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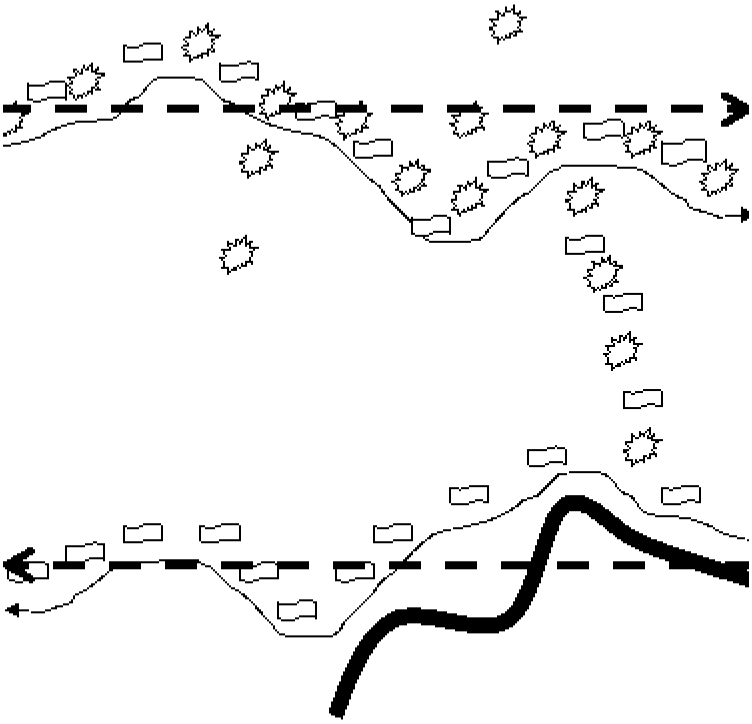

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# AMPHIBIANS AS MODEL ORGANISMS FOR STUDY ENVIRONMENTAL GENOTOXICITY

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**Abstract.** Animals, the silent sentinels, stand watch over the world's environmental health. Everyday, animals demonstrate intricate connections between them, us and our surroundings. Amphibians are vertebrates and include approximately 4400 existing species. Amphibians are in most cases, small, diverse and sensitive to environmental variability. They can be good indicators of habitat diversity, biological variety and local stressors on the environment. They are bathed in both water and air. They live outdoors on land and water and their skin, larvae and unshelled eggs are constantly exposed and in contact with the substances in their surroundings. We searched Web of Science and references of relevant publications to evidence the application of amphibians, especially *Xenopus laevis* as model animal in ecotoxicology.

**Keywords:** *amphibians, genotoxicity, ecotoxicology*

## Introduction

Environmental toxicology studies of environmental toxicants on the health of all organisms and on the different compartments of the environment. Its concern involves the fact that human survival depends on the preservation of other animal and plant species and on the environmental resources such food, water and fresh air which are menaced mostly by anthropogenic chemicals that alter living organisms and ecological balance.

World Health Organization statistics report that 80% of human diseases are related to environmental pollution (Pesch et al., 2004; Neubert, 2002; Zhanfen and Xiaobai, 2006). In recent years, there are increasing reports on endocrine disorder, reproductive dysfunction, sexual reversal, environmental deterioration and biodiversity alteration. In this context, ecotoxicology has become in modern times one of the focused issues (Colborn, 2004; Hoyer, 2001). Ecotoxicology is defined by integrating the ecological and toxicological effects of chemical pollutants on biosphere, including humans (Unger, 2003).

Procedures, protocols and testing organisms are important components of environmental technology. The recent establishment of a procedural paradigm for ecological risk assessment (e.g. EPA., 1991) constitutes a technological advance as well as a contribution to ecotoxicology practical goals. General methods to biomonitor (use of organisms to monitor contaminants and to imply possible effects to biota or sources of toxicants to humans (Goldberg, 1986) and apply biomarkers (cellular, tissue, body fluid, physiological or biochemical changes in individuals that are used quantitatively during biomonitoring to imply presence of significant pollutants or as early warning systems for imminent effects) are also important technologies developed in the last several decades.

One of the major problems in biomonitoring genotoxic pollutants is the choice of test organisms. Unequal sensitivity among species caused by metabolic rates, physiological conditions and target organs can yield misleading results.

Conventional test animals used in ecotoxicology include alga, earthworm, fish, avian species and mouse. Corresponding test methods of these animals are relatively mature and have definite application range. For example, alga test is suitable for assessing toxicities of organic pollutants in water environment, and fish test is suitable for assessing toxicity of more pollutants, while rat/mouse can provide plentiful toxicological data on pollutants. In recent years, more and more scholars have focused on ecotoxic effects of pollutants on amphibians.

## ***Amphibians, sentinel animals for environmental genotoxicity***

Amphibians are important model animal species in biology and they are used in studies on early embryonic development and cell biology. Amphibians have played a key role in the elucidation of the mechanisms of early development over the last century. Much of our knowledge about the mechanisms of vertebrate early development comes from studies using *Xenopus laevis*. The recent development of a remarkably efficient method for generating transgenic embryos is now allowing the study of late development and organogenesis in *Xenopus* embryos. *Xenopus* is a major contributor to our understanding of cell biological and biochemical processes, including: (1) chromosome replication; (2) chromatin, cytoskeleton and nuclear assembly; (3) cell cycle progression and intracellular signaling. Amphibian embryos remained the embryos of choice for experimental embryologists for many decades (Burlibaşa et al, 2005). European embryologists have predominantly used urodele embryos (such as *Triturus*) and embryos of the frog *Rana temporaria*, which is related to the North American species *Rana pipiens.* Amphibian embryos are large, can be obtained in large numbers and can be maintained easily and inexpensively in the laboratory. They are relatively easy to manipulate with microsurgical instruments, and they heal readily after surgery.

In recent years, this model animal species has gradually attracted the attention of more and more ecotoxicologists.

Concerns arising in 1990s about amphibian decline and instances of amphibian malformations have led to an increase in ecotoxicological studies of amphibians. Both aquatic and terrestrial stages are subject to contaminant effects (Unger, 2003).

Amphibians are believed to be sensitive to pollutants because of their highly permeable skin, and their varied lives, which maximize their exposure: they dwell on land and water, and eat both plants and animals at various stages of their life cycle (Conrad, 2010)

The reasons why amphibians and more specifically *X. laevis* is favored by numerous researchers are as follows (Zhanfen and Xiaobai, 2006):

1. *Living in water and easily being raised.*
2. *Ovulating throughout the year and numerous eggs.*
3. *Large size of eggs* – the eggs can be easily observed, collected, transferred and micromanipulated.
4. *Fast external development* – the process of embryonic development is easily controlled and observed.

5. *Easily manipulation and culturing in vitro* – during the embryo period it is relatively easy to isolate specific regions with determined functions and maintain explants in culture media.
6. *Transgenic technology availability in Xenopus* – many of the experiments using *Xenopus* in the study of early development have made use of injected mRNA, antibodies, or antisense oligonucleotides. These methods are transient, however, as the genome is not altered and the injected substance decays in time. The biggest leap forward in the establishment of *Xenopus* as a model organism beyond the limits of early development has been the development of techniques for generating transgenic embryos, by Enrique Amara and Kristen Kroll (1996, 1999, cited by Beck and Slack, 2001). A key advantage of *Xenopus* transgenesis is the ability to study transgene expression in living embryos using green fluorescent protein (GFP) as a reporter allowing quick and easy promoter analysis.

   Fini and his co-workers (2009) developed a real-time flow-through system, based on Fountain Flow cytometry, which measures in situ contaminant-induced fluorescence in transgenic amphibians larvae immersed in water sample. The system requires minimal human effort. This system is portable and self-contained, allowing on-site measurements.
7. *High sensitivity to environmental pollutants* – many laboratories have introduced *X. laevi* in ecotoxicological studies.

   Due to external fertilization and development, embryos and larvae are more susceptible to environmental pollution because of the direct exposure. Therefore amphibians are regarded as a good sentinel animals for environmental pollution. Martin Ouellet assembled a comprehensive review of the literature on amphibian malformations and from his study we can conclude that most amphibian malformations are frog and toad malformations. Ouellet's search found data on malformations occurring in more frog and toad species, sites, and specimens (*Table 1.*)

***Table 1.*** *Summary of Martin Ouellet's data showing most amphibian malformations are frog and toad malformations (Ouellet et al., 1997; Ouellet, 2000)*

| ***Frog versus Salamander Ratio*** | |
|---|---|
| Species affected | 2.6:1 |
| Sites where found | 3.7:1 |
| Numbers collected | 4.5:1 |

According to the U.S. Geological Survey's National Wildlife Health Center, malformations in amphibians arise from environmental factors that affect individuals at the larval stage of development. Scientists suggest that multiple causes are probably to blame for the malformation instances that have been reported worldwide, and that factors leading to malformations at a particular site may be different from those causing malformations at another site. At this time, the four major environmental factors identified as the causes of malformations are contaminants (chemicals and UV-B radiation, nutritional deficiencies, parasites, and injuries from predators (www.nwhc.usgs.gov).

As an amphibian species used in laboratory for many years, *Xenopus laevis* naturally attracts ecotoxicologists' attention. *X. laevis* embryos have been used to assess early developmental toxicity of environmental pollutants for many years (Frog Embryo Teratogenesis Assay – *Xenopus*, FETAX)(American Society for Testing and Materials (ASTM), Standard Guide for Conducting the frog Embryo Teratogenesis Assay – Xenopus (FETAX), ASTM E1439-98 in Annual Book of ASTM Standards, Philadelphia, 1998)(Zhanfen and Xiaobai, 2006).

*Sensitivity to endocrine disruptors*

There is mounting concern in the scientific, environmental and governmental sectors on a wide range of substances, known as endocrine disruptors, that may interfere with the normal functioning of a living organism's endocrine system. Endocrine disruption has the potential to cause reproductive, immunological and neurological problems and in most instances, tumors.

Endocrine disruptors are naturally occurring compounds or man-made chemicals that may interfere with the production or activity of hormones of the endocrine system leading to adverse health effects.

There is some evidence that endocrine disruptors may not only impact the individual directly exposed, but also future generations (Brown and Lamartiniere, 1995).

In 1999, Professor Werner Kloas and his colleagues from Karlsruhe University, Germany, first reported endocrine disruption of phenol substances on *Xenopus laevis* in 1999 and concluded that this species is suitable for studying endocrine disruption (Lutz and Kloas, 1999; Kloas et al., 1999).

In 2010, Hayes and colleagues published a review of the possible causes of a worldwide decline in amphibian populations, concluding that atrazine (one of the most widely used pesticides all over the world) and other hormone-disrupting pollutants are among likely contributors because they affect the reproductive function and make amphibians more susceptible to disease (Hayes et al., 2010). Many other studies demonstrated that atrazine interferes with endocrine hormones, such as estrogen and testosterone – in fish, amphibians, birds, reptiles, laboratory rodents and even human cell lines at part per billion levels (Hussein et al., 1996; Wilhelms et al., 2005; Solomon et al., 2008).

Atrazine causes adverse effects in amphibians through 1) estrogen-mediated mechanisms, 2) androgen-mediated mechanisms, 3) thyroid-mediated mechanisms, 4) adverse effects on gonadal development in amphibians, or 5) adverse effects at the population level in exposed amphibians (Solomon et al., 2005).

As a result of these studies, the Environmental Protection Agency (EPA) is reviewing its regulations on the use of pesticides. Several states are considering banning atrazine, and six class action lawsuits have been filed seeking to eliminate its use. The European Union has already barred the use of atrazine.

Hayes's studies were the first to show that the hormonal effects of atrazine disrupt sexual development in amphibians. Working with the *X. laevis*, Hayes and his colleagues showed that tadpoles raised in atrazine-contaminated water become hermaphrodites and they develop both female and male gonads (Hayes et al., 2002).

Subsequent studies showed that leopard frogs (*Rana pipiens*) collected from atrazine-contaminated streams from areas where atrazine was applied, often had eggs in their testes. And many males had lower testosterone levels than normal females and smaller than normal voice boxes, presumably limiting their ability to call mates (Hayes et al., 2003).

To understand the process by which endocrine-disrupting chemicals work we must look to the genes. Likein many instances in nature where there are complex interferences between living organisms, a gene is not expressed in isolation but rather in the context of other genes and their products, cells and tissues. This might be thought of as "the ecology of gene expression" (Crews and McLachlan, 2006). It is well known that these chemicals can act on a gene's developmental mechanisms, altering genotype expression. The mechanism of these phenotypic changes is probably epigenetic. In fact, endocrine-disrupting chemicals do not act on genes alone but on developmental mechanisms that integrate genetic and epigenetic interactions, resulting in a particular phenotype.

Hormones are known to epigenetically imprint genes in nonmammalian vertebrates. Working on epigenetic memory with the vitellogenin gene in frogs it was shown that the hormonal treatments applied early in life alter the response of hormonally regulated genes to the same or different hormones later in their life (Edinger et al., 1997). The first hormonal experience epigenetically alters the set point for the later hormone response. This process can be determined, in frogs, by methylation (Andres et al., 1984). The term epigenetic imprinting has also been used to describe a process in which estrogens in development cause persistent alterations in gene expression and reprogram cell fate (Alworth et al., 2002, Huang et al., 2005; Fei et al., 2005; Crews and McLachlan, 2006).

Epigenetic imprinting by endocrine-disrupting chemicals or other hormones represents one potential mechanism for Waddington's concept of genetic assimilation, a direct outgrowth of his research in epigenetics. Waddington speculated that environmentally induced changes in phenotype could become incorporated into the genome as evidenced by the persistence of the phenotype even after the original selection pressure is relaxed (Waddington, 1942, 1953). In this manner the selection might act on developmental pathways leading to adaptive change in the genome in conjunction with genetic mutation. Evidence has emerged in recent years that epigenetically mediated changes in phenotype can be stable over many generations (Crews and McLachlan, 2006). Those epigenetic mechanisms may play a role in endocrine disruption, which helps explain the transgenerational effects of some hormonally active chemicals.

## *The most used methods for genotoxicity testing on amphibians*

A growing interest in genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicants in the air, water and soil. Amphibians provide a suitable model for monitoring aquatic genotoxicity and wastewater quality.

### *Chromosome aberration assay*

Chromosomal mutation is a macrodamage of chromosomes. Chromosome aberration include structural aberrations such as fragments or intercalations and numerical aberrations (unequal segregation of homologous chromosomes during cell divisions, which leads to a loss or surplus of chromosomes (aneuploidy and polyploidy). Cytogenetic effects can be studied either in whole animals ("in vivo") or in cells grown in culture ("in vitro"). Usually, the cell culture is exposed to the test substance and afterwards treated with a metaphase-arresting substance (Colcimide). Following suitable staining the cells in metaphase are analyzed microscopically for the presence of chromosomal aberrations.

### *Micronucleus assay*

Micronuclei are generated from chromosome fragments or whole lagging chromosomes that were not incorporated in the daughter cell nuclei and remained in the cytoplasm after the nuclear envelope of doughter nuclei was reassambled. Micronuclei result either from chromosome breaks or dysfunction of the spindle apparatus or centromere kinetochore complexes, with subsequent elimination of whole chromosomes (aneugenic effects) (Campana et al., 2003). The measurement of micronuclei the cell division must be allowed to continue up to the interphase. Amphibian micronucleus procedure has been standardized (ISO 21427-1:2006). Micronucleus formation along with the sister chromatid exchanges and chromosome aberration assays is considered as a clastogenic endpoint. The principle of flow cytometric measurement of micronuclei was made possible (Kohlpoth et al., 1999, Sánchez et al., 2000) but equipment costs are high. Environmental biomonitoring with micronucleus assays has usually been performed "*in vivo*" by exposure of relevant aquatic organisms for several days followed by microscopic analysis.

Many results on the Micronucleus assay used in genotoxicity test on amphibians are available (Campana et al., 2003; Mouchet et al., 2009; Fernandez et al., 1993; Arkhipchuk et al. 2000; Krauter et al. 1987; Jaylet et al. 1986; Godet et al. 1996; Djomo et al. 2000).

### *SCE assay*

The sister chromatid exchange (SCE) assay detects reciprocal exchanges of DNA segments between two sister chromatids of a duplicated chromosome. SCEs represent the interchange of DNA at apparently homologous loci. This process involves DNA breakage and repair but as this process does not necessarily lead to permanent mutations. Some researchers classify the SCE assay as a genotoxicity test. Although little is known about its molecular basis, the SCE frequency is elevated under some pathogenic conditions in humans (e.g Bloom Syndrome) and the influence of mutagenic agents and therefore serves as a model for mutagenicity. The detection of sister chromatids is achieved by incorporation of e.g. bromodesoxyuridine into chromosomal DNA for two cell cycles followed by Hoechst staining and analysis in fluorescence microscopy.

### *Comet assay*

In recent years the Comet assay has gained broad attention, because the test is relatively easy to handle and can be applied with cells from different organisms and tissues. The alkaline version of the comet assay has been developed by Singh et al. (1989).

The comet assay, also known as the single-cell gel electrophoresis test, is a method of detecting DNA strand breakage (double, single, and alkali-labile sites expressed as single strand breaks) in virtually any nucleated cell. Significant advantages of the comet assay over other genotoxicity tests are its fairly straight forward technique, sensitivity, requirement for small numbers of cells (making the assay conductive to non-lethal testing) and rapid production of data (Tice et al., 2000). Cells are mixed with low-melting agarose, placed on microscope slides and lysed by an alkaline buffer with ionic detergents. The liberated DNA is resolved in an electrophoresis chamber, stained and evaluated by fluorescence microscopy. Cells with increased DNA damage display increased migration from the nuclear region towards the anode. The resulting comet-like structure is quantified by measuring the length of the tail and/or the tail moment (the intensity of the migrated DNA multiplied by the respective tail length (integral) with respect to the nuclear DNA). A review about the applicability of the comet assay in environmental monitoring was provided by Mitchelmore and Chipman (1998). The test has been applied to a broader range of aquatic organisms such as algae (Erbes et al., 1997), mussels (Mitchelmore et al., 1998; Pavlica et al. 2001), amphibians (Ralph and Petras 1998) and fish (Pandrangi et al., 1995; Devaux et al., 1997; Belpaeme et al., 1998; Mitchelmore and Chipman, 1998; Villarini, 1998; Risso-de Faverney et al., 2001; Schnurstein et al., 2001). The advantages of the test are the possibility to choose a broad range of test organisms and tissues, the use of even non-proliferating cells, and the fact that results can be obtained within one day. On the other hand there are still no standard test protocols and a certain degree of handling skills is a necessary prerequisite to routinely performe the test . Although no international accepted standard exists many researchers refer to a test protocol of Tice (1998).

### *Recent developments*

In the field of genotoxicity evaluation of environmental samples similar developments as in classical toxicology have been undertaken. Fini and coworkers (2009) developed a real-time flow-through system, which measures in situ contaminant-induced fluorescence in transgenic amphibians larvae immersed in water sample. The amplification of DNA by the Polymerase Chain Reaction technique enabled the detection of mutations at specific sites and the development of electrochemical DNA-based biosensors (Kennerley and Parry, 1994; Parsons and Heflich, 1998; Mascini et al., 2001; Picco and Collins, 2008).

Our unpublished yet results revealed that ChIP assay (Chromatin Immunoprecipitation Assay) may offers important informations regarding epigenetic alterations in male newt (*Triturus cristatus*) spermatogenesis due to environmental exposures.

The micronucleus test and the single cell gel electrophoresis (comet assay) are two most extensively used methods in the detection of genotoxicity of chemicals in the environment. Compared to other assays, they are sensitive, rapid and easy to handle.

Techniques in genetic ecotoxicology are in a rapidly evolving state. Threfore, reliable tools are now available for addressing more complex environmental problems. The increasing availability of reliable diagnostic tools will greatly improve our ability to assess the sublethal effects of exposure to hazardous substances. We must envision their promise for addressing these problems and identify the most urgent directions for future research.

## *Link between Genotoxic Responses and Reproductive Success*

In the early 1990s, studies began to associate environmental contamination with altered reproductive performance in wild populations of fish, amphibians, reptiles and birds (Colborn et al., 1993).

Genotoxic exposure can act as a selective force by eliminating sensitive genotypes, or by reducing the number of offspring that they contribute to the next generation. The result is a reduction in the total genetic variation within that population or a shift in genotypic frequencies. Genetic variation provides the requisite flexibility for a population to persist in the face of variable biotic and abiotic selective forces over time. Reduced variation can thus lead to increased rate of extinction.

Little has been done to assess the relationships between biomarkers of biologically effective lose and any measure of reproductive success. However, evidence that relates alterations in reproductive success to changes at the population level indicates the need for such markers. Numerous specific avenues of research could strengthen the use of biomarkers to predict reproductive effects. For example, promising future research might include measures of alterations in specific genes which are known to result in dysfunctional gametes or abnormal embryonic development. In general, there is a need for studies of linkages between exposure to contaminants, increases in frequencies of biomarkers, and reduced reproductive success with a select array of contaminants, biomarkers, and species.

The health effects of pesticide exposures on male reproduction represent a topic of considerable concern in environmental, occupational and reproductive epidemiology. In recent years, scientists have become more aware of the fact that human-made chemicals may disrupt reproductive function in both wildlife and humans (Colborn et al., 1993; Moline et al., 2000).

The research to understand the relationships between genotoxic responses and measures of reproductive success has its roots in an extensive literature on nonmammalian animal models in radiobiology and chemical carcinogenesis. With the development of new animal models in genetic ecotoxicology, new relationships between genotoxic responses and measures of reproductive success begin to emerge.

Genotoxic effects on amphibians are being considered in laboratory from an ecological perspective. Although other investigators evaluated genotoxic effects in amphibia exposed to mutagenic chemicals (Siboulet et al., 1984), none has determined whether genotoxic responses are predictive of detrimental reproductive effects. Studies are underway to determine whether there are correlations between frequencies of micronuclei in circulating erythrocytes, DNA adducts in liver, wet weight at metamorphosis, and time to metamorphosis of *Xenopus laevis* tadpoles following the exposure to benzo-$\alpha$-pyrene.

Spermatogenesis is a remarkable process that requires exquisite control and synchronization of germ cell development. It is prone to frequent error, as paternal infertility contributes to 30-50% of all infertility cases; yet, in many cases, the mechanisms underlying its causes are unknown. Germ cell development is a critical period during which epigenetic patterns are established and maintained. The progression from diploid spermatogonia to haploid spermatozoa involves stage- and testis-specific gene expression, mitotic and meiotic division, and the histone-protamine transition. All are postulated to engender unique epigenetic controls. Underscoring the importance of understanding how epigenetic marks are set and interpreted is evidence that abnormal epigenetic programming of gametes and embryos contributes to heritable instabilities in subsequent generations. Numerous studies have documented the existence of transgenerational consequences of maternal nutrition, or other environmental exposures, but it is only now recognized that there are sex-specific male-line transgenerational responses in humans and other species. Epigenetic events in the testis have just begun to be studied. New work on the function of specific histone modifications, chromatin modifiers, DNA methylation, and the impact of the environment on developing sperm suggests that the correct setting of the epigenome is required for male reproductive health and the prevention of paternal disease transmission (Godman et al., 2009).

Epigenetics provides a means of understanding how environmental factors might alter heritable changes in gene expression without changing DNA sequence, and hence the origin, of some diseases that are not explained by conventional genetic mechanisms. Various animal models have been described which lend themselves particularly well to studying this link between epigenetics and development abnormalities, because particular changes in DNA methylation patterns can be linked to a broad spectrum of heritable pathologies (Rosenfeld, 2010).

When the term epigenetics was originally introduced by Waddington (1939), it referred to any causal mechanisms that act on genes to govern a resulting phenotype. This definition was refined by Holliday (1987) to implicate DNA methylation changes that result in altered gene expression and later broadened to explain how the expression of a gene might be changed and then stably maintained by any modification that does not mutate the nucleotide sequence of the gene itself (Egger et al., 2004; Esteller, 2003; Feil, 2006; Serman et al., 2006). However, even this more expansive definition has needed further refinement as the term epigenetics began to be employed to include any manner of change that caused alterations in gene expression. A more restricted definition of epigenetics is a mitotically or meiotically heritable change in gene expression that occurs independently of an alteration in DNA sequence (Younson and Whitelaw, 2008).

Jean-Baptiste Lamarck has become famous for his theory regarding the inheritance of acquired characteristics, which suggests that individuals can pass on certain features that they acquired during their lifetime to their offspring (Costa, 2008). Some of the examples he chose, including the gradual lengthening of the neck of the giraffe as a result of its foraging lifestyle, fell into disfavor, first as a result of Darwin's theory of natural selection and later by the implications of Mendelian inheritance and the notion of the gene. However, inheritance of acquired characteristics has gradually acquired new currency. Thus, there are several examples of transgenerational inheritance of phenotype, which elude conventional genetic inheritance patterns and can probably be explained on the basis of inherited epigenetic modifications of the genome. Such inheritance patterns do not involve a change in DNA sequence but survive meiosis and can be passed through the maternal or paternal germ lines. One hypothesis to account for why transgenerational effects have evolved, is the idea that the transfer of epigenetic information across generations might confer “memory” of environmental stresses experienced in earlier generations, thereby preserving a rapid response to this stressor in subsequent generations (Molinier et al., 2006; Rosenfeld, 2010).

Exposure to a range of toxicants, including vinclozolin (Crews et al., 2007; Nilson et al., 2008), diethylstilbestrol (DES) (Newbold et al., 2006; Walker and Haven, 1997; Newbold et al., 2000), methoxychlor (Anway et al., 2005), and chromium (Cheng et al., 2004), can result in transgenerational disease states, including testis defects, kidney disease, reproductive cancer development, and immune abnormalities, which in many cases are due to epigenetic alterations within the male germ line, but the transgenerational effects of DES (Walker and Haven, 1997 ) and vinclozolin (Nilsson et al., 2008) can also be passed through the female germ line.

All these studies show that environmental factors may influence the epigenetic state and that these epigenetic modifications may be inherited through the male germ line and passed onto more than one generation. Paternal effects have further highlighted the importance of research into epigenetic regulation and male fertility. The concept that untransmitted alleles passed through the male germ line can affect the phenotype of the next generation is a new and exciting area of research.

The 2007 Summit on "Environmental Challenges to Reproductive Health and Fertility" convened scientists, health care professionals, community groups, political representatives and the media to hear presentations on the impact of environmental contaminants on reproductive health and fertility and to discuss opportunities to improve health through research, education, communication and policy. Environmental reproductive health focuses on exposures to environmental contaminants, particularly during critical periods of development, and their potential effects on future reproductive health, including conception, fertility, pregnancy, adolescent development and adult health (Woodruff et al., 2008).

The Summit provided a view of critical scientific information that underscored the need for further efforts in areas to improve reproductive health. One common theme throughout the Summit was communication and collaboration. Scientists bring unique and important contributions to studying the impact of environmental contaminants on reproductive health.

## Conclusions

The ecological assessment of territories exposed to anthropogenic influences must involve evaluation of the mutagenic potential of the environment. The assessment of genetic effects of environmental pollution on man is methodologically difficult and expensive; hence it is expedient to use indicator animal species for ecogenetic monitoring. Mass species of anurans are promising for this purpose, as specific features of their life cycle make them convenient for assessing the state of both terrestrial and aquatic ecosystems.

Amphibians are considered uniquely sensitive to man-made changes in the environment. Their porous skin is vulnerable to water borne toxins and infections, and their reliance on two habitats (water and land) means they cannot survive properly without both. Embryos and larvae of amphibians with external fertilization and development are susceptible to environmental pollutants due to direct exposure to the environment. Therefore, amphibians are regarded as good sentinel animals for environmental pollution. In addition, sex differentiation and sex organ development of *X. laevis* are sensitive to sex hormones and endocrine disruptors with sex hormone activities, which enable *X. laevis* to be used in studies on sex hormone disruption and reproductive toxicity of endocrine disruptors. Metamorphic development of *X. laevis* is very sensitive to thyroid hormones and thyroid disruptors, which enables *X. laevis* to be used for evaluating thyroid disruptors. Also, *X. laevis* ecotoxicology can be linked with amphibian population declines and malformed frog occurrence, being one of the hotspots in ecology. Thus, more and more laboratories have introduced *X. laevis* to ecotoxicological study.

Russell Mittermeier, President of Conservation International, said: "*Amphibians are one of nature's best indicators of overall environmental health. Their catastrophic decline serves as a warning that we are in a period of significant environmental degradation*." (Conor, 2004)

Researchers must be committed to providing long-term monitoring and rigorous investigation of the causes of declines in the especially sensitive amphibian component of the aquatic and terrestrial ecosystems worldwide. Scientists need to move forward with multi-disciplinary research that recognizes the value of animals as sentinels of environmental health. We also need to recognize that environmental protection measures that protect animal health often directly and indirectly protect our health as well.

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# RESPONSE OF *LEMNA MINOR* CLONES TO MICROCYSTIS TOXICITY

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**Abstract**. Tests based on the growth inhibition of *Lemna minor* are widely used in ecotoxicology. The assessment endpoint is the development of new fronds, which is not only a rapid response but is also of ecological significance. At present this is the only standard ecotoxicological test in Europe which uses a higher aquatic plant species, so toxic effect of different contaminants should be extrapolated to other macrophytes. As it might be suspected that cyanobacteria and macrophytes are in a competitive interaction, more and more studies are targeted to reveal the mechanisms via cyanobacterial toxins explicate inhibitory effect. In order to find out what actual risk cyanobacterial toxicity might pose on co-existing Lemna populations, Lemna growth inhibition tests were carried out using duckweed clones collected from different natural habitats. Striking differences were found, identifying clones showing normal reproduction in the presence of *Microcystis aeruginosa* but on the contrary, other clones showed not only reproduction inhibition but visual symptoms such as chlorosis and necrosis.

**Keywords:** *Lemna minor, ecological risk assessment, ToxAlert, microcystin*

## Introduction

The *Lemna minor* growth inhibition test is widely used in ecotoxicology, several standard test protocols are available. In addition to national protocols, international standards also apply: OECD Guidelines for the Testing of Chemicals: *Lemna* sp. Growth Inhibition Test, Draft Guideline 221 (OECD 2002) and the ISO/FDIS 20079 standard which has been recently introduced in Hungary (ISO/FDIS 20079: Water quality - Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) - Duckweed growth inhibition test). The aim of using a standard protocol is to quantify the ecological effect of a given toxicant (in this case cyanobacterial toxins), to make results comparable and if required, to extrapolate the results to other taxa. At present this test is the only bioassay in Europe which uses a higher plant species and should be able to provide reliable data for assessing risk posed on macrophytes. *Microcystis – Lemna* interaction is of significance because real-world interaction (competition) might be suspected. As such, the main target of this study was to reveal the uncertainty factors the standard *Lemna* test might have. Our null hypothesis was that in eutrophic habitats where toxic blooms occur, naturally co-existing *Lemna* populations might develop some resistance against cyanobacterial toxins and will show reduced sensitivity in comparison to previously non-exposed cultures.

## Review of literature

Of cyanobacterial toxins, the hepatotoxin microcystin produced by *Microcystis, Planktothrix (Oscillatoria)* and *Anabaena* sp. is probably posing the highest environmental risk. Up to now over 60 variants of this toxin have been identified, of them microcystin-LR seems to be the most widespread (e.g. Sivonen and Jones, 1999). These variants differ in their toxic potential, microcystin-LR being one of the most toxic, with an $LD_{50}$ µg/kg bw (based on the mouse assay). The WHO Guideline prescribes an 1 µg/l limit value for this variant in treated drinking water.

However, different other toxic compounds have been isolated from *Microcystis* such as cyanopeptolins (e.g. von Elert et al., 2005), or micropeptins (e.g. Ishida et al., 1997). Jungmann (1995) reported that aqueous extacts of *Microcystis* that were free of microcystins were proven toxic in bioassays.

It is a rather general phenomenon that microcystins do have inhibitory effects on growth of different duckweed species. Jang et al. (2007) gave evidence about reciprocal allelopathic responses between *Microcystis aeruginosa* and *Lemna japonica*. Various ecological symptoms have been investigated, such as biochemical responses (e.g. Yi et al., 2009), reduction of chlorophyll-a content and photosynthetic capacity (e.g. Weiss et al., 2000) and growth impairment. Romanowska-Duda and Tarczynska (2002) used *Spirodela oligorrhiza* and found that in the presence of 0.2 and 0.1 µg/l of MC-LR the reduction in the number of fronds was apparent after 24 hours of exposure. In a study conducted by Mitrovic et al. (2005), not only symptoms such as reduction in weight and frond number were recorded but bioaccumulation was also observed. Although most studies have been concentrating on planktonic strains, Mohamed and Al Shehri (2010) demonstrated that toxin production in epiphytic cyanobacteria can also affect aquatic plants.

Studies conducted on other aquatic plants have also shown clear response to Microcystis toxicity (e.g. Pflugmacher et al., 2001a; Pflugmacher, 2004; Yin et al., 2005). Pflugmacher (2002) tested microcystin-LR for its allelopathic power on aquatic macrophytes such as *Ceratophyllum demersum* and *Myriophyllum spicatum*, and found that exposure results in growth inhibition, reduction in photosynthetic oxygen production, and changes in pigment pattern. Szigeti et al. (2010) reported growth inhibition in *Ceratophyllum demersum* via MCY-LR-induced inhibition of cell elongation. It has been shown that microcystins inhibit photosynthetic activity in various plants (e.g. Pflugmacher et al., 2001b; Wiegand et al., 2002). Máthé et al. (2007). established the histological effects of exposure to microcystin-LR (MC-LR), a cyanotoxin on axenic *Phragmites australis* plantlets, describing e.g. aerenchyma obturation, the premature development of lateral roots, even root necrosis. Leaf necrosis was also reported (Babica et al., 2006). In addition to microcystins, several other metabolites produced by *Microcystis* were shown to have inhibitory effect on photoautrophs (e.g. Wiegand et al., 2002).

Some of the mechanisms via plants might develop resistance to cyanobacterial toxicity have been documented. It has been demonstrated that some species of green algae e.g. *Scenedesmus* sp. coexist and even flourish in the presence of either toxic cyanobacteria or their toxins (Sedmak and Kosi, 1998). Mohamed (2008) investigated the possible adaptive response of two representatives of green algae, *Chlorella vulgaris* and *Scenedesmus quadricauda* to pure microcystin-LR (MCYST-LR) and crude MCYSTs. During the first 3 days of exposure, both pure and crude MCYSTs significantly decreased the growth of the two algae compared to control cultures. Meanwhile, increases in glutathione-S-transferase (GST), glutathione peroxidase (GPX) and lipid peroxidation were also observed in toxin-treated cultures, finally growth and biochemical variables were restored and remained at levels near to those of control cultures. These results may suggest that polysaccharides might function as a protective agent in some microalgae against MCYST- induced oxidative stress. Stüven and Pflugmacher (2007) tested the effect of cyanobacterial toxins as well as cyanobacterial crude extract containing microcystins-LR and provided evidence of oxidative stress response in *Lepidium sativum* seedlings, manifested by lipid peroxidation, elevation of alpha- and beta-tocopherol concentrations and elevated activities of antioxidative enzymes like the glutathione peroxidase, glutathione S-transferase and glutathione reductase. Kamara and Pflugmacher (2007) suggested that in aquatic macrophytes increase in antioxidative enzyme levels can be part of the protection strategy of against oxidative stress.

## Materials and methods

### *Sample collection*

Ecotoxicity testing was carried out using liophilised *Microcystis aeruginosa* samples which were collected in the Kis-Balaton Water Protection System.

For preparing test samples, first a 2 mg liophilised *Microcystis*/ml test solution was prepared, than a 1:2:4:8:16 dilution series was set.

### *Lemna minor growth inhibition test*

#### *Lemna clones*

5 duckweed clones were used for comparison, collected from natural wetlands and semi-natural ponds as follows: Lemna1 from a slow-flowing stream, Lemna2 from an oligotrophic pond, Lemna3 and 4 from a slightly eutrophic pond and its tributary, and finally, Lemna5 test population was collected from the Kis-Balaton Water Protection System (a restored wetland in fact), where co-existence with *Microcystis* is proven.

#### *Experimental procedure*

The tests were carried out in accordance with the OECD Guideline 221 (*Lemna* sp. Growth Inhibition Test). Duckweeds consisting of 2 fronds were selected and 10-10 colonies were transferred to each test vessel, thus the starting frond number was 20. Frond numbers and the appearance of the colonies were recorded on Day4 and Day7. The test was terminated 7 days after the plants were inoculated into the test vessels.

Controls and test vessels were kept in an incubator illuminated by continuous warm fluorescent lighting. Light intensity was 8000 (± 100) lux as measured at the surface of the test solution. Temperature in the test vessel was 23 ± 2°C. Growth medium was prepared and sterilised according to the OECD Guideline. Erlenmeyer flasks of 150 ml volume were used as control and test vessels, with a randomised location.

### *Analysis of the data*

From the natural logarithm of number of fronds (lnF) specific growth rate (µ) and percent inhibition of growth rate (Ir) were calculated as stated in the Guideline.

### ***Microcystin determination***

Microcystin content of the liophilised test material was determined by capillary electrophoresis (Vasas et al., 2006).

## Results

Microcystin content of the liophilised test material was 2.836mgg-1.The different test populations have shown striking differences in their response to Microcystis toxicity. In case of Lemna1 100% inhibition was recorded in the highest concentration, 2 mg/ml, the next concentration, 1 mg/ml was still rather toxic, causing 45.24 % inhibition (*Fig. 1*). Lemna2 proved more sensitive, with total (100%) inhibition in the 2 mg/ml and 1 mg/ml concentrations, but then a rapid decrease was experienced in the ecological response, the next concentration of 0.5 mg/ml caused only 8.81 % inhibition (*Fig. 2*). In case of Lemna3, considerable toxicity (66.02 and 43.78 % inhibition) was experienced in the concentrations of 0.125 and 0.25 mg/ml, respectively (*Fig. 3*). Ecological response induced by the other (higher) concentrations did not exceed 20% (a sample is considered toxic if ecological response is above 20% /Suter, 1993/). Lemna4 did not show response, the highest inhibition was 6.65 % in the 2 mg/ml concentration, but this is well below the toxic threshold (*Fig. 4*). Lemna5 showed toxic response (31.11 and 41.63 % inhibition) in the two lowest concentrations (*Fig. 5*).

Not only sensitivity of the test populations differ but also, concentration-response relationships show different patterns. Lemna1 and Lemna4 can be characterised by an almost ideal concentration-response relationship, meaning that the response steadily increases for each higher concentration (*Fig. 1* and *Fig. 4,* respectively). Lemna2 (*Fig. 2*) shows an “all or nothing” response pattern, which is characterized by a transition from no significant effect at one effluent concentration to a complete effect (100% mortality) at the next higher concentration. Even more interesting is the pattern shown by Lemna3 and Lemna5 (*Fig. 3* and *Fig. 5*). These are typical inverse concentration-response relationships in which adverse effects decrease with increasing concentration. Most often, presence of nutrients may mask the toxic effect.

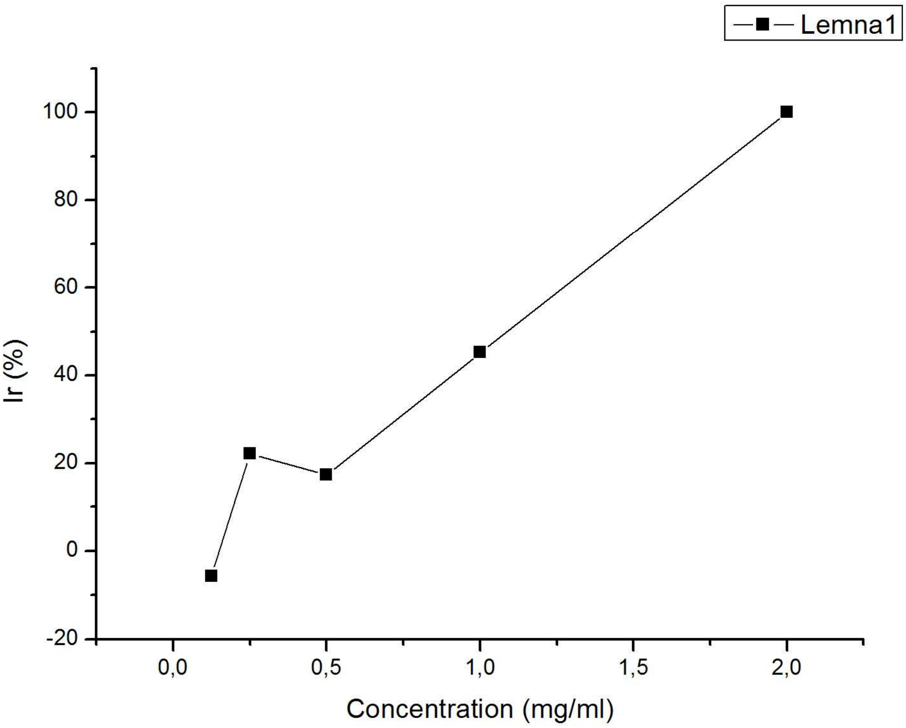

***Figure 1.*** *Concentration-response relationship for Lemna1*

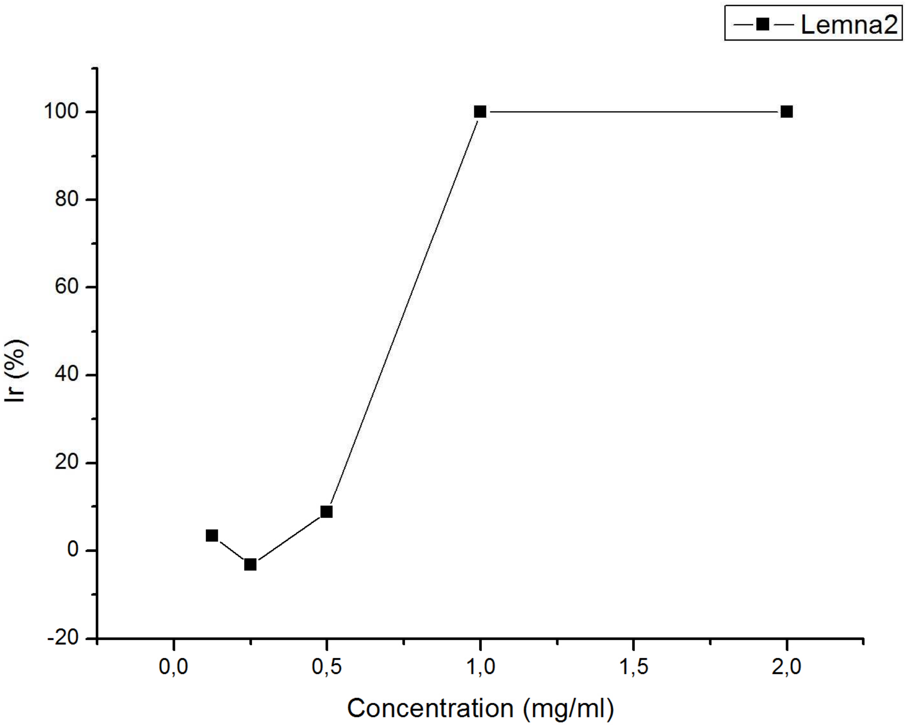

***Figure 2.*** *Concentration-response relationship for Lemna2*

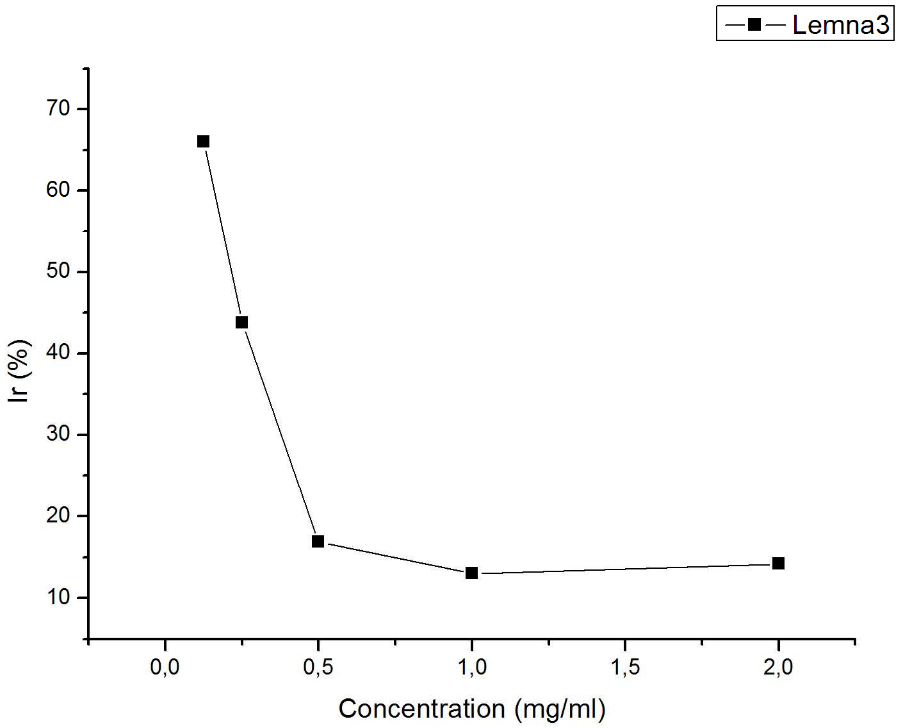

***Figure 3.*** *Concentration-response relationship for Lemna3*

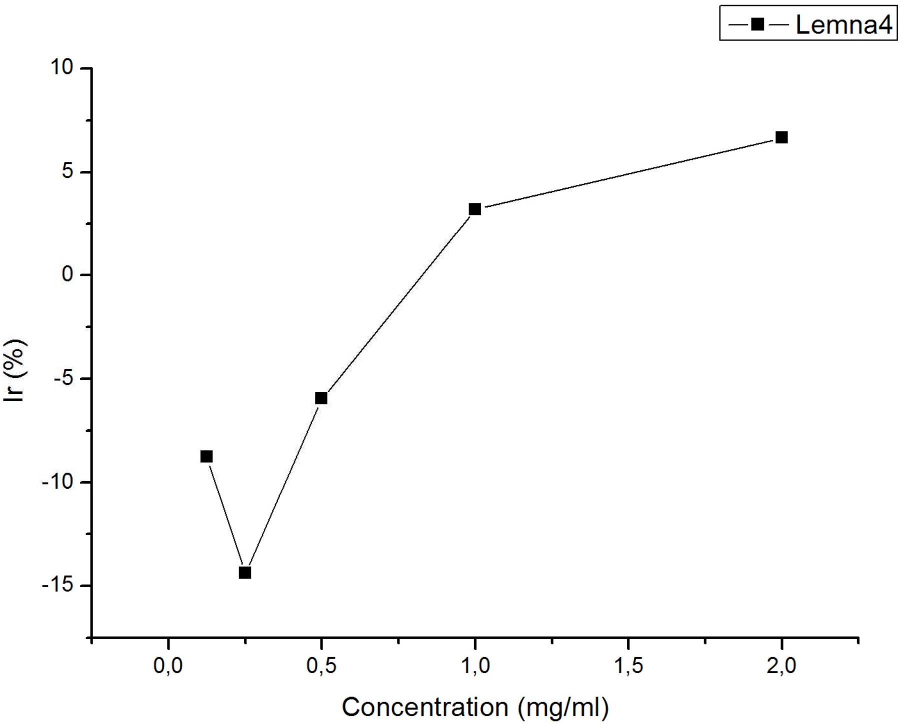

***Figure 4.*** *Concentration-response relationship for Lemna4*

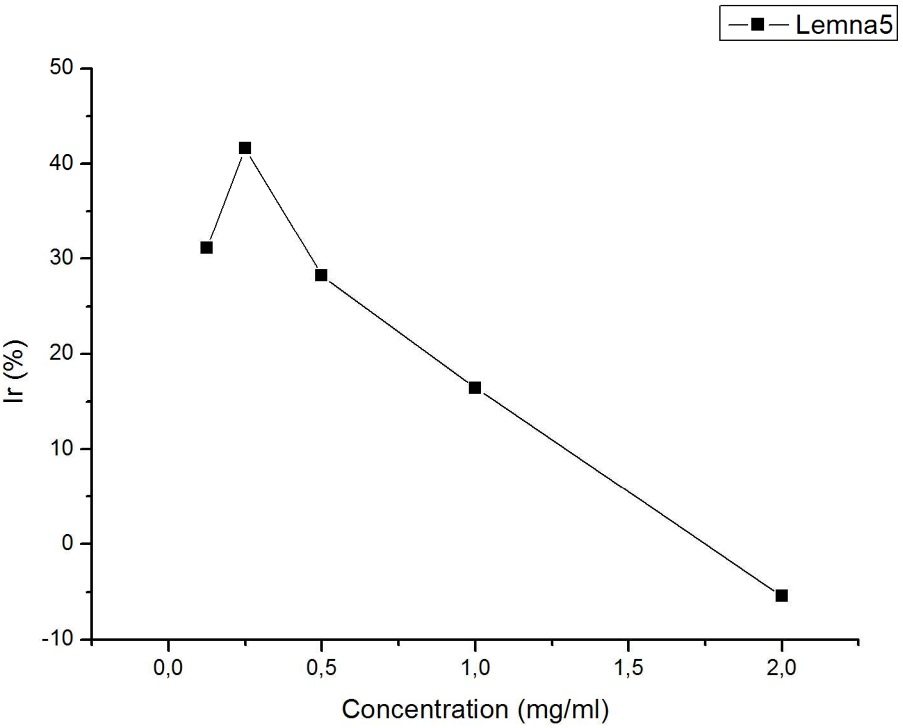

***Figure 5.*** *Concentration-response relationship for Lemna5*

## Discussion

Considering sensitivity, our null hypothesis was partially proven. We assumed that tolerance can be experienced in the case of Lemna clones which live in eutrophic habitats, and on the contrary, clones living in relatively pristine habitats would show sensitivity. Lemna2 (collected from an oligotrophic pond) showed the most significant response, as inhibition was 100% in the 2 mg/ml and 1 mg/ml concentrations. In the highest concentration, all fronds died by the end of the test and number of living fronds was lower than the original 20 in the 1 mg/ml concentration. It was followed by Lemna1 (collected from an oligotrophic, slow-flowing stream), expressing 100% inhibition in the highest concentration, 2 mg/ml, where number of living fronds were also reduced in comparison to the initial 20. It has to be noted that not only inhibition was experienced but visible syptoms: chlorosis and necrosis as well.

Lemna4 (collected from a slightly eutrophic pond) is also a rather clear case to analyse. Its concentration-response relationship is close to ideal (there is a slight anomaly in the second lowest concentration). It shows stimulating effects in concentrations 0.125, 0.25 and 0.5 mg/ml, than a slight inhibitory effect in concentrations 1 and 2 mg/ml. However, the highest inhibition amounts to only 6.65 %, therefore it can be concluded that Microcystis did not have any toxic effect on this test population., seemingly it showed practically complete resistance.

Our null hypothesis had assumed that Lemna3 would show the highest tolerance, as this clone was collected from the same habitat where toxic *Microcystis* bloom was experienced and where the sample *Microcystis* was collected. There is some indication that this clone had developed resistance, as higher concentrations of *Microcystis* during the test (0.5, 1 and 2 mg/ml) exerted rather low response, staying well below 20% of growth inhibition. However, the fact that lowest concentrations caused such high reproduction inhibition is difficult to interpret.

Lemna3 and Lemna5, however, as it was mentioned before, both show inverse concentration-response relationships but their sensitivity differ. For Lemna3, the 0.125 mg/ml *Microcystis* sample had a very toxic effect, causing reproduction inhibition of 66.02 %. The second lowest concentration of 0.25 mg/ml still proved to be rather toxic, having an inhibitory effect of 43.78 %. The concentrations of 0.5, 1 and 2 mg/ml caused a somewhat uniform response of 16.87, 12.96 and 14.17 % reproduction inhibition. The pattern is rather similar for Lemna 5, though extreme values are less extreme.

Biochemical explanation of resistance of higher plants is discussed in the Review of Literature. Duckweed is also capable of developing resistance, for example Saqrane et al. (2007) demonstrated that *L. gibba* could bio-transform microcystins and detected a suspected MC degradation metabolite. Mitrovic et al. (2004) measured the peroxidase activity (POD) of *L. minor* after exposure to several concentrations of the cyanotoxin, anatoxin-a. POD activity significantly increased after 4 days of exposure to an anatoxin-a concentration of 25 µg/ml. Activity of a detoxication enzyme, glutathione S-transferase (GST) was also elevated at anatoxin-a concentrations of 5 and 20 µg/l.

From ecological point of view these results clearly indicate that *Lemna* populations living in different habitat show very different response to *Microcystis* toxicity. We can come to the conclusion that it is rather impossible to estimate the response of an idealised *Lemna minor* to *Microcystis* toxicity – in fact, standard tests carried out on laboratory stock cultures might either underestimate or overestimate the actual risk. In case the actual risk of a field exposure by cyanobacterial toxins is to be assessed, we can advise to use *Lemna* clones which are collected from the vicinity of the bloom and which might well represent the actual recipients. Also, our results show that *Lemna* clones coexisting with cyanobacteria might acquire some "protection", by developing resistance.

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# USING THE DEAD TO MONITOR THE LIVING: CAN ROAD KILL COUNTS DETECT TRENDS IN MAMMAL ABUNDANCE?

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**Abstract.** Counts of animal corpses resulting from road traffic collisions can give useful information on changes in animal abundance if there is a correlation between the population density of the species in neighbouring habitats and the number of road kills observed. Collection of data on mammal road casualties can be carried out by untrained volunteers; it can be collected across large areas; and it is cost effective in terms of time and expense. We carried out a study to determine if road casualty data can be used to monitor mammal abundance and distribution using one of the most commonly recorded road casualty species in the UK, the rabbit (*Oryctolagus cuniculus*), as an example. We found a direct relationship between the numbers of rabbit road casualties and the numbers living in the wider landscape. Nearly 60% of the deviance in the live rabbit density index could be explained using only rabbit road casualty, landclass group and traffic flow data. Therefore the use of road casualty data is a cost effective method of monitoring rabbits and, by implication, other species over a large area in the UK, and is a highly effective means of monitoring terrestrial mammals.

**Keywords:** *road-kill, abundance, monitoring, rabbit, oryctolagus cuniculus*

## Introduction

There are a number of conservation and management issues associated with terrestrial mammals worldwide. Some are classified as rare or in serious decline (UK examples include the common dormouse (Bright and Morris, 1996); Eurasian otter (Mason and Macdonald, 2004) and red squirrel, *Sciurus vulgaris* (Gurnell et al., 2004), and the identification of trends in the abundance of these species is critical to their conservation. Others are considered problem species and require population management as a result. Red foxes (*Vulpes vulpes*), for example, are considered pests to livestock (Greentree et al., 2000); European badgers are thought to be linked to outbreaks of tuberculosis in cattle (Krebs, 1997); and brown rats (*Rattus norvegicus*) can transmit disease to humans (Kobayashi, 2001). Furthermore there is the increasing problem of non-indigenous species (e.g., grey squirrels, *Sciurus carolinensis*, and American mink, *Neovison vison*) that have important impacts on local biodiversity (Clout and Russell, 2008; Roy et al., 2009). Only by monitoring population changes regularly and over a long period can important trends be identified and robust priorities set for conservation and management action.

Obtaining accurate estimates of mammal numbers in diverse habitats and landscapes is the key to successful monitoring (e.g. National Bat Monitoring Programme, UK (Battersby and Greenwood, 2004); Biobasis Programme in north East Greenland (Schmidt et al., 2008; Meltofte, 2006). The fact that mammals are generally elusive and crepuscular or nocturnal means that it can be extremely difficult to count actual numbers living in the wider landscape, so measures of population size are almost always based on indices, based on counts of field signs such as faeces (Bailey and Putman, 1981), tracks (Beier and Cunningham, 1996), damage caused (Bryce et al., 1997) or game bag records (Tapper, 1987).

Before an index of abundance can be used to make quantitative assessments of population trends, it must first be calibrated, ideally, against an absolute measure of abundance. However, since this is generally not practical for mammals, an alternative option is to calibrate two indices of abundance against one another. A strong association between the two indices indicates that they can be considered reliable reflections of actual abundance, particularly if the association is maintained in a variety of habitats (Krebs, 1998). For example Drennan et al. (1998) calibrated track station counts of Aberts squirrels (*Sciurus aberti*) against estimates from capture-mark-recapture methods, and in Tasmania, eastern barred bandicoot (*Perameles gunnii*) road casualty numbers have been calibrated using population data that was obtained from live trapping grids in fields adjacent to the roads surveyed (Mallick et al., 1998).

To aid in the calibration of sign surveys, the validity of pellet and dung counts in different habitat and weather conditions has been studied extensively (Taylor, 1956; Angerbjorn, 1983; Iborra and Lumaret, 1997; Palomares, 2001) and all findings suggest that differences in habitat and weather should be taken into account. Limitations on when and where data can be collected mean that such surveys may be invalid, impractical or very expensive over large areas (i.e. countrywide). New methods are therefore needed that can provide indices of abundance for very large areas, without too many constraints on data collection. In particular, they should be practical on a large scale and be suitable for use by volunteers, as this is highly cost effective (Macdonald et al., 1998).

Approximately one million wild animals are killed on roads each year in the UK alone (Underhill and Angold, 2000). Counts of road traffic casualties are a potential means of monitoring changes in the abundance of several mammal species. Collection of data on mammal road casualties can be carried out by untrained volunteers; it can be collected across large areas; and it is cost effective in terms of time and expense. However it is vital to first determine the relationship between animal density and numbers of road traffic casualties (Baker et al., 2004; Brockie et al., 2009).

The aim of the present study was to calibrate a road-kill-based index of rabbit abundance against actual numbers in the wider landscape. Rabbits were selected as the study species because they are frequently sighted and easily identifiable road casualties (Gibb and Flux, 1983; Holsbeek et al., 1999; Caro et al., 2000), and because their status as a pest species (Thompson and King, 1994) means that much work has been conducted on methods by which to assess their abundance (Trout et al., 1986; Moller et al., 1996; Poole et al., 2003).

During the 1950s an outbreak of myxomatosis in Britain reduced the rabbit population to less than 1% of its previous size (Thompson and Worden, 1956). Since that time, attenuation of the myxoma virus (Ross et al., 1989) and increased genetic resistance within the rabbit population (Ross and Sanders 1984) have together resulted in steadily increasing numbers. The UK rabbit population is now estimated at more than 37 million individuals (Harris et al., 1995) and they are an important prey species for many predators and scavengers, including foxes, stoats (*Mustela erminea*) and mink (Corbet and Harris, 1991). Monitoring of rabbit abundance across the UK is thus of considerable importance in relation to conservation and management programmes. If road casualty data can be reliably linked to actual rabbit population density then surveys of rabbit road-kill numbers could potentially be used to monitor fluctuations in the rabbit population across different regions and habitats throughout the UK, with minimal effort and cost.

## Methods

### *Road casualty counts*

During July, August and September 2002, 29 transects were surveyed by car. Each transect was approximately 97km (60 miles) in length, and was situated in one of two landclass groups as classified by the CEH (Bunce et al., 1996). Fourteen transects were surveyed in areas classified as ‘pastoral V’ and 15 in ‘arable II’. All transects included only non-urban roads (i.e., only those that did not pass through ≥3.2km of built up land), and each covered ‘A’, ’B’ and ‘Minor’ roads in the ratio 4:3:1, respectively. A record was taken of the nearest town, county, odometer reading and road number at the start and end of each transect, and at approximate 16km intervals along each. Traffic flow was measured for each road type on each transect by recording the total number of oncoming vehicles.

Upon sighting a mammal road casualty, the surveyor identified it to species level (or recorded it as unidentifiable if this was not possible) and recorded the nearest town, county, road number, and odometer reading at the casualty site. The time of each sighting was also recorded.

The 29 transects driven had also been surveyed for road casualties in a separate study conducted during the same months of the previous year (2001). The survey protocols used were identical, and hence rabbit road casualty data were directly comparable between the two years.

### *Measuring live abundance*

Surveys to determine live rabbit abundance were conducted along each road transect, in each case within eight randomly selected 1km sample squares that were spaced approximately 12km apart along the route. All sampled areas were immediately adjacent to the road. If a selected 1km square contained an urban area, a new square was selected as close to the original as possible.

The owner of the land within each survey square was asked multiple-choice questions about rabbit and predator control (*Table 1*). When the landowner could not be located, an observer answered as many of these questions as possible whilst surveying the square, and noted that the landowner had not been questioned.

***Table 1.*** *Data collected during landowner surveys*

| Questions asked | Responses recorded |
|---|---|
| Predators present? | Fox / Stoat or Weasel / Other |
| Predators controlled? | Yes / No / Unknown |
| Area of square where predators removed | None / Part / All / Unknown |
| Predator removal effort | None / Little / Seasonal / Full Time / Unknown |
| Pheasant release pens present? | Yes / No |
| Estimate of pheasant numbers released | [figure quoted] |
| Predator traps present? | Yes / No [plus details] |
| Rabbit-ting carried out? | Yes / No / Unknown |
| How frequent is rabbit control? | None / Some years / Annual / Unknown |
| Seasonality of rabbit control | None / All year / Summer / Winter / Unknown |
| Rabbit control effort | None / Little / Hotspots / Widespread / Unknown |
| Rabbit control method | [method quoted] |

Surveyors walked across each 1km square twice, in either a north-south or east-west direction, following linear habitat features (*Figure 1*). Each half of the grid transect was approximately 200m from the parallel edge of the square and located sufficiently far from the second grid transect to avoid double counting of live mammals. Linear features were followed because they provide boundaries between different habitats and/or fields and because they are potential denning and breeding areas for rabbits. In addition, foxes tend to travel along linear habitat features, and so following them facilitated the counting of predator signs. The presence/absence of rabbits droppings, live sightings, burrows and scrapes within 5m of either side of the transect line was recorded for every 100m section of it. Since the total transect distance walked within each square was 2km, this resulted in a count out of 20 for the presence of each sign.

Additionally, within each 100m segment surveyors recorded the major type of land-use, the presence/absence of roads and water features, and the presence/absence of predator signs within 5m of the transect line.

The timing of the survey (July, August and September) was selected so that it coincided with the Mammals on Roads survey (Battersby, 2005), and so that results from the two were comparable. However, this is not an ideal seasonal period for rabbit sign surveying because it tends to be associated with high variance in rabbit numbers and with increased vegetation height and density (which make field signs harder to detect).

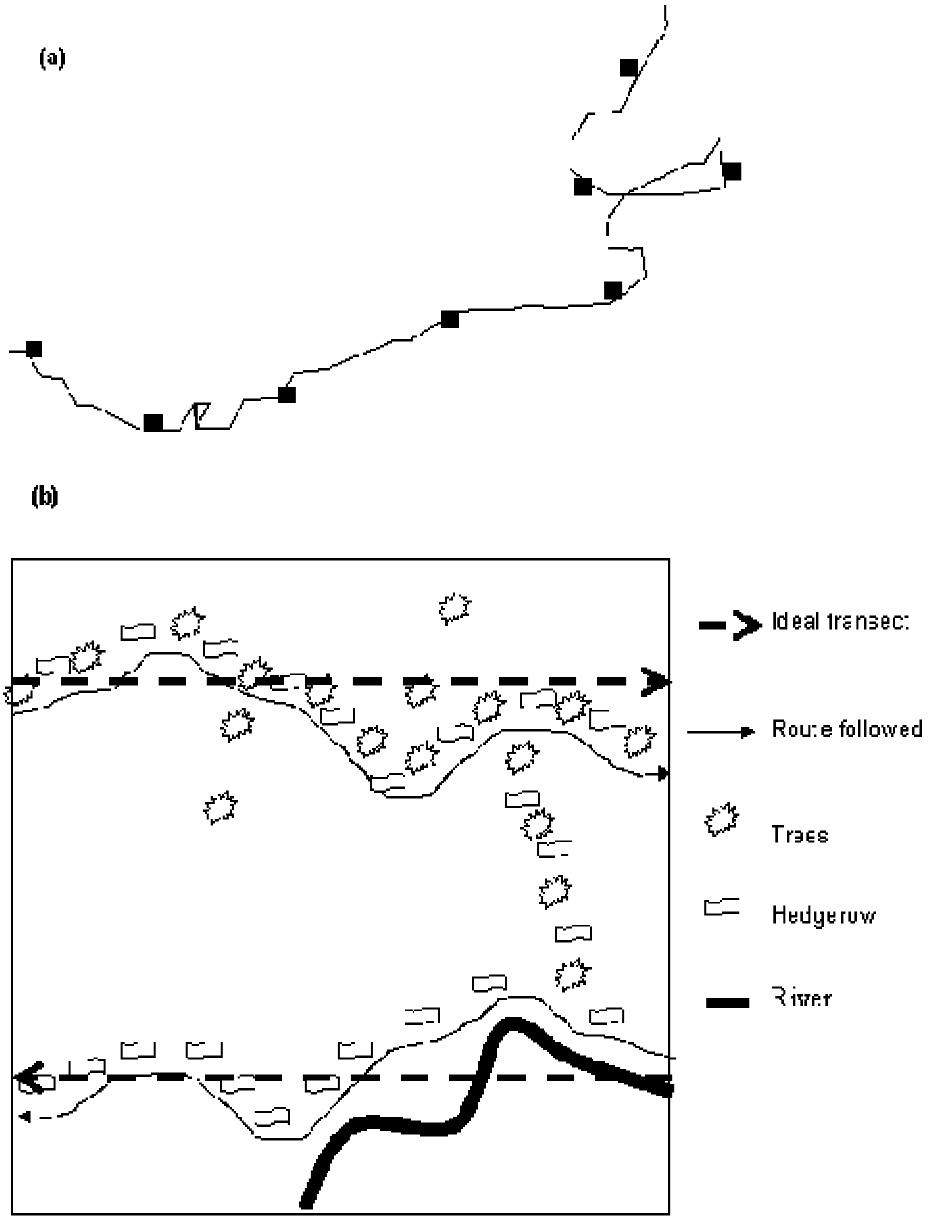

***Figure 1.*** *Examples of (a) a road transect (black line) with 1km squares (black squares) surveyed along the road transect route, and (b) ideal and actual 1km grid square transects*

### *Data analysis*

As it was not possible to ensure that all road transects were identical in length, counts of rabbit road casualties were standardised as the number per 100km of road surveyed.

For each 1km square surveyed, counts of rabbits and their signs were used to calculate a rabbit density index ($y$) using a previously calibrated regression equation (MAFF, 1982); *Equation 1*). This equation was previously produced following calibrations of rabbit sightings to live rabbit abundance (Poole et al., 2003), and hence has been validated as a method for accurately determining rabbit density.

For each 100m segment of every 2km grid transect, counts of the following were recorded: rabbits (V1) and hares (V2 ≈ 0.25), both living and dead; holes/forms (V3); scrapes/digs (V4); tracks/runs (V5 ≈ 8.70); dropping patches (V6); hair/fleck

(V7≈ 0.76); grazing/grazing damage (V8 ≈ 2.3); barking (V9 ≈ 0.39). The values recorded for each parameter in each 1km square were substituted into *Equation 1* to calculate a density index for live rabbit abundance within each survey area.

$$y = K + 0.531\ V1 + 0.120\ V12 + 0.455\ V2 + 0.078\ V3 + 0.351\ V4 - 0.323\ V5 + 0.387\ V6 + 0.508\ V7 - 0.129\ V8 - 0.404\ V9 \qquad \text{(Eq 1.)}$$

Where equation parameters were unknown, mean data for England and Wales from a survey in 1982 (MAFF, 1982) were substituted (shown in brackets above). Inclusion of a constant (*K*) in the equation ensures that all indices are positive.

A generalised linear model (GLM) with a Poisson error structure and $\log_e$ link function was used to model the relationship between live rabbit density, rabbit road casualties and a number of habitat-related variables. The response variable was the mean live rabbit density index for each road transect, calculated from the corresponding eight survey squares. were used as explanatory variables. A total of 24 potential explanatory variables were considered, including habitat, rabbit/predator-control variables, oncoming traffic count and the number of rabbit road casualties per 100km of transect driven. Values for edge habitat and land-use were recorded as percentages and hence were subject to the unit sum constraint. To ensure independence, the data were log-ratio-transformed before analysis (Aebischer et al., 1993). The most parsimonious model was obtained via stepwise removal of non-significant explanatory variables by $\chi^2$ deletion (Crawley, 1993).

In order to determine whether the GLM could accurately predict inter-annual changes in rabbit road casualty numbers, road-kill counts were compared for the years 2001 and 2002. Data used in this analysis were collected on the same transect routes, in the same seasonal period and using identical recording methods.

## Results

On average, more rabbit road casualties were sighted per 100km in the arable II landclass group than in the pastoral V landclass group (Mann Whitney test: $U = 58.0$, $P = 0.041$, $n_1 = 15$, $n_2 = 14$; *Figure 2*).

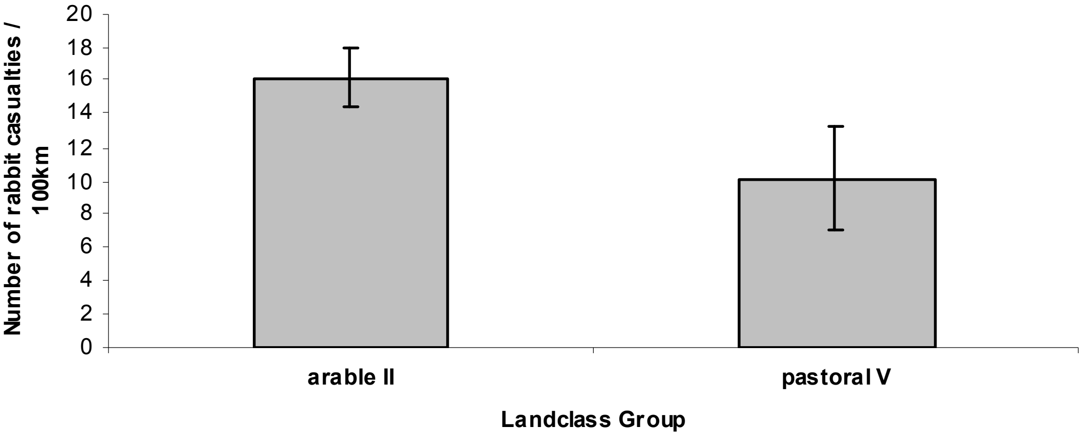

***Figure 2.*** *The number of rabbit road casualties per 100km in arable II and pastoral V landclass groups (mean ± SE)*

Overall, the live rabbit density index tended to be higher in arable II areas than in pastoral V. The mean live rabbit density in arable II areas was 10.45 (SE = 1.040, n = 120), compared to 6.45 in pastoral V areas (SE = 1.165, n = 111), and this difference was significant (Mann Whitney: U = 3740.5, $n_1$ = 120, $n_2$ = 111, P = <0.001; *Figure 3*).

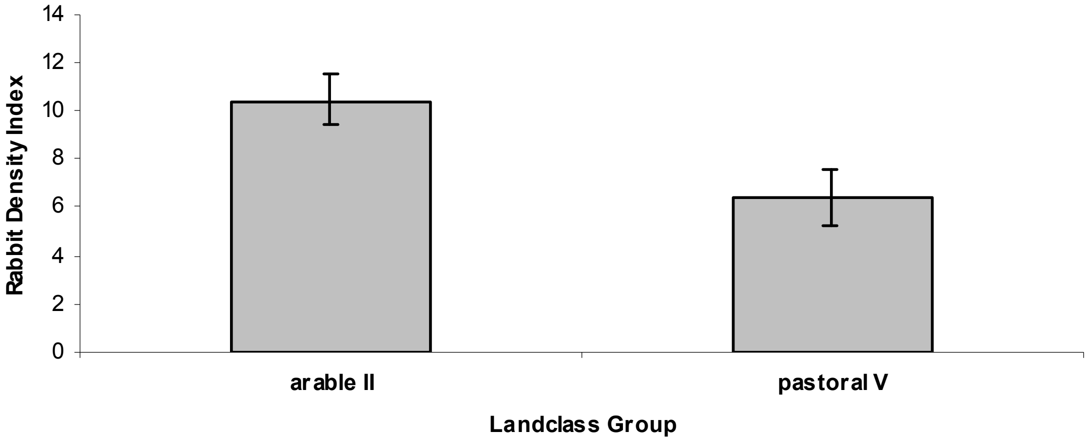

***Figure 3.*** *Live rabbit density indices calculated for survey areas classified as arable II and pastoral V (mean ± SE)*

During simplification of the generalised linear model, variables removed included all those relating to rabbit and predator control, pheasant management and habitat. Three explanatory variables and two interactions remained in the minimum adequate model, which explained 57.76 % of the deviance in the live rabbit density index (P < 0.001, df = 5, *n* = 28; *Table 2*).

***Table 2.*** *Generalised linear model of live rabbit density against traffic flow, rabbit road casualties per 100km and landclass group*

| Variable | Parameter Estimate | SE | p | Deviance | % deviance explained |
|---|---|---|---|---|---|
| Constant | 2.566 | 0.456 | - | - | - |
| Traf_km | -0.0613 | 0.0757 | <0.001 | 29.458 | 23.14 |
| Rab_km | 0.32 | 1.68 | 0.001 | 2.044 | 1.61 |
| LCG arable II | 0 | - | - | - | - |
| LCG pastoral V | -0.460 | 0.519 | 0.387 | 1.570 | 1.23 |
| Traf_km & LCG | -0.2610 | 0.0967 | 0.012 | 14.845 | 11.66 |
| Rab_km & LCG | 8.53 | 2.41 | 0.002 | 25.614 | 20.12 |
| **Total Deviance Explained** | | | | | 57.76 |

The majority of the variation in live rabbit density between the 1km survey squares was explained by traffic flow (Traf_km = 23.14 %), and the interaction between the number of rabbit road casualties per 100km and the landclass group (Rab_km.LCG = 20.12 %). This indicates that the relationship between live rabbit density and rabbit road casualty numbers can be explained using the traffic flow and landclass group variables (*Figure 4a*).

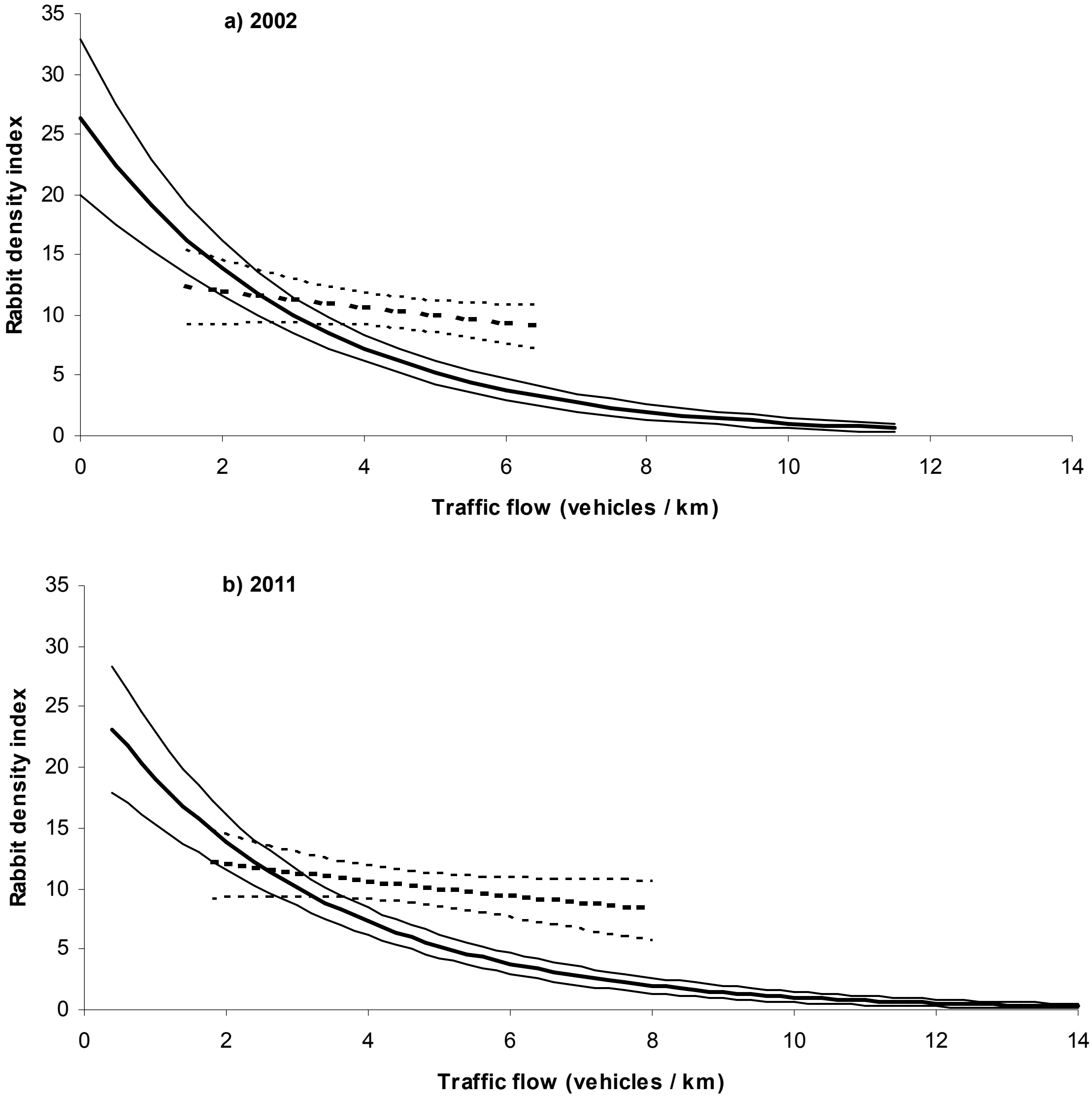

*Figure 4. Relationships between traffic flow and live rabbit density indices in arable II (dotted lines) and pastoral V (solid lines) landclass groups (one outlier excluded from calculations): (a) shows mean traffic flows recorded in 2002 (b) shows predicted traffic flow in 2011 (bold lines = mean values, light lines = ±SE)*

With regards to both landclass groups, the model indicates a negative correlation between live density and traffic flow (Traf_km parameter estimate = - 0.0613; *Figure 4a*), and a positive correlation between live density and road casualty numbers (Rab_km estimate = 0.32). Road casualty counts and traffic flow showed greater variance within pastoral V land, and therefore the predictions of the model (*Figures 5 and 6*) are given only between the parameters that would be found in the relevant landclass group.

Based on the results of this study, a regression equation (*Equation 2*) was devised to quantitatively explain the relationship between rabbit density and the three key variables, that is, traffic flow on neighbouring roads, road casualty counts, and landclass group. **A** is the live rabbit density index in a 1km square; **B** is the number of vehicles counted per 100km on the respective road transect; **C** is the number of rabbit road casualties per 100km of road surveyed on the respective transect; and **D** is the landclass group (0 = ‘arable II’ land; 1 = ‘pastoral V’ land).

$$A = 2.566 + (-0.0613B) + (0.32C) + (-0.460D) + (-0.2610(BD))+(8.53(CD)) \quad \text{(Eq. 2)}$$

Across the UK as a whole, traffic flow was approximately three percent higher in the third quarter of 2002 than in the same period in 2001 (DTLR, 2003). Since traffic flow and rabbit density were found to be negatively related (*Figure 6*), our model would thus predict a decrease in rabbit road casualties from 2001 to 2002 (see *Figures 5 and 6*). There was, however, no significant change in the number of rabbit road casualties per 100km in either of the two landclass groups between the two years (Mann Whitney test: arable II: U = 109.0, $n_1$ = 15, $n_2$ = 15, P = 0.902; pastoral V: U = 89.0, $n_1$ = 14, $n_2$ = 14, P = 0.701).

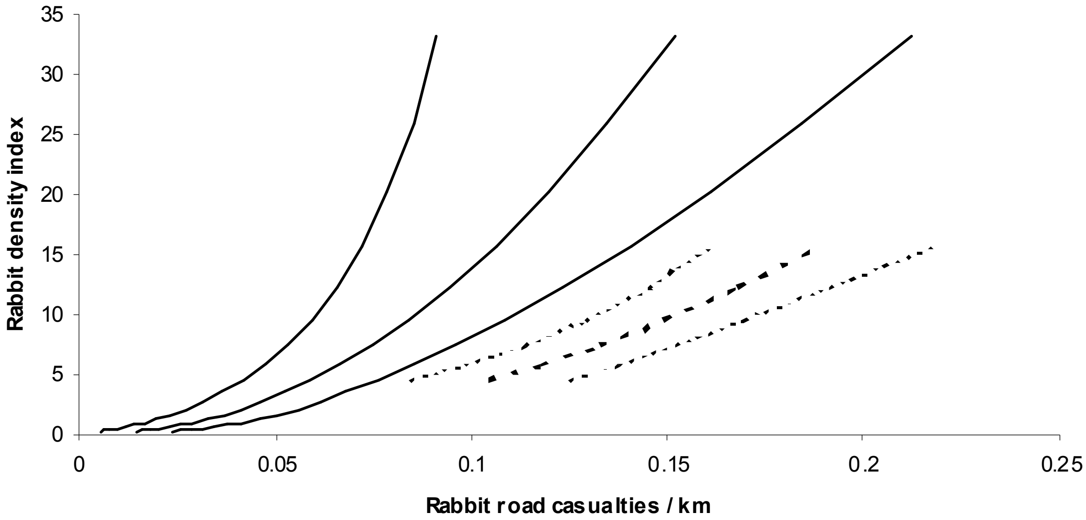

***Figure 5.*** *Predicted relationships between rabbit density and road casualty counts in arable II (dotted line) and pastoral V (solid line) landclass groups (mean = heavy lines; ±SE = light lines)*

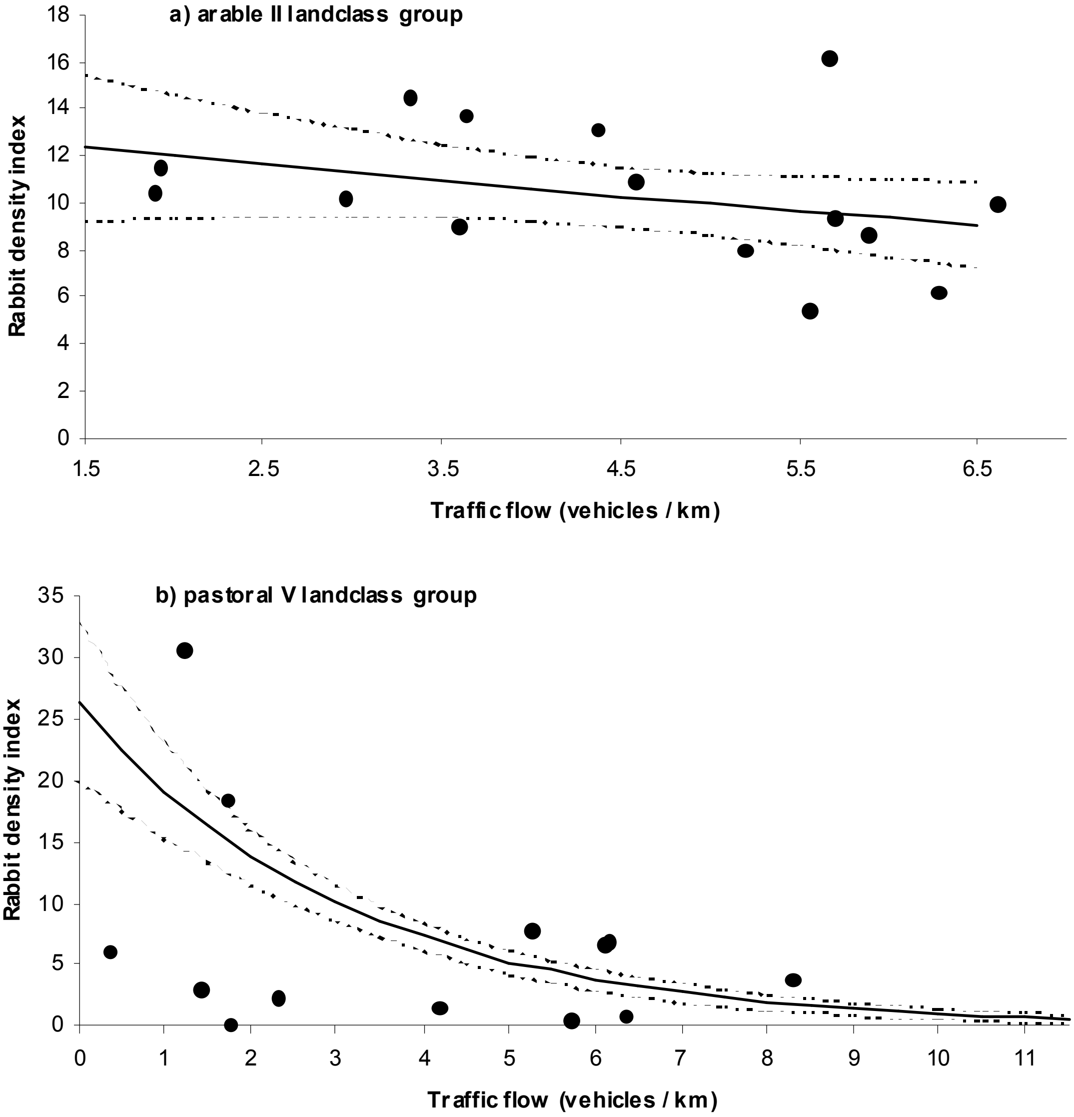

***Figure 6.*** *Recorded (dots) and predicted (solid lines) live rabbit density indices in relation to changing traffic flow in (a) arable II and (b) pastoral V landclass groups (broken lines = ±SE)*

## Discussion

This study demonstrates a strong relationship between rabbit road casualty numbers and the actual abundance of rabbits living in adjacent areas. Furthermore it shows that, so long as a small number of other variables are taken into consideration (namely traffic flow and landclass group), an index of abundance based on road-kill-counts (e.g., number of casualties per 100km) could be employed to monitor rabbit populations. Such an index of abundance based on counts of road casualties would be easy, cheap and practical to obtain across wide geographic areas.

Counting the numbers of rabbit road casualties as one travels in a vehicle is considerably cheaper than other methods of calculating indices of abundance that require trained personnel, and a larger area can be covered in the time available. The timing of the survey is also not restricted to times of day when rabbits are active, as are surveys utilising live counts which are usually limited to dawn or dusk surveys (Moller et al., 1996; Poole et al., 2003).

The number of rabbit road casualties found in both arable II and pastoral V landclass groups was high compared to indices recorded for rabbits in other studies (Sleeman et al., 1985). There was great variability in the numbers of rabbit road casualties per 100km recorded on each transect, however there were still significantly more rabbit road casualties recorded in the arable II than the pastoral V landclass group. The larger range in both the number of rabbits per 100km and the rabbit density index in the pastoral landclass group in comparison to the arable landclass group suggests that the arable landclass group as a whole is a more favourable environment for rabbits, and that there are only patches of suitable environment in the pastoral landclass group. This may account for the greater effect an increase in traffic flow is predicted to have on rabbit densities in the pastoral V landclass group than in the arable II landclass group (*Fig 3 and 4*). In the pastoral V landclass group rabbits may be more highly dispersed with fragmented populations, therefore any changes in the environment such as increased traffic flow may have a greater affect upon the localised rabbit populations (Forys and Humphrey, 1996).

The model presented here predicts a decrease in the live rabbit density index with increasing traffic flow. Similar conclusions were drawn from a study on frogs and toads in Ottawa Canada (Fahrig et al., 1995) where a negative relationship was found between the number of live amphibians and traffic flow.

Between 2001 and 2002 the traffic flow on UK roads increased by approximately 3% (DTLGR, 2003), however no change in the numbers of rabbit road casualties were recorded per 100km travelled in the two landclass groups in this chapter. This lack of correlation between the predictions of the model and the numbers of rabbit road casualties recorded is likely to be because only two years of data was available and it is unlikely that such a small timescale would show trends. Annual rabbit abundance fluctuates quite widely, consequently many years of data are required to show trends over time and also, rabbits may be continuing to increase their numbers since the population crash in the 1950s due to myxomatosis (Ross and Sanders, 1984).

The live rabbit density index was originally calibrated using counts of rabbits by the min of ag and fisheries using data from England and Wales. This explained 83-91% of the variance in live rabbit numbers (MAFF, 1982). This was validated by Poole et al. (2003) who showed that the relationship between counts of rabbits and live rabbit abundance still stands. It is therefore not necessary to recalibrate this equation for the landclass groups not studied here. However, the model presented here is based on data collected from two landclass groups (arable II and pastoral V) and extrapolation to include other land-types may not be justified, particularly since landclass was an important explanatory variable in the model. Factors such as predator densities and climate are likely to vary between landclass groups and may affect the relationship between rabbit road casualty rates and live abundance. Therefore, further calibration studies within the remaining five landclass groups will be required. Extension of this work should include calibration of road-kill indices for other UK mammal species using similar methods. Additional factors that may influence road casualty rates, including local road density and traffic speed should be considered when using such work to generate models to predict abundance from road-kill numbers.

The months of July and August 2001 were much wetter than the same period in 2002 (MetOffice, 2003). Therefore, since higher rainfall levels would be expected to increase the rate of disintegration of road casualties (de Carvalho and Linhares, 2001), it is possible that the higher casualty numbers recorded in the drier year of 2002 reflected climatic factors rather than rabbit population density.

The amount of rainfall may have affected the collection of the signs data as faecal pellets may be washed away during periods of heavy rain (Iborra and Lumaret, 1997; Palomares, 2001). To overcome this, surveys were not carried out on days when there was heavy rain; however there is a possibility that more pellets were counted in areas that had been drier for longer periods than others. The habitat in the surveyed 1km squares may have also influenced the number of faecal pellets recorded, as some squares had tall grasses or arable crops which were difficult to see through to ground level. Both these factors could have reduced the final live rabbit density estimates for the areas concerned.

Using traffic flow, numbers of rabbit road casualties and landclass group 57.75% of the deviance in the live rabbit density index could be explained using the model described here. The model could therefore be used over both time and large areas to monitor changes in the index of abundance of rabbits. If the limitations of the data are considered, the model could also be used as a method of calculating absolute abundance of rabbits living in the arable II and pastoral V landclass groups on an annual basis.

The model presented here fits well to both high and medium rabbit density areas. Road casualty data has been shown to correlate with the densities of other mammal species such as eastern barred bandicoot, racoons (*Procyon lotor)* and foxes (Mallick et al., 1998; Gehrt et al., 2002). This method should therefore be investigated further by calibrating other live populations of mammal species to the numbers of road casualties.

Road casualties of most mammal species are highly visible and easily recognisable and therefore are a ready source of data to be used for monitoring mammals. One of the main benefits of using road casualty data rather than other measures of rabbit abundance is the speed with which the data can be collected. Moller et al. (1996) found that using daytime transect counts of rabbits to get an index of abundance within 20% accuracy would require 20 counts, and for 10% precision would need 80-90 transect counts. It is not practical to carry out transect counts of this nature over large areas, especially if 20 or more counts are required at each site. We found that the numbers of rabbit road casualties can be directly linked to the numbers living in the wider landscape using only rabbit road casualty, landclass group and traffic flow data. There is therefore a huge potential for monitoring rabbits and by implication other species over a large area by using data collected in this manner.

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# IMPACTS OF CLIMATE CHANGE ON LEPIDOPTERA SPECIES AND COMMUNITIES

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**Abstract.** In this review, the impacts of climate change on Lepidoptera species and communities are summarized, regarding already registered changes in case of individual species and assemblies, and possible future effects. These include changes in abundance, distribution ranges (altitude above sea level, geographical distribution), phenology (earlier or later flying, number of generations per year). The paper also contains a short description of the observed impacts of single factors and conditions (temperature, atmospheric $CO_2$ concentration, drought, predators and parasitoids, UV-B radiation) affecting the life of moths and butterflies, and recorded monitoring results of changes in the Lepidoptera communities of some observed areas. The review is closed with some theoretical considerations concerning the characteristics of "winner" species and also the features and conditions needed for a successful invasion, conquest of new territories.

**Keywords:** *butterflies, moths, abundance, distribution, phenology*

## Introduction

Changes in climatic conditions greatly influence the development and range of insects. According to current estimates, the annual average temperature of the Earth increases with 1°C by 2025 and the probable rise in temperature by the end of the century is expected to reach 3°C (IPCC, 2007). Additionally, forecasts point to higher atmospheric $CO_2$ levels and changing patterns of UV radiation. The impacts of climate change must be studied from various aspects, such as forestry or agriculture (e.g.: Spencer et al., 2009; Ladányi and Horváth, 2010), human medicine (Kearney et al., 2009; Kiritani, 2006; Takken and Knolsm, 2007), phytogeography (Uniyal and Uniyal, 2009) etc. This vast work requires the interdisciplinal cooperation of research from many different fields (Hilker and Westerhoff, 2007; Strand, 2000), and it must be noted that the effects of human interference, ecosystem-climate control and feedbacks should also be studied (Drégelyi-Kiss et al., 2008).

The order of Lepidoptera is the fourth largest order of insects in Europe with 8470 species living in the area of 35 countries (Karsholt and Razowski, 1996). Due to this species richness, the taxon list of a given area provides an extremely detailed view of the environmental conditions of the site, and this is also true vice versa: butterflies and moths show a sensitive reaction to the change of abiotic factors. Thus, butterflies and moths can be considered as good indicator species in monitoring climate change (Ronkay, 2004).

Researchers started to study the potential effects of climate change on Lepidoptera in the early 1990's (Hedden, 1989; Peters, 1990; Watt et al., 1990; Dennis and Shreeve, 1991; Porter et al., 1991; Dennis, 1993; Williams and Liebhold, 1995; Harrington and Woiwod, 1995; Harrington and Stork, 1995). According to Woiwod (1997), climate change has three main impacts on Lepidoptera species:

1. Changes in abundance
2. Changes in range, distribution or area
3. Changes in phenology

It is also necessary to study the potential interactions of these effects. For instance, there is a well documented relation between abundance and distribution, since (temperate) species with the largest number of individuals generally have a large area as well (Lawton, 1995). It is also understood that phenological changes can also affect abundance (via the synchronization of predators and parasitoids with Lepidoptera as prey organisms and also the synchronization of larvae and their host plants (Watt and Woiwod, 1999), or the appearance of one or more extra generations in a year). According to Porter et al. (1991) and Logan et al. (2003) the following possible impacts can be expected in the near future: increasing rate of overwintering, prolonged development stage, changes in the synchronization of host plant and pest, changes in interspecific interactions including modifications in the relation of Lepidoptera and their natural enemies, increasing severity of invasions of migrant pests, changes in the frequency of damages due to gradation and general decrease in biodiversity.

According to Bale et al. (2002) the following problems have to be resolved to clarify the direct impacts of climate change on Lepidoptera:

1. Lepidoptera species already encounter and cope with vast temporal and spatial variability among natural conditions, therefore their reactions to climate change can significantly differ depending on their habitat location (e.g. altitude), feeding regime (e.g. poliphagous or monophagous) and host plant (good or inferior quality) not to mention other biotic interactions. It is hard to decide which effects can be attributed definitely to climate change, and often it is very complicated to separate direct and indirect effects.

2. Even some single factors can often have quite different effects depending on the extent of change and the affected species (e.g. increasing temperature can be harmful for the overwintering of some species by decreasing the snow cover and weakening the synchronization with the host plant, but in a warmer climate the development of some species can accelerate and some species will be able to colonize new areas (Ayres and Lombardero, 2000).

3. The niche width of many species is probably determined, at least in part, by temperature regime. Although we often know which species replace each other along climatic gradients, there is much less information on why one species replaces another.

## Changes in abundance: decreasing or increasing number of individuals

There are many problems to cope with in monitoring abundance changes caused by climate change. First of all, appropriate monitoring requires long-term data series since the individual numbers of Lepidoptera species can show great variability in short term and that can easily lead to misinterpreted short-term trends (Woiwod, 1991). The other problem is that even if we had long-term data, the observed changes can not be attributed definitely to climate change because the size of Lepidoptera populations is affected by many factors. These problems can only be overcome by very thorough, long-term study of some species or communities, but such data series are quite rare (Woiwod, 1997).

### *Decreasing number of individuals*

Since the 1960's the Rothamsted Insect Survey have been collecting light-trap data over 430 points of Great Britain (the number of traps varies from year to year because many light-traps have been operated by volunteers). More than 730 Macrolepidoptera species has been caught since the system started operation, and more than 10 million data have been recorded. In 1995, Pollard et al. predicted a further increasing trend in the area and abundance of the prevalent Lepidoptera species of the British Isles, since the results of the study showed that higher summer temperatures increase the number of Lepidoptera individuals. The results calculated with regression models by Roy et al. (2001) were quite similar: hot summer increased the number of individuals in 28 of the 31 studied Lepidoptera species, especially when the last year was rainy and the current year dry. Forecasts predict increasing abundance of most species, only the *Pieris brassicae* was seen declining. In contrast with these results, Conrad et al. (2004) conducted a TRIM analysis (Pannekoek and Van Strien, 2001; Van Strien et al, 2001) on a 35-year time series of 338 Macrolepidoptera species and showed that the individual numbers of 54°% of the species had decreased. 24°% of the species showed a stable population and strong increase in the individual numbers was shown by only 6 species. It was also stated that most species feeding on deciduous trees, shrubs, grass and low growing plants showed decreasing populations, while most lichen-feeders and conifer-feeders were increasing. The number of individuals of most species overwintering in egg stage decreased and fared worse than species overwintering as larvae or pupae, which are also mostly decreasing. Species overwintering as adults, on average, have increased, but the number of species was small and quite variable. The timing of the adult flight period showed an interesting relationship with abundance trends. Only those species which fly through the autumn, winter and spring showed populations which are, on average, increasing. Species which fly at other times of the year showed average (declining) population trends with species that fly only in the autumn or the summer and autumn having the greatest average decrease. In a later study, Conrad et al. (2006) analysed the Rothamsted Insect Survey (RIS) data for 337 species, each of which was represented by more than 500 individuals captured over the 35-year sampling period (1968–2002). Half of the studied species experienced a 10-year decline of at least 12%, and results suggest that British macro-moths have undergone declines at least as severe as British butterflies: the percentage of moth species declining (66%) was similar to the proportion of butterflies declining (71%) and greater than the proportion of birds (54%) or plants declining (28%).

Conrad et al. (2002) studied the population dynamics of *Arctia caja* on the British Isles between 1968 and 1999. It was shown that the average number of catches (4.2) rapidly fell after 1983 to 3.0 which is a 28°% decline. Many sources reported a declining number of individuals (Rotschild, 2000; Waring, 2000; Conrad et al., 2001) and later the spatial changes were also analysed by the SADIE (Spatial Analysis by Distance Indices) method (Conrad et al., 2006). Three different stages were observed in the spatial distribution: 1969-78 spatial distribution with a strong structure, 1979-90 almost stochastic distribution, and 1991-99 strong structure again with a powerful decrease in abundance). Conrad et al. (2003) pointed out that besides winter precipitation and spring temperatures the abundance of the species is also affected by the EA-index (East Atlantic teleconnection pattern). No relation was found however between the population dynamics and the NAO (North-Atlantic Oscillation) index significantly determining the winter climate of North Europe. Since the value of EA-index has been considerably growing in the last 5 decades, further decrease in the abundance of *Arctia caja* can be prognosticated according to the model mentioned above.

Conrad et al. (2003) analysed the Rothamsted Insect Survey (RIS) data between 1968 and 2003 for those species which were represented by at least 500 individuals. It was pointed out that the abundance of 71 species decreased in an extent that these species must be regarded as endangered according to IUCN criteria. Since in the case of these species we do not know about long-range migration, it is quite improbable that these decreasing populations might be "saved" by individuals from the continent.

Roberts et al. (1993) and Gray (1994) observed that the populations of *Lymantria dispar* can collapse in the United States after winters where the number of warm days was high. The probable reason for this is that warm temperature induces faster rate of metabolism in the individuals and the nutrient reserves needed for overwintering will be exhausted earlier.

### *Increasing number of individuals*

Salama et al. (2007) studied Macro-moths caught in a Rothamsted trap, operating from 1968 to 2003 as part of the Rothamsted Insect Survey. These data were used to investigate the long-term population trends of moth populations on East Loch Lomondside, Scotland. In total, 367 species of macro moths were recorded during this study. Over the 35 years of this study, an increase was recorded both in the overall number of individuals and moth diversity (Fischer's α: 19°% growth compared to 1968). Four of the most consistently abundant species, collectively constituting 27% of the average annual catch, were subjected to more detailed analysis. The three species that emerge during the summer months *Eulithis populata* (the northern spinach), *Hydriomena furcata* (july highflier) and *Idaea biselata* (the small fan-footed wave) became more abundant throughout the study period, (although for the latter species not significantly so). For *Eulithis populata* and *Idaea biselata* their emergence time became earlier, over the study period and in *Eulithis populata* and *Hydriomena furcata*, the flight duration also became longer. In contrast, the species that emerges as an adult during autumn and winter, *Epirrita dilutata* (the november moth) did not exhibit a significant change in abundance, emergence date or flight duration in this study.

## Changes in area (changes in latitude/altitude of dispersion)

The number of individuals per unit area (density) of Lepidoptera species decreases with higher geographical latitude – Gaston and Williams (1996), Wilf and Labandeira (1999) – and a similar trend can be observed in the case of increasing altitude over sea level. It can be expected that as a result of warming, the majority of Lepidoptera species living in the temperate zone will colonize areas with higher altitude and latitude (Hickling et al., 2006), although this can be limited by the mobility of the species and natural barriers (e.g. host-plant availability, sea, high mountains).

## *Horizontal changes in the range of distribution*

Fossil findings show that insects also had to react to climatic changes in the past (Coope, 1970, 1987, 1995; McGavin, 1994; Ashworth, 1997). Wilf and Labandeira (1999) observed increased damage intensity, frequency and diversity on late Paleocene host plant leaves in Southwest Wyoming. These observations prove that damage intensity and diversity of phytophagous insects living on the same latitude increased as a consequence of warming. Change in precipitation patterns can also heavily affect the change in area, though this impact is realized indirectly (via host plants and natural enemies), and it is very difficult to prognosticate. According to Bale et al. (2002), species which currently have wide latitudinal ranges, already encounter considerable temperature variation and are, in a sense, preadapted to cope with temperature change. Loss of habitats or changes in the microclimate of the habitats are severely affecting factors, but these are very difficult to attribute definitely to climate change since the role of human activity seems to be more determining. (E.g. according to Asher (2001), 70 % of semi-natural habitats have been lost in the British Isles since 1940 due to the intensification of agriculture.) Studying 46 butterfly species in Great Britain, Warren et al. (2001) showed a marked difference between the distribution and abundance of habitat specialist and habitat generalist species: generally area expansion was observed by the generalists and the specialist species showed contraction. Consequently those species whose distribution is primarily limited by climatic conditions (like *Polygonia c-album,* which appears 220 km to the North compared to 1970) can be expected to further expand their area. Specialist species with low mobility (like *Plebejus argus*) will be limited in their colonization of new areas with suitable climate by the great distances between their isolated habitats. Fox et al. (2003) reports a dramatic drop in the number of individuals of low-mobility, specialist species since 1970 (*Argynnis adippe, Leptidea sinapis, Boloria euphrosyne, Euphydryas aurinia, Coenonympha tullia, Plebeius argus, Hamearis lucina, Erynnis tages, Boloria selene*). Only one habitat specialist species with area expansion has been found (*Limenitis camilla*). Thomas et al. (2004) studied the distributional changes of 58 Lepidoptera species endemic for Great Britain. Results showed that 71 % of the species suffered area contraction and in the last four decades these species became extinct from 13 % of the 10 km$^2$ quadrates inhabitated in 1970. Two species become entirely extinct and the extent of area loss significantly exceeded that of plants and birds recorded in the British Isles.

Expansion towards higher latitudes and altitudes are supported by the observations below:

Parmesan (1996) monitored the distribution of *Euphydryas editha* on the ground of historical data sets at 151 sites in Canada, US and Mexico. Results showed that the species contracted from the southern border of its area and the decrease in individual numbers in low altitudes significantly exceeded that in higher altitudes. This was the first survey which studied the abundance of a Lepidoptera species on the full area of its distribution.

Parmesan et al. (1999) studied the distributional changes of 35 Lepidoptera species in Europe (none of them migratory species). According to their results, 63 % of the species expanded towards North (the Northern border of distribution moved 35-240 km to the North) and only 3 % of the species showed a southward expansion.

Parmesan and Yohe (2003) monitored 1700 taxa (insects, mammals, fish species, trees, birds and lichens) and observed that their distribution expanded annually 6,1 km to the North or vertically 6,1 m to higher altitude and events in spring (first singing of birds, arrival of migrant birds, appearance of butterflies, choruses and spawning in amphibians, shooting and flowering of plants) will commence 2.3 days earlier per decade.

Saarinen et al. (2003) reports northward shift of 7 butterfly species based on data between 1991 and 2000 of the National Butterfly Recording Scheme in Finland. Mitikka et al. (2008) constructed a model for the range shift of the map butterfly *(Araschnia levana)* in Finland for the period of 2000-2004. A significant relationship between annual maximum dispersal distance of the species and late summer temperature was detected, and empirical data showed that between 2000 and 2004 the range of the species moved continuously towards the North, especially in East Finland. Parmesan (2006) also concludes that primarily warm summers induced the northward shift of many North-European Lepidoptera species.

Kuchlein and Donner (1993) reported that *Phyllonorycter platani* (Gracillariidae) was first caught in 1965 in the Netherlands but since then it became the most common species in the country. Stigter and Frankenhuyzen (1991) reported that since the first catch (1985) of *Phyllonorycter leucographella* in the Netherlands, the species spread rapidly in the country. According to Vos and Zumkehr (1995), *Omphaloscelis lunosa* (Noctuidae) was caught only in the Southern part of the Netherlands until 1980, but by 1994 the species became common in the Northern part of the country and even appeared in the relatively cool Frisian Islands (number of annual catches increased from 10 to 260).

Kiritani (2006) cited reports on the northward range expansions of more than 50 butterfly species in Japan, and establishment of permanent populations of 10 butterfly species which had been considered to be migrant species before. Kiritani attributes the increasing abundance of *Helicoverpa armigera* and *Trichoplusia ni* to climate change, however his results show that global warming is more favourable for the natural enemies (with the exception of spiders) of the pests, since they are able to develop more generations annually than their preys.

Hill et al. (1999) forecast that *Pararge aegeria* will colonize all suitable habitats in the next 50 years in the British Isles.

According to one of the theories of theoretical ecology, when the climate of a given area changes, then the areas of the species living in this area and limited by climate in their distribution will also change. This theory was tested by Hellmann et al. (2008) by comparing the northward shift of a small-bodied specialist (*Erynnis propertius*) and a large-bodied generalist (*Papilio zelicaon*). Both species showed a positive reaction to warming and both species can expand their areas northwards, but in line with the expectations, the abundance and number of larvae of the large-bodied generalist showed a more powerful growth at the northern edge of distribution than the small-bodied specialist.

According to Thomas et al. (2001) both evolutionary and ecological processes react to climate change, thus playing a role in area shifts. In their study, 4 Lepidoptera species continuously increasing their area in the British Isles during the past 20 years were observed. It was observed that two species *(Hesperia comma, Aricia agestis)* colonized new types of habitats. *Aricia agestis* changed its main host plant (among experimental circumstances females participating in the colonization laid two-third of their eggs on *Geranium molle* which is more prevalent on the newly colonized territories, and only one-third of the eggs was laid on the former main host, *Helianthemum chamaecistus*).

Braschler and Hill (2007) pointed out alternative use of host plants by the British Isles populations of the (polyphagous) *Polygonia c-album* species (*Ulmus glabra* and *Urtica dioica* instead of the formerly consumed *Humulus lupulus*). Nylin et al. (2009) compared the populations of *Polygonia c-album* in Norway, Sweden, Great-Britain, Belgium and Spain, and found that the host plant preference shifted from *Salix caprea* to *Urtica dioica* facilitating rapid development (more generations per year), in regions where the host plant was available.

Species without diapause or overwintering in active form the rate of overwintering individuals will grow as winters become warmer. With the increase in winter average temperatures these species can shift northwards and higher altitudes.

The quiescence of most temperate Lepidoptera species is in winter which is spent in inactive pupa or larva form accompanied by slowing metabolism in order to increase frost tolerance. In most of the species the temperature required to finish winter diapause is lower than the temperature needed for the development, active stage and these reflect the summer and winter temperatures of the given area. Geographical range of some species is limited by the cold tolerance of their winter form, therefore their northern limit of distribution follows the isotherm of the winter minimum temperatures (Uvarov, 1931; Danilevskii, 1965). In case of a warming climate it is expected that the northern distribution of these species will be expanded.

As a consequence of warm winters, at the southern distribution border of some species the temperature will exceed the threshold required for the induction of diapause, so the southern border will shift northwards. For example the rate of survival of individuals in quiescence of *Inachis io* and *Aglais urticae* decreases significantly at 10°C compared to that at 2°C (Pullin and Bale, 1989), and the decrease in the rate is more marked in case of *Inachis io*. Therefore the southern range of *Inachis io* follows the isotherm of the 10°C mean January temperature, and the southern boundary of distribution of *Aglais urticae* can be found at a bit lower latitude (Bryant et al., 1997). If winters become warmer, the southern border of distribution will shift northwards, but since *Aglais urticae* is already present at the northernmost point of Norway (Nordkapp, North Cape, 71°10’ N, 24°11’ E) and can not move to the North because of the sea, its area will certainly decrease. Hill et al. (2002) monitored 51 Lepidoptera species in the British Isles and observed that 11 out of 46 southerly distributed species have expanded in the northern part of their distributions. For a subset of 35 species, they modelled the role of climate in limiting current European distributions and predicted potential future distributions for the period 2070-2099. Results show that most northerly distributed species will have little opportunity to expand northwards and will disappear from areas in the south, resulting in reduced range sizes. Southerly distributed species will have the potential to shift northwards, resulting in similar or increased range sizes. However, 30 out of 35 study species have failed to track recent climate changes because of lack of suitable habitat.

### *Vertical changes in the range of distribution*

Hodar and Zamora (2004) monitored the damages of the polyphagous *Thaumatopoea pityocampa* between 1992-2001 and found that the pest damaged plants at higher and higher altitudes. The vertical distribution of the species is greatly affected by winter temperatures determining the development of larvae. While the upper limit of distribution was around 1500 m above sea level, the species damaged mainly the *Pinus nigra* pine species. Following the very warm winters of 1997 and 1998 the moth damaged plants at 2000-2100 m above sea level, where populations of the boreal relict *Pinus sylvestris nevadensis* can be found. This raises conservation concerns, especially because it is expected that the intensity and frequency of damages and defoliation will both increase due to warmer winters.

Larval development period can become shorter in species where the development/temperature reaction curve grows rapidly, like *Saturnia pavonia*. This decreases the exposition time to predators/parasitoids (Bernays, 1997). These species are going to expand both in sense of geographical latitude and altitude above sea level. Species however which can be characterized by long larval period, slowly growing development/temperature curve, narrow ranged and low optimum temperatures (e.g. *Lasiocampa quercus callunae*), will face the challenge that habitats in the South and at low altitudes become too hot and these species are forced to shift their ranges to the North and higher altitudes (Bale et al., 2002). A similar reaction can be expected by species with two or more years of life cycle, where slow development decreases the opportunity of quick adaptation.

According to Butterfield and Coulson (1997), those species can be considered vulnerable which live among heavily limited climatic conditions, especially mountainous and cold-adapted species. Wilson et al. (2005) studied the Lepidoptera fauna of central Spain. It was observed that the lower elevational limits of 16 butterfly species had risen on average by 212m between 1967 and 2004, accompanying an increase of 1.3 1C in the mean annual temperature (equivalent to an approximate 225m uphill shift in isotherms). The vertical changes in these Lepidoptera species mean the loss of approximately one-third of suitable habitats, and this is expected to increase to 50-80 % during the 21st century.

Vertical changes had also been observed in the tropical areas: Chen et al. (2009) studied the Geometrid population of Mount Kinabalu in Borneo and reported that the area of 102 montainous species shifted upwards by 67 metres in average in the last 42 years.

## Models, forecasting changes in area using climate scenarios

“Climate envelope” models are used to forecast future distribution of species. “Climate envelope” is the set of environmental and climatic variables. Populations of species are able to live, feed and propagate between the upper and lower limits of these variables. This principle is applied by many computer programs, for instance CLIMEX, HABITAT, BIOCLIM and DOMAIN. Other researchers use linear models and GIS-techniques to forecast area shifts. These models are frequently used to prognosticate future distributions of artificially colonized, introduced species, e.g. *Chiasmia inconspicua* and *C. assimilis* (Palmer et al., 2007) and *Euclasta whalleyi* (Mo et al., 2000) in Australia, or to forecast the global distribution of species, like in the case of *Cactoblastis cactorum* (Legaspi and Legaspi, 2010). Petrányi et al. (2007) used the most widely accepted scenarios (IPCC, 1990, 1995, 2001, 2007; Murphy, 1995) of global general circulation models, Hungarian meteorological data of OMSZ (Hungarian Meteorological Service) and the CRU grid database of IPCC to create a forecast of the future changes of the Hungarian Lepidoptera fauna with a method applying geographical analogies. Results showed that moving southward on the Balkan peninsula, the share of members of Hungarian fauna gradually decreased and the proportion of species leaving or becoming extinct in Hungary and also the proportion of species potentially migrating to Hungary because of changing climate increased. Data obtained for the whole Lepidoptera order were compared with similar data gained for Macrolepidoptera and Microlepidoptera. It was observed that Macrolepidoptera data were significantly higher and Microlepidoptera data were significantly lower than those obtained for the entire order. Supposedly, this can be attributed to better flying and migratory characteristics of Macrolepidoptera. By studying individual species of Macrolepidoptera families it was found that Sphingidae species (with the best flying abilities) had the least endemic species in Hungary, while Geometridae species showed a relatively high proportion of endemic species, therefore these species are appropriate indicators of climate change in Hungary. As a summary, it was concluded that in case of realization of the studied scenarios, their effect would not impact 55-81 % of the Hungarian Lepidoptera fauna. Loss of the current fauna in species would attain 19-45 % at most, and these are species living on the northern limit of their distribution. The appearance of new species can be expected at most in a proportion of 19-31 % of the current fauna, and the area of these species is mainly of South-eastern character.

Some ecologists heavily criticize climatic mapping models and also call the attention that the area shift of a given species is the result of the complicated effects of many different factors; and currently we don’t have appropriate knowledge on interspecific relations. What’s more in many cases we have only scarce understanding on the most important physiological parameters of a single species (Davis et al., 1998; Lawton, 1998; Hodkinson, 1999; Baker et al., 2000). Araujo and Luoto (2007) stated by monitoring the European distribution of *Parnassius mnemosyne* with the “climate envelope” method, that biotic interactions heavily affect the predictive power and reliability of such models. Pearson and Dawson (2003) determined three general non-climatic factors fundamentally affecting the distribution of a given species:

1. biotic interaction like competition and predation
2. fast local evolution inducing area shift without environmental changes
3. limiting effect of barriers.

However, even the hardest critics of climate mapping models recognize that these methods can be applied with great benefits as a “first approach” or “null models”.

Thomas et al. (2004) applied a model created on the basis of the „climate envelope” method on 20 % of the Earth’s terrestrial area and used three different scenarios (slight, medium and drastic change) to prognosticate the number of species (mammals, birds, Lepidoptera, reptiles, frogs and plants) becoming extinct as a consequence of climate change. Results show that by 2050, 18-35 % of the studied taxa can disappear depending on the pace of change.

Zalucki and Furlong (2005) made forecasts with the CLIMEX model on the expansion of *Helicoverpa punctigera* and *H. armigera* in Australia. Using Australian climate data as a starting point, it was also determined where future expansion of *H. armigera* can be expected around the globe.

Beaumont and Hughes (2002) simulated the future area of 24 Australian Lepidoptera species by using BIOCLIM climate models. In case of a very conservative climate scenario (0.8-1°C rise in temperature by 2050), 88 % of species with relatively wide temperature optimum showed area contraction, and in 54 % of the species this contraction exceeded 20 % of their current area. When applying an extreme scenario (2.1-3.9°C rise in temperature by 2050), it was found that 92 % of he species showed contraction in their distribution, and 83 % of the species the extent of contraction exceeded 50 % of their current area.

Gutierrez et al. (2006) applied four different climate scenarios to forecast the future distribution of *Pectinophora gossypiella*, a pest damaging cotton plantations in California and Arizona. The scenarios of the GIS-based simulation reflected 1, 1.5, 2, and 2.5°C rise in daily maximum and minimum temperatures, and models also contained further variables representing values of solar radiation, precipitation, nitrogen content of soil, relative humidity and wind speed. Practically no impact was found as a consequence of 0.5 and 1°C rise in average temperature, but when the rise in average temperature reached 1.5°C the size of population surviving the winter grew significantly and the area of feeding (damaging) also expanded. This species is not considered yet as a serious pest in the South-Eastern states of the US. Venette and Hutchinson (1999) studied in a CLIMEX simulation that what areas can be expected to be colonized by the pest. Results show that the area expansion and serious damages of the species are inhibited by high amounts of precipitation and cold summer and winter temperatures. The model shows that there is 80 % chance that by rising temperatures, the species can appear and can cause severe damages in some parts of Arkansas, Louisiana, Mississippi, Alabama, Florida and Georgia.

The model by Fleming and Candau (1998) forecasts increasing severity of defoliation by *Choristoneura fumiferana* (Tortricidae) in Canadian pine forests due to decreasing number of late spring frosty days. The model by Hassell et al. (1993) suggests that if the tortricid larvae serving as hosts of parasitoids hatch earlier and earlier, they can avoid the appearance of adult parasitoid forms. As a consequence of the “refuge in time” phenomenon, it is possible that larvae can entirely avoid the threat of parasitoids. Based on the CGCM3-B1 climate scenario, Gray (2008) forecasts that between 2081 and 2100 (atmospheric carbon-dioxide level reaches 550 ppm) the defoliation damage by *Choristoneura fumiferana* will increase by 15 % in East-Canadian pine forests, the length of gradation periods will increase due to drier and warmer climate and the damages caused by the species will shift northwards.

Some researchers use life-cycle based classic eco-physiological models to study the impacts of climate change (Kingsolver, 1989; Logan et al., 1976; 2003). These models consider the metabolism of the larvae, thermoregulation and effects of temperature exerted on the insects via host plants. Yamamura and Kiritani (1998) used heat unit models for rapid estimation of potential changes in number of generations per year. Morimoto et al. (1998) prognosticated the future expansion of three damaging Lepidoptera species *(Plutella xylostella, Chilo suppressalis, Ephestis kühniella)* and a Coleoptera (Tenebrionidae) species *(Tribolium confusum)* by a model using heat units, photoperiodic conditions, maximum and minimum temperatures. Results showed that in case of a 2°C increase in annual average temperature, *P. xylostella* could develop 2 extra generations per year, and the distribution of *C. suppressalis* can spread northwards by 300 km.

## Phenology

### *Earlier flight*

According to Woiwod (1997), many factors can influence the beginning of the flight period, but temperature and precipitation immediately preceding the flight have the greatest effects. Since temperature (heat units) is of fundamental importance, the start of flight is one of the climate change related phenomena which can be studied easiest.

Studying the effects of climate change does not solely include monitoring of the beginning of the flight, but also the length of the flight period of adults and the time elapsed between generations and number of generations per year. Brakefield (1987) studied the flight of *Maniola jurtina* and *Pyronia tithonus* and showed that the extent of flight asynchronities can be different in species depending on habitat preference. Wing spotting and body size (smaller) of *Maniola jurtina* populations flying later in the year were significantly different from those individuals flying earlier (Brakefield, 1987).

Forister and Shapiro (2003) studied the first appearance of flying adults of 23 Lepidoptera species in California on a 31-year time series. It was observed that the first observation dates of individuals of species flying significantly earlier in this area of Mediterranean climate became earlier by 42 days in general.

Earlier flight of Macrolepidoptera was observed by Stefanescu et al. (2003) when analyzing trap data from 1988-2002 in Spain. The average date of the first flight of 8 species among the 19 species caught in large numbers advanced significantly.

Kearney et al. (2010) studied the appearance of *Heteronympha merope* in Southeast Australia, near to Melbourne by using data from 1941-2005. In the last 65 years the mean date of flight advanced by 1.5 days per decade – the average increase in temperature in this period had been 0.16°C per decade.

Kuchlein and Ellis (1997) monitored 104 Microlepidoptera species in the Netherlands which had been caught at least 30 individuals per year. It was observed that the first date of appearance of these species advanced by 11.6 days between 1975 and 1994. This was attributed primarily to warmer springs, the influence of warmer summers was found less determining.

Since it had been observed in Great Britain that mites usually appeared earlier on agricultural fields than in the 1960's, Woiwod (1997) studied at six sites whether this phenomenon could be observed at Lepidoptera species. For the sake of simplicity, only univoltine species were studied only at those sites where at least 20 individuals per year had been caught, and 5, 25, 50, 75 and 95 percentile of the appearances were studied. Results showed that *Orthosia gothica* flew almost a month earlier than in 1976 with all percentiles being significant. As a result of expanded monitoring, Woiwod (1997) found significant differences at 93 species, 88 showing earlier and 5 later mean dates of appearance. These results were quite similar to those found at mites by Zhou et al. (1996). It was concluded that during the next 20-30 years the first appearance dates of most mite and Lepidoptera species will advance depending on the pace of warming. Roy and Sparks (2000) prognosticated that 1°C rise in the annual average temperature of the British Isles moves the start and peak of flight period earlier by generally 2-10 days.

### *Later flight*

Woiwod (1997) observed that the species *Xestia xanthographa* caught at Cockayne Hatley light-trap behaved in a very interesting way, since this has been the only species at which the appearance of the flying adults slid to a later date during the past 20 years. The appearance of this species is relatively late, so it was supposed that the appearance can move to an even later date as a response of further warming. A similar phenomenon was described by Buse and Good (1996) during experimental conditions by *Operophtera brumata*.

### *Appearance of extra generations*

Future warming will affect species with different generations per year in a different way and extent. Higher temperatures (ceteris paribus) can result in faster development and consequently the appearance of extra generations in multivoltine species, like *Pieris brassicae* (Pollard and Yates, 1993). The area of most of these species might expand to higher latitudes and altitudes (Pollard et al., 1995; Hill et al., 1999; Parmesan et al., 1999).

Altermatt (2009) studied 355 bi- or multivoltine Lepidoptera species in Central Europe and observed augmented frequency of second and subsequent generations relative to the first generation in a warm period since 1980, and 44 species even increased the number of generations after 1980.

In case of 2°C increase in mean temperature, Morimoto et al. (1998) forecast the appearance of two new generations per year by *Plutella xylostella* in Japan.

Tobin et al. (2008) studied the phenology of the multivoltine *Paralobesia viteana* and found that the importance of warmer winters and springs is especially high, since the species begins diapause 2-3 weeks after the summer equinox (therefore the increase of heat units after this date has a limited effect on voltinism). An increase of above 2°C in mean temperatures however, can significantly bring forward the egg-laying of the second generation, so the probability of appearance of a third generation grows.

## Effects of influencing factors, climatic components and interspecific relations (temperature, atmospheric CO$_2$ content, UVB radiation, wind, precipitation, predators and parasitoids)

Undoubtedly, temperature can be studied and measured easiest among all the environmental conditions, this is why the decisive majority of the already published studies deal with this factor and its effects. Climate change results however not only in the change of temperature conditions, but affects the metabolism, phenology and distribution of Lepidoptera in many ways.

### *Changes in temperature*

Change of temperature can modify the life of Lepidoptera in many ways: the length of the life-cycle (development rate), voltinism, density and size of population, the genetic structure of the population, the extent of host plant exploitation, colonization of new areas, geographical distribution (presence-absence) can be altered. In most of the species, the limiting facto is the lack of the summer warmth (heat units), and not the appearance of the lethal heat stress. Bale et al. (2002) states however, that the effects of temperature change are frequently contradictory. Higher temperatures can help to accelerate the development of individuals and increase the survival rate but these4 can accompanied by lower adult body mass and lower fertility. There are some examples that the development rate of some species showed temperature-independence during examinations conducted in transects including areas from different altitudes, but laboratory trials detected temperature-dependence in these species, e.g.: (Butterfiled, 1976; Coulson et al., 1976; Fielding and Tatchell, 1995). The reason for this is that insects are able to actively manipulate their thermal micro-environment (May, 1979; Heinrich, 1977; Porter, 1982; Bryant et al., 2000).

### *Change in atmospheric $CO_2$-content: Impact of changing atmospheric $CO_2$-content on Lepidoptera through host plants*

The effect of increasing atmospheric $CO_2$-levels to Lepidoptera can be quite various (Bezemer and Jones, 1998; Whittaker, 1999). According to Coviella and Trumble (1999), increasing atmospheric $CO_2$-levels can have four main consequences to insect populations:

1. Local disappearance of some species.
2. Changes in the grading of some insect species (endangered, pest).
3. The area of some species will be changed, limited to the areas where exploitable host plants can be found.
4. Population dynamics of some species can be changed, and this will affect the interactions with other insects and plant species.

High $CO_2$ levels are unfavourable for the larvae, primarily because of the altered physiological attributes of the host plant. Almost all studies agree that the survival of the larvae decreases due to the unfavourable content of the food (lower water and nitrogen content, increased C:N ratio, higher phenolic compound and tannin content, and their development period becomes longer. In these cases compensatory feeding is quite characteristic: larvae consume more (up to 25 % more) from the inferior food. Contradictory conclusions have been drawn from the studies: according to forestry trials, increasing atmospheric $CO_2$ content (ceteris paribus) can lead to decreased level of defoliation (Knepp et al., 2005), while others state that due to compensation feeding, the intensity of damages is expected to grow (Lincoln et al., 1984).

Plants where eggs are to be laid on can be more hardly found by females of some Lepidoptera species at high atmospheric $CO_2$ levels.

Results of studies conducted on Lepidoptera and Orthoptera larvae at high atmospheric $CO_2$ levels (Lincoln et al., 1984, 1986, 1993; Lincoln and Couvet, 1989) showed decreasing populations, however the extent of decrease was not significant in every case.

Agrell et al. (2003) studied the effect of normal (387±8 µl/l) and elevated (696±2 µl/l) atmospheric $CO_2$ content on *Orgyia leucostigma* larvae on three tree species among different light conditions. Higher $CO_2$-concentration had a massive impact on the chemical composition of the foliage of the tree species: reduced water and nitrogen content with higher starch, phenolic glycoside (salicortine and tremulacine) and tannin content. The survival rate of larvae decreased by 62 %, development time increased and larval mass significantly decreased.

Goverde and Erhardt (2003) studied the development of *Coenonympha pamphilus* (Satyridae) larvae at elevated $CO_2$ concentration on four host plants. It was observed that due to the changes in the nutrient content of the plants, the development of larvae took 2 days longer in average, and plants gave different reactions on the change in $CO_2$-content which can affect host plant selection in the future.

Johns and Hughes (2002) studied the development of *Dialectica scalariella* (Gracillariidae) introduced to Australia for the control of the weed *Echium plantagineum* (Boraginaceae) on host plants grown at elevated $CO_2$ level. Results showed that the mortality of the larvae increased, the body-mass of the adults decreased and their development required longer periods. Lincoln et al. (1984) studied the development of *Pseudoplusia includens* (Noctuidae) at three different atmospheric level of $CO_2$ on soy host plants. Due to decreasing nitrogen content of the leaves, consumption of the larvae increased at higher $CO_2$ levels, therefore authors concluded that increasing damages of herbivore insects can be expected at elevated $CO_2$ levels.

Slower development and higher mortality of *Junonia coenia* (Nymphalidae) larvae fed by *Plantago lanceolata* host plants grown in atmosphere with elevated atmospheric $CO_2$ content (350 and 700 ppm $CO_2$) have been observed by Fajer et al. (1989). Authors concluded that this was caused by lower water and nitrogen content and not by the change in the concentrations of defensive metabolites (glucosides). However, in a following series of studies, slower development of larvae was not observed (Fajer et al., 1991).

According to the results of Dury et al. (1998), larval development of *Operophtera brumata* was detrimentally affected by increased phenolic content and decreased nitrogen content of oak leaves due to higher $CO_2$ levels. Those Lepidoptera species that are not able to feed longer than normal (Slansky, 1989) – in order to compensate lower nitrogen content – become vulnerable for their natural enemies (Hochuli, 1996). According to Woodward (1992) the effect of elevated $CO_2$ on the quality of foliage can vary between plant species, and Lloyd and Farquhar (1996) state that the nutrient content of the soil also has a great impact in this respect. The reaction of different Lepidoptera species to elevated $CO_2$ levels can also be different, e.g. the performance of *Lymantria dispar* improved on *Quercus rubra* while *Malacosoma disstria* did not show any changes. At elevated $CO_2$ levels the development of *Lymantria* was unchanged while that of *Malacosoma disstria* decreased on *Acer saccharum* (Lindroth et al., 1993). This is supported by the observation of Traw et al. (1996), that the productivity of *Lymantria dispar* in an elevated $CO_2$ atmosphere depends heavily on the host plant. Buse et al. (1998) also states that the effect of $CO_2$ on larval development is not unanimous.

Stiling and Cornelissen (2007) studied the development of 6 leaf-miner species in a 9-year series of studies at elevated $CO_2$ levels on 3 different host plants. Results showed that high $CO_2$-content decreased abundance of species by 21.6 %, increased relative food utilization by 16.5 % and development time by 3.87 % and total consumption by 9.2 %. The feed conversion of individuals and pupal mass decreased significantly (-19.9 % and -5,03 % respectively).

Stange (1997) observed that the $CO_2$-emission of *Opuntia stricta* host plants play an important role in the egg-laying of *Cactoblastis cactorum* females. Released $CO_2$ is captured by special receptors of the females and eggs are laid on the healthiest plants. At elevated $CO_2$ levels, females tend to find host plants more slowly and number of laid eggs also showed a significant decline.

### *Changes in the intensity of UV-B radiation*

According to the results of the (relatively small number of) available studies, increasing UV-B radiation is very unfavourable for Lepidoptera species. This is because the defensive mechanisms of host plants are unfavourable for the Lepidoptera, and some data describing tritrophic interactions are also state that parasitoids prefer the weakest larvae.

Hatcher and Paul (1994) studied the effect of elevated UV-B radiation on *Autographa gamma* larvae feeding on pea. According to their results the phenolic compound and nitrogen content of plants increased significantly, but this affected the feeding of the larvae only in a small extent. Due to increased nitrogen content, larvae utilized the consumed plant parts more effectively, however their total consumption decreased slightly. McCloud and Berenbaum (1994) observed definitely unfavourable effects when studying the survival of *Trichoplusia ni* larvae on *Citrus jambhiri* leaves exposed to UV-B radiation, because the plant metabolized toxic fumarocumarines as a response to radiation.

Caputo et al. (2006) observed no significant reactions of *Plutella xylostella* larvae on *Arabidopsis thaliana* host plant when exposed to UV-B radiation, female adults however laid significantly less eggs in case of stronger radiation. According to the results of the studies conducted by Foggo et al. (2007), scars done by *Plutella xylostella* larvae on irradiated plant were smaller and more shallow, though the number of scars was higher (but not significantly). When females could choose between irradiated and untreated plants, most of them preferred the untreated plants for egg laying. It was also observed that females of the parasitoid *Cotesia plutellae* species preferred those larvae that were feeding on irradiated plants.

### *Changes in precipitation, impacts of drought*

Drought periods are expected to become more frequent which is going to increase the intensity of damages by herbivore insects due to various reasons. First, according to Mattson and Haack (1987) the temperature of plants exposed to drought stress is 2-4°C higher than those with appropriate water balance (closure of stoma inhibits transpiration cooling), therefore these plants provide a favourable microhabitat for pests, this way facilitating their fecundity (Sanders et al., 1978) and accelerating their development (Lysyk, 1989). Second, the concentration of metabolites facilitating the fecundity of female Lepidoptera (carbohydrates, protein-hydrolizates) in the host plant with water deficit stress increases (Martinat, 1987). This is the reason why the frequency of total defoliation and a northward shift in the geographical distribution of damages by defoliators was prognosticated in North America by Williams and Liebhold (1995), Hanson and Weltzin (2000), Volney and Fleming (2000) and Logan et al. (2003), though in some Lepidoptera species (e.g. *Aglais urticae*) drought decreased fertility (Pollard et al., 1997).

### *Changes in the activities of predators and parasitoids*

The most important factor determining the efficacy of natural enemies are: weakening of prey-predator (parasitoid) synchronization i.e. the „refuge in time” effect (Fleming and Candau, 1998), variability of the climate, extreme weather phenomena and conditions (Stireman et al., 2005), changes in the foliage structure of host plants (Chen and Welter, 2007), proximity of host plants of alternative prey species and the presence or absence of the regional source population (Bell et al., 2006).

Fleming and Volney (1995) observed that the tortricid *Choristoneura fumiferana* propagates earlier and earlier due to increasing temperature in Canada, and this way the species might avoid the parasitoids in time.

Hance et al. (2007) studied the effects of extreme temperatures on parasitoids and reported that due to the difference in thermal preferences the synchronization of host-parasitoid relation can weaken both in space and time, which can lead to gradations. Thomson et al. (2010) monitored the distribution of natural enemies of the polyphagous tortricid *Epiphyas postvittana* in Australia and New Zealand and called the attention of the plant protection experts to the importance of alternative host plants of parasitoids.

Visser and Holleman (2001) described and Walther et al. (2002) also mentioned that due to warmer springs, the budburst of oaks and the synchronization of *Operophtera brumata* had weakened, and as a consequence the moth is less available for its predator (*Parus major*). This causes severe disturbance in the propagation of the predator (Visser et al., 1998).

## Changes in species numbers, biodiversity and migrations

### *Observed changes in some communities*

Panigaj and Panigaj (2008) monitored the Lepidoptera population of the Temnosmrecinská valley (Slovakia, High Tatra) at seven sites and compared the catch data with those 55 years before. 15 members of the earlier species list had disappeared, but 6 new species have been caught. The main reasons for this were the changes in abiotic factors and plant communities. Number of species which were caught at more than one site simultaneously decreased significantly, and the number of species which were caught at only one sight has grown. Kulfan and Zach (2006) also reported about the change of abundance and structure of local Lepidoptera communities in Skalnatá valley (High Tatra, Slovakia), which can be traced back to the change of climatic conditions.

Franzen and Johannesson (2007) monitored both nocturnal and diurnal Macrolepidoptera communities on approx. 100 km$^2$ in the Kullaberg Nature Reserve, Sweden. The findings were compared with a species list from 1950. According to the results, out of 597 species, 159 (22 %) formerly on the list disappeared and 22 new species appeared. The extent of decrease in species numbers was higher in case of diurnal butterflies and moths than in nocturnal and other Macrolepidoptera species. Individual numbers of disappeared species showed a strong decline in Finland as well and individuals of species observed in Kullaberg were also caught in Finland. In case of species not showing any change in abundance, Finnish trap data remained constant. 70 % of habitat specialist species disappeared and not just from the nature reserve but also the Finnish traps were not able to catch them, so it was concluded that this change of the latest 50 years affected a large area.

Bolotov (2004) reported that abundance and dominance characteristics of the taiga Lepidoptera fauna of the Western Russian Plains has changed significantly due to warming that started in the early 1990s. Species with formerly southern distribution appeared in the area (*Gonopterix rhamni, Euphydryas maturna, Melitataea athalia, Limenitis populi, Argynnis paphia, Argynnis aglaja*), and some migrant species also appeared who were not able to adapt to the conditions in an extent to settle down, but were regularly observed in the area (*Vanessa atalanta, Vanessa cardui, Inachis io, Colias hyale*). Following the warm years of 1977-1978 and 1983, settling of southern species was observed on the southern part of the Jamal-peninsula by Bogacheva and Olschwang (1978), Olschwang (1992) and Bogacheva (1986), and also on the north-western part of the Kola-peninsula by Isakov and Gromov (1997), but in the latter case, the settled population had been decimated by the first hard winter.

The GIS-based model processing data on 292 Lepidoptera species by Kerr (2001) shows the importance of habitat heterogeneity. Results showed that different types of plant cover present in the same quadrate heavily affected the species richness of the given area.

## *Appearance of invasive species*

Invasive or introduced species can severely affect the native biodiversity of the area. 40 % of North America's main insect pests are introduced (Niemela and Mattson, 1996), this ratio in Great-Britain is around 30 %, and what's more, 62 % of the 29 main defoliators damaging forests are not indigenous (Pimentel, 2002). The success of invasion is determined by various factors which can be modified by climate change in different directions, therefore it is quite complicated to forecast the final impact (Ward and Masters, 2007)

By studying some species, predisposing attributes which facilitate the species to become invasive can be defined. The most important attributes are as follows:

1. A wide range of diet (generalist/specialist)
2. Phenological plasticity (voltinism)
3. Life-cycle strategy (overwintering, frost tolerance)
4. Mobility and fast reproduction ability, introduction pressure

### *Width of diet range (generalist/specialist species, mono-/polyphagy)*

Invasion success is often linked with the width of diet range (Ehrlich, 1986; Simberloff, 1989). Generalist species have greater chance to find some kind of suitable food plant on a new area than specialist species feeding on only one or just a few plant species. However, taxonomic generalism can mean nutrient specialism in many cases (McNeill and Southwood, 1978; Lawton and McNeill, 1979; Bernays and Minkenberg, 1997), and it is also possible that the reason why a species is a generalist is that it has such a mixed nutrient requirement which can only be satisfied by consuming various plant species (Bernays et al., 1994). A specialist species often consumes only one plant species or just plant species belonging to the same order or family, but it is also possible that a specialist species become able to cope with the changing nutrient concentration of the plant (McNeill and Southwood, 1978). It is also possible that though generalists have a broader food-plant range, they can not cope with the increasing phenolic compound and decreasing nitrogen concentration of the plants which is expected due to increasing $CO_2$-levels (Bezemer and Jones, 1998). This is especially true in case of species belonging to such a guild the members of which do not conduct compensation feeding (Hamilton et al., 2004). And finally, it is also possible that a given species changes food plant in a new area, or increases its diet range (Pearson and Callaway, 2003).

### *Phenological plasticity*

A tight synchrony can be observed between herbivore insects and their food plants (those periods when the food plant is available). A good example for this is the *Lymantria dispar* (Hunter and Elkington, 2000), feeding on *Quercus rubra* and *Quercus velutina* species. If there is no alternative food plant and larvae hatch before budburst, they won't be able to find food and if they hatch well after budburst, the quality of the food will not be appropriate, so the fecundity of the species decreases. Phenological events of the spring (budburst, flowering) are becoming earlier and earlier (Sparks et al., 1997, 2000; Fitter and Fitter, 2002), which can severely affect the insect feeding on a given plant species.

Due to earlier springs, the length of the active period increases, which can be quite favourable for multivoltine species. A given food plant can become available for more Lepidoptera species than before. Therefore invasive species are expected mostly to require less tight synchronization with the given host plant (including multivoltine species).

### *Life-cycle strategy, overwintering, frost tolerance*

Detailed knowledge on the biological processes of overwintering and frost tolerance can provide a good indication on whether the survival of a species is possible or not in the new area (Bale and Walters, 2001). However, we do not possess these information in case of many species. Overwintering strategies of individual species are quite well known on the other hand. Most temperate species have some kind of winter diapause (Leather et al, 1993), which is generally obligate in univoltine and facultative in multivoltine species, where diapause is induced by some abiotic or biotic triggers. According to Bale et al. (2002) the area of those species will be expanded which are multivoltine, developing quickly, have no diapause, and those species which do not require low temperatures to induce diapause. Fast individual development can also be a decisive factor of the success of invasion (Lawton and Brown, 1986; Crawley, 1987).

### *Mobility and fast reproduction ability, introduction pressure*

Introduction pressure is a function of the number and reproduction rate of propagules introduced to the new area, and these factors are determined by the mobility of the species and the size of the new area and the distance from the source habitat. (Lockwood et al., 2005; Memmott et al., 2005). Changes in climate can provide better conditions for those species expanding in their flying form, since they can reach new areas in times when conditions are suitable for colonization, e.g. developing a new generation.

The success of colonization is heavily affected by the reactions of local communities to newly arriving populations (nutrients, niches). Various theories tried to explain the success of invasion and the extent of the newly establishing diversity, e.g. the theory of fluctuating resources (Davis et al., 2000), and the intermediate disturbance hypothesis (Grime, 1973; Horn, 1975; Connell, 1987; Roxburgh et al., 2004). Human impacts must not be disregarded and these can be either unfavourable (destruction of habitats etc.) or favourable (introduction of species, establishment of protected environment for example with glasshouses or production of new host plants).

## Summary and outlook

As a consequence of climate change, changing conditions exert various effects on Lepidoptera communities. Results of faunistical studies decisively show decreasing number of individuals, however the role of human impacts must be huge in this respects. A number of studies showed the horizontal and vertical expansion of butterflies and moths. Temperate Lepidoptera species show a general trend of expanding towards higher latitude and altitude, which is also supported by the forecasts of the applied models. Higher mean temperatures induce earlier flight of adults, appearance of new generations and due to area changes, food plant changes also can be observed in case of some species. One of the reasons for this is the weakened synchronization with the food plant. Increasing $CO_2$ levels are usually unfavourably affecting larvae, causing lower body mass and worse feed conversion. Intensification of UV-B radiation is also detrimental for the development of the larvae due to the enhanced defensive mechanisms and tritrophic interspecific interactions. Drought reduces the reproduction success of many Lepidoptera species, though defensive mechanisms of plants are expected to weaken, therefore the intensity of damages by some species is prognosticated to grow.

Predicting and monitoring the impacts of climate change have many pitfalls and challenges, which can fundamentally affect the results, e.g. human activity causing the destruction of habitats etc. According to Bale et al. (2002) future research must consider the following aspects:

1. By predicting the direct impact is of climate change on Lepidoptera and other insect species, the phenotipical and genotipical flexibility of individual species must also be considered.
2. Much greater attention has to be paid to the interactions of climatic factors. For example the direct effect of temperature can be modified by changes in precipitation, and this can affect relative humidity which greatly determines physiological functions (e.g. reproduction, fertility). Besides, direct impacts of climate change on the development of Lepidoptera and other insects must be interpreted in a broader context, especially in regard of natural enemies and host plants.
3. We have only little knowledge on the long-term population level responses and reactions of Lepidoptera and other insects to global environmental changes.
4. Scientific studies should be extended to other biological systems (e.g. terrestrial and sweetwater systems). The majority of currently available research results deal with terrestrial insects.

These ideas support the need for cooperation of researchers from various fields of science in order to clarify the individual effects and their complex resultant impacts.

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# IMPACTS OF SOLIDAGO GIGANTEA, PRUNUS SEROTINA, HERACLEUM MANTEGAZZIANUM AND FALLOPIA JAPONICA INVASIONS ON ECOSYSTEMS

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**Abstract**. The world's biodiversity and ecosystems are threatened by the invasion of alien plant species. They have been found to impact the structure in function of ecosystems in temperate, tropical and subtropical areas (Holm et al., 1977; Pysek, 1997; Byers et al., 2002; Ehrenfeld, 2003). We reviewed studies to summarize the impact of four plant species invasions on European ecosystems: *Solidago gigantea, Prunus serotina, Heracleum mantegazzianum* and *Fallopia japonica*, because they are amongst a number of invasive species which have spread over the last centuries, and are still increasing their range in Europe. They cause negative impacts on both native biodiversity and ecosystem functioning (including productivity, nutrient cycling and soil organic matter dynamics).

**Keywords:** *invasive plant species, natural and managed ecosystem, species effect, soil nutrients*

## Introduction

The spread of invasive alien plant species is an increasing concern in temperate (Pysek, 1998; Weber, 2001) as well as in subtropical and tropical areas (Holm et al., 1977; MacFadyen Cruttwell et al., 1996). An invasive plant species are species that adversely affect the habitats they invade economically, environmentally or ecologically. The majority of invaders reproduce by seed dispersal (Pysek, 1998), although an extremely successful minority relies exclusively on vegetative reproduction. The comparison between invasive and native plant species they displace has shown that invasive plant species tend to have a greater specific leaf area and higher nutrient concentrations in biomass and litter (Daehler, 2003; Ehrenfeld, 2003).

Invasive species can negatively impact native biodiversity and can be detrimental to economic interests as well as to public health, biodiversity; economic damage (Vitousek, 1997). Several authors: (Weber, 2001; Pairon et al., 2006; Vitousek et al., 1997; Byers et al., 2002; Ehrenfeld, 2003) have shown that invasion by an exotic or an alien plant is a serious threat to global biodiversity, severely affecting native species and communities. Range expansion of non-native alien species is likely to accelerate in response to global change (climate change, habitat modification, eutrophication, global transport), and has been described as one of the major threats to native communities (Maskell et al., 2006). Alien species invasions are often enhanced by anthropogenic disturbances (Hobbs and Huenneke, 1992). This may cause further changes to fundamental ecosystem processes in forests (MacMahon and Holl, 2001) and other ecosystems (Tickener et al., 2001; Weber, 2001; Ehrenfeld, 2003).

Some invasive species spread aggressively and their whole scale eradication may be difficult and have a high cost. To effect restoration of native plants their removal at specific sites is a necessary step. Eradication is especially important since the restoration process usually proceeds on a site-by-site basis across large area (Wiens et al., 1993). As human population and travel has increased the number of introductions of non-native alien organisms has increased in the past three centuries in both temperate (Weber, 2001) and tropical areas (Holm et al., 1977; MacMahon and Holl, 2001; Goodall and Erasmus, 1996).

***Table 1.*** *The four studied invasive plant species (Sg = Solidago gigantea; Ps = Prunus serotina; Hm = Heracleum mantegazzianum; Fj = Fallopia japonica)*

| **Species / Species variables** | | **Sg** | **Ps** | **Hm** | **Fj** |
|---|---|---|---|---|---|
| **1. Original use** | Ornamental use | + | + | + | + |
| | Soil quality improvement | +/- | + | | |
| **2.Type of vegetation** | Herb | | | | |
| | Schrub | + | | + | + |
| | Tree | | + | | |
| **3. Rapid population growth** | | + | + | + | + |
| **4. Mechanisms of invasion** | Seeds | + | | + | |
| | Vegetative reproduction | | | | + |
| **5. Soil changes** | pH | - | | | |
| | Nutrients | + | | | |
| | Soil fauna | | | | |
| | Soil organic matter | + | + | - | - |
| **7. Impacts on biodiversity** | | - | - | - | - |
| **8. Economical impacts** | | - | - | -- | --- |
| **9. Impacts on public welfare** | | -/+ | | --- | -- |

+ = positive effect and - = negative effect, -- = strong negative effect, --- = very strong negative effect.

There are general reviews on the effects of invasive exotic plant on soil nutrient cycling processes (Ehrenfeld, 2003; Levine et al., 2003). Dassonville et al. (2008) examined more specifically the impact of seven highly invasive plant species in North West Europe. Authors found that invaded plots had higher aboveground biomass and nutrient stocks in standing biomass compared to uninvaded resident vegetation, and concluded that the enhanced nutrient uptake may be a key trait of highly invasive plant species in Belgium. The authors also found that strong positive impacts (higher topsoil nutrient concentrations in invaded plots compared to uninvaded ones) were most often found in sites with initially low nutrient concentrations in the topsoil while negative impacts were generally found in the opposite conditions. The four plant species (*Solidago gigantea*, *Prunus serotina*, *Heracleum mantegazzianum* and *Fallopia* *japonica)* presented in this review are amongst those studied by (Dassonville et al., 2008), and amongst the more invasive species which have spread over Europe during the last centuries (Pysek, 1998; Weber, 2001; Dassonville et al., 2008), (*Table 1).* Their impacts are represented over the Europe.

## Impacts of *Solidago gigantea* invasions on ecosystems

### *Life history treats*

*Solidago gigantea* (Asteraceae) is a rhizomatous perennial herb native to North America and introduced into Europe about 250 years ago (Weber and Jacobs, 2005). The genus *Solidago* includes 100 species and almost all of these endemic to North America, although one species (*S. virgaurea*) is native to Europe (Gleason and Conquist, 1991). *S. gigantea* is present both in wet and dry habitats and colonizes mainly disturbed areas such as roadsides, but fewer populations are found adjacent to rivers and lakes (Jacobs et al., 2004).

### *Mechanism of invasion and ecosystem attribute that can be modified by S. gigantea*

After its introduction, S. gigantea has become widespread and has invaded a number of different ecosystems (Weber, 2001). Field survey results show increased growth performance of S. gigantea in its introduced range compared to conspecifics in their native range (Jacobs et al., 2004). Furthermore, Güsewell et al. (2006) found that S. gigantea often forms dense monospecific stands, eliminating all indigenous competitors in Europe, while in its native range, it is better integrated in diverse herbaceous vegetation. In fact, after comparison of 20 European (introduced) and 22 American (native) populations of S. gigantea (Güsewell et al., 2006) found that introduced S. gigantea populations tend to produce more shoots through clonal growth than the American native populations, even though there was no difference in shoots size and leaf traits. The authors argued that this may increase the ability of S. gigantea to compete against established species in dense stands or nutrient-poor sites. Meyer et al. (2005) tested the evolution of increased competitive ability hypothesis and found that European (introduced) S. gigantea are less resistant to American foliar pathogens and do not perform as well in the presence of insect herbivores as American (native) S. gigantea. Weber (2001) found evidence that strongly suggested that the spread of S. gigantea has not reached its limits and that its range expansion will continue. However the authors argued that S. gigantea is confined to moist soils while S. altissima which has spread less widely over Europe than S. gigantea, prefers drier sites, the latter might become more frequent along disturbed river banks and other moist soils (Weber, 2001).

### *Changes in soil properties and nutrient contents*

Even though it is considered as an invasive species, *S. gigantea* may be used for environmental management in disturbed and contaminated areas. In an area where the chemical industry was active until 1999 in northern Italy, Vallino (2006) found that *S. gigantea* was the only plant able to counteract the severely harmful effects of industrial chemicals because of its arbuscular mycorrhizal fungi state. Arbuscular mycorrhizal fungi provide plants with mineral nutrients in exchange for carbon compounds and protect them against diverse abiotic and biotic stresses (Newsham et al., 1995). Vallino et al. (2006) also found a high density of arbuscular mycorrhizal fungi biodiversity in the cover soil of a hill. However, the authors argued that fungal presence and biodiversity did not seem to be the key factors that promote plant diversity at a recovering site.

Studies in Belgium demonstrated an impact of *S. gigantea* on soil properties and biogeochemical cycles. Vanderhoeven et al. (2006) found that nutrient stocks in aboveground biomass were twice as high in plots invaded by *S. gigantea* than in resident uninvaded vegetation. This suggests that *S. gigantea* might enhance nutrient uptake (especially phosphorus) mainly in topsoil (0-10 cm), since it does not root much deeper than the indigenous population. When considering soil phosphorus, Chapuis-Lardy et al. (2006) found that the plots invaded by *S. gigantea* generally had higher concentrations of labile P (resin extractable inorganic P and bicarbonate extractable and organic P) than adjacent uninvaded soils. Furthermore soil $CO_2$ production and alkaline and acid phosphormonoesterase activities were also higher, suggesting that the higher labile inorganic P concentration was due to enhanced mineralization. Additional studies have been made on soil pH and phosphorus fractions. Herr et al. (2007) showed that invaded plots had lower soil pH and 20-30% higher labile P fractions and this different remain consistent across seasons. This higher P availability in invaded soil could be due to the active acidification of the rhizosphere and/or by a rapid turnover of fine roots in winter (Herr et al., 2007). Study on soil organic matter status has shown high C contents of particulate organic matter fractions and a high soil C mineralization in soils colonized by *S. gigantea* compared to plots with native vegetation (Koutika et al., 2007).

## Impacts of *Prunus serotina* invasions on ecosystems

### *Life history treats*

*Prunus serotina* (Rosaceae) is native to eastern North America, and was first introduced in Europe in the 17th Century as an ornamental tree. From the end of 19th century onwards, *P. serotina* has been used in forestry as an auxiliary tree (e.g. Germany, the Netherlands and Belgium). *P. serotina* was widely cultivated in forest to improve soil quality and to prevent fire around pine plantations. As an indigenous species in North America, *P. serotina* may be dominated by other alien plant species [16]. The authors found that *P. serotina* was among the species which survived to *Lonicera maackii*, a nonindigenous shrub and invasive species, in plots where *L. maackii* was eradicated by cutting or by method using stem glyphosate injection.

### *Mechanism of invasion and ecosystem attribute that can be modified by P. serotina*

*P. serotina* became invasive after its introduction and was called for long time “wood pest” (Lequercq, 1960). Currently *P. serotina* has spread across much of Europe i.e., from Denmark to Italy as well as from north France to Poland, Hungary and Rumania. This invasive plant species grows on sandy soil, but also grows on other soils from peaty to sandy.

To determine the factors controlling the abundance of *P. serotina* in the understory strata, Godefroid et al. (2005) conducted a study in Belgium and concluded that adoption of silvicultural system of irregular stands with mixed species with a dense canopy might hamper the development of *P. serotina* and allow a better conservation of indigenous forest species. The authors also argued that *P. serotina* shows a differential response to light intensity in relation to its development stage i.e., the species is heliophilous at the seedling stage and becomes a shade plant at the sapling stage.

To determine the main factors explaining *P. serotina* invasive success in Belgium, (Pairon et al., 2006) analysed flowering fruit production, seed rain, dispersal and viability and survival of seedling at different ages and individuals in pine plantations. The authors found that fruit production was high (up to 8,940 fruits per tree) as trees produced huge quantities of flowers, while both flower and fruit production were highly variable between years and among individuals. Furthermore, using a stochastic stage-structured population model to describe the dynamics of *P. serotina* in northern France, (Sebert-Cuvillier et al., 2007) studied the local demographic explosion of a species in a suitable stand where it had initially established. However the model could not predict the further spread of the species in the surrounding forest landscape.

### *Changes in soil properties and nutrient contents*

Reinhart et al. (2003) used the plant soil biota interactions and spatial distribution of *P. serotina* in its native and invasive ranges, and presented evidence showing that, in its native range, the soil community that develops in the rhizosphere of *P. serotina* inhibits the establishment of neighbouring conspecifics and reduce the performance of seedlings in greenhouse. In contrast in its introduced range, *P. serotina* readily establishes in close proximity to conspecifics, while the soil community enhances the growth of the seedlings. Reinhart et al. (2003) suggested that *P. serotina* may have escaped the direct negative effects of host-specific soil pathogens and its growth is facilitated by the soil community in its introduced range while being inhibited by the soil community in its native range. However the authors used the ‘whole soil’ inoculum, which tests the net effect of the soil community on survival and growth of *P. serotina* and did not test for the specific effect of soil pathogens on seedling establishment.

Koutika et al. (2007) evaluated soil organic matter dynamics under *P. serotina* stands in Belgium and found high C contents of particulate organic matter fractions and high soil C mineralization in *P. serotina* invaded areas. However, the authors argued that since soil texture was different at non-invaded and invaded sites in two out of three sites, changes occurring after *P. serotina* invasion might not be solely due to exotic plant invasion. Dassonville et al. (2008) also found that *P. serotina* tends to increase the pH of invaded soils.

## Impacts of *Heracleum mantegazzianum* invasions on ecosystems

### *Life history treats*

*Heracleum mantegazzianum* (Apiceae) is a Caucasian species invasive in Europe, which is regularly listed in global overviews of noxious invasive species (MacMahon and Holl, 2001; Vallino et al., 2006; Weber, 2004). *H. mantegazzianum* was introduced to the British Isles as a garden plant during the 19th century, and has since become widespread, particularly along rivers and waterways (Collingham et al., 2000). Other species of *Heracleum* were introduced into Europe from South East Asia in the 19th century, and are now also widespread in many countries. *H. mantegazzianum* (otherwise known as giant hogweed) has long been recognized as a prominent example of an invasive alien with a wide distribution, significant impact and remarkable dynamics of spread in Europe (Nielsen et al., 2005).

### *Mechanism of invasion and ecosystem attribute that can be modified by H. mantegazzianum*

*H. mantegazzianum* has higher gene flow between the invasive species ranges, possibly due to more frequent independent introductions (Jahodova, 2007). *H. mantegazzianum* does not reproduce vegetatively, but only by seeds (Pysek and Pysek, 1995; Tiley et al., 1996). Moravcovà et al. (2005) found that 90% of *H. mantegazzianum* seeds germinated regardless of the vigour of the mother plant or where it was produced on the plant. The authors concluded that the high fecundity and opportunistic behaviour associated with high percentages of germination of cold-stratified seed, as well as high seed production compared to other species (Pysek and Pysek, 1995; Tiley et al., 1996), may account for the successful invasion of *H. mantegazzianum* in Central Europe, where the species has negative effects on biodiversity, the economy and public health.

Using aerial photographs from 11 sampling dates between 1947 (before *H. mantegazzianum* invasion started) and 2000, Müllerovà et al. (2005) found that pastures and fields contributed 84.7 % to *Heracleum* total cover, forest and shrub 13.7% and human settlements 1.6 % at later stages of invasion. The authors found that as invasion proceeded, the populations spread from linear habitats to the surrounding landscape with the mean rate of aerial spread of 1,261 $m^2$ per year and linear spread of 10.8 m per year. The authors also argued that knowledge of rate of spread and habitat vulnerability to invasion facilitates the identification of areas at highest risk of immediate invasion.

Using an individual-based modelling technique, Nehrbass and Winkler (2007) illustrated that the invasion status of *H. mantegazzianum* and found that populations are still expanding in space. Moreover, the authors found that although the long term average population growth rate is larger than that of native species and populations generally expand, there are years in which populations of invasive species declined (actual growth rate $R<1$). The authors were able to validate their statistical data on local dynamics of *H. mantegazzianum* and estimated the long-term development of the examined populations of *H. mantegazzianum* to characterize invasive behaviour of the species. Short term empirical observations recorded decreasing individual numbers of the species and the deterministic matrix model approach. This result has been used and projected as future development. Furthermore, Nehrbass and Winkler (2007) found that *H. mantegazzianum* is still an invasive species, but choice of sampling site and date may lead to large variations in results of invasive potential. Analysis of the model results showed that more than a third of the invasive populations could have an annual growth rate below one.

### *Changes in soil properties and nutrient content*

Considering soil mineral element composition of invasive species (including *H. mantegazzianum*), Vanderhoeven et al. (2005) found an increase of concentrations of exchangeable essential nutrients under the canopy of exotic invasive plants mainly for K and Mn compared to native species. The authors concluded that their results fit in well with reports of an enhancement of N dynamics in invaded sites, probably due to higher primary productivity in exotic invasive plants compared to native vegetation. Koutika et al. (2007) showed that the soils under the plots invaded by *H. mantegazzianum* had lower particulate organic matter weight (POM), C and N contents and C mineralization. The authors suggested that soil organic matter dynamics slow in the plots invaded by *H. mantegazzianum.*

## Impacts of *Fallopia japonica* invasions on ecosystems

### *Life history treats*

The genus *Fallopia* (Polygonaceae) contains c. 24 species world wide of which seven are considered weeds, among them *Fallopia japonica* var. *japonica* (Houdt), the most invasive clone (Bailey, 1994). *Fallopia japonica* is a rhizomatous dioecious perennial (geophyte) native to Japan, Korea and Taïwan. In its native areas *F. japonica* grows in sunny places on hills, high mountains, road verges and ditches, river gravels and managed pastures on wide range of soils from sea level to 2400 m a.s.l. (Child and Wade, 2000). *F. japonica* was introduced into Europe as an ornamental plant in the 1820s and spread exponentially throughout Europe during the 1900s on disturbed areas, roadsides, and river banks via accidental transport of rhizome fragments (Sheppard et al., 2005).

The *Fallopia* species are invasive throughout the temperate world including much of North America and more recently Australia. They are on the IUCN 100 worst invasive species list, are the second most damaging alien plant in Germany (land holder poll) and are amongst the top 10 invasive plant species in France and UK (Bailey, 1994; Manchester and Bullock, 2000). In these countries *F. japonica* var *japonica* is one of three species recognised as having significant impacts on biodiversity (Manchester and Bullock, 2000). The species increase risk of flooding and the deep rhizomes hinder construction projects and cause physical damage to drainage structures, building foundations and road surface (Beerling, 1991). Shaw and Seiger (2002) believe that biological control is the only long-term, sustainable solution to *Fallopia* species.

### *Mechanism of invasion and ecosystem attribute that can be modified by F. japonica*

*F. japonica* forms dense stands that prevent other species from growing (Pysek, 1998). Tickener et al. (2001) argued that relatively little research has been undertaken on competitive interactions between alien and native riparian plant species such as *Fallopia or Heracleum.* The authors also observed that with the increase of biological invasion, riparian habitats which are considered to be particularly prone to invasion by alien plants, have been the focus of most studies.

### *Changes in soil properties and nutrient contents*

Kappes et al. (2007) evaluated the impact of *Fallopia* on soil dwelling fauna and concluded that the structure of the soil fauna shifted from a plant-based to detritus-based food chain. The *Fallopia* invasion also benefits predators that profit from his simplified vegetation structure. Therefore, Gerber et al. (2008) showed that riparian habitats invaded by *Fallopia*_support lower number of plant species and lower overall abundance and morphospecies richness of invertebrates as compared to native grassland and bush habitats. The authors also found that the biomass of invertebrates in bush was almost twice as high as that in *Fallopia*_invaded habitats. Finally Gerber et al. (2008) concluded that large-scale invasion by exotic *Fallopia* species is therefore likely to seriously affect biodiversity and reduce the quality of riparian ecosystems for amphibians, reptiles, birds and mammals whose diets are largely composed of arthropods.

Soil organic matter status has been evaluated in plots invaded by *F. japonica* invasion compared to plots with native vegetation in the central Belgium (Koutika et al., 2007). The authors showed the changes in soil organic matter status through a reduction of soil organic matter (lower particulate organic matter weight and C and N contents and lower C mineralization) in the invaded fields compared to fields under the native vegetation. Dassonville et al. (2007) examined the impacts of *Fallopia* on topsoil chemistry and nutrient stocks. They found that soils under *Fallopia* generally had higher exchangeable nutrient concentrations (Cu, K, Mg, P and Zn) compared to soils under native vegetation. Standing biomass was 3 to 13-fold higher depending on site, and 4-5-fold larger nutrient stocks in aboveground biomass of *Fallopia* were found. According to Dassonville et al. (2007), the higher nutrient concentration in soil invaded by *F. japonica* could be explained by a nutrient uplift mechanism (sensu Jobbagy and Jackson, 2004).

## Conclusions

We reviewed the literature to investigate four plant species which are amongst a number of invasive species which have spread over the last centuries, and are still increasing their range in Europe: *Solidago gigantea*, *Prunus serotina*, *Heracleum mantegazzianum* and *Fallopia japonica*. We can conclude that for all the four species: (i) Invasive expansion is continuing (ii) the four plant species are threats to biodiversity of natural and managed ecosystems; and (iii) *Solidago gigantea*, *Prunus serotina* and *Fallopia japonica* do not have a direct negative impact on public health, but *Heracleum mantegazzianum* is dangerous to humans.

The invasion mechanisms of both *S. gigantea* and *F. japonica* are quite similar, i.e. *S. gigantea* often forms dense monospecfic stands, eliminating all indigenous competitors (Güsewell et al., 2006), while *F. japonica* forms as well dense stands that prevent other species from growing (Pysek, 1998). The invasion mechanisms of *P. serotina* and *H. mantegazzianum* differ from those of *S. gigantea* and *F. Japonica*. In fact, the invasion mechanisms of *P. serotina* and *H. mantegazzianum* have some similarities in fruit seed production but *H. mantegazzianum* is more fecund and *P. serotina* has more flowers (Pairon et al., 2006; Pysek and Pysek, 1995; Tiley et al., 1996).

Three major characteristics of the impact of the four species on soil properties are: (i) the invasion of *S. gigantea* induces changes in soil properties mainly in the topsoil (0-10 cm) compared to the soils under the native vegetation, i.e., increased phosphorus availability (Chapuis-Lardy et al., 2006; Herr et al., 2007; Vanderhoeven et al., 2005; Vanderhoeven et al., 2006), and changes in soil organic matter dynamics mainly in active particulate organic matter and C respiration (Koutika et al., 2007); (ii) after invasion by *P. serotina*, soil properties change compared to soil under the native vegetation with an increase of C in particulate organic matter fraction and C respiration in the topsoil (Koutika et al., 2007); (iii) in contrast to the other two species, the invasions of *H. mantegazzianum* and *F. japonica* reduced the soil C respiration and created soil organic matter which decomposed slowly (Koutika et al., 2007).

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# HUMAN IMPACTS ON MACROPHYTE DIVERSITY, WATER QUALITY AND SOME SOIL PROPERTIES IN THE MADIKANE AND DUFUYA WETLANDS OF LOWER GWERU, ZIMBABWE

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**Abstract.** This study investigated how the exploitation of wetlands, locally known as dambos, changes their vegetation composition, soil properties and how that in-turn affects the water quality. We therefore compared diversity and soil conditions between a protected (Madikane) and exploited (Dufuya) dambo impacted by communal agriculture and grazing in Lower Gweru, Zimbabwe. Species diversity was higher in Madikane (H' = 2.52) than Dufuya (H' = 2.14). Species that were present in Madikane indicated a permanent or semi-permanent wetness compared to species tolerant to arid conditions, reflecting disturbance in Dufuya. Dambo utilization also indicated a change in dominance from perennials to annuals and an increase in exotic species. There was no significant difference in the physical structure of the soil between the rather pristine and exploited sites (% clay and % silt, $p > 0.05$). A significant difference was recorded in the chemical properties of the soil (pH, phosphorus, nitrate-N, ammonium-N and organic carbon content). Water quality was good in the protected dambo than the exploited dambo as indicated by the differences in calcium ions and conductivity. The protection of wetlands is shown to be important in conserving biological diversity.

**Keywords:** *dambo, species diversity, agriculture, soil condition*

## Introduction

African wetlands are an important resource base and are actively utilised by rural communities for socio-economic activities (Kirstern, 2005). The good water quality in wetlands allows a rich assemblage of plant and animal life to develop, hence they are a rich source of macrophyte biodiversity (Mitsch and Gosselink, 1993). The rapid loss of species from wetlands has led to a decline in productivity, nutrient retention and resistance to invasion by introduced plant species (Tilman, 1996; Loreau, 2000; Naeem et al., 2000). Despite their importance, wetlands are being continuously modified or reclaimed for agricultural purposes.

In Zimbabwe, wetlands are locally known as dambos (Whitlow, 1984; 1985b). They cover about 1.28 million ha or 3.6 % of the country's land area and 84% of them are located towards the north of the country on the undulating highveld plateau above 1200 m, where the mean annual rainfall is above 800 mm. The dispersed distribution of dambos has made them accessible to a high proportion of the rural population (Dambo Research Unit, 1987). The use of dambos as pasture and cultivation area has increased due to the growing rural population and economic pressures (Whitlow, 1983). As a result, dambo cultivation has become a well-established tradition amongst rural farmers in Zimbabwe such that their gardens provide a regular supply of crops (Whitlow, 1983; Acres, 1985), which is especially important during drought years.

The ecological value of dambos in Zimbabwe has been taken for granted because of incorrect public perceptions, poor legislation and conservation strategies that are not backed by adequate scientific research (Matiza, 1994). This makes it difficult to plan for dambo conservation and to integrate conservation and development goals at a local level. Much of what is known about local dambos relates to their agricultural uses (Whitlow, 1985a). There is a need to continue research on dambos and their biodiversity, especially in view of the growing level of human impacts that are contributing to their destruction. This study investigated how the exploitation of dambos may change their vegetation composition, soil properties and how that will in-turn affect the surface water quality by comparing a dambo disturbed through agriculture with a relatively undisturbed one.

## Materials and Methods

### *Study Area*

Madikane and Dufuya dambos are located 90 km west of Gweru in the Zimbabwean Midlands *(Figure 1)*. The area is overlaid by Kalahari Sands with gentle relief that has allowed the extensive development of dambos. The soil in the wetter parts of the dambos tends to be waterlogged and rich in organic matter and they support areas of grassland interspersed amongst the woodland. The average rainfall of the area is 650 mm as it is throughout Zimbabwe. Rainfall is strongly seasonal with one rainy season lasting from November to March.

The Dufuya system is located at 19°16’S, 29°19’E at an altitude of 1274 m next to Dufuya Primary School along the Sogwala road and drains into the Somkamba stream, a tributary of the Vungu River. The dambo area is 63.3 ha in extent, 28.2 ha is cultivated with about 212 gardens (mean area = 0.127 ha). Water drains into the dambo from a large spring, which is covered by a dense growth of vegetation. An intermittent stream meanders southwards from the spring creating a perennially damp marshy strip that bisects the system with two similar sets of gardens flanking either side of the open marshy strip. The gardens near the central drainage area are wetter than those towards the edge of the dambo that are drier and have sandier soils.

Madikane dambo is located at 19°14’S, 29°15’E at an altitude of 1256 m and is about 15 km from the Dufuya dambo and it also drains into the Somkamba stream. It is protected by the Environmental Management Agency as well as by the local villagers to whom the area is sacred and therefore cultivation is not permitted. Water drains from a spring into the dambo and meanders south into Somkamba stream.

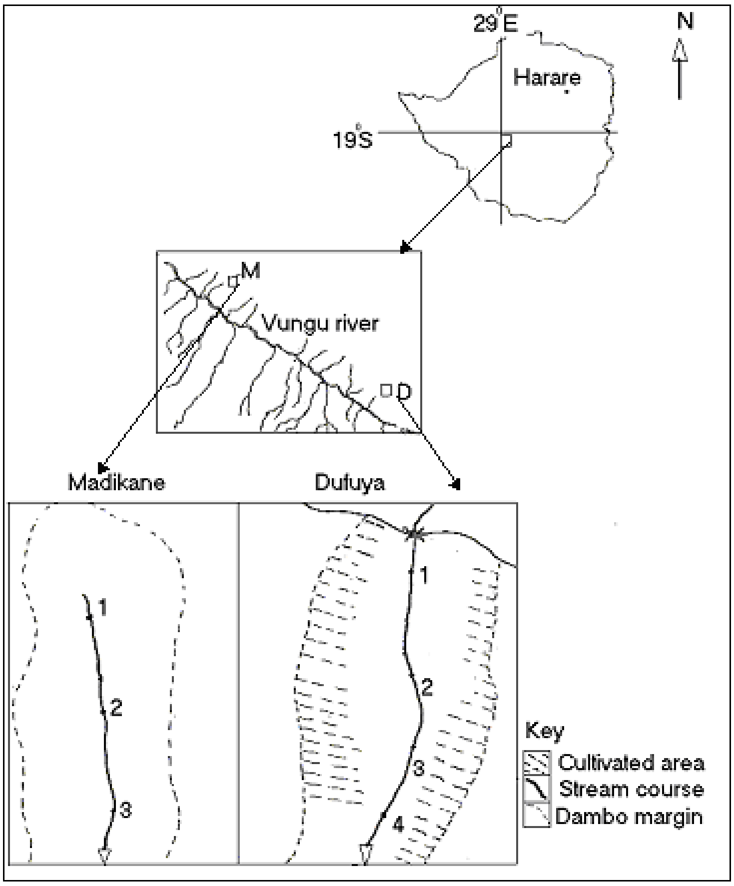

***Figure 1.*** *The location of the study sites showing the spatial relationship of the dambos Madikane (M) and Dufuya (D) and sampling stations*

### *Diversity assessment*

Plots that covered the outer and the central dambo zones were selected for the sampling of macrophytes. The two zones in the wetland were identified according to the amount of standing water in them. The outer area was marshy whereas the central zone was swampy with open water zones. Plants were sampled by means of quadrats. Line transects of 80 m were laid at 15 m intervals in Madikane dambo and at 50 m intervals in Dufuya. This was done so as to have an equal number of quadrats from both dambos. Plants were sampled along each transect by laying a 25 cm × 25 cm quadrat at 10 m intervals and recording the species composition, species abundance, and percentage cover in each quadrat. The plants encountered were identified with the help of the national herbarium using standard identification keys for the flora of Southern Africa (Bennet, 1980; Chapano, 2002).

### *Soil characteristics*

Soil sampling was done in November 2004. Soil samples were taken with a soil auger measuring 5 cm in diameter and 20 cm in depth. Fifteen surface (0-20 cm) and five subsurface (20-100 cm) cores were taken from each dambo. The samples were placed into self-sealing plastic bags and stored in a cooler until laboratory analysis. The pH of the soil was determined with a Philips digital pH meter (PW 9419) in a 2:1 water to soil ratio. The soil samples intended for the determination of total organic carbon were air dried, homogenized with a pestle and mortar and passed through a 2 mm sieve. The samples were then oxidized with a mixture of 1N potassium dichromate ($K_2Cr_2O_7$) and concentrated sulphuric acid ($H_2SO_4$) and the soil digest was titrated against a 0.2 M ferrous ammonium sulphate solution (Schnitzer, 1982).

Exchangeable ammonium was determined from undried samples by extracting with 0.5M solution $K_2SO_4$, and filtration through Whatman 42 filter paper followed by colorimetric estimation. A similar procedure was used for the determination of nitrate-nitrogen except that filtration was followed by cadmium reduction (Keeney and Nelson, 1982). Total nitrogen and total phosphorus were determined by digestion of soil at 110 °C for 1 hour in concentrated sulphuric acid followed by colorimetric estimation. Plant available phosphate was determined by the Bray method (Bray and Kurtz, 1945) in which 2.5 g of air dried soil was extracted with Bray P2 solution (0.03 N $NH_4F$ and 0.1 N HCl) followed by colorimetric estimation. Particle size distribution was determined by the hydrometer method in which 50 g of air dried soil was saturated with distilled water. The soil suspension was mixed with 300 ml tap water in a graduated cylinder where hydrometer readings and the temperature of the suspension were taken after 40 seconds and after 2 hours (Gee and Bouder, 1986).

### *Water quality*

Water samples were taken in November 2004, January 2005 and March 2005 from 5 sampling points in Dufuya dambo and 3 sampling points in Madikane dambo (*Figure 1*). At each sampling point, the following physical and chemical variables were measured with a Horiba UU 23 multi-probe meter (Horiba Ltd, Japan): temperature (°C), dissolved oxygen (mg $l^{-1}$), turbidity (NTU), conductivity ($\mu$S $cm^{-1}$) chloride, calcium and pH. Duplicate samples were taken at a depth of 10 cm with a plastic water sampler and placed in 1L polyethylene bottles and stored in an icebox at 4°C. The samples were transferred to the laboratory for the determination of total nitrogen, total phosphorus and ammonium nitrogen. The samples were analysed within 24 hours using HACH ER/ 04 kits. Total phosphorus was determined by the acid persulfate digestion method which measured soluble reduced phosphate in the digested solution (method 8190). Total nitrogen was determined by alkaline persulfate digestion (method 10071) while ammonia was determined by the salicylate method (method 10023).

### *Data analysis*

The plant data collected were used to compute community indices such as Sorensen's similarity index, Shannon-Wiener's diversity index, and Shannon index of evenness so as to evaluate the differences in the plant diversity of the two dambos (Sorensen, 1948; Shannon and Wienner, 1963). Cluster analysis (Minitab 13.0) was used to compare the two dambos in terms of their species composition by grouping quadrats with more similar species into the same clusters. Cluster analysis is an agglomerative method that starts with small groups of few samples, and progressively groups them into larger homogeneous units until the whole data set is sampled (Everitt, 1993). ANOVA was used to test for the significant differences in the soil physical and chemical parameters and water variables between the dambos (Minitab, 1995).

## Results

### *Plant diversity*

A total of 51 species were recorded in the two dambos *(Table 1).* Madikane had 39 species belonging to 17 families, while Dufuya had 29 species belonging to 10 families. Thirty one percent of the species occurred in both dambos. Grasses made up 18% of the total in Madikane and 14% in Dufuya and only one tree species, *Ficus thonningii*, was recorded (in Madikane). Exotic species were present in both dambos but they were more numerous in Dufuya with five species, amounting to 17% of the total, compared to Madikane with only three species, or 5% of the total.

Species diversity was higher in Madikane (H' = 2.52) than in Dufuya (H' = 2.14). The dominant species in Madikane were *Thelypteris confluens, Kyllinga erecta, Carex cognata, Cyperus rotundus,* and *Cyperus articulatus*, which made up 75% of the total. In Dufuya, the dominant species were *Fimbristylis dichotoma, Kyllinga erecta* and the unidentified Asteraceae Species A, which made up 70% of the total. There was a significant difference in species composition between Madikane and Dufuya (t-test, $p < 0.05$).

The central region of Madikane was dominated by *Thelypteris confluens* (15.87 %), *Phragmites australis* (15.84 %) and the *Cyperus articulatus* (36.08 %) while the central region of Dufuya was dominated by *Kyllinga erecta* (29.79 %), *Fimbristylis dichotoma* (22.4 %) and the Asterceae Species A (23.02 %). The Sorensen similarity index for the central regions was 11%. The outer region of Madikane was dominated by *Cyperus rotundus, Kyllinga erecta* and *Thelypteris confluens* while the comparable regions of Dufuya were characterized by species of disturbed ground, *Cleome gynandra* (2%), *Bidens pilosa* (1%), *Amaranthus hybridus* (4%) and *Cynodon dactylon* (7%) a species that occurs widely in overgrazed areas.

A cluster analysis of species composition separated the dambos into three distinct clusters with Madikane and Dufuya being distinctively separated *(Figure 2).* The outer zone of Madikane was widely separated from the central zone and was rather closer to the Dufuya cluster. This was a result of 22 species that were present in Madikane but absent in Dufuya, 12 species present in Dufuya but absent in Madikane and 10 species that were common to Dufuya and the outer region of Madikane. The central region of Madikane was separated from the rest of the regions because it was dominated by *Thelypteris confluens* and *Phragmites australis* suggesting a wet environment in that region.

***Table 1.*** *The mean composition (% by numbers) of macrophytes in two different zones of the two dambos (O = outer, C = central, M = Madikane, D = Dufuya). Species marked * are exotics and † are herbs.*

| Family | Species | MO | MC | DO | DC |
|---|---|---|---|---|---|
| Acanthaceae | *Hypoestes forskalei* † | | | 0.44 | 0.05 |
| Amaranthaceae | *Amaranthus hybridus** † | | | 3.51 | |
| Anacardiaceae | *Pyroides pyroides* | | 0.11 | | |
| Apiaceae | *Hydrocotyle bonariensis** † | 3.37 | 3.42 | | |
| Asteraceae | *Conyza welwitschii* † | 1.35 | 0.15 | | |
| | *Pseudognaphalium album* † | 0.03 | | | |
| | *Osteospermum monocephalum* | 0.38 | | 0.08 | 0.32 |
| | *Senecio strictifolius* † | | 0.04 | | |
| | *Blumea solidaginoides* † | 0.10 | | | |
| | *Conyza sumatrensis** † | | | 0.65 | |
| | *Lactuca indica* † | | | 0.13 | 0.11 |
| | *Bidens pilosa* † | | | 1.30 | |
| | *Otomeria elatior* † | | | 4.75 | |
| | Species A | 10.73 | 1.75 | 19.36 | 23.02 |
| | Species B | 0.75 | | | |
| Capparidaceae | *Cleome gynandra* † | | | 1.19 | |
| Cyperaceae | *Cyperus rotundus* | 19.34 | | 0.43 | |
| | *Kyllinga alba* | 4.76 | | 4.54 | 2.95 |
| | *Fuirena pubescens* | 0.02 | | | |
| | *Kyllinga erecta* | 14.08 | 2.33 | 35.10 | 29.79 |
| | *Carex cognate* | 7.63 | 12.89 | 0.08 | 3.46 |
| | *Fimbristylis dichotoma* | 0.10 | | 13.65 | 22.40 |
| | *Cyperus articulates* | 8.82 | 36.08 | 0.15 | 0 |
| Fabaceae | *Tephrosia* spp. † | 0.02 | | | |
| Fumariaceae | *Chironia palustris* † | 0.03 | | | |
| Malvaceae | *Sida rhombifolia* † | | 0.04 | | |
| Moraceae | *Ficus thonningii* | | 0.07 | | |
| Musaceae | *Musa caventish** | | | | 0.03 |
| Onagraceae | *Epilobium salignum* † | 0.10 | | | |
| | *Ludwigia stolonifera* † | 0.13 | | | |
| Orobanchaceae | *Cycnium tubulososum* † | 0.48 | 0.55 | 0.79 | 3.17 |
| Poaceae | *Panicum repens* | 5.37 | | 1.23 | 7.76 |
| | *Cynodon dactylon* | 1.87 | | 7.34 | 0.99 |
| | *Eragrostis inamoena* | 0.38 | | | |
| | *Paspalum scrobiculatum* | 0.25 | | | 1.29 |
| | *Andropogon eucomus* | 0.02 | | 0.32 | 0.35 |
| | *Sporobolus pyramidalis* | 0.01 | | 0.87 | |
| | *Eragrostis cylindriflora* | | | 0.08 | |
| | *Eriochloa macclounii* † | | | 0.08 | |
| | *Eragrostis heteromera* | | | 2.60 | |
| | *Paspalum urvillei** | | | 0.43 | 3.14 |
| | *Phragmites australis* | 1.47 | 15.84 | | |
| | Species C | | 4.88 | | |
| | Species D | 4.27 | 3.39 | | |
| | Species E | 0.15 | | 0.22 | 0.24 |
| Rosaceae | *Potentilla* spp.* † | | 0.07 | | |
| Rubiaceae | *Richardia scabra* † | | 0.22 | | |
| Solanaceae | *Physalis peruviana* † | | 0.11 | | |
| Thelypteridaceae | *Thelypteris confluens* † | 13.06 | 15.87 | | |
| Tyhaceae | *Typha capensis* † | 0.92 | 2.22 | 0.65 | 0.91 |
| Verbenaceae | *Lantana camara** | 0.01 | | 0.02 | |
| Species diversity (H’) | | 2.48 | 1.92 | 2.09 | 1.88 |
| ($E_H$) | | 0.72 | 0.65 | 0.63 | 0.66 |

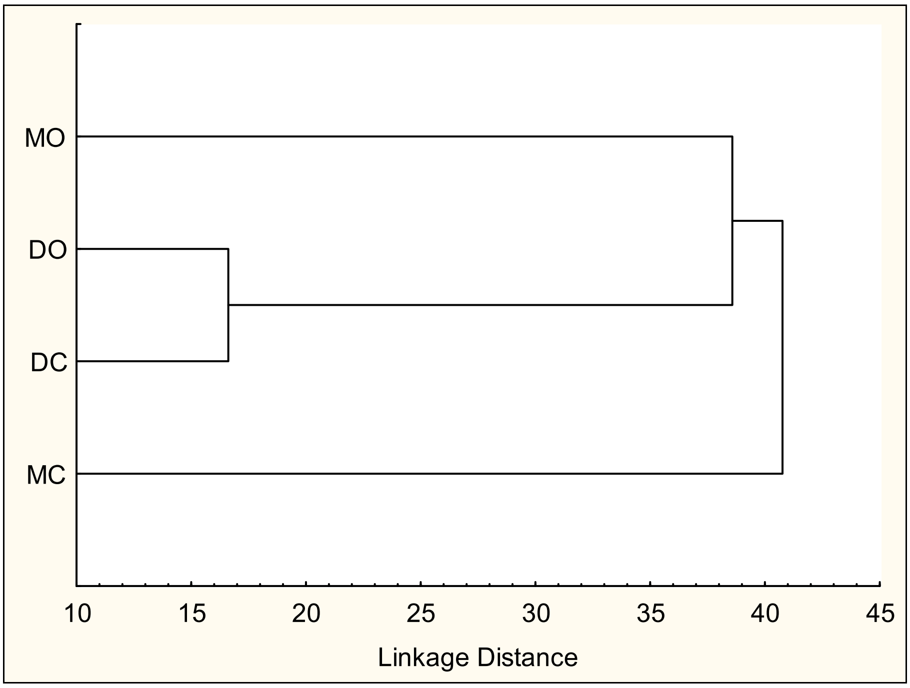

***Figure 2.*** *A dendrogram of species composition in the study areas (O = Outer and C = central; M = Madikane and D = Dufuya)*

### *Soil Properties*

The concentrations of ammonia, nitrate-nitrogen and total nitrogen in the soil was significantly higher in the surface layers of Madikane than in Dufuya *(Figure 3)*. This was also true for ammonium and total nitrogen in the deeper layers. Nitrate-nitrogen was much higher in the deeper layers of Dufuya. The differences between the two dambos were significant (ANOVA, $p < 0.05$) in each case.

The concentration of phosphorus was higher in Dufuya than Madikane *(Table 2)*. Soil pH was relatively higher in Madikane than Dufuya with a range of 4.98 – 10.15 and 5.26 – 7.86 respectively. The slight acidity of the soil in some parts of Dufuya and Madikane suggest the release of humic acids from organic matter decomposition.

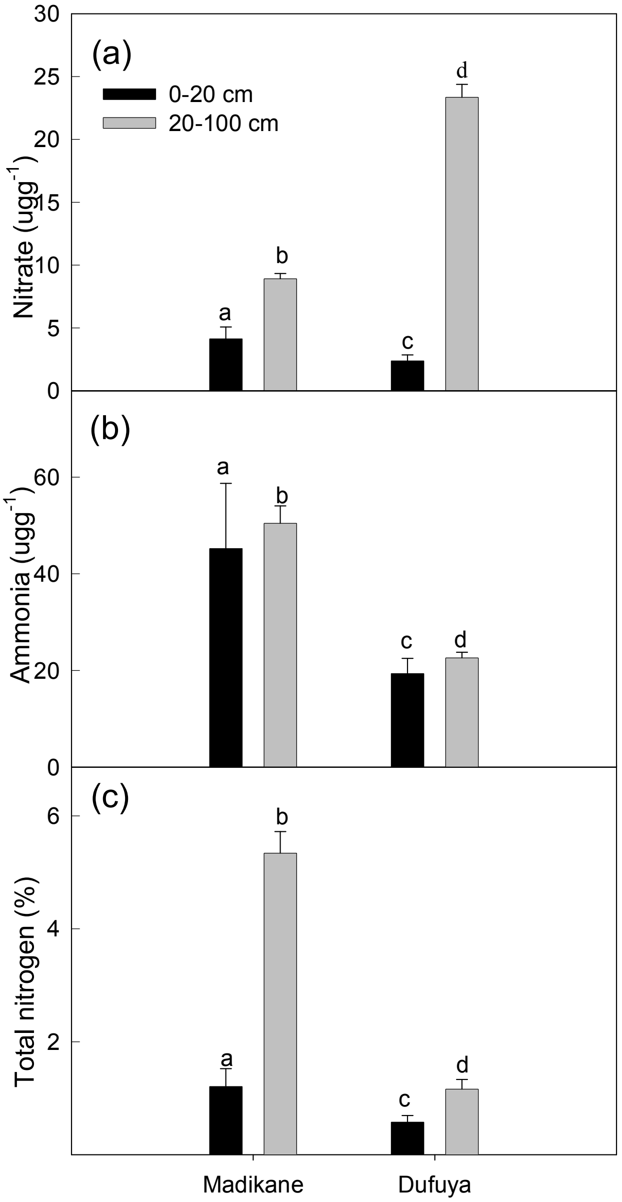

***Figure 3.*** *Mean concentrations of (a) nitrate nitrogen (µg $NO_3$ $g^{-1}$ soil), (b) ammonium nitrogen (µg $NH_4^+$ $g^{-1}$ soil) and (c) total nitrogen (%), in surface (0-20 cm) and subsurface (20-100 cm) samples. Values presented are means ± standard deviation. Values with the same superscript are not significantly different ($p > 0.05$)*

***Table 2.*** *Mean concentration of total phosphorus (P), plant available phosphorus ($P_2O_5$) and pH in Madikane and Dufuya with significantly different ($p < 0.05$) values highlighted in bold*

| | **Madikane** | **Dufuya** |
|---|---|---|
| PH | 6.9 | 6.3 |
| P (%) | 0.03 | 0.04 |
| $P_2O_5$ (µg $g^{-1}$) | **66.48** | **79.25** |

The concentration of organic carbon in both dambos was higher in the central zone than in the outer zone although it was significantly lower in Dufuya *(Figure 4)*. The concentrations in the drier outer zones were much lower than in the central zones and although the differences between the two dambos were relatively small they were still significant. Moisture content in the central and outer zones ranged between 60-90% and 1-45% respectively.

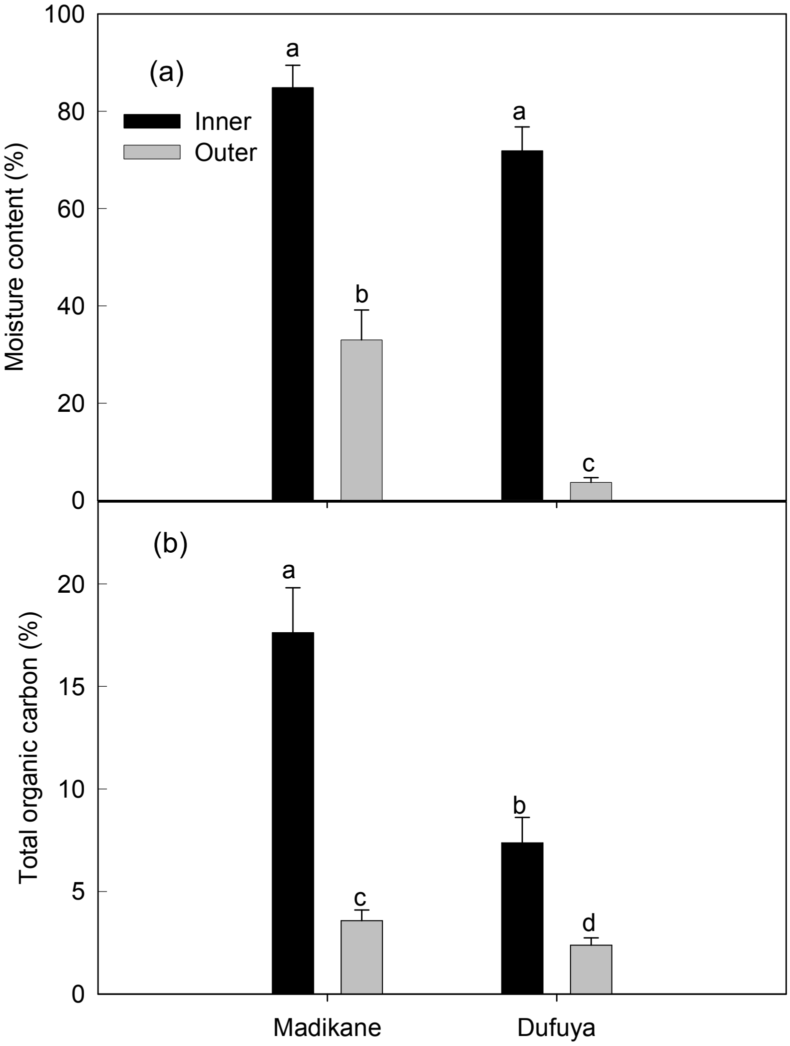

***Figure 4.*** *Mean content of (a) moisture (%) and (b) total organic carbon in the outer and in the central zone of Madikane and Dufuya. Values presented are mean ± standard deviation. Values with the same superscript are not significantly different (ANOVA: $p > 0.05$)*

There was a significant correlation between the amount of total organic carbon and moisture content in Madikane (Pearson correlation, $r = 0.74$) and Dufuya ($r = 0.88$). The amount of total organic carbon significantly increased with the increase in moisture content *(Figure 5)*. The increase in organic carbon with moisture content was more pronounced in Madikane ($r^2 = 0.78$) than Dufuya ($r^2 = 0.54$).

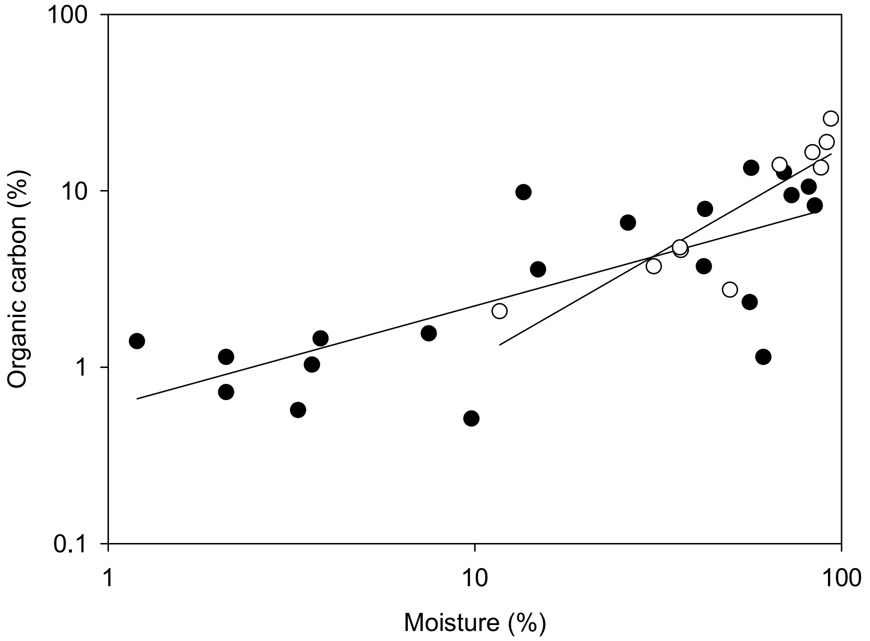

***Figure 5.*** *The relationship between moisture content and organic carbon in Dufuya (●) and Madikane (○). Regression lines were fitted as follows: $y = -0.22 + 0.57x$, $r^2 = 0.54$ (Dufuya) and $y = -1.15 + 1.20x$, $r^2 = 0.78$ (Madikane)*

The majority of soils sampled in the two dambos were loamy sand or sandy loam with clay content ranging from 3-29% in Dufuya and 5-19% in Madikane *(Table 3)*. Clay loam soils were only present in two sites in Dufuya. There were no significant differences detected in particle size composition between the two dambos, % silt (t-test, $p > 0.05$) and % clay ($p > 0.05$).

***Table 3.*** *The physical structure of soil in Madikane and Dufuya. Values presented are means ± standard deviations. Values with the same superscript are not significantly different (t-test: $p > 0.05$)*

| | **Sand** | **Clay** | **Silt** |
|---|---|---|---|
| Dufuya | $72.2 \pm 14.75^a$ | $13.0 \pm 7.01^b$ | $14.8 \pm 9.34^c$ |
| Madikane | $77.8 \pm 9.19^a$ | $10.7 \pm 4.33^b$ | $11.5 \pm 6.82^c$ |

## *Water quality*

The concentration of total nitrogen was relatively low in both dambos ($1.30 \pm 0.23$ mg $L^{-1}$ in Madikane dambo and $1.17 \pm 0.25$ mg $L^{-1}$ in Dufuya) but there was no significant difference between them (ANOVA, $p > 0.05$). The concentrations of ammonium nitrogen levels ranged from 0.01 to 0.05 mg $L^{-1}$ in both dambos and the differences between them were also not significant *(Table 4)*. The concentration of phosphorus was significantly higher ($p < 0.05$) in Dufuya ($1.30 \pm 0.26$ mg $L^{-1}$) than in Madikane ($0.66 \pm 0.12$ mg $L^{-1}$). Calcium was significantly higher in Dufuya than in

Madikane but there was no significant difference ($p > 0.05$) in the concentration of chloride. The pH of water in Madikane varied from 6.71 to 10.0 while it was nearly neutral in Dufuya (6.70 to 7.07). The conductivity was significantly ($p < 0.05$) higher in Dufuya (287 ± 36.18 µS $cm^{-1}$) than in Madikane (125 ± 3.61 µS $cm^{-1}$) but the concentration of dissolved solids was the same in both wetlands with a range of 0.1 to 0.2 g $L^{-1}$. The concentration of dissolved oxygen in Madikane ranged from 4.8 – 6.3 mg $L^{-1}$ and in Dufuya from 6.6 – 8.1 mg $L^{-1}$.

***Table 4.*** *Water quality variables of Madikane and Dufuya*

| | **Madikane** | **Dufuya** |
|---|---|---|
| AN (mg $L^{-1}$) | 0.05 ± 0.01 | 0.03 ± 0.02 |
| TDS (g $L^{-1}$) | 0.13 ± 0.05 | 0.12 ± 0.03 |
| N (mg $L^{-1}$) | 1.30 ± 0.23 | 1.17 ± 0.25 |
| P (mg $L^{-1}$) | 0.66 ± 0.12 | 1.30 ± 0.26 |
| Ca (µg $L^{-1}$) | 1.21 ± 0.42 | 2.23 ± 0.34 |
| Cl (mg $L^{-1}$) | 1.77 ± 0.17 | 2.05 ± 0.25 |
| pH | 6.76 ± 0.04 | 7.01 ± 0.04 |
| Conductivity (µS $cm^{-1}$) | 125 ± 3.61 | 287 ± 3.18 |
| DO | 5.66 ± 0.54 | 7.11 ± 0.38 |

## Discussion

The impact on vegetation structure was reflected by the differences in species composition between the rather pristine Madikane and impacted Dufuya sites. The species that were present in Madikane indicate a permanent or semi-permanent wetness (*Phragmites australis*, *Ludwigia stolonifera, Cyperus articulatus, Carex cognata, Typha capensis, Epilobium salignum* and *Thelypteris confluens*) (Everett, 1982a, 1982b) compared to species that require well-drained soils and can tolerate arid environments (*Amaranthus hybridus*, *Cleome gynandra and Hypoestes forskalei*), as in Dufuya. The dominance of species adapted to an arid environment especially in the outer region of Dufuya are indicative of the shrinking size of the dambo with increasing drought stress on the local biota. A continuous cultivation in Dufuya may therefore result in its disappearance.

The central regions of the two dambos were characterized by low species abundance but a different species composition. Competition with *Phragmites australis* and *Cyperus articulatus* in the centre of Madikane, *Kyllinga erecta* and *Fimbristylis dichotoma* in the centre of Dufuya could be strong and hence contribute to the low species diversity. These species tend to grow and colonize rapidly (Everett, 1982a, 1982b). The low species diversity in the central zones can also be attributed to high moisture content as few species are adapted to survive in areas where the soil is permanently waterlogged (Acres, 1985). Variation in competitive ability due to variation in soil characteristics is one possible mechanism allowing the local coexistence of plant species (Heather et al., 1997).

Dambo utilization resulted in a decrease of the indigenous species and facilitates the settlement of introduced and exotic species. *Cleome gynandra* and *Amaranthus hybridus,* indicator weeds for disturbance (Everett, 1982a) were only present in Dufuya. Fertilisation of natural ecosystems with phosphorous promotes common species at the expense of rare ones (Guseswell et al., 2005).

The use of dambos can also change the vegetation growth form. This was shown by the presence of species indicating disturbance in Dufuya. *Cynodon dactylon* is a perennial species and an indicator for grazing. It has high cover abundance but no dominating effect because of its short growth form. *C. dactylon* had lower cover abundance in Dufuya compared to Madikane. *Panicum repens*, a species which grows in the transition zone between land and water is also preferred by herbivores (Skarpe, 1997). A shift of species composition from perennials to annuals due to dambo utilization was observed in Dufuya dambo. The annual species that dominated in Dufuya especially the outer regions were *Eragrostis cylindriflora*, *Paspalum urvillei, Cleome gynandra*, *Amaranthus hybridus, Panicum repens* and *Bidens pilosa*. In Madikane, there was a mixture of annual and perennials. The change in species composition through extinction is thought to alter the way solar energy is captured and the rate at which matter is cycled in an ecosystem (Chapin et al., 2000; Cardinale et al., 2002).

The levels of soil nitrogen were generally higher in Madikane than Dufuya. This is not an unusual feature in peat soils as it is explained by decomposition and mineralization of plant material which releases nitrogen back into the soil (Zedler, 2000). Fertilization of agricultural land led to an accumulation of phosphorus and nitrogen (Compton and Boone, 2000). The slightly higher level of phosphorus in Dufuya suggests the influence of external sources. The low levels of phosphorus in Madikane are also explained by uptake with rapidly growing dambo vegetation (Bruland et al., 2003) since flooded soils are associated with reducing conditions which increase the solubility and mobility of phosphorus (Zedler, 2000). A change in the levels of nitrogen and phosphorus may shift the composition of a plant community by promoting a rapid growth of species that are best adapted. Low nutrients and high base concentrations have been found to be responsible for higher plant diversity in wetlands (Wheeler and Shaw, 1995). Hence some of the observed differences in species composition may be explained by the differences in nutrient composition. Species richness was raised in wetlands through phosphorus fertilisation when the initial N: P ratio of vegetation was above 2.0 (Gusewell et al., 2005).

Agricultural activity in hydric soils does not always result in the depletion of soil organic carbon (Shaffer and Ernst, 1999). In our study, it was shown that the wetness of the soil, which was influenced by the position in the dambo, had an effect on total organic content *(Figure 5)* since organic carbon increased with an increase in moisture content. Few micro organisms that breakdown organic matter are adapted to live in relatively wet environments (Mclatchey and Reddy, 1988). The slight differences in organic carbon between Madikane and Dufuya may be attributed to cultivation since organic carbon is assumed to be 58 % of total organic matter. Significant changes in soil physicochemical properties characterized by high bulk density and low organic matter were observed in meadows and pastures under utilisation (Kulik et al., 2007).

In Dufuya, the pH in water rapidly increased as the soil pH increased. This suggests a loss of $Ca^{2+}$ and other cat-ions from the soil. Lime ($CaCO_3$) that is used to control soil pH act as a source of $Ca^{2+}$. The effect of liming is also indicated by the small range of water pH in Dufuya than in Madikane. The increase of $Ca^{2+}$ was also shown by a high conductivity of water in Dufuya than Madikane. There was a significant difference in the water quality between Madikane and Dufuya as evidenced by the differences in nutrient concentrations of phosphorus and nitrogen. The ability to improve water quality is often high in the presence of species like *Phragmites australis* and *Typha capensis*

(Finalyson et al., 1986; Kadlec and Knight, 1996), which were more dominant in Madikane than in Dufuya. The high concentrations of total nitrogen recorded in water from both dambos (*Table 4*) are not an unusual feature as it is a characteristic of water from peat soils. The levels of nitrogen recorded in this study are close to those reported in Carolina and coastal wetlands (Bruland et al., 2003; Walbridge and Richardson, 1991).

Most of the nitrogen recorded in water could have been derived from peat soils through nitrification of ammonia produced from organic matter decomposition (Avinmelech et al., 1978) as evidenced by the high levels of nitrogen in the soil. The higher conductivity in Dufuya (287 $\mu$S cm$^{-1}$) compared to Madikane (125 $\mu$S cm$^{-1}$) is also explained by nutrient loading. Lime ($CaCO_3$) that is used to control soil pH act as a source of calcium. Leaching of calcium ions and other cations tend to increase the conductivity of water. A long term monitoring of the physical and chemical variables is needed so as to assess the extent of the impact of agriculture.

As dambo cultivation has to be fully recognized as an economic activity especially in the rural community, the question on dambo utilization is mainly how they should be used in a sustainable way. The current policy on dambo utilization has to be changed since it has allowed degradation to progress unchecked. Dambo farming under proper management emphasizing on community responsibility can result in sustainable use since dambos are a common property with no specific person responsible for their use or misuse. The management strategy has to be ecologically sound, economically viable, socially just, humane and adaptable.

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