Small mammal – heavy metal interactions in contaminated floodplains

Bioturbation and accumulation in periodically flooded environments

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Cover front: Sunrise in the 'Afferdensche en Deestsche Waarden' (ADW) in winter.
Cover back: Sunrise in the ADW in summer.
Microtus arvalis (Common vole).

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As water spins in circles twice
Spiders, snakes and the little mice
Get twisted around and tumble down
When Nature calls we all shall drown

J. Edlund, 1994


By Erica Nabben-van Montfort & Annelies Vogels

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Ecosystems can be regarded as dynamic complexes of interactions between biota on one hand and the environment on the other (Noss, 1996). At the same time inter- and intra-species interactions take place, while environmental characteristics also affect each other. Changing one factor can affect the whole system. A factor that has received a great deal of attention in scientific research during the last decades is that of contaminants in ecosystems. Although contaminants are part of the natural environment when they are natural components (Crommentuijn et al., 2000) they are considered components of pollution when their concentrations increase within a short time (in terms of geographical or biological timescales), often due to human activities, and effects on ecosystems can be expected. In Western Europe, and many other parts of the world, floodplain ecosystems have received large contaminant inputs, especially during the twentieth century (Vink et al., 1999; Schouten et al., 2000). However, this was not the sole factor influencing natural ecosystems, as there were numerous other anthropogenic influences such as agricultural activities, sand and clay excavations, construction of embankments and recreation in floodplains (Middelkoop and Van Haselen, 1999). In recent decades, efforts are being made to restore floodplain ecosystems, by supporting river and floodplain dynamics and the natural development of the morphology and vegetation (Nienhuis et al., 2002). Attempts are made to reduce anthropogenic influence as much as possible within the restrictions imposed by safety and economic considerations. However, the role and impact of contaminants in floodplain ecosystems has remained largely unclear (SSEO, 1999). Since it can be questioned whether it is possible to restore natural floodplain ecosystems that are under the influence of these contaminants, it is important to obtain a better understanding of the current interactions between contaminants, biota and the abiotic environment. In view of their persistent character, possible impact on ecosystems, and the large quantities present in the environment within floodplains, this thesis focusses on heavy metals, especially Zn, Cu, Pb and Cd, and their interactions with small mammals. The study concentrated on common vole, mouse, shrew and mole species as they are abundant, fulfil an important role in floodplain food webs and landscape engineering, and differ in terms of ecological niches, characteristics and functioning.

The interactions between biota, the environment and contaminants can be described by processes affecting distribution patterns of mammal and metal species and floodplain characteristics (e.g. patterns, behaviour, speciation) (Fig. 1). Processes causing the interactions are (1) bioturbation, (2) heavy metal accumulation and toxic effects, (3) habitat suitability, (4) landscaping, (5) heavy metal distribution and speciation, (6) competition and toxic effects.

1) Bioturbation

Small mammals directly and indirectly affect heavy metal distribution and speciation, as their burrowing activities redistribute substrate with heavy metals in a vertical or horizontal direction. Burrowing activities result in the mixing of substrate layers with different metal concentrations and/or displacement within the soil by tunnelling. This results in the surfacing of substrate from tunnels as hillocks or surface runs, and the redistribution of substrate at the surface by digging to create burrows and obtain food. At the same time, soil parameters such as fractionation in particles and contact between air and/or water and substrate particles are influenced by surfacing or changes in the degree of compaction. Changing soil parameters will affect metal speciation.

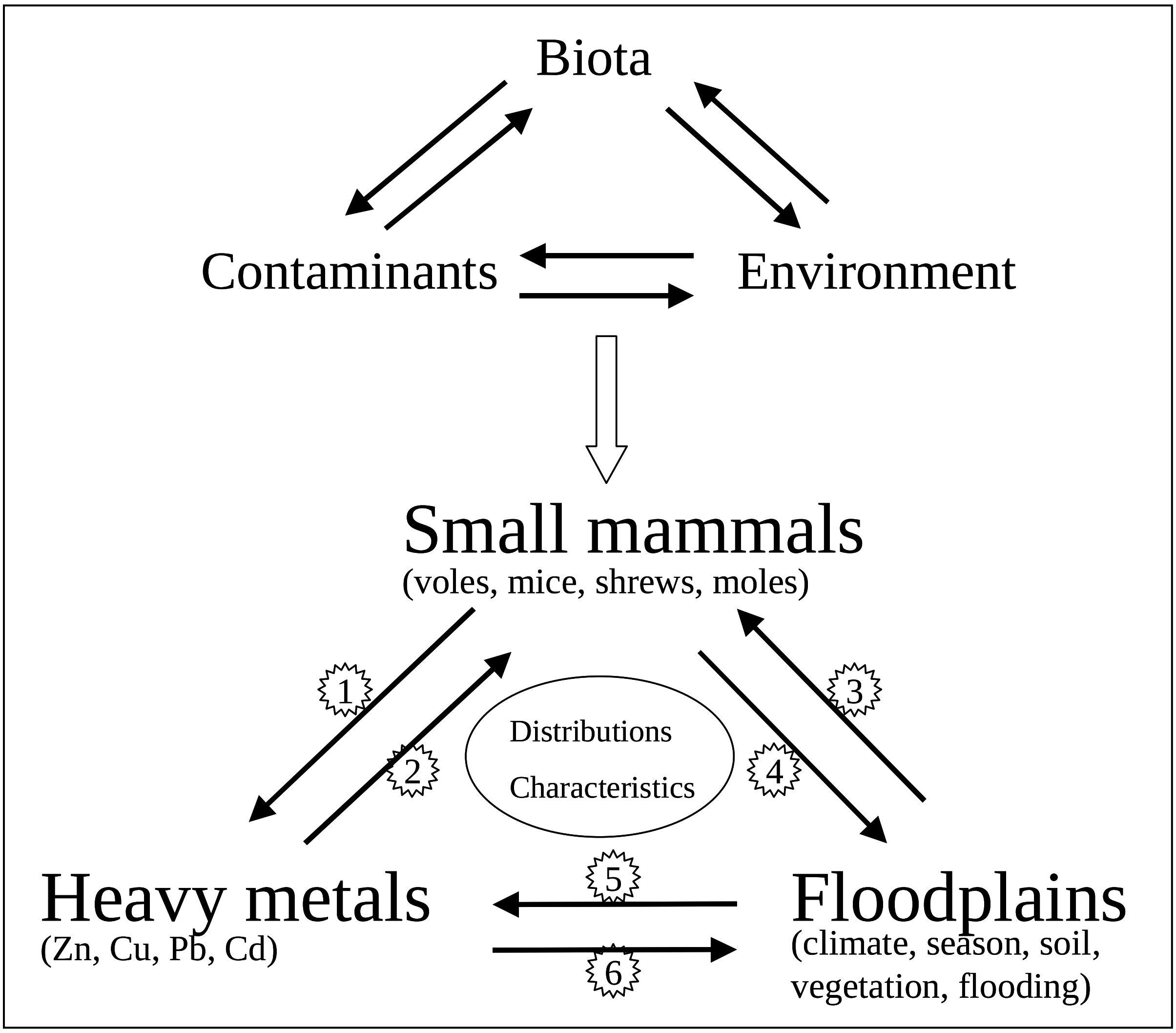

Fig. 1: Schematic representation of the interactions between small mammals and heavy metals in floodplains as a specific case of the general biota – contaminant – environment interactions which can be described for every contaminated system. Numbers indicate different processes and interactions; 1. bioturbation; 2. heavy metal accumulation and toxic effects; 3. habitat suitability; 4. landscaping; 5. heavy metal distribution and speciation; 6. competition and toxic effects.

2) Heavy metal accumulation and toxic effects

Small mammals are exposed to heavy metals, directly via drinking water or substrate intake, but more importantly via their food (plants and/or macro-invertebrates). At the various trophic levels, metals can be stored in specialised tissues or organs, or excreted. A certain proportion enters the small mammals, where it can also be stored or excreted. When critical concentrations are reached during exposure, toxicological effects on individual specimens can be expected. In case of severe heavy metal exposure, ecotoxicological effects can even be observed on population level.

3) Habitat suitability

The characteristics of the floodplain landscape determine the distribution and composition of the small mammal community. Each species needs a particular vegetation structure, soil type and other specific conditions for all aspects of their lives, like shelter, feeding and nesting. The configuration of suitable and unsuitable habitats also indirectly influences the temporal distribution of species. A factor with major impact is flooding, which periodically turns most of the floodplain into an unsuitable area, as well as causing high mortality in small mammal populations. This phenomenon makes connectivity of elevated areas in the floodplain important as recolonisation after floods is a process of re-emergence. Abiotic factors like temperature and rainfall influence population development directly (e.g. by initiating the onset of breeding and by causing mortality) or indirectly, for instance via food availability.

4) Landscaping

Small mammals, especially in large numbers, can have a significant effects on the landscape by grazing, manuring and fertilising, and by preying, or being preyed upon by other animals. By grazing, herbivorous small mammals can affect the species composition and biomass of vegetation. By feeding, storing food, making nests and burrowing, they introduce organic matter, nutrients and air into the soil, thus promoting plant growth and macro-invertebrate activity. Insectivores can also indirectly affect vegetation by preying on herbivorous macro-invertebrates, as well as earthworms which are important for landscape structuring. The availability of prey can attract large predators which can indirectly also have an impact on the landscape, as these predators will also landscape their environment. In fact, bioturbation is also a form of landscaping.

5) Heavy metal distribution and speciation

Landscape structure and other characteristics help determine for the distribution and speciation of heavy metals. Heavy metals enter the floodplain during floods and are distributed according to the flow patterns, which are influenced by elevation and the presence of vegetation and other obstacles. Contaminants in soil deposited in the past can be covered by either clean or contaminated sediments, which influences soil parameters and therefore speciation of metals. Factors like substrate type, but also the presence of vegetation affecting pH or organic matter input, affect the speciation of metals in the environment, and this also goes for abiotic factors like temperature and rainfall. Redistribution of surfaced substrate with heavy metals as a result of bioturbation occurs during floods, or by weathering.

6) Competition and toxic effects

Heavy metal contamination in floodplains can influence the vegetation composition as some species are favoured by contaminated environments while others are less competitive in such conditions. At high concentrations, contaminants can even have direct toxic effects on certain plant species.

The processes described above also affect one another. For instance, the surfacing of heavy metals by bioturbation will also affect the exposure risk of biota to the heavy metals. Another example is the high mortality of small mammals in a flood, which leads to a reduction of bioturbation, reducing the rate of redistribution of heavy metals. Further, interactions and processes all differ at temporal and spatial scale. As several of the variables show some periodicity (e.g. seasonality in climatic factors, population development and flooding cycles), various patterns can be distinguished. This makes it possible to calculate, measure or assess the relative importance of these processes.

1.2 Floodplains

Natural floodplains are characterised by recurrent flooding and erosion, sedimentation and succession cycles, resulting in heterogeneous landscapes and dynamic interactions between aquatic and terrestrial systems (Sheppe and Osborne, 1971; Leuven and Poudevigne, 2002; Robinson et al., 2002; Hughes et al., 2005). Thanks to the combination of continuing sedimentation, the input, export and redistribution of organic matter and nutrients, and the recurrent changes in gradients of conditions and environmental characteristics (Baldwin and Mitchell, 2000) these systems are among the most biodiverse in the world (Schuyt and Brander, 2004). Riverine areas are important from an ecological point of view, but have also been of specific human interest throughout the ages. Several present and former floodplain areas are in use as residential areas for industrial and agricultural purposes, and the larger rivers themselves have important transport functions (Van der Velde et al., 2004; Van Stokkom et al., 2005). Most floodplains along large rivers in Western Europe, as well as in other parts of the world, have become embanked and narrowed (Hughes et al., 2005). Rivers have become regulated and straightened (Middelkoop and Van Haselen, 1999; Nienhuis et al., 2002), as frequent floods are undesirable for most societal functions, and a certain permanent minimal river depth and width are necessary for shipping. Notwithstanding the resulting reduction in hydromorphodynamics and the area available for flooding, Western European floodplains still have an important function in nature conservation (Poudevigne et al., 2002; De Nooij et al., 2004), harbouring endangered species and forming ‘blue-green’ corridors through inhabited areas (Foppen and Reijnen, 1998; Baptist et al., 2004). As these important functions for nature conservation are increasingly recognised, various ecological rehabilitation projects are planned or in progress or have already been implemented to restore the hydromorphodynamics (Nienhuis et al., 2002), though within more restricted and sharply delimited areas than in intact floodplains.

Important processes determining species composition, densities and distribution in floodplains are flood events, which can vary in frequency, timing and duration. Species in floodplains have to cope with inundations and show various life strategies, such as maintenance, avoidance and/or mortality followed by recolonisation from non-flooding areas (Stelter et al., 1997; Junk, 1999; Andersen et al., 2000; Klok et al., 2006). Species assemblages are also determined by the characteristics of the landscape, consisting of a mosaic of habitats differing in suitability (Robinson et al., 2002; Van Looy et al., 2005; Ernault et al., 2006). Factors determining the suitability of habitats include the vegetation structure, soil characteristics, elevation, environmental conditions, food availability and shelter opportunities. The importance of these environments and environmental conditions as suitable habitats is different for each species. Besides the presence of habitat of various qualities within the landscape, their configuration in the landscape is also important (Kozakiewicz, 1993; Vos and Chardon, 1998), as connectivity provides opportunities for species to survive or avoid floods or to recolonise. The habitat configuration influences the time necessary to reach a certain population size or distribution from a reduced number of specimens during or after suboptimal conditions. All these factors affect species turnover and the spatial and temporal dimensions of food web composition. Hence, flood defence measures and ecological rehabilitation in floodplains, including excavations and the lowering of floodplain levels and embankments, as well as changes in management regime like grazing or mowing, can influence local species distribution patterns, and may also have favourable or unfavourable effects on organisms in other areas within the floodplain. As floods cause mortality and recolonisation, they are assumed to strongly affect the viability of metapopulations in flood-prone areas. Anthropogenic activities influencing connectivity are assumed to cause larger effects on species distribution in floodplains than in inland areas.

1.3 Diffuse pollution of riverbanks with heavy metals

The large Western European rivers have been and continue to be polluted by potentially toxic substances due to industrial and communal discharges and agricultural activities (Vink et al., 1999). Huge loads of these substances are deposited in the floodplains during high water discharges. Since the water quality in several parts of the rivers has improved during the last decades (Beurskens et al., 1993; Schröder, 2005) and some of these substances are gradually breaking down (Vorenhout et al., 2000), lower concentrations are currently found in the floodplain topsoils. However, several contaminants, including all heavy metals, are persistent and are at best redistributed, transported or mixed, or covered with clean sediments (Middelkoop, 2002). As heavy metals mostly enter the floodplains attached to suspended sediments during floods (Thonon, 2005), there is a diffuse process of contamination across the floodplain areas (Middelkoop, 1997). However, concentrations can vary greatly, even over small distances, due to natural or anthropogenically created sharp borders in terms of elevation or vegetation structure affecting sedimentation patterns (Asselman and Middelkoop, 1995). Other causes of major heterogeneity in contaminant distribution include excavations, point sources from industrial activities and local sources from agricultural activities. Thus, large quantities of heavy metals are still present in Western European floodplains, with Zn, Cu, Pb and Cd often being highlighted with respect to risks of accumulation in floodplain food webs, ecotoxicological effects on floodplain ecosystems and floodplain rehabilitation (Hendriks et al., 1995; Kooistra et al., 2001a; Notten et al., 2005a).

Heavy metals in floodplains can be present in various forms causing differences in solubility, transportability, extractability or chemical availability, as well as biological availability, including possible toxicological availability after uptake (Peijnenburg et al., 1997; Allen, 2002; Vijver, 2005). The distribution over different fractions, from the solid to the dissolved phase, either in complexes or as free ions, depends on the soil characteristics and other environmental conditions, and differs for different metals (Schröder, 2005). Important factors determining metal partitioning in floodplain topsoils are organic matter, clay and calcium carbonate content and factors like pH, redox potential and water content (Sauvé, 2002). The partitioning of metals in floodplain soils is chemically and physically influenced by environmental factors and conditions like flood events, rainfall, temperature, water table and biological activity by micro-organisms, plants and animals (Hesterberg, 1998; Eijsackers and Doelman, 2000; Vorenhout et al., 2000). The distribution of the metals across the different forms over time, influences the fate of metals in both the vertical and horizontal distributions in soil layers, and influences the potential for accumulation in food webs.

Generally speaking, the availability and solubility of Zn, Cu, Pb and Cd in soils can be characterised as follows:

1. In pristine or contaminated soils about 0.001 to 5% of the total zinc is in solution. This percentage is highly affected by pH, and the Zn concentration in solution is positively related to the total Zn concentration (Sauvé, 2002). Zn is an essential metal for plants and animals. It is readily taken up by plants, where it can be phytotoxic at concentrations often below the levels posing risks to animals. This means that the exposure route via herbivory is often not problematic (McLaughlin, 2002). The most available metal pools (e.g. soil solution or weak extracts) appear to be better predictors of phyto-availability than total metal concentrations (McLaughlin, 2002). Most soil invertebrates probably actively regulate Zn as internal concentrations are generally independent of soil concentrations. Isopoda and Diplopoda seem to maintain the highest internal concentrations (Heikens et al., 2001; Peijnenburg, 2002), although Vijver (2005) recorded much higher levels in Lumbricidae than in Isopoda.

2. Approximately 0.001 to 0.01% of the total copper content in soils is dissolved. The amount of Cu in solution is not as dependent on pH, as those of Zn, Pb and Cd, and is more closely related to the organic matter content than to the total Cu content of the soil (Sauvé, 2002). Cu is an essential metal, and as with Zn, the soil-plant barrier

generally protects the herbivores and higher food chain levels as phytotoxicity is more likely to occur (McLaughlin, 2002). Soil solutions or weak extracts might be better predictors of phyto-availability than total metal concentrations (McLaughlin, 2002). Cu might also be actively regulated by soil invertebrates (Peijnenburg, 2002), but internal concentration seem to increase slightly with increasing soil concentrations over a broad range (Heikens et al., 2001). As with Zn, Isopoda and Diplopoda show more accumulation than other soil invertebrates (Heikens et al., 2001; Vijver, 2005).

3. Similar to Cu, about 0.001 to 0.01% of the total lead content in soils is dissolved. The pH sensitivity of Pb solubility is intermediate between those of Zn and Cu. The Pb concentration in solution is more closely dependent on the total Pb concentrations than those for Zn, Cu and Cd. For Pb, the total concentration generally has a larger effect on the solubility than the organic matter content (Sauvé, 2002). On the other hand, Pb is also highly sorbed by soil colloids, so in floodplain soils, clay content might be an even better predictor of Pb solubility than organic matter content. Pb may be absorbed by plant roots but is generally not readily transferred to aboveground tissues (McLaughlin, 2002). It is therefore assumed that Pb especially affects subterranean fauna grazing on underground plant tissues (roots), and the connected food chains. In high concentrations, Pb can be highly phytotoxic. The increase in internal metal concentrations in soil invertebrates in relation to increasing external metal concentrations seems to be greatest for Pb, compared to Zn, Cu and Cd (Peijnenburg, 2002), but this has not been found in all invertebrate groups (Heikens et al., 2001). Exceptions include Isopoda and Collembola, which show higher internal concentrations than several other soil invertebrate groups (Heikens et al., 2001). Gastropoda also seem to regulate Pb very well (Notten et al., 2005b).

4. As regards cadmium, 0.05 to 15% of the total content in pristine and contaminated soils is in solution. The effect of total concentrations on the variation in dissolved Cd concentrations is generally negligible as dissolved concentrations are more closely related to organic matter content and pH (Sauvé, 2002). Cadmium, which is not essential for metabolic processes, generally poses animal health risks at levels that are not phytotoxic (McLaughlin, 2002). The soil-plant-animal route can therefore be of importance for the exposure to potential toxic levels of herbivores and the food chain linked to herbivores. The most available metal pools seem to be better predictors of phyto-availability than total Cd concentrations (McLaughlin, 2002). Internal concentrations in soil invertebrates are positively related to soil concentrations for several invertebrate groups, with Lumbricidae showing the greatest accumulation (Heikens et al., 2001; Peijnenburg, 2002). However, no such relation has been found for other groups like Isopods (which have the highest internal concentrations of all soil invertebrates), Formicidae and Chilopoda.

Floodplains are special environments in terms of metal pollution and toxic risks. In certain soils present in the floodplains, inorganic fractions like clay influence the solubility of metals (Sauvé, 2002; Van Vliet et al., 2005). Another special characteristic is that pH values vary only within a limited range, as was shown by Schröder (2005) in a screening of pore water characteristics at depths of 15 and 45 cm in 48 Dutch floodplains. The average pH values there were 7.01 ± 0.26, 7.03 ± 0.29 and 6.92 ± 0.32 in spring, summer and autumn, respectively. In the same study, the organic carbon content was shown to be always below 14%, and total metal concentrations in the 5 – 60 cm top layer ranged from approximately 40 to 1100, 4 to 110, 10 to 800 and 0.04 to 25 mg kg^{-1} dry weight (DW), for Zn, Cu, Pb and Cd respectively. The different modes of exposure, uptake processes and uptake mechanisms in various taxonomic groups (Vijver, 2005), as well as differences in exposure times, can result in deviations from general accumulation patterns in inland areas, as several parameters are not necessarily correlated to the total metal concentrations.

1.4 Small mammals

Small mammals are a very heterogeneous group of species. When small mammals are mentioned, generally species from two orders are meant: the Rodentia and the Insectivora, although smaller Carnivora like Mustelidae are sometimes also included. In this study we focus on the common small mammal species frequently trapped in our research with Longworth live traps in natural environments (not in buildings), including voles, mice and shrews. Although they were not trapped, moles are also discussed in this thesis.

Small mammals are assumed to be important animals in floodplains, as they are numerous (Jacob, 2003), show fast population growth, can play a role in landscape engineering by their feeding and burrowing behaviour (Mace et al., 1997; Edwards et al., 1999; Andersen and Cooper, 2000), and form important prey species for several larger omnivores, raptors and other predatory species (Erlinge et al., 1983; Thirgood et al., 2003). They are good model organisms, as they live in spatially well-defined small areas, live short lives (except for moles, 1 year is very old for these species in the wild), and can be studied relatively easily as they can be live-trapped and marked (Barrett and Peles, 1999; Flowerdew et al., 2004). This group of species is also interesting as they co-exist in small areas, while at the same time differing greatly in terms of life history, habitat preference and feeding behaviour, as they are either herbivores, omnivores or carnivores/insectivores. The greatest differences are between the four family groups:

1. **Voles** (Cricetidae) belong to the rodents (Rodentia). Voles have stout bodies, blunt snouts and small rounded ears. Their tail is always shorter than the head and body, and the eyes are relatively large. They are active during both day and night and throughout the year. Voles have short legs, and their feet have claws; most species are good diggers, creating burrow systems and above-ground trails. They have a thick fur, whose texture changes with the seasons, and the tail is also covered with hairs (Lyneborg and Den Hoed, 1972; Broekhuizen et al., 1992; Poor, 2005a). Representatives of the voles are *Microtus arvalis* (Common vole; Fig. 2.1), *Microtus agrestis* (Short-tailed field vole) and *Clethrionomys glareolus* (Bank vole). Voles generally have several litters a year, up to seven, of between three and 13 young. Females can become pregnant at ages of 14 days to two months. The population sizes can fluctuate greatly and are cyclic, especially at high latitudes (Lidicker, 2000; Huitu et al., 2005). Several species have the potential to become pests (Jacob, 2003; Briner et al., 2005). Voles are predominantly herbivores and can influence plant community composition through their grazing activity (Andersen and Cooper, 2000; Zhang et al., 2003). Some species, for instance *C. glareolus*, also eat insects during certain periods of the year. Most voles live for only a few months in the wild (Lyneborg and Den Hoed, 1972; Poor, 2005a). They provide a staple food source for many other species (Masman et al., 1988; Thirgood et al., 2003), and have been found to influence the population dynamics of predatory species (Beemster and Vulink, 2001).

2. **Mice** (Muridae) belong to the rodents. Compared to voles they are more slender and have a pronounced snout. Mice usually have prominent ears, and their tails can be long. The tail often has a naked appearance but can also be hairy, and is (semi-) prehensile. Among the mice there are good runners and climbers, often foraging

above-ground. Several species burrow or dig holes (Lyneborg and Den Hoed, 1972; Broekhuizen et al., 1992; Poor, 2005b). Representatives of mice are *Apodemus sylvaticus* (Wood mouse; Fig. 2.2) and *Micromys minutus* (Harvest mouse) and the cosmopolitan *Mus musculus* (House mouse). There is a large variation in the number of litters per year, ranging from a few, especially for seasonal breeders, to ten for certain species under favourable conditions. These productive species can also have seven to 13 young per litter, though others only have a few. Mice do usually not live more than a few months in the wild (Lyneborg and Den Hoed, 1972; Poor, 2005b). Among the mice there are strict herbivores as well as omnivores. Some of them are nocturnal. Several species are important food items for predators (Southern and Lowe, 1968; Jędrzejewski et al., 1995).

Fig. 2: Photos of small mammals including representatives of each of the family groups; 1. *Microtus arvalis* (Common vole); 2. *Apodemus sylvaticus* (Wood mouse); 3. *Sorex araneus* (Common shrew); 4. *Talpa europaea* (European mole). Photo 3 by Wim Dimmers.

3. **Shrews** (Soricidae) belong to the insectivores (Insectivora). Compared to other small mammals, shrews are small and have tiny eyes. They can be recognised by the pointed shape of their snout, and their relatively small ears. They have a high metabolic rate and must therefore eat very frequently, which means they are active throughout the day and night. They primarily feed on macro-invertebrates. Most species burrow while searching for food and also create tunnels. Representatives of the shrews are *Sorex* *araneus* (Common shrew; Fig. 2.3) and *Crocidura russula* (White-toothed shrew). Most species have several nests per year containing four to six young, some species up to ten (Lyneborg and Den Hoed, 1972; Ciszek and Meyers, 2002). Soricine shrews show a remarkable decline in body size and organ and skeletal weight during winter (Ochocińska and Taylor, 2003). Shrews are important food items for several predatory species in certain areas and during certain periods (Love et al., 2000; Norrdahl and Korpimäki, 2000).

4. **Moles** (Talpidae) belong to the insectivores. Most species are fossorial, digging underground tunnels in which they live and forage on subterranean macro-invertebrates (Edwards et al., 1999). Their bodies are fusiform, their eyes are tiny, the legs are short, and external ears are lacking. The forelimbs are specialised for digging; they are rotated in such a way that the elbows point dorsally and the palms of the front feet face backwards and terminate in claws. The fur of moles can be flattened equally well in any direction, which allows easy movement in the burrows backward as well as forwards. They have one litter of three to seven young a year. The most common species in Western Europe is *Talpa europaea* (European mole; Fig. 2.4). Moles have relatively high metabolic rates and are therefore active at all times of the day and night, throughout the year (Macdonald et al., 1997). Besides macro-invertebrates, they sometimes forage on small mammal species. The maximum lifespan of moles is seven years, but on average they live to about three years of age in the wild (Lyneborg and Den Hoed, 1972; Broekhuizen et al., 1992; Ciszek and Meyers, 2000).

1.6 Context of the research project

The research effort reported on in this thesis was part of the Stimulation Programme on System-oriented Ecotoxicological Research (SSEO) of the Netherlands Organisation for Scientific Research (NWO). The aims of this programme are to promote scientific knowledge and understanding of the way ecosystems respond to chemical pollution in situations of chronic and diffuse exposure. Secondly, it aims to make use of fundamental and other relevant knowledge to assist in formulating and implementing policy on the ecological risks of chronic and diffuse pollution of the environment by a mixture of substances (SSEO, 1999). The SSEO programme started in 1998. To achieve synergy in the research results of the various projects and to support the implementation of the findings in general models and policies, three research sites were selected. These were (1) the 'Ronde Venen' fens situated in the mid-western part of the Netherlands, where a peaty grassland has been filled in with urban waste materials over the last centuries; (2) the 'Biesbosch' wetlands, an estuary situated in the south-western part of the Netherlands, with research sites mainly influenced by fresh water, which has functioned as a sink for polluted river sediments supplied by the river Meuse throughout the previous century; and (3) The 'Afferdensche en Deestsche Waarden' (ADW) floodplains, the research area for the studies reported on in this thesis.

Knowledge about interactions between ecosystems and diffuse pollution is of increasing importance in view of past and recent policy changes towards floodplain management, and future plans for the Western European riverine areas. During the last decades, floodplains have generally been used for agriculture as well as for industrial activities, especially brick factories, and the exploitation of natural resources, mainly clay, sand and gravel. These activities were combined with the main task of the floodplains, that is harbouring water and stimulating drainage during high discharge periods. In the past, the main river channel was stabilised and deepened to facilitate shipping activities. Since these activities are hampered by large discharge fluctuations and by meanders in the river, the rivers have been regulated, normalised and canalised.

Although the anthropogenically influenced floodplains were ecologically deteriorated systems, they still had important ecological functions, maintaining populations of endangered and protected plant and animal species, and were important wetlands functioning as foraging areas for seasonal visitors like thousands of water birds. These important functions have been recognised and highlighted in several directives and conventions (Lenders et al., 2001; De Nooij et al., 2004). The large rivers and the adjacent semi-terrestrial areas provide opportunities to create large interconnected nature areas and may function as 'blue-green' (i.e. combining wetlands and green spaces) corridors between existing conservation areas and isolated populations (Foppen et al., 1998). Their was also a need to increase the water storage capacity of floodplains along rivers to handle the current increased water discharges by the rivers and the expected further increase in the future due to global climate changes. The activities to create more water storage capacity could also be considered an opportunity to stimulate habitat development (Smits et al., 2000; Van Stokkom et al., 2005) by excavating floodplains and removing obstacles. The excavated clay, sand and gravel was sold to finance these expensive operations. In addition, floodplains have been ecologically rehabilitated by restoring river dynamics where possible, creating side channels and lakes, and introducing a form of floodplain management with minimum anthropogenic influence, known in Dutch as 'nature development'.

However, a crucial aspect in this new management of Dutch floodplains is that ecosystems, and the development of habitats in particular, must not be hampered by the presence of pollutants in the systems. When negative effects of pollutants on ecosystems are present, it is important to know if these are of a temporary nature, and if they can be minimised by taking them into account in floodplain management. Since there are many uncertainties about the effects of pollutants on ecosystems, promising ecological rehabilitation plans are currently being delayed or cancelled, as expensive decontamination or remediation actions, or excavation and storage are compulsory when intervention values are exceeded (Visser, 1993; Schouten et al., 2000). Hence, it is important to extend the available knowledge about ecosystem – pollutant interactions in floodplains, and about the fate of pollutants in these dynamic environments. The results of the present study will therefore not only be of importance from the point of view of basic science. The study will deliver important input data and increase our understanding of underlying processes, thus providing the basis for ecological and ecotoxicological assessment and modelling that can be used to develop strategies for floodplain control and management.

This study was part of an NWO-SSEO project with the working title 'The role of bioturbators in the purifying capacity of floodplains'. The project was carried out in close cooperation between the Radboud University Nijmegen and the Vrije Universiteit Amsterdam, and involved several experts and institutions. The project included two sub-projects. One sub-project mainly focussed on bioturbation by earthworms and their interactions with pollutants in floodplains (Zorn, 2004), while the present PhD project mainly focussed on interactions between small mammals and pollutants in floodplains. However, since these two topics are closely related, their processes interact and the two subprojects involved combined experiments and measurements. The current thesis does not focus solely on small mammals, but also tries to compare and combine results of both studies.

1.7 Research area

The research underlying the present thesis was largely implemented in the 'Afferdensche en Deestsche Waarden' (ADW) area, a floodplain situated in the eastern part of the Netherlands (Fig. 3). The area, with a size of approximately 280 ha, is situated on the south bank of the river Waal, the main branch of the river Rhine in the Netherlands (longitude 51°54′N, latitude 5°38′E). The larger part of the area is protected on the river side by ‘summer dikes’, which are lower embankments to prevent the area from flooding during high water in summer. The floodplain area consists of agricultural land (maize fields and pastures), and areas without agricultural activities, where natural development of the vegetation has been promoted since 1995.

Fig. 3: Location of the ‘Afferdensche en Deestsche Waarden’ (ADW) and the ‘Millingerwaard’ (MW) research areas along the river ‘Waal’ in the Netherlands.

Large parts of the non-agricultural area are extensively grazed by horses and cattle, and consist mainly of grassland vegetation. Ungrazed parts consist of rough and softwood forest vegetation. As the floodplain is the subject of an ecological rehabilitation programme, clay excavations have taken place in order to stimulate natural wetland development. The coming years, summer dikes will be lowered, a side channel will be constructed, and the remaining parts will become a nature reserve (Zandberg, 1999; Zorn, 2004). Part of the research was also done in the Millingerwaard (MW) floodplains, which differ from the ADW floodplains by having a larger section designated as conservation area. In addition, ecological rehabilitation measures in the MW floodplains started earlier, viz. in 1990 (Helmer and Smeets, 1990).

Fig. 4: Images of the 'Afferdensche en Deestsche Waarden' floodplains (ADW); 1. traditional floodplain management with agricultural grasslands; 2. extensively grazed conservation area favouring ecological rehabilitation; 3. ungrazed area consisting of rough vegetation and softwood forest bordering the non-flooding area of a former brick factory; 4. river water transgressing the summer dike during a flood; 5. the floodplains during an inundation.

High water discharges in the river Rhine generally occur in late winter or early spring, as the snow in the Alps starts to melt, while heavy rainfall in the Rhine tributaries sometimes also cause floods in the ADW in other seasons. The area on average floods once a year, but in some years there is more than one flood event or none at all (www.waterbase.nl). Once the water transgresses the summer dike, the entire area, except for some elevated parts that are never flooded, is inundated within two or three days, with water depths of more than two metres being reached in most of the area. After such an inundation, it usually takes several weeks before the floodplain is dried up again - even though high water discharge generally only lasts a few days - as the water mainly leaves the floodplain by seepage towards the river channel (Thonon et al., 2005).

The area can be characterised as a moderately polluted floodplain. Early studies found average total metal concentrations in the 0-10 cm topsoil of 474 ± 342, 77 ± 49, 2.7 ± 2.0 mg kg^{-1} DW (n = 32) for Zn, Cu and Cd respectively (Kooistra et al., 2001a). Later studies confirmed the average concentrations for Zn and Cu, and showed that the Pb concentrations range between 60 and 300 mg kg^{-1} DW (Kooistra et al., 2005; Van Vliet et al., 2005). When areas not investigated previously were included, Cd concentrations appeared to be somewhat higher, ranging from 0.1 to 11.1 with an average of 3.7 mg kg^{-1} DW (Kooistra et al., 2005). Measurements in two areas within the ADW showed a range of 7.7 to 15.1% organic matter (OM) and 11.1 to 34.0% clay content (Van Vliet et al., 2005). Clay contents measured by Kooistra et al. (2001b) were slightly lower (below 25%), but OM contents were similar. Zorn (2004) recorded average OM contents of 15% and clay contents of 20%. This means that the Pb and Cd concentrations in ADW are below the maximum permissible concentrations (MPC) at which no direct toxicologic effects are to be expected, according to the Dutch quality standards for soils (Crommentuijn et al., 1997). MPCs are calculated taking the availability of the total metal concentrations into account, by correcting for the binding capacity of the substrate, viz. organic matter and lutum contents. The MPCs are sometimes exceeded for Zn, but are probably frequently exceeded for Cu, as MPCs are 620, 73, 530 and 12 mg kg^{-1} DW for Zn, Cu, Pb and Cd, respectively, in a standard soil containing 10% OM and 25% clay. The pH values in the ADW show a limited range from 7.0 to 7.5 (Zorn, 2004), as has also been found in other floodplains (Schröder, 2005).

The ADW is representative of Dutch large river floodplains in terms of pollution level, its embankments, landscape structures, the presence of agricultural and conservation areas, historic and current management and future plans. As explained, contaminants are diffusely distributed, originating from similar sources as in other Dutch floodplains, and released and distributed on a similar timescale as in other floodplain areas. The metals we studied, together with a mixture of organic substances, are expected to be the most problematic ones in most floodplains in terms of their amounts and properties. Most Dutch floodplains have been narrowed by embankments, between which water levels rise fast during floods. Obstacles like elevated areas and sizeable forests are usually removed as much as possible, because floodplain management is focused on keeping the hydraulic roughness low (Baptist, 2004). As a result, Dutch floodplains are dominated by agricultural fields and grasslands. The most important function of these floodplains is to increase discharge rates during high water, thus protecting the (inhabited) inland areas against floods, and maintaining the river's shipping functions (Van Stokkom et al., 2005). Besides being important for agricultural production, Dutch floodplains used to feature major industrial activities, especially brick factories, including the excavation of raw materials, which is why they often still feature infrastructure elements (Middelkoop and Van Haselen, 1999). In recent decades, their ecological values, as well as recreational functions, have received more attention (Nienhuis et al., 2002). In 1995, an ecological rehabilitation programme started at the ADW, which has now been partly implemented. Similar ecological rehabilitation programmes have been started in several other floodplain areas. Besides being representative of Dutch floodplains, the situation at ADW is probably also representative of most Western European lowland river floodplains.

1.8 Aim of the studies and outline of the thesis

The aim of the studies reported on in this thesis was to analyse the interactions between biota and contaminants in a dynamic floodplain environment, particularly the interactions between small mammals and heavy metals. The following research questions were formulated:

1. Where and when does exposure of small mammals to heavy metals occur?
2. What is the impact of bioturbation by small mammals on heavy metal distribution in floodplains?
3. Are there risks of accumulation of heavy metals in food webs within diffusely contaminated floodplains, and if so, what factors explain such accumulation?

This thesis is roughly divided into three parts, relating to the three research questions:

Chapters 2 and 3 identify the patterns in small mammal distribution. The most important factors determining temporal and spatial patterns are discussed and extrapolated to general rules. Chapter 2 presents the results of a live trapping study of the small mammal populations in the ADW in one year between two successive floods. Trapping data are combined with geomorphological and vegetation characteristics to determine the suitability of habitats. These results were used to prepare habitat suitability maps for the six most common vole, mouse and shrew species making it possible to calculate the numbers, densities and relative densities (densities weighted for the suitability of the landscape), in zones of distance from the non-flooded areas, throughout the year. Further results regarding the recolonisation of the ADW by small mammal species after floods are presented in Chapter 3, which reports on an additional year of monitoring. Although recolonisation is determined by different characteristics for each species, these characteristics can be described by habitat suitability patterns in a landscape. A limited number of parameters for all species allowed recolonisation to be described by relatively simple regression models. The predictive power of the recolonisation models was tested in a different floodplain (Millingerwaard). An attempt is made to classify the species on the basis of their recolonisation patterns.

In Chapters 4 and 5 the potential impact of bioturbation on the redistribution of heavy metals in floodplains is discussed. In the study reported in Chapter 4, we tested the hypothesis that heavy metals can be redistributed under the influence of turbation, as it affects the mobility of the contaminants. The hypothesis was tested in an experimental setting with a Zn-contaminated topsoil using manual turbation, with or without experimental inundation and/or rain. We tried to relate the observed differences in Zn distribution between treatments and soil parameters, in order to unravel the underlying processes. Bioturbation in the field (ADW) and in field enclosures was quantified in the study discussed in Chapter 5 for Common voles and European moles. We also calculated the amounts of substrate and heavy metals surfaced by bioturbation by small mammals on an annual basis, and the amounts available just before a flood. The results are compared with the effects of bioturbation by earthworms, as extrapolated from column experiments to field densities and with deposition during floods as calculated from sediment traps. The results allowed us to determine the share of bioturbation, especially that by small mammals, in the annual deposition and redistribution of substrate and heavy metals in floodplains.

The Chapters 6 and 7 discuss the potential risks of accumulation of heavy metals in food chains by relating soil concentrations to potential exposure concentrations and actual levels in small mammals. Chapter 6 relates the total heavy metal concentrations in the topsoil of the ADW area to $CaCl_2$-extractable concentrations, as a measure of potential availability to biota, as well as to concentrations in two important exposure routes in floodplain food webs, viz. vegetation and earthworms. As substantial segments of the floodplain food webs and small mammal populations are present in areas not annually flooded, the study focussed on differences in heavy metal concentrations and accumulation risks between flooding and non-flooding areas. Chapter 7 reports on measurements of the current heavy metal concentrations in the common small mammal species from the ADW floodplain. It is being hypothesised that accumulation would be highest for insectivores/carnivores and lowest for herbivores, with omnivores somewhere in between. Species-specific differences in metal accumulation in small mammals were related to environmental characteristics of trapping locations and species characteristics, as the actual exposure of species to soil-total or $CaCl_2$-extractable metal concentrations may differ, and population composition can influence exposure due to varying exposure time and foraging behaviour. Variables taken into account are the animals' presence in flooding or non-flooding areas in various seasons, and life-stage, sex and fresh weight of the specimens.

In Chapter 8 the results of the research project are discussed and integrated in a conceptual framework for interactions between small mammals and heavy metals in dynamic floodplains. Special attention is given to the implications of our findings for ecological risk assessment in polluted floodplains and for floodplain management.

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Chapter 2

Flooding ecology of voles, mice and shrews: The importance of geomorphological and vegetational heterogeneity in river floodplains

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Checking live traps

Abstract

Since voles, mice and shrews are important animals in food chains of river floodplains, there is a need for data on their spatial and temporal distribution in periodically flooded areas. During a live trapping study between two successive floods in an embanked river floodplain, the ‘Afferdensche en Deestsche Waarden (ADW)’, six species were frequently observed, viz, *Microtus arvalis* (Pallas 1778), *Clethrionomys glareolus* (Schreber 1780), *Sorex araneus* (Linnaeus 1758), *Crocidura russula* (Hermann 1780), *Micromys minutus* (Pallas 1771) and *Apodemus sylvaticus* (Linnaeus 1758). Ungrazed rough herbaceous vegetation appeared to be rich in numbers and species, whereas no spoors of small mammals were observed in large parts of the ADW floodplain (e.g. bare substrates and maize fields). Vegetation structure seemed to be very important in guiding the recolonisation process after flood events. Throughout the year the highest numbers of small mammals were captured on and near the non-flooding elevated parts functioning as refugia during inundation. Poor habitat connectivity, sparseness of non-flooding recolonisation sources and small numbers of survivors led to slow recolonisation. The time between two successive floods (eight months) was not long enough for entire recolonisation of ADW. Small mammal densities at more than 30 m from the non-flooding areas were always lower than in non-flooding areas.

2.1 Introduction

The floodplains of pristine rivers harbour a great diversity of habitats (Andersen et al., 2000; Ward et al., 2001; Van der Velde et al., 2004). The hydromorphodynamic nature of these floodplains, with their flood pulses, alternating erosion and sedimentation processes and vegetation processes like colonisation, succession and rejuvenation, creates a broad spectrum of habitats for animals (Junk et al., 1989).

It is assumed that small mammals play an important role in the food chain (Erlinge et al., 1983), also in floodplain ecosystems (Hendriks et al., 1995; Kooistra et al., 2005; Leuven et al., 2005). However, flooding events here influence the seasonal population development cycles of small mammals, as generally found in temperate zones. Flooding means that large parts of the floodplain are cleared of individuals, after which populations redevelop and the floodplain is recolonised. Knowledge of recolonisation and data on the occurrence, habitat preferences and spatial and temporal distribution of voles, mice and shrews in floodplains are limited. This is especially true in embanked floodplains, where the impact of flooding seems to be more severe than in natural floodplains.

Several small mammal ecotopes can be distinguished in floodplains, based on vegetation structure and habitat characteristics, functioning as suitable, marginal, or unsuitable habitats for the various species. The development of small mammal densities is assumed to reflect habitat suitability of the ecotopes for each species (Hansson, 1997). In floodplains, the process of recolonisation is also important, because individuals are forced to survive in refugia on the non-flooding parts during floods. Short periods of inundation can possibly be survived by certain species in vegetation above the water level. In periodically inundated floodplains, individuals may be present in the so-called marginal habitats for substantially longer periods of time than in areas without flooding. The densities in marginal habitats are expected to remain low in comparison with those in suitable habitats on non-flooding parts (Wolff, 1998). Marginal habitats may therefore play an important role in population dynamics in floodplains (Stelter et al., 1997).

In the vicinity of the non-flooding areas, the suitable habitats are expected to be recolonised first when floodwaters recede. The numbers of small mammals in these suitable habitats remain stable or increase during the period after recolonisation. Marginal habitats may also be recolonised relatively soon after the floodwater has receded, but numbers remain low or even drop after colonisation by the first individuals. These areas may function as transition habitats or stepping-stones for dispersal. How long it takes before the first individuals of a species can be found in the flooding parts after receding of the floodwater is dependent of the species-specific recolonisation rate, the quality of the non-flooding areas (refugia) and the connectivity of habitats with source populations in the refugia. As the time till the arrival of the first individuals is dependent of several aspects, this is not always a good measure of habitat suitability.

The goal of our study was to analyse which environmental variables determine the spatial and temporal distribution of small mammals (i.e. voles, mice and shrews) in an embanked river floodplain. The research questions were:
(a) Which habitat characteristics are related to the occurrence of small mammal species?
(b) What does the density pattern development of small mammals between two successive annual floods look like?
(c) Do habitat suitability and connectivity affect the recolonisation of floodplains by small mammals after a flooding event?
(d) Are recolonisation patterns of small mammals species-specific?

2.2 Study area

The 'Afferdensche en Deestsche Waarden' (ADW) is a floodplain with an area of 280 ha, embanked by means of summer and winter dikes (Fig. 1). It is situated 20 km west of the city of Nijmegen along the river Waal, the main distributary of the Rhine river in the Netherlands. The area is the subject of an ecological rehabilitation programme in which safety precautions against high river discharges are combined with the conversion of agricultural land into natural floodplain ecotopes. A programme of floodplain and summer dike lowering, clay excavation, construction of a side channel and removal of buildings and roads started during 1995, and will last until 2007 (Zandberg, 1999). The present study at ADW included areas with and without agricultural activities, the latter favouring natural development of the vegetation and offering a wide range of habitats. The floodplain area between the summer and winter dikes (about two-thirds of the total floodplain) is periodically flooded during times of high water discharges of the river. Flooding of the diked part (in general once or twice a year) occurs between November and May. Once the water transgresses the summer dike, the entire floodplain is inundated within two or three days, except for two areas with somewhat higher elevation, housing remnants of brick factories, two smaller areas of just a few square metres, and the winter dike (Fig. 1a). Within this short time, the water in the floodplain raises, to more than two metres above ground level at most locations. Water mainly leaves the floodplain by seepage towards the river channel, which means that inundations last much longer than the high water discharge period itself. Once inundated, it usually takes several weeks before the floodplain is dried up again; inundations of two or three months are not unusual.

The ADW floodplain was classified into four zones based on the distance to non-flooding areas (Fig. 1a) as follows: (a) non-flooding areas, (b) 0–30 m from non-flooding areas, (c) 30–120 m from non-flooding areas and (d) more than 120 m from non-flooding areas, this being the largest area. The distances were chosen such that similar numbers of monitoring sites were present in each zone.

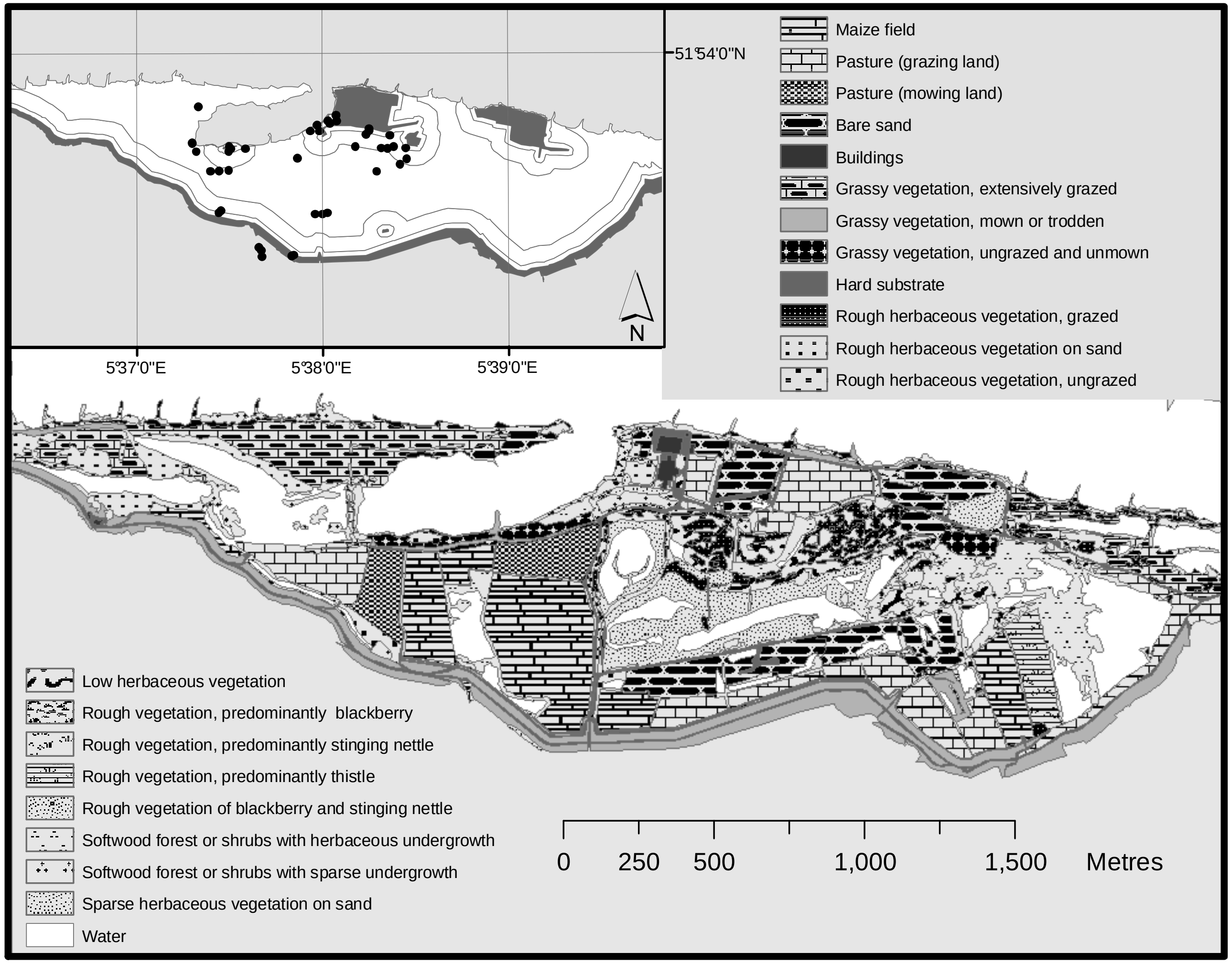


Fig. 1: (a) Positioning of the ADW floodplain, in which the monitoring locations are indicated by dots. Non-flooding areas are shown in grey, and the parallel lines indicate the borders of the other three distance zones (viz. 0-30 m, 30-120 m, and more than 120 m from non-flooding areas). (b) Classification of the ADW floodplain into small mammal ecotopes.

2.3 Material and methods

Flooding patterns during 2001-2002

Investigations were carried out during the period between two successive flooding events, one in March 2001 and one in February 2002. The flooding of ADW in the spring of 2001 started on 16 March, and the first inundated areas started to dry up on 6 April. The first metres adjoining the non-flooding areas had dried up by 18 April, while the entire floodplain had fallen dry on 5 May. In the winter of 2002, the water level in the river reached the summer dike level on 29 January, and a second time on 15 February. This caused an inundation of about 65% of the floodplain, followed by a complete flooding on 24 February.

Vegetation monitoring and live trapping of small mammals

A total of 40 plots (10 x 5 m) were selected, reflecting the wide range of vegetation structures present at various distances from non-flooding areas. These plots were used to monitor vegetation developments, using the method developed by Braun-Blanquet et al. (1932) to describe plant species composition and vegetation cover and height.

Four live trap sessions were held during May, July, October and December to monitor small mammal distribution patterns in ADW. Recorded small mammal densities are often derived from trapping studies (using pitfalls, live traps or snap traps) and are based on estimated trapping ranges, which depend on the animals' action diameters, daily movement distances or home ranges. Measuring home range sizes or animal movements is most accurately achieved using radio tracking, beta lights or fluorescent powders, but such studies commonly include a limited number of individuals, and results can be influenced by the observation technique (Boyce and Boyce, 1988a; Jorgensen, 2002). Trapping studies are usually executed in trapping lines or grids. The advantage of grids is that fewer calculations and assumptions are necessary to calculate densities of small mammals. A disadvantage of this approach is, however, that fewer trapping localities can be examined, as grids require more traps and effort per locality. Since we wanted to study the entire floodplain, and include as many relevant structures as possible at various distances from the elevated areas, we decided to use traps in lines, taking the disadvantages of a less accurate estimation of real densities for granted. Trap lines consisting of 10 Longworth live traps spaced at 5 m intervals were installed through each of the 40 plots and checked every 4 hours for 72 hours, after a two-day prebaiting period. The traps were baited with apple, carrot and tinned meat, and stuffed with tissue paper and hay. Trapped animals were individually marked by means of fur clipping, so they could be recognized at recapture (Gurnell and Flowerdew, 1990), and released.

The research plots were positioned using a GARMIN GPS 12 Personal Navigator, and the coordinates were plotted on a digital aerial photograph of the research area made available by the Geometric Services of the Directorate-General of Public Works and Water Management. The distances from trap lines to non-flooding areas and the surface areas of the homogeneous vegetation units were estimated using ArcView GIS for Windows (version 3.1).

Regression analyses

To identify the habitat preferences of small mammal species, the small mammal numbers were related to environmental variables using the Canoco for Windows package (version 4) (Ter Braak and Smilauer, 1998). As the trapping numbers appeared to be very low in spring, the data of the monitoring session in May were not included. Since distances to non-flooding areas, recolonisation rates and connectivity are assumed to be major factors in explaining the small mammal distribution patterns in floodplains, only the non-flooding parts and areas situated within 30 m of non-flooding parts were included in this analysis. The number of combinations of environmental parameters with trapping results equals 42 (viz. 14 monitoring sites x 3 monitoring moments). Van Apeldoorn et al. (1992) studied *Clethrionomys glareolus* (Schreber, 1780) and found that in areas where food is not the limiting factor, habitat suitability for small mammal species is mainly determined by structure variables, rather than by the species composition of the vegetation. We also focused our analysis especially on vegetation structure or plant species groups representing a particular structure, to make interpretation of the trapping results for the total floodplain possible. The environmental parameters incorporated were vegetation cover (%), vegetation height (cm), management (pastures; habitat creation areas; waste land; other (paths and roads)), shape of vegetation unit (rectangular shaped; line shaped; other (patchy)), vegetation type (stinging nettle; blackberry; blackberry and stinging nettle; rough herbaceous vegetation; shrubs; other (grass dominated), soil humidity (dry soil; humid soil; marshy land; other (water)), mowing regime (mowing land; other (unmowed)), grazing regime (grazing land; other (ungrazed)), positioning related to inundation (inland areas, periodically flooded areas, other (high water free areas)), soil texture (sandy soil; loamy soil; clayey soil; other (hard substrate)). Also the trapping period (week number of the year) was included in the analyses as a measure for seasonality and available time for population development. The classes mentioned as ‘other’ were not separately taken into the analyses, to minimize co-linearity. When a species is negatively related to the incorporated classes of an environmental variable this means that the species is especially related to the class ‘other’. As the gradient length for the dataset was found to be smaller than three (by means of a Detrended Correspondence Analysis (DCA)), a Redundancy Analysis (RDA) was conducted (ordination of small mammal species with an optimal environmental basis). The data were log-transformed before analysis to prevent that a few high values influence the ordination, as species abundances often display a highly skewed distribution. As log(0) is undefined we used the transformation model $Y'=\log(Y+1)$. (Ter Braak and Smilauer, 1998).

Calculation of densities

The ADW was classified in 21 easy to observe homogeneous vegetation units based on those environmental variables incorporated in the RDA. The 21 structure classes are referred to below as small mammal ecotopes (Fig. 1b). The presence of species in the trap lines within small mammal ecotopes on elevated areas and in the 0-30 metres zone was decisive for our attempts to verify the hypothesis of suitability of the ecotopes. If a species was present during each trapping period after the first observation in a trap line, the trap line was assumed to be in a suitable ecotope. If it was only occasionally present, the trap line was assumed to be in a marginal habitat. If a species was only present in a trap line located on an elevated part in December (the final trapping session), while there were neighbouring ecotopes that were assumed to be suitable, the trap line was assumed to be in a marginal habitat. For those classes in which no trap lines were present, the most important environment variables as determined from the RDA plot were decisive for the suitability of that small mammal ecotope class. We verified our expectations by observations in the field (spoor inventory; including burrowing activities and feeding trails).

The numbers of each species observed at the 40 monitoring sites were converted into average numbers per standard trap line per distance zone (consisting of a total of 30 traps, with 10 traps in each of the three suitability (suitable, marginal, unsuitable) classes; 72 hours of trapping, checking every 4 hours). These values are referred to below as the relative densities per distance zone. However, these relative densities are not completely independent of the suitability of the available habitats, since relative densities are influenced not only by distances but also by connectivity (the quality of the intervening habitats).

For comparison of the density development between zones, the species-dependent trapping range of a trap line must be considered, for which it is necessary to know the home range size. The general definition of home range in small mammal studies is ‘the area traversed by the individual in its normal activities of food gathering, mating and caring for young’ (Kikkawa, 1964). This means that it can be assumed that an animal will encounter an unoccupied trap in its home range within the three trapping days. Our own observations showed that after a three-day trapping period, the number of newly trapped individuals was approaching zero. A weakness of the method is the fact that the trapping efficiency for some trap-shy individuals may be reduced, even though their home range overlap the trap line. There will also be individuals living at larger distances from the trap line that occasionally sally outside their home ranges and encounter an unoccupied trap. Also dispersing individuals passing by can be included in the trap numbers. This is inherent to the use of live traps (even when they are positioned in grids) but deliver some uncertainty to the density estimations. However, it is expected that this uncertainty is comparable for the various monitoring sites. A wide range of species-specific mean home range sizes has been recorded in West- and North-European studies, as is shown in the Appendix. Besides the method of sampling and calculating, the home range size depends on factors like habitat quality, developmental stage of individual animals, season, population density and geographical region (Randolph, 1977; Pusenius and Schmidt, 2002).

The calculated home range size also depends on the method of calculation (Gurnell and Flowerdew, 1990). The species-dependent trapping ranges we used were calculated from the mean home range sizes derived from the literature. A circular home range shape was assumed, although also elliptic shapes (Andrzejewski and Babińska-Werka, 1986) and elongated shapes with preferred directions have been suggested (Randolph, 1977). Elliptic or linear home ranges might have been more realistic for the linear structures in the study area, especially for territorial species, but the assumption of circular home ranges seems reasonable for most biotopes. Possible overestimations or underestimations of densities were expected to be similar in the various zones of the area, justifying comparison of these zones.

Table 1: Action diameters and home range sizes of the various species as estimated from literature data (Appendix 1).

Species	Home range (m^2)	Action diameter (m)	Trap range of trap line (m^2)
Common vole *Microtus arvalis*	400	22.6	1530
Bank vole *Clethrionomys glareolus*	1000	35.7	2780
Common shrew *Sorex araneus*	600	27.6	1980
White-toothed shrew *Crocidura russula*	125	12.6	760
Harvest mouse *Micromys minutus*	400	22.6	1530
Wood mouse *Apodemus sylvaticus*	2500	56.4	5320

As the ecological niche of *Microtus agrestis* (Linnaeus, 1761) in England and Scandinavia is similar to that of *Microtus arvalis* (Pallas, 1778) in other West-European countries (Myllymäki, 1977b), studies of the former were used to estimate the mean home range size of *M. arvalis* at 400 m^2 (Table 1). The smallest mean home range size is that of *Crocidura russula* (Hermann, 1780), measuring 125 m^2, while the largest is that of *Apodemus sylvaticus* (Linnaeus, 1758), at 2500 m^2. This means that the trapping range equalled

$50d+\pi(d/2)^2$ square metres (Fig. 2), in which d is the action diameter of the expected mean circular home ranges in metres. The suggested trap range of the trap line was largest for *A. sylvaticus* (5320 m^2) and smallest for *C. russula* (760 m^2). Thus, equal relative densities of these two species would mean that the real densities would be about 7 times higher for *C. russula* than for *A. sylvaticus*. The trapping range and the available surface area of each habitat suitability class allowed us to estimate the total number of individuals in the floodplain for each species. Calculated total numbers divided by the total surface area of each zone (Fig. 1a) allowed us to compare species densities between zones.

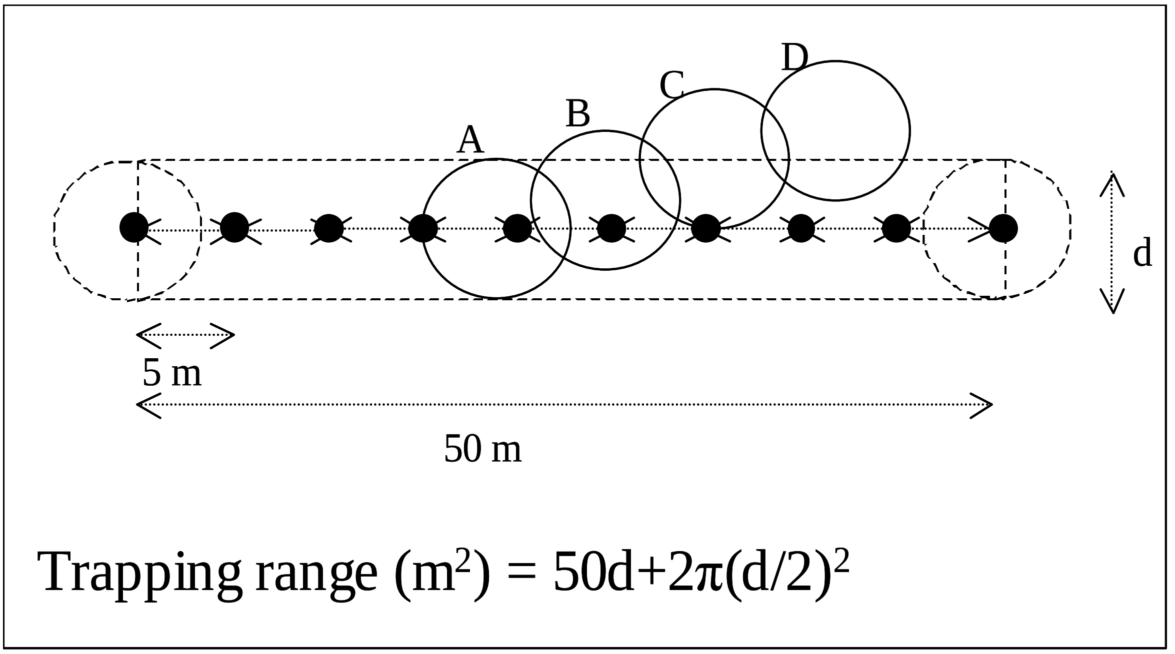


Fig. 2: Calculation of the trapping range. A trap line was 50 m long and consisted of 10 traps at 5 m intervals. d = diameter of a circular home range. The species-specific trapping range is the area lying within a distance of half a diameter from the trap line, assuming circular home ranges. All individuals for which the centres of their home range lie within a distance of half a diameter from the trap line will encounter a trap (A and B). Although these individuals can forage outside this expected trapping range (B), this is compensated for by the fact that other individuals can forage within the trapping range without encountering a trap (D).

2.4 Results

Species diversity and habitat suitability

The classification of the ADW floodplain into small mammal ecotopes (Fig. 1b) based on the most important environmental variables as shown in figure 3, shows that the southern and south-western parts of the floodplain were characterised by large rectangular homogeneous units. These rectangular homogeneous units are bordered by linear structures like verges and ditches, which is typical for agricultural landscapes. The small mammal ecotopes in the eastern and north-western parts showed a patchier pattern, which is typical for habitat rehabilitation areas. The patchiness of this area has become more obvious since habitat rehabilitation preparations started a few years ago, and is being promoted by extensive grazing by cattle or horses. The trapping program resulted in a total 572 captures of 271 individuals representing seven species (*M. arvalis*, *M. agrestis*, *C. glareolus*, *Sorex araneus* (Linnaeus, 1758), *C. russula*, *Micromys minutus* (Pallas, 1771) and *A. sylvaticus*). During the whole monitoring, 11 animals died in a trap. As only 4 individuals of *M. agrestis* were captured during the research period, this species was excluded from the analyses. The numbers of species trapped during July, October and December related to environmental parameters in a Redundancy Analysis (RDA) are shown in figure 3.

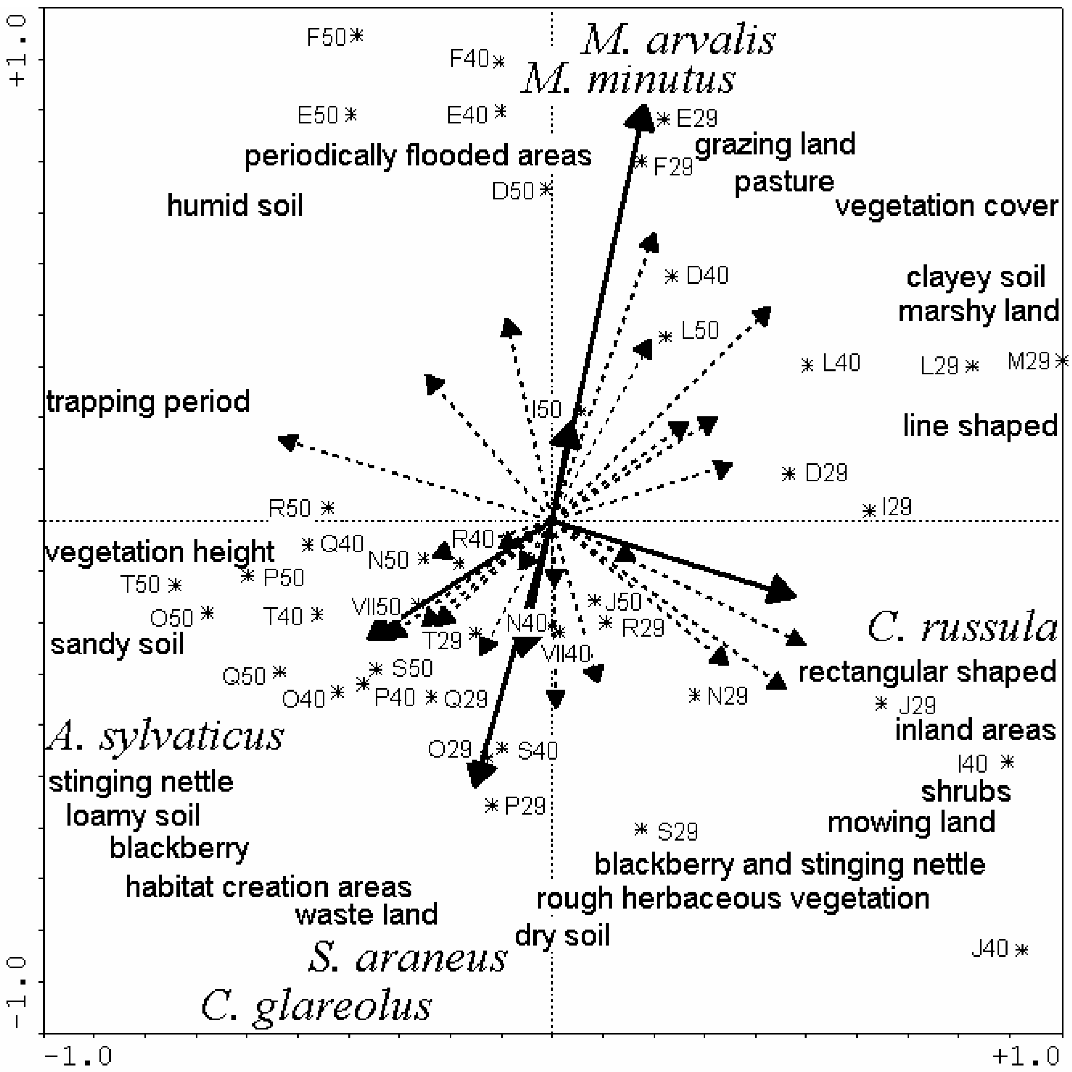


Fig. 3: Redundancy Analyses (RDA) of trapping results of small mammal species related to environmental variables. The plot shows the results of the analysis for the July, October and December monitoring data from the non-flooding areas and the areas located at 0-30 m from non-flooding areas. The ordination with an optimal environmental basis is shown. Ordination axes are aggregates of environmental variables that best explain the species data. The length of a species vector corresponds to the strength of regression to the environmental variables. The positioning of the individual monitoring sites at the three monitoring moments are shown as well (the letter indicates the monitoring site, the number shows the monitoring date in weeks after the drying up of the floodplain). The monitoring sites are described by the following environmental variables: vegetation cover (%), vegetation height (cm), trapping period (week number of the year) management (pastures; habitat creation areas; waste land; other (paths and roads)), shape of vegetation unit (rectangular shaped; line shaped; other (patchy)), vegetation type (stinging nettle; blackberry; blackberry and stinging nettle; rough herbaceous vegetation; shrubs; other (grass dominated), soil humidity (dry soil; humid soil; marshy land; other (water)), mowing regime (mowing land; other (unmowed)), grazing regime (grazing land; other (ungrazed)), positioning related to inundation (inland areas, periodically flooded areas, other (high water free areas)), soil texture (sandy soil; loamy soil; clayey soil; other (hard substrate)).

The distribution patterns of the most commonly trapped species, *M. arvalis* and *C. glareolus*, were best explained by the variables measured. As had also been found in other studies, *M. arvalis* was associated with grazed grasslands with a high vegetation cover. The pastures at ADW seemed to provide more suitable habitats than the meadows and grasslands of the habitat creation areas. The distribution patterns of *C. glareolus* were more closely related to vegetation height than to coverage. This species tended to prefer vegetation dominated by blackberry (*Rubus caesius* L) and stinging nettle (*Urtica dioica* L), as this ensures a certain vegetation height and probably opportunities for climbing. These vegetation types are often described as wasteland. *C. glareolus* was not found in pastures or large rectangular shaped homogeneous vegetation units. The relations between the distribution pattern and the ecological parameters were less significant for *S. araneus*, and *A. sylvaticus*, due to the smaller numbers trapped. These species showed the strongest positive regression with rough vegetation types like those dominated by *U. dioica* alone, by *U. dioica* and *R. caesius*, and wasteland. Both species appeared to be more common in taller vegetation, and they seemed to have a preference for dry substrates. The two species seemed to avoid pastures and natural grasslands. The opposite pattern was seen for *M. minutus*. Trapping period, which included only three monitoring moments, did not show a positive relation with one of the species, indicating that other factors (seasonality) did already reduce the increase of the populations before a new flooding event. No species seemed to be specifically linked to the habitat restoration areas. *C. russula* was found to be especially related to shrubs and line shaped structures along mowing land.

Table 2 shows the habitat suitability of the ecotope classes distinguished at ADW, based on the trapping results. Although buildings can provide good habitats for several species, they were not taken into account. Many small mammals were found especially in ungrazed rough herbaceous vegetation, which provided a suitable habitat for five of the six species, and represented a marginal biotope for *A. sylvaticus*. Large parts of the floodplain, viz. those with sparse herbaceous vegetation on sand, bare sand, maize fields and hard substrates, did not harbour any small mammals at all. These unsuitable biotopes form potential (partial) barriers to recolonisation, especially if they were large rectangular homogeneous units.

Table 2: Suitability of ecotope classes for the various small mammal species. The distribution of small mammal ecotopes is shown in Fig. 1a. 1: Rough herbaceous vegetation, ungrazed; 2: rough herbaceous vegetation, grazed; 3: low herbaceous vegetation; 4: rough herbaceous vegetation on sand; 5: sparse herbaceous vegetation on sand; 6: bare sand; 7: softwood forest or shrubs with herbaceous undergrowth; 8: softwood forest or shrubs with sparse undergrowth; 9: rough vegetation of blackberry and stinging nettle; 10: rough vegetation, predominantly blackberry; 11: rough vegetation, predominantly stinging nettle; 12: rough vegetation, predominantly thistle; 13: grassy vegetation, ungrazed and unmown; 14: grassy vegetation, extensively grazed; 15: grassy vegetation, mown or trodden; 16: pasture (mowing land); 17: pasture (grazing land); 18: maize field; 19: hard substrate; 20: buildings, not included and 21: water, not included.

Species	Suitable classes	Marginal classes	Unsuitable classes
Microtus arvalis	1, 2, 13	3, 14, 15, 16, 17	4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 19
Clethrionomys glareolus	1, 9	7, 10	2, 3, 4, 5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19
Sorex araneus	1	2, 4, 7, 9, 10	3, 5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19
Crocidura russula	1, 2, 14	9, 13, 15	3, 4, 5, 6, 7, 8, 10, 11, 12, 16, 17, 18, 19
Micromys minutus	1, 4	2, 9, 10, 13	3, 5, 6, 7, 8, 11, 12, 14, 15, 16, 17, 18, 19
Apodemus sylvaticus	8, 9, 10	1, 2, 7, 11, 12	3, 4, 5, 6, 13, 14, 15, 16, 17, 18, 19

Small mammal densities

Trapping numbers in May appeared to be very low. Irrespective of habitat quality, relative densities of *M. arvalis* showed an increase in all distance zones from May till October, followed by a decrease at the start of winter (Fig. 4). Relative densities were highest in the 0–30 m zone. Unlike those of *M. arvalis,* the relative densities of *C. glareolus* were relatively stable throughout the year for the whole floodplain. No individuals of *C. glareolus* were observed in areas situated more than 30 m from non-flooding parts. *S. araneus* showed a slight tendency to increase its relative densities from spring to summer, followed by a decrease, but the densities seem to be low. No individuals of this species were observed at more than 120 m from non-flooding parts. *C. russula* also showed relatively low densities

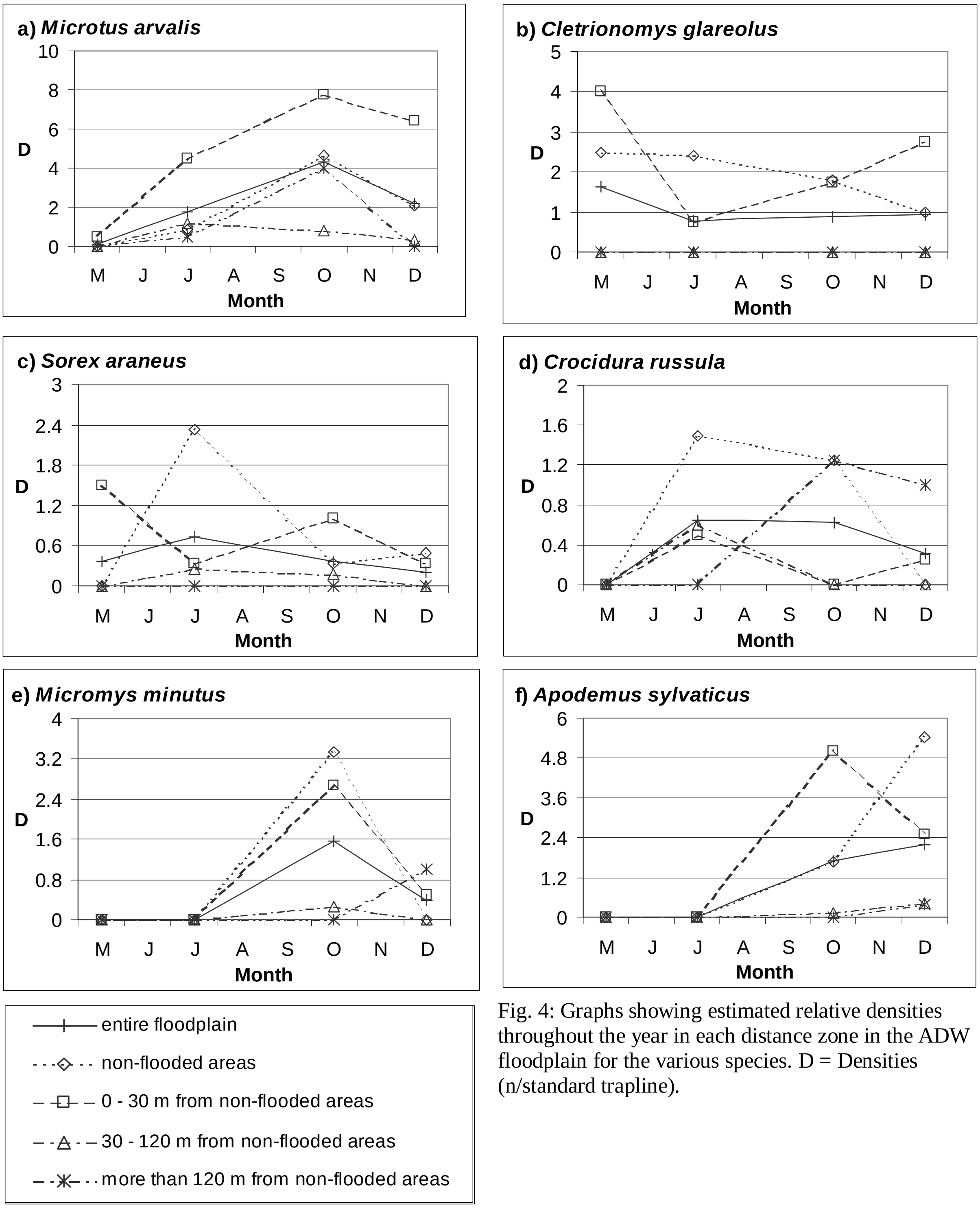
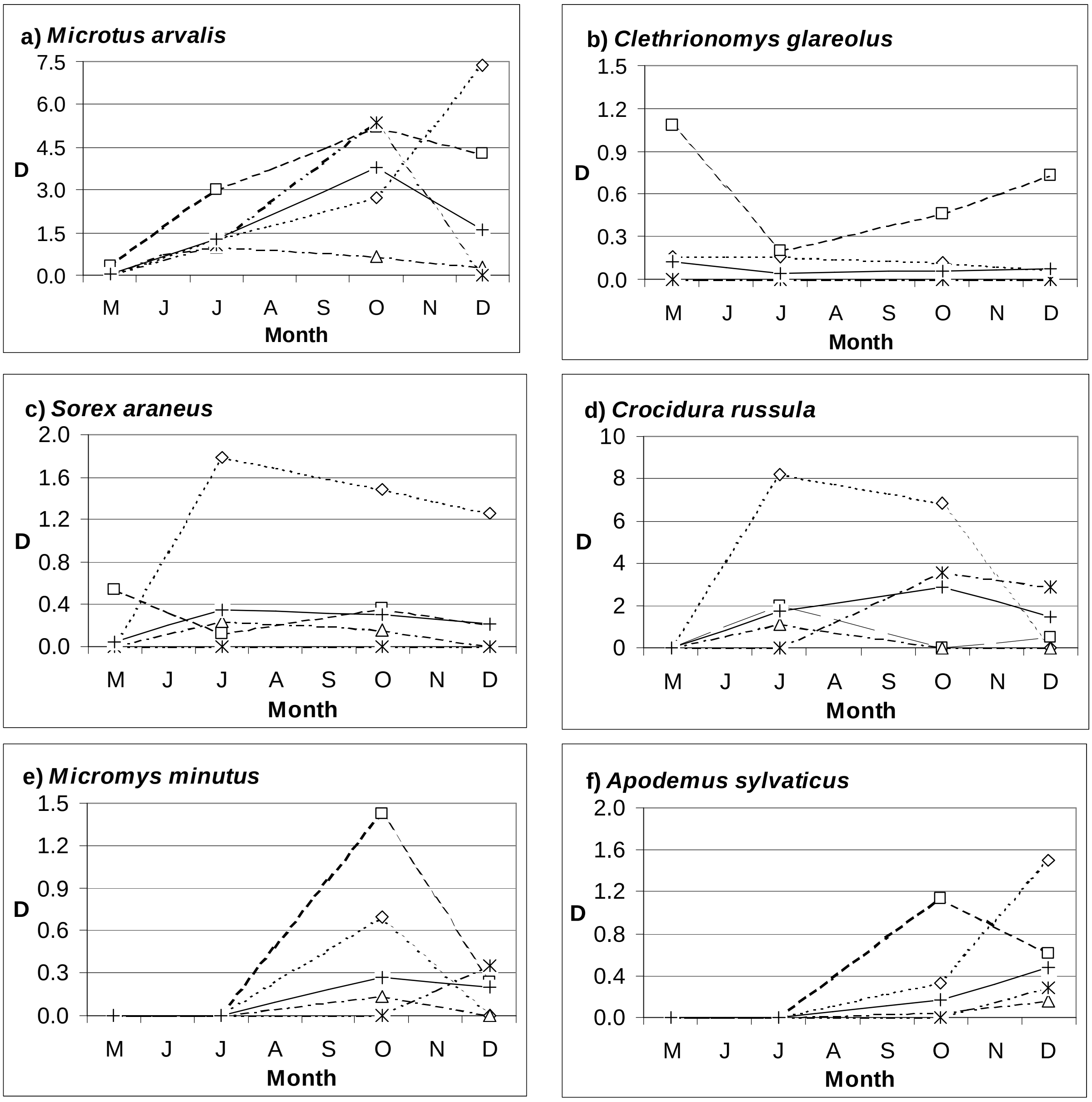


Fig. 4: Graphs showing estimated relative densities throughout the year in each distance zone in the ADW floodplain for the various species. D = Densities (n/standard trapline).

throughout the year, with an increase from almost zero in spring to peak densities from summer to autumn (depending on the distance from non-flooding parts), followed by a decrease at the start of winter. This species was found all over the floodplain at the end of the year. *M. minutus* and *A. sylvaticus* were both not trapped until autumn. The relative densities of *A. sylvaticus* in the total floodplain were still increasing towards winter, when *M. minutus* densities were decreasing, except for the areas located at the greatest distance from non-flooding parts. Both species were present throughout the floodplain in December.



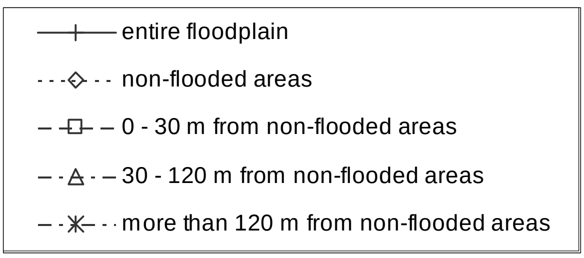


Fig. 5: Estimated population development in densities per distance zone in the ADW floodplain. D = Densities (n/ha).

Calculated peak autumn densities of *M. arvalis* of about 4 individuals per hectare were found in the entire ADW floodplain, but densities in particular zones sometimes rose to more than 7 individuals per hectare by December (Fig. 5). The densities of *C. glareolus* were highest in the zone located 0–30 m from the non-flooding areas, where densities of more than 0.7 individuals per hectare were found in May and December. Densities of *C. glareolus* in the non-flooding parts remained relatively low. Only in the non-flooding areas were *S. araneus* densities higher than 1 per hectare. The rest of the floodplain harboured fairly constant numbers of between 0 and 0.5 individuals per hectare. The densities of *C. russula* were estimated to peak at about 8 individuals per hectare in July in the non-flooding parts. *M. minutus* was only observed in October and December, showing a clear dispersal pattern from non-flooding and adjoining parts towards the more distant areas at the end of the year, but estimated total densities were again low. *A. sylvaticus* was also observed only at the end of the year, showing an increase in total densities in December.

2.5 Discussion

Habitat characteristics

Important habitat characteristics for the presence of small mammal species are found to be structure-related parameters, instead of plant species composition (Van Apeldoorn et al. 1992). We found that the presence of *C. glareolus* was related to wasteland, often with tall vegetation types. Such biotopes probably provide sufficient shelter and climbing opportunities (Geuse et al., 1985). Stinging nettle or the combination of blackberry and stinging nettle dominated this vegetation. *M. arvalis* is associated with herbaceous grasslands with dense vegetation cover, probably providing adequate shelter, which was more commonly present in the periodically flooded parts of the floodplain. Like *M. arvalis*, *M. minutus* also seemed to be more closely associated with the agricultural parts of the area, while the other species were more abundant in rough vegetation, not present in large connected areas, but especially found in smaller patches and wasteland areas.

Impact of flooding

A relation between the abundance of certain small mammal species and the trapping period, as has been observed in several other studies (Erlinge et al., 1983; Crespin et al., 2002) was not found, due to the low number of monitoring moments included in the analyses (Fig. 3). Reproduction of small mammals generally starts in spring or early summer, leading to an increase in numbers and densities. Peak densities are normally reached in autumn, after which a decrease in numbers can be observed, as reproduction stops, but predation and other mortality continue (Churchfield, 1980; Hansson, 1997). This general pattern depends on predator presence, food availability and climatic factors, which means that winter breeding or peak densities in spring may be found under particular conditions (Boyce and Boyce, 1988b; Hanski et al., 2001). However, we did not find that the presence of species was negatively related to the periodically flooded areas, which means that the impact of flooding is not so severe that small mammals do not return to these areas. Recolonisation of the periodically flooded parts was indeed observed.

Trapping numbers in May appeared to be extremely low. This indicates that after floods, the numbers in flooding parts are reduced to zero or almost zero for all species. It is known that small mammals in lower parts of the floodplain run a great risk of drowning (Andersen et al., 2000). Even if they reach non-flooding areas, mortality remains high, due to hypothermia (Pachinger and Haferkorn, 1998), stress and exhaustion or predation. The predation risk in such a new environment will be high just after arrival, as the environment is unfamiliar and often unsuitable, and several predators (e.g. crows, herons and gulls) are waiting for the arrival of this easy to catch prey. Flooding is most common during winter, increasing mortality due to low temperatures. Recolonisation of the largest area of the floodplain must occur from the small populations surviving in refugia on the elevated parts.

As the populations grow, the animals will disperse over the non-populated parts. The moment at which this dispersal starts differs among species. When flooding occurs in spring, the increase in small mammal numbers may be delayed due to a shortage of suitable habitats. In addition, food availability, for both herbivorous and insectivorous species, will also be influenced by the flooding event.

If the influence of floods on small mammal distribution is only temporary, relative densities, which are supposed to be independent of habitat quality as this is standardised, might be expected to be similar in the various zones shortly after inundation. Since relative densities, except for *M. minutus* and *C. russula*, were lower in areas more than 30 m from the recolonisation source populations (Fig. 4), this means either that recolonisation through suitable habitats is a slow process, or that these areas are less accessible due to partial or temporal recolonisation barriers. This indicates that the floodplain was not completely recolonised over a period of 8 months between the two successive floods. However, just after the initial flood has subsided, higher relative densities of *M. arvalis*, *C. glareolus* and *S. araneus* were observed in the zone between 0 and 30 m from the non-flooding areas than on the elevated parts. This may have been the result of differences in food availability and quality. The presence of young leaves emerging after inundation could have been important for the herbivorous vole species. The insectivorous *S. araneus* is probably especially attracted by the great abundance of macro-invertebrates in the debris at the flood mark, and by the presence after flooding of relative inactive earthworms (Zorn et al., 2005), which are their main food (Rudge, 1968; Pernetta, 1976). *C. glareolus* was in ADW never observed at distances of more than 30 m from the non-flooding areas.

The trapping results may not directly reflect their densities, especially for those species for which the trappability depends on the season. We assume that the numbers of *S. araneus* recorded at ADW are especially underestimated in May, as this species shows reduced surface activity in winter and early spring (Churchfield, 1980). The trappability of *M. minutus* was probably also reduced until autumn. This species is known to forage especially in the aboveground vegetation, and will only be trapped when vegetation is falling over, and seeds, their preferred food items, have dropped onto the ground. Failure to trap a species does not mean that this species is absent, but may also reflect very low densities. Like *M. minutus*, *C. russula* was not trapped in May. This was probably because *C. russula* is known to hibernate in buildings (Lange et al., 1994), where we did not trap. The densities of *A. sylvaticus* appeared to be low until autumn, which has been found in other studies as well (Montgomery, 1989), and populations were still growing in December, especially on the elevated parts and their surroundings, showing the recolonisation source of the population. Peak densities in winter and winter breeding have been recorded for this species as well (Southern, 1965; Smyth, 1966).

Habitat suitability

For all species, suitable habitats seemed to cover only small areas within the ADW floodplain. As unsuitable ecotopes can be potential partial or temporal barriers to recolonisation, the present vegetation structures may impact the recolonisation process. However, linear biotopes suitable for small mammals can enhance connectivity in the landscape by providing corridors, and patchiness can provide stepping-stones. It is only for the Microtidae, in this case especially *M. arvalis*, that large parts of the ADW floodplain seem to function at least as marginal habitats, as the species is also found in the low grassland-related ecotopes (Myllymäki, 1977b). An exception is *A. sylvaticus*, which can be found in the ecotope types with little coverage or undergrowth (Geuse et al., 1985). Other species are associated with denser and rougher vegetation. These species either need a certain amount of coverage (*C. russula* and *M. minutus*) or have a need for climbing opportunities (*C. glareolus*) or rough vegetation (*C. glareolus* and *S. araneus*) (Geuse et al., 1985; Van Apeldoorn et al., 1992). These scarce ecotopes were present only in a patchy pattern of small pieces in the habitat restoration parts of our area, predominantly along ditches and roadside verges, as well as along the edges of pastures in the agricultural part of the floodplain.

Except in December, the densities of *M. arvalis* were lower on the elevated non-flooding parts than in the lower parts (Fig. 5). This indicates that the suitable habitat for this species, that is various types of grassy vegetation, was more abundant in the lower parts. Other species, especially *S. araneus* and *C. russula*, showed the opposite pattern. The densities of these shrews were highest on the non-flooding parts, indicating that more suitable habitat (rough herbaceous vegetation types) was present there. According to the species densities graph, the suitable habitat for *C. glareolus*, which is dominated by blackberry and stinging nettle, was more common in the 0–30 m zone than on the elevated parts.

Small mammal densities and their implications

The occurrence, and especially the densities, of small mammals are season- and habitat-dependent, and also vary per geographical region. Pristine and non-regulated rivers have vast flooding zones where water levels in flooding zones do not rise that much as water levels in floodplains of embanked rivers during floods. This because the embanked floodplains are often narrow and sometimes even excavated, which give them a bath-tube shape. The natural variation in elevations in pristine river floodplains normally creates a whole range of non-flooding biotopes. Water levels rise more gradually, and have lower peaks, leaving fauna more opportunities to escape. It has been shown in an American study that small mammal species do not leave the lower parts of floodplains before a flood (Andersen et al., 2000). The small mammal densities recorded in the present study seem to be much lower than those recorded in the literature for inland areas (Appendix 1). This could be explained by the impact of flooding, the low recolonisation rate, and the poor connectivity, but can also be a result of our trapping methodology, and on home range based calculations of densities. However, much lower densities at distances more than 30 m from the recolonisation sources on the non-flooding parts are observed in most of the ADW area. This is a strong indication that densities within the area have the potential to be much higher when flooding events are absent. As we did not intensively monitor in grids or use radio-tags to observe the dispersal of individuals, we cannot conclude that species are absent in those areas where we did not trap them. However, it is clear that densities were low in 2001 in large parts of the ADW floodplain.

Frequency, duration and timing of floods are thought to influence recolonisation, due to the impact on small mammal populations, habitat structures and food availability. Therefore it has been suggested that monitoring in different years can provide valuable information. The present study has shown that in the ADW, a semi-natural Dutch floodplain, with a periodical flooding regime, the time between two successive floods seems to be not nearly long enough for recolonisation of the entire floodplain. Although the area is relatively small, resulting in short recolonisation distances of no more than a few hundred metres, densities in the lower parts of the floodplain were always lower than on the elevated parts. We found that the quantity and quality of elevated areas in floodplains and the connectivity of habitat structures were important factors determining the recolonisation process, with quality and connectivity values proving to be species-dependent.

Conclusions

In spite of small distances between non-flooding and flooding suitable habitats, it took more than 8 months for small mammals to recolonise suitable habitats in an embanked floodplain. Two of the six common species were never observed farther than 120 metres from non-flooding recolonisation sources. Densities of small mammals at more than 30 metres from elevated areas were always lower than those in the non-flooding parts. The recolonisation process appeared to be slow due to the poor habitat connectivity, the limited number of recolonisation sources, and the small number of survivors from which populations must recover. Suitable habitats for the small mammals included especially rough herbaceous vegetation, which was mostly present in small areas of wasteland. Five of the six investigated species (*M. arvalis*, *C. glareolus*, *S. araneus*, *C. russula* and *M. minutus*) were found there frequently. There were no spoors of small mammals observed in large areas with sparse herbaceous vegetation or maize fields. Vegetation structure parameters like coverage and height appear to be decisive for the small mammal composition. Active dispersers like *A. sylvaticus*, *M. minutus* and *C. russula* were observed throughout the floodplain after 8 months, but their densities were lower there than in the elevated areas, or were still increasing, indicating incomplete recolonisation. The recolonisation of the floodplain by *S. araneus* and *C. glareolus* appeared to be hampered, as suitable habitats at larger distances from sources of recolonisation were not inhabited after 8 months. The recolonisation by *M. arvalis*, driven by population expansion into adjoining habitats, was too slow to result in similar densities throughout the Afferdensche en Deestsche Waarden floodplain.

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Appendix 1: Home ranges and densities of small mammals. All data are from West- and North-European studies in environments comparable to floodplain habitats. Symbols indicate where specific data about males (♂), females (♀) or juveniles (juv) have also been incorporated. Besides general data, specific data on biotopes comparable to those found in the ‘Afferdensche en Deestsche Waarden’ floodplain have been incorporated when available between brackets, which means data on grassland for *M. arvalis*, *M. agrestis* and *S. araneus*; deciduous and mixed forests for *C. glareolus*, *S. araneus* and *A. sylvaticus*; pine forests for *C. glareolus*; scrubs for *S. araneus*; gardens for *C. russula* and plantations for *A. sylvaticus*. Specific data for summer and winter were also available for *C. glareolus*, *S. araneus* and *A. sylvaticus*.

Species	Recorded mean size of home ranges in m^2 (total recorded range)	Recorded densities (n/ha) (recorded densities specific for floodplains)	References
Microtus arvalis (♀, ♂, juv)	161 – 1350 (118 – 1500)	up to >1000 (64.1)	Myllymäki, 1977b; Boyce and Boyce, 1988b; Lange et al., 1994
Microtus agrestris (♀, ♂)	250 – 500 (200 – 700)	8 - 350	Myllymäki, 1977a&b; Tapper, 1979; Gurnell and Flowerdew, 1990; Erlinge et al., 1983; Agrell et al., 1992; Nelson, 1995
Clethrionomys glareolus (♀, ♂)	260 – 4100 (200- 11000)	some – 250 (1.7 – 139)	Kikkawa, 1964; Bergstedt, 1966; Crawley, 1969; Zejda and Pelikan, 1969; Bujalska, 1970; Pelikán, 1974; Zejda, 1976; Gurnell and Flowerdew, 1990; Alibhai and Gipps, 1985; Geuse et al., 1985; Wolton and Flowerdew, 1985; Andrezejewski and Babińska-Werka, 1986; Korn, 1986; Szaki, 1987; Gliwicz, 1989; Zejda, 1991; Lange et al., 1994; Pachinger and Haferkorn, 1998; Krištofik, 1999
Sorex araneus (♀, ♂)	360 – 1400 (90 – 2800)	a few – 50 (0 – 114.4)	Pelikán, 1974; Dickman, 1975; Churchfield, 1980; Gurnell and Flowerdew, 1990; Lange et al., 1994; Pachinger and Haferkorn, 1998; Krištofik, 1999; Ma and Talmage, 2001
Crocidura russula	50 - 200	up to 100	Lange et al., 1994 ; Favre et al., 1997
Micromys minutus	200 - 900	up to 250	Lange et al., 1994
Apodemus sylvaticus (♀, ♂)	230 – 12200 (230 – 21772)	<1 – 190 (1.7 – 19)	Kikkawa, 1964; Randolph, 1977; Gurnell and Flowerdew, 1990; Erlinge et al., 1983; Geuse et al., 1985; Montgomery, 1989; Zejda, 1991; Lange et al., 1994; Pachinger and Haferkorn, 1998; Krištofik, 1999

Chapter 3

Modelling recolonisation of heterogeneous river floodplains by small mammals

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Holes and above-ground trails of Microtidae

Abstract

Riverine landscapes are characterised by recurrent flooding events and successional landscape mosaics with high habitat heterogeneity, providing species-specific patterns of suitable and unsuitable biotopes. Landscape characteristics, like distance, barriers and their specifications (e.g. cumulative barrier width, barrier number) and the spatial arrangement of suitable habitat areas, are expected to affect the dispersal of animals in landscapes. The distribution of voles, shrews and mice in a floodplain was monitored for two years using live traps. Recolonisation was found to be a slow process, resulting in a heterogeneous distribution of small mammals in the floodplains. *Microtus arvalis* was found just after the floods in low densities and on or near non-flooding areas only. From summer to autumn densities gradually increased, and specimens could be observed on larger distances from the non-flooding areas. The density development pattern of *Crocidura russula* was similar to that of *M. arvalis*, but densities increased faster. In contrast, *Clethrionomys glareolus* and *Sorex araneus* could immediately be observed in the former flooded areas just after the floods, but throughout the year these species were not trapped on distances further than 120 metres from the non-flooding areas. *Micromys minutus* and *Apodemus sylvaticus* were trapped only occasionally in spring and summer after which those species could be found throughout the floodplain in larger densities in autumn. To analyse the influence of landscape characteristics on recolonisation, the floodplain was classified into suitable, marginal and unsuitable habitat landscape units for each of the small mammal species, based on the trapping results. Landscape characteristics relating to monitoring sites were measured from an aerial photograph using a geographic information system. After that, presence and recolonisation time of small mammals at monitoring sites could be described by multiple regression models based on these measured landscape characteristics. The predictive power of these models was tested in another floodplain by determining the species distribution after 35 weeks. Multiple regression models appeared to be useful in analysing recolonisation patterns and determining the importance of landscape characteristics for recolonisation by small mammals after flooding events. Available distribution data suggest three different types of recolonisers: (1) Gradual, density induced colonisers (2) Active dispersers (3) Long-distance dispersers after a lag. Results of regression models confirmed that *M. arvalis* could be characterised as a type 1 and *A. sylvaticus* as a type 3 species. The classification of the other species was not possible due to the relatively short time available for recolonisation.

3.1 Introduction

Small mammals (e.g. voles, shrews and mice) in floodplains have to cope with periodic floods. Flooding has a strong impact on their populations, resulting in high mortality, and restricting their presence to refugia on elevated terrains after inundation (Pachinger and Haferkorn, 1998; Andersen et al., 2000). After the water has retreated, the floodplain landscape has to be recolonised from these sources (Robinson et al., 2002). Small mammals are important animals in riverine ecosystems, because of their feeding and burrowing activities and their role in food webs (Wijnhoven et al., 2005). They are important prey items for several endangered or protected birds of prey and carnivorous mammals (Erlinge et al., 1983; Jongbloed et al., 1996; Hanski et al., 2001; Leuven et al., 2005), for which many floodplains are assumed to function as important conservation areas (Andersen et al., 2000; Wike et al., 2000; Robinson et al., 2002; Van den Brink et al., 2003; De Nooij et al., 2004). As predators predominantly forage in areas with abundant prey, it is important to understand the temporal and spatial distribution patterns of small mammals.

Several small mammal species are known to form potentially fast-growing populations (Erlinge et al., 1983; Delattre et al., 1999; Hanski et al., 2001). However, connectivity of landscapes is very important for a rapid dispersal through, or recolonisation of, such landscapes (Zhang and Usher, 1991; Wolff, 1999). Riverine landscapes are dynamic, and biologically and spatially complex, characterised by a successional landscape mosaic with high habitat heterogeneity (Robinson et al., 2002). Characteristics of the landscape elements between small mammal source populations and unoccupied suitable habitats, like distance, the presence and characteristics of barriers and the spatial arrangement of suitable habitat areas, are expected to affect the dispersal (in terms of speed and direction) of animals (Van Apeldoorn et al., 1992; Diffendorfer et al., 1999; Peles et al., 1999; Matthiopoulos, 2003). The role of various landscape characteristics in guiding dispersal and recolonisation may be species-specific, while landscape suitability may also differ for various species (Kozakiewicz, 1993; Bowers and Barrett, 1999; Robinson et al., 2002). Unsuitable areas can function as temporary or permanent barriers to dispersal (Bondrup-Nielsen, 1985; Peles et al., 1999), whereas relatively small suitable parts can influence the connectivity (e.g. by forming corridors or stepping-stones; Kozakiewicz, 1993; Wolff, 1999) and therefore the recolonisation speed. Models based on landscape characteristics have been used to study the impacts of habitat fragmentation on species distribution patterns (Vos and Chardon, 1998; Verboom et al., 2001; Verbeylen et al., 2003), and such models might be useful for floodplain conservation and management (Wolff, 1999).

The factors that determine the recolonisation process by small mammal species in various diversified floodplains are expected to be similar, although the relative importance of landscape characteristics may depend on the species. In this study, we investigated (1) The relative importance of various landscape characteristics for the recolonisation of floodplains by small mammals; (2) the existence of different patterns of dispersal behaviour (species-specific recolonisation patterns); (3) the usefulness of multiple regression models of floodplain recolonisation by small mammals as tools to analyse the importance of various landscape characteristics; and (4) the usefulness of multiple regression models in predicting the time to recolonisation and the presence of species at certain locations after flooding events.

3.2 Materials and methods

Research areas

The research project took place at the Afferdensche en Deestsche Waarden (ADW) and Millingerwaard (MW). Both floodplains are located on the left bank of the river Waal, the main branch of the river Rhine in the Netherlands (Fig. 1). The ADW, with an area of 280 ha, is situated 20 km west of the town of Nijmegen and consists of conservation areas and farmland. The ADW includes elevated areas left over from former brick factories, as well as clay excavations, small water bodies and side channels of the river. Similar structures can also be found in the MW, situated 20 km east of Nijmegen. The MW includes a larger section (including the entire 41.2 ha research area) designated as conservation area than the ADW. Both floodplains are included in ecological rehabilitation programmes involving natural and semi-natural wetlands, grasslands, shrub-dominated vegetation and softwood forests. Ecological rehabilitation measures started in 1990 at the MW (Helmer and Smeets, 1990), and after 1995 at the ADW (Zandberg, 1999). Because of this time difference, the vegetation at the MW is generally denser and rougher than that at the ADW, due to natural succession processes (Fig. 1). Both areas are embanked and include areas inside and outside the summer dikes (the dikes closest to the river that protect land against summer flooding). The summer dikes in the MW nowadays mostly resemble natural elevations like river dunes. Both research

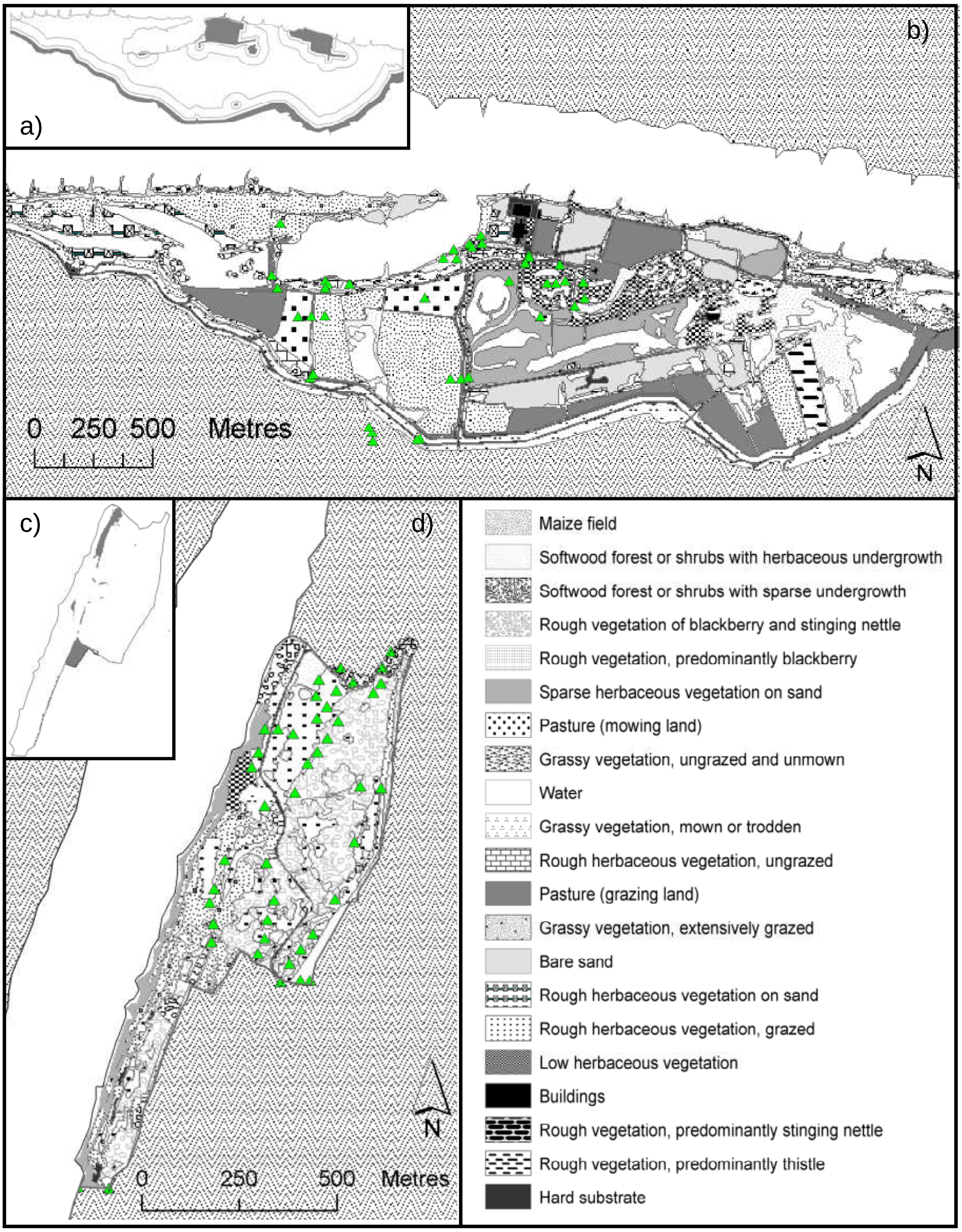

Fig. 1: Characteristics of the research areas. (a) Locations of the non-flooding areas (shaded) in the Afferdensche en Deestsche Waarden floodplain (ADW), with the borders of the distance zones (0-30 m; 30-120 m; >120 m) indicated as lines. (b) Classification of the ADW into small mammal ecotopes. (c) Locations of the non-flooding areas (shaded) in the research area at the Millingerwaard floodplain (MW). (d) Classification of MW into small mammal ecotopes. The small mammal monitoring plots are shown as triangles.

areas are regularly inundated. The flooding frequency at the ADW is more than once a year, while the MW is not flooded every year, due to its elevation. Small mammals in the ADW were monitored between April 2001 and January 2003 (Fig. 2). During this monitoring

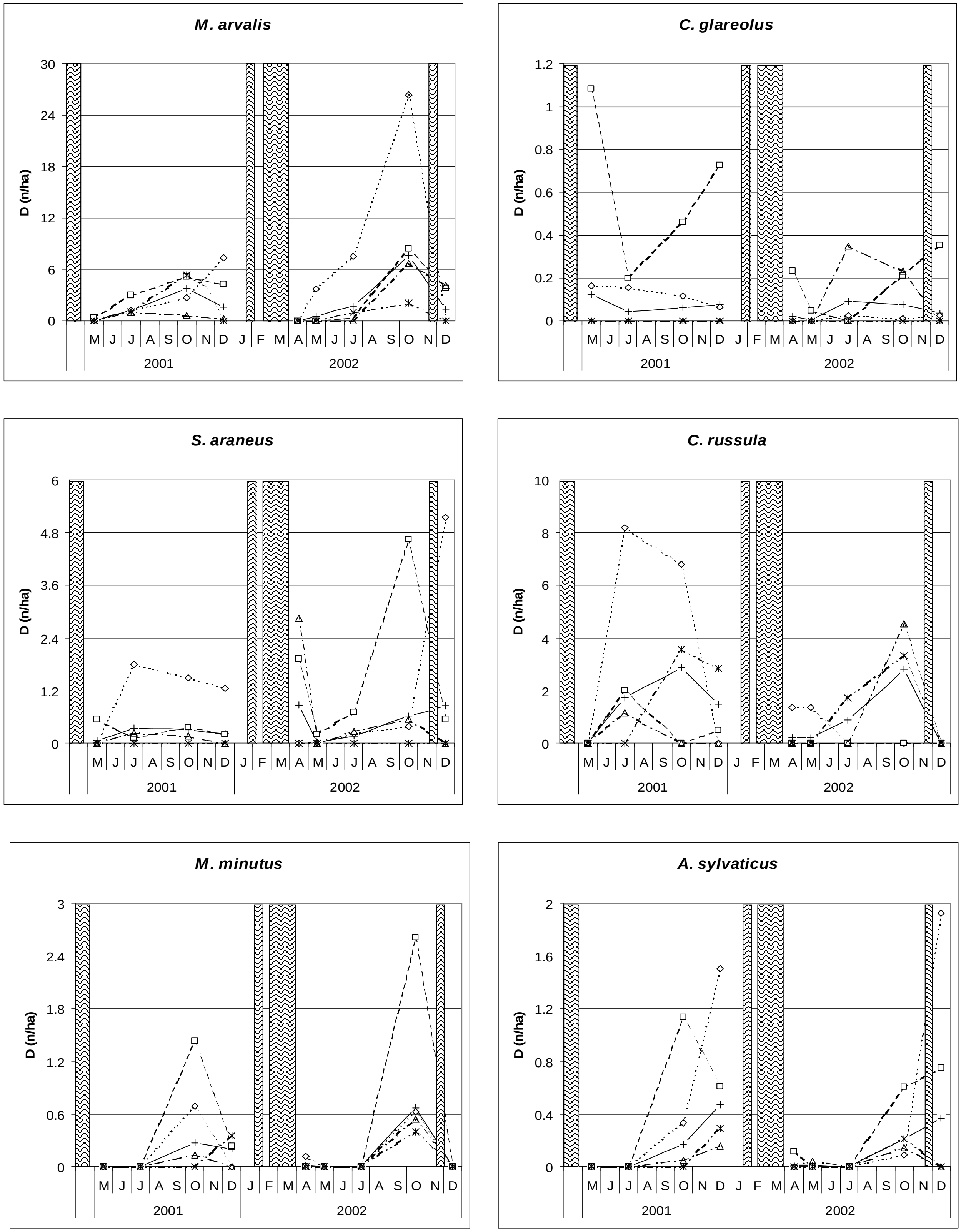

Fig. 2: Species densities (n/ha) at various distances from non-flooding areas in the Afferdensche en Deestsche Waarden floodplain through the years 2001 and 2002. Periods of inundation of the flooding parts are indicated in grey.

period, approximately 194 ha was flooded (followed by a gradually retreat of the water) between 16 April 2001 and 5 May 2001, between 24 February 2002 and 30 March 2002 and between 14 November 2002 and 5 December 2002. Approximately 126 ha was also flooded from 29 January 2002 to 24 February 2002. In the MW, small mammals were monitored between a flood event just before the spring of 2002 (after which the water had retreated by 18 March) and one in the first week of 2003.

Measuring small mammal distribution

In the ADW, the small mammal distribution patterns were monitored using trap lines with 10 live traps each, distributed over 34 selected monitoring plots, as indicated in Figure 1, and as described in Wijnhoven et al. (2005). The live traps were checked every 4 hours for 72 hours after a two-day prebaiting period, and were monitored at 7, 13, 24 and 34 weeks after the water had retreated in 2001, and at 2, 6, 16, 27 and 36 weeks after the water had retreated in 2002. During weeks 33 and 34 after the water had retreated in 2002, some of the monitoring sites were flooded again. Small mammals could only survive in refugia on the elevated non-flooding areas during inundation of the remaining parts of the area. In the two years of live trapping, nine small mammal species (voles, shrews, mice and mustelids) were trapped in the ADW, viz. *Microtus arvalis* (Pallas, 1771) (Common vole), *Clethrionomys glareolus* (Schreber, 1780) (Bank vole), *Sorex araneus* (Linnaeus, 1758) (Common shrew), *Crocidura russula* (Hermann, 1780) (White-toothed shrew), *Micromys minutus* (Pallas, 1771) (Harvest mouse), *Apodemus sylvaticus* (Linnaeus, 1758) (Wood mouse), *Microtus agrestis* (Linnaeus, 1761) (Short-tailed field vole), *Sorex minutus* (Linnaeus, 1766) (Pygmy shrew) and *Mustela nivalis* (Linnaeus, 1766) (Weasel). Between 46 and 247 individuals were trapped for each of the first six of these species. The other three species were trapped only occasionally (less than ten individuals per species), which was insufficient to analyse the recolonisation patterns. The species densities were calculated for zones based on the distance from non-flooding areas (0 – 30 m, 30 – 120 m, >120 m), and for the non-flooding areas themselves. Trapping numbers per trap line were converted into densities per habitat suitability class (suitable, marginal, or unsuitable habitats, as shown in Table 1). This meant that the species-specific trapping range of the trap lines had to be taken into account. The total area per habitat class in each zone was used to calculate the total density (n ha^{-1}) (Wijnhoven et al., 2005). The trapping results were used to calculate the time to recolonisation for 68 situations (two years of monitoring at 34 sites) assuming that the first trapping of a species at a monitoring site corresponds to the recolonisation time (in weeks after the retreat of the water) for that site.

In an attempt to cover a larger area with less effort, the populations of *M. arvalis*, *C. glareolus*, *S. araneus* and *C. russula* at the MW were monitored using ‘food traps’. These traps were positioned in lines of five across the monitoring plots (Fig. 1), and were checked and refreshed daily for three days. These traps were constructed in such a way that small mammals could freely enter and leave, while larger animals could not. Different types of bait (apple, carrot and tinned meat) were offered together in each trap to analyse the feeding patterns. The method was also combined with live trapping several times at the ADW, and observations were made in terrariums, allowing species to be identified from the feeding patterns. The specific feeding patterns were as follows. *M. arvalis* had a preference for apple (often gone), ate most of the meat and also ate carrot. *C. glareolus* preferred carrot, ate apple and ate some meat. *S. araneus* ate all the meat and took tiny pieces of apple. *C. russula* preferred carrot and ate small pieces of meat and apple. *A. sylvaticus* ate meat and apple and took tiny pieces of carrot. *M. agrestis* ate carrot and apple in similar amounts, and also ate from the meat. No data were available on the feeding patterns of *M. minutus*. When one of the vole species was present, all the bait in a trap was sometimes gone. As we made 15 observations per monitoring site, we were also able to ascertain if more than one species had been present, as there were always cases in which only one of them visited a trap. The characteristic pattern of *M. agrestis* was not observed in the MW, and we did not find the pattern characterising *A. sylvaticus,* although the species was assumed to be present. Monitoring sites were located at distances (shortest linear distance) of 0, 50, 100, 150, 250 and 400 metres from non-flooding areas, covering the entire research area in the MW. In total, 42 monitoring sites were analysed, yielding the distribution of four frequently observed species in the MW, 35 weeks after inundation.

Table 1: Suitability of riverine ecotopes for six small mammal species, determined from the 2001 trapping results in the 'Afferdensche en Deestsche Waarden' floodplain (derived from Wijnhoven et al., 2005).

Small mammal ecotopes	*M. arvalis*	*C. glareolus*	*S. araneus*	*C. russula*	*M. minutus*	*A. sylvaticus*
Maize field	U	U	U	U	U	U
Softwood forest or shrubs with herbaceous undergrowth	U	M	M	U	U	M
Softwood forest or shrubs with sparse undergrowth	U	U	U	U	U	S
Rough vegetation of blackberry and stinging nettle	U	S	M	M	M	S
Rough vegetation, predominantly blackberry	U	M	M	U	M	S
Sparse herbaceous vegetation on sand	U	U	U	U	U	U
Hay meadow	M	U	U	U	U	U
Grassy vegetation, ungrazed and unmown	S	U	U	M	M	U
Water	U	U	U	U	U	U
Grassy vegetation, mown or trodden	M	U	U	M	U	U
Rough herbaceous vegetation, ungrazed	S	S	S	S	S	M
Pasture (grazing land)	M	U	U	U	U	U
Grassy vegetation, extensively grazed	M	U	U	S	U	U
Bare sand	U	U	U	U	U	U
Rough herbaceous vegetation on sand	U	U	M	U	S	U
Rough herbaceous vegetation, grazed	S	U	M	S	M	M
Low herbaceous vegetation	M	U	U	U	U	U
Buildings	U	U	U	U	U	U
Rough vegetation, predominantly stinging nettle	U	U	U	U	U	M
Rough vegetation, predominantly thistle	U	U	U	U	U	M
Hard substrate	U	U	U	U	U	U

Suitability of ecotopes: U = unsuitable ecotope; M = marginal ecotope; S = suitable ecotope. Trap lines were assumed to be situated in a suitable ecotope when a species was present during each trapping period after the first observation. If the species was only occasionally present, the trap line was assumed to be in a marginal ecotope. If a species was only present in a trap line located on a non-flooding part in December, while there were neighbouring ecotopes which were assumed to be suitable, the trap line was assumed to be in a marginal habitat. In all other cases, the trap lines were assumed to be situated in unsuitable ecotopes.

Landscape characteristics

The landscape structure of the two research areas was classified into 21 structure classes to analyse the landscape characteristics (Fig. 1). The structure classes were based on vegetation and soil characteristics and were called small mammal ecotopes (Wijnhoven et al., 2005). In the ADW, this classification was based on intensive monitoring of vegetation development during fieldwork. The MW was classified into small mammal ecotopes using the vegetation map by De Ronde (2003). On the basis of the small mammal ecotope maps, species-specific habitat suitability maps according to the classification shown in Table 1 were drawn for the six small mammal species investigated.

All research plots were positioned using a GARMIN GPS 12 Personal Navigator. To measure the values of the landscape characteristics of each of the monitoring sites, we defined the recolonisation routes. We assumed that species had taken the shortest recolonisation route through suitable and marginal habitats from potential sources of recolonisation to the monitoring sites. When no direct route through suitable or marginal habitats was available, unsuitable habitats (potential barriers) were assumed to be crossed, and we used the shortest cumulative distance (when more than one potential barrier had to be crossed). As potential sources of recolonisation we defined those non-flooding areas on which species were indeed observed, or on which species were assumed to be present, as they were caught in suitable habitats that were located within 10 m from non-flooding areas and were directly connected by suitable habitats. The source of recolonisation was defined as the potential source with the shortest recolonisation route. All measurements of distances and areas between recolonisation sources and monitoring sites were done in ArcMap 8.0.

Landscape characteristics such as distances, surface areas and suitability of landscape structures have often been used in various forms in regression models predicting or evaluating species distribution (King et al., 1998; Mörtberg, 2001; Carignan and Villard, 2002; Chase et al., 2003; Chardon et al., 2003). We analysed a number of landscape characteristics for the various small mammal species. Distance (D) is the length in metres of the recolonisation route. Cumulative barrier width (W_{bar}) (referred to below as barrier width) is the summed length in metres of the recolonisation route through unsuitable habitats. Number of barriers (N_{bar}) is the number of barriers counted along the recolonisation route. Area of suitable and marginal habitat influencing recolonisation (A_{hab}) was calculated by first taking the shortest linear distance between the source and the monitoring site as the radius of a circle with the source as its centre, and then calculating the area of suitable and marginal habitats within this circle in square metres. Habitat suitability (S_{hab}) of the monitoring site was scored as 1, 2 or 3 for suitable, marginal and unsuitable habitats, respectively, as shown in Table 1.

Calculations and statistics

To compare the importance of landscape characteristics for the small mammal species in the two research areas, five models were calculated for each species, based on the monitoring results in the ADW. Two logistic regression models of the type $\ln(Y)=aX_1+bX_2+cX_3+...$, calculated using SPSS 11.5, predicted the presence (P) of species, with presence being validated as 1, and absence as 0 (Table 2). Comparable models have been used by Mörtberg (2001), Carignan and Villard (2002) and Chardon et al. (2003) for various species. Model I included the data of all monitoring sites, while Model II included only the data of monitoring sites in suitable and marginal habitats. The other three models were linear regression models of the type $Y=aX_1+bX_2+cX_3+...$, calculated using Microsoft Excel 2000, predicting the recolonisation time (RT) in weeks. These linear models were based on the data of suitable and marginal habitats only. Linear models have been used in several studies on species distributions, for instance those by King et al. (1998) and Chase et al. (2003), and yielded stronger regressions (in terms of R^2) than logistic models.

Monitoring sites where species were never observed were excluded from Model III. In the other two models, the absence of species was scored with a recolonisation time of 45 (Model IV) and 90 (Model V) weeks, respectively. Although these values are arbitrary, being merely used to analyse their effect on the model, 45 weeks was approximately the time between two successive floods and therefore the period available for recolonisation. When regression equations are based on data of suitable and marginal habitats only, this will automatically result in a weaker regression for habitat suitability. All measured landscape characteristics were included in the model, including those which did not show significant regression, as we wanted to analyse changes in regression factors between models.

Table 2: Regression coefficients of multiple regression models (least squares method) for the recolonisation of monitoring sites in the Afferdensche en Deestsche Waarden floodplain. Two models explain the presence (P = 1) or absence (P = 0) of six small mammal species based on landscape characteristics, calculated by logistic regression from the total data set (Model I) or from data for suitable and marginal habitats only (Model II). Three models calculate recolonisation time using linear regression for suitable and marginal habitats only. In model III, the absence of species is omitted (A = /), in model IV recolonisation time (RT) for the absence of species is set on 45 weeks (A = 45), in model V on 90 weeks (A = 90). If the regression coefficient is found significant this is indicated in bold underlined (p<0.05) or underlined (p<0.25).

		D	W_{bar}	N_{bar}	A_{hab}	S_{hab}	c	n	R^2
M. arvalis									
I	ln(P)	$-4.63*10^{-3}$	$-1.11*10^{-5}$	$-5.76*10^{-1}$	$-2.24*10^{-6}$	$-6.16*10^{-1}$	$\underline{\mathbf{+1.91}}$	68	0.25
II	ln(P)	$-4.62*10^{-3}$	$\underline{\mathbf{+0.20*10^{-1}}}$	$\underline{\mathbf{-3.31}}$	$-2.19*10^{-7}$	-1.17	$\underline{+3.21}$	47	0.15
III	RT	$+4.37*10^{-2}$	$-1.53*10^{-1}$	$+3.09$	$-1.34*10^{-4}$	$+6.38$	$+8.47$	27	0.14
IV	RT	$+3.35*10^{-2}$	$\underline{\mathbf{-1.13}}$	$\underline{\mathbf{+20.8}}$	$-4.24*10^{-5}$	$\underline{+9.97}$	$+8.82$	47	0.29
V	RT	$+7.76*10^{-2}$	$\underline{\mathbf{-2.94}}$	$\underline{\mathbf{+49.9}}$	$-4.04*10^{-5}$	$\underline{+18.8}$	$+4.73$	47	0.30
C. glareolus									
I	ln(P)	$-1.06*10^{-2}$	$-1.76*10^{-2}$	$+1.29*10^{-1}$	$+1.26*10^{-4}$	$\underline{\mathbf{-1.61}}$	$\underline{\mathbf{+3.30}}$	68	0.36
II	ln(P)	$+2.11*10^{-2}$	$-1.12*10^{-2}$	-1.72	$+4.95*10^{-6}$	$+6.92*10^{-1}$	$+5.56*10^{-1}$	35	0.21
III	RT	$+4.80*10^{-1}$	$+3.22*10^{-2}$	-59.2	$-2.61*10^{-3}$	-9.87	$\underline{+24.5}$	16	0.14
IV	RT	$+1.14*10^{-2}$	$+9.62*10^{-2}$	$+4.66$	$-1.74*10^{-4}$	$-4.60*10^{-1}$	$\underline{\mathbf{+22.1}}$	35	0.40
V	RT	$+3.32*10^{-2}$	$+1.93*10^{-1}$	$\underline{+12.3}$	$-3.38*10^{-4}$	$-2.65*10^{-1}$	$\underline{+31.0}$	35	0.46
S. araneus									
I	ln(P)	$-7.71*10^{-3}$	$\underline{-2.76*10^{-2}}$	$+1.41*10^{-1}$	$+1.64*10^{-5}$	$-4.71*10^{-1}$	$\underline{+1.78}$	68	0.28
II	ln(P)	$-6.79*10^{-3}$	$-1.99*10^{-2}$	$+5.99*10^{-2}$	$+1.71*10^{-5}$	$-4.39*10^{-1}$	$+1.60$	48	0.10
III	RT	$-1.36*10^{-1}$	$+1.54*10^{-1}$	$+6.89*10^{-1}$	$+1.18*10^{-3}$	$\underline{+6.54}$	$+3.80$	24	0.18
IV	RT	$+1.90*10^{-2}$	$\underline{+1.67*10^{-1}}$	$-3.13*10^{-1}$	$+4.17*10^{-5}$	$\underline{+8.85}$	$+10.2$	48	0.21
V	RT	$+5.52*10^{-2}$	$\underline{+3.71*10^{-1}}$	$-3.21*10^{-1}$	$-5.40*10^{-5}$	$+15.5$	$+14.7$	48	0.19
C. russula									
I	ln(P)	$-6.04*10^{-3}$	$\underline{-1.52*10^{-2}}$	$\underline{+4.39*10^{-1}}$	$+3.42*10^{-5}$	$-4.57*10^{-1}$	$-3.97*10^{-2}$	68	0.07
II	ln(P)	$-6.84*10^{-3}$	$\underline{-1.66*10^{-2}}$	$\underline{+8.36*10^{-1}}$	$+3.65*10^{-5}$	$+5.48*10^{-1}$	$\underline{-1.81}$	50	0.05
III	RT	$+8.27*10^{-3}$	$-8.80*10^{-2}$	$-9.17*10^{-2}$	$-7.34*10^{-6}$	$\underline{-14.4}$	$\underline{\mathbf{+39.4}}$	15	0.46
IV	RT	$+2.82*10^{-2}$	$+4.43*10^{-2}$	$\underline{-3.24}$	$-1.49*10^{-4}$	$\underline{-6.38}$	$\underline{\mathbf{+46.7}}$	50	0.06
V	RT	$+8.00*10^{-2}$	$+1.88*10^{-1}$	$\underline{-10.8}$	$-4.25*10^{-4}$	-11.0	$\underline{\mathbf{+86.8}}$	50	0.08
M. minutus									
I	ln(P)	$+7.34*10^{-3}$	$-1.28*10^{-2}$	$-4.64*10^{-1}$	$\underline{-9.59*10^{-5}}$	$\underline{-1.08}$	$\underline{+1.75}$	68	0.18
II	ln(P)	$+2.09*10^{-3}$	$+1.36*10^{-2}$	$\underline{-5.52*10^{-1}}$	$-6.83*10^{-5}$	$-2.62*10^{-1}$	$+4.42*10^{-1}$	49	0.09
III	RT	$\underline{+5.52*10^{-2}}$	$-6.60*10^{-2}$	$\underline{-3.35}$	$+3.37*10^{-4}$	-4.50	$\underline{\mathbf{+30.4}}$	16	0.48
IV	RT	$+3.89*10^{-2}$	$-1.20*10^{-1}$	$+2.08$	$+1.36*10^{-4}$	$-3.07*10^{-1}$	$\underline{\mathbf{+35.5}}$	49	0.16
V	RT	$+6.35*10^{-2}$	$-3.07*10^{-1}$	$+3.40$	$+3.45*10^{-4}$	$+2.72$	$\underline{\mathbf{+54.6}}$	49	0.14
A. sylvaticus									
I	ln(P)	$-6.27*10^{-4}$	$-3.37*10^{-3}$	$\underline{\mathbf{-9.75*10^{-1}}}$	$+3.08*10^{-5}$	$\underline{\mathbf{-1.54}}$	$\underline{\mathbf{+4.43}}$	68	0.31
II	ln(P)	$\underline{-9.39*10^{-3}}$	$\underline{+3.25*10^{-2}}$	$\underline{\mathbf{-1.01}}$	$\underline{+9.30*10^{-5}}$	$\underline{\mathbf{-2.55}}$	$\underline{\mathbf{+5.75}}$	47	0.19
III	RT	$+3.56*10^{-2}$	$\underline{-1.24*10^{-1}}$	$\underline{-4.88}$	$+4.44*10^{-5}$	$\underline{+8.32}$	$\underline{\mathbf{+19.9}}$	24	0.20
IV	RT	$\underline{+3.36*10^{-2}}$	$\underline{-1.17*10^{-1}}$	$+1.58$	$-2.50*10^{-4}$	$\underline{\mathbf{+10.5}}$	$\underline{\mathbf{+17.1}}$	47	0.28
V	RT	$\underline{+1.00*10^{-1}}$	$\underline{-3.43*10^{-1}}$	$\underline{+9.39}$	$\underline{-9.38*10^{-4}}$	$\underline{\mathbf{+29.0}}$	-14.2	47	0.34

Landscape characteristics measured between the sources of recolonisation and the monitoring sites: D = distance (m); W_{bar} = cumulative barrier width (m); N_{bar} = number of barriers; A_{hab} = area of intervening suitable and marginal habitat (m^2); S_{hab} = habitat suitability of monitoring site (suitable habitat =1; marginal habitat =2; unsuitable habitat =3); c = constant; n indicates sample size; R^2 indicates the explained variance by the models.

We report the significance of the calculated regression coefficients based on the calculated t-values, as well as the positive or negative influence of the characteristic on recolonisation. To evaluate the importance of the landscape characteristics for the recolonisation process we consider the significant related parameters of the models which explain more than 25% of variance in the data as potentially important. The relation of these landscape characteristics should be consistent (negative or positive relation) in those models which explain more than 25% of the variance.

Table 3: Comparison between measured and predicted recolonisation of the Millingerwaard floodplain. Predictions are based on the multiple regression models derived from the Afferdensche en Deestsche Waarden floodplain data for various small mammal species. Table shows average +/- standard deviation for the measurements and the models, and the number of measurements included (n). The predictive power is shown as the percentage of predictions that are similar to the trapping results; when the predicted value $x \geq 0.5$, the prediction is equal to P, and when $x < 0.5$, the prediction is equal to A in models I and II; when $x \geq 35$, the prediction is equal to P, and when $x < 35$, x is similar to A in models III, IV and V.

			M. arvalis		*C. glareolus*		*S. araneus*		*C. russula*	
			Measured	Model	Measured	Model	Measured	Model	Measured	Model
model I	total data, P=1, A=0	average	0.595	2.87	0.238	0.684	0.262	1.83	0.195	1.84 $*10^2$
		sd	0.497	4.67	0.431	4.19	0.445	1.62	0.401	7.48 $*10^2$
		n		42		42		42		41
		predictive power		57.1		76.2		40.5		46.3
model II	habitat data, P=1, A=0	average	0.636	1.70 $*10^8$	0.278	1.98	0.333	2.18	0.235	1.94 $*10^3$
		sd	0.492	7.97 $*10^8$	0.461	7.03	0.485	1.38	0.437	7.20 $*10^3$
		n		22		18		31		33
		predictive power		77.3		50.0		32.3		42.4
model III	habitat data, P=w, A=/	average		19.5		-27.2		34.8		16.8
		sd		2.58		45.1		26.3		7.96
		n		22		18		31		33
		predictive power		63.6		38.9		64.5		21.2
model IV	habitat data, P=w, A=45	average		26.8		26.7		32.3		31.9
		sd		13.6		4.43		3.37		7.79
		n		22		18		31		33
		predictive power		77.3		27.8		48.4		51.5
model V	habitat data, P=w, A=90	average		40.3		46.8		53.1		55.5
		sd		36.0		10.7		5.3		21.4
		n		22		18		31		33
		predictive power		63.6		55.6		71.0		66.7

P=presence of a species, A=absence of a species; the first two models are based on presence – absence data; in the other three models, the first observation of a species at a monitoring site is given as recolonisation time (RT) in weeks (w), with the absence of species either ignored (A=/), or set at 45 or 90 weeks.

The predictive power of the regression models was evaluated by applying the regression equations to the spatial data for the MW and comparing the predicted recolonisation data with the measured data for each of the monitoring sites. A predicted value ≥ 0.5 (for models I and II), and a value < 35 (for models III, IV and V) was interpreted as presence (Table 3). The predictive power was the percentage of predictions that corresponded to the observations. Average values ± standard deviations for various landscape characteristics were calculated for the different study sites, data sets and species (Table 4), as they can explain observed differences in recolonisation and/or differences in model outcomes.

Table 4: Values (average +/- standard deviation) of the landscape characteristics data for the various small mammal species in the Afferdensche en Deestsche Waarden floodplain (ADW) and the Millingerwaard floodplain (MW) used to predict recolonisation. Table shows values of monitoring data for all sites (ADW_t and MW_t) or for the sites in the suitable and marginal habitats only (ADW_h and MW_h).

		ADW_t Average	+/- sd	MW_t Average	+/- sd	ADW_h Average	+/- sd	MW_h Average	+/- sd
M. arvalis	D	219.2	185.8	95.5	91.2	138.5	131.2	83.7	92.9
	W_{bar}	21.4	30.7	12.2	18.8	4.4	8.7	5.6	14.0
	N_{bar}	1.1	1.3	0.9	0.9	0.5	0.7	0.4	0.6
	A_{hab}	39217.1	41164.2	15083.4	24556.6	32022.4	43853.4	12736.9	23746.5
	S_{hab}	1.8	0.9	2.1	0.9	1.3	0.5	1.4	0.5
C. glareolus	D	248.5	242.1	171.9	141.6	169.2	207.6	160.9	129.3
	W_{bar}	109.6	124.8	32.1	34.0	59.0	69.1	17.1	15.2
	N_{bar}	1.4	1.1	1.4	0.9	0.8	0.9	1.1	0.8
	A_{hab}	15412.5	18252.7	18322.7	20914.0	9974.3	14419.5	20226.0	22587.6
	S_{hab}	2.1	0.9	2.2	1.0	1.3	0.5	1.1	0.3
S. araneus	D	124.6	126.0	126.1	111.1	93.2	104.4	134.5	107.9
	W_{bar}	53.2	82.4	7.6	11.5	24.8	33.1	6.3	12.0
	N_{bar}	1.4	1.6	0.7	0.6	1.4	1.8	0.5	0.5
	A_{hab}	7054.8	9970.4	27975.9	34325.1	6732.6	11024.6	29633.2	34657.3
	S_{hab}	2.1	0.7	2.2	0.5	1.7	0.5	2.0	0.2
C. russula	D	235.5	169.3	135.7	92.3	222.0	182.3	139.2	84.2
	W_{bar}	47.3	42.4	7.2	22.7	34.8	37.9	6.6	24.8
	N_{bar}	1.9	1.2	0.6	0.5	1.7	1.2	0.5	0.5
	A_{hab}	22999	22303.4	51592.5	53129.9	23501.6	24051.8	49128.4	50031.1
	S_{hab}	1.8	0.8	1.8	0.7	1.3	0.5	1.6	0.5
M. minutus	D	152.3	125.7	n.a.	n.a.	120.0	104.8	n.a.	n.a.
	W_{bar}	41.6	54.8	n.a.	n.a.	21.4	25.4	n.a.	n.a.
	N_{bar}	1.7	1.6	n.a.	n.a.	1.6	1.7	n.a.	n.a.
	A_{hab}	12491.0	11501.3	n.a.	n.a.	10179.9	10644.5	n.a.	n.a.
	S_{hab}	2.1	0.7	n.a.	n.a.	1.7	0.5	n.a.	n.a.
A. sylvaticus	D	227.7	231.5	n.a.	n.a.	161.2	178.2	n.a.	n.a.
	W_{bar}	50.2	47.7	n.a.	n.a.	34.6	36.5	n.a.	n.a.
	N_{bar}	2.1	1.5	n.a.	n.a.	1.7	1.3	n.a.	n.a.
	A_{hab}	17109.2	18733.5	n.a.	n.a.	12635.9	15720.7	n.a.	n.a.
	S_{hab}	2.1	0.7	n.a.	n.a.	1.8	0.4	n.a.	n.a.

D = distance (m); W_{bar} = cumulative barrier width (m); N_{bar} = number of barriers; A_{hab} = area of intervening suitable and marginal habitat (m^2); S_{hab} = habitat suitability (1=suitable habitat; 2=marginal habitat; 3=unsuitable habitat); n.a.=not available.

3.3 Results

Figure 2 shows the development of the densities of small mammal species in the ADW. Differences between 2001 and 2002 were observed, but general trends in population development (e.g. total densities and timing of density peaks) of the species were similar for the two years. The densities of *M. arvalis* increased gradually in spring, and faster towards autumn. The densities of *M. arvalis* were generally highest on non-flooding areas throughout the year (especially in 2002), while densities in the other zones increased later in the year, an increase which was related to the distance to non-flooding areas. The densities of *C. glareolus* were highest immediately after the retreat of the water in the zone at a distance of 0 to 30 m from the non-flooding areas. Their total densities in the ADW were relatively stable throughout the year, and *C. glareolus* was not trapped at distances of more than 120 m from the non-flooding areas. *S. araneus* was also present immediately after the water's retreat in the lower flooding parts, after which densities fluctuated. This species was not observed at more than 120 m from the non-flooding areas either. In spring, *C. russula* was only trapped in non-flooding areas, after which the species gradually appeared at greater distances from these areas, while its total densities increased. Both *M. minutus* and *A. sylvaticus* were not trapped or only occasionally until autumn, when they appeared with greatest densities in the 0 to 30 m zone, but also at larger distances. In October 2002, both species were observed at more than 120 m from the non-flooding parts, and they were also observed in this zone in December 2001. *M. arvalis*, *C. russula* and *M. minutus* showed a decrease in total densities towards December, while the densities of *A. sylvaticus* were still increasing, and the densities of *C. glareolus* and *S. araneus* were at least stable.

Combining Table 1 and Figure 1 shows that the research areas were of different quality (in terms of suitability and connectivity) for the different species. This is also shown in Table 4, which evaluates the landscape characteristics in relation to monitoring sites. Some of the landscape characteristics, like cumulative barrier width, barrier number and recolonisation distance, generally had higher values in the ADW than in the MW. The area of suitable habitats between the source of recolonisation and a randomly chosen monitoring site was, however, similar for the two floodplains, or larger at the MW than at the ADW. The barrier width was greater for *C. glareolus* than for the other species, and recolonisation distances were also large for this species, as well as for *C. russula* and *A. sylvaticus*. The recolonisation distance for *M. arvalis* was large in the ADW, but small in the MW, unlike what we found for *S. araneus*. The area of suitable habitats in the vicinity was large for *C. russula* in both research areas, and also large for *M. arvalis* in the ADW. This was also reflected in the monitoring sites, which were often found in suitable habitats (i.e. with low S_{hab}).

When data for unsuitable habitats were excluded, the presence or recolonisation time of *M. arvalis* was most related to barrier number and barrier width. Recolonisation time was also related to habitat suitability for the models IV and V which explain more than 25% of the variance in the data. Significant relations were found between habitat suitability and the presence of *C. glareolus* (model I), and the number of barriers and recolonisation time (model V). These models explained 36 and 46% of the variance in data, respectively. However relations between these landscape characteristics and the presence or recolonisation time were only found in a singular occasion. Only one model (I) appeared to explain more than 25% of the variance in the presence data of *S. araneus*, at which a significant relation was found with barrier width. For *C. russula* this was only the case for model III where a significant relation between recolonisation time and habitat suitability was found, and for *M. minutus* for the same model where relations with distance and the number of barriers were found. The models I, IV and V explained more than 25% of the variance of presence and recolonisation time data for *A. sylvaticus*. For this species, habitat suitability was of significance in all of the three models, and the number of barriers, the barrier width and the distance were of significance in two of the three models.

It was found that the variance explained by the model (comparison between model I and II) improved for all species when the data of the unsuitable habitats were also taken into account. For *M. arvalis*, *C. glareolus* and *A. sylvaticus* it is found that the variance in recolonisation time explained by the model is much larger when absence data are included in the model. For *C. russula* and *M. minutus* this is just the other way around.

The presence – absence models for the ADW had greater predictive power for the MW observations for *M. arvalis* and *C. glareolus* than for the other species. The predictive power for *M. arvalis* was greatest when unsuitable habitats were excluded, while that for *C. glareolus* was greatest when these were included (Table 3). However, there was a huge variance in the models for *M. arvalis* and *C. russula* in particular. All of the species were expected to occur on more MW monitoring sites than where they were actually observed. Except for *M. arvalis*, the predictive power of the models decreased when unsuitable habitats were excluded. The predictive power of each of the models for *M. arvalis* based on recolonisation time was more than 60%, the greatest predictive power being shown by Model IV. The predictive power of the models for *S. araneus* and *C. russula* was greatest when absence meant a recolonisation time of 90 weeks.

3.4 Discussion

Small mammal distributions

Small mammals, like other animal species, are not homogeneously distributed over a floodplain. Although this fact is well known to ecologists (Leuven and Poudevigne, 2002; Poudevigne et al., 2002; Robinson et al., 2002; De Nooij et al., 2004), it is rarely taken into account in impact assessments of physical reconstruction and floodplain management plans. At best, these tend to take account of habitat suitability (Kooistra et al., 2005) for different species, assuming that species will be present when habitat structures and properties (e.g., vegetation, substrate, moisture and size) are suitable. However, frequent inundations are a serious problem for several species, which have to return after a flood. The recolonisation of habitats which are at first sight suitable habitats may then be a slow process, especially when landscapes are patchy, and lack large connected areas of suitable habitats. Figure 2 shows that the recolonisation of the ADW floodplain by small mammals was a slow process in both years of monitoring. The densities of all species were generally highest on the non-flooding areas or in the neighbouring zones (at distances of 0 – 30 m), and after seven to nine months, several species were still not present at distances of more than 120 metres from the non-flooding areas, or were present there in lower densities than in other zones.

Landscape characteristics and recolonisation

We selected five landscape characteristics which we expected to potentially affect recolonisation. The distance between a source population and a monitoring site is assumed to be important, as each species has its action radius, and the potential to disperse over a certain distance in time (Wolff, 1999; Wijnhoven et al., 2005). Distance is also assumed to be positively related to recolonisation time (Diffendorfer et al., 1999), and negatively related to the ‘logarithm of presence’ ln(P), in the absence of interference by other factors. For *A. sylvaticus*, increasing distance was indeed significantly related to absence or an increase in recolonisation time (in two of the three models which explain more than 25% of the variance in the data), and the same was true for *M. minutus* (in model III) (Table 2). For all other species, other landscape characteristics appeared to be more important in determining recolonisation, and in fact, *A. sylvaticus* also showed stronger regressions with other landscape characteristics. This indicates that distance in itself is not the major factor explaining the relatively slow recolonisation.

Potential barriers (unsuitable habitats) which have to be crossed can slow down a recolonisation process. How long it will take before barriers are crossed is related to barrier characteristics, and increasing densities within the source area can function as a trigger to cross barriers (Montgomery et al., 1991). It is often assumed that individuals (active dispersers more so than non-active dispersers) regularly sally outside suitable habitats, which is often encouraged by crowding (Gaines and McClenaghan, 1980; Bondrup-Nielsen, 1985; Bondrup-Nielsen and Karlsson, 1985; Van Apeldoorn et al., 1992; Diffendorfer et al., 1999). These movements through unsuitable habitats are more likely to result in the crossing of a partial barrier when its width is smaller. However, barriers can also accelerate recolonisation (distance per time) (Peles et al., 1999), as individuals may travel across them faster or in a straighter line, and are less tempted to settle in these unsuitable habitats. The number of barriers can therefore have either a positive or a negative effect on recolonisation speed.

Barrier width was found to be a significant factor for *M. arvalis* and *A. sylvaticus* (2 of the 3 models which explain more than 25% of the variance in the data) as wider barriers delayed recolonisation. By contrast, the recolonisation speed of *S. araneus* (in the only model which explains more than 25% of the variance in the data) was positively related to barrier width. The number of barriers, which also had implications for barrier width as we measured it, was positively related to recolonisation time (in the sense of delaying recolonisation) for *M. arvalis* (2 of the 3 models) and *C. glareolus* (1 of the 3 models) and for *A. sylvaticus* (2 of the 3 models) and *M. minutus* (the only model which explains more than 25% of the variance in the data). In the last case, model III showed a significant effect, which indicates that the number of barriers was not important for the areas that were recolonised, but was problematic for those monitoring sites that were not recolonised.

Another important characteristic is the surface area of available suitable habitats in the vicinity, as it can influence the direction and distance of dispersal. It is assumed that individuals are more likely to disperse in the direction of a connected suitable habitat than through an unsuitable habitat. If the source of recolonisation is a large connected area of suitable habitat, it will take longer before individuals are forced to disperse through unsuitable habitats due to crowding (Boyce and Boyce, 1988; Briner et al., 2005), resulting in a positive relation between the available area of suitable habitat and the recolonisation time. A larger area of suitable habitat can also mean an increase in connectivity over longer distances, by offering corridors or stepping stones. This should result in a negative relation between the area of suitable habitat and the recolonisation time. A larger area of suitable habitats, only had a significant accelerating effect on *A. sylvaticus* (1 of 3 models) and therefore did not seem to be a good descriptive factor for recolonisation the way we measured it.

Finally, habitat suitability will be of importance, as suitable habitats will probably be colonised sooner than marginal habitats with the same connectivity. We found that suitable habitats were colonised significantly sooner than marginal and unsuitable habitats for *A. sylvaticus* (all of the 3 models), *M. arvalis* (2 of the 3 models) and *C. glareolus* (1 of the 3 models), except for *C. russula*, for which we found the opposite regression for the only model which explain more than 25% of the variance in the data. This probably means that habitat suitability has not been very accurately described for *C. russula*, or that marginal habitats are not colonised later than suitable habitats at similar connectivity, but that suitability is only reflected by the densities it can carry.

The fact that the constant factor in several regression equations was also significant means that not all important landscape characteristics for recolonisation were included, or that the variation in the models of recolonisation time was relatively small, which would be expected to improve if recolonisation was monitored for longer than approximately 36 weeks. Especially for *C. glareolus*, only the constant factor appeared to be significantly related in the 3 relevant models, which indicates that the recolonisation of the habitats occured independent of the landscape characteristics measured by us. This could be the result of the fact that suitable habitats near the sources were colonised soon, while others were not reached throughout the monitoring period.

Types of recolonisation

Based on the density development patterns, we expect that three types of recolonisers can be distinguished, which should be reflected in the importance of the landscape characteristics determining the recolonisation of floodplains by small mammal species. The three different types distinguished are schematised in figure 3. For species of the first type, the ‘gradual, density induced colonisers’, population densities first have to increase in suitable areas (sources) before barriers are crossed. When densities increase, individuals are forced to move into unsuitable habitat searching for suitable habitats, at which suitable habitats in the

Fig. 3: Three types of recolonisation by small mammal species shown in six comparable time intervals for each of the types, as indicated by letters. The arrows indicate the dispersal movements between suitable habitats (rectangles), in which the populated parts are shown in grey. Unsuitable habitats in between suitable patches can form temporary barriers to recolonisation.

surroundings will be colonised first. Each extra barrier will delay recolonisation, while wider barriers to a certain extent can accelerate recolonisation, as individuals will not settle in unsuitable habitats. Just after a flood these species are only observed on and near non-flooding areas after which the floodplain will be colonised gradually. Species of type 2, the 'Active dispersers', frequently sally outside the suitable habitats. They can be found at several sites within a short time, and colonisation is often driven by habitat quality at a particular moment. Their dispersal is not impeded by areas of unsuitable habitats or potential barriers to a certain extent. A typical recolonisation pattern for these species involves rapid recolonisation of several adjacent sites, followed by a very slow or gradual colonisation of sites with poor connectivity. For species of type 3, the ‘Long-distance dispersers after a lag’, small stretches of unsuitable habitats initially prevent colonisation of suitable habitats. After longer periods, suitable habitats at large distances are also colonised. A certain increase in the population density in source areas is necessary before individuals start to cross barriers. Once dispersal through unsuitable habitats has been initiated, these species can travel over large distances. Therefore, recolonisation time is typically negatively related to barrier width.

The presence of *M. arvalis* was related to barrier number and barrier width and is a typical species of type 1. *M. arvalis* and also *C. russula* populations grew faster than those of the other species, gradually recolonising areas at larger distances. Both species recolonised areas at distances of more than 120 m. Therefore the density development pattern of *C. russula* also looked like type 1, but this could not be confirmed by the regression analyses. The fact that a negative relation between habitat suitability and recolonisation time was observed for *C. russula* indicates that the habitat is not described well or that the connectivity of the suitable habitats was poor.

Based on the density development patterns *C. glareolus* and *S. araneus* are expected to belong to type 2. They show a rapid recolonisation of several adjacent sites, followed by a very slow or gradual colonisation of sites with poor connectivity. Fast recolonisation of adjacent monitoring sites could be the result of favourable conditions in the lower parts just after the flood, probably relating to food availability and quality. Fresh plant shoots and relatively immobile earthworms are plentiful in the formerly inundated parts, and invertebrates are abundant in the debris at the flood mark. In addition, competition is minimal and there are plenty of shelter opportunities just after inundation. The total densities of *C. glareolus* and *S. araneus* were relatively stable throughout the year, and both species were not observed in about 50% of the total area (viz. the zone at a distance of more than 120 m from the non-flooding areas). It seems that for these species the research area had a poor connectivity and there was a lack of time for complete recolonisation. Although a positive relation between barrier width and recolonisation time was found for *S. araneus*, more data on distributions in floodplains are actually necessary to discriminate if *S. araneus* is indeed a type 2 species. Also the classification of *C. glareolus* in one of the recolonisation types is unclear from the regression models. For *C. glareolus* the number of barriers and recolonisation time are related, which is in line with publications referring to this species as a species with low dispersal activity, not very frequently crossing barriers (Bondrup-Nielsen and Karlsson, 1985; Kozakiewicz, 1993). However this could also be the result of several suitable habitats laying out of reach for this species within the research period.

For species of type 3, small stretches of unsuitable habitats initially prevent colonisation of suitable habitats. After longer periods, suitable habitats at large distances are also colonised. A certain increase in the population density in source areas is necessary before individuals start to cross barriers. Once dispersal through unsuitable habitats has been initiated, these species can travel over large distances. Therefore, recolonisation time is typically negatively related to barrier width. *A. sylvaticus* is a typical representative of this type. Also *M. minutus* shows a similar density development pattern, but can not be classified due to the poor regressions. Poor trappability during a large part of the year may also influence the pattern for this species (Lange et al., 1994). As a result, *M. minutus* suddenly appeared at several monitoring sites in autumn, making it look like a type 3 species, although it was most probably already present earlier in the year at some monitoring sites.

Predictive capacity of the regression models

Although regression models based on ecological data, and especially trapping results of small mammals, show a great deal of unexplained variance (low R^2 values, as shown in Table 2), they show valuable information, especially in relation to heterogeneously structured landscapes or species with different recolonisation strategies. Specifically, these models can give valuable information on the relative importance of landscape characteristics from different levels of significance, and show trends in positive or negative influence on species recolonisation. We decided to include all landscape characteristics in the regression models (even those which were not found to be significant), as the regression models were based on the data of only one floodplain with its own characteristics. Retaining all parameters in the model can give valuable information about the importance of landscape characteristics, when predictions for another floodplain (MW) are combined with its landscape characteristics. The R^2 values show that the absence of *C. russula* and *M. minutus* from several sites was not adequately explained by the landscape characteristics, as the regressions were much stronger when absence data were omitted. It is also shown that unsuitable habitats often explained the absence of species, as the regressions of model I were generally much better than those of model II. The presence and recolonisation time appeared to be described best by the chosen landscape characteristics for *M. arvalis* and *A. sylvaticus*, as for those species the constant value was not the only or best descriptive parameter, and the different models showed similar results.

The presence or absence of species at a monitoring site is easier to measure than the time until recolonisation, and this may be sufficient for several applications. The use of several monitoring sessions reduces the risk of missing a species which is actually present. Predicting the presence of species will be less accurate when floods occur in various seasons, as the development of small mammal populations will vary over the year (Southern, 1965; Montgomery et al., 1989), and the impact on species survival is different (Van der Velde et al., 2004). As the flooding intervals in the ADW and the MW were similar in the years in which we monitored them, we feel justified in using the presence – absence models of the ADW to make predictions for the MW. Except for individuals dispersing or occasionally sallying out of the suitable habitats, species are not expected to occur in unsuitable habitats. Therefore, we expect the predictive power of a model to increase when the trap lines in unsuitable habitats are ignored. Having monitoring sites in unsuitable habitats in the dataset will increase the deviation of the regression coefficients. This is actually not the case for all species which means that the unsuitable habitats also often had a poor connectivity in those cases.

Predictions of the presence of species in variously structured floodplains with different flooding regimes actually necessitate the use of regression models based on recolonisation time. A problem of these regression models is that of handling data on species absence. If these data are ignored, less information about decelerated recolonisation is incorporated. These regression models are expected to underestimate the actual recolonisation time in a landscape. It is possible to allocate a value to the absence data which is larger than the maximum recolonisation time recorded. Some uncertainty will remain, however, which can lead to underestimation or overestimation. The relative importance of model parameters for predictions about different species will not be changed by varying the value of the recolonisation time at monitoring sites where species are absent. In ideal situations, the regression models are based on a much longer monitoring time than is available in the area of prediction. This makes it unnecessary to add the value of the recolonisation time to monitoring sites that are not recolonised, as the predictive power of model III will be improved. The predictive power of similar models such as IV and V will also be improved, as a larger value for recolonisation time (>90) can be given to the sites where species are absent.

Species-specific landscape characteristics should be compared between the two research areas to interpret the predictive capacity of models, as values of the landscape characteristics in the area of prediction should lie within the range of values for the monitoring area. The predictions for the presence of *M. arvalis* in the MW were reasonable (predictive power of 57.1 and 77.3%; Table 3), while the landscape characteristics for *M. arvalis* in the ADW and the MW were also similar. This was especially the case for the most important factors determining the presence of *M. arvalis*, viz., barrier number and width (Table 4). Only the distances and the area of suitable habitats in the MW seem to be smaller, which does not greatly influence the predictions. The presence of all species, but especially that of *S. araneus* and *C. russula*, in the MW was, however, overestimated by the models (leading to a poor predictive power of between 32.3 and 46.3%). In general, barriers in the MW are narrower and greater areas of suitable habitats are present than in the ADW, for all species except for *M. arvalis*. Barriers may prevent the recolonisation of the MW by *C. glareolus* and *C. russula* to a greater extent than expected. For *S. araneus*, the barrier number and width are smaller in the MW, while the area of suitable habitat is larger. Barriers may prevent recolonisation by this species as well, or the much larger suitable area may slow down the recolonisation process. While the predictive power of the recolonisation model of *M. arvalis* was good (77.3%) when the recolonisation time for the suitable habitats where the species was assumed to be absent was set at 45 weeks (Table 3), the models for the other species had better predictive power at a mean recolonisation time of 90 weeks. This suggests that most of the suitable habitats for *M. arvalis* are inhabited when there is no flooding for a whole year, while for the other species, the complete recolonisation of the MW is probably a matter of years.

3.5 Conclusions

The results of this study confirm that the recolonisation of floodplains by small mammal species after flooding events is a relatively slow process. Floodplains in which linear distances between non-flooding areas and potential habitats never exceed 1 km, are found to be not entirely recolonised within one year. As the time between two successive floods is generally not long enough for a complete recolonisation of the floodplain, species distribution within floodplains is generally far from homogeneous, even when only suitable habitats are considered. The recolonisation patterns are species-specific. Three general types were distinguished based on recolonisation patterns. Species behaviour towards landscape characteristics as shown by regression equations confirmed the positioning of *M. arvalis* and *A. sylvaticus* in these types.

This study also showed that multivariate regression models are useful in determining the importance of landscape characteristics for recolonisation. The predictive power of the models looks promising, but can be improved using data from a few other research areas, especially those where longer recolonisation times are available. This would improve especially the value of models based on recolonisation time.

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Chapter 4

The effect of turbation on zinc relocation in a vertical floodplain soil profile

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Sampling microcosms

Abstract

Turbation is hypothesized to affect the redistribution of heavy metals in polluted floodplain soils by effects on mobility. This hypothesis was tested in microcosms by turbation of zinc-spiked sediment top layers. Manual turbation caused a fast decrease of the zinc content in the upper 15 cm of the soil, even though turbation was only applied to the upper two centimetres. It was especially zinc attached to colloid and organic matter particles that was redistributed from the top layer. Percolation processes resulted in attached zinc being drained to depths of more than 15 cm. The decrease in zinc content of the topsoil was even stronger in combination with inundation. No indications were found for the redistribution of zinc as a result of an increase of the extractability with 0.01 M $CaCl_2$ or changes in pH. The findings suggest that mechanical turbation and bioturbation may redistribute heavy metals from topsoils in polluted floodplains just after inundation as observed in these turbation experiments.

4.1 Introduction

European river catchments are often densely populated and industrialised (Leuven and Poudevigne, 2002; Van der Velde et al., 2004). Due to a lack of water pollution control and wastewater treatment facilities, many river floodplains have become polluted in the past (Admiraal et al., 1993; Middelkoop, 1997; Albering et al., 1999; Vink et al., 1999b; Mertens et al., 2001). The effects of these pollutants on ecosystems are largely unknown, but local extinctions or population declines due to deteriorated river water, sediment and floodplain soil quality have been suggested in several studies (Balk et al., 1993; Kerkhofs et al., 1993; Hendriks et al., 1995; Kooistra et al., 2001). Deposited pollutants are subject to hydromorphodynamics (e.g. flooding, erosion and sedimentation processes) and turbation by animals. However, knowledge on the fate of heavy metals, especially in relation to turbation and inundation, is scarce.

Floodplain soils often abundantly harbour burrowing animals, so-called bioturbators, including various mammals (e.g. voles, mice and moles) and soil macro-invertebrates, like earthworms and insects and their larvae (Mitchell, 1988; Müller-Lemans, 1996; Tyler et al., 2001). Bioturbation processes include digging, casting, and construction of nests and burrows. Bioturbation occurs especially in the upper 20 cm of soils, where the most recently deposited pollutants are present (Middelkoop, 1997). Some species like epigeic earthworms (e.g. *Lumbricus rubellus*) are especially active in the upper 3 cm topsoil (Zorn, 2004), and all species burrowing deeper but frequently surfacing (e.g. endogeic and anecic earthworm species and underground dwelling small mammal species) or species burrowing from the surface to deeper layers (e.g. rabbits and voles searching for food) turbate the topsoil as well. Zinc is a widespread heavy metal in river systems, occurring in elevated and potentially toxic quantities all over Europe (Balk et al., 1993; Kalbitz and Wennrich, 1998).

The hypothesis tested in the present study was that turbation influences the vertical distribution of pollutants in floodplain soils in two ways: 1) by mixing polluted and clean layers (Mitchell, 1988; Müller-Lemans, 1996; Tyler et al., 2001), and 2) by influencing directly the physical and indirectly the chemical soil processes which affect pollutant speciation and redistribution of certain soil fractions and compounds (Newson, 1995; Nielsen et al., 1996; Eijsackers and Doelman, 2000; Vorenhout et al., 2000; Langmaack et al., 2001). In the present study microcosms containing a zinc-polluted topsoil were used to study the effect of turbation on contaminated floodplain soils and the driving processes. Mechanical turbation was used, either combined with or without inundation, as water retention is expected to be important in contaminant redistribution and inundation is an important process affecting water fluxes in floodplain soils under natural conditions. Before execution of these experiments our hypothesis was first tested in a pilot experiment in which also the experimental duration was varied and also rain treatments were tested in all possible combinations. These pilot experiments will show if turbation is potentially important for the process of contaminant redistribution, or if various other treatments overrule possible effects.

The purpose of the experiments is to determine if turbation can affect heavy metal redistribution in the vertical profile, other than by the physical process itself. If turbation influences the mobility of heavy metals, this could also happen with bioturbation in floodplains. In the experiments, mechanical turbation was used, as we want to standardize our turbation treatment to diminish the variation between the replicates, and make interpretation of the observations easier. The research questions were: (1) does turbation cause substantial redistribution of zinc, and if so, how is this redistribution influenced by inundation? and (2) what is the mechanism underlying the possible zinc redistribution under the influence of turbation?

4.2 Materials and methods

The microcosms

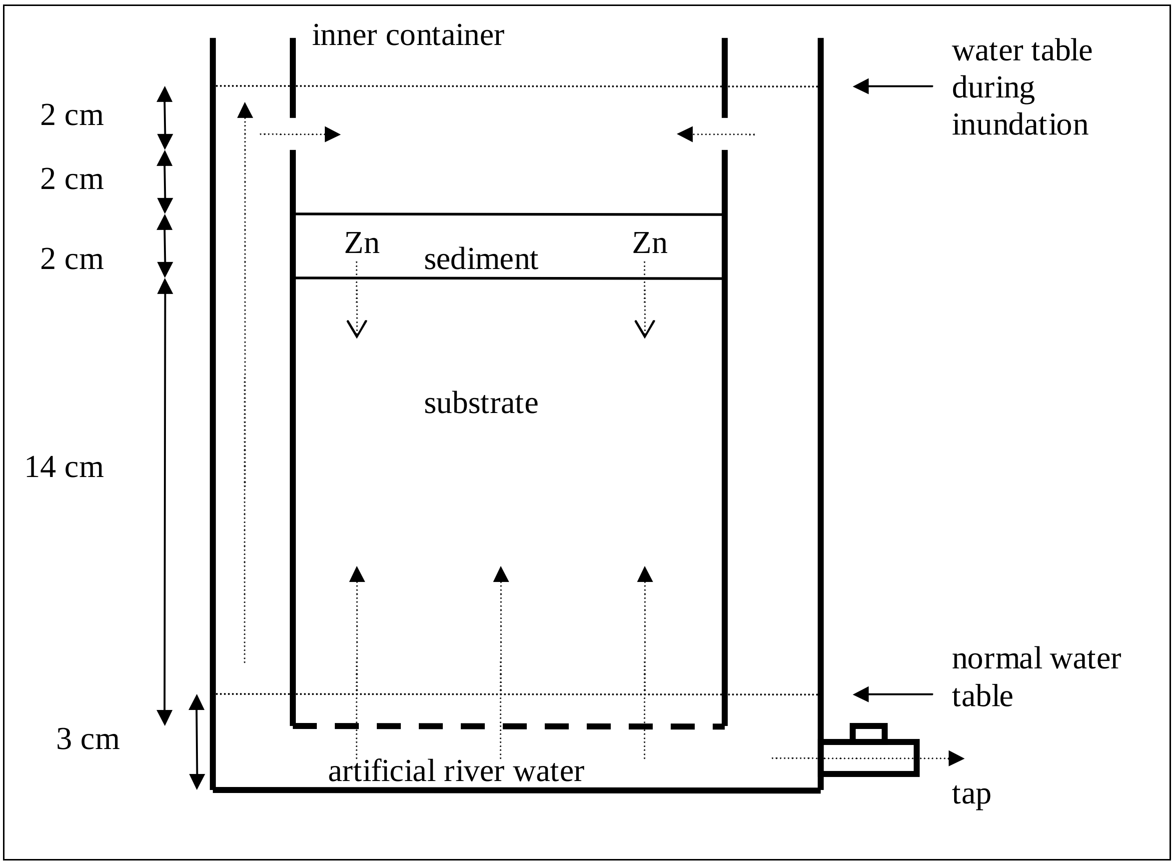

Fig. 1: Schematic representation of a microcosm.

The microcosms consisted of double-walled containers of iodised polyethylene (Fig. 1), which were bathed in 0.1 M HCl and rinsed with distilled water before use. The inner containers were provided with water-permeable bottoms and were filled with 14 cm of a relatively clean sandy clay substrate (pH_{CaCl2} = 7.1 ± 0.05; organic matter content of 1.6 ± 0.66% (DW); clay/silt (<53 µm) content of 38.9 ± 1.15% (DW), containing 61.3 ± 26.7 mg/kg (DW) zinc (n=3)). This substrate was obtained from the Afferdensche and Deestsche

Waarden (ADW floodplain), a floodplain along the River Waal, the main branch of the River Rhine in the Netherlands (Kooistra et al., 2001, 2005; Leuven et al., 2005). Water tables could be regulated between double walls. Rising water tables caused the water to percolate upwards through the soil, as soon as the substrate level was reached, the water also started to flood the substrate through a few holes in the inner wall. This water was prepared from distilled water to reflect the mean chemical composition of Rhine water as measured at Lobith (RIWA, 1999), by adding 242.3 mg/l $NaHCO_3$ (p.a., min. 99.5%, Merck), 235.4 mg/l $CaCl_2$ (anhydrous, min 97%, Fluka), 57.4 mg/l $MgSO_4$ (p.a., min. 99.5%, J.T. Baker), 13.9 mg/l K_2SO_4 (p.a., min. 99%, Merck) and 9.7 mg/l Na_2SO_4 (p.a., anhydrous, min. 99%, Merck) and establishing a pH of 7.8 using NaOH (p.a., min. 99%, Merck), as salts. The water table was maintained at a level of three centimetres in the outer container (evaporation was compensated for), causing the bottom centimetre of the experimental substrate to be saturated with river water, and the total substrate column to be moist. All microcosms were provided with a top layer of polluted sediment reflecting the composition of the sediment newly deposited after the flood of March 2001 at the ADW floodplain (our own measurements), which was prepared by mixing a sandy and a clayey floodplain soil (both from the ADW floodplain). To raise the organic matter and water contents, potting compost and artificial river water were added. The sediment was composed of 20.0% sand (>53 µm), and 80.0% clay/silt (<53 µm), plus 29.0% organic matter and 60.0% river water. $ZnCl_2$ (p.a., min. 98%, Merck) was added as a salt to a final content of 2269 ± 29 mg/kg (DW) Zn (n=3). The sediment was mixed each day for a period of 5 weeks before use. Measurements showed that at the start of the experiments the 0.01 M $CaCl_2$-exchangeable fractions of zinc, as a percentage of the total content in mg/kg, were 1.79 ± 0.12% (n=3), which is similar to levels observed for freshly deposited sediments in a moderately polluted floodplain in the Netherlands (e.g. the ADW floodplain; 1.40 ± 1.29% (n=15)). All microcosms were contaminated at t=0, by adding 1.2 kg (=2 cm) of polluted sediment on top.

Experimental treatments and analyses

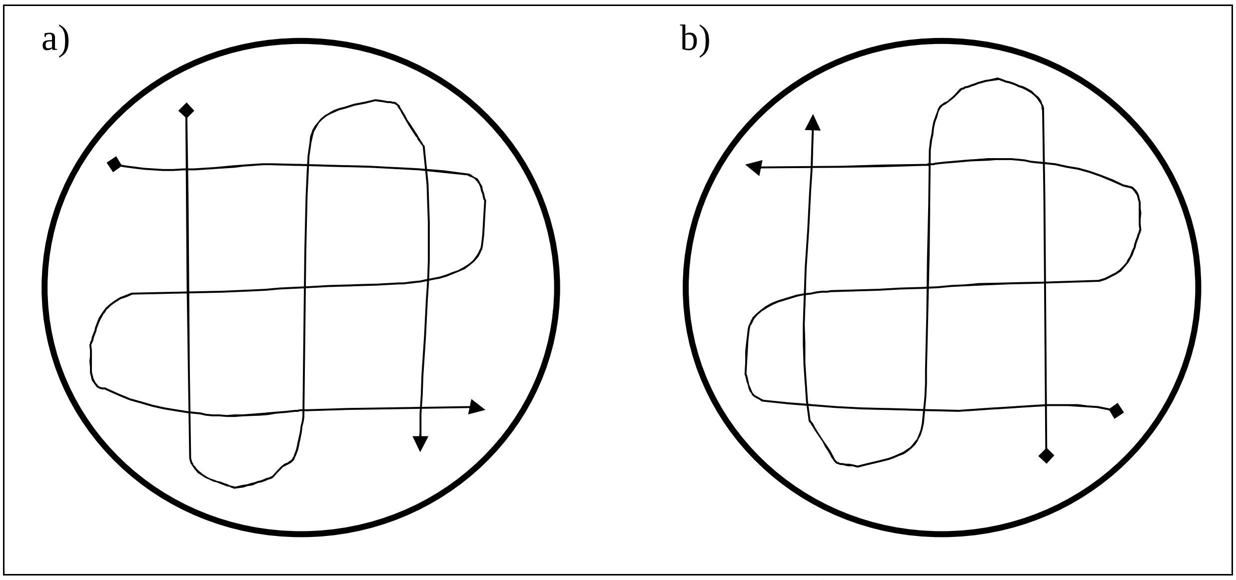

Fig. 2: Standardised turbation; direction in which the top segment (2 cm) of the microcosms was raked on a) even, and b) odd days. The sweeps of the rake overlapped to ensure that the entire surface was turbated.

The experiments were carried out under controlled conditions in a glasshouse. Four different treatments were executed, either with or without turbation, and with or without inundation in the possible combinations. Turbation was executed daily and consisted of mechanical turbation of the upper two centimetres of the soil, except in inundated situations. A three-toothed rake with two centimetres long teeth was pulled through the top layer in a standardised way, changing the stirring direction each day (Fig. 2). To simulate a typical river flooding of an embanked floodplain, the water table in the outer container was gradually raised from three centimetres to 22 cm (Fig. 1). The inundation period started five days before the end of the experiment that took 15 days in total. One day before termination of the experiment the water table was gradually reduced to the initial level by allowing the water to flow out through a tap.

After 15 days the vertical profiles of the substrate were sampled using a soil core sampler with a 2.5 cm diameter. Samples were taken at three spots in each of the microcosms. Three soil cores were taken, which were subdivided into four depth segments; 0 to 2.5 cm, 2.5 to 5.0 cm, 5.0 to 10.0 cm and 10.0 to 15.0 cm. Corresponding segments of the three soil cores taken at each spot were mixed before analysis.

The moisture content, organic matter content, clay/silt to sand distribution, pH_{CaCl2}, total zinc content, and 0.01 M $CaCl_2$-exchangeable zinc content of all soil samples were analysed. The moisture content was determined by drying five grams of wet soil (FW) for 24 h at 105 °C to measure the dry weight (DW); moisture content (%) was than calculated as ((FW-DW)*100)/DW. The organic matter content (OM) was calculated after scorching the dry soil for 4 h at 550 °C, after which the mineral weight (MW) was measured; OM (%) = ((DW-MW)*100)/DW. The clay/silt to sand distribution was estimated by mixing three 3.33 g portions of dry substrate and adding 50 ml of 35% H_2O_2, to disrupt particle aggregations by destruction of $CaCO_3$ and organic matter. After two days incubation, the suspension was boiled while adding distilled water to keep the substrate in suspension. Sieving over 53 µm separated the clay/silt and sand fractions, and the suspensions were dried at 105 °C, after which the fractions were weighted.

Total zinc content was measured after microwave destruction (using the Milestone 1200) of 0.2 mg DW substrate in a mixture of 3.0 ml 65% HNO_3 and 1.5 ml 37% HCl. The samples were topped up to 50 ml, after which the zinc content was measured using an Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES). The 0.01 M $CaCl_2$-exchangeable fraction was determined, as a measure of zinc mobility. The mobility of zinc can also have implications towards the bioavailability. Although bioavailability is dependent on the uptake processes by organisms (Peijnenburg et al., 1999a; 1999b), a fairly close relation between uptake and 0.01 M $CaCl_2$-extractable contents, has been found for several species (Janssen et al., 1997b; Vink et al., 1999a). Six grams (FW) of substrate to which 0.01 M $CaCl_2$ had been added in a 1:10 (m(DW)/v) ratio, was mixed for two hours, after which the suspension was centrifuged at 12000 rpm for 15 minutes. After the pH_{CaCl2} had been measured in the substrate suspension in 0.01 M $CaCl_2$, the supernatant was filtered over 0.45 µm. A pH of 2 was established with a few droplets of 65% HNO_3, and the zinc content of the sample was subsequently measured on the ICP-AES.

Differences in zinc content between the microcosms were tested using Student’s t-test, after possible differences in variance were tested using the F-test.

Pilot experiments

The experiments as described here were preceded by a series of pilot experiments in 15 microcosms, to get a first indication of our hypothesis on the effect of turbation, to check if our expectations are possibly not irrelevant as other treatments could have a much stronger influence, and to determine how long experiments should last. Here the above-mentioned treatments were executed combined either with or without rain treatment, and treatments lasting for 15 or 30 days. The above mentioned rain treatment consisted of a shower corresponding to 4.4 mm of rainfall every other day (two times the daily mean for the

Netherlands (KNMI, 2002)). The rainwater was prepared to reflect the mean composition of rainwater as measured at IJsselsteyn in the year 2000 (A.W. Boxman, personal communication), by adding to distilled water 8.33 mg/l NH_4NO_3 (p.a., min. 99%, Sigma), 2.42 mg/l Na_2S (from Na_2SxH_2O p.a., min. 99%, Merck), 1.66 mg/l $CaCl_2$ (anhydrous, min. 97%, Fluka), 1.32 mg/l K_2S (p.a., min. 99%, Merck), 1.07 mg/l NH_4Cl (p.a., min. 99.8%, J.T. Baker) and 0.86 mg/l $MgCl_2$ (from $MgCl_2x6H_2O$ p.a., 99.0-102.0%, Merck) as salts and establishing a pH of 6.0 by means of 30% HCl (p.a., Merck). Other treatments were comparable to those described for this study, as was the sampling and analysing procedure. All possible combinations were executed, except for the 30 days without any treatment, making 15 different treatments executed one-fold. As all combinations of treatments were only executed in one-fold, the pilot-experiments are not suitable to establish differences in zinc concentrations between treatments. But the measurements in a variety of treatments give a large dataset of zinc concentrations related to soil parameter data (15 treatments x three soil cores x four depth segments), within a wider range of each of the parameters. To identify the most distinguishing treatments related to differences in zinc contents and other soil parameters in the microcosms, a Principal Component Analysis (PCA) was performed after the gradient length for the dataset had been specified by means of a Detrended Correspondence Analysis (DCA), using the Canoco for Windows package (version 4) (Ter Braak and Smilauer, 1998).

Multiple regression analyses were done in Microsoft Excel 2000, based on the least squares method. In this procedure, the calculated squares of the regression coefficient and F- and t-values (using F-test and t-test) with corresponding degrees of freedom are compared with the critical F- and t-values. All measured soil parameters were initially included, but were excluded when t-levels were below the critical level at a significance level of 0.05. Logarithmic regressions were used, as the data of the parameters were not normal distributed.

4.3 Results

Pilot experiments

Multiple regression analyses to describe the relations between zinc content and soil parameters showed a decrease of the total zinc contents in the top segment after the treatments, which appeared to be related to a decrease in clay content, as shown by equation 1 (Table 1). The 2.5 - 5.0 cm depth segment showed no clear changes in total zinc contents, in contrast to the 5.0 - 10.0 cm segment, which showed total zinc contents positively related to the total contents in the top segment but also the $CaCl_2$-exchangeable fraction (Table 1: equation 2). Measurements in all depth segments of all the microcosms revealed a very close relation between the total zinc content and the clay/silt and organic matter contents (Table 1: Equation 3). In the top segment, the 0.01 M $CaCl_2$-exchangeable zinc content was correlated with the clay contents (Table 1: Equation 4). Measurements from all depth segments showed that pH, moisture content and clay content did not play a significant role in the exchangeability with 0.01 M $CaCl_2$, as organic matter content and total zinc content were the most important factors (Table 1: Equation 5).

The analyses of the depth segments indicated that a decrease in total zinc content related to the turbation treatment was visible in the upper 2.5 cm segment, even when inundation or rainfall were absent. Total zinc content at t=0 was 1828 ± 28 mg/kg (DW) (n=3), while after the turbation treatments, the measured range was between 428 and 861 mg/kg (DW). The microcosms not subjected to turbation also showed a downward displacement of zinc, with final contents between 1195 and 1755 mg/kg (DW). In all microcosms, the 0.01 M $CaCl_2$-exchangeable contents in the top depth segment (range between 1.4 and 15.7 mg/kg (DW)) were greatly decreased when compared to the initial situation (with a 0.01 M $CaCl_2$-exchangeable zinc content of 32.9 mg/kg (DW)). This phenomenon was again clearest in the turbated microcosms (1.4-3.7 mg/kg (DW) and 8.9-15.7 mg/kg (DW) of 0.01 M $CaCl_2$-exchangeable zinc in microcosms with and without turbation, respectively).

Table 1: Equations relating the metal content in the microcosms from the pilot study to the soil parameters.

$$Zn_{tot(0-2.5)}=64.64*OC_{(0-2.5)}+16.77*CC_{(0-2.5)}-449.8 \quad (1)$$
R^2=0.635, n=45, F= 36.5, p<0.001

$$Zn_{tot(5-10)}=35.80*10^{-3}*Zn_{tot(0-2.5)}+36.50 \quad (2)$$
R^2=0.380, n=45, F= 12.9, p<0.001

$$Zn_{tot(0-15)}=68.75*OC_{(0-15)}+19.84*CC_{(0-15)}-696.6 \quad (3)$$
R^2=0.787, n=180, F=326.6, p<0.001

$$Zn_{CaCl2(0-2.5)}=24.25*10^{-2}*CC_{(0-2.5)}+38.70*10^{-4}*Zn_{tot(0-2.5)}+11.62 \quad (4)$$
R^2=0.766, n=45, F=44.6, p<0.001

$$Zn_{CaCl2(0-15)}=24.65*10^{-4}*Zn_{tot(0-15)}+70.09*10^{-3}* CC_{(0-15)}-93.84*10^{-2}*pH+ 24.19*10^{-3}*MC_{(0-15)}+20.83*10^{-2}*OC_{(0-15)}+33.71*10^{-1} \quad (5)$$
R^2=0.866, n=180, F=225.2, p<0.001

Zn_{tot}= total zinc content (mg/kg dry weight), Zn_{CaCl2}= 0.01 M $CaCl_2$-exchangeable zinc content (mg/kg dry weight), OC= organic matter content (% of dry weight), CC= clay content (% of dry weight), MC= moisture content (% compared to dry weight), pH= pH_{CaCl2}, $_{(0-2.5)}$= substrate segment at depths of 0 - 2.5 cm depth, $_{(5-10)}$= substrate segment at depths of 5.0 - 10.0 cm depth, $_{(0-15)}$= all sampled segments between 0 and 15 cm taken into account separately.

Testing significant effects of time on zinc distributions (after testing the variance with an F-test), showed that the amount of zinc in the 0 to 2.5 cm depth segment cannot be expected similar (significant time effects in two out of eight cases with n=3), however regression shows that zinc levels were only slightly decreased (regression coefficient = 1.087; F = 20.0, df = 23). In the second layer the variance (F-test) was not comparable for all treatment groups (in two out of eight cases), but no significant time effects were observed, a strong regression was however found between the time treatments (regression coefficient = 0,619; F = 13,2, df = 23). As zinc concentrations did not differ a lot in most cases, and relative differences in zinc concentrations between treatments seem to be comparable, we chose to test the 15 days treatment only in the experiments, to minimize efforts in case of a four-fold execution of the various treatments. The differences in amounts of zinc redistributed were mainly caused during the first 15 days.

These results seem to indicate that the three treatments turbation, inundation and rainfall had an effect on the redistribution of zinc in the vertical profile. These indications were strongest for downward zinc redistribution under influence of turbation and weakest under the influence of rainfall. Turbation was probably the most important treatment causing differences in zinc distribution as shown by the PCA plot, clearly separating all microcosms with various treatments in the two groups, with or without turbation (Fig. 3). Non-turbated microcosms showed more variability in the soil parameters than the turbated ones. Organic matter content, soil moisture content, 0.01 M $CaCl_2$ exchangeable zinc content and particle size do vary between the treatments, but the pH_{CaCl2} always ranged between 6.7 and 7.7, and seems not to be important to distinguish the treatment groups of microcosms.

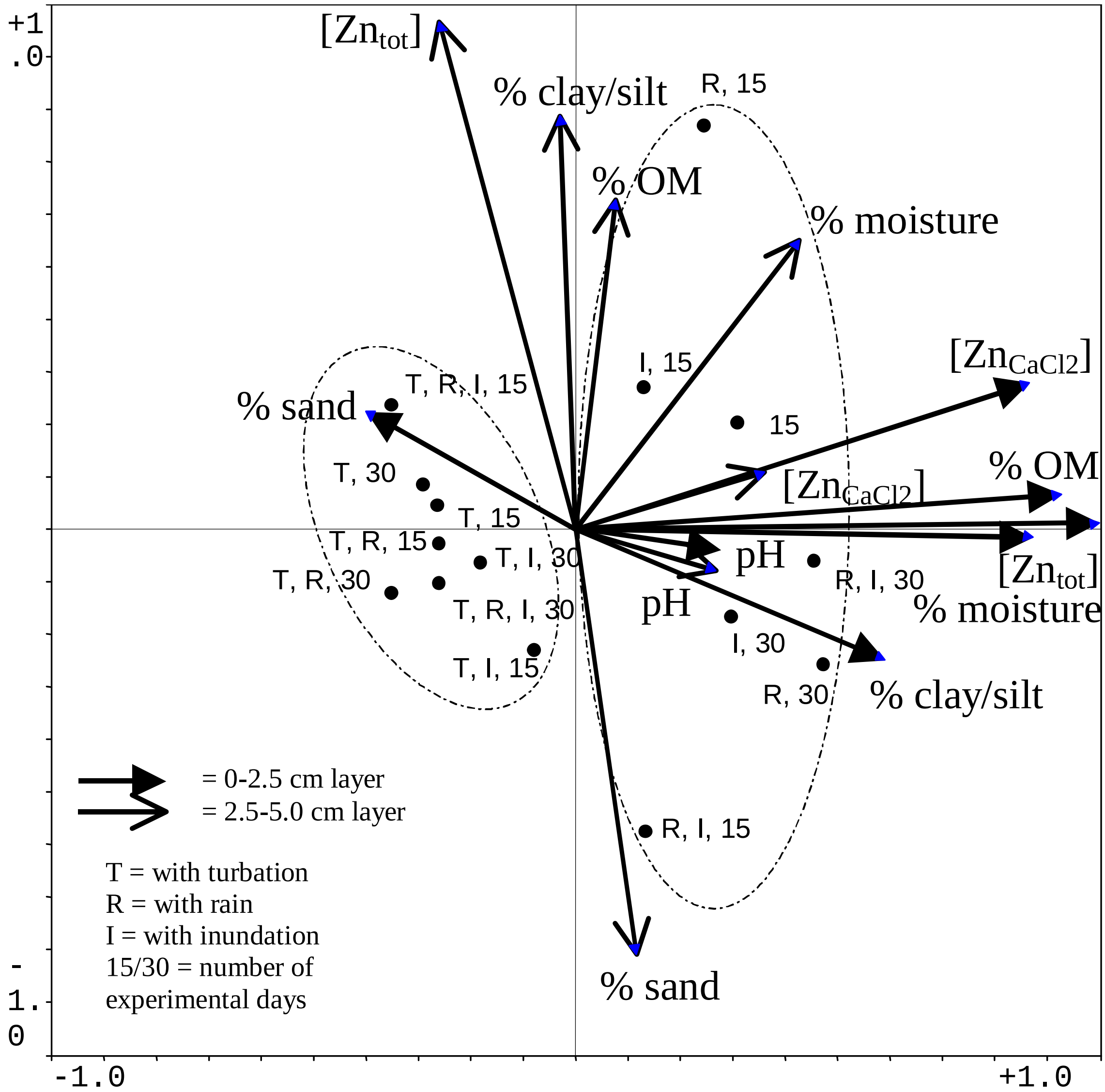

Fig. 3: Principal Component Analysis (PCA) of the microcosms from the pilot experiments, based on soil parameters measured in the two top substrate segments (0 - 2.5 cm and 2.5 - 5.0 cm). [Zn_{tot}] = total zinc content; [Zn_{CaCl2}] = 0.01 M $CaCl_2$-exchangeable zinc content; % OM = organic matter content; % moisture = soil moisture content; % clay/silt = substrate fraction <53µm; % sand = substrate fraction >53µm.

Main experiments

The top layers of the microcosms subjected to turbation had more marked relief than those of the other microcosms. Microcosms subjected to inundation showed smoothed surfaces at the end of the experiment. All microcosms appeared to feature a dried surface at the end of the experiments, while microcosms subjected to inundation without turbation showed some soil cracks.

Figure 4 shows that the total zinc content of the top segment was decreased compared to the starting situation (control, t=0) for all the treatments. However, the group subjected only to inundation was found to have a higher zinc content in the upper 2.5 cm compared to the control group (t=15, no treatment), while the content in the turbation plus inundation group was significantly reduced. After 15 days, the 0.01 M $CaCl_2$-exchangeable zinc contents in the upper segments were decreased in the control group compared to the control at t=0. A substantial part of the 0.01 M $CaCl_2$-exchangeable fraction had been transported to deeper segments or was bound to substrate components and not exchangeable by 0.01 M $CaCl_2$ anymore. The content of 0.01 M $CaCl_2$-exchangeable zinc in all the treatment groups significantly decreased ($p<0.05$, n=12) compared to the control group (t=15); this was true for all depth segments of the two groups subjected to turbation, and in the two upper segments of the inundation treatment group.

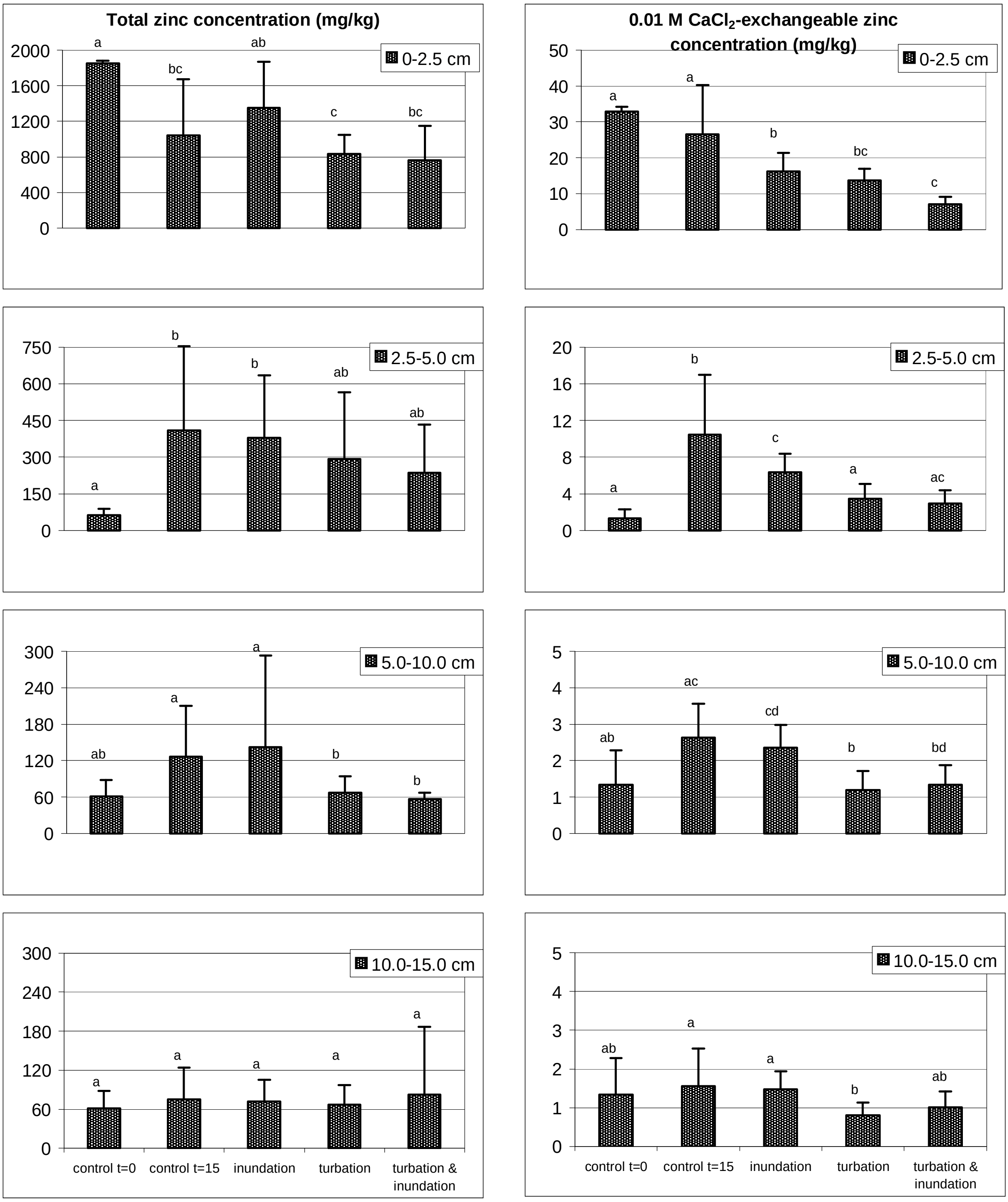

Fig. 4: Total and 0.01 M $CaCl_2$-exchangeable zinc content (mg/kg DW) in the different depth segments of the microcosms of the experiments. The 3 different treatments (with n=4) were compared with the control group (without treatment) after 15 days. The starting situation (control t=0) is also shown. Standard deviations are shown; different letters indicate the significant differences; identical letters indicate no significant differences between the treatment groups ($p<0.05$); n=12 in all treatment groups, except for the control t=0 group, where n=3.

4.4 Discussion

We found a clear effect of turbation on the zinc distribution in a vertical soil profile. Although a decrease in zinc content in the polluted sediment segment, causing an increase in the deeper soil segments, was also found in the t=15 control group after the experimental period, the decrease was always larger in the groups with turbation. The increase was small or even absent in the segments at depths between 2.5 and 15 cm. Redistribution of zinc in the control group (t=15) indicates that this is either (a) due to the effect of gravity on soil particles containing zinc, or (b) due to an increased mobility of zinc (free zinc ions or zinc attached to DOM) in a chemical gradient, both being transported with the ground water. If process (a) is dominant, zinc redistribution should be related to either organic matter or clay content, as the smallest particles are probably most easily transported with the groundwater, and positively charged molecules (e.g. several heavy metals) have a great affinity for those particles (Benedetti et al., 1996; Hesterberg, 1998; Plette et al., 1999). If process (b) is dominant, differences in 0.01 M $CaCl_2$-exchangeable fractions of zinc are likely. In addition, changes in pH and moisture content may be observed, as these have been shown to be important for metal solubility (Tack et al., 1996; Martínez and Motto, 2000; Zoumis et al., 2001). Turbation accelerates and increases at least one of the above processes. The results show that fixation of zinc or rearrangement of particles containing zinc may also affect segments deeper than 15 cm, as there are strong indications that zinc moved from the total column to the groundwater in all groups, but especially in the turbated groups. The total zinc content of the entire column seems to be less influenced in the group subjected only to inundation than groups subjected to other treatments, when compared to the starting situation. Moreover, the content in the top segment of the inundation group seems to be even higher than that in the control group (t=15), indicating the importance of the groundwater. Transport in a chemical gradient of dissolved zinc cannot explain the observed effect. The main process is therefore an upward water flux at inundation (percolation), leading to transport of sorbed zinc with soil particles, which partially compensates for the downward redistribution observed in the control microcosms.

Differences in total zinc content of the top segment were largest between the groups with and without turbation (Fig. 3), and were related to differences in organic matter and moisture content, and to the 0.01 M $CaCl_2$-exchangeable fraction of zinc. However, the redistribution of organic matter and clay/silt appeared to be the driving force behind the redistribution of zinc, as shown in the equations for the soil top segment alone and the whole column (Table 1) during the pilot experiments. In the 5 - 10 cm segment, the total zinc content reflects the presence of zinc in the top segment, which indicates that when more zinc disappeared from the top segment, and becomes redistributed to deeper segments, it was transported to the groundwater (Fig. 4). The absence of a relation between the zinc content in the 2.5 - 5.0 cm segment and turbation can be explained by the settlement effect, which was observed during the experimental treatments but was not taken into account while sampling, as settlement might be variable with depth. This settlement effect, characterised by compaction of the total soil column (by up to 1 cm), was present in the turbation and inundation treatment, but was strongest in the multiple treatments. Settlement leads to reduced porosity near the surface. This is stimulated by greater water content, resulting from inundation, and can also lead to sealing of the surface (Guebert and Gardner, 2001; Langmaack et al., 2001), which will influence water fluxes later on.

The 0.01 M $CaCl_2$-exchangeable content of zinc in the top segment decreased with decreasing clay/silt contents (Table 1). It has often been reported that both total zinc content and pH are important for the exchangeability of zinc with 0.01 M $CaCl_2$ (Spurgeon and Hopkin, 1996; Ge et al., 2000), and an increase of this zinc fraction would be expected under the influence of bioturbation (e.g. Vorenhout et al., 2000). In our experiment pH appeared to be not important for the observed changes (Table 1), as only a small pH range was observed within the columns of our microcosms. The observed range, between 6.6 and 7.4, is comparable to that in most floodplains along large European rivers; reported values including 7.0 - 7.8 at the ADW floodplain (our own measurements); 6.9 ± 0.6 along the Meuse (Albering et al., 1999); around 6.6 in the Mulde reservoir near the Elbe (Zoumis et al., 2001). Large differences in exchangeability of zinc with 0.01 M $CaCl_2$ are not expected in environments strongly buffered by the presence of large quantities of carbonate-rich clay, which are under permanent influence of river water, and have a stable pH of around 7.0 (Tack et al., 1996; Hesterberg, 1998; Martínez and Motto, 2000; Yin and Allen, 2000). An increase of the 0.01 M $CaCl_2$-exchangeable fraction could also suggest an increase of the bioavailability for several species, as mobile or weakly bound fractions are expected to be more bioavailable than almost irreversibly bound metals (Janssen et al., 1997b; Vink et al., 1999a). As there were no indications for an increase of the zinc exchangeability during the experiments, we neither expect an enlarged bioavailability of zinc, due to turbation activities as executed in our experiments.

Another factor affecting the exchangeability with 0.01 M $CaCl_2$ that is possibly influenced by turbation is redox potential (Wood and Shelley, 1999; Vorenhout et al., 2000; Olivie-Lauquet et al., 2001). This factor is also expected to be unimportant in our system, as it showed little variation, and there are no indications that it had changed during the 15 days experimental time. Large differences were to be expected then, between the treatments with inundation and without inundation. Increased zinc relocation was not expected, as the 0.01 M $CaCl_2$-exchangeable content had decreased in all segments after 15 days. It cannot be excluded that there was an increase of the exchangeability with 0.01 M $CaCl_2$, in the form of free ions as well as zinc attached to DOM, shortly after the start of the turbation, followed by fixation in deeper segments. However, the correlation between zinc redistribution and clay and organic matter redistribution does not suggest this. Part of the redistributed organic matter content could also belong to the mobile fraction, that is, the zinc attached to DOM. Kalbitz and Wennrich (1998) found that DOM is unimportant for the mobilization of zinc, as zinc appeared to have a low affinity for DOM, but they measured within a wide pH range, where mobilisation of zinc was related to pH. Wood and Shelley (1999) also showed in their model that the movement of particles resulting from turbation has little impact on metal mobility. Our results suggest that the downward redistribution process due to turbation, whether or not in combination with flooding, is strongest shortly after sediment deposition and turbation. After a few weeks, the redistribution is reduced to very small quantities.

Our results could be relevant towards various forms of turbation in floodplains. For instance mechanical disturbances like ploughing or harrowing in floodplains during the first days after the drying up of the floodplain could induce similar effects as described for our experiments. Mechanical turbation activities will, however, be uncommon just after inundation. Bioturbation may be very intense locally for certain bioturbating species (e.g. earthworms, ants, moles or voles), but probably also throughout the floodplain for all turbating species together. The impact of various types of bioturbation in floodplains depends on the type of organism, and their numbers present. Therefore turbation intensity is dependent of the season, or the timing of flooding events, and subsequent recolonisation processes (Wijnhoven et al., 2005). Bioturbators will have various ways of influencing the substrate segments at different depths (Mitchell, 1988), but as a rule, bioturbation processes take place mainly in the top 20 cm (Müller-Lemans, 1996). The manual turbation we used can be seen as a simulation of bioturbation, but than only of the top two centimetres of soil and that during the first days of drying up of the new deposited sediment just after flooding events. The turbation executed in this study is probably not very different from burrowing activities as shown locally by rabbits and voles scraping the topsoil in search for surface roots. Flooding has been found to reduce the numbers of for instance the epigeic earthworm species *Lumbricus rubellus*, which is especially active in the top three centimetres (Zorn, 2004; Zorn et al., 2005b). However, after flooding densities of 10 g (FW) per m^2 were still observed in the ADW floodplain along the River Waal, the Netherlands. These amounts of earthworms of this species are assumed to produce 20 grams of cast at the surface during the first ten days after the drying up of the floodplain. Cast produced in its tunnels is even not taken into consideration in this calculation. Other earthworm species, which are found to be more resistant to flooding, burrow to various depths, including the top two centimetres. The mean densities of these species of 130 g (FW) per m^2 as observed in the ADW floodplain (Zorn, 2004; Zorn et al., 2005b) are expected to produce about 260 g cast at the surface within the first ten days after flooding. Combining this with activities of Enchytraea, ants, spiders and insects including their larvae, with often the highest burrowing activity in the topsoil, we expect that bioturbation in the two centimetres topsoil in floodplains is generally intense, especially just after the drying up of the floodplain. To what extent our mechanical turbation simulates the combined turbation by various bioturbators in the field remains unclear from the experiments described. Therefore follow up field experiments and measurements will be necessary, as are experiments with longer inundation periods and higher water columns during flooding, with possible larger effects on the redox potential.

Local circumstances can be expected to determine the importance of factors like percolation (water table), anoxic conditions (duration of inundation, and water column depth), and rainfall (water infiltration and flows) for the redistribution of metals by bioturbation. Floodplain substrates, which are clayey, are generally buffered by a fair amount of carbonates such as calcite, as in our experiments, since clay has a large binding capacity for cations. Working with spiked sediments is sometimes criticised, as it is thought to ignore the effect of aging (Spurgeon and Hopkin, 1996; Sauvé et al., 2000). The 0.01 M $CaCl_2$-exchangeable contents as a percentage of the total zinc content were within a range of 0.14 to 2.38% (mean = 0.69 ± 0.43%, n=192) in the pilot study, and 0.14 to 7.05% (mean = 1.57 ± 1.10%, n=190) in the experiments. These percentages are in agreement with observations on fresh sediments a few days after flooding in the ADW floodplain (between 0.38 and 5.73%, mean = 1.40 ± 1.29%, n=15). We do not prove that the solubility of stronger bound zinc in our mixed sediment was comparable to freshly deposited sediment under field conditions, but one should expect an increase in the $CaCl_2$-exchangeable fraction, when the total solubility becomes larger with stronger extracts.

In our experiment, the top segment was contaminated. Water quality in several rivers has been improved in recent years, which means that polluted soils are often covered by clean soil nowadays (Middelkoop, 1997). Our experiments showed that the redistribution of contaminants under the influence of turbation was also measurable in deeper soil segments. Further experiments in which the polluted segment is present at various depths would seem to be useful.

The hypothesis that turbation will affect the heavy metal distribution of contaminated topsoils by an increased metal mobility, was tested using zinc as a contaminant. Processes responsible for changes in the vertical distribution of other heavy metals can vary (Van Straalen and Bergema, 1995; Tack et al., 1996; Janssen et al., 1997a; Sauvé, 2002). But a general trend to downward redistribution is to be expected, as shown in studies of Mace et al. (1997) and Zorn et al. (2005a) on the effects of bioturbation by rodents and earthworms on lead and zinc distribution. To clarify if our observations on effects of turbation are similar to processes resulting from bioturbation, measurements in field experiments, and observations in the field are necessary. These measurements will be complicated as effects coexist with influences of inundation and sedimentation. The present study shows that it is important to obtain a better understanding of both the quality (various ways to various depths) and quantity (the amount, intensity and frequency) of bioturbation in floodplains. Bioturbation is a very common process in floodplains, and the findings of this study indicate that a potential effect of biota on the fate of heavy metals in floodplains cannot be neglected.

4.5 Conclusions

Microcosm experiments showed that artificial soil turbation causes a downward redistribution of zinc attached to fine grain clay and organic matter particles moving from the top layer to deeper soil segments. Although only the upper two centimetres were turbated, the effects were measurable at soil depths of at least 15 cm. The redistribution is a gravity-driven process promoted by water fluxes and soil porosity. Turbation appeared to be the most important factor explaining the observed zinc redistribution, while inundation increased the downward redistribution of zinc. Differences in pH appeared to be of minor importance for the effects of turbation in our experiments, due to the buffering capacity provided by the large quantities of carbonate-rich clay and the influence of the river type water. To what extent our findings are comparable to effects of bioturbation on heavy metal contaminated floodplain topsoils is still unclear. However, as turbation by biota is a very common process in floodplains, a potential effect of these burrowing activities on heavy metal distribution, has to be taken into account under natural conditions.

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Chapter 5

The impact of bioturbation by small mammals on heavy metal redistribution in an embanked floodplain of the river Rhine

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Measuring molehills in the ADW floodplains by Mariëlle van Riel

Abstract

Floodplains along large European rivers are diffusely polluted with heavy metals due to emissions in the past. Because of low mobility of heavy metals in floodplain soils and improvements of water quality, these pollutants will remain in place, and can gradually become covered with less contaminated sediments. Bioturbators, especially earthworms, can play an important role in the mixing and surfacing of contaminated substrate. Surfaced substrate can be redistributed by recurrent flooding events, even to areas outside the floodplain. The question remained to what extent bioturbation by small mammals contributes to the redistribution of heavy metals from river sediments in floodplains. Extensive fieldwork on bioturbators such as voles, moles and earthworms and their distribution patterns, as well as on sediment deposition and bioturbation, was conducted at the 'Afferdensche en Deestsche Waarden' floodplain over the years 2001 – 2003. Field data were combined with data of experiments in field enclosures and substrate columns to calculate the amounts of sediment and heavy metals (Zn, Cu, Pb and Cd) redistributed during the floods as well as on an annual basis. Moles and voles surfaced considerable amounts of substrate and heavy metals, but not as much as earthworms which contribute a substantial proportion of the total deposition and redistribution during floods. Although the impact of moles and voles on the redistribution during floods was only locally important, on an annual basis the bioturbation activity of especially moles in floodplains cannot be neglected. The annual amounts of substrate and heavy metals surfaced by all investigated bioturbators were even larger than the total amounts of substrate and heavy metals deposited during floods.

5.1 Introduction

Many floodplains along large rivers in Europe are contaminated with pollutants, including a range of heavy metals (e.g. Cd, Cr, Cu, Hg, Ni, Pb and Zn), due to the deposition of contaminated sediments during flooding events in the past (Schröder, 2005). The sediments were especially contaminated in the 1960s and 1970s, due to unbridled emissions and a lack of integrated water pollution control (Vink et al., 1999; Middelkoop, 2000). Especially Zn, Cu, Pb and Cd are present in levels posing risks towards floodplain ecosystems (Kooistra et al., 2001, 2005; Leuven et al., 2005; Van Vliet et al., 2005). As retention, mobility and ecological implications of heavy metal contaminants in floodplain soils under changing environmental conditions are poorly understood, there are currently restrictions on digging activities, excavations and hydraulic engineering works in floodplains. This often interferes with ecological rehabilitation plans intended to reduce toxicological risks by removing contaminated soils. This may result in increased costs, delays and even cancellation of ecological rehabilitation activities.

Since the water quality of several large rivers has improved over the last decades, recently deposited soil layers are often less polluted than older layers in the subsoil (Vink et al., 1999; Ciszewski, 2002; Middelkoop, 2002). However, quite a number of animal species dig, root, grub or burrow through these soil layers, as they live partly or permanently underground, create nests, tunnels or hillocks, or search for food in the soil (Mitchell, 1988; Robinson et al., 2002). These soil dwellers can therefore mix the more polluted subsoil with the less polluted topsoil. In addition, they bring the subsoil to the surface, where the soil contaminants can be redistributed. The impact of these so-called bioturbators on the redistribution of heavy metals in floodplains remained largely unclear (Wijnhoven et al., 2006a). Some studies suggest that the impact of certain species on soil redistribution can be substantial. Earthworm and ant species are assumed to be important bioturbators when they are numerous (Scheu, 1987; Müller-Lemans, 1996; Tyler et al., 2001), which is the case in a large variety of ecotopes. Also several small mammal species could potentially have a strong impact on soil horizoning (Mitchell, 1988; Edwards et al., 1999). Examples are the European mole (*Talpa europaea*), which is known to create tunnels up to several hundreds of metres in length (Godfrey and Crowcroft, 1969; Haeck, 1969) and the Common vole (*Microtus arvalis*), a good burrower that is sometimes present in densities of up to 1000 individuals per ha (Lange et al., 1994). The burrowing activities of both species are accompanied by the creation of hillocks of excavated substrate. Since this substrate originates from tunnels in the more contaminated subsoil (Verbeke, 1997; Witte, 1997), heavy metals will be exposed at the surface, where weathering of the soil redistributes them with the substrate to the immediate vicinity of the former hillock. Floods can erode the soil hillocks and redistribute the soil and the associated contaminants over larger distances, not only within but also outside the floodplain. At the same time, floods also influence the spatial and temporal distribution of the bioturbators and have a strong impact on their population size (Andersen et al., 2000; Wijnhoven et al., 2005, 2006b) and burrowing activities.

The aim of this study was to estimate the contribution of bioturbation by small mammals to the distribution of the metals Zn, Cu, Pb and Cd in floodplains. In this paper the following research questions are addressed:

a) What amounts of substrate and heavy metals are surfaced by Common voles and European moles in a moderately polluted floodplain?
b) How do the amounts surfaced by small mammals compare to bioturbation by earthworms?
c) What is the share of bioturbation in the deposition of substrate and heavy metals during a flood?
d) What is the importance of bioturbation in the redistribution of substrate and heavy metals compared to the annual deposition during floods?

To answer these questions, we estimated vole- and molehill densities. Amounts of soil and heavy metal concentrations in vole- and molehills were estimated and related to soil concentrations. Further vole- and molehill turnover was monitored in an enclosure and in the open field. The heavy metal loads surfaced by voles and moles were compared to the loads surfaced by bioturbation by earthworms as established in column experiments, and the loads of heavy metals deposited during two floods in 2002.

5.2 Materials and methods

Research area

The ‘Afferdensche en Deestsche Waarden’ (ADW) (longitude 51°54′N, latitude 5°39′E) is a floodplain with an area of 280 ha, situated along the river Waal, the main distributary of the river Rhine in the Netherlands (Fig. 1). The research area (160 ha) is embanked by a summer dike (lower embankment near the river to prevent the area from flooding during most of the high waters), and a winter dike (major embankment at its southern border to prevent the hinterland from flooding). The ADW floodplain is a typical moderately polluted floodplain of a large lowland river in the Netherlands (Van Vliet et al., 2005), which has been the subject of an ecological rehabilitation programme since 1995 (Zandberg, 1999). It consists of natural and agricultural areas, including some elevated areas, clay excavations, small water bodies and side channels. The research area predominantly consists of grassland and ruderal vegetation types with low grazing intensity (Wijnhoven et al., 2005). The top soil consists of loamy clay deposited by the rivers with an average organic matter content of 7.3 ± 3.3%, a clay/silt content of 51.7 ± 19.1%, and a pH_{CaCl2} of 7.3 ± 0.2. Where excavations have take place, sandy soils can be found near the surface.

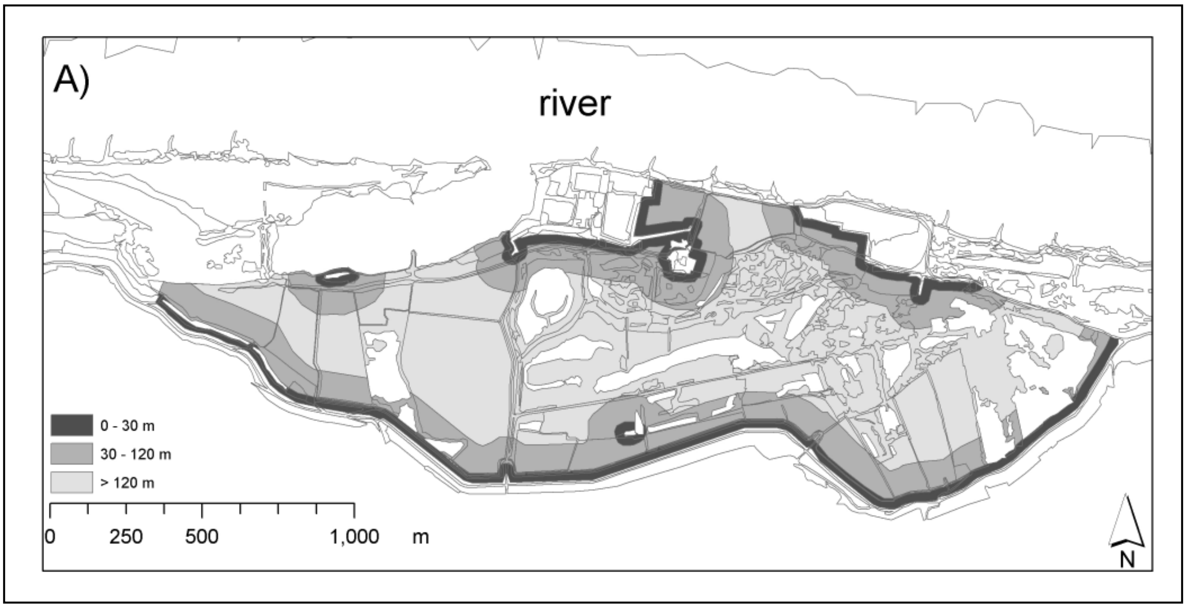
Fig. 1: Location of the 'Afferdensche en Deestsche Waarden' floodplain (ADW) along the river Waal in The Netherlands.

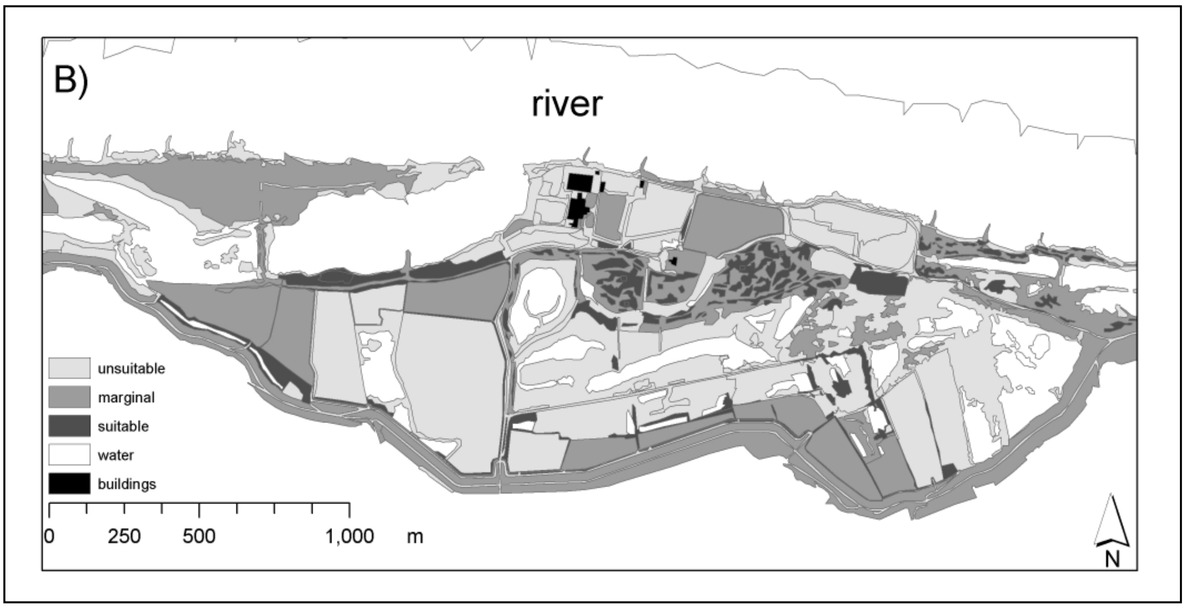
The area is subject to periodical inundations, at water discharges of the Rhine ≥ 6300 m^3/s at Lobith (Fig. 1). Between 1901 and 2004, the ADW floodplain flooded 77 times. During the last ten years (1995-2004) it flooded 10 times (http://www.waterbase.nl/). The water level in the river rises above the summer dike 4 days per year on average (http://www.waterbase.nl/). During floods, water levels rise to more than 3 m in the lower parts of the floodplain (about three quarters of the total area). The experiments and monitoring activities were executed in the period, between a flood in March 2001 and a flood in January 2004. During this period the area flooded 3 times (i.e. in February 2002, November 2002 and January 2003), of which a flood in February 2002 was preceded by a partial inundation in January. As the research area is bordered by embankments, water leaves the floodplain after flooding mainly by seepage towards the river channel. Once flooded, it takes about two to three weeks for the floodplain to fall dry entirely after the water level in the river has dropped below the height of the summer embankments. Average annual rainfall is 750 mm, and average annual air temperature is 9.6 °C for this region (as measured between 1971 and 2000; http://www.knmi.nl/).

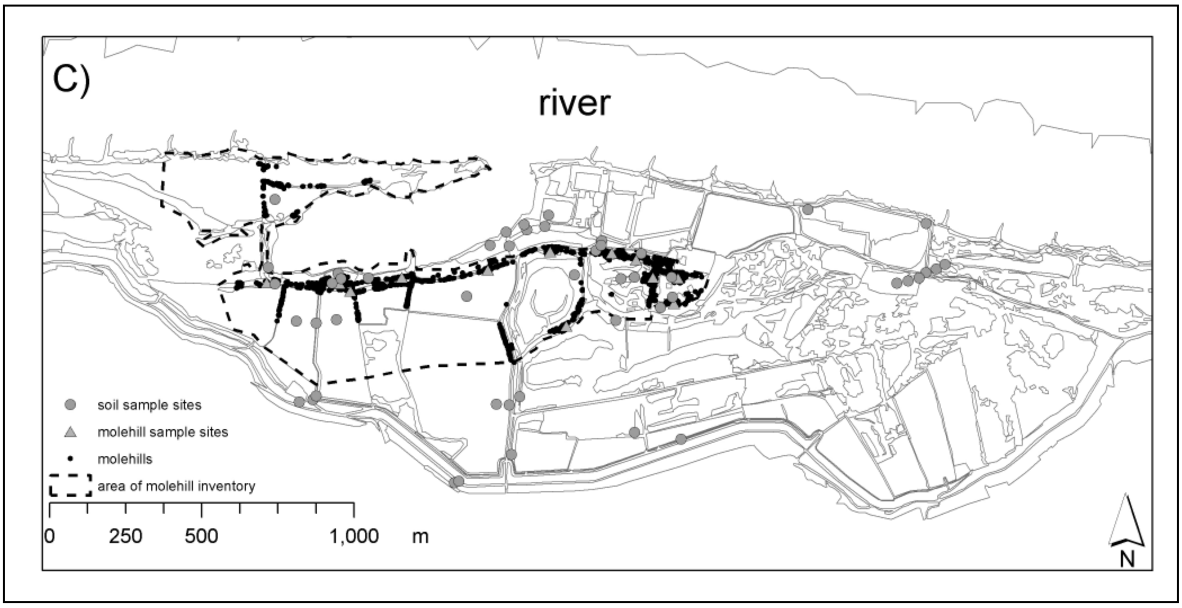
Density estimations

To estimate the total bioturbation in the ADW different methods were used. Hillocks from moles are easier to investigate in larger areas than hillocks from voles, due to their size. Vole populations, however, are easier to monitor by live trapping than mole populations due to differences in home range sizes and trappability. Therefore calculations of bioturbation by common voles are based on density estimations of the animals and bioturbation activity in field enclosures. Bioturbation by moles is calculated from density estimations of molehills (Table 1).

Wijnhoven et al. (2005) described the recolonisation of the ADW by small mammals after flooding events. In this study the research area was subdivided into four zones with similar numbers of monitoring sites: the non-flooding areas and zones at distances of 0 – 30, 30 – 120 and more than 120 m from these non-flooding parts.







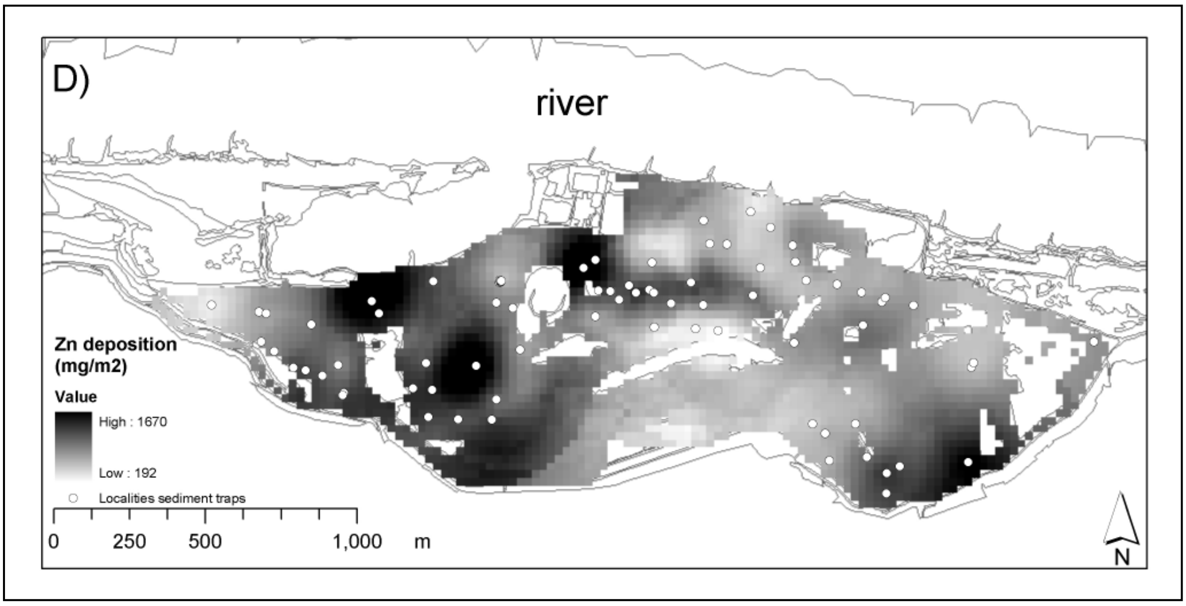


Fig. 2: Maps of the 'Afferdensche en Deestsche Waarden' floodplain, showing the research area, which is the embanked part. (a) Classification of the research area into zones at distances of 0 – 30, 30 – 120 m and more than 120 m from non-flooding areas. (b) Suitability of the research area for *M. arvalis*, showing the classification into suitable, marginal and unsuitable ecotopes. (c) The distribution of 5080 molehills over an area of 564 000 m^2 in November 2003, just before a flooding event, and the locations where the composition and metal concentrations in the molehills and in the 0 – 10 cm top soil layer were measured. (d) Zinc deposition during the floods of 2002 visualised in a 25x25 m grid, ranging from 192 to 1670 mg/m^2, as calculated from the results of sediment traps.

The calculations of bioturbation by voles and moles are therefore based on this subdivision. Earlier observations showed that the densities of small mammals significantly differ between the various zones. We focus on the three periodically flooding zones, as indicated in figure 2a for the embanked part of the ADW floodplain, as we want to estimate the share of bioturbation within the total sediment deposition. Wijnhoven et al. (2005) distinguished 21 small mammal ecotopes based on vegetation structure, soil characteristics and management regime. Based on trapping results these ecotopes were classified into suitable, marginal or unsuitable areas for small mammal species. The habitat suitability of our research area for

Common voles is presented in figure 2b. Wijnhoven et al. (2006b) calculated the densities of *M. arvalis* during the years 2001 and 2002 in the different zones, during nine trapping sessions. For each session an average density was calculated for each habitat suitability class in each zone. To calculate the amount of surfaced substrate by Common voles, the average density just before a flood and the average annual density per zone is calculated (Table 1). The average density is multiplied with the average amount of substrate surfaced by a vole as calculated from enclosure experiments.

Throughout 2001 to 2003, large areas of the ADW, including the zones at variable distances to non-flooding terrains described above, were checked for the presence of molehills. The position of each molehill, was determined using a GARMIN GPS 12 Personal Navigator, after which the positions were plotted on maps of the floodplain using ArcMap 8.0. As surface runs with an elevated ridge contribute to the total amount of surfaced soil, each half metre was also recorded. In November 2003, just before a flooding event, the number of molehills was counted over an area of 564000 m^2 (35.0% of the total research area; Figure 2c). The data from this inventory were used to calculate the amount of substrate redistributed during flooding. To calculate the amount of substrate surfaced annually, we assessed the recolonisation by moles by their molehill distribution within the same area, during the first four months after a flood in January 2003 (six counts at regular intervals). A count was also undertaken in large areas of the ADW in January 2002 (16.0% of the total research area) and in June 2002 (13.2% of the total research area), which were periods before and after a flooding event respectively.

Estimating surfaced substrate

An important factor for the estimation of surfaced substrate during a certain period (e.g. during the period between two floods) is the turnover rate of mole- and volehills. Dependent of the size, new hillocks gradually erode by weathering or trampling, and substrate becomes distributed by which the hillocks disappear. Existing molehills can also increase as new substrate is added from below periodically. Therefore also for the estimation of surfaced substrate different methods were used for voles and moles (Table 1). Field enclosure experiments at known vole densities were executed, to measure the amount of substrate surfaced and to establish the volehill turnover at known vole densities. Larger areas are necessary to monitor moles, so measurements for this species were done in the open field, which is less a problem as populations are expected to be more stable than for voles due to larger territories and less fluctuating densities under stable conditions.

For the vole studies two field enclosures (5 x 5 x 1.5 m with soil surface at 0.75 m) were built in 2003. One was situated in a sandy soil area and one in a clayey area. After the grassland vegetation had recovered from treading during the construction of the enclosures, 5 individuals of *M. arvalis* were introduced into each enclosure on August 6th. The animals were kept there for three months. Each month, we checked the presence of the animals and recorded their burrowing activities (volehills and holes). After three months all volehills were weighed with a field balance, taking all the substrate present above the soil surface. Subsequently, homogenised sub-samples were taken to measure moisture content and to calculate dry weight (DW), which allowed calculating the amount of substrate surfaced per vole in kilograms dry weight per individual (kgDW/ind; Table 1).

To estimate the amount of soil surfaced by moles we selected 12 locations within the floodplain with a high density of molehills, on various soil types and with a different vegetation cover (Figure 2c). At these locations ten molehills were weighed with a field balance, taking all the substrate above the soil surface. At each location, we took samples from three molehills, homogenised these, and determined the dry weight in the laboratory. An average molehill weight for the floodplain was calculated (Table 1).

Table 1. Input parameters, values and equations with their units and origin, used for the estimations and calculations.

Variable	Symbol	Units	Subdivision	Method	Average	Variation	n	Data from
- area	a	ha	0-30 m	ArcMap 8.0	18.24	n.a.	1	[g]
	a	ha	30-120 m	ArcMap 8.0	51.22	n.a.	1	[g]
	a	ha	>120 m	ArcMap 8.0	91.08	n.a.	1	[g]
	a	ha	ADW	ArcMap 8.0	160.45	n.a.	1	[g]
- flood recurrence period	y	weeks	ADW	2001/2002 [a]	45	n.a.	1	[g]
- metal concentration 10 cm topsoil	$[Zn]_{0-10}$	mg/kgDW	0-30 m	[a]	357	169 [e]	10	[g]
	$[Zn]_{0-10}$	mg/kgDW	30-120 m	[a]	570	409 [e]	11	[g]
	$[Zn]_{0-10}$	mg/kgDW	>120 m	[a]	428	216 [e]	13	[g]
	$[Cu]_{0-10}$	mg/kgDW	0-30 m	[a]	53.7	24.6 [e]	10	[g]
	$[Cu]_{0-10}$	mg/kgDW	30-120 m	[a]	77.2	50.5 [e]	11	[g]
	$[Cu]_{0-10}$	mg/kgDW	>120 m	[a]	61.0	29.5 [e]	13	[g]
	$[Pb]_{0-10}$	mg/kgDW	0-30 m	[a]	109	48 [e]	10	[g]
	$[Pb]_{0-10}$	mg/kgDW	30-120 m	[a]	175	122 [e]	11	[g]
	$[Pb]_{0-10}$	mg/kgDW	>120 m	[a]	131	69 [e]	13	[g]
	$[Cd]_{0-10}$	mg/kgDW	0-30 m	[a]	1.62	1.32 [e]	10	[g]
	$[Cd]_{0-10}$	mg/kgDW	30-120 m	[a]	2.73	2.31 [e]	11	[g]
	$[Cd]_{0-10}$	mg/kgDW	>120 m	[a]	2.32	1.42 [e]	13	[g]
- metal concentration in enclosure	$[Zn_{en}]_{0-10}$	mg/kgDW		[b]	722	27 [e]	6	[g]
	$[Zn_{en}]_{0-5}$	mg/kgDW		[b]	607	38 [e]	6	[g]
	$[Zn_{en}]_{0-35}$	mg/kgDW		[b]	674	64 [e]	6	[g]
	$[Cu_{en}]_{0-10}$	mg/kgDW		[b]	96.5	4.1 [e]	6	[g]
	$[Cu_{en}]_{0-5}$	mg/kgDW		[b]	81.7	3.4 [e]	6	[g]
	$[Cu_{en}]_{0-35}$	mg/kgDW		[b]	77.6	6.7 [e]	6	[g]
	$[Pb_{en}]_{0-10}$	mg/kgDW		[b]	203	12 [e]	6	[g]
	$[Pb_{en}]_{0-5}$	mg/kgDW		[b]	173	10 [e]	6	[g]
	$[Pb_{en}]_{0-35}$	mg/kgDW		[b]	210	15 [e]	6	[g]
	$[Cd_{en}]_{0-10}$	mg/kgDW		[b]	4.26	0.41 [e]	6	[g]
	$[Cd_{en}]_{0-5}$	mg/kgDW		[b]	3.52	0.56 [e]	6	[g]
	$[Cd_{en}]_{0-35}$	mg/kgDW		[b]	3.50	0.97 [e]	6	[g]
Voles:								
- vole density before flood	DVf	n/ha	0-30 m	$(DVf_{Dec01} + DVf_{Oct02})/2$	6.37	3 [e]	2	[h]
	DVf	n/ha	30-120 m	$(DVf_{Dec01} + DVf_{Oct02})/2$	3.49	4.6 [e]	2	[h]
	DVf	n/ha	>120 m	$(DVf_{Dec01} + DVf_{Oct02})/2$	1.01	1.5 [e]	2	[h]
- vole density in week w	DVw	n/ha	0-30 m	$DVw = 0.0006(w)^2 + 0.167(w)$		0.618 [f]	9	[h]
	DVw	n/ha	30-120 m	$DVw = -0.0007(w)^2 + 0.0928(w)$		0.186 [f]	9	[h]
	DVw	n/ha	>120 m	$DVw = -0.0044(w)^2 + 0.1895(w)$		0.307 [f]	9	[h]
- average annual vole density	DVa	n/ha	0-30 m	$DVa = ({}_0\int^y (-0.0006(w)^2 + 0.167(w)))/y$	4.19	n.a.	1	[g]
	DVa	n/ha	30-120 m	$DVa = ({}_0\int^y (-0.0007(w)^2 + 0.0928(w)))/y$	1.59	n.a.	1	[g]
	DVa	n/ha	>120 m	$DVa = ({}_0\int^y (-0.0044(w)^2 + 0.1895(w)))/y$	0.75	n.a.	1	[g]
- substrate at surface per vole	SVf	kgDW/ind	after 13 w	[b]	6.111	n.a.	5	[g]
- weekly increase number of volehills		%		[b]	9.56	n.a.	2	[g]
- weekly decrease number of volehills		%		[b]	6.49	n.a.	2	[g]
- increase of substrate at surface per vole			5 to 13 w	volehill and hole counts [b]	linear	n.a.	2	[g]
- substrate at surface per vole in week w	SVw	kgDW/ind	after initial 5 w	$SVw = 0.344(w) + 3.131$ [b]		n.a.	5	[g]

- weekly surfaced substrate per vole	SSVw	kgDW/ind*w		(344/1.0307) * 1.0956	0.366	n.a.	5	g
- substrate at surface by voles before flood	SSVf	kgDW	0-30 m	SSVf = DVf * a * SVf	710	n.a.	1	g
	SSVf	kgDW	30-120 m	SSVf = DVf * a * SVf	1090	n.a.	1	g
	SSVf	kgDW	>120 m	SSVf = DVf * a * SVf	593	n.a.	1	g
- annually surfaced substrate by voles	SSVa	kgDW	0-30 m	SSVa = DVa * y * a * SSVw	1250	n.a.	1	g
	SSVa	kgDW	30-120 m	SSVa = DVa * y * a * SSVw	1360	n.a.	1	g
	SSVa	kgDW	>120 m	SSVa = DVa * y * a * SSVw	1180	n.a.	1	g
- metal in volehill related to 10 cm topsoil			Zn	$[Zn]_{vh} = 0.98 * [Zn]_{0\text{-}10}$ [b]		0.95 [f]	78	g
			Cu	$[Cu]_{vh} = 0.98 * [Cu]_{0\text{-}10}$ [b]		0.95 [f]	78	g
			Pb	$[Pb]_{vh} = 1.01 * [Pb]_{0\text{-}10}$ [b]		0.96 [f]	78	g
			Cd	$[Cd]_{vh} = 0.94 * [Cd]_{0\text{-}10}$ [b]		0.83 [f]	78	g
- metal at surface by voles before flood	ZnSVf	kg		ZnSVf = SSVf * (0.98 * $[Zn]_{0\text{-}10}$)		n.a.	1	g
	CuSVf	kg		CuSVf = SSVf * (0.98 * $[Cu]_{0\text{-}10}$)		n.a.	1	g
	PbSVf	kg		PbSVf = SSVf * (1.01 * $[Pb]_{0\text{-}10}$)		n.a.	1	g
	CdSVf	kg		CdSVf = SSVf * (0.94 * $[Cd]_{0\text{-}10}$)		n.a.	1	g
- annually surfaced metals by voles	ZnSVa	kg		ZnSVa = SSVa * (0.98 * $[Zn]_{0\text{-}10}$)		n.a.	1	g
	CuSVa	kg		CuSVa = SSVa * (0.98 * $[Cu]_{0\text{-}10}$)		n.a.	1	g
	PbSVa	kg		PbSVa = SSVa * (1.01 * $[Pb]_{0\text{-}10}$)		n.a.	1	g
	CdSVa	kg		CdSVa = SSVa * (0.94 * $[Cd]_{0\text{-}10}$)		n.a.	1	g
<u>Moles:</u>								
- molehill density before flood	DMf	n/ha	0-30 m	inventory of 1.4 ha (Nov03) [a]	1209	n.a.	1	g
	DMf	n/ha	30-120 m	inventory of 12.2 ha (Nov03) [a]	174	n.a.	1	g
	DMf	n/ha	>120 m	inventory of 42.8 ha (Nov03) [a]	27.7	n.a.	1	g
- molehill density in week w	DMw	n/ha	0-30 m	$DMw = 0.00008(w)^2 - 0.0007(w)$ [a]		0.982 [f]	9	g
	DMw	n/ha	30-120 m	$DMw = 0.00001(w)^2 + 0.0002(w)$ [a]		0.551 [f]	9	g
	DMw	n/ha	>120 m	$DMw = 0.000005(w)^2 - 0.00007(w)$ [a]		0.731 [f]	9	g
- weight molehill	WM	kgDW	ADW	measured 10 hillocks at 12 localities [a]	0.948	0.630 [e]	120	g
- height molehill	HM	cm		existing and newly built hillocks [c]	6.7	3.15 [e]	132	g
- weekly decrease molehill height		cm		monitored for 66 days [c]	0.43	0.323 [e]	132	g
- weekly decrease molehill height		% HM		[c]	6.42	5.33 [e]		g
- annual number of molehills	DMa	n/ha	0-30 m	$DMa = (y)^{1.064} * (0.00008(y)^2 - 0.0007(y))$	21400	n.a.	1	g
	DMa	n/ha	30-120 m	$DMa = (y)^{1.064} * (0.00001(y)^2 - 0.0002(y))$	47900	n.a.	1	g
	DMa	n/ha	>120 m	$DMa = (y)^{1.064} * (0.000005(y)^2 - 0.00007(y))$	11400	n.a.	1	g
- substrate at surface by moles before flood	SSMf	kgDW	0-30 m	SSMf = DMf * a * WM	20900	n.a.	1	g
	SSMf	kgDW	30-120 m	SSMf = DMf * a * WM	8430	n.a.	1	g
	SSMf	kgDW	>120 m	SSMf = DMf * a * WM	2390	n.a.	1	g
- annually surfaced substrate by moles	SSMa	kgDW	0-30 m	SSMa = DMa * a * WM	370000	n.a.	1	g
	SSMa	kgDW	30-120 m	SSMa = DMa * a * WM	233000	n.a.	1	g
	SSMa	kgDW	>120 m	SSMa = DMa * a * WM	98700	n.a.	1	g
- metal in molehill related to 10 cm topsoil			Zn	$[Zn]_{mh} = 0.26 * [Zn]_{0\text{-}10} + 120$ [a]		0.44 [f]	90	g
			Cu	$[Cu]_{mh} = 0.26 * [Cu]_{0\text{-}10} + 16.3$ [a]		0.39 [f]	90	g
			Pb	$[Zn]_{mh} = 0.26 * [Zn]_{0\text{-}10} + 33.5$ [a]		0.47 [f]	90	g
			Cd	$[Zn]_{mh} = 0.18 * [Zn]_{0\text{-}10} + 0.76$ [a]		0.15 [f]	90	g
- metal at surface by moles before flood	ZnSMf	kg		ZnSMf = SSMf * (0.26 * $[Zn]_{0\text{-}10}$ + 120)		n.a.	1	g
	CuSMf	kg		CuSMf = SSMf * (0.26 * $[Cu]_{0\text{-}10}$ + 16.3)		n.a.	1	g
	PbSMf	kg		PbSMf = SSMf * (0.26 * $[Zn]_{0\text{-}10}$ + 33.5)		n.a.	1	g
	CdSMf	kg		CdSMf = SSMf * (0.18 * $[Zn]_{0\text{-}10}$ + 0.76)		n.a.	1	g
- annually surfaced metals by moles	ZnSMa	kg		ZnSMa = SSMa * (0.26 * $[Zn]_{0\text{-}10}$ + 120)		n.a.	1	g
	CuSMa	kg		CuSMa = SSMa * (0.26 * $[Cu]_{0\text{-}10}$ + 16.3)		n.a.	1	g

	PbSMa	kg		$PbSMa = SSMa * (0.26 * [Zn]_{0\text{-}10} + 33.5)$		n.a.	1	g
	CdSMa	kg		$CdSMa = SSMa * (0.18 * [Zn]_{0\text{-}10} + 0.76)$		n.a.	1	g
<u>Earthworms:</u>								
- earthworm density	DE	gFW/m^2	*A. chlorotica*	12 monitoring sessions in 3 years [a]	18	8 [e]	12	i, j
	DE	gFW/m^2	*A. caliginosa*	12 monitoring sessions in 3 years [a]	78	26 [e]	12	i, j
	DE	gFW/m^2	*L. rubellus*	12 monitoring sessions in 3 years [a]	34	22 [e]	12	i, j
	DE	gFW/m^2	*L. terrestris*	lowest estimation during flooding [a]	200	n.a.	2	i, j
- activity zone		cm	*A. chlorotica*		0-10	n.a.		i
		cm	*A. caliginosa*		0-10	n.a.		i
		cm	*L. rubellus*	(might be between 0-3)	0-5	n.a.		i
		cm	*L. terrestris*	(could be deeper; 0 up to 300)	0-35	n.a.		i
- cast turnover		weeks		[c]	1-2	n.a.		g
- cast at surface by earthworms before flood	SEf	gDWs/gFWe	*A. chlorotica*	10 days [d]	1.41	1.65 [e]	4	i
	SEf	gDWs/gFWe	*A. caliginosa*	10 days [d]	2.88	1.94 [e]	4	i
	SEf	gDWs/gFWe	*L. rubellus*	10 days [d]	2.18	0.59 [e]	4	i
	SEf	gDWs/gFWe	*L. terrestris*	10 days [d]	2.01	0.56 [e]	4	i
- cast surfaced by earthworms before flood	SSEf	kg	*A. chlorotica*	SSEf = SEf * a * DE * 10	40700	n.a.	1	g
	SSEf	kg	*A. caliginosa*	SSEf = SEf * a * DE * 10	361000	n.a.	1	g
	SSEf	kg	*L. rubellus*	SSEf = SEf * a * DE * 10	119000	n.a.	1	g
	SSEf	kg	*L. terrestris*	SSEf = SEf * a * DE * 10	645000	n.a.	1	g
- cast production by earthworms			increases linear after 40 days (data from 10, 20, 40 and 80 days available)				4	g, i
- annual cast production by earthworms	SEa	gDWs/gFWe	*A. chlorotica*	SEa = 0.0082 * ((y * 7) - 40) + 3.5	5.84	n.a.	1	g
	SEa	gDWs/gFWe	*A. caliginosa*	SEa = 0.028 * ((y * 7) - 40) + 5.97	13.95	n.a.	1	g
	SEa	gDWs/gFWe	*L. rubellus*	SEa = 0.101 * ((y * 7) - 40) + 6.95	35.74	n.a.	1	g
	SEa	gDWs/gFWe	*L. terrestris*	SEa = 0.026 * ((y * 7) - 40) + 3.98	11.39	n.a.	1	g
- annually surfaced cast by earthworms	SSEa	kg	*A. chlorotica*	SSEa = SEa * a * DE * 10	169000	n.a.	1	g
	SSEa	kg	*A. caliginosa*	SSEa = SEa * a * DE * 10	1750000	n.a.	1	g
	SSEa	kg	*L. rubellus*	SSEa = SEa * a * DE * 10	1950000	n.a.	1	g
	SSEa	kg	*L. terrestris*	SSEa = SEa * a * DE * 10	3660000	n.a.	1	g
- metal in worm cast related to 10 cm topsoil			*A. chlorotica*	$[Me]_{wc} = [Me]_{0\text{-}10}$ [b]		n.a.	6	g
			A. caliginosa	$[Me]_{wc} = [Me]_{0\text{-}10}$ [b]		n.a.	6	g
			L. rubellus	$[Me]_{wc} = ([Me_{en}]_{0\text{-}5} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10}$ [b]		n.a.	6	g
			L. terrestris	$[Me]_{wc} = ([Me_{en}]_{0\text{-}35} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10}$ [b]		n.a.	6	g
- metal at surface by earthworms before flood	MeSEf	kg	*A. chlorotica*	$MeSEf = SSEf * [Me]_{0\text{-}10}$		n.a.	1	g
	MeSEf	kg	*A. caliginosa*	$MeSEf = SSEf * [Me]_{0\text{-}10}$		n.a.	1	g
	MeSEf	kg	*L. rubellus*	$MeSEf = SSEf * (([Me_{en}]_{0\text{-}5} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10})$		n.a.	1	g
	MeSEf	kg	*L. terrestris*	$MeSEf = SSEf * (([Me_{en}]_{0\text{-}35} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10})$		n.a.	1	g
- annually surfaced metal by earthworms	MeSEa	kg	*A. chlorotica*	$MeSEa = SSEa * [Me]_{0\text{-}10}$		n.a.	1	g
	MeSEa	kg	*A. caliginosa*	$MeSEa = SSEa * [Me]_{0\text{-}10}$		n.a.	1	g
	MeSEa	kg	*L. rubellus*	$MeSEa = SSEa * (([Me_{en}]_{0\text{-}5} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10})$		n.a.	1	g
	MeSEa	kg	*L. terrestris*	$MeSEa = SSEa * (([Me_{en}]_{0\text{-}35} * [Me_{0\text{-}10}]) / [Me_{en}]_{0\text{-}10})$		n.a.	1	g

$_{0\text{-}10}$ = 10 cm topsoil; $_{0\text{-}5}$ = 0-5 cm topsoil; $_{0\text{-}35}$ = 0-35 cm topsoil; $_{en}$ = enclosure; w = time after flood in weeks; $_{Dec01}$ = December 2001; $_{Oct02}$ = October 2002; $_{vh}$ = volehill; $_{mh}$ = molehill; $_{wc}$ = earthworm cast; Me = metal; distr. = distribution; [Me] = metal concentration; [a] = field measurement; [b] = enclosure; [c] = field experiment; [d] = laboratory experiment; [e] = standard deviation; [f] = R^2 ; [g] = this study; [h] = Wijnhoven et al. (2006b); [i] = Zorn et al. (2004); [j] = Zorn et al. (2005).

The turnover time for molehills was calculated by measuring the decrease in molehill height of 132 hillocks (existing and newly built) at regular intervals of 11 days over a period of 66 days, within an area (150 m^2) where moles were active. Sometimes an increase in molehill height was observed. When the hillock had increased in size, we regarded this measurement as a new starting point to calculate the trend of decrease.

Estimating surfaced metals

Of the weighed and homogenised hillocks (volehills from enclosures and molehills from the selected locations), metal (Zn, Cu, Pb, and Cd) concentrations were determined. In the enclosures, three soil cores were taken within an area of 900 cm^2, after the animals had been removed after their stay for three months. Corresponding core segments with similar depths for 0 – 5 cm, 5 – 10 cm, 10 – 15 cm, 15 – 25 cm and 25 – 35 cm layers were mixed. In each enclosure, we took six of these aggregated samples per core segment for measuring moisture content and metal concentrations. For each of the four metals, a regression coefficient was calculated between the concentration in the top 10 cm soil and the concentration in the volehills. Assuming that the average depth to which voles burrow is relatively constant, we can calculate the amount of surfaced metals by using the regression equations, as we estimated the metal load of the top 10 cm of the research area (Table 1). Therefore we took three soil samples at 34 periodically flooded locations of the top 10 cm of soil in order to determine the moisture content and metal contents (Figure 2c). In this sampling we included the vole monitoring sites and the selected molehill locations. Mean contaminant levels (Zn, Cu, Pb and Cd) within the top 10 cm for the different zones were calculated. The correlation between the metal concentrations in the top 10 cm of soil, and the metal concentrations in the molehills was calculated. This allowed us to estimate the total amount of substrate and metal loads per zone surfaced by moles, based on molehill densities (Table 1).

The dry weight (DW) and moisture content was determined by drying 5 g of wet soil (FW) from depth segments, topsoils and vole- or molehills, for 24 h at 105°C. Metal concentrations (Zn, Cu, Pb and Cd) were measured after microwave destruction (using a Milestone 1200 microwave oven) of 0.2 mg DW substrate in a mixture of 3.0 ml 65% HNO_3 and 1.5 ml 37% HCl. The samples were diluted with demineralised water to 50 ml, and used to measure metal concentrations by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

Estimating bioturbation by earthworms

Zorn et al. (2005) monitored earthworm densities for three years in ADW in natural grassland with a clayey substrate. Multiple samples were taken and used to establish species composition, number of individuals and biomass. Four species were commonly found: *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Lumbricus rubellus* and *Lumbricus terrestris*. Zorn et al. (2004), measured the cast production of these species in experimental columns with a similar type of substrate for 80 days. The amount of substrate redistributed by various earthworm species was calculated using mean densities and weights recorded by Zorn et al. (2005) throughout the monitoring period, as densities and weights were not only negatively influenced by flooding events, but also by drought. To calculate the amount redistributed during a flood, a 10-day cast production (available from Zorn et al., 2004) was assumed to be representative, as during winter, the period in which generally floods occur, marked casts could still be recognised after a week, but no longer after two weeks. The annual production (in 45 weeks, which was the time between two floods in 2001/2002) could be calculated by extrapolating the cast production for 40 and 80 days (Table 1). We assumed that the metal distribution over the depth samples within the whole research area was similar to those measured in the enclosure on clay. From the average metal concentration at 0 – 10 cm depth in the three zones, the average metal concentration in earthworm surfaced cast could be calculated taking the species specific total cast production and the depth distribution of metals into account. Therefore we assume that the casts evenly originate from each horizontal layer between the soil surface and the maximum activity depth.

Estimating total deposition

Sediment and heavy metal deposition during floods (comprising redistributed floodplain sediment and influx of river sediment) were measured during the partial and complete inundations of the ADW in February and March 2002. Embankments largely prevent sediment transfer out of the floodplain and turn the floodplain into a settling tank where sediment settles easily. We therefore assumed that substrate surfaced by bioturbation is redistributed within the floodplain itself during floods and is not transported out of the floodplain. This is supported by observations during inundations of the ADW floodplain, showing rapid settlement of suspended sediments within the floodplain (Thonon and Van der Perk, 2003; Thonon et al., 2005). For this purpose, we placed 72 sediment traps in a stratified random pattern before the February flooding. Forty-three of those traps fell dry in between the two floods and were replaced by new ones before the March flooding. The sediment traps measured 50 by 50 cm and consisted of artificial grass tufts 2 cm in height, and were also used by Lambert and Walling (1987) and Middelkoop and Asselman (1998). Dry weights and heavy metal loads of trapped sediment were measured as described before. We interpolated the dry weights and heavy metal loads by kriging with Gstat (Pebesma and Wesseling, 1998) with 25 by 25 m grid cells. Multiplying the metal (Zn, Cu, Pb and Cd) load (in mg/m^2) by the floodplain area yielded the total annual metal load deposited during flooding (Table 1). All calculations for this study are executed in Microsoft Excel 2000, except for calculations of areas and densities of molehills, which are executed in ArcMap 8.0.

5.3 Results

Surfacing by moles and voles

The average density of *M. arvalis* for the period just before a flooding event was found to be almost twice as high in the 0 – 30 m zone as in the 30 – 120 m zone (Table 1), and almost six times higher than in the >120 m zone. The average density development for the whole period after the river water had receded can be described by second order polygons (Table 1). The variance explained by the model was more than 61% for the 0 – 30 m zone, but densities in the two other zones were generally lower, and the variation between the years and monitoring dates appeared to be irregular. Molehill densities just before a flood in 2003 were also differing a lot, with approximately seven times more hillocks in the 0 – 30 m zone than in the 30 – 120 m zone, and 44 times more hillocks than in the >120 m zone (Table 1). The regression models for the zone-dependent density development throughout the year showed high R^2 values.

The surfaced amount of substrate per individual vole in the enclosure experiments was 6111 gDW/ind. This leads to a total of 2393 kgDW surfaced substrate by voles for the research area, of which 46% originates from the 30-120 m zone (Table 1). Weekly surfaced substrate by voles was measured to calculate the amount of substrate surfaced annually. The weekly increase in the number of vole hills was found to increase linearly with 6.5%, after the initial 5 weeks with high burrowing activity. Each week 3.1% of the hills could not be found back anymore. It can be concluded that the weekly surfaced amount of substrate was 9.6% of the final 6111 gram surfaced per individual. This means that after 5 weeks, 3130.5 grams substrate (DW) was present at the surface, which weekly increased with 343.8 grams. The weekly amount of substrate surfaced will then be 365.5 gDW/ind/week (Table 1). This figure we multiplied with the estimated densities of voles for each week after the recede of the water. The time between two successive floods was 45 weeks (Table 1). Taking density development and area into account, this yielded the annual amount of substrate surfaced per zone. The annual amount of substrate surfaced in each of the zones lies in the same range (between 1100 and 1400 kgDW per zone; Table 1).

The average weight (gDW ± sd) of molehills was 948 ± 630 g (n=120; Table 1). Sixty-six percent of the approximately 31700 kgDW surfaced substrate by moles that is present just before a flood is originating from the 0-30 m zone. The turnover time for molehills was estimated for 73 molehills, which were already present at the start of the measurements, and another 59, which were newly built during the observation period. The average decrease in molehill height was 0.43 cm per week, whereas the average height was 6.7 cm. This means that after 16 weeks, the average molehill was totally refreshed, and could be regarded as a new hill. Each week, 6.4% of the substrate surfaced by moles was redistributed to the near vicinity due to erosion. The cumulative molehill densities per zone and the mean weight of a molehill (948 g), gives the annual surfaced amount of substrate of 701700 kgDW in the whole area of which 53% originates from the 0 - 30 m zone (Table 1).

The amounts of surfaced metals can be calculated from the average metal concentrations in the research zones. A large variability in average metal concentrations was found within the zones, consequently no significant differences between the zones could be established. However, for all metals a similar trend was observed with the lowest concentrations in the 0 – 30 m zone, and the highest in the 30 – 120 m zone. Average metal concentration in the first mentioned zone is almost half the concentration in the 30 – 120 m zone, and combined with uncertainties in other values such as estimated densities it seems to be justified to use these average values and not to calculate average concentrations for the whole floodplain. Metal concentrations of the volehills related to those of the 10 cm topsoil (Table 1) were used to calculate the amount of metals surfaced. We assumed that substrate in hillocks is originating in similar amounts from the depth layers between the surface and the maximum depth of burrowing activity. The regression was therefore forced through zero, as no contaminants in the topsoil will than result in no contaminants in the volehills. We checked if the soil cores taken were representative for the column of which the hillocks originated by investigating the heterogeneity of contaminant distributions in the enclosures. On a small scale, a few square metres, the variation in the distribution of contaminants in the floodplain soil was found to be low, justifying regression analyses between concentrations in soil samples and substrate from the volehills. Strong correlations with the upper 10 cm of substrate were found for the amounts of heavy metals within the volehills (Table 1). The amounts of heavy metals available at the surface just before a flood were approximately 1.11 kg Zn, 0.15 kg Cu, 0.37 kg Pb and 0.0052 kg Cd for the total floodplain (Fig. 3). The total metal loads surfaced on an annual basis were approximately 1.70 kg Zn, 0.24 kg Cu, 0.57 kg Pb and 0.0080 kg Cd (Fig. 4).

The heavy metal concentrations within the molehills could also be related to the concentrations in the top 10 cm of substrate (Table 1). The regression coefficients were lower than for the case of the volehills, explaining 39 to 47% of the observed variance. Just before the flood, 7.33 kg Zn, 1.02 kg Cu, 2.11 kg Pb and 0.035 kg Cd is available at the surface of the floodplain (Figure 3). On an annual basis this amounts approximately 165 kg Zn, 23.0 kg Cu, 47.7 kg Pb and 0.80 kg Cd, which is much more than surfaced by the Common vole (Fig. 4).

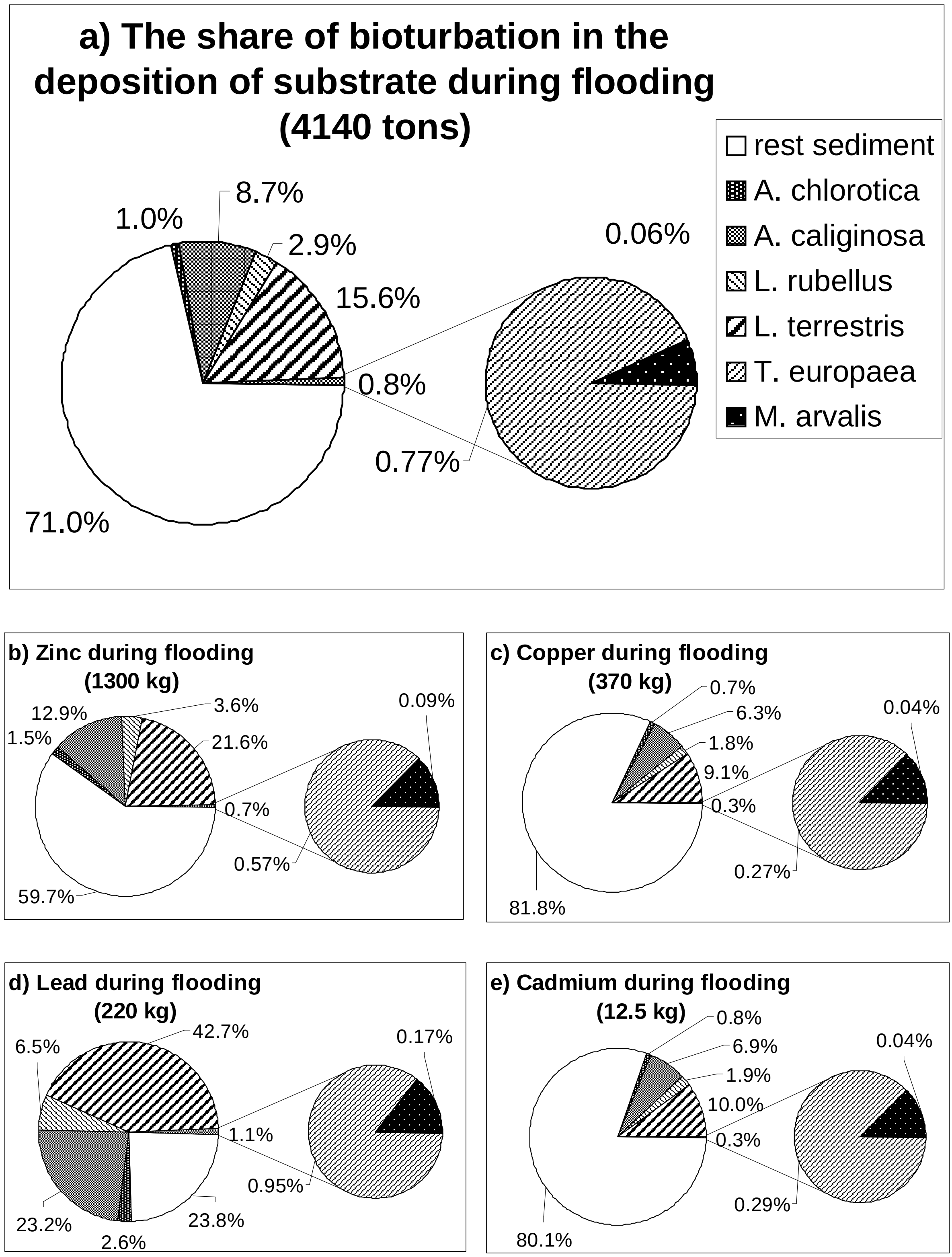


Fig. 3: Share of bioturbation by small mammals (*Talpa europaea* and *Microtus arvalis*) and earthworms (*Allolobophora chlorotica*, *Aporectodea caliginosa*, *Lumbricus rubellus* and *Lumbricus terrestris*) in the deposition of (a) substrate, (b) zinc, (c) copper, (d) lead and (e) cadmium, during flooding of the embanked part of the 'Afferdensche en Deestsche Waarden' floodplain.

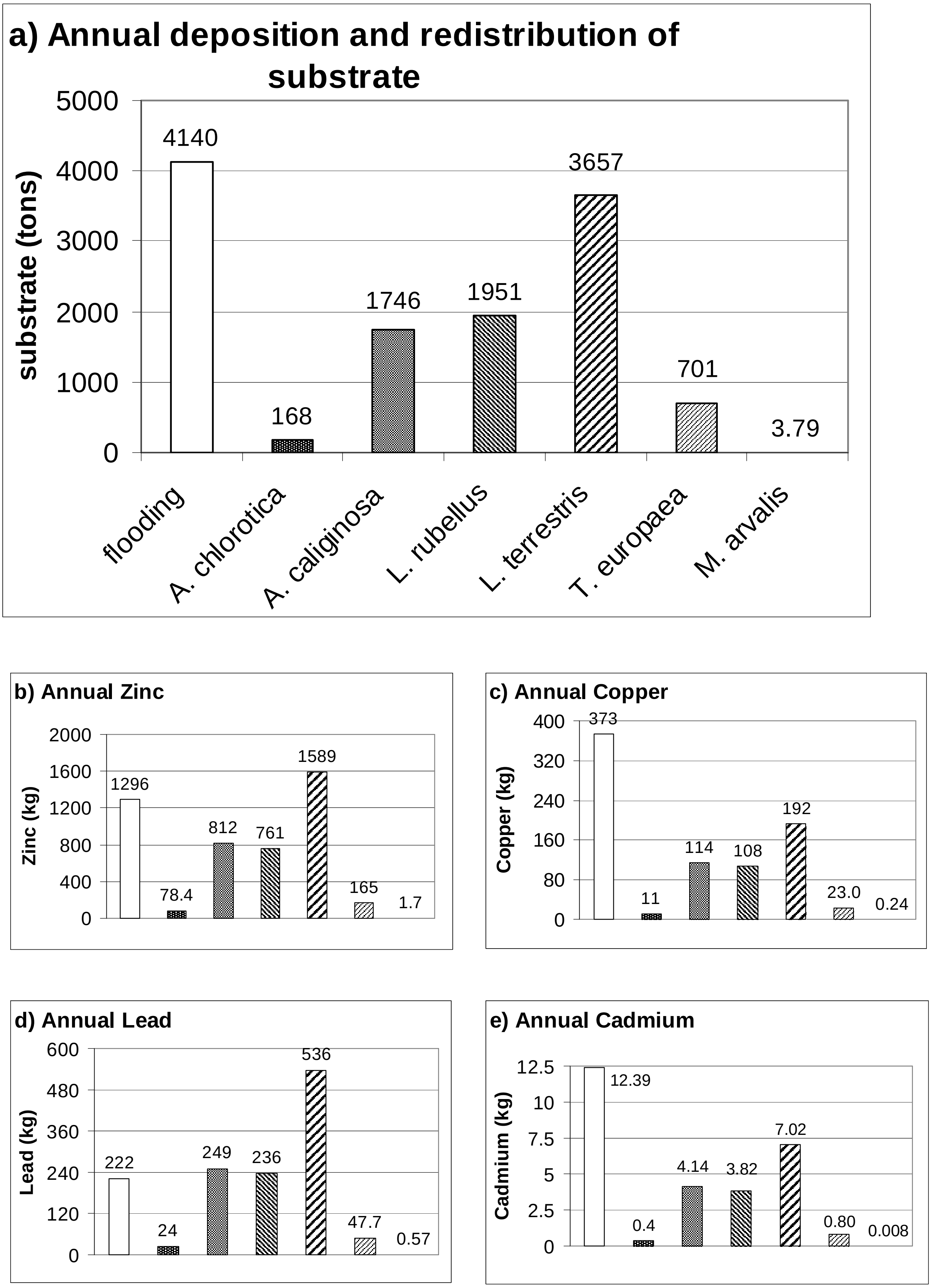


Fig. 4: Annual redistribution of (a) substrate, (b) zinc, (c) copper, (d) lead, and (e) cadmium due to bioturbation by small mammals (*Talpa europaea* and *Microtus arvalis*) and earthworms (*Allolobophora chlorotica*, *Aporectodea caliginosa*, *Lumbricus rubellus* and *Lumbricus terrestris*) compared to the annual deposition during floods, calculated for the embanked part of the ‘Afferdensche en Deestsche Waarden’ floodplain.

Surfacing by earthworms

Table 1 shows that the four earthworm species cast approximately 7523 tons of substrate at the surface, of which approximately 1166 tons will be redistributed during a flood. Measurements of metal concentrations within the depth profile on clay showed that the metal contamination is not evenly distributed. The highest concentrations are found in the 10 – 15 cm depth layer, while the 5 cm top soil is less contaminated than the deeper layers for all metals (Table 2). All earthworm species together cast approximately 3241 kg Zn, 425 kg Cu, 1046 kg Pb and 15.4 kg Cd on an annual basis in the research area (Fig. 4) with 514 kg Zn, 66.7 kg Cu, 166 kg Pb and 2.42 kg Cd present in casts at the surface just before a flood (Fig. 3).

Table 2. Relative contaminant distribution over the depth segments in percentages of the total content of the 0 and 35 cm soil layer, as measured from soil cores taken in an enclosure in a clayey area.

depth segment	% Zn	% Cu	% Pb	% Cd
0 – 5 cm	12.8	15.0	11.8	14.4
5 – 10 cm	17.8	20.5	16.0	20.8
10 – 15 cm	21.6	22.4	20.4	25.3
15 – 25 cm*	33.7	29.3	34.7	29.5
25 – 35 cm*	14.1	12.7	17.1	10.0

* The volume of these depth segments is double the volume of the others.

Deposition of locally redistributed floodplain- and river sediment

Extrapolation of the sediment trap results (for example figure 2d for the results of Zn deposition) showed that in 2002, about 4140 tons of sediment were deposited during floods in the embanked part of the ADW floodplain, containing about 1300 kg Zn, 370 kg Cu, 220 kg Pb, and 12.5 kg Cd (Fig. 3 and 4). The deposition of sediment and heavy metals during floods was about 100 times larger than the amounts available for redistribution just before the flood due to bioturbation by small mammals (Fig. 3). The proportion of heavy metals made available by *T. europaea* was about 5.5 to 7.5 times larger than the amount made available by *M. arvalis*, while the substrate surfaced by *T. europaea* was 13 times more (Fig. 3). Earthworm activity, especially bioturbation by *A. caliginosa* and *L. terrestris*, accounted for a substantial part of the sediment and heavy metals deposited in the floodplain during floods. Most striking are the results for Pb, as about three quarters of the amounts deposited probably resulted from the redistribution of soil surfaced by bioturbators (Fig. 3).

Over 2002, the amounts of substrate surfaced by *L. terrestris* approached the amounts deposited during floods (Fig. 4). Taking the six bioturbator species studied (four earthworms and two small mammal species) into account, the amounts of substrate surfaced were more than twice the total deposition during floods during that year. The share of *T. europaea* in the total amount of substrate surfaced by bioturbators seemed to be larger (8.5%) than just before the floods, where *T. europaea* only accounts for 2.7% of the total amount of substrate at the surface due to bioturbation. The share of moles in the total bioturbation on an annual basis was larger than that of the earthworm *A. chlorotica*, while the role of *M. arvalis* was minimal for both substrate and heavy metal redistribution (Fig. 4). The earthworms *L. terrestris*, *A. caliginosa* and *L. rubellus* surfaced more lead on an annual basis than was added by floods, while the amount of zinc surfaced by these species was similar to the annual total deposition during floods.

5.4 Discussion

The results show that large differences are present in the amounts of substrate and metals redistributed by the investigated bioturbating species. Given the uncertainties in the calculations (by incorporating averages or up-scaling results of a selection of samples) differences of a factor 5 to 12 between the two mammal species in the share of the deposition of substrate and heavy metals during a flood may be called considerable. This is even more the case for differences of a factor 83 to 185 between the share of the two mammals in the annual deposition of substrate and heavy metals. This also accounts for observed differences between the mammals and the earthworms. The amounts of substrate and heavy metals surfaced just before a flood differ a factor 35 to 68, while on an annual basis the amounts differ a factor 10 to 22.

Our calculations show that substantial proportions of the deposition of substrate and heavy metals during floods originate from bioturbation. It is not possible to measure the exact proportion of hillocks or cast that is redistributed during a flood, as this depends on factors like flow velocity of the water, vegetation and soil type. However, it is clear that a large part of the heavy metal deposition comes from redistributed floodplain soil. In particular the earthworm species appear to be important for this redistribution. The share of *T. europaea* and *M. arvalis* in the deposition of heavy metals by redistribution of surfaced substrate during floods was less than 1%. As it seems that the worm casts and the mole- and volehills were less contaminated with these elements than the new deposits introduced from outside the floodplain the share of bioturbators in the Cu and Cd deposition is smaller than their share in substrate deposition. Pb concentrations in river sediments, however, have been declined considerably compared to the past, which means that the Pb deposition could largely be attributed to the redistribution of surfaced material.

Moles redistributed 13 times more substrate than Common voles, but only 5.5 to 7.5 times more heavy metals. This is related to the depth at which the species are active in the floodplain soil, combined with the different heavy metal concentrations at different depths. We assumed a constant relation between the metal concentrations in the 10 cm topsoil and in the hillocks. For the excavated areas where the top clay layer, including most contaminants, has been removed several years ago, this results in a slight overestimation of the surfaced amounts of especially Zn, Cu and Pb by bioturbators as we found that here the top 10 cm is the most contaminated layer. However, considering the total floodplain area, the impact of this overestimation is small as the metal concentrations in the clayey unexcavated areas are much higher. Depending on the annual sediment deposition, in clayey areas, the highest Zn, Cu, Pb and Cd concentrations were found between 10 and 15 cm below the surface. The concentrations were often also increased at depths between 5 and 20 cm, sometimes the whole top layer (0 – 20 cm) contained high heavy metal concentrations. Pollutant levels were always much lower at depths of more than 25 cm below the surface (Table 2), which is in agreement with observations at similar sites in embanked floodplains along the Waal river (Middelkoop, 2002). Species burrowing deeper than 25 cm consequently also brought large amounts of cleaner soil to the surface. Moles are known to burrow to depths of up to 1 m (Verbeke, 1997; Witte, 1997). This is reflected by the low regression coefficients (± 0.4) for the ratio between metal concentrations in hillocks and in the 0 - 10 cm top soil (Table 1). Half of the substrate surfaced by moles originated from depths below 25 cm, since heavy metal concentrations were highest at depths of 10 – 15 cm. This means, assuming similar quantities of substrate originating from each of the upper layers, that the activities of the moles did indeed reach depths of 50 to 60 cm. However, from the high regression coefficients (± 0.97) for the relation between metal concentrations in volehills and the 0 – 10 cm soil layer we concluded that the major part of the substrate surfaced by the Common vole originated from the top 10 cm soil. This was confirmed when using Ca, Fe, Al, Mn and organic matter as tracers. These elements also showed strong correlations with the amounts found at depths of 0 – 10 cm (data not shown). It has been shown that in the floodplain soil, with pH values around 7 – 8 and a high clay content, the mobility of these elements and the heavy metals is low (Gäbler, 1997; Eijsackers and Doelman, 2000; Wijnhoven et al., 2006a), so leaching of elements to deeper layers can be neglected.

Earthworms were found to be important bioturbators. Amounts calculated for these species are estimations based on densities measured on a few monitoring sites. These monitoring sites were representative for the remaining part of the floodplain. Also at much lower densities than recorded, their role in the redistribution of substrate and metals during floods will be considerable. Earthworms have quiescence and diapause periods, during which their burrowing activities will be reduced. Therefore extrapolation of the column experiments with earthworms will lead to some overestimation of the amounts of surfaced substrate. However, earthworms in the field will show more burrowing behaviour as their corridors are continuously destroyed by weathering, trampling and flooding, which processes were absent in the columns. Since the origin of the surfaced substrate is important for the assessment of the amounts of heavy metals redistributed, the proportional impact of *L. terrestris* in heavy metal redistribution could have been overestimated, as we assumed an activity depth of 35 cm. However, burrowing depths below 35 cm, even up to 3 m below ground level, have also been reported (Zorn et al., 2004). This could reduce the estimates for metal concentrations considerably, as soils at these depths are unpolluted (Middelkoop 2000, 2002). However, it is expected that the species will not be active at depths below the water table, which is generally shallow in the ADW floodplain. We are aware that earthworm bioturbation was calculated from cast production in laboratory experiments. Intensive burrowing activity by earthworms is expected in the field immediately after flooding, as flooding probably destroyes a large percentage of the earthworm burrows. Initially the number of burrows increase rapidly. Later the production of burrows gradually stabilizes when equilibrium between formation and redistribution is reached (Zorn et al., 2004). Besides, earthworms extend or divert their burrows, newly hatched individuals create new burrows in the field, and burrows are destroyed by weathering and trampling. Under field conditions, the epigeic and endogeic earthworm species will be mainly present in the rooted zone and burrow less deep than Zorn et al. (2004) observed in their experimental setting. Therefore the depth of burrowing activity used for our calculations for *A. chlorotica* (0 – 10 cm), *A. caliginosa* (0 – 10 cm) and *L. rubellus* (0 – 5 cm) was based on field observations.

Our results show that the impact of small mammals on the redistribution of heavy metals in floodplains is much smaller than that of earthworm species. This is the result of the relatively low densities of small mammals in floodplains due to the frequent inundations, and the temporary absence of small mammals in large areas of the floodplain after the water has receded, as recolonisation is a gradual and slow process (Haeck, 1969; Wijnhoven et al., 2005, 2006b). Other bioturbating small mammal species in the floodplain, like *Clethrionomys glareolus*, *Apodemus sylvaticus*, *Sorex araneus* and *Crocidura russula*, have even lower densities than *M. arvalis*, especially in the parts of the floodplains that become inundated (Wijnhoven et al., 2005, 2006b). In addition, the amounts of substrate surfaced are assumed to be smaller for those species, especially for the shrews (*S. araneus* and *C. russula*) (Lange et al., 1994). The bioturbating capacities of *Microtus agrestis* are assumed to be similar to those of *M. arvalis*, but the two species seldom co-exist. It is therefore to be expected that the amounts of substrate surfaced by Microtidae in floodplains are similar and independent of the species (or combination of species) present. In contrast, large quantities of earthworms survive the floods, resulting in bioturbation throughout the floodplain (Zorn et al., 2005).

Small mammal bioturbation activities are potentially important on a local scale; in about 6% of the research area (the 0 – 30 m zone) they were responsible for more than a quarter of the heavy metals redistributed during floods in the ADW (Table 1). In these areas, the bioturbation activity was 2.6 to 5.3 times (voles) or 4.4 to 18.6 times (moles) higher than in the other parts, on an annual basis (Table 1). The impact of other species with bioturbating capacities, like rabbits and ants, has not been investigated in this project.

Bioturbation plays a quantitatively important role in the redistribution of heavy metals in river floodplains. Part of the measured deposition by floods is related to bioturbation. In addition, 2002 was an exceptional year with two floods, whereas normally one flood per 1.3 years occurs. This means that the average annual deposition of sediment and heavy metals is generally smaller than found in 2002. When no floods occur during a particular year, the bioturbation activity of the small mammals will be considerably larger. We found that the decrease in heavy metal loads in rivers has less influence on the deposited sediment and soil profiles because of bioturbation, due to the continuous mixing of polluted and clean layers by bioturbation.

Substrate originating from the floodplain itself is largely redistributed within the floodplain itself. This is caused by the embankments, which prevent sediment transfer out of the floodplain and turn the floodplain into a settling tank, where sediment settles easily. As an essential part of several ecological rehabilitation programmes now being planned along the large European rivers, the summer dikes will be partially removed (Silva et al., 2001; Nienhuis et al., 2002). In addition, more frequent flooding is expected as a consequence of climate change (Kwadijk and Middelkoop, 1994). When flooding occurs more frequently and embankments are removed, the river will wash away most of the surfaced substrate and heavy metals. This will have a cleansing effect on the floodplain topsoils in the longer term, especially in combination with improved water quality. This will be a very slow process as substrate can be surfaced in one floodplain and settle in another floodplain downstream. Further, the results have implications for the estimation of floodplain aggradation using sediment trap results (e.g. Middelkoop, 2000; Thonon, 2006). As substantial parts of the sediment originate from the floodplain itself, aggradation will be substantially overestimated if not corrected for bioturbation activity. Also for dating of sediment layers by tracers as radionuclides or heavy metals, bioturbation activity should be taken into account (Tyler et al., 2001; Ciszewski, 2002), because tracers from layers will be mixed, and spread over a larger soil column.

5.5 Conclusions

A substantial proportion of the deposition of substrate and heavy metals during floods in embanked Rhine floodplains originates from bioturbation. Especially earthworm species are found to be important in this, due to their high density in floodplains throughout the year. The four most common earthworm species together accounted for approximately 28, 40, 18, 75 and 20% of the 4140 tons substrate, 1300 kg Zn, 370 kg Cu, 220 kg Pb and 12.5 kg Cd respectively, deposited during a flood in the embanked part of the ‘Afferdensche en Deestsche Waarden’ floodplain. Moles and voles only accounted for 0.3 to 1.1% of the share in substrate and metal deposition. On an annual basis, the amounts of substrate and heavy metals surfaced by bioturbators are much larger than the amounts of sediment and metals deposited during floods at present. Although the impact of small mammals was found to be smaller than that of earthworms, the role of especially moles cannot be neglected. Bioturbation activities of moles and voles in the periodically flooded parts on an annual basis were largest in the zone close to the non-flooding areas. In this zone (6% of the total area), 2.6 to 5.3 and 4.4 to 18.6 more substrate and metals were surfaced by voles and moles respectively, than they surfaced in the remaining parts of the floodplain. Our data imply that bioturbation may strongly affect vertical distribution profiles of contaminants.

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Chapter 6

Metal accumulation risks in regularly flooded and non-flooded parts of floodplains of the river Rhine: Extractability and exposure through the food chain

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Monitoring vegetation development in the ADW floodplains

Abstract

Ecotoxicological risks of sediment contamination in floodplains are supposed to be highest in the regularly flooding parts. Therefore in risk assessments the non-flooding parts are neglected or considered to be reference areas. We investigated the metal extractability and levels in important food sources for vertebrates, viz. grass shoots and earthworms, in flooding as well as non-flooding parts and compared those to total metal concentrations. A comparison of these areas in the moderately polluted 'Afferdensche en Deestsche Waarden' floodplains along the river Rhine showed that total Zn, Pb and Cd concentrations were highest in the regularly flooding parts. However, $CaCl_2$-extractable Zn concentrations were highest in non-flooding areas and those of Pb and Cd were equal in both areas. Total Cu concentrations were not significantly different between the two areas, but $CaCl_2$-extractable Cu concentrations were highest in the regularly flooding areas. The metal concentrations in grass shoots of non-flooding areas were equal to (Zn, Cu, Cd) or higher than (Pb) those in regularly flooding areas. Zn concentrations in earthworms in regularly flooding areas were higher, but concentrations of Cu, Pb and Cd were not. Ecotoxicological risk assessments require analysis of the total and potentially bioavailable metal concentrations in soils as well as concentrations in biota. This study shows that the less contaminated non-flooding areas in moderately polluted floodplains cannot be neglected in metal accumulation studies nor can be used as pristine reference areas.

6.1 Introduction

Due to emissions in the past, the sediments of many large European rivers and their floodplains have become polluted with a variety of organic and inorganic substances. Although the quality of the river water and suspended matter have recently improved (Vink et al., 1999), large quantities of metals (e.g. zinc, copper, lead and cadmium) are still present in the floodplain soils due to the persistent character of these pollutants and the large amounts adsorbed to suspended matter deposited in recent decades (Ciszewski, 2003; Leuven et al., 2005). It has often been hypothesised and shown that metals accumulate in the floodplain food webs, where they can cause toxic effects at different trophic levels (Balk et al., 1993; Hendriks et al., 1995; Van den Brink et al., 2003). Vertebrates (mammals and birds of prey) might be particularly at risk due to their positions in food webs. Traditionally, research on contaminant loads in substrates and biota, ecotoxicological risk assessments and floodplain management as regards contaminants have focused on the regularly flooding parts, as contaminants are largely supplied during floods. The highest total metal quantities are often found at low elevations in floodplains, or in parts with low water velocities during floods, as this is where the clayey sediments deposit (Middelkoop, 1997; Schouten et al., 2000; Kooistra et al., 2001, 2005).

It is known that the risk of heavy metal accumulation in food webs is not necessarily simply related to total metal concentrations in soils, as bioavailability, and the exposure of and uptake by biota, are also important (Houba et al., 1996; Brun et al., 1998; Conder et al., 2000; Torres and Johnson, 2001; Sahuquillo et al., 2003; Vijver, 2005). However, environmental regulations in most industrialised countries are based on total soil concentrations (an exception being Switzerland) (Visser, 1993). In ecotoxicological risk assessment, as well as for management purposes and priority assessment for sanitation, the binding capacity of the substrate is partly taken into account by including special rules for certain pH, organic matter and/or clay levels (Houba et al., 1996; Visser, 1993; Vink et al., 1999; Van Straalen, 2001). For instance, according to Dutch regulations, total metal concentrations are calculated relative to a standard soil containing 10% organic matter and

25% lutum (particles <2 μm) on a dry weight basis (Ministerie van VROM, 2000) to correct for binding capacity and hence for availability. Such calculations to determine the risk to biota probably yield useful estimations for many soil types, but the outcomes are highly uncertain in the clay-rich soil types with pH values around 7 to 8 (Vink et al., 1999; Van de Brink et al., 2003; McBride et al., 2004) which are found in the floodplains of the large lowland rivers in the Netherlands and elsewhere (Verkleij, 2000; Vijver, 2005). In these situations, soluble or extractable concentrations of metals in soils could be more relevant to the accumulation risk (Aten and Gupta, 1996; Houba et al., 1996; Pueyo et al., 2004). Moreover correlations between total and extractable concentrations might be weak or absent under those conditions. The $CaCl_2$-extractable concentration is often mentioned as a better indicator of site-specific accumulation risks than the total concentration, as it is positively related to the bioavailability of heavy metals for several organisms (Sanders et al., 1987; Posthuma et al., 1998; Vink et al., 1999).

As the non-flooding parts of floodplains are generally expected to contain lower total metal concentrations than the regularly flooding parts, these non-flooding parts are often assumed to be unimportant in ecotoxicological risk assessment. They are assumed to contain natural background concentrations (Kooistra et al., 2001), or are not taken into account at all Balk et al., 1993; Hendriks et al., 1995). However, these elevated parts may also be polluted to some extent, especially in floodplains where they consist of constructed elevations, often built from substrates excavated from the floodplain itself (Ministerie van V&W et al., 1997). In addition, these areas have no deposition of sediments rich in clay and organic matter, which would reduce the chemical and biological availability of metals. Constructed elevations, including dikes (embankments) and non-flooding parts used for industry or housing, are numerous in the semi-natural and constructed floodplains along the large lowland rivers in the Netherlands.

Since the lower parts of floodplains are frequently inundated (often more than once a year), the elevated areas function as important refuges for many animal species (Robinson et al., 2002; Van der Velde et al., 2004; Wijnhoven et al., 2005). Substantial proportions of the populations of several species are assumed to forage on or inhabit the elevated areas for at least part of their lives. Recently, it has been shown that the densities of small mammals (e.g. voles, shrews and mice) within Dutch floodplains were highest on the elevated parts throughout the year. Total numbers in the lower parts only started to exceed those on the elevated parts several months (up to more than half a year) after the floodplain fell dry following a flood (Wijnhoven et al., 2005, 2006). Small mammals are important prey species for several predators (Balk et al., 1993; Jongbloed et al., 1996; Mertens et al., 2001), which generally forage in the areas with the highest prey densities. Hence, substantial parts of the total exposure to metal pollution of several vertebrate species, will take place in the non-flooding areas. This makes it relevant to investigate to which concentrations of metals vertebrates are exposed.

To this end, we have compared the importance of the exposure of biota to metal concentrations in the topsoil of elevated non-flooding areas and regularly flooding areas. We expected that total concentrations of the investigated metals Zn, Cu, Pb and Cd would be higher in the regularly flooding parts of the floodplain than in the non-flooding areas. We investigated whether the risks of metals accumulation to vertebrates were indeed highest in these regularly flooding parts, by comparing the regularly flooding and non-flooding areas in terms of total and $CaCl_2$-extractable soil concentrations. For most vertebrates, the available fraction in soils is only indirectly relevant, as food sources form the links for accumulation. Food species deal with contaminants in various ways: various species along the food chain may excrete contaminants, concentrate them in certain tissues or immobilise them (McLaughlin, 2002; Peijnenburg, 2002). It is therefore useful to analyse heavy metal concentrations in important food sources, in addition to total or extractable soil concentrations. Two major exposure routes in the food chains of vertebrates are grasses and earthworms which are also analysed. The following research questions were addressed in the present study:

(1) Are there differences in total metal concentrations between flooding and non-flooding areas?

(2) Are there differences in potentially bioavailable concentrations between these areas?

(3) Are there differences in the metal concentrations in grasses and earthworms between regularly flooding and non-flooding areas?

The findings are discussed in relation to exposure risks of vertebrates in regularly flooding and non-flooding parts of floodplains.

6.2 Materials and methods

Research area and sampling sites

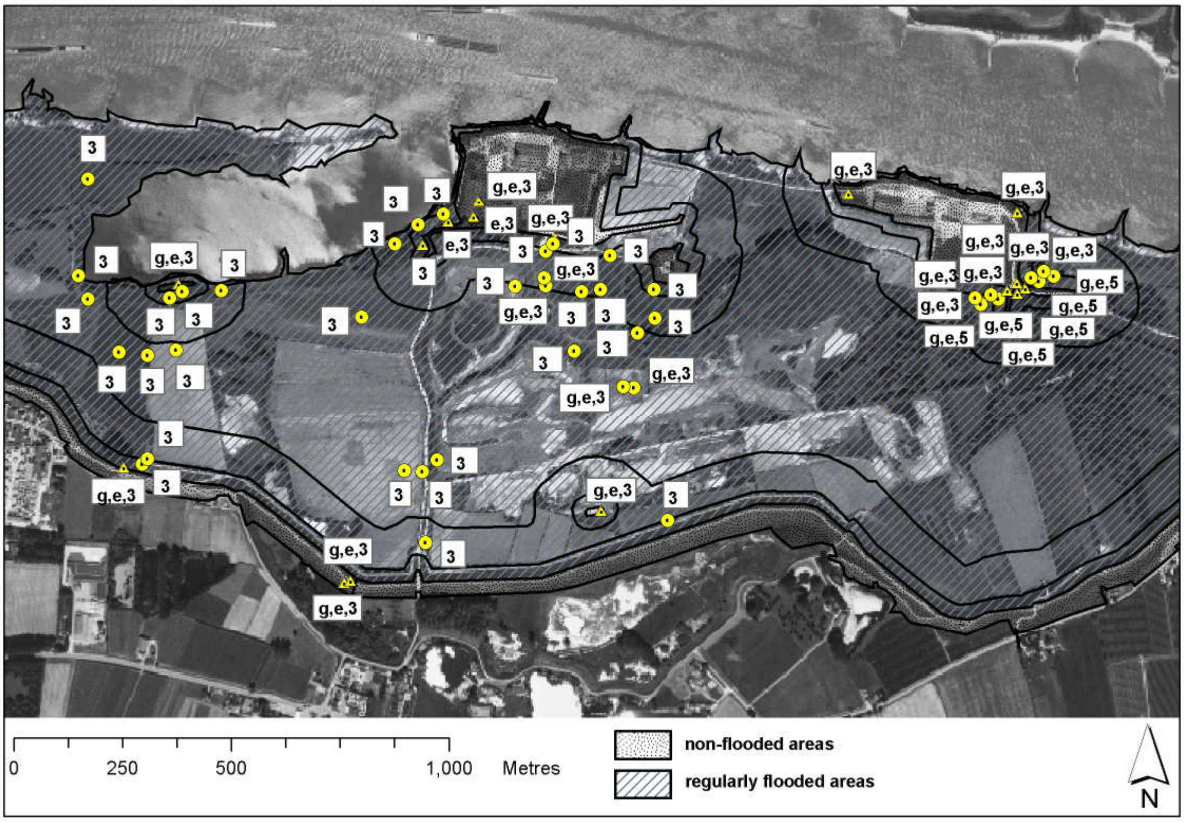

Fig. 1: Location of the sampling sites at the ‘Afferdensche en Deestsche Waarden’ (ADW) floodplains, which are bordered on the north side by the river Waal and on the south side by the outer dike. The non-flooding areas (¼ of the total area of 280 ha) are dot-shaded (sampling sites indicated by triangles), while the regularly flooding areas are hatch-shaded (sampling sites indicated by circles). The number of samples taken at each sampling site is indicated. When earthworms or grasses were sampled, this is indicated by ‘e’ and ‘g’, respectively. The digital aerial photograph was put at our disposal by the Geometric Service of the Directorate-General of Public Works and Water Management.

The research project was carried out at the ‘Afferdensche en Deestsche Waarden’ (ADW) floodplains (51˚54´N, 5˚38´E), situated 20 km west of the town of Nijmegen along the river Waal (the main distributary of the river Rhine in the Netherlands). These floodplains are characteristic of diffusely and moderately polluted floodplains in the Rhine delta, and were a focal area in an extensive ecotoxicological research programme by the Netherlands Organisation for Scientific Research (NWO-SSEO programme) (Zorn, 2004; Kooistra et al., 2005; Vijver, 2005). Between May 2002 and November 2003, soil cores were taken from the top 10 cm soil layer at 58 sampling sites (Fig. 1). These sampling sites, 16 of which were situated in non-flooding areas, were characterised by various types of vegetation, soil and land use (Wijnhoven et al., 2005). At each of the sites, three or five samples were taken with line intervals of at least 10 m. Each sample was prepared from three soil cores taken within a 1 m^2 plot. The sites were initially selected for the monitoring of small mammal distribution and recolonisation, generally based on vegetation structure, without prior information on contaminant levels. As the site selection method was similar in non-flooding and regularly flooding areas, we assumed that our sampling scheme resulted in a representative comparison of the contaminant levels, distribution and variability between the two areas. Both the non-flooding and the regularly flooding parts of the ADW floodplains included relatively sandy and more clayey sampling sites. The sandier parts are generally located in the excavated lower parts, and on the elevated grounds on which brick factories used to stand. The more clayey parts were generally the unexcavated lower parts, and the elevated dikes. All these areas were included in the sampling, because the sampling areas were randomly selected based on vegetation structure without prior knowledge about substrate parameters.

At 27 of the sampling sites (15 non-flooding and 12 regularly flooding sites), we also collected all vegetation from 625 cm^2 plots ($n \geq 3$) situated at the sites where the soil cores were taken (Fig. 1). All grass shoots from the vegetation samples were selected to obtain mixed samples of all Gramineae. The dominant grass vegetation at the sampling sites was always dominated by one or several of the following species: *Poa pratenses*, *Agrostis stolonifera*, *Elytrigia repens*, *Lolium perenne* and *Phleum pratense*. Thirteen grass samples of non-flooding areas were available, as grasses were absent from two of the sites. At the same 27 sites, all earthworms (Lumbricidae) were collected from 625 cm^2 plots ($n \geq 3$), down to a depth of 25 cm. The dominant earthworm species in the ADW floodplains are *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Allolobophora chlorotica* and *Octolasion cyaneum*, which were also found in our samples in various combinations. Also *Lumbricus terrestris* is a common species in the ADW floodplains (Zorn, 2004). However, this species was not observed in the analysed earthworm samples. All grass and earthworm samples were collected in autumn, when no flooding has occurred for more than half a year, as both season and flooding events have large effects on species compositions and age assemblages, and therefore on metal concentrations (Zorn, 2004). All soil and grass samples were stored at 5°C and treated (either oven-dried or suspended in $CaCl_2$ solution) within three days after collection. All earthworms were stored in 70% ethanol. Although preservation can have effects on dry weights (Leuven et al., 1985) and metal concentrations (Hendrickx et al., 2003), these effects should be similar for the samples from different sampling sites, as all earthworm samples were treated in the same way.

Analyses and measurements

We measured the moisture content, organic matter content, clay-silt to sand ratio, pH_{CaCl2}, total metal (Zn, Cu, Pb and Cd) concentrations and 0.01 M $CaCl_2$-extractable metal concentrations in all soil samples. The moisture content was determined by drying 5 g of wet soil (FW) for 24 h at 105°C to measure the dry weight (DW). The moisture content (%) was subsequently calculated as ((FW-DW)×100)/DW. The organic matter content (OM) was determined by scorching the dry soil for 4 h at 550°C, after which the mineral weight (MW) was measured; OM (%) = ((DW-MW)×100)/DW. The clay-silt to sand ratio was estimated by adding 50 ml of 35% H_2O_2 to 10 g dry soil, to disrupt particle aggregations by digesting $CaCO_3$ and organic matter. After two days of incubation, the suspension was boiled while adding distilled water to keep the substrate in suspension. Sieving over 53 µm mesh separated the clay-silt and sand fractions, and the suspensions were dried at 105°C, after which the fractions were weighed.

Total metal concentration of the soil was measured after microwave extraction (using a Milestone 1200 microwave oven) of 0.2 mg DW substrate in a mixture of 3.0 ml 65% HNO_3 and 1.5 ml 37% HCl. The samples were topped up to 50 ml, after which the metal concentration was measured using Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES). The 0.01 M $CaCl_2$-extractable fraction was determined as a measure of the potential metal availability. Six grams (FW) of substrate was mixed with 0.01 M $CaCl_2$ for two hours in a soil:solution ratio of 1:10, after which the suspension was centrifuged at 12 000 rpm (5000 g) for 15 minutes. After pH_{CaCl2} had been measured in the substrate suspension in 0.01 M $CaCl_2$, the supernatant was filtered over 0.45 µm mesh. A pH of 2 was obtained with a few droplets of 65% HNO_3, and the metal content of the sample was subsequently measured on the ICP-AES.

The grass shoot samples were oven-dried at 70°C, after which they were cut into small pieces, ground in liquid nitrogen and homogenised. The earthworm samples were also oven-dried at 70°C. Approximately 0.2 g of grass shoots per sample, and the entire earthworm samples (ranging between 0.2 and 0.6 g) were digested and analysed for metal concentrations as described for the soil samples.

Calculations and statistics

We calculated the location-specific reference value for the total metal concentrations in the soil, which is common practice for standard ecotoxicological risk assessment and priority determination for soil management purposes. This means taking availability into account by compensation for the binding capacity of the substrate. The location-specific reference value was calculated correcting for organic matter and lutum contents (Crommentuijn et al., 1997; Vink et al., 1999; Ministerie van VROM, 2000):

$$RV_{ls} = \frac{RV_{ss} \times (a + (b + L) + (c \times OM))}{a + (b \times 25) + (c \times 10)}$$

RV_{ls} = location-specific reference value for a metal (mg kg^{-1} DW); RV_{ss} = reference value of a metal for standard soil (mg kg^{-1} DW); L = % clay-silt (<53 µm) of DW; OM = % organic matter of DW; a, b, c = metal-specific values (‘a’ values are 50, 15, 50 and 0.4; ‘b’ values are 3, 0.6, 1 and 0.007; ‘c’ values are 1.5, 0.6, 1 and 0.021, for Zn, Cu, Pb and Cd respectively). Reference values used for standard soil were: 140 (Zn), 36 (Cu), 85 (Pb) and 0.8 (Cd) mg kg^{-1} DW (Vink et al., 1999; Van Straalen, 2001), at an organic matter content of 10%, and a lutum content of 25%. Instead of the lutum content (<2 µm), we used the clay-silt content in the calculations of the location-specific reference value, which yields higher values. However, we assume that there is a positive correlation between the lutum and the clay-silt content.

To assess correlations between the potentially bioavailable metal concentrations and the total metal concentrations and soil parameters, a principal component analysis (PCA) was performed on the $CaCl_2$-extractable concentrations, after the gradient length for the dataset had been specified by means of a detrended correspondence analysis (DCA) using the Canoco for Windows software package (version 4) (Ter Braak and Smilauer, 1998) including all individual samples (n=186). To confirm the indications shown by the PCA, we executed multiple regressions on the log-transformed data using the stepwise method for each of the metals. As flooding is a categorical variable the way we measured it, this variable was excluded from the multiple regressions (results not shown). As grass shoots and earthworms were not collected at all of the sampling sites, we tested the homogeneity of variances using Levene statistics and possible differences in average total or $CaCl_2$-extractable concentration of metals and soil parameters using one-way ANOVA's between the sub-set of sampling sites where these biota were collected and the total set of sampling sites, using SPSS 11.5 for Windows. Average values and variances of metal concentrations and soil parameters were compared between regularly flooding and non-flooding areas with t-test and F-test, using the average values per sampling site.

6.3 Results

Soil characteristics

For none of the soil parameters or metal concentrations tested were significant differences ($p \geq 0.05$) found between the sub-set of sampling sites where biota were collected and the total dataset of sampling sites. This was true both for regularly flooding and non-flooding areas. This means that the sub-sets were representative, in terms of average and variance, of the total datasets, for all parameters. The pH_{CaCl2} was significantly higher (t-test; $p<0.001$) in the regularly flooding areas than in the non-flooded areas (Table 1). The organic matter content was significantly higher (t-test; $p<0.05$) in the flooding areas (based on the total dataset). The variation in the observed moisture content in particular was found to be higher in the regularly flooding areas than in the non-flooding areas (f-test; $p<0.05$). The total metal concentrations in the soil were significantly higher in the flooding areas than in the non-flooding areas for all metals, except for Cu in the sub-set comparison. Differences in the $CaCl_2$-extractable concentrations were found for Zn, with a higher concentration ($p<0.001$) in the non-flooding areas, and for Cu, with a higher concentration ($p<0.05$) in the regularly flooding areas.

Table 1: Characterisation and comparison of sets of soil samples (and sub-sets of samples from which grass and earthworm samples were taken or not) taken in the ADW floodplains. Average values ± standard deviation for soil parameters and metal concentrations are shown, based on the average values per sampling site. Significant differences in variance (f) and average (t) value between non-flooding and flooding areas are shown for the total sample set and the sub-set of samples, with * $p<0.05$, ** $p<0.01$, *** $p<0.001$, - not significant ($p \geq 0.05$).

	Non-flooding		Flooding		f-test		t-test	
	Total (n=16)	Sub-set of sample sites (n=15)	Total (n=42)	Sub-set of sample sites (n=12)	Total	Sub-set	Total	Sub-set
OM (%)	6.05 ± 2.65	6.19 ± 2.68	8.22 ± 3.77	8.26 ± 4.74	-	*	*	-
Clay (%)	49.4 ± 20.7	51.2 ± 20.1	53.6 ± 19.6	49.8 ± 27.1	-	-	-	-
pH_{CaCl2}	7.08 ± 0.27	7.05 ± 0.23	7.41 ± 0.12	7.38 ± 0.15	***	-	***	***
Moisture (%)	23.1 ± 5.27	23.1 ± 5.27	28.3 ± 12.2	28.3 ± 12.2	*	*	-	-
$[Zn]_{tot}$ (mg kg^{-1})	218 ± 148	222 ± 152	441 ± 282	395 ± 265	**	-	***	*
$[Zn]_{CaCl2}$ (mg kg^{-1} 10)	11.6 ± 4.91	11.8 ± 4.95	2.87 ± 2.05	2.99 ± 2.36	***	**	***	***
$[Cu]_{tot}$ (mg kg^{-1})	30.9 ± 21.0	30.9 ± 21.7	61.0 ± 36.9	51.8 ± 39.0	*	*	***	-
$[Cu]_{CaCl2}$ (mg kg^{-1} 10^3)	63.6 ± 34.6	64.4 ± 35.6	94.8 ± 52.6	114 ± 59.3	-	-	*	*
$[Pb]_{tot}$ (mg kg^{-1})	79.9 ± 42.2	81.2 ± 43.4	138 ± 85.1	130 ± 74.9	**	-	**	*
$[Pb]_{CaCl2}$ (mg kg^{-1} 10)	3.27 ± 3.62	2.70 ± 2.94	3.69 ± 2.56	4.20 ± 2.43	-	-	-	-
$[Cd]_{tot}$ (mg kg^{-1})	1.34 ± 0.97	1.36 ± 1.00	2.75 ± 2.24	3.75 ± 3.08	***	***	**	*
$[Cd]_{CaCl2}$ (mg kg^{-1} 10)	18.8 ± 17.4	15.9 ± 13.4	21.4 ± 14.8	25.6 ± 13.7	-	-	-	-

Corrected metal concentrations relative to site-specific reference values

Total concentrations of each of the metals in the top 10 cm layer varied across the sampling sites in the ADW floodplains. The average concentrations for each sampling site for Zn ranged from 57 to 1166 mg kg^{-1} DW, those for Cu from 7.9 to 147 mg kg^{-1} DW, those for Pb from 13 to 359 mg kg^{-1} DW and those for Cd from 0.07 to 8.3 mg kg^{-1} DW. The metal concentrations at many sampling sites exceeded the reference values (Fig. 2). This was the case at 64, 41, 55 and 72% of the sites for Zn, Cu, Pb and Cd, respectively. For Cu and Pb, these were all, except for one, regularly flooding sampling sites. All sampling sites situated to the right side of the graph for Zn and Cd were also from regularly flooding areas. The values for sampling sites in the non-flooding areas were generally situated to the left side of the plot, which means that only 33, 7, 7 and 67% of the sampling sites were above the reference values for Zn, Cu, Pb and Cd, respectively. A comparison of the $CaCl_2$-extractable concentrations shows that the positions in the plot of the sampling sites in the non-flooding areas were similar to those of the regularly flooding areas for Pb and Cd (Fig. 2; Table 1). The $CaCl_2$-extractable concentrations for Zn were actually higher for the non-flooding sampling sites than for the regularly flooding sampling sites.

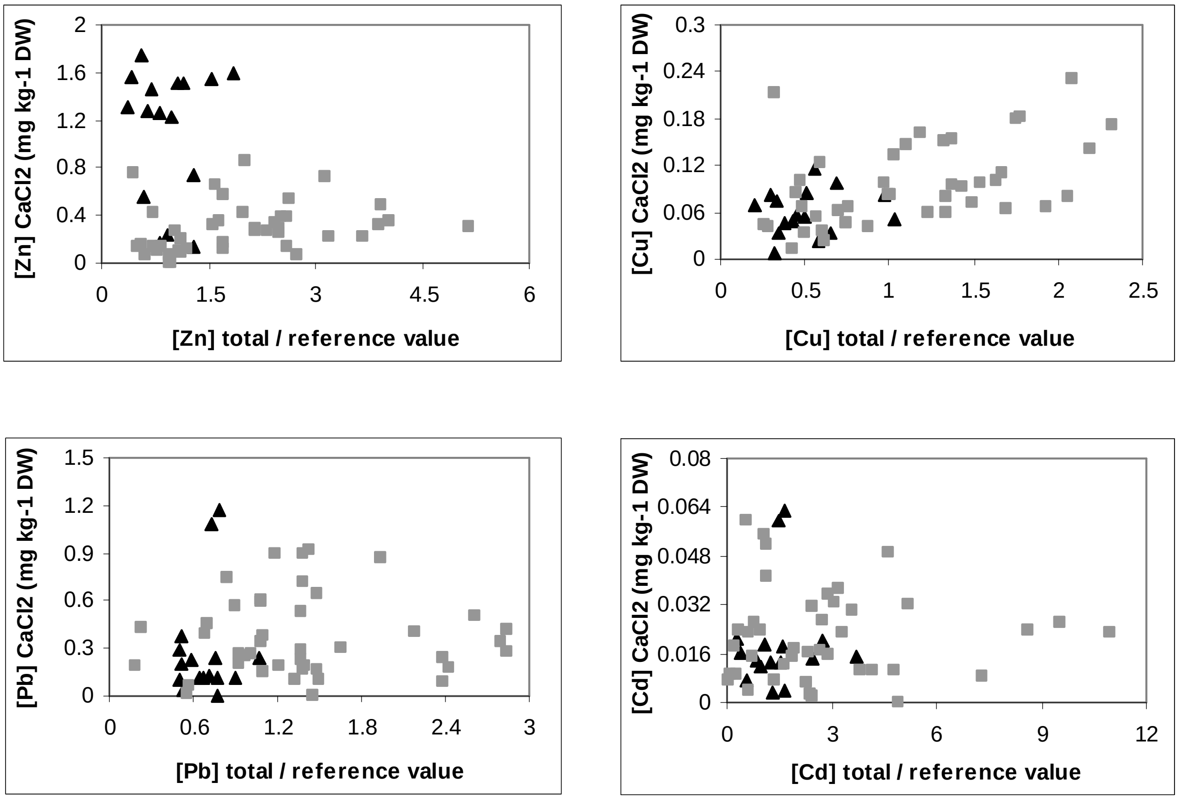

Fig. 2: Scatter plots showing the $CaCl_2$-extractable concentration (in mg kg^{-1} DW) of each of the metals (Zn, Cu, Pb and Cd) related to the total metal concentration divided by the location-specific reference value. This reference value is the background value of each metal for the Netherlands corrected for the organic matter and lutum contents. The non-flooding sampling sites are indicated by black triangles, the regularly flooding sampling sites by grey squares.

Metal concentrations related and compared

The principal component analysis (PCA) shows that the $CaCl_2$-extractable Zn concentrations are almost entirely explained by the horizontal axis, an aggregation of the 'flooded' and pH parameters (Fig. 3). The higher $CaCl_2$-extractable Zn concentrations were found at the non-flooding sites, and a negative correlation with pH was found. The total Zn concentration, a parameter more dominant in the vertical axis was of no importance for the $CaCl_2$-extractable Zn concentrations. The $CaCl_2$-extractable Pb concentrations appeared to be positively correlated to the total concentration, while relations for the other metals (Cu and Cd) were weaker. The results for Zn and Pb were confirmed by multiple regression analyses.

Significant correlation was found between the $CaCl_2$-extractable concentrations and the independent variables for which the t-values of the independent variables were largest for the above mentioned variables. An exception is the factor 'flooded' for Zn, as we did not include this factor in the multiple regressions. This factor is in the significant multiple regression equation partly substituted by clay (-) and $[Zn]_{tot}$ (+).

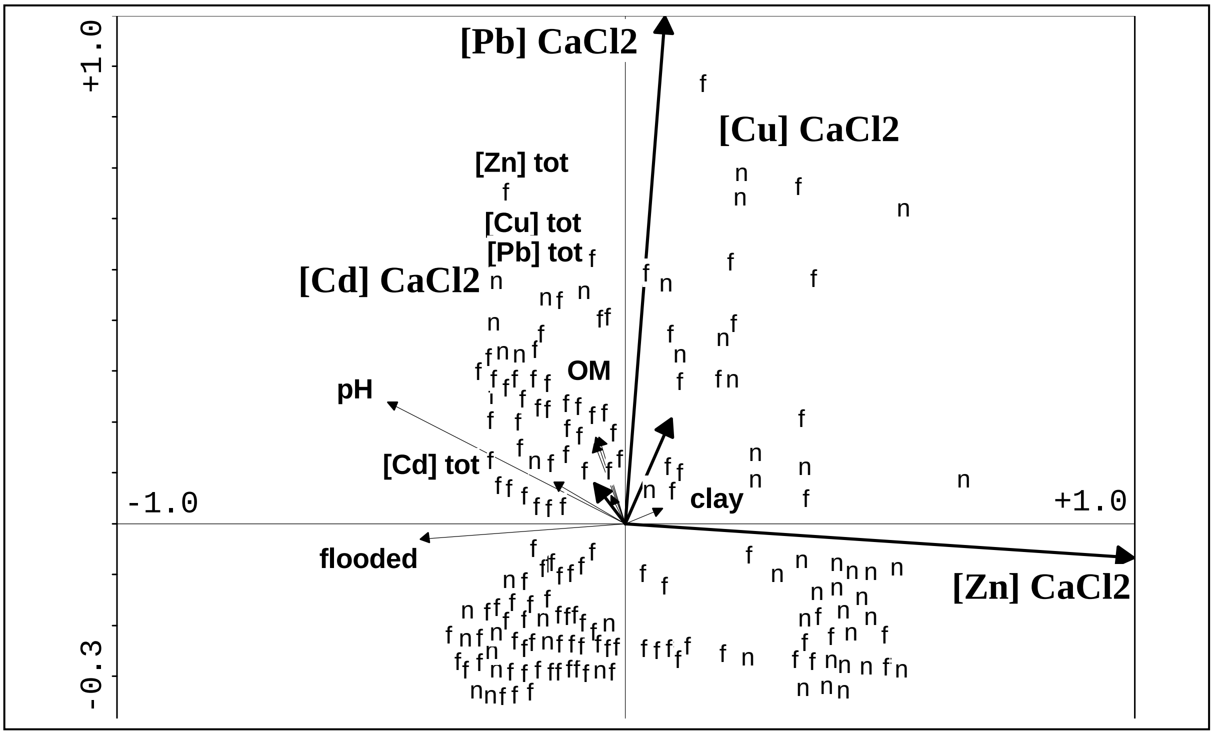

Fig. 3: Principal Component Analysis (PCA) of the $CaCl_2$-extractable soil concentrations ($[Me]_{CaCl2}$) of 186 samples. Explanatory variables are $[Me]_{tot}$ = total metal concentration in the soil, shown for Zn, Cu, Pb and Cd; OM = organic matter content; clay = clay content (<50 µm); pH = pH_{CaCl2}, flooded = parameter which equals 1 for regularly flooding sampling sites (f) and 0 for non-flooding sampling sites (n).

In general, organic matter and clay content appeared to be poor indicators of the presence of high $CaCl_2$-extractable concentrations. Figure 3 also shows that for Zn, the non-flooding sampling sites are clustered at the high $CaCl_2$-extractable concentrations, while the flooding sampling sites are clustered at the low $CaCl_2$-extractable Zn concentrations. However, several sampling sites show the opposite positioning in the graph, and trends are less clear for the other metals.

The total metal concentrations were significantly ($p<0.05$) higher in the regularly flooding areas than in the non-flooding areas (Fig. 4; Table 1). This difference remained when we related the total concentrations to the calculated location-specific reference values. The average uncorrected metal concentrations were close to the corrected reference values, but average Zn and Cd concentrations in regularly flooding areas were, respectively, 1.9 and 4.1 times the location-specific reference values. Of the $CaCl_2$-extractable metal concentrations, only those for Cu were significantly higher in the regularly flooding areas than in the non-flooding areas ($p<0.05$). The opposite was found for Zn, with significantly higher $CaCl_2$-extractable concentrations ($p<0.001$) in non-flooding areas than in regularly flooding areas. The metal concentrations in grass shoots were similar in non-flooded and regularly flooded areas, except for the lead concentrations, which were higher in non-flooding areas ($p<0.05$). Significant differences in metal concentrations in earthworms were only found for Zn, with the highest concentrations in the earthworms from regularly flooding areas ($p<0.05$).

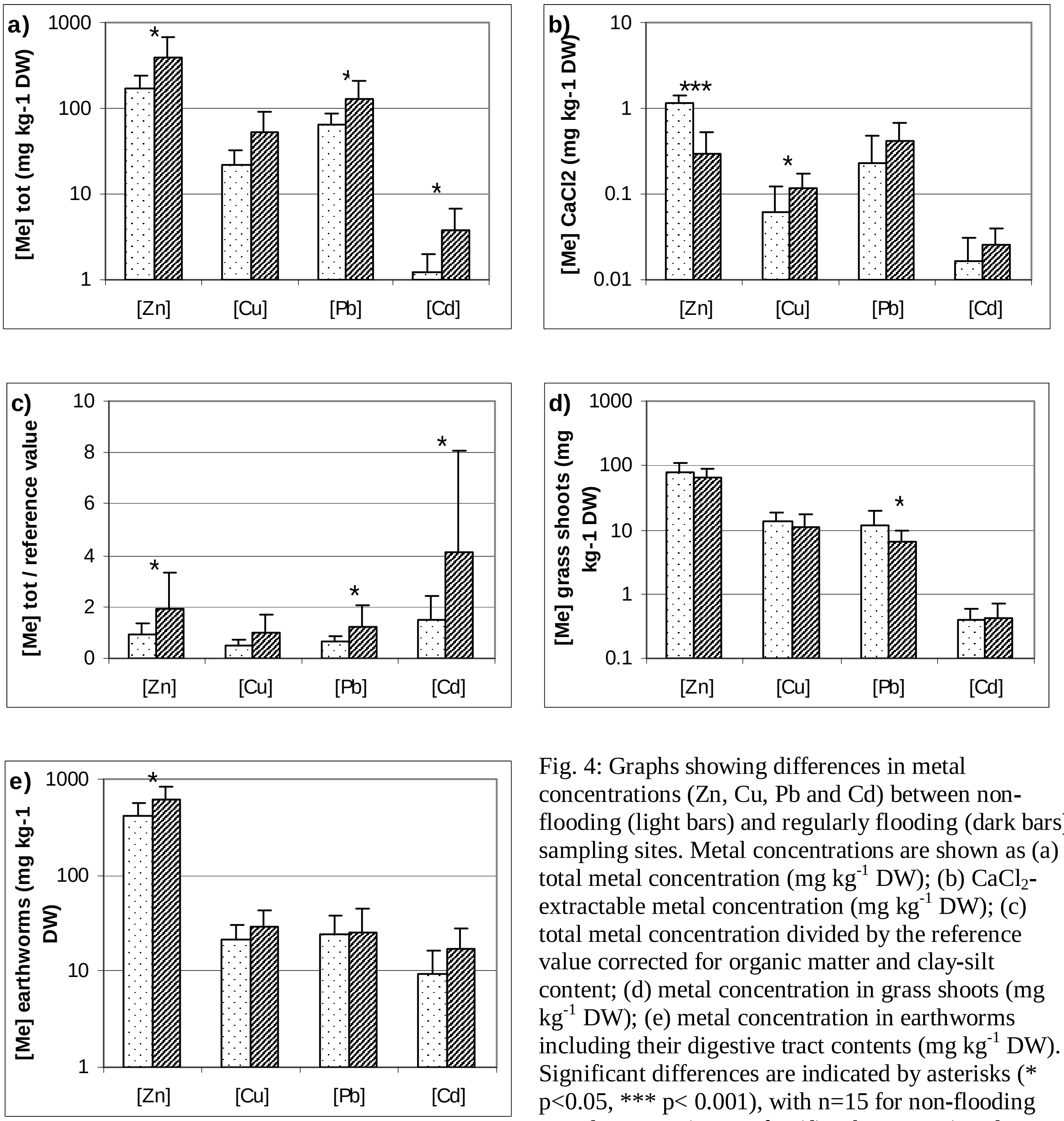

Fig. 4: Graphs showing differences in metal concentrations (Zn, Cu, Pb and Cd) between non-flooding (light bars) and regularly flooding (dark bars) sampling sites. Metal concentrations are shown as (a) total metal concentration (mg kg^{-1} DW); (b) $CaCl_2$-extractable metal concentration (mg kg^{-1} DW); (c) total metal concentration divided by the reference value corrected for organic matter and clay-silt content; (d) metal concentration in grass shoots (mg kg^{-1} DW); (e) metal concentration in earthworms including their digestive tract contents (mg kg^{-1} DW). Significant differences are indicated by asterisks (* $p<0.05$, *** $p< 0.001$), with n=15 for non-flooding sampling sites (except for (d), where n=13) and n=12 for regularly flooding sampling sites.

6.4 Discussion

Heavy metals are not distributed homogeneously over floodplains, and human interference in the form of constructions and excavations has increased the variability in soil metal concentrations in floodplains even more (Kooistra et al., 2001, 2005). Our measurements confirm that the ADW floodplains are moderately polluted, as the average topsoil concentrations for all metals were below the testing level (Classes 0-2 according to the Dutch soil quality standards (Vink et al., 1999; Vijver, 2005). Only three sampling sites were Class 3 contaminated, that is, had contaminant levels between the testing and intervention levels. Two of these sites were contaminated with Cd, one with Zn. Our comparison of the regularly flooding and non-flooding sites showed that in spite of the large variation in total metal concentrations within both areas, the regularly flooding areas were significantly more polluted with Zn, Pb and Cd than the non-flooding areas (Fig. 4). For all metals, including Cu, the most polluted sites were situated in the regularly flooding areas (Fig. 2).

When metal pollution is the result of deposition of contaminated sediments, the most contaminated areas generally also contain higher clay and organic matter contents (Middelkoop, 1997). As these metal-binding substances reduce the chemical availability of metals, and therefore possibly the bioavailability, we compared the regularly flooding and non-flooding areas relative to the site-specific reference values used in the Dutch system of soil legislation (Vink et al., 1999; Van Straalen et al., 2001). This correction yielded results similar to those obtained with total soil concentrations. This means that, in agreement with risk assessment studies and the focus of policy making, any ecotoxicological effects are expected to be generally the result of exposure of biota in the regularly flooding areas, rather than in non-flooding areas.

On the other hand, the $CaCl_2$-extractable soil concentrations did not show a significant positive correlation with the total metal concentrations divided by the location-specific reference values (Fig. 2; Fig. 3). Only for Cu might there be a positive trend, but Zn showed the opposite tendency, with the highest $CaCl_2$-extractable concentrations at the sites with the lowest total concentrations. The sites with the highest $CaCl_2$-extractable Zn concentrations were all situated in the non-flooding areas.

Other factors than the total concentration might be more important for the potential bioavailable concentration of heavy metals in moderately polluted floodplain soils. For Zn in particular, pH might be of importance (Fig. 3), as pH was significantly lower in the non-flooding areas than in the regularly flooding areas (Table 1). As regards Zn, Pb and Cd, the non-flooding areas showed a wide range of values, including the highest levels, of $CaCl_2$-extractable concentrations, even though the total concentrations were significantly lower (Fig. 2; Fig. 4). This indicates that either cleaner substrates were used for the construction of the non-flooding terrains, or that there was little or no input of contaminants after the construction of the non-flooding areas. At several elevated locations, however, the conditions were different in such a way that the percentage chemically available metals of the total concentration were higher than in the flooding areas. This might be due to drier substrates and slightly lower organic matter contents (Table 1), but this could not be verified by our data. Another reason might be a weaker influence of river water (pH around 8) with its pH-stabilising effect, and a greater influence of rainwater (pH values around 5-6; Stolk, 2001), but this hypothesis was not tested in this study.

Two important exposure routes to heavy metals in floodplains for first-, second- or third-order consumers in food webs are those via vegetation and via earthworms (Van den Brink et al., 2003; Zorn et al., 2005). The metal concentrations in the grass shoots were not higher in the regularly flooding areas than in the non-flooding areas, and lead concentrations in the grass shoots were even highest in the non-flooding areas. Hence, the risk of heavy metal accumulation in vegetation and herbivorous species feeding on aboveground plant parts seems to be at least similar, or even higher, in the non-flooding areas than in the regularly flooding areas. On the other hand, the metal concentrations observed in grass shoots were generally lower than the lowest recorded critical tissue concentrations for vegetation (Verkleij et al., 2000). The differences in $CaCl_2$-extractable concentrations of Zn and Cu observed in the soil were not reflected in the vegetation. This could be the result of $CaCl_2$-extractable concentrations not being very high, as is generally the case in Dutch floodplain soils. In addition, Zn and Cu, essential elements for plant species, are probably regulated to a certain extent (Lorenz et al., 1997; McLaughlin, 2002). Our measurements cannot explain the higher Pb concentrations in grass shoots from non-flooding areas, though poor correlations between salt extractions and Pb uptake by vegetation have been recorded before (Aten and Gupta, 1996; McLaughlin, 2002).

As we were interested in exposure and accumulation risks to vertebrates, we analysed heavy metal concentrations in earthworms. We included the substrate in their digestive tract, as species preying on earthworms will be exposed to the metal burdens in the bodies of the earthworms plus the amounts in the digestive tracts. The differences between flooding and non-flooding areas in metal concentrations in earthworms were not as large as those observed for the total soil concentrations, and were only significant for Zn. The risk of accumulation of heavy metals in predators of earthworms thus seems to be higher in regularly flooding areas than in non-flooding areas, especially for Zn, but did not differ as much as would be expected from the total soil concentrations. It has been established that heavy metal uptake generally takes place through the skin of earthworms, and should therefore be related to pore water concentrations (Spurgeon and Hopkin, 1996; Peijnenburg et al., 1997; Vijver et al., 2003; Vijver, 2005). $CaCl_2$-extractable concentrations should thus reflect the accumulation risk for earthworms better than total concentrations. However, as we analysed earthworms containing substrate in their digestive tracts, the risk of accumulation of heavy metals in the earthworms themselves is probably similar in the non-flooding areas as in regularly flooding areas.

Total metal concentrations can also be relevant to direct exposure of vertebrates. Several species ingest substantial amounts of soil in floodplains and are thus exposed to the total concentrations in their digestive tracts. Herbivores, for instance, will ingest certain amounts of substrate attached to plant materials (especially to the roots but also the above-ground parts). Insectivores and carnivores ingest substrate attached to subterranean preys, to preys killed on the ground and to furs, or substrate ingested with the water they drink or substrate that may be present in the digestive tracts of prey species, as is the case with preying on earthworms. It will depend on their internal environment which fraction is available to the organism itself and whether a weak or strong extraction method reflects the availability of heavy metals ingested with soil.

In this study, the soil was sampled by taking cores from depths of 0-10 cm. For most species, this is the relevant contact zone in which exposure or uptake takes place in the case of direct exposure. This soil layer is, however, not always the most polluted layer. It has been shown that the most polluted layers in several floodplains can nowadays be found at depths between 10 and 25 cm (Ciszewski, 2003; Zorn, 2004). Grasses root to depths of below 10 cm (Verkleij et al., 2000), and some variation in the vertical distribution of pollution occurs, introducing some variation in the relation with the concentrations measured in soil and grass. In addition, the rooting depth of grasses is probably influenced by soil characteristics. Similar aspects may have played a role in the heavy metal concentrations we measured in earthworms (Peijnenburg, 2002). Most earthworms and species are active in the top layer, but there will also be some exposure below 10 cm (Zorn, 2004). The earthworm species composition and the depths at which they are active are especially influenced by the presence of vegetation, soil characteristics and the groundwater table (Zorn et al., 2005).

A comparison between metal concentrations in soil and relatively immobile biota such as plants and earthworms is justified because heavy metal exposure of them will take place in the immediate vicinity of the sampling sites (Mertens et al., 2001). To minimise the risk that any difference in concentrations in plants we found between regularly flooding and non-flooding areas is due to variations in heavy metal uptake between plant species, we collected only Gramineae. Most factors influencing the metal concentrations in grass shoots, such as the species, the age of the plants and metal metabolism with storage in different parts, were assumed to be more or less similar between the sampling sites. We also assumed this for earthworms, realising that a certain variation in species metabolism differences and age distributions will be present.

Focussing floodplain risk assessments and soil management for metal contamination on the regularly flooding parts alone means that an important part of the exposure risk in food webs is ignored. Calculations assuming background levels of metals in non-flooding areas, or the assumption that risks are minimal as total concentrations are much lower than in regularly flooding areas, underestimate the risks to several floodplain species, at least in moderately polluted floodplains. This is even truer in view of the recent finding that important terrestrial species in the floodplain food webs are exposed for extensive periods mainly in the non-flooding areas, since that is where they are most numerous (Wijnhoven et al., 2005, 2006). As $CaCl_2$-extractable concentrations of Zn were higher in the non-flooding areas than in the regularly flooding areas, and there were only minor differences in Pb and Cd concentrations, it is especially the ecotoxicological risks for plant species, herbivores and predators foraging on herbivores which may be underestimated if these areas are not taken into account in risk assessments and floodplain soil management. This is supported by the similar metal concentrations, and even higher Pb concentrations in grass shoots from non-flooding areas as in those from the regularly flooding areas. Even the ecotoxicological risk to the food chains based on earthworms and insects should be reconsidered, as an important part of the exposure may also take place in non-flooding areas, and differences in accumulation appear to be not as large as expected from the total soil concentrations of Cu, Pb and Cd. The regularly flooding areas in moderately polluted floodplains probably only offer significantly higher ecotoxicological risks for vertebrates when they ingest soil in substantial amounts.

6.5 Conclusions

Our results show that, as expected, the regularly flooding parts of the ADW floodplains have significantly higher total Zn, Pb and Cd concentrations in the top soils than the non-flooding areas. The $CaCl_2$-extractable Zn concentrations are, however, significantly higher in the non-flooding parts, whereas the $CaCl_2$-extractable Pb and Cd concentrations are not significantly different between the two areas. Significant differences in Cu were only found for the $CaCl_2$-extractable concentrations, which were highest in the regularly flooding areas. As regards soil parameters, the pH was significantly lower in the non-flooding areas than in the regularly flooding areas, which might partly explain differences in availability of metals. Our comparison of major exposure routes for food webs showed that the metal concentrations in grass shoots were similar in the regularly flooding and non-flooding areas, while those for Pb were actually significantly higher in the non-flooding areas. Significant differences in metal concentrations in earthworms, analysed including their digestive tract contents, were only detected for Zn. The present study doubts the usefulness of corrected total metal concentrations in contaminated floodplain soil policies, in spite of the correction for binding capacity (lutum and OM content). Risk assessments and management of heavy metal contamination in floodplains should consider both total and extractable concentrations in the soil and concentrations in biota. Furthermore, the study shows that exposure to metals in non-flooding areas of moderately polluted floodplains cannot be neglected.

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Chapter 7

Heavy metal concentrations in small mammals from a diffusely polluted floodplain: Importance of species- and location-specific characteristics

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Taking the length of a Short-tailed field vole *Microtus agrestis* by Aafke Schipper

Abstract

The soil of several floodplain areas along large European rivers shows elevated levels of heavy metals as a relict from past sedimentation of contaminants. These levels may pose risks of accumulation in food webs and toxicological effects on flora and fauna. However, for floodplains data on heavy metal concentrations in vertebrates are scarce. Moreover these environments are characterised by periodical flooding cycles influencing ecological processes and patterns. To investigate whether the suggested differences in accumulation risks for insectivores/carnivores, omnivores and herbivores are reflected in the actual heavy metal concentrations in the species, we measured the current levels of Zn, Cu, Pb and Cd in 199 specimens of seven small mammal species (voles, mice and shrews) and in their habitats in a diffusely polluted floodplain. The highest metal concentrations were found in the insectivorous/carnivorous shrew *Sorex araneus*. Significant differences between the other shrew species *Crocidura russula* and the vole and mouse species was only found for Cd. The Cu concentration in *Clethrionomys glareolus*, however, was significantly higher than in several other vole and mouse species. To explain the metal concentrations found in the specimens, we related them to environmental variables at the trapping locations and to certain characteristics of the mammals. Variables taken into account were soil total and $CaCl_2$-extractable metal concentrations at the trapping locations; whether locations were flooding or non-flooding; the trapping season; and the life stage, sex and fresh weight of the specimens. Correlations between body and soil concentrations and location or specimen characteristics were weak. Therefore we assume that exposure of small mammals to heavy metal contamination in floodplains is significantly influenced by exposure time, which is age related, as well as by dispersal and changes in foraging and feeding patterns, under influence of periodical flooding.

7.1 Introduction

Industrial and communal wastewater discharges and agricultural activities have caused large-scale soil contamination of a majority of the floodplains along the large European rivers (Nienhuis et al., 1998). The heavy metals cadmium, copper, lead and zinc are present in large amounts in these diffusely polluted floodplains (Middelkoop and Van Haselen, 1999; Vink et al., 1999). Several studies have suggested that the present contaminant levels pose risks to floodplain ecosystems, through accumulation of heavy metals in food webs and possible toxicological effects in a variety of species (Hendriks et al., 1995; Van den Brink et al., 2003; Kooistra et al., 2001, 2005; Leuven et al., 2005).

Studies of contaminant levels in floodplain species have been scarce, and generally focused on lower trophic levels, like vegetation (Schröder, 2005) and macro-invertebrates like earthworms, snails, spiders and insects (Hobbelen et al., 2004; Notten et al., 2005; Van Vliet et al., 2005). Exceptions are studies including the Common shrew *Sorex araneus* (Hendriks et al., 1995), the Little owl *Athene noctua vidalli* (Van den Brink et al., 2003) and the European badger *Meles meles*, which were expected to forage in floodplains (Van den Brink and Ma, 1998). Assessments of the risk of contaminant accumulation in vertebrates (e.g. mammals and birds) in floodplains have generally been based on soil contaminant levels combined with accumulation factors, consumption rates and life expectancies (Jongbloed et al., 1996; Pascoe et al., 1996; Kooistra et al., 2001, 2005). Except for the soil contaminant levels, these parameters for the calculation of accumulation risks are generally derived from literature data from inland area or laboratory studies. However, floodplains are highly dynamic environments, with periodical flooding affecting species distribution, life expectancy and mortality, habitat suitability patterns within the landscape, food availability and recolonisation processes from the non-flooding areas (Robinson et al., 2002; Klok et al., 2006; Wijnhoven et al., 2006).

Small mammals (voles, mice and shrews) play an important role in floodplain food webs, acting at different trophic levels. They include predominantly herbivorous as well as insectivorous/carnivorous species. They are prey to a whole range of predatory mammals and birds of prey (Erlinge et al., 1983; Jongbloed et al., 1996). Since these small mammal species can be numerous in certain areas within the floodplains, and are often mentioned as species at risk of toxicological effects of the current contaminant levels in floodplains themselves, they are suitable as monitors of contaminant levels in floodplain ecosystems. It has been shown that the distribution and densities of the common small mammal species are highly influenced by flooding events, resulting in the highest densities throughout the year occurring on and near the non-flooding areas (Wijnhoven et al., 2005, 2006). Densities were also found to be much higher at the end of summer and in autumn, compared with winter and spring. This is a result of the gradual population growth afterwards increased mortality in winter especially during floods, which is reflected in the age distribution. Total metal concentrations in the soil are generally higher in the lower, frequently flooding areas, than in the non-flooding areas, but $CaCl_2$-extractable concentrations do not show a similar pattern (Wijnhoven et al., 2006). It is assumed that metal concentrations in small mammals are reflected not only by trophic level but also by the exposure time and metal concentrations in the exposure areas (Hunter et al., 1989; Torres and Johnson, 2001). Furthermore, different contaminant levels in small mammal species can occur because of seasonal variation (Greville and Morgan, 1989; Ma et al., 1991) as well as differences in size and sex (Dodds-Smith et al., 1992; Damek-Poprawa and Sawicka-Kapusta, 2004). We hypothesized that, of the seven common small mammal species in our research area (Wijnhoven et al., 2005), the highest average metal concentrations would be found in the insectivores/carnivores, (e.g. Common shrew *Sorex araneus* and White-toothed shrew *Crocidura russula*), while the lowest ones were expected in predominantly herbivorous species (e.g. Common vole *Microtus arvalis*, Short-tailed field vole *Microtus agrestis*, Wood mouse *Apodemus sylvaticus* and Harvest mouse *Micromys minutus*), with the more omnivorous Bank vole *Clethrionomys glareolus* in between. Species-specific metal concentrations were expected to be positively related to the metal concentrations in the soil of the trapping locations. We investigated whether these patterns of species- and location-related body metal concentrations were present in a diffusely polluted floodplain. The study tried to answer the following research questions:

(1) Are there differences in average metal concentrations between species and can these differences be explained by feeding behaviour (herbivory, omnivory and/or insectivory/carnivory)?

(2) Are such differences in metal concentration between species similar for each of the investigated metals?

(3) Are the possible interspecific and intraspecific differences in body metal concentrations related to soil metal concentrations or $CaCl_2$-extractable concentrations at the trapping locations?

(4) Are there intraspecific differences in metal concentrations which may be related to sex, life stage or size of the animals, the time of the year or the positioning of the traps?

The article discusses the consequences of our findings for heavy metal exposure and accumulation risks in floodplain food webs.

7.2 Materials and methods

Data collection

All data were collected at the ‘Afferdensche en Deestsche Waarden’ (ADW), a moderately to heavily polluted embanked floodplain area along the river Waal, the main distributary of the Rhine in the Netherlands (Fig. 1). The research area consists of lands inside and outside the summer dikes. The summer dikes are the lower inner embankments protecting agricultural areas in the floodplain against summer floods. Large parts of the floodplain are periodically flooded, on average once a year, predominantly in winter. The floodplain includes areas with and without agricultural activities. Those without agriculture feature naturally developed vegetation and offer a wide range of habitats. Detailed descriptions of the research area are given in Wijnhoven et al. (2005, 2006).

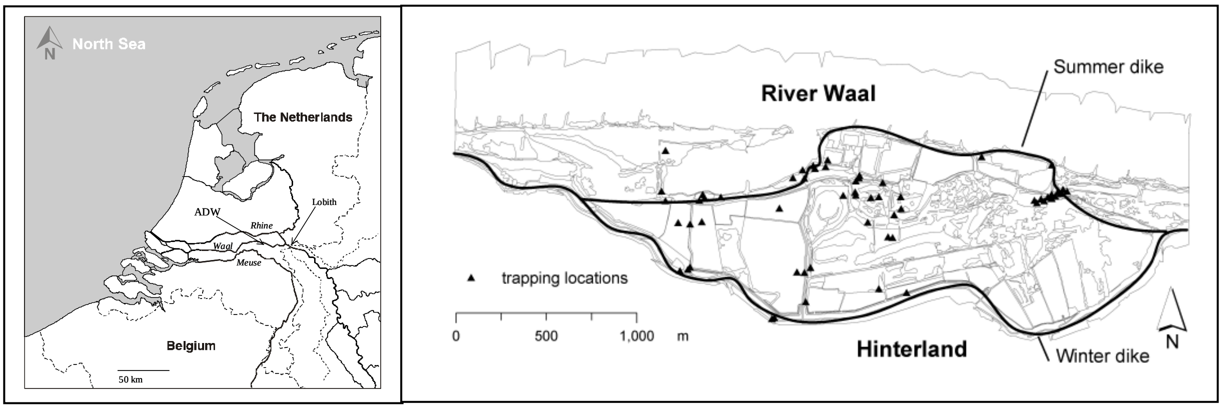


Fig. 1. Location of the ‘Afferdensche en Deestsche Waarden’ floodplain area in the Netherlands, and positions of the trapping locations (with 5 to 10 traps each) within this floodplain.

Small mammals from the ADW floodplain were collected at 58 sites (Fig. 1) between 2001 and 2003, using Longworth live traps in lines of 5 to 10 traps at each site. The traps were baited with apple, carrot and rinsed meat, and were stuffed with hay and tissue. Specifically for this study, there were three sessions of two 3-day trapping trials, all traps were checked twice a day in August 2002 and again in June and October 2003. Furthermore, all trapping casualties from monitoring studies were included, especially specimens trapped in winter and spring because mortality in that time of the year is higher (Wijnhoven et al., 2005, 2006). Trapping locations were originally selected to monitor recolonisation of the floodplain after flood events (Wijnhoven et al., 2005), so they were chosen based on habitat characteristics (vegetation structure, soil type and management type) without previous information on the levels of contamination. Therefore, trapping sites covered the whole range from non-flooding parts to flooding locations situated far from the non-flooding areas, and were expected to show a representative variation in contaminant levels for the study area. At each of the sites, three soil cores prepared from 3 or 5 soil samples from a 1 m^2 plot were taken with line intervals of at least 10 m. A 5 g portion of soil from each sample was oven–dried for 24 h at 105°C. The total metal content of 0.2 mg dry weight (DW) substrate in a mixture of 3.0 ml 65% HNO_3 and 1.5 ml 37% HCl was measured after microwave destruction using a MLS-1200 MEGA microwave oven (Milestone, Sorisole, Italy). The samples were topped up to 50 ml, after which the metal content was measured using inductively coupled plasma – atomic emission spectrometry (ICP-AES; Spectro Analytical Instruments, Kleve, Germany). The 0.01 M $CaCl_2$-exchangeable fraction was determined, as a measure of the potential metal solubility. A 6 g fresh weight portion of substrate, to which 0.01 M $CaCl_2$ had been added in a 1:10 (m(DW)/v) ratio, was mixed for two hours, after which the suspension was centrifuged at 12000 rpm (5000 g) for 15 minutes. After the pH_{CaCl2} had been measured in the substrate suspension in 0.01 M $CaCl_2$, the supernatant was filtered over a 0.45 µm pore filter. A pH of 2 was obtained by adding a few droplets of 65% HNO_3, and the metal content of the sample was subsequently measured on the spectrometer.

Fresh weights (FWs) of the mammals were determined, and the liver and kidneys of each specimen were weighed (FW). Parts of these organs and flank muscles were oven-dried for 24 h at 105ºC, after which DWs were measured. The metal contents of the animal tissues were measured after microwave extraction of approximately 0.01 to 0.25 mg DW with HNO_3 and HCl, and analysed on the ICP-AES as described above. The metal concentrations in whole animals were calculated from the concentrations in the liver, kidneys and muscle tissue. Concentrations in muscle tissue were assumed to reflect the concentration in the animal's remaining tissues, i.e., the entire animal minus liver and kidneys. Calculations were based on the species-specific weighed average distributions in percentage DW for liver, kidneys and other tissues (tissue to total body ratios), and the tissue-specific DW to FW ratios derived from our own data.

Statistical analysis

Inter-specific differences in metal concentration distributions were statistically tested at $p<0.05$, using the two-sample Kolmogorov-Smirnov-test (KS-test) in Systat for Windows 11 (Systat Software Inc., Richmond, CA, USA) because the metal content values within the species did not show a normal distribution (per one-sample KS-test). To search for possible explanations for interspecific differences in metal concentrations, metal concentrations in the soil (average total and $CaCl_2$-extractable concentrations at the locations where individuals were trapped) were compared also with the two-sample KS-test. Two-sample KS-tests are used as concentration distribution variation ('shape') and average or median concentration distribution ('location') will affect exposure of populations (Sokal and Rohlf, 1995). To calculate functional relations of metal concentrations in species, regressions between metal concentrations and mammal characteristics and environmental factors were calculated making use of Microsoft Excel, according to the stepwise-method with a critical F-value for the regression equations calculated at $p<0.05$. A significance level of $p<0.05$ for the individual regression coefficients was used. Parameters included were the total and $CaCl_2$-extractable metal concentrations at the trapping sites, the trapping locations in either flooding or non-flooding areas, the trapping times (for which the dates were divided into the four seasons); and the sexes (male vs. female), life stages (juvenile vs. adult); and size and condition of the animals (recorded as mg FW). Data of specimens were only included if all of the information for the selected parameters was available, which means that the sample size in these analyses was sometimes slightly smaller than the total number of specimens collected. Similarity in sex ratio for each of the species and the numbers of a species trapped in flooding and non-flooding areas was tested with the binomial test (with a two-tailed significance level of 5%) (Sokal and Rohlf, 1995). Interspecific differences in the distribution of DW to FW ratios, organ to total body ratios, sex ratio and life stage composition of the populations, as well as the proportional distribution of the populations over the flooding and non-flooding areas and the numbers trapped in each season were also tested in Systat 11 (two-sample KS-test), after checking for possible normal distributions (one-sample KS-test).

7.3 Results

Differences in heavy metal concentrations between species

In total, 199 specimens of seven small mammal species were collected (Table 1). The variance in organ to total body ratio within the species groups is not large, with the exception of *S. araneus* (Table 2). Comparing these ratios between species, the two shrew species show heavier livers and kidneys than the other species. Distributions in DW to FW ratios of especially liver, but also muscle tissue, also differed between species. Differences for this ratio in kidneys were found only between *A. sylvaticus* and *M. agrestis*. In all species the DW to FW ratios were somewhat higher for livers than for kidneys and lowest for muscle tissue.

Table 1. Characteristics of the small mammals collected. The numbers per species are shown with average values (± SD) of fresh weights, sex ratios (1 = male; 2 = female), life stages (1 = juvenile; 2 = adult), trapping locations (Flood: 1 = flooding; 2 = non-flooding) and trapping seasons (1 = spring; 2 = summer; 3 = autumn; 4 = winter). Unequal intraspecies distributions of sex ratio and flooding were tested ($p<0.05$) using binomial test (indicated when unequal; ns = no significant unequal distribution). Interspecies differences were tested when relevant using the two-sample Kolmogorov-Smirnov-test ($p<0.05$); significant differences are indicated by different superscript letters.

Species	n	FW_{body} (g)	Sex ratio		Life stage	Flood		Season
Apodemus sylvaticus	21	16.06 ±2.42	1.30 ±0.47	more males	1.52 ±0.51	1.33 ±0.48^{a}	ns	2.95 ±0.50^{a}
Clethrionomys glareolus	56	19.68 ±4.62	1.55 ±0.50	ns	1.68 ±0.47	1.79 ±0.41^{b}	more non-flooding	2.32 ±0.64^{bd}
Crocidura russula	11	10.97 ±1.04	1.45 ±0.52	ns	2	1^{ac}	more flooding	2.82 ±0.40^{ab}
Microtus agrestis	9	21.58 ±8.72	1.33 ±0.50	ns	1.67 ±0.50	2^{bde}	more non-flooding	2.44 ±0.53^{bc}
Microtus arvalis	31	19.48 ±6.03	1.55 ±0.51	ns	1.71 ±0.46	1.06 ±0.25^{cf}	more flooding	2.61 ±0.50^{b}
Micromys minutus	4	4.53 ±0.18	1	ns	2	1^{adf}	ns	3^{ab}
Sorex araneus	67	8.32 ±2.07	1.45 ±0.50	ns	1.66 ±0.48	1.55 ±0.50^{ae}	ns	2.18 ±0.85^{cd}

The average whole body Zn concentration was highest in *S. araneus* with 126 mg kg^{-1} DW, followed in descending order by *C. glareolus*, *C. russula*, *A. sylvaticus*, *M. arvalis*, *M. agrestis* and *M. minutus* (Fig. 2). The differences between *S. araneus*, and *M. arvalis*, between *S. araneus* and *A. sylvaticus* and between *S. araneus* and *C. glareolus* were significant ($p<0.05$). The variation in Zn concentrations in *S. araneus* was large. The highest Zn concentration was, however, observed in a specimen of *C. glareolus*, which contained 866 mg kg^{-1} DW, more than twice as much as the highest concentration measured in a specimen of *S. araneus* (416 mg kg^{-1} DW).

Average Cu concentrations were also highest in *S. araneus*, followed in descending order by *M. arvalis*, *C. glareolus*, *C. russula*, *M. minutus*, *A. sylvaticus* and (lowest) *M. agrestis*. The average of 15.6 mg kg^{-1} DW in *S. araneus* is significantly higher than in all the other species except *C. russula* and *M. minutus*. The average Cu concentration of 6.71 mg kg^{-1} DW in *C. glareolus* was also significantly higher than those found in *A. sylvaticus* and *M. agrestis*, with 4.05 and 3.38 mg kg^{-1} DW, respectively. The highest Cu concentration was measured in a specimen of *S. araneus*, with 88.9 mg kg^{-1} DW, but an individual of *M. arvalis* also had a high concentration, i.e. 62.0 mg kg^{-1} DW.

The order from high to low in average Pb concentrations was *S. araneus*, *C. russula*, *C. glareolus*, *M. agrestis*, *M. arvalis*, *A. sylvaticus* and *M. minutus*. The average value of 32.6 mg Pb kg^{-1} DW in *S. araneus* was significantly higher than the average concentrations in *A.*

sylvaticus and *C. glareolus*. For all species, only a few individuals had increased Pb levels, as was indicated by the median concentration of < 1.60 mg kg^{-1} DW for all species. The highest concentrations in individuals were found in *C. glareolus* (270 mg Pb kg^{-1}) and *S. araneus* (264 mg Pb kg^{-1}).

Table 2. Species and tissue specific DW to FW ratios (± SD) and species specific tissue to total body ratios (± SD) based on DWs, used to calculate whole body metal concentrations of specimens. Interspecies differences were tested using the two-sample Kolmogorov-Smirnov test ($p<0.05$); significant differences are indicated by different superscript letters.

Species	n	DW to FW ratio			Tissue to total body ratio (in DW)		
		Liver	Kidney	Muscle	Liver	Kidney	Remaining tissue
Apodemus sylvaticus	21	0.316 $\pm 0.057^{ac}$	0.253 $\pm 0.027^{a}$	0.235 $\pm 0.057^{a}$	0.0648 $\pm 0.0103^{a}$	0.0152 $\pm 0.0027^{a}$	0.920 $\pm 0.011^{a}$
Clethrionomys glareolus	56	0.272 $\pm 0.081^{b}$	0.243 $\pm 0.043^{ab}$	0.218 $\pm 0.066^{c}$	0.0701 $\pm 0.0123^{a}$	0.0165 $\pm 0.0029^{a}$	0.913 $\pm 0.014^{c}$
Crocidura russula	11	0.315 $\pm 0.041^{a}$	0.246 $\pm 0.022^{ab}$	0.204 $\pm 0.056^{abc}$	0.0904 $\pm 0.0191^{b}$	0.0213 $\pm 0.0031^{b}$	0.888 $\pm 0.021^{b}$
Microtus agrestis	9	0.251 $\pm 0.038^{b}$	0.228 $\pm 0.034^{b}$	0.210 $\pm 0.044^{abc}$	0.0701 $\pm 0.0115^{a}$	0.0149 $\pm 0.0027^{ac}$	0.915 $\pm 0.012^{ac}$
Microtus arvalis	31	0.287 $\pm 0.034^{cd}$	0.253 $\pm 0.029^{ab}$	0.235 $\pm 0.033^{ac}$	0.0678 $\pm 0.0097^{a}$	0.0127 $\pm 0.0013^{c}$	0.919 $\pm 0.010^{ac}$
Micromys minutus	4	0.264 $\pm 0.012^{bd}$	0.244 $\pm 0.032^{ab}$	0.173 $\pm 0.088^{abc}$	0.0679 $\pm 0.0104^{a}$	0.0250 $\pm 0.0060^{b}$	0.907 $\pm 0.014^{abc}$
Sorex araneus	67	0.305 $\pm 0.056^{ad}$	0.239 $\pm 0.046^{ab}$	0.178 $\pm 0.072^{b}$	0.106 $\pm 0.084^{b}$	0.0241 $\pm 0.0239^{b}$	0.869 $\pm 0.107^{b}$

The average Cd concentration was significantly higher in *M. agrestis* than in *S. araneus*, *C. russula* and *M. arvalis*; however, this is largely the result of one individual, which contained 63.9 mg Cd kg^{-1} DW. The average Cd concentration of 3.49 mg kg^{-1} in *S. araneus* was significantly higher than the average concentrations in all other species, except *C. russula*. The median concentrations were highest in *S. araneus* with 1.82 mg Cd kg^{-1} DW, followed by *C. russula* and *M. agrestis* with median concentrations of 0.58 and 0.57 mg Cd kg^{-1} DW, respectively, and the other species with median concentrations < 0.20 mg Cd kg^{-1} DW.

Exposure of small mammals to heavy metals

Exposure of species to total metal concentrations showed similar patterns for each of the metals. The total metal concentrations at the trapping sites of *C. glareolus* and *M. agrestis* were significantly lower than those at the sites of most of the other species, depending on the investigated metal (Fig. 3). Total Zn concentrations at the trapping locations of *A. sylvaticus* (398 mg kg^{-1} DW), *C. russula* (392 mg kg^{-1} DW) and *M. arvalis* (377 mg kg^{-1} DW) were significantly higher than those at the trapping locations of *C. glareolus* (200 mg kg^{-1} DW) and *M. agrestis* (110 mg kg^{-1} DW), and higher at the trapping locations of *S. araneus* (289 mg kg^{-1}

DW) than at those of *M. agrestis*. For Cu and Pb, significant differences were found between *A. sylvaticus*, *M. arvalis*, *S. araneus* and *C. russula*, all of which had high exposure concentrations, and *C. glareolus* and *M. agrestis*, which had low exposure concentrations. With regard to Cd, *A. sylvaticus* and *S. araneus* were exposed to significantly higher concentrations than were *C. glareolus* and *M. agrestis*.

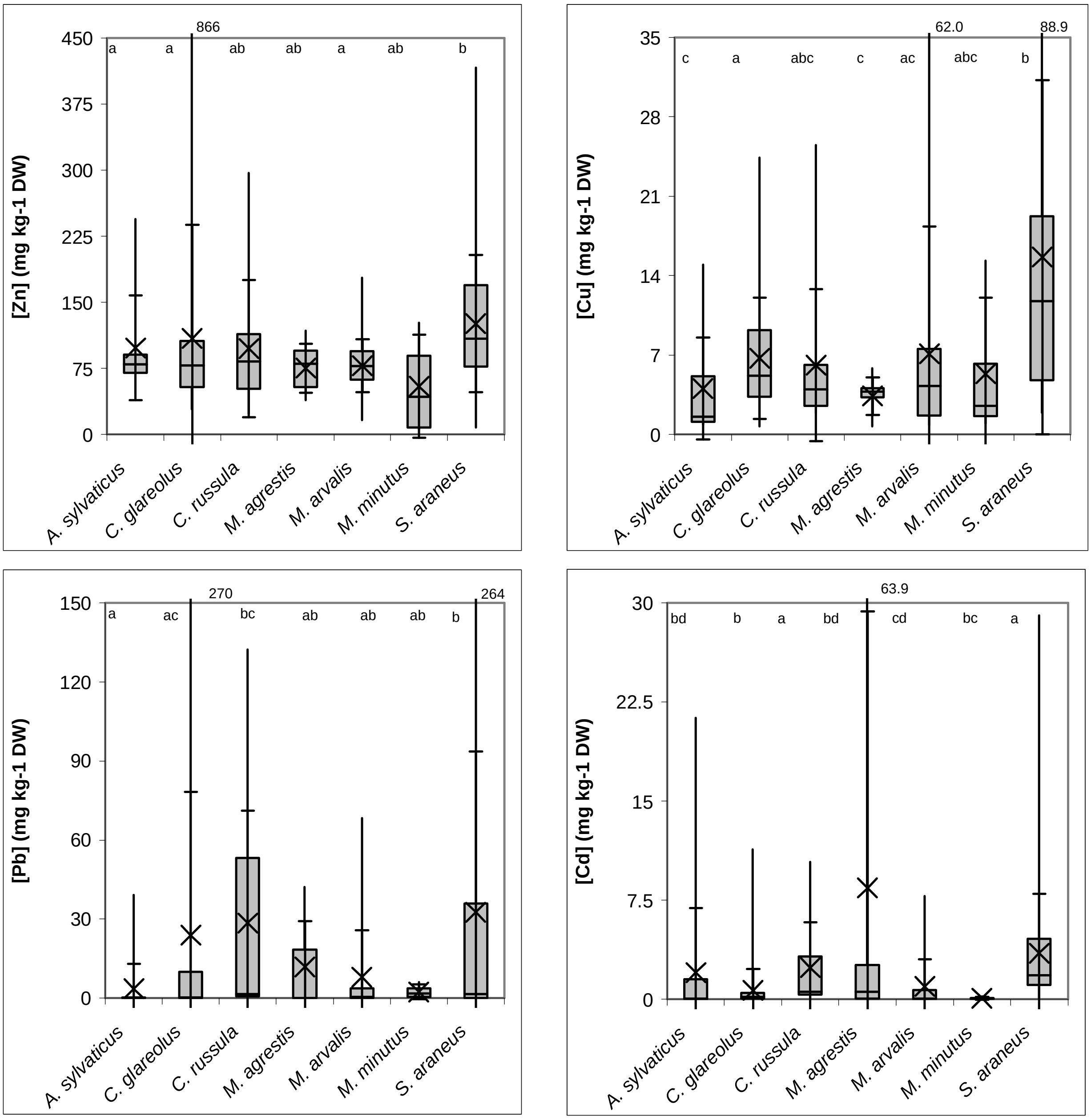


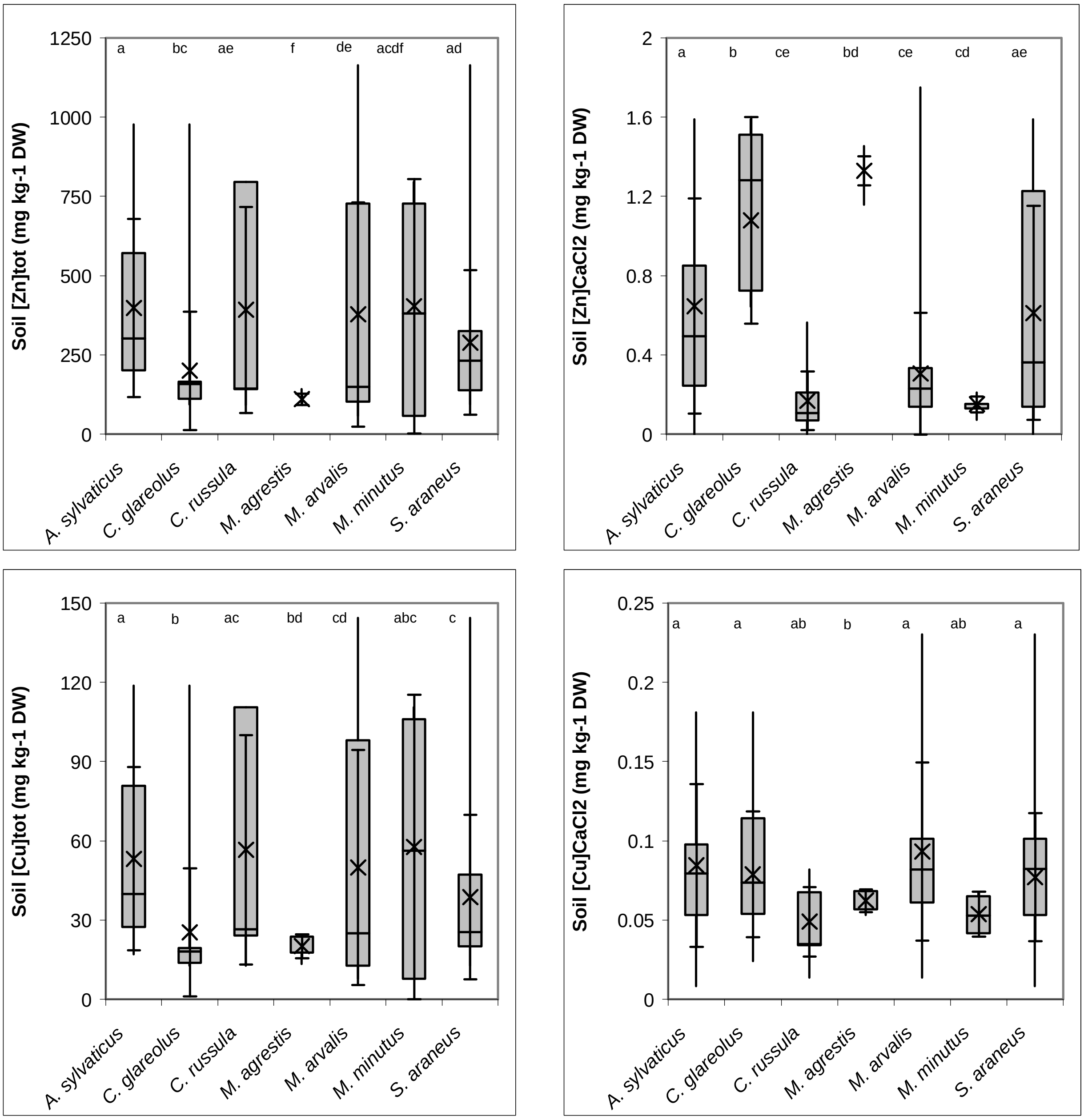
Fig. 2. Metal concentrations in various small mammal species trapped in the 'Afferdensche en Deestsche Waarden' floodplain. Average metal concentrations are indicated by crosses; error bars show SDs; total range (minimum and maximum observations) is shown as a vertical line. The columns indicate the 25% and 75% percentiles, with the median in between. Different letters indicate significant differences (p<0.05) in concentration distributions; identical letters indicate no significant differences between species.

Exposure to $CaCl_2$-extractable Zn was highest for *M. agrestis* and *C. glareolus*, which is significantly higher than for the other species (Fig. 3). Of these species *A. sylvaticus* was exposed to significantly higher concentrations than *M. arvalis*, *C. russula* and *M. minutus*. Differences in $CaCl_2$-extractability of Cu and Pb were smaller, but *A. sylvaticus*, *C. glareolus*, *M. arvalis* and *S. araneus* were exposed to higher Cu levels than *M. agrestis*, whereas *A.*

sylvaticus, *C. glareolus*, *M. arvalis* and *S. araneus* were exposed to higher Pb levels than *C. russula* and *M. agrestis*. Exposure to $CaCl_2$-extractable Cd concentrations was similar for all species.

Regression of metal concentrations

Significant regressions between metal concentrations in the various species on one hand and the species characteristics and/or environmental variables on the other hand were found for *A. sylvaticus* with respect to Zn, Cu and Pb, respectively (Table 3). Of the variance in Zn concentrations in this species, 58% was explained by the parameters taken into account: season, total Zn concentration in the soil, locations being flooding or non-flooding, and $CaCl_2$-extractable Zn concentration in the soil. Cu concentrations were related to life stages, with higher concentrations in adults. Pb concentrations were related to the sizes of the animals, with the highest concentrations in the smallest specimens. Significant regressions of metal concentrations in *C. glareolus* were found for Zn, with the highest concentrations



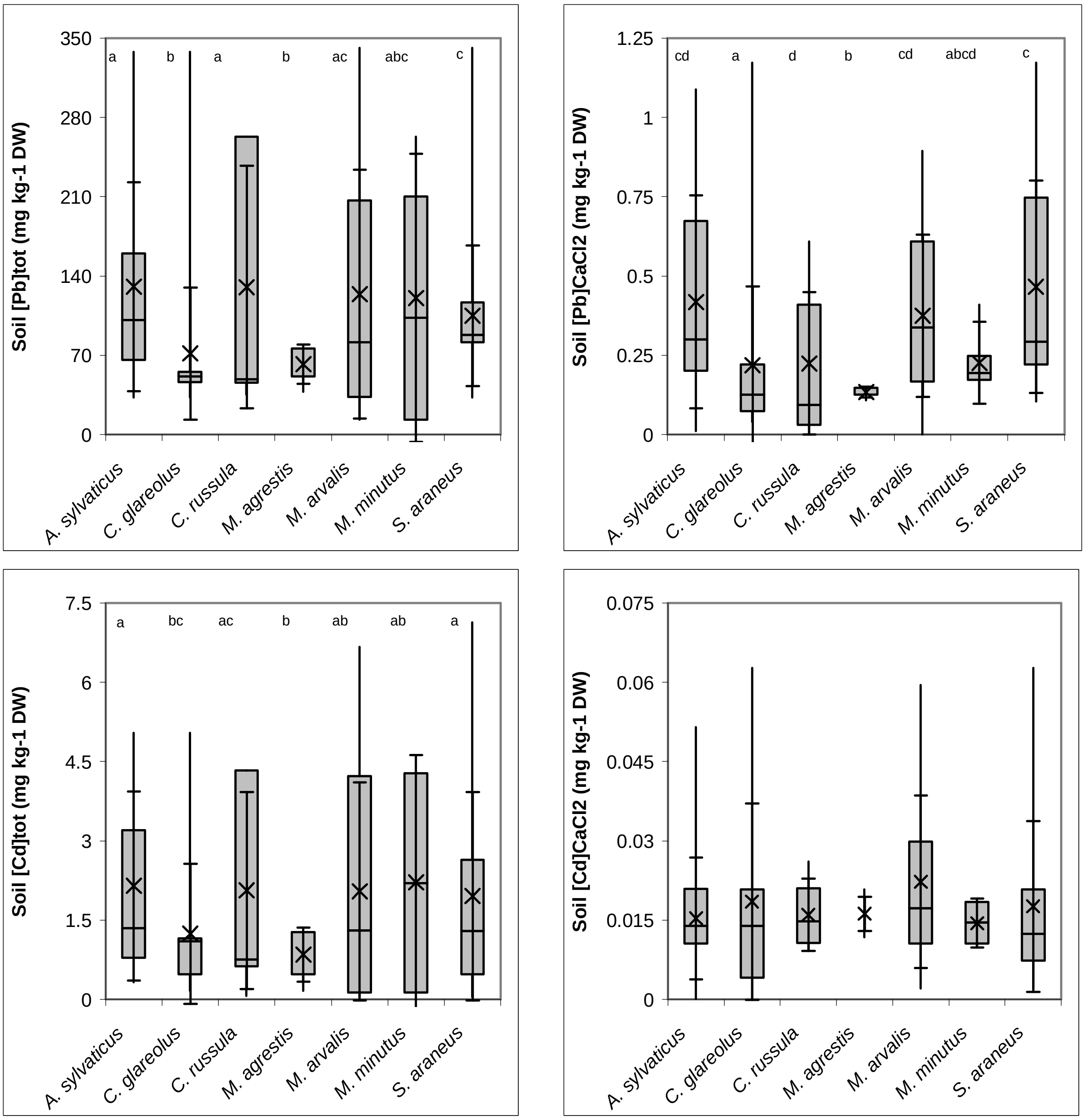


Fig. 3. Total and $CaCl_2$-extractable metal concentrations in the upper 10 cm of the soil at the 'Afferdensche en Deestsche Waarden' floodplain area trapping locations. Average metal concentrations are indicated by a cross; error bars show SDs; total range (minimum and maximum observations) is shown as a vertical line. The columns indicate the 25% and 75% percentiles, with the median in between. Different letters indicate significant differences ($p<0.05$) in concentration distributions; identical letters indicate no significant differences between metal concentrations in the soil.

found in the adults trapped in spring at locations with high $CaCl_2$-extractable Zn concentrations, and for Cd, with the highest concentrations found in the larger animals trapped toward winter. Cu concentrations in *C. russula* were positively related to the $CaCl_2$-extractable concentrations, and negatively to the total Cu concentrations in the soil; they were highest in the larger female animals. Cd concentrations in this species were more closely related to the season (highest toward winter) and were also positively related to the $CaCl_2$-extractable concentrations in the soil; again, they were highest in female individuals. A significant regression ($p<0.05$) for Zn concentrations in *M. arvalis* was only found with the sexes, with the highest concentrations in female animals.

Differences in population structure

Because differences in metal concentrations between species may result from differences in population structure or distribution, the characteristics of each species were analysed. For all species, more adults than juveniles were trapped, but for none of the species was the adults-to-juveniles ratio significantly higher than for another species. The fact that only adults were trapped from two species (*C. russula* and *M. minutus*) was reflected in the FWs of these species, which showed a relatively narrow range, with an SD < 10% of the average weight. Comparable numbers of males and female individuals were trapped for nearly all species, except for *A. sylvaticus*, where significantly more (i.e., twice as many) male than female animals were trapped. With regard to seasonal differences, *S. araneus* and *C. glareolus* were trapped significantly earlier, because half of those individuals had already been collected in early summer, than *A. sylvaticus*, in which more individuals were collected throughout fall. *S. araneus* was trapped earlier than *M. arvalis*, *M. agrestis*, *C. russula*, and *M. minutus*.

With regard to the numbers trapped in flooding and non-flooding areas, significantly ($p<0.05$) more specimens of *C. glareolus* and *M. agrestis* were trapped in non-flooding areas, whereas significantly more specimens of *C. russula* and *M. arvalis* were trapped in the flooding areas.

7.4 Discussion

Differences in average metal concentrations between species can be the result of differences in population structure between the species. The data set also showed differences in the relative numbers of each of the species trapped in each of the seasons. Nevertheless, the trapping results were in accordance with the distribution patterns of the species in the ADW floodplain found during the 2001 and 2002 monitoring programme (Wijnhoven et al., 2006). *C. glareolus* and *S. araneus* showed rather stable densities throughout the year. Other species showed density peaks during late summer and autumn. The highest densities of *A. sylvaticus* and *M. minutus* were found during late autumn and winter. The male dominated trapping results for *A. sylvaticus* seem to be a common phenomenon (Randolph, 1977). Kikkawa (1964) suggests that it results from a larger home range size and therefore greater trappability of male individuals.

With regard to the numbers of species trapped in flooding and non-flooding areas, the trapping results of *A. sylvaticus* and *M. minutus* in this study confirm the patterns found earlier by Wijnhoven et al. (2006), with more individuals of these species found in the flooding than in the non-flooding areas throughout the year. Wijnhoven et al. (2006) also found higher numbers of *M. arvalis* and *C. russula* in the flooding areas. Only the fact that the largest proportion of *C. glareolus* was trapped in non-flooding areas is not in agreement with the 2001 and 2002 monitoring results, in which the reverse was found. We collected 56 specimens of *C. glareolus* for this study, which is much more than for most of the other species. This is not in line with the relatively low densities of this species found in 2001 and 2002.

Our measurements of metal concentrations in small mammal species are representative of the values in the floodplain as a whole, and the previously mentioned differences between the data sets for the various species are generally in line with expectations for the species and the research area. Data for *M. minutus* should be interpreted with care because only four specimens were included in the analyses. *C. glareolus* might be exposed to different average metal concentrations in years of lower densities or when a larger proportion of the animals is present in the flooding areas. Variable factors - such as the frequency, timing and duration of flooding and climatic variations, and management measures such as mowing and grazing in the area - can also influence the accumulation risks through effects on habitat suitability and connectivity and therefore on species abundance and distribution.

In accumulation and risk assessment studies, most attention is given to shrews, in particular *S. araneus,* because these are the small mammals expected to show the highest accumulation of heavy metals in view of their insectivorous/carnivorous diet. However, with regard to the risks of accumulation and possible toxic effects to top predators, vole species are more relevant, because they generally occur in much higher densities and are the dominant prey for several species (Jongbloed et al., 1996). In addition, the fact that a particular small mammal species accumulates larger amounts of heavy metals does not necessarily mean that this is the species most at risk of toxic effects from pollutants. Some species could be more sensitive to heavy metals than others, and storage of heavy metals in organs, such as the liver, could be a good mechanism to cope with toxicants (Shore and Douben, 1994). In our study, *S. araneus* did indeed show the highest heavy metal concentrations of the seven small mammal species in the ADW floodplain area. For Cd, a maximum difference of a factor of 5.2 was found between *S. araneus* and the vole and mouse species. The differences are not as large as those found in inland areas or as those that have sometimes been suggested. A study by Ma et al. (1991) in polluted and relatively clean inland areas, found that estimated differences, by factors of 26 to 57, in Cd intake concentrations resulted in a difference of factors of 46 to 182 in kidney and 83 to 812 in liver concentrations. Hunter et al. (1989) recorded differences of factors of 2.1 to 12.1, 8.6 to 28.2, and 6.2 to 9.5 in kidney, liver, and muscle tissue, respectively, in control and polluted inland areas. The difference in bioaccumulation of Cd from soil to *S. araneus* and the voles (Microtidae) is a factor of 32 in the model described by Jongbloed et al. (1996). The estimated difference in exposure risk for shrews and voles, as calculated by Kooistra et al. (2001) for areas in the ADW floodplain, are factors of 2.1 to 9.8, which is more in line with our findings. For Pb, the difference between *S. araneus* and the vole and mouse species varied between 2.7 and 9.3 in our study. The difference of factors of 6.2 to 11.0 in Pb intake by Ma et al. (1991), resulting in a difference of factors of 4.9 to 22.3 in kidney and 1.6 to 7.1 in liver concentrations is in line with our results. However, concentrations of Pb in *C. russula* and *C. glareolus* were not significantly different in our study. For Cu, the difference between *S. araneus* and the vole and mouse species varied by factors of 2.2 to 4.6 in our study. These values are similar or even slightly higher than the differences of 1.6 to 2.0, 1.6 to 4.1, and 1.2 to 1.7 in kidney, liver, and muscle tissue, respectively, as recorded by Hunter et al. (1989). The average Zn contents in *S. araneus* in our study did not differ by more than a factor of 2 from those in the Microtine rodent species. In our study, the metal concentrations in, for example, *C. glareolus* and *M. agrestis* did differ less from those in *S. araneus* than recorded in the literature (for Cu and Cd) or were similar to them (for Zn and Pb). In our study, levels, except for Cd, in the shrew *C. russula* were similar to those in *C. glareolus* and also to those in several other vole and mouse species.

We expected that other than differences in metal concentrations between species of different trophic levels, the actual exposure concentrations would also be reflected in the metal concentrations in small mammal species and individuals (inter- and intraspecific variation). Because *C. glareolus* and *M. agrestis* were predominantly trapped in the non-flooding areas, their exposure to total Zn concentrations in the soil was generally lower than that of the other species, whereas their exposure to $CaCl_2$-extractable Zn concentrations was higher. This was, however, not reflected in the Zn concentrations in these two species. This suggests that the accumulation of Zn in these vole species is not determined by total or by $CaCl_2$-extractable Zn fractions alone, indicating that Zn is regulated by either the food species (Heikens et al., 2001) or by the small mammals themselves (Mertens et al., 2001), which influences observed tissue values. With regard to exposure to Cu, Pb, and Cd, only exposure to total concentrations was different, i.e., lower for *C. glareolus* and *M. agrestis,* than for some of the other species depending on the investigated metal, which was not reflected in the metal concentrations in the species either.

Although exposure concentrations in the soil for *C. russula* were generally similar to those for other species, the metal concentrations show a trend toward concentrations being lower than in *S. araneus* and similar to those in voles. This could be the result of differences in feeding patterns between the two shrew species. Earthworms, which are known to be strong accumulators of heavy metals (Hobbelen et al., 2004; Van Vliet et al., 2005), are more important food items for *S. araneus* than for *C. russula*. It is also possible that macro-invertebrates are more often eaten by vole species, in particular, *C. glareolus* and *M. agrestis*, than was thought previously.

Table 3. Significant regressions ($p<0.05$) between metal concentrations in small mammal species, characteristics of the species (see Table 1), and environmental variables (critical F value calculated at $p<0.05$).

Metal		F	n	R^2	P
	Apodemus sylvaticus				
Zn	$\ln([Zn_{body}]) = 1.41(\text{season}) + 0.00107([Zn_{tot}]) +1.11(\text{flood}) –0.722([Zn_{CaCl2}]) –1.24$	4.51	18	0.581	0.017
Cu	$\ln([Cu_{body}]) = 0.918(\text{life stage}) –0.490$	6.01	18	0.273	0.026
Pb	$\ln([Pb_{body}]) = -0.000610(FW_{body}) +7.30$	4.94	18	0.236	0.041
Cd	ns		18		
	Clethrionomys glareolus				
Zn	$\ln([Zn_{body}]) = 0.693(\text{life stage}) –0.554(\text{season}) +0.\ 295([Zn_{CaCl2}]) +6.58$	5.95	49	0.284	0.002
Cu	ns		49		
Pb	ns		49		
Cd	$\ln([Cd_{body}]) = 0.000236(FW_{body}) +1.48(\text{season}) –10.5$	6.49	49	0.220	0.003
	Crocidura russula $^{(s2, f1)}$				
Zn	ns		10		
Cu	$\ln([Cu_{body}]) = 45.1([Cu]_{CaCl2}) –0.0187([Cu_{tot}]) +0.828(\text{sex}) +0.000247(FW_{body}) –3.57$	19.9	10	0.941	0.003
Pb	ns		10		
Cd	$\ln([Cd_{body}]) = 4.87(\text{season}) +199([Cd]_{CaCl2}) +1.52(\text{sex}) –19.2$	17.2	10	0.896	0.002
	Microtus agrestis $^{(f2)}$				
Zn	ns		8		
Cu	ns		8		
Pb	ns		8		
Cd	ns		8		
	Microtus arvalis				
Zn	$\ln([Zn_{body}]) = 0.342(\text{sex}) +3.73$	4.65	29	0.147	0.040
Cu	ns		29		
Pb	ns		29		
Cd	ns		29		
	Sorex araneus				
Zn	ns		45		
Cu	ns		45		
Pb	ns		45		
Cd	ns		45		

Micromys minutus is not included because only four animals were trapped. $[Me_{body}]$ = metal concentration in animal (mg kg^{-1} DW). Species characteristics and environmental variables initially included are: sex = 1 for male and 2 for female; life stage = 1 for juveniles and 2 for adults; FW_{body} = fresh weight of animal (mg); $[Me_{tot}]$ = total metal concentration in the soil (mg kg^{-1} DW); $[Me_{CaCl2}]$ = $CaCl_2$-extractable metal concentration in the soil (mg kg^{-1} DW); flood = 1 for flooding and 2 for non-flooding trapping locations; season = 1 for animals trapped in spring, 2 for summer, 3 for autumn, 4 for winter. ^{s2} = all animals were adults; ^{f1} = all animals were trapped in flooding areas; ^{f2} = all animals were trapped in non-flooding areas. ns = non-significant.

In addition to the influence of soil metal concentrations for accumulation of heavy metals, several interfering factors might be of importance. For several combinations of small mammal species and metals tested in multiple regressions, no regressions with measured parameters explaining a significant part of the variance ($p<0.05$) in the observed metal concentrations in the animals were found. For *M. agrestis*, this can be ascribed to the small sample size and the small variance, but also for *S. araneus*, with 45 specimens, no significant regressions were found. None of the parameters were substantially more often found to be related to the metal concentrations in the small mammals. This means that the total metal concentrations in the soil at the trapping location are not a good predictor of the metal concentrations in the small mammals found there. Using $CaCl_2$-extractable concentrations in the soil hardly improved the relation with the accumulated metals in the small mammals. We conclude that the soil metal concentrations at the trapping locations do not necessarily reflect the exposure concentrations throughout the animals' life history. This could be caused by (1) large variations in exposure time (or age of the animals); (2) heterogeneity of the soil concentrations, which means that exposure within an animal's home-range may not be similar to that at the trapping location; or (3) movements, dispersal and/or shifts in feeding patterns, making such correlations irrelevant.

Life stage and FW of the small mammals only occasionally showed significant regressions. This could reflect a poor relationship between FW and age because condition may play a role. *S. araneus* are known to show a decrease in body mass during winter (Ochocińska and Taylor, 2003), which is probably also reflected as a larger variance in tissue to total body ratio (Table 2), but such a phenomenon is not assumed for the other species. Life stage could be a poor predictor, because small mammals remain juvenile for only a short period, causing the greatest variation in body metal concentrations within the group of adults. It seems most likely that the poor relations of soil metal concentrations with body metal concentrations result from the migration of species from one area to another or from shifts in diets. However, we found a relationship between the sex and body metal concentrations in three cases, with higher concentrations being found in female than in male individuals, suggesting that there are differences in feeding patterns or home-range structures between the sexes, or pregnancy effects on weight, for *C. russula* and *M. arvalis*.

It is difficult to assess the exposure risk and contaminant levels of individual small mammals in the highly dynamic floodplain environment. However, based on our results it is nevertheless possible to calculate average risks for species within ecological units or floodplains as a whole. It is important that the animals collected reflect the species distribution and densities throughout the year, making it crucial to combine measurements of metal accumulation with population monitoring.

Metal concentrations in wild small mammals are often measured in the target organs (e.g. liver and kidneys) (Hunter and Johnson, 1982; Hunter et al., 1984; Ma et al., 1991; Damek-Poprawa and Sawicka-Kapustra, 2003, 2004). Although this is relevant from a toxicological point of view, total body concentrations may be more relevant when considering small mammals as prey animals. The observed differences in metal concentrations between small mammal species have consequences for the risks to predators in floodplains. Species preying on *S. araneus*, for instance, run greater risks of heavy metal accumulation than species preying on voles and mice. However, *S. araneus* is generally not the most important prey species for predators (Jongbloed et al., 1996), because the densities of other species are often higher (Wijnhoven et al., 2006). Predators will forage where their preferred prey is available in large numbers, which means that in the ADW floodplain, exposure of predators specialising in Bank voles mainly occurs in non-flooding areas, whereas exposure of predators of *M. arvalis* occurs especially in flooding areas. This leads to differences in exposure between predators, such as the Weasel (*Mustela nivalis*) and the Eurasian kestrel (*Falco tinnunculus*), as a result of their different foraging behaviour (Erlinge et al., 1983), distribution of their prey, and contaminant levels within the prey. During floods, as well as for quite some time after an area has been flooded, exposure of predators of small mammals to heavy metals will generally occur in the non-flooding areas. Predators are probably sparse in the area during this time because they are forced to move away, because of low prey availability, or, in the case of generalists, forced to prey on other food sources (Van den Brink et al., 2003).

Differences in metal concentration ranges between the small mammal species seem to be small for Zn, which is probably to a certain extent regulated. Extremely high levels of metals were found in a few individual voles: Zn and Pb in *C. glareolus*, Cd in *M. agrestis* and Cu in *M. arvalis*. Although these extreme values could not be directly related to soil concentrations, they can possibly be explained by point sources of pollution and by foraging in other areas than the sample sites alone. All individuals with extreme body concentrations were trapped at or near non-flooding areas. Only in a few cases were the metal concentrations in the mammals related to soil metal concentrations, either total or extractable. We know that this floodplain is diffusely polluted, which implies heterogeneous contamination patterns. However, large differences in contaminant levels over short distances are not to be expected, unless there are certain point sources in the non-flooding areas where industrial activities have taken place in the past, or near the borders between the flooding and non-flooding parts. It is more likely that in this dynamic environment, exposure of small mammals at or near the trapping location only occurs during a part of their life history. Movements and dispersal probably interfere with the relations between soil metal concentrations and the concentrations in the bodies of the mammals. In addition, the metal concentrations in the small mammals could also be affected by shifts in feeding patterns, seasonal and flood-related aspects of food availability, habitat suitability and connectivity, and life stage related food preference, combined with the same variations in the metal contents in the food items themselves. Finally, exposure time, and therefore the age of the animals, might be an explanatory factor, as was indicated in a few cases by the relation between metal concentrations in small mammals and life stage, FW, or season.

7.5 Conclusions

Heavy metal concentrations in seven small mammal species from a diffusely polluted floodplain along the river Rhine differed between species. These differences can be partly explained by the trophic level of the species, because metal concentrations were highest in the carnivorous/insectivorous shrew, *S. araneus*. However, average metal concentrations in *S. araneus* differed from those in the vole and mouse species at the maximum by factors of 2.0, 4.6, 9.3 and 5.2 for Zn, Cu, Pb and Cd, respectively, which is less than has been reported by several studies of inland areas. No significant differences were found in average Zn, Pb and Cd concentrations between *S. araneus, M. agrestis* and *C. russula*. The vole *C. glareolus* and the shrew *C. russula* had similar concentrations of Cu, Pb and Zn. Although possible differences in accumulation between life stages and sexes or size- and season-related differences were corrected for, relationships between total or $CaCl_2$-extractable soil metal concentrations at the trapping locations and the metal concentrations in the mammals were poor or absent. We suspect that exposure time, dispersal and changes in foraging behaviour might be important factors influencing the exposure of small mammals in highly dynamic environments, such as floodplains. The observed differences in metal concentrations between small mammal species will have consequences in terms of risks to predators in floodplains and should therefore be considered in risk assessments.

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Chapter 8

Synthesis and conclusions

Measuring the weight of a small mammal during windy conditions by Aafke Schipper

8.1 Introduction

Floodplains along the large European rivers have become polluted over a number of decades (Middelkoop, 2002). Although the water quality in several rivers has improved significantly (Vink et al., 1999; Bij de Vaate et al., 2006), ecosystems are still coping with the heritage from the past (Nienhuis et al., 2002; Nienhuis, 2006). Although ecological rehabilitation programmes are being planned and implemented all over Europe, it is still uncertain whether ecological rehabilitation of floodplain ecosystems is possible at the current pollutant levels (Leuven et al., 2005). The fate of heavy metal pollutants in floodplain ecosystems is largely unclear, and the way ecosystems react to chronic and diffuse exposure to chemical pollution is unknown. It is especially the lack of field data on the results of a combination of processes and interactions that seems to be problematic. A better understanding of the ecological, ecotoxicological and environmental processes is therefore relevant when formulating and implementing policy with respect to the ecological risks of chronic and diffuse pollution of floodplains (SSEO, 1999). The aim of the studies reported on in this thesis was to deliver relevant input data on and improve our understanding of these underlying processes to provide a basis for ecological and ecotoxicological assessment and modelling and improve floodplain management. The studies therefore focussed on interactions between biota and contaminants in dynamic floodplain environments. In particular, we tried to quantitatively and qualitatively analyse the interactions between small mammals and heavy metals. The following research questions were formulated:

1. Where and when does exposure of small mammals to heavy metals occur?
2. What is the impact of bioturbation by small mammals on heavy metal distribution in floodplains?
3. Are there risks of accumulation of heavy metals in food webs within diffusely contaminated floodplains, and if so, what factors explain such accumulation?

8.2 Distribution of interactions between small mammals and heavy metals in floodplains

To investigate processes and interactions between contaminants and biota, it is important to know where and when these take place, which means that the temporal and spatial distributions of both contaminants and biota have to be investigated. The spatial and temporal distributions of small mammals were studied by monitoring with live traps at the ‘Afferdensche en Deestsche Waarden’ floodplains (ADW), and comparing with live trap adjusted observations with food traps at the ‘Millingerwaard’ floodplain (MW) (Chapters 2 and 3). The impact of flooding on the small mammal densities and distribution was found to be large for all investigated species. Effects of floods can still be observed after one year, and are estimated to last approximately two years for all investigated species, except *M. arvalis*. Because the ADW, like most embanked lower Rhine floodplains, is flooded every year, or at least at two-year intervals (RIZA/RIKZ, 2006), it can be concluded that current small mammal distributions are still determined by previous floods. Throughout the year, small mammal densities in the floodplain were significantly higher on and near non-flooding areas than at distances of more than 120 m from the non-flooding areas. Two species, *Clethrionomys glareolus* and *Sorex araneus*, did not even disperse as far as this in our study.

Another determinant of species distribution, next to flooding, is the structure of the landscape mosaic of biotopes that are suitable or unsuitable for the various species. The landscape pattern of mixed vegetation structures plays an important role in the slow recolonisation we observed, since ideal corridors for fast dispersal are generally scarce in present-day floodplain landscapes. These landscapes are characterised by areas with either a poor connectivity, or with large homogeneous suitable areas, which slow down recolonisation over the whole area as specimens settle and stay there (unpublished observations; RIZA/Geodon, 2006). The vegetation type favouring most small mammal species and having the potential to harbour large numbers, viz. ungrazed rough herbaceous vegetation, is generally scarce in floodplains, as people prefer to use as much of the land as possible, especially for agricultural purposes (Jongman, 1992). Rough vegetation is also considered undesirable because it increases hydraulic resistance during floods (Baptist et al., 2004; Peters et al., 2006). As a result, most of the floodplain is grazed, including the nature conservation areas. Vegetation types with low densities of small mammals, or harbouring no small mammals at all, like maize fields, most pastures, and low, sparse or trodden vegetation types, are often covering large parts of floodplains (Middelkoop and Van Haselen, 1999; RIZA/Geodon, 2006).

The combination of a dramatic reduction of the small mammal populations during floods, the concentration of the survivors in non-flooding areas, the slow population growth and the poor connectivity of the landscape slows down the recolonisation of the areas that re-emerge after a flood. The recurrent flooding frequency of approximately once a year for most embanked Rhine floodplains results in about half of the small mammal population being present on or within 30 m of the non-flooding areas, which generally represents only a small part of the total floodplain area (in the ADW 35%). Several studies (e.g. Kooistra, 2004; Leuven et al., 2005; Thonon, 2006), including our own (Chapters 5 and 6) have shown that the average heavy metal concentrations in the floodplain topsoil are much higher in the regularly flooding areas, although there are large variations as shown in Chapters 3, 5 and 6. As a contact area between small mammals and heavy metal contamination, the first few dozens of metres surrounding the non-flooding areas are generally most important, as the total metal concentrations there are higher than in the non-flooding areas, while small mammal numbers are higher than in other regularly flooding areas. However, local circumstances can qualify this general rule, through differences in elevations and vegetation structure influencing the distribution patterns of both, contaminants (Middelkoop, 2002; Thonon, 2006) and small mammals (Chapters 2 and 3), as well as through anthropogenic influences like excavations (Van Stokkom et al., 2005), local contaminating sources, or specific management regimes affecting the vegetation (Vulink, 2001). Other conditions can also be of importance for the heavy metal exposure of small mammals or for the impact of small mammals on heavy metal contamination. These include metal speciation, but also the characteristics of the small mammal species like burrowing and/or feeding behaviour. These conditions and processes might change the importance of certain floodplain areas as contact zones between heavy metal pollution and small mammals. We can conclude that during a flood, the contact areas are solely the non-flooding areas, after which regularly flooding areas at larger distances gradually become more important as contact areas. Since flood events generally occur in winter (RIZA/RIKZ, 2006), we can conclude that from spring to winter, the regularly flooding areas gradually become more important as contact areas, with a sudden return to the non-flooding areas during a (winter) flood. One comment we have to make about gradual recolonisation is that immediately after the retreat of the water (Chapter 2), rapid recolonisation by small mammals can be observed in the few dozen metres immediately surrounding the non-flooding areas, mainly due to the high quality and quantity of food just after a flood.

We can conclude that the environment, i.e. the floodplain and its characteristics, has a large impact on the small mammal distribution, by determining habitat suitability. The floods have the greatest impact, reducing numbers of small mammals and concentrating them in non-flooding areas. Other landscape characteristics are dominant in guiding the recolonisation of the floodplains, as a result of which the small mammals are not equally distributed over the floodplain. The heterogeneity of the landscape also guides such colonisation processes in inland areas, but the factor of flooding is absent there. Distribution patterns in floodplains are different from those in similar inland areas for at least two years after each flood, which means permanently for most Rhine floodplains. As a consequence, processes like bioturbation and landscaping by small mammals are concentrated in space and time, as are the risks of accumulation and possible toxic effects on small mammals and their predators.

8.3 The fate of heavy metals in contaminated floodplains

Bioturbation is assumed to be a common process in floodplains, which may affect the vertical distribution of heavy metals in floodplain soils by influencing soil conditions. Several small mammal species (as well as other animals) may be important bioturbators (Mitchell, 1988; Mace et al., 1997). As described in Chapter 4, we investigated the potential effect of bioturbation on heavy metal mobility by applying mechanical turbation to zinc-spiked top layers of floodplain soils. We found a decrease in the zinc content of the top 15 cm, although only the top 2 cm was disturbed. Since our results did not show changes in the extractability of zinc or changes in pH under the influence of mechanical turbation, we do not expect that bioturbation affects the solubility of metals by changing soil conditions, although bioturbation is important in the redistribution of metals in floodplains. It was found that zinc attached to colloid and organic matter particles was particularly redistributed from the top layer, which means that bioturbation affecting percolation processes is the dominant process for zinc redistribution. These findings show that bioturbation in floodplains under wet conditions, i.e. in soils with high groundwater levels (i.e. at depths of less than 15 cm), or in rainwater-soaked soils, can have an important effect on the zinc distribution. The redistribution of other metals might be proportionally more related to clay or to organic matter, but there are no reasons to assume that our findings are only valid for zinc. Such wet conditions can be expected during the first period after inundation or just before inundation, when groundwater levels are high. Depending on the conditions in the terrain, including local depressions or 'bathtub'-shaped floodplains, and the rate at which water levels in the river and therefore groundwater levels fall, such wet soil conditions can last for weeks. In these conditions several animals are present and show burrowing activities, for instance earthworms, most of which are flood-tolerant, and become active after flooding (Zorn et al., 2005). Several small mammal species have also been found to appear soon after or even during the retreat of the water. The most important bioturbator species are the mole *Talpa europaea* and other small mammal species like *C. glareolus* and *S. araneus*, which are attracted by the high food availability and quality in the formerly flooded areas (non-published observations and Chapters 2 and 3).

The water level in the river outside the 'summer dikes' is often higher than certain parts of the embanked floodplain (www.waterbase.nl). As therefore locally the water tables frequently rise to the surface level, wet conditions regularly occur throughout the year. As a result of (1) the concentration of small mammal populations in the non-flooding areas during floods, (2) the rapid recolonisation by some species of the first few dozen metres around the non-flooding areas, and (3) the slow recolonisation of the remaining larger parts of the floodplains, the effect that small mammals exert on the vertical metal contamination distribution in floodplain soil profiles by stimulating downward percolation is most significant in these zones immediately surrounding the non-flooding areas. Earthworms are generally present throughout the floodplain, regardless of the distance to the non-flooding areas, which means that bioturbation effects of earthworms are more or less evenly distributed over the floodplain (Zorn et al., 2005). The relative importance of this process is determined by soil type and vegetation structure, especially for earthworms but also for small mammals. As bioturbation affecting percolation processes and thereby the metal redistribution in contaminated soils is only significant under wet soil conditions, it is not important in the non-flooding areas.

Even more important for the redistribution of heavy metal contaminants than the influence of bioturbation on the physical and chemical properties of the soil, is the actual mixing of polluted and clean layers, and the surfacing of contaminated soil by bioturbators. Chapter 5 reports on the quantities of substrate and metals surfaced by Microtidae (*M. arvalis* in ADW) and *T. europaea*, the most important small mammal bioturbators in the floodplains along the river Rhine. Calculations for *M. arvalis* are based on the seasonal distribution patterns described in Chapters 2 and 3, combined with measurements on burrowing activities by this species in field enclosures. We surveyed the distribution and sizes of molehills, which were extrapolated to the total floodplain area. For both *T. europaea* and *M. arvalis*, we took account of the species-specific burrowing activity depths and the metal distributions in a vertical profile as well as the horizontal metal distribution, according to the rough division into three zones for the regularly flooding parts of the floodplain.

In the ADW floodplains approximately 43.7 and 0.24 kg substrate per m^2 are annually surfaced by moles and voles, respectively. These are substantial amounts, implying that the vertical distribution of metal contaminants in floodplain soils shows considerable dynamics. Again, the few dozen metres immediately surrounding the non-flooding areas show the greatest bioturbation effects by small mammals. Although these zones represent only a few percent of the total floodplain area (6% at ADW), much more substrate is surfaced there than in the remaining parts of the floodplains. Differences in sedimentation rates of the metals in recent decades, and probably also differences in the percentages of the metals bound to certain matrix fractions, have resulted in slight differences in the vertical distribution of the metals. For instance, Pb is more concentrated in the soil layers at depths of more than 15 cm compared to the other metals. This results in slight differences between the amounts of metals surfaced, but whereas the amounts of substrate surfaced by moles and voles differ by a factor of 200, the difference for the metals is a factor of 100 (Chapter 5).

Bioturbation by small mammals cannot be ignored, as on an annual basis, the amounts of substrate redistributed represent at least 17% of the total amounts deposited during floods. This only concerns substrate being surfaced by two species, with subsoil redistribution not taken into account. The impact of small mammal bioturbation for the various heavy metals, in terms of the amounts redistributed compared to those redistributed by floods, ranges from approximately 6.5% for Cu and Cd to 13 for Zn and 22% for Pb. This means that bioturbation significantly delays the process of topsoil cleansing in floodplains by the deposition of cleaner sediment resulting from the improved water quality. Earthworms are even more important as bioturbators, in view of their numbers, bioturbating activities and flood tolerance. In fact, a substantial part of the amounts of metals deposited in embanked floodplains originate from the floodplains themselves, due to bioturbation activity. For example, this is true for up to 75% or even more of the total Pb deposition. This means that bioturbating small mammals, together with other burrowing animals, are important constructors of the floodplain landscape, as they influence soil conditions, soil turnover and soil relief, as well as contaminant distribution, not to mention their influence through feeding activities.

8.4 Heavy metal accumulation risks in diffusely and moderately polluted floodplains

Chapters 6 and 7 discussed the risks of metal accumulation in floodplain food webs, and in small mammals and their predators in particular. A new aspect compared to other studies in floodplain environments is that the risks were not only modelled on the basis of soil concentrations, but were identified for the floodplain as a whole by measuring actual concentrations in soil, in vegetation and earthworms as major food sources, and in various species of small mammals, also including spatial and temporal distributions. Species distribution patterns (Chapters 2 and 3) showed that the non-flooding areas are important as exposure sites due to the large numbers of small mammals foraging there on an annual basis, in comparison with the regularly flooding areas. Although significantly higher total metal concentrations were found in the soil of regularly flooding areas, the risks of metal accumulation were not substantially lower in the non-flooding areas in terms of $CaCl_2$-extractable concentrations in the soil and measured concentrations in vegetation and earthworms. We did find differences relating to the investigated metals and to the exposure routes via vegetation and earthworms, leading to higher risks of lead accumulation in herbivores in non-flooding areas and higher risks of zinc accumulation in species feeding on earthworms. On the whole however, the accumulation risks were similar. Extractable concentrations might be important for the risk of accumulation in certain soil animals and their predators (Van Straalen et al., 2005; Vijver, 2005), which might include small mammal species which partially, locally or temporarilly feed on these soil animals. Significantly higher risks of accumulation in small mammals in regularly flooding areas may be expected when soil ingestion is an important route of exposure, which remains an uncertain component in risk assessments.

Chapter 7 focusses specifically on metal concentrations in small mammals. The study involved a comparison between the species, as risk assessment models are generally based on differences in feeding behaviour, after which body burdens are calculated from total soil concentrations (Jongbloed et al., 1996; Pascoe et al., 1996; Kooistra et al. 2005). We found that differences in average metal concentrations in small mammals could be partly explained by their trophic level. Average metal concentrations were highest in the carnivorous/ insectivorous shrew *S. araneus*, while concentrations in the other shrew, *C. russula*, were generally lower and were similar to those in the vole *C. glareolus*. For several metals, they were also similar to those in other vole and mouse species. This may suggest that the diet of *C. russula* includes fewer earthworms than that of *S. araneus*, as earthworms are effective metal accumulators (Hobbelen et al., 2004; Van Vliet et al., 2005). *C. glareolus* seemed to be less herbivorous than has sometimes been suggested (Hjalten et al., 2004; Hamers et al., 2006), and insects and spiders might be a significant part of their diet (Wijnhoven et al., unpublished). This is also suggested by Eccard and Ylonen (2006). The fact that huge intra-specific differences in metal concentrations in small mammals were found indicates that other factors are also involved.

The regression correlations between total or $CaCl_2$-extractable soil concentrations and the concentrations in individual small mammals were low or absent. This does not mean that there is no relation between soil and body concentrations. The metal concentrations at the trapping sites do not necessarily closely reflect the exposure concentration, and exposure time is also important. Other factors tested for their possible relation with the observed metal concentrations in small mammals were life stage, sex, size and trapping season. All factors were found to be significantly correlated to a few metal – mammal species combinations, but no single dominant factor could be extracted for the majority of mammal species or metals. This study proves that metal accumulation risks in floodplains are different from those in inland areas. Flood events have a large impact on species distribution patterns, resulting in large variations in food availability. These in turn lead to foraging for different foods in different areas, as well as differences in dispersal and in patterns and rates of recolonisation. Average exposure times will also be shorter in floodplains due to high mortality during flood

Table 1: Overview of recorded effect concentrations recorded in the literature for the effects of heavy metals in small mammals.

Effect concentrations (EC's) in laboratory experiments and in the wild:

Metal	EC whole body (mg/kg) FW	EC whole body (mg/kg) DW	EC kidney (mg/kg) FW	EC kidney (mg/kg) DW	EC liver (mg/kg) FW DW	Observed effect	Mammal species	Reference
Zn	130	533[#]					*Rattus norvegicus*	1
Cu	12	49.2[#]				NOEC[*]	*Sorex araneus*	1
Pb				25			Small mammals	2, 3
				50.8			*Peromyscus leucopus*	4
				1506		Lesions	*Blarina brevicauda*	4
Cd			30	119			*R. norvegicus*	5
				120			Small mammals	3
		3-24				Loss of body weight, renal injury	*Oryctolagus cuniculus*	6, 7
				105		Cellular damage	Small mammals	8
			100-300	350-1000		LOAEL[*]	Small mammals	8
				33	15	Reduced fetal weight	*Mus musculus*	9
				26	25	Lesions	Small mammals	10
				26	25	Lesions	*S. araneus*	11
			200	800		Onset of damage	*S. araneus*	12, 13
	26	87				NOEC[*]	*S. araneus*	1
	35	120				Effects on growth	*S. araneus*	1
					0.87	Effects on growth	*R. norvegicus*	1
			0.85	3.49[#]			*R. norvegicus*	1

Combi-toxicity observed in the wild:

Tissue	Concentration (mg/kg DW) Zn	Pb	Cd	Examples of observed effects	Species	Reference
Kidney	81.5	0.44	1.19	Cytosolic inclusion bodies	*Apodemus flavicollus*	14
	72.6	1.43	1.16	Swelling of glomeruli, proximal tubules	*A. flavicollus*	14
	89.6	2.51	6.59	Light chains of immunoglobulin	*A. flavicollus*	14
	73.3	93.2	23.58	Hyperplasia to tubules, atrophy	*A. flavicollus*	14
	92.7	1.93	7.23	Blood inflow, thicker distal tubule walls	*Clethrionomys glareolus*	15
	133.2	17.81	32.98	Atrophy of glomeruli, adhesion of Bowman's capsule	*C. glareolus*	15
Liver	117.7	0.18	0.34	Fibrosis, unevenly distributed glycogen	*A. flavicollus*	14
	123.6	0.22	0.25	Cells swollen, vacuoles in cytoplasm	*A. flavicollus*	14
	115.2	0.81	4	Progressive interstitial fibrosis	*A. flavicollus*	14
	99.9	17.61	8.66	Pyknotic nuclei, necrosis, fibrosis	*A. flavicollus*	14
	95.9	1.95	16.39	Nuclei polymorphism, vacuolisation	*C. glareolus*	15

FW =fresh weight; DW = dry weight
[#]Calculated from a 1:4.1 (DW:FW) ratio for whole animals and for kidneys (own data)
[*]Concentrations with no effect: NOEC = No Observed Effect Concentration; LOAEL = Lowest Observed Acceptable Effect Level
References: 1 = Hendriks et al. (1995); 2 = Ma (1989); 3 = Ma et al. (1991); 4 = Stansley and Roscoe (1996); 5 = Chmielnicka et al. (1989); 6 = Samarawickrama (1979); 7 = Nomiyama et al. (1973); 8 = Shore and Douben (1994); 9 = Webster (1988); 10 = Hunter and Johnson (1982); 11 = Hunter et al. (1984); 12 = Bremner (1979); 13 = Dodds-Smith et al. (1992); 14 = Damek-Poprawa and Sawicka-Kapusta (2003); 15 = Damek-Poprawa and Sawicka-Kapusta (2004)

events, with recolonisation by new generations, which also live short lives. This leads to smaller differences in accumulation between species at different trophic levels.

Little is known about effect levels of heavy metals for wild small mammals. Most information has been gathered from laboratory experiments, where a particular daily intake causes toxic effects. The metal concentrations measured in the small mammals from the ADW floodplains were generally below the critical body burdens recorded in the literature (Table 1). In only 1.8% of the specimens of *C. glareolus* was the recorded limit of 130 mg/kg

FW Zn (533 mg/kg DW) for rats (*Rattus norvegicus*) exceeded (Table 2). The 12 mg/kg FW Cu limit recorded for shrews was exceeded in 3.2 and 3.0% of the specimens of *M. arvalis* and *S. araneus* respectively.

Total Pb concentrations regularly exceeded the critical renal concentration of 25 mg/kg DW given by Ma (1989). As for the three vole species, *A. sylvaticus* and *C. russula* between 9.5 and 18.2% of the specimens exceeded this effect concentration for kidney tissue. For *S. araneus* and *M. minutus* even a higher proportion of the specimens exceeded the 25 mg/kg DW, and the 50.8 mg/kg DW at which effects have been recorded for White-footed mice (*Peromyscus leucopus*). It has to be noticed that renal effects of lead have been recorded at much higher concentrations for Shorttail shrews (*Blarina brevicauda*), so shrews might be less sensitive to lead.

Table 2: Percentage of specimens exceeding the lowest recorded effect concentrations for small mammals listed in Table 1.

		Species (n) EC	*A. sylvaticus* (21)	*C. glareolus* (56)	*C. russula* (11)	*M. agrestis* (9)	*M. arvalis* (31)	*M. minutus* (4)	*S. araneus* (67)
Whole	Zn	533 mg/kg DW[1]	0	1.8	0	0	0	0	0
body	Cu*	49.2 mg/kg DW[2]	0	0	0	0	3.2	0	3.0
	Cd	3 mg/kg DW[3]	19.0	3.6	27.3	22.2	9.7	0	30.0
	Cd	24 mg/kg DW[3]	0	0	0	11.1	0	0	0
Kidney	Pb	25 mg/kg DW[4]	9.5	14.3	18.2	11.1	9.7	50.0	26.7
	Pb	50.8 mg/kg DW[5]	0	5.4	9.1	11.1	6.5	25.0	23.3
	Cd	3.49 mg/kg DW[1]	9.5	37.5	100	22.2	3.2	25.0	88.3
	Cd	26 mg/kg DW[2,4]	0	1.8	18.2	0	0	0	21.7
	Cd	33 mg/kg DW[6]	0	0	18.2	0	0	0	16.7
Liver	Cd	0.87 mg/kg DW[1]	4.8	39.3	90.9	0	6.5	25.0	100
	Cd	15 mg/kg DW[6]	0	0	9.1	0	0	0	35.0
	Cd	25 mg/kg DW[2,4]	0	0	0	0	0	0	18.3

* = NOEC; [1] = EC for rats (*Rattus norvegicus*); [2] = NOEC for Common shrews (*S. araneus*); [3] = EC for rabbits (*Oryctolagus cuniculus*); [4] = EC for small mammals; [5] = White-footed mice (*Peromyscus leucopus*); [6] = House mice (*Mus musculus*)

Cadmium might be the most problematic heavy metal in the ADW floodplains, in view of the effect concentration measured in rabbits (*Oryctolagus cuniculus*) (3-24 mg/kg DW) (Table 1). We found concentrations in this critical range in 27.3 and 30.0% of the shrew species *C. russula* and *S. araneus*, respectively, while *M. agrestis* and *A. sylvaticus* also frequently showed Cd concentrations within this range. For *C. glareolus* and especially *C. russula* and *S. araneus*, the measured average concentrations were approaching or even exceeded the critical levels of 26 and 33 mg/kg DW recorded for the kidneys of small mammals in general and those for shrews (Hunter and Johnson, 1982; Hunter et al., 1984) and House mice (Webster, 1988). They also exceeded the 25 and 15 mg/kg DW critical levels recorded for liver tissue in the same studies. It should be noted that higher critical renal levels, but also much lower critical levels of 3.49 mg/kg DW are recorded in literature (Table 2). Critical renal levels of 3.49 mg Cd/kg DW were exceeded by individuals of each species.

In our study, as is generally the case in the wild, small mammals were exposed to a combination of several heavy metals, and possibly also other toxic compounds. In the studies by Damek-Poprawa and Sawicka-Kapustra (2003, 2004), toxic effects were observed at Pb, Cd and Zn concentration combinations in liver and kidney tissues of Yellow-necked mice (*Apodemus flavicollus*) and Bank voles (*C. glareolus*) (Table 1), similar to what we found for the whole body. Hence, it seems realistic to assume that toxic effects on small mammals may result from heavy metal contaminant loads present in the ADW floodplains. This would also be true for other Western European floodplains, as the ADW floodplains are representative of floodplains along the lower Rhine in terms of pollution. Further, higher heavy metal loads are frequently found along other larger Western European rivers like the Meuse, the Oder, and the Elbe (Middelkoop and Van Haselen, 1999; Vink et al., 1999).

It thus seems likely that specimens suffering toxicological effects are present at the ADW, and indications for this were indeed found (unpublished analyses of histological work on the specimens trapped for this study). We do not expect, however, that there will be effects at population level, as life expectancies are short and species reproduce at young ages, viz. a few weeks to a few months. Environmental conditions, especially periodical flooding, have a much stronger impact on the populations. On the other hand, top predators, like Little owl, European badger, European white stork and European common weasel, foraging on small mammals might be at risk from the current contaminant levels. Ecotoxicological risks for predators are especially high when *S. araneus* is an important food species, or when predators are foraging in relatively small areas including sites with very high metal concentrations (hotspots probably related to local point sources). However, as small mammal preys are not very abundant, at least at certain times of the year, we assume that the number of predators in floodplains is limited. It is also assumed that predators in floodplains include especially generalists, which feed on a whole range of mammals, and probably also invertebrates (Kruuk and Parish, 1981; Van den Brink et al., 2003), and that they forage across large areas (Village, 1990; Lange et al., 1994). Many of the foraging areas of predators in Rhine floodplains probably include floodplain as well as hinterland areas, as the embanked floodplains are often narrow and bordered by pastures, farmland and/or conservation areas in the hinterland (Van den Brink and Ma, 1998). This might also reduce exposure to metal contaminants.

Metal accumulation risks to small mammals in floodplains, and to their predators foraging where preys are abundant, are determined by the status of the main exposure sites, which form only a fraction of the total floodplain area. The sites at which exposure takes place are of course not necessarily the sites with high contaminant levels, but are those sites with suitable habitats for small mammals, especially in and near non-flooding areas, or with a good connectivity with such areas.

8.5 Small mammal ‘friendly’ floodplain management

Floodplain environments have the potential to harbour large numbers of various small mammal species, despite frequent flood events. A reduction of the flooding frequency of a floodplain will have a favourable effect on the total numbers of small mammals. However, flood events can support small mammal populations by stimulating the development of rough vegetation types due to high nutrient input and temporarily reduced grazing by cattle, horses, rabbits and herbivorous insects. Further, they may stimulate certain groups of macro-invertebrates which are present as eggs or in larval stages, or are attracted after flooding, and which can provide food for insectivorous/carnivorous species. A crucial factor favouring small mammals, as well as their predators and other species that are not very flood-tolerant, is the presence of enough non-flooding areas to act as refugia. These should offer suitable habitats with a good connectivity between suitable areas in the non-flooding parts and the regularly flooding parts. Small mammals will also be favoured by a gradual flooding of the area and relatively short periods of inundation, rather than fast flooding and long inundation periods. These conditions can often been found in pristine flooding zones (Fig. 1).

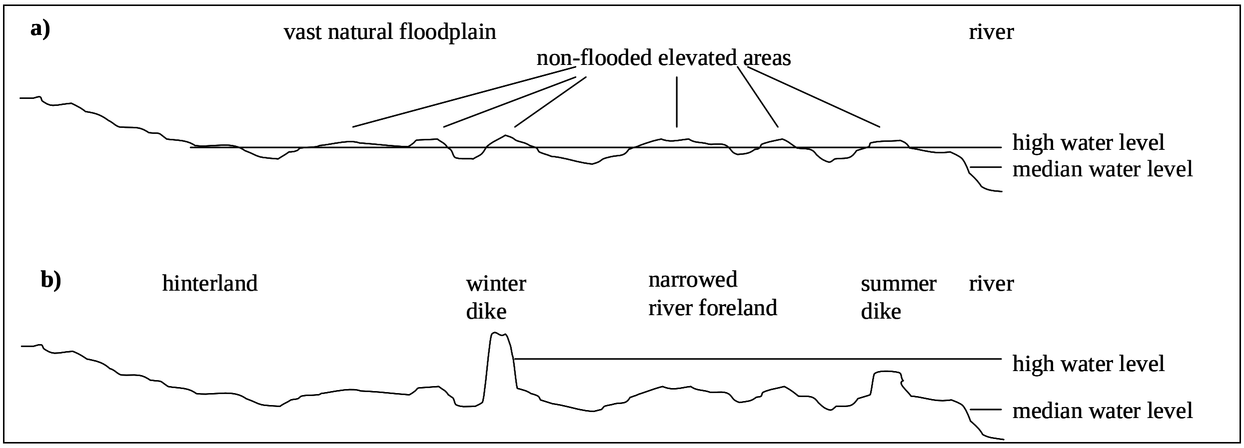

Fig. 1: (a) Pristine vast flooding zones, and (b) embanked floodplains, during high water discharges. As the water-carrying surface of the vast flooding zones is much larger than that of the narrow embanked systems, the water level there rises less during times of high water. The natural floodplain with its varied relief is characterised by the presence of more non-flooding areas where small mammals can survive, and from which recolonisation of the area can start after the flood.

The availability of large areas that can carry water results in water levels which rise more gradually and to lower heights, which means that areas with some relief, which riverine areas usually are, provide more non-flooding areas. In addition, vegetation, e.g. shrubs or trees, can provide more opportunities for temporary survival of small mammals. Inundations will not last as long in such natural floodplains, as water can freely flow back to the river as river water levels there fall. Moreover, the water is in contact with the earth over a larger surface area, so more of it can sink into the soil and the larger water surface causes more evaporation.

However, most of the Rhine floodplains are currently embanked and narrow, creating ‘bathtubs’ between the lower and higher embankments. As water flows over the lower embankment, these ‘bathtubs’ fill up relatively fast with water levels soon rising to several metres. In such narrowed floodplain areas (sometimes known as ‘river forelands’), the chances of survival of small mammals, especially in winter, are very low. It is important for the survival and recolonisation of the floodplain area to have several sources of recolonisation in the form of suitable non-flooding areas with good connectivity. Within the current embanked floodplains, the typical agricultural landscapes have the potential to favour fast recolonisation by several small mammal species. To this end, they should include many linear structures like ditches and field margins, consisting of narrow ridges of approximately 0.5 to 1.5 metres of ungrazed vegetation. Ridges of rough herbaceous vegetation favour all small mammal species we studied, and the presence of ridges of grassy vegetation may favour the development of dense populations of *Microtus arvalis*, *Crocidura russula* and *Micromys minutus*, while that of blackberry dominated vegetations favours *C. glareolus*, *Apodemus sylvaticus*, *S. araneus* and *M. minutus*. Linear structures can support the recolonisation of ‘gradual, density-induced colonisers’, ‘active dispersers’ and ‘long-distance dispersers after a lag’ (Chapter 3), although the third of these types recolonises faster in a patchy environment with separate patches of suitable habitats within an unsuitable matrix. Although the main part of the floodplain, e.g. the pastures and especially maize fields, will harbour low densities of small mammals or no small mammals at all, wasteland parts can function as important habitats for small mammal species. Nature conservation areas can harbour large numbers of small mammals, but here it is important that the densities of large grazers, which are often used in the management of habitat development areas, are kept to a minimum to favour the development of rough vegetation, and rough grassland. In these environments, recolonisation is generally guided by ‘stepping stones’, which should be numerous to favour ‘active dispersers’ and ‘gradual, density-induced colonisers’, although for the last type, the matrix can often function as a suitable habitat. The ‘long-distance dispersers after a lag’ require fewer stepping stones. In nature conservation areas, ungrazed parts can function as important habitats for small mammal species. During the first years after the construction of such habitat development areas, certain parts could be kept free of large grazers, to create rough vegetation habitats within the extensively grazed grasslands to favour small mammals.

Over the last decade, measures have been taken, to increase the water carrying capacity of the Rhine stream corridor to handle the increasing water discharges during periods of high water, and more of such measures are planned. The interventions that are implemented in individual floodplains for this purpose include dike repositioning to widen the floodplain, digging of secondary channels, lowering the level of the embanked floodplain, removing minor embankments such as the summer dikes and removing obstacles like elevated non-flooding areas (Nienhuis et al., 2002; Baptist et al., 2004; Kooistra, 2004). From a habitat management point of view, and for small mammal densities in particular, repositioning the ‘winter dike’ to widen the floodplain is the best option, but this is often out of the question because of human settlements just behind the winter dike or other important uses of the land there. Removing lower embankments like summer dikes and river dunes and elevated non-flooding areas will have a negative effect on small mammal densities, and consequently on predator densities.

Figure 2a schematically shows the situation in an average Dutch floodplain with an elevated non-flooding area and a summer dike as is the case at the ADW floodplains. The area between the summer dike and the river channel is most frequently flooded and the floodplain is occasionally inundated, which results in highest average annual mammal densities being concentrated in the elevated non-flooding areas, including the winter dike. From there, the floodplain is gradually recolonised by small mammals as the flood recedes, but before the entire floodplain is recolonised a new flood arrives. The annual average densities at larger distances in particular will never reach the annual average densities of the elevated non-flooding areas. When the lower embankments are removed, the entire floodplain is more frequently flooded, which leads to lower average annual densities of small mammals within the floodplain In addition, recolonisation will reach less far from the elevated non-flooding areas (Fig. 2b). When an elevated non-flooding area is removed, a source of recolonisation disappears, leading to a situation in which a smaller part of the total floodplain is recolonised (Fig. 2c). A combination of the two measures has the strongest negative effect on the small mammal populations (Fig. 2d). Digging a secondary channel connected to the river actually means partially removing a minor embankment. If a secondary channel is unconnected to the river it can be regarded as a local lowering of the floodplain level. The effect of the lowering of the level of a floodplain is relevant to small mammals, as the floodplain remains inundated for longer periods, which has consequences for the survival of small mammals on small elevations or in trees during floods. This has only a small effect on recolonisation. A temporary side channel periodically holding water and connected to the river channel will probably increase the habitat diversity, and can therefore even have a positive effect on small mammal densities and recolonisation, provided its barrier effect is not too strong.

Floodplains and large river stream corridors play an important role in maintaining or improving Western European biodiversity. Riverine areas function as habitats, as foraging areas and as green corridors between nature conservation areas, and harbour several endangered species (Foppen and Reijnen, 1998). Now and in the near future, as a result of the European Water Framework Directive, and within the context of improving the safety precautions for high water discharges, important decisions will be made on how to construct and manage our floodplains and rivers. There is a trend towards favouring water birds and fish species, as the needs of these species fit in best with floodplain landscapes with high water carrying capacities and low hydraulic roughness. But in the densely populated and intensively cultivated Western European countries, floodplains are also very important for other aquatic, semi-aquatic and terrestrial species. These species, including small mammals as well as amphibians and meadow birds, require good connectivity and heterogeneity in terms of elevation and vegetation with plenty of suitable habitats in non-flooding areas. This will also favour the predators of these species, which include mustelids, birds of prey, herons and storks, making the ecosystem more complete.

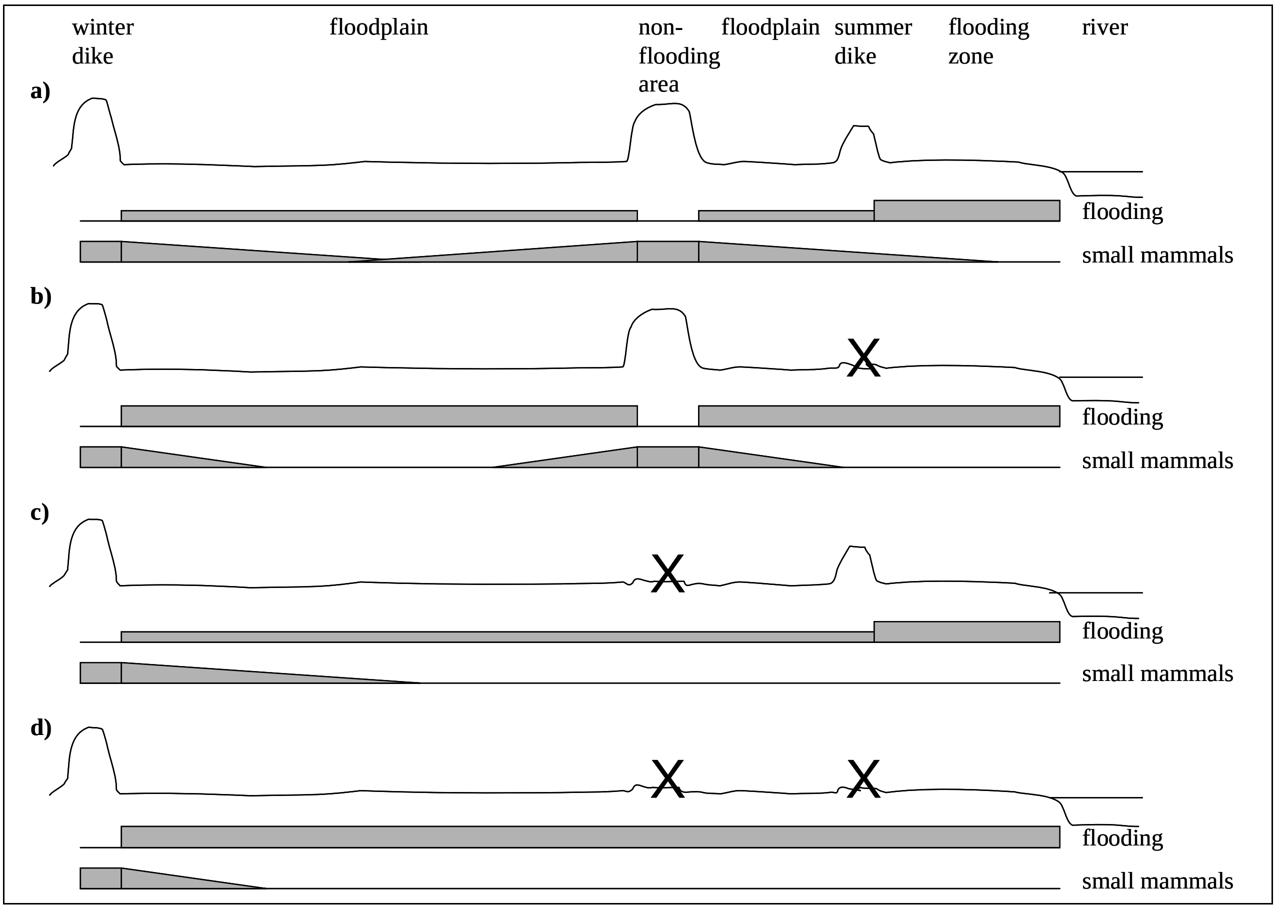

Fig. 2: The effects of measures to increase water carrying capacity enlarging measures on flooding frequencies and small mammal densities in embanked floodplains. The height of the dark bars represents the annual average flooding frequency and the numbers of small mammals: (a) Traditional embanked floodplain with major and minor embankments and one or more elevated non-flooding parts. (b) The effect of removing the lower embankments. (c) The effect of removing non-flooding elevated parts. (d) Removing both the lower embankments and non-flooding parts. **X** means removed elevated area.

8.6 Bioturbation and the implications for management

Metals cannot be degraded but only relocated to environments where they are less harmful, and/or be changed in such a way that ecotoxicological risks are minimised, by complexation, i.e. binding to less reversible or extractable matrix fractions. As the solubility of metals in floodplains is very low, due to the high clay and organic matter contents and a relatively high pH of around 7-8, seepage/leakage of metal contaminants to lower layers is not a major process. Under wet conditions, however, turbation activities such as bioturbation or mechanical turbation (digging or ploughing) may transport metals suspended in soil water attached to small clay and organic matter fragments. Although this process can be temporarily and regionally important, it is uncertain whether the effect is still present at depths of more than 15-20 cm below the bioturbation activity zone. On the scale of a floodplain, it is assumed that about 98-100% of the total metal content in the subsoil remains in place if it is not physically transported by animal or human activities. Only the top layers are transported by weathering or flooding, which can transport metals in a horizontal direction. This means that floodplain topsoil could gradually become cleaner, and metal-contaminated layers gradually become covered with cleaner layers of increasing thickness if the quality of the river water improves. Bioturbation and mechanical turbation cause subsoil and topsoil layers to be mixed, and bring deeper layers to the surface. In view of the considerable amounts of substrate involved, this process will mix contaminated layers with cleaner layers, slowing down the cleansing process. This is clearly exemplified by our findings on lead, for which the sedimentation in floodplains can actually largely be attributed to redistributed material from the floodplain itself. The small amounts of Pb entering the floodplain are probably originating from floodplains upstream. On the other hand, the constant mixing of more and less contaminated layers will slowly reduce the metal concentrations resulting in ecotoxicologically less risky average total concentrations.

Contaminated sediment is moving downstream, stimulated by bioturbation from one floodplain to another, finally accumulating in the sedimentation zones at the transition between rivers and the sea, usually the estuaries. For the river Rhine, this means the North Sea in the Rotterdam harbour area and the ‘Haringvliet’ and ‘Hollands Diep’ freshwater basins. Due to the present ‘bathtub’ shape of Dutch floodplains, this is only a significant process for the areas outside the lower embankments (‘summer dikes’). At these sites, bioturbation activities are low due to the higher flooding frequency. Flooding will become increasingly important when summer dikes are removed in the context of the new river management plans. In view of natural mixing and transport processes such as sedimentation, erosion and bioturbation, the current rules for handling contaminated soils, which lead to (1) delays or cancellations of interventions like ecological rehabilitation plans, (2) the creation of side channels and minor maintenance work on groins and (3) increased costs, are somewhat too strict.

8.7 Biota in contaminated floodplains

Since flooding is the dominant process in floodplains, there is a large difference between the structure and functioning of ecosystems at flooding and non-flooding sites. Figure 3 shows a diagram of the complex of processes, interactions and connections in floodplains, between flooding and non-flooding areas in particular, and between floodplains and their surrounding environments. It should be realised that such a diagram is a simplification of reality, which ignores less important aspects. If a particular aspect changes, however, this can affect the whole diagram, and impact on the importance of various aspects. One of the crucial processes in floodplains is flooding, which makes it important to discriminate between flooding and non-flooding areas, whether one is studying toxic risks, the fate of contaminants or species composition and distribution.

The flooding and non-flooding areas in the diagram only differ in terms of the connections with the river compartment. These connections are, however, crucial, and close similarity between the two types of area will only be found when floods are rare. It is also important to consider that temporal and spatial components cause variability in the relative importance of processes. The food web shown on the left (flooding areas), which is not further specified but exists of a complex web of species and interactions, will quickly become

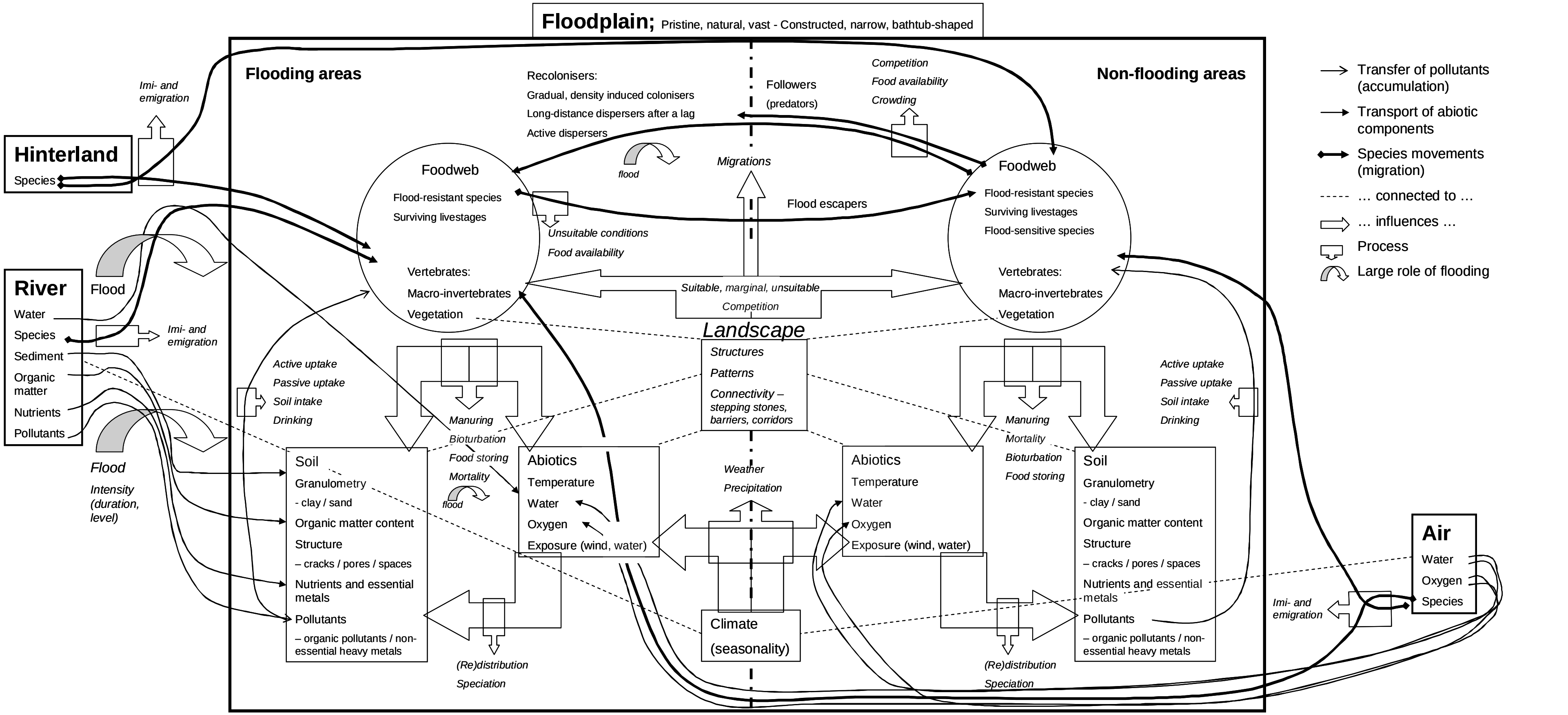

Fig. 3: A ‘simplified’ diagram showing the dominant processes, interactions, connections and movements in contaminated floodplains. Food web interactions and transfer of pollutants are not further specified.

very different when a flood event occurs. Mortality will then be particularly large, leading to a reduction of, or in many cases total absence of, several groups and species. Several flood-resistant species or surviving life stages might, however, temporarily have an advantage and increase, as the competition is reduced and the flood supplies other elements like water, nutrients and organic matter, wood debris and dead plant and animal materials. Flood escapers, especially the larger and more mobile organisms, fill the spaces in the non-flooding areas, extending the food web there even further. This might lead to crowding and increased competition. As the water recedes, this crowding together with the increased suitability of the emerging sites for certain species due to favourable conditions or reduced competition, results in recolonisation. For many species, this recolonisation is a gradual or slow process, guided by the connectivity of the landscape. Other, potentially fast, colonisers also expand slowly, as they follow their prey. When floods are frequent and non-flooding areas scarce, depending on whether the floodplain is closer to the 'pristine' or to 'bath tube-shaped' situation, the food webs in the flooding areas will differ more from those in the non-flooding areas at greater distances to the non-flooding areas and for shorter time intervals to the next flood event.

The recolonisation is guided by the landscape structure, which is determined by abiotic conditions, soil and vegetation. These three aspects are, however, also influenced by the species in the food webs, e.g. through grazing, manuring and bioturbation, and through anthropogenic land use. Colonising or recolonising species in the flooding areas may also originate from outside the floodplain. For species which come from the hinterland and are flood-sensitive, frequently inundated floodplains might function as a sink for their populations. Conversely, communities in the hinterland can benefit from the floodplains, especially the mobile species which find temporarily suitable conditions in floodplains.

All of the above processes have major consequences for accumulation risks and the fate of pollutants. The largest share of the pollutants in floodplains is, or at least used to be supplied by flooding, but nowadays the river may also reduce the risks to floodplain organisms by covering polluted soil layers with clean sediments and even redistributing pollutants from the floodplains seawards. Further availability of pollutants can be influenced by the abiotic conditions which can be changed by flooding and biotic activities. The above diagram, however, shows that temporal and spatial components in accumulation studies and risk assessments cannot be neglected, as the food webs are highly variable.

Some aspects which are of some importance to the processes and the fate of pollutants in particular are not presented in the diagram. These include the specification of the foodwebs including species, which might play an important role releasing nutrients and probably also pollutants from dead organisms back into the soil, and thus influencing the availability of pollutants. Other aspects like compartmentalisation, accumulation of pollutants in certain tissues and focal feeding on special tissues can also have major consequences for the risks of pollutants. Besides the differences between essential and non-essential metals, each element has its specific characteristics determining transfer through the compartments and the food webs.

A factor which is not included in Figures 3, but is generally very dominant, as it can interfere at almost every level and in almost every process, is anthropogenic activity. Examples of human influence include the direct impact on floodplain or landscape structures by digging and building activities and the indirect impact of floodplain management measures, like grazing regime. Anthropogenic activities may even impact on the climate, as is increasingly being realised. We should realise that if we even change one aspect or process of the floodplain ecosystem, a cascade of reactions might follow, which can result in a complete different system. Further we should realise that the models we use will only describe a part or a simplification of the system, and we should always beware that relatively unimportant aspects might become important in other situations.

8.8 Ecotoxicological risks in floodplains and how to reduce them

Total metal concentrations only partly reflect the ecotoxicological risks in floodplains, as was demonstrated in the present study and by others (Houba et al., 1996; Conder and Lanno, 2000; Sahuquillo et al., 2003; Van Straalen et al., 2005; Vijver, 2005). However, this conclusion has not yet been integrated in floodplain policies (Visser, 1993), which are still in use nowadays. Besides total metal concentrations, the first step of prioritisation of sanitation measures for metal-contaminated soils or the selection of the key spots for ecotoxicological risks should also take extractable metal concentrations and concentrations in biota into account. If only total metal concentrations are considered, with or without correction for clay content and organic matter content, there is a major risk of missing the sites with the highest ecotoxicological risks.

As the potentially bioavailable or extractable metal concentrations in floodplains are low, the ecotoxicological risks in areas with relatively high total metal concentrations can be low. However, the risks of accumulation are potentially higher in areas with relatively larger available fractions, e.g. sites where clay layers are being excavated and especially non-flooded areas with sandy soils. When higher total metal concentrations are present in these areas, for instance due to point sources or local spills, these might be the most sensitive spots from a risk assessment point of view and, hence, high-priority areas for sanitation.

Another aspect of ecotoxicological risk assessment, which is generally not, or only partially, taken into account, is the distribution of species in floodplains. Whereas outside floodplains, it is habitat suitability which determines where species may be present, in floodplains flooding is a dominant ecological process. Flood events have a huge impact on the survival of species and concentrate the populations in non-flooding areas. After the flood recedes, populations in these non-flooding areas grow gradually, leading to a slow density-dependent recolonisation of the previously flooded parts, depending of the connectivity of the landscape. Food availability is also influenced by the flood events, causing changes in macro-invertebrate species assemblages and variations in the development of vegetation, the two main food sources for small mammals. This means that specific sites, often comprising only a small fraction of the total area, are much more important as exposure sites for small mammals than the rest of the area. Hence, the most polluted sites are not necessarily the ones which should receive the greatest attention in risk assessment and floodplain policies on metal contamination. Elevated concentrations, either total or extractable, in non-flooding areas or in suitable habitats near non-flooding areas should receive the greatest attention in floodplain policies on metal contaminant risks for small mammals, as this is where the highest annual densities can be found. This is especially relevant for the qualitative and quantitative assessment of risks to small mammals and their predators. The situation might be slightly different for flood-tolerant species and species mainly preying on flood-tolerant animals, but even for these species, habitat suitability and connectivity should be taken into account.

As floodplain areas are highly dynamic environments, in which the suitability of areas in terms of survival opportunities, food availability and shelter opportunities change throughout the year, factors like mortality, shifts in feeding patterns, and shifts in foraging area occur more frequently and show more variation than in inland areas. This has consequences for ecotoxicological risk assessment, as exposure times vary with the age of the animals, exposure routes vary with food items, and exposure sites are also variable. Several parameters in ecotoxicological models should therefore be given different values in floodplains than in inland areas. Specific floodplain models should be created, or ‘inland’ models for risk assessment should be adapted for use in floodplains.

The sources of recolonisation of flooding sites by small mammals in floodplains are relatively easy to identify. As regards habitat suitability and connectivity, the most important exposure sites for species can be identified for existing floodplains, but also for future situations, based on floodplain reconstruction plans. Taking the existing distribution of contaminants and their potential availability into account, the exposure of small mammals and their predators can be minimised by carefully planning the reconstruction measures and the later management of the area.

8.9 Targets of floodplain management

We have demonstrated that areas within and outside the major embankments (‘winter dikes’) differ greatly in terms of physical, chemical, biological and ecological processes and their relative importance, which is reflected in the species assemblages and the changes therein. Hence, these areas should also be treated differently in management and policymaking. The Dutch policy on metal-contaminated soils should be reconsidered. As floodplains are highly dynamic environments characterised by a constant redistribution and mixing of contaminated soils, and as the potential availability of these contaminants is low due to the environmental conditions, it should be allowed to transport or relocate contaminated soils, after taking the necessary precautions, even if the contamination level is assessed as class 4. Under the current floodplain policy, habitat restoration projects involving excavating or relocating contaminated soils are frequently delayed or even cancelled, because the law requires certain costly sanitisation measures. There is limited capacity for such measures and few options are available for using or reusing the soil removed. This makes implementing such plans expensive and time-consuming. Such unnecessary delays and costs do not always benefit nature conservation and rehabilitation, and reduce opportunities for it. Provided certain precautions are taken, the risks of relocating contaminated soils are minimal. As a rule of thumb, one can say that as long as the contaminated soils are not transported to non-flooding or inland areas where the river water influence is minimal, and where soil conditions in the longer term could change due to falling pH and a lack of clay input, the ecotoxicological risks from the metal contaminants will not change.

Another factor that must be taken into account in floodplain management is that of species distribution patterns. This might even require the debate on the targets of habitat development in floodplains to be re-opened. If we envisage frequently flooding marshy areas favouring water birds and several fish species, we are moving in the right direction. If, however, we prefer a place suitable for a whole range of small mammal, amphibian and meadow bird species, including their predators like mustelids and birds of prey, we should provide areas with low flooding frequency by maintaining or extending the number and size of non-flooding areas, and by optimising the spatial configuration of floodplains.

8.10 Conclusions

1. The floodplains along the major Western European rivers are typical narrow, embanked and ‘bathtub’-shaped, which are characterised by a limited number of non-flooding areas and whose appearance is largely determined by agricultural land use or extensively grazed habitat development areas. In these floodplains, small mammals are much more abundant on and near non-flooding areas (i.e. within 120 m) than in the larger remaining part of the floodplains. This distribution is basically the result of the recurrent floods, with a frequency of about once a year, leading to high mortality and concentration of the few survivors in the non-flooding areas. After a flood recedes, recolonisation of the flooding areas is a slow and gradual process, due to the generally poor connectivity

between the non-flooding habitats and the suitable habitats in the flooding areas. As metal pollutants have been deposited as contaminated sediments during flood events over the last century, the highest concentrations are found in the flooding areas. Therefore, exposure of small mammals to heavy metals occurs especially in the areas surrounding the non-flooding sites. However, since the potential bioavailability of heavy metals is greater in the non-flooding areas, due to increased extractability and since non-flooding areas cannot be regarded as unpolluted, a large proportion of the exposure of small mammals leading to uptake of metal contaminants also takes place in the non-flooding areas.

2. The impact of bioturbation by small mammals on the redistribution of sediment and metal contaminants is substantial, especially in places where small mammals are most abundant, that is, in the areas surrounding the non-flooding parts of the floodplain. Compared to the annual deposition during floods, moles and common voles, the most important small mammal bioturbators, annually surface almost 1/5 of the amounts of substrate deposited by floods, and almost 1/15 to 1/4 of the most important heavy metal contaminants (Cu, Cd, Zn and Pb). And the amounts of substrate and heavy metals annually surfaced by earthworms even exceed by far the amounts deposited during floods. Surfaced contaminated substrates can be redistributed locally by weathering and redistributed over the entire floodplain during floods. Although the improved quality of the river water, which means that cleaner sediments are now being deposited in floodplains, the self-cleansing process in the floodplain topsoil is significantly delayed by bioturbation, as cleaner and contaminated layers are mixed. Bioturbation can also result in an increased redistribution of metal contaminants attached to colloid and organic matter particles to deeper soil layers by affecting the percolation process. However, this process is only significant under wet conditions when groundwater levels are high (e.g. just before and after inundations), and is therefore only relevant in relation to bioturbation by small mammals near non-flooding sites.
3. Taking species and metal distributions and potential metal extractability into account, the risks on heavy metal accumulation are comparable in flooding and non-flooding areas. Risks of metal accumulation in small mammals can partly be explained by the trophic level, but foraging behaviour and exposure time are also important. Tissue concentrations in small mammals from moderately polluted floodplains reach and frequently exceed critical concentrations, especially those for cadmium. Toxicological effects on small mammal specimens in moderately polluted floodplains are therefore realistic, but effects at population level are not to be expected, as especially in floodplains natural life expectancies are short and species reproduce at young ages. Top predators foraging on small mammals in moderately polluted floodplains might be at risk of toxicological effects, especially when Common shrews are an important food source. Metal accumulation risks for small mammals and their predators are determined by the status of the main exposure sites, which form only a fraction of the total floodplain area.

8.11 Recommendations for further research

This study aimed to provide a better understanding of the interactions between biota and pollutants in floodplains, focussing on the interactions between small mammals and heavy metals. As the study included ecological cycles, the time allotted for a PhD study is rather short to answer all questions. Moreover new questions arose during the research work, and gaps in knowledge were revealed that could not be investigated within the given time span.

1. It was difficult to relate current metal concentrations in mammals to soil concentrations. This is partly the result of the uncertainty about the locations where exposure of specimens to contaminants takes place. In addition, the exposure time, which equals the age of the specimens or the number of days they forage in contaminated areas, could not be determined in detail. Finally, the exposure route, in other words the food taken, was not determined. More data on metal accumulation risks to small mammals could be obtained by means of stable isotope techniques (Baugh et al., 2004). Stable isotope analyses of mammal tissue and possible food sources from several locations could probably determine the exposure sites and the fractional distribution over food items. This could also reveal shifts in food selection with age and in reaction to food availability, seasonality and/or flood events. The age of animals could possibly be determined by eye-lens size (Gliwicz and Jancewicz, 2001), but this would require a reference series of specimens of the relevant species, of known age. This can be achieved by permanent tagging in the field, or in mesocosm studies. Our studies have already indicated that little is known about food selection, preference and availability for small mammals. Predominantly insectivorous/carnivorous species in particular will encounter changes in food availability over time, in terms of quality and quantity. What is also unknown is the flooding ecology of most macro-invertebrate species. Trapping studies combined with pit-falls to catch macroinvertebrates can provide information about food availability and the consequences of changes in macro-invertebrate assemblages for the exposure of small mammals, as is recently shown by Schipper et al. (2007b). Another uncertainty in risk assessment is the role of soil intake in the metal exposure of small mammals (Beyer et al., 1994), which could be tested in laboratory experiments and feeding trials.
2. The recolonisation models for the various small mammal species could be significantly improved as more monitoring data become available from different floodplains. Data from floodplains with intervals between floods longer than two to three years would be of particular interest, as they would make it possible to determine how long it takes before all suitable habitats in floodplains are recolonised. For management purposes, it would be of interest to determine minimal sizes and spatial configurations of suitable non-flooding areas to maintain populations large enough to recolonise the regularly flooded areas after inundation. Live trapping of small mammals in trees could also give valuable information about survival rates during floods. The use of tags or tracers in foods offered, like dyes which can be detected in fur after they have been consumed, might be very useful in determining the numbers of small mammals escaping to non-flooding areas and areas in the hinterland.
3. It was found to be very difficult to relate histo-pathological abnormalities in small mammal tissues to metal concentrations in the field, and to determine if these represented toxicological effects. A brief preliminary field enclosure experiment provided indications of toxicological effects from the current contaminant levels in moderately polluted floodplains on liver and kidney tissues from shrews and voles (Wijnhoven et al., unpublished). Linking these effects and concentrations would require long-term exposure experiments in field enclosures, possibly in combination with feeding trials in the laboratory, using food collected in the field.
4. Habitat suitability, flooding ecology, recolonisation processes, shifts in species assemblages, foraging behaviour and species-specific accumulation all affect the exposure risks of model organisms (e.g. predators) to metal contaminants. Ecotoxicological and risk assessment models can be improved by including the available data and knowledge. Integrating floodplain-based data and taking spatially and temporally variable ecological aspects into account in ecotoxicological modelling and

risk assessment may be a step forward as is shown by Schipper et al. (2007a). The significance of integrating these aspects should be analysed and compared with traditional models based on broad average floodplain concentrations and accumulation factors.

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Summary

Floodplains are heterogeneous landscapes whose appearance is determined by recurrent cycles of flooding, erosion, sedimentation and succession. They are dynamic transitions between aquatic and terrestrial systems. In Western Europe, however, most floodplain areas have become deteriorated as a result of various human activities, which have transformed them into less dynamic, narrowed, sharply bordered, cultivated, regulated and impoverished systems with embankments, resulting in a 'bathtub' profile. Nevertheless, floodplains still have important functions for nature conservation, harbouring endangered species and forming 'blue-green' corridors through urbanised environments. Interventions to maintain and improve the ecological values of the Western European floodplain landscapes are being implemented. The restoration of the hydromorphodynamics of deteriorated floodplains in ecological rehabilitation projects is a popular approach and is among the most promising of the interventions which are currently being planned or implemented, or have already been completed.

One of the uncertainties in the ecological rehabilitation of floodplains is that many riverine areas are characterised by large quantities of heavy metal contaminants, which were deposited there during floods in the past. These heavy metals, which largely originate from human activities, are persistent substances that have the potential to accumulate in floodplain food webs and can cause toxic effects at various levels. Therefore, the question is whether rehabilitation of floodplain ecosystems is possible in view of current contaminant levels in the floodplain soils. Answering this question requires a better understanding of the processes and interactions between biota and contaminants in natural and cultivated floodplains, as this is where much knowledge is still lacking.

Our studies focused on the interactions between biota, heavy metal contaminants and the environment in floodplains, specifically those relating to small mammals, as they were assumed to be important animals in floodplain food webs. Small mammals (voles, mice and shrews) are ideal for such studies as they are numerous, show fast population growth, are landscape engineers through their feeding activities and burrowing behaviour, and form important prey species. In addition, they are relatively easy to study in trapping studies, live in relatively confined areas and have short lives. Also, various species which differ considerably in terms of life history, habitat preference and feeding behaviour co-exist in small areas. The interactions between small mammals, heavy metals and floodplains can be described by six bi-factorial processes, which differ in importance for ecosystem functioning, but co-occur and interact: (1) bioturbation, (2) accumulation and toxic effects, (3) habitat suitability, (4) landscaping, (5) metal distribution and speciation and (6) competition and toxic effects. Covering these processes, this thesis first focuses on where and when contacts between small mammals and heavy metal contaminants take place. Since little is known about the fate of heavy metals in floodplains, we studied the impact of bioturbation on their redistribution, as well as the risk of accumulation of the current heavy metal loads in the floodplain food webs.

Monitoring the small mammal populations in floodplains showed that the presence of small mammals can to some extent be predicted by the mosaic of landscape structures, if habitat suitability and connectivity are taken into account. Large parts of the floodplains studied can be characterised as unsuitable for small mammals, such as maize fields, most pastures and low, sparse or trodden vegetation types. On the other hand, a vegetation type which often covers only small areas within floodplains, viz. ungrazed rough herbaceous vegetation, can harbour large numbers of several species of small mammals. The dominant process in floodplains, however, is the occasional flooding of most of the area. It was found that the effects of floods on small mammal densities and distributions were still visible after more than a year, or even up to two years, depending on the species concerned and the

connectivity and suitability of the landscape. For an average floodplain, this means that the populations are permanently reduced and restricted to certain areas, as floods occur almost every year. This phenomenon can be explained by the greatly reduced population sizes during floods, the concentration of the survivors in the few non-flooding areas, the gradual population growth and the poor connectivity of the landscape, which slows down the recolonisation of flooded areas as they re-emerge. Hence, small mammal densities are always higher on and near the non-flooding parts than at greater distances from these parts, showing significant differences with densities in areas more than 120 m removed from the non-flooding areas. This was found for all investigated small mammal species. As a consequence, processes like bioturbation and landscaping by small mammals are concentrated in space and time, just like the risks of accumulation and possible toxic effects on small mammals and their predators.

Microcosm experiments with zinc-spiked top layers of floodplain soils showed a decrease in the zinc content of the upper 15 cm, although only the top 2 cm had been turbated. It was especially zinc attached to colloid and organic matter particles which was redistributed from the top layer, while no changes in the extractability of zinc or changes in pH under the influence of turbation were observed. The experiments showed that bioturbation might affect percolation processes under wet conditions, resulting in metal being redistributed to deeper soil layers. Such wet conditions can be expected in floodplains during the period immediately following inundation or at high groundwater levels. The amounts of metals redistributed by this process can be substantial, in view of the numbers, burrowing activities and flood tolerance of earthworms. Several small mammal species, like moles, but also vole and shrew species, were also observed during these wet conditions, especially in the areas adjoining non-flooding areas. Calculations on the amounts of substrate and metal contaminants surfaced in the field, however, show that it is particularly the physical mixing and redistribution by small mammals, especially moles and voles, which substantially influences the fate of heavy metals in floodplains. Depending on the metal being investigated, small mammals redistribute one-twentieth to more than one-fifth of the amounts currently being depositing during floods. If earthworms are also taken into account, bioturbation is a more important determinant of the amounts of metals found in top layers of floodplain soils than the current annual deposition during floods. Consequently, the vertical distribution of metal contaminants in floodplain soils is far from being in a steady state.

Risks of metal accumulation in floodplain food webs were identified for the entire floodplain by measuring actual concentrations in soil, in vegetation and earthworms as major food sources, and in various species of small mammals, taking spatial and temporal distributions into account. As expected, higher total metal concentrations were found in the soils of regularly flooding areas. This is why ecotoxicological risk assessment and floodplain management as regards contaminants traditionally focuses on the regularly flooding areas. However, the risks of metal accumulation were not found to be substantially lower in the non-flooding areas, based on $CaCl_2$-extractable concentrations in the soil and concentrations in vegetation and earthworms. Taking the distribution patterns of small mammals and their predators into account, it can be concluded that the contamination status of the non-flooding areas determines a large part of the risk of accumulation of heavy metals in floodplain food webs. Furthermore, both total and extractable concentrations in the soil and concentrations in biota should be considered in the process of risk assessment and prioritisation of heavy metal contaminated areas for floodplain management.

Compared to laboratory experiments and measurements in inland areas, and the ecotoxicological models based on their results, metal concentrations measured in floodplains show smaller differences between the carnivorous/insectivorous shrews and the predominantly herbivorous voles and mice. Significantly higher concentrations were found in

Common shrews, but metal loads in White-toothed shrews do not differ greatly from the concentrations found in species like Bank voles, Short-tailed field voles and Wood mice the first species. The findings might be explained by differences and variations in exposure time and foraging behaviour. Although some small mammals might suffer toxicological effects from the current contaminant status of the Western-European floodplains, effects at population level are not expected, as ‘natural’ life expectancies in floodplains are short and species reproduce at young ages.

The findings can be used to support decisions about spatial planning and management strategies for floodplain ecosystems in Western Europe. Floodplain management could favour species or communities and reduce ecotoxicological risks.

Samenvatting

Uiterwaardsystemen worden gekenmerkt door periodieke overstromingen, erosie, sedimentatie en successie cycli die een grote variatie binnen het landschap opleveren. Ze zijn de dynamische overgangen tussen het aquatische en het terrestrische milieu. Door talrijke menselijke activiteiten die rond en in uiterwaarden worden ontplooid, zijn veel West-Europese uiterwaarden ecologisch verarmd, en verworden tot ingeperkte, scherp begrensde, gecultiveerde, weinig dynamische en gevarieerde systemen met een 'badkuip profiel'. Desondanks zijn veel uiterwaarden nog steeds belangrijke natuurgebieden, waarin beschermde en bedreigde soorten leven. De aaneengeschakelde natuurgebieden langs de grote rivieren vervullen een belangrijke functie als 'blauw-groene' corridors door de verstedelijkte wereld. Er wordt steeds meer actie ondernomen om de natuurwaarden van uiterwaarden te behouden of te vergroten. Het herstellen van de hydromorphodynamiek in natuurontwikkelingsprojecten is een veelbelovende maatregel die meer en meer wordt uitgevoerd.

Een grote onzekerheid bij de uitvoer van natuurontwikkelingsprojecten is echter de aanwezigheid van grote hoeveelheden zware metalen in de uiterwaardbodems. Deze zijn gebonden aan rivierslib en daar afgezet tijdens overstromingen. De metaalverontreinigingen zijn grotendeels afkomstig van industriële en huishoudelijke emissies en diverse diffuse bronnen (o.a. landbouw en verkeer), en hebben een persistent karakter, en de potentie om in het voedselweb te accumuleren, waardoor toxische effecten kunnen optreden. De vraag is dan ook, of de huidige verontreinigingsniveau's het behoud en herstel van natuurwaarden in de uiterwaarden belemmeren. Om deze vraag te beantwoorden, is eerst meer inzicht nodig in de processen en de interacties die tussen biota en verontreinigingen optreden in meer natuurlijke en gecultiveerde uiterwaarden.

De biota – zware metalen verontreinigingen – milieu interacties in uiterwaarden zijn specifiek onderzocht voor kleine zoogdieren, daar het belangrijke schakels in het voedselweb van de uiterwaarden zijn. Kleine zoogdieren, waartoe de woelmuizen, ware muizen, spitsmuizen en mollen behoren, komen vrij algemeen voor in uiterwaarden, het zijn belangrijke prooien, ze planten zich relatief snel voort, en vormen het landschap door hun graas- en graafactiviteiten. Verder zijn ze goed te bestuderen met behulp van 'live trap' vallen, maken ze gebruik van relatief kleine leefgebieden, en zijn er grote verschillen in gedrag en levenspatroon tussen de verscheidene soorten die overigens ook vaak voorkomen binnen hetzelfde gebied. De interacties tussen kleine zoogdieren, zware metalen en het uiterwaardenmilieu zijn beschreven door zes processen die tegelijkertijd aanwezig zijn en elkaar beïnvloeden. Dit proefschrift behandelt de volgende processen: (1) Bioturbatie, (2) Accumulatie en toxische effecten, (3) Habitatgeschiktheid, (4) Landschaps vorming, (5) Verspreiding en speciatie van metalen, (6) Competitie en toxische effecten. Dit proefschrift beschrijft eerst waar en wanneer contact tussen kleine zoogdieren en zware metaalverontreinigingen optreedt. De rol van bioturbatie in de herverspreiding van zware metalen is onderzocht, om het lot van de verontreinigingen in het milieu te verhelderen. Vervolgens is het risico op accumulatie van zware metalen in het voedselweb bij de huidige verontreinigingsgraad van de uiterwaarden onderzocht.

De aanwezigheid van kleine zoogdiersoorten in de uiterwaarden kan worden voorspeld aan de hand van de landschapspatronen, door rekening te houden met habitatgeschiktheid en connectiviteit van deze habitats. Grote delen van de uiterwaarden kunnen worden gekarakteriseerd als ongeschikt voor kleine zoogdieren, zoals alle maisvelden, de meeste weilanden, lage en schaarse vegetatie en tredvegetatie. Onbegraasde kruidenrijke ruigtevegetatie daarentegen kan grote aantallen kleine zoogdieren van verschillende soorten herbergen. Dit vegetatietype bedekt echter veelal niet meer dan een paar procent van het totale

uiterwaardenoppervlak. De periodieke overstroming van het grootste gedeelte van de uiterwaarden is echter de belangrijkste factor die de verspreiding van de kleine zoogdieren bepaalt. De effecten van overstromingen op kleine zoogdierdichtheden en -verspreiding zijn na een jaar nog waarneembaar, soms zelfs nog na bijna twee jaar, afhankelijk van de onderzochte soort, de connectiviteit, de aanwezigheid van hoogwatervrije gebieden en de geschiktheid van het landschap. Daar overstromingen bijna jaarlijks voorkomen, betekent dit voor een gemiddelde uiterwaard dat de populaties permanent gereduceerd zijn, en zich in hun verspreiding beperken tot bepaalde gebieden. Dit als gevolg van de grote mortaliteit ten tijde van een overstroming, de concentratie van de overlevenden op de niet overstromende hooggelegen delen, de relatief langzame groei van de populaties en de gebrekkige connectiviteit van het landschap wat de rekolonisatie van de voorheen overstroomde delen afremt. Hierdoor zijn de kleine zoogdierdichtheden altijd hoger op en in de omgeving van de hooggelegen niet overstromende delen, dan op grotere afstand van deze terreinen. Voor alle onderzochte soorten kleine zoogdieren zijn significante verschillen gevonden tussen niet overstromende delen en gebieden op meer dan 120 meter afstand daarvan. Als gevolg hiervan zijn processen als bioturbatie en landschap engineering door kleine zoogdieren in tijd en ruimte beperkt, evenals de risico's op accumulatie en mogelijke toxische effecten in kleine zoogdieren en hun predatoren.

Experimenten in proefbakken met uiterwaardbodems met een met zink gecontamineerde toplaag, liet na turbatie van de bovenste 2 cm een afname in de zinkconcentratie zien in de bovenste 15 cm. Met name aan colloide en organisch materiaal gebonden zink werd als gevolg van turbatie herverspreid, terwijl geen veranderingen in extraheerbaarheid of pH veranderingen werden geconstateerd. De experimenten tonen aan dat onder natte omstandigheden bioturbatie wellicht de uitspoeling van metalen naar diepere lagen stimuleert. Deze natte omstandigheden, komen in uiterwaarden voor gedurende een aantal weken na een overstroming, en wanneer het grondwaterniveau hoog staat.

Rekening houdend met de grote populatiedichtheden, hun aanzienlijke graafvermogen en hun overstromingstolerantie, kan worden gesteld dat de herverspreiding van metalen in uiterwaarden door bioturbatie van regenwormen substantieel is. Ook kleine zoogdiersoorten zoals mollen, woelmuizen en spitsmuizen worden al weer vrij snel onder nog natte omstandigheden in de gebieden rond hoogwatervrije terreinen aangetroffen. Berekeningen van substraat en zware metalen die in het veld naar het oppervlak worden gewerkt, laten zien dat met name het fysieke mixen en herverspreiden door kleine zoogdieren, mollen en woelmuizen in het bijzonder, een substantieel effect heeft op de verspreiding van metaalverontreinigingen in uiterwaarden. In vergelijking met de sedimentatie gedurende een overstroming, werken kleine zoogdieren in verhouding een twintigste tot een vijfde deel daarvan, afhankelijk van het onderzochte metaal, boven de grond. Als gevolg van bioturbatie door regenwormen komen meer zware metalen in de toplaag van de uiterwaardenbodems terecht vanuit diepere lagen dan jaarlijks sedimenteert gedurende overstromingen. Zodoende kan worden gesteld dat de zware metalen niet vast liggen in het bodemprofiel, maar dat de verontreinigingen constant in beweging zijn.

De risico's op accumulatie van zware metalen in het voedselweb zijn vlakdekkend in de uiterwaarden geïdentificeerd door de actuele concentraties in de bodem, de vegetatie en regenwormen als belangrijke voedselbronnen, en in verschillende kleine zoogdiersoorten te meten, rekening houdend met de spatiële en de temporele verspreiding. Zoals te verwachten valt, worden hogere totaalconcentraties aan metalen gevonden in de overstromende bodems dan in die van de hoogwatervrije gebieden. Ecotoxicologische risicoanalyses en uiterwaardenbeheer met betrekking tot verontreinigde bodems richt zich traditioneel dan ook op de overstromende delen. De risico's op metaalaccumulatie blijken echter zeker zo groot te zijn in de hoogwatervrije gebieden wanneer het actuele metaalgehalte in de vegetatie en de regenwormen en de $CaCl_2$-extraheerbare fractie in de bodem in ogenschouw wordt genomen. Rekeninghoudend met de verspreiding van de kleine zoogdieren en hun predatoren in uiterwaarden, wordt geconcludeerd dat de verontreinigingsgraad van de hoogwatervrije terreinen, een groot gedeelte van het risico op accumulatie van zware metalen in het voedselweb van de uiterwaarden bepaalt. Het is aan te bevelen om zowel totale als extraheerbare metaalconcentraties als concentraties in biota mee te nemen in risico-analyses en in prioriteitsanalyses aangaande verontreinigingen in uiterwaarden. In vergelijking tot laboratoriumtestresultaten, metingen buiten de uiterwaarden, en modelberekeningen, blijken in de uiterwaarden kleinere verschillen in metaalconcentraties gevonden te worden tussen de carnivore/insectivore spitsmuizen en de voornamelijk herbivore woelmuizen en ware muizen. Er worden significant hogere metaalconcentraties gevonden in bosspitsmuizen, maar de concentraties in huisspitsmuizen zijn niet veel hoger dan die in soorten als de rosse woelmuis, de aardmuis en de bosmuis. Deze bevindingen worden wellicht verklaard door verschillen en variatie in blootstellingsduur en foerageergedrag. Het is zeer aannemelijk dat toxische effecten optreden bij individuen van verschillende kleine zoogdiersoorten bij de huidige verontreinigingsstatus van de West-Europese uiterwaarden. Effecten op populatieniveau worden echter niet verwacht, aangezien de ‘natuurlijke’ levensverwachting in uiterwaarden niet hoog is, en de soorten reeds na enkele weken tot maanden tot reproductie in staat zijn.

De studie biedt diverse vernieuwende feiten en inzichten voor een (hernieuwde) discussie over de inrichting en het beheer van de West-Europese uiterwaarden. Door middel van de inrichting en uiterwaardenbeheer kunnen soorten en gemeenschappen worden bevoordeeld, en kunnen de ecotoxicologische risico’s worden beperkt.

Acknowledgements

So here I am. ‘From the gutter to the stage[*]’, or from fieldwork in the floodplains to the moment when I can defend this thesis. It all started in the summer of 2000. Four years of trapping, collecting, measuring and sampling between the winter dikes and the river Waal. In these years I tried to put myself in the position of the small mammals. The river surprised me several times leaving me searching for traps under 1 m of 4°C water. Truckloads of contaminated substrate were transported to the laboratory, weighed on the field balance and turned inside-out for extractions and analyses, and holes were dug to construct enclosures and to take samples. I tended to look for a central and relatively unexposed spot to camp out on, at least after my first windy and cold experiences on the winter dike. In a mist, I looked for and followed the shadowy outlines of the vegetation to find my way and find back the traps. I checked my area every few hours, following the trails I created by my frequent walks. Further, I searched for my ‘prey’ where they were most abundant. In fact, I became ………… a small mammal!

But I could not do it on my own. I know that I have an unbridled interest in investigating nature and natural processes, but the population of interested students and trainees allowed me to study a wide range of aspects. To start with, there were the ‘mousebusters’, Sara Finote, Mark-Jan Verbruggen and Martijn Oostendorp. We had to learn everything the hard way; trapping non-stop for five days and nights during heavy rain, storm and cold, ending up with only four catches, which were actually only two specimens. After this experience, every trapping session was a success! No matter what, the three of you remained enthusiastic, and I will never forget the great time I had with you in the field and elsewhere. In the meantime, Dennis van den Berge and Eefke Lievaart were the brave students who ventured to act as the turbators in the experimental settings. Such physico-chemical research was not very popular. Eefke followed up Dennis and both did a session which involved a lot of chemical analyses. They both did a great job and kept up their motivation at least till we had the results. Then Alexandra Lequien and Cécile Doukouré came over for the summer. They reinforced our field team and started using the ‘food-trap’ techniques. Alexandra and Cécile, you gave cycling in the floodplains a whole new dimension and the French terms you introduced in the field journals became the standard for all subsequent trainees. Sjors Tonk was so enthusiastic that he insisted on a trapping study, even though I did not have enough time to help him much. Camping out in his mother’s car in the Afferdensche en Deestsche Waarden (ADW) area, he was the first to cover the whole floodplain, supplying valuable information on the distribution of the small mammal species. The accumulation and ecotoxicology studies started with Franciska de Vries. Besides the small mammals, she studied the food sources, for which she even cycled to the ADW during weekends on her sports bike. We discovered that slugs like spirits, but Franciska also laid the basis for a paper, as can be seen elsewhere in this thesis. Joost Smets insisted on doing a graduation project with me. As was to be expected, he was highly creative in using shells and pizza-box pieces to find out which holes were still in use by small mammals, and which were not. Yes Fiepke, I now believe that some people are born to haunt the floodplains during the night. And there was another student who was highly independent, delivering valuable work while I was working on other topics. Marinke van de Ven-Stassen compared our findings in the ADW with those in another floodplain (The Millingerwaard). She was like a mother to the small mammals in feeding trails, which ate from her hand. She also fine-tuned the food-trap methodology. She proved that she deserved to have her own project, which she organised in Bolivia, together with Max. Erika Koelemij even did two projects, starting with accumulation studies, and then identifying relations with histo-pathology in her second project. Thank you

Erika, for all the laboratory work you did. You analysed more than I could publish, and laid the basis for follow-up articles after this thesis. Gerrit Jungheim also did an accumulation-related project, and worked out everything to perfection, from laboratory methodology to reporting, using his own systematic approach. Gerrit, I'm sorry you had to leave us all so soon! It was your critical appraisal and your well-considered opinion, which made me learn at least as much from you as the other way around.

In addition to my immediate buddies in the field and the laboratory, there were several people who occasionally helped me out with checking traps (Eva de Hullu, Martijn Dorenbosch and Marten Geertsma), measuring molehills (Mariëlle van Riel) and building enclosures (Dirk Kruijt and Mariëlle van Riel). Of course, there were also the colleagues at the various departments where I was stationed or where I was allowed to use of the laboratory facilities. When I started my PhD, I was officially a member of the Plant Ecology team, had a working space at the Department of Aquatic Ecology and moved to Environmental Sciences after a while. Later on, as the status of Nature Conservation of Stream Corridors changed into the Centre for Water and Society (CWS) and after that into the Centre for Sustainable Management of Resources (CSMR; 'as dynamic as a natural floodplain'), I followed them, after some delay, to their new accomodation, which is already old nowadays. To make a long story short, I enjoyed working with all the colleagues I met at the various departments and centres, and wish to thank them all very much for the pleasant times I had. I would like to mention a few names in particular. Starting at the Department of Environmental Sciences, where I stayed the longest, I shared a room with Lammert Kooistra. I think we had very useful discussions, in which you were the experienced PhD student, who taught me to look at the world through environmental glasses and whose organising skills were an important example to me. The name of Lammert brings me to GIS, and this means I have to thank Gertjan Geerling, who always helped me out when I got fed up with this programme. I also had valuable discussions on methodology and statistics with Mark Huijbregts and Ad Ragas. I would like to thank Piet Nienhuis, who welcomed me into the laboratory community and introduced me to environmental-style staff meetings, and Jan Hendriks who allowed me to stay a little longer. I also want to thank my later room mate Anne Hollander. Karin Veltman, thanks for the pleasant collaboration. Further, I greatly enjoyed the contacts during coffee breaks, meetings, accidental encounters in the corridors and other activities, including the active runners' group we had at certain stages. In this I also want to include the colleagues at Stichting Bargerveen, RAVON, SOVON and VOFF.

When I was given a place in the CWS river room, the number of room mates increased. As writing became more and more my main task, pleasant interruptions also became more important to me. Martijn Bellemakers, Jeuf Spits, Emiel Kater, Mara Hauck, Marta Wozniak, Jos Brommer and Arie Vonk were always prepared to deliver these, as were the frequent coffee drinkers located elsewhere. Besides writing my thesis, I enjoyed the pleasant cooperation with Emiel Kater, Aafke Schipper and Gertjan Geerling, in the Beuningse floodplains research. This not only yielded valuable results, which will be or are already being published, but the work and discussions also gave me new insights, some of which I have incorporated in this thesis.

Laboratory work and chemical analyses formed an important part of my work. Many thanks are due to the colleagues of the Ecology departments, especially Roy Peters, Germa Verheggen, Martin Versteeg and Marij Orbons who were always helpful to me and my students. I am also indebted to the staff at the Botanical Gardens who were kind enough to accomodate my microcosm experiments and were always helpful, and to Gerard van der

Weerden in particular for discussing the experimental set-up. I also found out that grain-size analyses in columns are time-consumming, and I want to thank Lex Kempers and Gerard Bögemann for their information and cooperation. Of course many thanks are also due to Jelle Eygensteyn, Liesbeth Pierson and Rien van der Gaag, who saw thousands of samples entering their laboratory for metal analyses, and to Tony Coenen, who assisted me in staining slides for microscopy. This brings me to the histological and light microscopy work. Although this is not reported on in this thesis, we (me and my students) did a lot of work in this area as well, and a publication will follow. We could not do this work without the help of Jenny Copius Peereboom-Stegeman. She had straightforward explanations for the strange things we saw (often artefacts), and we learned and laughed a lot at the microscopy sessions.

At the start of my PhD project, I had to learn a lot in a very short time. Part of this was achieved during meetings and discussions at VZZ Arnhem (Dennis Wansink, Maurice de la Haye), RIZA Lelystad (Jos Vink, Jan Hendriks, Piet Bergers), CDL Nijmegen (Fred Poelma) and Alterra Wageningen (Nico van den Brink); I want to thank all of you very much for your time and effort. Albert Corporaal introduced me to the floodplains and provided me with much inspiration for the research plans. Then there were the SSEO colleagues, many of whom were starting PhD students like me. We exchanged ideas, had interesting informal meetings and field visits and met each other during courses and symposia. I joined forces with several of them in collective measurements, experiments and fieldwork. Of course I must mention Mathilde Zorn, who participated in the same bioturbation project. We often walked across the floodplains together, collected all kinds of samples, analysed samples together, discussed our findings, and prepared joint presentations. It was always great to work with you, Mathilde. Furthermore, I would like to thank all Animal Ecology colleagues of the Vrije Universiteit (VU), and especially Kees van Gestel, who was often involved in the joint work. Joint fieldwork was done with Diane Heemsbergen and with Ivo Thonon, the most interactive researcher within SSEO, even though he was not a participant. This joint fieldwork significantly reduced my workload, and in the end led to a valuable publication. Joint experiments were also started with Petra van Vliet and Wim Ma. Although we had a lot of start-up problems, which finally led to skipping the small mammal-related experiments, at least I learned a lot from the trapping and analysing of specimens outside the ADW floodplains. Part of the ecotoxicological research was done together with Timo Hamers, another example of a pleasant SSEO cooperation. And of course we couldn’t have done the monitoring work without the traps we borrowed from Erik Schellekens (Arcadis) and Alterra. I found the exchange of trapping experiences with Paul de Bie and Wim Dimmers always very valuable. Further I would like to thank Jan Klerkx for his linguistic comments. He saw this whole work pass through his hands in pieces, and at that time the Chapters were definitely less readable than they are now, after his corrections. Martijn Dorenbosch, thanks for your help with the cover. Then I would also like to thank the State Forestry Services (SBB) and Stichting Ark for their permission to do research in the ADW and Millingerwaard floodplains. Thanks are due to all the visitors of the floodplains who left the traps untouched. I had pleasant conversations with several of them, and occasionally they pulled my van out of the floodplain mud.

Of course many, many thanks are due to those who made the whole project, and my PhD studies in particular, possible. They had the original ideas, and saw opportunities for me. Hours and hours of discussions about planning, experiments, results, theories and manuscripts followed, not to mention the reading and correction of my ‘brews’. First of all, my supervisor Toine Smits. As he once said; ‘At first we had to sniff each other out’ as we had quite different characters, but the coalition worked. I very much appreciate the freedom he gave me to choose the research direction, and to incorporate aspects which had my special interest. Toine often responded to new ideas with great enthusiasm, and he knew how to encourage me. Although I did not meet him weekly, the e-mails I sent to my second supervisor Herman Eijsackers were always promptly answered. He very often pinpointed the weak points in my manuscripts, and if there is one person who is able to come to the point and summarize a discussion or meeting in one sentence, it is Herman, as he demonstrated at our meetings in Chinese restaurants and railway stations. Besides these two world-travelling supervisors with full diaries, I had two secondary supervisors, who, although they had similarly full diaries, always made time for me. I have known Gerard van der Velde for a long time, as he was once my buddy during my first tropical dive in Indonesia, where he also taught me all about the routines of Dutch comedians from the 1960s and 70s, and introduced new card-shuffling techniques. Later on, I worked with him as a voluntary researcher in projects on gammarids and molluscs. In many ways, he was/is my personal trainer. I am not sure if this is also true for our running, but the progress of my research, as well as all kinds of other subjects often less serious, was discussed weekly in the forest, while we were chuming out the kilometres. Gerard, I am sure our collaboration will not stop here, as there is still a lot to investigate, for which I will always be in need of a running humoristic ecological encyclopaedia. Rob Leuven can be characterised by his words during the last stage of the writing of my thesis, when I also had a new job; ‘Sander, don’t work too much during the weekends: weekends are for your girlfriend’. Although Rob is a passionate researcher, who knows how to encourage PhD students, he always has an eye for the person behind the researcher, and knows his students personally. Also, together with Gerard, he did an incredible job in commenting on and rephrasing my lines. He always found regressions and general rules where I saw only a chaos of measurements. Rob, you were always a great audience for my ideas and were able to solve many of my scientific problems. Rob and Gerard, without your participation, the manuscripts would not have been publications now. I also thank the manuscript commission, Prof. Dr. J.M. van Groenendael, Prof. Dr. A.J. Hendriks and Prof. Dr. N.M. van Straalen for their work.

But I will not forget where it all began. Mum, dad, you instilled in me an interest in nature. Even when I was very young you pointed out to various fascinating organisms and showed me the world by taking me for walks in the forests, and by visiting other countries. Where do you think my interest in nature comes from, with a Nijmegen biologist for a father? I should also mention my grandmother and grandfather in this respect, as they went bird-watching with me, took me fishing, and taught me to recognise the most important plants. Mum, dad, you encouraged me to go to university. Actually, you encouraged me in everything I did and wanted, whether it was travelling to the other side of the world, looking for a job in science, or running marathons. Thank you for everything.

Then there are a lot of people who are very important to me, and who played an important role in my producing of this thesis. There are those who diverted my thoughts, while not having a clue what I was doing during the week, and with whom I principally talked about other things. First of all, they include the ‘Duveltjes’; if you are one or if you are related to them, you will know who I mean! Our connections lay in bars, in music, in festivals, in holidays, in activities, nowadays even in sports. I enjoy hanging out with you, and hope we will have lots more gatherings in the future. All the people with whom I went running, cycling and diving over the years, thank you for the pleasant time, the exercise and the competition; tomorrow we will continue them all. Of course, I will always remember the great time we had during our ‘Autumn trekkings’, so they will be on the agenda every year as well. I don’t know if I will have the time to travel to the tropics every year, but my trips to countries like Indonesia and Tanzania were great experiences, not least because of the people I travelled

with, or whom I met. I am certainly planning to travel again and new destinations are on the cards!

Last, but definitely not least, I want to thank you, Dunja. When I met you, all the practical work for this thesis had actually been completed. You only saw me writing and calculating day in day out, and later also in the evenings and several weekends. You gave me the opportunity to complete my thesis, even though this meant that I had less time for you. I cannot promise that I will never work in the evenings again, as science is an important part of my life. But I can promise that we will live together again soon, whether it will be in Zeeland, in Malden, or at the other end of the world. Dunja, you are the best support I could have wished for. I love you.

Sander Wijnhoven,
Malden, Juni 2007

By Martijn Dorenbosch

*Album by Savatage (1995)

Curriculum vitae

Sander Wijnhoven was born on 5 August 1973 in Lieshout, the Netherlands. From 1978 till 1987 he grew up in the village of Bergeyk, in the farming country at the heart of the Brabantse Kempen region. After emigrating to the countryside near the small Belgian town of Lommel (a migration route of about 9 km), Sander was getting more and more interested in hiking through forests, canoeing in all sorts of waters and camping in the wild; his interest in nature was born. At that time he was going to secondary school (Rythovius College) in Eersel, the Netherlands, which meant that cycling (e.g. to school, bars or friends) became a hobby. It must have been around 1986 that he got interested in 'Metal' for the first time. In 1992, Sander took part in his first running contest, completing the 13.6 km 'Fire Brigade Border Run' within an hour.

In 1992, Sander started to study biology at the University of Nijmegen (KUN; later renamed Radboud University; RU), the Netherlands. He got a room at the 'Vossenveld' hall of residence, where he much later celebrated his 12.5 year anniversary of living in student accomodation. During the years of commuting between Lommel and Nijmegen, Sander climbed several 'Cols' with his fully packed 'Gazelle Tour de France' bike during the holidays, and discovered the 'Seven Hills' run.

His biology studies included three six-month graduation projects. The first project was on the general stress response of salmonids (Salmonidae), for which he worked at the Department of Animal Ecology (KUN) and did experiments at the Norwegian Institute for Freshwater Research (NIVA) in Bergen. During his second project, he investigated the temperature-dependent growth of perch (*Perca fluviatilis*) in the field and in the laboratory, a study which was implemented at the Centre for Limnology of the Netherlands Institute of Ecology (NIOO), and at the Department of Aquatic Ecology and Environmental Biology at the KUN. For his third project, Sander went to Indonesia for five months to work on the importance of seagrass meadows for reef fishes, in cooperation with the Universitas Hassanudin (UNHAS) in Ujung Pandang (South Sulawesi) and the KUN Department of Environmental Studies. Of course, this was a great opportunity to start SCUBA diving, and this was also where he lost his heart to this beautiful country. He was also active at the university as a mentor to help new first-year students find their way around the university and as a member of the committee organising the introduction programme for new students. He received his master's degree in biology in 1997.

Between 1997 and 2000, Sander worked several times as a teaching assistant at student practicals at the KUN and worked for a year as a voluntary researcher on the ecology of invasive species (Gammaridae) in the river Rhine, at the Department of Aquatic Ecology and Environmental Biology (KUN). He also worked for a year on a temporary contract as researcher at the Department of Evolutionary Microbiology (KUN), on the genetic diversity and biogeography of bivalves and aquatic snails. Between 1998 and 2000, Sander also worked as a travel guide for SNP Nature Travels, guiding walking and hiking tours in several countries (Turkey, Thailand, Poland, Spain, Italy and Switzerland).

In the year 2000, Sander started a new challenge in the form of a PhD study on 'The role of bioturbators in the purifying capacity of floodplains' at KUN, in the context of the NWO-SSEO programme, supervised by Prof. Dr. A.J.M. Smits, Prof. Dr. H.J.P. Eijsackers, Dr. R.S.E.W. Leuven and Dr. G. van der Velde, one of the results of which is this thesis. During this period, Sander supervised 14 students in their MSc projects and research traineeships, assisted several students with scientific presentations and reports, gave several lectures and organised field trips during courses as well as giving presentations at several international symposia (e.g. NWO-SSEO, IGBP, Soil & Water, Rodens et Spatium, SETAC). In the meantime, Sander improved his PRs on the half marathon (1 hour 27 min. 28 sec.;

Eindhoven) in 2001, and over 15 km (58 min. 07 sec.; ‘Seven Hills’ run) in 2004. Another new challenge started in 2003, when Sander could afford to buy a sports bike. The annual ‘Diekirch-Valkenswaard’ cycle race (250 km) led to a PR of 9.5 hours in 2006, and in 2005 he completed the ‘Amstel Gold Race’. In 2005 and 2006, Sander worked as a Junior Researcher at the Centre for Sustainable Management of Resources (CSMR) at Radboud University Nijmegen (RU), on ecological impact assessment and monitoring for the cyclic rejuvenation plans for the ‘Beuningse Uiterwaard’ floodplains. In this period, he moved to the quiet village of Malden, where he is currently still living.

Since April 2006, Sander has been working as a researcher at the Monitor Taskforce of the Centre for Estuarine and Marine Ecology (NIOO-CEME) in Yerseke. His research there focusses on the macrozoobenthos and fish assemblages of Dutch marine and estuarine waters with their historic trends and current developments.

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By Erica Nabben-van Montfort & Annelies Vogels

Room for notes:

Even more room for notes: