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Erratum

Erratum to "An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators Part 2: The knowledge base of LIMPACT" [Ecological Indicators 2 (2002) 239–249][☆,☆☆]

Michael Neumann[a,*], Joachim Baumeister[b], Matthias Liess[c], Ralf Schulz[a]

[a] *Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092 Braunschweig, Germany*
[b] *Department of Artificial Intelligence and Applied Computer Science, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany*
[c] *Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318 Leipzig, Germany*

The publisher regrets that in the previous issue of the journal of Ecological Indicators, Volume 2, Issue 3, Part 2 of a two-part paper by Michael Neumann et al., was published without Part 1. Both Part 1 and Part 2 now follow together in their correct form.

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* Corresponding author. Tel.: +49-3641-64-2742; fax: +49-3641/64-3325.
E-mail address: m.neumann@uni-jena.de (M. Neumann).

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An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators
Part 1. The database of LIMPACT

Michael Neumann[a,*], Matthias Liess[b], Ralf Schulz[a]

[a] *Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092 Braunschweig, Germany*
[b] *Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318 Leipzig, Germany*

Abstract

We developed an expert system (LIMPACT) to estimate the pesticide contamination of streams using macroinvertebrate indicators. Here, we present the database consisting of 157 data sets obtained from 1992 to 2000 through investigation of 104 small headwater streams with an agricultural catchment area. The contamination by pesticides (insecticides, fungicides and herbicides) during rainfall events varied greatly in both water and suspended-particle samples, occasionally reaching ecotoxicologically relevant levels. On the basis of standardised toxicities, the data sets were grouped into Not Detected ($n = 55$), Low (34), Moderate (42) and High (26) contamination with pesticides. Additionally, nine water-quality and morphological parameters were evaluated with regard to their influence on the fauna and are used to exclude unsuitable streams from LIMPACT. The benthic macroinvertebrate fauna data were divided into four time frames (March/April; May/June; July/August; September/October) and analysed regarding the abundance and the abundance dynamics of the 39 most common taxa. In contaminated streams, lower abundance for negative and higher for positive indicator taxa were observed. The number of taxa was significantly lower (unpaired *t*-test $P < 0.015$) in the most severely contaminated streams. Information abstracted from this empirical approach was used to create rules indicating or not indicating contamination and to build up the heuristic knowledge base of LIMPACT as shown in the Part 2 paper (M. Neumann, J. Baumeister, M. Liess, R. Schulz, An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrate as bioindicators. Part 2. The knowledge base of LIMPACT, Ecological Indicators, this issue).

Keywords: Agricultural non-point pollution; Surface water; Insecticides; Fungicides; Herbicides; Effect; Runoff

1. Introduction

Small streams in agriculturally used catchment areas are subject to various stressors. During heavy rainfall, runoff from agricultural fields may introduce soil, nutrients and pesticides and increases the discharge (Cooper, 1993; Neumann and Dudgeon, 2002). It has been shown that the impact of pesticides is an important parameter which influences the aquatic fauna (Liess and Schulz, 1999; Schulz and Liess, 1999). Small streams sum up to an enormous length on the landscape level and therefore the conservation and protection of their aquatic community should be a major concern. Consequently, a tool is needed to monitor water quality on the landscape level.

The indication of non-point source contamination via chemical analysis is costly. Because of its short-term character (Kreuger, 1995), only rainfall

* Corresponding author. Tel.: +49-531-3913180;
fax: +49-531-3918201.
E-mail address: m.neumann@tu-bs.de (M. Neumann).

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event-controlled sampling methods can reflect such transient contamination (Liess and Schulz, 2000; Liess et al., 1999). Hence, the indication via benthic macroinvertebrate bioindicators could give evidence over a longer period and therefore would be more cost-efficient. Furthermore, it would indicate the toxicity of the contamination and not only the concentration of chemicals.

Our group has undertaken investigations of a large number of agricultural headwater streams during the last 10 years. Event-controlled sampling methods and repeated sampling of the benthic macroinvertebrate fauna in the streams feature in the data sets. The aim of the present study was to use these data to develop a biological indicator system based on an expert system that estimates the pesticide contamination of small streams. The input parameters of the expert system are benthic macroinvertebrate abundance data and basic water-quality and morphological parameters. The output is an estimation of the pesticide contamination according to four classes. We name this expert system LIMPACT and in this paper we present the database. In Part 2 (Neumann et al., 2003), we present the development and the structure of the knowledge base of LIMPACT.

2. Materials and methods

2.1. Data pool and considered streams

All of the 104 investigated streams are located in Germany within regions of intensive agricultural land use. The largest numbers of sampling points were located around the city of Braunschweig. Other sampling regions were close to Hamburg, Hannover, Kassel and Mannheim. All streams were carefully selected to ensure that the impact from agricultural land use is the major stressor. None of the sampling points was located in urban areas or received industrial discharge or animal farm waste. Only seven streams received water from a sewage treatment plant.

All sampling sites were unshaded and represent lowland streams with low gradient (maximum slope: 3°) and with substrate of mixed sand, loam and silt. Most streams had a current velocity lower than $0.5\,m\,s^{-1}$; only three streams had a maximum between 0.5 and $0.9\,m\,s^{-1}$. Water depths varied between 5 and 70 cm, with only eight streams at their maximum deeper than 50 cm. The width of the streams ranged from 25 to 400 cm, with only eight streams at their maximum wider than 200 cm.

The streams were investigated by the Zoological Institute, Department of Limnology at the Technical University of Braunschweig, Germany between the years 1992 and 2000. Some streams were investigated repeatedly in different years. Consequently, we named the resulting data sets as “investigations per stream and year”. An overview of the data sets is given in Liess (1993), Schulz (1997), Wogram (2001), and Neumann et al. (2002). Here, we present the database application development. All data had to be gathered, classified in a common design and validity controlled. The process of data entry and acquisition was the most time-consuming part of the development of LIMPACT.

2.2. Contamination with pesticides

In all streams samples of both water and suspended particles were taken, by either a suspended-particle sampler (Liess et al., 1996) or a rainfall event-controlled water sampler (Liess et al., 1999). For 35 investigations per stream and per year, both suspended-particle and water analysis were available. The analysis was done at the Institute for Ecological Chemistry of the Technical University of Braunschweig with the method described by Liess et al. (1999). The analysis included a changing spectrum of pesticides (insecticides, herbicides and fungicides) because of differences in spraying programs. The mean detection limit for water samples was $0.05\,\mu g\,l^{-1}$ and ranged between 0.02 and $1\,\mu g\,l^{-1}$. For suspended-particle samples it was $1\,\mu g\,kg^{-1}$ and ranged up to $5\,\mu g\,kg^{-1}$. In this paper, we present an overview using the summed concentrations within the three pesticide classes, with water and suspended particles treated separately.

To represent the measured contamination with regard to its toxic potential, we calculated a specific value for each of the investigations per stream and year by extending the idea of Peterson (1994) and Wogram (2001). The concentration of each chemical agent is weighted for its toxic potential by the 48 h LC_{50} toxicity of the well-investigated species *Daphnia magna*. By summing up all samples for each investigation per stream and year, we calculated the Toxic Units annual sum ($TU_{Sum\ year}$) using formula (1). By doing this, we postulate an additive toxic effect of single pesticide exposures, which has also been suggested by Warne and Hawker (1995).

$$\mathrm{TU}_{\mathrm{Sum\ year}} = \log\left(\sum_{j=1}^{n}\left(\sum_{i=1}^{n}\frac{C_{ji}S_{ji}}{\mathrm{LC}_{50ij}}\right)\right) \quad (1)$$

where $\sum_{i=1}^{n}$: sum of all pesticides within one sample, $\sum_{j=1}^{n}$: sum of all samples within one investigation per stream and year, C: concentration of pesticide ($\mu g\,l^{-1}$) or ($\mu g\,kg^{-1}$),

$$S = \begin{cases} 1 & \text{for water samples} \\ \text{Solubility of pesticide } (mg\,l^{-1}) & \text{for suspended-particle samples} \end{cases}$$

and LC_{50}: *Daphnia magna* 48 h LC_{50} of pesticide ($\mu g\,l^{-1}$).

The $TU_{Sum\ year}$ was calculated for water and suspended-particle contamination separately. For suspended-particle samples, we additionally weighted each pesticide with its water solubility. The water solubility for the investigated insecticides is between 0.0029 and 12.4 $mg\,l^{-1}$, for fungicides between 2 and 110 $mg\,l^{-1}$ and for herbicides between 0.3 and 1050 $mg\,l^{-1}$.

In 35 investigations per stream and year, a $TU_{Sum\ year}$ for both water and suspended-particle samples was available. Fig. 1 shows the linear regression for those 19 investigations per stream and year with positive readings for both water and suspended particles. On the basis of this regression, we calculated the water $TU_{Sum\ year}$ value for those investigations per stream and year for which only suspended-particle samples were obtained. This procedure made all investigations per stream and year available for the data analysis.

2.3. Water-quality and morphological parameters

In addition to the pesticide contamination and the aquatic fauna, other water-quality and morphological parameters were measured in the streams. To indicate the organic pollution we used the saprobic index (Friedrich, 1990), which is calculated by weighted index-values for each occurring species according to its specific oxygen requirement. During the process of data acquisition, we compared only those information common to all investigations per stream and year and had to group the information on a higher level. Our aim was to give LIMPACT information about those parameters that may influence the aquatic macroinvertebrate fauna the most.

(1) Organic pollution according to the German saprobic index (see Friedrich, 1990) (mean per year).
(2) Morphological structures in the stream as the percentage of stream bed area covered by submerse and emerse plants, woody debris and tree roots (maximum per year).
(3) Stream bed area consist of sand (maximum per year).

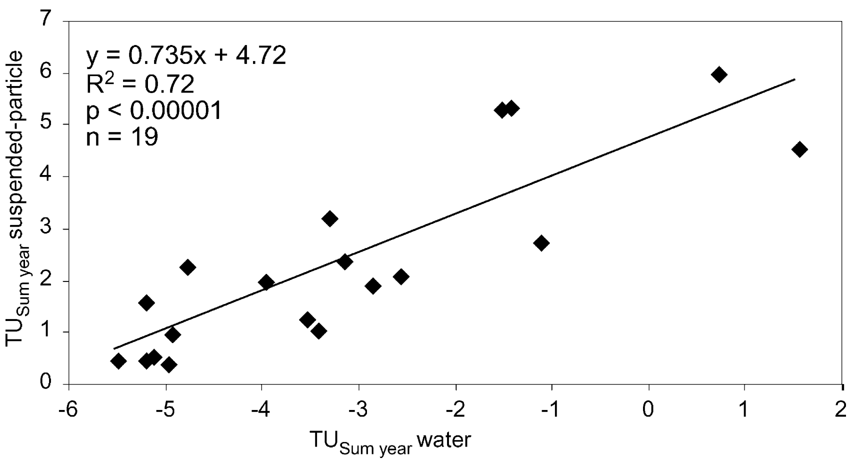

Fig. 1. Correlation between the summarised standard toxicity value $TU_{Sum\ year}$ for water samples and for suspended-particle samples.

(4) Current velocity (maximum per year).
(5) Cross-sectional area of stream: width in centimetres multiplied by the depth in centimetres (maximum per year).
(6) Drying out in summer (number of dry months).
(7) Conductivity of the stream water, to reflect the general soil type (mean per year).
(8) pH-value (mean per year).
(9) Carbonate water hardness to differentiate between silicate and carbonate streams (mean per year).

2.4. Benthic macroinvertebrate fauna

The animal sampling was done with four to six replicate Surber samplers (area of 0.125 m^2) per site and sampling date. Abundance data are given as the mean of individuals per square meter. When possible, the animals were identified at the species level. However, during the process of data acquisition we had to group some taxa on higher taxonomic levels.

3. Results and discussion

3.1. Database of LIMPACT

After data acquisition and database application development, we had access to biological data and pesticide contamination data from 104 sampling sites. Because some sampling sites were investigated repeatedly in different years, 157 investigations per stream and year were available. Animal sampling and measurement of water-quality and morphological parameters was done between one and seven times per year. Overall, our database had 660 sampling dates available.

3.2. Contamination with pesticides

For each investigation per stream and year, we selected only those water and suspended-sediment samples that followed heavy rainfall (>10 mm per day). A total of 555 samples were analysed. A total of 286 samples were contaminated and 269 were below detection limit. Of the 317 water samples, 64% (202) were contaminated with pesticide, whereas of the 238 suspended-particle samples only 35% (84) were contaminated. In water samples, the level of contamination increased from insecticides to fungicides and herbicides (Fig. 2). For suspended-particle samples the low herbicide contamination reflects the lower tendency to become bound to particles.

In USA, the Clean Water Act (CWA) of 1972 demands no emission of toxic substances into watercourses. The US EPA developed Water Quality Criteria (WQC) and Sediment Quality Criteria (SQC) and distinguishes between criteria for maximum concentration (CMC) and criteria for chronic concentration (CCC) (USEPA, 1991, 1999). None of the substances with a SQC (USEPA, 1992) and with a WQC are investigated here. In Germany, the Federal Environmental Agency has recently published a

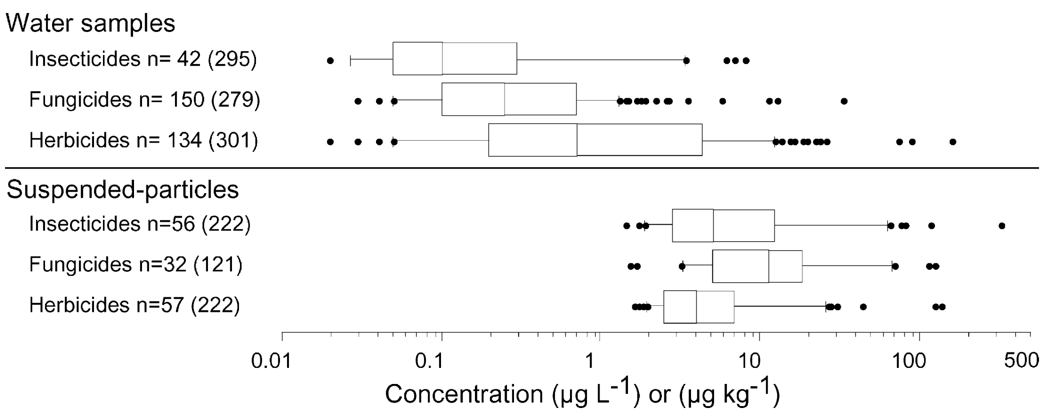

Fig. 2. Boxplots illustrating the distribution of pesticide concentrations in contaminated samples. Values in brackets are the total number of samples. Within each sample the sums for herbicides, fungicides and insecticides are presented separately.

proposal with quality targets for 35 pesticides (UBA, 1999). Of the 30 pesticide agents investigated here, only nine have a quality target. We found that seven (Chloridazon, Bromacil, Diuron, Isoproturan, Lindan, Metazachlor, Paration-ethyl, Simazin, Terbutylazin) of these exceeded the quality target. For drinking water the European Union has generally a target level of $0.1\,\mu g\,l^{-1}$ for each single pesticide. The stream water contamination we observed exceeds this level for 25 of the 30 pesticides. The pesticide load is above loads that have been shown to affect the benthos in microcosm studies (Liess and Schulz, 1996; Schulz and Liess, 2000). For suspended particles in stream water no target value is available in Germany at all. Sorption is known to decrease toxicity, but microcosm studies showed effects (Schulz and Liess, 2001a,b) at the same contamination level we found in the streams. We can state that the available data for the pesticide contamination of the streams show that some levels are above those that would be expected to affect the macroinvertebrate community.

Fig. 3 indicates for which month pesticide samples (a) or an animal sample (b) are available and shows that the most pesticide samples were taken in the months of April, May, and June. This period is within the main application period for pesticides in Germany, which lasts from late April to early August.

Fig. 4 shows the grouping of all 157 investigations per stream and year according to their $TU_{Sum\ year}$ value. In the first class ND (not detected) streams

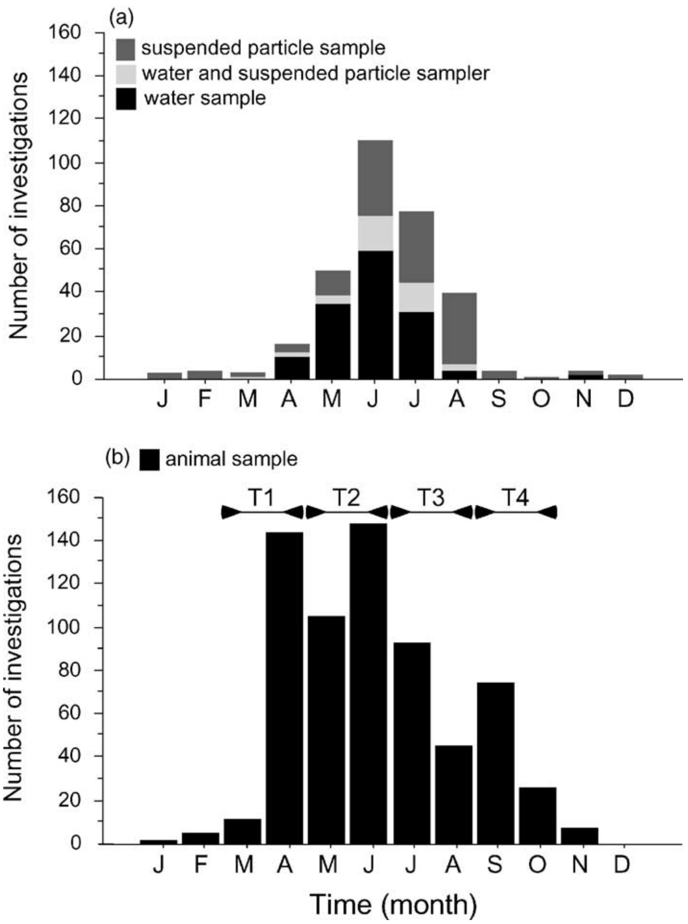

Fig. 3. Number of investigations in each month with (a) pesticide samples and (b) macroinvertebrate samples for the 157 investigations per stream and year.

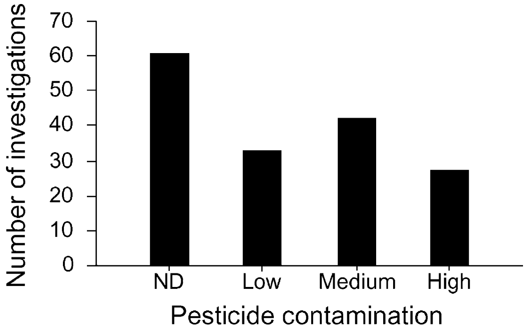

Fig. 4. Distribution of the 157 investigations per stream and year over the four pesticide contamination classes.

without any contamination above detection limit were grouped. The other three groups contain streams with detectable pesticide contamination: L stands for low contamination ($TU_{Sum\ year} < -4$), M for moderately contaminated ($TU_{Sum\ year} < -2$) and H for highly contaminated ($TU_{Sum\ year} \geq -2$).

The reason for grouping the streams rather than using the $TU_{Sum\ year}$ as a continuous variable is that this makes the expert system less sensitive to errors. Here, we choose a very conservative approach rather than overinterpreting the precision of our field measurements. These four groups of contamination are the basis for the development of LIMPACT and constitute the diagnoses which a user of LIMPACT will obtain as result (see Part 2, Neumann et al., 2003).

3.3. Water-quality and morphological parameters

Bioindicators can only work within a defined range of influencing factors. Consequently, we excluded from the expert system LIMPACT streams with extreme parameter values suggesting that other stressors than the pesticide contamination could influence the macroinvertebrate fauna. Of the nine parameters we considered, the five most important ones are presented in Fig. 5. The range in which a stream would be excluded from LIMPACT is labelled as suspected (S) or established (E). For details, refer to Part 2 (Neumann et al., 2003).

The organic pollution (Fig. 5 (1)) showed a normal distribution around a mean saprobic index of 2.2. According to (Friedrich, 1990) an influence on the macroinvertebrate fauna is expected for values higher than 2.3. LIMPACT does not accept streams with a saprobic index higher than 2.6. For values between 2.3 and 2.6, we only suspect (S) an unsuitable stream for LIMPACT. The mean over the four pesticide contamination classes shows a significant increase between the uncontaminated class and the two highest contamination classes (unpaired *t*-test, $P < 0.05$). However, the absolute increase of the sabrobic index from 2.15 to 2.25 is within a very small range and thus rather negligible.

The morphological structures in the streams (Fig. 5 (2)) varied over the whole range. According to (Sabarth, 1999), streams with values below 20% are suspected (S) as unsuitable for LIMPACT, because a small amount of morphological structures may be an influencing factor for the macroinvertebrate fauna and may, e.g. indicate a recent stream clearance. Most of the streams are maintained for the purpose of drainage, which consequently reduces the structure in the stream. However, no trend is found in the mean amount of morphological structures over the contamination classes.

The proportion of stream bottom covered with sand (Fig. 5 (3)) varied over the whole range from 0 to 100%. The mean over the four classes shows a significant (unpaired *t*-test, $P < 0.05$) decline from 45 to 22%. More severely contaminated streams have less sand, which could be caused by the soil type of the catchment area. The risk of runoff is lower for sandy soils. LIMPACT regards streams with values smaller than 20% or higher than 80% as unsuitable, because extreme values may influence the macroinvertebrate fauna (Sabarth, 1999; Wagner, 1987).

The maximum current velocity in the stream (Fig. 5 (4)) shows clearly that we considered small streams. Only three streams had a current velocity high enough to be considered unsuitable, along with the extremely low current velocities. The strong influence of current velocities on stream macroinvertebrates has been reported for example by Statzner (1981). The distribution of the means shows no correlation with contamination.

The cross-section of the stream (Fig. 5 (5)) also reflected our main focus on small streams. Streams with cross-sectional areas greater than 8000 cm^2 are suspected to be unsuitable for LIMPACT. It is known that stream typology influences the aquatic community

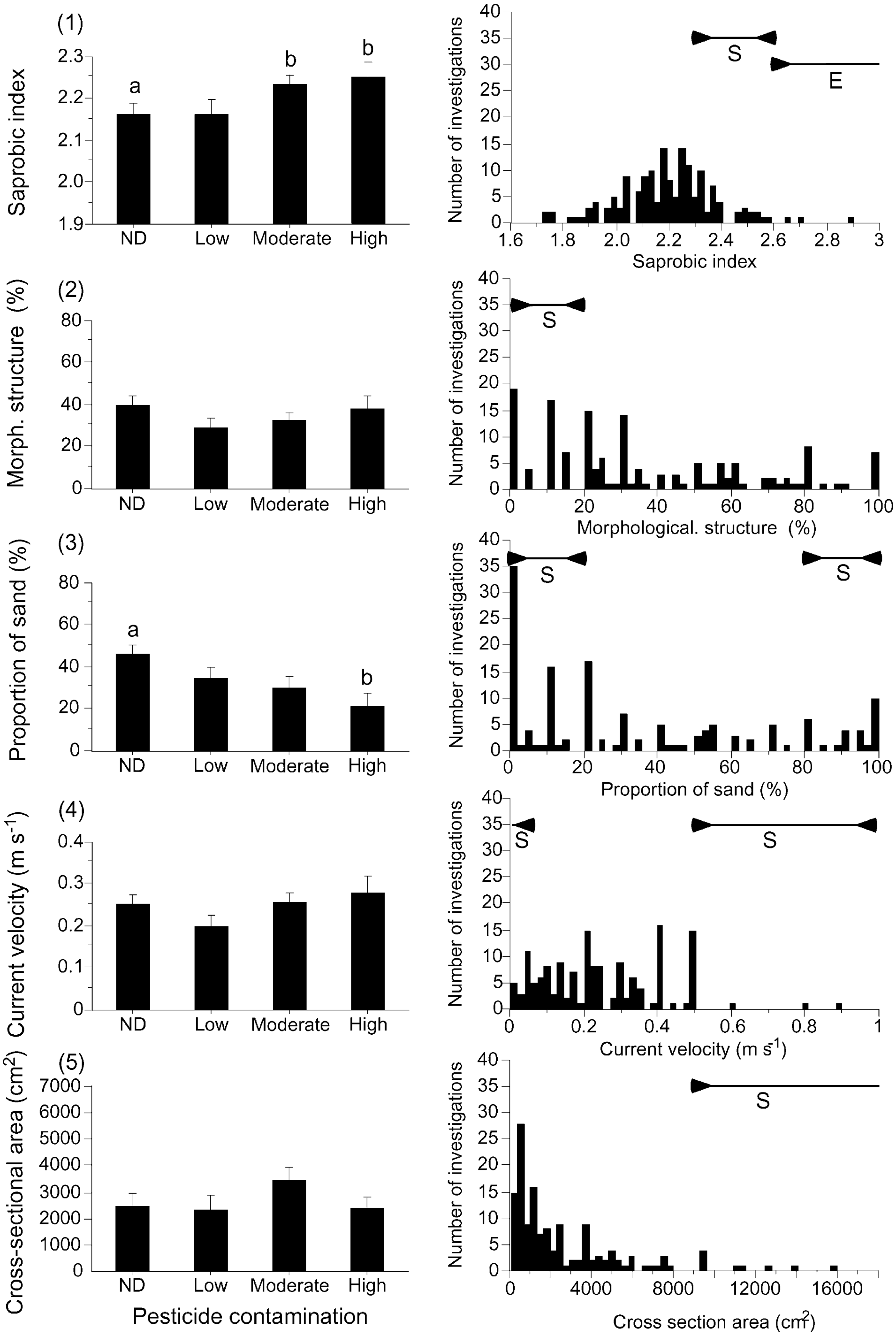

Fig. 5. Mean (±S.E.; $n = 26$–55) values of selected water-quality and morphological parameters over the four contamination classes (left graphs) and the distribution of the values in the database (right graphs). The suspected (S) or established (E) exclusion of data sets with extreme values from LIMPACT is indicated. Different letters indicate significant differences.

structure (Verdonschot, 1992). No trend is seen in the data, but there is a slight increase of mean size for moderately contaminated streams.

In small streams with an agricultural catchment area in Germany, the parameters (6) number of dry months, (7) conductivity, (8) pH-value and (9) carbonate water hardness are seldom stressors to the benthic macroinvertebrate fauna. None of these showed significant trends in the data set that could mask the effect of pesticide contamination on the macroinvertebrate fauna. In our data, no extreme values occurred; thus these parameters are not illustrated here. However, streams with extremely low and/or extremely high values for these parameters are also not accepted for the use in LIMPACT, because this may influence the aquatic fauna (Braukmann and Pinter, 1997).

3.4. Benthic macroinvertebrate fauna

To interpret the abundance dynamics of the benthic macroinvertebrate taxa within 1 year, we established four time frames for which information about abundance is essential. These are T1: March/April, T2: May/June, T3: July/August and T4: September/October (Fig. 2). This approach adjusts the various investigations per stream and year. With regard to the expert system this reduces the number of necessary sampling dates that the user of LIMPACT has to provide. In our database, 98% of the 157 investigations per stream and year provided sampling data during the main application period (Fig. 2).

The macroinvertebrate fauna was dominated by Trichoptera, Diptera, Oligochaeta, and Amphipoda (mainly *Gammarus pulex*). Oligochaeta and Gastropoda were found in 30% of the samples. A total of 386 taxa were found in the 104 sampled sites. Since rare taxa are liable to high sampling variability, for the development of the knowledge base of LIMPACT we used only the 39 most common taxa, representing 90.4% of the total abundance. A detailed list of these taxa is given in Part 2 (Neumann et al., 2003).

3.5. Benthic macroinvertebrate fauna as indicator

The detailed analysis of the abundance data and the abundance dynamics of each of the 39 taxa is presented in Part 2 (Neumann et al., 2003). As part of an empirical approach we classified the taxa as a negative indicator (NI) or as a positive indicator (PI) (Murtaugh, 1996) for pesticide contamination. A negative indicator is a species with an abundance negatively correlated with the pesticide contamination. The abundance of a positive indicator is positively correlated with the contamination. High abundance of a negative indicator therefore indicates low contamination, while high abundance of positive indicator suggests high contamination. In this paper, we used this grouping to analyse differences in number of taxa and abundance data for negative and positive indicators at variously contaminated sites.

Fig. 6 presents for all taxa, for negative indicator taxa and for positive indicator taxa the mean (±S.E.) of number of individuals and the mean (±S.E.) of number of taxa. The results are given separately for the four classes of pesticide contamination during March and April (T1; before main spraying period) and July and August (T3; following main spraying period).

The number of individuals showed a non-significant tendency to lower numbers with increasing contamination. Negative indicator taxa showed a significantly lower abundance at increased contamination. This difference was even more pronounced in the period T3, with the mean number of individuals reduced from T1 to T3 by 57% in the most strongly and 33% in the two intermediately contaminated classes. The PI taxa showed the opposite trend, with higher numbers in contaminated streams. From T1 to T3, the mean number was nearly unchanged in uncontaminated and strongly contaminated streams and increased in the two middle classes.

The total number of taxa, the number of NI taxa and the number of PI taxa were always lower in the most severely contaminated streams. In the period T3, the number of NI taxa was higher in the uncontaminated streams while the number of PI taxa was higher in the two middle classes. From T1 to T3 the number of NI taxa was reduced by 35% in the highest contamination and in the two middle classes by over 20%. The number of PI taxa remained nearly unchanged from T1 to T3. Overall the data showed a strong correlation between the abundance data and the pesticide contamination but not between the number of taxa and pesticide contamination. Consequently, we focused on the abundance data and the abundance dynamics while developing LIMPACT.

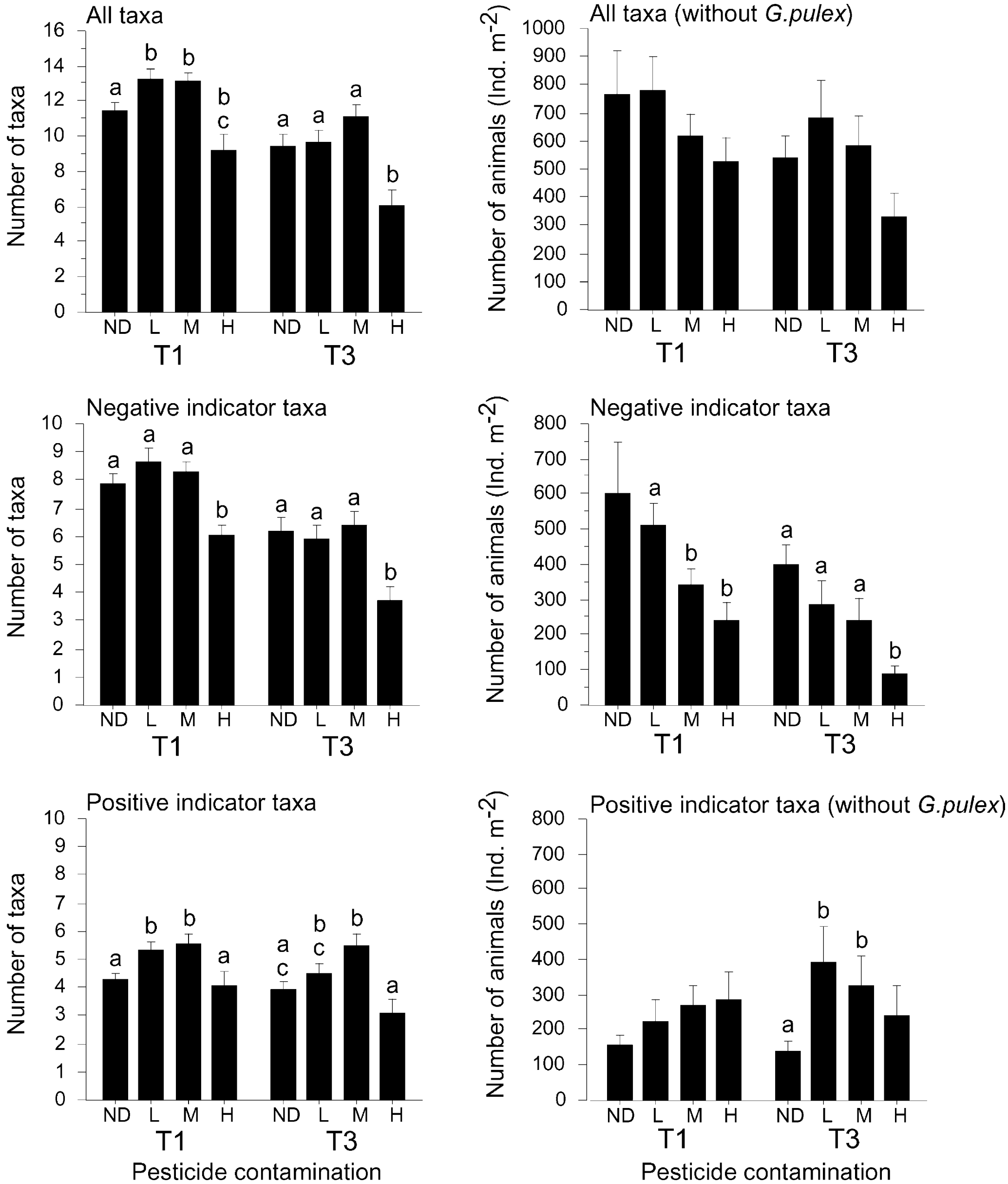

Fig. 6. Mean (±S.E.; $n = 26$–55) number of animals and number of taxa for the group of all taxa, negative indicator (NI) taxa and positive indicator (PI) taxa for each of the time frames T1 and T3 and each of the contamination group Not Detected, Low, Moderate and High. Different letters indicate significant differences within each time frame.

4. Conclusions

- The pesticide contamination of small headwater streams with an agricultural catchment was represented by its toxic potential, was classified into four pesticide contamination classes and will be used as the diagnoses of LIMPACT.
- Abundance data and data on abundance dynamics are suitable parameters to indicate the pesticide contamination classes of LIMPACT.

- High abundances of positive indicator (PI) taxa indicate a high pesticide contamination and high abundances of negative indicator (NI) taxa indicate only slightly contaminated streams.

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An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators
II. The knowledge base of LIMPACT[☆,☆☆]

Michael Neumann[a,*], Joachim Baumeister[b], Matthias Liess[c], Ralf Schulz[a]

[a] Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092 Braunschweig, Germany
[b] Department of Artificial Intelligence and Applied Computer Science, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany
[c] Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318 Leipzig, Germany

Abstract

The development and the evaluation of a biological indicator system for pesticide pollution in streams are presented. For small headwater streams with an agricultural catchment area, the expert system LIMPACT estimates the pesticide contamination according to the four classes: Not Detected (ND), Low (L), Moderate (M) and High (H) contamination without any specification of the chemical agents. The input parameters are the abundance data of benthic macroinvertebrate taxa within four time frames in a year (March/April, May/June, July/August, September/October) and nine basic water-quality and morphological parameters. The heuristic knowledge base was developed with the shell-kit D3 and contains 921 diagnostic rules with scores either to establish or to de-establish a diagnosis. The 418 rules had less than three symptoms, and only 47 rules had more than four symptoms in their rule condition. We differentiate between positive indicator (PI) taxa, which indicate contamination by high abundance values and positive abundance dynamics, and negative indicator (NI) taxa, a high abundance of which rules out contamination and indicates an uncontaminated site. We analysed 39 taxa and found 13 positive and 24 negative indicators. The database comprises 157 investigations per stream and year with rainfall event-controlled pesticide sampling and repeated benthic sampling as described in Part 1 [Ecol. Indicators, this issue]. For the evaluation of LIMPACT, we used the same cases. The correct class for the 157 investigations per stream and year is established by LIMPACT in 66.7–85.5% of the cases, with better results for uncontaminated sites. The overall alpha error probability (false positive) is 9.6% while the beta error probability (false negative) varied between 0 and 8% depending on the contamination class. If each stream is considered only once in the system ($n = 104$), the correct diagnosis is established by LIMPACT in 51.9–88.6% of the cases. In most of the remaining cases no diagnosis is established instead of a wrong one.

Keywords: Ecological indicator; Pesticide contamination; Small streams; Heuristic knowledge base; Model

1. Introduction

Small streams form the beginning of the water circuit. Simply because their lengths add up to a large total, they represent an important habitat for the aquatic fauna on the landscape level. After heavy rainfall,

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* Corresponding author. Tel.: +49-531-3913180;
fax: +49-531-3818201.
E-mail address: m.neumann@tu-bs.de (M. Neumann).

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these habitats are influenced by short-term impact from non-point sources, involving factors such as hydraulic stress and the input of nutrients and pesticides (Cooper, 1993; Neumann and Dudgeon, 2002). Usually, no regular monitoring systems are established for these agricultural non-point sources of pollution.

In Germany, the only recurrent monitoring in small streams done by governmental environmental agencies considers the contamination by biodegradable organic pollutants, monitored with the bioindicator-based saprobic system (Friedrich, 1990). After reviewing a wide range of ecological evaluation systems for running waters, Braukmann and Pinter (1997) proposed an expert system for evaluation purposes. Systems to monitor the influence of organic chemicals and pesticides are not in regular use, even though these substances are known to be very important stressors for the aquatic fauna (Schulz and Liess, 1999).

The main advantage of bioindicator systems is their easy and cost-efficient application. When they are used to monitor toxic contamination, they additionally indicate the ecotoxicological effect of the contaminant. They provide long-term information, whereas information from each chemical measurement applies at only one point in time. Consequently, a bioindicator system should be able to indicate agricultural short-term impact from non-point sources with low acquisition effort.

There are various approaches to evaluate the water quality of streams (Böhmer and Kappus, 1997), but no bioindicator system is known to indicate the pesticide contamination of small streams. In order to consider the ecological complexity and the uncertain knowledge in this domain, we used an expert system shell-kit as a tool. The advantages are that expert systems utilise the uncertain expert knowledge and ideally come to the same solution as the expert would do. The user has full control over the expert system, can scrutinise the solution, and if he does not want to follow the given question trail, can select the next questions by himself.

Our aim was to develop a bioindicator system in form of an expert system that estimates the pesticide contamination of small streams. We name this expert system LIMPACT (from limnology and impact) and will make it available over the Internet. The input parameters of LIMPACT are benthic macroinvertebrate abundance data and basic water-quality and morphological parameters. The output is an estimation of the pesticide contamination according to four classes without any specification of the chemical agents. The database of LIMPACT is documented in Part 1 (Neumann et al., 2003). In this paper, we present the development and the structure of the knowledge base of LIMPACT and the 39 benthic macroinvertebrate bioindicators it utilises, together with a first evaluation of the system.

2. Materials and methods

2.1. Introduction to expert systems

Expert systems are programs for reconstructing the expertise and reasoning capabilities of qualified specialists within their domains. The preliminary basic assumption is that experts construct their solutions from single pieces of knowledge, which they select and apply in a suitable sequence. For diagnostic tasks, they have to specify a set of solutions (diagnoses) and a set of observations (symptoms) and the knowledge for interweaving these two sets.

Experts are able to express their knowledge in various ways. Consequently, there are various types of knowledge of which expert systems can be built. Three common types are heuristic, set-covering and case-based. Whereas set-covering knowledge requires different fault models for each possible solution implemented by the expert (Baumeister et al., 2001), case-based reasoning is appropriate when there is a large collection of successfully solved cases plus domain knowledge available (Puppe, 1998). Heuristic classification is suitable for problems in which the expert is able to express diagnostic ratings on the basis of observations or a combination of observations (Puppe, 1998).

2.2. The shell-kit D3

The shell-kit D3 (http://www.d3web.de) was utilised for the development of the expert system LIMPACT. The shell-kit D3 is applicable for diagnostic tasks, provides a web-based user interface (d3web) and offers a visual knowledge acquisition component for a wide range of knowledge types. After a 1-day tutorial, most experts are able to construct expert systems by themselves. D3 has been already used in many medical, technical or service-support domains (Puppe, 1998; Puppe et al., 1996).

2.3. The knowledge representation

In the domain of interest here, we had the stream contamination as diagnoses, the abundance of taxa as observations and 157 investigations per stream and year as cases. Our aim was to build up a knowledge base to establish a diagnosis according to various possible observations. The domain expert was able to give certain scores (negative or positive) to types of stream contamination on the basis of given abundance data or combinations of them. For this reason, we chose the heuristic knowledge type for implementing LIMPACT. Heuristic classification is based on rules of the following kind:

"IF observation *X* then give diagnosis *Y* the score *Z*"

The observations *X* were clearly defined as the abundance of taxa, whereas the diagnoses are the graded amount of pesticide contamination in the stream. D3 provides a fixed range of seven positive and seven negative scores, which has been approved in previous different applications of D3 (Puppe, 2000). Reasoning with scores is easy and understandable for so the expert: given a true condition, the corresponding rule fires and adds the stated score to the specified diagnosis. When defining a rule, the expert can choose between the seven categories N1 (−5%) to N7 (−100%) for negative scoring and the seven categories P1 (+5%) to P7 (+100%) for positive scoring. The sum of two equal categories results in the next higher category (e.g. P3 + P3 = P4). A diagnosis is established (confirmed), if the sum of the given scores exceeds the category PS.

2.4. The applied diagnoses

The database contained a chemical pesticide measurement for all 157 investigations per stream and year. As described in Part 1 (Neumann et al., 2003), we calculated an annual toxic sum and grouped it into four classes. Therefore, the vital diagnoses of LIMPACT are four classes of pesticide contamination named: Not Detected (ND); Low (L); Moderate (M) and High (H) contamination.

Since LIMPACT is only designed to estimate the pesticide contamination of small lowland headwater streams within an agricultural area, we implemented the diagnosis "unsuitable stream". If the water-quality and morphological parameters are out of a specified range, LIMPACT establishes the diagnosis "unsuitable stream" (see Table 1). This causes all derivation rules for assessing the level of contamination not to fire and no contamination diagnosis will be established.

2.5. The observations

The most important parameters in the knowledge base are the abundances of taxa. We established four time frames for which information about abundance is requested. The time frames are: T1, March/April; T2, May/June; T3, July/August and T4, September/October. For each taxa, LIMPACT allows abundance values to be entered for these four periods of the year. Additionally, LIMPACT interprets the increasing

Table 1
Water-quality and morphological parameters and a schematic view of the 30 rules to establish the diagnosis "unsuitable stream"

Rule no.	Parameter	Score for the diagnosis "unsuitable stream"				
		P7	P3	No score	P3	P7
1	Organic pollution (saprobic index)	–	–	≤2.3	≤2.6	>2.6
2	Morphological structure (%)	–	<20	20–100	–	–
3	Proportion of sand (%)	–	>20	20–80	>80	–
4	Maximum current velocity ($m\,s^{-1}$)	–	<0.05	0.05–0.5	>0.5	>1
5	Cross-sectional area (cm^2)	–	–	≤8000	>8000	>20000
6	Number of dry months	–	–	0	≤3	>3
7	Conductivity ($\mu S\,cm^{-1}$)	≤50	≤150	150–2000	>2000	>3000
8	pH-value	≤6	≤7	7–9	>9	>10
9	Carbonate water hardness (mg $CaCO_3$ l^{-1})	≤100	–	100–550	–	>550

or decreasing abundance dynamics of a taxa by calculating the difference between the values for different time frames. Both the time frame information and the abundance dynamics are used to build up rules.

Besides administrative information, such as stream name or stream location, LIMPACT evaluates water-quality and morphological parameters like stream size or conductivity of the water to characterise a given stream. For simplification, these parameters are abstracted to qualitative values, which are used by the derivation rules.

2.6. The cases

The database holds 157 investigations per stream and year, produced from several investigations of 104 streams between the years 1992 and 2000. A total of 555 chemical pesticide analysis, 660 benthic macroinvertebrate samples and a characterisation of the streams according to nine water-quality and morphological parameters are available. The 157 cases are grouped into the four contamination classes according to their measured pesticide contamination. Additionally, they provide abundance data for the four time frames defined. This is described in detail in Part 1 (Neumann et al., 2003).

2.7. The streams considered

The classification system LIMPACT is designed to consider only small lowland streams with an agricultural catchment. Streams with any interfering factors are excluded to ensure that the impact of pesticide is the main stressor to the aquatic macroinvertebrate fauna. At this stage, we exclude streams with any industrial waste impact. No high organic contamination or strong chloride or pH-values are acceptable, and no highland streams or larger streams are considered. For details see Part 1 (Neumann et al., 2003).

3. Results and discussion

3.1. The implementation of the rules

We differentiated between two kinds of rules for the implementation of LIMPACT. Firstly, we designed rules to establish or de-establish the diagnosis "unsuitable stream". This procedure tests the suitability of a stream for a classification with LIMPACT. Altogether, we used 30 rules as suitability rules. Table 1 shows the parameters and a schematic view of the rules. Depending on the value of the parameter, either no rule fires or the score for the diagnosis unsuitable stream is set to P3 or P7. Within the normal range, no score is given. If the parameters are within a range in which only a minor effect on the benthic fauna is expected, the diagnosis unsuitable stream is only suggested (P3) and all other rules will fire. If more than four minor criteria score with P3, the diagnosis unsuitable stream is established, which prevents LIMPACT from firing other rules, i.e. no contamination diagnosis can be established. This will happen in either case, if P7 is scored.

The major part of the development of LIMPACT was to find and to implement the rules to estimate the stream contamination. During a first step, we selected those species and taxa that LIMPACT should consider. Rare taxa are liable to random and uninterpretable variations in abundance. Consequently, we analysed the 39 most common species and taxa representing 90.4% of the total abundance of all taxa. Table 2 gives an overview of the 39 taxa including the frequency of their occurrence and the relative abundance.

The database was used to analyse the 157 cases for the 39 taxa regarding the grouping within the four contamination classes. We searched for a trend in the abundance data suitable to create rules with certain scores (positive or negative) to establish or de-establish the four diagnoses for pesticide contamination. Those taxa with low abundances in polluted streams, we named negative indicators (NI). An NI is thus a taxon with an abundance negatively correlated with the pesticide contamination. The abundance of a positive indicator (PI) taxon is positively correlated with the contamination. High abundances of NI therefore indicate low or no contamination, while high abundance of PI suggests high contamination. All 39 taxa were analysed and classified (Table 2), with NI subdivided into those with clear, sensitive population dynamics (NI1) and without clear dynamics (NI2). PIs were differentiated into those with population dynamics suggesting tolerance against pesticide pollution (PI1), those with no changes in abundance over time (PI2) and those that are stimulated by moderate contamination (PI3). Two taxa could not be grouped according to this scheme and were labelled as unsure.

Table 2
List of the 39 benthic macroinvertebrate taxa considered for LIMPACT, with frequency of occurrence and relative abundance at the 660 sampling dates as well as the classification as positive (PI) or negative indicator (NI) taxa

Order	Taxon	Classification	Frequency (*n*/660)	Relative abundance (%)
Turbellaria	*Dugesia gonocephala*	PI1	142	0.78
Oligochaeta	*Erpobdella octoculata*	PI2	403	1.30
	Glossiphonia complanata	NI2	307	0.38
	G. heteroclita	PI2	144	0.09
	Thbificidae	PI2	307	3.34
	Oiigocbaeta	NI1	98	0.47
Gastropoda	*Pisidium* sp.	PI3	220	3.25
	Potamopyrgus antipodarum	PI2	74	2.43
	Radix ovata	PI2	213	1.75
Amphipoda	*Gammarus pulex*	PI1	587	60.00
Isopoda	*Asellus aquaticus*	NI1	213	1.46
Plecoptera	*Nemoura cinerea*	NI2	60	0.49
Coleoptera	*Dytiscidae*	PI3	135	0.24
	Agabus sp.	NI1	89	0.11
	Platambus maculatus	NI1	60	0.04
	Elmis sp.	PI2	180	0.6
	Haliplus sp.	NI1	79	0.08
	Helodes sp.	PI1	308	2.46
Diptera	Ceratopogonidae	PI2	82	0.11
	Chironomidae "white"	NI2	450	4.21
	Chironomidae "red"	NI2	396	6.06
	Limoniidae	Unsure	139	0.13
	Ptycbopteridae	NI1	65	0.58
	Simuliidae	NI1	183	1.68
	Tipulidae	NI2	111	0.11
	Other Diptera	Unsure	89	0.34
Ephemeroptera	*Baetis vemus*	NI1	62	0.23
	Baetis sp.	NI1	113	0.78
	Ephemera danica	NI1	68	0.4
Megaloptera	*Sialis lutaria*	NI2	93	0.16
Trichoptera	*Hydropsyche angustipennis*	NI1	60	0.14
	Anabolia nervosa	NI2	99	0.44
	Chaetopteryx villosa	NI1	158	0.85
	Halesus radiatus/digitatus	PI3	84	0.14
	Ironoqula dubia	NI1	69	0.13
	Limnephilus lunatus	NI1	379	3.68
	Limnephilus extricatus	NI1	145	0.28
	Limnephilus rhombicus	NI2	65	0.13
	Plectrocoemia conspersa	NI2	104	0.16

This approach may be influenced by general ecological principles. While the high abundance of a NI clearly indicates low contamination, a low abundance does not automatically indicate high contamination. The same is true for PI: high abundances usually found in contaminated streams but PI may also occur in uncontaminated streams. As a result, the analysis of the abundance data was done with regard to nine water-quality and morphological parameters of the streams. Furthermore, the analysis of the population dynamics of the taxa gives valuable information.

The heuristic diagnosis score pattern (Puppe, 2000) is able to deal with uncertain knowledge. Rules do not exclude one another but can fire at the same time. If the data did not count explicitly for one diagnosis, it was possible to score more than one diagnosis. We chose a conservative approach, with the strongest score set to P3. Consequently, a diagnosis needs at least five fired rules to be established. Up to now the knowledge base of LIMPACT has 921 diagnosis rules.

The taxon Chironomidae "red" is an example of a NI. In time frame 3 (T3), high abundances were found only in uncontaminated or slightly contaminated streams (Fig. 1a). Consequently, we created the rules 1 and 2 exemplified in Table 3, which fire simultaneously with the score P2. At the same time a high abundance of Chironomidae "red" at T3 is a strong indicator against a Moderate and High contamination, which is expressed by score N4 in rules 3 and 4. No rule with a positive score for High or Moderate contamination is used here.

The species *Glossiphonia heteroclita* is given as an example of a PI taxon (Fig. 1b). A high abundance indicates Moderate or High contamination (rules 5 and 6) and counts with negative score against no or low contamination (rules 7 and 8). This confirms the common view that oligochaetes are relatively tolerant taxa (Meller et al., 1998).

Chaetopteryx villosa as a NI species had population dynamics that may indicate less contamination

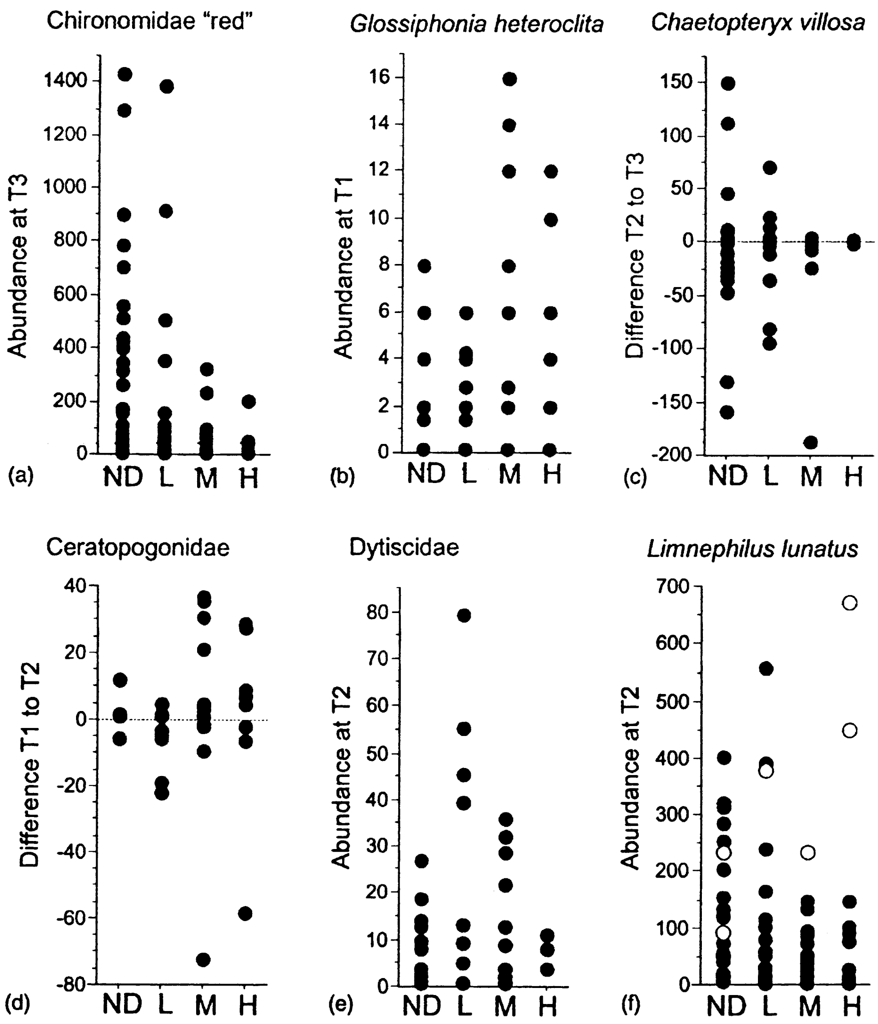

Fig. 1. Abundances or abundance dynamics of indicator taxa to illustrate the creation of rules. In (f) the open circles indicate sites with a large amount of morphological structure (>80%) in the streams.

Table 3
Schematic view of rules following the analysis of exemplary taxa in Fig. 1

Rule no.	Rule syntax
1	If Chironomidae “red” at T3 > 370 then give diagnosis ND the score P2
2	If Chironomidae “red” at T3 > 370 then give diagnosis Low the score P2
3	If Chironomidae “red” at T3 > 370 then give diagnosis Moderate the score N4
4	If Chironomidae “red” at T3 > 280 then give diagnosis High the score N4
5	If *G. heteroclita* at T1 > 8 then give diagnosis Moderate the score P2
6	If *G. heteroclita* at T1 > 8 then give diagnosis High the score P2
7	If *G. heteroclita* at T1 > 8 then give diagnosis ND the score N4
8	If *G. heteroclita* at T1 > 8 then give diagnosis Low the score N4
9	If *C. villosa* T2 to T3 10 to 75 then give diagnosis ND the score 12
10	If *C. villosa* T2 to T3 10 to 75 then give diagnosis Low the score P2
11	If *C. villosa* T2 to T3 > 75 then give diagnosis ND the score P3
12	If *C. villosa* T2 to T3 > 75 then give diagnosis Low the score N4
13	If *C. villosa* T2 to T3 > 10 then give diagnosis Moderate the score N4
14	If *C. villosa* T2 to T3 > 10 then give diagnosis High the score N4
15	If *C. villosa* T2 to T3 (−10) to 10 then give diagnosis Moderate the score P2
16	If *C. villosa* T2 to T3 (−10) to 10 then give diagnosis High the score P2

(Fig. 1c). An increasing abundance from T2 to T3 indicates Not Detected or Low contamination (rules 9 and 10) and counts against moderate and strong contamination (rules 13 and 14). Trichoptera are known to be sensitive to pesticide impact (Schulz and Liess, 1995, Stuijfzand et al., 2000). A strong growth can explicitly indicate an uncontaminated stream, which is scored with P3 (rule 11) and counts against a Low contamination with score N4 (rule 12). Nearly unchanged dynamics are used to score P2 to High or Moderate contamination (rules 15 and 16). Even though the figure suggests that a strong decline indicates less contamination, no rules are created because this is not justifiable from the autecology of this species. The reason for this phenomenon is a generally lower abundance in contaminated streams at T2, which makes a strong decline highly improbable. In this case, the rules need to combine both abundance and abundance dynamics characteristics.

Ceratopogonidae showed population dynamics from T1 to T2 as a PI (Fig. 1d). An increase was found only at contaminated streams and counts at the same time against less contamination. Meng and Lok (1985) found a starvation survival strategy for this group which indicates a tolerance to extreme situations. The abundance of Dytiscidae at T2 (Fig. 1c) is typical of a tolerant taxon that has selectively high abundance at Low and Moderate contamination. A high abundance rules out the not and the highly contaminated streams. This taxon was found to be tolerant to high temperatures (Velasco and Millan, 1998). On the other hand, it seems to be sensitive to strong pesticide contamination.

The last example (Fig. 1f) illustrates how water-quality and morphological parameters could influence the creation of complex rules. *Limnephilus lunatus* is a NI taxon. High abundance is typical of uncontaminated streams and in contaminated streams, the database showed mostly low abundance. An exception occurs in streams with extremely rich morphological structures (>80%) covering the stream bed (open circles: “good” structures). This large amount of morphological structures is associated with high abundance of *L. junatus*. This is also described by (Gower, 1967) and may mask the pesticide contamination so that no effect is observable. Consequently, the rules have to include the structural information in the following way:

If *L. lunatus* at T2 > 180 and structure is not “good” then give diagnosis ND the score P3.

Most rules in the knowledge base are complex rules. Four hundred eighteen rules had less than 3 symptoms, but 457 had 3 or 4 symptoms and 47 rules had up to 7 symptoms in their rule condition. None of these rules combining two time frames, a time frame and one or two measures of population dynamics are presented here. Overall, 622 rules have a positive score and count for a diagnosis while only 299 rules have a negative score against a diagnosis. The number of rules is nearly equally distributed over the four diagnoses with 226 for High contamination and 251 rules for Not Detected. However, the diagnosis ND has 83% positive scored rules while the diagnosis High contamination has only 48%. This proves that it is much easier to identify an uncontaminated stream because of its large number of taxa and the high abundance of NI taxa.

3.2. Evaluation of LIMPACT

For the evaluation of the expert system, we present the classification result (Table 4) of those 157 investigations per stream and year (cases) that have been used to build up LIMPACT. The evaluation showed a very good classification result, however, it is not independently obtained. The correct diagnosis was established in 66.7–85.5% of cases. A high percentage of cases were not classified. Because of our conservative approach, LIMPACT established no diagnosis instead of a wrong one for cases with less data availability. As standard diagnostic measures we calculated the alpha and beta error probabilities for those cases that were classified, only. The overall alpha error probability (false positive) is 9.6% (13 out of 135). It varied from 0% (H class) to 18.7% (L class). Most classification errors occur between ND and Low and on the other hand between Moderate and High contamination. The fact that there are only very few cases existing with a false prediction between ND and Low on the one hand and Moderate and High contamination on the other hand, gives further evidence for the ability of LIMPACT to provide a reliable rough estimation of pesticide contamination in streams based on macroinvertebrate data. The beta error probability (false negative) indicates how often the system failed to reject the hypotheses Ho when it actually should reject it. Here the best result shows the class Low (0%) and Moderate (0.9%). For the class High, we calculated a beta error of 4.4% and for Not Detected 8%.

The 157 cases in our database refer to investigations at a total of 104 sampling sites. Consequently, we also estimated the classification result for these 104 investigations per sampling site (Table 5). We always selected the most recent investigation. The results are comparable. The wrong classification rate is almost at the same level while the not classified rate is higher. The overall alpha error probability calculated for those cases with an established diagnosis only, is 12.5% (11 out of 88). The beta error probability varied from 0 to 8.9%. Both measures are comparable. We conclude from this that the repeated investigations have no significant influence on the quality of the classification result and consequently can be treated as independent investigations.

Possible reasons for classification errors and not classified data sets are the count of taxa at a sampling site and the number of sampling dates within a year. The more data the user provides, the more rules can

Table 4
Result of the classification of 157 investigations per stream and year at 104 sample sites using LIMPACT

Real contamination	Classification result (%)				
	Not Detected	Low	Moderate	High	Not classified
Not Detected (55)	**85.5** (47)	0 (–)	1.8 (1)	0 (–)	12.7 (7)
Low (34)	17.6 (6)	**76.5** (26)	0 (–)	0 (–)	5.9 (2)
Moderate (42)	2.4 (1)	0 (–)	**66.7** (28)	11.9 (5)	19.0 (8)
High (26)	0 (–)	0 (–)	0 (–)	**80.8** (21)	19.2 (5)

For each of the 157 cases, the measured real contamination is given according to the four classes and compared with the percentage of cases classified by LIMPACT into the four groups. Correct classifications are indicated by bold values. The number of cases per contamination class is given in brackets.

Table 5
Result of the classification of 104 investigations per stream and year at 104 sample sites using LIMPACT

Real contamination	Classification result (%)				
	Not Detected	Low	Moderate	High	Not classified
Not Detected (35)	**88.6** (31)	0 (–)	2.9 (1)	0 (–)	8.6 (3)
Low (29)	13.8 (4)	**75.9** (22)	0 (–)	0 (–)	10.3 (3)
Moderate (28)	3.7 (1)	0 (–)	**51.9** (15)	18.5 (5)	25.9 (7)
High (12)	0 (–)	0 (–)	0 (–)	**75.0** (9)	25.0 (3)

For each of the 104 cases, the measured real contamination is given according to the four classes and compared with the percentage of cases classified by LIMPACT into the four groups. Correct classifications are indicated by bold values. The number of cases per contamination class is given in brackets.

be activated. In our data set not every investigation per stream and year provided data for all four time frames. T1 and T2 data were available in 98% of the cases, while T3 data exist in 78% and T4 in 72% of the cases. This is one reason why the rate of cases that were not classified is quite high. As mentioned previously, to give no classification result rather than a wrong one reflects our conservative approach while developing LIMPACT.

We are not yet able to evaluate LIMPACT with an independent data set. As soon as new investigations are available, we shall use this data set for an independent evaluation and a refinement of the knowledge base. However, the knowledge base was built up by statistical data analysis and was adapted by domain knowledge of experts. This guarantees the high quality of the knowledge base of LIMPACT although it is based on a rather small number of cases.

3.3. Concluding discussion

A wide range of biological indicator systems to evaluate water-quality parameters is known. RIVPACS in Great Britain predicts the macroinvertebrate fauna to be expected at a site in the absence of environmental stress (Wright et al., 1998) and can be used to evaluate the present fauna. In The Netherlands, a similar approach is used for STOWA (Peeters et al., 1994). In Scotland, the integrated evaluation system SERCON (Boon, 2000) and in the USA the Rapid Bioassessment Protocols (Resh et al., 1995) were developed. The last one has been successfully used to locate stream contamination in large regions but is not able to specify the cause. In Germany, the saprobic index is well established to evaluate the biodegradable organic pollution in running waters (Friedrich, 1990). Systems to monitor heavy metals (Wachs, 1991), and acidification (Brakke et al., 1994) have been developed. However, no biological indicator system has yet estimated the pesticide contamination of small streams via benthic macroinvertebrate indicators.

Recently, a few expert systems were developed for stream water-quality evaluation. The ecological condition was estimated by expert system for rivers in agricultural landscapes (Sterba et al., 1997) and forest was found to be the main factor for river restoration. In Canadian watersheds, a water-quality model was coupled with an expert system to simulate the movements of pollutants (Ghosh et al., 2000) and in Korea, an expert system was used to determine stream water quality from uncertain and imprecise ecological information (Lee et al., 1997). An integrated model (Jia et al., 1998) including an expert system was applied for sustainable development of river basins and water-quality planning in China. To evaluate the thermal pollution of rivers, Kontic and Zagorc (1992) presented an expert system and applied it to a nuclear power plant. However, no expert system is available to estimate the water quality of streams by using the aquatic fauna as indicator. van Der Werf and Zimmer (1998) presented an expert system to estimate the environmental impact of pesticides by using the pesticide properties.

A general restriction of biological indicator systems is that indicator species must be present at the investigation site. This is also a limitation of LIMPACT. Until now, LIMPACT considers 39 species or taxa. If none of these taxa is present at a sampling site, none of the rules of the knowledge base of LIMPACT can fire. Consequently, such a sample site cannot be classified. In the evaluation process of LIMPACT presented in this paper, an average of 5 of the 39 indicator taxa was present in each case.

For the process of refinement, we have three major aims. First, we want to extend the knowledge base by adding more species. This will increase the precision and improve the classification result. Second, we intend to enhance the general applicability of LIMPACT. This should be achieved by analysing higher taxonomic levels, which would cause more generalised rules and a wider practicability of LIMPACT without limitations imposed by the presence or absence of single species. Third, we want to reduce the number of essential sampling dates. LIMPACT considers four time frames within 1 year. An extended version of LIMPACT should give sufficient results based on a lower number of sampling dates. This would again increase the simplicity and reduces the effort expended on invertebrate sampling.

The potential application of LIMPACT could be a yearly monitoring of streams and would reduce chemical analysis to the mandatory cases. Furthermore, it could be used to evaluate the success of risk mitigation strategies in the catchment to reduce the impact of pesticides.

4. Conclusions

- The biological indicator system LIMPACT will be available over the Internet.
- LIMPACT could be utilised to monitor the water quality of small streams via benthic macroinvertebrates and could thus reduce chemical monitoring to the necessary limit.
- An analysis of higher taxonomic levels could further increase the practicability of LIMPACT.

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