usage of veterinarians due to their indispensability and ability to diagnose issues and prescribe medications. However, veterinarians and biologists need the support that can be provided by technicians.

Veterinary technicians, or veterinary nurses, are usually thought of when your pets get sick, but they can be found volunteering or working for animal hospitals, research centers, zoos, circuses, sanctuaries, and even rehabilitation facilities. Veterinary technicians, especially licensed ones who have a degree and have passed national certifications, are able to perform duties some may compare to those of a Registered Nurse for humans. They are knowledgeable in many duties from animal handling to phlebotomy, diagnostic imaging, and laboratory techniques. Essentially, the only things a veterinary technician can't do are make a diagnosis, make a prognosis, prescribe medications, or actually perform a surgery. With the specific skill sets veterinary technicians have, it would be detrimental not to have them assist in conservation efforts and wildlife veterinary forensics.

Companion animal research is taking protocols in human medicine and translating or modifying them to help jump start research. These protocols can be translated or modified further to advance wildlife and conservation research. With the knowledge, skillset, and expertise veterinary technicians

Wildlife Veterinary Forensics: A New Opportunity for Veterinary Technicians in Conservation

Continued from page 11

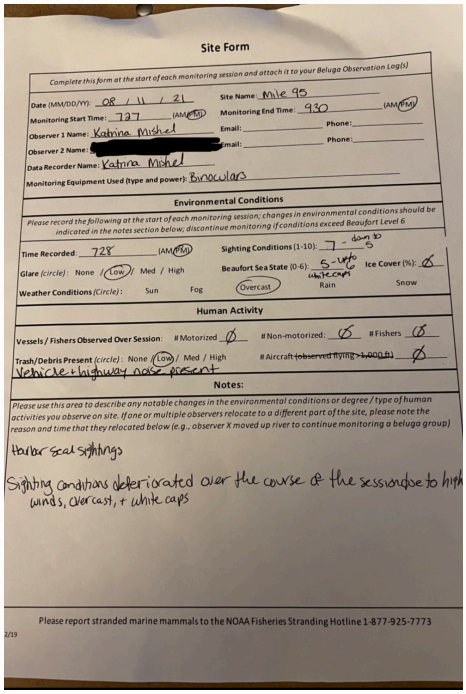
already have in companion animal medicine, it would be easiest for them to transfer these skills over to assisting conservation efforts.

Veterinarians and veterinary technicians are usually the primary sources that face animal cruelty firsthand, and often become involved in the legal investigations, conducting examinations, and collecting and analyzing evidence. Due to the increased awareness of animal cruelty and the lasting effects it has on everyone, veterinary forensics has been recognized as an important application to investigation, documentation, and prosecution of crimes against animals and led to the rapid growth of the veterinary forensics field. As with people, there is a broad range of criminal activities that need expert witnesses and research to rely on for evidential purposes.

Necropsies are how veterinary technicians and veterinarians find the evidence they need. Veterinary technicians assist in necropsies by taking notes, extensive photographs, samples, and aiding in dissection. A newer way to acquire evidence is through imaging techniques like CT and MRI scans. In human medicine, it is typical for technicians to conduct these scans, and veterinary technicians can perform these procedures for veterinarians. The use of CT scans (and other modern imaging techniques) can help create optimal planning of the necropsy to increase the likelihood of identifying the relevant changes in the body and thus optimize gathering the relevant evidence needed for prosecution. Depending on the funding available, wildlife forensic veterinarians and their technicians can utilize portable CT scanners in more semi-rural areas.

There are documented cases where conservationists are taking advantage of veterinary forensics. For example, the use of the rapid, non-destructive, reliable, eco-friendly ATR-FTIR spectroscopy to accurately identify what species a claw belongs to. In this situation, the veterinary technicians are often the ones to operate the spectrometers and analyzers. Veterinary technicians also assist in the necropsy, collection of materials, and prepping of samples for marine animal cases. Without the obvious presence of fishing gear or wounds, these cases need to be ruled out as drownings.

Since the need and interest for veterinary forensics has happened recently, theories and techniques are still evolving in the field. If veterinary technicians are able to get in on the ground floor, they can aid in testing and creating these set guidelines of evidence collection, especially since it is the technician's responsibility to collect and help analyze evidence. However, a lot of veterinary professionals have almost no training in forensic crime scene analysis, but at least have some



Site Form

Complete this form at the start of each monitoring session and attach it to your Beluga Observation Log(s)

Date (MM/DD/YY): 08 / 11 / 21 Site Name: Mile 95

Monitoring Start Time: 727 (AM) PM Monitoring End Time: 930 (AM) PM

Observer 1 Name: Katrina Mishel Email: Phone:

Observer 2 Name: [redacted] Email: Phone:

Data Recorder Name: Katrina Mishel

Monitoring Equipment Used (type and power): Binoculars

Environmental Conditions

Please record the following at the start of each monitoring session; changes in environmental conditions should be indicated in the notes section below; discontinue monitoring if conditions exceed Beaufort Level 6

Time Recorded: 728 (AM) PM Sighting Conditions (1-10): 7 - down to 5

Glare (circle): None / (Low) / Med / High Beaufort Sea State (0-6): 5 - up to 6 w/ caps Ice Cover (%): 0

Weather Conditions (Circle): Sun Fog (Overcast) Rain Snow

Human Activity

Vessels / Fishers Observed Over Session: # Motorized 0 # Non-motorized: 0 # Fishers 0

Trash/Debris Present (circle): None (Low) / Med / High # Aircraft ~~(observed flying >1,000 ft)~~ 0

Vehicle + highway noise present

Notes:

Please use this area to describe any notable changes in the environmental conditions or degree / type of human activities you observe on site. If one or multiple observers relocate to a different part of the site, please note the reason and time that they relocated below (e.g., observer X moved up river to continue monitoring a beluga group)

Harbor seal sightings

Sighting conditions deteriorated over the course of the session due to high winds, overcast, + white caps

Please report stranded marine mammals to the NOAA Fisheries Stranding Hotline 1-877-925-7773

2/19

understanding of forensic body collection through necropsies. In order to be successful, cooperative learning and cross training is advised since many veterinary professionals are not trained in forensic science and procedure. Veterinary technicians have already perfected being a "Jack of all Trades", learning crime scene analysis basics will add to the repertoire.

PTB Update for SWFS Autumn 2021 Newsletter

Author: Brian C. Hamlin

The Proficiency Test Board (PTB) members and myself want to give a shout out to the SWFS membership. Given the diversity of species encountered across membership labs and unique challenges faced by our global SWFS body, we recognize the need to continually develop new testing schemes, and to improve upon existing ones. We value your input and will work to remain open and sensitive to membership needs. Therefore, in the coming months we will be sending out a survey asking a range of questions related to the SWFS Proficiency Test Program and proficiency testing in general.

Areas that we are seeking feedback on include the use of synthetic DNA, which could provide a viable option for many SWFS member labs. The nature of this approach may allow tests to efficiently meet the varied permitting requirements of our international SWFS membership. Also for consideration is the addition of one or two domestic species (e.g., cat and dog) to our current mammal testing scheme in an effort to offer additional species diversity.

For those labs that currently participate in our mammal testing scheme (which includes potential White-tailed deer, Mule deer, N.A. Black bear, and N.A. Elk samples), or are looking to in the future, we plan to provide detailed guidance (via the SWFS website) to validated and published methods to assist analysts and labs with testing of these species. Look for this information to show up on the SWFS website in the Proficiency Testing Section later this year.

Lastly, we have been working to revise the current Proficiency Testing Charter and will be reaching out to membership for comment later this year.

Much has been going on with the PTB behind the scenes and we are excited to be moving forward. However, feedback from our membership is critical for making informed decisions as we progress. Please keep an eye out for the survey and we encourage you to participate.

Proficiency Director
Brian C. Hamlin

Proficiency Testing Board
Greta Frankham
Matthew Brick
Ashley Spicer
Joy Bruno

How might the practice of deliberately poisoning wildlife with pesticides affect local insect communities?

Authors: Isabel Fernández Verón and Irene Zorrilla, Center for Analysis and Diagnosis of Wildlife /Environmental and Water Agency of Andalusia, Environment and Sustainability Directorate.

In parts of Europe, Africa and Asia, people may use pesticides to deliberately poison wildlife in response to various resource-based conflicts, or as a means of capture for subsistence or trade. Repercussions of this largely illegal practice are well-documented in macro-scavengers, particularly vultures.

In southern Spain, since 2008, environmental agents processing suspected scenes of environmental

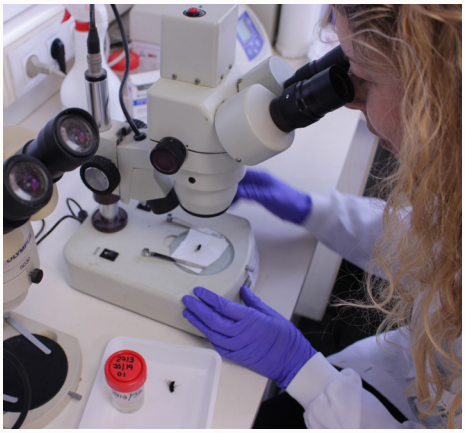
continued on 14

How might the practice of deliberately poisoning wildlife with pesticides affect local insect communities?

Continued from page 13

crime have collected representative insect specimens as both potential evidence and victims. The insect specimens are identified taxonomically to the degree possible and analyzed for a suite of pesticides and toxic substances. Between 2008 and 2019, pesticide residues were detected in a subset of pooled or uniquely insect samples (n = 46 instances; 12 consisting solely of arthropods). Four carbamate pesticides and/or associated metabolites were detected: aldicarb/aldicarb sulfoxide, carbofuran/3-hydroxycarbofuran, methomyl/methiocarb, either qualitatively (thin layer chromatography) or quantitatively (liquid/gas chromatography), reaching concentrations of 3.05 mg/kg (aldicarb) and 9.98 mg/kg (methiocarb). A range of species have been collected, ranging from beetles to blowfly to, in one case, European honeybees.

It is currently beyond the resources available for agents to quantify the extent of observed insect mortality in relation to poisoned bait or carcasses of poisoned animals. Nor is it within the scope of an investigation to record the identity of the living and deceased insect species encountered while searching a scene for evidence. However, this presents an opportunity for further entomological and ecological monitoring, as highlighted in a recent paper published in the Journal of Insect Conservation, titled:



'Is deliberate pesticide poisoning of wildlife impacting local insect communities? Wildlife and environmental forensic investigations in southern Spain present an opportunity for collaborative entomological monitoring'.

The aims of the paper were to:

- Generate awareness of the deliberate wildlife poisoning practice as it may affect local necrophagous and associated insect communities
- Share our process of sample collection, species identification and toxicological analyses to meet wildlife/environmental crime investigation requirements
- Stimulate interest in collaborative studies to more systematically examine/document any potential effects of the practice on susceptible arthropods species relative to their population status and ecosystem services provision
- Outline how this additionally gathered entomological information could also be used to strengthen wildlife/environmental forensics investigations.

We hope that colleagues will contact us to discuss potential collaborations that can benefit

Are Rapid Detection PCR Methods Forensically Useful?

Mary K. Burnham-Curtis, Lucy Webster, Greta Frankham

Wildlife inspectors and border agents continue to seek reliable and rapid methods to confirm legally declared species, detect misidentified or illegal species, and prevent invasive species incursions. Such rapid identification and detection methods can help international wildlife law enforcement agencies improve both detection of illegal wildlife in transit and identification of trade routes, as well as maintain efficient flow of legal commercial trade. The application of rapid-response, point-of-use methods for species detection has been on the minds of wildlife biologists since the development of real-time polymerase chain reaction (RT-PCR) technology in the early 1990s (1). This novel technology was applied early on to detect microbes, viruses, and pathogens in environmental samples (2,3).

More recently, RT-PCR has been used to identify specific target species in food products, such as commercial fish shipments (4) and processed meats (5,6). As a result, the ability to screen large numbers of samples for rare variants made RT-PCR a desirable method for monitoring animal species distributions (7–9). High profile invasive species events have highlighted the utility of RT-PCR methods on environmental DNA (eDNA) analyses to be applied to detection of invasive (10,11) as well as rare and endangered species (12–14).

The use of DNA analyses for species identification is well established in wildlife forensic science, although an RT-PCR method tends only to be applied as a presumptive test. RT-PCR offers a highly sensitive amplification method that can be applied to intact as well as compromised samples, enabling species identification through amplification with species-specific primers (15), differential species detection with melting point analysis (16), or simply direct sequencing of RT-PCR products with traditional Sanger sequencing.

Recently, RT-PCR techniques have been developed for identification of shark fins (17–19), Anguillid eels (8,20,21), lion and tiger bones (22), and rare turtle species (23), with the ultimate goal of deploying point-of-use analytical capabilities – for example at Border Control points. To date, at least seven manufacturers are producing operational portable RT-PCR instruments, primarily in response to the need for rapid SARS-Covid-2 testing. Their utility in the area of wildlife species detection, however, has not gone unnoticed. While this new technology has great potential as an investigative tool for identifying illegally trafficked wildlife, its use in forensic testing has some significant limitations which need to be recognized and addressed by anyone wishing to develop these tools.

The advantages of RT-PCR methods for law enforcement are primarily connected to rapid detection of illegal or invasive/injurious species at ports of entry. As an investigative tool, rapid species detection methods can help to facilitate legal commerce as well as detain suspicious shipments that otherwise may go undetected. But does this mean these results can be used as evidence in court?

The goal of wildlife forensic science is to apply science to the rule of law, and the development of validated protocols for species identification is critically important to that effort. The biggest concerns about rapid detection methods for wildlife forensics are related to species specificity, the frequency of false positives and false negatives, and intended users' ability to defend the interpretation of the results in a court of law. Given that the outcomes of forensic testing can result in the loss of liberty, false-positives may have a higher price in the forensic arena than when applied as a diagnostic tool.

Rapid detection with RT-PCR or similar Loop-Mediated Isothermal Amplification (LAMP) methods must be species specific because the results are visualized as positive amplicon formation (34). Mixtures of different primer sets that are designed to be highly specific for different species can be multiplexed if fluorescently labelled (35), and the products can be discriminated, such as with a Taqman assay (15). LAMP assays are increasing in popularity for rapid PCR detection because they are used at ambient temperature. While LAMP assays provide more opportunity for specificity, primer design and validation can become complex (36,37). Even when assaying known species, labs seldom have enough intraspecific data to develop robust assays that do not need repeated redesign and tweaking to be effective. More recently, increased eDNA research has resulted in the development of a wide range of species-

Are Rapid Detection PCR Methods Forensically Useful?

Continued from page 15

specific RT-PCR assays, so there are more available, but validation remains an issue (38), especially for forensic applications (39,40). In addition, transitioning an assay from the lab to an enforcement setting (such as at different ports with users of varying expertise) will be challenging.

The robustness of the test validation is of particular importance if these assays are to be applied in a forensic context. Species-specific tests need to account for the potential presence of other non-target organisms in the source collection location or mixed during transit that might cross-amplify and generate a false positive result. Without doing comprehensive tests for the potential suite of species, the final assay results could be suspect, rendering them open to interrogation if they proceed to court.

A RT-PCR method recently developed for shark fin ID appears to be useful for initial screening for species of concern (41), but the test development only targeted CITES-listed species, and did not test other congeners that may co-occur in shipments. The false positive error rate for this test is unknown without additional research, and the authors indicate that the application of this test should only be used to flag samples for further forensic testing. Another publication reports the development of a "species specific" RT-PCR test for Anguilla anguilla (21), but primer development for the method used sequences mined from GenBank rather than a full empirical test prior to testing on market samples. As a result, this particular test misses 3 of 13 A. anguilla haplotypes that have 1-3 base pair differences in the species-specific primer binding region (MBC, personal observation). An in-silico study of the published primers on 143 Anguilla sp. sequences suggested a minimum false negative detection rate of 7%, but the rate would vary with the composition of the shipment. Estimates of a false positive rate are also unknown, as the study only reported results from market samples and not from voucher specimens.

If these tests are validated robustly and rigorously according to forensic protocols similar to those established for traditional sequencing, they have the potential to be useful, but addressing the error rate will be complex. The presence of unknown species that may cross-amplify with the species-specific test will always be an issue. Sequence analysis will always provide confidence as a follow-up confirmatory test.

The interpretation of test results by individuals who are not trained forensic scientists may present the most significant barrier to implementation of rapid tests away from the forensic lab. If the end user's interpretation of results is questioned in court, the fact that they are not a trained forensic scientist could be an issue. In the US, for example, a wildlife inspector could use an on-site RT-PCR test as a "presumptive test," but likely would not qualify as an "expert witness" in court and thus could only testify to fact, and not interpretation. This does not mean the tests are not useful – in reality, rapid results tests are highly informative as investigative tools. Such tests can reduce the laboratory case load and increase inspection efficiency by allowing shipments wrongly suspected of violations to proceed if the rapid test is negative. Diverting "negative" shipments then allows the laboratory to focus on "positive" rapid results, which are more likely violations of the law.

We encourage further discussion on this topic among our forensic colleagues. The application of rapid detection PCR techniques to species identification for investigative purposes is recognized as a useful tool, however confirmation of species identity with DNA sequence analysis remains the confirmatory step for cases that may be equivocal or contested.

This article is the product of discussions among members of the SWFS Technical Working Group, and benefits greatly from contributions and reviews from M. Katherine Moore and members of the SWFS-TWG. We welcome additional commentary, as well as suggestions for other topics of discussion that the SWFS-TWG could address for the benefit of the SWFS membership.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Are Rapid Detection PCR Methods Forensically Useful?

Continued from page 16

References

1. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. Bio/Technology [Internet]. 1993;11(9):1026–30. Available from: https://doi.org/10.1038/nbt0993-1026
2. Sharkey FH, Banat IM, Marchant R. Detection and quantification of gene expression in environmental bacteriology. Appl Environ Microbiol. 2004;70(7):3795–806.
3. Spackman E, Senne DA, Bulaga LL, Myers TJ, Perdue ML, Garber LP, et al. Development of real-time RT-PCR for the detection of avian influenza virus. Avian Dis. 2003;47(SPEC. ISS.):1079–82.
4. Trotta M, Schönhuth S, Pepe T, Cortesi ML, Puyet A, Bautista JM. Multiplex PCR Method for Use in Real-Time PCR for Identification of Fish Fillets from Grouper (Epinephelus and Mycteroperca Species) and Common Substitute Species. J Agric Food Chem [Internet]. 2005 Mar 1;53(6):2039–45. Available from: https://doi.org/10.1021/jf048542d
5. Köppel R, Ruf J, Zimmerli F, Breitenmoser A. Multiplex real-time PCR for the detection and quantification of DNA from beef, pork, chicken and turkey. Eur Food Res Technol. 2008;227(4):1199–203.
6. Salihah NT, Hossain MM, Lubis H, Ahmed MU. Trends and advances in food analysis by real-time polymerase chain reaction. J Food Sci Technol [Internet]. 2016;53(5):2196–209. Available from: http://dx.doi.org/10.1007/s13197-016-2205-0
7. Dias PJ, Sollelis L, Cook EJ, Piertney SB, Davies IM, Snow M. Development of a real-time PCR assay for detection of Mytilus species specific alleles: Application to a sampling survey in Scotland. J Exp Mar Bio Ecol [Internet]. 2008;367(2):253–8. Available from: http://dx.doi.org/10.1016/j.jembe.2008.10.011
8. Fahmi MR, Solihin DD, Soewardi K, Pouyaud L, Shao Z, Berrebi P. A novel semi-multiplex PCR assay for identification of tropical eels of genus Anguilla in Indonesian waters. Fish Sci. 2013;79(2):185–91.
9. Rovatsos M, Kratochvíl L. Molecular sexing applicable in 4000 species of lizards and snakes? From dream to real possibility. Methods Ecol Evol. 2017;8(8):902–6.
10. Jerde CL, Mahon AR, Chadderton WL, Lodge DM. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv Lett. 2011;4(2):150–7.
11. Mahon AR, Jerde CL, Galaska M, Bergner JL, Chadderton WL, Lodge DM, et al. Validation of eDNA Surveillance Sensitivity for Detection of Asian Carps in Controlled and Field Experiments. PLoS One. 2013;8(3):1–6.
12. Rodgers TW, Dysthe JC, Tait C, Franklin TW, Schwartz MK, Mock KE. Detection of 4 imperiled western North American freshwater mussel species from environmental dna with multiplex qpcr assays. Freshw Sci. 2020;39(4):762–72.
13. Hulley EN, Tharmalingam S, Zarnke A, Boreham DR. Development and validation of probe-based multiplex real-time PCR assays for the rapid and accurate detection of freshwater fish species. PLoS One. 2019;14(1):1–20.
14. Franklin TW, McKelvey KS, Golding JD, Mason DH, Dysthe JC, Pilgrim KL, et al. Using environmental DNA methods to improve winter surveys for rare carnivores: DNA from snow and improved noninvasive techniques. Biol Conserv [Internet]. 2019;229(August 2018):50–8. Available from: https://doi.org/10.1016/j.biocon.2018.11.006
15. Wilcox TM, McKelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, et al. Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity. PLoS One. 2013;8(3).
16. Wittwer CT. High-resolution DNA melting analysis: Advancements and limitations. Hum Mutat. 2009;30(6):857–9.
17. Caballero S, Cardeñosa D, Soler G, Hyde J. Application of multiplex PCR approaches for

Are Rapid Detection PCR Methods Forensically Useful?

Continued from page 17

shark molecular identification: Feasibility and applications for fisheries management and conservation in the Eastern Tropical Pacific. Mol Ecol Resour. 2012;12(2):233–7.

18. Cardeñosa D, Fields A, Abercrombie D, Feldheim K, Shea SKH, Chapman DD. A multiplex PCR mini-barcode assay to identify processed shark products in the global trade. PLoS One. 2017;12(10):3–11.

19. Domingues RR, Bunholi IV, Pinhal D, Antunes A, Mendonça FF. From molecule to conservation: DNA-based methods to overcome frontiers in the shark and ray fin trade. Conserv Genet Resour [Internet]. 2021;13(2):231–47. Available from: https://doi.org/10.1007/s12686-021-01194-8

20. Espiñeira M, Vieites JM. Genetic system for an integral traceability of European eel (Anguilla anguilla) in aquaculture and seafood products: authentication by fast real-time PCR. Eur Food Res Technol. 2016;242(1):25–31.

21. Cardeñosa D, Gollock MJ, Chapman DD. Development and application of a novel real-time polymerase chain reaction assay to detect illegal trade of the European eel (Anguilla anguilla). Conserv Sci Pract. 2019;1(5):e39.

22. Dalton DL, Kotzé A, McEwing R, De Bruyn M, Mnisi C, Mwale M. A tale of the traded cat: development of a rapid real-time PCR diagnostic test to distinguish between lion and tiger bone. Conserv Genet Resour. 2020;12(1):29–31.

23. Cardeñosa D, Chapman DD, Robles YL, Ussa DA, Caballero S. Rapid species and river-of-origin determination for matamata turtles (Chelus sp.) using real-time PCR: Facilitating rapid return of trafficked specimens back to the wild. Aquat Conserv Mar Freshw Ecosyst. 2021;(January):1–8.

24. Xiu L, Binder RA, Alarja NA, Kochek K, Coleman KK, Than ST, et al. A RT-PCR assay for the detection of coronaviruses from four genera. J Clin Virol [Internet]. 2020;128:104391. Available from: https://www.sciencedirect.com/science/article/pii/S1386653220301335

25. Laverick MA, Wyn-Jones AP, Carter MJ. Quantitative RT-PCR for the enumeration of noroviruses (Norwalk-like viruses) in water and sewage. Lett Appl Microbiol. 2004;39(2):127–36.

26. Donaldson KA, Griffin DW, Paul JH. Detection, quantitation and identification of enteroviruses from surface waters and sponge tissue from the Florida Keys using real-time RT–PCR. Water Res [Internet]. 2002;36(10):2505–14. Available from: https://www.sciencedirect.com/science/article/pii/S0043135401004791

27. Jothikumar N, Kang G, Hill VR. Broadly reactive TaqMan® assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. J Virol Methods [Internet]. 2009;155(2):126–31. Available from: https://www.sciencedirect.com/science/article/pii/S0166093408003571

28. Frezza D, Giambra V, Chegdani F, Fontana C, Maccabiani G, Losio N, et al. Standard and Light-Cycler PCR methods for animal DNA species detection in animal feedstuffs. Innov Food Sci Emerg Technol [Internet]. 2008;9(1):18–23. Available from: https://www.sciencedirect.com/science/article/pii/S1466856407000628

29. Cavender WP, Wood JS, Powell MS, Overturf K, Cain KD. Real-time quantitative polymerase chain reaction (QPCR) to identify Myxobolus cerebralis in rainbow trout Oncorhynchus mykiss. Dis Aquat Organ. 2004;60(3):205–13.

30. Hermes R, Saragusty J, Moser I, Barth SA, Holtze S, Lecu A, et al. Differential detection of tuberculous and non-tuberculous mycobacteria by qPCR in lavage fluids of tuberculosis-suspicious white rhinoceros. PLoS One. 2018;13(11):1–15.

31. Wozney KM. Species Identification for Highly Processed Samples Associated. 2009. 76 p.

32. Darling JA, Blum MJ. DNA-based methods for monitoring invasive species: A review and prospectus. Biol Invasions. 2007;9(7):751–65.

33. Pie MR, Ströher PR, Agostinis AO, Lopes RB, Sfeir MZT, Ostrensky A. Development of a real-time

Are Rapid Detection PCR Methods Forensically Useful?

Continued from page 18

PCR assay for the detection of the golden mussel (limnoperna fortunei, mytilidae) in environmental samples. An Acad Bras Cienc. 2017;89(2):1041–5.

34. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. 2005;39(1):75–85.

35. Bustin SA. Real-time, fluorescence-based quantitative PCR: a snapshot of current procedures and preferences. Expert Rev Mol Diagn [Internet]. 2005 Jul 1;5(4):493–8. Available from: https://doi.org/10.1586/14737159.5.4.493

36. But GWC, Wu HY, Shao KT, Shaw PC. Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use. Sci Rep. 2020;10(1):1–14.

37. Spielmann G, Ziegler S, Haszprunar G, Busch U, Huber I, Pavlovic M. Using loop-mediated isothermal amplification for fast species delimitation in eels (genus Anguilla), with special reference to the European eel (Anguilla anguilla). Food Control [Internet]. 2019;101(November 2018):156–62. Available from: https://doi.org/10.1016/j.foodcont.2019.02.022

38. Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FSB, Olsvik PA, et al. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. BMC Mol Biol. 2010;11.

39. Harper LR, Lawson Handley L, Hahn C, Boonham N, Rees HC, Gough KC, et al. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). Ecol Evol. 2018;8(12):6330–41.

40. Veldhoen N, Hobbs J, Ikonomou G, Hii M, Lesperance M, Helbing CC. Implementation of novel design features for qpcr-based eDNA assessment. PLoS One. 2016;11(11):1–23.

41. Cardeñosa D, Quinlan J, Shea KH, Chapman DD. Multiplex real-time PCR assay to detect illegal trade of CITES-listed shark species. Sci Rep. 2018;8(1):1–10.

Recent publications:

In this section we provide a list of recent wildlife forensic publications pulled from web of science. This list covers the period from April 2021 to September 2021. We are not commenting on their quality or advocating their application, hopefully you will have you own opinions on this. If you know we have missed something, particularly one of your papers, please let us know and we will include it in the next edition.

Linacre, A (2021) Animal Forensic Genetics. GENES,2073-4425;DOI 10.3390/genes12040515

Smart, U, Cihlar, JC, Budowle, B (2021) International Wildlife Trafficking: A perspective on the challenges and potential forensic genetics solutions. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 1872-4973; DOI 10.1016/j.fsigen.2021.102551

Sim, Z, Monderman, L, Hildebrand, D, Packer, T, Jobin, RM (2021) Development and implementation of a STR based forensic typing system for moose (Alces alces). FORENSIC SCIENCE INTERNATIONAL-GENETICS. 1872-4973. 1878-0326; DOI 10.1016/j.fsigen.2021.102536

Veron, IF, Zorrilla, I, Richards, NL (2021) Is deliberate pesticide poisoning of wildlife impacting local insect communities? Wildlife and environmental forensic investigations in southern Spain present an opportunity for collaborative entomological monitoring. JOURNAL OF INSECT CONSERVATION. 1366-638X. 1572-9753; DOI 10.1007/s10841-021-00319-6

Beasley, J, Shorrock, G, Neumann, R, May, CA, Wetton, JH (2021) Massively parallel sequencing and capillary electrophoresis of a novel panel of falcon STRs: Concordance with minisatellite DNA profiles from historical wildlife crime. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 1872-4973. 1878-0326; DOI 10.1016/j.fsigen.2021.102550

Ghosh, T, Sharma, A, Mondol, S (2021) Optimisation and application of a forensic microsatellite panel to combat Greater-one horned rhinoceros (Rhinoceros unicornis) poaching in India. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 1872-4973. 1878-0326 DOI 10.1016/j.fsigen.2021.102472

Carrothers, KL, Goodmiller, LE, McLellan, MJ, Spicer, AM (2021) A novel approach to combatting proboscidean ivory trafficking using a multiplex High-Resolution Melt (M-HRM) assay. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 53; DOI 10.1016/j.fsigen.2021.102511

Vasiljevic, N, Lim, M, Humble, E, Seah, A, Kratzer, A, Morf, NV, Prost, S, Ogden, R (2021) Developmental validation of Oxford Nanopore Technology MinION sequence data and the NGSpeciesID bioinformatic pipeline for forensic genetic species identification. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 53; DOI 10.1016/j.fsigen.2021.102493

Andersson, AA, Tilley, HB, Lau, W, Dudgeon, D, Bonebrake, TC, Dingle, C (2021) CITES and beyond: Illuminating 20 years of global, legal wildlife trade. GLOBAL ECOLOGY AND CONSERVATION. 26; DOI 10.1016/j.gecco.2021.e01455

continued on 21

Recent publications:

continued from page 20

Zhang, HX, Gharavi, N, Wong, SHF, Deng, Y, Bahadori-Jahromi, A, Limkatanyu, S, Qiao, Y, Kuang, JS (2021) Effect of concentrated Butt-Joints on flexural properties of laminated Bamboo-Timber flitch beams. JOURNAL OF SANDWICH STRUCTURES & MATERIALS. 1099-6362. 1530-7972; DOI 10.1177/10996362211040103

Priyambada, P, Jabin, G, Singh, A, Ghosh, A, Singh, SK, Chatterjee, M, Venkatraman, C, Chandra, K, Sharma, LK, Thakur, M (2021) Digging out the keys in the heap of seized pangolin scales: up scaling pangolin conservation using wildlife forensics. FORENSIC SCIENCE INTERNATIONAL. 323; DOI 10.1016/j.forsciint.2021.110780

Andersson, AA, Gibson, L, Baker, DM, Cybulski, JD, Wang, S, Leung, B, Chu, LM, Dingle, C (2021) Stable isotope analysis as a tool to detect illegal trade in critically endangered cockatoos. ANIMAL CONSERVATION. 1367-9430. 1469-1795; DOI 10.1111/acv.12705

Davitkov, D, Vucicevic, M, Glavinic, U, Skadric, I, Nesic, V, Stevanovic, J, Stanimirovic, Z (2021)POTENTIAL OF INTER- AND INTRA-SPECIES VARIABILITY OF CHD1 GENE IN BIRDS AS A FORENSIC TOOL. ACTA VETERINARIA-BEOGRAD. 71; DOI 10.2478/acve-2021-0013

Kriangwanich, W, Nganvongpanit, K, Buddhachat, K, Siengdee, P, Chomdej, S, Ponsuksili, S, Thitaram, C (2021) Mammalian species identification using ISSR-HRM technique. SCIENCE PROGRESS. 104; DOI 10.1177/00368504211026163

Oury, N, Jaquemet, S, Simon, G, Casalot, L, Vangrevelynghe, G, Landron, F, Magalon, H (2021) Forensic genetic identification of sharks involved in human attacks. FORENSIC SCIENCE INTERNATIONAL-GENETICS. 54; DOI 10.1016/j.fsigen.2021.102558

Chanyandura, A, Muposhi, VK, Gandiwa, E, Muboko, N (2021) An analysis of threats, strategies, and opportunities for African rhinoceros conservation. ECOLOGY AND EVOLUTION. 2045-7758; DOI 10.1002/ece3.7536

Kitin, P, Espinoza, E, Beeckman, H, Abe, H, McClure, PJ (2021) Direct analysis in real-time (DART) time-of-flight mass spectrometry (TOFMS) of wood reveals distinct chemical signatures of two species of Afzelia. ANNALS OF FOREST SCIENCE. 1286-4560. 1297-966X; DOI 10.1007/s13595-020-01024-1

Singh, A, Ghosh, A, Dolker, S, Chinnadurai, V, Sharma, LK, Chandra, K, Thakur, M (2021) Species identification from seized animal oil: a case study of suspected Gangetic dolphin (Platanista gangetica). INTERNATIONAL JOURNAL OF LEGAL MEDICINE. 135; DOI - 10.1007/s00414-021-02574-3

Cardenosa, D, Chapman, DD, Robles, YL, Ussa, DA, Caballero, S (2021) Rapid species and river-of-origin determination for matamata turtles (Chelus sp.) using real-time PCR: Facilitating rapid return of trafficked specimens back to the wild. AQUATIC CONSERVATION-MARINE AND FRESHWATER ECOSYSTEMS. 31; DOI 10.1002/aqc.3613

Hyun, JY, Pandey, P, Kim, KS, Chon, A, Jeong, D, Bhak, J, Yu, M, Song, HK, Singh, R, Min, MS, Goyal, SP, Bayarkhagva, D, Marchenkova, T, Vitkalova, A, Lee, H (2021) Whole genome survey of big cats (Genus: Panthera) identifies novel microsatellites of utility in conservation genetic study. SCIENTIFIC REPORTS. 11; DOI 10.1038/s41598-021-92781-0

Recent publications:

continued from page 21

Schneider, J, Mas-Carrio, E, Jan, C, Miquel, C, Taberlet, P, Michaud, K, Fumagalli, L (2021) Comprehensive coverage of human last meal components revealed by a forensic DNA metabarcoding approach. SCIENTIFIC REPORTS. 11; DOI 10.1038/s41598-021-88418-x

Won, EJ, Yun, HY, Lee, DH, Shin, KH (2021) Application of Compound-Specific Isotope Analysis in Environmental Forensic and Strategic Management Avenue for Pesticide Residues. MOLECULES. 26; DOI 10.3390/molecules26154412

Willette, DA, Navarrete-Forero, G, Gold, Z, Lizano, AMD, Gonzalez-Smith, L, Sotil, G (2021) Characterizing Industrial and Artisanal Fishing Vessel Catch Composition Using Environmental DNA and Satellite-Based Tracking Data. FOODS. 10; DOI 10.3390/foods10061425

Blecher, AS, Ganswindt, A, Scheun, J (2021) Scales of our lives: Sex identification of Temminck's pangolin (Smutsia temminckii) using scales retrieved out of the illegal wildlife trade. GENERAL AND COMPARATIVE ENDOCRINOLOGY. 308; DOI 10.1016/j.ygcen.2021.113782

Krestoff, ES, Creecy, JP, Lord, WD, Haynie, ML, Coyer, JA, Sampson, K (2021) Mitochondrial DNA Evaluation and Species Identification of Kemp's kempii) Bones After a 3-Year Exposure to Submerged Marine and Terrestrial Environments. FRONTIERS IN MARINE SCIENCE. 2296-7745.8; DOI 10.3389/fmars.2021.646455

Engasser, EL, Stone, RL, Jameson, ML (2021) Habitat Associations of Carrion Beetles (Coleoptera: Silphidae) Across a Full Annual Cycle. ENVIRONMENTAL ENTOMOLOGY. 0046-225X. 1938-2936. 50; DOI 10.1093/ee/nvab011

Zhang, LN, Wang, YM, Bian, HY, Wang, LR, Li, H (2021) Optimal wavelengths selection from all points for blood species identification based on spatially resolved near-infrared diffuse transmission spectroscopy. INFRARED PHYSICS & TECHNOLOGY. 117; DOI 10.1016/j.infrared.2021.103865

Herrero-Villar, M, Sanchez-Barbudo, IS, Camarero, PR, Taggart, MA, Mateo, R (2021) Increasing incidence of barbiturate intoxication in avian scavengers and mammals in Spain. ENVIRONMENTAL POLLUTION. 284; DOI 10.1016/j.envpol.2021.117452

Karamanlidis, AA, Kopatz, A, Hernando, MD (2021) Dispersal patterns of a recovering brown bear (Ursus arctos) population in a human-dominated landscape. JOURNAL OF MAMMALOGY. 102; DOI 10.1093/jmammal/gyaa173

Rial-Berriel, C, Acosta-Dacal, A, Perez, MAC, Suarez-Perez, A, Melian, AM, Zumbado, M, Hernandez, LAH, Ruiz-Suarez, N, Hernandez, AR, Boada, LD, Montes, AM, Luzardo, OP (2021) Intensive livestock farming as a major determinant of the exposure to anticoagulant rodenticides in raptors of the Canary Islands (Spain). SCIENCE OF THE TOTAL ENVIRONMENT. 768; DOi 10.1016/j.scitotenv.2020.144386

Cabral, AE, Ricardo, F, Patinha, C, da Silva, EF, Correia, M, Palma, J, Planas, M, Calado, R (2021) Successful Use of Geochemical Tools to Trace the Geographic Origin of Long-Snouted Seahorse Hippocampus guttulatus Raised in Captivity. ANIMALS. 11; DOI 10.3390/ani11061534

Recent publications:

continued from page 22

Tian, LL, Han, F, Fodjo, EK, Zhai, WL, Huang, XY, Kong, C, Shi, YF, Cai, YQ, Samanidou, VF (2021) An Effective and Efficient Sample Preparation Method for 2-Methyl-Isoborneol and Geosmin in Fish and Their Analysis by Gas Chromatography-Mass Spectrometry. INTERNATIONAL JOURNAL OF ANALYTICAL CHEMISTRY. 1687-8760. 1687-8779; DOI 10.1155/2021/9980212

Sacks, BN, Hu, TY, Kierepka, EM, Vanderzwan, SL, Hickey, JR (2021) Development of 79 SNP markers to individually genotype and sex-type endangered mountain gorillas (Gorilla beringei beringei). CONSERVATION GENETICS RESOURCES. 1877-7252; DOI 10.1007/s12686-021-01217-4

Shen, XS, Liu, ML, Kong, C, Zhai, WL, Fodjo, EK, Cai, YQ (2021) Chitosan and nafion assisted modification of enzyme-free electrochemical immunosensors for the detection of tetrodotoxin. MATERIALS EXPRESS. 11; DOI 10.1166/mex.2021.1936

Chaitae, A, Rittiron, R, Gordon, IJ, Marsh, H, Addison, J, Pochanagone, S, Suttanon, N (2021) Shining NIR light on ivory: A practical enforcement tool for elephant ivory identification. CONSERVATION SCIENCE AND PRACTICE. 2578-4854; DOI 10.1111/csp2.486

Martin, AA, Fodjo, EK, Eric-Simon, ZV, Gu, Z, Yang, GX, Albert, T, Kong, C, Wang, HF (2021) Cys-AgNPs modified gold electrode as an ultrasensitive electrochemical sensor for the detection of 3-chloropropane-1,2-diol. ARABIAN JOURNAL OF CHEMISTRY. 1878-5352; DOI 10.1016/j.arabjc.2021.103319

Somaweera, R, Udyawer, V, Guinea, ML, Ceccarelli, DM, Clarke, RH, Glover, M, Hourston, M, Keesing, J, Rasmussen, AR, Sanders, K, Shine, R, Thomson, DP, Webber, BL (2021) Pinpointing Drivers of Extirpation in Sea Snakes: A Synthesis of Evidence From Ashmore Reef. FRONTIERS IN MARINE SCIENCE. 2296-7745; DOI 10.3389/fmars.2021.658756