# Conservation biology of the European hamster (Cricetus cricetus)

Mathilde Tissier

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**THÈSE** présentée par :

**Mathilde TISSIER**

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# Conservation biology of the European hamster (*Cricetus cricetus*)

## *Nutritional effects of crops on hamsters fitness and evaluation of their antipredatory behavior to upgrade wildlife underpasses.*

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A toi mon Fifi, mon pilier…

Quelques perceptions d’un monde que tu as trop tôt quitté

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👊 👋

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# Publications

## Studies presented in this manuscript

- Submitted or published

**Study 1: Tissier M. L.**, Handrich Y., Robin J.-P., Weitten M., Pevet P., Kourkgy C., Habold C. (2016). How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the verge of extinction. *Scientific reports,* doi: 10.1038/srep25531.

**Study 2: Tissier M.L.**, Handrich Y., Dallongeville O., Robin J-P. & Habold C. Diets derived from maize monoculture cause maternal infanticides in the endangered European hamster due to a vitamin B3 deficiency. *Proc. R. Soc. B,* **284**: 20162168*.*

**Study 3: Tissier M.L.**, Kletty F., Handrich Y., Habold C. Experiments in outside enclosures highlight that organically-grown monoculture crops reduce invertebrate and plant species richness and impair the fitness of a critically endangered farmland rodent. *Under review – Oecologia*.

**Study 5: Tissier M. L.***, Jumeau J.*, Croguennec C., Petit O., Habold C. & Handrich Y. (2016). An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features. *Ecological Engineering*, ***95***, 738-742*.*
* Co-first author

**Study 6: Tissier M. L.**, Bousquet C., Croguennec C., Fleitz J., Habold C., Petit O. & Handrich Y. Inter-individual differences in risk assessment and anti-predatory behaviour in an endangered species, the European hamster. Under review – *Animal behaviour.*

- In preparation

**Tissier M.L.**, Kourkgy C., Robin J.P., Eidenschenck J., Handrich Y. and Habold C. Habitat and nutrition of the European hamster in France – a Review (including **Box 1**).

- **Box 1:** Diet selection in the European hamster, daily macronutrient and energy intakes.
- **Table 6:** Effects of the strip-till technique on the diversity of collembolla in French farmland.

**Box 2**: **Tissier M.L.**, Habold C., Handrich Y., Zahn S., Criscuolo F. and Smith S. Telomere dynamics in the European hamster during hibernation and reproduction. In preparation for the *Journal of Experimental Biology*.

**Study 4: Tissier M.L.**, Handrich Y. and Habold C. Effects of 6 crop-based diets on the hibernation and the reproduction of the European hamster (*Cricetus cricetus*). In preparation for *Hormones and Behavior*.

**Box 3: Tissier M.L.**, Handrich Y., Habold C., and Petit O. A behavioral syndrome linking boldness and exploration in the European hamster (*Cricetus cricetus*). In preparation for *Ethology, Ecology & Evolution*.

**Box 4: Tissier M.L.**, Bousquet C., Fleitz J., Calibre S., Jumeau J., Habold C. and Handrich Y. An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (II) Tests with the European hamster in semi-natural conditions. In preparation for the *Journal of applied ecology*.

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## Studies not presented in this manuscript

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Weitten M., **Tissier M.L.**, Robin J.P. and Habold C. Diet influence on the annual cycle of a food-storing hibernator, the European hamster. *In preparation*.

**Tissier M.L**. and Habold C. (2017) Adaptations métaboliques et digestives des espèces hibernantes. *Cahiers de Nutrition et de Diététique*. https://doi.org/10.1016/j.cnd.2017.03.003.

Stier A., Massemin S., Zahn S., **Tissier M.L.** and Criscuolo F. (2015) Starting with a handicap: effects of asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free-living great tits. *Oecologia, 179, 4, pp 999-1010.*

**Tissier M.L.**, Williams T.D., Criscuolo F. (2014) Maternal effects underlie ageing costs of growth in the zebra finch (*Taeniopygia guttata*). *PLoS One*, doi: 10.1371/journal.pone.0097705.

# Scientific oral and poster presentations

**Tissier M.L.**, Handrich Y. & C. Habold. Effects of crop-based diets on the hibernation and the reproduction of the European hamster. 23rd « Meeting of the Hamster WorkGroup », October 2016, Heidelberg, Germany.

**Tissier M.L.**, Handrich Y. & C. Habold. Effects of cropland-based diets on the hibernation of a food-storing rodent. 15th « International Hibernation Symposium », August 2016, Las Vegas, US.

**Tissier M.L.**, Handrich Y., Robin J.P, Habold C. Infanticide and siblicide in maize-fed common hamsters: the nicotinamide deficiency responsible. Poster presentation, ICCPB, August 2015, Krakow, Poland.

**Tissier M.L.**, Handrich Y., Petit O., Robin J.P, Habold C. Cannibalism and reduced body conditions: why would corn cause reproductive failure in the Common hamster? Poster presentation, ICCB/ECCB, August 2015, Montpellier, France.

**Tissier M.L.**. Diet and reproduction in the Common hamster (*Cricetus cricetus*): would maize be a major cause in the decline of the species in France? *3 oral presentations*
- Lab communication, July 2014, IPHC-CNRS, France
- 21st meeting of the hamster workgroup, November 2014, Gelnhausen, Germany.
- Journées scientifiques de la ZAEU, December 2014, Strasbourg, France.

**Tissier M.L.**, Petit O., Croguennec C., Cornejo S. & Handrich Y. How do captive Common hamster perceive and react to predator's scent? *2 poster presentations*

- 7th European conference on behavioural biology (ECBB), July 2014, Prague, Tchek Republic.
- 21st meeting of the hamster workgroup, November 2014, Gelnhausen, Germany.

Jumeau J., Handrich Y. & **Tissier M.L.**. Use of wildlife underpasses by common hamster throughout a road interchange. Poster presentation, 20th meeting of the hamster workgroup, November 2013, Poznan, Poland.

**Tissier M.L.**, Williams T.D., Criscuolo F. Maternal effects underlie ageing costs of growth in the zebra finch (*Taeniopygia guttata*), CEPA, November 2013, Lyon, France.

Stier A.*, **Tissier M.L.***, Zahn S., Criscuolo F., and Massemin S. Hatching asynchrony uncovers a trade-off between growth and telomere erosion rate in great tit chicks. *Poster co-presenters, 9th *Ecology & Behaviour* meeting, 22-26 April 2013, Strasbourg, France.

## Popular publications and oral presentations

Chaigne A., **Tissier M.L.**, Habold C., Eidenschenck J., Ulrich B. 2015. Le Grand hamster (*Cricetus cricetus*) en Alsace, quel devenir? Publication in the Scientific journal of the SFEPM – 37th French symposium of mammalogy.

Eidenschenck J. and **Tissier**. **M.L.** « Le Grand hamster, emblème de la petite faune de la plaine d'Alsace ». *2 oral presentations*
- Conference of the '*Mardi Nature*' (GEPMA), January 2016, Strasbourg
- Conference of the '*Jardin des Sciences*', April 2016, Barr, France.

**Tissier M.L**. 2015. Tests en conditions contrôlées de l'impact des cultures sur la biologie du Grand hamster. Oral presentations to farmers at the Chambre d'Agriculture de la Région Alsace (CARA), March 2015, Strasbourg, France.

**Tissier M.L.,** Chaigne A., Uhlrich B. « Le Grand hamster (*Cricetus cricetus*) en Alsace, quel devenir? » Oral presentation at the 37th French symposium of mammalogy, October 2014, Morvan, France.

**M.L. Tissier**, C. Habold. Sous la terre d'Alsace : zoom sur le Grand Hamster. Oral presentation at the conference of the '*Jardin des Sciences*', October 2013, Strasbourg, France.

# Foreword

The ability of organisms to cope with changing environments and the constraints that they pose is at the base of natural selection and evolution. For decades, evolutionary biologists sought to understand how the evolution of organisms' physiology, ecology and behavior allow them to adapt to their environment. Human induced global change pose major and multi-scales constraints onto natural resources, including landscapes and biodiversity. The 6th crisis of biodiversity extinction is currently under way and appear as being mainly caused by anthropogenic actions. In the face of these decline in biodiversity, new research areas have emerged in the past decade, namely conservation physiology and conservation behavior. These research areas aim at improving the scientific knowledge on the underlying mechanisms that are at the base of species decline, in order to improve their conservation strategies. Such areas of research can be applied to highly anthropogenic and rapidly changing environments such as urban or sub-urban habitats and farmland.

In this context, the main aim of my thesis was to bring scientific knowledge regarding the underlying mechanisms responsible of the collapse in the French population of the European hamster (*Cricetus cricetus*), a rodent endangered across its European range. These results would ultimately serve to upgrade the current conservation strategies for this species. First, I sought to understand how crops currently cultivated in France can affect hamsters' biology, while trying to find non-cultivated crops favoring their fitness and of economic interest (**Thematic I**). Secondly, I investigated for the hamsters' antipredatory behavior and developed an anti-predation device to upgrade wildlife underpasses in the French distribution area of the species, in order to ultimately reconnect wild populations (**Thematic II**).

This manuscript is divided into 8 main sections. Following a general introduction replacing the decline of this species into the current 6th mass extinction, results of the Thematic I are presented. They are divided into three different sections (**chapters 1-3**), whereas the limits, scientific perspectives and applications to the improvement of monoculture farming are developed in the **chapter 4**. Then, results of the Thematic II are presented and distributed into two sections (**chapter**

**5 and 6**), *whereas the limits, perspectives and application to the improvement of wildlife underpasses are discussed in the* **chapter 7**. *In the last part of this manuscript, I conclude on the results presented and I express my opinions regarding the importance of policymakers, farmers and citizen for the hamster conservation. Finally, I replace this work into a broader context linking global change, loss of biodiversity, human health, and inequalities in the costs and benefits faced by human populations under the current environmental crisis.*

# Table of content

# THEMATIC I

## THEMATIC II

# Abreviations List

| | |
|---|---|
| **APT** | Tube Anti-Prédation (Anti-Predation Tube) |
| **CARA** | Chambre d’Agriculture de la Région Alsace, maintenant CAA pour Chambre d’Agriculture d’Alsace (Agricultural Agency of the Alsace) |
| **CNRS** | Centre National de la Recherche Scientifique (French National Center For Scientific Research) |
| **CRA** | Chambre Régionale d’Agriculture |
| **DREAL** | Direction Régionale de l’Environnement, de l’Aménagement et du Logement (Regional Environmental, Development and Housing office). |
| **GEPMA** | Groupe d’Etude et de Protection des Mammifères d’Alsace (Association For the Study and the Protection of Mammal species in the Alsace) |
| **LPO** | Ligue pour la protection des oiseaux (The League For Bird Protection) |
| **MEDDE** | Ministère de L’Ecologie, du Développement Durable et de l’Energie (French Ministery for Ecologie, Sustainable Development and Energy) |
| **NAD** | Nicotinamide adénine dinucléotide |
| **NADP** | Nicotinamide adénine dinucléotide Phosphate |
| **ONCFS** | Office National de la Chasse et de la Faune Sauvage (French Hunting and Wildlife Agency) |
| **Trp** | Tryptophane (Tryptophan) |
| **Vitamin B3** | Niacine ou Nicotinamide |

# Introduction

*“Nothing in Biology makes sense except in the light of evolution”*

Christian Theodosius, 1973

Content

Drawing: *Cricetus frumentarius*; © www.BioLib.de

# I. Living the 6th mass extinction crisis

## 1. The current extinction crisis *versus* the 'Big Five': the implication of human populations

Although biologists cannot precisely say how many species inhabit the Earth – this is especially true for invertebrates and fungi, largely under-described taxa (Monastersky, 2014; Thomas, 2009) – or exactly how many have gone extinct in the past (Ceballos et al., 2015), it is mostly agreed that the 6th mass extinction crisis is currently under way (Ceballos and Ehrlich, 2002; Monastersky, 2014). Indeed, many studies – even those using the most conservative modeling scenarios of current biodiversity loss (Barnosky et al., 2011; Ceballos et al., 2015) – highlight the great magnitude (% of species that have gone extinct (Barnosky et al., 2011)) and the rapid rate (% of species extinct/time over which the extinction occurred (Barnosky et al., 2011; Ceballos et al., 2015; Pimm and Raven, 2000; Sala and Sala, 2009)) of species extinction. Current biodiversity loss is such that by the year 2200-2500, a predicted 75% of known species will have gone extinct. After the Ordovician-Silurian, the Devonian, the Permian, the Triassic-Jurassic and the Cretaceous-Tertiary (K-T) mass extinctions crises (Barnosky et al., 2011), the current crisis would therefore officially be considered as the 6th mass extinction crisis of our planet's history.

This crisis is estimated to have been intensified by the exponential 'boom' (from 600 million to 6.3 billion people) human population experienced from 1700 to 2003 (Cohen, 2003). This represents a 10-fold increase in just over three centuries. The first 2 billion people milestone was reached in 1927, the 4 billion milestone in 1974, and the 6 billion milestone in 1999. To put this into perspective, it took some 195 to 160 thousand years for modern human *Homo sapiens* populations to reach the 2 billion milestone, and a mere 75 years to triple this figure to 6 billion. By 2050, it is estimated that 8.9 billion humans will be inhabiting the Earth (Cohen, 2003). These trends are currently verified, since the human population just reached 7.43 billion ("Countrymeters," n.d.) in 2016. This unprecedented growth in human population has been intrinsically linked to increasing pressures on natural resources (**Figure 1**), leading to the reduction of both non-renewable and potentially renewable resources (i.e. renewable to a certain extent, but some of these resources could soon reach the 'non-renewable' stage). These pressure and decline of natural resources are a key point of this 6th extinction crisis.

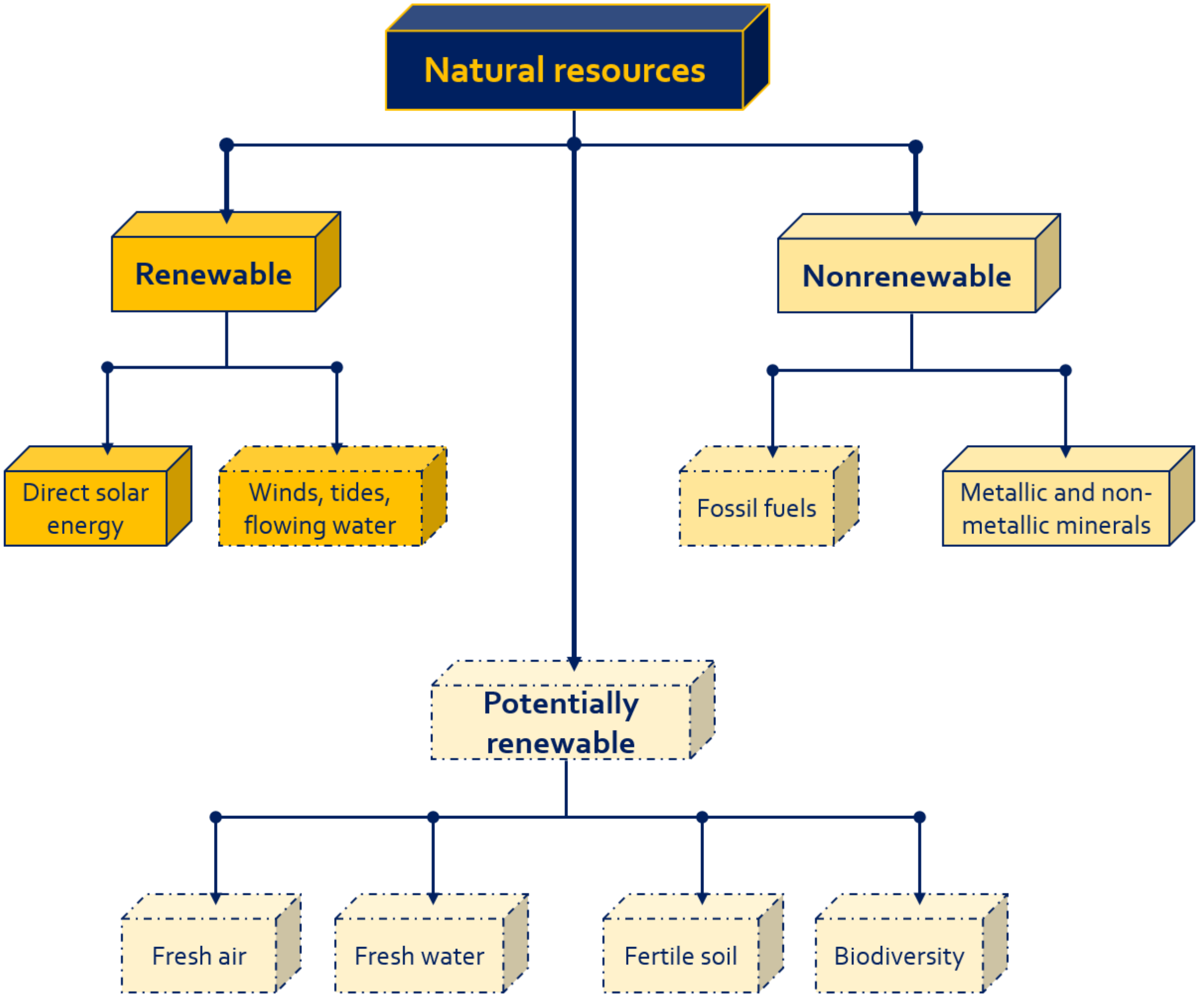

**Figure 1: Renewable, nonrenewable and potentially renewable natural resources**. Dotted lines represent the natural resources affected by human activities (adapted from Wadsworth Publishing Company/ITP, 1998).

## 2. The five main anthropogenic pressures on biodiversity

This human demographic trend and the parallel development and intensification of urbanization, terrestrial and marine exploitations (Vitousek et al., 1997) all constitute strong pressures negatively affecting biodiversity. Subsequently, the 6th mass extinction crisis has mainly been imputed to anthropogenic changes and their effects on biodiversity (Butchart et al., 2010; Ceballos and Ehrlich, 2002; Díaz et al., 2006; Pimm and Raven, 2000; Vitousek et al., 1997). Anthropogenic activities that constitute a threat to biodiversity can be regrouped under five main categories related to global change: habitat loss (fragmentation and loss of natural habitats), habitat degradation (e.g. pollution, modification of soil biochemical cycles), climate change, biological invasions (introduction of alien[1] invasive species) and overexploitation of natural resources (e.g. overfishing, overhunting, oil overexploitation) (Butchart et al., 2010; CBD and WHO, 2005; Myers et al., 2013; Pimm and Raven, 2000; Sala and Sala, 2009; Vitousek et al., 1997) (see Figure 2). Recent estimations reveal that 37% of the decline in animal populations is imputable to overexploitation, 31% to habitat degradation, 13% to habitat loss, 7% to climate change, 5% to invasive species, 4% to pollution and 2% to disease (Monastersky, 2014). Agriculture intensification, urbanization and overharvesting (i.e. overfishing and overhunting) represent the three major causes of biodiversity decline (Cohen, 2003; Foley et al., 2005; Lande, 1998; McKinney and Lockwood, 1999; Monastersky, 2014). As a consequence of these pressures, 20-43% of coral, amphibian, bird and mammal described species are currently threatened (Barnosky et al., 2011; Monastersky, 2014), whereas several millions of species remain to be described and their status assessed (Monastersky, 2014); and some will most likely disappear before even being discovered.

In recent years, researchers have argued that the number of species declining or going extinct *per se* is not the best proxy to compare extinction crises (Barnosky et al., 2011; Ceballos et al., 2015; Celâl Sengör et al., 2008). Rather, they highlight that other parameters are central indicators of extinction crises and have been under-estimated in the analysis of the current extinction. The first example is the *extinction rate*, estimated to be faster than all rates that would have produced the Big Five extinctions over hundreds of thousands or millions of years (Barnosky et al., 2011). Another example is the *number of populations going extinct* which is much greater than the number of species going extinct (Barnosky et al., 2011) and directly threatens ecosystem functioning (Ceballos and Ehrlich, 2002; Ceballos et al., 2015)). Finally, a third example is the product of the magnitude and the intensity of the extinction crisis, known as the *'greatness (G)'* (see (Celâl Sengör et al., 2008)). Moreover, considering solely the magnitude of species extinction at a global scale neglects the central idea that biodiversity is not only a number, but also a relative abundance, a composition, a spatial distribution, a range of functional traits and an interaction of genotypes, populations, species, functional types, functional traits and landscape units in a given system ((Díaz et al., 2006); see Figure 2*)*.

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[1] The term 'alien' refers to species or organisms occurring outside their natural (past or present) range and dispersal potential, whose presence and dispersal is due to intentional or unintentional human actions. It is opposed to the term 'native' referring to organisms that has originated in a given area without human involvement (Roques and Robinet, 2009).

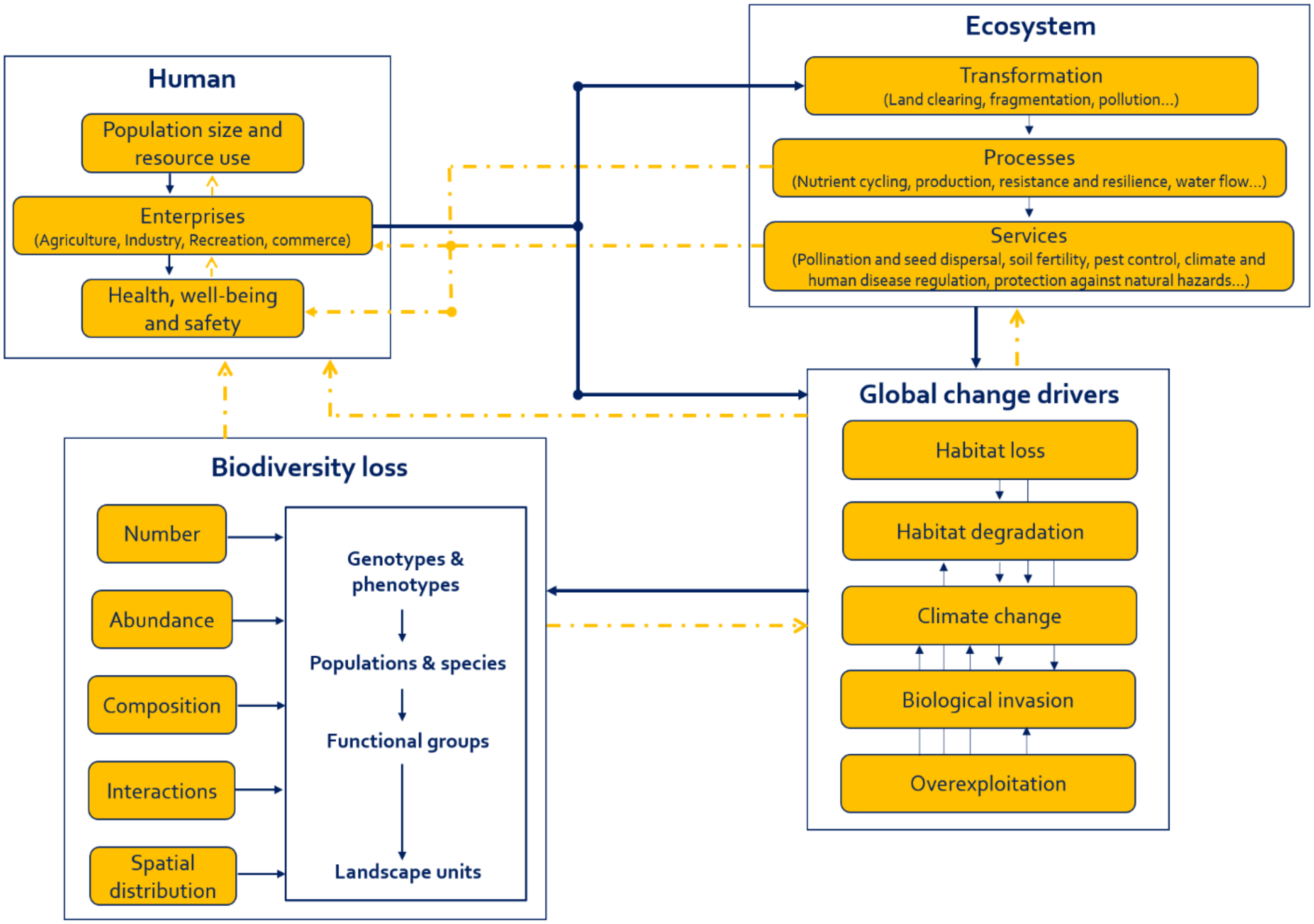


**Figure 2: Human influence on biodiversity loss through its effects on ecosystem characteristics and function, and its impact on global change**. Causal links are represented by blue solid lines whilst yellow dotted lines represent the feedback from one category to another. (adapted from (Colautti et al., 2017; Díaz et al., 2006; Vitousek et al., 1997)).

The complexity of this 6th mass extinction crisis – that ‘resembles none of the earlier ones and may end up being the greatest of all’ (Celâl Sengör et al., 2008) – and the uncertainty regarding its future (Monastersky, 2014; Pimm, 2008) are well recognized. This urges researchers to devote extensive attention to evaluating current and future risks to biodiversity. Considering the multifactorial nature of biodiversity loss and the uncertainty around ecosystem resilience to change, two important concepts have emerged. The first is that different species do not respond in the same way to global change: some species invade, some adapt and some collapse (CBD and WHO, 2005; Colautti et al., 2017; Jeschke and Strayer, 2008; McKinney and Lockwood, 1999;

Medan et al., 2011; Siriwardena et al., 1998), leading to clear ‘winners and losers’ (Díaz et al., 2006; McKinney and Lockwood, 1999). The second is that biodiversity loss threatens global economy but also human health and safety (Bayles et al., 2016; CBD and WHO, 2005; Díaz et al., 2006; Myers et al., 2013; Palumbi, 2001), leading to the massive awareness that it needs to be protected (Edwards and Abivardi, 1998).

## 3. Winners and losers in the face of global change: ultimate causes and consequences

### *Risks of extinction are population – not species – specific*

It is now well recognized that biodiversity loss does not occur randomly (Cardillo et al., 2005; McKinney and Lockwood, 1999), which tends to homogenize the biosphere and the diversity at the local, regional and global scales. As indicated above, the winners and losers have been considered as being family- or species-specific, with few species spreading and many collapsing on a global scale (CBD and WHO, 2005; McKinney and Lockwood, 1999; Medan et al., 2011). The ultimate causes of the differences between invading or collapsing species have been intensively investigated, and many investigations have focused on the shared life-history traits and strategies (Cardillo et al., 2005; Koh et al., 2004; McKinney and Lockwood, 1999) or on the shared ecological characteristics (Kotiaho et al., 2005) of declining/threatened species in opposition to alien or native invasive species. For instance, many researchers argued that endangered/declining species have opposite ecological characteristics (e.g. narrow niche breadth, restricted resource distribution (Kotiaho et al., 2005)) and life-history traits and strategies (e.g. large body size/mass, small clutch/litter size or number of offspring per year; slow pace-of-life) than invasive species (large niche breadth, small body mass/size, fast pace-of-life). However, a recent meta-analysis on 1813 species of birds and fish (Jeschke and Strayer, 2008) did not find evidence that species with a ‘slow’ history were ‘extinction-prone’ species whilst species with a ‘fast’ history were ‘invasive-prone’ species. They rather highlighted that threat status is mostly affected by specific ecological or life-history traits (body mass/size and life-history variables). Therefore, they argued that invasion success is rather positively linked to specific characteristics such as association with humans, herbivory and diet generalists, but that these traits or characteristics would not be at the opposite side of a same continuum. However, it is worthy to be noted that most of these studies are predictive or correlative and are based on data from the IUCN (International Union for

Conservation of Nature) (Jeschke and Strayer, 2008; Kotiaho et al., 2005; McKinney and Lockwood, 1999), which can be incomplete, not-updated or biased towards well-described and large body-size taxa (mostly birds, mammals, amphibians) and neglect less-described taxa (invertebrates, microbes…) (Barnosky et al., 2011; Ceballos and Ehrlich, 2002; Monastersky, 2014). Moreover, it is important to remind ourselves that correlation is not causation (Ksir and Hart, 2016; Thornhill, 1991). There are also evidence that populations of some well-known successful generalist species such as the European starling (*Sturnus vulgaris*) are affected (Mennechez and Clergeau, 2006) and suggest that behavioral innovation – rather than being a habitat or diet generalist – is more important to successfully cope with environmental change (Demeyrier, 2016; Mennechez and Clergeau, 2006).

The hypothesis assuming that endangered/declining and invasive species are at two opposite sides of a same ‘continuum of extinction’ because of differences in life-history strategies or ecological characteristics therefore appears unlikely. Because different characteristics can be favored at different stages of development or invasion (Capellini et al., 2015; Colautti et al., 2017), what appears more likely, is individuals’ characteristics may be under different selective pressures depending on the prevailing anthropogenic conditions in the environment at a given time. Recently, a second hypothesis has emerged accounting for the fact that even closely-related species (sharing many ecological and life-history characteristics) can differ from being invasive to being of conservation concern (Colautti et al., 2017). This hypothesis states that ‘more transient ecological and genetic factors are responsible for the varied ecological success of species in nature’ (Colautti et al., 2017). Indeed, it appears that endangered species suffer from greater genetic constraints and stronger negative biotic interactions (e.g. with pathogens and competitors) than invasive species (Colautti et al., 2017). This leads to a ‘spectrum of ecological success’ (Colautti et al., 2017), with at one end extinction and at the other invasion, with most species affected, independently of their life-history pace (‘fast’ or ‘slow’), their size/mass or their diet. Therefore, although some species or families appear to have been more affected than others, risk of extinction is indeed not family- or species-specific but rather population-specific. Populations of a same species can be expanding, stable or declining (Blaustein and Kiesecker, 2002; Byrne et al., 2015; Molfetti et al., 2013; Oli and Armitage, 2004; Ozgul et al., 2010; van der Zee et al., 1992) depending on the prevailing environmental conditions, specifically depending on the different abiotic and biotic factors (which interact in a context-dependent manner) to which they are subjected (Blaustein and Kiesecker, 2002; Koh et al., 2004).

### *Global change, phenotypic plasticity and extinction risk of a population*

The response of a population (expansion, stabilization or decline) therefore ultimately depends on the number and strength of the environmental pressures acting on it, and the population's plasticity and capacity of response to these pressures. It is now well-recognized that a given set of genotypes[1] may converge on the same phenotypes[2] or express different phenotypes depending on the environment (e.g. genotype x environment interaction, G x E; also called phenotypic plasticity (Stearns, 1989)). Moreover, a deleterious mutation can have no phenotypic effects under a 'neutral' environment but be amplified in a stressful environmental context (i.e. inbreeding x environment interaction, I x E (Colautti et al., 2017). It is now well recognized that the consequences of inbreeding and of the expression of the genotype on the phenotype, on the fitness and therefore on natural selection depend on the environment (Colautti et al., 2017). Given that natural selection acts on the phenotype (Stearns, 1989; West-Eberhard, 2005), it appears that the environment and the plasticity of a population are at the core of the differences of proliferation/extinction between populations of a same species, or closely-related species. Therefore, they can be considered as the main drivers of the place of a population (not a species) on the 'spectrum of ecological successes'.

Intra-individual phenotypic plasticity corresponds to the 'ability of an organism to react to an […] external environmental input with a change in form, state, movement or rate of activity' (West-Eberhard, 2003). Changes in the phenotype are numerous and can be (to name a few) morphological (e.g. changes in body mass/size (Ozgul et al., 2010; Smith et al., 1998; Yom-Tov, 2001)), physiological (e.g. endocrine and metabolic (Ricklefs and Wikelski, 2002)) or behavioral (e.g. plasticity of phenology (Charmantier et al., 2008), of personality (Dingemanse et al., 2010) or of mating (Mayer et al., 2017)). They will influence (in a positive or negative way) the performances of the individuals, their life-table variables (e.g. birth/death rates, fecundity, survival) and therefore their fitness. If the given phenotypic plasticity increases the fitness (1) of the individuals in a given environment, then natural selection will occur and the phenotype (and therefore the associated genotype) will be selected (i.e. adaptive phenotypic plasticity) (Colautti et al., 2017; West-Eberhard, 2003). If not, then the phenotype will disappear from the population. This led to the emergence of two distinguishable terms: (1) phenotypic plasticity which is a modification of the phenotype (at a given time)  arising directly from resource limits or other stresses, but that may not be increasing fitness whereas (2) adaptive phenotypic plasticity corresponds to changes in the phenotype that are a consequence of selection from previous generations, have a genetic basis and will maintain fitness across a range of environment (Colautti et al., 2017; West-Eberhard, 2003). Phenotypic plasticity – either adaptive or non-adaptive, irreversible or reversible – has been the topic of many investigations in the past four decades (Charmantier et al., 2008; Ghalambor et al., 2007; Hendry et al., 2008; Lane et al., 2012; Réale et al., 2003; Ricklefs and Wikelski, 2002; Valladares et al., 2014; West-Eberhard, 2003) and has been determined to be at the core of (micro)evolution (Colautti et al., 2017; Ricklefs and Wikelski, 2002; West-Eberhard, 2005, 2003).

### *Synergic effects of global change drivers and maladaptive plasticity*

However, data onto the synergic effects of global changes drivers on adaptive phenotypic plasticity are lacking, and it is likely probable that an adaptive phenotypic plasticity might become maladaptive (i.e. reducing fitness on the long-term basis, more than it increased it (Robertson et al., 2013) under rapidly changing environments in the face of global change. For instance (see theoretical examples in **Figure 3**), if an individual shows plasticity under the pressure of a given global change driver (e.g. climate change), plasticity which appears to increase its fitness, then selection occurs and the plasticity becomes adaptive. If the environment in which this plasticity occurred remain stable over time or if the changes remain constant, the population will therefore have a higher probability to cope and low risks of extinction (Population A, **Figure 3a**). However, if the environment do not remain stable or if the individuals of a population in which a phenotypic plasticity has been selected are under several global change threats, they will have subsequently lower chances to cope and the population might (strongly) decline (Population B, **Figure 3a**). In this case, the previously adaptive plasticity towards a given global change driver has a higher probability to become maladaptive. Such theoretical example of a potential maladaptive plasticity is represented in **Figure 3b**: an individual presents a phenotypic plasticity (e.g. change in its breeding phenology) under climate change pressures **(1)**, which increases its fitness and is therefore selected over time and observed at the population level (adaptive phenotypic plasticity). Given that adaptive phenotypic plasticity has a genetic basis, and that individual's behavioral, physiological, morphological and ecological are intrinsically related (Réale et al., 2007; Ricklefs and Wikelski, 2002; Sih et al., 2004), it is very likely that this plasticity in breeding phenology will be genetically related to other traits (e.g. sensitivity to fragmentation, stress response, body size, cognition, personality…) that will vary accordingly. In the **Figure 3b**, a theoretical correlation between plasticity in breeding phenology and boldness is represented (i.e. assuming that only bold individuals displayed this plasticity in breeding phenology, because of a theoretical common genetic basis between these two traits). Bold individuals are generally more exploratory (Fraser et al., 2001; Smith and Blumstein, 2008), and they are advantaged in (i) environment with low predation pressure (Réale et al., 2007) and ($i_2$) in their capacity to colonize anthropogenic habitats (Sih et al., 2004; Sol et al., 2013). However, they are disadvantaged in (ii) habitats with high predation pressures and ($ii_2$) have more chances to be trapped in rapidly-changing or low-quality habitats (Demeyrier, 2016; Robertson et al., 2013). Global change drivers such as biological invasions and habitat degradation (**Figure 3b**, **(2)** and **(3)**) can influence the colonization capacities of these bold individuals or the predation pressure in the colonized habitat, therefore negatively impacting plastic-bold individuals' fitness. This theoretical example is an illustration of a maladaptive phenotypic plasticity resulting from (a) an initial adaptive plasticity towards a given global change driver, (b) an intrinsic correlation between and individual's traits and (c) the synergic effects of varying global change drivers on the capacity of an animal to adapt.

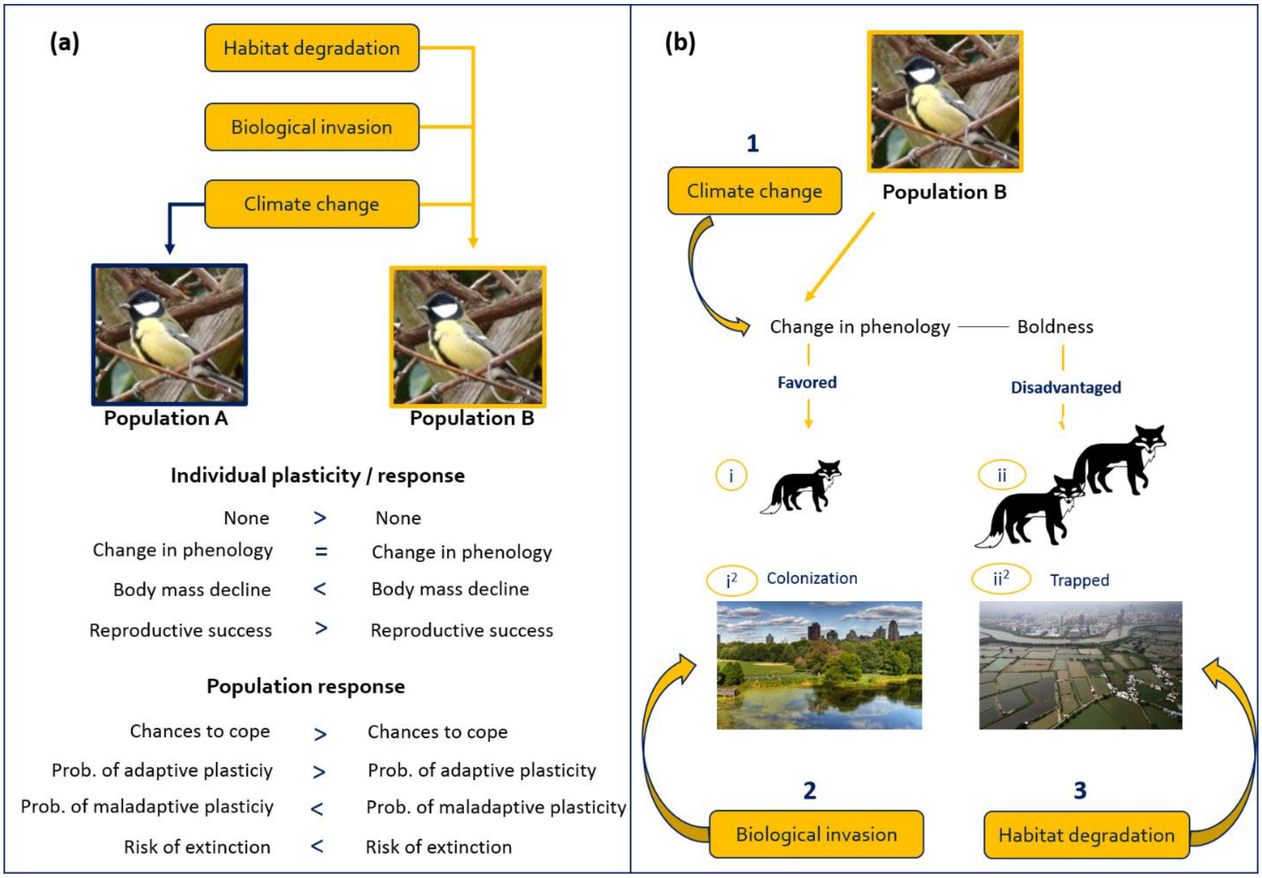


**Figure 3: Theoretical framework highlighting the potential effects of global change onto populations' responses.** In **(a)** the framework is adapted to a fictive population A only exposed to climate change in comparison to population B, exposed to several global change drivers. In **(b)**, a theoretical example leading to maladaptive plasticity is shown for population B.

Given that human activities are currently modifying the 'phenotype/fitness relationship' (Hendry et al., 2017), and that we still don't know precisely to which extent this will affect population dynamics (population size, age, growth, age structure), communities structure (number and diversity of species, food web structure), ecosystem processes (primary productivity, decomposition, nutrient cycling…) (Díaz et al., 2006; Hendry et al., 2017) or human health and safety (Bayles et al., 2016), the ecological and evolutionary costs of human activities on biodiversity loss still requires intensive investigations. This claim has triggered scientific – and more recently public and politic (McEuen, 2014; Trowborst et al., 2016) – awareness in regards of the tremendous effects of biodiversity loss on global economy and on human health and safety (Bayles et al., 2016; CBD and WHO, 2005; Díaz et al., 2006; Myers et al., 2013; Palumbi, 2001; Rogalski et al., 2017).

This ultimately leads to the massive approbation that biodiversity as a whole needs to be protected from global change, even though some people still remain skeptical (see (Budiansky, 1993; Lomborg, 2003)).

## 4. The value of nature and biodiversity.

Two main approaches can be used in conservation biology: conserving ecosystems (habitats) or species. Although both approaches have been intensively used, the most used has been the conservation of species, easier to implement than the conservation of ecosystems, but not always efficient and highly expensive (Barua, 2011; Simberloff, 1998; Vitousek et al., 1997). This approach has sparked many debate (Barua, 2011; Kontoleon and Swanson, 2003; Maslo et al., 2016; Simberloff, 1998) on the different strategies to be used. That concerns the choice of the species to protect (keystone species, umbrella, rare, charismatic or most vulnerable species) (Barua, 2011), on the way to protect it (re-introduction, reinforcement, translocation, establishment of natural reserves, supplemental feeding…), or on the lack of knowledge on the biology of some species to implement effective conservation measures. Another last and central point is the fact that if habitat is not suitable/adapted, every effort to protect the species will result in a failure (e.g. protected area too small, habitat not protected…). Therefore, the protection and restoration of whole ecosystems represent in most cases a better approach to protect biodiversity and natural resources as a whole (Blaustein and Kiesecker, 2002; Vitousek et al., 1997). Finally, one major difficulty was the absence of biodiversity or nature integration in decision making before the 80s, leading to many decisions ignoring its existence and many decision-maker assuming its unimportance (Edwards and Abivardi, 1998). Therefore, some conservationists have started to argue about the necessity to give a price/value to biodiversity so it would be integrated in political and economical decisions and better protected. Different values can be given to biodiversity and nature (see **Figure 4**): the total value can be divided into the instrumental (useful or use value) and the moral/existence (virtual or non-use value) values (the latter being harder to economically evaluate (Edwards and Abivardi, 1998)).

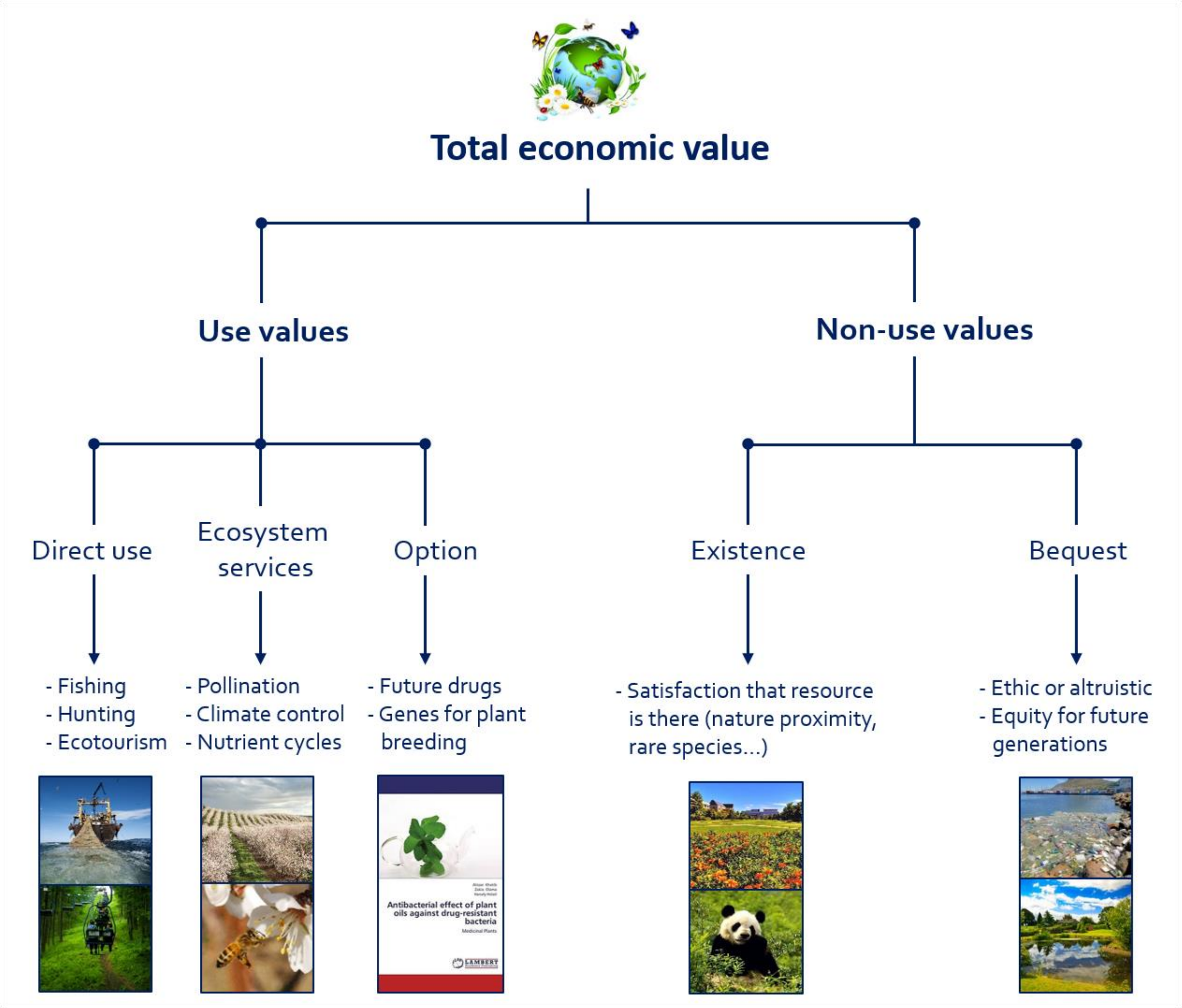


**Figure 4: Economic value of nature and biodiversity** (adapted from (Edwards and Abivardi, 1998)).

One of the most common current approach is to estimate the replacement costs of ecosystem services (Bullock et al., 2011), organized into four general categories by the Millennium Ecosystem Assessment (CBD and WHO, 2005). They are: (1) **Provisioning services**, such as food production for nutrition; (2) **regulating services**, through which ecosystems affect natural processes like vector-borne disease transmission or exposure to natural hazards; (3) **cultural services**, including recreational or psychosocial benefits derived from the environment; and (4) **supporting services**, which are not directly used by people but are instrumental in the delivery of other services (e.g. soil fertility, which underpins the provisioning service of food production, is an example). It appears easier to evaluate the replacement costs of the services rendered by an ecosystem than the exact value of this ecosystem.  For example, it is easier to evaluate the cost of hand-made pollination following the disappearance of bees in Maoxian (China's border) than to evaluate the total value of the ecosystem. In the same way, it is easier to economically evaluate the costs of Mangrove degradation (on human health or on human safety because of increased risks to tsunami without Mangrove protection (Bayles et al., 2016)) than to evaluate the total value of Mangrove ecosystems. Obviously, these approaches are only producing partial estimations of the value of nature and biodiversity, which is a great limit. Indeed, many decision-making regarding human safety and future or regarding the future of the planet are based on these approximations. In 1988, Norton (Norton, 1988) stated 'It is one thing to treat valuation of biodiversity as a guessing game or as a set of very interesting theoretical problems in welfare economics. It is quite another thing to suggest that the guesses we make are to be the basis of decision making that will affect the functioning of ecosystems on which we and our children will depend for life'. Moreover, there is a clear ethical limit associated to the fact that valuing biodiversity or the services it provides is highly anthropogenic. Therefore, the focus is often given to ecosystem services or species of value (of use and non-use) for humans and other species/resources are neglected. This is true for species or ecosystems of direct use (e.g. wildfowl, fish), for those producing ecosystem services (e.g. taxa such as bees or earthworms (Beynon et al., 2012; de Vries et al., 2013; Vanbergen, 2013; Wratten et al., 2012), and ecosystems such as wetlands or forests (CBD and WHO, 2005)), but also for species or ecosystems of non-use value (from which humans enjoy the existence) such as charismatic species (e.g. giant pandas (*Ailuropoda melanoleuca)* (Kontoleon and Swanson, 2003)) or biodiversity 'hotspots' (i.e. areas with high concentrations of endemic species that are undergoing major loss of habitats and species) (Myers et al., 2000; Pimm and Raven, 2000). This anthropogenic approach of biodiversity conservation (i.e. this concern for species/resource valuable to humans, the massive interest for charismatic or rare species…) has led to an important bias illustrated by the carelessness towards not-charismatic species, species in conflict with human activities (that can even be intensively exterminated), or ecosystem with common and endemic species, leading to the emergence of 'cold-spots' (Gomulkiewicz et al., 2000). Nonetheless, even though they might be imperfect, three major benefits have resulted from these approaches:

(1) valuing biodiversity has allowed to include it in decision-making;

(2) the implementation of natural reserves – qualified as '**living laboratories**' (Edwards and Abivardi, 1998) – has allowed to keep tracks of the roles of biodiversity in functioning ecosystems and of what landscapes previously were;

(3) species protection (when implemented on keystone or umbrella species) has allowed to maintain parts of biodiversity for future conservation plans (as an emergency measure) and in some cases to restore major ecosystems (e.g. of keystone species such as grey wolves (*Canis lupus*) in Yellowstone (Ripple and Beschta, 2012, 2003) or the European otter (*Lutra lutra*) (Bifolchi and Lodé, 2005)).

However, another limit (much more complicated to overcome) has emerged regarding species or ecosystems for which the intensive exploitation is highly lucrative: even if they are given an economic value, the need of their exploitation or the price extracted from it is, in most cases perceived as higher than their value of use or non-use. This is for example the case of species exploited for subsistence (e.g. bushmeat consumption in Madagascar (Bayles et al., 2016) or in Nunavut (Johnson-Down and Egeland, 2010)) or for commerce (poaching) and recreation (trophies) (Graham et al., 2010; Lindsey et al., 2006). This can also be the case of natural ecosystems such as grasslands, wetlands or forests that are continuously converted to farmlands or urban areas (Foley et al., 2005; Stoate et al., 2001) despite the efforts to preserve these ecosystems.

## II. Conflict of use between agriculture, urbanization and biodiversity.

### 1. Urbanization and agriculture intensification: history and consequences with the emergence of a major conflict of land use

Agriculture, by creating a food surplus and allowing new labor, allows for the expansion of new activities and industries, resulting into permanent human settlements and population growth (Antrop, 2004). Therefore, both agriculture expansion and urbanization are intrinsically linked, and are, with protected/recreational lands, the main characteristics of land use intensification, which have changed 'the world's landscapes in pervasive ways' (Foley et al., 2005). This is specifically true since the second half of the 20th century, during which both agriculture and urbanization greatly intensified (Antrop, 2004; Cohen, 2003; Evans, 1997; Matson et al., 1997; Naylor, 1996).

### *Human population, urbanization and road infrastructures fragmenting the landscape*

Urbanization can be broadly defined as a complex process transforming rural or natural landscapes into urban, sub-urban or industrial areas, made accessible by the development of road infrastructures (Antrop, 2004). Its worldwide intensification began at the beginning of the 19th century with roughly 2% of people living in cities in 1800 (Cohen, 2003). At this period, agglomerations started to spread out towards the countryside (Antrop, 2004). Urbanization then largely intensified from 1900 to 2000 (with 12% and 47% of people living in cities, respectively) (Cohen, 2003) and its almost exponential growth was intrinsically linked to the development of new modes of transportation, especially after World War II (Antrop, 2004). The increasing use of automobiles then led to the expansion of motorways, which replaced railroads and railways, and the emergence of new era of transports. By the end of the 20th century, high speed railways were also developed in Europe. These new railroads, associated to the already developed motorways, progressively created strong barriers fragmenting the landscape (Antrop, 2004). Moreover, the generalized use of cars associated with the development of technology have naturally augmented people's ability to commute between geographically distant areas. This phenomenon led to the formation of sub-urban areas, metropolitan villages and edge cities (Antrop, 2004; Berry and Plaut, 1978). With an increasing number of people living in cities (Cohen, 2003) and using automobiles as a mean of transportation, congestion problems have led to the construction of yet more road infrastructures (Antrop, 2004), further increasing landscape urbanization and fragmentation. Between patches of urban development (Antrop, 2004; Berry and Plaut, 1978) remnants of countryside have been in most cases converted to farmland or places of high recreational value, ironically because of the absence of urban environments nearby (Antrop, 2004). This created massive areas of human dominated landscapes. This phenomenon of urban sprawl appears to be more problematic for the landscape than the cumulative urban areas per se (Berry and Plaut, 1978) causing major problems of landscape fragmentation (Jaeger and Fahrig, 2004; Seiler and Folkeson, 2006). Thus, the effects of urbanization on the environments are quite factual: reduced vegetation cover with climatic outcomes, increased air, soil and water pollutions from light, sound, gas emissions and other chemical sources, modifications of the microclimate (Dominoni et al., 2013; Foley et al., 2005; Grimm et al., 2008; Hölker et al., 2010; Mage et al., 1992). On the other hand, the effects of urbanization on landscapes appears to be strongly dependent on geographical region, features and time (Antrop, 2004; Verburg et al., 2010). However, it remains uncertain which of urbanization or agriculture will continue to expand the most in the future (Verburg et al., 2010). Curent trends (Verburg et al., 2010) suggest that urbanization and agricultural intensifications are diametrically opposed and that their becoming will be region-dependent. Several scenarios predict that agricultural abandonment will be the dominant land use change in Southern Europe at the profit of urbanization and all the scenarios show that, in parallel, large areas will display major expansion of agriculture in order to meet the food, livestock feed and fuel demands of human populations (Verburg et al., 2010). Indeed, as indicated by (Foley et al., 2005), 'the annual addition of 77 million people poses formidable challenges of food, housing, education, health, employment, political organization and public order.'

### *Agriculture intensification: the Green Revolution.*

At the beginning of the 19th century, as human population grew and expanded, so did the need for food, leading to major and fast agriculture intensification over the world, entitled the 'Green Revolution' (Evans, 1997; Matson et al., 1997; Naylor, 1996). This agriculture intensification started in the US during the 30s, and by the 50s already had a major impact on American farms, notably with the introduction of seed hybrids for maize and the disappearance of both crop rotation and horses to the benefit of machinery (Naylor, 1996). Agriculture intensification started slightly later in Europe: during the 40s or the 50s depending on the countries considered (Björklund et al., 1999; Evans, 1997), and from mid- to late 60s in other parts of the world (i.e. in developed and developing countries) (Naylor, 1996). This 'Green Revolution' is declinable through four inter-related dimensions:

1) the remarkable rise in yields, e.g. the global production of cereal crops tripled from 40s to 50s (Naylor, 1996);

2) the intensive development of technologies, e.g. seed hybrids, seeds resistant to pests and disease, mechanization, chemical fertilization, irrigation… (Evans, 1997; Matson et al., 1997; Naylor, 1996);

**3)** the generalization of crop monoculture associated to a major increase in plot size and reduction in crop diversity (Björklund et al., 1999; Evans, 1997; Matson et al., 1997; Sotherton, 1998);

**4)** the displacement of several crops beyond their previous range (Naylor, 1996). This is notably the case for rice, maize and wheat, the three crops that gathered almost all efforts in agricultural intensification (Naylor, 1996).

Indeed, intensive work has focused on improving the performance, the nutritional qualities and the resistance to pathogens of maize, wheat and rice (Naylor, 1996; Ortiz-Monasterio et al., 2007). These crops have started to be grown in non-adapted ecosystems (Naylor, 1996), increasing the need to extensively control water, temperature and nutrient requirements. For example, crop irrigation and external nitrogen inputs have increased dramatically from the 60s to the 90s (Matson et al., 1997) resulting in a major rise in crop yields (Matson et al., 1997; Naylor, 1996). Irrigated corn has benefited the most (Matson et al., 1997) with yields that have risen from ~2500 kg/ha in the 40s to 4000 kg/ha in the 60s and 10 000 kg/ha in the 90s. More broadly, by the 90s, the Green Revolution had allowed to double the annual grain production in the developing world (Naylor, 1996). This rise in yields allowed encompassing for the loss of land towards urbanization. In Sweden for instance, 20% of the agricultural land was reallocated from 1951 to 1992: 10% towards reforestation and the restoration of bushes, and the other 10% were lost at the profit of urbanization. Despite this 20% removal of land from production, total harvest of cereals has increased by about 85% in this country (Björklund et al., 1999), highlighting how strongly the Green Revolution, namely through technologies development, has favored yields.

### *The limits of the Green Revolution and the costs of maize monoculture*

The Green Revolution has begun to face many limits from the 80s onward (Naylor, 1996), namely regarding the depletion of both non-renewable and potentially renewable natural resources. Such examples are soil fertility (potentially renewable), energy (e.g. fossil fuel used for machinery; nonrenewable) and fossil water (over-pumped in an effort to maintain irrigated crop yields; potentially renewable) (Björklund et al., 1999; Naylor, 1996; Vitousek et al., 1997). Indeed, three times more energy is needed for 1 ha of irrigated maize than for the same area of rain-fed maize in the US (Naylor, 1996). Moreover, across the world, agriculture alone is estimated to use

70% of the freshwater (Vitousek et al., 1997). The rate at which freshwater and soil have been exploited surpasses the natural rate of their regeneration (Naylor, 1996), and, associated with the depression in fossil fuel, are highly threatening agricultural productivity on a long-term basis. Indeed, the consequences of agriculture intensification at a local scale mainly concern the major decline in crop yields observed over the past 40 years. In the 90s, already 2 billion ha of cropland and pastures were degraded because of soil erosion throughout the world. This led to a loss in agricultural productivity of 10-50% depending on the severity of the degradation. Moreover, 0.5% of these degraded land were estimated as definitely unreclaimable. The cause of these declines in yields are numerous, and include problems of waterlogging, nitrate leaching, soils destructuration, alkalinization or salinization and loss of fertility, pesticide resistance, reduced genetic diversity of crops, modified host-parasites relationships and climate change (Foley et al., 2005; Hawkins et al., 2013; Matson et al., 1997; Naylor, 1996; Turcotte et al., 2016; van der Velde et al., 2012; Vitousek et al., 1997). Regarding maize, yields decline have started at the end of the 20th century, and even reached 80% from the 80s to 90s in some areas of the Philippines (Naylor, 1996). The decline in maize yields is now estimated at 14% worldwide (Fargione et al., 2009) and is expected to further increase because of climate change (Hawkins et al., 2013; van der Velde et al., 2012). Maize is currently the most produced crop worldwide (slightly beating wheat and rice; 32%, 31% and 22% of the world's cereal crop production (Nuss and Tanumihardjo, 2010)) but largely less consumed than these two other crops (human consumption: maize = 12%, wheat = 44% and rice = 37% (Nuss and Tanumihardjo, 2010)). This difference between production and consumption is explained by the diversified use of maize (or corn) (Fargione et al., 2009; Nuss and Tanumihardjo, 2010). Indeed, maize is particularly interesting from its fatty acid composition (especially adapted for livestock diets) and its high starch content (for ethanol production and the development of industrial and pharmaceutical industries). Therefore, it has been intensively used during the expansion of first generation biofuels, especially in the US (Fargione et al., 2009; Martin et al., 2008). The consequences of agriculture intensification transcend the decrease in yields and can also be measured at the regional and global scales through the pollution of groundwater, the eutrophication of lakes and rivers and the impact on atmospheric constituents and climate (Matson et al., 1997; Naylor, 1996). Growth in agricultural production has continued to outpace population growth on a global basis (Naylor, 1996) and with the recent boom in biofuel production (Fargione et al., 2009; Martin et al., 2008), crop monoculture – especially maize – is increasingly threatening natural resources (except fossil fuel) leading to great economic losses, human health problems (toxicity of synthetic organic chemicals, acid rain and photochemical smog…) and to a major decrease in biodiversity and ecosystem (Björklund et al., 1999; Turcotte et al., 2016; Vitousek et al., 1997).

### *Urban areas and farmland: conflict of land use*

Given that urban areas and cropland are usually developed in the same geographical areas (in lowlands, such as alluvial valleys), this has led to the emergence of a major conflict of land use between intensive agriculture and soaring urbanization (Antrop, 2004; Berry and Plaut, 1978). Therefore, not much place is left for nature and biodiversity in these highly cultivated and urbanized areas, which often leads to human-wildlife conflicts (Barua, 2011; Baruch-Mordo et al., 2014; Henle et al., 2008). Finally, given the observed and expected decline in crop yields, we can easily predict that in the future, and if nothing is effectively done to reverse these trends, more land will be required to maintain the annual global productions of major crops such as maize, wheat, soybean and rice, thereby increasing the competition with urbanization, nature and wildlife for available land. Interestingly, the perception of a conflict between intensive agriculture and urbanization has largely evolved. In the 70s, urbanization was perceived as a direct threat to agriculture and therefore human population food sustainability (Berry and Plaut, 1978). For instance, Berry and Plaut stated that 'land use controls [were constantly implemented] to redirect urban intrusions away from agricultural land'. However, it appeared that twice more cropland has been lost because of decreased productivity (Berry and Plaut, 1978) (i.e. a direct consequence of intensive agriculture) than because of urbanization in the US (Berry and Plaut, 1978). Much more recently, Verburg et al. (Verburg et al., 2010) highlighted that in Europe, more and more attention 'is given to policy interventions that are designed to counteract some of the negative consequences of land use change, such as the protection of designated areas to avoid conversion to (intensive) agriculture'. In parallel, several cities with important proportions of urban green spaces have been shown to be 'refuges of biodiversity'(Goddard et al., 2010), especially for species threatened by intensive agriculture, such as bees (Goulson et al., 2002; Hall et al., 2016). This phenomenon is a direct consequence of increased conservation friendly management in urban areas with in parallel the reduced quality of rural places owing to intensive agriculture (Goddard et al., 2010; Hall et al., 2016). In Europe, farmland is now encompassing for 40-70% of land cover (Antrop, 2004; Foley et al., 2005), the highest percentages being encountered in Western Europe. Roughly 14-17% of these land are devoted to crop monoculture (Sotherton, 1998) and France is largely on top of the list regarding the surface area covered by cereals (Stoate et al., 2001). Therefore, intensive agriculture appears as the biggest threat to natural landscapes and natural resources, including biodiversity, of the Old-Continent (Benton et al., 2003). 'The drive to squeeze ever more food from the land has sent Europe's farmland wildlife into a precipitous decline'. This alarming statement from Krebs et al. in *The Second Silent Spring?* (Krebs et al., 1999) summarizes the seriousness of the situation regarding farmland biodiversity in Europe.

## 2. Farmland wildlife in Europe

### *Wildlife decline in European farmland illustrated by the collapse of farmland bird populations*

The decline of wildlife in European farmland has been more sever and faster than in any other habitat (Stoate et al., 2001; Wilson et al., 2005), and has largely been illustrated by the collapse of farmland bird populations, that already largely took place by the end of the 90s (Benton et al., 2003; Evans, 1997; Stoate et al., 2001). In the UK, the decline of farmland bird populations between 1969 and 1994 ranged from 17 to 89% depending on the species (Evans, 1997), with species such as the grey partridge (*Perdix perdix*), the tree sparrow (*Passer montanus*), the corn bunting (*Milaria calandra*) and the turtle dove (*Streptopelia turtur*) showing the most alarming declines (of up to 82%, 89%, 85% and 77%, respectively (Evans, 1997; Stoate et al., 2001)). It appeared that farmland bird specialist species declined at the profit of generalist species (Siriwardena et al., 1998). In 1999, Björklund et al. (Björklund et al., 1999) revealed that 42-45% of farmland species were endangered in Sweden and that invertebrates had dramatically declined in regions with intensive agriculture between the 50s and the 90s (by 60% for wild pollinators and by 75% for cereal invertebrates). The same trend was observed much more recently for invertebrates in the UK (Thomas, 2009), even highlighting that butterfly species have been far more affected than plants or birds.

For decades, research on the effects of agriculture intensification on the decline of farmland wildlife has strongly been biased towards birds and invertebrates (especially regarding species of interest to agriculture, e.g. pollinators and earthworms) (Benton et al., 2003; Buckingham et al., 1999; Evans, 1997; Fuller et al., 1995; Sotherton, 1998; Stoate et al., 2001; Thomas, 2009; Wilson et al., 2005, 1999), whilst considerably less studies have investigated how it has affected farmland amphibians, reptiles and small mammals (Boothby, 1995; Tapper and Barnes, 1986); see (Hole et al., 2005; MacDonald et al., 2007; Stoate et al., 2001) for reviews of the literature underlining the bias). This bias can be explained by the fact that birds are well-described and easy to monitor, providing excellent indicators of environmental change (Stoate et al., 2001). Moreover, many small mammals (especially rodents) are still considered, in the 21th century, as pest species and therefore present few interests and even a threat to agricultural sustainability (Capizzi et al., 2014; Jacob, 2003; Zhang, 2011). However, rodents - since they greatly contribute to the complexity of food webs in croplands and are crucial for nutrient cycling and soil structure - are starting to be recognized as major actors in agricultural ecosystems and as ideal models to study how agricultural changes might impact biodiversity (Medan et al., 2011). Therefore, in the last decade, the number of studies on these taxa and their decline in farmlands has risen (Báldi and Faragó, 2007; Curado et al., 2011; Reid et al., 2014). Recent studies reveal similar trends as those observed in invertebrates and birds: populations of the European or brown hare (*Lepus europaeus*), the European hamster (*Cricetus cricetus*), the water vole (*Arvicola terrestris*), the harvest mouse (*Micromys minutus*) and many farmland bat species (*Chiroptera spp*.) have experienced drastic declines because of agriculture intensification (MacDonald et al., 2007; O'Brien, 2015; Weinhold, 2008). Such example is the collapse of the European hamster that shows a decline of roughly 75% in all its European range (Figure 5).

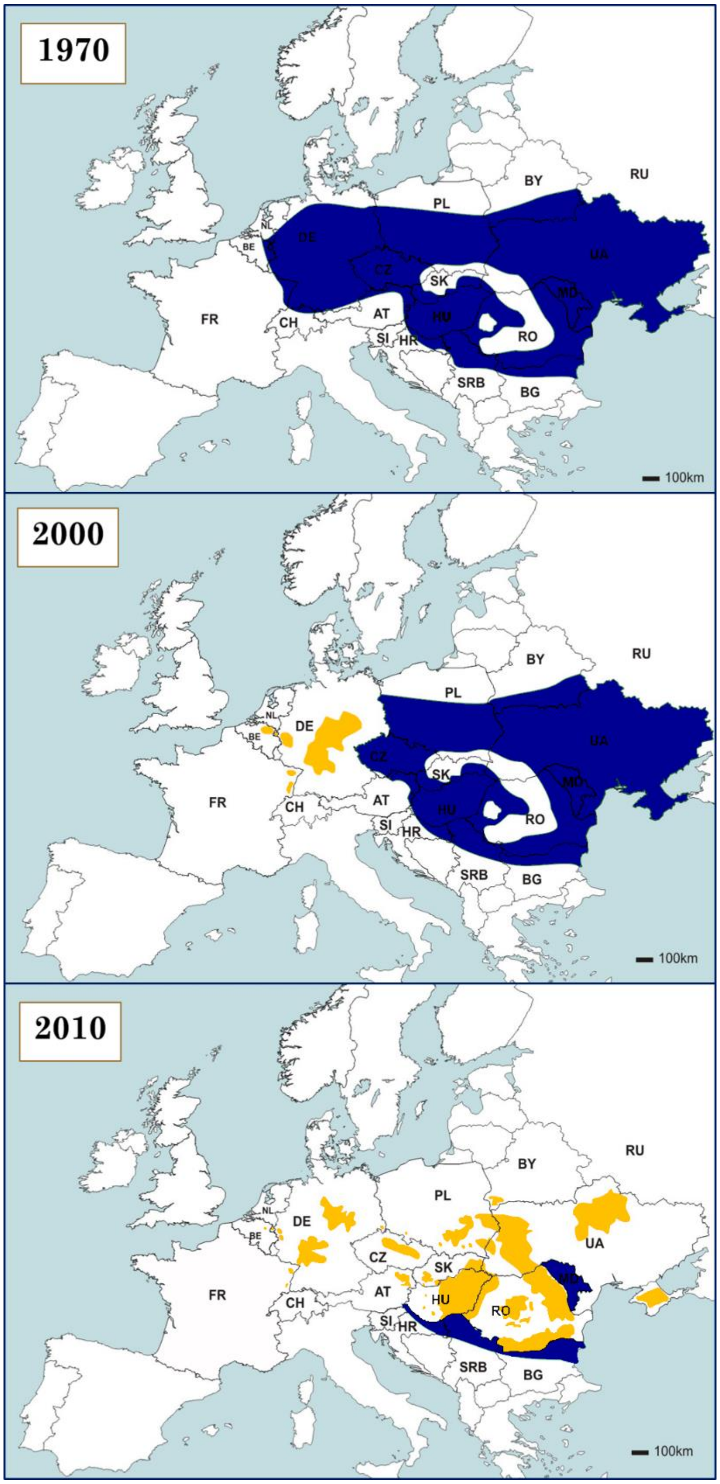


**Figure 5: Overview of the European area of distribution of *Cricetus cricetus* in 1970 (top), 2000 (middle) and 2010 (bottom).** Light orange represents updated data and highlights a fragmentation of the European area of distribution of the species. Dark blue represents the ‘initial’ area of distribution (in 1970) or a lack of updated data (2000 and 2010). (adapted from (Banaszek, 2015)).

It is important to highlight that the Figure 5 only gives an approximation of the fragmentation of the European hamster's distribution area. Indeed, precise data on the dates of decline in each European regions are not known, and not represented for the 'initial' map in 1970. Therefore, we cannot draw any conclusions regarding the dates at which the decline has started. However, these maps can be used to illustrate that the decline has not only occurred on the Western-Part, but on the totality of the distribution range of the species in Europe.

A recent review of the literature on this species (Surov et al., 2016) argues that hamsters decline throughout Europe has been caused by an apparent reduction in reproduction, ant not a reduction of survival. Authors state that this reduction in reproduction seems to have started in the 50s (Surov et al., 2016), but the Figure 7b of this study highlights an important gap in the data at this period. Therefore, their conclusions on the dates of the decline in reproduction seem overstated. They also argue that hamsters' populations collapsed between 1975 and the 1990 in Europe and therefore that 'modern agriculture' is unlikely to be responsible of the species decline, since it occurred later on. However, they give no dates or definition of 'modern agriculture' (Surov et al., 2016). Given the data on other farmland taxa showing similar trends (i.e. with a decline starting from the 50s or the 60s and being major in the 70s (Benton et al., 2002; Evans, 1997; Fuller et al., 1995), and given that agriculture intensification started between the 40s and the 50s in Europe (see section II.1.)), the conclusion of Surov et al. (Surov et al., 2016) on the implication of agriculture in the decline of the European hamster appears largely unfounded. Nevertheless, the other suggested causes of decline in this review (e.g. global warming, light pollution associated to urbanization) (Surov et al., 2016) remain to be investigated. Indeed, it is highly probable that the species suffered from several environmental pressures, which strength and diversity likely varied according to geographical localization (i.e. Western and Eastern Europe, urban areas or farmland…) and likely changed differently over time. These rapid population declines (either caused by human trapping and poisoning, intensive agriculture, urbanization or climate change (Kayser et al., 2003; Korbut and Agata, 2016; Monecke, 2013; Surov et al., 2016; Ulbrich and Kayser, 2004; Weinhold, 2008) have led to a partial or complete loss of genetic diversity in some of the Western populations (La Haye et al., 2011; Melosik et al., 2017; Neumann et al., 2004; Smulders et al., 2003). For instance, a study has revealed that the totality of the MHC (i.e. major histocompatibility complex, essential for the acquired immune system functioning) diversity has been lost in the Netherland population (Smulders et al., 2003). These loss of genetic diversity could greatly impair the dynamics of the concerned populations and any conservation efforts.

### *The main causes of farmland wildlife decline*

As indicated above, the ultimate causes of agriculture intensification on wildlife decline are numerous, and can be direct (e.g. increased tillage, land use change, major use of pesticides, reduced food diversity…) or indirect (e.g. modification/selection of the phenotype of domesticated species resulting into increased selection pressures on wildlife) (Turcotte et al., 2016). Agriculture intensification has in particular resulted into extreme changes in the structure and composition of landscapes, two major changes that have the most affected wildlife (Benton et al., 2003; Wilson et al., 2005) since they greatly impair species ecology (at all life-history stages) and allow few chances for adaptations. Indeed, intensive agriculture creates homogenized and simplified landscapes with greatly modified vegetation (e.g. crop density, vegetation architecture and diversity, total mass of vegetation per unit of time…) and associated to the loss of non-cropped habitats such as grassland, field margins, hedges, water-courses and trees (Benton et al., 2003; Stoate et al., 2001; Wilson et al., 2005). This modification of the vegetation has different consequences: reduction of the protective cover against predators, exposition to extremes of weather, reduction of foraging efficiency (by reducing the diversity, abundance and accessibility of food items) which will all result in a fitness reduction. This reduction could happen **directly** (through an increased predation rate, or a reduced reproductive success because of insufficient food availability) or **indirectly** (e.g. through the modification of the trade-off between time allocated to foraging, to vigilance against predators and to reproduction). Diversity of food, cover and more broadly of habitats is essential to the ecology and seasonality of many farmland vertebrates (Stoate et al., 2001). For instance, the brown hare is feeding on different crops according to the period of the year (Tapper and Barnes, 1986). Seed-eating birds highly depend upon remaining stubble and seed access during fall and winter (Evans, 1997). Moreover, males and females habitat requirements might differ (Stoate et al., 2001). Indeed, many bird species switch from one crop or one habitat to another during the breeding season, as part of their reproductive strategy and their changing food requirements throughout the year (Stoate et al., 2001)). If their food or habitat requirements are not met, individuals will disperse, then facing increased risks of predation or the risk of dispersing into poor-quality habitat in these intensively-cultivated landscapes (Benton et al., 2003). Furthermore, given that intensive farmland are also highly fragmented due to soaring road infrastructures development (Antrop, 2004; Foley et al., 2005; Forman and Alexander, 1998; Lesbarrères and Fahrig, 2012), wildlife can be affected in several ways. First, traffic noise has been shown to affect a variety of traits and the fitness of several vertebrate species (Reijnen et al., 2008; Troïanowski et al., 2017, 2015). Second, dispersion might be obstructed by road infrastructures. Given that wildlife underpasses (initially created to restore ecological corridors and reconnect isolated populations (Mata et al., 2008)) are used both by prey-species *and* their predators (Carsignol, 2006; Jumeau and Handrich, 2016; Little, 2003; Little et al., 2002; Mata et al., 2008), they might become 'ecological traps' (Little et al., 2002). Indeed, an increased risk of predation in the underpasses could lead to its avoidance by prey-species or an increased mortality-by-predation at proximity or inside the facilities. This phenomenon has been recorded for rodents, being predated by cats or foxes in French wildlife underpasses (Jumeau and Handrich, 2016). Even though these cases of predation appear to be context- and species dependent (Little et al., 2002), they could be particularly damaging to small or endangered populations.

### *Farmland wildlife: after three decades, the trend has not been reversed*

Management of agriculture should therefore focus onto increasing heterogeneity in the agricultural landscape and to reverse the trend towards dense, simplified and homogeneous structures that have characterized recent agricultural intensification (Wilson et al., 2005). This would allow to restore wildlife food resources, habitat diversity essential for nesting, foraging and protection from predators and would ultimately limit unnecessary dispersion. In parallel, there is a strong need to restore proper habitat connectivity through ecological corridors, namely to improve the efficiency and safety of wildlife underpasses for a broad diversity of animals.

However, it is now well known (i.e. for more than 30 years) that intensive agriculture and habitat fragmentation are a major cause of biodiversity and ecosystem service decline. Yet and approaching 2020, the trend in European farmland decline still has not been reversed (Inger et al., 2015; Newbold et al., 2015; van Strien et al., 2016). First, conservation efforts have often targeted some species at the expense of others (see above). Second, the time needed to recover biodiversity and functionality of intensive agro-ecosystems is long. Indeed, the pervasive effects of agriculture on biodiversity are known to last between 25-30 years (Baeten et al., 2010; Naylor, 1996). A recent study highlights that rarer bird species at the core of many conservation efforts (Sotherton, 1998) have been recently increasing in Europe whilst common farmland bird species are now showing alarming declines (Inger et al., 2015). These results suggest that conservation efforts have been effective for some targeted species, but that non-targeted species have suffered from the effects of intensive agriculture. Another explanation of this absence of reversed trend despite intensive efforts for wildlife conservation in Europe could be that for several decades, conservation approaches have been opposed to economic and industrial development (Edwards and Abivardi, 1998).

## 3. Conciliating economy, human development and farmland biodiversity

### *The agri-environmental schemes and the European directives for wildlife protection*

In 1992, the European Common Agricultural Policy (CAP) was reformed to stimulate the development of agri-environmental schemes in Europe, with 50-75% funding from the European Community (Stoate et al., 2001; Woldehanna et al., 2000). These agri-environmental schemes were mainly destined to reduce the pervasive effects of intensive agriculture on farmland wildlife and include 'more friendly' management such as the restoration of margins, hedges, crop rotations or delayed harvest (La Haye et al., 2010; Marshall and Moonen, 2002; Petrovan et al., 2013). The adoptions of such measures by farmers were supported by financial compensations. However, given the inadequacy of some of these compensations, the general reluctance of farmers to abandon traditional practices, this approach has rarely been a success (Stoate et al., 2001). Another reform of this CAP occurred in 2000, known as the 'Agenda 2000' reform, and includes conditions to the payments of financial compensations. Under this reform, payments are 'conditional on compliance with certain environmental restrictions on arable management' (Stoate et al., 2001). For instance, in the Netherlands, the introduction of mechanical weed control as an alternative to herbicides is a condition for payment on the two most intensively cultivated crops: maize and potatoes. The more recent Rural Development Regulation (1257/99) now provides farmers with the opportunity (on a voluntary basis) to adopt 'further ecologically, economically and socially sustainable management practices and systems within European arable systems'.

In parallel of these CAP reforms, a number of European Commission Directives were developed with the main aim of conserving European nature as a whole (Stoate et al., 2001; Trowborst et al., 2016) but these directives also have implications for farmland: this is the case of both the Birds Directive (79/409/CEE, the 2nd of April 1979) and the Habitats Directive (92/43/EEC, the 21st of May 1992) (Muséum national D'Histoire naturelle, 2007). These directives commit each member state to the maintenance and/or the restoration of species qualified as of 'community interest' in a proper conservation state. They have led to the development of different tools destined at the conservation of biodiversity, such as the 'Natura 2000' Network and the National Plans of Actions (PNAs) (Muséum national D'Histoire naturelle, 2007).

### *The Natura 2000 Network and the National Plan of Actions*

The 'Natura 2000' mainly aims at ensuring the long-term survival of highly threatened species and habitats of strong conservation priorities in Europe (Muséum national D'Histoire naturelle, 2007) and has mainly been implemented through the development of both special protection areas (ZPS) and special conservation area (ZSC). The French 'Natura 2000' Network currently protects 57% of the habitats of 'community interest' listed in the habitat directive, 17% of the species listed in the habitat directive and 63% of the bird species listed in the bird directive. Regarding the PNAs, they aim at defining the necessary actions for the conservation and the restoration of the most vulnerable species at the National Scale and are usually implemented on a five-year period (Muséum national D'Histoire naturelle, 2007). Regarding the number of PNAs in France, it appears that bird and plant species are the most represented, reptiles and amphibians following and then mammals, fish and mollusks being the least represented (Ministère Ecologie, 2012). Recently, multi-species PNAs have been implemented, namely for bats, reptiles and amphibians, allowing to increase the number of represented species (Ministère Ecologie, 2012). However, PNAs are therefore species-centered, and rarely integrate the conservation or the restoration of an entire habitat, or rarely conciliate conservation with economy (Ministère Ecologie, 2012; Muséum national D'Histoire naturelle, 2007). Nonetheless, they have allowed the implementation of 'emergency conservation measures' for species critically endangered and might therefore have prevented the extinction of these targeted species in the country. This is for example the case for the European hamster:, which has indeed beneficiated from 3 successive

PNAs (2002-2017) (Chaigne et al., 2015). These PNAs contained measures such as restocking programs (i.e. reinforcement of natural populations by releasing captive-bred animals in areas close to the remaining populations), implemented in ‘unharvested wheat’ to provide released hamsters with a protective cover against predators (Villemey et al., 2013). The 4th PNA (for the 2018-2023 period) is currently being written. These measures have prevented the extinction of the species in France, but can only be implemented on a short-term basis, and the French European hamster population has still not recovered (Chaigne et al., 2015; O’Brien, 2015).

### *Assessment of these measures: a lack of conciliation between society, economy and biodiversity*

Whilst the implementation of agri-environmental schemes has rarely been efficient (see (Stoate et al., 2001)), it appears that the Habitats and Birds directives have not properly been implemented by the member states (Trowborst et al., 2016). Therefore, after a re-evaluation of these directives (with a massive participation of citizens), the European Commission recently announced a decision to renew the evaluation  of an effective implementation of these directives in all the European member states (Trowborst et al., 2016). A perfect example of this defective implementation is the case of the European hamster in France: in 2013, the European Commission threatened France of a 17 million fine if rapid efforts were not allocated to the protection of the species and its habitat. Indeed, despite 3 PNAs for the species in France and the implementation of protected areas under the ‘Natura 2000’, the species (listed at the annex IV of the Habitats Directive) is still alarmingly declining and its habitat degraded by intensive agriculture and urbanization. This is a clear example of a conservation failure. However, as indicated above, this failure probably occurred because of the clearly opposed targets of conservation policies and economic and industrial development in the region (O’Brien, 2015). Indeed, biodiversity conservation (as agriculture sustainability, see (Naylor, 1996)), has three dimensions: biological, economic and social, and there is a strong need to reconcile the three (Edwards and Abivardi, 1998; McEuen, 2014).

In 1992, a powerful tool for biodiversity conservation has been developed at the European scale: the LIFE program, supported by the European commission. LIFE is described as ‘the EU’s financial instrument supporting environmental, nature conservation and climate action projects throughout the EU’ (“LIFE,” 2017): it promotes multi-partnership approaches that develop actions on climate and energy, food waste management, conservation biology, environmental restoration… ("LIFE," 2017). Since 1992, four phases have been completed (corresponding to the LIFE I: 1992-1995, LIFE II: 1996-1999, LIFE III: 2000-2006 and LIFE+: 2007-2013 which continues from 2014-2020) and already 4306 projects have been launched throughout the EU ("LIFE," 2017). During the same period, in France, 343 projects have been co-funded by LIFE, 219 of which focused on environmental innovation, 117 on nature/biodiversity conservation and 7 on information and communication. These projects represented a €772 million total investment, of which €270 million were funded by the European Union ("LIFE," 2017). On the 1st July 2013, a LIFE+ biodiversity project was launched for a 5 years period in Alsace (France), destined to the preservation of the European hamster and the associated farmland biodiversity, entitled '**A**lsace **LI**fe ham**STER**: Demonstration project to preserve European biodiversity in Alsace' (i.e. LIFE ALISTER). This project of €3.096 million, is co-funded by the European Union (50% of the total eligible budget), and the Ministère de l'Ecologie, du Dévelopement durable et de l'Energie (MEDDE). It is coordinated by the Alsace Region, now called the 'Grand-Est'. The ALISTER project involves different partners, both non-academic and academic: the Agricultural Chamber of the Alsace Region (the CARA), an association for mammalian studies and protection in the Alsace region (the GEPMA), a technical consulting office for the environment (Actéon), the French Wildlife and Hunting Agency (the ONCFS) and the French National Center for Scientific Research (CNRS). This project is declined into four axes conciliating the biological, economical and sociological aspects of conservation. This project aims at improving the knowledge on hamsters' biology, finding innovative and long-term solutions for its conservation in France and Europe, whilst ensuring good agricultural/economical profits in the Region. My PhD thesis is an integral part of both the axes 1 and 2 of this project. The 4 axes correspond to 4 different objectives to be achieved:

1. **Improving the habitat of the common hamster,** by identifying, testing and evaluating promising and long-term sustainable farming practices adapted to the hamster and to local pedo-climatic conditions. These practices must be nutritionally and seasonally favorable to hamsters and also compatible with farmer's technical and socio-economic constraints. These objective will be achieved through studies in controlled and semi-natural conditions, followed by the establishment

of a participatory process for selecting and testing practices with the highest potential in natural conditions.

2. **Reconnecting hamster populations** by developing a device to improve the efficiency of wildlife underpasses crossing. Three innovative actions are proposed, (i) the establishment of anti-predation systems to be placed in wildlife underpasses in order to improve hamster crossing, (ii) the testing of this anti-predation system in semi-natural conditions (in an exclosure built on both sides of a motorway equipped with a wildlife underpass) and natural conditions and (iii) the dissemination of recommendations for transport infrastructure development in the zones of presence of the species in Europe.

3. **Creating new development opportunities for hamster populations** by testing the introduction of hamsters in urban/suburban areas. Four actions are proposed, aiming at (i) identifying the required conditions for the development of hamster populations in urban areas (such as in Vienna, Austria or Prague, Czech Republic (Franceschini and Millesi, 2003; Hufnagl et al., 2010; Surov et al., 2016); (ii) releasing 60 hamsters in the selected favorable sites; (iii) monitoring their development and fitness and (iv) identifying pre-conditions and recommendations for viable urban-suburban hamster populations.

4. **Strengthening citizens' awareness and social acceptance** regarding the 'conservation value' of hamsters and their importance for biodiversity. This will be achieved through three main actions, first (i) the implementation of specific events which will mobilize the public, combined with (ii) the regular interaction regarding farmland biodiversity and the umbrella characteristic of the European hamster and finally (iii) the monitoring of regional media to evaluate the change in the citizen's perception regarding the species and its protection.

## III. The European hamster, from its biology to its conservation

In its review on the European hamster in 1977, Nechay stated 'Although the hamster is one of the longest known rodents, there are hardly any data regarding it as compared with the immense literature on other commonly occurring rodent species. Regarding many problems (e.g. taxonomy, reproduction, hibernation, stores), much is unknown, or only contradictory information is available. There is no knowledge of its population dynamics and demographic parameters, which is important for developing and organizing measures of control. Little is known of its territorial and social behaviour, or of its circadian and seasonal activity, migration, nutritional requirements and feeding habits'. Since then, intensive work has been carried out on hamsters' biological rhythms (Masson-Pévet et al., 1994; Monecke and Wollnik, 2005; Monecke et al., 2014, 2006; Sáenz De Miera et al., 2014; Wollnik and Schmidt, 1995), agriculture management for conservation purposes (Kuiters et al., 2007; La Haye et al., 2010; Out et al., 2011; Villemey et al., 2013; Weinhold, 2008), activity patterns and food habits in urban environments (Franceschini and Millesi, 2003; Franceschini et al., 2007; Hufnagl et al., 2010; Schmelzer and Millesi, 2003), population dynamics modelling (La Haye et al., 2014; Tkadlec et al., 2015; Ulbrich and Kayser, 2004) and more recently on the genetic diversity of different populations across Europe (Melosik et al., 2017; Neumann et al., 2005, 2004; Reiners et al., 2014; Smulders et al., 2003). However, exactly 40 years later from Nechay's statement, there is still a huge gap regarding the scientific knowledge in many aspects of hamsters' ecology, eco-physiology and behavior in farmland habitats.

## 1. The European hamster: its biology, its ecology and its history in the Alsace

### *Morphological and biological characteristics*

The European hamster is one of the largest rodents in the *Cricetidae* family. On average, males are longer and larger than females (27-32 cm and 350g, and 22-25 cm and 260g, respectively) (Fenyk-Melody, 2012; Nechay et al., 1977). Both sex show a marked seasonal cycle (**Figure 6**): they roughly hibernate from October to March and are active from April to September (Lebl and Millesi, 2008; Ulbrich and Kayser, 2004), although strong variations occur depending on individual sex and age. Adult males are the first to immerge in their burrow for winter and the first to hibernate, followed by adult females (**Figure 6**). Sub-adult individuals of both sexes only immerge in their burrow at the end of September-beginning of October (Ulbrich and Kayser, 2004). Therefore, adult females hibernate for shorter periods than adult males and spend more time active in their burrow before emerging from hibernation (Siutz et al., 2016). Slight variations regarding hibernation duration have also been recorded according to the geographical area considered and between lab and wild conditions (Schmelzer and Millesi, 2003; Thomas, 2004; Ulbrich and Kayser, 2004). Hamsters' body mass varies throughout the annual cycle: individuals gain weight for the summer, with adult males weighing up to 650 g (Fenyk-Melody, 2012; Nechay et al., 1977). The regulation of both the seasonal cycle and the body mass of hamsters are under the control of an endogenous circannual clock (Canguilhem et al., 1988; Masson-Pévet et al., 1994). This circannual clock is controlled by clock genes that defines a biological cycle on an annual basis, even under constant obscurity conditions (Masson-Pévet et al., 1994). A phase of sensitivity to the shortening natural photoperiod occurs around mid-July and results in the synchronization of the circannual clock with natural photoperiod leading to marked circadian and hormonal changes (e.g. namely the pineal melatonin) that induce gonadal regressions (Masson-Pévet et al., 1994; Monecke et al., 2014, 2006; Sáenz De Miera et al., 2014).

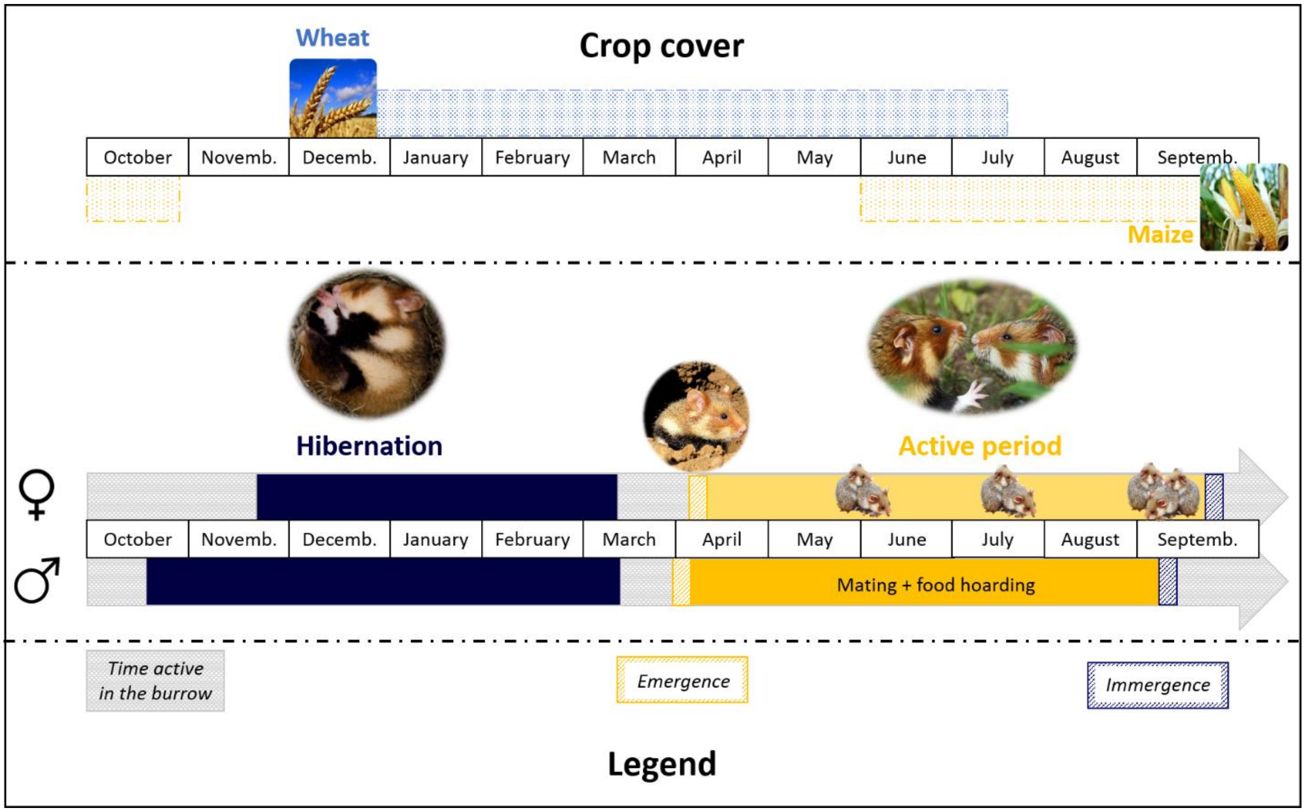


**Figure 6**: Seasonal cycle of the European hamster (adapted from (Nechay et al., 1977; Siutz et al., 2016; Ulbrich and Kayser, 2004) and timing of the protective cover for the two main crops cultivated in the Alsace (wheat and maize).

### *Behavior*

Studies on the behavior of the European hamster are very rare: only two studies have described the behavioral characteristics of the species (Eibl-Eibesfeldt, 1953; Ziomek et al., 2009). In 1953, V.I. Eibl-Eibesfeldt described the ethology of the species, based on direct observations, video-recording and pictures under captive and wild conditions (Eibl-Eibesfeldt, 1953). Much more recently, Ziomek et al. described the behavior of the species under zoo conditions and show that hamsters display much higher levels of non-social (94%) than social behaviors (~6%; mostly observed in pups) (Ziomek et al., 2009). Their findings echo the ancient literature, which describes the European hamster as 'territorial' and 'aggressive' (Eibl-Eibesfeldt, 1953; Nechay et al., 1977). The inter-individual interactions of hamsters inhabiting Vienna have been described (Siutz and Millesi, 2005) and two studies have characterized the sexual behavior of the species under laboratory conditions (Reznik-Schuller et al., 1974; Vohralik, 1974). However, apart from these descriptive studies, experimental studies on the behavior of the hamster, or comparative analyses of its behavioral ecology in different conditions and environments, are severely lacking.

### *Life-history strategy*

The European hamster is a species with a fast pace-of-life, i.e. with a short lifespan and a high reproductive rate that should compensate for a high predation rate (Capellini et al., 2015; Fenyk-Melody, 2012; Nechay et al., 1977). On average, hamsters life-expectancy is of 4 years in the wild, but recent data indicate a lower lifespan in several European populations (Franceschini-Zink and Millesi, 2008a; La Haye et al., 2014) and elevated predation in Western-Europe (Kayser et al., 2003; Villemey et al., 2013). The reported reproductive rate of this species in Europe is of 2-3 litters per year, composed of 4-7 pups each (Franceschini-Zink and Millesi, 2008a; Nechay et al., 1977), although recent field investigations in France report a very low reproductive success (2.5±1.4 pups per litter and 0.76 litter/female in 2014 (Kourkgy and Eidenschenck, 2015)). Therefore, it appears that their reproductive rate does not allow to compensate their mortality rate, although the ultimate causes for this observation are unknown. Several studies have highlighted the negative effects of harvesting and more broadly of a lack of protective cover on the survival of the species (La Haye et al., 2014; Villemey et al., 2013). Moreover, field reports suggest an important predation rate in the early season (i.e. in April-May in maize fields and after harvest in July in wheat fields) before hamsters may produce a viable litter (Kourkgy and Eidenschenck, 2015; Rostaing, 2011).

As indicated above, hamsters hibernate during the winter period. Hibernation is widely known as a strategy used to face periods of low energy availability and cold ambient temperatures (Geiser, 2013; Humphries et al., 2003a; Love et al., 2008; Ruf and Geiser, 2015). However, it also appears as a strategy to reduce annual mortality, partially through reduced predation rates (Turbill et al., 2011), and to minimize mammalian extinction (Geiser and Turbill, 2009). The European hamster is a food-storing hibernator (Humphries et al., 2003b), which means that it needs to hoard very large amounts of food (several kg) in its burrow to feed during periods of low food availability. (Wall, 1990). This can be during winter arousals (thanks to the maintenance of a fully functional digestive system during hibernation in this species (Weitten et al., 2016)), but also during the active period when above-ground foraging is not possible (Wall, 1990). It has been highlighted that the food hoarded during the fall is particularly crucial for food-storing hibernators when food availability is low on emergence: they can rely on the hoard they established in the fall for up to 11 months in the wild (Munro et al., 2008; Wall, 1990). Given that species at high risk of facing periods of food scarcity are those that rely the most on their hoarded food (Wall, 1990), the survival and reproductive success of wild hamsters might greatly depend on the nutritional value of these hoards. Indeed, only females weighing more than 200 g on emergence from hibernation are able to reproduce in the spring. Therefore, if hamsters' hoards are of low-quality, this might greatly reduce their over-winter survival or their body condition on emergence, and therefore their fitness. In the past, hamsters were able to hoard up to 50-90 Kg of seeds and tubers (Nechay et al., 1977; Wall, 1990). The reduced period in which hamsters may gather grains before hibernation (as a consequence of agriculture intensification) has been advanced as a cause of decline of the population in the Netherlands (van Oorschot and van Mansvelt, 1998 in (Stoate et al., 2001)). Therefore, the synergic effects of decreased survival during hibernation because of hoards of low-quality, reduced reproductive success on emergence and increased mortality by predation because of a lack of protective cover would substantially reduce hamsters' fitness. However, these different points remain to be further investigated.

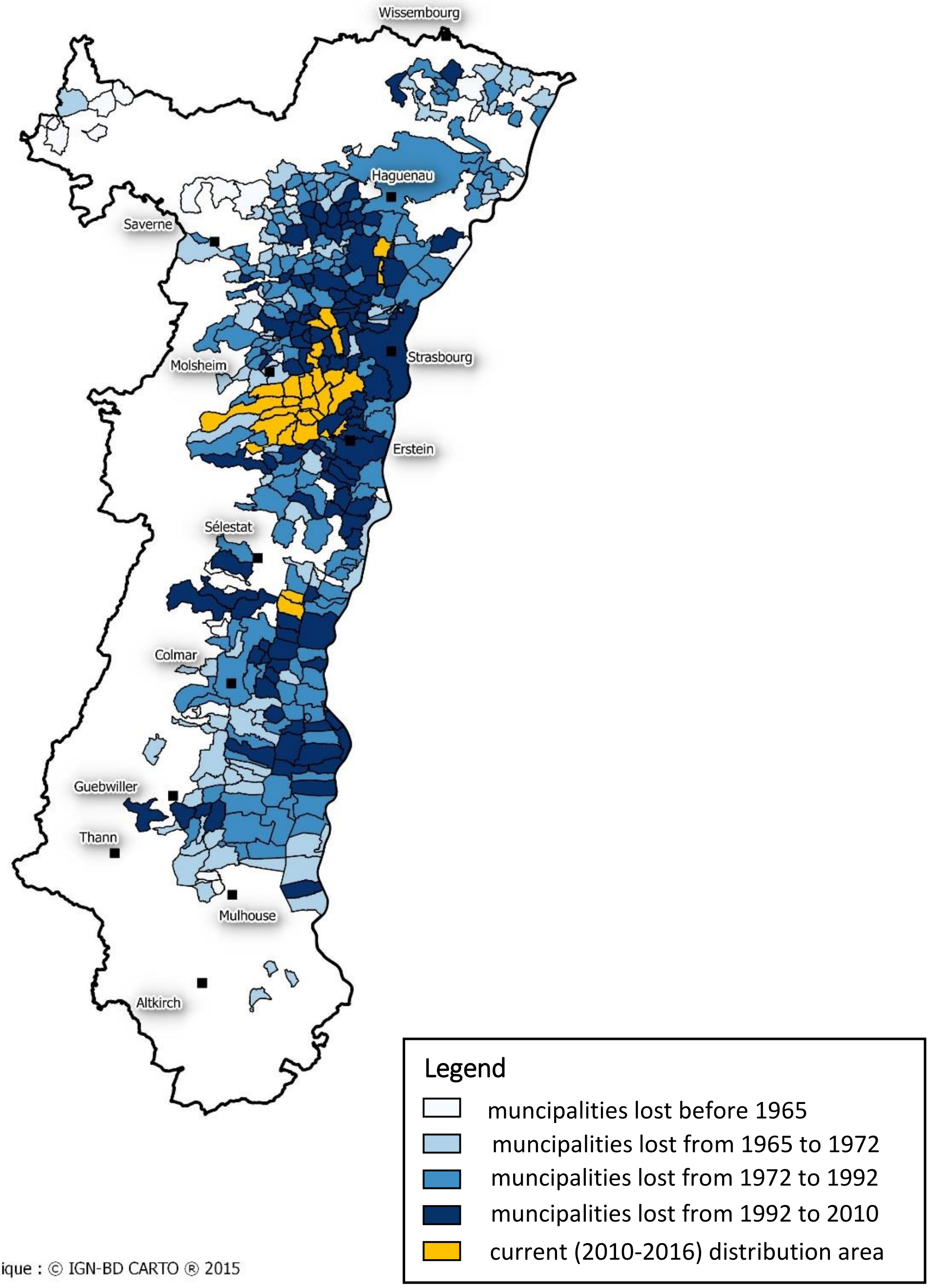
### *Ecology*

The European hamster lives in stable soils that are primarily composed of loess (very fertile and dense soils made up of detritic sedimentary deposits) and are thus particularly adapted to crops. It can dig burrows up to 2-meters deep, composed of several galleries and chambers (Figure 7) (Grulich, 1981; Nechay et al., 1977). These burrows have an important role for soil dynamics and soil fauna activity as they evolve in krotovinas, i.e. the result of burrowing activities on a geological scale (Pietsch et al., 2014). The species is omnivorous, and therefore 80% of its optimal diet is composed of vegetables such as green parts of plants, seeds, roots and fruits, with the remaining 20% made up of insects, annelids, mollusks and small rodents. It can consume crops (alfalfa, corn, cabbage, beets, wheat, rape…) as well as weeds such as dandelions, plantains or poppies (Gorecki and Grygielska, 1975; Nechay et al., 1977). However, no study has yet investigated the change in diet in farmland since the late 70s, paralleling changes in agricultural practices, nor how such changes might affect hoarding behavior, hibernation or reproduction. The home range of the species extends from 0.2 ha for females to 1.8 ha for males (Ulbrich and Kayser, 2004), in which hamsters should be able to forage and to find a partner. Males are polygamous, and the territory of one male usually covers home ranges of three or more females (Fenyk-Melody, 2012). However, in low densities/relict populations (< 5 individuals/ha), the home range of males does not extend far enough to reach the burrows of some females, and the latter therefore remain unfertilized (Ulbrich and Kayser, 2004).

**Figure 7: Schematic representation of a hamster burrow (several chambers and exits) in a wheat field**. © *Deutsche Wildtier Stiftung*.

### *A brief history of the species in the Alsace: from pest to strictly protected status*

European hamsters have long been considered as pests due to the damage they could cause to crops when populations are plentiful. Therefore, they have been actively hunted and poisoned from 1964 to 1990 in the Alsace, France (Chaigne et al., 2015). Despite the drastic population decline recorded in the late 1960s, the species was only protected from 1993-onwards, under the Habitat Directive and the Bern Convention (Chaigne et al., 2015; O'Brien, 2015). Its habitat started to be protected much later, in April 2007, under an inter-ministerial decree. However, despite these protections and the three successive PNAs implemented for its conservation from 2002-2016, the species continued to dramatically decline in the Region. It is estimated to have shown a 7-fold decline between 2007 and 2012 (ONCFS, personal comm.). In total, the species decreased by 94% (Chaigne et al., 2015) in its French distribution area from 1972 to 2010 (Figure 8). However, the protection of its habitat associated to the restocking programs of the PNAs (with approximately 300 hamsters released every year in unharvested wheat fields surrounded by electrified fences (ACTeon, 2012; Villemey et al., 2013)) have allowed to stabilize the French population between 500 and 800 individuals during the past seven years. Moreover, genetic diversity has been preserved in the wild as in the French captive units (Reiners et al., 2014). This number is below the survival threshold of this population, estimated to be of ~1500 individuals (ACTeon, 2012; Chaigne et al., 2015; Villemey et al., 2013).



Sources :
Fond cartographique : © IGN-BD CARTO ® 2015

Figure 8: Map showing the reduction of the distribution area of the hamster in the Alsace Region. Municipalities where the species has disappeared between 1897 and 1965 (white), between 1965 and 1972 (light blue), between 1972 and 1992 (medium blue) and between 1992 and 2010 (dark blue) are represented. In orange, the municipalities where the species has been recorded from 2010 to 2016 are represented. *Adapted from ONCFS data by Nicolas Volkringer and myself.*

## 2. The dilemma of the European hamster in the Alsace: find food fast but don't become fast-food

The Alsace region (France) is characterized by a great diversity of landscapes: the Vosges Mountains, the limestone reliefs, the Ried wetlands and the Alsace plain (Heuacker et al., 2015). This Alsace plain accounts for 1/3 of the Region and is dominated (at 90%) by farmland (with up to 80% of maize monoculture (O'Brien, 2015)) whilst the remaining territory (10%) is covered by urban areas and forests (Heuacker et al., 2015). About 91% of the species that depend on this habitat are threatened, including the European hamster (Heuacker et al., 2015). Indeed, the current French relict population of the species accounts for less than 1500 individuals and entirely depends on the agricultural ecosystems of this plain (Reiners et al., 2014; Villemey et al., 2013). Major landscape changes have been observed from the 1950 to 2010 in this region (see **Figure 9a**), echoing the described effects of agriculture intensification and urbanization on the landscape throughout Europe (**section II**). The average size of an agricultural field in 2010 is about 1.4 ha, which is well below the recorded size in other European countries (Björklund et al., 1999). However, this sized field (1.4 ha) corresponds to the home range of a male and is seven times larger than the home range of a female (Ulbrich and Kayser, 2004), suggesting that wild hamsters are strongly constrained in their ability to access a wide range of food items and would mainly have to rely on the main crop cultivated in their area of presence (see **figure 9b**). Moreover, given that the plain of Alsace is highly fragmented by road infrastructures (Heuacker et al., 2015; O'Brien, 2015), it appears that hamster dispersion is constrained by the associated barrier effect (see ***section II.2***). More worryingly, a recent study (Jumeau and Handrich, 2016) reveals a predation risk from cats and foxes on small mammals in the wildlife underpasses destined to reconnect wild hamster populations in the Alsace plain. Therefore, the European hamster seem to be facing a major dilemma in this highly anthropogenic landscape that could be summarized as follows: *find food fast but don't become fast-food*. Given that the hamster is an umbrella species (Heuacker et al., 2015), its conservation (if carried-out through the restoration of a more suitable habitat) should allow the preservation of a broad diversity of living organisms in European farmland. For instance, in France, 91% of the species living in the same farmland habitats than the hamster are endangered (Heuacker et al., 2015).

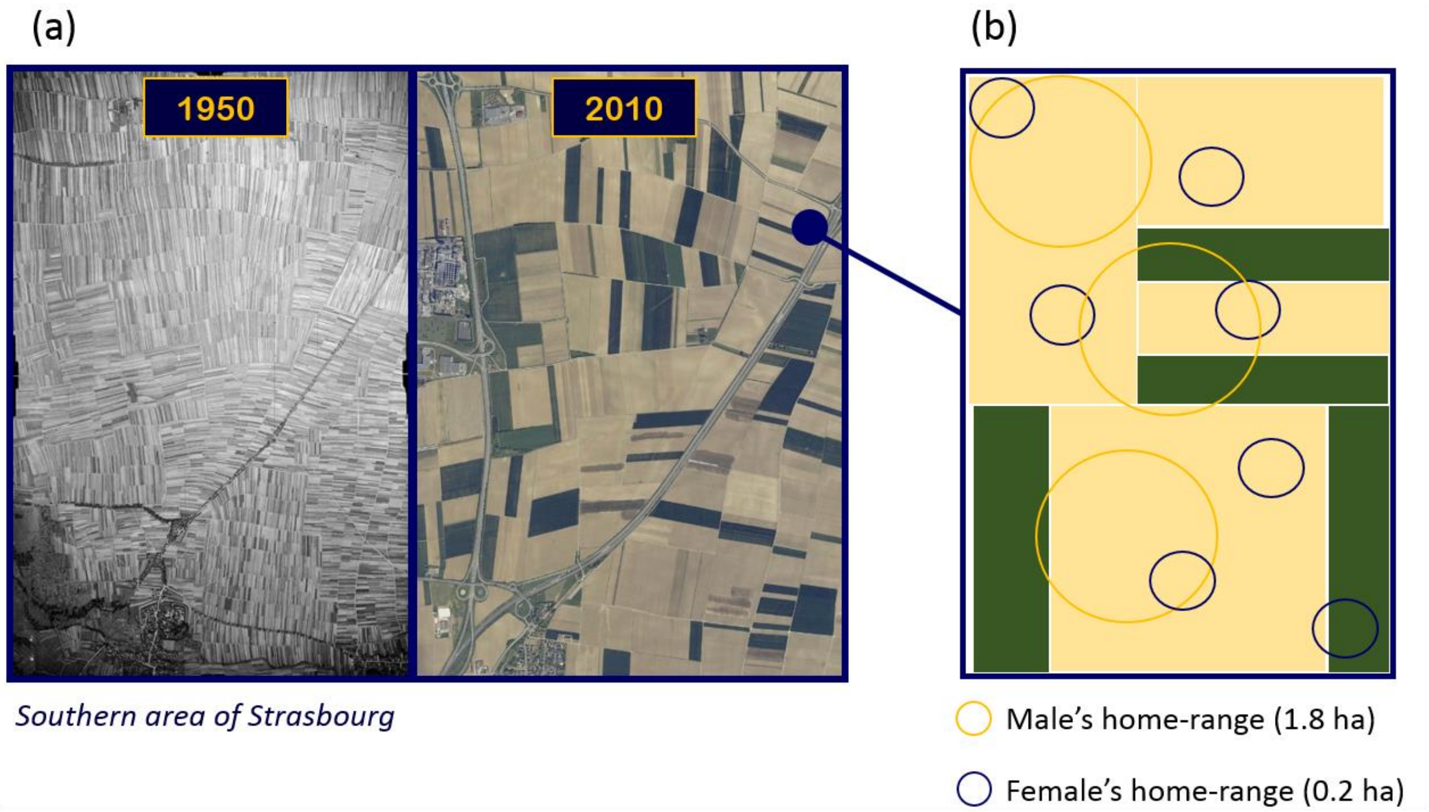


**Figure 9: (a) Landscapes changes in the Southern area of Strasbourg (Alsace, France), from 1950 to 2010 and (b) scheme representing the home range of male and female hamsters in comparison to the size of the plots in the Alsace**. In (a), both pictures (adapted from Archives Geoportail) are at the same scale and therefore highlight the marked increase in plot size as well as in the number and the size of the roads. In (b), the scheme is adapted from (a) and (Kayser et al., 2003).

Therefore, given the effects of both agriculture intensification and urbanization on landscape modifications, it is very likely that these observed changes in Alsace have had similar effects on hamster populations than those of similarly affected landscapes on farmland European birds (section II.2). For example, intensive monoculture could **directly** affect hamsters' fitness through a reduction of reproduction or survival (e.g. death of malnutrition, mechanization-associated mortality, pathogens or predation). However, whilst previous focus was given to the effects of predation on mortality in this species (owing to a reduced protective cover during the active period), there is a lack of data on the effects of crop monoculture on the reproduction or the hibernation of this species. In parallel, intensive monoculture could also **indirectly** affect fitness, e.g. if hamsters are in a habitat of poor quality, they might disperse to find a more suitable habitat, leading to an increased risk of predation, therefore indirectly affecting fitness.

The lack of updated data on the ecology of the species is jeopardizing the implementation of adapted conservation plans. Therefore, before being able to successfully conserve the European hamster, there is a strong need: (i) to better understand the diversity and the strength of the pressures operating on the populations of the species throughout Europe, (ii) whether these populations show phenotypic plasticity or not, and (iii) how global change is affecting each population. Specifically, that would mean to seek whether the problem is mostly related to a decrease in reproduction (as suggested in (Surov et al., 2016)), a decrease in survival because of increased predation rates (as suggested in (Ulbrich and Kayser, 2004; Villemey et al., 2013) or more likely an inter-relation between reduced reproductive success and increased mortality (as suggested by the reviews in farmland European birds (Benton et al., 2003; Wilson et al., 2005)).

## 3. Aims of my PhD

My PhD is an integral part of the first and second axes of the ALISTER project that ultimately aim at improving the French habitat of the species through the development of 'friendly agriculture practices' and the defragmentation of the landscape with the enhancement of wildlife underpasses. Most of the data on how nutrition impacts life-history traits of the European hamster using an ecophysiological and behavioral approach have been carried-out in urban areas, specifically in Vienna (Franceschini-Zink and Millesi, 2008a; Hufnagl et al., 2011, 2010; Schmelzer and Millesi, 2003; Siutz and Millesi, 2012, 2005). However, even though present in some cities (e.g. Vienna, Prague, Simferopol), hamsters are mostly found in farmland across their European range (Surov et al., 2016; Weinhold, 2008), and we are still lacking date relating nutrition and life-history traits of the species in its main habitat. The aim of my PhD is therefore to fill this gap by bringing new information on the farmland European hamster for the implementation of adapted conservation plans and the restoration of a more suitable habitat in the Alsace. The results should ultimately allow: 1) to implement innovative agricultural techniques to ensure a protective and nutritive cover during the totality of the hamster's life cycle and 2) to restore ecological corridors improving hamsters' dispersion and gene flow. These two points will be implemented in collaboration with the CARA, the ONCFS and the regional office of the MEDDE (i.e. the Direction

Régionale de l'Environnement, de l'Aménagement et du Logement, the DREAL). These measures are intrinsically related to the observation that with agriculture and urbanization intensifications, associated to habitat loss, degradation and fragmentation, hamsters face the same dilemma than most farmland animals (see above), namely '*find food fast but do not become fast-food*'. However, before being able to properly restore the hamsters' habitat, we need more information on its biology and on the pressures that intensive agriculture poses on hamsters' reproduction and those that road infrastructures pose to its dispersion.

Recent field investigations in the Alsace, revealed that less than one female produce a litter per year (against more than 2 litters/female in older literature and across Europe (Franceschini-Zink and Millesi, 2008b; Nechay et al., 1977)). This suggests that hamsters' reproduction has been dramatically reduced in France. However, we are severely lacking of data on the environmental factors that could have affected their reproduction. Specifically, we are lacking of information on how the monotonous diets usually imposed by monoculture have affected hamsters' fitness. The 1st part of my thesis therefore focused on this thematic (**Thematic I: Monoculture, nutrition and fitness**) and aimed at answering the following questions:

**1) Which environmental factors have the most affected the fitness-related traits of the species in the Alsace?** (Chapter 1)

Seeking to understand which pressures were responsible of the crash in the hamster population in the Alsace, I have gathered a body-mass data-set of 1527 wild-caught hamsters (from 1937 to 2014) looking at old records and old literature in the Alsace. I have then investigated for the effects of agriculture and climate changes on the decline in body mass observed from 1937 onwards (**study 1**). This study therefore brings new insight onto the pressures faced by the French hamster population during the past century.

**2) Are maize and wheat nutritionally adapted for hamsters' reproduction?** (Chapter 2)

As indicated above, maize and wheat are the two main monoculture crops in the Alsace. Seeking to understand how monotonous diets based on these two crops were affecting the hamsters' reproduction, I have conducted eco-physiological studies under controlled (**Box 1** and **Study 2**) and semi-wild (**Study 3**) conditions. With these studies I bring new insight onto the mechanistic effects of monoculture on the biology of the hamster. More specifically, this approach allows to pinpoints how monoculture per se, apart from the effects of mechanization or pesticides, can affect the fitness of the hamster.

**3) Which favorable crops could be associated to maize and wheat to ensure a good hibernation and a proper reproductive success in this species?** (Chapter 3)

Seeking to understand how to improve maize and wheat monoculture, I then investigated the effects of 6 crop-associations (3 wheat-based associations and 3 maize-based associations) on both the hibernation and the reproduction of the species (Study 4). I have also investigated for the physiological costs of these diets, looking at their effects on the hamsters' telomere (i.e. the protective caps of chromosomes) dynamics during hibernation (Box 2). Results of these last studies bring new information onto the effects of food hoards on the hibernation of this food-storing rodent. Moreover, the beneficial crop-associations found in this study will benefit to the hamsters' conservation.

**4) How the results of this Thematic I can be applied to the hamsters' conservation and the improvement of its habitat?** (Chapter 4)

In this chapter, I discuss the limits and scientific perspectives of my studies and expose some recommendations for the conservation of the species in the Alsace and in Europe. More specifically, based on the results of the chapter 1-3 and a review of the literature, I am making suggestions and recommendations for the Agricultural chamber and the ONCFS to improve the agro-ecosystems of the region for the hamster and the associated biodiversity.

In parallel of these reproductive issues recorded in the French population, a recent report highlights cases of predation by cats and foxes on small mammals (e.g. common voles and field mice) in the wildlife underpasses recently built for the hamster (Jumeau and Handrich, 2016). Given the fragility of the French hamster population, such cases of predation could be very harmful for this species. It therefore appears crucial to improve the safe crossing of hamsters, and more broadly small mammals, in these underpasses. However, before being able to improve hamsters' crossing, we need more information on their behavior, namely onto how they perceive and react to predators and in which way it will affect their use of any anti-predation system that we could implement. Therefore, the second part of my PhD focused on this thematic (**Thematic 2: Hamster** andipredatory behavior and anti-predation device: towards an improvement of wildlife underpasses) and aimed at answering the following questions:

**5) What is the optimal anti-predation device to secure hamsters' crossing? And how hamsters' perception and reaction towards predation pressures will influence their use of such a device? (Chapter 5)**

Seeking to secure and facilitate hamsters' crossing in wildlife underpasses, I have developed an anti-predation device to be placed in wildlife underpasses, with optimal characteristics for the hamster (study 5). In order to know whether hamsters would avoid this device in case of predation cues (such as urine) on it, or whether the presence of an actual predator will indeed push hamsters to use the device as a refuge, I have conducted three experiments in controlled conditions. These experiments (study 6), bring new information on the behavior of captive European hamsters when faced to several predation cues. However, results of both of these studies revealed important inter-individual differences in the response towards predation stimuli that were not explained by the sex of the individuals. Moreover, these studies were conducted in captivity and do not confirm the efficiency of the anti-predation device in the wild.

**6) How to explain the differences of behavioral reactions when faced to predation cues at the intra-species level? Will the results obtained in captivity be confirmed inside an actual underpasses? (Chapter 6)**

Seeking to understand how to explain the inter-individual differences in the behavioral responses observed in our captive population, I have conducted several tests to evaluate the personality (Careau et al., 2012; Martin and Réale, 2008; Réale et al., 2007) of the species (Box 3). Several personality traits are known to be related to reaction towards predators (i.e. boldness) and to dispersion (i.e. exploration/activity) (Réale et al., 2007). Given that boldness and dispersion appear to be intrinsically linked in several species (Réale et al., 2007; Wolf et al., 2007) I was seeking to understand whether a boldness-dispersion syndrome could explain the results observed in the chapter 5. In a last study (Box 4), I have investigated whether the anti-predation device was effective under semi-wild conditions, when hamsters were placed in an actual wildlife underpass with a predator at proximity.

**7) How the results of the thematic 2 can be applied to the hamsters' conservation and the improvement of its habitat?** (Chapter 7)

In this last chapter, I discuss the limits and scientific perspectives of the chapters 5 and 6. Moreover, based on the results of these two chapters, I am making recommendations regarding the implementation of the anti-predation device in the wildlife underpasses of the Alsace.

# Thematic I: monoculture, nutrition and fitness of the European hamster

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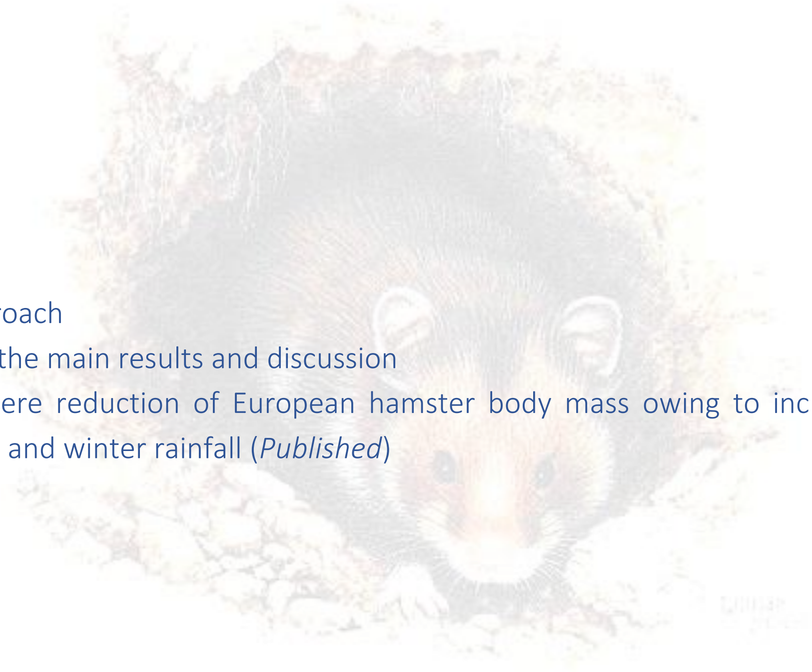
# Chapter 1

« *The most beautiful experience we can have is the mysterious. It is the fundamental emotion that stands at the cradle of true art and true science.* »

Albert Einstein, *The world As I see It*, 1931

# Take a look back on the causes of the French population decline: what can we learn from ancient data?



**Content:**

1. General approach
2. Summary of the main results and discussion
3. Study 1: Severe reduction of European hamster body mass owing to increasing maize monoculture and winter rainfall (*Published*)

## 1. General Approach

Global change have been shown to affect the phenotypes of wild vertebrates in many different ways, e.g. through modifications of their behavior, their physiology, their morphology or their reproduction. Specifically, climate (Smith et al. 1998; Yom-Tov 2001; Réale et al. 2003; Charmantier et al. 2008; Ozgul et al. 2010; Campbell et al. 2012, 2013; Byrne et al. 2015) and agricultural change (Rioux Paquette et al. 2014; Byrne et al. 2015) have been shown to affect the body mass, the breeding phenology and the reproductive success of several vertebrate species, both indirectly or directly related to individuals' fitness and to population dynamics (Ozgul et al. 2010; Milner et al. 2013; Rioux Paquette et al. 2014). Seeking to understand which drivers were responsible for the crash in the hamster population in the Alsace, we investigated the changes in body mass of 1527 wild-caught hamsters on emergence from hibernation (in April and May) during the last century (from 1937 to 2014). In that aim, I have gathered body mass data from the Reports of the French Society of Physiology in Strasbourg, wrote by A. Malan and C. Kayser (CNRS) until the 60s. I have also searched the archives of both the regional office of the MEDDE and the ONCFS for hamsters' body mass in April and May during the past decade. Finally, I have collaborated with Paul Pévet, a French researcher from the CNRS that captured hamsters in the Alsace during the 90s to study their biological rhythms and that had at his disposal body mass data from this decade.

Hamster females become fertile after their first winter only if their body mass exceeds 200 grams (Vohralik 1974; Nechay et al. 1977). Therefore, hamsters' body mass on emergence is a good proxy of the quality of their hibernation and of their body condition for spring reproduction. These changes in body mass were then correlated to environmental change, including agriculture (i.e. annual production and acreage allocated to 7 crops in the Alsace since 1989) and climate (i.e. mean annual temperature and mean annual rainfall since 1937) changes.

## 2. Summary of the main results and discussion

Hamsters' body mass on emergence from hibernation decreased by 21% from 1937 onwards. The decline seems to have occurred between the 70s and the early 90s, although data were not available for this period (resulting in two significantly different plateaus: one before the 70s and one from the 90s onwards). When looking at the effects of climate changes on body mass, no clear pattern appeared. However, when decomposing climate variables (temperature and rainfall) into seasonal variables (i.e. average summer temperatures, summer rainfall, winter temperatures and winter rainfall), we found that only increasing winter rainfall had a significant effect on hamsters' body mass change. The negative effects observed could be explained by an associated reduction of soil insulation with augmented winter rainfall (replacing snowfall), therefore increasing the cost of hibernation. Increasing winter rainfall could also result in the deterioration of the food hoards through increased soil moisture, therefore reducing the quality of the diet and ultimately hamsters' body mass.

When looking at the effects of agricultural change (from the 90s onwards), we found that maize monoculture intensification strongly and negatively affected hamsters' body mass on emergence, i.e. when the production and the acreage dedicated to maize monoculture were high the year n, body mass of hamsters were subsequently reduced the year n+1. This could result from the fact that maize is harvested in October, and thereafter the plots are left uncovered, therefore potentially affecting the microclimate, which could result in increased costs of hibernation for hamsters and ultimately on reduced body mass on emergence. Another possibility is that a year with high maize production and important surfaces allocated to this cereal might push hamsters to rely mostly on maize grains to spend the winter (i.e. store large amounts of this cereal and having a hard time finding other grains to store) and would therefore suggest that maize is not a proper food resource for the hibernation of this species, and is reducing hamsters' body condition on emergence. The correlative results of this study did not allow us to conclude on whether it is monoculture (i.e. change in the landscape and associated pedo-climatic conditions) or the maize itself (i.e. as being inappropriate for hamster's hibernation) or both of these elements combined that were affecting body mass on emergence. However, the nutritional properties of maize and their effects on hamsters' body condition and reproductive success will be developed in the chapter 2, whereas I present in the chapter 3 the results of my studies on the effects of maize-based diets on the hibernation of the species.

To conclude, this study pinpoints how both increasing winter rainfall (as a result of global warming) and maize monoculture intensification have been involved in the critical reduction of hamsters' body mass on emergence, which could have impaired their reproductive success in pervasive ways during the past 50 years. Moreover, our results strongly suggest that the intensification of maize monoculture – even though it has started later and therefore has affected hamsters for a shorter period – had a stronger effect on hamsters' body mass than increasing winter rainfall. Therefore, even though more studies are needed to better understand the inter-relation between global change and population dynamics in this species, given the rate of maize monoculture intensification in the Alsace, this study supports that agriculture management is at the core of the conservation of this species in France.

# Study 1 – Severe reduction of European hamster body mass on emergence from hibernation due to increasing maize monoculture and winter rainfall

*Published* [1]

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# How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the verge of extinction

**Mathilde L. Tissier[1,2], Yves Handrich[1,2], Jean-Patrice Robin[1,2], Mathieu Weitten[1,2], Paul Pevet[3], Charlotte Kourkgy[4] & Caroline Habold[1,2]**

**Over the last decades, climate change and agricultural intensification have been identified as two major phenomena negatively affecting biodiversity. However, little is known about their effects on the life-history traits of hibernating species living in agro-ecosystems. The European hamster (*Cricetus cricetus*), once a common rodent on agricultural land, is now on the verge of extinction in France. Despite the implemented measures for its protection, populations are still in sharp decline but the reasons for it remain unclear. To investigate how environmental change has affected this hibernating rodent, we used a data set based on 1468 recordings of hamster body mass at emergence from hibernation from 1937 to 2014. We reveal the adverse effects of increasing winter rainfall and maize monoculture intensification on the body mass of wild hamsters. Given the links that exist between body mass, reproductive success and population dynamics in mammals, these results are of particular importance to understand the decline of this species. In view of the rates of maize monoculture intensification and the predicted increase in winter rainfall, it is of the utmost importance to improve land management in Western Europe to avoid the extinction of this species.**

There is a consensus that agriculture and global warming are increasingly affecting wildlife[1–3]. Indeed, there has been growing evidence over the last two decades that climate change is affecting the demography and life-history traits of vertebrates[4–12]. Some species demonstrate a high phenotypic plasticity[6,8], whilst others fail to adapt and consequently suffer a reduction in fitness[13] and population decline[5]. However, climate change is just one of the numerous threats currently faced by wildlife, and species' response to climate change depend on their distribution, their life-history strategies and whether or not they are affected by additional pressures such as pollution, fragmentation, invasive species or habitat loss[9,14,15]. Parallel to climate change, croplands and pastures have greatly expanded and now cover almost 40% of the land on Earth[1]. This phenomenon is associated with changes in agricultural practices[1] and is currently supported through intensive cereal monocropping, mainly of maize[16,17]. The intensification of maize monoculture is known to cause soil degradation, the pollution of groundwater and biodiversity decline, affecting all species living in agricultural ecosystems[11,18–20]. Climate change and agriculture are directly (albeit partly) inter-related[1]: agricultural intensification is known to emit the greenhouse gases involved in climate change, which in turn directly affect agricultural production and sustainment. As a result, maize yields are expected to decrease by approximately 12% in the coming years[21,22]. Cumulatively, these elements directly alter the habitat of farmland species and make it less diversified and more unpredictable[16,23]. This stochasticity is even more damaging for small populations or species with a fast pace of life (*i.e.* a short lifespan and a high reproductive rate) and low dispersion capacities. Indeed, these animals are known to be less able to cope with "bad years"[24] and are highly susceptible to the Allee effect, *i.e.* reduced fitness at low population density[25–27]. The few existing studies linking climate change, agricultural intensification and life-history traits of farmland vertebrates have been carried out on birds or on stable populations of badgers[11,28]. However, nothing is known to

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date about the effects of climate and agricultural changes on the life-history traits of hibernating species, nor have any studies investigated how these factors affect fast pace of life mammals living in agro-ecosystems. Here, we investigate for the first time the combined role played by climate and agricultural changes in the alarming decline of the European hamster (*Cricetus cricetus*), a hibernating rodent that is now endangered in almost all Eurasian range states and even locally extinct[29,30]. The extinction threat is greater in the Western-part of its distribution area (*i.e.* Netherlands, France, Germany and Belgium)[31]. It is widely recognized that peripheral populations (*i.e.* at the edge of the distribution) are genetically more differentiated than central ones, but are however facing a higher stochasticity in demographic processes[32]. Consequently, their conservation deserves high priority and might require specific measures[33,34]. However, it has been difficult to implement efficient conservation measures without a clear understanding of what causes the decline of the European hamster–especially in France, where it has been considered as a pest until the 90s[35]. During the two last decades, the focus has been on hamster population dynamics, and studies based on burrow surveys and genetics have highlighted the deleterious effects of insufficient protective cover on the mortality rate of wild hamsters[29,30,36–39]. It has recently been suggested that other factors could be involved in the decline of the species across Europe, including an effect of climate change on life-history traits or an overall decrease in reproductive success[35,40]. Yet nothing is known to date about the environmental factors that could affect the body condition, lifespan or reproductive success of wild individuals. In mammals, body mass–known to be related to fitness, predation risk and thus population dynamics[26,41–43]–is highly dependent on habitat suitability in agro-ecosystems[28]. In European hamsters, females only become fertile after the first winter if their body mass exceeds a threshold of 200 g[44]. Poor body condition at emergence may therefore greatly impair their reproductive success in spring, and consequently affect the population dynamic of the species. We thus used data recorded from 1937 to 2014 to investigate how climate change and agricultural modifications could have affected this species, focusing on the impact of these drivers on the body mass of wild individuals at the period of emergence from hibernation. We first looked at the trend in body mass and climate since 1937 and changes in crop diversity since 1989 in the French area of distribution of the species. We then focused on inter-annual fluctuations in body mass between 1992 and 2014 and looked for a correlation with variations in environmental parameters that could account for decreasing body condition. Finally, we focused on the impact of climate on body mass since 1937 to disentangle effects of temperature from those of rainfall on the trend observed in body mass.

## Results

**Trend in body mass change since 1937.** Hamster body mass at the period of emergence decreased by around 21% between 1937 and 2014 (Fig. 1a). Two plateaus can be observed: one showing a significantly higher body mass before the 70s, and the other from the 90s onwards, with significantly lower body mass ($F_{3,1467} = 2.912$, $p = 0.033$). Post-hoc analyses supported the findings in previous literature, showing that males are heavier than females, whatever the decade (384 ± 15 g and 259 ± 15 g respectively, $F_{2,1467} = 668.2$ and $p < 0.001$). Results also indicated a higher weight in May than in April (312 ± 7 g and 298 ± 8 g respectively, $F_{1,1426} = 11.208$, $p = 0.001$). We found no effect of an interaction between sex, month and decade on body mass ($p > 0.2$).

On the examination of changes in body mass from 1992 to 2014, we found significant variations between successive years, both in males and females (Fig. 1b, $F_{9,740} = 3.348$, $p < 0.001$ and Fig. 1c, $F_{5,672} = 4.476$, $p < 0.001$ respectively). Males showed significantly lower body mass at the period of emergence in 1994 and 2014 (Fig. 1b; data unavailable for males in 2013). Females were significantly lighter in 1994 (Fig. 1c) and 2013 (Fig. 1c), but they did not show any sign of weight loss in 2014.

**Changes in environmental variables and impact on body mass.** When looking at inter-annual climate variation since 1937, we observed a slight increase in average temperatures during hibernation (October-March) and the active period (April-September) of the species (Fig. 2a, $R^2 = 0.171$, $p < 0.001$ and $R^2 = 0.385$, $p < 0.001$, respectively). Although there was no variation in the average annual rainfall, a significant increase was observed in rainfall averages during hibernation (Fig. 2b, $R^2 = 0.272$, $p < 0.001$). Changes in the acreage for different crop types were due to the expansion of maize and triticale crops from 1989 onwards (57% and 1233% respectively, Fig. 2c,d), whilst the amount of land used for other crops such as wheat, rapeseed, barley, rye and sunflower decreased by 12%, 56%, 74%, 49% and 91% respectively (Fig. 2c,d).

To investigate the effects of agricultural change on body mass, we first carried out PCA analysis of fourteen agricultural variables (surface and production of seven crops; see methodology for details); (Fig. 3). The PCA extracted two components which explained 91.1% of the total variance: the first opposed maize monoculture (negative values) to polycultural farming (*i.e.* wheat, rye, sunflower, rapeseed and barley; positive values, Fig. 3, X axis). The second component mainly opposed two cultures: rye (positive values) and triticale (negative values, Fig. 3, Y axis).

Secondly, we used path analysis to see how agriculture (components of the PCA) and climate (temperature and rainfall) affected the body mass of hamsters at emergence from hibernation (see methodology for details). The first path analysis (Model 1, Fig. 4a,b) revealed that body mass (from 1992 onwards) was strongly and positively related to polycultural farming (wheat, rye, sunflower, rapeseed and barley) and therefore negatively related to maize monoculture in both sexes (Fig. 4a,b, $p < 0.05$). We did not find any significant relationships between component 2 of the PCA and body mass in males or in females (Fig. 4a,b, $p > 0.1$). Temperatures and rainfall had differential effects on body mass, depending on the sex. Whilst male body mass at emergence was strongly and positively related to average temperature in year n-1, it did not seem to be affected by annual rainfall (Fig. 4a, $p = 0.01$ and $p > 0.1$ respectively). Conversely, female body mass was positively related to annual rainfall (year n-1), but not to average temperature (Fig. 4b, $p = 0.031$ and $p > 0.1$ respectively).

The second path analysis (Model 2, Fig. 4c) concerned the impact of inter-annual change in climate on body mass at the period of emergence (from 1937 onwards), and revealed that this trait was significantly and negatively

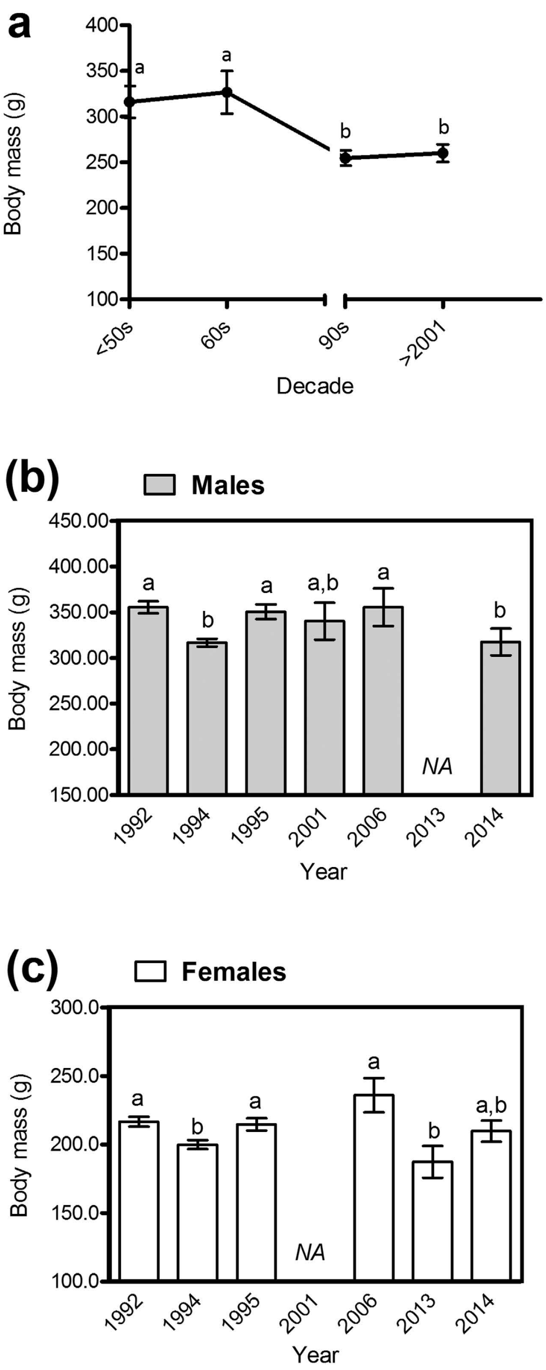

**Figure 1. Change in body mass of wild hamsters (males and females) at the period of emergence from hibernation.** (**a**) Body mass (g) is represented per decade from 1937 onwards (N = 1468; <50s corresponds to the period from 1937 to 1949, while >2001 represents the period from 2001 to 2014). Body mass (g) is represented per year since 1992 (**b**) in males (N = 720) and **(c)** in females (N = 672). Geometric means are represented ± SEM and different letters highlight significant differences (Multifactorial ANOVA, $p < 0.05$). See methodology section 3. for statistical details.

related to rainfall during hibernation in both sexes (Fig. 4c, $p < 0.009$). This was the only direct link found between climatic variables and body mass in males or females (Fig. 4c, $p > 0.09$).

## Discussion

The European hamster is a species with a fast pace of life, *i.e.* a short lifespan and a high reproductive rate that should compensate for a high predation rate[27,44]. It is widely recognized that introduction effort and the size and number of litters are the most important parameters for mammal population growth from small numbers[27]. Yet despite the theoretical high reproductive rate of these mammals and the strong reinforcement measures applied to protect these populations, the species has shown a decrease of 94% in its French distribution area since 1972[30].

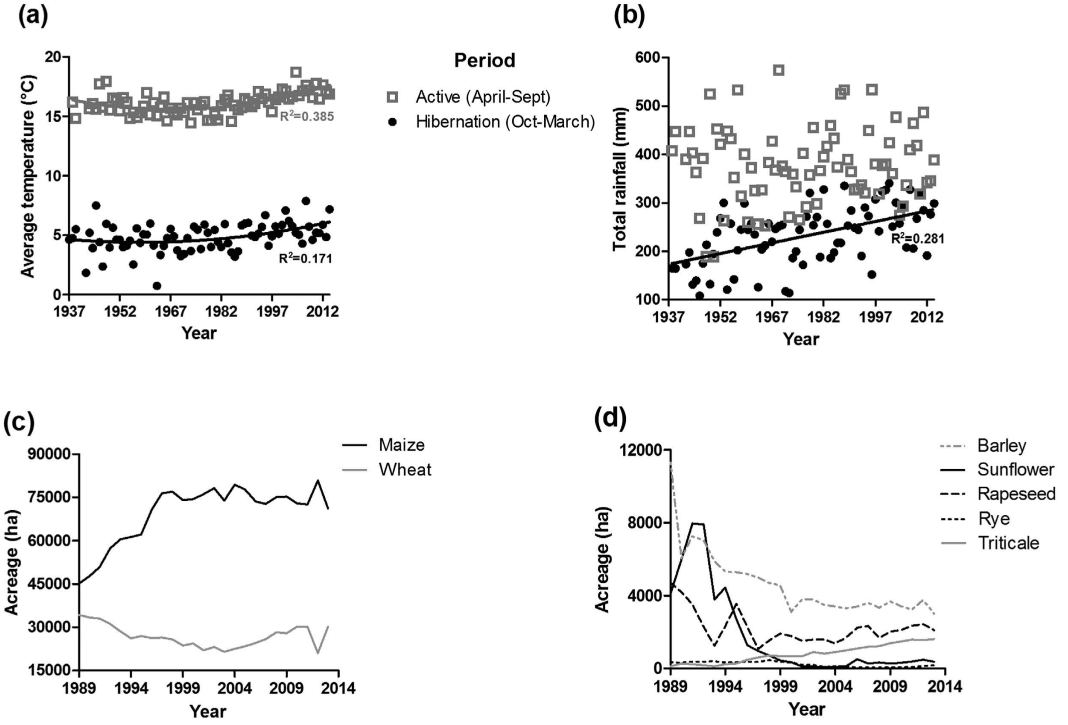

**Figure 2. Inter-annual change in temperature, rainfall and crop acreage in the Bas-Rhin (Northern Alsace, France) since 1937 (climate change) and 1989 (crop acreage).** (**a**) Average temperature (°C) and (**b**) total rainfall (mm) are shown according to the year and the period of the biological cycle of the common hamster (active period and hibernation). (**c**) Crop acreage (ha) of the main cereals (wheat and corn) and of (**d**) five other crops (rapeseed, barley, rye, sunflower and triticale) according to the year.

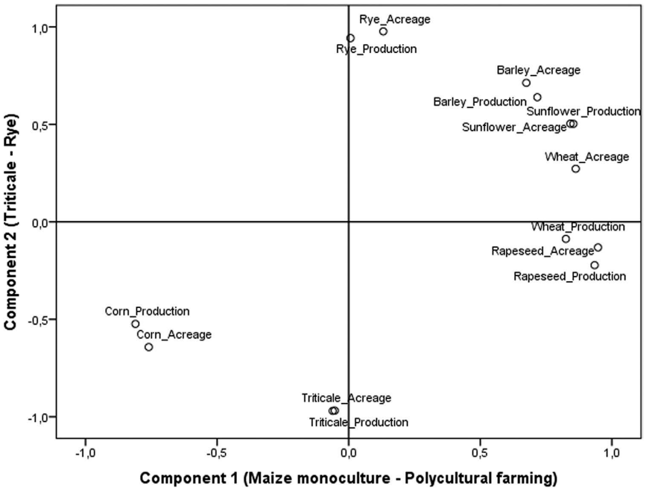

**Figure 3. Factorial map of PCA analysis on agriculture variables.** Variables include production (per 100 Kg) and acreage (in ha) of seven crops (rye, corn, triticale, barley, sunflower, wheat and rapeseed). The component 1 axis opposes maize monoculture ($<-0.5$) to polycultural farming ($>0.5$), while the component 2 axis mainly opposes triticale and rye crops. See methodology section 3. for statistical details.

Interestingly however, genetic diversity has been preserved[39]. Given the importance of genetic diversity of margin populations for the long-term conservation of species[32], French populations thus justify a high conservation priority. To reverse the decline and enhance the overall viability of these local populations, the French government has launched successive Conservation Plans for the period 2000–2016[31]. These plans include reinforcement operations and the reconstruction of a network of alfalfa or wheat plots covering several acres, which are partially harvested. Although these measures have helped to slow down the decline, they do not appear to be sufficient to increase the hamster population. The evaluation report concerning these action plans underlines the serious lack of knowledge about how multiple threats affect the biology and demographic parameters of hamster populations.

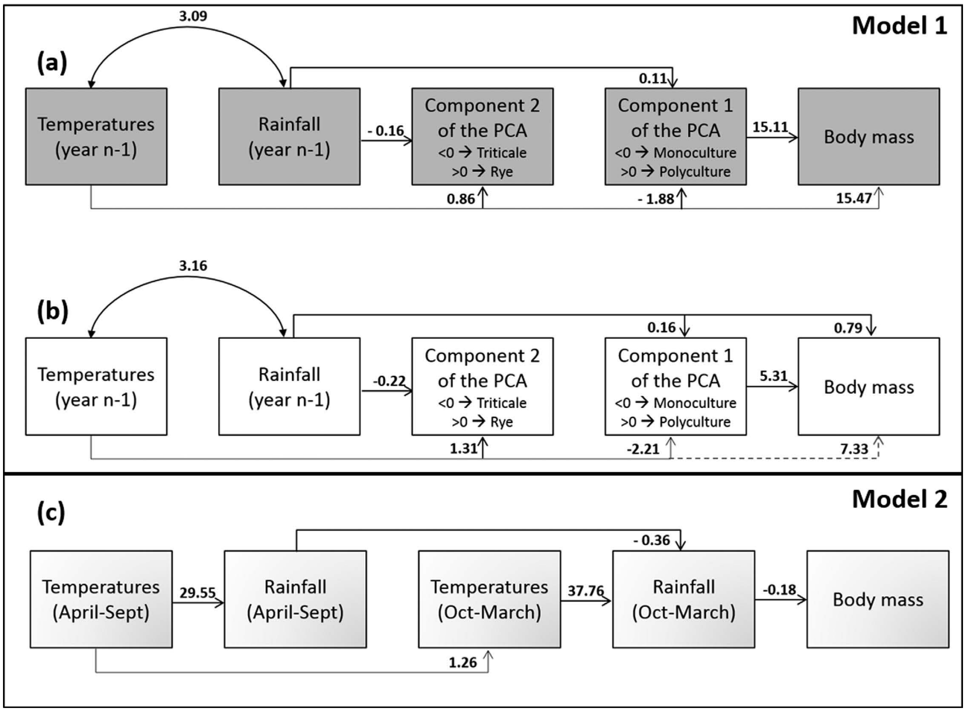

**Figure 4. Path analysis diagrams showing the impact of climate and agriculture (PCA components) on the body mass of wild hamsters.** Model 1 represents multiple relationships between temperatures, rainfall, agricultural variables and body mass of (**a**) males and (**b**) females from 1992 to 2014, and model 2 (**c**) shows multiple relationships between climatic variables and body mass of males and females from 1937 to 2014. Arrows indicate significant directed links between variables. Unstandardized estimates (which can be positive or negative) are indicated along their respective paths. Significant paths are indicated by solid arrows while the dashed arrow represents a non-significant path remaining in the selected model ($0.05 < p < 0.1$). See methodology for statistical details.

Our study reveals that climate change and maize monoculture have played a combined role in reducing the body mass of wild hamsters by up to 21% since 1937. Given the existing links between body mass, predation risks and reproductive success[26,41–43], it is very likely that this high decrease in body mass has affected the reproduction and life expectancy of hamsters. Increased predation rates[29,36,45] and reduced life expectancy (from 4 to 2 years[29,44]) have been recently reported in this species. Given that individuals gain mass throughout their life[44], this decrease in the average age of wild populations could also partly explain the decline in body mass that we observed. Our study sheds new light on how environmental change might have affected reproduction, a subject that was little understood until now[40]. We found that female body mass at emergence reached extremely low values in particular years (*e.g.* 186.47 ± 13.2 grs in 2013). As we indicated earlier, female European hamsters only become fertile after the first winter if their body mass exceeds a threshold of 200 g[44]. This overall decrease in body mass could therefore greatly impair the reproductive success of females by delaying the first reproductive event and reducing the number of females that produce a litter. This is supported by recent unpublished data on the reproductive success of the species in France[46]. Litter size-recorded as 5–8 pups per litter across Europe[44,47]-is currently estimated at 2.4 ± 1.6 pups in France[46]. More importantly, litter number, recorded as 2–3 litters/female/year[44,47] is currently less than 1 litter/female/year in France[46].

The affirmation that intensive agriculture is the main cause in the decline of the European hamster has recently been questioned, and global warming has been proposed as another cause of hamster population decline in Western Europe[40]. In our analysis, Model 1 revealed a strong negative effect of maize monoculture (which has increased by 57% since 1989) over the last two decades ($\beta = 15.11$, Component 1 of the PCA), and Model 2 revealed that from 1937 to 2014, winter rainfall (which had increased by 28% during this period) had a slightly negative effect on body mass ($\beta = -0.18$), which had declined by 21% during the same period.

Changes incurred by maize are thus relatively recent (with an intensification at the end of the 80s) and seem to have had a strong effect on body mass over a relatively short timescale. Conversely, winter rainfall started to increase at an earlier date (1937), and its effect on body mass seems to be less abrupt but to have had an effect over a longer period of time. These links between environmental variables and hamsters' body mass can be illustrated by two extreme examples. First, the 2012 explosion in maize production (following an extremely cold winter in 2011–2012 that damaged wheat production) was followed by a significantly reduced female body mass at emergence in 2013, associated with a high decrease in the number of burrows the same year[48]. This higher production of maize–associated with an increased allocation of land to this cereal–implies that less agricultural land was used for other crops. This might ultimately lead to less varied food resources for hamsters during the winter hibernation period. Given the importance of food stores for this species[44] and the poor nutritional quality of maize[49], this could negatively affect hibernation quality and thus hamster body mass at emergence. Moreover, as the stubble is removed from maize fields after harvest in France, the microclimate (*i.e.* ground temperature and humidity) in these bare plots during winter might be different to that found in wheat plots, which remain covered by crops throughout the winter[50]. Given that hibernation is known to be optimal at a given temperature[51], animals in these plots may increase their energy expenditure, leading to greater loss of body mass. These non-mutually exclusive hypotheses could explain the decline in body mass that we observed in animals emerging after years with high maize production. These ideas are supported by the observation that hamsters emerging in a field of maize ultimately leave the plot in the spring. However, they might remain on the maize plot if it had been previously seeded with cereals (*personal com*.). In Germany (where maize occurrence ranges from 12% to 19%, compared to 55 to 80% in Alsace, France), it has been demonstrated that the occurrence of hamsters decreases as the presence of maize increases, with no hamsters found in the areas where more than 18% of land was covered by maize[52]. Researchers in the Netherlands do not prospect in maize fields that they consider as an unfavourable crop for the species[29]. The second example of links between environmental variables and hamster body mass is the negative link between winter rainfall and body mass, illustrated by the reduced body mass of males emerging after the particularly warm and wet winter in 2013–2014 (*i.e.* 44% more rainfall than in 2006). In contrast to studies on non-hibernating vertebrates highlighting that increasing temperatures have an effect on a variety of species[4–6,28], our study reveals that European hamsters seem to be more affected by increasing winter rainfall than by temperatures. However, our model revealed that increasing winter rainfall is associated with a warmer climate, which thus indirectly affects hamster body mass. This finding echoes recent studies in hibernating mammals stating that winter conditions (*i.e.* winter Pacific Decadal Oscillation) and warmer climates can negatively affect the duration of hibernation and the survival of individuals[53,54]. We can imagine that increasing winter rainfall would increase soil moisture in depth (by percolation and rising of groundwater, located two to three meters underground in Alsace). During winter, hamsters live in a ~2 metre-deep burrow[44] and would thus probably experience changes in soil moisture more than changes in above-ground temperatures. Increased rainfall could lead to higher levels of soil moisture during hibernation and wet the animal's fur, ultimately reducing the insulation it provides and increasing the cost of hibernation through higher heat loss and greater energy expenditure, inducing faster body-mass loss. Another hypothesis is that wet soils could lead to the fast deterioration of food hoarded in the burrow, resulting in lower stores of intact food and/or a lower quality of available food reserves for the winter. A study in kangaroo rats[55] has shown that individuals can remodel their burrow and increase the number of exits in response to high rainfall, presumably to increase the evaporation rate and avoid the deterioration of seed caches. No study to date describes precisely how European hamsters manage the different stocks of food inside their burrow, *i.e.* whether the entire stock is stored at the same place and depth and if it is therefore equally affected by soil moisture. The effect of winter rainfall on body mass at emergence could be of particular importance to start disentangling the reasons behind the decline of Central populations of the species (*i.e.* in Eastern Europe, where maize monoculture has not expanded to the same extent as in Western Europe). This idea is supported by a recent study highlighting that the current shrinkage of the species range in Europe may be a response to the oceanic climate gradient extending eastwards in Europe[56]. Regarding the negative impact of maize monoculture on body mass at emergence, our results suggest that it could have played a major role in the decline of the European hamster in France, covering 55 to 80% of its natural habitat. However, the multiple relationships presented above do not allow us to determine whether it is the maize itself (*i.e.* its composition), monoculture (*i.e.* a lack of food diversity/availability or a particular micro-climate) or both of these elements combined that negatively affect body mass at emergence. We will thus further investigate the nutritional value of maize for this species, and in parallel, look at how the fitness of wild individuals is affected by maize monoculture in Alsace.

Our results ultimately suggest that the overall decrease observed in body mass over the last century might have affected the reproduction and survival of this species, and thus population dynamics. A recent study in the European badger revealed positive links between global warming, increasing quality of habitat in agricultural lands, body weight and population size in this species[28]. Although the direction of the link is in the direct opposite of what we observed in the European hamster, the links between environmental parameters, body mass and population dynamics are consistent. Our results also provide objective knowledge that is underpinning concrete management proposals for the sustainment of European hamster populations in France. Further studies are needed to extend our understanding of the underlying mechanisms that explain the impact of maize or winter rainfall on the fitness of this species. Given the high rates of maize monoculture intensification and climate change, we can expect the decline of European hamster populations to continue. This is especially true if nothing is done to improve land management and inverse the predominance of intensive maize monoculture in Western Europe. One solution would be to manage agricultural landscapes by managing field plots with a combination of sharing and sparing strategies[57] in order to improve soil quality, the abundance of invertebrates and thus habitat suitability for the European hamster.

## Material and Methods

### Species and study site descriptions.

The European hamster lives in loose and stable soils, which are also particularly adapted to crops. Individuals have a marked seasonal body mass cycle in which they gain weight in summer[44]. On average, males are longer and larger than females (27–32 cm and 350 g, and 22–25 cm and 260 g, respectively). Hamsters are 'food-storing' hibernators: they store large amounts of food in their burrow before hibernating[44] and feed on these stocks during their winter arousals. Hibernation lasts from late September to April, with variations occurring according to sex, age and body condition[38,44].

In France, the current relict population of the species (<1500 individuals) is solely found in the agricultural ecosystems of the Alsace plain[30,39], dominated by maize monoculture (that covers up to 80% of all cropland). In this study, we focused on a "central" population representing 80% of total European hamster population in Alsace[39], located in the vicinity of Blaesheim (Alsace, France, N48°30′14.044" and E7°36′28.414, elevation: 154 m).

The average size of agricultural plots in this area has tripled since 1971 (1.4 ha in 2010, compared to 0.54 ha in 1971). This surface area is equivalent to the home range of a male, and is seven times the size of the home range for a female[38].

**Trend in body mass change since 1937.** We used individual body mass data collected between 1937 and 2014 by CNRS research teams (C. Kayser, B. Canguilhem, A. Malan and P. Pévet) and by the National Hunting and Wildlife Agency (ONCFS). This experiment was in accordance with EU 270 Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory 271 and wild animals. It was approved by the Ethical Committee (CREMEAS) under agreement 272 numbers 00624-01 and 00305-01. Wild hamsters were captured and weighed from 1937 to the 60s by CNRS research teams (C. Kayser, B. Canguilhem and A. Malan) for captive studies on hibernation. During the 90s, P. Pévet (CNRS) captured, weighed and then used hamsters for the establishment of a breeding unit and studies on biological rhythms. Finally, from 2001 to 2014, the ONCFS captured and weighed individuals before releasing them immediately. Data concerning the reproductive success of wild females have only been available since 2014. The different teams followed the same protocol for capture (always starting from the 1–5th of April and ending on 30th of May) and body mass measurement. We focused on body mass in April and May as a proxy measure to evaluate the body condition of individuals emerging from hibernation, as body mass at this period is a key factor in survival and reproductive performances[44]. Data included body mass at the period of emergence for 1468 individuals: 660 females, 742 males and 66 individuals for which the sex was unknown. Analyses were run both with and without these 66 individuals, but since the trend was equivalent in both cases, we retained data for these individuals in our analysis. Data were spread over 19 years between 1937 and 2014 and computed to period class (*i.e.* "decades"): <50s (up to and including 1949), the 60s, the 90s and 2001–2014. We tested the possible impact of (i) sex, (ii) decade, (iii) month (April or May) and interactions between (iv) sex*decade and (v) sex*month on body mass.

We then focused on the inter-annual fluctuations of body mass between the early 90s and 2014, the period for which we had the most detailed data set. This enabled us to look at the impact of environmental variables on this trait while excluding confounding factors such as hunting (the species has been protected since the early 90s but was previously actively trapped and poisoned[30,35,39]). Analyses were run separately for each sex due to the sexual dimorphism in this species and because data was missing for males (2013) and females (2001). We then tested whether (i) year and (ii) month (April or May) had an impact on body mass.

**Changes in environmental variables and impact on body mass.** To investigate the impact of environmental change on body mass from 1992 to 2014, we focused on the relationships between body mass at emergence (year n), climate (year n-1) and crop diversity/availability (year n-1). We first looked at how inter-relations between rainfall, temperatures and agriculture (year n-1) could impair body mass at emergence (year n). The climatic parameter was composed of (i) average annual temperature and (ii) total annual rainfall (data from Météo France, Entzheim station). The agricultural parameter was derived from data on the production (per 100 Kg) and acreage (ha) of seven crops: wheat (*Triticum aestivum*), maize (*Zea mays*), sunflower (*Helianthus annuus*), barley (*Hordeum vulgare*), rye (*Secale cereal*), triticale (*Triticosecale sp*.) and rapeseed (*Brassica napus*).

In a second analysis (Model 2), we tried to understand whether body mass on emergence (year n) had been more affected by the climate during the winter (rainfall and temperatures from October to March, year n-1 to year n) or by the climate during the active period (from April to September, year n-1) from 1937 to 2014. The climatic parameter was composed of (i) the average temperature and ($i_2$) the total rainfall during the active period, and (ii) average temperature and ($ii_2$) total rainfall during hibernation.

**Statistical analyses.** Data for body mass change were analysed using multifactorial ANOVAs. Normality was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using a non-parametric Levene test. Body mass variables were log-transformed to fulfil normality conditions. Multiple comparisons were analysed via post-hoc LSD testing. We back-transformed the data using the antilog[58] for representation of body mass (rather than $\text{Log}_{10}$ of body mass) in Fig. 1: data represented are geometric means ± SEM.

Data for climate change (temperature and rainfall) were analysed using quadratic and linear regressions, respectively. We carried out a principal component analysis (PCA) to reduce the number of agricultural variables and to extract the main ones.

Path analysis[59] was then used to test for the presence, nature and strength of multiple relationships between environmental variables (rainfall, temperature, components of the PCA) and body mass. The most parsimonious model was selected by removing insignificant paths one by one. We only removed a path if the Chi-square (which tests the null-hypothesis that the reduced model fits the data as well as the saturated model) value of the model remained $>0.1$. Analyses were conducted using IBM SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp; SPSS-AMOS for path analysis), and the significance threshold was set at $p < 0.05$.

## References

1. Foley, J. A. *et al.* Global consequences of land use. *Science* **309,** 570–574 (2005).
2. Monastersky, R. Life-a status report. *Nature* **516,** 158–161 (2014).
3. Willis, K. J. & Bhagwat, S. A. Biodiversity and climate change. *Science* **326,** 806–807 (2009).
4. Yom-Tov, Y. Global warming and body mass decline in Israeli passerine birds. *Proc. R. Soc. B Biol. Sci.* **268,** 947–52 (2001).
5. Reading, C. J. Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia* **151,** 125–31 (2007).
6. Charmantier, A. *et al.* Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320,** 800–3 (2008).
7. Levinsky, I., Skov, F., Svenning, J. C. & Rahbek, C. Potential impacts of climate change on the distributions and diversity patterns of European mammals. *Biodivers. Conserv.* **16,** 3803–3816 (2007).

8. Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. Genetic and plastic responses of a northern mammal to climate change. *Proc. Biol. Sci.* **270,** 591–596 (2003).
9. Dormann, C. F., Gruber, B., Winter, M. & Herrmann, D. Evolution of climate niches in European mammals? *Biol. Lett.* **6,** 229–32 (2010).
10. Campbell, R. D., Newman, C., Macdonald, D. W. & Rosell, F. Proximate weather patterns and spring green-up phenology effect Eurasian beaver ( *Castor fiber* ) body mass and reproductive success: the implications of climate change and topography. *Glob. Chang. Biol.* **19,** 1311–1324 (2013).
11. Rioux Paquette, S., Pelletier, F., Garant, D. & Belisle, M. Severe recent decrease of adult body mass in a declining insectivorous bird population. *Proc. R. Soc. B Biol. Sci.* **281,** 20140649 (2014).
12. Nielsen, A. *et al.* Are responses of herbivores to environmental variability spatially consistent in alpine ecosystems? *Glob. Chang. Biol.* **18,** 3050–3062 (2012).
13. Lane, J. E., Kruuk, L. E. B., Charmantier, A., Murie, J. O. & Dobson, F. S. Delayed phenology and reduced fitness associated with climate change in a wild hibernator. *Nature* **489,** 554–557 (2012).
14. Schneider, M. F. *et al.* Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecol. Lett.* **13,** 720–735 (2010).
15. Mendenhall, C. D., Karp, D. S., Meyer, C. F. J., Hadly, E. A. & Daily, G. C. Predicting biodiversity change and averting collapse in agricultural landscapes. *Nature* **509,** 213–7 (2014).
16. Fargione, J. E. *et al.* Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation. *Bioscience* **59,** 767–777 (2009).
17. Williams, N. Questions on biofuels. *Curr. Biol.* **17,** 617 (2007).
18. Medan, D., Torretta, J. P., Hodara, K., de la Fuente, E. B. & Montaldo, N. H. Effects of agriculture expansion and intensification on the vertebrate and invertebrate diversity in the Pampas of Argentina. *Biodivers. Conserv.* **20,** 3077–3100 (2011).
19. Gelade, G. *et al.* Farmland Biodiversity and the Footprint of Agriculture. *Science* **315,** 825–828 (2007).
20. Goulson, D., Nicholls, E., Botías, C. & Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, **347,** 1255957 (2015).
21. Van der Velde, M., Tubiello, F. N., Vrieling, A. & Bouraoui, F. Impacts of extreme weather on wheat and maize in France: Evaluating regional crop simulations against observed data. *Clim. Change* **113,** 751–765 (2012).
22. Hawkins, E. *et al.* Increasing influence of heat stress on French maize yields from the 1960s to the 2030s. *Glob. Chang. Biol.* **19,** 937–947 (2013).
23. Wilson, J. D., Whittingham, M. J. & Bradbury, R. B. The management of crop structure : a general approach to reversing the impacts of agricultural intensification on birds? *Ibis (Lond. 1859).* **147,** 453–463 (2005).
24. Sæther, B. *et al.* Life History Variation Predicts the Effects of Demographic Stochasticity on Avian Population Dynamics. *Am. Nat.* **164,** 793–802 (2004).
25. Courchamp, F., Clutton-Brock, T. & Grenfell, B. Inverse density dependence and the Allee effect. *Trends Ecol. Evol.* **14,** 405–410 (1999).
26. De Roos, A. M., Persson, L. & McCauley, E. The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. *Ecol. Lett.* **6,** 473–487 (2003).
27. Capellini, I., Baker, J., Allen, W., Street, S. & Venditti, C. The role of life history traits in mammalian invasion success. *Ecol. Lett.* **18,** 1099–1107 (2015).
28. Byrne, A. W., Fogarty, U., O'Keeffe, J. & Newman, C. *In situ* adaptive response to climate and habitat quality variation: spatial and temporal variation in European badger (Meles meles) body weight. *Glob. Chang. Biol.* **21,** 3336–3346 (2015).
29. La Haye, M. J. J., Swinnen, K. R. R., Kuiters, A. T., Leirs, H. & Siepel, H. Modelling population dynamics of the Common hamster (Cricetus cricetus): Timing of harvest as a critical aspect in the conservation of a highly endangered rodent. *Biol. Conserv.* **180,** 53–61 (2014).
30. Villemey, A., Besnard, A., Grandadam, J. & Eidenschenck, J. Testing restocking methods for an endangered species: Effects of predator exclusion and vegetation cover on common hamster (Cricetus cricetus) survival and reproduction. *Biol. Conserv.* **158,** 147–154 (2013).
31. Weinhold, U. *Draft European action plan for the conservation of the common hamster (Cricetus cricetus L., 1758). Technical report.* (2008) Available at: http://www.zoogdierenwerkgroep.be/sites/default/files/zwg/EuropeanPlanConservationHamster.pdf (Accessed: 5th November 2014).
32. Eckert, C. G., Samis, K. E. & Lougheed, S. C. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Mol. Ecol.* **17,** 1170–1188 (2008).
33. Hampe, A. & Petit, R. J. Conserving biodiversity under climate change: The rear edge matters. *Ecol. Lett.* **8,** 461–467 (2005).
34. Telleria, J. L. The decline of a peripheral population of the European robin Erithacus rubecula. *J. Avian Biol.* **46,** 159–166 (2015).
35. O'Brien, J. Saving the common hamster (Cricetus cricetus) from extinction in Alsace (France): potential flagship conservation or an exercise in futility? *Hystrix, Ital. J. Mammal.* **26,** doi: 10.4404/hystrix-26.2-11230 (2015).
36. Kuiters, L., La Haye, M., Muskens, G. & Van Kats, R. Survival analysis to predict the predation risk in reintroduced populations of the common hamster (Cricetus cricetus) in the Netherlands. Paper presented at *V European Congress of Mammalogy*, Siena, Italy. *HYSTRIX The Italian Journal of Mammalogy: Associazione Teriologica Italiana* (2007, September 21–26).
37. La Haye, M. J. J., Neumann, K. & Koelewijn, H. P. Strong decline of gene diversity in local populations of the highly endangered Common hamster (Cricetus cricetus) in the western part of its European range. *Conserv. Genet.* **13,** 311–322 (2011).
38. Ulbrich, K. & Kayser, A. A risk analysis for the common hamster (Cricetus cricetus). *Biol. Conserv.* **117,** 263–270 (2004).
39. Reiners, T. E., Eidenschenk, J., Neumann, K. & Nowak, C. Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the Common hamster of the French Alsace region. *Mamm. Biol.* **79,** 240–246 (2014).
40. Monecke, S. All things considered? Alternative reasons for hamster extinction. *Zool. Pol.* **58,** 41–57 (2013).
41. Ozgul, A. *et al.* Coupled dynamics of body mass and population growth in response to environmental change. *Nature* **466,** 482–5 (2010).
42. Campbell, R. D., Newman, C., McDonald, D. W. & Rosell, F. Proximate weather patterns and spring green- up phenology effect Eurasian beaver ( Castor fiber ) body mass and reproductive success : The implications of climate change and topography. *Glob. Chang. Biol.* **19,** 1311–1324 (2013).
43. Dobson, F. S., Risch, T. S. & Murie, J. O. Increasing returns in the life-history of Columbian ground squirrels. *J. Anim. Ecol.* **68,** 73–86 (1999).
44. Nechay, G. & Grulich, I. The Common Hamster ( Cricetus cricetus [L.]) ; a Review. *EPPO Bull.* **7,** 255–276 (1977).
45. Kayser, A., Weinhold, U. & Stubbe, M. Mortality factors of the common hamster Cricetus cricetus at two sites in Germany. *Acta Theriol. (Warsz).* **48,** 47–57 (2003).
46. Kourkgy, C. & Eidenschenck, J. *Rapport annuel de présentation des données collectées et premières analyses. Technical report*. (2015) Available at: http://www.oncfs.gouv.fr/Plan-de-restauration-du-Grand-Hamster-ru82/Mission-Hamster-a-lONCFS-ar1098 (Accessed: 12th February 2016).
47. La Haye, M. J. J., Koelewijn, H. P., Siepel, H., Verwimp, N. & Windig, J. J. Genetic rescue and the increase of litter size in the recovery breeding program of the common hamster ( *Cricetus cricetus* ) in the Netherlands. Relatedness, inbreeding and heritability of litter size in a breeding program of an endangered rodent. *Hereditas* **149,** 207–216 (2012).

48. Chaigne, A., Tissier, M. L., Habold, C., Eidenschenck, J. & Uhlrich, B. In *Les mammifères sauvages, Recolonisation et réémergence*, Vol. 10, 312–322 (Bourgogne Nature, 2015).
49. Nuss, E. T. & Tanumihardjo, S. A. Maize: A paramount staple crop in the context of global nutrition. *Compr. Rev. Food Sci. Food Saf.* **9,** 417–436 (2010).
50. Rosenberg, N. J., Blad, B. & Verma, S. B. *Microclimate: The Biological Environment*, *2nd edn* (John Wiley & Sons, 1983).
51. Heldmaier, G., Ortmann, S. & Elvert, R. Natural hypometabolism during hibernation and daily torpor in mammals. *Respir. Physiol. Neurobiol.* **141,** 317–329 (2004).
52. Albert, M., Reiners T. E. & Encarnação J. A. Distribution of Common hamsters (Cricetus cricetus) in relation to landscape scale crop composition in Hesse (Central Germany). *Poster presented at 18th Meeting of the International Hamster Workgroup, Strasbourg, France* (2011, October). Available at: https://www.researchgate.net/publication/234061410_Distribution_of_Common_hamsters_Cricetus_cricetus_in_relation_to_landscape_scale_crop_composition_in_Hesse_Central_Germany (Accessed: 15th March 2016).
53. Patil, V. P., Morrison, S. F., Karels, T. J. & Hik, D. S. Winter weather versus group thermoregulation: What determines survival in hibernating mammals? *Oecologia* **173,** 139–149 (2013).
54. Turbill, C. & Prior, S. Thermal climate linked variation in annual survival rate of hibernating rodents: shorter winter dormancy and lower survival in warmer climates. *Funct. Ecol.* doi: 10.1111/1365-2435.12620 (2015).
55. Edelman, A. J. Kangaroo Rats Remodel Burrows in Response to Seasonal Changes in Environmental Conditions. *Ethology* **117,** 430–439 (2011).
56. Korbut, Z. & Agata, B. The history of species reacting with range shifts to the Oceanic-Continental climate gradient in Europe. The case of the common hamster (Cricetus Cricetus). *Kosmos* **65,** 69–79 (2016).
57. Butsic, V. & Kuemmerle, T. Using optimization methods to align food production and biodiversity conservation beyond land sharing and land sparing. *Ecol. Appl.* **25,** 589–595 (2015).
58. Bland, J. M. & Altman, D. G. *Transformations, means, and confidence intervals*. **312,** 1996 (1996).
59. Shipley, B. *Cause and Correlation in Biology. A User's Guide to Path Analysis, Structural Equations and Causal Inference (*Cambridge University Press, 2000).

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## Author Contributions

M.L.T., C.H. & Y.H. conceived and designed the study. M.L.T. performed data analyses and prepared the figures. M.L.T. wrote the first draft of the manuscript, C.H. and Y.H. made the first corrections and C.K., M.W., P.P. and J.-P.R. contributed substantially to revisions.

## Additional Information

**Competing financial interests:** The authors declare no competing financial interests.

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# Chapter 2

« *The Devil is in the details* »

# Nutritional effects of cereal monoculture on the reproductive success of the European hamster

Content:

## 1. General approach

With agriculture intensification and the expansion of monoculture, farmland species are often constrained by the lack of food diversity and quality (Evans 1997; Goulson et al. 2015). In this context, and with the main aim of filling the gap regarding how the diet of the European hamster in intensive farmland can affect its biology, we designed three studies in captive (box 1 and study 2) and semi-natural conditions (study 3). More precisely, the common goal of these studies was to understand how the major increase in maize and wheat monocultures recorded in the study 1 (Tissier et al. 2016) can impair hamsters' reproduction through a modification/restriction of their diet. This chapter is therefore declined into three sections:

- In the first section (box 1; two diet selection experiments), I present the results of a study investigating for food preferences in captive female hamsters. First, we compared maize and wheat (i.e. the two main crops currently cultivated in the Alsace (O'Brien 2015; Tissier et al. 2016) to other crops that were important components of hamsters' diet in the 70s across European farmland (Gorecki & Grygielska 1975; Nechay et al. 1977). We have then verified whether hamsters spontaneously consumed clover and earthworms, as a prerequisite for the study 2.

- Secondly, I present the results of a study (study 2) looking at the effects of wheat and maize on the reproduction of hamster females under controlled conditions. The omnivorous diet of the species in current and intensive French farmland was simulated by supplementing maize and wheat with either clover or earthworms (i.e. protein-rich items), leading to four diets: maize-clover, maize-worm, wheat-clover and wheat-worm. I then looked at how these diets affected females' fecundity (number of pups at parturition), change in body mass, oxytocin plasma levels (i.e. the main maternal hormone in mammals) as well as pups' survival and body mass at weaning.

- In the last part of this chapter (study 3), I have investigated whether the results observed in the study 2 (i.e. in captive females) were confirmed under semi-natural conditions. More precisely, I was seeking to understand whether hamsters were able to have a sufficiently diverse diet by consuming invertebrates and adventive plants to ensure a good reproduction, depending on the diversity of the sowing. I therefore designed a study in semi-wild enclosures, to compare for the effects of organic monoculture crops (enclosures seeded with either wheat or maize) and mixed organic crops (enclosures seeded with four crops each) on hamsters' fitness. I recorded hamsters' survival and reproductive success (number of pups emerging from the burrow) as well as the adventive plant and invertebrate species richness in each enclosure.

## 2. Summary of the main results discussion

All the food items (i.e. wheat and maize grains, potato tubers, maize leaves, alfalfa roots and alfalfa leaves) given to the hamsters in the first diet selection experiment (Box 1, groups A and B) were consumed, in varying proportions. Hamsters preferentially consumed wheat grains (5.31±0.41 g/day), maize grains (5.10±0.41 g/day) and potato tubers (5.47±0.56 g/day). However, their choice towards these food items vary according to the other items in the diet, likely to meet their specific daily energy and macronutrient requirements (i.e. protein = 1.07±0.01 g/day, lipid = 0.43±0.03 g/day and energy = 204.1±0.15 KJ/day). Therefore, hamsters' food choice seem to vary depending on their food preference (i.e. palatability of the food item), their physiological requirements (i.e. required daily protein, lipid and energy intakes) and the composition of the food items that they have at their disposal. The second diet selection experiment (Box 1; group C) first reveals that hamsters displayed a strong food preference towards clover and earthworms (compared to the conventional diet and wheat grains). It also pinpoints that hamsters' daily protein, lipid and energy intakes strongly increase between March and May, likely to meet increased daily requirements during this reproductive period (Speakman 2008).

Results of the study 2 revealed that females fed with maize-based diets showed a reduction of up to 75% of their reproductive success compared to females fed on the wheat-worm diet, mainly caused by a high rate of infanticide during the first day after parturition. This reduced reproductive success in maize-fed females was associated to hyperactivity and aggressive behaviors compared to wheat-fed females. However, despite these reduced maternal behaviors and this greater aggressivity, females fed with maize did not show a reduction in their body mass that is often correlated with litter reduction in rodent (Schneider & Wade 1989). They did not show either a significant reduction in their plasma levels of oxytocin that usually related to aggressivity and reduced maternal behaviors in rodents (McCarthy 1990; Harmon et al. 2002).

Finally, the few surviving pups in the maize groups displayed reduced body mass at weaning (of up to 50%) and two cases of siblicides were observed. We found no significant differences in macronutrients, energy or mineral contents (see Appendix 1 for minerals) of the diets that could explain these results. We rather showed that the tryptophan and vitamin B3 (i.e. niacin or nicotinamide) deficiencies in maize were responsible of these high rates of infanticides. This vitamin is crucial for red-ox reactions (through its implication in NAD and NADP synthesis, see Figure 10). Therefore, tryptophan and vitamin B3 deficiencies ultimately lead to the damage of many tissues and a variety of symptoms (diarrhea, dermatitis and dementia in humans (Hegyi et al. 2004; Wan et al. 2011), the black-tongue syndrome in dogs (Ammerman et al. 1995) or aggressivity in rodents (Walz et al. 2013). Given the complexity of vitamin B3 synthesis *in vivo* (Figure 10), and given that tryptophan is also essential to the synthesis of serotonin (involved in aggressiveness and depression (Ernandes et al. 1996); 5-HIAA, Figure 10), the detrimental effects of a predominance of maize in the diet are extremely difficult to counteract with other food items (Ernandes et al. 1996).

Results of the study 3 (in semi-wild enclosures) confirm that hamsters were not able to compensate for this deficiency by consuming other food items such as invertebrates or adventive plants. Indeed, hamsters' reproductive success was 82% lower in monoculture crops than in mixed crops. In parallel, monoculture crops reduced by 38% and 28% adventive plant and invertebrate species richness compared to mixed crops. Although the link between reduced hamsters' reproductive success and decreased plant and invertebrate species richness in this 3rd study is only correlative, results strongly suggests that hamsters were suffering from nutritional deficiencies in monoculture crops that strongly reduced their reproductive success. This study therefore pinpoints how monoculture per se (i.e. even when excluding the use of pesticide and mechanization) impairs hamsters' reproduction. Moreover, these results highlight the importance of restoring diverse sowing in agricultural landscapes. However, replacing monoculture by 4-crops sowing appears extremely difficult to implement in the current agricultural schemes in the Alsace. Therefore, the next step will be to investigate for two-crop-associations favorable to the hamster and that could be of agronomic/economic interests or more easily implemented by French farmers than the sunflower-maize-wheat-alfalfa association.

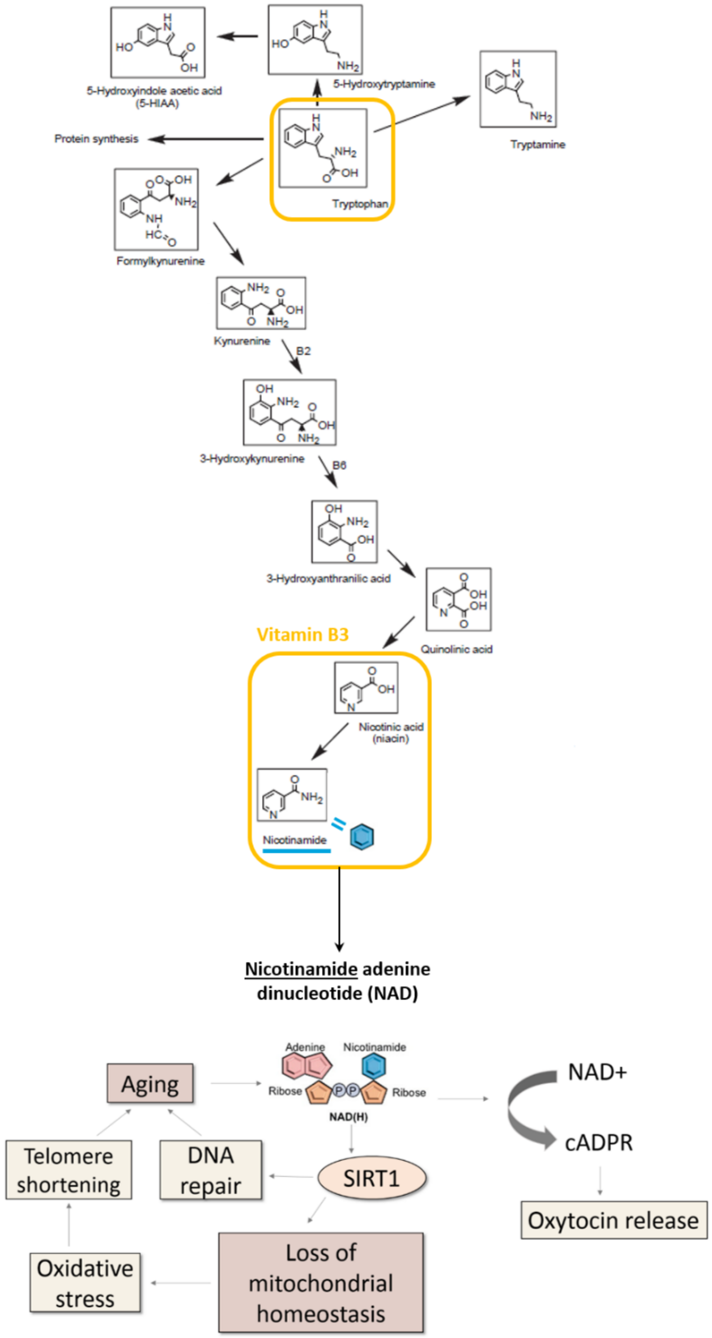

**Figure 10: diagram representing the metabolism of tryptophan to vitamin B3 and sumarizing the pathways between nicotinamide, ocytocin, oxidative stress and aging in mammals** (adapted from (Itagaki 2005; Herranz & Serrano 2010; Wan et al. 2011; Imai & Guarente 2014)). 5-HAA: serotonin, B2: riboflavin, B6: pyridoxine and SIRT1: sirtuin 1, telomeres: protective caps of the chromosomes.

# Box 1 - Diet selection in the European hamster, daily macronutrient and energy intakes

*Unpublished data* [1]

## Short introduction

The last studies on the diet of the European hamster in farmland date back to the 70s and report that the species was feeding on grains (wheat, poppy, maize), invertebrates, green parts of plants (wheat, clover, rape, beet, potatoes and alfalfa) and even on vertebrates (Gorecki & Grygielska 1975). In summer (June and July), wheat (75.5%) and maize starch (13.1%) were predominantly found in the stomachs of farmland hamsters. However, in the fall (October, November), potato starch (44.4%) and maize starch (22.2%) were the most represented. Regarding the green parts of plants, wheat crop was the most consumed in summer (31.6%) whereas in the fall green parts of clover and potatoes were the most represented (25% and 22.5%, respectively). Therefore, the authors conclude that wheat is the predominant plant (60.6%) in the diet of the hamster during the whole vegetation period, and that corn and potatoes are following (approximately 14.5% of occurrence for each). However, this ranking in food consumption is very likely caused by a predominance of wheat in the field (i.e. hamsters were captured in wheat fields during the summer and in clover during the fall) and do not really represent a food choice or any food preference. Moreover, agro-ecosystems have greatly changed since this study (carried-out in Poland in 1974), and in the Alsace, farmlands mostly do not contain poppies, rape, beans or oats anymore. Maize and wheat are predominant (see (O'Brien 2015; Tissier et al. 2016)), with occasional clover inter-crops (ACTeon 2012) and frequent plots of alfalfa (from 3% to 25% (ACTeon 2012)), considered as a favorable crop for the species ((Out et al. 2011; ACTeon 2012) but see (Villemey et al. 2013)).

Therefore, we carried-out these diet selection experiments to investigate: **1)** whether hamsters spontaneously consumed and/or show a preference between maize and wheat grains in captive conditions, **2)** for the predominance of these grains in their diet when supplemented with other food items and **3)** the palatability of earthworms and clover given to the females in the study 2.

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[1] These data will be part of the review: Tissier M.L., Kourkgy C., Robin J.P., Eidenschenck J., Handrich Y. and Habold C. Habitat and nutrition of the European hamster in France – a Review, in collaboration with the ONCFS – *In preparation*

## Methodology

### *Animals and housing conditions*

Hamsters were housed in large cages (W x H x D: 380 x 257 x 590 mm) and maintained in controlled environmental conditions (temperature 20±2°C; humidity 35-55%; summer photoperiod, 16L: 8D) throughout the experiment. They were fed *ad libitum* on a conventional diet (pellets 105, from Safe, Augy, France, composed of 19.3% protein, 54.9% carbohydrates, 5.1% lipids, 4.2% cellulose, 5.0% minerals, and 11.5% water) *prior* to the experiment.

### *Experimental protocol*

Two diet selection experiments were carried-out. The first experiment started 20 days after the transition from winter photoperiod (8L: 16D; ~ march) to summer photoperiod and was performed on 30 one year-old female hamsters. These 30 females were divided into two groups (A and B) with different food items (**Table 1**). The second experiment started later (8 weeks after the transition from winter photoperiod) and was performed on 10 one-year old females (group C, **Table 1**). Regarding the experimental set-up, females were first exposed to the food items of each experimental group (A, B or C) during a 40-hour period of habituation, during which they had also access to conventional food items (pellets 105, see above). The totality of the food was then removed for 8 hours before the start of the food selection experiment. Hamsters were then maintained either on the group A (15 females), B (15 females) or C (10 females; in this group, the pellets 105 represented one of the four items) for 24 hours, to estimate their daily food intake. After this period, the totality of the food remaining in the cage was collected, dried and weighed to evaluate hamsters' daily food intake (g day$^{-1}$ of dry matter).

| Group | Item 1 | Item 2 | Item 3 | Item 4 |
|---|---|---|---|---|
| A | Maize grains | Wheat grains | Maize leaves | Alfalfa roots |
| B | Maize grains | Wheat grains | Alfalfa leaves | Potatoes |
| C | Wheat grains | Earthworms | Clover | Pellets 105 |

**Table 1: composition of the experimental diets.** The three diets were composed of four items and hamsters received 8 g (fresh material) of each item, except in the diet C in which they received 10 g of each food item.

### *Macronutrient content of the food items*

We measured the total protein (Kjeldhal method (Folch et al. 1957)), lipid (Folch method (Folch et al. 1957)) and energy (using a PAAR calorimeter calibrated with benzoic acid) content for each food item. Each sample was carried out in duplicate in each of the three techniques (More details can be found in (Tissier et al. 2017), study 2). We were not able to measure the protein and lipid content of the maize leaf because of a lack of fresh material.

### *Ethics*

The experimental protocol followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and was approved by the Ethical Committee (CREMEAS) under agreement number 00624-01.

### *Data analyses*

Data presented are means ± SEM. Normality of the residuals was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using a Levene test. In the first diet selection experiment, to investigate for food preference, we looked at the effect of the diet (A or B) and the item identity on the specific daily food intake for each food items, using a Linear Model (LM). The diet and the item identity were included as fixed factors and the total daily intake was included as a covariate. In the second selection experiment, a LM was also used and only the item identity was included as fixed factor, with the daily intake as a covariate. Multiple comparisons were analysed via post-hoc LSD (least significant difference) testing. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA).

## Results

In each group, four items given to hamsters were consumed, but in varying proportions. Results of the first diet selection experiment revealed that hamsters displayed a strong selection (food item effect, $F_{5,111}= 11.32$, $p=7.8.10^{-9}$). They ingested significantly more potato tubers (5.47±0.56 g/day), maize grains (5.10±0.41 g/day) and wheat grains (5.31±0.41 g/day) than alfalfa leaves (2.02±0.56 g/day), maize leaves (2.62±0.60 g/day) and alfalfa roots (2.14±0.6 g/day; $p<0.05$). We also found a group*food item interaction ($F_{1,111} = 6.55$, $p = 0.012$). Post-hoc analyses highlighted that in the group A, hamsters selected for wheat grains over maize grains, whereas maize leaves and alfalfa roots were the least consumed food items (**Figure 11a**). In the group B, however, hamsters ingested the most potatoes and maize grains, whereas wheat grains were slightly (but not significantly, $p = 0.071$) less consumed. Alfalfa leaves were the least consumed among all the food items (**Figure 11b**).

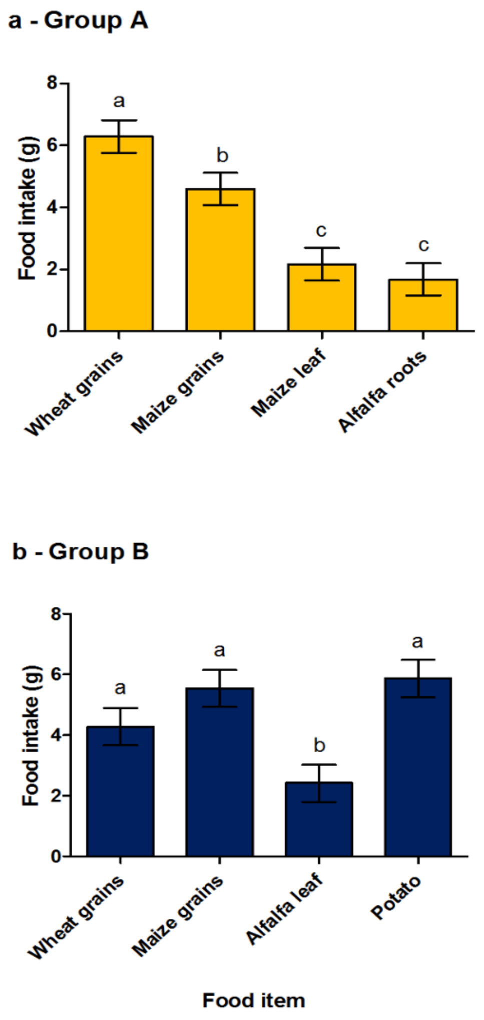


**Figure 11: daily food intake of each food item in the group A (a) and the group B (b)**. The specific daily food intake is represented for each food item in grams. Different letters mean significant differences between the food items ($p<0.05$).

In the second food experiment, hamsters also consumed the four food items available but they displayed a selection (effect of the food item, $F_{3,35} = 26.65$, $p < 0.001$) towards clover and earthworms (**Figure 12**).

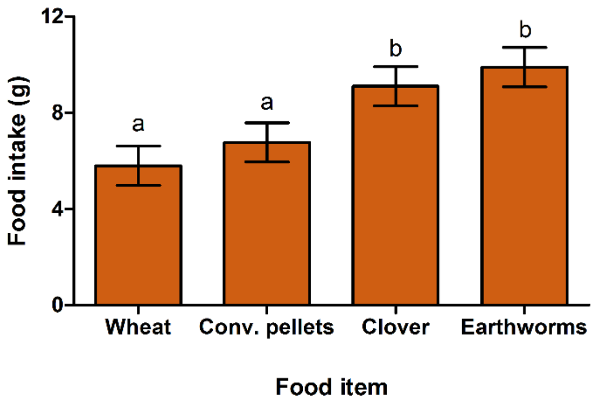


**Figure 12: daily intake of each food item in the group C**. The specific daily food intake of each food item is represented in grams. Different letters mean significant differences between the food items ($p<0.05$).

Comparing the group A and B highlights that the daily intakes in proteins (~ 1.07±0.01 g/day), lipids (~0.43±0.03 g/day) and energy (~204.1±0.15 Kj/day) are extremely similar between these two groups, despite some differences in the total daily food intake (g of food/day) (**Table 2**). When looking at the group C, we can observe up to a 2-fold increase in the daily intakes in food and energy compared to the groups A and B. As a result, individuals from the group C ingested 3 times more proteins and 2 times more lipids than in the groups A and B.

| Group | Food item | Ingested quantity (g of FM/day) | Ingested energy (KJ/day) | Ingested proteins (g/day) | Ingested lipids (g/day) |
|---|---|---|---|---|---|
| A | Wheat (grains) | 6.285 | 99.932 | 0.622 | 0.226 |
| A | Maize (grains) | 4.597 | 79.528 | 0.382 | 0.170 |
| A | Alfalfa (roots) | 1.685 | 11.627 | 0.101 | 0.067 |
| A | Maize (leaves) | 2.169 | 13.079 | NA | NA |
| A | Total | 14.736 | 204.166 | 1.105 | 0.463 |
| B | Wheat (grains) | 4.278 | 68.020 | 0.424 | 0.154 |
| B | Maize (grains) | 5.536 | 95.773 | 0.459 | 0.205 |
| B | Potato (tubers) | 5.868 | 25.819 | 0.088 | 0.018 |
| B | Alfalfa (leaves) | 2.414 | 14.243 | 0.111 | 0.022 |
| B | Total | 18.096 | 203.855 | 1.082 | 0.399 |
| C | Wheat (grains) | 5.796 | 92.156 | 0.574 | 0.209 |
| C | Earthworms | 9.896 | 46.69 | 1.534 | 0.228 |
| C | Clover | 9.101 | 26.50 | 0.257 | 0.106 |
| C | Pellets 105 | 6.770 | 102.3 | 1.476 | 0.366 |
| C | Total | 31.563 | 267.65 | 3.841 | 0.909 |

**Table 2: Daily food, energy, protein and lipid intakes according to the group and the food items**. Values are given in fresh matter (FM), kilojoules (KJ) and grams (g). Pellets 105: conventional diet

given to the hamsters. NA indicate missing values. We therefore used data from (AFZ et al. 2011) to estimate the total protein and lipid intakes in the group A.

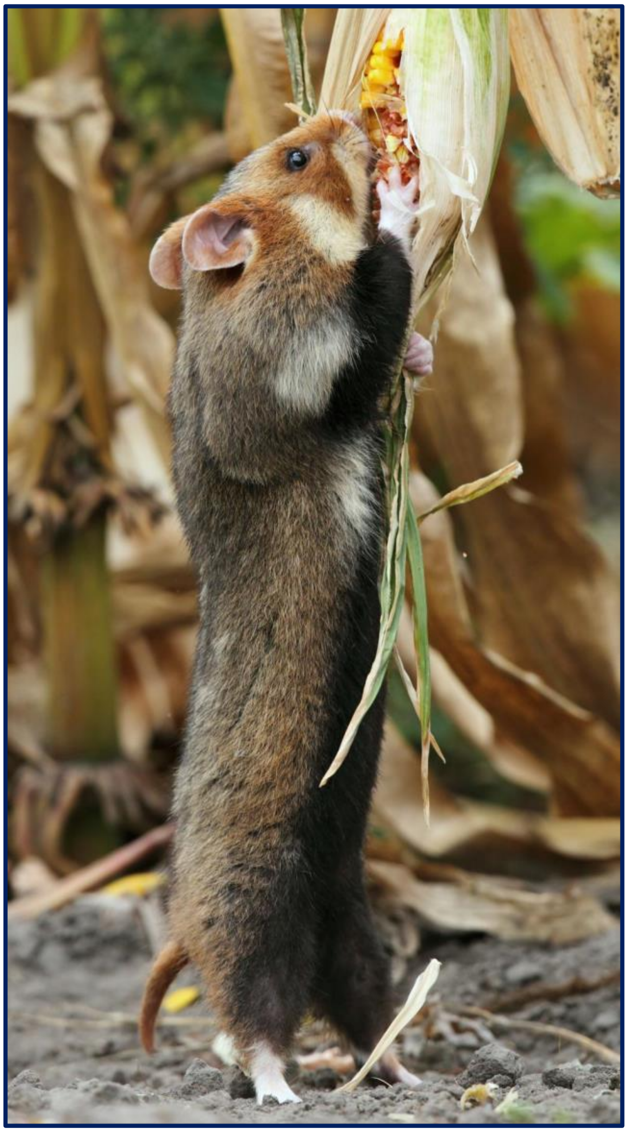
### Short discussion

These results show that hamsters consumed all the food items available. However, they display important food selection, which varies according to the items available. Indeed, some food items (i.e. potato, earthworms, clover, maize grains and wheat grains) were consumed in significantly greater quantities. However, hamsters' food consumption varies according to the diversity of the food items provided (*e.g*. wheat grains were preferentially consumed in the group A, but not in the two other groups). When looking at the ingested levels of macronutrients and energy in the different groups, it appears that in the groups A and B (tested in March), female hamsters ingested the same amount of energy (~204 Kj/day), lipids (~0.4 g of lipids/day) and proteins (~1.08 g of proteins/day) in the two groups, but not the same quantity of fresh mass (FM intake of ~14.7 g in the group A and ~18.1 g in the group B). In the latter, hamsters ingested important levels of potato (not given to the group A), which is rich in water but poor in lipids and proteins. Therefore, animals of the group B were able to ingest great amount of this food item, without significantly increasing their daily energy and macronutrient intakes. These results therefore suggest that hamsters of these two groups modify their consumption in function of their food preference but also to meet their physiological needs, which is only possible if their diet allow them to do so (*i.e*. if it is diversified enough).

In the group C (tested in late May), we can observe that hamsters greatly increase their daily food intake (i.e. twofold to threefold increase compared to groups A and B). In the group C, they ingested ~31.6 g of FM per day, leading to consequent increase in their daily energy, lipid, and protein intakes. This increase in food, energy and macronutrient intakes is a probable consequence of the physiological states of hamsters, that were in the reproductive period in late May, known to be associated to increased daily requirements (Schneider & Wade 1989, 1991; Schneider 2004; Speakman 2008). Now that the spontaneous consumption of maize and wheat grains, as well as earthworms and clover have been confirmed, it remains to investigate whether these items influence the reproduction of the species.

# Study 2 - maize consumption, vitamin B3 deficiency and high rates of maternal infanticides

*Published* [1]



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[1] Tissier ML, Handrich Y, Dallongeville O, Robin J-P, Habold C.2017. Diets derived from maize monoculture cause maternal infanticides in the endangered EuropeanhamsterduetoavitaminB3 deficiency. *Proc.R.Soc.B* 284: 20162168. http://dx.doi.org/10.1098/rspb.2016.2168

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# Diets derived from maize monoculture cause maternal infanticides in the endangered European hamster due to a vitamin B3 deficiency

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From 1735 to 1940, maize-based diets led to the death of hundreds of thousands of people from pellagra, a complex disease caused by tryptophan and vitamin B3 deficiencies. The current cereal monoculture trend restricts farmland animals to similarly monotonous diets. However, few studies have distinguished the effects of crop nutritional properties on the reproduction of these species from those of other detrimental factors such as pesticide toxicity or agricultural ploughing. This study shows that maize-based diets cause high rates of maternal infanticides in the European hamster, a farmland species on the verge of extinction in Western Europe. Vitamin B3 supplementation is shown to effectively restore reproductive success in maize-fed females. This study pinpoints how nutritional deficiencies caused by maize monoculture could affect farmland animal reproduction and hence their fitness.

## 1. Introduction

In recent decades, there have been major changes in agricultural practices, directly associated with the increasing demand for food, plastics and biofuels [1–3]. This increased demand is currently satisfied by intensively managed monoculture of cereal crops [2,3]. Cereal monoculture has been associated with an increase in the mortality of farmland species because of pesticide toxicity, agricultural mechanization or higher predation rates [4–9]. The resulting decline in biodiversity has led to a drastic reduction in ‘ecosystem services’, which are currently threatening human safety and nutrition worldwide [1,10–12]. These nutritional threats notably occur through a reduction in access to micronutrients (minerals, amino acids and vitamins) by pollinators [13].

Intensive monoculture has also been linked to the increase in the size of plots, the loss of edge zones and of green corridors associated with marked environmental stochasticity in agroecosystems [1,2]. In parallel, the intensive use of inputs is reducing the diversity and abundance of adventive species, soil fauna and microbial communities [10,14,15]. Taken together, these phenomena are making food availability unpredictable and poorly diversified for farmland wildlife, which are thus restricted to a more monotonous and stochastic diet [16–18]. Goulson *et al*. [16] recently stated that ‘it seems certain that bees inhabiting intensive farmland have a more monotonous diet than they would have experienced in their evolutionary past, but how this impacts upon their fitness remains unclear’. Indeed, the lack of flower diversity in intensively managed farmland with the predominance of flowers in the form of mass-flowering crops such as wheat, maize or canola strongly constrain pollinators in their diet [16,19]. More generally, all species with small home ranges that live in agricultural landscapes appear to be constrained in their diet by thousands of hectares dominated by one or two intensively cultivated crops. However, studies are still lacking on how crop-based diets with varying macronutrient, mineral or vitamin contents and amino acid composition influence the physiology, behaviour or key life-history traits—such as reproduction—of farmland animals. This is especially true for species with small home ranges, a marked seasonal cycle or for rare or endangered species that are complex to monitor in the wild without threatening their survival. Indeed, studies including the effects of crop-based diets on wildlife in agricultural landscapes are still scarce and limited to invertebrates [16,19] or mammals with large home ranges [20].

The European hamster (*Cricetus cricetus*)—critically endangered in Europe [21–23]—is particularly threatened by the expansion of wheat and maize monocultures in Western Europe [22,24]. The highly fluctuating food availability (with crop rotation and harvest) associated with the strong seasonality of the species (which hibernates from October–April and reproduces from April–September) put it at high risk of facing periods of food scarcity, even during the reproductive period (e.g. after harvest). The European hamster belongs to the food-hoarding hibernators [25–27], known to hoard very large amounts of food in their burrow to feed during winter arousals but also during the active period (for up to 11 months in some species [27,28]) when aboveground foraging is not possible [27]. Given that species at high risk of facing periods of food scarcity are those that rely the most on their hoarded food [27], the survival and reproductive success of wild hamsters might greatly depend on the nutritional value of their hoards. However, up-to-date data on the link between nutrition and fitness of wild hamsters in the agroecosystems of Western Europe are severely lacking, most current studies on this aspect involve hamsters living in urban habitats [29–32]. The most recent data on the nutrition of the species in agroecosystems date back to the 1970s–1980s [26,33] and revealed that hamsters mostly feed on cereal crops (wheat, corn, rape), tubers and invertebrates. We therefore designed this experimental study specifically to analyse the impact of natural-based diets composed of cereals supplemented with natural protein-rich items on the reproductive investment and ultimately on the reproductive success of captive female hamsters. This design is based on the study of Gorecki & Grygielska [33], and on the postulate of Nechay [34] which states that wild female hamsters do not emerge from their burrow before their first gestation. They thus rely on the food they hoarded the previous summer (mainly seeds) until gestation occurs, then supplement their diet with fresh food items, including plants available in spring, such as clover or invertebrates such as earthworms during gestation and lactation.

Maize appears to be slightly energy richer than wheat but contains marginally less proteins [35], whereas earthworms are significantly richer in proteins than clover [35]. Consequently, and given the importance of protein and energy supplies during reproduction in vertebrates [36–41], we were expecting to observe (i) slightly larger litters or larger pups at parturition by females fed maize-based diets, (ii) slightly higher growth rates of pups of females fed wheat-based diets compared with maize-based diets, and (iii) a significantly greater growth rate in pups of females fed with diets supplemented with earthworms than those fed with diets supplemented with clover. Following the results of a study dating from 1945 [42] reporting that the vitamin B3 deficiency (i.e. niacin or nicotinamide) in maize was responsible for delayed growth in rats, we designed a second experiment in which hamsters were fed on maize-earthworms diets, one of which included a vitamin B3 supplement. Given the positive effects of this vitamin on growth parameters in livestock and rats, we were expecting (iv) a significantly greater growth rate and body mass in pups at weaning in the group fed with the vitamin B3-supplemented diet. By focusing on the direct impact of crop-based diets on reproduction, rather than the indirect impact of monoculture on mortality, we provide new insights into the impact of food on the life-history traits of farmland animals. This could thus help explain how the expansion of intensive monoculture is affecting wildlife fitness in agroecosystems.

## 2. Material and methods

### (a) Animal care and breeding protocol

Hamsters were maintained in controlled environmental conditions (temperature 20°C–23°C; 35–55% humidity; summer photoperiod, 16 L : 8 D) and housed individually (W × H × D: 265 × 237 × 420 mm) until breeding (i.e. two weeks after the beginning of the experiment). The first experiment lasted from April to July 2014 and used 29 one-year-old primiparous females born in 2013 in our captive breeding unit (CNRS, IPHC-DEPE, Strasbourg, France). The second experiment lasted from April to July 2015 and used 14 one-year-old primiparous females from our captive breeding unit. Prior to the experiments, the females in both experiments were fed a conventional diet (pellets 105, from Safe, Augy, France, composed of 19.3% protein, 54.9% carbohydrates, 5.1% lipids, 4.2% cellulose, 5.0% minerals and 11.5% water). During the experiments, they were bred with 1-year-old males from our breeding unit (29 males in 2014 and 14 in 2015), which were fed the conventional diet until being paired with the females. Breeding pairs were placed in large cages (W × H × D: 380 × 257 × 590 mm) equipped with a shelter box (W × H × D: 140 × 230 × 230 mm) for two weeks.

### (b) First experiment: effect of natural-based diet on the maternal investment in reproduction

#### (i) Experimental protocol: diets and food intake

In the first experiment (2014), at emergence from hibernation, the 29 females were fed ad libitum with either wheat (*Triticum* spp.) or maize (*Zea maize*) grains and supplemented with either clover (*Trifolium pretense*) or earthworms (*Lumbricus terrestris*) after mating. Before parturition, the complement consisted of 5 g $\text{female}^{-1}\ \text{d}^{-1}$ of either earthworm or clover. It was then increased by 1 g $\text{young}^{-1}\ \text{d}^{-1}$ between parturition and weaning (30 days after parturition). This led to four different diets: wheat-worm (WW, $n = 7$), wheat-clover (WC, $n = 8$), maize-worm (MW, $n = 7$) and maize-clover (MC, $n = 7$). Water was provided ad libitum throughout the experiment.

Females' daily intake of maize and wheat grains was recorded along with the total protein, lipid and energy content of each diet. Grains were freeze-dried to constant mass and ground under liquid nitrogen to obtain a homogeneous powder for analysis. Just before analysis, the powder was lyophilized for 48 h to eliminate any remaining traces of water. Nitrogen content was determined in triplicate using 150–200 mg aliquots according to the Kjeldahl method [43]. *Protein content* was calculated as nitrogen content × 6.25 [44]. *Lipid content* was determined in duplicate using 1 g aliquots according to a procedure adapted from the Folch method [45] with a chloroform/methanol (2/1, v/v) solution as extraction solvents. Ash content was determined gravimetrically in duplicate from 1 to 2 g samples ignited in a muffle furnace at 400°C for 24 h. Total body water was then calculated by subtracting total dry body mass from fresh body mass. Finally, *energy content* was determined on dry 0.7–1.4 g aliquots by using an isoperibol bomb calorimeter Parr 6200 with benzoic acid as standard. The carbohydrate ration was equal to 100% of energy value minus the lipid and protein percentages.

### (ii) Litter size, reproductive success and body mass

Twice a day (at 08.00 and 19.00), we monitored the number of females that initiated parturition. Females that did not give birth or did not raise their litter were subjected to a second mating event. Their body mass (±0.01 g) was recorded 1 day before pairing, 8 days after parturition and at the end of the experiment (i.e. 30 days after parturition if they initiated parturition and raised their litter or 30 days after the second reproductive attempt if they did not). These three periods are hereafter referred to as 'before', 'middle' and 'end' of the experiment. We monitored the size of the litters every day to obtain the early survival rate of pups (from birth to weaning). The body mass of the pups (±0.01 g) was recorded at 8, 14 and 30 days of age.

### (iii) Oxytocin plasma levels of mothers

Blood sampling: approximately 200 µl of blood was sampled from the sublingual vein under 2% isoflurane anaesthesia before pairing, 8 days after parturition and at weaning. Plasma levels of oxytocin (i.e. an important maternal hormone [46,47]) were measured, using commercially available ELISA kits (Enzo Life Sciences, ADI-900-153A). This ELISA kit was formulated for the measurement of rat plasma. All the assays were validated with serial dilutions of hamster plasma showing linear changes in sample values that were parallel with standard curves produced according to the manufacturer's standards. All measurements were made in duplicate. The average intra-assay variation coefficient was 2.5%.

## (c) Second experiment: effects of vitamin B3 supplement on maternal investment in reproduction

In the second experiment (2015), the 14 females were fed ad libitum with maize at emergence from hibernation and supplemented with earthworms after mating. Before parturition, the supplement consisted of 5 g of earthworm per female per day. It was then increased by 1 g young$^{-1}$ d$^{-1}$ between parturition and weaning.

The vitamin B3 solution was prepared by dissolving 3 g of nicotinamide powder (greater than or equal to 99.5% (HPLC), 72340 Sigma) in 1 l of saline solution (NaCl, 9 g l$^{-1}$), leading to a concentration of 3 g l$^{-1}$. Earthworms were injected with 100 µl of vitamin B3 solution and immediately given to the females ('maize-worm-B3' group, $n = 7$). The non-supplemented females ('maize-worm' group, $n = 7$) were given earthworms injected with 100 µl of NaCl. Each female in the 'maize-worm-B3' group was thus supplemented with 0.3 mg of vitamin B3 per day, meeting the estimated levels in the wheat-earthworm diet from the first experiment. It was the increased by 0.05 mg per pup at parturition and until weaning.

Twice a day, we checked parturition and litter size. Females were weighed (±0.01 g) 1 day prior to pairing and at the end of the experiment (see §2b). Pups were weighed (± 0.01 g) at 8 and 30 days old.

## (d) Data analyses

Data presented are means ± s.e.m. Normality was tested, using a Kolmogorov–Smirnov test and variance homogeneity was checked using a Levene test. We first looked at the effect of the diet on the *average number of pups per female* at parturition and at weaning, including females that did not give birth. This variable was analysed using a GLM (probability distribution: Poisson, link function: log) with diet as a fixed factor. Data concerning the two reproductive attempts for each female were grouped in this first analysis and tested separately for 2014 (four diets) and 2015 (two diets, one supplemented with vitamin B3). We performed a Cox regression to check for an effect of the diet on the *early survival of the pups*. *The body mass of the pups* was analysed, using linear mixed models. The diet, the age, the sex of the pups and the interactions between these variables were included as fixed factors, and the litter size was included as a covariate in this LMM model. The identity of the pups nested in the identity of the litter was included as a random factor for repeated measurements on the same individuals and the same litter. Data on pups' body mass collected in 2014 and 2015 were analysed separately. Regarding mothers, *body mass and plasma levels of oxytocin* in 2014 were also analysed using an LMM. The diet, the period and the diet × period interaction were included as fixed factors, and the identity of the females as the random factor for repeated measurements on the same individual (for body mass analysis). Finally, the number of pups was included as a covariate. Multiple comparisons were analysed via *post hoc* least significant difference testing. Final model selection was based on the best Akaike information criterion for small samples value. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for WINDOWS v. 21.0. Armonk, NY: IBM Corp.), and the significance threshold was set at $p < 0.05$. Figures were prepared, using GRAPHPAD PRISM software (v. 5, La Jolla, CA, USA).

# 3. Results

## (a) Macronutrient content of the diets, average number of pups per female at parturition and early survival of the pups

Females ingested on average 14.5 ± 1.2 and 15.8 ± 4.8 g d$^{-1}$ of grains in the maize- and wheat-based diets, respectively. The whole 5 g of supplements (both of earthworm and clover) was entirely consumed each day. Both the earthworm diets, and both the clover diets, had similar macronutrient and energy contents (figure 1*a*), respectively. The wheat-worm diet was composed of 42% proteins, 43% carbohydrates and 15% lipids with a dry food energy content of 17.7 kJ g$^{-1}$, whereas the maize-worm diet was composed of 46% proteins, 40% carbohydrates and 14% lipids with a dry food energy content of 18.3 kJ g$^{-1}$. The wheat-clover diet was composed of 19% proteins, 69% carbohydrates and 12% lipids with a dry food energy content of 16.5 kJ g$^{-1}$, whereas the maize-clover diet was composed of 17% proteins, 71% carbohydrates and 12% lipids with a dry food energy content of 17.2 kJ g$^{-1}$.

We found no effect of the diet on the average number of pups per female at parturition (figure 1*b*; Wald $\chi^2 = 2.60$, $p = 0.46$). However, we found a strong effect of the diet on the early survival of the pups (Wald $\chi^2 = 43.77$, $p = 1.7 \times 10^{-9}$). Pups born to females fed the wheat-worm diet had a survival rate of about 80% at weaning, which was significantly higher than pups born to females fed the other diets (survival rate lower than 12%, figure 1*c*, $p = 7.3 \times 10^{-11}$). We found an effect of diet ($F_{3,23.6} = 21.4$, $p = 6.5 \times 10^{-7}$) and age (i.e. 8, 14 and 30 days, $F_{2,49.9} = 135.11$, $p = 7.2 \times 10^{-21}$) on the body mass of the surviving pups, as well as an effect of the age × diet interaction (electronic supplementary material, figure S1, $F_{6,47.4} = 13.2$, $p = 1 \times 10^{-8}$). *Post hoc* analyses indicated that at 8 days (electronic supplementary material, figure S1*a*), the body mass of the pups born to females fed the wheat-based diets was significantly higher than the body mass of the pups born to females fed both maize-based diets ($p < 0.009$). At 14 days (electronic supplementary material, figure S1*b*), the pups born to females fed the wheat-worm diet weighed

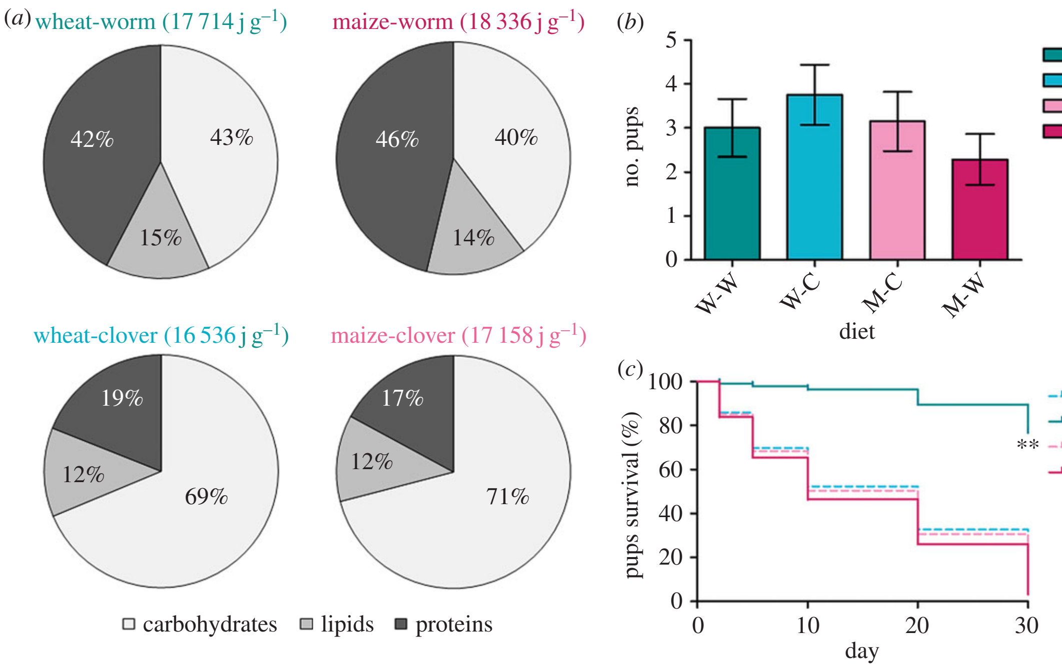

**Figure 1.** Gross energy and macronutrient content of the four diets, average number of pups per female at parturition and survival of the pups according to diet. (*a*) Protein, lipid and carbohydrate contents are represented as a percentage (%) of the energy content (in j $g^{-1}$ of DM). (*b*) Average number of pups per female at parturition (i.e. including females that did not gave birth) according to the diet and (*c*) early survival (%) of the pups (i.e. from birth to 30 days old = weaning) according to the diet. Solid lines correspond to diets supplemented with earthworms and the dashed lines correspond to diets supplemented with clover. (*b*,*c*) W-W, wheat-worm; W-C, wheat-clover, M-C, maize-clover and M-W, maize-worm. The asterisk indicates a significant difference in survival between the wheat-worm and the three other diets ($p = 0.003$). See methods section for details. (Online version in colour.)

significantly more than the pups born to females fed the three other diets ($p = 2.9 \times 10^{-5}$), and the pups born to females fed the wheat-clover diet weighed more than the pups born to females fed the maize-worm diet ($p = 0.037$), but did not significantly differ from the pups born to females fed the maize-clover diet ($p > 0.2$).

### (b) Maternal body mass and plasma levels of oxytocin

Concerning variations in body mass of the mothers during reproduction, we found an effect of period ($F_{2,64} = 26.98$, $p = 3.2 \times 10^{-9}$), diet ($F_{3,64} = 7.107$, $p = 3.4 \times 10^{-4}$) and the diet × period interaction ($F_{6,64} = 2.97$, $p = 0.013$). *Post hoc* analyses revealed that females fed the maize-worm diet lost significantly less body mass than females fed the three other diets (figure 2*a*, $p < 0.001$), whereas females fed the wheat-clover diet lost more body mass than females fed the wheat- and maize-worm diets (figure 2*a*, $p < 0.03$). We found no effect of the reproductive attempt (first or second) on the body mass of females ($p > 0.1$). We found no effects of the diet or of the period on maternal plasma levels of oxytocin ($p > 0.27$). However, we found an effect of the diet × period interaction ($F_{5,47} = 2.99$, $p = 0.020$) and *post hoc* analyses indicated that, 8 days post-parturition, females fed the wheat-worm and the maize-worm diets had significantly higher oxytocin plasma levels than females fed the wheat-clover diets ($p = 0.014$ and $p = 0.044$, respectively), whereas females fed the maize-clover diet were between the two (figure 2*b*).

### (c) Average number of pups per female at weaning, pups' body mass and effects of the vitamin B3 supplement

In the first experiment (2014) and as a consequence of the differences in pups' survival rates between the diets, the number of pups at weaning was significantly higher in the wheat-worm diet than in the three other diets (figure 3*a*, 2014, Wald $\chi^2 = 17.37$, $p < 0.001$). The body mass of the pups at weaning was also significantly higher in pups born to females fed the wheat-worm diet than in pups born to females fed the three other diets (figure 3*b*, 2014, $F = 15.16$, $p = 2 \times 10^{-5}$). In the second experiment (2015), the vitamin B3 supplement significantly increased the average number of pups per female in the group fed the maize-worm-B3 diet compared with the group fed the maize-worm diet (figure 3*a*, 2015, Wald $\chi^2 = 10.94$, $p < 0.001$). Vitamin B3 supplementation also significantly affected pups body mass at weaning ($F = 15.157$, $p < 0.001$): pups in the group with the vitamin B3 supplement were heavier than pups in the group with the maize-worm diet (figure 3*b*, 2015, $F = 10.795$, $p = 0.041$).

## 4. Discussion

Contrary to our original expectations, maize caused reproductive failure in captive European hamsters. Females were not affected in their capacity to give birth, but in their ability to wean their litters. Indeed, the pups' survival was severely reduced in both groups fed the maize-based diets, caused by litter suppression the first day after parturition (in 95% of the cases). In the group fed the wheat-based diets, significant reductions in litter size were observed in the group fed the wheat-clover diet but not in the group fed the wheat-worm diet, where the survival rate of the pups at weaning was 80%. When comparing the wheat-worm and maize-worm diets—similar in macronutrient and energy content—it appeared that the litter suppressions observed in the maize-worm diet could not be explained by looking at these factors. In the second experiment, we demonstrated that adding a vitamin B3 supplement restored reproductive success similar

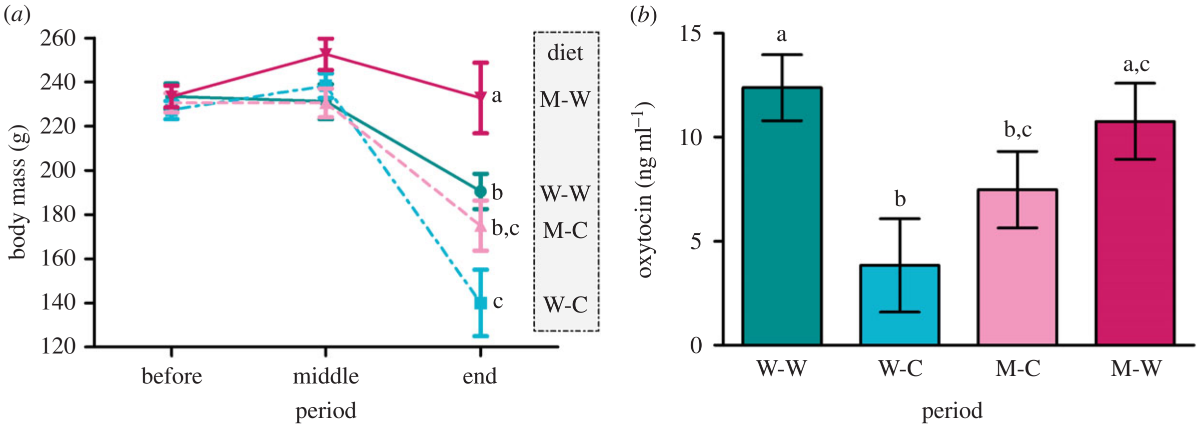

**Figure 2.** Variations in body mass and oxytocin plasma levels of mothers. (*a*) Changes in body mass during the first experiment according to the diet: the three periods 'before', 'middle' and 'end' correspond respectively to 1 day prior to pairing, 8 days after parturition and 30 days after parturition (or 30 days after the second reproductive attempt for females that did not give birth). The solid lines represent diets supplemented with earthworms and the dashed lines represent diets supplemented with clover. (*b*) Oxytocin plasma levels of mothers 8 days post-parturition, according to the diet. Different letters indicate significant differences between the diets ($p < 0.05$). (Online version in colour.)

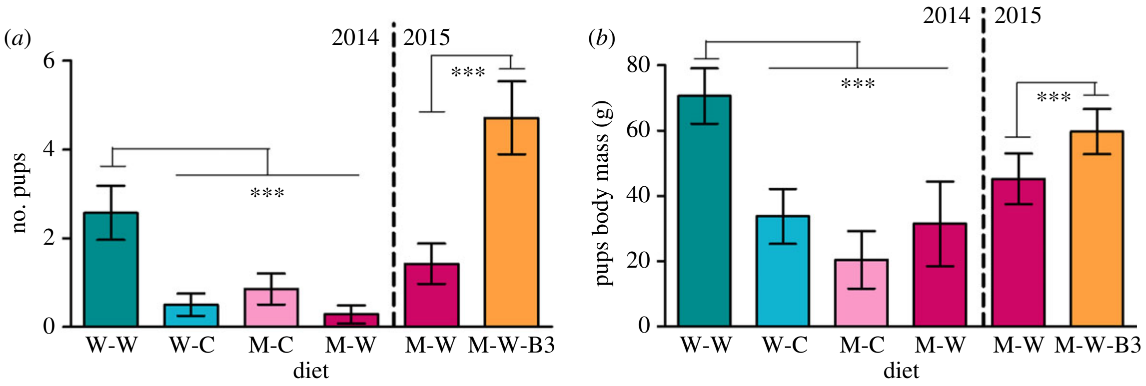

**Figure 3.** Effects of the diet and the vitamin B3 supplement on the number of pups and on pups' phenotype. Average number of pups per female (*a*) and body mass of the pups (*b*) at weaning depending on the diet. Data represent the first experiment (2014) and the vitamin-B3-supplement experiment (2015). W-C, wheat-clover; W-W, wheat-worm; M-C, maize-clover, M-W, maize-worm and M-W-B3, maize-worm + vitamin B3 supplementation. Stars indicate significant differences between the diets ($p < 0.001$). See methods section for details. (Online version in colour.)

to that recorded for the group fed the wheat-worm diet in the first experiment.

## (a) Macronutrient and energy content of the diets and maternal investment in reproduction

The two clover-supplemented diets had lower protein and energy content than the two earthworm-supplemented diets. Lack of proteins or energy could explain the low survival rates of pups observed in the group fed the clover-supplemented diets [36,37]. This hypothesis was supported by the fact that the oxytocin plasma levels of females fed the maize- and wheat-clover diets were lower at 8 days post-parturition, which could explain the abandonment of their litters [46,47]. Moreover, females fed on these two diets also displayed the highest body mass loss throughout reproduction. However, despite similar results in terms of body mass loss and oxytocin plasma levels between females of these two diets, we observed discrepancies in maternal behaviours. Females fed the wheat-clover diet gave birth in the nest and remained with their pups during the first 7 days. We then observed a gradual reduction in the litter, to 3, 2 and then 1 or ultimately zero pups and the pups' growth gradually decreased from 7 to 30 days. In contrast, most of the mothers in the group fed the maize-clover diet did not display maternal behaviour: they did not gave birth in the nest (pups were spread out in the cage) and then placed their pups on top of their hoard of maize grains before eating them. This litter suppression always occurred on the first day after parturition. Only one female weaned a litter (in her second reproductive attempt), composed of four pups. In this litter, two cases of siblicides were observed at the age of 34 days (the two males cannibalized their female siblings while they were still alive), echoing the dementia found in humans fed on maize [48,49].

Regarding the maize-worm and wheat-worm diets, we found they had very similar macronutrient and energy contents. However, the pups' survival rate and body mass were respectively 75% and 45% lower in the group fed the maize-worm diet compared with the group fed the wheat-worm diet. The extremely low survival rate of the pups in the group fed the maize-worm diet was associated with the same 'abnormal' behaviours of mothers as in the group fed the maize-clover diet (see above). These observations suggest a suppression of maternal behaviours and association of the pups with food items. Only one female weaned a litter (of two pups) on the maize-worm diet (also during her second reproductive attempt). However, females on the maize-worm diet—which displayed suppression of maternal behaviours—lost significantly less body mass than females fed the three other diets. Surprisingly, this lower investment in reproduction was not associated with reduced oxytocin plasma levels 8 days post-parturition in females fed the maize-worm diet, as we might have expected, given the high rate of infanticide [50]. This suggests that the modifications in maternal behaviour in maize-fed females are not a consequence of reduced maternal hormones but rather of a modification of the neural system, inducing dementia-like behaviours like that recorded in humans feeding intensively on maize [48].

## (b) Vitamin B3/tryptophan deficiencies in maize, related pathologies in animals, including humans and effects of the supplementation

Maize is known to be lacking of several micronutrients: calcium, tryptophan (trp), lysine, riboflavin and bioavailable vitamin B3 (i.e. niacin, nicotinamide or vitamin PP) [51,52]. Most of these micronutrients were expected to be complemented by clover or earthworms, which are rich in minerals [35] but contain low levels of trp and vitamin B3 [35,53]. Daily supplementation with 0.3 mg of vitamin B3 in females fed the maize-worm diet restored reproductive success, with an 85% increase in the number of weaned pups compared with the group fed the maize-worm diet. This highlights the fundamental role of vitamin B3 in European hamsters' reproductive success.

Deficiencies in trp and vitamin B3 have been linked to growth retardation in rats [42], the 'black-tongue' syndrome in dogs [54] and pellagra in humans (i.e. three-Ds disease: diarrhoea, dementia and dermatitis) [48,49]. Trp is an essential amino acid, precursor of vitamin B3 (i.e. nicotinamide, involved in the synthesis of NAD and NADP, crucial for cell functioning [49]) and serotonin (involved in aggressiveness and depression [55]). The only way for animals to access trp and vitamin B3 is through their diet, and while trp levels in maize are particularly low [52], vitamin B3 is present in a tightly bound form, not bioavailable for animals [52]. Tryptophan metabolism *in vivo* is extremely complex [49,55] and therefore, the detrimental effects of a predominance of maize in the diet are extremely difficult to counteract with other food items [55]. This is why improperly cooked [56,57] maize-based diets have been associated with higher rates of homicide, suicide and cannibalism in humans [55,58] and have caused pellagra [48,49], which decimated three million people in North America and Europe from 1735 to 1940. The high propensity of maize in our experimental diets caused 'abnormal' maternal behaviour (pups stored with maize stores), infanticide and siblicide associated with diarrhoea and skin/fur problems in pups; these symptoms resemble those found in humans affected by pellagra [48] (see electronic supplementary material, table S1 for details and frequencies of the observed symptoms in our experiment). Although there is a vast—though ancient—literature on the effects of maize on human, livestock or rats [42,55,56,59–62], this is the first study revealing such a strong negative effect (i.e. 95% reduction in reproductive success) of maize-based diets and vitamin B3 deficiency on such an important fitness-related life-history trait as reproduction.

## (c) Maize, vitamin deficiencies and farmland wildlife

Only a few studies have investigated how maize monoculture could influence the diet of wildlife or how vitamin deficiencies could harm farmland animals [9,20,63]. Black bears living in agricultural landscapes consume corn (i.e. maize), sunflower and oats but females with cubs appear to exhibit a risk-aversion towards these crops and rather feed on other food items during reproductive periods [20]. Regarding invertebrates, wild bees are known to be threatened by monotonous diets imposed by mass-flowering crops [16] and some species rely intensively on maize pollen [64]. Given that proper trp and vitamin B3 dietary intakes appear to be crucial for bees [65,66] and that both pollen quality and diversity influence their longevity, physiology and resistance/tolerance to disease [16], we argue that maize monoculture is probably strongly impairing the fitness of these endangered pollinators because of nutritional deficiencies. However, data are still lacking and experimental studies are needed to confirm this hypothesis. Finally, regarding the European hamster, given that wild populations of this species are surrounded by 55–80% of intensively managed maize monoculture in Alsace (France), with sized field of 1.4 ha that corresponds to seven times the home range of a female, extremely low crop rotations (i.e. sometimes more than seven successive years of maize cultivated in the same plot) and high use of herbicides—dramatically reducing the proportion of adventive species—wild hamsters are undoubtedly constrained in their diet. Indeed, a hamster would need to ingest 22–45 g of weeds to obtain 0.3 mg of vitamin B3 per day (see electronic supplementary material, table S2 for details of the calculation). These numbers equal or surpass the daily food intake of female hamsters, which was of 14 ± 5 grs $d^{-1}$ in our experiment.

In a previous study [24], we revealed the detrimental effects of the intensification of maize monoculture on the body mass of wild hamsters in France, which has decreased by 21% since 1937. We reported that years with high maize production and high acreage allocated to this cereal led to lower hamsters' body mass upon emergence from hibernation. We were not able to conclude on whether this negative effect was caused by monoculture (i.e. a lack of food diversity/availability or a particular microclimate) or by maize itself (i.e. its composition), but we hypothesized that maize was not an adequate food resource for this species. In this study, we found that maize is nutritionally inadequate for this species during reproduction. However, regarding the maize-worm diet, the slight differences in pups' body mass and reproductive success between 2014 and 2015 is explained by two females having successfully raised a litter of four and six pups, respectively, in 2015. This is likely revealing an interindividual difference of sensitivity and symptom expression to the trp and vitamin B3 deficiencies, previously recorded in humans [58,61], and potentially reflecting differences of individual quality [67].

Taken together, our results therefore strongly suggest that an over-abundance of maize in the diet of the European hamster could be particularly detrimental for reproduction in the wild and support the hypothesis of a decline in the reproductive success of wild hamsters in France [68]. The average number of pups per female (2.4 ± 1.2 pups per litter) and number of litters per female (0.93 litter $\text{female}^{-1}$) in our experiment echoes data recorded in the wild in France in 2014 (2.5 ± 1.4 pups per litter and 0.76 litter female$^{-1}$ [69]). Knowing that we observed a 94% decrease in French populations of the species in recent decades [70], it is now crucial to determine how the fitness of wild hamsters could be affected by maize monoculture, and whether it is possible to improve their reproductive success through better management of agriculture and the inclusion of feeding patches.

## 5. Conclusion

Here we demonstrated that—independently of macronutrient and energy content—a vitamin B3 deficiency in maize grains is responsible for reproductive failure and abnormal maternal behaviour in captive-fed European hamsters. More eco-physiological studies are needed to better understand how monoculture can influence the fitness of farmland species by modifying their diet, and particularly how vitamin deficiencies can affect the reproduction, longevity, and hence the evolution of farmland wildlife. However, given the intensification of maize monoculture across the globe—associated with a reduction in both the diversity and the abundance of other plants, soil fauna and microbial communities—we argue that an over-abundance of maize compared with other food items in the diet of farmland animals could be particularly detrimental for their fitness. Knowing that these species already face many threats and that most of them are in danger of extinction, it is urgent to restore a diverse range of plants in agricultural schemes, to ensure that farmland animals have access to a more diversified diet.

Ethics. The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and was approved by the Ethical Committee (CREMEAS) under agreement no. 00624-01.

Authors' contributions. M.L.T. and C.H. conceived the study and M.L.T., C.H. and Y.H. designed the study. M.L.T. collected the data with the help of O.D. in 2014, performed data analyses and wrote the first draft of the manuscript. C.H. made the first corrections and Y.H., O.D. and J.P.R. contributed substantially to revisions.

Competing interests. The authors declare no competing interests.

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## References

1. Foley JA *et al.* 2005 Global consequences of land use. *Science* **309**, 570–574. (doi:10.1126/science.1111772)
2. Fargione JE *et al.* 2009 Bioenergy and wildlife: threats and opportunities for grassland conservation. *Bioscience* **59**, 767–777. (doi:10.1525/bio.2009.59.9.8)
3. Williams N. 2007 Questions on biofuels. *Curr. Biol.* **17**, 617. (doi:10.1016/j.cub.2007.07.054)
4. Kayser A, Weinhold U, Stubbe M. 2003 Mortality factors of the common hamster *Cricetus cricetus* at two sites in Germany. *Acta Theriol.* (*Warsz*). **48**, 47–57. (doi:10.1007/BF03194265)
5. Villemey A, Besnard A, Grandadam J, Eidenschenck J. 2013 Testing restocking methods for an endangered species: effects of predator exclusion and vegetation cover on common hamster (*Cricetus cricetus*) survival and reproduction. *Biol. Conserv*. **158**, 147–154. (doi:10.1016/j.biocon.2012.08.007)
6. Jacob J. 2003 Short-term effects of farming practices on populations of common voles. *Agric. Ecosyst. Environ*. **95**, 321–325. (doi:10.1016/S0167-8809(02)00084-1)
7. Rands MRW, Sotherton NW. 1986 Pesticide use on cereal crops and changes in the abundance of butterflies on arable farmland in England. *Biol. Conserv*. **36**, 71–82. (doi:10.1016/0006-3207(86)90102-3)
8. Wolansky MJ, Harrill JA. 2008 Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. *Neurotoxicol. Teratol*. **30**, 55–78. (doi:10.1016/j.ntt.2007.10.005)
9. Gill RJ, Raine NE. 2014 Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Funct. Ecol*. **28**, 1459–1471. (doi:10.1111/1365-2435.12292)
10. Björklund J, Limburg KE, Rydberg T. 1999 Impact of production intensity on the ability of the agricultural landscape to generate ecosystem services: an example from Sweden. *Ecol. Econ*. **29**, 269–291. (doi:10.1016/S0921-8009(99)00014-2)
11. Allan E *et al.* 2015 Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecol. Lett*. **18**, 834–843. (doi:10.1111/ele.12469)
12. Díaz S, Fargione J, Chapin FS, Tilman D. 2006 Biodiversity loss threatens human well-being. *PLoS Biol*. **4**, 1300–1305. (doi:10.1371/journal.pbio.0040277)
13. Chaplin-Kramer R, Dombeck E, Gerber J, Knuth KA, Mueller ND, Mueller M, Ziv G, Klein A-M. 2014 Global malnutrition overlaps with pollinator-dependent micronutrient production. *Proc. R. Soc. B* **281**, 20141799. (doi:10.1098/rspb.2014.1799)
14. Tsiafouli MA *et al.* 2015 Intensive agriculture reduces soil biodiversity across Europe. *Glob. Change Biol*. **21**, 973–985. (doi:10.1111/gcb.12752)
15. Altieri MA. 1999 The ecological role of biodiversity in agroecosystems. *Agric. Ecosyst. Environ*. **74**, 19–31. (doi:10.1016/S0167-8809(99)00028-6)
16. Goulson D, Nicholls E, Botías C, Rotheray EL. 2015 Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**, 1–16. (doi:10.1126/science.1255957)
17. Medan D, Torretta JP, Hodara K, de la Fuente EB, Montaldo NH. 2011 Effects of agriculture expansion and intensification on the vertebrate and invertebrate diversity in the Pampas of Argentina. *Biodivers. Conserv*. **20**, 3077–3100. (doi:10.1007/s10531-011-0118-9)
18. Butler SJ, Vickery JA, Norris K. 2007 Farmland biodiversity and the footprint of agriculture. *Science*. **315**, 825–828. (doi:10.1126/science.1136607)
19. Lebeau J, Wesselingh RA, Van Dyck H. 2016 Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proc. R. Soc. B* **278**, 3465–3473. (doi:10.1098/rspb.2016.0455)
20. Ditmer MA, Garshelis DL, Noyce KV, Laske TG, Iaizzo PA, Burk TE, Forester JD, Fieberg JR. 2015 Behavioral and physiological responses of American black bears to landscape features within an agricultural region. *Ecosphere* **6**, part28. (doi:10.1890/ES14-00199.1)
21. Monecke S. 2013 All things considered? Alternative reasons for hamster extinction. *Zool. Pol*. **58**, 41–57. (doi:10.2478/zoop-2013-0004)
22. O'Brien J. 2015 Saving the common hamster (*Cricetus cricetus*) from extinction in Alsace (France): potential flagship conservation or an exercise in futility? *Hystrix, Ital. J. Mammal*. **26**, 89–94. (doi:10.4404/hystrix-26.2-11230)
23. Franceschini-Zink C, Millesi E. 2008 Population development and life expectancy in common hamsters. In *The common hamster: perspectives on an endangered species* (eds E Millesi, H Winkler, R Hengsberger), pp. 45–59. Vienna, Austria: Austrian Academy of Sciences Press.
24. Tissier ML, Handrich Y, Robin J-P, Weitten M, Pevet P, Kourkgy C, Habold C. 2016 How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the

verge of extinction. *Sci. Rep*. **6**, 25531. (doi:10.1038/srep25531)

25. Humphries MM, Thomas DW, Kramer DL. 2003 The role of energy availability in mammalian hibernation: a cost–benefit approach. *Physiol. Biochem. Zool*. **76**, 165–179. (doi:10.1086/367950)
26. Nechay G, Hamar M, Grulich I. 1977 The common hamster (*Cricetus cricetus* [L.]); a review. *EPPO Bull*. **7**, 255–276. (doi:10.1111/j.1365-2338.1977.tb02727.x)
27. Wall SB. 1990 *Food hoarding in animals*. Chicago, IL: University of Chicago Press.
28. Munro D, Thomas DW, Humphries MM. 2008 Extreme suppression of aboveground activity by a food-storing hibernator, the eastern chipmunk (*Tamias striatus*). *Can. J. Zool*. **86**, 364–370. (doi:10.1139/Z08-008)
29. Hufnagl S, Siutz C, Millesi E. 2010 Diet composition of common hamsters (*Cricetus cricetus*) living in an urban environment. *Säugetierkundl. Inf*. **7**, 57–66.
30. Franceschini C, Millesi E. 2005 Reproductive timing and success in common hamsters. In *Int. Hamsterworkgroup, Strasbourg*, pp. 63–66.
31. Hufnagl S, Franceschini-Zink C, Millesi E. 2011 Seasonal constraints and reproductive performance in female common hamsters (*Cricetus cricetus*). *Mamm. Biol. - Zeitschrift für Säugetierkd*. **76**, 124–128. (doi:10.1016/j.mambio.2010.07.004)
32. Franceschini-Zink C, Millesi E. 2008 Reproductive performance in female common hamsters. *Zoology* **111**, 76–83. (doi:10.1016/j.zool.2007.05.001)
33. Gorecki A, Grygielska M. 1975 Consumption and utilization of natural foods by the common hamster. *Acta Theriol*. (*Warsz*). **20**, 237–246. (doi:10.4098/AT.arch.75-20)
34. Nechay G. 2000 *Status of hamsters Cricetus cricetus, Cricetus migratorius, Mesocricetus newtoni, and other hamster species in Europe*, vol. 106. Strasbourg, France: Council of Europe.
35. AFZ, INRA, CIRAD, FAO. Feedipedia, Animal Feed Resources Information System. 2011 http://www.feedipedia.org/. (accessed 5 September 2016)
36. Speakman JR. 2008 The physiological costs of reproduction in small mammals. *Phil. Trans. R. Soc. B* **363**, 375–398. (doi:10.1098/rstb.2007.2145)
37. Koskela E, Jonsson P, Hartikainen T, Mappes T. 1998 Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. Lond. B* **265**, 1129–1134. (doi:10.1098/rspb.1998.0408)
38. Skibiel AL, Hood WR. 2015 Milk matters: offspring survival in Columbian ground squirrels is affected by nutrient composition of mother's milk. *Front. Ecol. Evol*. **3**, 1–10. (doi:10.3389/fevo.2015.00111)
39. Schneider J, Wade G. 1989 Effects of maternal diet, body weight and body composition on infanticide in Syrian hamsters. *Physiol. Behav*. **46**, 815–821. (doi:10.1016/0031-9384(89)90042-5)
40. Criscuolo F, Monaghan P, Nasir L, Metcalfe NB. 2008 Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proc. R. Soc. B* **275**, 1565–1570. (doi:10.1098/rspb.2008.0148)
41. Zambrano E, Rodríguez-González GL, Guzmán C, García-Becerra R, Boeck L, Díaz L, Menjivar M, Larrea F, Nathanielsz PW. 2005 A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *J. Physiol*. **563**, 275–284. (doi:10.1113/jphysiol.2004.078543)
42. Krehl WA, Teply LJ, Sarma PS, Elvehjem CA. 1945 Growth-retarding effect of corn in nicotinic acid-low rations and its counteraction by tryptophane. *Science* **101**, 489–490. (doi:10.1126/science.101.2628.489)
43. Kjeldahl J. 1883 A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem*. **22**, 366–382. (doi:10.1007/BF01338151)
44. Campbell RR, Leatherland JF. 1980 Estimating body protein and fat from water content in lesser snow geese. *J. Wildl. Manage*. **44**, 438. (doi:10.2307/3807975)
45. Folch J, Lees M, Sloane Stanley GH. 1957 A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem* **226**, 497–509.
46. McCarthy MM. 1990 Oxytocin inhibits infanticide in female house mice (*Mus domesticus*). *Horm. Behav*. **24**, 365–375. (doi:10.1016/0018-506X(90)90015-P)
47. Witt DM, Carter CS, Walton DM. 1990 Central and peripheral effects of oxytocin administration in prairie voles (*Microtus ochrogaster*). *Pharmacol. Biochem. Behav*. **37**, 63–69. (doi:10.1016/0091-3057(90)90042-G)
48. Hegyi J, Schwartz RA, Hegyi V. 2004 Pellagra: dermatitis, dementia, and diarrhea. *Int. J. Dermatol*. **43**, 1–5. (doi:10.1111/j.1365-4632.2004.01959.x)
49. Wan P, Moat S, Anstey A. 2011 Pellagra: a review with emphasis on photosensitivity. *Br. J. Dermatol*. **164**, 1188–1200. (doi:10.1111/j.1365-2133.2010.10163.x)
50. Harmon AC, Huhman KL, Moore TO, Albers HE. 2002 Oxytocin inhibits aggression in female Syrian hamsters. *J. Neuroendocrinol*. **14**, 963–969. (doi:10.1046/j.1365-2826.2002.00863.x)
51. Nuss ET, Tanumihardjo SA. 2010 Maize: a paramount staple crop in the context of global nutrition. *Compr. Rev. Food Sci. Food Saf*. **9**, 417–436. (doi:10.1111/j.1541-4337.2010.00117.x)
52. Burkholder PR, Mc Veigh I, Moyer D. 1944 Niacin in maize. *Yale J. Biol. Med*. **16**, 659–663.
53. Warne MA, Lenz EM, Osborn D, Weeks JM, Nicholson JK. 2001 Comparative biochemistry and short-term starvation effects on the earthworms *Eisenia veneta* and *Lumbricus terrestris* studied by $^1$H NMR spectroscopy and pattern recognition. *Soil Biol*. **33**, 1171–1180. (doi:10.1016/S0038-0717(01)00021-9)
54. Ammerman CB, Baker DH, Lewis AJ. 1995 *Bioavailability of nutrients for animals: amino acids, minerals and vitamins*. London, UK: Academic Press Limited.
55. Ernandes M, Guardia M, Giammanco S. 1996 Maize based diets and possible neurobehavioural after-effects among some populations in the world. *Hum. Evol*. **11**, 67–77. (doi:10.1007/BF02456990)
56. Katz SH, Hediger ML, Valleroy LA. 1974 Traditional maize processing techniques in the new world. *Science*. **184**, 765–773. (doi:10.1126/science.184.4138.765)
57. Sefa-Dedeh S, Cornelius B, Sakyi-Dawson E, Afoakwa EO. 2004 Effect of nixtamalization on the chemical and functional properties of maize. *Food Chem*. **86**, 317–324. (doi:10.1016/j.foodchem.2003.08.033)
58. Mawson AR, Jacobs KW. 1978 Corn consumption, tryptophan, and cross-national homicide rates. *J. Orthomol. Psychiatry* **7**, 227–230.
59. Carter EGA, Carpenter AJ. 1982 The available niacin values of foods for rats and their relation to analytical values. *J. Nutr*. **112**, 2091–2103.
60. Carpenter KJ, Schelstraete M, Wall S, Vilicch VC. 1988 Immature corn as a source of niacin for rats. *J. Nutr*. **118**, 165–169.
61. Goldsmith GA, Sarett HP, Register UD, Gibbens J. 1952 Studies of niacin requirement in man. I. Experimental pellagra in subjects on corn diets low in niacin and tryptophan. *J. Clin. Invest*. **31**, 533–542. (doi:10.1172/JCI102638)
62. Hundley JM. 1947 Production of niacin deficiency in rats. *J. Nutr*. **34**, 253–262.
63. Vanderplanck M, Moerman R, Rasmont P, Lognay G, Wathelet B, Wattiez R, Michez D. 2014 How does pollen chemistry impact development and feeding behaviour of polylectic bees? *PLoS ONE* **9**, e86209. (doi:10.1371/journal.pone.0086209)
64. Danner N, Härtel S, Steffan-Dewenter I. 2014 Maize pollen foraging by honey bees in relation to crop area and landscape context. *Basic Appl. Ecol*. **15**, 677–684. (doi:10.1016/j.baae.2014.08.010)
65. de Arruda VAS, Pereira AAS, de Freitas AS, Barth OM, de Almeida-Muradian LB. 2013 Dried bee pollen: B complex vitamins, physicochemical and botanical composition. *J. Food Compos. Anal*. **29**, 100–105. (doi:10.1016/j.jfca.2012.11.004)
66. Fengkui Z, Baohua X, Ge Z, Hongfang W. 2015 The appropriate supplementary level of tryptophan in the diet of *Apis mellifera* (Hymenoptera: Apidae) worker bees. *J. Insect Sci*. **15**, 161. (doi:10.1093/jisesa/iev142)
67. Hamel S, Côté SD, Gaillard JM, Festa-Bianchet M. 2009 Individual variation in reproductive costs of reproduction: high-quality females always do better. *J. Anim. Ecol*. **78**, 143–151. (doi:10.1111/j.1365-2656.2007.0)
68. Surov A, Banaszek A, Bogomolov P, Feoktistova N, Monecke S. 2016 Dramatic global decrease in the range and reproduction rate of the European hamster *Cricetus cricetus*. *Endanger. Species Res*. **31**, 119–145. (doi:10.3354/esr00749)
69. Kourkgy C, Eidenschenck J. 2015 Délivrable Action D1. Rapport annuel de présentation des données collectées et premières analyses, année 2014.
70. Reiners TE, Eidenschenk J, Neumann K, Nowak C. 2014 Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the common hamster of the French Alsace region. *Mamm. Biol*. **79**, 240–246. (doi:10.1016/j.mambio.2013.10.004)

**Electronic Supplementary Material –** MS: 'Diets derived from maize monoculture cause high rates of infanticide in the endangered European hamster due to a vitamin B3 deficiency' by Mathilde Tissier, Yves Handrich, Odeline Dallongeville, Jean-Patrice Robin and Caroline Habold – *Proceedings of the Royal Society B*

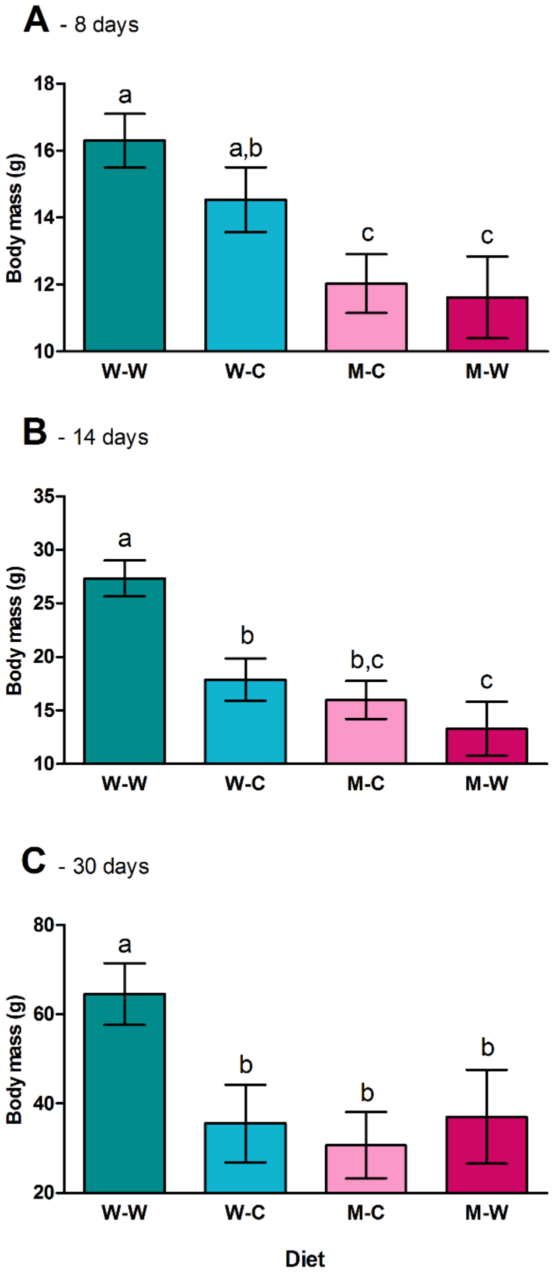

**Figure S1: Pups body mass at 8 (A), 14 (B) and 30 (C) days according to the diet**. Data are represented for the first experiment (2014). Different letters highlight significant differences between the diets ($p<0.037$). W-W=Wheat-Worm, W-C=Wheat-Clover, M-C=Maize-Clover and M-W=Maize-Worm.

| Age-class | Symptoms | Details | Frequency |
|---|---|---|---|
| Adults (mothers, N=14) | Hyperactivity | Females were running in circle in the cage, climbing and pounding their feeders when we entered in the room. | 100% |
| | Infanticide | Females stored their pups with their hoards of maize before eating them. Pups were still alive at that time. | 95% |
| | ' black tongue' | We noticed that females had swollen and dark tongue when we carried out the blood sampling; their blood was thick and difficult to sample. | 70% |
| Pups (surviving pups, N=6) | Diarhea | | 100% |
| | Growth retards | See results section of the manuscript | 50% |
| | Skin and fur problems | Pups displayed hairs deficiency and rash problems (looking like eczema), mostly on the back, until the age of 20 days. The hairs then started to grow, but at weaning they still displayed lower density of hairs than pups supplemented with the vitamin B3 (see Figure S2). | 50% |
| | Cannibalism | Two male pups cannibalized their female siblings while steel alive. | 30% |
| | Dental growth problem | One female pups had extreamly long and twisted teeth. | 16,6% (1 of 6) |

**Table S1: symptoms observed in mothers and pups of the maize-based diets**. Data are represented for the first experiment (2014) only and include individuals from both the maize-clover and maize-worm diets.

**Figure S2**: **Ventral fur of two pups at weaning (*i.e.* 30 days), from the maize-worm diet and from the maize-worm-B3 diet**.

| Plant species | Amount of free vitamin B3 (mg/g of plant DM) | Quantity (g) to ingest for a 0.3 mg vitamin B3 daily intake |
|---|---|---|
| Dandelion (1) | 0.4 mg / 55 g | 41,25 |
| Poppy (1) | 0.1 mg / 9 g | 22,5 |
| Pigweed (2) | 6.72 / 100 g | 44,78 |

**Table S2: Weeds commonly found in maize fields (3), amount of free vitamin B3 they contain and dry mass of these plants for hamster to ingest to reach a 0.3 mg vitamin B3 daily intake**. The calculation has been done using literature and internet sources ([1-3], see below).

## References

1. SR-21 U (2016) SelfNutrition Data. Available at: http://nutritiondata.self.com/ [Accessed June 10, 2016]
2. J.A. D, Ayensu ES *Medicinal Plants of China* Available at: http://www.pfaf.org/user/plant.aspx?LatinName=Portulaca+oleracea [Accessed September 10, 2016].
3. Karim SMR, Ismail AA Weeds as plant genetic resources for production of nutritious supplementary food in agriculture 1. (May 2012):1–16. [Accessed September 10, 2016]

# Study 3 - organic monoculture crops impair the reproductive success of the hamster in semi-wild enclosures

*Under review* [1]

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[1] Tissier ML, Kletty F, Handrich Y, Habold C. Experiments in outside enclosures highlight that organically-grown monoculture crops reduce invertebrate and plant species richness and impair the fitness of a critically endangered farmland rodent – *Under review in Oecologia*

# Experiments in outside enclosures highlight that organically-grown monoculture crops reduce invertebrate and plant species richness and impair the fitness of a critically endangered farmland rodent.

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Author contributions: MLT and CH designed the study. MLT and FK collected the data and FK performed invertebrate and plant species identification. MLT carried out statistical analyses and wrote the manuscript. YH, FK and CH contributed significantly to manuscript revisions and gave final approval for publication.

## Abstract

Intensive cereal monoculture is currently the main cause of biodiversity decline in Europe. However, it is difficult to disentangle the effects of intensive monoculture (e.g. pesticides use, mechanical ploughing and reduced protective cover), let alone evaluate how far the reduction of crop diversity affects biodiversity. Therefore, it remains unclear to which extent farmland biodiversity, and especially vertebrates, are affected by a reduction of their food resources. We therefore designed this study in semi-wild enclosures to investigate the effects of organic monoculture crops (wheat or corn) and mixed organic crops (a combination of wheat, corn, sunflower and alfalfa) on (1) adventive plant and invertebrate species richness and (2) on the reproductive success of the European hamster (*Cricetus cricetus*), a critically endangered umbrella species of European farmlands. We found that plant and invertebrate species richness were respectively 38% and 28% lower in monoculture compared to mixed crop enclosures. In addition, hamsters' effective reproductive success was reduced by 82% in monoculture enclosures. These results highlight that even before taking the use of pesticide and mechanization into account, monoculture *per se* can be detrimental for biodiversity. Furthermore, this study pinpoints how it strongly reduces the fitness of a critically endangered farmland rodent. We believe that future research should promote the integration of how food reduction in agroecosystems affect farmland wildlife. Moreover, given the conservation status of the European hamster, and indeed the majority of farmland animals in Europe, we argue that conservation plans must focus on restoring plant and invertebrate diversity on farmland.

**Keywords**: biodiversity, nutritional deficiencies, reproduction, agriculture, conservation

## Introduction

Agriculture intensification has been synonymous to a rise in the use of fertilizers, pesticides, irrigation and deep ploughing (Foley et al., 2005; Wilson, Whittingham, & Bradbury, 2005) and has also entailed the generalization of intensive monoculture, leading to extended areas of homogenized and simplified agro-ecosystems dominated by one or two crops (Benton, Vickery, & Wilson, 2003; Goulson, Nicholls, Botías, & Rotheray, 2015; Wilson et al., 2005). The detrimental effects of agricultural intensification on the collapse of bird and pollinator populations have been well documented (Björklund, Limburg, & Rydberg, 1999; Rands & Sotherton, 1986; Rioux Paquette, Pelletier, Garant, & Belisle, 2014), and many studies have revealed the negative effects of pesticides, fertilizers or mechanical ploughing on the decrease in adventive species, soil fauna and microbial communities (Altieri, 1999; Björklund et al., 1999; Changguo, Pingjiu, Genxing, Duosheng, & Qiuhua, 2006; Tsiafouli et al., 2015). However, given the difficulty involved in disentangling the effects of low crop diversity from the direct and indirect effects of pesticide use and agricultural ploughing on adventive plants and wildlife (Benton et al., 2003), data describing the effects of monoculture crops *per se* (i.e. the decrease in crop diversity) on vertebrate species are still scarce. An increasing number of studies show that invertebrate species, such as pollinators, are strongly affected by a lack of food in crop monocultures (Dance, Botias, & Goulson, 2017; Goulson et al., 2015; Wood, Holland, & Goulson, 2016). Nonetheless, it remains unclear how far decreased crop diversity *per se* – without considering the effects of fertilizer, pesticides or ploughing – could impair the fitness of wildlife and especially vertebrates living in agro-ecosystems. In this study, we investigate the effects of wheat and corn monoculture crops on the fitness of the European hamster (*Cricetus cricetus*), a critically endangered species throughout the continent (O'Brien, 2015; Weinhold, 2008). We also investigate how these monoculture crops affect adventive plant and invertebrate species richness that are part of hamsters' diet. Although several causes have been suggested (Monecke, 2013; Surov, Banaszek, Bogomolov, Feoktistova, & Monecke, 2016), intensive agriculture is considered to be the main reason for the decline of this omnivorous farmland rodent (M. J. J. La Haye, Swinnen, Kuiters, Leirs, & Siepel, 2014; M. La Haye, Müskens, Van Kats, Kuiters, & Siepel, 2010; O'Brien, 2015; Tissier et al., 2016; Villemey, Besnard, Grandadam, & Eidenschenck, 2013; Weinhold, 2008). First, intensive crop monoculture lead to increased predation pressure caused by a reduction of the protective cover (Villemey et al., 2013; Weinhold, 2008). Secondly, the intensification of Corn monoculture has negatively affected wild hamsters body mass during the last century in France, therefore likely impairing reproduction (Tissier et al., 2016). Finally, Corn-based diets have been shown to strongly reduce hamsters reproductive success under captive conditions, because of a major deficiency in vitamin B3 (Tissier, Handrich, Dallongeville, Robin, & Habold, 2017). Seeking to understand how crop monoculture affects hamsters fitness from a nutritionally perspectives, and whether hamsters were able to compensate for the vitamin B3 deficiency under more natural conditions, we designed this study in outside enclosures. The effects of organic monoculture crops (enclosures seeded with either wheat or Corn) were compared with those of mixed organic crops (enclosures seeded with four crops each). This design allowed us to exclude confounding effects such as agricultural ploughing, the use of pesticides and fertilizer or the increased predation rates generally associated with intensive monoculture (Fargione et al., 2009; Foley et al., 2005; Wilson et al., 2005). In view of the absence of herbicide use and the short timeline of this experiment (4 months) in comparison to the time generally required to observe an effect of sowing diversity on the unsown plant species richness in previous studies (Van der Putten et al., 2000), we expected to observe a similar adventive plant species richness in mixed- and monoculture crop enclosures. As the number of crops sown is positively related to invertebrate richness (Asteraki, Hart, Ings, & Manley, 2004), we also expected to find a higher invertebrate species richness in mixed enclosures than in those containing one crop variety. Then, we expected the higher food diversity in mixed crop enclosures to result in better reproductive success than in monoculture enclosures. Finally, based on the results of a previous study in captive conditions (Tissier et al., 2017), we were expecting that hamsters would not be able to compensate for the nutritional deficiencies of Corn by ingesting sufficient amount of invertebrates and adventive plants. Indeed, they would need to ingest 22-45 g of weeds (which is superior to the daily food intake of this species) to meet their daily requirement in vitamin B3 (Tissier et al., 2017) and compensate for the deficiency caused by Corn consumption.

## Material and methods

### 1. Study site, animals and husbandry conditions

This study was carried out from April to August 2015 (Fig. 1A) in outdoor enclosures located in the moat of a Fort (see Fig. 1B). Loess soil was brought from outside the Fort to allow hamsters to dig a burrow and to mimic the type of soil usually found in its natural habitat (Pietsch, Kühn, Lisitsyn, Markova, & Sinitsyn, 2014). Each enclosure had a surface area of 16m$^2$ and was closed by a fence (beginning 1.5m underground and reaching 1 meter above ground level). A 30 cm-high galvanized steel plate was added to the top of the fence to prevent hamsters from climbing over the top. The 60 enclosures used in this experiment were divided into 3 groups (hereafter referred as 'crop type'): wheat (*W*; N=20), Corn (*C*; N=20), and mixed crop (*MX*; N=20; see Fig. 1B). To limit edge effects and to avoid that monoculture enclosures would benefit from the adjacent proximity of mixed enclosures (e.g. by increasing invertebrate diversity owing to a more diverse sowing), the 20 enclosures of each crop type were adjacent (see Fig. 1B) and monoculture crop enclosures were separated from mixed crop enclosures by an uncultivated enclosure (Fig. 1B, numbers 34 and 55). Given that Corn-based diets were found to profoundly affect hamsters' reproduction in captive conditions (Tissier et al., 2017) and that the detrimental effects of a predominance of Corn in the diet are extremely difficult to counteract with other food items (Ernandes, Guardia, & Giammanco, 1996), the *MX* enclosures were seeded with four crops (wheat, Corn, sunflower and alfalfa) to prevent the apparition of the deficiency and to give hamsters the opportunity to select their food items according to their physiological needs. Wheat (*Triticum sp*.) and Corn (*Zea mays*) were sown in February and April 2015, respectively. A quarter of each *MX* enclosure was seeded with wheat in February 2015 and the remaining plots of each *MX* enclosure were sown with Corn, alfalfa (*Medicago sativa*) and sunflower (*Helianthus annuus*) in April 2015 (see Fig. 1A for the experiment timeline). Weeds were removed from each enclosure by hand before sowing. We used organic seeds and no chemical treatment was applied in any of the three crop types. The enclosures were all covered by a net (10x10 cm mesh, 2.5m above the enclosures) in order to protect the hamsters from predators.

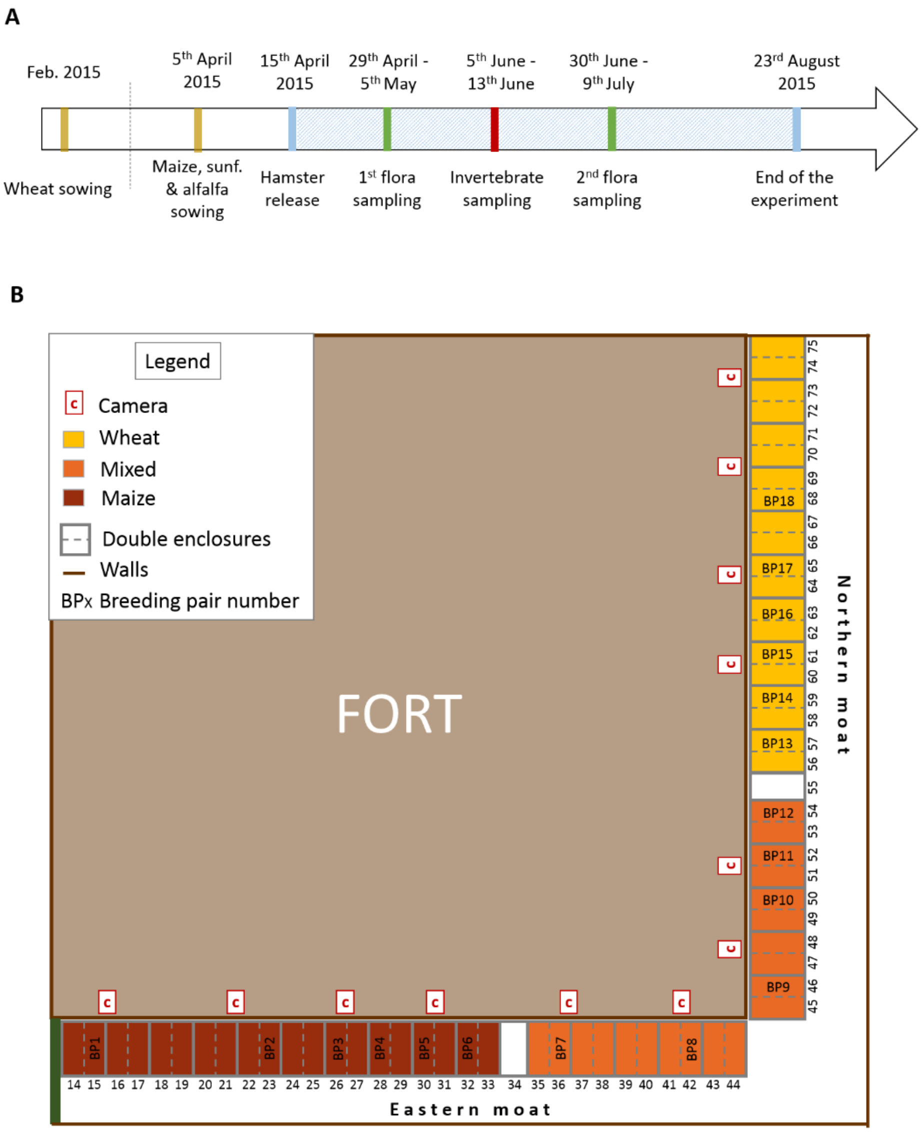


**Fig. 1: Experiment timeline (A) and layout of the enclosures (B).** In (A), the hatched part of the timeline indicates the period during which hamsters were monitored. In (B), the enclosures are schematized according to their position in the moats and to their crop type: Corn (left, yellow), mixed (middle, blue) and wheat (right, green). The 20 adjacent Corn enclosures were located in the eastern moat and the 20 adjacent wheat enclosures in the northern moat. Ten of the mixed crop enclosures were localized in the eastern moat and the remaining ten were in the northern moat, which allowed to investigate for a moat effect. Solid lines indicate the fence between

different enclosures. Dashed lines show the passage created in July between two adjacent enclosures (see text below for further details).

The experiment was carried out on 36 one-year-old captive-born hamsters (18 males and 18 females). Hamsters were grouped in breeding pairs (6 breeding pairs per crop type) and each pair was housed in two juxtaposing enclosures (the second remaining inaccessible until July in order to allow the cover to grow). As hamsters are food-hoarding animals (Wall, 1990), we provided each group with supplements of either wheat grain (in *W* enclosures), corn kernels (in *C* enclosures) or a mix of sunflower seeds, corn kernels  and wheat grains (in *MX* enclosures) to mimic the hoards they would have made during the previous autumn in the same conditions. Indeed, food-hoarding animals are known to hoard large amounts of seeds in autumn for their nutritional requirements during their winter arousal from torpor and during reproduction (Humphries, Thomas, & Kramer, 2003; Munro, Thomas, & Humphries, 2008; Nechay, Hamar, & Grulich, 1977; Wall, 1990).

## 2. Adventive plant sampling

Adventive plants were sampled twice in all the enclosures, with sampling of the entire enclosure surface: once between April 29th and May 5th and a second time between June 30th and July 9th 2015. Every recorded specie s was numbered, pictured and identified to the species (or the genus when species identification was impossible). This provided details of the adventive plant species richness (number of species = $N_{plant}$) in each enclosure for the two sampling periods, depending on the presence/absence of hamsters in the enclosure.

## 3. Invertebrate sampling

The soil-surface active invertebrates were sampled using pitfall traps (Ward, New, & Yen, 2001). These traps consisted of small containers (Ø5cm and 8cm deep) placed in the ground with the opening placed at the surface (Baker & Dunning, 1975). They were filled with 2cm of liquid (salt, dishwashing liquid, 70° alcohol and water) designed to kill and conserve the invertebrates falling into the trap (adapted from (Baker & Dunning, 1975)). Pitfall traps were placed in each enclosure for a 13-day period (5th - 17th June 2015). When collected, invertebrates were transferred to a 70° alcohol solution to guarantee tissue conservation until identification. We recorded only mesofauna and macrofauna (*i.e*. ≥4 mm) species. This provided the aboveground invertebrate species richness (number of species = $N_{invertebrates}$) and the invertebrate abundance (total number of invertebrate individuals in the enclosure = $n_{invertebrates}$) for each enclosure.

### 4. Hamster survival and reproductive success

The reproductive success of each hamster was estimated using network cameras (permanent recording; ref. DS-2CD3226F-I, HikVision, China) overlooking the enclosures (see Fig. 1 for the position of the cameras), camera traps (placed at the burrow entrance) and trapping. If pups were observed on video camera or camera trap pictures, 3-5 traps were placed in the enclosure immediately after they started to explore the outside of the burrow. Once trapped, the pups were weighed and tagged (with 1.4 x 8mm RFID chips, Biolog-ID, France) and their sex was determined. We were thus able to estimate the total number of pups produced per enclosure/breeding pair.

### 5. Data analyses

Data presented are means ± SEM. Normality of the residuals was tested using a Kolmogorov-Smirnov test, and variance homogeneity was checked using a Levene test. The effect of the crop type on the (i) $N_{invertebrates}$ and (ii) $n_{invertebrates}$ was analysed using linear models (LM) with the crop type ('Crop': Corn, Mixed or Wheat), the presence of hamsters in the enclosure ('Hamster', binary: Yes or No) and the Crop*Hamster interaction as fixed factors. $N_{plant}$ and $N_{invertebrates}$ were respectively included as covariates in the analysis of (i) $N_{invertebrates}$ and (ii) $n_{invertebrates}$. The effect of the crop type on (iii) $N_{plant}$was analysed using a linear mixed model (LMM) with the crop type ('Crop' variable), the presence of hamsters in the enclosure ('Hamster' variable), the sampling period ('Sampling' variable: first or second) and the following interactions as fixed factors: Crop*Hamster and Crop*Sampling. In all the models, (1) the enclosure number was included as a random factor for repeated measures on the same enclosure and (2) the moat (Northern or Eastern) was included to correct for a moat effect. Finally, we looked at the effects of the crop type on the number of pups produced per breeding pair using a generalized linear model (GLM, Probability distribution: Poisson, Link function: Log), with the crop type as fixed factor.

Model selection was based on the best AICc (Akaike information criterion for small samples) value for the LM and LMM models. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Fig.s were prepared using GraphPad prism software (Version 5, La Jolla, USA).

## Results

### a) Adventive plant species richness ($N_{plant}$)

A total of 76 adventive plants were recorded: 53 plants were identified as species, 10 were linked to a genus and 13 plants were only identified as belonging to a family (recorded as "Indeterminate number 1 to 13"; five of these thirteen species were unidentified thistles). We found no effects of the moat, the sampling or the hamster presence on this variable (p>0.1; see Tab. S1). Adventive plant species richness was significantly affected by the presence of hamsters ($F_{1,71.6}$ = 94.14, p<0.001), the crop type ($F_{2,53.8}$ = 10.18, p<0.001) and the three following interactions: Hamster*Crop ($F_{2,70.2}$ = 3.21, p = 0.046), Hamster*Sampling ($F_{1,60}$=78.27, p<0.001) and Crop*Sampling ($F_{2,53}$ = 3.37, p = 0.042). Post-hoc analyses highlighted that in the first sampling period, regardless of whether the hamsters were present or not (Hamster*Crop interaction, $F_{2,53}$ = 1.002, p = 0.37), *MX* enclosures exhibited a higher plant species richness than *C* enclosures (Mean difference = 5.0±1.4, p = 0.001), with *W* enclosures being intermediate (Fig. 2A; mean difference with *MX* = 1.3±1.3, p = 0.317 and mean difference with *C* = 3.6±1.9, p = 0.056). In the second sampling period (Fig. 2B), post-hoc analyses highlighted that when no hamsters were present, $N_{plant}$ was higher in the *MX* enclosures (24.0±1.1 per enclosure) compared to the *C* or *W* enclosures (Fig. 2B, *No hamster*; *C* = 16.4±1.5, *W* = 16.7±1.6 and p=0.012 and p=0.022, respectively). However, no differences were found between the crop type when hamsters were in the enclosures (Fig. 2B, *Hamster*, *MX* = 6.2±1.6, *C* = 5.1±1.8 and *W* = 5.3±2.1, p>0.1).

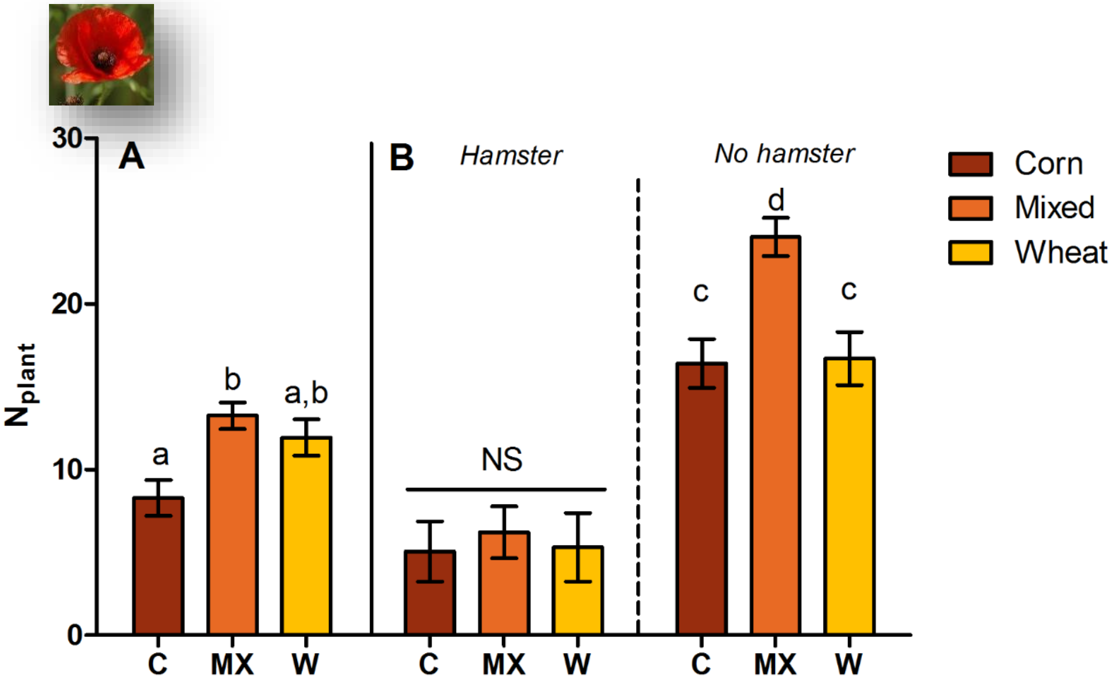


**Fig. 2: Adventive plant species richness ($N_{plant}$ per enclosure of 16m$^2$) according to the crop type and the sampling period.** (**A**) The first sampling period according to the crop type and (**B**) the second sampling period according to the crop type and the presence or absence of hamsters in the enclosure. C=Corn, MX = Mixed crop (Corn, Wheat, Sunflower and Alfalfa) and W=Wheat. Different letters indicate significant differences between the groups ($p<0.001$); NS represents non-significant differences between the groups ($p>0.05$).

### *Invertebrate species richness ($N_{invertebrates}$) and abundance ($n_{invertebrates}$)*

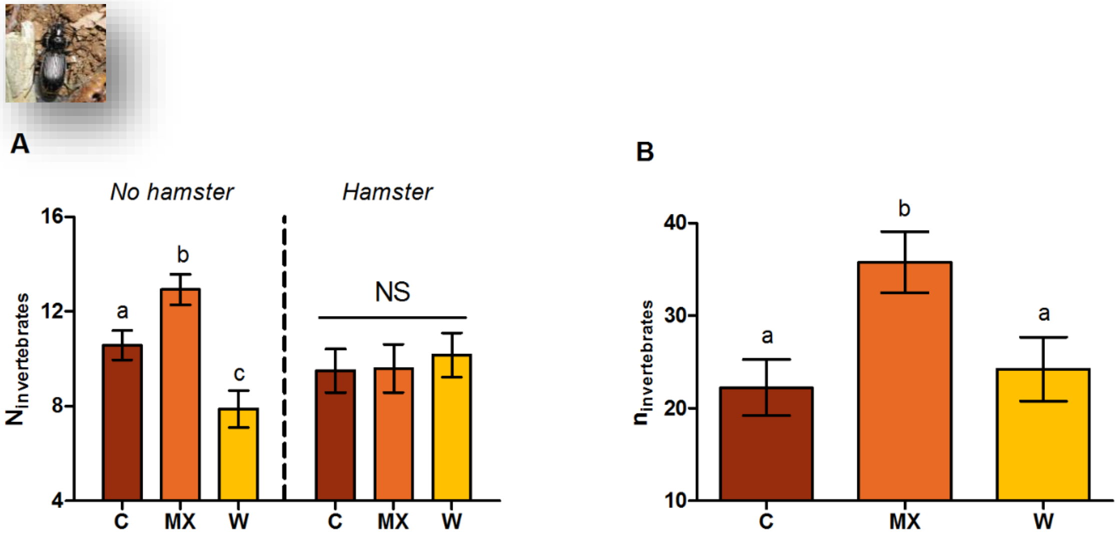
Regarding the soil-surface active invertebrates ($N_{invertebrates}$), we recorded a total of 69 invertebrate species, most of which were insects (see Tab. S2). Concerning the crop-type effect on $N_{invertebrates}$ (Fig. 3A), the best model (with the lowest AICc) was the reduced model (Tab. S3) that included crop type, hamster presence and the Crop*Hamster interaction. We found an effect of crop type ($F_{2,47} = 3.4$, $p=0.041$) and the Crop*Hamster interaction ($F_{2,47}=5.4$, $p = 0.008$) on $N_{invertebrates}$. Post-hoc analyses indicated that in the absence of hamsters, $N_{invertebrates}$ was greater in *MX* enclosures than in *C* and *W* enclosures, and *C* enclosures exhibited higher $N_{invertebrates}$ values than *W* enclosures (Fig. 3A, *No hamsters;* mean $N_{invertebrates}$: *MX* = 12.9±0.6, *C* = 10.6±0.6 and *W* = 7.9±0.8 species, p <

0.013). However, no effect of the crop type was seen when hamsters were present in the enclosure (Fig. 3A, *Hamster,* mean $N_{invertebrates}$: *MX* = 9.6±1.0, *C* = 9.5±0.9 and *W* = 10.2±0.9; p>0.8). Regarding the invertebrate abundance ($n_{invertebrates}$), we also found an effect of the crop type ($F_{2,46}$ = 4.82, p=0.013; see model in Tab. S4) with *MX* enclosures exhibiting significantly higher $n_{invertebrates}$ values than *C* and *W* enclosures (Fig. 3B, means: *MX* = 35.8±3.3, *C* = 22.2±3.0, *W* = 24.2±3.4; p = 0.005 and p = 0.023, respectively). However, $n_{invertebrates}$ was not found to be affected by hamster presence, $N_{invertebrates}$ or the Crop*Hamster interaction (Tab. S4, p>0.11).

**Fig. 3: Invertebrate (A) species richness and (B) abundance according to the crop type.** Data represent the average number (Mean±SEM) recorded per enclosure of 16m$^2$**.** C=Corn, MX=Mix (Corn, Wheat, Sunflower and Alfalfa) and W=Wheat. Different letters indicate significant differences between the groups (p<0.03); NS represents non-significant differences between the groups (p>0.05).

b) Hamster reproductive success

From the 23rd of August, some individuals from the *C* and *W* enclosures (mostly males) started to disperse and were recaptured in *MX* enclosures; they had escaped from their own enclosures by destroying the fence and then colonized the *MX* enclosures. This led to the dispersion of individuals from the mixed enclosures to *C* enclosures. Consequently, the experiment was stopped at this point and subsequent data (after August 23) are not included in the results. Reproduction occurred



in all three crop types. The number of pups produced per breeding pair (Wald $\chi^2 = 15.7$, $p<0.001$) was found to be affected by the crop type, with significantly more pups produced in the *MX* enclosures (3.3±0.8 pups per breeding pair on average) than in the other crop types (Fig. 4; with on average 0.5±0.3 and 0.7±0.3 pups per breeding pair in *C* and *W* enclosures, $p = 0.002$ and $p = 0.003$, respectively). Twenty pups were born in MX enclosures compared to three and four pups in *C* and *W* enclosures, respectively (Tab. S5). Informative data on the survival of adults in each crop type group (before August 23) is also shown in Tab. S5.

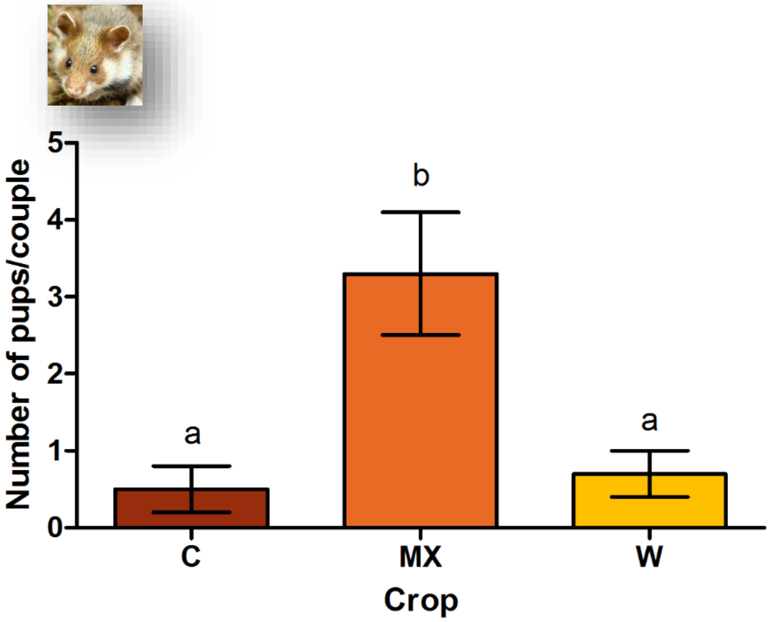


**Fig. 4: Total number of pups per breeding pair according to the crop type**. C=Corn, MX=Mixed crop (Corn, Wheat, Sunflower and Alfalfa) and W=Wheat. Different letters (a;b) indicate significant differences between the groups ($p<0.003$).

## Discussion

Although we were expecting the short timeline of the experiment to result in similar adventive plant species richness for mixed and monoculture enclosures, our results actually reveal that the crop type affected the adventive plant species richness, even within this short time-lapse. Regarding our other hypotheses, monoculture crops were confirmed to negatively affect invertebrate species richness and the reproductive success of hamsters.

### *Effects of crop diversity on adventive plant species richness ($N_{plant}$)*

Monoculture enclosures exhibited significantly lower adventive plant species richness ($N_{plant}$) than mixed crop enclosures when no hamsters were present in the enclosure. The low $N_{plant}$ recorded in Corn enclosures during the first sampling period (April 29th-May 5th) could be partially explained by the manual weed removal carried out before sowing Corn at the beginning of April. This situation is representative of what hamsters face in the wild when they emerge in a field of Corn after hibernation (O'Brien, 2015). However, the higher $N_{plant}$ recorded in mixed crop enclosures compared to both wheat and Corn enclosures in the second sampling period (June 30th-July 9th, when no hamsters were present) appeared to be entirely explained by the difference in crop type. The higher crop diversity in mixed enclosures therefore seems to have led to the settlement of a higher diversity of adventive plants within a short time-lapse (i.e. 2-3 months). The effects of sowing diversity on plant species richness appear to be complex (Finn et al., 2013). Indeed, some results reveal that sowing a highly diverse mix (more than 5 species) is more likely to suppress weeds than a low-diversity mix (5 species) (Van der Putten et al., 2000). In contrast, another study showed that simple mix sowing (3 species) resulted in lower plant species richness than complex mix sowing (Asteraki et al., 2004). It is now well established that several parameters might influence the richness of unsown species by modulating the dynamics of the seed bank, influencing interspecific interactions or affecting niche complementarity. These factors include soil type and moisture, the diversity, density and species composition of the seeds, and the presence of copses or field boundaries nearby (Altieri, 1999; Asteraki et al., 2004; Finn et al., 2013; Nyfeler et al., 2009; Van der Putten et al., 2000). Consequently, the mixed crop type of our experiment (with the four following crops: alfalfa, Corn, wheat and sunflower) could have favored the germination of a greater variety of seeds, for example through the modification of plant density, microclimate or nutrient inputs.

### *Effects of crop diversity on invertebrate species richness ($N_{invertebrates}$) and abundance ($n_{invertebrates}$)*

When hamsters were not present, monoculture enclosures exhibited significantly lower invertebrate species richness ($N_{invertebrates}$) and lower invertebrate abundance ($n_{invertebrates}$) compared to mixed crop enclosures. These results suggest a higher attractiveness of *MX* enclosures for invertebrates compared to *C* and *W* enclosures. Given that plant species richness and diversity are known to be positively related to the diversity and abundance of invertebrates

(Asteraki et al., 2004; Webb, Clarke, Nicholas, Journal, & Jan, 1984), this could be explained by the higher plant species richness in *MX* enclosures compared to that of monoculture enclosures.

*Crop type, food consumption and reproductive success of hamsters*

The crop type effect was buffered by hamsters' presence (all enclosures exhibited a low $N_{plant}$). This strongly suggest that hamsters in mixed enclosures benefitted from a food supply composed of numerous adventive plant species. In *C* enclosures, hamsters consumed the majority of the Corn grains sown before they had germinated, whilst in *W* enclosures, they consumed the sprouted grains of wheat after having uprooted the seedling. These phenomena were not observed in mixed enclosures, where hamsters ate all the green parts of sunflower and alfalfa plants but did not consume the wheat or corn, which were left untouched until the summer. The flowers of the corn plants were eaten in July (in both *C* and *MX* enclosures) whilst hamsters 'harvested' ears of wheat when they reached the milk stage (at the end of June, in both *MX* and *W* enclosures). All the ears of wheat had been collected in all these enclosures within approximately one week. When looking at all the enclosures, we observed that hamsters rarely consumed the green parts (leaves and stem) of certain plants, which systematically remained in the enclosures. These were mainly sown wheat and corn and unsown Poaceae, i.e. bluegrass (*Poa annua, Poa trivialis* and *Poa nemoralis)*, the nettle (*Urtica dioica*) and the hemp-nettle (*Galeopsis tetrahit*). The crop type effect on $N_{invertebrates}$ was also buffered when hamsters were present in the enclosure. This could either suggest that the diet of the hamsters was improved by a higher invertebrate species richness or that the effects of hamsters on the vegetation buffered the effects of the crop type on the diversity of invertebrate species.

However, the results generally show that hamsters suffered from eating a less diverse diet (i.e. a more limited variety of sown and unsown plants, less diversity of supplemented seeds and potentially lower richness and abundance of invertebrates) in the monoculture compared to the *MX* enclosures. Given the importance of food diversity to meet protein and energy requirements for reproduction in small mammals (Schneider, 2004; Speakman, 2008; Wade & Schneider, 1992), this could explain the differences observed in terms of reproductive success between *MX* and monoculture enclosures. Indeed, it appears that hamsters were not able to compensate for a low crop diversity in monoculture enclosures by consuming other food items such as invertebrates or weeds, both of which were also impaired by the monoculture crop, leading to a low effective reproductive success (number of pups emerging from the burrow) in these enclosures. Taken altogether, these results highlight how low crop diversity can impair the fitness of this vertebrate species, even before considering the impact of pesticides, ploughing and predation pressures.

### *Consequences for hamsters and farmland biodiversity as a whole*

The strong effects of organic crop monoculture on biodiversity shown in this study highlight that monoculture itself – apart from other detrimental effects associated to this agricultural practice – has a negative effect on biodiversity. The intensive use of pesticides in conventional farming is known to drastically decrease the diversity of adventive plants and invertebrates (Altieri, 1999; Labruyere, Ricci, Lubac, & Petit, 2016; Rands & Sotherton, 1986) and is highly likely to cause nutritional deficiencies in the hamster and other farmland vertebrates with small home ranges, thus affecting their reproduction. This is supported by previous studies highlighting how the reduction of food diversity in intensively managed farming regimes impairs the fitness of seed-eating birds when compared to mixed crop farming (Evans, 1997; Vickery et al., 2001; Wilson, Morris, Arroyo, Clark, & Bradbury, 1999; Wilson et al., 2005). It appears that bees are also suffering from a lack of food diversity, mainly because of the increasing lack of flowers in farmlands (Decourtye, Mader, & Desneux, 2010; Goulson et al., 2015; Vanderplanck et al., 2014).

It is very likely that hamsters living in monoculture crop fields increase their rate of dispersion to find adequate food resources, and this hypothesis is supported by our observations of hamsters dispersing from monoculture to *MX* enclosures in late August. However, in Europe, the average field size corresponds to the home range of a male and is seven times the size of the home range for a female (O'Brien, 2015; Ulbrich & Kayser, 2004). Individuals have to disperse over a great distance to find proper food resources - from 350m to 1km, which is incompatible with the ecology of this species (Ulbrich & Kayser, 2004) - consequently increasing their risk of predation. Predation appears to be one of the main cause of decline of the species across Europe (Kayser, Weinhold, & Stubbe, 2003; Ulbrich & Kayser, 2004). Reproduction also appears to have decreased over the past century (Surov et al., 2016). For instance, the number of litters recorded to be of 2-3 litters in older literature (Nechay et al., 1977), is currently estimated to be lower than 2 litters/female/year in European farmlands (Surov et al., 2016) and to be as low as 0.76 litter/female/year in France

(Kourkgy & Eidenschenck, 2015). We therefore argue that it is of the upmost importance to increase crop diversity at the plot level through mixed crop farming or wildflower margins, to improve food diversity and thus increase the reproductive success of this species. This is supported by a recent study in Germany which highlights that wildflower fields are ideal habitats for hamsters (Fischer & Wagner, 2016). This policy would also offer farmland vertebrates shelters to protect from predators and should thus reduce mortality by predation (Whittingham & Evans, 2004; Wilson et al., 2005). From an agronomical perspective, the sowing of mixed crops appears to maintain a resistance to weed invasion (Finn et al., 2013) while sustaining yields under the appropriated N fertilization and sowing composition (Finn et al., 2013; Nyfeler et al., 2009). Sowing an appropriate variety of seeds in agro-ecosystems could thus allow a reduction in the use of herbicides whilst favoring biodiversity. Finally, one of the main current conservation measures of the species in France is to reintroduce hamsters to conventional unharvested wheat plots (Villemey et al., 2013) surrounded by electric fences. Given the low reproductive success of hamsters in the wheat enclosures of our experiment, we suggest improving this conservation measure by reintroducing hamsters to plots containing a variety of crops.

## Supporting information

Tab. S1: statistical output of the crop type effect on the plant species richness.

Tab. S2: number of species recorded and species occurrences for each order (total and per crop type).

Tab. S3: statistical output of the crop type effect on the invertebrate species richness ($N_{invertebrates}$).

Tab. S4: statistical output of the crop type effect on the invertebrate abundance ($n_{invertebrates}$).

Tab. S5: total number of pups produced, adult mortality and adult emigration/death according to the crop type (N = 6 breeding pair per crop type).

Tab. S6: data used to perform statistical analyses ($N_{invertebrates}$, $n_{invertebrates}$, $N_{plant}$ and reproductive success of hamsters).

## Authors contribution

MLT and CH designed the study. MLT and FK collected the data and FK performed invertebrate and plant species identification. MLT carried out statistical analyses and wrote the manuscript. YH, FK and CH contributed significantly to manuscript revisions and gave final approval for publication.

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## References

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems & Environment*, *74*, 19–31.

Asteraki, E. J., Hart, B. J., Ings, T. C., & Manley, W. J. (2004). Factors influencing the plant and invertebrate diversity of arable field margins. *Agriculture, Ecosystems and Environment*, *102*(2), 219–231. doi:10.1016/j.agee.2003.07.003

Baker, A. N., & Dunning, R. A. (1975). Some effects of soil type and crop density on the activity and abundance of the epigeic fauna, particularly Carabidae, in sugar-beet fields. *Journal of Applied Ecology*, *12*(3), 809–818.

Benton, T. G., Vickery, J. A., & Wilson, J. D. (2003). Farmland biodiversity: Is habitat heterogeneity the key? *Trends in Ecology and Evolution*, *18*(4), 182–188. doi:10.1016/S0169-5347(03)00011-9

Björklund, J., Limburg, K. E., & Rydberg, T. (1999). Impact of production intensity on the ability of the agricultural landscape to generate ecosystem services : an example from Sweden. *Ecological Economics*, *29*, 269–291.

Changguo, X., Pingjiu, Z., Genxing, P., Duosheng, Q., & Qiuhua, C. (2006). Changes in diversity , protein content , and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. *Acta Ecologica Sinica*, *26*(6), 1667–1674.

Dance, C., Botias, C., & Goulson, D. (2017). The combined effects of a monotonous diet and exposure to thiamethoxam on the performance of bumblebee micro-colonies. *Ecotoxicology and Environmental Safety*, *139*, 194–201.

Decourtye, A., Mader, E., & Desneux, N. (2010). Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie*, *41*(3), 264–277. doi:10.1051/apido/2010024

Ernandes, M., Guardia, M. La, & Giammanco, S. (1996). Maize based diets and possible neuro- behavioural after-effects among some populations in the world. *Human Evolution*, *11*(1), 67–77.

Evans, A. (1997). The importance of mixed farming for seed-eating birds in the UK. In *Farming and birds in Europe* (p. Chapter 12).

Fargione, J. E., Cooper, T. R., Flaspohler, D. J., Hill, J., Lehman, C., Tilman, D., … Oberhauser, K. S. (2009). Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation. *BioScience*, *59*(9), 767–777. doi:10.1525/bio.2009.59.9.8

Finn, J. A., Kirwan, L., Connolly, J., Sebastià, M. T., Helgadottir, A., Baadshaug, O. H., … Lüscher, A. (2013). Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *Journal of Applied Ecology*, *50*(2), 365–375. doi:10.1111/1365-2664.12041

Fischer, C., & Wagner, C. (2016). Can agri-environmental schemes enhance non-target species? Effects of sown wildflower fields on the common hamster (Cricetus cricetus) at local and landscape scales. *Biological Conservation*, *194*, 168–175. doi:10.1016/j.biocon.2015.12.021

Foley, J. A., Defries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., … Snyder, P. K. (2005). Global consequences of land use. *Science (New York, N.Y.)*, *309*(5734), 570–574. doi:10.1126/science.1111772

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites , pesticides , and lack of flowers. *Science*, *347*(6229), 1–16. doi:10.1126/science.1255957

Humphries, M. M., Thomas, D. W., & Kramer, D. L. (2003). The Role of Energy Availability in Mammalian Hibernation : A Cost-Benefit Approach. *Physiological and Biochemical Zoology*, *76*(2), 165–179.

Kayser, A., Weinhold, U., & Stubbe, M. (2003). Mortality factors of the common hamster Cricetus cricetus at two sites in Germany. *Acta Theriologica*, *48*(1), 47–57.

Kourkgy, C., & Eidenschenck, J. (2015). *Délivrable Action D1. Rapport annuel de présentation des données collectées et premières analyses, année 2014.* Strasbourg. Retrieved from www.oncfs.gouv.fr/Plan-de-restauration-du-Grand-Hamster-ru82/Mission-Hamster-a-lONCFS-ar1098

La Haye, M. J. J., Swinnen, K. R. R., Kuiters, a. T., Leirs, H., & Siepel, H. (2014). Modelling population dynamics of the Common hamster (Cricetus cricetus): Timing of harvest as a critical aspect in the conservation of a highly endangered rodent. *Biological Conservation*, *180*, 53–61. doi:10.1016/j.biocon.2014.09.035

La Haye, M., Müskens, G., Van Kats, R., Kuiters, A., & Siepel, H. (2010). Agri-environmental schemes for the Common hamster (Cricetus cricetus). Why is the Dutch project successful ? *Aspects of Applied Biology*, *100*, 117–124.

Labruyere, S., Ricci, B., Lubac, A., & Petit, S. (2016). Crop type, crop management and grass margins affect the abundance and the nutritional state of seed-eating carabid species in arable landscapes. *Agriculture, Ecosystems & Environment*, *231*, 183–192. doi:10.1016/j.agee.2016.06.037

Monecke, S. (2013). All things considered? Alternative reasons for hamster extinction. *Zoologica Poloniae*, *58*(November), 41–57. doi:10.2478/zoop-2013-0004

Munro, D., Thomas, D. W., & Humphries, M. M. (2008). Extreme suppression of aboveground activity by a food-storing hibernator, the eastern chipmunk (Tamias striatus). *Canadian Journal of Zoology*, *86*(5), 364–370. doi:10.1139/Z08-008

Nechay, G., Hamar, M., & Grulich, I. (1977). The Common Hamster ( Cricetus cricetus [ L .]) ; a Review. *EPPO Bull.*, *7*(2), 255–276.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, *46*, 683–691. doi:10.1111/j.1365-2664.2007.0

O'Brien, J. (2015). Saving the common hamster (Cricetus cricetus) from extinction in Alsace (France): potential flagship conservation or an exercise in futility ? *Hystrix, the Italian Journal of Mammalogy*, *26*(2). doi:10.4404/hystrix-26.2-11230

Pietsch, D., Kühn, P., Lisitsyn, S., Markova, A., & Sinitsyn, A. (2014). Krotovinas, pedogenic processes and stratigraphic ambiguities of the Upper Palaeolithic sites Kostenki and Borshchevo (Russia). *Quaternary International*, *324*, 172–179. doi:10.1016/j.quaint.2013.05.036

Rands, M. R. W., & Sotherton, N. W. (1986). Pesticide use on cereal crops and changes in the abundance of butterflies on arable farmland in England. *Biological Conservation*, *36*(1), 71–82. doi:10.1016/0006-3207(86)90102-3

Rioux Paquette, S., Pelletier, F., Garant, D., & Belisle, M. (2014). Severe recent decrease of adult body mass in a declining insectivorous bird population. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140649. doi:10.1098/rspb.2014.0649

Schneider, J. E. (2004). Energy balance and reproduction. *Physiology & Behavior*, *81*(2), 289–317. doi:10.1016/j.physbeh.2004.02.007

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *363*(1490), 375–98. doi:10.1098/rstb.2007.2145

Surov, A., Banaszek, A., Bogomolov, P., Feoktistova, N., & Monecke, S. (2016). Dramatic global decrease in the range and reproduction rate of the European hamster Cricetus cricetus. *Endangered Species Research*, *31*, 119–145. doi:10.3354/esr00749

Tissier, M. L., Handrich, Y., Dallongeville, O., Robin, J., & Habold, C. (2017). Diets derived from maize monoculture cause maternal infanticides in the endangered European hamster due to a vitamin B3 deficiency. *Proceedings of the Royal Society B*, *284*(1847), 1–8. doi:http://dx.doi.org/10.1098/rspb.2016.2168

Tissier, M. L., Handrich, Y., Robin, J.-P., Weitten, M., Pevet, P., Kourkgy, C., & Habold, C. (2016). How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the verge of extinction. *Scientific Reports*, *6*(April), 25531. doi:10.1038/srep25531

Tsiafouli, M. a., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkhofer, K., … Hedlund, K. (2015). Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology*, *21*(2), 973–985. doi:10.1111/gcb.12752

Ulbrich, K., & Kayser, A. (2004). A risk analysis for the common hamster (Cricetus cricetus). *Biological Conservation*, *117*(3), 263–270. doi:10.1016/j.biocon.2003.12.006

Van der Putten, W. H., Mortimer, S. R., Hedlund, K., Van Dijk, C., Brown, V. K., Lep&#x000E4;, J., … Smilauer, P. (2000). Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. *Oecologia*, *124*(1), 91–99. doi:10.1007/s004420050028

Vanderplanck, M., Moerman, R., Rasmont, P., Lognay, G., Wathelet, B., Wattiez, R., & Michez, D. (2014). How does pollen chemistry impact development and feeding behaviour of polylectic bees? *PLoS ONE*, *9*(1), 1–9. doi:10.1371/journal.pone.0086209

Vickery, J. A., Tallowin, J. R., Feber, R. E., Asteraki, E. J., Atkinson, P. W., Fuller, R. J., & Brown, V. K. (2001). The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology*, *38*, 647–664.

Villemey, A., Besnard, A., Grandadam, J., & Eidenschenck, J. (2013). Testing restocking methods for an endangered species: Effects of predator exclusion and vegetation cover on common hamster (Cricetus cricetus) survival and reproduction. *Biological Conservation*, *158*, 147–154. doi:10.1016/j.biocon.2012.08.007

Wade, G. N., & Schneider, J. E. (1992). Metabolic fuels and reproduction in female mammals. *Neuroscience and Biobehavioral Reviews*, *16*(2), 235–272. doi:10.1016/S0149-7634(05)80183-6

Wall, S. B. Vander. (1990). *Food Hoarding in Animals*. (University of Chicago Press, Ed.). Retrieved from http://books.google.com/books?hl=fr&lr=&id=AhlvmkSkxeYC&pgis=1

Ward, D. F., New, T. R., & Yen, A. L. (2001). Effects of pitfall trap spacing on the abundance, richness and composition of invertebrate catches. *Journal of Insect Conservation*, *5*(1), 47–53. doi:10.1023/A:1011317423622

Webb, A. N. R., Clarke, R. T., Nicholas, J. T., Journal, S., & Jan, N. (1984). Invertebrate diversity on fragmented Calluna-Heathland: Effects of surrounding vegetation. *Journal of Biogeography*, *11*(1), 41–46.

Weinhold, U. (2008). Draft European action plan for the conservation of the common hamster (Cricetus cricetus L., 1758). In: Convention on the conservation of European wildlife and natural habitats. In *Standing Commitee. Council of Europe, Strasbourg, France,* (pp. 1–36).

Whittingham, M. J., & Evans, K. L. (2004). The effects of habitat structure on predation risk of birds in agricultural landscapes. *Ibis*, *146*(SUPPL. 2), 210–220. doi:10.1111/j.1474-919X.2004.00370.x

Wilson, J. D., Morris, A. J., Arroyo, B. E., Clark, S. C. S. C., & Bradbury, R. B. (1999). A review of the abundance and diversity of invertebrate and plant foods of granivorous birds in northern Europe in relation to agricultural change. *Agriculture, Ecosystems and Environment*, *75*(1-2), 13–30. doi:10.1016/S0167-8809(99)00064-X

Wilson, J. D., Whittingham, M. J., & Bradbury, R. B. (2005). The management of crop structure : a general approach to reversing the impacts of agricultural intensification on birds ? *Ibis*, *147*, 453–463.

Wood, T. J., Holland, J. M., & Goulson, D. (2016). Providing foraging resources for solitary bees on farmland: Current schemes for pollinators benefit a limited suite of species. *Journal of Applied Ecology*. doi:10.1111/1365-2664.12718

**Supplementary information** - Experiments in outside enclosures highlight that organically-grown monoculture crops reduce invertebrate and plant species richness and impair the fitness of a critically endangered farmland rodent.

| Variable | Numerator df | Denominator df | F value | Significance |
|---|---|---|---|---|
| **Intercept** | **1** | **6.94** | **37.33** | **0.001** |
| **Hamster** | **1** | **71.60** | **94.14** | **<0.001** |
| **Crop** | **2** | **53.83** | **10.18** | **<0.001** |
| Sampling | 1 | 53.12 | 2.79 | 0.101 |
| Moat | 1 | 52.03 | 0.81 | 0.373 |
| **Hamster*Crop** | **2** | **70.23** | **3.21** | **0.046** |
| **Hamster*sampling** | **1** | **59.97** | **78.27** | **<0.001** |
| **Crop*sampling** | **2** | **53.01** | **3.37** | **0.042** |
| Hamster*crop*sampling | 2 | 59.16 | 1.11 | 0.338 |

**Table S1: statistical output of the crop type effect on adventive plant species richness.** The best model (with the lowest AICc) is shown, i.e. the full model. Removing the triple interaction did not change the results but increased the AICc by 10. This interaction was therefore kept in the model. Significant p-values (α<0.05) are in bold print.

| Class | Order | N | Occurrences (per type of enclosures) |
|---|---|---|---|
| Insecta | Hymenoptera | 11 | **93** (32 Mix - 32 Maize > 29 Wheat) |
| | Diptera | 10 | **71** (31 Mix > 22 Maize > 18 Wheat) |
| | Hemiptera | 9 | **44** (16 Mix - 16 Wheat > 12 Maize) |
| | Coleoptera | 9 | **110** (48 Mix > 44 Maize > 18 Wheat) |
| | Orthoptera | 2 | **19** (10 Mix > 6 Maize > 3 Wheat) |
| | Lepidoptera | 3 | **6** (3 Maize > 2 Mix > 1 Wheat) |
| | Raphidioptera | 1 | **1** (Maize) |
| | Neuroptera | 1 | **2** (Maize) |
| Malacostraca | Isopoda | 2 | **60** (23 Mix > 22 Maize > 15 Wheat) |
| Arachnida | Araneae | 10 | **82** (36 Maize > 26 Mix > 20 Wheat) |
| | Opiliones | 1 | **11** (9 Maize > 2 Mix) |
| Chilopoda | Lithobiomorpha | 2 | **2** (1 Maize - 1 Mix) |
| Diplopoda | | 1 | **5** (Maize) |
| Gastropoda | Pulmonata | 3 | **26** (19 Mix > 6 Wheat > 1 Maize) |

**Table S2: Number of species recorded and species occurrences for each order (total and per crop treatment)**. The total occurrence, in bold print, represents the sum of the occurrence of all species of an order in all the enclosures. The occurrence per crop type represents the number of enclosures of each crop type containing a species from each order.

| Variable | Numerator df | Denominator df | F value | Significance |
|---|---|---|---|---|
| **Intercept** | **1** | **47** | **876.59** | **<0.001** |
| Hamster | 1 | 47 | 1.07 | 0.307 |
| **Crop** | **2** | **47** | **3.41** | **0.041** |
| **Hamster*Crop** | **2** | **47** | **5.42** | **0.008** |

**Table S3: statistical output of the crop treatment effect on the invertebrate species richness ($N_{invertebrates}$).** The best model (with the lowest AICc) is shown. Significant p-values (α<0.05) are indicated in bold print.

| Variable | Numerator df | Denominator df | F value | Significance |
|---|---|---|---|---|
| Intercept | 1 | 46 | 2.99 | 0.090 |
| Hamster | 1 | 46 | 1.67 | 0.203 |
| **Crop** | **2** | **47** | **4.82** | **0.013** |
| $N_{fauna}$ | 1 | 46 | 2.66 | 0.110 |
| Hamster*Crop | 2 | 46 | 0.71 | 0.495 |

**Table S4: statistical output of the crop treatment effect on the invertebrate abundance ($n_{invertebrates}$).** The best model (with the lowest AICc) is shown. Significant p-values (α<0.05) are indicated in bold print.

| **Crop type** | ***Corn*** | ***Mix*** | ***Wheat*** |
|---|---|---|---|
| **Number of pups** | 3 | 20** | 4 |
| **Adult mortality** | Two (17%) | One (8%) | Two (17%) |
| **Adult emigration/death** | Five (42%)<br>Date: April-May | Five (42%)<br>Date: July-August | Five (42%)<br>Date: April-May |

**Table S5: total number of pups produced, adult mortality and adult emigration/death according to the crop type (N = 6 breeding pair per crop type).** The two asterisks indicate a statistical difference between the Mix group and both the Maize and Wheat groups. See the Results section of the manuscript for further details. 'Adult mortality' shows the number and the percentage of adults that were found dead in the enclosure. 'Adult emigration/death' indicates animals that disappeared from their respective enclosures but for which no corpse was found. Data do not include observations after August 23; see the methodology section of the manuscript for further details.

# Chapter 3

*"We are what we eat, and what we evolve to eat"*

# Which crops are favorable to the hamster when associated to maize and wheat?

Content:

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## 1. Goal of this study

This study mainly aims at bringing solutions to the decreased reproductive success of the European hamster observed in the previous studies (study 2 and study 3) caused by wheat and maize monoculture crops. I also wanted to investigate for the effects of these two crops on the hibernation of the species, since up-to-now I have been focusing on the hamsters' reproductive success. I therefore explored for crops that could either prevent from vitamin B3 deficiency when associated to maize, or for crops that would ensure a good hibernation and reproductive success when associated to wheat. The idea was to find favorable crop to be associated either with wheat or maize, to be included in the agricultural schemes of the Alsace.

## 2. Approach

The experiment was carried-out on 84 captive hamsters (42 ♂ and 42 ♀), that were fed either on maize-radish (Mrad), maize-soybean (Msoy), maize-sunflower (Msunf), wheat-radish (Wrad), wheat-soybean (Wsoy) or wheat-sunflower (Wsunf), see Figure 13.

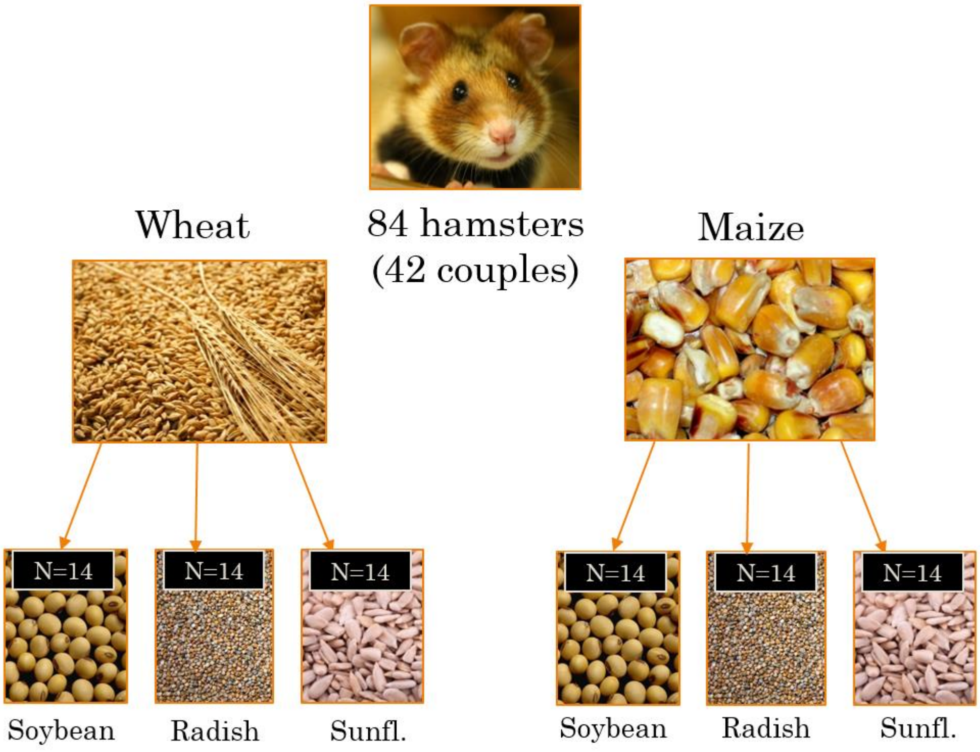

**Figure 13: Experimental diets and sample size of the study** (study 4 and box 2).

The experiment started in November 2015 and terminated after the first reproductive period in late May 2016. Hamsters were weighed and sampled (blood and ear tissues) four times: 1) prior and 2) after hibernation and 3) prior and 4) after reproduction. I recorded their levels of activity and their food intake during the whole hibernation period (November-March) as well as the litter size at parturition and at weaning during the early reproductive period (April-May).

## 3. Summary of the main results

Hamsters fed on Wsoy, Mrad and Msunf were significantly more active during hibernation and have lost significantly less body mass (up to twice less). They also had a better reproductive success than hamsters from the three other diets.  Sunflower and radish therefore appear to be favorable when associated to maize whereas they are unfavorable when associated to wheat. In contrast, soybean is favorable when associated to wheat but unfavorable when associated to maize. The main conclusion of this study would therefore be that there is not one 'favorable' crop for the species, but rather favorable crop-associations (see **Figure 14**). Furthermore, it appears that crop-associations favorable for hibernation are for most also favorable for reproduction.

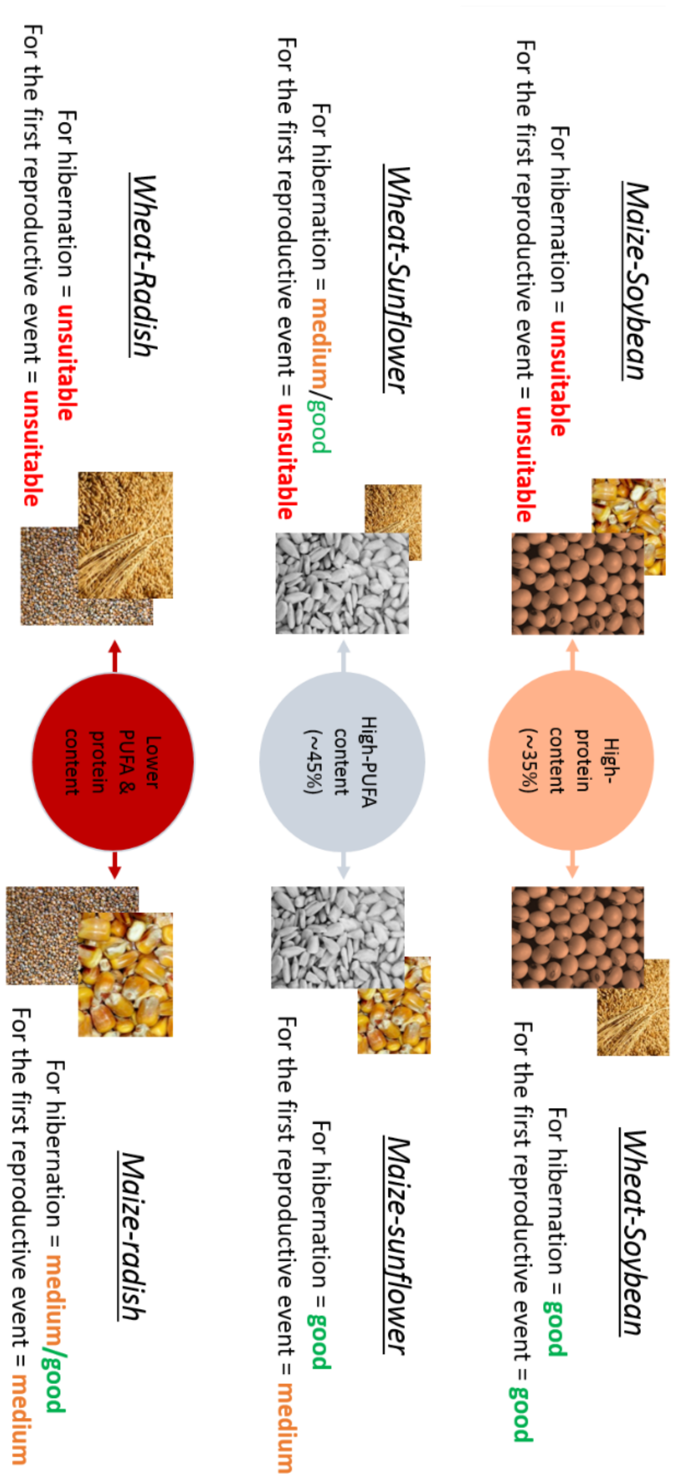

**Figure 14: summary of the crop-based association effects on the hibernation and the reproduction of the European hamster**. The content in PUFA and proteins is estimated following available data (AFZ et al., 2011). The size of the seed pictures are representative of hamsters' food preferences in each diet (See Figure 12 (c) of the study 4).

# Study 4 - effects of 6 crop-based diets on the hibernation and the reproduction of the European hamster (*Cricetus cricetus*).

*In preparation* [1]

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[1] These data will be part of the article: Tissier M.L., Kletty F., Robin J.P., Handrich Y. and Habold C. Effects of 6 crop-associations based on wheat and maize on the hibernation and the reproduction of the European hamster (*Cricetus cricetus*) – *In preparation*

## Short introduction

In the previous studies, we showed that European hamsters (*Cricetus cricetus*) fed on maize-rich diets displayed high rates of infanticide, with 95% of the pups killed during the first day after parturition. We later showed that an experimental supplementation of vitamin B3 restored a good reproductive success in hamsters fed on a maize-worm diet, compared to hamsters fed on the same diet without the vitamin supplement (study 2). In another study, we pinpointed that monoculture crops of maize and wheat were impairing hamsters' reproductive success (with less than 1 pups/couple) under semi-wild conditions (i.e. in outside enclosures, study 3). We argued that this reduced reproductive success was due to a strong reduction in the diversity of hamsters' diet. Moreover, this suggests that hamsters were not able to compensate for the vitamin B3 deficiency in maize by ingesting invertebrates or adventive plants in these semi-wild conditions.

However, up-to-now, we are still lacking of data on the effects of crop-based diets on the hibernation of this food-hoarding hibernator and on how they will affect hamsters body condition on emergence on which greatly depend their reproductive performances (Nechay et al., 1977; Vohralik, 1974). Therefore, with the double aim of understanding how food stores might affect hibernation and subsequent reproduction, but also of finding crops that could be associated with maize and wheat to improve the reproductive success of the species in French farmland, we have investigated the effects of 6 new crops-based diets on the hibernation and the reproduction of 42 pairs of captive hamsters: three maize-based diets (maize-radish, maize-soybean and maize-sunflower) and three wheat-based diets (wheat-radish, wheat-soybean and wheat-sunflower). These data will also bring new information on the effects of the diet on the hibernation of food-storing hibernators. Indeed, most studies conducted on this group of hibernators have been carried-out on Eastern chipmunks (*Tamias striatus*) (Humphries et al., 2011; Munro et al., 2008, 2005). Results of these studies seem to indicate different patterns than the one observed in fat-storing hibernators, in which diets rich in polyunsaturated fatty acids (PUFA (Arnold et al., 2015)) are positively related to the depth and duration of torpor bouts and therefore to energy saving during hibernation (Geiser et al., 1994; Jastroch et al., 2016; Munro and Thomas, 2004). However, in the food-storing Eastern chipmunk, it appears that PUFA-rich diets are associated with a reduction of torpor use, which can be interpreted as a strategy to avoid oxidative stress (Munro et al., 2005). Indeed, an excess of PUFA in the diet can also be detrimental, as these fatty acids are more prone to autoxidation (Frank et al., 1998; Munro and Thomas, 2004). Therefore, a lack of PUFA in the diet would lead to a hibernation of reduced quality, whereas an excess would cause oxidative stress.

## Methodology

### *Animals and housing conditions*

This study was carried-out on 84 one-year-old hamsters (42 ♂ and 42 ♀) from November 2015 to the beginning of June 2016 in our captive breeding unit (CNRS, IPHC-DEPE, Strasbourg, France). They were fed *ad libitum* on a conventional diet (pellets 105, from Safe, Augy, France, composed of 19.3% protein, 54.9% carbohydrates, 5.1% lipids, 4.2% cellulose, 5.0% minerals, and 11.5% water) and maintained in controlled environmental conditions (temperature 20 °C to 23 °C; 35%-55% humidity; summer photoperiod, 16L: 8D) *prior* to the experiment. Hamsters were housed individually (W x H x D: 265 mm x 237 mm x 420 mm) throughout the hibernation period. Cages were enriched with paper and plastic shelters during the whole experiment. In April, hamsters were housed in breeding pairs in large cages (W x H x D: 380 mm x 257 mm x 590 mm) equipped with a shelter box (W x H x D: 140 mm x 230 mm x 230 mm) for two weeks. After this two-week period, males were removed and females remained in the cage until they weaned their litter (i.e. 30 days post-parturition).

### *Experimental protocol: design, timetable and diets composition*

Three crops were selected to be associated with wheat and maize: sunflower (*Helianthus annuus*), soybean (*Glycine max*) and fodder radish (*Raphanus sativus oleiformis*). Sunflower and soybean were selected for their great tryptophan and vitamin B3 contents, but also for their respective PUFA and protein contents (see Table 3). Regarding fodder radish, it was selected because researchers in the Netherlands have recorded good population dynamics for hamsters living in radish fields (La Haye M.J.J, *personal comm.*). Moreover, this crop appears as a good intercultural crop for wheat and especially favorable for invertebrates and farmland birds (CARA Bourgogne, 2012; Heckenbenner and De Pontbriand, 2011).

| Analysis | Unit | Food item | | | | |
|---|---|---|---|---|---|---|
| | | *Wheat* | *Maize* | *Sunflower* | *Soybean* | *Pellets 105* |
| **Main analysis** | **Unit** | **Avg** | **Avg** | **Avg** | **Avg** | **Avg** |
| Dry matter | % as fed | 87.0 | 23.4 | 92.8 | 88.8 | 88.5 |
| Crude protein | % DM | 12.6 | 9.4 | 16.6 | 39.6 | 21.8 |
| Lipids | % DM | 3.6 | 4.4 | 47.9 | 21.3 | 5.8 |
| Total sugars | % DM | 3.2 | 2.1 | 2.7 | 8.7 | 62.0 |
| Starch | % DM | 69.1 | 73.4 | 1.3 | 6.4 | / |
| Gross energy | MJ/kg DM | 18.2 | 18.7 | 28.7 | 23.6 | 13.5 |
| **Minerals** | **Unit** | **Avg** | **Avg** | **Avg** | **Avg** | **Avg** |
| Calcium | g/kg DM | 0.7 | 0.5 | 2.6 | 3.2 | 8.5 |
| Magnesium | g/kg DM | 1.2 | 1.2 | 3 | 2.4 | 1.5 |
| Manganese | mg/kg DM | 40 | 5 | 19 | 29 | 79.1 |
| Zinc | mg/kg DM | 31 | 21 | 50 | 43 | 88.1 |
| Copper | mg/kg DM | 6 | 5 | 18 | 19 | 19.2 |
| Iron | mg/kg DM | 78 | 37 | 52 | 121 | 285.9 |
| **Amino acids** | **Unit** | **Avg** | **Avg** | **Avg** | **Avg** | **Avg** |
| Leucine | % protein | 6.5 | 12 | 6.0 | 7.5 | / |
| Lysine | % protein | 2.9 | 3.1 | 3.9 | 6.2 | / |
| Tryptophan | % protein | 1.2 | 0.7 | 1.6 | 1.3 | / |
| **Fatty acids** | **Unit** | **Avg** | **Avg** | **Avg** | **Avg** | **Avg** |
| Palmitic | % Lipids | 25 | 11 | 6 | 10 | / |
| Palmitoleic | % Lipids | 1 | Traces | Traces | Traces | / |
| Stearic | % Lipids | 1 | 2 | 5 | 4 | / |
| Oleic | % Lipids | 12 | 28 | 18 | 20.7 | / |
| Linoleic | % Lipids | 55 | 56 | 69 | 55 | / |
| Linolenic | % Lipids | 5.4 | 1 | 0.3 | 7.3 | / |
| **Vitamins** | **Unit** | **Avg** | **Avg** | **Avg** | **Avg** | **Avg** |
| B3 | mg/100 g | 1.8 | <1 | 8.3 | 0.4 | 41,8 |

Table 3: macronutrient and micronutrient composition of the crop selected for this experiment. Data are given for the grains/seeds of each crop; data on fodder radish are not available. Avg: average, pellet 105: conventional food (from SAFE) given to the hamsters in our breeding unit. (This table is based on (AFZ et al., 2011; USDA SR-21, 2016)))

A total of 6 diets were composed: maize-radish (7 ♂ and 7 ♀), maize-soybean (7 ♂ and 7 ♀), maize-sunflower (7 ♂ and 7 ♀), wheat-radish (7 ♂ and 7 ♀), wheat-soybean (7 ♂ and 7 ♀) or wheat-sunflower (7 ♂ and 7 ♀); see Table 4 below. During reproduction (8th march-10th June), hamsters were supplemented in proteins by receiving 2g of earthworm, which roughly corresponds to one worm (*Lumbricus terrestris*), every two days. Hamsters were maintained under controlled environmental conditions mimicking their seasonal cycle in the wild (Table 4): the photoperiod was based on the natural ephemerid of Strasbourg (48° 34′ 24″ North and 7° 45′ 08″ East) and the room temperature was about ~20°C in summer, 12°C in the fall and 10°C in winter (approaching the temperature of the burrow in the wild).

| Date | Period | Group | Diet | | Photoperiod | $T_a$ | Humidity |
|---|---|---|---|---|---|---|---|
| Birth-5th November 2015 | Before experiment | 1 | Conventional diet (pellets 105) | | Natural (summer, fall) | 20±2°C | 35%-45% |
| | | 2 | | | | | |
| | | 3 | | | | | |
| | | 4 | | | | | |
| | | 5 | | | | | |
| | | 6 | | | | | |
| 5th Nov. – 10th Dec. 2015 | Sampling OH (blood, ear tissues) | 1 | Conventional diet (pellets 105) | | Natural (fall, winter) | 12±1°C | 45%-60% |
| | | 2 | | | | | |
| | | 3 | | | | | |
| | | 4 | | | | | |
| | | 5 | | | | | |
| | | 6 | | | | | |
| 10th -16th December 2015 | Food habituation | 1 | Conv. diet + maize + soybean | | Natural (winter) | 10±1°C | 55%-65% |
| | | 2 | Conv. diet + maize + sunflower | | | | |
| | | 3 | Conv. diet + maize + radish | | | | |
| | | 4 | Conv. diet + wheat + soybean | | | | |
| | | 5 | Conv. diet + wheat + sunflower | | | | |
| | | 6 | Conv. diet + wheat + radish | | | | |
| 16th December - 8th March 2016 | Hibernation | 1 - Msoy | Maize + | Soybean | Natural (winter) | 10±1°C | 55%-65% |
| | | 2 - Msun | | Sunflower | | | |
| | | 3 - Mrad | | Radish | | | |
| | | 4 - Wsoy | Wheat + | Soybean | | | |
| | | 5 - Wsun | | Sunflower | | | |
| | | 6 - Wrad | | Radish | | | |
| 8th march - 10 June | Reproduction | 1 - Wsoy | Maize + | Soybean | Natural (spring) | From 10 to 20±1°C (+2°C/day) | 50%-60% |
| | | 2 - Wsun | | Sunflower | | | |
| | | 3 - Wrad | | Radish | | | |
| | | 4 - Wsoy | Wheat + | Soybean | | | |
| | | 5 - Wsun | | Sunflower | | | |
| | | 6 - Wrad | | Radish | | | |

**Table 4: timetable and details of the experimental design and diets**. Sampling OH corresponds to ear and blood samplings at the onset of hibernation. More details can be found in the text.

### *Body mass, food intake and food preferences*

Hamsters were weighed at the onset of hibernation (OH, from the 25th November to the 9th December 2015), post-hibernation (PH, between the 23-25th March 2016) and post-reproduction (PR, from the 1rst to the 10th of June). These data were used to calculate changes in hamsters' body mass over the totality of the experiment. At each cage cleaning, we collected the litter and sieved it to gather the totality of the uneaten food. This food was then dried and weighed to calculate hamsters' food intake over the hibernation period. This was then used to estimate hamsters' daily food intake (g $day^{-1}$ of dry matter) and food preferences.

### *Activity index*

The use of torpor was estimated three times a week between 3 and 5 pm, starting on the 21st of December and ending on the 8th of March. A score was attributed to each hamster (based on (Concannon et al., 1989)) according to their breath movements and their position in the cage. A score of 1 was assigned to hamsters with the body tightly curved and less than 1 breath in 30 seconds (i.e. likely in deep torpor). A score of 2 was assigned to hamsters with the body curved and breathing from 1-7 times in 30 seconds (i.e. in shallow torpor or in a deep sleep). Finally, a score of 3 was attributed to hamsters that where either active in the cage or displaying more than 7 breaths during 30 seconds (i.e. active or in a light sleep). Over the entire hibernation period, we therefore disposed of an index of activity for each hamster, ranging from 1 (mostly inactive, or in torpor) to 3 (mostly active, never in torpor).

### *Reproductive success and litter size*

Twice a day (at 8 am and 7 pm) between the end of April and the end of May, we monitored the number of females that initiated parturition and the litter size. The body mass of the pups (± 0.01 g) was recorded at 8 and 30 days of age.

### *Blood sampling*

Hamsters were sampled three times over the entire experiment: at the onset of hibernation (OH), at emergence from hibernation (PH) and finally post-reproduction (PR). We collected blood (~200 µL from the sublingual vein) under 2% isoflurane anaesthesia.

### *Data analyses*

Data presented are means ± SEM. Normality of the residuals was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using a Levene test. We first looked at the effect of the diet on the hamsters' *activity index* between December and the end of March using a Generalized Linear Mixed Model (GLMM), with the diet, the sex and the diet*sex interaction as fixed factors. The identity of the individuals and the date were included as random factors. We then investigated how the diet affected the *body mass loss* and *average daily food intake* during hibernation. These variable were analysed using a linear model (LM) with diet, sex and the diet*sex interaction as fixed factors. The body mass at the onset of hibernation and the average activity index of each hamster were included as covariates in this model. Regarding hamsters' food preferences during hibernation, we looked at the daily food intake for each of the two items using a Linear Mixed Model (LMM) with the diet, the sex, the type of the food item (base or supplement) and the diet*type of food item interaction as fixed factors. We also included the body mass at the onset of hibernation and the activity index as covariates in this model. The identity of the individuals was included as a random factor for repeated measures on the same individual. Multiple comparisons were analysed via post-hoc LSD (least significant difference) testing. Final model selection was based on the best AICc (Akaike information criterion for small samples) value. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA).

*Ethics*

The experimental protocol followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and was approved by the Ethical Committee (CREMEAS) under agreement number 00624-01.

## Results

*Activity index, food intake, food preferences and body mass loss during hibernation*

All hamsters displayed torpor (index of 1) during the hibernation period of this experiment, except one female from the Maize-radish group. The *activity index* was significantly affected by the diet (**Figure 15 (a)**, $F_{5,81.4} = 1.9$, $p = 0.047$) and the sex ($F_{1,81.4} = 6.1$, $p = 0.015$; females were more active than males: $\text{index}_{♀} = 2.01 \pm 0.35$ and $\text{index}_{♂} = 1.77 \pm 0.35$). However, we found no effect of the body mass at the onset of hibernation ($F_{1,81.4} = 0.8$, $p = 0.4$) or the sex*diet ($F_{5,81.4} = 1.3$, $p = 0.3$) interaction on this variable. The daily food intake (g of dry matter/day) was affected by the diet (see Figure 15 (b); $F_{5,43} = 2.8$, $p = 0.029$) and the activity index ($F_{1,43} = 93.5$, $p<0.001$; $R^2 = 0.69$). However, we found not effect of the sex ($F_{1,43} = 0.47$, $p = 0.5$) or the sex*diet interaction ($F_{5,43} = 0.70$, $p = 0.6$) on this variable. Regarding hamsters' food preferences, we found a significant Diet*Type of food item interaction (Figure 15 (c); $F_{1,97} = 6.3$, $p < 0.001$), highlighting that in four diets (Msoy, Msunf, Wsoy, Wsunf) hamsters consumed significantly more the supplement (soybean and sunflower), whereas in the two other diets (Mrad and Wrad) they preferentialy consumed the 'base" (maize and wheat, respectively). For information, average body-mass are given in the **Table 5** according to the period, the diet and the sex of the individuals.

| PERIOD | DIET | M-soy | M-sunf | M-rad | W-soy | W-sunf | W-rad |
|---|---|---|---|---|---|---|---|
| OH | | 252.9±14.2 | 253.1±14.2 | 252.6±14.2 | 253.6±14.2 | 253.9±14.2 | 254.1±14.2 |
| PH | | 194.1±11.9 | 231.3±11.9 | 216.9±11.9 | 234.6±11.9 | 212.9±11.9 | 188.4±11.9 |
| PR | | 224.3±12.5 | 256.1±13.6 | 229.7±13.2 | 257.3±13.0 | 240.2±12.5 | 203.2±13.2 |
| PERIOD | SEX | Males | | | Females | | |
| OH | | 271.7±8.2 | | | 235.0±8.2 | | |
| PH | | 230.3±6.9 | | | 195.8±6.9 | | |
| PR | | 257.0±7.1 | | | 213.3±7.9 | | |

**Table 5: Hamsters' average body mass according to their diet, their sex and the period.** Three periods are represented: OH = Onset of Hibernation, PH = Post-Hibernation and PR = Post-Reproduction. Means+SEM are recorded, in grams.

Hamsters' *specific body mass loss* was significantly affected by the sex ($F_{1,70} = 27.5$, $p<0.001$), the diet (Figure 15 (b); $F_{5,70} = 2.9$, $p = 0.021$), the activity index ($F_{1,70} = 69.5$, $p<0.001$, $R^2 = 0.34$) and the body mass at the onset of hibernation ($F_{1,70} = 83.8$, $p<0.001$; $R^2 = 0.33$) but not by the sex*diet interaction ($F_{5,70} = 1.2$, $p = 0.3$).

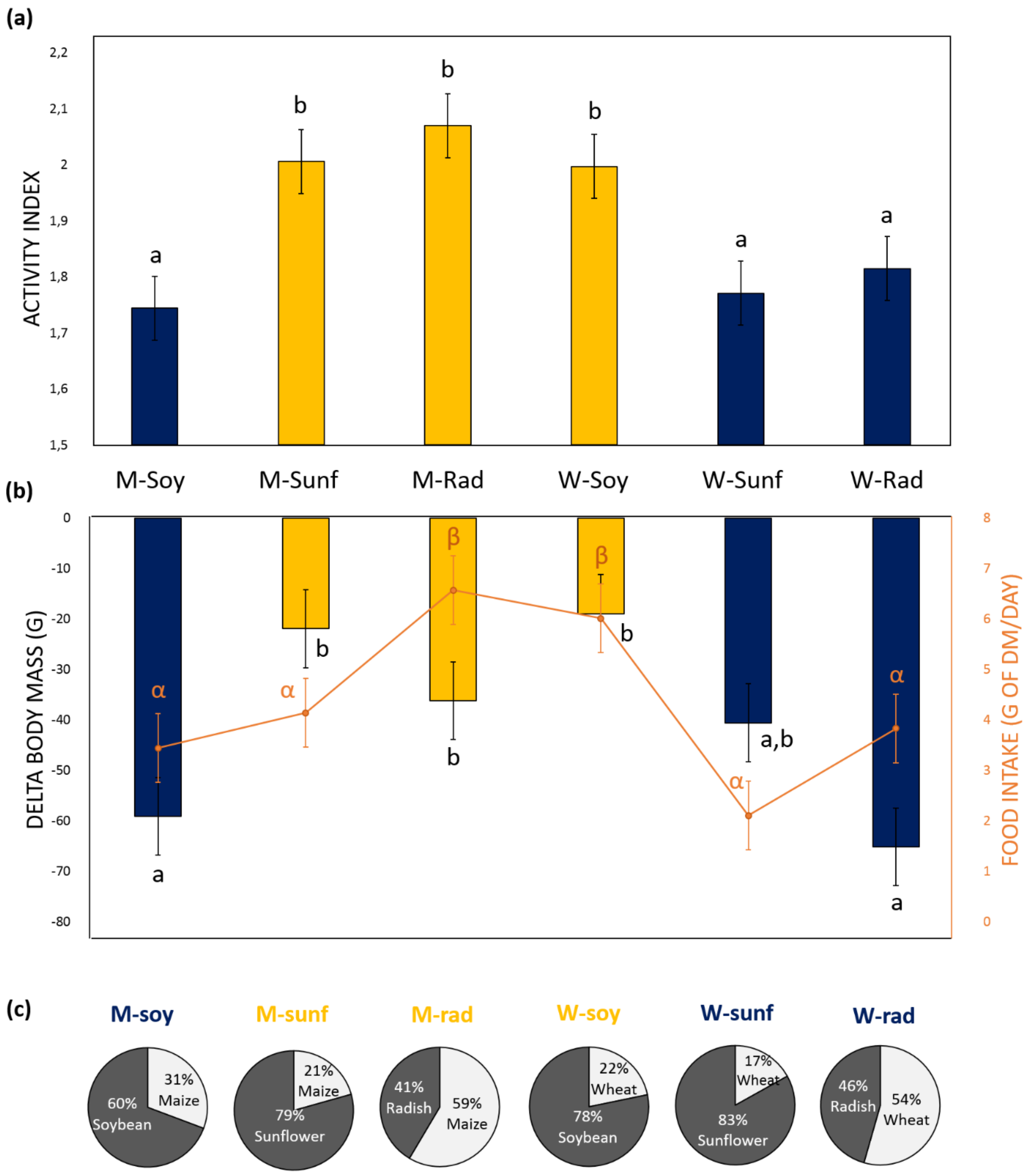

**Figure 15: Hamsters' activity index (a), body mass loss and daily food intake (b) and food preferences (c) during hibernation according to their respective diet**. In (b) the body mass loss (in grams) and the daily food intake (in grams of dry matter per day) are represented according to the diet. Different letters mean significant differences between the diet groups, $p<0.05$ (greek letters are used for food intake).

Finally, regarding hamsters' reproductive success, informative data are presented below and seem to suggest an effect of the diet on this variable, with three diets (Msunf, Mrad and Wsoy) that would tend to more favorable than the three others (Msoy, Wsunf and Wrad; Figure 16).

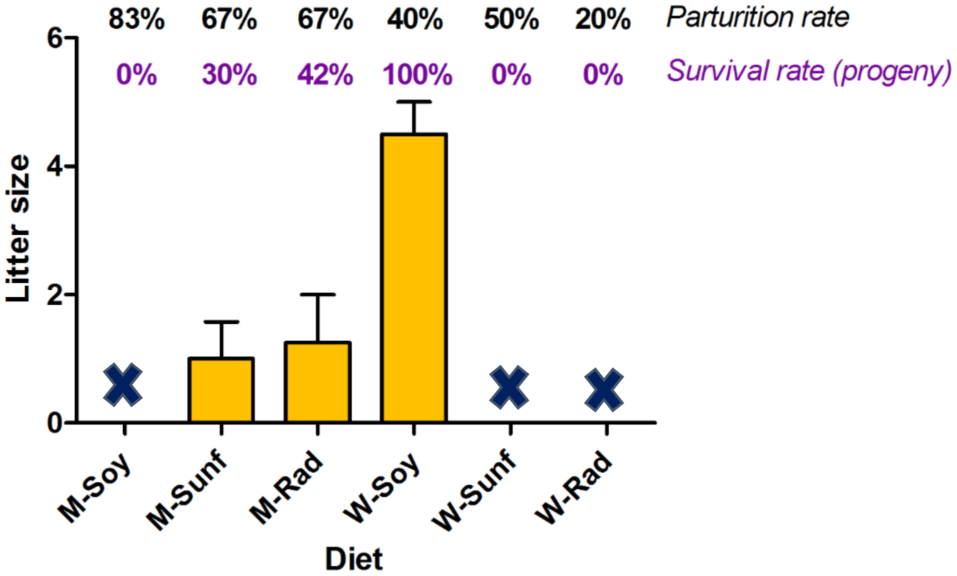

**Figure 16: Females' litter size at weaning (30 days post-parturition) according to the diet**. Parturition rate (i.e. fecundity) and pups' survival rate are given in %. The crosses mean no weaned pups in these diet groups.

## Discussion

The results of this study reveal two different hibernation strategies used by European hamsters: 1) either to remain mainly active (i.e. elevated activity index) and greatly rely on food hoards or 2) to spare energy through the use of torpor (i.e. low activity index) and therefore rely less on food hoards. It also appears that the composition of the hoards influences the use of one or the other strategy, with hamsters less torpid in three diet groups (Maize-Sunflower, Maize-Radish and Wheat-Soybean) than in the three others (Maize-Soybean, Wheat-Sunflower and Wheat-Radish).

When looking at the body mass loss compared to the amount of food ingested (see Figure 15), the Wsoy, Msunf and Wsunf diets appear to be more 'efficient' than the three others. Indeed, in the Wsoy and Msunf, hamsters have spared the most in terms of body mass compared to the three other groups. In comparison, hamsters from the Mrad diet needed to eat large amounts of food and however lost more body mass than hamsters from the Msunf and Wsoy groups. This result is probably explainable by differences in energy and protein contents between these diets. Indeed, the Wsunf diet is particularly rich in energy (see Table 15), whereas the WSoy is rich in proteins. Therefore, hamsters from these two diets could remain active, eat less and still reduce their body mass loss. In comparison, we expect that the MRad would be less rich in both energy and proteins than these two diets. Macronutrient analyses of these diets are ongoing to confirm this hypothesis. In the Wsunf diet, hamsters have lost intermediate body mass, but whereas eating less than in the Mrad diet, which was possible thanks to an increased use of torpor (low activity index). Since hamsters would not necessarily have access to *ad-libitum* food in the wild, this diet could be especially appropriated under food restrictive environmental conditions. Regarding the two remaining diets (Msoy and Wrad), hamsters' body mass loss was the highest of the 6 groups and roughly corresponded to 25% of hamsters' body mass at the onset of hibernation.

Taken altogether, the Wheat-Soybean, Maize-Sunflower and Wheat-Sunflower associations therefore appear to be more appropriate for the hibernation of this species. The Maize-Radish appears appropriate under *ad-libitum* food conditions, which is unlikely in the wild. Finally, results also highlight sex differences in terms of activity and body mass loss during hibernation, echoing what has been found in other species (Michener, 1992) and in a recent study on the European hamster (Siutz et al., 2016). Indeed, in our study, females were less torpid and have lost more body mass than males, independently of the diet. However, they have lost significantly less mass in the Wsoy, Msunf and Mrad diets than in the three others. Therefore, given that females weighing less than 200g on emergence from hibernation are not able to reproduce (Nechay et al., 1977; Vohralik, 1974), the quality/composition of the hoards appear particularly important for females' hibernation and subsequent reproduction.

Regarding hamsters' reproductive success, the tendency observed suggests that the crop-associations favorable for hibernation were also favorable for reproduction: hamsters that have displayed the lowest body mass loss have had a subsequent better reproductive success. This could also be due to the fact that in these three diet groups, animals were more active and potentially earlier ready for reproduction. Indeed, in the three 'more torpid' groups, several animals were still torpid by the 10th of April, whereas on the 27th of March, males from the 'active groups' all had well-developed testes suggesting that they already were in a reproductive state. However, since I only present informative data and preliminary results here, deeper statistical and data analyses are needed to be able to conclude. Moreover, hormonal analyses will be performed to bring more information on the reproductive state of the individuals according to their respective diet. They will first include leptin analysis, which is related to hibernation success, individuals' energetic conditions and maternal investment into reproduction (Dark, 2005; French et al., 2009; Weitten et al., 2013)). We will also perform analyses on plasma levels of testosterone and estradiol, for their respective role in mammalian reproduction (Wade and Schneider, 1992; Yohannes et al., 2016; Zambrano et al., 2005), their implications in tryptophan metabolism to vitamin B3 (Shibata and Toda, 1997) and in aging (Barrett and Richardson, 2011; Breu et al., 2011; Calado et al., 2009). Interestingly, the effects of the diets on hamsters' hibernation and reproduction do not seem to be explained by the macronutrient content of the diets (see summarizing figure 11 above). For example, the two protein-rich diets (Maize-Soybean and Wheat-Soybean), in which hamsters ingested approximately the same proportion of Soybean compared to wheat and maize (see Figure 12 (c)) had opposite effects on both hibernation and reproduction. Furthermore, in the Maize-Soybean diet, high rates of infanticides (as those described in **study 2**) have been recorded, suggesting an imbalance between tryptophan and other amino acids such as leucine or lysine in this diet. Therefore, if verified, this would suggest that the quantity of protein or fatty acids per se does not explain the differences in hibernation strategies and reproductive success between the different groups of our study. Rather, the type of proteins (e.g. representativeness and ratio between all the essential amino acids), the type of fatty acids (e.g. representativeness and ratio between the different fatty acids) or the content in vitamins/antioxidants would be more important. Measurements of the macronutrient and micronutrient contents of these diets are therefore ongoing.

Finally, the diet is a key component of the trade-offs between reproduction, self-maintenance and growth in many taxa (Aiken et al., 2016; Shine, 2013; Stearns, 1992; Zera and Harshman, 2001). for instance, diet's contents in PUFA or antioxidant are known to influence aging, by modulating oxidative stress or telomerase activity, ultimately leading to an increased attrition of telomeres (i.e. the protective caps of chromosomes (Das, 2014; Giroud et al., 2014; Hoogenboom et al., 2012; Muhammad Anjum et al., 2012; Munro and Thomas, 2004). Therefore, oxidative stress measurements are ongoing and data on telomere dynamics in the hamsters of this study are presented in Box 2.

## Conclusion

Interestingly, sunflower and radish therefore appear to be globally (i.e. when taking into account hibernation and reproduction) favorable when associated to maize, whereas they are unfavorable when associated to wheat. In contrast, soybean is favorable when associated to wheat but unfavorable when associated to maize. The main conclusion of this study would therefore be that there is not one 'favorable' crop for the species, but rather favorable crop-associations.

# Box 2 – Telomere dynamics in the European hamster during hibernation and reproduction

*Unpublished data*[1]

## Short introduction

Hibernation is widely known as a strategy used to face periods of low energy availability and cold ambient temperatures ($T_a$) (Humphries et al., 2003a; Ruf and Geiser, 2015). However, it has also been identified as part of a "slow-paced" mammalian life-history tactic and associated to retarded physiological ageing, low rates of fecundity and increased maximum longevity (Ruf et al., 2012; Turbill et al., 2011). Most hibernators – *i.e*. fat-storing hibernators – fatten *prior* to hibernation season and rely to a large extent on stored fat as an energy source in winter (Geiser, 2013; Humphries et al., 2003b). Conversely, food-storing hibernators (some hamster species and chipmunks) hoard very large amounts of food in their burrow *prior* to winter and rely exclusively on these hoards as an energy source throughout winter (Humphries et al., 2003b; Munro et al., 2008; Nechay et al., 1977; Wall, 1990). Finally, some species are intermediate and use one or the other strategy depending on the environmental conditions (Humphries et al., 2003b).

Hibernation is defined as a succession of torpor phases, during which animals decrease their metabolism and their body temperature to a large extent ($T_b$~$T_a$+1°C) (Geiser, 2013; Ruf and Geiser, 2015). Although torpor is expressed throughout winter, hibernators do not remain constantly torpid. Bouts of torpor are indeed interrupted by periodic rewarming (arousals), followed by euthermic phases ($T_b$~37°C). Arousals and euthermic phases together use most of the energy required during hibernation (Geiser, 2013; Ruf and Geiser, 2015). However, rewarming also triggers transient and high levels of oxidative stress (Orr et al., 2010), known to negatively affect telomere length (*i.e*. the protective caps of chromosomes, involved in cell and whole organism ageing processes (Bize et al., 2009; Harley et al., 1990; Heidinger et al., 2012; Monaghan and Haussmann, 2006). Recently, the duration of the euthermic phases and the number of arousals during hibernation have been positively correlated to the erosion of telomeres in fat-storing hibernators (Giroud et al., 2014; Hoelzl et al., 2016). These studies therefore suggest that both the time spent euthermic (Giroud et al., 2014) and the number of arousals (Hoelzl et al., 2016) have deleterious impact on the ageing rate of individuals.

Interestingly, fat- and food-storing hibernators exhibit important variations in their pattern of hibernation (Humphries et al., 2003a, 2003b; Ruf and Geiser, 2015). Fat-storing hibernators express long (~ 200h) and deep ($T_b$ min<1°C) bouts of torpor compared to food-storing hibernators (~ 90h and $T_b$ min~4°C). Consequently, food-storing hibernators arouse more frequently (~ 21 times (Humphries et al., 2011)) and spend more time euthermic (>24h) than fat-storing hibernators (that arouse ~12 times (Humphries et al., 2011) and for a mean duration <12h). Therefore, we could expect that food-storing hibernators would display faster rates of aging compared to fat-storing hibernators, whereas they should display lower aging rates than non-hibernating species. Up-to-date, no studies have investigated for the telomere dynamics of food-storing hibernators. We therefore conducted this study on 84 hamsters (42 females and 42 males from the study 4, fed on 6 crop-based diets), seeking to understand 1) whether the telomere dynamics of food-storing hibernators was similar to the one observed in fat-storing hibernators and 2) whether the diets provided to hamsters could influence this dynamic (Das, 2014; Giroud et al., 2014; Hoelzl et al., 2016).

## General approach and Methodology

### *Sampling*

A total of 84 hamsters (42 ♂ and 42 ♀) were sampled (i.e. ear tissue punches), at three periods: 1) at the onset of hibernation (OH), post-hibernation (PH) and post-reproduction (PR). The ear tissue punches (~2mm$^2$) were collected in the same time than blood (**study 4**) under 2% isoflurane anaesthesia. Before sampling, the area was cleaned with ethanol and scissors were burnt at 200°C between each sampling, to prevent from any DNA contamination for later telomere analyses. Hamsters were distributed into 6 diet groups: Maize-Radish, Maize-Soybean, Maize-Sunflower, Wheat-Radish, Wheat-Soybean and Wheat-Sunflower (for more details, see Methodology section of the **study 4**).

### *Relative Telomere Length measurements*

Telomere measurements were carried out on DNA extracted from the ear tissue punches using the DNeasy Blood & Tissue Kit, according to the manufacturer's protocol (Qiagen, Germany). The Relative Telomere length measurements were conducted by quantitative real-time amplification (Cawthon, 2002) adapted for ear tissues and for the European hamster. This measurement is based on the determination of a number of amplification cycles necessary to detect a lower threshold of fluorescent signal. It estimates the quantity of telomere repeated DNA sequences relative to a reference gene (c-Myc) that was predetermined to be non-variable in copy number (non-VCN) among the sample genomes (Smith et al., 2011). Primer sequences for the non-VCN gene (5’-GAG GGC CAA GTT GGA CAG TG-3’, and 5’-TTG CGG TTG TTG CTG ATC TG-3’) amplified a 54 bp portion of the c-Myc proto-oncogene. Forward and reverse telomeric primers were 5’-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3’ and 5’- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3’, respectively.

Briefly (see Supplementary Material and Methods in (Turbill et al., 2013) for details): 20 ng of DNA was added to 10 µl of Sensifast no-ROX 2 x mastermix (Bioline, Germany); with an extra 200 µM final concentration of dNTPs; and forward and reverse primers at 0.3 µM. Cycling conditions included: 1) an initial hold at 95°C for 5 mins; 2) 40 cycles of 94°C for 10 secs, 58°C (telo) or 61°C (c-Myc) for 20 secs, and 72°C for 20 secs with fluorescence readings recorded at this step; and 3) a final melt step was at 56°C to 95°C in 1°C increments. Cycling was performed on a Rotorgene Q real-time PCR instrument (Qiagen, Germany). Measurements were carried-out in triplicates (for each of the three time points, OH, PH and PR) and associated to a no-template control (water) and five replicates of the control DNA sample. Multiple single-use aliquots of the extracted DNA from the control sample were stored at -20°C until use in each run (all nine runs were conducted over 14 days). As a measure of repeatability, the mean coefficient of variation (CoV) of the three replicate reactions for each sample was 5.0% for the telomere reaction and 5.4% for the c-Myc reaction.

Calculation of RTL was carried-out using the software LinRegPCR (V.12.5) to estimate the mean PCR efficiency of 82.8% for telomeres and 89.2% for c-Myc, and the following formula:

$$RTL_S = (E^{CT}\wedge Cq^{CT} / E^{ST}\wedge Cq^{ST}) / (E^{CN}\wedge Cq^{CN} / E^{SN}\wedge Cq^{SN})^{*}$$

* Where: $RTL_S$ = relative telomere length for a given sample; $E^{CT}$ = mean efficiency of the control telomere reactions; $Cq^{CT}$ = mean cycle threshold of the control telomere replicates; $E^{ST}$ = mean efficiency of all sample telomere reactions; $Cq^{ST}$ = mean cycle threshold of within-sample telomere replicates; $E^{CN}$ = mean efficiency of the control non-VCN gene reactions; $Cq^{CN}$ = mean cycle threshold of the control non-VCN gene replicates; $E^{SN}$ = average efficiency of all sample non-VCN gene reactions; and $Cq^{SN}$ = average cycle threshold of within-sample non-VCN gene replicates (Supplementary Material and Methods in (Turbill et al., 2013).

*Data analyses*

Data presented are means ± SEM. Normality of the residuals was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using a Levene test. We first looked at the effect of the diet on the hamsters' *relative telomere length* during hibernation and reproduction using a Linear Mixed Model (LMM), with the diet, the period (OH, PH and PR), the sex and the diet*sex interactions as fixed factors. The identity of the individuals was included as a random factor for repeated measures on the same individuals. We then investigated how the diet affected the *changes in telomere length (delta telomere length, ΔTL)* during hibernation ($\Delta TL_{hibernation}$ = telomere length$^{PH}$ - telomere length$^{OH}$) and during reproduction ($\Delta TL_{reproduction}$ = telomere length$^{PH}$ - telomere length$^{OH}$) using linear models (LM). We included the diet, the sex and the diet*sex interaction as fixed factors. The 'previous' telomere lengths (i.e. at OH for $\Delta TL_{hibernation}$ and at PH for $\Delta TL_{reproduction}$) were included as covariates in these models. Final model selection was based on the best AICc (Akaike information criterion for small samples) value. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA).

### Preliminary results

The relative telomere length was significantly affected by the period ($F_{2,108}$ = 42.5, $p < 0.001$), but not by the sex ($F_{1,75}$ = 0.6, $p = 0.5$), the diet ($F_{5,73}$ = 0.2, $p = 0.9$) or the sex*diet (F = 5,74 = 0.4, p = 0.8) interaction. Post-hoc analyses indicated that hamsters elongated their telomere between PH and PR (i.e. during reproduction; Figure 17A).

Regarding the $\Delta TL_{hibernation}$ (i.e. the change in telomere length during hibernation) was significantly affected by the diet (**Figure 17B**, $F_{5,43} = 2.7$, $p = 0.035$) and by the Telomere Length at OH ($F_{1,43} = 49.3$, $p < 0.001$; $R^2 = 0.48$). However, we found no effect of the sex on this variable ($F_{1,43} = 1.9$, $p = 0.18$).

Finally, when looking at the $\Delta TL_{reproduction}$ (i.e. the change in telomere length from PH to PR), we found a significant effect of the Telomere length at PH ($F_{1,34} = 24.2$, $p < 0.001$), but no effects of the diet (**Figure 17C**, $F_{5,34} = 0.3$, $p = 0.9$), the sex ($F_{1,34} = 0.8$, $p = 0.4$) or the sex*diet interaction ($F_{4,34} = 0.1$, $p = 0.9$) on this variable.

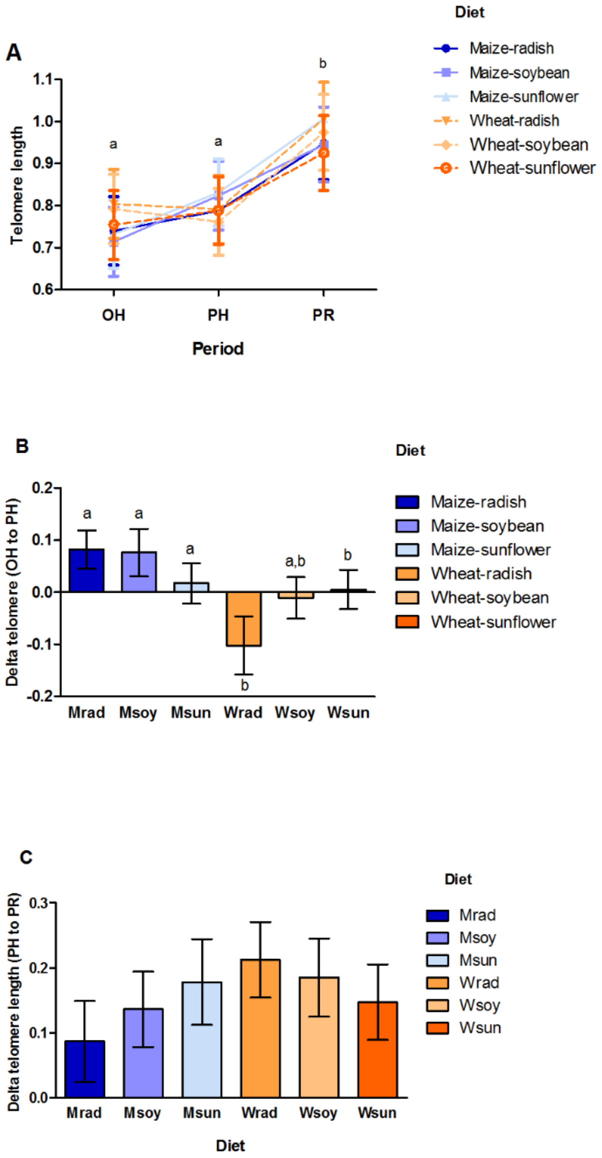

**Figure 17: Hamsters telomere dynamics during hibernation and reproduction.** Different letters indicate significant differences between the periods (in A) or the diet groups (in B), $p < 0.05$.

## Short discussion

Preliminary results show that the diet had a slight effect on telomere dynamics in the European hamster. Indeed, it has affected the *Δ*telomere during hibernation (especially in the Wrad group, which showed the greatest body mass loss during hibernation). However, the diet had no effect on the *Δ*telomere during reproduction, period during which all the hamsters displayed an elongation of their telomeres, independently of their respective diet. Therefore, at the end of the experiment (post-reproductive period), hamsters displayed the same telomere length between the different diet groups.

When looking at the *Δ*TL during hibernation, we can observe that in the Wheat-Radish diet, hamsters have lost significantly more telomeres than in the 5 other diets. Moreover, in two of these 5 diet groups (Maize-Radish and Maize-Soybean, see **study 4** for more details), hamsters have even elongated their telomeres. Interestingly, the telomere dynamics do not seem to follow the dynamic of body mass loss or activity index recorded in this experiment (Figure 12A and 12B, **study 4**), conversely to what has been found in the fat-storing Edible dormouse (*Glis glis*) (Turbill et al., 2013). This could be explained by recent findings, suggesting that it is not the duration spent in torpor per se that would affect the most telomere shortening during hibernation, but the rate of re-warming (arousal) from torpor (Hoelzl et al., 2016). However, given that our activity index only provides an estimation of the use of torpor, we would not be able to investigate for this correlation.

Surprisingly, we found no sex differences in telomere dynamics in the European hamster, which contrasts with the tremendous literature on the sex differences in telomere length and telomere shortening in many vertebrates, including mammals ((Barrett and Richardson, 2011; Benetos et al., 2001; Cherif et al., 2003; Mayer et al., 2006; Nawrot et al., 2004; Tissier et al., 2014); but see (Bize et al., 2009; Heidinger et al., 2012)). Deeper data analyses are needed to better understand these results as well as further investigation on the diets composition in micro- and macronutrients to better understand the diet effect on the $\Delta TL_{\text{hibernation}}$.

Regarding the elongation of telomeres observed during reproduction, this could be an extreme example of the shielding hypothesis stating that mothers protect themselves from oxidative stress during fetal and lactating periods in order to protect their offspring (Blount et al., 2015).

Because hibernating species have the ability to restore their telomeres, this protection may favor telomere elongation during other periods of the year. This finding echoes what has previously been found in adults Edible dormice, where a telomere elongation has also been recorded during the active period (Hoelzl et al., 2016; Turbill et al., 2013).  However, as indicated by Hoelzl (Hoelzl et al., 2016), such an elongation was observed in dormice when they received extra food in a year of low natural food abundance. Therefore, it is likely that this pattern in our study is caused by the presence of *ad-libitum* food and the absence of foraging necessity to collect this food. Further studies under wild conditions and on a long-term basis are therefore needed to better understand the telomere dynamics of this food-storing rodent.

# Chapter 4

“Le Grand hamster est un grand stimulant de l’intelligence collective car sa préservation questionne les modes de développement agricole, urbain et économique d’aujourd’hui et de demain” J. Eidenschenck 2017

# From Eco-physiology to conservation: limits, perspectives and recommendations

Content:

1. Summary of the main results and advantages of our set-ups
2. Limits to our studies, discussion and scientific perspectives
3. Applied perspectives and recommendations for the conservation of the European hamster: how to improve maize and wheat monocultures?

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## 1. Summary of the main results and advantages of our set-ups

Results of the **study 1** bring new information on the drivers that affect the French population of European hamster, especially regarding the implication of winter rainfall. It also brings new leads on the effects of maize monoculture on this species. However, the correlative results presented in this study cannot be used to explain the underlying mechanisms by which both increasing winter rainfall and maize monoculture could affect the body mass of the species. Therefore, we carried-out experimental studies in captive (**study 2** and **study 4**) and semi-captive conditions (**study 3**) to further investigate for the effects of maize on the biology of the hamster. These studies are contributing to the understanding of how maize can affect hamsters' fitness from a nutritional perspective. For instance, in the **study 2**, pups from females fed on maize-based diets had reduced body mass at weaning compared to pups from females fed on wheat-worm or maize-worm-vitamin B3. Moreover, in the **study 4**, females fed on maize-based diets have lost on average more body mass than females fed on wheat-based diets, except for females fed on the maize-sunflower diet. However, we reported in the **study 1** that the production of sunflower collapsed in the Alsace in favor of maize and wheat (**study 1**). These body mass reduction could partially explain the 21% decrease of wild hamsters' body mass during the last century, reported in the **study 1.** Therefore, taken altogether, results of these studies support our initial hypothesis that maize is nutritionally unsuitable for hamsters. Because these studies were conducted under controlled conditions and on a short-term basis, they allow to rapidly produce results applicable to the hamsters' conservation.

## 2. Limits and scientific perspectives to our studies

### a) The limits to our studies

*Short-term studies*

Nonetheless, this short-term approach can also have some disadvantages. Indeed, we are lacking of information of the long-term effects of being fed on monotonous diets of maize and wheat. Specifically, we do not know how it could affect the life expectancy or the long-term fitness of the individuals, and more specifically of the population. For example, two females were able to wean proper litters of 4-6 pups when fed on a maize-worm diet in 2015 (**study 2**). We could assume that on a long-term basis, these two females would be favored over females that could not wean any litters or over females that weaned litters of pups showing harsh and diarrhea problems and cases of siblicides. Indeed, the plasticity of these two females when fed on maize-based diets compared to the other females that were greatly affected by the vitamin B3 deficiency allowed them to produce more pups, and if heritable, this plasticity could allow pups to be less susceptible to these kind of monotonous diets. If these pups are also able to reproduce properly when fed on maize-based diets, the negative effects of maize-based diets at the population level would consequently be reduced. The strong selection that would occur in such case would select these phenotypes in the population, producing a case of adaptive phenotypic plasticity (Ghalambor et al. 2007). Nonetheless, pups of these females had hair deficiency and lower body mass at weaning, which could affect their survival or reproductive rate, even more under wild conditions. Moreover, on the 21 females tested (for a total of 42 reproductive attempt), only 2 females displayed such cases of plasticity towards maize-based diets, and only during their second reproductive attempt. Moreover, females were fed ad-libitum and were in controlled conditions with reduced maintenance and foraging costs. Therefore, even though these females could be favored on a long-term basis under controlled conditions, this would be very unlikely under wild conditions when they have to face high immunity, stress and foraging costs.

### *Choice of the food items*

Moreover, our studies were mostly based on seeds and as suggested by the quite low reproductive success of the **study 4** (even in the best crop-association groups), it appears that feeding hamsters with two types of seed, even when supplemented with earthworms, is not nutritionally sufficient during the reproductive period. Indeed, even in the 3 best crop-associations (Wheat-Soybean, Maize-Radish and Maize-Sunflower), the reproductive success was quite low for this species, given the low parturition rates of females in the W-Soy diet (40%), and the small litter-sizes of females in the M-Sunf and M-Rad diets (2 pups/litter at weaning). This suggests that a food item is potentially missing, likely green parts of plants (i.e. 'weeds') given their intensive consumption in spring/summer recorded in older literature in the wild (Gorecki & Grygielska 1975). This assumption is supported by the results of the **study 3** where hamsters consumed a very large proportions of adventive plants during reproduction and where the greater diversity observed in the mixed enclosures (compared to monoculture enclosures) was associated to a better reproductive success.

### *Artificial mating system and studies under controlled or small-scale conditions*

Another point that needs to be considered is the artificial mating of our experiment, with each male being randomly paired with a female. In the wild, males are polygamous and visit the burrow of the females (Nechay et al. 1977; Fenyk-Melody 2012) starting at emergence from hibernation and until late July. If females are receptive, then mating occur (Vohralik 1974). Otherwise the male is chased out of the burrow (Nechay et al. 1977). Given that mate choice and males quality affect reproductive success (Davies 1989; Insel & Hulihan 1995; Webster et al. 1995; Bergstrom & Real 2000), the artificial mating process implemented in captive conditions might therefore impact hamsters' reproductive success. However, given that the same set-up was used between the different diet groups, this should not have affected the differences observed between the diets.

Results of all these studies would therefore benefit to be confirmed by large-scale experiments in the wild. Indeed, the experiments carried-out in outside enclosures (study 3) highlight that, despite what was found in captive conditions in the study 2, wheat was not more favorable than maize for hamsters' reproductive success.  This result is potentially due to increased costs associated to varying time and energy budgets in semi-wild compared to captive conditions, that could affect hamsters' trade-offs between reproduction and self-maintenance (Stearns 1992). Therefore, results obtained in the study 4 – regarding the favorable crop-associations to the hamster – would ultimately have to be complemented by semi-wild or wild experiments to confirm their benefic effects onto the hamsters' fitness.  Regarding results of the study 3, they would gain to be confirmed by large-scale experiments. Indeed, the scale (i.e. the size of the plots) and the spatial structure in this experiment are not entirely representative of the agro-ecosystems. Given that insects and the European hamster can be expected to strongly respond to landscape structure and processes (Benton et al. 2003; Marshall et al. 2006), this could affect, and likely compound with the results obtained in the study 3.  However, even though large-size field experiments could allow to deepen the results observed in these studies and to investigate and better understand the underlying mechanisms and the complexity of the biotic and abiotic factors affecting hamsters' population in the French agro-ecosystems, data of recent/current field investigations already tend to support our findings.

### b) Data from the field supporting our results

#### *Maize consumption and maternal infanticide*

The French National Hunting & Wildlife Agency (ONCFS) is conducting survey on hamsters' population in the Alsace since 1999. They have mostly worked on investigating for burrow number to estimate the population size of the species and since 2013, with the launch of the ALISTER program, they started to investigate for the effects of farming practices on females' reproductive success. Recent data from their field investigations bring several evidence of females and pups feeding on maize, specifically from pictures taken by camera-traps (Figure 18). These pictures highlight that hamsters consume as well green part of the plants (Figure 18a) as seeds (Figure 18b, c and d). They also pinpoint that maize can be consumed at different stage of the life-cycle and at different seasons (spring and summer) and that it is hoarded in later summer/fall (Figure 18c and d). They are also known to feed on maize seeding in April (Gorecki & Grygielska 1975; Nechay et al. 1977).

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**Figure 18: Pictures of hamsters eating maize in the fields (Alsace, France).** In **(a)**, a female eating the green parts of the plant (spring). In **(b)** a corn cob excavated by a hamster from its burrow after harvest (July 2016) in a wheat field. In **(c)** an adult and in **(d)** a juvenile bring corn cobs into their burrow (late summer). © Charlotte Kourkgy – ONCFS.

Although the quantity of maize in hamsters' diet in French farmland remain to be determined, these pictures undoubtedly show that hamsters are feeding on maize at different periods of the year and are also hoarding maize cobs in their burrow. Recently, some cases of cannibalism/infanticides have been recorded in a maize field in the Alsace (Figure 19). The observation shown in this figure 19 is obviously anecdotal and does not give insight on the propensity of this behavior in maize fields but still pinpoints that the results of the study 2 appear to concord, at least partially, with some events observed in French farmland.

**Figure 19: Female hamster eating her progeny in a maize field in Alsace (France).** © Charlotte Kourkgy – ONCFS.

*Reproductive success of wild hamsters in the Alsace*

The average litter size of wild hamster females in 2014 in the Alsace region was of 2.5±1.4 pups/litter (Kourkgy & Eidenschenck 2015) which echoes the average litter size of females from the study 2 (2.4±1.2/litter), fed on diets mimicking the ones of wild hamsters in current French farmland. However, the litter-size might not be the most reliable proxy to compare results in captivity and in the wild. Indeed, in the field, the litter size is currently estimated by camera-traps (see Figure 20) at proximity of the burrows and might under-estimate the number of pups per litter (Kourkgy & Eidenschenck 2015). Indeed, each burrow has several exits (see Figure 7, page 36) and the pups, that are not morphologically distinguishable, do not necessarily exit altogether, and often disperse before being all captured to be marked. Therefore, the number of litters/female appears to be more reliable to compare reproductive rate between captive and field studies. It is estimated to be of 0.75 litter/female in Alsace (Kourkgy & Eidenschenck 2015) and ranged from 0.7-0.9 litter/female in our studies, which remains extremely similar and highlight a reproductive rate well below the rate of the species in Europe (historically: 2-3 litters/female (Nechay et al. 1977), currently from 1.6 to 2.2 litter/female) or of captive females fed on a conventional diet (1.8-2.2 litter/female in our breeding unit). However, given that we rarely reproduce females three times in our breeding-unit, this last number needs to be interpreted cautiously.


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**Figure 20: Camera trap device (left) placed at the entrance of the burrow to detect the pups exit (right).** Extracted from (Kourkgy & Eidenschenck 2015)).

### c) Research perspectives

As indicated above, some analyses from the study 4 are ongoing, namely regarding hamsters' hormonal and oxidative status during hibernation and reproduction depending on their respective diets. These data should provide more information which would allow to have better idea of the trade-offs faced by hamsters during this experiment.

### *Seed conservation in the burrow*

Regarding the crop food items *per se*, studies investigating for seeds conservation in wild conditions are essential, even more given the increasing winter rainfall recorded in the study 1 that could affect food moisture in the burrow. For example, moldy maize is containing mycotoxins such as the T2-toxin (Schoental 1978), which is known to cause important cardiovascular lesions and tumors of the digestive organs and of the brain in rats, similar to those described in Pellagra. Moreover, the symptoms caused by this mycotoxin are aggravated in individuals of low nutritional status (Schoental 1978). Therefore, seed conservation appear to be a major parameter of hoard quality and it would be important to favor food items that can prevent the deterioration of the hoards to guaranty a good hibernation. Further studies should also focus onto integrating other food items in the "hoards" during hibernation, such as tubers (potatoes, beets…) known to be part of hamsters' diet and hoards during winter from historical records (Nechay et al. 1977). There is also a need for more information on the nutritional properties of germinated parts of plants ant their importance for the hamster, especially in spring. Therefore, future studies should aim at investigating this aspect and namely in which extent weeds such as dandelion (greatly consumed by the hamster in the past (Gorecki & Grygielska 1975) could improve the reproductive success of the species.

### *Diet composition of wild hamsters in the Alsace*

Regarding studies in field conditions, the ONCFS is currently conducting investigation on the effects of the protective cover on the behavior and the mortality rates of the species and on the effects of innovative agronomic techniques (aiming to increase the diversity of the cover in farmland) on its reproductive success. In this context, research should also aim at investigating for the diet of the species, using investigation of pouches composition (Hufnagl et al. 2010) or more reliably using stable isotopes (Ben-David et al. 1997; Dalerum & Angerbjörn 2005). Another central parameter that should be investigated is the effects of agricultural techniques and monotonous diets on the immune system of the hamsters, which has been largely uninvestigated up-to-now. Intensive agriculture and monotonous diets are known to have reduced the immunity of bees and bats, leading to major collapse in their populations (Blehert et al. 2008; Allen et al. 2009; Goulson et al. 2015). Given that blood sampling are carried-out at each capture (~300µL), I would recommend to use part of this blood to investigate for antimicrobial capability of blood (Kriengwatana et al. 2014) or the immune challenge faced by the hamsters (Lochmiller & Deerenberg 2000). However, even though more studies are needed on the effects of monoculture crops on the fitness of this species, and onto how it can affect its reproduction, its immunity, its behavior or its mortality rates, given the size of hamsters' populations in France it appears urgent to implement some of the results in the studies 1-4 into the current conservation plans for the species.

## 3. Applied perspectives and recommendations for the conservation of the European hamster: how to improve maize and wheat monocultures?

In this section I will develop some recommendations for the conservation of the European hamster based on the results of the studies 1-4. More specifically, I will present different options to improve maize and wheat monocultures and more broadly the agricultural landscape in the Alsace.

### a) Conventional maize and wheat monocultures in the Alsace

Maize is sown by mid-April in the Alsace and only starts to present a cover between May and June (**Figure 21a**). Therefore, when hamsters emerge from hibernation (in April), they have no protective and feeding covers, for up to 2 months. Maize is subsequently harvested in October and the plots are usually left uncultivated during winter and in most of the cases, they are sown again with maize in spring (in 73% of the cases, 6-7 years of maize are cultivated successively (Revel-Mouroz 2015)). Wheat, on the other hand is usually sown in December (i.e. hamsters hibernate at this period) and by the end of March, it presents a cover ranging from 30 to 60 cm height (see **Figure 21b**). Wheat is harvested during the first half of July (i.e. during the hamsters' reproductive period) and in most cases the plot is left uncultivated for maize sowing the next spring.

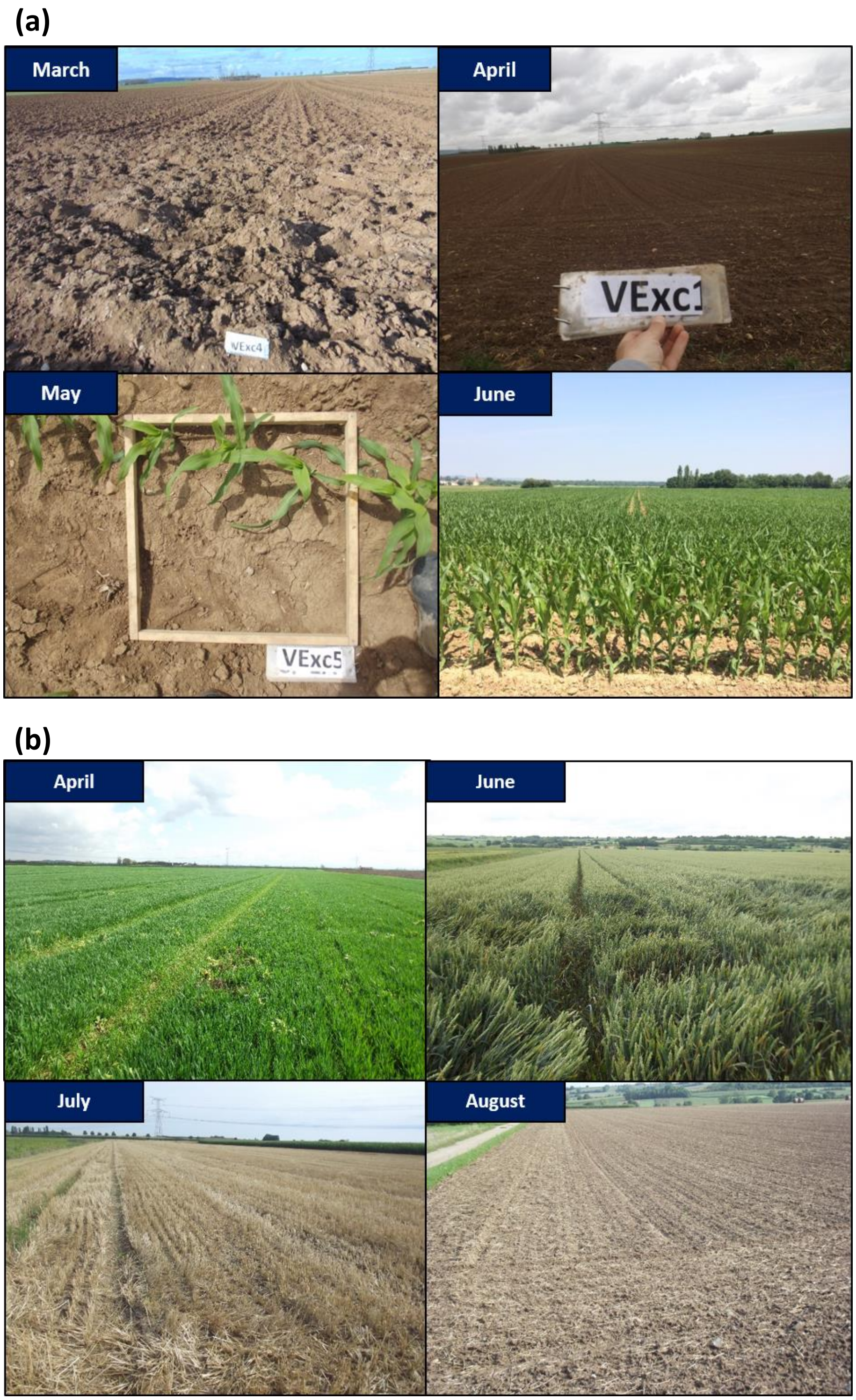


**Figure 21: Pictures of maize fields (a) and wheat fields (b) in the Alsace.** Pictures are represented from March to June for maize in **(a)**, and from April to August for wheat in **(b)**, to highlight the critical periods for the hamster in both crops. © Charlotte Kourkgy – ONCFS

### b) Improving these monocultures: different solutions

Several options/approaches can be used to improve maize and wheat monocultures and to prevent for the occurrence of the behavioral and reproductive problems recorded in the studies 2-4. These approaches could consist into **i)** restoring crop rotations and inter-crop covers, **ii)** restoring field margins or **iii)** developing techniques allowing to sow maize or wheat inside another cover.

#### *Restoring crop rotations and inter-crop cover*

Crop rotations have disappeared from agricultural landscapes during the Green Revolution (Naylor 1996; Evans 1997). In the Alsace, the three main cropping system are: a) no rotation (7 years of maize in a row), b) 6 years of maize + one year of wheat and c) 6 years of maize + 1 year of beat (Revel-Mouroz 2015). Given that rotational cropping systems are beneficial for farmland biodiversity, but also for fixing soil Nitrogen and for soil stability and fertility (Heckenbenner & De Pontbriand 2011; Kremen & Miles 2012; de Vries et al. 2013), the restoration of crop rotation is therefore a central approach of ecological restoration in agro-ecosystems.

Regarding wheat, based on the results of the **study 4** and on data on inter-crops cover (Heckenbenner & De Pontbriand 2011; CARA Bourgogne 2012), I would recommend the rotation described in **Figure 22** (adapted from (Heckenbenner & De Pontbriand 2011; CARA Bourgogne 2012), which would allow hamsters to make hoards similar to the favorable wheat-soybean crop-association in the study 4, whereas benefiting farmland wildlife as a whole. Moreover, given the low reproductive successes observed in the study 4 suggesting that hamsters need adventive plants during reproduction, work needs to be done to increase their occurrence in farmland. They could be derived from either organic farming or agro-ecology, where the use of herbicides to destroy the intercrop covers is replaced by mechanical destruction.

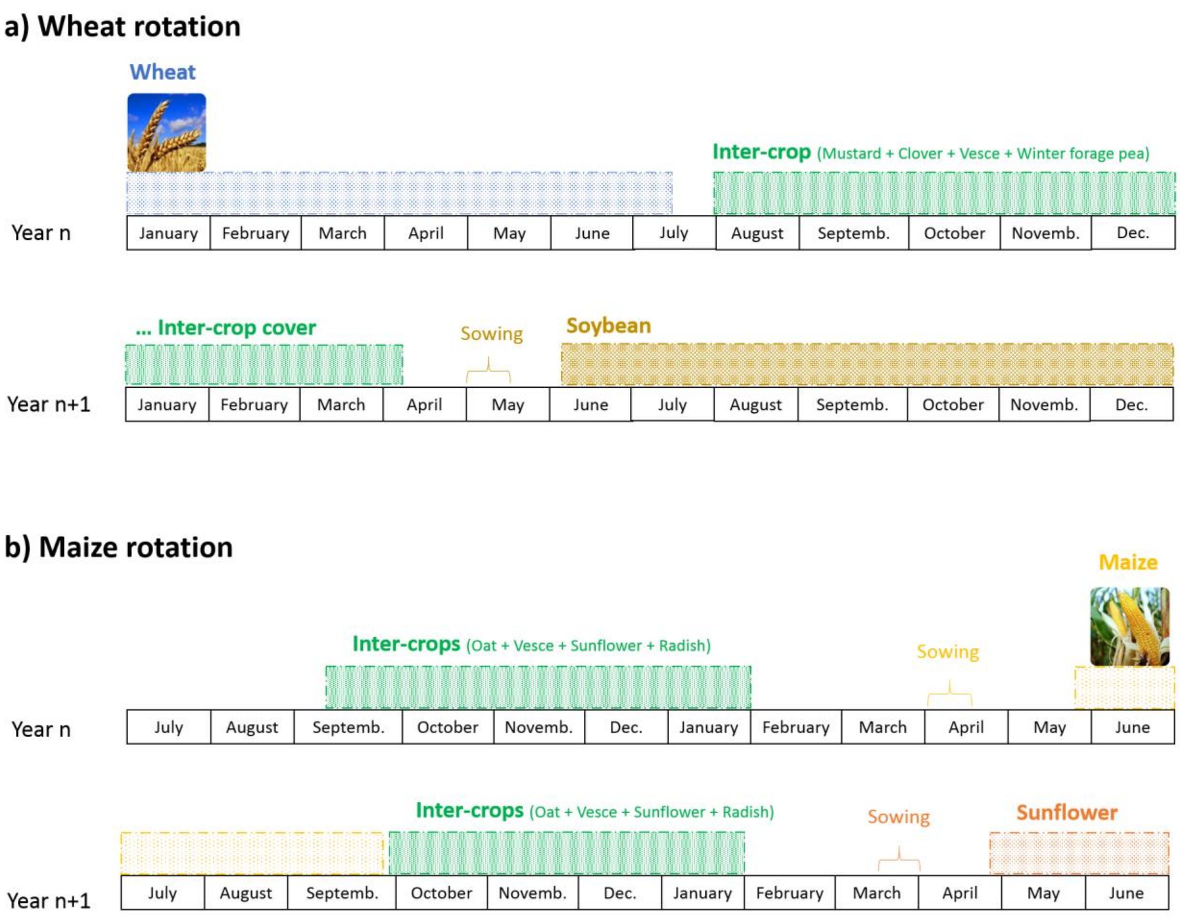


**Figure 22**: **Examples of recommended crop rotations with wheat (a) and maize (b).** Dates of sowing are taken from (Acta - Les institus techniques Agricoles 2017) and inter-crop cover compositions are adapted from results of the study 4 and (Heckenbenner & De Pontbriand 2011; CARA Bourgogne 2012).

However, even though these crop rotations associated to inter-crops would allow to increase the crop diversity at the landscape level or throughout the year, they do not fix the problems of a lack of cover in spring before maize sowing or in summer after wheat harvest (Figure 22). This is mostly owing to the fact that the inter-crop cover has to be destructed 2 months before sowing spring crops (Heckenbenner & De Pontbriand 2011), which takes several weeks to grow. Regarding wheat, these inter-crops are generally sown in July, after harvest, and by the time they grow, farmland wildlife would already face predation or feeding issues. Therefore, crop-rotations are not sufficient in solving the problem of a lack of protective and feeding cover for farmland wildlife and would need to be associated to the restoration of field margins (that would allow both protective and feeding covers after harvest or until the crop grows) or the sowing of spring crops inside the winter inter-crop cover.

### *Restoring field margins*

In order to enhance biodiversity in farmland, many research recommend to restore edges (Young 2000; Rey Benayas et al. 2008) and field margins (Bäckman & Tiainen 2002; Marshall & Moonen 2002; Vickery et al. 2009), beneficial for a wide range of species. However, these margins are known to be both beneficial and damageable to crop yields. Indeed, they can be a source of weeds (that would spread into crops) or of faunal pest species. Nonetheless, if diverse, they might also be reservoirs of a variety of pollinators  or of natural enemies to pests, both beneficial to crop yields (Altieri 1999; Marshall & Moonen 2002). However, despite their important role in providing nesting sites and protective cover from predators for many farmland birds and small mammals (Wilson et al. 1999; Marshall & Moonen 2002), field margins can also, in some cases, attract predators (Altieri 1999) and would therefore become detrimental. Therefore, it is of the upmost importance to adapt the field margins to: a) the field size, b) the landscape structure (mainly the mosaic of crops already implemented in the landscape), c) the type of crop and d) the known interaction/competition between the targeted monoculture crops to improve (e.g. maize or wheat) and the sowing to use for the margins.

Regarding wheat and maize monocultures in the Alsace, the recommendations made below take into account the results of the **studies 3 and 4** of this manuscript, associated to the results of several studies/reviews on the brown hare (Petrovan et al. 2013), pollinators (Bäckman & Tiainen 2002; Decourtye et al. 2010) and farmland birds (Vickery et al. 2009). Based on all these studies, and given the relatively small size of the fields in the Alsace (i.e. 1.5 ha on average), I would recommend the implementation of field margins ranging from 3-5 meters width, with a minimum of 4-5 species in the margin-sowing (e.g. see Table 6). The sowing should *at minima* contain soybean for wheat field margins, and sunflower + radish for maize field margins.

| Monoculture | Plot's size | Margin's width | Sowing 1 | Period 1 | Sowing 2 | Period 2 |
|---|---|---|---|---|---|---|
| Wheat | > 1 ha | 5 meters | Radish, Alfalfa, Winter oat and forage pea. | Fall & Winter | Soybean, Clover, Poppy, Sorghum & Sunflower | Spring Summer |
|  | < 1 ha | 3 meters |  |  |  |  |
| Maize | > 1 ha | 5 meters | Radish, Sunfl., Buckwheat, Vesce and Rape | Summer & Fall | Barley, Radish, Alfalfa & Winter forage pea | Winter Spring |
|  | < 1 ha | 3 meters |  |  |  |  |

**Table 6: Examples of sowing for wheat and maize field margins**. Recommended minimum margin's width are given for plots of less than 1 ha and for plots of more than 1ha.

Nonetheless, given the important inter-relations between crop management, margins quality and the fauna and flora diversity, the relatively small size of these margins, or the importance of landscape structures in ecological processes, the restoration of margins is just one of many approaches to improve biodiversity at the local scale. Moreover, one type of margin cannot cover the need for all taxa at all season (Benton et al. 2003; Vickery et al. 2009). Therefore, it appears undeniable that the restoration of field margins cannot be a substitute to other approaches aiming at improving agro-ecosystems by re-implementing techniques more respectful of the environment, and which would allow to sow a crop directly inside another crop (e.g. maize inside a winter inter-crop cover, or a summer inter-crop cover inside wheat).

### c) A promising technique to sow maize and wheat inside another cover

#### *Approach: the strip-till technique*

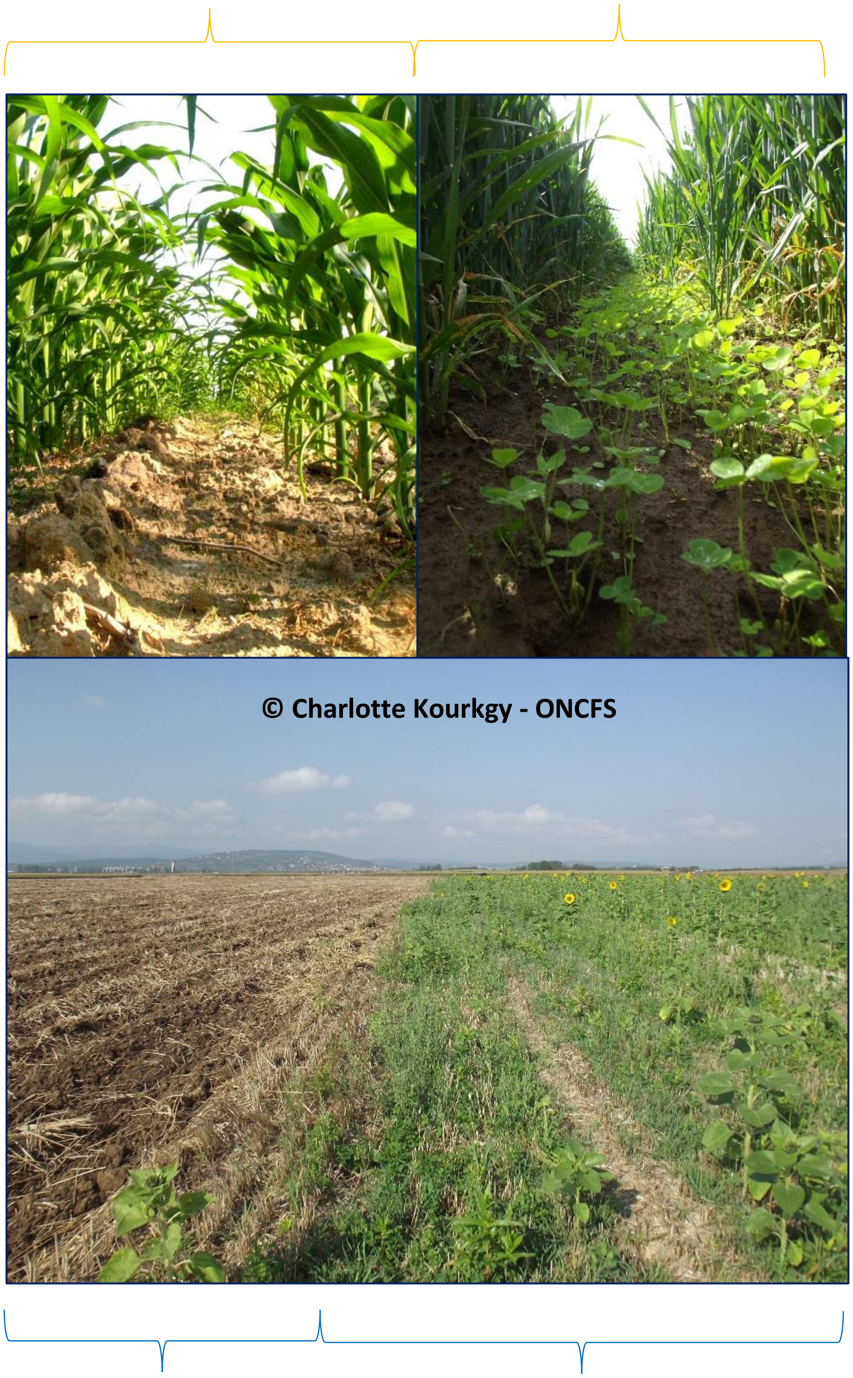
Such an example of technique is the strip-tillage, or 'strip-till'. This technique, consists into plowing the soil only on the sowing line (**Figure 23**) (Dobrecourt et al. 2012). Several variants of this technique exist, with different depth and period of tillage. This technique, developed in the US, has been largely used to sow a spring crop (corn or soybean) into a winter crop (Baker & Griffis 2005; Halvorson et al. 2011). In the fall, a tillage operation takes place during which a narrow strip in the center of each row is tilled, whereas the residues are left in place. In the next spring, seeds are planted into the bare, tilled strips (Baker & Griffis 2005). In addition, localized application of fertilizer can be implemented, or a vegetative cover can be sown in the inter-lines. The implementation of this technique need to be studied and adapted to each crops, soil types and climates to find proper associations of crops that would not concur for soil nutrients and water.

**Figure 23: The strip-till technique**. © ALISTER - CARA

In the framework of the ALISTER project, the Alsacian Agricultural Agency (CARA) has worked onto the development of the strip-till to improve maize and wheat monocultures in the Alsace. For 3 years now, they have conducted agronomic testing to associate clover or a mix between oat and clover (and in some cases sunflower) to maize and wheat. For maize, these associations have been conducted using the strip-till technique. For wheat, they have been conducted by implementing clover directly inside wheat or by sowing a multi-species inter-crop just after harvest (see Figure 24). Part of this work has been done in collaboration with the French Wildlife Agency (ONCFS), responsible of hamsters monitoring in the wild. One of the main goal was to investigate whether these approaches would benefit the hamster in terms of reproductive success and survival. Therefore, the ONCFS monitored hamsters inside 'improved field plots' and inside 'conventional field plots' (see examples on the Figure 24).

Conventional
maize (June. 2016)

‘Strip-till’ maize
(June. 2016)



© Charlotte Kourkgy - ONCFS

Conventional
wheat (Sept. 2016)

‘Strip-till’ wheat
(Sept. 2016)

**Figure 24**: Pictures illustrating conventional maize (top-left) and improved maize by the strip-till technique (top-right) as well as conventional wheat (bottom-left) and improved wheat (bottom-right), in which a multi species intercrop has been implemented (< than 10days after harvest). © ONCFS

Data regarding the effects of this strip-till approach on the hamsters' fitness are currently under analyses. In parallel, the group of protection of mammalian species in the Alsace (GEPMA) has conducted, in partnership with the league for bird protection (LPO), a survey of the farmland skylark (*Alauda arvensis*) to investigate whether the strip-till could benefit this bird species. Finally, in collaboration with the ONCFS and the CARA, I have also developed a survey on collembolla, soil invertebrates that are important eco-indicator of the soil quality and fertility. This analysis was developed in collaboration with Cédric De Vigne (Université Catholique de Lille), specialist of collembolla and carapid species. Data are shown below (**Table 7**).

| **FIELD SITE** | **SPECIFIC RICHNESS** | | **ABUNDANCE** | |
|---|---|---|---|---|
| | Conventional | Strip-till | Conventional | Strip-till |
| ALTORF | 5 | 12 | 13 | 52 |
| ERNOLSHEIM | 5 | 13 | 12 | 81 |
| OBERNAI | 12 | 13 | 30 | 84 |
| OBERNAI 2 | 5 | 13 | 7 | 53 |

**Table 7**: Specific richness and abundance of collembolla in conventional and 'strip-till' maize fields in 2016.

These data highlight the major benefits of the strip-till on these arthropod species. Indeed, we can observe that the specific richness of collembolla is up to 2-fold greater whereas collembolla abundance is 3-fold to 4-fold greater in strip-till compared to conventional maize fields.

### *Applications and recommendations for the strip-till*

Based on the results of the studies 3 and 4, and in order to improve wheat monoculture, the strip-till can be used to implement the wheat-soybean association. This approach is currently being tested by the CARA, in collaboration with a farmer: wheat has been sown in 3x3 m plots in the winter 2016-2017. In the early spring 2017, the strip-till will allow to plow lines in this winter wheat, in preparation for soybean sowing in April. If the technique appears successful (allowing good wheat and soybean yields), this will be applied onto real-size plots and associated to a hamster monitoring. The advantage of this association is to provide: **i)** an agronomic and economic interest for farmers, **ii)** a benefit for hamsters (and potentially other farmland animals) and **iii)** a good combination of crops (one winter crop and one spring crop), easily combinable and likely not in competition for nutrients and water.

Regarding maize, based on the results of the studies 2-4 and as indicated above in this chapter, I would recommend to sow a mixed-cover between the lines of maize containing at minima sunflower and forage radish. One example of mix-sowing could be the association between forage radish + oats + white mustard + phacelia + sunflowers to be sown in September. This sowing has been developed for spring sunflower by SLY France and a conservation agriculture association named 'Biodiversity, Agriculture, Soil and Environment' (BASE) (Zinkand 2012). This example is illustrated on the adjacent **Figure 25**.

**Figure 25: picture of a strip-tillage in a cover of forage radishes, oats, white mustard, phacelia, sunflowers and legumes adapted to spring crops.** © SLY France

*The three sisters*

Another option for improving maize would be to focus on the implementation of the strip-till based on the 'Three Sisters'. This ancestral technique (see **Figure 26**) developed and used by Native Americans to grow corn consists into associating corn (maize), beans and squash. From a mechanistic point-of-view, the beans develop by climbing onto corn, which offers corn with a better stability. In parallel, the squash covers the soil which benefits corn by providing an ecological 'lute' against weeds and maintaining a soil humidity (preventing corn from desiccation during periods of drought). Moreover, beans and squashes provide natural nitrogen inputs avoiding the use of fertilizers. Finally, the association of these three crops also led to the creation of the 'Three Sisters Squash' soup, which associates beans, maize and squashes. This meal has likely been developed by Native Americans to prevent the occurrence of Pellagra (Brenton & Paine 2000).

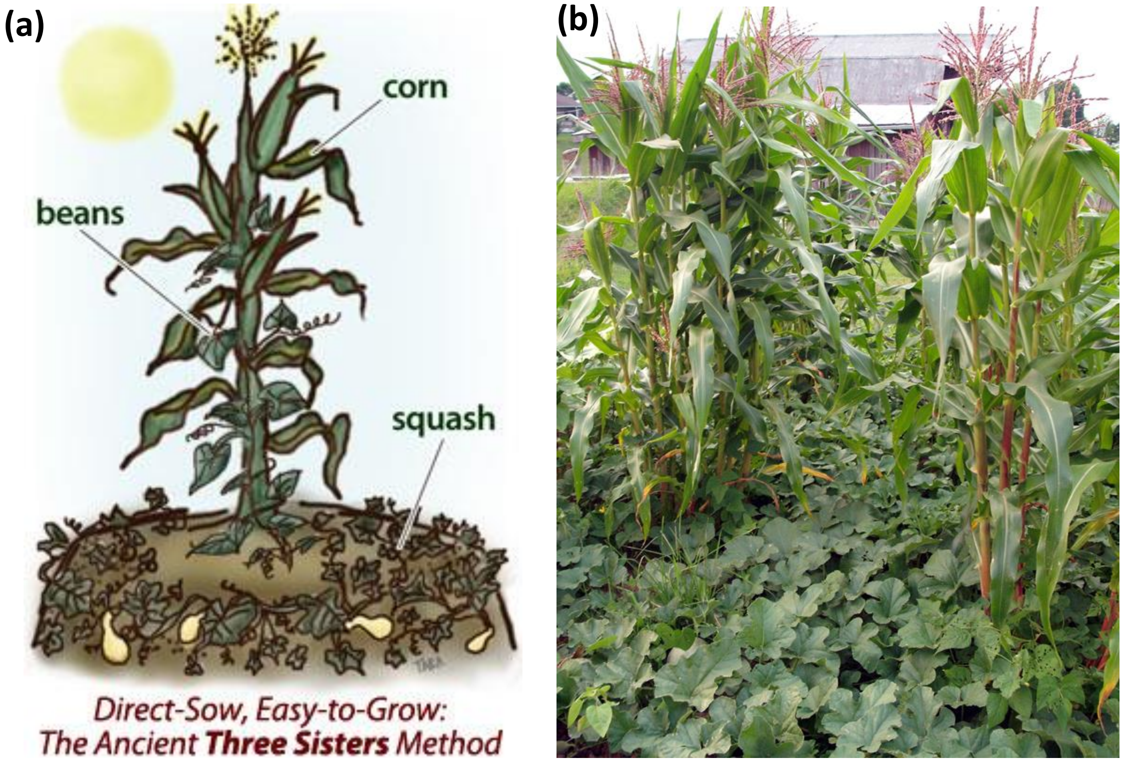
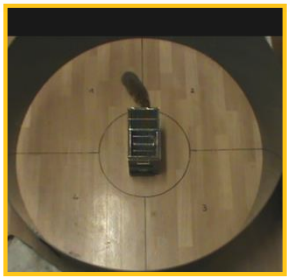


Figure 26: **The Three-Sisters (corn, beans and squash), an ancestral agronomic method (a) and a potential innovative agro-ecological technique (b).** © google images.

This method has largely been considered as 'un-adapted' to current agricultural approach, as it is often associated to 'companion planting' or 'companion gardening'. However, the strip-till approach provides a good opportunity to try associating these crops in a same field, with some variants from the original Native American technique. For example, in Southern France, corn is cultivated in association with white beans (called the 'corn beans' (Coopérative du Haricot Tarbais 2012)). In Mexico, a recent outbreak of this approach has recently been observed, under the 'Milpa' designation (Birol et al. 2007; Bermeo et al. 2014). Interestingly, the Rodale Institute (which promotes organic agriculture) also introduces sunflower as the optimal 'Fourth sister' to be associated with beans, squash and corn (Rodale Institute 2013). This opens the path to promising experimental tests regrouping these four crops to preserve the European hamster and other farmland biodiversity in the Alsace.

# Thematic II – Hamster antipredatory behavior and the anti-predation device

## Towards an improvement of wildlife underpasses



# Chapter 5

*« Antipredatory behaviors have great potential as management tools that may significantly improve conservation and management programs»*

Berger et al. 2016.

## An anti-predation device to facilitate and secure hamsters' crossing in wildlife underpasses

Content:

© Drawing – LeenZuydgeest2015

## 1. General approach

With soaring habitat fragmentation, ecologists are striving to find solutions to restore ecological corridors and to reconnect isolated populations. One measure consists in building wildlife underpasses or bridges to allow animals to safely cross motorways. But the size and shapes of these passages are generally not adapted to the crossing of small mammals. Moreover, wildlife underpasses and culverts are known to be used by these species but also by their predators. Therefore, in order to prevent these passages to become ecological traps, we built an anti-predation device to ensure the safe crossing of small mammals. This device was specifically developed to facilitate and secure the crossing of the European hamster (*Cricetus cricetus*). The implementation of this kind of new prototypes could be divided into different phases: 1) the lab tests of basic design features (**study 5**); 2) the validation of the prototype efficiency under controlled conditions (**study 6**); 3) the real-size field tests and the large scale tests under field conditions and 4) post-construction monitoring. I address the point 3) in the **chapter 6** (**box 4**) whereas the points 4), which is developed in collaboration with Jonathan Jumeau (a PhD student) is briefly developed in the **chapter 7** of this manuscript.

In the **study 5**, I present the prototype of our anti-predation device, its spontaneous use by captive hamsters and how it could serve as a 'specific passage' facilitating the safe crossing of small animals in wildlife underpasses. Three batches of tests were conducted to select for the anti-predation prototype which maximized the crossing of hamsters of various body sizes and body masses (see **Figure 27**). The **study 6** features European hamsters' perception of predation and confirms the efficiency of our anti-predation prototype under controlled condition. In this study, the hamsters' response towards cats' urine and the presence of a predator (the European ferret, *Mustela putorius furo*) was investigated. We also tested whether the presence of this predator indeed pushed hamster to use the anti-predation device as a refuge. In that aim, two experiments were carried-out: one with a ferret in a cage, and the second with a mobile ferret).

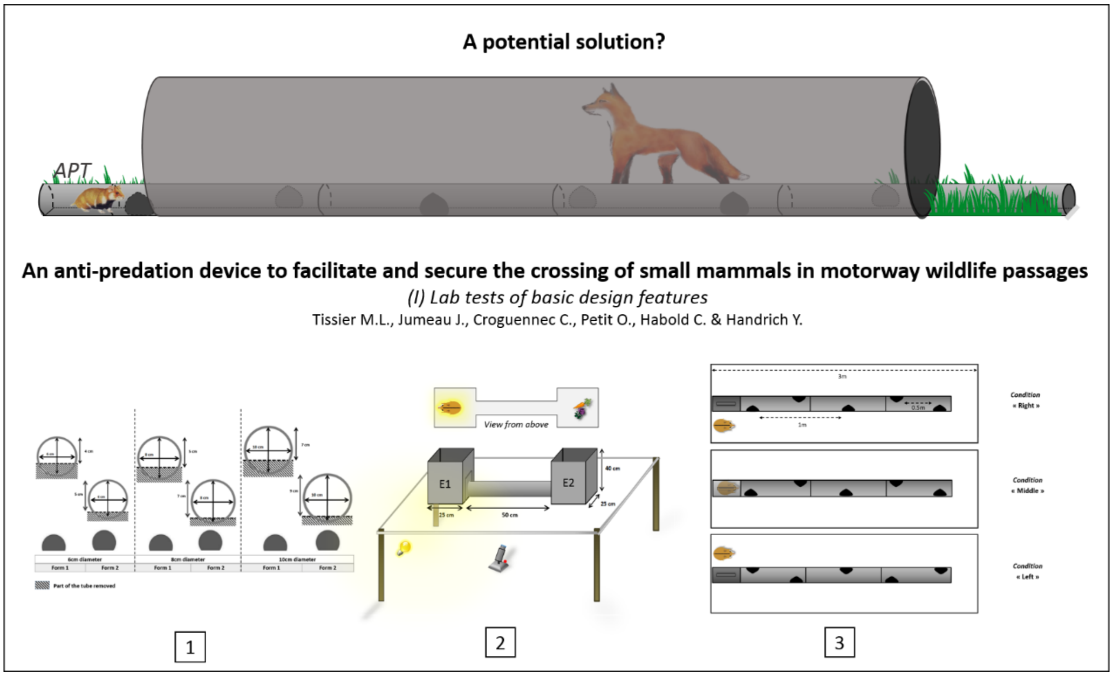


**Figure 27: graphical abstract summarizing the approach and the tests carried-out in the study 5.** In a first step (1 and 2), we investigated for the best features (shape and size) of the anti-predation device. Then (3), we studied whether hamsters spontaneously used the device in an arena mimicking a wildlife underpass under a predator-free condition. All these tests were carried-out under controlled conditions.

## 2. Summary of the main results and short discussion

The anti-predation device developed and described in the **study 5** is of the sub-tunnel type, mimicking the galleries of the European hamster in the wild (Marquet 2014). The different tests conducted allowed us to select for an anti-predation tube ('APT') with a 10 cm-diameter, a curved shape and lateral openings, optimizing the crossing (number, speed and turn-over) of a large body-size range of hamsters. The different tests conducted in the **study 6** highlight that hamsters showed many behavioral characteristics of boldness (Réale et al. 2007): inspection of the predator, aggressiveness, mobbing, direct attacks towards the predator (only in males) and finally high latency before going into a refuge (i.e. the APT). We found great inter-individual differences in the use of the APT by hamsters (which could not solely be explained by the sex of the individuals). However, most of the individuals did not use the APT when the predator (i.e. the European ferret) was in a cage at proximity. They rather spent significantly more time close to the predator. These behavioral responses could highlight that the hamsters did not perceive an actual risk of predation, possibly because the predator was 'non-mobile' (limited by the cage). Moreover, a study has shown that golden hamsters were able to perceive whether the predator were previously fed on hamsters, mice or chicken (Apfelbach et al. 2015). Given that in this experiment, the ferret was fed on a conventional diet, hamsters' response could also be an artefact of this methodology issue. Therefore, we conducted another experiment in which the ferret was mobile, and therefore able to pursue the hamster along the passage. To prevent from any aggressions between the ferret and the hamsters, they were always separated by a horizontal grid (implemented all along the passage). Moreover, the ferret was fed with a piece of hamster corpse every morning prior to the experiment. In this last experiment, we found that the hamsters' response towards the ferret has changed. Indeed, under these conditions, hamsters significantly increased their use of the APT compared to the predator-free condition, although they still displayed aggressive behaviors and a consequent latency before entering the APT. Taken altogether, these results highlight that the hamsters' behavioral responses towards predation are sex and context dependent, obviously influenced by the predator's diet and mobility, which are likely influencing its motivation to trap hamsters.

The results of these two studies therefore bring new information on the hamsters' response towards predation in captive conditions, and highlight the boldness of this species. However, the observed responses of the hamsters in these captive conditions might not reflect their response in an actual wildlife underpass. Moreover, we failed to explain some of the inter-individual differences observed in our population by the sex or the mass of the individuals. Therefore, two main questions arise: 1) how to explain the differences of behavioral reactions when faced to predation cues at the intra-species level? And 2) would the APT really allow the restoration of a connectivity in the wild, i.e. increase the dispersion rates throughout the underpasses and reduces the predation rates? These two questions will be addressed in the chapter 6 (box 3 and box 4) of this manuscript.

# Study 5 – The anti-predation tube (ATP): lab tests of basic design features.

*Published* [1]

*A domestic cat eating a common vole captured inside a wildlife underpass – Alsace (France)*

*A European hamster nesting inside a wildlife underpass – Alsace*

© Jonathan Jumeau

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[1] Tissier M. L., Jumeau J., Croguennec C., Petit O., Habold C. & Handrich Y. (2016). An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features. *Ecological Engineering*, *95*, 738-742

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Short communication

# An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features

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ABSTRACT

A great number of wildlife underpasses are used to mitigate the environmental impact of urbanization and road infrastructure expansion, thus restoring ecological connectivity. However, the simultaneous use of these structures by small mammals and their predators could result in increased predation rates in these passages or lead small mammals to avoid using them. This would be particularly harmful to small populations or threatened species such as the European hamster (*Cricetus cricetus*). To overcome this problem and to provide lateral escape opportunities along the length of the underpasses, we developed an anti-predation tube. We tested the features (shape and size) of this device under laboratory conditions and validated its use by captive European hamsters. Our results reveal that the optimal anti-predation tube has a diameter of 10 cm, a curved shape and lateral openings. This device will be tested under field conditions to validate its efficiency to protect small mammals using wildlife underpasses. If confirmed, this system could considerably improve crossing conditions in bigger tunnels and on bridges such as agricultural under- or overpasses, which have been unsuitable for small animals until now.

## 1. Introduction

The high demographic growth of human populations has produced soaring urbanization and road infrastructure development since the beginning of the 20th century (Seiler and Folkeson, 2006), causing substantial habitat loss. The development of the road infrastructure entails the accidental killing of animals by vehicles and causes fragmentation, leading to the isolation of wild populations, the loss of genetic diversity and border effects with a consequent repercussion on population dynamics and survival (Coffin, 2007; Frankham et al., 2002; Haddad et al., 2015). These negative effects are particularly harmful to endangered or small populations (Frankham et al., 2002; Jaeger and Fahrig, 2004), which are highly sensitive to environmental stochasticity (Courchamp et al., 1999; De Roos et al., 2003).

The European hamster (*Cricetus cricetus*) is one such species. It is critically endangered in Western-Europe (Villemey et al., 2013), and the French area of this species (*i.e.* in the Alsace Region) has decreased by 94% since 1972 to current levels of less than 1500 individuals (Reiners et al., 2014). The road transport network developed at an alarmingly high rate during the same period (Carsignol, 2006, 2005; Saussol and Pineau, 2007). In Alsace, a major motorway project is currently underway in one of the relict population core areas of this species (Dantec, 2014). In order to avoid the isolation of wild individuals and take mitigation and compensation measures for road construction, wildlife under- and overpasses have been built to restore connectivity in Alsace (DREAL, 2011; Gilbert-Norton et al., 2010; Saussol and Pineau, 2007). These structures – and other non-specific passages such as culverts, which are known to be suitable for the crossing of small mammals (Mata et al., 2008) – allow the dispersion and migration of a wide range of species (Carsignol, 2006; Forman et al., 2002; Mata et al., 2008). The simultaneous presence of prey species (*e.g.* small mammals such as rodents and shrews) and their predators (*i.e.* fox, cats, mustelids) in wildlife underpasses (Carsignol, 2005; Grilo et al., 2008; Little, 2003; Little et al., 2002; Mata et al., 2008) entails an

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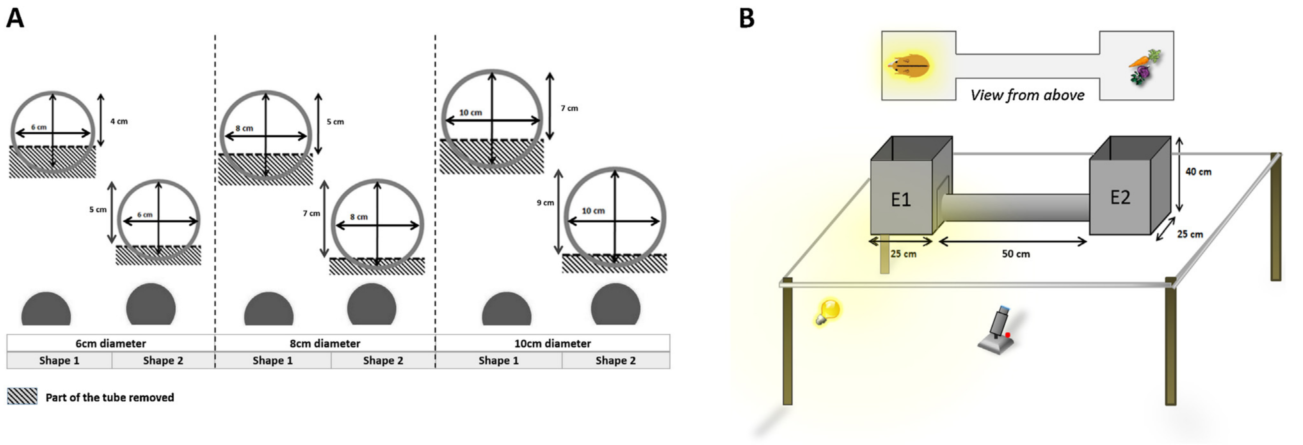

**Fig. 1.** Experimental design to validate the optimal shape and size of the APT. The three diameters and two shapes tested in Experiment 1 (6 combinations) are shown in **A**. The design in which each hamster was tested for the 6 combinations is shown in **B**.

increased predation risk for small mammals using these infrastructures. Consequently, small mammals may avoid these underpasses (Ruiz-Capillas et al., 2013) which might become potential ecological traps (Little, 2003). Although this idea has been widely debated (Little et al., 2002), particularly with regard to large mammals, very little is known regarding rodents. In the specific case of endangered species, the risk of possible predation cannot be ignored. A variety of road-crossing structures are available, and those targeting aquatic organisms and amphibians are apparently much more prevalent than those designed for terrestrial animals (Ward et al., 2015). More attention should thus be paid to providing suitable crossing structures for terrestrial animals. As indicated by Mata and collaborators (Mata et al., 2008), the adaptation or enrichment of culverts should not be ignored given the significance of these structures for certain species (such as badgers or small mammals) and their relatively low cost.

In this context, we developed a “sub-tunnel” type anti-predation device, *i.e.* a small tube to be placed inside the passages that mimics the natural galleries used by wild European hamsters. This article presents the tests carried out in captivity to determine the optimal features of this anti-predation tube (APT) and determine whether hamsters use it spontaneously. This is the preliminary step before field tests and the potential recommendation to implement this type of device on a large scale. The APT should ultimately enable hamsters and other small mammals to avoid or escape any predators they encounter in the passage by either using the APT for the entire crossing (avoidance) or by entering this tube through lateral openings when in danger in the underground passage (escape). This APT has been developed as part of a conservation program (LIFE+ Alister) for the European hamster in France which aims at restoring the connection of wild populations of the species in Alsace, France.

## 2. Material and methods

### 2.1. Animals and husbandry conditions

The experiment was performed on 18 unrelated captive European hamsters (9 males and 9 females). Males weighed on average 443 ± 139.9 g and females 352.8 ± 66.9 g. Individuals were housed in transparent Plexiglas cages (420*265*180 mm, D*W*H) and their environment was enriched with wood and shredded paper. Animals were provided with an *ad libitum* supply of water and food pellets (N° 105, from Safe, Augy, France). The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and were approved by the Ethical Committee (CREMEAS) under agreement number 02015033110486252 (A PA FIS#397). 01.

### 2.2. Experiment 1: shape and size of the APT

The goal of this first experiment was to find the ideal size and shape of the APT. We aimed to create a device that would not affect the crossing of animals larger than hamsters, and would be inaccessible to relatively small predators. Consequently, it had to be as small as possible whilst allowing the crossing of small mammals of various sizes, including the European hamster, which is one of the largest rodents in France (Fenyk-Melody, 2012). The device should also be low cost and easy to clean for a widespread use in wildlife underpasses and culverts. European hamster galleries in the wild vary in shape, and diameters range from 4 to 10 cm (Marquet, 2014). We therefore tested two shapes and three diameters of plastic PVC tubes (Fig. 1A) using the device shown in Fig. 1B (based on the Chiaroscuro tests in rodents). This first experiment used unperforated 50 cm lengths of tube. A sample of 10 hamsters (5 males and 5 females) of varying corpulence (from 249 g to 608 g) was used for this experiment. Each hamster was randomly tested for the 6 combinations of tubes for 5 min in each tube (60 tests in total) and were never tested twice on the same day. Each subject was placed at one end of the tube (E1, see Fig. 1B) while appetizing food items (onions and carrots) were placed at the other end (E2, see Fig. 1B) to motivate the animals to cross the tube. The device was cleaned with ethanol after each trial. The experimental design was set up on a transparent table to enable filming during the tests, which were carried out in low light conditions (20W-light bulb) and at ambient temperature (19 °C ± 2 °C).

### 2.3. Experiment 2: spontaneous use of the APT

The goals of this second experiment were to test whether hamsters spontaneously used the APT and the lateral entrance/exits. Our APT prototype consisted of 2.78m-long sections of PVC tubing with a diameter of 10 cm (see 3.1 of Results section for further details). Holes of the same diameter were cut on alternate sides of the tube every 1 m to allow the lateral entrance/exit of individuals. The device was then placed in an artificial enclosure that reproduced the shape of a classic wildlife underpass (1 m wide × 0.40 m high × 3 m long; Fig. 2). Eight hamsters (4 males and 4 females) were randomly placed in the enclosure for habituation one day before the trials. Each individual was then randomly tested for 12 min in 3 conditions (see Fig. 2): left (**L** = the individual was placed in the enclosure, on the left of the anti-predation device), right

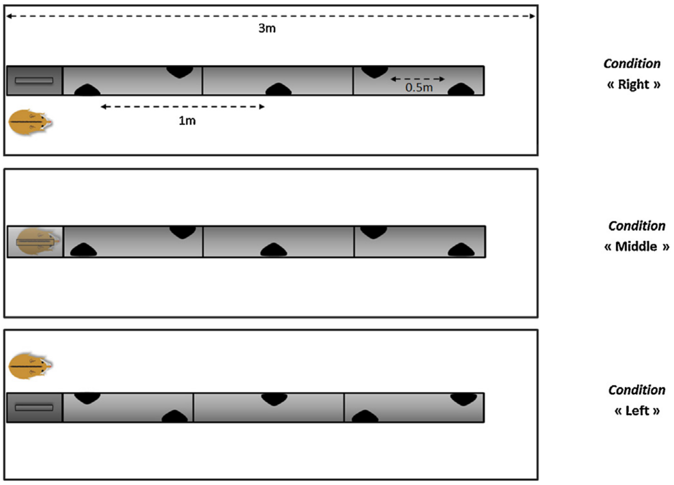

**Fig. 2.** Experimental design to validate the use of the APT by European hamsters (Experiment 2).

(**R** = the individual was placed on the right of the anti-predation device) and middle (**M** = the individual was placed inside the anti-predation device). After each trial, the enclosure and the device were cleaned with ethanol and the room was aired. Experiments were carried out and filmed under low light conditions and at an ambient temperature of 22 °C.

### *2.4. Data analyses*

Four variables were considered when selecting the optimal form and diameter of the APT (test 1, Fig. 1), namely **(i)** the latency between the beginning of the test and the first arrival of the hamster at E2; **(ii)** the duration of the first crossing from E1 to E2 (time between the first entrance in the tube and the first exit in E2); **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (representing the degree of use of the device) and **(iv)** the number of partial crossings from the end of the tube (*i.e.* only the head or the two front paws entered the tube, indicating a reluctance to cross). Variable **(ii)** was log-transformed to guarantee the normality of the residuals. Data were analyzed using a linear mixed model (LMM) composed of four fixed factors: the diameter and the shape of the tube, the sex of the individuals and the consumption of food at E2. The testing order (to control for possible habituation to the device) and the age of the individuals were included as covariates. As body mass is strongly correlated to the sex and the age of the individuals in this species (Fenyk-Melody, 2012), this variable was not included as a covariate in our models to avoid multicollinearity. We controlled for repeated measures on the same individual by including its identity as a random factor in our models.

When looking at the use of the APT by the hamsters (Test 2, Fig. 2), we considered 3 different variables: ($\mathbf{i}_2$) the time spent in the tube per hour, ($\mathbf{ii}_2$) the latency between the beginning of the test and the first entrance to the tube via a lateral opening (representing the speed of decision to use the device) and ($\mathbf{iii}_2$) the number of times an individual uses the lateral entrances in the tube per hour (representing the degree of use of the device). Variables ($\mathbf{i}_2$) and ($\mathbf{ii}_2$) were analyzed using GEE models (Generalized Estimation Equations for variables with residuals that did not follow a normal distribution; *i.e.* with binary responses or enumeration). The ($\mathbf{iii}_2$) variable was analyzed using a linear mixed model (LMM), using the identity of the individual as a random factor. Three fixed factors were included in these models, namely the test condition ("left", "right" and "middle"), the order of the tests and the sex of the individuals. For the "middle" condition (in which the hamster was placed inside the APT), the time the individual spent in the tube before the first exit was excluded from the analyses.

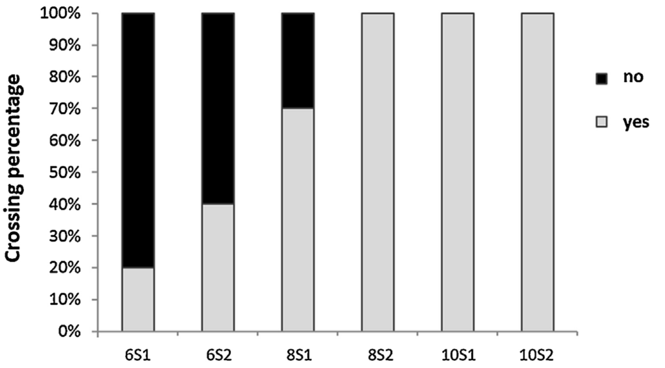

**Fig. 3.** Crossing percentage from end E1 to end E2 according to the diameter (6 cm, 8 cm and 10 cm) and the shape (S1 and S2) of the tube.

Normality was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using the Levene test. Multiple comparisons were analyzed via post-hoc LSD (least significant difference) testing. Model selections were carried out parsimoniously using an ascendant stepwise procedure combined with AICc verification (Akaike information criterion corrected for small samples). Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0., IBM Corp., Armonk, NY, released 2012), and the significance threshold was set at $p < 0.05$.

## 3. Results

### *3.1. Shape and size of the APT (Experiment 1)*

A total of 60 tests were carried out on 10 individuals (10 trials for each type of tube, Fig. 1). Hamsters only used the 6 cm diameter tube in 1/3 of the 20 tests (Fig. 3, 6S1 and 6S2). For subsequent analyses we therefore only considered tubes with diameters of 8 cm and 10 cm (*i.e.* 8S1, 8S2, 10S1 and 10S2 tubes). When the hamsters successfully crossed from E1 to E2, they did so without stopping in 98% of cases. Two females turned back, and this occurred four times in the 10 cm diameter tubes.

The diameter of the tube significantly affected **(i)** the latency between the beginning of the test and the first arrival of the hamster at E2 (Fig. 4A, $W = 269$, $p < 0.01$), which was significantly shorter in the 10 cm diameter tube. It significantly increased **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (Fig. 4B, $F_{1,22} = 82.629$, $p < 0.01$). Finally, it also had an effect on **(iv)** the number of partial crossings (indicating a reluctance to cross), which was higher in the 8 cm diameter tube than in the 10 cm diameter tube (7.1 ± 1.9 and 5.3 ± 1.9 respectively; $F_{1,18} = 7.893$, $p = 0.012$).

The shape of the tube affected **(i)** the latency before the first arrival of the hamster at E2 ($F_{1,33} = 5.333$, $p = 0.027$), which was shorter in the S2 tube than in the S1 tube (66.9 ± 12.7 s and 111.1 ± 14.4 s respectively) and **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (Fig. 4B; LMM, $F_{1,22} = 21.027$, $p < 0.01$). Finally, **(ii)** the duration of the first crossing from E1 to E2 was not affected by the diameter or the shape of the tube (LMM, $p > 0.05$). We found no effects of sex, age or testing order on the four variables (LMM, $p > 0.05$).

### *3.2. Spontaneous use of the APT and the lateral entrances/exits (Experiment 2)*

A total of 24 trials were carried out on 8 individuals in this experiment. The eight individuals entered the tube in every trial, with one male refusing to enter the tube in two of its three trials. We

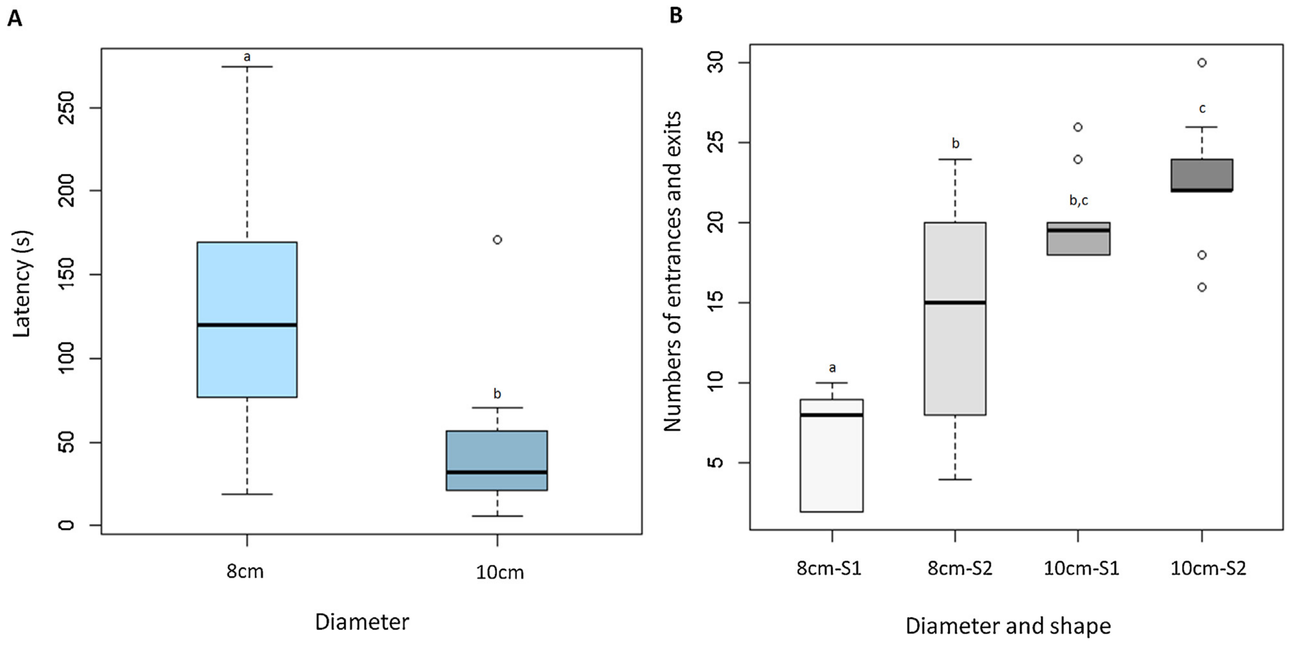

**Fig. 4.** Efficiency of the APT according to its diameter and shape (Experiment 1). **(A)** Latency between the beginning of the test and the first arrival of the hamster at E2 according to the diameter of the tube (8 cm and 10 cm) and **(B)** Number of times individuals entered or exited the tube via E1 and E2 according to the diameter (8 and 10 cm) and the shape (S1 and S2) of the tube. The different letters highlight significant differences between the groups ($p < 0.05$).

found inter-individual differences, with individuals entering the tube on average 351 ± 224 s after the start of the trial (total duration of 720s, see Section 2.3). Hamsters spent a total of 34 ± 32 s inside the tube (9% of the total test time) and made 8 ± 7 complete crossings between the tube and the artificial enclosure. We found an effect of sex on (**i**$_2$) the time spent in the tube per hour (GEE, $p = 0.035$), (**ii**$_2$) the latency between the beginning of the test and the first entrance to the tube via a lateral opening ($F_{1,6} = 29.128$, $p < 0.01$) and (**iii**$_2$) the number of times an individual uses the lateral entrances in the tube per hour (GEE, $p = 0.004$). Females spent more time inside the tube than males did (230 ± 133 s and 112.5 ± 133 s respectively), entered the tube more frequently (hourly frequency of 22.45 ± 7.4 s for females and 8.79 ± 2.5 s for males) and entered the tube sooner than males did (163.5 ± 49.7 s and 480.5 ± 50.6 s respectively for females and males). We found an effect of condition (M, R and L) on the number of times lateral entrances were used (GEE, $p < 0.01$): this number was significantly higher in the M condition than in the two others (Wilcoxon tests, $p < 0.05$).

## 4. Discussion

The results of these laboratory tests reveal that the 10S2 tube (*i.e.* diameter of 10 cm and curved shape) is the most suitable tube for the anti-predation device. Although the diameter of European hamsters galleries in the wild varies from 4 to 10 cm (Marquet, 2014), we found that 70% of the animals failed to enter the 6 cm diameter tubes, and 30% did not enter the 8S1 tube. Individuals that failed weighed more than 300 g and 400 g respectively. We can therefore conclude that only juveniles or small adults could use such small tubes. We also found that whatever the shape of the tube, individuals crossing those with a 10 cm diameter did so faster and more frequently than individuals using the 8 cm tubes. Regardless of the diameter, Shape 2 appears to be more appropriate than Shape 1, and increases the speed of the decision to cross and the frequency with which individuals use the tube.

As the European hamster is one of the biggest rodents in France (Fenyk-Melody, 2012), this tube could thus also be used by small rodents (*e.g.* voles, shrews. . .). However, the two smallest mustelids – the Stoat (*Mustela ermine)* and the least weasel (*Mustela nivalis*) – can enter galleries with diameters of less than 4 cm (Dayan and Simberloff, 1994; Gliwicz, 1988) and would therefore probably use this device as well. In this case, the APT would not entirely suppress predation pressure but would still reduce it by preventing the predation of small rodents by larger predators such as cats and foxes. Such cases of predation (*e.g.* domestic cats preying on up to 12 voles in one night) have recently been observed in monitored underpasses in the Alsace (*unpublished data*). We thus hypothesize that small mammals would globally be favored by the presence of the APT, which would allow them to avoid predators (even mustelids) by using the lateral holes to avoid any such predators in the passage. This hypothesis is currently being tested in captive and semi-natural conditions.

The second experiment reveals that all the individuals spontaneously used the device when placed in an artificial enclosure that mimicks the shape of a wildlife underpass. It was often used to cross from one side of the enclosure to the other. Results also reveal that females use the APT more than males and enter it more quickly. This could be explained by a difference in personality, as females are generally more anxious than males (Réale et al., 2007). They may therefore have sought refuge in a confined space such as the tube. It would be of interest to see whether this difference persists under natural conditions. Our next studies will investigate whether hamsters increase their use of the APT in presence of predation cues (*e.g.* proximity of predator urine or a cage containing a predator).

Following the results of our two experiments, the 10S2 tube has been selected to be placed in several wildlife underpasses and culverts in the Alsace (France). These devices will be monitored to validate the use of the APT by the European hamster and other small mammals. We will also investigate whether the use of the APT by hamsters in wildlife underpasses – which can be up to 50 m long in the Alsace – confirms the findings of this laboratory experiment. The length of the APT will be extended to one meter beyond the end of the main underground passages, with lateral exits, to reduce the risk of cats and foxes catching small mammals leaving the APT. We will also observe whether other small species use it to cross the passages.

## 5. Conclusion

This anti-predation tube is a potential tool to provide a specific passage facilitating the safe crossing of small animals within wildlife underpasses and culverts. It could also be used to enrich bigger passages (*e.g.* agricultural overpasses or wildlife bridges), that are currently unsuitable for small animals (Mata et al., 2008).

## Acknowledgments

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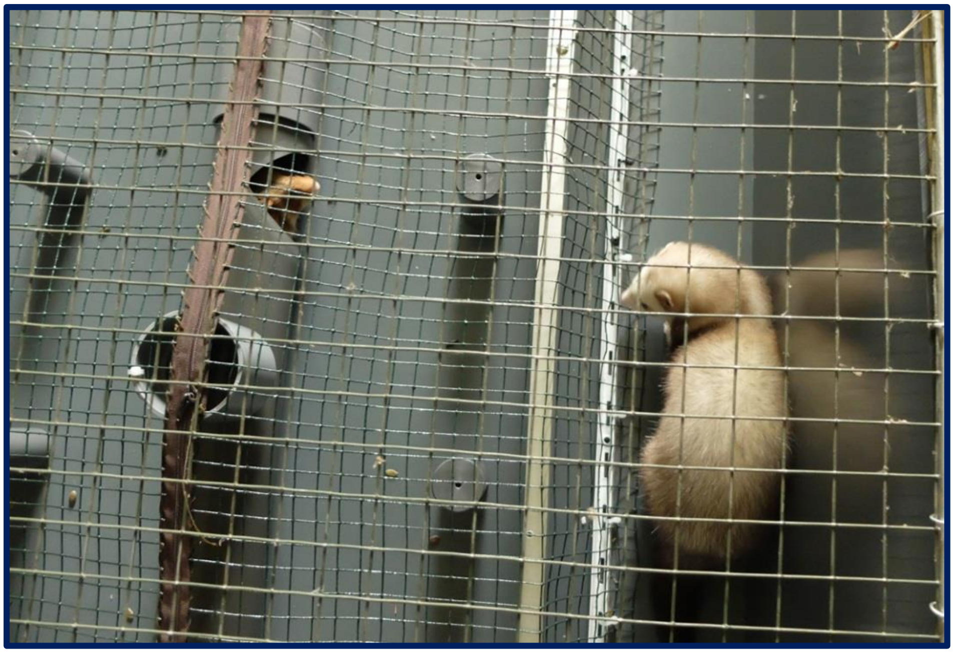
## References

Carsignol, J., (2005). Aménagements et mesures pour la petite faune.
Carsignol, J., (2006). Routes et passages à faune: 40 ans d'évolution.
Coffin, A.W., 2007. From roadkill to road ecology: a review of the ecological effects of roads. J. Transp. Geogr. 15, 396–406.
Courchamp, F., Clutton-Brock, T., Grenfell, B., 1999. Inverse density dependence and the Allee effect. Trends Ecol. Evol. 14, 405–410, http://dx.doi.org/10.1016/S0169-5347(99)01683-3.
DREAL, 2011. Modalités de mise en oeuvre des mesures compensatoires.
Dantec, Y., (2014). Autoroute A355 Grand Contournement Ouest de Strasbourg.
Dayan, T., Simberloff, D., 1994. Character Displacement, Sexual Dimorphism, and Morphological Variation among British and Irish Mustelids. Ecology 75, 1063–1073.
De Roos, A.M., Persson, L., McCauley, E., 2003. The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. Ecol. Lett. 6, 473–487, http://dx.doi.org/10.1046/j.1461-0248.2003.00458.x.
Fenyk-Melody, J., 2012. The European hamster. In: The Laboratory Rabbit Guinea Pig, Hamster, and Other Rodents. Elsevier Inc., pp. 923–933, http://dx.doi.org/10.1016/B978-0-12-380920-9.X0001-6.
Forman, R.T.T., Sperling, D., Bissonette, J.A., Clevenger, A.P., Cutshall, C.P., Dale, V.H., Fahrig, L., France, R., Goldman, C.R., Heanue, K., Jones, J.A., Swanson, F.J., Turrentine, T., Winter, T.C., 2002. Road Ecology Science and Solutions. Isl. Press.
Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge University Press.
Gilbert-Norton, L., Wilson, R., Stevens, J.R., Beard, K.H., 2010. A Meta-Analytic Review of Corridor Effectiveness. Conserv. Biol. 24, 660–668, http://dx.doi.org/10.1111/j.1523-1739.2010.01450.x.
Gliwicz, J., 1988. Sexual Dimorphism in Small Mustelids: Body Diameter Limitation. Oikos 53, 411–414.
Grilo, C., Bissonette, J. a., Santos-Reis, M., 2008. Response of carnivores to existing highway culverts and underpasses: implications for road planning and mitigation. Biodivers. Conserv. 17, 1685–1699, http://dx.doi.org/10.1007/s10531-008-9374-8.
Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A., Nicholls, A.O., Orrock, J.L., Song, D., Townshend, J.R., 2015. Habitat fragmentation and its lasting impact on Earth ecosystems. Sci. Adv., 1–9, http://dx.doi.org/10.1126/sciadv.1500052.
Jaeger, J.A.G., Fahrig, L., 2004. Effects of road fencing on population persistence. Conserv. Biol. 18, 1651–1657, http://dx.doi.org/10.1111/j.1523-1739.2004.00304.x.
Little, S.J., Harcourt, R., Clevenger, A., 2002. Do wildlife passages act as prey-traps? Biol. Conserv. 107, 135–145.
Little, S., 2003. The Influence of Predator-prey Relationships on Wildlife Passage Evaluation. ICOET, pp. 277–292.
Marquet B., (2014). Le Grand hamster *Cricetus cricetus* (Linnaeus, 1758): contribution à l'étude de l'animal et de son statut en Alsace.
Mata, C., Hervas, I., Herranz, J., Suarez, F., Malo, J.E., 2008. Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. J. Environ. Manage. 88, 407–415, http://dx.doi.org/10.1016/j.jenvman.2007.03.014.
Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol. Rev. 82, 291–318, http://dx.doi.org/10.1111/j.1469-185X.2007.00010.x.
Reiners, T.E., Eidenschenk, J., Neumann, K., Nowak, C., 2014. Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the common hamster of the French Alsace region. Mamm. Biol. 79, 240–246, http://dx.doi.org/10.1016/j.mambio.2013.10.004.
Ruiz-Capillas, P., Mata, C., Malo, J.E., 2013. Community Response of Mammalian Predators and Their Prey to Motorways: Implications for Predator-Prey Dynamics. Ecosystems 16, 617–626, http://dx.doi.org/10.1007/s10021-013-9634-7.
Saussol, J.N., Pineau, C., 2007. Biodiversité et infrastructures de transports terrestres. Sétra: note d'information. Econ. Environ. Concept 79, 1–15.
Seiler, A., Folkeson, L., (2006). Habitat fragmentation due to transportation infrastructure: COST 341 national state-of-the-art report Sweden.
Villemey, A., Besnard, A., Grandadam, J., Eidenschenck, J., 2013. Testing restocking methods for an endangered species: effects of predator exclusion and vegetation cover on common hamster (*Cricetus cricetus*) survival and reproduction. Biol. Conserv. 158, 147–154, http://dx.doi.org/10.1016/j.biocon.2012.08.007.
Ward, A.I., Dendy, J., Cowan, D.P., 2015. Mitigating impacts of roads on wildlife: an agenda for the conservation of priority European protected species in Great Britain. Eur. J. Wildl. Res. 61, 199–211, http://dx.doi.org/10.1007/s10344-015-0901-0.

# Study 6 – European hamsters' perception and reaction to predation and their consequent use of the APT as a refuge.

*Under review* [1]



*In this rebuilding of a wildlife underpass, a domestic ferret is free to pursue the hamster all along the passage and to cross the anti-predation tube by climbing a horizontal fence preventing from any aggression between the individuals.*

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[1] Tissier M. L., Bousquet C., Croguennec C., Fleitz J., Habold C., Petit O. & Handrich Y. Inter-individual differences in risk assessment and anti-predatory behaviour in an endangered species, the European hamster. *Under review in Animal Behaviour.*

# Inter-individual differences in risk assessment and anti-predatory behaviour in an endangered species, the European hamster

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## ABSTRACT

Understanding the inter-individual differences in perception and reaction to predation cues are at the base of many research in animal behaviour. More recently, anti-predatory behaviour has also been shown as being an important tool in conservation biology. It is often assumed that prey species display offensive strategies only if they have no chances of escaping the predator. However, the anti-predatory behaviour of some species remain to be studied. Here, we investigated for the first time the perception and reaction to predation of captive-reared European hamsters (*Cricetus cricetus*) towards varying predation cues. When exposed to the urine of a cat and a goat in a Y-maze test, hamsters spent more time close to the cat's scent than to the goat's scent. Then, when exposed to a non-mobile European ferret (maintained in a cage), some males displayed threatening behaviours towards the ferret. Moreover, hamsters increased the time they spent close to the ferret's cage and did not take refuge in the anti-predation tube placed (APT) in the apparatus. This APT has previously been designed to upgrade wildlife underpasses and reconnect wild populations of European hamsters, which are highly endangered throughout the continent. Finally, when exposed to a mobile ferret previously fed with hamster's corpse, hamsters displayed mobbing and aggressive behaviours towards the ferret, before taking refuge inside the APT. Taken altogether, results of this study highlight that, even with a possibility to take refuge from the predator, European hamsters first adopt a defensive strategy and suggest that the motivation of the ferret is an important driver of their decision to flee. Moreover, these results will benefit the conservation of this endangered species across Europe.

**Keywords**: boldness, mobbing, conservation, rodent, predation, prey species

## INTRODUCTION

Predation is a strong selective force that has led prey species to evolve adaptations and behavioural strategies to minimize predation risk (Abrams & Matsuda, 1997). When exposed to a predation cue, prey-species generally display a risk-assessment phase. Depending on the perceived risk (De Franceschi, Vivattanasarn, Saleem, & Solomon, 2016), animals will freeze (to avoid being spotted by the predator) or display responses that can be characterized as defensive (i.e. fleeing, predator/area avoidance, decreased locomotion and foraging activity or increased vigilance), or more rarely, offensive (the prey species attack or mob the predator). It is generally considered that animals fight the predator only if they have no chances of freezing or fleeing (Eilam, 2005). Investigating the use of one or the other strategy by a broad diversity of species has been at the base of many studies in behavioural ecology (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005; Apfelbach, Soini, Vasilieva, & Novotny, 2015; Carlson, Healy, & Templeton, 2017; Eilam, 2005). Additionally, understanding the inter-individual variations in the response to predation cues has gained interest in the past decades, given the fitness consequences of such variations (Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, & Johnson, 2004; Smith & Blumstein, 2008). For instance, perhaps counterintuitively, it has been shown in guppies (*Poecilia reticulata*) that individuals approaching their natural predator were less at risk of being attacked than their non-approaching congeners (Godin & Davis, 1995). Understanding the consequences of inter-individual variations is even more important for endangered species for which the anti-predatory strategies are not well understood, which is jeopardizing their conservation (Berger et al. 2016). For instance, ensuring that the perception of predation has not been lost in captive-reared animals (Blumstein, Holland, & Daniel, 2006; Ward, MacDonald, Doncaster, & Mauget, 1996) that are part of restocking programs (in which individuals are released every year to sustain wild populations) appears extremely important.

The European hamster is one of the most endangered mammal in Europe (Weinhold, 2008). Nonetheless, despite some descriptive studies on hamsters behaviour in a zoological park (Ziomek, Zgrabczyńska, & Poradzisz, 2009), on sexual interactions (Reznik-Schuller, Reznik, & Mohr, 1974; Vohralik, 1974) or on inter-individual interactions in an urban environment (Siutz & Millesi, 2005), studies on the behaviour of the species are severely lacking. This is particularly true regarding its anti-predatory behaviour. Apart from the descriptive study on the ethology of the European hamster by Eibl-Eibesfeldt (Eibl-Eibesfeldt, 1953), characterizing it as 'territorial' and 'aggressive', there is currently no information on the anti-predatory behaviour of this species. European hamsters are currently benefiting from several conservation measures throughout the continent, including restocking programs (with several hundred hamsters released in the fields every year in each country) and reconnection of wild populations through the improvement of wildlife underpasses. In that aim, an 'Anti-Predation Tube (APT)' has recently been developed (Tissier, Jumeau, et al., 2016) to guarantee the safe-crossing of hamsters and other rodents in wildlife underpasses, by reducing the risks of predation. The features and use of this device by captive-reared hamsters have been validated under controlled predator-free conditions (Tissier, Jumeau, et al., 2016). However, the efficiency of the APT still needs to be validated in the presence of a predator.

In this study, we thus investigated for the first time the perception and reaction of European hamsters towards predation cues, with three main aims: (I) understanding whether captive-reared hamsters perceive predation cues, (II) how they respond to them (i.e. which one of the defensive or offensive strategy they display) and (III) whether this could affect their decision to take refuge in the APT. To that purpose, we carried-out three different experimental studies: we first investigated whether captive-reared hamsters were able to discriminate both the odour of a predator from a non-predator scent, as these are sufficient conditions to confirm the existence of a recognition mechanism (Monclús, Rödel, Von Holst, & De Miguel, 2005). To do so, we conducted a Y-maze test (April-May 2014) with a predator's urine (domestic cat, *Felis silvestris catus*) and an herbivore's urine (goat, *Capra hircus*). We predicted that hamsters would display escaping behaviours or spend less time near the predator odour as most rodents and lagomorphs do (Apfelbach et al., 2005; McPhee, Segal, & Johnston, 2010; Monclús et al., 2005; Zhang, Sun, Bruce, & Novotny, 2008). In a second experiment, we evaluated the efficiency of the APT in presence of a 'non-mobile' European ferret (*Mustela putorius furo*). This test aimed at investigating the perception of the predator's presence by hamsters, their reactions and their consequent use of the APT. Based on the literature, we were expecting that hamsters will spend more time at distance from the ferret (i.e. avoidance) (Apfelbach et al., 2005) and will increase their use of the APT in the presence of this predator. In a third experiment, we aimed at emulating wild conditions more closely by using the same design of APT, but with a mobile ferret. Furthermore, given the results of a recent study highlighting that the behavioural responses of the dwarf hamsters (*Phodopus campbelli*) to odour cues of the European ferret were stronger when the ferret was fed with hamsters (Apfelbach et al., 2015), in addition to being mobile, the ferret was previously accustomed to be fed with hamster corpses in this third experiment. We again expected hamsters to avoid the predator and increase their use of the APT.

## METHODOLOGY

### *Hamsters and housing conditions*

The experiments were performed in April-May 2014 and 2016 on 32 two-year old captive European hamsters: 9 for the Y-maze test (2014, 5 ♂ and 4 ♀), 8 for the APT efficiency test with a non-mobile ferret (2014, 4 ♂ and 4 ♀) and 16 for the APT efficiency test with a mobile ferret (2016, 6 ♂ and 10 ♀). European hamsters are one of the largest hamster species in the world (Nechay, Hamar, & Grulich, 1977; Reznik, Reznik-Schüller, & Ulrich, 1978). Males and females weigh on average 350 and 250 g, respectively, with important seasonal variations and the localization in Europe (Nechay et al., 1977; Reznik et al., 1978; Tissier, Handrich, et al., 2016). Hamsters are omnivorous and feed on seeds, roots, green parts of plants, invertebrates and small vertebrates (Gorecki & Grygielska, 1975; Nechay et al., 1977). This species is described as territorial and very aggressive (Franceschini, Siutz, Palme, & Millesi, 2007; Nechay et al., 1977; Ziomek et al., 2009), although no studies have investigated the behavioural responses of hamsters towards predators. Adult European hamsters are predated by red foxes (*Vulpes vulpes*), stoats (*Mustela erminea*), raptors/birds of prey (e.g. common buzzard *Buteo buteo*), domestic cats, badgers (*Meles meles*) and dogs (*Canis lupus f. familiaris*) (Kayser, Weinhold, & Stubbe, 2003; La Haye, Müskens, Van Kats, Kuiters, & Siepel, 2010). Juveniles are also predated by common kestrels (*Falco tinnunculus*), long-eared owls (*Asio otus*), grey herons (*Ardea cinerea*), crows (*Corvus corone corone*) and rooks (*Corvus frugilegus*) (Kayser et al., 2003; *personnal comm*.)

Hamsters were housed in transparent Plexiglas cages (420*265*180 mm, D*W*H) prior to the experiments and their environment was enriched with wood and shredded paper. Animals were provided with an *ad libitum* supply of water and food pellets (N° 105, from Safe, Augy, France). The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and were approved by the Ethical Committee (CREMEAS) under agreement number 02015033110486252 (A PA FIS#397). 01.

*The Y-maze test*

We used urine from adult non-castrated female domestic cats as the predator odour. For the herbivorous odour, we chose to use urine from adult female goats, which do not represent a competitive species for the hamster. Regarding cats, the urine was collected by a veterinarian (N=15) before castration and as for goats, it was collected in a cup during urination (N=5). Within the hour after urine collection, we mixed the 15 cats urine to obtain a homogenous 'predator urine' and to avoid inter-individual differences. The same was done for the goat urine. We then prepared compresses with 1 ml of either 'predator urine' or 'herbivorous urine' that were immediately frozen at -28°C. Both a 'predator compress' and a 'herbivorous compress' were unfrozen 15 minutes prior to each experiment. To that end, we placed them inside two separated and closed petri dishes, at $T_a$=22°C.

The petri dishes with the 'predator compress' and 'herbivorous compress' were placed in the ***b*** or ***c*** extremities of the Y-maze, behind a metal grid (Figure 1A) and were opened 3 minutes before starting the experiment. The maze was then closed with a transparent plastic plate at the bottom, in order to let predator and herbivorous odours diffuse into their respective branches. Approximately 2 g of earthworms (*Lumbricus terrestris*), an appetent food for the hamster, were placed close to the grids (see Figure 1A). Each hamster was placed in a box at the extremity ***a*** of the maze. Once it got out from the box, we closed a trap to maintain it in the maze and started the 5 minutes recording (hamsters were videotaped from the bottom). The beginning of the experiment thus corresponds to the closing of the trap. One day prior to the beginning of the experiment, each hamster was acclimated to the maze. We used compresses with 1 mL of water for these acclimations. To control for a potential side effect as well as for the time of the day, each hamster was randomly tested 4 times (36 tests, with one day of interval between each test): (i) predator odour on the right and herbivorous odour on the left, (ii) predator odour on the left and herbivorous odour on the right, (iii) in the morning and (iv) in the afternoon. Test order was randomized. The device was cleaned with ethanol 70° and aired for 7 minutes whilst compresses and worms were renewed after each trial. All the tests were carried out in low-light conditions (20W-light bulb) in a room at $T_a$=16°C±1° C.

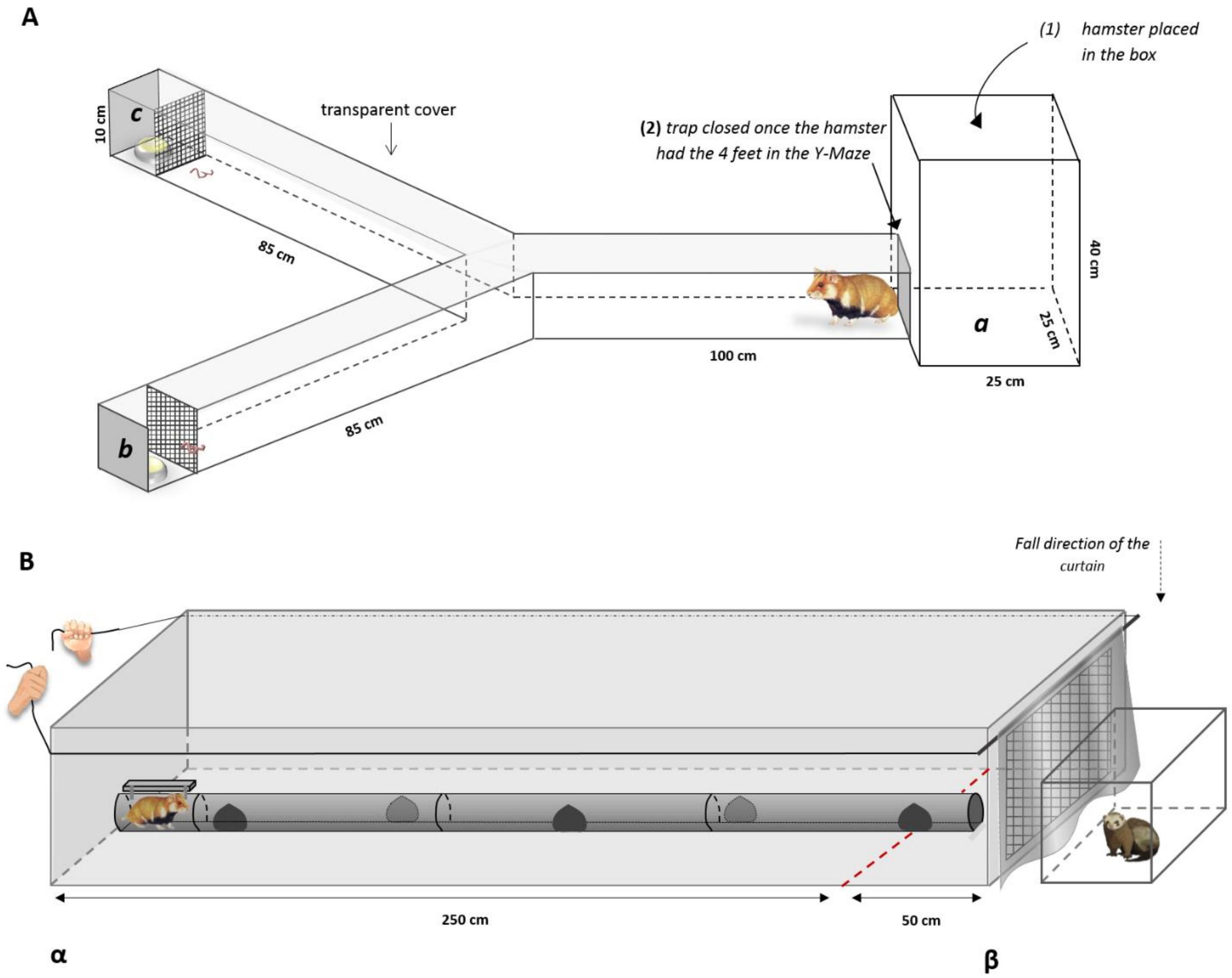

**Figure 1: Schematic representation of the experimental designs for (A) the Y-Maze test, (B) the APT efficiency test with a non-mobile ferret**. The associated methodology is described in the text, sections '*The Y maze test*' and '*The APT efficiency test with a non-mobile ferret*'.

### *The APT efficiency test with a non-mobile ferret*

A rectangular PVC enclosure of 3x1x0.4 meters (Length*Width*Height) with a metal grid (wire mesh: 25*25 mm) at one extremity (see Figure 1B) was created, to mimic the shape of classical wildlife underpasses in the French area of distribution of the species (Jumeau & Handrich, 2016).

A prototype of 2.78 meters length of the anti-predation tube (APT) previously developed for this species (Tissier, Jumeau, et al., 2016) was placed in the centre of this enclosure. An opaque curtain was masking the grid at the extremity of the enclosure and a line was drawn on the floor at 50cm from the grid. In this experiment, the predator condition (P) consisted of placing a female European ferret in a cage (0.8x0.4x0.4) behind the curtain (extremity β, Figure 1B) prior to placing the hamster in the extremity α. When the hamster first crossed the line, the curtain was smoothly lowered by the experimenter (see figure 1B) to reveal the ferret to the hamster. Hamsters' behaviour was then videotaped for 5 minutes. Between each trial, the APT, the enclosure and the ferret's cage were cleaned with ethanol 70° whereas the room was aired. Between the trials, the ferret was kept in a cage with hay, litter, water and food *ad libitum*. Two hours before each session, its food was removed. Hamsters were previously tested under three 'predator-free' conditions to validate their spontaneous use of the device: they were either placed on the left, on the right or inside the APT. Given that we found no side effects (see (Tissier, Jumeau, et al., 2016) for detailed results) and that the order of these three conditions was randomized, we grouped the results of the three 'predator-free' conditions to create a control 'predator-free' condition ($P_{free}$) to which we will compare the P condition. All the experiments were carried out in low-light conditions at $T_a$=22°C.

*The APT efficiency test with a mobile ferret*

The same rectangular PVC enclosure as the one described in the previous section was used, with some modifications. First, the ferret was not outside, but inside the enclosure. We therefore needed to build a separation ensuring the safety of both the hamster and the ferret, while allowing for physical proximity and potential predatory attempts from the ferret. To do so, we firmly attached a metal grid with small wire mesh (25*25 mm) inside the enclosure, disposed horizontally and all along the APT (10cm diameter), 12cm above the bottom of the apparatus. Therefore, both the hamster and the ferret could freely see, smell and move towards each other, but could not directly interact. We could easily add and remove the hamster from the inner part of the apparatus by opening and closing a zip. In order to avoid the ferret from escaping the enclosure, we added (after introducing the animals) two additional metal grids (1.6*1.1 m) covering the whole apparatus.

Unlike in the previous setup, hamsters were food deprived in the evening preceding the test and we added food rewards in all the four corners of the enclosure (but still inside the inner metal grid). Each food reward consisted of 10 pumpkin seeds and ¼ of a carrot slice placed directly on the apparatus floor. Thus, the hamster was motivated to feed and/or to hoard food. The protocol had two conditions, with each condition consisting of three phases. In the 'predator' condition (P), the hamster was first introduced in the inner part of the apparatus for 10 minutes (pre-treatment phase). Then, in a second step, we added the ferret in the apparatus for 5 minutes (treatment phase). Finally, we removed the ferret and left the hamster for another 10 minutes in the apparatus (post-treatment phase). In the 'predator-free' condition ($P_{free}$), the hamster was also first introduced in the inner part of the apparatus for 10 minutes (pre-treatment phase). Then, the metal grid was opened by the experimenter, mimicking the introduction of the ferret. The hamster was then left the hamster alone in the apparatus for 5 minutes (treatment phase). Finally, the experimenter entered the room, opened the upper metal grid, mimicked the removal of the ferret and left the hamster for another 10 minutes in the apparatus (post-treatment phase). Each hamster underwent both conditions, with half of the individuals passing the P condition first and the other half passing the $P_{free}$ condition first. Both conditions were separated by one week. Every step was videotaped in a different video file. If the hamster consumed food in any of the protocol steps, we replenished the food rewards so that 10 pumpkin seeds and a quarter of a carrot slice were always present at the beginning of a step. Thus, the maximal reward a hamster could consume was 3*4*10 = 120 pumpkin seeds and 3*4*1 = 12 quarters of carrot slices. None of the hamsters participating to the 'mobile ferret' experiment took part in any previous experiments made with this apparatus.

### *Statistical analyses*

Regarding the Y-maze, the *cumulated time spent in each branch* of the maze (in seconds) was analysed using a Linear Mixed Model (LMM) whereas the *number of body-shaking (i.e. snorting) episodes* (as a measure of disturbance (Kleiman, 1973)) and the *number of visits in each branch* of the Y-maze were analysed using Generalized Linear Mixed Models (GLMM, Probability distribution: Poisson, Link function: Log). We included the type of odour, the sex, the order of the trial (to test for an eventual habituation), the side where the predator odour was presented and the sex*odour interaction as fixed factors. The identity of the individuals was included as a random factor for repeated measures on the same individual.

Regarding the APT efficiency test with a non-mobile ferret, given that the $P_{free}$ tests lasted 12 min compared to the P test that lasted 5 min (to avoid an habituation from the hamster or the ferret), all the variables were analysed per unit of time (i.e. per min). We looked at the effect of the condition ($P_{free\ or}$ P) on the *proportion of time spent in the APT*, *the proportion of time spent after the threshold* (i.e. close to the grid), the *U-turns frequency* from the extremity with the grid to the other extremity, the *scraping frequency* and the *rearing frequency* (i.e. when the individual raises the upper part of the body, which corresponds to an exploratory behaviour) using the Wilcoxon matched-pairs signed rank test.

Regarding the APT efficiency test with a mobile ferret, videos were blind-analysed (i.e. the experimental condition was not known by the experimenter). We then calculated the difference between the value in the post-treatment phase and the value in the pre-treatment phase for the following seven variables: *time spent inside the APT*, *number of entrances in the APT*, *distance travelled within the APT* (calculated by noting the distance in centimetres between the entrance hole and the exit hole), *time spent in the middle of the apparatus*, *time spent in the corners of the apparatus*, *number of eaten/hoarded pumpkin seeds and carrot slices*, and *time spent eating/hoarding*. The distributions of these seven differences did not differ from the normal distribution (normality was assessed via a Shapiro test). Therefore, all the variables were analysed using LMMs. For each model, we included the sex of the hamster, the presence or the absence of the ferret during the treatment period, the order of the ferret presentation (i.e. whether the 'predator' condition was first or second), as well as all two-way interactions, as fixed factors. The identity of the hamsters was included as a random factor for repeated measures on the same individual. The models were then simplified by backward omission of non-significant terms until a minimal model was found. During the treatment phase of the P condition (i.e. when the ferret was present), we also noted the number of mobbing events displayed by the hamster towards the ferret. We analysed this variable using a GLM (probability distribution: Poisson, link function: log), with the sex of the hamster as a fixed factor.

Data presented are means ± SEM. Normality of the residuals of every model was tested using a Kolmogorov-Smirnov test or a Shapiro-Wilk test. Analyses were conducted using R (R-3.2.3) with the RStudio interface (RStudio, Inc., 0.99.491.0), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA) or the R package ggplot2 (ggplot2.org).

## RESULTS

### *The Y-maze test*

In total, 36 tests were carried out, but one video was missing because of a camera issue (N=35 recorded tests, for a total of 175 min). The 9 individuals did not show a side bias: the right and left branches were respectively chosen first on 34 and 36 occasions. We thus did not use the position of the odour in the subsequent analyses. We found an effect of the type of odour on the time spent in each branch ($F_{1,58} = 4.37$, $p = 0.041$): hamsters spent significantly more time in the branch with the predator urine than in the branch with the herbivorous urine (Figure 2A, 101.9±6.3 and 85.5±6.3 seconds for predator and herbivorous scents, respectively). We found no effect of the other variables or the interaction on the time spent in each branch ($p>0.2$). Regarding the number of body-shaking episodes, we found no effect of the type of odour (Wald $\chi^2 = 0.5$, $p = 0.5$). However, this variable was significantly affected by the sex (Wald $\chi^2 = 8.5$, $p = 0.003$) and the sex*odour interaction (Wald $\chi^2 = 6.1$, $p=0.014$). Post-hoc analyses revealed that females displayed a higher mean number of body-shaking episodes (Figure 2B) when faced with the predator urine than when faced with the herbivorous urine (mean difference = -0.65±0.18, $p<0.001$). No differences were found for males between the two odours (Figure 2B, mean difference = 0.04±0.04, $p=0.28$). No effects of the other variables were found on the number of body-shaking episodes ($p>0.1$). Finally, regarding the number of visits in each branch, we found no effect of the odour (Wald $\chi^2 = 0.4$, $p=0.6$) nor the sex (Wald $\chi^2 = 1.1$, $p=0.3$) but an effect of the sex*odour interaction (Figure 2C, Wald $\chi^2 = 7.96$, $p=0.005$). Females visited the branch with the predator scent significantly more often than the branch with the herbivorous scent (4.3±0.4 and 3.7±0.2 times, respectively; $p=0.035$). We found no differences in the number of visits between the two odours for males (which visited the predator and the herbivorous branches on average 4.2±0.2 and 4.7±0.5 times, respectively; $p=0.16$).

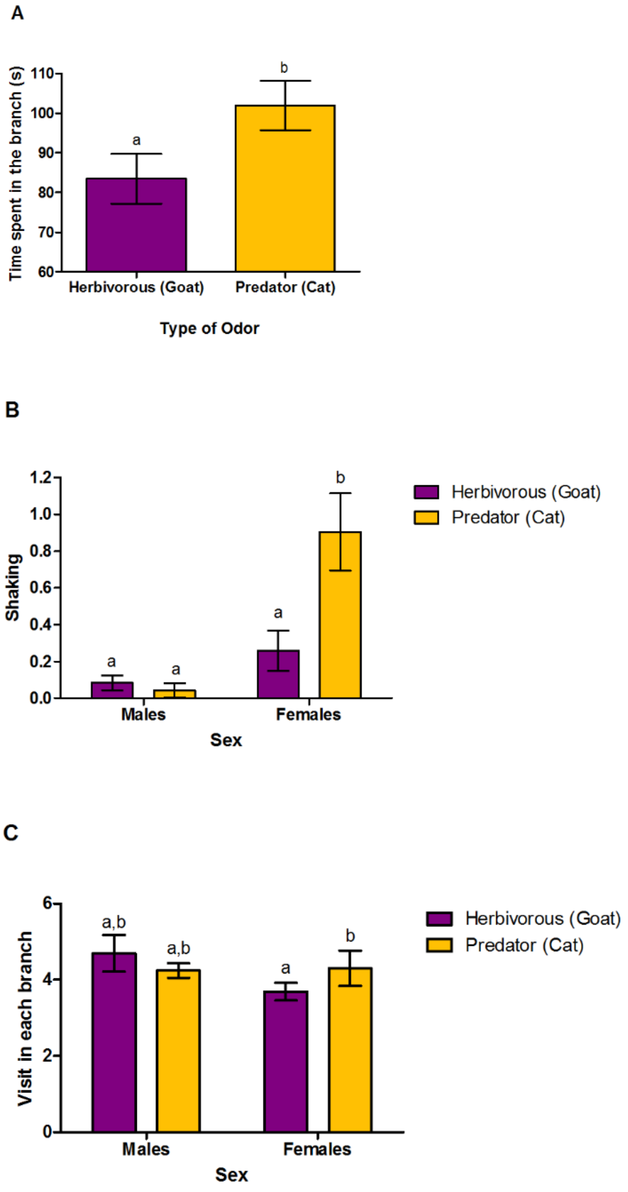

**Figure 2: Effects of the type of odour (Predator or Herbivorous) on (A) the time spent in each branch, (B) the mean number of body-shaking episodes and (C) the number of visits in each branch of the Y-maze**. In (B) and (C), mean ± SE are represented according to the sex and the type of odour (because of the significant sex*odour interaction). Different letters mean significant differences between the groups. In (C), we found no differences regarding the number of visits in each branch between males and females tested with herbivorous odour at the α=0.05 level (p = 0.055 and p=0.058, respectively).

*The APT efficiency test with a non-mobile ferret*

A total amount of 32 tests were carried out (328 min of recording) on the 8 individuals (P and $P_{free}$ conditions). We found significant differences between the P and $P_{free}$ conditions for four variables (see Table 1): the *U-turns frequency* (Wilcoxon signed rank test, T = 27, p = 0.03, N = 8), the *rearing frequency* (T = 0, p = 0.02, N = 8), the *scraping frequency* (T = 0, p = 0.03, N = 8) and the *proportion of time spent after the threshold* (T = 27, p = 0.03, N = 8). However, we found no differences between the P and the $P_{free}$ conditions when considering the *body-shaking frequency* (T = 11, p =

0.4 and T = 0, p = 0.2; N = 8) and the *proportion of time spent in the APT* (T = 13, p = 0.9, N = 8). In the P condition, agonistic behaviours (spitting, grunting and posture attack; see (Ziomek et al., 2009)) were recorded in 3 of the 4 males. These behaviours were never observed in the $P_{free}$ conditions. However, this difference was not significant (T = 6, p = 0.2, N = 8).

| Variable | Condition | Mean±SE | Predator effect |
|---|---|---|---|
| U-turns frequency (number/min) | $P_{free}$ | **0.01±0.01** | ↗ |
| | P | **0.28±0.08** | |
| Rearing frequency (number/min) | $P_{free}$ | **0.63±0.08** | ↘ |
| | P | **0.23±0.07** | |
| Scraping frequency (number/min) | $P_{free}$ | **0.07±0.02** | ↘ |
| | P | **0** | |
| Proportion of time spent after the threshold (s/min) | $P_{free}$ | **0.30±0.03** | ↗ |
| | P | **0.51±0.09** | |
| Body-shaking frequency (number/min) | $P_{free}$ | 0.05±0.02 | — |
| | P | 0.09±0.04 | |
| Proportion of time spent in the tube (s/min) | $P_{free}$ | 0.05±0.01 | — |
| | P | 0.06±0.04 | |
| Agonistic behaviors' frequency (number/min) | $P_{free}$ | 0 | — |
| | P | 0.1±0.05 | |

**Table 1: Effects of the presence of the predator on hamsters' behaviour in the APT efficiency test with a non-mobile ferret**. Mean±SE are represented according to the Predator condition (P) and to the Predator-free condition ($P_{free}$). Means in bold represent significant differences between the two conditions (Wilcoxon signed rank test). The arrows represent the direction of the difference (only when significant). See methodology for details and results section for statistics.

*The APT efficiency test with a mobile ferret*

The exposure to a mobile ferret increased the time spent in the APT in the post-treatment phase compared to the pre-treatment phase (Figure 3A, $F_{1;15} = 4.55$, df = 15, p = 0.049). Similarly, hamsters exposed to a mobile ferret during the treatment phase increased their time spent in the middle of the apparatus (Table 2 & Figure 3B, $F_{1;13} = 6.01$, $p = 0.03$). As a corollary, the time spent in the corners of the apparatus decreased after hamsters were exposed to a mobile ferret (Figure 3C, $F_{1;13} = 8.16$, $p = 0.01$). Means+SEM and a summary of these results are represented in Table 2.

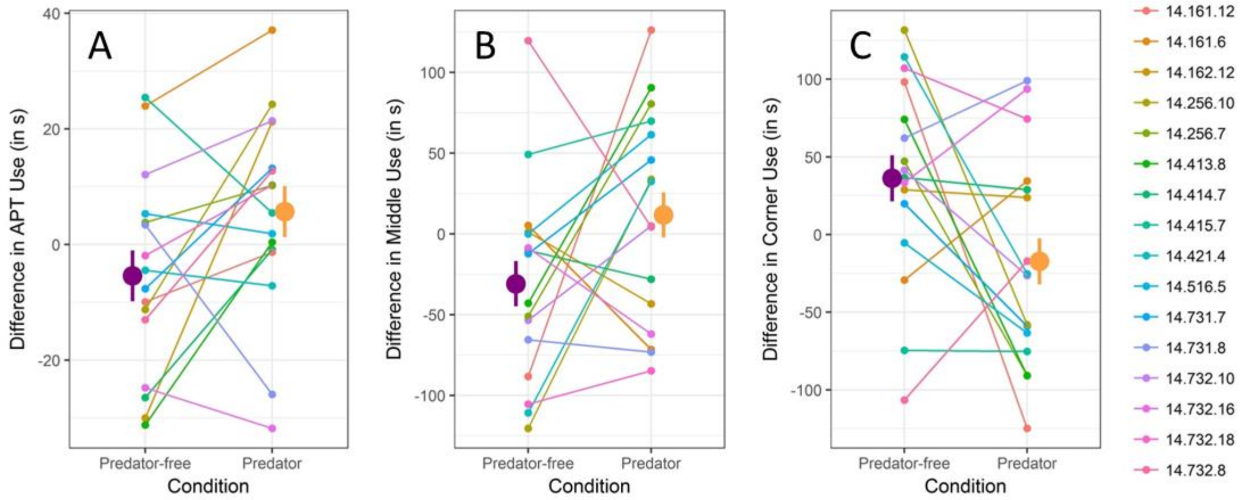

**Figure 3: Effects of the presence (Predator) or not (Predator-free) of the ferret during the 5-min treatment phase.** The effect of the presence of the predator on (A) the difference between post- and pre-treatment time spent in the APT, (B) the difference between post- and pre-treatment time spent in the middle of the apparatus and (C) the difference between post- and pre-treatment time spent in the corners of the apparatus are represented. Orange points and error bars represent the mean and its associated standard error for the P condition, purple points and error bars represent the mean and its associated standard error for the $P_{free}$ condition. Coloured points and lines are individual data of the 16 hamsters used in the experiment. Individual identities are recorded on the right: the first number (i.e. 14) indicates the birth year; the second number indicates the litter identity and the third number indicates the identity of the individual.

The number of entries and distances travelled in the APT were higher in the post-treatment phase compared to the pre-treatment phase for the P condition, but not for the $P_{free}$ condition (Table 2, $F_{1;13.8} = 15.8$, $p = 0.001$ and $F_{1;12.9} = 6.65$, $p = 0.023$, respectively). The distance travelled per entry was not affected by the presence of the ferret in the treatment phase ($p > 0.2$). The time spent feeding/hoarding and the number of eaten/hoarded food items during the post-treatment phase were not affected by the presence of the ferret (Table 2, $p > 0.1$). For the time spent in the corners and in the middle of the apparatus, the test session number interacted with the sex of the hamster (Figure S1): males in the second session (irrespective of whether the second session was the "predator" or the "predator-free" condition) had a different behaviour than other sex-session combinations. Indeed, males spent more time in the middle and less time in the corners in the second session (Figure S1, $F_{1,13} = 6.3$, $p = 0.026$ and $F_{1,13} = 6.8$, $p = 0.022$ respectively). Finally, only females travelled longer distances inside the APT during the post-treatment phase compared to the pre-treatment phase in the first test session compared to the second session (Figure S2, $F_{1,12.88} = 17.3$, $p = 0.001$).

| Variable | Condition | Mean ± SE | Predator effect |
|---|---|---|---|
| Time spent in Middle (s) | $P_{free}$ | **-30.9 ± 14.0** | ↗ |
| | P | **+11.7 ± 14.0** | |
| Time spent in Corner (s) | $P_{free}$ | **+36.2 ± 14.8** | ↘ |
| | P | **-17.3 ± 14.8** | |
| Time spent in Tube (s) | $P_{free}$ | **-5.4 ± 4.4** | ↗ |
| | P | **+5.7 ± 4.4** | |
| Number of entries in Tube* | $P_{free}$ | **+0.3 ± 0.7** | ↗ |
| | P | **+2.1 ± 0.7** | |
| Distance traveled in Tube (cm) | $P_{free}$ | **+5.6 ± 90.9** | ↗ |
| | P | **+210.6 ± 90.9** | |
| Time spent Eating (s) | $P_{free}$ | **-9.9 ± 15.2** | — |
| | P | **-18.1 ± 14.1** | |
| Number of consumed Food items | $P_{free}$ | **+5.6 ± 4.4** | — |
| | P | **-4.1 ± 5.2** | |

**Table 2: Effects of the presence of the predator on hamsters' behaviour in the APT efficiency test with a mobile ferret**. Mean±SE are represented according to the Predator condition (P, orange) and to the Predator-free condition ($P_{free}$, purple). The arrows represent the direction of the difference (only when significant). See methodology for details and results section for statistics.

Within the 5 minutes of the ferret's presence, 11 hamsters displayed mobbing behaviours towards this predator (Figure 4). We found that, on average, females mobbed the ferret significantly more than males (♂ = 1.7±0.5 and ♀ = 5.0±0.7; Wald $\chi^2$ = 10.1, p = 0.002, N = 16).

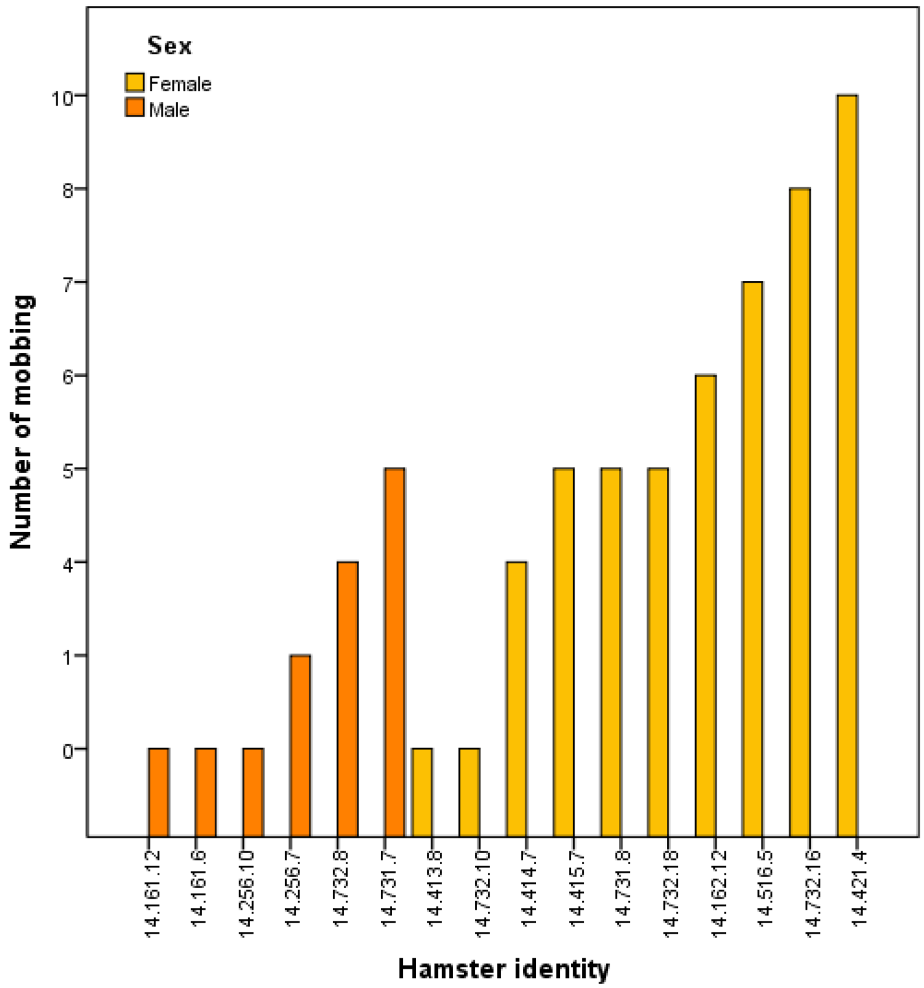

**Figure 4: number of mobbing behaviour displayed by hamsters in the APT efficiency test with a mobile ferret.** Males are represented in dark orange whereas females are represented in light orange. Mobbing behaviour include running towards the predator, grunting, spitting and direct attacks. Hamster identity: the first number (i.e. 14) indicates the birth year; the second number indicates the litter identity and the third number indicates the identity of the individual.

## DISCUSSION

Contrary to our initial predictions, captive European hamsters do not escape when faced with predator odours or to the predator itself. They rather significantly increase the time spent near the predator or its odour, whilst displaying disrupting (body-shaking) or aggressive behaviours, highlighting that they do perceive predation cues (odours and predator’s presence). Therefore, they seem to display a bold personality type (Réale et al., 2007) as they do not avoid or immediately escape when faced with predation cues, in contrast to what was observed in mice, voles, rabbits, golden hamsters and rats (Apfelbach et al., 2005; McPhee et al., 2010; Monclús et al., 2005). However, results from the third experiment highlight that they perceive the increased predation risk when exposed to a mobile European ferret and consequently take refuge in the APT.

*Y-maze test*

The results of this test highlight that predator-naïve hamsters are able to discriminate urine of a cat (the predator model in our study) from the urine of a goat (the neutral scent). However, they spent more time close to the cats’ than to the goats’ odour, which is counter-intuitive based on the literature on the reaction of rodent prey-species to predators’ odour (Apfelbach et al., 2005; McPhee et al., 2010; Monclús et al., 2005). However, females displayed a higher number of body-shaking episodes when facing the predator odour than when facing the herbivorous odour. This is a characteristic reaction to disturbances in rodents, usually considered as an agonistic behaviour (Kleiman, 1973; Williams, 1999).

The reaction of prey species to predators appears to be species dependent (Apfelbach et al., 2005). Most prey species – from mice to ungulates – display a ‘defensive strategy’ (escaping behaviour, decreased locomotion, predator or area avoidance…) (Apfelbach et al., 2005; Banks, Hughes, & Rose, 2003; Camp, Rachlow, Woods, Johnson, & Shipley, 2012). However, some species rather display an ‘offensive strategy’ (showing aggressive behaviours, mobbing and no direct escape) in response to a first exposure to predation cues (Eilam, 2005). Taken altogether the results of the Y-maze test suggest that European hamsters are rather using the offensive strategy compared to other rodents that generally show predator-urine avoidance (Apfelbach et al., 2005; McPhee et al., 2010; Monclús et al., 2005). However, it has been shown that the predator’s diet influences the perception and strength of reaction of the dwarf hamster when faced with predator’s urine (Apfelbach et al., 2015). Therefore, it is possible that European hamsters would have reacted differently to cats’ urine if they were previously fed with hamsters. They could also have reacted differently to the urine of another predator.

*The APT efficiency test with a non-mobile ferret*

The results of this test, revealing significant behavioural differences between the Predator (P, i.e. in the presence of the European ferret) and the 'Predator-free' ($P_{free}$) conditions, highlight that hamsters perceived the presence of this predator species, even though they have been reared in captive conditions. However, contrary to our predictions, they spent more time after the threshold (i.e. close to the predator) in the P compared to the $P_{free}$ condition. This reinforces the results of the Y-maze test, regarding the use of an 'offensive strategy' in this species. In parallel, we observed a behavioural shift in hamsters: exploratory (rearing) and grooming behaviours were significantly decreased or even suppressed in favour of U-turns (significantly increased in the P condition) and agonistic behaviours (recorded only in males). Taken separately, the increased number of U-turns in presence of the predator would indicate an avoidance. However, if we consider this greater number of U-turns associated to the increased time spent close to the predator, this rather suggests a 'risk assessment' phase (Blanchard, Blanchard, Rodgers, & Weiss, 1990) which precedes decision-making of escaping and/or taking refuge in a secure area (Kramer & Bonenfant, 1997). Nonetheless, hamsters do not increase their use of the APT in the presence of the predator, which could be explained in several ways. First, hamsters may not have perceived the ferret as a real predator (after this phase of risk assessment) but rather displayed an attraction for novelty (Hughes, 2007). Although possible, this hypothesis is unlikely, given the aggressive behaviours displayed by three of the four males and the suppression of exploratory/grooming behaviours in all the individuals. However, the ferret's presence might not have represented an immediate risk of predation, strong enough to push hamsters to use the APT. Indeed, the ferret being in a small cage, it was limited in its movements and was consequently rather inactive/non-mobile. Moreover, hamsters were separated from the ferret by two grids (the one of the cage and the one of the enclosure), which could have prevented them from searching for a secure area.

*The APT efficiency test with a mobile ferret*

The test with the mobile ferret confirms that captive European hamsters are using an offensive strategy towards the predator. They mainly faced the direct threat of the predator by mobbing it. However, some hamsters also used the APT in order to protect themselves from the predator's presence. We therefore observed two distinguished strategies in the presence of the predator: a defensive strategy (only 4 hamsters) and an offensive strategy (11 hamsters). One individual neither attacked the predator nor used the tube to take refuge. Results of this third experiment also highlight that these captive-reared hamsters still perceive the increased predation risk when exposed to a predator such as the European ferret, when mobile and previously fed with hamster's corpse. Indeed, hamsters used more frequently the APT and spent more time inside after (compared to prior) the predator exposure. They also avoided the corners of the apparatus, which are the furthest positions from the APT. However, both the number of eaten/hoarded food items and the time spent eating/hoarding food were not significantly affected by the predator's presence. Given that each hamster was tested only once with the ferret, we cannot assess whether the strategy a hamster uses is repeatable over time. It is possible that the choice of strategy depends on subtle cues delivered by the ferret on its immediate capture intentions (De Franceschi et al., 2016).

*Reaction to predation, personality and ecological implications*

Taken altogether, the results of these three experiments show that European hamsters display several signs of risk evaluation and bold behaviours before escaping cats' urine or the ferret's presence. Moreover, most individuals even displayed mobbing behaviours (or even direct attacks) towards the predator, revealing that they adopted an offensive strategy. As indicated by Eibl-Eibestfeldt (1953), the offensive strategy is more important when the predator managed to closely (~2 meters) approach the hamster. European hamsters usually mock the predator (Eibl-Eibesfeldt, 1953), but if the later continues to approach, then the hamster attacks and can even harm the predator by sinking its teeth into its legs. In this study, however, we highlighted that different individuals of a same population display different types of response towards the predator (i.e. offensive or defensive). These reaction-types, consistent across situations, have been characterized as 'boldness' in many taxa (Mafli, Wakamatsu, & Roulin, 2011; Réale et al., 2007). Therefore, our results suggest that the hamster is rather a bold species, with inter-individual variations occurring in our captive population, suggesting the existence of personality in this species, which would need to be confirmed by repeated tests of boldness on the same individuals. Boldness has been related to other traits of personality such as exploration or aggressiveness (Ariyomo & Watt, 2012; Mafli et al., 2011; Réale et al., 2007; Sih et al., 2004; Wolf, van Doorn, Leimar, & Weissing, 2007). Given the implications of these traits for animals' dispersion, foraging efficiency, parental behaviour and more broadly for survival and reproductive success (Boon, Réale, & Boutin, 2007; Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010; Réale et al., 2007; Sinn, Gosling, & Moltschaniwskyj, 2008), it is very likely that this great boldness in hamsters would have major ecological implications for wild populations. For instance, in several taxa, bold individuals face higher risk of predation (Réale et al., 2007) and might therefore be disadvantaged in environments with great predation risk (Abbey-Lee, Mathot, & Dingemanse, 2016). Moreover, bold individuals could be less prone to use the APT as a refuge than shy individuals. Nonetheless, despite their offensive strategy and elevated latency before avoiding the predator, hamsters finally showed an increase in their use of the APT when the predator was mobile.

The European hamster is one of the largest rodent in Europe with adult males weighing up to 650g (Fenyk-Melody, 2012; Nechay et al., 1977). Hamsters possess long teeth and have often been described as very aggressive, especially females (Eibl-Eibesfeldt, 1953; Nechay et al., 1977; Reznik et al., 1978). Therefore, similar to what has been observed in fish (Godin & Davis, 1995), the offensive strategy might be beneficial under some conditions for adults facing relatively small predators such as the European ferret. Nonetheless, it is likely that the benefits of such strategy would be reduced when facing larger predators such as foxes. Therefore, hamsters might observe a different strategy towards such big predators (Graw & Manser, 2007). Nonetheless, direct attacks against dogs and humans have been recorded under wild conditions (Eibl-Eibesfeldt, 1953; *personnal comm*.) and several studies report that aggressiveness is generally reduced in captive compared to wild individuals (Fenyk-Melody, 2012; Nechay et al., 1977). Given the importance of experience (Carlson et al., 2017), it would be interesting to investigate for the differences in behavioural responses of both captive-reared and wild hamsters towards bigger predators than cats and ferrets or towards variations in ground cover levels. Moreover, it has been shown in several taxa that individuals are generally bolder, more exploratory and more aggressive in highly anthropogenic environments (Sih et al., 2004; Sih, Ferrari, & Harris, 2011; Sol, Lapiedra, & González-Lagos, 2013). Given that European hamsters have evolved in farmlands in the past centuries (Nechay et al., 1977; Weinhold, 2008), and are now frequently found in urban areas (Surov, Banaszek, Bogomolov, Feoktistova, & Monecke, 2016), these bold-reaction types might reflect an adaptation to such environments that have been maintained under recent captive conditions. However, individuals that are more exploratory, bold and aggressive have reduced capacities to exploit new resources in changing or stochastic environments compared to shy individuals (Demeyrier, 2016). Indeed, the latter are more precautious and attentive to external stimuli and are better to adapt to changing environmental conditions (Demeyrier, 2016; Robertson, Rehage, & Sih, 2013; Sih et al., 2004). Further research, comparing hamsters from different breeding units, as well as wild hamsters from urban areas and farmland is therefore needed to better understand the environmental effects and fitness consequences of these bold behaviours.

## CONCLUSION

Taken altogether, these three experiments highlight that captive-reared European hamsters rather display a bold response towards the European ferret (no direct escaping, mobbing and in some cases directly attacking). Nonetheless, despite their bold behavior, hamsters used the APT when perceiving an imminent risk of predation (i.e. with a mobile ferret in the setup). This is therefore the first study to bring information on the risk-assessment and inter-individual differences in perception and reaction towards predation cues in this species. Nonetheless, investigation regarding the reaction of hamsters towards bigger (e.g. foxes) or avian (e.g. birds of prey) predators, or towards varying ground cover are now needed. Regarding the APT, we have equipped and monitored several wildlife underpasses in the French area of distribution of the European hamster to validate its efficiency under wild conditions.

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## References

Abbey-Lee, R. N., Mathot, K. J., & Dingemanse, N. J. (2016). Behavioral and morphological responses to perceived predation risk: A field experiment in passerines. *Behavioral Ecology*, *27*(3), 857–864. doi:10.1093/beheco/arv228

Abrams, P. A., & Matsuda, H. (1997). Prey Adaptation as a Cause of Predator-Prey Cycles. *Evolution*, *51*(6), 1742. doi:10.2307/2410997

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A., & McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: A review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews*, *29*(8), 1123–1144. doi:10.1016/j.neubiorev.2005.05.005

Apfelbach, R., Soini, H. a., Vasilieva, N. Y., & Novotny, M. V. (2015). Behavioral responses of predator-naïve dwarf hamsters (Phodopus campbelli) to odor cues of the European ferret fed with different prey species. *Physiology & Behavior*, *146*(June), 57–66. doi:10.1016/j.physbeh.2015.04.014

Ariyomo, T. O., & Watt, P. J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Animal Behaviour*, *83*(1), 41–46. doi:10.1016/j.anbehav.2011.10.004

Banks, P. B., Hughes, N. K., & Rose, T. A. (2003). Do native Australian small mammals avoid faeces of domestic dogs? Responses of Rattus fuscipes and Antechinus stuartii. *Australian Zoologist*, *32*(3), 406–409. doi:10.7882/AZ.2002.018

Blanchard, R. J., Blanchard, D. C., Rodgers, J., & Weiss, S. M. (1990). The characterization and modelling of antipredator defensive behavior. *Neuroscience and Biobehavioral Reviews*, *14*(4), 463–472. doi:10.1016/S0149-7634(05)80069-7

Blumstein, D. T., Holland, B. D., & Daniel, J. C. (2006). Predator discrimination and “personality” in captive Vancouver Island marmots (Marmota vancouverensis). *Animal Conservation*, *9*(3), 274–282. doi:10.1111/j.1469-1795.2006.00033.x

Boon, A. K., Réale, D., & Boutin, S. (2007). The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecology Letters*, *10*(11), 1094–1104. doi:10.1111/j.1461-0248.2007.01106.x

Camp, M. J., Rachlow, J. L., Woods, B. A., Johnson, T. R., & Shipley, L. A. (2012). When to Run and When to Hide: The Influence of Concealment, Visibility, and Proximity to Refugia on Perceptions of Risk. *Ethology*, *118*(10), 1010–1017. doi:10.1111/eth.12000

Carlson, N. V., Healy, S. D., & Templeton, C. N. (2017). Hoo are you? Tits do not respond to novel predators as threats. *Animal Behaviour*, *128*, 79–84. doi:10.1016/j.anbehav.2017.04.006

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., & Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). *Proceedings. Biological Sciences / The Royal Society*, *277*(1687), 1571–9. doi:10.1098/rspb.2009.2128

De Franceschi, G., Vivattanasarn, T., Saleem, A. B., & Solomon, S. G. (2016). Vision Guides Selection of Freeze or Flight Defense Strategies in Mice. *Current Biology*, *26*(16), 2150–2154. doi:10.1016/j.cub.2016.06.006

Demeyrier, V. (2016). *Habitats urbanisés : des trappes écologiques potentielles pour les oiseaux sauvages ?*

Eibl-Eibesfeldt, V. I. (1953). Zur Ethologie des Hamsters (Cricetus cricetus L.). *Ethology*, *10*(2), 204–254.

Eilam, D. (2005). Die hard: A blend of freezing and fleeing as a dynamic defense - Implications for the control of defensive behavior. *Neuroscience and Biobehavioral Reviews*, *29*(8), 1181–1191. doi:10.1016/j.neubiorev.2005.03.027

Fenyk-Melody, J. (2012). The European Hamster. In *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* (pp. 923–933). Elsevier Inc. doi:10.1016/B978-0-12-380920-9.X0001-6

Franceschini, C., Siutz, C., Palme, R., & Millesi, E. (2007). Seasonal changes in cortisol and progesterone secretion in

Common hamsters. *General and Comparative Endocrinology*, *152*(1), 14–21. doi:10.1016/j.ygcen.2007.02.008

Godin, J.-G. J., & Davis, S. A. (1995). Who Dares, Benefits: Predator Approach Behaviour in the Guppy (Poecilia reticulata) Deters Predator Pursuit. *Proceedings of the Royal Society B: Biological Sciences*, *259*(1355), 193–200. doi:10.1098/rspb.1995.0028

Gorecki, A., & Grygielska, M. (1975). Consumption and Utilization of Natural Foods by the Common Hamster. *Acta Theriologica*, *20*(09), 237–246.

Graw, B., & Manser, M. B. (2007). The function of mobbing in cooperative meerkats. *Animal Behaviour*, *74*(3), 507–517. doi:10.1016/j.anbehav.2006.11.021

Hughes, R. N. (2007). Neotic preferences in laboratory rodents: Issues, assessment and substrates. *Neuroscience and Biobehavioral Reviews*, *31*(3), 441–464. doi:10.1016/j.neubiorev.2006.11.004

Jumeau, J., & Handrich, Y. (2016). *Analyse expérimentale des traversées d'une infrastructure routière par la petite faune : Suivu photo et vidéo des hamstéroducs*. Retrieved from https://hal.archives-ouvertes.fr/hal-01406451/document

Kayser, A., Weinhold, U., & Stubbe, M. (2003). Mortality factors of the common hamster Cricetus cricetus at two sites in Germany. *Acta Theriologica*, *48*(1), 47–57.

Kleiman, D. G. (1973). Maternal behaviour of the green acouchi (Myoprocta pratti Pocock), a South American caviomorph rodent. In *Behaviour* (Vol. 43(3), pp. 48–84).

Kramer, D. L., & Bonenfant, M. (1997). Direction of predator approach and the decision to flee to a refuge. *Animal Behaviour*, *54*(2), 289–295. doi:10.1006/anbe.1996.0360

La Haye, M., Müskens, G., Van Kats, R., Kuiters, A., & Siepel, H. (2010). Agri-environmental schemes for the Common hamster (Cricetus cricetus). Why is the Dutch project successful ? *Aspects of Applied Biology*, *100*, 117–124.

Mafli, A., Wakamatsu, K., & Roulin, A. (2011). Melanin-based coloration predicts aggressiveness and boldness in captive eastern Hermann's tortoises. *Animal Behaviour*, *81*(4), 859–863. doi:10.1016/j.anbehav.2011.01.025

McPhee, M. E., Segal, A., & Johnston, R. E. (2010). Hamsters use predator odors as indirect cues of predation risk. *Ethology*, *116*(6), 517–523. doi:10.1111/j.1439-0310.2010.01766.x

Monclús, R., Rödel, H. G., Von Holst, D., & De Miguel, J. (2005). Behavioural and physiological responses of naïve European rabbits to predator odour. *Animal Behaviour*, *70*(4), 753–761. doi:10.1016/j.anbehav.2004.12.019

Nechay, G., Hamar, M., & Grulich, I. (1977). The Common Hamster ( Cricetus cricetus [ L .]) ; a Review. *EPPO Bull.*, *7*(2), 255–276.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291–318. doi:10.1111/j.1469-185X.2007.00010.x

Reznik, G., Reznik-Schüller, H., & Ulrich, M. (1978). *Clinical anatomy of the European hamster (Cricetus cricetus)*.

Reznik-Schuller, H., Reznik, G., & Mohr, U. (1974). The European hamster (Cricetus cricetus L.) as an experimental animal: breeding methods and observations of their behaviour in the laboratory. *Zeitschrift Versuchtierkunde*, *16*(1), 48–58. Retrieved from https://hal.archives-ouvertes.fr/hal-01406451/document

Robertson, B. A., Rehage, J. S., & Sih, A. (2013). Ecological novelty and the emergence of evolutionary traps. *Trends in Ecology and Evolution*, *28*(9), 552–560. doi:10.1016/j.tree.2013.04.004

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution*, *19*(7), 372–378. doi:10.1016/j.tree.2004.04.009

Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*, *4*(2), 367–387. doi:10.1111/j.1752-4571.2010.00166.x

Sinn, D. L., Gosling, S. D., & Moltschaniwskyj, N. A. (2008). Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Animal Behaviour*, *75*(2), 433–442. doi:10.1016/j.anbehav.2007.05.008

Siutz, C., & Millesi, E. (2005). Social interaction in European Hamsters. In *13th Meeting of the International Hamster workgroup*. Illmitz/Vienna.

Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology*, *19*(2), 448–455. doi:10.1093/beheco/arm144

Sol, D., Lapiedra, O., & González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Animal Behaviour*, *85*(5), 1101–1112. doi:10.1016/j.anbehav.2013.01.023

Surov, A., Banaszek, A., Bogomolov, P., Feoktistova, N., & Monecke, S. (2016). Dramatic global decrease in the range and reproduction rate of the European hamster Cricetus cricetus. *Endangered Species Research*, *31*, 119–145. doi:10.3354/esr00749

Tissier, M. L., Handrich, Y., Robin, J.-P., Weitten, M., Pevet, P., Kourkgy, C., & Habold, C. (2016). How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the verge of extinction. *Scientific Reports*, *6*(April), 25531. doi:10.1038/srep25531

Tissier, M. L., Jumeau, J., Croguennec, C., Petit, O., Habold, C., & Handrich, Y. (2016). An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features. *Ecological Engineering*, *95*, 738–742. doi:10.1016/j.ecoleng.2016.07.012

Vohralik, V. (1974). Biology of the reproduction of the Common hamster Cricetus cricetus (L.). *Vest. Cs. Spol. Zool.*, *38*(3), 228–240.

Ward, J. F., MacDonald, D. W., Doncaster, C. P., & Mauget, C. (1996). Physiological response of the European hedgehog to predator and nonpredator odour. *Physiology and Behavior*, *60*(6), 1469–1472. doi:10.1016/S0031-9384(96)00245-4

Weinhold, U. (2008). Draft European action plan for the conservation of the common hamster (Cricetus cricetus L., 1758). In: Convention on the conservation of European wildlife and natural habitats. In *Standing Commitee. Council of Europe, Strasbourg, France,* (pp. 1–36).

Williams, J. L. (1999). Effects of conspecific and predator odors on defensive behavior, analgesia, and spatial working memory. *Psychological Record*, *49*(3), 493–536.

Wolf, M., van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, *447*(7144), 581–4. doi:10.1038/nature05835

Zhang, J. X., Sun, L., Bruce, K. E., & Novotny, M. V. (2008). Chronic exposure of cat odor enhances aggression, urinary attractiveness and sex pheromones of mice. *Journal of Ethology*, *26*(2), 279–286. doi:10.1007/s10164-007-0060-1

Ziomek, J., Zgrabczyńska, E., & Poradzisz, A. (2009). The behaviour of the common hamster (Cricetus cricetus) under zoo conditions. *Der Zoologische Garten*, *78*(4), 221–231. doi:10.1016/j.zoolgart.2009.08.006

# Chapter 6

> *« Personality is likely to exert an important influence on many aspects*
> *of animal ecology and evolution »*
> *Denis Réale, 2007*

# European hamsters' personality, implications for the use of the APT and tests under semi-natural conditions

Content:

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## 1. General approach

As indicated above, two main questions or perspectives arise from the results of the chapter 5. These questions and perspectives will be addressed in this chapter. First (Box 3), I aimed at understanding what could explain the inter-individual differences observed in the study 6 regarding hamsters' behavioral reactions when facing a predator. Indeed, whilst we investigated for the effects of the predator's presence on the sample of hamsters tested, and of their global use of the device, these tests reveal important differences in hamsters' response, with some hamsters that displayed aggressive behaviors, mobbing or even attacked the predator where other hamsters displayed a more passive reaction of defense (see the summarizing Figure 28 below).

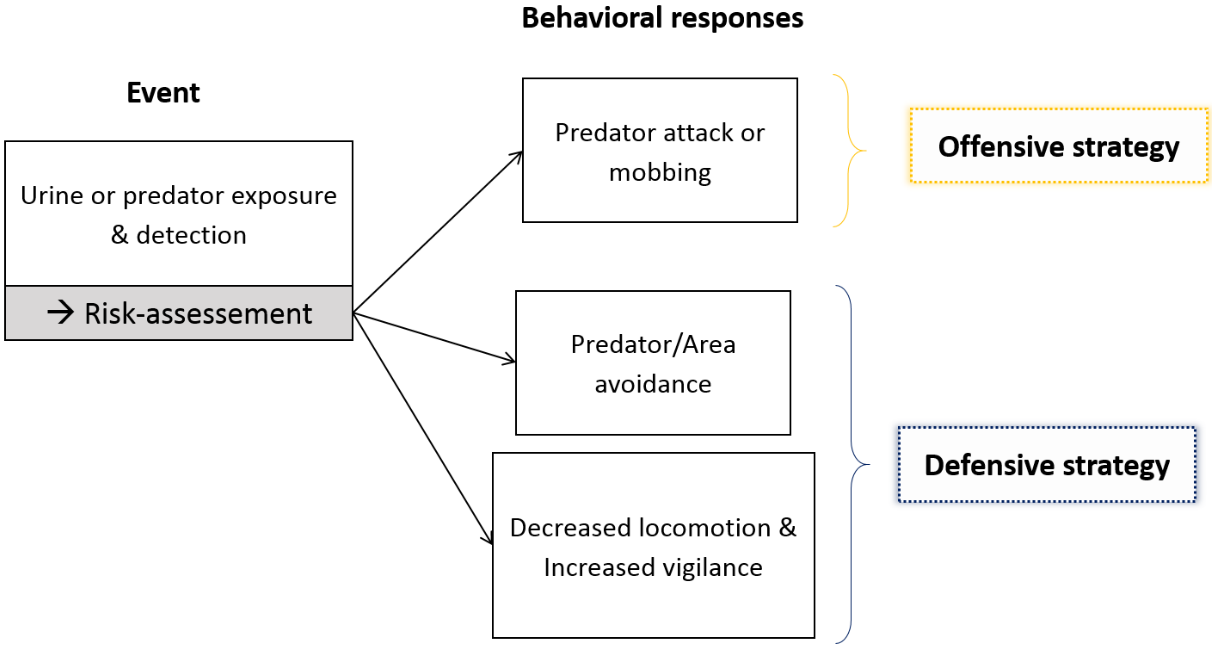

Figure 28: Summary of hamsters' behavioral responses towards the European ferret in the study 6. Some hamsters displayed behavior characteristics of an active defense whereas other rather displayed a passive defense. The figure is adapted from (Croguennec 2015).

Seeking to understand how to explain these differences at the intra-population level, I conducted several tests to investigate for the repeatability of hamsters' behavioral responses to different situations in our captive unit. In other words, I characterized some traits of their personality (Réale et al. 2007, 2010). Given the existing links between personality and some traits such as dispersion, reaction towards predators and ultimately survival (Réale et al. 2007), I also aimed at investigating how the potential personality differences in our captive population could affect hamsters' decision to consider and use the APT as a refuge.

In a second time (Box 4), I aimed at testing for the efficiency of the APT under semi-natural conditions and to investigate whether it was indeed used by hamsters in case of a predators' presence under this condition, i.e. in an actual wildlife underpass. This last study is declinable into several experiments, carried-out in a semi-wild enclosure of 3000m$^2$ built in the area of presence of the hamster in the Alsace (see Figure 29). For the purpose of the two experiments described in the Box 4, two small enclosures were built on both extremities of a wildlife underpass.

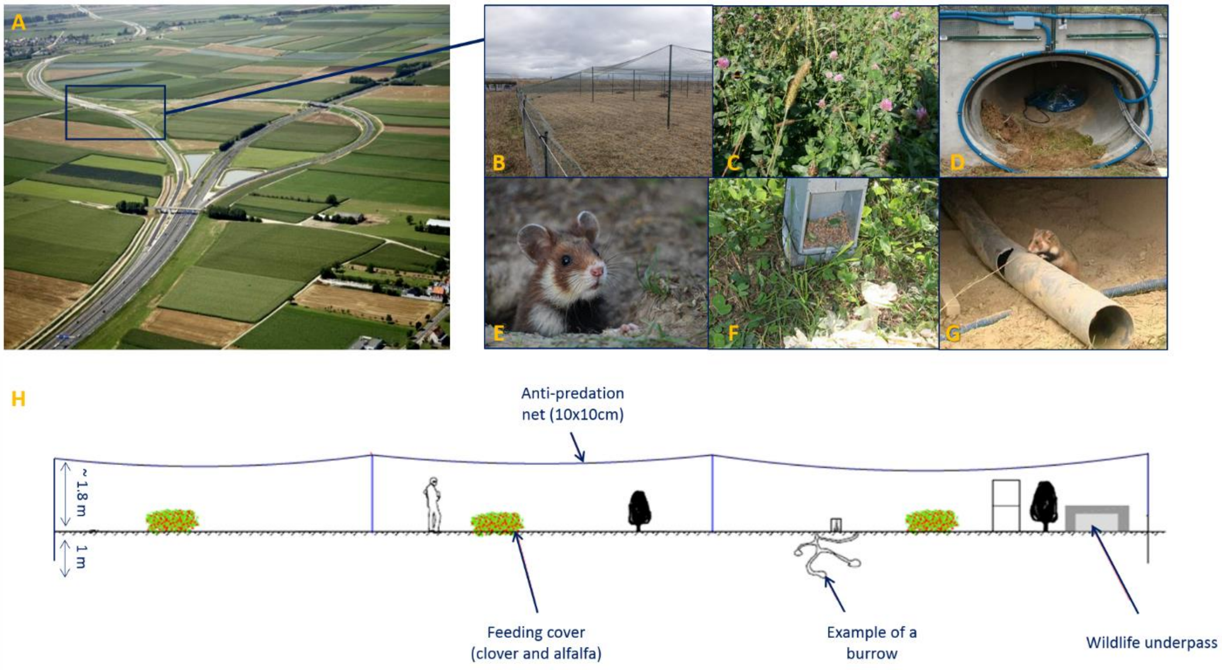

**Figure 29: Pictures and schemes summarizing the semi-wild enclosure of 3000 m$^2$ described in the Box 4**. In **A**, the area of presence of the enclosure is represented (i.e. at the Voie Rapide du Piémont des Vosges, VRPV). The pictures represent: the enclosure in **B**; the vegetative and feeding cover present in the enclosure in **C**; the wildlife underpass in **D**; a hamster emerging from a burrow in **E**; the feeders placed in the enclosure in **F** (x3) and the APT placed inside the wildlife underpass in **G**.

The first experiment carried-out in this enclosure aimed at investigating whether the presence of the APT improves the attractiveness of the underpass, under a predator-free ($P_{free}$) condition. In other terms, would the APT could serve as an enrichment to be placed inside wildlife underpasses and to favor hamsters' dispersion? The second experiment aimed at verifying that the APT was indeed use as a refuge for hamsters when a predator was present inside the underpass.

# Box 3 - A behavioral syndrome linking exploration and boldness in the European hamster

*In preparation* [1]

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[1] These data will be part of an article entitled: Tissier M. L., Handrich Y., Habold C., & Petit O. A behavioral syndrome linking exploration and boldness in the European hamster. – *In preparation*

## Short introduction

Inter-individual differences in the behavior of animals of a same population have been described in several taxa. When these differences are maintained over time and across different situations, they can refer to the animal personality or temperament (Réale et al. 2007, 2010). An individual's personality is a composite of several traits, namely boldness, exploration, activity, aggressiveness and sociality (note that the latter only concerns social species). For each trait, the individual will vary along a continuum (Réale et al. 2007) between two extremes (e.g. for boldness, the continuum varies from shy to bold). There is a tremendous literature highlighting the existing links between personality, physiological and fitness-related traits (Boon et al. 2007; Biro & Stamps 2008, 2010; Careau et al. 2008; Careau & Garland 2015). The ecological consequences of personality are also well recognized, with many research and review highlighting the associations between personality traits and dispersion, foraging and antipredator behavior (Réale et al. 2007; Dingemanse et al. 2010; Fürtbauer et al. 2015). Finally, the correlations and trade-offs between different personality traits, i.e. behavioral syndromes, (Wolf et al. 2007; Smith & Blumstein 2008; Petelle et al. 2015) have gathered a lot of attention from behavioral ecologists in the past 10 years. For instance, the boldness-exploration behavioral syndrome has been extensively studied (Fraser et al. 2001; Wolf et al. 2007; Réale et al. 2007; Smith & Blumstein 2008) and suggests that these two personality traits have co-evolved (Wolf et al. 2007). This behavioral syndrome can help to explain the expression of behaviors that appear non-adaptive in some context, e.g. an inappropriately high activity when predators are present (Sih et al. 2004), which would be an indirect expression of/correlated to the animal's boldness. Therefore, seeking to understand 1) whether such behavioral syndrome might exist in the European hamster, 2) how it would affect its reaction towards predators and 3) how it might influence its use of an Anti-predation Tube (APT (Tissier et al. 2016)) developed to upgrade wildlife underpasses and reconnect wild populations of this endangered species. We therefore carried-out several personality tests under controlled conditions. Previous studies carried-out on a variety of taxa (e.g. in lizards (Rodriguez-Prieto et al. 2011), fishes (Fraser et al. 2001; Cote et al. 2010) and in the American red squirrel (Boon et al. 2008)) revealed that bold animals are in general also more exploratory compared to shy animals. Moreover, Wolf et al. (Wolf et al. 2007) highlight that, given the adjustment of risk-taking behaviors to the expected future fitness and the longevity of the individuals, animals with 'high expectations' (i.e. long lifespan and preservation for future reproduction) will rather be risk-averse (i.e. shy). Therefore, they predict that animals with 'low expectations' would instead be 'risk-prone' (i.e. bold). Taken altogether, we were therefore expecting to find a positive behavioral syndrome linking boldness and exploration in the European hamster, with the boldest individuals being also the most exploratory.

## Methodology

### 1. Animals and husbandry conditions

Hamsters were housed individually in transparent Plexiglas cages (420*265*180 mm, D*W*H) and maintained in controlled environmental conditions (temperature 20 °C to 23 °C; 35%-55% humidity). They were submitted to a winter photoperiod (8L: 16D) from July 2013 to January 2014 and then to a summer photoperiod (16L: 8D) starting on the 1$^{st}$ of January. Their environment was enriched with wood and shredded paper. They were provided with an *ad libitum* supply of water and food pellets (N° 105, from Safe, Augy, France). The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and were approved by the Ethical Committee (CREMEAS) under agreement number 02015033110486252 (A PA FIS#397). 01.

### 2. Exploration

We quantified hamsters' exploratory behavior using the Open-field test adapted from (Archer 1973). The set-up consisted of an opaque circular arena in PVC (diameter: 1.25 m, height: 0.80 m) divided into 5 zones by lines drawn on the ground. Four peripheral zones of equal volume were delimiting a central zone (Ø = 41 cm, see **Figure 30**).

Each hamster was transferred from its cage (with some of its own litter) to a small box (40 L×15 W×15H cm), covered with a metal grid. This box was then directly placed on the center of the arena (**Figure 30**) and remained closed for 3 min. At expiration of the 3 min, the box was opened and we started the video recording. Hamsters were filmed for 5 min using a Sony Camcorder. After testing, each hamster was immediately transferred back in its cage whereas the arena and the box were cleaned with 70% ethanol. When possible (i.e. 50% of the cases), hamsters were tested twice: the first test was carried-out between the 30 January and the 5th march 2014 (~4-8 weeks after the passage on the summer photoperiod; Open-field 1, N = 100) and the second test was carried out 2 months later (to prevent from any habituation to the arena; Open-field 2, N = 50). Videos were analyzed by three experimenters: each experimenter analyzed 2-3 variables in all the 150 videos. The experimenter A recorded (i) the latency between the opening of the box and the exit of the hamster from the box, (ii) the number of marking (i.e. urine, feces or fur rubbing against the wall of the arena) and (iii) the time spent self-grooming. The experimenter B recorded (iv) the number of grooming sequences, (v) the number of body-shaking and (vi) the time spent in the box. Finally, the experimenter C recorded (vii) the number of transitions between the different zones and (viii) the number of rearing (i.e. when the hamster raised the upper part of the body). Rearing and transitions are the most used variables to characterize exploration in rodents (Réale et al. 2007; Montiglio et al. 2010, 2012, 2013; Careau et al. 2015).

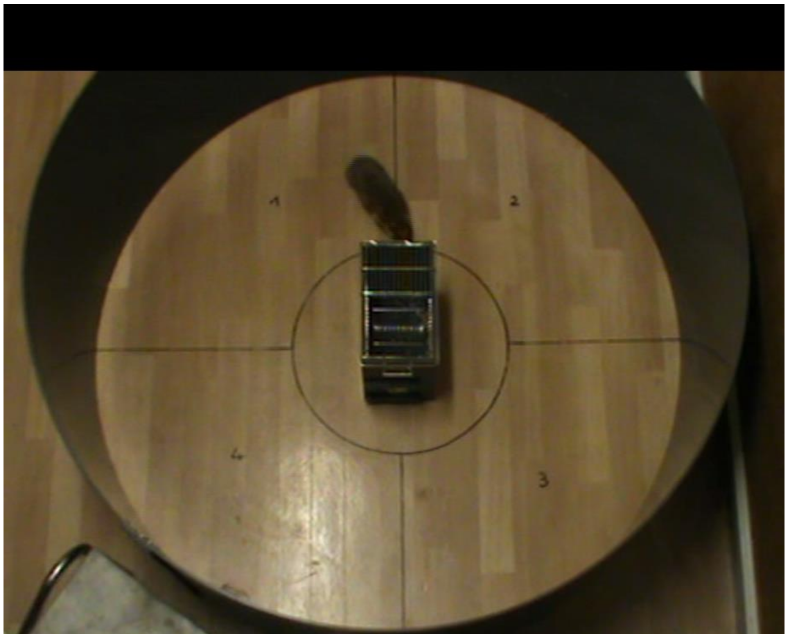

**Figure 30: circular arena used in the Open-field tests**.

### 3. Boldness

Data from labyrinth experiments (the Y-maze test, where hamsters were confronted to cats' urine) and the test of efficiency of the ATP with a non-mobile ferret (study 6, thereafter called the APT efficiency test) were confronted to the exploratory data of the present study. Namely, for each individual, we used (a) the time spent in the branch with the predator odor (Y-maze test), (b) the time spent close to the predator (APT efficiency test) and (c) the number of U-turns (APT efficiency test) as measures of boldness. The variables (a) and (b) are reflecting boldness whereas the (c) is reflecting shyness.

### 4. Data analyses

We first investigated for the effects of the age, the sex and the age*sex interaction on both the *number of rearing* and the *number of transitions* in the Open-field (i.e. measures of exploration), using a Linear Model (LM). Normality of the residuals was tested using a Kolmogorov-Smirnov test. Multiple comparisons were analyzed via post-hoc LSD (least significant difference) testing. Final model selection was based on the best AICc (Akaike information criterion for small samples) value. The links between exploration (data from the Open-Field) and boldness (data from the Y-maze test, N = 8 and data for the APT efficiency test with a mobile ferret, N = 8) were analyzed using Linear Regressions or Spearman correlations when normality was not respected. Data presented are means ± SEM. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA).

## Preliminary results

A total of 95 videos have been analyzed up-to-now (Open-field 1). These preliminary results therefore do not include 5 videos from the Open-field 1 and all the data from Open-field 2. We found an effect of the age on the number of rearing in the Open-field ($F_{1;91} = 4.3$, $p = 0.04$), showing that on average two-years-old individuals performed significantly less rearing than one-year-old individuals (**Figure 31 (a)**). However, sex ($F_{1;91} = 0.8$, $p = 0.4$) and sex*age interaction ($F_{1;91} = 0.1$, $p = 0.8$) had no effect on this variable. Regarding the number of transitions in the Open-field, no effects of age ($F_{1;91} = 1.0$, $p = 0.3$) and sex ($F_{1;91} = 0.8$, $p = 0.4$) were found, but we found an effect of the sex*age interaction ($F_{1;91} = 7.4$, $p = 0.01$). Post-hoc analyses revealed that one-year-old females performed more transitions than one-year-old males, and the trend was reversed when looking at two-years-old individuals (**Figure 31 (b)**).

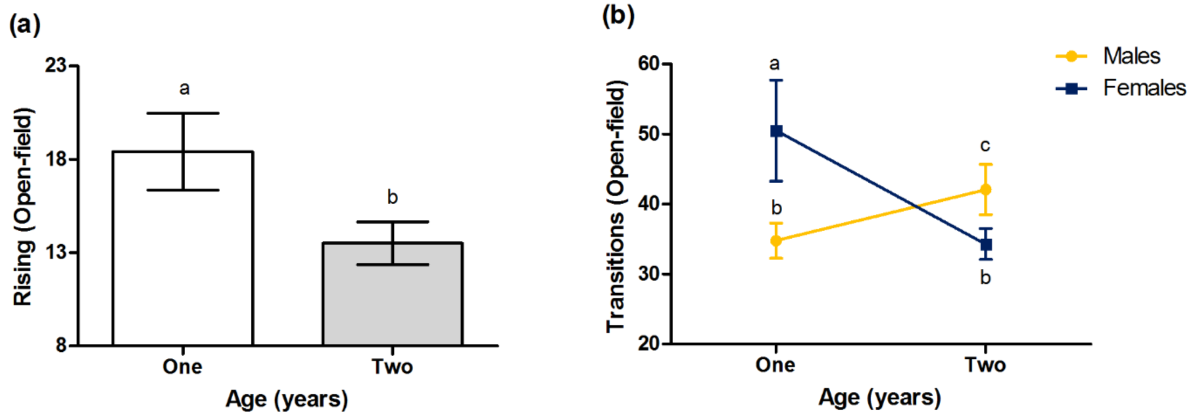

**Figure 31: Hamsters levels of exploration in the Open-field according to their age and sex**. In **(a)** is represented the average number of rearing according to the age ($N_{one\text{-}year\text{-}old} = 65$ and $N_{two\text{-}year\text{-}old} = 30$). In **(b)** is represented the average number of transitions according to the sex ($N_{males} = 55$ and $N_{females} = 40$) and age of the individuals. Different letters mean significant differences between the groups, $p < 0.05$.

Results from the regressions revealed that the number of rising (exploration, Open-field) was significantly and positively related (Figure 32 (a); $R^2 = 0.791$, $p = 0.003$) to the time spent in the branch with the cats' urine (boldness, Y-maze test). The number of transitions (exploration, Open-field) was significantly and negatively related (Figure 32 (b); $R^2 = 0.726$, $p = 0.007$) to the U-turn frequency (boldness, APT efficiency test with a non-mobile ferret). However, I found no significant links between the other recorded variables ($R^2 < 0.4$ and $p > 0.1$).

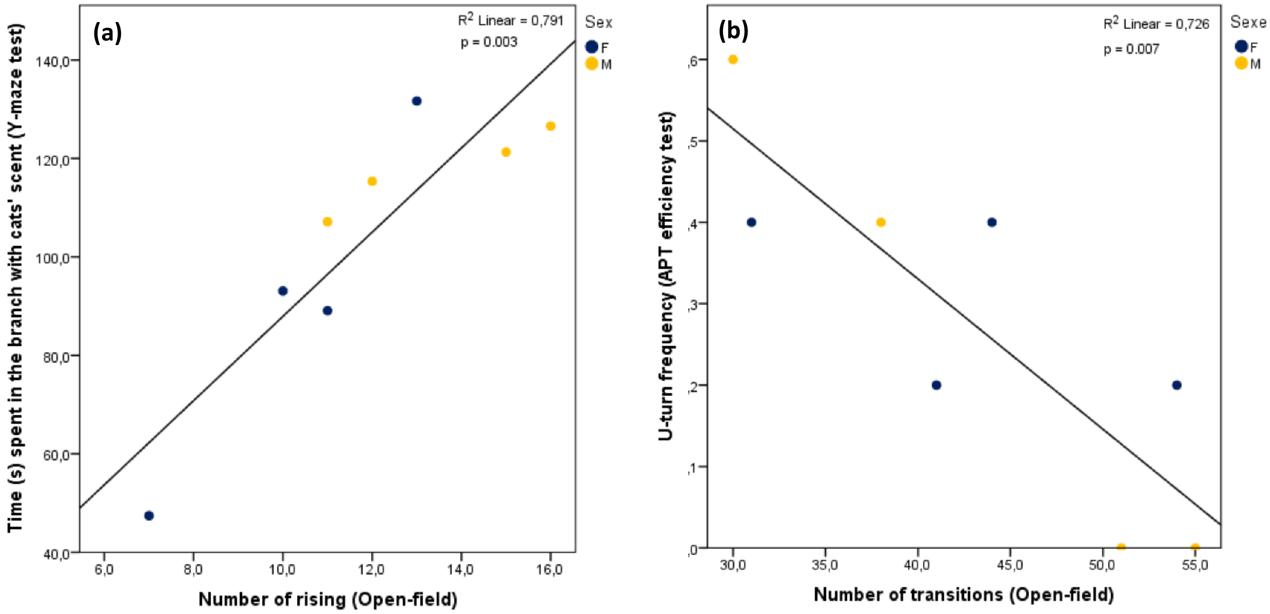

**Figure 32: Linear regressions showing the links between exploration and boldness in the European hamster.** In **(a)** is represented the regression between the number of rearing (exploration, Open-field test) and the time spent in the branch with the predators' urine (boldness, Y-maze test). In **(b)** is represented the regression between the number of transitions (exploration, Open-field test) and the U-turn frequency (boldness, APT efficiency test with a non-mobile ferret). Males (yellow) and females (dark blue) are represented for information.

## Short discussion

These preliminary results highlight the effects of hamsters' sex and age onto exploration. On average, 2-years-old individuals are less exploratory than one-year-old ones. However, this trend seems to be sex-specific. Indeed, females are more exploratory when older whereas the reverse link is observed for males. In the wild, individuals are generally expected to live for 4 years (Nechay et al. 1977; Reznik et al. 1978; Fenyk-Melody 2012) but recent data highlight that life expectancy is less than 2 years in the European range of the species (Franceschini-Zink & Millesi 2008; La Haye et al. 2014). Given that exploration is linked to dispersion (i.e. more exploratory individuals disperse more than less exploratory ones) (Fraser et al. 2001; Dingemanse et al. 2003), these results could have major implications regarding hamsters' population dynamics in the wild. However, to my knowledge, no study has investigated for the interaction between sex and age on hamsters' dispersion in wild conditions.

These results also highlight the existence of a behavioral syndrome linking boldness and exploration in the European hamster. The positive links between these two personality traits echoes what has been found in other species ((Fraser et al. 2001; Boon et al. 2008; Cote et al. 2010; Rodriguez-Prieto et al. 2011); however, see (Wolf et al. 2007) which predicts a negative link between boldness and exploration). Therefore, hamsters that were the most exploratory were also the boldest. Indeed, hamsters that displayed the highest number of rearing and transitions (Open-Field test) either spent the greatest time close to cats' urine (in the Y-maze test) or displayed the less U-turns (i.e. as a measure of shyness in the APT efficiency test with a non-mobile ferret). Given the well-known link between exploration and dispersion (Fraser et al. 2001; Dingemanse et al. 2003), it is very likely that these individuals would also be the most dispersing in wild conditions. They would therefore face the highest risks of predation in wildlife underpasses. We can assume that the shyest individuals would always use the APT developed in study 5 as a secure area to cross the underpasses, whereas boldest individuals would be expected to use it only under imminent threats. However, these assumptions remain to be tested under wild conditions.

## Box 4 - The APT as an anti-predation device or an enrichment under semi-natural conditions?

*In preparation* [1]

Hamster using the APT in presence of a ferret in a cage - © CNRS

Ferret in a cage inside a wildlife underpass, no APT - © CNRS

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[1] Tissier M.L., Bousquet C., Fleitz J., Calibre S., Jumeau J., Habold C. and Handrich Y. An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (III) Tests with the European hamster under semi-natural conditions.

## Short introduction

Wildlife underpasses and bridges are often un-adapted to the crossing of small animals (Mata et al. 2008) and often leads to increased risks of predation (Jumeau & Handrich 2016). Therefore, we built an anti-predation tube (APT, **study 5**) to facilitate the safe crossing of small animals within wildlife underpasses and culverts. Moreover, this APT could be adapted to bigger passages (*e.g.* agricultural overpasses or wildlife bridges), and serve as an 'enrichment' to make these passages more attractive to small mammals. The efficiency of the APT has been tested under controlled conditions with captive European hamsters (**study 6**). This last study revealed that hamsters use of the APT as a refuge when facing an imminent risk of predation. However, before generalizing the implementation of the APT in wildlife underpasses, we need to ensure that it will indeed increase hamster dispersion and allow them to safely cross wildlife underpasses. In other terms, we need to validate its efficiency under wild/semi-wild conditions. Indeed, the environmental factors to which hamsters are confronted to could affect its crossing or its use of the APT. Such examples are the length of the wildlife underpasses (~50m long, against 3m long in the controlled conditions), the traffic noises, the presence of odors or other small-mammals (e.g. voles or shrews). We therefore designed this study in semi-wild conditions, in which we built two small enclosures at both extremities of a wildlife underpasses in the French area of distribution of the hamster. We were therefore able to manipulate the encounter between hamsters and a predator in this underpass, with and without the presence of the APT. In this last study, we investigated whether the presence of the APT would:

1) Improve the attractiveness of the underpass, under a predator-free condition. In other terms, would the APT could serve as an enrichment to be placed inside wildlife underpasses and to favor hamsters' dispersion?
2) Improve hamsters crossing and serve as a refuge for hamsters when a predator was present inside the underpass?

Given the spontaneous use of the APT observed in the **study 5** and its use as a refuge in the **study 6**, we were expecting to observe increased hamsters' crossing when the APT was placed inside the underpass under both the predator and the predator-free conditions.

## Methodology

### 1. Animals and husbandry conditions

A total of 28 two-years-old hamsters were used in this study, among which 14 captive females, 12 captive males and 2 semi-wild males (born from captive hamsters released in 2014 inside an enclosure of 3000m$^2$). These 2 hamsters were not included in the analyses but were used to verify that they did not show a marked different behavioral response than captive individuals. Prior and after the experiments, hamsters were housed individually in transparent Plexiglas cages (420*265*180 mm, D*W*H) in our captive unit (CNRS, IPHC, Strasbourg). They were maintained in controlled environmental conditions (temperature 20 °C to 23 °C; 35%-55% humidity; photoperiod: 16L: 8D). Their environment was enriched with wood and shredded paper. They were provided with an *ad libitum* supply of water and food pellets (N° 105, from Safe, Augy, France). The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and were approved by the Ethical Committee (CREMEAS) under agreement number 02015033110486252 (A PA FIS#397). 01.

### 2. Experimental protocol

*Geographical area and experimental design*

Both studies have been carried-out near Blaesheim (Alsace, France) at the intersection of the 'Voie Rapide du Piémont des Vosges'. In the summer 2014, a small enclosure (6 m$^2$, enclosure A, **Figure 33**) was built at one extremity of a wildlife underpass (~50m long), whereas a large enclosure (3000m$^2$, not represented on the picture) was built at the other extremity (see **Figure 30** above). For the purpose of the two experiments detailed below, we then built a smaller enclosure in the 3000m$^2$ enclosure (enclosure B, **Figure 33**).

Thanks to this design, we were able to release hamsters (all equipped with a RFID transponder) in the enclosure A and to recapture them after a 16h-period. Basically, hamsters were released at 6 pm and re-captured the next morning at 10 am. The wildlife underpass was equipped with 4 video cameras, 2 camera-traps and 2 RFID (Radio Frequency Identification) antenna (schematized in **Figure 33**). We were therefore able to record hamsters' activity in the wildlife underpass during the entire 16h-period. A food bait was placed inside the enclosure B, in order to motivate hamsters to cross the underpass. We first investigated for the effects of the APT on the attractiveness of the underpass by manipulating for the presence of the APT inside the underpass under a Predator-free condition ($P_{free}$ condition). In a second time, we tested for the efficiency of the APT as a predation-device by carrying-out the same manipulation, but this time in the presence of a predator inside the underpass (P condition).

**Figure 33: Graphical abstract and pictures representing the experimental design used in the experiments 1 and 2.**

*Predator-free condition*

This experiment was carried-out from the 16th September to the 28th October 2015. A total of 12 hamsters were tested, with two tests per hamster, namely with and without the APT inside the underpass. The order of the tests were randomized for each individuals whereas ensuring for a minimum of 15 days between the two tests. These tests were carried-out in the total absence of a predator and therefore just aimed at testing for the effects of the APT on the attractiveness of the underpass, and to act as a 'control'.

*Predator condition*

This experiment was carried-out from the 18th July to the 15th September 2016. A total of 16 hamsters were tested in this experiment. Each hamster was tested twice: with and without the APT inside the underpass. The order of the tests were randomized for each individuals whereas ensuring a minimum of 15 days between the two tests. In all the tests, a ferret was placed in a cage inside the wildlife underpass just before introducing the hamster into the enclosure A.

### 3. Data analyses

Video and camera-traps data are currently being analyzed. Therefore, solely data from the RFID antenna in are presented in this box. I have investigated for the effects of the presence of the APT (with and without) on hamsters' crossing frequency (i.e. number of crossing per hour). The analyses were conducted separately for the $P_{free}$ and P conditions using Linear Mixed Models (LMM; normality of the residuals was tested using a Kolmogorov-Smirnov test). I included the APT presence, the sex, the order of the test (first or second) and the sex*APT presence interaction as fixed factors. Finally, the identity of the individuals was included as a random factor for repeated measures on the same individual in both LMM. Model selection was based on the lowest AICc values. Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), and the significance threshold was set at $p<0.05$. Figures were prepared using GraphPad prism software (Version 5, La Jolla, USA).

## Preliminary results

Under the $P_{free}$ condition (fall 2015), hamsters crossed the underpass 0.45±0.15 times/hour on average. However, I found no effects of any of the variables on the crossing frequency under this condition (LMM; see **Table 8**, left). The model with the best AICc is provided.

| Source | Df | F | p-value |
|---|---|---|---|
| Intercept | 1;2 | 10.78 | 0.13 |
| Sex | 1;12 | 1.11 | 0.31 |
| Condition | 1;10 | 1.99 | 0.19 |
| Sex*Condition | 1;10 | 3.26 | 0.10 |

| Source | Df | F | p-value |
|---|---|---|---|
| Intercept | 1;6 | 0.68 | 0.44 |
| Sex | 1;14 | 0.01 | 0.96 |
| Condition | 1;11 | 0.89 | 0.37 |
| **Sex*Condition** | **1;11** | **10.83** | **<0.01** |
| Test | 1;10 | 0.75 | 0.41 |

**Table 8: output of the Linear Mixed Models on hamsters' crossing frequency.** The output is represented for the $P_{free}$ condition (left, grey) and the P condition (right, yellow).

Under the P condition (summer 2016), hamsters crossed the underpass 0.11±0.14 times/hour on average. I found a significant effect of the sex*condition interaction on this variable, but no effects of the sex, the order of the test or the condition (LMM; Table 8, right). Post-hoc analyses revealed that the presence of the APT significantly increased males crossing frequency but had no effects on females (see Figure 34).

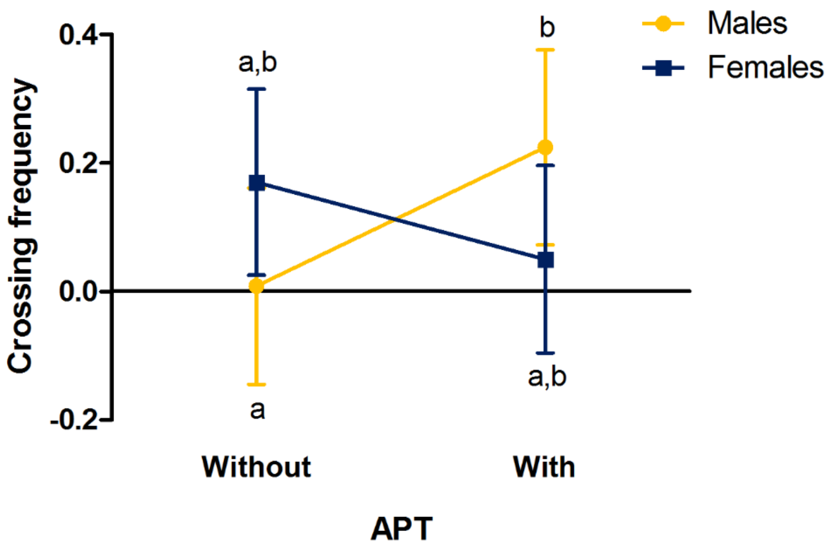


**Figure 34: crossing frequency according to the presence of the APT and the sex of the hamsters under the Predator condition**. Different letters mean significant differences between the groups, $p<0.05$.

## Short discussion

Contrary to our initial predictions, the APT do not seem to make the underpass more attractive, and therefore do not serve as an enrichment. Indeed, neither males of females crossed more the underpass in the presence of the APT. However, this conclusion cannot be extended to all the underpasses or to other small mammals. Indeed, the underpass used in this study was ~50 m long, and therefore one of the longest in the area of presence of the hamster. Moreover, the underpasses in this area have different shapes (oval, rectangular) and diameters (from 80 cm to 2 meters). All these parameters could likely influence hamsters' decision to cross and the importance of the APT as an enrichment. Regarding other species, we highlighted in the study 6 that the hamsters was a bold species (or risk-prone), and did not display the same behavioral reactions than other small mammals when faced to predation cues. We also highlighted that hamsters did not increase their use of the APT in presence of a non-mobile predator whereas they increased it in presence of a mobile predator (i.e. an imminent threat). Therefore, the APT might not be of use for them in absence of predation risks. In contrast, shyness species that are rather risk-averse (Wolf et al. 2007; Réale et al. 2007; Fürtbauer et al. 2015), might benefit more of the presence of the APT inside the underpass, even under a $P_{free}$ condition. This assumption remains to be tested by investigating the use of the APT by other small mammals (e.g. voles and shrews) under natural conditions and by analyzing in parallel their position on the shyness-boldness continuum.

Nonetheless, although the APT did not serve as an enrichment, these preliminary results tend to confirm its efficiency as an anti-predation device. Indeed, its presence significantly increased males' crossing. Given the results of the box 4, males are the individuals that would benefit the most of the presence of the APT. Indeed, this experiment was carried-out on two-years-old individuals and results of the box 4 highlighted that two-years-old males are more exploratory than two-years-old females and therefore likely more dispersing (Fraser et al. 2001; Cote et al. 2010)). Very likely, these results would be reversed in one-year-old individuals, in which females (more exploratory than males at that age; box 4) would the most benefit of the presence of the APT when a predator is present in the underpass. Taken altogether, these results therefore support the implementation of the APT in the French area of distribution of the hamster.

Finally, it is important to be noted that hamsters' crossing frequency was three times greater under the $P_{free}$ condition (0.45±0.15 crossing/hour) than under the P condition (0.11±0.14 crossing/hour). However, since the samples of hamsters changed between the two experiments and given that they were not carried-out at the same period (i.e. summer and fall), these results are hardly comparable. However, this observation would benefit of another experiment to investigate whether this is an artefact of the season or the pool of hamsters tested, or indeed an effect of the predators' presence onto the hamsters' decision to cross.

# Chapter 7

« Behavioral ecology has been slower than other fields in responding to the current conservation crisis and its changing research needs »

Bruce A. Robertson, 2013

# From behavioral ecology to conservation: limits, perspectives and recommendations

Content:

1. Limits and scientific perspectives to our studies (Thematic 2)
2. Applied perspectives and recommendations for the conservation of the European hamster: how to improve road infrastructures and beyond?

© Drawing - Dominique Pain

Carrying out behavioral studies under controlled conditions allowed us to conceive a new anti-predation prototype (APT). We then tested for its optimal characteristics for the hamster (study 5) and its efficiency in the presence of a predator (study 6). The results of the study 6 also bring new information on the behavioral reaction of the European hamster toward different predation cues. Finally, the chapter 6 (boxes 3 and 4) bring some insight onto the underlying mechanisms explaining the boldness of the European hamster (box 3) and confirm the efficiency of the APT under semi-natural conditions, but for males only (box 4). Because these studies were conducted under controlled conditions, they allow to produce results that improve the understanding of hamsters' behavior, some of which are directly applicable to its conservation. However, our set-ups also face some limits that are described and discussed below.

## 1. Limits and scientific perspectives to our studies (Thematic 2)

### *Captive-reared animals*

Despite Blumstein et al. highlighted the maintenance of antipredatory behavior in captive-reared marmots (Blumstein et al. 2006), some researches have revealed a decrease in the response of captive hedgehogs to predation cues (Ward et al. 1996). This 'inconsistency' might arise from the variance existing in rearing conditions, the time that the colony has spent in captivity or could species-specific. Nonetheless, these observations imply that the results obtained in our studies would benefit of being carried-out with wild individuals. As indicated in the box 4, two semi-wild males were included in our study aiming at investigating the efficiency of the APT as a refuge for hamsters. Given the reduced sample-size, these two males were not included into statistical analyses. However, they did not show any apparent difference with captive individuals in the frequency at which they crossed the underpass. Nonetheless, further studies are needed to investigate whether this can be generalized to a larger sample of semi-wild, or even wild individuals. Moreover, futures studies should also investigate how semi-wild or wild hamsters respond to the presence of a predator and whether they show the same mobbing and attacks than captive-reared individuals. These studies should pay attention to the latency of response to predation cues of captive-reared compared to wild individuals, which could be an important determinant of the survival of captive-reared hamsters after being released in the wild.

### *Small sample-size*

Broadly, the mall sample-size of our experiments is the major limit faced in our studies (studies 5 and 6, box 4). Indeed, given the great inter-individual differences observed in the response to the predator, we obtained several 'specific responses' that can hardly be generalized to a population. For example, in the APT efficiency test with a non-mobile ferret (**study 6**), 3 males displayed mobbing behaviors and even attacked the predator, whereas the 4th male and the 4 females did not. Therefore, we observed no changes in the frequency of agonistic behaviors when comparing the Predator and Predator-free ($P_{free}$) conditions at the level of the sample studied (N=8). However, we could imagine that further increasing the sample-size would increase the number of males displaying this behavior, reveal a sex*predator interaction or would show that some females also display this behavior. For instance, in the second APT efficiency test (N=16), this time with a mobile ferret (**study 6**), females also displayed mobbing and attacks towards the ferret, and at a higher frequency than males. Therefore, when looking at results of both of these experiments, it is hard to conclude on the potential sex effects regarding the use of the offensive or defensive strategy towards a predator such as the European ferret. These small sample-size were justified by technical and ethical reasons. First, we wanted to prevent from any habituation from the ferret towards hamsters. Second, the available individuals in our breeding unit for these experiments were limited. Third, the 3-R rule (Reduce, Replace and Refine) implies to reduce the number of used individuals as much as possible. This sample-size reduction is especially important for animals used in experiments carried-out under stressful conditions (which was the case here, in these experiments carried-out under predation risks). Nonetheless, given the important inter-individual differences, I would recommend to at minima: i) increase the sample size to 15 males and 15 females in each experiment, ii) including wild or semi-wild individuals in the study and iii) if possible, including individuals of different age in future experiments.

### *Daylight conditions of our studies*

For technical reasons, but also because European hamsters have been observed crossing wildlife underpasses in the Alsace during the day (adjacent **Figure 35**), our studies on hamsters' antipredatory behavior and personality were carried-out during the day. Given that the hamster is reported to be a nocturnal or crepuscular species during the active period (Fenyk-Melody 2012), this could have created a bias in the results that we obtained (i.e. on the reaction towards the ferret or regarding hamsters' exploration). Nonetheless, the population of hamsters in Vienna (Austria) is mostly active during the day (Schmelzer & Millesi 2003). In the Alsace, recent data reveal that hamsters shift their daily pattern of activity and become mostly active during the day after wheat harvest in July (Albert et al. 2014). Therefore, hamsters seem to be rather flexible in terms of activity patterns in the wild, which are not restricted to nocturnal/crepuscular periods unlike in captive conditions (Wollnik et al. 1991; Monecke & Wollnik 2005). Therefore, hamsters are not typical nocturnal rodents that would display patterns of activity only during this period of the day (Fenyk-Melody 2012).

**Figure 35: A European hamster crossing a wildlife underpass during the day.** © Jonathan Jumeau

*Choice of the predator*

Regarding the choice of the predator, our studies were conducted using cats' urine (Y-maze test, study 6) and the European ferret (APT efficiency tests, study 6). These choice were made for two reasons. First, most of the cases of predation observed in wildlife underpasses in the Alsace were imputed to cats (Jumeau & Handrich 2016). Second, the European ferret was chosen because this species is known to enter hamsters' burrow in the wild (see Figure 36) and could therefore pose a threat to hamsters inside the APT. However, preys' behavior towards the presence of a predator might depend onto the type of predator (Apfelbach et al. 2005).

**Figure 36: polecat exiting from a hamster burrow.** © ONCFS

Therefore, hamsters might not react in the same way towards foxes than they reacted towards ferrets. Indeed, it is very unlikely that they will benefit from the bold behavior/mobbing that they displayed in the study 6 regarding big predators such as foxes. Therefore, hamster's response towards such predators might be consistently different and remain to be investigated. This is even more justified given the important predation pressure operating onto recently released captive-reared hamsters (Villemey et al. 2013), especially by foxes (see Figure 37 below).

**Figure 37: Foxes predating adult hamsters.** In **(a)**, a case of predation at the burrow entrance is shown. In **(b)**, a fox that managed to penetrate inside a releasing unharvested field of wheat (surrounded by fences) and that captured two newly released hamsters. © ONCFS

### *Personality*

Regarding exploration, and as indicated in the box 4, part of the data remain to be analyzed, namely regarding the open-field 2. These data will allow to ensure for the repeatability of exploration in our analyses, which is a prerequisite to validate the existence of personality (Réale et al. 2007). Indeed, to be able to consider the pattern that we observed as personality traits, the inter-individual differences observed need to be repeatable across time and situations (Réale et al. 2007; Dingemanse et al. 2010). Given the existing links between personality and fitness (see Figure 38 below), a better understanding of hamsters' personality, at the scale of the population in our breeding unit could allow a better understanding of the survival and reproductive success of newly released hamsters.

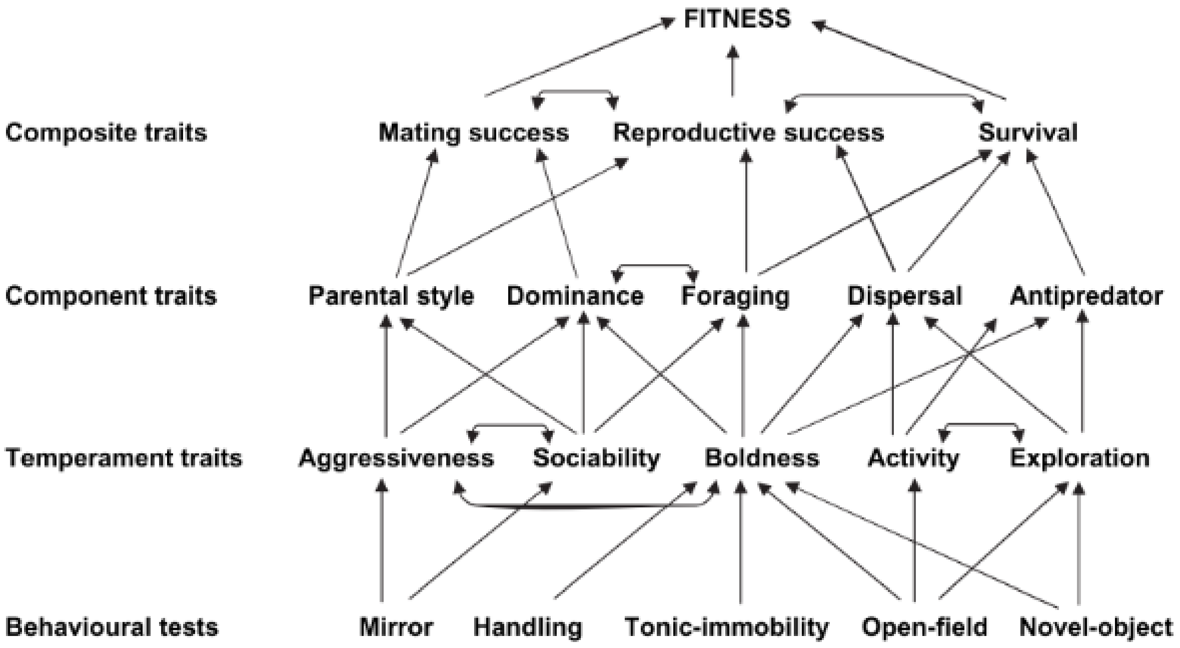


**Figure 38: Diagram illustrating the proposed framework of (Réale et al. 2007) for the ecology of personality (or temperament) traits**. The arrows represent the main (but not all possible) links between the illustrated variables.

## 2. Applied perspectives and recommendations for the conservation of the European hamster: how to improve road infrastructures and beyond?

### *Hamsters' personality and conservation*

Results shown in the box 4 highlight the importance of understanding the inter-relation between exploration and boldness in hamsters regarding their use of the APT developed in the study 5. However, these results could be more broadly applied to the conservation of the species. Indeed, restocking methods take place every year in the Alsace (Villemey et al. 2013), with between 200-400 one-year old hamsters released every year. In the box 4, we highlighted a sex*age interaction effect on hamsters exploration, as well as a positive relation between exploration and boldness in two-years-old hamsters. Given the existing links between exploration, boldness and dispersion or survival in several taxa (Dingemanse et al. 2003; Réale et al. 2007; Boon et al. 2008; Cote et al. 2010), it is very likely that the specific release of one-year old hamsters creates a bias affecting hamsters' population dynamics (De Roos et al. 2003). Moreover, many other traits such as parental style, foraging efficiency or more broadly reproductive success are also associated to individuals' age and personality types (Broussard et al. 2003; Boon et al. 2007; Réale et al. 2007). Therefore, restocking programs (also developed in other European countries (Müskens et al. 2008; Sander & Weinhold 2008), should ensure to release hamsters of varying age and sex, while trying to ensure for a diversity of behavioral phenotypes/personalities. Indeed, exploratory or bold individuals usually face a higher risk of predation but have greater dispersion and foraging capacities (Fraser et al. 2001; Réale et al. 2007; Cote et al. 2010). However, some studies have also revealed a greater reproductive success in bold, compared to shy individuals (Réale et al. 2009; Ariyomo & Watt 2012). Therefore, ensuring for the behavioral diversity of the released pool of hamsters could allow to maximize the fitness at the population level.

### *The APT efficiency and road infrastructures improvement*

Given the limits of captive conditions explained above, there is a need to pursue the tests regarding the efficiency of the APT under wild conditions. To that aim, I have collaborated with Jonathan Jumeau on this project in the past 2 years. This collaboration will allow to ensure for the efficiency of the APT on a large-scale basis. It will also offer the opportunity to investigate whether the refuge function of the APT is indeed greater for shyer prey-species than hamsters (e.g. shrews or voles).

## Validating the efficiency of the APT under wild conditions

Jumeau J., Tissier M.L., Handrich Y.

**Approach**:

In order to investigate for the efficiency of the APT for hamsters and other small mammals, a study started in 2015 in the Alsace. In the framework of this study, 8 wildlife underpasses have been monitored, among which 4 were equipped with the APT. The main goals were to:

1. Find the best localization (i.e. central or lateral, with external extremities or not) to place the APT whereas taking into account the natural movements of wildlife inside the underpasses.
2. Monitor the behavior of wildlife towards the APT. Specifically, to investigate for its efficiency as an anti-predation device for small mammals whereas ensuring that it does not impair the crossing of bigger animals.
3. Investigate for the role of the APT as a device improving the attractiveness of the underpass for small mammals, in the absence of a predator.

**Progress**:

Data analyses are ongoing. However, up-to-date analyses suggest an important use of the APT by small mammals (voles and field mice) and by their smallest predators (stoats and weasels).

One paper is expected to be published on this first study. The writing is expected to start in the summer 2017.

**Perspectives**:

The study continues in 2017, with the implementation of the APT in agricultural underpasses (see Figure 39). These structures are too large and therefore not-adapted to the crossing of small mammals. The implementation of the APT in these structures could therefore allow to improve their attractiveness for small mammals, which could use the APT as a specific underpass.

Figure 39: An agricultural underpass in the Alsace.

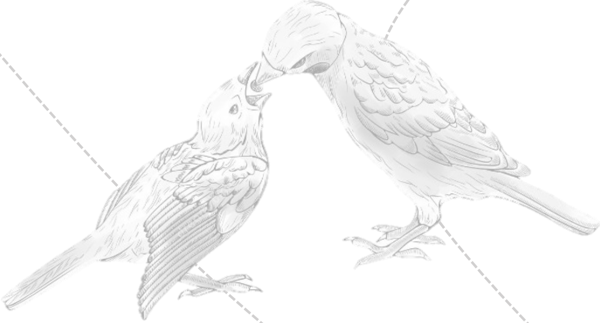
The trends observed in this study carried-out in wild conditions seem to confirm the positive function of the APT observed under captive and semi-wild conditions and to generalize it to other species than hamsters. Therefore, given the state of hamster populations in the Alsace, I would recommend to implement the APT in all the wildlife underpasses present in the French area of distribution of the species, before further investigations. In that aim, recommendations on the implementation of the APT will be given to road builders and the Ministerial organization (DREAL) in the Alsace. Basically, the recommendations for the APT implementations are based on three points:

1. A 10 cm-diameter PVC tube with lateral openings every 1-meter on both side of the device.
2. The low costs (in time and money) of the implementation of such device. Indeed, the average cost of the APT is estimated at 1.65€/meter, which corresponds to a price of 49.5€ for a wildlife underpass of 50m.
3. I would recommend to let the APT protrude from the underpass with several exits to give hamsters the opportunity to escape a predator that could wait at the exit.

These recommendations will be discussed with urban/road developers. Then, first recommendations will be given under the form of 'Action sheets' that will be adapted to the size and shapes of the different wildlife underpasses in the Alsace (see Appendix 2). Moreover, in the framework of the ALISTER project, I will write a French report to be distributed to road builders and local authorities, including recommendations based on the main results of my studies.

# Conclusion

> « *In a very real sense, the world is in our hands - and how we handle it will determine its composition and dynamics, and our fate.* »
>
> *Peter M. Vitousek 1997*



## Global conclusion & extension to a broader context

1. Improvement of the French habitat of the European hamster: conclusions from the data presented in this manuscript

2. Conciliating economy, human development and biodiversity: is it feasible?

## 1. Improvement of the French habitat of the European hamster: conclusions from the data presented in this manuscript

### *Urge to improve hamsters' habitat in France*

As indicated in the **chapters 4 and 7**, investigations are still needed to better understand to which extant the results presented in this manuscript could be applied to wild populations. However, some of these results can already be implemented to improve its French habitat. Given the highly threatened status of the species in France (with less than <1000 individuals in the wild), and the up-to-now maintenance of a good genetic diversity (Reiners et al. 2014), some results can already be implemented to improve the French hamsters' habitat and to preserve the species in the Alsace. First, there is an urgent need to reduce cereal monoculture in the Alsace as well as non-cultivated fields, either during summer for wheat, or during winter and spring for maize. Instead, mixed-farming, fine-scale mosaic of crops in the landscape and the restoration of adventive plants need to be promoted. Secondly, regarding wildlife underpasses, the implementation of the APT should be started before the research investigations validating the efficiency of the device (described in **chapter 7**) are terminated. Indeed, given the low costs of the APT, its ease of installation, its refuge function for hamsters in the presence of a predator and given that it is not impairing the crossing of bigger mammals (Jumeau J., *personal comm.*), wildlife underpasses in the French area of distribution of the species should all be quickly equipped, and ideally monitored.

### *The importance of farmers, policymakers and local authorities*

The improvement of French farmland cannot be achieved without farmers. Up-to-now, this work has been realized thanks to a close collaboration implemented by the CARA and the ONCFS with farmers, which needs to continue and to be reinforced. Moreover, there is therefore a strong need to inform farmers of the ongoing work, research findings and to include them in the different processes. For instance, the CARA gave me the opportunity to present results of the **Study 2** (revealing the major negative effects of maize consumption onto hamsters' reproduction) in front of a group of 30-40 farmers in 2015. This provided me the opportunity to exchange about my work with them and I received a very good feedback. Moreover, the discussions that we had following this presentation allowed me to include some of the requests in my experiments, namely regarding the integration of soybeans in the study 4. Later on, I had the opportunity to discuss the results of the study 4 with some of these farmers and one was very enthusiastic regarding the implementation of the wheat-soybean associations, currently ongoing. These are just personal and specific examples regarding the benefits of sharing research with the farming community.

However, even though an increasing number of farmers are involved in benefic actions towards hamsters and farmland biodiversity in the Alsace, a great complexity regarding the implementation of promising agricultural techniques favorable to the hamster remains. Indeed, many farmers often suffer from retards in the payments of the CAP compensations, such as unharvested wheat. These retards put them at economic difficulties, especially for farmers with small-size exploitations. Moreover, many farmers start to be concerned about the fact that for many years (i.e. since 2002), many actions have been implemented for hamsters in the framework of the 3 PNAs. Nonetheless, despite this great investments (in terms of money and work), populations of hamsters are still not increasing. Finally, many of them have an impression of inequity regarding the 'costs to pay' for hamsters conservation. Indeed, agriculture and farmers are pointed as responsible for hamsters decline, and they are urged to take actions to remedy to it. But, as a farmer once asked 'Among all the people concerned by the fate of the hamster and the biodiversity, how many do actually impute part of their salary into conservation? Because that is what you all ask us to do.'

Regarding the implementation of the APT, in order for it to be effective, discussions need to be rapidly initiated with road-builders. These discussions would allow to inform them of the characteristics, benefits and advantages of the APT. This would also be the opportunity to present and distribute the action sheets (Appendix 2) for the implementation of the APT depending on the characteristics of the wildlife underpass. These sheets could then be made available by local authorities, such as the DREAL and the Direction Départementale des Territoires (DDT), through their respective website pages. These local authorities should also have the responsibility to ensure for the maintenance of the APT once installed in the wildlife underpasses in the Alsace. This maintenance for examples includes to ensure for the correct installation and fixing of the APT or for its cleaning (mostly during period of elevated rainfall or mud flows in spring). Indeed, there is a risk that the APT would become clogged or that with mud and rain it would be displaced. I will further develop these problems and offer potential solutions in a report that I will write – in the framework of the ALISTER project – for the European commission and these local authorities.

### *Benefits to other species and adaptation of our results in other European countries*

As indicated above, the European hamster is an umbrella species and these measures destined to improve its habitat should therefore benefit other species. In the Alsace, according to the last published Red List, 91% of the (described and monitored) species inhabiting the same farmland habitat than the hamster are threatened or endangered. This percentage includes two plant-species (the common Corncockle, *Agrostemma githago* and *Arnoseris minima*); two bird-species (the Yellowhamer, *Emberiza citrinella* and the Eurasian stone-curlew, *Burhinus oedicnemus*); two odonate species (the Southern damselfly, *Coenagrion mercurial* and the Ornate bluet, *Coenagrion ornatum*); one solitary-bee (*Colletes hylaeiformis*), one fish (the spined loach, *Cobitis taenia*) and one mammal species (the European hamster). The butterfly *Arethusana arethusa* has already gone extinct in the Alsace Region. All these species could benefit from the restoration of adventive plants - as it has already been shown in solitary-bees (Wood et al. 2016) – and from the features of a more heterogeneous farmland habitat, which namely provides wildlife with more diverse diets and various habitats to reproduce, to nest and to hide from predators (Stoate et al. 2001; Benton et al. 2003; Wilson et al. 2005).

Moreover, results of the studies 2-6 could be applied to other European countries where conservation measures are implemented for hamsters. For instance, thanks to the exchange that takes place at the Annual Meetings of the International Hamster Workgroup (IHWG), some results of the study 4 are already used in other countries. For instance, researchers from Bavaria (Germany) and conservationists in Austria, will include the maize-sunflower and wheat-soybean crop-associations in the recommendations that they make towards local authorities for the hamster conservation.

## 2. Conciliating economy, human development and biodiversity: is it feasible?

### *The Biodiversity and agricultural crises*

In the introduction, I presented and discussed the advantages and limits of estimating the price of biodiversity. One of the main advantages is that this approach has allowed to include biodiversity conservation into policy making. Given the high costs associated to its decline, biodiversity therefore became a central point in research during the past decades, and more and more citizens and politicians start to be concerned by this biodiversity crisis. However, things are moving on too slowly, and many politicians and policymakers still remain hermetic/skeptical to global change, or to their implication into biodiversity loss. Nonetheless, human populations are also directly and increasingly affected by global change. For instance, climate change has already caused massive emigrations and the emergence of violent conflicts because of heat stress, which could threaten human security (Barnett & Adger 2007; Reuveny 2007; Mueller et al. 2014). The currently rising global temperatures have also caused several Anthrax outbreaks in Northern Siberia (the two latest occurred in 1941 and 2016) because of the 'reactivation' of the bacterium *Bacillus anthracis*, which is mortal for humans and wildlife (Guarino 2016; The Siberan Times Reporter 2016). Finally, human-induced climate changes also increasingly threaten crop yields (Fargione et al. 2009; van der Velde et al. 2012; Hawkins et al. 2013). This could impact food security at a global scale and would consequently increase problems of undernutrition across the world (Myers et al. 2016). Current investigations to reduce this trend in crop yields reduction include: 1) crop diversification, 2) generalization of agro-ecology, 3) restoration of landscape heterogeneity and 4) restoration of crop rotations, which echoes the recommendations made in the chapter 4 for hamsters and biodiversity preservation (Cirad 2009; INRA 2016).

Regarding agriculture intensification, it is now well-known that the associated uses of pesticides are responsible of humans neurodegenerative diseases or cancers (Senanayake & Karalliedde 1987; Wesseling et al. 2002; Alavanja et al. 2003; Baltazar et al. 2014). Moreover, an elevated consumption of corn syrup seems to be partly involved in the high prevalence of obesity in the US (Bray et al. 2004). Important maize consumption would also be linked to the chronic diseases and carcinoma epidemics recorded in African populations (Isaacson 2005; Dutton 2009). Furthermore, recent Pellagra (the 3D diseases caused by a niacin deficiency) outbreaks have been recorded in Nepal, Angola, Zimbabwe, Malawi, Mozambique and South-Africa (Wan et al. 2011). More broadly, many populations still suffer from important nutritional deficiencies across the world, namely of essential micronutrients such as iron, zinc, omega-3 fatty-acids and vitamins A and E (Millennium ecosystem assessment 2005; Cheung et al. 2016; Golden et al. 2016). Currently, 17% of the world population is zinc deficient, whereas 20% of pregnant women worldwide are iron deficient and 30% are Vitamin A deficient (Cheung et al. 2016). Moreover, another 11% of the current global human population is expected to become deficient in one of these micronutrients because of fish overexploitation (Golden et al. 2016). Deficiencies in these micronutrients can impair child growth and women pregnancies, but can also result in increasing hear-diseases and cancers rates (Millennium ecosystem assessment 2005; Vanbergen 2013; Daily & Karp 2015; Golden et al. 2016). These studies therefore strengthen the conclusions made in the study 2 regarding the negative effects of intensive monoculture crops on the reproduction of the hamster, and more broadly on animal species, including humans. Recently, researchers have started to link the decrease in ecosystem services such as pollination and soil functioning to a decrease in these essential micronutrients (i.e. iron, vitamins A and E) (Brittain et al. 2014; Chaplin-Kramer et al. 2014; Smith et al. 2015). Many of the most pollinator-dependent crops are also among the richest in micronutrients essential to human health (Chaplin-Kramer et al. 2014) and human micronutrient deficiencies are more likely to occur in areas with high pollination dependence for vitamin A and iron (Chaplin-Kramer et al. 2014). We are therefore facing massive global modifications of food resources, which are expected to become more and more important with the observed decrease in yields, soil depletion, and decrease in the micronutrient contents of many food items.

*Therefore, we need to remind ourselves that we do not need biodiversity, but rather that we are part of it and to start reconsidering our perceptions of global change and of the current crisis of mass extinction.*

The concept of Ecosystem Services (ES), which is too simplistic, not entirely accurate (e.g. do not represent to a full extent the global motivations to protect biodiversity and highly anthropocentric), starts to become outdated (Comberti et al. 2015; Gunton et al. 2017)) Very recently, a new framework which goes beyond the ecosystem services approach has been proposed (Gunton et al. 2017): the Ecosystem Valuating Framework (EVF). Authors propose to value ecosystems throughout a suite of aspects integrating ecological, cognitive, communal and ideological categories into the framework of this approach (Figure 40, (Gunton et al. 2017)). The core of this framework is to estimate the value of an ecosystem by including all the aspects of it and integrating the specific value given by all the stakeholders. Each stakeholder will value any given ecosystem to varying degrees (negatively or positively) for each of the 12 aspects presented on the Figure 40 below. The final evaluation consists in attributing relative scores on (as many as possible) these axes of valuation. Gunton et al. (Gunton et al. 2017) also highlight that this framework could be applicable to developing countries or non-Western cultures, that are often found in parts of the world of great conservation importance (Comberti et al. 2015) and generally the most affected by conservation efforts under the ES concept and hot-spot approaches (Millennium ecosystem assessment 2005; Comberti et al. 2015). Moreover, these populations also face many inequities and are paying the highest costs of global change and biodiversity loss.

**Figure 40: The Ecosystem Valuating Framework (EVF).** The suite of aspects for valuing ecosystems is shown as rays emanating from an ecosystem (site) of interest. The ecological category is indicated in green, cognitive in blue, communal in red and ideological in purple. Examples of stakeholders specialized in each kind of valuation are named in boxes, and some categories of value associated with each aspect around the periphery. An assessment ought to consider all the aspects for (i) a specific place and relevant stakeholders, or (ii) specific stakeholders and all relevant places. *Extracted from (Gunton et al. 2017).*

### *Inequality in the costs and benefits between human populations in the face of these crisis*

There are strong disparities between developed countries and poorest populations in terms of costs and benefits of conservation efforts on the short-term and long-term bases (Edwards & Abivardi 1998; Myers et al. 2013; Bayles et al. 2016). Indeed, populations from developing countries face high inequalities compared to people from Westernized Countries and are facing higher risks of heat stress, undernutrition or 'payback' of ecosystem alteration (e.g. Mangrove degradation) (Myers et al. 2013; Bayles et al. 2016). And unfortunately, these disparities and inequalities are expected to further increase with climate changes in these developing countries (Díaz et al. 2006), since when protein and vitamins from local food resources (e.g. crops and hunting), decrease as a result of habitat loss, climate change and reduced pollination services, the richest can still purchase them, whereas the poorest cannot.'

In Westernized Countries, inequalities also exist between whom is ensuring the costs of biodiversity conservation and whose it benefits at the intra-population level. Indeed, biodiversity often benefits the society as a whole whereas the costs and efforts of preserving it generally fall upon the individual (Edwards & Abivardi 1998). I gave such an example on the previous paragraph regarding the benefits of changing conventional farming (i.e. benefits for the society as a whole) and the costs (i.e. that usually fall upon farmers). In order for agricultural changes to be efficient, the approach needs to integrate the society, the media, the educators, the policymakers as well as the scientific community: everyone needs to be involved and integrated as stakeholders, and not only concerned about global change or the biodiversity crisis. For instance, if citizens want to benefit from an improved farming approaches, they need to be prepared to pay a higher price for it and to change their way of eating. This should be supported by policymakers. In parallel, educators and media play a major role by informing citizens and teaching the young generations. This integrated approach appears feasible under the EVF framework described above (**Figure 40**), but would greatly depends on policymakers and citizens. To my opinion, only such integrated approach can be efficient on a long-term basis, and should allow reducing human-induced global change as well as inequities between the costs and benefits of these efforts.

A good example to close this thesis is the organic canteen in the municipality of Barjac (in the Gard Region, Southern France). For 10 years now, organic and mostly local meals are provided to children at the school of this French municipality. The mayor, Mr. Edouard Chaulet, is at the base of this decision, aiming at supporting local farmers and producers, favoring organic farming, providing healthy meals for the children and teaching them about the importance of good food choices and habits. The meals cost 8.5€, but only 2.5€ are asked to the families, and the municipality pays the difference. Through this approach, and by educating the children that will be the policymakers, citizen, scientists, journalists and educators of tomorrow, I believe that this decision would reveal to be very efficient and expect that it would be generalized to other municipalities soon.

# References

## A

Acta - Les instituts techniques Agricoles, 2017. Terresinovia [WWW Document]. URL www.terresinovia.fr/ (accessed 2.1.17).

ACTeon, 2012. Plan National d'actions en faveur du hamster commun Cricetus cricetus.

AFZ, INRA, CIRAD, FAO, 2011. Feedipedia [WWW Document]. Anim. Feed Ressources Inf. Syst. URL http://www.feedipedia.org/ (accessed 5.9.16).

Aiken, C.E., Tarry-Adkins, J.L., Ozanne, S.E., 2016. Transgenerational effects of maternal diet on metabolic and reproductive ageing. Mamm. Genome. doi:10.1007/s00335-016-9631-1

Alavanja, M.C.R., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A., 2003. Use of agricultural pesticides and prostate cancer risk in the agricultural health study cohort. Am. J. Epidemiol. 157, 800–814. doi:10.1093/aje/kwg040

Albert, M., Kourkgy, C., Reiners, T.E., Eidenschenck, J., 2014. We are watching you! Detection of Common hamsters (Cricetus cricetus) surface activity using wildlife camera traps, in: 21st Annual Meeting of the International Hamster Workgroup. pp. 37–38.

Allen, L.C., Turmelle, A.S., Mendonça, M.T., Navara, K.J., Kunz, T.H., McCracken, G.F., 2009. Roosting ecology and variation in adaptive and innate immune system function in the Brazilian free-tailed bat (Tadarida brasiliensis). J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 179, 315–323. doi:10.1007/s00360-008-0315-3

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosyst. Environ. 74, 19–31.

Ammerman, C.B., Baker, D.H., Lewis, A.J., 1995. Bioavailability of Nutrients For Animals: Amino Acids, Minerals and Vitamins, United Kin. ed. Academic Press Limited.

Antrop, M., 2004. Landscape change and the urbanization process in Europe. Landsc. Urban Plan. 67, 9–26. doi:10.1016/S0169-2046(03)00026-4

Apfelbach, R., Blanchard, C.D., Blanchard, R.J., Hayes, R.A., McGregor, I.S., 2005. The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci. Biobehav. Rev. 29, 1123–1144. doi:10.1016/j.neubiorev.2005.05.005

Apfelbach, R., Soini, H. a., Vasilieva, N.Y., Novotny, M. V., 2015. Behavioral responses of predator-naïve dwarf hamsters (Phodopus campbelli) to odor cues of the European ferret fed with different prey species. Physiol. Behav. 146, 57–66. doi:10.1016/j.physbeh.2015.04.014

Archer, J., 1973. Tests for emotionality in rats and mice: A review. Anim. Behav. 21, 205–235. doi:10.1016/S0003-3472(73)80065-X

Ariyomo, T.O., Watt, P.J., 2012. The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. Anim. Behav. 83, 41–46. doi:10.1016/j.anbehav.2011.10.004

Arnold, W., Giroud, S., Valencak, T.G., Ruf, T., 2015. Ecophysiology of Omega Fatty Acids: A Lid for Every Jar. Physiology 30, 232–240. doi:10.1152/physiol.00047.2014

## B

Bäckman, J.P.C., Tiainen, J., 2002. Habitat quality of field margins in a Finnish farmland area for bumblebees (Hymenoptera: Bombus and Psithyrus). Agric. Ecosyst. Environ. 89, 53–68. doi:10.1016/S0167-8809(01)00318-8

Baeten, L., Hermy, M., Van Daele, S., Verheyen, K., 2010. Unexpected understorey community development after 30 years in ancient and post-agricultural forests. J. Ecol. 98, 1447–1453. doi:10.1111/j.1365-2745.2010.01711.x

Baker, J.M., Griffis, T.J., 2005. Examining strategies to improve the carbon balance of corn/soybean agriculture using eddy covariance and mass balance techniques. Agric. For. Meteorol. 128, 163–177. doi:10.1016/j.agrformet.2004.11.005

Báldi, A., Faragó, S., 2007. Long-term changes of farmland game populations in a post-socialist country (Hungary). Agric. Ecosyst. Environ. 118, 307–311. doi:10.1016/j.agee.2006.05.021

Baltazar, M.T., Dinis-Oliveira, R.J., de Lourdes Bastos, M., Tsatsakis, A.M., Duarte, J.A., Carvalho, F., 2014. Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases-A mechanistic approach. Toxicol. Lett. 230, 85–103. doi:10.1016/j.toxlet.2014.01.039

Banaszek, A., 2015. The species that shift their ranges in response to the oceanic-continental climatic gradient in Europe: is the Common hamster one of them?, in: The 22nd Annual Meeting of International Hamster Workgroup. Olomouc, p. 7.

Barnett, J., Adger, W.N., 2007. Climate change, human security and violent conflict. Polit. Geogr. 26, 639–655. doi:10.1016/j.polgeo.2007.03.003

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B., Ferrer, E. a, 2011. Has the Earth's sixth mass extinction already arrived? Nature 471, 51–57. doi:10.1038/nature09678

Barrett, E.L.B., Richardson, D.S., 2011. Sex differences in telomeres and lifespan. Aging Cell 10, 913–21. doi:10.1111/j.1474-9726.2011.00741.x

Barua, M., 2011. Mobilizing metaphors: The popular use of keystone, flagship and umbrella species concepts. Biodivers. Conserv. 20, 1427–1440. doi:10.1007/s10531-011-0035-y

Baruch-Mordo, S., Wilson, K.R., Lewis, D.L., Broderick, J., Mao, J.S., Breck, S.W., 2014. Stochasticity in natural forage production affects use of urban areas by black bears: Implications to management of human-bear conflicts. PLoS One 9, 1–10. doi:10.1371/journal.pone.0085122

Bayles, B.R., Brauman, K.A., Adkins, J.N., Allan, B.F., Ellis, A.M., Goldberg, T.L., Golden, C.D., Grigsby-Toussaint, D.S., Myers, S.S., Osofsky, S.A., Ricketts, T.H., Ristaino, J.B., 2016. Ecosystem Services Connect Environmental Change to Human Health Outcomes. Ecohealth 1–7. doi:10.1007/s10393-016-1137-5

Ben-David, M., Flynn, R.W., Schell, D.M., 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111, 280–291. doi:10.1007/s004420050236

Benetos, a, Okuda, K., Lajemi, M., Kimura, M., Thomas, F., Skurnick, J., Labat, C., Bean, K., Aviv, A., 2001. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension 37, 381–385. doi:10.1161/01.HYP.37.2.381

Benton, T.G., Bryant, D.M., Cole, L., Crick, H.Q.P., 2002. Linking agricultural practice to insect and bird populations: A historical study over three decades. J. Appl. Ecol. 39, 673–687. doi:10.1046/j.1365-2664.2002.00745.x

Benton, T.G., Vickery, J.A., Wilson, J.D., 2003. Farmland biodiversity: Is habitat heterogeneity the key? Trends Ecol. Evol. 18, 182–188. doi:10.1016/S0169-5347(03)00011-9

Bergstrom, C.T., Real, L.A., 2000. Towards a theory of mutual mate choice: Lessons from two-sided matching. Evol. Ecol. Res. 2, 493–508.

Bermeo, A., Couturier, S., Galeana Pizaña, M., 2014. Conservation of traditional smallholder cultivation systems in indigenous territories: Mapping land availability for milpa cultivation in the Huasteca Poblana, Mexico. Appl. Geogr. 53, 299–310. doi:10.1016/j.apgeog.2014.06.003

Berry, D., Plaut, T., 1978. Retaining agricultural activities under urban pressures: A review of land use conflicts and policies. Policy Sci. 9, 153–178. doi:10.1007/BF00143740

Beynon, S.A., Mann, D.J., Slade, E.M., Lewis, O.T., 2012. Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. J. Appl. Ecol. 49, 1365–1372. doi:10.1111/j.1365-2664.2012.02210.x

Bifolchi, A., Lodé, T., 2005. Efficiency of conservation shortcuts: An investigation with otters as umbrella species. Biol. Conserv. 126, 523–527. doi:10.1016/j.biocon.2005.07.002

Biro, P. a., Stamps, J. a., 2008. Are animal personality traits linked to life-history productivity? Trends Ecol. Evol. 23, 361–368. doi:10.1016/j.tree.2008.04.003

Biro, P.A., Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol. Evol. 25, 653–659. doi:10.1016/j.tree.2010.08.003

Birol, E., Villalba, E.R., Smale, M., 2007. Farmer Preferences for Milpa Diversity and Genetically Modified Maize in Mexico: A Latent Class Approach.

Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L., Monaghan, P., 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proc. Biol. Sci. 276, 1679–83. doi:10.1098/rspb.2008.1817

Björklund, J., Limburg, K.E., Rydberg, T., 1999. Impact of production intensity on the ability of the agricultural landscape to generate ecosystem services : an example from Sweden. Ecol. Econ. 29, 269–291.

Blaustein, A.R., Kiesecker, J.M., 2002. Complexity in conservation: Lessons from the global decline of amphibian populations. Ecol. Lett. 5, 597–608. doi:10.1046/j.1461-0248.2002.00352.x

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J.C., Rudd, R.J., Ward, B., 2008. Bat White-Nose Syndrome : An Emerging Fungal Pathogen ? doi:10.1126/science.1163874

Blount, J.D., Vitikainen, E.I.K., Stott, I., Cant, M.A., 2015. Oxidative shielding and the cost of reproduction. Biol. Rev. 91, 483–497. doi:10.1111/brv.12179

Blumstein, D.T., Holland, B.D., Daniel, J.C., 2006. Predator discrimination and “personality” in captive Vancouver Island marmots (Marmota vancouverensis). Anim. Conserv. 9, 274–282. doi:10.1111/j.1469-1795.2006.00033.x

Boon, A.K., Reale, D., Boutin, S., 2008. Personality , habitat use , and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus*. Oikos 117, 1321–1328. doi:10.1111/j.2008.0030-1299.16567.x

Boon, A.K., Réale, D., Boutin, S., 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. Ecol. Lett. 10, 1094–1104. doi:10.1111/j.1461-0248.2007.01106.x

Boothby, J., 1995. Sustaining a Threatened Landscape : Farmland Ponds in Cheshire. J. Environ. Plan. Manag. 38, 561–568. doi:10.1080/09640569512823

Bray, G.A., Nielsen, S.J., Popkin, B.M., 2004. Consumption of high-fructose syrup in beverages may play a role in the epidemic of obesity. Am. J. Clin. Nutr. 79, 537–543.

Brenton, B.P., Paine, R.R., 2000. Pellagra and Paleonutrition : assessing the diet and health of maize horticulturists through skeletal biology. Nutr. Anthropol. 23, 2–9.

Breu, A., Sprinzing, B., Merkl, K., Bechmann, V., Kujat, R., Jenei-Lanzl, Z., Prantl, L., Angele, P., 2011. Estrogen reduces cellular aging in human mesenchymal stem cells and chondrocytes. J. Orthop. Res. 29, 1563–71. doi:10.1002/jor.21424

Brittain, C., Kremen, C., Garber, A., Klein, A.M., 2014. Pollination and plant resources change the nutritional quality of almonds for human health. PLoS One 9. doi:10.1371/journal.pone.0090082

Broussard, D.R., Risch, T.S., Dobson, F.S., Murie, J.O., 2003. Senescence and age-related reproduction of female Columbian ground squirrels. J. Anim. Ecol. 72, 212–219. doi:10.1111/j.1461-0248.2005.00739.x

Buckingham, D.L., Evans, A.D., Morris, A.J., Orsman, C.J., Yaxley, R., 1999. Use of set-aside land in winter by declining farmland bird species in the UK. Bird Study 46, 157–169. doi:10.1080/00063659909461127

Budiansky, S., 1993. The doomsday myths. US News World Report, 13 81–91.

Bullock, J.M., Aronson, J., Newton, A.C., Pywell, R.F., Rey-Benayas, J.M., 2011. Restoration of ecosystem services and biodiversity: Conflicts and opportunities. Trends Ecol. Evol. 26, 541–549. doi:10.1016/j.tree.2011.06.011

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K.E., Carr, G.M., Chanson, J., Chenery, A.M., Csirke, J., Davidson, N.C., Dentener, F., Foster, M., Galli, A., Galloway, J.N., Genovesi, P., Gregory, R.D., Hockings, M., Kapos, V., Lamarque, J.-F., Leverington, F., Loh, J., McGeoch, M.A., McRae, L., Minasyan, A., Morcillo, M.H., Oldfield, T.E.E., Pauly, D., Quader, S., Revenga, C., Sauer, J.R., Skolnik, B., Spear, D., Stanwell-Smith, D., Stuart, S.N., Symes, A., Tierney, M., Tyrrell, T.D., Vie, J.-C., Watson, R., 2010. Global Biodiversity: Indicators of Recent Declines. Science (80-. ). 328, 1164–1168. doi:10.1126/science.1187512

Byrne, A.W., Fogarty, U., O'Keeffe, J., Newman, C., 2015. In situ adaptive response to climate and habitat quality variation: spatial and temporal variation in European badger (Meles meles) body weight. Glob. Chang. Biol. 21, 3336–3346.

## C

Calado, R.T., Yewdell, W.T., Wilkerson, K.L., Regal, J. a, Kajigaya, S., Stratakis, C. a, Young, N.S., 2009. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. Blood 114, 2236–43. doi:10.1182/blood-2008-09-178871

Campbell, R.D., Newman, C., McDonald, D.W., Rosell, F., Macdonald, D.W., Rosell, F., 2013. Proximate weather patterns and spring green-up phenology effect Eurasian beaver ( *Castor fiber* ) body mass and reproductive success: the implications of climate change and topography. Glob. Chang. Biol. 19, 1311–1324. doi:10.1111/gcb.12114

Campbell, R.D., Nouvellet, P., Newman, C., McDonald, D.W., Rosell, F., Macdonald, D.W., Rosell, F., 2012. The influence of mean climate trends and climate variance on beaver survival and recruitment dynamics. Glob. Chang. Biol. 18, 2730–2742. doi:10.1111/j.1365-2486.2012.02739.x

Canguilhem, B., Vaultier, J., Pvet, P., Coumaros, G., Masson-pvet, M., Bentz, I., 1988. Photoperiodic regulation of body mass , food intake , hibernation , and reproduction in intact and castrated male European hamsters , Cricetus cricetus 549–557.

Capellini, I., Baker, J., Allen, W., Street, S., Venditti, C., 2015. The role of life history traits in mammalian invasion success. Ecol. Lett. 1099–1107. doi:10.1111/ele.12493

Capizzi, D., Bertolino, S., Mortelliti, A., 2014. Rating the rat: Global patterns and research priorities in impacts and management of rodent pests. Mamm. Rev. 44, 148–162. doi:10.1111/mam.12019

Cardillo, M., Mace, G.M., Jones, K.E., Bielby, J., Bininda-emonds, O.R.P., Sechrest, W., Orme, C.D.L., Purvis, A., 2005. Multiple causes of high extinction risk in large mammal species. Science (80-. ). 309, 1239–1241.

Careau, V., Garland, T., 2015. Energetics and behavior: many paths to understanding. Trends Ecol. Evol. 30, 365–366. doi:10.1016/j.tree.2015.04.007

Careau, V., Montiglio, P.-O.O., Garant, D., Pelletier, F., Speakman, J.R., Humphries, M.M., Réale, D., R??ale, D., 2015. Energy expenditure and personality in wild chipmunks. Behav. Ecol. Sociobiol. 69, 653–661. doi:10.1007/s00265-015-1876-2

Careau, V., Réale, D., M., H.M., Thomas, D.W., 2012. The Pace of Life under Artificial Selection: Personality, Energy Expenditure, and Longevity Are Correlated in Domestic Dogs. Am. Nat. 18, 3–19. doi:10.1675/1524-4695(2008)31

Careau, V., Thomas, D., Humphries, M.M., Réale, D., 2008. Energy metabolism and animal personality. Oikos 117, 641–653. doi:10.1111/j.0030-1299.2008.16513.x

Carsignol, J., 2006. Routes et passages à faune : 40 ans d'évolution.

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nuc. Acids Res. 30.

CBD, WHO, 2005. Connecting Global Priorities: Biodiversity and Human Health, A State of Knowledge Review, in: Convention on Biological Diversity and World Health Organization. Geneva. doi:10.13140/RG.2.1.3679.6565

Ceballos, G., Ehrlich, P.R., 2002. Mammal Population Losses and the Extinction Crisis. Science (80-. ). 296, 904–907. doi:10.1126/science.1069349

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M., Palmer, T.M., 2015. Accelerated modern human – induced species losses: entering the sixth mass extinction. Sci. Adv. 1, 1–5. doi:10.1126/sciadv.1400253

Celâl Sengör, A.M., Atayman, S., Ozeren, S., 2008. A scale of greatness and causal classification of mass extinctions: implications for mechanisms. Proc. Natl. Acad. Sci. U. S. A. 105, 13736–13740. doi:10.1073/pnas.0805482105

Chaigne, A., Tissier, M.L., Habold, C., Eidenschenck, J., Uhlrich, B., 2015. Le Grand hamster (Cricetus cricetus) en Alsace, quel devenir ?, in: Bourgogne Nature - Les Mammifères Sauvages, Recolonisation et Réémergence. pp. 312–322.

Chaplin-Kramer, R., Dombeck, E., Gerber, J., Knuth, K.A., Mueller, N.D., Mueller, M., Ziv, G., Klein, A.-M., 2014. Global malnutrition overlaps with pollinator-dependent micronutrient production. Proc. R. Soc. B Biol. Sci. 281, 20141799. doi:10.1098/rspb.2014.1799

Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B., Sheldon, B.C., 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science 320, 800–3. doi:10.1126/science.1157174

Cherif, H., Tarry, J.L., Ozanne, S.E., Hales, C.N., 2003. Ageing and telomeres: A study into organ- and gender-specific telomere shortening. Nucleic Acids Res. 31, 1576–1583. doi:10.1093/nar/gkg208

Cheung, W.W.L., Jones, M.C., Reygondeau, G., Stock, C.A., Lam, V.W.Y., Frölicher, T.L., 2016. Structural uncertainty in projecting global fisheries catches under climate change. Ecol. Modell. 325, 57–66. doi:10.1016/j.ecolmodel.2015.12.018

Cirad. 2009. La Recherche Agronomique Pour Le Développement. Available from http://www.cirad.fr/.

Cohen, J.E., 2003. Human population: the next half century. Science (80-. ). 302, 1172–1175. doi:10.1126/science.1088665

Colautti, R.I., Alexander, J.M., Dlugosch, K.M., Keller, S.R., Sultan, S.E., 2017. Invasions and extinctions through the looking glass of evolutionary ecology. Philos. Trans. R. Soc. B Biol. Sci. 372, 20160031. doi:http://dx.doi.org/10.1098/rstb.2016.0031

Comberti, C., Thornton, T.F., Wylliede Echeverria, V., Patterson, T., 2015. Ecosystem services or services to ecosystems? Valuing cultivation and reciprocal relationships between humans and ecosystems. Glob. Environ. Chang. 34, 247–262. doi:10.1016/j.gloenvcha.2015.07.007

Concannon, P.W., Fullman, L. a, Baldwin, B.H., Tennant, B.C., 1989. Effects of induction versus prevention of hibernation on reproduction in captive male and female woodchucks (Marmota monax). Biol. Reprod. 41, 255–61. doi:10.1095/biolreprod41.2.255

Coopérative du Haricot Tarbais, 2012. Le Haricot Tarbais [WWW Document]. URL https://www.haricot-tarbais.com/histoire_haricot_tarbais.html

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., Sih, A., 2010. Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). Proc. Biol. Sci. 277, 1571–9. doi:10.1098/rspb.2009.2128

Countrymeters [WWW Document], n.d. URL http://countrymeters.info/ (accessed 12.8.16).

CRA Bourgogne, 2012. Cultures Intermédiaires [WWW Document]. URL http://agriculture-de-conservation.com/sites/agriculture-de-conservation.com/IMG/pdf/cipan-bourgogne.pdf (accessed 1.1.17).

Croguennec, C., 2015. Reconnexion des populations de Grand hamster (Cricetus cricetus) : Perception de la prédation et mise en place de systèmes anti-prédation au niveau des passages à faune. Strasbourg.

Curado, N., Hartel, T., Arntzen, J.W., 2011. Amphibian pond loss as a function of landscape change - A case study over three decades in an agricultural area of northern France. Biol. Conserv. 144, 1610–1618. doi:10.1016/j.biocon.2011.02.011

# D

Daily, G.C., Karp, D.S., 2015. Nature's bounties: Reliance on pollinators for health. Lancet 386, 1925–1927. doi:10.1016/S0140-6736(15)61244-2

Dalerum, F., Angerbjörn, A., 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144, 647–658. doi:10.1007/s00442-005-0118-0

Dark, J., 2005. Annual lipid cycles in hibernators: integration of physiology and behavior. Annu. Rev. Nutr. 25, 469–97. doi:10.1146/annurev.nutr.25.050304.092514

Das, U.N., 2014. Telomere length and polyunsaturated fatty acids. Nutrition 30, 1218–21. doi:10.1016/j.nut.2014.04.001

Davies, N.B., 1989. Sexual conflict and the polygamy threshold. Anim. Behav. 38, 226–234.

De Roos, A.M., Persson, L., McCauley, E., 2003. The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. Ecol. Lett. 6, 473–487. doi:10.1046/j.1461-0248.2003.00458.x

de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. a, Bjørnlund, L., Bracht Jørgensen, H., Brady, M.V., Christensen, S., de Ruiter, P.C., d'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., Bardgett, R.D., 2013. Soil food web properties explain ecosystem services across European land use systems. Proc. Natl. Acad. Sci. U. S. A. 110, 14296–301. doi:10.1073/pnas.1305198110

Decourtye, A., Mader, E., Desneux, N., 2010. Landscape enhancement of floral resources for honey bees in agro-ecosystems. Apidologie 41, 264–277. doi:10.1051/apido/2010024

Demeyrier, V., 2016. Habitats urbanisés : des trappes écologiques potentielles pour les oiseaux sauvages ?

Díaz, S., Fargione, J., Chapin, F.S., Tilman, D., 2006. Biodiversity loss threatens human well-being. PLoS Biol. 4, 1300–1305. doi:10.1371/journal.pbio.0040277

Dingemanse, N.J., Both, C., van Noordwijk, A.J., Rutten, A.L., Drent, P.J., 2003. Natal Dispersal and Personalities in Great Tits. Proc. Biol. Sci. 270, 741–7. doi:10.1098/rspb.2002.2300

Dingemanse, N.J., Kazem, A.J.N., Réale, D., Wright, J., 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol. Evol. 25, 81–89. doi:10.1016/j.tree.2009.07.013

Dobrecourt, J.., Hilaireau, J.M., Michel, A., Ballot, R., 2012. Pratiquer le strip-till [WWW Document]. URL http://agropeps.clermont.cemagref.fr/mw/index.php/Pratiquer_le_strip-till

Dominoni, D., Quetting, M., Partecke, J., 2013. Artificial light at night advances avian reproductive physiology. Proc. Biol. Sci. 280, 20123017. doi:10.1098/rspb.2012.3017

Dutton, M.F., 2009. The African Fusarium/maize disease. Mycotoxin Res. 25, 29–39. doi:10.1007/s12550-008-0005-8

# E

Edwards, P.J., Abivardi, C., 1998. The value of biodiversity: Where ecology and economy blend. Biol. Conserv. 83, 239–246. doi:10.1016/S0006-3207(97)00141-9

Eibl-Eibesfeldt, V.I., 1953. Zur Ethologie des Hamsters (Cricetus cricetus L.). Ethology 10, 204–254.

Ernandes, M., Guardia, M. La, Giammanco, S., 1996. Maize based diets and possible neuro- behavioural after-effects among some populations in the world. Hum. Evol. 11, 67–77.

Evans, A., 1997. The importance of mixed farming for seed-eating birds in the UK, in: Farming and Birds in Europe. p. Chapter 12.

## F

Fargione, J.E., Cooper, T.R., Flaspohler, D.J., Hill, J., Lehman, C., Tilman, D., McCoy, T., McLeod, S., Nelson, E.J., Oberhauser, K.S., 2009. Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation. Bioscience 59, 767–777. doi:10.1525/bio.2009.59.9.8

Fenyk-Melody, J., 2012. The European Hamster, in: The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. Elsevier Inc., pp. 923–933. doi:10.1016/B978-0-12-380920-9.X0001-6

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem 226, 497–509.

Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E. a, Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570–574. doi:10.1126/science.1111772

Forman, R.T.T., Alexander, L.E., 1998. Roads and Their Major Ecological Effects. Annu. Rev. Ecol. Syst. 29, 207–231. doi:10.1146/annurev.ecolsys.29.1.207

Franceschini, C., Millesi, E., 2003. Influences on population development in urban living European Hamsters ( Cricetus cricetus ) 2001–2003.

Franceschini, C., Siutz, C., Palme, R., Millesi, E., 2007. Seasonal changes in cortisol and progesterone secretion in Common hamsters. Gen. Comp. Endocrinol. 152, 14–21. doi:10.1016/j.ygcen.2007.02.008

Franceschini-Zink, C., Millesi, E., 2008a. Population development and life expectancy in Common Hamsters, in: Millesi, E., Winkler, H., Hengsberger, R. (Eds.), The Common Hamster: Perspectives on an Endangered Species. Austrian Academy of Sciences Press, pp. 45–59.

Franceschini-Zink, C., Millesi, E., 2008b. Reproductive performance in female common hamsters. Zoology (Jena). 111, 76–83. doi:10.1016/j.zool.2007.05.001

Frank, C.L., Dierenfeld, E.S., Storey, K.B., 1998. The Relationship Between Lipid Peroxidation, Hibernation, and Food Selection in Mammals. Amer. Zool. 38, 341–349.

Fraser, D.F., Gilliam, J.F., Daley, M.J., Le, A.N., Skalski, G.T., 2001. Explaining Leptokurtic Movement Distributions: Intrapopulation Variation in Boldness and Exploration. Am. Nat. 158, 124–135. doi:10.1086/321307

French, S.S., Greives, T.J., Zysling, D. a, Chester, E.M., Demas, G.E., 2009. Leptin increases maternal investment. Proc. Biol. Sci. 276, 4003–4011. doi:10.1098/rspb.2009.1199

Fuller, R.J., Gregory, R.D., Gibbons, D.W., Marchant, J.H., Wilson, J.D., Baillie, S.R., Carter, N., 1995. Population declines and range contractions among lowland farmland birds in Britain. Conserv. Biol. 9, 1425–1441. doi:10.1046/j.1523-1739.1995.09061425.x

Fürtbauer, I., Pond, A., Heistermann, M., King, A.J., 2015. Personality, plasticity and predation: Linking endocrine and behavioural reaction norms in stickleback fish. Funct. Ecol. 29, 931–940. doi:10.1111/1365-2435.12400

## G

Geiser, F., 2013. Hibernation. Curr. Biol. 23, 188–193. doi:10.1016/j.cub.2013.01.062

Geiser, F., Mcallan, B.M., Kenagy, G.J., 1994. The degree of dietary fatty acid unsaturation affects torpor patterns and lipid composition of a hibernator 299–305.

Geiser, F., Turbill, C., 2009. Hibernation and daily torpor minimize mammalian extinctions. Naturwissenschaften 96, 1235–1240. doi:10.1007/s00114-009-0583-0

Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21, 394–407. doi:10.1111/j.1365-2435.2007.01283.x

Giroud, S., Zahn, S., Criscuolo, F., Chery, I., Blanc, S., Ruf, T., B, P.R.S., Turbill, C., 2014. Late-born intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to hibernation: consequences for ageing processes. Proc. R. Soc. B 281, 20141131. doi:10.1098/rspb.2014.1131

Goddard, M.A., Dougill, A.J., Benton, T.G., 2010. Scaling up from gardens: biodiversity conservation in urban environments. Trends Ecol. Evol. 25, 90–98. doi:10.1016/j.tree.2009.07.016

Golden, C.D., Allison, E.H., Dey, M.M., Halpern, B.S., McCauley, D.J., Smith, M., Vaitla, B., Zeller, D., Myers, S.S., Cheung, W.W.L., Dey, M.M., Halpern, B.S., McCauley, D.J., Smith, M., Vaitla, B., Zeller, D., Myers, S.S., 2016. Fall in fish catch threatens human health. Nat. News 534, 317–320. doi:10.1038/534317a

Gomulkiewicz, R., Thompson, J.N., Holt, R.D., Nuismer, S.L., Hochberg, M.E., 2000. Hot Spots, Cold Spots, and the Geographic Mosaic Theory of Coevolution. Am. Nat. 156, 156–174. doi:10.1086/303382

Gorecki, A., Grygielska, M., 1975. Consumption and Utilization of Natural Foods by the Common Hamster. Acta Theriol. (Warsz). 20, 237–246.

Goulson, D., Hughes, W.O.H., Derwent, L.C., Stout, J.C., 2002. Colony growth of the bumblebee, Bombus terrestris, in improved and conventional agricultural and suburban habitats. Oecologia 130, 267–273. doi:10.1007/s004420100803

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites , pesticides , and lack of flowers. Science (80-. ). 347, 1–16. doi:10.1126/science.1255957

Graham, N.A.J., Spalding, M.D., Sheppard, C.R.C., 2010. Reef shark declines in remote atolls highlight the need for multi-faceted conservation action. Aquat. Conserv. Mar. Freshw. Ecosyst. 20, 543–548. doi:10.1002/aqc.1116

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bal, X., Briggs, J.M., 2008. Global Change and the Ecology of Cities.

Science 319, 756–760. doi:10.1126/science.1150195

Grulich, I., 1981. Die Baue des Hamsters (Cricetus cricetus, Rodentia, Mammalia). Folia Zool. 30, 99–116.

Guarino, B., 2016. Anthrax sickens 13 in western Siberia, and a thawed-out reindeer corpse may be to blame [WWW Document]. Washington Post. URL https://www.washingtonpost.com/news/morning-mix/wp/2016/07/28/anthrax-sickens-13-in-western-siberia-and-a-thawed-out-reindeer-corpse-may-be-to-blame/?utm_term=.5bd43f864bf5 (accessed 2.26.17).

Gunton RM, van Asperen EN, Basden A, Bookless D, Araya Y, Hanson DR, Goddard MA, Otieno G, Jones GO. 2017. Beyond Ecosystem Services: Valuing the Invaluable. Trends in Ecology & Evolution:1–9. Elsevier Ltd. DOI: 0.1016/j.tree.2017.01.002. Available from http://linkinghub.elsevier.com/retrieve/pii/S0169534717300150.

## H

Hall, D.M., Camilo, G.R., Tonietto, R.K., Ollerton, J., Ahrné, K., Arduser, M., Ascher, J.S., Baldock, K.C.R., Fowler, R., Frankie, G., Goulson, D., Gunnarson, B., Hanley, M.E., Jackson, J.I., Langellotto, G., Lowenstein, D., Minor, E.S., Philpott, S.M., Potts, S.G., Sirohi, M.H., Spevak, E.M., Stone, G.N., Threlfall, C.G., 2016. The city as a refuge for insect pollinators. Conserv. Biol. 14 Septemb, doi: 10.1111/cobi.1284. doi:10.1111/cobi.12840.This

Halvorson, A.D., Del Grosso, S.J., Jantalia, C.P., 2011. Nitrogen Source Effects on Soil Nitrous Oxide Emissions from Strip-Till Corn. J. Environ. Qual. 40, 1775. doi:10.2134/jeq2011.0194

Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.

Harmon, A.C., Huhman, K.L., Moore, T.O., Albers, H.E., 2002. Oxytocin Inhibits Aggression in Female Syrian Hamsters. J. Neuroendocrinol. 14, 963–969.

Hawkins, E., Fricker, T.E., Challinor, A.J., Ferro, C. a T., Ho, C.K., Osborne, T.M., 2013. Increasing influence of heat stress on French maize yields from the 1960s to the 2030s. Glob. Chang. Biol. 19, 937–947. doi:10.1111/gcb.12069

Heckenbenner, B., De Pontbriand, S., 2011. CIPAN : quand l'outil réglementaire devient un atout agronomique et faunistique. Faune Sauvag. 2e trimest, 11–19.

Hegyi, J., Schwartz, R. a., Hegyi, V., 2004. Pellagra: Dermatitis, dementia, and diarrhea. Int. J. Dermatol. 43, 1–5. doi:10.1111/j.1365-4632.2004.01959.x

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B., Monaghan, P., 2012. Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. U. S. A. 109, 1743–8. doi:10.1073/pnas.1113306109

Hendry, A.P., Farrugia, T.J., Kinnison, M.T., 2008. Human influences on rates of phenotypic change in wild animal populations. Mol. Ecol. 17, 20–29. doi:10.1111/j.1365-294X.2007.03428.x

Hendry, A.P., Gotanda, K.M., Svensson, E.I., 2017. Human influences on evolution, and the ecological a nd societal consequences. doi:10.1098/rstb.2016.0028

Henle, K., Alard, D., Clitherow, J., Cobb, P., Firbank, L., Kull, T., McCracken, D., Moritz, R.F.A., Niemelä, J., Rebane, M., Wascher, D., Watt, A., Young, J., 2008. Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe-A review. Agric. Ecosyst. Environ. 124, 60–71. doi:10.1016/j.agee.2007.09.005

Herranz, D., Serrano, M., 2010. Impact of sirt1 on mammalian aging. Aging (Albany. NY). 2, 315–316. doi:v2/n6/full/100156.html [pii]

Heuacker, V., Kaempf, S., Moratin, R., Muller, Y., 2015. Livre Rouge des espèces menacées en Alsace. Strasbourg.

Hoelzl, F., Cornils, J.S., Smith, S., Moodley, Y., Ruf, T., 2016. Telomere dynamics in free-living edible dormice (Glis glis): the impact of hibernation and food supply. J. Exp. Biol. 219, 2469–2474. doi:10.1242/jeb.140871

Hole, D.G., Perkins, A.J., Wilson, J.D., Alexander, I.H., Grice, P. V., Evans, A.D., 2005. Does organic farming benefit biodiversity? Biol. Conserv. 122, 113–130. doi:10.1016/j.biocon.2004.07.018

Hölker, F., Wolter, C., Perkin, E.K., Tockner, K., 2010. Light pollution as a biodiversity threat. Trends Ecol. Evol. 25, 681–682. doi:10.1016/j.tree.2010.09.007

Hoogenboom, M.O., Metcalfe, N.B., Groothuis, T.G.G., de Vries, B., Costantini, D., 2012. Relationship between oxidative stress and circulating testosterone and cortisol in pre-spawning female brown trout. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 379–87. doi:10.1016/j.cbpa.2012.07.002

Hufnagl, S., Franceschini-Zink, C., Millesi, E., 2011. Seasonal constraints and reproductive performance in female Common hamsters (Cricetus cricetus). Mamm. Biol. - Zeitschrift für Säugetierkd. 76, 124–128. doi:10.1016/j.mambio.2010.07.004

Hufnagl, S., Siutz, C., Millesi, E., 2010. Diet composition of Common hamsters (Cricetus cricetus) living in an urban environment. Säugetierkundl. Inf.

Humphries, M., Thomas, D., Kramer, D., 2011. Torpor and digestion in food-storing hibernators. Physiol. Biochem. Zool. 74, 283–292. doi:10.1086/319659

Humphries, M.M., Thomas, D.W., Kramer, D.L., 2003a. The role of energy availability in Mammalian hibernation: An Experimental Test in Free-Ranging Eastern Chipmunks. Physiol. Biochem. Zool. 76, 165–179. doi:10.1086/367950

Humphries, M.M., Thomas, D.W., Kramer, D.L., 2003b. The Role of Energy Availability in Mammalian Hibernation : A Cost-Benefit Approach. Physiol. Biochem. Zool. 76, 165–179.

I

Imai, S., Guarente, L., 2014. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 24, 464–471.

Inger, R., Gregory, R., Duffy, J.P., Stott, I., 2015. Common European birds are declining rapidly while less abundant species ' numbers are rising 28–36. doi:10.1111/ele.12387

INRA. 2016. Agriculture Durable. Available from http://www.inra.fr/Grand-public/Agriculture-durable.

Insel, T.R., Hulihan, T.J., 1995. A gender-specific mechanism for pair bonding: Oxytocin and partner preference formation in monogamous voles. Behav. Neurosci. 109, 782–789.

Isaacson, C., 2005. The change of the staple diet of black South Africans from sorghum to maize (corn) is the cause of the epidemic of squamous carcinoma of the oesophagus. Med. Hypotheses 64, 658–660. doi:10.1016/j.mehy.2004.09.019

Itagaki, T., 2005. Opposing effects of oestradiol and progesterone on intracellular pathways and activation processes in the oxidative stress-induced activation of cultured rat hepatic stellate cells. Gut. doi:10.1136/gut.2004.053728

## J

Jacob, J., 2003. Short-term effects of farming practices on populations of common voles. Agric. Ecosyst. Environ. 95, 321–325.

Jaeger, J. a G., Fahrig, L., 2004. Effects of road fencing on population persistence. Conserv. Biol. 18, 1651–1657. doi:10.1111/j.1523-1739.2004.00304.x

Jastroch, M., Giroud, S., Barrett, P., Geiser, F., Heldmaier, G., Herwig, A., 2016. Seasonal Control of Mammalian Energy Balance: Recent Advances in the Understanding of Daily Torpor and Hibernation. J. Neuroendocrinol. 28, 1–10. doi:10.1111/jne.12437

Jeschke, J.M., Strayer, D.L., 2008. Are threat status and invasion success two sides of the same coin? Ecography (Cop.). 31, 124–130. doi:10.1111/j.2007.0906-7590.05343.x

Johnson-Down, L., Egeland, G.M., 2010. Adequate nutrient intakes are associated with traditional food consumption in nunavut inuit children aged 3-5 years. J. Nutr. 140, 1311–1316. doi:10.3945/jn.109.117887

Jumeau, J., Handrich, Y., 2016. Analyse expérimentale des traversées d’une infrastructure routière par la petite faune : Suivu photo et vidéo des hamstéroducs.

## K

Kayser, A., Weinhold, U., Stubbe, M., 2003. Mortality factors of the common hamster Cricetus cricetus at two sites in Germany. Acta Theriol. (Warsz). 48, 47–57.

Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C., Smith, V.S., 2004. Species Coextinctions and the Biodiversity Crisis. Science (80-. ). 305, 1632–1634. doi:10.1126/science.1101101

Kontoleon, A., Swanson, T., 2003. The Willingness to Pay for Property Rights for the Giant Panda: Can a Charismatic Species Be an Instrument for Nature Conservation? Land Econ. 79, 483–499. doi:10.2307/3147295

Korbut, Z., Agata, B., 2016. The history of species reacting with range shifts to the Oceanic-Continental climate gradient in Europe. The case of the common hamster (Cricetus Cricetus). Kosmos 65, 69–79.

Kotiaho, J.S., Kaitala, V., Komonen, A., Päivinen, J., 2005. Predicting the risk of extinction from shared ecological characteristics. Proc. Natl. Acad. Sci. 102, 1963–1967. doi:10.1073/pnas.0406718102

Kourkgy, C., Eidenschenck, J., 2015. Délivrable Action D1. Rapport annuel de présentation des données collectées et premières analyses, année 2014. Strasbourg.

Krebs, J.R., Wilson, J.D., Bradbury, R.B., Siriwardena, G.M., 1999. The second Silent Spring? Nature 400, 611–612. doi:10.1038/23127

Kremen, C., Miles, A., 2012. Ecosystem Services in Biologically Diversified versus Conventional Farming Systems: Benefits, Externalitites, and Trade-Offs. Ecol. Soc. 17, 1–23. doi:10.5751/ES-05035-170440

Kriengwatana, B., Wada, H., Macmillan, A., MacDougall-Shackleton, S. a, 2014. Juvenile nutritional stress affects growth rate, adult organ mass, and innate immune function in zebra finches (Taeniopygia guttata). Physiol. Biochem. Zool. 86, 769–81. doi:10.1086/673260

Ksir, C., Hart, C.L., 2016. Correlation still does not imply causation. The Lancet Psychiatry 3, 401. doi:10.1016/S2215-0366(16)30005-0

Kuiters, L., La Haye, M., Muskens, G., Van Kats, R., 2007. Survival analysis to predict the predation risk in reintroduced populations of the common hamster (Cricetus cricetus) in the Netherlands, in: V European Congress of Mammalogy. HYSTRIX The Italian Journal of Mammalogy, Siena.

## L

La Haye, M., Müskens, G., Van Kats, R., Kuiters, A., Siepel, H., 2010. Agri-environmental schemes for the Common hamster (Cricetus cricetus). Why is the Dutch project successful ? Asp. Appl. Biol. 100, 117–124.

La Haye, M.J.J., Neumann, K., Koelewijn, H.P., 2011. Strong decline of gene diversity in local populations of the highly endangered Common hamster (Cricetus cricetus) in the western part of its European range. Conserv. Genet. 13, 311–322. doi:10.1007/s10592-011-0278-x

La Haye, M.J.J., Swinnen, K.R.R., Kuiters, a. T., Leirs, H., Siepel, H., 2014. Modelling population dynamics of the Common hamster (Cricetus cricetus): Timing of harvest as a critical aspect in the conservation of a highly endangered rodent. Biol. Conserv. 180, 53–61. doi:10.1016/j.biocon.2014.09.035

Lande, R., 1998. Anthropogenic, Ecological and Genetic Factors in Extinction and Conservation, in: Extinction Risk and Life History Evolution. 18th Symposium of the Society of Population Ecology. pp. 259–269.

Lane, J.E., Kruuk, L.E.B., Charmantier, A., Murie, J.O., Dobson, F.S., 2012. Delayed phenology and reduced fitness associated with climate change in a wild hibernator. Nature 489, 554–557. doi:10.1038/nature11335

Lebl, K., Millesi, E., 2008. Lebl, Yearling male Common Hamsters and the trade-off between growth and reproduction.pdf, in: Millesi, E., Winkler, H., Hengsberger, R. (Eds.), The Common Hamster (Cricetus Cricetus): Perspectives on an Endangered Species. Austrian Academy of Sciences Press, Vienna, pp. 115–125.

Lesbarrères, D., Fahrig, L., 2012. Measures to reduce population fragmentation by roads: What has worked and how do we know? Trends Ecol. Evol. 27, 374–380. doi:10.1016/j.tree.2012.01.015

LIFE [WWW Document], 2017. . last Updat. - January 2017. URL http://ec.europa.eu/environment/life/ (accessed 1.15.17).

Lindsey, P.A., Alexander, R., Frank, L.G., Mathieson, A., Roma??ach, S.S., 2006. Potential of trophy hunting to create incentives for wildlife conservation in Africa where alternative wildlife-based land uses may not be viable. Anim. Conserv. 9, 283–291. doi:10.1111/j.1469-1795.2006.00034.x

Little, S., 2003. The influence of predator-prey relationships on wildlife passage evaluation, in: ICOET. pp. 277–292.

Little, S.J., Harcourt, R., Clevenger, A., 2002. Do wildlife passages act as prey-traps? Biol. Conserv. 107, 135–145.

Lochmiller, R.L., Deerenberg, C., 2000. Trade-Offs in Evolutionary Immunology: Just What Is the Cost of Immunity? Oikos 88, 87–98. doi:doi:10.1034/j.1600-0706.2000.880110.x

Lomborg, B.E.M. the R.S. of the W., 2003. The Skeptical Environmentalist: Measuring the Real State of the World. Cambridge University Press, Cambridge, UK. doi:10.1038/scientificamerican0502-14

Love, O.P., Wynne-Edwards, K.E., Bond, L., Williams, T.D., 2008. Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. Horm. Behav. 53, 104–11. doi:10.1016/j.yhbeh.2007.09.007

## M

MacDonald, D.W., Tattersall, F.H., Service, K.M., Firbank, L.G., Feber, R.E., 2007. Mammals, agri-environment schemes and set-aside - What are the putative benefits? Mamm. Rev. 37, 259–277. doi:10.1111/j.1365-2907.2007.00100.x

Mage, D., Ozolins, G., Peterson, P., Webster, A., Orthofer, R., Vandeweerd, V., Gwynne, M., 1992. Urban Air Pollution in Megacities of the World 30.

Marquet, B., 2014. Le Grand hamster Cricetus cricetus (Linnaeus, 1758) : contribution à l'étude de l'animal et de son statut en Alsace.

Marshall, E.J.P., Moonen, A.C., 2002. Field margins in northern Europe: Integrating agricultural, environmental and biodiversity functions. Agric. Ecosyst. Environ. 89, 5–21. doi:10.1016/S0167-8809(01)00315-2

Marshall, E.J.P., West, T.M., Kleijn, D., 2006. Impacts of an agri-environment field margin prescription on the flora and fauna of arable farmland in different landscapes. Agric. Ecosyst. Environ. 113, 36–44. doi:10.1016/j.agee.2005.08.036

Martin, C., Independence, E., Act, S., Bank, W., Webb, P., 2008. Biofuel boom High life. Curr. Biol. 20, 128–129.

Martin, J.G.A., Réale, D., 2008. Temperament, risk assessment and habituation to novelty in eastern chipmunks, Tamias striatus. Anim. Behav. 75, 309–318. doi:10.1016/j.anbehav.2007.05.026

Maslo, B., Leu, K., Faillace, C., Weston, M.A., Pover, T., Schlacher, T.A., 2016. Selecting umbrella species for conservation: A test of habitat models and niche overlap for beach-nesting birds. Biol. Conserv. 203, 233–242. doi:10.1016/j.biocon.2016.09.012

Masson-Pévet, M., Naimi, F., Canguilhem, B., Saboureau, M., Bonn, D., Pevet, P., 1994. Are the annual reproductive and body weight rhythms in the male European hamster (Cricetus cricetus) dependent upon a photoperiodically entrained circannual clock? J. Pineal Res. 17, 151–163. doi:10.1111/j.1600-079X.1994.tb00127.x

Mata, C., Hervas, I., Herranz, J., Suarez, F., Malo, J.E., 2008. Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. J. Environ. Manage. 88, 407–415. doi:10.1016/j.jenvman.2007.03.014

Matson, P.A., Parton, J.W., Power, A.G., Swift, M.J., 1997. Agricultural Intensification and Ecosystem Properties. Science (80-. ). 277, 504–509. doi:10.1126/science.277.5325.504

Mayer, M., Künzel, F., Zedrosser, A., Rosell, F., 2017. The 7-year itch: non-adaptive mate change in the Eurasian beaver. Behav. Ecol. Sociobiol. 71, 32. doi:10.1007/s00265-016-2259-z

Mayer, S., Brüderlein, S., Perner, S., Waibel, I., Holdenried, A., Ciloglu, N., Hasel, C., Mattfeldt, T., Nielsen, K.V., Möller, P., 2006. Sex-specific telomere length profiles and age-dependent erosion dynamics of individual chromosome arms in humans. Cytogenic Genome Res. 112, 194–201.

McCarthy, M.M., 1990. Oxytocin inhibits infanticide in female house mice (Mus domesticus). Horm. Behav. 24, 365–375. doi:10.1016/0018-506X(90)90015-P

McEuen, A.B., 2014. Embrace New Conservation. Front. Ecol. Environ. 12, 322–323. doi:doi:10.1890/14.WB.009

McKinney, M.L., Lockwood, J.L., 1999. Biotic homogeneization: a few winners replacing many losers in the next mass extinction. Trends Ecol. Evol. 14, 450–453. doi:10.1126/science.285.5435.1834

Medan, D., Torretta, J.P., Hodara, K., de la Fuente, E.B., Montaldo, N.H., 2011. Effects of agriculture expansion and intensification on the vertebrate and invertebrate diversity in the Pampas of Argentina. Biodivers. Conserv. 20, 3077–3100. doi:10.1007/s10531-011-0118-9

Melosik, I., Ziomek, J., Winnicka, K., Reiners, T.E., Banaszek, A., Mammen, K., Mammen, U., Marciszak, A., 2017. The genetic characterization of an isolated remnant population of an endangered rodent (Cricetus cricetus L.) using comparative data: implications for conservation. Conserv. Genet. 0, 1–17. doi:10.1007/s10592-017-0925-y

Mennechez, G., Clergeau, P., 2006. Effect of urbanisation on habitat generalists: starlings not so flexible? Acta Oecologica 30, 182–191. doi:10.1016/j.actao.2006.03.002

Michener, G.R., 1992. Oecologia Sexual differences in over-winter torpor patterns of Richardson ' s ground squirrels in natural hibernacula 397–406.

Millennium ecosystem assessment, 2005. Ecosystems and Human well-being: Health Synthesis, Washington, DC. doi:10.1196/annals.1439.003

Milner, J.M., van Beest, F.M., Solberg, E.J., Storaas, T., 2013. Reproductive success and failure: the role of winter body mass in reproductive allocation in Norwegian moose. Oecologia 172, 995–1005. doi:10.1007/s00442-012-2547-x

Ministère Ecologie, 2012. Plans Nationaux d'actions en faveur des espèces menaces - Objectifs et exemples d'actions [WWW Document]. URL http://www.developpement-durable.gouv.fr/-Especes-menacees-les-plans-.html (accessed 1.15.17).

Molfetti, É., Torres Vilaça, S., Georges, J.Y., Plot, V., Delcroix, E., Le Scao, R., Lavergne, A., Barrioz, S., dos Santos, F.R., de Thoisy, B., 2013. Recent Demographic History and Present Fine-Scale Structure in the Northwest Atlantic Leatherback (Dermochelys coriacea) Turtle Population. PLoS One 8. doi:10.1371/journal.pone.0058061

Monaghan, P., Haussmann, M.F., 2006. Do telomere dynamics link lifestyle and lifespan? Trends Ecol. Evol. 21, 47–53. doi:10.1016/j.tree.2005.11.007

Monastersky, R., 2014. Life - a status report. Nature 516, 158–161. doi:10.1038/516158a

Monecke, S., 2013. All things considered? Alternative reasons for hamster extinction. Zool. Pol. 58, 41–57. doi:10.2478/zoop-2013-0004

Monecke, S., Malan, A., Wollnik, F., 2006. Asymmetric control of short day response in European hamsters. J. Biol. Rhythms 21, 290–300. doi:10.1177/0748730406290315

Monecke, S., Wollnik, F., 2005. Seasonal variations in circadian rhythms coincide with a phase of sensitivity to short photoperiods in the European hamster. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 175, 167–183. doi:10.1007/s00360-005-0472-6

Monecke, S., Wollnik, F., Pévet, P., 2014. The Circannual Clock in the European Hamster: How Is It Synchronized by Photoperiodic Changes?, in: Annual, Lunar, and Tidal Clocks. pp. 277–308.

Montiglio, P.O., Garant, D., Pelletier, F., Réale, D., 2012. Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunks, Tamias striatus. Anim. Behav. 84, 1071–1079. doi:10.1016/j.anbehav.2012.08.010

Montiglio, P.O., Garant, D., Thomas, D., R??ale, D., 2010. Individual variation in temporal activity patterns in open-field tests. Anim. Behav. 80, 905–912. doi:10.1016/j.anbehav.2010.08.014

Montiglio, P.-O.O., Garant, D., Bergeron, P., Messier, G.D., R??ale, D., Dubuc Messier, G., Réale, D., 2013. Pulsed resources and the coupling between life-history strategies and exploration patterns in eastern chipmunks (Tamias striatus). J. Anim. Ecol. 83, 720–728. doi:10.1111/1365-2656.12174

Mueller, V., Gray, C., Kosec, K., 2014. Heat stress increases long-term human migration in rural Pakistan. Nat. Clim. Chang. 4, 182–185. doi:10.1038/NCLIMATE2103

Muhammad Anjum, F., Nadeem, M., Issa Khan, M., Hussain, S., 2012. Nutritional and therapeutic potential of sunflower seeds: a review. Br. Food J. 114, 544–552. doi:10.1108/00070701211219559

Munro, D., Thomas, D.W., 2004. The role of polyunsaturated fatty acids in the expression of torpor by mammals: A review. Zoology 107, 29–48. doi:10.1016/j.zool.2003.12.001

Munro, D., Thomas, D.W., Humphries, M.M., 2008. Extreme suppression of aboveground activity by a food-storing hibernator, the eastern chipmunk (Tamias striatus). Can. J. Zool. 86, 364–370. doi:10.1139/Z08-008

Munro, D., Thomas, D.W., Humphries, M.M., 2005. Torpor patterns of hibernating eastern chipmunks Tamias striatus vary in response to the size and fatty acid composition of food hoards. J. Anim. Ecol. 74, 692–700. doi:10.1111/j.1365-2656.2005.00968.x

Muséum national D'Histoire naturelle, 2007. Inventaire National Du Patrimoine Naturel [WWW Document]. URL https://inpn.mnhn.fr/ (accessed 1.15.17).

Müskens, G.J.D.M., Van Kats, R.J.M., Kruiters, A.T., 2008. Reintroduction of the common hamster, Criterus cricetus, in the Netherlands. Preliminary results.

Myers, N., Mittermeier, R., Mittermeier, C., DaFonesca, G., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Conserv. Biol. 403, 853. doi:10.1038/35002501

Myers, S.S., Gaffikin, L., Golden, C.D., Ostfeld, R.S., Redford, K.H., Ricketts, T.H., Turner, W.R., Osofsky, S.A., 2013. Human health impacts of ecosystem alteration. Proc. Natl. Acad. Sci. U. S. A. 110, 18753–60. doi:10.1073/pnas.1218656110

Myers, S.S., Smith, M.R., Guth, S., Golden, C.D., Vaitla, B., Mueller, N.D., Dangour, A.D., Huybers, P., 2016. Climate Change and Global Food Systems : Potential Impacts on Food Security and Undernutrition 1–19. doi:10.1146/annurev-publhealth-031816-044356

## N

Nawrot, T.S., Staessen, J. a, Gardner, J.P., Aviv, A., 2004. Telomere length and possible link to X chromosome. Lancet

(London, England) 363, 507–10. doi:10.1016/S0140-6736(04)15535-9

Naylor, R.L., 1996. Energy and Resource Constraints on Intensive Agricultural Production. Annu. Rev. Energy Environ. 21, 99–123. doi:10.1146/annurev.energy.21.1.99

Nechay, G., Hamar, M., Grulich, I., 1977. The Common Hamster ( Cricetus cricetus [ L .]) ; a Review. EPPO Bull. 7, 255–276.

Neumann, K., Jansman, H., Kayser, A., Maak, S., Gattermann, R., 2004. Multiple bottlenecks in threatened western European populations of the common hamster Cricetus cricetus (L.). Conserv. Genet. 5, 181–193. doi:10.1023/B:COGE.0000030002.01948.b3

Neumann, K., Michaux, J.R., Maak, S., Jansman, H.A.H., Kayser, A., Mundt, G., Gattermann, R., 2005. Genetic spatial structure of European common hamsters (Cricetus cricetus) - A result of repeated range expansion and demographic bottlenecks. Mol. Ecol. 14, 1473–1483. doi:10.1111/j.1365-294X.2005.02519.x

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D., Choimes, A., Collen, B., Day, J., De Palma, A., Diaz, S., Echeverria-Londono, S., 2015. Global effects of land use on local terrestrial biodiversity. Nature 520, 45. doi:10.1038/nature14324

Norton, B.G., 1988. Commodity, amenity, and morality, Wilson, EO. ed.

Nuss, E.T., Tanumihardjo, S. a., 2010. Maize: A paramount staple crop in the context of global nutrition. Compr. Rev. Food Sci. Food Saf. 9, 417–436. doi:10.1111/j.1541-4337.2010.00117.x

## O

O’Brien, J., 2015. Saving the common hamster (Cricetus cricetus) from extinction in Alsace (France): potential flagship conservation or an exercise in futility ? Hystrix, Ital. J. Mammal. 26. doi:10.4404/hystrix-26.2-11230

Oli, M.K., Armitage, K.B., 2004. Yellow-Bellied Marmot Population Dynamics : Demographic Mechanisms of Growth and Decline Published by : Ecological Society of America MARMOT POPULATION DYNAMICS : DEMOGRAPHIC. Ecology 85, 2446–2455.

Orr, A.L., Lohse, L.A., Drew, K.L., Hermes-Lima, M., 2010. Physiological oxidative stress after arousal from hibernation in Arctic ground squirrel. Comp. Biochem. Physiol. 153, 213–221. doi:10.1016/j.cbpa.2009.02.016.Physiological

Ortiz-Monasterio, J.I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R., Pe??a, R.J., 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci. 46, 293–307. doi:10.1016/j.jcs.2007.06.005

Out, M.E., Van Kats, R.J.M., Kuiters, L., Müskens, G.J.D.M., La Haye, M.J.J., 2011. Hard to stay under cover: seven years of crop management aiming to preserve the common Hamster (Cricetus cricetus) in the Netherlands., in: Angermann, R., Görner, M., Stubbe, M. (Eds.), Proceedings of the 16th and 17th Meeting of the International Hamster Workgroup, 2009, Ranis, Germany, 2010, Gödollo, Hungary. Säugetierkundliche Informationen. pp. 37–50.

Ozgul, A., Childs, D.Z., Oli, M.K., Armitage, K.B., Blumstein, D.T., Olson, L.E., Tuljapurkar, S., Coulson, T., 2010. Coupled dynamics of body mass and population growth in response to environmental change. Nature 466, 482–5. doi:10.1038/nature09210

## P

Palumbi, S.R., 2001. Humans as the World's Greatest Evolutionary Force. Science (80-. ). 293, 1786–1790. doi:10.1017/CBO9781107415324.004

Petelle, M.B., Martin, J.G. a, Blumstein, D.T., 2015. Heritability and genetic correlations of personality traits in a wild population of yellow-bellied marmots (Marmota flaviventris). J. Evol. Biol. 1–9. doi:10.1111/jeb.12700

Petrovan, S.O., Ward, A.I., Wheeler, P.M., 2013. Habitat selection guiding agri-environment schemes for a farmland specialist, the brown hare. Anim. Conserv. 16, 344–352. doi:10.1111/acv.12002

Pietsch, D., Kühn, P., Lisitsyn, S., Markova, A., Sinitsyn, A., 2014. Krotovinas, pedogenic processes and stratigraphic ambiguities of the Upper Palaeolithic sites Kostenki and Borshchevo (Russia). Quat. Int. 324, 172–179. doi:10.1016/j.quaint.2013.05.036

Pimm, S.L., 2008. Biodiversity: Climate Change or Habitat Loss — Which Will Kill More Species? Curr. Biol. 18, R116–7. doi:10.1016/j.cub.2007.11.054

Pimm, S.L., Raven, P., 2000. Extinction by numbers. Nature 403, 843–845. doi:10.1038/35002708

## R

Réale, D., Dingemanse, N.J., Kazem, A.J.N., Wright, J., 2010. Evolutionary and ecological approaches to the study of personality. Philos. Trans. R. Soc. B Biol. Sci. 365, 3937–3946. doi:10.1098/rstb.2010.0222

Réale, D., Martin, J., Coltman, D.W., Poissant, J., Festa-Bianchet, M., 2009. Male personality, life-history strategies and reproductive success in a promiscuous mammal. J. Evol. Biol. 22, 1599–1607. doi:10.1111/j.1420-9101.2009.01781.x

Réale, D., McAdam, A.G., Boutin, S., Berteaux, D., 2003. Genetic and plastic responses of a northern mammal to climate change. Proc. Biol. Sci. 270, 591–596. doi:10.1098/rspb.2002.2224

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol. Rev. 82, 291–318. doi:10.1111/j.1469-185X.2007.00010.x

Reid, N., Dingerkus, S.K., Stone, R.E., Buckley, J., Beebee, T.J.C., Marnell, F., Wilkinson, J.W., 2014. Assessing Historical and Current Threats to Common Frog (Rana temporaria) Populations in Ireland. J. Herpetol. 48, 13–19. doi:10.1670/12-053

Reijnen R, Foppen R, Ter Braak C, Thissen J. 2008. The Effects of Car Traffic on Breeding Bird Populations in Woodland . III . Reduction of Density in Relation to the Proximity of Main Roads. Journal of Applied Ecology **32**:187–202.

Reiners, T.E., Eidenschenk, J., Neumann, K., Nowak, C., 2014. Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the Common hamster of the French Alsace region. Mamm. Biol. 79, 240–246. doi:10.1016/j.mambio.2013.10.004

Reuveny, R., 2007. Climate change-induced migration and violent conflict. Polit. Geogr. 26, 656–673. doi:10.1016/j.polgeo.2007.05.001

Revel-Mouroz, A., 2015. Conception et evaluation de systemes de culture innovants, performants et favorables a la petite faune. Mémoire de fin d'étude, diplôme d'ingénieur agronome, option Production Végétale Durable. Montpellier.

Rey Benayas, J.M., Bullock, J.M., Newton, A.C., 2008. Creating woodland islets to reconcile ecological restoration, conservation, and agricultural land use. Front. Ecol. Environ. 6, 329–336. doi:10.1890/070057

Reznik, G., Reznik-Schüller, H., Ulrich, M., 1978. Clinical anatomy of the European hamster (Cricetus cricetus).

Reznik-Schuller, H., Reznik, G., Mohr, U., 1974. The European hamster (Cricetus cricetus L.) as an experimental animal: breeding method sand observations of their behaviour in the laboratory. Zeitschrift Versuchtierkd. 16, 48–58.

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends Ecol. Evol. 17, 462–468. doi:10.1016/S0169-5347(02)02578-8

Rioux Paquette, S., Pelletier, F., Garant, D., Belisle, M., 2014. Severe recent decrease of adult body mass in a declining insectivorous bird population. Proc. R. Soc. B Biol. Sci. 281, 20140649. doi:10.1098/rspb.2014.0649

Ripple, W.J., Beschta, R.L., 2012. Trophic cascades in Yellowstone: The first 15years after wolf reintroduction. Biol. Conserv. 145, 205–213. doi:10.1016/j.biocon.2011.11.005

Ripple, W.J., Beschta, R.L., 2003. Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. For. Ecol. Manage. 184, 299–313. doi:10.1016/S0378-1127(03)00154-3

Robertson, B.A., Rehage, J.S., Sih, A., 2013. Ecological novelty and the emergence of evolutionary traps. Trends Ecol. Evol. 28, 552–560. doi:10.1016/j.tree.2013.04.004

Rodale Institute, 2013. The three sisters and that fourth sister non one really talks about [WWW Document]. URL http://rodaleinstitute.org/the-three-sistersand-that-fourth-sister-no-one-really-talks-about/

Rodriguez-Prieto, I., Martin, J., Fernandez-Juricic, E., 2011. Individual variation in behavioural plasticity: direct and indirect effects of boldness, exploration and sociability on habituation to predators in lizards. Proc. R. Soc. B Biol. Sci. 278, 266–273. doi:10.1098/rspb.2010.1194

Rogalski, M.A., Gowler, C.D., Shaw, C.L., Hufbauer, R.A., Duffy, M.A., 2017. Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. Philos. Trans. R. Soc. B 372, 20160043. doi:http://dx.doi.org/10.1098/rstb.2016.0043

Roques, A., Robinet, C., 2009. Alien species in a warmer world 24, 1–7.

Rostaing, S., 2011. Renforcement des populations de Grands hamsters en Alsace. Evaluation de l'impact des cultures d'accueil.

Ruf, T., Bieber, C., Turbill, C., 2012. Survival, aging, and life-history tactics in mammalian hibernators, in: Living in a Seasonal World. Springer, Berlin, pp. 123–132.

Ruf, T., Geiser, F., 2015. Daily torpor and hibernation in birds and mammals. Biol. Rev. 90, 891–926. doi:10.1111/brv.12137

S

Sáenz De Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M.P., Pévet, P., Hazlerigg, D.G., Simonneaux, V., 2014. A circannual clock drives expression of genes central for seasonal reproduction. Curr. Biol. 24, 1500–1506. doi:10.1016/j.cub.2014.05.024

Sala, O.E., Sala, O.E., 2009. Global Biodiversity Scenarios for the Year 2100  Science (80-. ). 287, 1770–1774. doi:10.1126/science.287.5459.1770

Sander, M., Weinhold, U., 2008. The reintroduction project of Cricetus cricetus near the city of Mannheim, Baden-Württemberg, Germany - first results and experiences, in: Proceedings of the 11th, 14th, 15th Meeting of the International Hamster Workgroup.

Schmelzer, E., Millesi, E., 2003. Activity patterns in a population of European hamsters (Cricetus cricetus) in an urban environment, in: Proceedings of the 11th Meeting of the International Hamsterworkgroup. pp. 19–22.

Schneider, J., Wade, G., 1991. Effects of Ambient Temperature and Body Fat Content on Maternal Litter Reduction in Syrian Hamsters. Physiol. Behav. 49, 135–139.

Schneider, J., Wade, G., 1989. Effects of Maternal Diet , Body Weight and Body Composition on Infanticide in Syrian Hamsters. Physiol. Behav. 46, 815–821.

Schneider, J.E., 2004. Energy balance and reproduction. Physiol. Behav. 81, 289–317. doi:10.1016/j.physbeh.2004.02.007

Schoental, R., 1978. Mouldy grain and the aetiology of pellagra : the role of toxic metabolites of Fusariurn. Biochem. Soc. Trans. 8, 147–150.

Seiler, A., Folkeson, L., 2006. Habitat fragmentation due to transportation infrastructure: COST 341 national state-of-the-art report Sweden.

Senanayake, N., Karalliedde, L., 1987. Neurotoxic Effects of Organohosphorus Insecticides. N. Engl. J. Med. 316, 761–763. doi:10.1056/NEJM198703263161301

Shibata, K., Toda, S., 1997. Effects of sex hormones on the metabolism of tryptophan to niacin and to serotonin in male rats. Biosci. Biotechnol. Biochem. 61, 1200–2. doi:10.1271/bbb.61.1200

Shine, R., 2013. Ecology: The Lunch of a Lifetime. Curr. Biol. 23, R615–R617. doi:10.1016/j.cub.2013.06.004

Sih, A., Bell, A., Johnson, J.C., 2004. Behavioral syndromes: An ecological and evolutionary overview. Trends Ecol. Evol. 19, 372–378. doi:10.1016/j.tree.2004.04.009

Simberloff, 1998. Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? Biol. Conserv. 83, 247–257.

Siriwardena, G.M., Baillie, S.R., Buckland, S.T., Fewster, R.M., Marchant, J.H., Wilson, J.D., 1998. Trends in the abundance of farmland birds: A quantitative comparison of smoothed Common Birds Census indices. J. Appl. Ecol. 35, 24–43. doi:10.1046/j.1365-2664.1998.00275.x

Siutz, C., Franceschini, C., Millesi, E., 2016. Sex and age differences in hibernation patterns of common hamsters: adult females hibernate for shorter periods than males. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 186, 801–811. doi:10.1007/s00360-016-0995-z

Siutz, C., Millesi, E., 2012. Effects of birth date and natal dispersal on faecal glucocorticoid concentrations in juvenile Common hamsters. Gen. Comp. Endocrinol. 178, 323–329. doi:10.1016/j.ygcen.2012.06.009

Siutz, C., Millesi, E., 2005. Social interaction in European Hamsters, in: 13th Meeting of the International Hamster Workgroup. Illmitz/Vienna.

Smith, B.R., Blumstein, D.T., 2008. Fitness consequences of personality: A meta-analysis. Behav. Ecol. 19, 448–455. doi:10.1093/beheco/arm144

Smith, F.A., Browning, H., Shepherd, U.L., 1998. The influence of climate change on the body mass of woodrats Neotomu in an arid region of New Mexico , USA. Ecography (Cop.). 21, 140–148.

Smith, M.R., Singh, G.M., Mozaffarian, D., Myers, S.S., 2015. Effects of decreases of animal pollinators on human nutrition and global health: A modelling analysis. Lancet 386, 1964–1972. doi:10.1016/S0140-6736(15)61085-6

Smith, S., Turbill, C., Penn, D.J., 2011. Chasing telomeres, not red herrings, in evolutionary ecology. Heredity (Edinb). 107, 372–373.

Smulders, M.J.M., Snoek, L.B., Booy, G., Vosman, B., 2003. Complete loss of MHC genetic diversity in the Common Hamster (Cricetus cricetus) population in The Netherlands. Consequences for conservation strategies. Conserv. Genet. 4, 441–451. doi:10.1023/A:1024767114707

Sol, D., Lapiedra, O., González-Lagos, C., 2013. Behavioural adjustments for a life in the city. Anim. Behav. 85, 1101–1112. doi:10.1016/j.anbehav.2013.01.023

Sotherton, N.W., 1998. Land use changes and the decline of farmland wildlife: An appraisal of the set-aside approach. Biol. Conserv. 83, 259–268. doi:10.1016/S0006-3207(97)00082-7

Speakman, J.R., 2008. The physiological costs of reproduction in small mammals. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 375–98. doi:10.1098/rstb.2007.2145

Stearns, S., 1989. Trade-offs in life-history evolution. Funct. Ecol. 3, 259–268. doi:10.2307/2389364

Stearns, S.C., 1992. The evolution of life histories, Oxford Uni. ed. Oxford University Press, Oxford.

Stoate, C., Boatman, N.D., Borralho, R.J., Carvalho, C.R., de Snoo, G.R., Eden, P., 2001. Ecological impacts of arable intensification in Europe. J. Environ. Manage. 63, 337–365. doi:10.1006/jema.2001.0473

Surov, A., Banaszek, A., Bogomolov, P., Feoktistova, N., Monecke, S., 2016. Dramatic global decrease in the range and reproduction rate of the European hamster Cricetus cricetus. Endanger. Species Res. 31, 119–145. doi:10.3354/esr00749

## T

Tapper, S.C., Barnes, R.F.W., 1986. Influence of farming practise on the ecology of the brown hare (Lepus europaeus). J. Appl. Ecol. 23, 39–52.

The Siberan Times Reporter, 2016. First anthrax outbreak since 1941: 9 hospitalised, with two feared to have disease. Sib. Times.

Thomas, J.A., 2009. Comparative Losses of British Butterflies, Birds, and Plants and the Global Extinction Crisis. Science (80-. ). 1879, 1879–1881. doi:10.1126/science.1095046

Thomas, W., 2004. INFLUENCE OF HOUSING CONDITIONS ON THE HIBERNATION PATTERNS OF EUROPEAN HAMSTERS

(CRICETUS CRICETUS) 1–4.

Thornhill, N.W., 1991. An evolutionary analysis of rules regulating human inbreeding and marriage. Behav. Brain Sci. 14, 247–293.

Tissier, M.L., Handrich, Y., Dallongeville, O., Robin, J., Habold, C., 2017. Diets derived from maize monoculture cause maternal infanticides in the endangered European hamster due to a vitamin B3 deficiency. Proc. R. Soc. B 284, 1–8. doi:http://dx.doi.org/10.1098/rspb.2016.2168

Tissier, M.L., Handrich, Y., Robin, J.-P., Weitten, M., Pevet, P., Kourkgy, C., Habold, C., 2016. How maize monoculture and increasing winter rainfall have brought the hibernating European hamster to the verge of extinction. Sci. Rep. 6, 25531. doi:10.1038/srep25531

Tissier, M.L., Jumeau, J., Croguennec, C., Petit, O., Habold, C., Handrich, Y., 2016. An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features. Ecol. Eng. 95, 738–742. doi:10.1016/j.ecoleng.2016.07.012

Tissier, M.L., Williams, T.D., Criscuolo, F., 2014. Maternal effects underlie ageing costs of growth in the zebra finch (Taeniopygia guttata). PLoS One 9, e97705. doi:10.1371/journal.pone.0097705

Tkadlec, E., Losik, J., Bendova, M., Petrova, I., 2015. Applying matrix population models to life history of the Common hamster: from models to further field studies, in: 22nd Annual Meeting of the International Hamster Workgroup. p. 28.

Troïanowski M, Dumet A, Condette C, Lengagne T, Mondy N. 2015. Traffic noise affects colouration but not calls in the European treefrog (Hyla arborea). Behaviour **152**:821–836. Available from http://booksandjournals.brillonline.com/content/journals/10.1163/1568539x-00003255.

Troïanowski M, Mondy N, Dumet A, Arcanjo C, Lengagne T. 2017. Effects of traffic noise on tree frog stress levels, immunity and color signaling. Conservation Biology:1–24. Available from http://doi.wiley.com/10.1111/cobi.12893.

Trowborst, A., Chapron, G., Fleurke, F., Epstein, Y., Lopez-Bao, J.V., 2016. Europe's biodiversity avoids fatal setback. Science (80-. ). 355, 140.

Turbill, C., Bieber, C., Ruf, T., 2011. Hibernation is associated with increased survival and the evolution of slow life histories among mammals. Proc. Biol. Sci. 278, 3355–63. doi:10.1098/rspb.2011.0190

Turbill, C., Ruf, T., Smith, S., Bieber, C., Turbill, C., Ruf, T., Smith, S., Bieber, C., 2013. Seasonal variation in telomere length of a hibernating rodent Seasonal variation in telomere length of a hibernating rodent. Biol. Lett. 9, 20121095.

Turcotte, M., Araki, H., Karp, D., Poveda, K., Whitehead, S., 2016. The eco-evolutionary impacts of domestication and agricultural practices on wild species. Philos. Trans. R. Soc. B. doi:10.1098/rstb.2016.0033

## U

Ulbrich, K., Kayser, A., 2004. A risk analysis for the common hamster (Cricetus cricetus). Biol. Conserv. 117, 263–270. doi:10.1016/j.biocon.2003.12.006

USDA SR-21, 2016. Nutrition Data [WWW Document]. URL http://nutritiondata.self.com/ (accessed 9.1.16).

## V

Valladares, F., Matesanz, S., Araujo, M.B., Balaguer, L., Benito-Garzon, M., Cornwell, W.K., Gianoli, E., Guilhaumon, F., van Kleunen, M., Naya, D., Nicotra, a B., Poorter, H., Zavala, M., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol. Lett. 1351–1364. doi:10.1111/ele.12348

van der Velde, M., Tubiello, F.N., Vrieling, A., Bouraoui, F., 2012. Impacts of extreme weather on wheat and maize in France: Evaluating regional crop simulations against observed data. Clim. Change 113, 751–765. doi:10.1007/s10584-011-0368-2

van der Zee, F.F., Wiertz, J., Ter Braak, C.J.F., van Apeldoorn, R.C., Vink, J., 1992. Landscape change as a possible cause of the badger Meles meles L. decline in The Netherlands. Biol. Conserv. 61, 17–22. doi:10.1016/0006-3207(92)91203-5

van Strien, A.J., Meyling, A.W.G., Herder, J.E., Hollander, H., Kalkman, V.J., Poot, M.J.M., Turnhout, S., van der Hoorn, B., van Strien-van Liempt, W.T.F.H., van Swaay, C.A.M., van Turnhout, C.A.M., Verweij, R.J.T., Oerlemans, N.J., 2016. Modest recovery of biodiversity in a western European country: The Living Planet Index for the Netherlands. Biol. Conserv. 200, 44–50. doi:10.1016/j.biocon.2016.05.031

Vanbergen, A.J., 2013. Threats to an ecosystem service: Pressures on pollinators. Front. Ecol. Environ. 11, 251–259. doi:10.1890/120126

Verburg, P.H., van Berkel, D.B., van Doorn, A.M., van Eupen, M., van den Heiligenberg, H.A.R.M., 2010. Trajectories of land use change in Europe: A model-based exploration of rural futures. Landsc. Ecol. 25, 217–232. doi:10.1007/s10980-009-9347-7

Vickery, J.A., Feber, R.E., Fuller, R.J., 2009. Arable field margins managed for biodiversity conservation: A review of food resource provision for farmland birds. Agric. Ecosyst. Environ. 133, 1–13. doi:10.1016/j.agee.2009.05.012

Villemey, A., Besnard, A., Grandadam, J., Eidenschenck, J., 2013. Testing restocking methods for an endangered species: Effects of predator exclusion and vegetation cover on common hamster (Cricetus cricetus) survival and reproduction. Biol. Conserv. 158, 147–154. doi:10.1016/j.biocon.2012.08.007

Vitousek, P.M., Mooney, H. a, Lubchenco, J., Melillo, J.M., 1997. Human Domination of Earth’ s Ecosystems. Science (80-. ). 277, 494–499. doi:10.1126/science.277.5325.494

Vohralik, V., 1974. Biology of the reproduction of the Common hamster Cricetus cricetus (L.). Vest. Cs. Spol. Zool. 38, 228–240.

## W

Wade, G.N., Schneider, J.E., 1992. Metabolic fuels and reproduction in female mammals. Neurosci. Biobehav. Rev. 16, 235–272. doi:10.1016/S0149-7634(05)80183-6

Wall, S.B. Vander, 1990. Food Hoarding in Animals.

Walz, J., Stertz, L., Fijtman, A., Santos, B., Almeida, R., 2013. Tryptophan diet reduces aggressive behavior in male mice.

Psychol. Neurosci. 6, 397–401. doi:10.3922/j.psns.2013.3.18

Wan, P., Moat, S., Anstey, a., 2011. Pellagra: A review with emphasis on photosensitivity. Br. J. Dermatol. 164, 1188–1200. doi:10.1111/j.1365-2133.2010.10163.x

Ward, J.F., MacDonald, D.W., Doncaster, C.P., Mauget, C., 1996. Physiological response of the European hedgehog to predator and nonpredator odour. Physiol. Behav. 60, 1469–1472. doi:10.1016/S0031-9384(96)00245-4

Webster, M.S., Pruett-Jones, S., Westneat, D.F., Arnold, S.J., 1995. Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution (N. Y). 49, 1147–1157.

Weinhold, U., 2008. Draft European action plan for the conservation of the common hamster (Cricetus cricetus L., 1758). In: Convention on the conservation of European wildlife and natural habitats, in: Standing Commitee. Council of Europe, Strasbourg, France,. pp. 1–36.

Weitten, M., Oudart, H., Habold, C., 2016. Maintenance of a fully functional digestive system during hibernation in the European hamster, a food-storing hibernator. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 193, 45–51. doi:10.1016/j.cbpa.2016.01.006

Weitten, M., Robin, J.-P., Oudart, H., Pévet, P., Habold, C., 2013. Hormonal changes and energy substrate availability during the hibernation cycle of Syrian hamsters. Horm. Behav. 64, 611–7. doi:10.1016/j.yhbeh.2013.08.015

Wesseling, C., Keifer, M., Ahlbom, A., McConnell, R., Moon, J.D., Rosenstock, L., Hogstedt, C., 2002. Long-term neurobehavioral effects of mild poisonings with organophosphate and n-methyl carbamate pesticides among banana workers. Int. J. Occup. Environ. Health 8, 27–34. doi:10.1179/oeh.2002.8.1.27

West-Eberhard, M.J., 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. U. S. A. 102 Suppl, 6543–6549. doi:10.1073/pnas.0501844102

West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution, Press, Oxf. ed.

Wilson, J.D., Morris, A.J., Arroyo, B.E., Clark, S.C.S.C., Bradbury, R.B., 1999. A review of the abundance and diversity of invertebrate and plant foods of granivorous birds in northern Europe in relation to agricultural change. Agric. Ecosyst. Environ. 75, 13–30. doi:10.1016/S0167-8809(99)00064-X

Wilson, J.D., Whittingham, M.J., Bradbury, R.B., 2005. The management of crop structure : a general approach to reversing the impacts of agricultural intensification on birds ? Ibis (Lond. 1859). 147, 453–463.

Woldehanna, T., Lansink, A.O., Peerlings, J., 2000. Off-farm work decisions on Dutch cash crop farms and the 1992 and Agenda 2000 CAP reforms. Agric. Econ. 22, 163–171. doi:10.1016/S0169-5150(99)00046-8

Wolf, M., van Doorn, G.S., Leimar, O., Weissing, F.J., 2007. Life-history trade-offs favour the evolution of animal personalities. Nature 447, 581–4. doi:10.1038/nature05835

Wollnik, F., Breit, A., Reinke, D., 1991. Seasonal Change in the Temporal Organization of Wheel-Running Activity of the European Hamster, Cricetus cricetus. Naturwissenschaften 78, 419–422. doi:10.1007/BF01133417

Wollnik, F., Schmidt, B., 1995. Seasonal and daily rhythms of body temperature in the European hamster (Cricetus cricetus) under semi-natural conditions. J. Comp. Physiol. B 165, 171–182. doi:10.1007/BF00260808

Wood, T.J., Holland, J.M., Goulson, D., 2016. Providing foraging resources for solitary bees on farmland: Current schemes for pollinators benefit a limited suite of species. J. Appl. Ecol. doi:10.1111/1365-2664.12718

Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E., Desneux, N., 2012. Pollinator habitat enhancement: Benefits to other ecosystem services. Agric. Ecosyst. Environ. 159, 112–122. doi:10.1016/j.agee.2012.06.020

## Y

Yohannes, E., Gwinner, H., Lee, R.W., Schwabl, H., 2016. Stable isotopes predict reproductive performance of European starlings breeding in anthropogenic environments. Ecosphere 7, 1–14.

Yom-Tov, Y., 2001. Global warming and body mass decline in Israeli passerine birds. Proc. R. Soc. B Biol. Sci. 268, 947–52. doi:10.1098/rspb.2001.1592

Young, T.P., 2000. Restoration ecology and conservation biology. Biol. Conserv. 92, 73–83. doi:10.1016/S0006-3207(99)00057-9

## Z

Zambrano, E., Rodríguez-González, G.L., Guzmán, C., García-Becerra, R., Boeck, L., Díaz, L., Menjivar, M., Larrea, F., Nathanielsz, P.W., 2005. A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. J. Physiol. 563, 275–284. doi:10.1113/jphysiol.2004.078543

Zera, A.J., Harshman, L.G., 2001. The Physiology of Life History Trade-Offs in Animals. Annu. Rev. Ecol. Syst. 32, 95–126. doi:10.1146/annurev.ecolsys.32.081501.114006

Zhang, J., 2011. China’s success in increasing per capita food production. J. Exp. Bot. 62, 3707–3711.

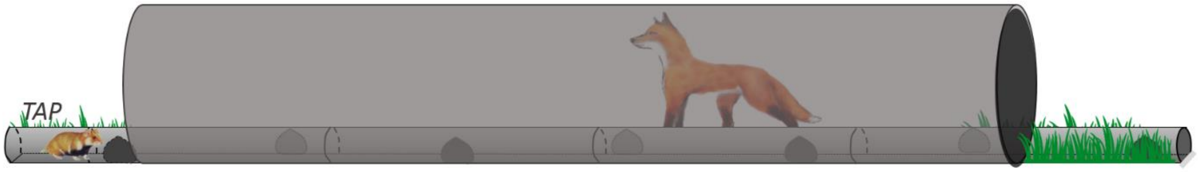
Zinkand, D., 2012. Strip-Tillers Exploring Cover Crops in France [WWW Document]. URL https://www.striptillfarmer.com/articles/124-strip-tillers-exploring-cover-crops-in-france

Ziomek, J., Zgrabczyńska, E., Poradzisz, A., 2009. The behaviour of the common hamster (Cricetus cricetus) under zoo conditions. Der Zool. Garten 78, 221–231. doi:10.1016/j.zoolgart.2009.08.006

# APPENDIX I – Fiches actions pour l’amélioration des passages à faune des infrastructures de transport.

## 2017

**Le tube anti-predation – TAP – une solution pour sécuriser la traversée des micro-mammifères, dont le Grand hamster, dans les passages à faune en Alsace.**



Un système à adapter aux différents passages à faune

__Conception du TAP__ : Mathilde L. Tissier, Jonathan Jumeau, Julie Fleitz, Yves Handrich.

__Rédaction des fiches-action__ : Julie Fleitz, en collaboration avec Mathilde L. Tissier et Yves Handrich.

## FICHE A : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « GRAND »

Surface au sol ≥ 50cm de chaque côté des TAP

Coefficient d'ouverture ≥ 1

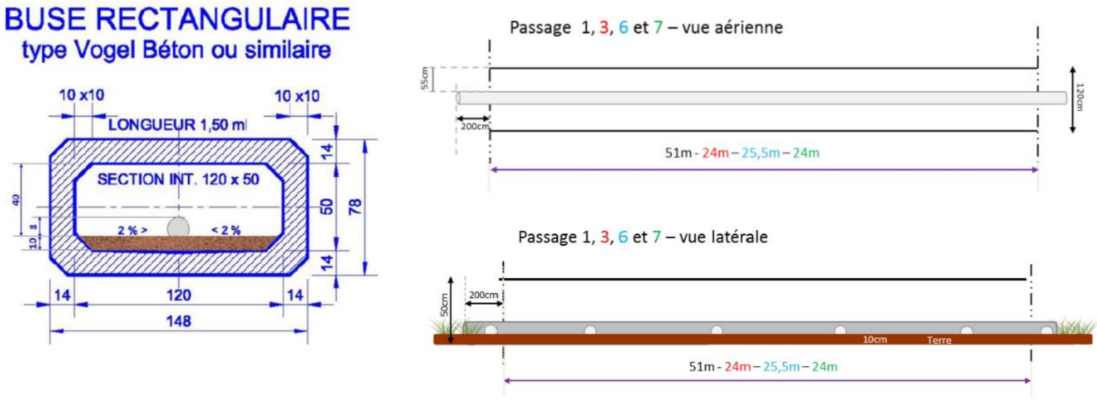
→

Tubes de forme F2

Diamètre de 10cm

Positionnés au centre du passage

Passages concernés : Passage 1 (VRPV), Passage 3 (A35), passage 6 (A35) et passage 7 (VRPV)



Schémas généraux avec dimensions, distances et positions du dispositif anti-prédations pour les passages rectangulaires 1, 3, 6 et 7

### POINTS CLEFS

- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Surface plate et large de chaque côté des TAP** qui facilite le guidage et la progression des autres espèces notamment des amphibiens
- ✓ **La dimension et la forme de ces passages à faune** permettent la mise en place des tubes anti-prédation les plus adaptés au Grand hamster

### PRECONISATIONS

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des TAP.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa rectitude tout au long du tunnel

**Des vérifications régulières doivent être effectuées** : aussi bien aux abords que dans les passages à faune afin de garantir une efficacité maximale des tunnels et des dispositifs anti-prédation sur le long terme.

### VISUEL

## FICHE B : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « MOYEN »

Surface au sol ≤ 50cm de chaque côté des TAP

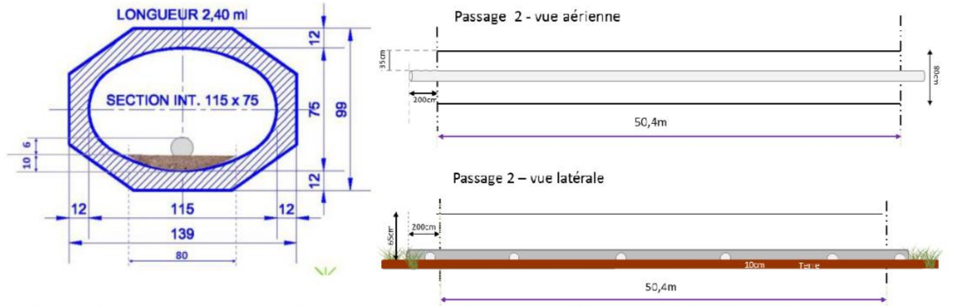
Coefficient d'ouverture ≥ 1

Tubes de forme F2

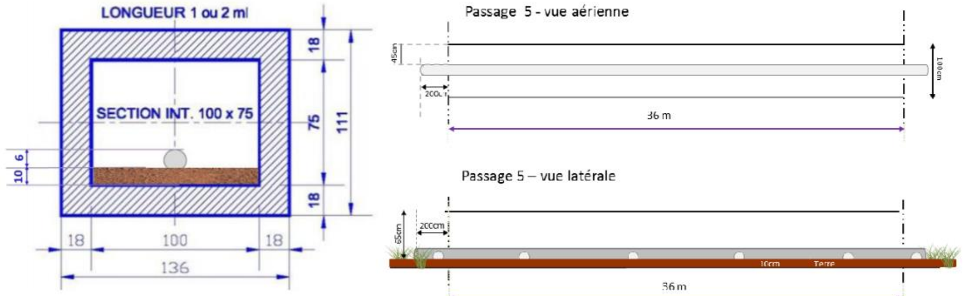
Diamètre de 8cm

Positionnés au centre du passage

Passages concernés : Passage 2 (VRPV) et passage 5 (A35)



Schémas généraux avec dimensions, distances et positions des tuyaux en béton type Moduloval ou similaire pour le passage 2



Schémas généraux avec dimensions, distances et positions des cadres en béton pour le passage 5

### POINTS CLEFS

- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Diamètre des TAP légèrement réduit,** ce qui incitera peut être davantage d'autres micromammifères à utiliser le dispositif tout en laissant une surface au sol suffisante pour les autres espèces

### PRECONISATIONS

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des tubes anti-prédation.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa linéarité dans le tunnel.

**Des vérifications régulières doivent être effectuées** : la surface au sol disponible étant réduite, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

## FICHE C : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « MINI »

Surface au sol ≤ 50cm de chaque côté des TAP

Coefficient d'ouverture < 1

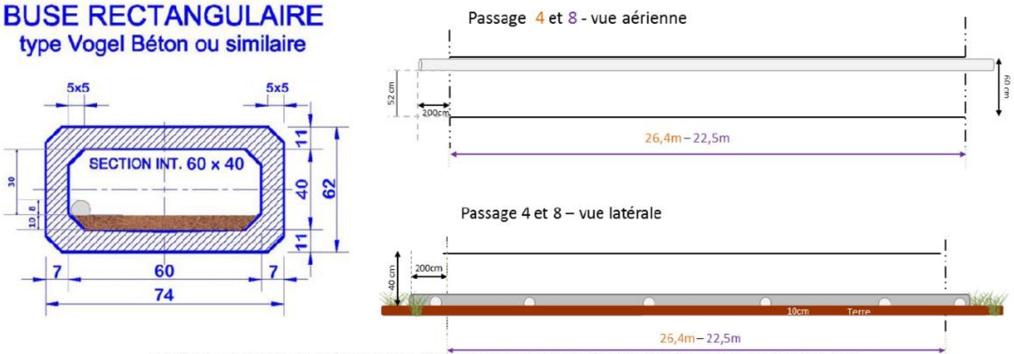
→

Tubes de forme F2

Diamètre de 8cm

Positionnés sur un des côtés du passage

Passages concernés : Passage 4 (A35) et passage 8 (VRPV)



Schémas généraux avec dimensions, distances et positions du dispositif pour les passages 4 et 8

### POINTS CLEFS

- ✓ **TAP sur un des côtés** afin de laisser un espace suffisant aux autres espèces à l'intérieur du passage à faune
- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Diamètre des TAP légèrement réduit** ce qui incitera peut être davantage d'autres micromammifères à utiliser le dispositif

### PRECONISATIONS

**Les TAP peuvent être fixés aux parois du passage à faune** : pour éviter que les tubes ne bougent au centre du tunnel et bloquent le passage des animaux.

**Mise en place de tubes non coupés (forme ronde)** : au cas où le sol du passage serait trop irrégulier.

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des tubes anti-prédation.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa rectitude tout au long du tunnel

**Des vérifications régulières doivent être effectuées** : la surface au sol disponible étant réduite, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

## FICHE D : ENRICHISSEMENT DES OUVRAGES AGRICOLES

Surface au sol ≥ 50cm de chaque côté des TAP

Coefficient d'ouverture ≥ 1

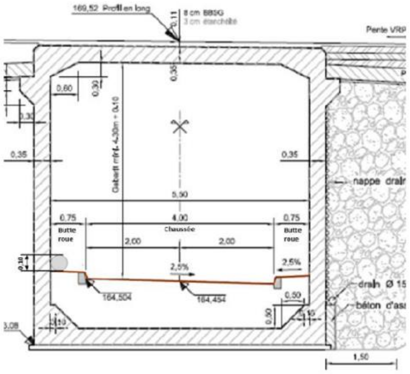
→

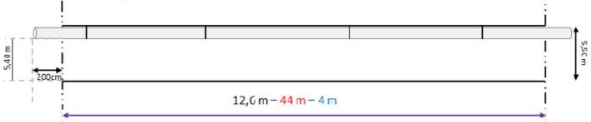
Tubes de forme F2

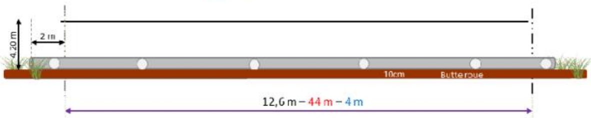
Diamètre de 10cm

Positionnés sur un des côtés du passage

Passages concernés : Ouvrage agricole 1 (A35), 2 (VRPV) et 3 (A35)




Passage agricole 1, 2 et 3 - vue aérienne


Passage agricole 1, 2 et 3 – vue latérale

### POINTS CLEFS

- ✓ **TAP sur un des côté** afin de ne pas gêner le passage des engins agricoles.
- ✓ **Fixation des TAP contre la paroi des passages agricoles** pour éviter qu'ils ne soient déplacés et dégradés.
- ✓ **Diamètre des TAP de 10 cm**, la taille des ouvrages agricoles permet la mise en place du dispositif le plus adapté au Grand hamster.

### PRECONISATIONS

**Utilisation de tubes en béton** : le passage étant facilement accessible et fréquenté par des machines agricoles, l'utilisation du béton est préconisée pour permettre une meilleure résistance aux dégradations naturelles et au vandalisme.

**Mise en place de tubes pleins (non coupés)** : au cas où le sol du passage serait trop irrégulier.

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et son bon alignement aux parois du tunnel

**Des vérifications régulières doivent être effectuées** : l'accessibilité du dispositif par les usagers (humains) pose crucialement le problème de la régularité des contrôles. Dans ce cas, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

# FICHE E : CONSEILS POUR L'AMENAGEMENT DE NOUVELLES ROUTES

## Choix et installation du passage à faune

- ✓ **Buse rectangulaire 120 x 50cm** type Vogel Béton ou similaire.
- ✓ **Surface de parcours naturelle, plate et large** à l'intérieur du passage pour faciliter le guidage et la progression des animaux.
- ✓ **Installation systématique tous les 300m**.
- ✓ **Installation du passage en pente** pour permettre un drainage naturel.

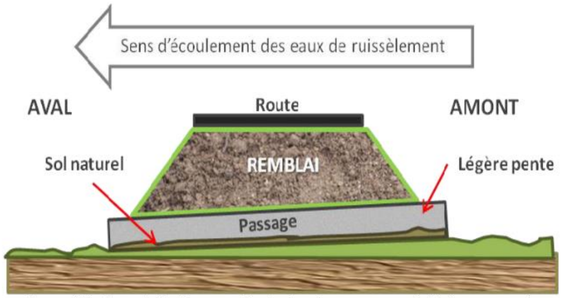


**Figure 21**: Caractéristiques principales des passages inférieurs pour la petite faune (16)

## Choix et installation du dispositif anti-prédation

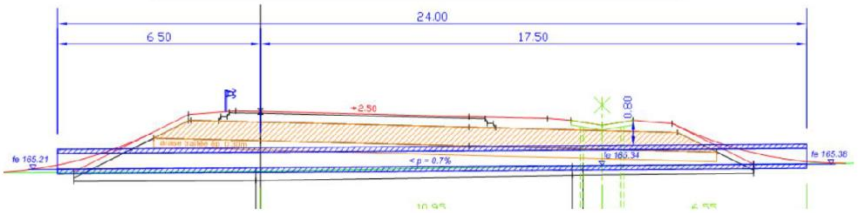
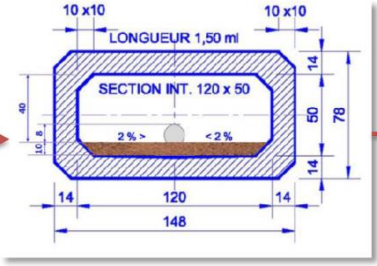


**Figure 22**: Profil en travers d'un hamstéroduc de type rectangulaire (passage en bleu et route en noir) (8)

Surface au sol ≥ 50cm de chaque côté des TAP

Coefficient d'ouverture ≥ 1



Tubes de forme F2

Diamètre de 10cm

Positionnés au centre du passage

### PRECONISATIONS GENERALES

**L'entrée doit être préservée des nuisances humaines** : pas de lumière artificielle, pas de route ou piste à proximité des entrées…

**Le passage doit être facile d'accès pour la faune et les équipes d'entretien** : dégager l'entrée des obstacles éventuels qui pourraient gêner le passage des animaux + favoriser les surfaces planes ou en pente faible.

**Les dimensions des passages à faune doivent tenir compte de l'effet tunnel** : plus le passage est long et plus sa section doit être grande.

**La mise en place des TAP doit se faire précisément pour être efficaces** : les tubes doivent être assemblés les uns derrière les autres, former une ligne droite à l'intérieur des passages et toujours être en contact avec un sol naturel.

**Pour les grands ouvrages de type ouvrage agricole ou pont, favoriser la mise en place de tubes en béton** plus solides sur le long terme que le PVC et pouvant résister aux dégradations et au vandalisme.

**Former (et communiquer avec) les personnes chargées de l'entretien de ces structures** : des inspections courantes (2 à 10 fois par an) doivent être mises en place afin d'évaluer l'état général des infrastructures, gérer l'entretien à l'intérieur et aux abords des tunnels et rapporter les dysfonctionnements éventuels.

# APPENDIX II – Fiches actions pour l'amélioration des passages à faune des infrastructures de transport.

2017

## Le tube anti-predation – TAP – une solution pour sécuriser la traversée des micro-mammifères, dont le Grand hamster, dans les passages à faune en Alsace.

Un système à adapter aux différents passages à faune

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## FICHE A : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « GRAND »

Surface au sol ≥ 50cm de chaque côté des TAP

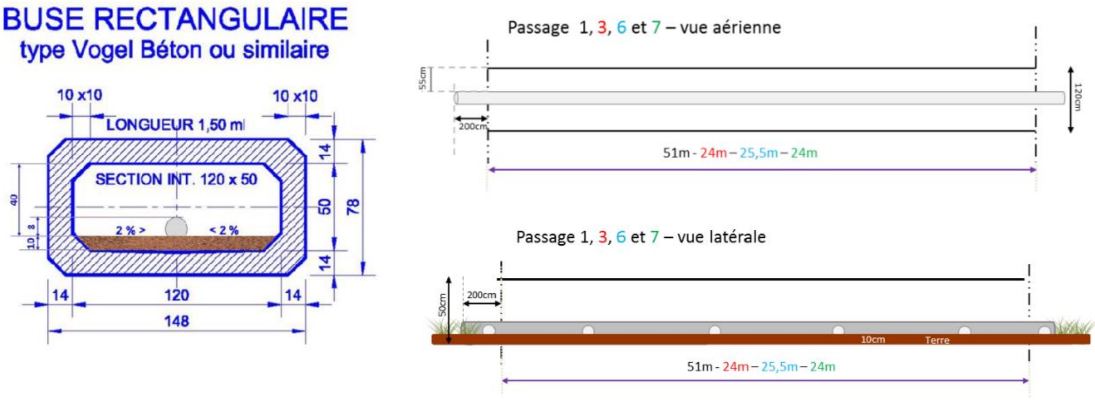
Coefficient d'ouverture ≥ 1

Tubes de forme F2

Diamètre de 10cm

Positionnés au centre du passage

Passages concernés : Passage 1 (VRPV), Passage 3 (A35), passage 6 (A35) et passage 7 (VRPV)



Schémas généraux avec dimensions, distances et positions du dispositif anti-prédations pour les passages rectangulaires 1, 3, 6 et 7

### POINTS CLEFS

- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Surface plate et large de chaque côté des TAP** qui facilite le guidage et la progression des autres espèces notamment des amphibiens
- ✓ **La dimension et la forme de ces passages à faune** permettent la mise en place des tubes anti-prédation les plus adaptés au Grand hamster

### PRECONISATIONS

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des TAP.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa rectitude tout au long du tunnel

**Des vérifications régulières doivent être effectuées** : aussi bien aux abords que dans les passages à faune afin de garantir une efficacité maximale des tunnels et des dispositifs anti-prédation sur le long terme.

### VISUEL

## FICHE B : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « MOYEN »

Surface au sol ≤ 50cm de chaque côté des TAP

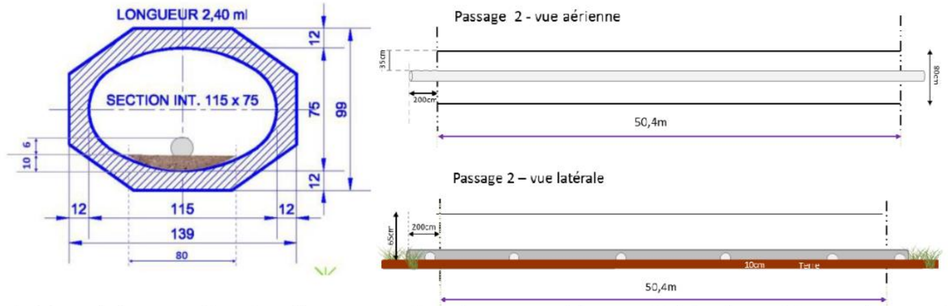
Coefficient d'ouverture ≥ 1

Tubes de forme F2

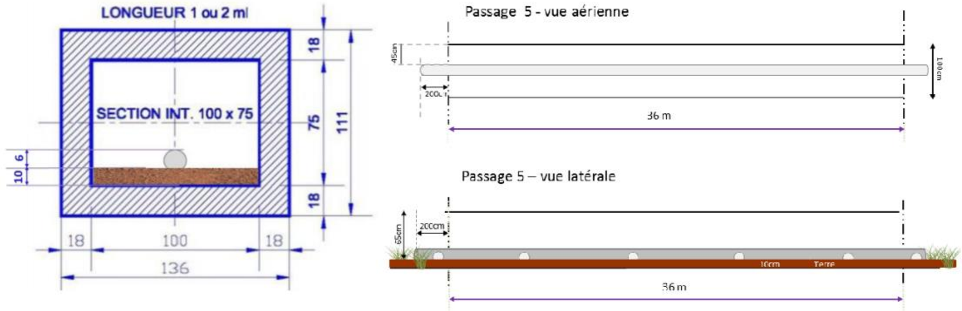
Diamètre de 8cm

Positionnés au centre du passage

Passages concernés : Passage 2 (VRPV) et passage 5 (A35)



Schémas généraux avec dimensions, distances et positions des tuyaux en béton type Moduloval ou similaire pour le passage 2



Schémas généraux avec dimensions, distances et positions des cadres en béton pour le passage 5

### POINTS CLEFS

- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Diamètre des TAP légèrement réduit,** ce qui incitera peut être davantage d'autres micromammifères à utiliser le dispositif tout en laissant une surface au sol suffisante pour les autres espèces

### PRECONISATIONS

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des tubes anti-prédation.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa linéarité dans le tunnel.

**Des vérifications régulières doivent être effectuées** : la surface au sol disponible étant réduite, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

## FICHE C : ENRICHISSEMENT DES PASSAGES INFERIEURS TYPE « MINI »

Surface au sol ≤ 50cm de chaque côté des TAP

Coefficient d'ouverture < 1

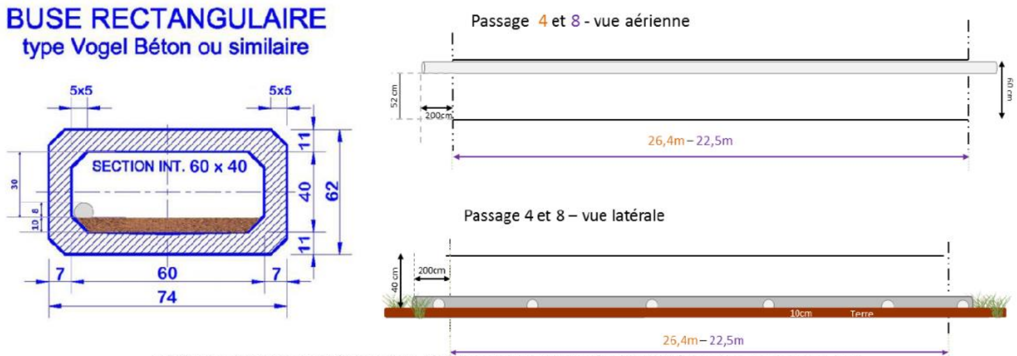
→

Tubes de forme F2

Diamètre de 8cm

Positionnés sur un des côtés du passage

Passages concernés : Passage 4 (A35) et passage 8 (VRPV)



Schémas généraux avec dimensions, distances et positions du dispositif pour les passages 4 et 8

### POINTS CLEFS

- ✓ **TAP sur un des côtés** afin de laisser un espace suffisant aux autres espèces à l'intérieur du passage à faune
- ✓ **Présence d'un sol naturel** au contact duquel les animaux pourront se déplacer dans le tunnel et dans le dispositif anti-prédation
- ✓ **Diamètre des TAP légèrement réduit** ce qui incitera peut être davantage d'autres micromammifères à utiliser le dispositif

### PRECONISATIONS

**Les TAP peuvent être fixés aux parois du passage à faune** : pour éviter que les tubes ne bougent au centre du tunnel et bloquent le passage des animaux.

**Mise en place de tubes non coupés (forme ronde)** : au cas où le sol du passage serait trop irrégulier.

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Le sol naturel du tunnel doit être le plus régulier possible** : vérifier que le sol en terre ne présente pas de fortes irrégularités ce qui diminuerait l'efficacité des tubes anti-prédation.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et sa rectitude tout au long du tunnel

**Des vérifications régulières doivent être effectuées** : la surface au sol disponible étant réduite, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

## FICHE D : ENRICHISSEMENT DES OUVRAGES AGRICOLES

Surface au sol ≥ 50cm de chaque côté des TAP

Coefficient d'ouverture ≥ 1

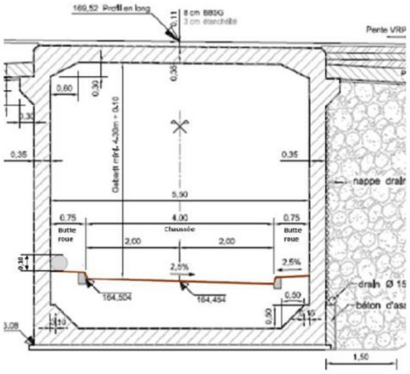
→

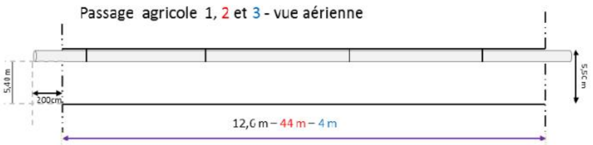
Tubes de forme F2

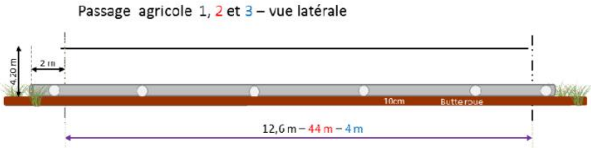
Diamètre de 10cm

Positionnés sur un des côtés du passage

Passages concernés : Ouvrage agricole 1 (A35), 2 (VRPV) et 3 (A35)







### POINTS CLEFS

- ✓ **TAP sur un des côté** afin de ne pas gêner le passage des engins agricoles.
- ✓ **Fixation des TAP contre la paroi des passages agricoles** pour éviter qu'ils ne soient déplacés et dégradés.
- ✓ **Diamètre des TAP de 10 cm**, la taille des ouvrages agricoles permet la mise en place du dispositif le plus adapté au Grand hamster.

### PRECONISATIONS

**Utilisation de tubes en béton** : le passage étant facilement accessible et fréquenté par des machines agricoles, l'utilisation du béton est préconisée pour permettre une meilleure résistance aux dégradations naturelles et au vandalisme.

**Mise en place de tubes pleins (non coupés)** : au cas où le sol du passage serait trop irrégulier.

**Le dispositif anti-prédation dépasse du tunnel** : il doit être signalé aux équipes d'entretien des accotements.

**Les tubes anti-prédation doivent être raccordés les uns avec les autres**: lors de la mise en place, il est important de vérifier la continuité du dispositif et son bon alignement aux parois du tunnel

**Des vérifications régulières doivent être effectuées** : l'accessibilité du dispositif par les usagers (humains) pose crucialement le problème de la régularité des contrôles. Dans ce cas, il est d'autant plus important de vérifier qu'aucun obstacle n'entrave le déplacement des animaux.

### VISUEL

## FICHE E : CONSEILS POUR L'AMENAGEMENT DE NOUVELLES ROUTES

### Choix et installation du passage à faune

- ✓ **Buse rectangulaire 120 x 50cm** type Vogel Béton ou similaire.
- ✓ **Surface de parcours naturelle, plate et large** à l'intérieur du passage pour faciliter le guidage et la progression des animaux.
- ✓ **Installation systématique tous les 300m**.
- ✓ **Installation du passage en pente** pour permettre un drainage naturel.

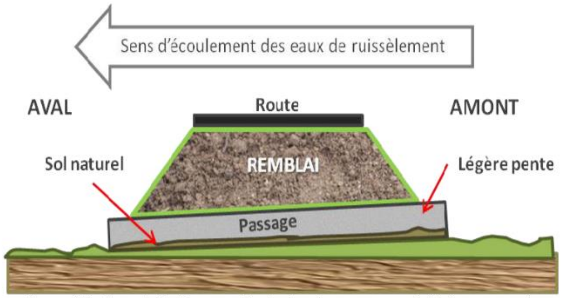


**Figure 21**: Caractéristiques principales des passages inférieurs pour la petite faune (16)

### Choix et installation du dispositif anti-prédation

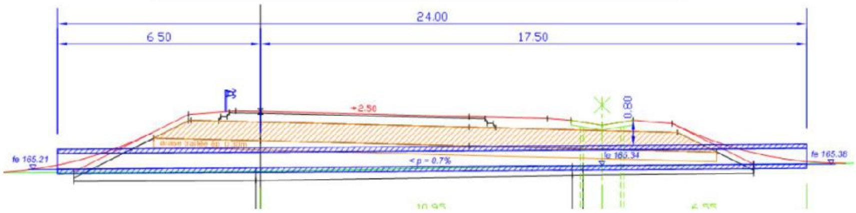
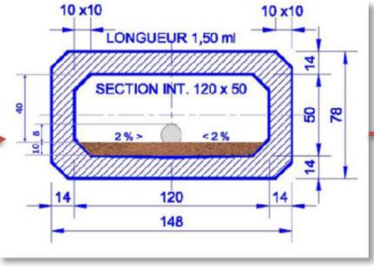


**Figure 22**: Profil en travers d'un hamstéroduc de type rectangulaire (passage en bleu et route en noir) (8)

Surface au sol ≥ 50cm de chaque côté des TAP

Coefficient d'ouverture ≥ 1



Tubes de forme F2

Diamètre de 10cm

Positionnés au centre du passage

### PRECONISATIONS GENERALES

**L'entrée doit être préservée des nuisances humaines** : pas de lumière artificielle, pas de route ou piste à proximité des entrées…

**Le passage doit être facile d'accès pour la faune et les équipes d'entretien** : dégager l'entrée des obstacles éventuels qui pourraient gêner le passage des animaux + favoriser les surfaces planes ou en pente faible.

**Les dimensions des passages à faune doivent tenir compte de l'effet tunnel** : plus le passage est long et plus sa section doit être grande.

**La mise en place des TAP doit se faire précisément pour être efficaces** : les tubes doivent être assemblés les uns derrière les autres, former une ligne droite à l'intérieur des passages et toujours être en contact avec un sol naturel.

**Pour les grands ouvrages de type ouvrage agricole ou pont, favoriser la mise en place de tubes en béton** plus solides sur le long terme que le PVC et pouvant résister aux dégradations et au vandalisme.

**Former (et communiquer avec) les personnes chargées de l'entretien de ces structures** : des inspections courantes (2 à 10 fois par an) doivent être mises en place afin d'évaluer l'état général des infrastructures, gérer l'entretien à l'intérieur et aux abords des tunnels et rapporter les dysfonctionnements éventuels.

# French Summary

## Introduction

Le hamster commun (*Cricetus cricetus*), ou Grand hamster, est une espèce emblématique d'Alsace, seule zone de présence française. Malgré son statut légal d'espèce protégée et les actions spécifiquement menées depuis 2000 en faveur de sa sauvegarde, sa population et son aire de répartition ne cessent de décliner. Les expérimentations visant à sa conservation, menées ces dernières années dans d'autres pays européens, couplées aux travaux de recherche nationaux, ont permis d'identifier un certain nombre de pratiques qui pourraient garantir à moyen terme le maintien de l'espèce en France. Cette thèse est partie intégrante du projet ALISTER (programme Life+ Biodiversité financé par l'Union Européenne) qui vise à tester et à démontrer, dans plusieurs zones d'habitat, la pertinence, au niveau régional, d'actions préalablement identifiées comme potentiellement favorables au hamster.

Ma thèse s'articulait autour de deux objectifs : 1) Améliorer l'habitat du Grand hamster, sur la base d'un recensement exhaustif de l'existant, en expérimentant les pratiques agricoles les plus prometteuses et leurs effets sur la biologie de l'espèce. En effet, le modèle démographique de Leirs (2002) montre que seule une augmentation de son succès reproducteur permettra de stabiliser ses populations en Europe de l'Ouest de manière pérenne. Or, le succès reproducteur est conditionné par les apports alimentaires avant l'hibernation, mais aussi pendant la gestation et la lactation des femelles et la croissance des jeunes (Franceschini-Zink & Millesi, 2008). 2) Reconnecter les populations. Le morcellement de l'habitat du Grand hamster est lié à deux phénomènes différents : l'intensification d'une agriculture industrielle et la fragmentation inhérentes des zones favorables à sa survie (alimentation/prédation) ; l'urbanisation galopante et les infrastructures associées, augmentant plus encore le cloisonnement des populations. Afin de remédier à la forte densité d'infrastructures routières en Alsace, certaines d'entre elles ont été équipées de passages pour la faune sauvage, qui semblent être utilisés à la fois par les petits carnivores et par le Grand hamster. La fonctionnalité de ces passages peut être compromise s'il existe un risque renforcé de prédation au moment de leur traversée.

L'objectif et les approches étaient donc doubles :

**I)** Le premier volet visait à expérimenter, en captivité ou semi-captivité (en s'affranchissant donc des problèmes de prédation qui dépendent souvent de la qualité du couvert végétal), quelles espèces végétales sont les plus favorables au Grand hamster en termes d'apports nutritionnels : 1/ au moment de la constitution des réserves pré-hivernales, et ce afin de lui permettre une hibernation optimale, la meilleure condition corporelle à l'émergence et un succès reproducteur optimal ; 2/ pendant la lactation des femelles, qui est une période particulièrement coûteuse en énergie ; 3/ pendant la croissance des jeunes qui requiert des apports nutritionnels spécifiques. L'objectif étant de déterminer quelles plantes permettraient au Grand hamster d'avoir une hibernation optimale et le meilleur succès reproducteur en fonction de la qualité des apports alimentaires disponible en milieu naturel, tout au cours de la réalisation du cycle reproductif.

**II)** Le second volet de cette thèse consistait à évaluer l'efficacité des dispositifs anti-prédation lors du franchissement de passages à faune par les hamsters. La création des dispositifs a été réalisée en milieu contrôlé où la personnalité des hamsters a également été évaluée. Les dispositifs ont ensuite été testés en milieu semi-naturel et évalués via un suivi vidéo complet. En parallèle, des hamsters ont été relâchés dans un enclos afin de provoquer expérimentalement la rencontre avec différentes espèces de prédateurs (chat, renard, furet). L'utilisation des systèmes anti-prédation a ainsi pu être corrélée à la personnalité des individus.

## Résultats et discussion

<u>I) Premier volet</u> :

1. Une première étude analysant l'effet des changements environnementaux en Alsace sur la masse des hamsters en sortie d'hibernation (proxy de leur condition corporelle et de leur future capacité à se reproduire) a été réalisée. Basée sur un jeu de données de 1468 individus entre 1937

et 2014, cette analyse révèle que la masse des hamsters en Alsace a diminué de 21% en un siècle (Figure 1).

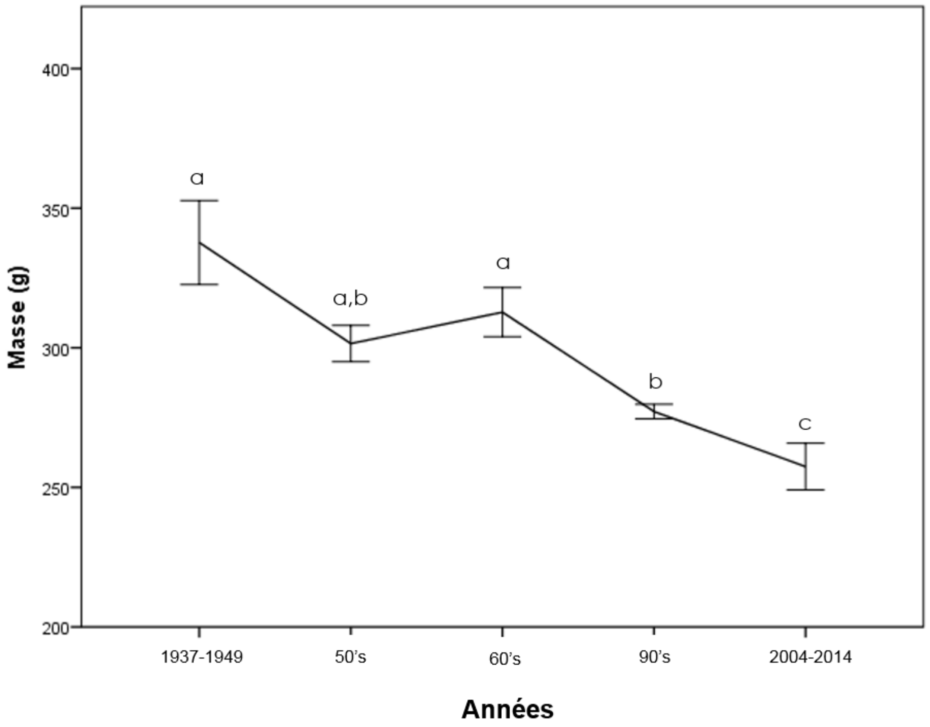


**Figure 1 : Evolution de la masse de hamsters sauvages à l'émergence d'hibernation (Avril-Mai) en Alsace depuis 1937**. Les lettres mettent en évidence des différences significatives entre les décennies (Two-way ANOVA).

Cette baisse s'avère principalement causée par l'intensification de la monoculture de maïs (illustrée en Figure 2A) et l'augmentation des pluies hivernales (conséquence des changements climatiques ; Figure 2B).

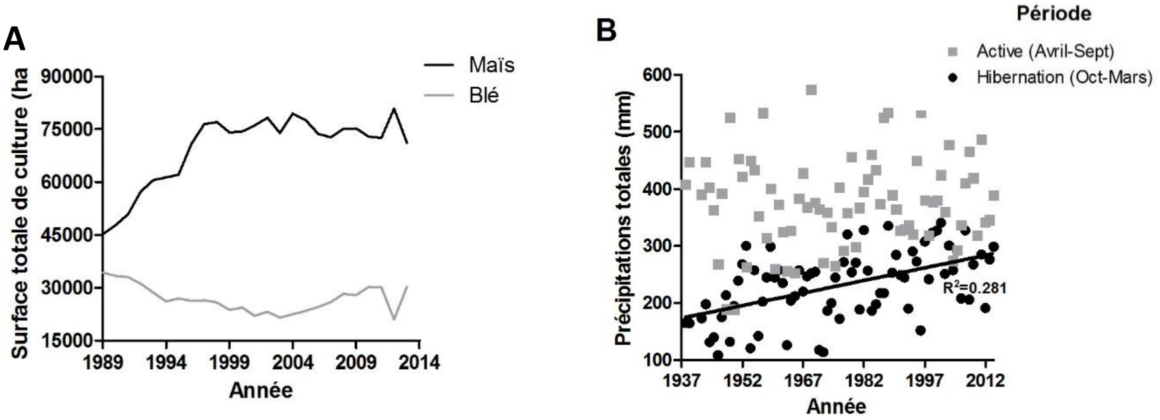


**Figure 2 : Augmentation de la surface totale allouée au maïs et au blé depuis 1989 (A) ainsi que des précipitations pluvieuses saisonnières depuis 1937 (B).**

*Cette étude (‘****study 1****’ de ce manuscrit) a été publiée en 2016 dans la revue Scientific reports.* Elle a été relayée dans la presse régionale et nationale (ci-dessous, un exemple dans le JDD du 5 Juin 2016).

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JDD | 5 juin 2016

## Le grand hamster sauvera-t-il sa peau ?

**ILS SONT AUJOURD'HUI MOINS D'UN MILLIER** en France, regroupés en Alsace. Et malgré les mesures de protection, « *les effectifs de grands hamsters ne cessent de diminuer. Il est impossible de stabiliser les populations à long terme* », constate Caroline Habold, écophysiologiste à l'Institut pluridisciplinaire Hubert-Curien (CNRS/université de Strasbourg). On croise désormais, en moyenne, moins de trois individus par hectare en France, en Allemagne et aux Pays-Bas. Bon indicateur de la qualité de l'écosystème agricole, ce rongeur a même disparu en Belgique. Quels sont les responsables de ce déclin qui pourrait aboutir à une extinction ? On ne sait pas précisément. Pour y voir plus clair, une équipe de l'Institut Curien, en collaboration avec l'Institut des neurosciences cellulaires et intégratives et l'Office national de la chasse et de la faune sauvage, a donc étudié l'évolution de la masse corporelle du grand hamster au cours du siècle passé ; 1.468 individus au sortir de l'hibernation, entre 1937 et 2014, sont « *passés sur la balance* ». Résultat : selon l'étude publiée dans Scientific Reports, ce mammifère a perdu sur cette période 21 % de sa masse corporelle (un mâle adulte pèse environ 400 g). Une chute de poids corrélée à l'augmentation des pluies, à l'intensification de la monoculture de maïs et à l'urbanisation. « *L'augmentation des épisodes pluvieux hivernaux, directement liée au changement climatique, peut être néfaste à la bonne conservation des aliments stockés dans les terriers*, constate Caroline Habold. *Qui plus est, le couvert végétal offert par le maïs, qui pourrait protéger le grand hamster des renards et des rapaces, n'est pas disponible au sortir de l'hibernation.* » Conséquences : moins de nourriture et plus de prédateurs…

La réhabilitation du grand hamster (son seuil de viabilité en Alsace est estimé à 1.500 individus) n'est pas pour demain. Certes, quelques parcelles de blé, contre indemnités, ne sont pas récoltées afin d'offrir au rongeur un couvert végétal. Mais cette mesure d'urgence « *n'est pas tenable à long terme*, estime Caroline Habold. *Il faut trouver des mesures agro-environnementales pérennes.* » Exemple : le sous-semis de trèfle entre les rangs de maïs et de blé.

*Grand hamster commun d'Alsace.* S. CORDIER/JACANA

**RICHARD BELLET** @richardbellet1

2. Une seconde étude menée en 2014 portant sur l'effet des céréales (blé et maïs) sur la reproduction du hamster a révélé que la consommation de maïs, qu'elle soit associée à des végétaux (trèfle) ou des invertébrés (vers de terre), est responsable d'infanticides maternels chez le Grand hamster (à hauteur de 95%). Afin de comprendre ce comportement, nous avons mesuré de nombreux paramètres : niveaux plasmatiques d'ocytocines, contenus en macronutriments (lipides, protéines et glucides) et en énergie des régimes, contenus en minéraux ainsi que niveaux de pesticides. La composition en macronutriment nous a indiqué que les régimes supplémentés en trèfle (que ce soit sur la base du blé ou du maïs) étaient légèrement appauvris en protéines et pouvaient expliquer un mauvais succès reproducteur. Toutefois, aucun des paramètres étudiés ne permettaient d'expliquer la différence entre les régimes blé-vers et maïs-vers, tous deux très similaires.

Une troisième étude menée en 2015 a permis de démontrer que ce comportement maternel était causé par une carence en vitamine B3 dans le maïs (vitamine présente sous forme non bio-disponible dans cette plante). En effet, une supplémentation en vitamine B3 chez des femelles nourries au maïs et vers de terre a permis de restaurer le comportement maternel des femelles et la survie des jeunes (Figure 3).

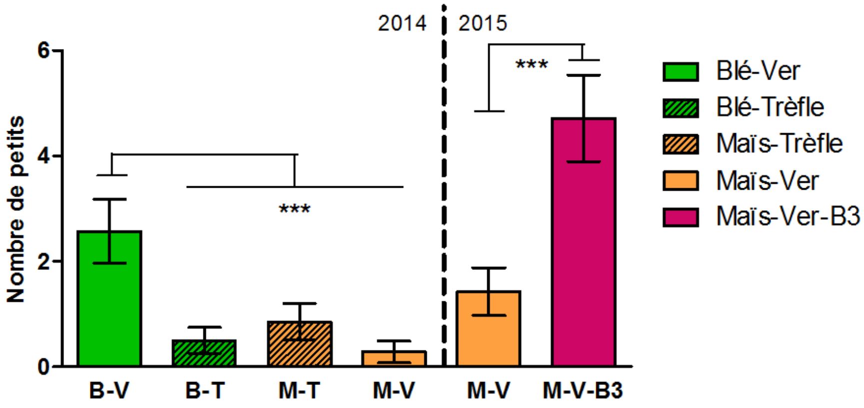


Figure 3 : nombre moyen de petits par portée au sevrage en fonction du régime.

Dans la discussion de cette troisième étude, le parallèle a été fait avec l'humain, chez qui la consommation importante de maïs cuisiné de manière inappropriée a décimé des millions de personnes pendant 2 siècles, et avec les abeilles, donc le sens de l'orientation peut être affecté par de telles carences.

*Ces deux études ont été regroupées ('**study 2**' de ce manuscrit) et publiées dans un même article dans la revue Proceedings B : Biological Sciences. Cette étude a également été relayée dans la presse régionale, nationale et internationale, comme dans Le Monde.*

3. Dans une quatrième étude réalisée en enclos, nous avons cherché à savoir si les hamsters pouvaient compenser la carence en vitamine B3 du maïs en consommant des invertébrés ou des plantes adventives. Cette étude, réalisée au Fort Joffre, a permis de mettre en évidence que dans les enclos avec une seule culture (blé ou maïs), le taux de reproduction était 82% inférieur à celui des hamsters qui étaient dans des enclos contenant 4 cultures (blé+maïs+tournesol+luzerne) comme représenté dans le Tableau 1 ci-dessous.

| **Paramètres \| Culture** | **Blé** | **Maïs** | **Mixte** |
|---|---|---|---|
| Nombre de petits | 4 | 1 | 19 |
| Mortalité adulte | 17% | 17% | 8% |
| Emigration/mort | 42% (pré-reproduction) | 42% (pré-reproduction) | 42% (**post**-reproduction) |

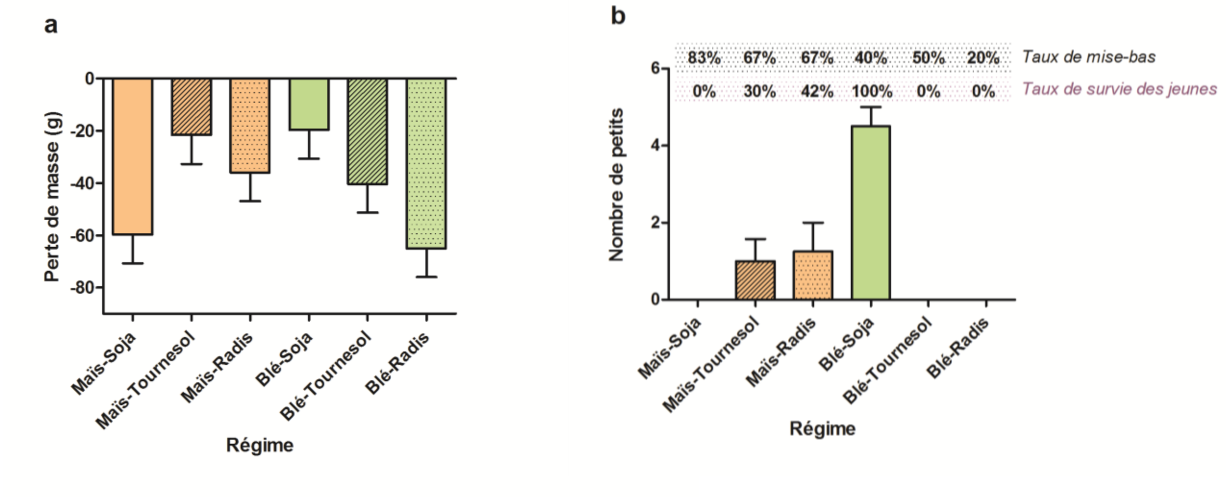
Tableau 1 : nombre de petits par couples et mortalité/émigration (%) des adultes en fonction du type de culture dans les enclos.

Dans cette étude, nous avons également démontré que le fait de cultiver une seule céréale, même de manière biologique, diminue (de 38% et 28% respectivement) la richesse spécifique en plantes adventives en invertébrés. Ces résultats démontrent donc l'importance d'avoir une diversité culturale en plaine Alsacienne, pour le hamster comme pour le reste de la biodiversité végétale et animale.

*Cette étude ('**study 3**' de ce manuscrit) est actuellement en cours d'évaluation dans la revue Oecologia*.

4. En vue de proposer des mesures agronomiques concrètes bénéfiques à la conservation du hamster, une cinquième étude a été réalisée pour tester un supplément en graines naturellement riches en vitamine B3 (tournesol, radis fourrager, soja) sur l'hibernation (hiver 2015-2016) et la reproduction 2016 de hamsters nourris soit au blé soit au maïs. Nous avions donc 6 associations différentes : maïs-soja, maïs-tournesol et maïs-radis puis blé-soja, blé-tournesol et blé-radis.

Il a été mis en évidence que les femelles ont perdu plus de masse spécifique (perte de masse rapportée à la masse initiale de l'animal) que les mâles au cours de l'hibernation (51.1±5.9 grs et 34.1±6.0 grs respectivement) de manière générale. Les associations apparaissant comme les moins favorables au cours de l'hibernation sont les associations *maïs-soja*, *blé-tournesol* et *blé-radis* (pertes de masse spécifique d'environ 60grs, et échecs de reproduction ; voir Figure 4a et 4b). Les régimes *maïs-tournesol*, *maïs-radis* et *blé-soja* sont les plus favorables avec des pertes de masse spécifique de l'ordre de 20 grs et une meilleure reproduction que dans les 3 régimes précédents (Figure 4a), le régime blé-soja assurant le meilleur succès reproducteur (Figure 4b).



<u>Figure 4</u> : **a.** perte de masse spécifique au cours de l'hibernation en fonction du régime et **b.** nombre moyen de petits par femelle au sevrage (avec indication des taux de mise-bas et de survie des jeunes pour chaque régime).

Cette étude suggère donc que la perte de masse au cours de l'hibernation est un élément très important chez cette espèce en ce qui concerne le succès reproducteur à l'émergence. Nous pouvons aussi en déduire 3 associations favorables (par ordre décroissant) : blé-soja, maïs-tournesol et maïs-radis à la fois pour l'hibernation et la reproduction de l'espèce.

*Cette étude ('**study 4**' de ce manuscrit) est actuellement en cours de préparation.*

Ces associations favorables sont en cours d'inclusion dans les schémas agricoles en Alsace. A titre d'exemple, l'association blé-soja est testée par un agriculteur impliqué dans le programme LIFE+ ALISTER, en collaboration avec la Chambre d'Agriculture d'Alsace et l'Office National de la Chasse et de la Faune Sauvage. Le but de ces tests est double : s'assurer de l'intérêt agro-économique de cette association en termes de rendements, et évaluer l'effet sur les hamsters sauvages en Alsace.

<u>II) Deuxième volet</u> :

1. Une sixième étude a été réalisée en conditions contrôlées et a permis de développer un système anti-prédation en forme de sous-tunnel en PVC, à installer dans les passages à faune (Figure 5). Ces

tests ont mis en évidence la forme et le diamètre les plus adaptés au passage des hamsters (maximisant l'utilisation du dispositif) : un tube de forme arrondie (simulant les galeries du hamster), de 10cm de diamètre, avec des ouvertures latérales tous les mètres de chaque côté.

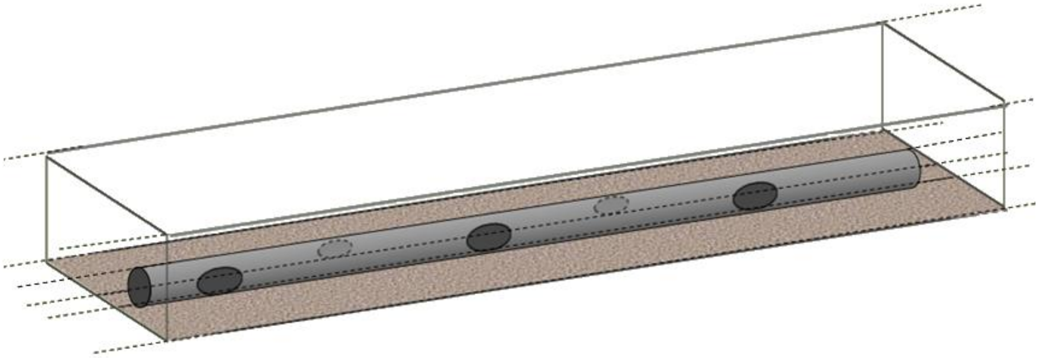


**Figure 5 : système anti-prédation avec ouvertures latérales développé pour sécuriser la traversée des hamsters et autres micromammifères dans les passages à faune.**

*Ce système anti-prédation a été « breveté » par une publication méthodologique en 2016 ('**study 5**'), dans la revue Ecological Engineering.*

2. Trois autres études ont été réalisées (en 2014, 2015 et 2016) afin de mieux appréhender la perception de la prédation, la réaction face à différents stimuli de prédation (odeur, présence d'un prédateur derrière une grille) et l'utilisation du système anti-prédation mis en place. Ces études révèlent que le hamster a un comportement audacieux et a tendance à passer plus de temps vers l'odeur de prédateur, à montrer des comportements agressifs voire à attaquer le prédateur lorsque celui-ci est immobile (voir tableau 2 ci-dessous).

| Variable | Condition | Moy±SE | Effet prédateur |
|---|---|---|---|
| Fréquence de demi-tours (nb/min) | $P_{sans}$ | **0.01±0.01** | ↗ |
| | Prédateur | **0.28±0.08** | |
| Fréquence de redressement (nb/min) | $P_{sans}$ | **0.63±0.08** | ↘ |
| | Prédateur | **0.23±0.07** | |
| Fréquence de grattage (nb/min) | $P_{sans}$ | **0.07±0.02** | ↗ |
| | Prédateur | **0** | |
| Proportion de temps passé proche du prédateur (s/min) | $P_{sans}$ | **0.30±0.03** | ↗ |
| | Prédateur | **0.51±0.09** | |
| Fréquence d'ébrouement (nb/min) | $P_{sans}$ | 0.05±0.02 | - |
| | Prédateur | 0.09±0.04 | |
| Proportion de temps dans le tube (s/min) | $P_{sans}$ | **-5.4 ± 4.4** | ↗ |
| | Prédateur | **+5.7 ± 4.4** | |
| Fréquence de comportements agressifs (nb/min) | $P_{sans}$ | 0 | ↗ |
| | Prédateur | 0.5±0.05 | |

L'utilisation du système anti-prédation par les hamsters n'a pas augmenté en présence d'un prédateur immobile. En revanche, lorsque le prédateur pouvait se déplacer (dans une enceinte close, et séparé du hamster par une grille), les hamsters (après avoir montré des comportements agressifs) ont significativement plus utilisé le système anti-prédation.

*Cette étude ('**study 6**') a été soumise dans la revue Animal behaviour.*

3. Deux autres études ont été réalisées pour valider l'efficacité du tube anti-prédation en milieu naturel, l'une en enclos et l'autre en milieu naturel (en collaboration avec Jonathan Jumeau). Ces deux dernières études sont actuellement en cours d'analyse.

## Conclusion

Les apports de cette thèse permettront, en collaboration avec la Chambre d'Agriculture, l'Office National de la Chasse et de la Faune sauvage et les collectivités territoriales d'améliorer l'habitat du hamster en diversifiant les parcelles agricoles et de diminuer la fragmentation en reconnectant les populations sauvages de hamster.

# Abstract

The European hamster (*Cricetus cricetus*) is one of the most endangered mammal in Europe. In France, less than 800 individuals are currently inhabiting farmlands of the 'Grand-Est' Region. However, despite the specific actions undertaken since 2000 in favor of hamsters' conservation, the French population is still highly threatened. My thesis is part of the project ALISTER (Life + Biodiversity program funded by the European Union), which aims at testing the relevance (at the regional level) of some actions previously identified as potentially favorable to the species. Nonetheless, there is still a huge gap in understanding the underlying mechanisms that could affect hamsters' fitness. Specifically, this work therefore aimed at bringing scientific knowledge regarding hamsters' eco-physiology and behavioral ecology, in order to improve conservation strategies of this targeted species. This has been done throughout two main approaches:

1. Investigating for the nutritional effects of crops on the fitness of the hamster and finding which crops, of economic interest, are the most favorable for the hamster in terms of nutritional inputs. In that aim, the effects of several crops on the hibernation and the reproductive success of captive and semi-captive hamsters have been investigated. The main results highlight that elevated maize consumption is severely reducing hamsters' reproduction because of a major deficiency in vitamin B3. This deficiency appears extremely difficult to compensate with other food items, and only the association with sunflower allow hamsters to have a proper hibernation and first reproduction under captive conditions. More broadly, results highlight the negative effects of wheat and maize monoculture on the fitness of this species and pinpoint that the implementation of crop associations such as wheat-soybean and maize-sunflower need to be implemented in the Alsace, as a conservation measure for this species.

2. Evaluating the antipredatory behavior of the European hamster and developing an anti-predation tube (APT) that will serve to upgrade wildlife underpasses in the Alsace and ultimately allow to reconnect wild populations. The tests have been carried out under controlled and semi-captive conditions and the main results reveal that hamsters display an offensive strategy (e.g. mobbing, threatening and attacking the predator) when facing cats' urine or a real predator (i.e. the European ferret). In these context, the efficiency of our anti-predation device has been validated since it allowed hamster to take refuge when facing an actual predation risk. The APT will now be implemented in wildlife underpasses in the Alsace, whereas its effectiveness will now be evaluated via a comprehensive video monitoring under natural conditions. The results obtained regarding the antipredatory behavior of this species bring new perspective avenues that could be applied to the hamster conservation.

**Keywords**: corn, farmland wildlife, monoculture, mixed farming, fragmentation, predation, conservation

**Une action coordonnée par :**

**En partenariat avec :**

**Financée par :**

Mathilde TISSIER

# Conservation biology of the european hamster (*Cricetus cricetus*)

*Nutritional effects of crops on hamsters fitness and evaluation of their antipredatory behavior to upgrade wildlife underpasses*

## Résumé

Le Grand hamster (*Cricetus cricetus*), l'un des mammifères les plus menacés d'Europe, est en voie d'extinction en France. Toutefois, nous manquons d'information sur les causes de son déclin et sur comment améliorer sa conservation. Durant ma thèse, je me suis intéressée à l'effet des cultures sur la reproduction du hamster. Les principaux résultats indiquent qu'une consommation importante de maïs conduit à une diminution drastique du succès reproducteur en raison d'une carence en vitamine B3. Une autre étude démontre que des associations de cultures (blé-soja ou maïs-tournesol) sont favorables au hamster et devraient être mises en place en Alsace. En parallèle, j'ai développé un tube anti-prédation (TAP) pour améliorer les passages à faune et reconnecter les populations sauvages. Des tests comportementaux ont révélé que les hamsters présentent des comportements audacieux face au prédateur, mais utilisent tout de même le TAP comme refuge, validant sa fonction anti-prédation. Le TAP sera maintenant mis en place dans plusieurs passages à faune en Alsace. Les résultats de cette thèse vont maintenant bénéficier à la conservation du hamster en France et en Europe.

**Mots-clé**: maïs, agri-faune, monoculture, polyculture, fragmentation, prédation, conservation

## Résumé en anglais

The European hamster (*Cricetus cricetus*), one of the most endangered mammal in Europe, is on the verge of extinction in France. However, we are still lacking information on the causes of its decline and on how to improve its conservation. During my PhD, I therefore investigated for the nutritional effects of crops on hamsters' fitness. The main results highlight that elevated maize consumption is severely reducing hamsters' reproduction because of a major deficiency in vitamin B3. Then, I found that crop associations such as wheat-soybean and maize-sunflower are favorable to the species and should be implemented in the Alsace. I also developed an anti-predation tube (APT) that will serve to upgrade wildlife underpasses and ultimately allow to reconnect wild populations. Moreover, behavioral tests presented in this thesis reveal that hamsters display bold behaviors when facing a predator. Nonetheless, they use the APT as a refuge in such cases, which validated its anti-predatory function. Therefore, the APT will now be implemented in wildlife underpasses in the Alsace. Results of this PhD will now benefit the conservation of the species in France and in Europe.

**Keywords**: corn, farmland wildlife, monoculture, mixed farming, fragmentation, predation, conservation

www.grand-hamster-alsace.eu