

# ISoWiF 2017

BOOK OF ABSTRACTS

Book of Abstracts  
of the Xth  
International  
Symposium on  
Wild  
Fauna (ISoWiF  
2017)

## X<sup>th</sup> INTERNATIONAL SYMPOSIUM ON WILD FAUNA

21 to 23 September 2017

UTAD, Vila Real , PORTUGAL



# BOOK OF ABSTRACTS

ISoWiF 2017

X<sup>th</sup> INTERNATIONAL SYMPOSIUM ON WILD FAUNA

21<sup>st</sup> – 23<sup>rd</sup> SEPTEMBER, VILA REAL, PORTUGAL

ORGANIZED BY



PARTNERS



**TITLE**

Book of abstracts of the X<sup>th</sup> International Symposium on Wild Fauna (ISOWiF 2017), 21<sup>st</sup> and 23<sup>rd</sup> of September 2017, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal.

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## WELCOME MESSAGE

Dear ISoWiF2017 participants,

Wild Animals Vigilance Euromediterranean Society (WAVES), have a pleasure of welcoming all participants in the *X<sup>th</sup> International Symposium on Wild Fauna (ISoWiF2017)* and express its feeling of honor to organize this conference in the *University of Trás-os-Montes and Alto Douro (UTAD)*, in Vila Real, a city located in the North of Portugal, in the Douro Region, World Heritage listed by UNESCO.

For WAVES and UTAD, Wild Fauna subjects represent a common topic of major interest. Therefore, organize this ISoWiF edition, it was of main significance, being selected important topics transversal to this area that should be transparently and pragmatically presented during this two conference days:

- A - Wild fauna management and conservation;**
- B - Wild fauna photography seen by professional eyes and researchers;**
- C - Wild fauna diseases under *One Health* approach;**
- D - Effects of environmental changes on wild fauna and habitats.**

Around 150 participants (students, researchers, technicians, representatives of government authorities and wild fauna management companies) from different countries are expected to attend this symposium, with more than 80 presentations, providing the opportunity of interacting with an interdisciplinary group, exchange experience, improve innovation and knowledge, fortifying relationships and network and participating in the discussion about the future of wild fauna, under an integrated perspective and ONE HEALTH approach.

On this occasion we also would like to extend our sincere thanks to our partners and sponsors that believe in this project and support this important event. Also, a special thanks to the Scientific Committee that was of extreme importance in the program definition and to all the authors that will share with us their research studies and experiences – Without your contributions this conference would not exist.

We are looking forward to seeing and welcoming you to UTAD for ISoWiF2017.

On behalf of ISoWiF2017 Organizing Committee

  
**Prof.ª Madalena Vieira-Pinto**

(Convenor and chairman of the international and local committees)





## PROGRAM Pre-Symposium Workshops

21<sup>st</sup> of September 2017

<b>WORKSHOP 1</b>	<b>Wild fauna photography (14:00-18:00)</b>
<b>Coordination</b>	Craig Jones – Wildlife Photographer, UK João Carrola – UTAD, DeBA, Portugal Pedro Rêgo – Professional Wildlife Photographer – Bragança, Portugal André Brito – Centro de Ciência de Vila Real, Portugal

<b>WORKSHOP 2</b>	<b>Genetics as an essential tool in the wild fauna conservation and management (9:00-13:00)</b>
<b>Coordination</b>	Raquel Chaves and Filomena Adegas – Genetic Department – UTAD, Portugal

<b>WORKSHOP 3</b>	<b>Wild birds' pathology diagnosis (14:00-18:00)</b>
<b>Coordination</b>	Gerry M. Dorrestein - Diagnostisch Pathologie Laboratorium NOIVBD – Netherland



# PROGRAM - ISoWiF2017

22<sup>nd</sup> of September 2017- morning

## A – Topic: Wild fauna management and conservation

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9:00 – 9:15	<b>Opening conference</b>
9:15 – 10:00	<b>Plenary lecture – Actions for the conservation and management of wild fauna in protected natural areas of Castilla y León</b> Jesús Palacios (Natural Areas and Protected Species Section (Zamora-Spain). Territorial Service for the Environment of Junta de Castilla y León)
10:00 – 10:20	<b>Invited lecture –The reintroduction of <i>Gyps fulvus</i> in Sicily: a successful experience</b> Domenico Vicari (Istituto Zooprofilattico Sperimentale della Sicilia, Italy)
10:20 – 10:40	<b>Invited lecture – The management of invasive and pest species in a EU perspective</b> Andrea Amici (University of Tuscia Dipartimento di Scienze Agrarie e forestali - Department of Agriculture and Forestry Science, Italy)
10:40 – 11:00	<b>DISCUSSION</b>
11:00 – 11:40	<i>Coffee-break and Poster Session</i>
11:40 – 11:55	<b>LIFE Imperial: a project for the conservation of the Iberian Imperial Eagle (<i>Aquila adalberti</i>) in Portugal.</b> Liliana Barosa (LPN – Liga para a Protecção da Natureza, Castro Verde, Portugal)
11:55 – 12:10	<b>Potential use of lactic acid bacteria as probiotics to control TB in wildlife.</b> María Bravo (Dept. de Sanidad Animal, Facultad de Veterinaria, Universidad de Extremadura, Spain)
12:10 – 12:25	<b>Estimation of the foxes (<i>Vulpes vulpes</i>) consistency in the ATC of Avellino Province.</b> Luigi Esposito (University of Naples Federico II / Dept. of Veterinary Medicine and Animal Production, Napoli, Italy).
12:25 – 12:40	<b>Wildlife bromethalin poisoning reported at CAHFS, USA: a future threat for European wildlife?</b> Marcos Pérez-López (Toxicology Area and INBIO G+C Research Institute, Faculty of Veterinary Medicine (UEX), Caceres , Spain)
12:40 – 13:10	<b>DISCUSSION</b>
<i>LUNCH - 13:10 – 14:30 (University canteen)</i>	

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22<sup>nd</sup> of September 2017 - afternoon

**B – Topic: Wild Fauna photography seen by professional eyes and researchers**

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14:30 – 15:15	<b>Plenary lecture – Conservation with a camera – giving a voice to the voiceless using the medium of photography</b> Craig Jones (Professional wildlife photographer, Manchester, United Kingdom)
15:15 – 15:30	<b>Invited lecture – African wildlife photography</b> Licinia Machado (Wildlife Photographer, Portugal)
15:30 – 15:50	<b>Invited lecture – Biodiversity Program of Vila Real and Wildlife Conservation</b> Darinka Gonçalves (Science Centre of Vila Real, Environmental Service of CMVR/UTAD-CITAB, Vila Real, Portugal)
15:50 – 17:00	<i>Coffee-break and Poster Session</i>
17:00 – 17:15	<b>Invited lecture – Photography and video as central research and conservation tools in modern times</b> João Soares Carrola (UTAD, Deba/CITAB, Vila Real, Portugal)
17:15 – 17:40	<b>Invited lecture – From The Arctic to the Serengeti plains - Perspectives and a photographic look of some endangered species in the World.</b> Pedro Rêgo (Wildlife Photographer, GO WILD, Bragança, Portugal)
17:40 – 17:55	<b>DISCUSSION</b>
18:00 – 18:40	<b>Opening Ceremony</b>

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**Photography Exposition**

19:00 – 21:00      **– Wildlife Conservation Photography: the Beautiful, the Rare and the Ugly–**

**ICE BREAK - “Espumante” of Honour – Restaurant Panorâmico - UTAD**

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23<sup>rd</sup> of September 2017- morning

## C – Topic: Wild fauna diseases under One Health approach

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9:00 – 9:45	<b>Plenary lecture – Arthropod vectors as a link for pathogens shared by wildlife, livestock and humans</b> Francisco Ruiz-Fons (Wildlife and Vector-borne disease epidemiologist. Instituto de Investigación en Recursos Cinegéticos IREC – Ciudad Real. Spain)
9:45 – 10:05	<b>Invited lecture – Wild fauna diseases under One Health approach: The example of large game in Portugal</b> Madalena Vieira Pinto (Dept. Veterinary Science,/CECAV, University of Trás-os-Montes e Alto Douro, Portugal)
10:05 – 10:25	<b>Invited lecture – Monitoring of radionuclides in the environment</b> Martin Tomko (University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic)
10:25 – 10:55	<b>DISCUSSION</b>
10:55 – 11:40	<i>Coffee break and Poster Session</i>
11:40 – 11:55	<b>MRSA in wild animals?</b> Margarida Sousa (Dept. of Veterinary Sciences, Veterinary and Animal Science Research Center (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal)
11:55 – 12:10	<b>Report of <i>Giardia spp.</i> and <i>Cryptosporidium spp.</i> in Iberian Wolf (<i>Canis lupus signatus</i>) in NW of Iberian Peninsula.</b> Ana Luisa Pereira (Dept. de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal)
12:10 – 12:25	<b>First reports and spatiotemporal aspects of the distribution of <i>Philophthalmus lucipetus</i> and <i>Philophthalmus lacrymosus</i> in gulls in Portugal.</b> Maria Casero (Wildlife Rehabilitation and Investigation Center (RIAS) – Associação ALDEIA, Ria Formosa Natural Park, Olhão, Portugal)
12:25 – 12:40	<b>Spatial analysis and modeling of wildlife tuberculosis in a multi-host pathogen system.</b> Nuno Santos (Life and Health Sciences Research Institute (ICVS), School of Health Sciences, CIBIO, University of Minho, Braga, Portugal)
12:40 – 13:10	<b>DISCUSSION</b>
<b>LUNCH - 13:10 – 14:30 (Building “Complexo Laboratorial”)</b>	

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23<sup>rd</sup> of September 2017- afternoon

## D – Topic: Effects of environmental changes on wild fauna and habitats

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14:30 – 15:15	<b>Plenary lecture - The effects of modern agriculture on farmland birds in Britain: can we change their fate through science-based management?</b> Carlos Sánchez (Game & Wildlife Conservation Trust)
15:15 – 15:35	<b>Invited lecture – Spatial-Temporal Analysis of large game and tuberculosis within Idanha-a-Nova County for the years of 2006 – 2016</b> José Aranha (University of Trás-os-Montes e Alto Douro, CITAB, Dept. of Forest and Landscape, Vila Real, Portugal)
15:35 – 16:00	<b>DISCUSSION</b>
16:00 – 16:40	<i>Coffee break and Poster Session</i>
16:40 – 17:00	<b>Invited lecture – The increase of ungulates due to environmental changes: meat quality of hunted meat</b> Claudia Russo (University of Pisa - Dept. of Veterinary Science, Italy)
17:00 – 17:15	<b>Atomic study using hair from faeces of <i>Canis lupus signatus</i> as an environmental bio-indicator for monitorization of Ecosystems</b> Miguel Costa (Dept. de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal)
17:15 – 17:30	<b>Diversification of the Spanish grey partridge (<i>Perdix perdix</i> ssp. <i>hispaniensis</i>) habitat in the natural protected Area of the “Lago de Sanabria y sierras Segundera y de Porto” (Zamora, NW Spain)</b> Pablo Santos (Natural Areas and Protected Species Section (Zamora-Spain). Territorial Service for the Environment of Junta de Castilla y León)
17:30 – 17:45	<b>Wild rabbits (<i>Oryctolagus cuniculus</i>) in shrubby environments: correlation between habitat factors and the species presence</b> António Crespí (Dept. of Biology and Environment (DEBA), School of Life Sciences and Environment, CITAB, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal)
17:45 – 18:00	<b>Threatened fish and mussel populations in Douro basin (Northern Portugal): in-situ and ex-situ conservation measures</b> Amilcar Teixeira (CIMO-ESA-IPB — Mountain Research Centre, School of Agriculture, Polytechnic Institute of Bragança, Portugal)
18:00 – 18:30	<b>DISCUSSION</b>
18:30 – 19:00	<b>Conclusions – Closing ceremony</b>
20:00	<b>CONFERENCE DINNER</b>

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# ORAL ABSTRACTS





## Topic A - Wild fauna management and conservation

### ACTIONS FOR THE CONSERVATION AND MANAGEMENT OF WILD FAUNA IN PROTECTED AREAS OF CASTILLA Y LEÓN.

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#### Abstract

One of the hallmarks of the 21st century society is the concern with the preservation of the environment.

In Spain the National State Administration has the authority to establish the basic legislation on environmental protection, while the Autonomous Communities have the capacity to establish additional rules of protection.

Furthermore, the European Union had enacted a set of directives in the field of environmental protection that have shaped a basic normative framework.

In Castilla & León, the Law 4/2015 over the Natural Heritage of Castilla & León, established a network of Natural Protected Areas, with three complementary networks; the Natura 2000 network, the network of Protected Areas and the network of Natural Areas of Particular Interest. The strategic planning of Natura 2000 network includes a set of instruments with different approaches and contents. This instruments are:

- The Master Plan for implementation and management of Natura 2000 network.
- The Basic Plans for management and conservation of Natura 2000 values.
- The Basic Plans for management and conservation of Natura 2000 areas.

The network of Natural Protected Areas in Castilla & León comprises parks (national, regional and natural), natural reserves, natural monuments and protected landscapes.

**Key words:** Natura 2000; wild fauna; Castilla & León; environment; parks

## **The reintroduction of *gyps fulvus* in Sicily: a successful experience**

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### **Abstract**

Since 1999 the Nebrodi Park has been carrying on a reintroduction project of Griffon Vulture *Gyps fulvus*, a bird extinct in Sicily in the 1965. Since 2005, the project has been carried on only in the Nebrodi and has been managed directly by the Park. In the Park about 40 vultures were released; at the end of this period, a group of 11 individuals was established in Nebrodi. Seventy-nine more griffons were released in 17 different times. In 2005 the first reproduction events were recorded, which have continued each year to 2012; 54 nesting events were verified and 43 fledglings at five different nesting sites have been identified. Individuals released in the Nebrodi were observed in Calabria, Abruzzo and in the French Alps, and there are numerous reports in other parts of Sicily. In the first half of 2012, we counted 12 breeding pairs and more than 60 individuals, of which at least 38 had been released in Nebrodi, 5 were immigrants from other regions (Spain, Croatia, Calabria) while about 20 did not hold rings or other marks. In 2013, other 80 griffons were released. Nowadays the colony is composed by 130 griffons, with about 30 couples. The colony of griffon vultures is integrated in the ecological context of the Park, as shown by frequent observations of individuals feeding on carcasses. The project includes the monitoring of the colony and carries on environmental education and tourism promotion activities. The main threats to the griffons in Sicily are the use of poisoned baits and collisions with power lines and wind turbines. To ensure the success of the reintroduction of griffons in Sicily we envisage the establishment of new colonies on other sites through the activation of reintroduction projects on a regional scale and more feeding stations in strategic areas of the island.

**Key words:** Griffon Vulture, Reintroduction, Sicily, *Gyps Fulvus*, Parco dei Nebrodi

## The management of invasive and pest species in a EU perspective

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### Abstract

An alien species (AS) is usually defined as “a species introduced outside its normal distribution area”. Large part of AS are unable to survive in the new environment and usually die. According to Sundseth (2014), AS can be defined as Invasive alien species (IAS) when their establishment and spread modify ecosystems, habitats, or species. IAS include animals and plants that “are introduced accidentally or deliberately into a natural environment where they are not normally found, with serious negative consequences for their new environment”. They represent a major threat to native plants and animals in Europe, and to biodiversity conservation. In addition, they cause damage worth billions of euros to the European economy every year (Sundseth 2014). On a total of 12,000 alien species registered in Europe, around 10–15% can be considered “invasive”. AS and IAS occur in all taxonomic groups; fungi, bacteria, micro-organisms, invertebrates, plants, amphibians, reptiles, fish and, in a limited number, also mammals. AS/IAS enter the EU in many different ways. Some of these are introduced intentionally; farming, forestry, aquaculture, game, horticulture, but large part as pets and garden plants. Large part of AS/IAS enter the EU unintentionally; contaminants, ‘hitchhikers’ and ‘stowaways’. AS/IAS are a major threat to Europe’s biodiversity conservation, the EU published a new Regulation on IAS under target 5 of the EU’s Biodiversity Strategy to 2020. The aims of the Regulation are to prevent, minimize and mitigate the adverse impacts of IAS on biodiversity and ecosystem services, and limit their damage to the economy and human health. The Regulation strategy against IAS imply; a) Prevention, b) Early warning and rapid response, c) Management of already established invasive alien species. An AS is included in the list of IAS of Union Concern the basis of risk assessment. Three different sources are available to obtain list of IAS; a) Delivering Alien Invasive Species Inventories for Europe (DAISIE), b) Global invasive species database - GISD, c) CABI (Centre for Agriculture and Biosciences International). World’s Worst 100 Invasive Alien Species (IAS) of Terrestrial Vertebrates according to DAISIE in EU are: *Branta Canadensis*, *Cervus nippon*, *Lithobates catesbeianus*, *Mustela vison*, *Myocastor coypus*, *Nyctereutes procyonoides*, *Ondatra zibethicus*, *Oxyura jamaicensis*, *Procyon lotor*, *Psittacula krameri*, *Rattus norvegicus*, *Sciurus carolinensis*, *Tamias sibiricus*, *Threskiornis aetopicus*, *Trachemys spp.*

**Key words:** invasive; alien species; biodiversity; vertebrates; Europe

## **LIFE Imperial: a project for the conservation of the Iberian Imperial Eagle (*Aquila adalberti*) in Portugal**

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### **Abstract**

The Iberian imperial eagle (*Aquila adalberti*) is one of the most threatened birds of prey in the world. Currently the species only breeds in the Iberian Peninsula, where it has suffered a major decline during the last century. In Portugal, this trend culminated with the disappearance of the species breeding population. The return of the Iberian imperial eagle to Portugal as a breeding population was confirmed only in 2003 and since then it has been slowly re-colonizing the country. Currently the species breeding population is mostly concentrated in 4 Special Protection Area's including Castro Verde, Vale do Guadiana, Mourão/Moura/Barrancos and Tejo International, Erges and Pônsul. In 2016, the national breeding population reached 15 couples. The Project LIFE+ “Conservation of the Iberian imperial eagle (*Aquila adalberti*) in Portugal, LIFE13 NAT/PT/001300” aims to create conditions to increase the population of Iberian Imperial Eagle by applying a set of conservation measures targeted at reducing the threats to the species. The Project is coordinated by Liga para a Protecção da Natureza (LPN) and has as partners the Instituto da Conservação da Natureza e das Florestas (ICNF); the Câmara Municipal de Castro Verde (CMCV); the Faculdade de Ciências da Universidade de Lisboa (FCUL); the Guarda Nacional Republicana (GNR); the EDP Distribuição – Energia S.A.; the Sociedade Española de Ornitologia (SEO/BirdLife) and the TRAGSATEC S.A.. The most important threats to the Iberian Imperial Eagle are habitat loss and degradation, the decline of wild rabbit populations, electrocution in power lines, shooting, the illegal use of poisons and disturbance to nesting areas. The Project includes actions such as improving habitat quality, surveillance and control of threats in the species territories, minimizes the impact of power lines, screening for poisoning cases with canine units for the detection of poisons, and training and environmental education. The breeding population in Portugal has been increasing and reached 15 couples in 2016. The project already corrected 23km of dangerous power lines in the species territories. The canine units for poison detection are operating and have performed more than 100 patrols. The training and educational activities reached more than 1000 participants including students, different stakeholders, authorities and the general public. The implementation of these conservation measures contributes to consolidate the return of the Iberian Imperial Eagle to Portugal.

**Key words:** *Aquila adalberti*; Biological conservation; birds of prey; LIFE Program; Natura 2000 network

### Potential use of lactic acid bacteria as probiotics to control TB in wildlife

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#### Abstract

Bovine tuberculosis (bTB) is a chronic bacterial disease mainly caused by *Mycobacterium bovis* that leads to significant economic losses worldwide. bTB has been eradicated from many European nations but it is still very prevalent in some countries where wildlife reservoirs of *M. bovis* have been confirmed. This is the case of wild boar (*Sus scrofa*) in Spain, and the European badger (*Meles meles*) in the UK. Lactic acid bacteria (LAB) have been proposed as a new alternative for controlling bTB due to their probiotic properties, which include their ability to: (1) inhibit the growth of *Mycobacterium* species; and (2) trigger beneficial host immune responses. The main objective of this collaborative study between Spain and the UK was the probiotic characterization of LAB isolated from faeces of wild boar and badgers to evaluate their potential as an environmentally friendly alternative against bTB. LAB have been isolated and identified as *Pediococcus* spp., *Lactobacillus* spp., *Enterococcus* spp. and *Weissella* spp. Overall, the isolates show significant antimycobacterial activity and seem to be associated with innate immunomodulation. Some of the isolates have induced NF-κB activation in M1-like macrophages, suggesting a potential role as vaccine adjuvants or boosters of Th1 immune response; whereas other isolates have showed potential anti-inflammatory properties as they suppress NF-κB activation in the macrophages. Further studies such as whole-genome sequencing and antibiotic resistance tests will confirm the potential use of our LAB isolates as probiotics in wildlife before any *in vivo* confirmatory experimental studies with virulent *M. bovis*. Nevertheless, our current *in vitro* data suggest that these isolates could be used as an early therapy and/or vaccine adjuvants to reduce or prevent infection but also as a tool to reduce inflammation and the amount of viable excreted mycobacteria in highly infected animals. These measures could indeed lead to a long-term decrease in the prevalence of bTB in wild boar and badgers.

**Key words:** probiotics; lactic acid bacteria; bovine tuberculosis; wildlife

## Estimation of the foxes (*Vulpes vulpes*) consistency in the ATC of Avellino Province

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### Abstract

The Avellino Province is situated between the latitude 41°17'10"N - 40°42'22"N and longitude 15°34'25"E - 14°33'34"E. Its territory occupies a total area of 279,164 ha representing 20.54% of the Campania regional area (13,596 km<sup>2</sup>). The entire bibliography indicates a ubiquitous fox distribution throughout the Italian country and describes a species that occupies all habitats, both in protected and in the hunting areas, without exclusion of urban centers. Although the fox population is considered by the IUCN as Least Concern (LC 2008) and the species is not contemplated neither by the Berne Convention nor by CITES nor by the Habitat Directive (DPR 357/1997), and that Italian legislation (Law 157/1992), according to regional hunting schedules, includes the species in the hunting list, it is not easy to find accurate censuses that identify a reliable numerical consistency. The fox census and monitoring actions are indispensable when one of the objectives of Hunting Management Authority is to ensure a stable presence of wild or restocking smaller game (hare and pheasant). The estimation of fox presence was commissioned by "ATC of the Avellino Province", into its competence areas of 199,247 ha. On an area of 5,908 ha 7 different habitat types were identified. The fox presence percentages were calculated on 8 itineraries and 112 routes. The results related to the total area surveyed are derived from the autumn-winter counts performed at night by using a line-transect sampling scheme, and from the spring and summer census by evaluating the dead found along the routes used for the corvids' census. Data showed that fox density obtained from spotlight census was 7.31 head/100 ha and that foxes' carcasses found on the roads amount to 10.42% of the previous one.

Habitat	Itineraries	dead	foxes	dead	spotlight
	km	n.	n.	KAI	KAI
Urbanized	3.650	3	42	0.27	11.51
Rural - Urban	8.373	2	63	0.24	7.52
Wood - Urban	9.592	3	58	0.21	6.05
Rural - Wood - Urban	9.164	5	61	0.22	6.66
Rural - Wood - Industrial	15.510	6	57	0.19	3.68
Wood	14.412	4	32	0.21	2.22
Rural - Wood	37.698	12	64	0.13	1.70
Rural	19.763	10	55	0.20	2.78
Total	118.162	45	432	0.19	3.66

**Key words:** fox; hunting monitoring; geographical distribution; population; ATC Avellino

**Acknowledgement:** This work was realized with a financial contribute of ATC Provincia di Avellino.

## **Wildlife bromethalin poisoning reported at CAHFS, USA: a future threat for European wildlife?**

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### **Abstract**

In 2008, the U.S. Environmental Protection Agency (EPA) requested to phase out second-generation anticoagulant rodenticides, which led to an increase in the production of bromethalin-based rodenticides in USA. In the European Union, this compound has not been approved yet according to the EU Pesticides Database (2017), but new restrictions to the use of anticoagulant rodenticides may change this situation. The main objective of the present communication is to evaluate the epidemiology of bromethalin-related cases of wildlife poisoning recorded at the California Animal Health and Food Safety Laboratory (CAHFS) during the past three years, in order to determine the environmental relevance of this compound, and the potential threats to the European fauna if it is approved in the future.

A retrospective study on bromethalin-confirmed cases of wildlife species recorded at CAHFS between January 2013 and December 2016 was conducted. Both species and tissue specimens submitted for analysis were considered. According to the inclusion criteria, 261 bromethalin-suspected events were recorded during the period between 2013 and 2016 related to wildlife species. Of these, 55 were confirmed positive for bromethalin exposure. With a clear increase in the number of positive samples observed during the considered period (1 in 2013, more than 30 in 2016), striped skunks and raccoons represented the highest percentage of positive cases (43.6 and 40.0 %, respectively). It is important to indicate that neither diurnal nor nocturnal raptors were affected by this pesticide. With respect to toxicological analysis, most of the positive cases were identified with adipose tissue (36/55) or brain (17/55), demonstrating the relevance of these two tissues for confirming exposure.

The present study provides useful information on the epidemiology of bromethalin poisoning in mammalian species in USA and highlights the risk this pesticide could pose to European wildlife if commercialized in the future, although the possibility of secondary or relay poisoning in wild carnivores that ingest bromethalin-intoxicated prey remains unknown. Moreover, the rapidity of action compared to traditional anticoagulant rodenticide compounds together with the lack of an appropriate antidote makes bromethalin potentially more toxic to our wildlife.

**Key words:** bromethalin; wildlife; rodenticide; epidemiology; poisoning.





## Topic B - Wild Fauna photography seen by professional eyes and researchers

### Conservation with a camera" giving a voice to the voiceless using the medium of photography

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#### Abstract

The natural world can be communicated within images, creating a unique and artistic reflection of what the photographer see with the camera. Images are a simplified visions of this seen through eyes, with the emphasis on composition, lighting and colour at the very heart of each picture, capturing their beauty, fascination and graceful expression with each image.

Images represent an event that occurred in the wild something that the photographer witness and record with cameras (mainly sophisticated digital cameras). Skill lies in interpreting and presenting this in a way that invokes beauty, mood and emotion with each moment captured, and aiming that these images can be strong to directly or indirectly be a contribution to conserve nature, habitat, plants, invertebrates and all the beautiful fauna that exists.

Wildlife photography's power rests on the belief that it represents an event that occurred naturally in the wild; something witnessed and recorded by the photographer with his camera at that given time. Clever use of friendly animals, hot spots, bait and the per-arranged perches or props along with digital technology has forced everyone to re-evaluate and question of the validity of images we see now. What are the limits?

As a responsible photographer of wildlife I capture my images as seen on the ground. I always put the welfare of the subject's life and care of the environment above any photograph I take. I never use flash, props, live bait or any bait that will adversely affect the behaviour of an animal. I am always honest in declaring the circumstances under which a photograph has been taken by myself. I never use digital manipulation to misrepresent a subject or mislead the viewer.

I also try and help wildlife the best I can by putting together conservation and photography. This can be very powerful, combining these two elements they can have a profound impact that can move people to such a degree that change can and does happen.

It is my intention to use my photographs of the natural world to bring people's awareness of what beautiful wildlife we have on our doorstep and all around us and the importance of conservation and the need to preserve our national heritage.

**Key words:** photography; wildlife; conservation; habitats; ethics

## **African wildlife photography**

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### **Abstract**

Photography is a powerful means of communication that can be used in wildlife conservation in many ways.

In times Confucius said: “A picture is worth a thousand words”. Showcasing the beauty of the subject or calling attention to problems, photography reaches everyone, regardless of language, religion, culture, education or schooling. Africa is the wildlife photographer’s paradise.

The variety of mammals in Africa is higher than in Europe and Asia together, from the little duiker to the great elephant, the amazing amount of birds and reptiles, and the different landscapes makes Africa a big playground for any wildlife photographer.

Therefore there is also the responsibility to take care of this unique continent, and lot of different landscapes and climate, and his rich and precious wildlife. When preparing a Safari there are a some things to consider: choosing when and where to go, how to behave, what to avoid; making a Safari with respect for the animals and environment, in an ethical way, and taking into account also the native communities, they culture, traditions, etc. and try to preserve nature as they always did.

**Key words:** photography; Africa; wildlife; respect; conservation

## **Biodiversity program of Vila Real and wildlife conservation**

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### **Abstract**

Vila Real city has focused his environmental actions on biodiversity divulgation since 2008. Supported by a program called "Biodiversity Program", numerous projects and initiatives had occurred since then. The Centre of Science of Vila Real, in the last years implemented these actions mainly with activities with kids from schools of the region, but also with intense photography and video work on wild species from all Vila Real region.

Aiming to have a comprehensive vision of this subject, all the work done so far has allowed to gain knowledge of the species present in this territory. At the same time and with a strong concern for the awareness of the society towards the conservationist attitudes and the preservation of the wild flora and fauna, several public campaigns to promote biological heritage were made.

The entrepreneurship of the Biodiversity Program has counted with a strong investment of several public institutions, such as the University of Trás-os-Montes and Alto Douro (UTAD), private institutions, several non-governmental environmental organizations, schools, local restoration organizations and other entities.

In the area of biodiversity knowledge, we must highlight the monitoring activities on the wild species of the territory. This global work allowed the construction of an exhaustive archive of data, but also of images of the species, which constitute an invaluable collection for species identification and for the production of various promotional, recreational, educational materials, and of course precious elements for environmental education and wildlife conservation. The awareness for that issues is the main goal of the program, not only for kids and students but also for adults in order to take into account the nature and wildlife treasure they have in their vicinity and how is important to preserve it, for the next generations

At this point we can say that photography and video are one of the most effective means to contribute to spread awareness of environmental issues, but also for conservation of species and their habitats.

**Key words:** photography; biodiversity; Vila Real; Centre of Science; conservation

## Photography and video as central research and conservation tools in modern times

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### Abstract

Photography takes on several components, such as affective, cognitive, aesthetic, conservationist but also scientific. The images are an important element of communication between the photographer/researcher and the observer, and depends, therefore, on a visual observation and its correct reading. This binomial is processed simultaneously and stored in the brain and stay longer for stronger message or impact.

Images are fundamental for the registration of ongoing research, taking into account its explanatory and confirmation of the observation. It facilitates the dissemination of the results obtained to researchers, students but also to the general public. Science has been advancing throughout the history and the greatest advances have occurred in line with the advances of technology and equipment's, including the cameras used to image or video capture.

But the photography and video can be also powerful tools that can used to empower conservation of nature, all kinds of wildlife, but also habitats (e.g. creation of National Parks). Science underlined the need of nature conservation, but we have to be effective and quick in communicating that to the public, and documentary video and photography can provide a good tool using scientific knowledge to generate public awareness and promote mind changes, with actual information overload (worse by “data smog”).

Modern societies live distant, or aware, from nature thus the photography and video are fundamental to promote conservation, showing the *Beautiful*, the *Bad* and also the *Ugly* (vast negative impacts of human activities like poaching, illegal wildlife trade, massive habitat and prey loss, etc.). But to be effective it should reach general public but also, national agencies, big companies and also government policies, etc..

In Australia, *Peter Dombrovski* turned is camera against the construction of hydroelectric dams in the wild rivers of Tasmania, and in 1983 published the acclaimed book “Wild Rivers”. *Wang Zijang* produce the film “Paradise of birds” with *Xi Zhinong*, but also do an intense efforts to protect the snub-nosed monkey habitat.

**Key words:** Photography; video; conservation of wildlife, research.

## **From the Arctic to the Serengeti plains- perspectives and a photographic look of some endangered species in the world**

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### **Abstract**

Earth is changing, it is and it will always be changing...this is part of its life cycle, it's part of a whole that is generated and regenerated. But something is different in this change, something is changing at a high pace. The climate is changing fast, and all this alterations are affecting in large scale wildlife but with higher impact the endangered animals.

Nowadays in the arctic, hunting is no longer the primary danger for animals like the Polar Bear. The migrations in the Serengeti in Africa are starting to be affected by the lack of regularity of the rains. In Portugal, the increasing of months without rain is affecting a huge number of wildlife species too.

Global climate changes and global warming are affecting severely wildlife species placing into a high risk the ecosystems and consequently their important ecological services.

In this wildlife photography presentation, we will see some images of the reality of this places, the animals and some stories about those ecosystem situation, namely in the Arctic, Africa and Europe, particularly in Portugal, showing wildlife forced to change their habits and increasing their life situation and populations survival, and relevant ecosystem perturbations.

This is a first person view, through the eyes and the lens of a wildlife photographer, who is really concerned with all that human impacts and consequences, for short and long term.

**Key words:** photography; wildlife; Arctic; Serengeti; climate changes



## Topic C - Wild Fauna diseases under One Health approach

### Arthropod vectors as a link for pathogens shared by wildlife, livestock and humans

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#### Abstract

Along the last five decades Europe was experiencing big socioeconomic, agricultural and environmental changes that had consequences for wildlife population dynamics and thereby for pathogens shared with humans and domestic animals. Several wildlife species benefited from those changes and drastically increased both in distribution and numbers, carrying over severe changes in the dynamics of the pathogens and arthropod vector populations they host. Climate change also influenced European arthropod vector populations and, together with changes in animal host and human demographics, promoted the spread of vector-borne diseases of relevance to wildlife, domestic animal and human health. Along this period Europe suffered severe epidemics of vector-borne diseases, e.g. Bluetongue and Schmallenberg in domestic ruminants, West Nile fever in horses and humans and Tick-borne Encephalitis and Crimean-Congo haemorrhagic fever in humans, among others, whose links to wildlife hosts and wildlife vectors have not been properly traced back.

European wildlife communities host a high number of vector species and vector-borne pathogens that are shared or may be potentially shared with livestock, pets and humans. The vector species linking vector-borne pathogens from domestic and wild cycles are unknown for several relevant vector-borne diseases of Europe. However, the role that wildlife plays in vector population and vector-borne pathogen dynamics are still poorly understood for several relevant vector-borne diseases in Europe. A good example supporting wildlife research needs is Crimean-Congo haemorrhagic fever (CCHF): i) Ticks of the genus *Hyalomma* transmit CCHF virus; ii) Both pathogen and vector are hosted by wild animal species; but iii) How wild host population and community dynamics shape vector and pathogen dynamics has not been properly addressed to date. Therefore, pathogen surveillance in wildlife as well as wildlife and vector population monitoring would aid in promoting science-based preventive strategies to counteract the impact of endemic vector-borne diseases and forecast future health threats from exotic vector-borne diseases.

**Key words:** Prevention; Vector ecology; Wildlife disease surveillance; Wildlife Livestock-Human interface; Wildlife population monitoring



## Wild fauna diseases under One Health approach: The example of large game in Portugal

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### Abstract

One health is a concept that aims to bring together human, animal, and environmental health, demanding an interdisciplinary approach and a collaborative effort to address important public health issues, which include emerging infectious and zoonotic diseases.

Zoonosis are diseases and / or infections that occur in animals (wild and domestic) and are transmitted naturally to humans. Of the approximately 1415 pathogens that can affect humans, it is estimated that around 61% are zoonotic, pointing out zoonosis as the most critical risk factor to human health and well-being, with regard to infectious diseases

Under zoonosis context, in the last decades, the role of wildlife has evidenced an increasing importance, being referred by OIE General Director (Dr. B. Vallat) in 2008 that “Surveillance of wildlife diseases must be considered equally as important as surveillance and control of diseases in domestic animals”.

Within wildlife, large game species, which may be in closer contact with man, may constitute a greater threat in the transmission of zoonotic diseases. For that, it is important to understand the factors that increase contact between large game and humans in developing predictive approaches to disease emergence.

According to official data, red deer and wild boar are the two main species of large game hunted in Portugal, representing a relevant economic and social activity. Zoonotic diseases that can affect these species can be directly transmitted to man (contact with organic fluids, ingestion of meat) or indirectly through the infection of other animals (e.g. cattle, dogs) or through the environment contamination. Associated to source of infection, some peculiarities in the circuit of game meat production can also precipitate situations of risk for public health. In these cases, training and education of hunters, must be considered an essential strategic measure to mitigate the occurrence zoonotic diseases.

In large game species, at present, Tuberculosis, mainly caused by *Mycobacterium bovis*, assumes the most important sanitary issue. Nevertheless more diseases, with public and/or animal health concern, have been found in large game species in Portugal. Those will be highlighted during the presentation, under one health perspective, reinforcing the importance of implementing multidisciplinary scientific-based control programs.

**Keywords:** Zoonosis; large game; red deer; wild boar; tuberculosis

## Monitoring of radionuclides in the environment

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### Abstract

The aim of our work is to discuss the consequences of the food chain contamination with radionuclides, especially focusing on the radiocaesium impact after the Chernobyl nuclear accident. In particular the  $^{137}\text{Cs}$  isotope still represents risk. Some plants have a high ability of  $^{137}\text{Cs}$  uptake from the environment and thus are considered to be bioindicators. Among them especially some mushrooms and European blueberry - bilberry (*Vaccinium myrtillus*) play an important role. Up today, the  $^{137}\text{Cs}$  isotope can be detectable in the meat of game, mainly in wild boars, however in elk and reindeer as well. Although the occurrence of highly contaminated food in most of the European countries is currently limited, the activity concentration of the  $^{137}\text{Cs}$  isotope in the meat of wild boars from the German-Czech border line (Šumava region) in some cases still several times exceeds acceptable limit.

**Keywords:** wild boar; contamination; radiocaesium; Europe

## MRSA in wild animals?

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### Abstract

When addressing the methicillin-resistant *Staphylococcus aureus* (MRSA) issue we all trigger the common knowledge to connect them to clinical isolates. The truth is that this worrisome organism is everywhere: in human clinical isolates, in healthy people that work inside of clinical or care facilities, is often associated to livestock animals and to respective handlers, is found in food or in slaughter lines, in wastewaters, garbage and recently, as been associated to wild animals. This is new but we couldn't say that we were not expecting that. In Portugal, with one of the highest rates of clinical MRSA isolates (>47%) in Europe, this MRSA flow problem was a little neglected. Our investigation group found zoonotic staphylococci species, including MRSA, in various wild animals: birds of prey, little mammals, wild rabbit and wild boars. We hope our findings could lead the authorities to start thinking and acting in this field. It's urgent to take a better control over the antibiotic prescription in the clinical practice (human and animal) and in the farm environment because our mistakes are already endangering wild animals. We all know that bacteria spread by anthropogenic sources, in farm handling, by wastewaters or garbage but how it aims the wild animal populations is not completely understood. Human and animal sources of antimicrobial-resistance determinants as untreated sewage or manure might contaminate the areas surrounding wild habitats, being many times used as food source for wild animals. With our recent results we may say that, in Portugal, we are facing a serious problem regarding MRSA. One more time, wild animals are pointed as hosts of clinically important antimicrobial resistant bacteria and more studies should take place to understand how this is happening.

**Key words:** MRSA; wild animals; Portugal; one health.

## Report of *Giardia* spp. and *Cryptosporidium* spp. in Iberian Wolf (*Canis lupus signatus*) in NW of Iberian Peninsula

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### Abstract

The Iberian Wolf (*Canis lupus signatus*), one of the major wildlife predator of the Iberian Peninsula, carries a cultural and historical stigma that contributed to its historical decline, although currently their populations are increasing across Europe. It is recognized that wolves can be a reservoir of many zoonotic diseases. *Giardia* spp. and *Cryptosporidium* spp. are considered ubiquitous protozoan parasites and can be a cause of gastrointestinal diseases in many mammals. Both protozoan are included in the 2004 WHO Neglected Diseases Initiative, with special concern for the species of *Giardia duodenalis* and *Cryptosporidium parvum* that can be a cause of human morbidity and mortality. Thus, the study aimed to assess the presence of *Giardia* spp. and *Cryptosporidium* spp. in the wolf populations from the North of Spain. The fifty faecal samples were collected in the Northwest of Spain, from May until October of 2013 and 2014 and were maintained at -20 °C, until analysis through a commercial direct immunofluorescence assay (Cellabs® Pty Ltd, Brookvale, Australia). The test was considered positive, if one or more oocysts and cysts were present. Ten samples (20.0 %) were positive for both *Giardia* spp. and *Cryptosporidium* spp. From positive samples, *Giardia* cysts and *Cryptosporidium* oocysts were detected in simple infections with more frequency (90.0 %), with seven (14.0 %) samples positive for *Giardia* spp., and two (4.0 %) samples positive for *Cryptosporidium* spp. Our results show that *Giardia* spp. were more frequently found than *Cryptosporidium* spp. The lower prevalence as compared with previous studies may be due to the fact that our samples were frozen and thus the test sensitivity may be lower. However, this study reveals that Iberian wolf is susceptible to the presence of these agents and at the same time can potentially play a role on dissemination. As human and wildlife domains are getting increasingly intertwined, parasitic diseases should be considered a serious threat to wildlife. Conversely, humans and their livestock could be more susceptible to emerging diseases.

**Keywords:** *Canis lupus signatus*; *Cryptosporidium* spp.; *Giardia* spp.; Iberian Peninsula

## First reports and spatiotemporal aspects of the distribution of *Philophthalmus lucipetus* and *Philophthalmus lacrymosus* in gulls in Portugal

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### Abstract

Eye trematodes of the genus *Philophthalmus* Loos, 1899 are eye parasites of birds and mammals (including humans), which use invasive freshwater snails as intermediate hosts. Here we examined the presence of philophthalmi in a total of 1515 gulls (589 *Larus fuscus* and 926 *Larus michahellis*) admitted between January 2010 and October 2016 for rehabilitation at the Wildlife Rehabilitation and Research Center - RIAS in Olhão (Southern Portugal). We recorded the first infected *L. fuscus* and *L. michahellis* in September and November 2015, respectively. Morphological and molecular analysis reveal the presence of two species: *Philophthalmus lacrymosus* and *Philophthalmus lucipetus*. These species present different locations and clinical signs, while *P. lucipetus* was detected under the nictating membrane and cause no clinical signs, *P. lacrymosus* was found in the conjunctival sac and cause serious eye damage in the same host species. The migratory character of *L. fuscus* could be the key to the appearance of *Philophthalmus* spp. in Portugal, since the occurrence of this trematode has been confirmed in countries where this species breeds. We speculate that the trematode could establish locally, utilizing for example the invasive freshwater snail *Melanooides tuberculata*, which is already known in southern Spain and other surrounding regions. The outbreak of *Philophthalmus* spp. in gulls in south Portugal was a highly dynamic event, since the first six cases, found between July and October 2015, was originated from a single municipality, and only later more cases started to be retrieved from other municipalities and within 15 months, the philophthalmi affected almost the entire Algarve coast. These findings represent the first record of both philophthalmi in the Iberian Peninsula, their first record in *L. michahellis* and the first record of *P. lacrymosus* in *L. fuscus*. Considering the low host specificity of these trematodes, the invasive nature of intermediate hosts of *Philophthalmus* spp. and the zoonotic potential of both trematode species, examination of other potential hosts within the study area and the follow-up of the outbreak are needed.

**Keywords:** Digenea; eye trematodes; gulls; philophthalmosis; *Philophthalmus lucipetus*; *Philophthalmus lacrymosus*; Portugal

## Spatial analysis and modeling of wildlife tuberculosis in a multi-host pathogen system

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### Abstract

Spatial epidemiology aims to describe, explain and predict the spatial heterogeneity in disease distribution. The goal of this study was to investigate the spatial epidemiology of bovine tuberculosis (bTB) in wildlife in the multi-host pathogen system of Iberian Peninsula, using the wild boar (*Sus scrofa*) as a sentinel species. Wildlife bTB distribution was surveyed in mainland Portugal from 2006-2013 using serology from hunted wild boar. Disease mapping, cluster analysis and modeling were performed using areal spatial generalized linear mixed models with conditional autoregressive priors. Blood collected in absorbent papers proved to be a valid substitute for serum in bTB serological surveys, with an almost perfect agreement between the two biological matrixes (Kappa=0.818). Antibodies against bPPD were detected in 2.4 % (CI<sub>95</sub> 1.5 – 3.8 %) of 674 wild boar, with 2 geographical clusters identified in Central- and South-Eastern Portugal. At the national scale, the final conditional autoregressive model with bTB presence as dependent variable included, as explanatory variables, historical population dynamics of wild boar, red deer (*Cervus elaphus*) hunting bag, hunting management intensity and the interaction between the latter two variables. The risk map predicted by these models confirms the strong spatial structure of wildlife bTB and shows a good agreement with independent reports of *M. bovis* isolation from wildlife. This study demonstrates that serological tests coupled with blood collection in absorbent paper are a valid strategy for large-scale bTB surveys in wild boar. Data reported here further support that bTB is an emerging disease of wildlife in the Iberian Peninsula, stressing the need to implement control programs to prevent further geographical spread and increase in prevalence.

**Keywords:** bovine tuberculosis; wildlife; spatial epidemiology



## Topic D - Effects of environmental changes on Wild fauna and habitats

### The effects of modern agriculture on farmland birds in Britain: can we change their fate through science-based management?

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#### Abstract

It is widely accepted that the overall decline of farmland birds in Britain and Europe since the 1950s is mainly driven by changes in farming intensity. In Britain, long-term monitoring and research on the formerly numerous grey partridge *Perdix perdix* L. revealed the negative effects of herbicides and insecticides on chick survival, together with the effects of poor or absent cover on nesting success and overwinter survival. Aiming to reverse the decline of grey partridges and other farmland birds, the GWCT has conducted applied research to develop practical solutions and hence stimulate grey partridge recovery.

The selective exclusion of pesticides and herbicides from the edges of cereal fields to protect chick-food insects through 'Conservation Headlands' proved that it is possible not only to increase insects, but also increase plant species and richness. Providing proper nesting cover through Grassy strips along field margins and hedgerows was shown to increase breeding densities, as partridges need the old grass stems and dead leaves for nesting. As a result of this research, 'Beetle-banks' were later developed, which can be described as a strip with perennial grass to host predatory invertebrates all year round and provide partridge nesting cover. Beetle-banks are normally 0.4 metres high and 2 metres wide and should be laid across large fields. The GWCT has also shown that partridges and songbirds are more likely to be found in 'Game and wildlife crops', than arable crops nearby. These crops aim to provide overwinter cover and supplementary feed, including insect-rich habitats. Recent studies have shown that gamebirds and songbirds use Feeders (hoppers) frequently when natural food is scarce, though more research is needed. These research-based options have been introduced in the English agri-environment schemes, so farmers are paid to help wildlife.

After the implementation of these options and predator removal as a 'complete package' in 'demonstration or restoration projects' in England and Europe, success for grey partridges and other farmland birds has been achieved.

**Key words:** beetle-bank; cover; grey partridge; insects; songbirds



## **Spatial-Temporal Analysis of large game and tuberculosis within Idanha-a-Nova County for the years of 2006 – 2016**

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### **Abstract**

Between 2006 and 2016, within Idanha-a-Nova County, 3963 wild boars and 5881 red deer were hunted. By analysing the annual variation of hunted R. deer in relation to W. boars, it was noticed that R. deer numbers present an alternating variation and that the variation of W. boars presented an increasing behavior.

Within Idanha-a-Nova Parishes, it was noticed that in 11.8% of them only W. boars were hunted and in 64.7% of them both species were hunted. The variation in hunted animals reveals that R. deer were located mainly in Penha Garcia (24.4%), Rosmaninhal (62.3%) and Segura (8.4%) and W. boars were located almost everywhere in the County, mainly in Penha Garcia (28.4%). Achieved results lead us to assume that from 2006 to 2016 a high density of both species was observed in Rosmaninhal and Penha Garcia as well a particular spatial coexistence. Analysing land cover cartography and satellite images, it was noticed that, not only Northern parishes are relatively isolated from the others by a vast and dense forested area, but also the South county are partially linked to Spain by an area where Tejo River uses to have little water in summer, which could interfere with the spatial distribution of the wild ungulates. Concerning 2006 to 2016 tuberculosis (TB) spatial-temporal distribution, this study revealed that, during this period, 647 (16.3%) W. boars and 570 (9.7%) R. deer were declared unfit for consumption after detection of TB-compatible lesions (TBCL). The overall prevalence of TBCL in large game ranged from 9.9 ( $\pm$  9.37, 95% CI) in 2006 to 17.9 ( $\pm$  10.18, 95% CI) in 2016 for W. boars and from 7.9 ( $\pm$  6.48, 95% CI) in 2006 to 8.0 ( $\pm$  3.02, 95% CI) in 2016 for R. deer. TB prevalence was higher in the southern County, which presented more than 50% of the hunted animals with TBCL and the high density of both species that cohabit and share resources. Statistical analysis of the data shown that a positive nonlinear relationship can be established between hunting animals and the prevalence of TBCL, for both species: W. boar  $R = 0.448$  ( $p = 0.048$ ) and R. deer  $R = 0.617$  ( $p = 0.012$ ).

**Keywords:** Wild game; Tuberculosis; GIS; Spatial analysis, Wild boar; Red deer

## The increase of ungulates due to environmental changes: meat quality of hunted meat

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### Abstract

In Europe and in Italy wild ungulate populations are increasing dramatically mainly due to the increase of marginal lands and to the lack of predator; in recent decades, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and mainly wild boar (*Sus scropha*) have expanded their home range and increased in abundance (Ramanzin et al., 2010). Nowadays, the presence of ungulates has exacerbated conflicts with human activities (damage to crops and garden, road accidents, etc), so it is necessary to limit the population growth mostly through hunting, with a consequent increase of meat disposable on the market. As regards quality and hygiene assurance, Regulations EC no 178/2002, 853/2004 and 854/2004 must be observed. Most interesting are the studies on meat quality parameters, both chemical and physical, because there is an enormous variability due to season, feed in the wild, age, etc. In central Italy wild boar meat is traditionally consumed for its high nutritional value and particularly sensory properties, which are desired by consumer (Strazdina et al., 2014). Due to the well know problems related to obesity, hypercholesterolemia and some cancer (Wood et al., 2003), it seems interesting to report some data on fat content and fatty acid composition. Recently a study was conducted on 42 meat quality samples, analysed for fatty acid and microbiological profiles (Russo et al., 2017). Gender and hunting age showed little differences on fat quality while the hunting month (from October to January) mostly influences the fatty acid profile of meat, which is probably due to the fact that, in the wild, there is no feed control. Generally, fatty acid content is not well balanced for human diet: nevertheless fat content is very low, so, even the possible negative effects are negligible. Wild boar meat seems to be of good microbiological quality, with health risks for the consumer comparable to those associated with meat obtained from farm animals. Some data are available even on fallow deer (*Dama dama*): even in this case, gender and hunting age did not modify fatty acid profile but, on the contrary, fatty acid content is well balanced (Russo et al., 2007). Actually, we are deepened our study on meat quality of roe deer (*Capreolus capreolus*), but we still don't have a sufficient number of samples. Considering that wild meat is a sporadic component of the diet, we think that it may take part of a well-balanced diet for human without any risk.

**Key words:** environmental changes; game; meat; quality; Tuscan

## **Atomic study using hair from faeces of *Canis lupus signatus* as an environmental bio-indicator for monitorization of Ecosystems**

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### **Abstract**

Heavy metals are important environmental contaminants and the hair may be used as a bio-indicator of these contaminants. Wolf is a natural predator seen as one of the mammals in the top of the trophic chain in Iberian Peninsula. The aim of this study was to assess the presence of elements with regard to heavy metals in the hair obtained from faeces of Iberian wolves (*Canis lupus signatus*) that were collected in different areas of northern Spain. For this purpose, faecal samples were collected directly from the environment (n=37) and then, at the laboratory, washed with acetone to ensure the removal of all external contaminants and to help getting hair separated from faeces. After the washing procedure, each sample was first dried with paper and secondly with a hair dryer until water was completely removed. Analysis was done by X-Ray fluorescence spectroscopy (XFS) with a machine SEA6000VX. XFS permitted us to assess heavy metals qualitative identification by ionisation of elements and recognition of energetic transitions giving form to unique signatures. Samples were submitted to two different electromagnetic waves for ionisation of elements. One electromagnetic wave with 15 KeV was used for assessing heavy metals with less ionisation energy and the other with 50 KeV was for heavy metals with higher ionisation energy. The elements sulphur, calcium, phosphorus, potassium, chlorine, copper, zinc, iron and bromine were identified in these samples. The elements identified in samples were different according to the studied area, suggesting that diet habits can be different between packs or that there were different environmental contaminations.

The information that we obtained can just indicate a possibility of an environmental contamination with heavy metals like bromine or copper in some groups. Other elements can be interpreted as normal in samples.

**Key words:** Environmental contamination; Hair; Iberian wolf; Bromine; Copper

**Diversification of the Spanish grey partridge (*Perdix perdix ssp. hispaniensis*) habitat in the natural protected Area of the “Lago de Sanabria y sierras Segundera y de Porto” (Zamora, NW Spain)**

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**Abstract**

The Iberian grey partridge (*Perdix perdix ssp. hispaniensis*) is one of the most threatened species in Castilla y León region (Spain). The population living in the “Parque Natural del Lago de Sanabria y sierras Segundera y de Porto” (NW Zamora, Spain) is the most important in the region and the most southern in the Iberian Peninsula. The population of Iberian grey partridge live in the area above one thousand eight meters. This population is very relevant at regional, national and international level.

The main threats for the species is the loss of scrub-pasture mosaic due to loss of grazing and the excessive scrubbing of its habitat. During the last 10 years, the Natural Park Direction have been developed actions for the diversification of the habitat of the species mainly consisting in:

- 1) Scrub clearance area and recovery of mountain pastures.
- 2) Harrowing and rye sowing in order to increase the availability of food for partridges during the adverse periods.
- 3) Cleaning and adaptation of natural water points.

The annual censuses carried out in the Natural Park during the last fifteen years have allowed us to discover that the implementation of these measures have contributed to the stability of the grey partridge populations trend in the working area. At the same time, this kind of measures have contributed to the traditional livestock activity, to the prevention of the fire forest and to get better the population trend of the other hunting and protected species in the high mountains of the Natural Park.

**Key words:** Iberian grey partridge; habitat; diversification; conservation; population

## **Wild rabbits (*Oryctolagus cuniculus*) in shrubby environments: correlation between habitat factors and the species presence**

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### **Abstract**

The interior areas of Portugal had experience a drastic change in the traditional patterns of land use, in part because of the aging of the population and exodus to the urban environment. This trend towards urbanization is relatively recent, with the urban population rising from 38.8 % to 63.5% between 1970 and 2015. The increase of shrubby landscape has been the most relevant consequence of the rural abandonment.

The study was carried in two hunting areas, Fafião and Cabril, located in the National Park of Peneda-Gerês (Portugal). The aim of our analysis is to understand the influence of this landscape transformation on the wild rabbit (*Oryctolagus cuniculus*), and the habitat's preferential characteristics involved in its biological needs, in order to find guidelines to improve the habitat. Wild rabbit distribution was assessment into each grid of 500x500 m with faecal pellets. Vegetation cover was categorised into six different types. The habitat suitability for several biological needs (food, tranquillity, shelter) was evaluated in each cell using the HSI, habitat model described by Carmo (1986) with some modifications. All model components are considered as independent variables. The structural analysis of the vegetation was carrying out with the transept methodology. The principal components analysis (PCA) was performed in order to analyze the influence vegetation variables with environmental variables involved the wild rabbit distribution. The first axis opposes rural areas to shrub communities and the second component is positively related with the mosaic landscape. The results are exposed based on the floristic approach. Three groups of species were detected into nanofanerophytes with different signification by rabbit. Classification tree modelling showed homogeneous groups using combinations of independent variables. The variables that discriminated between cells with wild rabbit presence/absence were the shrubs (cover type that provide better shelter) and leguminous shrub as food. Vegetation structural development is an important factor to considerer in the presence of this species.

**Key words:** *Oryctolagus cuniculus*; shrubby environments; Portugal; habitat preferences

## **Threatened fish and mussel populations in Douro basin (Northern Portugal): impacts of environmental changes and *in-situ* and *ex-situ* conservation measures**

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### **Abstract**

Freshwater ecosystems are threatened by human activities and their biodiversity declines far greater than those on marine and terrestrial ecosystems. Several impacts such as habitat loss and fragmentation, pollution, river regulation, overexploitation, introduction of invasive species and environmental changes are responsible for increasing the extinction risk of native species and for the disruption of important ecosystem functions and services. Freshwater mussels (Bivalvia: Unionoida) are among the most threatened faunistic groups in these freshwater ecosystems. These mussels depend on fish to complete their life cycle, where mussel larvae use a specific range of fish hosts to metamorphose. Therefore, the persistence of freshwater mussel species will ultimately depend on the conservation of their fish hosts. The Iberian Peninsula holds a high level of spatially restricted species and endemisms. Many native fish and mussel species of Iberia are listed as vulnerable, endangered or critically endangered and their populations are declining. In this study, we will use data collected in the last 5 years in the main tributaries of River Douro in Portugal to assess the conservation status of native freshwater mussel and fish species and, in particular, the impact of environmental changes. Despite the low human density in three basins (Sabor, Tâmega and Tua), some river stretches are suffering of habitat loss and degradation. Furthermore, the increase in intensity and magnitude of extreme climatic events are inducing higher mortality rates in fish and mussel populations. There are reports of massive die-offs of mussel populations due to a succession of irregular drought and flood events. For all these reasons, several *in-situ* and *ex-situ* conservation measures have been developed to protect endangered native species of NE Portugal. In addition, other actions were oriented to the training and public awareness for the conservation of threatened species and habitats.

**Key words:** bivalves; fishes; conservation; threats; impacts



# POSTERS ABSTRACTS





## Topic A - Wild fauna management and conservation

### Assessing gastrointestinal parasites of Iberian wolves in northern Spain through environmental fecal samples

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#### Abstract

Although multiple studies were conducted in the diverse areas occupied by wolves, the majority of those conducted in the Iberian Peninsula so far used data collected *post-mortem*. The use of fecal samples is an excellent alternative to study wolf parasites and can lead to results that tell us about wolf feeding habits and, indirectly, about the parasitic profile of their preys. The major threats to the Iberian Wolf (*Canis lupus signatus*) include habitat degradation and fragmentation due to the expansion of human occupation, ultimate changes to agricultural practices and territory management, serious decline of preys and poaching but it is known that parasitic infections severely influence wildlife populations, Iberian Wolf being no exception. The aim of this study is to evaluate the prevalence and diversity of gastrointestinal parasites among wolves in northern Spain. For this purpose, fecal samples were collected directly from the environment (n=398) in predetermined transects in Los Ancares (n=249), Picos de Europa (n=129) and Costa da Morte (n=20) between July and October 2012 and were analyzed using a qualitative coprological technique (Willis), followed by microscopy. The overall prevalence was 57.5%. At least ten different parasites have been identified with prevalence ranging between 0.3-37.7%: *Trichuris* spp. (37.7%), Ancylostomatidae (13.8%), Taeniidae (6.0%), *Toxocara* spp. (6.0%), *Capillaria* spp. (3.8%), *Spirocerca lupi* (1.3%), *Dicrocoelium* spp. (1.0%), *Cystoisospora* spp. (0.8%), *Hymenolepis diminuta* (0.5%) and *Nematodirus* spp. (0.3%). Despite the environmental origin of the samples, the prevalence has been high as well as the diversity of the parasites found. In addition to the wolf health threat, some of them have zoonotic potential, underlying the need to think from the perspective of One Health. This is an ongoing survey.

**Key words:** Iberian wolf; parasites; *Trichuris*; Taeniidae; One Health

## Comparing the alleged and estimated abundance of the Wolf (*Canis lupus*) in the province of Rieti (Italy)

Adriani, S.<sup>1</sup>, Fosso, F.<sup>1</sup>, Longhi, E.<sup>1</sup>, Rampini, E.<sup>1</sup>, Roncarà, T.<sup>1</sup>, Sconci, S.<sup>1</sup>, Valentini, E.<sup>1</sup>.

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### Abstract

In recent years, the idea that the Wolf (*Canis lupus*) is a species that has become overabundant is growing rapidly in Italy. In fact, for the Apennine population there are two distinct estimates. The first indicates a numeric presence of 1600/1900, the other one of 1070/2472 wolves. On this basis, a national numerical containment plan is being prepared without defining a target density. In addition, the proposed containment plan does not take into account the real impact of the predator on animal husbandry, the extent and dynamics of the poaching to which Wolf is subjected, nor the consistency and all the problems associated with stray dogs. The most optimistic density data for the Apennine area (2.99 wolves/100km<sup>2</sup>), shared in 2014 by the scientific world, allows the estimated population of about 80 wolves in the province of Rieti. So that in the largest municipality of this area, just that of Rieti (207km<sup>2</sup>), it is estimated a presence that does not exceed 10 wolves. In small municipalities, the estimated number decreases proportionally. To investigate the population perception on the presence of wolves in their communities, a human dimension survey has been conducted by administering standardized questionnaires. It involved a random sample of 664 citizens distributed in 62 of the 72 municipalities in the Province. 573 citizens (86.30% of the interviewed) expressed an opinion on this topic. Results were submitted to statistical analysis. In relation to the perception of the number of wolves considered to be present in the municipality of residence, the sample thus stands in relation to the estimates conducted with scientific technical criteria:

- 1 to 10 wolves: *compatible* - 57.77%;
- 10 to 50 wolves: *moderately higher* - 30.54%;
- 50 to over 100 wolves: *enormously higher* - 11.69%.

Some scientists argue that adopting the Wolf national containment plan is a political answer to some of the hegemonic lobbies. This survey helps to highlight the inflexibility of the basic elements on which a large part of the population bases their instances.

**Key words:** Wolf; *Canis lupus*; density; estimate; perception

**Estimation of the spread of poaching of the Wolf (*Canis lupus*) in the province of Rieti.  
Basic knowledge to discuss the numerical control plan proposed in Italy**

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**Abstract**

In recent years, has been heightened in Italy the presumption that the Wolf (*Canis lupus*) has become an overabundant species, and consequently unsustainable for the coexistence with animal husbandry and wild game. This conjecture, supported by some breeders and hunting organizations, is accompanied by intense poaching. The support of some political parties is leading to the launch of a containment program on national basis that was never adopted for a specially protected species. The data and knowledge acquired for the province of Rieti do not collude with some basic assumptions of the proposed numerical containment plan. Such criticism prompted the launch of a human dimension survey in the province of Rieti, to obtain data on poaching. Considering that poaching is a violation punishable by law, there was a real risk that the interviewees would not report the known events. Therefore, anonymous questionnaires were submitted. The survey was conducted in 86% of the municipalities of the province of Rieti. The random sample of respondents consists of 573 hunters operating in the same Province. The results indicate that 25.15% of respondent are aware of cases of poaching during the 2012-2016 five-year period, but none cases is known to law enforcement agencies or prosecutors. During the year 2016, 98 cases of Wolf poaching were reported. The poaching of wolves was registered in 21 of the 62 municipalities investigated. As large part of poaching take place during drive hunting, some authors estimate the annual amount of Wolf poaching between 10 and 20% of the population. Already in 2002, in the *National Action Plan for the Conservation of the Wolf*, the ending of poaching was hoped. However, despite the persistence of poaching, the population is steadily growing. Whilst hypothesizing the end of poaching, the amount of culling by derogation proposed in the containment plan (up to 5% of the total population) would be irrelevant to the Wolf's population containment in Italy. Effective species management requires actions other than numerical control.

**Key words:** Wolf; *Canis lupus*; poaching; Rieti; management

## **The hypothesis of Wolf repopulations in Italy: A case study in the province of Rieti (Italy) to evaluate the public perception of this myth**

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### **Abstract**

It's a common view in Italy that the current occurrence of the Wolf is partly due to alleged repopulation measures carried out by Protected Areas, State Forestry Corps, Environment Ministry and/or Environmentalist Associations. These alleged procedures used in the release processes are, among others, helicopter throwing and on-site ground transportation by an off-road vehicle. Moreover, the supposed released wolves come from national breeding or by importing from abroad populations. Nevertheless, ruling out the possibility of illegal releases by individuals and/or cases of accidental dispersal of wolves legally and/or illegally kept; there are no Wolf projects and records of restocking. In 1982, the *Gruppo Lupo Italia* committee officially intervened to clarify this delicate issue (Protocol 3187 of July 2<sup>nd</sup> 1982). The information was scarcely disclosed by the media and the idea of repeated repopulations continued to spread and root into the present situation. This study aims to define the statistical profile of supporters in favour (A) and against (B) on the hypothesis of Wolf repopulations. The data were extracted from a complex human dimension survey conducted by questionnaires to a random sample of 665 citizens distributed in 62 of the 72 municipalities in the province of Rieti. 68.72% of respondents expressed an opinion on the question, but only 65.11% provided complete and useful data for statistical processing. In respect of the latter sample, the following results were obtained:

- A. *existence of repopulation* 37.14%: mean age 52.12 years (S.D. = ± 17.25). Education level: university degree 4.01%, high school diploma 35.77%, middle school diploma 47.08%, elementary school 13.14%;13.14%;
- B. *non-existent repopulation* 23.91%: mean age 42.87 years (S.D. = ± 16.06). Education level: university degree 8.18%, high school diploma 47.17%, middle school diploma 38.36%, elementary school 6.29%.

In conclusion, those who believe that the Italian populations of wolves have been subject to repopulation over time are statistically older and with a lower educational level than those who claim that repopulations have never occurred.

**Key words:** Wolf; *Canis lupus*; Rieti; repopulation; environmental education

## Is bioaccumulation of metallic pollutants in wild boar influenced by the season of the year?

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### Abstract

Within the field of Ecotoxicology, bioaccumulation of heavy metals in different animal species has generated great scientific interest, since it has been shown that chronic exposure to these compounds can cause, among others, alterations on the reproductive and nervous systems, which have been related to population declines in many species. Furthermore, the consumption of meat with high levels of contaminants could be considered a human risk factor. Wildlife animals, especially those of hunting interest as the wild boar (*Sus scrofa*), may be suitable bioindicators, due to its large geographical distribution, food and social habits, sexual dimorphism, life expectancy and opportunity for regular sampling during hunting activities. The objective of this study was to analyze the concentration of two toxic metals, lead and cadmium, and two essential ones, copper and zinc, in liver of wild boar from different parts of Galicia (Northwest Spain) during the season 2011/2012. A total number of 130 wild boars were sampled in different hunting activities. Liver samples were dried and wet digested using acids, and subsequent quantitative analysis of heavy metals content on the mineralized samples was performed by anodic stripping voltammetry with mercury electrode.

The average hepatic concentrations (expressed in mg/kg dry weight) were  $0.361 \pm 0.512$  for Pb;  $0.295 \pm 0.495$  for Cd;  $24.17 \pm 9,384$  for Cu; and  $54.00 \pm 36.24$  for Zn. Statistically significant differences were found related to the Cd concentrations depending on the time of sampling ( $p < 0.001$ ), with both males ( $p < 0.05$ ) and females ( $p < 0.001$ ) showing lower levels of Cd in the early hunting season (October to December) when compared to the end (January and February). These differences for Cd could be attributed to the reproductive ecology of the species (i.e., the needs of gestation). No influence of the period of the year was observed for the rest of the considered metals. The quantified values can be indicative of normal levels of exposure, within those that could be considered as baseline in similar ecosystems, thus assuming no risk of exposure to high concentrations of the referred metals.

**Keywords:** Bioaccumulation; ecotoxicology; metals; wild boar; liver

## Hepatic biomarkers: enzymatic activities for assessment of organophosphorus exposure on seagull (*Larus michaellis*)

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### Abstract

The yellow-legged gull *Larus michaellis* can be found in coastal cities and landfill sites, where they have a very wide feeding spectrum, including areas where pollutants (e.g. pesticides) can be accumulated. The inhibition of acetylcholinesterase (AChE) activity has been the most widely used biomarker to confirm exposure to organophosphate (OP) and carbamate (CB) pesticides. Furthermore, other B-esterases such as carboxylesterase (CbE) have been increasingly used as complementary indicators of anticholinesterase compounds exposure and have a potential role in pesticide metabolism. In that regard, also the glutathione S-transferase (GST) enzymes play an important role in xenobiotics detoxification, presenting particularly high levels of activity in liver. The main aim of the present study was to determine hepatic levels of AChE, CbE and GST enzymes activities in *L. michaellis* sampled in Northwest Spain during the years 2013 and 2014. In addition, a potential specific OP poisoning was assessed by means of the evaluation of AChE reactivation. The influence of gender, age (adults and juveniles) and sampling mode [from control population campaigns (CPC) or received at the Wildlife Recovery Center (WRC)] were also assessed. The results showed no significant gender-related differences in enzyme activities (mean  $\pm$  SEM, mU/mg of protein), whereas some age-related differences in CbE activity were found, being lower in adults. AChE activity in animals from CPC was lower ( $5.08 \pm 0.6$ ) than in those from WRC ( $8.38 \pm 0.5$ ), and an OP-related AChE reactivation was higher in animals from CPC ( $46.58 \pm 6.6\%$ ). In contrast, hepatic CbE and GST activities were lower in WRC animals (CbE:  $\alpha$ -NA=216.38 $\pm$ 16.8, 4-NPA=210.30 $\pm$ 20.04; GST=187.90 $\pm$ 13.01) than in animals from CPC (CbE:  $\alpha$ -NA=295.80 $\pm$ 29.1, 4-NPA=455.60 $\pm$ 32.3; GST=241.30 $\pm$ 14.4). Taking in consideration the results, it can be concluded that the studied animals were exposed to OP compounds at levels that might cause acute/chronic health effects and enzymatic sub-lethal effects. Results indicate that gulls may be considered in biomonitoring programs as a sentinel species to assess the impact of some pesticides in non-target organisms.

**Key words:** *Larus michaellis*; organophosphorus; B-esterases; glutathione S-transferase; biomonitoring.

## Admittance of shot animals in wildlife rehabilitation centers in the Iberian Peninsula

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### Abstract

While shooting protected species is considered an illegal act in the Iberian Peninsula, the admittance of wounded and dead individuals in wildlife rehabilitation centers due to this threat is frequent. In this work, we analyze the data regarding admitted animals due to shooting in seven wildlife rehabilitation centers in Portugal between 2010 and 2015 and in 22 centers in Spain between 2014 and 2015. Regarding the data collected in Portugal between 2010 and 2015, it was concluded that 486 animals were admitted with evidence of being shot. A slight reduction in the total number of admittances by shooting was observed between 2010 and 2014 (average of 73 admittances per year) and an accentuated increase in 2015 (119 admittances). In these centers it was observed an increase in the level of admittance between August and January, which is a period consistent with the hunting season.

Analyzing the data of the admitted animals due to shooting in the 22 centers in the Iberian Peninsula (49% of the existing centers), a total of 757 animals affected by this plight were recorded in 2014 and 2015. Animals pertaining to 16 orders and 78 different species were affected in total. The 5 most affected species by this plight were the buzzard (n=165), the sparrowhawk (n=71), the common kestrel (n=55), the eagle owl (n=54) and the goshawk (n=52). Out of the 78 species admitted in the reviewed centers, only 9 of the species are huntable in the Iberian Peninsula.

While this data represents an overview, it does not reflect the total number of animals affected by this cause. The detectability of injured or dead animals in the field is extremely difficult and the fact that it is an illegal act makes the authors of these crimes to quickly discard the animals. In wildlife centers, mostly due to lack of means, it is not always possible to verify suspicion of shooting resorting to radiography, which also decreases the presented values.

While most of the affected animals present protection status of minor concern, in this work it was observed the illegal shooting of priority species such as the Iberian lynx, Spanish imperial eagle, Bonelli's eagle, Montagu's harrier and wildcat. Since data analysis establishes a correlation between hunting season and the months with the highest admittance rate in the wildlife rehabilitation centers, urgent and improved supervision of hunting activity is necessary in order to effectively control this threat.

**Keywords:** Shotgun; wildlife; Iberian Peninsula; wildlife rehabilitation centers



## Status of rock partridge (*Alectoris graeca*) in Sibillini Mountains National Park, Italy

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### Abstract

The rock partridge (*Alectoris graeca*) is a mountain's bird (*galliformes*) with a European wide distribution. In Italy, there are three subspecies. In the Apennines, is present *A. g. graeca subsp.* Although this population is genetically related to the Balkan one, it should be considered a distinct Management Unit (MU) of specific conservation interest. The species, listed in Annexes I and II/A of the Birds Directive (147/2009/EC) lives in an unfavourable conservation *status* (SPEC 1) and its Italian populations have been classified as Vulnerable (VU) by the Italian IUCN Committee. In the Apennines, the species persists in fragmented small isolated populations. A monitoring program was developed in 2016 in the *Sibillini Mountains National Park*. Pre-reproductive monitoring was performed with the *playback census* using 191 stations along 44.2 km of sample transects. Post-reproductive monitoring was performed by counting the coveys on sample strips (78.1 km) or areas (1,977.25 ha) with the help of pointing dogs. Pre-reproductive density resulted in  $2.54 \pm 1.39$  territorial males (TM)/100 ha of nesting suitable area (mean  $\pm$  95% conf. lim.). The number of breeding pairs was estimated in  $189 \pm 56$ . Post-reproductive density results in  $1.24 \pm 0.16$  coveys/100 ha with average size of  $7.1 \pm 1.2$  individuals. A density of  $6.4 \pm 2.5$  individuals/100 ha was estimated suggesting a consistence of  $1.496 \pm 378$ . Reproductive success was estimated as young/adult ratio ( $J/A = 1.95 \pm 0.9$ ). According to the Institute for Environmental Protection and Research (ISPRA), the recorded parameters suggest the feasibility of a limited capturing of founders for *ex situ* conservation programs. However, a preliminary Population Viability Analysis described a "vulnerable" population with 66% extinction probability in 100 years, suggesting to pick up eggs rather than removing mature birds. Other guidelines are: to plan a long lasting and continuative monitoring program, to increase the *carring capacity* principally by planning and monitoring meadows to avoid over and under grazing, to increase knowledge on population genetics and limiting factors, to start a meta-population approach for future experimental design and management actions.

**Key words:** rock partridge; *Alectoris graeca*; *status*; *ex situ* conservation; monitoring

## **Effect of hunting selective pressure on morphological traits of roe deer (*Capreolus capreolus*) in the Province of Viterbo (Italy)**

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### **Abstract**

The selective pressures of human harvesting can determine desirable or undesirable exterior morphological changes on the harvested populations, faster than it does under natural ecological pressures. In this view, the constant biometric monitoring of a harvested population can help in the evaluation of the correctness of the hunting plan and suggesting adaptive adjustments. In the present study, we analyzed exterior morphological data recorded on 881 roe deer (*Capreolus capreolus*) harvested in four hunting district (HD) in the north of Viterbo province (ATC VT1, Lazio Region, Italy) in the period between June 2010 and March 2017. Six morphological traits were recorded: full weight (FW), length of the right trophy (LT), height at the withers (HW), total length (TL), foot length (FL), and chest circumference (CC). Five classes (age x sex) were considered; C0 (from born to 12 months), M1 (males from 12 to 24 months), M2 (males from 24 months onwards), F1 (females from 12 to 24 months), F2 (females from 24 months onwards). We tested the effect of the hunting district (HUD), hunting season (HUS), hunting period (HUP), and class (CLA), on the morphological traits, with the non-parametric *Kruskal-Wallis* test, for multiple comparisons, and *Mann-Whitney U* test for pairwise comparisons. Our results suggest that the factor HUD has a significant effect on LT ( $p = 0.042$ ) and on CC ( $p = 0.017$ ). As expected, the CLA significantly affects all the morphological traits. The higher measurements were recorded on M2 and the lower one on C0. No differences were recorded between F1 and M1 in FW, FL and CC. F2 results not different from M1 and F1 in FL and CC. The HUP results in higher FW ( $p = 0.02$ ) during the summer. Differently, CC is higher ( $p = 0.001$ ) in February and March. The HUS, as measurement of the time since harvesting beginning, has significant effects on all the morphological traits. During the last three years of hunting a stabilization of FW, LT and CC around the maximum values registered on the observation period, occurred. This evidence suggests that the harvesting and management plan adopted in the four hunting districts is correct and is promoting the maintenance of a healthy population.

**Key words:** hunting; management; monitoring; morphology; directional selection

## **A deterministic model for predicting and managing damage risk by wild boar (*Sus scrofa*) on agricultural crops**

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### **Abstract**

In Italy, as well as in Central and Western Europe, wild boar (*Sus scrofa*) populations are expanding also because of the increase of wild land – agriculture interface, consequently leading to conflicts with human activities. In Viterbo province (Central Italy), between the years 2007 and 2013, wild boar was responsible for 85.5% of the overall damage caused by wildlife to agriculture. The average yearly damage caused by wild boar accounts for 490.000,00 € (min = 430.000,00; max = 610.000,00). To mitigate the conflicts, the “*damage compensation policy*” is no longer sustainable. The present study aims modelling the agronomic risk in Viterbo province, identifying and quantifying, with a deterministic approach, the ecological and environmental factors affecting the spatial behaviour of the wild boar and the sensitivity and use of different agricultural areas. Specific questionnaires were administered to skilled experts to obtain, for hunting and non-hunting periods, data on distribution, core-areas, movements, habitat selection, resistance and friction to movement and types of crops damaged. The sensitivity of different agricultural areas was determined on the basis of the Agricultural Average Value. This approach, avoiding considering the effective location of actual damages, allows for the evaluation of the potential risk and is able to identify areas deducted from crop succession. All these data enabled us to implement a GIS model for agronomic risk. During the hunting season, 40.5% of the arable land, 40.2% of vineyards, 41.04% of hazelnuts and chestnuts, 69.79% of pasture meadows, 37.78% of the remaining crops, fall into medium to very high-risk classes. During the non-hunting season, 40.6% of the arable land, 42.0% of vineyards, 28.7% of hazelnuts and chestnuts, 71.11% of pasture meadows, 39.00% of the remaining crops, fall into medium to very high-risk classes. The locations of actual damage events were used for the external validation of the deterministic model. The 71% of damage locations fall in the medium – very high-risk classes, thus suggesting a good prediction power of the model. We intend to improve the model with data from hunters and GPS applied to hunting dogs during the tracking and locating phases.

**Key words:** wild boar; *Sus scrofa*; damage risk; prediction; deterministic model

## **Prediction of body weight of the European wild boar (*Sus scrofa*) from morphometric measurements using image analysis**

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### **Abstract**

Body weight (BW) is an important trait in monitoring growth, nutritional adequacy of habitat or to detect disease outbreaks in wild animals. However to obtain this trait using weighing scales is unrealistic in species where handling is impractical. Take advantage from image analysis techniques it is feasible achieving models using non-invasive methods that decreases the need to handle or restrain animals. Thus, this study aimed the prediction of BW of the European wild boar (*Sus scrofa*) from morphometric measurements using image analysis. For this work, 38 animals with a BW of  $46 \pm 21$  kg (ranging from 15 to 85 kg) were measured. These animals resulted from nine driving hunt in the region of Trás-os-Montes and Alto Douro, Portugal. The BW was determined with a dynamometer scale (Kern HUS 150K50) with 50 g precision. A high-resolution digital camera (Nikon D3100) featuring 14.2-megapixel sensor was used for image acquisition. With the animals in lateral decubitus position, two images were acquired. One from lateral and other from dorsal side views. During image acquisition process a ruler was used for scale and care was taken to place the animals in the same position. From images morphometric measures of height, body length, hind foot length and heart girth were obtained using Fiji software. Multiple linear regression stepwise models combined with k-fold cross validation was used to predict BW. The estimates was based on the k-fold coefficient of determination (k-fold-R<sup>2</sup>), the root mean square error of the cross-validation (RMSE) and the ratio of prediction to deviation (RPD) calculated as the ratio SD/RMSE. The developed k-fold cross-validation model was capable of predicting BW with a k-fold-R<sup>2</sup> of 0.878 and a RPD of 8.5. This result suggest that the model optimized with k-fold cross-validation can operate accurately for other data. Also, the RPD value greater than 2.5 indicate excellent model prediction. The results of the present study showed that morphometric measurements obtained with image analysis offer potential for predicting BW of wild boar. Further studies with more animals are necessary for calibration improvement and robustness of the model.

**Key words:** wild boar; body weight; image analysis; prediction model

## Canine Distemper: an actual old issue for wildlife conservation

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### Abstract

Canine distemper is a worldwide infectious disease caused by a *Morbillivirus*, related to the *Paramyxoviridae* family, along with rinderpest virus, peste-des-petits-ruminants virus, measles virus, phocid distemper virus and cetacean *Morbilliviruses*. Canine distemper virus (CDV) is an enveloped, negative-sense RNA virus, which infects different cell types of various organs and tissues. CDV is mainly known as the etiological agent of the severe systemic disease characterized by a variety of symptoms in dogs. In affected dogs, CDV infection is characterized by a systemic and/or nervous clinical course and viral persistence in selected organs involving the central nervous system (CNS) and lymphoid tissue. Symptoms include fever, respiratory, enteric and neurologic disorders. Domesticated dogs, unvaccinated or improperly vaccinated populations, are the main *reservoir* of CDV, which is a multi-host pathogen. In fact, the virus has been reported in all members of the *Canidae* and *Mustelidae* families and in some members of the *Procyonidae*, *Hyaenidae*, *Ursidae* and *Viverridae* families. It has been also described in large felids and marine mammals. More recently, CDV was detected in non-human primates with high mortality rates. CDV is highly contagious and has been cause of drastic population declines in terrestrial and aquatic wildlife. In our study, we have analysed samples from carnivorous wildlife in central Italy, and we also had the possibility to perform analysis on infected and symptomatic animals, hosted in a Wildlife Rescue Center. Our study shows a wide CDV circulation involving different ecotypes and species in our investigated area, confirming the fact that the spread and incidences of CDV epidemics in wildlife are still worldwide increasing. Motivated from a lack of knowledge in the pathogenesis and wildlife dynamics of the CDV and the recently infections in primates that have raised concerns of a potential zoonotic risk in humans, the aims of this study are to review the distemper disease in wildlife and to assess the epidemiology of the disease in central Italy where its impact in endangered species should be evaluated for specific protection programs.

**Key words:** canine distemper; morbillivirus; wildlife conservation; epidemiology; central Italy

## ***Pseudogymnoascus destructans* and the White Nose Syndrome in bat populations of Northern Portugal**

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### **Abstract**

White-nose syndrome (WNS) is an emerging disease caused by a white psychrophilic fungus named *Pseudogymnoascus destructans* (Pd), which colonizes the ears, nose and wing membranes of hibernating bats. In the US, this colonization leads to pathological lesions and is the cause of bat mass mortality nation-wide, earning the differentiation between WND (White-nose Disease) in the US and WNS in Europe, where there is no related significant bat mortality. Despite this, the presence of the fungus across this continent, including Portugal, where it was first isolated in 2013 in the Trás-os-Montes region, remains a potential threat to bats, given the significant decline in hibernating populations, which in Portugal is of particular concern given the unfavorable conservation status of at least 8 different species.

To understand the distribution of the agent in Northern Portugal, a screening for Pd in bats of the districts of Vila Real and Bragança was performed. Nose and wing membrane swabs were made from 34 specimens of *Barbastella barbastellus*, *Miniopterus schreibersii*, *Myotis blythii*, *M. emarginatus*, *M. myotis*, *Plecotus austriacus*, *Rhinolophus euryale*, *R. ferrumequinum* and *R. hipposideros* during the monitoring campaigns for hibernating bats in 2014 (License 05/2014/CAPT, ICNF). Manipulation of the animals was performed under the “Guidelines of European Community Directive 92/43/EEC” and the “Agreement on the Conservation of Populations of European Bats”. The samples were inoculated into appropriate media and the obtained isolates were identified by direct methods.

Pd was identified in 20.6% (7<sup>+</sup>/34) of bats, with detection in two counties of Vila Real district (Mondim de Basto and Vila Real) and in the county of Carrazeda de Ansiães, Bragança district. Pd isolates were obtained from specimens of the *Myotis*, *Rhinolophus* and *Miniopterus* genus. Its presence in both districts is of concern given the endangered conservation status of the species of these genera in Portugal, reason why it's important to monitor these populations and enforce epidemiological surveillance of diseases with mortality potential, in order to preserve the ecosystem's dynamics and biodiversity.

**Keywords:** Bats; *Pseudogymnoascus destructans*; White nose disease; WND; Portugal

## **Education and Environmental Awareness - “Nature interpretation paths in the Douro Valley agroforestry landscapes”**

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### **Abstract**

There is a currently evident need to alert society to the importance of agriculture and forests as economic activities in the preservation of wildlife, as well as to the regulating role that orderly hunting activities play in its maintenance. These primary and complementary activities, perfectly integrated in the ecosystems to which they belong, make up the greater part of the socio-economic and entertainment culture of rural areas – Douro’s winemaking region, for instance, where the Association for Hunting and Fishing “Socalcos do Douro” operates in the management and exploration of hunting areas.

It’s clear that environmental education will be a decisive tool in spreading awareness, as well as in accessing the symbiotic relationship between wildlife and agricultural systems, and the importance of resource management and agroforestry for the maintenance of natural preserves and the promotion of human well-being. For this purpose, a framework of procedures and strategies was developed, based on establishing pedestrian interpretative paths and targeted at school-age children, as well as at the increasingly affluent tourism in the region.

The project was outlined for a period of three years. The expected positive impact incorporates local development, for the rural tourism brought in, gastronomy, landscape, and other leisure activities.

With the project “nature interpretation paths in the Douro Valley agroforestry landscapes”, the previously mentioned Association for Hunting and Fishing “Socalcos do Douro”, with hunting resources management and exploration responsibilities, hopes to contribute to the acknowledgement of the role the hunter and the farmer play in the conservation of Nature, as well as bring attention to orderly hunting, and its all-encompassing place in Portuguese society as an ancestral activity, perfectly integrated in the traditional uses of soil, as well as in the Mediterranean agroforestry and its ecosystems.

The general goals are in line with the development strategy for activities associated with the appreciation of agricultural ecosystems’ biodiversity and the local development of the Tua Valley Regional Natural Park.

**Key words:** environmental education; wildlife; hunting; agroforestry landscape; wellness activities



## **Monitoring the carnivorous mammals' community in the Faia Brava Reserve: A structural analysis to support conservation measures**

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### **Abstract**

Monitoring carnivorous mammals is essential for the management and implementation of conservation measures. These species are a globally threatened group, but if efforts in the correct management of their habitats are made, this trend can be reversed. In the Faia Brava Reserve (RFB), managed by Associação Transumância e Natureza (ATN), the monitoring of these mammals has the main target of knowing the species present, their distribution and abundance, and to understand the influence of the land management in RFB, as well as identify the most significant areas for the conservation of carnivorous mammals.

Phototrapping and search for tracks and other signs of presence were the two techniques used for sampling. The area was divided into 13 grid cells of 1x1 km UTM (WGS 84), from which 8 were selected to carry out the study, excluding those with the largest part of their area outside of RFB. Fieldwork was carried out in two sampling periods: in Winter - February to April - and Spring – April to May. Two transects of 500 m were done in each grid cell, with a total of 16 transects per sampling period. Concurrently, 3 cameras were placed per grid cell, with at least 300 meters' radius between them, resulting in 24 cameras locations per sampling. All data was cataloged and processed with PAST software and was represented using ArcGis 10.1.

The results of these monitoring methods allowed us to estimate a carnivore species richness value of 7 and detect 2 hotspots, as well as a new record for the weasel (*Mustela nivalis*) occurrence in the RFB. The management plan of RFB seems to benefit the presence and abundance of carnivores, where 7 of the 14 species cataloged in Portugal were recorded in RFB, including priority species for conservation, such as the wildcat (*Felis silvestris*). The management plan in RFB attending with the conservation of habitats and the management of plant biomass by large herbivores in the wild seems to be a promising model to be considered and tested in new projects that aim the conservation of carnivorous mammals.

**Key words:** Carnivores' conservation; species richness; phototrapping; abundance; distribution



**Biochemistry profile and proteinogram of adult tufted capuchin monkey (*Cebus spp.*,  
Erxleben, 1777) kept in captivity in the Paraíba State, Brazil**

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**Abstract**

Biochemical tests and plasma proteins have been extensively used in Veterinary Medicine for the clinical assessment of animals and when properly interpreted, represent an important tool for the veterinarian in establishing diagnosis, prognosis and treatment of diseases that affect wild and domestic animals.

The objective of this work was to determine serum reference values for the main biochemical constituents and proteinogram from 50 clinically healthy monkeys (*Cebus spp.*) adults of both sexes (25 males and 25 females), kept in captivity in the State of Paraíba/Brazil, subject to food handling, health and environmental similar. Significant differences ( $P < 0.05$ ) were found for levels of urea, creatinine, GGT, CK, albumin and beta-globulin between males and females.

In conclusion, factors such as gender may influence some biochemical constituents and some variables of the proteinogram of the capuchin monkeys and therefore should be considered in the moment of the interpretation of the test in this species.

**Key words:** Biochemistry; serum proteins; electrophoresis; nonhuman primates

## Secondary poisoning by anticoagulant rodenticides: a serious threat for wildlife

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### Abstract

Anticoagulant rodenticide (AR) have been manufactured to suppress pest populations in agricultural or urban settings. However, secondary poisoning by these compounds has emerged as a significant concern for conservation and management of non-target wildlife. Generally, the mechanism of AR function is to bind and inhibit enzyme complexes responsible for the recycling of vitamin K1, thus creating a series of deleterious clotting and coagulation impairments. Being divided into two classes (first and second-generation compounds), the second ones are more acutely toxic, often requiring only a single dose to cause intoxication and persist in tissues and in the environment. Wildlife are thought to be at greatest risk of exposure to ARs in agricultural, urban or peri-urban settings, where large quantities of these compounds are often used. At the present study, a relevant case of second-generation AR poisoning related to a red fox is showed. The nutritional state as well as the postmortem condition of the animal were poor. Histopathological lesions were associate with extensive hemorrhage in pulmonary lobules, the interstitium and the alveoli filled with hemolyzed red blood cells and homogenous eosinophilic material. No other remarkable lesions could be detected in any other tissues at sites of section. Toxicological analysis revealed Difenacoum at a concentration of 108 ppb. In absence of any other clinical data concerning this case, and taking in consideration the feeding behavior of the affected animal, a secondary poisoning with anticoagulant rodenticides must be considered, showing the relevance of this problem for wildlife. It must be noted that, in addition to the risk from lethal toxicosis, sublethal AR exposure may compromise wild animals through a reduction in the function of normal clotting.

**Key words:** rodenticide; anticoagulant; secondary poisoning

### Genetic analysis of an intersex in *Capreolus capreolus*

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#### Abstract

In mammals, sex determination underlies a complex network of pathways driven by multiple genes. Any failure in these genes and/or pathways can trigger a cascade of events that may lead to intersexuality. A buck (*Capreolus capreolus*) hit and killed by a car, with an intersex phenotype, was admitted to the UTAD Veterinary Hospital and analysed by a multidisciplinary team. The karyotype of the individual could not be determined due to technical limitations. Hermaphroditism was suspected due to the fact that the individual had: (a) developed antlers and a male appearance, but it also had a vulvar opening; (b) testis- and ovary-like structures; (c) the ovary-like structures appeared to be functional but the testis did not produce sperm. Similar cases have been reported for dogs, cats, goats, horses, pigs but rarely in wild mammals, such as roe deer. On the basis that this intersexuality may have had genetic origin, the objective of this work was the study, classification and investigation of this case in roe deer. Three molecular approaches were optimized: AMELOGENIN (X and Y), SRY (Sex-determining Region on the Y chromosome) and PIS (Polled Intersex Syndrome). DNA was extracted from muscle tissue of the case animal, and from blood of a healthy male buck and a healthy female doe (positive controls). To our best knowledge, this work presents the third case of a well-studied true hermaphroditism in roe deer. Using molecular genetics methodologies, it was possible to confirm that the animal was SRY negative, AMEL-X positive and AMEL-Y negative and was free of the PIS deletion, being diagnosed with the SRY negative XX hermaphroditism syndrome. This study has great value, not only for the rarity of cases, but it also emphasizes the importance of genetics in the sexual identification of wild animals and is a promising way to gain a more comprehensive view of sex determination. The results can gain importance when complemented with approaches in other candidate genes, such as SOX9, to clarify the regulatory mechanism of sex determinism.

**Keywords:** Intersex; hermaphroditism; *Capreolus capreolus*; Sex-reversal; Molecular Sexing

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## Understanding the cross-talk between microbiota, host fitness and the environment using Egyptian mongoose (*Herpestes ichneumon*) as a model

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### Abstract

The Egyptian Mongoose (*Herpestes ichneumon*, Linnaeus, 1758) is a medium-sized carnivore from the *Herpestidae*. It is an opportunist feeder, consuming mostly rabbits, but also reptiles, other small mammals, amphibians, birds, crayfish, eggs or carrion. In Iberia Peninsula, it was conventionally considered as an introduced species during the Muslim Invasions. However, a recent study based on mitochondrial DNA suggested that it was naturally dispersed during the Late Pleistocene sea-level fluctuations. The species was historically restricted to the south of the Tagus River, nonetheless, in the last three decades, it has gradually expanded into central and north-eastern regions of Portugal. This expansion was mostly driven by land-use changes in shrub-dominated ecosystems, forest clearing, agricultural practices, and climate change.

Several aspects of mongoose' biology remain ill-defined. In this work, we thoroughly investigated the gut microbiota of Egyptian mongoose sampled in Portugal, since the gut microbiota is being progressively acknowledged as a fundamental component of mammals' ecology, being crucial for energy recruitment, immunity and molding eco-biological features such as behavior.

Extensive culture-dependent methods and culture-independent microbial profiling, based on single molecule, real-time sequencing of the full 16S rRNA gene, were performed, allowing for phenotypic and phylogenetic characterizations and the semi-quantification of microbial communities within mongoose microbiota. Molecular fingerprinting of cultivable bacterial isolates by RAPD was also completed.

We generated for the first time extended baseline information on the microbiome of mongoose, enabling the exploitation of microbial community differences between sexes and exploring the influence exerted by diet and the ecological context of each host in its microbiome signature. This work sets the ground for more comprehensive studies on the microbiome of Mediterranean wild carnivores, including sympatric threatened species.

**Keywords:** Egyptian Mongoose; Gut Microbiota; Microbial Profiling; Carnivores; Wildlife Management

## Parasitological survey in wild birds kept for rehabilitation in Central Eastern Portugal

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### Abstract

The study of parasites in the wild fauna may have several objectives, namely finding the most prevalent species, relate their presence with the feeding behaviour, assessing the ones that can be related with ecosystem changes as biological tags and diagnosing the most pathogenic harmful ones. Wildlife rehabilitation centers can be a helpful way of performing this kind of studies, since using faecal samples, most of the gastrointestinal parasite fauna can be assessed.

A parasitological survey was performed at CERAS, a Wildlife Rehabilitation Center located in Castelo Branco, Portugal, from January 2016 till May 2017. A total of 65 birds were examined, being 50 birds of prey (27 diurnal; 23 nocturnal) and 15 of other families. Fecal samples were collected in all birds, being performed flotation and natural sedimentation coprological methods. From 65 samples, 24 showed parasitic forms (36.9%), although only 16 (24.6%) showed patent infections, being the remaining 8 positive for mite eggs (12.3%) and 1 of them even showed a mouse nematode egg (*Aspicularis* sp.) (1.5%) considered as pseudoparasites. From the 50 birds of prey, 13 (26%) showed at least 1 oocyst and/or helminth egg. Regarding protozoans, 5 birds showed *Eimeria* spp. oocysts (10%) and concerning helminth eggs, only 1 had infection by Cestodes (2%), 6 by Trematode (12%) and 9 had nematode eggs (18%), being the most prevalent group of parasites, and the most biodiverse one (Anisakidae, *Syngamus* sp., *Tetrameres* sp. and *Capillaria* sp, being this last one the most prevalent, with 9 out of 9 positive for these helminths). Diurnal birds showed 9 positive in 27 (33.3%), while nocturnal showed 4 positive in 23 (17.4%). Concerning the other birds, 3 out of 15 (20%) were positive (1 corvid, 1 gull, 1 egret) with a less diverse parasite fauna (*Eimeria* spp., Trematoda, *Capillaria* spp.).

As preliminary conclusions, we can state that birds of prey are the most representative group kept in this animal facility, being also the group with higher parasitic prevalence and most biodiverse composition, particularly the diurnal species, probably due to a more diverse feeding behaviour of eagles, falcons and hawks compared with owls.

**Key words:** Wild birds; Rehabilitation Center; Gastrointestinal Parasites; Survey; central Portugal

## Feeding and excretion characteristics of wild (*Oryctolagus cuniculus algirus*) and domestic (*Oryctolagus cuniculus cuniculus*) rabbits

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### Abstract

The aim of this study was to evaluate the feed intake and excretion characteristics of two types of rabbits, wild (*O. cuniculus algirus*) vs. domestic (*O. cuniculus cuniculus*). Ten wild (W; mean LW=927g) and ten domestic (D; mean LW=4645g) adult rabbit does were fed *ad libitum* with a control diet (C) with 15% of dehydrated alfalfa, 229,7 particle index. The feed ingestion and faeces excretion were controlled. The samples of this material, the chemical composition and granulometrie were analysed. Wild does dry matter (DM) intake per kg live weight (LW) was 55% higher ( $P<0.001$ ) than the intake of the D ones (58 vs. 37g DM per kg BW, respectively). However, no difference ( $P>0.05$ ) was found when intake was expressed per metabolic weight ( $\text{kg}^{0.75}$  BW). The dry matter excretion, expressed by live weight, were also higher ( $P<0.05$ ) in wild than domestic rabbits (20.5 vs. 12.5 g MS/kg LW) but were similar when expressed by metabolic weight. In the chemical composition of faeces was observed higher ( $P<0,05$ ) dry matter and NDF in wild rabbits. The organic matter, crude protein and crud fat were similar. Domestic rabbit does showed a higher ( $P<0.05$ ) DM, organic matter, crude energy and NDF total tract apparent digestibility (3; 2; 3; 3 percentage points, respectively) than wild does. The particle index and modulus fitness of faeces of wild rabbits were lower (309 vs. 345 and 0,9 vs. 1,1, respectively) than domestic rabbits. This study suggests that wild rabbits exhibit a higher intake per kg LW and a lower digestibility than their domestic counterparts, but the feed ingestion by metabolic weight were similar. The digestive tract degradability of feed differs by type of rabbits because faeces particle size was lower in wild rabbits.

**Key words:** Rabbits; wild; domestics; ingestion; excretion

## **FRESHCO: Multiple implications of invasive species on Freshwater Mussel decline and coextinction processes**

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### **Abstract**

Freshwater mussels (FM) are among the most threatened faunistic groups worldwide. FM depend on fish to complete their life cycle, where mussel larvae (glochidia) use a specific range of fish hosts. This trait makes them particularly threatened by invasive alien species (IAS) since changes on the host fish populations may eventually lead to the decline or co-extinction of the dependent species. The Iberian Peninsula holds a high level of threatened freshwater species, including fish and mussels. Furthermore, Iberian freshwater systems are among the most heavily invaded ecosystems in the world. Under those considerations, FRESHCO project aims to estimate the direct and indirect impacts of selected highly IAS belonging to distinct functional groups (from plants to vertebrates) on the native Iberian FM and its fish host fauna. Five tasks (T) will be executed: T1 will use field and lab experiments to assess the possible competition between the IAS *C. fluminea* and native FM; T2 will be composed of a series of lab experiments to assess host fish suitability of native FM; T3 will use molecular and classical detection tools to analyze the diets of selected IAS and assess their predation on FM larvae, juveniles and adults; T4 will use a battery of field and lab experiments to assess the impact of massive annual die-offs of the invasive aquatic plant *E. crassipes* on the native FM; and in T5, 150 sites of the Douro river basin will be surveyed where mussel and fish assemblages and their habitats will be characterized, in order to evaluate and distinguish the impacts of IAS and habitat degradation on the native fauna. From an ecological point of view, expected results from this project would help to elucidate the real impacts of the most prominent IAS on the threatened native FM and fish species in Iberia. From a management point of view, this project will facilitate a more effective allocation of resources spent on both native species conservation and IAS management.

**Key words:** invasive alien species; host fishes; native mussels; rivers; impacts



## KAI calculation for hunting and non-hunting species in a game reserve of Castilla y León (Spain)

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### Abstract

The “Coto de Caza Valle de Vidriales” (Coto) is situated between the latitude 42°0'50.77"N-42°6'16.38"N and longitude 5°52'22.25"O-5°55'56.05"O. Its territory occupies a total area of 5,493 ha subdivided into 6 Municipalities (Granucillo, Grijalba de Vidriales, Moratones, Pozuelo de Vidriales, Bercianos de Vidriales, Villaobispo), all located in the Zamora Province (northwestern Spain). The “Coto” is managed by the “Club Deportivo de Caza Valle de Vidriales” for hunting activities. Before and after the hunting season (October-February) direct counts are made for estimating the relative abundance of game animals. According to Habitat Directive, game animals used for shooting are classified into “big game species” (Red Deer, Roe Deer, Wild Boar, Wolf, Fox) and “small game species” (Red Partridge, Pheasant, Pigeon, Aquatic birds, Quail, Wild Rabbit, Hare). Our work was developed on 5 itineraries of about 20 km representative of the entire areas on which Kilometric Abundance Index (KAI) were repeated 4 times in two months. The aim of this work was to describe the abundance of animal biodiversity (not only game animals) in an area in which there are hunting pressure. The results are presented in the table below:

	KAI 1		KAI 2		KAI 3		KAI 4		KAI 5		KAI tot	
	n	n/km	n	n/km	n	n/km	n	n/km	n	n/km	n	n/km
Hen harrier	0,00	0,00	1,00	0,05	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,01
Great bustard	1,00	0,05	0,00	0,00	11,00	0,44	0,00	0,00	0,00	0,00	12,00	0,11
Long eared owl	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Common kestrel	1,00	0,05	8,00	0,40	11,00	0,44	2,00	0,10	0,00	0,00	22,00	0,21
E. Nighthjard	12,00	0,60	4,00	0,20	1,00	0,04	4,00	0,19	2,00	0,10	23,00	0,22
Deer	17,00	0,85	1,00	0,05	0,00	0,00	6,00	0,29	18,00	0,90	42,00	0,40
Quail	4,00	0,20	3,00	0,15	1,00	0,04	1,00	0,05	0,00	0,00	9,00	0,08
Rabbit	1,00	0,05	15,00	0,75	0,00	0,00	1,00	0,05	13,00	0,65	30,00	0,28
Carrion crow	17,00	0,85	45,00	2,25	43,00	1,72	18,00	0,86	27,00	1,35	150,00	1,42
Roe deer	35,00	1,75	14,00	0,70	5,00	0,20	11,00	0,52	28,00	1,40	93,00	0,88
Common raven	0,00	0,00	1,00	0,05	1,00	0,04	0,00	0,00	0,00	0,00	2,00	0,02
Gray Heron	0,00	0,00	1,00	0,05	1,00	0,04	1,00	0,05	1,00	0,05	4,00	0,04
Domesticus cat	1,00	0,05	0,00	0,00	1,00	0,04	0,00	0,00	0,00	0,00	2,00	0,02
Wild boar	1,00	0,05	0,00	0,00	0,00	0,00	4,00	0,19	2,00	0,10	7,00	0,07
W. marsh harrier	0,00	0,00	0,00	0,00	1,00	0,04	0,00	0,00	0,00	0,00	1,00	0,01
Barn owl	1,00	0,05	2,00	0,10	0,00	0,00	1,00	0,05	0,00	0,00	4,00	0,04
Hare	9,00	0,45	9,00	0,45	0,00	0,00	6,00	0,29	14,00	0,70	38,00	0,36
Common blackbird	0,00	0,00	3,00	0,15	0,00	0,00	1,00	0,05	1,00	0,05	5,00	0,05
Little owl	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,05	0,00	0,00	1,00	0,01
Pigeon	52,00	2,60	36,00	1,80	43,00	1,72	0,00	0,00	14,00	0,70	145,00	1,37
C. wood pigeon	0,00	0,00	0,00	0,00	0,00	0,00	9,00	0,43	0,00	0,00	9,00	0,08
Ducks	0,00	0,00	0,00	0,00	120,00	4,80	0,00	0,00	0,00	0,00	120,00	1,13
Red Partridge	44,00	2,20	33,00	1,65	9,00	0,36	6,00	0,29	0,00	0,00	92,00	0,87
C. buzzard	4,00	0,20	4,00	0,20	12,00	0,48	2,00	0,10	0,00	0,00	22,00	0,21
E. turtle dove	20,00	1,00	17,00	0,85	7,00	0,28	3,00	0,14	1,00	0,05	48,00	0,45
EA Magpie	8,00	0,40	5,00	0,25	2,00	0,08	4,00	0,19	0,00	0,00	19,00	0,18
Fox	8,00	0,40	5,00	0,25	1,00	0,04	8,00	0,38	4,00	0,20	26,00	0,25

**Key words:** hunting monitoring; KAI; game population; hunting reserve

**Acknowledgement:** This work was realized with a financial contribute of DM 976/2014 of MIUR (Young Students Mobility of Federico II University of Naples, Italy), the agreement with University of León (Spain), and the courtesy of “Coto de Caza Valle de Vidriales”



## Estimation of the numerical corvids consistency for population control in the ATC of Avellino Province

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### Abstract

The Hunting Management Authority "ATC of the Avellino Province", with the aim of setting up population control plans for species of hunting interest, causing damage to agricultural crops or other fauna-hunting species, has realized a census program for Corvids population (Hooded crow/*Corvus coronae cornix*; Jay/*Garrulus glandarius*; Magpie/*Pica pica*; Rook/*Corvus frugilegus*, Jackdraw/*Coloews monedula*). In order to produce a valid estimate for the entire Province of Avellino, the territories belonging to the various administrative bodies were considered: 119 Municipalities; 5 Mountain Communities; 2 Regional Protected Areas; the Natura 2000 areas (15 SIC and 2 ZPS). Lastly, it was considered the total Agricultural-Forestry-Pastoral Area of the Avellino ATC, which, on a total area of 279,164 has, occupies 199,247 has. Based on the interception of visible nests from the road, 8 itineraries have been selected and 782 kilometres were travelled, from which 112 routes (of about 1000 x 500 m) were checked, totalizing 118.162 km (15.11%). Routes were classified per dominant type of habitat, and checked during the reproduction and post-reproduction period (March-September), and in the winter (October-February). Species, Nests, and Number of birds were noted and counted. The survey area amounts to 5,908 has (2.12% of the total Province). Finally, kilometric abundance index (KIA) were calculated for each species and type of habitat. The number of corvids reported results the first one in the hunting area of Avellino Province, and the esteem is the milestone of the management and conservation of the game animals.

Habitat	Jackdraw (KAI)		Eurasian jay (KAI)		Magpie (KAI)		Rook (KAI)		Hooded crow (KAI)	
	Nest	Birds	Nest	Birds	Nest	Birds	Nest	Birds	Nest	Birds
Urbanized	0.00	7.40	0.00	0.00	4.66	3.01	0.00	0.55	1.37	2.74
Rural - Urban	0.00	6.21	0.00	0.00	5.02	3.94	0.00	1.79	1.91	5.49
Wood - Urban	0.00	2.81	0.10	0.42	3.65	1.98	0.00	2.92	2.71	3.23
Rural - Wood - Urban	0.00	6.00	0.00	0.00	3.27	4.58	0.00	1.53	1.96	12.66
Rural - Wood - Industrial	0.00	6.51	0.00	0.00	3.29	3.68	0.00	0.97	2.00	14.44
Wood	0.00	0.14	0.62	2.64	7.63	3.61	0.00	2.15	0.97	0.83
Rural - Wood	0.00	4.19	0.45	0.93	6.45	2.41	0.00	7.00	4.64	5.76
Rural	0.00	6.38	0.05	0.10	6.07	3.49	0.00	1.82	2.07	10.47
Total	0.00	4.64	0.24	0.67	5.48	3.17	0.00	3.43	2.76	7.30

**Key words:** corvids; hunting monitoring; geographical distribution; population; ATC Avellino

**Acknowledgement:** This work was realized with a financial contribute of ATC Provincia di Avellino.

## Fauna presence in the Hunting Reserve of “Obora” (Slovak Republic)

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### Abstract

The “Obora Hunting Reserve” is collocated on Rozhanovce (Košice) between 48°46'40.95"N - 48°44'22.61"N and Longitude 21°23'35.82"E - 21°23'1.56"E. Its territory occupies a total area of 494 hectares completely fenced and subdivided in three different areas: 1) Fallow deer one (100 heads); 2) Fallow deer two (100 heads); 3) Mouflon (50 heads). The “Obora” is managed by “University of Veterinary Medicine and Pharmacy in Košice” for hunting and research activities. Each year the hunting activity is programmed by a plan which define number of big-game animals available to be shot during one year. The number of shot animals ensure that the total number of animals is the same at the beginning (1<sup>st</sup> March) and at the end (28<sup>th</sup> February) of each hunting season. Our work was developed on 3 itineraries of about 5 kilometers used for make a direct count and calculating Kilometric Abundance Index (KAI) repeated 15 times in one month (October), in addition a two hours observation was made every day (20 days) from watchtowers present in each area. Aim of the work is to describe the presence of not hunting animals in a hunting reserve.

	Area Fallow Deer 1				Area Fallow Deer 2			Area Mouflon			
	KAI 1 (5km)		Watchtower		KAI 2 (5 km)		Watchtower	KAI 3 (5 km)		Watchtower	
	n	n/km	1 A n	1 B n	n	n/km		2 A n	n	n/km	3 A n
Fallow deer	39	7.8	67	14	52	10.40	37	4	0.80	5	3
Roe deer	0	0.00	0	0	0	0.00	0	1	0.20	1	1
Mouflon	3	0.60	6	0	0	0.00	0	17	3.40	21	53
Fox	2	0.40	0	0	3	0.60	0	6	1.20	0	0
Squirrel	19	3.80	0	0	18	3.60	0	11	2.20	0	0
Domestic cat	0	0.00	0	0	0	0.00	0	1	0.20	0	0
Badger	1	0.20	0	0	1	0.20	0	0	0.00	0	0
Weasel	0	0.00	0	0	1	0.20	0	1	0.20	0	0
Pine marten	1	0.20	0	0	2	0.40	0	0	0.00	0	0
Beech marten	0	0.00	0	0	1	0.20	0	0	0.00	0	0
Hooded crow	3	0.60	2	1	6	1.20	1	2	0.40	1	0
C. wood pigeon	18	3.60	5	0	22	4.40	4	5	1.00	2	1
C. buzzard	4	0.80	1	0	6	1.20	0	2	0.40	0	0
Ural owl	1	0.20	1	0	2	0.40	0	0	0.00	0	0
EA Magpie	4	0.80	3	0	3	0.60	2	6	1.20	2	0
EA Jay	1	0.20	2	1	0	0.00	0	4	0.80	1	3
Fieldfare	0	0.00	7	0	0	0.00	4	0	0.00	8	5
Redwing	0	0.00	3	2	0	0.00	7	0	0.00	6	10

**Key words:** hunting monitoring; KAI; game population; hunting reserve

**Acknowledgement:** This work was realized with a financial contribute of DM 976/2014 of MIUR (Young Students Mobility of Federico II University of Naples, Italy) and the courtesy of University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic).

## Correlation between signalment data and densitometry of long bones in the Red Fox (*Vulpes Vulpes*)

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### Abstract

The bone density of wild animals has already shown to be useful in examination of several species. The aim of this study was to verify the possible correlations between the signalment data of Red Foxes, originating from two different regions of Italy (Picentini mountains – Campania region; Cerveteri – Lazio region), and the body mass density (BMD) of long bones. The BMD (g/cm<sup>2</sup>) was measured through the non-invasive technique of Dual-Energy X-Ray Absorptiometry (DEXA). Therefore, geographical origin, age, sex and weight of 14 duly hunted Red Fox carcasses were recorded. The age has been evaluated through the dental wear method. Intact long bones have been skeletonized, and dried at 25 ° C for 35 days. The BMD was assessed by densitometer Lunar Piximus (GE Medical Systems) at level of diaphysis and epiphysis of each bone. Data have been averaged, in order to obtain one value of BMD for each bone. Correlations between BMD and age and weight were assessed using the Pearson coefficient. Differences between sexes and geographical origins were evaluated through ANOVA. Significance was set at P<0.05. The BMD of all bones was significantly correlated with age particularly for the tibia (R<sup>2</sup>=0.284; P=0.022). The BMD of femur was significantly correlated with the weight (R<sup>2</sup>=0.817; P=0.034). The BMD was significantly higher for the foxes coming from Cerveteri (P=0.038). No differences were found between the two sexes. Further studies are needed to confirm or disprove the correlations found, and as well as possibly to propose reliable predictive model for the assessment of the age.

**Keywords:** Fox; DEXA; weight; age; geographical differences

## **Hunting and Wildlife Conservation**

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### **Abstract**

Hunting it's a great conservation and wildlife management tool. It gives economic value to wildlife, the hunters' presence prevents poaching, and hunting can be used for wildlife population management. There are some excellent projects regarding hunting and conservation all over the world. Wildlife population management in the Great Limpopo Trans Frontier Park. CAMPFIRE the first communal area management and conservation program in Africa. Marromeu Game Reserve where hunting and the local communities achieved high levels on anti-poaching. Hunting, wildlife conservation and tourism speak at one voice in Namibia. Hunters support the Giant Sable Project in Angola. Local hunters and the scientific community recovered Roe Deer in Bornes Mountain. Where there is no sustainable hunting, wildlife is in danger, like in Kenia a wildlife conservation disaster, since 1977.

**Key words:** hunting; wildlife; conservation; management

## **Traditional and geometric morphometric study of bat species killed by Wind Farm turbines in the North of Portugal**

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### **Abstract**

Geometric morphometries (GM) is useful to analyse the interspecies differences as shape changes and improved characterization of species relative to traditional morphometric methods. The movement of wind turbines is a cause of bat mortality. These bats carcasses can be a useful source of information on bat populations in a defined region, without capturing living specimens and thus avoiding ethical and welfare constraints. The aim of this study is the characterization of three bat species analysed by traditional morphological parameters and using landmark-based GM of their tail-wing to visualize patterns of variation among species. 41 specimens, 25 *Pipistrellus pipistrellus*, 8 *Pipistrellus pygmaeus* and 8 *Nyctalus leisleri* were measured with a calliper and were taken photos using a digital camera (Nikon 1200D) featuring 18 megapixel, mounted at a height of 47 cm. Photos were taken by fixing bats with its dorsal side held against a plasticised millimetre paper. The head, wings and tail were carefully extended, and fixed with transparent adhesive tape. To GM evaluation, 17 landmarks were manually digitized in ImageJ 1.50e software with Point Picker plugin. Landmarks were replicated of previous works and these points are morphological well defined on the ventral-view, in the left side of each specimen. The GraphPad Prism 6.01 was used to traditional morphological parameters and the GM landmarks were analysed using MorphoJ 1.06d software. The wingspan and the lengths of body, tail, arm, forearm, hand and 5<sup>th</sup> digit are bigger in the *N. leisleri* and do not differ between *Pipistrellus*. The tail-wing shape of the three species was compared using Canonical Variates Analysis and Principal Component Analysis, which showed also a significant difference between means to *N. leisleri*. As far as we known, this is the first study to assess wing variation in size and shape by GM in carcasses of bats. Despite the lack of knowledge of the time after death, dehydration and destruction of parts of the carcasses, the standardized collection of photographs for GM seems to be a methodology recommended to routinely use in order to deepen the morphological characterization of the bats.

**Key words:** bat species; external morphology; new fauna record; photogrammetry; geometric morphometrics

**Monitoring of wild boar presence in the SIC IT9340086 “Lago dell’Angitola”:  
 five years of observations**

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**Abstract**

Aquatic habitats are very complex, and communities closely linked to aquatic environments are highly influenced by the opportunistic species and even more by alien species. The complexity of food nets involves a large number of living beings and the balance that keeps them can be threatened by species such as wild boar. The Regional Nature Park “Serre” has benefited of the "Angitola FISH<sub>2</sub>O" project as a sustainable management model for a SCI and Ramsar area. This work is the result of the ongoing ex-post monitoring project but is able to provide the first numerical results about the presence of wild boar in the various habitats that characterize the Angitola Lake. The FPC method is one of the methods used to estimate the number of wild boars per hectare within the environments of SIC IT 9340086 and contribute to verifying the hypothesis of the "Management Plan". Six different habitats have been identified within which, 10 transects were traversed 6 times in the April-May period in 5 years (2013-2017) for a total of 250 sampling. The results obtained are reported in the table and, allow to estimate the population density of wild boars within the protected area. The differences between habitats and over the years are described with increased presence of animals in the swamp-mead habitat and a trend that is increasing from 2013 to 2014, decreasing in 2015 and stable in 2016 and 2017.

Habitat	Swamp-Mead	Pine Forest	Oak tree	Cypress	Agrarian	Coniferous-Mead
2013	13,30	9,00	8,73	8,07	6,74	2,97
2014	13,45	11,25	10,21	9,25	8,42	3,28
2015	12,80	6,76	7,24	6,03	5,06	2,65
2016	13,28	8,13	8,15	7,89	7,11	3,01
2017	13,56	9,17	8,88	8,17	6,14	2,89
Average	13,28	8,86	8,64	7,88	6,69	2,96

**Key words:** wild boar; monitoring; habitat distribution; Site of Community Interest, Natura 2000

**Acknowledgement:** This work was realized with a financial contribute of FEP Calabria 2007-2013 misura 3.2 Cod. 02/BA/12 “Angitola FISH<sub>2</sub>O

## Butterfly Species Richness and Diversity in the municipality of Vila Real, Portugal

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### Abstract

Butterflies can be sensitive to ecosystems disturbance due to changes in composition and abundance over time as a consequence of weather conditions, intensification of agriculture and habitat fragmentation. Thus, the use of butterflies as eco-indicators to assess species traits, habitat quality and to establish ecological strategies in landscape conservation management is a growing research area.

The Centre of Science of Vila Real evaluated the species-habitat relationships of 60 butterfly species across five habitat types (Mediterranean forest, pine forest, marsh, shrub and agricultural areas) in the municipality of Vila Real. Sampling records were made using 500m walking transect counts from April to July of 2017.

A total of 935 individuals belonging to 60 species, 40 genera and 5 families of Lepidoptera (Rhopalocera) were recorded. Family-wise distribution of butterflies showed that members of Nymphalidae dominated the sampling (30 species and 562 individuals) followed by Lycaenidae (14 species and 230 individuals).

From the pooled data, 15 species are listed as moderately threatened species, one as endangered species, and the rest as common species, in “The Butterflies of Portugal”. Butterfly diversity parameters showed variations for the sampling transects. The results showed high values of species richness and diversity of butterflies in the forest transect, followed by shrubs, agricultural and marsh transects. On overall, the dominance of species was low and evenness of distribution was moderate. Species richness followed the same pattern of the diversity parameters with exception to the pine forest. Additionally, butterflies species comparison between the different transects revealed that both forest habitat (Mediterranean and pine) have most of the species in common, especially from Nymphalidae and Pieridae families.

**Key words:** butterfly assessment; species diversity; species richness

## **Topic B - Wild fauna photography seen by professional eyes and researchers**

### **The use of nest cameras as a tool for conservation management of Bonelli's eagle (*Aquila fasciata*) in some natural parks in Barcelona**

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#### **Abstract**

This work shows some examples related to the use of nest cameras as a tool for short and long-term management of this and eventually similar raptor species.

The reproduction period of four pairs of Bonelli's eagles (*Aquila fasciata*) that breed in two nature reserves in the region of Catalonia, Spain, has been monitored for 15 years by using nest cameras. The installation of these cameras in some of the eagle's nests was providing live streaming of images covering most of the activities, including the reproductive period. These live images allow obtaining information about direct actions and behavior, avoiding inconveniences that could damage the eggs' incubation and chick's development processes. On the other side the record of all images generated an important set of data about some poor known facts in this species, such as nutrition, and some behavioral aspects in the nest, and relations between individuals, and parents - chicks. Along a number of recorded years, changes in selected prey species, show a substantial adaptive capacity for the selection of prey, according field availability. It was also evaluated the dedication time of adult male and female concerning different labors such as the nest preparation, eggs incubation, and feed and care of chicks.

**Keywords:** nest camera; Bonelli's eagle; management; conservation



## Photography techniques and images in Biological Sciences and wildlife conservation

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### Abstract

Photography represents a very important tool to the Biological Sciences, and at its beginning it revolutionized the scientific world. The different photographic techniques and the capture of images are fundamental, not only to document, communicate and spread new discoveries and advances in research in the various areas of biological sciences but also in teaching the diverse biology subjects. One of the most recent techniques in Photography is *Stacking*, that consists on the assemblage of several digital images using specific software. The final image, which consists of tens (or hundreds) of stacked images, allows a virtually unlimited field depth. This technique is used to reveal tiny insects or parts of them with a great detail, all along the subject. On the other hand, it will help to complement and validate research, aiming to publish them on numerous international scientific journals in such different areas as Zoology, Microbiology, Ecology, Botanic, among others.

It's also useful to sensitize the general public on species conservation, environmental education, including alerting and sensitizing the general population, as well as institutions and political entities anthropogenic impacts, and also for climate change. The presentation of images in various areas and in different times, can help to show the biodiversity loss, extent of areas destruction, ice melting areas in Artic Sea, habitat destruction, caused by anthropogenic activities. Putting together, conservation and photography can be a powerful tool, because it's possible to produce strong images that have a profound impact on people. They have the power to bring positive changes, showing destructed habitats, while allowing endangered animals a voice further afield from that place, where was captured their plight and story. Is a useful tool to show the huge biodiversity we still have, confirm new species but also alert for endangered species.

Therefore, it is possible to implement and demonstrate through the different techniques used and consequent images obtained, the importance that Photography still have in biological sciences, higher education, and of course to environmental and wildlife conservation, in a way that the natural resources will be correctly used without jeopardizing their supply for future generations.

**Key words:** conservation; preservation; photography; biological sciences; wildlife

## Photography as an additional tool for the conservation of amphibians

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### Abstract

Amphibians are one of the most vulnerable vertebrate class in the region of Vila Real. This group is very sensitive to environmental changes due to their biological characteristics and ecological needs, such as their type of respiration or the particularity of having a life cycle with a aquatic and a terrestrial. Thus, the knowledge of the life cycle is important to establish conservation and preservation measures for this group of endangered animals all over the world.

The municipality of Vila Real created a pond next to the Science Center facilities, as a supplementary measure to reinforce the conservation of the amphibians that occur in the region and at the same time to create community awareness for the need of amphibian's conservation and their habitat preservation. This pond is integrated in practical activities projected for kids that visit the Science Centre, and is a support for the common laboratory activities for environmental education, animal conservation and biodiversity projects of Vila Real region.

This artificial pond has unique features, such as a glass window that allows visitors to directly observe the amphibians in their habitat without disturbing them.

We are monitoring the natural colonization of the pond as well as the development of amphibian species throughout their different life cycle stages, since the eggs until the adults.

In this context a photographic work was carried out, which consists of photographing all the larval stages of the various species. This work is extremely important due to the scarce information and characterization of the larval stages of amphibians occurring in Portugal.

Until now, we have recorded and photographed four species, two belonging to order Urodela (*Triturus marmoratus* and *Lissotriton boscai*) and to order Anura (*Pelophylax perezi* and *Hyla arborea*). For all of these species except for *H. arborea*, we have documented the entire life cycle, including the larvae fase, inside an aquarium for better image quality.

**Key words:** Amphibians; larvae; artificial pond; metamorphosis, identification, conservation



## Topic C - Wild fauna diseases under One Health approach

### First report of molecular detection of *Rickettsia* spp in ticks collected in hedgehogs from North of Portugal

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#### Abstract

European hedgehog (*Erinaceus europaeus* Linnaeus, 1758) is a small nocturnal insectivorous mammal commonly found in rural, suburban and urban habitats. Hedgehogs are frequently parasitized by fleas and ticks which are important vectors of several pathogens that can cause disease in both animals and humans. The aim of this study was to assess the presence of *Rickettsia* from a total of 100 ticks present in four hedgehogs kept in the Rescue and Rehabilitation Center in the North of Portugal.

DNA was extracted from individual ticks, and further analysed in DNA pools of five ticks. *Rickettsia* spp was detected by conventional PCR using specific primers targeting the outer membrane protein (*ompB*) gene. Ticks were identified by species using taxonomic keys. The majority of ticks were *Ixodes* spp and *Rickettsia* was detected in 10 (50%) pool samples of ticks. To proceed with *Rickettsia* species characterization the amplicons will be sequenced and analysed through Blast (NCBI). The European hedgehog are exposed to *Rickettsia* through infected ticks but it is still unknown their role as a potential reservoir of *Rickettsia*. Future studies need to be address in order to investigate if they are able to develop rickettsiemia and infect other ticks. Humans are likely to be in contact with *Rickettsia*-infected ticks when manipulating hedgehogs or their nests in garden or parks, thus these mammals could be involved in the transmission cycle of *Rickettsia*. We highlight the zoonotic potential of these bacteria, and the need for raising awareness about this issue.

**Key words:** Hedgehog; One Health; *Rickettsia*; ticks; zoonosis

## Environmental contamination with Ancylostomatidae eggs from faecal samples of Iberian Wolf (*Canis lupus signatus*) in Spain

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### Abstract

Wild canids are under several pressures in human landscapes, and parasitism is an important factor regarding the conservation of biodiversity, not only from an environmental contamination perspective but also with the presence of zoonotic parasites that may pose a risk to human health. The aim of this study was to evaluate the occurrence of Ancylostomatidae in faecal samples of Iberian Wolf collected in the North of Spain. For this purpose 528 Iberian wolf faecal samples were collected from the environment and were frozen at -20 °C until analyzed by natural sedimentation, a qualitative coprological technique. Briefly describing the method, feces were mixed with tap water in a container and left to settle for approximately one hour after which the supernatant was decanted. These procedures were repeated until the supernatant appeared clear. One drop of sediment from each sample was then taken and placed on a microscope slide along with a drop of 0.1 % methylene blue (Panreac ®, Barcelona, Spain). Ancylostomatidae eggs were found in 15.5 % (n=82) of the samples. Despite the samples origin (environmental) and the fact that they were not fresh, a high positive frequency was observed, taking into account the high sensitivity of the eggs at the freezing stage, which could result in false negatives. *Ancylostoma caninum* is a zoonotic parasite and can cause cutaneous larva *migrans*, but at the same time, could be a threat for endangered species in the field. *A.caninum* is more frequently transmitted by milk, although horizontal transmission can occur (e.g. percutaneous or oral transmission) by ingestion of third-stage larvae from the environment. Although we do not know if these eggs are *A. caninum* or *U. stenocephala*, both could be fatal for young cubs exacerbated by other stress factors. The non-invasive collection and analysis of frozen faeces samples from living animals can provide, even with simple techniques, valuable information. Vigilance and routine surveillance of wildlife ultimately aiming at the concept of “One Health” is needed.

**Keywords:** Ancylostomatidae; Helminths; Parasites; Iberian Wolf; Iberian Peninsula

## Wild birds as a source of multidrug-resistant and ESBL/AmpC-producing *E. coli* isolates in the northwest of Spain

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### Abstract

The increasing *emergence* of multidrug-resistant (MDR) *Escherichia coli* worldwide has become a major public health concern as well as the global spread of Extended Spectrum and plasmid encoded class AmpC Beta-lactamases (ESBL and pAmpC, respectively).

The aims of this study were to determine whether wild birds of the northwest of Spain (Galicia) are carriers of MDR *E. coli* and also if ESBL/pAmpC-producing isolates are present within the normal microbiota of these animals.

The cecal content of 44 wild birds (40 *Buteo buteo*, 2 *Milvus migrans*, 1 *Garrulus glandarius*, 1 *Pica pica*) was sampled for *E. coli* isolation. Susceptibility patterns to eighteen antimicrobial drugs were determined by the standard disk diffusion method. ESBL and AmpC producers were confirmed by standard double-disk synergy test, and typed by PCR/sequencing. Finally, the serotypes, phylogroups, sequence types (STs), PFGE, and virulence-gene profiles of all ESBL/pAmpC-producing *E. coli* isolates were established.

Fifteen of the 44 (34%) wild birds analyzed were carriers of *E. coli* isolates resistant to at least one antibiotic and 11 (25%) of MDR *E. coli*. The most prevalent resistances were to nalidixic acid (29.45%), ampicillin (25%), doxycycline (20.45%), chloramphenicol (20.45%) and ciprofloxacin (20.45%). Moreover, 7 of the 44 (15.9%) birds carried ESBL/pAmpC-producing strains with 10 isolates recovered characterized as: SHV-12 (5); CTXM-1, CMY-2 (2); SHV-12, CMY-2 (1); CTX-1, SHV-12, CMY-2 (1) and CMY-2 (1).

The 10 ESBL/pAmpC-producing isolates showed high diversity, belonging to different phylogroups (A, B1, E, F), serotypes (O101:H10, O9:H4, O57:H4, O45:H45, O153:HNT, O9:H10, O101:HNT, O11:HNT) and STs. Importantly, 2 isolates were ST10-A and 1 ST117-F, being clonal groups associated with MDR infections in humans. Furthermore, all ESBL/pAmpC-producing *E. coli* isolates carried virulence genes associated with extraintestinal pathogenic *E. coli* (ExPEC) and two of them satisfied the ExPEC status.

In conclusion, this study provides evidence that wild birds in the north-west of Spain may play an important role in the dissemination of MDR *E. coli* including clonal groups potentially zoonotic for humans.

**Keywords:** ESBL; pAmpC; MDR; *Escherichia coli*; wildlife

## **The illegal use of poison in Portugal and the link between the health of wildlife, human beings, domestic animals and ecosystems**

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### **Abstract**

Poisoning is currently one of the greatest threats to numerous wild species, many of them with high conservation status, such as the Iberian imperial eagle, vultures, Iberian wolf and Iberian lynx. The use of poisons is often associated with game and agricultural activities, but conflicts between people are also the source of many cases of poisoning; the use of poisons is also frequent in rodents and wild birds control.

The ease with which chemical products can be acquired and applied combined with the number of individuals it can affect and its non-selectivity, makes poison use unpredictable and with uncontrollable consequences. Once toxic substances enter the food chains, it can take on uncontrollable proportions that might even reach humans and domestic animals and, thus, constitutes a serious public health problem. The risk of contamination of soils, water and even food crops exists as some toxics can remain in the environment for long periods at doses high enough to be dangerous.

To assess the illegal use of poison, within Project LIFE Imperial we collected information from 3 different data sources (Programa Antídoto de Portugal (2003-2015), wildlife recovery centres (2010-2015) and Guarda Nacional Republicana (2013-2015)). In this study, we compiled over five hundred cases of possible poisoning affecting domestic and wild species (43 species). The percentage of domestic animals poisoned ranged between 7% and 81% of the cases, depending on the data source. The data suggested that the use of poison is wide spread across Portugal with a higher incidence of cases in the interior. The poisoned baits used to attract animals were very diverse and included different types of meat, dog food or fish. The poison substances used included Carbamates, rodenticides, Organophosphorates, Organochlorides or strychnine.

Wildlife mortality acts as “sentinel” for larger environmental contamination with consequences on the entire ecosystem, including humans and domestic animals. Therefore, monitoring the illegal use of poisons should be enhanced in an integrated "One Health" perspective in order to correctly evaluate and minimize the impact of this practice on public health, wildlife and ecosystems.

**Key words:** poisoning; wildlife; public health; ecosystems; One Health

## **Are Portuguese hunting dogs spreading gastrointestinal parasites to wildlife?**

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### **Abstract**

Hunting activities play an important role in parasites epidemiology and hunting dogs are very important concerning the One Health studies, as they may contribute to parasite spillover and spillback. When humans fail in the proper management of domestic animals and wildlife, parasites can be transferred from the sylvatic to the domestic environment, favouring transmission to humans. Hunting dogs are therefore important for the management of all: environmental, animal (domestic and wild) and human health. The aim of this survey was to assess the prevalence and diversity of the gastrointestinal parasites in hunting dogs from Portugal. For this purpose we collected 215 faecal samples from 28 packs of 14 municipalities from the north and centre of Portugal. The samples were analysed through a qualitative coprological flotation technique. Gastrointestinal parasites were found in 133 (61.9%) samples, from 22 (78.6%) packs, namely: Ancylostomatidae (43.7%), *Trichuris* spp. (24.7%), *Toxocara canis* (11.2%), *Toxascaris leonina* (6.0%), *Cystoisospora* spp. (4.7%) and Taeniidae (0.9%). Samples with Taeniidae eggs were saved for further molecular studies. Multiple parasitic forms (up to five) have been found in the same sample. The overall prevalence has been high as well as the diversity of parasites found. We identified at least six different parasites and five of them are recognised as zoonotic, so the human risk is present. We highlight the identification of Taeniidae eggs, because they are immediately infective and the spreading of these eggs in the domestic/wild environment may contribute to the development of diseases caused by metacestode in domestic/wild animals and humans. Hunting dogs are indeed spreading gastrointestinal parasites to wildlife. The high level of environmental contamination found calls for a greater awareness of the problem among hunters. Health education and risk communication should be developed to hunters and their families, in a perspective of One Health. This is an ongoing survey.

**Key words:** hunting dogs; Ancylostomatidae; Taeniidae; One Health

**Gastrointestinal parasites in wild ungulates – need to raise awareness towards potential zoonotic parasites**



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### Abstract

Diseases shared with wildlife, particularly zoonoses, are of increasing concern in Europe. Although the annual harvest of wild ungulates, especially of wild boars (*Sus scrofa*), little is known about the gastrointestinal parasites of these animals in Portugal. To add current knowledge on the parasite fauna of wild ungulates in Idanha-a-Nova, Portugal, faeces have been recovered during the meat inspection of these animals, done after hunting in February 2017, with the aim to assess the prevalence and diversity of the gastrointestinal parasites in wild ungulates. We collected 70 faecal samples from wild boar (*Sus scrofa*) (n=45), red deer (*Cervus elaphus*) (n=15), mouflon (*Ovis ammon musimon*) (n=7) and fallow deer (*Dama dama*) (n=3). The samples were analysed through two different qualitative coprological techniques – flotation and natural sedimentation. Gastrointestinal parasites were found in 57.1% of the samples, namely: strongyle (52.9%), *Nematodirus* spp. (5.7%), *Capillaria* spp. (2.8%), *Ascaris suum* (2.8%), *Paramphistomum* spp. (1.4%), *Trichuris suis* (1.4%), *Metastrongylus* spp. (1.4%) and *Eimeria* spp. (1.4%). Comparing with the survey carried out in 2016 in the same area, the overall prevalence has been higher in deer and mouflon, but lower in wild boars. We highlight the identification of *Paramphistomum* spp., by the authors' knowledge, for the first time in wild ungulates in Portugal. The multiple infections were less frequent in wild boars, nevertheless, zoonotic helminths have been identified, *Ascaris suum* and *Trichuris suis*. Wild boars could be involved in the epidemiology of these zoonotic parasites acting as a reservoir host, in a region where free range domestic pigs are frequently bred, there is the need to raise awareness towards zoonotic parasites in local populations and also together with hunters, in a perspective of One Health. This is an ongoing survey.

**Key words:** Wild boar; deer; mouflon; *Ascaris suum*; *Paramphistomum* spp.

## What about virulence genes in ESBL-producing *Escherichia coli* from European free-tailed bats?

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### Abstract

The *Chiroptera* order inhabits a multitude of diverse ecological ecosystems and has an essential role in the health and economics ecosystems. *Escherichia coli* is a commensal microorganism of the intestinal flora of mammals with ability to acquire multiple antibiotic resistant and virulence genes representing a human health problem. Bacteria produce virulence factors that are molecules that promote effectiveness of infection and enable them to: colonization of a niche in the host, immunoevasion, immunosuppression, entry into and exit out of cells and obtain nutrition from the host. In this work we characterized virulence factors in *Escherichia coli* isolated from European free-tailed bat (*Tadarida teniotis*) in Portugal.

The presence of virulence genes (*fimA*, *cnf1*, *papC*, *papGIII* and *aer*) was analysed in 19 extended-spectrum beta-lactamase (ESBL) producing *E. coli* isolates from free-tailed bats using specific primers. The DNA was extracted using the boiling method and the concentration and purity was determined using the ND-1000 spectrophotometer. The *fimA* gene was detected in 13 isolates, the *cnf1* in 7 isolates, the *aer* gene in 3 isolates and the *papGIII* in 2 isolates. Genes encoding fimbrial adhesive systems (*fimA*) are the most commonly occurring virulence genes in *E. coli* that cause urinary tract infections. The adhesion factors promote colonization (*papC*, *papGIII*), invasion (*aer*), and replication within uroepithelial cells and precede the expression of other bacterial products such as toxins (*cnf1*), iron acquisition systems, and host defence avoidance mechanisms. In this sense, the present study represents a step forward on understanding how *T. teniotis* might be a reservoir of ESBL producing *E. coli* strains harboring this type of virulence genes highlighting the need for additional studies in order to establish the clonal lineages circulating in this ecosystem.

**Key words:** ESBL producing *E. coli*; *T. teniotis*; virulence factors

## Relation between virulence factors and phylogenetic groups in *Escherichia coli* from European free-tailed bat in Portugal

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### Abstract

*Tadarida teniotis* is one of the species of bats present in Portugal, with distribution in the western Palearctic mainly associated with the Mediterranean. *Escherichia coli* is a commensal microorganism of the intestinal flora of most animals that may be responsible for a variety of diseases such as diarrhea. *E. coli* strains can be classified into four distinct phylogenetic groups (A, B1, B2, D). Commensal isolates are generally associated with groups A and B1, whereas in most cases enteropathogenic isolates are assigned to group D. It has been reported that isolates from phylogenetic group B2 frequently accumulate extra-intestinal virulence factors. The cumulative impact of one or more virulence factors is what distinguishes potential pathogens from harmless intestinal strains. They are responsible by helping bacteria to colonize the host and circumvent its defenses to allow invasion and its survival to environmental attacks. In this work we analyze the relation between virulence factors and phylogenetic groups in 43 *E. coli* strains from *T. teniotis* in Portugal. The distribution were as follow: Group A (16): *fimA* (12), *CnfI+fimA* (1), *papGIII+fimA* (1), *aer+fimA* (1), *aer+papGIII+fimA* (1) Group D (9): *fimA* (6), *papGIII* (1), *cnfI+fimA* (1), *aer+fimA* (1); Group B1 (7): *fimA* (3), *cnfI* (1), *cnfI+fimA* (2), *aer+fimA* (1); Group B2 (1): *papGII+fimA* (1). It is important to point that almost 35% of the *E. coli* isolates belonged to the phylogroup D. The other isolates belonged to phylogroups A (44.2%), B1 (16.2%) or B2 (2.3%). Different reports have revealed that these phylogroups differ in the presence of virulence factors, ecological niches and life-history and some characteristics, such as their ability to exploit different sugar sources, antimicrobial-resistance profiles and growth rate. The majority of the *E. coli* isolates that carried *fimA* gene belonged to phylogroup A. Several reports showed that strains belonging to phylogroups B2 and D contained more virulence factors than strains from the phylogroups A and B1. Future researches should report whether virulence factors genes could persist in the environment or circulate in animals for long periods and be disseminated by wildlife and other vectors.

**Key words:** *Escherichia coli*; bat; virulence factors; phylogenetic groups

## Isolation of *Klebsiella oxytoca* in yemen chameleon (*Chamaeleo calypttratus*) with pericarditis

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### Abstract

An adult male yemen chameleon (*Chamaeleo calypttratus*) from Gaia Biologic Park (Avintes, Portugal) presented, at *post mortem* examination, a suppurative pericarditis. Additionally, liver was enlarged, pale and soft, kidneys were large, pale with whitish deposits compatible with urate crystals (visceral gout) and the lungs presented an heterogeneous colour and condensed areas suggesting pneumonia. Histological examination confirmed macroscopic results. The bacteria specie determination was confirmed using the VITEK® system (2 GP ID Car REF 21342). The isolated agent was identified as *Klebsiella oxytoca*. It is one of the most common microorganisms isolated from healthy and ill captive reptiles. *Klebsiella oxytoca* can also be found in a wide range of environments, as the mucosal membranes of mammals as part of the commensal flora, being commonly referred as ubiquitous and opportunistic. This microorganism can be responsible for a variety of nosocomial infections in humans that may lead to hospital outbreaks. Although often found in reptiles, in chameleons this is the first report of *Klebsiella oxytoca* as etiologic agent of purulent pericarditis, to the knowledge of the authors. The main portal of entry of the agents in heart is haematogenous. *K. oxytoca*, normally present has a commensal in the oral and digestive flora, may have become an opportunistic agent and spread in to the organism. So, we cannot exclude a previous immunodeficient status of the animal, due to captivity or other systemic diseases caused by metabolic problems or others factors. This report reinforce the importance to improve knowledge on pathogens in reptiles, not only because the lethal consequences of the infections in these animals but also because some (as the one reported in this study) can be zoonotic.

**Key words:** Pericarditis; *Chamaeleo calypttratus*; *Klebsiella oxytoca*; Reptile

## Prevalence of gastrointestinal parasites in wild common buzzards (*Buteo buteo*)

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### Abstract

Gastrointestinal parasites are very common among wild birds. Some of these parasites parasitic agents may be commensal, but in stressful situations, like hospitalization and illness, they can become pathogenic, due to immunodepression.

The main objectives of this work were to study the prevalence of parasite infections and to identify the most common types of parasites in wild common buzzards (*Buteo buteo*).

This study included 72 birds which were admitted to the Wildlife Recovery Center of the Veterinary Hospital of University of Trás-os-Montes e Alto Douro (CRAS-HVUTAD) between the years of 2012 and 2016. For all the birds, fresh fecal samples were collected within the first 24 hours of hospitalization for coprological examination, by a NaCl (30%) flotation method, at the Laboratory of Parasitology of the Department of Veterinary Sciences of UTAD. Classification of the age of birds was defined according to morphological and phenotypic criteria, such as the molt pattern and iris coloration.

The prevalence of infection with gastrointestinal parasites was 91.7%. Ten different genera of parasites were identified. The most common parasitic agents were *Capillaria* spp. (73.6%), trematodes (51.4%) and *Eimeria* spp. (12.0%). Thirty three percent of the animals were infected with three or more different parasites. Adult and subadult birds had a higher prevalence of infection than juveniles and chicks. This study demonstrates a high prevalence of birds infected with intestinal parasites of a considerable diversity, thus contributing to the characterization of the gastrointestinal parasites found in one of the most common birds of prey in Portugal.

Further parasitological studies are required to ascertain the real impact of endoparasites in the health status of this species.

**Keywords:** *Buteo buteo*; *Capillaria*; endoparasites; trematodes; Portugal

## Serum protein electrophoretic values in wild common buzzards (*Buteo buteo*)

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### Abstract

Common Buzzard (*Buteo buteo*) is a species of the *Accipitridae* family, widespread in Europe, with a conservation status considered as Least Concern (LC) by the IUCN Red List. It is one of the most common species of diurnal raptors that is admitted in wildlife rehabilitation centers in Portugal. The main admission causes are unknown trauma, hunting and illness.

Serum protein distribution by electrophoresis is a valuable tool to reach a diagnosis, monitoring response to treatment and establishing a prognosis. It helps to differentiate acute from chronic inflammatory process, as well, globulin distribution patterns may serve as a means of screening for infectious diseases. To help the clinical evaluation of diurnal raptors in a near future, reference values must be established. The main purpose of this study is to present protein electrophoretic values for healthy Common Buzzards in Portugal.

Twenty healthy Common Buzzards admitted to the Wildlife Rehabilitation Centre of University of Trás-os-Montes e Alto Douro were selected for this study. Blood samples were collected from basilar vein into heparin tubes, and centrifuged to obtain serum. Serum samples were analyzed by capillary electrophoresis and the obtained results were statistically treated with JMP<sup>®</sup> 11.0.0. Mean electrophoretic values were total protein 3.54g/dL, albumin 1.52g/dL, alpha 1 globulin 0.35g/dL, alpha 2 globulin 0.48g/dL, beta globulin 0.94g/dL, gamma globulin 0.25g/dL, albumin/globulins ratio 0.81. This work presents the first serum protein electrophoresis reference values for this species in Portugal, giving wildlife veterinarians a valuable tool for better screening of subclinical infectious diseases in this species.

**Keywords:** serum proteins; electrophoresis; Common Buzzards

**Parasitic fauna of the Red deer (*Cervus elaphus*) in the Tatra National Park (TANAP) and preventive measures for its reduction**

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**Abstract**

Parasitic diseases of wild animals represent more than 2/3 of all diseases. With constant rising of the cloven-hoofed game in the recent period (concentration rise), there is an assumption that the prevalence of diseases will rise. In our study we focused on Tatra National park area (TANAP). The cooperation between the University of Veterinary Medicine and Pharmacy in Košice and the TANAP Administration (ancestor of the TANAP State Forestry) was established in 1970. Since 1971, based on the mutual cooperation agreement, the project "Game Health Control and Game Management in the TANAP State Forestry" was initiated. One of the priority objectives of this cooperation was the deworming of the game based on the results of coprological examinations of faeces from separate protective circuits and/or feeding areas using preparations based on ivermectin and mebendazolium/rafoxanidum. In the relatively small area of the TANAP (74,111 ha, of which 68% is represented by forests) there are present all the main, but also the rare species of the Central European fauna. This territory belongs to the red-deer hunting area J XXVI Vysoké Tatry (High Tatras). From a climate point of view, the JXXVI hunting area is located in a mildly cold, cold and ice-cold mountain climatic region with an average annual air temperature of 0 to 6 °C. Between 2000 – 2017 we examined 2690 faecal samples from red deer using standard coprological techniques (flotation, larvoscopic and sedimentation method). The overall prevalence of endoparasites in faecal samples from TANAP red deer population was 76.3 %, from which 69.6 % were larvae of deer lung worms (f. *Dictyocaulidae* and *Protostrongylidae*). Species from family *Trichostrongylidae* were most prevalent gastrointestinal nematodes (21.2 %). Long-term monitoring and reduction of the most serious parasites of game in TANAP hunting grounds showed, that coprological examinations and application of anthelmintics play an important role especially in areas with a high intensity of infection. As the national park represents the highest degree of protection (from 3rd to 5th degree), ecological aspect of the treatment is particularly important. This results in a necessity of a proper management of this area while preserving its natural biodiversity.

**Key words:** endoparasites; red deer; dehelminthisation; Tatra National Park; Slovakia



## Ectoparasites in cloven-hoofed game

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### Abstract

Wild ruminants are often infected by wide spectrum of parasites. Various ectoparasites cause health problems and also they can cause death. There are two types of bots in Slovakia – warble fly (f. *Hypodermatidae* - *Hypoderma Diana*, *Hypoderma actaeon*) and botfly (f. *Oestridae* - *Cephenemyia stimulator*, *Cephenemyia auribarbis*, *Pharyngomyia picta*). In 2009 – 2016 ectoparasites were monitored in the cloven-hoofed game. In accordance with the Hunting Act 274/2009 and amendments to certain laws 821 roe deer and 316 red deer were examined for warble fly within the cooperation with hunting associations in Prešov and Košice region. Also 154 roe deer and 54 deer were examined for botfly. After removal, larvae were held in 70% alcohol and then they were identified. In roe deer the prevalence of the warble fly was 51.77 % and the prevalence of botfly was 46.10 %. In red deer the prevalence of warble fly was 48.42 % and the prevalence of botfly was 37,04 %. During the monitoring the weight of shot animals positively diagnosed with parasites the weight loss of roe deer was 20,3 % lower than the weight loss of those with negative diagnosis. The weight loss in red deer was considerably lower (12.5 %). We also noticed ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) in 73 % of examined animals and *Lipoptena cervi* in 86 % of examined animals. These ectoparasites are vectors of important pathogens which can be dangerous for humans. Based on the observed facts we concluded that ectoparasites monitoring in game has great importance especially for influencing the overall health of the game and human.

**Key words:** warble fly; botfly; cloven hoofed game; Slovakia



### The occurrence of *Dirofilaria* infections in wild carnivores

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#### Abstract

A blood filarioses has been recently associated with warm climate countries, but several factors, such as climate and global changes, contributed to the spread of these parasites in the northern areas. Due to these changes, many of exotic diseases occur in temperate conditions, including Slovakia. *Dirofilaria immitis* and *D. repens* are the causal agents of cardiopulmonary and subcutaneous dirofilariosis. The present work was to find the prevalence of *Dirofilaria* spp. in wild carnivores. During 2015 – 2017 were examined 61 blood samples and spleen samples of wild carnivores from districts of Košice and Prešov regions: 39 red foxes (*Vulpes vulpes*), 18 european badgers (*Meles meles*), 2 beech marten (*Martes foina*), 2 racoon dogs (*Nyctereutes procyonoides*). Additionally, peripheral blood was taken from 21 red foxes (11 foxes during anaesthesia, 10 foxes after hunting). Microfilariae were detected by means of modified Knott test. All positive samples were submitted to PCR analyses for species identification. . No blood sample taken during anaesthesia was positive. Because it has been difficult to obtain blood from these wild animals, the research on wild carnivores focused also on molecular analysis after previous DNA isolation from the spleen. In total 40 spleen samples were examined. The overall prevalence of dirofilariosis in wild carnivores reached 27,87 %. Molecular analysis confirmed the presence of the species *D. repens* in 17 out of 39 red foxes (43,59 %). No european badger, beech marten and racoon dogs were positive for dirofilariosis. Species *D. immitis* was not recorded. The prevalence was significantly higher in foxes coming from the districts of Košice region districts in comparison with the northern districts of Prešov region. This study highlights the importance of red foxes as reservoirs of dirofilariosis in eastern Slovak regions pointing to the necessity to continue with monitoring programmes of dirofilariosis not only in domestic carnivores, but also in wild carnivores (mainly red foxes) that can play an important role as reservoirs as well as in spreading of disease through mosquitoes.

**Key words:** wild carnivores; dirofilariosis; *Dirofilaria repens*; *Dirofilaria immitis*; Slovakia

## Use of Multiple-locus variable-number tandem repeat analysis to differentiate human and animal *Mycobacterium bovis* isolates with identical spoligotyping profile

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### Abstract

*Mycobacterium bovis* is the main causative agent of tuberculosis (TB) in animals and has a wide range of hosts, including humans. The value of Multiple-locus variable-number tandem repeat analysis (MLVA) as a method for the molecular characterization of *Mycobacterium bovis* has been confirmed in different epidemiological scenarios. The aim of this study was to compare *Mycobacterium bovis* isolates from human and animal (cattle and wild boar) origin which shared identical spoligotype to identify the possible source of infection in these patients, and contribute to the knowledge of the epidemiology of human TB caused by *Mycobacterium bovis* in Extremadura. This comparative analysis was performed by PCR amplification of 19 loci VNTR on 141 *Mycobacterium bovis* isolates (including *Mycobacterium bovis* BCG isolates) with the spoligotypes SB0120, SB0121, SB0134 and SB0295. Eight isolates from human patients, 85 from cattle and 48 from wild boar. VNTR analysis revealed a high degree of genetic diversity among Extremadura *Mycobacterium bovis* population. It should be noted that one of the human isolates had an identical MLVA-18 profile as three isolates from animal origin. However, there was no evidence of recent animal-to-human transmission, so they were probably the result of endogenous reactivation of an infection acquired in the past.

**Key words:** *Mycobacterium bovis*; Tuberculosis; One health; Molecular epidemiology; Genotyping

### Antibiotic Resistances of Python's oral microbiota: a study case

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#### Abstract

Reptiles, in particular snakes, have been one of the major attractions in public exhibitions, documentaries, ZOOs, and others. However, snakebites are a huge public health concern, mostly due the infections caused. Snake's oral microflora is composed by several bacterial agents, and some of them associated with human's infections. Therefore, it becomes necessary a full characterization of the oral microbiota of such animals, but more important than this, is to investigate the microorganism's antibiotic resistance and their impact on the environment, animal and public health (One health approach). Take in consideration this information, the aim of this study was to isolate, identify the oral microbiota of different Python's species (2 *Python reticulatus*, 1 albino *Python reticulatus* and 2 *Python regius*), and characterize the microflora for antibiotic resistance.

Oral microbiota was collected, before feeding, with a sterile cotton-swab and cultured in 3 different and selective mediums, incubated for 48 h at 37 °C. Thus 16 isolates were selected based on their different colony morphologies and samples, and characterized using catalase, oxidase and Gram tests. Based on biochemical techniques (API20 NE), species belonging to the *Aeromonas*, *Flavobacterium*, *Pseudomonas* and *Vibrio* were identified, and a molecular identification based on the 16S rRNA sequencing is under progress. These species were then characterized for the antibiotic resistance profile using the disc diffusion method with 27 antibiotics belonging to several groups such as B-lactamases (penicillins, cephalosporins, monobactams, carbapenems), aminoglycosides, quinolones, chloramphenicol, and tetracycline. Based on the EUCAST breakpoint tables we detected multiple drug resistance (MDR) in all tested bacteria, and 2 isolates exhibited intermediate and resistance phenotypes to imipenem, an antibiotic only used in hospital environment. The above findings suggest the acquirement of resistance mostly due the indiscriminate and global use of antibiotics.

**Key words:** Python; Oral-microbiota; Antibiotics; Multi-drug resistance; One Health

### **Single dose vaccination against porcine circovirus type 2 (PCV2) reduces severity of tuberculous lesions in wild boar (*Sus scrofa*)**

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#### **Abstract**

Wild boar Tuberculosis (TB) is a chronic bacterial disease mainly caused by *Mycobacterium bovis*. TB prevalence and severity in wild boar have been related recently with the existence of other concomitant pathogens that frequently infect these animals. Thus, TB prevalence has been positively associated with high prevalence of porcine circovirus type 2 (PCV2). Furthermore, TB-affected wild boar and co-infected with pathogens such as PCV2 or *Metastrongylus* spp., have shown more probabilities of suffering severe TB lesions. So that, the aim of this study was to explore whether a single-dose vaccination against PCV2 targeting on young animals affects TB prevalence and severity in wild boar. The study was conducted on a game estate with a well-known history of high prevalence of TB located in central Spain. Two groups of animals were compared (a control group and a PCV2 vaccinated group). To do it, three different capturing events were conducted in the study area in August of 2013, 2014 and 2015. Captured animals were individually identified using a microchip and were vaccinated against PCV2 with one dose of a commercial vaccine (Suvaxyn PCV2, Zoetis, Spain) (vaccinated group). Animals included in this study were sampled during the hunting events celebrated between 2013-2015. TB diagnosis was based on the presence of TB-like lesions and a microbiological confirmation. In order to assess whether PCV2 vaccination reduced both, PCV2 prevalence and viral load, a Real-Time PCR to diagnose PCV2 was carried out in some of the studied animals. A total of 70 animals were captured, identified, vaccinated and finally released. The prevalence of TB found in vaccinated animals was similar than those found in control animals. However, a higher percentage of TB positive animals showing a generalized TB pattern was detected in the control group, in contrast to the vaccinated group. No statistical significant differences were found in the percentage of animals infected by PCV2, neither in the mean PCV2 load between vaccinated and control animals. In conclusion, results obtained in this work show that PCV2 vaccination could be a useful tool to reduce TB severity in wild boar game estates.

**Keywords:** Tuberculosis; wild boar; PCV2; co-infection; vaccination

## **A case study –wildlife risk mitigation programs for bovine tuberculosis**

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### **Abstract**

Animal Tuberculosis (TB) is a chronic zoonotic disease caused by mycobacteria belongs to the *Mycobacterium tuberculosis* complex (MTC).

Some countries have great economic TB-related losses in cattle and disease eradication programs have been developed, which is the case of Portugal. In some regions of the country, like the southeast of central region, difficulties in further progressing of the programme may be related with potential transmission from wildlife, mainly red deer and wild boar, which happens particularly when food and water are scarce resources.

Understanding the risk factors associated with TB infection in livestock and wildlife and both-direction transmission, is fundamental to develop effective and feasible strategies scientific-based to mitigate risks of wildlife transmission and finally control TB in cattle.

In this study, a TB risk assessment protocol was applied to some cattle farms. This protocol includes some steps: contact and inquiry of farms' owners, exploration boundary mapping, characterizing in field of TB transmission risky points and reporting the main information with recommended measures that have to be implemented to reduce the risk for interspecific contacts. Additionally, to complete the report is necessary to include information on health status of the farm's animals, based on official data, and cohabitant large game species which information is obtained through hunted animals' sanitary inspection data.

In our case study, the main risk points were essentially associated with how the animals are fed and watered, how food is stored and also with the permeability for wildlife of exploration's fences. Details about recommended measures will be provided. Our experience suggests that it is needed to design farm specific biosecurity plans since a standard one does not work equally for all of the explorations where TB risk is evident. It is needed to involve farmers, hunters and health authorities to have progress in this fight against TB infection in game and farm animals.

**Keywords:** Bovine Tuberculosis; large game; cattle; risk factors

## Morphological and morphometric analysis of the Italian honeybee

### *(Apis mellifera ligustica)* spermatozoa: a preliminary study for pathological assessments

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#### Abstract

Since few decades, the world is facing important losses in the number of honeybees, with great threat to the agro-zootechnic economics and to the global biodiversity. It is well known that stressors, such as pathogens, agrochemicals, environmental changes, flower-poor habitats, can affect the female and male reproductive system, impairing queens' and drones' fertility. The aim of this study was to assess the morphological features and morphometric parameters of the Italian honeybee (*Apis mellifera ligustica*) spermatozoa in order to define its standard characteristics and to compare them with any pathological changes. Morphological and morphometric analysis were performed on 250 spermatozoa collected from sexually mature drones. Samples were stained with Hematoxylin-Eosin and observed with a Nikon ECLIPSE 80i microscope (100X objective and 10X ocular). Morphological features taken in consideration were: shape of the head, integrity of the tail, abnormal nucleus staining. Sperm total length (ToL), nucleus length (NL), width (NW) and area (NA), tail (TaL), perforator (PL) and head length (HL) were measured using an image analysis software (Nikon® NIS Elements 4.0). Results were expressed as mean and standard deviation. The mean morphometric parameters of spermatozoa were: ToL 189,28±5,88 µm, NL 3,66±0,38 µm, NW 0,63±0,09 µm, NA 1,6±0,28 µm, TaL 182,66±5,82 µm, PL 3,50±0,30 µm and HL 7,16 ±0.45 µm.

As the head is considered the main transporter of the male genome and its modifications may reflect anomalies of DNA content, correlations between the mean morphometrical parameters of the head were analyzed. The HL was found to be positively correlated both to the PL and to the NL ( $p < 0,01$ ) while the NW was negatively correlated to the HL ( $p < 0,05$ ). Moreover, the NL is negatively correlated to the PL. Morphologically, some honeybee spermatozoa (7%) revealed visible defects, such as split and broken tails, irregularity of nuclear chromatin condensation, possibly due to physiological and/or pathological changes. However, more detailed studies are necessary to interpret these data and to standardize the normal morphology and morphometry of the spermatozoa in this subspecies.

**Key words:** honeybee; spermatozoa; morphometry; morphology

## **Tuberculosis spatial-temporal analysis within Idanha-a-Nova country for 2006 up to 2016 in domestic cattle and larger game hunting**

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### **Abstract**

In Portugal, few epidemiological studies regarding tuberculosis (TB) infection have been done regarding the relationship between cattle raised for human consumption and the large game. Thus, analyzing and modeling the geographical distribution of these animals and the disease, as well as identifying possible interspecific interactions may be extremely important in order to better understand the transmission between wild animal reservoirs and domestic cattle populations, which cohabite in the same area. The present research quantify and analyzes TB spatial variation in cattle farms within Idanha-a-Nova county for 2010-2014, and evaluate the relationship between spatial its distribution both for large game and domestic cattle, since most of the herds are raised in extensive regime Which facilitates the coexistence with wild ungulates such as wild boar and deer. Achieved results show that every year, the positive holdings in Idanha-a-Nova are located mostly in the central and southern part of the county. New infections appear to be more widely dispersed compared to persistent or reinfected farms that are more localized in the southern part of the county and less expressive in central areas.

Results from disease temporal and geographic dispersion shown that, for 2010, 2011 and 2013, the estimated prevalence of TB in large game within positive farms geographical areas was higher compared to negative farms geographical areas and these differences were significant ( $P < 0.05$ ) both for R. deer and W. boar. Analysing all achieved results, it was noticed that the problem of TB is more serious in large game animals than in cattle. The spread of the disease in large game seems to be independent of the improvement of cattle farms' health status.

As conclusion, we can state that wild species seem to have the capability to maintain the disease in the absence of cattle that, in conjugation of some environmental factors, such as the scarcity of water and green natural vegetation due to hot and dry springs, will easily pass to cattle when wild species seek artificial water reservoirs and irrigated fields.

**Key words:** Wild game; Tuberculosis; GIS; Spatial analysis; W. boar; R. deer



## Serological findings of *Borrelia burgdorferi* sensu lato in wild boars of the Trás-os-Montes region: preliminary results

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### Abstract

Lyme borreliosis (LB) is an emerging zoonosis with global distribution caused by infection with pathogenic spirochetes of *Borrelia burgdorferi* sensu lato (*B.b.s.l.*) complex, transmitted through the bite of infected *Ixodes* ticks, with *I. ricinus* as the main vector in Europe.

These ticks feed on a range of vertebrates, domestic and wild, including wild boar (*Sus scrofa*), a large ungulate of great economic interest in Portugal due to hunting activities and the commercial value of its meat. The role of wild boar in the epidemiological cycle of *B.b.s.l.* has been recently investigated in the Trás-os-Montes region, with the first ever detection of borrelial DNA in sera of hunted wild boars (*B. afzelii*, GenBank Accession KF990318), and the outcome knowledge that *I. ricinus* ticks are the second most prevalent ticks in these animals, right after *Dermacentor* specimens, reinforcing the need for further studies. In that context, sera from 73 animals hunted in Vila Real and Bragança districts (hunting seasons: 2012/13, 2014/15, 2015/16) were tested for the presence of anti-*B.b.s.l.* antibodies by Indirect Immunofluorescence Assay (IFA), using the antigen produced from cultures of reference strains of *B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. burgdorferi* sensu stricto. The results were analysed statistically (JMP® 10.0 SAS) according to district, sex and age by nominal logistic regression, and the strength of association between variables was estimated by the calculation of odds ratio (OR) [95% confidence interval (CI)]. A seropositivity rate of 46.6% (34<sup>+</sup>/73) (titres: 1:64-1:256) was obtained. Only the variable “age” showed statistical significance ( $P<0.05$ ), meaning that aging might constitute a risk factor for the presence of antibodies to *B.b.s.l.* (OR=2,786; 95%CI=1,013-7,664). These findings provide arguments to the importance of wild boars as a potential reservoir for these spirochetes, raising public health concerns, mainly towards occupational groups linked to hunting, as well as the need to develop integrative epidemiological tools rooted in the “One Health” concept, that take into consideration climate, land use and ecosystem changes in the spatio-temporal context.

**Key words:** Wild boar; hard ticks; *Borrelia burgdorferi* sensu lato; Portugal; One Health



**Skin bacterial isolates in pool frogs (*Pelophylax lessonae*) living in two different aquatic habitat.**

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**Abstract**

Few studies have attempted to fully describe the resident bacterial flora of frog skin. The aim of the present study was to investigate the microflora change of pool frogs (*Pelophylax lessonae*) living in two different aquatic environments present near the Angitola lake in the Regional Natural Park of Serre (Vibo Valentia, Calabria, Italy).

To better discern the relationship between bacterial communities from the same site but in different individuals, we performed bacterial investigation by using swabs collected from different sites of frog's body. Furthermore, we cultured bacteria from the water that housed the frogs.

By means of the culturing methods, *Aeromonas hydrophila* and *Enterococcus durans* were detected in the majority of the collected samples. Only from an aquatic environment, both from the skin and the water samples, the opportunistic pathogen *Acinetobacter baumannii* was isolated. This last bacterial identification deserves special attention, since this bacterium is often isolated in the human clinical samples causing nosocomial infections difficult to treat for the documented increased resistance to antibiotics.

We also noticed that the resident bacteria on the skin of frogs were the same present in their own aquatic environment, except the pool water sample containing chlorine. This underlines that the skin flora is susceptible to the belonging environment.

Future researches are required in order to better characterize the interaction between animal and habitat. Furthermore, such surveys can be useful to highlight the role of frog as sentinel organism, and to aim at identifying the bacteria with influence on animal and human health.

**Keywords:** Skin; Bacteria; Frog (*Pelophylax lessonae*)

## **$^{137}\text{Cs}$ Occurrence in the Wild Boar meat in Slovakia 30 years after the Chernobyl catastrophe**

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### **Abstract**

According to screening measurement performed in European countries, high radioactivity levels were reported in the wild boars muscles from Sumava (Czech Republic). Seasonal fluctuation of  $^{137}\text{Cs}$  activity in the wild boar meat samples was observed in the forests on the southern Rhineland. Monitoring of  $^{137}\text{Cs}$  activity in the wild boar meat samples in the hunting grounds in Slovakia was initiated based on the reports on exceeding limits of the content of radiocaesium in the meat of wild boar from the surrounding countries.

The aim of this study was to determine the  $^{137}\text{Cs}$  post Chernobyl contamination of wild boars population in different hunting districts of Slovakia during 2013-2014. A total of 50 samples of thigh muscle samples from wild boars of different age categories (4 month – 2 years) were evaluated.  $^{137}\text{Cs}$  activity was measured by gamma spectrometry (Canberra).

Despite the fact Slovakia is closer to Chernobyl as Czech Republic and Germany, the  $^{137}\text{Cs}$  activity measured was very low and far below the permitted limit (600 Bq.kg<sup>-1</sup>). Radiocaesium activity in examined samples was very low and therefore consumption of wild boar meat do not represent a health risk problem concerning to this chemical hazard.

**Keywords:** wild boar; contamination; radiocaesium; Slovakia

## **Impact of the dispersion of *Thelazia callipaeda* infection in wild animals: reporting cases in 34 foxes from Lugo and Ourense (Galicia-NW Spain)**

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### **Abstract**

The aim of this communication is to report the occurrence of *Thelazia callipaeda* infection in foxes from Northwestern Spain. Cases of thelaziosis in wild animals have been described for the first time in 46 foxes in Italy. Since then, other cases have been described in Switzerland, Spain, Portugal, Bosnia and Herzegovina and Romania.

From January to February of 2016 (n=241) and 2017 (n=144) a total of 385 carcasses of red foxes (*Vulpes vulpes*) were examined from 46 municipalities of Galicia where canine thelaziosis is highly prevalent. At necropsy, all foxes were subjected to an ocular examination few hours after death. *Thelazia* spp. eyeworms were collected from the conjunctival sac of infected foxes using sterile cotton swabs or flushing with physiological saline solution. Out of 385 examined animals, 34 (8.83%) results positive by *T. callipaeda* infection. Positive foxes were detected only from 9 municipalities, located in the province of Lugo (6) and Ourense (3). This is the first report of the presence of *T. callipaeda* in foxes from NW Spain.

In the last few years many authors have described the importance of the wild cycle of this ocular nematode in the dispersion of the illness. Although other wildlife species were demonstrated to act as definitive hosts for *T. callipaeda*, foxes are probably the most suitable wild carnivores due to their peri-urban habits, which may favor a closer contact with the vector.

**Key words:** *Thelazia callipaeda*; foxes; NW Spain; emerging disease

Acknowledgements: Wildfauna Recovery Centers of Galicia, Dirección Xeral de Patrimonio Natural (Xunta de Galicia, Spain).

### **Wolves with irreversible lameness. Can they survive?**

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#### **Abstract**

This work makes a review of three clinical cases of Iberian wolves from the recovery centers of Galicia and Asturias that presented good corporal condition in spite of suffering permanent lameness with functional inability in an extremity:

*Clinical case 1:* complete amputation of the thoracic limb in the proximal part of the forearm. The osseous and skin tissues were healed perfectly.

*Clinical case 2:* ancient unconsolidated tibial fracture (malunion), caused by shot and osteomyelitis, with angulatory and rotary deformity. Bone appears healed with callus formation, but its either malaligned and rotated.

*Clinical case 3:* premature physis closure of the distal ulna (following trauma) that has caused disproportional growth of the bones, shortening and lateral rotation of the forearm.

The surprising of these cases is that the corporal condition, corporal weight and fat deposits were good. It supposes that these wolves were able to survive and keep in good conditions in spite of the chronic injuries that caused functional inability of one limb.

How this is possible occur in wild animals? In our experience this is possible because the area of study (NW Spain) has a sum of several favorable conditions:

- 1.- The abundance and availability of food (domestic and wild animals)
- 2.- The territory of the pack is small
- 3.- The favorable effect of living in pack

Acknowledgements: The authors wish to thank the Wildfauna Recovery Centers of Galicia and Asturias; Dirección Xeral de Patrimonio Natural (Consellería de Medio Ambiente e Ordenación do Territorio. Xunta de Galicia, Spain); and Consejería de Agroganadería y Recursos Autóctonos de Caza y Pesca (Principado de Asturias, Spain) for authorizing this study and the continuous collaboration.

**Key words:** Wolf; *Canis lupus signatus*; survival; fracture; lameness

## **Q Fever: seroprevalence study in wild ungulates in the autonomous community of Castilla y León**

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### **Abstract**

Q fever is an emerging zoonosis produced by the bacterium *Coxiella burnetii*, it could be a severe public health problem and awareness of the disease must be promoted worldwide. Domestic ruminants are considered as the main reservoir of bacteria, the main clinical sign associated with *C. burnetii* infection is late-term abortion in small ruminants, and fertility problems in cattle. The aim of this study was to evaluate by serology analyses (ELISA) the prevalence of *C. burnetii* in wild ungulates in The Autonomous Community of Castilla y León (Spain). This study was based on the analysis serum samples to determine the level of exposure to *C. burnetii* in wild ungulates population. The study took place during the period 2009-2016. Sera were collected from wild ungulates (872 media per year). Thus, through ELISA technique (ID Screen®, Q Fever Indirect Multi-species) it was observed a moderated individual seroprevalence (2.16%) in wild ungulates. These results showed that *C. burnetii* seroprevalence in wild ungulates evaluated was moderated widespread. There is a need to build awareness among farmers and veterinarians of *C. burnetii* infection in farmed ruminants, including risk factors for spillover from domestic ruminant populations to humans.

**Key words:** Castilla y León; *Coxiella burnetii*; Q fever; seroprevalence; wild ungulates.

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## Virulence genes among vanA-*Enterococcus faecium* from wild Red-legged partridges: a threat to public health?

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### Abstract

Enterococci are generally considered a harmless commensal of the mammalian gastrointestinal tract. However, the emergence of vancomycin-resistant *enterococcus* (VRE) is one of the major causes of nosocomial infections. *Enterococcus* spp. may acquire virulence genes. Genes encoding virulence, which are present in enterococci, have been found in both human and animals. The red-legged partridge (*Alectoris rufa*) is a typical farmland and wild game bird common in Portugal. The meat of partridges is consumed in several countries; however, no study has reported the presence of virulence genes in partridges in Portugal. Thus, the aim of this study was to analyse the prevalence of virulence genes in vanA-containing *Enterococcus* in faecal samples of partridges in Portugal.

From the 305 faecal samples of red-legged partridges, 6 vanA-*E. faecium* isolates were detected. The detection of genes encoding the virulence factors, including enterococcal surface protein (*esp*) and hyaluronidase (*hyl*) was determined in all *Enterococcus* spp. isolates using PCR. The *hyl* gene was found in 5 of the 6 isolates and *esp* gene was detected in all of them.

The enterococci ability to produce invasive infections has been related to virulence factors. Virulent strains can colonize the intestinal tract and spread to other organs, such as the urinary tract. The *esp* and *hyl* genes are the most frequently found in hospital outbreaks caused by VRE strains and have also been detected in food. The *esp* protein may contribute to the colonization and persistence of enterococci in urinary infections. This gene is also associated with biofilm formation, which enhances the bacterial resistance, which may lead to chronic infections. The *hyl* protein, encoded by the *hyl* gene, helps the spread of the bacteria since it seems to be responsible for the destruction of mucopolysaccharides and cartilage. Nevertheless, the mechanisms of animal infections caused by enterococcal virulence genes are not quite understood yet therefore further studies are required.

**Key words:** wild birds; red-legged partridge; virulence genes; enterococci



## Topic D - Effects of environmental changes on Wild fauna and habitats

### Camera traps as a tool to define the monitoring base for the jaguar and other mammal species in the Chaco Seco, Paraguay

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#### Abstract

The Gran Chaco Trinacional (Bolivia, Paraguay and Argentina) is the largest ecoregion in the western hemisphere after the Amazon. It holds an important biodiversity (more than 3400 species of flora, some 500 species of birds, 150 species of mammals, 120 species of reptiles and 100 of amphibians) and some of them are under threat. The importance of this ecoregion and the high anthropic pressure the states have defined key priority areas for conservation. The area of this research is the property of Victoria Paraguay which holds this characteristic of ecosystems and biodiversity. One of the most important species is jaguar (*Panthera onca*), which is also an umbrella species and the largest cat and predator in the region. Its conservation allows the conservation of the species under the trophic chain. Camera trapping methods were selected because of its biology nature (cryptic, with low density and nocturnal habits) that makes highly difficult to detect and monitoring to collect data and information to learn more about this species, its environment and preys (inter and intraspecific). This study is carried on since March 2016. The main activity in this property is cattle raising. Land use, climatic conditions are also analyzed. Important preliminary data as abundance and density of jaguar and its preys allows to analyze the conflict levels between this big cat and cattle and to reduce these levels of conflict. An Action Plan for the management of the jaguar for this area is proposed, taking in consideration that this activity and conflicts will remain for the long term.

**Key words:** *Panthera onca*; camera trap; density; abundance; action plan



## The occurrence of thorny-headed worms parasitising in birds of prey (Accipitriformes, Falconiformes, Strigiformes) in Slovakia

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### Abstract

The prey of carnivorous birds is very often the source of parasitic infection. Birds of prey can host a wide spectrum of heteroxenous parasites including acanthocephalans. Within this research 286 individuals of birds of prey belonging to 23 species from the orders Accipitriformes, Falconiformes and Strigiformes were examined for the presence of thorny-headed worms. All carcasses were subjected to helminthological autopsy and parasites were determined according to morphological characters.

The results confirmed the occurrence of the genus *Centrorhynchus* in the territory of Slovakia, of which three species were identified in our samples – *C. buteonis*, *C. aluconis* and *C. globocaudatus*. Overall 6 out of 23 bird species (26.1 %) and 34 out of 286 bird individuals (11.9 %) were infected with thorny-headed worms. The lowest prevalence (2.4 %) was in the order Falconiformes in which only 1 out of 5 bird species was positive for the presence of *C. globocaudatus*. The worms were found in two individuals of *Falco tinnunculus*. In the order Accipitriformes all three species of the genus *Centrorhynchus* were confirmed. The acanthocephalan infection was found in two (*Buteo buteo*, *B. rufinus*) out of 11 bird species. The prevalence in this bird order was 8.3 %. *C. aluconis* was the only species identified in the order Strigiformes, but the prevalence in this group was the highest (42.2 %) and also the number of infected bird species (4/7), namely *Asio otus*, *Strix aluco*, *S. uralensis* and *Tyto alba*. The highest number of examined and also positive birds belongs to the species *S. uralensis* (15/15) with 100 % prevalence of *C. aluconis*.

The study was supported by the Science Grant Agency VEGA No. 1/0080/15.

**Key words:** *Centrorhynchus*; helminthes; raptors; owls

## Evaluation of the Adverse Effects of Glyphosate on the Development of Zebrafish – Preliminary Results

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### Abstract

The glyphosate use has dramatically increased in recent years, being currently, the most used herbicide worldwide. Residues of glyphosate have been detected in water and soil near of its application sites. Despite its longer half-life, between 7 and 140 days in water, the Environmental Protection Agency (EPA) classifies glyphosate formulations as low or non-toxic to birds and mammals, and as practically non-toxic to moderately toxic to aquatic invertebrates. However, most recent studies indicate that glyphosate can disturb vertebrate systems, although these works are not sufficient to elucidate the underlying biological mechanisms. In this regard, the assessment of glyphosate effects to environment and human health is a priority. The zebrafish embryo is a promising unconventional vertebrate model for *in vivo* developmental studies, with several advantages in the assessment of the contaminants effects to the aquatic organisms. Thus, zebrafish embryos were collected after fertilization, washed and exposed for 72 h (from 3 to 75 h post-fertilization) at several concentrations of glyphosate, doses ranging from 2 to 15 µg/mL (Roundup Ultra Max®). During the exposure, lethality and developmental anomalies were evaluated in a concentration-response manner. Exposure to increasing doses of glyphosate induced a higher mortality during the early development of zebrafish, showing a LC50 of 8.53 µg/mL. The subsequent exposure to sublethal doses of 2 and 5 µg/mL induced a slight decrease in the hatching rate and heart rate. After the exposure period, several parameters, related to oxidative stress, were evaluated. The dose of 5 µg/mL induced a superoxide dismutase inhibition while the remaining oxidative stress biomarkers were only slightly affected. These results confirm glyphosate action as an oxidative stress agent. Additional studies should be performed to confirm the potential changes in the biochemical parameters analysed and to understand the underlying mechanisms of toxicity and its implications in animal and human health, as well in the environment.

**Key words:** Glyphosate; ecotoxicity; aquatic wildlife; zebrafish; embryos

**Evaluation of biotic stress on *Carassius auratus* and *Squalius cephalus*  
 by biochemical antioxidant markers**

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**Abstract**

One of the evaluation methods applied to the Angitola Lake’s healthy control (Regional Natural Park of "Serre" Calabria, Italy) was to test biodiversity state of stress due to the environmental pressure. The water component is one of the ecological substrate of the protected area and fishes presence depends on quality and alternation of the water level. Both are influenced by opening or closing of a dam. Biochemical pro and antioxidant markers have been evaluated on some tissue targets, as liver and muscles, on two fish species, that are an integral part of the typical biodiversity of the lake: the *Carassius auratus* and the *Squalius cephalus*. Specimens from 30 *Carassius auratus* and 30 *Squalius cephalus* were sampled during the stressed period of Autumn season, period in which consequently to the dam opening, water reaches the minimum level of the year. From each animal liver, dorsal and caudal muscles were collected to assess their oxidant and antioxidant status. Lipid peroxidation, intracellular ROM (Reactive Oxygen Metabolites) and antioxidant barrier (fast and slow antiROM) were measured by kits purchased from *Diacron International s.r.l.* (Grosseto, Italy). Preliminary results underlined higher lipid peroxidation in the *Carassius auratus* liver respect to the *Squalius cephalus* liver (P <0.001). Differences are reversed if considering ROMs and Anti-ROMs in the liver (P <0.001).

Furthermore, the levels of antioxidant barrier in the liver and in the muscles resulted always lower in *Carassius auratus* respect to *Squalius cephalus* (P<0.001), and only Anti-ROMI resulted different in the Caudal muscle of *Carassius Vs Squalius* at (P<0.01).

In conclusion, the results indicate that these biochemical indexes can be considered as one of the reliable biomarkers of oxidative stress influenced by the environment and lifestyles in these animals.

	<i>Carassius auratus</i>				<i>Squalius cephalus</i>			
	Lipotiss (μEq/L)	dROMs (U CARR)	Anti-ROMI (μEq/L)	Anti-ROMII (μEq/L)	Lipotiss (μEq/L)	dROMs (U CARR)	Anti-ROMI (μEq/L)	Anti-ROMII (μEq/L)
<b>Liver</b>	19±4.10*	26±4.89*	2064±80*	3474±116*	1±0.52*	81±4.84*	3805±101*	4772±81*
<b>Dorsal muscle</b>	0	24±2.85*	217±30*	2118±54*	0	43±2.91*	640±31*	3359±379*
<b>Caudal muscle</b>	0	32±16.16*	547±37**	2563±256*	0	61±14.24*	517±32**	3222±531*

1 U CARR = 0.08 mg% of Hydrogen peroxide - \*P≤0.001; \*\* P≤0.01

**Key words:** Oxidative stress; ROM; *Carassius auratus*; *Squalius cephalus*; protected area

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## Climate change impacts on toxicity and its effects in fish

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### Abstract

Global warming caused mainly by the increase of greenhouse gases emissions refers to a slight but constant increase in atmospheric temperature across the entire Earth (expected to rise between 1,8 °C and 4 °C until the end of this century) inducing abrupt climate changes, related with the rise in severity and frequency of meteorological phenomena, such as heavy rains (that cause floods and sediment resuspensions with toxics), long snowfalls, prolonged droughts and water shortage, increased wildfires, soil erosion, among others, which occur all over the planet.

Thus, climate changes can affect water chemistry (namely the acidification of the oceans), toxics bioavailability and effects (remobilization and transport of the sediments releasing toxic compounds), biotransformation of contaminants and the variation of food availability for aquatic organisms. On the other hand, the amount of water dissolved oxygen decreases, which disturbs basic fish metabolism. All these factors together will have severe implications in the majority of aquatic organisms, in particular to ichthyofauna.

The limits of fish tolerance at higher temperatures are lower, and even more in the presence of toxic compounds (stress on stress), both together can cause genetic, biochemical, histological and physiological problems, with impacts in the development, reproduction and behaviour. Therefore, histopathological lesions are considered good biological markers for environmental biomonitoring studies under exposure of cocktails of pollutants or simple compound that can be evaluated in the gill, liver, gonad, kidney and intestine of fishes.

Therefore, complex interactions occurs between toxics and water chemistry parameters changes with impacts in fish. This synergy is tricky to be studied and understood, but it is important to take that into account in biomonitoring studies as low non-effective level of pollutants can caused severe impacts in wildlife under climate changes – stress on stress response. For riverine fish that problem is of major concern under abnormal variations in the river flow all along the year, and its impacts in water flow velocity and consequences in water physicochemical properties, increasing fish perturbations at different biological levels.

**eywords:** climate changes; rivers; toxicity; fish; histopathology biomarkers

## **Preliminary behavioral data in Mozambique tilapia (*Oreochromis mossambicus*) under the combined effect of copper sulphate and temperature**

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### **Abstract**

Climate changes are a big problem not only for human activities but also for wildlife survival, namely aquatic organisms, including fish, though the alteration of the behaviour, development and reproduction. Copper sulphate (CuSO<sub>4</sub>) is a versatile chemical extensively used in industry, agriculture and fish farming. Likewise, the objective of this study was to evaluate behavioural responses of animals exposed to CuSO<sub>4</sub> at different concentrations and temperatures.

In this study, 42 Mozambique tilapia (*Oreochromis mossambicus*), divided in six aquariums were exposed for 28 days long to CuSO<sub>4</sub> at concentrations of 1,1 mg/L (*lc*) and 3,6 mg/L (*hc*) at two different temperatures: 25°C (*T1*) and 32°C (*T2*). Behavioural observations (5 min each) were made twice a day during the exposure period.

At the middle of the experiment (14 days), all exposed fish present slow swimming, mainly fish under higher temperature (*T2*). On the 22<sup>nd</sup> day of exposure, in *T2/hc*, most of the fishes were nipping at the surface (trying to get more oxygen) or stationary on the bottom. In *T1/hc* fish were mostly stationary on the bottom while in *T2/lc*, higher numbers of fish (near 50%) were nipping at the surface. These preliminary data show that *hc* leads to more animals (around 60%) mumbling in the water column.

As overall conclusion, we can say that fish exposed to copper and higher temperature present considerable changes in the social and reproductive behaviour, like reduced dominance and aggression display (presenting normal silvery colour compared with black colour of bigger dominant male – alpha male), lower frequency of courtship acts, reduced swimming activities and feeding, which can affect the survival of the fish. Video-record tracking will be evaluated to obtain more accurate data and results.

**Keywords:** temperature; copper sulphate; tilapia; behavior; interaction

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