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# Julius-Kühn-Archiv

Jens Jacob, Alexandra Esther (Editors)

## 8<sup>th</sup> European Vertebrate Pest Management Conference

Berlin, Germany, 26-30 September 2011

- Book of Abstracts -



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Erwin-Baur-Str. 27, 06484 Quedlinburg,

Tel.: 03946 47-200, E-Mail: [GFF@jki.bund.de](mailto:GFF@jki.bund.de)

Internet: <http://www.jki.bund.de/> Bereich "Über das JKI"

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**Editors:**

Jens Jacob and Alexandra Esther

Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppheideweg 88, 48161 Münster, Germany

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## **Preface**

Following the tradition of biennial meetings, that was established with the 1<sup>st</sup> European Vertebrate Pest Management Conference in 1997 in York, UK, the 8<sup>th</sup> European Vertebrate Pest Management Conference aimed to provide a platform for scientists, management staff and legislators involved in vertebrate pest issues.

The 2011 meeting was organized by the Vertebrate Research Group of Julius Kühn Institute, Federal Research Centre for Cultivated Plants and the Faculty of Agriculture and Horticulture of Humboldt University. The organizers thankfully acknowledge the financial support provided by the Federal Ministry of Food, Agriculture and Consumer Protection, the Federal Ministry of Health, the Federal Environment Agency, the German Phytomedical Society, and the German Research Foundation.

Former meetings have been held in York, United Kingdom (1997), Braunschweig, Germany (1999), SeKibbutz Ma'aleh Hachamisha, Israel (2001), Parma, Italy (2003), Budapest, Hungary (2005), Reading, United Kingdom (2007) and Lyon, France (2009). In Berlin 2011, circa 150 delegates met for a week of conferencing (about 130 talks and posters, 1 workshop), social interaction (conference tour) and discussions about the latest research, developments, opportunities and achievements in vertebrate pest management.

The organizers paid special attention to include presentations from countries beyond Europe and paid particular attention to countries with developing and emerging economies. As a result, participants from 25 European countries and 13 countries from Africa, Asia, Australia, north-America and south-America contributed talks and posters.

The presentations were held in 9 symposia on fertility control, invasives, birds, new tools incl. a workshop, mammal population dynamics, rodenticide resistance, vertebrates in developing/ emerging countries, wild boar, and zoonoses. The 130 conference contributions are summarized in this book of abstracts. In addition, a special issue of the international scientific journal *Pest Management Science* will be dedicated to the 8<sup>th</sup> European Vertebrate Pest Management Conference. Publication of the special issue will be in early 2012 and contain full papers of about 20 invited, peer-reviewed articles.

All summaries in this book of abstracts are available online via [www.jki.bund.de/en](http://www.jki.bund.de/en) and can also be accessed through the conference website [www.evpmc.org](http://www.evpmc.org).

The abstracts presented here demonstrate the diversity of regions, economic systems, and vertebrate species that matter in the context of vertebrate pest management. It seems that vertebrate damage to European agriculture, forestry, horticulture and infrastructure exceeds 1 billion € per year. This is likely to increase because the value of many crops is growing as a result of rising global demand for food, timber and bio-fuel. The monetary loss due to vertebrate borne disease in Europe is barely known.

There is a range of management methods available to minimize vertebrate damage in plant production, health protection and conservation. The major challenges for the future are 1) the development of management approaches that are environmentally benign, effective and economical and 2) to maintain and expand the diversity of methods in the management tool box to appropriately control problems caused by overabundant vertebrates.

The next European Vertebrate Pest Management Conference will be held in Finland organized by Otso Huitu and Heikki Henttonen of the Finnish Forest Research Institute. We are looking forward to the meeting in 2013 in northern Europe.

We sincerely thank all colleagues that were involved in organizing the 8<sup>th</sup> European Vertebrate Pest Management Conference. We are indebted to organizers of the symposia, the plenary speakers and all other presenters. Thank you for making the conference such a productive and enjoyable meeting.

Münster, September 2011

Jens Jacob and Alexandra Esther

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## What we know and don't know about invasive vertebrates in Europe

Jeschke, J.M.

Ludwig-Maximilians-University Munich, Department of Biology II, Ecology, Planegg-Martinsried, Germany,

jonathan.jeschke@gmx.net

Cary Institute of Ecosystem Studies, Millbrook, NY 12545, USA

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### Introduction

Invasive species are those that have been introduced to regions beyond their native range, established in the wild, and spread substantially from their point of introduction (Blackburn et al., 2009; Lockwood et al., 2007). They can diminish biodiversity, introduce diseases, and cause further ecological problems and economic costs (Kettunen et al. 2009). Although a few early publications about invasive species date back to the 19<sup>th</sup> century (Darwin, 1859; reviewed in Cadotte, 2006), publications have only become numerous since the late 20<sup>th</sup> century (Richardson and Pyšek, 2008). Many hypotheses about invasive species have been proposed, but only recently has it become possible to test them adequately, as sufficient studies and data were not available before. A prominent example for a project that collected data on invaders in Europe is DAISIE (2009). With sufficient studies and data now available, invasion biology has entered a new stage where existing hypotheses can be rigorously tested, and those that fail these tests may be modified or replaced. Another challenge that invasion biology is currently facing is the need to build bridges with related disciplines (Davis, 2009). To illustrate these challenges and their implications for invasive species management (Clout and Williams, 2009), I outline to what degree we can currently answer the following questions, which have guided research on invasive species for decades (Drake et al., 1989): (1) How many species become invasive in a given region? (2) Which species become invasive? (3) Which regions are especially susceptible to invasive species? I will focus on invasive vertebrates in Europe, but for comparison, I will also mention other taxonomic groups and continents.

Keywords: biotic resistance hypothesis, exotic species, fast life histories, invasion biology, non-native species, propagule pressure, tens rule

### How many species become invasive in a given region?

If policy makers are faced with the question whether or not to invest in border controls against accidental species introductions, they need to know what fraction of introduced species will become invasive. This fraction can then be used to parameterize cost-benefit models. A hypothesis that predicts this fraction is the tens rule, which says that of 100 introduced species, about 10 will establish themselves and 1 will become invasive (Williamson, 1996; Williamson and Brown, 1986). Recent studies, however, suggest that this hypothesis lacks empirical support, especially for vertebrates (Jeschke, 2008; Jeschke and Strayer, 2005).

### Which species become invasive?

If a company plans to introduce a vertebrate species, e.g. for food production, it would be helpful to have a probability estimate that this species will become invasive. Having this goal in mind, invasion biologists are looking for the characteristics of invasive species that discriminate them from other species. There are many hypotheses about the characteristics of invasive species. A classical hypothesis says that species with a fast life history are more often invasive than species with a slow life history, i.e. invasive species tend to reproduce early, have a high fecundity, and a short lifespan (Lodge, 1993). Among mammals and birds, species with larger brains are supposedly more successful in invading new environments, due to their supposedly higher behavioural flexibility (Sol and Lefebvre, 2000; Sol et al., 2008). Besides these rather species-specific traits, there are also traits that characterize the association of species with humans and that also potentially influence invasiveness. Such traits were largely ignored in early studies on biological invasions but are now in the focus of many researchers. One such trait is propagule pressure, a composite trait that reflects how often individuals of a given species are introduced to a given region, and how many individuals are introduced each time (Blackburn et al., 2009; Lockwood et al., 2005). Recent studies about invasive vertebrates suggest that propagule pressure and other traits

characterizing their association with humans are stronger determinants of invasiveness than life-history traits, brain size, and other species-specific traits (e.g. Jeschke and Strayer, 2006).

### **Which regions are especially susceptible to invasive species?**

Comparing different regions, e.g. different European countries, it is apparent that some regions host more invasive species than others. The classic idea to explain these differences is the biotic resistance hypothesis which says that regions with relatively low biodiversity and high human impact are more susceptible to invasions than regions with relatively high biodiversity and low human impact (Elton, 1958). However, recent studies of invasive vertebrates do not support this hypothesis (Chiron et al., 2009; Jeschke and Genovesi, 2011; Leprieur et al., 2008).

### **Conclusions**

Many hypotheses about invasive vertebrates and other invaders lack empirical support. They are thus potentially misleading when designing management strategies against invaders. It is time to revise these hypotheses.

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## **Ungulate impact on forest regeneration and dynamics and its implications for forest management and conservation – long-term data from Bialowieza primeval forest, Poland**

Jędrzejewska, B.

Mammal Research Institute, Polish Academy of Sciences, 17-230 Białowieża, Poland, bjedrzej@zbs.bialowieza.pl

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A natural food chain found throughout European forests includes three trophic levels: forest plants, ungulates, and large carnivores. However, the natural structuring of that system has been affected by humans for so long and in so many ways that nowadays the prevailing notion of the ungulates' role in forests is that of damage agents to tree regeneration. One of the very few places that could help us understand the original role of ungulates in forest regeneration and dynamics is Białowieża primeval forest (Poland), which preserves the last remnants of European temperate forests with five species of ungulates (red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, moose *Alces alces*, European bison *Bison bonasus* and wild boar *Sus scrofa*) and two species of large predators (wolf *Canis lupus* and Eurasian lynx *Lynx lynx*). Historical and contemporary human impact on Białowieża primeval forest have been diverse but much smaller compared to other European woodlands.

The impact of ungulates (mainly red deer, the species dominating in the community) on regeneration and dynamics of natural forests of Białowieża primeval forest was investigated at three time scales: 10-year experimental study, 70 years of observational study, and 150-200-year data on forest, ungulate, and carnivore inventories. The experimental study demonstrated that ungulates strongly affected the density and species diversity of tree regeneration in height class >50 cm. Data on 70 years of natural dynamics in tree recruitment showed that total recruitment of all tree species was negatively correlated with ungulate density. Yet, the variation in response among tree species was related to the preferences of herbivores: the most preferred forage species, hornbeam *Carpinus betulus*, showed high recruitment in periods of abundant deer, due to its browsing-tolerance. In the longest perspective (150-200 years), ungulate impact on forest dynamics was analyzed together with other factors such as climate change, human-caused changes in wolf and lynx densities (major agents of ungulate mortality) and forest fire history. At that longest time-scale, the primary force driving the observed changes in dynamics and species composition of Białowieża primeval forest was the abrupt decline in forest fires in the early 19<sup>th</sup> century. It triggered the change from *Pinus*-dominated forests (18<sup>th</sup> century), to *Picea*-dominated stands (1850-1950), to deciduous (*Carpinus*, *Tilia*) forests (after 1950). Ungulates appeared a secondary – but still important – agent modifying the densities and species composition of tree regeneration and recruitment. All data evidenced a highly dynamic and variable time structure of natural forests, in contrast to the rather static view which prevails in both the silviculture and the conservation approach.

## **Fertility control for invasive pest mammals – Fare we making progress?**

Hinds, L.A.

CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, ACT 2601, Australia, lyn.hinds@csiro.au

Invasive Animals CRC, University of Canberra, ACT, Australia

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Management of exotic or native invasive mammals at a landscape scale is complex and time-consuming. Current conventional techniques require repeated application and may or may not be species specific, and some methods (shooting, poisoning) are becoming increasingly unacceptable to the general public, particularly in urban and peri-urban environments. Fertility control was proposed as another potential tool for vertebrate pest management more than 3 decades ago. Since then the approach has gained public acceptance because it is perceived as a more species specific and humane approach. However, so far, no fertility control products have been developed which can be easily delivered to wildlife populations on a broad-scale. Ideally, a fertility control agent needs to induce permanent sterility leading to reduced recruitment in the pest population. It also must be easily delivered to reach an appropriate proportion of the target population, be species specific with minimal side-effects (behavioural or social structure changes), and be environmentally benign and cost effective.

Reproductive targets for fertility control include disrupting either the hormonal feedback associated with the hypothalamic-pituitary-gonadal axis, the function of the gonads, fertilisation, and/or implantation. Later stages of pregnancy and lactation could also be targeted but these raise animal welfare concerns.

The use of steroidal (e.g. synthetic progesterone) and non-steroidal hormone implants (e.g. agonists against gonadotrophin releasing hormone, GnRH) to disrupt hormonal regulatory feedback has been quite successful. However, their delivery is problematic and expensive at the population level because each individual must be captured for treatment.

Another approach has been the development of immunocontraceptive vaccines in which the body's immune response targets a self hormone (e.g. GnRH) or other reproductive antigen (such as follicle or egg coat proteins, sperm proteins, implantation or other uterine or oviduct proteins). While GnRH and porcine egg coat (zona pellucida) injectable immunocontraceptive vaccines have been shown to be very effective, their delivery also requires individual capture and, in some cases, booster immunisations. Remote delivery using darts has been successful for some vaccines, but cost and time are limiting application at the population level.

Using viruses to deliver immunocontraceptive vaccines was extensively researched for rabbits, foxes and mice in Australia, but this work ceased for technical reasons. In New Zealand, recombinant vaccinia virus is being assessed for delivery of disease vaccines and immunocontraceptive vaccines for possums.

Many plant extracts have been screened for their effects on gonadal function, implantation and/or the subsequent progress of a pregnancy. Some effects (abortions, suppression of lactation) raise welfare concerns. However, the main problem is the rapid reversibility of their effects after treatment ceases, and poor palatability at the required doses.

Chemosterilants have always been of interest, with one chemical, 4-vinylcyclohexene diepoxide (VCD) currently being tested for its sterilising effects in rodents. VCD causes depletion of ovarian primordial follicle populations, but it is not species specific and requires delivery over a prolonged period (>10 days). Although formulation for oral delivery is likely to be feasible, the challenge remains to specifically target the chosen pest species at a population level. The inclusion of a plant extract in food baits which also contain a chemosterilant could enhance the effects of both agents and lead more rapidly to infertility. Ovarian-specific phage peptides may also have potential here.

Oral delivery of any of the above agents, particularly of immunocontraceptive vaccines, remains a major challenge – protecting the reproductive antigens from degradation in the gut, stimulating uptake via mucosal immune sites and generating sufficient antibody responses to inhibit reproductive processes is extremely difficult.

Will fertility control work in all species? Certainly the potential of fertility control as a management tool is considered high for species with high fecundity, high natural adult mortality rates and rapid turnover and where the effects of sterilisation may exceed an increase in juvenile or adult survival due to a lowering of birth rates.

It could also be used to prevent or reduce population growth after other techniques have been applied to reduce numbers, particularly in long-lived species. If only fertility control was applied in long-lived species, then until natural mortality reduced population size, the infertile animals would continue to cause as much impact as fertile animals. This would not be of value to the land manager in the short term.

In conclusion fertility control has potential and obviously appeals to the public as a humane approach. However, considerably more needs to be done to develop fertility control approaches which can be delivered efficiently and cost effectively at a population level.

## **Eradication of invasive birds from tropical oceanic islands: lessons learned from studies of common mynas *Acridotheres tristis***

Feare, C.<sup>1</sup>, Greenwell, P.<sup>2</sup>, Edwards, H.<sup>3</sup>, Taylor, J.<sup>4</sup>, Van der Woude, J.<sup>5</sup>

<sup>1</sup>WildWings Bird Management, 2 North View Cottages, Grayswood Common, Haslemere, Surrey GU27 2DN, UK, feare\_wildwings@msn.com

<sup>2</sup>13, Tuscany House, Durdham Park, Bristol BS6 6XA, UK

<sup>3</sup>222 Buchanan Street, Balfour, G63 0TE, Scotland

<sup>4</sup>18 Bredbury Green, Romiley, Stockport, Cheshire SK6 3DN, UK

<sup>5</sup>Animal Ecology Group/BESO, Centre for Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC Groningen, Netherlands

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### **Abstract**

Many tropical oceanic islands host unique taxa that have evolved in isolation from non-indigenous influences. Some of these endemic taxa are now threatened by man-induced changes that include the introduction of predators, competitors, vegetation and seed dispersers. While considerable progress has been made to eradicate alien mammals from islands, methodologies for the eradication of alien birds lag behind. This paper will discuss findings of recent and on-going attempts to develop eradication techniques for common mynas *Acridotheres tristis*, invasive birds that have been widely introduced on tropical oceanic islands and are suspected of negative impacts on endemic and indigenous fauna and flora.

During the course of these studies, features of common myna behavior and demography on small islands have been discovered that can influence preferred control methodologies; these discoveries also highlight the lack of available basic knowledge of alien invasive bird biology at the inception of most eradication attempts. On Denis Island, Seychelles, mensural and age/sex data have been collected from all caught mynas, revealing new demographic information about the population on this small island, and supporting inferences made from indirect information elsewhere. Experiences with different control methods will be described, highlighting their benefits and disadvantages and how these may vary between island types, and possible risks to endemic taxa from their use.

These studies have revealed fundamental requirements for the eradication of populations involving hundreds of mynas and will contribute to the further development of appropriate methodologies, but how widely applicable they will be for other invasive bird species remains to be determined.

Keywords: *Acridotheres tristis*, common myna, eradication, invasive birds, tropical oceanic islands

### **Introduction**

Common mynas have been introduced, deliberately or accidentally, to many oceanic islands in the tropics. In addition to damaging crops, they are alleged, with some evidence, to have negative impacts on some endemic bird species and to be involved in the dispersal of plants, especially alien invasives. Techniques are needed to eradicate common mynas where they are demonstrably compromising biodiversity.

### **Materials and methods**

Traps and toxicants have been investigated as potential eradication methods on some islands in the Indian and Atlantic Oceans, and inferences have been made from behavioral and morphological data obtained from living and dead mynas on the efficacy of different control techniques and on the demography of small island populations.

### **Results**

Trapping, using a variety of techniques, appears to be the most efficient technique for achieving a sustained depletion of numbers, can be selective and provides large samples of birds for subsequent analysis. The avian toxicant 'Starlicide' is toxic to common mynas but it promotes bait aversion after repeated exposure and has even been seen to promote aversion to the site where most birds on one island

exhibited symptoms of illness before dying. The toxicity of Starlicide to non-target taxa, especially herpetofauna, is unknown.

Various measures of population age structure suggested that myna productivity on small tropical islands is low but we still do not know whether reduction in numbers relieves constraints on productivity. On Denis Island, Seychelles, > 90 % of the myna population has now been removed by trapping and the impact of this removal on the productivity of endemic birds will be assessed.

### **Discussion**

These projects have revealed fundamental requirements for the eradication of populations involving hundreds of mynas: adequate highly motivated staff devoted to eradication, unqualified open-ended support of island owners, and the acquisition of demographic and behavioral data to improve eradication prospects of these projects and future attempts elsewhere. These studies will contribute to the development of island myna eradication methodologies but how widely applicable they will be for other invasive bird species remains to be determined.

## Retrieving and retaining older and advancing novel rodenticides-as alternatives to anticoagulants

Eason, C.T.<sup>1,4</sup>, Henderson, R.<sup>2</sup>, Murphy, E.<sup>3</sup>, Shapiro, L.<sup>4</sup>, MacMorran, D.<sup>4</sup>, Blackie, H.<sup>1</sup>, Brimble, M.<sup>5</sup>, Conole, D.<sup>5</sup>, Rennison, D.<sup>5</sup>, Gibson, T.J.<sup>6</sup>, Gregory N.G.<sup>6</sup>

<sup>1</sup>Centre for Wildlife Management and Conservation, Faculty of Agriculture and Life Sciences, Department of Ecology, Lincoln University, New Zealand, charles.eason@lincoln.ac.nz

<sup>2</sup>Pest-Tech, Leeston, New Zealand

<sup>3</sup>Department of Conservation, Christchurch, New Zealand

<sup>4</sup>Connovation Ltd, Auckland, New Zealand

<sup>5</sup> University of Auckland, Auckland, New Zealand

<sup>6</sup>Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, UK

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### Abstract

Anticoagulant compounds are likely to play an important role in the control of commensal rodents for crop protection and conservation for the foreseeable future. However there are concerns regarding their persistence and the development of more widespread resistance. We are seeking to retrieve and retain older alternatives and develop novel rodenticides. Our three pronged approach is firstly to improve the performance of older non-anticoagulant rodenticides such as zinc phosphide, secondly to optimise the performance of 1<sup>st</sup> generation anticoagulants and thirdly to identify alternatives to anticoagulant rodenticides with the same mode of action as paraminopropiophenone (PAPP), which was registered in New Zealand as a predacide in April 2011.

Keywords: anticoagulants, paraminopropiophenone, synergists, zinc phosphide

### Introduction

The most prolific period of rodenticide development occurred between the 1940s and the 1980s. First generation anticoagulant rodenticides and zinc phosphide were developed in the 1940s, 50s and 60s, with cholecalciferol, bromethalin and second generation anticoagulant rodenticides developed in the 1970's and 80's, partly to overcome resistance to the less potent anticoagulants (Buckle and Smith, 1994). During this period it was recognised that it was important to have two classes of rodenticides, both anticoagulants and alternatives to anticoagulants. In recent times the need for toxicants for field use that are effective but less persistent than second-generation anticoagulants, and therefore likely to be less hazardous to non-target bird species and other non-target species has been highlighted. Ironically registration requirements in Europe and around the world have reduced the number of options available for rodent management. We believe it is important to retain and refine the use of rodent control tools for conservation, disease control and agricultural protection and develop new alternatives to anticoagulants. Ideally alternatives to existing anticoagulants would combine limited persistence and humaneness, however this is a significant challenge. A microencapsulated form of zinc phosphide has been developed and a low dose of cholecalciferol combined with diphacinone or coumatetralyl is being re-evaluated (Eason et al., 2010a) to provide three low residue alternatives. In April 2011 para aminopropiophenone (PAPP), a methaemoglobinaemia inducer was registered for the control of predators in New Zealand. PAPP is humane in its mode of action and does not bioaccumulate. It has an antidote and is highly toxic to species like stoats (Eason et al., 2010b) but unfortunately not toxic to rodents. Approximately 50 compounds with the same mode of action including analogues of PAPP have recently been screened to assess their potency as rodenticides.

### Methods

Groups of caged rats have been presented with a microencapsulated form of zinc phosphide containing 1.5% in a palatable paste bait. Coumatetralyl (0.03%) combined with cholecalciferol (0.015%) and diphacinone (0.05%) also combined with cholecalciferol (0.015% and 0.03%) have been tested on caged rats in the same bait matrix. Analogues of PAPP have been screened for their potency as rodenticides. *In vitro* work was carried out using a methaemoglobin assay involving hepatic microsomes and rat erythrocytes. The toxicity of the most promising candidates from the *in-vitro* screening has recently been assessed *in vivo* in laboratory rats by oral gavage.

## Results

A microencapsulated form of zinc phosphide containing 1.5% has been shown to be 100% effective in caged rats. A combination of coumatetralyl (0.03%) and cholecalciferol (0.015%) has also been confirmed as having high potency in rats and similar to that achieved by diphacinone (0.05%) and cholecalciferol (0.15%). Diphacinone (0.05%) was partially effective as a single dose rodenticide when combined with cholecalciferol (0.015%) and more effective when combined with a higher dose of cholecalciferol (0.03%). PAPP and sodium nitrite have been developed as vertebrate pesticides in New Zealand and Australia (Eason et al., 2010b). In laboratory rats neither compound is sufficiently potent to be an effective rodenticide. Approximately 50 compounds with the same mode of action including analogues of PAPP have been screened for their potency as rodenticides. This screening has identified a compound with an LD50 of approximately 40-50mg/kg. Further derivatives of this more potent analogue are being synthesized and screened.

## Conclusion

It has been suggested that product innovation needs to be stimulated to encourage alternatives to the current suite of rodenticides, as a number of these are associated with secondary poisoning or bioaccumulation or they are viewed as inhumane (Mason and Littin, 2003). We have advanced an improved formulation of zinc phosphide and are confirming the synergistic effects of cholecalciferol when co-administered with first generation anticoagulants. These developments may provide partial solutions and help provide products that break the cycle of rodenticide resistance. However to produce completely new rodenticides a new level of innovation is needed. Our current approach is to attempt to build on the platform created by PAPP. We are part way through a programme of research, development and registration activity and further in vitro and in-vivo testing is scheduled over the next 3 years on novel candidates as well as field trials. Any new tools that emerge would most likely need to be integrated with anticoagulant rodenticides which are likely to play an important role in the control of rodents for the foreseeable future.

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## Current situation of human vector-borne diseases in European wildlife

Zeller, H.

European Centre for Disease Prevention and Control, Stockholm, Sweden, herve.zeller@ecdc.europa.eu

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Several human diseases originate from wildlife, and some of them are of major public health importance. Wildlife may act as a reservoir of human pathogens as shown in the last decades with the emergence of Nipah or SARS diseases in Asia involving bats as reservoirs or the re-emergence of West Nile fever (WNF) or Crimean-Congo Hemorrhagic fever (CCHF) in Europe. Human diseases originating from wildlife can be divided in two groups: diseases contracted by humans by direct or indirect (excreta) contact with wild animals and diseases transmitted to humans by an arthropod vector as mosquito, tick or biting midge previously infected from wildlife (arthropod-borne diseases). Transmission of pathogens from wildlife to domestic animals and then humans may also occur.

A coordinated approach for vector-borne disease surveillance, prevention and control in Europe was initiated to identify potential threats for humans by the European Centre for Disease Prevention and Control (ECDC), which is in charge of risk assessment of communicable diseases. ECDC provides financial support to an European network of laboratories for emerging viral diseases (ENIVD) for an early detection of human pathogens and a network of medical entomologists (VBORNET) which provides information about the present distribution of arthropod vectors and surveillance activities undertaken in Europe. In addition, ECDC initiated collaboration with the European Agency for Food Safety regarding risks linked to wildlife.

Prevention and control of human diseases originating from wildlife require a multi-disciplinary approach to understand the mechanisms of transmission of pathogens and determine predictive indicators of potential (re)emergence of pathogens. Environmental and human behavior changes can increase the human-wildlife interface and the risk of transmission of pathogens. Surveillance of absence or presence or variations of incidence of pathogens in wild animals would be appropriate to better define the potential risks of transmission to humans.

Currently several vector-borne diseases are under surveillance in Europe but most of these disease are reported under passive surveillance and information provided by the Member States are published in epidemiological reports. Other diseases are under more active surveillance such as WNF or CCHF with an early reporting information system of human cases. Surveillance of wildlife involved in the maintenance of these viruses *in natura* (birds for WNF virus, lagomorphs, rodents or birds for CCHF virus) is difficult. Detection of presence of virus in arthropods (mosquitoes for WNF, ticks for CCHF) can be performed but cost-effectiveness and sustainability are questionable. The use of sentinel birds for WNF or domestic ungulates for CCHF can be an alternative to monitor active transmission in areas where competent arthropod vectors are present. Hantaviruses which cause hemorrhagic fever with renal syndrome in humans are transmitted by rodents *Apodemus* and *Myodes* sp. Monitoring of rodent population dynamics and hantavirus excretion may provide useful information about any increasing risk of transmission to humans but this is not used for an early warning system.

In Europe several studies of wildlife to detect the distribution of pathogens of human importance are undertaken. Other projects e.g. the EDENext FP7 project - *Biology and Control of vector-borne infections in Europe* will provide the understanding and modelling the mechanisms of introduction, establishment, and spread of vectors and human vector-borne diseases, and the improvement of intervention and control strategies for vector populations for the benefit of partners such as human and veterinary public health agencies and other groups involved in risk assessment, control and prevention.

## Alien mammalian species in Russia: ancient and modern invasions

Khlyap, L.

AN Severtsov Institute of Ecology and Evolution of the RAS, 33 Leninskij prosp., Moscow, 119071, Russia, khlyap@mail.ru

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### Abstract

There are about 70 alien species of mammals in Russia. Some species had occupied wide ranges before the 19th century, but most ones have penetrated into the new areas in the 20th century. Ancient invasions (before the 19th century) are known for rodents of human settlements (*Mus musculus*, *Rattus rattus*, *Rattus norvegicus*) and arable lands (*Microtus levis*, *Microtus arvalis*). In the European part of Russia these rodents are now common animals that cause harm to humans. In some regions they are continuing to expanding their ranges. The modern invasions are usually caused by human activities, and to a lesser extent by climate change. About half of the modern invasions represents self-spreading, intentional introductions constitute 23%, 16% are reintroductions, and 13% are accidental introductions. In the second half of the 20th century intentionally introduced mammals: (*Neovison vison*, *Ondatra zibethicus*, *Nyctereutes procyonoides*) occupied the largest areas. Among the self-spreading mammals, the greatest enlargements in geographical range were by *Sus scrofa*, *Martes foina*, and *Pipistrellus kuhlii*. These species have high reproductive potential and the ability to make long-distance movements. The distributions of invading mammals in Russian territory in the second half of the 20th century are illustrated in maps.

Keywords: alien species, ancient and modern invasions, mammal, Russia

### Introduction

There are about 70 species of mammal that are invasive aliens in different regions of Russia. Some had occupied extensive ranges before the 19th century (archeoinvaders), but most ones have penetrated into the new areas in the 20th century (neoinvaders). The aim of our research is to identify the causes of ancient and modern invasions of mammal species to help prevent further invasions.

### Materials and methods

The main criterion used to classify species as alien is an extension of their area, that is, the appearance of a species in places that it did not inhabit previously. Distribution maps, including original GIS Rodent and Pika Populations of Russia (<http://www.sevin.ru/vertebrates/>; Tupikova et al., 1999; Khlyap et al., 2000), and literature data were used. Additionally, information was received from 37 Russian biosphere reserves that are located throughout the country and have a long history of protecting biodiversity and monitoring different environment components. Some results of analysis of these materials have been published (Bobrov et al., 2008; Khlyap et al., 2010; Khlyap and Warschavsky, 2010; Neronov et al., 2008).

### Results

Ancient invasions (before the 19th century) are known for rodents of human settlements (*M. musculus*, *R. rattus*, *R. norvegicus*) and arable lands (*M. levis*, *M. arvalis*, and perhaps *Apodemus agrarius*). Stable populations of house mice and black rats existed in modern Russia in ancient times (BC) in the ancient states located in the northern Caucasus, at the mouth of the Don and the adjacent coasts of Azov Sea. In late 18th century they occupied most of the European part of Russia and *M. musculus* had penetrated to the south of Eastern Siberia. *R. norvegicus* penetrated the European part of Russia in the 17th century, occupied most of it by the early 19th century and reached Western Siberia at the end 19th the century. Agrophilic rodents reached the boundaries of their geographical range at the end of the 19th century. In some regions synanthropic and agrophilic rodents are expanding their ranges even today. Range expansions of all other alien mammal species (neoinvaders) have occurred since the mid 19th century. The modern invasions are usually caused by a desire to transform the fauna (intentional introduction, 18 species); to restore endangered species (reintroduction, 15 species); human environment transformations

and (rarely) climate changes (self-spreading, 42 species); casual delivery, domestic animals and mammals that escaped from captive (accidental introduction, 9 species).

### Discussion

A few species of mammals were invasive in ancient times, particularly synanthropic and agrophilic rodents. Synanthropic rodents are accidental introduced mammals. They settled over all continents accompanying people. Expansions of agrophilic rodents occurred with increasing areas occupied by crops. The modern ranges of agrophilic rodents are limited by the placement of arable land (Neronov et al., 2001). In the European part of Russia these rodents are now common animals which cause harm to humans. The number of neoinvader species is an order of magnitude greater. Most are self-spreading species. This means that the most important factor for modern invasions is an anthropogenic transformation of the environment. In the second half of the 20th century the largest areas were invaded by the intentionally introduced mammals: *N. vison* (Fig. 1), *O. zibethicus*, and *N. procyonoides*. Among the self-spreading mammals, the greatest enlargements in geographical range have been by *S. scrofa*, *M. foina*, *P. kuhlii*. These species have high reproductive potential and the ability to make long-distance movements.



**Fig. 1** Modern area of *N. vison* in Russia (by Khlyap et al., 2011)

Questions of limitations of mammal invasions are far from being solved. People can and should impose bans on the intentional introduction of mammals and restrictions on reintroductions. But much more effort is needed to curb self-spreading and accidental introductions of mammal species.

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## Turning back the tide of American mink invasion at unprecedented scales in partnership with communities

Lambin, X., Atkinson, S., Bryce, R., Davies, L., Gray, H., Oliver, M.K, Urquhart, J.  
School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, AB24 2TZ,  
Scotland, UK, x.lambin@abdn.ac.uk

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### Abstract

Successful eradications of harmful invasive species have been mostly confined to islands while control programs in mainland areas remain small, uncoordinated and vulnerable to recolonisation. We took an adaptive approach to achieve large scale eradication of invasive American mink in a mainland area in North East Scotland. Capitalising on the convergent interests of a diverse range of local stakeholders, we created a coordinated coalition of trained volunteers to detect and trap mink. Starting in montane headwaters, we systematically moved down river catchments, deploying mink rafts, an effective detection and trapping platform. Volunteers took increasing responsibility for raft monitoring and mink trapping as the project progressed. Within 3 years, all breeding mink had been removed from 10,570 km<sup>2</sup> with the involvement of 186 volunteers. Capture rate within sub-catchments increased with connectivity to mink in other sub-catchments and with proximity to the coast where there is more productive habitat. The main factor underpinning the success of this project was functional volunteer participation.

Keywords: adaptive management, *Arvicola terrestris*, Cairngorms, compensation, dispersal, genotyping, *Neovison vison*, participation

### Introduction

Eradication of invasive vertebrates has hitherto been restricted to islands, has nearly invariably been performed by paid professionals and has rarely been achieved on a significant scale in mainland areas. As a result, native biodiversity in mainland areas remains severely affected by nefarious invasive species. To allow the recovery of threatened native species, innovative management strategies are required to remove invasives from large areas durably. We describe the strategy adopted and achievements to date of a project that has implemented the largest mainland invasive species eradication effort worldwide and, through an adaptive management approach, use the convergent interests of local communities to maximum benefit to secure a pest-free area at such a scale as to considerably reduce recolonisation.

### Methods

The strategy of the project consisted of coordinating and optimising the efforts of an existing, local and skilled workforce with convergent interests to deliver coordinated, systematic sub-catchment by sub-catchment eradication and monitoring of American mink, so as to achieve maximum conservation benefit on a scale not previously attempted anywhere worldwide. A key component of the strategy was to promote the systematic use of mink rafts, floating platforms with a footprint-recording plate made of moist clay and sand under a wooden tunnel. Mink rafts record footprints and are designed to act both as a monitoring device and as a trapping site for American mink. Raft monitoring also provides feedback on the impact of trapping, which helps to motivate volunteers.

The strategy of the project was to expand mink control spatially and establish a ‘rolling carpet’, deploying rafts and recruiting volunteers to operate them in each sub-catchment, moving downstream from the headwaters of the 5 main river catchments that flow from the Cairngorms National Park but retaining the network of rafts behind the expanding control front to ensure detection and removal of immigrants. Our long-term management goal was to achieve sustainable catchment-wide removal of mink, hence creating suitable conditions for the recovery of the focal native species on a large scale by promoting ownership of biodiversity resources by local communities.

The project was initiated with only partial knowledge of upland mink populations. Specifically, we did not know how large an increase in mink trapping effort beyond the baseline level was required to bring about a sustained decline in the local mink population. We thus chose to use an adaptive management approach, with information gained in the early stages used to optimise the project’s conservation benefit,

sustainability and cost effectiveness. It was thus essential to systematically collect data from all aspects and participants of the projects to inform management. Volunteers were trained in the use of rafts. Project staff monitored mink rafts where volunteers were unavailable. Volunteers were instructed to set cage live-traps on rafts whenever mink footprints or sightings were recorded and/or contact a project officer or named volunteer to carry out trapping and dispatch of mink. Those willing were trained to dispatch mink humanely using air rifles or pistols of sufficient power. Culled mink were aged based on X-ray and cross-sectioning of canines. They were genotyped at twelve microsatellite (Zalewski et al., 2009) and parentage was reconstructed. From this, we inferred rich information on natal dispersal and compensatory immigration.

## Results

Within 3 years, the project removed 376 mink from 10,570 km<sup>2</sup> with the involvement of 186 volunteers. By the 4<sup>th</sup> year, the number of mink removed had exceeded 600 and 4 river catchment were effectively cleared of breeding mink (Bryce et al., 2011). The proportional contribution of volunteers to the project increased steadily over time. By the end of 3 years, volunteers monitored 86% of all rafts and caught 51 % of mink. The overall probability that a volunteer remained actively involved in the project per 6 month period was 86.8 % but this varied according to profession. Fisheries staff had the highest retention, game keepers had the lowest and the retention rates of wildlife conservation professionals, local residents and land managers varied over time with evidence of gradual improvement.

Mink capture rate within a focal sub-catchment was affected by the intensity of mink control and erosion of mink numbers in the rest of the catchment. Connectivity, reflecting the distance to and the number of mink remaining in surrounding sub-catchments, was the dominant effect predicting within sub-catchment mink capture rate, with proximity to the coast where there is more productive habitat also contributing. Genotyping and parentage assignment of culled mink revealed exceptionally long natal dispersal (mean=19 km max: 138 km), hence the need to work over very large scales and prevent mink born in lowland areas from impacting biodiversity in the uplands. Localised upland sub-catchments characterised by the presence of sheep hill farms and short swards suitable for rabbits had high ratios of immigrants (Oliver et al., 2009), highlighting how concentrating trapping efforts on those prey rich area and turning them into attractive sinks can negatively influence wider scale mink dynamics.

## Discussion

The main factor underpinning the success of this project was functional volunteer participation. Optimising the effectiveness of the volunteer workforce was thus central to the use of functional participation. The technical simplicity of mink raft method is conducive to its use in a community conservation project. Given the need to maintain a level of over-watch through the area cleared of mink to guard against a decreasing risk of reinvasion, it will remain essential that project officers continue supporting, motivating and engaging volunteers for the long term. A developing understanding of habitat selection by recolonising dispersers will help focussing long term monitoring as will the gradual expansion of the project area to cover 20,000 km<sup>2</sup> by 2014. The defining factors underpinning the success of the project are strong volunteer involvement, efficient and systematic methods of monitoring and control, an adaptive approach to suit local conditions, the strategic use of topography to minimise recolonisation and an ambitious vision; elements that are applicable to other invasive species and areas. It is a strong testament to what can be achieved when empowering local communities to take a stake in their local biodiversity and thus reason for optimism that the tide of invasion can be rolled back on a large scale where the convergent interest of local communities can be harnessed.

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## Aliens attack – population dynamics and density control of American mink *Neovison vison* in four National Parks in Poland

Niemczynowicz, A.1, Brzeziński, M.2, Zalewski, A.<sup>1</sup>

<sup>1</sup>Mammal Research Institute, Polish Academy of Sciences, Waszkiewicza 1c, 17-230 Białowieża, Poland, aniemczynowicz@zbs.bialowieza.pl

<sup>2</sup>Department of Ecology, University of Warsaw, Banacha 2, 02-097 Warsaw, Poland

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### Abstract

American mink (*Neovison vison*) is a semi-aquatic species, endemic to North America, which was introduced to the wild in Europe in the 1930s. In many introduced areas, the American mink is a significant predator of waterfowl and riparian mammals, leading to a marked decrease in their density. The aim of this study is to analyze adaptation of mink in their introduced range and to determine factors affecting population dynamics and colonization rate after eradication in the 4 National Parks of Poland (Biebrza, Narew, Warta Mouth and Drawa National Park). Studies have shown that it is possible to reduce the number of mink in protected areas and gain greater knowledge of the biology and ecology of invasive species. Fieldwork and trapping were conducted in each park over 5 days twice a year (March and November 2009, 2010 and 2011). In each park, trapping took place at two sites: an experimental area [EA] (from which the mink were removed) and a control area [CA] where mink were marked and released. Live-traps were set on the banks of rivers. During the spring and summer 20 rafts were deployed (10 in the EA and 10 in the CA in each park) for population monitoring and to determine the effectiveness of mink trapping. In total 248 mink were caught (125 in the CA and 123 in the EA). The ratio of males to females was 1.6:1. Preliminary results show large differences in morphological traits and population parameters between the various national parks. The average body weight of males and females from the West of Poland was 1.35kg and 0.6kg, respectively, and from the East of Poland 2.0kg and 0.8kg, respectively. The lowest density of mink was in the Drawa (2.5 inds./10 km watercourse), and the highest was in the Narew NP (9 inds./10 km watercourse). The rate of recolonisation was related to the time since trapping and density of the mink in the NP. The frequency of mink sign found in the EA after 6 months of mink trapping was similar to that in the CA, suggesting a fast recolonisation of the eradication area in all parks. Our results underscore the need for continuing research and further analysis to underpin eradication programs, as well as monitoring and controlling populations of invasive predators in protected areas.

Keywords: American mink, biometrics, invasion, monitoring, *Neovison vison*, Poland, recolonisation

## **Project Halo – predator control for native bird recovery in rural and urban areas**

Simmons, J.H.

Group Manager Biosecurity-Heritage, Waikato Regional Council, Hamilton, New Zealand,  
john.simmons@ew.govt.nz

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### **Abstract**

Project Halo's aim is to assist in habitat restoration and biodiversity. Within its initial years the focus was on increasing sightings of Tui within urban areas of Hamilton city. The reason initial an effort focussed on this species was because a viable breeding population was known to exist within a 20 kilometre winter feeding range of the bird. Research had shown that Tui, a nectarivorous species, do not need 'corridors' unlike other wildlife and they are known to commute into the city to feed on abundant exotic plants and, more increasingly with the assistance of volunteer groups, native plant species.

Keywords: habitat restoration, multi-agency approach, predator control

### **Introduction**

An intensive predator control programme was initiated in 2007 to facilitate the re-introduction of endemic song bird species back into Hamilton city. Named the Hamilton Halo Project (Project Halo), it was initiated by Waikato Regional Council's Biosecurity-Heritage Group and is aimed at bringing iconic native bird species, such as the Tui, Bellbird and Kereru back into the urban areas of Hamilton city. These three species are both important pollinators and dispersers of native plants, and highly valued by residents of the city for their bird song and theatrics. Recent studies had shown that tui nest success was only 27 percent, mostly due to high populations of ship rats in the Waikato. To increase the number of Tui visiting the city, summer breeding success needed to be enhanced within the 20 kilometre feeding range around Hamilton city.

### **Methodology**

In conjunction with the project's strategic partners – the Department of Conservation and Landcare Research – seven sites within a 20 km radius of Hamilton City were initially identified as being known tui nesting sites and ear-marked to receive intensive rat, possum and mustelid control. The control programme for these sites sees them receiving three year's predator control over a five year cycle. In 2009/10 six of the seven Halo sites, totalling 1,024 hectares, were controlled and achieved less than 5% rat tracking index over the bird nesting period. In addition to the intensive control being undertaken, other organisations and volunteer groups have undertaken planting of native trees over many years which assists in providing the Tui with an increased food source within Hamilton City.

### **Discussion**

Project Halo has been undertaking intensive predator control for a total of five years. The success of the control has been dramatic in terms of both nesting success, sightings in the city and in public enthusiasm and support. The public have been very proactive in contacting the Council with Tui sightings in and around Hamilton City. Public enthusiasm and support has been aided with Project Halo launching a social networking campaign in October 2009 by way of Facebook and Twitter. A recent survey was undertaken with 39% of the sites' active monthly users responding with positive feedback supporting the objectives of Project Halo.

The successful reduction of predators at breeding sites, through intensive control, led to Waikato Regional Council collaborating with Landcare Research and the University of Waikato in the release of 50 bellbirds at Hamilton Gardens within the city's bounds. Leg bands and transmitters were used to identify the birds for the first three weeks. The release occurred in May 2009 and attracted much media and public attention with reported sightings proving invaluable to the project post release. Public assistance again proved invaluable as the project team relied on sightings to track the birds with an unexpected and welcome side effect being previously unsighted unbanded bellbirds being reported.

The success of this project could not have been achieved without a multi-agency approach. The expertise of the Department of Conservation in undertaking many predator control and habitat restoration programmes combined with Landcare Research's extensive knowledge in the behaviour and ecology of the Tui and other native bird species has improved the chances of this project succeeding and its focus being broadened to include other native bird species.

Community volunteer groups are essential for continuing Halo objectives into the long-term. Throughout the seven control sites, bait stations networks have been installed and the likes of volunteer groups can supplement Council-funded control through refilling these stations. In addition, the ongoing native planting initiatives by other organisations and volunteer groups will result in providing an increased food source for the city's increasing populations of native birds.

## Spatial use and interaction of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in central Europe – competition or coexistence?

Drygala, F.<sup>1</sup>, Zoller, H.<sup>2</sup>

<sup>1</sup>Dep of Animal Ecology, University of Potsdam, Maulbeerallee 1, 14469 Potsdam, Germany, drygala@gmx.net

<sup>2</sup>Institute of Biodiversity, University of Rostock, Universitätsplatz 2, 18055 Rostock, Germany

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Keywords: habitat use, interaction, *Nyctereutes procyonoides*, population density, raccoon dog, red fox, spatial use, telemetry, *Vulpes vulpes*

### Introduction

Invasive alien species have many ecological effects and may threaten biological diversity. They can alter habitat, and prey on or compete with native fauna (e.g., Ebenhard, 1988; Hulme, 2007; Vilà et al., 2010). The raccoon dog is native to eastern Asia, but was introduced as a fur game species to the western parts of the Soviet Union in the 1930s-1950s. It is widespread in Northern and Eastern Europe, is still spreading in Central Europe (Drygala et al., 2010) and was recently listed in the top 100 most damaging invasive species in Europe by the DAISE project (<http://www.europealiens.org/speciesTheWorst.do>).

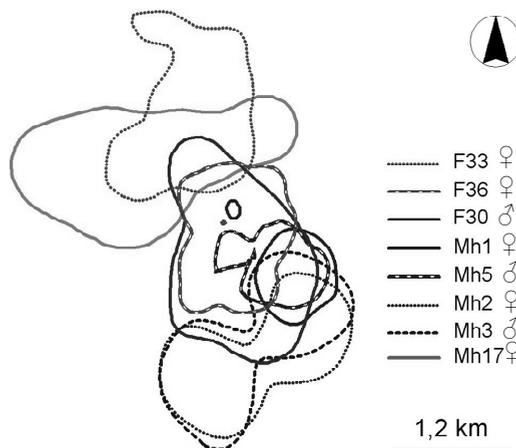
Between June 2004 and September 2006, we carried out a telemetry study in the intensively used agricultural landscape of northeast Germany (Mecklenburg-Western Pomerania, district of Güstrow) into inter-specific interference competition between red foxes and raccoon dogs. The non-native raccoon dog has been present throughout the area since the end of the 1990s. This is the first investigation on potential interaction of the two canids in Central Europe.

### Materials and methods

We used VHF-telemetry to record 6.627 location data from 15 red foxes and 20 raccoon dogs during the 28 month field study project. 23 stable home ranges (both MCP 100 and Kernel analyses) were calculated. We analysed home range sizes, home range overlap, interaction and habitat indices use using ArcView GIS 3.2a and Ranges6 v1.2.

### Results

The average annual home range sizes based on 23 seasonal home ranges (95% kernels) of red foxes and raccoon dogs were  $177.22 \pm 36.82$  ha (n=10) and  $161.09 \pm 74.62$  ha (n=13), respectively. The home ranges of the two species overlapped by up to 93% (Figure 1).



**Fig. 1** Home range shape (K95) and overlap for red foxes (F) (n=3) and raccoon dogs (Mh) (n=5)

We found slightly positive intra-specific interaction indices ( $I_i$ ) (Jacobs, 1974) for red fox ( $I_i=0.12$ ) and raccoon dog ( $I_i=0.13$ ) but a neutral inter-specific interaction index ( $I_i=0.01$ ). There was a significant ( $p=0.03$ ) difference between intra- and inter-specific interaction pattern. On the basis of estimated home range sizes and reproduction and mortality rates a population density of 3.1 red foxes/ km<sup>2</sup> and 3.4 raccoon dogs/km<sup>2</sup> was calculated in autumn. The raccoon dog differed significantly ( $p<0.05$ ) from the red fox in its use of habitat types ( $H_{pi}$ =habitat preference index), with preference of dense vegetation cover ( $H_{pi}=0.66-0.8$ ) and avoidance of open areas ( $H_{pi}=-0.58--0.79$ ). The red fox displayed significantly less preference for or avoidance of specific habitat types ( $H_{pi}=-0.33-0.27$ ). Unlike those of the red fox, the home ranges of the raccoon dog shifted significantly with changes in habitat type and state. Areas of maize and rape were only used intensively when the crops offered sufficient cover.

### Discussion

The population densities of both species are very high by international standards, and seem to result from the abundance of resources and different use of habitats that reflected both differences in food preferences and morphologically-determined differences in hunting and feeding strategies between species. There was no evidence of strong interference competition between the two canids. However, VHF- telemetry may not reveal specific behaviour patterns and predation of red fox on raccoon dogs (esp. juveniles) and vice versa, as recorded in Eastern Germany (Drygala unpublished data).

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## **The even darker side of the eastern gray squirrel (*Sciurus carolinensis*): a review of global introductions, invasion biology, and pest management strategies**

Huynh, H.M.<sup>1,2</sup>, Bertolino, S.<sup>3</sup>, Lurz, P.W.W.<sup>4</sup>, Koprowski, J.L.<sup>5</sup>, Williams, G.R.<sup>6</sup>, Thompson, C.W.<sup>1</sup>, McAlpine, D.F.<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, Texas Tech University, Lubbock, TX, 79409, USA, howard.huynh@ttu.edu

<sup>2</sup>Department of Natural Science, New Brunswick Museum, Saint John, NB, E2K 1E5 Canada

<sup>3</sup>University of Turin, DIVAPRA Entomology and Zoology Via L. da Vinci 44, 10095 Grugliasco (TO), Italy

<sup>4</sup>Lurzengasse 3, D-97236 Randersacker, Germany

<sup>5</sup>Wildlife Conservation and Management, School of Natural Resources and the Environment, 306 Biological Sciences East, University of Arizona Tucson, AZ, 85721, USA

<sup>6</sup>Department of Biology, Dalhousie University, Halifax, NS, B3H 4J1, Canada

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### **Abstract**

The eastern gray squirrel, *Sciurus carolinensis*, is one of the world's most recognized sciurids and a highly successful human commensal. Historically restricted to eastern North America, gray squirrel populations are now established in Italy, U.K., South Africa, and Australia, and squirrels continue to expand their geographic range globally. Successful introductions of *S. carolinensis* often result in significant negative impacts on native ecosystem integrity. As a result, countries have devised and implemented unique pest management strategies to reduce or eradicate *S. carolinensis* populations, but with differing levels of success. We review accounts of historical global introductions of *S. carolinensis*, discuss its invasive biology and impacts in non-native habitats, and recommend specific management strategies that should successfully curtail establishment of this species in non-native habitats.

Keywords: establishment, introduction, management, non-native, *Sciurus carolinensis* G.

### **Introduction**

The eastern gray squirrel (*Sciurus carolinensis* G.) is a highly adaptable arboreal sciurid native to eastern North America (Flyger, 1999). It is also a successful human commensal, and has established populations in non-native habitats around the world as a result of introductions by people (Bertolino, 2009). Here, we review its historical introductions, comment on why it has been such a successful invasive species, and recommend possible strategies and plans for effectively managing introduced populations of this species.

### **Materials and Methods**

Historical accounts from publications and reports were reviewed for data pertaining to *S. carolinensis* introductions around the world. Life history information was used to make inferences on the invasive species biology of gray squirrels, and to determine why they are such successful alien species. Current management plans were also reviewed to determine what elements of control were most effective.

### **Results and Discussion**

Introductions of *S. carolinensis* into non-native habitats around the world have occurred since the early 1800s. In North America, regional introductions were made into previously unoccupied Canadian provinces (e.g., Nova Scotia; Huynh et al., 2010) and numerous western U.S. states, while introductions also occurred in South Africa (Davis, 1950) and Australia (Peacock, 2009). The U.K. (Lloyd, 1983) and Italy (Currado et al., 1987) have had multiple introduction events associated with the successful establishment of *S. carolinensis*. Hence, it appears that successful establishment of *S. carolinensis* is partly dependent on high propagule pressure generated by repeated introductions by people.

Trophically, gray squirrels are ecological generalists. Their highly adaptable nature in terms of resource exploitation provides them with intrinsic advantages that promote population establishment and growth. For example, though *S. carolinensis* primarily inhabit broad-leaved, mixed deciduous woodlands with mast species, they are able to thrive on food sources found in conifer-dominated stands (Koprowski, 1994). Such adaptable foraging behavior on the part of *S. carolinensis* can significantly increase survival and reproductive success, especially in the context of interspecific interactions with native species (e.g., competition – Gurnell et al., 2004; disease transmission – Sainsbury et al., 2000).

Though the availability of trees (mast, shelter) appears to be a limiting factor in restricting population expansion, the ability of *S. carolinensis* to thrive in human-modified landscapes facilitates the species establishment in introduced environments. Urban areas in particular often have high population densities of *S. carolinensis* and can function as refugia and patch sources in dispersal dynamics.

Gray squirrels are often considered attractive and benign by local communities, which may enhance the probability of successful establishment. Indeed, human enamourment with gray squirrels appears to be an important factor in determining the fate of an introduced population – the novelty of introduced *S. carolinensis* along with the associated emotional attachment generated by their charisma may function as intractable hindrances to development and proper implementation of pest management and eradication programs (e.g., Bertolino et al., 2000).

Effective management plans for introduced gray squirrels should include: detection and monitoring of introduced *S. carolinensis* (Tattoni et al., 2006); understanding the ecological interactions and impacts of *S. carolinensis* in non-native habitats (Gurnell et al., 2004); collating spatial data on the environment (i.e., landscape structure, connectivity and composition; Lurz et al., 2001); and eradication of potential founders before population establishment (Tattoni et al., 2006).

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## Changes in the impact and control of the grey squirrel (*Sciurus carolinensis*) as determined from regional surveys in Great Britain

Mayle, B.<sup>1</sup>, Broome, A.<sup>2</sup>

<sup>1</sup>Centre for Human and Ecological Sciences, Forest Research, Farnham, Surrey, GU10 4LH, UK, [brenda.mayle@forestry.gsi.gov.uk](mailto:brenda.mayle@forestry.gsi.gov.uk)

<sup>2</sup>Centre for Human and Ecological Sciences, Forest Research, Northern Research Station Roslin Midlothian EH25 9SY

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### Abstract

The grey squirrel (*Sciurus carolinensis*) was introduced to approximately 30 sites in England and Wales from the USA between 1876 and 1929, to three sites in Scotland from Canada between 1892 and 1920, and to one site in Ireland in 1911 (Middleton, 1931). Soon after its introduction damage to trees due to seasonal bark stripping activity by grey squirrels was reported (Middleton, 1931). Despite the formation of the National Anti-Grey Squirrel Campaign' in 1931, aimed at exterminating the 'pest', grey squirrel populations continued to increase and expand in distribution.

Research suggests that damage is triggered by high numbers of squirrels, but particularly when high numbers of juveniles enter the population in early summer (Gurnell, 1989, Kenward et al., 1996). By 1967 it was recognised that eradication was no longer feasible and, to limit the risk of bark stripping damage, populations should be reduced just prior to and during the damage period (Mayle et al., 2007, Pepper and Currie, 1998, Rowe, 1967).

Surveys in state and private forests since 1952 have monitored grey squirrel distribution and impacts. Two of these (1983 and 2000) also gathered information on control efforts used to minimise damage. We report on the results of these surveys and changes in relation to changes in squirrel distribution, along with efficacy of control efforts.

Keywords: grey squirrel, bark-stripping, damage,

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## Assessment of invasive muskrat *Ondatra zibethicus* distribution and impacts on ecosystems in Lithuania

Butautytė-Skyrienė, G.<sup>1</sup>, Paulauskas, A.<sup>1</sup>, Ulevičius, A.<sup>2</sup>

<sup>1</sup>Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT- 44404 Kaunas, Lithuania, butautyte@gmail.com

<sup>2</sup>Faculty of Natural Sciences, Vilnius University, M. K. Čiurlionio 21/27, LT- 03101 Vilnius, Lithuania

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### Abstract

After acclimatization muskrats spread over almost all Lithuania and increased to about 40 000 individuals in the 1980s. But since then the number of muskrats has decreased to about 2000 individuals. We used BINPAS (Bioinvasion Impact/Biopollution Assessment System) for terrestrial ecosystems, which is usually using for water ecosystems, to assess the impact of *Ondatra zibethicus* on native species and communities, on habitats, on ecosystems and the biopollution level (BPL). The impact of muskrats varied between different regions of Lithuania. Widespread and high numbers of muskrats, and strong biopollution of ecosystem functioning were identified in four regions of Lithuania – Nevėžis and Nemunas Rivers, Šalčia River, Varėnė river, Vištytis Regional Park.

Keywords: BINPAS, bioinvasion impact, *Ondatra zibethicus*, muskrat, distribution

### Introduction

The muskrat *Ondatra zibethicus* was introduced to Lithuania from Archangelsk in 1954 and from Kazakhstan in 1956 (Lavrov, 1957). After acclimatization, they have spread over almost all of Lithuania. The aim of this paper is to describe the distribution, abundance and the impact of muskrats on native species and communities, habitats and ecosystems in Lithuania.

### Materials and methods

Muskrat distributions were analysed in 11 forest enterprises: Alytus, Valkininkai, Kaunas, Šiauliai, Anykščiai, Varėna, Šalčininkai, Zarasai, Nemenčinė, Utena and Ignalina. The abundance of muskrats was assessed by the numbers of individuals, lodges and burrows. To evaluate the biopollution level (BPL) of muskrats we used the method of Bioinvasion Impact/Biopollution Assessment System (BINPAS) proposed by Olenin et al. (2007) and available at <http://www.corpi.ku.lt/databases/binpas>. Invasive species impacts were scored at five levels ranging from: no impact (0), weak impact (1), moderate impact (2), strong impact (3) and massive impact (4). We similarly assessed muskrat abundance impacts on native communities (C0-C4), habitats (H0-H4), ecosystem functioning (E0-E4), and biopollution level in the period 1986-2011 over 16 biotopes in Lithuania.

### Results

Muskrat distribution and abundance during the last 10 years has been highly variable. For example, in 2002 muskrats were most abundant in forest enterprises of Zarasai district (400 individuals), Valkininkai (333 individuals), Nemenčinė (293) and Utena (278). In 2005, however, they were most abundant in Valkininkai (220) and Ignalina (124), respectively.

The highest abundance (E) from the overall assessment occurred in five different Lithuania regions. But in many cases, muskrats occurred in low numbers in several localities (A). The impacts on native species or communities ranged from none (C0) to moderate (C2) and no sites with strong or massive impacts were detected.

A strong impact level on ecosystem functioning (E3) was evident only on the Šalčia river, upstream of Žygantiškės village, Šalčininkai district. In other cases, the impact levels were weak or nonexistent. The impact was on habitats was also low (H0-H2), but a strong biopollution level (BPL=3) was noted in 5 regions.

## Discussion

In this study we examined the distribution and bioinvasion situation in Lithuania of the invasive species *O. zibethicus*. Numbers of muskrats during the investigation period in Lithuania were quite variable. During 1991-2000 five rivers (Varėnė, Šalčia, Merkys, Šventoji, Virinta) were surveyed along their banks to establish muskrat distribution and abundance. In the Šalčia, Merkys after eight years muskrat became practically extinct. In Varėnė the relative abundance was similar in all 9 years, as were the Šventoji and Virinta (Ulevičius and Balčiauskas, 2002). Compared to the period 1967-1975 when muskrats were abundant in Lithuania and the numbers were estimated at about 40.000, currently their numbers have fallen to 2.000-2.500 individuals (Žiemienė and Paulauskas, 2005).

The impact of muskrats on native species and communities (C0-C2) were negative for amphibians, fish and mollusks. Negative impacts also occurred on herbal and woody plants, and new growth was reduced in areas densely populated with muskrats. Muskrat impacts on waterfowl macrophytes (reeds, rushes etc.) degraded the protective properties of vegetation in coastal waters. Muskrats also impacted on the semi-aquatic rodent guild species composition, particularly the indigenous semi aquatic rodent *Arvicola terrestris* (Danilov, 2009). They caused moderate impacts on riparian vegetation structure by feeding on it, and also burrowing in the banks of water bodies (Sokolov and Lavrov, 1993). They also affected species composition, population size and age structure of freshwater unionid mussel communities (Owen et al., 2011). Muskrats impacts extended beyond habitat damage to effects on ecosystems (Danell, 1996; Nentwig et al., 2009). In Nevėžis and Nemunas Rivers, Šalčia River, Varėnė river, and Vištytis Regional Park muskrats were assessed as having a strong impact (E3) on ecosystem functioning and energy flow by consuming riparian vegetation, and releasing of the subsurface ground by burrowing and thus influencing chemistry and physical properties of water (Sokolov and Lavrov, 1993).

## Acknowledgments

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## Approaches to deal with the coypu (*Myocastor coypus*) in urban areas - an example of practice in southern Brandenburg, Germany

Walther, B.<sup>1,3</sup>, Lehmann, M.<sup>2</sup>, Fuelling, O.<sup>1,3</sup>

<sup>1</sup>Erminea GmbH, Mendelstr. 11, D-48149 Muenster, bernd.walther@erminea.com

<sup>2</sup>LELF, Plant Protection Service, Vom-Stein-Str. 30, D-03050 Cottbus

<sup>3</sup>University of Muenster, Institute of Landscape Ecology, Robert-Koch-Str. 26-28, D-48149 Muenster

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### Abstract

During the last 10 years the coypu population (*Myocastor coypus*) in the city of Cottbus caused increasing problems by gnawing on plants, destroying river banks, attacking pets and even pedestrians. The legal situation in the federal state of Brandenburg, the competence of different authorities as well as public opinion made it difficult to find a simple solution. To protect river banks in the city and the waterways of park Branitz, a UNESCO landscape park, the institutions involved and an external consultant bureau developed a specific package of measures. Prohibition of feeding coypu in the city area led to a rapid disintegration of the coypu colonies and stopped the stream bank destruction. Eradication of the park population was successful but a few individuals immigrated within one year, as not all the identified dispersal routes could be closed.

Keywords: agency cooperation, damage, eradication, feeding prohibition, population management

### Introduction

After escapes and releases from fur farms in the early 1990s the increasing coypu population in Southern Brandenburg became a serious pest problem, especially in urban areas. In Cottbus, coypus live in the city park along the river Spree and in small canals and ponds in the UNESCO landscape park Branitz. Coypus were especially attracted to locations where they were fed by people. At such places complete destruction of ground vegetation, damage to trees, massive erosion of pond and stream banks and small islands occurred, as well as undermining and tilting of sculptures. As well as the coypus, several other rodent species were attracted to the feeding places, posing an increased risk of disease transmission to people. The coypus even attacked and bit pets and pedestrians.

Since the legal and administrative responsibilities of the city administration and the relevant authorities were unclear, it was difficult to find a solution. The Nature Conservation Act, Game Law and Plant Protection Act did not provide adequate control measures. The Animal Welfare Act provided a range of possibilities to control the coypu but the public would not tolerate an extensive reduction by lethal methods. The Veterinary Office as the authority in charge therefore contracted the wildlife consultants Erminea GmbH to map the destruction in the park Branitz and to work out practical measures for an effective population management.

### Materials and methods

A first meeting and on-site inspection involving representatives from the veterinary office, regulatory office, gardens office, nature conservation department, water department, water and land communities, park Branitz administration, plant protection service and Erminea GmbH took place in March 2008. The coypu population in the park Branitz was assessed in October 2008 ahead of planned landscape rehabilitation measures. Individuals were counted, their main activity periods determined, feeding and resting places detected and damage documented. In the outer park areas all tributaries and water discharges were checked to identify potential dispersal routes for coypus. Based on the findings, an action plan was developed and adjusted to the needs and possibilities of the institutions involved. The proposed measures were carried out by authorities, park administration and the water and land communities between November 2008 and March 2009.

### Results

A total of 26 coypus - 4 males, 4 females, 18 pups - were found in the park Branitz. The colony consisted of four family groups using contiguous territories in the main canal and its ponds. Their main activity periods were in the late morning and in the evening, before sunset. The coypus fed on riverine

vegetation, reed beds, plantations and grassland. Feeding by people occurred in three places. For resting the animals preferred broad bush vegetation on the islands and steep banks. Where coypus occurred frequently damage was observed, such as destroyed vegetation cover, peeled bushes or trees, erosion of banks and islands and undermining of sculptures. Along the park borders nine potential gaps for invading coypus were detected - all of these involved passages under roadways. Five of them were already closed by fences in the waterway and on the embankment.

Based on these observations the action plan recommended a rapid eradication of family groups of coypu, and the closing of all channels and creeks leading in and out of the park to prevent new invasions. According to the action plan, the veterinary office authorised trappers of the land and water communities to eradicate the population in the park Branitz. Trapping was finished successfully within two weeks. The fencing of channels should be done as a cooperation of the park Branitz, the neighbouring zoological garden, the gardens office and the water department. Two individuals immigrated into the park during spring 2009 as the fencing of channels had not all been completed.

For the city area prohibition of feeding of coypus was recommended by Erminea and implemented by the regulatory office, and coypu abundance on former feeding places declined quickly. Animals abandoned these places individually or in small family groups. Consequently no further damage was reported.

### **Discussion**

Relations between humans and wildlife are regulated by many different and sometimes even contrary laws. Animal welfare, wildlife conservation, hunting or pest control are controlled by different authorities. In case of a pest problem it is difficult to find the leading authority to take action. Another major problem is the costs of necessary actions. Both of these issues played a major role in Cottbus and the park Branitz. As coypus are furry mammals, public opinion was an additional factor that had to be taken into account. As there was a potential risk of injuries and infection of people the veterinary office took the lead in Cottbus. Once the project was started effective measures were not too difficult to identify and execute. Within the city boundaries along the river Spree coypus occurred as individuals or small family groups. As long as no new feeding places are established, the coypu problem in the city area will remain under control.

In the park Branitz the problem was slightly different and lethal methods were employed. Coypus cannot be tolerated in the park at all as the artificial waterways and ponds have to keep their original design due to its status as an international heritage. Recolonisation from outside the park must be prevented and this is the responsibility of different authorities. Barriers to prevent reinvasion have not yet been fully installed and coypus are present in the park again. The responsibility for dealing with invasive and neo-zoic species should be in the hands of a single agency no matter where and what kind of control is needed.

## Best practice fox management in Australia

Saunders, G., McLeod, L.

Vertebrate Pest Research Unit and Invasive Animals CRC, NSW Department of Primary Industries, Forest Rd., Orange, NSW, Australia, glen.saunders@industry.nsw.gov.au

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### Abstract

Red foxes (*Vulpes vulpes*) impact on populations of many prey species in Australia, and so are the targets of widespread management programs. In this study we monitored fox management programs already operating across 4.5 million hectares of regional New South Wales (NSW) to compare the impact of varying fox baiting effort on the survival of lambs as a major prey species. The spatial coverage and frequency of fox baiting were both correlated with lamb survival. Lamb survival was higher in areas where fox baiting was done twice a year, in autumn and late winter/spring. Properties that had near neighbours participating in group fox baiting programs had higher survival of lambs than properties that did not. These results support the development of landscape-scale fox management programs, incorporating a high level of group participation to reduce the impact on vulnerable species.

Keywords: Australia, fox management, group control, predation, seasonal timing, *Vulpes vulpes*, 1080 baiting

### Introduction

Since its establishment across Australia in the late nineteenth century, the European red fox, *Vulpes vulpes*, has been one of the most destructive introduced pest species, threatening the survival of many native animals as well as impacting on the livestock industry (Saunders et al., 1995). Poisoning, using 1080 bait is currently the most widespread and effective method used (Saunders and McLeod, 2007). Shooting and trapping, sometimes encouraged through the offer of bounties have historically been popular but are less efficient and cost-effective than poisoning. Exclusion fencing, den fumigation and destruction, and guard animals such as dogs and alpacas are also used.

A major research effort to develop an immunocontraceptive vaccine for fertility control could not overcome technical constraints associated with a product suitable for field release (Strive et al., 2007). Ongoing research efforts have been directed into registering a new toxin, para-aminopropiophenone (PAPP) and an alternative toxin delivery system, the spring-loaded M-44 mechanical ejectors (Marks et al., 2004, Marks and Wilson, 2005). Current research has also focussed on best practice programs to reduce fox impacts on both native wildlife (conservation programs) and livestock production. The culling of foxes reduces the impact of predation at a local level, however immigration, compensatory breeding and juvenile survival allow fox populations to recover quickly (e.g. Gentle et al., 2007). Hence there has been a movement towards landscape approaches with group participation to increase the effectiveness of fox management programs. Despite their popularity, there is little experimental evidence of their effectiveness due to the difficulty in conducting long-term, broad-scale ecological field experiments (McLeod et al., 2008; Rushton et al., 2006). This study was undertaken to determine if there was a correlation between the frequency and spatial coverage of fox control programs and the survival of lambs (McLeod et al., 2010).

### Materials and methods

Experiments designed to detect the impacts of foxes require a large number of replicates to detect the relatively small effect (Greentree et al., 2000). This study took advantage of existing fox management programs on 5740 properties distributed across 4.5 million hectares in central west NSW to determine if lamb survival on a particular property was affected by fox baiting effort on that property or neighbouring properties. Initially we used the 'LambAlive' component of the 'GRAZPLAN' decision support system (Donnelly et al., 1997) to estimate the level of lamb mortality that was independent of predation. Resulting lamb survival figures were then analysed using a linear mixed effects model that incorporated the covariates of varying distance and times of neighbouring baiting, and the fixed effects of sheep breed, rainfall, year and season, their interactions, and individual property effects.

## Results

Most control programs were conducted in early autumn, and late winter/ early spring, which coincided with the dispersal and breeding periods for foxes. The Sheep breed factor was found to be highly significant, along with three covariate interactions: i) baiting six months apart on the lambing property only, ii) near neighbour baiting just prior to lambing and baiting on the lambing property, and iii) near neighbours baiting both just prior to lambing as well as 6-9 months prior to the lambing. The model predicted significant increases in lamb survival of up to 20% could be achieved when all near neighbours participated.

## Discussion

Landholders who participated with their neighbours in coordinated baiting programs were likely to have greater survival of lambs than landholders who did not. Furthermore, as the proportion of the adjoining properties that coordinate their fox baiting program increased, so did the survival of lambs on those properties. The frequency of fox control was also positively correlated with the survival of lambs even without neighbour support, with more frequent baiting (twice a year compared with once or no baiting) correlated with higher lamb survival. In Australia baiting of foxes is currently a valuable control tool to protect and improve the survival of species vulnerable to predation. Coordinated control of foxes over a large spatial scale and increasing the frequency of control to at least twice a year may further enhance protection by reducing fox immigration and any compensatory breeding and juvenile survival.

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## Why 0.02%? A review of the basis for current broadscale control of rabbits in New Zealand

Nugent, G.<sup>1</sup>, Warburton, B.<sup>1</sup>, Fisher, P.<sup>1</sup>, Twigg, L.<sup>2</sup>, Cowan, P.<sup>3</sup>

<sup>1</sup>Landcare Research, PO Box 40, Lincoln 7640, New Zealand, nugentg@landcareresearch.co.nz

<sup>2</sup>15 George Road, Lesmurdie, WA 6076, Australia

<sup>3</sup>Landcare Research, Private Bag 11052, Palmerston North 4442, New Zealand

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### Abstract

Rabbit (*Oryctolagus cuniculus*) populations in many parts of New Zealand have begun to increase dramatically as the impact of rabbit haemorrhagic disease wanes, and aerial poisoning control operations have resumed. Aerial 1080 poisoning of rabbits has historically used high prefeed and toxic bait sowing rates and low toxic loading. We review the extent to which this practice is based on a good mechanistic understanding of the process, or has evolved by trial and error to find a workable and affordable system. Current operational practices appear to have resulted from attempts to provide solutions to poor bait quality and concerns about the welfare of livestock. Current research on 1080 use on rabbits in Australia and possums (*Trichosurus vulpecula*) in New Zealand suggests that higher toxic loading and decreased sowing rates may be as effective as current practice, but with substantive reductions in the costs associated with control methods and the amount of toxin applied to the environment.

Keywords: aerial poisoning, operational practice, *Oryctolagus cuniculus*, rabbits, sodium fluoroacetate, sowing rates, toxic loading, 1080

### Introduction

Aerial poisoning of rabbits (*Oryctolagus cuniculus*) emerged in the latter half of the 20<sup>th</sup> century as a crucial tool for reducing the economic cost to pastoral agriculture in New Zealand. However, the need for aerial poisoning declined dramatically in 1997 with the illegal release of rabbit haemorrhagic disease (RHD), a viral disease lethal to rabbits. The impact of RHD now appears to be waning and land managers have resumed aerial application of 1080 (sodium fluoroacetate) baits (primarily carrot), using practices established in the mid-1990s. Those practices include use of prefeeding, a low toxic loading, and high sowing rates. In contrast, aerial 1080 poisoning of possums (*Trichosurus vulpecula*) in New Zealand now uses a relatively high toxic loading, and considerably less prefeed and toxic bait, and is at least partly a result of research and operational refinement over the decade in which aerial poisoning of rabbits was in abeyance. This disparity prompted a review of the basis for the practices used for rabbits (Twigg, 2010). Here we assess whether current best practice for aerial poisoning of rabbits with 1080-laden carrot bait is based on a good mechanistic understanding of the process, or has evolved by trial and error to find a workable and affordable system.

### Methods

We combine a literature review with new primary data to ask two interlinked questions; why do managers use the historically favoured 1080 toxic loadings of 0.02% for carrots and 0.04% for oats (now formalised in current registrations), and why do they apply bait at the rates and manner that they do? We then explore (focusing on carrot bait) what the optimal loading and sowing rates might be, based on the data available on rabbit densities, movement and foraging behaviours, and susceptibilities to 1080. This assessment is important because poison programmes remain the only realistic backstop for regaining control of burgeoning rabbit populations beyond the reach of other follow-up or secondary control measures. The greatest concern is the potential resurgence of bait and poison shyness problems that arose from historically poor practice during the final years of the pest boards in the 1980s (Lough, 2009).

### Results

Our review suggests that low toxic loading of 1080 appears not to be based on experimental optimisation using New Zealand rabbits, but on early (1960s) Australian toxicity assessments. A low toxic loading is also favoured to minimise risk to livestock, particularly sheep (*Ovis aries*), and destocking periods (McIntosh, 1958). Further, despite long-standing concerns about carrot bait quality, current practices still

appear to produce a large number of sub-lethal bait fragments (Batcheler, 1982). Rabbits that ingest few sub-lethal fragments may become ill and stop feeding (within 30 min – 3 hours) prior to obtaining a lethal dose. This phenomenon has major implications for increasing bait shyness in rabbit populations. Thus high sowing rates appear to be an attempt to overcome poor bait quality by providing rabbits with many opportunities to encounter and ingest sufficient toxic bait to ensure a lethal dose. We suggest an alternative approach is to increase toxic loading of 1080 (up to a max. of 0.15%), such that the number of baits that need to be encountered and ingested to obtain a lethal dose is reduced. Using this approach, we deduce that the amount of toxic bait that would need to be sown to ensure the ingestion of a lethal dose could be substantively reduced, perhaps by as much 80%. We argue that previous research indicates that undesirable non-target and environmental impacts of higher 1080 concentrations are minimal.

### **Discussion**

The ‘traditional’ use of multiple prefeeds and high toxic sowing rates may be an unintended counter to poor bait quality and the subsequent need for rabbits to find multiple baits. Whereas low toxic loading of 1080 appears to be a tradition spawned by concern about the risk to sheep rather than a consideration relating to efficacy of use against rabbits. Thus formalised current registrations pertaining to 1080 use have arisen from operational trial and error, and historical research that has attempted to find effective solutions to operational problems. We deduce from both parallel experience in possum poisoning (where there has been a shift to individually lethal baits and reduced sowing rates) and to the success of the one-shot-oat technique in Australia, that an approach based on use of individually lethal baits has the potential to be as effective as current practice and yet open the door to a substantive reduction in the amount of toxic and nontoxic bait used, and in the amount of 1080 applied to the environment.

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## Plans to eradicate invasive mammals on an island inhabited by humans and domestic animals (Corvo, Azores, Portugal)

Oppel, S.<sup>1</sup>, Beaven, B.M.<sup>2</sup>, Bolton, M.<sup>1</sup>, Bodey, T.W.<sup>1</sup>, Gerales, P.<sup>3</sup>, Oliveira, N.<sup>3</sup>, Hervias, S.<sup>3</sup>, Henriques, A.<sup>3</sup>, Silva, C.<sup>3</sup>

<sup>1</sup>Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, United Kingdom, steffen.oppel@rspb.org.uk

<sup>2</sup>Department of Conservation, Stewart Island Field Centre, PO Box 3, Stewart Island, New Zealand

<sup>3</sup>Sociedade Portuguesa para o Estudo das Aves, Av. João Crisóstomo 18 - 4<sup>o</sup> dto, 1000-179 Lisboa, Portugal

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### Abstract

Non-native invasive mammal species have been eradicated from many islands to conserve native species diversity. The Azores (Portugal) previously hosted very large seabird colonies, but since human colonization and the introduction of rodents (*Rattus rattus* and *R. norvegicus*) and cats (*Felis catus*) many seabird colonies have declined or disappeared as a result of predation. Because the Azores are also inhabited by humans and livestock, we reviewed the challenges associated with the eradication of invasive mammals from inhabited islands in order to plan for a seabird restoration project on the island of Corvo. Detailed analyses of the social, cultural, and economic costs and benefits of eradication are required to increase the probability of the local community supporting the eradication campaign. However, the ecological benefits of eradication are difficult to trade-off against social and economic costs due to the lack of a common currency. Local communities may oppose an eradication campaign because of perceived health hazards, inconvenience, financial burdens, religious beliefs, or other cultural reasons. Besides these social challenges, the presence of humans and domestic animals also complicates eradication and biosecurity procedures. For example, houses, garbage-disposal areas, and livestock-feeding areas can provide refuges for many synanthropic species and so decrease the probability of a successful eradication. Transport of humans and goods to an island increases the probability of inadvertent reintroduction of invasive mammals, and the establishment of permanent quarantine measures is required to minimize the probability of unwanted recolonization after eradication. Most of these challenges exist on Corvo, and continued work with the community is required before an eradication project can be initiated.

Keywords: Azores, cats, Corvo, eradication, invasive mammals, island, rats

### Introduction

The introduction and spread of non-native mammals on islands has become a major threat to native island species that evolved in the absence of mammals (Blackburn et al., 2004; Jones et al., 2008). Over the past 40 years, eradications of mammals have reduced the negative effects of non-native mammals on native species (Howald et al., 2007; Towns and Broome, 2003). Most eradication campaigns have been conducted on islands that are not permanently inhabited by humans partly because such operations are less complicated. However, more than half of islands (55%, n=38) where eradication of non-native mammals has a high conservation benefit-to-cost ratio are inhabited permanently by humans (Brooke et al., 2007). Thus, we examined the operational challenges associated with eradications on islands with permanent human populations, in order to prepare a plan for the eradication of Ship rats (*R. rattus*) and feral cats (*F. catus*) from the island of Corvo.

### Major challenges for mammal eradication on inhabited islands

Mammal eradications on inhabited islands require more than the consideration of the biological and technical pre-requisites to remove all individuals of a target species. While these basic prerequisites (i.e. by what means a target species can be killed, and whether it is possible to kill all individuals) for successful eradication have received much attention (Zavaleta, 2002), permanent human settlements require the consideration of additional factors including how an eradication campaign will affect human inhabitants, their domestic animals, and human activities. The eradication project must be socially acceptable to the community involved, and social and ecological benefits must outweigh the social and ecological costs. Moreover, the probability of recolonization must be reduced to near zero despite regular

traffic transporting goods that support the island's human population. The removal of all individuals of an invasive target species is also made more difficult when target species can seek refuge in buildings or escape poison bait by accessing alternative anthropogenic food sources.

### **Lessons from the island of Corvo**

Corvo is a 1700 ha volcanic island with ~400 human inhabitants, most of whom make a living through farming, with the principal land use being livestock grazing. Due to inaccessible steep cliffs, eradication of rodents on Corvo would only be feasible through the aerial distribution of poison bait pellets, which may put livestock and feral ungulates at risk of accidental ingestion of poison. In addition, food provided to domestic animals is widely available for invasive rodents, and may reduce attractiveness of poison bait to target animals and thus reduce the probability of successful eradication. Corvo is dependent on imported goods and building materials, which are transported by boat and plane and are currently not inspected or treated for hidden rodents prior to shipment. Biosecurity measures that prevent the accidental re-introduction of invasive mammals would be required at all ports of departure of boats and planes for Corvo, on boats themselves, and around the harbour and airport on Corvo. These quarantine measures would have to be maintained in perpetuity and could significantly increase the cost of living on Corvo.

Most people on the island favour the idea of eradicating rats from Corvo. Because rats serve as prey to both domestic and feral cats, the eradication of rats alone may increase the adverse impact of cats on seabirds (Bergstrom et al., 2009; Caut et al., 2007), and seabird populations would therefore benefit from a combined rat and cat eradication. Many people support eradication of feral cats, but are opposed to removing domestic cats. This increases the operational complexity of feral cat eradication because it is often difficult to distinguish feral and domestic cats, and more labour-intensive methods would be required for feral cat eradication. Ongoing research and community involvement will decide whether rat and cat eradication on Corvo is feasible.

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## **Welfare and ethical issues in invasive species management**

Cowan, P.<sup>1</sup>, Warburton, B.<sup>2</sup>, Fisher, P.<sup>2</sup>

<sup>1</sup>Landcare Research, Private Bag 11052, Palmerston North 4442, New Zealand, cowanp@landcareresearch.co.nz

<sup>2</sup>Landcare Research, PO Box 40, Lincoln 7640, New Zealand

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Keywords: animal ethics, animal welfare, humaneness, invasive species management, uncertainty

### **Introduction**

Increasingly, invasive species management is being challenged by changing attitudes in society and greater restrictions on the use of control methods. Invasive species management usually involves the deaths of many animals, mostly the target pest(s) but often other non-target species as inadvertent by-kill. Management usually involves a variety of lethal methods such as traps, toxins and hunting, which may differ in their animal welfare costs, their risks to non-target species and, for toxins, of environmental contamination and spread through food webs. The welfare issues include the pain and suffering caused directly by the control method used (to both target and non-target) and any flow on effects (e.g. trophic cascades), while the ethical costs include the consideration of the justification and outcomes of the control or eradication programme. Recently there have been a number of developments that address some of the concerns raised about invasive species management. The development of a framework for humaneness assessment of control tools, modelling of management strategies that minimise numbers of animals killed, and improved definition of management outcomes that incorporate uncertainty are significant steps forward in providing invasive species managers with means of informed choice of the extent to which they can mitigate the welfare and ethical costs of invasive species management.

### **A framework for humaneness assessment**

A welfare assessment framework was developed recently that produces a ranking of the relative welfare impacts of vertebrate toxic agents on their mammalian targets and other non-target mammals (Sharp and Saunders, 2008). This was modified to improve general utility and applied to a range of invasive species management traps and toxins used for invasive species management in New Zealand (Fisher et al., 2010). Examples are presented to demonstrate the application of the framework, and to highlight some issues with application of the framework identified, particularly the assumption of best-practice application and availability of data. Overall, the welfare impact assessment framework was a useful approach to providing invasive species managers with information to allow selection of control tools on the basis of welfare as well as cost and efficacy, and should provide a future consistency in relative comparisons between control tools for a range of vertebrate pest species.

### **Minimising numbers of animals killed**

Research, policy and regulation have most often focused on the welfare impacts (humaneness) of the management tools used (Shivak et al., 2005; Warburton et al., 2000). Efforts to mitigate welfare impacts have thus been focused at the individual animal level rather than at the population level. For invasive species management, however, there is a population issue because often large numbers of animals are involved and so the total welfare cost of a control programme may be significant. An example of rodent control for biodiversity protection is presented to show how a modelling approach can be taken to compare the number of animals killed and the operational costs of two different control strategies, and so allow managers to take total welfare cost into account in their choice of control strategy.

### **Management outcomes and uncertainty**

From a welfare perspective, control operations that fail to manage invasive species effectively may have high costs and little benefit, and so are of major concern (Cowan and Warburton, 2011). Such failures may mean that tens to thousands of the target pest have been killed without achieving the goal of the operation and, in the worst case where there is no further management of the pest species, those animals have died to no good purpose, or at best for a temporary reduction in their impacts. Such uncertainty can be reduced by better definition of proposed outcomes of management by asking questions such as (1) do the perceived benefits actually justify the large-scale killing of the pest species?; (2) is the risk of failure

too high?; (3) will perverse outcomes result in minimal benefits?; (4) will the management fail because of cessation of funding or because of unforeseen technical problems?; and (5) will the benefits of successful management be lost if reinvasion cannot be minimised? These issues all contribute uncertainty to management operations, and failure highlights the welfare and ethical issues, and makes future management more difficult. To address this, programmes must identify uncertainties at the planning stage and develop appropriate mitigation strategies. Such approaches to reducing the risk of failure should be complemented by a learning-based strategy and the adoption of an adaptive management framework that has as its first tenet the need to learn and reduce uncertainty (Walters and Holling, 1990; Warburton and Norton, 2009).

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## Fallow deer (*Dama dama* Linnaeus, 1758) in the province of Rieti (central Italy): origin and first data on the competition with native red deer and roe deer

Bonanni, M.<sup>1</sup>, Adriani, S.<sup>2</sup>, Cecchini, C.<sup>3</sup>, Morbidelli, M.<sup>4</sup>, Amici, A.<sup>2</sup>

<sup>1</sup>Via F. Martinelli 34, Roma, Italy, bonanni\_m@libero.it

<sup>2</sup>Università della Tuscia, Dipartimento di Produzioni Animali, Via C. De Lellis, snc, 07100 Viterbo, Italy

<sup>3</sup>Via delle Cese 6, 67020 Sant'Eusanio Forconese, L'Aquila, Italy

<sup>4</sup>Via S. Di Muzio 9, 02010 Borbona, Rieti, Italy

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### Abstract

The most recent literature describing the distribution and abundance of fallow deer (*Dama dama*) in Italy does not indicate the presence of this species in the province of Rieti. Some reports indicate the presence of the species in the middle valley of the river Velino. To test the reliability of those reports a field study was undertaken using three different techniques: fixed points census, line transects, and snow tracking. The area investigated was about 3,500 hectares. Given the topography of the study area these techniques were carried out opportunistically. Fallow deer were located, and the population was estimated to be about 70-90 individuals. Investigation in the Archives of the Provincial Command of the State Forestry Corps of Rieti allowed the origin of the population to be traced. The fallow deer escaped from a group of individuals reared on an estate called Santogna in 2001-2002. The animals came from the Presidential Estate of Castelporziano (Roma). The population of this invasive species is problematic because it dominates the native red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), and restricts their natural expansion.

Keywords: allochthonous, competitor, *Dama dama*, distribution, Rieti province

### Introduction

The fallow deer (*Dama dama*) is a species native of the eastern portion of the Mediterranean. The current distribution is considered almost entirely man-made, with the exception of the population in Turkey in the National Park Termessos (Carnevali et al., 2009; Pedrotti et al., 2001). The species is quite common in Western Europe, particularly in England. The populations derived from groups kept in captivity for ornamental and hunting purposes, introduced in estates and game reserves, and escaped from pens (Apollonio, 2003; Scalera, 2001). The species is social and this characteristic limits its ability to dispersal. The deer is classified as a ruminant, of the intermediate type with the ability to use a variety of pasture and vegetation types (Perco, 1987). These features allow it to adapt easily to different environments, except for arid areas and those where the snow cover is persistent. The high level of interspecific competition with other deer species and the dominance of fallow deer in deer killed for venison (Carnevali et al., 2009; Perco, 1987; Scalera, 2001) poses management problems in areas of sympatry, particularly where expansion of native species is possible. For these areas ISPRA (Institute for Protection and Environment Research) proposes drastic steps to contain or eradicate the fallow deer (Carnevali et al., 2009). The Italian distribution area of the species (about 2,700 km<sup>2</sup>) is very fragmented and comprises: Tuscany, Umbria, Tuscany and Romagna Apennines, the area between the Ligurian Apennines and the provinces of Alessandria and Pavia. Fallow deer are also spread in the Alps, provinces of Belluno, Treviso and Pordenone, and present in the plains or low hills of Veneto, Friuli-Venezia Giulia and Piemonte. Isolated nuclei, sometimes significant, occur in peninsular Italy and on islands. Overall fallow deer occur in 60 out of 113 provinces (53%) (Carnevali et al., 2009). No data are available for Rieti. To fill this gap a study has started to acquire the first data on a group of deer reported in the middle valley of the Velino (province of Rieti). The aim of survey was threefold: 1 - define the distribution and abundance of the population, 2 - identify the source of the population, 3 - determine potential interference between fallow deer and other deer species.

## Materials and methods

The surveys were preceded by a preliminary phase aimed at verifying the presence of the species. Successful outcome of this phase was followed by investigations of the distribution and origin of the nucleus. The study area, identified according to reports, is mountainous with altitudes between 850 m a.s.l. and 1,500 m a.s.l. The area is characterized by extensive mixed forest with a predominance of *Quercus* spp. at lower altitudes and *Fagus sylvatica* at higher ones. The woods are interspersed with large areas of natural pasture. The snow is sporadic and shallow with an average snowpack and low persistence. The field surveys were conducted from September 2008 to March 2011 using fixed points census (after finding 10 sites), line transects (for detection of signs of presence), and snow tracking (technique applied in an "opportunistic" way on routes identified in conjunction with the snow deposition). The area of distribution was estimated using the MCP (minimum convex polygon), with geo-referenced signs of presence. To trace the origins of the population we investigated information held by the State Forestry Department (CFS) of the middle valley of the Velino (Post, Bournemouth), the Archives of the Provincial Command of the CFS of Rieti, and a fenced area of about 600 hectares called Santogna near the town of L'Aquila in which the CFS has maintained a herd of fallow deer. Interviews were conducted with some operators employed at Santogna in the 1980s and 1990s.

## Results

A total of 36 sessions of fixed point census were performed (18 at sunrise, and 18 at sunset). Transects were searched 13 times for a total of 546 km. Six snow tracking sessions were carried out for a total of 42 km. The analysis of data collected showed that the population of fallow deer numbers 70–90 deer, and the area used is about 3,500 hectares in the municipalities of Borbona and Posta. The nucleus of deer, the only one present in the province of Rieti in the wild, is derived from the herd reared by CFS in the area named Santogna (Leonessa municipality, province of Rieti). The oldest data refer to 1964, when the CFS of Rieti acquired from the Presidential Estate of Castelporziano (Rome) 6 deer (2 ♂ and 4 ♀) to be placed in the enclosure of Santogna. In 1970, 20 fallow deer were introduced (6 ♂ and 14 ♀) to an area of about 70 ha of the estate of Santogna fenced with metal mesh 3 m high. The documents show that the breeding of deer was uncontrolled and, because of inbreeding, the population condition weakened, especially the female component. The farm was managed until December 1992 at which time approximately 150 deer were present, about 100 of which were males. It was not possible to find documents that describe the development of breeding after 1992. In 1998, during inspections conducted in the drafting of legislation of the Province of Rieti Wildlife, approved by the Lazio Region in 2001 (Del Zoppo, pers. comm.), the fenced enclosure still housed animals. Between 2001 and 2002 the population was reduced to about 120 animals due to escapes. For at least a couple of years, deer, including escapees, continued to frequent the compound, in which they continued to be fed. The animals gradually began their dispersal giving rise to the wild population censused in this study (Durante, pers. comm.).

## Discussion

The area currently occupied by fallow deer also has a population of roe deer. This area also lies along the lines of expansion of two different populations of red deer, one coming from the reserve of the Velino-Sirente through the Regional Natural Reserve of the Duchessa and the Mountains National Park Gran Sasso Monti della Laga. Knowing the competition and the dominance of fallow deer over red deer and roe deer, which are native species of great conservation concern, a rapid decision needs to be made on the most appropriate management arrangements to control fallow deer. The aim must be to prevent the fallow deer adversely affecting the natural expansion and consolidation of the roe deer. The fallow deer population should also be monitored over the next few years to assess its effects on the roe and red deer populations.

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## Study on the presence and perception of coypu (*Myocastor coypus* Molina, 1782) in three areas of Lazio region (Italy)

Adriani, S.<sup>1</sup>, Bonanni, M.<sup>2</sup>, Amici, A.<sup>1</sup>

<sup>1</sup>Università della Tuscia, Dipartimento di Produzioni Animali, Via C. De Lellis, snc, 07100 Viterbo, Italy, adrianisettimo@libero.it

<sup>2</sup>Via F. Martinelli 34, Roma, Italy

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### Abstract

The coypu (*Myocastor coypus*) is native of sub Patagonian region, the temperate areas of Chile and Argentina. In Italy the first animals were introduced in 1928 for farming for fur production. Released and escaped coypu have altered the ecosystems colonized and impacted on agriculture and they are now considered as an invasive species. Three study areas were chosen in the Lazio region. The first area was a protected natural reserve characterised by extensive agriculture and backyard farming, the second was characterised by intensive and specialised agriculture, and the third was characterised by intensive agriculture. In order to understand whether there are problems caused by coypu in these areas, a questionnaire was prepared and distributed to 574 farmers/peoples living/working close to the investigated areas, and 389 completed questionnaires were obtained. The results covered the legal status of the species, the problem of damage to crops, and the negative effect on native species, and highlighted that the removal of this invasive species should be accompanied by an information program involving farmers and people living close to areas suitable for coypu.

Keywords: eradication, introduction, invasive species, *Myocastor coypus*, public survey, rodents

### Introduction

The coypu (*Myocastor coypus*) is native of sub Patagonian region, the temperate areas of Chile and Argentina (Cocchi, 2002). The species was introduced to North America, England, Asia, Africa and Europe (Soccini and Ferri, 2001; Cocchi and Riga, 2001). In Italy the first animals were introduced in 1928 by the National Institute of Rabbit husbandry in Alessandria, for farming for fur production (Vicini et al., 2003). The project was successful for a few decades and then declined. Inadequate housing and voluntary release by the farmers (to avoid the disposal of animal carcasses) were the main causes of dispersal of coypu (Soccini and Ferri, 2001). Naturalised groups have occurred since the 1970s (Soccini and Ferri, 2001). At the end of the 20th century Lazio, Umbria, Tuscany, Lombardy, Emilia Romagna, Veneto and Piedmont were the Italian regions with the highest density of free living individuals (Santini, 1983). The high reproductive potential and adaptability of the coypu have allowed the rapid colonization of new territories (Scalera, 2001). At the national level there are two large distribution areas: the Po Valley and northern Adriatic coast, and the Tyrrhenian coast between the Arno and the Tiber (Cocchi and Riga, 2001). The presence of a rich network of waterways and ponds has facilitated the natural dispersion and settlement of the species (Cocchi and Riga, 2001; Soccini and Ferri, 2001; Vicini et al., 2003). Coypu (Perco and Lovari, 2002) have greatly altered the ecosystems colonized, impacted on agriculture and are now considered invasive. In many areas they are now controlled or eradicated (Bertolino et al., 2001, 2005; Panzacchi et al., 2007). Little is known about the perception of the species by the farmers and what activities can be adopted for species containment. To address this, during a study on the distribution a questionnaire on the species was given to farmers and stakeholders.

### Materials and methods

Three study areas were chosen on the basis of the first results on coypu distribution and on the basis of a deterministic model of habitat suitability of the species (unpublished results). The study area identified in the province of Rieti (hereinafter referred to as area A) coincides with a portion of river Aia of 3.6 km, a tributary of the Tiber, where hunting is allowed. The territory lies in the municipalities of Torri in Sabina, Selci and Tarano and is characterized by intensive agriculture. There is no coypu containment program and no information provided to stakeholders. The study area identified in the province of Viterbo (hereinafter referred to as area B) is a protected area (Natural Reserve of Tuscania) where a portion of 3.8 km of river Marta was studied. Information was available to stakeholders but there were no

containment programs. The study area identified in the province of Latina (hereafter referred to as C) lies in the Municipality of Latina, and is a hunting area of with 4.8 km. A containment program has been in place for three years.

In order to understand whether there are problems caused by coypu in these areas, a questionnaire was developed and given to 574 farmers/peoples living/working close to the investigated areas (207 in area A, 168 in B, 199 in C). The researchers helped the farmers to complete the questionnaires or asked them to return them within fifteen days. A first evaluation was the completeness and relevance of responses. The statistical analysis of responses was carried out only on questionnaires completed in full and relevant.

## Results

Of a total of 574 questionnaires 389 were returned (67.8%), 148 in A, 111 in B, and 130 in C. Of these 358 were complete (141 in A, 99 in B, 118 in C). The following results were considered relevant from a social and technical point of view:

1. the coypu is a protected species (62% in A, 12% B, 14% C);
2. the coypu causes damage to the agricultural crops (96% in A, 39% B, 48% C);
3. the coypu interferes with native species (35% in A, 74% B, 55% C);
4. Nutria is poached (91% in A, 12% B, 13% C);
5. Nutria carcasses are destroyed illegally and without veterinary supervision (67% in A, 0% B, 0% C);
6. Nutria meat is consumed (77% in A, 0% B, 0% C).

## Discussion

The farmers were unclear about legal status of coypu. Its classification as an invasive species depends on information and containment programs. The problem of damage to crops is spread across all three areas. The negative effect on native species is not known by most of the people, both in protected and non-protected areas. Poaching was thought to exist mainly because of ignorance of the legal status of the species. The research concluded that the removal of coypu should be accompanied by an information program directed at farmers and people living close to areas suitable for the species.

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## Genetic variability of raccoon dogs and their impacts on the environment in Lithuania

Pūraitė, I.<sup>1</sup>, Gričiuvienė, L.<sup>1</sup>, Paulauskas, A.<sup>1</sup>, Sruoga, A.<sup>1</sup>, Gedminas, V.<sup>2</sup>, Butkauskas, D.<sup>3</sup>

<sup>1</sup>Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT- 44404 Kaunas, Lithuania, a.paulauskas@gmf.vdu.lt

<sup>2</sup>Kaunas Tadas Ivanauskas Zoological Museum, Laisvės 106, LT-44253 Kaunas, Lithuania

<sup>3</sup>Institute of Ecology of Nature Research Centre, Akademijos str. 2, LT-08412 Vilnius, Lithuania

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### Abstract

The raccoon dog (*Nyctereutes procyonoides*) is a prominent example of a pest with a wide distribution in Europe and significant ecological impacts. Alien species may have an effect on various levels of biological organisation: genetic, population, community and habitat/ecosystem. Using basic information on abundance, distribution (Bioinvasion Impact/Biopollution Assessment System (BINPAS) and genetic diversity of raccoon dogs, we identified their impact on the environment in Lithuania. Genetic variation of 269 individuals of *N. procyonoides* from Lithuania was analyzed using RAPD and D-loop analysis. RAPD analysis showed that there were 70 polymorphic loci and the number of fragments varied among the primers. The genetic polymorphism was most with ROTH – 180 – 05 and ROTH – 180 – 06 (100%) and least with ROTH – 180 – 10 (25%). Using Rac-1F and Rac-1R primers were amplified D-loop fragments of individuals from different locations in Lithuania. The genetic data suggest that raccoon dogs colonised Lithuania from different neighbouring countries Belarus and Latvia. The raccoon dog impact on native species and communities (C0-C2) were moderately negative for amphibians, mollusks, rodents, birds, insects, and reptiles and for transmission of pathogens.

Keywords: genetics, impacts, invasion, *Nyctereutes procyonoides*, RAPD, raccoon dog

### Introduction

During 1929 to 1955 the raccoon dog (*Nyctereutes procyonoides*) was introduced to 82 locations in the European area of former Soviet Union from the Amur-Ussuri region in far-eastern Russia and has spread from the southern districts to boreal forests, steppe and semi-desert. They were not introduced to Lithuania but were first observed there in 1948, in the eastern part of the country. It is believed that the raccoon dog came from neighboring countries since it was introduced in 1936 to Belarus and Latvia in 1948. Since 2000, raccoon dogs have spread in widely in Europe, observed in 2002 in Macedonia, 2005 in Italy and 2008 in Spain. The raccoon dog population situation in Lithuania can be divided into five periods, during which population size varied. Since 1970 the species has been declared invasive and hunting is permitted throughout the year. Invasive alien species are often a significant threat to ecosystems and, in Lithuania, the raccoon dog impacts on various levels of biological organisation: genetic, population, community and habitat/ecosystem and populations of native rodents, birds, insects, amphibians, and reptiles. In this study we examined raccoon dog genetic variability in relation to the origin of invasion and the consequences of raccoon dog bioinvasion of Lithuania.

### Materials and methods

Between 2007 and 2011 hunters sampled tissue from 269 raccoon dogs from different locations in Lithuania. Genetic variation was analyzed using RAPD and D-loop analysis. Genomic DNA was extracted from frozen liver and muscles using “Genomic DNA Purification Kit#KO512” (Fermentas, Lithuania). PCR for RAPD was performed on a Mastercycler gradient (Eppendorf) in a 21 µl reaction mix and 4 µl template DNA (~25-50 ng). Thermocycling parameters after predenaturation step at 94°C for 4 minutes were 44 cycles with denaturation step at 94°C for 1 minute, annealing step at 49.9 – 60.1°C for 1 minute, elongation step at 72°C for 2 minute and final elongation step at 72°C for 5 minutes.

PCR for D-loop analysis using primers Rac-1F 5'-tcgtgcattaatggcttc-3' and Rac-1R 5'-ccattgactgaatgacaccttg-3' was performed in 20 µl reaction mix and 5 µl template DNA (~50 ng) and thermocycling parameters after predenaturation step at 95°C for 5 minutes were 35 cycles with denaturation step at 94°C for 45 s, annealing step at 55°C for 45 s, elongation step at 72°C for 1 minute and final elongation step at 72°C for 7 minutes. PCR product of D-loop were prepared for sequencing using “GeneJET™ Gel Extraction Kit#K0691” (Fermentas, Lithuania).

RAPD results were analyzed using “TREECON for Windows” (Van de Peer et al., 1994) program. Relationships among individuals were represented in a UPGMA cluster tree and we also calculated degree of polymorphism among individuals. GenBank blastn search algorithm was used (Altschul et al. 1990) to confirm that sequences were from *N. procyonoides*. To determinate haplotypes we used “CLC Sequence Viewer 6.4”. Haplotypes phylogenetic relationships were assessed through haplotypes network using “Network” program.

To evaluate the biopollution level (BPL) of *N. procyonoides* we used the method of Bioinvasion Impact/Biopollution Assessment System (BINPAS), available at <http://www.corpi.ku.lt/databases/binpas> (Olenin et al. 2007). Invasive species impacts were scored at five levels ranging from: no impact (0), weak impact (1), moderate impact (2), strong impact (3), massive impact (4). We similarly assessed raccoon dog impact on native communities (C0–C4), habitats (H0–H4), ecosystem functioning (E0–E4) and biopollution.

## Results

Raccoon dog DNA was amplified with ten primers (ROTH-180-01-ROTH-180-10) and 70 fragments were obtained ranging from 110 to 2000 base pairs. The primer ROTH – 180 – 09 amplified the most polymorphic bands (n = 16). Genetic polymorphism was highest for ROTH – 180 – 05 and ROTH – 180 – 06 (100%) and least for ROTH – 180 – 10 (25%). Using the Rac-1F and Rac-1R primers we successfully amplified D-loop fragments of individuals from different locations in Lithuania.

## Discussion

Following translocation of raccoon dogs by people from Far East to North West European Russia during 1929 to 1955, they began to invade neighbouring areas. However, unlike most other invading species, understanding of the specific dynamics of invasion by raccoon dogs is facilitated by the known date of the European invasion and its geographical source, which is the NW region of the former Soviet Union. Thus, theoretical predictions regarding the general population development seemed fairly simple (Pitra et al., 2010). The genetic data suggest that raccoon dogs colonised Lithuania from the neighbouring countries of Belarus and Latvia, beginning some time after their introduction to Latvia in 1948 and Belarus in 1936) with westward expansion into the wide open ecological niches. However, raccoon dogs were introduced to Latvia and Belarus from different original populations. This situation suggests the likelihood of a second genetic bottleneck because the invaders would only represent a subsample of the originally translocated populations (Pitra et al., 2010).

The raccoon dog impacts on native species and communities (C0-C2) were moderately negative for amphibians, mollusks, rodents, birds, insects, and reptiles, and for transmission of pathogens.

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## **Results of four years of digital urban monitoring of *Rattus norvegicus* with RatMap in Hamburg including data on infestation near the surface and in underground sewers**

Plenge-Bönig, A., Zickert, A., Baumgardt, K., Sammann, A.

Institute of Hygiene and Environment, Free and Hanseatic City of Hamburg, Germany,  
anita.plenge-boenig@hu.hamburg.de

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### **Background**

The digital monitoring system RatMap is used to monitor urban rat populations in Hamburg, Germany. In addition to the analysis of surface data, data from infestations in underground sewers are compared for the first time in Germany.

### **Methods**

Infestation was identified systematically according to a case definition for rat infestation. Descriptive analysis of the data was done with EpiInfo (CDC), and the MySQL data-base RatMap linked to ArcGis (ESRI), which allows analysis of rat infestations in space and time, and the extent and outcome of control measures. However, activities of the Institute are limited to the occurrence of rats near surface of public property. Therefore, data from the sewer system provided by Hamburg water public works were entered into the geo-database. Results of evaluation over a period of four years are demonstrated. If applicable, 95 % confidence intervals were calculated.

### **Results**

In the period from 2007 to 2010, 8,770 rat indications were notified to the database. Following the case definition for above-ground rat infestation, 7,278 were rated as true infestations, with 2,214 cases in 2007, 2,507 in 2008, 1,194 in 2009, and 1,393 in 2010 respectively. Rat incidence peaked significantly in the summer months in 2008, and decreased significantly in the summer months of 2009. Annual frequencies show at least two peaks in April and in July, where patterns may differ related to seasonal temperature cycles. The number of cases from a private pest control company roughly reflects the cases on governmental property. The frequencies of the numbers of rat notifications over the last 17 years show similar patterns compared to an index of small wild rodents monitored by a forest department in northern Germany. The geographic reflection of data from above ground infestations of *Rattus norvegicus* is not always consistent with the one from underground infestations.

## **Practical rat control in the city of Zürich – there is more than just baiting!**

Schmidt, M.E.

Beratungsstelle Schädlingsbekämpfung, Umwelt- und Gesundheitsschutz Zürich, Walchestrasse 31, Pf. 3251, 8021 Zürich, Switzerland, marcus.schmidt@zuerich.ch

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The Urban Pest Advisory Service (UPAS) is responsible for controlling rats and mice in public areas in the city of Zürich. For rat problems on private properties, the house owner has to contact a private pest control company. Rats however do not care about property boundaries and, usually, nobody feels responsible for solving the rat problem and paying for it. UPAS conducts a survey of the area concerned to ensure a quick solution in such cases. If rats appear repeatedly in the same surroundings near houses, and water (lakes or rivers) is not close by, it is important to check sewer plans in relation to the position of the rats' burrows. If they overlap, the sewer system has to be checked with a sewer camera and if damage is found, repairing the sewer pipe is the only effective solution.

I present a practical rat management case with the control measures used and how the case was finally solved. This includes several minutes of professional film sequences of the rat management case.

Rats appeared for two consecutive years in the backyards of two houses. By the time the UPAS was informed, high numbers of rats had established themselves. In one of the houses there was a bakery that kept old bread in paper bags next to the backdoor. In August 2009, direct baiting into the rat burrow openings was conducted with 1 kg difenacoum grain bait and three chlorophacinone wax blocks (100 g). During a follow-up visit nine days after the placement of bait, two moribund rats were seen. No further rat activity was observed after 15 days and the bait blocks showed no sign of fresh gnawing. Comparing the position of the rat burrows with the sewer plans of the backyard we found no overlaps. The baker was ordered to install a rat safe box to store his old bread.

In August a year later UPAS was called again and an even bigger rat population of approximately 30 rats was evident in the same areas of the backyard. Again the rat burrows were baited with 1 kg of difenacoum grain bait. In addition, six coumatetralyl bait bags (100 g) were placed in safe positions. It was found that the rats could easily enter the newly-installed bread box of the bakery. The baker was contacted again and storage of bread in the backyard was forbidden. 11 days after the first baiting the follow-up visit showed no signs of fresh rat activity. The house with the bakery had steps covered with an iron grill leading into a small, dark underground room. A leak in the sewage pipe of the house was found by inspecting this room. It was there that rats were able to come to the surface from the sewer system. The house owner was contacted and had to renew this sewage pipe and pay for the rat control. The rat problem was finally solved with these measures.

## **Occurrence of Norway rat (*Rattus norvegicus*) in above-ground and underground habitats in Budapest, Hungary**

Bajomi, D., Kiss, Z., Papp, G.

Bábolna Bio Ltd., Budapest, Hungary, igazgato@babolna-bio.com

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In 1971-1972 a rat control program was implemented throughout Budapest and continuous maintenance work has been going on since 1973. In order to ensure efficacy, all rat occurrence related data have been recorded since 1974. Using the special code system developed by us, it is possible to determine which are the macro-habitats and, within those the micro-habitats, where Norway rats occur most frequently.

It is assumed that during rat control in urban areas (i.e. large cities) or maintenance of rat-free conditions, it is not necessary to perform rodenticide treatments in the sewer system. To assess the importance of control in the sewer system and to test this assumption, the authors conducted a separate analysis of the data related to Norway rat occurrence in Budapest between 2000 and 2009.

Considering the average of the 10 years in question – 63% of the rats occurred in buildings and related habitats, 30% in the sewer system while 7% occurred at places other than in these habitats. With rats living in a given building, the frequency of their occurrence in the sewer system near that building was also determined.

In the light of the data obtained, rat control in the sewer system is considered to be of primary importance in terms of efficacy within a long term urban rat management program.

## Accumulation of chlorophacinone in susceptible and resistant Norway rat strains

Berny, P., Caillis, P., Vey, D.

Université de Lyon, VetAgro Sup, USC1233, INRA, F-69280 Marcy L'Etoile, France, p.berny@vetagro-sup.fr

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Keywords: anticoagulant, bioaccumulation, non-target exposure, rodenticide, secondary poisoning, toxicity

### Introduction

Anticoagulant rodenticides (AVK) are known to accumulate substantially in the liver of exposed animals. Their half-lives are very long; up to 350 days for brodifacoum in rats (Erickson and Urban, 2002). As a consequence, secondary poisoning occurs in predators and scavengers feeding regularly on poisoned animals (Berny et al., 1997, Shore et al., 1999). This has been observed in many species, including endangered species such as the red kite (*Milvus milvus*) (Berny and Gaillet, 2008). In Europe, rodenticide resistance appears to be widely distributed. There is strong evidence supporting the role of the *Vkorc1* gene in the resistance of the Norway rat (*Rattus norvegicus*) (Rost et al., 2004) and several SNPs' associated with resistance have been identified (Pelz et al., 2005). The objective of this work is to analyze the potential for bioaccumulation of given anticoagulant compounds in resistant rats from various European strains. The hypothesis tested was that resistant strains should accumulate anticoagulants to a larger extent than susceptible rats.

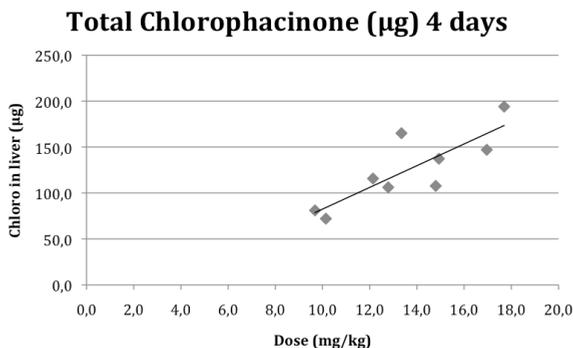
### Materials and methods

Resistant rats were obtained from Germany (Dr. Jens Jacob), United Kingdom (Dr. Alan McNicoll) and France, carrying respectively the Y139C, L120Q (Berkshire strain) and Y139F resistance mutations. Susceptible rats were obtained from our own breeding colony. The genetic status of each strain was verified with DNA sequencing (homozygous resistant animals). The resistance status of each strain has already been published (Pelz et al., 2005). Rats were fed *ad libitum* on a wheat-bait containing chlorophacinone (50 mg/kg). This anticoagulant was chosen because it is one of the most commonly used in France and also because all strains are known to be resistant to a significant extent to this AVK. Four animals of each strain (2 males, 2 females) were euthanized after 1, 4, 9 or 14 days of continuous exposure. A second series of exposure was conducted on rats exposed 3 days on day 3, 8 and 13 and euthanized at days 4, 9 and 14. PT time was determined on the day of euthanasia for each rat. Chlorophacinone and its major hydroxyl metabolites were analyzed by HPLC with UV detection in plasma and liver samples for all animals.

### Results

The resistance status of the strain had a definite effect on the survival rate at day 14 and on the time-to-death. Resistance was L120Q > Y139C=Y139F, as confirmed by mean time to death and elevation of PT. Daily consumption of chlorophacinone was fairly consistent for all strains and during the entire study period with a mean of 3.0 mg/kg/day (LD50: 2.1 mg/kg). Accumulation of chlorophacinone was clearly dose-dependent for the first week (i.e. day 4), at least for the parent compound (Figure 1).

Accumulation seemed to stop during the second week in all strains except L120Q. No significant differences could be detected between strains after 4 days. At Day 9 or 14, differences were noted, especially related to the time of death, which was clearly strain dependent. Overall, L120Q rats accumulated higher concentrations of chlorophacinone in the liver than other resistant strains and susceptible rats (incomplete data at the time of abstract submission). Metabolites accumulated somewhat in the liver but with no apparent dose-dependent relationship. Among the four different durations of exposure (days 1, 4, 9 and 14) accumulation occurred to a less significant extent. (Plasma and some liver samples are still being analyzed at the time of submission of this abstract).



**Fig. 1** Liver accumulation of chlorophacinone (Chloro) after 1 week in resistant rats (data combined for all strains).

### Discussion

Published work regarding AVK accumulation in resistant rats is very limited so far. Atterby et al. (2005) compared whole-body residues of the L120Q strains (both Hampshire and Berkshire, the latter being resistant to difenacoum) and found no significant difference between susceptible and resistant strains. In their study, the authors showed that whole body concentrations peaked after 3 days and remained constant over 20 days of feeding. These results are quite different from ours, but the study design was also very different: we chose chlorophacinone, because all the strains were known to be strongly resistant and we analyzed only the liver, known to be the cumulative site of contamination of many mammalian species, rather than whole bodies. Since the liver is also rapidly consumed by predators, this choice appeared more appropriate to investigate potential ecotoxicological impacts of the anticoagulant. Our results clearly show that resistant rats may carry more AVK in their body than susceptible rodents, therefore they represent a greater risk to predators and scavengers feeding on them.

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## Resistance as a factor in environmental exposure of anticoagulant rodenticides - a modelling approach

Daniells, L., Buckle, A., Prescott, C.V.

School of Biological Sciences, Harbourne Building, University of Reading, Berkshire, RG6 6AS, UK, l.j.daniells@pgr.reading.ac.uk

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### Abstract

Anticoagulant rodenticide (AR) resistance in Norway rat populations has been a problem for fifty years, however its impact on non-target species, particularly predatory and scavenging animals has received little attention. Field trials were conducted on farms in Germany and England where resistance to anticoagulant rodenticides had been confirmed. Resistance is conferred by different mutations of the VKORC1 gene in each of these regions: tyrosine139cysteine in Germany and leucine120glutamine in England. A modelling approach was used to study the transference of the anticoagulants into the environment during treatments for Norway rat control. Baiting with brodifacoum resulted in lower levels of AR entering the food chain via the rats and lower numbers of live rats carrying residues during and after the trials due to its lower application rate and efficacy against resistant rats. Bromadiolone and difenacoum resulted in markedly higher levels of AR uptake into the rat population and larger numbers of live rats carrying residues during the trials and for long periods after the baiting period. Neither bromadiolone nor difenacoum provided full control on any of the treated farms. In resistant areas where ineffective compounds are used there is the potential for higher levels of AR exposure to non-target animals, particularly predators of rats and scavengers of rat carcasses. Thus, resistance influences the total amount of AR available to non-targets and should be considered when dealing with rat infestations, as resistance-breakers may present a lower risk to wildlife.

Keywords: anticoagulant rodenticides, environmental exposure, residues, resistance

### Introduction

Anticoagulant rodenticides (ARs) are used globally to control pest rodent infestations. Resistance to ARs first appeared against warfarin and diphacinone in Scotland in 1958 (Boyle, 1960). In the wake of this, more potent ARs were produced but resistance has since developed to some of these (RRAG, 2010). Monitoring residues in carcasses of predators and scavengers in the UK has shown that ARs may affect a range of non-target species, including some of high conservation value (Burn and Carter, 2002; Shore et al., 2005). The risk to wildlife depends on several factors, including AR loading in individual rats and total AR residue in rat populations. The impact of resistance on these factors is less well understood. Resistant rats are able to consume some rodenticides without dying and so may carry body burdens of active substance at higher levels and for longer than rats that are susceptible to the poisons (Atterby et al. 2005; Brakes and Smith, 2005). Using data from field trials against resistant rats and modelling the movement of active substance in the rat population we show that this is indeed the case and rodenticide-resistant areas could be a particular hazard with regard to non-target exposure.

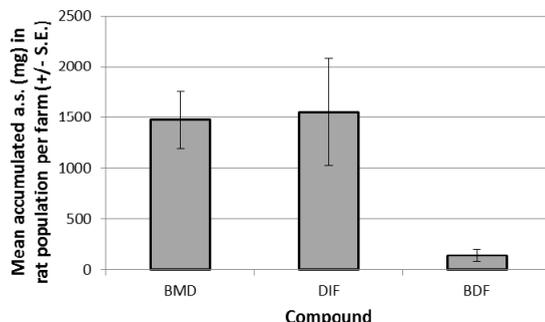
### Materials and methods

Field trials of three ARs (brodifacoum, bromadiolone and difenacoum) were conducted on farms in North-west Germany and Southern England between 2005 and 2010 where resistance to bromadiolone and difenacoum in Norway rats (*Rattus norvegicus*) was confirmed. Rat population censuses were carried out pre- and post-baiting. AR baits were applied according to product labels, pulsed-baiting was used for brodifacoum and surplus baiting for bromadiolone and difenacoum (Buckle, 1994). All baits contained 0.005% w/w of the respective active substances. A model was created utilizing the data obtained from these trials to predict the amounts of the AR active substances in different environmental compartments through the course of the trials.

### Results

Across all trials brodifacoum was the most effective compound reducing rat populations to less than 1% of their original size. Bromadiolone and difenacoum had lower rates of success as would be expected in

resistant areas (29% reduction to population growth and 13-84% reduction per farm respectively). Our model showed that bromadiolone and difenacoum use in these trials resulted in much higher levels of AR entering the food chain via the rats than the use of brodifacoum (Figure 1). It also showed that AR continued to enter the rat population at a much higher rate throughout the trials where control was less effective. The model predicted that after the end of baiting live rats carrying residues of AR were present for more than 10 times as long for bromadiolone and difenacoum trials than for brodifacoum trials.



**Fig. 1** Mean amounts of anticoagulants taken up by rat populations baited with each compound: bromadiolone (BMD, n=6 trials), difenacoum (DIF, n=4) and brodifacoum (BDF, n=2), over the whole farm. BMD and DIF trials showed much higher uptakes over the course of the trials than BDF trials.

## Discussion

The use of potent AR ‘resistance-breakers’ (including brodifacoum and flocoumafen) is sometimes avoided due to their higher toxicity and potential to be more hazardous in the environment (Eason et al., 2002). However in areas where practitioners seek to control resistant rodent infestations, their use may pose less of a risk than applications of ineffective baits. Compounds to which rodents are resistant to do not provide effective control and create a long-term source of AR to enter the environment. The higher quantities of AR used show that using ineffective compounds may extend both the period and severity of exposure to non-target animals to ARs. Conversely the use effective use of resistance-breakers to control anticoagulant rodenticide-resistant rat populations results in lower environmental exposure of ARs for non-targets. Of course, the relative toxicity of the different ARs will also play an important part in overall risk assessments.

## Acknowledgements

The data from the field trials used in the computer modelling were kindly provided by the Rodenticide Resistance Action Committee and Syngenta AG.

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## VKOR and anticoagulant resistance – mutations, models and mechanisms

Müller, C.R., Rost, S.

Department of Human Genetics, University of Wuerzburg, Wuerzburg, Germany

crm@biozentrum.uni-wuerzburg.de

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Coumarin derivatives, e.g. warfarin, are in world-wide use for rodent pest control since they effectively repress blood coagulation. However, rodent populations developed resistance soon after the introduction of such compounds. Today, in many countries, effective pest control is hampered by the rapid spread of coumarin-resistant rodent populations. Chemically related compounds to those using in rodent control are the main class of drugs used for treatment and prevention of thrombo-embolic events in humans.

VKORC1, the warfarin-sensitive enzyme active in the reduction of vitamin K epoxide has been identified as the key component of the vitamin K redox cycle and the target of coumarin drugs (Li et al., 2004; Rost et al., 2004). Mutations in VKORC1 have been shown to confer resistance (*in vivo* and *in vitro*) to anticoagulants in humans as well as in laboratory and wild-caught *R. norvegicus* and *M. m. domesticus* (Pelz et al., 2005; Rost et al., 2009). Mutant animals and populations have been found world-wide. Apparently, VKORC1 mutations affecting different amino acid positions have arisen independently in different resistance areas. A single sequence variant in the VKORC1 promoter has been identified as the major genetic determinant of coumarin dosage requirement in humans (Oldenburg et al., 2007; Rieder et al., 2005).

Recently, X-ray crystallography has allowed delineating the three-dimensional structure of a bacterial homologue of VKOR. The resulting model can explain the topology of this membrane-bound protein and the mode of action of most mutations observed so far (Li et al., 2010).

VKORC1-like genes and proteins are present in organisms from all kingdoms of life. Apparently, vitamin K, and VKOR activity, are not only used for the carboxylation of proteins. Kinetic and expression studies of VKORC1-L1, the human paralogue of VKORC1, have shown that ancestral VKORs may play an important role in neutralizing reactive oxygen species which are generated during all oxidative reactions (Westhofen et al., 2011).

The presentation will review and update our present understanding of VKOR and anticoagulant resistance.

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## **Anticoagulant resistance in the UK and a new guideline for the management of resistant infestations of Norway rats (*Rattus norvegicus* Berk.)**

Buckle, A.

School of Biological Sciences, Harbourne Building, University of Reading, Berkshire, RG6 6AS, UK,  
Chairman, UK Rodenticide Resistance Action Group, a.p.buckle@reading.ac.uk

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### **Abstract**

Anticoagulant resistance was first discovered in UK Norway rats (*Rattus norvegicus* Berk.) in 1958 and has been present ever since. The possible detrimental impact of resistance on effective rodent control was quickly recognised and, for almost three decades, extensive research was conducted on the geographical distribution and severity of anticoagulant resistance in UK rats. Various schemes for the eradication of resistant rats were also implemented. At first, surveys showed resistance only to the first-generation anticoagulants, such as warfarin, chlorophacinone and coumatetralyl, but later resistance to the more potent second-generation anticoagulants, such as difenacoum and bromadiolone, was also discovered. Unlike some European countries, where only one or two resistance mutations occur, virtually all known rat resistance mutations occur in the UK and five are known to have significant impacts on anticoagulant efficacy. Little is currently known of the geographical extent of anticoagulant resistance among Norway rats in the UK because no comprehensive survey has been conducted recently. At an operational level, anticoagulants generally retain their utility for Norway rat control but it is virtually impossible to control resistant rats in some areas because of restrictions on the use of the more potent resistance-breaking compounds. This paper describes the development of resistance in Norway rats in the UK, outlines the present situation for resistance management and introduces a new resistance management guideline from the UK Rodenticide Resistance Action Group (RRAG, 2010).

Keywords: anticoagulant resistance, resistance management, Norway rat, resistance mutations:Y139C, Y139F, Y139S, L120Q, L128Q

### **Background**

Effective rodent control in the UK relies upon the anticoagulant rodenticides but resistance to them is widespread. Early surveys of resistance in Norway rats (*Rattus norvegicus* Berk.) revealed foci scattered across much of England, as well as parts of Scotland and Wales. One such survey, conducted in the years to 1972, showed resistance in 14 separate locations (Greaves and Rennison, 1974). The largest of these was an area on the Anglo-Welsh border, which became one of the most well-known and extensive UK resistance foci. Another substantial focus was first discovered in 1968 in Kent and East Sussex. It remained at least until 1974, when field trials of difenacoum were conducted there, but then was lost to sight. Among the other locations only one, first discovered in Hampshire in 1969, came to be a practical problem over a significant area (Greaves and Cullen-Ayres, 1988). It was thought that many of the other resistance foci recorded in 1974 had either died out naturally or had been removed by efforts at eradication (Greaves, 1995) but subsequent events have shown that neither was likely the case.

### **Resistance testing and resistance mutations in the UK**

Laymen who hear the term 'resistant' often think that it is synonymous with the word 'impervious'. In other words rats said to be resistant to a particular anticoagulant cannot be killed by it. This is very rarely so and all resistance testing requires a degree of qualitative interpretation. This was certainly the case with the feeding test used to distinguish resistant from susceptible rats in the early work of Greaves and Rennison (1974) and is even more so with the blood-clotting response (BCR) test which came to replace it (Prescott et al., 2007). A degree of certainty was provided, however, when a new genetical test was developed for resistance by workers based in Germany (see Pelz et al., 2005). For the first time it became possible to examine the DNA of individual rats and know for certain if they possessed a genetical resistance mutation. However, detailed biological work is still required to determine the practical impacts on operational rat control of a resistance mutation identified by DNA sequencing. Nevertheless, this test has revolutionised the study of anticoagulant resistance in the UK, and world-wide. A list of DNA mutations found at UK rat resistance foci, and locations of some of their foci, is given in Table 1. Knowledge of resistance in UK continues to increase and a new focus, or more likely an old one that had

remained undetected for many years, was recently identified in Kent with a DNA mutation not previously found in the UK (Prescott et al., 2011).

**Tab. 1** Known anticoagulant resistance mutations in Norway rats in UK (from Pelz et al., 2005; Prescott et al., 2011)

Resistance mutation	Abbreviated mutation name	Where present
Leucine128Glutamine	L128Q	Central Southern Scotland, Yorkshire, Lancashire
Tyrosine139Serine	Y139S	Anglo-Welsh border
Leucine120Glutamine	L120Q	Hampshire, Berkshire
Tyrosine139Cysteine	Y139C	Gloucestershire, Norfolk, Lincolnshire, Yorkshire, SW Scotland
Tyrosine139Phenylalanine	Y139F	Kent
Phenylalanin63Cysteine	F63C	Cambridge/Essex
Arginine33Proline	N33P	Nottinghamshire

### UK Rodenticide Resistance Action Group (RRAG) Guidelines

Laboratory and field studies conducted previously in the UK, and elsewhere, provide an understanding of the effects of some of these mutations on operational rat control. The practical impacts of two (F63C, N33P) are unknown. Among the remainder, two mutations (L128Q, Y139S) confer resistance to the first-generation anticoagulants but are largely susceptible to compounds of the second-generation, such as difenacoum and bromadiolone. These compounds are recommended for use against them and, more generally, anticoagulants are effective for rat control in the UK. However, three widely-distributed mutations (L120Q, Y139C, Y139F) confer a degree of resistance to bromadiolone and difenacoum. Resistance management is compromised at sites with these mutations because UK regulations, unlike those proposed under the Biocidal Products Directive, restrict potentially effective anticoagulants, brodifacoum, difethialone and flocoumafen, to use 'indoors', making them virtually useless for rat control. The reason for this restriction is concern about secondary poisoning of wildlife (Carter and Burn, 2000). Consequently, rat control practitioners continue to use bromadiolone and difenacoum at sites where they are resisted. This situation, in existence for about 25 years in the UK, has probably exacerbated the severity of anticoagulant resistance and promoted its spread (Greaves, 1995). RRAG Guidelines (RRAG, 2010) state the obvious, namely that anticoagulant compounds should not be used where there is resistance to them. Where bromadiolone and difenacoum resistance occurs, and rats cannot be controlled by other means, a procedure is proposed whereby those wishing to control resistant rats apply to the UK Health and Safety Executive to use brodifacoum, difethialone or flocoumafen 'in and around buildings'. It remains to be seen whether this proposal is acceptable to UK authorities and whether the applications can be administered in a timely and cost-effective manner.

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## Investigation of the current status of anticoagulant resistance in UK Norway rats by VKORC1 genotyping

Clarke, D.J.<sup>1</sup>, Prescott, C.V.<sup>2</sup>

<sup>1</sup>Department of Chemical & Biological Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK, d.j.clarke@hud.ac.uk

<sup>2</sup>School of Biological Sciences, University of Reading, Berkshire, RG6 6AS, UK

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### Abstract

Anticoagulant rodenticide resistance in Norway rats was first reported in the 1950s in Scotland (Boyle et al., 1960) and has been recorded in a number of other foci in England and Wales from the 1960s to present. Up until the mid-1990s resistance was monitored in trapped live rats using lethal feeding period (LFP) and blood clotting response (BCR) tests (Myllymaki, 1995). However, in 2004 the identification and sequencing of the vitamin K epoxide reductase (VKORC1) gene that confers resistance in Norway rats and House mice (Rost et al., 2004) made it possible to identify resistant animals in the laboratory using the new molecular DNA sequencing technology. As DNA can be extracted from tissue taken from the tip of the tail of recently killed animals, there is no longer a requirement for trapping and testing live animals. Pilot testing of small numbers of UK rats by Pelz and colleagues in 2005 and 2009 (Pelz et al., 2005; Rost et al., 2009) revealed 5 mutations associated with resistance from samples of frozen tissue archived from wild rats caught in the 1990s and a further 2 mutations from lab strains generated from wild founder animals caught in the 1960s and 1990s. Substantially higher numbers of animals have been genotyped in Belgium, Denmark, France and Germany (Grandemange et al., 2009; Rost et al., 2009). In contrast to France and Germany where spatial mapping of hundreds of resistant animals has occurred using this new technique, in the UK we have a very limited current knowledge of the distribution and prevalence of the different genotypes that cause resistance. Only two areas (Cambridge and Kent) have had more than 2 animals analyzed (Prescott et al., 2011; Rost et al., 2009). This report describes the VKORC1 genotyping of over 150 wild rats in the UK from 2007 to date, from areas close to known resistance foci as well as new locales where resistance has not been reported before. The results reveal a more detailed spatial mapping of resistance mutations in the UK that will contribute to the future management of rat populations in the UK.

Keywords: anticoagulant resistance, DNA sequence, mutations, Norway rat, UK

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## Distribution and consequences of VKORC1 polymorphisms in Germany

Runge, M.<sup>1</sup>, Von Keyserlingk, M.<sup>1</sup>, Braune, S.<sup>1</sup>, Freise, J.<sup>2</sup>, Eiler, T.<sup>3</sup>, Plenge-Bönig, A.<sup>4</sup>, Becker, D.<sup>4</sup>, Pelz, H.-J.<sup>5</sup>, Esther, A.<sup>5</sup>, Rost, S.<sup>6</sup>, Müller, C.R.<sup>6</sup>

<sup>1</sup>Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Veterinary Institute Hannover, Germany, martin.runge@laves.niedersachsen.de

<sup>2</sup>LAVES, Task Force, Pest Management, Oldenburg, Germany

<sup>3</sup>Landwirtschaftskammer, Oldenburg, Germany

<sup>4</sup>Institute for Hygiene and Environment of the Free and Hanseatic City of Hamburg, Germany

<sup>5</sup>Julius Kühn-Institut, Institut für Pflanzenschutz in Gartenbau und Forst, Wirbeltierforschung, Münster, Germany

<sup>6</sup>Department of Human Genetics, University of Würzburg, Germany

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### Abstract

Derivates of 4-hydroxycoumarin have been used worldwide as rodenticides since the 1950s. These rodenticides inhibit blood clotting by repression of the vitamin K-reductase reaction. Only a few years after the first application of anticoagulant rodenticides resistant Norway rats were detected. Nucleotide polymorphisms within the vitamin K-reductase complex subunit 1 (VKORC1) locus were found to provide the genetic basis for resistance to anticoagulants within Norway rats and other commensal rodents throughout the world (Rost et al., 2004, Pelz et al., 2005). The occurrence of rodents resistant to anticoagulants during the last decades led to increasing difficulties in the control of rodent populations, with consequent associated hygiene problems and possible increased risk of infection of humans and livestock by epizootic and zoonotic pathogens. Therefore, different monitoring programs were conducted in Germany and the consolidated results of these studies are presented here.

Since 2004, samples of Norway rats were screened for the Tyr139Cys polymorphism to define resistance areas in the German Federal States of Lower Saxony, Saxony-Anhalt, Berlin, Schleswig-Holstein, Hamburg and North Rhine-Westphalia. A special monitoring program was implemented in Lower Saxony to obtain more detailed information on the occurrence of resistant rats. Therefore, since 2008, Norway rats obtained as by catch of muskrat trapping, as well as rats caught purposely for the study from rural or urban areas, were investigated for sequence changes in the gene VKORC1. At some sites rat feces were also examined for resistance polymorphisms.

The Tyr139Cys polymorphism, within the VKORC1 locus, was detected by a newly-developed real-time PCR method using minor groove binding probes. In total, about 660 samples from Lower Saxony were investigated. 3.8% of these samples showed a heterozygous Tyr139Cys mutation within the VKOR gene and 4.7% showed an equivalent homozygous mutation.

Sequencing of all samples confirmed the prevalence of Tyr139Cys as the most abundant polymorphism in Germany. However, two further polymorphisms, Ala26Thr (also known from England) and Ser79Phe, were identified in single populations in Saxony-Anhalt and Berlin. The resistance effects of these polymorphisms have yet to be investigated. In the present paper we present the geographical distribution and consequences of the Tyr139Cys polymorphisms in Norway rats in terms of management issues and the risk of outbreaks of zoonotic diseases.

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## Antidotal potential of specific diets in Norway rats

Jacob, J.<sup>1</sup>, Freise, J.F.<sup>2</sup>

<sup>1</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppheideweg 88, 48161 Münster, Germany, jens.jacob@jki.bund.de

<sup>2</sup>LAVES Lower Saxony, Veterinary Task-Force, Department of Pest Control, PF 39 49, 26029 Oldenburg, Germany

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Some commensal rodent species can cause significant damage to stored produce and infrastructure and transmit pathogens to humans, livestock and companion animals. The most common approach to manage over-abundant populations of commensal rodents is the use of anticoagulant rodenticides. In several rodent species, including Norway rats (*Rattus norvegicus*), genetic resistance to anticoagulant compounds occurs throughout the world. This can hamper the success of control operations. In addition, predators and scavengers can be at elevated risk because poisoned, resistant prey individuals can carry considerable amounts of anticoagulant residues.

Some nucleotide polymorphisms in the vitamin K reductase complex subunit 1 (VKORC1) gene are associated with rodenticide resistance. They are also related to several pleiotropic effects such as increased vitamin K requirement in Norway rats. It is possible that the uptake of dietary vitamin K from food sources that are present on farms mitigate these effects and/or acts as an antidote to anticoagulant compounds.

In this study the preference for food rich in vitamin K1 was assessed in Norway rats that were either susceptible or resistant to bromadiolone due to the homozygous nucleotide polymorphism Tyr139Cys (Y139C). In addition, the effect of vitamin K1 consumption from several food sources on blood clotting in bromadiolone-resistant and susceptible adult male Norway rats was assessed. In preliminary trials, the effect of the consumption of corn silage on blood clotting time was tested in bromadiolone-dosed Y139C rats.

Rats did not generally prefer the consumption of food sources rich in vitamin K1. Interestingly, there was no effect of vitamin K1 uptake on blood clotting times. However, the uptake of four out of five corn silages prevented a rise in blood clotting times that otherwise occurred in vitamin K-deficient bromadiolone resistant Y139C rats. This was apparently due to compounds with vitamin K activity present in silage.

Preliminary trials indicated that the effect on blood clotting of a dose of bromadiolone equivalent to nine times the ED<sub>50</sub> was significantly lower in bromadiolone-resistant rats that consumed silage versus than in resistant rats that were fed standard rodent pellets. This effect was not evident in bromadiolone-susceptible individuals.

Possible antidotal effects, and the prevention of vitamin K related pleiotropic effects in Y139C rats, may contribute to the maintenance and spread of nucleotide polymorphisms related to rodenticide resistance. Corn silage is becoming increasingly available to rats due to the expansion in bio-energy generation from corn silage. Therefore, there may be an increase in the occurrence of anticoagulant-resistant Norway rats and associated problems in agro-ecosystems in the future.

## **Distribution and frequency of VKORC1 sequence variants conferring resistance to anticoagulants in *Mus musculus***

Pelz, H.-J.<sup>1</sup>, Rost, S.<sup>2</sup>, Müller, E.<sup>2</sup>, Esther, A.<sup>1</sup>, Ulrich, R.G.<sup>3</sup>, Müller, C.R.<sup>2</sup>

<sup>1</sup>Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppeideweg 88, 48161 Münster, Germany, hans-joachim.pelz@jki.bund.de

<sup>2</sup>Department of Human Genetics, University of Würzburg, Biozentrum, Am Hubland, 97074 Würzburg, Germany

<sup>3</sup>Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, 17493 Greifswald - Insel Riems, Germany

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House mouse tissue samples from 30 populations in Germany, Switzerland and the Azores were analyzed for sequence changes in the gene VKORC1, which potentially confer resistance to anticoagulant rodenticides. Except for one population originating from south Germany, sequence variants were found in individuals from all locations analyzed (29 out of 30 sites surveyed), with less than 10 % of the individuals matching the wild-type genotype. The most frequent and widespread amino acid substitutions were Leu128Ser, Tyr139Cys and a group of linked sequence changes (Arg12Trp/Ala26Ser/Ala48Thr/Arg61Leu). These three genotypes occurred either alone or in combination with each other or with other less frequent sequence changes. Where they occurred as the sole variant, the proportion of homozygous animals was 72-83 %, suggesting a high selection pressure due to permanent pest control in these populations.

An evaluation of published data revealed that the three frequent sequence changes found are associated with a substantial loss of rodenticide efficacy of first generation anticoagulants (e.g. warfarin, coumatetralyl) as well as the second generation compound bromadiolone and most probably also difenacoum. Further studies are required to investigate the effect on compounds of higher potency, in particular, where combinations of sequence changes occur in one individual.

## Adaptive introgressive hybridization with the Algerian mouse (*Mus spretus*) promoted the evolution of anticoagulant rodenticide resistance in European house mice (*M. musculus domesticus*)

Song, Y.<sup>1</sup>, Endepols, S.<sup>2</sup>, Klemann, N.<sup>3</sup>, Richter, D.<sup>4</sup>, Matuschka, F.-R.<sup>4</sup>, Shih, C.-H.<sup>1</sup>, Nachman, M.W.<sup>5</sup>, Kohn, M.H.<sup>1</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology, Rice University, Houston, Texas 77005, USA, hmkohm@rice.edu

<sup>2</sup>Bayer CropScience AG, Environmental Science, D-40789 Monheim, Germany

<sup>3</sup>Spillenweg 3, D-48231 Warendorf, Germany

<sup>4</sup>Division of Pathology, Department of Parasitology, Charité Universitätsmedizin, Berlin, Germany

<sup>5</sup>Department of Ecology and Evolution, University of Arizona, Tucson, Arizona, USA

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### Abstract

Adaptive introgressive hybridization refers to the natural transfer of genes after interspecific mating between species, and subsequent expression of these genes in the recipient species as a new trait that confers selective advantages. Conceptually, the process harnesses immense potential to explain rapid evolution of traits. However, the process requires that reproductive isolation be overcome. Here we present a case of the adaptive introgression of anticoagulant rodenticide resistance from the Algerian mouse (*Mus spretus* Lataste, 1883) to the Western European house mouse (*M. musculus domesticus* Linnaeus, 1758). These once allopatric species have come into secondary geographic contact and hybridize occasionally. However, only for an approximately ~20.3 megabase-sized genomic fragment, carrying the vitamin K 2,3-epoxide reductase subunit 1 gene (*vkorc1*) of *M. spretus* (*vkorc1<sup>spr</sup>*), has hybridization resulted in introgression into the *M. m. domesticus* genome.

This *vkorc1<sup>spr</sup>* allele carries amino-acid substitutions, conferring resistance to anticoagulants, has evolved under positive selection in *M. spretus* ( $Ka/Ks=1.54-1.93$ ) and displays adaptive population genetic dynamics in *M. m. domesticus* populations. Since its natural inception <60 years ago, this novel form of pesticide-resistant mice has spread far into the range of *M. m. domesticus*. Other *vkorc1* resistance alleles now known to occur in European house mice originated either by *de novo* mutation or from standing genetic variants. Recombinants between these and introgressed alleles are now emerging, but the role of such novel alleles in resistance has yet to be established. Our snapshot of the ongoing adaptive introgression of *vkorc1<sup>spr</sup>* illustrates how hybridization, coinciding with strong selection with anticoagulants, resulted the breakdown of reproductive barriers between *M. spretus* and house mice. Pest control should anticipate the possibility of horizontal gene transfer between closely related rodent species as a mechanisms leading to rodenticide resistance.

Keywords: genetic introgression, hybridization, vitamin K epoxide reductase subcomponent 1, *vkorc1*

### Introduction

Anticoagulant rodenticides inhibit the vitamin K 2,3-epoxide reductase complex and thereby block blood coagulation, such that susceptible house mice (*M. m. domesticus*) consuming the bait succumb to haemorrhage. Anticoagulant rodenticides, notably warfarin, which have been in use since 1950, locally have lost their effectiveness. Single point mutations in the vitamin K epoxide reductase subcomplex 1 (*vkorc1*) gene are known to form the basis of this resistance in mice, as well as in Norway rats (*Rattus norvegicus* Berkenhout, 1769) (Li et al., 2004; Pelz et al., 2005; Rost et al., 2004, 2009). Warfarin resistance is a classic mammalian case of microevolution by mutation that can be observed directly in the field. Newer rodenticides, including bromadiolone, were developed to counteract such resistance. Here we report on a population level survey in Europe that discovered that, in addition to point mutations in *vkorc1*, anticoagulant resistance in European house mice has evolved within the last 60 years by genetic introgression of *vkorc1* from *M. spretus* (*vkorc1<sup>spr</sup>*) into the genome of house mice. We characterize the *vkorc1<sup>spr</sup>* introgression, show that it can mediate resistance, and we report on its selective advantage among field populations of house mice.

## Materials and methods

To determine the genetic mutations, we conducted DNA sequencing of the *vkorc1* gene in >100 mice from Europe following methods as described in Song et al. (2008). We determined the amount of genetic material in house mice derived from *M. spretus* by conducting a scan of chromosome 7 and sequencing 18 genes, including the *vkorc1* gene and its 5' region. We conducted population genetics tests to infer the selection history of the introgression. Finally, we conducted molecular evolutionary analyses of the *vkorc1* gene in mice. In order to assess the susceptibility of mice to anticoagulants, feeding trials were conducted with wild-derived strains of house mice from Germany carrying the complete version of *vkorc1<sup>SPR</sup>*. The testing protocol followed OPP 1.204 (US EPA, 1991) standards and was conducted under the German equivalent of IACUC (Institutional Animal Care and Use in Research) protocols.

## Results

We showed that genetic variants have been introgressed from *M. spretus* into *M. m. domesticus* over ~20.3 megabases (Mb) on chromosome 7, including *vkorc1*. The introgressed *vkorc1<sup>SPR</sup>* (and recombinants thereof) has high frequency (>80%) in areas where *M. spretus* and house mice occur sympatrically (e.g. Spain), and remains abundant in areas where only house mice occur (e.g. Germany, ~33%). The *vkorc1<sup>SPR</sup>* was not detected in mice from England, Scotland, Italy and Greece. House mice carrying the complete *vkorc1<sup>SPR</sup>* displayed reduced susceptibility to three anticoagulant rodenticides when compared to house mice carrying the wild-type copy of the gene (mortality rates to coumatetralyl and bromadiolone were 20% and 9%, respectively). In contrast, *M. m. domesticus* carrying wild-type *vkorc* displayed mortality rates of 84-100% to coumatetralyl and 85% to bromadiolone. Finally, 20% of *M. m. domesticus* carrying complete *vkorc1<sup>SPR</sup>* survived difenacoum trials, whereas all *M. m. domesticus* with wild-type *vkorc1* died when fed on difenacoum bait.

The *vkorc1* is one of the fastest-evolving genes in the *M. spretus* genome, and we showed that this rapid evolution took place after *M. spretus* had split from its congeneric species of *Mus*. A Ka/Ks ratio >1 indicates that this rapid evolution was due to positive selection on amino acids in the *M. spretus* lineage.

## Discussion

Adaptive protein evolution of *vkorc1* in the *M. spretus* lineage was driven by unknown ecological factors, but the combination of amino-acids in *vkorc1<sup>SPR</sup>* protect *M. spretus* against anticoagulants (Baumler and Asran, 1987), i.e. is have resistance as a pleiotropic effect. Horizontal transfer of *vkorc1<sup>SPR</sup>* to house mice transfers this trait. Apparently, low levels of inter-specific gene flow (through fertile female hybrid offspring) between *M. spretus* and house mice has been sufficient to enable the transfer of the anticoagulant rodenticide resistance gene during the past 60 years since the introduction of anticoagulant rodenticides.

The geographic origin of the introgression likely is in Southwestern Europe or in Northern Africa, where *M. spretus* is sympatric and on occasion apparently forms hybrids with *M. musculus* (Orth et al., 2002). Two independent evolutionary trajectories, both involving the *vkorc1* gene, have led to the adaptation to selection pressure applied by anticoagulant rodenticides in house mice: point mutation and adaptive introgressive hybridization.

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## Field trials to assess resistance to warfarin and difenacoum of house mice in relation to the occurrence of variants in the *vkorc1*-gene before and after the treatments

Endepols, S.<sup>1</sup>, Klemann, N.<sup>2</sup>, Song, Y.<sup>3</sup>, Kohn, M.H.<sup>3</sup>

<sup>1</sup>Bayer CropScience AG - Environmental Science - Innovations, 40789 Monheim, Germany, stefan.endepols@bayer.com

<sup>2</sup>Warendorf, Germany

<sup>3</sup>Rice University, Houston, USA

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### Abstract

House mice (*Mus musculus domesticus*) vary considerably in their susceptibility to anticoagulants, and several non-synonymous sequence variants in the coding region of the vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*) were found in Germany (Rost et al., 2009). It was the aim of our study to characterize the degree of resistance in relation to *vkorc1* genotypes in local mouse groups, and to test whether certain genotypes were selected by sequential treatments with the two anticoagulant rodenticides warfarin and difenacoum.

Two successive treatments were conducted, the first with bait containing warfarin followed by a second using difenacoum. Their effects were determined on local sub-groups of mouse infestations in different sub-units on two livestock farms in Westphalia, Germany. The frequency of different *vkorc1* genotypes, as determined by Sanger sequencing, was considered relative to the rodenticide treatment results for each sub-group and sampling period.

Three tolerance types were identified on farm one: A=warfarin-susceptible, B=resistant to warfarin, but susceptible to difenacoum, C=approx. one half of animals resistant to both anticoagulants. On farm 2, only type A and B were identified. A high degree of resistance was observed in *vkorc1* wild-type mice. In all cases, only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice. Hence, in these mouse infestations, practical resistance to anticoagulants was not accompanied by any identifiable *vkorc1* resistance marker.

The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International.

Keywords: difenacoum, house mouse, *Mus musculus domesticus*, resistance to anticoagulants, rodent control, rodenticide treatment, *vkorc1*, warfarin

### Introduction

House mice (*Mus musculus domesticus*) vary considerably in their susceptibility to anticoagulants. In contrast to rats (*Rattus norvegicus*) in Northern Germany, where virtually all anticoagulant resistant rats carry a Y139C mutation in vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*), several other non-synonymous coding sequence variants of the *vkorc1* appear to segregate in mouse populations from Germany including the Y139C variant (Rost et al., 2009). However, with the exception of the Y139C mutation, little information from field studies is available on the susceptibility to anticoagulants in mouse strains marked by these variants, and on the effect of practical treatments with rodenticides. In the present study, the effectiveness of two anticoagulant rodenticides was investigated in two populations of the house mouse suspected to harbor resistant animals. Genetic analysis of *vkorc1* was used to provide information about type and frequency of *vkorc1* sequence variants occurring in sub-units of the populations before and after the treatments. The aim was to characterize the degree of resistance in relation to genotypes in local mouse groups, and to test whether certain *vkorc1* genotypes were selected by the treatments, i.e. increased or decreased in allele frequency as a result of rodenticide use.

The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International and MHK and YS were partially funded by National Institute of Health grant R01.

## Materials and Methods

Two successive treatments with anticoagulant rodenticides were conducted and monitored for their effect on local sub-groups of mouse infestations in the different sub-units on two livestock farms in Westphalia, Germany. Grain baits containing 0.05% warfarin for the first treatment, and 0.005% difenacoum for the second treatment, were systematically distributed at certain structural elements according to the standard rodent control program BayTool for 28 days ([www.baytool.info](http://www.baytool.info)). Bait consumption was recorded during regular visits to the treated sites, and the effect of the treatments was determined by census baiting. Mice were trapped prior to, between the two treatments, and after the treatments for tissue sampling (tail clips). Since most non-synonymous mutations of *vkorc1* in mice were found in the first and third exons (Rost et al., 2009), the two segments were amplified using the primers and conditions described in Song et al. (2008). All PCR products were cleaned by ExoSAP-IT (USB, Cleveland, OH) and sequenced with the Sanger-method for the detection of coding sequence variants in the gene. The frequency of *vkorc1* genotypes was put into relation to the rodenticide treatment results for each sub-group and sampling period.

## Results

Mice consumed 3.0 kg of warfarin bait and 1.2 kg of difenacoum bait during the first trial, and 3.7 kg and 1.6 kg respectively of the two baits during the second trial. The level of resistance differed markedly between sub-units on both farms (Table 1). Three tolerance types were identified on farm one: A=warfarin-susceptible, B=resistant to warfarin, but susceptible to difenacoum, C=approx. one half of animals resistant to both anticoagulants. On farm 2, only type A and B were identified.

**Tab. 1** Results of anticoagulant treatments according to sub-units in 2 trial sites.

Site 1			Site 2		
Tolerance type (sub-unit No.)	Survival rate (%)		Tolerance type (sub-unit No.)	Survival rate (%)	
	Warfarin	Difenacoum		Warfarin	Difenacoum
A (1)	7.2	0	A (1)	7.1	0
B (2)	92.9	0	B (2)	104.0	7.2
C (3)	59.3	94.5	B (3)	92.0	5.7

We obtained *vkorc1* sequences for 41 mice on farm 1 and 54 mice on farm 2. Only one non-synonymous mutation R58G was found in exon 1 in all the sequenced mice from the two farms. On farm 1 almost all mice (n=39) were *vkorc1* wild-type, and only 2 mice carried the heterozygous mutation R58G (i.e. allele frequency 2.4%). On farm 2, all mice were homozygous for R58G in tolerance type A (n=18, warfarin-susceptible, sub-unit 1). 12 mice trapped in sub-unit 2+3 were wild-type, 6 were homozygous and 18 were heterozygous for R58G. Thus, we observed a high degree of resistance in *vkorc1* wild-type mice. Only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice.

## Discussion

Resistance to warfarin was present in both mouse populations studied. Resistance to difenacoum occurred only in one sub-unit on one farm. Even there, no *vkorc1* variant was found that could explain *vkorc1*-mediated resistance, at least at the level of protein coding mutations. We therefore conclude that resistance to anticoagulants can be manifested in field populations of mice without the presence of *vkorc1* coding mutations. In addition to the comparatively well-understood *vkorc1*-mediated resistance (e.g., Y139C variant), our results suggest the possible involvement of other genes and/or other genetic mechanisms (e.g., gene regulatory or epigenetic changes) in anticoagulant resistance in mice. It is also conceivable that food rich in vitamin K mediated a fully or partially non-genetic form of resistance.

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## Fluctuation and fixation of rodenticide resistance alleles in *Rattus norvegicus*

Berthier, K.<sup>1</sup>, Benoit, E.<sup>2</sup>, Berny, P.<sup>2</sup>, Lasseur, R.<sup>2</sup>, Merville, A.<sup>2</sup>, Peigneaux, F.<sup>2</sup>, Cosson, J.-F.<sup>1</sup>

<sup>1</sup>Centre de Biologie pour la Gestion des Populations, INRA, Campus International de Baillarguet, CS30016, 34988, Montpellier-sur-Lez, France, karine.berthier@supagro.inra.fr

<sup>2</sup>Ecole Vétérinaire de Lyon, INRA, BP 83, 69280, Marcy l'Etoile, France

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### Abstract

Worldwide control of commensal rodents relies on anticoagulant rodenticides (AVKs). As with many other pesticides used continuously over long periods of time, the target species, in particular Norway rats (*Rattus norvegicus*) and House mice (*Mus musculus domesticus*), are increasingly becoming resistant. Several allelic forms of the gene involved in the resistance (VKORC1) have been recently identified in *R. norvegicus* (Grandemange et al., 2007, 2009, 2010). The occurrence and frequency of resistance alleles in rodent populations depends on selection pressures, such as the frequency and intensity of anticoagulant rodenticide treatments, and rodent population dynamics, including spatial structure, population size and dispersal. In commensal situations, the spatial distribution of favorable conditions for the establishment of rat infestations depends on the density and configuration of the human habitat. In an agricultural landscape rats will occur as a metapopulation, with sub-populations mainly located within the farmsteads, where resources are most easily available. The mean dispersal distance for commensal rats across non-commensal habitats is known to be highly restricted, i.e. 300 meters (Le Louarn and Quéré, 2003). Passive dispersal may however occur over longer distances due to human transportation (Le Louarn and Quéré, 2003). In such a context, the genetic structure of rat populations will be shaped by interplay between migration (gene flow), genetic drift and local selection (cost and benefit of the resistance). To disentangle the relative influence of these three evolutionary forces on the fluctuation and fixation of resistance alleles, we compared the genetic structure of rat populations at the VKORC1 gene and microsatellite markers (Bryja et al., 2007). This comparison was firstly done in a metapopulation context, considering two groups of seven and eleven farms, respectively, located near the city of Lyon in France. Rats were trapped and genetic material collected within each farm before a rodenticide treatment was carried out at one of the farms. A second sampling was then conducted to follow the restoration/recolonisation of the rat population that had undergone the rodenticide treatment. In a further experiment, the same comparisons will be carried out for an isolated population, using samples collected from an island in Brittany, before and after the population was treated with rodenticides.

Keywords: microsatellites, population dynamics, *Rattus norvegicus*, resistance, rodenticides, selection pressure

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## Characteristics of the local distribution of the Y139C resistance gene in Norway rats (*Rattus norvegicus*) in a focus of resistance in Westphalia, Germany

Klemann, N.<sup>1</sup>, Esther, A.<sup>2</sup>, Endepols, S.<sup>3</sup>

<sup>1</sup>Spillenweg 3, D-48231 Warendorf, Germany, nicole.klemann@t-online.de

<sup>2</sup>Julius-Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Münster, Germany

<sup>3</sup>Bayer CropScience AG - Environmental Science, Monheim, Germany

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### Abstract

The Westphalian resistant Norway rat strain is characterised by the possession of the Y139C variant of the *vkorc1* gene, and practical resistance occurs in rat infestations at different frequencies to the anticoagulants warfarin, coumatetralyl, bromadiolone and difenacoum. Within the present study we investigated whether there was an obvious pattern in the distribution of resistance in relation to the distance to an identified hot spot of resistance from the site of sampling and whether the frequency of the resistance gene was connected with local conditions, such as rodent control history.

Rats were trapped at a single infested site in each of 12 1km x 1km squares in a line including a resistance hot spot. Tissue samples were taken from all trapped rats, and genotyped for the Y139C variant of the *vkorc1* gene. The frequency of the resistance gene was determined for each site sampled. Data were also collected about rodent control measures applied in the past and other relevant local conditions.

The frequency of the resistance gene varied considerably between < 20% and > 80%. There was no obvious correlation of the frequency of the resistance gene and the distance to the hot spot, and there was no increase or decrease of the gene frequency in west-east direction. Permanent baiting and poor rodent control practice seemed to increase the incidence of resistance in the respective site. The implementation of good rodent control practice is recommended to prevent an increase in the frequency of resistance.

The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International.

Keywords: anticoagulant resistance, Norway rat, *Rattus norvegicus*, rodent control, rodenticide treatment

### Introduction

The Muensterland/Westphalia focus of anticoagulant resistance has been well investigated in terms of the extent of the area, where resistant Norway rats may appear, the genetics of the resistance gene, and the impact of resistance on the practical outcome of treatments using bromadiolone and difenacoum (Pelz et al., 1995; Rost et al., 2004; Endepols et al., 2011). The Westphalian resistant rat strain is marked by the Y139C variant of the *vkorc1* gene, and practical resistance occurs at different frequencies to the anticoagulants warfarin, coumatetralyl, bromadiolone and difenacoum. Studies investigating the nature of resistance were performed only on farms, which were peculiar for their rat control problems, obviously being hot spots of resistance. Such hot spots might be centers from where resistant rats disperse. In the present study, it was investigated whether there was an obvious pattern of the distribution of resistance correlating with the distance to such a hot spot. Further, it was investigated whether the frequency of the resistance gene was connected with local conditions such as geography and rodent control history in the respective site. The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International.

### Materials and Methods

A livestock farm known to be a hot spot of resistance was selected and situated at the centre of 12 squares, each measuring 1km x 1km, distributed in a west-east line near a small town in the eastern part of the Muensterland resistance area. In every square, a site was located with a rat infestation which permitted the trapping of at least 10 rats. We tried to avoid the selection of sites with extensive rat problems, but to find sites with less conspicuous rat infestations.

Tissue samples were taken from all trapped rats, and genotyped for the Y139C variant of the *vkorc1* gene by the amplification refractory mutation system (ARMS)-PCR test. The frequency of the resistance gene was determined for each site. Data were collected about rodent control measures applied in the past, local geographical conditions, characteristics of livestock keeping and other relevant criteria.

## Results

The Y139C *vkorc1* gene was found at all of the sites investigated. The frequency of the resistance gene varied considerably between < 20% and > 80%. There was no obvious correlation between the frequency of the resistance gene and the distance from the site of sampling to the central hot spot, and there was no increase or decrease of the gene frequency in either a westward or eastward direction from the hot spot.

Of all parameters noted for each site, those of rat control practice appeared to be most connected with the frequency of resistance. On one livestock farm, permanent baiting was performed, and all rats there carried the resistance gene. Those sites with good rodent control practice employed showed the lowest frequency of resistance.

## Conclusions

The frequency of Y139C anticoagulant resistance varies much within short distances between infested sites within the Muensterland/Westphalia focus of anticoagulant resistance, e.g. less than one kilometer, which is less than the distance a rat may move in one night (Taylor and Quay, 1978). It is therefore difficult to make any conclusion about the incidence of resistance at one site based on resistance tests conducted on a site nearby. Most of the sites sampled in this study were livestock farms. Permanent baiting and poor rodent control practice seemed to increase the incidence of resistance at the respective site. The implementation of good rodent control practice is recommended to prevent an increase in the frequency of resistance. It is recommended that, if an anticoagulant is applied and found to be not fully effective due to resistance, surviving rats at treated sites should be removed entirely using one of the more potent and effective anticoagulants.

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## Small mammal communities in agricultural landscapes in Germany: review of field data over the last decade

Von Blanckenhagen, F., Städtler, T.

RIFCon GmbH, Im Neuenheimer Feld 517, 69120 Heidelberg, Germany, felix.vonblanckenhagen@rifcon.de

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### Abstract

Little is known about general composition of small mammal communities in agricultural land in Germany. Most published data represent only a few months' data in a specific habitat type focussing on a small region. This presentation will review data from several studies performed in the last decade of almost every year in agricultural land across different regions in Germany. Data on the distribution of small mammal species in landscapes dominated by agricultural land including cropped fields, meadows and adjacent field structures like hedgerows, woodland and field margins are presented. The data were collected during different investigations regarding diverse topics studied and are summarized for this presentation for the first time. The presented data may help to interpret the spatial and temporal composition and distribution of small mammal communities in agricultural landscapes.

Keywords: agricultural landscape, Germany, small mammals, species composition, species distribution,

### Introduction

Published information about small mammal communities in agricultural landscapes in Germany is mostly restricted to a short time and a small spatial scale. Therefore, a large data set collected during different investigations spread over Germany is summarized. The presented data will show spatio-temporal patterns and distribution of small mammal communities in agricultural landscapes in Germany.

### Materials and methods

The data set consists of 111 independent trapping grids distributed over 7 federal states in the years 2001-2010 (excluding the years 2004 and 2009). 11 of these trapping grids were especially designed for trapping of shrews and did not provide data of small rodent species. Trapping was mainly conducted with multi-capture live traps (Ugglan traps, Grahnab), which were partially adapted to the specific topics of some studies. The data set covers the months January-November. To get comparable values across all studies conducted, trapping efficiency (TE; captured ind./100 trap checks), was calculated. Trapping during all studies was performed in agricultural fields and adjacent off-crop habitats. For the presentation these data were assigned to the following habitat types: (1) agricultural field, (2) skirt of wood, (3) shrubbery (including hedge structures) and (4) grassland (including meadows, pastures and the ground plant cover in orchards).

More than a quarter of a million trap checks were conducted, >150,000 in agricultural fields, >60,000 in grassland and >40,000 in shrubbery and skirts of wood. The temporal scale of the data will be presented according to meteorological seasons.

### Results

The trapping data revealed 18 different species found in agricultural landscapes. The habitat which contains most species was the skirt of wood (14 species), followed by agricultural fields (13 species) and shrubbery and grassland (9 species each). Thirteen species belonged to the order *Rodentia*, 4 to *Insectivora* and 1 to *Carnivora*. Considering mean TE, the four most abundant species were bank vole (*Myodes glareolus*, TE 14.2), yellow-necked mouse (*Apodemus flavicollis*, TE 10.6), common vole (*Microtus arvalis*, TE 6.7) and wood mouse (*A. sylvaticus*, TE 4.6). For further considerations and analysis only these species were evaluated, providing a sufficient data base.

In the skirt of woods, bank vole and yellow-necked mouse were the key species. At spring, bank voles, yellow-necked mice and also the wood mouse showed similar trapping efficiencies (6.7, 7.3 and 7.7 respectively). But in summer and autumn, bank vole (TE 38.2) and yellow-necked mouse (TE 25.8) were dominating in 'skirt of woods'.

The habitat category shrubbery with diverse microhabitats showed high numbers of bank voles (TE 15.5) and wood mice (TE 17.1) in spring, which changed in summer and autumn when bank vole (TE 27.1 and 21.9, respectively) yellow-necked mouse (TE 15.3 and 19.0, respectively) showed highest abundance.

Grassland habitats were dominated by the common vole. In autumn, also the other species were trapped but TEs were much lower.

In agricultural fields the trapping efficiency was about a tenth compared with the other habitats. The common vole and the wood mouse showed the highest trapping efficiency here. In agricultural field in spring the wood mouse had the highest TE and in summer, TE of the common vole (TE 3.0) was twice as high compared to the wood mouse (TE 1.3). In autumn, the numbers of common vole (TE 2.1) and wood mouse (TE 2.0) were nearly equal. The yellow-necked mouse and the bank vole showed their highest TE in summer in agricultural fields (TE 1.0 and 0.8, respectively).

The wood mouse population showed a decline in TE in summer in the habitats shrubbery and skirt of woods but an increase in agricultural fields. In grassland, the wood mouse was trapped in spring and summer only occasionally.

Considering the population dynamics (i.e. yearly maximum TE) common vole, bank vole, yellow-necked mouse and wood mouse followed the same general pattern. Furthermore, for the year 2005 TEs of the common vole of 3 distant regions were available, which show strong regional differences within the same season.

## Discussion

Based on the data set, the small mammal community in agricultural landscapes in Germany was dominated by four rodent species. As expected, grassland habitats are dominated by common voles, and in agricultural field in spring the wood mouse had the highest TE and the second highest following the common vole in summer and autumn. The small mammal communities described are in concordance with data from the Czech Republic (Heroldova et al., 2007), where the dominating species in forest were the yellow-necked mouse and the bank vole. Also for 'windbreaks' Heroldova et al. (2007) showed the same species (yellow-necked mouse, wood mouse and bank vole) as in the similar habitat 'shrubby' in our data set. The dominance of common voles and wood mice in agricultural habitats is confirmed by results of Heroldova et al. (2007) and Boye (2003), and our findings about small mammal communities reflect the species habitat preferences given e.g. in Braun and Dieterlen (2005). Interestingly, the populations of the wood mouse (i.e. TEs in 'shrubby' and 'skirt of woods') declined in summer, whereas TE in agricultural habitats increased. This finding supports the hypothesis of Ouin et al. (2000) that dispersal towards crops could be the main factor explaining population drops in hedge like habitats. The yearly population development had the same tendency for the herbivorous common vole and the pronounced granivorous bank vole, wood mouse and yellow-necked mouse, as known from Flowerdew et al. (2004), Giraudoux et al. (1994), Flowerdew (1985), which have different habitat and food preferences. This might be an indication for species unspecific factors influencing the whole small rodent community. However, on the regional scale difference in yearly common vole abundance can be strong.

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## Population dynamics and dispersal patterns of common voles (*Microtus arvalis*)

Leukers, A.<sup>1,2</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppeideweg 88, 48161 Münster, Germany, angela.leukers@jki.bund.de

<sup>2</sup>Institute for Landscape Ecology, Westphalian Wilhelms-University Münster, Robert-Koch-Straße 26-28, Germany, 48149 Münster

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### Abstract

Outbreaks of common voles (*Microtus arvalis*) can cause significant losses in agriculture and forestry. To minimise damage it is useful to prevent voles from dispersing from refuges to arable land (Lidicker, 1975; Singleton et al., 2003). To apply timely and spatially targeted management methods, sound knowledge about the distribution patterns of voles at field-refuge-boundaries is required. Field sites for this study, funded 2009-2012 by the German Federal Environmental Foundation (DBU) are located in Saxony-Anhalt, Germany. Circular grassland areas below wind energy plants are used as replicated experimental refuges. Barrier fences were installed in 10 of 16 refuges that allow immigration but prevent emigration. Capture-mark-release revealed a population expansion August 2010. Individual number rose higher in refuges without barrier fences. Mowing in June and September 2010 had a negative effect on vole abundance. Aerial pictures and telemetry studies are used to detect vole distribution onto the field. The analysis of dispersal dynamics will be continued and appropriate management methods will be tested in the last year of the study.

Keywords: capture-mark-release, common vole, dispersal pressure, distribution patterns, management methods, *Microtus arvalis*

### Introduction

At high abundances, common voles (*Microtus arvalis*) can cause significant losses in agriculture and forestry, because they can disperse from refuges (e.g. field edges) to arable land. This study, funded 2009-2012 by the German Federal Environmental Foundation (DBU), aims to investigate population dynamics and distribution patterns of common voles at field-refuge-boundaries as a basis to develop methods for sustainable vole management. Field sites are located in Saxony-Anhalt, Germany. Grassland areas (320 m<sup>2</sup>) below wind energy plants, from which common voles invade fields, are used as experimental refuges (n=16). To measure dispersal pressure, barrier fences were installed at 10 refuges that allow immigration but prevent emigration. Since October 2009, population dynamics and dispersal rate from refuges to fields are surveyed monthly.

### Materials and methods

Capture-mark-release was used to measure population size; body weight, sex and reproductive status of each individual were assessed. Additionally, weather parameters, vegetation height and cover were measured. Radio-telemetry was applied to reveal individual dispersal dynamics to the field. With the help of aerial pictures, possible vole activity on adjacent fields was surveyed to analyze distribution patterns on the population level.

### Results

More than 800 individuals have been marked so far. Recapture probability within a trapping session was >50%. Extrapolated vole density in refuges with barrier fence averaged 150–300 ind./ha (October 2009 to July 2010). In May, reproductive activity started and resulted in up to 850 ind./ha in August 2010 (extrapolated). As predicted by the Chitty-effect (Krebs, 1978), average body weight was highest in the phase of high population density (Burthe, 2010). Population size in refuges without barrier fence was slightly lower in spring, but higher in autumn compared to the fenced areas. Mowing in June and September 2010 reduced vole abundance both in refuges with and without barrier fence remarkably, whereas effects were more distinctive for the latter. In 2010, 20 adult voles were equipped with a radio-collar and released outside the refuge. However, all returned to the refuge within two days. Consequently, no vole activity could be noticed in the field so far by means of aerial pictures. Over-

wintered voles in spring 2011 were on average 5 g heavier compared to voles in the previous year, while the proportion of juveniles increased to 20%.

### Discussion

A reasonable population increase could be detected in August 2010, but did not lead to a dispersal of voles onto the field so far. It can be assumed that the maximum population density in the refuges is not yet reached. The observed negative influence of shortening vegetation on the number of individuals has been shown in other studies before (Jacob and Hempel, 2003). Higher population sizes in refuges without barriers compared to refuges with barrier fence in autumn 2010 could be a result of higher immigration rates. Subsequent DNA-analyses (microsatellites, parentage exclusion) will reveal new individuals in refuge populations. Influence of field cultivation (ploughing, fallow) could be one reason for the lack of established vole populations on the field so far (Jacob and Halle, 2001). The analysis of dispersal dynamics will be continued and appropriate management methods (barrier fences and -furrows, pit fall traps) will be tested in the last year of the study.

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## **Economic evaluation of biological rodent control using barn owls *Tyto alba* in alfalfa**

Motro, Y.

Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, POB 78, Bet Dagan, 50250, Israel, yoavmot@moag.gov.il

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### **Abstract**

Rodents are common pests in various agricultural cultivations. Utilization of barn owls for rodent pest control has long been used. In Israel, the indirect effect of barn owl predation pressure on alfalfa crop yield has been examined. Using radio-telemetry, barn owls were tracked to form a density-distance function, which was later used to estimate predation pressure on whole fields. This function was utilized on all barn owls nesting in the vicinity to assess accumulated predation pressure on fields, which was compared to crop yield in contemporary alfalfa harvests. Results show that barn owl presence has a positive effect on alfalfa crop yield, enhancing crops by 3.24% and allowing a net profit of 30 US\$/hectare-year. These results are important since they encourage farmers to use this environmentally friendly, healthy method owing to agricultural-economical considerations, thereby evading the environmental-financial conflict.

Keywords: alfalfa, barn owl, biological control, economy, Israel, predation pressure, rodent,

### **Background**

Rodents are major pests to a variety of agricultural crops (Stenseth et al., 2003). Of the rodent pests in Israel, levant voles (*Microtus guentheri*) are major pests in field crops, with alfalfa (*Medicago sativa*) being, perhaps the main victim due to a number of reasons: Year-round fresh vegetation, nutritiousness, perennality, summer irrigation and lack of soil cultivation during crop growth period (Moran, 2003). Vole populations can accumulate in alfalfa to thousands of burrow openings per hectare and harm crops severely (Motro et al., 2010). Chemical poisoning is dangerous and quite inefficient due to the palatability of the crop itself. The use of wild barn owls (*Tyto alba*) in artificial nesting boxes for the control of rodents has been suggested a few decades ago and is implemented in many regions of the world (Charter et al. 2010; Meyrom et al., 2009; Motro et al., 2010; Taylor, 1994). Alas, it has never been shown before that this environmentally friendly control regime is cost effective.

### **Methods**

The study site was in Kibbutz Sde-Eliyahu, Israel (32°30'N, 35°30'E). 58 nesting boxes for barn owls were erected in 1983-1996, and their annual occupation and success have been monitored since. A total of 429 alfalfa harvests were examined in 21 fields over 10 years (1999-2008). The predation pressure was calculated by fitting 16 of the owls with radio-telemetry and tracking their movements (White and Garrott, 1990) to form a function of their occurrence probability by distance from the nest (Venables and Ripley, 2002). The predation pressure was integrated on all the fields' area and accumulated for all active nesting boxes during the specific harvest period. This gave a total predation pressure estimate for a certain field in a certain point in time. The total predation pressure was then compared to the crop yield of the harvest of the same field at the same time.

### **Results**

In this study, the effect of predation pressure on rodents by barn owls was shown to have significant positive effect on alfalfa crop yield and on the financial income for the farmer. Among other factors analyzed, predation pressure was found to have a statistically significant positive effect on alfalfa crop yield, enhancing crops by 440 kg/hectare-year which consists of 3.24% of the annual production. The associated revenue increase amounted to 100 US\$/hectare-year. Attributing the costs associated with installation and maintenance of all the 58 nesting boxes to the 41.8 hectares assigned in an average year to alfalfa production at Sde-Eliyahu, one obtains a net benefit of 30 US\$/hectare-year. Further modeling studies have shown that alfalfa outputs would convexly increase with predation pressures, meaning that better nest box layout may have an even greater effect on rodents and crops.

## Discussion

These results show that despite the apparently low contribution in terms of alfalfa yields, and the fact that the potential contribution of the nesting boxes to the yields of other crops is completely ignored, rodent control by barn owls is found to be profitable. The use of rodenticides was not calculated as a substitute, since their ineffectiveness discourages farmers from using them. Two other important elements that have not been considered here are the environmental and health issues. The reason for this is that the aim of this research is to verify whether this control method is profitable for the farmer, not for the environment or the society. These results are important because they encourage farmers to utilize this method and gain profit even without the intervention of the authorities (by subsidies, laws or fines) and avoiding the environmental-financial conflict from their aspect: they use an environmentally friendly control method – and save money.

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## **Voles and boreal silviculture – overview of damage and options for management**

Huitu, O.<sup>1</sup>, Henttonen, H.<sup>2</sup>

<sup>1</sup>Finnish Forest Research Institute, Suonenjoki Unit, Juntintie 154, FI-77600 Suonenjoki, Finland, otso.huitu@metla.fi

<sup>2</sup>Finnish Forest Research Institute, Vantaa Unit, P.O. 18, FI-01301 Vantaa, Finland

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Voles of the genera *Microtus* and *Myodes* have become increasingly severe pests in Finnish silviculture during recent decades. During this time, the level of damage caused by voles has clearly surpassed that caused by ungulates, the previously most notorious of vertebrate forestry pests. It was estimated that during the most recent population density peak in the winter of 2008/09 voles destroyed ca. 36 million tree seedlings in the southern half of Finland. The collective area of total damage encompassed 20,000 hectares, and that of minor damage, such as eating the apical buds of conifer seedlings, roughly three times more. Financial losses due to replanting were estimated to near 20 million euros. The degree of damage caused by voles is first and foremost influenced by vole density. In Finland, vole populations fluctuate cyclically in three-year periods, albeit not synchronously over the whole country. Damage to seedlings occurs almost exclusively during winters immediately succeeding peak vole densities. The functional explanation for this appears to be that high density vole populations deplete their preferred winter food resources, grasses and herbs, and thereafter resort to consuming tree seedlings despite their poorer dietary quality. Silvicultural practices also affect the relative susceptibility of seedling stands to vole damage. For example, different tree species are differentially preferred by voles, small seedlings are more prone to damage than larger seedlings, and heavily fertilized seedlings are of higher nutritive value to voles, thus predisposing them to consumption. Intensive mechanical practices such as soil preparation, removal of slash and ground vegetation control all tend to reduce the quality of seedlings stands as habitat for voles, thus reducing the likelihood of population densities reaching destructive levels. Despite the severity of damage caused by voles, few management actions are currently widely employed by forest owners. The currently most often recommended modes of management are 1) timing of planting to years when a vole population peak is not imminent, 2) adequate soil preparation prior to planting, 3) mechanical or chemical control of grasses in seedling stands, 4) protection of seedlings by shelters and/or repellents, and 5) reduction of vole numbers in late autumn by trapping or poisoning. The Finnish Forest Research Institute is carrying out extensive laboratory and field experimentation on the applicability of each of these management actions, as well as on a suite of other topics with potential to contribute to the reduction of vole-induced damage in boreal managed forests.

Keywords: population cycle, seedling, silviculture, vegetation control, vole damage

## Trap-tubs as a means of vole-damage reduction in afforestations

Krüger, F., Jarchow, D.

Department of Forest Protection, Northwest German Forest Research Station, D-37079 Göttingen, Germany, frank.krueger@nw-fva.de

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Trap-tubs are unbaited plastic buckets with one-way doors that allow voles to enter the tub and predators to consume the voles. Trap-tubs have been suggested as a means of vole control by Niemeyer et al. (1996; 1999). They reported a considerable amount of voles caught throughout the year. However, there was no proof that constant trapping positively affected the survival rates of trees in the forest-plantation.

At different sites in Lower Saxony, in 2003 three newly planted and fenced afforestation areas were chosen as trial plots. Each plot was divided into two halves, each  $\geq 1$  ha. In each pair, one of these halves was equipped with trap-tubs on a 30 x 30 m grid, resulting in a promedium of 11 tubs per ha and the other halves were untreated controls.

Trap-tubs were installed adjacent to a resting-perch for birds of prey, thus fixing the tubs to the ground and to facilitate removal of caught voles by those birds as well as inspection and maintenance by the investigators.

Twice a year the tubs were cleaned from debris and the entrances were checked for functionality. In autumn, fresh cuttings of apple twigs were placed in the plots to estimate the abundance of epigeous voles according to bite marks (Krüger 1996). In spring, a sample of 100 forest plants was checked for vole damage. In the last inspection (2011) all remaining trees were counted to establish survival rates.

The trap-tubs had a measurable effect on trees' survival rates in general. Yet more importantly: the disappearance of those tree-species considered highly preferred by voles, and generally present only as rare admixtures, was reduced a great deal in the treated areas (survival rate treatments: 55-90%; survival rate untreated control: 17-61%). Economically, the investment paid off at 5% interest rate or just with a small positive result at 3% interest rate already after a period of 8 years. In the case of a necessary replanting of the damaged trees the investment in this management system is even more justified.

Keywords: afforestation, *Microtinae*, *Microtus*, *Myodes*, permanent trapping, trap-tub, vole damage

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## Surveys of Scottish farmers and their vertebrate pests – case study from a long running dataset

Hartley, G., Campbell, S.

Pesticides and Wildlife, Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh EH12 8NJ, UK, gill.hartley@sasa.gsi.gov.uk

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### Abstract

Data on perceived pests to farmers were collected annually since 1998, during predominantly face-to-face interviews with the farmer as an adjunct to routine pesticide usage surveys. During each year of the survey, particular farm types were selected for surveying, and data are presented for arable and grassland and fodder crop farms. A case study examines the impact of the withdrawal of strychnine, previously used as a method of mole (*Talpa europea*) control, on the pest status of the mole in Scotland. The paper also discusses the advantages and disadvantages of this long-running data set.

Keywords: agriculture, moles, pests, strychnine, surveys

### Introduction

Since 1998, as an adjunct to pesticide usage surveys, SASA has been interviewing farmers in Scotland to ask them about their vertebrate pest problems. This has yielded a long-term data set of the perceptions of farmers regarding those species they consider pests, the areas where problems most often occur, and their approach to control. While there are several limitations to this data set, there are some advantages, not least being the consistent approach to questioning and the time scale over which surveys have been conducted. This can allow an assessment of the changing pest status of a species over time, and has particular importance when a key method of control is lost.

Strychnine was once the main method of mole control in the UK. However, in September 2006 approval for the use of this pesticide was lost. This data set was used to assess changes in the perception of farmers to moles, both regionally and nationally, and also examines changes to the methods used to control moles as a consequence of the loss of strychnine.

### Materials and methods

Due to differences in the use of pesticides according to the farm type, biennial surveys of arable farmers were conducted since 1998, but only three surveys of grassland and fodder crop farmers conducted in 2002, 2005 and 2009. Farms were selected on a randomised-stratified design based on land-use region and size group. Farmers were presented with a list of mammalian and avian species and asked “which of these are a pest on your farm and why; which of these identified pests do you attempt to control, and what methods do you use?” Finally, farmers were asked to rank their top three worst pests. Regions were amalgamated into three super-regions to allow for small sample sizes. Logistic regression analysis for binomial data was applied to the number of farmers who reported a particular feature (e.g. those that applied mole control); the model has allowed for over-dispersion in the data.

### Results

There was no evidence of a widescale increase in the distribution of mole problems across the two farming sectors, either since the surveys began, or since the withdrawal of strychnine in 2006. There was weak evidence of an increase in the seriousness of mole problems within both farming sectors, although the role of strychnine withdrawal in this effect was equivocal. With respect to management methods, the use of fumigation and trapping increased substantially over recent years, although trapping was more popular than fumigation, especially among fodder crop farmers. There was also an increase in the proportion of farmers (in both sectors) with a mole problem, who chose not to control moles subsequent to the withdrawal of strychnine.

On average, more farmers in Central and Southern regions (East Fife, Lothian, Central Lowlands, Tweed Valley, Southern Uplands and Solway) reported mole problems than farmers from the other two regions. In Northern areas of Scotland (Highlands and Islands, Orkney and Caithness and Moray Firth) the

perception of mole problems by farmers was most variable, while in Eastern areas of Scotland (Aberdeenshire and Angus), fewer farmers on average reported mole problems in the period up to 2006. However, the number of farmers reporting mole problems increased dramatically in Eastern areas subsequent to the loss of strychnine.

### **Discussion**

The results from these surveys indicate the pest status of the mole based on the perception and experience of the farmer being interviewed. This opinion can reflect the economic importance of the mole, which along with the cost, availability and simplicity of control methods, will influence the farmers' decision to undertake control. However, the data should be interpreted with caution, since other factors can also play a role in forming opinions and the need to control a species.

It has been speculated that the withdrawal of strychnine would lead to an increase in mole problems, particularly since the alternative methods are either more expensive or more difficult to apply (Quy and Poole, 2004). However, there was no overwhelming evidence to support this in the current analysis, and farmers were recorded as increasing their use of traps, and to a lesser extent fumigation, as methods of control. Nonetheless, the greater number of farmers choosing not to control moles may indicate a reluctance or inability of farmers to make use of alternative, but more costly, management methods. In the long-term, the effects of reduced management may result in palpable evidence of an increase in the distribution and seriousness with which moles are perceived within key agricultural sectors.

There are several reasons that may explain the regional differences in reported mole problems across Scotland. Future surveys will allow an assessment of the long-term impacts of the loss of strychnine on mole management and on any changes to the importance of the mole as an agricultural pest, regionally and nationally.

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## **Distribution, abundance and damages caused by European beavers (*Castor fiber*) in Polish forests**

Borowski, Z., Borkowski, J.

Department of Forest Ecology, Forest Research Institute, Braci Lesnej 3, 05-090 Raszyn, Poland,  
Z.Borowski@ibles.waw.pl

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Based on data from the National Beaver Census, carried out in Poland in 2000-2001, and using data from Polish State Forestry and National Parks we analysed the distribution and abundance of the beavers in Poland and damages caused by this species in Polish forests. The beaver has been recorded in 252 Forest Districts (out of 413) and 13 National Parks (out of 19). Based on collected data we estimate the approximate size of beaver population to be 14,500 individuals. Tree damage caused by cutting and debarking by beavers show clear seasonal pattern, they were recorded in the autumn-winter period. Intensity of such damage was correlated with the number of recorded beavers. Tree damage caused by cutting and debarking occurred in 25% of forest districts settled by beavers, while flooding was observed in 29%. Tree species that beavers preferred the most were: oak (*Quercus* sp.), willow (*Salix* sp.) and birch (*Betula* sp.), whereas black alder (*Alnus glutinosa*), Scotch pine (*Pinus sylvestris*) and lime (*Tilia* sp.) were the least preferred. Based on low intensity and spatial distribution of damages we concluded that this species does not generate a strong negative impact on Polish riparian forests.

Keywords: beaver abundance, beaver distribution, *Castor fiber*, forest ecosystem, tree damage

## Behavioral responses of voles along fences patrolled by natural predators

Fuelling, O., Buehler, E., Airoidi, J.-P., Nentwig, W.

Division of Community Ecology, Institute of Ecology and Evolution, Baltzerstrasse 6, CH-3012 Bern, Switzerland, olaf.fuelling@uni-muenster.de

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### Abstract

In a two year field experiment in Switzerland we tested if vole barriers combined with traps was a suitable tool to protect meadows and orchards from vole damage. We used a special kind of vole live trap that could be opened by terrestrial predators to feed on the captured animals. The experiment was designed to compare fences with traps, fences without traps and control lines without fences or traps. Predators preferred to move along fences with traps, which has been presented elsewhere. Voles on the other hand showed a preference for control lines as a general pattern but clear effects were masked by other factors. In practice, however, self service traps will attract natural predators and can therefore enhance the effect of simple fences to stop invading voles.

Keywords: *Arvicola*, damage, *Microtus*, orchards, plant protection, predation, rodents

### Introduction

In European landscapes voles (*Microtus ssp. Arvicola ssp.*) are abundant and highly reproductive mammals. They can cause severe damage in agricultural areas (Walther et al., 2008) and are therefore often regarded as pests. Vole control measures, biological as well as conventional ones, can be locally effective but voles re-colonise the attractive habitats within a short time. Barriers to stop invading voles have been tested with different results (Witmer, 2007; Walther and Pelz, 2006). These rodents, however, are not only a pest to farmers but also an important resource for numerous predators (Halle, 1993). Consequently the question arises whether the combined effects of physical vole barriers and the threat by natural predators are able to protect high value crops (Malevez and Schwitzer, 2005). To test this hypothesis a two year field trial in Switzerland was done.

### Materials and methods

The experiment was carried out from November 2006 until December 2008 at three sites in central and western Switzerland. Eschenbach (E) and Oensingen (O) are located in the Swiss Midland whereas Saignelégier (S) is in the Jura Mountains. All three sites had grassland mainly used for pasture, as well as grass and hay production. At each site three lines of 150 m length were defined. The first and the second lines' fences were made of 12x12 mm wire mesh, reaching 40 cm above and 20 cm below ground. The third line was a control with no major obstacle for moving voles. The difference between the first and the second line was a set of custom made live traps. The traps had two entrance doors for voles and a top which could be opened by terrestrial predators to take the captured voles as easy prey. 20 of these self service traps were set on both sides of the fences at each trial site. To measure the vole activity above ground, 10 cm high obstacles were placed right angled to the fences and control lines. 22 obstacles were set along each line. An obstacle had two tubes to count vole passages (according to Halle and Lehmann, 1987). Below ground activity of the voles was measured by heat sensors carefully placed in the vole tunnels.

The above and below ground activity counts were pooled to 15-day intervals for all three sites and analysed by the programme Permanova (Anderson, 2001), a non-parametric ANOVA method based on permutations of the data. Analyses were performed to determine the effect of the factors site (three levels: E, O, S; fixed), fence type (three levels: fence+traps, fence alone, control; fixed), year (two levels: year 1, year 2; fixed) and time of day (two levels: day, night; fixed) on aboveground activity of voles and activity of predators.

### Results

Signs and trapping results revealed that *Arvicola amphibius* was dominant in Eschenbach, *Microtus arvalis* in Oensingen, and in Saignelégier both species were abundant. The Permanova analyses showed that the recorded above ground activity was significantly different between all sites and line types, with

$p=0.001$  in an a posteriori test for sites and  $p=0.004$  for line types, respectively. The highest activity was measured in Oensingen, followed by Saignelégier and Eschenbach. Significantly more activity was measured at controls compared to fences with traps and fences alone. In addition, the interaction term of site x line type was also significant ( $p=0.001$ ). For below ground activity the same procedure was applied. There were no significant differences between sites or fence types for below ground activity.

In general the above ground vole activity increased from the first to the second year. This pattern was most pronounced at the control lines. In Saignelégier the activity along fences with traps and fences alone slightly decreased from the first to the second year and increased along control lines during the same period. The a posteriori test revealed that the decrease of activity at fences alone was statistically significant ( $p=0.001$ ).

## Discussion

During this field experiment in Switzerland the behaviour of predators and prey was manipulated by traps and fences set in a grassland habitat. Vole activity was highest along control lines, whereas predators, in the same experimental trial, moved significantly more often along fences with traps than along fences without traps or control lines (Fuelling et al., 2011). This pattern can be explained by assuming that the predators were attracted along fences because of a better hunting success and especially along fences with self service traps. Voles react to this increased predation risk with a decreased above ground activity. Below ground the fences had no effect on the predation risk and therefore no differences in below ground activity of voles could be observed. For voles the interaction between site and line type, however, was significant too but when each location was analysed separately, the pattern was not clear any longer. This might be explained by local differences, e.g. the occurrence of *Arvicola* and/or *Microtus* voles. Furthermore, vole behaviour is driven by more than just predator avoidance. According to Halle (1993), timing of vole activity is probably primarily driven by other factors than predators. His main argument was that in natural conditions no predator free time seems to exist during a day. Additionally, the above ground activity increased from 2007 to 2008. As vole populations are known to show multiannual fluctuations (Krebs, 1996), a population increase might explain this pattern. Nevertheless, the general pattern of increase was not consistent as in Saignelégier vole activity along the two fences decreased while it increased along the control line.

Local species composition, changing population densities in two consecutive years and other natural and anthropogenic factors may have masked the behavioural response of voles. Nevertheless, we conclude that for practical issues fences and especially fences equipped with self service traps are beneficial to protect valued crops like orchards. The physical fence barrier combined with the increased predation risk is a serious obstacle for voles to enter such a protected orchard (Walther and Pelz, 2006; Fuelling et al., 2011) even if the effects on vole behaviour are minor.

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## Recent change in patterns of vole dynamics – for better or for worse?

Gliwicz, J.<sup>1</sup>, Jancewicz, E.<sup>2</sup>

<sup>1</sup>Museum and Institute of Zoology, PAS, Warsaw, Poland, gliwicz@miiz.waw.pl

<sup>2</sup>Dept. Forest Zoology and Game Management, SGGW, Warsaw, Poland

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Over the last decade, several reports on arvicolid rodent populations have presented evidence of a change in lemming and vole dynamics, from regular high-amplitude density oscillations to acyclic fluctuations at relatively low level. Most studies have focused on the arctic region of Europe (above 60° N), and they indicate that the fading of multiannual cycles has been caused by climate warming, which is most pronounced in the winter season. Less persistent snow cover might be responsible for dramatic declines in the numbers of wintering voles. In this report we present data indicating similar changes in open habitats of Poland (49°-54°N). The population dynamics of two vole species, *Microtus oeconomus* and *M. arvalis*, examined over the last 25 years, exhibit marked changes in the pattern of density fluctuation: the fairly regular population fluctuations observed until the late 1980s or early 1990s have subsequently become more erratic, reaching significantly lower levels, on average. When searching for the most probable causes of these changes, we examined which winter climate factors had the greatest influence on the successful overwintering of a population of the root vole *M. oeconomus* in the open marshland of eastern Poland. Using long-term weather data from a local meteorological station, and precise data on root vole dynamics collected over a 12-year period, we found that the duration of snow cover, combined with winter severity and the duration of the thermal winter were the best predictors of the number of winter survivors. According to 48-year weather records, all of these variables indicate a gradual decrease in the severity of winters, with the most apparent change in the duration of snow cover at the end of 1980s. This indicates that climate warming could be responsible for changes in vole dynamics also in more temperate regions of Europe. The main positive effect of the change to lower-level, erratic fluctuations in vole density would be a reduction in the damage they cause to field crops, while the main negative effect may be a decrease in animal species diversity in farmland and in more natural open habitats. We present indirect evidence suggesting that decreasing availability of voles - the main prey species of many predators - has increased predator pressure on alternative prey, mostly small and mid-sized birds and mammals.

Keywords: alternative prey, climate warming, fading cycles, *Microtus arvalis*, *M. oeconomus*, species diversity, winter

## Long-term population dynamics of the field vole from the Czech Republic

Tkadlec, E.<sup>1,2</sup>, Bejček, V.<sup>3</sup>, Flousek, J.<sup>4</sup>, Šťastný, K.<sup>3</sup>, Zima, J.<sup>2</sup>, Sedláček, F.<sup>2</sup>

<sup>1</sup>Department Ecol Envir Sci, Fac Sci, Palacky University Olomouc, Czech Republic, emil.tkadlec@upol.cz

<sup>2</sup>Inst Vertebrate Biology, Ac Sci Czech Rep, Brno, Czech Republic

<sup>3</sup>Fac Env. Sci, Czech Agric Univ, Prague, Czech Republic

<sup>4</sup>Administration of Krkonoše National Park, Vrchlabí, Czech Republic

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Cyclic population dynamics of voles from central Europe have traditionally been documented using data collected in the common vole (*Microtus arvalis*) which is a more relevant vole to farming in this region. However, because this species does not occur in northern Europe, direct comparison of central European dynamic patterns with those in Fennoscandia has never been possible. However, this does not apply to the field vole (*Microtus agrestis*) whose distribution range covers much of Europe from central to northern regions, including Great Britain and Fennoscandia. Here we present long-term data on field vole dynamics from two mountain locations collected regularly twice a year by snap trapping over a period of 25 years from 1986 to 2010. The first time series data come from a study plot in the Ore Mountains (Erzgebirge) situated at the altitude of about 800 m a.s.l., the other one from that in the Giant Mountains (Riesengebirge) situated at the altitude of almost 1,100 m. There were two important features in their dynamic behaviour. First, both populations exhibit second-order dynamics with peaks about at intervals of 4 to 5 years. Second, in both of them there is a declining trend in mean density and cycle amplitude suggesting that cyclic behaviour in central European field voles is fading out in a way similar to voles in Fennoscandia. We tested for the effects of several climatic variables but the results are not consistent. These findings emphasize that population dynamics of central and northern European voles are influenced by the same mechanism which is able to operate on a large geographic scale.

Keywords: climate effects, *Microtus agrestis*, population cycles, time series analysis

## Dynamics and reproduction of small rodents in Germany

Jacob, J.

Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppeideweg 88, 48161 Muenster, Germany, jens.jacob@jki.bund.de

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### Abstract

Analyses of time series of population dynamics and reproduction of small rodent species in Germany show that 1) first order effects prevail and higher order effects occur, 2) that within and across species abundance can fluctuate synchronously and 3) that part of the density dependence may be explained by intra-specific mechanisms related to reproduction.

Keywords: *Microtus arvalis*, *Microtus agrestis*, *Myodes glareolus*, population dynamics, reproduction

### Introduction

The size of populations of small rodents such as common voles (*Microtus arvalis*), field voles (*M. agrestis*) and bank voles (*Myodes glareolus*) fluctuates with a period of about 2-5 years in Germany and in other parts of Europe (e.g., Jedrzejewski and Jedrzejewska, 1996; Luque-Larena et al., this volume; Tkadlec and Stenseth, 2001). During population peaks, these species can cause considerable damage to agricultural crops and forest trees. Crop losses can exceed several 100 million € in Europe per year (Jacob and Tkadlec, 2011). In addition, they can carry and transmit disease to humans and livestock. One recent example is an outbreak of human hantavirus infections in Germany in 2010, when more than 2,000 people were infected (Faber et al., 2010). A thorough understanding of biological and ecological features of fluctuating small rodent populations is vital to improve early warning systems be it for the protection of crops or for the protection of human health. Timely forecasts of rodent outbreaks can raise awareness by stakeholders and aid farmers, foresters as well as health care staff to engage in early management actions.

### Methods

The multi-annual nature of the population dynamics of the rodents involved makes it necessary to use time-series to study patterns and mechanisms of abundance fluctuations and associated phenomena including damage to plants and transmission of zoonotic diseases. Therefore, the hard work of the States' plant protection staff and of the forest workers in obtaining abundance estimates during many decades is highly relevant and highly appreciated.

Data were collected in several locations and pooled by Federal State. For common voles, counts of reopened tunnel entrances were available that were collected by State Plant Protection Institutions in the Federal States of Mecklenburg-Western Pomerania (1990-2008), Saxony-Anhalt (1956-1995) and Thuringia (1971-1998). For bank voles and field voles, data were available from snap-trapping in Bavaria (1980-1989; data kindly provided by W. Bäumler) and trapping conducted by forestry administration in Lower Saxony (1971-2010). In addition, a data set was used that combined snap trap data of common voles and field voles from forestry administration in North-Rhine Westphalia (1972-2005). Information about reproductive performance in spring and autumn (%pregnant, %lactating, number of embryos) was limited to common voles in Saxony-Anhalt (1977-1994). Partial autocorrelations (time series), pair-wise correlations (synchrony of fluctuations) and REML multiple correlations were calculated using JMP 8.0 (SAS Institute 2008).

### Results

In all areas and species concerned, small rodent populations fluctuated considerably. The *s*-index was  $0.41 \pm 0.05$  for common voles,  $0.34 \pm 0.01$  for bank voles and  $0.29 \pm 0.02$  for field voles. Auto-correlation patterns for common voles suggested 3-4 year outbreak periods in Saxony-Anhalt and in combined common vole/field vole abundance from North-Rhine Westphalia but not in Mecklenburg-Western Pomerania. There was also a tendency for bank voles and field voles in Lower Saxony for outbreaks with a 3-4 year period. Time series from Bavaria were too short to extract reliable information.

The rate of increase of vole populations of the same species fluctuated synchronously in adjacent states but there was also synchrony in field vole and bank vole populations that were several hundred km apart. Also, the rate of increase of bank vole and field vole populations fluctuated synchronously within and between states and there was also clear correlation of the rate of increase of field vole and common vole populations across states. The only negative correlation found was between growth rates of *Microtus* voles (no separation of species) in the northwest of Germany and common vole populations in the northeast and in central Germany.

Reproductive parameters within autumn or spring were positively correlated in common voles in the central German state of Saxony-Anhalt where high pregnancy rate was related to high lactation rate and high numbers of embryos. High reproductive activity in spring was negatively correlated to abundance and reproductive performance in the following autumn and the same was true for the relation of reproductive activity in the previous autumn and abundance in the current autumn. The negative relation of reproductive activity and future breeding and dynamics seemed to be more pronounced between previous and current autumn than between spring and autumn and there was a negative correlation between abundance in the previous autumn and population growth to the following autumn.

## Discussion

In the time-series analyzed, examples for first order effects in the population dynamics of common voles, field voles and bank voles in Germany are common. In addition, in some time-series, higher order effects occurred such as in common voles in Saxony-Anhalt and *Microtus* voles in Lower Saxony. This is in concord with other studies of the population dynamics of small rodents in Europe (Luque-Larena et al., this volume; Tkadlec and Stenseth, 2001). Delayed effects did not occur in all time-series of bank vole or field vole abundance. Despite the differences in the cycle pattern, there was generally synchronous fluctuation within and among some species indicating that similar ecological processes govern changes in abundance. These may include several extrinsic and extrinsic factors.

The analyses of breeding and abundance in common voles in Saxony-Anhalt suggest that high breeding performance is negatively correlated to future breeding and abundance. This indicates that at least part of the density dependence may be explained by intra-specific mechanisms related to reproduction. Interestingly, delayed density dependence in fluctuating bank vole populations is related to reproductive phenomena including senescence (Tkadlec and Zejda, 1998) that might also explain the pattern found for common voles in this study.

The results may aid the development of predictive models for forecasting the outbreak risk of small mammal species based on climate (Imholt et al., in press) and weather (Blank et al., in press) by including biological predictors. Such models are relevant for crop protection and/or the protection of human and livestock health.

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## Large-scale range expansion and eruption of common vole (*Microtus arvalis*) outbreaks in agricultural plains of NW Spain: historical reconstruction and novel impacts

Luque-Larena, J.J.<sup>1</sup>, Mougeot, F.<sup>2</sup>, Arroyo, B.E.<sup>3</sup>, Viñuela, J.<sup>3</sup>, Jareño, D.<sup>3</sup>, Arroyo, L.<sup>1,3</sup>, Lambin, X.<sup>4</sup>

<sup>1</sup>Depto. CC Agroforestales, ETSIIAA, Universidad de Valladolid, Campus La Yutera, 34004, Palencia, Spain, j.luque@agro.uva.es

<sup>2</sup>Estación Experimental de Zonas Áridas (EEZA, CSIC), Ctra. de Sacramento s/n La Cañada de San Urbano, 04120 Almería, Spain

<sup>3</sup>Instituto de Investigación en Recursos Cinegéticos (IREC) (CSIC-UCLM-JCCM), Ronda de Toledo s/n 13005, Ciudad Real, Spain

<sup>4</sup>Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Avenue, AB24 2TZ, Aberdeen, UK

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### Abstract

By the end of last century the distribution range of *Microtus arvalis* in NW Spain greatly expanded and outbreaks began to be reported in recently-colonised agricultural habitats. Ever since, novel impacts to regional farming, biodiversity conservation and public health have recurrently been associated to vole outbreaks. Here we present the first attempt to reconstruct recent changes of common vole dynamics and distribution in the NW of the Iberian Peninsula. We compile published information for the last 50 years and describe a new methodological approach based on semi-quantitative data obtained from complementary sources. Our results show how, from late-1970s, the entire lowland plains of Castilla y León region were rapidly (< 20 years) filled in by expanding populations. Analyses of outbreaks also suggest a 5-year cyclic pattern from late-1970s onwards, contrasting with the typical 3-year cycle described in most populations of this species. Our results contribute to improve the current knowledge of rodent dynamics in Europe and may serve as a baseline to local pest management programmes.

Keywords: agriculture, Castilla y León, cycles, *Microtus arvalis*, outbreaks, range expansion, Spain

### Introduction

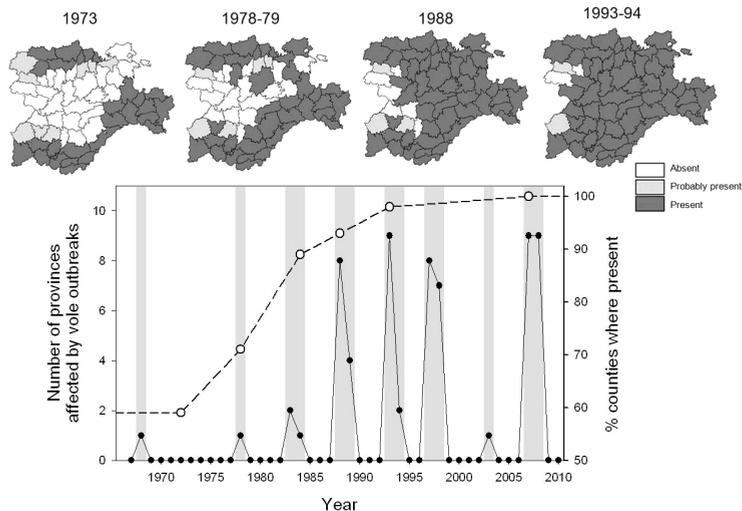
In Europe, *Microtus arvalis* is a major vertebrate pest for plant production that can cause important economical losses to farming during population outbreaks (Jacob and Tkadlec, 2010). Recently, hitherto unseen dynamics of common vole populations have erupted in agricultural areas of NW Spain (Castilla y León region, CyL hereafter). By the end of 20<sup>th</sup> century, periodic vole outbreaks in agricultural plains followed a massive regional-scale colonisation event in CyL (González-Esteban and Villate, 2007). Unprecedented socio-economical impacts are now endemic in recently colonised habitats, including significant crop damage episodes and zoonotic outbreaks. Time series of vole fluctuations are essential for assessing the mechanisms behind outbreaks and planning management practice that could mitigate outbreaks. Unfortunately, the absence of any long-term vole monitoring data impedes quantitative analyses of vole outbreaks in NW Spain. In this study, we build a spatial-temporal model for both range expansion (*regional colonisation process*) and population fluctuations (*outbreak dynamics*) of common voles in Spain. In addition, we evaluate patterns of zoonotic outbreaks and environmental (chemical control campaigns) impacts in the region and their relation to rodent dynamics. We describe a new methodological approach to tackle the historical reconstruction of recent vole dynamics.

### Methods

Reconstruction of vole dynamics from 1960 onwards was based on both quantitative and semi-quantitative data from scientific and other sources, provided they reported explicit, spatial (province, sub-province (i.e., *comarca*)) and/or temporal (year, month), information on common vole presence and/or occurrence of outbreaks (unusually high vole densities). Data were compiled from three different sources: (a) scientific papers published in local (Spanish) peer-reviewed journals (Spanish Scientific Journals: SSJ); (b) national technical reports from annual series on plant protection and pest control (i.e., *Reuniones Anuales de los Grupos de Trabajo Fitosanitarios*) published by the Ministry of Agriculture (Ministry of Agriculture Reports: MAR); and (c) digital archives of daily issues of the main regional newspaper, *El Norte de Castilla*, which is one of the oldest (>150 years) in the country (Norte de Castilla News: NCN).

## Results

*Regional colonisation process:* Maps from SSJ allowed recreating vole expansion dynamics in sub-provinces. Up to early-1970s, *M. arvalis* was limited to mountainous peripheral landscapes in CyL. However, between late-1970s and mid-1990s the entire region was rapidly filled in by expanding populations (>85% of sub-regions with voles during first large-scale outbreak in 1988) (Figure 1). *Outbreak dynamics:* Outbreak data from SSJ, MAR and NCN were highly correlated. Eruption of periodic outbreaks in CyL correlated in time with the range expansion process (Figure 1).



**Fig. 1** Reconstruction of colonisation process and outbreak dynamics in CyL based on SSJ, MAR and NCN

Major outbreaks affected 6 to 9 of the 9 CyL provinces since 1988, although the extent of reported damage varied between provinces. Wavelet analysis based on semi-quantitative data suggests a 5-year cyclic pattern from late-1970s onwards. Spectral and autocorrelation analyses confirmed 5-year outbreak cycles. *Impacts related to rodent dynamics:* Data from NCN show a strong association between outbreaks and rodenticide campaigns, whose effects (e.g., secondary poisoning of non-target fauna) seem to expand for 1-2 years post-outbreak. NCN data also show that the two tularaemia outbreaks in humans officially declared in Spain (1997 and 2007) were both immediately preceded by large vole outbreaks in the region.

## Discussion

Analyses of semi-quantitative data including newspaper archives yielded the following new findings: (1) the first description of long-term time series (> 40 years) of vole fluctuation dynamics in the Iberian peninsula; that represents a new southern limit for outbreaks within the latitudinal range (40°-60°N) where heaviest rodent damage to plant production are most often described in temperate Europe (Jacob and Tkadlec, 2010); (2) evidence that Iberian vole outbreaks are a recent phenomenon that have paralleled a rapid (<20 years) and large-scale (ca.  $5 \times 10^6$  ha) range expansion, and are almost exclusive to recently-colonised agricultural plains with a climate characterized by aestival droughts. We hypothesise that causes for the massive expansion of voles from (humid) mountains to (dry) plains include the increase of irrigated crops in the region; and (3) Iberian outbreaks apparently fit a 5-year cyclic pattern, which contrasts with the typical 3-year cycles described elsewhere (Lambin et al., 2006); common vole populations from CyL offer thus new opportunities to address global mechanisms and causation of rodent cycles (Lambin et al., 2002). We also suggest that common vole outbreaks in NW Spain are a further example of the common link between pest rodent and disease outbreaks in highly altered ecosystems (Ostfeld and Mills, 2007). Finally, we showed a repeated pattern of rodenticide use and environmental impact after each outbreak since 1988. Both science and common sense urges Spain

to upgrade from traditional control strategies towards more scientifically-oriented management paradigms (i.e., *ecologically-based rodent management*).

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## Development of a forecast model for outbreaks of common voles (*Microtus arvalis*) in Germany

Imholt, C.<sup>1</sup>, Blank, B.<sup>2</sup>, Esther, A.<sup>1</sup>, Perner, J.<sup>3</sup>, Volk, T.<sup>4</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppheideweg 88, 48161 Muenster, Germany, christian.imholt@jki.bund.de

<sup>2</sup>Nordhornstr. 55, 48161 Muenster, Germany

<sup>3</sup>U.A.S. Umwelt- und Agrarstudien GmbH, Ilmstraße 6, 07743 Jena, Germany

<sup>4</sup>proPlant Gesellschaft für Agrar-und Umwelteinformatik mbH, Albrecht-Thaer-Straße 34, 48147 Münster, Germany

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Intensely fluctuating abundances are a profound part of the population dynamics of common voles (*Microtus arvalis*). Though peak numbers can vary widely, during gradation events the population density can simultaneously rise up to several thousand individuals per hectare at a national or even continental scale and cause massive crop losses in agriculture and forestry. Consequently, common voles are of great concern for crop protection and so far no reliable method is established to predict such population outbreaks. Measures to combat vole outbreaks are usually taken when the damage is already clearly visible.

The aim of this project is to identify potential predictors and to develop a predictive model for the population dynamics of common voles based on weather parameters as well as landscape factors. Such a model would enable farmers to take spatially and temporally targeted preventive measures that could minimise the effects of vole outbreaks. Additionally, such an approach would lead to an overall reduction in the use of rodenticides and eventually reduce the contamination risk for non-target species.

To achieve this aim, long-term historic datasets on population dynamics in common voles were located and digitalized from the archives of various institutions. A total of nearly 4,500 single abundance measures spanning more than 25 years were used for subsequent analyses. Potential predictors were readily available extrinsic factors such as weather (monthly mean of temperature, snow cover, precipitation and sunshine duration) as well as landscape factors (elevation, soil type, groundwater fluctuations, etc.). Both analyses were done separately to identify potential predictors of both parameter types. Non-parametric methods such as boosted regression tree- (BRT) and classification and regression tree- (CART) analyses were used to quantify the influence of single predictors (BRT) as well as identity predictor constellations and their thresholds leading to differences in vole densities (CART).

For landscape factors site elevation and soil type played a key role in shaping the distribution of common vole outbreak risks. Additionally, this study demonstrated that weather parameters were closely related to the variation in regional outbreak risk of common voles. Mostly weather parameters in winter and early spring were identified to be highly important. For perennial grassland in autumn for example the snow days in January as well as the sunshine duration in March and the temperature of the previous October seemed to be decisive factors. Validation of the deduced set of rules showed that the predictive model based on weather parameters can successfully predict around 70% of the historic population fluctuation. Validation on external data to assess potential extrapolation of the model to other areas is currently ongoing. Through a combination of both approaches (landscape and weather parameters) but also through increasing the forecast resolution through interpolated weather data such a forecast model might be as reliable as more complex but often impractical biological models.

## Foraging in risk-homogeneous landscapes – a spatial model for pest species distribution and damage in agriculture?

Eccard, J.A.

Animal Ecology, Maulbeerallee 1, University of Potsdam, 14469 Potsdam, Germany, eccard@uni-potsdam.de

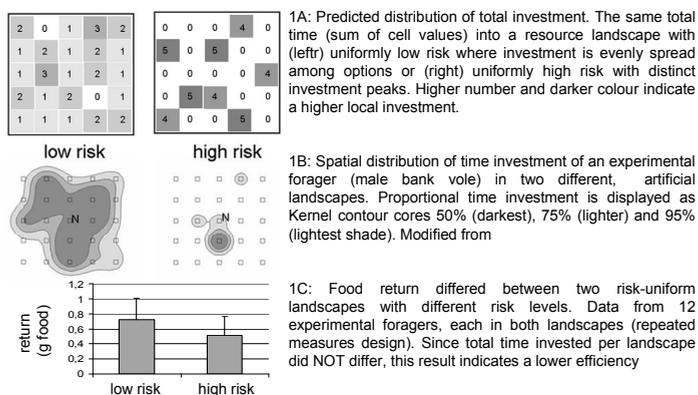
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Keywords: behavioural ecology, predation risk, risk-taking, rodents

Behaviour is shaped by evolution to maximise fitness by balancing gains and risks. Decision making models in biology, psychology or economy have investigated choices among options which differ in gain/risk. A foraging animal has to trade off food and safety, according to its biological state.

In some landscape these compromises are difficult, because predation risk is uniformly distributed in space. Agricultural landscapes, especially in large scale monocropping, are homogeneous in structure. Depending on the movement scale of species using landscapes, fields or parts of fields, individuals may perceive such structures as uniform in risk and gain. Adequate predictions for the emerging foraging patterns in risk-uniformity, especially under an overall, high risk, are missing.

Based on the existing models on local decision making in risk-heterogeneity we test predictions extrapolated to a landscape level with uniform risk distribution, comparing among independent high-risk landscapes and low-risk landscapes (Figure 1A) We provide experimental support for our hypotheses, investigating the foraging behaviour of voles in artificial landscapes (Figure 1B). In high risk uniform landscapes animals invested their foraging time in fewer options and accepted lower total returns, i.e. reduced foraging efficiency (Figure 1C), compared to their behaviour in low risk-uniform landscapes (Eccard and Liesenjohann, 2008; Eccard et al., 2008, Liesenjohann and Eccard, 2008)



**Fig. 1** Hypotheses and results for a foraging experiment in experimental, risk-homogeneous landscapes (Eccard and Liesenjohann, 2008)

Agricultural landscapes differ in field size and farming practice and some provide animals with risk-uniformity on a large scale. We suggest, that risk-uniformity affects the species distribution in such landscapes and also the distribution of damage by foraging animals in agricultural landscapes.

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## Synchronous population fluctuations of forest and field voles: implications for population management

Tkadlec, E.<sup>1,3</sup>, Suchomel, J.<sup>2</sup>, Purchart, L.<sup>2</sup>, Heroldová, M.<sup>1</sup>, Čepelka, L.<sup>2</sup>, Homolka, M.<sup>1</sup>

<sup>1</sup>Institute of Vertebrate Biology AS CR, Brno, Czech Republic

<sup>2</sup>Mendels University, Brno, Czech Republic

<sup>3</sup>Palacky University, Olomouc, Czech Republic, emil.tkadlec@upol.cz

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### Abstract

High population densities of field and forest voles cause economic losses by consuming crop or slowing down forest regeneration by damaging the bark of young trees. Consequently, programs to monitor abundances are often implemented as part of population management. Whereas abundances of the common vole (*Microtus arvalis*) in the Czech Republic has regularly been checked by the State Phytosanitary Administration for decades, no monitoring program has so far been invented to monitor forest voles. Because population numbers of different vole taxa are often observed to fluctuate in phase, we explored the possibility whether the monitoring data for the common vole can be used to forecast numbers of forest voles. Since 2002, we have monitored by snap-trapping bank vole (*Myodes glareolus*) populations in three forests situated in southern Moravia, Czech Republic. Correlation analysis of time series of yearly population changes for the common and bank vole revealed that populations of field and forest voles in southern Moravia fluctuate in a close synchrony, the correlations being consistently higher than 0.8. This result provides the reasonable possibility of exploiting the data from the extensive common vole population monitoring program for prediction in population management of forest voles.

Keywords: bank vole, common vole, *Microtus*, *Myodes*, correlation of population fluctuations

### Introduction

In forestry, small rodents cause damage by chewing of the young tree bark and consuming seeds (Gill, 1992). The voles, primarily of the genera *Myodes* and *Microtus* are known for their temporal regular fluctuations in population numbers called population cycles (Hansson, 2002; Tkadlec and Zejda, 1998). The impact of vole populations on forest ecosystems should vary accordingly, with the highest damage at peak density and the lowest one at population lows. Long-term regular monitoring program is a prerequisite for a well-informed population management capable of forecasting vole numbers in the next year. However, collection of these data on a larger spatial scale is not simple because the methods of vole trapping are fairly laborious. On the other hand, there is already an extensive program in the Czech Republic for monitoring common vole populations in farmland organized by the State Phytosanitary Administration. It is based on a burrow index which is very easy to be estimated by pure inspection with no need of trapping. There is suggestive evidence that populations of different rodent taxa largely fluctuate in a close synchrony (e.g., Hansson, 2002). This phenomenon thus offers the possibility for rodent pest population management of exploiting data on abundances of one species for predicting population numbers of the others. In this study, we measure the degree of spatial synchrony between population dynamics of the common vole from open farmland and those of the bank vole from forest areas in southern Moravia between 2002 and 2010 to explore whether monitoring data for the common vole can be used for prediction in population management of forest voles.

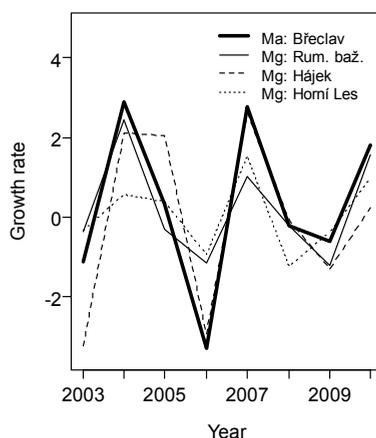
### Materials and methods

To assess the degree of synchrony between the bank and common vole, we monitored regularly in spring and autumn the abundances of the bank vole by snap-trapping in three forest complexes in southern Moravia: Rumunská bažantnice, Hájek and Horní les. The first forest, Rumunská bažantnice, was the most variable forest with mixed broad-leaved and coniferous trees. The second was a typical production forest dominated oak and hornbeam, with rich herb stratum. The third was a semi-natural lowland oak forest. Five lines of 20 traps were laid 5 m apart for three days. Relative abundance rA was calculated as the number of voles per 100 trap nights. For the common vole, we used a burrow index collected twice a year within a monitoring program organized by the State Phytosanitary Administration for a district Břeclav, southern Moravia. The index is estimated as the number of active burrow entrances per hectare.

In brief, the active burrow entrances are counted by walking across the field along four 100-m strips each 2.5 m wide. Hence, the entrances were counted for a total area of 1,000 m<sup>2</sup>. Each year, 20 to 60 fields with different crop (mostly lucerne, winter wheat and rape) were sampled and the mean index then calculated for the district. Prior to analysis, the data on autumn abundances of both species were log-transformed and then converted by differencing to series of yearly population growth rates per capita  $r_t$  obtained as  $r_t = \ln N_t - \ln N_{t-1}$ . This is because we are interested in synchrony of processes rather than population numbers. Then the Pearson coefficients of correlation were calculated between 3 series of bank vole populations and one series of the common vole population.

## Results

Population dynamics of 3 bank vole populations were highly synchronous with the growth rate of the common vole population from the district Břeclav (Figure 1, population rate), with correlations being all  $>0.8$  ( $p < 0.05$ ). The correlations for Rumunská bažantnice, Hájek and Horní les were 0.88, 0.86 and 0.82, respectively. The mean correlation was 0.85 which is a reasonably high value suggesting that the data on population change in the common vole have a high predictive value for management of forest voles.



**Fig. 1** Population growth rate of the bank vole (Mg) and the common vole (Ma) in southern Moravia.

## Discussion

We confirmed that populations of forest and field voles fluctuate in a close synchrony and that the data collected for the common vole can be of great predictive value for forest vole management. Similar correlations were observed for the bank vole and the field vole (*Microtus agrestis*) in Sweden (Hansson, 2002). The underlying mechanisms behind the observed synchrony remain unclear. In bank voles, it is generally accepted that the multiannual changes in numbers can well be driven by variability in food resource availability (e.g. Pucek et al., 1993). It is known that seed masting can stimulate population growth in several forest rodents, such as voles and mice. However, there is no information at all what kind of resource could affect population growths in common vole populations during the same period of time. This remains the important task for future studies.

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## Plant biomass and prediction of debarking caused by rodents in artificial regeneration of forest stands

Homolka, M.<sup>1</sup>, Heroldová, M.<sup>1</sup>, Kamler, J.<sup>1,2</sup>

<sup>1</sup>Institute of Vertebrate Biology, AS CR, 60365 Brno, Czech Republic, homolka@ivb.cz

<sup>2</sup>Mendel University of Agriculture and Forestry, 61300 Brno, Czech Republic

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### Abstract

In the last ten years a strong intention was observed to replace spruce monocultures with mixed coniferous-broadleaved stands close to natural composition. This was accompanied by much higher rodent impact on the broad-leaved plantations than previously. To protect planted trees on forest clearings, we conducted the research in Drahaný Highland (350-550 m a.s.l.) and monitored the bark damage in young beech trees, rodent density dynamics, broad-leaved trees seed harvest and a snow cover in four years period. Intensity of bark damage was not related to the rodent abundance but was influenced by high seed crop and above the average snow cover. Primary production dynamic might be a good predictive factor of broad-leaved bark damage by voles. Applicability of this method should be proved for long periods and in various natural conditions.

Keywords: density, seed crop, snow cover, voles

### Introduction

In forestry practice, prediction of rodent impact would be most helpful tool to protect young trees in reforestation against bark damage. The main pre-requisite for a correct forecasting is the long-term monitoring of rodent dynamic, of their impact, their food supply and other related data. However, there is paucity of such informations. The main cause of bark damage is insufficient food supply, especially in winter.

Forecasting of damage by rodents in agriculture is realized by monitoring of common vole density (Zapletal et al., 2001). In forestry, monitoring of rodent population dynamic is complicated and not sufficiently effective (Gill, 1992). Moreover intensity of bark damage might be very variable and area specific (Suchomel et al., 2009). In general, bark damage on plots were influenced above all by quality of food supply and shelter conditions (Kamler et al., 2011).

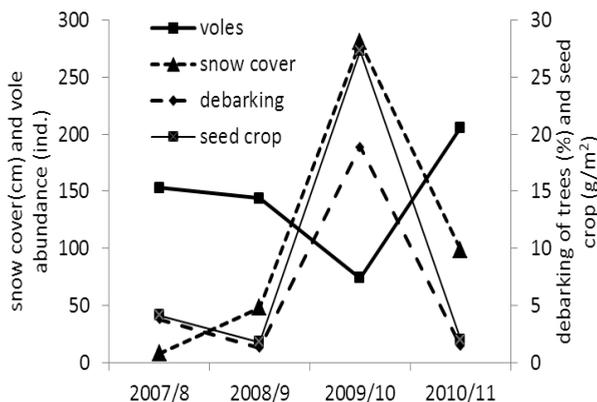
The key problem would be then to predict deficient food supply on which bark damage arise. In this study, we analyzed the factors, which may influence the winter bark damage and its extent on the broad-leaved forest young trees.

### Materials and methods

In Drahaný Highland (Central Moravia, Czech Republic) monitoring was realized in mixed coniferous-broad-leaved stands environment in altitude 350-550 m a.s.l. From autumn 2007 to spring 2011 on 11 clearings with planted beech plots research was concentrated on autumn rodent density (*Myodes glareolus*, *Microtus arvalis* and *M. agrestis*; 610 individuals), the broad-leaved trees seed harvest in the surrounding environment, and spring bark damage extent on beech (1,300 controlled individuals).

### Results

The highest extent of bark damage was in winter with the lowest previous autumn vole number. Just an opposite situation was in winter with highest vole density (Figure 1). The intensity of bark damage was related to snow cover. After winter with low snow cover damage was rare, after winter with high and long lasting snow cover bark damage was about ten times higher. The winter with high bark damage was preceded by rich autumn seed harvest.



**Fig. 1** Relations between debarking young beech tree, abundance of voles, seed crop and snow cover (snow cover represented by the sum of snow height in pentads from November until March)

## Discussion

Our results from highland indicate that here are two main factors influencing the beech bark damage: good seed harvest and high and long lasting snow cover. Good years reflect favorable conditions leading to high primary production (Hilton and Packham, 1997) on which voles are dependent (Tast and Kalela, 1971). Snow cover restricted the vole spatial activity and their access to good quality food. After a good primary production year rodents overwintered in good condition with a low mortality to the end of winter. In early spring, with still some snow cover, vole populations turned to bark consumption. In the year after good overwintering vole population graduated. Generalization of these conclusions should be verified by further research in various environments.

## Acknowledgements

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## Vole impact on tree regeneration: insights into forest management

Heroldová, M.<sup>1</sup>, Homolka, M.<sup>1</sup>, Tkadlec, E.<sup>1</sup>, Kamler, J.<sup>1</sup>, Suchomel, J.<sup>2</sup>, Purchart, L.<sup>2</sup>, Krojerová, J.<sup>1</sup>, Barančeková, M.<sup>1</sup>, Turek, K.<sup>2</sup>, Baňář, M.<sup>3</sup>

<sup>1</sup>Institute of Vertebrate Biology, AS CR, 60365 Brno, Czech Republic, heroldova@ivb.cz

<sup>2</sup>Mendel University of Agriculture and Forestry, 61300 Brno, Czech Republic

<sup>3</sup>Forestry and Game Management RI, Jiloviště, Czech Republic

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### Abstract

In Europe, much of the damage to tree seedlings in plantations is caused by rodents, such as common vole (*Microtus arvalis*), field vole (*M. agrestis*) and bank vole (*Myodes glareolus*). To understand the relationships between the environmental variability which may influence the rodent impact in forest plantations we conducted a study in 12 forest regions of the Czech Republic. In total, we recorded cumulative damage in 19,650 young trees of 8 species at 393 plots. Broadleaves were far more affected by gnawing than conifers (mean 10% and 3%, respectively). Of the monitored species, beech was damaged most often (24% of individuals). The intensity of browsing differed between the regions (6-60% of browsed individuals) with the lowest damage at altitudes below 400 m a.s.l. The proportion of the trees damaged increased with age of the plantation up to 6 years. The factors which principally influenced the impact on trees were herb layer plant cover, its height, its species composition, litter size and presence of weedy species.

Keywords: environmental variables, forest regeneration, vole impact

### Introduction

Rodents represent an important natural component in forest ecosystems. The typical feature of rodents is high reproduction rate and related fluctuation in their abundance within seasons of the year and within several-year periods (Hansson, 2002a; Stenseth et al., 2002; Tkadlec and Zejda, 1998). This ability is, from the forestry point of view, the most significant problem. Rodents at high population densities at localities with favourable conditions (open areas) cause damage to vegetation especially in artificial regeneration of forest stands (Hansson, 2002b). The biggest problems are caused by species that consume mainly the vegetative parts of plants; i.e. the field vole and the common vole. Bank voles as a forest rodent having a broader feeding niche are less damaging in forest plantations (Hansson, 2002b). At the times of food shortage, these species feed on bark and under certain conditions they are able to destroy young trees at whole planted areas (Gill, 1992). In spite of the damage small mammals cause to woody species, little attention is paid to research of their ecology in relation to the forest environment in central Europe. This is because in the former forestry practice coniferous trees were favored over other broadleaved tree species and forest damage was low and neglected. In the last ten years there was a strong intention to replace spruce monocultures by mixed coniferous-broadleaved stands. These are close to natural composition and suffer much higher impact on the broad leaved plantations (Gill, 1992).

The aim of our study was a survey on the extent of damage caused by rodents to artificial forest plantations within the Czech Republic and to demonstrate possible factors which may influence the intensity of damage. Our results may route to practical applications as to predict and prevent rodent damage and help in tree planting and planting plots management.

### Materials and methods

Within the Czech Republic, we selected 12 regions representing forests in various altitudes. In each of these regions, we assessed the extent of rodent-caused bark browsing at 15 to 40 plots. The monitored plantations were 3 to 15 years old and of various tree species. The plots were chosen with respect to the prevailing group of forest types in the region. At each plantation plot we examined 50 individuals of the selected tree species. In each tree, we took record of its height, stem diameter at soil surface and extent of bark damage over the last 4 to 5 years. Damage was estimated on the basis of the debarked area size, distance of the lower margin of the browsed area from soil surface and share of the damaged circumference of the stem. At each tree the microhabitat was checked as to percentage of herb layer, dicotyledonous herbs, grasses and weedy species cover. Special attention was given to the presence of

the litter and its thickness categorized increasingly as 1 to 3 levels. The same factors as in microhabitats were checked in plantation plot surroundings. All considered factors were analysed by fitting a generalized linear model.

## Results

In total 19.650 trees of the 8 species at 393 plots were checked for cumulative damage by rodents. Broadleaves were more affected by gnawing than conifers (mean 10% and 3%, respectively). Of the monitored species, beech was damaged most often (24% of individuals). The intensity of browsing differed between the regions (6-60% of browsed individuals) with the lowest damage at altitudes below 400 m a.s.l. The proportion of the trees damaged increased with age of the plantation ( $\chi^2=44.55$ ,  $p<0.001$ ) (up to 6 years). Regarding tree microhabitat quality the most important factors were herb layer plant cover ( $\chi^2=272.38$ ,  $p<0.001$ ) and its height ( $\chi^2=380.10$ ,  $p<0.001$ ). Also important was its species composition. Impact is high if grasses prevail ( $\chi^2=14.28$ ,  $p<0.001$ ) and lower if dicotyledonous species prevail ( $\chi^2=76.55$ ,  $p<0.001$ ). Very important was the presence of the litter and its thickness (which is mostly connected with grass invasion) ( $\chi^2=116.16$ ,  $p<0.001$ ) and invasion of weedy species ( $\chi^2=116.21$ ,  $p<0.001$ ). Also the surrounding plots parameters were important and correspond to the particular plot parameters. The rate of damage was higher if surrounding plots were invaded by grasses ( $\chi^2=96.41$ ,  $p<0.001$ ) with thick litter size ( $\chi^2=10.93$ ,  $p<0.001$ ). Fencing of the plots also increased rodent damage ( $\chi^2=9.27$ ,  $p<0.01$ ).

## Discussion

Our results show the importance of habitat structure on the level of tree damage. Succession of vegetation at forest clearings changes the living conditions (amount of food, shelter) of rodents and their species composition. The share of damaged trees (of the attractive species) increased with age of the felled area and may be due to accumulation of browsing in the first five years after planting. As an example Ferguson et al. (2003) reported nine years old planting of pines with low ground vegetation to be not suitable for herbivorous voles. In our study, local conditions of the trees were of great importance for the degree of the tree bark damage and tree survival. Thick grass litter, higher herb layer cover and its height were clearly related to increased damage of the trees on clearings. Grassy clearings and meadows are suitable biotopes for vole species (Birney et al., 1976). Vegetation removal with herbicides, grazing or cutting are widely recognized control techniques for many rodent species (Gill, 1992). Also the weed control in tree microhabitats is known to decrease vole damage (Davies and Pepper, 1989). All of these negative factors can be considered and directly managed.

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## Is the Italian strategy to face the problem of stray dogs sustainable? A case study of two small municipalities in central Italy

Adriani, S.<sup>1</sup>, Bonanni, M.<sup>2</sup>, Amici, A.<sup>1</sup>

<sup>1</sup>Università della Tuscia, Dipartimento di Produzioni Animali, Via C. De Lellis, snc, 07100 Viterbo, Italy, adrianisettimo@libero.it

<sup>2</sup>Via F. Martinelli 34, Roma, Italy

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### Abstract

The Italian law for pet animal protection and prevention of stray dogs (L. 281/91) requires municipalities to solve the problem of stray dogs. According to this regulation the municipalities are obliged to capture free dogs which have to be put in adequate kennels. As a priority, the animals should be housed in public kennels, if they do not exist, also private kennels are acceptable. If the legitimate owner is not identified, the municipalities are obliged to maintain the animal lifelong. Large part of small Italian municipalities are not able to build and manage public kennels. In compliance with the law they are forced to enter into agreements with private facilities and to cover the costs. Currently the average cost for maintaining a dog in a kennel is approximately € 3.50/head/day, for an annual cost of € 1,250.00/head. In the present study we evaluated the economic sustainability of the law in two small municipalities of central Italy. Under the hypothesis that all stray dogs would be housed in the kennel the total expense would be € 19,493.00/year. It is interesting to note that this amount corresponds to the sum of the costs arising from the annual fees for human welfare (indigents support € 5,532, school meals € 6,780, support for elderly € 7,497).

Keywords: kennel, municipality budget, Rieti, small municipalities, stray dog

### Introduction

The problem of stray dogs is present worldwide (Butcher, 1999). In Italy it is very important and well known since ancient times (Cassina and Fico, 1990; Virga, 1991). It is difficult to define and update quantitative data of the phenomenon because of its peculiar characteristics (Beck, 1973) and different ways of manifestation (Coman and Robinson, 1989) in different areas of the country. Studies performed in Italy in the last three decades show that of a total of 2,762,862 dogs present, about 220,140 were stray dogs (7.96% of total) and 79,112 feral dogs (2.86% of total) (Boitani and Fabbri, 1984; Boitani, 1992). In the same studies stray dogs were divided into four different categories, depending on the relationships with humans, from which directly or indirectly they depend. In this study, the term includes all the stray dogs that wander in uncontrolled human settlements, with or without a master. The existing law provides that if the Government fails or neglects to adopt measures and/ or precautions that tend to remove and eliminate the potential danger posed by stray dogs, it is liable for damages caused by stray dogs (Beck, 1975; Vučinić et al., 2008). This implements the general principle of “*neminem laedere*” art. 2043 of the Civil Code. Therefore, the municipalities should be an active part in the actions of identifying, capturing and housing stray dogs in suitable kennels (Blanchard, 2009; Butcher, 2009) by the burden of maintenance lifetime. In this regard the Italian law for pet animals protection and prevention of stray dogs (L. 281/91), also enacted to prevent, control and eradicate the phenomenon of stray dog; in paragraph 1 of Art. 4 it imposes that “municipalities, individually or associated, ensure the rehabilitation of existing municipal kennels and build kennels for dogs in accordance with the criteria established by regional law and using the contributions for that purpose from the region”. Nowadays 1,650 Italian municipalities (of 8,094 in 2010) are not equipped with their own kennels and must draw agreements with private ones. In the present study we evaluated the economic viability of the law 281/91 in small towns. We investigated the budget of two municipalities in the province of Rieti (central Italy) in order to compare public expense for human welfare and stray dog housing.

### Materials and methods

During the period between March 1 and December 31 2010, a survey was conducted to count stray dogs present in those municipalities (Boscagli et al., 2010). We counted only the dogs present inside the town (Santamaria et al., 1990; Natoli et al., 2009). These animals should not exist on the basis of the Italian

law 281/91. The municipalities' budget, including the expenses for stray dog housing, and the daily cost per dogs was recorded in the administrative offices of the municipalities on the basis of the agreement with kennels. Human welfare expenditures were also recorded (support for indigents, sea stays for elderly peoples).

## Results

Both municipalities were not equipped with a kennel, public or private. In the municipality A (20 villages with less than a total of 2,000 residents) were counted 17 stray/wandering dogs. In town B (69 villages with a total of about 4,000 residents) 43 stray/wandering dogs were counted. In January 1999, the municipality A subscribed to an agreement with a private kennel, updated several times for the economic aspects. Nowadays the municipality pay a fix fee € 8,000/year to house up to a maximum of 8 dogs. For any unit in excess a daily rate of € 3.50 (VAT included) is applied, for an annual fee of € 1,277/head. If the 17 stray dogs were caught and housed in conformity with the law the municipality should pay an annual cost of € 11,493 and a total expense of € 19,493/year. It is interesting to note that this amount corresponds to the sum of the costs arising from the annual fees for human welfare such as indigents support (€ 5,532), meat for school meals (€ 6,780) and sea stays for elderly and two assistants (€ 7,497). The municipality B subscribed an agreement with a private kennel. Nowadays the municipality pays a price of € 3.50/head/day (VAT included), for an annual fee of € 1,277/head. If the 43 stray dogs were caught and housed in conformity with the law the municipality should pay an annual cost of € 54,932.50/year. This amount is twice the costs arising from the annual fees for human welfare (€ 7,581 for indigents support, € 9,567 for meat for school meals, € 11,532 for sea stays for elderly and two assistants).

## Discussion

The presence of stray dogs shows the failure of municipalities in the application of law 280/91. The analysis of the overall economic status of these institutions indicates, however, the real impossibility to fulfil the constraints of the law. The scarcity of economic resources induces the institutions to omit the application of the law not to overcharge their budget. So the law cannot achieve the aim to solve the problem of stray dogs because small municipalities are unable to finance facilities and dog support.

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## **A reward strategy for hunters to pursue the control programs of red fox (*Vulpes vulpes* Linnaeus, 1758)**

Adriani, S.<sup>1</sup>, Bonanni, M.<sup>2</sup>, Primi, R.<sup>1</sup>, Amici, A.<sup>1</sup>

<sup>1</sup>Università della Tuscia, Dipartimento di Produzioni Animali, Via C. De Lellis, snc, 07100 Viterbo, Italy

<sup>2</sup>Via F. Martinelli 34, Roma, Italy, bonanni\_m@libero.it

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### **Abstract**

A novel strategy for pest control is described in the present study. Together with the implementation of the normal control program of antagonists of hunting species a “reward strategy” aimed at involvement of hunters was implemented to enhance the containing effects on red fox (*Vulpes vulpes* L.). Exploiting the concept “more foxes killed - more game for restocking”, the territorial hunting areas of Rieti Province (Central Italy), developed two methods to stimulate the killing of foxes by hunters during the hunting season. Five years of application showed good results in terms of foxes killed, with an increased number of foxes killed in the first year of the application of one of the methods implemented.

Keywords: carnivore, pest control, Rieti province, *Vulpes vulpes*, wildlife management

### **Introduction**

The European red fox (*Vulpes vulpes* Linnaeus, 1758) is a wild carnivore which diet includes a wide variety of food resources (Hartova-Nentvichova et al., 2010) depending on its broad geographic range and covering any point along the specialist – generalist continuum (Panzacchi, 2008). Several studies point out the evidence that foxes have negative impacts on a very broad range of wild vertebrates, but also poultry and livestock, and that these impacts are mediated directly by predation and by other direct and indirect processes (competition, transmission of diseases, etc.) (Saunders et al., 2010). In vulnerable ecosystems the predator-prey imbalance can lead to excessive loss of biodiversity and to the local extinction of species of particular conservation concern (Wallach et al., 2009). The common perception of hunters that the red fox excessively preys on hunting species has meant that this carnivore was always considered a pest (Boitani and Vinditti, 1987). It is well known, in fact, that predation by foxes affect partridge (*Perdix perdix*), hare (*Lepus* spp.), rabbit (*Oryctolagus cuniculus*) (Knauer et al., 2010) and pheasant (*Phasianus colchicus*) populations (Draycott et al., 2008). In order to reduce predation on game species, hunters put in place a strong pressure to achieve control programs of fox population (Toso and Giovannini, 1991; Toso and Genovese, 2003). However, the results of studies on the effects of fox control on population dynamics of prey species, were ambiguous (Salek et al., 2010; Reynolds et al., 2010; Knauer et al. 2010; Panek, 2009). In Italy the red fox is a hunting species, but since it is not a coveted prey, the normal practice of hunting does not contribute to its containment. As a result many institutions have begun autonomous control activity. To encourage the removal of this predator, control plans include a “reward strategy” to compensate hunters with “valuable” wild game (hare, pheasant, grey partridge) in proportion to the number of foxes removed. This study was designed to ascertain the quantitative aspects of containment regularly conducted by the Territorial Hunting Areas (ATCs) in the province of Rieti (Central Italy) in the period 2005-2010.

### **Materials and methods**

The province of Rieti is divided into two ATCs, identified as ATCRI1 and ATCRI2. Each has developed a plan to control the fox, with different rewarding strategies. To stimulate the killing of foxes by hunters the ATCRI1 plan provides the reward of one hare for every three foxes culled. The ATCRI2 plan, instead, provides a scoring system (1 fox = 25 points), with two distinct reward combinations: 1) per hunter resident in the province of Rieti, 2) re-stocking. The first included: 100 bonus points = 1 year insurance policy for hunting (value € 85.00), 75 points = 1 card access to ATCRI2 in the next hunting season, 75 points = 1 free permit for the use of training dog areas. The second provides: 1 hare = 50 points, 1 pheasant = 10 points, 1 grey partridge = 5 points. Although differently articulated both projects are based on the concept of the exchange of killed fox in wild game animals for restocking. Furthermore, since the aim of the ATCs was only to test the procedures in terms of acceptance by hunters and other

stakeholders (farmers, common citizens, etc.), in this paper parameters of fox population and prey species were not investigated.

## Results

The following table (Table 1) shows, in detail, the data of containment activities conducted in the ATCRI1 and ATCRI2 in the 2005-2010 period.

**Tab. 1** Foxes killed in each ATC in the period 2005-2010

ATC	2005/06	2006/07	2007/08	2008/09	2009/10	Total
RI1	262	245	346	421	652	1,926
RI2	282	348	298	180	510	1,618
Total	544	593	644	601	1,162	3,544

During the five years examined 3,544 foxes were killed (1,926 and 1,618 in the ATCRI1 and ATCRI2 respectively). The province of Rieti covers about 2,750 km<sup>2</sup>, the average culling intensity was 1.3 foxes/km<sup>2</sup>.

## Discussion

The sustainable management of natural resources together with the guarantee for their preservation relies on the continued participation of the people living in the area (Swanson and Barbier, 1992). Reward strategies that consider many aspects of the workplace in order to both attract and keep high quality people doing the right things may be expected for conservation and protection aims. In this case the local administrators responsible for wildlife management suggested the application of a rewarding system to the control of a carnivore pest, with good results in terms of foxes killed. It is not known whether the increase of the annual levy from 544 foxes in 2005/2006 to 1,162 in 2009 /2010, is due to an optimization strategies, a broader commitment of operators to capture or, more simply, the increasing attractiveness of the reward. This was independent of criteria of proper wildlife management. It is hoped that the recent activation of a monitoring plan for the species throughout the province and the simultaneous indication of the target density can help to control activities of the fox, and, simultaneously, to assess the results.

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## **Impact of the fat dormouse (*Glis glis* Linnaeus 1766) on hazel orchards in the area of Alta Langa and Belbo, Bormida, Uzzone Valleys (province of Cuneo, Italy): a preliminary assessment of agricultural damage**

Ghirardi, M., Tizzani, P., Dematteis, A.

Faculty of Veterinary Science, University of Turin, via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy  
University Foundation Ce.Ri.Ge.Fa.S. (Centro Ricerca e Gestione Fauna Selvatica), Frazione Rore 17, 12020 Sampeyre (CN), Italy, marco.ghirardi@libero.it

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### **Abstract**

In the summer of 2008, the fat dormouse (*Glis glis* Linnaeus 1766), pest animal in the Langhe agricultural territory (Province of Cuneo, Italy), was studied with the aim of understanding its night invasion and feeding exploitation capacity on hazel orchards, in order to assess the resulting loss of marketable hazelnuts. Using 9 hazel orchards as study samples, dormice density, damage in cultivations and exploitation time were calculated and the results obtained were correlated. Damages happened mainly between July (when fruits start to grow) and late August (when fruits are harvested). Density (estimated by 18 nocturnal survey sessions) reached values between 4.13 and 247 vocalizing animals/hectare in the central hours of the night. The fruits loss, evaluated taking into consideration 33 plots placed inside the cultivations, accounted for 61% of the total of collected hazelnuts. Density and damage values were connected with the physical features of hazel orchards, their position in relation to wooded areas and the study period.

Keywords: *Glis glis*, hazel orchards, hazelnuts, damage, nocturnal survey, density, fruits loss

### **Introduction**

In the Langhe territory (Province of Cuneo, Italy), over the past few years, the fat or edible dormouse (*Glis glis* Linnaeus 1766), normally present in wooded areas, has become a pest species for agriculture. It is responsible for enormous damages on hazelnuts crops which are typical cultivations of Piedmont. The decreased productivity of faulty hazel orchards required a specific study aimed at understanding dormice feeding behavior in agricultural areas.

### **Materials and methods**

The research was carried out in the summer of 2008 (from late June to late September) in 9 hazel orchards (with different sizes and distances from wooded areas, located in five typical Langhe villages). The following cultivation parameters were analyzed: age and rows of hazel trees, spacing among these plants, number of cultivation sides bordering on wooded areas, closure of the leaf canopy of hazel groves, number of contacts between hazel trees and other trees of the wooded area. These cultivations were used as study samples for the assessment of dormice density inside the cultivations and neighboring wooded areas (Burgess et al., 2003).

The same hazel orchards were also studied in order to evaluate the loss of hazelnuts, due to feeding and fruit handling by rodents.

The dormice density was estimated by means of 18 sessions of nocturnal survey, based on detection of vocalizing animals along 2 to 4 transects, inside both the hazel orchards and the ecotonal orchard-wood zone (total transects: 210), in three standard times per night (22.30-24.00; 1.30-3.00; 4.30-6.00) (Hoodless and Morris, 1993; Jurczynszyn, 1995).

The damage was evaluated using 33 plots on the ground (plots mean area: 19.8 m<sup>2</sup>). The plots varied in number in every hazel orchard and they were arranged gradually away from the ecotonal zone. From July to September 2008, 44,847 hazelnuts were collected in the plots and divided into the following categories: "gnawed", "untouched" (but fallen prematurely due to dormice handling), "decayed/not grown" and "ripe" (ready for harvesting). The first three categories described the loss of marketable fruits.

## Results

On average, the density values ( $D$  = number of dormice/hectare) were 1.5 to 36 times higher in hazel orchards than in wooded margins. The peak dormice activity was reached in central hours (mean value of  $D$ : 106) and between the end of July and the first half of August ( $D$  minimum value: 4.13, on July 23<sup>rd</sup>;  $D$  maximum value: 247, on August 15<sup>th</sup>). These values, recorded in specific areas for a limited period of time, are not comparable with those recorded in nature (0.6 to 36 dormice/ha) (Kryštufek et al., 2003).

The fruits loss accounted for 61% of collected hazelnuts (27,523 damaged fruits out of 44,847 total fruits). Gnawed hazelnuts represented about a quarter of this percentage. The ripe fruits yield was <40%. The mean number of gnawed fruits was high in the plots close to the ecotonal zones and it diminished as distance from the wooded areas increased (correlation coefficient  $r=-0.62$ ;  $p<0.01$ ).

Several features of hazel orchards (i.e. distance from wooded areas, number of sides bordering on wooded areas, closure of the leaf canopy of hazel trees) and the study period (more or less the advanced growth phase) were statistically correlated with density (multiple correlation coefficient  $R=0.93$ ;  $p<0.05$ ) and damage values (multiple correlation coefficient  $R=0.80$ ;  $p<0.05$ ).

Damage values were also correlated with dormice density values (correlation coefficient  $r$  varying from 0.66 to 0.81), although data were insufficient for a correct statistical correlation.

## Discussion

The data analysis emphasized the relevance of ecotonal orchard-wood zones: in these zones, branches providing contact between hazels and wooded areas trees represent preferential transition points for dormice during the night invasions of cultivations (Capizzi et al., 2007). Information on these contact points and other orchard features are important for mapping possible environmentally benign strategies for the reduction of fruits loss: buffer zone around hazel orchards ("no-tree zone"), physical barriers, chemical deterrents, live trapping and translocation of dormice in minor-risk areas. These strategies differ in cost, arrangement time and maintenance; therefore, their effectiveness should be tested.

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## Capture traps as a method to minimize damage by red deer (*Cervus elaphus*) in golf courses

Farfán, M.A.<sup>1,2</sup>, Duarte, J.<sup>1,2</sup>, Vargas, J.M.<sup>2</sup>

<sup>1</sup>BioGea Consultores, Calle Navarro Ledesma 243, Málaga, Spain, jddbiogea@gmail.com

<sup>2</sup>Dpt. of Animal Biology, University of Málaga, Faculty of Sciences, Campus Teatinos, 29071 Málaga, Spain

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### Abstract

We tested the effectiveness of capture traps to control red deer populations in golf courses. We found that capture traps are useful to reduce populations of red deer and, therefore, to manage its presence and damage in urban areas. Nevertheless, we used capture traps with manual closing but this system is time consuming and ineffective. It is advisable to change this method to an automatic locking system.

Keywords: *Cervus elaphus*, golf course, red deer, urban environment, wildlife management

### Introduction

The land use changes happening in Spain in the second half of last century have had a substantial impact on landscape and as consequence have involved important modifications in species distribution. In Andalusia (southern Spain) the land abandonment and the agricultural intensification have given rise to the disappearance of mosaics of pastures or agricultural lands with a high proportion of natural vegetation that existed several decades ago. This resulted in a homogenization of the landscape since the most productive areas were devoted to the intensive agriculture and the less fertile were abandoned followed by the regeneration of scrubland and woodland (Fernández-Alés et al., 1992; de Andrés et al., 2002). The effect of changes and transformations of landscape caused by human activity for the distribution of wild species is in some cases detrimental for some species (Smith et al., 2005; Delibes-Mateos et al., 2010) but positive for others (Acevedo et al., 2006; Falcucci et al., 2007; Acevedo et al., 2011). In the case of red deer, the distribution range has increased considerably in Andalusia affecting urban areas as golf courses where they are causing damage.

### Materials and methods

We installed a capture trap in a golf course located in the Costa del Sol (Andalusia-southern Spain), with 10x10x2.10m dimensions. The door was closed manually with a pulleys and ropes system. The person in charge of this task was hidden 25 m from the capture trap. During three consecutive months we visited the capture trap every three days, baited the capture trap with broad beans periodically and used an attractive substance for red deer. We controlled the visits of red deer to the capture trap using two camera traps. After three months we started with captures in the morning and at night during six consecutive days.

### Results

Red deer groups visited the capture trap on 15 different occasions. The first visit occurred two months after finishing the installation of capture trap. All visits were by adult females and juveniles and occurred mainly at night and early morning. There were no visits by deer to the trap after a person was placed close to the trap to operate the closing mechanism.

### Discussion

The results have shown that a capture trap can be a useful method to control populations of red deer in urban areas. This is in accordance with the results obtained by others authors in different regions. In fact, red deer groups have not feared the presence of a capture trap and they entered the trap to forage. However, manual closing of the capture trap is not effective because it needs many waiting hours and the presence of people around capture trap can scare away red deer. Therefore, it is advisable to change this method to an automatic locking system.

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## Landfill habitat restoration can reduce the incidence of vertebrate pest species

Duarte, J.<sup>1,3</sup>, Zurita, F.<sup>2</sup>, Farfán, M.A.<sup>1,3</sup>, Vargas, J.M.<sup>3</sup>

<sup>1</sup>BioGea Consultores, Calle Federico García Lorca 14, 29400 Ronda, Spain, jddbiogea@gmail.com

<sup>2</sup>Consorcio Provincial de RSU de Málaga, Diputación Provincial de Málaga, Calle Pacífico 54, 29004 Málaga, Spain

<sup>3</sup>Dpto. Animal Biology, University of Málaga, Faculty of Sciences, Campus Teatinos, 29071 Málaga, Spain

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### Abstract

We monitored over one annual cycle the abundance of vertebrate species in three landfills, one of them still in use and the other two abandoned since 2004. We found that the presence of vertebrate pest species or their abundance was lower in the abandoned landfills than in the one in use. Restoration actions and habitat management in sealed up landfills can reduce pest incidence and related damage or health risks in the surrounding habitats or urban areas.

### Introduction

Pest species can be abundant in landfills (Camiña and Montelío, 2005). In the context of a landfill we considered a species as a pest if it uses the landfill as a habitual habitat or takes temporal advantage of their resources and can therefore produce damage to landfill facilities, surrounding crops, livestock or game harvesting; or if it can carry germs or act as a vector for diseases or be harmful for humans. We have monitored during one year the presence, use and abundance of vertebrate species in three landfills, one of them still in use and the other two abandoned and sealed up.

### Material and methods

The study area was located in the south of Spain (Málaga province, Andalucía). The landfill in use was the Complejo Medioambiental de la Costa del Sol (Casares). The two abandoned were Ronda landfill and Viñuela landfill, partnerships of the Sulfanet4EU project (European Community) for the sustainable use of abandoned landfills. These two landfills have been sealed up and restoration actions carried out. We tried to compare the incidence of vertebrate pest species between landfills.

### Results

We found seven species that fulfil the criterion of pest: the yellow-legged gull (*Larus cachinnans*), the black kite (*Milvus milvus*), the cattle egret (*Bubulcus ibis*), the wild boar (*Sus scrofa*), the red fox (*Vulpes vulpes*), the feral cat (*Felis catus*) and the feral dog (*Canis familiaris*). Presence of gulls and egrets follow an annual pattern in the landfill in use. Gulls are frequent in the landfill all year round except in summer when they disappear. The gull population size using the landfill is estimated at 1,614 to 2,646 birds (95% confidence interval). Egrets are also frequent every season except in spring. Their population size is estimated between 250 and 466 birds (95% confidence interval). Gulls are completely absent from the abandoned landfills (even when they are present in a surrounding water reservoir) while egrets on some occasions use the abandoned landfills. Black Kites are present in the landfill in use in spring and early summer while they use the abandoned landfills only in autumn. Kites use the landfill in use as settling places during their prenuptial migration, staying for 15-30 days and looking for food. The abandoned landfills are used only to settle for one or two nights during the postnuptial migration.

The use of the landfills by carnivorous mammals and wild boars is high. Boars, feral dogs and cats and red foxes are frequent in both types of landfills. However, we find significant differences in the abundance of these species between in-use and abandoned landfills, as they are more abundant in the first type of landfill.

### Discussion

The abandonment and sealing up of a landfill accompanied by habitat restoration actions can reduce the incidence of some vertebrate pest species. Restoration actions and habitat management can help to reduce investments on pest species management (Álvarez and Chico, 2003) even in active landfills. Wild boars cause damage to crops and golf courses while carnivores can limit some game species. In the case

of gulls it is important to consider habitat restoration in landfills. Gulls are in direct contact with rubbish and they tend to nest in buildings. Gull populations are increasing in coastal areas causing health risk and attacks to humans during their breeding season. Indirect management of pests through habitat manipulation can also help to improve the habitat quality for other endangered species (Orueta, 2007).

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## **Fertility control in Europe: applications for an overcrowded continent**

Massei, G.<sup>1</sup>, Cowan, D.<sup>1</sup>, Miller, L.A.<sup>2</sup>

<sup>1</sup>Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom, giovanna.massei@fera.gsi.gov.uk

<sup>2</sup>National Wildlife Research Center, Fort Collins, 4101 LaPorte Avenue, Fort Collins, CO 80521-2154 USA United States

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Current trends in human and wildlife population growth and landscape development indicate that human-wildlife conflicts are likely to increase worldwide in the near future. This is particularly important for Europe where the density of the human population is relatively high. For instance, although Europe and the US have a similar land mass, twice as many people live in Europe compared to the US. Lethal control to mitigate human-wildlife conflicts can be ineffective in the long term, unfeasible, or may be unacceptable for its impact on the environment and on animal welfare. Among the non-lethal methods to manage overabundant wildlife, fertility control offers a humane, publicly acceptable method to reduce the size of a population. In parallel, public interest in alternatives to surgical sterilization for companion animals and livestock has fostered investment into the development of fertility control agents. Recent advances in research and development have led to the registration of novel fertility control agents for wildlife. Species-specific systems to deliver baits containing oral contraceptives to target species are now available. In addition, the development of new software and mathematical models has allowed researchers to make predictions of the effects of fertility control on population size. We present experimental data on the effectiveness of fertility control agents on model wildlife species and we illustrate examples of species-specific bait delivery systems. We discuss applications of fertility control to feral dogs and cats and zoo animals; we provide examples of how fertility control could be used for disease management.

## Administration of the GnRH-targeted immunocontraceptive vaccine 'GonaCon™' to the tammar wallaby, *Macropus eugenii*: side effects and welfare implications

Snappe, M.A.<sup>1,2,3</sup>, Hinds, L.A.<sup>1,2</sup>, Miller, L.A.<sup>4</sup>

<sup>1</sup>Invasive Animals CRC, University of Canberra, Bruce, ACT, 2617, Australia, melissa.snappe@csiro.au

<sup>2</sup>CSIRO Ecosystem Sciences, GPO Box 1700, Canberra ACT 2601, Australia

<sup>3</sup>Research School of Biology, Australian National University, Acton, ACT, 0200, Australia

<sup>4</sup>USDA National Wildlife Research Centre, Fort Collins, Colorado, USA

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Fertility control is being investigated as an alternative, non-lethal method of managing overabundant wildlife where conventional lethal techniques are no longer considered acceptable based most often on concerns for animal welfare. Gonadotrophin releasing hormone (GnRH) targeted immunocontraception has been shown to disrupt reproductive function in numerous mammal species by preventing GnRH-stimulation of gonadotrophin release from the pituitary, which results subsequently in a loss of gonadal activity in both sexes. GonaCon™, a single shot, injectable GnRH vaccine developed by the USDA National Wildlife Research Centre, has achieved long-lasting infertility in males and females of a number of species. Despite this success, it is necessary to evaluate all of the potential effects treatment with this vaccine may have on both individuals and social structure within a targeted population to avoid detrimental impacts on animal welfare and failure of a management strategy due to unforeseen changes in population dynamics. The effects of GonaCon™ on reproduction and behaviour have been assessed over the last 5 years in both short- and long-term experiments in the male and female tammar wallaby, a model macropodid marsupial species. From these studies, we have shown that this method causes 100% infertility in males vaccinated as adults (>2 years duration) or juveniles (≥2 years) and in adult females (>4 years). During this time data has also been collated for behavioural changes, body condition, effects on lactation and the formation of vaccination site reactions in the injected muscle block.

Males treated with the GnRH-vaccine as juveniles demonstrated significantly reduced rates of sexual behaviour compared to Control males, whilst those treated as adults demonstrated a statistically comparable number of mating attempts but failed to produce a copulatory plug. When held with vaccinated animals, experimental control males showed a decreased rate of agonistic behaviour compared to that observed when they were grouped with other untreated males. Despite this overall decrease in agonistic interactions, control males showed significantly more agonistic behaviours than did vaccinated animals during dominance observations, and achieved a higher dominance rank. Control males also showed sexual-type behaviours towards vaccinated males during dominance observations at a comparable rate to that demonstrated by intact males towards non-receptive females. Body condition was also altered in immunised animals compared to controls, with immunised animals generally having a greater amounts of mesenteric and kidney fat at autopsy. Lactation was not affected by administration of GonaCon™ to females with pouch young. The majority of animals immunised with GonaCon™ develop one or more granulomas at the site of injection, although signs of disrupted muscle function or discomfort were not apparent. Despite the various changes observed in the male and female tammar following immunisation, GonaCon™ does not apparently impact negatively on their welfare. Further studies at a population level are required to determine the effects of modified behaviour on social structure and individual wellbeing.

## Field evaluation of the immunocontraceptive vaccine GonaCon<sup>TM</sup> in free-living mammal populations

Cowan, D.<sup>1</sup>, Massei, G.<sup>1</sup>, Ward, A.<sup>1</sup>, Miller, L.A.<sup>2</sup>

<sup>1</sup>Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom, dave.cowan@fera.gsi.gov.uk

<sup>2</sup>National Wildlife Research Center, Fort Collins, 4101 LaPorte Avenue, Fort Collins, CO 80521-2154 USA United States

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There is increasing demand for benign approaches to the resolution of conflicts between human interests and wildlife. One potential non-lethal approach is fertility control, although effective tools have only recently begun to emerge. A major technology breakthrough has thus been the development of single-dose injectable immunocontraceptive vaccines that inhibit the fertility of individual animals for several years. This has culminated in the GonaCon<sup>TM</sup> vaccine being registered in the USA for use on white-tailed deer (*Odocoileus virginianus*). Here we describe the further evaluation of this vaccine in free-living populations of three other mammal species, wild boar (*Sus scrofa*), European badger (*Meles meles*) and feral goats (*Capra hircus*). These studies used remote monitoring techniques, including GPS collars, behavioural and physiological measures to quantify the impacts of single-dose vaccination on individuals and population consequences. The vaccine was effective in reducing the fertility of females of all three species. Furthermore, no negative welfare consequences were observed and no measurable effects found with respect to ranging or social behaviour. An 89% reduction in female feral goat fertility was maintained for at least two years post-vaccination and fertility control has now been incorporated into the long-term management plan for this population. This is the first active use of fertility control in the management plan for any mammal population in Europe. We anticipate that this example will be repeated with increasing frequency, across a range of potential target species, as opportunities for this emerging technology are realised, particularly to complement rather than necessarily replace more traditional wildlife management.

## Proposed strategic management of fallow deer to conserve endemic red deer in the Mesola forest, Ferrara, Italy

Ferri, M.<sup>1</sup>, Ferraresi, M.<sup>1</sup>, Gelati, A.<sup>1</sup>, Vitturi, M.<sup>2</sup>

<sup>1</sup> AUSL, Servizio Veterinario (Local Health Authority, Veterinary Service), 41122 Modena, Via Finzi 211, Italy, m.ferri@ausl.mo.it

<sup>2</sup> LAV (Antivivisection League) Lega Antivivisezione O.n.l.u.s., Viale Regina Margherita 177, 00198 Roma, Italy

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### Abstract

The Mesola Forest (1,058 ha), located in the Ferrara province in the north of Italy, is completely enclosed and is home to a small group of endemic red deer (*Cervus elaphus*) that used to be widespread across all of North Italy. The Forest also contains a large population of fallow deer (*Dama dama*) re-introduced in the 1950s-1960s. Since 1982, the fallow deer have been managed by shooting however the population has continued to increase. Therefore shooting of the fallow deer is not only ineffective but is also unpopular and may cause stress in both red deer and fallow deer. The Modena Veterinary Service and the Antivivisection League suggested adoption of a fertility control programme based on the use of an immunocontraceptive vaccine (GonaCon<sup>TM</sup>) registered in the USA for white-tailed deer. The use of the vaccine could help to reduce the fertility of the fallow deer population and lead to an increase in the red deer population which is currently too small to be safe from extinction. The fertility control program for the fallow deer population is proposed as part of a strategy to maintain the biodiversity of the Mesola Forest.

Keywords: *Dama dama*, fallow deer, fertility control, GonaCon<sup>TM</sup>, immunocontraception, killing, multi-year contraception, shooting

### Introduction

The Mesola Forest is located in the Ferrara province, in the North of Italy. This 1,058 hectares is one of the last remnants of the primordial plain forest in the 'Delta del Po' Park. Currently, the forest hosts around 120 red deer (*C. elaphus*). These animals are the remnant of a once vast, endemic population that was widespread in all the forests of the North of Italy. The Forest is also home to a large fallow deer population (*D. dama*) which was re-introduced in the 1950s and 1960s because the original population introduced by the Estense family during the Renaissance became extinct in 1945. The fallow deer population has rapidly increased and caused excessive over-browsing and over-grazing in the Mesola Forest. After 20 years the overpopulation of fallow deer resulted in the adoption of handling and shooting plans (1982). This approach did not solve the problem since, after 3,180 killings in twenty years (most of them in the last few years), the fallow deer population in 2010 remains around 950-1,000 compared to 602 in 2006 (Gallo, 2008; Lovari and Nobili, 2010). In order to resolve this problem the Modena Local Veterinary Service (AUSL) and the Antivivisection League have proposed to the Government Administration responsible for the forest reserve safeguard, to adopt a population management programme based on immunocontraception (Cooper and Larsen, 2006). This type of contraceptive control consists in the administration of a single shot vaccine to obtain multi-year infertility. The project which will last for 8-15 years is regarded as feasible and could substitute existing, ineffective culling management.

### Methods

The Authors proposed to the Puntamarina Biodiversity Office (Army Forestal Corp) a cooperation programme which aims at controlling the fertility in the fallow deer population using a contraceptive vaccine called (GonaCon<sup>TM</sup>) already licensed for use in North American white-tailed deer (Killian et al., 2008). The implementation of a management programme, based on existing subdivisions of the internal areas of the fenced Forest is under scrutiny. These sub-enclosures would make it possible to handle female fallow deer to treat them with the contraceptive vaccine and equip them with an ear-tag and to sample blood to monitor the antibodies levels. The UK's Food and Environment Research Agency has been contacted to finalise the research programme and to provide the contraceptive. The vaccination plan, which must be approved by the Italian Health Authority, could have the first positive effects after

2-5 years by allowing an initial stabilisation, followed by an effective numerical reduction of the fallow deer population without the need to continue with shooting schemes. The Authors propose to set up an interdisciplinary team (veterinarians and wildlife technicians) to manage the vaccination activities and the provision of supplementary food to the two populations of deer.

### **Predicted benefits**

Thanks to this programme in a few years it should be possible to vaccinate the vast majority of fallow deer living in the Mesola Forest. The main target of the project is to reduce that population and also to increase the number of red deer in the reserve. If the number of red deer increases it could be reintroduced to other suitable sites thus enhancing the survival of this endemic deer. If shooting was maintained, immunocontraception could still be used after an initial reduction through culling to manage the residual herd of fallow deer and to keep the planned maximum number (20-40) of an allochthonous species of historic interest in this Reserve. Moreover, the experience gained in using the GonaCon<sup>TM</sup> vaccine in the Mesola Forest would be extremely useful to extend the approach to small deer enclosures maintained in many Italian parks, reserves and farms as wildlife exhibits for tourists. All these structures often house excessive number of animals (such as deer, wild-boar) that, due to public opposition, cannot be managed through culling. Another important aspect of the program proposed has been the creation of a team composed of vets and wildlife experts who will be responsible for the vaccinations and the serological monitoring of the fallow deer. They will also provide essential advice on nutrition and animal welfare matters related to the two ungulates species of the Reserve and thus help to restore the biodiversity of the area.

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## Assessing recombinant vaccinia virus as a delivery system for fertility control vaccines in the brushtail possum (*Trichosurus vulpecula*)

Duckworth, J.<sup>1</sup>, Cross, M.<sup>1</sup>, Fleming, S.<sup>2</sup>, Scobie, S.<sup>1</sup>, Whelan, E.<sup>2</sup>, Prada, D.<sup>3</sup>, Mercer, A.<sup>2</sup>, Cowan, P.<sup>4</sup>

<sup>1</sup>Landcare Research, PO Box 40, Lincoln 7640, New Zealand, duckworthj@landcareresearch.co.nz

<sup>2</sup>Virus Research Unit, University of Otago, PO Box 56, Dunedin, New Zealand

<sup>3</sup>Ecogene Laboratory, Landcare Research Auckland 1142, New Zealand

<sup>4</sup>Landcare Research, PO Palmerston North 4442, New Zealand

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### Abstract

The introduced brushtail possum (*Trichosurus vulpecula*) is a major threat to native biodiversity and agricultural production in New Zealand. Research on non-lethal management methods is focussed on fertility control, and aims to develop zona pellucida (ZP) vaccines suitable for bait delivery to free-living possums. Vaccine delivery remains a challenge. One highly successful oral wildlife vaccine which has been widely used to control rabies in wildlife in the US and Europe, is based on a replication-limited recombinant vaccinia virus (rVV). The potential of rVV as a vaccine delivery system has yet to be tested in any Australian marsupial species. In the present study we evaluated the infectivity of rVV, as well as cell-mediated and antibody immune responses, in the marsupial brushtail possum. Possums were exposed to a model recombinant vaccinia construct (rVV399, which expresses the Eg95 antigen of the hydatid disease parasite *Echinococcus granulosus*) or the parent vaccinia virus strain (Lister) by applying 10<sup>8</sup> pfu of virus, drop wise onto the external surface of the nose and into the oral cavity. Both the recombinant and parent viruses persisted in the mucosal epithelium around the palatine tonsils for up to 2 weeks post-exposure. The parent vaccinia and the rVV399 construct induced peripheral blood lymphocyte reactivity against viral antigens in possums by 4 weeks post-exposure, and were still detectable at 4 months post-exposure. Serum antibody reactivity to the antigen Eg95 was recorded in 7/8 possums which received a single dose of the rVV399 construct and in 7/7 animals which received triple-dose delivery, with titre end-points in the latter case exceeding 1/4000 dilution. This study demonstrates that vaccinia virus will readily infect possums via an oronasal route and is capable of generating immune reactivity against both viral and heterologous antigens. This highlights the potential of recombinant vaccinia as a wildlife delivery system for fertility control vaccines for the brushtail possum and potentially other marsupial species.

Keywords: bait delivered vaccines, brushtail possum, fertility control, marsupial, vaccinia, wildlife

### Introduction

The introduced brushtail possum (*Trichosurus vulpecula*) is a major threat to native biodiversity and agricultural production in New Zealand. Research on non-lethal management methods is focussed on fertility control, and aims to develop zona pellucida (ZP) vaccines suitable for bait delivery to free-living possums. We have shown that bacterial ghosts (BG) (particulate vaccines derived from non-living empty cell envelopes of gram-negative bacteria) expressing the c-terminal residues of possum ZP protein 2 (ZP2-C) significantly reduced the fertilisation rate of artificially inseminated possums and conception rates of naturally bred possums when delivered by oral or eye/nose routes (Walcher et al., 2008). Despite developing new constructs capable of expressing the ZP antigen at higher levels and encapsulated formulations to prevent proteolysis and gastric acid degradation in the gastrointestinal tract, we have not been able to improve immune response intensity and longevity sufficiently to make the vaccines practical for field application. A review of potential delivery systems for fertility control vaccines in possum (Cross et al., 2011) identified replication-limited poxviruses such as recombinant vaccinia virus (rVV) as the basis of a potential oral wildlife vaccine for possums. rVV has been widely used to control rabies in wildlife in the US and Europe, but has never been evaluated in an Australian marsupial. In the present study we evaluated the infectivity of rVV as well as cell-mediated and humoral immune responses, in the marsupial brushtail possum.

## Materials and methods

Adult female possums, housed under Physical Containment Level 2 conditions, were exposed to a model recombinant vaccinia construct (rVV399, expressing the Eg95 antigen from the hydatid disease parasite *Echinococcus granulosus*, Marsland et al., 2003) by applying 10<sup>8</sup> pfu of virus drop wise onto the external surface of the nose and into the oral cavity. Under ketamine anesthesia, eight possums were treated with a single dose of rVV339; seven animals with three weekly doses of rVV339 and eight with a single dose of the parent-strain vaccinia virus (Lister). The mucosa around the palatine tonsil was swabbed for DNA prior to treatment and at week 1, 2, and 4 post-infection to assess viral persistence by PCR analysis (Reubel et al., 2005; Sandvik et al., 1998). Blood samples were collected at week 0, 4, 8, 12 and 16 to measure immune reactions. Thymidine incorporation was used to assess lymphocyte proliferation responses to viral antigens, and possum serum antibody binding to Eg95 protein was measured by ELISA (Duckworth et al., 2007).

## Results

A single dose of rVV339 infected 3/8 possums, and 7/7 animals were infected in the triple-dose group. No rVV339-treated animals showed overt signs of viral pathology. Following a single dose with the parent-strain vaccinia virus, 8/8 treated possums were infected and two animals exhibited small facial lesions that were positive for presence of vaccinia virus by PCR and *in vitro* culture. Both the recombinant and parent viruses persisted in the mucosal epithelium around the palatine tonsils for up to 2 weeks post-exposure. Parent strain vaccinia and the rVV399 construct induced peripheral blood lymphocyte reactivity against viral antigens, first apparent at 4 weeks post-exposure and still detectable at 4 months post-exposure. Serum antibody reactivity to Eg95 was recorded in 7/8 possums which received a single dose of the rVV399 construct, and in 7/7 animals which received triple-dose delivery and was detectable for at least 4 months. Maximum antibody titers were 1:2048 for possums which received a single dose or rVV339 and 1:4096 for animals that received multi-doses of the construct. Possums that received parent-strain vaccinia virus and non-exposed control possums remained non-responsive to Eg95.

## Discussion

This study demonstrates that vaccinia virus can establish an infection in brushtail possums following application into the oral cavity and onto the external surface of the nose; a route of delivery designed to simulate the natural feeding behavior of possums. This is the first report in an Australian marsupial species demonstrating an immune response to a recombinant antigen in a poxvirus construct. While the potency and longevity of vaccinia-based vaccines expressing an immunocontraceptive antigen in possums is yet to be confirmed, these initial results, and the extensive safety and efficacy precedent set by the Raboral<sup>TM</sup> oral rabies vaccine in eutherian wildlife, provide encouragement for such an approach to be used as an oral vaccine delivery system for possums and potentially other marsupial species.

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## The use of DiazaCon™ to limit fertility in grey squirrels

Mayle, B.<sup>1</sup>, Ferryman, M.<sup>1</sup>, Peace, A.<sup>1</sup>, Yoder, C.A.<sup>2</sup>, Miller, L.A.<sup>2</sup>, Cowan, D.<sup>3</sup>

<sup>1</sup>Centre for Human and Ecological Sciences, Forest Research, Farnham, Surrey, GU10 4LH, UK, [brenda.mayle@forestry.gsi.gov.uk](mailto:brenda.mayle@forestry.gsi.gov.uk)

<sup>2</sup>National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA

<sup>3</sup>The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK

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### Abstract

The grey squirrel is an invasive alien species introduced to Great Britain in the late 19<sup>th</sup> Century and to Northern Italy during the early 20<sup>th</sup> Century. As well as displacing the native European red squirrel, grey squirrels cause significant impact to trees and woodlands through bark-stripping activity, and are implicated in the decline of woodland bird populations. In Britain, eradication is no longer an option at a regional scale, but fertility control offers a possible approach to minimise negative impacts. The cholesterol mimic DiazaCon™ has been successfully used to inhibit reproduction in some species as it reduces cholesterol available for steroid reproductive hormone synthesis. Results are presented from enclosure studies investigating whether DiazaCon™ would reduce cholesterol levels enough and for a sufficient period to reduce fertility in female grey squirrels.

Keywords: cholesterol, contraception, DiazaCon™, fertility control, grey squirrel, 20, 25-diazacholesterol dihydrochloride

### Introduction

Since its introduction to Britain in the late 19<sup>th</sup> Century the grey squirrel (*Sciurus carolinensis*) has been of increasing concern to woodland and conservation managers due both to seasonal bark stripping activity and its impact on woodland biodiversity, particularly the native red squirrel (*S. vulgaris*). Estimates of annual costs to the British timber industry of damage to trees through bark stripping by grey squirrels are £3.40 (€3.84) per ha of vulnerable conifers, and £5 (€5.65) per ha for the three most vulnerable broadleaf timber tree species; sycamore (*Acer pseudoplanatus* L.), beech (*Fagus sylvatica* L.) and oak (*Quercus* sp.) (Williams et al., 2010). As tree damage is believed to be triggered in part by an increase in juveniles in the population during late spring, following breeding in January/February, fertility control offers a non-lethal approach to minimising impacts.

DiazaCon™ is a cholesterol mimic that inhibits cholesterol production and blocks steroid hormone production and thus can reduce fertility in both males and females. A reduction in cholesterol of approximately 40% will reduce reproduction in many bird species and rats (Yoder et al., 2005). An initial study (Yoder et al., in press) showed that DiazaCon™ treatment over 8 days reduced cholesterol ≥40% for 2 months in grey squirrels. As grey squirrels will not breed in cages in captivity, enclosure studies were used to investigate whether DiazaCon™ would reduce cholesterol levels enough and for a sufficient period to reduce fertility in female grey squirrels.

### Materials and methods

DiazaCon™ was provided by the Avitrol Corporation (Tulsa, OK) via National Wildlife Research Center (NWRC). Enclosure studies were carried out in 2007 and 2009. In each year 32 female grey squirrels were caught from the wild and held in individual cages to acclimatize before the treatment feeding period. Sixteen females were allocated each to treatment or control. For both studies DiazaCon™ was presented on whole grain wheat coated with corn oil and sugar to achieve a dose of 55mg/kg bodyweight (assuming 20g bait was eaten each day over a 10 day feeding period). Control animals received untreated bait, and daily bait intake was monitored for both control and treated groups.

After treatment the females were released into enclosures with males and monitored monthly thereafter for reproductive success. Treatment females were allocated six each to 2 large and two each to 2 small enclosures, based upon mean daily DiazaCon™ dose intake. The same numbers of control females were allocated to the enclosures based upon pre-feeding period bodyweight, with animals of similar weight allocated to each enclosure where possible. Before the feeding period all females were weighed, a

vaginal smear taken to check the stage of the oestrous cycle and a blood sample was taken for cholesterol and desmosterol testing at NWRC and for progesterone assay. At each recapture females were visually assessed for lactation (raised, hairless nipples), weighed, and blood samples and vaginal smears taken. DNA testing was used to confirm relatedness of juveniles/litters found to females.

## Results

In 2007 the DiazaCon™ dose levels achieved ranged from 8.9 to 25.7 mg/kg (mean 16.6 mg/kg) (n=16). Plasma cholesterol reduced in control and treated groups post treatment, then returned to above pre-treatment levels for the control group and remained at this level until the end of the study. For treatment females plasma cholesterol levels remained below 50% of that of the control group for 68 days. By the end of the 4 month study period there was no significant difference between treatment and control groups. Desmosterol levels were correlated with dose of DiazaCon™. As only 2 control females had litters, both with 2 kittens, results in terms of effect of DiazaCon™ on reproduction were inconclusive.

In 2009 a greater range of dose intake was achieved with a mean intake of 28.7 mg/kg and range 9.0-66.2 mg/kg (n=13). The target dose of 55 mg/kg was achieved for 2 females. Cholesterol response showed a significant increase in cholesterol for control animals (n=16) on release into the enclosures, as observed in 2007. The decline in cholesterol post feeding was less pronounced than observed in 2007. A significant reduction in cholesterol was observed for the treatment group up to 4 months post treatment with treatment cholesterol at only 66% of the control group at this time. Mean desmosterol response reflected the observed decrease in cholesterol. Although more pregnancies were observed during the 6 months that females were held in the enclosures only one control female bred soon after release into the enclosures and the rest bred between May and late June. Nine control females bred (7 litters found) and 5 treatment females bred (3 litters found). One treatment female bred after the end of the study.

## Discussion

These studies demonstrate that DiazaCon™ fed over a 10 day period is effective at reducing cholesterol levels by  $\geq 40\%$  in female grey squirrels for up to 4 months. A lack of information on seasonal changes in plasma cholesterol for grey squirrels limits interpretation of the results. As most females bred after cholesterol levels in treated females had returned to pre-treatment levels the results are inconclusive in terms of effect of DiazaCon™ on fertility in grey squirrels.

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## Reproductive inhibition with gossypol in the lesser bandicoot rat, *Bandicota bengalensis*

Singla, N., Meenu, M.

Department of Zoology, Punjab Agricultural University, Ludhiana-141004, Punjab, India,

neenasinglal@gmail.com

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### Abstract

Different concentrations of pure gossypol (PG), cottonseed extract containing 8.3% gossypol (CSE) and crude cotton seed oil containing 0.01% gossypol (CCSO) were fed to *Bandicota bengalensis* of both sexes to determine their effects on reproduction. Single oral administration of either 40 or 80mg PG/kg body weight (bw), or 100 or 200 mg CSE/kg bw did not affect reproductive parameters. Feeding of bait containing 0.05% PG for 4 days; 0.01 or 0.02% PG for 16 days and 0.2 or 0.5% CSE for 7 or 15 days each caused dose dependent decreases in weights of reproductive organs and accessory sex glands and motility, viability and density of sperm in cauda epididymal fluid. There was a dose dependent increase in sperm abnormalities. Gossypol-specific sperm abnormalities were also observed. No breeding was observed in untreated cyclic female rats paired with male rats treated with 0.05 or 0.02% PG for 4 or 16 days respectively, or 0.5% CSE for 15 days. A significant ( $p \leq 0.05$ ) increase in oestrous cycle length was observed in female rats fed bait containing 0.01% PG for 18 days or 0.5% CSE for 15 days.

No effect on the oestrous cycle was observed in female rats fed bait containing 5 or 10% CCSO for 13 days. Feeding of bait containing 10% CCSO for 15 or 30 days to male rats also had no significant effect on reproductive parameters. Treatment of farmer's sugarcane fields with 2% zinc phosphide followed by treatment with 10% CCSO for 15 or 30 days had no significant effect on rodent activity. Our results reveal that reproduction of *B. bengalensis* can be inhibited with multiple doses of gossypol but the effects are dose and time dependent.

Keywords: cotton seed extract, crude cotton seed oil, gossypol, reproductive inhibition

### Introduction

Gossypol produces species specific and dose dependent antifertility effects in mammals. Yu and Chan (1998) emphasized the need for continuing studies on gossypol. The lesser bandicoot rat, *B. bengalensis* is the major rodent pest of South Asia inflicting heavy losses on agricultural crops. The success of this species lies in its highly adaptable nature coupled with high reproductive capacity. Reproductive inhibition is a non-lethal alternative that has the potential to provide long lasting control. The present study was conducted to evaluate the potential of gossypol in inhibiting reproduction of *B. bengalensis*.

### Materials and methods

Mature and healthy *B. bengalensis* of both sexes were trapped from crop fields and acclimatized to laboratory conditions. Gossypol (PG) was obtained from MP Biomedicals, USA. Cotton seed extracted compound (CSE) was obtained by etheral extraction of *G. hirsutum* seeds for 24 hr. Crude cotton seed oil (CCSO) was procured from an oil refinery in Ludhiana. Gossypol content in CSE and CCSO was estimated according to the method described by AOCs (Singh, 1991). Male rats were given single oral doses of 40 or 80mg PG /kg bw (n=4/group), or 100 or 200 mg CSE/kg bw (n=3/group), or fed multiple doses in bait (cracked wheat: powdered sugar: powdered milk: groundnut oil, 85:5:5:5) of 0.05% PG for 4 days (n=3/group), 0.01 or 0.02% PG for 16 days (n=3 /group), 0.2% CSE for 7 or 15 days (n=3 per group), 0.5% CSE for 7 or 15 days (n=3/group) or 10% CCSO for 15 or 30 days (n=7/group). Female rats were fed 0.01% pure gossypol for 18 days (n=3/group), 0.2% CSE for 7 or 15 days (n=3/group), 0.5% CSE for 7 or 15 days (n=3/group) and 5 or 10% CCSO for 13 days (n=5/group).

For each treatment group there were equivalent numbers of untreated controls. Fifteen days after the end of treatment, all treated and untreated male rats were bred with untreated cyclic female rats for 15 days after which all the male rats were autopsied to record the effect of treatment on weights of reproductive organs and accessory sex glands, sperm motility, viability, density and morphology in cauda epididymal fluid and histomorphology of testes. Female rats were observed for pregnancy and delivery of pups. Vaginal fluid of treated and untreated female rats was observed during pre- and post-treatment periods

twice each day to determine effects on the oestrous cycle. Sugarcane fields at village Iraq, district Ludhiana, Punjab (India) were treated with 2% zinc phosphide followed by treatment with 10% CCSO for 15 or 30 days to compare rodent activity, based on pre- and post-treatment period census bait consumption, with that in untreated fields and fields treated with only 2% zinc phosphide.

## Results

Single oral administration of either 40 or 80mg PG/kg bw, or 100 or 200 mg CSE/kg bw to male *B. bengalensis* did not affect the weights of reproductive organs and accessory sex glands and sperm parameters in cauda epididymal fluid. Feeding of bait containing 0.05% PG for 4 days; 0.01 and 0.02% PG for 16 days and 0.2 and 0.5% CSE for 7 and 15 days each caused a dose dependent decrease in the weight of the testis, epididymis and seminal vesicles and motility, viability and density of sperm in cauda epididymal fluid, and a dose dependent increase in sperm abnormalities.

In rats treated with PG, gossypol-specific sperm abnormalities (missing axial fibers and sheaths in the flagellar region of spermatozoa) were observed. No breeding was observed in untreated cyclic female rats bred with male rats treated with 0.05 or 0.02% PG for 4 and 16 days respectively or with 0.5% CSE for 15 days. There was a significant ( $P \leq 0.05$ ) increase in duration of the oestrous cycle from  $4.51 \pm 0.02$  to  $4.58 \pm 0.03$  ( $t=2.74$ ,  $df=4$ ) and from  $4.52 \pm 0.02$  to  $4.64 \pm 0.07$  days ( $t=2.33$ ,  $df=4$ ) in female rats fed on bait containing 0.01% PG for 18 days and 0.5% CSE for 15 days, respectively.

Feeding of bait containing 5 and 10% CCSO for 13 days to female rats did not have any effect on the duration of the oestrous cycle. Feeding of bait containing 10% CCSO for 15 or 30 days to male rats also had no significant effect on the weights of reproductive organs, sperm parameters, histomorphology of testis and reproductive success of rats. No significant difference in rodent activity was observed among sugarcane fields treated with only 2% zinc phosphide and those treated with 2% zinc phosphide followed by treatment with 10% CCSO for 15 and 30 days.

## Discussion

The absence of effects on reproduction following single oral doses of PG and CSE may be due to the fast elimination of gossypol from the body (Othman and Abou-Donia, 1988). In our studies, a greater increase in oestrous cycle length was observed in females treated with CSE than in those treated with PG. This greater effect has been attributed to other factors present in seed extracted samples (Eagle and Bialek, 1952). A dose dependent decrease in reproductive organ weights and sperm quality and quantity in rats after gossypol treatment was also observed by Taylor et al. (1991).

The reduction in sperm concentration in gossypol-treated small rodents has been attributed mainly to the damage caused to the germinal epithelium leading to low production of sperm (Hoffer, 1983). Spermatocyte apoptosis during the meiosis of spermatogenesis is the main reason for the antifertility (Chang et al., 2010). Gossypol specific abnormalities in sperm have been reported previously (Nadakavkaren et al., 1979). Longer oestrous cycle lengths in gossypol-treated rats have also been reported (Lin et al., 1985). The present results reveal that reproduction of *B. bengalensis* can be inhibited with multiple doses of gossypol but the effects are dose and time dependent.

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## Effects of the combination of levonorgestrel and quinestrol on reproductive hormone levels and their receptor expression in female Mongolian gerbils (*Meriones unguiculatus*)

Ly, X.<sup>1</sup>, Guo, Y.<sup>2</sup>, Shi, D.<sup>1</sup>

<sup>1</sup>College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, China, shidazhao@cau.edu.cn

<sup>2</sup>National Agricultural Technology Extension and Service Center, Beijing 100125, China

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The effects of treatment with a combination of levonorgestrel and quinestrol (EP-1; ratio of 2:1) on reproductive hormone levels and their receptor expression in female Mongolian gerbils were examined. In the time-dependent experiment, fifteen gerbils were given a single intragastric dose of EP-1 (50 µg/g body weight) at dioestrus and killed 7, 14, or 21 days later (n=5 animals per group). Control animals (n=5) received peanut oil at dioestrus and were killed on Day 0. The ovaries and uteri were collected for RNA extraction. Blood samples were collected before euthanasia. In the second, dose-dependent experiment, another twenty gerbils (n=5 per treatment group) were given EP-1 intragastrically once at 0, 2, 10, or 50 µg/g body weight at dioestrus. The control group was given peanut oil. Blood samples were collected at 7 days after administration. The gerbils were killed 21 days after treatment. The ovaries and uteri were collected for RNA extraction.

The effects of EP-1 treatment were time- and dose-dependent. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) decreased, whereas serum estradiol (E2) and progesterone (P4) increased after EP-1 treatment compared to control treatment. EP-1 down-regulated the mRNA expression of the follicle-stimulating hormone receptor (FSHR) and the estrogen receptor (ER)  $\beta$  in the ovary. EP-1 up-regulated the mRNA expression of the luteinizing hormone receptor (LHR) and the progesterone receptor (PR) in the ovary as well as ER $\alpha$  and PR in the uterus of Mongolian gerbils. However, EP-1 had no obvious effects on ER $\alpha$  mRNA expression in the ovary.

The current study has demonstrated that the effect of EP-1 on the expression of ER subtypes is tissue-specific in Mongolian gerbils. EP-1 disrupted the reproductive endocrinology of the Mongolian gerbil. The findings suggest that the effects of EP-1 on reproductive hormone levels and their receptor expression in Mongolian gerbils may be a result of the synergistic actions of levonorgestrel and quinestrol with quinestrol playing the major role.

Keywords: EP-1, reproductive hormone, reproductive hormones receptors

## **Quinestrol treatment induces testicular damage via oxidative stress in male Mongolian gerbils (*Meriones unguiculatus*)**

Shen, W.<sup>1</sup>, Shi, D.<sup>1</sup>, Wang, D.<sup>1</sup>, Guo, Y.<sup>2</sup>, Hai, S.<sup>1</sup>, Yue, Z.<sup>3</sup>

<sup>1</sup>College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, China, shidazhao@cau.edu.cn

<sup>2</sup>National Agro-tech Extensions and Service Center, Beijing 100125, China

<sup>3</sup>College of Veterinary Medicine, China Agricultural University, Beijing 100193, China

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The hypothesis that quinestrol exerts testicular damage via oxidative stress was investigated in male gerbils using a daily oral gavage of 3.5 mg/kg body weight for 2 weeks (the multiple-dose treated group, n=15) or 35 mg/kg body weight (single-dose treated group, n=15). The testicular histological morphology, antioxidant capacity and malondialdehyde (MDA) concentration in testicular tissue and plasma were assessed at 15, 30, and 60 days following the single or multiple treatment. Five gerbils per group were killed at the different time points. The results showed that the activity of the antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) in testicular tissue decreased at 15 days after treatment. These effects led to increased MDA concentrations while at the same time germ cells were reduced and showed an irregular distribution in the seminiferous tubules of quinestrol-treated gerbils. At 30 days, the testicular weight and antioxidant capacity had continued to decrease, while the MDA concentrations continued to increase and testicular histopathological changes were more pronounced. Single-dose and multiple-dose treatment had a similar effect on the antioxidant enzymes and MDA, but testicular damage was more severe at 15 and 30 days after the end of the multiple-dose treatment. By 60 days after treatment withdrawal, however, the above parameters had recovered to control levels. The results show that quinestrol causes reversible damage to gerbil testes and this may be caused by oxidative stress. Furthermore, multiple-dose treatment has greater disruptive effects on testicular morphology compared to a single higher dose treatment.

Keywords: oxidative damage, quinestrol, reversibility, testicular damage

## The management of non-native birds in the United Kingdom

Allan, J.

Head of Wildlife Programme, Food and Environment Research Agency, Sand Hutton, York, UK,  
john.allan@fera.gsi.gov.uk

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In common with many other parts of the world, the UK has a long history of bird species establishing themselves either naturally, such as the collared dove (*Streptopelia decaocto*) and little egret (*Egretta garzetta*) in the last 50 years; or as a result of deliberate or accidental introductions. Introduced species such as the little owl (*Athene noctua*) and Canada goose (*Branta Canadensis*) have been present for hundreds of years and are regarded by many as part of the 'natural' avifauna of the country. Debate continues about whether other species, such as the eagle owl (*Bubo bubo*), have at some time been naturally present and should be regarded as native rather than introduced, even though the individual birds now breeding in the UK are almost certainly escapees from captivity. Others, such as common pheasant (*Phasianus colchicus*), continue to be released in huge numbers every year for sport shooting.

The impact of these species on the ecosystems that they occupy is poorly understood. For example limited information is available on the impact that hundreds of thousands of released common pheasants have on other ground feeding birds or on the impact of the growing population of Canada Geese on UK wetlands.

The legal status of non-native birds in the UK is also complex. All wild birds are subject to the EU Birds Directive, and the UK Wildlife and Countryside Act, which protects all wild birds and permits management of particular species by the issue of licenses. Game birds, such as common pheasant and red-legged partridge (*Alectoris rufa*) are subject to the Game Act, however, and are not regarded as 'wild birds'. Recently arrived species, which are not on the official UK list of wild birds, and which have not arrived by natural means, are not protected at all

Against this background, the UK government and its conservation agencies need to make decisions about the management of non-native birds. Historically, the approach has been inconsistent and subject to pressures from interest groups such as the hunting lobby, bird conservation organizations and animal rights groups. Financial considerations have also played an important part in decision making, with some species now regarded as simply too expensive to manage with current population sizes. In an effort to bring more consistency to the process of managing non-native species (both plants and animals) the UK has established the non-Native Species Secretariat, which commissions expert risk assessments for non-native species and these are used to help for policy on whether, or how, these species are managed. These risk assessments, combined with logistical feasibility and cost are used to inform decisions about future management actions.

This paper describes the complexities involved in the management of non-native birds in the UK and uses a number of examples ranging from do nothing to licensed control to complete eradication to show how different decision making processes have resulted in different outcomes for the UK's avifauna.

## Current situation and problems of management of pest birds in the cities of Ukraine

Gavris, G.

I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of the Ukraine, Vul. B. Khmelnytskogo, 15, Kyiv, 01601, Ukraine, gavris@izan.kiev.ua

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Data are reported of research conducted over the last ten years in some large cities of the Ukraine (Kyiv, Odessa, Dnipropetrovs'k, Simferopol', Chernihiv, etc.). The main pest bird species are feral pigeon *Columba livia* and house sparrow *Passer domesticus*. Pigeons can cause damage to buildings, especially to historical architectural monuments but practical management of this problem is not undertaken in the Ukraine at present. Also pigeons cause big problems at premises used to store and process grain. Catching of birds and release in distant regions is now the only method of control used by some manufactures (beer factories, etc.). Sparrows constitute a menace for large supermarkets and bakeries, due to damage to foodstuffs and fouling with feces. Control includes catching of birds and their release outside. Cases of visitation of other species of *Passeriformes* to supermarkets have been noted in migration and wintering periods. Also rooks *Corvus frugilegus* create some discomfort for people during nesting or at winter roosts in city parks and squares. Management of rooks is not undertaken. Other traditional pest bird species (swallows, gulls, starlings, etc.) create only small local problems. Thus management of birds in the cities of Ukraine is at an unsatisfactory level and this problem should be addressed at governmental level.

Keywords: cities of Ukraine, *Columba livia*, management pest birds, *Passer domesticus*

## Hide-and-seek in Europe: highly pathogenic avian influenza H5N1

Globig, A.<sup>1</sup>, Staubach, C.<sup>2</sup>, Harder, T.<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Suedufer 10, 17493 Greifswald-Insel Riems, Germany, anja.globig@fli.bund.de

<sup>2</sup>Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Wusterhausen, Germany

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When in winter 2005/2006 the highly pathogenic avian influenza virus (HPAIV) H5N1 of Asian lineage entered Europe for the first time, the public was highly alarmed and ‘bird flu’ became a number one topic in the media for several weeks. Between February and May 2006 several hundred wild birds were found dead and infected with the highly pathogenic strain in a total of fourteen EU countries (Martinez et al., 2008), and public health concerns were mounting when domestic cats were found to have succumbed to the disease after feeding on infected bird carcasses (Klopfleisch et al., 2007). Several projects were subsequently enrolled in order to explore the source of the virus and the status of infection among apparently healthy wild birds associated with the risk of transmission to and further spread among poultry.

Now, 5 years later, ‘bird flu’ has almost completely vanished from the public agenda. However, the assumption that ‘bird flu’ has disappeared is quite contrary to the actual situation: In Africa (Egypt) and vast parts of Asia the HPAI H5N1 virus is still circulating in poultry with occasional spill-over transmissions to wild birds and even humans. The last occurrence of HPAI H5N1 in Europe dates back to March 2010 when poultry was infected in the Romanian Danube Delta. At the same time a common buzzard was affected in Bulgaria indicating, again, an involvement of wild birds (Reid et al., 2011).

The presentation will give an overview on our current knowledge on HPAIV H5N1 in wild birds. The important aspects of AIV ecology in wildlife (monitoring techniques, surveillance, habitat use and migration patterns) will be discussed.

Keywords: highly pathogenic avian influenza H5N1, surveillance, wild birds

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## Environmental impacts of the control with organophosphate pesticides and explosions of the red-billed quelea bird *Quelea quelea* in Africa

Cheke, R.A.<sup>1</sup>, Van der Walt, E.<sup>2</sup>, Mbereki, C.<sup>3</sup>, Mtobesya, B.N.<sup>4</sup>, Magoma, R.N.<sup>5</sup>, Farman, D.I.<sup>1</sup>, Adranyi, E.<sup>1</sup>, McWilliam, A.<sup>6</sup>

<sup>1</sup>Natural Resources Institute, University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK, r.a.cheke@greenwich.ac.uk

<sup>2</sup>Agricultural Research Council, Plant Protection Research Institute, P. Bag X134, Queenswood, 0121, Pretoria, Republic of South Africa

<sup>3</sup>Plant Protection Division, Ministry of Agriculture, P/Box 0091, Gaborone, Botswana

<sup>4</sup>Plant Health Services, Ministry of Agriculture Food Security and Cooperatives, P.O. Box 9071, Arusha, Tanzania

<sup>5</sup>Plant Health Services, Ministry of Agriculture Food Security and Cooperatives, P.O. Box 9071, Dar es Salaam, Tanzania

<sup>6</sup>The Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH, U

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### Abstract

Both spraying with pesticides and using explosions to kill red-billed quelea (*Quelea quelea*) may affect non-target organisms and leave hazardous contaminants in the environment. Results of monitoring such effects in Botswana and Tanzania showed that although few non-target mortalities were noted, indirect effects in terms of depressed cholinesterases in birds and small mammals were marked and pesticides and contaminants from products used for the explosions persisted at controlled sites at unacceptable levels and for long periods (at least 188 days for sprays and at least 11 months for petroleum products and plastics).

Keywords: cholinesterases, explosions, non-target organisms, organophosphate sprays, *Quelea quelea* L., red-billed quelea, residues

### Introduction

The red-billed quelea *Quelea quelea* is a major pest of small grain cereals in sub-Saharan Africa (Bruggers and Elliott, 1989). The birds nest colonially and also roost in dense concentrations, providing targets that can be controlled by spraying with organophosphate pesticides, such as fenthion and cyanophos, or by explosives. Some environmental impacts of these lethal methods used against *Q. q. lathamii* in Botswana and *Q. q. aethiopica* in Tanzania were investigated. Mortalities of non-target organisms were recorded after control operations. The blood cholinesterase levels of non-target birds and small mammals were monitored and soil residue analyses were conducted on samples after sprays with fenthion or cyanophos and after explosions. Pesticide droplet sizes and deposition were also studied.

### Materials and methods

(a) *Sprayed sites*. Soil samples of approximately 200 g (maximum depth 10 cm) were collected at 7 sprayed sites in Botswana and 4 in Tanzania and residues of fenthion or cyanophos detected by GCMS. As blood cholinesterases are depressed by the action of organophosphates, we used custom-made field kits using the Ellman reaction (Ellman et al., 1961, Test-mate kits, EQM Research Inc., Cincinnati, USA) to analyse erythrocyte cholinesterase (acetylcholinesterase, AChE) and plasma cholinesterase (butyrylcholinesterase, PChE) levels in the blood of birds and small mammals caught before and after spraying. Droplet samples were collected on magnesium oxide (MgO) coated glass slides placed on metal sampling masts 1 m above ground level. (b) *Sites of explosions*. Soil samples were collected at 10 sites in Botswana before and after explosions of diesel and petroleum mixtures in plastic containers and levels of total petroleum hydrocarbons and plastics were detected by GCMS. Any dead non-targets were recorded and effects on the vegetation assessed visually.

### Results

Spraying operations' effects on non-target birds were confirmed by direct associations between non-target morbidity and the depression of blood cholinesterase levels by as much as 90%. Small mammal populations examined 6 weeks after aerial sprays in northern Botswana had significantly reduced blood cholinesterases. Fenthion had a half life of 47 days in soil, sometimes remaining at unacceptably high

levels (11.7 µg/g) for up to at least 188 days. Both soil analyses and pesticide droplet analyses showed that the distribution of fenthion was very uneven at sites sprayed in Botswana. The droplets per cm<sup>2</sup> were nearly always below the recommended value of 44 which, together with other operational problems, such as excessively high droplet volume median diameters, lower than recommended numerical median diameters and poorly positioned spray nozzles, led to poor quelea kills. With one exception due to a misidentification of the target birds, no excessive non-target mortalities were noted out of all of the control methods monitored. Small mammals were the most numerous victims of explosions, after which both residues of petroleum products (up to 131 µg/g of total petroleum hydrocarbons, TPH; mean=7.51, SD=22.13, n=43) and plastics (up to 3.2 µg/g of total phthalates; mean=0.32, SD=0.61, n=43) were detected in soil samples. The mean concentrations for TPHs and phthalates were, respectively, 49 and 9 times greater than background levels and likely to be responsible for ecological disruption including secondary poisoning. The explosions also damaged the soil by leaving craters covering >1% of the controlled areas, where the highest concentrations of hydrocarbons and phthalates were found, and led to burnt vegetation. However, the plants involved recovered within 1 year unless their trunks were split. Residues of TPHs (up to 1.3 µg/g) and of phthalates (up to 0.854 µg/g) were still present 11 months after explosions.

### Discussion

Although fewer non-target mortalities were recorded than expected on the basis of other studies (McWilliam and Cheke, 2004), the environmental effects were nevertheless unacceptable in terms of soil residues and morbidity of wildlife. Recommended mitigation measures for explosions include (a) removing plastic remains of petroleum product containers for disposal at safe containment facilities rather than burning them in situ after explosions; (b) investigating the possibility of replacing the plastic containers used for the explosions with 'tetra pack' cartons; for ground sprays (c) ensuring that cut-line orientation and layout is conducted with knowledge of the prevailing wind direction and wind speed to minimize human health and environmental impacts; (d) ensuring that all spraying equipment is serviced, calibrated and well maintained; (e) ensuring that sprayer calibration is performed at regular intervals and includes droplet analysis to ensure proper pesticide deposition; (f) ensuring that appropriate droplet calibration is performed for each type of pesticide formulation to be used; for aerial sprays (g) satellite navigation systems should be available; (h) target sites should be marked clearly and indicate where within the target site the spray swaths are required; (i) two way radios (VHF and CB) could aid in demarcating the layout of target areas if the pilot is enabled to contact the ground crew for important visual information; for both explosions and sprays (j) removing dead quelea and other carcasses after control operations and disposing of them in safe containment facilities; (k) wearing of protective gear during control operations; (l) training in bird identification methods to ensure that only quelea birds are targeted; (m) consider using mass-trapping in cases where this could serve as a control measure and provide food.

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## Assessing the effects of three potential chemical repellents to prevent bird damage to corn seeds and seedlings

Esther, A.<sup>1</sup>, Tilcher, R.<sup>2</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Federal Research Institute for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppeideweg 88, 48161 Münster, Germany, alexandra.esther@jki.bund.de

<sup>2</sup>KWS SAAT AG, Grimsehlstraße 31, 37555 Einbeck, Germany

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Bird damage to seeds and seedlings of corn and other crops is widespread in organic farming because no adequate seed protection is available. In this study we systematically tested the effect of seed treatments with three synthetically-produced substances known to affect bird feeding behavior (anthraquinone, pulegone and methylantranilate) in different concentrations. There was no negative effect on the germination of seeds for all seed treatments. Their repellent effect was tested by food and seedling choice (treated *versus* untreated) experiments with pigeons (*Columba livia*) in aviaries. The best treatment variants in these trials were additionally tested regarding plant growth in the field where wild birds had unlimited access.

In aviaries, untreated seeds were clearly preferred over treated seeds by pigeons. The highest feeding deterrence effect occurred with the treatment variants 7.5% pulegone and 0.5% methylantranilate. In these cases less than 5% of the daily consumption was treated seeds. In contrast, however, there was no treatment effect on seedlings. The same number of seedlings grown from treated and untreated seeds remained intact.

Further replications and field experiments with additional treatment variants are being conducted at the moment. The repellent effects of some of the treatment variants indicate that further studies with the same compounds but based on plants and benefit-cost-analyses should be done to yield a bird repellent to be used for organic seed protection.

## **Control of the urban pigeon *Columba livia* population and the preservation of common swift *Apus apus* and bats *Chiroptera* during the restoration of the Ghirlandina tower in the city of Modena (Italy)**

Ferri, M.<sup>1</sup>, Ferraresi, M.<sup>1</sup>, Gelati, A.<sup>1</sup>, Zannetti, G.<sup>4</sup>, Domenichini, A.<sup>1</sup>, Ravizza, L.<sup>3</sup>, Cadignani, R.<sup>2</sup>

<sup>1</sup>AUSL, Servizio Veterinario (Local Health Authority, Veterinary Service), 41122 Modena, Via Finzi 211, Italy, m.ferri@ausl.mo.it

<sup>2</sup>Head of Historical Building Service Modena Municipal Authority, Modena, Italy

<sup>3</sup>Municipality of Melegnano (MI), Italy

<sup>4</sup>Department of Animal Health University of Parma, Parma, Italy

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### **Abstract**

The problem of the excessive abundance of the urban pigeon *Columba livia* population in the city of Modena has been solved thanks to the cooperation between the Modena Municipal authority and the Local Veterinary Service (AUSL). They have followed an integrated program that contains the following points: use of an oral veterinary licensed drug (Ovistop<sup>®</sup>, ACME, 1997-2002) in order to limit the pigeons fertility; use of architectural devices in order to limit the access of the pigeons in private and public structures, periodic monitoring of the colonies of pigeons and public information regarding the treatments and the integrated program. In the year 2008 Modena Municipality started the restoration of the Ghirlandina Tower. The above-mentioned authorities decided to exclude the pigeon population from the many scaffold holes existent on the tower. However, rather than completely remove all the scaffold holes to limit the pigeon reproduction, they decided to reduce the size of the scaffold holes, to allow access only to smaller animals, like Common swifts *Apus apus* and bats. In conclusion, the Modena Municipal Authority and the Local Veterinary Service have reached two important results: the first one was the effective reduction in the number of urban pigeons and the second one was the preservation of some animal species that are essential components of biodiversity in the city of Modena.

Keywords: bat, biodiversity, fertility control, Nicarbazine, pigeon, scaffold holes, swift

### **Introduction**

The Ghirlandina tower is the most important monument in the city of Modena. It is the bell tower of the Cathedral, standing at 89.35 m and it is part of the UNESCO World Heritage site. Its stone cover structure was badly damaged by atmospheric pollution and the daily corrosion caused by urban pigeons that have lived in the tower and to nest in its recesses for centuries. In addition to the pigeons, swifts and bats have always found a safe haven in the niches of the external walls without causing additional damages to the tower. At the beginning of the tower restoration, the Veterinary Service, that is part of the Department of Public Health, proposed to the Ghirlandina Scientific Committee an intervention in order to control the breeding population of pigeons and the consequential biological damage (1<sup>o</sup> target). At the same time, the veterinarians proposed to maintain favorable living conditions for the survival of the other species nesting in the same external walls used by the pigeons, such as swifts and bats, (2<sup>o</sup> target).

### **Materials and methods**

In order to reach the first target, to reduce the pigeon population, the pigeons in the most critical areas of the centre of Modena were treated with Nicarbazine that represents the best choice of treatments to reduce fertility of pigeons (Martelli et al., 1992; Gelati et al., 2000; Zucconi et al., 2003) thanks to its high versatility of use and its physical formulation that makes the drug unpalatable to most other avian species. The group of pigeons of the Dome (and Tower) and of other 6 most critical sites in central Modena, were administered with special maize grains (Ovistop<sup>®</sup>, ACME) each containing 800 ppm of Nicarbazine, attached to the grains by means of a patented process that prevents its release into the environment and it is delivered in doses of 8-10 g per pigeon per day. The Nicarbazine treatment was applied in different sites of the city, especially in the surrounding areas of the Ghirlandina in accordance with the following guidelines (Ferri et al., 2009). The choice of the colonies to be treated with Nicarbazine was based on the evaluation of their impact on urban hygiene. Therefore there was a preliminary identification followed by mapping of the sites and then finally a quantitative assessment of

the urban pigeons and their colonies was undertaken. At this point, the drug was administered to pigeons at regular intervals in selected locations. The drug was distributed at the dose of 6.4-8 mg of Nicarbazine per pigeon per day, 5 days a week, from mid March to mid October, thus covering the whole reproduction cycle of the urban pigeons. Since March 2008 each year the number of pigeons in the treated colonies has been estimated. (Table1). In addition, the public were given comprehensive information concerning the integrated programme by local newspapers, leaflets and internet sites.

**Tab. 1** Annual count of the pigeons in 6 sites treated with nicarbazine, in the center of the city of Modena, Italy; 2008-2011

site	Modena downtown pigeons abundance (in critical sites)			
	March 2008 n°	March 2009 n°	March 2010 n°	March 2011 n°
Porta S. Agostino	100	60	45	49
P.Redecocca	300	190	70	57
Rua Frati Minori	250	200	80	29
Rua Frati Minori	70	0	0	1
Piazza Grande	180	70	40	21
Piazza Matteotti	100	40	70	17
Piazza Pomposa	60	15	35	31
<b>Total</b>	<b>1060</b>	<b>575</b>	<b>340</b>	<b>205</b>

To reach the second target, the maintenance of acceptable living conditions for other species like swifts and bats, the height of the scaffold holes was reduced to 3,5 -5 cm by fixing a wedged piece of a brick to obtain a narrower entrance. Both this wedge and the reduction in the size of the hole (Figure 1) prevent the entrance of pigeons but allow access to swifts and bats. 200 scaffold holes of the medium and upper part of the tower were reduced in this way while a few scaffold holes in the lower part were closed with a steel net to prevent access to birds and to guarantee an adequate air circulation. In addition, prior to the restoration program, the Ghirlandina tower was used by Peregrine Falcons to roost. An artificial nest was erected on the north side of the top balcony of the tower in the hope of attracting Peregrines to nest, since these birds may help to keep the pigeon population under control.



**Fig. 1** A scaffold hole modified to be inaccessible for pigeons and allow swifts and bat enter; a wedged piece of brick is shown before the use in the hole

## Results and Discussion

The results obtained following the integrated program are summarized in the Table 1, showing the yearly trends of reduction of the pigeons in all the sites treated with Nicarbazine (Ovistop<sup>®</sup>, ACME). In the site 'Piazza Grande', Nicarbazine was given to pigeons while, since 2008, all the scaffold holes of the belltower Ghirladina were inaccessible to the pigeons due the impenetrable curtain that covers the

shelves because work in progress. In the remaining sites only Nicarbazine was given. Another aspect of the veterinary intervention has been the attention given to the need to effectively decrease the number of nesting sites used by the pigeons. To do that, it was reducing the shape and size of the entrance of the ancient niches to facilitate the access of swifts and bats. It will be possible for the swifts and bats to use the scaffold holes since the spring of 2012 (the tower is still undergoing renovation and is totally covered with an impenetrable sheet) but at Melegnano (Italy) it has been verified that the church bell tower (Lombardy), with the scaffold holes treated (Ferri and Ravizza) in the same way last winter, in 2011 seems already used by swifts. The restoration of ancient buildings and monuments needs great efforts by public authorities in order to limit the risks and damage caused by a combination of biological, physical and chemical factors. Biological damage caused by pigeons must be minimized. But on the other hand there is the need to keep at an acceptable level the presence of other insectivorous species in the urban and monumental habitats because those animals are an integral part of the city scenario.

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## Providing incentives to encourage a control program of hooded crows (*Corvus corone cornix* L., 1758): a case study in Rieti province (Italy), 2005-10

Amici, A.<sup>1</sup>, Adriani, S.<sup>1</sup>, Bonanni, M.<sup>2</sup>, Serrani, F.<sup>1</sup>

<sup>1</sup>Università della Tuscia, Dipartimento di Produzioni Animali, Via C. De Lellis, snc, 07100 Viterbo, Italy

<sup>2</sup>F. Martinelli 34, Roma, Italy, bonanni\_m@libero.it

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### Abstract

The hooded crow is allowed to be hunted according to Italian law. This bird is characterized by a very high reproductive potential and is considered a pest species. In this paper we present the results of a control program of hooded crow (*Corvus corone cornix* L.) undertaken in Rieti province (central Italy) in 2005-10. The Hooded crow is considered as a pest species by hunters due to its wide distribution and abundance, and to the impact on small game species. The program was organized by Local Hunting management Authorities (ATC), in compliance with Italian rules. These plans are intended to reduce the hooded crow population to reduce predation on eggs and/or small game, particularly *Lepus europaeus*, *Perdix perdix* and *Phasianus colchicus*.

Keywords: containment, *Corvus corone cornix* L., Larsen trap, letter box, Rieti province

### Introduction

The hooded crow (*Corvus corone cornix* L.) is present throughout Italy, including islands, with the exception of the eastern and western portions of the Alps, where it is replaced by the carrion crow (*C. corone corone*) (Incerpi et al., 1980). In the sympatric areas the two populations hybridize, producing fertile offspring with intermediate plumage color. This bird is characterized by a very high reproductive potential (Loman, 1980) and is considered a pest species. The species frequents a wide range of habitats ranging from the plains to the mountains, both in wooded and open areas. Nest sites are in the upper parts of tall trees. This species is particularly common in populated areas and especially where food resources are available in high amounts (e.g. garbage dumps). The gregarious and omnivorous hooded crow lives in mixed flocks with the jackdaw (*Corvus monedula*) and finds food resources in agricultural fields (Incerpi et al., 1980). Predation of eggs and chicks of wild bird species has been documented (Valle, 1998; Dei et al., 2000; Scarton et al., 2004; Gargiano and Guerrini, 2005; Mostini, 2007), although the intensity of the impact on bird populations has not been measured (Meinig and Boye, 2001). Incerpi et al., (1980) reported the predation of some mammals, especially if young and debilitated. In Rieti province the predation of hare puppies has been reported (Adriani, 2009). This behavior and/or the predation of game species, together with the numerical increase in hooded crow populations that took place in recent decades persuaded hunters that it is a major factor limiting the increase of game species (mainly partridge, pheasant and hare). The species is included in the list of hunted species but is not regarded as a game species. This does not contribute to its containment and has led many institutions to undertake monitoring and/or control activities, usually conducted using the Larsen trap (Cocchi, 1996). This study aims to provide a comprehensive picture of measures to reduce the hooded crow conducted in the province of Rieti by the Local Hunting Authorities (ATC) through the provision of incentives to hunters.

### Materials and methods

The institutions for wildlife management (ATC) in Rieti province have implemented a plan to contain the hooded crow, which provided incentives represented by game animals (brown hare, pheasant, grey partridge). To the hunters a number of wild animals were assigned on the basis of crows eliminated. The R11 ATC assigns a hare for every 3 foxes killed and a pheasant for every 5 crows; the assigned game animals were used for restocking in the municipality of residence of the hunter. The ATC R12 in the hunting season 2009/2010 has activated a program for predator control which awarded the participants a certain score according to the animals captured. The scores were as follows: 1.5 points per crow shot, and 0.75 points if captured in a trap provided by the ATC. The hunters can spend the score accumulated in different ways: a) 100 bonus points give 1 per year's insurance policy for hunting (value € 85.00); b) 75 points for one hunting permit in the following hunting season; c) 75 points allowed to use the dog

training area in the next season; d) wild animals to repopulate the municipality, 1 brown hare = 50 points, 1 pheasant = 10 points, 1 grey partridge = 5 points.

## Results

The programs put in place by the hunting organizations (ATC RI1 and RI2) induced an intense killing of hooded crows throughout the Province. In table 1 the overall number of hooded crows captured in the period 2005-2010 is reported separately for ATC (RI1 and RI2).

**Tab. 2** Trend of the overall number of hooded crows captured in the hunting districts RI1 and RI2 in 2005-2010

Year	05/06	06/07	07/08	08/09	09/10	Total
RI1	430	609	740	1,332	1,322	3,733
RI2	536	938	526	863	1,536	4,399
Total	966	1547	1,266	2,195	2,858	8,132

If we consider the average withdrawal per km<sup>2</sup> the results is about three crows/km<sup>2</sup>. By the social point of view the 95% of hunters chose the reimbursement of insurance policy, and 5% the possibility to train dogs as the first choice. As a second choice the 36% of hunters chose the training dog opportunity and 64% the release of hunting species.

## Discussion

The containment program has been conducted only in the hunting areas. For this reason, the program was affected by the presence of crows in undisturbed areas. The removal of hooded crow was zero in some areas (eg. protected areas) and well above average in others. It is not easy to explain the cause of the increase in crow removal, from 966 birds in 2005-6 to 2,858 in 2009-10. The increase might have been due to the improvement of the capture strategies, or more simply to the attractiveness of the incentives provided to the participants. It is hoped that the recent initiation of a monitoring plan for the species throughout the province (from 2010 in ATC RI2 and from 2011 in the ATC RI1) and the simultaneous indication of the density of the target species in different geographical areas of Rieti province (Adriani and Bonanni, 2010; Adriani et al., 2011) can give an indication of the efficacy of the control activities of the hooded crow. The monitoring of the species now begun makes it possible to assess the results that will be pursued in future operations.

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## Spiking buildings to avoid house martin (*Delichon urban*) nesting: is it a good choice?

Duarte, J.<sup>1,2</sup>, Farfán, M.A.<sup>1,2</sup>, Vargas, J.M.<sup>2</sup>

<sup>1</sup>BioGea Consultores, Calle Federico García Lorca 14, 29400 Ronda, Spain, jddbiogea@gmail.com

<sup>2</sup>Dpt. of Animal Biology, University of Málaga, Faculty of Sciences, Campus Teatinos, 29071 Málaga, Spain

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### Abstract

We tested the effectiveness of installing spikes (® EcoPic E-5) on building eaves and windows to avoid the nesting of house martins. We found that spikes cannot prevent the nesting or reduce colony size. The first year after installing the spikes the colony may be partially affected. However house martins are able to overcome the spikes in the next years and rebuild the nests even on the spikes. We conclude that spikes are ineffective as a deterrent system.

Keywords: deterrents, house martin, nesting, spikes, urban wildlife control

### Introduction

House martins (*Delichon urbica*) are migratory birds legally protected in Spain. However, their breeding colonies produce dirt and act as parasite reservoirs causing aesthetic problems and a likely health risk (Yamauchi, 2005). Every year communities of owners try to clean the building eaves, removing nests, repainting and looking for solutions and deterrents to avoid any new nesting.

### Material and methods

The study area was located in a 13-building complex named "Residencial El Lago" at Los Arqueros Golf and Country Club (Benahavis, Andalucía, south of Spain). In February 2009 (before the pre-nuptial bird migration from Africa) we removed martin nests and installed spikes in all the places where they had nested.

We monitored the reconstruction of the colony during the 2009 breeding season, recording the construction of nests on spikes and the displacement of nests to new spike-free locations. In February 2010 we removed all the nests again and monitored the colony. In both years we used a number of buildings as controls, removing nests but without installing spikes.

### Results

We did not find significant differences in the average numbers of nests between treated and control buildings (with and without spikes installed) (ANOVA test;  $F_{2,36}=1.509$ ;  $p=0.235$ ). In the control we found  $3.07\pm 1.04$  nests per building while in treated buildings we found  $1.23\pm 0.52$  (2009) and  $1.76\pm 0.65$  (2010). The average nests per building did not significantly differ between control and 2009 (Mann-Whitney test;  $Z=-1.400$ ;  $p=0.161$ ), between control and 2010 (Mann-Whitney test;  $Z=-0.829$ ;  $p=0.407$ ) or between both treated years 2009 and 2010 (Mann-Whitney test;  $Z=-0.783$ ;  $p=0.434$ ).

The spikes displaced 56.25% (2009) and 17.39% (2010) of new nests forcing martins to rebuild them on locations without any spikes installed. However, 43.75% (2009) and 82.61% (2010) of new nests were rebuilt on the spikes. The spikes compared with the control had a significant effect in 2009 ( $\chi^2=26.75$ ;  $df=8$ ;  $p<0.001$ ) but not in 2010 ( $\chi^2=14.05$ ;  $df=8$ ;  $p=0.0803$ ). Considering both years the effect was not significant either ( $\chi^2=3.69$ ;  $df=16$ ;  $p=0.0747$ ). The frequency of nests installed on spikes was not significantly different between years ( $\chi^2=14.05$ ;  $df=5$ ;  $p=0.5945$ ).

### Discussion

As it has been shown with other type of deterrents (Duarte et al., 2011), these may have a significant effect on the colony during the first year after the installation, with a nest displacement rate of 50% and a relative decrease in colony size. However martins are able to overcome the deterrent, managing to nest and rebuild the colony as shown by the high percentage of nests installed on spikes during the second year. We conclude that spikes are not a good choice to avoid martin nesting on buildings as their efficacy is relative.

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## Prevalence of anticoagulant rodenticide poisoning in France: human and animal data

Berny, P.<sup>1</sup>, Velardo, J.<sup>1</sup>, Pulce, C.<sup>2</sup>, D'Amico, A.<sup>2</sup>, Kammerer, M.<sup>3</sup>, Lasseur, R.<sup>1</sup>, Belhadj, A.<sup>4</sup>, Mastain, O.<sup>5</sup>

<sup>1</sup>Université de Lyon, VetAgro Sup, USC1233, INRA, F-69280 Marcy l'Etoile, France, p.berny@vetagro-sup.fr

<sup>2</sup>Centre antipoison - Centre de Pharmacovigilance, Lyon, France

<sup>3</sup>CAPAE-Ouest - Oniris-Nantes, Atlapole La Chantrerie, BP40706, F-44704 Nantes Cedex

<sup>4</sup>Liphatech, F-47280 Pont du Casse, France

<sup>5</sup>Office National de la Chasse et de la Faune Sauvage, Domaine de St Benoist, F-78120 Auffargis, France

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### Abstract

Anticoagulant rodenticides have been used for over 50 years to control rodent populations. Since their first introduction, resistance developed in rodents, and second generation products, more active but also more toxic, have been marketed. These compounds are currently being reviewed under the European Regulation and the purpose of this work was to describe anticoagulant poisoning based on the retrospective data from French human and animal poison control centers. Cases from 2004 to 2007 were collected. Overall, the proportion of anticoagulant exposure as reported to Lyon's Poison Control Center appeared very limited and mostly occurred in young children, with no or very limited clinical severity. Some cases also occurred after the intentional use of anticoagulants in adults. But circumstances of exposure are usually accidental in human beings (77%).

In animals, both domestic and wild species, anticoagulant exposure is more common and often accompanied by clinical signs. Among domestic species, dogs represent over 60% of the cases: in wildlife hares and rabbits account for almost 50% of the submitted cases, followed by predators and scavengers. The compounds involved are quite representative of the market share of anticoagulants, at least for human beings and domestic animals. In wildlife, bromadiolone and chlorophacinone were by far the most important products, being the only ones registered for field use (until 2011). There is no report of mortality in the human data, and less than 1% of all exposure cases in domestic animals.

Keywords: animal exposure, anticoagulant, human exposure, rodenticide, secondary poisoning, toxicity

### Introduction

In France, rodenticides are currently licensed for use against household and field rodent species. All anticoagulants are available against household rodents. Only chlorophacinone and bromadiolone were licensed for use outdoors and in the field in France (prior to 2011). Anticoagulants are toxic to human beings as well as to many vertebrate species and very thorough reviews of their toxic doses and effects are available (Petterino and Paolo, 2001). Few surveys or reports are available, unfortunately, to evaluate the reality of poisoning or exposure to these compounds in humans as well as in animals.

### Materials and methods

A retrospective survey of human and animal poisoning cases with anticoagulant rodenticides recorded between January 1<sup>st</sup>, 2004 and December 31<sup>st</sup>, 2008 was conducted. Three different sources of data were used to retrieve suspected and confirmed anticoagulant poisoning incidents (anticoagulant drugs excluded):

- 1) human data were accessed via the Lyon Poison Control Center, which accounts for 16% of all calls in France,
- 2) domestic animal data from the CAPAE-Ouest (Nantes College of Veterinary Medicine) were retrieved,
- 3) wild animal toxicovigilance data were obtained from the Toxicology Laboratory of VetAgro Sup, which is part of the SAGIR network (for wildlife disease surveillance).

### Results

Among the 124,897 calls received regarding suspected poisoning cases in humans, only 770 concerned anticoagulant rodenticides (0.6%), and only 280 were considered as potential poisoning cases. In domestic animals, anticoagulant rodenticides (n=1,269) represented 9% of all cases (n=14,145) and

severe cases represented 8.5% of these calls (n=105). Mortality was reported in 8 cases. Dogs represented 75.1% of all cases, cats 9.1%. In the laboratory, 1,750 wildlife suspected poisoning cases were reported and 476 were suspected anticoagulant poisoning cases (27.2%). Anticoagulant poisoning was eventually confirmed in 185 cases (10.6%). Hares and rabbits represented almost 40% of the cases; predators, scavengers were also commonly found (22%).

In humans, circumstances of exposure were mostly accidental, but 20% of the cases related to voluntary exposure (suicidal attempts). The active substances involved were mostly 1) difenacoum, chlorophacinone in human beings, 2) difethialone, difenacoum, brodifacoum in domestic animals, 3) bromadiolone and chlorophacinone in wildlife. The outcome is seldom known (except in wildlife, but only dead animals are collected). In the 280 cases considered as potential poisoning problems in humans no death was reported. In animals, 0.6% mortality was observed at the time of call.

## Discussion

Anticoagulant poisoning in human beings appears to be of moderate importance in our sample (less than 1% of the cases recorded), as it is commonly observed in many countries around the world. Children under the age of 4 years are most commonly exposed. In France, regulatory authorities request the use of bittering agents to reduce accidental bait intake by children (Bronstein et al., 2007; Bronstein et al., 2008; Watson et al., 2005). The situation is completely different in animals: anticoagulant poisoning is a major issue in veterinary clinical toxicology, as described elsewhere (Wang et al., 2007). Highly susceptible species appear more at risk: dogs, hares/rabbits, wild boars and foxes (Petterino and Paolo, 2001; Berny et al., 1997). In domestic species, the most toxic 2<sup>nd</sup> generation anticoagulants (brodifacoum, difethialone) are more involved than their market share would suggest over other compounds such as bromadiolone, or difenacoum. Since many cases are reported only when clinical signs occur, logically more toxic compounds will be overrepresented.

In wildlife, only bromadiolone and chlorophacinone were licensed for use against field rodents in France (prior to 2011). Most cases of exposure in human beings are not severe and do not result in any harmful outcome (98% in a review of US poison control center data (Bronstein et al., 2007; Bronstein et al., 2008; Caravati et al., 2007; Watson et al., 2005). It has recently been stated that anticoagulants do not necessitate systematic gastric decontamination or prophylactic Vitamin K administration (Caravati et al., 2007; Ingels et al., 2002), and follow-up is even considered optional for most cases received at the Lyon Poison Control Center. It is noteworthy that no case of death could be attributed to anticoagulant rodenticide exposure in this study. In animals, there is a general lack of information on the circumstances of poisoning and, with a delayed onset of signs, poisoning is often suspected in animals only when clinical signs occur, hence the severity.

As a conclusion, we would like to point out that anticoagulant exposure in France is quite uncommon in human beings and usually benign based on poison control center data and the potentially serious cases followed-up in this 5-year retrospective survey. In domestic animals, clinical cases are more common, especially because exposure is not detected early. In wildlife, our study reveals that products other than the licensed ones are sometimes detected and that inappropriate use of anticoagulants in the field exists.

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## High exposure rates of anticoagulant rodenticides in carnivorous birds and mammals in Danish landscapes

Elmeros, M.<sup>1</sup>, Christensen, T.K.<sup>1</sup>, Lassen, P.<sup>2</sup>

<sup>1</sup>Dept. of Wildlife Ecology and Biodiversity, National Environmental Research Institute, Aarhus University, Grenåvej 14, DK-8410 Rønde, Denmark, elm@dmu.dk

<sup>2</sup>Dept. of Environmental Chemistry and Microbiology, National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark

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### Abstract

We analyzed anticoagulant rodenticides (ARs) in birds of prey, owls and small mustelids to assess exposure rates and levels in Denmark. ARs were detected in 84-97% of individuals in all species. High AR prevalences and concentrations were recorded in all seasons in both birds and mammals. Prevalence of ARs was considerably higher than reported elsewhere with similar exposure scenarios. Further research on the potential effects of these high AR exposure levels on population levels of non-target species is warranted.

Keywords: HPLC, mustelids, non-target species, owls, raptors, secondary poisoning

### Background

Use of anticoagulant rodenticides (AR) is an efficient and widely used method to control rodent populations (WHO, 1995). The use of ARs also poses a risk of secondary poisoning of non-target carnivorous species (Eason et al., 2002, Laasko et al., 2010). Incidents of fatal poisoning have been documented in a wide range of species but relatively few studies have addressed the exposure rates and concentrations in free-ranging carnivorous birds and mammals (Shore et al., 2003, Walker et al., 2008). We surveyed the AR exposure rates and concentrations in non-target carnivorous species from Danish landscapes dominated by intensively managed agricultural lands. In Denmark rodent control is almost exclusively done as chemical control in bait boxes using primarily the most toxic and persistent second generation ARs.

### Materials and methods

Carcasses of raptors, owls and small mustelids were collected opportunistically, e.g. as road kills. The carcasses were collected in typical Danish agricultural landscapes. Residues of five commonly used ARs (bromadiolone, brodifacoum, coumatetralyl, difenacoum and flocoumafen) were analyzed by HPLC coupled with a fluorescence and photodiode array detector in liver tissues (Jones, 1996; Shore et al., 2003). Residues were extracted from homogenized tissue with acetone/dichloromethane and analyzed using a Hypersil 5 $\mu$  C18 column. The mobile phase consisted of a gradient of 0-9 min methanol:ammonium acetate:acetonitrile; 9-45 min ammonium acetate:acetonitrile and 45-55 min water:acetonitrile. Blank and spiked control samples were processed and analyzed for quality assurance.

### Results

ARs were detected in 84-97% of individuals of each species (Tab. 1). ARs were also detected in all individuals in a small number (<10) of red kite (*Milvus milvus*), marsh-harrier (*Circus aeruginosus*), short-eared owl (*Asio flammeus*), Eurasian eagle-owl (*Bubo bubo*), and little owl (*Athene noctua*). More than 60% of birds and 80% of mustelids had detectable levels of more than one substance. No differences in AR prevalences and concentrations were detected between gender, age, season or cause of death in birds. In stoats and weasels the highest concentrations were found in animals from autumn and winter (negative binomial regression;  $\chi^2=18.92$ ,  $p<0.001$ ). A similar trend was also seen amongst some of the birds. Potentially fatal concentrations (>200 ng/g ww) were recorded in all seasons in birds and mammals. AR concentrations were highest in stoats and weasels with unknown cause of death ( $\chi^2=11.32$ ,  $p<0.01$ ) and body condition was weakly but significantly correlated with total AR concentration ( $r=0.28$ ,  $p<0.05$ ).

**Tab. 1** Frequency of occurrence of anticoagulant rodenticides and total mean, median and range of concentrations in carnivorous birds and mammals from Denmark with detectable levels of anticoagulant rodenticides. Hepatic AR concentrations above 200 ng/g ww have been associated with mortalities in raptors and small mustelids.

Species	n	Occurrence %	Mean concentration ng/g ww	Median concentration ng/g ww	Proportion >200 ng/g %	Range ng/g ww
Weasel	69	97.1	141.9	70.9	17.4	0-1,660
<i>Mustela nivalis</i>						
Stoat	61	96.7	149.4	59.0	18.0	0-1,419
<i>M. erminea</i>						
Common kestrel	66	89.4	99.0	46.0	13.6	0-690
<i>Falco tinnunculus</i>						
Common buzzard	141	93.6	74.5	50.0	5.7	0-721
<i>Buteo buteo</i>						
Rough-legged buzzard	31	83.9	40.8	26.5	0.0	0-139
<i>B. lagopus</i>						
Tawny owl	44	93.2	78.4	39.0	9.1	0-534
<i>Strix aluco</i>						
Barn owl	80	93.7	114.1	71.0	13.7	0-1,092
<i>Tyto alba</i>						
Long-eared owl	38	94.7	19.4	13.5	0.0	0-84
<i>Asio otus</i>						

## Discussion

The prevalence of ARs in Danish birds of prey, owls and mustelids were markedly higher for most species than reported elsewhere. After correcting for differences in analytic detection limits prevalence was approximately twice the levels reported in e.g. Great Britain (Shore et al., 2003; Walker et al., 2008). Similar exposure levels have only been reported in small mustelids during widespread field campaigns of rodents in New Zealand (Murphy et al., 1998). Possible explanations for the high exposure levels in Denmark may include intensive human land use, mandatory control of rats and the use of ARs by private householders. Abundance of most raptors and owls has been stable or increased during the past decades in Denmark. Thus, the high exposure levels by ARs does not have a crucially adverse effect on the population level for raptors and owls, but locally intensive use of ARs may cause increased chick mortality in some birds (unpublished data). Stoats and weasels are assumed to be widespread but in decline due to habitat destruction and fragmentation in Denmark. The high AR exposure rates and concentrations suggest that the present AR use and application methods and policies may pose an additional significant risk to the conservations status for stoats and weasels in Denmark. Further studies to determine the impact of AR use and secondary poisoning of individual animals on a population level, and risk assessments of different AR application scenarios are urgently needed.

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## **Anticoagulant rodenticides: exposure and residues in non-target rodents and their predators**

Broll, A.<sup>1</sup>, Esther, A.<sup>1</sup>, Schenke, D.<sup>2</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppheideweg 88, 48161 Muenster, Germany, anke.broll@jki.bund.de

<sup>2</sup>Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Straße 19, 14195 Berlin, Germany

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The use of first and second generation anticoagulant rodenticides to control rodent pests is widespread. The delayed action of anticoagulant rodenticides (ARs) causes a potential risk for predators that ingest poisoned rodents (secondary poisoning). Moreover, non-target rodents and other small mammals may also inadvertently feed on bait (primary poisoning). Studies from other countries (e.g. U.K. and New Zealand) indicate that ARs are transferred through the food chain, but due to the different regional conditions and farming practices these studies can only be used as an indicator for Germany, where systematic research on the exposure path of ARs does not exist. One aim of our study is to analyze the contents of ARs in target species, non-target rodents and owl pellets, as an example of non-target exposure to predators. To investigate this question experiments are carried out on farms close to Muenster (North-Rhine Westphalia, Germany), where barn owls (*Tyto alba*) and/or little owls (*Athene noctua*) are present. From both links of the potential food chains (target rodents – predators/ non-target rodents - predators) samples are taken. To assess AR exposure of owls, we collect spit pellets of barn owls and little owls. Rat snap traps are placed on rat trails or at places with potential rat occurrence on farms. Mouse snap traps are set in two transects extending from the farm to explore the relationship between distance from bait and poison distribution in non-target rodents. Using HPLC, fresh pellets and liver samples of rats and mice will be analyzed in terms of contents of eight ARs (brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, floucomafen and warfarin). AR-residues within the food chain and composition of the predators' prey will be used to assess the potential risk that the two owl species may ingest poisoned prey. The potential of different rodent species (target and non-target) to induce secondary poisonings will also be analyzed. The composition of owl food is analyzed by identifying rodent craniums and invertebrates within the pellets. First data of our fieldwork will be presented. An overview of further work including records of AR-residues in predators found dead in Germany will be given. (This study is funded by the German Federal Environment Agency grant number 3710 63 401.).

Keywords: anticoagulant rodenticides, Germany, non-target, owl, rodent, secondary poisoning

## Diphacinone and coumatetralyl persistence in deer and implications for wildlife management

Eason, C.T.<sup>1,2</sup>, Murphy, E.<sup>3</sup>, Ross, J.G.<sup>1</sup>, Hix, S.<sup>2</sup>, Arthur, D.<sup>4</sup>, MacMorran, D.<sup>2</sup>, Broome, K.<sup>3</sup>, Fairweather, A.<sup>3</sup>

<sup>1</sup>Centre for Wildlife Management and Conservation, Lincoln University, Lincoln, New Zealand, charles.eason@lincoln.ac.nz

<sup>2</sup>Connovation Research Ltd, Auckland, New Zealand

<sup>3</sup>Department of Conservation, Christchurch and Hamilton, New Zealand

<sup>4</sup>Selwyn Rakaia Vet Services Ltd, Dunsandel, New Zealand

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### Abstract

Because of the concerns regarding wildlife contamination following the field use of anticoagulants the hepatic persistence of diphacinone and coumatetralyl has been compared in deer. Initial coumatetralyl concentrations in liver following oral dosing with 8.25 mg/kg coumatetralyl were similar on day 1 to those achieved by administration of diphacinone at 1.5 mg/kg. Coumatetralyl was more slowly eliminated ( $t_{1/2}$  14 days) than diphacinone ( $t_{1/2}$  6 days) and residues were still present in liver tissue after 50 days versus 12 days for diphacinone. Bioaccumulation on repeated field use is unlikely.

Keywords: coumatetralyl, deer, diphacinone, persistence, pharmacokinetics, residues

### Introduction

The pharmacokinetics of rodenticides determines their tendency to bioaccumulate on repeated exposure. Pharmacokinetic research involves dosing animals and taking blood or tissue samples for analysis. In this study liver samples were taken for analysis of diphacinone or coumatetralyl since it is well known that the highest concentration of these compounds occurs in the liver. Diphacinone is reported to have a short hepatic half-life of 3 days in rats (Fisher et al., 2003), and shorter than the 55 days reported for coumatetralyl in rats (Fisher et al., 2003). The aims of this study were to investigate the comparative persistence of diphacinone versus coumatetralyl in deer. The persistence of anticoagulants has not previously been reported in deer which could come into contact with rodenticide baits if they are used on or near deer farms or in conservation settings to kill predators of ground dwelling birds.

### Methods

Six young male red deer weighing from 77 to 89 kg were split into two groups of three. Deer were fed palatable feed containing 8.25 mg/kg coumatetralyl or 1.5 mg/kg diphacinone. The doses of 8.25 mg/kg coumatetralyl and 1.5 mg/kg diphacinone represented  $\frac{1}{2}$  the published single dose LD50 in rats for coumatetralyl, namely 16.5 mg/kg and for diphacinone, 3.0 mg/kg (Buckle and Smith, 1994). No acute toxicity data exists for either of these compounds in deer. Liver biopsies were undertaken with the same lobe of the liver sampled on each occasion with repeat samples taken in very close proximity to each other. The incision site was located through the 11th intercostal space on the right hand side of each deer. A 1-2 g liver biopsy was taken. To minimise trauma a sedative and local anaesthetic was administered as outlined in West and Vermut (1995). Analyses of residue concentrations were undertaken on liver samples by an established hplc technique. Animal Ethic Approvals were obtained. The half-lives ( $t_{1/2}$  days) for diphacinone and coumatetralyl were determined from the regression as  $(\log_e(2)/b)$ , where b is the slope of the regression of  $\log_e(\text{concentration})$  against time.

### Results

Diphacinone concentrations deer liver decreased to below or at the level of detection of 0.05  $\mu\text{g/g}$  within 29 days (see Table 1). The depletion half-life for diphacinone in liver tissue in deer was 6.3 days (+0.8 SEM). Initial coumatetralyl concentrations in deer liver following dosing with 8.25 mg/kg coumatetralyl were similar on day 1 to those achieved by administration of diphacinone at 1.5 mg/kg. However residues of coumatetralyl were still present after 50 days in one animal (see Table 2). The depletion half-life for coumatetralyl in deer was 14.4 days (+3.6 SEM). MDL=0.02  $\mu\text{g/g}$ .

**Tab. 1** Liver concentrations following oral administration of diphacinone (1.5 mg/kg).

Day after dosing	Individual conc. in µg/g	Average concentration µg/g
1	0.48, 1.26, 0.22	0.69
5	0.24, 0.40, 0.29	0.31
12	0.41, 0.00, 0.00	0.14
29	0.00, 0.00, 0.00	0.00

**Tab. 2** Liver concentrations following oral administration of coumatetralyl (8.25 mg/kg).

Day after dosing	Concentration in µg/g	Average concentration µg/g
1	0.39, 0.66, 0.37	0.47
8	0.49, 0.49, 0.41	0.46
15	0.47, 0.09, 0.09	0.22
29	0.50, 0.09, 0.00	0.20
50	0.08, 0.00, 0.00	0.03
85	0.00, 0.00, 0.00	0.00

## Discussion

Assessments of the persistence of diphacinone and coumatetralyl have been completed in deer and the depletion half-life calculated in liver tissue. Coumatetralyl was more slowly eliminated in deer ( $t_{1/2}$  14 days) than diphacinone ( $t_{1/2}$  6 days) and residues were still present in liver tissue after 50 days versus 12 days for diphacinone. Both compounds appear to be more suited for repeated field use versus the more persistent second generation anticoagulants with diphacinone being less likely to bioaccumulate than coumatetralyl. In contrast with the results obtained with these compounds in deer brodifacoum and all other second-generation anticoagulants have unusually long hepatic half-lives in liver in all species tested (Laas et al., 1985; Parmar et al., 1987; Huckle et al., 1989a and b). Not surprisingly, given the unusual persistence of brodifacoum, residues have been found in game animals such as pigs and deer and a range of avian species (Eason et al., 2001). This paper confirms that coumatetralyl and diphacinone are quickly eliminated from mammals including deer, which is important in areas where deer may be game for hunters. An understanding of the persistence of rodenticides is important when considering rodenticides for field use for conservation and in an agricultural setting. Furthermore we believe that the idea of revisiting synergists such as low dose cholecalciferol with diphacinone and coumatetralyl, whilst presenting registration challenges, has scientific merit in terms of producing rodenticides as potent as brodifacoum with reduced hazards to non-target wildlife resulting from bioaccumulation.

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## Welfare assessment of fatal methaemoglobinaemia in adult rats (*Rattus norvegicus*)

Gibson, T.J.<sup>1</sup>, Gregory, N.G.<sup>1</sup>, Quy, R.J.<sup>2</sup>, Eason, C.T.<sup>3</sup>

<sup>1</sup>Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, UK,

tgibson@rvc.ac.uk

<sup>2</sup>The Food & Environment Research Agency, UK

<sup>3</sup>Lincoln University, New Zealand and Connovation Ltd, New Zealand

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### Abstract

This study aimed to assess the welfare of rats poisoned with a lethal dose of a methaemoglobin (MetHb) inducing compound. Twenty rats were dosed orally with either the active compound (treated) or the vehicle only (control). Blood samples were collected post mortem for analysis of MetHb%. Female and male rats received a mean dose of 263 (SD 6) and 199 (SD 13) mg/kg respectively. Mean time to death was 67 (SD 36) and 354 (SD 158) minutes for female and male rats respectively. Control animals did not show any signs of intoxication. The time to death from methaemoglobinaemia in rats was significantly shorter than that previously reported for anticoagulants with no obvious signs of distress or pain.

Keywords: cerebral depression, death, methaemoglobinaemia, methaemoglobin (MetHb), welfare

### Introduction

Methaemoglobin-inducing compounds are being evaluated for use as vertebrate pesticides for the control of feral pigs (Anon, 2010), stoats (Fisher et al., 2005; Eason et al., 2010; Dilks et al., 2011), ferrets (Fisher and O'Connor, 2007), bushtail possums (Fisher et al., 2008) and feral cats (Murphy et al., 2007) with the aim of improving the humaneness of pest control. They target red blood cells and induce the formation of MetHb, which reduces the capacity of blood to carry oxygen to tissues and causes depressed consciousness, respiratory depression and leads to death over a shorter time period than anticoagulant agents. Rodents have previously been demonstrated to have a high MetHb reductase activity after treatment with sodium nitrite (Stolk and Smith, 1966) or *p*-aminopropiophenone (PAPP) (Scawin et al., 1984). Newly synthesised analogues of PAPP have shown promise during in-vitro and in-vivo testing as being more toxic in rodents. Currently there has been no comprehensive assessment of the welfare of rodents poisoned with MetHb-forming compounds. It is important to evaluate the welfare problems that could or do arise when killing by this means prior to further testing for efficacy. The aim of the study was to objectively examine the behaviour of rats during fatal methaemoglobinaemia. The observations will be used to develop a rat-specific ethogram for use during efficacy testing.

### Materials and methods

Twenty Wistar-strain, laboratory rats (*Rattus norvegicus*) (10♂, 10♀) were fasted overnight prior to experimentation. Rats were randomly selected and dosed with either the active compound (treated) or the vehicle only (control). Animals were matched for dosing time and sex. Treated male (5) and female (5) rats received the active compound in a PEG:TEA (9:1 ratio) vehicle at a mean dose of 231 (SD 35) mg/kg via oral gavage with matched controls receiving the vehicle only. The dosing volume was 1 ml.

Post dosing, rats were immediately placed in individual cages and observed under white lighting. After dosing the time to onset of sickness behaviours, the duration and frequency of abnormal behaviours, changes in posture, and the time to unconsciousness and death were recorded. As treated animals died, the matched controls were dispatched by cervical dislocation. Immediately post mortem, blood samples were collected to assess circulating MetHb% at death (CO-oximeter, Instrumentation Laboratories Ltd, Warrington, UK). Post-mortem examinations were conducted on each animal to examine for gross pathological signs of methaemoglobinaemia. All procedures were carried out under the provisions of the Animals (Scientific Procedures) Act 1986 and with the approval of the institute's Ethical Review Panel.

### Results

Female and male rats received a mean dose of 263 (SD 6) and 199 (SD 13) mg/kg respectively. All treated rats died, with mean time to death of 67 (SD 36) and 354 (SD 158) minutes for female and male rats respectively. Treated animals showed a general cascade of signs of methaemoglobinaemia with

cyanosis of the hind feet and nose, ears became pale, whisker twitching stopped or was reduced all by 9 (SD 2) minutes post dosing. All treated animals then became lethargic (often in a prostrate posture), body movements became uncoordinated and slowed by 26 (SD 19) minutes. The animals then became unresponsive to air being blown on the back or face, followed by no response to a manual tail or ear pinch at 147 (SD 112) or 163 (SD 133) minutes respectively. When animals were close to death they became unresponsive to handling and lost their righting reflex. Immediately prior to death the corneal reflex was absent and the respiration rate dropped below 48 breaths per minute (normal respiration rate was 85-110 breaths per minute). Control animals did not show any signs of intoxication. In both the control and treated animals there were no signs of laboured breathing, excessive salivation or vocalisation. Post-mortem the blood of all treated rats was dark brown in colour with MetHb levels of 76% (SD 4) and 74% (SD 7) in female and male rats respectively, compared with controls at 0%.

## Discussion

The time to death from methaemoglobinaemia in rats was significantly shorter than that previously reported for anticoagulants (Littin et al., 2000), and was dose-related rather than sex-related. Initially following intoxication rats appeared asymptomatic, then 9 minutes post-dosing cyanosis was observed followed by lethargy and ataxia (26 minutes). In humans it has been reported that consciousness becomes increasingly depressed with MetHb concentrations of between 45-55%, and at levels of 55-70% there is circulatory failure, cardiac arrhythmias and coma (Hall et al., 1986). Initial results from a separate pilot study suggest that rats dosed with 30 mg/kg of the active compound had MetHb levels of 56% at 15 minutes, peaking to 69% at 30 minutes. Based on these findings, the behavioural observations and the human literature it was concluded that the rats experienced depressed consciousness from 26 minutes onwards. The mean time to un-responsiveness to pain was 147 minutes and total unconsciousness was achieved by 163 minutes. It appears from these results that the events leading to death from methaemoglobinaemia are relatively more humane than those from anticoagulant intoxication, based on the reduced time to death, hypoxia-induced cerebral depression and absence of obvious signs of distress or pain.

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## Automatic and permanent rodent-monitoring - a proper method to evaluate rodenticide effects?

Fuelling, O.<sup>1</sup>, Klemann, N.<sup>2</sup>, Endepols, S.<sup>3</sup>

<sup>1</sup>Institute of Landscape Ecology, University of Muenster, Robert-Koch-Str. 26, D-48149 Münster, Germany, olaf.fuelling@uni-muenster.de

<sup>2</sup>Warendorf, Germany

<sup>3</sup>Bayer CropScience AG – Environmental Science, Monheim, Germany

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### Abstract

During an evaluation trial to test the efficiency of a rodenticide product we compared the standard feeding census method with a new activity monitoring tool. This tool uses wireless motion sensors to register movements of free ranging rats or mice. With both methods a survival rate after the treatment was calculated and the results (less than 3% survival) matched closely. The permanent monitoring, however, gave an additional benefit as the animal behavior could be observed throughout the whole trial, especially the time delay between treatment and effect.

Keywords: EPPO guideline, monitoring, *Mus musculus*, *Rattus norvegicus*, registration, rodenticide, rodents

### Introduction

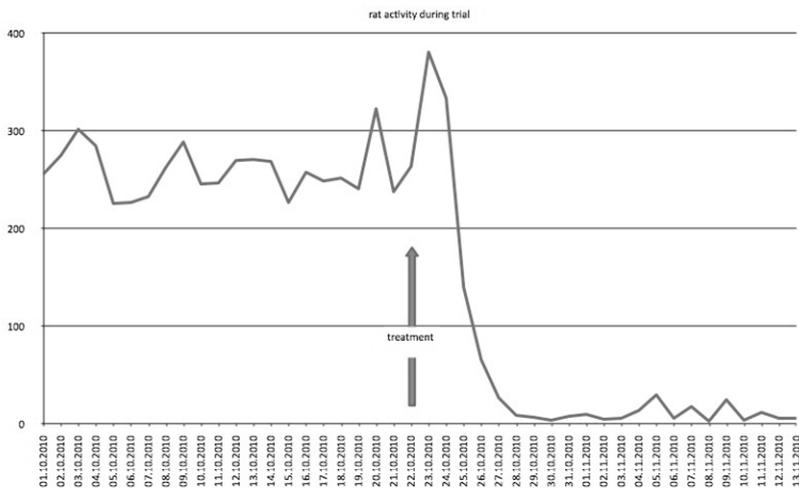
For the registration of rodenticides in Europe, efficiency tests according to the guideline of the European and Mediterranean Plant Protection Organisation (EPPO) have to be conducted. The EPPO-guideline to test against synanthropic rodents (Anonymous 2004) asks for proper methods to determine the percentage mortality following a treatment. The most widespread method is the feeding census. To do so, feeding points using un-poisoned broken wheat or rolled oats have to be established. A feeding census should be done twice, one census before treatment and one census after treatment. The guideline allows alternative methods mentioning live-trapping as one possibility. However, pre- and post-treatment census methods do not allow a permanent observation of the rodent population during the treatment, furthermore they might have a misleading effect on animal behaviour, e.g. bait or trap shyness. Other methods like tracking plates are less accurate. Therefore, we compared the new automatic monitoring device with the most reliable census, the feeding census, during an efficacy test with a rodenticide product.

### Materials and methods

The field trial was conducted at the research centre Neu-Ulrichstein, near Homberg (Ohm), Hessa, Germany. At the site of the research centre an abandoned pig stable was infested with a minimum of 80 Norway rats. No rat control has been conducted for the last four or five years. The rats concentrated their activities in the basement of the building, where left over cereals and rare human activities provided perfect conditions for rats. Two weeks before the application, a feeding census was conducted. As a second, independent, method to observe the effects of the rodenticide, the new electronic device was used. The Rodent Monitoring Tool (by Erminea GmbH, Germany) consists of movement sensors wireless connected to a base station to collect the data. During the trial four movement sensors were used to observe the rat population. The position of each sensor was near by, but not on top of the four feeding trays. Movement data were recorded to a digital storage card and sent via e-mail for daily monitoring. The monitoring tool was programmed with a delay time of five to six minutes after recording a movement. With such a delay a maximum of eleven records per hour was possible. To match the feeding census data a 24 h-average of movement records was calculated for each census day. A single rodenticide treatment with coumatetralyl was chosen for an optimal assessment of the product efficiency. The post-treatment feeding census was performed 21 days after the treatment and covered 48 hours. In contrast to the two disjunctive feeding counts, the activity monitoring was done permanently for 44 days covering pre- and post treatment census feeding.

## Results

During two days pre-treatment census, an average of 2,339 g of rolled oats was taken. Assuming that rats consume between 20 and 40 g per night, at minimum 80 Norway rats were present. The automatic monitoring system allowed a permanent recording of the rat activity (Figure 1). To compare permanent activity with the feeding census, averages were calculated for each census day. During the pre-treatment period an average of 2.47 movements per hour was measured. A single rodenticide treatment was applied at the 22<sup>nd</sup> of October. First dead rats were found three days after. 21 days after the treatment a post treatment feeding census was started and only 69 g oats per day were consumed. Taking pre- and post census data, we calculated the survival rate of 2.95 %. The recorded activity at the same days was 0.06 hourly movements. The activity values were taken as well to calculate a survival rate of 2.43 %.



**Fig. 1** The permanent activity recording allowed monitoring the rat population not just at some days before and after the treatment, but throughout the whole trial. After the treatment the activity increased slightly and then dropped drastically to almost zero.

## Discussion

Both census methods applied during this trial were suitable to evaluate the rodenticide effects of the tested product. In fact both very different methods resulted in similar survival rates of less than 3%. As the feeding census is a well established and accepted method, the permanent monitoring can now be seen as a suitable tool too. The feeding census allows an estimate of the number of animals using the bait stations by the amount of taken food. The activity monitoring on the other hand is a non-invasive tool that does not influence the animal behaviour. Therefore, it could be used during the whole trial even while the treatment was applied. In the current trial the recorded activity and the discovery of dead rats showed that the rodenticide effect occurred within three to five days after treatment. Immediately after the treatment the activity increased and then dropped drastically. A similar pattern was found in a second trial to test a rodenticide product on house mice in a pig stable (unpublished data). This test, however, had to be abandoned as the monitoring tool detected a second activity increase eight days after the treatment which turned out to be an invasion of Norway rats. However, the short activity increase after rodenticide treatments is an interesting pattern and needs further investigation. The combination of feeding census and the Erminea Rodent Monitoring Tool provides an estimation of the rodent density as well as of diurnal activity patterns. They deliver an estimation of rodent control efficacy by two independent estimations. As rodenticide evaluation tests are usually conducted with two independent census methods, we consider the combination of both methods to be very reliable and useful.

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## Integrating ecology and technology to create innovative pest control devices

Blackie, H.<sup>1</sup>, MacMorran, D.<sup>2</sup>, Shapiro, L.<sup>2</sup>, Woodhead, I.<sup>3</sup>, Diegel, O.<sup>4</sup>, Murphy, E.<sup>5</sup>, Eason, C.T.<sup>1,2</sup>

<sup>1</sup>Centre for Wildlife Management and Conservation, Faculty of Agriculture and Life Sciences, Department of Ecology, Lincoln University, New Zealand, helen.blackie@lincoln.ac.nz

<sup>2</sup>Connovation Research Ltd, Auckland, New Zealand

<sup>3</sup>Lincoln Ventures Ltd, PO Box 133, Lincoln, New Zealand

<sup>4</sup>Creative Industries Research Institute, Auckland University of Technology, Auckland, New Zealand

<sup>5</sup>Department of Conservation, Christchurch, New Zealand

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### Abstract

The development of innovative pest management and monitoring tools requires the integration of animal ecology, toxicology and design engineering. Resetting, multi-kill devices offer substantial advantages over current baiting or trapping techniques. This research outlines the development and testing of a long-life, resetting, toxin delivery systems for predator control, which has recently been laboratory and field trialed on stoats (*Mustela erminea*) and weasels (*M. nivalis*). Results of laboratory trials showed similar responses to stoats and weasels after a 40% PAPP paste was delivered onto the sternum. Both species groomed the paste off within approximately 4 minutes of application and death occurred after an average of 40 minutes for stoats and 32 minutes for weasels. A pilot field trial was also conducted with proof of concept demonstrated and no non-target interference recorded. Findings provided valuable feedback to design engineers which lead to the development of an improved 'Mark II' design. Long-life toxin delivery systems could be deployed to control a variety of pest species and further developments of these tools are ensuring their use for widespread field applications.

Keywords: *Mustela erminea*, para-aminopropiophenone, resetting toxin delivery systems

### Introduction

Pests such as black rats (*Rattus rattus*), stoats (*Mustela erminea*), ferrets (*M. furo*) and possum (*Trichosurus vulpecula*) continue to cause major harm to New Zealand's biodiversity. To ensure the survival of native species on the mainland, continued predator control is vital. However, current control strategies rely largely on labor intensive trapping. Current traps are costly in terms of dollars per kill due to the need to frequently check and, where necessary, reset them. There is a need for reliable, cost-effective and safe devices which enable a large number of kills in a manner that eliminates environmental and ecological problems, eliminates the need for costly repeated applications of bait and substantially reduces the frequency of consumable resupply. In a new fusion of ecology and engineering skills we are looking beyond current toxic baits and control methods to develop novel resetting toxin delivery systems which take advantage of an animal's natural behavior (such as grooming).

### Materials and methods

Resettable toxin delivery systems were developed by Connovation Ltd, which dispensed 1 gram of a toxic spray (containing 40% PAPP) to animal's sternum after they triggered a treadle mechanism. Each device was capable of approximately 100 doses and was also fitted with a counter and a 2-minute 'timeout' to allow animals to escape following dosage (therefore clearing the device for the next individual).

Initial trials focused on stoat and weasel (*Mustela nivalis*) interactions with devices and their success in triggering systems to get a non-toxic dose of spray. After proof of concept was achieved para-aminopropiophenone (PAPP) was included within the system as a humane toxin. Resettable tunnels were placed in the animal's cage with fresh meat as a lure. Animals were monitored with motion sensing video cameras for the following information: times until device triggered, time until grooming, onset of symptoms, ataxia, coma and death.

Following the success of lab trials these resettable toxin delivery systems were then trialed at a field site in the Blue Mountains, West Otago, New Zealand. Results of these trials were used to provide feedback to design engineers to ensure optimisation of the devices

## Results

Laboratory trial results demonstrated that both stoats and weasels triggered the toxin delivery systems and were successfully dosed with PAPP. The average time until grooming after toxin delivery was 4.2 min for both species. Average time until coma was 24 min (range 20-33 min) for stoats and 18 min (range 14-58 min) for weasels. Death occurred after an average of 40 min for stoats (range 29-59 min) and 32 min for weasels (range 20-77 min).

The pilot field trial results confirmed that stoats interact and trigger devices in a natural setting, while non-targets were affected. Initial engineering problems associated with deploying a long-life device have been fed back to design engineers, which lead to the production of a 'Mark II' device with improved effectiveness.

## Discussion

Results of laboratory trials showed similar reactions for weasels and stoats to PAPP delivered in a resettable toxin delivery system. Both species groomed PAPP off shortly after it was dispensed on to fur and responses suggested that PAPP dispensed in this manner was effective and humane. Similar efficacy has been achieved with PAPP in meat baits, with trials showing stoats died quickly after eating the PAPP bait with first symptoms occurring from six to 40 min after ingestion and death between 15 and 85 min (Eason et al., 2010).

The pilot field trial demonstrated that these toxin delivery devices were capable of providing a resettable, multi-kill device with a design which prevented non-target interference. Devices could be left unattended in the field for long periods, hence eliminating the need for costly repeated applications of bait or trap resetting.

In order to meet the challenges facing pest vertebrate pest control in New Zealand and overseas, a strategic approach needs to be taken which incorporates the development of new baits and toxins with resetting, multi-kill, long-life tunnel toxin delivery systems. Resetting systems using PAPP have proven extremely effective at killing both stoats and weasels. Systems are now being modified to target other mammalian pests such as brushtail possums and feral cats (*Felis catus*).

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## Searching for alternative methods for a sustainable population management of the common vole (*Microtus arvalis*) in Saxony-Anhalt

Eggert, J.<sup>1</sup>, Wolff, C.<sup>2</sup>, Richter, K.<sup>1</sup>

<sup>1</sup>Anhalt University of Applied Sciences, Department of Agriculture, Ecotrophology and Landscape Development; Strenzfelder Allee 28, D-06406 Bernburg, Germany, j.eggert@loel.hs-anhalt.de

<sup>2</sup>Regional Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt, Plant Protection Department, Strenzfelder Allee 22, D-06406 Bernburg, Germany

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### Abstract

High crop damage can occur due to gradations by *Microtus arvalis*. After the gradation years 2004/2005 and 2007 the Ministry of Agriculture and Environment Saxony-Anhalt initiated a research project to find new ways of managing *Microtus arvalis* on farmland. At the moment, the conclusion of this project is that deep tillage is the most effective method to minimize the *Microtus* populations. The impact of weather conditions such as rain and ground frost without snow is also significant. Because of their non-selective toxicity against other vertebrates rodenticides should only be used as a last option.

Keywords: common vole, gradation, *Microtus arvalis*, Sachsen-Anhalt, Saxony-Anhalt, tillage, weather

### Introduction

In times of high population density the common vole (*Microtus arvalis*) causes high crop damage on agricultural land. The damage caused by common vole gradation can in some areas reach 70% or even 80% of the harvest (Jacob and Tkadlec, 2010; Plant protection Department Saxony-Anhalt pers. comm., 2008). After the last gradations in 2004/2005 and 2007 the Ministry of Agriculture and Environment Saxony-Anhalt initiated in 2008 the research project on the common vole in Saxony-Anhalt. Part of the project is to develop methods for a sustainable management of *Microtus arvalis*. Establishing monitoring systems for the risk prediction of common vole gradations is another part of the project.

### Materials and methods

Areas with a high density of common vole were monitored regularly. On infested fields two areas of 250 m<sup>2</sup> each were selected. On these fields the *Microtus arvalis* activity was controlled using the common method of closing all tunnel entrances. After 24 and 48 hours the number of reopened tunnel entrances was counted.

Another field of research was exploring the effect of different land management systems on the development of the *Microtus arvalis* abundance. For this purpose a tillage experiment with five tillage types was used, each one with a width of 24 m and two replicates. Plot one was ploughing to a depth of 21 cm, plot two grubbing with a cultivator to a depth of 18 cm, plot three shallow grubbing with a cultivator to a depth of 10 cm, plot four disking with a disk harrow to a depth of 6 cm and plot five was no-till/direct drilling.

### Results

During the past three years the regional development of the *Microtus arvalis* abundance and its state of gradation was monitored. With this regional data farmers are enabled protect their crops in time.

It could clearly be shown that the type of tillage has great influence on the progression of a possible gradation. However, also weather was an important factor. The results of the tillage experiment showed that a deep tillage, whether through ploughing or deep cultivating, has the greatest influence on the *Microtus arvalis* population and decreases the rate of activity to nearly zero. The shallow tillage variants showed a decrease of vole activity, however, a measurable activity remained. On the no-tillage plots a rather high population of *Microtus* survived and started spreading after the abundance ascended.

However, the spring population is not only affected by the type of tillage but also depends on previous weather conditions as stated by Herold (1954). Not being an objective of this study, a simple comparison of spring populations with the climate of the previous winter shows that even no-till/direct drilling fields show no/nearly no activity under certain conditions: Whether there has been any tillage or not, in years

following winters with heavy frost (and only slight snow) followed by rainy conditions (on frozen soil) no gradation has to be expected and no measures are necessary to protect the harvest.

### **Discussion**

Forecast models will be useful to predict the regional infestation risk, but they never acquit the farmer from monitoring his fields. It is also important to establish a monitoring system through the whole state to gather information. Basic research about the mechanisms which cause gradations of the common vole is still needed to discover prevention methods.

If there is high population density, deep tillage is the best way to control the population at the affected site. Therefore, it is necessary to start the tillage as soon as possible post-harvest. It might be necessary to repeat the treatment before sowing. The key factor is the depth of the tillage.

If there is a large population of *Microtus arvalis* post-harvest, no-till should not be the sowing method. The use of rodenticides should be only the last option because of their non-selective toxicity against other vertebrates. It is also important to keep refuges of *Microtus* managed. This includes mowing ditches and soft shoulders of roads, which makes them accessible for predators such as kestrels, buzzards, red- and black-kite, herons as well as foxes and mustelids.

The massive influence caused by weather conditions needs more research to allow better predictions.

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## The development of a light-weight, long-life diphacinone rodent bait

Ross, J.G.<sup>1</sup>, Eason, C.T.<sup>1,2</sup>, Sam, S.<sup>1</sup>, Shapiro, L.<sup>2</sup>, Blackie, H.<sup>1</sup>, MacMorran, D.<sup>2</sup>, Aylett, P.<sup>2</sup>, Tucker, N.<sup>3</sup>, Razzaq, H.<sup>3</sup>

<sup>1</sup>Centre for Wildlife Management and Conservation, Department of Ecology, Lincoln University, Lincoln, New Zealand

<sup>2</sup>Connovation Research Ltd, Manukau, Auckland, New Zealand

<sup>3</sup>Plant and Food Research, Lincoln, New Zealand, james.ross@lincoln.ac.nz

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### Abstract

Rodents introduced into mammal-free New Zealand seriously impact our vulnerable native flora and fauna. As a result, considerable research effort has focused on developing control techniques for reducing and/or eradicating rodents with excellent success in the eradication of both Norway and ship rats from many offshore islands. This control work has now created numerous pest-free sanctuaries thus enabling the translocation of many endangered native bird species. Whilst this research work has generally been positive, there are still numerous examples where control has failed to successfully eradicate mouse populations. Another problem is that there has been reliance on bait containing brodifacoum for rodent control and this can create major secondary poisoning risks for non-target predators and scavengers. Recent research has suggested that low bait palatability and/or poor bait delivery systems are the most likely reasons for unsuccessful mouse control. This purpose of this research project is to develop a novel bait for rodents involving extruded paste technology. This technology enables us to enhance the geometric shape of the bait with the emphasis on increasing attractiveness for mice. This bait has also been designed to be lightweight, easy to apply in the field and has an added advantage of a natural waterproof coating to lengthen field durability and palatability. Preliminary trials with captive mice indicate that the new bait is significantly more palatable than a standard rodent bait for both rats and mice. Weathering and toxic field trials of the new bait are currently underway and the results of this research will be presented.

Keywords: diphacinone, long-life bait, mice, rats, rodent control

## Avian predators as a biological control system of common vole (*Microtus arvalis*) populations in NW Spain: experimental set-up and preliminary results

Jareño, D.<sup>1</sup>, Paz, A.<sup>2</sup>, Arroyo, L.<sup>1</sup>, Viñuela, J.<sup>1</sup>, Arroyo, B.E.<sup>1</sup>, Mougeot, F.<sup>3</sup>, Luque-Larena, J.J.<sup>4</sup>, Fargallo, J.A.<sup>5</sup>

<sup>1</sup>Instituto de Investigación en Recursos Cinegéticos, Ronda de Toledo s/n, 13005 Ciudad Real, Spain, javier.vinuela@uclm.es

<sup>2</sup>GREFA, Apdo. 11, 28220 Majadahonda, Madrid, Spain

<sup>3</sup>Estacion Experimental de Zonas Aridas, 04001 Almeria, Spain

<sup>4</sup>Depto. CC Agroforestales, ETSIIAA, Universidad de Valladolid, Campus La Yutera 34004, Palencia, Spain

<sup>5</sup>Museo Nacional de Ciencias Naturales, José Gutierrez Abascal 2, 28006 Madrid, Spain

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### Abstract

Ecologically-based pest management using biological control has been successfully tested elsewhere, but it has never been evaluated for cyclic vole plagues in Europe. We report the first large-scale replicated experiment to study the usefulness of artificially increasing populations of Common kestrels (*Falco tinnunculus*) and Barn owls (*Tyto alba*) to control common vole populations in agricultural habitats where nesting sites for raptors are scarce. The first preliminary results suggest: 1) population density of both predator species may be increasing in response to both nest-site availability and rodent density; 2) voles are a major prey; and 3) vole density during an increase phase of the population cycle may be reduced near nest-boxes.

Keywords: *Microtus arvalis*, outbreaks, biological control, *Falco tinnunculus*, *Tyto alba*, agriculture, Spain

### Introduction

In Europe, *Microtus arvalis* is a major agricultural pest that can produce important crop damage during population outbreaks (Jacob and Tkadlec, 2010) as well as sanitary problems (Vidal et al., 2009). In Spain this pest is usually controlled by environmentally aggressive campaigns based on large-scale and massive rodenticide use, which can have an impact on non-target species such as the red kite (*Milvus milvus*) (Olea et al., 2009; Mougeot et al., in press). The development of alternative environmentally-friendly control strategies is essential. Artificially increased populations of barn owls have been tested in some areas as a biological control method of rodent pests. These can have similar efficacy to rodenticides at lower cost (Brown et al., 2006, Muñoz-Pederos et al., 2010). Common kestrels may have a regulatory effect on common vole population density (Fargallo et al., 2009) and it has been suggested that increasing predator density could be a promising technique to control this pest (Pelz, 2003). However, the efficacy of this control method has yet to be properly tested (Jacob and Tkadlec, 2010).

### Methods

The study took place in three areas of Castilla y León (NW Spain) where vole populations reached high densities during the last vole outbreak of 2007. Within each study area, we selected a control and an experimental plot of 2000 ha each. In the experimental plots, 100 nest boxes were installed, 50 of these specifically designed for kestrels, and 50 others designed for barn owls. We monitored occupancy and breeding success of raptors using nest boxes, and estimated the abundance of both rodents and diurnal avian predators in each area. The abundance of diurnal avian predators was measured using a Kilometric Index of abundance from eight road transects in each study area (four in each control and four in each experimental area). The abundance of rodents was measured using Sherman LFAHD traps, three times per year (March, July and November) on twelve trapping plots stratified by habitat (alfalfa, cereal, and uncultivated), each trapping plot with 35 traps. Additionally, an indirect abundance index of the presence of *M. arvalis* based on the presence of fresh droppings of and/or vegetation clipping was used in one of the study areas (Jareño, 2010). Diet of kestrels and barn owls was evaluated by analyzing fresh pellets and food remains at nests.

## Results

The abundance of common kestrels observed during road transects increased more in the experimental areas than in control areas. Kestrel breeding population reached 1.75 pairs/100 ha in the area with highest overall vole density. Barn owls have occupied nest boxes later, coinciding with increasing rodent densities during spring 2011, and reaching by now 0.8 pairs /100 ha. The pattern of nest-box occupancy apparently matched, spatially and temporally, that of rodent density. Voles were a staple prey for both raptor species, but barn owls also consumed large amounts of wood mouse (*Apodemus sylvaticus*) and other small mammals. We found a significant positive relationship between distance to the nearest nest box, occupied or not, and the abundance of common voles (Wald Chi-square=8.554, df=1, p=0.003).

## Discussion

Supplying nest boxes for avian predators produced an important increase in the numbers of kestrels and barn owls in the experimental areas; this was expected due to the scarcity of nesting places (no trees, few buildings) in the agricultural habitats of NW Spain. Common voles were a main prey of avian predators in the study areas, even at vole densities that were relatively low compared with those of peak-abundance years. Preliminary results suggest that kestrels may be selecting nest boxes near higher vole density areas. Vole density was positively associated to distance to nest-boxes, but independently of its occupancy status, while the effect of kestrel occupancy on vole density around the nest box was only near significance. Perhaps we were detecting a possible effect of the presence of nest boxes, not occupancy, as these can be used as perching sites by kestrels and other raptors breeding in the area, such as common buzzards. These preliminary results are encouraging, but obviously an experimental set-up on 6000 ha will not be able to stop a rodent plague that may affect  $3 \times 10^6$  ha in Castilla y León (Jacob and Tkadlec 2010), but may have local effects. Thus, caution must be kept until a new vole peak is reported in the study area, to assess relative vole density and crop damages in experimental areas as compared to areas without nest boxes.

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## Smell you later - the repelling effect of secondary plant compounds against water voles and common voles

Fischer, D.<sup>1</sup>, Prokop, A.<sup>2</sup>, Wink, M.<sup>3</sup>, Mattes, H.<sup>4</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forestry, Vertebrate Research, Toppheideweg 88, 48161 Muenster, Germany, daniela.fischer@jki.bund.de

<sup>2</sup>W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany

<sup>3</sup>University of Heidelberg, Institute of Pharmacy and Molecular Biotechnology, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

<sup>4</sup>University of Muenster, Institute of Landscape Ecology, Robert-Koch-Straße 26, 48149 Muenster, Germany

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Water voles (*Arvicola spec.*) and common voles (*Microtus arvalis*) are abundant in most parts of Germany and are known to cause enormous damage in fruit growing and horticulture as well as in agriculture.

There is a clear need for sustainable measures to manage vole damage. This study aimed to develop a vole repellent on the basis of secondary plant metabolites. A prerequisite for the future repellent is that the plant species required for obtaining the active substance would need to be widely available and sustainable in their production. Potentially an effective water vole repellent could help reduce crop damage but also minimise the use of kill traps and rodenticides. An additional effect would be the reduction of impacts on non-target species. In this project voles were exposed to various secondary plant metabolites (pure substances and essential oils) to study their repulsive olfactory effect on the animals.

The effect of volatile substances on water voles was tested in a T-maze. The voles could choose between a test box including a test substance and a control box without odour. The substances were considered to be a potential repellent if the test box was avoided. Three essential oils, pepper oil (black) (Mann-Whitney-U-test,  $p=0.005$ ), geranium oil (Chinese) ( $p=0.046$ ) and onion oil ( $p=0.046$ ) as well as one pure plant compound (methyl nonyl ketone,  $p=0.006$ ) repelled voles. Different combinations of these substances did not significantly increase the repelling effect compared to the use of single substances. The two most effective single substances, pepper oil (black) and methyl nonyl ketone, are currently being tested in enclosure and field trials with water voles and common voles. Recent results of these trials testing the effect of burrow application with foam and spray carriers will be presented.

## **Workshop on new tools and methods - alternatives to rodenticides and environmental implications**

Schmolz, E.<sup>1</sup>, Eason, C.T.<sup>2</sup>

<sup>1</sup>Federal Environment Agency, Sect. IV 1.4 Health Pests and their Control, new-rodenticides@uba.de

<sup>2</sup>Centre for Wildlife Management and Conservation, Faculty of Agriculture and Life Sciences, Department of Ecology, Lincoln University, NZ

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The authorization and evaluation of anticoagulant rodenticides for annex 1 inclusion after EG biocide directive 98/8 has revealed that most of these substances exhibit environmental properties which are undesirable or intolerable. However, from a health perspective, the need to control rats and mice with biocides in urban habitats and on farms is undisputed. Commensal rats and mice transmit infectious diseases, and rodent control is in many cases a statutory measure. Thus, from a human and animal health perspective effective rodenticides must be available.

At the moment, no alternatives to anticoagulants are on the biocide market – with the exception of chloralose, carbon dioxide, hydrogen cyanide and aluminium phosphide, all of which are only applicable under special circumstances and mostly only against specific target organisms. The development of new rodenticides is challenging, since uptake of baits is dependent on the complex social behavior of the target organisms, and their use must be safe for humans and non-target organisms.

The workshop will

- address regulatory concerns against the authorization of anticoagulants
- describe problems and successes in the development of new rodenticides
- point to possible economic hindrances

Contributions will cover

- Environmental concerns for authorization of anticoagulant rodenticides in the EU from a regulatory perspective
- Humaneness of rodenticides from a regulatory perspective
- Development of new rodenticides: research perspectives
- Development of new rodenticides: industry perspectives
- Sustainable rodent control: definition of control objectives and long term eradication

## **Rodent management in urban and rural ecosystems: experiences from central Argentina**

Cavia, R.

CONICET and Dpto. de Ecología, Genética y Evolución, FCEN, UBA, Cdad Universitaria, PB II, 4to piso, (C1428EHA) Nuñez, Buenos Aires, Argentina, rcavia@ege.fcen.uba.ar

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### **Abstract**

Rodent control based only on the use of rodenticides is known to be ineffective over the long-term, although it is the main method applied by farmers and public authorities in central Argentina. This paper presents field results and then discusses the successes and failures of three rodent control experiences based on ecological knowledge. The rodent control experiences discussed here are the following: 1) chemical control combined with environmental management in a shantytown, 2) reduction of vegetation height along farm perimeters and chemical control in poultry sheds, and 3) prevention of rodent reinfestation using physical barriers in poultry sheds. In the first two experiments rodent infestation decreased in the treated areas, although in the shantytown infestations levels increased 90 days after the last application of rodenticide. In the third experiment the variation in rodent infestation was similar in sheds with and without physical barriers. A combination of rodenticide application and environmental management reduced rodent abundance.

Keywords: environmental management, habitat manipulation, physical, rodent control exclusion

### **Introduction**

Historically, the control of rodents has largely relied on the use of rodenticides, being sometimes a palliative measure applied when the problem is already well established (Colvin and Jackson, 1999). Rodent control using poison alone is not an effective long-term solution (Singleton et al., 1999), however, farmers and public authorities choose to rely solely on rodenticides to control rodents in rural and urban ecosystems in Argentina. This study shows and discusses the successes and failures of rodent control experiences carried out in these ecosystems where chemical control was combined with environmental management measures, habitat manipulation, or exclusion by physical barriers.

### **Methods and results**

- 1) 'Implementation and Evaluation of an Integrated Program for Rodent Control in a Shantytown.' The efficiency of an integrated program for the control of rodents in a shantytown of Buenos Aires City, Argentina was evaluated. This program lasted one year and was divided in a preparatory phase, an execution phase and a monitoring phase. During the preparatory phase, an environmental survey of public spaces and dwellings was conducted in the test area to identify factors favoring the presence of rodents and to determine strategies and management measures to be implemented in the execution phase. Rodent abundance was monitored using nontoxic bait stations during four periods: the preparation phase, the execution phase, immediately after the execution phase, and 90 days after the end of the execution phase. According to the results of the preparatory phase, control measures proposed for the execution phase were as follows: (1) to carry out poisoning campaigns in dwellings and public spaces, (2) to remove weeds and pave vacant lots within and around the neighborhood, (3) to increase the frequency of garbage collection and use rodent-proof garbage containers, and (4) to conduct a health education campaign on sanitation improvement. The last two control measures failed. The garbage collection frequency did not increase and containers were not changed. The education campaign consisted in awareness meetings for neighbors and the distribution of flyers by community leaders to householders which provided sanitation measures to maintain rodent abundance at low levels, however only in 6.2% of the dwellings (n=104) neighbors implemented at least one of the proposed activities. In the experimental area, the proportion of dwellings and public spaces with signs of rodent activity decreased significantly from the beginning of the trial to immediately after the end of the execution phase, but rodent activity increased 90 days after the execution phase. No differences were found when comparisons were made for the control area.
- 2) 'Experimental assessment of rodent control on two poultry farms of central Argentina.' Previous studies carried out on poultry farms in this region revealed that rodent infestation is positively

associated with the percentage of the perimeter of the farm covered with vegetation (Gómez Villafañe et al., 2001), the amount of plant cover above 20 cm in height, and the fact that sheds located at the perimeter of the farm show higher rodent abundance than those located between other sheds on the same farm (Gómez Villafañe et al., 2003). We experimentally assessed the effect of controlling vegetation height along farm perimeters on the abundance of rodents in 2 broiler poultry farms in central Argentina. We carried out an experimental design based on the before-after-control-impact method. After vegetation treatments, there was a significant decrease in rodent abundance at the perimeter of the farm, particularly of the Pampean grassland mouse, *Akodon azarae*. In poultry sheds, there was a significant decrease in rodent abundance on non affected farms due to the reductions in abundance of the house mouse *Mus musculus*, possibly because of a major application of rodenticide.

- 3) 'Rodent experimental exclusion from breeding sheds in poultry farms.' In order to evaluate the effect of exclusion by physical barriers on rodent re-infestation in poultry sheds, two sheds of 60 m x 12 m (one in each of two different farms) were enclosed with sheets of zinc 80 cm above ground and 40 cm below ground. The remaining sheds in each farm were used as experimental controls. After building the enclosure and during the five following weeks we conducted an intensive removal of rodents by trapping and poisoning in all sheds of both farms. The cost of the enclosures included 496 man/hours, USD 3,115 in materials and USD 1,200 in fuel. A total of 264 rodents were removed on both farms with an effort of 992 cage trap-nights, 2063 Sherman trap-nights and 2118 snap trap-nights. *M. musculus* was the dominant species followed by *Rattus norvegicus* and *A. azarae*. Relative abundances of mice and rats were estimated in all sheds on five occasions over eight months using a tunnel track index calibrated with rodent capture data. The variation in rodent relative abundance was similar in enclosed and not enclosed sheds. The relative abundance of mice and rats decreased from the beginning of the experiment towards the end of the rodent removal period, when it reached values of zero or close to zero. After this period, mice relative abundances increased to the initial values, while rat abundance remained low in both enclosed and not enclosed sheds.

## Discussion

The combination of rodenticide application and environmental sanitation reduced rodent abundances in both dwellings and public spaces of the shantytown. However, the fact that the rodent population increased when control measures were discontinued suggests that food and shelter for rodents were still available. In broiler poultry farms, the control of vegetation growth at the perimeters and the appropriate timing of rodenticide applications are more effective if both control measures are applied simultaneously, preventing re-infestation from perimeters. Finally, the application of physical barriers was ineffective to prevent rodent re-infestation of the breeding poultry sheds. Although this could have been due to the failure of the farmers to keep the doors of the sheds closed, this method may be inappropriate for places where laborers have to go in and out many times a day as in breeding poultry sheds.

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## Current status of bird pest species in agroecosystems of Buenos Aires province, central Argentina

Codesido, M., Bilenca, D.

Grupo de Estudios sobre Biodiversidad en Agroecosistemas (GEBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Pab. II, Buenos Aires C1428EGA, Argentina. CONICET, Rivadavia 1917, Buenos Aires C1033AAJ, Argentina, dbilenca@ege.fcen.uba.ar

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### Abstract

We carried out the first assessment of abundance and distribution of bird pest species (eared dove *Zenaida auriculata*, monk parakeet *Myiopsitta monachus*, picazuro pigeon *Patagioenas picazuro* and spot-winged pigeon *P. maculosa*) in the pampas of Buenos Aires province, Argentina, and analyzed their association with the presence of crops and/or introduced exotic woodlots in the rural landscape. We surveyed 35 transects located along secondary roads. Bird pest abundance was significantly higher at those sites with presence of woodlots ( $p < 0.0001$ ). Species-specific analyses revealed that abundance of both monk parakeet and picazuro pigeon was sensitive to the frequency of woodlots of tall and perennial tree species (*Eucalyptus* spp.;  $p < 0.001$ ), whereas eared dove abundance increased significantly with the frequency of woodlots composed by short and perennial tree species (*Pinus* spp., *Casuarina* spp.;  $p = 0.002$ ). Most bird pest species in the study area are more sensitive to the presence of woodlots rather than to the presence of croplands in the rural landscape. These results have practical implications, suggesting that an effective control of bird pest species in Buenos Aires province could be attained by managing exotic perennial tree species woodlots.

Keywords: Argentina, Buenos Aires, eared dove, habitat management, monk parakeet, Pampas

### Introduction

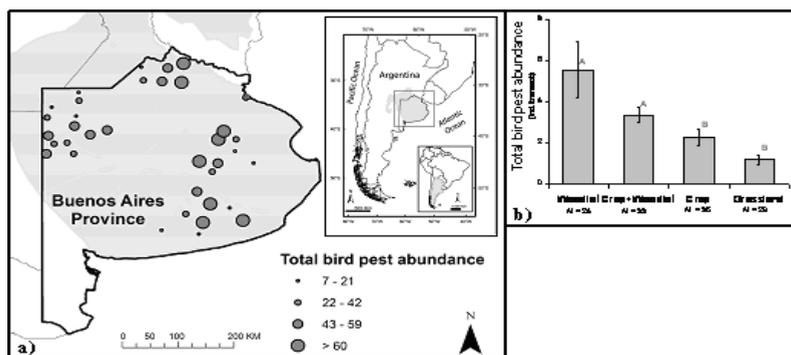
Eared dove (*Z. auriculata*), monk parakeet (*M. monachus*) and pigeons (including picazuro and spot-winged pigeon, *P. picazuro* and *P. maculosa*, respectively) are among major bird pests of Argentina (Bruggers et al., 1998). Population increase of these species has been associated to a mosaic landscape where food patches (croplands) and breeding habitats (patches of woodland) are present in suitable proportions (Bucher and Ranvaud, 2006). Most of these studies have been carried out in the region of the Espinal, where agriculture expanded into areas previously covered by dry woodland (Murton et al., 1974), whereas studies in the grasslands of the Pampas region are lacking. Trees were originally absent in the Pampas, even though nowadays woodlands of both native and exotic species have been establishing as riparian vegetation or along roadsides, or by the introduction of exotic tree species around rural buildings and as shading woodlots for cattle (Ghersa et al., 2002). The introduction of trees in Buenos Aires province has been followed by the expansion of doves, parakeets and pigeons (Daguerre, 1936; Gibson, 1919). In addition, recent concern of farmer associations due to crop losses in Buenos Aires province, where rangelands have been increasingly replaced by crops (Baldi and Paruelo, 2008) has prompted a survey of abundance and distribution of these bird pest species.

### Materials and methods

Bird surveys were conducted during summer 2006-2008 along 35 transects located on secondary roads of Buenos Aires province (Fig. 1). Each transect was 20 km long, with permanently marked survey points located every kilometer. Surveys were performed following the point-count method (Bibby et al., 2000). All pest birds seen or heard within a 200 m radius around each point during a 5 min period were identified. In addition, the presence/absence and the identity of crops and/or woodlots were recorded at each point. Thus, each transect was considered as a block and the combination of the presence/absence of crops and/or woodlots were considered as treatments, resulting in 4 treatments of a block design (Zar, 1996): 1) crop+woodlot; 2) crop; 3) woodlot; and 4) grassland (control). The abundance of bird pest species was used as the response variable (data were log transformed). In addition, linear regressions were performed between the abundance of birds and the relative frequency of woodlots along transects.

## Results

There was a significant difference of total bird pest abundance among transects (i.e., blocks), revealing a higher abundance towards the East of Buenos Aires province (Figure 1a;  $F=1.88$ ;  $p=0.01$ ). Tukey's contrasts among treatments revealed that bird pest abundance was significantly higher at those sites with presence of woodlots (Figure 1b;  $F=13.61$ ;  $p<0.0001$ ). Species-specific analyses revealed that abundance of both parakeets and pigeons was sensitive to the frequency of woodlots of tall and perennial tree species (*Eucalyptus* spp.;  $p<0.001$ ), whereas dove abundance increased significantly with the frequency of woodlots composed by short and perennial tree species (*Pinus* spp. and *Casuarina* spp.;  $p=0.002$ ). In addition, eared dove was the only species whose abundance was significantly higher at those sites with the joint presence of crops and woodlots ( $F=9.03$ ;  $p<0.0001$ ).



**Fig. 1** (a) Study area (Buenos Aires province, Argentina, 307,571 km<sup>2</sup>), showing total bird pest species abundance sampled at each transect; (b) total bird pest species abundance according to the presence/absence of crops and/or woodlots in the rural landscape. Different letters show statistically significant differences (ANOVA and Tukey's test;  $p<0.0001$  and  $p<0.05$ , respectively).

## Discussion

To our best knowledge, this is the first comprehensive assessment of abundance and distribution of bird pest species in the Pampas of Buenos Aires province. Our results show that, overall, bird pest species in the study area are more sensitive to the presence of woodlots with exotic perennial tree species rather than to the presence of croplands in the rural landscape. The high affinity of parakeets with tall *Eucalyptus* trees should be related to the reduction of predation risk (and human control) on well-built nests located above a certain threshold height (Daguerre, 1936; Gibson, 1919). These results have practical implications, suggesting that an effective control of bird pest species in the study area could be attained by managing exotic perennial tree species woodlots, either by the reducing woodlots near to crops or by planting short and deciduous trees instead of tall and perennial trees.

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## **The Ecorat project: development of ecologically-based rodent management for the southern African region**

Mulungu, L.S.<sup>1</sup>, Belmain, S.R.<sup>2</sup>, Dlamini, N.<sup>3</sup>, Eiseb, S.<sup>4</sup>, Kirsten, F.<sup>5</sup>, Mahlaba, T.<sup>3</sup>, Makundi, R.<sup>1</sup>, Malebane, P.<sup>5</sup>, Von Maltitz, E.<sup>5</sup>, Massawe, A.<sup>1</sup>, Monadjem, A.<sup>3</sup>, Taylor, P.<sup>6,7</sup>, Tutjavi, V.<sup>4</sup>

<sup>1</sup>Pest Management Centre, Sokoine University of Agriculture, P.O. Box 3110, Chuo Kikuu, Morogoro, Tanzania

<sup>2</sup>Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom, s.r.belmain@gre.ac.uk

<sup>3</sup>Department of Biological Sciences, University of Swaziland, Private Bag 4, Kwaluseni, Swaziland

<sup>4</sup>National Museum of Namibia, P O Box 1203, Windhoek, Namibia

<sup>5</sup>Agricultural Research Council – Plant Protection Research Institute, P/bag X134, Queenswood, Pretoria 0121, South Africa

<sup>6</sup>Durban Natural Science Museum, P. O. Box 4085, Durban, 4000, South Africa

<sup>7</sup>Dept of Ecol. and Resource Man., Univ. of Venda, P Bag X5050, Thohoyandou, 0950, South Africa

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### **Abstract**

The aim of this study was to carry out basic ecological research on rodent pests within subsistence-level agricultural communities in Africa. A range of techniques were used to collect baseline ecological knowledge on the temporal and spatial dynamics of rodent populations within rural farming communities in Tanzania, Swaziland and Namibia. These techniques included habitat surveys using removal trapping, capture-mark-recapture grids, and radio tracking of individually tagged animals. We also studied the local communities' knowledge, attitudes and practices with respect to rodents and their control, the current cost of rodent damage and the costs/benefits of rodent control. Based on these data, a case-control trial was implemented to evaluate an ecologically-based rodent management (EBRM) intervention using intensive trapping coordinated at the community level. Results showed that intensive trapping using community based rodent management was cost-beneficial for rural farming communities, and these EBRM strategies are ecologically sustainable. Our research has shown that efficacy is more than 75% when compared to what farmers normally do to reduce rat populations. Farmer training and community cooperation are essential, and expertise in social anthropology to develop appropriate knowledge dissemination platforms must be supported.

Keywords: disease, intensive trapping, population dynamics, rodent damage

### **Introduction**

Rodents cause a myriad of problems for African households. Diseases incl. plague, Lassa fever, leptospirosis, typhus and food contamination caused by rats means that their disease burden alone makes rodents one of the most important problems facing African families (Meerburg et al., 2009). Nearly any crop can be damaged by rats. In any year a farmer may face crop losses of 5-20% but this can approach 100% during an outbreak, reaching epidemic proportions through aseasonal rainfall or other environmental changes (Normile, 2010, Singleton et al., 2010). Rat damage does not stop when crops are harvested, and many subsistence farmers continue to suffer serious losses when on farm storage is not rat proof. Despite serious damage, most African farmers do little to control rats. Repeated unsuccessful attempts to control rats have ingrained a sense of apathy and defeatism. In most cases, a farmer's measurement of success is having a few dead rodents as opposed to noticing that they have more food or that their families are in better health through fewer rodents. Research over the last two decades has refocused around an integrated approach to rodent management. EBRM strategies aim to understand the behaviors and breeding patterns of different rodent species and to use this knowledge to pinpoint problems and solutions. This rodent management paradigm has now taken centre stage in many parts of the world as a more sustainable solution (Brown et al., 2006; Jacob et al., 2010; Singleton et al., 2007; Sluydts et al., 2009; Stenseth et al., 2003).

### **Material and Methods**

From January 2007 to December 2009, the ECORAT project carried out research on rodent ecology, rodent biology and rodent-human interactions. We studied the local agricultural communities' knowledge, attitudes and practices with respect to rodents and their control, the current cost of rodent

damage and the costs/benefits of rodent control. The multidisciplinary research consortium was drawn from institutions in Namibia, South Africa, Swaziland and Tanzania, with central technical input provided by the Natural Resources Institute of the University of Greenwich in the UK. The ECORAT project based its research within rural agriculture communities to study how rodents affect people's livelihoods. A range of techniques were used to collect baseline ecological knowledge on the dynamics of rodent populations in rural African farming communities. These techniques included habitat surveys by removal trapping, capture-mark-recapture, and radio tracking of individually tagged animals. An intervention program was carried out in 12 villages across the 3 countries; half of the villages followed their indigenous rodent management practice and half followed the ECORAT method. Indigenous rodent management is defined as what farmers in these villages normally do to manage rats: essentially occasionally using acute poisons. The ECORAT method of intensive daily kill trapping was organized at the community level, with traps rotating around the community to share the costs. This ensured that the rodent population was reduced at a large enough scale to limit the effects of immigration back into the intervention zone. The scientific team monitored and compared the indigenous and ECORAT method by assessing changes in the rodent population abundance as well as effects on rodent damage, particularly assessing differences in grain storage loss. The number of rats killed by intensive trapping in the six ECORAT villages was recorded, which acted as one form of monitoring as we compared this to monthly monitoring of the rat population in the six indigenous villages by three nights of kill trapping in a small number of homesteads. We also made further comparisons on what was going on with the rodent populations using tracking tiles.

## Results and Discussion

Community based rodent management through intensive trapping was shown to be cost-beneficial for rural farming communities, and these EBRM strategies are ecologically sustainable. Our research has shown that efficacy is more than 75% when compared to what farmers normally do to reduce rat populations. Thus, considerable financial, health and food security benefits accrue to households that take part in ECORAT-style rodent management. However, challenges remain to promote widespread adoption. The problems that rats cause depend on local agro-ecological and socio-economic conditions, which must be understood in context. Farmer training and community cooperation are essential, and expertise in social anthropology to develop appropriate knowledge dissemination platforms must be supported (Palis et al., 2005). Research on rodent pests is relatively neglected due to a lack of awareness about the extent of the problem and 'new' EBRM solutions. We believe that strengthening the capacity of research and knowledge extension across African institutions to deliver EBRM would have major positive impacts on poverty and economic development across Africa.

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## Is a native rodent competitively dominant over an invasive rodent in lowland agro-forest habitat of the Philippines?

Stuart, A.M.<sup>1</sup>, Prescott, C.V.<sup>1</sup>, Singleton, G.R.<sup>2</sup>

<sup>1</sup>School of Biological Sciences, The University of Reading, Berkshire, RG6 6AS, UK, a.stuart@reading.ac.uk

<sup>2</sup>International Rice Research Institute, DAPO 7777, Manila, Philippines

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### Abstract

In the lowland agro-forest of the Sierra Madre Biodiversity Corridor (SMBC), it is considered that a native rodent species, *Rattus everetti* is competitively dominant over an invasive pest species, *Rattus tanezumi*. The main aim of this study was to assess the response of *R. tanezumi* following short term removal of *R. everetti*. We tested this experimentally by trapping and removing *R. everetti* from two treatment sites in agro-forest habitat on three occasions over three consecutive months. This was followed by three months of non-removal trapping. Two non-treatment sites were trapped for comparison. Following *R. everetti* removal, *R. everetti* individuals rapidly immigrated into the treatment sites and a significantly higher proportion of *R. tanezumi* females were in breeding condition in the treatment sites than in the non-treatment sites. The results from this study provide evidence of competition between native and invasive rodent species in complex agro-ecosystems. We were also able to demonstrate that *R. everetti* populations are able to recover rapidly from the non-target effects of short-term lethal control in and around agro-forest.

Keywords: interspecific competition, microhabitat use, pest management, Philippines, *Rattus everetti*, *R. tanezumi*, removal experiment

### Introduction

In the Philippines, little is known about the interactions that take place between native and non-native rodent species in complex agro-ecosystems. Previous studies suggest that non-native pest species of rodents are restricted to heavily disturbed areas except where native species are absent (Balete et al., 2009; Heaney et al., 1989; Rickart and Heaney, 1991). In the lowland agro-forest of the Sierra Madre Biodiversity Corridor (SMBC), the abundance of the non-native pest species *Rattus tanezumi* is low relative to nearby agricultural habitats, whereas, the abundance of the native rodent *Rattus everetti* is high (Stuart et al., 2008). It is considered that *R. everetti* may block or inhibit *R. tanezumi* from establishing within the agro-forest areas because of interspecific competition (Stuart et al., 2008). The main aim of this study was to assess the response of *R. tanezumi* and the other rodent species in the community following short term removal of *R. everetti* from an agro-forest habitat.

### Methods

Trapping grids of 42 cage-traps were placed in two treatment and two non-treatment sites in agro-forest habitat. At each site, a trapping grid of 42 (6 x 7) locally-made single-capture live cage-traps (300 mm x 140 mm x 140 mm) was used. Traps were spaced 15 m apart, giving a grid area of 6750 m<sup>2</sup>. Trapping sites were at least 500 m apart. Trapping was conducted over six sessions from May to October. In the treatment sites, three sessions of *R. everetti* removal trapping was followed by three sessions of non-removal trapping. At each trap station, the vegetation structure ('microhabitat') was assessed by measuring the ground vegetation cover, understorey vegetation cover and canopy cover within a circular quadrat of one metre radius, centred on the trap entrance.

### Results

Following *R. everetti* removal, *R. everetti* individuals rapidly immigrated into the treatment sites and a significantly higher proportion of *R. tanezumi* females were in breeding condition in the treatment sites than in the non-treatment sites. Irrespective of the treatment, there was a clear contrast in the use of canopy cover by *R. tanezumi* and *R. everetti*. *R. tanezumi* preferred microhabitat with less canopy cover, which one would associate with severely disturbed habitat with few trees, whereas, *R. everetti* preferred microhabitat with a dense canopy.

## Discussion

These findings support the hypothesis that *R. everetti* has a negative effect on female *R. tanezumi* reproductive activity in the non-treatment sites due to interspecific competition. This study thus provides evidence of a native rodent, *R. everetti*, out-competing an invasive rodent, *R. tanezumi*, in a complex agro-ecosystem in the Philippines. Ong and Rickart (2008) suggest that non-native pest rodents predominate in severely disturbed habitat and that practices that minimise habitat disturbance, and instead encourage the regeneration of second-growth forest, would be an effective management action against non-native pest rodents. The results from this study support this suggestion. We were also able to demonstrate that *R. everetti* populations are able to recover rapidly from the non-target effects of short-term lethal control in and around agro-forest.

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## **Rodent outbreaks and extreme weather events: a southeast Asian perspective**

Singleton, G.R., Htwe, N.M., Nelson, A.D.

International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines, g.singleton@cgiar.org

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### **Abstract**

In recent reviews of rodent outbreaks (Singleton et al., 2010a,b), three general systems were identified that influence the food supply of rodents in significantly different ways. One is life-cycle- or evolution-driven in the form of plant masting events. Outbreaks triggered by masting, including bamboo and beech forests, are examples of this system. The second is climatic; these include outbreaks driven by changes in abiotic conditions alone (aseasonal or unusual rainfall events, or major climatic events such as El Niño or La Niña). These are irregular and rodent populations respond rapidly to the peaks in increased food availability. The third is anthropogenic responses associated with extreme climate events or market forces with outbreaks driven by changes in cropping systems. These are driven directly by anthropogenic responses to calamitous events such as cyclones, high rainfall, and drought, or responses to shortfalls of production of staple crops.

In Southeast Asia a massive outbreak of rodents in the Ayeyarwaddy delta in 2009 and 2010 was associated with a calamitous weather event, cyclone Nargis, which occurred 15 months prior to the outbreak. We present findings that support the association between the effects of cyclone Nargis and the subsequent rodent outbreaks. These rodent populations response appeared to be associated with an extended period of available high quality food, which was caused by asynchronous and aseasonal planting of rice during the 2008 monsoon season. We contend that climate change and extreme climatic events will increase the impacts of rodents on agricultural production in coming years.

Keywords: extreme climatic events, Outbreaks, Rodents, Southeast Asia

### **Introduction**

Since 2007, rodent outbreaks in Asia, from bamboo masting, have led to severe food shortages in Mizoram (India), Chin State (Myanmar), Chittagong Hill Tracts (Bangladesh), and upland provinces of Lao PDR. In Laos, emergency food assistance was required for 85,000-145,000 people. These outbreaks have affected highly vulnerable and food insecure families. In 2009-2011, high rodent losses also occurred in lowland irrigated rice-based systems in the Philippines, Myanmar and Indonesia, not related to bamboo masting (Singleton et al., 2010a). In 2009 in the Ayeyarwaddy delta, a major outbreak of rodent populations occurred in the monsoon rice season 15 months after cyclone Nargis, a calamitous event that led to the death of 140,000 people and destroyed more than 450,000 houses. This paper will examine the hypothesis that the outbreak of rodents in the Ayeyarwaddy delta was driven by asynchronous and aseasonal planting of rice post-Nargis, which extended the period when high quality food (rice from the booting to ripening stage) was available for rodents.

### **Methods**

In July 2010, a household survey was conducted of 103 farmers from 5 village tracts in Bogale Township in the Ayeyarwaddy delta. The survey focused on the main production constraints, the cropping systems, the time of planting and the yields obtained during 2007 to 2009.

A two-stage remote sensing analysis was performed to derive an independent estimate of the rice planting dates for each year. The first stage identified the area planted to rice in each monsoon season; pixels were classified as rice or non-rice using a slightly modified version of the rice paddy detection algorithm by Xiao et al. (2006). The second stage estimated the date of the start of the season for each rice pixel using the approach developed by Jönsson and Eklundh (2004). Moderate Resolution Imaging Spectroradiometer (MODIS) Surface Reflectance was used to estimate planting dates. An 8-day composite (MOD09A1) provided the best observations to create a high quality composite image. This method enabled compilation of rice area maps, which were then used to select the rice pixels for the detection of the 'start of season' using the TIMESAT software (Jönsson and Eklundh, 2004).

Data on the numbers of rats in the Bogale Township in 2008 and 2009 were obtained from local government agencies which conducted bounty campaigns; 50 Kyatt were paid per rat tail (900 Kyatt=1USD).

### Results

In the 2008 monsoon season, the major constraints for farmers for rice production were lack of money for inputs (12.4%), and limited time for cultivation due mainly to lack of labour (34.4%); rats were ranked as the major pest by 6.7% of farmers. In the 2009 monsoon season, rats were ranked by 19.1% of farmers as their major constraint.

In 2009, in the Ayeyarwaddy delta, 2.6 million rats were collected in 3 months through community action in five townships.

In Bogale Township, the TIMESAT analysis indicated that the time of planting of the monsoon rice crop in 2008 was extended by on average 24-32 days compared to the 2007 and 2009 monsoon seasons. This pattern was confirmed by the data collected from the households' surveys.

### Discussion

In Southeast Asia a massive outbreak of rodents in the Ayeyarwaddy delta in 2009 and 2010 was associated with a calamitous weather event, cyclone Nargis, which occurred 15 months prior to the outbreak. Our findings support the association between the effects of cyclone Nargis and the subsequent rodent outbreaks. The extended planting season in the 2008 monsoon season would have led to an extension of the main breeding season by around 4 weeks. The main rodent pest species have a peak in breeding activity from the booting stage of rice through to its harvest (Sudarmaji et al., 2010). The first litter of the main breeding season does not usually breed during that crop. However, an extension of the breeding season by 3-4 weeks would enable them to breed and provide a platform for an exponential increase in the rodent population. Because the rodent population would be at a low density following the 3-5 m tidal surges associated with cyclone Nargis, it would take at least two seasons for the rodents to build up to high densities. We contend that climate change and extreme climatic events will increase the impacts of rodents on agricultural production in coming years. The delays between these events and population outbreaks of rodents may be greater than 1 year.

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## **Rat floods and water floods: the ecological and sociological dynamics of rodent management in Bangladesh**

Chakma, N.<sup>1,3</sup>, Belmain, S.R.<sup>2</sup>, Sarker, N.J.<sup>1</sup>, Sarker, S.U.<sup>1</sup>, Kamal, N.Q.<sup>3</sup>, Sarker, S.K.<sup>3</sup>

<sup>1</sup>Department of Zoology, Dhaka University, Dhaka-1000, Bangladesh, nikhil\_forestry@yahoo.com

<sup>2</sup>Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom

<sup>3</sup>Association for Integrated Development-Comilla, Raghupur, Comilla Sadar, Comilla-3500, Bangladesh

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### **Abstract**

Rodent pests in Bangladesh are ubiquitous and cause severe damage to agricultural production and human health. Pest problems are typically chronic in the lowland flood plains, with some minor seasonal variation in the types of problems occurring. In the upland areas, rodents are not normally a severe chronic problem because agriculture is less intense and highly seasonal. However, the uplands face serious acute problems with rodent outbreaks every 50 years. Until now, the dynamics of these outbreaks have been poorly documented and studied. Our research in Bangladesh has focused on studying this outbreak phenomena as well as mitigating the chronic problems of rodents by introducing agricultural communities to ecologically-based rodent management strategies.

### **Introduction**

Bangladesh suffers from both acute and chronic rodent pest problems. Acute outbreaks of rodents are confined to the uplands of the Chittagong Hill Tracts and related to the 50-year flowering and seed masting cycle of the bamboo species, *Melocanna baccifera*. These acute outbreaks lead to regional famine over 2-5 consecutive years, with longer term problems affecting an entire generation of children caused by household indebtedness incurred during the famine years (Singleton et al., 2010). The most recent bamboo flowering cycle spread southwards from India into Bangladesh in 2007 and has continued each year since up to the present. This has provided the opportunity to collect basic scientific evidence that links gregarious bamboo flowering to rodent population outbreaks (Belmain et al., 2010).

Chronic rodent pest problems are found throughout the Brahmaputra-Ganges flood plain where rice production is nearly continuous with 2-3 crops per year and only disrupted during the monsoon floods when most agricultural land is put temporarily under water. Seasonal flood water limits rodent harborage and food resources but also drives rodents into villages where rodent damage to food storage and disease transmission to livestock and people are exacerbated. Despite these acute and chronic problems, farmers and householders in Bangladesh do relatively little to try to control their rodent pest problems. We argue that commonly held beliefs such as 'rodents are too clever to be controlled' can be overcome through scientifically-driven programs that provide farming communities with appropriate knowledge and tools. Research in Bangladesh has taken place in both upland and lowland agro-ecosystems with a view of helping communities and extension services to implement ecologically-based rodent management (EBRM) strategies.

### **Materials and methods**

In the uplands, we started removal trapping of rodents in March 2009 in several habitats (bamboo forest, rice fields, villages and houses) in Ruma Upazilla, Bandarban District on a monthly basis using a combination of kill and live traps (Sherman single capture, multicapture cage, single capture cage). Breeding and taxonomic data were collected from all captured animals. At the same time and in the same forests where rodents were collected, data on bamboo phenology were collected regarding seed fall timing, abundance, germination, and seed damage by rodents using 30 plots (1 m<sup>2</sup>), 10 m apart arranged in three separate transects. In the second season of data collection, bamboo phenology data were collected in forests that were cut, burned or left undisturbed to understand how such treatments potentially affect bamboo seed production rates.

To prevent rodent migration from bamboo forests to rice fields, experimental trials were established using trap-barrier system of fences (TBS), comparing indigenous bamboo constructed fences embedded with kill traps against the typical TBS design construction from plastic sheeting embedded with multicapture traps. Damage to growing rice was measured in unfenced rice fields and compared to the two types of fenced field by counting cut tillers in 1m<sup>2</sup> plots, 6 plots per field, 3 replicates per fence treatment. In the lowlands, case-control studies were implemented to evaluate a program of EBRM which focused on community-based intensive trapping inside households. Household trapping was coupled with environmental management to reduce rodent harborage in the village area, particularly by rodent-proofing haystacks used to feed livestock and to reduce rodent access to stored food by rodent-proofing granaries situated within houses. The efforts of communities implementing EBRM were scientifically monitored and compared to non-intervention communities using footprint tracking tiles to understand the effect of this intervention on rodent numbers, and using controlled trials to monitor the levels of post-harvest damage to household rice stores. Community members collected monitoring data in personal diaries on a weekly basis across a range of rodent management and rodent damage factors.

## Results

In the uplands, the dominant species of rodents in all habitats belonged to the *Rattus* species and *Mus* species complexes. Rodent captures were generally very high in village households and very low in outdoor environments, including bamboo forests. Breeding females in forest habitats were apparent throughout the bamboo masting period, appearing soon after bamboo seed initiation. Surveys of bamboo phenology showed that rodents partially damaged bamboo seeds from very early on when seeds were small and still developing, with damage continuing through seed development, after the seed has fallen to the ground and during early stages of germination.

The amount of bamboo seed produced was estimated at 30-80 tonnes per hectare of forest; lower yields were found from forests that had been cut or burned around the time of flower initiation. TBS fences were able to significantly reduce rodent damage to rice fields; however, the cost-benefits were unfavorable for widespread adoption. In the lowland plains of Bangladesh, research on the ecological dynamics of rodents showed that rodent impacts on rice and vegetable production, food storage loss and contamination, and physical damage to houses and personal possessions were severe, chronic and ubiquitous.

Rodent damage was exacerbated by continuous and asynchronous crop production, diverse and fragmented habitats, and poor environmental sanitation and food storage practices. Scientific and community monitoring activities showed that the case-control trials to implement EBRM in lowland agricultural communities reduced rodent populations and the damage caused to people's livelihoods by up to 80% compared to traditional rodent management practices (typically *ad hoc* poison use). Trapping at the village-wide scale, particularly during the monsoon season, reduced the potential for rodents to recolonize rice fields after flood waters subsided. Efforts to scale up the extension of EBRM across Bangladesh are ongoing, and data will be presented that show sustainable EBRM implementation is possible at a sizeable scale without large government or donor support.

## Discussion

Previously, the Western scientific community was skeptical about the scale and impact of rodent outbreaks in the bamboo forests of the Himalayan foothills across India, Bangladesh and Myanmar. This skepticism was fuelled by historical accounts and descriptions of rodent outbreaks that fell into the realm of the improbable if not the mythological. Our research in the Chittagong Hill Tracts has provided clear evidence that rodents do eat bamboo seeds and increase their breeding in response to this superabundant food resource.

We can also confirm that there is variability in the flowering, with some areas flowering all in one season, whilst others take 2-3 years, with the entire masting event possibly taking >5 years to complete in a given locality due to the patchy nature of bamboo stands. Our ongoing trapping in non-masting bamboo forests will help clarify and confirm this evidence of linkages between gregarious bamboo flowering, rodent outbreaks and regional famine.

Chronic rodent damage in the lowlands of Bangladesh is severe and routinely accepted by local communities due to poor awareness of appropriate methods and tools. EBRM can make significant reductions in rodent numbers and damage, particularly when communities are encouraged to work together to intensively trap rodents during the monsoon season. Communities that receive appropriate knowledge about EBRM and experience it first-hand do change their behavior and practice. Adoption of community-based trapping remains stable in the majority of communities two years after their initial experience.

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## Are rodent population eruptions in southeast Asia associated with quantity or quality of food?

Htwe, N.M.<sup>1,4</sup>, Singleton, G.R.<sup>1</sup>, Sluydts, V.<sup>2</sup>, Hinds, L.A.<sup>3</sup>

<sup>1</sup>International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines, nhtwe@cgiar.org

<sup>2</sup>Evolutionary Ecology Group, University of Antwerp, Groenenborgerlaan 171, B-2020, Antwerpen, Belgium

<sup>3</sup>CSIRO Sustainable Ecosystems, GPO Box 284 Canberra, ACT, 2601, Australia

<sup>4</sup>Myanma Agriculture Service, Plant Protection Division, Bayintnaung Road Insein PO 11011, Yangon, Myanmar

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### Abstract

Rice field rat's population occasionally undergoes widespread eruption in Indonesia, Vietnam, the Philippines, and Myanmar following extreme weather event. Asynchronous or aseasonal planting of crops in response to unusual weather events can extend the period that high quality food is available to rodents. Consequently, rodents may extend their breeding season and a population eruption is more likely to occur. However, it is unclear the association between the quality and quantity of food and the reproductive success of female rice field rats. An improved understanding of the effects of food availability and quality on rodent reproduction could enable better forecasts of rodent outbreaks in response to unusual weather events which could lead to asynchronous or aseasonal planting of crops. We studied how the breeding performance of the rice field rat, *Rattus argentiventer*, responded to food supply at different stages of the rice crop in the Philippines. Our results suggest that rice plants at the booting to ripening stages provided high quality food for rice field rats and it drove higher conception rate of female rats at these stages of the rice crop. We contend that the extension of the growing season by 3 to 4 weeks provides high quality food for rodents for an extended period, which in turn provides sufficient conditions for a population eruption. Therefore we recommend that synchronous planting is the effective proactive action for rodent management.

Keywords: asynchronous planting, breeding, *Rattus argentiventer*, rice field rats, rodent outbreaks

### Introduction

Food quantity and quality influence the reproductive ability of female rodents (Bomford, 1987; Leirs et al., 1994). The main drivers leading to rodent outbreaks in Southeast Asia seem to be increasing cropping intensities, or changes in agricultural cropping systems related to unusual weather events (Singleton et al., 2010). This can provide high quality food for rodents for longer periods of time per year and lead to an extended breeding season of rodents. However, knowledge about the effect of food availability and quality on reproductive success of rice field rats is still lacking. A better understanding of the factors that influence the breeding dynamics of rice field rats could lead to better forecasts of rodent population eruptions. In turn, this could provide smallholder farmers in Southeast Asia with the opportunity of taking proactive actions for rodent management before high populations of rodents occur. The objective of this study was to determine the importance of food quantity and quality for the reproductive success of *R. argentiventer*.

### Materials and methods

The study was conducted in irrigated rice cropping systems in Mindoro, Philippines. Kill-trapping was conducted at different crop stages - tillering, booting (early stage of flowering), ripening (two weeks before harvest), and stubble (two weeks after harvest) - to collect adult female *R. argentiventer*. The estimated time of conception of breeding females, and litter size were assessed. Stomach contents of females were collected to identify their diet at each crop stage. The quality of food was estimated by assays of protein content of rice plants and grain sampled at the main crop stages. Grain which was spilled in the rice field during harvest was sampled two weeks after harvest to estimate the availability of food during the stubble stage.

### Results

Conception rates were highest at the early booting to ripening stages (51.2 and 36.4% respectively) and lowest at tillering and stubble stages (2.7 and 15.2% respectively). The litter size was highest at ripening

and lowest at the tillering stage ( $12.33 \pm 0.89$  and  $9.22 \pm 0.86$  respectively). The majority of stomach contents at the various crop stages were as follows: (i) tillering stage: rice plant parts, the golden apple snail, and dicotyledonous leaves (ii) booting stage: leaf sheaths and young panicles of rice plant, (iii) ripening stage: rice plant parts and rice grains (iv) stubble stage: rice grains. The highest protein content of rice plants was at the early flowering stage followed by the milky stage. The amount of spilled grain in the rice field was  $400 \pm 20.3$  kg/ ha.

## Discussion

The highest conception rates were occurred at early flowering stage (booting stage) and ripening stage. The litter size was also highest at these times. The main stomach content of *R. argentiventer* at the early flowering stage was leaf sheaths and young panicles of rice plants. At the ripening stage, the main stomach contents were rice plant parts and rice grains. The highest protein content was detected at those two stages as was the highest rate of conceptions. Therefore, we conclude that the quality of the rice plant at the early flowering to ripening stage strongly influences the breeding success of *R. argentiventer*. There was plenty of spilled grain ( $400 \pm 20.3$  kg/ ha) inside the rice field at stubble stage. However, breeding success at this stage and at the tillering stage, based on estimated time of conception, was low. Therefore the quality of food appears to be a more important factor driving reproductive success of rice field rats than quantity of food. Our study supports the contention (Lam, 1983; Leung et al., 1999) that the quality of food is the main driver for rodent population eruptions in Southeast Asia. Further, an extension of the period of planting by just 3 to 4 weeks would provide high quality food for rodents for a longer time. Maintaining synchronous planting is the simplest and most effective strategy to prevent rodent population eruptions.

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## Population dynamics and breeding patterns of *Mastomys natalensis* Smith 1932 in irrigated rice in eastern Tanzania

Mulungu, L.S.<sup>1</sup>, Ngowo, V.<sup>2</sup>, Mdangi, M.<sup>3</sup>, Katakweba, A.S.<sup>1</sup>, Tesha, P.<sup>2</sup>, Mrosso, F.P.<sup>4</sup>, Mchomvu, M.<sup>2</sup>

<sup>1</sup>Pest Management Centre, Sokoine University of Agriculture, P.O. Box 3110, Morogoro, Tanzania, lothmulungu@yahoo.co.uk

<sup>2</sup>Rodent Control Centre, Ministry of Agriculture, Food Security and Cooperatives, Morogoro, Tanzania

<sup>3</sup>MATI-Ilonga, Box 66, Kilosa-Morogoro-Tanzania,

<sup>4</sup>Ilonga Agricultural Research Institute P.O. Box 33 Kilosa, Tanzania

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### Abstract

The population dynamics and breeding patterns of the Multimammate rat, *Mastomys natalensis*, were investigated in irrigated rice cropping systems in eastern Tanzania in 2010/ 2011. Population abundance varied with habitat and crop growth stages. In both rice fields and fallow land, the highest population peak was observed during the dry season from July to October. The results show that *M. natalensis* is sexually active throughout rice cropping season in the study area, although it reaches the highest level at maturity stage of crop growth. Breeding occurred in the dry and wet seasons, and suggests that it was highly influenced by the presence of a rice crop in both seasons. More juvenile individuals were recorded at transplanting stage in each season and few in the subsequent crop growth stages in all habitats. Breeding, therefore, was not seasonal and seemed not to be associated with rainfall patterns. The sex ratio of *M. natalensis* was not skewed to either males or females.

Keywords: breeding, fallow land, population, rice fields

### Introduction

In Tanzania, crop production of cereals is increasing. In these cropping systems, rodent pest cause serious losses and widespread food shortage. In 2004, there was an outbreak of *Mastomys natalensis* populations in lowland irrigated rice in Mvomero district, Morogoro region. Also, over the past 5 years, extension agents in rice-growing districts have reported significant rodent problems (pers. comm. Victoria Ngowo, Rodent Control Centre, Morogoro). There is little known about the rodent species, and their population dynamics in irrigated areas where rice is a major crop. The occurrence of rodent outbreaks in Tanzania in mosaic habitats is influenced by the rainfall pattern (Leirs, 1995; Linn, 1991). Rodents breed during the long rains and usually starts one month after the usual peak rainfall, lasting until dry season (Leirs, 1995). Neonates grow slowly and normally do not mature before the next rainy period. Unless abundant rains appear before March and April the following year, they will be at least six months old before they begin to breed (Leirs, 1995). However, if the short rains are abundant, sub-adults mature and may breed as early as January. Neonates in such early breeding seasons grow fast and mature in their third month, starting to breed during the main breeding period. This additional generation allows the development of high densities later in the year (Leirs et al., 1996). This situation, however, could be different in irrigated rice agro-ecosystems.

### Material and Methods

Four (70 m x 70 m each) permanent trapping grids in rice field and fallow mosaics were laid out in Hembeti village, Mvomero District at 06°16'S, 37° 31'E. Trapping was conducted from June 2010 to May 2011. Sherman live traps (H.B. Sherman Traps Inc., Tallahassee, FL, USA) were used and were set for three consecutive nights at intervals of four weeks. A single trap was placed at each trapping station making it 49 traps per grid. Traps were baited with peanut butter mixed with maize bran/maize flour and placed afternoon and were inspected in the morning. Captured animals were taken to the field laboratory for processing. Animals were identified to genus or species level, toe clipped for new animals, weighed and reproductive status recorded. They were later released at the station of capture. The data were recorded and entered into a CMR data input program for analysis. Population size was estimated for each 3-day trapping session using the M(h) estimator of the program CAPTURE for a closed population, which allows for individual heterogeneity in trapping probability (White et al., 1982).

## Results

Species composition and Discussion: A total of 1520 individual animals belonging to 5 species of rodents and one shrew (*Crocidura sp.*) were captured in a total of 5292 trap nights (28.7% trap success). The rodent species comprised of *M. natalensis*, *Rattus rattus*, *Dasmys sp.*, *Acomys spinnosissimus*, and *Gramomys sp.* *M. natalensis* comprised more than 92% of the total capture.

Rodent population abundance: Temporal variations in population density changes were observed between habitat and crop growth stages. In both rice fields and fallow land, the highest population peak was observed during the dry season from July to October, when >200 animals per ha were captured.

Breeding patterns: Breeding occurred in both the dry and wet seasons, and suggests that it was highly influenced by the presence of a rice crop in both seasons. More juvenile individuals were recorded at transplanting stage in each season and few in the subsequent crop growth stages in all habitats. Breeding, therefore, was not seasonal and seemed not to be associated with rainfall patterns as suggested by Leirs (1995) and Linn (1991) in maize-based cropping systems. The sex ratio of *M. natalensis* was not skewed to either males or females.

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## Man-eating and cattle-lifting by tigers and conservation implications in India

Chauhan, N.P.S.

Wildlife Institute of India, P.O. Box 18, Chandrabani, Dehradun 248001, India, npsc@wii.gov.in

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### Abstract

We investigated the incidences of human casualties and cattle-lifting by tigers and circumstances of attacks and suggested mitigation strategies. In India, tigers mostly survive in small numbers in protected areas which are isolated due to habitat fragmentation and disturbances. Man-killing behaviour and predation on cattle are the main issues of confrontation, and as a result, the conservation efforts are adversely affected. In the states of Uttar Pradesh, Uttarakhand, Rajasthan, Madhya Pradesh, Orissa and West Bengal, tigers have caused 161, 51, 7, 133, 25 and 445 human casualties respectively during 1990-2009. In Madhya Pradesh, 133 human casualties occurred in and around 6 national parks, 14 sanctuaries and 20 forest divisions. Male casualties were more (n=656) than female casualties (n=166). Out of 822 human casualties, most human casualties (n=376) occurred in the age group of 25-30 years. Most tiger casualties of humans (51%) occurred between 16:00-20:00h. Tigers in Uttar Pradesh, Uttarakhand, Bihar, Orissa and West Bengal killed 511, 1938, 1899 and 733 cattle, respectively, during this period. In Madhya Pradesh, tiger and leopard killed 18736 cattle and a majority of cattle kills occurred within protected areas and reserve forests (92.6%) than in cattle sheds and outside the forests (7.4%). This has led to a strong resentment among people and they showed increased apathy and antagonism towards tiger conservation. Recommendations for reducing the human-tiger conflict include are suggested.

Keywords: cattle killings, conflict, human deaths, injuries, man-eater, mitigation, Tiger, tiger reserves

### Introduction

Tiger, being a large and magnificent carnivore, have attracted considerable attention the world over. Tigers mostly survive in small numbers in protected areas which are isolated due to habitat fragmentation and disturbances (Panwar, 1987; Johnsingh et al., 1991). Presently, the tiger population is threatened due to increasing biotic pressure, leading to habitat loss, degradation and fragmentation, which make such populations very unstable (Soule, 1986). Poaching activity is adversely affecting tiger numbers in reserves. Since inception of the Indian Wildlife (Protection) Act 1972 and the 'Project Tiger', a considerable effort has been made towards the conservation of tigers in India (Panwar, 1987). Over 60% of the world's tiger population survives in the Indian sub-continent and the future of this endangered cat lies in India. But large carnivores like tigers, lions and leopards which attack humans and kill cattle have always been the concern of the wildlife manager. The problems of man-killing and livestock depredation by tigers have been studied by Khaire et al. (1994), Koppikar and Sabnis (1989), Sawarkar (1986), and Chauhan and Rajpurohit (1998). An attack on a human has been considered as an aberrant form of behavior of tigers. In recent years, the problem of human-tiger conflict is on the increase in and around the sanctuaries. Little scientific information is available on the genesis of human-tiger conflict and mitigation strategies from different tiger areas in India. For conservation of tigers on a long term basis, mitigation of human-tiger conflict is necessary. This paper deals with assessment of the problems of human-tiger conflicts in and around the tiger reserves and suggests mitigation strategies.

### Methods

Well designed questionnaire formats were used for collecting information on human-tiger conflicts from field areas and from victims or their families. Based on the information gathered, affected areas were visited to collect the information on the area profile, level of the conflict, human casualties, livestock killings, place, time and seasonality of incidences and causes of conflict. Selected villages located on the edge of the forest were surveyed to determine the areas frequented by tigers. Information on compensation paid for the losses was also recorded.

## Results and discussion

**Human casualties:** Human deaths and cattle lifting by tigers mostly occur outside the protected area boundaries because the agricultural fields offer continuity of habitat out of the forest areas and good conditions for resting, hiding and ambush cover for tigers. A total of 822 cases of human casualties by tiger were reported from six States. In Uttar Pradesh, Uttarakhand, Rajasthan, Madhya Pradesh, Orissa and West Bengal, tigers have caused 161, 51, 7, 133, 25 and 445 human casualties, respectively, during 1990-2009. In Dudhwa and Corbett tiger reserves, 23 and 56 human casualties respectively occurred. In Madhya Pradesh, 133 human casualties occurred in and around 6 national parks, 14 sanctuaries and 20 forest divisions. Male casualties were higher (n=656) than female casualties (n=166). Among males, there were 435 human killings and 221 injuries. Among females, there were 86 killings and 80 injuries. Human casualties showed a decreasing trend during 1990-2001. Males had an increased likelihood of casualties because they moved extensively inside forests for the collection of non timber forest produce, and in agricultural fields for farming activities. During 1990-2001, most of the human casualties by tiger occurred in the periphery of the sanctuary and few occurred inside. We observed marked monthly variation in these years. The monthly and diurnal patterns of occurrence of human casualties can be correlated with the activities of people and movement of tigers in and around the reserves. Of 822 human casualties by tiger, the age of 695 cases was recorded. Most human casualties (n=376) occurred in the age group of 25-30 years. Tiger were responsible for most human casualties (51%) between 16:00-20:00h, followed by 23% of cases between 12:00-16:00 h.

**Cattle killings:** From 1990 to 2008, tigers in Uttar Pradesh, Uttarakhand, Bihar, Orissa and West Bengal killed 511, 1938, 1899, 733 and 2111 cattle, respectively. In Madhya Pradesh, tigers and leopards killed 18736 cattle during this period. In Palamau tiger reserve, on average 268 cattle were lifting every year. A majority of cattle kills occurred within protected areas and reserve forests (92.6%) than in cattle sheds and outside forests (7.4%). A high density of humans and livestock gives rise to a high probability of encounters. In the Sundarbans, high human casualties were related to people collecting non-timber forest produce. A man-eating tiger is actually very rare (Siddiqi and Choudhury, 1986). Chakarbarti (1984) believed that the ferocity of the tigers in the Sundarbans was related to the salinity of the surrounding water. Cattle in these areas serve as a supplement to natural prey. All the same, it contributes significantly to the human-tiger conflict. Recommendations for reducing the human-tiger conflict include: 1. People should be alert and vigilant, 2. Ameliorative measures for protection and habitat improvement, 3. Restoration of prey base, 4. Restriction on livestock grazing in tiger reserves, 5. Cattle should be attended by graziers, 6. Compensation for losses needs to be settled promptly, 7. Man-eaters should be captured and kept in zoos or destroyed, 8. Relocation of villages outside tiger reserves, 9. Involvement of people in planning and implementation of mitigation strategies, and 10. Public education and awareness.

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## Human-leopard conflict in Mandi district, Himachal Pradesh, India

Kumar, D., Chauhan, N.P.S.

Wildlife Institute of India, P.O. Box 18, Chandrabani, Dehradun 248001, India, dev@wii.gov.in

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### Abstract

We studied the nature and extent of human-leopard conflict and circumstances of attacks in Mandi district during 1987 to 2007. There were 162 human casualties in Mandi district by leopards, out of which 37 casualties were in Mandi forest division (FD), 71 in Sundernagar FD, 34 in Jogindernagar FD, 10 in each Nachan FD and Karsog FD. Maximum cases occurred in the vicinity of villages (36%), followed by in crop field (19%) and grassland (14%). During 1992, 1993, 1998, 2001 and 2002, number of human casualties were 11, 12, 13, 12 and 22 respectively. Maximum human casualties occurred in November (n=21), followed by 18 casualties in January, 16 in October and 15 in July. Attacks on males were higher (n=99) than on females (n=53). Out of 162 cases, 13 persons were killed and 149 were injured. A maximum of 91 (56%) casualties occurred between 16:01-22:00 hours and 37 (23%), 22 (14%) and 12 (7%) cases occurred each during the morning (04:01-10:00), daytime (10:01-16:01) and night (22:01-04:00). Compensation for human casualties was paid for 68% cases within 3 month of occurrence of incidence and 27% cases within 3-6 months. Livestock killings were 8,905 in 4,967 attacks. Recommendations have been made to reduce the human casualties and livestock depredation by leopards in the Mandi district.

Keywords: human killing, injuries, Leopard, livestock killings, recommendation

### Introduction

The leopard (*Panthera pardus*) is the most widely distributed of all the world's large cats (Bailey, 1993). Leopards are adapted to live well in savannah, rain forest, mountain elevation, dense vegetation, low scrub and thickets and even quite close to human habitation (Bailey, 1993). Leopards are widely distributed in India. But due to expansion of human influence and ever-increasing pressure on natural resources has greatly intensified the issue of human-leopard conflict in a wide variety of situations. Carnivore-human conflict is a worldwide problem for the wildlife management (Chauhan and Rajpurohit, 1996; Karanth and Madhusudan, 2002; Chauhan et al., 2002; Treves and Karanth, 2003). Carnivores are involved in a wide range of conflicts including predation on human and livestock and carnivore mortality by people (Chakrabarti, 1992; Chellum and Johnsingh, 1993). The problem of human-leopard conflict has recently increased in the Indian Himalayan region due to changes in land use patterns (Chauhan et al., 2002; Singh et al., 2008).

### Methods

We examined casualties and predation activity throughout Mandi district by the common leopard (*Panthera pardus*) on humans and a variety of livestock types using data gathered over the last twenty years (1988-2007) from a compensation scheme for human casualties and livestock losses. Field data were collected using a combination of qualitative methods (unstructured interviews, participatory observation and focus group discussions) and quantitative methods (structured interviews). Information on human casualties, age and sex of victims, activity of victims, place of casualty, seasonal variation, mode of attack and nature of injuries etc. was collected in pre-designed questionnaire formats. This study aimed to investigate the nature and extent of injuries and deaths of humans and livestock kills by leopard and leopard deaths by human beings, spatial and temporal patterns of conflict between humans and the leopard, financial losses caused by leopard and local perceptions and tolerance towards the leopard and its conservation.

### Result and discussion

Human casualties: A total of 162 human casualties by leopards were reported in all five forest divisions of Mandi district during 1988-2007. The highest number of attacks by leopards was 71 with 2 killing and 69 injuries in the Sundernagar forest division followed by Mandi forest division with 37 attacks comprising 2 killing and 35 injuries, Jogindernagar forest division with 34 attacks comprising 8 killings (highest recorded) and 26 injuries. Karsog and Nachan forest divisions had 10 attacks each - all 10 were

injuries in Karsog and there was 1 killing and 9 injuries in Nachan. The total number of casualties showed an increasing trend over these years. The number of male casualties (n=99) was higher than the female casualties (n=63). Out of the total of 162 cases, 13 people were killed and 149 people were injured. Among 13 killings, 6 were male and 7 were female. Whereas, of the 149 injuries, 93 were male and 56 were female. The highest number of human casualties (n=22), including 1 killed and 21 injured occurred in 2002, followed by 13 human casualties which (all injured) in 1998. Leopard attacks on human vary across the season as 38.9%, 30.2% and 30.9% cases were reported during winter, monsoon and summer season, respectively. A maximum number of 28 (17.2%) casualties occurred in the age group of 25-30 years, followed by 23 (14.2%) casualties in the age group of 40-48 years. A maximum of 91 (56%) casualties occurred during the evening (16:01-22:00 hours). There were 37 (23%), 22 (14%) and 12 (7%) cases occurred each during morning (04:01-10:00), daytime (10:01-16:01) and night (22:01-04:00) hours. A total of 36% of human casualties by leopard were reported in villages followed by crop field (19%), grassland (14%) and other places like nallah/ravine, school playground etc. (12%). 10% human casualties were reported from forest and a lowest of 9% from cowsheds.

**Livestock killings:** A total 4,967 attacks and 8,905 killings of livestock by leopards were reported in five different forest divisions of Mandi district during 1987-2007. The maximum attacks were 2,222 with 3710 livestock killed in Sundernagar forest division followed by Jogindernagar forest division with 17,02 attacks and 2,545 killed. The lowest number of attacks was 224 in which 683 livestock got killed in Karsog forest division. Livestock killing showed much variation over the years; it showed increasing pattern from 1987 onwards and reached a maximum in 1991 and then declined to a minimum in 2007. Goats, sheep and cows suffered maximum killings, i.e. 3,043, 2,598 and 1,677, respectively, followed by ox and buffalo with 855 and 328, while 182 calves, 165 horse/mule with and 57 donkey/mares also were killed. Out of the total 8,905 numbers, most number of killings i.e. 8,723 were adults while only 182 were young. Out of the total 4,967 cases, 83% occurred in the cowshed followed by forest, village and grassland, and crop field and other (nallah/ravine etc.) with 5%, 4% each and 2% each, respectively. A maximum of 74% of casualties occurred in night time (22:01-04:00 hours) followed by 15% in the afternoon/evening (16:01-22:00 hours). Some 8% and 3% of cases occurred during the daytime (10:01-16:01) and morning (04:01-10:00) hours. We propose the following management recommendations 1. Community awareness programs, 2. Introduction of prey species in forest, 3. Establishment of insurance scheme in the targeted villages, 4. Regulated livestock grazing, 5. To avoid leopard attacks, clearing of bushes at village fringes, 6. Restricting activities especially in morning, evening and night time, 7. To scare away leopard, lighting or fire or light in nights. 8. Habitat management, 9. Artificial and natural barriers, 10. Confirmed man-eaters should be captured and kept in zoos or killed.

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## Rodents as carriers of tick-borne zoonotic diseases and their ecological impact

Paulauskas, A.<sup>1</sup>, Radzijeuskaja, J.<sup>1</sup>, Rosef, O.<sup>1,2</sup>

<sup>1</sup>Department of Biology, Vytautas Magnus University, Vileikos str. 8, Kaunas, LT-44404, Lithuania, a.paulauskas@gmf.vdu.lt

<sup>2</sup>ATP-Innovation AS, 3800 Bø i Telemark, Norway

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### Abstract

Rodents as pests are known to be reservoirs of tick-borne zoonotic infection of viral, bacterial and protozoan origin and are important hosts of the immature stages of *Ixodes* ticks. A total 493 rodents were live-trapped in different habitat in two biogeographical regions Lithuania and Norway. The ecological impact of different species of rodents in particular habitats was evaluated according to their infestation with ticks and presence of *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum* infection. Results of the present study demonstrate that although the infestation rate varied between rodent species it was dependent on sampling location and not correlated with infection prevalence in rodents. We observed that mice in Lithuania were more frequently infested with ticks than voles, but, the higher prevalence of *B. burgdorferi* s.l. infection was detected in voles, than in mice. The overall prevalence of infection in Lithuania was higher than in Norway. In Norway, prevalence of infection in *A. flavicollis* and *M. glareolus* was not significantly different. The present study confirmed evidence that the rodent impact on maintenance of Lyme borreliosis depends on biogeographical region and habitat type, and that the zoonotic reservoirs of *B. afzelii* are *M. arvalis*, *M. glareolus*, and *A. flavicollis* in Lithuania, and *A. flavicollis*, *A. sylvaticus* and *M. glareolus* in Norway. In our present study *A. phagocytophilum* was not detected in any of the rodents.

Keywords: *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., PCR, rodents, tick-borne zoonosis

### Introduction

Rodents as pests are known to be reservoirs of tick-borne zoonotic infection of viral, bacterial and protozoan origin and are important hosts of the immature stages of *Ixodes* ticks. Different rodent species, however, vary in their susceptibility to infection, infestation with ticks, propensity to infect feeding vectors and consequently vary in their importance for pathogen transmission and impact on human and animal health. Recent studies in Europe have demonstrated a role of rodents, especially *Apodemus* mice and *Myodes* voles, in the epidemiology of tick-borne bacterial zoonoses, such Lyme borreliosis and anaplasmosis (Humair et al., 1999; Hanincová et al., 2003; Sinski et al., 2006; Bown et al., 2006). However, the knowledge concerning abundant zoonotic reservoirs is still limited and scarce in Lithuania and in other European countries. The aim of present study was to assess the importance of different species of rodents as carriers for both the ticks and bacteria and their ecological impact.

### Materials and methods

Rodents were live-trapped in deciduous, pine and mixed forest, and ecotonal areas in two different biogeographical regions of Lithuania and Norway in 2005, 2006, 2009 and 2010. Attached engorged and all moving ticks were carefully removed from rodents, counted and identified. DNA from ticks and rodent ear tissue samples was extracted. The presence of bacteria *B. burgdorferi* s.l. and *A. phagocytophilum* were identified by polymerase chain reaction (PCR). *B. burgdorferi* s.l. genospecies was identified using Multiplex PCR, nested PCR and sequencing. Targets for amplification of *Borrelia* DNA were the *fla* gene, the *rrs-rrlA* intergenic spacer (IGS) and the *ospA* gene. For detection of *A. phagocitophilum*, fragments of *msh2* and 16 rRNA genes in *A. phagocytophilum* genome were amplified. The prevalence of infestation, abundance of infestation with ticks and the prevalence of infection in different rodent species were estimated.

### Results

A total 493 (248 in Lithuania and 245 in Norway) small rodents belonging to ten species (*Apodemus flavicollis*, *A. agrarius*, *A. sylvaticus*, *Microtus arvalis*, *M. agrestis*, *Myodes glareolus*, *Rattus norvegicus*, *Mus musculus* and *M. oeconomus*) were collected. In Lithuania, the overall prevalence of

infestation with *I. ricinus* was 52% for *A. flavicollis*, 40% for *A. agrarius*, 31% for *M. arvalis*, and 28% for *M. glareolus*. Abundance of infestation was higher on *A. flavicollis* (2.4), a little lower on *M. glareolus* (2.3), followed by *A. agrarius* (1.7) and *M. arvalis* (0.9). In Norway, the overall prevalence of infestation with *I. ricinus* was 79.2% for *A. flavicollis*, 58.5% for *A. sylvaticus* and 94% for *M. glareolus*. The ranges of numbers of immature *I. ricinus* tick infested individual hosts in Norwegian locations were 1–108 for *A. flavicollis*, 1–13 for *A. sylvaticus* and 0–73 for *M. glareolus*. Rodent infestation between sampling sites ranged from 0% to 92% and from 33.3% to 98% in Lithuania and Norway, respectively. The overall prevalence of *B. burgdorferi* s.l. in rodents from Lithuania was 23.4% (58 out of 248) and 8.3% (24 out of 245) in Norway. The prevalence of *B. burgdorferi* s.l. in Lithuania varied considerably between species: 53% of *M. arvalis*, 22% of *M. agrestis*, 21% of *M. glareolus*, 11% of *A. flavicollis*, 7% of *A. agrarius*, and in addition the single captured *M. musculus*, were infected. In Norway, the prevalence of infection of *B. burgdorferi* s.l. was 9.2% in *A. flavicollis*, 4.3% in *A. sylvaticus*, and 10.9% in *M. glareolus*. Genotyping of *B. burgdorferi* s.l. revealed that *B. afzelii* was the single genospecies detected in rodents from Norway. In Lithuania almost all rodents harbored only *B. afzelii*, but *B. garinii* was detected in *M. musculus*, *M. glareolus* and *M. arvalis*. *A. phagocytophilum* was not detected in any of the tested rodents.

## Discussion

In Lithuania, *A. flavicollis* and *A. agrarius* were more frequently infested with immature *I. ricinus* ticks than *M. arvalis* and *M. glareolus*. However, a higher prevalence of *B. burgdorferi* s.l. infection was detected in voles, than in mice. In Norway, rodent infestation with ticks was depended on sampling sites than on rodent species. The prevalence of *B. burgdorferi* s.l. infection varied between species and sampling sites in both countries. The overall prevalence of infection in Lithuania was higher than in Norway. In Norway, infected rodents were captured only in locations situated in the southern part. The prevalence of infection in *A. flavicollis* and *M. glareolus* not significantly different, but in *A. sylvaticus* was at least twice lower. Some other studies conducted in Europe, in contrast with our, reported higher *B. burgdorferi* s.l. prevalence in mice, than in voles, or suggested only limited participation of these species of rodents in the circulation of the pathogen (Gray et al., 1999; Hanincová et al., 2003; Siński et al., 2006). These findings suggest that the impact of rodents on maintenance of Lyme borreliosis depends on habitat type and biogeographical region. Although our present study did not recognize rodents as carriers of anaplasmosis, the study conducted in UK showed that rodents can maintain *A. phagocytophilum* in woodland habitats (Bown et al., 2006). *A. flavicollis* are natural hosts for tick-borne encephalitis. Although the TBE virus was detected in *M. glareolus* and *M. agrestis*, the experimental data show that bank voles develop lower viremias and transmit TBE viruses to a lesser extent than do rodents of the genus *Apodemus* (Dobler, 2010; Tonteri et al., 2011).

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## Evaluation of bait uptake by ricefield rats using Rhodamine B as a bait marker under enclosure conditions

Tung, T.T.<sup>1,2</sup>, Henry, S.<sup>1</sup>, Cowan, D.<sup>3</sup>, Sudarmaji<sup>4</sup>, Hinds, L.A.<sup>1</sup>

<sup>1</sup>CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, Act, 2601, Australia, thanhtung.tran@csiro.au

<sup>2</sup>Research School of Biology, the Australian National University

<sup>3</sup>Wildlife Management Programme, the Food and Environment Research Agency, Sand Hutton, York YO41 1LZ UK

<sup>4</sup>Indonesian Center for Rice Research, Jl Raya 9, Sukamandi, Subang, 41256, Indonesia

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### Abstract

Oral delivery of fertility control agents requires information on rates of bait consumption by the target species. In this study, the consumption of cereal bait by wild ricefield rats (*Rattus argentiventer*) was assessed under enclosure conditions using Rhodamine B (RB) as a bait marker in two baiting rounds. Total bait consumption increased during the first 4 to 5 days of the initial feeding period. After the first round of RB application, high proportions (84.6-100%) of animals were consuming baits in all enclosures. The average daily consumption per individual was around 10.5g/100g body weight. Similarly, following the second round of RB application (14 days after the 1<sup>st</sup> round) high proportions (93.8-100%) of animals were consuming the bait, and the average daily consumption per individual of 9.8g/100g body weight. The acceptance of bait with respect to sex was not significantly different ( $p > 0.05$ ) between male and female rats.

Keywords: bait-marker, bait uptake, enclosure, *Rattus argentiventer*, Rhodamine B

### Introduction

Ricefield rats (*Rattus argentiventer*), one of the most important pests in rice production areas in SE Asia, are considered a potential target for the application of fertility control (Jacob et al., 2008). The efficacy of an orally delivered anti-fertility agent in the field depends largely on bait acceptance by the target species. Trials to assess bait consumption under field conditions can help to define the parameters for effective delivery of anti-fertility agents in baits. This study was conducted to assess the bait uptake of wild ricefield rats using Rhodamine B as a bait marker under enclosure conditions.

### Materials and methods

This study was conducted at the Indonesian Centre for Rice Research at Sukamandi, West Java, using 3 enclosures (25 m x 50 m), which were under fallow conditions. Water was freely available, the soil was moist and weeds were allowed to grow during the course of the experiment. Ricefield rats were caught from the fields, and weighed, sexed and tagged before release into the enclosures (24 females, 16 males per enclosure). The bait used in this study had been defined from a series of laboratory trials and contained 25% vegetable oils, 5% sugar, 10% broken rice, 30% wheat flour, and 30% rice flour. After preparation, this bait was coated with Rhodamine B (0.3% w/w). In each enclosure, 18 bait stations (covered plastic boxes, 30 cm x 21 cm x 12 cm) were employed. Initially, rats were acclimatized for 3-4 days with unhulled rice available in the bait stations. The cereal bait was then offered and uptake monitored until the daily consumption reached an asymptote (>5 days). After that, bait coated with RB was offered overnight. To obtain an estimate of the proportion of animals consuming bait (Eberhard, 1982; Cowan et al., 1987), approximately 50% of the animals from each enclosure were caught (population sampling) and killed to collect whiskers for detecting the presence of RB. The remaining animals were fed with cereal bait again for another 4 days, and daily bait consumption was recorded. For 2 enclosures, a second round of RB application was then conducted. At the end of the experiment, all animals were caught and killed to collect whiskers for RB detection using a fluorescence microscope. The method of RB detection in whiskers was as described by Fisher (1998).

## Results

The daily bait consumption by ricefield rats increased during the first 4 to 5 days of the feeding period and reached an asymptote thereafter. As expected, after population sampling to remove animals, bait consumption by the remaining animals sharply declined in all enclosures. After the first round of RB exposure, analysis of RB bands in whiskers indicated high proportions of animals (84-100%) were consuming baits in all enclosures. The average daily bait consumption per individual was 10.5g/100g body weight. Following the second round of RB application in 2 enclosures, a higher proportion of animals (93.8-100%) were shown to consume RB bait and bait consumption per individual (9.8 g/100 g body weight) remained high. The acceptance of bait with respect to sex was not significantly different ( $p > 0.05$ ) between male and female rats.

## Discussion

Increases in bait consumption by ricefield rats occurred during the first 4-5 days of exposure in each enclosure. Similar increasing bait uptake over the first 5-8 days of exposure has also been reported in rabbits (Cowan et al., 1987). The initial rise in bait consumption may be due to all animals eating baits from the time of first exposure and thereafter increasing their consumption over time. Alternatively, it is possible that, over time, an increasing number of animals began to eat the bait. We are unable to determine which behavioural response is occurring in this study, but can conclude, on the basis of estimated quantities consumed per day once asymptote consumption was achieved, that the majority of rats were eating approximately 10% of their body weight per day.

As rodent species are known to exhibit neophobic responses (Barnett, 1958; Mitchell, 1976; Lund, 1988), increasing the exposure period to a new food could reduce the neophobic effect on feeding behaviour. Therefore it is important to understand changes in bait uptake in order to determine the optimal pre-baiting period that might be required prior to application of baits containing control agents. This will maximise the bait uptake by target species. In all enclosures, there were no differences in consumption of cereal bait alone and the cereal bait coated with RB (0.3% w/w). These results suggest that RB did not influence bait acceptance by ricefield rats, and therefore can be successfully used for further bait uptake studies in ricefield rats. The results from this study suggest that the tested bait was highly preferred by ricefield rats and therefore could potentially be used as a carrier of control agents.

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## The possibility of use of some essential oils in rodenticidal baits

Jokić, G.<sup>1</sup>, Vukša, M.<sup>1</sup>, Đedović, S.<sup>1</sup>, Stojnić, B.<sup>2</sup>, Kataranovski, D.<sup>3,4</sup>

<sup>1</sup>Institute of Pesticides and Environmental Protection, Banatska 31b, POB 163, 11080 Belgrade-Zemun, Serbia, jokicg@ptt.rs

<sup>2</sup>Faculty of Agriculture, University of Belgrade, Serbia

<sup>3</sup>Institute for Biological Research 'Sinisa Stankovic', Department of Ecology, University of Belgrade, Serbia

<sup>4</sup>Faculty of Biology, Institute of Zoology, University of Belgrade, Serbia

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### Abstract

The effect of essential oils of ten different plant species, at rates of one and two ml per kg of plain bait, on bait acceptance and palatability in choice feeding tests for Swiss mice was studied under controlled laboratory conditions. Our aim was to determine the possibility of use of the tested essential oils in rodenticidal baits as potential attractants at rates which prevent mould development and which are applicable in humane medicine. Also, the use of these oils would be to prolong utility value of baits and reduce the use of antifungal ingredients, which usually reduce bait acceptance. Cinnamon and anise essential oils, commonly used as attractants, and clove oil, showed the best effects, because that they did not negatively affect bait acceptance and palatability, while fenchel and bergamot oils showed repellent action which became more pronounced as the content of oil in baits increased.

Keywords: bait acceptance, essential oil, palatability, Swiss mice

### Introduction

In human medicine, differently applied essential oils, alone or in combination, show insecticidal, antifungal, antibacterial, antioxidant, antimutagenic and antitrombotic activity. Likewise, in protection of food and raw materials, particularly cereal products, essential oils prevent the development of *Aspergillus flavus*, *Penicillium spp.* and *Fusarium graminearum*, which produce aflatoxin and mycotoxin. Being widely available and important dietary elements for the majority of rodent species, cereals are commonly used as base materials in rodenticide baits. Depending on conditions at the place of exposure, and particularly under conditions of increased humidity and temperature, mould growth occurs and can significantly reduce bait acceptance.

### Materials and methods

Adult males and females of Swiss mice (Institute for Medical Research, Military Medical Academy, Belgrade, Serbia), weighing from 20 to 25 g were used in the study. The animals were housed in plastic cages, under standard laboratory conditions, 21-24 °C, 12/24-hours light/dark cycle, controlled 45-70% relative humidity, and water available *ad libitum*. The effect of essential oils from ten plant species was investigated: bergamot orange (*Citrus aurantium* ssp. *bergamia*), scots pine (*Pinus silvestris*), eucalyptus (*Eucalyptus globulus*), clove (*Eugenia caryophyllata*), lavender (*Lavandula officinalis*), fenchel (*Foeniculum vulgare*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomi zeylanicum*) and anise (*Pimpinella anisum*). Plain baits were prepared according to EPPO standard (EPPO/OEPP, 1999), by mixing 90% of coarsely-cut cereal, 5% of corn oil and 5% of medium-ground oatmeal, and 25 ml/kg of pure alcohol was added as a solvent for essential oils, while oil baits were obtained by adding one or two ml of diluted essential oil in alcohol per one kilogram of plain bait. Bait acceptance and palatability were determined according to Johnson and Prescott (1994), in choice feeding test.

### Results

The cinnamon, anise and clove oils showed the highest level of acceptance. With the increase in cinnamon oil content, the growing trend of bait acceptance was recorded from 51.1% to 59.0%, as the growing trend of palatability also, from 1.04 to 1.44, while with the increase in anise oil content, the level of bait acceptance decreased from 49.5% to 41.6%, as did palatability, from 0.98 to 0.71. Also, by the increase in content of clove oil in baits from one to two ml/kg of plain bait, the growing trend of bait acceptance, from 47.0 to 50.4, and palatability, from 0.88 to 1.01, was recorded. The lowest level and

declining trend of bait acceptance and palatability with an increased content of oil in the baits, was recorded for fenchel from 26.2% to 8.0%, and from 0.35 to 0.08 and bergamot from 9.0% to 2.6%, and from 0.09 to 0.03.

### Discussion

The amount of essential oils in the baits applied in this study was similar to the quantity of attractants which are added during the preparation of rodenticide bait. It is known that in rodenticidal bait preparation process, the addition of cinnamon and aniseed can improve bait acceptance by rodents (Buckle, 1994; Marsh, 1988). The results of our study show that cinnamon, anis and essential oils in tested quantities do not act as attractants, but also do not negatively affect bait acceptance and palatability for Swiss mice. For now, antifungal activity of cinnamon and clove essential oils on *Aspergillus flavus*, *Fusarium graminearum* and *Penicillium ssp.* (Aldred et al., 2008; Bluma and Etcheverry, 2008; Marin et al., 2004; Salmeran and Pozo, 1991) and of anise oil (Bluma and Etcheverry, 2008) on *Aspergillus flavus* has been confirmed. Likewise, it is known that by adding paranitrophenol and dehydroacetic acid or paraffin, it is possible to postpone or absolutely prevent mould development, but at the same time, frequently, reduce bait acceptance by rodents (Buckle, 1994).

Based on the results of our study, we believe that cinnamon, anise and clove essential oils at tested application rates will not have a negative impact on acceptance and palatability of cereal-based baits. Also, based on the findings of other authors, we believe that by adding these oils, mould development on baits can be delayed or prevented.

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## **A successful control of the invasive Indian house crows (*Corvus splendens*) in Jeddah, Saudi Arabia**

Felemban, H.M.

Dept. of Biological Sciences, Faculty of Science, King Abdul-Aziz University, P.O.Box 80056, Jeddah 21589, Saudi Arabia, hfelemban@kau.edu.sa

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The Indian house crow (*Corvus splendens*) has successfully invaded many cities and areas along the sea coast of the Arabian Peninsula. Populations of the Indian house crow are well established in Jeddah, Saudi Arabia, and its numbers have increased rapidly since 1980. Its numbers have exceeded 70,000 birds in the late 1990s.

A control program was undertaken by the Municipality of Jeddah. Crow population dynamics, their ecology, dispersal, and behavior were considered for this study. Regular nesting and roosting surveys were conducted. Also information on mechanical and chemical control, and monitoring the crows' movement along the coast and inland were recorded during the period of October 2007 - October 2010.

A program is planned to cull up to 45% of crow populations during the first two years. The mechanical methods of management include: removing nests and collecting eggs and chicks from nesting sites during the breeding season. Cutting and clearing the outer side of trees is an effective method to prevent crows from building their nests. Also behavioral observations on feedings, roosting areas, breeding and movements of crows were conducted at different times of the day. Chemical control was limited to using baits containing DRC-1339 (3-chloro-p-toluidine hydrochloride).

This study has implications for the management of pest bird populations. An increase of the geographical range of crows to other areas may lead to an exacerbation of the problems caused by crows.

## **Monitoring and control of rodent pests in Albania**

Çota, E.

Agricultural University of Tirana, Albania, ejupcota@gmail.com

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Monitoring of the population density and the associated damage of pest rodents was carried out in two districts of Albania. The major crops were alfalfa and potato.

The damage caused by rodents is occasionally very high. The population density was monitored by closing the holes in the evening and counting the active holes early in the next morning. The active holes per 100 m<sup>2</sup> were counted and provided an index of population density (The *hole blocking census* method). Although, different rodent control techniques are used in Albania, trapping, and rodenticides are the main techniques applied. An economic threshold based on the index of rodent density was used to decide when to treat the populations. We use two different thresholds; in spring it is 5-10 holes for 100 m<sup>2</sup>; in autumn it is 20-30 holes per 100 m<sup>2</sup>. The monitoring process starts 10 days before possible treatment with rodenticides.

The results show that there is higher rodent damage in alfalfa than in other crops.

Keywords: control, economic threshold, monitoring, rodenticides, rodents

## Agricultural crop depredation by nilgai antelope (*Boselaphus tragocamelus*) and mitigation strategies: challenges in India

Chauhan, N.P.S.

Wildlife Institute of India, P.O. Box 18, Chandrabani, Dehradun 248001, India, npsc@wii.gov.in

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### Abstract

In India, problems associated with locally overabundant wildlife species have emerged as important management issues for reason of some species losing their natural habitat and adapting themselves to the man-altered situation. Crop-raiding by locally overabundant populations of nilgai antelopes (*Boselaphus tragocamelus*) has been widely reported in many parts of the country. Due to prolonged breeding activity and lacks of potential predators, numbers of nilgai have increased considerably and become locally overabundant in the states of Gujarat, Uttar Pradesh, Haryana, Punjab, Rajasthan, Madhya Pradesh and Delhi. The extent of human-nilgai conflict varied from place to place within these states. Nilgai were found to be capable of causing extensive damage to most agricultural crops. Damage to wheat (*Triticum aestivum*), gram (*Cicer arietinum*) and mustard (*Brassica campestris*) crops was caused not only by foraging but also through trampling, resting in field and daily movement of the animals. In low density nilgai areas, losses to wheat, gram and moong (*Phaseolus mungo*) crops were 20-30%, 40-55% and 40-45%, respectively. Damage to guar (*Cyamopsis tetragonoloba*) and cotton (*Gossypium arboreum*) was 20-35% and 25-40%, respectively. Whereas in high density nilgai areas, damage to wheat, gram and moong was 35-60%, 50-70% and 45-60%, respectively. Mustard was seldom eaten by nilgai but it was damaged by trampling. There were also increased incidences of road mishaps (7-12 cases/state/year) due to vehicular collisions. Though people considered nilgai as a sacred animal, conflict between nilgai and farmers is on the increase, and which is adversely affecting the conservation ideals. Options for damage control and managing nilgai populations are available but each of them has their advantages and limitations. Possible management strategies to reduce crop damage are suggested.

Keywords: *Boselaphus tragocamelus*, agricultural crops, damage, road mishaps, mitigation strategies

### Introduction

In India, problems associated with locally overabundant wildlife species have emerged as important management issues for reason of some species losing their natural habitat and adapting themselves to the man-altered situation. Crop-raiding by locally overabundant populations of nilgai (*Boselaphus tragocamelus*) has been widely reported in many parts of the country. Although people considered nilgai as a sacred animal, conflict between nilgai and farmers is on the increase, which is adversely affecting the conservation ideals.

In India, after the introduction of the Wildlife Protection Act (1972) and through associated management actions, the populations of many wildlife species have increased considerably, and a few of them have decidedly become locally overabundant. Due to disparate and often incompatible land use practices, these species have become ecological dislocates. Those that have been successful in adjusting to the man-altered habitats have thrived, and in many places such species have become serious pests of agricultural crops and are competing for resource utilization with domestic stock (Caughley, 1981; Howard and Dutta 1982; Ghosh et al., 1987). Nilgai, an antelope, is afforded holy and sacred rites by Hindus, and has rapidly grown in numbers outside protected areas. Agricultural crop damage by nilgai and blackbuck has been widely reported from almost all corners of India (Prater, 1980; Majupuria, 1982; Schultz, 1986, Rajpurohit, 1988).

Rural societies existing on subsistence agriculture can ill afford to have their crops raided by nilgai. Realizing the seriousness of the problem, poor farmers are now becoming increasingly intolerant to damage to their crops. Some have developed outright hostile attitudes toward the animals. It has now become important that administrators and wildlife managers take the initiative to actively control the wildlife damage to mitigate this problem, which is also in the larger conservation interest. During 2006-2010, extensive survey work was conducted in different states, and information was collected on the

occurrence and abundance of nilgai, and on their habitat and crop depredation patterns in the affected areas.

### Results and discussion

Nilgai is a highly adaptive antelope. Nilgai was recorded in 114 protected areas in 16 states, namely, Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Uttarakhand, Uttar Pradesh, Jharkhand and West Bengal in the country. Out of these, Bihar, Uttar Pradesh, Rajasthan, Gujarat, Haryana, Punjab, Madhya Pradesh and Uttarakhand states have an estimated population of 5,500, 254,449, 20,974, 97,004, 41,434, 10,312, 60,677 and 7,728 animals, respectively, and they are the worst affected. They occur in human dominated landscapes and crop fields outside protected areas.

Nilgai populations have increased considerably due to prolonged breeding activity and a high rate of multiple births and lack of potential predators. They have become locally overabundant in these states, thereby causing serious problems which include damage to crops, economic losses and increased incidence of road mishaps due to vehicular collisions. Nilgai caused extensive damage to most agricultural crops. Naturally diurnal, nilgai raid crops after dusk. Damage to wheat (*Triticum aestivum*), gram (*Cicer arietinum*) and mustard (*Brassica campestris*) crops was caused not only by foraging but also due to trampling of the crop during resting and movements of the animals. In low density nilgai areas, losses to wheat, gram and moong (*Phaseolus mungo*) crops were 20-30%, 40-55% and 40-45%, respectively. Damage to guar (*Cyamopsis tetragonoloba*) and cotton (*Gossypium arboreum*) was 20-35% and 25-40%, respectively. Whereas in high density nilgai areas, damage to wheat, gram and moong was 35-60%, 50-70% and 45-60%, respectively. Mustard was seldom eaten by nilgai but it was damaged by trampling. The extent of crop damage varied considerable, depending upon the animal numbers and crop protection strategy followed in the area. Mustard and cotton are grown extensively in the affected region and were found to provide excellent hiding cover to these animals. There were also increased incidences of road mishaps due to vehicular collisions in these states. The accidents ranged from 7 to 12 cases per State every year.

### Recommendations

Understanding animal damage problems and their control is the prerequisite of resource management in most man-altered habitats to which wildlife species adapt successfully (Howthorne, 1971). Large number of options for damage control and managing nilgai populations are available but each of them has their advantages and limitations. Nilgai cannot be killed due to religious reverence. Possible mitigation strategies to reduce crop damage include use of fear provoking stimuli, chemical repellents, fencing agricultural areas, capture and translocation, sustained harvesting, and reproductive management of nilgai populations.

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## Human casualties and agricultural crop raiding by wild pigs and mitigation strategies in India

Chauhan, N.P.S.

Wildlife Institute of India, P.O. Box 18, Chandrabani, Dehradun 248001, India, npsc@wii.gov.in

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### Abstract

We investigated the nature and extent of human-wild pig conflict in northern and central India. Wild pigs (*Sus scrofa*) surviving in disturbed and fragmented habitats were responsible for many human casualties and extensive damage to agricultural crops. Information on human casualties, place of attack, sex of victims, and agricultural crop raiding was collected from the records of the forest department and by interviewing villagers and ocular estimation of crop damage in 11 states. In total, there were 927 human casualties by wild pigs in these states during 1990-2010; out of which 4.2% were death cases and 95.8% injury cases. Male casualties were more (81.2%) than females (18.8%). Maximum cases (77.9%) occurred in forests, followed by 18.3% cases in crop fields and 3.8% cases in the vicinity of villages. Damage to agricultural crops by wild pigs was enormous and widespread. They fed on all phenological stages, but tender stages and matured crops were highly susceptible to damage. Damage to *Triticum vulgare*, *Oryza sativa*, *Zea mays*, *Pennisetum typhoides*, *Saccharum officinarum*, *Arachis hypogea*, *Cicer arietinum*, *Hordeum vulgare*, *Sorghum vulgare* and *Brassica campestris* was 5-20%, 5-15%, 10-30%, 5-15%, 5-20%, 10-30%, 5-15%, 5-15%, 5-10% and 5-10% respectively in these states. *Eleusine coracana*, *Phaseolus mungo*, *Glycine max*, *Sesamum indicum*, *Lens esculenta*, *Ipomoea batatas* and *Lythyrus sativum* showed damage to varying extent (5-10%). In some areas, pulses and vegetables were damaged to 5-25%. Damage was very high in crop fields close to wildlife areas. Today, these problems have aggravated beyond tolerable limits and have resulted into direct conflict between people and wild pigs. This has also adversely affected the conservation ideals. Mitigation strategies for mitigation of these conflicts have been suggested.

Keywords: agricultural crops, damage conflict, human deaths, injuries, mitigation, wild pigs

### Introduction

The wild pig (*Sus scrofa*) is one of the most widely distributed large mammals. It has always been associated itself with man, and successfully utilises the human altered landscape (Ahmed, 1991; Fadeev, 1981; Erkinaro et al., 1982). The wild pigs notoriety as a crop pest is universal (Tisdell, 1982). Wild pigs raid crops and utilises the agro-ecosystem for food resource and shelter. There has been increasing trend in the wild pigs-man conflict in and around protected areas, managed forests and human settlements throughout the country. People have developed an antagonistic attitude towards the wild pigs. Pigs also adversely affect conservation ideals. We investigated the human-wild pig conflict in the state of Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Uttarakhand, Rajasthan, Gujarat, Madhya Pradesh, Bihar, Maharashtra and Chhattishgarh located in northern and central India.

### Methods

Information on human casualties, circumstances of attacks, place of attack and sex of victims, and agricultural cropping pattern, and nature and extent of crop damage by wild pigs was collected from the records of the forest department and using questionnaire survey of villages located in and around protected areas and managed forests and interview of the victims or their families and analysis of human casualty cases in different states. Agricultural crop fields were surveyed and crop damage assessment was done using a visual estimation method in few randomly selected crop fields. Information on compensation paid for the losses and yearly payments was also collected.

### Results

In the state of Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Uttarakhand, Rajasthan, Gujarat, Madhya Pradesh, Bihar, Maharashtra and Chhattishgarh, wild pigs accounted for 927 human casualties during 1990-2010. Maximum cases occurred in Himachal Pradesh (16.5%), followed by Maharashtra (15.7%), Madhya Pradesh (12.7%) and Rajasthan (11.4%). There were 4.2% death cases as compared to

888 (95.8%) injury cases. Male casualties were more (n=753) than female casualties (n=174). Maximum cases i.e. 722 (77.9%) occurred in forests, followed by 170 (18.3%) cases in crop fields and 35 (3.8 %) cases in the vicinity of villages. Most of these attacks were accidental and occurred when these victims ventured into the forests for collection of non-timber forest produce in forests, fuelwood, fodder, medicinal plants, or to graze their livestock and while working in their crop fields

There was marked monthly variation in human casualties by pigs during 1990-2010. Out of 927 cases, the highest number of casualties occurred in November (n=183, 19.7%), followed by December (n=132, 14.2%), January and August (n=93, 10% each), October (n=87, 9.4%), September (n=69, 7.4%), March (n=63, 6.8%), July (n=57, 6.1%) and so on. The age group of 867 cases was recorded in these states during 1990-2010. Among these cases, the highest number of 276 human casualties occurred in the age group of 41-50 years. There were 186, 144 and 132 casualties in the age group of 31-40 years, 21-30 years and 51-60 years, respectively. Out of 816 human casualties, the highest number of cases by wild pigs occurred between 08:01-12:00h (40.6%), followed by 16:01-20:00h (34.3%), 04:01-08:00h (14.2), 12:01-16:00h (10.9%)

Wild pigs were found to damage variety of agricultural crops, namely, *Saccharum officinarum*, *Zea mays*, *Arachis hypogaea*, *Hordeum vulgare*, *Triticum aestivum*, *Oryza sativa*, *Cicer arietinum*, *Pennisetum typhoides*, *Sorghum vulgare*, *Phaseolus mungo*, *Ipomoea batatas* and *Lythyrus sativum*. Damage to these crops varied from 5 to 36% in different states. Other oilseed and legume crops damaged by pigs were found to be mustard (*Brassica campestris*), til (*Sesamum indicum*), moth (*Vigna aconitifolius*), guar (*Cyamopsis psoralioides*), matira (*Citrullus vulgaris*), tinda (*Citrullus vulgaris*), jeera (*Cuminum cyminum*), isabgol (*Plantago ispaghula*), methi (*Trigonella corniculata*), raira (*Brassica juncea*), chili (*Capsicum annum*) and pea (*Pisum sativum*).

## Discussion

Wild pig's attacks on human beings varied in different states, and most of these were accidental. Maximum cases occurred in forests than in crop fields and vicinity of villages. These human casualties occurred when villagers ventured into the forests for collection of fuelwood, fodder, medicinal plants, grazing their livestock or when they were working in their crop fields. Male casualties were more than females, and this could be due to more involvement of men in field activities. Monthly variation in human casualties could be correlated with activities of villagers in crop fields, forests and villages. Wild pigs caused extensive damage to variety of agricultural crops. Mature crops were highly susceptible to damage by pigs. Damage to crops was reported to increase when there was less natural food available in forest, and artificial feeding of wild pigs could reduce crop damage (Mackin, 1970; Andrejewski and Jezierski, 1978; Genov, 1981). Mitigation strategies include use of local protective methods, co-operative guarding of matured crops, people should be alert and vigilant in crop fields, wire fences with flying, flashing ribbons or plastic strips that produce scaring sounds and other frightening devices and creation of education and awareness among people.

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**A novel hepatitis E virus-like agent in wild Norway rats (*Rattus norvegicus*) from Germany**

Ulrich, R.G.<sup>1</sup>, Plenge-Bönig, A.<sup>2</sup>, Schielke, A.<sup>3</sup>, Kindler, E.<sup>4</sup>, Dremsek, P.<sup>1</sup>, Gregersen, H.<sup>5</sup>, Rietschel, W.<sup>5</sup>, Groschup, M.H.<sup>1</sup>, Reetz, J.<sup>3</sup>, Guenther, S.<sup>6</sup>, Heckel, G.<sup>4</sup>, John, R.<sup>3</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Südufer 10, 17493 Greifswald-Insel Riems, Germany, rainer.ulrich@fli.bund.de

<sup>2</sup>Institute of Hygiene and Environment, Hamburg, Germany

<sup>3</sup>Federal Institute for Risk Assessment, Berlin, Germany

<sup>4</sup>University of Bern, Institute of Ecology and Evolution, Bern, Switzerland

<sup>5</sup>Wilhelma, Zoologisch-Botanischer Garten Stuttgart, Stuttgart, Germany

<sup>6</sup>Institute of Microbiology and Epizootics, Veterinary Faculty, Free University, Berlin, Germany

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Hepatitis E represents a rare human disease in developed countries. This disease is characterized by a self-limiting jaundice of varying severity and often accompanied by unspecific symptoms like fever, headache and pain of the upper abdomen. Autochthonous cases in Europe are caused by hepatitis E virus (HEV) genotype 3, which is most likely transmitted to humans from domestic pigs, wild boar or deer. Multiple reports on the detection of HEV-specific antibodies in rats suggested the presence of an HEV-related agent; however, infectious virus or a viral genome could not be demonstrated in these rodents so far. Recently, a nested broad-spectrum RT-PCR protocol was developed capable of detecting different HEV genotypes including those derived from wild boar and chicken. Using this novel assay, an initial screening of 30 fecal samples of wild Norway rats (*Rattus norvegicus*) from Hamburg (Germany) resulted in the detection of two sequences with similarities to human, mammalian and avian HEV. Investigation of liver tissue samples from additional rats from Hamburg resulted in the assessment of the complete nucleotide sequence showing a typical genome organization of HEV. Additional molecular and serological studies of Norway rats from Berlin and Stuttgart indicated a broad geographical distribution of this novel virus. Phylogenetic analyses of partial and complete genome sequences suggest this virus as separate HEV genotype tentatively designated as rat HEV. Moreover, the phylogenetic analyses of rat HEV sequences from different geographical origin demonstrated a geographical clustering suggesting an isolated long-term evolution of the different strains. Virus particles with morphology reminiscent of HEV could be demonstrated by immune electron microscopy in a fecal sample from an infected rat. Real-time RT-PCR and immunohistochemistry investigations of different tissue samples indicate the hepatotropism of the virus. Future investigations are dedicated to characterize the molecular evolution and host adaptation of the virus, to assess its zoonotic potential and to study its possible application in an animal model for human hepatitis E.

Keywords: hepatitis E virus, phylogeny, *Rattus norvegicus*, RT-PCR

## Lassa virus serology in rodents: spatial survey in Guinea, west Africa

Fichet-Calvet, E.<sup>1</sup>, Koulemou, K.<sup>2</sup>, Sylla, O.<sup>2</sup>, Soropogui, B.<sup>2</sup>, Kourouma, F.<sup>2</sup>, Doré, A.<sup>2</sup>, Becker-Ziaja, B.<sup>1</sup>, Koivogui, L.<sup>2</sup>, Günther, S.<sup>1</sup>

<sup>1</sup>Department of Virology, Bernhard-Nocht Institute of Tropical Medicine, 20359 Hamburg, Germany, ecalvet@club-internet.fr

<sup>2</sup>Projet de Recherches sur les Fièvres Hémorragiques en Guinée, Centre Hospitalier Universitaire Donka, BP 5680, Conakry, Guinée

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Lassa fever is a hemorrhagic fever, due to an arenavirus, and affects 150,000-300,000 persons per year in west Africa, leading to 5,000-10,000 deaths per year. The disease appears to have two geographically separate endemic areas: the Mano River region (Guinea, Sierra Leone and Liberia) in the west, and Nigeria in the east. Because of the recurrent public health problem in these countries, we made an extensive field study in Guinea, between 2002 and 2005. We then demonstrated that the multimammate mice *Mastomys natalensis* was the only host of the Lassa virus. The spatial survey conducted in 17 villages distributed in Coastal, Upper and Forest Guinea, showed that 3 villages were infested by the virus. Using the same collection done at this time, we investigate now the Lassa virus serology in the rodent population to verify if other species than *M. natalensis* could be potential carriers. A standardized trapping was conducted in different habitats: houses, cultivations and forest. The rodents were identified morphologically and molecularly by sequencing the *cytochrome b* gene. The screening of IgG antibodies was done by using the indirect immunofluorescence assay, with the strain Bantou 289 as antigen. The average seroprevalence was 8% (129/1551), distributed as follow: 18% (109/596) in *M. natalensis*, 3% (7/251) in *Mastomys erythroleucus*, 12% (4/32) in *Lemniscomys striatus*, 4% (4/112) in *Praomys daltoni*, 7% (3/40) in *Mus minutoides* and 1% (2/163) in *Praomys rostratus*. They were mainly found (122/129) in the 3 villages where the Lassa virus was previously described, suggesting that other species than *M. natalensis* could also serve as carrier in the circulation of the virus in high endemic zone. But 3 other villages located in coastal Guinea showed 6 seropositive animals, where the reservoir was absent from this area. These findings are discussed in the framework of ecoepidemiology of the disease.

Keywords: IgG, Lassa fever, *Mastomys natalensis*, rodents, serology, tropics, west Africa, zoonosis

## Biome-specific rodent dynamics and hantavirus epidemiologies in Europe

Henttonen, H.<sup>1</sup>, Leirs, H.<sup>2</sup>, Kallio, E.R.<sup>3,4</sup>, Tersago, K.<sup>2</sup>, Voutilainen, L.<sup>1,5</sup>

<sup>1</sup>Finnish Forest Research Institute, Vantaa, Finland, heikki.henttonen@metla.fi

<sup>2</sup>Univ. Antwerp, Belgium

<sup>3</sup>Inst. Integrative Biol., Univ. Liverpool, UK

<sup>4</sup>Dept Biol. and Environm. Sci, Univ. Jyväskylä, Finland

<sup>5</sup>Haartman Institute, Univ. Helsinki, Finland

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Among emerging diseases, rodent-borne (robo) viral infections provide a prime example. In Europe, the incidence is increasing and more countries report robo infections. In the large collaborative EU project EDEN (Emerging Diseases in a Changing European Environment), we studied robo diseases from several points of view. Our most important aim was to understand the differences in human epidemiology in nephropathia epidemica, the most common hantaviral disease in Europe, caused by Puumala hantavirus (PUUV), between boreal and temperate Europe. We documented fundamental differences in the transmission dynamics of PUUV between these two biomes. The patterns in temperate zone are greatly affected by masting events, of which the frequency may increase due to warmer summers, while in the snowy boreal zone predator driven vole cycles shape the dynamics. Consequently, the underlying top-down or bottom-up causes of rodent fluctuations are different. We have further documented the role of landscape patterns (homogenous taiga vs. fragmented temperate forests) in rodent/virus dispersal, and in the presence or absence of host threshold densities for the PUUV occurrence. In addition, local environmental conditions (e.g., temperature and moisture) affect the virus survival outside the host, which may cause variation in indirect transmission. These results are essential for human risk evaluation with regard to both long-term and seasonal occurrence of PUUV in the environment. In conclusion, it is important to realize that within the same host/virus system, biome specific PUUV epidemiologies occur, which highlights the importance of geographically comparative studies in Europe.

## Relationship between bank vole abundance, seroprevalence and human hantavirus infections

Reil, D.<sup>1,3</sup>, Imholt, C.<sup>1</sup>, Schmidt, S.<sup>2</sup>, Rosenfeld, U.M.<sup>2</sup>, Ulrich, R.G.<sup>2</sup>, Eccard, J.A.<sup>3</sup>, Jacob, J.<sup>1</sup>

<sup>1</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Toppeideweg 88, 48161 Muenster, Germany, daniela.reil@jki.bund.de

<sup>2</sup>Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, Suedufer 10, 17493 Greifswald - Insel Riems, Germany

<sup>3</sup>University of Potsdam, Institute of Biochemistry and Biology, Animal Ecology, Maulbeerallee 1, 14469 Potsdam, Germany

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In temperate zones, bank vole (*Myodes glareolus*) population dynamics show seasonal variations and multi-annual fluctuations (3-4 years) as a function of food conditions. In turn, food quality and quantity - here mast of forest trees, mainly beech (*Fagus sylvatica*) - are dependent on climatic conditions. Furthermore, the bank vole is the reservoir species for Puumala viruses in Germany, a hantavirus species that can cause haemorrhagic fever with renal syndrome (HFRS) in humans. Years with bank vole peak abundance can oscillate with a large number of human Puumala virus infections. Therefore, the aim of our study is to investigate the potential correlations between climate, beech mast, bank vole abundance and Puumala virus infections within the host species as well as in humans.

Population density and dynamics of bank voles are estimated in climatically different regions of Germany (North Rhine-Westphalia - NRW, Baden-Wuerttemberg - BW, Thuringia - THR and Mecklenburg-Western Pomerania - MVP) using live trapping (capture-mark-recapture). Trapping sites are three woodland areas per state which are sampled three times a year - April, July and October. Blood samples of captured individuals are collected for serological detection of Puumala virus infection and to determine the seroprevalence in the rodent host populations.

In 2010, bank vole abundances were high in NRW (60-100 individuals per hectare (ind/ha)), BW (70-110 ind/ha) and THR (50-100 ind/ha), but low in MVP (<20 ind/ha). Population densities in each state increased from spring to summer and decreased from summer to fall. Estimated Puumala virus seroprevalences in bank voles also fluctuated between trapping seasons (NRW 23-67%, BW 22-49%, MVP 0-5%, THR 0-2%), but unlike population densities, seroprevalences were highest in April 2010 and lowest in October 2010. Results of trapping sessions within year in 2010 show no positive correlation of bank vole abundances and Puumala virus seroprevalences in bank voles. On the contrary, human Puumala virus infections are positively correlated with estimated bank vole abundances in 2010 ( $R^2=0.37$ ).

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Keywords: abundance, bank vole, seroprevalence, human infection, Puumala virus

## The role of rodents as carriers of disease on UK farms: a preliminary investigation

Stuart, A.M.<sup>1</sup>, Prescott, C.V.<sup>1</sup>, MacIntyre, S.<sup>1</sup>, Sethar, A.<sup>1</sup>, Neuman, B.W.<sup>1</sup>, McCarthy, N.D.<sup>2</sup>, Wimalaratna, H.<sup>2</sup>, Maiden, M.C.J.<sup>2</sup>

<sup>1</sup>School of Biological Sciences, The University of Reading, Berkshire, RG6 6AS, UK, a.stuart@reading.ac.uk

<sup>2</sup>Department of Zoology, Peter Medawar Building, University of Oxford, Oxford, OX1 3SY, UK

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### Abstract

In the UK, *Campylobacter* spp. and Lymphocytic Choriomeningitis Virus (LCMV), an Old World arenavirus, cause two zoonoses of concern that may be transmissible from rodents to humans and livestock. The aims of this preliminary investigation were to examine the occurrence of *Campylobacter* spp. and LCMV in Norway rats *Rattus norvegicus* on UK farms and to identify and characterise the Sequence Types of the *Campylobacter* isolates. Samples were collected from wild Norway rats and fresh Norway rat faeces. Multi Locus Sequence Typing (MLST) was performed on *C. spp.* isolates and samples were tested for arenavirus RNA by RT-PCR. Six *C. spp.* isolates were identified. One isolate was *C. lari* and five isolates were *C. jejuni*. Following MLST profiling, three unique *C. jejuni* sequence types were identified. Two of which are novel and the third is typically associated with livestock and human infection. Nine positive results for LCMV were obtained giving an overall prevalence of 25% across four sites. This is higher than previously reported for this species.

Keywords: arenavirus, *Campylobacter*, disease, Lymphocytic Choriomeningitis, *Rattus norvegicus*, rodents

### Introduction

Rodents are known to be reservoirs of a large number of pathogens, many of which are transmitted to humans and their domesticated animals through contamination of stored produce and animal feed with rodent urine and faeces (Gratz, 1994). Two such zoonoses that may be transmissible from rodents to humans and livestock are *Campylobacter* spp. and Lymphocytic Choriomeningitis Virus (LCMV).

*C. spp.* is a common cause of gastro-intestinal infection in humans in the UK, with 57,772 cases reported for England and Wales in 2009. Transmission to humans is believed to be primarily through undercooked meat, from contaminated water, and through contact with pets. Ninety-seven percent of sporadic cases have been attributed to farm animals, and in particular the meat and poultry industry. Because Norway rats (*Rattus norvegicus*) commonly occur around UK farms and have previously been identified to carry *Campylobacter* strains (Meerburg et al., 2006), it is considered that they may be a potential source of infection to livestock.

Lymphocytic Choriomeningitis Virus (LCMV) is an arenavirus that is distributed worldwide, and is carried by rodents. It is carried as a life-long persistent infection (passing from mother to offspring), and is asymptomatic in persistently infected rodents. It is believed to be contracted by humans through breathing air that is contaminated with rodent excrements. Infections may therefore occur wherever infected rodent hosts of the virus are found. Little is known about the prevalence of this virus in wild rodents in the UK. However, recently this virus has been reported in a range of wild rodent species, with House mice (*Mus musculus*) more likely to be infected than other species (Blasdel et al., 2008).

The aims of this preliminary investigation were to examine the occurrence of *C.spp.* and LCMV in Norway rats on UK farms and to identify and characterize the strains of the *C. spp.* isolates.

### Methods

For *Campylobacter* analysis, samples were collected from four rural livestock farm sites. Initial samples were obtained by extracting intestinal content from freshly killed wild Norway rats into saline water; subsequently, samples were obtained from fresh Norway rat feces collected into peptone water.

The extracts were cultured within 24 h of collection in Bolton enrichment broth at 42°C within a microaerophilic environment and *Campylobacter* isolates were obtained using modified charcoal

cefoperazone deoxycholate agar plates (mCCDA; Oxoid). Multi Locus Sequence Typing (MLST) was then performed on *C. spp.* isolates using the method of Dingle et al. (2001).

For arenavirus analysis, 35 Norway rat samples were collected from four livestock farm sites. Liver and spleen tissue was collected into Trizol reagent from rats that had previously been stored at -21°C. Samples were tested for arenavirus RNA by RT-PCR using the methods described by Blasdell et al. (2008); RNA was extracted by using QIAamp viral RNA mini-kit (QIAGEN, Crawley, UK), first strand cDNA synthesis was carried out using primers complementary to the conserved genomic termini, and PCR was conducted using degenerate primers designed to bind conserved regions of all known arenavirus sequences.

## Results

A positive result for *C. spp.* was obtained from the only site where samples were collected from intestinal content, and positive results for *C. spp.* were obtained from three of four sites where samples were collected as fresh droppings. From these, six *C. spp.* isolates were identified. One isolate was *C. lari*, for which a partial MLST profile was obtained, and five isolates were *C. jejuni*, for which full MLST profiles were obtained. Following MLST profiling, three unique *C. jejuni* sequence types (STs) were identified. Two of these strains are novel, and have been designated ST 5129 and ST 5130. The third, ST 586, is a known ST, typically associated with chicken, cattle and human infection.

Nine positive results for LCMV were obtained (3/10, 3/10, 2/10, 1/5), giving an overall prevalence of 25% across all four sites. Many of the rats in this study also had enlarged spleens, another common sign of persistent LCMV infection.

## Discussion

The presence of *C. jejuni* in wild rats from UK farms correlates with previous findings in Denmark (Jensen et al., 2006) and the Netherlands (Meerburg et al., 2006). Through MLST analysis we were able to identify that the *C. jejuni* isolates included one strain that is known to infect humans and livestock and two that are usually rather ecologically separate. Rats can thus harbor a relatively wide range of *Campylobacter* strains. However, from these data it is not possible to establish whether the novel *Campylobacter* strains carried by rats are host-specific, or whether the rats were colonized by *Campylobacter* strains from a variety of sources. Further research will be conducted to clarify whether rats play a role in the transmission of *C. spp.* in the farm environment.

The high prevalence of LCMV in wild Norway rats found in this study was unexpected and is higher than previously reported for this species (Blasdell et al., 2008). With such a high incidence in wild Norway rats, as well as in wild House mice (Blasdell et al., 2008), the chance of human infection must be significant. However, because not all people exposed to the virus become ill and with symptoms similar to those of influenza, except in severe cases when it can cause meningitis or encephalitis, it is unsurprising that the disease has historically been under-reported. Further studies are needed to thoroughly investigate the prevalence levels of LCMV in wild rodents in both urban and rural areas so as to identify the risk posed to humans.

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## **Badgers, farm buildings and bovine tuberculosis (*Mycobacterium bovis*) in cattle: the practical importance of understanding host behavior**

Delahay, R., Judge, J.

Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK, dez.delahay@fera.gsi.gov.uk

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Management of disease in wildlife populations is challenging, not least because host behavior and ecological conditions present substantial practical constraints and can influence the outcome of interventions in unpredictable ways. Understanding the behavior of infected hosts may provide valuable insights into how to more effectively target management interventions. Badgers have been implicated as a wild maintenance host of *Mycobacterium bovis* and source of infection for UK cattle. Intensive behavioral studies provided evidence of widespread and frequent visits by badgers to a variety of farm facilities where they may contaminate cattle feed and come into close direct contact with cattle. Further evidence suggested that animals in advanced stages of disease progression may be more likely to visit farm buildings than uninfected individuals. On some farms visits to buildings could be the single most important risk factor for *M. bovis* transmission between badgers and cattle. An understanding of badger foraging behavior may provide insights into spatio-temporal variations in the likelihood of such visits, which may be influenced by the impact of weather conditions and habitat characteristics on the availability of natural foods. Experimental investigations identified practical measures that are highly effective in preventing badger visits to farm buildings, although their cost-effectiveness in terms of reduced disease risk is unknown. A better understanding of the relationship between host infection status, behavior and the likelihood that an animal will visit farm facilities may provide valuable information on where and when intervention measures are likely to be most effective.

## Evaluating selective culling with vaccination to control wildlife disease: badgers and bovine tuberculosis (bTB)

Smith, G.C., Wilkinson, D.

The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK, graham.smith@fera.gsi.gov.uk

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### Abstract

Emerging infectious diseases often originate in wildlife, but the complex dynamics of wild animal populations mean that disease control is a major scientific and policy challenge. Culling and vaccination can be effective but the ecological characteristics of wild animals may confound the outcomes of simple management programs. In Britain, badgers *Meles meles* are a recognized reservoir of *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), and are involved in its transmission to cattle. Experiments have shown that culling badgers can increase bTB incidence in badgers and cattle, by perturbing the social structure of badger populations, and increasing contact rates. Selective removal of infected badgers and vaccination of the remainder has recently been advocated as a potential solution. Simulation modelling suggests that this intuitively appealing policy could at best deliver only a minor advantage over thoroughly applying either vaccination or culling, but carries a risk of making the disease problem much worse. We suggest this counterintuitive outcome arises because: 1, not all animals can be caught and tested 2, some genuinely infected animals will be test negative 3, selective culling leaves a larger population of susceptible hosts than non-selective culling 4, some susceptible hosts will not respond to vaccination and 5, perturbation increases contact and transmission rates among a relatively high density population of infectious and susceptible animals.

Keywords: cattle, combined control strategies, *Mycobacterium bovis*, perturbation, spatial model

### Introduction

The selective culling of infected wild animals has been proposed as a more acceptable alternative to large-scale culling, and when combined with vaccination of the non-infected susceptible individuals, can be seen as a viable strategy for disease management. Here, we examine the case for selective culling of badgers to reduce bTB, by using an established simulation model, and taking account of the possible role of social perturbation that can occur with group living animals. Social perturbation, as a result of culling, in the badger has been responsible for negative consequences for bTB in cattle (Donnelly et al., 2003; 2006).

### Materials and methods

A spatially explicit, stochastic mechanistic simulation model of bTB dynamics in badgers and cattle was utilized. This comprises a farm landscape, with cattle herds subject to normal cattle management practices (movement, slaughter, skin testing and pre-movement testing) overlaid onto a badger population consisting of contiguous social group territories (Wilkinson et al., 2009). Following culling the badger population becomes perturbed, allowing individuals to move more widely, spreading infection and increasing disease prevalence. The level of perturbation in the model (Wilkinson et al., 2009) has previously been validated against results from a large-scale field experiment (Donnelly et al., 2007; Jenkins et al., 2008). Since it is not known how much culling triggers social perturbation, we tested the sensitivity of the model to a range of perturbation trigger points. We also simulated an optimum control strategy, where we used best-case management parameter values (trapping efficacy, bTB test-performance and vaccine efficacy). Selective badger culling was simulated once each year, when every captured badger was tested for bTB and either culled (test positive animals) or vaccinated (test negative animals). Badger perturbation was either switched on for any level of cull, or low level culling was assumed to avoid perturbation, and these were compared to culling or vaccination on its own. Vaccination on its own was assumed to avoid any perturbation effect.

## Results

Before any intervention in badger populations was applied in the model, the mean output parameters stabilized at: 7.5 adult badgers per social group, 1.3 infected badgers per social group, and 0.05 herd breakdowns per farm per year.

Comparing strategies involving culling or vaccination only, the effect of perturbation after culling resulted in a greater reduction in the number of infected badgers during the 5 years of vaccination. However, after 10 years, the culling strategy had resulted in fewer infected badgers than vaccination.

With a combined targeted strategy, the effect on the number of infected badgers was strongly dependent on the point at which perturbation was assumed to be invoked. When perturbation was triggered by one or more animals being culled, the number of infected badgers initially increased higher than that observed for the cull-only strategy, and remained worse than doing nothing for almost twice as long as culling. When perturbation was triggered by three or more culled badgers, a similar number of infected badgers remained as for the vaccination-only strategy, in both the short and the long term.

By limiting the number of badgers culled annually in any one group to below the threshold required to invoke perturbation all these targeted cull and vaccinate strategies resulted in fewer infected badgers than the vaccination-only strategy, but the mean difference was very small.

## Discussion

Modelling vaccination-only and culling-only strategies on *M. bovis* infection rates in badgers, show that vaccination-only is a more successful strategy in the short-term than culling, because of the adverse effects of social perturbation. In the longer term (beginning a few years after control ceases), badger culling appears more successful at reducing badger infection.

The intuitive appeal of combining badger vaccination with selective culling is that by removing fewer animals from each social group, perturbation effects are minimized. However, such a strategy would not necessarily prevent perturbation. It is not known how many badgers can be removed before perturbation occurs as it could be a response to mortality by any means. Triggering social perturbation during selective culling (with vaccination of test-negative, i.e. healthy animals) could produce the negative social effects, but with a higher density population. This leads us to the non-intuitive conclusion that selective culling is likely to be a higher risk strategy than vaccination-only or culling-only. Even a combined cull and vaccinate strategy in which the number of badgers removed was limited to below the threshold for inducing perturbation was only marginally more successful at reducing *M. bovis* infection in the badger population than a vaccination-only strategy. We suggest that these results are tested for generality in other species disease systems.

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## Assessing classical swine fever disease control measures using an individual-based model

Lange, M.<sup>1</sup>, Kramer-Schadt, S.<sup>2</sup>, Thulke, H.-H.<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research Leipzig – UFZ, Dept. of Ecological Modelling, Leipzig, Germany, martin.lange@ufz.de

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany

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### Abstract

Classical Swine Fever (CSF) is considered an endemic disease in European wild boar populations. Huge effort is paid on oral mass vaccination against CSF virus, but few is known about the efficacy of different application schemes of the control measures in space, or in dependence of the outbreak dynamics. We used an individual-based, spatially-explicit model to assess vaccination strategies under uncertain virulence. A preventive component of vaccination was found crucial for limiting disease spread and preventing disease endemicity.

Keywords: Classical Swine Fever, disease control, epidemiological modelling, individual-based model, oral mass immunization, uncertainty, virulence

### Introduction

Classical Swine Fever (CSF) is a viral disease in wild boars (*Sus scrofa*) and domestic pigs causing huge economic impact on individual farmers and national economies. The management of the disease became even more complicated in the last decades due to endemicity in wild boar populations in several European countries. Huge effort is paid on CSF control in wild boar populations by oral mass vaccination, but few is known about the efficacy of the applied measures to control or eradicate the disease. Furthermore, virulence as a crucial parameter for disease dynamics varies widely between CSF virus strains and is highly uncertain.

### Methods

We implemented a spatially-explicit, individual-based wild boar population model, coupled with a CSF virus model on the level of individual traits. The model accounts for social behavior of boar groups as well as individual variations in disease outcomes. Over a range of case mortality and duration of the infectious period (the virulence), we tested alternative spatial baiting strategies. We compared these scenarios regarding the performance of the management measured by final size of the infected area and long-term persistence.

### Results

Our analysis showed that artificial immunization can facilitate disease persistence under certain conditions. High success in virus eradication as well as prevention of disease spread was only possible with preventive vaccination in terms of baiting in front of the epidemic wave. Buffered vaccination effort was completely sufficient to exploit the effect of vaccination of the entire area, which translates strategic needs into a practical management plan. A buffer radius corresponding disease spread distance of one year revealed suitable to fully exploit the potential of oral mass vaccination.

### Conclusions

Although preventive baiting strategies are not yet implemented in the field due to EU legislation but with marker vaccines in sight, we recommend buffered baiting of the area with infected animals.

## Differences in genetic structuring of populations of the Argentine hemorrhagic fever reservoir, the rodent *Calomys musculus*, from endemic and non endemic zones

Chiappero, M.B.<sup>1,3</sup>, Piacenza, M.F.<sup>2,3</sup>, Gardenal, C.N.<sup>1,3</sup>, Calderón, G.E.<sup>4</sup>, Provensal, C.<sup>2</sup>, Polop, J.J.<sup>2</sup>

<sup>1</sup>Population Genetics and Evolution, FCEFYN, National University of Córdoba, Argentina, ngardenal@efn.unc.edu.ar

<sup>2</sup>Department of Natural Sciences, FCEFYQY, National University of Río Cuarto, Argentina

<sup>3</sup>National Council for Scientific and Technological Research (CONICET), Argentina

<sup>4</sup>National Human Viral Diseases Institute 'Dr. Julio I. Maiztegui' (INEVH), Pergamino, Argentina

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### Abstract

We estimated the effective population size and genetic structuring within populations of the rodent reservoir of Argentine Hemorrhagic Fever, *Calomys musculus*, from zones with different epidemiologic condition (non endemic, historic and epidemic). We found that populations from epidemic zones presented higher effective sizes, higher differentiation among subpopulations and higher levels of internal relatedness than in non-endemic zones. These results would explain the maintenance of the virus in populations from epidemic zones and the patchy distribution of infected rodents.

Keywords: Argentine Hemorrhagic Fever, *Calomys musculus*, endemic area, genetic structure, non endemic area, population effective size

### Introduction

*Calomys musculus* (Rodentia, Cricetidae, Sigmodontinae) is one of the most abundant native rodent species in agro-ecosystems of central Argentina, and the reservoir of Junin virus (JV; Arenaviridae), the etiological agent of Argentine Hemorrhagic Fever (AHF). This serious disease is endemic in a large part of central Argentina, where most of the agricultural and cattle production activities of the country concentrates. Since the discovery of the disease, the endemic area has been expanding gradually; however, the expansion rate has been considerably slower in the last years, suggesting some limiting mechanism (Mills and Childs, 1998; Sabattini and Contigiani, 1982). JV is transmitted horizontally among rodents, mainly during the breeding period so it can be enhanced by high effective sizes and high levels of gene flow among reservoir populations, which favor the close contact among infected and non infected individuals. Therefore, in order to contribute to understand the progression of the disease, in this work we estimate the effective size and genetic differentiation among individuals in populations from zones with different epidemiological situation: non endemic zones, epidemic areas (where the incidence of the disease is high), and historic areas (former epidemic zones where the incidence has declined to sporadic cases).

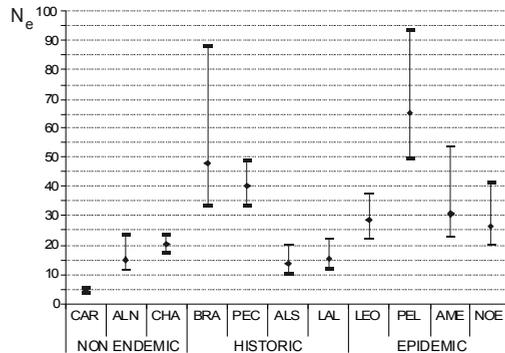
### Materials and methods

*C. musculus* individuals were captured using Sherman-like live traps in 13 geographic populations located in non-endemic (n=3), historic (n=4) and epidemic (n=6) zones in central Argentina. In each population, traps were arranged in two groups (subpopulations) of 3 lines of 20 traps, separated by 2 to 5 km. A total of 211 rodents were genotyped for 6 microsatellite loci following Chiappero et al. (2005). Population effective sizes ( $N_e$ ) were calculated using the Bayesian method implemented in the web-based program OneSamp (Tallmon et al., 2008). The genetic differentiation among pairs of rodents from different subpopulations within populations was estimated by the  $F_{ST}$  statistic (Rousset, 2000); values were averaged by population and means were compared among populations by a Friedman non-parametric test. Additionally, we estimated the internal relatedness (IR; Amos et al., 2001) of individuals, which measures the relatedness of an individual's parents. Mean IR values for the epidemic, historic and non endemic areas were compared using a Friedman test.

### Results

Population effective sizes for all populations are shown in Figure 1.  $N_e$  was lower in non-endemic populations compared to epidemic ones. Populations from the historic area showed different behaviors: BRA (located in the central historic area) and PEC (limiting geographically with the epidemic area) show

high  $N_e$ , while ALS and LAL (that limit with the non-endemic area) present low  $N_e$ . Mean  $\hat{a}$  values were significantly higher in populations from the non endemic area than in populations of the epidemic and historic areas ( $p < 0.001$ ), while IR was significantly lower in the non-endemic area populations ( $p = 0.012$ ).



**Fig. 1** Effective sizes estimates (filled diamonds) and 95% credible intervals (vertical bars) in 13 populations of *C. musculus* from non endemic, historic an epidemic zones of Argentine Hemorrhagic Fever.

## Discussion

Since the discovery of AHF, the endemic area has been expanding gradually, but prevalence of infection in reservoir populations can be very high in some localities and very low or absent at nearby sites. Within an epidemic locality, infected animals are clustered in a restricted area (focal distribution), yielding a patchy distribution of the JV (Calderón, 2004). We found that populations of the reservoir show a higher effective size, higher differentiation among subpopulations and higher levels of internal relatedness in epidemic areas than in non-endemic ones, which would be in line with the proposal of focal distribution of infected specimens. Polop et al. (2007) reported that the population abundance of *C. musculus*, and also the relative abundance of this species with respect to other species of the rodent assemblage, is higher in endemic sites compared to non-endemic ones. The high IR values found in endemic sites could be the result of mating occurring mostly among nearby individuals given the high levels of abundance in those sites which would, in turn, increase the genetic distance between subpopulations. The high effective size in epidemic populations would be enough to maintain the virus-cycle infection; on the contrary, populations with low effective sizes (non endemic areas) would be more prone to clear eventual infections, diminishing the probability of transmitting the virus to other conspecific rodents.

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## Natural hosts of different hantavirus genotypes in south America: who is who?

Gardenal, C.N.<sup>1,5</sup>, Gonzalez-Ittig, R.E.<sup>1,5</sup>, Rivera, P.C.<sup>1,5</sup>, Levis, S.<sup>2</sup>, Salazar-Bravo, J.<sup>3</sup>, Barquez, R.M.<sup>5</sup>

<sup>1</sup>Population Genetics and Evolution, National University of Cordoba, Argentina, ngardenal@efn.uncor.edu

<sup>2</sup>INEVH, Pergamino, Argentina

<sup>3</sup>Department of Biological Sciences, Texas Tech University, Lubbock, Texas, USA

<sup>4</sup>PIDBA, Miguel Lillo Institute, National University of Tucuman, Argentina

<sup>5</sup>National Council of Scientific and Technological Research (CONICET), Argentina

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### Abstract

Accurate species identification of the genus *Oligoryzomys* is particularly important because several species act as natural hosts of hantaviruses affecting humans. We used molecular phylogenetic and a phylogeographic approaches to assign the specific status to individuals from a wide geographical area of Central and South America. We summarize here the rodent-hantavirus relationship emerging from our results and from those available in the literature.

Keywords: Hantavirus, molecular phylogeny, natural hosts, *Oligoryzomys*, phylogeography

### Introduction

The genus *Oligoryzomys* (Rodentia, Cricetidae) groups American native rodents distributed from Mexico to the south of South America. Several species of the genus have been recognized as natural hosts of the etiological agents of Hantavirus Pulmonary Syndrome (HPS) (Levis et al., 1998; Padula et al., 2000). In some cases, two different viral lineages have been associated with the same species of *Oligoryzomys*, contradicting the hypothesis that hantavirus genotypes are host-related (Schmaljohn et al., 1985). These results could be explained by the confusing specific taxonomy of *Oligoryzomys*, mainly because of the high similarity in discrete morphological characters and the lack of revisions taking into account regional differences. To contribute to the knowledge of taxonomy, distribution and evolutionary relationships of *Oligoryzomys*, we analyzed sequences of mitochondrial (mt) DNA in individuals from a vast geographical area of Central and South America. We used standard phylogenetic methodologies but, when different well supported clades within a recognized species were detected, a phylogeographic approach was applied to infer their taxonomic status. We also integrated our results with host-parasite relationships reported for the genus *Hantavirus*.

### Material and methods

Tissues were collected from live-trapped animals from Argentina, Chile and Uruguay. The mtDNA control region and *cytochrome b* (*cyt b*) were used as genetic markers, following procedures described in Rivera et al. (2007) and González-Ittig et al. (2010). All the sequences of different species of *Oligoryzomys* from Central and South America available in GenBank were incorporated to the phylogenetic inferences, and when appropriate to the phylogeographic analyses. Data were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods. Regarding the phylogeographic analyses, we obtained a median-joining network, performed a hierarchical analysis of molecular variance (AMOVA), a mismatch distribution analysis and calculated standard diversity indices.

### Results and Discussion

The phylogenetic trees obtained with both mtDNA segments, using MP, ML and a Bayesian approach presented similar topologies. On the bases of the monophyletic clades recovered, their statistical node support, and in the context of previous available information from cytogenetics and geographical distribution, the following species of *Oligoryzomys* were validated: *O. chacoensis*, *O. destructor*, *O. fornesi*, *O. longicaudatus*, *O. microtis*, *O. nigripes*, *O. stramineus*, *O. moojeni*, *O. fulvescens*, *O. vegetus*, *O. delicatus* and *O. rupestris*. Recently, Hanson et al. (2011) compared *cyt b* sequences of specimens of *Oligoryzomys* captured in Panama and western Venezuela with those from GenBank assigned to *O. fulvescens*; the authors suggest that *O. delicatus* and *O. costaricensis* are separated species from *O. fulvescens*. The specific status of *O. magellanicus*, *O. griseolus*, *O. victus*, *O. andinus*, and *O. arenalis*

are still dubious and should be revised carefully in integrative studies based on molecular, cytogenetic and morphological data from samples covering a wider geographical range.

Regarding the nominal species *O. flavescens*, our phylogeographic analysis revealed the existence of at least four different lineages allopatrically distributed (three of them are present in Argentina). The species *O. flavescens sensu stricto* would have experienced a recent range expansion.

**Tab. 1** Species of *Oligoryzomys* acting as reservoirs of different hantavirus genotypes and geographic distribution of rodent natural hosts (\*according to Hanson et al., 2011)

Species	Distribution	Hantavirus genotype
<i>O. longicaudatus</i>	Southern Argentina and Chile	Andes
<i>O. flavescens</i> “West”	NW Argentina, SW Bolivia and W Paraguay	Bermejo
<i>O. flavescens sensu stricto</i>	E Argentina and S Uruguay	Lechiguanas
<i>O. nigripes</i>	NE Argentina, E Paraguay and S Brazil	Juquitiba
<i>O. microtis</i>	NE Bolivia, E Perú and W Brazil	Rio Mamore
<i>O. chacoensis</i>	N Argentina, SE Bolivia and Paraguay	Oran
<i>O. costaricensis</i> *	West Panama and Costa Rica	Choclo
<i>O. delicatus</i> *	Venezuela and Surinam	Maporal

The integrative analysis of the information on hantavirus genotypes recovered from humans or rodents, and the available data on species delimitation and geographic distribution in *Oligoryzomys*, supports the following relationships between natural hosts and viral genotypes:

The assignment of accurate relationships among different hantavirus genotypes and their hosts is essential to delimit areas in which the HPS could be endemic.

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## Hantavirus infections in forestry workers

Bjedov, L.<sup>1</sup>, Margaletić, J.<sup>1</sup>, Vucelja, M.<sup>1</sup>, Medved, M.M.<sup>2</sup>, Matijević, I.<sup>2</sup>, Krajinović, L.C.<sup>2</sup>, Markotic, A.<sup>2</sup>

<sup>1</sup>Department of Forest Protection and Wildlife Management, University of Zagreb, Faculty of Forestry, Svetošimunska 25, 10000 Zagreb, Croatia, lbjedov@sumfak.hr

<sup>2</sup>University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Mirogojska 8, 10000 Zagreb, Croatia

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### Abstract

Forestry workers are exposed to wild animals, vegetation and soil, which may contain pathogens harmful to humans. Infection can have different pathways from inhalation, direct contact and vector-borne inoculation through the skin injuries. The aim of our study was to determine infection rates in forestry workers caused by different rodent-borne hantaviruses (HTV) in the central Posavina region, Croatia. We tested sera of 295 forestry workers for the presence of specific IgG antibodies for Puumala, Dobrava-Belgrade and Hantaan. Additionally at two different locations also in Posavina region 105 wild rodents were trapped and tested for HTV using direct fluorescence antibody test. Only bank voles were screened for presence of PUU N antigen using Western blot method. Nova Gradiška and Slavonski Brod are - known as endemic regions for HTV. However, the high HTV seroprevalence of foresters (16.3%) was unexpected. 11.2 % of forestry workers were infected with Puumala hantavirus (PUUV) and 4.4% with Dobrava-Belgrade hantavirus (DOBV) and rest of 0.7% were positive for both viruses. Overall 14% of rodents trapped on two locations were tested positive for HTV. In lungs of 26.3% of bank voles virus antigen was detected. Such high infection rates in forestry workers show how important is to monitor rodent populations and to analyze prevalence of particular rodent born zoonoses.

Keywords: bank vole, Dobrava-Belgrade hantavirus, forestry workers, Puumala hantavirus, rodents

### Introduction

Hantaviruses, which are members of a genus within the family *Bunyaviridae*, can cause either of two human zoonoses: hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS) (Schmaljohn and Hjelle, 1997). Hantaviruses are primarily rodent-borne and their transmission from reservoir hosts to humans may occur through inhalation of aerosols of dried excreta, inoculation through the conjunctiva, or entry through broken skin or rodent bites (Hjelle et al., 1995). Forestry workers as well as farmers, soldiers, hunters, campers, hikers, veterinarians, biologists and laboratory workers have the highest risk of infection. The aim this study was to determine infection rates in forestry workers caused by rodent-borne hantaviruses (HTV) in the central Posavina region, Croatia.

### Materials and methods

In the central Posavina region, Croatia, sera of 295 forestry workers and 60 controls (volunteers from the same region) was tested in seven different administrative forestry units for the presence of specific IgG antibodies. The determination of specific IgG antibodies for Puumala and Dobrava-Belgrade virus was done using ELISA IgG test with antigen Hantaan 76-118, Puumala CG 18-20 and Dobrava 907/5. At two different locations also in Posavina region 105 wild rodents were trapped and tested for HTV. Rodents were captured during September using snap traps at two different locations: Okučani (45°22'28"N, 17°17'05"E) and Nova Gradiška (45°18'30"N, 17°17'10"E) (approximately 20 km apart). Sampling of small rodents was done at a hillside on Psunj mountain, 400 meters above sea level, in forests where common beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) predominate. Captured rodents were tested for HTV using direct fluorescence antibody test from kidney and lung tissue. Only bank voles were screened for presence of PUU N antigen using Western blot method described previously (Plyusnin et al. 1995). Total RNA isolated from rodent lung tissues was reverse transcribed followed by PCR amplification with primers specific for PUUV medium (M) or small (S) genome segments (data previously published and described in Cvetko et al. 2005).

## Results

High percentage of forestry workers (16.3%) was found to contain HTNV-specific antibodies. From total of 295 forestry workers in 11.2 % Puumala hantavirus (PUUV) and in 4.4% Dobrava-Belgrade hantavirus (DOBV) reactive antibodies were detected. The rest of 0.7% was positive for both viruses.

Rodents captured included bank voles (*Myodes glareolus*, n=57), yellow-necked mice (*Apodemus flavicollis*, n=35), and wood mice (*A. sylvaticus*, n=13). Overall 14.3% of rodents trapped on two locations were tested positive for hantaviruses. According to the direct fluorescence antibody test wood mouse (*A. sylvaticus*) had 23.1% positive individuals. Within bank voles (*M. glareolus*) there was 14.0% of infected animals and 11.4% within captured yellow-necked mice (*A. flavicollis*). Western blot method showed that *M. glareolus* as the most abundant species had antigen presence in lungs of 15 individuals (26.3 %). Positive *M. glareolus* were found at both locations (see detailed results in Cvetko et al. 2005).

## Discussion

Forestry workers are exposed to wild animals, vegetation and soil, which may contain eukaryotic parasites, fungi, bacteria and viruses harmful to humans. Rodent borne HTV are widely endemic in Europe and at least two different human - pathogenic species, PUUV and DOBV have been reported (Krüger et al., 2011). Our data shows high hantavirus seroprevalence in forestry workers, most like due to PUUV and DOBV infections. The natural reservoirs of hantaviruses are small rodents, and each of the various virus species is associated primarily with a single host species. Rodents captured in the same part of Croatia also show high infection rates. In Croatia the bank vole is considered to be the principal reservoir of PUUV, with yellow-necked field mice, long-tailed field mice, and striped field mice (*Apodemus agrarius*) playing minor roles in maintenance and transmission of other hantaviruses (Borčić et al., 1991; Markotić et al., 2002). The high percentage of infections with hantaviruses in forestry workers and rodents indicated that additional epidemiological measures as well as education of forestry workers about the risk of HTV transmission should be established. Further studies should be conducted, to address the area-, hantavirus- and rodent species specific modes of transmission, as well as mapping of endemic and hyperendemic foci.

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## Prevalence of *Toxoplasma gondii* in Belgian wildlife

De Craeye, S.<sup>1</sup>, Speybroeck, N.<sup>2</sup>, Baert, K.<sup>3</sup>, Ajzenberg, D.<sup>4,5</sup>, Dardé, M.L.<sup>4,5</sup>, Collinet, F.<sup>5</sup>, Tavernier, P.<sup>6</sup>, Van Gucht, S.<sup>7</sup>, Dorny, P.<sup>8,9</sup>, Dierick, K.<sup>1</sup>

<sup>1</sup>Scientific Institute of Public Health, Communicable and Infectious Diseases, National Reference Center for Toxoplasmosis, Engelandstraat 642, B 1180 Brussels, Belgium

<sup>2</sup>Institute of Health and Society (IRSS), Université Catholique de Louvain, Boite 3058, Clos Chapelle aux champs 30, B 1200 Bruxelles, Belgium

<sup>3</sup>Research Institute for Nature and Forest, Wildlife management, Gaverstraat 4, B 9500 Geraardsbergen, Belgium, kristof.baert@inbo.be

<sup>4</sup>Centre National de Référence (CNR) Toxoplasmose / *Toxoplasma* Biological Resource Center (BRC), Centre Hospitalier-Universitaire Dupuytren, Limoges, 87042, France

<sup>5</sup>Laboratoire de Parasitologie-Mycologie, EA 3174-NETEC, Faculté de Médecine, Université de Limoges, Limoges, 87025, France

<sup>6</sup>Wildsurv project, Operational Direction Interactions and Surveillance, Veterinary and Agrochemical Research Centre, Groeselenberg 99, B 1180 Brussels, Belgium

<sup>7</sup>Scientific Institute of Public Health, Communicable and Infectious Diseases, National Reference Center for Rabies, Engelandstraat 642, B 1180 Brussels, Belgium

<sup>8</sup>Department of Animal Health, Institute of Tropical Medicine, Nationalestraat 155, B 2000, Antwerp, Belgium

<sup>9</sup>Laboratory of Parasitology, Ghent University, Faculty of Veterinary Medicine, Salisburylaan 133, B 9820 Merelbeke, Belgium

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*Toxoplasma gondii*, an obligate intracellular protozoan parasite, has a worldwide high prevalence in most warm-blooded animals and humans. Few studies are available on the occurrence of this parasite in wild animals. In this study we investigated the prevalence of *T. gondii* in Belgian wildlife. We tested brain samples from red foxes (*Vulpes vulpes*), European polecats (*Mustela putorius*), European pine martens (*Martes martes*), raccoons (*Procyon lotor*), brown rats (*Rattus norvegicus*), muskrats (*Ondatra zibethicus*) and roe deer (*Capreolus capreolus*). The samples were tested by Real Time PCR for the presence of *T. gondii* brain cysts. The amplified DNA target was the 529 bp *T. gondii* 'repeat element' (AF146527). To check for inhibition, the cellular r18S gene was used. The prevalence was found to be: red fox: 57/304; European polecat: 2/2; European pine marten: 1/2; raccoon: 0/2; brown rat 19/335; muskrat 2/10 and roe deer 1/33. Twenty-six of the *T. gondii* positive DNA samples from foxes were genotyped: 25 were type II and one type III. In addition, 73 roe deer serum samples were tested by SAG1 ELISA for the presence of anti-*T. gondii* antibodies, 38 (52%) were positive.

## Surveillance of *Echinococcus multilocularis* in rodents in the vicinity of the finding of the first infected red fox (*Vulpes vulpes*) in Sweden

Olsson, G.E.<sup>1</sup>, Hörnfeldt, B.<sup>1</sup>, Ågren, E.<sup>2</sup>, Wahlström, H.<sup>2</sup>

<sup>1</sup>Swedish University of Agricultural Sciences, Umeå, Sweden, gert.olsson@slu.se

<sup>2</sup>National Veterinary Institute, Uppsala, Sweden

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### Introduction

The fox tape worm *Echinococcus multilocularis* (EM) was recently detected in Sweden for the first time. Increased surveillance of foxes have shown that the prevalence on country basis is low, approximately 0.1-0.2%. In an attempt to identify the intermediate host(s) included in the life cycle of EM in Sweden, local rodent populations were sampled in the vicinity of the first location (Västra Götaland county) of EM discovery.

### Methods

Sampling was performed in the south west part of Sweden, within a 50 km radius surrounding the finding of the first infected fox. The aim of the sampling was to maximise the number of likely fox prey of possible EM competent intermediate hosts, though without any particular statistical design.

### Results

In total, 236 rodents were trapped in April 2011. The most commonly trapped species was *Arvicola amphibius* (former *A. terrestris*), followed by *Myodes glareolus*, *Microtus agrestis*, *Apodemus sylvaticus*, and *A. flavicollis*. By May 31, a total of 152 rodents have been autopsied and no macroscopic lesions of EM had been detected. Final results are expected in June 2011.

### Discussion

*A. amphibius* was the most commonly caught rodent, is the largest in size and perhaps exhibits the highest biomass of voles per unit area where it occurs. *Microtus arvalis*, one of the main intermediate hosts for EM in southern Europe, does not occur in Sweden and *Ondatra zibethicus*, another known intermediate host for EM only occurs in the northern part of Sweden.

As the prevalence in rodents is expected to be lower than in foxes, the probability of finding EM in 200 rodents is probably rather low. Therefore, there is a need for additional, preferably targeted, sampling. One strategy could be to focus on species with high local densities and thus high probability of attracting foxes. In such favourable habitat patches, the probability of foxes defecating, and thus deposit EM eggs, may also be considerable although defecation otherwise is used for territorial markings. This may in turn render these rodent populations higher probability of EM egg exposure from fox faeces, and subsequent higher prevalence to EM infection. A parallel attempt to obtain rodent material, that has proven successful for other purposes, is to utilize the habit of for example different owl species to cache preyed rodents in artificial nest boxes, by collecting such rodents and replacing them with laboratory mice.

## **The rate of trematode infections in wild ungulates in Naryn State Nature Reserve of the Kyrgyz Republic**

Shermatov, S.

Departement of Agrotechnology, Naryn Staat University, Kyrgyz Republik, shermatov@rambler.ru

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Parasitic infections of wild ungulates frequently occur in mixed host populations. To study this, helminthological research has been carried in the Naryn State Nature Reserve in Kirgisia since 2001.

Complete helminthological necropsy of liver and digestive tract of ungulates was performed. In addition to this, 1,325 domestic animals from households surrounding the nature reserve were examined for trematodes.

Wild ungulates in the Naryn State Nature Reserve have the following trematode fauna: *Fasciola hepatica*, *Dicrocoelium lanceatum* and *Parafasciolopsis fasciolaemorpha*. The long-term investigations show that both the prevalence and intensity of *Dicrocoelium* are increasing in wild ungulates. In some years, prevalence in red deers was 100% and in roe deers 85%. On average, prevalence of *D. lanceatum* according to the last data is 53.7% and intensity 64.3 specimens.

Investigations of domestic animals which feed on highland pastures surrounding the nature park revealed that dominant helminths are *Dicrocoelium lanceatum* and *Fasciola hepatica* and less common are *Fasciola hepatica* and *Parafasciolopsis fasciolaemorpha*. Trematode prevalence in domestic cattle was 59.0%. The prevalence of *Fasciola* in cattle was 25-38% and intensity 62, dicroceliasis in sheep 80% and 79-117.

In the Naryn State Nature Reserve the main trematodosis of wild ungulates are dicroceliasis, fasciolosis and seldom paramphistomatosis. Multispecies infections are common. Epizootological situation of Naryn State Nature Reserve indicates that trematode infections afflict considerable number of wild and domestic animals in the nature reserve and in the surroundings. A special hazard is the flow of trematodes between wild and domestic species, like *D. lanceatum* from domestic animals to wild ungulates and *P. fasciolaemorfa vice versa*.

## **Evaluation of bait acceptance by wild boar and non-target species - test of different distribution modalities and seasonal variations - implication for oral vaccination efficiency against classical swine fever virus**

Sage, M., Hubert, P., Rossi, S.

Game and Wildlife Agency, Wildlife Sanitary Unit, Au Bord du Rhin, 67150 Gerstheim, France,

mickael.sage@oncfs.gouv.fr

4National de la Chasse et de la Faune Sauvage, France

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### **Abstract**

Field assessment of the proportion of target and non-target individuals that consume baits is crucial to evaluate and optimize the cost-efficacy of a baiting campaign. In our study, different pre-baiting and baiting systems were tested at a long time scale (12 months) to try to improve vaccination efficiency of wild boars against classical swine fever virus. Evaluation of seasonal variation in natural food resources (competition with bait consumption) and life cycle succession of wild boar from piglets to adults is included and consumption of baits by non-target species is discussed.

Keywords: bait consumption, classical swine fever, disease control, oral vaccination efficiency, wild boar

### **Introduction**

The European wild boar (*Sus scrofa*) is a major reservoir host for pathogens that affect humans and domestic animals. Bait delivery of vaccines to this target species is an essential component of disease management strategies worldwide. In spite of the long-term oral vaccination implemented in different European countries since the 1990s, classical swine fever virus (CSFV) may persist (von Rügen et al., 2008). To improve vaccination efficiency in the field, the utilisation of different pre-baiting and baiting systems have been suggested, but no data are available concerning the evaluation of such measures.

Recent studies based on animal immunity by examining the serological status of hunted wild boar from a vaccinated area, suggest a seasonal variability of vaccination efficiency. However, these previous field studies were limited in assessing vaccination efficiency (Rossi et al., 2010; von Rügen et al., 2008).

The objective of this study was to evaluate by camera trapping the bait consumption at a long time scale (12 months) according to different modalities of distribution. We aimed to include all seasonal variation in natural food resources (competition with bait consumption) and life cycle succession of wild boar from piglets to adults.

### **Materials and methods**

A field uptake study was carried out from autumn 2010 to summer 2011 in the Vosges mountains (north-eastern France). Baits were distributed on the ground every 15 days on different pre-baiting and baiting systems: on 6 regular feeding places (2 kg of maize distributed per day on 15 m<sup>2</sup>) and 3 occasional feeding places (5 kg of maize distributed every 15 days on a transect of 200 m); close to 3 boar's wallows (without maize distribution) and to 3 tracks (without maize distribution).

Bait uptake was determined from examining pictures taken with digital game cameras with infrared illumination. The relative frequency of visits by different animal species was reported at each site.

### **Results**

Up to now, only data from August to November 2010 were analyzed. These results suggest that whatever the site of bait distribution, non-target species such as Eurasian badgers (*Meles meles*), red foxes (*Vulpes vulpes*), mustelids spec. were frequently observed consuming baits (44 of the 305 distributed baits). Sixteen percent of young wild boars (<1 year) and 41% of sub-adults (1-2 years) were consuming baits when being in contact with the baits. Up to now, no consumption by adults was yet observed, but these data are based on very few contact occasions with baits to allow any conclusion (n=2).

Boar's wallows and regular feeding places appeared to be the most efficient areas for bait uptake while very little consumption was observed on occasional feeding places or close to tracks.

Baits consumed by wild boars were all eaten within 3 days after distribution and were mostly consumed within the first day (0.35 day; CI95% 0.08-0.69). As the CSFV vaccine is stable for 3-4 days at ambient temperature (Brauer et al., 2006), those results suggest an optimal vaccination after bait consumption.

### Discussion

These first results observed in autumn possibly arise because the natural and artificial food resources were generating moderate bait uptake by wild boar. Analyses of the last aspect is currently in progress. Definitive results including seasonal variation according to the availability of natural food and life cycle succession of wild boar will be presented during the symposium. These findings will provide a basis for the development of new solutions including time tables and different pre-baiting and baiting systems to improve vaccination efficiency. These new solutions may be transferable for the distribution of anti-parasitic drugs, toxicants or contraceptives to wild boar.

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## **Managing wild boar - considerations for wild boar management based on game biology data**

Keuling, O.

Institute for Wildlife Research at the Veterinary Medicine University of Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany, oliver.keuling@tiho-hannover.com

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### **Abstract**

The wild boar is a large ungulate which may produce serious economic problems. As the wild boar is highly reproductive, a fast dispersing species and flexible in behavior, management has to be adapted to the adaptive wild boar populations. On the other hand, the wild boar lives small scaled in family-groups, which enables management concepts on regional scale. Female wild boar of all age classes should be hunted in favour by comprehensive hunting methods for wild boar population regulation.

Keywords: game management, hunting management, radio-telemetry, reproduction, *Sus scrofa*, wild boar, wildlife biology

### **Introduction**

All over Europe hunting bags of wild boar *Sus scrofa* are increasing. Rising population densities and expanding distribution due to increased nutritional conditions, climate change and inefficient hunting require an effective and biologically based wild boar management to prevent economic problems (e.g. crop and grassland damage, transmitting zoonoses to livestock and humans). A lot of wild boar research has been done in the last decades. However, game managers were not able to incorporate this knowledge into the development of effective management strategies. Thus, further studies have to be accomplished aiming to combine wildlife biology and management.

Research results from radio-telemetric studies on wild boar ethoecology, estimation concepts, reproduction, mortality rates and efficiency of different hunting methods that provide a basis for management concepts.

### **Material and Methods**

I reviewed literature and own results on wild boar etho- and demecology and compared wild boar biology with hunting bag statistics, efficiency of hunting, and population models to describe adapted management concepts.

### **Results and Discussion**

Mean space use, especially of reproductive females within family groups, is small scaled; dispersal rates are low and male biased. Habitat use-availability-analyses showed that wild boar may prefer crop fields in summer. High damage of crops and grassland may occur. Moderate hunting has only small impact on wild boar behavior compared to seasonal influences. Changed space use patterns are mainly influenced by changed food availability. The wild boar is a flexible species, shown by high variation of all observed results between individual groups. Wild boar groups react flexible to several seasonal intrinsic and extrinsic factors. The omnivore wild boar easily adapts to various environments. Its wide eco-ethological plasticity allows the species to colonize new habitats and to enlarge its distribution.

Population estimations of wild boar are still insufficient. Thus further research has to be done to compare population data with hunting bag statistics to know exact harvest rates for regulation of populations. Some future attempts will be genetic analyses of hunting bags and phototrap counts.

Contrary to the high mean reproduction rate of wild boar with 262%, the mortality rate is in many European studies about 50%, the quota should be about 80%. Thus, the sustainable harvest rate was not exhausted. Life history models show that 80% of a year's piglets and additionally some older females have to be harvested to regulate the population. Single hunt from hides is the dominating and an efficient hunting method. Hunting efficiency should be raised by conducting more comprehensive drive hunts to

raise the proportion and amount of piglets and to shoot especially young adult females, which have the highest future reproductive success due to age class specific reproductive and mortality rates.

### **Conclusion**

All over Europe hunting rates seem to be lower than reproduction of wild boar. To reduce populations and thus, damages, hunting rates have to be increased especially for females, as all age classes of females are highly reproductive. The strong site fidelity of wild boar enables regional and even local management concepts. However, the high flexibility and learning aptitude require adapted flexible management strategies. Last but not least, game managers will have to incorporate willingness and ability of leisure hunters and stake holders into management concepts. The combination of different hunting methods is necessary to achieve efficient regulation or reduction in comprehensive areas.

## **Ecological impacts of feral pigs (*Sus scrofa*) on freshwater ecosystems in tropical Australia**

Mitchell, J.

Biosecurity Queensland, Natal Downs Rd. Charters Towers, Queensland, Australia, 4820,

jim.mitchell@deedi.qld.gov.au

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### **Abstract**

A range of ecological indicators found in northern Australian tropical freshwater habitats were used to quantify feral pig impacts on elements of biodiversity. These indicators were measured for two years in both unprotected ephemeral freshwater lagoons and those protected by fencing from pig impact. The sequential measurements of these ecological indicators as the lagoons drew down gave a guide to the consequences of feral pig impacts on biodiversity. Overall, feral pig activity had a negative impact on the ecological condition of the ephemeral lagoons studied with the major impacts related to destruction of macrophytes and a reduction in water clarity. The visual differences in the proportion of aquatic macrophytes between protected and unprotected lagoons were dramatic; protected lagoons had significantly more macrophyte coverage. The upheaval of wetland sediments in unprotected wetlands significantly reduced the water clarity and had subsequent effects upon key water quality parameters such as dissolved oxygen availability. Other water quality parameters such as nutrients were also strongly affected by pig activity contributing to an increase in nutrient levels in the unprotected lagoons. We have demonstrated that feral pigs do have significant impacts upon wetlands in the tropical environments we studied. However, we have also demonstrated that there are significant natural disturbances also operating in these ecosystems that should be taken into account when assessing impacts to wetlands.

Keywords: ecological impact, freshwater biodiversity, *Sus scrofa*

### **Introduction**

In Australia, the community general perception is that feral pigs are doing substantial ecological damage and pose a threat to ecological values of many regional ecosystems. There is a distinct lack of information in relation to the dry tropics seasonal freshwater habitats. A number of rare or endangered species and ecosystems regarded as threatened or suspected of being threatened by feral pig impacts occur in the dry tropics and in particular the tropical freshwater ecosystems. A range of ecological indicators found in freshwater habitats was used to quantify feral pig impacts on elements of biodiversity. These indicators were measured for two years in both unprotected ephemeral freshwater lagoons and those from which pig impact was excluded. The sequential measurements of these ecological indicators as the lagoons drew down gave a guide to the consequences of feral pig impacts on biodiversity.

### **Materials and methods**

This study was conducted in Lakefield National Park in the tropical savannas area of northern Australia, an area of high conservation values. Lakefield is renowned for its vast river systems and spectacular wetlands. In the wet season rivers and their tributaries join to flood vast areas. During the dry season, rivers and creeks leave large permanent waterholes, lakes and lagoons which attract a diversity of animals, particularly waterbirds. Large populations of feral pigs also inhabit this high value freshwater ecosystem; population densities of 4.3 pigs/km<sup>2</sup> have been calculated for this study site.

This study consisted of 'paired' lagoons containing a wide array of submerged, emergent and floating aquatic plants at each of four locations. At each location, one lagoon was enclosed by a pig-proof fence; the associated lagoon was fenced with a 4 plain wire fence to exclude feral cattle. Water quality was sampled over two years at approximate two month intervals. A multi probe was used to record water quality parameters at 30-min intervals for a 24-h period. Proximal lagoons were measured in tandem. Water was sampled for total and dissolved components of nitrogen and phosphorus, ammonia and turbidity. Between four and six permanent transects at 15-25 m intervals in each lagoon were used to measure species composition and abundance of emergent and submersed macrophytes. Sampling for aquatic invertebrates and freshwater fishes was also conducted.

## Results

Pig disturbance created an obvious disturbance of the lagoons. The entire substrate around the margin of all unfenced lagoons was turned over as a result of pig rooting activities. This pig rooting resulted in progressively decreasing aquatic plant cover and increasing amounts of open water and bare ground. There was a significant interaction between fencing treatment and time ( $F_{2,12} = 0.66$ ,  $p = 0.002$ ). This was due to a significant decrease in macrophyte coverage over time in the unfenced, but not fenced treatments. Although water clarity also naturally declines over the course of the dry season, this effect was further exacerbated as a result of pig disturbance. This loss of aquatic plant cover and decline in water clarity were the strongest effects detected in this study. Nutrient concentrations did increase over the course of the season as would be expected with decline in water level but the effects of pigs greatly increased the nutrient levels beyond their natural increase. Dissolved oxygen conditions progressively deteriorated over the course of the season but this effect was heightened in unfenced lagoons compared to fenced lagoons. Pig disturbance is implicated in negatively impacting dissolved oxygen availability and increasing harmful ammonia, nutrient and turbidity levels. No effect of pig rooting was observed on macroinvertebrate or fish species composition and abundance.

## Discussion

This study demonstrates that the foraging activities of feral pigs in these floodplain lagoons disrupt physical, chemical and biological environments. Pig-mediated disturbance in the unfenced lagoons significantly affected water clarity by dramatically increasing turbidity. The degree to which this may have altered primary productivity is unknown, however we have clearly linked pig foraging to the destruction of aquatic macrophytes, and the proliferation of bare ground and open (but turbid) water in these lagoons. Feral pigs pose a serious ecological and economic threat in many parts of the world, including Australia. We argue, however, that their true ecological effects might be best measured in a landscape-specific framework because their effects probably depend on the biology and disturbance history of the affected community and pigs are problematic in a very wide variety of wetlands across Australia.

We have demonstrated that feral pigs do have significant impacts on the water health of wetlands in these tropical wetland environments. Macrophyte populations and water clarity/nutrients are strongly influenced by pig foraging. We have also demonstrated that the level of impacts is also related to the pig population abundance.

## The impacts of feral boar on woodland flora and fauna in Great Britain

Mayle, B.<sup>1</sup>, Harmer, R.<sup>1</sup>, Kewitt, A.<sup>1</sup>, Peace, A.<sup>1</sup>, Straw, N.<sup>1</sup>, Williams, D.<sup>1</sup>, Upson, M.<sup>2</sup>

<sup>1</sup>Forest Research, Farnham, Surrey, GU10 4LH, UK, [brenda.mayle@forestry.gsi.gov.uk](mailto:brenda.mayle@forestry.gsi.gov.uk)

<sup>2</sup>MSc in Conservation and Forest Protection, Imperial College, Silwood Park, Ascot, Berkshire, SL5 7PY, UK

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### Abstract

Feral boar have relatively recently become established in woodlands in Great Britain, but the long-term effect of their presence, in particular rooting activity, on woodland habitats and biodiversity is unknown. Protocols to investigate boar presence, densities and impacts to inform policy makers and practitioners are being investigated within a collaborative project by Forest Research and The Food and Environment Research Agency, funded by Forestry Commission and Defra. Results are presented from initial studies on plants and invertebrates carried out in a series of woodlands in south east England.

Keywords: biodiversity, feral boar, impacts, *Sus scrofa*, woodland

### Introduction

Although feral boar (*Sus scrofa* L.) are well established throughout most of the rest of Europe, in Great Britain breeding populations have only recently become established in the wild through escapes/releases from captive and farmed populations (Spitz, 1999). In woodlands their rooting activity can be visually very obvious but the effect of this disturbance of soil and litter on woodland biodiversity and ecosystem services is unknown. Studies are ongoing to develop protocols with which to evaluate boar impacts on biodiversity (positive and negative) within specific habitats in woodlands in Britain.

### Materials and methods

Twelve broadleaved woodland sites in the south of England, with differing levels of boar activity, were selected. Invertebrates active on the ground and in the litter layer were sampled using 10 pitfall traps per site, in two transects of 5 traps placed at 10 m intervals. Transects were located in areas of differing rooting activity where possible, and protected to limit the effect of direct interference by boar on sample collection. Numbers of individuals in each of the main invertebrate groups were recorded, and ground beetles (Carabidae) identified to species. Ground vegetation was assessed during May and August in triplets of 2x2 m quadrates located at 10 m intervals along the transects used for pitfalls. The middle quadrate of each triplet was placed over the pitfall trap; an additional quadrate triplet was located 10 m beyond the last pitfall trap.

Stand characteristics and bluebells (*Hyacinthoides non-scripta*) were assessed during May using 30 4x4 m quadrates placed at 30 m intervals along parallel transects 30 m apart. The percentage of bluebell cover was assessed along with an index of level of presence of bramble (*Rubus fruticosus* agg.), bracken (*Pteridium aquilinum* L.), grasses and sedges (*Graminae* and *Cyperaceae*), rushes (*Juncaceae*), ericaceous (*Ericaceae*) species, shrubs <2 m, other herbaceous vascular plants. The dominant tree species in the under-storey and over-storey were recorded within 10 m of the quadrate centre, and the stand structure recorded.

Presence and level of rooting activity within the pitfall transects was recorded on alternate visits, during August for the quadrate triplets, and during assessment of the bluebell quadrates.

### Results

The sites were generally unmanaged coppice growing on clay soils with oak (*Quercus* sp.) and sweet chestnut (*Castanea sativa* Mill.) the main over-storey species, and hornbeam (*Carpinus betulus* L.) and sweet chestnut the under-storey. In May, the ground flora was dominated by herbs, mainly bluebell and anemone (*Anemone nemorosa*) which senesce in June, hence ground cover in summer for all sites was very sparse. Vegetation along pitfall transects in May was similar with mean site cover 50%, but by August this had declined to <10%. A total of 50 plant species (12-27 within a woodland) were recorded along the pitfall transects, with fewer species recorded in May than August. Bluebell and anemone were the most common species.

Extensive rooting activity was recorded during spring when sites and location of transects were decided and recently rooted areas were readily identified. It became progressively more difficult to distinguish between recent and old rooting. There was great variation between and within sites in the amount and distribution of rooting, in terms of numbers of quadrates rooted (13-100%) and area of rooting in each quadrate (<1-40%).

Most rooting was recorded beneath neglected coppice with standards. This was marginally significant ( $p \leq 0.05$ ), and probability of rooting was greater where oak and sweet chestnut were the dominant over-storey ( $p \leq 0.05$ ). There was no relationship with under-storey species. Although the frequency of rooting along the pitfall transects was low it reflected the general amount of rooting recorded across each site as a whole.

There were large differences within and between sites in the amount and distribution of bluebells. Presence of bluebells ranged from 20-100% of quadrates in a site, with cover within quadrates ranging from 2-45%. There was no significant association between the occurrence of overall disturbance within a quadrate and the presence or absence of bluebells.

Large numbers of invertebrates were captured with 67,449 being identified to species, family or order; ants were the most common (36% of individuals). Beetles were the next most abundant with >50% of these being ground beetles. Although 33 ground beetle species were caught (12,629 individuals) only *Abax parallelepipedus* and *Pterostichus madidus* were abundant. Between 10 and 20 ground beetle species occurred at each site with 4 species occurring at every site. Although the total number of beetles caught was related to the amount of rooting, the number of species found at a site was not. Total numbers of ground beetles were also related to the % cover of bluebells.

Total numbers of *Geotrupidae*, *Staphylinidae*, *Curculionidae*, and *Silphidae* were not related to % rooting or bluebell cover either at the site or trap level.

## Discussion

There is evidence for some significant associations between feral boar rooting and woodland structure and species, and the presence of bluebells. This is consistent with the expectation that rooting will be greater where the habitat contains species producing large fruits such as oak and sweet chestnut, and that rooting will have an adverse effect on bluebells. However, clear conclusions are not possible due to large variation within and between sites and the short term of the study.

The positive relationship between total numbers of ground beetles and percentage rooting at the site level probably reflects a tendency for boar to root more frequently in sites where large numbers of ground beetles are already present. The species richness and diversity of ground beetle community showed no relationship with rooting.

We have developed an effective method for sampling ground dwelling invertebrates in the presence of boar (Harmer et al., in press). Studies are continuing, focusing on other woodland habitats and priority species groups such as pollinators.

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## Factors affecting the level of damage by wild boar in farmlands in north-eastern Poland

Frąckowiak, W.<sup>1</sup>, Gorczyca, S.<sup>2</sup>, Merta, D.<sup>1</sup>, Wojciuch-Płoskonka, M.<sup>1</sup>

<sup>1</sup>Department of Ecology, Wildlife Research and Ecotourism, Biology Institute, Pedagogical University of Krakow, Podbrzezie 3, 31-054 Kraków, Poland, fracko@poczta.fm

<sup>2</sup>Parliamentary Team for Forestry, Environmental Protection and Hunting Traditions, Wiejska 6, 00-902 Warszawa, Poland

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### Abstract

The level of damage caused by wild boar in 33 forest districts was investigated. The study area covered 2.231 million ha including 0.662 million ha of forests. In the last six years (2005-2010) the wild boar population has increased from 15,200 to 24,100 individuals and harvesting from 10,700 to 20,700. In the same period, the area of farmland damaged by these animals increased from 1,470 ha to 2,800 ha. The level of damage results primarily from 5 variables of which the most important are population density ( $r=0.67$ ) and Simpson's biodiversity index ( $r=0.54$ ). The population density of wild boars, however, was affected by 5 variables. The most important was Simpson's biodiversity index ( $r=0.86$ ), proportion of oak and beech trees ( $r=0.80$ ) and amount of cereal food at baiting sites ( $r=0.68$ ). The highest level of wild boar-related damage was recorded in April (on meadows) and in August (in cereals). The financial performance of wild boar management indicates that the income from selling carcasses is lower than the damage compensation. The paper discusses the methods to reduce numbers of wild boars, based on reliable population census data and reproductive patterns.

Keywords: biodiversity index, damage compensation, management strategy, oak and beech proportion, relative population density

### Introduction

Over the last 20 or so years Poland has witnessed an uncontrolled increase in wild boar population numbers. Hunting bags have increased year by year, but according to the hunting statistics the wild boar population levels and the extent of damage to farmland have also risen. In the 2009/10 hunting season the number of wild boars harvested was 217,900 individuals, whilst the estimated population number after the hunting season stood at 249,900, and the compensation payments made to farmers for boar-related damage amounted to 12.4 million €. The aim of study was to learn about the factors affecting the level of damage exerted by wild boars in farmlands in north-eastern Poland.

### Material and methods

The data collected covers 6 hunting seasons (2005/2006-2010/2011) was obtained from 365 hunting districts, whose game management is supervised by 33 forest districts reporting to the Regional Directorate of State Forest in Olsztyn. The total area of all these hunting districts is 2.231 million ha including 0.662 million ha of forest. Data from hunting districts were pooled to obtain the total figures for each forest district. The length of forest-farmland border line was determined for each forest district using digital maps. The data on the structure of forest stands were used to calculate the biodiversity index (Simpson, 1949). In January 2011, the game statistics data regarding wild boar population numbers were verified in 3 forest districts by block count technique (Bobek et al., 2009; Kogenazawa et al., 1995) and a method for analyzing the results of collect hunts (Bobek et al., 2009).

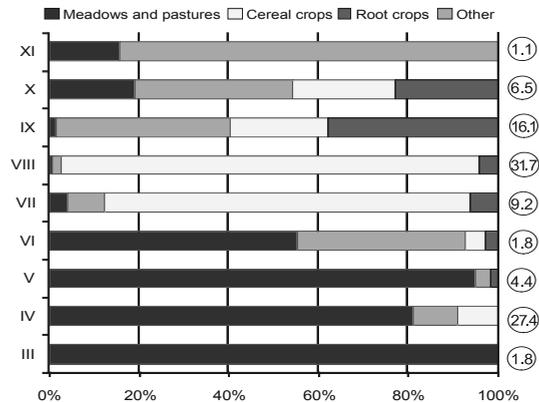
### Results

In 2005, the hunters estimated the wild boar numbers at 15,000 whereas in 2011 it stood at 24,000 individuals. The harvesting figures for the wild boar population increased systematically from 11,000 to 21,000 animals, but the area of farmlands affected by boar damage also increased from 1,470 ha to 2,800 ha. The data from the 33 forest districts show a lack of significant correlation between changes in population numbers and hunting bag ( $r=0.099$ ,  $p=0.607$ ). Verification of wild boar numbers reported by hunters indicated that boar numbers were 20.7-40.4% higher. Our calculations used the number of wild boars harvested per 1,000 ha of forest as a measure of relative population density.

The level of damage by wild boars in farmlands increased in line with the increases in population density of boars ( $r=0.67$ ), the density index in farmland-forest ecotones ( $r=0.51$ ), proportions of oaks and beech ( $r=0.50$ ), the Simpson biodiversity index for dominant tree species ( $r=0.54$ ), and age classes of forest stands ( $r=0.47$ ). However, the impact of the increased density index of feeding strips with corn protecting farmland against wild boar damage was not significant.

There was a significant positive correlation of the relative population density with the density index in the ecotone ( $r=0.47$ ), proportion of oak and beech in the stands ( $r=0.80$ ), the amount of cereal food at baiting sites ( $r=0.68$ ) and biodiversity index for dominant tree species in forest stands ( $r=0.86$ ). The wild boar population density was negatively correlated to the proportions of pine, larch and spruce in the forest stands ( $r=-0.83$ ).

The highest proportions of damage by boars occurred in August (31.5%) and in April (27.4%). The August damage affected mostly cereal crops, whereas in April, the main damage was inflicted in meadows and pastures (Figure 1).



**Fig. 1** Agricultural crops damaged by wild boar in north-eastern Poland. Percent of various crops damaged during every month is given in bars. Data in circles represent monthly distribution (%) of the total area damaged during growing season.

Table 1 shows that the revenues obtained from boar carcass sales did not exceed the damage compensation. Therefore, this shortfall had to be covered by revenues generated from the sale of roe and red deer meat.

**Tab. 1** Fiscal balance of wild boar management in northeastern Poland

Year	2006	2007	2008	2009	2010
Damaged area (ha×103)	1.47	1.47	1.99	2.52	2.82
Value of compensation (Euro×103)	452.5	477.5	712.5	900.0	872.5
Number of wild boar harvested (×thousand)	9.56	9.57	13.53	20.19	20.66
Income from wild boar carcass sale (Euro×103)	335.0	360.0	507.5	757.5	775.0
Fiscal balance (income-compensation (Euro×103))	-117.5	-117.5	-205.0	-142.5	-97.5

## Discussion

An uncontrolled increase in wild boar population numbers has resulted from the fact that objective methods for estimating population numbers have not been applied. The estimations made by hunters are based on guesstimates – estimations whose margin of error can be significant. Because of high population densities, the presence of feeding strips with crops for boars, does not affect the level of damage inflicted by boars in farmlands. Therefore, apart from the expensive and controversial fencing, the only available method of alleviating the damage in farmlands is to reduce boar population densities based on reliable data on population numbers, population net increase and the reproductive pattern of

local populations (Bieber and Ruf, 2005). In Poland, several methods for estimating wild boar population numbers have been already tested (Bobek et al., 2009; Fonseca et al., 2007). However, the implementation of these methods into practice is hampered by the law, which stipulates that all decisions concerning game management rest with the Polish Hunting Association.

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## **The wild boar *Sus scrofa* L. as neighbor in an agricultural landscape – a new project**

Herbst, C., Keuling, O.

Institute for Wildlife Research at the Veterinary Medicine University of Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany, coralieherbst@gmx.de

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With the increasing and spreading of wild boar populations the damage in agricultural fields and the threat of diseases like European swine fever are on the rise. In case of an outbreak of European swine fever the damage to the domestic pig breeding may be billions of Euros for Germany alone. Therefore, farmers and veterinary authorities claim that regulation and reduction of the wild boar population is required.

A management system is necessary, which integrates the social structure and behavior of the wild boar with hunting. A new project that started in 2011 in northern Germany will test the reduction of game damage and reducing the risk of an outbreak of European swine fever by means of hunting management.

Radio-marked wild boar will give new information about movement patterns and habitat utilization in a landscape dominated by agriculture. Data will be used to detect age dependent differences among the wild boars causing damage. The role of the leading sow in a wild boar group will be considered to reveal how shooting of the leading sow may affect the group. A better understanding of her role in the process of dispersal and hence the increasing risk of spreading diseases is needed to develop a management approach based on game biology data.

Keywords: agriculturally dominated area, European swine fever, leading sow, spatial behavior, *Sus scrofa*

## **Conundrum of the Eurasian wild pig *Sus scrofa* status on the island of Singapore: human-wildlife and environmental conflict**

Haridas, S.<sup>1</sup>, Diong, C.H.<sup>1</sup>, Seet, G.<sup>2</sup>, Lee, N.S.L.<sup>3</sup>

<sup>1</sup>Division of Natural Sciences and Science Education National Institute of Education, Nanyang Technological University, Singapore, cheonghoong.diong@nie.edu.sg

<sup>2</sup>Division of Mechatronics and Design, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore

<sup>3</sup>Vertta Technical Services, Singapore

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The Eurasian wild pig (*Sus scrofa*) is Singapore's largest, native terrestrial mammal. Once thought to extinction, recent records suggest that the pigs have recolonized the urbanized main island of Singapore from southern peninsula Malaysia and offshore islands. Despite the numerous sightings of wild pigs in the Western and Central parts of Singapore, no population density estimate is available. Apart from the spread of exotic species and alteration of community structure in fragmented forest patches, other impacts caused by wild pigs include conflicts with human activities. Intrusion of wild pigs into open spaces and recreational areas have caused damage to lawns, gardens, urban parks, turfs, fairways and greenery from golf courses to cemeteries. Wild pigs have contributed to military aircraft and vehicular accidents with recent evidence suggesting the propensity to extend the hazards to civilian road users. Injuries to humans were the consequence of encroachments into wild pig habitats mainly due to illegal hunting and military training activities. Unless systematic density surveys and ecological studies are conducted, lessening the magnitude of human-wildlife conflicts posed by the wild pig on native biodiversity and the environment will remain a challenge. Wild pig activities and their propensity for human-wildlife conflict in Singapore are discussed. A novel, safe and unobtrusive study method employing the use of wireless aerial thermal videography onboard a helium-inflated airship is also discussed.

## Wild boar population at the Vistula Spit – management of the species in forested and urban areas

Bobek, B., Fraćkowiak, W., Furtek, J., Merta, D., Orłowska, L.

Department of Ecology, Wildlife Research and Ecotourism, Biology Institute, Pedagogical University of Krakow, Podbrzezie 3, 31-054 Kraków, Poland, b.bobek@o2.pl

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### Abstract

The area of the spit of Vistula river spans 166 km<sup>2</sup> including 49 km<sup>2</sup> of forest. Population numbers of wild boars living in forests (n=290) and in urban areas (n=56-82) were determined. The city wild boars had a higher piglets/female ratio than forest wild boars (4.3 vs. 3.8). Among the culled forest wild boars (n=62), 20% of piglets had *corpora lutea* whereas 57.1% of subadult and adult females were pregnant. The size of the daily home range of city wild boars (n=7) was determined by radio-telemetry and found to be 3.5-5.8 ha. A questionnaire-based opinion survey was carried out among local residents and visitors regarding the conflict between city wild boars and humans. The paper also discusses possible solutions to the conflict.

Keywords: city wild boar, home range, human attitudes, population census, radio-telemetry, reproduction, Poland

### Introduction

Increased population numbers in wild boars result in invading urban areas. In Western Europe, the best known examples are wild boar populations living in the urban areas of Barcelona and Berlin (Cahill et al., 2003; Kotulski and Konig, 2008). These city wild boars occur also in a number of cities throughout Poland, which is the case particularly in the Tricity of Gdańsk-Gdynia-Sopot (Szramka and Karbownik, 2009), Katowice, and Kraków. On the Baltic coast, wild boars have also colonized summer holiday resorts in forests. One such area is the Vistula Spit. The aim of this study was to present the conflict between the wild boar and man and to suggest solutions.

### Methods

The area of the Vistula Spit is 166 km<sup>2</sup> of which 49 km<sup>2</sup> are covered primarily by coniferous and mixed coniferous forests. The local resident population is 14,900 people but in the summer season this number is increased by the 165,000 holiday-makers that visit the region. The estimation of population numbers of forest wild boars was carried out by analysis of collective hunting (Bobek et al., 2005). The population numbers of city wild boars were determined by direct observations. A certain number of culled animals (n=68) was examined, and their sex, age carcass mass, presence of *corpora lutea* and embryos, and the kidney fat index determined. The home ranges of city wild boars (n=7) were determined using the radio-telemetric technique. Questionnaire-based surveys regarding the conflict between city wild boars and humans were conducted among 300 residents and 300 visitors.

### Results

The population density of forest wild boars was 76.6 individuals/1,000 hectares, and the calculated population number was 290 individuals. In August, the number of city wild boar was estimated to be 82 individuals while in November the number dropped to 56 animals. Among forest wild boars, the number of piglets per sow was 3.8. In August, among city wild boars there were 5.3 piglets per sow, while in November that number was 4.3. The city wild boars that were captured, were ear tagged with radio-transmitters and taken 30-40 km from their place of capture had returned to their place of capture within 24-48 hours. The average daily home range in these wild boars was 3.6-5.8 ha.

The results of the surveys indicate that all of the permanent residents and 74% of visitors saw city wild boars. Conflict-type encounters (involving ripped clothing, attacking dogs, snatching shopping bags, pestering people on beaches, collisions with vehicles, and destruction of vegetable gardens and lawns) were experienced by 21% of resident respondents and 11% of holiday-makers. Among permanent

residents, 62% respondents would like to see the number of city wild boars reduced, and 55% of holiday-makers had no idea how the conflict could be solved (Table 1).

**Tab. 1** Human attitude regarding “urban” wild boar in Vistula Spit

Questions	Answers					
	Residents (n=300)			Visitors (n=300)		
	yes	no	no opinion	yes	no	no opinion
Urban wild boar encounter	300	0	-	222	78	-
Conflict Encounters	63	237	-	33	189	-
Harmful animals	282	12	6	102	51	147
Touristic attraction	141	150	9	135	60	105

Action	Proposed actions	
	Residents (n=300)	Visitors (n=300)
	Extermination	39
Reduce number of animals	186	18
Increase number of animals	0	15
No change in number	39	93
No opinion	36	165
TOTAL	300	300

## Discussion

During the most recent decade, the culling of wild boars in the study areas increased from 89-187 ind./y. This is probably due to the low food availability in forests and high population density in the habitat, from where wild boars to colonize urban areas. City wild boars have access to high-protein food of anthropogenic origin and therefore, the number of piglets per sow is higher than in forest wild boars. The decrease in the number of city wild boars between August and November could be due to emigration, caused by the low availability of food of anthropogenic origin (absence of visitors). It is thus highly probable that the animals migrated to the nearby conurbation of Gdańsk-Gdynia-Sopot, where a large population of city wild boars lives (Szramka and Karbownik, 2009). Illegal culling of these animals by locals might be another reason. The solution to the issue of the city wild boars should consist in reducing the population density of forest wild boars by half, which would probably alleviate the pressure of wild boars on urban areas. Killing captured animals would not be accepted by locals. Therefore, the only reasonable solution would be to erect a fenced enclosure covering a dozen or so ha, to house captured city wild boars in order to create a kind of local tourist attraction. The animals placed in such enclosures should be sterilized (Massei et al., 2008) in order to prevent reproduction.

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**Camera traps and activity signs to estimate density and population trends in wild pigs**Massei, G.<sup>1</sup>, Cowan, D.<sup>1</sup>, Lambert, M.<sup>1</sup>, Coats, J.<sup>1</sup>, Watola, G.<sup>1</sup>, Fox, S.<sup>1</sup>, Ward, A.<sup>1</sup>, Pietravalle, S.<sup>1</sup><sup>1</sup>Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom, giovanna.massei@fera.gsi.gov.uk

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Wild boar are often associated with damage to agriculture, traffic incidents and spread of diseases to humans and livestock. Controlling numbers of wild boar requires estimating local densities to measure the impact of any population management plan on actual numbers. However these densities are notoriously difficult to assess as wild boar are predominantly nocturnal, rely on dense vegetation for cover and avoid people, particularly when hunted. In many instances, indices of abundance based on activity signs such as tracks, pellet groups and rooting could be used instead of absolute numbers to monitor population trends. Animal population surveys based on camera trap surveys are increasingly employed in wildlife management due to the availability of recently developed, relatively inexpensive equipment suitable for field trials. This has been complemented by a growing literature aimed at establishing a conceptual framework for the optimal use of camera trap surveys. A novel approach using camera traps to estimate population density without the need for recognising individual animals has recently been developed and calibrated against known sizes of ungulate populations. The aims of this study, carried out at five sites in England, were to evaluate and compare the use of several activity indices to monitor population trends of wild boar and to estimate wild boar densities based on camera traps.

The results of this study showed that the method based on camera trap surveys could be used to detect differences in wild boar population abundance indexes between and within sites and to estimate absolute densities of wild boar in English woodlands. Surveys based on activity signs proved to have a low precision in estimating abundance indexes and were restricted to winter due to the persistence of activity signs which were more detectable in winter than in summer. Conversely, camera trap surveys were less likely to be affected by season and could be used any time of the year. The relatively small variation associated with the estimates derived from the camera trap surveys resulted in detectable differences, at least between some seasons or sites, in the relative indexes of abundance and in the estimated densities of wild boar.

## **Preliminary analysis of the diet of wild boar (*Sus scrofa* L., 1758) in an agro-ecosystem of central Punjab, Pakistan**

Hafeez, S.<sup>1</sup>, Ashfaq, M.<sup>2</sup>

<sup>1</sup>Department of Forestry, Range Management and Wildlife, University of Agriculture, Faisalabad, Pakistan, shahid\_frw@yahoo.com

<sup>2</sup>Department of Entomology, University of Agriculture, Faisalabad, Pakistan

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During the present study, 86 wild boars (*Sus scrofa*) were killed to examine their stomach contents in central Punjab, Pakistan. Stomach contents were found to be correlated with feeding sites, plant material, and the nature of food material and items consumed from both cultivated and non-cultivated lands. Stomach contents of these specimens revealed that 33 types of food items were consumed of which *Triticum aestivum*, *Saccharum officinarum*, *Zea mays*, *Oryza sativa*, earthworms and *Prosopis juliflora* were main staples. The major components of the wild boar's diet in central Punjab originated from cultivated crops (58%), non-cultivated crops (30%), animal matter (9%) and unidentified matter (3%). Wheat was the most intensively consumed item; it contributed 23% of the total dry weight of stomach contents. *O. sativa* was most intensively consumed item during autumn and followed by *Z. mays* and *S. officinarum*. In winter, the most important food items were *T. aestivum*, *S. officinarum* and earthworms. During spring, again wheat was the most intensively eaten item. *Prosopis juliflora* was the main food in summer; unidentified origin was regularly represented in the seasonal samples of stomach contents.

Keywords: central Punjab, food items, season, wild boar

## Carcass weight, condition and reproduction of wild boars harvested in north-western Poland

Orłowska, L.<sup>1</sup>, Rembacz, W.<sup>2</sup>, Florek, C.<sup>3</sup>

<sup>1</sup>Orłowska L., Department of Ecology, Wildlife Research and Ecotourism, Pedagogical University of Krakow, Podbrzezie 3, 31-054 Krakow, Poland, orłowska.lidia@gmail.com

<sup>2</sup>Rembacz W., os. Władysława Jagiello 22/5, 60-694 Poznan, Poland

<sup>3</sup>Florek C., Moryńska 1, 74-505 Mieszkowice, Poland

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### Abstract

During the period from October 2008 till January 2009, in the carcass material assembled from the collective hunting of wild boars (n=165), the age structure of population, body masses and conditions of wild boars were assessed. Among the harvested wild boars, piglets constituted 35.8%, and subadults 53.9%, whereas adults only 10.3%. In females of all age classes *corpora lutea* and embryos were found. The average number of *corpora lutea* was 6.3 per female. The average litter size was 5.9 embryos per female. When divided in age classes, in older wild boars the average was 9.0 embryos per female and only 2.0 embryos in the yearling class. Of the embryos whose sex could be determined (n=19), 63.2% were females and 36.8% males.

Keywords: age classes, litter size, pregnancy, wild boar

### Introduction

In recent years, increases in wild boar population numbers were observed in Poland as well as all over Europe (Keuling et al., 2008). At the same time the level of damage inflicted by this species increased significantly and compensation payments made to farmers in the 2009/2010 season amounted to 12.4 million €. Wild boars are increasingly often entering towns and cities and are frequently involved in road accidents. One of the reasons that the rapid growth in numbers of wild boars has gone unnoticed is the lack of reliable data on the population net increase, which should provide the basis for setting management plans for harvesting this species. This index can be calculated when the reproduction rate of a given population is known. Therefore, the objective of this study was to investigate the condition, age, and reproduction rate in the wild boar population occurring in north-eastern Poland, where intensive cultivation is pursued on its rich brown soils.

### Materials and methods

The material for this study was collected during collective hunting, organized in the period between October 2008 and January 2009 in the Mysliborz and Gryfino forest districts. In the course of each hunt the kidneys with surrounding fat and the lower jaw were dissected from all harvested males. The reproductive tracts, the kidneys with fat and lower jaws were collected from females. The uteri were assessed in a laboratory (i.e. the number of *corpora lutea* and embryos were counted) and the kidneys were weighed. The age of harvested wild boars was determined on the basis of tooth eruption patterns, which allowed determining the farrowing time pattern. In total 165 wild boars were examined for this study.

### Results

Piglets comprised 35.8% of harvested wild boars. The proportion of animals in the yearling class was 53.9%, and only 10.3% of the total number of animals harvested were in the older age class. Among the piglets bagged, 55.9% were male and 44.1% female. The great majority of harvested yearlings were females (76.4% vs. 23.6% of males). A similar trend was noted in older wild boars, where the predominance of females was also remarkable (64.7% vs. 35.3% of males).

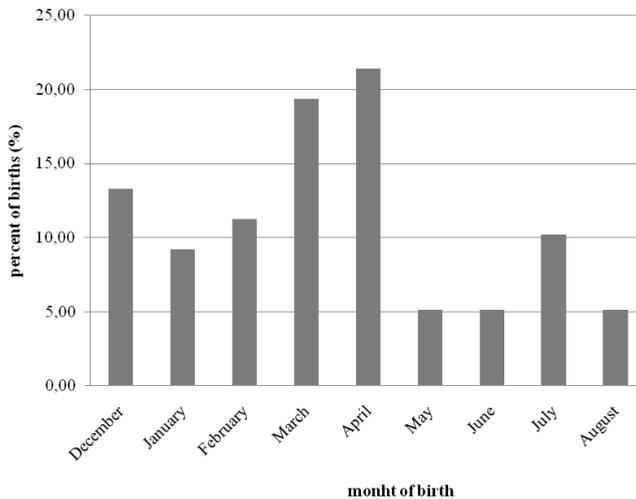
*Corpora lutea* and embryos were found in females of all age classes. Most of these, however, occurred in the yearling class (Table 1). The average number of *corpora lutea* per female was 6.3. The smallest average number of *corpora lutea*, i.e. 5.5 per female was found among the piglet age-class. The highest number, i.e. as many as 7.5 *corpora lutea*, was found in older wild boar females. The average litter size was 5.9 per female. Depending on the age class, an average of 9.0 embryos per female was found among

older wild boars, whereas in the yearling class it was only 2.0 embryos per female. Of the embryos whose sex could be determined (n=19), 63.2% were females and 36.8% males. The average body mass of wild boars harvested indicate clearly, that, except in the piglet class, males are heavier than females. The calculated kidney fat index (KFI) in males was lower than in females.

**Tab. 1** Body condition and reproduction of wild boar in north-western Poland

Age classes	Males						Females			
	Sample size	Carcass weight (kg) ±SE	KFI	Sample size	Carcass weight (kg) ±SE	KFI	Females with corpora lutea	Pregnant	Percent of fertilized (%)	Average litter size
Piglets	33	23.5± 1.8	1.68	26	25.1± 1.3	1.69	2	1	3.8	2.0
Subadults	21	64.2± 3.1	1.81	68	56.8± 1.1	2.14	18	12	17.6	6.2
Adults	6	106.7 ± 5.6	1.83	11	76.2± 2.1	2.20	4	1	9.1	9.0

The farrowing season in the study area lasted from December until August (9 months), with clear peaks in the months of March, April, July and December (Figure 1).



**Fig. 1** Fertility period of wild boar in north-western Poland

## Discussion

The area from which the wild boar carcasses were obtained for this study consists of fertile soils used to cultivate high-protein crops. Moreover, the oak-beech forests of the Myslibórz and Gryfino forest districts provide a rich food supply to wild boars. This availability of high-protein food is likely to result in earlier maturity in the boars from the youngest age class as well as resulting in the extension of the farrowing season to cover a larger part of the year (9 months) (Durio et al., 1995). The reproductive pattern found in this study coincides with that of other wild boar populations in Western Europe (Gaillard and Jullien, 1993, Gethöffer et al., 2007). Therefore, it is essential to determine the

reproductive pattern of wild boar females and to carry out a proper population census, which will allow devising a suitable strategy for harvesting wild boars.

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## **Reproductive parameters, birth date-effect and body condition of wild boars (*Sus scrofa*) inhabiting forest and forest-farmland environments in Poland**

Merta, D.<sup>1</sup>, Albrycht, M.<sup>1</sup>, Frąckowiak, W.<sup>1</sup>, Furtek, J.<sup>1</sup>, Mamok, T.<sup>2</sup>

<sup>1</sup>Department of Ecology, Wildlife Research, and Ecotourism, Institute of Biology, Pedagogical University of Krakow, Podbrzezie 3, 31-054 Kraków, Poland, dorota-zbl@o2.pl

<sup>2</sup>Rudziniec Forest District, Leśna 3, 44-160 Rudziniec, Poland

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### **Abstract**

The following parameters were measured in 315 wild boars harvested in the 2008/2009 hunting season in south-western Poland: carcass mass and length, body condition, age, and – in sows – the reproductive parameters. The material was collected from a large (720 km<sup>2</sup>), non-fragmented forest called Bory Dolnoslaskie (BD), and from several dozen small forests (SF) with a total area of 703 km<sup>2</sup> of forested areas surrounded by agricultural land. In the BD among female piglets harvested there, no sexually mature individuals were found. In the SF environment, some of the female piglets reached sexual maturity, and the percentage of pregnant sows was higher compared to animals from the BD environment. The farrowing season in the SF environment spanned 10 months whereas in the BD environment only 6 months. The paper discusses the use of the above-mentioned parameters to devise the strategy for management of wild boar in Poland.

Keywords: age class, agriculture damage, high protein forage, pregnancy, wild boar

### **Introduction**

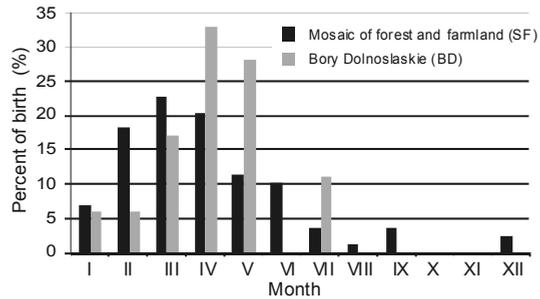
Over the last 15 years, the population of wild boars soared from 83,900 individuals in 1997 to as many as 249,800 in 2010. Although harvesting wild boars increased in the corresponding period by 198% this activity has failed to stabilize the population of this species. The aim of this study was to investigate the conditions, birth distribution and reproduction parameters for boars within areas that are different in terms of the potentially available food supply.

### **Methods**

Data were collected from October 2008 to January 2009 in south-western Poland during collect hunt. The study areas were a large non-fragmented coniferous forest called Bory Dolnoslaskie (BD) and several dozen of small deciduous forests (SF) surrounded by farmlands. The forest in BD and SF covers 720 and 7.3 km<sup>2</sup>, respectively. For each wild boar (n=315) data on body weight and length were collected and the lower jaw and one kidney with fat were dissected. The date and location of culling were recorded for each carcass. In addition, from each female the reproductive organs were dissected. The age of animals was assessed from tooth eruption and wear of the lower jaw teeth. Two indices of condition were calculated: carcass weight/length ratio (CWL) and kidney fat index (KFI).

### **Results**

The mean carcass weight in boars of particular age classes was always higher in animals living in the forest-farmland environment (piglets: 13.2 kg vs. 24.3; subadults: 37.5 kg vs. 46.9 kg; adults: 64.4 kg vs. 69.4 kg), with the differences among piglets and subadults being statistically significant. The mean kidney fat index (KFI) was also statistically significantly different in the boars harvested in these two environments (BD=1.96, SF=2.23, F(1.309)=16.63, p<0.01). A similar trend was demonstrated in terms of the CWL condition index. In the forest-farmland environment, the majority of births took place within three months (February, March, April), when 61.4% of piglets were born. The farrowing season in the BD forest lasted 6 months (from January till July) and the peak of piglets births fell in the months of March, April and May, where 78.0% of all piglets were born (Figure 1).



**Fig. 1** Fertility period in two environments: mosaic of forest and farmland (SF) and Bory Dolnoslaskie

In female piglets from BD, the presence of neither embryos nor *corpora lutea* was found, whereas in the forest-farmland mosaic 26.8% of animals in this age class had *corpora lutea*, and 12.5% females were pregnant (Table 1).

**Tab. 1** Body weight and reproductive parameters of wild boar females (n=184) inhabiting two different environments in south-western Poland

Habitat and age class	Sample size (n)	Carcass weight (kg)	Females with <i>corpora lutea</i> n (%)	Pregnant females n (%)	Average litter size (n)
<b>Bory Dolnoslaskie (BD)</b>					
Piglets	22	15.7	0 (0.0%)	0 (0.0%)	0.0
Subadults	20	39.3	6 (30.0%)	4 (20.0%)	3.0
Adults	26	61.6	6 (23.1%)	2 (7.7%)	5.5
<b>Mosaic of forest and farmland (SF)</b>					
Piglets	56	23.3	15 (26.8%)	7 (12.5%)	4.0
Subadults	39	45.8	21 (53.8%)	16 (41.0%)	5.1
Adults	21	67.0	19 (90.5%)	10 (47.6%)	6.8

Among the embryos (n=48) collected from the forest-farmland mosaic, 69.4% were male individuals, and the average litter size there was 4.8 embryos. The average body mass of the 3 lightest females which had *Corpora lutea*, was 32.0 kg in the Bory Dolnoslaskie, whereas it was 15.5 kg in the forest-farmland mosaic.

## Discussion

In coniferous forest stands (BD), the potential food for boars (soil invertebrate fauna, rodents, oak and beech nuts) is much poorer than that in deciduous forest stands (SF). Moreover, in the forest-farmland mosaic environment there is a high-protein additive to the wild boars' diet, primarily obtained from cultivated plants such as maize and rape. The reproductive pattern found in this study for the forest-farmland mosaic (SF) coincides with the results of similar field studies conducted in Western Europe (Gaillard and Jullien, 1993, Santos et al., 2006, Gethöffer et al., 2007). The early maturation of female piglets is a factor adversely affecting the quality of the population. The piglets born in summer and autumn are unable to attain the right body condition in order to survive the winter, and some of them die, whilst those that survive the winter often fail to reach the weight corresponding to their age. Therefore, in the areas where female mature piglets are found, the proportion of piglets in the hunting bag should be very high (Bieber and Ruf, 2005).

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## 8<sup>th</sup> European Vertebrate Pest Management Conference

The 8<sup>th</sup> European Vertebrate Pest Management Conference was held 26-30 September 2011 in Berlin, Germany. It was organised by the Vertebrate Research Group of Julius Kühn Institute, Federal Research Centre for Cultivated Plants and the Faculty of Agriculture and Horticulture of Humboldt University. The Conference is a biennial meeting of people interested in various aspects of vertebrate pest management. Overabundant vertebrate populations can be responsible for crop loss, public and animal health concerns, structural damage and conflicts with conservation interests. The conference is a forum for all involved in basic research in vertebrate biology, ecology, methodology, legislation and the application of these topics in wildlife management.

The intention of the meeting was to foster the interaction of experts from Europe and beyond specializing in different fields of applied and basic vertebrate research because thorough knowledge of all relevant aspects is a vital prerequisite to make informed decisions in vertebrate pest management.

This book of abstracts summarizes all contributions that were presented in 9 symposia: 1) Fertility control in vertebrates, 2) Invasive vertebrates, 3) Management of birds, 4) New tools and methods - alternatives to anticoagulants including a workshop, 5) Population dynamics and management of mammals, 6) Rodenticide resistance and management of commensal rodents, 7) Vertebrate management in developing/emerging countries, 8) Wild boar biology and management, and 9) Zoonotic diseases in vertebrates.

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