Bioavailability of Pesticides in Freshwater Sediments

The importance of Sorption and Uptake Routes

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2007

Acta Universitatis Agriculturae Sueciae
2007:3

ISSN 1652-6880
ISBN 91-576-7302-2

Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Åkerblom, N. 2007. Bioavailability of pesticides in freshwater sediments
– The importance of sorption and uptake routes.
Doctoral dissertation.
ISSN 1652-6880, ISBN 978-91-576-7302-2

In ecological risk assessment standardized sediment toxicity tests are used to predict the hazard of chemicals for sediment-living organisms. Feeding is a prerequisite in these long-term tests to avoid starvation of test organisms. Therefore, added food particles may act as vectors for the test compound. The importance of food particles as vectors, however, is dependent on several factors, for example, sorption and major uptake routes. In this thesis, laboratory experiments on the importance of pesticide sorption and uptake routes for the bioavailability to the midge *Chironomus riparius* in sediment toxicity test setups were performed.

A feeding selectivity study showed that larvae almost exclusively fed on added food particles, and highly neglected sediment particles. Additionally, experiments on the sorption of the insecticide lindane, showed that food and peat particles (used in artificial sediment) efficiently sorbed lindane (>95% after 48 h). The binding strength of lindane was weak, facilitating particulate uptake. However, the uptake from dissolved lindane was higher than the uptake from particles. From this we concluded that toxicity may be underestimated in spiked-sediment scenarios, where hydrophobic pesticides sorb to the sediment and larvae to a large extent feed on uncontaminated food particles. Conversely, in a spiked-water scenario, the food particles may act as vector, resulting in a facilitated particulate uptake, in addition to the uptake from water.

Sediment organic matter affects sorption, and thus bioavailability of pesticides. Pyrethroid toxicity was much higher in artificial sediment than in a natural sediment, indicating the simplicity and shortcomings of using artificial sediments. Interestingly, the sediment quality highly affected bioavailability in spiked-water. For example, *C. riparus* larvae in sediments with low organic matter content and exposed to spiked-water pyrethroids, showed lower survival, slower development, and increased adult size, than those in sediments with higher organic matter. The pyrethroid deltamethrin, showed an LC_{50}-value (28 d) for *C. riparius* larvae in artificial sediment of 16 pg/L and 11 µg/kg for water- and sediment exposures, respectively, i.e. toxic effects occurred at concentrations lower than the detection limits for high-tech analytical methods.

This thesis contributes to a wider understanding of processes affecting bioavailability in freshwater sediments, and in particular in standardized sediments used in toxicity testing. The understanding of test compound sorption and bioavailability is crucial for sound interpretations of toxicity tests and for the general credibility of such tests.

Keywords: artificial sediment, toxicity tests, *Chironomus riparius*, bioavailability, pesticides, sorption, desorption, uptake route

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Appendix

Papers I-IV

This thesis is based on the following publications, which are referred to by their Roman numerals:

I. Åkerblom, N. and Goedkoop, W. 2003. Stable isotopes and fatty acids reveal that *Chironomus riparius* feeds selectively on added food in standardized toxicity tests. *Environmental Toxicology and Chemistry 22 (7)*, 1473-1480.

II. Åkerblom, N., Goedkoop, W., Björklund, E., Nilsson, T., and Kylin, H. Sorption and desorption of lindane in standardized toxicity tests with *Chironomus riparius* - High resolution studies of compound-particle interactions and bioavailability. (Submitted manuscript)

III. Åkerblom, N., Arbjörk, C., Hedlund, M., and Goedkoop, W. Deltamethrin toxicity to the midge *Chironomus riparius* Meigen - Effects of exposure scenario and sediment quality. (Submitted manuscript)

IV. Goedkoop, W., Spann N., and Åkerblom, N. Sublethal cypermethrin concentrations affect mean development rate and adult size in the midge *Chironomus riparius* Meigen. (Manuscript)

Introduction

Pesticides in aquatic ecosystems

In contrast to many other man-made chemicals present in the environment, pesticides are manufactured to be harmful to specific target organisms and intentionally spread into the environment. Hence, there is an obvious risk that non-target organisms are affected, either at the application site, or due to unintentional spread, at nearby or distant areas. Pesticides are transported to aquatic environments through atmospheric deposition, surface run-off or leaching (e.g. Kreuger, 1999) and frequently accumulate in soft-bottom sediments and aquatic organisms (Kreuger, Peterson & Lundgren, 1999; Lehotay, Harman-Fetcho & McConnell, 1998; Miles & Pfeuffer, 1997). In all parts of the world pesticides have been detected in aquatic ecosystems (Adielsson, Törnquist & Kreuger, 2006; Hoffman, Capel & Larson, 2000; Kreuger, 1998; Kreuger, Peterson & Lundgren, 1999; Miles & Pfeuffer, 1997; Pérez-Ruzafa *et al.*, 2000) and information of how these pesticides affect organisms is often missing. For example, in canals in south Florida, more than 700 pesticide detections were made during a 4-year period (Miles & Pfeuffer, 1997). Atrazine and ametryn were most often detected in the water samples, whereas hydrophobic and highly persistent pesticides like DDE and ametryn, were more frequently found in sediments. In Sweden, a recent monitoring program for four small agricultural catchments detected 57 active pesticides ingredients (including 6 degradation products) in surface water and 11 (including 2 degradation products) in sediments (Adielsson, Törnquist & Kreuger, 2006). The most frequently detected pesticides were the herbicides bentazone (detected in all water samples) and glyphosate. Occasionally, also residues of pesticides that had been withdrawn from the Swedish market were detected, e.g. atrazine and lindane. These studies show that both currently used pesticides and pesticides withdrawn from the market years ago do reach and/or accumulate in aquatic ecosystems and thereby constitute a threat to all aquatic organisms.

Bioavailability to benthic invertebrates

Benthic communities play a key role in energy, nutrient, and contaminant fluxes and are functionally important in transferring environmental contaminants to higher trophic levels (Burton, 1991; Reynoldson, 1987). Pesticides in the sediments may hit key organisms/processes and thereby cause serious damage to community composition and function. The toxicity of a chemical, however, is totally dependent on the uptake and the concentration in the organism, or even the concentration at the target receptor in the organism. Only the bioavailable fraction of a pesticide can be incorporated in aquatic organisms and thereby reach the specific target receptors (Hamelink & Spacie, 1977). The bioavailability is therefore of critical importance and determines the ecological significance of contaminant accumulation in freshwater ecosystems.

The bioavailability of pesticides in benthic invertebrates is determined by several factors, for example the biology of the organisms, the surrounding

environment, and chemical properties (Fig. 1). For example, organism size (Bruner, Fisher & Landrum, 1994a), lipid content (Bruner, Fisher & Landrum, 1994a; Landrum & Fisher, 1998), and feeding behavior (Bruner, Fisher & Landrum, 1994b; Gossiaux, Landrum & Fisher, 1998; Leppänen, 1995) can dramatically affect the rate of a chemical's absorption from the environment. Sediment properties, like organic matter quality (Fleming, Holmes & Nixon, 1998; Gunnarsson *et al.*, 1999) and quantity (Fleming, Holmes & Nixon, 1998; Goedkoop & Peterson, 2003) and water properties, like pH (Ghillebaert *et al.*, 1996) and temperature (Kumaraguru & Beamish, 1981; Lydy, Belden & Ternes, 1999) are also important factors governing pesticide bioavailability. Additionally, chemical properties, like hydrophobicity and molecular structure (Calvet, 1989; Landrum, Harkey & Kukkonen, 1996) affect the bioavailability, and thus the toxicity, of pesticides in freshwater sediments.

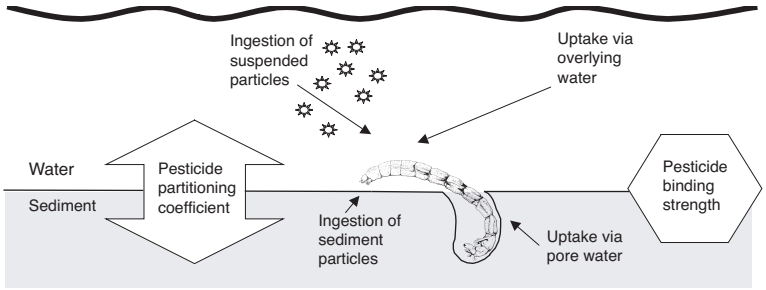

Fig. 1. Uptake routes, and factors affecting bioavailability of pesticides in benthic invertebrates. Depending on life strategy and feeding behavior, the organisms assimilate pesticides from different sources (e.g. phytoplankton, sediment, and water). Partitioning coefficients (e.g. K_{oc} and K_{ow}) and binding strength of the pesticides depend on physical and chemical properties of the chemical itself and of the substrates, and affect the bioavailability in these different sources.

Routes of pesticide exposure in aquatic invertebrates include uptake from pore- and overlying water across body walls and respiratory surfaces, and uptake from contaminated food/sediment particles via ingestion (Fig. 1) (Conrad, Fleming & Crane, 1999; Hamelink & Spacie, 1977; Power & Chapman, 1992; Reynoldson, 1987). Numerous studies have stressed the significance of the digestive uptake pathway for hydrophobic compounds (Forbes *et al.*, 1998; Harkey, Landrum & Klaine, 1994a; Leppänen, 1995; Selck, Palmqvist & Forbes, 2003; Sharpe & Mackay, 2000). Selck *et al.* (2003) for example, showed that sediment-bound fluoranthene contributed to at least 30% of the total uptake in the deposit-feeding polychaete *Capitella* sp. when exposed to both dissolved and particulate contaminants. In another study, Sharpe and Mackay (2000), used a fugacity model to estimate that sediment-dwelling organisms accumulate 96% of a simulated hydrophobic compound (log K_{ow}>6) from the sediment and only 4% from the water. The relative importance, however, of uptake via the digestive pathway or via pore and/or overlying water, respectively, is highly dependent on the sorptive behavior of the pesticide, i.e. if the pesticide is predominantly sediment-sorbed or

dissolved in water. Additionally, sediment-contaminant contact time, i.e. aging, is an important determinant for chemical bioavailability in sediments (Conrad, Comber & Simkiss, 2002; Landrum, Eadie & Faust, 1992; Leppänen & Kukkonen, 2000; Luthy *et al.*, 1997). Conrad *et al*. (2002), for example, showed that the bioavailability of pyrene in sediments decreased with sorption time for the oligochaete *Lumbriculus variegates*. Also, uptake mediated through the digestive pathway tends to be slower than when uptake occurs directly from the water phase (Forbes*, et al.*, 1998; Landrum & Fisher, 1998), due to a two-phase process in dietary accumulation involving both the desorption from food particles in the gut and the uptake through interstitial membranes.

Desorption depends on the binding strength of the pesticide to the sediment/food particles, and is thus also an important factor for bioavailability of sediment contaminants (Davies *et al.*, 1999; Lamoureux & Brownawell, 1999; Nilsson & Björklund, 2005). Lamoureux and Brownawell (1999) for example, showed a clear relationship (r^2>0.78) between biota-sediment factors (BSFs) and PAH and linear alkylbenzene desorption for deposit-feeding mussels *Yoldia limatula*. Similarly, desorption of contaminants from refractory food particles is low due to strong binding, leaving the contaminants largely unavailable for consumer animals (Davies*, et al.*, 1999; Decho & Luoma, 1994). Conversely, contaminants associated with labile organic matter has a higher bioavailability (Gossiaux, Landrum & Fisher, 1998; Gunnarsson*, et al.*, 1999; Lotufo, Farrar & Bridges, 2000; Selck, Decho & Forbes, 1999; Widenfalk, 2005). Lotufo *et al*. (2000), for example, showed that DDT is more bioavailable for the marine polychaete *Neanthes arenaceodentata* when associated with the fish food TetraMarin compared with sediment with the same carbon-normalized DDT concentration, indicating that organic carbon quality influence contaminant bioavailability. Similarly, Gunnarsson *et al*. (1999) showed that PCB (3,3´,4,4´-tetrachlorobiphenyl) accumulation in the infaunal brittle star, *Amphiura filiformis*, is higher in labile organic substrates than in more refractory. These studies show that processes like food selection, ingestion, digestion, and assimilation, in addition to sorption properties, also are important factors determining the bioavailability of sediment contaminants.

Sediment toxicity tests

Whole-sediment toxicity tests with benthic macroinvertebrates are important tools in risk assessments of pesticides and other chemicals (Burton, 1991; OECD, 2004a; OECD, 2004b; Streloke & Köpp, 1995). Although experimental conditions in laboratory microcosm studies are far from the complexity found in natural environments these studies have been used as efficient tools for predicting the bioavailability and toxicity of pesticides (e.g. Crane *et al.*, 1999). However, numerous sources of variation associated with toxicity tests performed in the laboratory may confound a sound interpretation of toxicity test results and the prediction of environmental safe concentrations, like predicted no effect concentrations (PNECs). For example, Goedkoop and Peterson (2003) showed that both *C. riparius* larval burrowing activity and sediment organic matter strongly

modified test conditions, and consequently the toxicity of lindane. Earlier, confounding effects of food additions on the toxicity test results have been addressed in various studies (Ankley *et al.*, 1993; Harkey, Driscoll & Landrum, 1997; Ristola, 1995; Ristola *et al.*, 1999). These confounding factors make it precarious to compare toxicity tests with different experimental designs, e.g. different test sediments, larval densities, exposure pathways, and feeding regimes. To meet this criticism a high degree of standardization is outlined in official guidelines, in which for example artificial sediment with known constituents is used (e.g. OECD, 2004a; OECD, 2004b). However, our knowledge of the fate and exposure pathways of test compounds in standardized toxicity tests is still poor, likely having strong implications for the interpretation of toxicity test results and for the overall credibility of these tests.

Objectives

The objective of this thesis was to contribute to a wider understanding of the processes affecting bioavailability of pesticides in freshwater sediments, in particular processes like sorption to particles and feeding behavior of test organisms in standardized sediment toxicity tests.

My major working hypotheses were:

1. that *Chironomus riparius* larvae selectively feed on added food particles in standardized sediment toxicity tests. (Paper I)

2. that hydrophobic pesticides efficiently sorb to organic particles present in standardized toxicity tests. (Paper II)

3. that desorption properties of hydrophobic pesticides differ, depending on the sorbent's organic matter quality, and that this has implications for particulate uptake in *C. riparius* larvae. (Paper II)

4. that pesticide toxicity, and thus bioavailability, to *C. riparius* larvae is affected by sediment quality (natural vs. artificial) and exposure scenario (spiked water vs. spiked sediment). (Paper III)

5. that pesticide sublethal effects to *C. riparius* larvae are dependent on pesticide sorption to organic matter in spiked water experiments. (Paper IV)

Materials and methods

In this section I give a brief overview of materials and methods used in the separate papers. For a more detailed description, I refer to the papers in appendix.

The test organism *Chironomus riparius* (papers I-IV)

The test organism *Chironomus riparius* is a surface deposit-feeding midge common in sediments where fine particulate organic matter accumulates, both in lakes and in areas of reduced current velocities in rivers and streams (Berg, 1995). The life cycle of *C. riparius* can be divided into three aquatic stages (egg, larvae, consisting of four instars, and pupae) and one aerial adult stage. During the first larval stage, 1st instar, the larvae are pelagic (Pinder, 1995). Thereafter, the larvae settle to the bottom and construct tubes of algae, sediment particles or other suitable material. *C. riparius* larvae feed by extending their head and anterior part of the body outside their tube while using the posterior prolegs to maintain contact with the inner surface of the tube (Berg, 1995). Oxygen uptake in *C. riparius* larvae mainly occurs through the abdominal tubules at the posterior part of the body (Cranston, 1995).

The species has been used in freshwater toxicity tests because of its relatively large body size and short generation time (easily cultured), and the fact that the larval stages live in close contact with the sediment as well as the water (Burton *et al.*, 2003; Nebeker *et al.*, 1984). The *C. riparius* larvae used in our experiments were obtained from a laboratory culture (60 L aquarium) with approximately 3 cm bottom substrate and a 15 cm deep water column. Larvae were regularly fed with pulverized aquarium fish food (Tetraphyll®) and adult midges were allowed to swarm in a cage above the aquarium.

Peat-based artificial sediment and artificial water (papers I-IV)

In standardized toxicity tests, artificial sediment with pulverized peat as organic fraction and artificial freshwater (e.g. M7-medium), are frequently used instead of natural sediments and water (OECD, 2004a; OECD, 2004b; Streloke & Köpp, 1995). Artificial sediment and water has several advantages, for example that tests can be initiated at any time during the year and there is no need to pretreat the sediment or the water to remove indigenous fauna. In addition, artificial sediment and water reduce inter-laboratory variability. One concern about the artificial sediment, however, is that it may be too simplistic to simulate true contaminant behavior of natural sediments and thus toxicity (Chapman *et al.*, 1999; Fleming, Holmes & Nixon, 1998; Goedkoop *et al.*, 2005).

Artificial sediment was prepared according to OECD guidelines (OECD, 2004a; OECD, 2004b), by mixing sand, *Sphagnum* peat (5% to 20%, <150 µm), kaolin clay, and $CaCO_3$ p.a. for buffering. The sediment was moisturized and added to glass test vessels. Artificial freshwater (M7-medium) was prepared by mixing deionised water, trace elements, macronutrients, and vitamins (OECD, 2004a; OECD, 2004b; Streloke & Köpp, 1995). M7-medium was carefully added to the test vessels, without disturbing the sediment surface, and test vessels where thereafter placed in a climate room for acclimation prior to the experiments (paper I, III, and IV).

Pesticides (papers II-IV)

In our studies *Chironomus* larvae were exposed to three different pesticides, lindane, deltamethrin, and cypermethrin (Table 1). All these are hydrophobic insecticides, known to be highly toxic to benthic organisms (Anderson, 1989; Beketov, 2004; Taylor *et al.*, 1993). Lindane is used on a wide range of crops, in stored product warehouses and storerooms, in public health applications, and in seed treatments. In our experiments we used ^{14}C-lindane (paper II) that was quantified by liquid scintillation counting (LKB Wallac 1217 Rackbeta). Deltamethrin (paper III) (purity 98.0%) is a photo-stable synthetic pyrethroid insecticide developed in the mid 1970s (Elliott *et al.*, 1974). It is mainly used for control of agricultural and household insect pests, but is also used in industry, forestry, product storage, and veterinary applications (Smith & Stratton, 1986; Tomlin, 1997), as well as against mosquitoes in malaria infested areas (Yáñez *et al.*, 2002). Cypermethrin (paper IV) is also a synthetic pyrethroid insecticide, used in similar applications as deltamethrin (Smith & Stratton, 1986; Tomlin, 2003). Cypermethrin is also used as a treatment against infestations by parasitic sea louse in intensive salmonid aquaqulture (Moore & Waring, 2001).

Table 1. *The pesticides used in the experiments, their active ingredients as IUPAC names, partition coefficients in octanol and water (log K_{ow}) and maximum permissible concentrations (MPC)*

Pesticide	Active ingredient	Log K_{ow}[a]	MPC[b] water (µg/L)	MPC[b] sediment (µg/kg)
Lindane	γ-hexachlorocyclohexane	3.5	0.77	190
Deltamethrin	(S)-α-cyano-3-phenoxybenzyl (1*R*)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate	4.6	0.0003	1.3
Cypermethrin	(RS)-a-cyano-3-phenoxybenzyl(1RS)-cis-trans-3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropanecarboxylate	6.6	0.00009	0.39

a. (Tomlin, 2003)
b. (Crommentuijn *et al.*, 2000)

Feeding selectivity of *Chironomus* riparius in standardized toxicity tests (Paper I)

In standardized long-term sediment toxicity tests with *C. riparius,* food additions are a prerequisite to avoid reduced survival, growth, and reproduction due to reasons other than toxicants (Ankley *et al.*, 1994; Ankley*, et al.*, 1993; Ristola*, et al.*, 1999). Selective feeding on uncontaminated food particles is therefore a possible confounding factor in these tests. Our objective with this study was to quantify the feeding selectivity among *C. riparius* larvae in standardized toxicity tests using stable isotopes of C and N and fatty acid analyses. Stable isotope ratios of carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) have become powerful and popular tools to study elemental and material cycles in ecology and ecotoxicology (Lajtha & Michener, 1994). In addition, specific fatty acids can be used as biomarkers, for

example to study differences in diet between *Chironomus* species (e.g. Goedkoop *et al.*, 1998; Napolitano, 1999). We combined stable isotope and fatty acid biomarkers to study feeding selectively among *Chironomus* larvae in standardized toxicity tests.

We used a factorial design where larvae were supplied with three different food resources, artificial sediment (sediment–no food treatment), artificial sediment and Tetraphyll (sediment+food), and sand and Tetraphyll (sand+food) (Fig. 2). Each experimental vessel was supplied with artificial sediment (10.3% peat) or sand with particles bigger than 200 µm to prevent ingestion by larvae (Davies*, et al.*, 1999) and M7–medium. The test vessels were placed in a climate room, and 25 larvae (1st instars) were added to each vessel. Both treatments fed Tetraphyll (sediment+food and sand+food) were terminated after 10 days, while the sediment–no food treatment was run for 28 days, since these unfed larvae had a very slow development, resulting in marginal body weight gain after 10 days. At termination, larvae were collected, counted, and weighted to determine larval individual biomass. Approximately 50% of the larvae from each vessel were randomly selected for dissection of the alimentary tracts according to Johnson (1985).

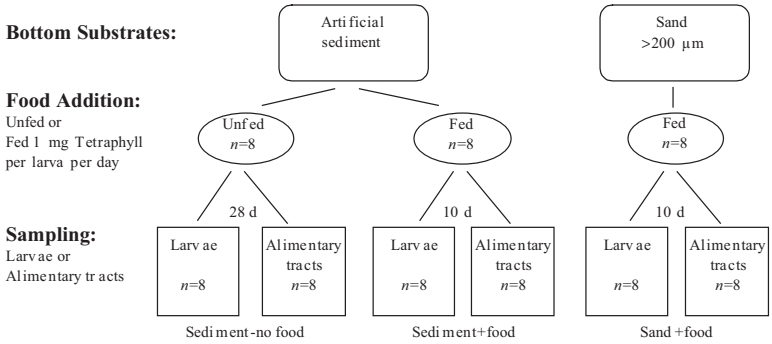

Fig. 2. Illustration showing the experimental setup for the feeding selectivity study with *Chironomus riparius*. Larvae in one treatment (sediment+food) could selectively feed on either sediment particles or added food, whereas larvae in the other two treatments (sediment–no food and sand+food) had access to only one of these potential food items.

The $\delta^{13}C$ and $\delta^{15}N$, as well as contents of carbon and nitrogen were analyzed using a gas chromatograph coupled to a mass spectrometer. The $\delta^{13}C$ values are reported relative to the V–PeeDee Belemnite (PDB) Standard, i.e. $\delta^{13}C$ in ‰, is the deviation of the isotopic ratio of the sample from that of the PeeDee Belemnite Standard; $\delta^{13}C$ (‰) = ($R_{sample}/R_{standard}$–1) x 1000, where $R = {}^{13}C/{}^{12}C$. A positive $\delta^{13}C$ value means that the sample has more of the heavier isotope (^{13}C) than the standard. $\delta^{15}N$ in ‰, is the deviation of the isotopic ratio of the sample from that of the atmospheric nitrogen standard; $\delta^{15}N$ (‰) = ($R_{sample}/R_{standard}$–1) x 1000, where $R = {}^{15}N/{}^{14}N$.

The contribution of Tetraphyll and artificial sediment to the carbon and nitrogen content in *Chironomus* larvae, was quantified using a traditional two–source mixing model, modified from Vander Zanden and Rasmussen (2001). The model was adjusted for fractionation (enrichment of $\delta^{13}C$ or $\delta^{15}N$ across trophic levels) according to Yoshii (1999):

$$TP\ (\%) = \frac{\delta^{13}C_{(larvae\ SeF)i} - F - \delta^{13}C_{Se}}{\delta^{13}C_{F} - \delta^{13}C_{Se}} \times 100$$

where *TP* is the contribution of Tetraphyll (in %) to carbon content in *Chironomus* larvae, F is the fractionation in ‰ calculated by:

$$F = \frac{(\delta^{13}C_{larvae\ Se} - \delta^{13}C_{Se}) + (\delta^{13}C_{larvae\ SaF} - \delta^{13}C_{F})}{2}$$

where $\delta^{13}C_{(larvae\ SeF)i}$ is the $\delta^{13}C$ in larvae from each replicate (i=1–4) in the sediment+food treatment, $\delta^{13}C_{Se}$ is the mean $\delta^{13}C$ in artificial sediment, $\delta^{13}C_{F}$ is the mean $\delta^{13}C$ in food (Tetraphyll), $\delta^{13}C_{larvae\ Se}$ is the mean $\delta^{13}C$ in larvae from the sediment–no food treatment, and $\delta^{13}C_{larvae\ SaF}$ is the mean $\delta^{13}C$ in larvae from the sand+food treatment.

Consequently, the contribution of artificial sediment (in %) to carbon content in *Chironomus* larvae could be calculated as 100 – *TP* (%). The mixing model was applied for both $\delta^{13}C$ and $\delta^{15}N$.

Fatty acid (FA) concentrations were quantified as methyl esters by using gas chromatography according to Ahlgren *et al*. (1994). Individually FA were identified and quantified by comparing their retention time and the area of the peaks on the chromatogram with those of known standards. Principal components analysis (PCA) was used to identify patterns in our data. PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components (PC).

Lindane sorption/desorption and uptake in *Chironomus riparius* (Paper II)

The understanding of test compound behavior in sediment toxicity tests is crucial for correct interpretations and for the general credibility of such tests. The objective of this study, therefore, was to quantify sorption and sequential desorption of lindane to various particle types present in standardized toxicity tests and uptake of particulate and dissolved lindane in *C. riparius* larvae. We run three separate experiments that followed the methods outlined in Fig. 3. Sorption was quantified by using a batch equilibrium approach with particles (peat, sand, kaolin clay, artificial sediment, or finely ground Tetraphyll) suspended in a spiked

solution, according to OECD guideline 106 (OECD, 2000). ^{14}C-lindane, dissolved in M7 and with the nominal concentration of 20 µg/L, was allowed to sorb to the particles for 4 to 48 h, while the test vessels (Teflon® tubes) were shaken on a mixing table. Subsequently, the vessels were centrifuged (3200 g), and a subsample of the supernatant was analyzed with liquid scintillation counting. The supernatant from the Tetraphyll treatment was first filtered to distinguish lindane sorbed to suspended particles from that dissolved in the M7-medium (Fig. 3).

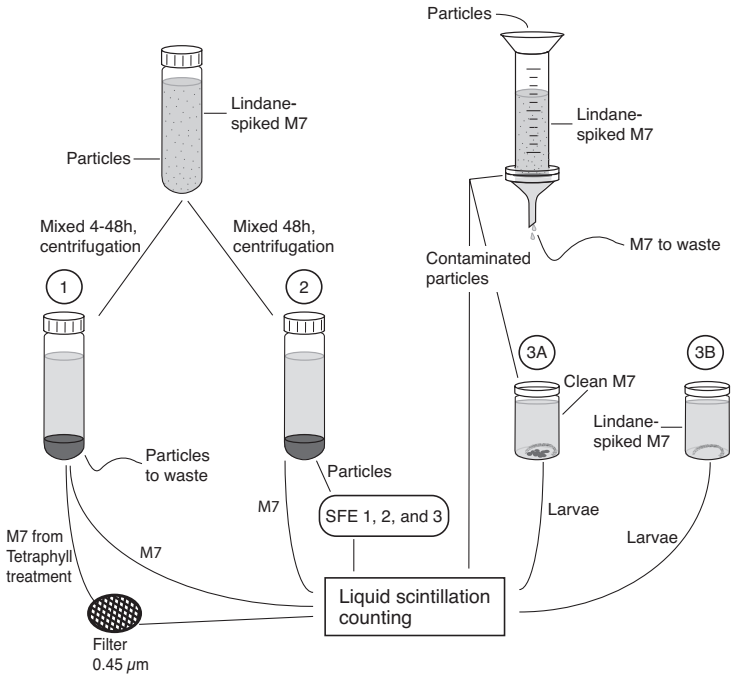

Fig. 3. The experimental setups for lindane experiments with respect to; **1.** Sorption to particles with batch equilibrium experiments, **2.** Desorption by supercritical fluid extractions (SFE1=40 °C and 100 bar; SFE2=40 °C and 340 bar; SFE3=100 °C and 340 bar), **3A.** *Chironomus riparius* larval uptake of lindane from contaminated particles, and **3B.** *C. riparius* larval uptake of dissolved lindane. For further details, see text.

Desorption behavior of lindane sorbed to peat, Tetraphyll, and artificial sediment was determined using a selective supercritical fluid extraction (SFE) method modified from Nilsson *et al.* (2002). Unlike conventional solid-liquid extraction methods, SFE utilises CO_2 in a supercritical condition to extract contaminants from e.g. sediment or soil samples. Particles were mixed in a ^{14}C-lindane solution (nominal concentration: 22.6 µg/L) during 48 h and then centrifuged, as described for the batch equilibrium experiment above (Fig. 3). A subsample of the supernatant was transferred to scintillation vials to quantify dissolved lindane. Aliquots of the wet particles (pellets) were mixed with Na_2SO_4, to get homogeneous dry mixtures, and transferred to SFE thimbles. Different binding-strength levels of particle-associated lindane were determined by sequentially running three 1-h extractions with consecutively harsher SFE conditions (weak: 40

°C and 100 bar, intermediate: 40 °C and 340 bar, and strong: 100 °C and 340 bar) on the same thimble (Hewlett-Packard 7680T SFE extractor). After each extraction, the analyte was trapped in Florisil® (60-100 mesh) and quantified by liquid scintillation counting.

In the batch equilibrium approach above we found that lindane efficiently and rapidly sorbed to both peat and Tetraphyll particles. Therefore, sorption of lindane to added food particles in standardized spiked-water toxicity tests was simulated, by allowing Tetraphyll to settle through M7-medium spiked with ^{14}C-lindane (nominal concentrations: 22 and 68 µg/L) (Fig. 3). Spiked M7-medium was added to glass filter holders loaded with nitrocellulose membrane-filters (0.45 µm). Finely ground Tetraphyll was added on top of the M7-surface, and after 8-min sedimentation the contaminated Tetraphyll particles were retained from the filters with a spatula and lindane sorption was quantified, as above. Similarly, sorption of lindane to peat particles was quantified using the same experimental set up as for Tetraphyll except that the peat particles were slightly moisturized prior to the sedimentation in the filter holders. This modification was necessary to facilitate peat particle sedimentation in the funnels.

Larval uptake of particulate Tetraphyll- and peat-associated lindane (as above) was quantified in glass vessels containing contaminated particles, sand (>200 µm to avoid ingestion (Davies*, et al.*, 1999)) and oxygen-saturated, uncontaminated M7-medium (Fig. 3). One 4th instar *C. riparius* larva was added to each vessel and exposed for 24 h. Larvae from half of the vessels/treatment were then sampled to quantify lindane uptake. Larvae from the remaining vessels were transferred to depuration vessels containing sand, oxygen-saturated M7-medium, and Tetraphyll. Depuration was run for 24 h, a time fully sufficient for gut purging in *C. riparius* (Davies*, et al.*, 1999). Upon sampling, larvae were rinsed, anaesthetized, and blotted dry, prior to liquid scintillation counting.

Active and passive uptake of dissolved lindane through respiratory surfaces and skin was quantified in living and formalin-killed larvae exposed for 24 h (Fig. 3). Depuration of living larvae and sampling followed to procedure described above for particulate uptake.

Sediment property effects on bioavailability of pyrethroids (Papers III and IV)

The toxicity of deltamethrin and cypermethrin, respectively, to *C. riparius* was studied in whole-sediment microcosms experiments with different exposure scenarios and/or different sediment qualities. Generally, the experiments followed the methods described in OECD guidelines 218 (spiked sediment) (OECD, 2004a) and 219 (spiked water) (OECD, 2004b), with the exception of some minor modifications.

In paper III, we tested effects of sediment quality (natural sediment from ponds on the Island of Gotland, Sweden, and standardized artificial sediment with 5%

peat) and exposure scenario (spiked sediment and spiked water) on deltamethrin toxicity. The nominal concentrations in the spiked sediments ranged 0 to 260 µg/kg (dw; artificial sediment) and 0 to 166 µg/kg (dw; natural sediment), respectively. In the spiked-water experiments, the nominal concentrations ranged 0 to 300 pg/L (artificial sediment) and 0 to 48 pg/L (natural sediment), respectively.

In paper IV, we studied effects of sublethal cypermethrin concentrations on mean development rate and adult size of *C. riparius*. We used a spiked-water scenario with a concentration range of 0 to 3.2 µg/L and artificial sediment with a gradient of organic matter content (0, 5, and 20% OM).

Experiments in paper III and IV were conducted in glass vessels (1 L) containing sediment and M7–medium (Fig. 4). At the start of the experiments, twenty 1st instar larvae were added to each test vessel. The larvae were fed regularly with 0.25 mg Tetraphyll per larva and day during the first 10 days, and 0.5 mg per larva and day thereafter. From day 10 daily checks were made to count and collect emerged midges. The experiments were terminated after 28 (paper III) or 29 (paper IV) days, when survival and sex were scored. Development rate (the reciprocal of development time; unit 1/day) was calculated as:

$$\overline{x} = \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$$

where $\overline{x}$ is mean development rate, i is the index of the inspection intervals, m is the maximum number of inspection intervals, f_i is the number of midges emerged in the inspection interval, n_e is the total number of midges emerged until the end of the experiment, and x_i is the development rate of midges emerged in interval i, calculated as:

$$x_i = \frac{1}{day_i - \frac{l_i}{2}}$$

where day_i is the inspection day, and l_i is the length of the inspection interval (as days, usually 1 day). In paper IV also the adult wing lengths were measured, as a proxi of adult size for controls, acetone controls, and concentrations of 0.05 and 0.8 µg/L.

A major concern in studies of toxicity of pyrethroids is the fact that toxicity may occur at concentrations that are below the detection limit for the most advanced analytical methods. In our studies, true deltamethrin exposure concentrations (paper III) were analyzed at the highest nominal spiked sediment concentrations (260 and 166 µg/kg dw, respectively) on day 0. Deltamethrin was extracted from the sediment by using a Soxtec Avanti extraction system. The extracts were cleaned and analyzed with gas chromatography (GC) with electron capture and/or mass spectrometry (MS) detectors. Deltamethrin in overlying M7-medium from the highest spiked sediment treatments was detected with GC-MS as in the

sediment extracts. In paper IV, the cypermethrin stock solution concentration was verified by GC-MS.

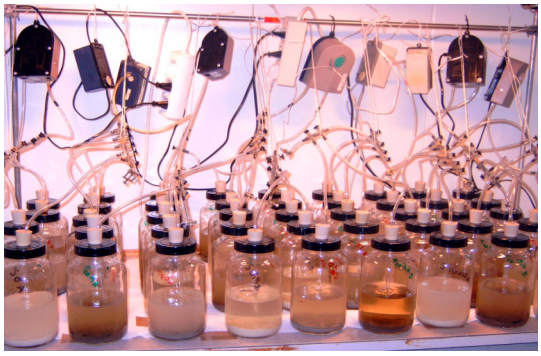

Fig. 4. Photo of experimental vessels in paper IV. The test vessels were aerated through Pasteur pipettes and supplied with lids to prevent water evaporation and the escape of emerged adult midges. Artificial sediments with 0, 5, or 20% organic matter were used.

Results and discussion

The role of feeding selectivity for bioavailability (Paper I)

In this study we showed that *C. riparius* has a high preference for Tetraphyll and ingests artificial sediment only to a minor extent in standardized toxicity tests. Two-source mixing models, based on stable isotopic analysis, revealed that Tetraphyll on average contributed to 94±6.9% of larval carbon content and 90±4.3% of larval nitrogen content, while artificial sediment contributed to only 5.9±6.9% and 9.6±4.3% of larval carbon and nitrogen content, respectively. As a consequence of larval feeding selectivity, test compound bioavailability and thus toxicity in standardized toxicity tests will be affected. A combination of decreased contaminant ingestion and increased elimination due to larval feeding on uncontaminated food particles results in a decreased bioavailability. Conversely, sorption of the test compound onto food particles, and subsequent larval feeding on these particles, results in increased contaminant ingestion, and thus a higher uptake.

The $\delta^{13}C$ ratio of larvae differed among all three treatments (Fig. 5), and ranged -23.34±0.56‰ (sediment-no food treatment) to -20.17±0.20‰ (sand+food treatment). The $\delta^{15}N$ ratio of larvae from the sediment-no food treatment was also markedly lower, 0.33±0.52‰, than that of larvae fed Tetraphyll, 7.45±0.36‰ (sediment+food) and 7.82±0.15‰ (sand+food). Larvae in both treatments fed Tetraphyll had similar $\delta^{15}N$ composition, but differed slightly in $\delta^{13}C$. The stable isotopic pattern for larvae supplied with a single type of food (artificial sediment or Tetraphyll) closely resembled that for the food they were raised upon (Fig. 5). Therefore, a very low average fractionation of $\delta^{13}C$, -0.07‰, and $\delta^{15}N$, 1.14‰, was observed between the larvae and their food. The ecological role of this low

fractionation is discussed in a separate paper (Goedkoop, Åkerblom & Demandt, 2006), not included in this thesis.

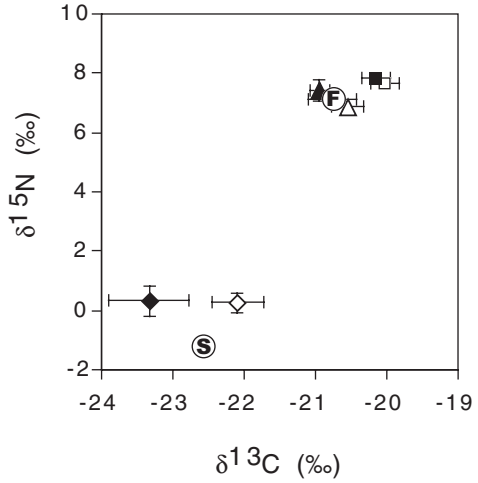

Fig. 5. Stable isotope ratios ($\delta^{13}C$ and $\delta^{14}N$) in whole *C. riparius* larvae (filled symbols), in alimentary tracts of *C. riparius* (unfilled symbols), and in food resources (S=artificial sediment; F=added food). Three different treatments corresponding to three different food resources: ◆/◇ = samples from the sediment-no food treatment; ▲/△ = samples from the sediment+food treatment; ■/□= samples from the sand+food treatment. Error bars denote ± 1 standard deviation.

Stable isotopic ratios in whole larvae may not per se reflect selective feeding but could also be a consequence of selective assimilation (Vander Zanden & Rasmussen, 2001). Tetraphyll is a better food source than peat due to its higher nutritious value and higher digestibility. Consequently, carbon and nitrogen from Tetraphyll is likely assimilated at a much higher efficiency than from peat. The isotopic composition of *C. riparius* alimentary tracts, however, was almost consistently similar to that of whole larvae. Additionally, the $\delta^{13}C$ and $\delta^{15}N$ ratios of alimentary tracts from sediment+food and the sand+food treatments were similar, indicating that larvae in both treatments exploited the same food resource, i.e. Tetraphyll. Hence, by analyzing stable isotopic ratios in both whole larvae and alimentary tracts, we eliminated the potential risk of misinterpretation caused by selective assimilation.

This preferential feeding on Tetraphyll particles was further confirmed by analyses of FA patterns of larvae, peat, and Tetraphyll. Principal components analysis clearly illustrated that *Chironomus* larvae that had access to both sediment and Tetraphyll showed almost similar FA patterns as those fed Tetraphyll (without sediment) (Fig. 6). As expected, larvae from the sediment-no food treatment had a very low content of FA and showed a slow growth, reflecting nutrient and/or elemental limitation. These results confirm that food additions indeed are necessary to avoid false positive results and maintain normal development among *Chironomus* larvae during long-term toxicity studies (Ankley*, et al.*, 1994; Ankley*, et al.*, 1993; Ristola*, et al.*, 1999) and point out Tetraphyll particles as potentially important vectors for test compound uptake.

Remarkably few studies have addressed the relationship between food additions and test compound bioavailability (Harkey, Driscoll & Landrum, 1997; Harkey, Landrum & Klaine, 1994b; Pascoe *et al.*, 1990; Wiederholm, Wiederholm &

Milbrink, 1987). Moreover, the results of such studies are contradictory, i.e. the bioavailability is both positively and negatively correlated with feeding, depending on the contaminant considered. Consequently, physical and chemical characteristics of the test compound (sorption characteristics), but also the exposure scenario (spiked sediment or spiked water) are important factors to determine the effects of selective feeding in standardized toxicity tests.

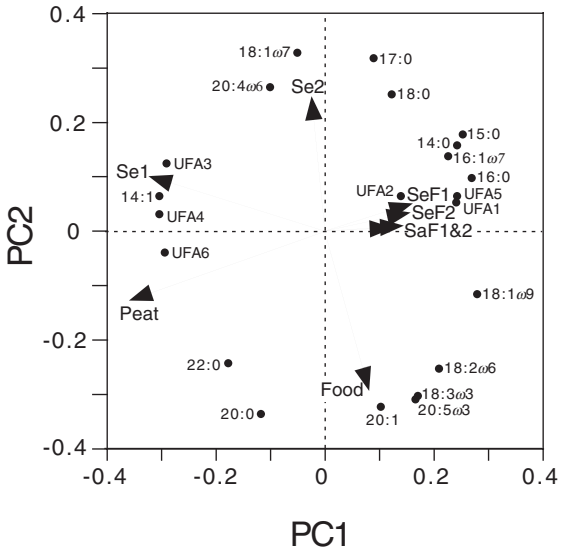

Fig. 6. PCA-plot. Principal component analysis plot for concentrations of fatty acids (dots) in *Chironomus riparius* larvae and food resources (Peat and Food). Notations adjacent to the vectors refer to the experimental treatments: Se1&2= the sediment–no food treatment; SeF1&2= the sediment+food treatment; SaF1&2= the sand+food treatment. UFA= unidentified fatty acids. The principal components (PC) for fatty acids have been divided with 20 to fit into the same figure as larvae and food resources.

Effects of sorption and binding strength on test compound bioavailability (paper II)

Lindane sorption to peat and Tetraphyll particles was efficient and reached 98±0.1% and 97±0.1%, respectively, after 48 h of mixing in a spiked solution. Sorption to artificial sediment was lower, 77±0.2%, and that to sand and kaolin only 9.6±1.3% and 8.3±0.8%, respectively. Most inorganic minerals are polar, and thus strongly favor interactions which allow them to form hydrogen bonds, such as with water. Consequently, the sorption of organic chemicals to these minerals requires displacement of tightly bound water molecules. Conversely, chemical sorption to organic-rich particles does not require displacement of water, and thus the sorption to these particles is more efficient than to minerals (Schwarzenbach, Gschwend & Imboden, 1995). Lindane's affinity for organic-rich particles is well documented (Goedkoop & Peterson, 2003; Just, Hawker & Connell, 1990; Kalsch *et al.*, 1998; Lee *et al.*, 2004), but the high-resolution approach used in our study provides new insight in the behavior of lindane, and compounds with similar characteristics, in standardized toxicity tests.

The desorption experiments with SFE showed that most lindane was loosely bound to artificial sediment, peat, and Tetraphyll, respectively (Fig. 7). The weak extraction desorbed 44±5.1% of initially added lindane from artificial sediment. For peat and Tetraphyll, the proportions of lindane removed with the weak SFE were higher, 73±3.8% and 76±9.3%, respectively, though not significantly different than that in artificial sediment. In fact, both the weak and the intermediate extraction strengths used in our study were milder than the extraction strength used by Nilsson and Björklund (2005) to selectively remove the bioavailable fraction of PCBs in contaminated lake sediments when studying uptake in *C. riparius*. Consequently, only the strong SFE in our study reveal the lindane fraction that is not available for the larvae. Consequently, 84% of lindane sorbed to artificial sediment, 86% of that sorbed to peat, and 90% of that sorbed to Tetraphyll, are likely bioavailable and can potentially be taken up in *C. riparius* larvae through the digestive pathway.

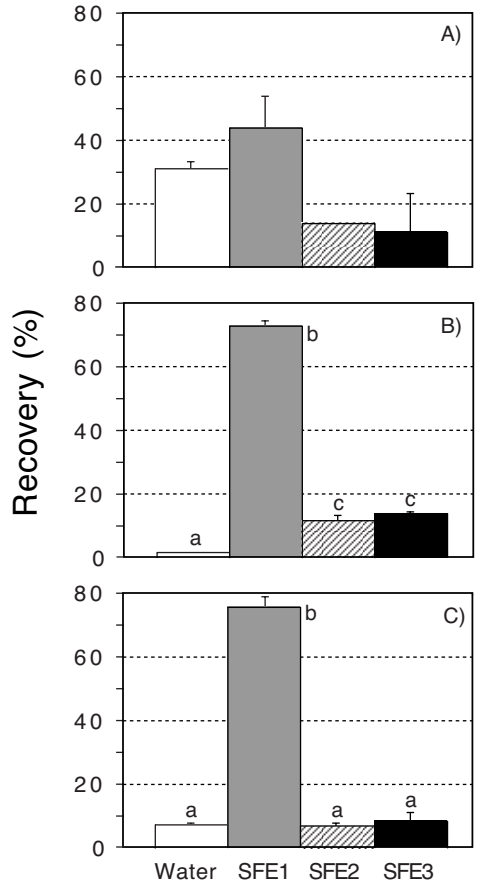

Fig. 7. Proportion of dissolved and desorbed lindane (mean±1SD) in treatments with (a) artificial sediment, (b) peat and (c) Tetraphyll extracted with different extraction strengths using supercritical fluid extraction (SFE). SFE1=40 °C and 100 bar; SFE2=40 °C and 340 bar; SFE3=100 °C and 340 bar. For further details, see text. Letters denote differences among the various fractions within each treatment (Bonferroni/Dunn; $p<0.0083$).

Our studies also showed that larval uptake of particle-associated lindane (in % of added amounts) was independent of the particle type that larvae were feeding upon, i.e. peat or Tetraphyll. Larvae fed contaminated peat or Tetraphyll reached a mean body concentration of 0.95-1.1 µg/g ww larvae (22 µg/L treatments) and 1.8-2.7µg/g (68 µg/L treatments), respectively (Table 2). This is in agreement with our desorption experiment, which showed that the binding strength of lindane to peat and Tetraphyll was the same, indicating a similar potential bioavailability. Another plausible explanation for the lack of difference in particulate uptake in our study is that a substantial part of the particle-associated lindane may desorbed from peat and Tetraphyll and hence was available as dissolved lindane in these test systems. By using the K_d-value obtained from the batch equilibrium experiment, the initial lindane water concentration, the proportion of lindane that sorbed to particles in the sedimentation test, and assuming that 50% of the lindane that could be desorbed with the mildest SFE condition is prone to desorb in clean M7, we estimate that the water concentrations in the test vessels where larvae were fed contaminated particles may range 2-9 µg/L. Larval uptake in treatments with dissolved lindane was 4.9 to 11 µg/g and consistently higher, than the uptake from particles, up to 2.7 µg/g (peat, 68 µg/L) (Table 2). These results indicate that water is an important exposure pathway for lindane. Hence, the assumed relatively large uptake from water may have obscured any possible differences in particulate uptake between peat and Tetraphyll.

Interestingly, passive uptake by dead larvae was very high, and reached 22% and 34% of the active uptake, in 22 µg/L and 68 µg/L treatments, respectively. The use of dead animals to determine cuticular partitioning may underestimate uptake, as there is no internal circulation. Hence, the high uptake into dead larvae in our study, also show that uptake from water is an important uptake pathway for lindane.

Table 2. *Uptake of dissolved and particulate lindane in Chironomus riparius larvae, as total body concentrations (µg/g ww) and as % of initially added lindane, respectively. Peat and Tetraphyll particles were contaminated by sedimentation through a spiked M7 column with the nominal lindane concentrations of 22 and 68 µg/L. All values are mean±1SD, and n=5*

	——Larval uptake (µg/g ww)——			———Larval uptake (%)———		
Nominal Conc.	Dissolved	Peat	Tetraphyll	Dissolved	Peat	Tetraphyll
22 µg/L	4.9±0.71	1.1±0.10	0.95±0.15	12±1.8	2.6±0.2	3.1±0.5
68 µg/L	10.8±1.16	2.7±0.21	1.8±0.25	11±1.2	2.1±0.1	2.0±0.3

Effects of sediment quality and exposure pathway on bioavailability (paper III)

In this study we found that the toxicity of deltamethrin to *C. riparius* larvae was highly variable, both between sediments (artificial and natural sediments) and between exposure scenarios (spiked water and spiked sediment) (Fig. 8). For

artificial sediment we obtained an LC_{50}-value of 16 pg/L in the spiked-water experiment, which is more than 600 times lower than LC_{50}-values reported for aquatic organisms (including fish) in other deltamethrin studies (Beketov, 2004; Bradbury & Coats, 1989; Ghillebaert*, et al.*, 1996; Hill, 1989; Mian & Mulla, 1992; Smith & Stratton, 1986) and way below the Maximum Permissible Concentration (MPC) of 300 pg/L calculated by Crommentuijn *et al*. (2000). Also the LC_{50}-value of 11 µg/kg (dw) obtained for the spiked artificial sediment is remarkably low, considering the proposed MPC value of 1.3 µg/kg (dw) (Crommentuijn*, et al.*, 2000). However, calculations of these environmentally safe concentrations are based on toxicity data determined for a few aquatic organisms, or, as for the MPC for sediment, extrapolated from results for water exposures by using the equilibrium-partitioning model. Based on our results, a recalculated MPC for sediment-exposed deltamethrin by using a safety factor of 100 (LC_{50}-value/100) would be twelve times lower, i.e. 0.11 µg/kg, whereas a recalculated MPC for water exposures would be more than three orders of magnitude lower, i.e. 0.16 pg/L than the original values. These findings underscore the need for more and better data on the toxicity of pesticides.

In contrast to the tests with artificial sediment, far lower toxicity was found in studies with natural sediment. High survival among *Chironomus* larvae was scored in both spiked-water and spiked-sediment experiments (Fig 8). The higher survival in natural sediment was in part attributed to the higher organic matter content of 12.5 ± 0.05%, which was some three-fold higher than that of the artificial sediments (4.1–4.8%). Hence, sorption to natural sediment was probably much more efficient than sorption to the artificial sediment, thereby decreasing the bioavailability and thus the toxicity. Additionally, deltamethrin degradation was much faster in the natural (50% left on day 0) than in the artificial sediment (more than 100% left on day 0), probably due to a lower microbial biomass in the artificial sediment than that commonly found in natural sediments (Goedkoop*, et al.*, 2005). Consequently, the nominal high-dose concentration of 166 µg/kg in spiked natural sediment corresponded to a calculated true exposure concentration of 83 µg/kg, i.e. still more than seven times higher than the LC_{50}-value of 11 µg/kg obtained in the test with spiked artificial sediment. The remaining high deltamethrin concentration in natural sediment, indicates that decreased bioavailability due to sorption to organic matter (Fleming, Holmes & Nixon, 1998; Ghillebaert*, et al.*, 1996; Hill, 1989; Maund *et al.*, 2002; Muir *et al.*, 1985; Yang *et al.*, 2006), and not compound degradation, was the main reason for the lack of deltamethrin toxicity to *Chironomus* larvae in this sediment.

One interesting aspect of this study was that sediment properties had a very strong impact on the outcome of the spiked-water tests. The toxicity in spiked-water exposures with artificial sediment was more than 8-times higher than in natural sediment. Apparently, the sorption of the test compound to the natural sediment or to dissolved organic matter in these test vessels was very efficient, thereby rendering deltamethrin largely unavailable. Additionally, similarly to the observed difference of deltamethrin degradation in artificial and natural sediments the degradation in the water column was likely much faster in test vessels with natural sediment, than in those with artificial sediment. Degradation of pyrethroids

in water is generally faster than in sediments (Muir, *et al.*, 1985). Consequently, both sorption and degradation may have contributed to the lack of toxicity of water-exposed deltamethrin in experiments with natural sediment.

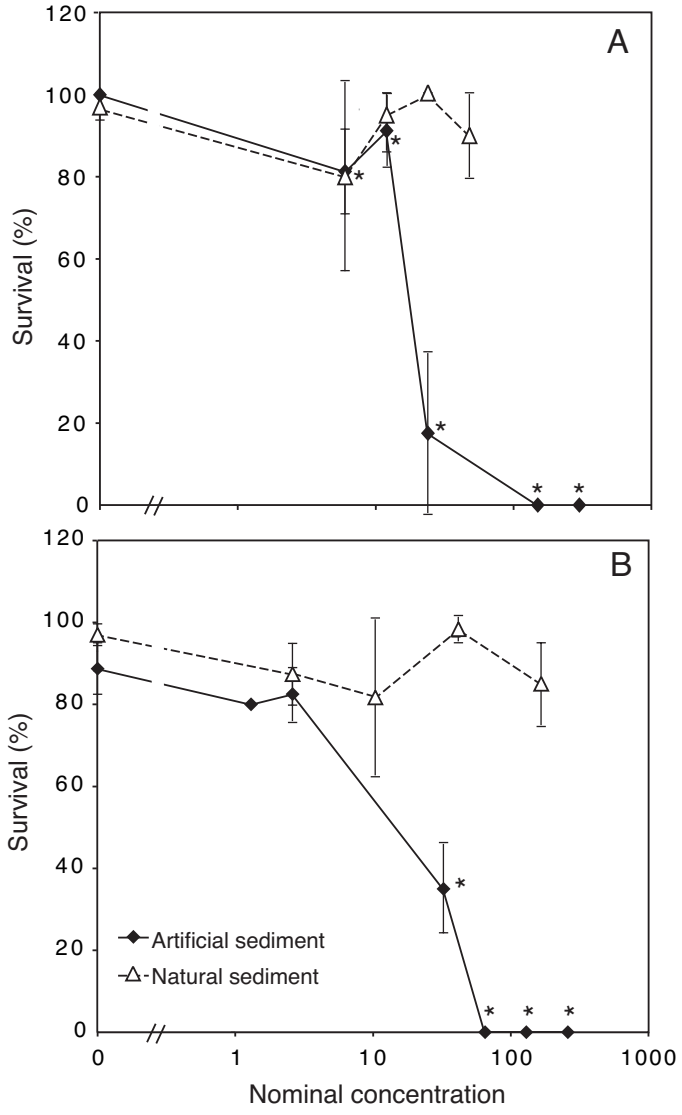

Fig. 8. Survival of deltamethrin exposed *Chironomus riparius* larvae in experiments with spiked water (pg/L) underlain with uncontaminated artificial or natural sediment (A) and in experiments with spiked artificial and spiked natural sediment (µg/kg) (B). Note that a significant decrease in survival across the deltamethrin concentration gradient only was detected in the experiments with artificial sediment. Values are mean ± SD and asterisks (*) denote significant differences from the controls.

The toxic action of deltamethrin in tests with artificial sediment was almost immediate, indicated by the lack of visual evidence of larval activity in the sediments (i.e. development of burrow structures) at concentrations exceeding 24 pg/L in spiked-water test and 33 µg/kg in the spiked-sediment test. Likely, there was a short time slot of pyrethroid action at the beginning of the test, i.e. shortly after the addition of the test compound, when 1st instar larvae were exposed (and killed) by exposure from water and/or pore water. Forbes and Cold (2005) found that even very brief exposures (1-h) to the pyrethroid esfenvalerate during early larval life affect *Chironomus* survival and development rate, lending support to this conjecture.

The high toxicity of deltamethrin (and many other pyrethroids) combined with its high detection limit relative to reported toxic concentrations, constitutes a serious problem for detecting toxic concentrations in the environment (e.g. in pesticide monitoring programs for inland waters). The detection limit for deltamethrin in sediment in our study, was 3-10 µg/kg (dw), which is slightly lower than our LC_{50}-value of 11 µg/kg dw. However, the quantification limit was 40 µg/kg, showing the difficulty in analyzing sediment-sorbed deltamethrin at concentrations close to the LC_{50}-value. Quantification of toxic deltamethrin concentrations in water is even more problematic, since the detection limit (30 ng/L) exceeds the LC_{50}-value of 16 pg/L by three orders of magnitude. Consequently, it is not possible to detect toxic water concentrations of deltamethrin using GC-MS techniques as in our study. A variety of alternative methods to detect deltamethrin have been developed (Fernández-Gutiérrez *et al.*, 1998; Lee *et al.*, 2002; van der Hoff *et al.*, 1996), however, none of these methods has yet a sensitivity that allows detection in the pg/L range. Consequently, only biological tests can provide the answers that chemical analyses and immunoassays fail to provide and are currently the only reliable tool to detect toxic concentrations of dissolved deltamethrin (and many other pyrethroids).

Exposure scenario effects on bioavailability (papers I-III)

In standardized sediment toxicity tests, either spiked-sediment or spiked-water is used as the exposure pathway, depending on the intended application of the test. The spiked-sediment scenario is intended to simulate accumulated levels of persistent chemicals in the sediment (OECD, 2004a) and the spiked-water scenario is intended to simulate pesticide spray drift events or accidental spills (OECD, 2004b). The pesticide distribution and behavior, and hence the bioavailability and the toxicity, will depend on which exposure scenario that is used (Fig. 9).

In a spiked-sediment scenario, the pesticide will be equally distributed in the sediment (Fig 9a). Hydrophobic pesticides will efficiently sorb to peat particles during the spiking process (paper II). Additionally, aging may occur during test vessel acclimating and continuously during the experiment (Conrad, Comber & Simkiss, 2002; Landrum, Eadie & Faust, 1992; Luthy, *et al.*, 1997) further decreasing the bioavailability and hence the uptake of dissolved lindane. In a spiked-sediment scenario, the water concentration, and hence the Tetraphyll

particle concentration, will therefore remain low due to sorption to sediment particles. Larvae will almost exclusively feed on uncontaminated Tetraphyll particles (paper I) and as a consequence, the major exposure pathway in these systems will be through pore water in the immediate surrounding microhabitat of the larvae (which probably occurs in paper III).

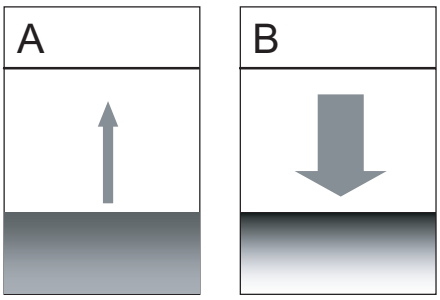

Fig. 9. (A) A spiked sediment scenario where the test compound is equally distributed in the sediment. The test compound continuously leaks from the sediment into pore water and overlying water. (B) A spiked water scenario, where a high concentration of the test compound sorbs to the surface layer of the sediment.

Conversely, in a spiked-water scenario a rapid redistribution of hydrophobic pesticides from the water phase to the very surface layer of the sediment (Fig. 9b) and to added food particles will occur (paper II and III). In paper III, the concentration of deltamethrin in the upper 1-mm surface layer of the sediment in a spiked-water scenario, was calculated to be 1 ng/kg dw (assuming that all deltamethrin sorbed to the surficial sediment, at the concentration of 16 pg/L = LC_{50}). This calculated sediment concentration is still more than three orders of magnitude lower than that at which toxic effects were detected in our experiment with spiked artificial sediment. The observed toxic response in the spiked-water experiment with artificial sediment is therefore probably not an effect of a toxic deltamethrin concentration of the surface sediment, but rather a consequence of uptake via water and/or pore water. Also for other pyrethroids, uptake via water is an important pathway (Conrad, Fleming & Crane, 1999; Fleming, Holmes & Nixon, 1998). Alternatively, added food particles could function as a vector for pesticide uptake when the test compound adsorbs to these food particles during sedimentation. This process will be quantitatively most important during the initial days of the test, when water concentrations are highest. In paper II, for example, it was calculated that 24% of initially added lindane sorbed to Tetraphyll particles and settle to the sediment surface during a 28 day spiked-water experiment (if 10 mg Tetraphyll is added per day according to OECD's guideline 219 (2004b)). Since most lindane is weakly bound to Tetraphyll, a large fraction is available for digestive uptake in *C. riparius* larvae. Therefore, Tetraphyll probably is an important vector for lindane uptake, since larvae almost exclusively feed on these particles (paper I), lending further support to these ideas. Consequently, spiked-water experiments result in two exposure pathways, since larval uptake from dissolved pesticides is important (paper II and III) and Tetraphyll may act as

vector (paper I and II). Hence, exposure scenario, i.e. spiked water or spiked sediment, is an important scene-setting factor for bioavailability in standardized toxicity tests and may have strong implications for the outcome of toxicity test results.

Effects and implications of sublethal pesticide concentrations on life history variables (Paper IV)

In this study we showed that sediment organic matter (OM) content and cypermethrin concentrations highly affected emergence, mean development rate, and adult size of *C. riparius* larvae. Emergence of adult midges ranged 70–100% and did not differ among controls and concentrations up to 0.8 µg/L in all OM-treatments. At the highest concentration tested, however, no adults emerged from treatment with 0% OM, while only two of twenty larvae made the hatch in a single replicate of the treatment with 5% OM. In the treatment with 20% OM, however, emergence at 3.2 µg/L was 26±11%. As expected, the cypermethrin toxicity was inversely related with the concentration of organic matter in the sediment (e.g. Fleming, Holmes & Nixon, 1998; Goedkoop & Peterson, 2003). Also mean development rate showed clear concentration effects at all three OM-levels, with lower mean development rates at 0.8 µg/L, than in lower concentrations across treatments. At concentrations of 3.2 µg/L only few larvae emerged and mean development rate was thus not calculated.

More interesting, however, are our observed effects on adult size (Fig.10). In the 0% OM treatment, adults of both sexes were larger at 0.8 µg/L than those in 0.05 µg/L and in controls. Also in the treatment with 20% OM, male midges were larger at concentrations of 0.8 µg/L than in controls. These larger adults co-occurred with significantly lower development rates at 0.8 µg/L, implying that the larvae had longer time to develop and thus could grow larger. These results show that exposure of low cypermethrin concentrations affects normal larval development, which have implications for later life stages. Males more easily respond by increased somatic growth than females, possibly due to a lower allocation of assimilated nutrients and energy to reproduction than females (Atchley 1971). Contradictory, allocation of assimilated toxicants to eggs will efficiently contribute to a higher tolerance of female larvae to toxicants (e.g. Medina *et al.*, 2002). We speculate that these sex-specific sublethal effects could contribute to a skewed distribution between sexes in natural populations, and subsequently decrease mating success and ultimately population densities. These scenarios call for the further development of sublethal response variables, like for example physiological biomarkers. However, the ecological significance of any such suborganismal, physiological responses to toxicants is relevant only when it can be related to the fitness of individuals and/or detrimental population-level effects.

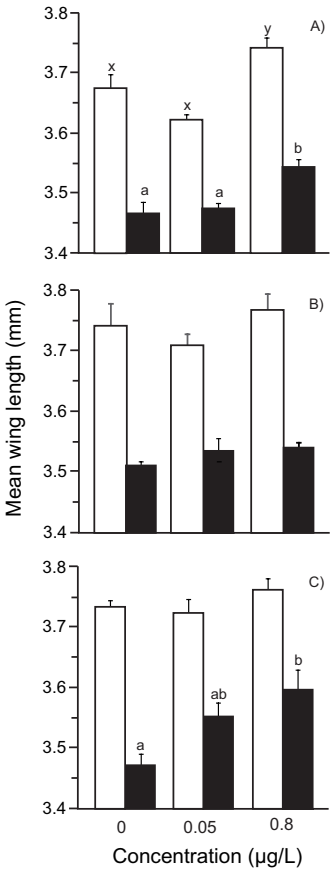

Fig. 10 Mean female (white bars) and male (black bars) wing length (mm) for *Chironomus riparius* larvae exposed to dissolved cypermethrin in artificial sediments containing 0 (A), 5 (B), and 20% organic matter content (C). Error bars represent standard errors (n=4). Letter codes denote significant differences, i.e. treatments not having letters in common differ significantly (Tukey-Kramer HSD tests).

Main conclusions and future perspectives

- Added food particles act as vectors for contaminant uptake in test organisms and confound the interpretation of sediment toxicity test results. Since spiked-sediment tests are intended to simulate accumulated levels of contaminants in the sediment, additions of plant material to the sediment prior to the start of the experiment, instead of regularly food additions, are recommended.

- Pesticide sorption to organic particles in standardized toxicity tests is fast and efficient. The binding strength to theses particles, however, is weak allowing for substantial desorption. Consequently, digestive uptake likely occurs simultaneously with uptake from water in spiked-water scenarios. In spiked-sediment scenarios, the test compound initially sorbs to the sediment, which then acts as a reservoir, continuously supplying the pore water with low pesticide concentrations.

- Low-dose pesticides, for example pyrethroids, are frequently toxic in concentrations lower than the detection limits for high-tech analytical methods, having implications for environmental monitoring of such compounds. Consequently, only biological tests can provide the answers that chemical analyses fail to provide and are currently the only reliable tool to detect toxic concentrations of deltamethrin (and many other pyrethroids).

- Sublethal pesticide concentrations affect *C. riparius* life history variables, having implications for mating success and ultimately population densities. A further development of sublethal response variables, like for example physiological biomarkers, is therefore important to be able to study the ecological significance of these effects.

Svensk sammanfattning

Bekämpningsmedel sprids, till skillnad från andra miljögifter, avsiktligt ut i naturen. De har dessutom en inneboende giftverkan. Avdunstning, ytavrinning, och läckage genom marken ned till grundvattnet gör att de sprids och transporteras vidare till sjöar och vattendrag och resthalter detekteras regelbundet, både i den fria vattenmassan och i sediment. De organismer som lever i sedimentet, t.ex. mygglarver, är betydelsefulla för ekosystemets funktion. De tar t.ex. vara på den näring som finns i sedimentet och återför den till organismer högre upp i näringskedjan. Även miljögifter kan på detta sätt tas upp i sedimentlevande organismer och föras vidare från sedimentet till t.ex. fisk och människa. Bekämpningsmedel som hamnar i sjöar kan därmed påverka hela ekosystemet genom att t.ex. slå ut viktiga sedimentlevande arter eller genom att transporteras uppåt i näringskedjan och därmed ge effekter på en högre nivå. Den biologiska effekten beror dock helt på biotillgängligheten av bekämpningsmedlet, d.v.s. hur effektivt det kan tas upp av organismer.

Jag har studerat biotillgängligheten av bekämpningsmedel i larver av fjädermyggor. I mina försök har jag undersökt hur larvernas ätbeteénde, bekämpningsmedlets bindningbenägenhet till sediment- och matpartiklar och sedimentets egenskaper påverkar biotillgängligheten. Jag har gjort laborationsstudier där faktorer som t.ex. ljus och temperatur kan styras noggrant, och jag har till stor del följt de standardiserade tester som finns tillgängliga för riskbedömningar av kemikaliers miljöpåverkan. Mina studier bidrar till en ökad förståelse för hur kemikalier uppför sig i dessa standardtester och därmed en ökad insikt i hur man bör tolka resultaten från dessa försök.

I den första studien visade jag att fjädermygglarver väldigt effektivt väljer vad de vill äta. I standardtesterna förses larverna regelbundet med näringsrikt akvariefisk-foder. I försöken visade det sig att larverna nästan uteslutande åt av fiskmaten och i princip inte alls ifrån testsedimentet, vilket skulle kunna innebära

att testkemikaliens effekter underskattas. Man kan också tänka sig att testkemikalien binder till fiskmaten, och att larverna på så sätt får i sig höga koncentrationer som resulterar i negativa effekter, t.ex. ökad dödlighet eller fördröjd utveckling.

I den andra studien visade jag att lindan (ett klassiskt bekämpningsmedel som ofta detekteras i sediment) mycket snabbt och effektivt binder till de organiska partiklar som finns närvarande i standardtesterna, d.v.s. till torv och fiskmat. Bindningen är dock väldigt svag, vilket innebär att larverna bör kunna ta till sig det lindan som finns på partiklarna när de äter, d.v.s. lindan lossnar och tas upp i mag-tarm-kanalen. Upptag via huden från det lindan som var löst i vattnet var dock större än upptaget via mag-tarm-kanalen från partiklar. Slutsatsen från detta försök var att om testkemikalien tillsätts till vattnet, så kan upptag ske både genom huden och mag-tarm-kanalen. Om testkemikalien tillsätts till sedimentet däremot, så kommer det att binda effektivt till sedimentpartiklarna vilket minskar biotillgängligheten av det lösta lindanet.

I den tredje och fjärde studien, visade jag att de moderna insektsmedlen deltamethrin och cypermethrin är giftiga i mycket låga koncentrationer. Giftigheten är dock helt beroende av vilket sediment som används i testerna. Sediment med hög organisk halt ger lägre giftighet, eftersom bekämpningsmedlet binder till de organiska sedimentpartiklarna. Vid låga koncentrationer överlever mygglarverna, men de utvecklas långsammare och de blir större än de som inte exponeras. Sådana effekter är svåra att upptäcka i naturliga populationer, men de kan ge långsamma förändringar som så småningom påverkar hela ekosystemet.

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Acknowledgement

Jag har tillbringat sju år på Institutionen för Miljöanalys (IMA). Ibland känns det som en väldigt lång tid och ibland känns det som om det har gått alldeles för fort. Det är lätt att trivas när man omringas av inspirerande och trevliga människor, och jag har många jag vill tacka för den tid som jag tillbringat på IMA.

Jag vill börja med att tacka min huvudhandledare **Willem Goedkoop**. Från första stund fick jag ett vänligt mottagande, och sedan dess har du alltid funnits till hands – en väldigt viktig egenskap som handledare! Du har uppmuntrat, stöttat, tipsat, rättat och ifrågasatt. Du är fantastiskt entusiastisk och alltid full med idéer. Om jag mot förmodan skulle skriva en avhandling till, så skulle jag välja dig som handledare, utan tvekan.

Tack också till **Henrik Kylin**, min biträdande handledare, som varit till stor hjälp när jag haft frågor om organisk miljökemi, och som gav mig chansen att få undervisa på organiska miljökemikursen.

Mina övriga medförfattare, **Christina Arbjörk** och **Mikael Hedlund**, för trevliga timmar i labbet, inte minst i klimatrummet i källaren, **Tobias Nilsson**, för att du tog väl hand om mig i Lund. **Nicole Spann** – Tausund Dank für viele schöne stunden im labor und für tolle zusammenarbeit. Hoffentlich sehen wir uns bald wieder!

Jag vill tacka alla **gamla och nya doktorander**, för gemenskapen, doktorandresan, alla spex, höghöjdsäventyr, trevliga fikastunder, osv, osv. Ett speciellt tack till mina forna rumskamrater, **Anneli Widenfalk**, under åren har vi delat rum, kämpat på lab, åkt på konferensresor, klättrat i träd och granskat manus tillsammans. Miljöanalysen är sig inte riktigt lik sedan du försvann och jag har saknat dig. Snart

blir vi kollegor igen! **Staffan Åkerblom**, vi har kämpat sida vid sida bokstavligt talat, och du hann först! Men jag kom inte långt efter. Tack för alla kappa-tips och för din spontanitet. **Anna Lundqvist**, för att du håller mig uppdaterad. Nu vet jag vad stana och messa är. Få har nog skrattat så mycket på en Mälaren-runt-tur som du och jag. Det var kul!

Staffan Bengtsson och **Markus Meili**, tack för att ni granskade mina manus inför halvtidsuppföljningen.

Tack också till alla hjälpsamma människor på IMA, hela **OMK** med **Åsa, Gunborg, Märit, Eva, Kerstin och Jenny**, under alla dessa år har jag sprungit hos er titt som tätt med prover och frågor och alltid fått snabb hjälp. Ni är fantastiska! Jag har också fått mycket hjälp av geokemilab. och av biologlab.- Tack! **Mikael Östlund**, jag är glad över att vi ”bott” mittemot varandra en tid. Tack för alla figurtips och för alla tusen svar på mina tusen frågor. **Ewa Bringmark**, en omtänksam och mycket sympatisk prefekt. Du står vid rodret och skeppet seglar vidare. För tillfället hoppar jag iland, men vem vet – kanske hoppar jag ombord igen.

Tack till Apollo Books som lät mig använda deras fina bild på *Chironomus* till framsidan på avhandlingen.

Det finns även många utanför IMA som jag vill tacka, **Magnus Peterson**, för ett trevligt plattfiskdyk och för att du tog väl hand om mig på Gotland Tack också för att du under lång tid därefter försåg mig med prover från Smöjen. **Erland Björklund**, för att du ställde upp till 100% när jag kom till Lund för att köra SFE. **Inger Juremalm**, som kämpade tappert med att få fram stabila isotoper från mina något udda larv- och larvtarmprover, **Elisabet Börjesson**, som alltid svarat på mina frågor om scinträknaren. **Bernt Andersson**, som var till stor hjälp på kurslaborationerna, **Ulla Edberg**, för att du var en perfekt mentor. När jag tänker tillbaka på våra samtal inser jag att du har gett mig en hel del insikt, om både det ena och det andra.

Livet består av mer än att skriva en avhandling, därför vill jag även tacka mina vänner och släktingar. **Sara Karlsson**, för att du fått med mig på som många dykäventyr. Krabborna i klipptaket på nattdyket i Strömstad och sköldpaddorna på Curacau kommer jag aldrig att glömma! **Karin Olsson**, för bra laxsamarbete och för alla trevliga arbetspass och samtal på kolonilotten, Stora familjen, **mamma** och **Leffe, pappa** och **Carina** och **brorsan** och **Malin**. Ni har alltid betytt mycket för mig och gör det fortfarande. Ibland önskar jag att ni fanns lite närmare. Ljusdals- och Sundsvallsfamiljen, **Birgitta** och **Bertil, Tord** och **Wenche** och **Jonas, Åsa, Oskar, Olle och Hilma**. Ni får mig alltid att känna mig hemma hos er.

Sist men inte minst, stort tack till min alldeles egna underbara familj! **Per, Elsa** och **Vera,** ni är de viktigaste i mitt liv, och jag lovar att ni ska få se mer av mig framöver. Jag älskar er!