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Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication

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SUMMARY

1. We analysed a large number of concurrent samples of macroinvertebrate communities and chemical indicators of eutrophication and organic pollution [total-P, total-N, NH_4–N, biological oxygen demand (BOD_5)] from 594 Danish stream sites. Samples were taken over an 11-year time span as part of the Danish monitoring programme on the aquatic environment. Macroinvertebrate communities were sampled in spring using a standardised kick-sampling procedure whereas chemical variables were sampled six to 24 times per year per site. Habitat variables were assessed once when macroinvertebrates were sampled.

2. The plecopteran *Leuctra* showed a significant negative exponential relationship ($r^2 = 0.90$) with BOD_5 and occurred at only 16% of the sites with BOD_5 above 1.6 mg L^{-1}. Sharp declines with increasing BOD_5 levels were found for the trichopteran families Sericostomatidae and Glossosomatidae although they appeared to be slightly less sensitive than *Leuctra*. Other plecopterans such as *Isoperla* showed a similar type of response curve to *Leuctra* (negative exponential) but occurred at sites with relatively high concentrations of BOD_5 up to 3–4 mg L^{-1}. In contrast, the response curve of the isopod *Asellus aquaticus* followed a saturation function reaching a plateau above 3–4 mg L^{-1} BOD_5 and the dipteran *Chironomus* showed an exponential increase in occurrence with increasing BOD_5 concentration.

3. Macroinvertebrate occurrence appeared to be related primarily to concentrations of BOD_5, NH_4–N and total-P whereas there were almost no relationships to total-N. Occurrence of a number of taxa showed a stronger relationship to habitat conditions (width and substrate) than chemical variables.

4. Important macroinvertebrate taxa are reduced at concentrations of BOD_5 that are normally perceived as indicating unimpacted stream site conditions. Our results confirmed sensitivity/tolerance patterns used by existing bioassessment systems only to some degree.

Keywords: bioassessment, biological oxygen demand, macroinvertebrates, nutrients, streams

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Introduction

Macroinvertebrates are the group of organisms most frequently used in biomonitoring of streams and rivers worldwide. Currently more than 50 different approaches for bio-monitoring using macroinvertebrates exist (e.g. A.F.N.O.R., 1982; De Pauw & Vanhooren, 1983; Wright, Sutcliffe & Furse, 2000; Friberg *et al.*, 2006). The main focus of traditional assessment systems using macroinvertebrates in streams has been organic pollution (e.g. Metcalfe-Smith, 1996), because the main pressures on streams and rivers have been organic pollution from untreated sewage or agricultural point sources (manure, silage). All assessment systems targeting organic pollution are derived from observational studies and based on oxygen demands of individual macroinvertebrates (Liebmann, 1951; Sladecek, 1973), building on the original concept of the saprobic system (Kolkwitz & Marsson, 1902).

While organic pollution tolerance of a wide range of macroinvertebrate taxa is established through numerous studies of macroinvertebrate occurrence along gradients in organic pollution, little is known on how macroinvertebrates respond to elevated nutrient levels. Considerable research effort has gone into describing and measuring nutrient transport in streams, primarily aiming at quantifying loading to downstream waters (e.g. Kronvang *et al.*, 2005) and impacts of excess nutrient loading on the overall ecology of both lakes and coastal areas have been documented in a large number of studies (e.g. Meeuwig, Kauppila & Pitkanen, 2000; Jeppesen *et al.*, 2005). While eutrophication, especially phosphorous, has been found to have impacts on primary producers and has been incorporated in a number of indices (e.g. Biggs, 2000; Kelly *et al.*, 2008), studies showing direct and indirect effects on higher trophic levels have been less conclusive. A number of studies report changes in macroinvertebrate and fish communities after nutrient additions in primarily oligotrophic environments (Johnston *et al.*, 1990; Perrin & Richardson, 1997; Biggs *et al.*, 2000; Robinson & Gessner, 2000) whereas to our knowledge only one study to date has tried to develop a nutrient biotic index for streams (Smith, Bode & Kleppel, 2007).

The EU Water Framework Directive (WFD; Directive 2000/60/EC – Establishing a Framework for Community Action in the Field of Water Policy) defines a framework for assessing waterbodies including streams and rivers. One of the indicator groups to be used in WFD monitoring of stream and rivers is macroinvertebrates. The long tradition of using macroinvertebrates in most European countries is likely to make them a focal biological element in the first reporting period of the WFD and they have been the element most comprehensively compared among member states in the recent inter-calibration of ecological status classes (European Commission, 2007). For this reason, it is essential that our knowledge of how macroinvertebrates respond to pressures is reliable.

The present study is based on a large data set in which both chemical and biological samples, using quality-assured and standardised protocols, were obtained simultaneously. This data set presents a unique opportunity to explore relationships between a range of different stream macroinvertebrate taxa and key chemical and habitat variables to increase our knowledge of the sensitivity/tolerance of macroinvertebrate taxa to various measures of water quality. Specifically we aimed to investigate how occurrence of macroinvertebrate taxa correlated to gradients in water chemistry and if some taxa exhibited distinct threshold or optimum values. We furthermore wanted to test if distribution of macroinvertebrate taxa along a gradient in organic pollution, expressed as BOD_5, showed sensitivity or tolerance responses that were comparable with existing knowledge.

Methods

Data sources and strategy

The data set consisted of macroinvertebrate community samples from 594 Danish stream sites over the entire country. For each macroinvertebrate sample there were analyses of chemical variables in the stream water which included BOD_5, total-P, NH_4–N and total-N. Biological oxygen demand (BOD_5) is a measure of the quantity of labile organic matter while the nutrients express the level of eutrophication. Channel width was measured at most sites ($n = 447$), while substrate composition was measured in little more than half of the sites ($n = 266$). Samples were taken during an 11-year time span as part of the Danish Monitoring Programme on the Aquatic Environment (Søndergaard, Skriver & Henriksen, 2006). Samples served different purposes in the monitoring programme and were used to assess nutrient transport, impacts of point source pollution and general water quality status, so not all variables were measured at all sites.

The entire data set was used in the analysis by pairing each macroinvertebrate sample with average values of the chemical variables sampled in the same year at that site. Habitat data were subsequently included in the analysis to elucidate how much variability that could be explained by physical variables. We assumed that influence of habitat variables at the 266 sites sampled are representative of the entire data set. Furthermore, we assume that macroinvertebrates at a given site responded to the chemical and physical environment at that site independently of spatial settings. Thus we do not consider location of the sites in the river network in our analysis.

Macroinvertebrate sampling

Macroinvertebrate sampling was undertaken according to the guidelines of the Danish Stream Fauna Index (DSFI; Skriver, Friberg & Kirkegaard, 2000). The sampling procedure is standardised, and includes, in principle, all microhabitats at a site. Sampling was undertaken using a standard hand net with a 25 × 25 cm opening and a tapering net bag with a mesh size of 0.5 mm (European Standard EN 27 828). Sampling was done at three transects across the stream spaced about 10 m apart; four standardised kick samples were taken at each transect 25%, 50%, 75% and 100% from one of the stream banks. The 12 kick samples were pooled for further analysis. The kick sampling was supplemented by 5 min of hand-picking from submerged stones and large wooden debris. The macroinvertebrates collected by hand-picking were included in the total taxa list.

The macroinvertebrates was sorted and identified in the laboratory to a pre-defined taxonomic level and only a few taxa were enumerated in accordance with the standard procedure (Skriver *et al.*, 2000). The pre-defined taxonomic level differs among taxonomic groups so that some, such as Plecoptera, were identified to genus whereas Ephemeroptera and Trichoptera were identified to family. In the present study we investigated 23 taxa identified to the taxonomic level of the DSFI method (Table 1). The number of species and their dominance in Denmark within each taxonomic group was derived using existing information from the literature (Wiberg-Larsen, 1984; Nilsson, 1996; Stoltze & Pihl, 1998; Wiberg-Larsen *et al.*, 2000) combined with a subset of taxa lists from the Danish national monitoring programme being fully identified (J. Skriver, unpubl. data). In most of the DSFI taxonomic groups only relatively few species occurs in Danish streams (all possibilities are listed in Table 1 with the most common species highlighted) reflecting a limited species pool and this enables interpretation of the results on a more detailed taxonomic level than the actual identifications.

Chemical analysis

Chemical samples were collected six to 24 times per year from the same reaches as the kick samples. They were obtained using glass or polyethylene bottles. Samples were transported and stored cool (0–4 °C) and dark prior to the chemical analysis. Sample were stored a maximum of 48 h (BOD_5 and total-N) or 24 h (total-P, NH_4–N). Analyses were undertaken using the following international standards by accredited laboratories: EN ISO 1899-2 (BOD_5) with a 0.5 mg L^{-1} detection limit, EN ISO 6878 (total-P) with a 0.01 mg L^{-1} detection limit, EN ISO 11732 (NH_4–N) with a 0.01 mg L^{-1} detection limit and EN ISO 11905-1 (total-N) with a 0.06 mg L^{-1} detection limit. In total, chemical data used in this study comprised 3068 BOD_5 samples, 6329 total-P samples, 5516 NH_4–N and 6108 total-N samples.

Habitat variables

Habitat variables were collected together with each macroinvertebrate sample. Stream width was measured to the nearest 0.1 m and substrate composition was visually assessed within the 20 m macroinvertebrate sampling reach. Substrate classes included stones (>6 cm), coarse gravel (2–6 cm), gravel (2 mm–2 cm), sand (0.01 mm–2 mm), silt/clay (<0.01 mm) and peat. Overlaying organic matter on the bed was classified as either mud (<1 mm) or coarse detritus (>1 mm). In addition, vegetation cover was quantified. Depending on coverage on the reach each substrate class was assigned a score (S) between 0 and 3: not present (0), sparsely present (1; coverage > 0–10%), moderately present (2; coverage 11–30%) and dominating (3; coverage > 30%). The

Table 1 Minimum level of identification in the Danish Stream Fauna Index (DSFI) and species occurring in Denmark within each DSFI taxonomic group

DSFI taxonomic group	Species within the taxonomic group
Oligochaeta ≥ 100*	*c.* 100 species
Hirundinae	
Erpobdella	*Erpobdella octoculata* (L.) *E. testacea* (Savigny)
Malacostraca	
Asellus	*Asellus aquaticus* (L.)
Gammarus	***Gammarus pulex*** (L.), *G. lacustris* Sars
Ephemeroptera	
Baetidae	*Baetis rhodani* (Pict.), *B. vernus* Curt. (+6 other species)
Ephemeridae	***Ephemera danica*** Mull., *E. vulgata* L.
Heptageniidae	***Heptagenia sulphurea*** (Mull.) *Kageronia fuscogrisea* (Retz.) (+3 other species),
Leptophlebiidae	*Leptophlebia marginata* (L.), *Paraleptophlebia submarginata* (Steph.) (+3 other species)
Plecoptera	
Amphinemura	***Amphinemura stanfussi*** (Ris), *A. sulcicollis* (Steph.)
Isoperla	***Isoperla grammatica*** (Klapalek), *I. difformis* (Poda)
Leuctra	*Leuctra hippopus* (Kempny), *L. nigra* (Oliv.), *L. digitata* Kempny, *L. fusca* (L.)
Nemoura	***Nemoura cinera*** (Retz.), *N. avicularis* Morton, *N. dubitans* Morton, *N. flexousa* Aubert
Megaloptera	
Sialis	***Sialis lutaria*** L., *S. fuliginosa* Pict., *S. nigripes* Ed. Pict.
Coleoptera	
Elmis	*Elmis aenea* (Mull.)
Limnius	*Limnius volckmari* (Panz.)
Trichoptera	
Goeridae	*Silo nigricornis* (Pict.), *S. palllipes* (Fab.), *Goera pilosa* (Fab.) (+1 species)
Glossosomatidae	*Agapetus fuscipes* (Curt.) (+2 other species)
Limnephilidae	Limnephilidae (*c.* 30 lotic species)
Sericostomatidae	***Sericostoma personatum*** sp., *Notidobia cilliaris* (L.)
Rhyacophilidae	*Rhyacophila nubila* (Zett.), *R. fasciata* (Hagen)
Diptera	
Chironomus	*Chironomus riparius* (Meg.), *C. plumosus* (L.)
Gastropoda	
Ancylus	*Ancylus fluviatilis* (Mull.)
Lymnaea	*Lymnaea peregra*† (Mull.) (+6 other species)

For each taxonomic group names of the most commonly found species are in bold. Rare species are not listed other than by the number of species occurring in Danish streams.
*Oligochaeta is only registered in a sample if they occur in 100 or more individuals
†*Lymnaea peregra* is due recent advance in taxonomy now considered to be *Radix balthica* L

substrate composition on the entire sampling reach was quantified using a substrate index. All substrates were assigned a coarseness index value (I); stones, coarse gravel and vegetation were given a value of 2, gravel was assigned a value of 1, sand and coarse detritus were given a value of 0, silt, clay and peat were given a value of −1 and mud a value of −2. The substrate index was calculated as the sum of index values multiplied by the score for the individual substrates on the site: Substrate Index $= \sum_{i=1}^{10} S_i \times I_i$ where i, the individual substrate and 10, the maximum number of substrate categories.

Data analysis

The occurrence of the 23 taxa was examined individually for intervals of BOD$_5$, total-P, NH$_4$–N and total-N along the entire gradient of these variables. Yearly average values of each chemical variable were compared to the macroinvertebrates sampled in the same year. Macroinvertebrate data were presence–absence of the 23 taxa, in each of the 594 samples. Chemical variables were grouped in heuristically set intervals: with regard to BOD$_5$, total-P and NH$_4$–N the number of predefined intervals was 12–13 and nine for total-N for all taxa. In the definition of the intervals we aimed for a relative large number of observations in each interval (typically 100–250) to eliminate large differences in values between intervals because of sporadic occurrence of macroinvertebrate taxa. For each interval the percentage of sites at which each of the 23 macroinvertebrate taxa occurred was calculated and curves were fitted to each data set with chemical intervals (midpoint) and the calculated percentages. Relationships were analysed statistically by exponential or polynomial regression (Snedecor & Cochran, 1989). The approach used here was selected in favour of logistic regression as the latter assumes sigmoid relationships (Agresti, 1990) which were not the case for the relationships in the present study.

We tested the validity of our analytical approach of dividing occurrence of taxa into pre-defined intervals by analysing how changing interval ranges would influence model outputs for each taxon and the four chemical variables. We ran more than 4000 randomisations for each combination of taxa and chemical variables by having 12 intervals with randomly chosen (two of each) lower and upper limits. The first interval had a fixed lower limit (zero) and the last interval a fixed upper limit. For each randomisation new model parameters were estimated and the mean, minimum and maximum were calculated. Changing the intervals did not distort any overall relationships found by the initial division because of the large number of data points (data not presented).

The relationship between occurrence of macroinvertebrate taxa and BOD_5 and the two habitat variables (width and substrate index score) was tested with multiple logistic regressions (Agresti, 1990). This allowed us to compare the response to BOD_5 in the analyses using the pre-defined interval as well as testing how much additional variability could be explained by including the two habitat variables. Here we only include analyses with BOD_5 as it was assumed to be the main chemical variable driving the distribution of macroinvertebrate taxa. Similar analyses with the other three chemical variables did not reveal different patterns (data not shown).

Comparison with saprobic values

The response of macroinvertebrate taxa to BOD_5 in this study was compared to saprobic values. These are derived on observational studies and reflect macroinvertebrate sensitivity/tolerance towards levels of dissolved oxygen in the water (Zelinka & Marvan, 1961). Saprobic values are used as an integral part of calculating the saprobic index but have also been used as in the development of biotic indices such as biological monitoring working party (Metcalfe-Smith, 1996). Saprobic values were obtained from http://www.freshwaterecology.info and here we used the Austrian saprobic values (Moog, 2002) to ensure consistency. However, saprobic values between countries are very comparable and it is unlikely that results would have differed by using another set of saprobic values. As saprobic values are on the species level, we used values of the dominating species within each taxon (Table 1). If multiple species were equally common in Danish streams, an average value was calculated. Relationship between occurrence of macroinvertebrate taxa in the present study and saprobic values were tested using linear regression.

Results

Characteristics of the data set

The number of samples with both macroinvertebrate and chemical variables ranged between 288 (total-N) and 555 (NH_4–N) (Table 2). All four chemical variables ranged widely in concentration (Table 3).

Responses to BOD_5

Occurrence of most taxa showed a negative exponential relationship with BOD_5 (Fig. 1). This was highly significant for *Leuctra*, *Amphinemura* and *Isoperla*. *Leuctra* only occurred in 16% of samples when BOD_5 was >1.6 mg L^{-1}. A number of taxa showed a similar type of response curve with an exponential decrease in occurrence with BOD_5 but the model fit was less significant (Fig. 1). Only one taxon, *Chironomus*, showed a positive exponential relationship with BOD_5.

For most taxa, second-degree polynomial models best fit the data but the response was highly variable (Fig. 1). Oligochaeta ≥ 100 and *Asellus* followed a saturation function where occurrence increased until reaching a plateau at 3–4 mg BOD_5 L^{-1} while *Erpobdella*, *Elmis* and *Gammarus* followed a unimodal function. *Erpobdella* had an optimum at 3.5 mg BOD_5 L^{-1} where it occurred at approximately 70% of samples while both *Elmis* and *Gammarus* had optima at 2 mg BOD_5 L^{-1}.

Table 2 Number of samples in which each taxa occurred, stratified by chemical variable

DSFI taxa group	BOD_5	P-total	NH_4–N	N-total
Oligochaeta ≥ 100	514	257	512	253
Erpobdella	235	140	231	140
Asellus	341	193	336	192
Gammarus	480	252	478	248
Baetidae	455	243	452	239
Ephemeridae	84	30	84	30
Heptageniidae	99	45	92	45
Leptophlebiidae	98	45	94	43
Amphinemura	141	47	141	44
Isoperla	85	34	81	34
Leuctra	132	44	132	40
Nemoura	319	154	320	150
Sialis	126	65	127	65
Elmis	295	148	292	146
Limnius	92	40	90	39
Goeridae	102	53	103	52
Glossosomatidae	30	14	31	12
Limnephilidae	498	255	497	251
Sericostomatidae	124	48	124	45
Rhyacophilidae	175	69	174	67
Chironomus	20	13	21	13
Ancylus	96	57	95	55
Lymnaea	138	76	138	74

Both Limnephilidae and Baetidae occurred in most samples (>90%) when BOD_5 was between 0.5 and 3 mg L^{-1} above which their occurrence began to decline until they were only found at approximately 50% of samples when BOD_5 was >5 mg L^{-1}.

Ephemeridae, *Sialis* and *Lymnaea* were the only taxa analysed that did not show a significant relationship between occurrence and BOD_5.

Response to nutrients

Overall, the relationship of individual taxa to both total-P and NH_4–N followed that of BOD_5 whereas this was not the case with total-N (Figs 2–4). Furthermore, taxa that were highly sensitive towards elevated levels of BOD_5 were generally also sensitive to elevated concentrations of total-P and NH_4–N. Occurrence of most taxa showed similar responses (negative logarithmic) as for BOD_5 to both total-P and NH_4–N (Figs 2 & 3) but generally no relationship to total-N. Relationships to total-P and NH_4–N were stronger for some taxa than with BOD_5 (e.g. *Nemoura*, Leptophlebiidae) but weaker for others (e.g. *Leuctra* and *Isoperla*). Only *Limnius* and Glossosomatidae showed significant negative logarithmic relationships to total-N. Most taxa showed no decrease in occurrence with increasing concentration of total-N and the likelihood of finding these taxa in any given sample was almost the same along the entire gradient. Occurrence of *Asellus* and *Elmis* showed a unimodal function with optima at approximately 6 mg N L^{-1} but were still found at 40% of sites with a total-N concentration of 11 mg N L^{-1} (Fig. 4).

Influence of habitat characteristics on relationships

Inclusion of stream width and substrate index score in the analysis revealed that one or both could explain additional variability for most but not all taxa (Table 4). However, BOD_5 still was significantly related to occurrence of 14 out of 23 taxa, confirming relationships shown in Fig. 1. However, occurrence of some taxa showed a stronger relationship with the two habitat variables than BOD_5. Examples included Heptageniidae (stream width) and *Elmis* (substrate index score). Overall, inclusion of two habitat variables did not alter the response to BOD_5 and only a few interaction terms ($BOD_5 \times$ width and $BOD_5 \times$ substrate) were significant (Table 4). BOD_5 explained most of the variation in occurrence for 10 of 23 taxa analysed versus six for substrate index score and six for stream width.

Table 3 Key values for habitat and chemical variables included in the analyses

	Width (m)	Substrate index score	BOD_5 (mg L^{-1})	Total-P (mg L^{-1})	NH_4–N (mg L^{-1})	Total-N (mg L^{-1})
No. sites	447	266	554	292	555	288
Average	4.4	4.7	1.8	0.17	0.17	5.20
SE	0.2	0.3	0.08	0.009	0.04	0.15
Max.	30	16	37.2	1.50	18.7	18.55
Min.	0.3	−12	0.5	0.01	0.004	0.33
Median	2.8	5	1.5	0.13	0.08	5.17
75%-fractile	5.6	9	2.1	0.20	0.16	6.73
25%-fractile	1.6	1	1.0	0.09	0.05	3.27

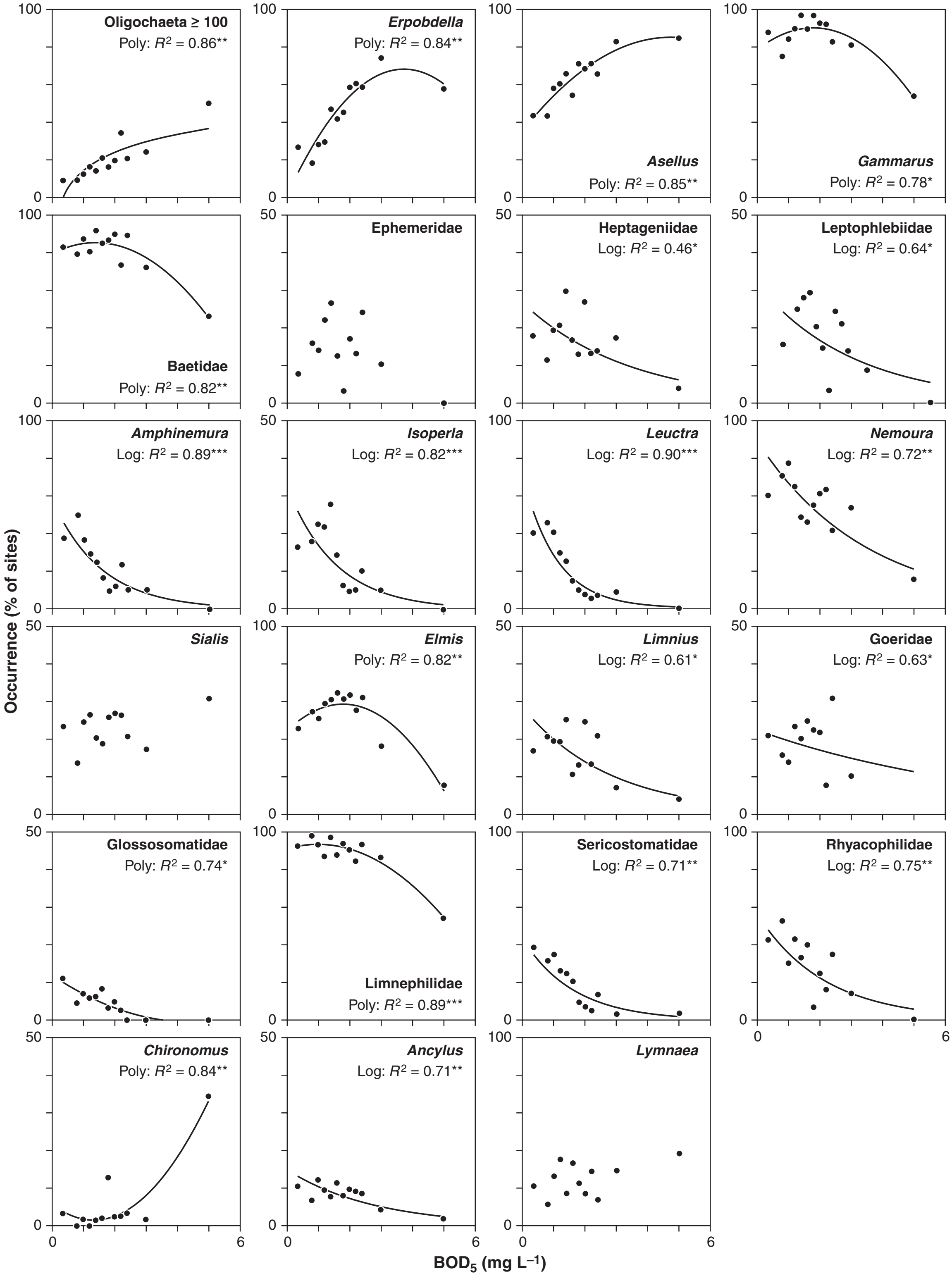

Fig. 1 Relationship between BOD$_5$ and occurrence of 23 macroinvertebrate taxa. Poly, second-degree polynomial model; Log, logarithmic model. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$.

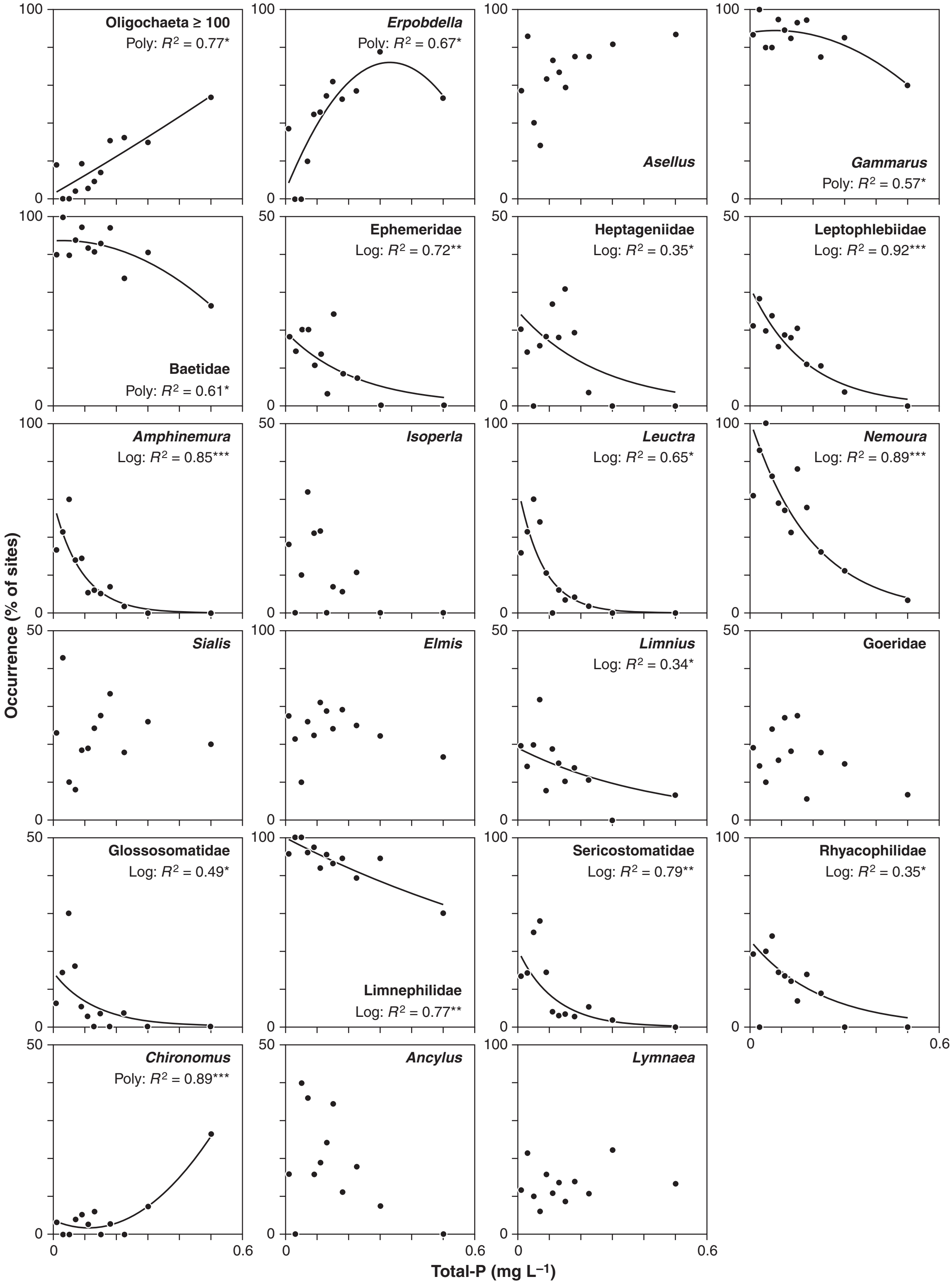

Fig. 2 Relationship between total-P and occurrence of 23 macroinvertebrate taxa investigated. Poly, second-degree polynomial model; Log, logarithmic model. Statistical significance: *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

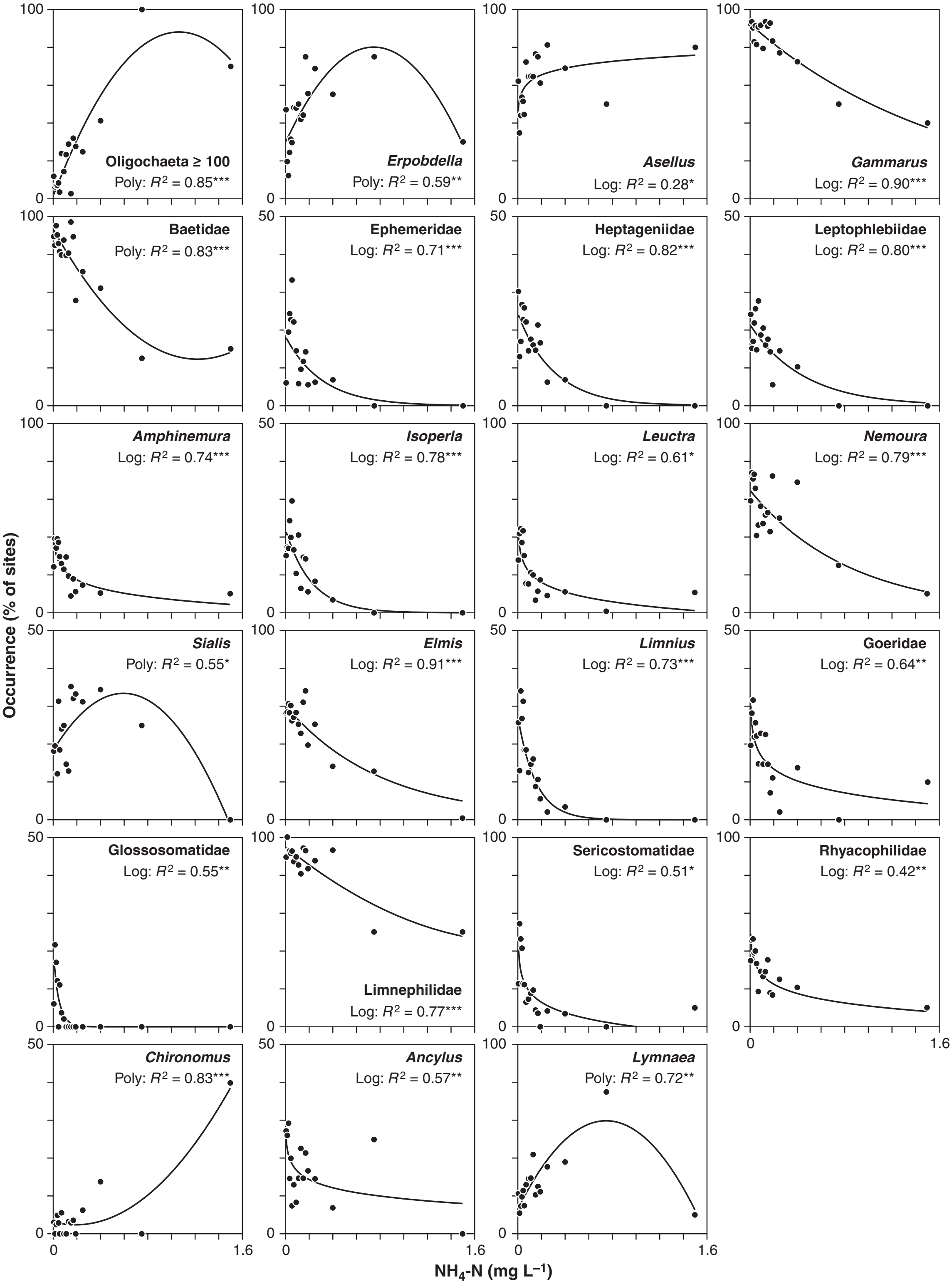

Fig. 3 Relationship between NH$_4$–N and occurrence of 23 macroinvertebrate taxa investigated. Poly, second-degree polynomial model; Log, logarithmic model. Statistical significance: $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$.

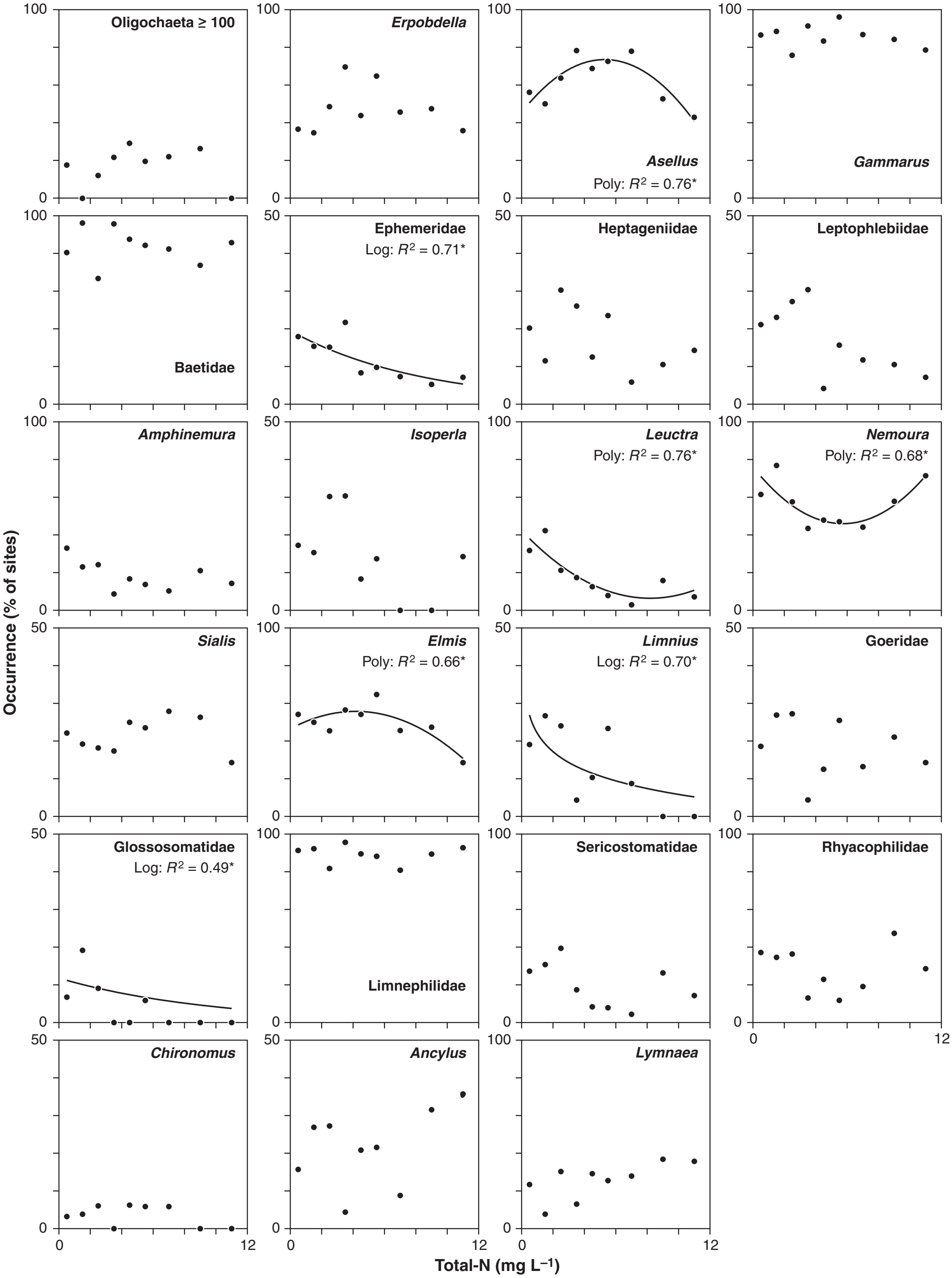

Fig. 4 Relationship between total-N and occurrence of 23 macroinvertebrate taxa investigated. Poly, second-degree polynomial model; Log, logarithmic model. Statistical significance: *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

Table 4 Multiple logistic regression of BOD_5 and the two habitat variables, stream width and substrate index score

	Multiple regression output			
Taxon	Interaction	BOD_5	Width	Substrate
Oligochaeta ≥ 100	ns	ns	ns	ns
Erpobdella	*($BOD_5 \times$ width)	***	**	ns
Asellus	ns	**	ns	*
Gammarus	ns	***	ns	ns
Baetidae	ns	*	*	*
Ephemeridae	ns	ns	*	ns
Heptageniidae	*($BOD_5 \times$ width)	ns	**	ns
Leptophlebiidae	ns	ns	ns	*
Amphinemura	ns	**	ns	ns
Isoperla	*($BOD_5 \times$ width)	ns	**	ns
Leuctra	ns	**	ns	ns
Nemoura	ns	*	*	ns
Sialis	ns	ns	ns	***
Elmis	**($BOD_5 \times$ width)	ns	***	***
Limnius	ns	*	ns	*
Goeridae	ns	*	*	*
Glossosomatidae	ns	*	*	ns
Limnephilidae	ns	*	*	ns
Sericostomatidae	ns	*	ns	ns
Rhyacophilidae	*($BOD_5 \times$ substrate)	ns	ns	*
Chironomus	ns	***	ns	ns
Ancylus	ns	ns	ns	*
Lymnaea	ns	*	ns	ns

Asterisks denote significance level: $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$; ns, non-significant. Variables that explained most of the variation in the multiple logistic regression are underlined. All possible interactions terms were included in the regression but usually were not significant. Only the term $BOD_5 \times$ width and $BOD_5 \times$ substrate were significant for a few taxa as indicated in the table.

Correlation between habitat and chemical variables

There was no significant correlation between width and substrate index score or between habitat and chemical variables (Table 5). BOD_5 were correlated strongly and positively to NH_4–N ($r = 0.86$) as well as total-P ($r = 0.52$) while there was no correlation with total-N.

Comparison with saprobic values

The number of sites in which selected taxa occurred at 1 and 2 mg L^{-1} BOD_5 was calculated using the best statistical models ($P < 0.001$ or better) developed on the BOD_5 – occurrence relationship (Fig. 1). The ratio between occurrence at 2 and 1 mg L^{-1} BOD_5 was then calculated as a relative measure of sensitivity/tolerance and compared with published saprobic values. Overall, there was a linear relationship ($r^2 = 0.65$, $P = 0.0048$) between the observed sensitivity/tolerance in this study and saprobic values (Fig. 5). However, *Leuctra* appeared clearly more sensitive to BOD_5 than indicated by its saprobic value while the opposite was true for *Elmis*.

Discussion

Stream macroinvertebrate taxa showed varied types of responses along the chemical gradients investigated. Relationships were in general similar for individual taxa with respect to BOD_5, total-P and NH_4–N whereas most showed no relationship between occurrence and the gradient in total-N. Some taxa were surprisingly sensitive to even slightly elevated levels of BOD_5 as well as total-P and NH_4–N. Habitat characteristics influenced occurrence of certain taxa but overall BOD_5 was the primary predictor. There was a relationship between sensitivity/tolerance of individual taxa found in this study and published saprobic values but also a considerable scatter.

Our findings show that occurrence of important macroinvertebrate taxa are reduced in occurrence at

Table 5 Correlation matrix for habitat and chemical variables used in the study

	Width	Substrate index score	BOD_5	Total-P	NH_4–N	Total-N
Width	–	ns	ns	ns	ns	ns
Substrate index score	ns	–	ns	ns	ns	ns
BOD_5	ns	ns	–	$r = 0.48^{***}$	$r = 0.86^{***}$	ns
Total-P	ns	ns	$r = 0.48^{***}$	–	$r = 0.52^{***}$	$r = 0.19^{**}$
NH_4–N	ns	ns	$r = 0.86^{***}$	$r = 0.52^{***}$	–	ns
Total-N	ns	ns	ns	$r = 0.19^{**}$	ns	–

r = Pearson's correlation coefficient with asterisks denoting significance level: $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$; ns, not significant.

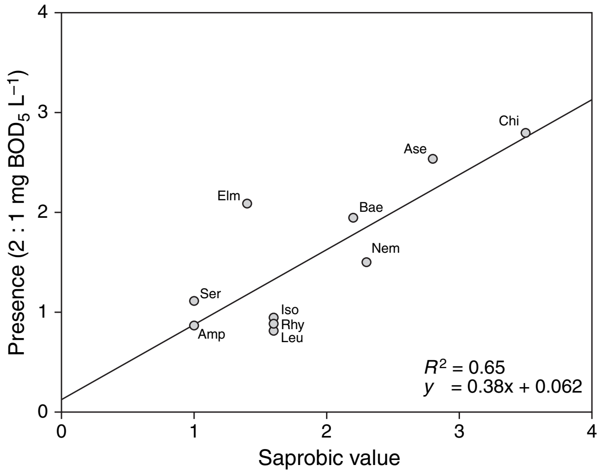

Fig. 5 The relationship between the ratio of occurrence at 2 and 1 mg L^{-1} BOD_5 and saprobic values. Ase, *Asellus*; Bae, Baetidae; Amp, *Amphinemura*; Iso, *Isoperla*; Leu, *Leuctra*; Nem, *Nemoura*; Elm, *Elmis*; Ser, Sericostomatidae; Rhy, Rhyacophilidae; Chi, *Chironomus.*

BOD_5 levels within a range that is normally perceived as indicating an unpolluted stream site. The European Environment Agency (EEA, 1991, 1994) compiled monitoring data from European Rivers and found average BOD_5 levels in near-pristine rivers to be about 1.6 mg L^{-1} and concluded from this that values below 2 mg L^{-1} represented only slightly affected watercourses. Often the boundary value between reference and impacted conditions is set to 2 mg L^{-1} BOD_5 by most EU Member States and recently the boundary value was set to 2.4 mg L^{-1} BOD_5 between high and good ecological quality as part of the intercalibration of macroinvertebrate methods in the Central-Baltic GIG (European Commission, 2007). Our results indicate that these boundaries should be reconsidered and that acceptable BOD_5 levels in small lowland rivers should be lowered to least 1.5 mg L^{-1} BOD_5 to secure that the composition of the macroinvertebrate communities reflect reference conditions.

Overall, our findings confirm tolerance patterns and indicator status of macroinvertebrates used in most bioassessment systems that target organic pollution. However, for individual taxa the tolerance to organic pollution was either less or greater that would have been predicted using saprobic values. *Leuctra* was more sensitive to high levels of BOD_5 than would be predicted from its saprobic value. Furthermore, neither of the two habitat variables strongly influenced the occurrence of *Leuctra*. However, the saprobic values used here were calculated as an average of the four species occurring in Denmark (Wiberg-Larsen, 1984). The two species that are less sensitive according to their saprobic value is *Leuctra fusca* (si = 2) and *L. digitata* (si = 1.7) which occur as nymphs in the summer/autumn. As samples analysed here are taken in spring, it is very likely that the majority of *Leuctra* found was *L. hippopus* or *L. nigra* which emerge in spring and are considered to be more sensitive (si = 1.2 and 1.3, respectively) to organic pollution. However, even if the average value is lowered, *Leuctra* as a genus still stands out as being highly sensitive and a good indicator organism. In contrast, *Elmis aenea* appears to be less sensitive than normally perceived. The strong positive correlation to substrate index score suggests that the occurrence of this species is driven primarily by the presence of coarse substrates and less so by BOD_5. Coarse substrate and high current velocities increase re-aeration and consequently reduce the impact of microbial degradation on dissolved oxygen content. Andersen (1994) found that an impact of BOD_5 on a macroinvertebrate-based index was reduced with increasing current velocities. That multiple stressors can act synergistically has been experimentally demonstrated (Folt *et al.*, 1999; Matthaei *et al.*, 2006). Therefore, one of the limitations of systems such as the saprobic index is that it was developed to detect a single stressor, organic pollution, across large gradients. However, we cannot determine if the limited accordance between levels of sensitivity/tolerance found here and the saprobic system is a true difference, or is related to occurrence of multiple stressors.

Insights into how macroinvertebrates respond to gradients in BOD_5 provided by our study still do not address causation, and more experimental work on oxygen metabolism in key indicator taxa could be very valuable. Today, inaccuracy might be an integral part of the majority of assessment systems because of a lack of understanding about underlying mechanisms controlling the distribution of macroinvertebrates in anthropogenically disturbed stream ecosystems.

Eutrophication as such is not likely to directly stress macroinvertebrate communities in lowland systems whereas reduced oxygen levels, which may result from high organic matter loadings from point source pollution and microbial breakdown of plant biomass in eutrophic systems, are extremely critical.

Biological oxygen demand as a proxy for dissolved oxygen is with all likelihood the primary driver of macroinvertebrate distribution across stream sites, and relationships to both total-P and NH_4–N is primarily caused by inter-correlation. That NH_4–N concentrations are linked to sewage input is long established (e.g. Hynes, 1960) and in our study were very strongly correlated with BOD_5. Furthermore, the significant correlation found between BOD_5 and total-P, albeit not as strong as for NH_4–N, would indicate that the main source of phosphorous is sewage. In densely populated catchments, up to 50–76% of the phosphorous load comes from point sources (EEA, 1999), whereas non-point pollution with phosphorous in European rivers ranges between 2% and 60% (Farmer, 2004).

There was clearly no strong negative impact on any of the taxa investigated of total-N, indicating that eutrophication in isolation did not change occurrence of macroinvertebrate taxa in a detectable manner. Reported impacts of increased nutrient levels on stream macroinvertebrates have mainly been indirect; i.e. densities of macroinvertebrates have increased as a consequence of increased periphyton biomass (e.g. Biggs *et al*., 2000). As our data set did not allow us to test for changes in macroinvertebrate abundance, this might explain why we detected only a very limited response to the observed total-N gradient despite its wide range.

Our contention that macroinvertebrates are poor indicators of eutrophication contrasts to a recent study by Smith *et al.* (2007) in which they developed a macroinvertebrate index sensitive to elevated concentrations of nutrients (total-P and NO_3^-). However, they did not measure BOD_5 and our results indicate that total-P might be linked to sewage input. Hence the responses found by Smith *et al.* (2007) could to some degree reflect oxygen depletion. We did not analyse for NO_3^- in our study but it should be linked to total-N and primarily indicate diffuse agricultural pollution. The reason that Smith *et al.* (2007) found a relationship, albeit fairly weak, might be that they covered indirect effects better by analysing a larger number of taxa (164) which would include more species belonging to the grazer guild that might respond positively to nutrient-induced increases in primary productivity.

Even though BOD_5 could partly explain the occurrence of most taxa, habitat variables explained more of the variability in half of the taxa investigated. Habitat features are very important for the distribution of macroinvertebrates (see Mackay, 1992; Hart & Finelli, 1999; Lake, 2000 for reviews). From a management perspective these results stress the importance of an integrated approach to monitoring where it is recognised that organisms respond to a range of natural features and a combination of pressures. Recent studies have indeed identified, using various multivariate approaches, that macroinvertebrates respond to combinations of natural environmental factors and anthropogenic pressures (e.g. Feld & Hering, 2007) and there has been development of number of approaches (sensitivity numbers, multimetric indices, additive models) that are sensitive to more than one stressor (e.g. Chessman & McEvoy, 1998; Barbour & Yoder, 2000; Yuan, 2004). However, assessments of ecological quality using stream macroinvertebrates are still highly skewed towards systems based on oxygen sensitivity/tolerance of individual taxa and although the present study clearly identified that BOD_5 was important in determining macroinvertebrate occurrence, more and better indicators need to be developed for other stressors such as habitat degradation. Despite the large number of papers on habitats and macroinvertebrates, many aspects of physical–biological coupling are still not understood (Hart & Finelli, 1999).

Our study indicates that tolerance to organic pollution by selected taxa should be used to assess ecological quality of streams and rivers. By using only metrics or indices some of the sensitivity introduced by individual taxa is lost, as are their more specific response curves to a given stressor. Occurrence of sensitive taxa that is easy to identify, such as the genus *Leuctra*, could be incorporated into multimetrics to increase assessment precision along stressor gradients and to detect organic pollution. The reason that communities are preferred to single taxa in bioassessment is the risk of not finding a given taxon at a site due to sampling effort (spatial and temporal constraints) or dispersal barriers/delays. To counteract these problems, *in situ* bioassays using sensitive species might be a helpful tool to determine ecological status of a site as the absences of certain taxa could reflect a historical exclusion due to pollution and that re-colonisation subsequently has not occurred.

Studies of freshwater macroinvertebrate respiration have not specifically targeted indicator taxa used in most biotic indices. Oxygen demands have been established indirectly from observations of occurrence and succession of individual taxa along gradients in organic pollution as in our study but very rarely with quantification of BOD_5. Our findings show that occurrence of important macroinvertebrate taxa are reduced at levels of BOD_5 that are normally perceived as indicating unimpacted stream site conditions. However, our findings confirm tolerance patterns and indicator status of macroinvertebrates used in most current bioassessment systems that target organic pollution. Our results also indicate that existing assessment systems could be modernised, including more rigorous testing of indicator organisms as well as improved understanding of the habitat–macroinvertebrate coupling. Eutrophication as such is not likely to directly stress macroinvertebrate communities in lowland systems whereas reduced oxygen levels appear to be extremely critical.

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