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Rank Ordering of Macroinvertebrate Species Sensitivity to Toxic Compounds by Comparison with That of *Daphnia magna*

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The community inhabiting a body of water represents the "ecological memory" of the habitat, and hence the composition of aquatic communities is used to monitor various stressors. A prerequisite for the development of useful indicator systems is that the species be ranked in order of their sensitivity to the stress parameter of interest. In the case of toxic xenobiotics, no existing classifications of aquatic macroinvertebrate species according to their specific sensitivities are sufficiently comprehensive to allow these organisms to be used as indicators for contamination of the water. However, this is precisely the area in which community-based indicator systems are particularly desirable. This is because precise information about contamination via chemical analysis of water samples is often difficult to obtain and laborious (Liess *et al.* 1999). Changes in aquatic communities, as a consequence of exposure to test substances, can be examined by contamination of experimental mesocosms. The results have repeatedly shown that, while various characteristics of the individual species tested are influential, the most important is the animal's specific physiological sensitivity to the contaminant in the acute toxicity test (Van den Brink *et al.* 1996; Sherratt *et al.* 1999). Therefore, the ranking of species should be based on the results of toxicity tests. For most species, however, hardly any information about their sensitivities is available (Notenboom *et al.* 1995). Thus, what is required, is a comprehensive compilation of sensitivities at such a taxonomic level, that from known species sensitivities, inferences can be made about species not yet subjected to toxicity tests.

Towards this end, a classification of macroinvertebrates according to their specific relative sensitivities to toxic substances is given in the present paper, using the order as basic taxonomic level. The data evaluated for this purpose, drawn from the literature, comprised LC50 and EC50 values for the exposure of various macroinvertebrate species to many substances. As information about any given species and substance is limited, the species cannot all be compared directly with one another; hence their relative sensitivities are calculated by comparison with toxicity data for the standard test species *Daphnia magna* (Cladocera), for which a large database is available. The species are ranked separately with respect to two groups of toxins: organic compounds (S_o) and metal compounds (S_m). Possible reasons for the particular sensitivities of the different species are discussed, as is the extent to which sensitivities measured in the laboratory reflect a species' *ecological* sensitivity in the field. This paper is part of a research programme aiming to develop a system that indicates community alterations corresponding to the contamination of the water by toxic substances.

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MATERIALS AND METHODS

The toxicity data were assembled from the database AQUIRE of the United States Environmental Protection Agency (United States EPA 2000). In order to check and interpret the findings, the corresponding original literature was examined as far as it was accessible. For the search in AQUIRE the following orders (according to the taxonomic system used in Kaestner 1965) were considered: Amphipoda, Basommatophora, Cladocera, Coleoptera, Copepoda, Decapoda, Diptera, Ephemeroptera, Heteroptera, Hirudinea, Isopoda, Lamellibranchia, Megaloptera, Monotocardia, Odonata, Oligochaeta, Ostracoda, Plecoptera, Trichoptera and Tricladida. The two gastropod orders Monotocardia and Basommatophora were not treated separately and hence are combined as "Gastropoda" in the remainder of the text. The search was conducted at the genus level, including all genera listed by Illies (1978) as abundant in Central Europe. The range of toxicity data was restricted to LC50 and EC50 values from freshwater laboratory tests with a duration of exposure between 1 and 96 hours. The parameters considered are categorized in AQUIRE as “immunity”, “intoxication”, “mortality” and “reproduction”. Basically, all the literature cited in AQUIRE that met the above criteria was included. When multiple references were found for a given test species and substance, the arithmetic mean of the effect concentration values was taken. When a value differed by more than a factor of ten from the closest one in a group of at least three values published by other authors, the aberrant value was discarded so as to remove outliers from the data set.

The objective was to compare the sensitivities of the individual macroinvertebrate orders, so that they could be ranked accordingly. However, the particular substances that had been tested for toxicity varied widely among the different orders, and where a given substance had been tested on species belonging to two orders, the experiments often used different endpoints. For this reason, direct comparisons of the orders with one another were impossible. Therefore *D. magna*, for which an unusually large set of data is available as it is commonly used in toxicity tests (Koivisto 1995), was chosen as the reference species: every individual effect concentration for a particular species and substance was divided by the corresponding value for *D. magna*. Comparisons were made only between those EC50 or LC50 values that were obtained with the same duration of exposure and the same endpoint. The logarithm of the quotient obtained for each species “i” was termed the relative physiological tolerance with respect to the tolerance of *D. magna* for the same substance (written below as relative tolerance T_{rel}).

$$T_{\mathrm{rel}} = \log\left(\frac{\mathrm{EC50}_{\mathrm{i}}}{\mathrm{EC50}_{D.\ magna}}\right)$$

A T_{rel} value of 0 thus indicates a relative tolerance equal to that of *D. magna*. For species more sensitive than *D. magna* T_{rel} is less than 0 and for less sensitive species it is greater than 0. The relative tolerances, with respect to a given substance, found for the various species were transferred to the taxonomic level of the order by taking the arithmetic mean of the T_{rel} values for all the test organisms belonging to a particular order, regardless of their species. This approach overemphasizes species that have been tested with an above-average number of substances. Nevertheless, this method of taking means was chosen because for a considerable number of the individual species test results were available for only one or a few substances. The error introduced by overweighting such individual test results, which would have resulted from taking a mean at species or genus level and subsequently taking a secondary mean at the order level, was considered to be greater than the error resulting from overweighting individual species.

Statistical tests were carried out with the SPSS® for MS Windows® software (Version 10.0). $\alpha = 0.05$ was chosen as the significance level for all tests. A comparison of the mean T_{rel} of each individual order with that of *D. magna* was undertaken with the *one sample t-test* with 0 as test value, after the data had been shown to be normally distributed by the Kolmogoroff-Smirnov test. The statistical relation between the T_{rel} for the two substance classes S_{o} and S_{m} was determined by linear regression. The dependence of T_{rel} on the order to which the test species belonged was checked by analysis of variance (ANOVA), using the individual T_{rel} values for each order as test variable and disregarding the species level.

RESULTS AND DISCUSSION

AQUIRE yielded toxicity data, allowing direct comparison with *D. magna,* for a total of 254 different substances (211 S_{o} and 43 S_{m}), which came from 472 publications (327 for S_{o} and 145 for S_{m}). Within the individual orders the number of substances for which comparable effect concentrations were available, both for the included taxa and also for *D. magna,* ranged from one (Ostracoda, S_{m}, and Megaloptera, S_{o}) to 118 (Cladocera, S_{o}). LC50 test results accounted for 80 % of the data and EC50 results the remaining 20 %. The exposure durations in the experiments considered were distributed as follows: 3 % of the individual values were associated with an exposure of less than 24 hours, 78 % between 24 and 48 hours, and 19 % between 48 and 96 hours. The number of species per order in this sample of toxicity data was between one (Ostracoda, S_{m} and Megaloptera, both substance groups) and 63 (Diptera, S_{o}). No data were found regarding the sensitivity of the order Coleoptera to S_{m}. Further, the sensitivity of the order Ostracoda to S_{m} and that of the order Megaloptera to both substance groups were not calculated, as there were insufficient data. A complete list of the species and substances per order is accessible on the Internet, as is a complete list of the original literature from which the data in this study was derived (Wogram *et al.* 2000).

The ANOVA with the T_{rel} values as dependent variable and the orders as factors showed that for each of the substance groups S_{o} and S_{m} the variances of T_{rel} between the orders were significantly higher than within the orders ($p < 0.0001$ for both substance groups). The T_{rel} values for the individual orders with respect to both S_{o} and S_{m} are shown in figure 1. The class Gastropoda (including the orders Basommatophora and Monotocardia), and the orders Coleoptera, Diptera, Heteroptera, Hirudinea, Isopoda, Lamellibranchia, Odonata, Oligochaeta and Turbellaria exhibited significantly higher mean tolerances towards the substance group S_{o} than does *D. magna* (one sample t-test). The order Lamellibranchia, with a T_{rel} of 1.95, was the most tolerant. The Amphipoda, Plecoptera and the Cladocera other than *D. magna* had significantly lower mean tolerances than *D. magna*; with $T_{\mathrm{rel}} = -0.36$, the order Plecoptera was the least tolerant of all. The remaining orders did not differ significantly from *D. magna* in their physiological tolerances towards S_{o}. There was a tendency for the Copepoda, Decapoda and Trichoptera to be less sensitive than *D. magna*, and for the Ostracoda and Ephemeroptera to be more sensitive.

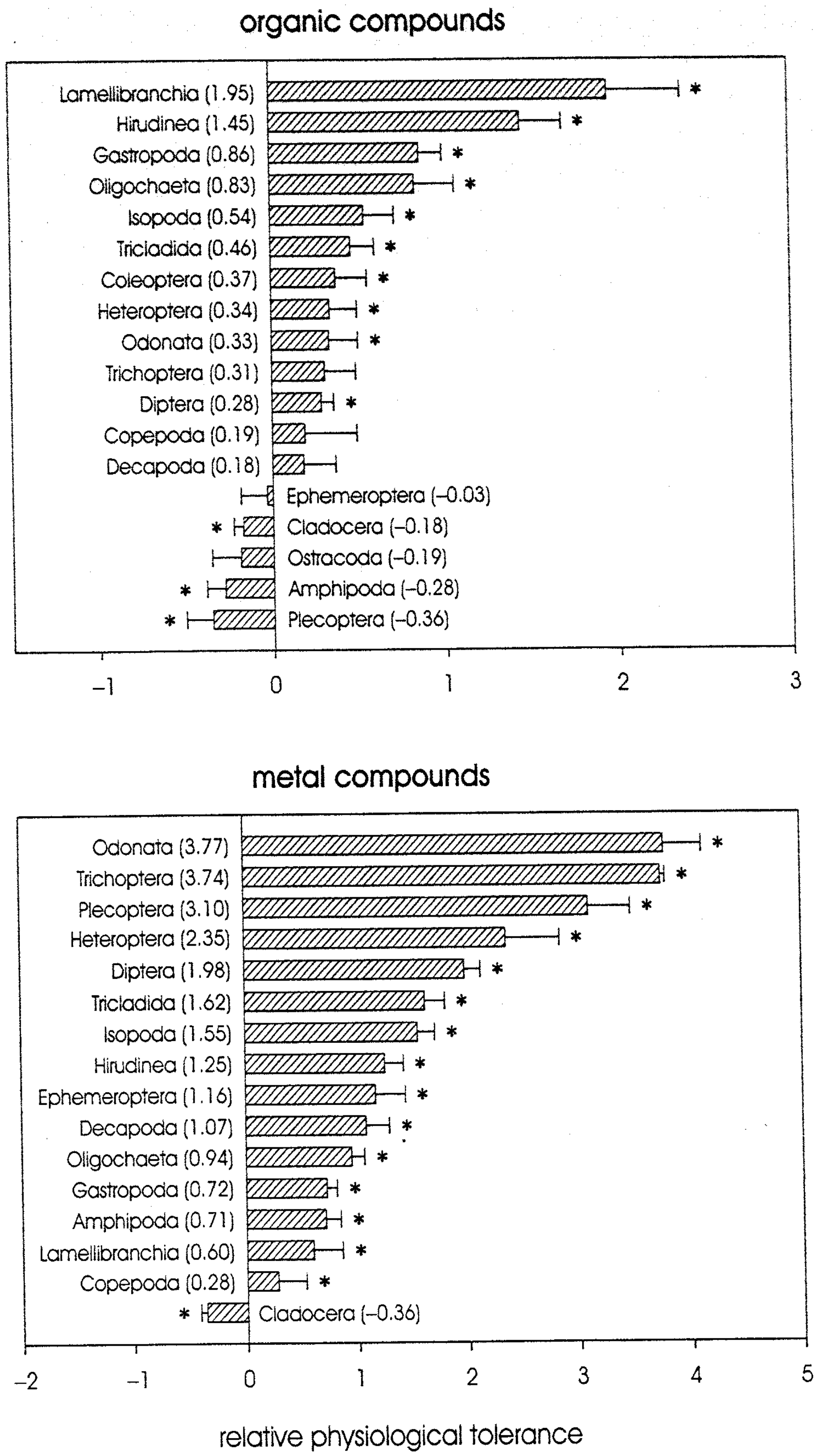

Figure 1. Differing relative physiological tolerances (T_{rel}) of macroinvertebrate orders with respect to organic compounds and metal compounds compared to *Daphnia magna* (arithmetic means of 5 to 460 individual values ± standard error; individual values given in parentheses). The vertical line at $x = 0$ represents the tolerance of *D. magna*, with which the other values are compared. The asterisks denote values significantly different from that for *D. magna* (one sample t-test, $p < 0.05$). The bar labelled "Cladocera" refers to cladoceran species other than *D. magna*. The bar labelled "Gastropoda" includes the orders Basommatophora and Monotocardia.

With respect to the substance group S_m, all but one of the orders considered here exhibited significantly higher mean T_{rel} values than *D. magna* (fig. 1). Only the order Cladocera (without *D. magna*) was significantly more sensitive than *D. magna.* The highest physiological tolerances were found for the insect orders Heteroptera, Odonata, Trichoptera, Plecoptera and Diptera; the lowest, apart from the Cladocera, were found for the Copepoda, Lamellibranchia, Amphipoda and Gastropoda. There was thus a considerable difference between the substance groups S_o and S_m in the tolerance ranking established on the basis of T_{rel}. The T_{rel} values of the orders with respect to the substance group S_m were not significantly correlated with their T_{rel} values with respect to S_o (linear regression, $n = 16$; $r^2 = 0.028$; $p = 0.378$). For this reason, in deriving indices from the present data substances belonging to the "organic" class must be treated entirely separately from the class "metal compounds".

The significance of the ANOVA for both substance groups, with the individual orders as factors and T_{rel} as dependent variable, shows that the physiological sensitivity of macroinvertebrates to a broad spectrum of substances does reflect, among other things, the order to which the animal belongs. For the group S_o, furthermore, sensitivity is discernibly influenced by the degree of kinship at a relatively high taxonomic level. For example, the twelve least physiologically tolerant orders all belong to the arthropod group, whereas the four least sensitive orders are members of other major taxa (Mollusca, Annelida; fig. 1). Similarly, the orders belonging to the Crustacea, with the exception of the Isopoda, are relatively close to one another when ranked according to T_{rel}. The same applies to the insect orders, apart from the Plecoptera, as well as to the groups Mollusca (Gastropoda and Lamellibranchia) and Annelida (Oligochaeta and Hirudinea). This finding is consistent with results published elsewhere: in a review of articles on interspecies toxicity relationships Slooff *et al.* (1986) concluded that the sensitivity of species depended on their taxonomic position and the nature of the toxic substance. Hoekstra *et al.* (1994), examining the literature regarding 26 chemicals, demonstrated that the variation of sensitivity between species within a class is usually less than the variation between classes.

One potential explanation for the tendency of arthropods to be more sensitive to organic compounds than are the oligochaetes and molluscs involves the oxygen consumption required for detoxification of organic xenobiotics. The decontamination of the organism by means of oxidation reactions utilizing P450 cytochromes (Steinberg *et al.* 1992) consumes substantial amounts of oxygen. The resulting increased breathing rate can thus be used as a biomarker for toxic substances (e.g., in fish: Evans & Wallwork 1988). Molluscs and oligochaetes, unlike arthropods, dispose a large proportion of organic xenobiotics not by metabolizing them but rather by simple passive excretion or by accumulation in the body's adipose tissue (Steinberg *et al.* 1992). However, a relationship between the greater sensitivity of arthropods, in comparison to non-arthropods, and these physiological differences remains speculative.

As would be expected, however, no sharp dividing line can be drawn between the sensitivity of arthropods on the one hand and that of molluscs and oligochaetes on the other on the basis of the present results. This is because, even within the arthropod group there are considerable physiological and morphological differences between the various orders. In particular, morphological differences of the respiratory organs should be considered as a possible explanation of sensitivity differences between the individual arthropod orders. For instance, the breathing of atmospheric oxygen (Coleoptera, Heteroptera, many Diptera) rather than absorbing oxygen through gills (other arthropods) might make an organism less sensitive to brief contamination with toxic substances. This is due to the area over which its body is in contact with the water is smaller. Such types of dependence of physiological sensitivity on the morphological features of a macroinvertebrate have not yet been investigated to any great extent. Another potential reason for the relatively high sensitivity of some arthropod taxa (e.g., Plecoptera, Ephemeroptera) is that they have a considerably higher oxygen consumption. Conceivably, species with a generally low oxygen requirement can compensate for the increased oxygen consumption during detoxification better than those requiring large amounts of oxygen in the first place.

The sensitivity to metal compounds, however, did not differ distinctly between arthropods and non-arthropods. Four out of five orders in the class Insecta did show higher physiological tolerances than all the remaining taxa, but the tolerances of crustaceans, molluscs, triclads and oligochaetes cannot be distinguished clearly according to the present results. This finding is consistent with the similarity among these taxa with respect to the mechanism of detoxification of heavy metals. Most macroinvertebrate groups inactivate heavy metal ions by way of metallothionein-like proteins (Steinberg *et al.* 1992). The reasons for the existing variations in sensitivity to metal compounds among the taxa, in contrast, are not clear.

As a possible explanation for variances of T_{rel} at *all* taxonomic levels, factors that can influence the results of standard toxicity tests for both substance classes of interest here deserve consideration. There are such factors for which no standard specifications exist, and which could not be taken into account for the present comparisons: for instance, the hardness and temperature of the water, the light intensity and quality, the volume of the test vessels or the life stage of the test organisms (Roshon *et al.* 1999).

In the course of the work on comparative toxicity, sensitivity comparisons usually have been undertaken at the taxonomic level of species or genus (e.g., Roshon *et al.* 1999; Versteeg *et al.* 1999). Conversely, there have been no previous, comprehensive classifications at the level of the order. Because the physiological and morphological similarities between organisms are further apart, the more distantly related the organisms are, the more the variances of T_{rel} were to be expected even *within* the individual orders. For present purposes it was nevertheless decided to rank the sensitivities at the relatively high taxonomic level of the order as the sparsity of data ruled out a ranking of lower-level taxa. The differences in sensitivity found between the various orders justify this approach. However, when employing the sensitivity values presented here to evaluate aquatic communities in the field, the interspecific sensitivity differences within a given order should be taken into account. For example, Guerold (2000) showed for several organism-based indices that as the taxonomic level at which the organisms were identified fell, the index values became progressively more erroneous. The mean T_{rel} values for individual orders accordingly represent a statistical trend arising from the large number of species and substances included in this study, and need not apply to every individual one of the substances and species. Thus, in the course of a toxicological evaluation of individual substances employing particular macroinvertebrate species, the ranking of physiological sensitivities at the level of the order cannot replace the standard toxicity test. Furthermore, effects of contamination on aquatic communities with individual substances that have a taxon-specific action (e.g., in the group of pyrethroids) should not be evaluated with the present database because the actual, specific sensitivities of the taxa to these substances can deviate considerably from the general sensitivities found here.

In the opinion of the authors the database presented here will be of use primarily where a comprehensive set of data (for example, when monitoring animal communities in the field) is to be put in relation to contamination with mixtures of substances. Such a situation is more often observed in the field than contamination with individual toxic substances (Boedeker *et al.* 1993; Steinberg *et al.* 1992). Complex pollution of water is the rule, for example, for plant pesticides (Liess *et al.* 1999) and heavy metals (Mueller & Furrer 1994; Ramos *et al.* 1999). In an attempt to demonstrate contamination with toxic substances with reference to the present data, it must be taken into account that the physiological sensitivity of an organism is only one of many influential factors that determine its *ecological* sensitivity to toxins. For example, the duration and temporal pattern of the generation cycle (Sherratt *et al.* 1999; Van den Brink *et al.* 1996) as well as the recolonization capacity (Liess & Schulz 1999; Schulz & Liess 1999) of a species can contribute crucially to its ecological tolerance of toxins. Furthermore, the ranking of sensitivities undertaken here should not be regarded as static, but is intended to be continually developed and diversified as scientific understanding of the subject increases. A separate consideration of the sensitivities of macroinvertebrates to substance groups with particular ecotoxicologic relevance (e.g., pyrethroids, organophosphates) in the course of subsequent studies would be desirable.

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