

Spiders as bio-indicators of anthropogenic stress in natural and semi-natural habitats in Flanders (Belgium): some recent developments

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Summary

Spiders have been extensively used as ecological indicators in nature conservation and management in Flanders (Belgium). Recently, research projects have been set up to assess the effects of heavy metal pollution and habitat fragmentation on spider populations. From the first results of these studies, it seems that spiders could be good bio-indicators for evaluating the impact of these anthropogenic disturbance factors on natural ecosystems.

Spiders as bio-indicators

Until recently, spiders were used only as ecological indicators. The term “ecological indicator” was defined by Blandin (1986) as a special case of bio-indication in which the absence or presence of a species and, in the latter case, its abundance are the bio-indicator. We have used spiders as ecological indicators to evaluate the nature conservation value and the biodiversity of particular sites or to evaluate the effects of changes in habitat structure brought about by nature-management measures. The approach is straightforward and simple (Maelfait & Baert, 1988; Maelfait, 1996). Situations differing in features assumed to be of importance for nature conservation or nature management are sampled for their spider fauna by means of pitfall traps. By means of multivariate techniques, the different sampling sites are ordinated or classified. From this analysis, the factors determining the composition of the spider communities are deduced. This approach has been applied for spiders of dune habitats (Maelfait *et al.*, 1997), woodlands (Maelfait *et al.*, 1991, 1995), marshlands (Maelfait *et al.*, 1992, 1993), grasslands (Maelfait & Seghers, 1986; Maelfait *et al.*, 1988; Maelfait & De Keer, 1990) and heathlands (Maelfait *et al.*, 1990;

Maelfait, 1993)—all in the northern part of Belgium.

Here we want to illustrate how we have tried to use spiders for other kinds of bio-indication, in which individuals and populations of spiders are analysed in more detail. Two anthropogenic disturbance factors which are generally considered to have important effects on wild organisms in densely populated and economically highly developed regions are pollution by heavy metals and habitat fragmentation. Although at first apparently of a quite different nature, they are both suspected of being able to cause bottlenecks in populations of wild organisms. That is why we have investigated the effects of both factors at the same time and why we report here the first results of these combined studies. The occurrence and the type of problems these two disturbance factors may cause to, amongst others, spider populations (as bio-indicators) are described in some detail.

Anthropogenic stress to natural and semi-natural ecosystems

A first factor of human disturbance which may affect spider populations is exposure to high concentrations of heavy metals. In the northern part of Belgium, as well as in places close to

metallurgical plants, high concentrations of these elements also occur along polluted rivers, along motorways and in forests.

Spiders and other invertebrates were collected in the marshes along the banks of the River Scheldt (Hendrickx *et al.*, 1998). Like most of the rivers in the northern part of Belgium, the Scheldt is heavily polluted by agriculture and industry; this is true for both water column and underwater soil (Stronkhorst, 1993; Van Gils *et al.*, 1993; Zwolsman & Van Eck, 1993). It therefore seemed worthwhile to investigate the degree of contamination by heavy metals occurring in the terrestrial habitats alongside that tidal river. At extremely high tides, these marshes are flooded by brackish or (higher up) by fresh water.

Another habitat type in which we looked for heavy metal contamination in spiders is forest. Only about 8% of the surface of Flanders is covered by forest. The few remaining older forests, as well as the more recent forest plantations, are very small and are therefore highly vulnerable to airborne pollution.

The density of roads in Flanders is one of the highest in the world. This also means a high total surface of roadside verges. These verges are considered to be of importance for nature conservation; in some parts of our region they are the only remaining more-or-less natural habitats. The motorway verges in Flanders are either planted with shrubs and trees or they are regularly mown in a nature-friendly management regime aimed at producing grassland vegetation rich in flowering plants. As shown by Dutch research, relatively large roadside verges may have an ecological function as a corridor for the dispersal of ground-living arthropods between otherwise isolated natural areas (Vermeulen, 1994; Vermeulen *et al.*, 1994). However, it has to be realised that if these roadside verges are too heavily polluted by, for instance, heavy metals, animals using these connecting strips may bring contamination into the natural areas they connect.

Fragmentation is a second environmental stress factor for whose effects we have tried to develop a bio-indication system. In almost the whole of Europe, intensification of agriculture and transport and urbanization has led to a situation in which all more or less natural areas are small or, at best, relatively small patches

distributed in a nature-unfriendly matrix. Flanders, the northern part of Belgium, could be a textbook example of the massive degradation of natural landscapes. Owing to rapid economic development after World War II, almost unconstrained by any rural planning, natural and semi-natural habitats in Flanders became highly fragmented. Populations bound to habitat types only occurring in these more natural areas are therefore small and isolated. This may lead to population dynamics problems: stochastic oscillations may cause local extinction of these small populations. As well as a reduction in average population size, habitat fragmentation also increases isolation of populations. This situation is expected to result in genetic impoverishment (Avisé, 1996) and a reduced capacity to adapt genetically to environmental changes: local or global, anthropogenic or natural. We have recently started a research project, in which several universities and institutes are collaborating, to investigate the extent to which loss of genetic biodiversity has occurred in the remaining natural areas of Flanders. The project seeks to contribute to the development of bio-indicators for the occurrence and the degree of genetic isolation and impoverishment. A main goal of modern nature conservation in our region is to try to enlarge the remaining natural areas and to connect them by corridors and stepping stones. This will result in larger populations or, at least, to small populations interconnected by dispersing individuals, in other words to metapopulations. Theoretically, both outcomes would result in larger populations with a lower risk of loss of genetic diversity. The extent to which these theoretical expectations are achieved will have to be checked by monitoring by means of bio-indicators for fragmentation/defragmentation effects.

Heavy metal concentrations in spiders

Tables 1–4 show concentrations for cadmium (Cd) and lead (Pb) in spiders and some other taxonomic groups, expressed in ppm. The animals were all captured by hand and killed in a freezer. After washing, they were dried for 48 hours at 70 °C. Metal concentrations were determined by means of Atomic Absorption Spectrometry (AAS) on a number of individuals with a total dry weight of between 20 and 100 mg.

Lead and cadmium, in particular, were determined because they are, as far as is known, two non-essential elements (Hopkin, 1989).

Table 1 lists the concentrations of Cd in animals captured in brackish marshes along the tidal River Scheldt. The concentrations in the investigated spider species are often very much higher than in the species of the other groups which are mainly detritus-feeding animals. In the first and the second marshes, the highest concentrations are measured in the spider species. In the third marsh, Saeftinghe, two of the three spider species and the lumbricids show the higher concentrations. In the fourth marsh, all spider species and three of the woodlice species are highly contaminated.

Table 2 lists concentrations of Cd found in spiders, two woodlouse species and a terrestrial amphipod living in the tidal marshes of the freshwater part of the Scheldt. Again, very high Cd concentrations are noted in the spider species, with very wide fluctuations between species and places.

Cd and Pb concentrations of spiders and woodlice captured in forest habitats are given in Table 3. Zoniën Leo is situated in the immediate vicinity of a very busy motorway near Brussels. Lead pollution is reflected by the very high concentrations of that metal in the woodlouse *Oniscus asellus*. In comparison with cadmium, lead seems to be have accumulated to a lesser extent in the spiders.

Another example of lead and cadmium pollution along motorways is presented in Table 4. Here, spiders and woodlice were captured on four roadside verges near Ghent, alongside the E17, a motorway with a very dense traffic. Here, relatively high lead concentrations were also noted in the spider species. Like the results for the animals captured in the beech forest, cadmium values are not that different for woodlice and spiders.

An important conclusion from the results above is that the degree of contamination on a particular site can differ considerably between different groups, between different species and even between developmental stages of the same species. Therefore, the same stage of the same species should be used to compare different sites with each other. Lumping different species in the same sample should be avoided.

	Cd
Groot buitenschoor	
<i>Carcinus maenas</i>	–
<i>Ligia oceanica</i>	2.5
<i>Orchestia gammarella</i>	2.1
<i>Tegenaria picta</i>	10
Paardeschor	
<i>Carcinus maenas</i>	2.8
<i>Sphaeroma rugicauda</i>	1.3
<i>Orchestia gammarella</i>	2.5
<i>Pardosa purbeckensis</i>	17
Saeftinghe	
<i>Lumbricus</i> sp.	17
<i>Porcellio scaber</i>	4.2
<i>Orchestia gammarella</i>	2.9
<i>Erigone longipalpis</i>	2.2
<i>Pachygnatha clercki</i>	8.5
<i>Pardosa purbeckensis</i>	11
Galgeschoor	
<i>Armadillidium vulgare</i>	4.8
<i>Ligia oceanica</i>	26
<i>Oniscus asellus</i>	17
<i>Philoscia muscorum</i>	27
<i>Sphaeroma rugicauda</i>	1.4
<i>Orchestia gammarella</i>	5.6
<i>Pachygnatha clercki</i>	23
<i>Pardosa purbeckensis</i> (juv.)	28
<i>Pardosa purbeckensis</i> (ad.)	28
<i>Trochosa ruricola</i> (juv.)	55
<i>Trochosa ruricola</i> (ad.)	21

Table 1: Cadmium concentrations (ppm) in spiders (in bold), crustaceans and lumbricids in four brackish marshes along the River Scheldt.

	Cd
Bazel (hoogspanning)	
<i>Pardosa amentata</i>	19
<i>Pirata piraticus</i>	–
<i>Tmeticus affinis</i>	–
Durme (28)	
<i>Centromerus sylvaticus</i>	–
<i>Clubiona phragmitis</i>	–
<i>Pardosa amentata</i>	5.6
<i>Pirata piraticus</i>	–
Berlare	
<i>Trachelipes rathkei</i>	2.8
<i>Clubiona phragmitis</i>	45
Moerzeke (15N)	
<i>Porcellio scaber</i>	3.6
<i>Orchestia cavimana</i>	–
<i>Clubiona phragmitis</i>	19
<i>Pirata piraticus</i>	21

Table 2: Cadmium concentrations (ppm) in spiders (in bold) and woodlice in four woodland habitats in Belgium.

	Cd	Pb
Enamebos B		
<i>Pardosa amentata</i>	7.7	–
<i>Oniscus asellus</i>	21	15
Walenbos F		
<i>Meta segmentata</i>	11	–
Spider spp.	7.5	–
<i>Philoscia muscorum</i>	15	15
<i>Oniscus asellus</i>	17	13
Zoniën Leo		
<i>Coelotes terrestris</i>	15	1.5
<i>Oniscus asellus</i>	21	49
Zoniën F		
<i>Coelotes terrestris</i>	15	1.4
<i>Oniscus asellus</i>	17	16

Table 3: Cadmium and lead concentrations (ppm) in spiders (in bold) and woodlice in four woodland habitats in Belgium.

The need for bio-indication is emphasized by the data brought together in Figure 1. In eight marshes along the freshwater part of the River Scheldt, litter and soil were sampled in a standardized manner; specimens of *Pirata piraticus* were also collected (> 20 mg of dry weight from each site). As can be seen, no simple relationship exists between litter and soil cadmium concentrations and those measured in *P. piraticus*. This is presumably because the concentration in the spider is dependent not only on the concentration of the element in the soil but also on the availability of that heavy metal to its prey items. It is known that the availability of ecotoxic substances to organisms living in a particular habitat is also influenced, in a rather complex manner, by environmental factors such as: soil texture, organic matter content of the soil, cationic exchange capacity, pH, etc. (Tack & Verloo, 1996). This leads to the conclusion that the degree of contamination in biota cannot be deduced in a simple manner from concentrations in their environment (e.g. soil concentrations): bio-indicators have to be used as well.

In spiders, cadmium in particular is often accumulated at relatively high concentrations. This correlates with the food chain experiment by Clausen (1989) in which he showed that the cadmium concentration in the spider *Steatoda bipunctata* was five to seven times higher than that in the contaminated fruit flies on which they were fed. As shown here and earlier (Clausen, 1986), very high concentrations of lead occur in

	Cd	Pb
UZ		
<i>Pardosa pullata</i>	7.3	24
<i>Porcellio scaber</i>	16	370
Eke		
<i>Pardosa pullata</i>	4.9	16
<i>Pardosa palustris</i>	4.2	16
<i>Porcellio scaber</i>	10	130
Lochristie		
<i>Pardosa palustris</i>	7.0	22
<i>Porcellio scaber</i>	29	170
Desteibergen		
<i>Pardosa palustris</i>	7.7	11
<i>Porcellio scaber</i>	8.7	110

Table 4: Cadmium and lead concentrations (ppm) in spiders (in bold) and woodlice in four verges along the motorway E17 in the vicinity of Gent.

spiders along motorways. We therefore think that, together with other animal groups (e.g. woodlice), spiders have good potential for use as indicators of the availability of these ecotoxic elements: in other words, as indicators of the effects of this kind of man-made disturbance to ecosystems.

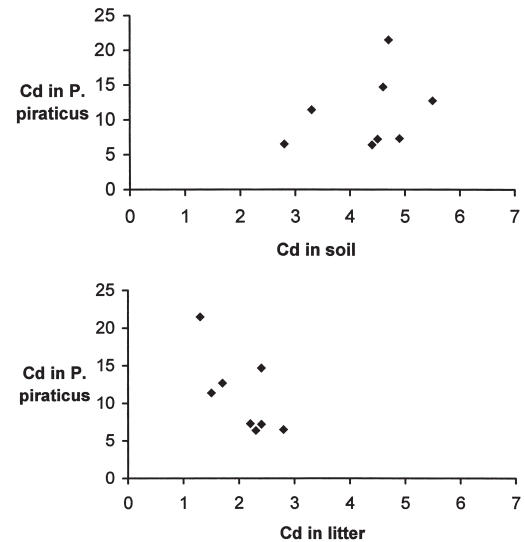


Fig. 1: Cadmium concentrations in adult *Pirata piraticus* (ppm) versus Cd concentration in soil and litter for eight marshes in the freshwater part of the River Scheldt.

Biological effects of heavy metals

The uptake of heavy metals in the food chain is one thing, but their incorporation does not necessarily imply that they exert any significantly harmful effect on individuals or populations. More important questions are: (1) do these substances cause changes in the development and the growth of organisms; and (2) do they cause a change in the fitness of populations or of particular segments of populations; in other words, do they exert selection and will they cause an evolutionary change?

Developmental instability

Genetic and environmental disturbance can result in developmental instability, which can be measured by fluctuating asymmetry (FA), i.e. the asymmetry that results from the inability of organisms to develop along precisely determined pathways, leading to differences between the two sides of bilateral organisms (Markov, 1995). These differences between left and right can be used as biological indicators of environmental quality (Markov, 1995).

To detect these levels of FA in the population of *Pirata piraticus* of the Galgenschuur, a highly polluted brackish marsh in the port area of Antwerp, we compared the distance between the two dorsal spines on tibia IV of 21 egg-sac-carrying females on the left and the right sides of these spiders. From a number of possibilities tried (e.g. total length of tibia, length of metatarsus), this measurement proved to have the highest reproducibility. To check that the differences between left and right side were not due solely to measuring errors, we conducted each measurement four times independently. To exclude the possibility that differences between left and right were the result of other types of asymmetry, or were due to measurement error, two statistical requirements have to be fulfilled (for details, see Palmer, 1994). Firstly, a Kolmogorov-Smirnov test for normality was conducted to distinguish FA from non-directional and directional asymmetry. Departure from normality was not significant, meaning that the differences can be considered as being due to FA. Secondly, to test for the possibility that differences between left and right are not due to measurement errors, a two-way ANOVA (sides, individuals) was conducted. This was highly

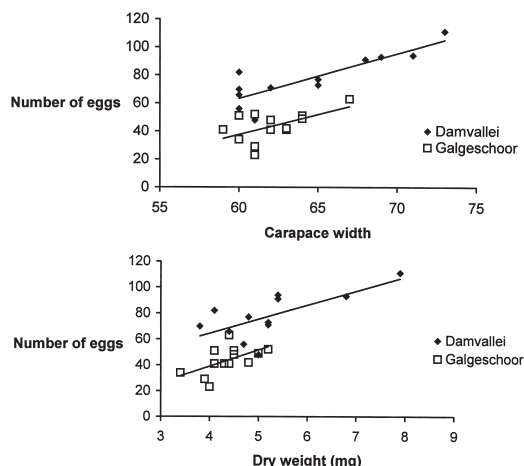


Fig. 2: Regressions of number of eggs versus size for two populations of *Pirata piraticus* differing in degree of heavy metal contamination.

significant ($P < 0.001$), meaning that left-right differences are not artefacts of measurement.

Although FA is mostly used as a population parameter, we used individual levels of FA of 13 individuals to relate them to internal Cd concentrations. To be able to determine concentrations in individual spiders, the much more sensitive electrothermal AAS with Zeeman correction was used. A significant regression (0.59 ; $P < 0.05$) was found between these two variables. This result suggests two important conclusions: (1) Cd is probably one of the main environmental stress factors affecting the population of *P. piraticus* from the Galgenschuur; and (2) internal Cd concentrations seem to be a good measure of the stress that this heavy metal causes.

Changes in reproductive effort

In Figure 2 we compare the regressions between the number of eggs in the egg sac of females of *P. piraticus* and the size of these females for two populations. The first one, from the Galgenschuur, is heavily contaminated (mean content in spiders expressed in ppm for Cd: 94; Cu: 513; and Zn: 838); the second, from the Damvallei, much less so (Cd: 8; Cu: 167; and Zn: 500). In the heavily polluted marsh, the Galgenschuur, we can observe that: (1) the

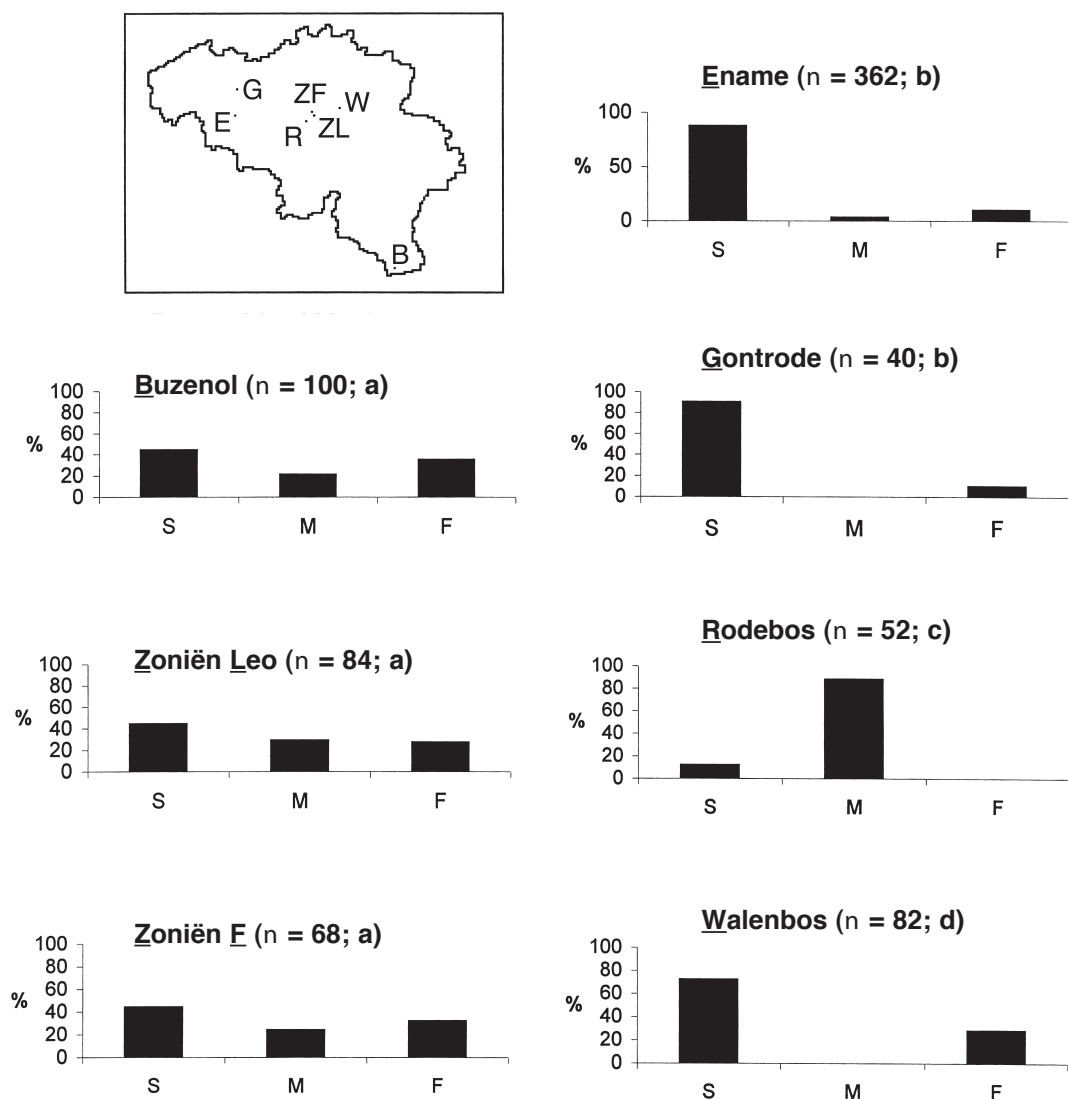


Fig. 3: Allelic percentages for the enzyme PGI for seven populations of *Coelotes terrestris* from different woodland habitats in Belgium (see text for explanation).

number of eggs is lower for the same size of the female; and (2) the females reproduce at smaller sizes. We also compared the mean size of the individual eggs for both populations by measuring the diameter of a number of undeveloped eggs under a stereomicroscope with a calibrated eyepiece. The diameters of 49 eggs from 6 cocoons from the Galgeschoor have as mean 0.840 mm (standard error of the mean (SE): 0.006), and 46 eggs (seven cocoons) from the

Damvallei: 0.698 mm (SE: 0.005). The eggs from the Galgeschoor population have a diameter that is about 20% larger than the diameter of the eggs from the other population. Of course, this preliminary result will be checked in the future by measuring much more freshly deposited eggs in both populations. It would be intriguing to find out how smaller reproducing females, producing fewer and larger eggs can better cope with heavy metal pollution. The

genetic background of the observed differences also remains to be investigated. It is interesting to note that the same suite of differences was also observed between a heavily contaminated and an uncontaminated population of the woodlouse *Porcellio scaber* (Donker *et al.*, 1992).

Population genetic effects of habitat fragmentation

To assess the population genetic effects of habitat fragmentation, we analysed the variation present in enzymes involved in the general metabolism of cells. This variation can be made visible using cellulose acetate gel electrophoresis (Hebert & Beaton, 1989). We applied this technique to populations of *Coelotes terrestris*, an agelenid spider restricted to the forest litter layer. For the populations we investigated, we found interpretable variation in the banding patterns of the enzyme PGI. The zymograms of that enzyme could be regarded as being caused by three alleles: F for Fast, M for Medium and S for Slow. Individual spiders can thus have one of the following genotypes: SS, SM, SF, MM, MF and FF. By determining the genotype of a sufficiently large number of individuals per population, the allelic frequencies of these populations can be estimated and compared with each other. Figure 3 shows the allelic percentages in *Coelotes terrestris* populations occurring in different woodlands. Also indicated is the number of observations these percentages are based on, and the results of χ^2 comparisons: for the populations having the same letter behind the number of observations, there is no statistical reason to assume that their allelic compositions are different. For populations having a different letter, the probability that they have the same allelic frequencies is lower than 1%. The three samples on the left were collected in forest complexes larger than 1000 hectares. The samples on the right came from populations living in woodland fragments smaller than 200 hectares. The populations occurring in the smaller forests seem to be genetically impoverished. They have allelic frequencies differing from those of the large forests and often differing from each other. These differences are probably caused by reproductive isolation. From these results, we can conclude that there is a good chance that spiders will also be useful in studying the effects of habitat

fragmentation and in monitoring the effects of habitat defragmentation in the future.

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