



Validating the NemaSPEAR[%]-index for assessing sediment quality regarding chemical-induced effects on benthic communities in rivers

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ABSTRACT

Fine, cohesive sediments provide a habitat for a very diverse fauna and considerably contribute to important ecosystem services of aquatic ecosystems. As fine sediments are often hotspots of chemical contamination, the benthic fauna has to be protected in order to maintain its ecological functioning and thereby the proper provision of important ecosystem services. However, in these habitats usually meio-faunal organisms prevail, which are, so far, neglected in biomonitoring studies, while routine benthos monitoring (e.g. according to the EU Water Framework Directive) is solely based on macroinvertebrates. The recently developed NemaSPEAR[%]-index filled this methodological gap by providing a monitoring tool using freshwater nematodes, which are one of the most abundant and species rich invertebrates in fine sediments. In the present study the NemaSPEAR[%]-index was revised and validated based on a larger data set of nematode species and physico-chemical properties in river sediments in order to increase its applicability. The larger data set led to a similar categorization of nematode species at risk (NemaSPEAR). Validation of the NemaSPEAR[%] with an independent test data set, as well as external field data published by other authors and experimental microcosm data confirmed its usefulness as a specific index detecting chemical induced changes in benthic communities, whereas the index also worked at a higher taxonomic level (genus; NemaSPEAR[%]_{genus}). A separation of the index for metal and organic pollution (NemaSPEAR[%]_{metal}, NemaSPEAR[%]_{organic}) showed no benefits for assessing sediments with mixed contamination, allowing the use of only one NemaSPEAR[%] for overall pollution. Moreover, based on its variance in lowly polluted reference sediments, class boundaries were set up for categorizing samples according to their ecological status, with a NemaSPEAR[%] higher or lower 30% indicating an acceptable or not acceptable ecological status, respectively. Overall, this study confirmed the robustness and relevance of the NemaSPEAR[%]-index for assessing the quality of fine sediments and using it as a line of evidence in a weight-of-evidence framework. Thus, the index can be a valuable tool for classification and prioritization of fine sediments supporting risk managers and regulators in making sediment management decisions.

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1. Introduction

Benthic invertebrates play an important role for the functioning of freshwater ecosystems (Covich et al., 1999; Majdi et al., 2014; Schmid and Schmid-Araya, 2002). They represent a significant food source for fish, thus linking benthic and pelagic food webs (Ellis and Coull, 1989; Spieth et al., 2011; Weber and

Traunspurger, 2015). Moreover, benthic invertebrates help to break down and recycle organic matter that has been deposited in the sediment, thus, considerably contributing to the nutrient cycling in water bodies (Palmer et al., 1997; Traunspurger et al., 1997). Beside their ecological relevance, which makes them an important protection goal in terms of environmental risk assessment, they possess high indicative power, for assessing quality deteriorations in benthic habitats (Patrício et al., 2012; Rosenberg and Resh, 1993; Wilson and Kakouli-Duarte, 2009). Therefore, the structure of benthic invertebrate communities is often used as a tool for defining the ecological status of and for detecting chemical induced changes in freshwater ecosystems (e.g. Hering et al., 2004; Munoz et al., 2012). Historically, in routine biomonitoring programs

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(e.g. in context of the EU WFD) mainly macroinvertebrate communities were assessed to evaluate the ecological status of inland waters, by using various indices to identify anthropogenic impact (e.g. SPEAR[%]-index, [Von der Ohe and Liess, 2004](#); Saprobic Index, [Rolauffs et al., 2004](#)). In fine, adhesive sediments, often hotspots of chemical pollution, however, only a limited number of macrobenthic species can be found, impeding an accurate quality assessment ([Lopez-Doval et al., 2010](#); [Wolfram et al., 2010](#)). In these habitats, meiofaunal invertebrates (e.g. nematodes) dominate the benthic communities, showing high abundances and diversity ([Michiels and Traunspurger, 2005](#); [Traunspurger, 2000](#)). Moreover, nematodes showed to be useful bioindicators in aquatic ecosystems ([Danovaro et al., 2009](#); [Hägerbäumer et al., 2015](#)) and to respond sensitively to environmental chemicals with changes in their community structure (e.g. [Brinke et al., 2010](#)).

Therefore, recently, an index was developed to assess chemical induced changes in freshwater nematode communities, the NemaSPEAR[%]-index, that classifies nematodes in species at risk (NemaSPEAR) and species not at risk (NemaSPEAR_{notAR}) ([Höss et al., 2011](#)). This classification was done using multivariate analysis on a large data set on freshwater sediments (mainly large rivers and lakes), including nematode species data, as well as data on sediment concentrations of selected contaminants. The NemaSPEAR[%]-index expresses the ratio of NemaSPEAR to total species in a sample and has been deduced separately for metal and organic pollution (NemaSPEAR[%]_{metal}; NemaSPEAR[%]_{organic}). The index has been validated using an independent data set and data from various microcosm studies and showed to be dose-dependently related to chemical contamination ([Hägerbäumer et al., 2016](#); [Höss et al., 2011](#)). Moreover, the NemaSPEAR[%] showed comparable results to the SPEAR[%]-index (based on macro-invertebrates) in a weight-of-evidence study on sediments from three different European river basins ([Wolfram et al., 2012](#)).

Nevertheless, several issues had to be clarified, before the NemaSPEAR[%]-index could be routinely used for assessing the quality of freshwater sediments:

- (1) An index requires boundaries that allows for distinguishing hazardous from non-hazardous sediments, acceptable from unacceptable ecological status. For the SPEAR[%]-index several boundaries were defined to categorize sites into five classes of ecological status, according to the EU WFD ([Beketov et al., 2009](#); [Von der Ohe et al., 2007](#)). Based on these approaches, class boundaries should also be set up for the NemaSPEAR[%]-index.
- (2) [Höss et al. \(2011\)](#) distinguished between metal and organic pollution for classification of NemaSPEAR, resulting in two indices specific for the type of pollution. Based on an enlarged data-base it had to be evaluated if this splitting provides advantages over the indication of overall pollution, which is usually experienced by benthic communities due to a mixed metal and organic contamination.
- (3) The classification of nematode species at risk was based on their occurrence at lowly polluted sites and their absence in polluted sites. Thus, only species that occurred in the “reference sites” of the underlying data set could be classified as NemaSPEAR. In the data set of [Höss et al. \(2011\)](#), the cleanest sediments were sampled at different sites of Lake Constance (i.e. from a lentic habitat). Although, the Lake Constance is strongly influenced by inputs of rivers (e.g. river Rhine) and, thus, many lotic nematode species can also be found here, some important nematode species, typical for pristine lotic habitats were missing in the data set.
- (4) The taxonomic identification of nematodes to species level is not trivial and requires the knowledge of experts, making it challenging to determine the NemaSPEAR[%]-index. Although, due to the progress in molecular taxonomy (e.g. [Vervoort et al.,](#)

[2012](#)), it can be expected that the analysis of nematode communities can be managed also by non-taxonomists, an index based on higher taxonomic levels (e.g. genus) might find a broader acceptance in biomonitoring studies.

Therefore, the aims of this study were (1) to revise the list of NemaSPEAR ([Höss et al., 2011](#)) by newly classifying the species using an enlarged training dataset (215 samples, including 63 new samples from lotic habitats) and the overall toxic potential of the sediments (no distinction of metal and organic pollution), (2) to validate the revised index with an independent test data set (73 sites, including 22 new samples from lotic habitats) and experimental data, (3) to re-evaluate the benefit of splitting the index according to the type of contamination (i.e. NemaSPEAR[%]_{metal}, NemaSPEAR[%]_{organic}), (4) to define class boundaries for the NemaSPEAR[%]-index that allow to classify sediments according to their ecological status, and (5) to classify nematodes in genera at risk and genera not at risk and, thus, to enable calculation of an index on a taxonomically less challenging level (NemaSPEAR[%]_{genus}).

2. Material and methods

2.1. Data sets

The sets of environmental and nematode community data, that has been used by [Höss et al. \(2011\)](#) were extended by analyzing 85 additional sediment samples collected from 73 sites in lotic freshwater systems from various river basins in Germany (see Table S1 in SI for a description of the sites). This yielded a total number of 288 data sets for nematode communities and environmental data.

According to [Höss et al. \(2011\)](#), the data were divided into one large training set (215 samples), used for deriving nematode species classification and one smaller test set (73 samples), used as an independent data set for validating the revised NemaSPEAR[%] index. The criteria for assigning samples to the subsets were set following [Höss et al. \(2011\)](#): (1) The training set contains >90% of the species that were found for the whole data set; (2) the gradient of sediment properties and contamination should be comparable in the two data sets; (3) lentic and lotic samples should be represented in both data sets; and (4) the various samples of one sampling site are not split between the two subsets to avoid pseudo replication.

2.2. Sediment sampling and characteristics

Sediments were sampled as described in [Heininger et al. \(2007\)](#), using a stainless steel van-Veen-type grab sampler for analysis of physico-chemical parameters (top 10-cm layer; 10 sub-samples), and by means of piston drill with an acrylic glass tube (5- or 8-cm diameter) for nematode analysis (top 2–3-cm layer; 3 sub-samples).

The analysis of physico-chemical properties and concentrations of pollutants was performed as described in previous studies ([Heininger et al., 2007](#); [Höss et al., 2011](#)). The extended sample set (see Table S1 in SI) was analyzed using gas chromatography/mass spectrometry for selected PAHs and chloro-organic compounds according to DIN CEN/TS 16181:2013-12 and DIN EN 16167:2012-11, respectively. Tributyltin was detected and quantified by gas chromatography with pulsed flame photometric detection according to DIN EN ISO 23161 2011-10 in conjunction with E DIN 19744:2003-08. The metals cadmium, chromium, copper, lead and the metalloid arsenic were analyzed by inductively coupled plasma optical emission spectrometry (ISO 11885-E22:1997-11). Zinc and mercury were measured using atom absorptions spec-

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