Identifying ecological effects of organic toxicants and metals using the SPEAR approach

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1. Schäfer, R.B., Gerner, N., Kefford, B.J., Rasmussen, J.J., Beketov, M.A., de Zwart, D., Liess, M., von der Ohe, P.C., 2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities? Environmental Science & Technology 47 (14), pp. 7996-8004, doi: 10.1021/es4014954.

2. Gerner, N.V., Koné, M., Ross, M.S., Pereira, A., Ulrich, A.C., Martin, J.W., Liess, M., 2017. Stream invertebrate community structure at Canadian oil sands development is linked to concentration of bitumen-derived contaminants. Science of the Total Environment 575, pp. 1005-1013,doi: 10.1016/j.scitotenv.2016.09.169.

3. Gerner, N.V., Cailleaud, K., Bassères, A., Liess, M., Beketov, M.A., 2017. Sensitivity ranking for freshwater invertebrates towards hydrocarbon contaminants. Ecotoxicology 26 (9), pp. 1216-1226, doi: 10.1007/s10646-017-1847-7.

4. Liess, M., Gerner, N.V., Kefford, B.J., 2017. Metal toxicity affects predatory stream invertebrates less than other functional feeding groups. Environmental Pollution 227, pp. 505-512, doi: 10.1016/j.envpol.2017.05.017.

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Summary

The aquatic environment is exposed to multiple environmental pressures and mixtures of chemical substances, among them petroleum and petrochemicals, metals, and pesticides. Aquatic invertebrate communities are used as bioindicators to reflect long-term and integral effects. Information on the presence of species can be supplemented with information on their traits. SPEAR-type bioindicators integrate such trait information on the community level.

This thesis aimed at enhancing specificity of SPEAR-type bioindicators towards particular groups of chemicals, namely to mixtures of oil sands-derived compounds, hydrocarbons, and metals.

For developing a bioindicator for discontinuous contamination with oil-derived organic toxicants, a field study was conducted in the Canadian oil sands development region in Northern Alberta. The traits 'physiological sensitivity towards organic chemicals' and 'generation time' were integrated to develop the bioindicator $SPEAR_{oil}$, reflecting the community sensitivity towards oil sands derived contamination in relation to fluctuating hydrological conditions.

According to the $SPEAR_{organic}$ approach, a physiological sensitivity ranking of taxa was developed for hydrocarbon contamination originating from crude oil or petroleum distillates. For this purpose, ecotoxicological information from acute laboratory tests was enriched with rapid and mesocosm test results. The developed $S_{hydrocarbons}$ sensitivity values can be used in SPEAR-type bioindicators.

To specifically reflect metal contamination in streams via bioindicators, Australian field studies were re-evaluated with focus on the traits 'physiological metal sensitivity' and 'feeding type'. Metal sensitivity values, however, explained community effects in the field only weakly. Instead, the trait 'feeding type' was strongly related to metal exposure. The fraction of predators in a community can, thus, serve as an indicator for metal contamination in the field.

Furthermore, several metrics reflecting exposure to chemical cocktails in the environment were compared using existing pesticide datasets. Exposure metrics based on the 5% fraction of species sensitivity distributions were found to perform best, however, closely followed by Toxic Unit metrics based on the most sensitive species of a community or *Daphnia magna*.

Zusammenfassung

Aquatische Ökosysteme sind einer Vielzahl an Umweltstressoren sowie Mischungen chemischer Substanzen ausgesetzt, darunter Petroleum und Petrochemikalien, Metalle und Pestizide. Aquatische Gemeinschaften wirbelloser Arten werden als Bioindikatoren genutzt, um Langzeit- sowie integrale Effekte aufzuzeigen. Die Information über das Vorkommen von Arten kann dabei um weitere Informationen zu Eigenschaften dieser Arten ergänzt werden. SPEAR-Bioindikatoren fassen diese Informationen für Artengemeinschaften zusammen.

Ziel der vorliegenden Doktorarbeit war es, die Spezifität von SPEAR-Indikatoren gegenüber einzelnen Chemikaliengruppen zu verbessern – speziell für Ölsand-Bestandteile, Kohlenwasserstoffe und Metalle.

Für die Entwicklung eines Bioindikators für diskontinuierliche Belastung mit organischen Ölbestandteilen wurde eine Freilandbeprobung in der kanadischen Ölsand-Abbauregion im nördlichen Alberta durchgeführt. Die Arteneigenschaften „physiologische Sensitivitiät gegenüber organischen Chemikalien“ sowie „Generationszeit“ wurden in einem Indikator, $SPEAR_{oil}$, integriert, welcher die Sensitivität der Artengemeinschaften gegenüber Ölsand-Belastung in Abhängigkeit von fluktuierenden hydrologischen Bedingungen aufzeigt.

Äquivalent zum $SPEAR_{organic}$-Ansatz wurde eine Rangliste der physiologischen Sensitivität einzelner Arten gegenüber Kohlenwasserstoff-Belastung durch Rohöl oder Petroleum entwickelt. Hierfür wurden Informationen aus ökotoxikologischen Kurzzeit-Laborversuchen durch Ergebnisse aus Schnell- und Mesokosmen-Tests ergänzt. Die daraus entwickelten $S_{hydrocarbons}$-Sensitivitätswerte können in SPEAR-Bioindikatoren genutzt werden.

Um Metallbelastung in Gewässern mittels Bioindikatoren spezifisch nachweisen zu können, wurden die Arteneigenschaften „physiologische Metallsensitivität“ und „Ernährungsweise“ von Artengemeinschaften in australischen Feldstudien ausgewertet. Sensitivitätswerte für Metalle erklärten die Effekte auf die Artengemeinschaften im Gewässer jedoch unzureichend. Die „Ernährungsweise“ hingegen war stark mit der Metallbelastung korreliert. Der Anteil räuberischer Invertebratenarten in einer Gemeinschaft kann daher als Indikator für Metallbelastung in Gewässern dienen.

Weiterhin wurden verschiedene Belastungsanzeiger für Chemikalien-Cocktails in der Umwelt anhand von Pestizid-Datensätzen verglichen. Belastungsanzeiger, die auf der 5%-Fraktion einer Species-Sensitivity-Distribution beruhen, eigneten sich am besten, gefolgt von Toxic Unit-Ansätzen, die auf der sensitivsten Art einer Gemeinschaft oder *Daphnia magna* beruhen.

Contents

Chapter 1: Introduction and objectives

1.1. Chemicals in the aquatic environment

Water – although recognized as the most important natural resource – is detrimentally affected by human activities (Vörösmarty et al. 2010). The list of pressures acting on aquatic ecosystems is long and ranges from depletion to degradation. Anthropogenic pressures result from population growth and increasing economic development and include land conversion, water withdrawal, eutrophication and pollution, overharvesting and overexploitation, and the introduction of invasive alien species (Millennium Ecosystem Assessment 2005a). The Millennium Ecosystem Assessment (2005b) revealed that the proportion of species threatened with extinction is higher in freshwater ecosystems than in other ecosystems. Eutrophication and pollution are deteriorating water quality, and with this, impairing aquatic species. One of the declared UN sustainable development goals (SDG) calls to “By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials [ ]" (UN SDGs, goal 6.3, adopted in 2015). However, to date, a wide range of hazardous substances enter the aquatic environment via diverse pathways and persist for long periods of time, among them are petrochemicals, metals, and pesticides (Harmon and Wiley 2011).

Petrogenic oil and petrochemicals, as a group of persistent organic pollutants, are toxicologically and ecotoxicologically of high importance. Their entry into the environment occurs, among other pathways, via accidents such as oil spills. This is mainly the case for marine ecosystems. Well-known large-scale accidents were, for instance, the Exxon Valdez and the Deepwater Horizon oil spills in 1989 and 2010, respectively, which lead to wide ranging deterioration of ecosystems (Peterson 2001). But also freshwater ecosystems are affected from oil pollution, for instance via leakages or seepage from pipelines (Douglas 2002). However, not only from pipelines, but also directly from the deposits and their development, oil can reach aquatic systems. Of particular importance are polycyclic aromatic hydrocarbons (PAHs) due to their cancerogenic and mutagenic properties (Canadian Environmental Protection Act 1994). PAHs are non-polar organic compounds composed of multiple aromatic rings containing only carbon and hydrogen. They can reach aquatic ecosystems also via atmospheric deposition from industrial activities and the burning of fossil

fuel and fires. Furthermore, PAH are washed into streams via run-off from traffiqued roads (Beasley and Kneale 2002).

PAHs are accumulated by aquatic organisms following uptake via water, sediment, and/or food. PAH uptake by aquatic organisms occurs faster in the solubilized form. Solubility of PAH substances depends on their size, i.e. number of aromatic rings and alkyl groups. Despite physico-chemical properties of the PAHs themselves, uptake is also influenced by environmental variables, such as binding agents like suspended and dissolved organic matter. Metabolization of PAHs in animals via the mixed-function oxygenase enzyme systems (MFOs) results in toxic, carcinogenic, and/or mutagenic intermediate products (Canadian Environmental Protection Act 1994). Despite acute toxicity, studies have also reported sublethal and chronic effects of PAHs to freshwater animals (Canadian Environmental Protection Act 1994, Christiensen 1975). Also PAH-induced reproductive impairment has been observed and is related to an altered estrogen receptor function by direct binding or activation of pathways via other receptors (Arens et al. 2017).

In water quality studies, hydrocarbons have received less attention than, for instance, heavy metals. Particularly, hydrocarbon research in freshwater systems is underrepresented compared to the marine environment (Beasley and Kneale 2002). Thus, to date, biomonitoring methods – particularly for freshwater – still lack indicators to specifically reflect impacts caused by hydrocarbons. The challenge here is to discriminate these effects from effects of confounding environmental stressors.

Another persistent and ubiquitously occurring group of toxicants in the aquatic environment are heavy metals. Besides natural background levels of metals stemming from the geological weathering of rocks, past and current mining activities (Nriagu and Pacyna 1988), landfills (Naveen et al. 2017) as well as urban areas (Sharley et al. 2016) contribute to their entry into the environment. Copper and zinc reach streams also via run-off from roofs (Marsalek 1990). Under the European Water Framework directive (WFD) (BMUB/UBA 2016), lead, cadmium and nickel are considered priority pollutants. In Germany, mercury is even declared an ubiquitous pollutant.

Several metals are elements essential to life, enabling biological functions, however, only in low bioavailable concentrations. They are toxic in concentrations higher than these requirements. As they are persistent, they accumulate in the tissue of organisms (Hormon 2011) with subsequent transfer within the food web. Acute and chronic toxicity comprises

sublethal as well as lethal effects (Beasley and Kneale 2002). The effects of metals on aquatic species depend on the concentration and speciation of the respective metal or metal mixture. A metal's speciation determines its bioavailability and is influenced by a number of environmental parameters (Wang 1987) such as pH, temperature, alkalinity and hardness as well as the presence of organic or inorganic ligands and fine particles in the water. Metal toxicity is, furthermore, dependent on the exposed species' tolerance, life stage and feeding type (Wang 1987). Despite this knowledge, the link between metal exposure and effects on aquatic communities is not yet satisfactorily understood. A broad range of acute laboratory test results exist (e.g. U.S. Environmental Protection Agency (US EPA) database ECOTOX 2013) as well as rankings of the sensitivity of macroinvertebrate taxa towards metals based on laboratory tests (Malaj et al. 2012, von der Ohe and Liess 2004, Wogram and Liess 2001). Acute sensitivities observed in laboratory studies, however, proved unsuitable for predicting effects in the field, where low concentrations prevail over longer periods and coincide with further biotic, abiotic and chemical stressors (Brix et al. 2011).

Contamination with pesticides, on the other hand, became an environmental problem with the beginning of industrial agriculture. Environmental effects have been described by Carson (1962). Since then, several pesticides have been banned while new ones were approved for usage. Every year, large amounts of pesticides are applied and enter the environment via diffuse and point sources (Leu et al. 2004). De et al. (2014) report a worldwide pesticide consumption of about two million tons per year. From these, 45 % and 25 % are consumed in Europe and the USA, respectively. Pesticide consumption has been increasing (Roser and Ritchie 2017) and is predicted to increase further due to climate change (Kattwinkel et al. 2011). Pesticides are applied to control unwanted plants, insects or microorganisms and fungi (herbicides, insecticides and fungicides, respectively) and are effective at low concentrations. However, concurrently non-target species are affected as well. During rainfall, pesticides applied to agricultural land or urban areas are washed into adjacent streams via runoff, exposing aquatic species to short-term pesticide pulses (Leu et al. 2004). Pesticides exhibit direct effects of acute poisoning on aquatic organisms. In addition, a variety of indirect ecological effects can result. Macroinvertebrate communities can, for instance, suffer from the application of herbicides that cause a reduction in their food supply. Such food web effects or other indirect effects can possibly be stronger than the direct toxicity.

1.2. Environmental monitoring via bioassessment

Adverse effects on ecosystems originating from chemical pollutants can be assessed via environmental monitoring. As ecosystems are complex, detected effects are usually provoked by multiple substances or stressors. In a study by Schäfer et al. (2016) it was found that 96.6% of the sites studied were exposed to more than one stressor. Assessing the risk originating from mixtures of chemicals requires knowledge about the environmental concentrations and toxicity of each of these chemicals individually and jointly. To assess the level of exposure and detect related effects in the aquatic environment, the most direct way is to identify chemical concentrations in water and sediment and observe how aquatic species respond to the presence of these contaminates in comparison to unimpaired reference sites. Such field assessments, however, face a number of challenges: Environmental systems are complex and abiotic and biotic stressors, not related to toxicants, contribute to the effects. Furthermore, interactions between stressors are possible and apart from direct effects also indirect effects can occur. If a predatory species in a food web is affected and its abundance reduced, this can result in increased abundances of their prey species, for instance. Effects can already occur after a single pulse exposure or only after long-term exposure and can appear on organism, population or community level. Furthermore, the above suggested exposure assessment via water and sediment sampling with subsequent chemical analysis, despite being expensive, reflects only the stressors at the time of sampling, and thus, depends on the timing of sampling.

To assess effects of long-term or infrequent toxicant exposure, aquatic species can be used as indicators of biological response (Holt and Miller 2011). Such bioindicators reflect the effects originating from combinations of chemicals and from multiple additional biotic and abiotic stressors (Holt and Miller 2011) on species integrated over time. When developing new bioindicators, combined exposure and effect assessments are conducted and the power of the bioindicators in explaining the observed exposure patterns in the field is determined. Suitable bioindicators can then be applied in environmental monitoring. They allow reducing the frequency of expensive and time consuming exposure assessment via chemical analyses. For freshwater ecosystems, macroinvertebrates – the group of invertebrate species visible with the naked eye which comprise insects, mollusks and crustaceans, among others – are used as bioindicators since decades. They possess several advantages as explained in Metcalfe (1989).

1.3. Trait based bioindicators

Aquatic communities consist of a variety of species with distinct physiological characteristics, behavior, ecological niches and functions – so called species traits. These comprise for example the way a species feeds (feeding type) or moves (migration ability), the ability to withstand high water flow (lotic or lentic), the habitat it prefers (habitat type), by which level of stress it is affected (physiological sensitivity) and can recover from stress pulses (e.g. generation time), to name a few. It has become widely acknowledged that considering such traits in biomonitoring has several advantages in comparison to traditional biomonitoring based on taxonomic information (Culp et al. 2011, van den Brink et al. 2011). Taxonomy based bioindication has the restriction that it is spatially dependent, as most species do not occur ubiquitously. As ecosystem functions at sites with comparable environmental parameters are often similar, the trait composition of communities – rather than the taxonomic community composition – can be extrapolated from one site to another (Culp et al. 2011). Furthermore, traits can help in identifying specific stressors in systems exposed to a multitude of stressors (Statzner and Beche 2010). Such stressor-specific traits can provide diagnostic information on causal relationships between observed exposure and effects. Thus, considering species' traits in biological communities, instead of solely relying on their taxonomy, renders trait based bioindicators more regionally independent (Schäfer et al. 2007, Schäfer et al. 2011b, von der Ohe et al. 2007) and more specific towards certain stressors. Therefore, it is of major importance to understand how a species responds to the exposure of toxicants or other stressors and how it recovers from it (van den Brink et al. 2010). Such information is usually determined in ecotoxicological studies. These are mostly laboratory based and establish dose-response relationships between single species and contaminants under controlled conditions. Such tests, conducted for the risk assessment of chemicals, have generated toxicity information, which is available via databases (e.g. ECOTOX database (US EPA)). Usually, the result is expressed as the concentration at which 50% of test organisms show sublethal (EC50) or lethal effects (LC50) (Calow and Forbes 2003).

A trait-based bioindication system that utilizes laboratory based toxicity information and further species trait information is the SPEAR system ("SPEcies At Risk"). Central element of SPEAR bioindicators is the physiological sensitivity of species towards chemicals. Selecting information on the sensitivity towards certain groups of chemicals, e.g. organics, renders the bioindicator specific towards this group of contaminants. By adding stressor related trait information, e.g. on generation time or migration ability, specificity can be

increased further (Liess and von der Ohe 2005) in cases of seasonal or infrequent exposure. Due to this specificity, SPEAR-type bioindicators are less influenced by confounding environmental stressors not related to the investigated traits (Liess et al. 2008, Liess and von der Ohe 2005, Schletterer et al. 2010). Existing SPEAR systems are $SPEAR_{pesticides}$ for pesticide exposure (Liess and von der Ohe 2005), $SPEAR_{organic}$ for organic toxicants in general (Beketov and Liess 2008), $SPEAR_{salinity}$ for salinity stress (Schäfer et al. 2011a) and $SPEAR_{habitat}$ for structural degradation of streams (von der Ohe and Goedkoop 2013).

Added mechanistic and diagnostic knowledge can, thus, be gained through trait based approaches, relying on the above described stressor-specificity. This specificity, however, is often restricted due to trait combinations (Bunzel et al. 2014). As traits often exist in sets, they also relate to overlapping, and thus, intercorrelating environmental parameters (Poff et al. 2006), which is often observed in the field (Bunzel et al. 2013). For this reason, traits may have similar explanatory power for confounding stressors even without causal relationship, rendering effect assessment difficult. For instance, Rasmussen et al. (2011) and Bunzel et al. (2013) observed an influence of habitat quality on $SPEAR_{pesticides}$, however, only under conditions of strong habitat degradation. Nevertheless, SPEAR bioindicators are quite robust towards minor changes in confounding environmental parameters. They are, furthermore, robust towards high abundances of single taxa, as abundances are expressed logarithmically in order to not overweigh high values.

1.4. Ecological risk assessment

Ecological risk assessment estimates the likelihood of detrimental effects in ecosystems resulting from the exposure to environmental stressors by linking the intensity of potential effects of stressors and the probability of occurrence of these stressors (Suter 2008). With regard to chemicals, the intensity of effects refers to the chemical's toxicity to single species and whole communities while the probability of occurrence refers to exposure patterns in the field. Toxicity information is obtained via a tiered approach, i.e. testing is classified into levels of different complexity and effort. While the first tier represents laboratory based toxicity tests with single species, in the second tier toxicity information for more than one test species is gathered. Examples for the latter are species sensitivity distributions (SSD) that determine a threshold concentration which is assumed to be protective for e.g. 95% of the species within a community. Higher tiers make use of micro- or mesocosm testing,

investigating population or entire community patterns, and eventually field studies. Higher tier systems are closer to reality and allow observing, for instance, indirect effects and biotic interactions (Fleeger et al. 2003). Concurrently, however, system complexity increases. As test effort increases from lower to higher tiers, toxicity data from higher tier systems is scarce. Figure 1 provides an excerpt of exposure and effect assessment approaches available for ecological risk assessment. For exposure assessment, examples of different tier levels are given.

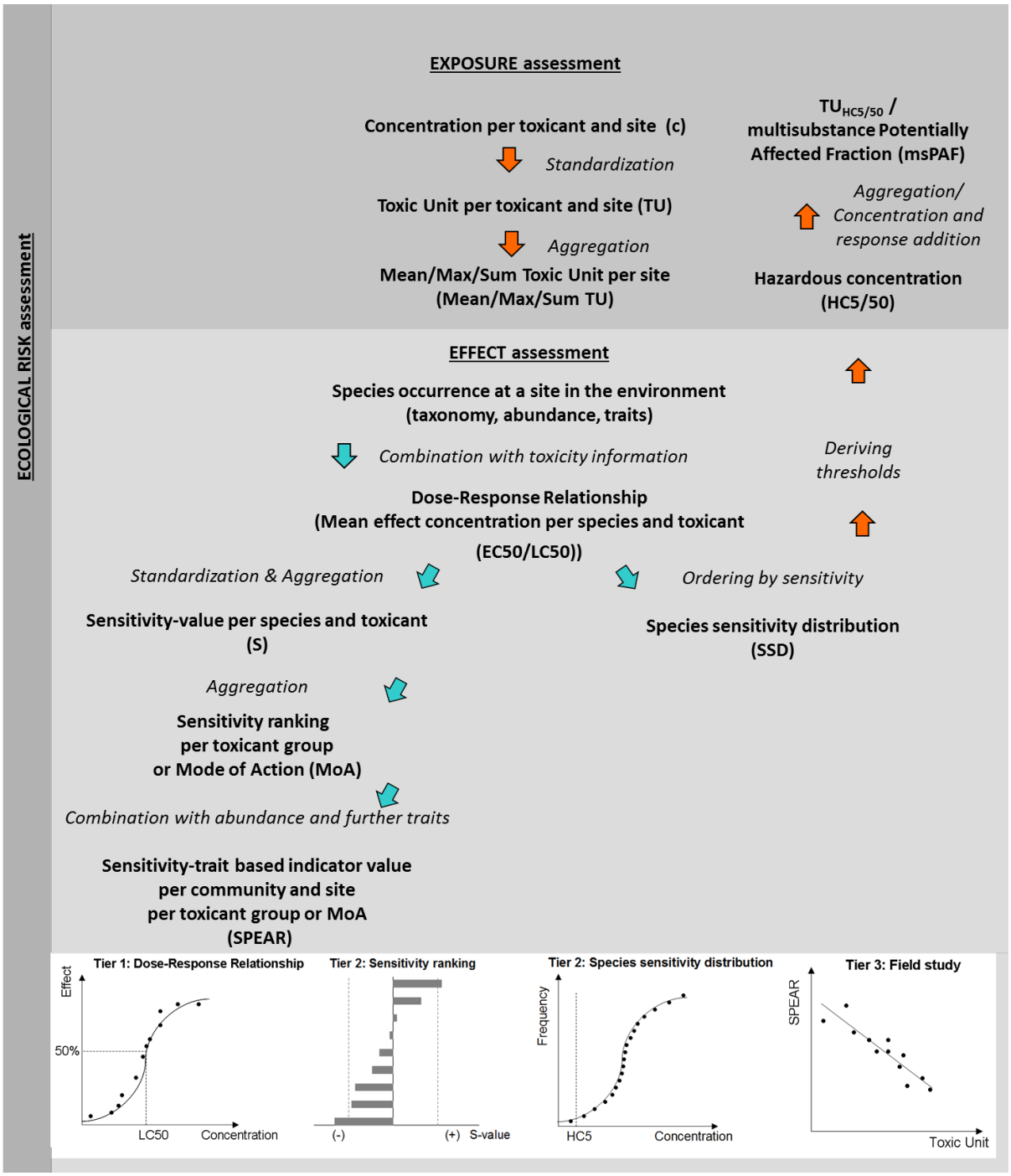

Figure 1. Scheme with an excerpt of available approaches on exposure and effect assessment on different tier levels that can be used for ecological risk assessment.

Effect assessment relies on the species assemblages encountered at a respective sampling site. Following the identification and abundance assessment of each taxon in the sampled macroinvertebrate community, the taxonomy and abundance information can be supplemented with trait information for each taxon – for instance the physiological sensitivity of a species towards certain toxicants. This information can be obtained from sensitivity (S) values that describe a species' physiological sensitivity towards a group of toxicants. $S_{organic}$ values are an example. They were developed for single taxa based on the LC50s of single organic toxicants to these species (Liess and von der Ohe 2005). These LC50s originate from dose-response relationships (Figure 1) determined in laboratory studies, as reported in literature and databases. As a way of standardization, the LC50 for each species-toxicant combination can be expressed in relation to the LC50 of the particular toxicant to a benchmark test organisms like the freshwater crustacean *Daphnia magna*. In a following step, for each species, the standardized LC50s of several toxicants are aggregated for groups of toxicants (e.g. organics), generating S-values. The S-values for all taxa in a community can be ordered by size, rendering a S-ranking for this community (Figure 1). S-rankings illustrate which species are most and least sensitive towards this group of toxicants. For SPEAR-type bioindicators, the S-values of those species occurring in a sampled community are applied to the log-transformed abundances of each species in that community. They can, additionally, be combined with further traits.

Another approach, alternative to the standardization of EC50/LC50 values with *D. magna*, is to arrange the respective effect concentrations (EC50/LC50) of single toxicants for each species of a community in ascending order, generating a cumulative density function graph. From such species sensitivity distributions (SSD, Figure 1), thresholds for acceptable concentrations in the environment can be derived. The environmental concentration at which 5% of the species are potentially affected (HC5), for instance, accepts the loss of 5% of the species. The information from SSD curves can again be aggregated across different toxicants and even across toxicants with different modes of action (MoA), allowing to derive a multisubstance potentially affected fraction of species (msPAF) (De Zwart and Posthuma 2005, Smetanová et al. 2014).

Environmental exposure assessment, on the other side, relies on a measured toxicant concentration in a stream, as depicted in Figure 1. If multiple toxicants occur at a sampling site, the concentrations of the single toxicants can be standardized, for instance, with regard to the respective lethal concentration of these toxicants to *D. magna.* In this way, the

concentration of each toxicant is reported in terms of its toxicity to one species and is expressed as Toxic Unit (TU). The TU approach (Sprague 1970) allows reporting mixed toxicity by summing up single TUs. Alternatively, the average or highest TU of all substances of the mixture can be determined.

1.5. Bioindicators in practice

The European WFD focusses not only on the concentration of pollutants or other stressors in the aquatic environment but also on the effects they generate. In that way, joint effects of the present stressors as well as the influence of other environmental parameters are acknowledged. The goal to be achieved by the WFD is for rivers and streams to reach the "good ecological status" or "potential" in terms of the quality of biological communities, hydrological characteristics, stream morphology and the chemical status. The assessment of the ecological status is, thus, a combination of chemical exposure and biological effect assessment. However, the measures of effect applied within the WFD are mainly taxonomy-based. The German Perlodes system (part of the ASTERICS software, Meier et al. 2006) evaluates macroinvertebrates as one of the five biological quality elements (being fish, invertebrates, diatoms, plants and phytoplankton) considered for the ecological status. The endpoints assessed, beside species abundances and community composition, are e.g. the abundance of EPT taxa as generalized representatives of sensitive species and the saprobic index as an indicator for organic pollution. The saprobic index can be considered a trait-related indicator. Habitat preferences resemble further trait information acknowledged in Perlodes. However, the trait of physiological sensitivity towards chemicals is not yet considered. Similarly, the sensitivity towards environmental stressors, for instance temperature, has not been considered until recently, when the LAWA working group empirically derived thresholds for sulphate, iron and temperature for macroinvertebrates based on species' tolerance ranges (Halle et al. 2015a, 2015b, 2016). Indicators were, subsequently, derived from these ranges (e.g. KLIWA indicator by Halle et al. 2016).

Similarly, this thesis aims at providing specific indicators for species' sensitivities towards chemicals and at demonstrating the applicability and usefulness of such indicators in environmental monitoring, as they might be of value for ongoing monitoring activities within the WFD. Until now, a vast number of actions has been undertaken to improve the ecological status of rivers and streams: organic loads have been reduced by improving the efficiencies of waste water treatment plants (WWTPs) and the morphological structure of many streams has

been restored or at least improved. Nevertheless, until now the good ecological status could be reached in only 8.2% of German water bodies (WasserBLicK/BfG 2016). Trait-based diagnostic tools could help in identifying prevalent stressors in multi-stressor systems, and thus, in informing about potential causes of degradation, which could be addressed by cause-directed restoration measures.

The lack of biomonitoring tools specific to single toxicants or toxicants groups can be attributed to the scarcity of toxicity information for a wide range of chemicals and species. To develop such specific tools, information from toxicity tests for a large number of species-toxicant combinations is required. The possibilities based on the current data availability are explored in this thesis. Furthermore, the option of supplementing the available data with additional information obtained from rapid or mesocosm testing is demonstrated. To date, no SPEAR-type bioindicators exist for the assessment of hydrocarbon and metal exposure, which was, thus, aimed at in this thesis. Based on the field assessments evaluated in this thesis, novel SPEAR-type bioindicators were developed, tested and validated for a later application.

1.6. Research questions and aim of the thesis

This thesis aimed at improving current approaches in exposure and effect assessment. On the exposure side, it was assessed which metrics for exposure assessment exist, how much explanatory power they have and if they are applicable in practice considering data availability. Our research aimed at identifying powerful metrics applicable in practice. With the hypothesis that the most sophisticated metrics are the most powerful, we conducted a comparative evaluation of exposure metrics using a set of field studies on pesticide contamination. More in detail, we compared currently applied exposure metrics for mixture toxicity with regard to their relationship with an effect metric for macroinvertebrate communities (**Chapter 2**). Thus, the thesis starts with a re-evaluation of a set of field studies by applying different exposure metrics for chemical mixtures. The chapter has the specific goal to give recommendation on how mixture toxicity can best be characterized and considered in environmental risk assessment. In this context, the quality of the often applied TU approach has been reconsidered in comparison to a set of more elaborate alternatives. The suitability of an aggregation of toxicants via standardization with *D. magna* is questioned by evaluating whether a bias is generated with this standardization. This could be due to unequal relative toxicities between *D. magna* and other species for different toxicants or toxicant

groups. Furthermore, it is assessed whether *D. magna* is a sufficiently sensitive species to represent toxic effects to entire aquatic invertebrate communities.

To this end, monitoring data of invertebrate communities and organic chemicals, mainly pesticides, from five studies in Europe and South-East Australia were re-analyzed. Nine exposure metrics were used for estimating the toxicity of the mixtures. The invertebrate communities were described with $SPEAR_{pesticides}$, which has proven suitable in many previous studies with pesticide exposure (Liess et al. 2008, Schäfer et al. 2012, von der Ohe and Goedkoop 2013). The relationship between all exposure metrics and $SPEAR_{pesticides}$ was determined.

The investigation is of high relevance, as it has been questioned whether *D. magna* is a sufficiently sensitive representative of aquatic communities concerning exposure towards different kinds of chemicals or whether inherent differences in toxicity between chemicals exist (Rubach et al. 2010). A comparison to alternative exposure metrics that do not standardize with *D. magna* has not been conducted to date. Previous studies have rather compared different metrics of effect than of exposure.

On the effect side, the main objective of this thesis was to develop bioindicators for specific pollutant groups, namely for oil sands-derived compounds (**Chapter 3**), hydrocarbons (**Chapter 4**) and metals (**Chapters 5**). **Chapter 3** reports on a field study that was conducted in the Canadian oil sands development region in Northern Alberta. Aquatic communities in this area are exposed towards a mixture of oil sands-derived chemicals mainly consisting of PAHs, metals and naphthenic acids (Kelly et al. 2010, Timoney 2007). The exposure pathways are diverse and can be natural or anthropogenic. Constant riverbed and shore erosion, similarly as groundwater flow into rivers, result in natural loading with bitumen or bitumen-derived substances in the streams flowing through the oil sands deposit. Additionally, anthropogenic sources related to oil sands development can contribute to the contamination in the streams. Aquatic species are exposed to these substances via contact with water and sediment or uptake of food (McElroy et al. 1989). Monitoring programs were put in place starting in 1997 but had several limitations (Main 2011). Thus, Environment Canada and the Province of Alberta started with an extended monitoring program in 2011 (Environment Canada 2011). Still, effective biomonitoring tools capable of identifying effects of toxicant exposure in the streams in the Athabasca region are needed.

In this study, we therefore aimed at developing a bioindicator capable of explaining species distribution in relation to exposure patterns – in this case fluctuating contamination levels of oil sands-derived organic toxicants. Thus, a SPEAR-type bioindicator was developed because it proved effective in the past (Liess et al. 2008, Schäfer et al. 2012, von der Ohe and Goedkoop 2013) and is not solely taxonomy based but considers trait information, rendering it regionally independent (Schäfer et al. 2007). To this end, combined exposure and effect monitoring was conducted in three consecutive years to unravel which toxicants are most relevant in shaping aquatic invertebrate communities. Exposure and effect patterns, thus, informed the selection of suitable species traits for a new bioindicator to improve future effect monitoring in the area.

The third study aimed at developing a physiological sensitivity ranking specific towards hydrocarbon contamination (**Chapter 4**) because sensitivity values for hydrocarbon contaminants, which could be used in SPEAR-type bioindicators, have not been derived yet. So far, SPEAR-type bioindicators have been based on information on species' sensitivity towards organic compounds in general ($S_{organic}$ values for $SPEAR_{pesticides}$ and $SPEAR_{organic}$) or salinity ($SPEAR_{salinity}$). We thus derived S-values from laboratory based information on hydrocarbon toxicity, using existing databases (ECOTOX database (US EPA)). This is challenging, as S-values are developed based on the LC50s of single toxicants for single taxa and LC50s are not available for all species-toxicant-combinations. Specifically for hydrocarbons, toxicity data is limited. Therefore, in this thesis, results from rapid test and mesocosm studies were added to the available laboratory toxicity studies. This extended database allowed deriving sensitivity values specifically reflecting hydrocarbon toxicity – at least for a set of taxa. A first validation of the ranking was conducted by re-evaluating a field study. Here, the newly generated $S_{hydrocarbons}$-values were applied as sole trait in a SPEAR-type bioindicator, here called $SPEAR_{hydrocarbons}$, following the approach for $SPEAR_{organic}$ (Beketov and Liess 2008).

Sensitivity values for metal contamination had already been developed (Malaj et al. 2012) but not yet applied to field datasets. Therefore, the last study investigated effect patterns caused by metal exposure and tested the S_{metal}-values in a SPEAR-type bioindicator (**Chapter 5**). However, as metal sensitivity has been shown to differ between laboratory and field exposure in various studies (Brix et al. 2011, Buchwalter et al. 2007, Clements et al. 2013), a simple

application of the S_{metal}-values in a SPEAR-type bioindicator without the acknowledgement of further traits, is not realistic. Thus, in addition to the physiological metal sensitivity, the trait feeding type was investigated. For this purpose, three datasets from intense metal mining areas in Australia and Tasmania were re-evaluated with regard to trait patterns.

1.7. References

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Chapter 2: "How to characterize chemical exposure to predict ecologic effects on aquatic communities?"

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2.1. Abstract

Reliable characterization of exposure is indispensable for ecological risk assessment of chemicals. To deal with mixtures, several approaches have been developed, but their relevance for predicting ecological effects on communities in the field has not been elucidated. In the present study, we compared nine metrics designed for estimating the total toxicity of mixtures regarding their relationship with an effect metric for stream macroinvertebrates. This was done using monitoring data of biota and organic chemicals, mainly pesticides, from five studies comprising 102 streams in several regions of Europe and South-East Australia. Mixtures of less than 10 pesticides per water sample were most common for concurrent exposure. Exposure metrics based on the 5% fraction of a species sensitivity distribution performed best, closely followed by metrics based on the most sensitive species and *Daphnia magna* as benchmark. Considering only the compound with the highest toxicity and ignoring mixture toxicity was sufficient to estimate toxicity in predominantly agricultural regions with pesticide exposure. The multisubstance Potentially Affected Fraction (msPAF) that combines concentration and response addition was advantageous in the study where further organic toxicants occurred. We give recommendations on exposure metric selection depending on data availability and the involved compounds.

2.2. Introduction

The characterization of chemical exposure in freshwater ecosystems is a crucial prerequisite for ecological risk assessments, but is hampered by practical and theoretical issues. Practically, it remains a challenge to sample and analyze all ecotoxicologically relevant substances that enter a water body (Schwarzenbach et al. 2006). Theoretically, even if a complete characterization of exposure would be obtained for a certain site, potential mixture effects and the limited availability of effects data for species in the target system render the assessment difficult (Strempel et al. 2012, Kortenkamp et al. 2009, Beketov and Liess 2012). Different approaches to (1) assess the risk from observed concentrations of chemicals and (2) deal with chemical mixtures have been developed. Toxic Units (TU) (Sprague 1970) are a relatively simple method to assess the risks from toxicant exposure for a group of organisms (e.g., invertebrates, plants, fish) and have been widely applied to standardize observed toxicant concentrations based on acute and/or chronic toxicity data from standard test

organisms (e.g., *Daphnia magna* and *Hyalella azteca* for invertebrates, *Pimephales promelas* for fish). While TUs are often calculated relative to one species only, they provide a benchmark for the toxicity to other parts of the community as long as the relative sensitivity of these organisms to a chemical remains similar (von der Ohe and Liess 2004). However, the use of TU for *D. magna* ($TU_{D.\ magna}$) has been criticized, as this species is not always the most sensitive species (Rubach et al. 2010). Equally relevant, cases exist where the relative sensitivity between *D. magna* and other aquatic invertebrates differs substantially among compounds. For example, compared to insects, *D. magna* exhibits much lower sensitivity to neonicotinoids and insect growth inhibitors (Brock and Wijngaarden 2012), while in general *D. magna* and other cladocerans tend to be more or similarly sensitive to other organic toxicants than many but not all freshwater insects (von der Ohe and Liess 2004).

Furthermore, species sensitivity distributions (SSDs) have been introduced to integrate acute and/or chronic toxicity data for several species into a concentration−effect relationship from which hazardous concentrations (HC_p) leading to effects on *p*% of species can be derived (Posthuma et al. 2002). This HC_p can be used in the TU approach to standardize the observed concentrations to a defined fraction of potentially affected species (*p*) instead of a particular species and could therefore provide a benchmark that is more robust to variations in the relative sensitivity of species. However, due to the scarcity of toxicity data, SSDs for different chemicals rely on differing sets of species, which may compromise the suitability of the derived HC_p to provide a consistent benchmark. Moreover, SSDs have been criticized because they rely on a number of assumptions that are generally not met, e.g., that the set of species used in a SSD is an unbiased sample of the target group of species or that the loss of any species is of equal ecological relevance (Forbes 2002). Consequently, for a given concentration the fraction of affected taxa in an ecosystem can differ from the estimated *p*% (Kefford et al. 2005).

By neglecting potentially synergistic effects between chemicals, concentration addition (CA) represents a conservative approach to deal with chemical mixtures (Kortenkamp et al. 2009). For TUs, CA corresponds to the sum of the TU (sumTU) of each chemical detected in a sample. By contrast, considering only the potential effects of the compound with the maximum toxicity and ignoring potential effects from all other compounds results in the so-called maximum TU (maxTU) indicating the minimum estimated toxicity of the most potent component of the mixture. This maxTU has successfully been applied to evaluate pesticide effects on stream macroinvertebrates and compared to the sumTU showed a similarly high association with macroinvertebrate-based effect metrics (Liess and von der Ohe 2005, Schäfer

et al. 2011). While CA relies on the theoretical assumption of the same mode of action (MOA), a second model of mixture toxicity, independent action, also called response addition (RA) and used here for consistency with De Zwart and Posthuma (2005), integrates effects from compounds with different MOAs (Kortenkamp et al. 2009). RA requires a concentration-response model, which is not available for the TU approach, but exists for SSDs. On theoretical grounds, a combination of CA and RA has been suggested for complex mixtures (Altenburger et al. 2004), and the multisubstance Potentially Affected Fraction (msPAF) was introduced (De Zwart and Posthuma 2005). The msPAF approach first applies CA to compounds with the same MOA and subsequently uses RA to aggregate the different MOAs. Previous studies examined effects of mixtures from different groups of organic and inorganic chemicals and found statistically significant associations with the abundances of 50% to 74% of taxa in communities (Posthuma and De Zwart 2006, 2012).

The aim of this study was to compare the relationship of different exposure metrics for summarizing the total toxicity of mixtures of organic chemicals (mostly pesticides) with an effect metric for stream macroinvertebrate communities. We used $SPEAR_{pesticides}$ (Liess and von der Ohe 2005), which indicates the fraction of pesticide-sensitive invertebrate taxa in a community based on their physiological sensitivity and biological traits such as generation time and dispersal capacity, as effect metric because it displayed a close relationship and high specificity to pesticide exposure in previous field studies from different regions of the world (Schäfer et al. 2012). We hypothesized that, due to the limited availability of ecotoxicological data, using $TU_{D.\ magna}$ would outperform SSD-based methods relying on toxicity data from differing sets of species. Moreover, based on previous studies we did not expect a relevant increase in predictive power for community effects from the consideration of mixture effects.

2.3. Material and methods

2.3.1. Study selection and description

We selected five studies for which data were available on an organic toxicant exposure gradient for multiple streams (>5) with concurrent macroinvertebrate community data that indicated effects of this exposure. Four of the five studies including their data have been described in a recent meta-analysis on thresholds for the effects of pesticides (Schäfer et al. 2012) and were complemented by a further study reporting effects of organic toxicants on macroinvertebrate communities (von der Ohe et al. 2009). The selected studies encompassed 14 to 28 streams in predominantly agricultural areas in different regions of Europe and in

South-East Australia (Table 1). In the following we refer to the individual studies with the country name, while the related freshwater ecoregions are given in Table 1. Between 10 and 153 individual organic compounds were measured and across all data sets 107 different compounds (Supporting Information (SI) Table S1) were detected. The toxicant exposure was expressed as $maxTU_{D.\ magna}$ (Equation 2) in these studies. The relationships between $maxTU_{D.\ magna}$ of the original studies and potential effects in terms of $SPEAR_{pesticides}$ were relatively high ($0.61 < r^2 < 0.89$) (Table 1) and was in some of the studies moderated by the availability of forested reaches upstream that may serve as landscape recolonization pools (Liess and von der Ohe 2005, Schäfer et al. 2007). The values for the effect metric ($SPEAR_{pesticides}$) were taken from the meta-analysis (Schäfer et al. 2012) and von der Ohe et al. (2009) and details on the calculation of this metric can be found therein. Furthermore, the chemical concentrations from the included studies were compiled and corrected for bioavailability by the total organic carbon (TOC) content based on the partitioning between water and organic carbon according to DiToro et al. (1991)

$$C_d = \frac{C_{\text{tot}}}{\left(f_{\text{OC}} K_{\text{OC}} + 1\right)} \qquad [1]$$

where Cd approximates the dissolved, bioavailable concentration, Ctot is the total concentration in the whole water sample, KOC is the dimensionless soil organic carbon−water partitioning coefficient, and fOC is the fraction of organic carbon that was approximated with the TOC content. We note that this correction may underestimate the ecotoxicologically active concentration since particle-adsorbed compounds can still exert toxic effects (Schulz and Liess 2001). Finally, the chemical concentrations were employed to calculate the different exposure metrics.

Table 1. Included Studies with Information on Number (No.) of Sites and Measured Compounds, Relationship between Exposure and Effect ($SPEAR_{pesticides}$) Metrics for Models Reported in the Original and in This Study

Region	No. of sites	Ecoregion[a]	No. of compounds measured	r^2 for relationship between exposure and effect metric		Model original/this study contained FUS[d]?	ref.
				Model original study[b]	Best-fit model this study[c]		
Brittany, France	16	Central and Western Europe	10	0.72	0.77	yes/yes	(Schäfer et al. 2007)
Central Germany	20	Central and Western Europe	21	0.75	0.73	yes/yes	(Liess and von der Ohe 2005)
Victoria, Australia	24	Bass Strait Drainages	97	0.68[e]	0.81[e]	no/yes	(Schäfer et al. 2011)
Denmark	14	Central and Western Europe	31	0.61	0.68	no/no	(Rasmussen et al. 2012)
Catalonia, Spain	28	Eastern Iberia	153	0.89	0.90	yes/no	(von der Ohe et al. 2009)

[a] According to Abell et al. (2008).
[b] $maxTU_{D.\ magna}$ was exposure metric.
[c] Exposure metric of best-fit model given in Table S3.
[d] Forested upstream sections.
[e] After removal of one outlier.

2.3.2. Calculation of exposure metrics

Four different metrics were employed to assess the exposure to organic toxicants: the $TU_{D.magna}$, the TU for the most sensitive organism for which toxicity data was available ($TU_{Sensitive}$), the TU based on the HC*p* from a SSD (TU_{HCp}) and the msPAF, also based on SSDs. The schematic calculation of metrics is displayed in Figure 1 and data and computer code for computation is given in the SI. Briefly, the TU for a chemical *i* is calculated as

$$TU_i = \frac{c_i}{LC50_{i,j}} \quad [2]$$

where *c* is the concentration measured in the environment and LC50*i*,*j* the median lethal concentration for species *j*, which is *D. magna* in case of $TU_{D.\ magna}$ and the most sensitive species in the case of $TU_{Sensitive}$, i.e., the species with lowest available LC50. To compute TU_{HCp}, the LC50 was substituted by HC*p* in Equation 2. We calculated the HC_5 and HC_{50} based on a SSD for each compound, which represent the concentrations potentially affecting 5% and 50% of the tested species, respectively. The SSDs were computed for individual

compounds with a minimum of 6 data points (i.e., species; see below for rationale) assuming a log-normal model. Note that we did not examine other model types (e.g., Weibull, Probit) for the individual SSDs (Ritz 2010) due to (1) the large number of compounds in our data set and (2) the statistical properties of the log-normal distribution that simplified computation of msPAF (De Zwart and Posthuma 2005). The slopes of the resulting SSDs were averaged per MOA for msPAF calculation, after assigning each compound to one of four MOAs (SI Table S1), depending on whether it affected (1) acetylcholinesterase, (2) the sodium channel, or (3) the electron transport chain or acted as (4) narcotic. We separated (1) and (2) following Stenersen (2004) and since the slopes were statistically significantly different (Welch two sample *t* test, $p = 0.014$). Four (cyanide, propargite, spinosynd, tebufenozide) of the 107 compounds that could not be assigned to any of these MOAs and that were ecotoxicologically negligible with respect to their concentrations were omitted in the calculation of all exposure metrics. The minimum requirement of 6 data points for SSD calculation was selected to provide robust estimates of the mean slope per MOA (see SI Figure S1), which is in agreement with results from another study (van Zelm et al. 2007). If less than 6 data points were available for a compound, which was the case for 72 of 103 compounds (SI Table S1), the average of the available data points was used as SSD midpoint (= HC_{50}) and combined with the mean slope of the related MOA to derive the HC_5. Note that the requirement of a minimum of 6 data points represented a compromise between the uncertainty related to the construction of individual SSDs from few data points and the uncertainty related to assigning the mean slope, but more data points are usually required to derive precise estimates of the HC_p for regulatory risk assessment (Newman et al. 2000).

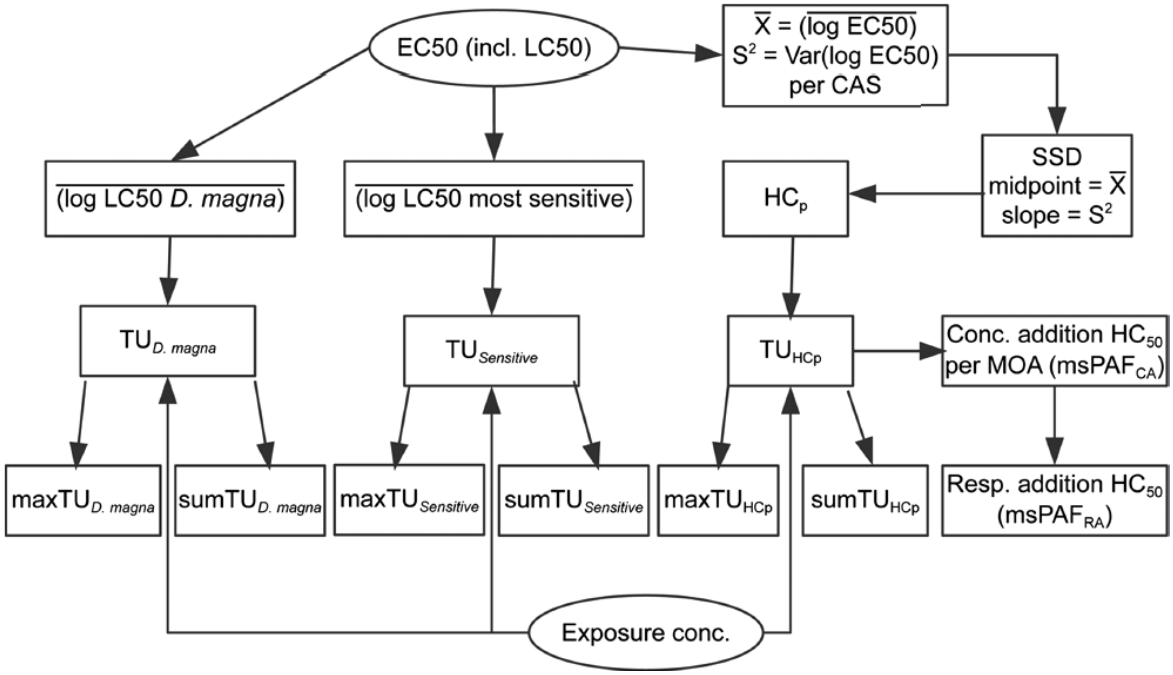

Figure 1. Schematic overview on the calculation of the exposure metrics. See paragraph "Calculation of exposure metrics" for explanation of the acronyms. An overline indicates the mean for the respective variable, Var refers to the variance. Conc. = Concentration; Resp. = Response.

For all exposure metrics different end points regarding chemical mixtures were computed including the sumTUs based on the CA approach and the maxTU based only on the single compound exhibiting the maximum expected toxicity as outlined above (Figure 1). Note that calculation of the sumTU for $TU_{Sensitive}$ and HC_p can lead to summation of effects related to different species (or sets of species), but was done for sake of completeness. Moreover, for 85 of 103 compounds a crustacean was the most sensitive species, thus related species would be pooled in most cases. For calculation of msPAF, in the first step, the TU_{HC50} of all compounds with the same MOA *k* were added up based on the CA approach. Subsequently, the estimated response ($msPAF_{CA,k}$) was derived using the mean slope related to *k*. These $msPAF_{CA,k}$ were then employed to compute $msPAF_{RA}$ based on RA:

$$msPAF_{RA} = 1 - \prod\left(1 - msPAF_{CA,k}\right) \quad [3]$$

where *k* = 1 to n different MOAs. In our study *n* = 4 since there were 4 different MOAs (SI Table S1). Note two limitations in our study with respect to the original protocol by De Zwart and Posthuma (2005) for calculation of $msPAF_{RA}$. Their protocol suggested assigning a new MOA when slopes of compounds with the same MOA would deviate by >10%. We classified compounds into 4 broad MOAs to guarantee availability of a sufficient number of compounds per MOA. Strict application of the protocol would have resulted in several MOAs with only

one or a few compounds to compute the slope of the related MOA. Given that for approximately 70% compounds no SSD could be computed and a mean slope of the related MOA was assigned, we decided to estimate this slope based on a high number of compounds to yield more robust estimates (van Zelm et al. 2007). Still only 13, 10, 7, and 1 compounds were available for calculation of the mean slope for the MOAs narcotic, acetylcholinesterase, sodium channel, and electron transport chain, respectively. Moreover, in the case of non-narcotic MOA, the protocol suggested to include in the SSD for a compound only taxa, which are known to respond to the specific MOA of this compound. Again, this rule was not adapted due to the low number of available data points (i.e., species) per compound for SSD calculation. However, we restricted the input data for SSDs to freshwater invertebrates, and since the effect metric was related to the freshwater macroinvertebrate community, this should be a minor issue.

The exposure metrics were calculated per site for each of the included studies (Table 1). As for the original studies, if different sampling methods or sampling dates for a site were available, the maximum exposure metric for this site was used in later analysis, based on the rationale that the highest toxic event determines the community effect (Schäfer et al. 2011). For 8 of 102 sites without quantifiable detections, no exposure metrics could be calculated and they were set to 1/10 of the minimum value for the related metric in that study.

2.3.3. Processing of acute toxicity data

Acute toxicity data for 48, 72, and 96 h exposure periods (for sources, see SI Text S1) were restricted to the taxonomic phyla of invertebrates in freshwater ecosystems. Only studies with LC50 as well as the median effect concentration (EC50) for the end points mortality or immobility were selected. All effect concentrations were converted into µg/L and the median was calculated for replicates (being defined as same species + compound + exposure duration + reference). Subsequently, this preprocessed toxicity data was limited to the 103 included compounds (SI Table S1), and was complemented by baseline calculations for compounds with missing toxicity data (SI Text S1). The whole data set was further processed applying the following rules: data for the shortest exposure period were selected if data from different exposure periods for the same species−compound combination were available; data for the same species−compound−exposure period combination were excluded as outliers if they differed by a factor of >30 from the group mean. In addition, the water solubility, the baseline toxicity, and toxicity data for closely related taxa were considered to check the plausibility of individual toxicity values. Due to the inherent variability in the data, no correction for

differences in the exposure periods was applied. If multiple data points per species−compound combination were available, the mean was calculated after log-transformation. Before TU calculation (Equation 2), the data was back-transformed using the antilogarithm.

2.3.4. Data analysis

Before data analysis, all exposure metrics were log-transformed. The intercorrelation and the relationship between the newly calculated and the original $maxTU_{D.\ magna}$ were checked using Pearson's correlation coefficient *r*. The performance of the exposure metrics when employed to explain ecological effects was examined by establishing separate linear models with $SPEAR_{pesticides}$ as response variable. Given that the selected studies reported mediation of toxicant effects by forested upstream sections (FUS) as defined in the original studies (Table 1), for each exposure metric two models with and without the variable FUS were built. This yielded a total of 90 models (5 countries × 9 exposure metrics × 2 levels for FUS), which were evaluated based on r^2 and Bayesian information criterion (BIC). No indication of a nonlinear relationship was found during visual checking of all models. The Wilcoxon rank-sum test on the BIC was used to decide on the inclusion of the variable FUS in the final model separately over all models from each study. Based on the Wilcoxon ranksum tests, the final models with or without FUS were selected and the models ranked per country based on the BIC. Subsequently, the ranks for each exposure metric were summed across countries irrespective of whether the model included the variable FUS. The lowest rank indicated the exposure metric with the lowest BICs across all countries. Furthermore, in accordance with Burnham and Anderson (2002) all models with a difference of ≤2 to the BIC of the best-fit model in terms of lowest BIC per country were selected and counted across all countries, again ignoring the variable FUS. Finally, we selected the best-fit model in terms of BIC for each country and compared the explained variance (r^2) to that of the original model of the respective study to explore potential improvements in the relationship between the exposure and effect metric. All computations and graphics were done using R (R Development Core Team 2013) and we provide the full code and all data except for the Australian study (SI) to enable reproducible research (Barnes, 2010).

2.4. Results

Fifty percent of the samples contained 2 to 6 individual compounds at quantifiable concentrations in Australia and Denmark, 4 to 7 in France, 2 to 4 in Germany, and 1 to 8 in Spain (Figure 2). The TU-based exposure metrics exhibited a high intercorrelation ($0.9 \leq r \leq 0.99$), whereas r was slightly lower for the correlation of $msPAF_{RA}$ with these metrics and ranged from 0.82 to 0.94 (SI Table S2). Similarly, there was a very high correlation (all $r \geq 0.96$) between the newly calculated $maxTU_{D.\ magna}$ and the $maxTU_{D.\ magna}$ reported in the original studies (SI Figure S2).

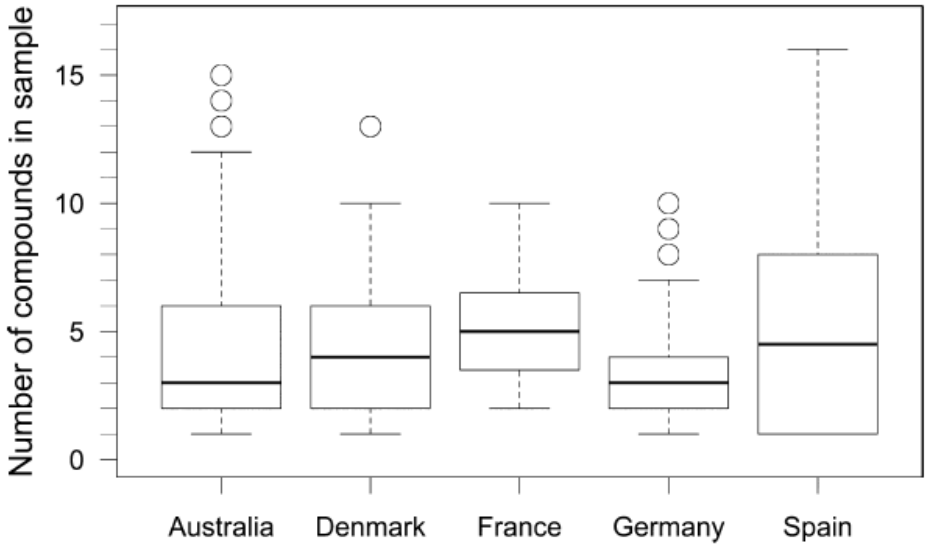

Figure 2. Box-and-whisker plot (Tukey 1977) of the number of compounds above the limit of quantification found in the different studies per water sample. Note the different number of sampled sites, sampling techniques, limits of quantification and measured compounds per study (Table 1).

The relationship between the exposure metrics and the effect metric $SPEAR_{pesticides}$ exhibited the lowest BIC when FUS were included as variables for Germany, France, Australia, and Spain (Table 3). Hypothesis testing indicated statistical significance of the inclusion of FUS for France and Australia and of the exclusion for Denmark (Wilcoxon rank sum test, all $p < 0.05$), whereas no statistical differences between the ranks of models with and without FUS were found for Germany ($p = 0.11$) and Spain ($p = 0.86$). Nevertheless, 6 of the 8 models with the lowest BIC for Germany contained the variable FUS (Table 3) and we therefore included this variable in the final models. Across all countries, the two metrics $TU_{Sensitive}$ and TU_{HC5} had the lowest ranks for the BICs and accounted for the best-fit models for all countries except Spain, where $msPAF_{RA}$ performed best (Table 2, Table 3). For Australia and Denmark the best-fit models involved sumTU, for Germany and France maxTU and for Spain msPAF

(Table 3). The models within a BIC range of 2 to the best-fit model exhibited a maximum reduction in r^2 of 0.04 for Denmark, and ≤0.02 for all other countries. The $TU_{Sensitive}$, TU_{HC5}, and $sumTU_{D.\ magna}$ were among these models for ≥3 of the 5 countries (Table 2). The best-fit model for the newly calculated metrics improved the relationship with the effect metric $SPEAR_{pesticides}$ by 1% to 13% in terms of explained variance, except for Germany with a 2% reduction in r^2 (Table 1).

Table 2. Rank Sums Across Countries and Selection for Set of Best Models for the Different Exposure Metrics[a]

Exposure metric	Rank sum of metric across countries	Number of times metric was among models within a range of 2 to BIC of best-fit model
$sumTU_{HC5}$	14	4
$sumTU_{Sensitive}$	18	3
$maxTU_{HC5}$	21	3
$maxTU_{Sensitive}$	21	3
$maxTU_{D.magna}$	22	2
$sumTU_{D.magna}$	26	3
$msPAF_{RA}$	32	1
$maxTU_{HC50}$	35	1
$sumTU_{HC50}$	36	1

[a] Results for the same exposure metric were pooled irrespective of whether the model included the variable "forested upstream sections".

2.5. Discussion

2.5.1. Composition of toxicant mixtures

The present results showed that, despite several hundreds of currently used pesticides in agriculture, mixtures of less than 10 pesticides for a water sample seem most common for the concurrent exposure of freshwater ecosystems in agricultural regions. Thus, although the studies were very different in terms of sampling, number of measured compounds, limits of quantification, and sampling intervals (Table 1), they yielded a remarkably similar number of compounds per water sample with 75% of samples having ≤7 compounds detected at quantifiable concentrations (Figure 2), except for Spain. However, the Spanish study involved further organic toxicants in addition to pesticides because the sites were not limited to mainly

agricultural influences as for the other studies. Our results are in agreement with a study on 83 pesticides in agricultural streams of the US that found 2 to 10 compounds in most water samples (Belden et al. 2007a). Less information is available for tropical regions, but a study on 11 streams in tropical northeast Australia similarly detected up to 10 and an average of 4 pesticides in event-driven water samplers (Smith et al. 2012, Kefford 2013).

2.5.2. Is *Daphnia magna* a sufficiently good benchmark for toxicity?

The results refuted our hypothesis that $TU_{D.\ magna}$ would outperform methods based on SSDs, because TU_{HC5} was ranked as best metric and was selected most frequently among the best-fit models (Table 2). The results of individual countries showed that either TU_{HC5} (in Australia), $TU_{Sensitive}$ (Denmark, France and Germany), or $msPAF_{RA}$ (Spain) performed best in terms of BIC (Table 3). Given that the SSDs were not checked individually for more appropriate models than the log-normal model (Ritz 2010), the SSD-based exposure metrics might still be enhanced (Newman et al. 2000). Nevertheless, compared to the best $TU_{D.magna}$ model, i.e., irrespective of max or sum and FUS, the best fit model improved the explained variance (r^2) only by 1% to 4% (Table 3), except for France (+8%). Moreover, the low performance of the TU_{HC50}, which was calculated as the mean of all toxicity data for a compound, demonstrates that indeed different sets of compounds used in SSD calculation can increase the noise. Finally, SSDs require model fitting and are often limited by the available toxicity data, whereas the $TU_{D.magna}$ relies on much simpler calculus and is less restricted by data limitations, since *D. magna* belongs to the most tested species. However, these characteristics hold as well for the $TU_{Sensitive}$, which outperformed $TU_{D.magna}$ (Table 2), despite the fact that for 58% of the compounds *D. magna* was also the most sensitive species (SI Table S3). This was largely because for 46% of compounds *D. magna* was the only freshwater invertebrate tested. For the 56 compounds where multiple freshwater invertebrates were tested, in more than 75% and 30% of these cases (43 and 18 compounds) another species was more sensitive and >1 log unit more sensitive than *D. magna*, respectively (SI Figure S3, Table S3). Hence, our results support the criticism (Rubach et al. 2010) regarding $TU_{D.magna}$ that depending on the mode of action of the compound *D. magna* is not always the most sensitive species. Nevertheless, the differences in r^2 between the best $TU_{D.magna}$ and $TU_{Sensitive}$ model were <4%, except for France and Spain with 8% higher $TU_{Sensitive}$ and $TU_{D.magna}$ models, respectively (Table 3). This ambiguous result is probably due to individual compounds, for which either *D. magna* is not sensitive, e.g., neonicotinoids or insect growth regulators (Brock and

Wijngaarden 2012), or for which the most sensitive species differ too much in their sensitivity to be a reliable benchmark for community effects. Moreover, if more toxicity data became available, this might increase the differences between effect metrics, which currently all heavily rely on *D. magna*. Overall, our study shows that the TU_{HC5} provides the most reliable exposure metric for streams draining agricultural catchments, but under data or resource constraints, both $TU_{Sensitive}$ and $TU_{D.magna}$ could be applied.

Table 3. Goodness of Fit Measures (r^2 and BIC) for the Different Exposure Metrics Used in the Linear Models with $SPEAR_{pesticides}$ as Response Variable for the Different Countries Sorted by BIC[a]

Country	Explanatory variables in model	r^2	BIC
Australia	$sumTU_{HC5}$ + FUS	0.69	-31.4
Australia	$maxTU_{HC5}$ + FUS	0.69	-31.2
Australia	$maxTU_{D.magna}$ + FUS	0.67	-29.9
Australia	$sumTU_{D.magna}$ + FUS	0.67	-29.7
Australia	$sumTU_{Sensitive}$ + FUS	0.63	-26.8
Australia	$maxTU_{Sensitive}$ + FUS	0.62	-26.5
Australia	$maxTU_{HC50}$ + FUS	0.62	-26.4
Australia	$msPAF_{RA}$ + FUS	0.61	-25.9
Australia	$sumTU_{HC50}$ + FUS	0.61	-25.5
Australia	$sumTU_{HC5}$	0.53	-24.6
Australia	$maxTU_{HC5}$	0.53	-24.5
Australia	$maxTU_{D.magna}$	0.47	-21.5
Australia	$sumTU_{D.magna}$	0.45	-20.6
Australia	$sumTU_{Sensitive}$	0.42	-19.2
Australia	$maxTU_{Sensitive}$	0.40	-18.4
Australia	$maxTU_{HC50}$	0.30	-14.9
Australia	$msPAF_{RA}$	0.30	-14.8
Australia	$sumTU_{HC50}$	0.24	-13.0
Denmark	$sumTU_{Sensitive}$	0.68	-40.5
Denmark	$sumTU_{HC5}$	0.67	-40.3
Denmark	$sumTU_{HC50}$	0.66	-39.9
Denmark	$maxTU_{Sensitive}$	0.66	-39.7

Denmark	$sumTU_{Sensitive}$ + FUS	0.71	-39.2
Denmark	$maxTU_{HC5}$	0.64	-39.1
Denmark	$sumTU_{D.magna}$	0.64	-38.9
Denmark	$maxTU_{HC50}$	0.63	-38.7
Denmark	$sumTU_{HC50}$ + FUS	0.69	-38.3
Denmark	$maxTU_{Sensitive}$ + FUS	0.68	-38.2
Denmark	$sumTU_{HC5}$ + FUS	0.68	-38.0
Denmark	$maxTU_{D.magna}$	0.61	-37.9
Denmark	$sumTU_{D.magna}$ + FUS	0.66	-37.0
Denmark	$maxTU_{HC5}$ + FUS	0.64	-36.5
Denmark	$maxTU_{HC50}$ + FUS	0.64	-36.4
Denmark	$maxTU_{D.magna}$ + FUS	0.62	-35.5
Denmark	$msPAF_{RA}$	0.49	-34.1
Denmark	$msPAF_{RA}$ + FUS	0.57	-33.9
France	$maxTU_{Sensitive}$ + FUS	0.77	-23.2
France	$sumTU_{Sensitive}$ + FUS	0.75	-22.4
France	$maxTU_{Sensitive}$	0.68	-21.2
France	$sumTU_{Sensitive}$	0.66	-20.1
France	$maxTU_{D.magna}$ + FUS	0.69	-18.5
France	$sumTU_{D.magna}$ + FUS	0.68	-18.4
France	$msPAF_{RA}$ + FUS	0.66	-17.5
France	$maxTU_{HC5}$ + FUS	0.63	-15.7
France	$sumTU_{HC5}$ + FUS	0.62	-15.6
France	$msPAF_{RA}$	0.53	-14.9
France	$sumTU_{HC50}$ + FUS	0.60	-14.7
France	$maxTU_{HC50}$ + FUS	0.60	-14.6
France	$maxTU_{D.magna}$	0.49	-13.5
France	$maxTU_{HC5}$	0.48	-13.4
France	$sumTU_{D.magna}$	0.48	-13.3
France	$sumTU_{HC5}$	0.48	-13.1
France	$sumTU_{HC50}$	0.44	-12.1
France	$maxTU_{HC50}$	0.44	-12.1

Germany	$maxTU_{Sensitive}$ + FUS	0.73	-34.6
Germany	$sumTU_{HC5}$ + FUS	0.72	-34.2
Germany	$sumTU_{Sensitive}$ + FUS	0.72	-34.1
Germany	$maxTU_{D.magna}$ + FUS	0.72	-33.8
Germany	$maxTU_{Sensitive}$	0.67	-33.7
Germany	$sumTU_{HC5}$	0.67	-33.7
Germany	$maxTU_{HC5}$ + FUS	0.71	-33.5
Germany	$sumTU_{D.magna}$ + FUS	0.71	-33.5
Germany	$sumTU_{Sensitive}$	0.66	-33.1
Germany	$maxTU_{D.magna}$	0.66	-33.1
Germany	$sumTU_{D.magna}$	0.66	-32.9
Germany	$maxTU_{HC5}$	0.65	-32.8
Germany	$maxTU_{HC50}$ + FUS	0.67	-30.9
Germany	$sumTU_{HC50}$ + FUS	0.67	-30.5
Germany	$msPAF_{RA}$ + FUS	0.67	-30.5
Germany	$maxTU_{HC50}$	0.59	-29.5
Germany	$sumTU_{HC50}$	0.59	-29.3
Germany	$msPAF_{RA}$	0.57	-28.3
Spain	$msPAF_{RA}$ + FUS	0.92	-75.7
Spain	$msPAF_{RA}$	0.90	-73.0
Spain	$sumTU_{HC5}$	0.90	-72.2
Spain	$sumTU_{HC5}$ + FUS	0.90	-69.7
Spain	$maxTU_{HC5}$	0.89	-68.4
Spain	$maxTU_{HC5}$ + FUS	0.89	-67.2
Spain	$maxTU_{D.magna}$	0.86	-61.9
Spain	$maxTU_{D.magna}$ + FUS	0.86	-59.0
Spain	$maxTU_{HC50}$	0.83	-57.8
Spain	$maxTU_{HC50}$ + FUS	0.84	-55.4
Spain	$sumTU_{D.magna}$	0.81	-54.1
Spain	$sumTU_{D.magna}$ + FUS	0.81	-51.6
Spain	$sumTU_{Sensitive}$	0.78	-49.6
Spain	$sumTU_{Sensitive}$ + FUS	0.80	-49.1

Spain	$maxTU_{Sensitive}$ + FUS	0.76	-44.9
Spain	$sumTU_{HC50}$	0.73	-44.4
Spain	$maxTU_{Sensitive}$	0.72	-43.1
Spain	$sumTU_{HC50}$ + FUS	0.75	-43.0

[a] FUS = forested upstream sections.

2.5.3. How should mixture toxicity be considered?

The differences between maxTU and sumTU were negligible both in terms of explained variance between best maxTU and sumTU models per country (<2% for all, Table 3) and in terms of counts, where sumTU and maxTU were 11 and 9 times among the best models across countries (Table 2). Moreover, for all metrics the according sumTU and maxTU were extremely highly correlated (all r = 0.99, SI Table S2). Furthermore, in 25 of 34 sites where acutely toxic concentrations occurred ($TU_{Sensitive}$ > 0.1, (Van Wijngaarden et al. 2005)), the $maxTU_{Sensitive}$ accounted for ≥87% of toxicity in terms of $sumTU_{Sensitive}$ (Figure S4). This is in agreement with a recent review of ecotoxicological mesocosm studies concluding that "the effects are mostly no larger than those of the most toxic substance" (Verbruggen and Van den Brink 2010). Our results are not in contrast with previous reviews highlighting the applicability of mixture toxicity models (i.e., CA and RA) for prediction of pesticide toxicity (Kortenkamp et al. 2009, Belden et al. 2007b, Coors and Frische 2011). They rather show that in agricultural regions the toxic effects are mainly driven by a single compound and consequently maxTU is often sufficient to predict toxicity on stream macroinvertebrate communities.

Despite $msPAF_{RA}$ relying on the most sophisticated theoretical grounds, it was only superior for the Spanish data with 2% and 0.3% gain in *r2* with and without FUS (Table S3). Although the improvement was minimal, it may result from mixtures of both pesticides and nonagricultural organic toxicants occurring in Spain, whereas in the other countries pesticides were the only relevant organic toxicants. Given that the SSD-based TU_{HC5} was the most reliable exposure metric, the lower performance of $msPAF_{RA}$ in the other countries seems not due to our simplified SSD approach, but can be explained by the higher noise associated with the HC_{50} on which msPAF relied and by accounting for specific MOAs. The msPAF is advantageous if inorganic and organic toxicants have to be considered, and future studies should investigate whether msPAF outperforms other methods under exposure of different classes of organic toxicants.

2.5.4. Potential limitations and outlook

(1) Calculation of exposure metrics

There were only minor differences between the $maxTU_{D.\ magna}$ calculated in the current study and those calculated in the original studies (SI Figure S2). These differences are due to newer data for this study and the fact that the original studies often used data sources such as the pesticide manual (Tomlin 2003) or the Pesticide Properties Database (FOOTPRINT 2006), which give only one acute toxicity value per compound based on data quality considerations. By contrast, we calculated the mean in the case of multiple values per species−compound combination owing to the large number of compounds and toxicity data, though the plausibility of individual values was checked. Since our best-fit models in most cases improved the relationship with the effect metrics (Table 1), this justifies the automated approach we employed. Nevertheless, for individual compounds a more thorough quality check of the input toxicity data might still lead to improvements.

(2) Measuring effects with $SPEAR_{pesticides}$

It could be argued that our results are restricted to the effect metric $SPEAR_{pesticides}$, which may be biased and not truly represent community change. However, several studies found $SPEAR_{pesticides}$ more indicative of pesticide-induced community change than other commonly used metrics (Liess et al. 2008, von der Ohe and Goedkoop 2013). Moreover, $SPEAR_{pesticides}$ showed high discriminatory power to non-toxicity gradients (Schäfer et al. 2011) and to our knowledge is the only metric that has been successfully validated for detecting pesticide stress across ecoregions (Schäfer et al. 2012). Finally, the effects detected by this metric have been shown to translate into losses of regional biodiversity (Beketov et al. 2013).

An alternative approach to effect metrics is multivariate statistical methods for biotic community data, but these entail the risk that a high association of an exposure metric with one of the many non-toxicity gradients present in community data, would be falsely interpreted as reliable exposure metric. In fact, the variation in communities due to toxicants can be very low compared to other non-toxicity gradients (Szöcs et al. 2012). Thus, a simulation model of toxicant-impaired ecological communities with a strong and known toxicity gradient would be needed, but such models are scarce (Hurst et al. 2008). We thus argue that $SPEAR_{pesticides}$ is currently the most suitable effect metric to evaluate toxicity exposure metrics, but we provide data and computer code so that the results can be scrutinized using other approaches.

(3) Data availability and effect thresholds

Our findings depend on the available toxicity data for freshwater invertebrates, and if more toxicity data for species other than *D. magna* become available, the superiority of exposure metrics such as $TU_{Sensitive}$ or TU_{HCp} could increase. Moreover, the msPAF approach could benefit from a finer consideration of different MOA and limiting SSDs to taxa specifically affected. Despite criticism on the over-reliance of ecotoxicology on a few test species over several decades (Cairns Jr. 1986), *D. magna* was the only tested freshwater invertebrate for almost half of the compounds in this study. This situation is not likely to change soon, unless testing methods are adopted that are specifically designed to accelerate the testing of many species (Kefford et al. 2005) and access to existing toxicity data is improved (Schäfer et al. 2013).

If exposure metrics based on species other than *D. magna* were more widely employed, this would pose the question of adaptation of effect thresholds. Currently, regulatory effect thresholds such as the first tier of the European Union Uniform Principles for the authorization of pesticides are partly defined with respect to standard toxicity tests with *D. magna* (EC 2002). Furthermore, reviews and meta-analyses have suggested effect thresholds for freshwater ecosystems with a strong focus on *D. magna* as benchmark organism (Van Wijngaarden et al. 2005, Schäfer et al. 2012). Hence, future studies should examine whether the formerly derived effect thresholds still apply for exposure metrics based on SSDs or other species than *D. magna*.

2.6. Acknowledgements

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Chapter 3: "Stream invertebrate community structure at Canadian oil sands development is linked to concentration of bitumen-derived contaminants"

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3.1. Abstract

In Canada, the Athabasca oil sands deposits are a source of bitumen-derived contaminants, reaching the aquatic environment via various natural and anthropogenic pathways. The ecological effects of these contaminants are under debate. To quantify the effects of bitumen-derived contaminants we monitored the aquatic exposure of PAHs, metals, and naphthenic acids as well as the invertebrate community in the Athabasca River and its tributaries. PAH concentrations over 3 consecutive years were related to discharge and were highest in the year with high autumn rainfall. In the year with the highest PAH concentrations, these were linked with adverse effects on the aquatic invertebrate communities. We observed relative effects of the composition and concentration of contaminants on the invertebrate fauna. This is reflected by the composition and abundance of invertebrate species via the use of the species' traits "physiological sensitivity" and "generation time". Applying the SPEAR approach we observed alterations of community structure in terms of an increased physiological sensitivity and a decrease of generation time for the average species. These effects were apparent at concentrations 100 times below the acute sensitivity of the standard test organism *Daphnia magna*. To rapidly identify oil sands related effects in the field we designed a biological indicator system, $SPEAR_{oil}$, applicable for future routine monitoring.

3.2. Introduction

Canada has the third largest petroleum reserves in the world, with 95% of this located in the province of Alberta, the Athabasca oil sands deposit being the largest reserve.

Via various natural and anthropogenic pathways, the input of bitumen-derived contaminants from oil sands deposits to the aquatic environment occurs. Naturally, the Athabasca River and its tributaries have incised into the bitumen deposit, the McMurray Formation, and therefore, they constantly receive a natural loading of bitumen or bitumen-derived substances by riverbank and riverbed erosion. Additionally, natural groundwater flow into rivers, including into the main stem Athabasca River, is known to contain bitumen-derived organic acids including naphthenic acids (NAs)) (Headley and McMartin, 2004, Ross et al. 2012). Neither the magnitude of the flux nor the toxicity of these natural bitumen sources to the river are known.

Anthropogenic sources also contribute to the contamination of surrounding aquatic systems with bitumen or bitumen-derived substances. In the Athabasca oil sands deposit, surface

mining activities as well as in situ extraction facilities are located. Accordingly, anthropogenic sources include run-off or wind-blown dust from surface mining and land disturbance, seepage from tailings ponds (Frank et al. 2014), volatilization and regional deposition from upgraders and tailings ponds (Kelly et al. 2009, Parajulee and Wania 2014). Surface deposition is often washed into aquatic systems by run-off (Parajulee and Wania 2014). Accidental pipeline spills cause input of oil constituents (Gosselin et al. 2010). Anthropogenic emission of airborne particles increases concentrations of soluble and particulate polycyclic aromatic hydrocarbons (PAH) in water and snow (Kelly et al. 2009).

The natural and anthropogenic input pathways are spatially overlapping in the investigated region because the main surface mining activities are concentrated where the Athabasca River and its tributaries are transporting naturally eroding material from the McMurray Formation (Alberta Geological Survey 2014, Hein and Cotterill 2006). A differentiation between pathways is challenging as both natural and anthropogenic sources (i.e. anthropogenically caused emissions of oil sands oil sands constituents before upgrading of the oil sands oil sands material (Kurek et al. 2013)) originate from the same oil sands deposit, and therefore, have similar chemical fingerprints.

The composition of bitumen-derived substances entering the aquatic environment is complex and highly variable. Major components are hydrocarbons, including PAHs, NAs and other polar organics, inorganic salts and trace metals (Kelly et al. 2010, Timoney 2007). PAHs are natural components of the bitumen in the oil sands (Neff and Stubblefield 1995, Sauer and Boehm 1991) and can be subdivided: Lower molecular weight (2- and 3-ring) PAHs are acutely toxic to aquatic organisms but are volatile, whereas high molecular weight (4- to 7-ring) PAHs, exhibiting low solubility and volatility, are known to be carcinogenic and mutagenic (Gill and Robotham 1989, Neff 1979, Prabhukumar and Pagilla 2010). Due to their hydrophobicity, PAHs entering the aquatic environment have a high affinity for suspended particles, thus, they occur mainly within the sediment compared to the aqueous phase (Moore and Ramamoorthy 1984, Neff 1979).

NAs are natural organic acids in bitumen and complex mixture consisting of alkyl-substituted cycloaliphatic carboxylic and acyclic aliphatic acids (Headley and McMartin 2004). NAs are water-soluble and are the main dissolved organic constituents of Mature Fine Tailings (MFT) and oil sands process-affected water (OSPW) following the hot water extraction of bitumen. They are a component within a larger family of organic acids that contributes to the acute toxicity of OSPW (MacKinnon and Boerger 1986). Metals are also a toxicologically relevant group of contaminants present in bitumen. Their bioavailability, and thus toxicity, depends on

a number of abiotic factors such as pH, water hardness, and availability of organic ligands (Hamelink et al. 1994).

Stream macroinvertebrates are exposed to bitumen-derived substances present in the aquatic environment via contact with water, sediment, and uptake of food (McElroy et al. 1989). Few studies on the effects of bitumen-derived substances on aquatic macroinvertebrate communities have been conducted *in situ*. Barton and Wallace (1979a) conducted a taxonomic survey of two tributaries of the Athabasca River in the oil sands region and studied long-term effects of oil spills and hydrocarbon exposure on invertebrate communities. Following this research, the Regional Aquatics Monitoring Program (RAMP), beginning in 1997, conducted regular macroinvertebrate monitoring in the region. This monitoring program, however, had several limitations (Main 2011). In 2011, Environment Canada and the Province of Alberta developed a comprehensive joint monitoring program (Environment Canada 2011).

To examine for potential environmental effects from mining activities, environmental monitoring needs sensitive bioindicators of effects and the present study intends to develop and apply such a bioindicator for aquatic effects based on the composition of macroinvertebrate communities. To this end, we applied the SPEAR (SPEcies At Risk) approach (Liess and von der Ohe 2005), which identifies those traits that characterize vulnerable species in relation to particular types of stressors or contaminants. Relevant traits are identified based on *a priori* ecotoxicological knowledge combined with field observations as described in (Liess and von der Ohe 2005). The system of SPEAR bioindicators currently includes the following indicators: $SPEAR_{pesticides}$ for pesticides (Liess and von der Ohe 2005), $SPEAR_{organic}$ for organic toxicants in general (Beketov and Liess 2008), and $SPEAR_{salinity}$ for salinity stress (Schäfer et al. 2011). The advantage of linking traits – and not species – with a specific environmental factor is the high degree of independence from geographical difference in species composition (Schäfer et al. 2012) and also from environmental factors that are not related to the investigated traits (Liess et al. 2008). The combination of several stressor related traits increases the specificity of the approach (Liess and von der Ohe 2005), making it a valuable tool.

The aim of the present study was to assess the composition and concentration of bitumen-derived contaminants in the Athabasca River and its tributaries and to quantify the associated ecological effects in the aquatic environment. Based on this information, we developed a sensitive biological indicator using the SPEAR approach, applicable to estimate the presence and magnitude of bitumen-derived aquatic pollution and associated ecological effects.

3.3. Material and Methods

3.3.1. Study area and sampling sites

Field monitoring was conducted in the McMurray Formation area (Figure S1) in September 2010, 2011, and 2012. In 2010, 9 sites were sampled for water, sediment, and macroinvertebrates; in 2011 and 2012, the sampling was repeated for these 9 sites and extended for an additional 10 sites (Figure S1). Sites were selected to cover most of the disturbed area in the surface mineable area north of Fort McMurray and also sites further upstream of the mineable area, including both the Athabasca river and its tributaries. Twelve sampling sites were located in areas with industrial development: ATR-1, ATR-2, ATR-3, BER, ELR, FOC, HAC, MAR-1, MAR-2, MUR, POC, and TAR according to the nomenclature of RAMP (Regional Aquatics Monitoring Program (RAMP) 2011). These sites were expected to exhibit higher concentrations of bitumen derived substances relative to other sampling sites because the natural oil sands deposit is closer to the surface (Alberta Geological Survey 2014, Hein and Cotterill 2006) and there is a potential of additional anthropogenic contributions (Kelly et al. 2009). Conversely, the two sites, ATR-5 and GUR, were located close to the outer margin of the deposit, further from industrial development, and were therefore expected to have lower concentrations of bitumen-derived substances. It was also assumed that the sites situated south of Fort McMurray (HOR, CHR, JAR, and SUC) would show lower concentrations of bitumen-derived substances, because the bitumen deposit lies deeper and there is no surface mining activity but only in-situ mining (Hein and Cotterill 2006). The Athabasca main stem was sampled at 2 sites upstream of Fort McMurray (ATR-4, ATR-5) and 3 sites downstream of the main development in and north of Fort McMurray (ATR-1, ATR-2, and ATR-3). Tributaries were sampled upstream (MAR-2) and downstream (BER, ELR, FOC, HAC, MAR-1, MUS, POC, and TAR) of local development where access by land was possible. All downstream sites are located close to the river mouths, while the upstream site is 50 km upstream of the river mouth. Sites were selected only within the oil sands area to ensure similar boundary conditions, excluding other types of pollution. Possible non-oil sources of pollution at the investigated sites are a pulp mill effluent close to site ATR-5 and a municipal wastewater treatment plant in Fort McMurray upstream of the sites ATR-3, ATR-2, and ATR-1. Pollution from agricultural sources is not present, as agriculture does not extend as far north as Fort McMurray.

3.3.2. Polycyclic aromatic hydrocarbons, naphthenic acids, and metals

Water samples were collected in hydrocarbon free bottles and stored at ~4°C. Sediment samples were collected in an I-Chem jar without headspace, stored at ~4°C and transferred to a -20°C storage facility after arrival to the laboratory.

Samples were analyzed for PAHs at the Biogeochemical Analytical Service Laboratory of the University of Alberta by gas chromatography mass spectrometry (GC-MS) analysis as detailed in Kelly et al. (2009). The list of PAH compounds analyzed is given in Table S1. Quality control was assured by quantification of the percentage recovery of parent PAH species and resulted in mean recovery of 95% (range from 86 to 102%).

Water samples were analyzed for NAs by HPLC-QTOF mass spectrometry as described in Ross et al. (2012) in 2010 and 2011; samples collected in 2012 were analyzed by HPLC-Orbitrap mass spectrometry described in Pereira et al. (2013) with on-line solid phase extraction (Pereira and Martin 2014). Results between sampling years can be compared semi-quantitatively.

For metals analysis, water samples were filtered (0.45 µm) and treated with nitric acid. Trace metals were measured by inductively coupled plasma mass spectrometry (ICP-MS) as detailed in Mahdavi et al. (2012).

For a comparison of the toxicity of the various compounds identified, toxic units (TU) were determined for PAHs, NAs and metals according to Sprague and Ramsay (1965). The TU approach expresses the concentration of single toxicants in relation to the sensitivity of *Daphnia magna* as a standard test organism in order to standardize and aggregate single substance's toxicities and define the overall toxicity at the sampling site (Liess and von der Ohe 2005). TU values are calculated according to formula [Equation 1]:

$$\text{TU } \textit{Daphnia magna} = \max_{i=1}^{n}\left(\log\left(\frac{C\,i}{\text{LC50}\,i}\right)\right) \qquad [1]$$

where TU $_{\textit{Daphnia magna}}$ is the maximum/highest toxic unit of the *n* PAH, NA, or metal compounds detected at a certain site, C*i* is the concentration (µg/L) of each compound *i* and LC50*i* (µg/L) is the 48h LC50 of compound *i* for *D. magna.*

To define the overall toxicity at a sampling site, compounds with same mode of action, i.e. on PAH, NA, and metal level, were aggregated. Accordingly, TU values were obtained for each parent PAH compound (alkylated PAHs were treated like their parent congeners due to data restrictions) and out of these TUs, only the one PAH with the maximum/highest TU of all detected PAHs at a given sampling site was considered. The same procedure was applied for

metals. For NAs, the total sum concentration of bitumen-derived NA homologues was derived for each sampling site. This value was divided by the median LC50 for *D. magna* 48h tests according to formula [Equation 1]. As the mode of action for the acute toxicity of both PAHs and NAs is narcotic (Di Toro et al. 2000, Di Toro et al. 1991, Frank et al. 2010), we also determined the sum of PAH TUs and NA TUs as an overall organic toxicant load. Further details about analytical methods and TU calculation can be obtained in the Supplementary material.

3.3.3. Water physico-chemical parameters

Temperature, pH, conductivity, oxygen content, current velocity, river width and depth, water color, sediment structure, the occurrence of surface oil, turbidity, phosphate, nitrate, nitrite, and ammonia were recorded. Details can be found in the Supplementary Material. In order to evaluate if high flows increase toxicant concentration in the river, river discharge recordings for all three years were obtained from RAMP (RAMP) for 10 sites in 2010 and 2011 and 12 sites in 2012. The sites sampled in 2010 and 2011 were ATR-1, BER, CHR, ELR, FOC, MAR-1, MAR-2, MUR, POC, and TAR; and in 2012 additionally JAR and SUC. A discharge ratio was calculated as the change of discharge in the week before and during the sampling campaign in September relative to the sum of discharge from beginning of July until mid September.

3.3.4. Invertebrate community

Sampling of invertebrate communities was performed in the various habitats available at each site and live sorting was conducted on site. The collected organisms were preserved and were identified following Clifford (1991). Details are available in the Supplementary material.

$SPEAR_{oil}$, the average community sensitivity towards oil contamination, is based on the trait 'physiological sensitivity', $S_{organic}$ (Wogram and Liess 2001), and the length of generation time (GT), representing the recovery potential from disturbance (Liess and von der Ohe 2005).

$SPEAR_{oil}$ as well as $SPEAR_{organic}$ are both based on $S_{organic}$ values (available via SPEAR Calculator on http://www.systemecology.eu/spearcalc/index.en.html). $S_{organic}$ reflects the taxon-specific sensitivity towards organic toxicants in general - comprising different modes of action (Beketov and Liess 2008), and are thus, calculated based on LC50 values of all organic compounds. They are usually determinable on species-level. If this is not possible due to data

restriction, they are calculated for genus or family level, which is a valid substitute according to Beketov et al. (2009). Since oil constituents are a mixture of substances, including hydrocarbons, PAHs, and NAs, we assume that they are well represented by organic toxicants in general, consisting of a large range of substance groups with a variety of modes of action. Long-term exposure with PAHs and NAs might cause excess toxicity (Lister et al. 2008, Scarlett et al., 2012), while narcosis is suggested as the primary mechanism for acute toxicity of PAHs (Di Toro et al. 2000, Di Toro et al. 1991) and NAs (Frank et al. 2009). Since $S_{organic}$ is based on LC50 values of all organic substances, it includes narcotic and non-narcotic mechanisms of toxicity. The LC50 values used stem from acute laboratory toxicity data available from the ECOTOX database (US EPA, http://cfpub.epa.gov/ecotox/data_download.cfm (status 2000 and 2002)). They are calculated as taxon-specific sensitivity in relation to the sensitivity of the reference species *D. magna*, according to formula [Equation 2].

$$Si = \frac{\log\left(LC50_{Daphniamagna}\right)}{LC50_i} \qquad [2]$$

with S_i being the physiological sensitivity of a taxon *i*

D. magna is a well-known sensitive test organism towards a variety of toxicants, therefore, it is one of the most widely used species in ecotoxicological tests, and thus, is the species with the highest number of test results for the highest number of substances. Chronic sensitivity information would have been prioritized as measurement of sensitivity in the field; however, chronic toxicity information is poorly available for many taxa and for many contaminants. Nevertheless, it has been shown in earlier work, that acute and chronic sensitivity are related in a linear way, which is also expressed via the Acute-Chronic Ratio (ACR) (Hoff et al. 2010). Furthermore, earlier SPEAR studies (Beketov and Liess 2008, Liess and von der Ohe 2005, Schäfer et al. 2011), in which the bioindicator is also based on acute sensitivity information, have shown high predictability of toxicant levels in the field.

Based on the S_i values of the taxa present at a sampling site and their weighted abundances, $SPEAR_{organic}$ can be obtained according to formula [Equation 3] (Beketov and Liess 2008).

$$SPEARorganic = \frac{\sum\left(\log(x_i + 1) * Sorganic\right)}{\sum(\log(x_i + 1))} \qquad [3]$$

with $SPEAR_{organic}$ as the average community sensitivity towards organic toxicants, $S_{organic}$ as the physiological sensitivity of a taxon *i* towards organic toxicants, and x_i as the abundance of taxon *i* out of all taxa *n* occurring at one sampling site.

In contrast to $SPEAR_{organic}$, $SPEAR_{oil}$ includes the trait generation time. As fluctuating environmental conditions favor short-lived organisms and we showed that hydrological conditions in the study area fluctuate strongly, the consideration of generation time for the bioindicator development is required. Species with short generation time are more capable in adapting to and recovering from a stressor or pollutant occurring in pulses. Thus, $SPEAR_{oil}$ was calculated according to formula [Eq. 4] with abundance (x), $S_{organic}$, and GT for each taxon *i* of all taxa (*n*) found at a given sampling site:

$$SPEARoil = \frac{\sum\left(\log(x_i + 1) * \left(\frac{Sorganic - 1}{GT} \right) \right)}{\sum(\log(x_i + 1))} \qquad [4]$$

An online software to calculate $SPEAR_{oil}$ is made publicly available as part of the SPEAR Calculator on http://www.systemecology.eu/spear/.

GT was determined based on information obtained from the SPEAR trait database (Liess and von der Ohe 2005) as well as from Schäfer et al. (2011), Clifford (1991), and Barton and Wallace (1980). A GT of 0.5 equals 2 generations per year, while a GT of 2 equals 1 generation in 2 years. According to formula [3], tolerant taxa with long GTs are more sensitive than tolerant taxa with short GTs. The same is true for sensitive taxa, which are even more vulnerable in case of long GTs compared to sensitive taxa with short GTs. In case no information on GT was available from the databases, the GT of the closest related taxon was applied.

Environmental factors such as e.g. temperature can result in minor changes in the GT, which are, however, not expected to change the classification of a taxon between the relatively rough classification of 0.5, 1 or 2. This trait classification is, thus, robust to minor changes due to confounding environmental factors.

It is to be noted that species data were entered into the formula logarithmically in order to not overweight high abundances - a common approach with biological metrics.

The results of the $SPEAR_{oil}$ calculation are given in Table S5. For the sites sampled in 2010-2012, $SPEAR_{oil}$ ranged between -3.07 and -1.49 with higher values reflecting a more sensitive community composition.

To investigate specificity and cross sensitivity of the new bioindicator, other bioindicators already available were also applied: $SPEAR_{organic}$ (Beketov and Liess 2008), the metal indicator S_{metal} (Malaj et al. 2012), and the conventional indices taxa richness, taxa richness of Ephemeroptera, Plecoptera, and Trichoptera, and Shannon's diversity index. The distinct metal sensitivity values (S_{metal}) were developed by Malaj et al. (2012) and are based on LC50

values of metals. For taxa for which no S_{metal} could be determined, the value for the taxonomically closest taxa was applied.

3.3.5. Statistics

We conducted two linear multivariate ordination analyses after Detrended Correspondence Analysis (DCA) confirmed linear length of gradients. Firstly, constrained Redundancy Analysis (RDA) was performed followed by unconstrained Principal Component Analysis (PCA).

RDA was conducted with log10(x+1) transformed species abundances. Taxa were aggregated to family level by summing up abundances of all taxa belonging to one family for each sampling site. Only taxa with scores >0.2 are marked with red arrows; TU metal and TU PAH are marked with blue arrows. The RDA shows the variance of species distribution as explained by the TUs. Monte Carlo permutation test (package vegan, function anova.cca) confirmed significance of the variation in species composition explained by the explanatory variables. The distribution of sites was checked; a horseshoe effect did not occur.

Analysis of variability and relations of traditional biological indices, species traits, and SPEAR indices to environmental factors between sites was performed via PCA. Environmental parameters were used as explanatory variables, biological indices as response variables. "$SPEAR_{organic}$" and "gewGT" represent the traits physiological sensitivity and GT, both weighted by abundance. The proportion of predators in the community is represented by "partPred". The proportion of predators is a trait possibly related to metal contamination, assuming higher contaminant uptake due to predatory feeding type (Liess and Gerner et al. in preparation). Available evidence suggests that diet is an important source of metal accumulation in insects even to date there have been no conclusive studies evaluating whether dietary metal accumulation causes toxicity (Brix et al. 2011). Prior to PCA analyses, the indices' values were standardized to zero population mean, with a standard deviation equal to one, as they do not share the same units. The first two ordination axes were identified as interpretable using the Broken Stick criterion.

Via non-parametric Spearman correlation the relationship between discharge ratio and TU PAHs was analyzed. Parametric Pearson correlations were performed to identify the relationships between biological indicators and chemical and/or environmental parameters.

All statistical analyses were conducted using the software R (version 2.15.2).

3.4. Results and discussion

3.4.1. PAH concentration and toxicity

The total dissolved PAH concentrations (Figure S2 A, Table S8) measured in 2010 are comparable to those measured in the Athabasca River and its tributaries in 2008 by Kelly et al. (2009). Also maximum dissolved parent and alkylated PAH concentrations measured in 2011 and 2012 by RAMP (Regional Aquatics Monitoring Program (RAMP) 2012, 2013) are comparable to the maximum values detected here.

Results indicate that the ∑C1-C4 alkylated congeners (i.e. up to 4 carbons as alkyl substituents) occurred in higher concentrations than the C0 (i.e. parent) congeners at the sites investigated (Figure S2 A-C). This is indicative of a predominantly petrogenic source, rather than a pyrogenic source (Sauer and Boehm 1991).

Among all sites investigated, the mean ratios of parent to ∑C1-C4 alkylated PAH concentrations were 0.03, 0.06, 0.16, 0.36, and 5.63 for fluorene, dibenzothiophene, phenanthrene/anthracene, chrysene, and fluoranthene/pyrene, respectively. Parent naphthalene was not detected, despite good recovery in reference (97.7 %) and quality control samples (100%). A similar profile of parent and alkylated PAHs in water was detected by Birks et al. (2013).

Additional indicators of predominantly petrogenic origin of PAHs are the bell shaped distribution curves for the C0 through C4 PAH (Figure S2), with peak concentrations at C1 or C2 (Akre et al. 2004, Gill and Robotham 1989) in 2010, 2011, and 2012.

A literature review shows that source identification is generally challenging, requiring to discriminate whether PAHs from petrogenic origin enter the water naturally or due to oil sands development; their chemical fingerprints are highly similar. Nevertheless, some studies attempted to identify the source of oil constituents in the aquatic environment in the oil sands region (Headley et al. (2001), Hall et al. (2012), Timoney and Lee (2011), Kelly et al. (2009)). When comparing composition and distribution patterns of PAH mixtures between sampling sites, following approaches by Hall et al. (2012) and Headley et al. (2001), two distinct groups were observed in 2010 (Figure S2 A). The sites POC, BER, MUR, ATR-3, and FOC were similar, with a prevalence of alkyl naphthalenes (C1-4), phenanthrenes/anthracenes (C0-4), dibenzothiophenes (C0-4). This first group of sites was located north of Fort McMurray where natural oil sands deposits occur close to the surface and large areas are exposed to surface mining and tailings storage. The profile of this group is similar to the distribution of dissolved PAHs in water of tributaries observed by Kelly et al. (2009).

Conversely, at ATR-4, CHR, SUC, and JAR phenanthrenes/anthracenes (C0-3), dibenzothiophenes (C0-3), fluorenes (C1-3), fluoranthene (C0), pyrene (C0), and very low levels of 4- to 6-ring PAHs were found; the proportion of fluorenes was higher compared to the first group. This second group of sites is located in and south of Fort McMurray, where the deposit lies deeper.

It could be expected that the invertebrate community structure is a representation of each group. This expectation is partly true (Figure 3): sites POC, BER, and MUR are represented by rather tolerant communities while sites ATR-4 and CHR are represented by sensitive communities. For the sites FOC, JAR, and SUC the trend is not clear.

In 2011 and 2012, no clear PAH distributions were observed among sampling sites. Thus, the observations from 2010 are yet to be confirmed, and therefore, no identification of sources or further interpretation was attempted.

The toxicity of PAHs in the water column, given as maximum TUs, varied substantially between the 3 sampling years. Results indicate that TUs were generally higher in 2010 than in 2011 and 2012 (Figure S3). In 2010, the majority of the sites were characterized by single maximum TU values above -3. As TUs are on a logarithmic scale, this translates into PAH concentrations from 1000 times up to only 10 times below the acute sensitivity of *D. magna*. In contrast, in 2011 and 2012 none of the sites had a TU above -3. In 2011, single maximum TU values were generally below -5; in 2012, TU values were below -4, corresponding to PAH concentrations at least 100.000 or 10.000 times below the acute sensitivity of *D. magna*.

3.4.2. Hydrology and PAHs

Results indicate that PAH concentrations are associated with the discharge ratio. Peak PAH levels occurred at times of high flows during the sampling period in autumn and comparatively low flows in the months before sampling (Figure S4). In 2010, river discharge was characterized by high flows during autumn sampling and low flows in the summer months prior to sampling. In 2011 and 2012, however, discharge was higher in the summer months compared to the discharge during sampling in autumn. Accordingly, PAH concentrations in the samples and the associated TUs of the sampling sites were also higher in 2010 compared to 2011 and 2012 (Figure S2, Figure S3). The consideration of hydrological history can greatly improve the prediction of PAH loading. The relevance of high flow rates has also been identified by Birks et al. (2013), Akre et al. (2004), and Timoney and Lee (2009, 2011). A regression between discharge ratio and TUs of PAHs of all three years

revealed a positive dependency of TU on the discharge ratio (non-parametric Spearman correlation rho = 0.67, $p < 0.05$, $n = 25$) (Figure S5). The correlation is also depicted excluding site POC (rho = 0.62, $p < 0.05$, $n = 22$) (see section "Further environmental parameters" for explanation).

For absolute values of discharge (Timoney and Lee 2011) such as the total and mean discharge during the whole year as well as discharge during the months before sampling or during sampling - we could not detect a relevant correlation with PAH concentration.

3.4.3. PAHs and macroinvertebrate community structure

The aquatic invertebrate taxa that were sampled at the sites during three years and their respective abundances are listed in Table S5.

The RDA (Figure 1, Table S10) model conducted for 2010, the year with the highest toxic units, is significant and shows the species distribution according to TU metals and PAH distribution. The RDA identifies which taxa occur at high and low TUs, which is similar for metals and PAHs. However, no general taxonomic consistency in the distribution of invertebrate families was observed. For instance, not all families of Ephemeroptera are clustering, but only Metretopodidae, Leptophlebidae, Ephemerellidae, and other Ephemeroptera while Baetidae, Baetiscidae, Heptageniidae, and Siphlonuridae are clustering in the opposite direction.

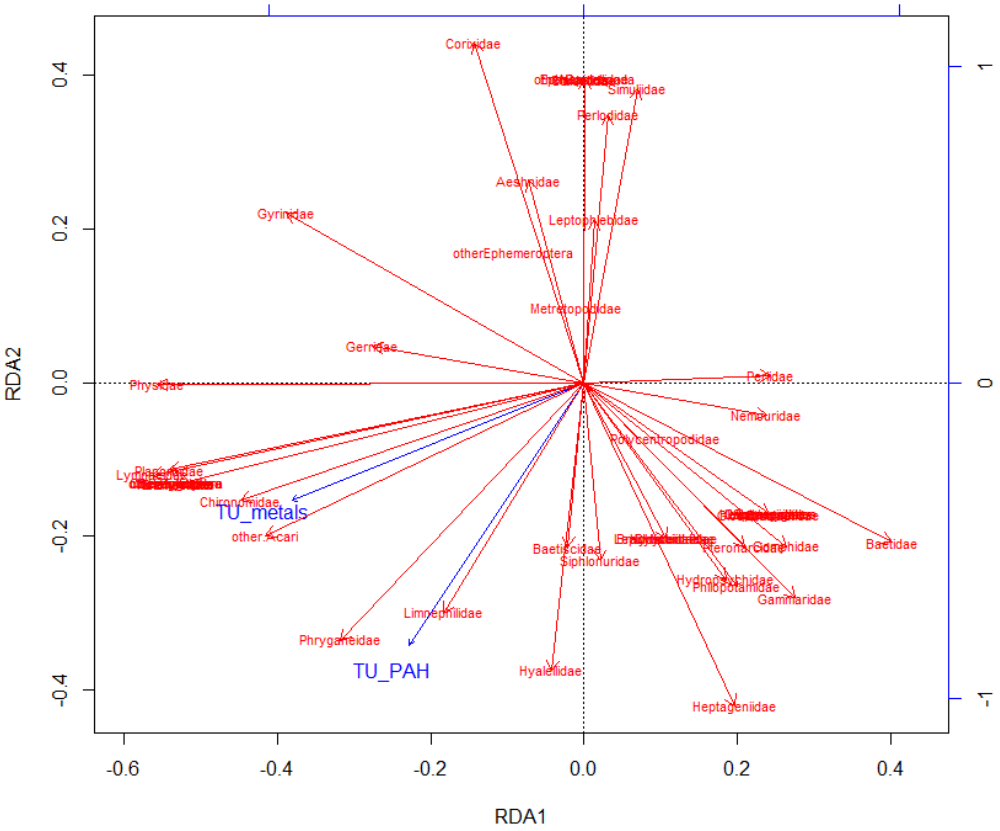

Figure 1. Ordination plot for Redundancy Analysis (RDA) on the variance of the species distribution in 2010 as explained by the TUs. Species abundances were log10(x+1) transformed; taxa were aggregated to family level; only taxa with scores >0.2 are marked with red arrows; TU metals and TU PAHs are marked with blue arrows. Clustering taxa are: Planorbidae with Eremaeidae, Haliphilidae, other Coleoptera, Sciomyzidae, Notonectidae, Lymnaeidae, Coenagrionidae and other Zygoptera; Simuliidae with Ephemerellidae, Nepidae, Corduliidae, Valvatidae and other Gastropoda; Lepidostomadidae with Dytiscidae, Brachycentridae and Hydrobiidae; Sphaeridae with Elmidae, Calopterygidae, Leptoceridae, Glossosomatidae, and Helicopsychidae.

As taxonomy did not explain species occurrence with respect to the degree of metal or PAH contamination, we analyzed patterns of species according to their traits. Traits were selected from the trait repertoire applied for $SPEAR_{pesticides}$ in terms of ecological relevance: "$S_{organic}$" is a reasonable explanatory trait as it describes the sensitivity of species towards organic contamination in general which may also be relevant for exposure towards oil constituents (for a list of $S_{organic}$ values see (von der Ohe and Liess 2004)). Due to the periodic nature of exposure towards oil constituents during high discharge periods, the length of GT may also be of importance describing species ability for recovery. "Migration ability" was not regarded meaningful, as exposure towards oil sands constituents is not restricted to single streams but to the entire area, and thus, recolonization from neighbor streams is not relevant for recovery.

Also the trait "presence of sensitive aquatic stages during the time of maximum exposure" is not a meaningful descriptor for the oil sands related contamination as peak exposure is not restricted to seasonal patterns.

The PCA conducted subsequently shows the relations between traditional biological indices, species traits, SPEAR indices and environmental factors, including TUs (Figure 2, Table S10). The first 2 Principal components (PC) are interpretable and explain 66% of the total variance. TU metals and PAHs are both negatively related to PC1, while $SPEAR_{oil}$ and $SPEAR_{organic}$ are positively related to PC1. The indices most strongly related to PC2 are TR, partPred, S_{metal}, and EPTperc. The traits $SPEAR_{organic}$ and gewGT are negatively related with TUs while partPred and S_{metal} are not related to TUs. The metal toxicity (TU metals) could not be explained by the bioindicator S_{metal} (Malaj et al. 2012). This indicates that the invertebrate community investigated was not primarily shaped by metals. The combination of the two relevant traits $SPEAR_{organic}$ and gewGT forms the $SPEAR_{oil}$ index and results in a strong negative relation to TU PAHs. The PCA also depicts that site POC has the highest TU metals and PAHs, while the sites ATR-4, FOC, JAR, and CHR are characterized by the lowest.

Jointly considering the results from the taxonomic lists (Table S5), the RDA and PCA, and the various indicators (Table 1), allows comparing the community structure between sites with high and low levels of contamination. For instance, site POC with high TU PAHs and TU metals is taxonomically represented by high abundances of Coleoptera, Diptera, and especially, Gastropoda - all located in the lower left square of the RDA (negative RDA1 and 2). At the same time, the PCA shows a negative relation to $SPEAR_{organic}$, S_{metal}, and gewGT for site POC. In this case, the $S_{organic}$ value is -0.79, S_{metal} is 0.56, and gewGT is 0.67. On the other hand, for instance at site ATR-4 with low TU PAHs and TU metals, mainly taxa of the orders Ephemeroptera, Plecoptera, and Trichoptera occur. In the PCA, ATR-4 is positively related to $SPEAR_{organic}$, S_{metal}, and gewGT. ATR-4 has an $S_{organic}$ of -0.18, a S_{metal} value of 1.26, and a gewGT of 0.84. This represents a community with high sensitivities towards organic toxicants and long generation times, likely to occur at less contaminated sites. S_{metal}, however, indicates a metal tolerant community, which is not reasonable here.

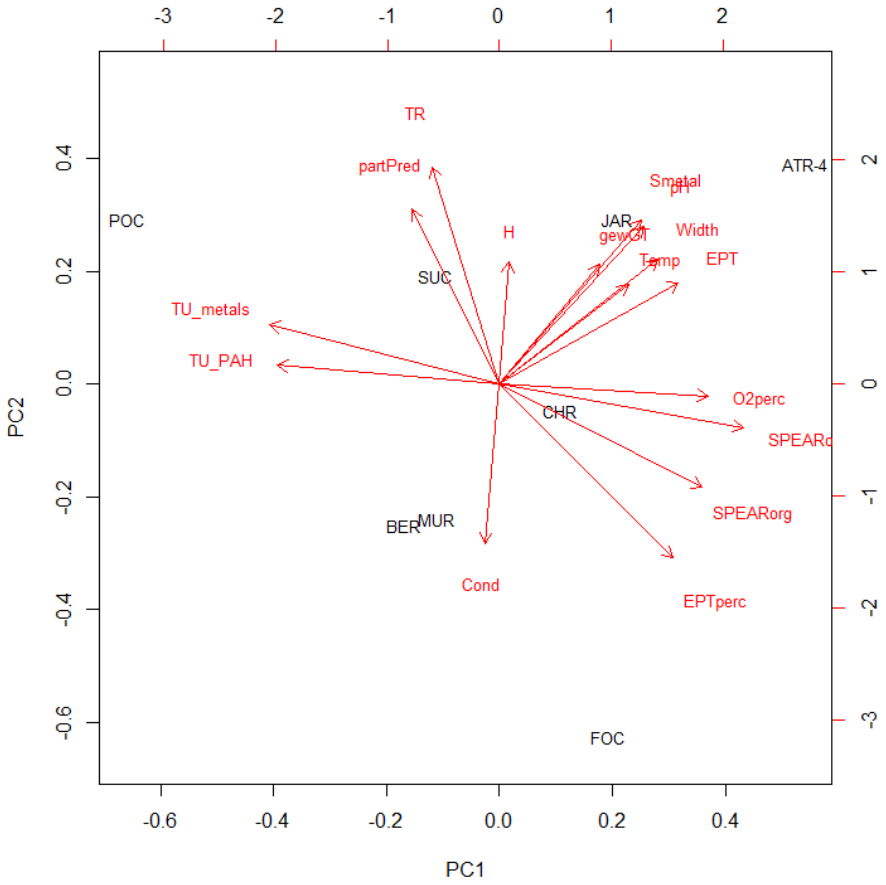

Figure 2. Ordination plot for Principle Components Analysis (PCA) of traditional biological indices, species traits, SPEAR indices and environmental factors, including toxic units from the samples taken in 2010. Indices' values were standardized to zero population mean, with a standard deviation equal to one.

Similarly as in the PCA, in the regression analysis, we found a strong relationship between TU PAHs and $SPEAR_{oil}$, (parametric Pearson correlation: $r^2 = 0.77$, $p < 0.005$, $n = 8$) (Figure 3) in the year with the highest concentrations of PAHs (2010). In the two subsequent years with lower PAH levels, no significant relationships were observed.

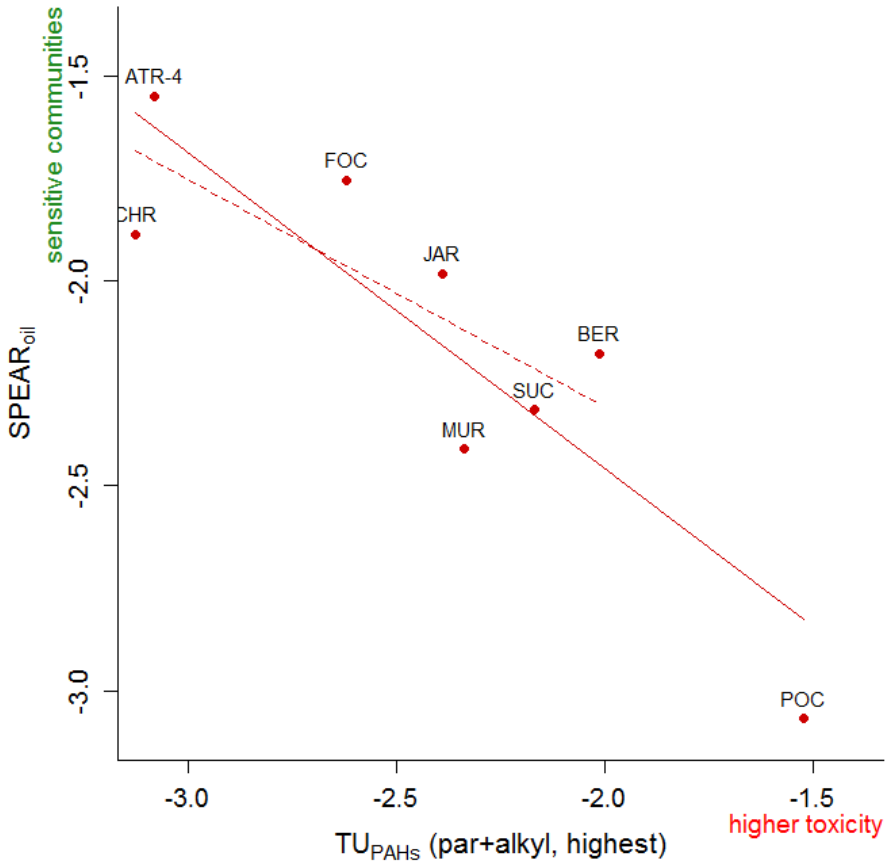

Figure 3. Linear regression model for the relationship between log Toxic Units of total PAHs (parent and alkylated forms, maximum TU) in water and *SPEAR*$_{oil}$ for the sites sampled in 2010. The linear model explained 77% of the variation (Pearson correlation: $p < 0.01$, $n = 8$). Performing the correlation without POC resulted in 60% of explained variation (Pearson correlation: $p < 0.05$, $n = 7$). Solid and dotted line indicate correlation with and without site POC, respectively.

The identification of the relationship between *SPEAR*$_{oil}$ and TU PAHs observed in 2010 reflects the fact that species' traits are linked to the ecological conditions of the species' habitat (Townsend and Hildrew 1994). Taxa found with high sensitivity (high $S_{organic}$) were mainly Plecoptera (Isoperla, Acroneuria, Pteronarcys, and Pteronacella) as well as Amphipoda (*Hyalella azteca* and *Gammarus lacustris)* and Ephemeroptera (Baetis, Centroptilum, Baetidae, and Pseudocloeon). Plecoptera and Anisoptera (Aeshna, Epitheca, Somatochlora, Gomphidae) are characterized by medium to long GTs. Hence, they are especially vulnerable to oil contamination.

In contrast to *SPEAR*$_{oil}$, traditional biological metrics of toxicant impact on communities, namely taxa richness (TR), the Shannon-Wiener diversity index (H') and taxa richness of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT), were only weakly correlated with PAH levels in all years. Also other indicators of the SPEAR family were not related to the

measured contamination. For $SPEAR_{organic}$, a non-significant trend was observed between the measured TU PAHs and the indicator response ($r^2 = 0.44$, $p > 0.05$, $n = 8$) (Table 1).

Table 1. Pearson correlation coefficients (r^2) of macroinvertebrate community metrics correlated with chemical and environmental parameters pH, O2 (%), conductivity (µS), current velocity (m/s) and stream width (m) for the sampling year 2010. Biological indicators are taxa richness (TR) and their classes (TR HK), taxa richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT) and in classes (EPT HK) and in percent (EPT%), Shannon-Wiener diversity index (H'), average community sensitivity towards oil ($SPEAR_{oil}$), average community sensitivity towards organic toxicants ($S_{organic}$) weighted by abundance which results in $SPEAR_{organic}$, average community sensitivity towards metals (S_{metal}), and generation time weighted by abundance. Significant correlations are indicated with *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, $n = 8$. Negative correlations are indicated by a (-) sign.

	TU PAHs	TU metals	pH	O_2	Conductivity	Current velocity	Width
***SPEAR*$_{oil}$**	(-) 0.77**	(-) 0.88***	0.22	0.57*	0.00	0.43	0.26
***SPEAR*$_{organic}$**	(-) 0.44	(-) 0.62*	0.04	0.29	0.12	0.16	0.15
S_{metal}	(-) 0.31	(-) 0.07	0.64*	0.02	(-) 0.1	0.06	0.68*
gewGT	(-) 0.1	(-) 0.13	0.34	0.43	(-) 0.24	0.44	0.08
TR	0.22	0.16	0.13	(-) 0.03	(-) 0.14	(-) 0.32	0.02
TR HK	0.23	0.22	0.12	(-) 0.1	(-) 0.06	(-) 0.33	0.06
EPT %	(-) 0.39	(-) 0.53*	(-) 0.02	0.31	0.18	0.46	0.02
EPT	(-) 0.13	(-) 0.31	0.18	0.45	(-) 0.07	0.11	0.29
EPT HK	(-) 0.08	(-) 0.22	0.22	0.30	(-) 0.01	0.01	0.44
Shannon H'	0.12	0.01	0.00	0.08	(-) 0.10	(-) 0.05	0.08

PAH concentrations monitored in river sediment revealed whether the invertebrate community was rather shaped by contaminants in the water column or in the sediment. The TUs, calculated as bioavailable dissolved fraction, had a lower range (median = -6.35, 1st quartile = -7.32, 3rd quartile = -5.56) than the water TUs (median = -4.58, 1st quartile = -5.84, 3rd quartile = -3.91). Furthermore, the PAH TUs in sediment did not correlate to any of the biological indicators. Accordingly, PAHs in the water column and not in the sediment seem to mainly determine the effect on macroinvertebrate organisms (Figure S2 D-F). It is assumed that aquatic invertebrates are exposed to oil sands constituents present in the aquatic environment via contact in the water column, uptake of water, contact to sediment, and uptake of food (McElroy et al. 1989). However, uptake of PAHs occurs more rapidly in the solubilized form (Obana et al. 1983), hence, pelagic organisms may be more at risk than benthic species.

3.4.4. Metals and naphthenic acids concentration and toxicity

Metal concentrations (Table S3) in water were positively correlated with PAHs (r^2 = 0.68, p < 0.05, n = 8) in 2010, and as a result, the TUs of metals (Table S4) and PAHs were equally negatively correlated with the $SPEAR_{oil}$ indicator (r^2 = 0.88, p < 0.001, n = 8) (Table 1). As was already observed in the PCA, there is no relationship between TU metals and the indicator for metal toxicity, S_{metal} (Malaj et al. 2012). The strong correlation between TU metals and $SPEAR_{oil}$ is, thus, presumably due to their interrelation with TU PAHs.

Toxic units of NAs, calculated based on NA profiles and their concentrations (Figure S6), were not related to any of the biological indices of the macroinvertebrate community. The sum of PAH and NA TUs were similarly correlated as PAH TUs with $SPEAR_{oil}$ (PAH TUs vs. $SPEAR_{oil}$: r^2 = 0.77, p < 0.005; PAH + NA TUs vs. $SPEAR_{oil}$: r^2 = 0.77, p < 0.005). Also in 2011 and 2012, where PAH exposure was lower, considering the NA TUs did not change the relationship between PAHs and $SPEAR_{oil}$. Accordingly, as there was no clear indication that at these concentrations NAs show a toxic effect on the invertebrate community, this study focuses on PAHs as the main toxicants of concern.

Despite the focus on PAH toxicity in this study, we want to highlight that to a certain extend all stressors may act in concert and cause the observed effect in combination (Liess et al. 2016). However, a dominant effect of metals is not likely, as we could not identify a relationship between toxicity (TUs) of metals and the S_{metal} indicator. Nevertheless, it may add to the toxic effect of PAHs.

3.4.5. Further environmental parameters

Apart from the dependency of invertebrate community descriptors on PAH contamination, $SPEAR_{oil}$ also correlated with dissolved oxygen in 2010 (Pearson correlation: r^2 = 0.57, p < 0.05, n = 8) and with pH in 2012 (r^2 = 0.24, p < 0.05, n = 8). However, the dissolved oxygen concentrations fluctuated around a median of 93.4 % throughout the three sampling campaigns in 2010-12 (1st quartile = 87.55 %, 3rd quartile = 97.6 %). Dissolved oxygen concentration exceeded 80% at all site except one with 75 % saturation (POC). According to existing autecological knowledge, such values do not indicate oxygen shortage for invertebrates occurring in the investigated sites (Canadian Council of Ministers of the Environment 1999). As shallow streams with a constant flow of water were investigated, we do not expect considerably lower oxygen concentrations in the micro zone over the sediment. Only the site POC is characterized by a dammed up stream section with very low flow rates.

This resulted also in only few rheophilic species sampled at POC in 2010. As POC differs from the other sampling sites with regard to this parameter, the correlation between $SPEAR_{oil}$ and PAH TUs for the year 2010 was also performed excluding POC (Figure 3), This exercise shows the advantage of the SPEAR bioindicator systems to be trait-based instead of taxonomy-based: Even with differences in habitat conditions (in this case at site POC), and thus, also different taxa forming the community, the trait information still serves to overcome these site-specific differences. The linear regression with and without sampling site POC does not differ considerably, showing that the trait composition at POC fits the relationship between contamination level and trait-related community composition observed at the other sampling sites.

Also the pH fluctuation in 2010-12, with a median of 7.9 (1st quartile = 7.5, 3rd quartile = 8.2), a minimum of 6.6 and a maximum of 8.8, is not expected to negatively affect the invertebrates investigated here (U.S. Environmental Protection Agency (US EPA) and Office of Water - Office of Science and Technology (4304 T) 2006).

Our results indicate that current guidelines for PAHs in water seem not to be protective for all substances. For example, the most toxic substance at many sites investigated in this study was indeno[1,2,3-c,d]pyrene, a high molecular weight parent PAH. The corresponding water toxicity calculated for the substance correlated well with the observed ecological effect, suggesting that occurrence of indeno[1,2,3-c,d]pyrene may to a great extent determine community composition. Community level effects were apparent at a concentration of 0.008 µg/L indeno[1,2,3-c,d]pyrene without other substances that added to the toxicity of the respective sites in 2010. This is a factor of 200 below the acute LC50 (1.6 µg/L) of this substance. The safe concentration for aquatic life is set to 0.21 µg/L in the surface water quality guidelines for use in Alberta (Alberta Environment 1999), which is a factor of 30 above the concentration that was observed to be effective in the field. As the setting of guidelines relies on a few laboratory test species, more research needs to be put into validating sensitivity of species in the laboratory and in the field in order to prevent detrimental long-term community effects, such as changes in the invertebrate communities observed here. Further research is also required on field effects of particle bound PAH, metal contamination and of naphthenic acids.

Furthermore, in areas with high natural input of contaminants like in the oil sands region, regionally specific guidelines may be considered, taking into account background contamination. Here, the possibility has to be acknowledged that benthic communities inhabiting regions of high PAH concentrations may be adapted and, as a result, their toxicity

thresholds may be higher here than in other regions. However, within species such adaptation is only possible to a certain degree. Other species which are physiologically more tolerant should still predominate, which will be reflected with the bioindicator.

3.4.6. Conclusions

To summarize, PAH concentrations in the Athabasca River and its tributaries were related to discharge and were highest in years with high autumn rainfall. Effects on the macroinvertebrate community structure were observed using the biological indicator system, $SPEAR_{oil}$. Alterations in terms of increased physiological sensitivity and decreased generation time were observed at a TU of around -2.5, which translates into PAH concentrations 100 times below the acute sensitivity of the standard test organism *D. magna*. Hence, increases of PAH exposure of up to 0.202 µg/L, as for example observed in tributaries from up- to downstream of oil sands development by Kelly and co-workers (Kelly et al. 2009), represent toxicologically relevant concentrations for aquatic communities.

The bioindicator $SPEAR_{oil}$, developed in this study, is applicable for future monitoring of oil sands related effects, allowing for further validation with independent data sets. Further application will also help defining the possible range of $SPEAR_{oil}$ values, allowing to develop categories of $SPEAR_{oil}$.

3.5. Acknowledgements

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Chapter 4: "Sensitivity ranking for freshwater invertebrates towards hydrocarbon contaminants"

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4.1. Abstract

Hydrocarbons have an utmost economical importance but may also cause substantial ecological impacts due to accidents or inadequate transportation and use. Currently, freshwater biomonitoring methods lack an indicator that can unequivocally reflect the impacts caused by hydrocarbons while being independent from effects of other stressors. The aim of the present study was to develop a sensitivity ranking for freshwater invertebrates towards hydrocarbon contaminants, which can be used in hydrocarbon-specific bioindicators. We employed the Relative Sensitivity method and developed the sensitivity ranking $S_{hydrocarbons}$ based on literature ecotoxicological data supplemented with rapid and mesocosm test results. A first validation of the sensitivity ranking based on an earlier field study has been conducted and revealed the $S_{hydrocarbons}$ ranking to be promising for application in sensitivity based indicators. Thus, the first results indicate that the ranking can serve as the core component of future hydrocarbon-specific and sensitivity trait based bioindicators.

4.2. Introduction

Hydrocarbons in all their forms are of utmost economical importance (BP 2013, US EIA (US Energy Information Administration) 2013). However, as most of the industrial chemicals, hydrocarbons may cause ecological impacts either due to accidents or inadequate transportation, use, and extraction (Gill and Robotham 1989). Therefore, assessment and prediction of the ecological impacts of hydrocarbons is highly relevant. Systematic biomonitoring is necessary for both aquatic and terrestrial ecosystems, and particularly required in the areas at risk (e.g. around production and transportation facilities) and after catastrophic contamination events (Phillips and Rainbow 1993).

For freshwaters, the current biomonitoring methods lack an indicator that unequivocally reflects the impacts caused by hydrocarbons, while being independent from effects of confounding environmental stressors like physico-chemical water quality parameters and physical river characteristics.

A bioindicator for organic toxicants in general, named $SPEAR_{organic}$, has been developed using freshwater invertebrates (Beketov and Liess 2008). Validation of this index showed that it reflects contamination of streams and rivers with petrochemicals and synthetic surfactants and at the same time it is independent of confounding factors (Beketov and Liess 2008, Schletterer et al. 2010). Schuwirth et al. (2015), however, demonstrated that $SPEAR_{pesticides}$ correlates

strongly with other indices. As intercorrelations between environmental parameters often occur in the field, distinct effect assessment can be difficult (Bunzel et al. 2013). Rasmussen and colleagues (2011) and Bunzel et al. (2013) found habitat quality to influence $SPEAR_{pesticides}$. This, however, only occurred when habitat degradation was strong. In Beketov et al. (2013) it showed that with the Toxic Units gradient of pesticides also other environmental parameters increased or decreased. Sensitivity values for specific chemicals or physico-chemical parameters have a stressor specificity. Research suggests that using traits-based information – such as applied in the SPEAR approach – may offer causal diagnosis assessments, going beyond traditional taxonomic-based information (Culp et al. 2011). However, this diagnosis does not indicate exclusive causal relationships.

$SPEAR_{organic}$ is part of a system of SPEAR (SPEcies At Risk) bioindicators. These bioindicators are based on taxa's bio-/ecological traits such as generation time and migration ability. The sensitivity towards different groups of toxicants can be seen as the result of a set of physiological traits and is dependent on the extent of stress factors like exposure towards chemicals. Piscart et al. (2006), for instance, observed changes in many of the traits studied with increasing levels of salinity stress. Nevertheless, trait-based approaches are regarded promising for environmental monitoring (Baird et al. 2011, Bonada et al. 2006, Statzner and Bêche 2010). They have the advantages of being independent of regional constraints (Bonada et al. 2006, Statzner and Bêche 2010) and can help discriminating individual effects of multiple stressors (Bonada et al. 2006, Statzner and Bêche 2010, Wooster et al. 2012). In Szöcs et al. (2014), for example, the trait-based evaluation yielded a higher explained variance compared to the taxonomy-based evaluation.

With different combinations of traits the SPEAR biodindicators are designed to be specific towards particular types of contaminants. Currently, the system includes the following indicators: $SPEAR_{pesticides}$ for pesticides (Liess and von der Ohe 2005), $SPEAR_{organic}$ for organic toxicants in general (Beketov and Liess 2008), and $SPEAR_{salinity}$ for salinity stress (Schäfer et al. 2011). The SPEAR indicators reflect the average community sensitivity ($SPEAR_{organic}$) or the part of sensitive species in a community ($SPEAR_{pesticides}$, $SPEAR_{salinity}$). The core trait that is used for creating SPEAR indices is the Relative Sensitivity (S), which is the taxon-specific toxicological sensitivity (i.e. $S_{organic}$ for organic toxicants). It is calculated as a taxon's sensitivity relative to the sensitivity of the benchmark test-species *Daphnia magna* (von der Ohe and Liess 2004, Wogram and Liess 2001). Calculation of such sensitivity values is based on existing ecotoxicological data and aims at creating a ranking system for all the major taxonomic groups according to their sensitivity towards certain

chemical groups. Such sensitivities are used in all SPEAR indices either in combination with other traits ($SPEAR_{pesticides}$) or alone ($SPEAR_{organic}$).

Extensive reviews have been conducted compiling information on the toxicity of hydrocarbons (Eisler 1987, Erben et al. 2003, Nagpal 1993). However, the first step necessary for creating such a SPEAR indicator for hydrocarbon contamination is the development of a sensitivity ranking ($S_{hydrocarbons}$) for freshwater invertebrates towards hydrocarbon contaminants, as such a ranking does not exist yet. This was done in the present paper. Such a $S_{hydrocarbons}$ ranking can serve as the core elements of future sensitivity trait based bioindicators, e.g. of the SPEAR-type, for hydrocarbon contaminants. It can be the basis for any biomonitoring index that relies on sensitivity information and focuses on this type of contamination. One bioindicator based on this ranking is developed here, the $SPEAR_{hydrocarbons}$ indicator. Such bioindicators may be applicable not only to field data but may also be useful for effect assessment of mesocosm studies involving hydrocarbons. The added value of the present study is the large set of experimental data reanalyzed, restructured, and combined into a ranking system generalizing taxa sensitivity to a group of compounds.

The authors chose the method of a sensitivity ranking as it is a direct and simple approach to measure and compare the sensitivity of diverse taxa to a specific type of contaminants. However, also alternative approaches to ranking systems are available (Ippolito et al. 2012, Piscart et al. 2006, Rico and Van den Brink 2015, Szöcs et al. 2014). For instance, Rubach et al. (2010) linked taxa's sensitivities to their traits, generating sensitivity–trait relationships which again allowed producing mode-specific sensitivity (MSS) rankings. MSS rankings were also produced for invertebrate families for different chemical classes (Rico and Van den Brink 2015). This method has the advantage of not only relying on *D. magna* as single species for standardizing toxicity. Relying only on one species has a risk related to the quality of the toxicity information for this species – especially when using only one or few studies to derive its LC50. However, *D. magna*, as the most tested species in ecotoxicology studies, is in practice most suitable for toxicity standardization (Wogram and Liess 2001). Furthermore, the standardization with *D. magna* has been successfully applied in studies using the SPEAR approach (Beketov and Liess 2008, Liess and von der Ohe 2005, Schäfer et al. 2011).

Aim of the present investigation was to development a sensitivity ranking ($S_{hydrocarbons}$) for freshwater invertebrates towards hydrocarbon contaminants based on available data sets of laboratory-based toxicity information. Additional mesocosm and rapid test data sets were included to extend the dataset. We hypothesize that this hydrocarbon-specific ranking can reflect the hydrocarbon contamination in field studies. It should be noted that the authors do

not intend to produce a sensitivity ranking from which certain thresholds can be derived, such as SSD curves. We rather provide the central element for combined sensitivity-trait-indicators to reflect community sensitivity.

4.3. Materials and methods

The $S_{hydrocarbons}$ sensitivity ranking proposed here was developed based on available datasets – highlighting the importance of data availability. $S_{hydrocarbons}$ is designed to indicate hydrocarbon/refinery effluent contamination. Calculation of the sensitivities is based on acute laboratory, rapid test and mesocosm toxicity values for single taxa within the major taxonomic macro-invertebrate groups. The $S_{hydrocarbons}$ ranking was calculated based on toxicity data (median lethal concentrations, LC50) for compounds found in crude oil or petroleum distillates in combination with toxicity data for middle distillate single blend mixed with water as a model compound representing contamination by refinery effluents. Middle distillate single blend is a fraction that corresponds to kerosene with regard to the composition of hydrocarbons. We reviewed results on the median concentrations of hydrocarbons in refinery effluents discharging to the freshwater environment in Europe (Cailleaud et al. 2015, Leonards et al. 2010), which showed that a middle distillate single blend contains similar hydrocarbons with regard to the median refinery effluent discharging to the freshwater environment. Furthermore, toxicity data from mesocosm experiments performed at Lacq by TOTAL for light distillate single blend, other middle distillates single blend, and xylene (unpublished data) were added to the dataset. Light distillate single blend corresponds to the hydrocarbon composition in gasoline while other middle distillates single blend include the faction corresponding to diesel. Results from the CONCAWE study (Cailleaud et al. 2015, Leonards et al. 2010) allowed us to select middle distillate single blend as a model compound representing the hydrocarbon content in most refinery effluents. As toxicity data availability is restricted, we additionally used toxicity information from available mesocosm studies (UFZ & TOTAL PETRO SPEAR project, unpublished results; TOTAL, a previous mesocosm study, unpublished results) testing light and middle distillate single blend hydrocarbon mixtures. These also contain hydrocarbon blocks found in refinery effluents. These compound groups were added to the list of single compounds found in crude oil or petroleum distillates as they reflect relevant information on mixture toxicity.

4.3.1. Data set description

Literature data

The sensitivity calculation is based on datasets of toxicity studies (Table S2) stemming from various sources of literature. Published toxicity data were brought together from the U.S. Environmental Protection Agency (US EPA) database ECOTOX (2013) (124 studies), as well as from the OECD (Existing Chemicals Database (2013)), Environment Canada (First Priority Substances List (2013)), and ESIS (European chemical Substances Information System (2013)) databases (25 studies), among others including data from the International Uniform Chemical Information Database (IUCLID) (formerly from the European Chemicals Bureau European Chemicals Agency (ECHA), now from the European Chemicals Agency (ECHA)), which again comprise single literature sources.

The US EPA ECOTOX database was accessed in 2011 and 346.276 studies were extracted and processed in the following way: Only freshwater studies testing aquatic macroinvertebrates were kept, studies with missing indication of test duration or concentration unit were disregarded, only the endpoints LC50, LD50, EC50 and ED50 with concentration units that are convertible into µg/L (this means, excluding dose units) and with test durations from 1-5 days (in 12 h steps) were used. In a next step, a data search according to CAS numbers was conducted for 52 hydrocarbon substances found in crude oil (Table S1). Data from the additional sources mentioned above were added. In total, results of 149 studies were included in the analyses. The test durations of the toxicity studies were 48 and 24 h, in 60.4 and 39.6 % of the studies, respectively. In order to obtain a dataset of toxicity data with homogeneous exposure times, the LC50s for the 24h test duration were extrapolated to 48 h by recalculation of the effect concentrations according to the equation of linear regression between the LC50s at 24 h and at 48 h. For this, substances with *D. magna* tests available for both 24 and 48 h were selected. The median effect concentration for each substance was determined both for 24 and for 48 h tests. These median concentrations were log10-transformed, tested for linearity, and plotted. In a next step, the equation of linear regression ($y = -0.1157 + 0.9606 * x$) was applied to predict 48h effect concentrations for those substances for which only 24h studies were available. This approach was conducted following von der Ohe and Liess (2004). The linear regression applied was $y = 0.905x+0.090$ with $r^2 = 0.69$ and $p < 0.05$ ($n = 66$) (Figure S1). The list of compounds included in the calculation of the $S_{hydrocarbons}$ values consisted of 29 petroleum compounds, 7 alkanes, 2 cycloalkanes, 6 alkenes, and 8 mono-aromatics (Table S1). Regarding the frequency of appearance of the

different compounds in the data set, the most frequent compounds were: Benzene (45), Toluene (22), Styrene (17), Ethylbenzene (11), and Cumene (11) (number of tests in parentheses). The mode of action of these organic compounds on macroinvertebrates is narcotic (Abernethy et al. 1986, Veith et al. 1983).

Polycyclic aromatic hydrocarbons (PAHs) were not included in the analysis. They have a much higher toxicity than all other compounds as shown in the present study (median LC50 PAHs = 272 µg/L, median LC50 alkanes = 11,921 µg/L, median LC50 other compounds = 13,559 µg/L). However, toxicity data is very limited, and thus, underrepresented. Therefore, inclusion of PAHs studies would result in a biased S-classification, as those taxa, for which PAH toxicity information is available, would demonstrate higher sensitivity in comparison to those taxa, for which no PAH values exist. This study developed a system for petrochemicals in general, without considering compound-specific toxic mode of actions. The consideration of PAHs would require further toxicity testing in the laboratory and was beyond our aims.

Rapid test data

The middle distillate single blend data were adopted from two rapid test studies (Schröttle et al., unpublished results and TOTAL, Lacq, France, unpublished results). These are studies in which a series of acute laboratory toxicity tests were performed with different invertebrate taxa previously collected in the field without having to cultivate these taxa in the laboratory. The method is in accordance with the rapid toxicity testing method suggested by Kefford et al. (2005). In both studies, toxicity of middle distillate single blend was determined as LC50 values in rapid tests with test durations from 24 to 72 (in data originating from Schröttle et al. UFZ – Helmholtz Centre for Environmental Research) or 96 h (in data originating from TOTAL). However, for the present study, only the LC50s from 24 and 48 h tests were used (Table S3). Macroinvertebrate organisms for the rapid tests were obtained directly from field pristine sites without previous culturing. Actual middle distillate single blend concentrations in the tests were determined analytically.

Mesocosm data

Additional middle distillate single blends, light distillate single blend and xylene toxicity information was obtained from mesocosm studies conducted at UFZ and at PERL (TOTAL) (unpublished results) (Table S4). These were mostly on the taxonomic level of family. The following substances were tested in the mesocosm: Middle distillate single blend 1 tested in the PETRO-SPEAR project at UFZ; Middle distillate single blend 1 tested in the PETRO-

SPEAR project at PERL (TOTAL); Middle distillate single blend 2 tested at PERL (TOTAL); Middle distillate single blend 3 tested at PERL (TOTAL); Light distillate single blend tested at PERL (TOTAL); Xylene tested at PERL (TOTAL) (unpublished data). Macroinvertebrate abundance data (i.e. number of individuals at different levels of contamination) were transferred into effect concentrations (i.e. LC50 values) via the Excel Makro REGTOX (Vindimian 2001, Vindimian 2005). REGTOX allows for the calculation of dose-response parameters by applying the models Hill equation, Log-Normal, or Weibull, of which we used the Log-Normal model. For Middle distillate 3, only chronic EC50s (lethal effect) could be derived. These chronic EC50s were then scaled to acute toxicity by applying an A/C ratio of 4.47 (Redman et al. 2012). For 6 substance-taxon-combinations, EC50 values could be obtained with 95% confidence intervals (CI). Only these EC50s were used in this study. For another 12 combinations, EC50s could be calculated but the determination of the 95% CI was not possible.

The mesocosm data have been obtained for individual taxa that have colonized the stream mesocosms with exposure to both the soluble and the insoluble fractions of distillate single blends. The reference toxicity threshold for *D. magna*, on the other hand, has been obtained for a water accommodated fraction (WAF) containing only the water soluble fraction of the hydrocarbon mixture (OECD 2000). In future studies these methods should be harmonized. The impact of using these values generated via different exposure methods could not be assessed in the present study due to data constraints. Because tests with different exposure methods were used, the approach and calculated ranks should be reanalyzed as the data base becomes larger and more consistent.

The distribution of all LC50 values (48 h) combined from the above described datasets is shown in Figure S2.

4.3.2. Calculation of the sensitivities

Essentially, the sensitivity ranking is a taxon-specific sensitivity relative to the sensitivity of the standard toxicological test species *D. magna* calculated following formula [1] (Wogram and Liess 2001):

$$S_i = \log \frac{LC50_{\ Daphnia\ magna}}{(E)LC50_i} \qquad [1]$$

with S_i = relative sensitivity of a taxon *i* towards a certain toxicant (i.e. $S_{hydrocarbons}$ value of taxon *i*), $LC50_{Daphnia\ magna}$ = experimental LC50 for *D. magna*, and $(E)LC50_i$ = experimental EC50 or LC50 for taxon *i* for a certain toxicant.

The authors chose to standardize the taxon-specific sensitivity with the sensitivity of *D. magna*, as this is the species for which most laboratory-based toxicity information is available and it is among the most sensitive aquatic macroinvertebrate species. However, our dataset demonstrates that *D. magna* toxicity test results for single substances can have high variation, which will directly influence the ranking (see exemplarily the test results for decane in Table S2). An alternative option could be a standardization with the average community sensitivity (HC50) (Rubach et al. 2010). However, toxicity information for entire communities is quite limited, rendering this approach difficult to apply in practice. Deriving the HC50 from a too small dataset will also result in uncertainty (Newman et al. 2000).

A value of zero thus indicates sensitivity equal to that of *D. magna*. For taxa more sensitive than *D. magna*, the *S*-value is greater than zero; for less sensitive taxa, the value is smaller than zero. Because the *S*-values are expressed logarithmically, a score of one means that the taxon in question is, 10-fold more sensitive than *D. magna* towards a particular substance.

When in the literature dataset a study occurred multiple times with the same taxon, substance, test duration, endpoint, and author, the median of the effect concentrations (i.e. (E)LC50 values) within each reference was determined. When multiple test values were found for one combination of taxon and substance, the median of the effect concentration values was taken, combining different references.

The LC50s stemming from the rapid tests (middle distillate single blend 1) and from the mesocosm tests (light distillate single blend, middle distillate single blend 1/2 and 3 and xylene) were added in a final step. The addition of rapid test LC50s expanded our data set by 21 toxicity values and the addition of mesocosm test EC50s by another 18 toxicity values.

In the next step, the relative sensitivity (S_i) was calculated for each taxon-substance combination in accordance to formula [1], reflecting each taxon's sensitivity towards each substance in relation to *D. magna*. Finally, the sensitivity values of all toxicants tested towards one taxon (*S*) were aggregated as the arithmetic mean, as shown in formula [2].

$$S = \overline{\sum_{i=1}^{n} S_i} \qquad [2]$$

with *S* = relative sensitivity of a taxon *i* towards all toxicants tested with this taxon, S_i = relative sensitivity of a taxon *i* towards a certain toxicant, and *n* = number of toxicants per taxon.

This was done on the taxonomic levels of genus, family, and order. Toxicity information on species level originates mainly from the literature sources listed in paragraph „Literature data“ (Table S2). Rapid test data were both on species and family level (with one taxon on order and one on class level) (Table S3). Mesocosm data, however, were (except for Oligochaeta) available solely on family level (Table S4). For the classifications on family and order level, different species and genus of the same family or order, respectively, were considered. As entries in taxa lists of field or mesocosm studies usually comprise various taxonomic levels, having at hand *S*-values for the taxonomic levels of genus, family, and order allows applying the value of the most appropriate taxonomic level.

As not every compound is tested with every taxon, the sensitivity of a taxon was calculated only when at least two different substances had been tested for the same taxon. This approach was chosen to avoid having no restriction for data use with consideration of all available information. In this way, substance-specific biases can partly be mitigated. The use of the restriction resulted in different sets of taxa and compounds included in the analyses at different taxonomic levels, since the maximum amount of accessible information was used at each level.

The S-values of the taxon “Planariidae” is reported in Figure 1 and Table S5 but was not applied in the validation. Due to the implausibly high S-value, this family level taxon was considered an artifact in the mesocosm experiments. Instead, the next higher taxonomic level “Tricladida” was applied.

A description of the toxicity information used and integrated into the S-values is shown in Table 1. The table demonstrates that the mesocosm data gets relevant only on family level. The order level is rater an integration of the families into fewer groups; only few additional tests are added on order level. Note that only taxa with >1 chemical are considered, except if a single rapid test value is available.

Table 1. Description of the toxicity information integrated into the S-values on genus, family, and order level. Indicated are the number of studies used and the respective number of chemicals tested in these studies. Within these studies, the number of rapid test and mesocosm studies is indicated. The number of genera or families on which the next level S-values are based is given.

Taxon	Number of studies	Number of chemicals	Number of rapid tests	Number of mesocosm tests	Number of genera	Number of families
Genus level						
Aedes	2	2	0	0	NR	NR
Amphimelania	2	2	0	0	NR	NR
Asellus	4	3	2	0	NR	NR
Baetis	1	1	1	0	NR	NR
Brachionus	3	3	0	0	NR	NR
Calopteryx	1	1	1	0	NR	NR
Ceriodaphnia	4	4	0	0	NR	NR
Chironomus	2	2	0	0	NR	NR
Cloeon	1	1	0	0	NR	NR
Corixa	1	1	0	0	NR	NR
Culex	2	2	1	0	NR	NR
Daphnia	23	22	2	0	NR	NR
Dugesia	1	1	0	0	NR	NR
Erpobdella	1	1	0	0	NR	NR
Gammarus	4	3	2	0	NR	NR
Hyalella	1	1	0	0	NR	NR
Hydra	1	1	0	0	NR	NR
Ischnura	1	1	0	0	NR	NR
Lymnaea	2	2	0	0	NR	NR
Nemoura	1	1	0	0	NR	NR
Orthocladinae	1	1	1	0	NR	NR
Family level						
Asellidae	4	3	2	0	1	NR
Baetidae	4	3	2	1	1	NR
Brachionidae	3	3	0	0	1	NR
Calopterygidae	1	1	1	0	1	NR
Chironomidae	4	3	1	1	2	NR
Coenagrionidae	1	1	0	0	1	NR
Ceratopogonidae	1	1	0	1	0	NR
Corixidae	1	1	0	0	1	NR
Culicidae	3	3	1	0	2	NR
Daphniidae	23	22	2	0	1	NR
Erpobdellidae	1	1	0	0	1	NR
Gammaridae	7	4	4	1	1	NR
Hyalellidae	1	1	0	0	1	NR
Hydridae	1	1	0	0	1	NR
Lymnaeidae	2	2	0	0	1	NR
Nemouridae	1	1	0	0	1	NR
Planariidae	2	2	0	1	1	NR
Simulidae	2	1	2	0	0	NR
Thiaridae	2	2	0	0	1	NR

Order level						
Amphipoda	6	3	4	0	NR	1
Arhynchobdellida	1	1	0	0	NR	1
Basommatophora	2	2	0	0	NR	1
Diplostraca	23	22	2	0	NR	1
Diptera	6	3	4	0	NR	3
Ephemeroptera	3	2	2	0	NR	1
Heteroptera	1	1	0	0	NR	1
Hydroida	1	1	0	0	NR	1
Isopoda	4	3	2	0	NR	1
Neotaenioglossa	2	2	0	0	NR	1
Odonata	1	1	0	0	NR	1
Plecoptera	1	1	0	0	NR	1
Ploima	3	3	0	0	NR	1
Tricladida	4	2	3	0	NR	1
Zygoptera	1	1	1	0	NR	1

4.3.3. Performance of $SPEAR_{hydrocarbons}$ assessment

A first validation was conducted with a previous field study (Beketov and Liess 2008) that assessed the sum of hydrocarbons in freshwater samples as well as the $SPEAR_{organic}$ values of macroinvertebrate communities sampled. Here, $SPEAR_{organic}$ was applied, which is derived from the $S_{organic}$ values, and thus, only based on literature data for organic toxicants in general without inclusion of rapid test or mesocosm data or other traits. In this study, $SPEAR_{organic}$ had shown high correlation to synthetic surfactants and petrochemicals. Other biological indices (EPT taxa richness, overall taxa richness, and Shannon's diversity index H') used in that study did not respond well to these contaminants. We applied the $S_{hydrocarbons}$ ranking to calculate a new version of the *SPEAR* indicator (see formula [3]). This new $SPEAR_{hydrocarbons}$ version is built with toxicity information specific for contamination by hydrocarbon refinery effluents. The original $SPEAR_{organic}$ indicator describes the average $S_{organic}$ sensitivity of the invertebrate community to organic toxicants in general, and likewise, the new version reflects the average $S_{hydrocarbons}$ sensitivity.

$$SPEARhydrocarbons = \frac{\sum_{i=1}^{n} \log(xi+1) * S_i}{\sum_{i=1}^{n} \log(xi+1)} \qquad [3]$$

where $SPEAR_{hydrocarbons}$ is the average community sensitivity towards contamination by hydrocarbon refinery effluents, x_i is the abundance of taxon *i*, S_i is the $S_{hydrocarbons}$ sensitivity of taxon *i*, and *n* is the number of taxa in the sample.

$SPEAR_{hydrocarbons}$ was determined with the $S_{hydrocarbons}$ values of (i) the lowest possible taxonomic level and (ii) family level. Similar as in the earlier $SPEAR_{organic}$ study, the relationship between toxicant concentration and $SPEAR_{hydrocarbons}$, was tested via linear regression analyses. This allows a later comparison between the two levels of identification, the latter of which would be more convenient to be used in future biomonitoring studies.

4.3.4. Statistical analyses

Linear regression was applied for the extrapolation of 24 h LC50 values to 48 h, after visually testing for linearity of the log10-transformed 24 h as well as 48 h values via histograms and of residuals via qqnorm-plots (Dormann and Kühn 2009).

Furthermore, Spearman's rank sum correlation coefficients between toxicant concentration and $SPEAR_{hydrocarbons}$ on the lowest level and family level of identification were determined.

Comparison of the $S_{hydrocarbons}$ values with the existing $S_{organic}$ values derived for organic toxicants in general (von der Ohe and Liess 2004) for each taxon was also performed by non-parametric Spearman correlation.

All statistical analyses were performed in the free and open source software R (R Development Core Team).

4.4. Results and discussion

The extrapolation of 24 h LC50 values to 48 h (Figure S1) facilitated the compilation of the toxicity data set from which the $S_{hydrocarbons}$ ranking was derived (Figure 1). The dataset included information from literature, rapid tests, and mesocosms studies testing middle distillate single blend 1/2, light distillate single blend, xylene, and middle distillate 3 single blend.

The most sensitivity taxon in the ranking was Daphnidae (when excluding Planariidae as an outlier) and the least sensitive taxa were Lymnaeidae and Ceratopogonidae.

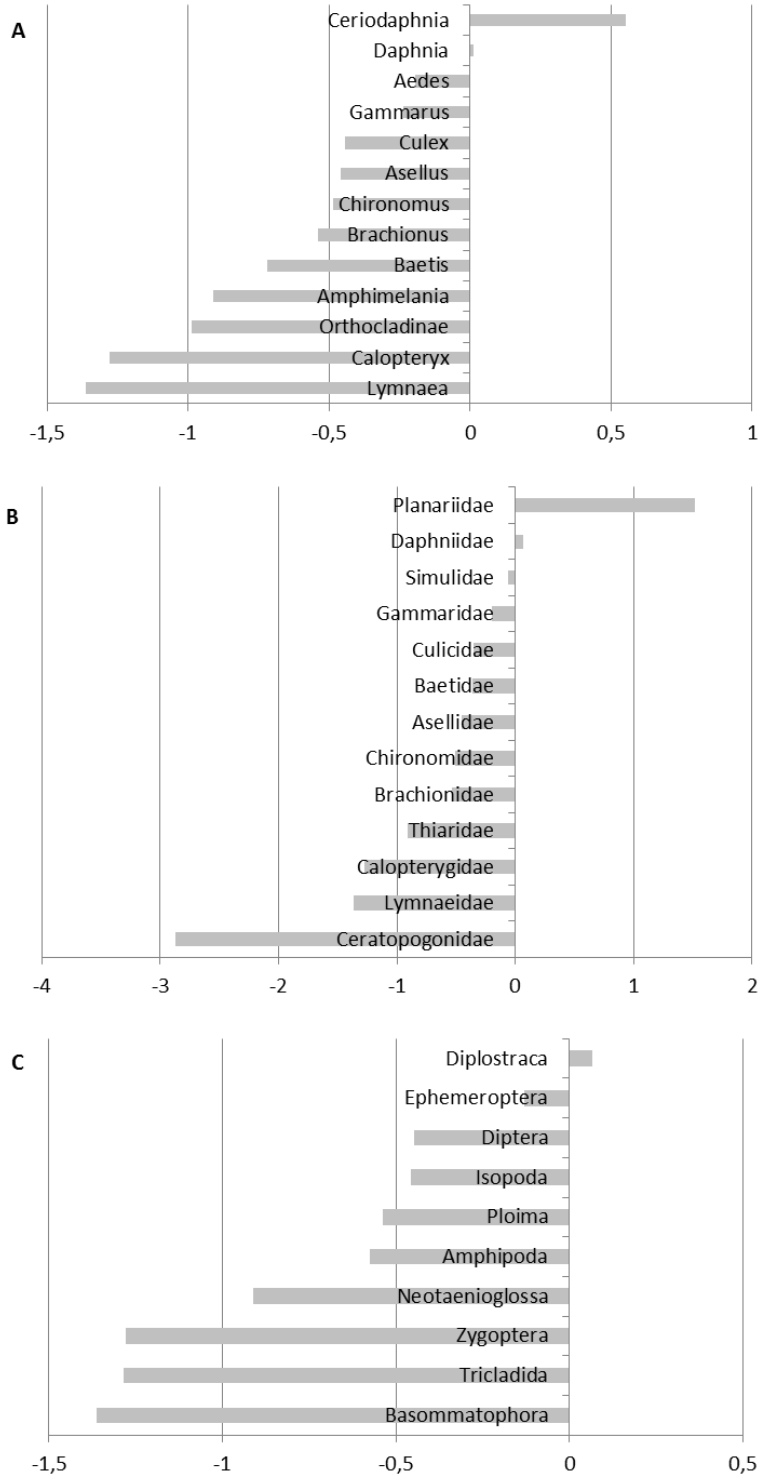

Figure 1. The sensitivity ranking $S_{hydrocarbons}$ for the genus (a), family (b), and order (c) levels based on literature, rapid tests, and mesocosm studies with middle distillate single blend, light distillate single blend, xylene, and middle distillate 3 single blend. A $S_{hydrocarbons}$ value of zero indicates a sensitivity equal to *D. magna*. For taxa more sensitive than *D. magna*, the *S*-value is greater than zero; for less sensitive taxa, the value is smaller than zero. Because the *S*-values are expressed logarithmically, a score of one means that the taxon is, on average, 10-fold more sensitive than *D. magna* towards a particular substance. The taxon "Planariidae" on family level should be considered an outlier.

The correlation coefficient between the toxicant concentrations and $SPEAR_{hydrocarbons}$ for the lowest level of identification was rho = -0.734, $p < 0.001$ (Figure 2) and rho = -0.769, $p < 0.001$ for family level of identification in the validation study (Beketov and Liess 2008). This demonstrates that $SPEAR_{hydrocarbons}$ shows good correlation with the field concentration of hydrocarbons even though the correlation between $SPEAR_{organic}$ and the concentration of hydrocarbons was higher in the original study ($r^2 = 0.68$, $p < 0.05$) (Beketov and Liess 2008).

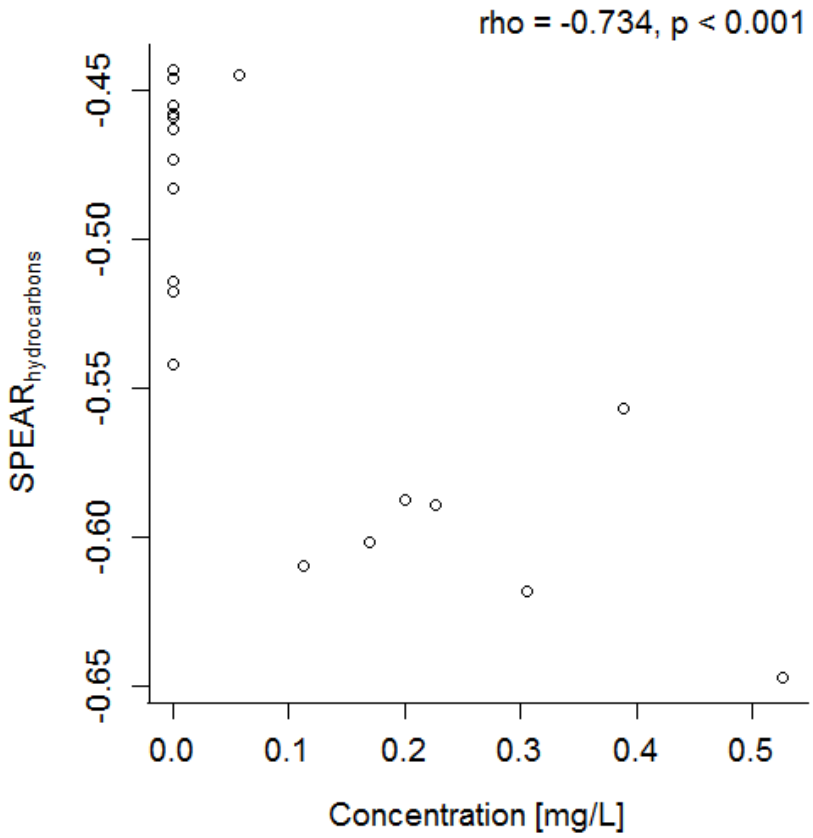

Figure 2. Comparison between the SPEAR values obtained for each sampling site based on the $S_{hydrocarbons}$ ranking (lowest taxonomic level) and concentrations of hydrocarbons [mg/L] for the validation study by Beketov and Liess (2008). Non-parametric Spearman's correlation gives a rho = -0.734, $p < 0.001$, $n = 19$. A $S_{hydrocarbons}$ value of zero indicates a sensitivity equal to *D. magna*. For taxa more sensitive than *D. magna*, the *S*-value is greater than zero; for less sensitive taxa, the value is smaller than zero. Because the *S*-values are expressed logarithmically, a score of one means that the taxon is, on average, 10-fold more sensitive than *D. magna* towards a particular substance. Reference conditions are plotted at a concentration of 0 mg/L.

Comparison of the new ranking with the previously computed general ranking for all organic toxicants, $S_{organic}$ (von der Ohe and Liess 2004) (Figure 3), shows that the distribution of the sensitivity to organic toxicants in general and to hydrocarbons is significantly but not strongly

correlated (rho = 0.662, $p < 0.005$). Thus, the $S_{hydrocarbons}$ values can hardly be predicted from the $S_{organic}$ values.

When comparing the ranking order of the taxonomic groups for the $S_{hydrocarbons}$ (excluding "Planariidae") and the $S_{organic}$ ranking, Daphnidae and Gammaridae are among the most sensitive taxa in both rankings. Zygoptera and Lymnaeidae/Basommatophora are the least sensitive taxa.

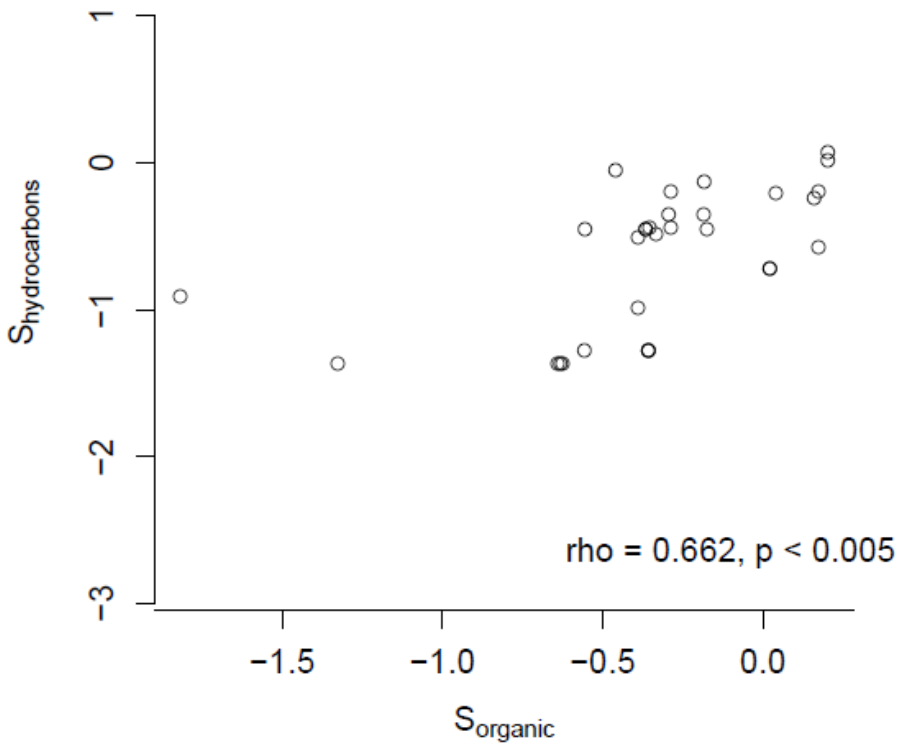

Figure 3. Non-parametric Spearman correlation between $S_{organic}$ values and $S_{hydrocarbons}$ values. One data point represents one taxon (species, genus, family or order level) from the $S_{hydrocarbons}$ ranking and its respective $S_{organic}$ value (rho = 0.662, $p < 0.005$, $n = 31$).

4.5. Conclusion

The present study presents the first sensitivity ranking for freshwater invertebrates towards hydrocarbons. This ranking, being based on extensive analysis of the existing ecotoxicological data, represent a basis for the future development of SPEAR-type bioindicators that can universally be applied to specifically assess contamination with hydrocarbons. To this end, we tested the newly developed S-ranking as central element of a SPEAR-type bioindicator.

The first version of such a SPEAR-type bioindicator based on the $S_{hydrocarbons}$ ranking only (i.e. without any additional eco- and biological traits), which is presented here as

$SPEAR_{hydrocarbons}$, showed good correlations with hydrocarbon contamination. $SPEAR_{organic}$, which was applied in the original study, still has a higher correlation with hydrocarbon contamination. This is expectedly due to the larger dataset that $SPEAR_{organic}$ is based on.

The newly developed $S_{hydrocarbons}$ ranking does not serve to distinguish between toxicity caused by hydrocarbons and toxicity caused by other organic contaminants. Instead, the $S_{hydrocarbons}$ was rather a first trial to develop a more specific sensitivity classification based on a more specific set of compounds. The mode of action of hydrocarbons is narcotic but also carcinogenic and mutagenic, resembling excess toxicity. Excess toxicity means that toxicity is higher than expected with narcotic processes (in case of PAHs) (Prabhukumar and Pagilla 2010). $S_{organic}$ on the other hand, based on toxicity data of organic contaminants in general, contains a large number of modes of action (others being e.g. effects on acetylcholinesterase, sodium channel or the electron transport chain) (von der Ohe et al. 2005). For this reason a more specific sensitivity classification is needed.

The present study, however, reveals that the existing database is still limited. It also shows that supplementing the available literature data with LC50s obtained from rapid and mesocosm tests can enhance the dataset.

The final evaluation of the sensitivity ranking developed here will require (i) further validation by applying the new S-values to other field studies and also to mesocosm experiments, (ii) validation of other bioindicators (besides $SPEAR_{hydrocarbons}$) based on the $S_{hydrocarbons}$ ranking, and (iii) improvements of the ranking with additional information like biological traits. Thus, further improvement of the sensitivity ranking presented here requires experimental studies with a wider range of taxonomic groups. The taxonomic groups that were well covered by the present study are Diptera, Gastropoda, and Crustaceans. The groups that were poorly present in the analyses were mainly among the insects: Ephemeroptera, Trichoptera, Zygoptera, Heteroptera, but also Isopoda and Tricladida/Planariidae. The groups that were not present in the analyzed data were e.g. Plecoptera, Anisoptera, Coleoptera, Bivalvia, and Oligochaeta. Especially, adding more toxicity data for such groups as Trichoptera, Ephemeroptera, Plecoptera, and other insects will improve reliability of the sensitivity values.

Also of importance is to gain knowledge about the toxicity of PAHs towards all groups of invertebrates in order to allow the inclusion of this group of toxicants into the rankings. With the current data available, an inclusion of this important group of toxicants is not possible, as argued in the Material and Methods section. All this missing information could be quickly and easily derived using the rapid toxicity test method with field-collected invertebrates

(Kefford et al. 2005, Schröttle et al. unpublished results). Furthermore, differences in exposure methods (emulsion versus WAF) in mesocosm tests should be harmonized between test species and reference species (*D. magna*) for application in future studies. To make existing data more available, we furthermore call for increased data sharing of toxicity test results.

4.6. Acknowledgements

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Chapter 5: "Metal toxicity affects predatory stream invertebrates less than other functional feeding groups"

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5.1. Abstract

Ecosystem effects of heavy metals need to be identified for a retrospective risk assessment, and potential impacts need to be predicted for a prospective risk assessment. In this study, we established a strong correlation between the toxic pressure of dissolved metals and invertebrate species. We compiled available data from a wide geographical range of Australian streams that were contaminated with heavy metals [mainly copper (Cu) and zinc (Zn)] and the corresponding invertebrate communities. Heavy metal toxicity is positively related to the proportion of predators within the invertebrate community, represented by the $predator_{ratio}$, with an effect threshold range of 2.6 µg/L - 26 µg/L for Cu and 62 µg/L - 617 µg/L for Zn. These effect concentrations are in the ranges of the concentrations identified in model ecosystems and other field investigations and are just above the existing guideline limits. Heavy metals also affects the taxa richness negatively. Other community measures, such as the evenness, number of EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa, SPEcies At Risk ($SPEAR_{pesticides}$ or $SPEAR_{salinity}$) were relatively poorly correlated with heavy metal toxicity in the streams. Therefore, we suggest applying the $predator_{ratio}$ within the community as a starting point for an indicator of the dissolved metal toxicity, the $SPEAR_{metals}$.

5.2. Introduction

Emissions, especially from mining (Nriagu and Pacyna 1988) but also from urban catchments (Sharley et al. 2016) and landfills (Naveen et al. 2017) cause heavy metal contamination of freshwater ecosystems. Streams receiving contamination generally reveal effects of heavy metals on the species composition and related ecosystem services. Examples include streams in Asia (Iwasaki et al. 2009, Qu et al. 2010), Australia (Edwards 2002, Norris et al. 1980), Europe (Ehrman et al. 2008, Kuzmanovic et al. 2016, Rehfeldt and Sochtig 1991), and North America (Clements 1994, Kiffney and Clements 1994). Accordingly, retrospective and prospective risk assessments require the ability to reveal the ecological effects of metals within ecosystems.

The current ability to link exposure of metals with effects on the community structure in streams is not satisfactory. A ranking of invertebrate sensitivity has been determined via acute laboratory tests (von der Ohe and Liess 2004, Wogram and Liess 2001). However, the acute sensitivity to the metals identified in standard laboratory tests does not predict the chronic effects of low doses in the field (Poteat and Buchwalter 2014) as generally supported by the

current study. The concentrations required to induce effects on laboratory populations are often higher than those that impact invertebrate communities in the field (Buchwalter et al. 2007). However, when considering feeding type of test organisms some investigations revealed that herbivore and detritivore species were more affected than predators by metal contamination (Clements 1994, Kiffney and Clements 1994, Leland et al. 1989, Qu et al. 2010).

Accordingly, the aim of the present investigation was to identify if functional feeding groups could be used to determine the field-relevant vulnerability of autochthonous species. We therefore aimed to establish a link between heavy metal pollution and various descriptors of community structures in stream ecosystems by considering a large number of sites with a wide geographical distribution by the re-evaluation of three existing datasets.

5.3. Methods

5.3.1. Field data sources

We collated data from three field studies including 35 sites across a 3500-km north-south gradient in Australia that ranged from tropical (Northern Territory) to temperate (Tasmania) latitudes (Edwards 2002, Norris 1986, Norris et al. 1980). The data included metal pollution measured in water and associated stream macroinvertebrate communities. These datasets were selected because the only anthropogenic disturbance was metal pollution, and the measured metal toxicity in the water and filtrate varied over 3 orders of magnitude between the sites in terms of toxic units (TUs) relative to the reference species *Daphnia magna* (Table S1). The most relevant heavy metals in terms of the TUs were copper (Cu) > zinc (Zn) > cadmium (Cd) > lead (Pb) for dataset 1 (Norris et al. 1980), Zn > Cu > Cd > Pb for dataset 2 (Norris 1986), and Cu > Zn > cobalt (Co), nickel (Ni) > iron (Fe) > manganese (Mn) for dataset 3 (Edwards 2002). An overview of the maximum and mean concentrations of each metal with the respective TU is given in Table S2. Site numbers (see also Figure 1) correspond to the sampling sites of datasets (details below) in the following order: Norris 1980 study sites 1-8, Norris 1986 study sites 1-10, and Edwards study sites EB2(A), EB2(B), EB4(A), EB4(B), EB4S(A), EB4S(B), EB5I(A), EB5I(B), EB8(A), EB8(B), EB8(C), FC(A), FC(B), FR5, FR6, LFR8, and LFR9. Note that sites 18, 25 and 26 are outside the axis limits. Site 18 has a strong positive relationship to HCO3, and sites 25 and 26 are related to the predator ratio.

Dataset 1 (Norris et al. 1980) contains eight sites along a 180-km section of the South Esk River in northeast Tasmania (41°S, E147°E). The climate in the region is maritime cool temperate, and the major land uses are native forestry and mixed farming with mostly cattle grazing. At each site, both heavy metals and invertebrates were sampled on 10 occasions over a 2-year period. Metal pollution originated from two tin and tungsten mines that commenced operations in 1892 and 1930 near the town of Rossarden. Ore was processed locally to separate tin and tungsten. Until 1959, the mines discharged tailings directly into tributaries of the South Esk River; then, the tailings were impounded in settling ponds. Of the eight sites, three were located in the South Esk River upstream of the tributary carrying metals from the mines, and the remaining five were located downstream of this tributary. The metal concentrations of Cd, Zn, Cu and Pb were measured in the water, filtered water and non-filterable residue at 10 sites using an atomic absorption spectrophotometer (Pye Unicam model SP 1950, Cambridge, UK) (Norris et al. 1981). Measurement of the mercury (Hg), Ni, Co and chromium (Cr) concentrations was initially performed but was discontinued because these metals were only detected at concentrations near zero or below the detection limits (Norris et al. 1981). We identified the mean and maximum concentrations of each metal recorded at each site over the duration of the study as shown in Table 1 of Norris et al. (Norris et al. 1981). The most relevant metals in this study were Cu and Zn, with ranges of 2.5 to 9 and 18.9 to 194 µg/L, respectively. The total alkalinity ranged from 21 to 43 mg/L of calcium carbonate ($CaCO_3$). Macroinvertebrate samples were collected concurrently at the eight sites with the metal samples using an airlift sampler and were mostly identified to the species level (Norris et al. 1982). All species collected at each site were used for the statistical analysis, as shown in Table 1 of Norris et al. (1982).

Dataset 2 (Norris 1986) is from the Molonglo River in New South Wales and the Australian Capital Territory near Canberra in southeast Australia (36°S, 149°E). The climate of the region is continental cool temperate. Mining began at Captains Flat in 1882, with major pollution of the Molonglo River chiefly from zinc occurring between 1938 and 1962 (Weatherley et al. 1967). Remediation commenced in 1974 and reduced the metal contamination. One site was upstream of the mine, whereas the other 9 sites were downstream. The dataset we used comprised the 10 sites, which were each sampled twice to measure Zn, Cu, Pb and Cd in the water and filtrate and analysed using an atomic absorption spectrophotometer. Stream macroinvertebrates were sampled with 5-min hand-net (500 µm) collections (Weatherley et al. 1967). The specimens were sorted and identified mostly to the species level in the laboratory. We calculated the total abundance of each invertebrate species

and the mean metal concentration of both sampling time points. The most relevant metals were Cu and Zn, which occurred in the range of 4.8 to 72.6 and 11.5 to 19,700 µg/L, respectively. For the second dataset, alkalinity in terms of $CaCO_3$ was extrapolated from the measured bicarbonate (HCO_3) concentration to correct the metal concentrations for alkalinity according to the Australian and New Zealand guidelines for fresh and marine water quality (ANZECC and ARMCANZ 2000). This extrapolation was based on the regression between the $CaCO_3$ and HCO_3 measurements of dataset 3 (Edwards 2002). A $CaCO_3$ range of 13.42 to 164.71 mg/L was determined.

Dataset 3 (Edwards 2002) originates from the Finniss River (13°S, 131°E) and its tributaries in the Northern Territory, Australia. Unlike the other sampling locations, this river system is located in the wet-dry tropics where most rain falls during the monsoonal summer. During the dry winters, the Finniss River typically disappears or dries to a series of water holes with no surface flow between them. The Finniss River East Branch was polluted by acid rock drainage from a copper and uranium mine at Rum Jungle commencing in the 1950's and continuing until the early 1970's (Jeffree et al. 2001). Remediation began in 1982 and resulted in a substantial decrease in the heavy metal concentrations and an improvement in the fish communities (Jeffree et al. 2001). The dataset analysis here consisted of 17 sites, 10 of which were upstream of any metal pollution; the remaining 7 sites were downstream of the mine and varied in the proportion of their flow originating from the polluted Finniss River East Branch. Each site was sampled and analysed to determine the total Fe, Cu, Mn, Zn, Co and Ni concentrations in the water and filtrate using an atomic absorption spectrophotometer (Appendix 2a of Edwards (Edwards 2002)). In this river system, the metals with the highest toxicity were Cu and Zn in most cases (Table S2), ranging from 1.5 to 1,800 and 3.8 to 3,900 µg/L, respectively. The stream invertebrate abundances were recorded for 8 sampling time points between August 1994 and September 1995. The invertebrates were mostly identified to the family level (except for Chironomidae to the sub-family level and Acarina, Oligochaete and Nematoda, which were not identified further). The statistical analyses were based on the average taxa abundances.

5.3.2. Calculating metal toxicity in streams

Each site from the three studies was characterised according to the toxic pressure of the metals. The environmental concentrations of the metals were scaled to the acute effects on *D. magna* measured in TUs (Sprague 1970) by dividing the metal concentration by the respective

48-h median effect concentration (EC50) for *D. magna*. Subsequently, the toxic pressure of the metals for each site was given as the mean TU (mean of the TU values across all metals measured at that site within this study), highest TU (maximum of the TU values across all metals measured), sum TU (sum of all TU values across all metals measured), and mean of the 3 highest TUs (mean of the TU across the 3 metals with the highest TU values). Because the highest TU was the best proxy when correlating the TU and the proposed SPEcies At Risk from the metal ($SPEAR_{metals}$) index (see Table S4), the results shown here relate only to the highest TU. Only water concentrations were available to relate to the community structure. Sediment concentrations, and especially metal concentration in food as the main source of the internal metal loads (Poteat and Buchwalter 2014), may have improved the relation to environmental contamination. Additionally, we did not use the corrected metal toxicity based on the hardness according to (ANZECC and ARMCANZ 2000) as this measure of metal toxicity is less related to the observed effects on invertebrates in the field. This is in line with the finding of (Markich et al. 2005) that the use of a generic hardness-correction is not assessing the toxicity of metals to freshwater species. Therefore, we only report statistical analyses based on the measured concentrations.

5.3.3. Grouping of taxa according to ecological traits

Ecological trait information on feeding type was collected from journal articles, online databases and identification keys, including the Australian Freshwater Invertebrates Guide of the Murray-Darling Freshwater Research Centre (http://www.mdfrc.org.au/bugguide/index.htm), the Digital Key to Aquatic Insects of North Dakota (http://www.waterbugkey.vcsu.edu/php/mainkey.php), Schäfer et al. 2011 (Schäfer et al. 2011), and the Operationelle Taxaliste (http://www.fliessgewaesserbewertung.de/en/download/bestimmung/). Each taxon was assigned a number between 0 and 10 to indicate the relevance of each feeding type. If the predatory type was ≤5, the taxon was classified as a predator. Additionally, we classified the taxon as a predator if it was classified as a predator in one of the listed sources. The grouping of taxa according to their feeding types is given in Table S3. Obviously, the categorisations for feeding type are not clear-cut borderlines as they also depend on the food available. Nevertheless, we believe that our grouping provides a first approximation to reality.

The physiological trait "sensitivity to metals" was based on the work of Wogram and Liess (Wogram and Liess 2001) in the updated version of Malaj et al. (Malaj et al. 2012). The respective sensitivity (S) values are based on acute laboratory data on metals obtained from the US Environmental Protection Agency (EPA) Ecotox database (http://cfpub.epa.gov/ecotox/). When acute laboratory data for Cu/Zn sensitivity for a taxon were not available then the value of the closest relative was applied as detailed in (von der Ohe and Liess 2004).

5.3.4. Calculation of biological indices

The invertebrate abundances for repeated sampling events were summarised to the mean abundance for each site and taxon. From this summary, the following biological indices were calculated: (1) Taxa richness (TR), which was the total number of taxa (mostly species) collected at a particular site; (2) EPT taxa richness (EPT), which was the number of Ephemeroptera, Plecoptera, and Trichoptera taxa (mostly species); (3) Shannon's diversity index (H'); (4) sensitivity to metals (S_{metal}) (Wogram and Liess 2001) (Malaj et al. 2012); (5) $SPEAR_{pesticides}$ (Liess and von der Ohe 2005); (6) $SPEAR_{organic}$ (Beketov and Liess 2008); (7) $SPEAR_{salinity}$ (Schäfer et al. 2011) (the SPEAR indices were calculated using the online SPEAR calculator; 2013 version; http://www.systemecology.eu/spear/); and (8) the proportion of predators of (a) all taxa and (b) Trichoptera alone, which was the most diverse group. The proportion of predators was calculated according to the following formula:

$$\mathrm{Pr}\,edator_{ratio} = \frac{\sum_{i=n}^{n} \log(x_i + 1) * y}{\sum_{i=n}^{n} \log(x_i + 1)} \qquad [1]$$

where *n* was the number of taxa, xi was the abundance of taxon *i* and y was 1 if taxon *i* was classified as a predator and otherwise was 0.

The taxon-specific metal sensitivity was calculated as the median lethal concentration (LC50) relative to the LC50 of the standard test species *D. magna* using the following formula (Wogram and Liess 2001):

$$S_i = \frac{\log(LC50_{Daphnia\ magna})}{LC50_i} \qquad [2]$$

where S_i was the relative sensitivity of taxon *i* towards a certain metal, $LC50_{Daphnia\ magna}$ was the LC50 for *D. magna* and $LC50_i$ was the LC50 for taxon *i* for a certain metal.

Subsequently, the Community S_{metal} can be calculated according to formula [3] based on the physiological S_{metal} sensitivity values determined by formula [2].

$$\text{Community } S_{metal} = \frac{\sum_{i=1}^{n} \log(xi+1) * S_i}{\sum_{i=1}^{n} \log(xi+1)} \quad [3]$$

where Community S_{metal} is the average community sensitivity towards contamination with dissolved metals, *xi* is the abundance of taxon *i*, Si is the S_{metal} sensitivity of taxon *i* and *n* is the number of taxa in the sample.

The sensitivity calculation was based on a dataset of toxicity studies from the US EPA ECOTOX database (2011). We extracted only studies that met the following criteria: freshwater test; availability of information on the test duration and concentration unit that could be converted into µg/L; test durations of 24 or 48 h; reporting of the LC50, LD50, EC50 or ED50 and testing of inorganic metal salts. The LC50s of studies with 24-h test durations were extrapolated to 48 h following the method of von der Ohe and Liess (2004) (von der Ohe and Liess 2004). Studies that occurred multiple times were averaged by applying the median (first level S-value). Multiple test values for the same combination of taxon and substances were also averaged using the median (second level S-value). Relative sensitivity (Si) values were calculated for each taxon-substance combination according to formula (2) to indicate a taxon's sensitivity towards a certain substance relative to *D. magna*. In the last step, the sensitivity values of all of the metals tested towards a single taxon (S) were averaged by the mean (third level S-value). The results are displayed in Table S3.

5.3.5. Statistical analysis

Pearson's correlation analysis was performed to determine correlations between the highest TU and the S_{metal}, predator to non-predator ratio ($predator_{ratio}$), taxonomic indices TR, EPT, and H' and $SPEAR_{salinity}$, $SPEAR_{organic}$ and $SPEAR_{pesticide}$. The latter three indices were tested to identify whether these indices, which were designed to identify salinity (Schäfer et al. 2011), pesticide contamination (Liess and von der Ohe 2005) and organic pollution (Beketov and Liess 2008), respectively, responded to metal contamination. The differences in proportion of predators in the total community between four groups of metal contamination were tested using ANOVA, followed by pairwise t-tests. All analyses were performed with the R software (http://www.r-project.org/, version 2.15.2).

Analyses of the responses of the H', TR and EPT indices and the $predator_{ratio}$ of all taxa to environmental factors were performed using a linear unconstrained multivariate ordination technique termed the principal components analysis (PCA). An ordination analysis was chosen because this method was specifically appropriate to describe continuous changes in multi-component systems (Leps and Smilauer 2003). The biological indices and environmental parameters were used as response and explanatory variables, respectively. The linear method was chosen due to the relatively short length of the gradients found by the preliminary detrended correspondence analysis. The unconstrained type of ordination (PCA of the biological indices with passive projection of environmental variables) was chosen because this method took into account the actual variability in the response variables (indices), including the variability not related to the explanatory variables. Prior to the analysis, the values of the indices were standardised to a zero population mean and a standard deviation of one because they did not share the same units of measurement. Environmental variables that were not recorded in at least 30% of the sites were excluded from the analysis. All ordination analyses were performed using the CANOCO 4.5 program for Windows (Wageningen, the Netherlands) according to the methods of Ter Braak and Smilauer (2002) and Leps and Smilauer (2003).

5.4. Results

5.4.1. Field metal exposure is related to community descriptors

We identified the community descriptors related to field metal contamination by applying a PCA. According to the ordination plot PC1, the proportion of predators ($predator_{ratio}$) was negatively related to the toxic pressure exerted by the highest toxicity of any metal at a site (Figure 1, Table 1). Of the other biological community descriptors, only the taxa richness and the number of EPT taxa showed weak relationships with metal contamination (Figure 1, Tables S5). Alkalinity, conductivity, pH and carbonate were not associated with PC1 and thus were not associated with metal toxicity or the $predator_{ratio}$ (Figure 1, Table S5). Only the magnesium, calcium and sulfate concentrations were positively associated with metal toxicity (Figure 1, Table S5). However, none of the investigated descriptors had a strong link to stream metal contamination comparable to the $predator_{ratio}$ (Table S2 and S5). From these results, we conclude that the $predator_{ratio}$ is a good proxy for Cu and Zn toxicity independent

of the other environmental parameters present at a site, which is in contrast to other measures of the community structure. We therefore apply the *predator*$_{ratio}$ as starting point for an indicator for the metal toxicity within the stream; the *SPEAR*$_{metals}$.

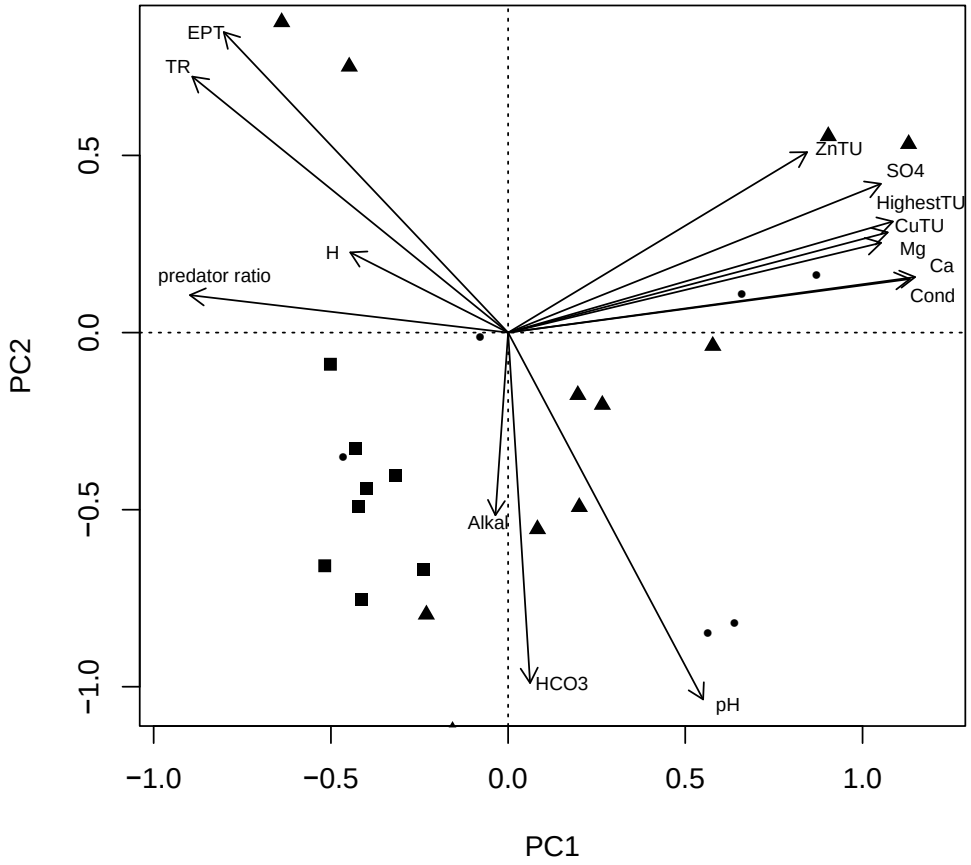

Figure 1. Ordination plot for the principal components analysis of the biological indices and environmental variables passively projected onto the ordination model. The data originated from 3 independent investigations as indicated by the data points (squares: Norris et al. 1980,; circles Norris 1986; and triangles Edwards 2002). TR: taxa richness, H: Shannon diversity, EPT: Ephemeroptera, Plecoptera, and Trichoptera taxa, Alkal = alkalinity, HighestTU = toxic unit of the most toxic metal, ZnTU = toxic unit derived from the Zn concentration, CuTU = toxic unit derived from the Cu concentration, Cond = conductivity, *predator*$_{ratio}$ = *SPEAR*$_{metals}$. For site number assignment see methods. For summary of the principal components analysis see Table S6.

Table 1. Pearson's correlation of heavy metal contamination with various community descriptors enabling to identify their respective specificity. S-values indicate sensitivity to metals (for details see the Methods). The community descriptors are taxa richness (TR), proportion of predators ($predator_{ratio}$), community S_{metal} as the mean S value of all metals, the S-values of the means of Cu and Zn, taxa richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT), Shannon-Wiener diversity index (H′), and the average community sensitivity towards organic toxicants ($SPEAR_{organic}$), pesticides ($SPEAR_{pesticides}$) and salinity ($SPEAR_{salinity}$). Negative correlations are indicated by a (-).

Biological indicator vs. Highest TU	***r***	***r²***	***p***
Taxa richness	-0.506	0.256	$p < 0.01$
$predator_{ratio}$	0.786	0.617	$p < 0.001$
Community S_{metal} (mean of all metals' S-values)	0.144	0.021	$p > 0.1$
Community S_{metal} (mean of Cu and Zn S-values)	0.406	0.165	$p < 0.05$
EPT	-0.384	0.147	$p < 0.05$
H	-0.183	0.034	$p > 0.1$
$SPEAR_{organic}$	0.084	0.007	$p > 0.1$
$SPEAR_{pesticides}$	-0.057	0.003	$p > 0.1$
$SPEAR_{salinity}$	-0.28	0.078	$p > 0.1$

5.4.2. Field metal exposure and predator ratios

The $predator_{ratio}$ of the invertebrate community was strongly related to the Cu and Zn metal toxicity at the investigated streams (Figure 2). This ratio was approximately 0.32 at low-contamination sites with a TU less than -1 and increased at highly contaminated sites to reach approximately 0.73 at TUs greater than 1. Although predators declined in absolute taxa numbers and abundance with increasing contamination, non-predators declined to a greater degree. The taxa number and the abundances of non-predators declined from the reference sites (TU <-1) to the highly contaminated streams (TU >1) from 22 to 9 species and from 1302 to 151 individuals in an average sampling, respectively. Predators declined from the reference sites to the highly contaminated streams from 9.6 to 6.6 species and 457 to 141 individuals, respectively. Hence, both feeding groups were affected by high Cu and Zn concentrations, but the non-predators were more affected than the predators. When only Cu or Zn toxicity was used to explain the observed predator ratio, the explained variance declined from $r^2 = 0.618$ and $p < 0.001$ for the predator ratio vs. the highest TU to $r^2 = 0.298$ and $p < 0.001$ or $r^2 = 0.36$ and $p < 0.001$, respectively. These results suggested that both metals were responsible for the observed effects.

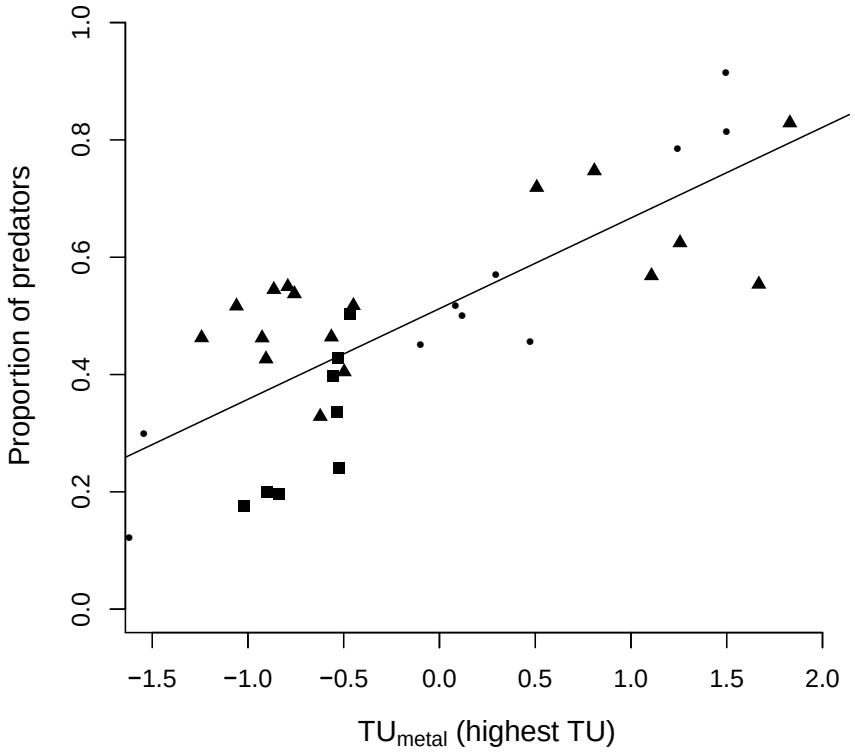

Figure 2. Linear regression of the maximum TUs in water and the proportion of predators (*predator*$_{ratio}$) (r^2 = 0.617, p < 0.001). The data originated from 3 independent investigations as indicated by the data points (squares: Norris et al. 1980; circles Norris 1986; and triangles Edwards 2002).

Within the order Trichoptera, the ratio of predators was related to the Cu and Zn toxicity. This ratio was approximately 0.3 at the reference sites (TU <-1) but increased with TUs less than 0 and reached levels up to 1 (i.e., only predators present) at highly contaminated sites with TUs of approximately 1. A linear regression of the maximum TU in water and the proportion of predators within the order Trichoptera revealed an r^2 = 0.38 and p < 0.001 (Figure S2). The proportion of Trichoptera predators within all Trichoptera increased with the increasing metal contamination when low-contamination sites (TU <-1) were compared with sites characterised by a TU between -1 and 0 or 0 and 1 or a TU >1 (ANOVA; Tukey's post hoc). The post hoc test revealed no significant differences between the <-1 reference group and the -1 to 0 and 0 to 1 groups (p = 0.62 and p = 0.58, respectively). However, the reference group was significantly different from the >1 group (p < 0.001).

5.4.3. Acute laboratory sensitivity of feeding groups to heavy metals

We investigated the extent to which the acute sensitivity of invertebrates to metals could be associated with the feeding group. Because Cu and Zn were the most relevant toxicants in our field dataset, we examined species sensitivity to these two metals. We applied the mean sensitivity of S(Cu) and S(Zn) for each species from Malaj et al. (2012) and identified the following ranking in order of decreasing sensitivity: filter feeder > shredder > gathering collector > grazer > predator (Figure 4). However, the average acute metal sensitivity of the taxa within the community (the Community S_{metal}) was only related to the metal contamination within sites to a minor extent (Table 1).

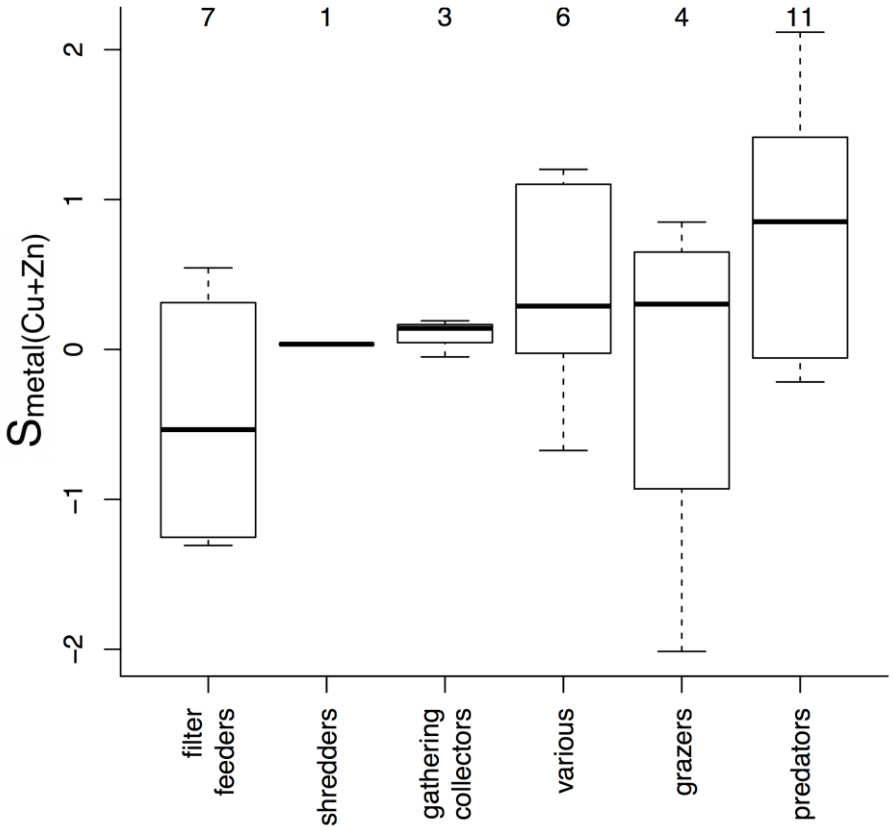

Figure 3. Acute sensitivity of functional feeding groups to Cu and Zn identified using acute laboratory data (LC50, 48 h). S_{metal} (Cu, Zn) was calculated as the mean of the Cu and Zn S-values. Negative S-values indicate higher sensitivity compared to *Daphnia magna*, and positive values indicate lower sensitivity. Predators are characterised by a significantly reduced acute sensitivity compared to the pooled other feeding groups (t-test: t = 2.328, df = 12.28, $p < 0.05$). Boxplots indicate the median within the box, the lower and upper quartiles are indicated by the box margins, and the maximum and minimum observations are indicated by the whiskers. The total number of taxa available to calculate S-values is given in Table S3. Figure 3 shows mean S(Cu)- and S(Zn)-values for all taxa reported in Malaj et al. (2012), aggregated on order level, and their respective feeding types.

5.5. Discussion

5.5.1. Standard toxicity tests are not indicative of field effects

The Community S_{metal}, which was indicative of the average acute metal sensitivity of the community, was only weakly related to the measured metal toxicity in the investigated streams (Table 1). This result is in line with other studies showing that the results of standard laboratory toxicity tests are not predictive of the field effects of metals. Recent explanations for this phenomenon include the following:

(i) The short duration of laboratory toxicity tests (Clements et al. 2013). Generally, invertebrates need several months to reach a steady state tissue concentration, and this duration by far surpasses the time allotted for most acute toxicity tests (Poteat and Buchwalter 2014).

(ii) When comparing dissolved vs. dietary acquisition of metals, diet often seems to be the prominent route of exposure (Brix et al. 2011, Poteat and Buchwalter 2014). Additionally, metals from dietary sources may be more toxic than water-derived metals (Xie and Buchwalter 2011).

(iii) Most laboratory tests do not evaluate the effects of metals on sensitive life stages, such as metamorphosis of larvae (Wesner et al. 2014) and latent effects after metamorphosis in the adult stage (Debecker et al. 2017).

(iv) Environmental stressors increase the effects of toxicants, including metals (Liess et al. 2016). These processes are not considered in standard test systems and may be of high relevance in metal-polluted communities.

5.5.2. Predatory feeding type as a community predictor of metal toxicity

The PCA indicated that the predator ratio was linked to the measured metal toxicity in the streams. The non-predatory taxa were affected to a much stronger extent than the predators. A tendency towards a higher tolerance of predators compared to taxa with other feeding types was observed in the acute standard laboratory tests (Figure 4). Nevertheless, the average sensitivity of taxa within a community (the Community S_{metal}) was only loosely related to the measured metal toxicity in the streams (Table 1). Accordingly, the acute sensitivity to metals only predicts the occurrence of taxa in the field to a minor extend. Instead, whether a taxon has a predominantly predatory lifestyle appears to largely determine their field sensitivity to

metals. Measures based on taxonomic classification (e.g. EPT) or other ecological traits (e.g. $SPEAR_{pesticides}$) were not related to metal concentration (Table 1).

No comparable investigation has linked the ratio of predators to heavy metal contamination within a multitude of streams that cover a wide geographical range. Nevertheless, several investigations revealed that herbivore and detritivore species were more affected than predators at metal concentrations similar to those in the present study (Clements 1994, Kiffney and Clements 1994, Leland et al. 1989, Qu et al. 2010). In a North American stream, the most metal sensitive group was heptageniid mayfly scrapers (Clements 1994). Herbivorous (Baetis) and detritivorous (*Pteronarcella badia*) mayflies were negatively affected by metal mixtures (Kiffney and Clements 1994). In streams in the mountainous area in China, increased Cu concentrations from mines induced a decrease in the scraper abundance (Qu et al. 2010). Additionally, experimental long-term dosing with Cu in a natural stream resulted in a decrease in many herbivorous and detritivorous insects at a dose of approximately 5 µg/L, whereas most predatory insects were not affected at a dose of 10 µg/L (Leland et al. 1989). Several potential mechanisms that are not mutually exclusive may be responsible for the low sensitivity of predators and the higher sensitivity of non-predators:

1) Predators may be better able to regulate their internal metal concentrations. Stream invertebrates efficiently regulate Cu and Zn (Goodyear and McNeill 1998). When comparing the relationships between Zn and Cu in water with the concentrations in aquatic macroinvertebrates, the correlation between the external and internal concentrations is steeper for collector-gatherers and scraper-grazers than for predators (Goodyear and McNeill 1999) (Kiffney and Clements 1993). This finding indicates that predators are more successful at regulating their internal Cu and Zn concentrations. Because food is the main source of the internal metal loads (Poteat and Buchwalter 2014), a lower metal load in invertebrate prey compared with that in algae and detritus may be responsible for this difference. This hypothesis is supported by the findings of Bossuyt et al. that *Daphnia magna* has a higher ability to regulate aqueous copper than algae (Bossuyt and Janssen 2005). Another mechanisms that may account for a lower internal metal concentrations of predators compared to non-predators would be a size related reduced metal uptake. Potentially bigger predators with a higher body mass / surface ratio could account for lower uptake rates of metals. This potential relevance of size is supported by a study investigating the effects of metals on three ephemeropterans and one plecopteran were metal related mortality was increasing with

decreasing body size (Kiffney and Clements 1996). However, the ecological relevance of such a process has not been identified.

2) Non-predators may be affected more by a combination of biotic stress and heavy metal contamination. Such combined effects of metals and predation can synergistically affect individuals, as reviewed and modelled by Liess et al. (2016). The authors showed that the presence of such environmental stressors, increased individual sensitivity to toxicants by a factor of up to 100. Mechanistic investigations showed that two species of net-spinning caddisflies (*Chimarra* sp. and *Hydropsyche morosa*) exposed to 6 µg/L of Cu were more susceptible to predation by the stonefly *Paragnetina media* than non-exposed individuals (Clements et al. 1989). The cause of this metal-induced susceptibility to predation may be impaired flight behaviour. For example, adaptive alarm behaviour (swimming speed) was impaired at a 5 µg/L Cu dose in juvenile salmon prey. This impairment led to an increase in the capture success rate of trout predators and eventually to reduced survival (McIntyre et al. 2012). As a result, the biomass of those groups of macroinvertebrate taxa that were highly available for salmonids were significantly reduced at the metal-polluted sites (Iwasaki et al. 2009). In the present investigation, this metal-induced susceptibility to predation may be even more relevant in streams where metals eliminated fish populations, resulting in invertebrate predators becoming the top predators and benefitting from reduced predation by fish. In this context, the polluted sections of the streams investigated here were characterised by a strong reduction in fish diversity and abundance as a result of metal pollution. The Molonglo River was fish-free (Norris 1986), and the polluted sections of the Finniss River prior to remediation had a reduced diversity and abundance of fish (Jeffree et al. 2001). The South Esk River showed a high diversity of fish upstream of the entry of metals, but nearly no fish were present within the metal-contaminated stream sections (Norris and Lake 1984). These results indicate that metal contamination causes a reduction in the length of the food chain by eliminating vertebrate predators.

For toxicants other than metals, only scattered investigations exist on the comparative sensitivity of feeding groups. However, these few also indicate a lower sensitivity of predators to toxicants. For insecticides, Brinke et al. (2010) found that nematode communities in control microcosm consisted of less than 50% predators and omnivors. When contaminated with the insecticide ivermectin more than 70% predators and omnivors were present in the communities. For salinity stress in in south-east Australian streams, Keffort et al. (2012) found that the proportion of predator species increased from 17% of species at salinities <

0.05 mS/cm to 57% of species at 35.5 mS/cm. Such findings suggest that if a general lower sensitivity of predators to toxicants will be confirmed the $predator_{ratio}$ of invertebrate communities will not be exclusively indicative for heavy metal contamination.

5.5.3. Effect thresholds

In this investigation, we revealed that the aquatic invertebrate community structure related to the predator ratio was affected by Cu or Zn concentrations in the TU range from -1 to 0. This concentration range corresponds to an effect threshold for dissolved Cu of 2.6 µg/L - 26 µg/L and for dissolved Zn of 62 µg/L - 617 µg/L. However, these values are only estimates, and the significance depends on the sample size, variability and presence of reference sites. Nevertheless, similar threshold concentrations were found in long-term bioassays and field investigations. For example:

For model ecosystems, exposure to dissolved Cu at a concentration of 9.3 µg/L resulted in a change in the structure and function of the invertebrate community (Hedtke 1984). Additionally, increased predation of caddisflies by stoneflies was observed in experimental streams with a dissolved Cu concentration of 5.5 µg/L, resulting in a strong decline in prey organisms (Clements et al. 1989). Similarly, microcosm macroinvertebrate communities showed reduced abundance and altered community compositions at a 5 µg/L Cu concentration (Clements et al. 2013). Finally, chronic effects of 3.16 µg/L of dissolved Cu on marine amphipods were observed in microcosms when environmental stress (UV-B) was present (Liess et al. 2001). Moreover, the emergence of subimagos and imagos of the mayfly *Centroptilum triangulifer* declined with dissolved Zn concentrations of approximately 100 µg/L (Wesner et al. 2014).

In field investigations, an effect threshold similar to that of the microcosms was identified for dissolved Cu. Dosed channels of a mountain stream showed a decline in the aquatic insect standing stock at a dissolved Cu concentration of 5 µg/L (Leland et al. 1989). Additionally, the distribution of aquatic insect taxa along a metal contamination gradient was altered by dissolved Cu concentrations in the range of 3-10 µg/L (Cain et al. 2004). An extended investigation of 400 sites in the UK, USA, and Japan revealed safe concentrations of 6.6 µg/L for dissolved Cu and 34 µg/L for dissolved Zn (Iwasaki and Ormerod 2012). A similar effect threshold of 50 µg/L was identified for the effects of elevated dissolved Zn concentrations on reduced Ephemeroptera richness across a range of streams receiving wastewater from various mining activities (Clements and Kiffney 1995).

When comparing existing microcosm and field investigations with our results, we can conclude that the predator ratio increases with the metal concentration within a range that is comparable with the lowest threshold concentrations identified for Cu and Zn. The application of the predator ratio provides the advantage that common a priori knowledge on the feeding type of taxa can be applied to identify the effects of metals on communities. This advantage is especially valuable when assessing and predicting the effects of metals on communities for which the detailed metal sensitivities of species are not known. When further validated, this indicator broadens the trait-based indicator approach of the SPEAR to also indicate the biological effects of heavy metals.

5.5.4. Water quality criteria

The current study suggests that the water quality criteria for Cu and Zn are protective related to the predator ratio for aquatic invertebrates. However, Cu affected the species composition at concentrations near the water quality criteria. We identified community effects when the Cu concentrations reached 2.6 µg/L and the Zn concentrations reached 62 µg/L. The EU maximum acceptable concentration of Cu in water for the EQS (Environmental Quality Standard) is 2.4 µg/L; the AA-EQS (annual average) is 0.7 (Wenzel et al. 2014). The US EPA sets a dissolved criterion maximum concentration (CMC) of 2.3 µg/L. The criterion continuous concentration (CCC) is 1.5 µg/L. In Australia, the 95% level of protection is set to 1.4 µg/L (ANZECC and ARMCANZ 2000). These guideline limits for Cu appear to be protective with regards to the effect threshold for Cu of 2.6 µg/L observed in this study. The EU maximum acceptable concentration for Zn in water for the EQS is 33 µg/L, and the AA-EQS is 10.9 (Wenzel et al. 2014). The US EPA set a dissolved CMC of 2.3 µg/L. The CCC is 1.5 µg/L. In Australia, the 95% level of protection is set to 8.0 µg/L. Accordingly, the guideline limits for Zn appear to be protective with regard to the effect thresholds for Zn of 62 µg/L observed in this study. Finally, the results of the present study suggest that the metal with the highest TU may be used as a proxy for the toxicity exerted by the mixture of all metals present at a sampling site.

The question remains, to which extent metal contamination, and the related increased predator ratio, impairs the functioning of ecosystems. To date the effect of metal related community structure on functional endpoints like leaf degradation has not been investigated. Therefore, comparisons may be drawn with the altered community composition following agricultural

pesticide stress in streams. Several investigations reveal that low pesticide contamination (TU ≤ -3) results in a 50% decrease in leaf litter degradation (Münze et al. in print, Münze et al. 2015, Schäfer et al. 2007). Similarly, direct effects of heavy metal contamination on fungal colonization and leaf decomposition rates have been identified in polluted streams (Ehrman et al. 2008). Based on these observations we expect that metal concentrations which cause an increased predator ratio will, similarly, impair functional endpoints such as leaf degradation.

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Chapter 6: General discussion

This thesis investigated the feasibility and appropriateness of trait-based bioindicators developed with laboratory derived sensitivity information on specific toxicant groups to reflect contamination in the field. To this end, specific sensitivity values towards hydrocarbon contamination to be applied in effect evaluation of refinery effluents were developed in this thesis (**Chapter 4**). Additionally, the $SPEAR_{organic}$ bioindicator for organic contaminants in general has been adapted to fluctuating concentrations of oil sands derived contaminants (**Chapter 3**). Sensitivity values for metal-specific bioindicators had already been developed (Malaj et al. 2012) but not yet applied to field datasets. This was, for the first time, conducted in this thesis (**Chapter 5**). Additionally, we have compared different approaches of exposure assessment with regard to their explanatory power (**Chapter 2**).

6.1. Toxic units based on *Daphnia magna* not the best but a reliable exposure metric

The first study (**Chapter 2**) revealed that mixtures containing less than ten pesticides were most common at most of the sampling sites in the five field studies. We found that exposure metrics based on the 5% fraction (HC_5) of a species sensitivity distribution (SSD) performed best, closely followed by metrics based on the most sensitive species and *Daphnia magna* ($TU_{D.magna}$). Considering only the compound with the highest toxicity (max) was sufficient to estimate toxicity in agricultural regions with pesticide exposure. At sites where further organic toxicants occurred, the multisubstance potentially affected fraction ($msPAF_{RA}$) performed better in reflecting the mixture exposure. However, the $msPAF_{RA}$, which is based on the HC_5 threshold obtained from SSDs under consideration of different Modes of Action (MoA), being the most sophisticated method, is the metric requiring most process steps.

Though the study revealed the above mentioned exposure metrics to best explain combined exposure, the selection of a metric is also strongly dependent on data availability. TUs are commonly calculated for one species only, *D. magna*, because it is one of the most tested aquatic invertebrate taxa. Accordingly, data availability is highest for *D. magna*. When applying SSD-derived metrics or TU of the most sensitive species, on the other hand, one requires toxicity data for a whole set of species towards a certain chemical; in the first case, to produce the SSD curve and in the latter case to determine the most sensitive out of the set of species. Our investigation, however, revealed that for 46% of the compounds re-evaluated, *D.*

magna was the only species tested. When multiple taxa where tested, other species were in 75% of cases more sensitive. Nevertheless, applying the most appropriate metric per study produced only minor improvements in the explanatory power in comparison to the original studies that applied $TU_{D.magna}$. It has to be noted, however, that unequal data-availability for the species-toxicant combinations can have influenced the outcome.

In conclusion, with regard to higher data and resource needs for more elaborate metrics, $TU_{D.magna}$ is a valid exposure metric to be applied. Based on this outcome, the other investigations in this thesis draw upon $TU_{D.magna}$ as a handable but also adequate metric to reflect field exposure.

6.2. Bioindicator $SPEAR_{oil}$ reflects community-level effects of exposure towards bitumen-derived contamination

The central observation in the second study (**Chapter 3**) was that the level of bitumen-derived contamination was not constant over time but was strongly fluctuating over the three sampling years. We found that hydrology plays a major role with regard to the level of contamination, as have earlier studies (Akre et al. 2004, Birks et al. 2013, Timoney and Lee 2009, 2011). Specifically, polycyclic aromatic hydrocarbons (PAH) concentrations were highest in the year with high autumn rainfall following low summer rainfall.

Based on this information on non-constant contamination, the trait generation time was regarded important, as fluctuating environmental conditions select for short-living taxa (Sherratt et al. 1999, Stark et al. 2004). The generation time represents a taxon's recovery potential after stress events. Additionally, the physiological sensitivity towards organic chemicals was regarded central to reflect the toxicity of this mixture of bitumen-derived organic substances. This physiological sensitivity is represented by $S_{organic}$-sensitivity values, which is the trait considered in the SPEcies At Risk (SPEAR) bioindicator $SPEAR_{organic}$ that was developed for continuous exposure towards organic toxicants (Beketov and Liess 2008). When combined with generation time, as we did here, sensitive taxa with long generation times become the most sensitive, while tolerant taxa with short generation times become the most tolerant group. As peak exposure periods caused by weather patterns in this area usually affect all streams, the recolonization potential from nearby streams is considered low. Recolonization from upstream areas less affected by high flow rates might be more relevant. Furthermore, no strict seasonal patterns are of importance here but a dependency on precipitation, rendering further traits as applied in $SPEAR_{pesticides}$ (like migration ability and

the presence of sensitive aquatic stages during the time of maximum exposure) inappropriate. Thus, only the two traits 'physiological sensitivity' and 'generation time' were combined in this study to generate $SPEAR_{oil}$. In the year with the highest PAH concentrations, $SPEAR_{oil}$ could reflect adverse effects on aquatic invertebrate communities' structure. Nevertheless, further validation is required in additional field studies before $SPEAR_{oil}$ can be applied for routine monitoring of oil sands related effects.

The use of a trait-based indicator was of great advantage in this study, as taxonomy-based approaches, for instance the Redundancy Analysis (RDA), reflecting the variance of species distribution in relation to TUs, demonstrated that invertebrate families of the same order were differently related to exposure. Accordingly, no toxicity-dependent occurrence of certain families and orders can be expected.

It has to be noted, however, that multivariate statistics just as SPEAR-type and other bioindicators have a risk of false association between exposure and effect due to potential intercorrelation with further toxicants or other environmental parameters. Environmental parameters can even show a stronger relationship to species occurrence patterns than chemicals, and thus, can mask effects caused by chemicals. Similarly, one of the sites sampled in the Athabasca region in this study was not only characterized by high organic contaminant levels but also by low flow rate and oxygen level. Nevertheless, the application of a trait-based indicator enhances the information obtained from monitoring activities and serves as a diagnostic tool, as described in Chapter 1.3 ("Trait-based bioindicators").

A source identification of oil sands contamination was challenging; this was also found by other researchers (Timoney and Lee 2009). The reason is that fingerprints of chemicals from petrogenic origin – whether they enter the aquatic ecosystem via natural or anthropogenic pathways – are nearly identical. However, the samples that we collected in 2010 could be divided into two groups with regards to their PAH composition and distribution patterns. One group of samples reflected sites located where the oil sands deposits naturally occur close to the surface and where surface mining takes place, while the other group reflected sites located where the oil sands can only be found in deeper layers and where in-situ-mining is conducted. This grouping, however, rather discriminated between areas differently exposed to weathering processes. A differentiation between natural and anthropogenic sources requires a different study design, comparing sites that only receive natural loadings and near-by sites that additionally receive loads due to mining activities. One option is to compare upstream and downstream sections of a same stream in those cases where mining takes place only at the

downstream sections. We did this at one site and observed elevated toxicity at the downstream site. Nevertheless, the downstream sections can also naturally contain high levels of bitumen, as the stream receives natural loading during its journey due to erosion processes. Alexander and Chambers (2016), however, showed that natural background variation could be ruled out as reason for longitudinal changes in concentrations because the concentrations they measured did not change as the rivers transitioned the oil sands formation. The authors compiled a 38-year dataset (1972 to 2010) to assess concentration patterns of three elements (dissolved selenium, dissolved arsenic, and total vanadium) along tributaries of the Athabasca and Clearwater rivers. They demonstrated that concentrations were higher at sites post-development than at reference sites with highest concentrations occurring during early developmental phases and land clearing. Further studies directed at source identification are urgently needed.

The fact that guideline limits were exceeded by those TU values, which we observed to affect invertebrate community structure emphasizes the need for a holistic evaluation for guideline setting. Effects not only on sensitive benchmark species but on entire community structures need to be considered. Intact communities are of high relevance for ecosystem functioning and the provision of ecosystem services, such as for instance nutrient cycling and the self-cleaning capacity of rivers (de Groot et al. 2002). For a holistic evaluation, also the enhancement of toxicity through additive and non-additive effects in mixtures needs to be considered. Even though we could identify PAHs to be the substance group primarily shaping the invertebrate communities in the sampling area, as shown by the high correlation between $SPEAR_{oil}$ and TU PAHs, we stress that toxicants and also non-toxic stressors cause ecological effects in combination (Liess et al. 2016). Furthermore, we point out that taxa occurring in areas with extreme natural background concentrations might have become more tolerant over time via adaptation. With regard to setting guidelines, this needs to be considered in order to make thresholds applicable in such regions. To this end, Berry (2016) has investigated the occurrence of local adaptation in response to oil sands development. He could, however, not find adaptation patterns. This might be due to the relatively short time that oil sands development exists. Future research can circumvent this challenge by testing for adaptation caused by oil sands occurrence irrespective of natural or anthropogenic origin.

Applying $SPEAR_{hydrocarbons}$ (**Chapter 4**), which is derived from toxicity information for hydrocarbons except for PAHs, to the Canadian dataset was tested but was not found effective in

explaining community composition. This supports the conclusion that the predominant group of contaminants was PAHs. A similar diagnosis was formulated by Arens et al. (2017) who report lower reproductive efforts in fish exposed to oil sands-related organic compounds. Molecular markers showed that exposure was dominated by PAHs and not by naphthenic acids, even though the authors found elevated concentrations of naphthenic acids in tributaries close to mining developments and elevated concentrations of cadmium, copper, nickel and selenium in fish. With ongoing sharing of toxicity information, a derivation of hydrocarbon S-values including PAHs might be feasible in the future. Then, it would be promising to again test $SPEAR_{hydrocarbons}$ in effect monitoring in the Athasbasca region.

6.3. Sensitivity ranking $S_{hydrocarbons}$ developed as core component of future sensitivity based bioindicators

This investigation (**Chapter 4**) aimed at developing a specific sensitivity ranking for hydrocarbon compounds found in crude oil or petroleum distillates representing contamination by refinery effluents. This was done using existing ecotoxicological databases enriched with rapid and mesocosm test results. It should be noted that also the extrapolation of tests with different durations to one common duration, in this case 48h, contributed considerably in extending the dataset. Applying the $S_{hydrocarbons}$-ranking in a SPEAR-type bioindicator demonstrated high predictive power in a first validation study. The investigation shows that by supplementing the toxicity data with results from rapid and mesocosm tests, the dataset can be extended sufficiently to allow deriving rankings applicable for bioindicators.
Following this first validation, further validation with other field or mesocosm studies will be the next step to test the bioindicator's applicability for future monitoring of petroleum contamination in aquatic ecosystems. The study demonstrates that the development of S-values for a specific group of compounds, in this case hydrocarbons, is possible. $S_{hydrocarbons}$ represents a first trial to develop a more specific sensitivity classification based on a particular set of compounds within the group of organic toxicants. A long-term goal for future developments in SPEAR-type bioindicators is to further increase stressor specificity also for other groups of toxicants. Currently, data availability is still hindering this development but increased data sharing will help to overcome this restriction. Additionally, fast and easy testing methods such as rapid testing (Kefford et al. 2005) can be applied to broaden the knowledge base - even for taxa that are not easy or impossible to cultivate in the laboratory.

6.4. Proportion of predators in a community ($SPEAR_{metals}$) reflects metal contamination

The last study (**Chapter 5**) investigated effect patterns caused by exposure towards dissolved heavy metals with regard to traits. The intention was to develop a SPEAR-type bioindicator for metal contamination based on an existing metal sensitivity ranking (S_{metal}-values). This study represents the first to apply S_{metal}-values in practice. However, the metal sensitivity values derived from acute laboratory tests could only weakly explain effects in the field. The physiological sensitivity, thus, does not serve as meaningful trait-information. This is in line with several studies that reported strong discrepancies in sensitivities towards metals observed in the laboratory and in the field (Brix et al. 2011, Buchwalter et al. 2007, Clements et al. 2013), making the derivation of ecologically meaningful thresholds difficult. Therefore, adapting the SPEAR approach to metals might not be a simple transfer of the $SPEAR_{organic}$ approach. Rather, we found that the trait feeding type was strongly correlated with metal exposure in the field. The re-evaluation of the three Australian and Tasmanian datasets with regard to the trait feeding type revealed an increased tolerance of predators towards metal contamination. The part of predators in a community, the predator ratio, was, thus, found to serve as a meaningful community descriptor explaining metal contamination in the environment. Also other studies observed an elevated sensitivity in non-predatory feeding groups, such as herbivore and detrivore species, with metal contamination (Leland et al. 1989, Qu et al. 2010). Nevertheless, further validation is required in other metal-contaminated areas. The physiological mechanisms for the enhanced tolerance of predators will need to be assessed in future studies. Possible mechanisms are discussed in **Chapter 5**, including a potentially higher regulatory capability for internal metal concentrations by predatory species and/or potentially lower metal uptake related to the body mass to surface ratio. Furthermore, non-predatory species might be more susceptible to additional stressors which again enhances the effect of metals. Another reason might be a metal induced reduction or eradication of the fish community with the result of predatory invertebrates becoming the top predators, feeding on prey invertebrate species without themselves being preyed on. All these processes can change the proportion of predators in a community. This community descriptor is suggested for application as an indicator reflecting metal contamination.

6.5. Conclusions, recommendations and future challenges

Throughout the presented studies, data scarcity with respect to certain taxonomic and chemical groups was perceived. In Chapter 2, for instance, we found that for 46% of compounds *D. magna* was the only freshwater invertebrate tested. Data are especially rare for aquatic insects such as Ephemeroptera, Plecoptera and Trichoptera as found in Chapter 4. The number of EPT taxa is an often applied metric in field assessments – also for the European Water Framework directive (WFD) (AQEM 2013) – as species of the orders Ephemeroptera, Plecoptera and Trichoptera are regarded as the most sensitive aquatic invertebrates (Meier et al. 2006). However, there is a need for more ecotoxicological knowledge on the sensitivity of aquatic insects. Added sensitivity information for insects will further improve reliability and will allow deriving sensitivity values on lower taxonomic levels. In particular, toxicity information with regard to PAHs is scarce. Toxicity information additional to that from standard laboratory tests can also be generated via rapid testing methods (Kefford et al. 2005) or be derived from mesocosm studies (Chapter 4). An often unused potential is the re-evaluation of existing datasets with novel methods and approaches (Chapter 2) as well as the evaluation of metadata. Concluding, more data sharing will increase data availability which will enhance the quality of bioindicators, and similarly, of exposure metrics like TUs.

In that way, future studies will be able to extend the approaches conducted here with a more solid database. This will improve the sensitivity values for specific groups of chemicals, for a wider number of taxa, for a lower taxonomic level and with a higher level of accuracy. A higher specificity towards certain stressors can ideally help to disentangle effects in systems with multiple stressors, and thus, to allocate the most influential stressor(s). Future studies and monitoring programs will need to show if such disentanglement is feasible.

Not only the data basis for toxicity information but also for traits remains to be improved, especially regarding data consistency (Culp et al. 2011). The identification of species traits in the field serves to assess functional groups, and thus, to evaluate the intactness of ecological functions and processes within aquatic ecosystems. Therefore, trait information allows for process-based monitoring of, for instance, functions responsible for nutrient cycling and the self-purification potential of streams. Alterations in ecosystem functions were, for example, observed by Roussel et al. (2008) who detected that breakdown rates were altered by copper exposure. This effect on an ecosystem function resulted from a shift of a gastropod-dipteran-crustacean to a dipteran dominated community (Joachim et al. 2017). The community lost sensitive species, which were grazers and shedders, with more tolerant species remaining,

which were collectors and deposit feeders. A similar shift in the ratio of feeding types in a community was demonstrated in Chapter 5. Such shifts can destabilize community structures and can affect ecological functions. Extending practical knowledge in this field is a task for future studies.

For the assessment of ecosystem functions and services, process-based monitoring, in addition to status-based monitoring, is coming more into focus (Lago et al. 2014). Status-based monitoring is conducted for the WFD in terms of taxonomic composition of communities (Hering et al. 2010). For assessing for ecosystem functions, however, taxonomy-based status is considered only as a weak proxy (Lago et al. 2014). Information on ecosystem functions might be crucial for policy and management decisions in human impacted environments, as ecosystem functions are pre-conditions for the provision of ecosystem services. These are services provided by ecosystems and that benefit human well-being (MEA 2005). Ecosystem services are gaining importance with regard to a sustainable and integrated management of landscapes and riverscapes, especially in urban areas (Maes et al. 2016).

Trait-based approaches might even gain in importance in the future due to the increasing presence of invasive species. As their presence is hardly avoidable, future water management will have to integrate this new reality into its evaluation practices. In the WFD, however, invasive species are not evaluated in a positive way (LAWA-AO 2014). Invasive species are often strong competitors, tolerant towards changing environmental conditions and have short reproduction rates. They can, thus, often outcompete native species (Lenz et al. 2011). Despite such potential adverse effects, invasive species can also conduct important functions in ecosystems. Trait-based approaches are able to account for invasive species' superior tolerance towards toxicants and stressful environmental conditions while similarly accounting for their fast recovery potential and contribution to ecosystem functions. Applying this knowledge when describing communities will be of high value compared to a solely taxonomy-based view.

Furthermore, the ability to characterize mixture toxicity will become even more relevant, as the number of man-made chemicals is likely to increase (e.g. Roser and Ritchie 2017) and with this also their entry into the environment. In parallel, additional environmental stressors related to climate change are augmenting (Bender et al. 2017). All in all, integrated water management practices need to consider multiple environmental stressors, which allows to identify hot-spots. These inform practitioners about priority areas for mitigation or

rehabilitation measures. Such integrated water management requires an interdisciplinary evaluation integrating chemistry, ecotoxicology, and ecology (Guasch et al. 2012).

Similarly, as anthropogenic development is expected to extend and cover more and more land, as was observed with the oil sands development in Northern Albert (CAPP 2010), it becomes increasingly relevant to have cheap, efficient and solid methods at hand to monitor these large-scale areas. Environmental monitoring is the first step in the process of bringing water bodies into a healthy state as demanded by the WFD and by several national water quality guidelines worldwide (e.g. Canadian Council of Ministers of the Environment 1999). Such directives and guidelines need to be adopted in all countries in order to operationalize the protection and restoration of rivers and streams. However, environmental impact assessment in developing countries frequently suffers from insufficient consideration of international standards – or is not conducted at all (Li 2008). Cumulative effects are hardly ever considered in developing countries (Glasson et al. 2005). These countries, however, are similarly confronted with oil (United Nations 2007) or oil sands (e.g. Venezuela) (Schenk et al. 2010) contamination, among others. Furthermore, areas with poor infrastructure for oil transport face high accident and spill rates. Pesticides, on the other hand, are being widely applied and brought into the environment in areas with high cotton production, e.g. in Pakistan (Government of Pakistan 1987-88 to 2005-06) and in countries oriented towards high agricultural productivity as in the United States of America (Ritter 1990) or European countries (Malaj et al. 2014). Of course, also metal contamination is a problem in developing countries – especially in areas of metal mining. In those countries facing severe pollution problems, it is of even higher importance to have affordable but reliable indicator systems at hand.

In the end, however, input reduction at the source and appropriate mitigation measures need to be adopted in the future, as the remediation of streams is costly and time-consuming (Vörösmarty et al. 2010). For achieving and maintaining a good ecological status in rivers and streams, a fundamental change in environmental policy and regulation is required with the aim to reduce pressures on ecosystems at the source. Furthermore, restoration expenses need to already be considered in the planning and approval phase of e.g. mining activities, because the legibility for the financial effort for restoration following mining activities needs to be guaranteed. In theory this is intended but often not practiced (Tenenbaum 2009).

Also pollutants present in industrial and domestic sewage such as microplastic, nanoparticles and pharmaceuticals need to be reduced at the source to prevent or at least reduce their input into the environment. This implies that their use is restricted to the extent necessary, their intentional input banned and their unintentional input avoided. The elsewise arising effort for removal, e.g. of plastic from the water surface and column, is extremely time and cost intensive. Likewise, the overconsumption of pharmaceuticals results in high removal costs in wastewater treatment plants. In parallel, current pesticide application in agriculture will need to be limited drastically when aiming at reducing exposure towards pesticides in streams. This is only feasible by a reformation of the conventional high-intensity farming practices. A continuation of the current practices under the face of climate change would result in increasing pesticide application (Kattwinkel et al. 2011). Also abiotic environmental stressors such as water temperature, erosion during heavy rain events or water shortfall in streams during periods of droughts (Sommerhäuser 1999) are expected to increase with climate change. Such elevated stress levels make aquatic organisms more susceptible towards further stressors like toxicants (Liess et al. 2016).

Mitigation measures for individual stressors may, thus, not be effective in reducing ecological risks. Integrating concepts as well as data from ecology, ecotoxicology and further disciplines, as well as the consideration of whole ecosystems and their functioning is essential to manage multiple stressors. A consideration of consequences will need to be conducted in all human interventions with nature to ensure a sustainable use of ecosystems and their services and for the conservation of biodiversity – both essential for human well-being.

6.6. References

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A Supplementary Information for Chapter 2

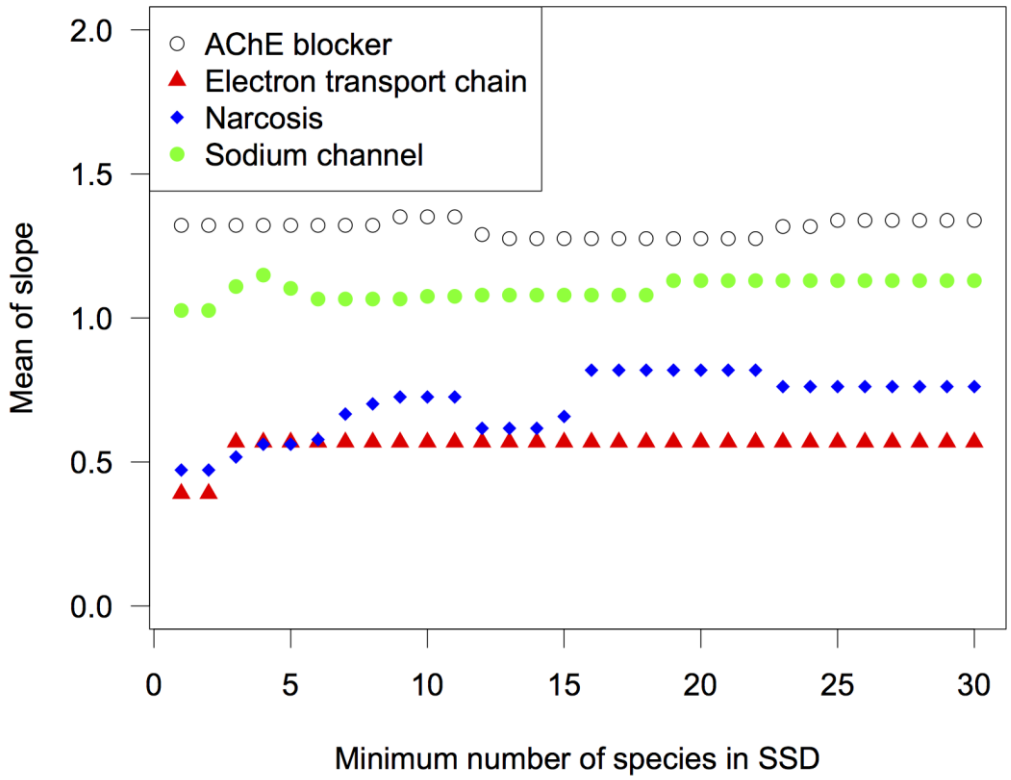

Figure S1. Mean of slope in terms of the standard deviation of individual species sensitivity distributions (SSD) for each mode of action in relation to the minimum number of species required for SSD calculation. Log_{10}-transformed toxicity data were used as input for the related SSD and centred to 0 prior to calculation.

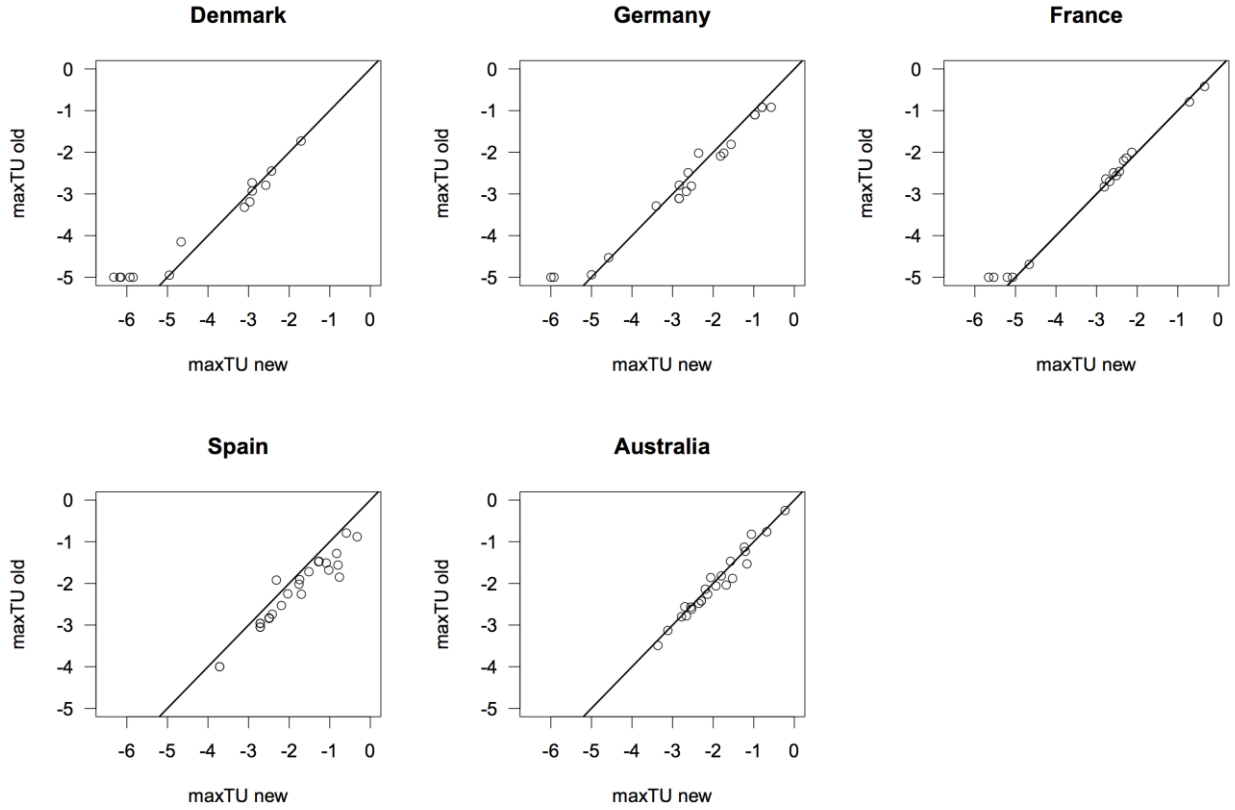

Figure S2. Correlation between the newly calculated $maxTU_{D.\ magna}$ and the $maxTU_{D.\ magna}$ reported in the original studies. Lines indicate the 1:1 relationship.

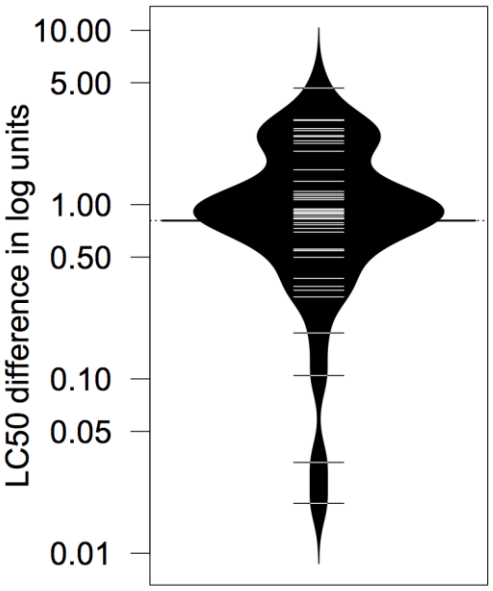

Figure S3. Beanplot for the differences in EC/LC50 between *Daphnia magna* and the most sensitive species, for cases where *Daphnia magna* was not most sensitive (cf. Table S4). The lines indicate individual observations, the black polygon gives the empirical density shape and the long line indicates the mean. For more information see Kampstra (2008).

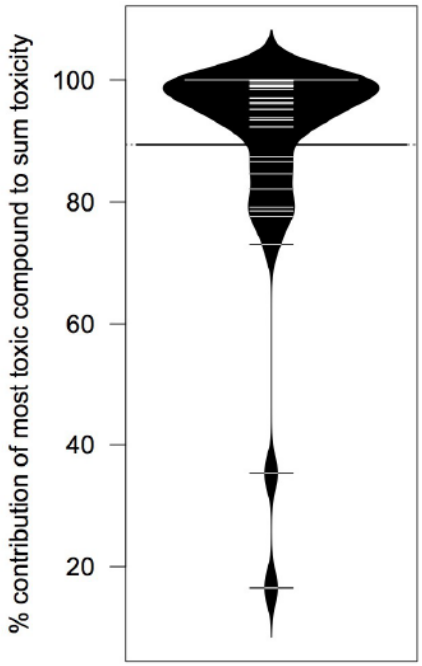

Figure S4. Beanplot for the % contribution of the most toxic compound ($maxTU_{Sensitive}$) to the $sumTU_{Sensitive}$ in sites with a $TU_{Sensitive} > 0.1$. The lines indicate individual observations, the black polygon gives the empirical density shape and the long line indicates the mean, for more information see Kampstra (2008).

Table S1. Detected compounds[a] in the studies with CAS, number (No.) of species available per species sensitivity distribution (SSD), mode of action and acute toxicity data (median effect/lethal concentration (EC/LC50)) for *Daphnia magna* (DM) and the most sensitive tested freshwater invertebrate species (MSS). Table first sorted by number of species for SSD then by acute toxicity data for DM. See Text S1 for sources of toxicity data.

CAS	Compound name	No. species for SSD	Mode of action	Mean log EC/LC50 DM (µg L-1)	Log EC/LC MSS (µg L-1)
52686	trichlorfone	1	AChE Blocker	-0.42	-2
82657043	bifenthrin	1	Sodium channel	0.29	-2.03
50293	p,p-DDT	1	Sodium channel	0.3	-0.77
118741	hexachlorobenzene	1	Narcosis	0.72	0.72
72548	p,p-DDD	1	Sodium channel	0.95	-0.17
141517217	trifloxystrobine	1	electron transport chain	1.13	1.13
175013180	pyraclostrobine	1	electron transport chain	1.2	1.2
23103982	pirimicarb	1	AChE Blocker	1.21	1.21
121552612	cyprodinil	1	Narcosis	1.62	1.62
2032657	methiocarb	1	AChE Blocker	1.73	0.85
72559	p,p-DDE	1	Sodium channel	1.9	1.04
120068373	fipronil	1	Sodium channel	1.94	-0.72
140669	t-octylphenole	1	Narcosis	1.95	1.95
104358	nonylphenol-1-ethoxylate	1	Narcosis	2.14	2.14
60571	dieldrin	1	Sodium channel	2.2	-0.05
83164334	diflufenican	1	Narcosis	2.38	2.38
143390890	kresoxim-methyl	1	electron transport chain	2.4	2.4
20427843	nonylphenol-2-ethoxylate	1	Narcosis	2.43	2.43
42576023	bifenox	1	Narcosis	2.68	2.68
79127803	fenoxycarb	1	AChE Blocker	2.7	2.7
67306007	fenpropidine	1	Narcosis	2.72	2.72
173584446	indoxacarb	1	Sodium channel	2.78	1.69
34123596	isoproturon	1	Narcosis	2.88	2.88
119446683	difenoconazole	1	Narcosis	2.89	2.89
40487421	pendimethaline	1	Narcosis	2.94	2.94
52888809	prosulfocarb	1	Narcosis	2.96	2.96
19670156	endosulfan II	1	Sodium channel	3.18	3.18
32809168	procymidone	1	Narcosis	3.26	3.26
74070465	aclonifen	1	Narcosis	3.27	3.27

103651	n-propylbenzene	1	Narcosis	3.32	3.32
60515	dimethoate	1	AChE Blocker	3.33	0.85
67564914	fenpropimorph	1	Narcosis	3.35	3.35
53112280	pyrimethanile	1	Narcosis	3.46	3.46
95636	1,2,4-trimethylbenzene	1	Narcosis	3.47	3.47
112281773	tetraconazole	1	Narcosis	3.48	3.48
107534963	tebuconazole	1	Narcosis	3.57	3.57
67747095	prochloraz	1	Narcosis	3.63	3.34
188425856	boscalid	1	Narcosis	3.73	3.73
23950585	propyzamide	1	Narcosis	3.75	3.75
108678	1,3,5-trimethylbenzene	1	Narcosis	3.78	3.78
60168889	fenarimol	1	Narcosis	3.8	3.8
66246886	penconazole	1	Narcosis	3.84	3.84
886500	terbutryne	1	Narcosis	3.85	3.85
34256821	acetochlor	1	Narcosis	3.91	3.91
106325080	epoxiconazole	1	Narcosis	3.94	3.94
43121433	triadimefone	1	Narcosis	3.95	3.95
55219653	triadimenole	1	Narcosis	4.05	4.05
88671890	myclobutanil	1	Narcosis	4.09	4.09
5915413	terbutylazine	1	Narcosis	4.17	3.48
94361065	cyproconazole	1	Narcosis	4.41	4.41
1007289	desisopropylatrazine	1	Narcosis	4.42	3.48
21087649	metribuzine	1	Narcosis	4.44	4.44
6190654	desethylatrazin	1	Narcosis	4.47	3.71
138261413	imidacloprid	1	AChE Blocker	4.48	-0.19
57837191	metalaxyl	1	Narcosis	4.58	4.58
30125634	desethylterbutylazine	1	Narcosis	4.62	4.62
110488705	dimethomorph	1	Narcosis	4.69	4.69
26225796	ethofumesate	1	Narcosis	4.74	4.74
1698608	chloridazon	1	Narcosis	4.97	4.97
41394052	metamitron	1	Narcosis	5.02	5.02
75274	bromodichloromethane	1	Narcosis	5.17	5.17
51235042	hexazinone	1	Narcosis	5.19	5.19
124481	dibromochloromethane	1	Narcosis	5.2	5.2
74953	dibromomethane	1	Narcosis	5.27	5.27
77732093	oxadixyl	1	Narcosis	5.72	5.72
470906	chlorfenvinphos	11	AChE Blocker	-0.6	-0.6
1897456	chlorothalonil	11	Narcosis	2.17	1.08
108952	phenole	120	Narcosis	4.19	3.3

15972608	alachlor	14	Narcosis	4.39	3.45
79016	trichloroethylene	16	Narcosis	4.41	4.38
122349	simazine	17	Narcosis	3.62	3.28
131860338	azoxystrobin	2	electron transport chain	2.13	2.13
319846	a-hexachlorocyclohexane	2	Sodium channel	2.92	2.92
98828	isopropylbenzene	2	Narcosis	3.74	3.74
7287196	prometryne	2	Narcosis	4.1	4.1
75252	bromoform	2	Narcosis	4.66	4.64
1912249	atrazine	22	Narcosis	4.55	2.1
1582098	trifluraline	23	Narcosis	2.53	1.7
1563662	carbofuran	27	AChE Blocker	1.72	0.36
104405	4-n-nonylphenole	3	Narcosis	1.96	1.58
36734197	iprodione	3	Narcosis	2.63	2.63
330552	linuron	3	Narcosis	2.72	2.72
19666309	oxadiazon	3	Narcosis	3.36	3.26
95476	o-xylene	3	Narcosis	3.72	3.72
100414	ethylbenzene	3	Narcosis	4.07	3.29
107062	1,2-dichloroethane	3	Narcosis	5.55	5
1031078	endosulfan sulfate	4	Sodium channel	3.12	0.08
333415	diazinon	43	AChE Blocker	0.14	-0.36
52645531	permethrin	43	Sodium channel	0.35	-1.67
56382	parathion	49	AChE Blocker	0.27	-0.64
127184	tetrachloroethylene	5	Narcosis	4.1	3.56
51218452	metolachlor	5	Narcosis	4.11	3.19
1330207	m(p)-xylene	5	Narcosis	4.77	4.77
58899	g-hexachlorocyclohexane	54	Sodium channel	3.21	0.48
87865	pentachlorophenole	59	electron transport chain	2.61	1.88
85018	phenanthrene	6	Narcosis	2.77	2.45
67663	trichloromethane	6	Narcosis	5.11	4.93
2921882	chlorpyrifos	66	AChE Blocker	-0.27	-1.46
83329	acenaphthene	7	Narcosis	3.53	2.38
60207901	propiconazole	7	Narcosis	3.79	2.95
63252	carbaryl	80	AChE Blocker	1.11	0.56
959988	endosulfane I	9	Sodium channel	2.91	-0.15
108883	toluene	9	Narcosis	4.52	4.52

[a] Except for cyanide, propargite, spinosynd and tebufenozide since not considered in calculation of exposure metrics (see “Calculation of exposure metrics” in paper for rationale)

Table S2. Intercorrelation (Pearson correlation coefficient *r*) between log-transformed exposure metrics across all studies. All correlations were statistically significant ($p < 0.001$).

	maxTU$_{D.magna}$	**sumTU$_{D.magna}$**	**maxTU$_{Sensitive}$**	**sumTU$_{Sensitive}$**	**maxTU$_{HC50}$**	**sumTU$_{HC50}$**	**maxTU$_{HC5}$**	**sumTU$_{HC5}$**
maxTU$_{D.magna}$	1							
sumTU$_{D.magna}$	0.99	1						
maxTU$_{Sensitive}$	0.95	0.94	1					
sumTU$_{Sensitive}$	0.96	0.95	0.99	1				
maxTU$_{HC50}$	0.95	0.96	0.91	0.91	1			
sumTU$_{HC50}$	0.94	0.96	0.89	0,9	0.99	1		
maxTU$_{HC5}$	0.94	0.92	0.93	0.92	0.94	0.92	1	
sumTU$_{HC5}$	0.94	0.94	0.93	0.93	0.96	0.94	0.99	1
msPAF$_{RA}$	0.83	0.82	0.86	0.85	0.86	0.84	0.94	0.94

Table S3. Number of compounds (NoC) where *Daphnia magna* (DM) or another species was most sensitive and information on the relationship between EC/LC50s (% with respect to all 103 compounds).

Compounds where	**NoC**	**NoC only DM toxicity data available**	**NoC additional toxicity data available**	**EC/LC50 for most sensitive species ≤ 1 log unit of LC50 for DM**	**EC/LC50 for most sensitive species > 1 of LC50 for DM**
DM most sensitive species	60 (58%)	47 (46%)	13 (13%)	Not applicable	Not applicable
DM not most sensitive species	43 (42%)	0	43 (42%)	25 (25%)	18 (17%)

Text S1: Data sources for experimental data

Experimental acute toxicity data (48, 72, and 96-h exposure periods, effect/lethal concentration 50%) for freshwater invertebrates were retrieved from the US EPA ECOTOX database (U.S. Environmental Protection Agency). These data were complemented by toxicity data from the RIVM e-toxbase database (De Zwart, 2002), the Umweltbundesamt ETOX database (Umweltbundesamt), the Pesticide Properties DataBase (The FOOTPRINT Pesticide Properties DataBase) and data associated with publications (Kühne et al, 2013; von der Ohe et al, 2005; von der Ohe et al, 2009). Baseline toxicity estimation was done using the software ECOSAR (U.S. Environmental Protection Agency). See raw data in Supporting Information for sources of toxicity data for individual compounds (File "Toxicity_data.csv"). The *K*oc values were taken from Schüürmann et al. (2006) or in case of pesticides from the Pesticide Properties DataBase (The FOOTPRINT Pesticide Properties DataBase). Predictions of missing values were performed by the software system ChemProp (Schüürmann et al., 1997) applying the most appropriate models among Schüürmann et al. (2006) and Sabljic et al. (1995).

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B Supplementary Information for Chapter 3

1. Methods

1.1. Quantification of PAHs

For PAH analysis, water samples (1L) were spiked with PAH surrogates (acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, and phenanthrene-d10; Accustandard, New Haven, CT, USA) and were extracted with 60 ml of dichloromethane (DCM) three times. The combined DCM was dried with combusted sodium sulfate and concentrated to 5 ml by rotary evaporator. To this concentrate, 10 ml hexane was added and the total extract was then rotary evaporated to ~1 ml. The extract was loaded onto a 3 g silica gel column for clean-up. The PAH fraction was eluted using 1:1 DCM/pentane and was concentrated to 1 ml by a nitrogen evaporator prior to GC-MS analysis. Gas chromatography mass spectrometry (GC-MS) analysis was performed on an Agilent 6890N gas chromatograph with a split/splitless inlet and 5975 mass spectrometer (Agilent Canada, Mississauga, ON, Canada). The column was a DB-5 capillary column (30 m x 0.25 mm, 0.5 um film, J&W Scientific, Agilent Canada, Mississauga, ON, Canada).
The method detection limits in 2010 ranged from 0.77 ng/L (for acenaphthylene) to 5.21 ng/L (for indeno[1,2,3-c,d]pyrene), in 2011 from 0.05 ng/L (acenaphthylene, acenaphthene) to 0.33 mg/L (dibenzo[a,h]anthracene), and in 2012 from 0.05 ng/L (acenaphthylene, biphenyl) to 0.33 mg/L (indeno[1,2,3-c,d]pyrene).

For PAH analysis of sediment via GC-MS, 3 g of homogenized sediment was mixed with 20 g of anhydrous sodium sulfate (EMD, Germany, muffled at 500°C overnight) to remove moisture, then transferred into a 33 ml stainless steel accelerated solvent extraction (ASE) cell, spiked with PAH surrogate (acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, and phenanthrene-d10; Accustandard, New Haven, CT, USA) and topped up with Ottawa sand (Fisher Scientific Canada). The extractions were carried out using dichloromethane (DCM) (Optima grade, Fisher) as the extraction solvent with a Dionex ASE 200 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA). The DCM extracts were then dried with combusted sodium sulfate, and concentrated down to 5 ml by nitrogen evaporator. The concentrated extracts were loaded on to the gel permeation chromatography system (GPC) (Automated Gilson GX-271GPC clean-up system, Canadian distributor - Mandel Scientific, Guelph, ON, Canada) equipped with an Envirosep ABC GPC column (350 x 21.2 mm, Phenomenex, Torrance, CA, USA) to remove lipids in the extracts. The PAH fractions collected from GPC were blown down under nitrogen gas near to dryness, reconstituted with 2 ml hexane (Optima grade, Fisher) and loaded onto the 30 cm silica gel column for further clean up. The PAH fractions were eluted using 1:1 DCM/pentane and concentrated to 1 mL by nitrogen evaporation prior to GC-MS analysis. The GC-MS analysis was performed on an Agilent 6890N gas chromatography with a split and splitless inlet and 5975 mass spectrometry (Agilent Canada, Mississauga, ON, Canada) using a DB-5 capillary column (30 m x 0.25 mm, 0.5 um film, J&W Scientific, Agilent Canada, Mississauga, ON, Canada).

1.2. Quantification of naphthenic acids

In fall 2010, water samples for NA analysis were collected by RAMP (Regional Aquatics Monitoring Program 2011) and were shipped to the University of Alberta for analysis. In 2011 and 2012, water samples for NA analysis were collected as part of the present study. Water samples from 2010 and 2011 were liquid/liquid extracted and analyzed by the HPLC-QTOF mass spectrometry method described by Ross et al. (2012), while samples collected in 2012 were analyzed by the HPLC-Orbitrap mass spectrometry method described by Pereira et al. (2013) with on-line solid phase extraction (Pereira and Martin 2014). Profiles of naphthenic acids detected in the samples taken in 2010 are shown in Figure S6.

1.3. Quantification of metals

Water samples were filtered (0.45 µm) and treated with nitric acid solution (1 ml nitric acid for 100 ml sample), and subsequently, were analyzed for trace metals by inductively coupled plasma mass spectrometry (ICP-MS) on an ELAN 9000 (PerkinElmer/SCIEX; Walthan, MA, USA) as described previously by Mahdavi et al. (2012). Standard solutions containing the same matrix as the samples were prepared at appropriate concentrations for each element. Samples were analyzed in duplicate, and analytical accuracy in the determination of metals was checked with reference standards.

1.4. Calculation of Toxic Units

The TU approach standardizes the toxicity of single compounds detected in an environmental sample in order to define the overall toxicity per sampling site. It puts in-stream toxicants concentrations in relation to the sensitivity of the standard test species, *Daphnia magna*.

LC50 values were obtained from the ECOTOX database (US EPA, http://cfpub.epa.gov/ecotox/data_download.cfm (accessed 09.14.2011)). If no toxicity values were available, values estimated with the Ecological Structure Activity Relationships Class Program ECOSAR (Nabholz and Mayo-Bean 2009) and with a read-across method (Schüürmann et al. 2011) as calculated with ChemProp (kindly provided by UFZ Department of Ecological Chemistry, 2011, http://www.ufz.de/index.php?en=6738; Schüürmann et al. 1997) were used.

For the calculation of PAH TUs, a lack of toxicity information exists for the alkylated PAH species. Therefore, LC50 values of the respective parent forms were applied, which is a conservative approach as alkyl derivatives are known to be more toxic than their unsubstituted congeners (Turcotte et al. 2011). At each sampling site, always the one PAH with the maximum/highest TU of all detected PAHs at that site was considered, instead of the sum of all PAH TUs for that site, as these were found to best reflect the link between exposure and effect. This means that typically the high molecular weight PAHs, which are the most toxic PAHs, and therefore, produced the highest TUs (around TU = -1.5), represented the maximum TU at a given sampling site. If no PAH species was detected at a site, the site was assigned a TU of −7, representing a TU that is lower than the lowest TU at sites with detectable PAHs (TU ~ -6.0). As TUs are on a logarithmic scale, a difference of 1 indicates a 10 times lower or higher concentration.

Water TUs of PAHs were calculated from the PAH concentrations in water following formula [Eq. 1]. Sediment TUs of PAHs were calculated from the bioavailable dissolved portion of the sediment concentrations according to a modification of the equilibrium partitioning approach (Di Toro et al. 1991, Schäfer et al. 2011), followed by the TU approach given in [Eq. 1].

TUs of NAs in water were calculated on the basis of single homologues (Figure S6), each of which is a distinct number of carbons bound by a number of double bonds, with the molecular formula $C_nH_{2n+Z}O_2$, where n is the number of carbons and Z is the hydrogen deficiency arising due to double bonds or rings. Ross et al. (2012) showed that bitumen-derived NA profiles in surface waters have a Gaussian-like distribution of homologues, centered around n = 16 and Z = −6. This distribution pattern distinguishes them from background fatty acids stemming from biological sources. For this reason, we considered only homologues with the formula n = 13 through n = 19 with Z = -4 and Z = -6. Area ratios of these single bitumen-derived NA homologues were converted into concentrations (µg/L) by determining their respective fraction of the total NA concentration. Subsequently, the total sum concentration of bitumen-derived NA homologues was derived for each sampling site. This value was divided by the median LC50 for *D. magna* 48h tests, being 54954 µg/L, according to formula [Eq. 1]. The LC50s for NAs were obtained from the ECOTOX database (US EPA, http://cfpub.epa.gov/ecotox/data_download.cfm (accessed 09.14.2011)) and the publications by Frank et al. (2008, 2009) and Jones et al. (2011).

TUs of metals were calculated as those for PAHs. Accordingly, TUs were obtained for each metal compound and out of these, the one compound with the maximum/highest TU was considered for further analysis.

1.5. Source identification for PAHs

The pattern of the composition of PAH compounds was compared between sampling sites according to the methods by Hall et al. (2012) and Headley et al. (2001). Hall and colleagues (2012) compared oil sands samples with reference river sediments and identified several PAHs with indicator qualities: C2-C4-dibenzothiophenes, C2-C4-fluoranthenes/pyrenes, and C2 benzo[a]anthracenes/chrysenes. These PAHs are indicative of bitumen origin and transport by Athabasca River floodwaters. Headley and co-workers (2001) compared the profiles of the alkylated PAH distributions, which were similar among all samples; also here, an indication for a common petrogenic source.

1.6. Measurement of water physico-chemical parameters

Temperature (°C), pH, conductivity (µS) and oxygen content (%, mg/L) were measured using portable field devices (WTW Multi 340i, Germany; Extech Instruments ExStik II, Germany). Current velocity was determined by drift method (Marotz and Minor 1971). River width and depth, water color, sediment structure and occurrence of surface oil were recorded by visual observation. In 2011 and 2012, turbidity was determined using a portable turbidity-meter (Turbiquant 1100 IR, Merck, Germany). Additionally, phosphate, nitrate, nitrite and ammonia (mg/L) were determined in 2011 and 2012 using visocolor® ECO tests (Macherey-Nagel GmbH & Co. KG, Germany).

1.7. Determination of invertebrate community

At each sampling site, all substrate types present (bedrock, cobbles and pebbles, sand and clay) were sampled for approximately five minutes each. Sampling was performed with a 500-µm mesh-size Surber Sampler (Hydro-Bios, Kiel, Germany) in central and shoreline areas in wadeable streams or along the shoreline in non-wadeable rivers. Additionally, approximately 5-10 stones were inspected for epifauna and invertebrates were collected by hand. Aquatic plants, algae and inundated grass at the river banks were also sampled with the Surber-sampler.

Live sorting of the macroinvertebrates was performed on site in white trays until all animals were collected (Liess and von der Ohe 2005) but at most for ~ 30 minutes. Only in case a species was found in very high abundances, not all specimens were collected but the number of specimens remaining in the tray was estimated. As the abundance enters the SPEAR formula in a logarithmic way, small estimation errors are not affecting the outcome of the index. Collected organisms were preserved with 70% ethanol in small plastic jars and were transported to the UFZ (Leipzig, Germany) where they were identified to the lowest possible taxonomic level, which was genus for most taxa, following the identification key of Clifford (1991).
All aquatic life stages present at the sampling time were sampled. As sampling took place in autumn, these were mainly larger specimens. Insects were mainly at larval stage, except for Hemiptera and Coleoptera being a combination of both larvae and adults.

1.8. Calculation of taxonomic indices

Several commonly used conventional biological indices for describing taxa richness and diversity in the invertebrate communities were determined, including taxa richness (TR), taxa richness of Ephemeroptera, Plecoptera, and Trichoptera species (EPT), and Shannon's diversity index (H').

An online software to calculate $SPEAR_{oil}$ is made publicly available as part of the SPEAR Calculator on http://www.systemecology.eu/spear/ (after publication).

$SPEAR_{organic}$ was applied based on the $S_{organic}$ values without the inclusion of further traits. $SPEAR_{organic}$ was developed by Beketov & Liess (2008) to identify effects originating from contamination with organic toxicants.

The metal indicator S_{metal} is based on the relative sensitivity values towards metals, which are generally contrary to the sensitivity values towards organics, i.e. insects are generally sensitive towards organics but tolerant towards metals and vice versa (Malaj et al. 2012, von der Ohe and Liess 2004).

2. Figures

Figure S1. Map of the study area in Northern Alberta. Study sites were ATR-1 (Athabasca River 1), ATR-2 (Athabasca River 2), ATR-3 (Athabasca River 3), ATR-4 (Athabasca River 4), ATR-5 (Athabasca River 5), POC (Poplar Creek), BER (Beaver River), MUR (Muskeg River), FOC (Fort Creek), MAR-1 (MacKay River 1), MAR-2 (MacKay River 2), ELR (Ells River), TAR (Tar River), HAC (Hartley Creek), HOR (House River), CHR (Christina River), SUC (Sunday Creek), JAR (Jackfish River), and GUR (Gull River). The red margins indicate surface mineable area, grey margins watershed boundaries; areas in yellow depict land disturbance and light blue areas within the yellow areas represent tailings ponds; sites sampled in all three years are depicted by red circles; yellow circles indicate sites sampled only in 2011 and 2012. (Sources: National Geographic base map, Surface mineable area map (kindly provided by Martin Davies, Hatfield Consultants), Land disturbance map (RAMP 2012), and Watershed boundaries map (RAMP 2012)).

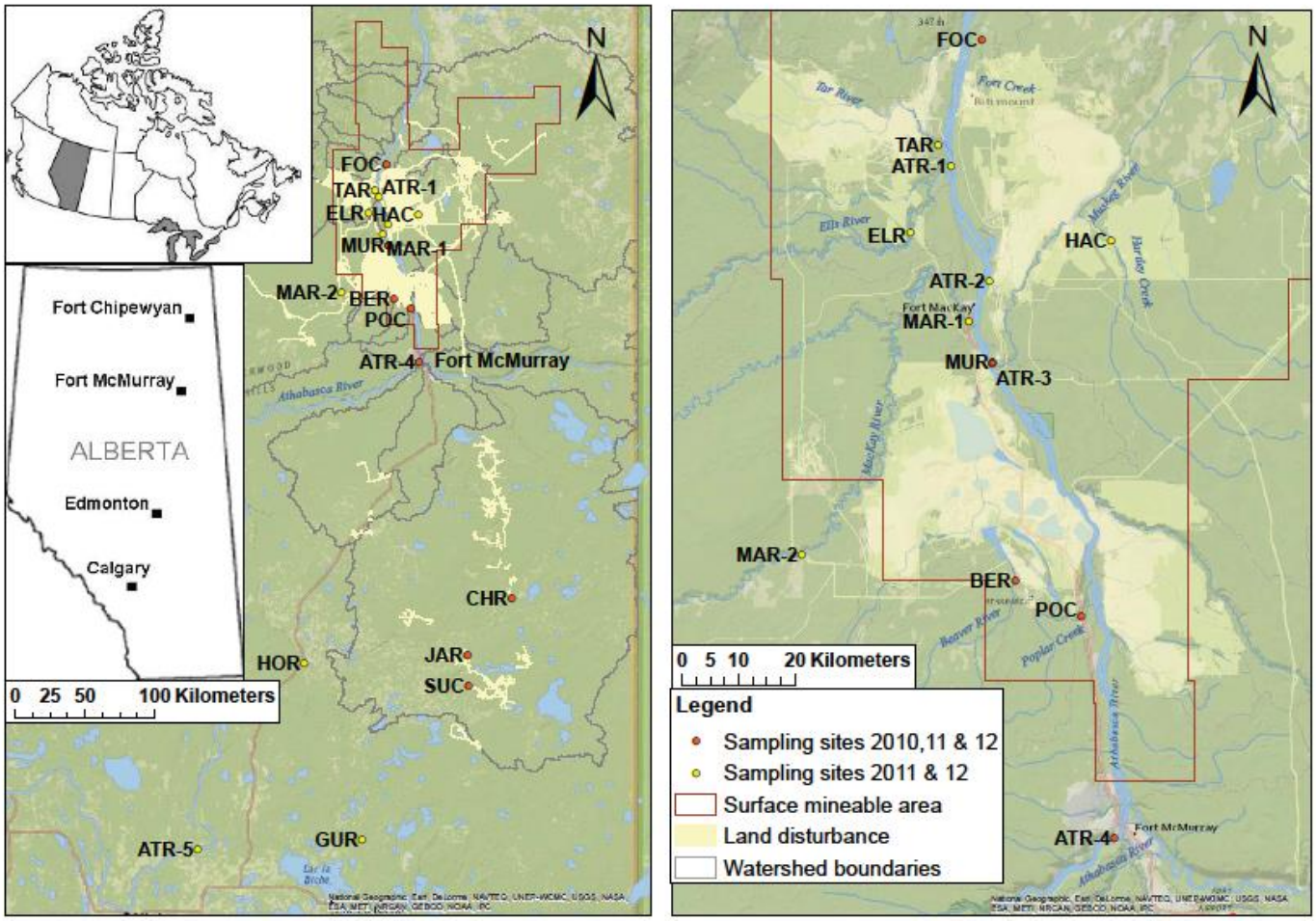
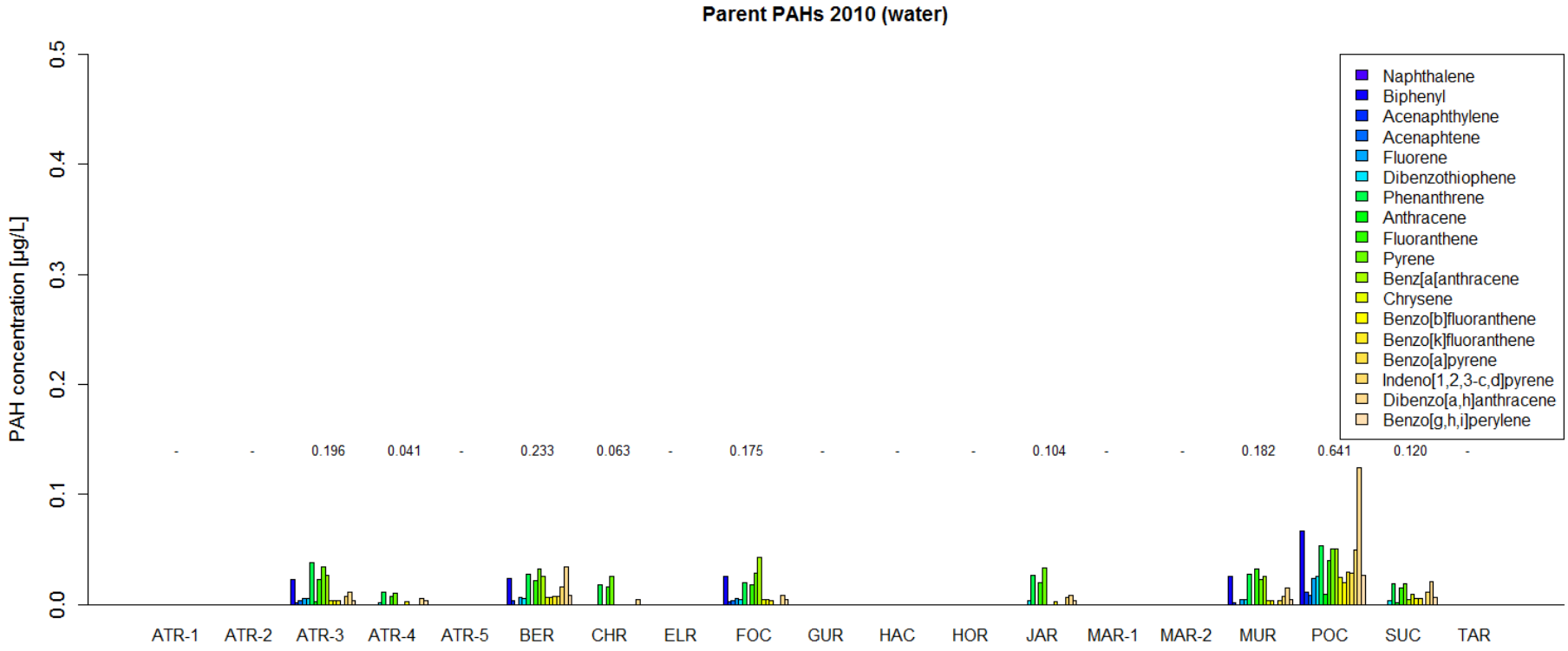
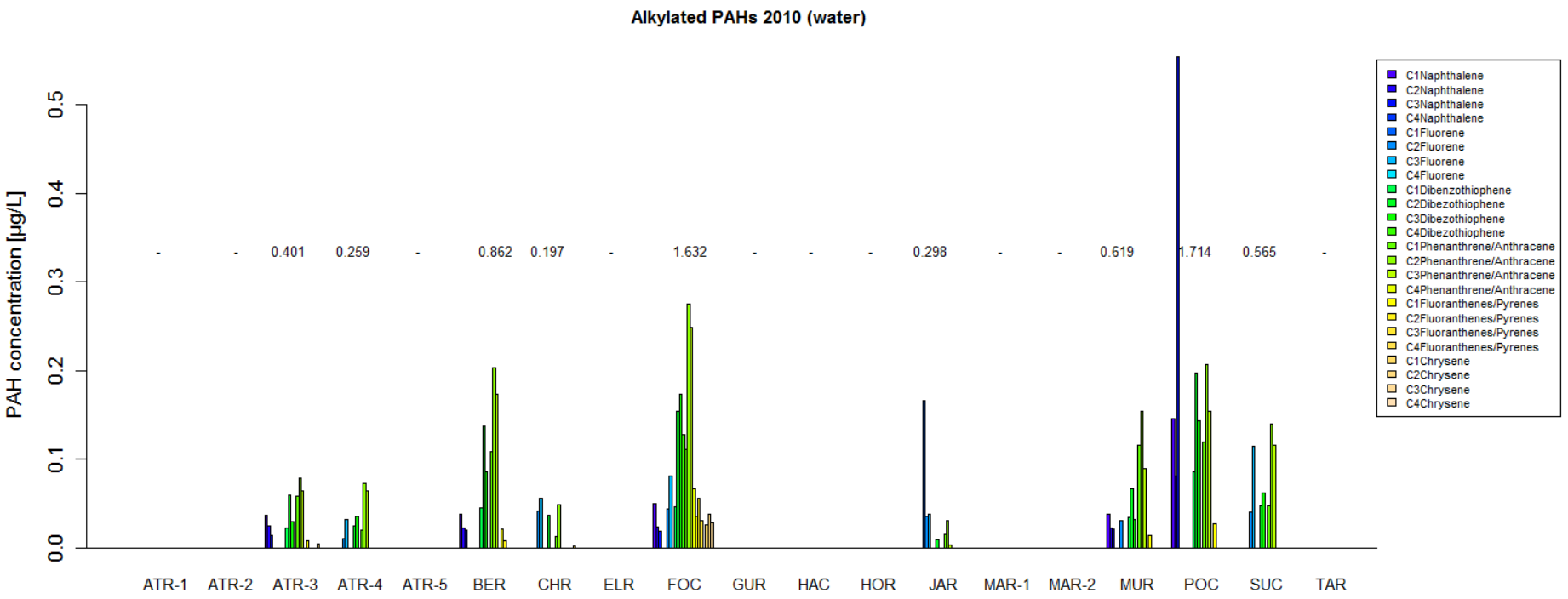


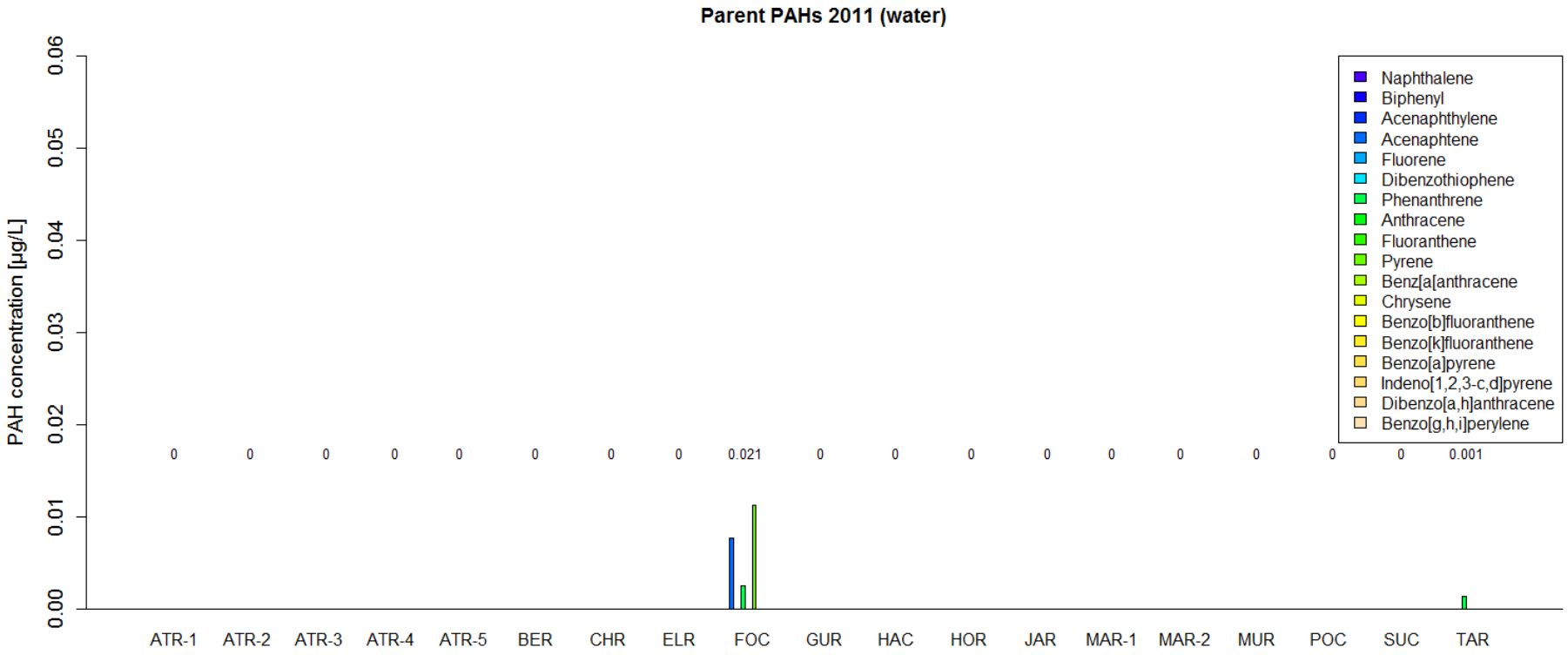
Figure S2. Concentrations (µg/L) of parent (upper graph) and ∑C1-C4 alkylated (lower graph) PAH compounds of samples taken in (A) 2010, (B) 2011, and (C) 2012 in water, (D) 2010 in sediment, (E) 2011 in sediment, and (F) 2012 in sediment. Note different scaling of the y-axes between the years. Total parent or alkylated PAH concentration per site given above each site [µg/L].

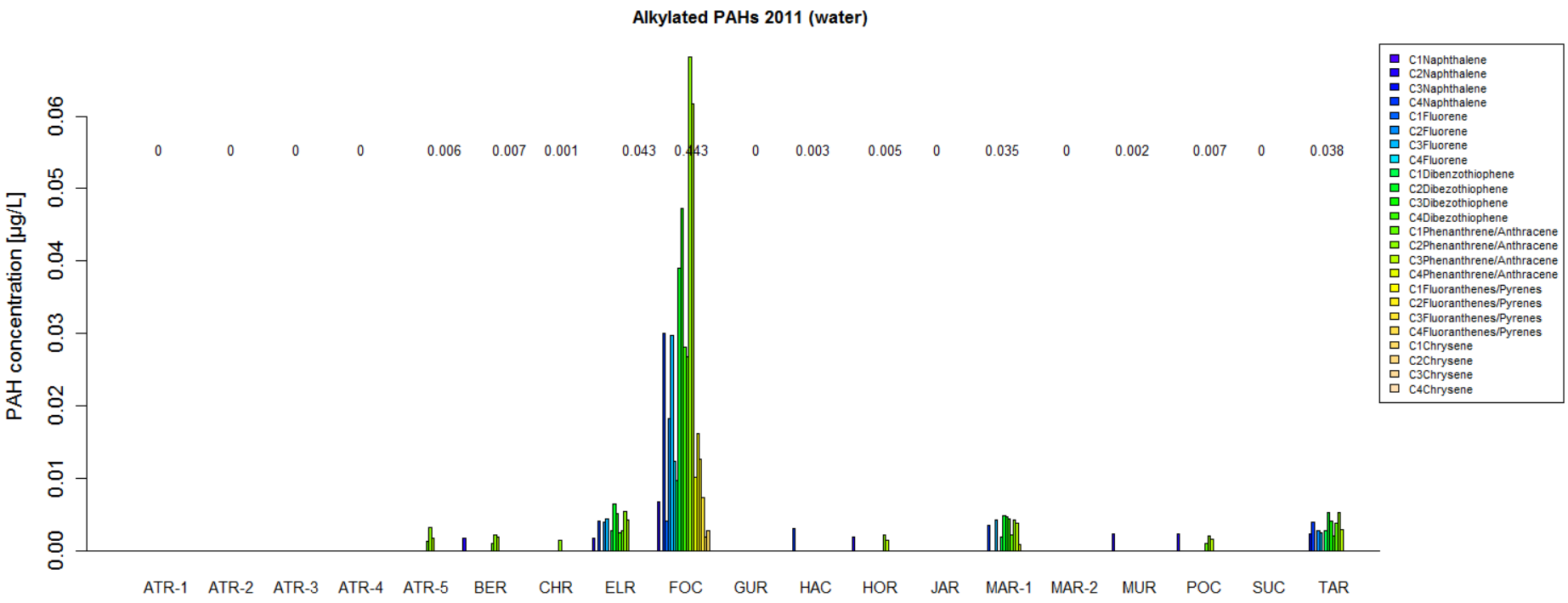
A)



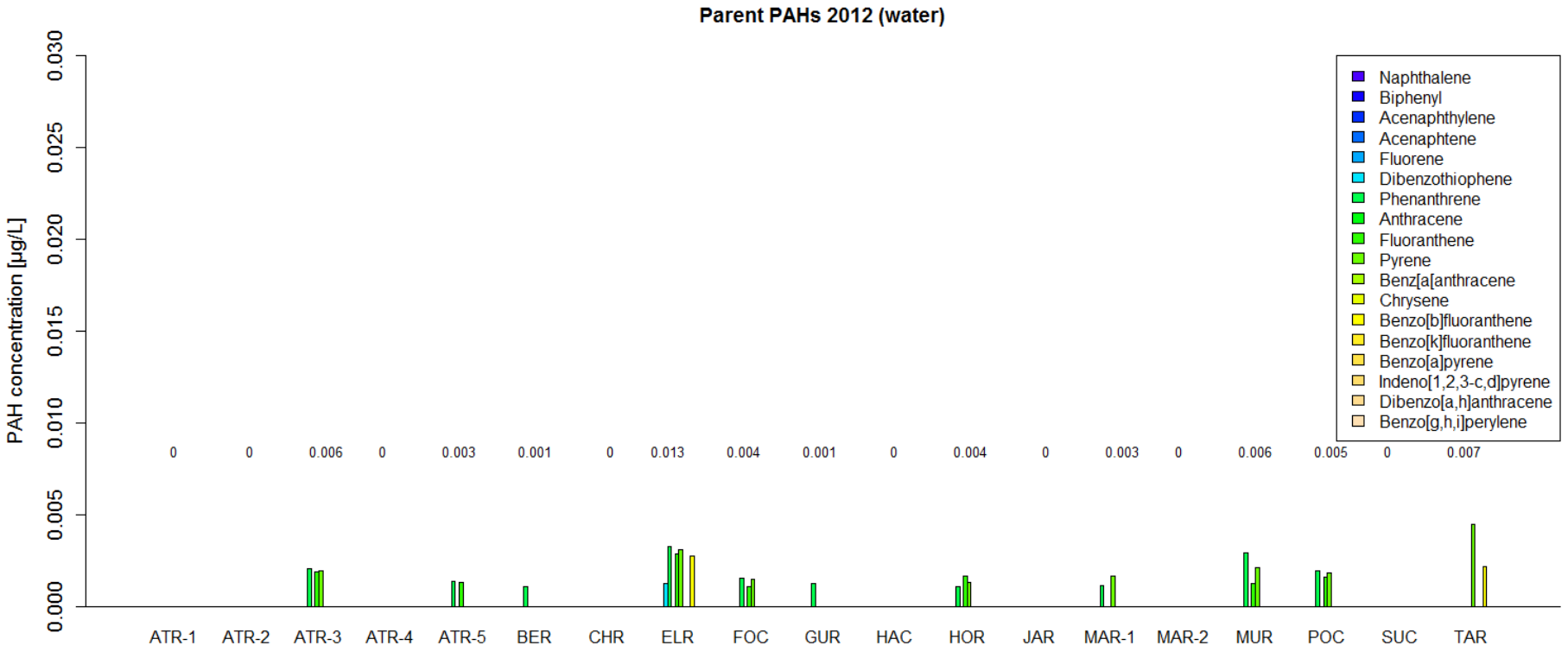

Alkylated PAHs 2010 (water)
PAH concentration [µg/L]
0.0
0.1
0.2
0.3
0.4
0.5
- - 0.401 0.259 - 0.862 0.197 - 1.632 - - - 0.298 - - 0.619 1.714 0.565 -
ATR-1 ATR-2 ATR-3 ATR-4 ATR-5 BER CHR ELR FOC GUR HAC HOR JAR MAR-1 MAR-2 MUR POC SUC TAR
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene

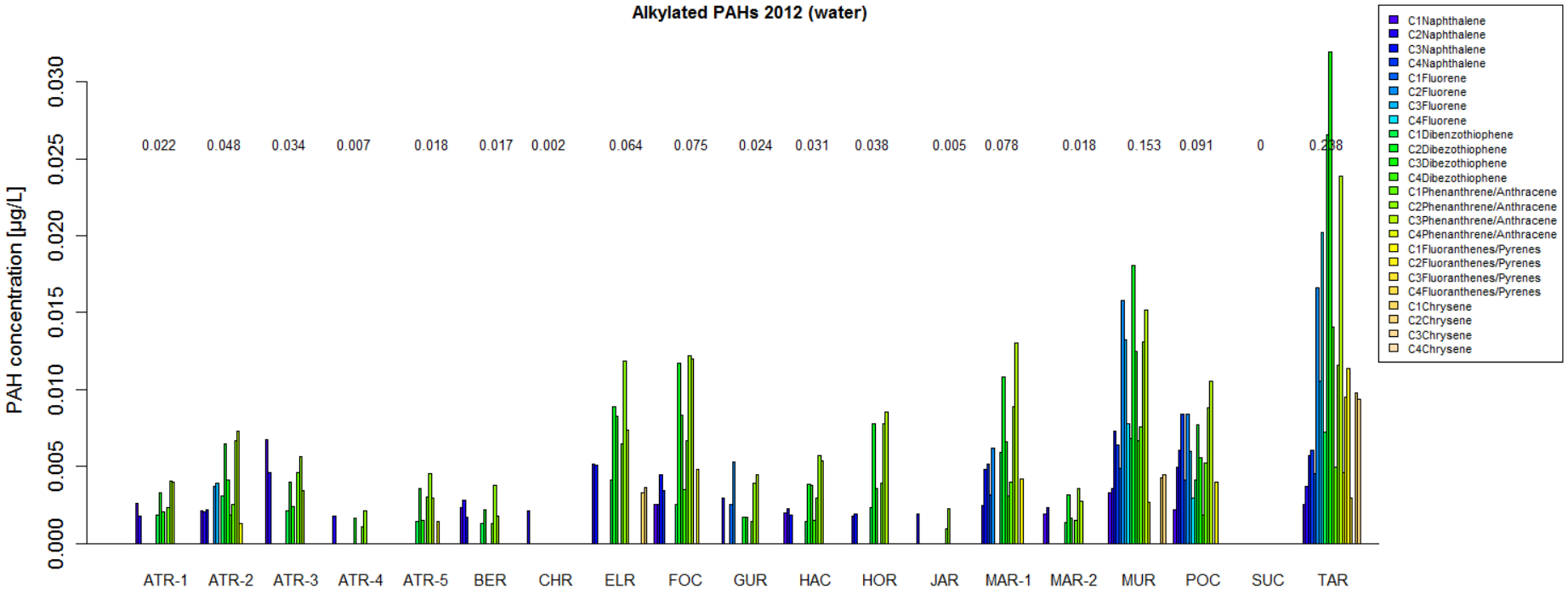
B)


Parent PAHs 2011 (water)
PAH concentration [µg/L]
0.00
0.01
0.02
0.03
0.04
0.05
0.06
Naphthalene
Biphenyl
Acenaphthylene
Acenaphtene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a[anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
0
0
0
0
0
0
0
0
0.021
0
0
0
0
0
0
0
0
0
0.001
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR

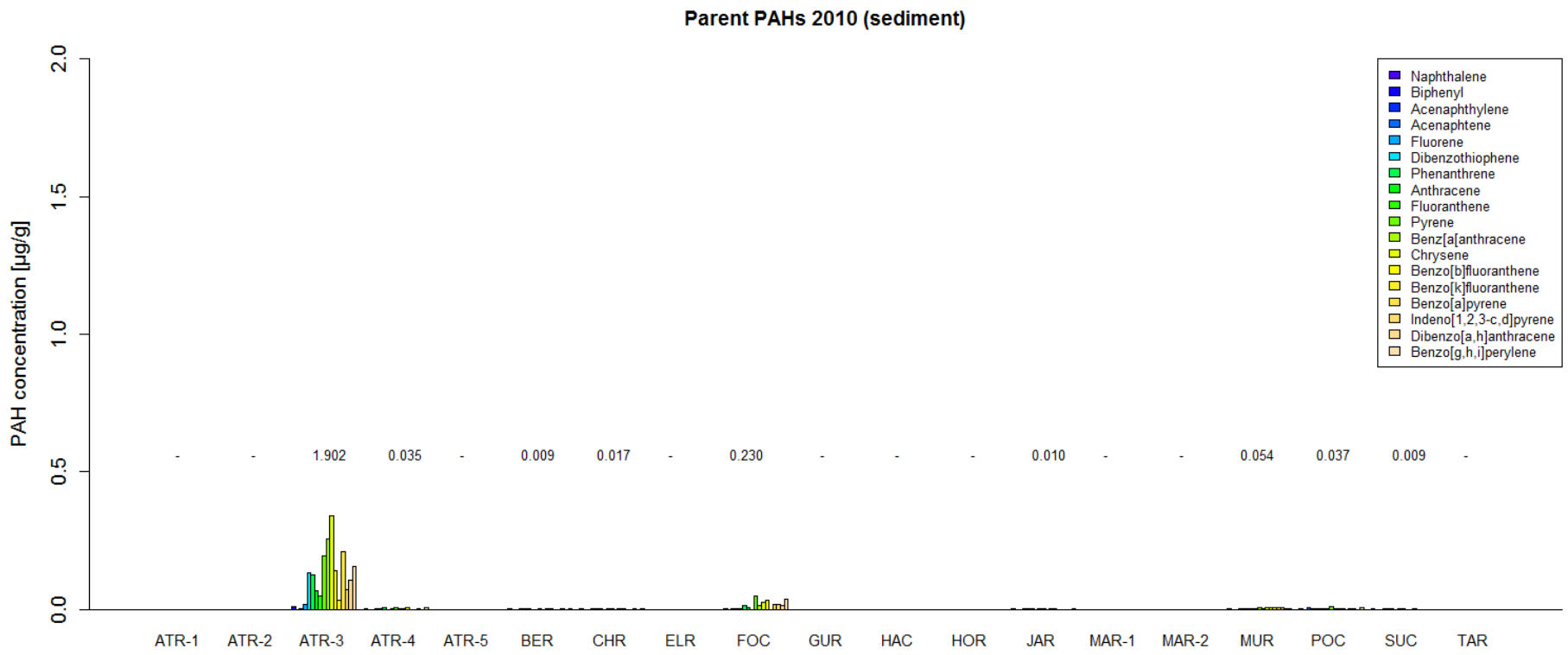

Alkylated PAHs 2011 (water)
PAH concentration [µg/L]
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0
0
0
0
0.006
0.007
0.001
0.043
0.443
0
0.003
0.005
0
0.035
0
0.002
0.007
0
0.038
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene

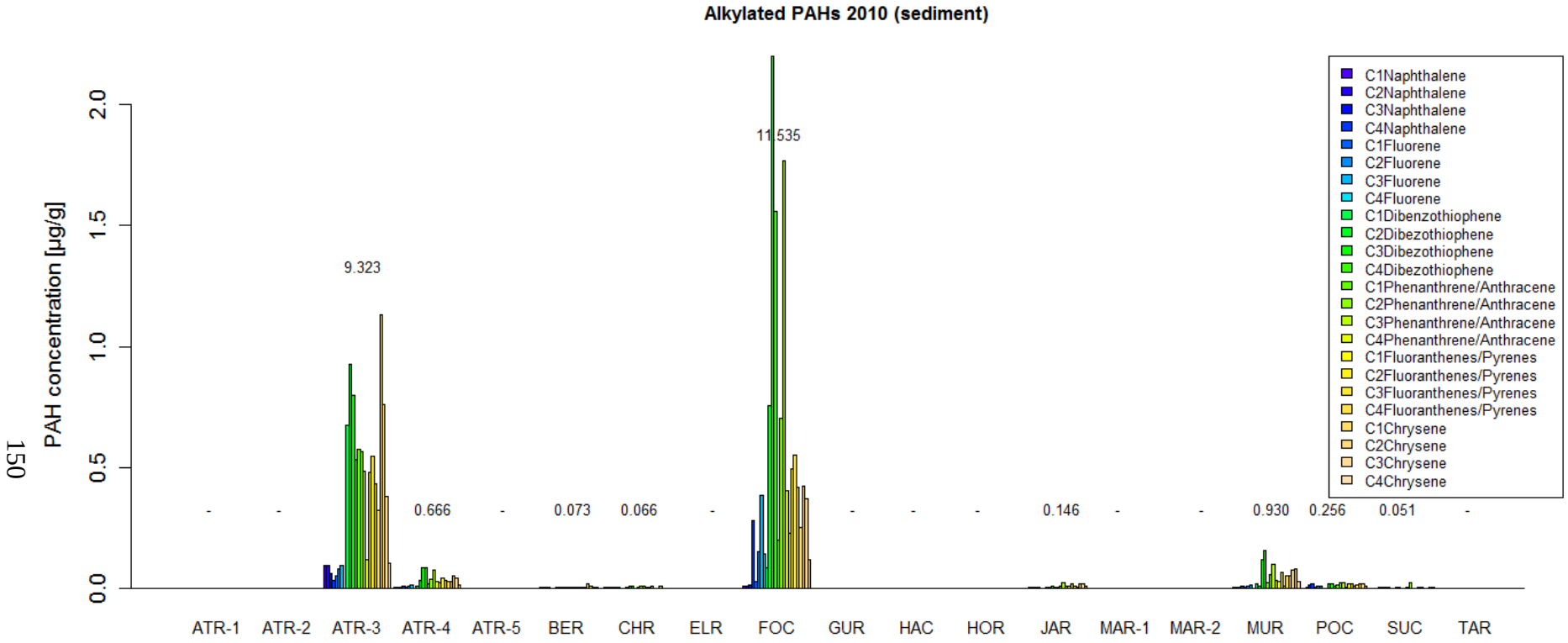
C)


Parent PAHs 2012 (water)
PAH concentration [µg/L]
0.000
0.005
0.010
0.015
0.020
0.025
0.030
Naphthalene
Biphenyl
Acenaphthylene
Acenaphtene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a[anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
0
0
0.006
0
0.003
0.001
0
0.013
0.004
0.001
0
0.004
0
0.003
0
0.006
0.005
0
0.007
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR


Alkylated PAHs 2012 (water)
PAH concentration [µg/L]
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.022
0.048
0.034
0.007
0.018
0.017
0.002
0.064
0.075
0.024
0.031
0.038
0.005
0.078
0.018
0.153
0.091
0
0.238
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene

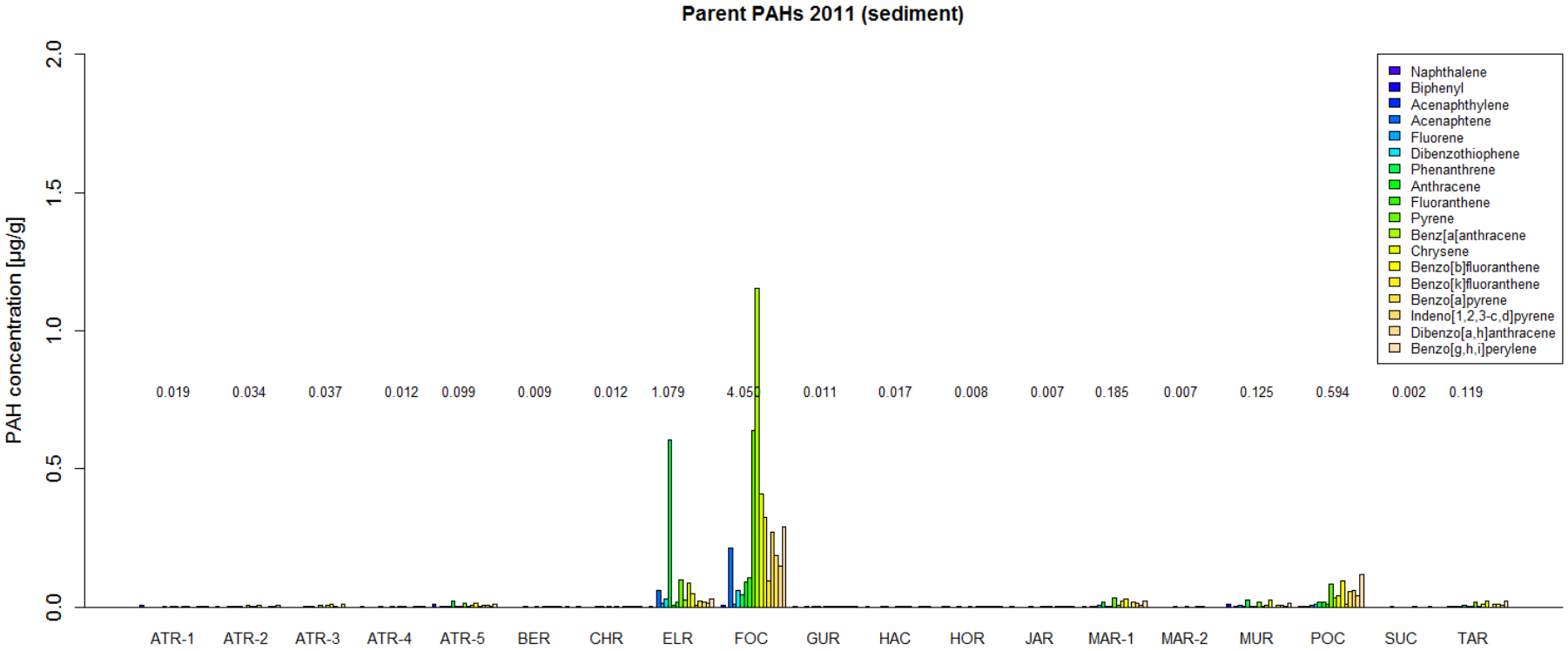
D)

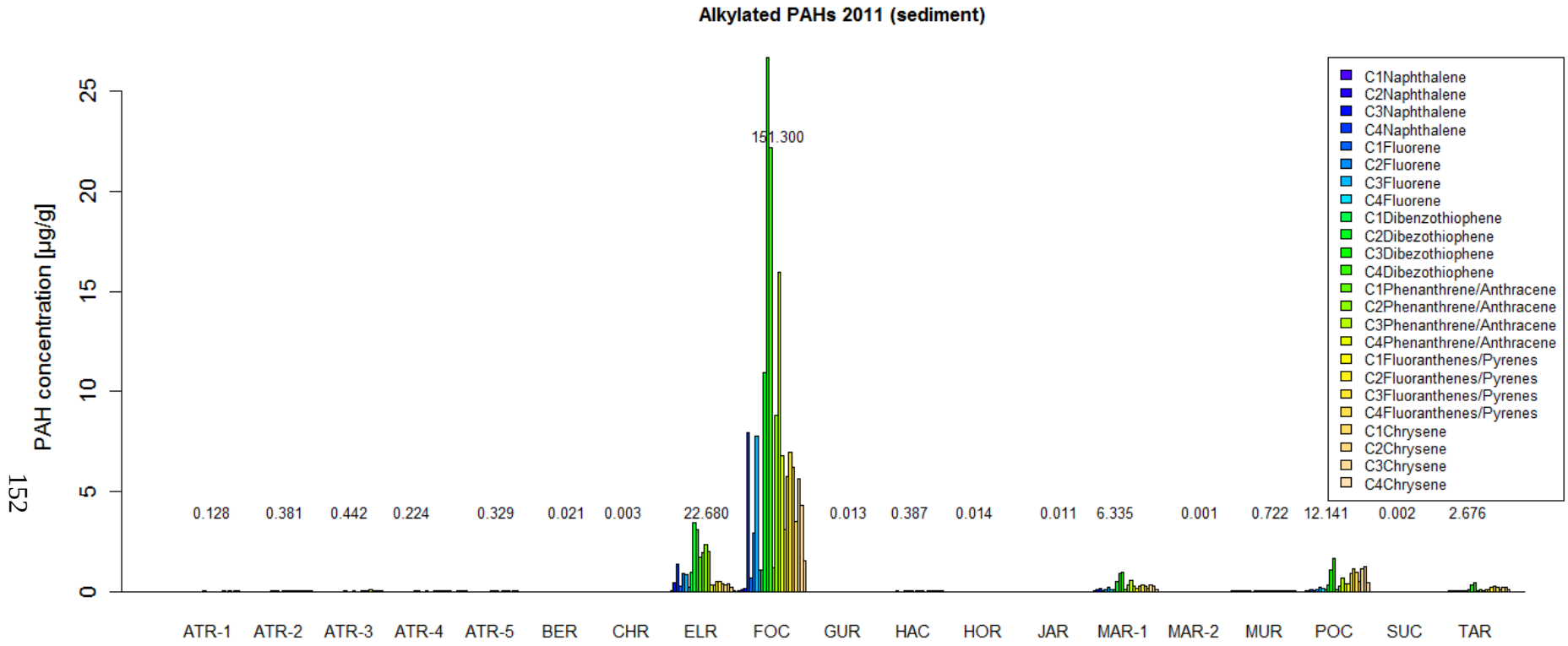

Parent PAHs 2010 (sediment)
PAH concentration [µg/g]
0.0
0.5
1.0
1.5
2.0
Naphthalene
Biphenyl
Acenaphthylene
Acenaphtene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a[anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
- - 1.902 0.035 - 0.009 0.017 - 0.230 - - - 0.010 - - 0.054 0.037 0.009 -
ATR-1 ATR-2 ATR-3 ATR-4 ATR-5 BER CHR ELR FOC GUR HAC HOR JAR MAR-1 MAR-2 MUR POC SUC TAR


Alkylated PAHs 2010 (sediment)
PAH concentration [µg/g]
0.0
0.5
1.0
1.5
2.0
9.323
11.535
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene
- - 0.666 - 0.073 0.066 - - - - 0.146 - - 0.930 0.256 0.051 -
ATR-1 ATR-2 ATR-3 ATR-4 ATR-5 BER CHR ELR FOC GUR HAC HOR JAR MAR-1 MAR-2 MUR POC SUC TAR

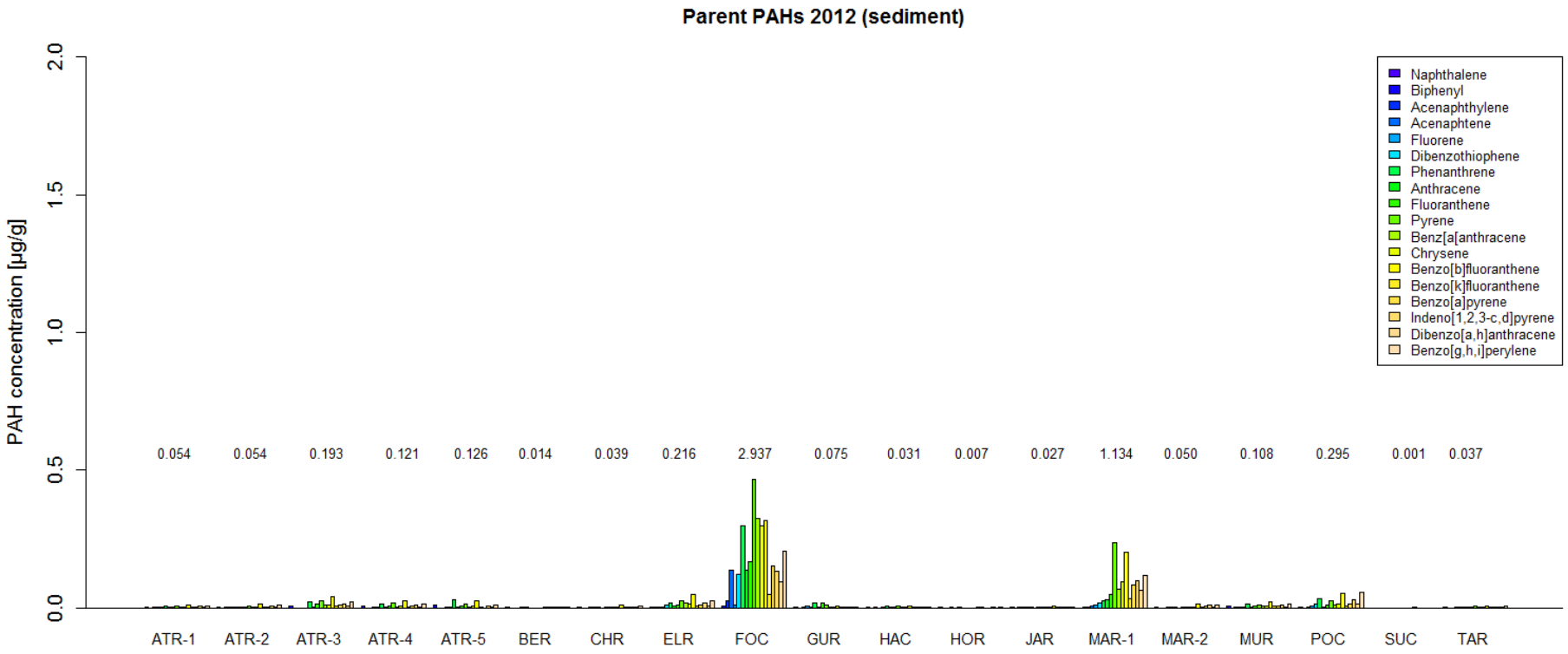
150

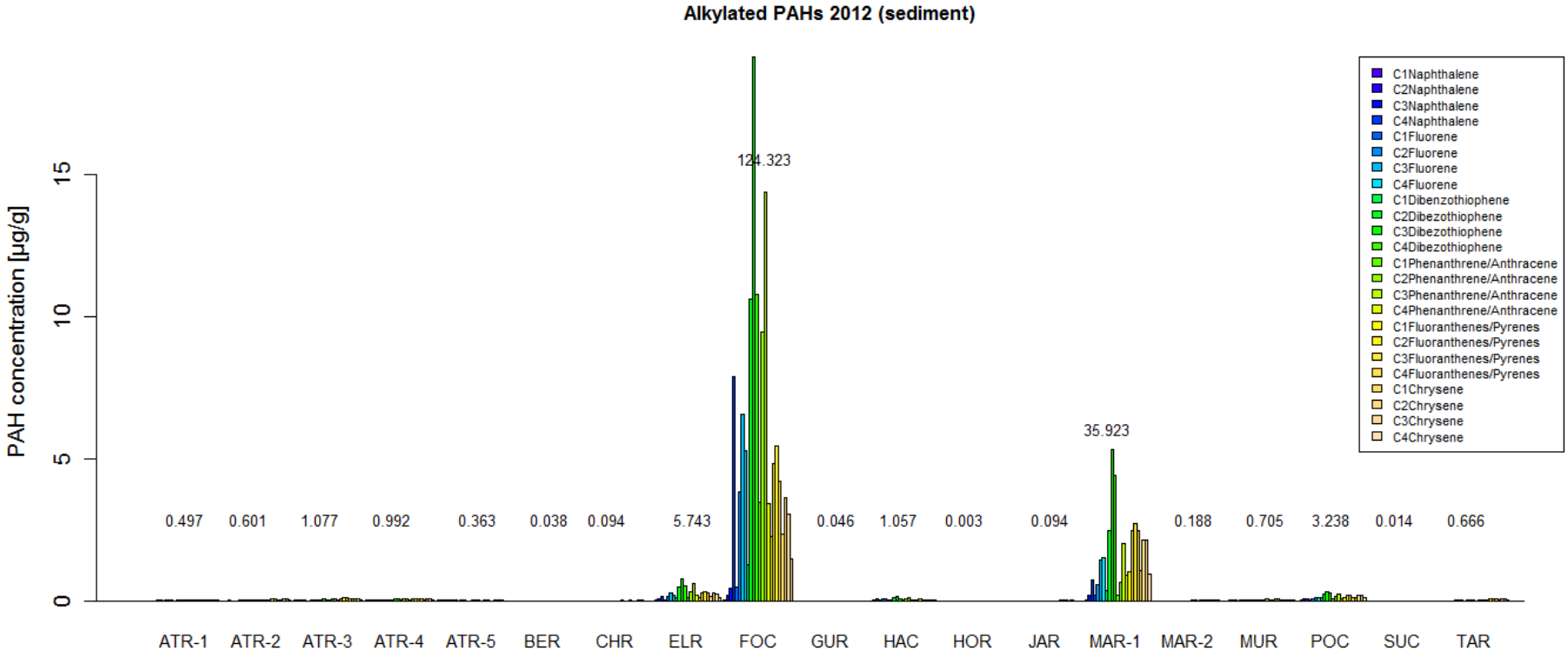
E)


Parent PAHs 2011 (sediment)
PAH concentration [µg/g]
0.0
0.5
1.0
1.5
2.0
0.019
0.034
0.037
0.012
0.099
0.009
0.012
1.079
4.05
0.011
0.017
0.008
0.007
0.185
0.007
0.125
0.594
0.002
0.119
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR
Naphthalene
Biphenyl
Acenaphthylene
Acenaphtene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a[anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene


Alkylated PAHs 2011 (sediment)
PAH concentration [µg/g]
0
5
10
15
20
25
151.300
0.128
0.381
0.442
0.224
0.329
0.021
0.003
22.680
0.013
0.387
0.014
0.011
6.335
0.001
0.722
12.141
0.002
2.676
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene

F)


Parent PAHs 2012 (sediment)
PAH concentration [µg/g]
0.0
0.5
1.0
1.5
2.0
Naphthalene
Biphenyl
Acenaphthylene
Acenaphtene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a[anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
0.054
0.054
0.193
0.121
0.126
0.014
0.039
0.216
2.937
0.075
0.031
0.007
0.027
1.134
0.050
0.108
0.295
0.001
0.037
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR


Alkylated PAHs 2012 (sediment)
PAH concentration [µg/g]
0
5
10
15
124.323
35.923
0.497
0.601
1.077
0.992
0.363
0.038
0.094
5.743
0.046
1.057
0.003
0.094
0.188
0.705
3.238
0.014
0.666
ATR-1
ATR-2
ATR-3
ATR-4
ATR-5
BER
CHR
ELR
FOC
GUR
HAC
HOR
JAR
MAR-1
MAR-2
MUR
POC
SUC
TAR
C1Naphthalene
C2Naphthalene
C3Naphthalene
C4Naphthalene
C1Fluorene
C2Fluorene
C3Fluorene
C4Fluorene
C1Dibenzothiophene
C2Dibezothiophene
C3Dibezothiophene
C4Dibezothiophene
C1Phenanthrene/Anthracene
C2Phenanthrene/Anthracene
C3Phenanthrene/Anthracene
C4Phenanthrene/Anthracene
C1Fluoranthenes/Pyrenes
C2Fluoranthenes/Pyrenes
C3Fluoranthenes/Pyrenes
C4Fluoranthenes/Pyrenes
C1Chrysene
C2Chrysene
C3Chrysene
C4Chrysene

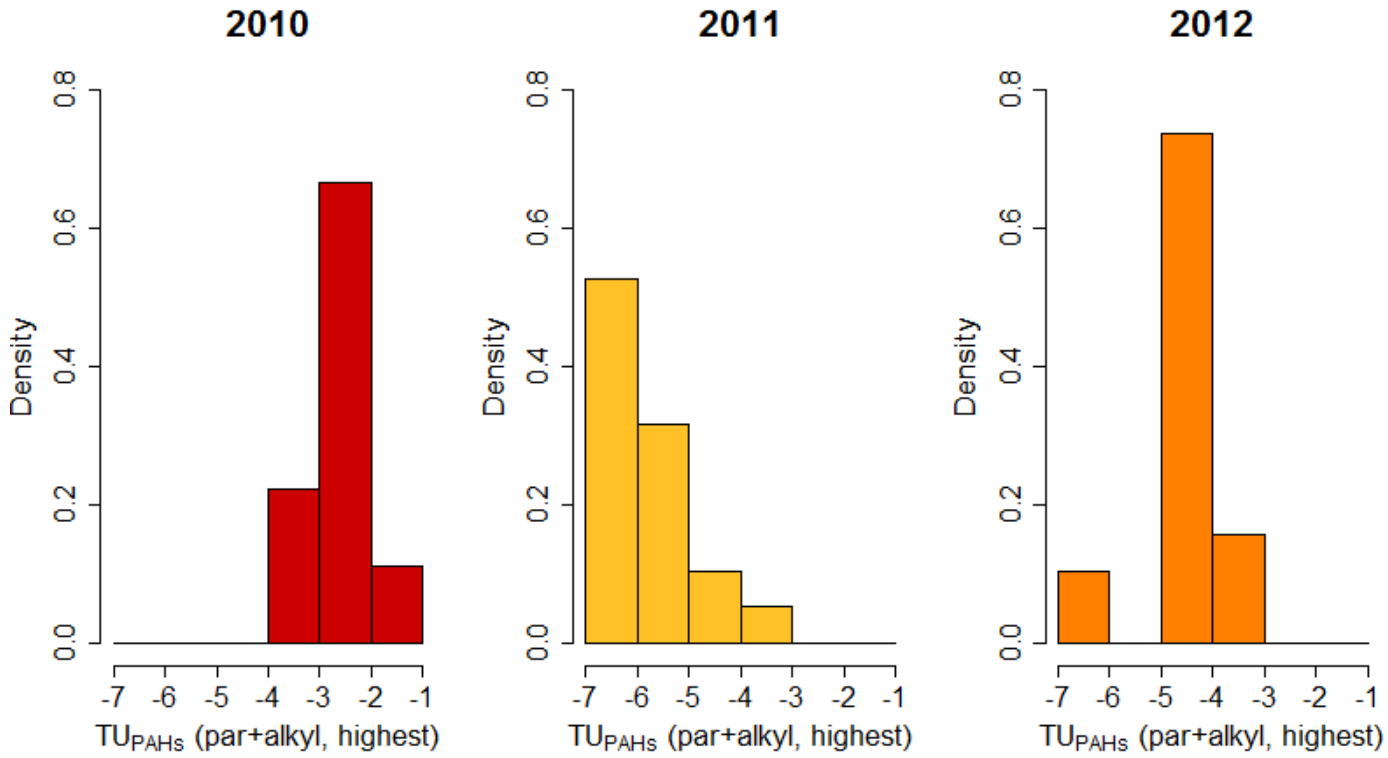


Figure S3. Frequency distribution of log Toxic Units of total PAHs (parent and alkylated forms, maximum TU) in water for all sites sampled in 2010, 2011, and 2012.

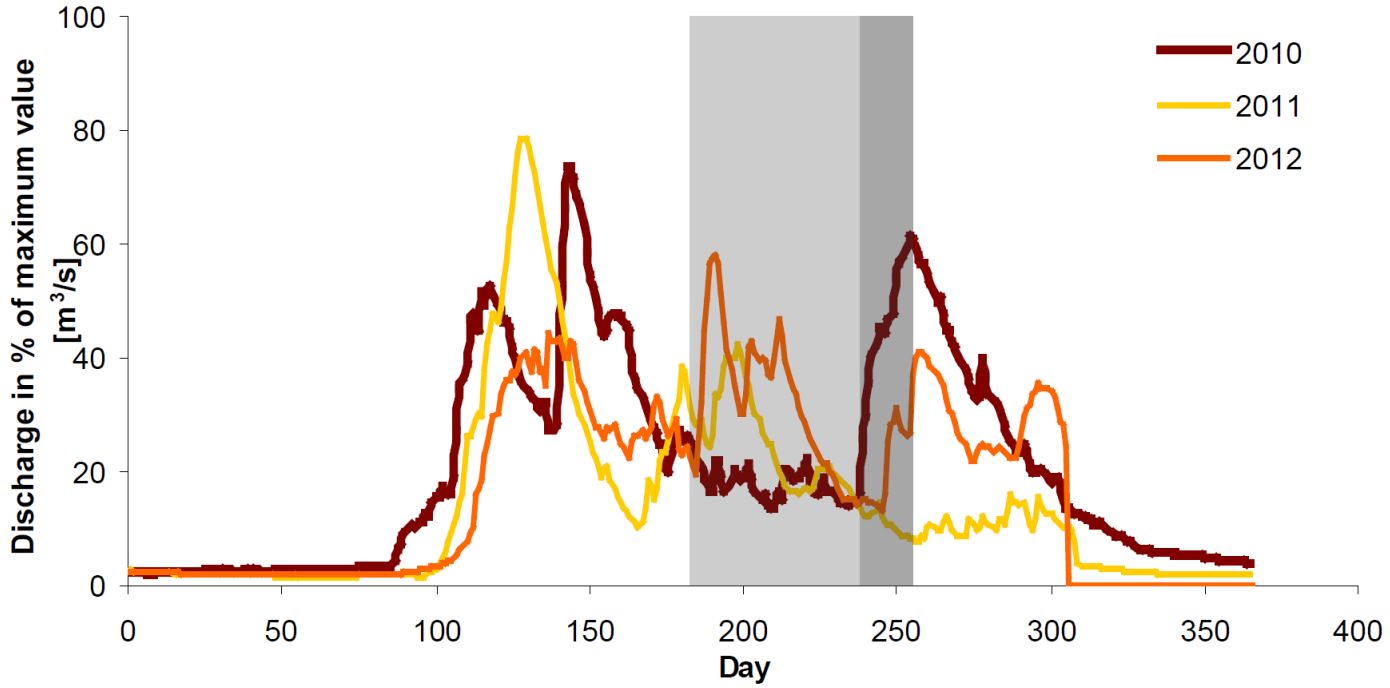


Figure S4. Discharge in % of maximum value [m^3/s] over the course of a year for the years 2010, 2011, 2012. Calculated as mean discharge of 10 sites (data source: RAMP, http://www.ramp-alberta.org/ramp/data.aspx). Discharge during the sampling time in autumn is indicated by the dark grey bar and discharge in the summer months is highlighted in light grey.

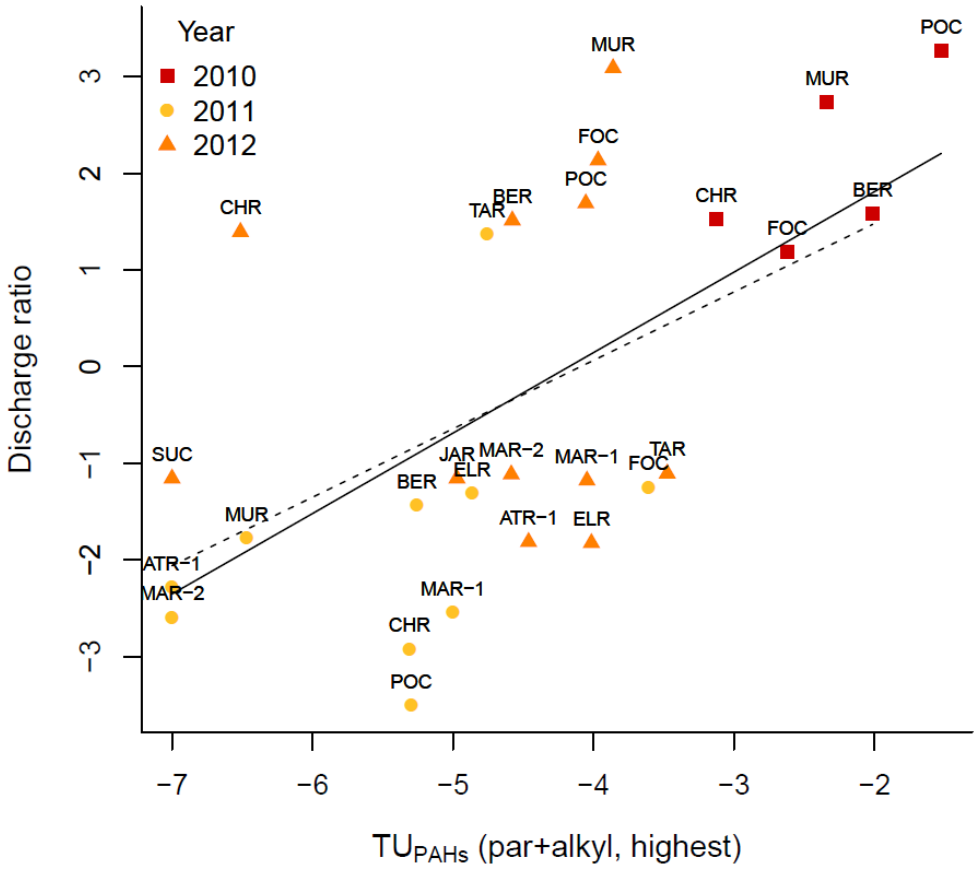
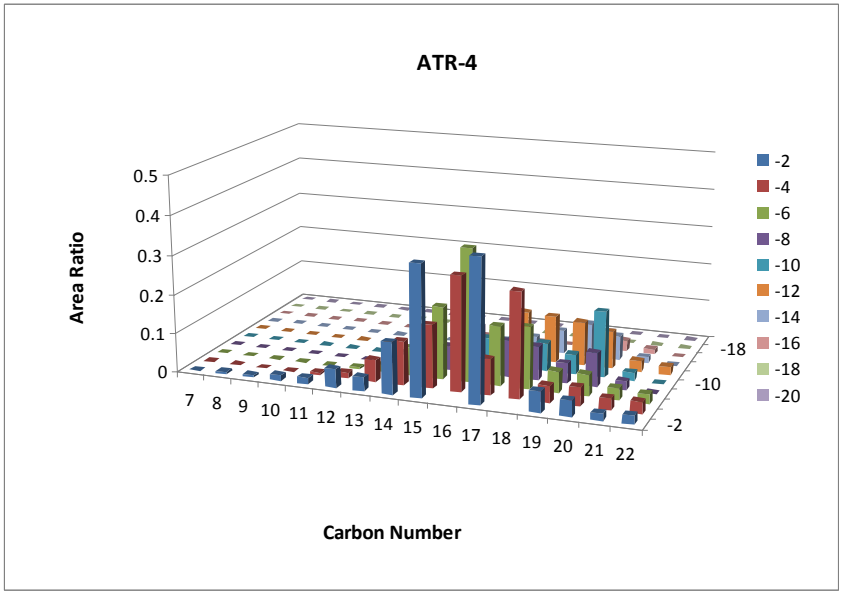
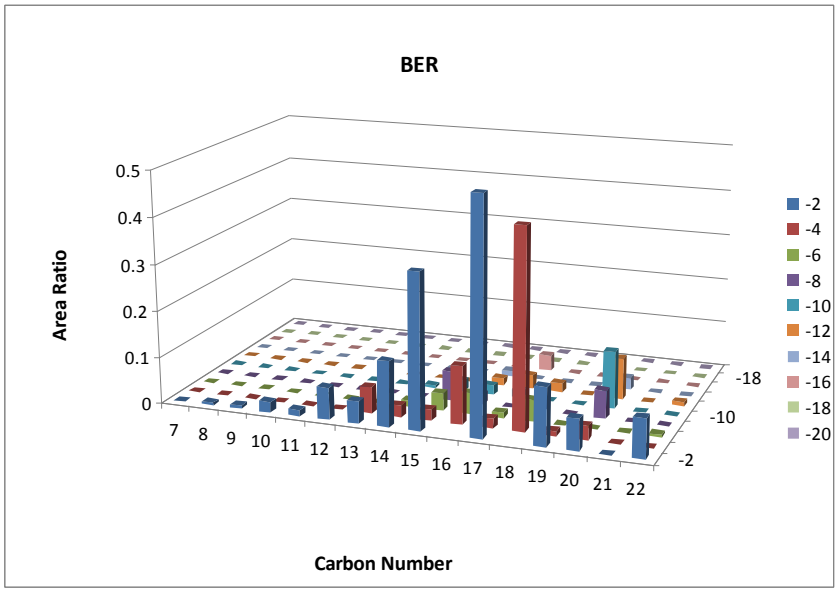
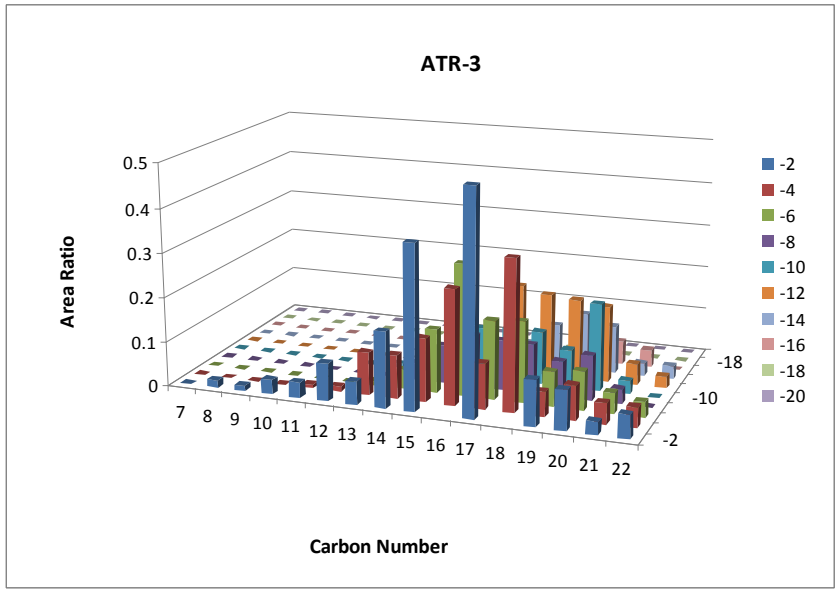
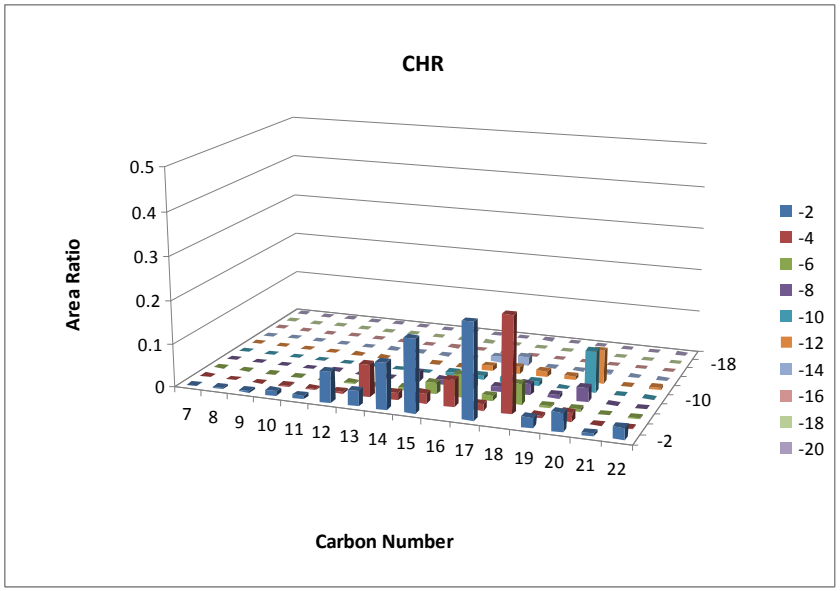


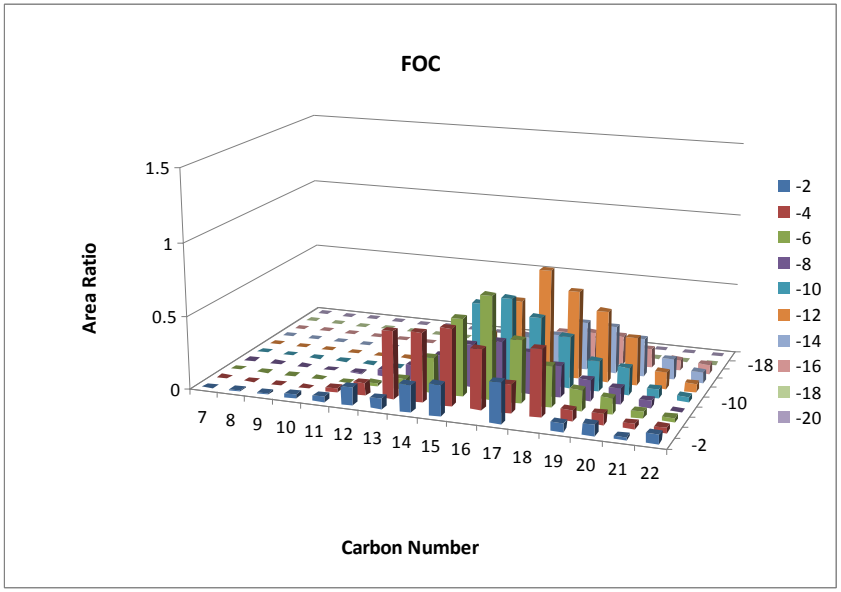
Figure S5. Discharge ratio, calculated as discharge during the sampling period in relation to discharge during summer, plotted against log Toxic Units of total PAHs (parent and alkylated forms, maximum TU) in water for 2010, 2011, and 2012. Solid and dotted line indicate correlation with site POC (Spearman correlation: rho = 0.67, $p < 0.001$, n = 25) and without (rho = 0.62, $p < 0.005$, n = 22), respectively.

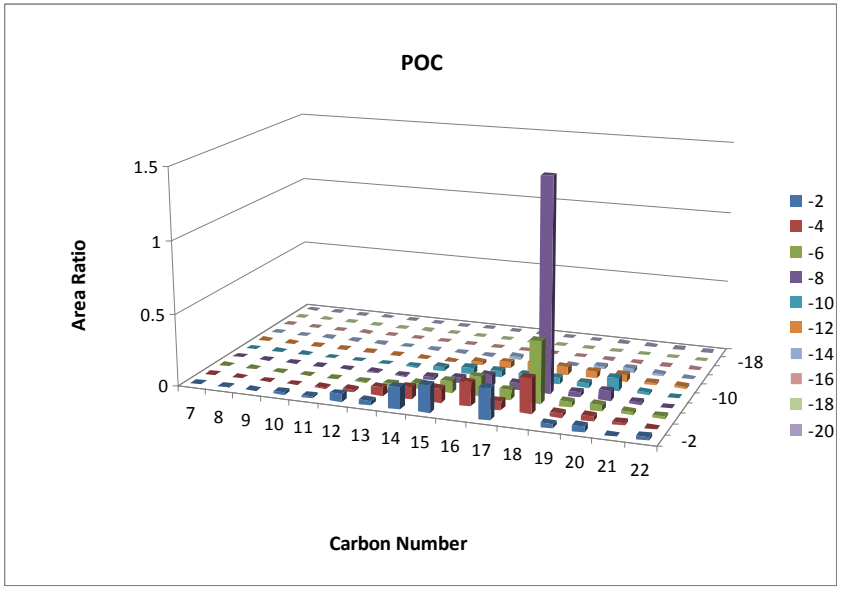

ATR-4
Area Ratio
0.5
0.4
0.3
0.2
0.1
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
-18
-10
-2
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
Carbon Number


BER
Area Ratio
0.5
0.4
0.3
0.2
0.1
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
-18
-10
-2
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
Carbon Number


ATR-3
Area Ratio
0.5
0.4
0.3
0.2
0.1
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
-18
-10
-2
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
Carbon Number


CHR
Area Ratio
0.5
0.4
0.3
0.2
0.1
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
-18
-10
-2
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
Carbon Number





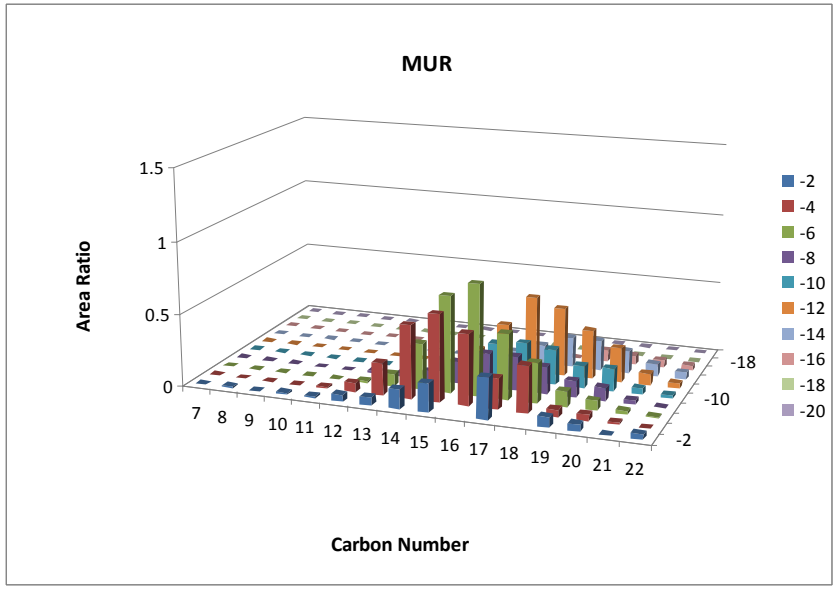


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Figure S6. Profiles of naphthenic acids in 2010, including typical patterns of bitumen-derived naphthenic acid homologues (ATR-4, FOC, ATR-3, MUR). The respective number of carbons (n) is indicated on the x-axis, the area ratio on the y-axis, and the hydrogen deficiency (Z) on the z-axis, according to the molecular formula ($C_nH_{2n+z}O_2$).

3. Tables

Table S1. Analyzed parent and alkylated PAH compounds according to the 16 PAHs of the list of "Priority Pollutants" by the US EPA. Also indicated are their respective LC50 values (µg/L) for *Daphnia magna*. Toxicity values were (a) calculated as median of 48h *D. magna* toxicity studies available in the ECOTOX database (US EPA, http://cfpub.epa.gov/ecotox/data_download.cfm (status 2011)) or (b) estimated using the ECOSAR (Nabholz and Mayo-Bean 2009) or read-across method (Schüürmann et al. 2011) for substances for which no toxicity studies existed. For C1-4 phenanthrene/anthracene and C1-4 fluoranthene/pyrene, the mean of the single parent compounds was applied.

parent PAH compounds	no. of rings	LC50 *Daphnia magna* of parent PAH compounds	alkylated PAH compounds	LC50 *Daphnia magna* of alkylated PAH compounds
Naphthalene	2	6900 (a)	C1-4 Naphthalene	6900 (a)
Acenaphthene	3	2362.5 (a)	-	-
Acenaphthylene	3	1953.7 (b)	-	-
Biphenyl	2	1497.45 (a)	-	-
Phenanthrene	3	535.18 (a)	C1-4 Phenanthrene/Anthracene	300.25
Dibenzothiophene	3	466 (a)	C1-4 Dibenzothiophene	466 (a)
Fluorene	3	430 (a)	C1-4 Fluorene	430 (a)
Benzo(*a*)pyrene	5	125.65 (a)	-	-
Chrysene	4	104.44 (b)	C1-4 Chrysene	104.44 (a)
Pyrene	4	91.02 (a)	-	-
Fluoranthene	4	78 (a)	C1-4 Fluoranthene/Pyrene	84.51
Benzo(*a*)anthracene	4	73.71 (a)	-	-
Anthracene	3	65.32 (a)	-	-
Benzo(*b*)fluoranthene	5	57.99 (b)	-	-
Benzo(*k*)fluoranthene	5	7.53 (b)	-	-
Benzo(*ghi*)perylene	6	7.38 (a)	-	-
Dibenzo(*ah*)anthracene	5	6.15 (a)	-	-
Indeno(1,2,3-*cd*)pyrene	6	1.66 (b)	-	-

Table S2. PAH compounds with highest Toxic Units (TU) per sampling site in 2010, their concentration, and the respective median lethal concentration (LC50) for *Daphnia magna*.

	2010			
Sampling site	Compound	Compound concentration (µg/L)	LC50 *D. magna* (µg/L)	TU
ATR-1	-	-	-	-
ATR-2	-	-	-	-
ATR-3	Indeno(1,2,3-CD)pyrene	0.00705	1.66	-2.37
ATR-4	Dibenzanthrecene	0.00511	6.15	-3.08
ATR-5	-	-	-	-
BER	Indeno(1,2,3-CD)pyrene	0.01612	1.66	-2.01
CHR	Dibenzanthrecene	0.0046	6.15	-3.13
ELR	-	-	-	-
FOC	Phenanthrene + Anthracene	0.72073	-2.34	-2.62
GUR	-	-	-	-
HAC	-	-	-	-
HOR	-	-	-	-
JAR	Indeno(1,2,3-CD)pyrene	0.00677	1.66	-2.39
MAR-1	-	-	-	-
MAR-2	-	-	-	-
MUR	Indeno(1,2,3-CD)pyrene	0.00764	1.66	-2.34
POC	Indeno(1,2,3-CD)pyrene	0.04969	1.66	-1.52
SUC	Indeno(1,2,3-CD)pyrene	0.01124	1.66	-2.17
TAR	-	-	-	-

Table S3. Metal concentrations (µg/L) in samples taken in 2010 and their respective LC50 values (µg/L) for *Daphnia magna*. Toxicity values were calculated as median of 48h *D. magna* toxicity studies available in the ECOTOX database (US EPA, http://cfpub.epa.gov/ecotox/data_download.cfm (status 2011)). Those compounds, for which no toxicity information is available, could not be considered in the TU value calcualation.

	2010											
Sampling site	Aluminum	Arsenic	Beryllium	Boron	Cadmium	Cesium	Chromium	Cobalt	Copper	Gallium	Iron	Lead
LC50 *D. magna*	38646.42	5815.00		141000.00	101.50		292.50	2506.94	38.55		10884.17	1815.00
ATR-1	-	-	-	-	-	-	-	-	-	-	-	-
ATR-2	-	-	-	-	-	-	-	-	-	-	-	-
ATR-3	350.3	0.1	0.05	<0.05	2.65	0.1	0.5	5.2	26.6	0.3	401.9	8.9
ATR-4	215.3	<0.05	0.25	<0.05	3.1	0.05	<0.1	5.3	13.95	0.1	111.45	4.65
ATR-5	-	-	-	-	-	-	-	-	-	-	-	-
BER	569.3	0.3	0.25	6.7	2.75	0.1	2	4.45	22.35	0.15	802.15	7.55
CHR	410.7	0.25	0.25	<0.05	2.5	0.05	<0.1	5	13.4	0.1	482.35	5
ELR	-	-	-	-	-	-	-	-	-	-	-	-
FOC	395.75	<0.05	0.15	<0.05	2.3	0.05	<0.1	5.3	12.65	0.1	212.25	4.7
GUR	-	-	-	-	-	-	-	-	-	-	-	-
HAC	-	-	-	-	-	-	-	-	-	-	-	-
HOR	-	-	-	-	-	-	-	-	-	-	-	-
JAR	335.85	0.1	0.5	<0.05	2.95	0.1	<0.1	4.85	13.95	0.05	21.1	4.85
MAR-1	-	-	-	-	-	-	-	-	-	-	-	-
MAR-2												
MUR	557.15	0.1	0.1	<0.05	2.55	0.1	<0.1	5.1	23	0.1	<2	8.15
POC	493.6	0.25	0.9	98.05	2.75	0.05	5	5.6	58.2	0.1	296.25	7.5
SUC	199.75	0.15	0.45	<0.05	2.35	0.05	<0.1	5.3	15.55	0.05	300.9	5.55
TAR	-	-	-	-	-	-	-	-	-	-	-	-

continued **Table S3**. Metal concentrations (µg/L) in samples taken in 2010.

	2010											
Sampling site	Manganese	Mercury	Molybdenum	Nickel	Rubidium	Selenium	Silver	Strontium	Thallium	Uranium	Vanadium	Zinc
LC50 *D. magna*	26193.11	8.00	320150.00	2257.90			5.84		61.00		2381.45	1100.00
ATR-1	-	-	-	-	-	-	-	-	-	-	-	-
ATR-2	-	-	-	-	-	-	-	-	-	-	-	-
ATR-3	0.6	0.0037	0.05	4	1.1	5.9	1.35	118.35	<0.05	0.1	0.15	<0.25
ATR-4	0.2	0.0048	0.55	2.8	0.5	<0.05	0.25	203.85	<0.05	0.3	<0.05	<0.25
ATR-5	-	-	-	-	-	-	-	-	-	-	-	-
BER	0.85	0.0054	0.15	2.25	0.45	1.7	0.6	151.85	<0.05	0.1	0.7	<0.25
CHR	1.05	0.0051	<0.05	5.8	0.3	0.3	0.4	81.75	<0.05	0.05	<0.05	409.85
ELR	-	-	-	-	-	-	-	-	-	-	-	-
FOC	<0.05	0.001	<0.05	5.4	0.65	1.95	0.15	201.05	<0.05	0.15	<0.05	337.95
GUR	-	-	-	-	-	-	-	-	-	-	-	-
HAC	-	-	-	-	-	-	-	-	-	-	-	-
HOR	-	-	-	-	-	-	-	-	-	-	-	-
JAR	<0.05	0.001	1.15	4.05	0.65	3.05	0.35	67.5	<0.05	<0.05	<0.05	<0.25
MAR-1	-	-	-	-	-	-	-	-	-	-	-	-
MAR-2	-	-	-	-	-	-	-	-	-	-	-	-
MUR	0.25	0.0026	0.25	5	1.35	<0.05	0.95	99.9	<0.05	0.1	<0.05	<0.25
POC	2.3	0.0013	1.5	<0.05	1	1.3	0.4	160.25	<0.05	0.1	4.1	270.05
SUC	<0.05	0.0021	0.05	2.3	0.35	7.1	0.35	63.95	<0.05	<0.05	<0.05	520.8
TAR	-	-	-	-	-	-	-	-	-	-	-	-

Table S4. Metals with highest Toxic Units (TU) per sampling site in 2010, their concentration (µg/L), and the respective median lethal concentration (LC50) for *Daphnia magna* (µg/L).

	2010			
Sampling site	Compound	Compound concentration (µg/L)	LC50 *D. magna* (µg/L)	TU
ATR-1	-	-	-	-
ATR-2	-	-	-	-
ATR-3	Copper	26.6	38.55	-0.16
ATR-4	Copper	13.95	38.55	-0.44
ATR-5	-	-	-	-
BER	Copper	22.35	38.55	-0.24
CHR	Zinc	409.85	1100.00	-0.43
ELR	-	-	-	-
FOC	Copper	12.65	38.55	-0.48
GUR	-	-	-	-
HAC	-	-	-	-
HOR	-	-	-	-
JAR	Copper	13.95	38.55	-0.44
MAR-1	-	-	-	-
MAR-2	-	-	-	-
MUR	Copper	23	38.55	-0.22
POC	Copper	58.2	38.55	0.18
SUC	Zinc	520.8	1100.00	-0.32
TAR	-	-	-	-

Table S5. Aquatic invertebrate taxa with their respective abundances in samples taken from all sampling sites in (A) 2010, (B) 2011, and (C) 2012. $S_{organic}$ and generation time (GT) are indicated for each taxon. $SPEAR_{oil}$ was calculated (bottom line) per sampling site as the sum of (log(abundance+1)* ($S_{organic}$ -1) / GT) of all taxa divided by the sum of the weighted abundance (log(abundance+1)) of all taxa, according to formula [Eq. 4].

A) 2010, sites POC, BER, FOC, ATR-3

Order	Suborder	Family	Subfamily	Genus	Species	*S organic*	GT	POC	BER	FOC	ATR-3
Acari						-1.64	1				
	Oribatida	Eremaeidae		Hydrozetes		-1.64	1	4			
Coleoptera						-1.26	1	20			
		Dytiscidae				-0.81	1				
		Elmidae		Optioservus		-1.15	2				
		Gyrinidae		Gyrinus		-1.15	1	65			
		Haliplidae		Haliplus		-1.83	1	1			
Diptera		Chironomidae	Chironominae			-0.39	0.5	25			
			Tanypodinae			-0.39	0.5	4			
		Sciomyzidae		Sepedon		-0.35	0.5	1			
		Simuliidae				-0.46	0.5			1	
Hemiptera	Heteroptera	Gerridae				-0.56	0.5	5	10		
		Corixidae				-0.29	0.5	5	10		
				Sigara/Callicorixa		-0.29	0.5				
		Nepidae		Nepa cinerea		-0.56	0.5				
		Notonectidae		Notonecta		-0.82	1	1			
Odonata	Anisoptera	Aeshnidae		Aeshna		-0.96	2		4		
		Corduliidae		Epitheca		-0.96	2				
				Somatochlora		-0.96	2				
		Gomphidae				-0.96	2				
				Ophiogomphus		-0.96	2				
	Zygoptera					-0.24	1	1			
		Calopterygidae		Calopteryx		-0.36	2				
		Coenagrionidae		Enallagma		-0.24	1	5			
Ephemeroptera						-0.14	1	4		6	
		Baetidae				0.02	0.5				
				Baetis		0.02	0.5	2	16	18	
				Centroptilum		0.02	0.5			6	

		Pseudocloeon		0.02	0.5			42
	Baetiscidae	Baetisca		-0.14	1		16	
	Ephemerellidae	Ephemerella		-0.30	1			
	Heptageniidae	Heptagenia		-0.30	0.5	5	8	6
		Stenonema		-0.30	0.5			
	Leptophlebidae	Leptophlebia		-0.30	0.5		8	
	Metretopodidae	Metretopus		-0.14	0.5			
		Siphloplecton		-0.14	0.5		31	
	Siphlonuridae	Analetris		-0.30	0.5		8	
Plecoptera	Nemouridae	Amphinemura		0.25	1			1
	Perlidae	Acroneuria		0.38	1			
	Perlodidae	Isoperla		0.38	1			
	Pteronarcyidae	Pteronarcys		0.31	2			
		Pteronarcella		0.31	2			4
Trichoptera	Brachycentridae	Brachycentrus		-0.06	1			
	Glossosomatidae	Glossosoma		-0.06	0.5			
	Helicopsychidae	Helicopsyche		-0.62	0.5			
	Hydropsychidae	Cheumatopsyche		-0.76	1			
		Hydropsyche		-1.03	1			
	Lepidostomatidae	Lepidostoma		-0.06	1			
	Leptoceridae	Ceraclea		-0.06	1			
	Limnephilidae			-0.06	1		1	
		Limnephilus		-0.06	1	20		
	Philopotamidae	Dolophilodes		-0.06	0.5			
	Phryganeidae	Ptilostomis		-0.06	1	2	1	
	Polycentropodidae	Neureclipsis		-0.06	1			
		Polycentropus		-0.06	1			
	Ueonidae/Limnephilidae			-0.06	1			
Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5			
	Hyalellidae	Hyalella	azteca	0.32	0.5	1	8	
Veneroida	Sphaeriidae	Pisidium		-2.09	1			
		Sphaerium		-2.09	0.5			
Gastropoda				-0.64	0.5			
	Hydrobiidae			-1.82	0.5			
	Lymnaeidae	Lymnaea/Stagnicola		-0.64	0.5			
		Stagnicola		-0.64	0.5	174		
	Physidae	Physa		-1.64	0.5	12		
	Planorbidae			-1.94	0.5	225		
		Helisoma		-1.94	0.5	13		

				Promenetus	umbilicatellus	-1.94	0.5			1		
		Valvatidae		Valvata	sincera helicoidea	-1.82	1					
							SPEARoil	**-3.07**	**-2.18**	**-1.76**		**NA**

2010 continued, sites MUR, ATR-4, CHR, JAR, SUC

Order	**Suborder**	**Family**	**Subfamily**	**Genus**	**Species**	***S organic***	**GT**	**MUR**	**ATR-4**	**CHR**	**JAR**	**SUC**
Acari						-1.64	1				1	
	Oribatida	Eremaeidae		Hydrozetes		-1.64	1					
Coleoptera						-1.26	1					
		Dytiscidae				-0.81	1		1			
		Elmidae		Optioservus		-1.15	2				1	
		Gyrinidae		Gyrinus		-1.15	1			65		1
		Haliplidae		Haliplus		-1.83	1					
Diptera		Chironomidae	Chironominae			-0.39	0.5				1	1
			Tanypodinae			-0.39	0.5		1		1	
		Sciomyzidae		Sepedon		-0.35						
		Simuliidae				-0.46	0.5					10
Hemiptera	Heteroptera	Gerridae				-0.56	0.5					10
		Corixidae				-0.29	0.5			65		65
				Sigara/Callicorixa		-0.29	0.5			3		
		Nepidae		Nepa cinerea		-0.56	0.5					1
		Notonectidae		Notonecta		-0.82	1					
Odonata	Anisoptera	Aeshnidae		Aeshna		-0.96	2	11		2		4
		Corduliidae		Epitheca		-0.96	2					2
				Somatochlora		-0.96	2					4
		Gomphidae				-0.96	2		1			
				Ophiogomphus		-0.96	2				32	
	Zygoptera					-0.24	1					

	Calopterygidae	Calopteryx	-0.36	2				1	
	Coenagrionidae	Enallagma	-0.24	1					
Ephemeroptera			-0.14	1		2			12
	Baetidae		0.02	0.5				8	
		Baetis	0.02	0.5	2	3	1	53	
		Centroptilum	0.02	0.5	5		1		6
		Pseudocloeon	0.02	0.5					
	Baetiscidae	Baetisca	-0.14	1	2				
	Ephemerellidae	Ephemerella	-0.30	1					12
	Heptageniidae	Heptagenia	-0.30	0.5	2	13	1		6
		Stenonema	-0.30	0.5	2	3		15	
	Leptophlebidae	Leptophlebia	-0.30	0.5					25
	Metretopodidae	Metretopus	-0.14	0.5	2				
		Siphloplecton	-0.14	0.5					18
	Siphlonuridae	Analetris	-0.30	0.5					
Plecoptera	Nemouridae	Amphinemura	0.25	1					
	Perlidae	Acroneuria	0.38	1			5	31	
	Perlodidae	Isoperla	0.38	1			2		
	Pteronarcyidae	Pteronarcys	0.31	2		31			
		Pteronarcella	0.31	2					
Trichoptera	Brachycentridae	Brachycentrus	-0.06	1		55			
	Glossosomatidae	Glossosoma	-0.06	0.5				3	
	Helicopsychidae	Helicopsyche	-0.62	0.5				1	
	Hydropsychidae	Cheumatopsyche	-0.76	1				1	
		Hydropsyche	-1.03	1		28		1	
	Lepidostomatidae	Lepidostoma	-0.06	1		14			
	Leptoceridae	Ceraclea	-0.06	1				2	
	Limnephilidae		-0.06	1			2		
		Limnephilus	-0.06	1		7			
	Philopotamidae	Dolophilodes	-0.06	0.5		14		2	
	Phryganeidae	Ptilostomis	-0.06	1				1	

	Polycentropodidae	Neureclipsis		-0.06	1		28			
		Polycentropus		-0.06	1					2
	Ueonidae/Limnephilidae			-0.06	1				16	
Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5		6		11	
	Hyalellidae	Hyalella	azteca	0.32	0.5		6			
Veneroida	Sphaeriidae	Pisidium		-2.09	1				4	
		Sphaerium		-2.09	0.5				9	
Gastropoda				-0.64	0.5					7
	Hydrobiidae			-1.82	0.5		1			
	Lymnaeidae	Lymnaea/Stagnicola		-0.64	0.5	4				
		Stagnicola		-0.64	0.5					
	Physidae	Physa		-1.64	0.5	4				1
	Planorbidae			-1.94	0.5					
		Helisoma		-1.94	0.5					
		Promenetus	umbilicatellus sincera	-1.94	0.5					1
	Valvatidae	Valvata	helicoidea	-1.82	1					1
					SPEARoil	**-2.41**	**-1.55**	**-1.89**	**-1.98**	**-2.31**

B) 2011, sites ATR-5, HOR, POC, MAR-2, BER

Order	**Suborder**	**Family**	**Genus**	**Species**	***S organic***	**GT**	**ATR-5**	**HOR**	**POC**	**MAR-2**	**BER**
Nematoda, Nemata											
Oligochaeta					-1.01	0.5					13
Hirudinea					-0.45	0.5			1		1
Arhynchobdellida, Pharyngobdellida		Erpobdellidae			-0.41	0.5					1
Rhynchobdellida		Glossiphoniidae	Placobdella/ Nata		-0.49	0.5					1
Araneae					-1.64						4
Acari		Hydrachnidia			-1.64	1	1	4	1	1	3
Collembola			Isotomus								
Coleoptera		Curculionidae			-1.15	1					

	Dytiscidae	Acilius	-0.81	1				
		Coptotomus	-0.81	1				
		Ilybus	-0.81	1		1		
		Liodessus	-0.81	1	2	1		24
		Oreodytes/ Potamonectes	-0.81	1				
		Oreodytes/ Potamonectes/ Deronectes	-0.81	1		1		1
	Elmidae	Narpus	-1.15	2	2			
		Narpus/ Dubiraphia	-1.15	2				4
		Optioservus	-1.15	2				
		Heterlimnius/ Optioservus	-1.15	2			1	
		Zaitzevia	-1.15	2				
	Gyrinidae	Gyrinus	-1.15	1			16	1
	Haliplidae	Haliplus	-1.83	1				
		Peltodystes	-1.83	1				3
	Hydraenidae	Hydraena	-1.15	1				1
Diptera			-0.37	0.5			1	
	Anthomyiidae		-0.37	0.5				
	Chironomidae		-0.39	0.5				
	Chrionomidae	Chironominae	-0.39	0.5	7	6	14	15
		Orthocladinae	-0.39	0.5		13	3	1
		Chironominae/ Orthocladiinae	-0.39	0.5				
		Tanypodinae	-0.39	0.5	2	3	4	10
	Culicidae		-0.29	0.5				
	Dixidae	Dixella	-0.35	0.5			1	
	Empididae	Hemerodromia	-0.35	0.5				
	Limoniidae, Tipulidae		-0.35	0.5			1	
	Rhagionidae, Leptidae, Athericidae	Atherix	-0.35	1	4			
	Simulidae		-0.46	0.5				
		Prosimulium	-0.46	0.5	1			
		Simulium	-0.46	0.5		273		
		Stegopterna	-0.46	0.5		108		
	Tabanidae	Crysops	-0.35	0.5				
	Tipulidae	Dicranota	-0.35	0.5				

			Hexatoma/ Limnophila		-0.35	0.5					
			Prinocera		-0.35	0.5		1	1		
Hemiptera					-0.56	0.5					
		Corixidae			-0.29	0.5					
			Arctocorixa/ Callicorixa		-0.29	0.5					
			Coenocorixa		-0.29	0.5	35				
			Hesperocorixa		-0.29	0.5			1		15
			Palmacorixa		-0.29	0.5				1	
			Sigara		-0.29	0.5		4			
			Sigara/ Callicorixa		-0.29	0.5	89				
		Gerridae			-0.56	0.5					
			Gerris/ Limnoporus		-0.56	0.5			2		
		Mesoveliidae	Mesovelia		-0.56	0.5			1		
		Nepidae	Nepa	cinerea	-0.56	0.5					1
		Notonectidae			-0.82	0.5					
			Notonecta		-0.82	1			2		12
Odonata	Anisoptera				-0.96	2					
		Aeshnidae	Aeshna		-0.96	2					2
		Corduliidae	Somatochlora		-0.96	2					
		Gomphidae	Ophiogomphus		-0.96	2	1	2			
	Zygoptera	Calopterygidae	Calopteryx		-0.36	2					3
		Coenagrionidae	Coenagrion/ Enallagma		-0.24	1					
Ephemeroptera					-0.14	0.5				3	
		Ametropodidae	Ametropus		-0.14	0.5					
		Baetidae			0.02	0.5				19	54
			Baetis		0.02	0.5	2		118	6	
			Baetis/ Centroptilum		0.02	0.5					
			Baetis/ Pseudocloeon		0.02	0.5					
			Baetis/ Centroptilum/Pseudocloeon		0.02	0.5					
		Baetiscidae	Baetisca		-0.29	1		1			1
		Caenidae	Caenis		-0.30	0.5					2
		Ephemeridae	Ephemera		-0.30	1					
		Ephemerellidae			-0.30	1					
			Drunella		-0.30	1		5			
			Seratella		-0.30	1					
		Heptageniidae			-0.30	0.5	11			3	

		Cinygma	-0.30	0.5				3	
		Heptagenia	-0.30	0.5		1		6	
		Rhitrogena	-0.30	0.5	3	1		3	
		Stenacron	-0.30	0.5					
		Stenonema	-0.30	0.5					
	Leptophlebiidae		-0.30	1					
		Leptophlebia	-0.30	1				46	12
		Paraleptophlebia	-0.30	0.5		9			
	Oligoneuriidae	Isonychia	-0.29	1					
	Metretopodidae	Siphloplecton	-0.56	0.5				31	1
	Siphlonuridae		-0.30	0.5					
		Ameletus	-0.30	0.5		1		9	
		Parameletus	-0.30	0.5					
	Tricorythidae	Tricorythodes	-0.56	0.5			5	19	
Plecoptera			0.31	1					
	Chloroperlidae		-0.36	1				2	
	Nemouridae	Amphinemura	0.25	1					
		Nemoura	0.25	1					
		Nemoura/ Podmosta	0.25	1				1	
		Zapada	0.25	1		1			
	Perlidae	Acroneuria	0.38	1				2	
		Claassenia	0.38	1					
			0.38	1	13				
	Perlodidae/ Chloroperlidae		0.38	1					
	Perlodidae	Isogenoides	0.38	1					
		Isoperla	0.38	1		2		1	
		Skwala	0.38	1					
	Pteronarcyidae	Pternarcella	0.31	2					
		Pteronarcys	0.31	2					
	Taeniopterygidae	Taeniopteryx	0.38	1					
Trichoptera			-0.62	1	12				
	case bearing		-0.62	1					
	caseless		-0.62	1					
	Brachycentridae		-0.06	1	1				
		Brachycentrus	-0.06	1	6	3	1	1	
		Micrasema	-0.06	1		2			
	Glossosomatidae		-0.06	0.5					
		Glossosoma	-0.06	0.5		2	4	3	

	Helicopsychidae	Helicopsyche		-0.62	0.5				72	
	Hydropsychidae			-1.03	1	2				
		Arctopsyche		-1.03	1		1			
		Cheumatopsyche		-0.76	1	29	4			
		Hydropsyche		-1.03	1	7	22	16	44	
	Lepidostomatidae	Lepidostoma		-0.06	1		4	13	1	
	Leptoceridae	Oecetis		-0.06	1		1			
	Limnephilidae			-0.06	1					
		Allomyia		-0.06	1					
		Limnephilus		-0.06	1			2	5	18
	Philoptamidae	Dolophilodes		-0.06	0.5					
	Polycentropodidae	Neureclipsis		-0.06	1		1			
	Phryganeidae			-0.06	1					
		Ptilostomis		-0.06	1				4	2
Cladocera	Daphniidae	Daphnia		0.20	0.5					122
		Simocephalus		0.20	0.5					
Amphipoda	Gammaridae			0.16	0.5					
		Gammarus	lacustris	0.32	0.5			3		
		Hyalella	azteca	0.16	0.5			10	1	2
Copepoda				0.16						1
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5					
		Sphaerium		-2.09	0.5					
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5				2	
	Lymnaeidae/ Physidae			-0.64	0.5					
	Lymnaeidae	Lymnaea		-0.64	0.5					
		Fossaria/ Bakerilymnaea		-0.64	0.5	9		2	1	
	Physidae			-1.64	0.5					
		Physa		-1.64	0.5			21	2	2
	Planorbidae	Gyraulus		-1.94	0.5					
		Promenetus	umbilicatellus	-1.94	0.5			8	4	4
Heterostropha	Valvatidae	Valvata	sincera sincera	-1.82	1					
					SPEARoil	**-2.06**	**-1.86**	**-2.68**	**-2.43**	**-2.27**

2011 continued, sites FOC, ATR-3, MUR, ELR, TAR

Order	Suborder	Family	Genus	Species	*S organic*	GT	FOC	ATR-3	MUR	ELR	TAR
Nematoda, Nemata							1				
Oligochaeta					-1.01	0.5	13		1	1	
Hirudinea					-0.45	0.5					
Arhynchobdellida, Pharyngobdellida		Erpobdellidae			-0.41	0.5					
Rhynchobdellida		Glossiphoniidae	Placobdella/ Nata		-0.49	0.5					
Araneae					-1.64						
Acari		Hydrachnidia			-1.64	1		1		5	6
Collembola			Isotomus								
Coleoptera		Curculionidae			-1.15	1	1				
		Dytiscidae	Acilius		-0.81	1				1	
			Coptotomus		-0.81	1					
			Ilybus		-0.81	1					
			Liodessus		-0.81	1					
			Oreodytes/ Potamonectes		-0.81	1					
			Oreodytes/ Potamonectes/ Deronectes		-0.81	1					
		Elmidae	Narpus		-1.15	2					
			Narpus/ Dubiraphia		-1.15	2					
			Optioservus		-1.15	2					
			Heterlimnius/ Optioservus		-1.15	2					
			Zaitzevia		-1.15	2	2				
		Gyrinidae	Gyrinus		-1.15	1					
		Haliplidae	Haliplus		-1.83	1					
			Peltodystes		-1.83	1					
		Hydraenidae	Hydraena		-1.15	1					
Diptera					-0.37	0.5					
		Anthomyiidae			-0.37	0.5					2
		Chironomidae			-0.39	0.5					
		Chrionomidae	Chironominae		-0.39	0.5	3		1	74	
			Orthocladinae		-0.39	0.5			2	17	15
			Chironominae/		-0.39	0.5					

			Orthocladiinae								
			Tanypodinae		-0.39	0.5	4		1	2	1
		Culicidae			-0.29	0.5					
		Dixidae	Dixella		-0.35	0.5					
		Empididae	Hemerodromia		-0.35	0.5					
		Limoniidae, Tipulidae			-0.35	0.5					
		Rhagionidae, Leptidae, Athericidae	Atherix		-0.35	1					
		Simulidae			-0.46	0.5					
			Prosimulium		-0.46	0.5			11		
			Simulium		-0.46	0.5					15
			Stegopterna		-0.46	0.5					
		Tabanidae	Crysops		-0.35	0.5					1
		Tipulidae	Dicranota		-0.35	0.5					
			Hexatoma/ Limnophila		-0.35	0.5					
			Prinocera		-0.35	0.5					
Hemiptera					-0.56	0.5					
		Corixidae			-0.29	0.5					
			Arctocorixa/ Callicorixa		-0.29	0.5					
			Coenocorixa		-0.29	0.5					
			Hesperocorixa		-0.29	0.5					
			Palmacorixa		-0.29	0.5					
			Sigara		-0.29	0.5					
			Sigara/ Callicorixa		-0.29	0.5					
		Gerridae			-0.56	0.5					
			Gerris/ Limnoporus		-0.56	0.5					
		Mesoveliidae	Mesovelia		-0.56	0.5					
		Nepidae	Nepa	cinerea	-0.56	0.5					
		Notonectidae			-0.82	0.5					
			Notonecta		-0.82	1					
Odonata	Anisoptera				-0.96	2					
		Aeshnidae	Aeshna		-0.96	2					1
		Corduliidae	Somatochlora		-0.96	2	1				
		Gomphidae	Ophiogomphus		-0.96	2		2	5	6	
	Zygoptera	Calopterygidae	Calopteryx		-0.36	2				2	
		Coenagrionidae	Coenagrion/ Enallagma		-0.24	1					
Ephemeroptera					-0.14	0.5			1		1
		Ametropodidae	Ametropus		-0.14	0.5		4			

	Baetidae		0.02	0.5		4		17	3
		Baetis	0.02	0.5	174			17	122
		Baetis/ Centroptilum	0.02	0.5			2	52	12
		Baetis/ Pseudocloeon	0.02	0.5			3	26	12
		Baetis/ Centroptilum/Pseudocloeon	0.02	0.5					
	Baetiscidae	Baetisca	-0.29	1					
	Caenidae	Caenis	-0.30	0.5			2		
	Ephemeridae	Ephemera	-0.30	1					
	Ephemerellidae		-0.30	1					
		Drunella	-0.30	1					
		Seratella	-0.30	1					
	Heptageniidae		-0.30	0.5		2		1	1
		Cinygma	-0.30	0.5					
		Heptagenia	-0.30	0.5				1	
		Rhitrogena	-0.30	0.5					
		Stenacron	-0.30	0.5					1
		Stenonema	-0.30	0.5				3	
	Leptophlebiidae		-0.30	1					
		Leptophlebia	-0.30	1			1	7	
		Paraleptophlebia	-0.30	0.5					
	Oligoneuriidae	Isonychia	-0.29	1					
	Metretopodidae	Siphloplecton	-0.56	0.5					
	Siphlonuridae		-0.30	0.5				2	
		Ameletus	-0.30	0.5					
		Parameletus	-0.30	0.5				2	
	Tricorythidae	Tricorythodes	-0.56	0.5				10	
Plecoptera			0.31	1					1
	Chloroperlidae		-0.36	1	6	2			
	Nemouridae	Amphinemura	0.25	1	9				
		Nemoura	0.25	1					
		Nemoura/ Podmosta	0.25	1					
		Zapada	0.25	1	17				
	Perlidae	Acroneuria	0.38	1					
		Claassenia	0.38	1					
			0.38	1				1	
	Perlodidae/ Chloroperlidae		0.38	1					

	Perlodidae	Isogenoides		0.38	1		6			
		Isoperla		0.38	1				3	
		Skwala		0.38	1					
	Pteronarcyidae	Pternarcella		0.31	2	5				
		Pteronarcys		0.31	2				9	
	Taeniopterygidae	Taeniopteryx		0.38	1			2	8	
Trichoptera				-0.62	1					
	case bearing			-0.62	1					
	caseless			-0.62	1					
	Brachycentridae			-0.06	1					
		Brachycentrus		-0.06	1					1
		Micrasema		-0.06	1					
	Glossosomatidae			-0.06	0.5					
		Glossosoma		-0.06	0.5					4
	Helicopsychidae	Helicopsyche		-0.62	0.5					
	Hydropsychidae			-1.03	1					
		Arctopsyche		-1.03	1					
		Cheumatopsyche		-0.76	1		2			
		Hydropsyche		-1.03	1					15
	Lepidostomatidae	Lepidostoma		-0.06	1					
	Leptoceridae	Oecetis		-0.06	1					
	Limnephilidae			-0.06	1					
		Allomyia		-0.06	1					
		Limnephilus		-0.06	1					
	Philoptamidae	Dolophilodes		-0.06	0.5					
	Polycentropodidae	Neureclipsis		-0.06	1					
	Phryganeidae			-0.06	1					
		Ptilostomis		-0.06	1					
Cladocera	Daphniidae	Daphnia		0.20	0.5					
		Simocephalus		0.20	0.5					
Amphipoda	Gammaridae			0.16	0.5					
		Gammarus	lacustris	0.32	0.5					
		Hyalella	azteca	0.16	0.5					
Copepoda				0.16						
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5					
		Sphaerium		-2.09	0.5					
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5					
	Lymnaeidae/ Physidae			-0.64	0.5					
	Lymnaeidae	Lymnaea		-0.64	0.5					

			Fossaria/ Bakerilymnaea		-0.64	0.5			1		
		Physidae			-1.64	0.5					1
			Physa		-1.64	0.5			1	2	
		Planorbidae	Gyraulus		-1.94	0.5					
			Promenetus	umbilicatellus	-1.94	0.5				1	
Heterostropha		Valvatidae	Valvata	sincera sincera	-1.82	1					
						SPEARoil	**-1.78**	**-1.57**	**-2.39**	**-2.12**	**-2.28**

2011 continued, sites ART-1, ATR-4, HAC, ATR-2, MAR-1

Order	**Suborder**	**Family**	**Genus**	**Species**	***S organic***	**GT**	**ATR-1**	**ATR-4**	**HAC**	**ATR-2**	**MAR-1**
Nematoda, Nemata											
Oligochaeta					-1.01	0.5			3		1
Hirudinea					-0.45	0.5					
Arhynchobdellida, Pharyngobdellida		Erpobdellidae			-0.41	0.5					
Rhynchobdellida		Glossiphoniidae	Placobdella/ Nata		-0.49	0.5					
Araneae					-1.64						
Acari		Hydrachnidia			-1.64	1			1		1
Collembola			Isotomus								
Coleoptera		Curculionidae			-1.15	1					
		Dytiscidae	Acilius		-0.81	1					
			Coptotomus		-0.81	1					
			Ilybus		-0.81	1					
			Liodessus		-0.81	1					
			Oreodytes/ Potamonectes		-0.81	1					
			Oreodytes/ Potamonectes/ Deronectes		-0.81	1					
		Elmidae	Narpus		-1.15	2					
			Narpus/ Dubiraphia		-1.15	2					
			Optioservus		-1.15	2	1		3		
			Heterlimnius/ Optioservus		-1.15	2					

		Zaitzevia	-1.15	2					
	Gyrinidae	Gyrinus	-1.15	1					
	Haliplidae	Haliplus	-1.83	1					
		Peltodystes	-1.83	1					
	Hydraenidae	Hydraena	-1.15	1					
Diptera			-0.37	0.5					
	Anthomyiidae		-0.37	0.5					
	Chironomidae		-0.39	0.5					1
	Chrionomidae	Chironominae	-0.39	0.5					5
		Orthocladinae	-0.39	0.5	1				1
		Chironominae/ Orthocladiinae	-0.39	0.5		35			
		Tanypodinae	-0.39	0.5			1		
	Culicidae		-0.29	0.5					
	Dixidae	Dixella	-0.35	0.5					
	Empididae	Hemerodromia	-0.35	0.5	1				
	Limoniidae, Tipulidae		-0.35	0.5		3			
	Rhagionidae, Leptidae, Athericidae	Atherix	-0.35	1					
	Simulidae		-0.46	0.5					
		Prosimulium	-0.46	0.5					
		Simulium	-0.46	0.5					1
		Stegopterna	-0.46	0.5					
	Tabanidae	Crysops	-0.35	0.5					
	Tipulidae	Dicranota	-0.35	0.5					
		Hexatoma/ Limnophila	-0.35	0.5					
		Prinocera	-0.35	0.5					
Hemiptera			-0.56	0.5					
	Corixidae		-0.29	0.5					
		Arctocorixa/ Callicorixa	-0.29	0.5					
		Coenocorixa	-0.29	0.5					
		Hesperocorixa	-0.29	0.5					
		Palmacorixa	-0.29	0.5					
		Sigara	-0.29	0.5					
		Sigara/ Callicorixa	-0.29	0.5					
	Gerridae		-0.56	0.5					
		Gerris/ Limnoporus	-0.56	0.5					
	Mesoveliidae	Mesovelia	-0.56	0.5					

		Nepidae	Nepa	cinerea	-0.56	0.5				
		Notonectidae			-0.82	0.5				
			Notonecta		-0.82	1				
Odonata	Anisoptera				-0.96	2				
		Aeshnidae	Aeshna		-0.96	2				
		Corduliidae	Somatochlora		-0.96	2				
		Gomphidae	Ophiogomphus		-0.96	2	2	23	20	11
	Zygoptera	Calopterygidae	Calopteryx		-0.36	2				
		Coenagrionidae	Coenagrion/ Enallagma		-0.24	1				
Ephemeroptera					-0.14	0.5				
		Ametropodidae	Ametropus		-0.14	0.5	1			
		Baetidae			0.02	0.5				
			Baetis		0.02	0.5				
			Baetis/ Centroptilum		0.02	0.5	2		46	2	101
			Baetis/ Pseudocloeon		0.02	0.5			33		43
			Baetis/ Centroptilum/Pseudocloeon		0.02	0.5					
		Baetiscidae	Baetisca		-0.29	1			1		
		Caenidae	Caenis		-0.30	0.5					1
		Ephemeridae	Ephemera		-0.30	1					1
		Ephemerellidae			-0.30	1		3			
			Drunella		-0.30	1			1		
			Seratella		-0.30	1				2	
		Heptageniidae			-0.30	0.5	2	17		8	1
			Cinygma		-0.30	0.5					
			Heptagenia		-0.30	0.5				1	2
			Rhitrogena		-0.30	0.5		23			
			Stenacron		-0.30	0.5					
			Stenonema		-0.30	0.5			1		
		Leptophlebiidae			-0.30	1				1	
			Leptophlebia		-0.30	1					4
			Paraleptophlebia		-0.30	0.5					
		Oligoneuriidae	Isonychia		-0.29	1					1
		Metretopodidae	Siphloplecton		-0.56	0.5					
		Siphlonuridae			-0.30	0.5				5	
			Ameletus		-0.30	0.5					
			Parameletus		-0.30	0.5					
		Tricorythidae	Tricorythodes		-0.56	0.5					3

Plecoptera			0.31	1					
	Chloroperlidae		-0.36	1					1
	Nemouridae	Amphinemura	0.25	1					
		Nemoura	0.25	1			1		
		Nemoura/ Podmosta	0.25	1					
		Zapada	0.25	1			1		
	Perlidae	Acroneuria	0.38	1					
		Claassenia	0.38	1			1		
			0.38	1					
	Perlodidae/ Chloroperlidae		0.38	1				1	
	Perlodidae	Isogenoides	0.38	1					
		Isoperla	0.38	1	3	4			2
		Skwala	0.38	1			1		
	Pteronarcyidae	Pternarcella	0.31	2					
		Pteronarcys	0.31	2	1	2	5	1	1
	Taeniopterygidae	Taeniopteryx	0.38	1			1		1
Trichoptera			-0.62	1					
	case bearing		-0.62	1					
	caseless		-0.62	1					
	Brachycentridae		-0.06	1					
		Brachycentrus	-0.06	1	2	2	9		
		Micrasema	-0.06	1			2		
	Glossosomatidae		-0.06	0.5					
		Glossosoma	-0.06	0.5			1		
	Helicopsychidae	Helicopsyche	-0.62	0.5	1				
	Hydropsychidae		-1.03	1					
		Arctopsyche	-1.03	1					
		Cheumatopsyche	-0.76	1		39	21	4	
		Hydropsyche	-1.03	1		54	45	2	
	Lepidostomatidae	Lepidostoma	-0.06	1			5		
	Leptoceridae	Oecetis	-0.06	1					
	Limnephilidae		-0.06	1					
		Allomyia	-0.06	1			3		
		Limnephilus	-0.06	1					
	Philoptamidae	Dolophilodes	-0.06	0.5					
	Polycentropodidae	Neureclipsis	-0.06	1				3	
	Phryganeidae		-0.06	1					
		Ptilostomis	-0.06	1					

Cladocera	Daphniidae	Daphnia		0.20	0.5					
		Simocephalus		0.20	0.5					
Amphipoda	Gammaridae			0.16	0.5					
		Gammarus	lacustris	0.32	0.5					
		Hyalella	azteca	0.16	0.5					
Copepoda				0.16						
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5					
		Sphaerium		-2.09	0.5				1	
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5					
	Lymnaeidae/ Physidae			-0.64	0.5					
	Lymnaeidae	Lymnaea		-0.64	0.5					
		Fossaria/ Bakerilymnaea		-0.64	0.5					
	Physidae			-1.64	0.5					
		Physa		-1.64	0.5					1
	Planorbidae	Gyraulus		-1.94	0.5			1		
		Promenetus	umbilicatellus	-1.94	0.5			1		
Heterostropha	Valvatidae	Valvata	sincera sincera	-1.82	1					
					SPEARoil	**-1.51**	**-1.84**	**-1.83**	**-1.89**	**-2.04**

2011 continued, sites CHR, JAR, SUC, GUR

Order	**Suborder**	**Family**	**Genus**	**Species**	***S organic***	**GT**	**CHR**	**JAR**	**SUC**	**GUR**
Nematoda, Nemata										
Oligochaeta					-1.01	0.5		1		
Hirudinea					-0.45	0.5				
Arhynchobdellida, Pharyngobdellida		Erpobdellidae			-0.41	0.5				
Rhynchobdellida		Glossiphoniidae	Placobdella/ Nata		-0.49	0.5				
Araneae					-1.64					
Acari		Hydrachnidia			-1.64	1		3	1	
Collembola			Isotomus							1
Coleoptera		Curculionidae			-1.15	1				
		Dytiscidae	Acilius		-0.81	1				
			Coptotomus		-0.81	1				2

		Ilybus	-0.81	1				
		Liodessus	-0.81	1				
		Oreodytes/ Potamonectes	-0.81	1				2
		Oreodytes/ Potamonectes/ Deronectes	-0.81	1				
	Elmidae	Narpus	-1.15	2				
		Narpus/ Dubiraphia	-1.15	2				
		Optioservus	-1.15	2				
		Heterlimnius/ Optioservus	-1.15	2				
		Zaitzevia	-1.15	2				
	Gyrinidae	Gyrinus	-1.15	1	2	12	3	12
	Haliplidae	Haliplus	-1.83	1				2
		Peltodystes	-1.83	1				
	Hydraenidae	Hydraena	-1.15	1				
Diptera			-0.37	0.5				
	Anthomyiidae		-0.37	0.5				
	Chironomidae		-0.39	0.5				
	Chrionomidae	Chironominae	-0.39	0.5	4	6	44	9
		Orthocladinae	-0.39	0.5	4	7	6	1
		Chironominae/ Orthocladiinae	-0.39	0.5				
		Tanypodinae	-0.39	0.5		2	3	3
	Culicidae		-0.29	0.5				
	Dixidae	Dixella	-0.35	0.5				1
	Empididae	Hemerodromia	-0.35	0.5		1		
	Limoniidae, Tipulidae		-0.35	0.5	1			
	Rhagionidae, Leptidae, Athericidae	Atherix	-0.35	1				
	Simulidae		-0.46	0.5				
		Prosimulium	-0.46	0.5				
		Simulium	-0.46	0.5		5	125	1
		Stegopterna	-0.46	0.5		5		
	Tabanidae	Crysops	-0.35	0.5			1	
	Tipulidae	Dicranota	-0.35	0.5			1	
		Hexatoma/ Limnophila	-0.35	0.5			1	
		Prinocera	-0.35	0.5				

Hemiptera					-0.56	0.5		30		
		Corixidae			-0.29	0.5				7
			Arctocorixa/ Callicorixa		-0.29	0.5	2			
			Coenocorixa		-0.29	0.5			13	
			Hesperocorixa		-0.29	0.5				
			Palmacorixa		-0.29	0.5		1		
			Sigara		-0.29	0.5				
			Sigara/ Callicorixa		-0.29	0.5		4		1
		Gerridae			-0.56	0.5		1		
			Gerris/ Limnoporus		-0.56	0.5				1
		Mesoveliidae	Mesovelia		-0.56	0.5				
		Nepidae	Nepa	cinerea	-0.56	0.5				
		Notonectidae			-0.82	0.5				
			Notonecta		-0.82	1				
Odonata	Anisoptera				-0.96	2				
		Aeshnidae	Aeshna		-0.96	2			1	
		Corduliidae	Somatochlora		-0.96	2				
		Gomphidae	Ophiogomphus		-0.96	2	9	10	3	
	Zygoptera	Calopterygidae	Calopteryx		-0.36	2		2		
		Coenagrionidae	Coenagrion/ Enallagma		-0.24	1				1
Ephemeroptera					-0.14	0.5				
		Ametropodidae	Ametropus		-0.14	0.5	44			
		Baetidae			0.02	0.5	53			
			Baetis		0.02	0.5				
			Baetis/ Centroptilum		0.02	0.5		31		81
			Baetis/ Pseudocloeon		0.02	0.5		13		
			Baetis/ Centroptilum/Pseudocloeon		0.02	0.5			147	
		Baetiscidae	Baetisca		-0.29	1	1			
		Caenidae	Caenis		-0.30	0.5				4
		Ephemeridae	Ephemera		-0.30	1		1		
		Ephemerellidae			-0.30	1				
			Drunella		-0.30	1		2	2	
			Seratella		-0.30	1	4	12		
		Heptageniidae			-0.30	0.5	8			
			Cinygma		-0.30	0.5				
			Heptagenia		-0.30	0.5				
			Rhitrogena		-0.30	0.5				
			Stenacron		-0.30	0.5				

		Stenonema	-0.30	0.5		18		4
	Leptophlebiidae		-0.30	1				
		Leptophlebia	-0.30	1	5	6	7	46
		Paraleptophlebia	-0.30	0.5		3		
	Oligoneuriidae	Isonychia	-0.29	1				
	Metretopodidae	Siphloplecton	-0.56	0.5			7	
	Siphlonuridae		-0.30	0.5				
		Ameletus	-0.30	0.5	1		5	
		Parameletus	-0.30	0.5				
	Tricorythidae	Tricorythodes	-0.56	0.5	1			
Plecoptera			0.31	1				
	Chloroperlidae		-0.36	1				
	Nemouridae	Amphinemura	0.25	1				
		Nemoura	0.25	1				
		Nemoura/ Podmosta	0.25	1				
		Zapada	0.25	1			11	
	Perlidae	Acroneuria	0.38	1		3		
		Claassenia	0.38	1				
			0.38	1				
	Perlodidae/ Chloroperlidae		0.38	1				
	Perlodidae	Isogenoides	0.38	1				
		Isoperla	0.38	1	8	5	7	
		Skwala	0.38	1				
	Pteronarcyidae	Pternarcella	0.31	2				
		Pteronarcys	0.31	2	7			
	Taeniopterygidae	Taeniopteryx	0.38	1	1			
Trichoptera			-0.62	1				
	case bearing		-0.62	1				
	caseless		-0.62	1				
	Brachycentridae		-0.06	1				
		Brachycentrus	-0.06	1	2		114	
		Micrasema	-0.06	1				
	Glossosomatidae		-0.06	0.5				
		Glossosoma	-0.06	0.5		2		
	Helicopsychidae	Helicopsyche	-0.62	0.5		7		
	Hydropsychidae		-1.03	1				
		Arctopsyche	-1.03	1				
		Cheumatopsyche	-0.76	1		2		

		Hydropsyche		-1.03	1	1	26	66	3
	Lepidostomatidae	Lepidostoma		-0.06	1		26	4	
	Leptoceridae	Oecetis		-0.06	1		9	13	
	Limnephilidae			-0.06	1	1			
		Allomyia		-0.06	1				
		Limnephilus		-0.06	1				4
	Philoptamidae	Dolophilodes		-0.06	0.5		22		
	Polycentropodidae	Neureclipsis		-0.06	1		4		1
	Phryganeidae			-0.06	1				
		Ptilostomis		-0.06	1				
Cladocera	Daphniidae	Daphnia		0.20	0.5				
		Simocephalus		0.20	0.5			1	
Amphipoda	Gammaridae			0.16	0.5				
		Gammarus	lacustris	0.32	0.5				101
		Hyalella	azteca	0.16	0.5		19	1	41
Copepoda				0.16					
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5				
		Sphaerium		-2.09	0.5				
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5				
	Lymnaeidae/ Physidae			-0.64	0.5				
	Lymnaeidae	Lymnaea		-0.64	0.5				3
		Fossaria/ Bakerilymnaea		-0.64	0.5				
	Physidae			-1.64	0.5				
		Physa		-1.64	0.5		1		24
	Planorbidae	Gyraulus		-1.94	0.5			1	2
		Promenetus	umbilicatellus	-1.94	0.5				
Heterostropha	Valvatidae	Valvata	sincera sincera	-1.82	1				
					SPEARoil	**-1.70**	**-1.99**	**-2.01**	**-2.37**

C) 2012, sites ATR-5, HOR, POC, MAR-2, BER

Order	Suborder	Family	Genus	Species	*S organic*	GT	ATR-5	HOR	POC	MAR-2	BER
Oligochaeta					-1.14	0.5			2		
Haplotaxida		Naididae			-1.10	0.5					
Lumbriculida		Lumbriculidae			-1.40	0.5					
Hirudinea					-0.45	0.5			6		
Pharyngobdellida		Erpobdellidae			-0.41	0.5			3		
Rhynchobdellida		Glossiphoniidae			-0.49	0.5			3		
		Piscicolidae	Piscicola	milneri	-0.60	0.5					
Acari					-1.64	1					2
Araneae					-1.64			1			
Coleoptera		Dytiscidae	Acilius		-0.81	1				1	
			Coptotomus		-0.81	1				1	2
			Laccornis		-0.81	1				1	
			Liodessus		-0.81	1				1	4
			Oreodytes/Potamonectes		-0.81	1					
		Elmidae	Optioservus		-1.15	1			3		1
		Gyrinidae	Gyrinus		-1.15	1		1	5		7
		Haliplidae			-1.83	1					
			Brychius		-1.83	1				1	
					-1.26	1					
Diptera		Anthomyidae			-0.37	0.5					
		Athericidae	Atherix		-0.35	1	1	4			
		Caenidae	Caenis		-0.30	0.5					
		Ceratopogonidae			-0.35	0.5					
		Chironomidae			-0.39	0.5					
			Chironominae		-0.39	0.5		13	3	31	7
			Chironominae/Orthocladiinae		-0.39	0.5	1				
			Orthocladiinae		-0.39	0.5		1			
			Tanypodinae		-0.39	0.5	1	1	6	2	6
		Empididae			-0.35	0.5					
		Simuliidae			-0.46	0.5			137		1
		Tabanidae			-0.35	0.5		2			
		Tipulidae	Hexatoma		-0.35	0.5					1
			Limoniidae/Limoniinae		-0.35	0.5		2			
			Prinocera		-0.35	0.5					
Hemiptera	Heteroptera	Corixidae			-0.29	0.5			1		
			Callicorixa		-0.29	0.5		1			

			Hesperocorixa		-0.29	0.5		1	1	35	
			Palmacorixa	buenoi	-0.29	0.5					
			Sigara		-0.29	0.5	69	4		71	14
		Gerridae	Limnoporus		-0.56	0.5			3		
		Hebridae	Merragata		-0.56						
		Nepidae	Nepa	cinerea	-0.56	0.5				1	1
		Notonectidae	Notonecta		-0.82	1				34	
Odonata	Anisoptera	Aeshnidae	Aeshna		-0.96	1					2
		Corduliidae	Somatochlora		-0.96	1			1		
		Gomphidae	Ophiogomphus		-0.96	1	1	2			8
	Zygoptera	Calopterygidae	Calopteryx		-0.36	1					5
Ephemeroptera		Ametropodidae	Ametropus		-0.14	1					
		Baetidae			0.02	0.5					2
			Baetis		0.02	0.5			40		
			Baetis/Centroptilum		0.02	0.5				9	21
			Baetis/Pseudocloeon		0.02	0.5		5			
			Callibaetis		0.02	0.5				9	
			Centroptilum		-0.25	0.5					
			Pseudocloeon		0.02	0.5					
		Baetiscidae	Baetisca		-0.14	1				1	
		Caenidae	Caenis		-0.30	0.5			2		
		Ephemerellidae	Drunella		-0.30	1		7			
			Ephemerella		-0.30	1					
			Serratella		-0.30	1					
		Ephemeridae	Ephemera		-0.30	1					1
			Hexagenia		-0.30	1				1	
		Heptageniidae			-0.30	0.5	2	2	37		2
			Heptagenia		-0.30	0.5	1		13		
			Rhithrogena		-0.30	0.5					
			Stenacron		-0.30	0.5		3	8		
			Stenonema		-0.30	0.5			15		
		Leptophlebiidae			-0.30	1		7		1	
			Leptophlebia		-0.30	1				2	
			Leptophlebia/Paraleptophlebia		-0.30	1					19
			Paraleptophlebia		-0.30	1		7			
		Metretopodidae	Siphloplecton		-0.14	0.5		2		5	9
		Oligoneuriidae	Isonychia		-0.14	1					
		Siphlonuridae			-0.30	0.5				1	
			Ameletus		-0.30	0.5		15			57

		Analetris		-0.30	0.5					
	Siphlonuridae/ Metretopodidae			-0.30	0.5					
	Tricorythidae	Tricorythodes		-0.14	0.5			2		2
				-0.14	0.5			1		
Plecoptera	Chloroperlidae			-0.36	1		1			
	Nemouridae	Nemoura		0.25	1					
		Zapada		0.25	1		1			
	Perlidae	Acroneuria		0.38	1					
		Claassenia		0.38	1		1			
	Perlodidae	Isoperla		0.38	1		1			8
		Skwala		0.38	1			1		
	Pteronarcyidae	Pteronarcella		0.31	1					
		Pteronarcys		0.31	1		6			1
	Taeniopterygidae	Taeniopteryx		0.38	1					1
	Taeniopterygidae/Nemouridae			0.38	1		3			
				0.31	1					
Trichoptera	Brachycentridae	Brachycentrus		-0.06	1	4	16			
		Micrasema		-0.06	1					
	Glossosomatidae	Glossosoma		-0.06	0.5		2			
		Glossosoma/Anagapetus		-0.06	0.5			3		
		Protoptila/Agapetus		-0.06	0.5					
	Helicopsychidae	Helicopsyche		-0.62	0.5			28		1
	Hydropsychidae			-1.03	1					
		Cheumatopsyche		-0.76	1	23	3			
		Hydropsyche		-1.03	1	19	24	10		
	Hydroptilidae			-0.89	0.5					
		Oxyethira		-0.89	0.5					
	Lepidostomatidae	Lepidostoma		-0.06	1		31	133		
	Leptoceridae	Ceraclea		-0.06	1			6		
		Mystacides		-0.06	1		1			
		Oecetis		-0.06	1		1			
		Triaenodes		-0.06	1					
	Limnephilidae			-0.06	1		1			
		Limnephilus		-0.06	1				3	2
	Philopotamidae	Dolophilodes		-0.06	0.5					2
	Phryganeidae	Ptilostomis		-0.06	1				3	1
	Polycentropodidae	Neureclipsis		-0.06	1			1		4
				-0.62	1				1	
Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5			2		

		Hyalella	azteca	0.16	0.5			2	10	1
Copepoda	Cyclopoida			0.16	0.5				1	
Cladocera				0.19	0.5				2	
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5	2		35		3
		Sphaerium		-2.09	0.5					3
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5					3
	Lymnaeidae	Bakerilymnaea		-0.64	0.5				1	
		Stagnicola		-0.64	0.5					
	Physidae			-1.64	0.5			1		
		Aplexa		-1.64	0.5				1	
		Physa		-1.64	0.5				1	3
	Planorbidae	Gyraulus		-1.94	0.5					6
		Promenetus	umbilicatellus	-1.94	0.5					
Heterostropha	Valvatidae	Valvata	tricarinata	-1.82	1					
					SPEARoil	**-2.37**	**-1.76**	**-2.61**	**-2.20**	**-2.50**

2012 continued, sites FOC, ATR-3, MUR, ELR, TAR

Order	**Suborder**	**Family**	**Genus**	**Species**	***S organic***	**GT**	**FOC**	**ATR-3**	**MUR**	**ELR**	**TAR**
Oligochaeta					-1.14	0.5					
Haplotaxida		Naididae			-1.10	0.5	2			7	1
Lumbriculida		Lumbriculidae			-1.40	0.5	1				
Hirudinea					-0.45	0.5					
Pharyngobdellida		Erpobdellidae			-0.41	0.5					
Rhynchobdellida		Glossiphoniidae			-0.49	0.5					
		Piscicolidae	Piscicola	milneri	-0.60	0.5				1	
Acari					-1.64	1	1			5	1
Araneae					-1.64						
Coleoptera		Dytiscidae	Acilius		-0.81	1					
			Coptotomus		-0.81	1					
			Laccornis		-0.81	1					
			Liodessus		-0.81	1					
			Oreodytes/Potamonectes		-0.81	1					
		Elmidae	Optioservus		-1.15	1	2				1
		Gyrinidae	Gyrinus		-1.15	1					
		Haliplidae			-1.83	1					

			Brychius		-1.83	1					
					-1.26	1					
Diptera		Anthomyidae			-0.37	0.5					1
		Athericidae	Atherix		-0.35	1					
		Caenidae	Caenis		-0.30	0.5					
		Ceratopogonidae			-0.35	0.5					
		Chironomidae			-0.39	0.5				1	1
			Chironominae		-0.39	0.5	4	2		6	2
			Chironominae/Orthocladiinae		-0.39	0.5	1				
			Orthocladiinae		-0.39	0.5	1	3		1	7
			Tanypodinae		-0.39	0.5				10	
		Empididae			-0.35	0.5	1				
		Simuliidae			-0.46	0.5					2
		Tabanidae			-0.35	0.5	1				
		Tipulidae	Hexatoma		-0.35	0.5					
			Limoniidae/Limoniinae		-0.35	0.5					1
			Prinocera		-0.35	0.5					
Hemiptera	Heteroptera	Corixidae			-0.29	0.5					
			Callicorixa		-0.29	0.5					
			Hesperocorixa		-0.29	0.5					
			Palmacorixa	buenoi	-0.29	0.5					
			Sigara		-0.29	0.5		10	1	1	
		Gerridae	Limnoporus		-0.56	0.5					
		Hebridae	Merragata		-0.56						1
		Nepidae	Nepa	cinerea	-0.56	0.5					
		Notonectidae	Notonecta		-0.82	1					
Odonata	Anisoptera	Aeshnidae	Aeshna		-0.96	1				2	
		Corduliidae	Somatochlora		-0.96	1					
		Gomphidae	Ophiogomphus		-0.96	1		18	1	1	
	Zygoptera	Calopterygidae	Calopteryx		-0.36	1					
Ephemeroptera		Ametropodidae	Ametropus		-0.14	1			1		
		Baetidae			0.02	0.5		4			
			Baetis		0.02	0.5	68	2			110
			Baetis/Centroptilum		0.02	0.5				20	2
			Baetis/Pseudocloeon		0.02	0.5					
			Callibaetis		0.02	0.5					
			Centroptilum		-0.25	0.5					
			Pseudocloeon		0.02	0.5				2	2
		Baetiscidae	Baetisca		-0.14	1					

	Caenidae	Caenis	-0.30	0.5				1	5
	Ephemerellidae	Drunella	-0.30	1					
		Ephemerella	-0.30	1					
		Serratella	-0.30	1		1			22
	Ephemeridae	Ephemera	-0.30	1					
		Hexagenia	-0.30	1					
	Heptageniidae		-0.30	0.5		25			1
		Heptagenia	-0.30	0.5		9			8
		Rhithrogena	-0.30	0.5					
		Stenacron	-0.30	0.5					
		Stenonema	-0.30	0.5					
	Leptophlebiidae		-0.30	1					
		Leptophlebia	-0.30	1	1	1	1	1	7
		Leptophlebia/Paraleptophlebia	-0.30	1					
		Paraleptophlebia	-0.30	1					
	Metretopodidae	Siphloplecton	-0.14	0.5				1	
	Oligoneuriidae	Isonychia	-0.14	1					
	Siphlonuridae		-0.30	0.5					
		Ameletus	-0.30	0.5		3			
		Analetris	-0.30	0.5					
	Siphlonuridae/ Metretopodidae		-0.30	0.5					1
	Tricorythidae	Tricorythodes	-0.14	0.5				3	1
			-0.14	0.5					
Plecoptera	Chloroperlidae		-0.36	1					
	Nemouridae	Nemoura	0.25	1					5
		Zapada	0.25	1	17				2
	Perlidae	Acroneuria	0.38	1					
		Claassenia	0.38	1					
	Perlodidae	Isoperla	0.38	1		10		7	11
		Skwala	0.38	1					
	Pteronarcyidae	Pteronarcella	0.31	1	1				
		Pteronarcys	0.31	1		2		1	
	Taeniopterygidae	Taeniopteryx	0.38	1			2	2	
	Taeniopterygidae/Nemouridae		0.38	1					
			0.31	1					
Trichoptera	Brachycentridae	Brachycentrus	-0.06	1		2			3
		Micrasema	-0.06	1					1
	Glossosomatidae	Glossosoma	-0.06	0.5					2
		Glossosoma/Anagapetus	-0.06	0.5					

		Protoptila/Agapetus		-0.06	0.5					
	Helicopsychidae	Helicopsyche		-0.62	0.5					
	Hydropsychidae			-1.03	1					
		Cheumatopsyche		-0.76	1		6			
		Hydropsyche		-1.03	1				13	6
	Hydroptilidae			-0.89	0.5					
		Oxyethira		-0.89	0.5					
	Lepidostomatidae	Lepidostoma		-0.06	1				1	2
	Leptoceridae	Ceraclea		-0.06	1					
		Mystacides		-0.06	1					
		Oecetis		-0.06	1					
		Triaenodes		-0.06	1				1	
	Limnephilidae			-0.06	1					
		Limnephilus		-0.06	1					
	Philopotamidae	Dolophilodes		-0.06	0.5					
	Phryganeidae	Ptilostomis		-0.06	1					
	Polycentropodidae	Neureclipsis		-0.06	1					
				-0.62	1					
Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5					
		Hyalella	azteca	0.16	0.5					1
Copepoda	Cyclopoida			0.16	0.5					
Cladocera				0.19	0.5					
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5					
		Sphaerium		-2.09	0.5					
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5					
	Lymnaeidae	Bakerilymnaea		-0.64	0.5					
		Stagnicola		-0.64	0.5					
	Physidae			-1.64	0.5					
		Aplexa		-1.64	0.5					
		Physa		-1.64	0.5	1				
	Planorbidae	Gyraulus		-1.94	0.5		1		5	
		Promenetus	umbilicatellus	-1.94	0.5					
Heterostropha	Valvatidae	Valvata	tricarinata	-1.82	1					
					SPEARoil	**-2.29**	**-2.10**	**-1.49**	**-2.41**	**-1.88**

2012 continued, sites ATR-1, ATR-4, HAC, ATR-2, MAR-1

Order	Suborder	Family	Genus	Species	*S organic*	GT		ATR-1	ATR-4	HAC	ATR-2	MAR-1
Oligochaeta					-1.14		0.5					
Haplotaxida		Naididae			-1.10		0.5	2				35
Lumbriculida		Lumbriculidae			-1.40		0.5					
Hirudinea					-0.45		0.5					
Pharyngobdellida		Erpobdellidae			-0.41		0.5					
Rhynchobdellida		Glossiphoniidae			-0.49		0.5					
		Piscicolidae	Piscicola	milneri	-0.60		0.5					
Acari					-1.64		1		1	4		2
Araneae					-1.64							
Coleoptera		Dytiscidae	Acilius		-0.81		1					
			Coptotomus		-0.81		1					
			Laccornis		-0.81		1					
			Liodessus		-0.81		1				1	1
			Oreodytes/Potamonectes		-0.81		1					
		Elmidae	Optioservus		-1.15		1			1		
		Gyrinidae	Gyrinus		-1.15		1	24				
		Haliplidae			-1.83		1					
			Brychius		-1.83		1					
					-1.26		1					
Diptera		Anthomyidae			-0.37		0.5					
		Athericidae	Atherix		-0.35		1					
		Caenidae	Caenis		-0.30		0.5					7
		Ceratopogonidae			-0.35		0.5					
		Chironomidae			-0.39		0.5					
			Chironominae		-0.39		0.5	3	1		3	7
			Chironominae/Orthocladiinae		-0.39		0.5	1			4	
			Orthocladiinae		-0.39		0.5	1	1			1
			Tanypodinae		-0.39		0.5			1		9
		Empididae			-0.35		0.5					
		Simuliidae			-0.46		0.5					
		Tabanidae			-0.35		0.5					
		Tipulidae	Hexatoma		-0.35		0.5					
			Limoniidae/Limoniinae		-0.35		0.5					
			Prinocera		-0.35		0.5			2		
Hemiptera	Heteroptera	Corixidae			-0.29		0.5					

			Callicorixa		-0.29	0.5				1	
			Hesperocorixa		-0.29	0.5	1	4		1	43
			Palmacorixa	buenoi	-0.29	0.5			1		
			Sigara		-0.29	0.5	38	23	4	3	43
		Gerridae	Limnoporus		-0.56	0.5					
		Hebridae	Merragata		-0.56						
		Nepidae	Nepa	cinerea	-0.56	0.5					
		Notonectidae	Notonecta		-0.82	1					
Odonata	Anisoptera	Aeshnidae	Aeshna		-0.96	1					
		Corduliidae	Somatochlora		-0.96	1					
		Gomphidae	Ophiogomphus		-0.96	1	1	1	5	5	14
	Zygoptera	Calopterygidae	Calopteryx		-0.36	1					
Ephemeroptera		Ametropodidae	Ametropus		-0.14	1		1			9
		Baetidae			0.02	0.5			3		
			Baetis		0.02	0.5	1	1	10		5
			Baetis/Centroptilum		0.02	0.5	7		16	6	
			Baetis/Pseudocloeon		0.02	0.5		1		7	
			Callibaetis		0.02	0.5					3
			Centroptilum		-0.25	0.5					2
			Pseudocloeon		0.02	0.5					8
		Baetiscidae	Baetisca		-0.14	1					
		Caenidae	Caenis		-0.30	0.5			3		
		Ephemerellidae	Drunella		-0.30	1			2		
			Ephemerella		-0.30	1		1			
			Serratella		-0.30	1	1				
		Ephemeridae	Ephemera		-0.30	1					
			Hexagenia		-0.30	1					
		Heptageniidae			-0.30	0.5		9		4	
			Heptagenia		-0.30	0.5	3	16		4	1
			Rhithrogena		-0.30	0.5		7			
			Stenacron		-0.30	0.5					
			Stenonema		-0.30	0.5			1		
		Leptophlebiidae			-0.30	1					5
			Leptophlebia		-0.30	1	1			2	10
			Leptophlebia/Paraleptophlebia		-0.30	1					
			Paraleptophlebia		-0.30	1					
		Metretopodidae	Siphloplecton		-0.14	0.5					
		Oligoneuriidae	Isonychia		-0.14	1					1
		Siphlonuridae			-0.30	0.5					

		Ameletus	-0.30	0.5					
		Analetris	-0.30	0.5	2	1	1	7	
	Siphlonuridae/ Metretopodidae		-0.30	0.5					
	Tricorythidae	Tricorythodes	-0.14	0.5					1
			-0.14	0.5					
Plecoptera	Chloroperlidae		-0.36	1					
	Nemouridae	Nemoura	0.25	1					1
		Zapada	0.25	1			6		
	Perlidae	Acroneuria	0.38	1					
		Claassenia	0.38	1					
	Perlodidae	Isoperla	0.38	1	22	5	2		15
		Skwala	0.38	1					
	Pteronarcyidae	Pteronarcella	0.31	1					
		Pteronarcys	0.31	1			2		1
	Taeniopterygidae	Taeniopteryx	0.38	1				1	4
	Taeniopterygidae/Nemouridae		0.38	1					
			0.31	1		2			
Trichoptera	Brachycentridae	Brachycentrus	-0.06	1	1	1	5	1	
		Micrasema	-0.06	1			10		
	Glossosomatidae	Glossosoma	-0.06	0.5			1		
		Glossosoma/Anagapetus	-0.06	0.5					
		Protoptila/Agapetus	-0.06	0.5			5		
	Helicopsychidae	Helicopsyche	-0.62	0.5			1		
	Hydropsychidae		-1.03	1				5	
		Cheumatopsyche	-0.76	1		7		3	
		Hydropsyche	-1.03	1	1	36	17	1	
	Hydroptilidae		-0.89	0.5	1				
		Oxyethira	-0.89	0.5		1			
	Lepidostomatidae	Lepidostoma	-0.06	1	1		1		
	Leptoceridae	Ceraclea	-0.06	1					
		Mystacides	-0.06	1					
		Oecetis	-0.06	1					
		Triaenodes	-0.06	1					
	Limnephilidae		-0.06	1			4		
		Limnephilus	-0.06	1					
	Philopotamidae	Dolophilodes	-0.06	0.5					
	Phryganeidae	Ptilostomis	-0.06	1					
	Polycentropodidae	Neureclipsis	-0.06	1		1			
			-0.62	1					1

Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5					
		Hyalella	azteca	0.16	0.5					
Copepoda	Cyclopoida			0.16	0.5					
Cladocera				0.19	0.5					
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5			6		2
		Sphaerium		-2.09	0.5					1
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5					
	Lymnaeidae	Bakerilymnaea		-0.64	0.5	1				
		Stagnicola		-0.64	0.5					
	Physidae			-1.64	0.5					
		Aplexa		-1.64	0.5					
		Physa		-1.64	0.5					14
	Planorbidae	Gyraulus		-1.94	0.5					2
		Promenetus	umbilicatellus	-1.94	0.5			2		
Heterostropha	Valvatidae	Valvata	tricarinata	-1.82	1					
					SPEARoil	**-2.15**	**-2.15**	**-2.18**	**-2.14**	**-2.62**

2012 continued, sites CHR, JAR, SUC, GUR

Order	**Suborder**	**Family**	**Genus**	**Species**	***S organic***	**GT**	**CHR**	**JAR**	**SUC**	**GUR**	
Oligochaeta					-1.14	0.5					
Haplotaxida		Naididae			-1.10	0.5	2	3	9		
Lumbriculida		Lumbriculidae			-1.40	0.5					
Hirudinea					-0.45	0.5					
Pharyngobdellida		Erpobdellidae			-0.41	0.5					1
Rhynchobdellida		Glossiphoniidae			-0.49	0.5					1
		Piscicolidae	Piscicola	milneri	-0.60	0.5					
Acari					-1.64	1		3	4		
Araneae					-1.64				2		
Coleoptera		Dytiscidae	Acilius		-0.81	1					
			Coptotomus		-0.81	1			1		
			Laccornis		-0.81	1					
			Liodessus		-0.81	1					
			Oreodytes/Potamonectes		-0.81	1					
		Elmidae	Optioservus		-1.15	1			1		
		Gyrinidae	Gyrinus		-1.15	1	21	4			

		Haliplidae			-1.83	1		2		
			Brychius		-1.83	1				
					-1.26	1	1			
Diptera		Anthomyidae			-0.37	0.5				
		Athericidae	Atherix		-0.35	1				
		Caenidae	Caenis		-0.30	0.5				
		Ceratopogonidae			-0.35	0.5			1	
		Chironomidae			-0.39	0.5				
			Chironominae		-0.39	0.5		40	17	1
			Chironominae/Orthocladiinae		-0.39	0.5	1	1		
			Orthocladiinae		-0.39	0.5			5	
			Tanypodinae		-0.39	0.5		4	5	
		Empididae			-0.35	0.5				
		Simuliidae			-0.46	0.5		2	16	
		Tabanidae			-0.35	0.5		1	1	
		Tipulidae	Hexatoma		-0.35	0.5				
			Limoniidae/Limoniinae		-0.35	0.5				1
			Prinocera		-0.35	0.5				
Hemiptera	Heteroptera	Corixidae			-0.29	0.5				
			Callicorixa		-0.29	0.5				
			Hesperocorixa		-0.29	0.5	68	1	3	3
			Palmacorixa	buenoi	-0.29	0.5				
			Sigara		-0.29	0.5	149	7	7	
		Gerridae	Limnoporus		-0.56	0.5				
		Hebridae	Merragata		-0.56					
		Nepidae	Nepa	cinerea	-0.56	0.5				
		Notonectidae	Notonecta		-0.82	1			2	
Odonata	Anisoptera	Aeshnidae	Aeshna		-0.96	1			1	
		Corduliidae	Somatochlora		-0.96	1				
		Gomphidae	Ophiogomphus		-0.96	1	5	9		
	Zygoptera	Calopterygidae	Calopteryx		-0.36	1		11	3	3
Ephemeroptera		Ametropodidae	Ametropus		-0.14	1		13	1	
		Baetidae			0.02	0.5		2		
			Baetis		0.02	0.5		42		
			Baetis/Centroptilum		0.02	0.5	18		16	
			Baetis/Pseudocloeon		0.02	0.5			12	
			Callibaetis		0.02	0.5				
			Centroptilum		-0.25	0.5		157		1
			Pseudocloeon		0.02	0.5				

	Baetiscidae	Baetisca	-0.14	1				
	Caenidae	Caenis	-0.30	0.5			3	2
	Ephemerellidae	Drunella	-0.30	1				
		Ephemerella	-0.30	1				
		Serratella	-0.30	1	1	120	1	
	Ephemeridae	Ephemera	-0.30	1				
		Hexagenia	-0.30	1		1	1	2
	Heptageniidae		-0.30	0.5	2	1		
		Heptagenia	-0.30	0.5	5	2		
		Rhithrogena	-0.30	0.5				
		Stenacron	-0.30	0.5				
		Stenonema	-0.30	0.5		2	5	2
	Leptophlebiidae		-0.30	1	2	4		
		Leptophlebia	-0.30	1	31	14	27	13
		Leptophlebia/Paraleptophlebia	-0.30	1				
		Paraleptophlebia	-0.30	1		1		
	Metretopodidae	Siphloplecton	-0.14	0.5	17		5	
	Oligoneuriidae	Isonychia	-0.14	1				
	Siphlonuridae		-0.30	0.5			1	
		Ameletus	-0.30	0.5	1			
		Analetris	-0.30	0.5	4			
	Siphlonuridae/ Metretopodidae		-0.30	0.5				
	Tricorythidae	Tricorythodes	-0.14	0.5	1			
			-0.14	0.5				
Plecoptera	Chloroperlidae		-0.36	1			1	
	Nemouridae	Nemoura	0.25	1				
		Zapada	0.25	1				
	Perlidae	Acroneuria	0.38	1		1		
		Claassenia	0.38	1				
	Perlodidae	Isoperla	0.38	1	16	3		
		Skwala	0.38	1		1	2	
	Pteronarcyidae	Pteronarcella	0.31	1				
		Pteronarcys	0.31	1	6			
	Taeniopterygidae	Taeniopteryx	0.38	1	1			
	Taeniopterygidae/Nemouridae		0.38	1				
			0.31	1				
Trichoptera	Brachycentridae	Brachycentrus	-0.06	1	2		1	
		Micrasema	-0.06	1		2	4	
	Glossosomatidae	Glossosoma	-0.06	0.5				

		Glossosoma/Anagapetus		-0.06	0.5				
		Protoptila/Agapetus		-0.06	0.5				
	Helicopsychidae	Helicopsyche		-0.62	0.5				
	Hydropsychidae			-1.03	1				
		Cheumatopsyche		-0.76	1			2	
		Hydropsyche		-1.03	1	3	2	2	
	Hydroptilidae			-0.89	0.5				
		Oxyethira		-0.89	0.5				
	Lepidostomatidae	Lepidostoma		-0.06	1		4	6	
	Leptoceridae	Ceraclea		-0.06	1				
		Mystacides		-0.06	1		1		
		Oecetis		-0.06	1		3	1	2
		Triaenodes		-0.06	1		2		
	Limnephilidae			-0.06	1			1	
		Limnephilus		-0.06	1		1	2	2
	Philopotamidae	Dolophilodes		-0.06	0.5		1		
	Phryganeidae	Ptilostomis		-0.06	1				2
	Polycentropodidae	Neureclipsis		-0.06	1		5		
				-0.62	1				
Amphipoda	Gammaridae	Gammarus	lacustris	0.32	0.5			11	30
		Hyalella	azteca	0.16	0.5			6	9
Copepoda	Cyclopoida			0.16	0.5				
Cladocera				0.19	0.5				
Veneroida	Sphaeriidae	Pisidium		-2.09	0.5			1	
		Sphaerium		-2.09	0.5		2	1	
Basommatophora	Ancylidae	Ferrissia	rivularis	-1.23	0.5			2	4
	Lymnaeidae	Bakerilymnaea		-0.64	0.5				
		Stagnicola		-0.64	0.5			2	1
	Physidae			-1.64	0.5				
		Aplexa		-1.64	0.5				
		Physa		-1.64	0.5		4	6	16
	Planorbidae	Gyraulus		-1.94	0.5				
		Promenetus	umbilicatellus	-1.94	0.5		17	1	
Heterostropha	Valvatidae	Valvata	tricarinata	-1.82	1				
					SPEARoil	**-2.02**	**-2.29**	**-2.44**	**-2.18**

Table S6. Classical biological indicators taxa richness (TR), taxa richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT, EPT%), Shannon-Wiener diversity index (H'), and part of predators (% Pred.) in samples taken from all sampling sites in 2010, 2011, and 2012.

	2010					2011					2012				
Sampling site	TR	EPT	EPT%	H'	% Pred.	TR	EPT	EPT%	H'	% Pred.	TR	EPT	EPT%	H'	% Pred.
ATR-1	-	-	-	-	-	8	7	87.50	1.99	18.60	21	13	61.90	2.06	43.73
ATR-2	-	-	-	-	-	14	11	78.57	2.07	25.34	17	11	64.71	2.59	23.32
ATR-3	NA	NA	NA	NA	NA	9	7	77.78	2.04	44.18	17	12	70.59	2.34	28.89
ATR-4	17	11	64.71	2.26	18.76	11	8	72.73	1.68	15.86	19	13	68.42	2.20	32.94
ATR-5	-	-	-	-	-	15	10	66.67	1.95	38.85	11	5	45.45	1.36	35.76
BER	13	8	61.54	2.24	37.38	32	7	21.88	2.38	40.32	36	16	44.44	2.86	38.64
CHR	10	6	60.00	1.19	81.23	20	14	70.00	2.10	21.37	23	16	69.57	2.07	46.49
ELR	-	-	-	-	-	26	16	61.54	2.43	18.89	25	13	52.00	2.68	32.23
FOC	8	7	87.50	1.49	12.52	12	5	41.67	1.11	21.57	13	4	30.77	1.22	12.62
GUR	-	-	-	-	-	27	7	25.93	2.20	22.21	18	8	44.44	2.21	22.53
HAC	-	-	-	-	-	26	18	69.23	2.39	12.55	29	19	65.52	3.01	17.80
HOR	-	-	-	-	-	27	17	62.96	2.83	29.72	34	22	64.71	2.90	25.18
JAR	21	12	57.14	2.26	32.96	35	18	51.43	3.09	32.29	41	25	60.98	2.36	25.64
MAR-1	-	-	-	-	-	21	13	61.90	1.55	18.48	30	13	43.33	2.68	34.70
MAR-2	-	-	-	-	-	37	22	59.46	2.73	20.28	28	11	39.29	2.26	44.27
MUR	9	6	66.67	1.98	27.59	13	5	38.46	2.19	17.48	4	2	50.00	1.33	43.62
POC	22	5	22.73	1.85	41.54	27	7	25.93	1.81	17.00	32	15	46.88	2.34	20.88
SUC	20	7	35.00	2.29	49.63	26	11	42.31	2.14	31.04	46	18	39.13	3.29	28.87
TAR	-	-	-	-	-	19	10	52.63	1.70	15.81	27	17	62.96	2.00	10.13

Table S7. Trait-based indicators average community sensitivity towards oil ($SPEAR_{oil}$), towards organic contaminats ($SPEAR_{organic}$) and towards metals (S_{metal}) in samples taken from all sampling sites in 2010, 2011, and 2012. Generation time weighted by abundance (gewGT) is given for samples in 2010.

	2010				2011			2012		
Sampling site	$SPEAR_{oil}$	$SPEAR_{organic}$	S_{metal}	gewGT	$SPEAR_{oil}$	$SPEAR_{organic}$	S_{metal}	$SPEAR_{oil}$	$SPEAR_{organic}$	S_{metal}
ATR-1	-	-	-	-	-1.51	-0.17	1.08	-2.15	-0.36	0.91
ATR-2	-	-	-	-	-1.89	-0.51	0.70	-2.14	-0.45	0.84
ATR-3	NA	NA	NA	NA	-1.57	-0.26	0.73	-2.10	-0.34	0.85
ATR-4	-1.55	-0.18	1.26	0.84	-1.84	-0.50	1.24	-2.15	-0.39	1.28
ATR-5	-	-	-	-	-2.06	-0.40	1.25	-2.37	-0.66	1.62
BER	-2.18	-0.31	0.31	0.62	-2.27	-0.58	0.34	-2.50	-0.55	0.60
CHR	-1.89	-0.40	0.80	0.76	-1.70	-0.25	0.60	-2.02	-0.32	0.81
ELR	-	-	-	-	-2.12	-0.29	0.45	-2.41	-0.48	0.70
FOC	-1.76	-0.09	0.32	0.68	-1.78	-0.18	0.67	-2.29	-0.36	0.44
GUR	-	-	-	-	-2.37	-0.47	0.41	-2.18	-0.38	0.52
HAC	-	-	-	-	-1.83	-0.42	0.98	-2.18	-0.41	0.90
HOR	-	-	-	-	-1.86	-0.45	1.23	-1.76	-0.28	1.05
JAR	-1.98	-0.42	0.72	0.85	-1.99	-0.35	0.83	-2.29	-0.49	0.69
MAR-1	-	-	-	-	-2.04	-0.27	0.30	-2.62	-0.55	0.63
MAR-2	-	-	-	-	-2.43	-0.40	0.85	-2.20	-0.36	0.58
MUR	-2.41	-0.61	0.12	0.85	-2.39	-0.44	0.29	-1.49	-0.19	1.04
POC	-3.07	-0.79	0.56	0.67	-2.68	-0.51	0.60	-2.61	-0.48	0.70
SUC	-2.31	-0.58	0.34	0.91	-2.01	-0.33	0.85	-2.44	-0.49	0.66
TAR	-	-	-	-	-2.28	-0.38	0.54	-1.88	-0.23	0.70

Table S8. Sum parent, sum alkylated and total PAH concentrations [µg/L] in water in samples taken from all sampling sites in 2010, 2011, and 2012.

	2010			2011			2012		
Sampling site	Parent	Alkyl	Total	Parent	Alkyl	Total	Parent	Alkyl	Total
ATR-1	-	-	-	0.000	0.000	0.000	0.000	0.022	0.022
ATR-2	-	-	-	0.000	0.000	0.000	0.000	0.048	0.048
ATR-3	0.196	0.401	0.597	0.000	0.000	0.000	0.006	0.034	0.039
ATR-4	0.041	0.259	0.300	0.000	0.000	0.000	0.000	0.007	0.007
ATR-5	-	-	-	0.000	0.006	0.006	0.003	0.018	0.021
BER	0.233	0.862	1.095	0.000	0.007	0.007	0.001	0.017	0.018
CHR	0.063	0.197	0.260	0.000	0.001	0.001	0.000	0.002	0.002
ELR	-	-	-	0.000	0.043	0.043	0.013	0.064	0.077
FOC	0.175	1.632	1.808	0.021	0.433	0.445	0.004	0.075	0.079
GUR	-	-	-	0.000	0.000	0.000	0.001	0.024	0.025
HAC	-	-	-	0.000	0.003	0.003	0.000	0.031	0.031
HOR	-	-	-	0.000	0.005	0.005	0.004	0.038	0.042
JAR	0.104	0.298	0.401	0.000	0.000	0.000	0.000	0.005	0.005
MAR-1	-	-	-	0.000	0.035	0.035	0.003	0.078	0.081
MAR-2	-	-	-	0.000	0.000	0.000	0.000	0.018	0.018
MUR	0.182	0.619	0.801	0.000	0.002	0.002	0.006	0.153	0.160
POC	0.641	1.714	2.355	0.000	0.007	0.007	0.005	0.091	0.096
SUC	0.120	0.565	0.685	0.000	0.000	0.000	0.000	0.000	0.000
TAR	-	-	-	0.001	0.038	0.040	0.007	0.238	0.244

Table S9. Chemical and environmental parameters pH, O_2 [%], conductivity [µS], current velocity [m/s], and stream width [m] in samples taken from all sampling sites in 2010, 2011, and 2012.

	2010					2011					2012				
Sampling site	pH	O_2	Cond.	Curr.	Width	pH	O_2	Cond.	Curr.	Width	pH	O_2	Cond.	Curr.	Width
ATR-1	-	-	-	-	-	8.6	100	310	0.15	150	8.01	97.6	247	0.2	150
ATR-2	-	-	-	-	-	8.35	-	233	0.4	100	7.93	88.1	218	0.8	100
ATR-3					125	8.23	105	262	0.6	150	8.1	91.6	255	0.3	150
ATR-4	7.4	99	230	1	125	8.7	-	279	0.7	150	8.11	95.1	276	0.3	150
ATR-5	-	-	-	-	-	8.5	96	260	1	150	8.11	89.9	298	0.5	150
BER	6.6	90	227	0.2	5	7.8	81	510	0.25	4	7.6	85	278	0.1	4
CHR	7.2	92	140	0.9	15	7.83	-	242	0.6	20	7.62	87.4	102.2	0.5	20
ELR	-	-	-	-	-	8.5	102.6	198	0.4	10	8.15	99.3	200	0.4	10
FOC	6.9	96	530	-	1.5	8.3	81.4	620	0.6	2	7.96	93.2	619	1	2
GUR	-	-	-	-	-	7.92	-	304	0.5	7	7.64	88	324	0.1	3
HAC	-	-	-	-	-	8.7	-	-	0.4	3.5	7.3	82	168	2	8
HOR	-	-	-	-	-	8.2	82	202	0.5	10	7.31	86.2	88.5	1	10
JAR	7.1	102.5	167		12	8.2	-	182	0.5	10	7.91	91.7	194.2	0.4	10
MAR-1	-	-	-	-	-	8.8	-	1002	0.9	15	7.94	97.6	194.1	0.15	15
MAR-2	-	-	-	-	-	7.9	105.1	199.2	0.7	15	7.97	97.7	183.7	0.6	15
MUR	6.7	96	260	1	8	8.53	94.7	428	0.3	4	7.43	85.7	214	2	8
POC	6.9	75	315	0.15	8	7.86	78	63.2	0.4	7	8.07	95.1	380	1	7
SUC	7.2	98	180	0.5	4.5	7.4	-	223	0.2	5	7.62	90.1	231	0.3	5
TAR	-	-	-	-	-	8.35	97	344	0.55	5	7.78	93.6	298	1	5

Table S10. Summary of the RDA and PCA results. Explained proportion of total variance in the RDA is 40.5%; the model was confirmed significant. In the PCA, the first two ordination axes being interpretable and explaining 66.3% of total variance.

RDA	RDA1	RDA2
Eigenvalue	12.6107	8.0459
Proportion Explained	0.2473	0.1578
Cumulative Proportion	0.2473	0.405
Proportion Explained	0.6105	0.3895
Cumulative Proportion	0.6105	1
Sum of all canonical eigenvalues	20.7	
PCA	PC1	PC2
Eigenvalue	6.448	4.158
Proportion Explained	0.403	0.26
Cumulative Proportion	0.403	0.663
Sum of all eigenvalues	10.606 14	

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C Supplementary Information for Chapter 4

Table S1. List of the 52 hydrocarbon substances found in crude oil for which the toxicity data search was conducted and their respective CAS number and hydrocarbon grouping.

CAS	Group	Name
8002059	Petroleum	Petroleum
8008206	Petroleum	Kerosine (petroleum)
8009038	Petroleum	Petrolatum
8030306	Petroleum	Naphtha
8042475	Petroleum	White mineral oil (petroleum)
63993737	Petroleum	5-[[2-(2-Butoxyethoxy)ethoxy]methyl]-6-propyl-1,3-benzodioxole mixt. with petroleum distillates and pyrethrins
64475850	Petroleum	Petroleum spirits
64741431	Petroleum	Gas oils (petroleum), Straight-run
64741884	Petroleum	Distillates (petroleum), Solvent-refined heavy paraffinic
64741895	Petroleum	Distillates (petroleum), Solvent-refined light paraffinic
64741975	Petroleum	Distillates (petroleum), Solvent-refined light naphthenic
64742478	Petroleum	Hydrotreated light distillates (petroleum)
64742536	Petroleum	Hydrotreated light naphthenic distillate (petroleum)
64742547	Petroleum	Distillates (petroleum), Hydrotreated heavy paraffinic
64742558	Petroleum	Distillates (petroleum), Hydrotreated light paraffinic
64742569	Petroleum	Solvent-dewaxed light paraffinic distillates (petroleum)
64742650	Petroleum	Solvent-dewaxed heavy paraffinic distillates (petroleum)
64742887	Petroleum	Solvent naphtha (petroleum), Medium aliph.
64742898	Petroleum	Solvent naphtha (petroleum), Light aliph.
64742945	Petroleum	Solvent naphtha (petroleum), Heavy arom.
64742956	Petroleum	Solvent naphtha (petroleum), Light arom.
68187586	Petroleum	Aromatic petroleum pitch
68334305	Petroleum	Fuels, diesel
68477316	Petroleum	Distillates(petroleum), Catalytic reformer fractionator residue, Low boiling
68602802	Petroleum	Distillates (petroleum), C12-30-arom.
68608264	Petroleum	Sulfonic acids, Petroleum, Sodium salts
72623848	Petroleum	Hydrotreated neutral oil-based lubricating oils (petroleum), C15-30 contg. solvent deasphalted residual oil
72623860	Petroleum	Hydrotreated neutral oil-based lubricating oils (petroleum), C15-30
72623871	Petroleum	Hydrotreated neutral oil-based lubricating oils (petroleum), C20-50
109660	Alkanes	Pentane
110543	Alkanes	Hexane
142825	Alkanes	Heptane
111659	Alkanes	Octane
111842	Alkanes	Nonane
124185	Alkanes	Decane
112403	Alkanes	Dodecane
287923	Cycloalkanes	Cyclopentane
110827	Cycloalkanes	Cyclohexane
109671	Alkenes	1-Pentene
109682	Alkenes	2-Pentene
592416	Alkenes	1-Hexene
4050457	Alkenes	2-Hexene
592767	Alkenes	1-Heptene
111660	Alkenes	1-Octene
71432	Monoaromatics	Benzene
108883	Monoaromatics	Toluene
100414	Monoaromatics	Ethylbenzene
106423	Monoaromatics	P-Xylene
108383	Monoaromatics	M-Xylene
95476	Monoaromatics	O-Xylene
98828	Monoaromatics	Cumene
100425	Monoaromatics	Styrene (vinylbenzene)

Table S2. LC50 values (in µg/L) for invertebrate taxa obtained from **literature studies.**

CAS	Substance	Class	Order	Family	Species	LC50 (µg/L)	Test duration	LC50 (µg/L) 48h	Source
71432	Benzene	Malacostraca	Amphipoda	Gammaridae	Gammarus fossarum	67000.0	48	67000.0	USEPA ref.no.13419
71432	Benzene	Malacostraca	Amphipoda	Gammaridae	Gammarus fossarum	76000.0	24	32146.7	USEPA ref.no.13419
71432	Benzene	Malacostraca	Amphipoda	Gammaridae	Gammarus pulex	42000.0	48	42000.0	USEPA ref.no.15788
71432	Benzene	Clitellata	Arhynchobdellida	Erpobdellidae	Erpobdella octoculata	320000.0	48	320000.0	USEPA ref.no.15788
71432	Benzene	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	230000.0	48	230000.0	USEPA ref.no.10574
71432	Benzene	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	230000.0	48	230000.0	USEPA ref.no.15788
71432	Benzene	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	230000.0	48	230000.0	USEPA ref.no.14863
71432	Benzene	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	940000.0	48	940000.0	USEPA ref.no.13419
71432	Benzene	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	1410000.0	24	451901.0	USEPA ref.no.13419
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	10154.3	48	10154.3	USEPA ref.no.18991
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	17200.0	48	17200.0	Niederlehner et al. 1998
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	18400.0	24	8905.5	USEPA ref.no.4343
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia cucullata	373000.0	48	373000.0	USEPA ref.no.2017
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	10000.0	24	5128.6	USEPA ref.no.6516

71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	10000.0	48	10000.0	USEPA ref.no.6516
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	10865.0	48	10865.0	USEPA ref.no.7069
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	18000.0	24	8730.1	USEPA ref.no.13142
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	18000.0	24	8730.1	USEPA ref.no.16968
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	31244.0	48	31244.0	USEPA ref.no.11936
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	97700.0	48	97700.0	USEPA ref.no.7069
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	200000.0	48	200000.0	USEPA ref.no.5184
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	250000.0	24	94435.6	USEPA ref.no.5184
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	406000.0	48	406000.0	USEPA ref.no.2017
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	682000.0	48	682000.0	USEPA ref.no.10060
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	1130000.0	24	369858.8	USEPA ref.no.5718
71432	Benzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia pulex	305000.0	48	305000.0	USEPA ref.no.2017
71432	Benzene	Insecta	Diptera	Culicidae	Aedes aegypti	59270.0	24	25669.4	USEPA ref.no.5700
71432	Benzene	Insecta	Diptera	Culicidae	Aedes aegypti	200000.0	48	200000.0	USEPA ref.no.10574
71432	Benzene	Insecta	Diptera	Culicidae	Aedes aegypti	200000.0	48	200000.0	USEPA ref.no.14863
71432	Benzene	Insecta	Diptera	Chironomidae	Chironomus riparius	100000.0	48	100000.0	USEPA ref.no.15788

71432	Benzene	Insecta	Diptera	Culicidae	Culex pipiens	71000.0	48	71000.0	USEPA ref.no.10574
71432	Benzene	Insecta	Ephemeroptera	Baetidae	Cloeon dipterum	34000.0	48	34000.0	USEPA ref.no.15788
71432	Benzene	Insecta	Heteroptera	Corixidae	Corixa punctata	48000.0	48	48000.0	USEPA ref.no.15788
71432	Benzene	Hydrozoa	Hydroida	Hydridae	Hydra oligactis	34000.0	48	34000.0	USEPA ref.no.10574
71432	Benzene	Hydrozoa	Hydroida	Hydridae	Hydra oligactis	34000.0	48	34000.0	USEPA ref.no.15788
71432	Benzene	Malacostraca	Isopoda	Asellidae	Asellus aquaticus	120000.0	48	120000.0	USEPA ref.no.15788
71432	Benzene	Malacostraca	Isopoda	Asellidae	Asellus aquaticus	440000.0	48	440000.0	USEPA ref.no.13419
71432	Benzene	Malacostraca	Isopoda	Asellidae	Asellus aquaticus	680000.0	24	233571.9	USEPA ref.no.13419
71432	Benzene	Gastropoda	Neotaenioglossa	Thiaridae	Amphimelania holandri	1360000.0	48	1360000.0	USEPA ref.no.13419
71432	Benzene	Gastropoda	Neotaenioglossa	Thiaridae	Amphimelania holandri	2550000.0	24	772536.2	USEPA ref.no.13419
71432	Benzene	Insecta	Odonata	Coenagrionidae	Ischnura elegans	10000.0	48	10000.0	USEPA ref.no.15788
71432	Benzene	Insecta	Plecoptera	Nemouridae	Nemoura cinerea	130000.0	48	130000.0	USEPA ref.no.15788
71432	Benzene	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	1000000.0	24	331131.1	USEPA ref.no.9385
71432	Benzene	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	1000000.0	24	331131.1	USEPA ref.no.17689
71432	Benzene	Turbellaria	Tricladida	Planariidae	Dugesia lugubris	74000.0	48	74000.0	USEPA ref.no.15788
78784	Isopentane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2300.0	48	2300.0	Adema and van den Bos Bakker 1986

95476	O-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	1000.0	24	638.3	USEPA ref.no.13142
95476	O-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	1390.0	48	1390.0	USEPA ref.no.7069
95476	O-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	3185.1	48	3185.1	USEPA ref.no.11936
95476	O-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	3820.0	48	3820.0	USEPA ref.no.12665
95476	O-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	17200.0	48	17200.0	USEPA ref.no.7069
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	601.0	48	601.0	USEPA ref.no.11936
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	1400.0	24	865.5	USEPA ref.no.13142
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	1400.0	24	865.5	USEPA ref.no.16968
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2000.0	24	1195.2	IUCLID
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4000.0	24	2238.0	Springborn Laboratories 1990c
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4000.0	48	4000.0	IUCLID
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	10600.0	48	10600.0	USEPA ref.no.7069
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	25200.0	48	25200.0	UB/TIB (McLean et al. 1989)
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	25400.0	48	25400.0	USEPA ref.no.7069
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	91000.0	24	37838.4	Bringmann et al. 1982
98828	Cumene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	95000.0	24	39340.5	USEPA ref.no.5718

100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	3200.0	48	3200.0	Neiderlehner et al 1998
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2123.4	48	2123.4	USEPA ref.no.11936
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2200.0	24	1302.8	USEPA ref.no.13142
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2200.0	24	1302.8	USEPA ref.no.16968
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2375.0	24	1396.3	USEPA ref.no.6984
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2950.0	48	2950.0	USEPA ref.no.7069
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	16150.0	48	16150.0	USEPA ref.no.7069
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	75000.0	48	75000.0	USEPA ref.no.5184
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	77000.0	24	32529.3	USEPA ref.no.5184
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	184000.0	24	71558.3	IUCLID
100414	Ethylbenzene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	190000.0	24	73666.8	USEPA ref.no.5718
100425	Styrene (vinylbenzene)	Malacostraca	Amphipoda	Gammaridae	Gammarus fossarum	64000.0	48	64000.0	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Malacostraca	Amphipoda	Gammaridae	Gammarus fossarum	72000.0	24	30611.6	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Malacostraca	Amphipoda	Hyalellidae	Hyalella azteca	13000.0	24	6503.1	USEPA ref.no.18326
100425	Styrene (vinylbenzene)	Malacostraca	Amphipoda	Hyalellidae	Hyalella azteca	13000.0	48	13000.0	USEPA ref.no.18326
100425	Styrene (vinylbenzene)	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	810000.0	24	273639.6	USEPA ref.no.13419

100425	Styrene (vinylbenzene)	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea stagnalis	6400000.0	48	6400000.0	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4700.0	48	4700.0	USEPA ref.no.18326
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	5000.0	24	2738.8	USEPA ref.no.18326
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	23000.0	48	23000.0	USEPA ref.no.5184
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	27000.0	24	12600.4	USEPA ref.no.5184
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	59000.0	48	59000.0	USEPA ref.no.15923
100425	Styrene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	182000.0	24	70854.0	IUCLID
100425	Styrene (vinylbenzene)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	255000.0	24	96143.3	USEPA ref.no.5718
100425	Styrene (vinylbenzene)	Malacostraca	Isopoda	Asellidae	Asellus aquaticus	76000.0	48	76000.0	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Malacostraca	Isopoda	Asellidae	Asellus aquaticus	99000.0	24	40836.6	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Gastropoda	Neotaenioglossa	Thiaridae	Amphimelania holandri	124000.0	48	124000.0	USEPA ref.no.13419
100425	Styrene (vinylbenzene)	Gastropoda	Neotaenioglossa	Thiaridae	Amphimelania holandri	143000.0	24	56961.2	USEPA ref.no.13419
106423	P-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	3600.0	24	2034.5	USEPA ref.no.13142
106423	P-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4880.0	48	4880.0	USEPA ref.no.7069
106423	P-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	8493.6	48	8493.6	USEPA ref.no.11936
106423	P-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	32300.0	48	32300.0	USEPA ref.no.7069

108383	M-Xylene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	2441.9	48	2441.9	USEPA ref.no.18991
108383	M-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4265.0	48	4265.0	USEPA ref.no.7069
108383	M-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	4700.0	24	2589.7	USEPA ref.no.13142
108383	M-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	9555.3	48	9555.3	USEPA ref.no.11936
108383	M-Xylene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	39650.0	48	39650.0	USEPA ref.no.7069
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	3780.0	48	3780.0	Niederlehner et al. 1998
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Ceriodaphnia dubia	9000.0	24	4662.2	USEPA ref.no.4343
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	6000.0	48	6000.0	USEPA ref.no.6516
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	6720.0	48	6720.0	USEPA ref.no.7069
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	7000.0	24	3713.8	USEPA ref.no.13142
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	7000.0	24	3713.8	USEPA ref.no.16968
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	8000.0	24	4190.8	USEPA ref.no.6516
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	11500.0	48	11500.0	Bobra, 1983
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	11517.5	48	11517.5	USEPA ref.no.11936
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	14900.0	48	14900.0	Hermens (Adema 1991)
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	19600.0	48	19600.0	USEPA ref.no.5087

108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	84000.0	24	35194.3	USEPA ref.no.847
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	92000.0	48	92000.0	USEPA ref.no.7069
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	310000.0	24	114731.4	USEPA ref.no.5184
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	310000.0	48	310000.0	USEPA ref.no.5184
108883	Toluene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	470000.0	24	167204.7	USEPA ref.no.5718
108883	Toluene	Insecta	Diptera	Culicidae	Aedes aegypti	21520.0	24	10261.8	USEPA ref.no.5700
108883	Toluene	Insecta	Diptera	Chironomidae	Chironomus riparius	47000.0	48	47000.0	USEPA ref.no.4072
108883	Toluene	Insecta	Diptera	Chironomidae	Chironomus riparius	108660.0	48	108660.0	USEPA ref.no.14396
108883	Toluene	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	113000.0	24	46029.5	USEPA ref.no.9385
108883	Toluene	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	113000.0	24	46029.5	USEPA ref.no.17689
108883	Toluene	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	113300.0	24	46140.1	USEPA ref.no.6002
109660	Pentane(/n-Pentane)	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2700.0	48	2700.0	Adema and van den Bos Bakker 1986
109660	n-Pentane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	9100.0	48	9100.0	Adema and Bakker 1986
109660	Pentane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	9740.3	48	9740.3	USEPA ref.no.11936
110543	Hexane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	3878.1	48	3878.1	USEPA ref.no.11936
110543	Hexane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	50000.0	24	22007.4	USEPA ref.no.5718

110543	Hexane	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	68000.0	24	29068.4	USEPA ref.no.9385
110543	Hexane	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	68300.0	24	29184.4	USEPA ref.no.6002
110543	Hexane	Monogononta	Ploima	Brachionidae	Brachionus calyciflorus	68300.0	24	29184.4	USEPA ref.no.17689
110827	Cyclohexane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	900.0	48	900.0	Adema and Bakker 1986
110827	Cyclohexane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	3787.2	48	3787.2	USEPA ref.no.11936
110827	Cyclohexane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	340000.0	24	124735.0	USEPA ref.no.5718
111659	Octane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	377.0	48	377.0	USEPA ref.no.11936
111660	1-Octene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	6500.0	48	6500.0	USEPA ref.no.63143
124185	Decane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	28.5	48	28.5	USEPA ref.no.11936
124185	Decane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	18000.0	48	18000.0	USEPA ref.no.5184
124185	Decane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	23000.0	24	10898.4	USEPA ref.no.5184
142825	Heptane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	50000.0	24	22007.4	USEPA ref.no.5718
287923	Cyclopentane	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	10521.0	48	10521.0	USEPA ref.no.11936
592416	1-Hexene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	30000.0	48	30000.0	Adema and van den Bos Bakker 1986
592416	1-Hexene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	45000.0	48	45000.0	USEPA ref.no.63143
592416	1-Hexene	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	230000.0	48	230000.0	Shell Research Group.SBGR. 85.182

8002059	Petroleum	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	36000.0	24	16347.6	IUCLID
8002059	Petroleum	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	38900.0	48	38900.0	IUCLID
8002059	Petroleum	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	42000.0	24	18794.9	IUCLID
64742945	Kerosine/ Solvent naphtha (petroleum), Heavy arom.	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	950.0	48	950.0	IUCLID
64742956	Kerosine/ Solvent naphtha (petroleum), Light arom.	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	6140.0	48	6140.0	IUCLID
64742956	Kerosine/ Solvent naphtha (petroleum), Light arom.	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	170000.0	24	66612.6	IUCLID
64742956	Kerosine/ Solvent naphtha (petroleum), Light arom.	Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	226000.0	24	86192.2	IUCLID

Table S3. LC50 values (in µg/L) for invertebrate taxa obtained from **rapid test** studies conducted with middle distillate single blend at the UFZ, Leipzig, Germany and at TOTAL, Lacq, France.

Class	Order	Family	Species	LC50 (µg/L)	Test duration	Source
Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	2520	48	Rapid tests UFZ
Branchiopoda	Diplostraca	Daphniidae	Daphnia magna	270	48	Rapid tests UFZ
Malacostraca	Amphipoda	Gammaridae	Gammarus pulex	5480	48	Rapid tests UFZ
Malacostraca	Amphipoda	Gammaridae	Gammarus pulex	2320	48	Rapid tests UFZ
Malacostraca	Isopoda	Asellidae	Asellus aquaticus	3580	48	Rapid tests UFZ
Malacostraca	Isopoda	Asellidae	Asellus aquaticus	3910	48	Rapid tests UFZ
Insecta	Diptera	Culicidae	Culex pipiens	9520	48	Rapid tests UFZ
Insecta	Ephemeroptera	Baetidae	Baetis rhodani	7340	48	Rapid tests UFZ
Insecta	Zygoptera	Calopterygidae	Calopteryx splendens	26440	48	Rapid tests UFZ
Malacostraca	Amphipoda	Gammaridae		9540	48	Rapid tests Total
Malacostraca	Amphipoda	Gammaridae		137870	48	Rapid tests Total
Mollusca				31530	48	Rapid tests Total
Mollusca				40560	48	Rapid tests Total
Mollusca				71870	48	Rapid tests Total
Insecta	Diptera	Simulidae		1580	48	Rapid tests Total
Insecta	Diptera	Simulidae		1610	48	Rapid tests Total
Insecta	Diptera	Chironomidae		13550	48	Rapid tests Total
Insecta	Ephemeroptera	Baetidae		1200	48	Rapid tests Total
Turbellaria	Tricladida			292800	48	Rapid tests Total
Turbellaria	Tricladida			18010	48	Rapid tests Total
Turbellaria	Tricladida			61150	48	Rapid tests Total

Table S4. LC50 values (in µg/L) for invertebrate taxa obtained from **mesocosm studies** conducted with middle distillate single blends, light distillate single blend, and xylene at UFZ and at PERL (TOTAL). Abundance data were transferred into LC50 values via the Excel Makro REGTOX (Vindimian 2001, Vindimian 2005).

Substance	Family	LC50 (µg/L)
Middle distillate 1/2 single blend	Chironomidae	1657.38
Middle distillate 1/2 single blend	Asellidae	791.20
Light distillate single blend	Polycentropodidae	8.75
Light distillate single blend	Baetidae	6.20
Light distillate single blend	Chironomidae	20070.98
Light distillate single blend	Gammaridae	26.47
Light distillate single blend	Planariidae	5.13
Light distillate single blend	Oligochaeta	2569.57
Xylene	Hydroptilidae	372116.00
Xylene	Baetidae	10333.33
Xylene	Corixidae	299307.22
Xylene	Chironomidae	15597177.69
Middle distillate 3 single blend	Baetidae	55.76
Middle distillate 3 single blend	Ceratopogonidae	3716.89
Middle distillate 3 single blend	Chironomidae	175.31
Middle distillate 3 single blend	Gammaridae	2.84
Middle distillate 3 single blend	Lymnaeidae	2011.32
Middle distillate 3 single blend	Planariidae	5667.89

Table S5. $S_{hydrocarbons}$ ranking for the taxa-levels genus, family, and order. The taxon "Planariidae" (*) was not used in the validation study, as this value is incomprehensibly high.

Taxon	S-value
Genus-level	
Ceriodaphnia	0.553
Daphnia	0.012
Aedes	-0.197
Gammarus	-0.238
Culex	-0.444
Asellus	-0.458
Chironomus	-0.486
Brachionus	-0.539
Baetis	-0.721
Amphimelania	-0.912
Orthocladinae	-0.987
Calopteryx	-1.278
Lymnaea	-1.363
Family-Level	
Planariidae *	1.516 *
Daphniidae	0.065
Simulidae	-0.058
Gammaridae	-0.199
Culicidae	-0.353
Baetidae	-0.359
Asellidae	-0.458
Chironomidae	-0.509
Brachionidae	-0.539
Thiaridae	-0.912
Calopterygidae	-1.278
Lymnaeidae	-1.363
Ceratopogonidae	-2.871
Order-Level	
Basommatophora	-1.363
Tricladida	-1.286
Zygoptera	-1.278
Neotaenioglossa	-0.912
Amphipoda	-0.574
Ploima	-0.539
Isopoda	-0.458
Diptera	-0.448
Ephemeroptera	-0.130
Diplostraca	0.065

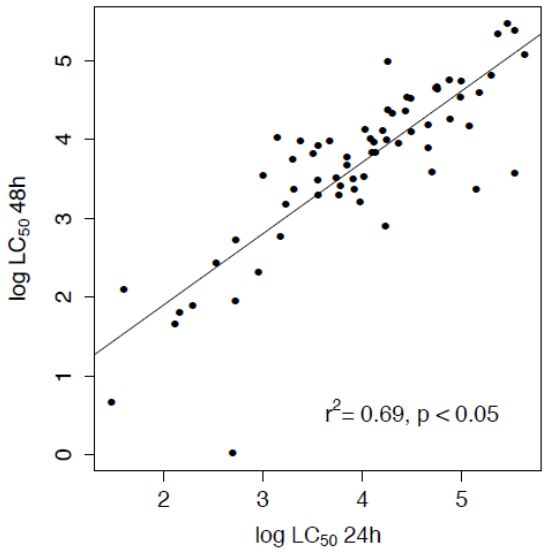

Figure S1. Extrapolation of LC50s (µg/L) from 24h tests to 48h performed by a linear model with log-transformed values. The dataset for the extrapolation includes *D. magna* tests with all petrochemical substances available in ECOTOX including crude oil constituents, anthropogenic petrochemicals, and PAHs (Polycyclic aromatic acids). Exclusion of 2 outliers from which the lower value is by a factor of 3.6 to 2.5 higher than the highest of the remaining values (for 24 and 48h values, respectively), regression formula y = 0.905x+0.090, r^2 = 0.69, p < 0.05, n = 66.

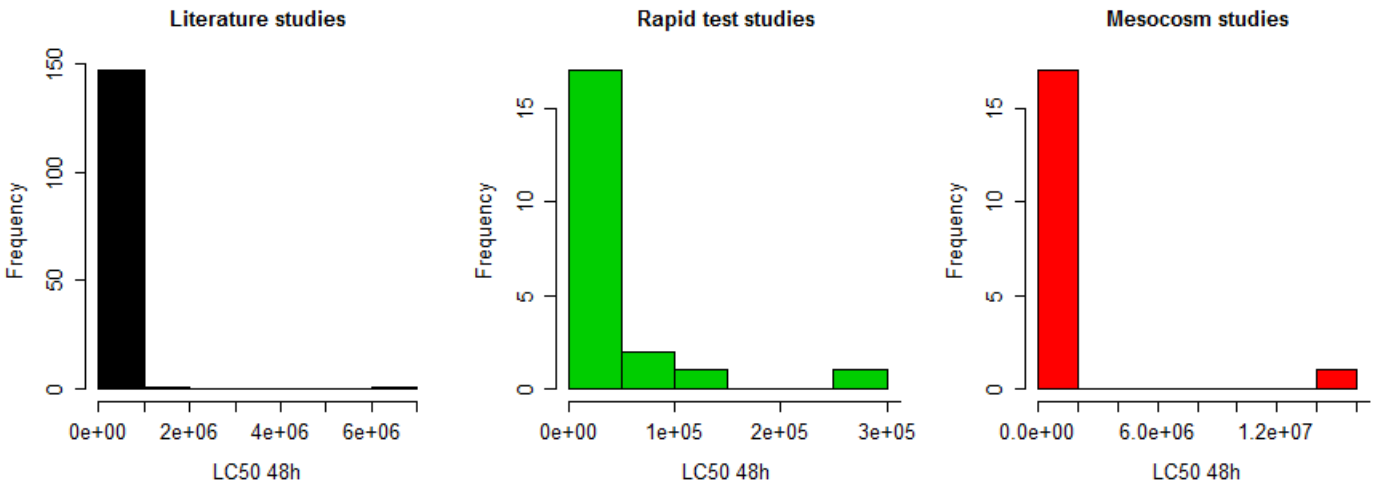

Figure S2. Frequency distribution of LC50 (48h) data from literature, rapid test, and mesocosm studies forming the dataset applied for the development of the S-ranking.

References

Vindimian, E., 2001. The biological monitoring of toxic impacts on the environment. Cellular and Molecular Biology 47 (8), pp. 1309-1318.

Vindimian, E., 2005. MSExcel macro REGTOX EV7.0.5.xls. Available from http://www.normalesup.org/~vindimian/en_index.html.

D Supplementary Information for Chapter 5

Table S1. LC50 values (48h) for Cu, Zn, Ni, Cd, and Pb for *Daphnia magna* were adopted from literature if available (Malaj 2012). Values for Fe, Mn, and Co were determined as the respective medians of all relevant LC50 or EC50 studies with mortality endpoints for these metals. Only studies testing the inorganic metal salt ions were included. Studies considered were of test durations of 48h and 24h, in which the latter ones were extrapolated to 48h values according to von der Ohe & Liess (2004).

Metal	**LC50 *D. magna* 48h (µg/L)**
Cu	26.3
Zn	617
Fe	10884
Mn	26193
Co	2507
Ni	2041
Cd	55
Pb	398

Table S2. Measured values for all sites of highest and mean concentration for each metal with respective TU´s. Concentrations in Norris 80: Mean of 2 years; in Norris 86: Mean of 2 sampling times; and in Edwards: Mean over all months. Copper and Zinc generally exert the highest toxicity in 90% of the sites investigated. At 5% of the sites Cadmium reveals the highest toxicity. However, in these cases Zinc exerts a toxicity that is only slightly lower. At another 5% of the sites Iron reveals the highest toxicity. However, here Copper exerts a toxicity that is only slightly lower.

Study	Site	Community S_{metal} (mean all metals)	Community S_{metal} (mean Cu, Zn)	$SPEAR_{metal}$ (Predator ratio)	Highest TU	Substance with highest TU	Conc of metal with highest TU (µg/L)	Conc Cu (µg/L)	Conc Zn (µg/L)	Conc Cd (µg/L)	Conc Fe (µg/L)	TU Cu	TU Zn	TU Cd	TU (Fe)	Alkal (CaCO3)
Norris 80	1	1.09	1.17	0.18	-1.02	Cu	2.50	2.50	18.90	n/a	n/a	-1.02	-1.51	n/a	n/a	21.00
Norris 80	2	1.02	1.10	0.20	-0.84	Cu	3.80	3.80	34.80	n/a	n/a	-0.84	-1.25	n/a	n/a	27.00
Norris 80	3	1.02	1.10	0.20	-0.90	Cu	3.30	3.30	27.60	n/a	n/a	-0.90	-1.35	n/a	n/a	35.50
Norris 80	4	1.21	1.34	0.34	-0.54	Cu	7.00	7.00	179.00	n/a	n/a	-0.57	-0.54	n/a	n/a	26.50
Norris 80	5	1.27	1.43	0.50	-0.47	Cu	9.00	9.00	194.00	n/a	n/a	-0.47	-0.50	n/a	n/a	28.00
Norris 80	6	1.02	1.21	0.40	-0.56	Cu	7.30	7.30	143.00	n/a	n/a	-0.56	-0.63	n/a	n/a	34.50
Norris 80	7	1.01	1.22	0.43	-0.53	Cu	7.80	7.80	135.00	n/a	n/a	-0.53	-0.66	n/a	n/a	43.00
Norris 80	8	0.88	1.05	0.24	-0.52	Cu	7.90	7.90	96.00	n/a	n/a	-0.52	-0.81	n/a	n/a	34.00
Norris 86	1	0.73	0.94	0.50	0.13	Zn	825.50	6.45	825.50	2.09	n/a	-0.61	0.13	-1.42	n/a	28.83
Norris 86	2	1.12	1.50	0.82	1.51	Zn	19742.00	61.37	19742.00	10.76	n/a	0.37	1.51	-0.71	n/a	13.42
Norris 86	3	1.04	1.25	0.92	1.50	Zn	19654.00	72.57	19654.00	13.37	n/a	0.44	1.50	-0.61	n/a	14.21
Norris 86	4	1.16	1.16	0.79	1.25	Zn	10964.50	4.79	10964.50	5.74	n/a	-0.74	1.25	-0.98	n/a	37.12
Norris 86	5	0.98	1.14	0.46	0.48	Zn	1867.00	<DL	1867.00	2.55	n/a	-2.00	0.48	-1.33	n/a	81.36
Norris 86	6	0.95	1.02	0.57	0.30	Zn	1234.00	<DL	1234.00	1.68	n/a	-2.00	0.30	-1.52	n/a	81.36
Norris 86	7	0.78	0.88	0.52	0.09	Zn	761.50	<DL	761.50	1.36	n/a	-2.00	0.09	-1.61	n/a	148.12
Norris 86	8	0.50	0.66	0.45	-0.09	Zn	500.00	<DL	500.00	1.32	n/a	-2.00	-0.09	-1.62	n/a	164.71
Norris 86	9	0.67	0.86	0.30	-1.54	Cd	1.60	<DL	11.50	1.60	n/a	-2.00	-1.73	-1.54	n/a	110.99
Norris 86	10	-0.38	-0.08	0.12	-1.61	Cd	1.34	<DL	14.00	1.34	n/a	-2.00	-1.64	-1.61	n/a	123.23
Edwards	EB2A	0.61	1.25	0.75	0.81	Cu	169.34	169.34	104.27	n/a	239.07	0.81	-0.77	n/a	-1.66	118.76
Edwards	EB2B	0.69	1.22	0.72	0.51	Cu	84.69	84.69	185.21	n/a	221.69	0.51	-0.52	n/a	-1.69	130.41

Edwards	EB4A	0.53	1.10	0.62	1.26	Cu	474.11	474.11	424.93	n/a	182.81	1.26	-0.16	n/a	-1.77	4.73
Edwards	EB4B	0.61	1.17	0.57	1.11	Cu	336.33	336.33	392.19	n/a	189.06	1.11	-0.20	n/a	-1.76	10.15
Edwards	EB4SA	0.51	0.91	0.46	-0.93	Cu	3.12	3.12	7.44	n/a	655.60	-0.93	-1.92	n/a	-1.22	17.84
Edwards	EB4SB	0.56	0.89	0.54	-0.76	Cu	4.60	4.60	3.88	n/a	836.00	-0.76	-2.20	n/a	-1.11	13.02
Edwards	EB5IA	0.33	0.97	0.55	1.67	Cu	1222.61	1222.61	2479.15	n/a	179.31	1.67	0.60	n/a	-1.78	1.85
Edwards	EB5IB	0.34	0.87	0.83	1.83	Cu	1775.31	1775.31	3886.54	n/a	153.06	1.83	0.80	n/a	-1.85	59.96
Edwards	EB8A	0.47	0.89	0.33	-0.62	Cu	6.28	6.28	12.93	n/a	688.13	-0.62	-1.68	n/a	-1.20	32.48
Edwards	EB8B	0.52	0.88	0.46	-0.56	Cu	7.17	7.17	7.56	n/a	615.20	-0.56	-1.91	n/a	-1.25	22.64
Edwards	EB8C	0.46	0.78	0.43	-0.91	Cu	3.27	3.27	11.13	n/a	1176.00	-0.91	-1.74	n/a	-0.97	52.70
Edwards	FCA	0.51	0.91	0.46	-1.24	Cu	1.51	1.51	3.84	n/a	138.00	-1.24	-2.21	n/a	-1.90	52.17
Edwards	FCB	0.56	0.93	0.52	-1.06	Cu	2.29	2.29	8.39	n/a	174.00	-1.06	-1.87	n/a	-1.80	83.51
Edwards	FR5	0.75	1.15	0.52	-0.45	Cu	9.35	9.35	8.23	n/a	325.75	-0.45	-1.87	n/a	-1.52	184.50
Edwards	FR6	0.68	1.00	0.40	-0.50	Cu	8.38	8.38	5.40	n/a	671.00	-0.50	-2.06	n/a	-1.21	189.13
Edwards	LFR8	0.58	0.93	0.55	-0.79	Fe	1757.50	2.32	7.88	n/a	1757.50	-1.05	-1.89	n/a	-0.79	47.08
Edwards	LFR9	0.61	0.9	0.54	-0.86	Fe	1486.25	2.64	7.73	n/a	1486.25	-1.00	-1.90	n/a	-0.86	8.39

Table S3. Grouping of taxa according to their traits "physiological sensitivity" (S-value) and "feeding type" (Predator). Predator category 1 refers to predatory feeding type, while Predator=0 refers to non-predatory feeding types.

Class	Order	Family	Species	Community S_{metal}	Predator
Annelida	Clitellata - Hirudinea	Glossiphoniidae	*Glossiphonia tasmaniensis*	0.003	1
	Haplotaxida	Megascolecidae	*Megodrilus sp.*	0.302	0
			Telmatodrilus multiprostatus	0.302	0
		Phreodrilidae	*Phreodrilus nudus*	0.302	0
	Lumbriculida	Lumbriculidae	*Lumbriculus variegatus*	-0.283	0
	Oligochaeta	Naididae	*Pristina proboscidea*	0.646	0
		Tubificidae	*Tubifex tubifex*	-0.295	0
			Limnodrilus hoffmeisteri	0.108	0
				0.302	0
Turbellaria	Tricladida	Dugesiidae	*Cura pinguins*	0.038	1
Bivalvia	Heterodonta/ Veneroida	Sphaeriidae	*Sphaerium tasmanicum*	-0.280	0
			Pisidium casertanum	-0.280	0
	Unionoida	Hydriidae		-0.405	0
Clitellata - Hirudinea	Arhynchobdellida	Richardsonianidae		0.003	1
Crustacea	Amphipoda	Ceinidae	*Austrochiltonia australis*	-0.451	0
	Decapoda	Atyidae	*Paratya australiensis*	-0.821	0
				0.4138	0
		Palaemonidae		-0.251	1
		Sundatelphusidae		0.133	1
	Isopoda	Phreatoicoidae	*Colubotelson sp.*	0.908	0
Gastropoda	Basommatophora	Planorbidae	*Physastra gibbosa*	0.757	0
		Ancylidae	*Ferrissia tasmanica*	0.602	0
			Ferrissia petterdi	0.602	0
				0.45	0
		Planorbidae	*Isidorella hainesii*	0.757	0
			Gyraulus tasmanicus	0.757	0
		Ancylidae		0.450	0
	Neotaenioglossa	Hydrobiidae	*Rivisessor gunnii*	0.613	0
			Potomopyrgus niger	0.613	0
Insecta	Diptera	Ceratopogonidae	*Nilobezzia sp*	0.145	1
		Chaoboridae		0.520	1
		Chironomidae	*Eukiefferiella sp.*	0.561	0
			Cardiocladius sp.	0.561	0
		Chironomidae - Chironominae	*Dicrotendipes sp.*	0.561	0
			Stempellina sp.	0.561	0
			Stenochironomus sp.	0.561	0
			Rheotanytarsus sp.	0.561	0
			Microspectra sp.	0.561	0

	Chironomidae - Orthocladiinae	*Cricotopus sp.*	0.561	0
		Cricotopus albitibia	0.561	0
	Chironomidae - Tanypodinae	*Procladius sp.*	0.561	1
		Coelopynia pruinosa	0.561	1
		Ablabesmyia notabilis	0.561	1
	Culicidae		1.088	1
	Dolichopodidae		0.520	1
	Empididae		0.520	1
	Muscidae	*Limnophora sp.*	0.520	1
	Simulidae	*Austrosimulium sp.*	0.520	0
	Tanytarsini	*Microspectra sp.*	0.561	0
	Tipulidae		0.520	0
Ephemeroptera	Baetidae	*Baetis baddamsae*	-0.059	0
		Tasmanophlebia lacustris	-0.059	0
		Baetis sp.	-0.059	0
	Caenidae	*Tasmanocoenis sp.*	0.303	0
	Leptophlebiidae	*Atalophlebia australis*	0.303	0
		Atalophlebia nr longicaudata	0.303	0
		Atalophlebioides sp.	0.303	0
		Atalophlebioides sp.	0.303	0
		Atalonella sp.	0.303	0
		Atalophlebia sp.	0.303	0
Hemiptera/ Heteroptera	Corixidae	*Micronecta sp.*	0.875	1
		Micronecta annae	0.875	1
	Mesovelidae		0.039	1
	Nepidae		-0.123	1
	Notonectidae	*Enithares woodwardi*	0.039	1
		Anisops doris	0.039	1
	Velidae		0.039	1
Megaloptera	Sialidae	*Austrosialis sp.*	2.673	1
Odonata - Anisoptera	Aeschnidae	*Aeschna longissima*	-0.348	1
Odonata - Anisoptera	Gomphidae	*Austrogomphus guerini*	-0.348	1
Odonata - Anisoptera	Libellulidae		-0.348	1
Odonata - Zygoptera	Coenagrionidae	*Ishnura sp.*	1.284	1
	Isosticidae		1.707	1
	Megapodagrionidae	*Argiolestes sp.*	1.707	1
	Protoneuridae		1.707	1
Plecoptera	Gripopterygidae	*Leptoperla varia*	1.395	0
		Dinotoperla sericauda	1.395	0
		Cardioperla nigrifons	1.395	0
		Riekoperla sp.	1.395	0
	Notonemouridae	*Tasmanocerca bifasciata*	1.395	1
		Austrocercella tillyardi	1.395	1
Trichoptera	Calamoceratidae	*Anisocentropus latifascia*	1.968	0
	Conoesucidae		1.968	0

Ecmonidae	*Ecnomus sp.*	1.968	1
Glossosomatidae	*Agapetus sp.*	1.968	0
Helicopsychidae	*Helicopsyche murrumba*	1.968	0
Hydropsychidae	*Cheumatopsyche sp.*	2.053	0
	Asmicridea sp.	2.053	0
Hydroptilidae	*Helyethira sp.*	1.968	0
Leptoceridae	*Oecetis sp.*	1.968	1
	Oecetis sp.	1.968	1
	Notalina sp.	1.968	1
	Notalina filva	1.968	1
	Triplectides sp.	1.968	1
Odontoceridae	*Atriplectides dubia*	1.968	0
Philorheithridae	*Aphilorheithrus sp.*	1.968	1
Polycentropodidae		1.968	1
Rhyacophilidae	*Taschorema ferulum*	1.869	1
	Apsilochorema sp.	1.869	1
Tasimiidae		1.968	0

Table S4. Pearson's correlation of SPEcies At Risk (reference) from metals ($SPEAR_{metal}$), being the Predator ratio and heavy metal contamination given by the four indices mean TU (=mean of the TU values across all metal measured at that site within this study), sum TU (=sum of all TU values across all metals measured), mean of the 3 highest TU (=mean of the TU across the 3 metals with the highest TU values), and highest TU (=maximum of the TU value across all metal measured).

$SPEAR_{metal}$ ($predator_{ratio}$) vs. measures of TU	*r*	r^2	*p*
Norris, 1980			
Mean TU	0.848	0.719	0.008
Sum TU	0.848	0,719	0.008
Mean of the 3 highest TU	0.862	0.743	0.006
Highest TU	0.817	0.667	0.013
Norris 86			
Mean TU	0.926	0.858	< 0.001
Sum TU	0.926	0.858	< 0.001
Mean of the 3 highest TU	0.937	0.879	< 0.001
Highest TU	0.952	0.906	< 0.001
Edwards, 1994			
Mean TU	0.666	0.444	0.004
Sum TU	0.666	0.444	0.004
Mean of the 3 highest TU	0.684	0.468	0.002
Highest TU	0.705	0.497	0.002
all			
Mean TU	0.535	0.286	< 0.001
Sum TU	0.325	0.106	0.057
Mean of the 3 highest TU	0.719	0.516	< 0.001
Highest TU	0.786	0.617	< 0.001

Table S5. Pearson's correlation of biological and environmental parameters and heavy metal contamination given by the highest TU.

Biological and environmental parameters vs. Highest TU	***r***	***r²***	***p***
$SPEAR_{metal}$ ($predator_{ratio}$)	-0.795	0.63	< 0.001
TR	-0.492	0.242	0.003
EPT	-0.382	0.146	0.024
Alkalinity	-0.007	0.0005	0.97
Conductivity	0.685	0.469	< 0.001
pH	-0.618	0.382	< 0.001
Carbonate	-0.36	0.129	0.065
Magnesium	0.64	0.41	< 0.001
Calcium	0.776	0.602	< 0.001
Sulphate	0.66	0.44	< 0.001

Table S6. Summary of the principle components analysis (see Figure 1 for the ordination plot). The first four ordination axes were interpretable according to the Kaiser-Guttman criterion and explained 86% of the total variance.

PCA	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	6.925	3.668	1.77	1.145
Proportion Explained	0.433	0.229	0.111	0.088
Cumulative Proportion	0.433	0.662	0.773	0.861
Sum of all eigenvalues	16			

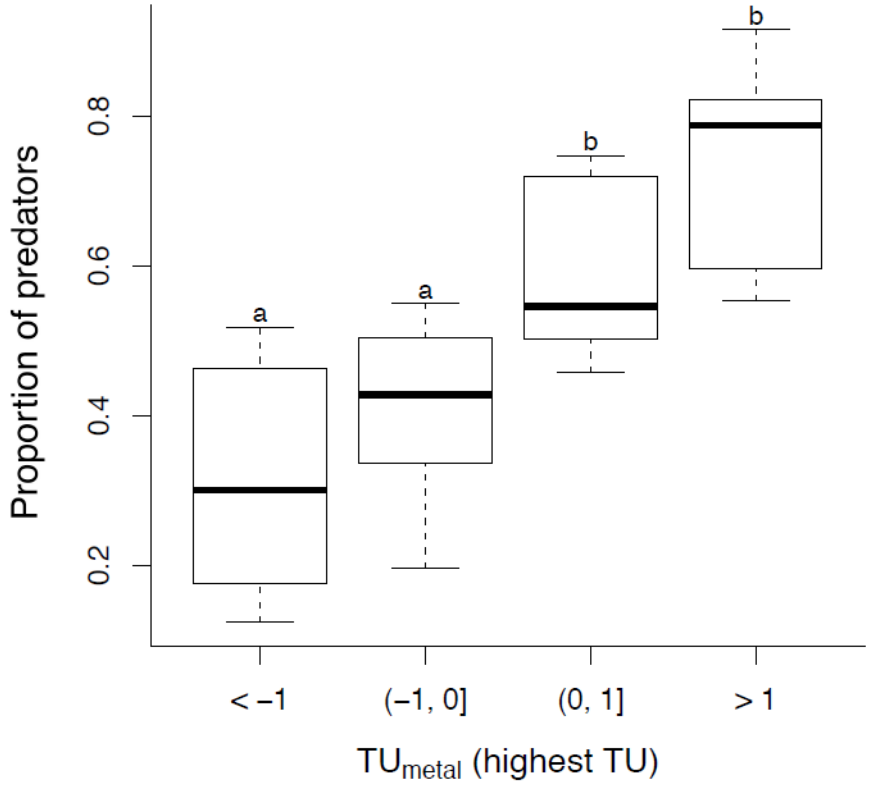

Figure S1. Proportion of predators in the total community compared between four groups of metal contamination: Sites with low TUs (<-1), TUs between -1 and 0, TUs between 0 and 1, and TUs >1. ANOVA ($p<0.001$) followed by Tukey's post hoc test. The post hoc test revealed significant differences between low impact sites (<-1) and sites with a TU of 0 to 1 and >1 ($p<0.01$ and $p<0.001$, respectively) (denoted as distinct groups a and b). The difference between the groups <-1 and -1 to 0 was not significant ($p=0.49$) (denoted as equal group a), nor the difference between groups 0 to 1 and >1 ($p=0.23$) (denoted as equal group b)

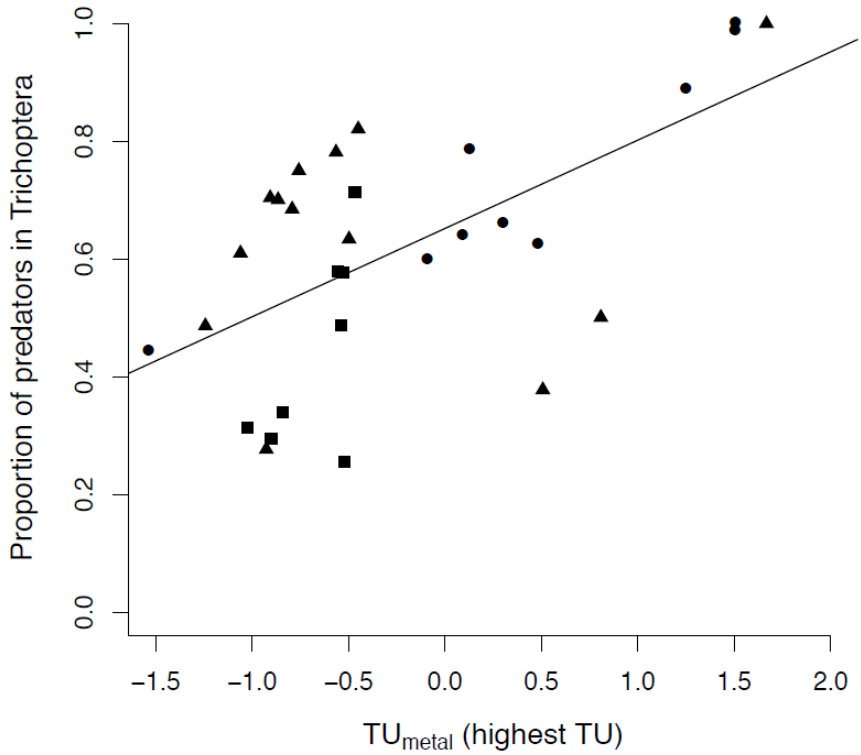

Figure S2. Linear regression of the maximum TU in water and the proportion of predators within the order Trichoptera (r2=0.38 and p<0.001). The data originated from 3 independent investigations as indicated by the data points (squares: Norris et al., 1980; circles Norris, 1986; and triangles Edwards, 2002).

Acknowledgements

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Author's Contributions

Title:	How to characterize chemical exposure to predict ecologic effects on aquatic communities?
Authors:	Schäfer, R.B., Gerner, N., Kefford, B.J., Rasmussen, J.J., Beketov, M.A., de Zwart, D., Liess, M., von der Ohe, P.C.
Status:	Published in 2013 in Environmental Science & Technology 47, pp. 7996-8004.
Contribution:	Study design: RBS (100%); provision of data: RBS (35%), PCO (35%), ML (20%), JJR (10%); calculation of exposure metrics: NVG (70%), PCO (20%), RBS (5%), DdZ (5%); data analysis: RBS (100%); discussion and interpretation of results: all (NVG 20%); drafting of manuscript: RBS (80%), BJK (10%), MB (10%); revising manuscript: all (NVG 20%).

Title:	Stream invertebrate community structure at Canadian oil sands development is linked to concentration of bitumen-derived contaminants
Authors:	Gerner, N.V., Koné, M., Ross, M.S., Pereira, A., Ulrich, A.C., Martin, J.W., Liess, M.
Status:	Published in 2017 in Science of the Total Environment 575, pp. 1005-1013.
Contribution:	Study design: ML (60%), NVG (40%); provision of data: NVG (50%), MK (20%), MSR (10%), AP (10%), ACU (5%), JWM (5%); calculation of exposure and effect metrics: NVG (100%); data analysis: NVG (80%), MK (20%); discussion and interpretation of results: NVG (50%), ML (30%), MK (10%), JWM (10%); drafting of manuscript: NVG (100%); revising manuscript: all (NVG 80%).

Title:	Sensitivity ranking for freshwater invertebrates towards hydrocarbon contaminants
Authors:	Gerner, N.V., Cailleaud, K., Bassères, A., Liess, M., Beketov, M.A.
Status:	Published in 2017 in Ecotoxicology 26(9), pp. 1216-1226.
Contribution:	Study design: NVG (30%), KC (30%), MAB (20%), AB (10%), ML (10%); provision of data: NVG (60%), KC (20%), MAB (20%); calculation of sensitivity values: NVG (100%); data analysis: NVG (40%), MAB (40%), KC (10%), AB (10%); discussion and interpretation of results: NVG (40%), MAB (20%), KC (20%), ML (10%), AB (10%); drafting of manuscript: NVG (50%), MAB (50%); revising manuscript: NVG (70%), MAB (10%), ML (10%), KC (10%).

Title:	Metal toxicity affects predatory stream invertebrates less than other functional feeding groups
Authors:	Liess, M., Gerner, N.V., Kefford, B.J.
Status:	Published in 2017 in Environmental Pollution 227, pp. 505-512.
Contribution:	Study design: ML (50%), NVG (25%), BJK (25%); provision of data: BJK (70%), NVG (30%); calculation of sensitivity values: NVG (100%); data analysis: NVG (100%); discussion and interpretation of results: ML 50%, BJK 30%, NVG 20%; drafting of manuscript: ML 70%, NVG 30%; revising manuscript: all (NVG 33%).

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Research experiences

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Education

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Oct 2008–Aug 2009	Diploma thesis at the IFM-Geomar in Kiel and the Leigh Marine Laboratory of the University of Auckland, New Zealand *Title: "Does stress tolerance differ between indigenous and non-indigenous marine species and between populations from pristine and anthropogenically impacted habitats?"*
2004-2009	Biology (diploma), Goethe-University Frankfurt/ Main, *main subjects: Microbiology, Ecology and evolution of plants and fungi, Ecology and evolution of animals*
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Scholarships

2008	Stipend of the Vereinigung von Freunden und Förderern der Universität Frankfurt for diploma thesis in New Zealand
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Publications

Gerner, N. V., Nafo, I., Winking, C., Wencki, K., Strehl, C., Wortberg, T., Niemann, A., Anzaldua, G., Lago, M., Birk, S., Submitted to Ecosystem Services. Large-scale river restoration pays off: A case study of ecosystem service valuation for the Emscher restoration generation project.

Anzaldua, G., Gerner, N. V., Lago, M., Abhold, K., Hinzmann, M., Beyer, S., Winking, C., Riegels, N., Krogsgaard Jensen, J., Termes, M., Amorós, J., Wencki, K., Strehl, C., Ugarelli, R., Hasenheit, M., Nafo, I., Hernandez, M., Vilanova, E., Damman, S., Brouwer, S., Rouillard, J., Schwesig, D., Birk, S., accepted. Getting into the water with the Ecosystem Services Approach: the DESSIN ESS Evaluation Framework. Ecosystem Services.

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Eigenständigkeitserklärung

Hiermit bestätige ich,

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Nadine Vanessa Gerner

Düsseldorf, 09.01.2018