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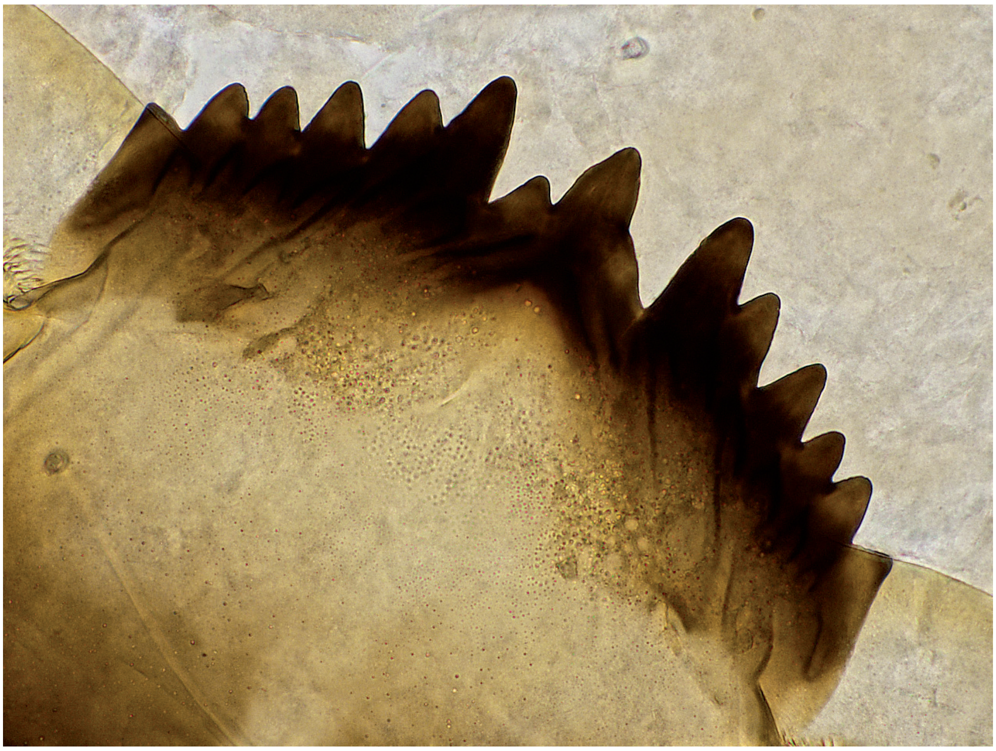
309

Johanna Salmelin

New applications of zoobenthos measurements for risk assessment of chemicals in aquatic ecosystems



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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2015

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ABSTRACT

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Benthic animals in aquatic ecosystems suffer from various anthropogenic pollutants. A challenge in risk assessment of chemicals in aquatic ecosystems (i.e. ecological risk assessment) has been to balance cost-efficiency and effectiveness in detecting early warning signals of chemicals. In this study, hyperspectral imaging and behavioural measurements were evaluated as novel applications for detecting chemical effects on benthic animals, and the reliability of the incidence of chironomid morphological deformities as a more traditional bioindicator of sediment ecotoxicity was assessed. Chironomid deformity experts (25) analysed an identical set of samples for deformities. Deformity assessments were highly subjective and inconsistent. Apparently, guidelines and criteria are needed to improve and ascertain the reliability of deformity analysis. Hyperspectral imaging was tested as a novel method to detect metal contamination of benthic macroinvertebrates using cadmium (Cd) as a model metal. No effects of Cd were observed on traditional toxicity endpoints, survival and number of morphological gill abnormalities in caddisfly larvae. In contrast, hyperspectral features revealed a weak association with Cd concentration indicating darkening of larval soft tissues at high Cd. Further development and testing of this method and of the imaging process in particular, are needed. Another novel method was based on a quadrupole impedance conversion-technique to record behavioural responses of animals. A Multispecies Freshwater Biomonitor (MFB), which utilizes this technique, enabled quantitative behavioural measurements of benthic larvae in sediment and *in situ* stream water exposures. MFB was found applicable for recording different behaviours, locomotion and ventilation of chironomid, mayfly and lamprey larvae, although no consistent behavioural responses in contaminated sediments or mine-impacted streams were detected.

Keywords: Behaviour; benthic macroinvertebrates; hyperspectral imaging; interrater reliability; morphological deformities.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

I have contributed significantly to planning, data collection, analyses and writing of each paper. I planned the studies with HH and K-MV (I, II, III, IV), together with AKK (II, III, IV) and MTL (III, IV). Hyperspectral data analysis was conducted by IP and H-HP (II). AV was responsible for cadmium analysis (II), and HK for analysis of PCDD/Fs and PCBs (III). Survival *in situ* was carried out by MTL and AKK, and MTL analysed the survival data (IV). All papers were finalised with co-authors.

I Salmelin J., Vuori K.-M. & Hämäläinen H. 2015. Inconsistency in the analysis of morphological deformities in Chironomidae (Insecta: Diptera) larvae. *Environmental Toxicology and Chemistry* 34: 1891–1898.

II Salmelin J., Pölönen I., Puupponen H.-H., Hämäläinen H., Karjalainen A.K., Väisänen A. & Vuori K.-M. 2015. Hyperspectral imaging of macroinvertebrates - a pilot study of a novel technique for detecting metal contamination in aquatic ecosystems. Manuscript.

III Salmelin J., Karjalainen A.K., Hämäläinen H., Leppänen M.T., Kiviranta H., Kukkonen J.V.K. & Vuori K.-M. 2015. Biological responses of midge (*Chironomus riparius*) and lamprey (*Lampetra fluviatilis*) larvae in ecotoxicity assessment of PCDD/F, PCB and Hg contaminated river sediments. Manuscript.

IV Salmelin J., Leppänen M.T., Karjalainen A.K., Vuori K.-M., Gerhardt A. & Hämäläinen H. 2015. Assessing ecotoxicity of biomining effluents in stream ecosystems by *in situ* invertebrate bioassays: a case study in Talvivaara, Finland. Submitted manuscript.

1 INTRODUCTION

1.1 Ecological risk assessment

Ecological risk assessment (ERA) is defined as the process that evaluates the likelihood of adverse ecological effects, which may occur as a result of exposure to one or more stressors (Anon. 1992). Chemicals are an example of anthropogenic stressors with potential adverse effects on the environment. ERA can provide information to identify environmental problems and aid decision making in environmental management and regulation. One aim of environmental policy is to protect both the environment and human health from the risks posed by chemicals, based on national and EU legislation. Hazard is usually defined as a situation with potential to cause harm, and risk is accordingly defined as the combination of the probability of occurrence of a defined hazard and the magnitude of the consequences of the occurrence (Fairman *et al.* 1998). ERA involves several steps from problem formulation to analysis and risk characterization (Anon. 1998). The analysis phase includes 2 steps: exposure assessment and effect assessment. In exposure assessment the goal is to evaluate exposure sources of contaminants and their distribution in environmental compartments, together with the evaluation of potentially affected species and exposure routes. However, chemical concentrations in water or sediment of aquatic ecosystems do not demonstrate effects on biota, so effect assessment is also needed. Effect assessment evaluates ecological effects and concentration-response relationships based on ecotoxicity testing in the laboratory or in the field. ERA is related to the risk assessment of human health, e.g. via toxic chemicals that accumulate into biota and biomagnify along food webs up to human consumers. Examples of these chemicals include lipophilic polychlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), as well as some metals like mercury (Hg) (Pereira 2004, Karjalainen *et al.* 2012, Lehnherr 2014).

Bioindicators at different levels of biological organizations (individual, population, community or ecosystem level) are used in ERA to assess ecological and ecotoxicological effects (Bartell 2006). Ecological effects of chemical stressors at the population and community level may be assessed using species diversity and other community structure metrics. Ecotoxicological indicators at the individual level may utilize measures of behavioural and morphological responses. Traditional ecotoxicity endpoints of aquatic macroinvertebrates are related to growth, survival, immobilization, development, emergence, and reproduction. According to Burger and Gochfeld (2001), the characteristics of a good bioindicator can be categorized into biological, methodological and societal relevance. A biologically relevant bioindicator is sensitive, specific, and has low natural variability. It should be measurable, and be related to biologically important changes. A methodologically relevant bioindicator is common and easy to monitor, has clear-cut objectives, clear-cut data gathering and analysis phases, and allows hypothesis-testing. A socially relevant bioindicator should take into account animal-welfare issues, and be easily understood, scientifically defensible, and cost-effective.

1.2 Morphological responses and their measurement

Anthropogenic chemicals as environmental stressors can affect organisms by inducing morphological deformities, which can be used as bioindicators of exposure, effect or both. Sublethal stress caused by environmental contamination can increase the incidence of structural deformities in aquatic insects, gastropods and fishes (Conroy *et al.* 1996, Carls *et al.* 1999). One commonly used indicator of sediment toxicity is deformities in the head capsule structures of Chironomidae (Insecta: Diptera) larvae (Warwick and Tisdale 1988). In addition, gill and anal papillae damage in caddisfly larvae (Insecta: Trichoptera: Hydropsychidae) have been used to indicate metal pollution and organic chemical contamination of freshwaters (Camargo 1991, Vuori 1994, Vuori and Parkko 1996, Leslie *et al.* 1999, Vuori and Kukkonen 2002, Ratia *et al.* 2012).

Hydropsychid larvae have 17–19 abdominal gill tufts depending on the species. Normal, undamaged gills are whitish and branching. According to Ratia *et al.* (2012), a gill tuft can be considered damaged if it is totally reduced or its basal or distal parts darkened, or if the gill tuft has dark spots on > 50 % of its branches. However, some studies consider only totally reduced gill tufts as damaged (Vuori 1994, Vuori and Kukkonen 2002). Also darkening of the ionoregulatory organs (anal papillae) of hydropsychid larvae has been observed as a response to metal exposure (Vuori 1994).

Chironomid larval deformities may occur in various head capsule structures like antennae, mandibles, premandibles, epipharyngeal pecten and labral lamellae (Warwick and Tisdale 1988). However, deformities of the mentum are most often and most easily examined. The mentum of *Chironomus* spp. is a double-walled labial plate with 13 sclerotized teeth located on the ventral side of the head capsule, posterior to the mouthparts. Warwick and

Tisdale (1988) have described mentum aberrations that are typically referred to as deformities including missing, additional, split and asymmetrical teeth, deep smooth-edged indentations called Köhn gaps, and a large deviation from the normal tooth configuration. Researchers apply variable evaluation criteria for mentum deformities. For instance, a split median tooth is counted as a deformity in some studies (Dias *et al.* 2008, Langer-Jaesrich *et al.* 2010a), but not in other studies (Arambourou *et al.* 2013). Similarly, mechanical aberrations of mentum teeth, such as breakage and wear, can be considered either as deformities (Gagliardi and Pettigrove 2013), or be excluded from the deformity analysis (Swansburg *et al.* 2002, Ochieng *et al.* 2008).

Chironomid larval deformities have been widely studied as a potential early warning indicator of pollution since the study of Hamilton and Sæther (1971). Larval developmental processes during larval ontogeny are suggested to be disrupted by xenobiotic chemicals, and an increase of deformities between moults as larvae grow has been observed (Vermeulen *et al.* 2000a). Contamination of aquatic environments by metals (Swansburg *et al.* 2002, Ilyashuk *et al.* 2003, Martinez *et al.* 2006, Di Veroli *et al.* 2012), endocrine-disrupting chemicals (Meregalli *et al.* 2001), and complex mixtures of xenobiotic substances (Hudson and Ciborowski 1996, Planelló *et al.* 2015) have been associated with the increased incidence of mentum deformities. Some studies, however, have indicated contradictory results with no effects of endocrine disruptors or metals on *Chironomus* spp. mentum deformity rate, including laboratory bioassays by Vermeulen *et al.* (2000b), Langer-Jaesrich *et al.* (2010a) and Arambourou *et al.* (2013).

The deformity response of chironomid larvae has been measured as the frequency of deformed individuals within a population (deformity incidence, DI), which is reported to be an adequate measure (Hämäläinen 1999) although there are several other indices which take into account the severity of deformities. Correspondingly the hydropsychid abnormality incidence describes the proportion of individuals with abnormalities (Vuori and Kukkonen 2002), whereas the hydropsychid gill abnormality index (HYI) represents the severity of damage within the population, described by the average number of abnormal tracheal gill tufts for all individuals. The latter is considered to be the more informative bioindicator of Cd induced stress (Vuori and Kukkonen 2002). Assessment of both chironomid mentum deformities and hydropsychid gill damage is potentially prone to subjectivity since they require expert interpretation. Lack of clear guidelines to assess deformities, and the broad definition of a deformity in chironomid larvae as “any morphological feature that departs from the normal configuration” (Warwick 1988, Vermeulen 1995), certainly invite subjectivity in assessments. Reliability in the context of inter-rater agreement studies refers to the degree to which different experts or raters agree in their evaluations (Uebersax 1988), and reliable results are achieved when several raters independently make similar assessments or judgements on an identical set of data.

1.3 Behavioural responses and their measurement

Behaviour can be defined as "the physical manifestation of the animal's internal neuronal, metabolic and endocrine processes, and at the same time the integrated physiological response to its environment" (Clotfelter *et al.* 2004, Baatrup 2009). Anthropogenic chemicals can affect the nervous and hormonal systems of animals, which regulate animal behaviour (Adkins-Regan and Weber 2002, Dell'Omo 2002, Clotfelter *et al.* 2004). Behavioural endpoints indicate an integrated physiological stress response of an animal to its external environment, when chemical-induced endocrine or nervous system impairment changes behaviour. Behavioural changes may result from direct toxicity and failure of adaptive mechanisms, or from adaptive responses to mitigate the effects of chemical exposure (Dell'Omo 2002). Behavioural stress responses of animals can be sensitive and rapid indicators of changes in the environment (Maradona *et al.* 2012, Melvin and Wilson 2013), since they may be detected earlier than changes in development or survival (De Lange *et al.* 2006, Gerhardt 2009, Denoël *et al*. 2013). Behavioural endpoints have potential as early-warning indicators of adverse impacts of chemicals on ecosystems. Behavioural changes may be linked to the changes at the population, community or ecosystem level if species interactions or metabolism of individuals are affected. Aquatic toxicity studies with behavioural endpoints have gained increased interest (e.g. Kirkpatrick *et al.* 2006, Sardo and Soares 2010, Bossus *et al.* 2014, Leonard *et al.* 2014), since they are considered integrative measures of toxicity also at low and varying chemical concentrations, and potentially offer a cost-effective way to detect toxicity and complete exposure evaluation in ecological risk assessment. In spite of increased interest in using behavioural endpoints in ecological risk assessment, there are currently no standardized test species or test protocols to be applied in behavioural aquatic toxicity testing.

Behavioural aquatic ecotoxicology studies have been focused on measuring basic behavioural phenomena, such as animal locomotor behaviour, which are simple and quantifiable (Bailey 2002). Measurement of more complex behaviour, such as swimming navigation or competition behaviour, often involves reducing behaviour to several quantifiable variables, such as swim path length or number of encounters (Lipp 2002). Examples of behavioural endpoints of aquatic invertebrates used in ecotoxicological studies include swimming activity, burrowing response, valve closure of molluscs, feeding rate, oviposition, predator-prey interactions and phototaxis (Boyd *et al.* 2002, Steele 2013).

Traditional behavioural studies have utilized visual observations to quantify behavioural responses of animals (Bae and Park 2014), but behavioural studies are prone to observer bias, and expectations of the observer have been shown to influence scoring results (Tuyttens *et al.* 2014). New unbiased methods based on computerized vision systems have been developed to replace visual observation, for example to measure complex fish courtship behaviour without human interpretation (Baatrup 2009). Other advanced techniques to measure behaviour include methods based on video-tracking and non-visual quadrupole impedance conversion technique (Gerhardt 2001, Bae and Park 2014).

1.4 Aims of the study

The main focus of this thesis was on developing new applications that might be used for effect characterization in ecological risk assessment. The aim was to test novel techniques like quadrupole impedance conversion and hyperspectral imaging to detect potential adverse effects of contaminated sediments or water on benthic animal behaviour and morphology. These novel applications potentially offer sensitive and cost-effective tools for ecological risk assessment. The reliability of one commonly used bioindicator of sediment toxicity, the deformity incidence in chironomid headcapsule mouthparts, was also evaluated. The objectives were:

1. To evaluate the interrater reliability and potential biases in chironomid deformity assessment (I).
2. To develop a novel application of hyperspectral imaging for detecting effects of metal pollution on benthic invertebrates (II). Specific aims were to study if hyperspectral imaging can be used to differentiate between metal contaminated and non-contaminated caddisfly larvae, and to study if metal body burden in larvae can be predicted from hyperspectral data.
3. To evaluate applicability of a new method utilizing a quadrupole impedance conversion technique to quantify behavioural responses of benthic animals in sediment in the laboratory exposures (III) and *in situ* (IV) exposures. More specifically, the aim was to assess potential ecotoxicity of multi-contaminated river sediments via biological responses of sediment-dwelling chironomid and lamprey larvae (III). Impacts of biomining effluents on water quality and aquatic invertebrates were assessed in streams using *in situ* exposures with survival and behavioural endpoints (IV).

2 MATERIALS AND METHODS

2.1 Novel applications

The extended depth of focus (EDF) technique combines microscope pictures taken at different focus levels (Z-series) into one image, and was utilized to prepare the material for the inter-rater reliability study (I). The test material consisted of microscope EDF images of menta of 211 *Chironomus* spp. larvae collected from the field and fixed on microscope slides with polyvinyl-lactophenol. The EDF technique provides images with high clarity, and e.g., also the lateral parts of the convex mentum of chironomid larvae are clearly visible, which is not possible with conventional microscope photographs. A digital camera (Nikon DS) connected to an optical microscope (Olympus BX41 System Microscope) was used to take the images. In total 3–13 images from different focus levels of each mentum were combined into one image with the laboratory image analysis software (NIS-Elements D 4.11.01 64-bit), and saved as TIFF (Tagged Image File Format) images, which were shared with the participants in the inter-rater reliability test.

Behavioural measurements of benthic animals were performed using a Multispecies Freshwater Biomonitor©-device, MFB (III, IV). MFB quantitatively records the behavioural patterns of an individual by a non-visual quadrupole impedance conversion technique (Gerhardt *et al.* 1998, Gerhardt 2001). Each MFB-test chamber has one pair of electrodes creating a weak electrical field of high-frequency alternating current within a chamber, and another pair of electrodes functioning as an impedance sensor, which measures the current changes due to the animal movements inside the test chamber (Fig. 1). The behavioural signals within the frequency range of 0.5–8.5 Hz are analysed via a stepwise discrete Fast Fourier Transform (FFT), an algorithm converting the original periodic sinusoidal signal into its component frequencies. The analysis yields a percentage of time (%) an animal is spending on movements with frequencies that can be associated with certain behavioural patterns like ventilation or locomotion. A calibration of the respective frequencies and a typical behaviour was done beforehand in the laboratory by simultaneous recording in the MFB oscilloscope and visual observation of larval locomotion such as crawling and swimming, and of ventilation. The MFB was operated in the field with a 12 V battery (IV). Recordings with empty chambers *in situ* before the recordings of the animals proved that there were no extraneous disturbances, for example from turbulence in the water (IV). The noise level was similarly adjusted also in the laboratory study (III). One larva was placed in each test chamber, and the chamber ends were sealed with a lid with a mesh size of 0.5 mm to prevent larvae from escaping while allowing water renewal inside the chambers. Test chambers were filled with 1 part sediment and 4 parts artificial freshwater, and were placed into a large glass beaker with artificial freshwater (III), or placed *in situ* into the stream so that test chambers were filled with stream water (IV). Larvae were acclimated in the test chambers for 30 min, and their behaviour was then recorded for 2 (III) or 3 h (IV) for 4 min at 10 min intervals, so that altogether 12 or 18 repeated measurements were obtained from each individual. Behaviour of mayfly larvae was measured after 3-h exposure *in situ* in 6 streams in September 2012 (IV). Behaviour of 4th instar chironomid larvae was measured once after the 10-d sediment exposure in the laboratory, and behaviour of lamprey larvae 5 times during the 28-d sediment exposure: at days 0, 3, 7, 14 and 28 (III).

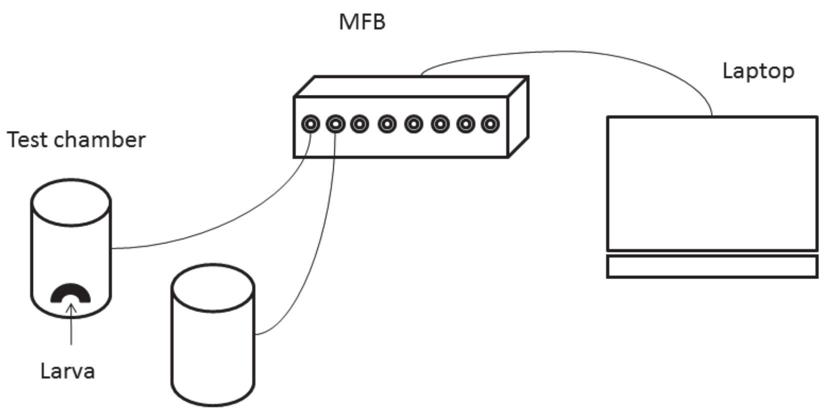

FIGURE 1 Multispecies Freshwater Biomonitor, MFB, for the behavioural measurements included 8 test chambers with an individual larva placed in each chamber, MFB-device, and the laptop.

Hyperspectral imaging (HSI) is spectroscopy coupled with imaging (II). In spectral analysis the goal is to recognize substances based on their spectral properties, since a given substance often has a unique spectral signature due to the differences between substances in reflecting and absorbing different wavelengths of light. HSI produces an image of pixel spectra, so that the entire spectrum is acquired at each pixel, which results in a 3-dimensional data cube with the reflectance spectrum forming the 3rd dimension. The technique can reveal changes which might be unseen by the human eye since the spectral analysis operates also in the wavelengths of infrared light. A wide range of HSI applications has already been developed in geology, mineralogy, agriculture and the steel industry (e.g. Riaza *et al.* 2011, Antonucci *et al.* 2012). The main instrument used in HSI in this thesis was a compact and lightweight hyperspectral imager operating with wavebands between 500 and 850 nm, developed by VTT Technical Research Centre of Finland. Spectral separation in this device was based on the piezo-actuated Fabry-Perot interferometer (II). HSI was mounted on a stand (Fig. 2 in II) and a macro objective used for sufficient magnification. Illumination was provided from both sides using 200 W broadband halogen lights. After 4 d exposure to Cd, each larva was prepared and mounted in a Petri dish with insect pins prior to imaging. The dish was on a diffuse grey surface and filled with water sufficient to fully immerse each larva. A workflow for HSI data processing included six steps: 1) data normalization to reflectance, 2) spectral unmixing to delineate the specimen in the image, 3) gathering of all spectra from the specimen and selection of endmembers, 4) calculation of inversion for selected endmembers using a filter vector algorithm and forming of abundance maps, 5) calculation of statistical features for abundance images, and 6) utilization of a manifold learning approach to classify specimens into different groups (II).

2.2 Chemicals

Experimental waters were spiked with anhydrous cadmium chloride, $CdCl_2$ (Alfa Aesar GmbH & Co KG), in the laboratory exposures with hydropsychid larvae in the hyperspectral imaging study (II). Cd was chosen as a representative model toxicant because it is one of the priority hazardous substances in the US and EU legislations, and it is known to induce morphological abnormalities in hydropsychids (Vuori 1994, Vuori and Kukkonen 2002). Cd has similar properties as zinc (Zn) and Hg, and it is mainly released locally into the environment from mining activity as a by-product from Zn, copper (Cu) and lead (Pb) ore mining (Anon. 2011). Other sources of Cd include industrial production of NiCd-rechargeable batteries, pigments, stabilizers and substances for corrosion prevention, land use for agricultural purposes, and fertilisers. Cd analyses of experimental waters and larval tissue samples (II) were performed in the Department of Chemistry, University of Jyväskylä. The ICP-OES measurements (larval Cd body burden and Cd in water samples of 10 and 100 $\mu g\ l^{-1}$) were performed with a Perkin-Elmer (Norwalk, CT, USA) model Optima 8300 inductively coupled plasma optical emission spectrometry. A Perkin Elmer Model AAnalyst 800 atomic absorption spectrometer with an AS-800 autosampler was used for GFAAS measurements (water samples of 0 and 1 $\mu g\ l^{-1}$).

River sediments that were used in the laboratory sediment bioassay (III) were analysed for PCDD/Fs, PCBs and Hg. 17 PCDD/F and 37 PCB congeners were analysed from frozen sediment and lamprey larvae tissue samples with an accredited method in the National Institute for Health and Welfare. The quantification was performed by gas chromatography with high resolution mass spectrometry (GC-HRMS). $WHO_{PCDD/F+PCB}TEQ_{2005}$, the toxic equivalency according to the World Health Organization (WHO) indicating the overall toxic potency of sediments for mammals, and $WHO_{PCDD/F+PCB}TEQ_{1998}$ indicating the overall toxic potency for fish were calculated (III). TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is one of the most toxic synthetic compounds. Of all the 210 PCDD/F and the 209 PCB congeners, those structurally similar to TCDD (17 PCDD/Fs and 12 dioxin-like PCBs) are considered the most toxic with established toxic equivalency factor (TEF) values. TEF describes their toxic potency in relation to TCDD (Van den Berg *et al.* 2006), and was used in calculations of TEQs. Very high log K_{ow} (octanol-water partitioning coefficient) of PCDD/Fs (4.3–8.0) and PCBs (4.5–8.0) indicate their high accumulation in lipophilic environmental matrices. This high affinity to lipids enables their accumulation into organisms and biomagnification along food webs up to humans (Pereira 2004, Karjalainen *et al.* 2012). The degradation rate of PCDD/Fs and PCBs is low, so these compounds are very persistent in the environment. PCDD/Fs are unintentional by-products from industrial processes and products, in contrast to the large-scale commercial production of PCBs before restrictions on production and use in Europe in the 1980s. Other sources of PCDD/Fs and PCBs include fossil fuel combustion and waste incineration (Pereira 2004).

Total and dissolved metals (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, Ti, U,V, Zn), selenium (Se), sulphur (S), phosphorus (P) and sulphate (SO_4^{2-}) were analysed from the stream waters to characterize water quality of mine-impacted and reference streams at *in situ* exposures (IV). Samples to represent the dissolved metal fraction were filtered (Whatman 25 mm GD/XP syringe filter, pore size 0.45 µm) in the field. Samples were preserved with 65 % suprapur nitric acid (HNO_3), and stored at 4 °C until elemental analysis with ICP-OES, ICP-MS, or Ion Chromatography (SO_4^{2-}) in the accredited Environmental Measurement and Testing Laboratory of the Finnish Environment Institute. Metal concentrations were compared to the national environmental quality standards (EQS) that are set for Cd, Ni and Pb. The site-specific background concentrations were taken into account by adjusting the EQSs for streams situated in the black shales geological region by adding background concentrations to the EQSs as following the practice in Kauppi *et al.* (2013).

2.3 Study species

In this thesis 6 taxa with a benthic larval phase were studied (Table 1).

TABLE 1 Study species, chemical exposure and measured endpoints.

Species	Chemical	Measured endpoints	Paper
Chironomus spp.		Interrater reliability of morphological deformities	I
Hydropsyche pellucidula	[a]Cd	Morphological deformities, survival, body burden, spectral properties	II
Chironomus riparius	[b]PCDD/Fs, PCBs, Hg	Behaviour, survival, growth, morphological deformities	III
Lampetra fluviatilis	[b]PCDD/Fs, PCBs, Hg	Behaviour, survival, growth, body burden	III
Heptagenia dalecarlica	[c]Metals, Se, S, P, SO_4^{2-}	Behaviour, survival	IV
Lumbriculus variegatus	[c]Metals, Se, S, P, SO_4^{2-}	Survival	IV

[a] spiked artificial freshwater
[b] field-collected sediment
[c] stream water

Chironomus spp. (Diptera: Chironomidae) samples used as the test material in the interrater agreement study (I) included field-collected *C. anthracinus*- and *C. plumosus*-type larvae.

5th instar larvae of the caddisfly species *Hydropsyche pellucidula* (Curtis, 1834) (Trichoptera: Hydropsychidae) used in Cd exposures and hyperspectral imaging (II) were collected from unpolluted Siikakoski rapid in the outlet of Lake Konnevesi, Konnevesi, Finland in November 2012. Abundant and widespread *H. pellucidula* is a net-spinning filter feeder, living in fast-flowing sections of rivers with 5 larval instars.

Chironomus riparius Meigen, 1804 (Diptera: Chironomidae) larval behaviour, survival and growth was measured in sediment bioassays (III). The larvae were from the laboratory-reared population from the culture sustained in standardized conditions in Finnish Environment Institute, Jyväskylä, Finland. Culture conditions were as follows: 1.0 mM artificial freshwater, pH 6–8, temperature 20 ± 2 °C, ammonium < 5 mg l^{-1}, oxygen saturation > 60 %, artificial sediment substrate, and 16:8 h light:dark cycle.

Lampetra fluviatilis (Linnaeus, 1758) (Petromyzontiformes: Petromyzontidae), European river lamprey larvae, young-of-the-year, length 17–22 mm, used in sediment bioassays (III) were collected from south-eastern Finland from the River Urpalanjoki, which is not affected by industrial effluents. Older and larger individuals (45 to 130 mm) were collected from R.

Urpalanjoki and R. Kymijoki to evaluate bioaccumulation of PCDD/Fs and PCBs (III). *L. fluviatilis* larvae live inside sediment burrows in slowly flowing parts of rivers as suspension feeders (Mallatt 1982).

Heptagenia dalecarlica Bengtsson, 1912 (Ephemeroptera: Heptageniidae), mayfly larvae, for which survival and behaviour were measured *in situ* (IV), were collected from the River Mustinjoki, Kainuu, Finland. This species occurs in running water sites, chiefly in riffles (Elliot *et al.* 1988) but it can also be found from stony shores of large lakes.

The oligochaete *Lumbriculus variegatus* (Müller, 1774) individuals used for *in situ* survival tests (IV) were from a laboratory-reared population from the culture sustained in standardized conditions in the Finnish Environment Institute, Jyväskylä, Finland. Culture conditions were as follows: 1.0 mM artificial freshwater, pH 6–8, temperature 20 ± 2 °C, ammonium < 5 mg l^{-1}, oxygen saturation > 60 %, soft unbleached paper tissue substrate, and 16:8 h light:dark cycle.

2.4 Study sites

The test material for interrater reliability study (I) consisted of microscope EDF images of *Chironomus* spp. larvae collected in 1996 from Lake Saimaa, south-eastern Finland. Most of the larvae (n = 154) were from a reference site upstream from a local pollution source, a paper and pulp mill. The rest of the larvae (n = 57) were collected from a site immediately downstream from the factory. The sediment of the impacted site was heavily contaminated with Hg, organohalogens and chlorophenolics (site A1 in Soimasuo *et al.* 1988). A possible preconception bias was evaluated by revealing the origins of the larvae, i.e. whether the larvae were from the reference or from the impacted site, to half of the raters. Thus they performed an open assessment, whereas the other half who lacked this information made a blind assessment.

Sediment samples used in sediment bioassays with *C. riparius* and *L. fluviatilis* larvae (III) were collected across a pollution gradient in the River Kymijoki and from the River Urpalanjoki not affected by industrial effluents. R. Urpalanjoki sediments were used as reference sediments in the bioassays. Sediments of R. Kymijoki have been polluted by PCDD/Fs from chlorophenol production (wood preservative Ky5), and by Hg that originated from the chloro-alkali plant and from the use of Hg in slime control in the paper industry (Salo *et al*. 2008, Verta *et al.* 2009). The loading of dioxins and Hg was significantly reduced already in 1970s and 1980s, due to closing of the industrial plants and changes in the industrial processes. Surface sediment samples were collected in 2 replicates from 6 sites, Kuusaansaari (sample codes 1A and 1B), Keltti (2A, 2B), Lopotti (3A, 3B), Koskenalusjärvi (4A, 4B), Ahvionkoski (5A, 5B) and Kyminlinna (6A, 6B) in R. Kymijoki, and from 1 site in R. Urpalanjoki (7A, 7B) in July 2012 (Fig. 1 in III).

River lamprey larvae for tissue residue analysis (III) were present in and collected from the lowest downstream sites of the R. Kymijoki (sites 5 and 6), and R. Urpalanjoki.

Behaviour and survival of mayfly larvae, *H. dalecarlica* and survival of the oligochaete *L. variegatus* (IV), were measured *in situ* at 6 streams (Fig. 1 in IV): in 3 sites receiving runoff from the Talvivaara multimetal mine (R. Kalliojoki, R. Tuhkajoki and R. Lumijoki), and 3 spatially matched reference sites streams not receiving mine effluents (R. Aittopuro, R. Kohisevanpuro and R. Joutenjoki).

Mine-impacted and reference streams were paired according to their catchment areas and watersheds as follows: i) R. Kalliojoki – R. Aittopuro, ii) R. Tuhkajoki – R. Kohisevanpuro, and iii) R. Lumijoki – R. Joutenjoki. Mine-impacted R. Lumijoki and the reference R. Joutenjoki are situated in the Vuoksi watershed, and the other streams in the Oulujoki watershed. R. Kalliojoki and R. Tuhkajoki belong to the same watercourse forming a gradient with R. Tuhkajoki and being more distant from the mine area. R. Kalliojoki and R. Aittopuro differ from the other streams in having catchment areas in the metalliferous black shales region with high background metal concentrations in the bedrock (Loukola-Ruskeeniemi *et al.* 1998, Gustavsson *et al.* 2011). These black shales are rich in Ni, Zn and Cu with naturally acidic and metal-rich surface water runoff, especially if the bedrock is exposed and in contact with water. Talvivaara Mining Company Plc, currently owned by the state of Finland via Terrafame Ltd, situated in Sotkamo, Finland, started biomining activities in 2008 with the main products of Ni and Zn. The construction of the mine continued and metal production was intermittent due to technical problems during 2008 and 2009 (Anon. 2013), but in 2010 and 2011 the production was running throughout the year. The mine applied a novel method in Finland and in Europe, the bioheapleaching technique, to extract Ni, Zn, Cu, Co and U from the low-grade ore (Riekkola-Vanhanen 2013).

2.5 Statistical analyses

Several different statistical analyses were used in the study (Table 2).

TABLE 2 The statistical analyses used in the study.

Statistical analysis	Paper
Cohen's kappa	I
Mann-Whitney U-test	I, III, IV
X^2-test	I
Odds ratio	I
One-way ANOVA	II, IV
Pearson correlation	III, IV
Spearman correlation	II, III, IV
GLM, general linear model	III
Kruskal-Wallis H-test	III
Friedman test	III
t-test	IV

3 RESULTS AND DISCUSSION

3.1 Reliability of deformity analysis

Inter-rater reliability of the chironomid deformity analysis was found to be low, indicating that experts (hereafter raters) evaluate deformities subjectively and inconsistently (I). All raters evaluated the same set of samples, but the total number of cases classified as deformed varied greatly from 23 to 140, and consequently DI from 11 to 66 % among raters. DI calculated separately for the reference site data was 4–65 %, and for the impacted site 27–70 % (Fig. 6 in I).

In most of the assessments the distributions of the dichotomous response (normal/deformed) differed between the reference and impacted sites (I). Conservative deformity assessments, that included only obvious or absolute abnormalities like missing and extra teeth and Köhn gaps (Madden *et al.* 1995) and excluded any wear or mechanical damage, were more likely to distinguish the reference and impacted sites than were non-conservative assessments. Those 6 assessments which did not differentiate between the sites had the highest number of deformities defined (Fig. 7 in I). This high number of assessed deformities arouse mainly from interpreting worn teeth as deformed. Conservative deformity assessments showed greater effect sizes measured as odds ratios, which was reflected in considerably smaller sample sizes required to detect a difference in DI between the impacted and the reference site. This means greater cost-efficiency, as smaller sample sizes are needed to discern an effect. The blind and open assessments for the reference site resulted in an average DI of 17 % and 19 %, respectively, with no statistically significant difference. The same was true for the impact site where the blind (DI = 35 %) and open (40 %) assessments did not differ indicating that the preconception in the form of background information about site contamination did not bias deformity evaluation.

Morphological deformities as a bioindicator have many advantages when considering the criteria of Burger and Gochfeld (2001) for useful indicators. For example, the DI and HYI indexes seem biologically relevant because they are related to biologically significant factors, such as larval growth and development (Gerhardt and Janssens de Bisthoven 1995, Vuori 1995, Hämäläinen *et al.* 1998, Martinez *et al.* 2006). In contrast, DI obviously also has disadvantages, which complicate its use in ecological risk assessment. One of the most significant limitations is the inconsistent evaluation of deformities, shown as a large variation in deformity assessments among experts (I), so the methodological relevance with clear-cut data gathering and analysis phases required by Burger and Gochfeld (2001) for a good bioindicator was not met. The highly differing criteria in deformity assignment have likely led to inconsistent results in midge larval deformity studies. Reliability and consistency could be increased by developing guidelines to be applied in the chironomid deformity assessment by standardization of the analysis. The results of this inter-rater reliability study may serve in the development of a CEN (the European Committee for Standardization) guidance standard for the criteria for identification of morphological deformities in chironomid larvae. Lack of blinding is one potential source of systematic error in animal toxicity studies (Krauth *et al.* 2013), so to avoid bias and to give the results greater credibility it is recommended conducting any deformity assessments blind. It might be useful to study inter-rater agreement again after the guidelines for the deformity analysis are available, to evaluate if the consistency increases when experts share the same criteria for the deformity interpretation. Inconsistency might also be reduced in the future by developing automated recognition methods for deformity identification, using computer based image analysis.

3.2 Hyperspectral imaging

Hyperspectral imaging was tested as a novel technique for detecting responses of aquatic insects to metal contamination. Results of the 4-d Cd exposure of *H. pellucidula* larvae indicated that larvae accumulated waterborne Cd into their tissues (II). Larval tissue Cd concentration was correlated to the actual water Cd concentrations, but was on average highest at the second highest exposure concentration. All larvae survived the exposures, so Cd concentrations 1–100 µg Cd l^{-1} were acutely in a sublethal range for *H. pellucidula*. *Hydropsyche* spp. are generally tolerant of metal exposure, and typically have efficient detoxification processes (Cain *et al.* 2004). The average number of damaged gills per larvae in the exposed population, the HYI, was low and varied from 1.47 (± 0.42) in the control to 1.63 (± 0.25) in the exposure concentration of 10 µg l^{-1} with no significant differences among exposure populations.

Whereas these conventional toxicity responses, survival and morphological abnormalities, did not differ among Cd exposures, the hyperspectral imaging data indicated that larvae exposed to high Cd concentrations may have different spectral properties than control larvae. Results indicated some concentration-response relationship of larval spectral features to the Cd exposure, but it was too weak for reliable automatic distinction between exposed and unexposed larvae. These data were captured from the dorsal side of the insect and indicated that soft abdominal parts of the larvae are darker when exposed to high Cd concentrations.

The observed association of larval spectral features and Cd exposure can be compared with 7 causal criteria summarized by Adams (2003): strength, consistency and specificity of association, and temporality, biological gradient, experimental evidence and biological plausibility. The strength of observed association of spectral features to Cd concentration was low, and further studies are needed to define the consistency of this association. Because this was a laboratory exposure, other potentially confounding factors were controlled and soft tissue darkening could be designated as a specific effect of Cd. However, some potential sources of error could arise from the preparation of larvae for HSI, and from the imaging protocol used. For example, in the current imaging system the larvae were immersed in water to avoid desiccation, but as water absorption can be an additional source of error in HSI, it would be advantageous to eliminate this step. The fourth criterion, temporality, considers if the cause precedes the effect, and if the effect decreases when the cause is decreased or removed (Adams 2003). The soft tissue darkening indicated by HSI was only observed in larvae exposed to high Cd. Temporality could be controlled better if the larvae were imaged before Cd exposures, but in the present imaging process it was not possible. Biological gradient and experimental evidence according to Adams (2003) refer to a dose-response relationship observed in the system and validated by experimental studies. HSI to detect responses of aquatic insects to metal contamination clearly needs reassessment to establish whether observed spectral features are also associated with metal pollution under more realistic field conditions with variable metal mixtures. Biological plausibility assumes a reasonable biological mechanism that links the cause and an effect. Physiological mechanism that could explain the soft tissue darkening in the high Cd concentration indicated by HSI is unclear. For example, darkening of the fish tail region has been reported as an effect of lead exposure (Mebane *et al.* 2008) but the mechanism responsible for a colour change was not known. Darkening of hydropsychid soft tissues at high Cd levels has been observed (Vuori 1994, Vuori and Kukkonen 2002), but this was due to heavy darkening and reduction of tracheal gills located on the ventral side of the larvae, and darkening of larval anal papillae. Colour change might result from some metabolic disturbances, or from the adsorption of Cd on body surfaces. Metals can be adsorbed on the body surfaces of aquatic insects by direct binding to chitin, which is the main component of abdominal cuticle of insects, or as oxide coating on the cuticle (Gonzalez-Davila *et al.* 1990, Hare 1992, Dittman and Buchwalter 2010).

3.3 Behavioural responses

Behavioural types of *C. riparius* larvae included: (i) ventilation, i.e. dorsoventral, undulating, regular movements approximately at the range of 1.0–3.5 Hz; (ii) other locomotory activity, mainly slower movements associated with foraging and crawling (0.5–1.0 Hz); and (iii) inactivity (Fig. 2) (III). Also some higher frequency (4.0–8.5 Hz) signals were recorded indicating faster movements in some measurement periods, and these were included in the data analysis, since exposure may not only affect the time spent in ventilating but possibly also the frequency of ventilation. Behaviour of larvae of the lamprey *L. fluviatilis* consisted of (i) locomotion activity like burrowing and swimming at ≤ 2.0 Hz, (ii) ventilation at approximately 2.0–3.5 Hz and (iii) inactivity (Fig. 2) (III). Some movements associated with swimming and burrowing were also quite fast with high frequency signals apparently overlapping with ventilation signals. Due to overlapping, all signals from 0.5 Hz to 8.5 Hz were analysed as a one composite measure of larval activity, although the majority of the behavioural signals were < 4.0 Hz. Behavioural patterns of larvae of the mayfly *H. dalecarlica* included: (i) high amplitude, low frequency signals of locomotory activity, such as crawling and swimming behaviour (< 2 Hz); (ii) low amplitude and higher frequency signals of gill ventilation (≥ 2.0 Hz); and (iii) inactivity (Fig. 2) (IV).

Ventilation behaviour of larval chironomids consists of undulating movements of their body to irrigate their sediment burrows with oxygenated water (Roskosch *et al.* 2012). Ventilation of lamprey larvae involves regular movements of ventilatory muscles in the velum and functions both in gas exchange and in feeding, as water with food particles and oxygen is pumped into the mouth and pharynx (Hill and Potter 1971, Yap and Bowen 2003). In contrast, ventilation of mayfly larvae *H. dalecarlica* involves beating the mobile gills. *H. dalecarlica* has 7 pairs of gills laterally on the abdomen, each single gill lamella with a tuft of filaments (Engblom 1996). Mayfly larvae take up dissolved oxygen from water by diffusion through body and gill surfaces (Williams and Feltmate 1992), but gas exchange can be enhanced by beating the gills. Chloride epithelia in mayfly larvae are located in the tracheal gills and on both sides of the abdomen, and are actively ventilated by gills for ion uptake. Gills are thus important not only in breathing but also for osmoregulation in mayfly larvae.

Chironomid larvae in the reference sediment spent approximately 45 % and 10 % of the measurement time on locomotion and ventilation, respectively (Fig. 4 in III). No differences were found among sediment exposures in time spent on locomotion or ventilation (III). However, some indication of behavioural changes in activity level were observed in the most contaminated sediments (2B), where larval activity was approximately 10 % lower than in other sediment exposures (Fig. 6 in III) although no statistically significant differences were found. The time chironomid larvae spent in locomotion and ventilation in the reference conditions was in general in accordance with results of Azevedo-Pereira and Soares (2010), Langer-Jaesrich *et al.* (2010b) and Kienle *et al.* (2013).

Larval lamprey behaviour was measured 5 times during 28-d exposure, but temporal differences were not found in any of the treatments (III). There was also no difference in lamprey larval locomotory or ventilatory activity across the sediment treatments within a measurement day. Lamprey larvae in the reference sediment were quite inactive. According to Mallat (1982), larval lampreys are normally very inactive in sediments, and increased activity is a sign of stress. In contaminated sediments the range of larval lamprey activity was greater than in the reference sediment, especially in the most polluted sediment after 28-d exposure. Behavioural responses differed among individuals, so that some larvae were inactive while some were highly active within the same sediment exposure and measurement day.

To conclude, no significant or consistent differences in behavioural responses among sediment exposures were detected for any species. This was the first time chironomid or lamprey larvae had been used in behavioural MFB measurements in the sediment, and their distinct behavioural patterns in MFB measurements indicated that their behaviour can be quantified in sediment bioassays. Measuring the behaviour of burrowing species in the sediment is ecologically relevant and is important especially for species like lamprey that express negative phototaxis (Binder *et al.* 2013). In order to develop the approach, the effects of measurement conditions, intervals, and duration need to be better understood. Other measured endpoints in these sediment bioassays included larval growth, survival, chironomid DI and lamprey larvae body burden (III). These multi-contaminated sediments did not affect survival of chironomid and lamprey larvae in laboratory exposures, nor DI of chironomid larvae, but chironomid larval growth was reduced in some of the contaminated sediments, larvae being smaller in the contaminated sediments 1A, 2B and 4B than in the reference sediment. In their review of behavioural ecotoxicity studies, Melvin and Wilson (2013) found that, although behavioural end points typically were more sensitive than developmental or reproductive end points, some studies suggested that behavioural responses occurred at greater concentration levels than effects on growth, which is probably due to differences in experimental conditions and depends on the species, chemicals and chemical concentrations studied. Tissue concentrations of PCDD/Fs and PCBs of field-collected lamprey larvae were over hundredfold and tenfold, respectively, in the R. Kyminjoki those in the reference site (III). This accumulation indicates biomagnification of dioxins and PCBs in food webs and risk for humans consuming the fish.

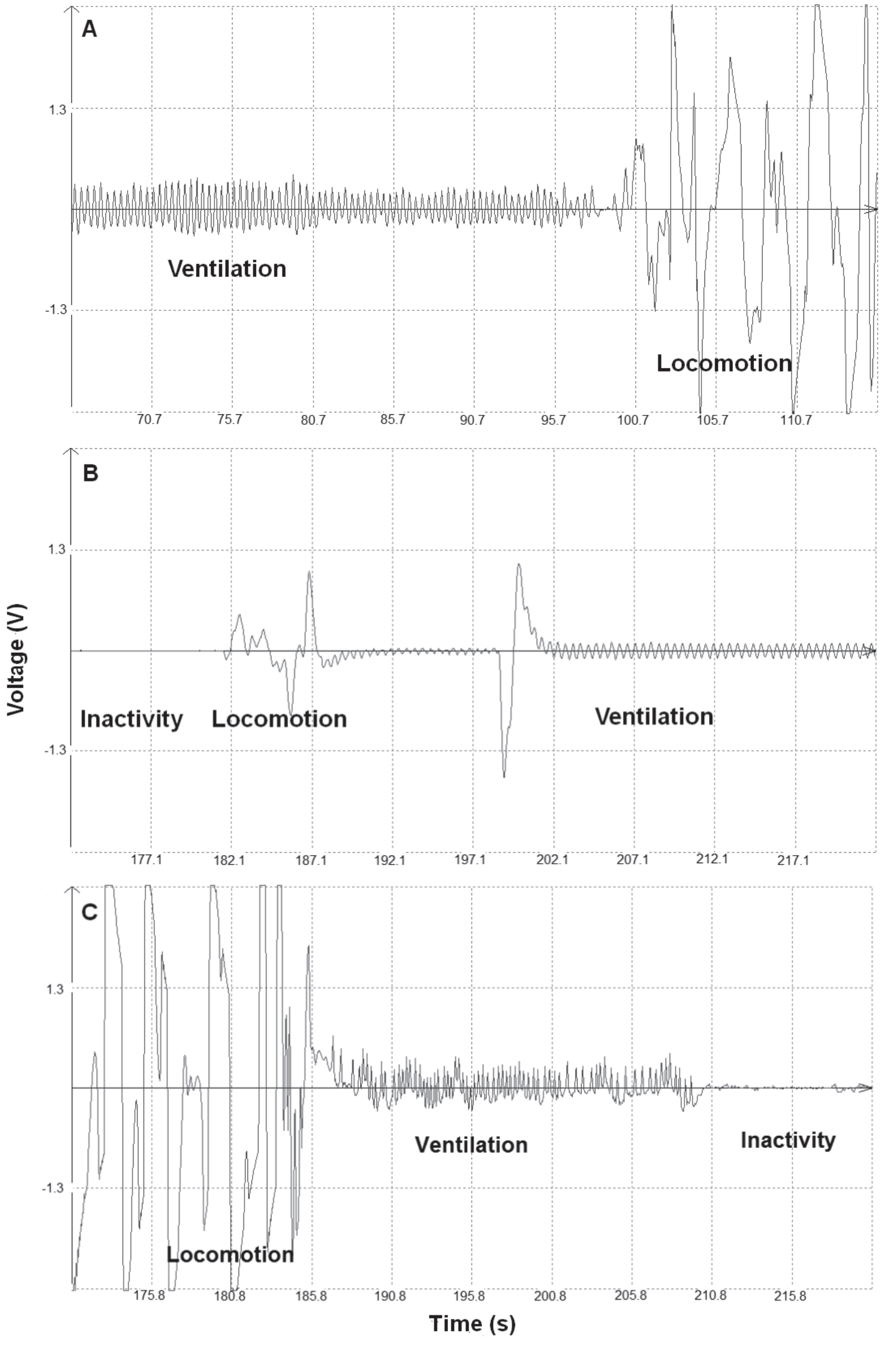

FIGURE 2 The 3 main behavioural patterns of *C. riparius* (A), *L. fluviatilis* (B) and *H. dalecarlica* (C) larvae detectable by MFB: high amplitude signals of locomotory activity, regular low amplitude and higher frequency signals of ventilation, and inactivity. Data acquired from the reference sediment exposures (*C. riparius* and *L. fluviatilis*) (III) and from the reference site R. Joutenjoki (*H. dalecarlica*) (IV).

Survival of oligochaete worms and mayfly larvae was not affected by biomining effluents, but behavioural changes in mayfly larvae were observed, although any consistent behavioural stress reactions typical of all 3 mine-impacted sites were detected (IV). Metal pollution and salinization of streams receiving biomining effluents was observed. In one mine-impacted stream larval locomotion and ventilation increased, while it decreased in another impacted stream with the highest salinity (Fig. 3 in IV). The high salinity of the R. Lumijoki might have affected the observed low ventilation rate, since an increase in water salinity has been shown to decrease ventilation rate and eventually the density of chloride cells over subsequent moultings in mayfly larvae (Wichard *et al.* 1973, Williams and Feltmate 1992). Mayfly larval locomotory activity within the reference streams varied on average between 6 and 19 %, and ventilation 1–3 %. This is consistent with the results of Gerhardt *et al.* (2005a) who found that behaviour of mayfly larvae *Choroterpes picteti* in the reference water typically consisted of long periods of inactivity, with locomotory activity up to 25 % of the time, and gill ventilation up to 5 %. Activity of *H. dalecarlica* larvae has been shown to be nocturnal in late summer with night-time foraging (Huhta *et al.* 1995) which could explain the measured low day-time activity level of these larvae. In mine-impacted streams the range of average time spent in ventilation and locomotion was greater than in the reference streams, between 0.2–3 % and 9–39 %, respectively. The unexpected indication of stress was pale coloration and slime excretion in oligochaete worms exposed at the mine-impacted R. Lumijoki, and higher moulting rate of mayfly larvae. Higher numbers of mayfly larvae exuviae, or shed exocuticles, in the R. Lumijoki (5) than in the reference R. Joutenjoki (0) after 3-day exposure was observed, which may suggest ionoregulatory or metal stress. Increased moulting rates of mayfly nymphs under acid stress have been observed (Rowe *et al.* 1988), and moulting has also been associated with metal exposure of hydropsychid larvae in the study of Vuori and Kukkonen (1996). Dittman and Buchwalter (2010) showed that moulting is a primary mode of elimination for both adsorbed and absorbed Mn in aquatic insects. Hence moulting rate might be worth further studies to evaluate its usefulness as an endpoint for waterborne metal and sulphate toxicity for aquatic insect larvae. Mayfly behavioural and survival data from one pair of rivers, mine-impacted the R. Lumijoki and the reference R. Joutenjoki, indicated that behavioural responses might be more sensitive than survival for detecting an effect of water contamination by metals and sulphate. Mayfly survival did not differ among these streams, but ventilation was significantly lower in the mine-impacted R. Lumijoki.

Large variability between individuals in their behavioural responses was observed in some of the sediment exposures with lamprey larvae (III), and also *in situ* measurements with mayfly larvae (IV). Within the same measurement period with the same exposure some individuals were highly active while other individuals remained inactive. This high individual variability may complicate extracting generalizations and straightforward conclusions for exposure effects. An exposure to a xenobiotic can widen the range of behavioural responses at first (Gerhardt and Palmer 1998). Increasing the number of individuals per treatment usually increases the power of the statistical tests to detect significant changes in variable data, but this may also increase the costs of the study. Another solution might be using each individual as its own control, so that behaviour of an individual is first measured in the control and then in the exposure conditions (Clotfelter *et al.* 2004). According to Craig and Laming (2004), averaging may not be the most effective way to handle the behavioural data when there is a large variation in behavioural activity among individuals, since this may reduce the sensitivity of behavioural measurement. It might be advantageous to consider alternative methods to handle the variable behavioural data, so that behavioural disruptions occurring beyond the normal behavioural variability could be discerned. Although behavioural changes can be detected rapidly within the first hours of exposure (Gerhardt *et al*. 2005a, De Lange *et al.* 2006), longer exposure time could have enabled detection of potential concentration-dependent shifts in behaviour as observed by Gerhardt and Palmer (1998) and Gerhardt *et al.* (2002). Stepwise stress response models (Gerhardt *et al.* 2005b, Zhang *et al.* 2012) indicate that invertebrates and fishes display a cascade of regulative behavioural responses in a time-dependent sequence. For example, *D. magna* exhibited decreased ventilation and locomotory activity followed by increased ventilation during acid mine drainage stress (Gerhardt *et al.* 2005b).

4 CONCLUSIONS

Chironomid larval deformities are widely used as bioindicators of sediment toxicity. The study of interrater agreement on deformity assessment (I) indicated that experts evaluate deformities subjectively and inconsistently, which weakens the indicator value of chironomid deformity analysis. Hence, the equivocal and partly contrasting results of studies concerning chironomid deformity incidence as a stress response to contamination might partly result from inconsistent interpretation of deformities. To increase the reliability, standardization of the method with clear assessment guidelines is needed. Inconsistency might also be reduced by developing automated recognition methods for deformity identification using computer-based image analysis. The results also suggested that more sensitive detection of effects of sediment toxicity can be obtained by taking into account only absolute deformities like missing and extra mentum teeth.

Results of the pilot study of hyperspectral imaging to detect responses of aquatic insects to metal contamination (II) showed that traditional toxicity endpoints, larval survival and the number of gill abnormalities, did not differ among Cd concentrations in the laboratory exposures. In contrast, HSI data indicated a weak association of larval spectral properties to the Cd exposure. These data suggest that soft tissues on the larval dorsal side are darker in high Cd. Further research is needed to clarify if this colour change could be interpreted as an early-warning signal of metal-induced stress. It is emphasized that this is a pilot study, and some improvements for future studies are suggested. First, the actual imaging process needs further development, especially concerning stabilization of larvae for spectral imaging in a standardized manner without damaging them. It is also important to acquire information of tissue Cd accumulation, gill damages, and specific spectral features from each larva to better evaluate the concentration-response associations. In this pilot study a workflow was developed for processing data from a novel application of hyperspectral imaging, but reassessment is needed to evaluate the potential of HSI for detecting metal contamination of aquatic environments. Further studies might also include field measurements, metal mixture studies, and utilization of a HS-camera operating with a greater spectral range.

MFB was found applicable for measuring behavioural responses of 3 benthic species. MFB enabled *in situ* measurements, and measurement of behavioural responses of animals living in sediments. Behavioural measurements in sediment, the natural habitat of burrowing animals, increase the ecological relevance of an effect assessment. Confounding factors in behavioural measurements included large behavioural variation among individuals in some exposures. Some results suggested that behavioural endpoints may be more sensitive than survival for detecting an effect of mine effluents on mayfly larvae. Future studies should focus on optimizing and standardizing behavioural measures to be used in ecological risk assessment.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Uusia pohjaeläinmittauksiin perustuvia menetelmäsovelluksia vesistöjen kemikaaliriskien arviointiin

Maa- ja metsätaloudessa, teollisuudessa ja kotitalouksissa käytettävistä kemikaaleista ja maankäytön seurauksena maaperästä vapautuvista raskasmetalleista osa päätyy jokiin ja järviin, missä ne voivat aiheuttaa merkittävää haittaa vesieliöstölle. Yksi vesiensuojelun keskeinen tavoite onkin haitallisista aineista aiheutuvien riskien vähentäminen. Riskien arvioimisen ja vähentämisen edellytyksenä on haittavaikutusten tunteminen. Perinteisesti vesistövaikutuksia arvioidaan vesi- tai sedimenttinäytteiden kemiallisilla pitoisuusmittauksilla. Erityisesti virtavesissä vesinäytteisiin perustuva arviointi on ongelmallista johtuen pitoisuuksien suuresta ajallisesta ja paikallisesta vaihtelusta. Lisäksi pitoisuuksiin perustuvat ympäristölaatunormit eivät huomioi vaihtelevien olosuhteiden merkitystä, aineiden yhteisvaikutuksia eivätkä ympäristön muita stressitekijöitä. Siksi pitoisuusmittausten rinnalle tarvitaan tapauskohtaisia ekologisten vaikutusten mittauksia.

Tämän väitöstutkimuksen päätavoitteena oli testata ja kehittää uusia selkärangattomien pohjaeläinten käyttäytymisvasteiden ja rakenteellisten muutosten mittausmenetelmiä haitallisten aineiden vesistövaikutusten arviointiin ja seurantaan. Kemikaalien aiheuttama ympäristöstressi voi ilmetä esimerkiksi eläinten käyttäytymismuutoksina ja rakenteellisina vaurioina. Nämä vasteet ja niiden taustalla oleva fysiologinen stressi voivat lisätä eläinten kuolleisuutta tai heikentää lisääntymismenestystä ja näin johtaa populaatiotason muutoksiin ja edelleen vaikutuksiin yhteisö- ja ekosysteemitasolla.

Tutkimuksen ensimmäisessä osatyössä selvitettiin pohjaeläinten rakenteellisten vaurioiden tulkintaa. Epämuodostuneiden surviaissääskien osuuden on havaittu kasvavan saastuneilla paikoilla, ja epämuodostumien esiintyvyyttä käytetään yleisesti ilmentämään järvien ja jokien sedimenttien myrkyllisyyttä. Epämuodostumatutkimusten luotettavuuden ja tulosten vertailtavuuden kannalta on olennaista, että tutkijat määrittävät epämuodostumat samalla tavalla. Epämuodostuma-arviointien yhtenevyyden tutkimiseksi järjestettiin kansainvälinen vertailukoe, jossa 25 alan asiantuntijaa teki surviaissääskitoukkien epämuodostuma-analyysin samasta mikroskooppivalokuva-aineistosta. Kävi ilmi, että asiantuntijoiden tulkinnat epämuodostumista ovat hyvin epäyhtenäisiä, mikä heikentää merkittävästi tämän bioindikaattorin käyttökelpoisuutta sedimenttien myrkyllisyyden ilmentäjänä. Arvioiden yhdenmukaisuutta voitaisiin kuitenkin parantaa ohjeistuksella. Tutkimuksen tulokset tarjoavat perustan ohjeistukselle ja määritysmenetelmän standardisoinnille sekä konenäkösovelluksen kehittämiselle vaurioiden automaattiseen arviointiin.

Tutkimuksen toisessa osatyössä tutkittiin hyperspektrikuvantamisen soveltuvuutta jokien pohjaeläinten metallisaastuneisuuden ilmentämiseen.

Menetelmä perustuu kuvattavien kohteiden eroihin valon eri aallonpituuksien heijastumisessa ja absorboitumisessa, jolloin erilaisten kohteiden ja materiaalien tunnistaminen on mahdollista niille ominaisten spektripiirteiden avulla. Laboratoriotutkimuksessa vesiperhostoukat altistettiin kadmiumille, jonka jälkeen ne kuvattiin hyperspektrikameralla. Yleisesti käytetyissä toksisuusvasteissa, kuolleisuudessa ja toukkien rakenteellisten vaurioiden määrässä, ei havaittu eroja eri altistuspitoisuuksissa. Sen sijaan tiettyjen spektrikuvantamisella erotettavien piirteiden havaittiin liittyvän toukkien altistustasoon. Spektripiirteiden perusteella ei kuitenkaan riittävän luotettavasti pystytty arvioimaan altistustasoa. Nämä alustavat tulokset ovat perustana jatkotutkimuksille. Menetelmää kannattaa edelleen kehittää, ja arvioida tarkemmin sen soveltuvuus vesistöjen metallikuormittuneisuuden havaitsemiseen.

Tutkimuksen kolmas osa-alue käsitteli pohjaeläinten käyttäytymisvasteiden mittaamista uudella menetelmällä. Tutkimuksessa käytettiin laitteistoa, joka rekisteröi eläimen liikkeen aiheuttamat sähköiset signaalit mittauskammiossa. Laitteisto on käytössä muualla Euroopassa vedenlaadun seurannassa sekä ekotoksikologisessa tutkimuksessa, mutta tutkimusta paikallisilla lajeilla ja paikallisissa ympäristöissä tarvitaan kehitettäessä menetelmän sovellusta haitallisten aineiden seurantaan Suomessa. Tutkimuksessa todettiin menetelmän soveltuvan sekä sedimentin sisällä elävien eläinten käyttäytymismittauksiin laboratorioaltistuksissa että paikan päällä joessa tehtäviin mittauksiin. Aiemmat tutkimukset osoittavat, että käyttäytymisen muutokset voivat olla nopeampi ja herkempi vaste haitallisille aineille kuin vaikutukset esimerkiksi eläimen kasvuun ja kehitykseen. Tässä tutkimuksessa surviaissääsken tai nahkiaisen toukilla ei havaittu selviä, yhdenmukaisia käyttäytymisvasteita niiden altistuessa PCDD/F- ja PCB-yhdisteiden ja elohopean saastuttamille sedimenteille. Nahkiaistoukkien lisääntynyt liikkumisaktiivisuus ja toisaalta yksilöiden välinen suuri liikkumisaktiivisuuden vaihtelu sedimentissä, jossa mitattiin korkeimmat haitta-ainepitoisuudet, viittaavat kuitenkin toukkien stressiin. Maastossa tehdyissä mittauksissa päivänkorennon toukilla havaittiin käyttäytymismuutoksia kaivosteollisuuden jätevesien vaikutusalueella. Vasteet eivät olleet yhdenmukaisia kaikilla vaikutuspaikoilla, mikä todennäköisesti johtui paikkojen toisistaan poikkeavasta vedenlaadusta. Päivänkorentojen käyttäytymisvasteet näyttivät olevan kuolleisuutta herkempi kaivosjätevesien vaikutusten ilmentäjä.

Kemikaalien aiheuttamien ympäristövaikutusten tunnistaminen ja ehkäiseminen on tärkeää vesiekosysteemien normaalin rakenteen ja toiminnan turvaamiseksi. Tämä väitöstutkimus toi uutta tietoa mittausmenetelmistä ja niiden soveltuvuudesta haitallisten aineiden vaikutusten arviointiin pohjaeläimistössä, ja sen tuloksia hyödynnetään ekologisen riskinarvioinnin menetelmien jatkokehityksessä.

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