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Invertebrate traits for the biomonitoring of large European rivers: an assessment of specific types of human impact

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1. The power of selected biological invertebrate traits for discriminating different types of human impact (heavy metal pollution and cargo-ship traffic) were tested using ecological reasoning and linear Discriminant Function Analysis (DFA).
2. Frequency distributions of individual traits and categories of traits from 68 least impacted river reaches (LIRRs) and 304 impacted river reaches were used to define simple assessment rules based on ecological reasoning for specific impairments in large European rivers. In calibration, a maximum of three variables with *a priori* predictions and two different impairment threshold levels were used. Similarly, DFA was performed on the same variables included in the ecological reasoning approach, but also on all available traits or trait categories.
3. Validation with an independent data set (40 LIRRs, 291 variously impacted river reaches) and using the ecological reasoning approach showed that 75–78% of the reaches were correctly assigned with rules on all impact types, 35–57% with rules on heavy metal pollution and 78–93% with rules on cargo-ship traffic. By comparison, validation showed that DFA performed globally poorer than the ecological reasoning approach. In addition, the performance of the rules based on ecological reasoning remained stable, whereas DFA performance changed between calibration and validation.
4. Although not defined for this purpose, our study provided alarming evidence regarding the impact of cargo-ship traffic on invertebrate communities in river reaches. Reaches with cargo-ship traffic were found to have more genera with long life cycles that reproduce repeatedly by ovoviviparity and have a sessile life.
5. The performance of our trait-based approach to correctly assign reaches to either least impacted or impacted conditions should promote further research on the topic across larger geographic areas (without regionalization) and across smaller stream types to provide a powerful biomonitoring tool that fulfils current European Union directives.

Keywords: benthos, biological traits, cargo-ship traffic, heavy metal pollution, human impacts

Introduction

For decades, large rivers have been used for transport, recreation, fisheries and industrial and drinking water supply. Consequently, in all European countries human pressures have threatened the ecological integrity of large rivers (Petts, Möller & Roux, 1993).

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For example, large rivers have been modified to allow navigation and power generation, resulting in multiple point and non-point discharges. These modifications have led to habitat degradation and loss of connectivity, resulting in alterations in flow and temperature regime as well as water quality (Whitton, 1984; Petts *et al.*, 1993). To develop more comprehensive water legislation, the European Union (EU) promotes integrated river basin management for Europe (Water Framework Directive) and fosters research on biomonitoring tools that enable the assessment of the effects of human activities on the ecological quality of all water bodies (Anonymous, 1999; European Commission, 2000). Such biomonitoring tools should at least meet the following requirements: (i) be operational on both local and larger scales (Ormerod, Pienkowski & Watkinson, 1999), which requires rescaling of reference conditions (e.g. Wright, Sutcliffe & Furse, 2000), a significant expansion of databases across Europe (e.g. Verdonschot & Nijboer, 2004; Pont *et al.*, 2006) and intercalibration (Sandin & Hering, 2004); (ii) address both the occurrence of taxa and the functioning of ecological processes (e.g. Dale & Beyeler, 2001); (iii) be predictive (e.g. Norris & Hawkins, 2000); and (iv) address deviations in ecological integrity as the difference between expected and observed (natural or impacted) conditions (e.g. Nijboer *et al.*, 2004).

Monitoring community-level responses is currently used for addressing the abiotic and biotic state of a given environment and usually improves the accuracy of the assessment compared to the use of a single indicator species. However, one problem of using communities as bioindicators is that the observed changes are the product of natural stochastic variation and independent deterministic changes associated with perturbation from human activities (Hodkinson & Jackson, 2005). Moreover, bioassessment that relies on richness-based indicators can underestimate true biological impairment (Cao & Hawkins, 2005).

An alternative approach and one being actively explored combines food and feeding habits of macroinvertebrate taxa with a wide variety of other biological traits such as body size, fecundity, voltinism and dispersion ability to describe functional community structure (e.g. Richards *et al.*, 1997; Minshall & Robinson, 1998; Statzner *et al.*, 2001a; Snook & Milner, 2002; Bremner, Rogers & Frid, 2003; Lamouroux, Dolédec & Gayraud, 2004) and functional

biodiversity (e.g. Bady *et al.*, 2005). In contrast to species that appear or disappear along geographical and downstream gradients, multiple biological traits (e.g. body size, life cycle, food) each described by multiple trait categories (e.g. small, intermediate, large body size), generally occur throughout a region, i.e. they could provide a 'multi-probe' biomonitoring tool for discriminating various types of human impact (e.g. Dolédec, Statzner & Bournaud, 1999; Statzner *et al.*, 2005). Most of these traits represent functional attributes that should be predictably affected by various types of human impact (Statzner *et al.*, 2005), thus supplementing existing biological indicator systems while fulfilling EU requests (Dziocck *et al.*, 2006).

Our purpose here is to use invertebrate traits, as advocated by Statzner *et al.* (2005), to discriminate impacted reaches of large European river reaches from near-natural reference conditions. In large European rivers, organic pollution seems to be no longer the main impairment (see e.g. ICPDR, 2001; Böhmer, Rawer-Jost & Zenker, 2004), and indeed our database included so few reaches with clear organic pollution that we could not address this issue. In contrast, our database included a sufficient number of reaches with heavy metal pollution, cargo-ship traffic and a mixture of both, so we focus our analyses on these types of human-generated impact.

According to ecological theory, human disturbance should potentially affect many of the biological traits of riverine biota (Statzner, Hildrew & Resh, 2001b). However, those traits conferring rapid population growth, and thus resilience to disturbances, should be especially favoured (e.g. small size, many descendants per reproductive cycle, short life cycles; see Townsend & Hildrew, 1994 and our predictions for associated trait category responses in Table 1). Contamination by heavy metals occurs through external contact or through food (e.g. Paul & Meyer, 2001), hence it should be particularly critical for large body surface-volume ratios of small-sized or gill-bearing forms or for higher trophic levels (Table 1). Finally, favouring the distribution of individuals among river reaches or the invasion of river reaches, cargo-ship traffic should act on traits facilitating the transport by ships, the establishment of new populations and/or the resistance to wave action caused by ships (Table 1). Thus, predictions may sometimes be contradictory. For example, the relative abundance of small-sized individuals should increase for all types of disturbances,

Table 1 Predictions for trait and for trait category responses to impacts assessed in this paper. Impacts may change specific trait (i.e. under impact, the deviation of these would increase), or may decrease (↓) or increase (↑) the relative abundance of a single trait category in comparison with a reference [the labels correspond to those used by Statzner *et al.* (2005) and are re-used in Table 6 and 7 below; traits are identified by a capital letter and trait categories are identified by the same capital letter plus a number]

Impact type	Trait	Category	Rationale
All types*	(A) Maximal size (B) Descendants per cycle (C) Voltinism		Rapid population growth and increase of resilience capacity
Heavy metal pollution	(A) Maximal size (M) Food (N) Respiration	(A1_2 [†]) small size (≤ 10 mm) ↓ (M7_9 [‡]) animal food of all sizes ↓ (N2) gill respiration ↓	Greater body surface-volume ratio favours metal uptake per unit volume. Contamination through food
Cargo-ship traffic	(C) Voltinism (D) Reproductive cycles per individual (F) Reproductive method (G) Parental care (I) Locomotion & attachment	(C3) long cycles (\geq semivoltine) ↑ (D3) several cycles (>2) ↑ (F1) single individual ↑ (F2) hermaphroditism ↑ (G1) bud production ↑ (G5 [§]) cemented aquatic eggs ↑ (G6) ovoviviparity ↑ (I4 [§]) temporary attachment* ↑ (I5 [§]) permanent attachment* ↑	Facilitation of foundation of new populations through individuals dispersed by ships and better resilience capacity. Facilitation of dispersal for asexual forms. Resistance to wave action. Facilitation of attached forms

*In addition, all impact types should affect the overall mean trait (see Methods section for its definition; abbreviated ALL in Table 6 and 7).

[†]Includes the two smallest size categories.

[‡]Animal food of size <1 mm, between 1–10 mm and >10 mm.

[§]These trait categories simultaneously facilitate transport by cargo-ship traffic and resistance against wave action resulting from cargo-ship traffic.

*i.e. short-term or long-term attachment.

whereas they should decrease at high heavy metal concentrations due to a larger surface to volume ratio. Alternatively, the relative abundance of individuals with short life cycles should increase for all types of disturbances due to their higher resilience capacity, whereas long life cycles should increase with cargo-ship traffic due to increased probability of successful reproduction of new colonizers of a river reach (Table 1). Moreover, interactions may occur among multiple stressors over space and time and impacts may affect communities in an additive way (e.g. Culp, Cash & Wrona, 2000). Further complications regarding the predictions of Table 1 arise from the existence of trade-offs among traits because there are different solutions for living under a given environmental constraint and species with different combinations of biological traits may withstand similar types of stress (Statzner, Dolédec & Hugueny, 2004).

Given the above complexities, the calibration and validation of impact assessment rules based on the occurrence of functional attributes should follow two obvious analytical approaches: ecological reasoning would consider, e.g. long life cycles *or* ovoviviparity as potential trait categories responding to cargo-ship

traffic and multivariate analysis would combine trait categories (e.g. long life cycles *and* ovoviviparity) implicitly searching for a structure based on correlations among attributes. Following the initial assessment of trait patterns in least impacted river reaches (LIRRs) of large rivers (having a width ≥ 40 m; Statzner *et al.*, 2005), we thus tested the discriminative power of selected community attributes for different types of human impact considering both a predictive, ecological reasoning (derived from Table 1) and a predictive, multivariate approach using linear discriminant analysis (see e.g. Moss *et al.*, 1999) to define impact assessment rules for all types of impact, heavy metal pollution and cargo-ship traffic. For both approaches, we subsequently validated these assessment rules on an independent data set.

Methods

Data

We developed a set of impact assessment rules using 68 least impacted river reaches (Statzner *et al.*, 2005). These reaches were not affected by cargo-ship traffic

Impact	Width (m)*	Longitude*	Latitude*	Richness [†]
(a)				
LIRRs [‡] (<i>n</i> = 68)	40–430	10.0W–20.9E	42.4–52.3N	30 ± 10.7
Metals (<i>n</i> = 65)	45–430	1.3W–6.0E	43.8–47.6N	27 ± 10.6
Metals & ships (<i>n</i> = 66)	112–310	4.7–12.4E	44.4–55.4N	13 ± 8.7
Cargo-ships (<i>n</i> = 142)	169–668	6.4–14.6E	48.0–53.4N	14 ± 8.1
(b)				
LIRRs (<i>n</i> = 40)	40–430	0.3W–8.1E	43.4–50.4N	26 ± 10.3
Metals (<i>n</i> = 60)	40–500	1.3W–5.7E	43.9–48.9N	29 ± 12.0
Metals & ships (<i>n</i> = 66)	60–371	4.7–12.4E	44.4–53.3N	15 ± 10.4
Cargo-ships (<i>n</i> = 139)	135–668	4.7–14.6E	44.4–53.4N	15 ± 8.4

*Range (Note that exact width was not available for all sites).

[†]Mean ± standard deviation.

[‡]Least impacted river reaches.

and had good water quality (class 1–2; see Table 2 in Statzner *et al.*, 2005). For heavy metal pollution, we considered a site as impacted if any metal values were higher than class two (Cd, Cu, Hg, Ni, Pb and Zn; for class limits see Table 2 in Statzner *et al.*, 2005). Quantitative information on cargo-ship traffic was not available for many reaches, so we simply assigned presence or absence of cargo-ship traffic to each reach. For our three groups of impact (heavy metal pollution, cargo-ship traffic, combined heavy metal pollution and cargo-ship traffic) we had 65, 142 and 66 impacted reaches respectively. These sites were used to define impact assessment rules; each group of sites covered a similar size range and largely overlapped geographically across Europe (Table 2a). In addition, we included 31 reaches with organic pollution (higher than class two; see Table 2 in Statzner *et al.*, 2005), heavy metal pollution and/or cargo-ship traffic for the assessment of all impact types. These reaches represented our calibration data set.

For validation of our impact assessment rules we used an independent data set consisting of 40 LIRRs, and 60, 139, 66 and 26 reaches impacted by heavy metal pollution, cargo-ship traffic, combined heavy metal pollution and cargo-ship traffic and organic pollution, heavy metal pollution and/or cargo-ship traffic, respectively, which had environmental characteristics similar to those of the calibration data set (Table 2b). The relatively large difference in the number of LIRRs between the two data sets was related to the availability of LIRR data and the intention by Statzner *et al.* (2005) to include LIRRs from many different European countries. To be consistent with the selection of our 68 LIRRs in the calibration data set (genus richness >10 and

Table 2 Some environmental characteristics and generic richness found in the four groups of rivers for (a) the calibration data set and (b) the validation data set

proportion of genera with alien species <0.1; see Table 3 in Statzner *et al.*, 2005), we constrained the LIRRs in the validation data set similarly, resulting in 40 LIRRs. By contrast, the similar number of impacted reaches in the two data sets indicated a good representation of (originally randomly selected) reaches of different rivers. Finally, reduction of generic richness for combined heavy metal pollution and cargo-ship traffic or cargo-ship traffic alone compared to LIRRs or heavy metal pollution alone indicated that stream macroinvertebrate communities were not discriminating effects of heavy metal pollution (Table 2a,b).

We worked with the trait database described in detail by Gayraud *et al.* (2003) and used in Statzner *et al.* (2005). The database contains affinity scores (ranging from ‘zero’ for no affinity to ‘three’ for a high affinity of a species to a given trait category; see Chevenet, Dolédec & Chessel, 1994) for 66 categories of 14 traits of invertebrate species occurring in large European rivers. Two maximal size categories (maximal size ≤5 mm and between 5 and 10 mm) were aggregated as maximal size ≤10 mm and predaceous foods (animal food type and size <1 mm, between 1–10 mm and >10 mm) were aggregated as animal food of all sizes (see Table 1) resulting in 63 trait categories.

Affinity scores for genera were derived by averaging species scores, and rescaled so the sum of a given trait equalled ‘one’. To obtain the proportion of a given trait category in the invertebrate communities, the affinity scores were weighted by the presence-absence of each genus, and these values were added for the given trait category [i.e. applying the metrics suggested by Gayraud *et al.* (2003)]. The resulting weighted categories of each trait were rescaled to sum to ‘one’ for a given community.

Table 3 Pearson correlation coefficients among one selected trait category indicative for heavy metal pollution (maximal size ≤ 10 mm) and one selected trait category indicative for cargo-ship traffic (\geq semivoltine cycle) and all other trait categories having impact predictions (see Table 1) across the invertebrate genera ($n = 217$) and across the invertebrate communities (presence-absence weighted categories, $n = 275$, including all LIRRs and reaches with heavy metal pollution or cargo-ship traffic in the calibration data set). Correlation coefficients with categories being indicative for the other types of impact are shown in italics

Categories	A1_2: Maximal size ≤ 10 mm		C3: \geq Semivoltine cycle	
	Genera	Communities	Genera	Communities
A1_2: Maximal size ≤ 10 mm	–	–	0.239	-0.497
M7-9: Animal food of all sizes	-0.317	-0.467	0.002	-0.156
N2: Gill respiration	-0.311	-0.063	-0.212	-0.322
D3: >2 Cycles per individual	-0.121	-0.729	0.342	0.778
F1: Single individual	-0.025	-0.083	0.049	-0.215
F2: Hermaphroditism	-0.118	-0.682	-0.045	0.386
G1: Bud production	-0.105	-0.136	0.037	-0.093
G5: Cemented aquatic eggs	0.190	0.594	0.036	-0.456
G6: Ovoviviparity	-0.140	-0.726	0.232	0.723
I4: Temporary attachment	-0.020	-0.039	0.201	0.176
I5: Permanent attachment	0.027	-0.575	0.196	0.658

Impact assessment rules based on ecological reasoning

To develop and validate the impact assessment rules based on the frequency distributions of selected traits and trait categories, we used the mean model previously defined for 68 LIRRs (Statzner *et al.*, 2005). For individual trait categories, we considered four groups of river reaches (i.e. LIRRs, reaches with heavy metal pollution or cargo-ship traffic only, and both types of impact) as frequency distributions of the difference between the observed values (i.e. relative abundance of trait categories) in the reaches of a given group and the average of all 68 LIRRs considered as the expected value (i.e. mean model of relative abundance of trait categories). Furthermore, half of the sum of the absolute difference between observed (p_j) and expected (p_{exp}) values for all categories (1 to i) of a trait provided the dissimilarity (known as Sorensen or Bray-Curtis dissimilarity) per trait for the j reaches of a grouping (i.e. $D_{ij} = 0.5 \sum |p_{ij} - p_{i,exp}|$; see Spellerberg, 1991; p. 132), and averaged over all traits provided a measure of the global deviation from the mean model or overall mean trait.

Developing impact assessment rules required the definition of discrimination thresholds by comparing the cumulative frequency distributions of the LIRRs and impacted reaches. As a first threshold, we used the intersection of the two distributions (threshold #I in Fig. 1; see e.g. Oberdorff *et al.*, 2002) assuming this would balance type I (assigning validation-LIRR to impacted groupings) and type II (assigning impacted

reaches to validation-LIRR) errors in subsequent validations (see e.g. Sandin & Johnson, 2000). In addition, we used another currently defined threshold (threshold #II in Fig. 1), which enveloped 90% of the values of the LIRRs (see e.g. Rosenberg, Reynoldson &

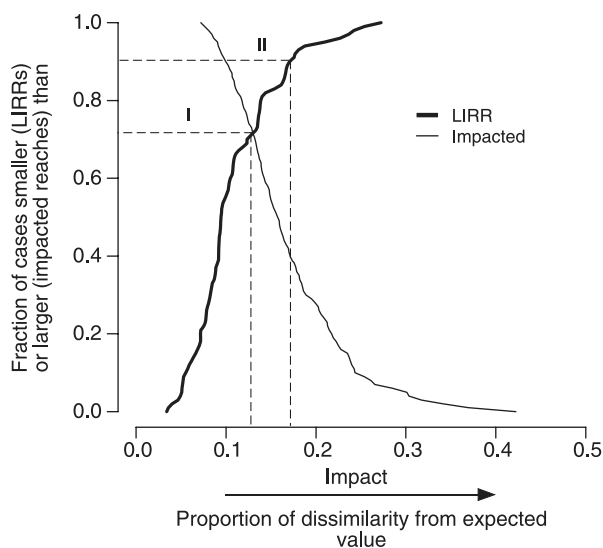


Fig. 1 Development of impact assessment rules based on ecological reasoning. Example of cumulative frequency distributions of the deviations from the expected trait value [e.g. mean of all categories of a trait in all Least Impacted River Reaches (LIRRs); see Statzner *et al.*, 2005;] of trait values in LIRRs and impacted river reaches, and two thresholds used for the discrimination between LIRRs and impacted river reaches, with (I) the intersection of the two frequency distributions, (II) the value that enveloped 90% of the LIRRs. Correct assignments of reaches using this methodology are provided in Tables 6 and 7.

Resh, 2000; Simpson & Norris, 2000). This threshold cut off the long tails in the LIRR distributions of many traits and their categories and apparently provided a more realistic definition of our LIRR conditions (see Statzner *et al.*, 2005). If threshold #II > threshold #I (which was often the case), the 90% envelope of LIRRs would provide an alternative in terms of type I and type II errors. We developed these rules for the sum of all impact types and separately for heavy metal pollution and cargo-ship traffic, and then validated the impact assessment rules using an independent data set (see Data section).

Impact assessment rules based on multivariate analysis

The predictive ability of our trait-based approach was tested using Discriminant Function Analysis (DFA); a method commonly used in biomonitoring studies for assigning cases to types of environmental conditions (e.g. Moss *et al.*, 1999; Reynoldson, Rosenberg & Resh, 2001; Bailey *et al.*, 2007). Groups of sites from the calibration data set were pre-assigned to LIRRs or a given impact. We then used DFA to determine the degree to which these site groups were discriminated by: (i) the 12 trait categories for which we had *a priori* predictions (listed in Table 1; column 'Category'), (ii) all 63 trait categories available in our database, (iii) the overall mean trait and nine traits for which we had *a priori* predictions (listed in Table 1; column 'Trait'), and (iv) the overall mean trait and the 14 traits available in our database. Finally, we used the DFA models obtained from the calibration data set to calculate the number of reaches (LIRRs or impacted reaches) correctly assigned in the validation data set.

Results

Correlations of trait categories across genera and communities

Pearson correlation coefficients that excluded (genera) and included (communities) presence-absence effects were used to assess if trait responses to human impact were related to potential evolutionary correlates among the trait categories according to our *a priori* predictions (Table 1). Significant correlations for both groupings would indicate that evolutionary correlates would be the cause of community trait patterns,

suggesting that a multivariate technique would be appropriate for our task, whilst higher correlations across communities than across genera would indicate that ecological reasoning should provide better descriptions and predictions of impact types.

From the trait categories used in the predictions of impact types, we selected one trait category indicative for heavy metal pollution (maximal size ≤ 10 mm) and one for cargo-ship traffic (\geq semivoltine cycle), and assessed how the other trait categories correlated with these two variables (Table 3). For maximal size ≤ 10 mm, correlations with trait categories indicative of cargo-ship traffic were often higher using community- than genera-level traits, whereas correlations with trait categories indicative of heavy metal pollution were relatively similar in both groupings (animal food) or higher in the genera (gill respiration). The negative correlation of these two trait categories with small size was presumably related to predation being limited by body size (predators are typically larger than their prey) and that small-sized invertebrates have a relatively high body surface-volume ratio that facilitates respiration (i.e. gills are not required). In contrast, for the \geq semivoltine category, correlations with trait categories also indicative of cargo-ship traffic were often higher for community- than for genera-level traits (Table 3). Thus, relations among these trait categories were generally stronger across communities than across taxa, despite that the number of communities (275) was slightly higher than the number of genera (217). These different patterns, between the trait categories indicative of heavy metal pollution and cargo-ship traffic, suggest the use of a multivariate analysis for the former and ecological reasoning for the latter.

Development of impact assessment rules based on ecological reasoning

Selection of traits From the four trait variables having a predicted response for all types of impact (overall mean trait, maximal size, number of descendants per reproductive cycle, voltinism, see Table 1), we selected one trait as being representative for all types of impact (overall mean trait) and one trait each as being representative of heavy metal pollution (maximal size) and cargo-ship traffic (voltinism, see Table 1) (see Fig. 2). All three trait variables (and the fourth, i.e. number of descendants per reproductive

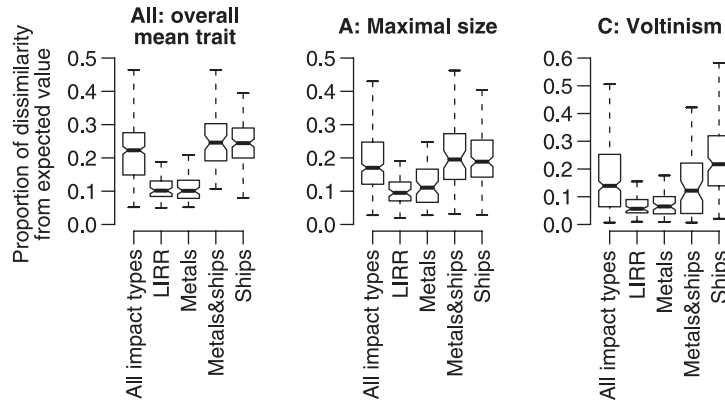


Fig. 2 Example of three trait variables indicative for all types of human impact (see Table 1). Box plots summarize the deviations (Bray-Curtis dissimilarity) from the expected trait value [e.g. mean of all categories of a trait in all Least Impacted River Reaches (LIRRs); see Statzner *et al.*, 2005] in 304 reaches having a mixture of impacts (organic pollution, cargo-ship traffic, and/or metal pollution, noted All impact types), 68 LIRRs (LIRR), 65 reaches with heavy metal pollution (metals), 66 reaches with both cargo-ship traffic and heavy metal pollution (metals & ships) and 142 reaches with cargo-ship traffic (ships) from the calibration data set. Notches represent 95% confidence intervals of the corresponding median.

cycle, not shown in Fig. 2) provided the best detection for cargo-ship traffic (alone and often in combination with heavy metal pollution), whereas the effect of heavy metal pollution was hardly detected in our calibration data set. Combining these two types of impact with organic pollution resulted in better discrimination of impact, slightly lower than with cargo-ship traffic (all impact types in Fig. 2).

Among the trait variables included in the predictions for all types of impact, the number of descendants per reproductive cycle was best correlated with

the overall mean trait ($r = 0.788$), whereas maximal size and voltinism were slightly less correlated to the overall mean trait ($r < 0.766$) and both were weakly correlated with each other (Table 4a). Consequently, as the number of descendants per reproductive cycle was best correlated with the overall mean trait, this variable was eliminated from the development rules. By contrast, all three traits indicative of heavy metal pollution (i.e. maximal size, food and respiration, see Table 1) were poor at discriminating the effects of heavy metal pollution alone (Figs 2 & 3). These

Table 4 Pearson correlation coefficients among variables (Bray-Curtis dissimilarity from the expected mean trait category values in all LIRRs) that were included in the predictions on given types of impact (see Table 1) with (a) all impact types (all data of the calibration data set, including 31 reaches with organic pollution, heavy metal pollution and/or cargo-ship traffic, $n = 372$), (b) heavy metal pollution (LIRRs and reaches having only heavy metal pollution in the calibration data set, $n = 133$) and (c) cargo-ship traffic (LIRRs and reaches having only cargo-ship traffic in the calibration data set, $n = 210$)

(a) All impact types	A: Maximal size	B: Descendants per cycle	C: Voltinism	
ALL: Overall mean trait	0.766	0.788	0.712	
A: Maximal size	–	0.570	0.431	
B: Descendants per cycle		–	0.482	
(b) Heavy metal pollution	M: Food	N: Respiration		
A: Maximal size	0.589	0.436		
M: Food	–	0.601		
(c) Cargo-ship traffic	D: Cycles per individual	F: Reproductive method	G: Parental care	I: Locomotion & attachment
C: Voltinism	0.719	0.169	0.756	0.736
D: Cycles per individual	–	0.463	0.907	0.691
F: Reproductive method		–	0.478	0.403
G: Parental care			–	0.833

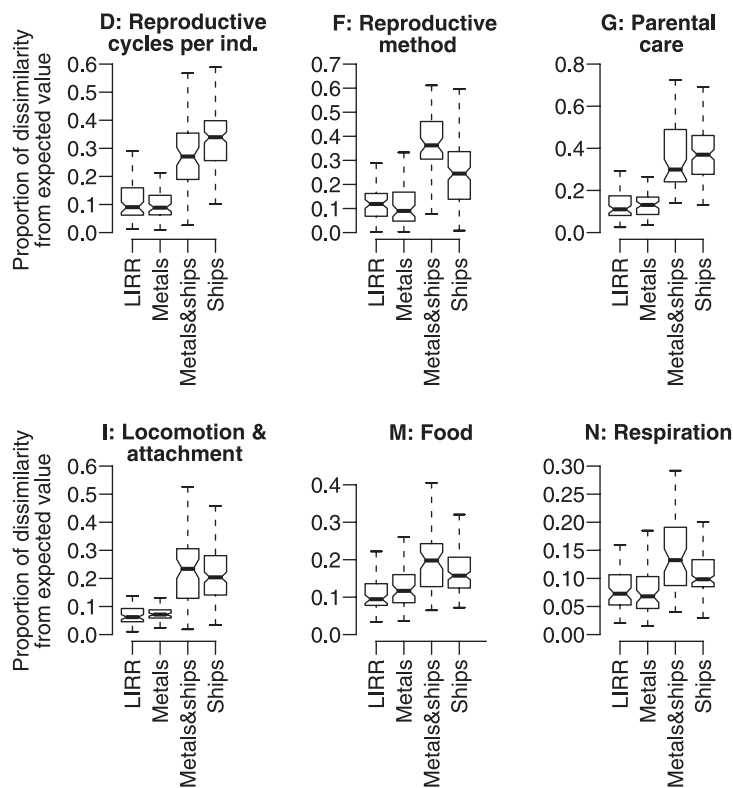


Fig. 3 Example of traits indicative for cargo-ship traffic or heavy metal pollution. See Fig. 2 for further details.

findings imply that better discrimination of heavy metal impact with the presence of cargo-ships was spurious (i.e. not related to heavy metals but to cargo-ships). However, because the three traits are indicative of heavy metal pollution and were not highly redundant (Table 4b) all three traits were included in the development of our rules to test their discrimination potential when combined together and for further comparison with DFA. Lastly, the five traits indicative for cargo-ship traffic (voltinism, number of reproductive cycles per individual, reproductive method, parental care, locomotion and attachment, see Table 1) provided better discrimination of this type of impact than the three traits used for discriminating the effects of heavy metal pollution (Figs 2 & 3). The highest correlation was found between the number of reproductive cycles per individual and parental care ($r = 0.907$), followed by the correlation between parental care and locomotion & attachment ($r = 0.833$) (Table 4c). Because of these high correlations and because parental care and locomotion & attachment were also highly correlated to voltinism ($r > 0.736$) these variables were removed, resulting in three traits (voltinism, number of reproductive cycles

per individual, reproductive method) used in the development of our assessment rules.

Selection of trait categories Visual inspection of the distributions of trait categories as predictors of heavy metal pollution (Fig. 4) and the relatively low correlations among them (Table 5a) indicated that all three trait categories were needed for the development of impact assessment rules for heavy metal pollution despite the poor discrimination of LIRRs from impacted reaches. Inspection of the distributions of trait categories as predictors of cargo-ship traffic resulted in the elimination of four trait categories (reproduction by a single individual, bud production, cemented aquatic eggs and temporary attachment to substrate; Fig. 5). In addition, two more trait categories were eliminated because they were highly correlated with each other as well as with other categories (\geq semivoltine cycle and ovoviviparity, Table 5b). As a result, three trait categories (>2 reproductive cycles per individual, hermaphroditism and permanent attachment to substrate) were used in the development of impact assessment rules for cargo-ship traffic.

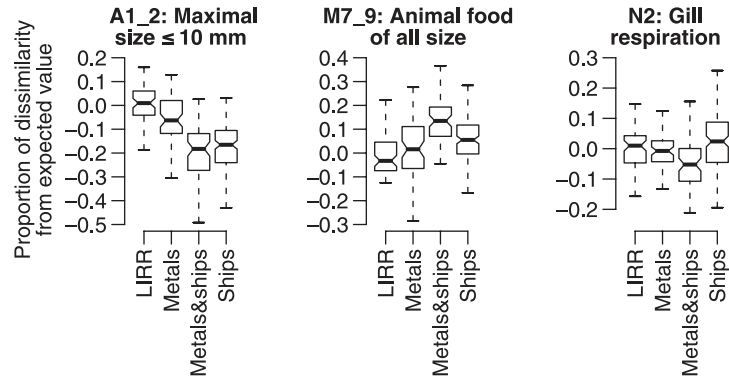


Fig. 4 Three trait categories indicative for heavy metal pollution. See Fig. 2 for further details.

Table 5 Pearson correlation coefficients among categories that were included in the predictions on (a) heavy metal pollution and (b) cargo-ship traffic (omitting four trait categories providing a poor discrimination of cargo-ship traffic from LIRRs, see Fig. 4 and text). See Table 4 for further details

(a) Heavy metal pollution		M7_9: Animal food of all sizes	N2: Gill respiration		
A1_2: Maximal size ≤10 mm		-0.655			0.013
M7_9: Animal food of all sizes		-			-0.393
N2: Gill respiration					-
(b) Cargo-ship traffic		D3: >2 Cycles per individual	F2: Hermaphroditism	G6: Ovoviviparity	I5: Permanent attachment
C3: ≥Semivoltine		0.771	0.321	0.704	0.630
D3: >2 Cycles per individual		-	0.553	0.953	0.687
F2: Hermaphroditism			-	0.601	0.477
G6: Ovoviviparity				-	0.792

Development of assessment rules The best (i.e. most correct) assignments were found by combining trait variables in various strategies in the development rules. However, using threshold #I, the category permanent attachment (I5 in Table 6) alone reduced the assignment error to 6% for both type I and type II errors (LIRRs and reaches with only cargo-ship traffic; Table 6), indicating that monitoring this impact type could be done using a simple approach. Correct assignment of LIRRs by variables having predictions for all types of human impact or for cargo-ship traffic exceeded 70%, contrasting with variables having predictions for heavy metal pollution for which assignment error could reach 57% (Table 6).

Correct assignment by variables having predictions for all types of human impact was lower for reaches with heavy metal pollution (Metals in Table 6) than for reaches with cargo-ship traffic (Cargo-ships in Table 6). Similarly, the correct assignment of reaches with only heavy metal pollution by variables

indicative for this impact type was lower than the correct assignment of reaches with only cargo-ship traffic by variables indicative for this impact type (Table 6). For the reaches having heavy metal pollution and cargo-ship traffic, the correct assignments by rules on heavy metal pollution was slightly lower than that by rules on cargo-ship traffic. Thus, the increased correct assignments of these reaches by heavy metal pollution rules (in comparison to reaches with only heavy metal pollution) were again spurious and related to the presence of cargo-ship traffic. Finally, the correct assignment across all reaches was again significantly higher for rules on all impact types and for rules on cargo-ship traffic than for rules on heavy metal (total in Table 6).

Validation of assessment rules based on ecological reasoning

Regression of correct assignments (% of reaches in Table 7) from validation (y) on correct assignments

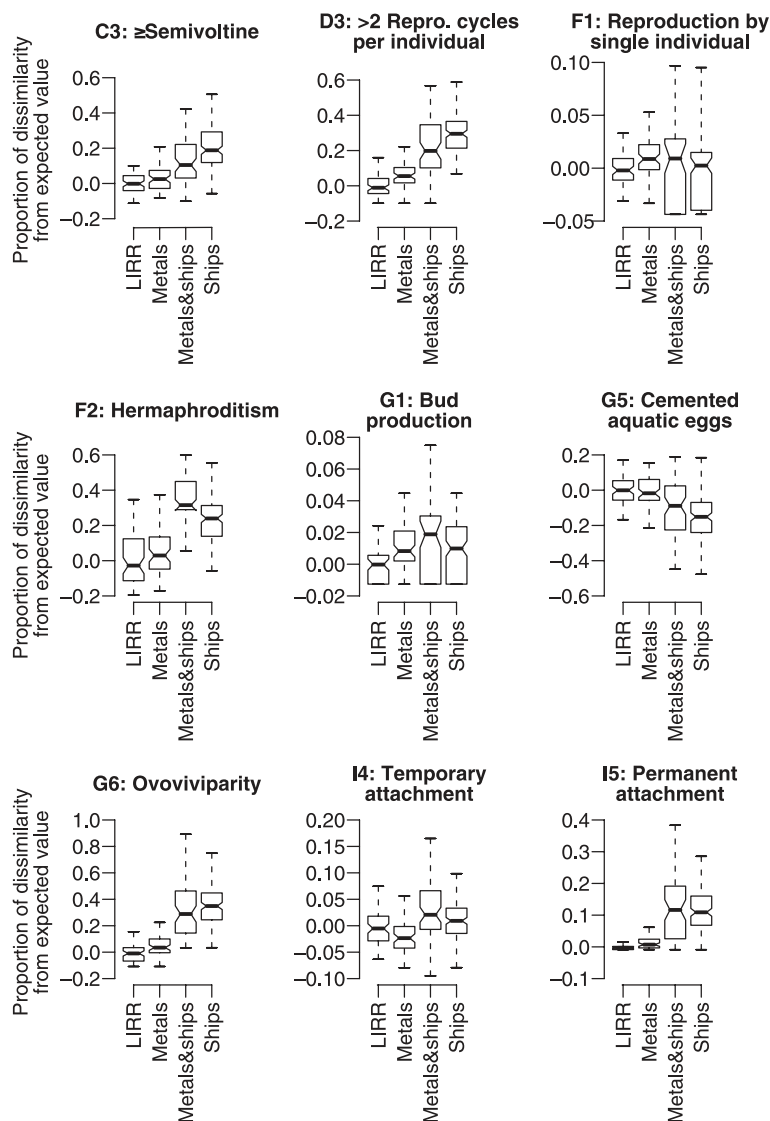


Fig. 5 Nine trait categories indicative for cargo-ship traffic. See Fig. 2 for further details.

(Table 6) from calibration (x) resulted in a robust relationship:

$$y = -8.82(\pm 1\text{SE} : 2.42) + 1.04(\pm 0.03)x; n = 75;$$

$$R^2 = 0.94; P < 10^{-15}$$

Hence, the relative performance of the rules was preserved in the validation (slope = 1) but the sensitivity decreased between calibration and validation (intercept < 0). This decrease was related primarily to the lower number of correct LIRR assignments in validation. As the detailed descriptions provided in the previous section also apply to the validations, we focus only on a few of the assessment rules here.

Obviously, the choice of an assessment rule for biomonitoring will depend on the aim of the monitoring programme. Our results showed that use of a simple rule was best for monitoring on all types of disturbance (using threshold #I); 78% of the reaches were correctly assigned (Table 6). However, use of a simple rule resulted in a 37% probability of type I errors (i.e. only 63% of the LIRRs were correctly assigned; Table 7). Accordingly, if the aim is to reduce type I errors, then either ALL&A or ALL&C and threshold #II would be recommended as these rules resulted in 90% of the LIRRs correctly assigned (Table 7). By contrast, two more complex rules (D3&F2 or I5&F2) were slightly better at discriminating impact of cargo-ship traffic (93% of all reaches

Table 6 Development of impact assessment rules based on ecological reasoning. Correct assignment of river reaches (in %, as integers) for different rules and variables having predictions for different types of human impact (see Table 1 for labels) for the two selected thresholds ordered by increasing potential risk of type I errors (assigning validation-LIRR to impacted groupings, increasing from threshold II to threshold #I)

Assessment rule	Impact	LIRRs (<i>n</i> = 68)	Metals (<i>n</i> = 65)	Metals & ships (<i>n</i> = 66)	Cargo-ships (<i>n</i> = 142)	Total (<i>n</i> = 341)
Threshold #II						
ALL&A or ALL&C*	All types	88	19	92	89	77 [†]
A&N or M&N	Metals	90	26	73	51	58
A1_2&N2 or M7_9&N2	Metals	93	8	32	90	64
C&F or D&F	Ships	90	80	97	87	88
D3&F2 or I5&F2	Ships	90	80	96	98	92
Threshold #I						
ALL	All types	78	25	96	91	78 [†]
ALL&A or ALL&C	All types	72	31	96	92	78 [†]
M	Metals	56	56	96	11	45
A&N or M&N	Metals	43	60	99	9	43
A1_2	Metals	63	63	96	6	46
A1_2&N2 or M7_9&N2	Metals	46	62	77	37	51
C	Ships	88	82	55	88	80
C&F or D&F	Ships	71	71	99	90	84
I5	Ships	94	74	80	94	87
D3&F2 or I5&F2	Ships	77	72	99	97	88

*ALL stands for overall mean trait.

[†]Includes 31 reaches with organic pollution, heavy metal pollution and/or cargo-ships, i.e. *n* = 372.

Table 7 Validation of the impact assessment rules based on ecological reasoning with independent data. Correct assignments of river reaches (in %, as integers) using different rules and variables defined in Table 6. See Table 6 for further details

Assessment rule	Impact	LIRRs (<i>n</i> = 40)	Metals (<i>n</i> = 60)	Metals & ships (<i>n</i> = 66)	Cargo-ships (<i>n</i> = 139)	Total (<i>n</i> = 305)
Threshold #II						
ALL&A or ALL&C	All types	90	13	85	89	75*
A&N or M&N	Metals	60	22	68	48	49
A1_2&N2 or M7_9&N2	Metals	73	3	28	90	57
C&F or D&F	Ships	90	80	99	93	91
D3&F2 or I5&F2	Ships	98	75	99	98	93
Threshold #I						
ALL	All types	63	22	99	93	78*
ALL&A or ALL&C	All types	60	27	99	93	78*
M	Metals	30	53	94	8	38
A&N or M&N	Metals	25	45	96	4	35
A1_2	Metals	38	58	97	7	41
A1_2&N2 or M7_9&N2	Metals	30	58	76	35	48
C	Ships	78	92	49	86	78
C&F or D&F	Ships	65	65	99	94	85
I5	Ships	93	73	77	99	88
D3&F2 or I5&F2	Ships	68	68	99	96	88

*Includes 26 reaches with organic pollution, heavy metal pollution and/or cargo-ships, i.e. *n* = 331.

were correctly assigned resulting a type I error frequency of 2%) than the simpler approach (88% of all reaches were correctly assigned). In contrast to all impact types and cargo-ship traffic, the discrimination

of heavy metal impact was poorer than predicted. At best, the small size category (A1_2; Table 7) at threshold #I correctly assigned 58% of the reaches with only heavy metal pollution. Moreover, using this

rule, 62% of the LIRRs and 93% of the reaches with only cargo-ship traffic would be classified as polluted by heavy metals.

Impact assessment based on multivariate analysis

Development of assessment rules The DFA with 10 variables (overall mean trait and nine traits; Table 8a) resulted in correct assignment of 71% of LIRRs, 40% of reaches impacted by heavy metal pollution, 77% of reaches impacted by cargo-ship traffic, 45% of reaches impacted by both heavy metal pollution and cargo-ship traffic and 45% of the reaches impacted in addition by organic pollution (Mixed in Table 8a), compared to correct assignments of 75%, 45%, 81%, 51% and 61%, respectively, using 15 variable DFA models.

Exclusion of the overall mean trait did not change the results in any of the models. Indeed, the overall mean trait was the best single predictor; this variable alone explained 46% of the overall variability. Among the traits that had *a priori* predictions (Table 1), two

variables (number of reproductive cycles per individual and parental care) each explained 43% of the variability. Of the five traits for which we did not have *a priori* predictions (not included in Table 1), body form was equally robust.

The DFA on the 12 selected trait categories having *a priori* predictions for heavy metal pollution and cargo-ship traffic (Table 1) resulted in correct assignment of 65% of LIRRs, 49% of reaches impacted by heavy metal pollution, 85% of reaches impacted by cargo-ship traffic, 50% of reaches impacted by both heavy metal pollution and cargo-ship traffic and 48% of reaches impacted by mixed human alterations (Table 8b); for all 63 trait categories the correct assignments were 85%, 80%, 92%, 71% and 90% respectively (Table 8b). Of the trait categories with *a priori* predictions, three (ovoviviparity, 50%, hermaphroditism, 43% and \geq semivoltine cycle, 38%) explained most of the overall variability. Of the 51 trait categories for which we did not have *a priori* predictions (not included in Table 1), 15 were shown to explain a similar amount of variability (range: 40–55%) as the *a priori* predictors, resulting in a considerable increase in the number of correctly assigned reaches by DFA on all 63 trait categories (in comparison to the 12 selected trait categories; Table 8b).

Validation of assessment rules Correct assignments (% of reaches in Table 9) in validation (*y*) were

Table 8 Global assignment of reaches (in %, as integers) used in developing assessment rules resulting from Discriminant Function Analysis (DFA) performed on the calibration data set with (a) 10 trait variables (first number, overall mean trait and nine specific traits; see Table 1) and all 15 variables (second number, overall mean trait and 14 traits available in our database) and (b) 12 selected trait categories (first number, see Table 1 for these categories) and all 63 trait categories (second number) available in our database. Note that the values in the columns correspond to the reaches of each group that were assigned to the row labels (correct assignments in bold)

Grouping	LIRR (n = 68)	Metals (n = 65)	Metals & ships (n = 66)	Cargo- ships (n = 142)	Mixed* (n = 31)
(a)					
LIRR	71–75	46–43	3–8	5–4	3–3
Metals	25–19	40–45	5–3	6–4	10–6
Metals & ships	1–1	6–5	45–50	9–9	19–19
Cargo-ships	3–3	6–8	33–27	77–81	23–10
Mixed	0–1	2–0	14–12	2–1	45–61
(b)					
LIRR	65–85	32–12	0–0	1–1	3–0
Metals	25–9	49–80	9–5	2–0	6–6
Metals & ships	0–3	5–2	50–71	11–7	29–3
Cargo-ships	7–3	14–6	29–14	85–92	13–0
Mixed	3–0	0–0	12–11	1–0	48–90

*Includes reaches with organic pollution, heavy metal pollution and/or cargo-ships.

Table 9 Global assignment of validation reaches by DFA. See Table 8 for further details

Grouping	LIRR (n = 40)	Metals (n = 60)	Metals & ships (n = 66)	Cargo- ships (n = 139)	Mixed* (n = 26)
(a)					
LIRR	53–60	45–35	9–14	4–3	4–8
Metals	43–30	45–55	8–9	6–9	12–12
Metals & ships	0–0	5–2	39–36	12–17	38–19
Cargo-ships	5–8	3–7	33–30	75–69	12–15
Mixed	0–3	2–2	11–11	4–2	35–46
(b)					
LIRR	38–50	33–17	2–2	0–1	4–0
Metals	15–23	45–72	11–14	4–1	4–8
Metals & ships	3–3	5–12	56–55	14–11	27–4
Cargo-ships	43–25	17–0	26–18	81–86	15–15
Mixed	3–0	0–0	6–12	1–1	50–73

*Includes reaches with organic pollution, heavy metal pollution and/or cargo-ships.

regressed on the correct assignments (Table 8) in calibration (x) to compare the predictive power of DFA model.

$$y = 11.1(\pm 8.8) + 0.69(\pm 0.13)x; n = 20$$
$$R^2 = 0.61; P < 10^{-4}$$

Our results showed that the relative performance of the DFA-model to assign reaches correctly in the calibration was not preserved in validation (slope < 1). Although the intercept was not significantly different from zero, its positive nature and the slope indicate that, in comparison to the calibration DFA, the correct assignments increased at the low and decreased at the high ends in the validation. In addition, the regression explained only 61% of the data variability using a relatively low number of observations ($n = 20$). Together, these findings show that the DFA model was less accurate than the assessment rules based on ecological reasoning.

Discussion

Trait-based approaches rely on taxon-free metrics (i.e. biological traits), which offer a quantification of evolutionary responses to environmental selective forces across broad geographic gradients and represent known biological processes. For example, size has implications for many ecological functions of lotic invertebrates (e.g. production/biomass; production/respiration; e.g. Statzner, 1987) and the allometry of physiological and life-history traits may control species composition and relative abundances, thus implying a functional link between body-size distributions and communities (Robson, Barmuta & Fairweather, 2005). According to Poff *et al.* (2006), trait-based approaches should focus on evolutionary labile traits that have low statistical correlations (i.e. are phylogenetically independent) and are linked in a mechanistic way to a specific human impact. Our study considered these key elements, thereby providing a critical step towards the development of a biomonitoring tool for large European rivers (Statzner *et al.*, 2005). Using invertebrate traits, we applied both a step-by-step approach based on ecological reasoning and a multivariate approach to quantify the amount of deviation of impacted river reaches from previously defined LIRR conditions, and assessed the performance of these approaches in

subsequent validations with an independent data set.

Correlations of trait categories across genera and across communities

In our study, we found that correlations of trait categories having *a priori* predictions with one trait category indicative for cargo-ship traffic (\geq semivoltine cycle) were typically stronger across communities than across taxa. Such higher correlations confirm the potential action of independent trait filters already observed by other authors, e.g. for terrestrial plants (Mabry, Ackerly & Gerhardt, 2000), marine fish (Jennings *et al.*, 2001) and invertebrates of smaller European streams (Statzner *et al.*, 2004). In contrast, for a trait category indicative for heavy metal pollution (i.e. small size), correlations with other trait categories indicative for heavy metal pollution were similar or even higher across genera than across communities, whereas correlations with trait categories indicative for cargo-ship traffic showed the reverse pattern. This was a second indication (see also the similar richness values of LIRRs and reaches with only metal pollution; Table 2) that it would be difficult to develop impact assessment rules for heavy metal pollution.

Development of impact assessment rules

Assessment rules based on ecological reasoning correctly assigned $>70\%$ of the reaches to LIRR conditions. Statzner *et al.* (2005), in a recent review of the literature, showed that *c.* 70% correct assignments to reference groupings were considered as robust models. Hence, our selection of traits and trait categories showed promise as predictors to discriminate impact. In one of our models, a single trait category (I5, permanent attachment) resulted in the correct assignment of 94% of the reaches to LIRR conditions. By comparison, models calibrated to discriminate the effects of heavy metal pollution performed poorly. However, we cannot exclude that the definition of classes of heavy metal pollution, dictated by available information, was inaccurate, because it is generally difficult to find a single human impact in large European rivers. Furthermore, the heavy metal pollution in our large river reaches was perhaps not strong enough to produce the predicted traits

patterns, since steps have been taken to reduce heavy metal pollution (Vink, Behrendt & Salomons, 1999; Middelkoop, 2000), though problems still occur in terms of bioaccumulation (e.g. Scholz *et al.*, 2005: p. 44). Overall, the response to heavy metal pollution can be very complex, especially because it influences biological interactions (Clements, 1999). For example, predation intensity may increase in metal-polluted streams (see review by Pollard & Yuan, 2006). Moreover, Rawer-Jost *et al.* (2000) showed that the proportion of predators (one of the metrics selected by us: animal food of all prey sizes) was a reliable metric for various trait categories of impairment, not only heavy metal pollution. In fact, very few studies have shown that invertebrate traits respond to heavy metal pollution (e.g. in smaller streams Archaimbault, 2003; N. Philips pers. comm.).

Current biomonitoring approaches generally suffer from a lack of specific responses to a given impact (Bonada *et al.*, 2006). For example, Fore, Karr & Wisseman (1996) underlined that using a multimetric approach, the discrimination of human impact types is generally difficult, because metrics that respond reliably to a given impact (e.g. logging) are likely to respond to other types of disturbances (e.g. grazing or urbanization). Using a similar approach, Böhmer *et al.* (2004) emphasized that most metrics they assessed respond to human impacts independently of the type of stressor, but that the metrics vary in the degree of reaction to stressor types. The relatively poor detection of moderate heavy metal pollution in our study thus calls for further search for trait categories that specifically respond to this impact.

Validation of impact assessment rules

An accurate assignment of reference conditions is a necessary component of many biomonitoring programmes, and is central to the European Water Framework Directive (e.g. Nijboer *et al.*, 2004; Vlek, Verdonschot & Nijboer, 2004). In our models, this was evaluated using independently defined LIRR and impacted conditions. Using rules based on ecological reasoning, we correctly assigned 75–78% of all reaches with rules on all impact types, 35–57% with rules on heavy metal pollution and 78–93% with rules on cargo-ship traffic. These values are largely competitive with the few validation tests on independent data available in the literature. For

example, using DFA models based on landscape stressors and community composition, Bailey *et al.* (2007) correctly assigned 51% of validation sites to appropriate groupings. Using more than a hundred metrics (including composition/abundance, richness/diversity, sensitivity/tolerance, functional groups), Vlek *et al.* (2004) could correctly classify 54% of their streams into four quality classes. However, compared to the rules based on ecological reasoning, the performance of DFA was globally poorer. Moreover, besides differences in overall assignment, the two approaches also differed considerably in their stability. By comparison, the relatively simple rules using only a few *a priori* predicted traits or trait categories remained stable in the validation. In other words, poor rules performed poorly and powerful rules performed powerfully in both calibration and validation. In contrast, the performance of the DFA models changed between calibration and validation, illustrating the unstable performance of this approach, and leading us to conclude that DFA is seemingly inappropriate for biomonitoring using trait-based approaches as we have done here.

Implications of cargo-ship traffic for the functional composition of invertebrate communities

Although our study was not designed to assess the implications of cargo-ship traffic for the functional trait composition of invertebrate communities in large rivers, the results of our analyses merit a brief discussion of this ostensibly ignored topic. Clear differences were noted regarding changes in the overall mean trait, both with cargo-ship traffic alone or in combination with heavy metal pollution. For example, reaches with cargo-ship traffic differed up to 75% in their overall mean trait from the mean in the LIRRs. Even more pronounced differences were noted for individual trait categories such as \geq semivoltine, $>$ two reproductive cycles per individual, and, particularly, ovoviviparity and permanent attachment to the substratum, i.e. reaches with cargo-ship traffic had considerably more genera with long life cycles, that reproduced repeatedly by ovoviviparity and had sessile life. Most studies on the effects of cargo-ship traffic on freshwater ecosystems have focussed on the establishment of non-indigenous species from commercial ship ballast water (e.g. Grigorovich *et al.*, 2002;

Zhulidov *et al.*, 2004) and/or the disturbance of benthic invertebrates by ship-induced waves (e.g. Garcia *et al.*, 2007). However, our results indicate that cargo-ship traffic can have profound effects on the functional composition of invertebrate communities in large rivers. Accordingly, this topic deserves more priority, particularly in the context of current research efforts focused on other impact types that obviously (e.g. heavy metal pollution) or presumably have less effect on the functional invertebrate community composition in large rivers.

Implications for impact assessment of lotic ecosystems

The calibration and validation of new tools to be used in biomonitoring is largely limited by data availability and quality (see Hering *et al.*, 2004). Determination of deviations from reference conditions (LIRRs), requires *a priori* knowledge on the type and level of impact. Our analyses provided a strong and reliable baseline for reference conditions (LIRRs) that could be used in the future for the biomonitoring of functional response in large rivers and floodplain systems (see Henle *et al.*, 2006). In our data set, the presence of cargo-ship traffic was readily detected, but quantification was not possible as we lacked data on frequency and size of cargo-ships. In contrast, none of our models were able to adequately discriminate the effects of heavy metal pollution. Our results imply the presence of multiple human impacts in large rivers, and that the detection of specific impacts can be complex. Consequently, future applied research using a trait-based approach should focus on two elements: first, are the results from well-known large rivers such as the Elbe, Danube, Rhine or Rhône transferable to other less studied large rivers? (e.g. Henle *et al.*, 2006) and second, further studies should encompass smaller streams to provide a complete biomonitoring tool that requires no regionalization, in contrast with other approaches (e.g. Moog *et al.*, 2004), and no modelling of expected values from environmental condition as in other existing indicator systems (Dziocck *et al.*, 2006).

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