Fate of contaminants in Baltic Sea sediment ecosystems: 
the role of bioturbation

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Till mamma och pappa
ABSTRACT

Aquatic sediments are of major importance for the cycling of environmental pollutants, acting as both sinks and secondary sources of contaminants to the ecosystem. Sediment-living organisms can affect the fate and transport of contaminants through activities like feeding and burrowing, collectively called bioturbation. Apart from high contaminant levels, the Baltic benthic ecosystem is affected by stressors such as eutrophication-induced anoxic conditions and invading alien species. The main objectives of this thesis were to determine the effects of bioturbation on contaminant fluxes in Baltic Sea sediments and to increase the understanding of how these other stressors act together upon contaminant fate in the benthic ecosystem.

Bioturbation affected contaminants in a species-specific way. The native species Monoporeia affinis and Macoma balthica increased the incorporation of BDE-99 and Cd deposited on the sediment surface, enhancing their retention in the sediment. The invasive polychaete Marenzelleria sp. did not contribute to the incorporation of surface-deposited contaminants, however, significantly increased the release of contaminants back to the water column. Reoxygenation of anoxic laminated sediments and bioturbation by Marenzelleria increased the sediment-to-water flux of dissolved organic contaminants. When the bioturbation-driven release of PCB was compared to the release caused by physical sediment resuspension, results indicated that the continuous activities of benthic infauna can be just as, or even more, important than physical disturbance for the remobilization of sediment-bound contaminants. Bioaccumulation was significantly higher when contaminants were deposited associated to phytoplankton compared to lignin or sediment, suggesting that there are likely seasonal differences in the mobilization of contaminants in the benthic ecosystem.

In summary, bioturbation is an important process influencing contaminant fate in Baltic Sea sediments, and the risk of remobilization of historically buried contaminants may increase with improved benthic redox conditions and the invasion of new deeper-digging species, such as Marenzelleria.
This thesis is based on the following five papers, referred to in the text by their roman numerals.


III  Hedman JE, Stempa Tocca J, Gunnarsson JS (Manuscript). Remobilization of PCB from Baltic Sea sediment: comparing the roles of bioturbation and physical resuspension. *Submitted to Environmental Toxicology & Chemistry*.


V  Hedman JE, Gunnarsson JS, Samuelsson G, Gilbert F. Sediment particle reworking and solute transport by two common polychaetes in the Baltic Sea, *Nereis diversicolor* and *Marenzelleria* sp. (Manuscript)

My contribution to the papers: (I) – experimental design and execution, major part of the chemical analyses, data analysis, most of the writing. (II) – experimental design and execution, participated in the chemical analyses and writing. (III) – experimental design, major part of the experimental execution, chemical analyses and data analysis, all writing. (IV) – contaminant analysis (GC-ECD) of extracts, participated in data analysis and writing. (V) – participated in the experimental execution, bromide analysis, most of the writing.

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INTRODUCTION

An extensive amount of organic and metal contaminants has been released into the environment as a consequence of the worldwide industrialization and urban development. To determine and predict the risk of adverse effects of these contaminants in the ecosystem, including human health, it is important to have a good understanding of the processes that affect their environmental fate, i.e. their distribution between media (e.g. air, water, soil/sediment) and ultimate removal from the ecosystem. Generally, the fate of a contaminant in the environment can be assessed by its physicochemical properties. However, the environment is inherently dynamic, both in space and time, with complex interactions between abiotic and biotic processes that make the fate of a contaminant increasingly difficult to predict (and a lot more interesting!) when including ecological factors.

Aquatic sediments are of major importance for the global cycling of environmental contaminants. Many persistent organic pollutants (POPs) and metals have a strong affinity for particles and tend to accumulate in the seabed. Their burial deeper into the sediment matrix is an important process in removing them from the ecosystem, and sediments are thus often their final deposit. Nevertheless, the large amount of contaminants stored in these sediments clearly also makes them potential secondary sources to the ecosystem. Sediment-living organisms have a profound influence on the biogeochemical properties of their habitat through activities such as feeding and burrowing, collectively called bioturbation (Aller 1982). This also significantly affects the bioavailability, degradation and transport of sediment-associated contaminants (Forbes & Forbes 1994).

Scope and aims of the thesis

This thesis focuses on the role of benthic macrofauna for the fate of contaminants in Baltic Sea sediment ecosystems. The Baltic Sea suffers from several anthropogenic stressors, including contamination by hazardous substances and eutrophication, as well as the invasion of non-indigenous species. Separately, each of these stressors has caused adverse effects in the Baltic ecosystem but there are also interactive effects that influence the fate of contaminants in the benthic system. The main aims of the thesis were to determine the effects of bioturbation on
contaminant fluxes in Baltic Sea sediments and to increase the understanding of the complex interactions between ecological (e.g. bioturbation, organic matter cycling) and physicochemical processes (e.g. contaminant partitioning) when assessing contaminant fate in aquatic ecosystems.

The thesis is based on 5 experimental studies, paper I-V. In the first two papers (I, II) the interactive effects of macrofauna activity (bioturbation and feeding) and settling organic matter for the fate of newly deposited contaminants were examined. The aim was to investigate how organic matter of different nutritional qualities would stimulate infaunal feeding and bioturbation and thereby affect the overall fate of the associated contaminants. Contaminants used in paper I and II were the metal cadmium and the brominated flame retardant BDE-99. The following two papers (III, IV) investigated the role of sediment as a source of historically buried contaminants. In paper III the effect of bioturbation by common Baltic infauna was compared to simulated physical resuspension for the sediment-to-water release of a polychlorinated biphenyl (PCB). In paper IV the aim was to study the effects of reoxygenation and recolonisation on the release of POPs from naturally contaminated sediments, imitating the recovery from eutrophication induced anoxic bottom water conditions. In paper I-IV, three common Baltic species with different functional traits were used: the amphipod Monoporeia affinis, the clam Macoma balthica, and the polychaete Marenzelleria sp. The polychaete genus Marenzelleria is invasive in the Baltic Sea and has changed the benthic community structure in many areas. In order to gain more knowledge about the mode and rate of bioturbation by this worm, the mechanistic transport of particles and solutes by Marenzelleria sp. and another polychaete, Nereis (Hediste) diversicolor, was investigated and compared in paper V.

1 In this thesis, fate refers to the distribution of contaminants between compartments making up the sediment ecosystem, simplified as sediment, overlying water and organisms. Since all work has been done with POPs or a trace metal (cadmium), a discussion of contaminant degradation or metabolism is not included here, although for many pollutants (even for POPs on longer time scales) it may be the most important process for detoxification and removal from the ecosystem.
Contaminants enter the aquatic environment through atmospheric deposition or via rivers, land run-off and direct discharges. Air-water exchange is the dominating process in the open ocean, and the association to sinking particulate organic matter is a driving mechanism of contaminant removal from the water column (Wania & Daly 2002). Contaminants entering with land discharges are often already sorbed to suspended particles and their immediate fate in coastal waters is thus affected by the hydrological transport and deposition of particles. The natural process of sedimentation and the high particle affinity of contaminants result in the accumulation of contaminants in aquatic sediments, often to concentrations several orders of magnitude higher than in water, which makes sediments important sinks.

**Contaminant properties and partitioning between media**

The behavior of a given contaminant in the environment is largely controlled by its physicochemical properties (Schwarzenbach et al. 2003). Based on these properties it will interact/react with various environmental constituents and partition between compartments (e.g. air, water, sediment). This is important for environmental transport processes and resistance to degradation, as well as for the bioavailability and potential toxicity to living organisms.

Many different compound classes belong to the group of POPs. Some of the most well-known members are chlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), dioxins, and organochlorine pesticides like DDT. Recently a range of brominated organic compounds has been added to the list, e.g. the polybrominated diphenyl ethers (PBDEs) commonly used as flame retardants (de Wit 2002). POPs have a high resistance to chemical and biological degradation and consequently a long environmental half-life. They are often nonpolar compounds with a low solubility in water but with a high affinity for solid phases (Schwarzenbach et al. 2003). In the aquatic environment, therefore, they rapidly escape the water phase by adsorbing to (adsorption) or diffusing into particles (absorption), both processes are jointly called sorption (Pignatello & Xing 1996). Organic matter (OM) is generally the most important sorbent for hydrophobic organic contaminants (HOCs) in the environment (Karickhoff et al. 1979). There is
a large variety in the sorption properties of OM for HOCs and much work has been
done to examine how the OM structure affects sorption/desorption processes
(Grathwohl 1990, Pignatello & Xing 1996, Cornelissen et al. 2005). Generally, it is
suggested that contaminants are more strongly associated with aged and/or
condensed OM than with fresh amorphous OM (Allen-King et al. 2002, Huang et
al. 2003). In addition, the sorption strength of HOCs generally increases with
increasing $K_{ow}$ - a measure of compound hydrophobicity (Karickhoff et al. 1979,
Chou & Griffin 1986), which has important implications for the environmental
transport and bioavailability of individual HOCs.

Partitioning of metal contaminants (such as Cd, Zn, Hg, Pb) in the aquatic
environment is a more complex process than for HOCs because metals can
associate with both organic and inorganic elements in several different ways, e.g. by
adsorption and complexation, as well as co-precipitate to form discrete particulates
(Santschi et al. 1997). All of these processes are highly sensitive to changes in the
abiotic environment, such as redox state, pH and salinity (Chapman et al. 1998). In
aerobic environments, adsorption to OM and iron and manganese
oxides/hydroxides is the predominant process, while under anoxic conditions the
formation of insoluble metal sulfides controls the dissolved fraction (Di Toro et al.
1990).

**Transport processes at the sediment-water interface**

**Chemical and physical processes**

Sedimentation and resuspension of particles are important processes affecting the
fate of contaminants in benthic environments. In the absence of mixing (physical or
biological), continuous deposition of new sediment layers will eventually isolate
contaminants in deeper layers and reduce the risk of remobilization and exposure to
organisms. If the newly deposited material is clean or holds significantly lower
contaminant concentrations, the sediment quality will gradually improve (Wang et
al. 2004). However, before reaching an area where the hydrographical conditions
are stable enough to promote long-term accumulation, particles usually go through
a series of resuspension and relocation events (Jonsson 2000). Physical
resuspension and advective transport of contaminated sediment particles are key
processes in the large-scale fate of contaminants, and numerous field studies have provided evidence for the importance of sediment resuspension for the transport and internal recycling of HOCs and trace metals in aquatic ecosystems (Baker et al. 1985, Ko et al. 2003). Apart from the mass transport of particles and associated contaminants, resuspension also increases the risk of contaminant exposure to pelagic organisms (Eggleton & Thomas 2004). Resuspended sediment particles are exposed to a new chemical environment which may promote desorption and release of associated contaminants into the aquatic phase as truly dissolved chemicals (Gong et al. 1998).

The release of dissolved contaminants from sediment to water involves three steps; 1) desorption from particle to pore water, 2) transport to the sediment-water interface, and 3) diffusive transport over the boundary layer (Fig 1). The slowest step in this sequential process determines the rate of release (Ortiz et al. 2004). For POPs, slow desorption is often the limiting factor in the release, particularly for very hydrophobic compounds, while for metals, partitioning is largely a function of the sediment redox condition. Undisturbed sediments usually consist of a thin (mm) oxidized surface layer over anoxic bulk sediment, which is due to the rapid consumption of oxygen penetrating from the overlying water by chemical and microbial processes in the sediment. The pore water concentration of metal contaminants in those sediments is thus largely controlled by the presence of sulfides and OM (Morse & Luther 1999). Adveective pore water transport of solutes within the sediment and to the sediment-water interface can be of great importance in permeable sediments, however, in fine-grained cohesive sediments, which is where contaminants often accumulate, adveective pore water transport is negligible and is instead dominated by molecular diffusion (Huettel & Gust 1992). Before reaching the overlying water, contaminants have to pass through the diffusive benthic boundary layer (DBL), a sub-layer close to the sediment surface where turbulent mixing of the overlying water is approaching zero and compounds can only pass this thin boundary layer by molecular diffusion (Boudreau 2001). Diffusion over the benthic boundary layer is driven by the concentration difference between the sediment pore water (C_{pw} [ng L^{-1}]) and the overlying water (C_w). The flux (N [ng m^{-2} d^{-1}]) of a dissolved contaminant from sediment to water can therefore be expressed by:
\[ N = K_f \cdot (C_{pw} - C_w) \]

Where \( K_f \) is the mass transfer coefficient (MTC [cm d\(^{-1}\)]) describing the rate of diffusion over the boundary layer, which is inversely proportional to the length of the diffusive path, i.e. here the thickness of DBL (usually < 1 mm). The boundary layer thickness is affected by several physical factors, such as current velocity and sediment surface roughness, thus also altering the rate of transfer. MTCs are determined empirically in laboratory experiments or field studies, and are often used in chemical-fate models to predict the flux of dissolved contaminants from sediment to water (Erickson et al. 2005).

![Fig 1. Schematic representation of soluble release of PCB from sediment to water.](image)

**Macrofauna activities**

There is a tight coupling between the organisms making up the benthic community and the biogeochemical properties of the sediment, also affecting the fate of deposited contaminants (Forbes & Forbes 1994). Sediment-living organisms have developed a wide variety of different behavioral and feeding strategies in order to adapt to and interact with their living environment. Bioturbation is the reworking of the upper sediment layer (typically top 10 cm) by infauna from activities like feeding, burrowing and the creation and ventilation of biogenic structures (e.g. tubes, burrows). This biological mixing and transport of particles and solutes profoundly affects various chemical and microbial processes within the sediment, such as organic matter decomposition and inorganic nutrient regeneration, as well as physical characteristics like porosity and sediment surface topography (Kristensen et al. 1992, Aller & Aller 1998, Reise 2002). Benthic organisms can be
divided into functional groups based on how they mix particles and on whether and how they irrigate the sediment (Francois et al. 2002, Gerino et al. 2003). Some species randomly mix the sediment as they freely move around searching for food (i.e. biodiffusors), whereas others are stationary and transport particles unidirectional from one place to another (‘non-local transport’), e.g. by feeding at depth and defecating digested material on the sediment surface (i.e. conveyors). This mixing and transport of particles generally increase the rate of burial of surface deposited contaminants and thus potentially enhance their retention in the sediment (Petersen et al. 1998, Sandnes et al. 2000). On the contrary, they may also bring back old pollutants to the top sediment layer, which slows down the burial and increases the risk of remobilization to the water (Ciarelli et al. 1999). Particle-biodiffusion can also significantly increase the erodability of the sediment and contribute to a higher release of contaminants even at low shear stress (Ciutat et al. 2006). Many species create permanent or semi-permanent tubes and burrows that extend well into the anoxic bulk sediment. To meet respiratory needs and to avoid toxic effects from exposure to sulfides in the anoxic pore water, these organisms must ventilate their burrows (i.e. bio-irrigators) with oxic water from above the sediment surface (Riisgård & Larsen 2005). This creates oxic micro-environments within the sediment matrix that can oxidize metal sulfides and trigger microbial mineralization of OM in the sediment (Heilskov & Holmer 2003), both of these processes can increase the release of sorbed contaminants. The creation of burrow structures extends the sediment-water surface area considerably and exposes previously buried particle-associated contaminants at depth, increasing the area available for diffusional processes (Aller & Aller 1998). Active burrow ventilation, in addition, intensifies this process by creating turbulent (eddy) diffusion within the burrow structures, and an advective transport of soluble contaminants over the sediment-water interface (Thibodeaux et al. 2001).

**Uptake in organisms**

Infauna are exposed to contaminants via several different routes, including pore water and overlying water, as well as through the ingestion of contaminated sediment particles. Generally, only truly dissolved contaminants can diffuse across the cell membrane and the bioavailable fraction is consequently positively related to the aqueous concentration of the compound (Landrum & Robbins 1990, Worms et
al. 2006). Uptake over the body surface or respiratory organs via interstitial or overlying water therefore depends a lot on the partitioning properties of the contaminant and the sediment OM content. For very hydrophobic (log $K_{ow} >6$) organic contaminants and many metals uptake via food is suggested to be the most important route for deposit-feeding organisms (Leppänen & Kukkonen 1998, Griscom et al. 2002). Dietary assimilation of contaminants by animals, however, also assumes that the contaminant desorbs from the ingested material in the digestive tract and is subsequently absorbed by the gut epithelium. Several factors within the gut can influence the rate and extent of desorption, e.g. pH and enzymatic activity of the digestive fluid, and contaminants that are not absorbed by the organism are transported back to the sediment with the feces (Weston & Maruya 2002). Bioaccumulation is the net accumulation of a compound as a result of uptake from all routes of exposure and loss from processes like metabolism and elimination (Landrum et al. 1996). The simplest way to determine bioaccumulation in infauna is to relate the steady state contaminant concentration in the animal to the total concentration in the sediment and express it as a bioaccumulation factor, BAF. This obviously does not explain any of the underlying mechanisms of bioaccumulation and several models based on the understanding of contaminant partitioning have been developed to increase the possibility to accurately predict bioaccumulation. For HOCs, the equilibrium partitioning theory (EPT) assumes that partitioning between the organism and the sediment is regulated by the organic carbon content of the sediment, pore water concentration and lipid content of the organism, which at equilibrium should result in a more or less constant biota-sediment accumulation factor (BSAF) (Di Toro et al. 1991). Recently, much attention has been given to the importance of also including the variability of organic matter composition in these models (Cornelissen et al. 2005). The partitioning and assimilation of metals is generally more complex than for HOCs and other steady state models have been developed for metals, e.g. the free ion activity model (FIAM) and the biotic ligand model (BLM), which include environmental variables like dissolved OM, cation concentrations and pH (Niyogi & Wood 2004, Luoma & Rainbow 2005).

Still, many models fail to accurately predict the bioaccumulation of contaminants by deposit-feeding infauna. This highlights the importance of other factors, such as
the feeding ecology and physiology of the organisms, as well as the coupling between their behavior and sediment biogeochemistry (cf. contaminant partitioning) (Forbes & Forbes 1994, Leppänen & Kukkonen 2006). Most deposit-feeders are selective feeders, choosing particles with high organic carbon content and nutritious value (Jumars & Wheatcroft 1989). Moreover, accumulation depends on e.g. the ingestion rate, digestive system and assimilation efficiency of the particular species (Mayer et al. 1996). Uptake and bioaccumulation of contaminants by benthic invertebrates can cause adverse effects, like behavioral changes, impaired reproduction or death (Bombardier & Bermingham 1999). In this thesis, however, bioaccumulation is not related to toxic effects but rather treated as a vector of contaminants re-entering the ecosystem.

**WHAT’S SO SPECIAL ABOUT THE BALTIC SEA?**

The semi-enclosed Baltic Sea is one of the world’s largest brackish water bodies and is characterized by unique hydro-geographical and ecological features. It consists of a series of basins separated by shallow sills and the average water depth is ca. 60 m. A stable salinity gradient progresses from almost limnic conditions (2-3 psu) in the north to near marine (>20 psu) in the Danish Straits, where the Baltic connects with the North Sea. The intrusions of dense saline water in the south and the large riverine input of fresh water creates a halocline at 60-80 m in most of the Baltic Sea (Matthäus & Schinke 1999). The water below the halocline is permanently or periodically subjected to hypoxia/anoxia as a consequence of infrequent saltwater intrusions, and the deep water residence time in the Baltic Sea is more than 30 years (Meier et al. 2006). The brackish water Baltic Sea has a low species diversity and functional complexity (Bonsdorff & Pearson 1999). The Baltic biota consists of mainly euryhaline species originating from either marine or freshwater environments. The macro-zoobenthic infauna is dominated by a few species, primarily small-size shallow dwelling organisms (Laine 2003). The benthic community structure has been described as being at an early successional stage (Rumohr et al. 1996, Bonsdorff 2006), i.e. it is largely dominated by opportunistic, stress-tolerant small organisms (Rosenberg 2001). For example, in the deeper soft bottoms there is a lack of deep burrowing and subsurface deposit feeders.
(Bonsdorff & Pearson 1999), and large taxonomic groups (such as echinoderms) important in marine environments are totally absent from the southern Baltic Proper and northwards (Elmgren 1984). As a consequence, the bioturbation mixing depth in the Baltic Sea is generally shallow (<5 cm).

During the last 50 years, the Baltic Sea ecosystem has been under severe anthropogenic stress, including hazardous chemicals, eutrophication and overfishing (Elmgren 2001). The Baltic Sea is enclosed by heavily industrialized countries and more than 85 million people live within its drainage area. Although monitoring indicates that the load of hazardous substances has been reduced considerably over the past 20-30 years, problems persist (HELCOM 2008). For example, the concentration of many contaminants in Baltic biota is high compared to organisms in other marine environments (Berggren et al. 1999), several tons of POPs and metals are stored in the Baltic sediments (Jonsson et al. 1990, Borg & Jonsson 1996), and current levels of planar-PCBs and dioxins in many Baltic fish species are exceeding the limit for human consumption set by the European Union (Isosaari et al. 2006). The present-day main sources for some of these pollutants are unclear, suggesting diffusive sources (Agrell et al. 2001). Although atmospheric deposition probably is a major route of entry for new contaminants, another potential source to the Baltic biota is remobilization from the large sediment deposits and via food-web transfer from predation on infauna. Eutrophication in the Baltic Sea has, as a secondary effect, led to an increased sedimentation rate of OM and to decreased oxygen levels in benthic environments when the OM is mineralized. Consequently, bottom areas with prevailing hypoxic/anoxic conditions have been extended, particularly in deep areas below the halocline but also in shallow coastal areas, leading to large-scale depletion of benthic infauna (Diaz & Rosenberg 1995, Karlson et al. 2002). Recently, attention has been given to a potentially new and increasing problem in the Baltic, i.e. the invasion of non-indigenous species (Leppäkoski et al. 2002). For example, the establishment of the deep-burrowing (>35 cm) polychaete genus *Marenzelleria* has significantly changed the benthic community structure in many areas (Zettler et al. 1995, Perus & Bonsdorff 2004). However, the question remains as to whether it will have a positive or negative effect on the Baltic Sea ecosystem.
METHODS AND STUDY ORGANISMS

The sampling of sediment, water and organisms for all studies (except IV) in this thesis was done in the southern Stockholm archipelago, NW Baltic Proper. *Monoporeia affinis* and *Macoma balthica* were collected with a benthic sledge (>20 m; I-III), *Marenzelleria* sp. and *Nereis diversicolor* were sieved out on-site from sandy sediment (1 m; I-III, V). Sediment for paper IV was collected in the inner Stockholm Archipelago at 30 m depth and *Marenzelleria neglecta* in the Darss-Zingst Estuary, Germany.

Experimental systems

All experiments were performed under controlled laboratory conditions using acrylic tube core microcosms (8 cm Ø) with either sieved sediment (I-III, V) or intact natural sediment cores collected from a ship and brought to the laboratory (IV). In paper I, II and V the systems were kept static. In paper III and IV individual water re-circulating systems were created, passing the overlying water through a series of filters; i.e. first two glass fiber filters (GFs) for the collection of particle-associated organic contaminants and then two polyurethane foam filters (PUFs) in order to retain the soluble fraction (Fig 2). The physical resuspension in paper III was generated by a motor-driven paddle, run twice a week.

![Fig 2. Schematic figure of experimental units used paper III.](image)
Contaminants, organic material and tracers

The studied contaminants were either artificially added (spiked) radio-labeled compounds (I-III) or ‘natural’ environmental pollutants present in the collected sediment (IV). In paper I and II, the brominated flame retardant $^{14}$C-BDE-99 (2,2’,4,4’,5-pentabromodiphenyl ether) and metal $^{109}$Cd were added to the sediment surface spiked to different types of organic matter (OM). In paper III, sediment was spiked with a tri-chlorinated PCB, $^{14}$C-PCB-32 (2,4’,6-trichlorobiphenyl), and added to each core as a 3 cm layer on top of underlying clean (un-spiked) sediment (III). The advantages of using radio-labeled compounds are e.g. the possibility to study the fate of a specific contaminant and to use concentrations well below reported toxic levels due to high analytic detection capacity. It is also a relatively cost- and time effective method compared to chemical analytical methods.

The different types of OM used in paper I and II were: 1) the microalgae *Tetraselmis* spp., 2) kraft-lignin derived from terrestrial wood, and 3) surface sediment. The different OM types represented a high (*Tetraselmis*), low (lignin) and medium (sediment) quality OM source to the benthic invertebrates. The quality was referred to as the nutritious value of the OM source, defined as the relative amount of total nitrogen (TN) to total carbon (TC) in the OM, and the amount of amino acids and fatty acids (Gunnarsson et al. 1999).

In paper V, inert particle (luminophores) and solute (Br-) tracers were added to each core. Luminophores were added on the sediment surface, whereas bromide was added to the overlying water column.

Study organisms

The organisms used in this thesis were chosen based on their ecological importance in the benthic communities of the Baltic Sea, as well as their functional differences (Fig 3). The amphipod *Monoporeia affinis* and the bivalve *Macoma balthica* are dominant members of the deeper soft sediments, both in terms of abundance and biomass (Bonsdorff et al. 2003, Laine 2003). *M. affinis* is a surface deposit-feeder mainly feeding on fresh organic matter (Byren et al. 2002). It burrows actively in the upper few cm of the sediment and displays a nocturnal swimming behavior (Lopez & Elmgren 1989, Lindström & Fortelius 2001). The clam *M. balthica* is less...
motile, normally positioned ca. 5 cm into the sediment protruding its siphons to the sediment surface, where it can switch between surface deposit- and suspension-feeding (Olafsson 1986, Rumohr et al. 1996).

The invasion of the three sibling species of the polychaete genus *Marenzelleria* has changed the community composition in many areas (Zettler et al. 2002, Perus & Bonsdorff 2004). This spionid polychaete is a surface-deposit feeder, creating J-shaped semi-permanent burrows down to 35 cm (Zettler 1996). Apart from the priapulid *Halicryptus spinulosus*, it is the only deep-burrowing species in the northern Baltic Sea (Bonsdorff & Pearson 1999). They inhabit both shallow sandy substrates as well as deeper more muddy sediments at abundances ranging from a few hundred individuals per m$^2$ up to 39 000 ind. per m$^2$ (Kube et al. 1996). It is very difficult to separate the sibling species by morphological identification. Based on the results of Blank et al. (2008), who used genetic tools to identify and map the species distribution in the Baltic Sea, it is most likely that the worms used in this thesis were exclusively *M. neglecta*. In shallow coastal areas *Marenzelleria* spp. are competing with the common ragworm *Nereis (Hediste) diversicolor*, that inhabits sediments down to ca 15 m depth (Rasmussen 1973). They are

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**Fig 3.** Graphic illustration of the position in the sediment of species used in the thesis.
opportunistic omnivores, although their main feeding strategies are surface-deposit and filter feeding (Scaps 2002). *N. diversicolor* lives in more or less permanent U or Y-shaped burrows, creating a complex network of burrows extending down to ca. 15 cm that they are known to actively ventilate (Davey 1994).

In all experiments, the number of organisms added to the cores corresponded to average field densities. Therefore, the density and biomass could differ between treatments and all results relate to the natural field density of each species. This choice was made to increase the ecological relevance of the studies to the field situation.

**Sampling and chemical analyses**

At the end of each experiment, the sediment was sectioned into ca. 1 cm slices and organisms were carefully picked out by hand and placed in brackish water to let them purge their gut before tissue residue analysis. In paper I-III, all samples were analyzed using liquid scintillation counting (LSC) for the detection of $^{14}$C and gamma spectrometry for $^{109}$Cd. Filters (GFs and PUFs) from experiments in III and IV, as well as sediment and organisms in IV, were Soxhlet-extracted before analysis. In paper III extracts were analyzed with LSC and in paper IV all extracts were analyzed using a $\mu$-electron capture detector capillary-gas-chromatograph (GC-ECD), identifying 14 different organic contaminants (IV).

Luminophore analyses were done under UV-light followed by quantification using image analysis software (V). Pore water bromide concentrations were determined spectrophotometrically (Presley 1971). Luminophore profiles were fitted to a gallery-diffusion model (Francois et al. 2002), quantifying the rate of particle-mixing by giving two coefficients: an apparent biodiffusion coefficient, $D_b$, and a non-local transport coefficient, $r$. 
**PAPERS IN BRIEF - RESULTS**

**Fate of surface deposited contaminants**

**Paper I and II: Interactive effects between bioturbation and settling OM**
BDE-99 and cadmium were deposited on the sediment surface associated with OM of different nutritional qualities: phytoplankton, terrestrial lignin and sediment. The burial, remobilization (I) and bioaccumulation (II) of the two contaminants by three species with diverse feeding and bioturbation strategies, *Monoporeia affinis*, *Macoma balthica* and *Marenzelleria* sp., were analyzed after 34 d.

**Paper I: Fate**
There were clear species-specific differences and complex interactive effects between the type of contaminant and OM. For example, the burial of BDE-99 generally decreased in the order *Macoma balthica* > *Monoporeia affinis* > *Marenzelleria*, while the highest amount of Cd was buried by *Marenzelleria*. OM type had no effect on the burial of BDE-99, but the addition of phytoplankton significantly increased the burial of Cd by *Marenzelleria*. BDE-99 is highly hydrophobic (log $K_{ow} >7$) and sorbs strongly to particles and OM (de Wit 2002). Cadmium, on the other hand, is probably less particle reactive and more mobile as a dissolved chemical element. Results from paper I suggest that: 1) *M. affinis* and *M. balthica* mix and bury particles and associated contaminants in a biodiffusive mode while *Marenzelleria* does not cause any down-ward transport of particles, 2) *Marenzelleria* can transport dissolved compounds in their burrow structures and over the sediment-water interface by bio-irrigation, 3) bioturbation by all species increase the retention of surface-deposited contaminants in the sediment, and 4) OM type only seemed to be important as a sorbent, e.g. more Cd was released from the labile OM (phytoplankton), and did not affect the intensity of particle and solute mixing through bioturbation.

**Paper II: Bioaccumulation**
OM quality significantly affected the bioaccumulation of both contaminants. For Cd, bioaccumulation by all species was in the order *Tetraselmis* >> lignin > sediment, while for BDE-99 the trend was *Tetraselmis* >> sediment ≥ lignin. The normalization to sediment TOC and animal lipid (BSAF) for the accumulation of
BDE-99, did not remove the observed differences between treatments, thus suggesting that other factors than simple partitioning were important. Hence, based only on the nutritious quality of the OM, bioaccumulation was as hypothesized highest when associated with the nutritious algae. Species-specific differences were only significant for Cd, following *Marenzelleria* > *Macoma* > *Monoporeia*, but for BDE-99 there were no general differences. The species-specific Cd accumulation could be attributed to differences in the feeding ecology of the organisms, such as feeding rate and particle selectivity. However, it may also be due to differences in additional exposure routes, i.e. uptake of dissolved Cd from pore water and overlying water, particularly for *Marenzelleria* sp. that was shown to transport Cd via bio-irrigation (I). Due to the high K$_{ow}$ of BDE-99, the primary route of exposure to the organisms was probably through the diet.

**Remobilization of sediment-associated contaminants**

*Paper III: Comparing bioturbation and physical resuspension*

Physical processes are generally considered more important than biological in the remobilization of sediment-associated contaminants. Two modes of bioturbation-driven release of PCB (biodiffusion by *Monoporeia affinis* and bio-irrigation by *Marenzelleria* sp.) were compared to the release due to physical sediment resuspension generated by a motor-driven paddle, aiming at simulating the transient and episodic events that are likely to occur in the deeper accumulation areas of the Baltic Sea. Fluxes of soluble and particle-associated PCB-32 (log K$_{ow}$ 5.4) were quantified weekly during 30 days. Both bioturbation and physical resuspension significantly increased the PCB fluxes compared to controls. The relative importance of remobilized dissolved vs. particle-associated PCB differed between treatments, suggesting differences in the driving mechanisms behind the flux. Bioturbation by *M. affinis* caused the highest flux of both PCB fractions compared to the other treatments, while *Marenzelleria* sp. only remobilized dissolved PCB. This could be related to their specific modes of bioturbation; amphipods actively mix the upper sediment layer as well as suspend sediment particles, and the polychaetes create and irrigate semi-permanent burrows. The physical disturbance resulted in a very high concentration of suspended particles in the water column immediately after each resuspension event (every 3-4 days), however, over the
whole experiment time the total flux of particles, and particle-associated PCB, was still lower than the flux caused by the continuous bioturbation by *Monoporeia affinis*. There was little difference in the flux of dissolved PCB caused by *Marenzelleria* sp. (1.7 times higher than controls) and by physical resuspension (2.1 times higher). In all treatments, the release of dissolved PCB was more than an order of magnitude higher than that of particle-associated PCB, indicating a significant potential exposure route to pelagic organisms of the most bioavailable PCB form. Calculated mass transfer coefficients (0.43-1.37 cm d\(^{-1}\)) corresponded to previously reported values for tri-chlorinated PCBs. Results in paper **III** indicate that the continuous activities of benthic infauna can be just as, or even more, important than the large, but intermittent, pulses of physically resuspended sediment for the remobilization of sediment-bound contaminants.

**Paper IV: The effect of oxygenation and recolonisation of laminated sediments**

Extended periods of anoxic/hypoxic conditions in bottom waters of the Baltic Sea have led to depletion of bioturbating macrofauna and subsequent spreading of laminated sediments, and it has been estimated that up to 30 tons of PCBs may be stored in these sediments. In paper **IV**, the effects of reoxygenation and subsequent bioturbation on the release of POPs from naturally contaminated and laminated Baltic Sea sediments were investigated. The sediment was bioturbated by the invasive polychaete *Marenzelleria neglecta*, that is tolerant to low oxygen levels and can potentially rapidly colonize areas after periodic anoxia/hypoxia. The flux of both soluble and particle-associated POPs from sediment to water, including HCHs, HCB, DDTs and PCBs, was quantified after 85 days. Oxygenation followed by bioturbation by *M. neglecta* significantly increased the flux of dissolved POPs (0.7-3 times) compared to hypoxic sediments but had no effect on the remobilization of particle-associated POPs. Only oxygenation slightly decreased the flux of POPs perhaps as a result of co-precipitation of DOM-associated POPs and ferrous oxides following aeration. As in paper **III**, the flux of dissolved PCB always exceeded the flux of particle-associated PCB by an order of magnitude. MTCs (\(K_f\)) were between 0.06-0.45 and 0.1-1.8 cm d\(^{-1}\) in the oxygenated only and bioturbated cores, respectively. There was a positive correlation between \(K_f\) and log \(K_{ow}\), explaining 75% of the variation in the oxygenated cores but only 44% in the bioturbated cores, suggesting that other factors than hydrophobicity regulated the soluble release of
contaminants from the bioturbated sediments, i.e. likely the creation and bio-irrigation of burrows exposing contaminants at depth. Colonization of laminated sediments by *M. neglecta* could generate a substantial release of bioavailable POPs from sediment to water.

**Bioturbation mechanisms**

*Paper V: Particle and solute transport by two polychaetes*  
The effects of bioturbation on sediment biogeochemistry have been shown to be highly species-specific, and can be linked to particular feeding and burrowing strategies. It is therefore of interest to characterize and quantify the bioturbation efficiency of individual species for a better prediction and understanding of processes such as cycling of nutrients and contaminants in the sediment compartment. *Paper V* investigated the particle-mixing and solute transport by *Marenzelleria* sp. and *Nereis diversicolor* in Baltic Sea sediments using inert particle (luminophores) and solute (Br⁻) tracers. To quantify the intensity of particle reworking, a gallery-diffusion model was used to calculate the biodiffusion coefficient, $D_b$, and non-local transport parameter, $r$. A higher percentage of luminophores was buried below the top 0.5 cm surface layer by *Nereis* (13%) than by *Marenzelleria* (6%), as well as to a greater depth (5 vs. 1.5 cm). $D_b$ did not differ between the two worms ($2.4 \times 10^{-3}$ cm$^2$ d$^{-1}$) indicating a similar mixing rate of the top 1-2 cm sediment. On the other hand, the model only detected non-local transport by *Nereis* ($r$: $2.5$ y$^{-1}$). Thus, results suggest that *Nereis* is a more efficient particle-reworker than *Marenzelleria*. Bioturbation by both worms significantly increased the sediment pore water Br⁻ concentrations compared to controls. The shape of the bromide profiles in the *Marenzelleria* cores indicated that the worms caused an enhanced diffusion (biodiffusion) in the upper 1-1.5 cm followed by an advective transport down to ca. 8 cm. In *Nereis* the bromide profiles were more varied but generally showed an enhanced diffusion throughout the whole core with sub-surface peaks. Compared to controls, *Marenzelleria* increased the net flux of bromide into the sediment 4-6 times and *Nereis* 3-5 times. The two polychaete worms displayed different modes and intensity of bioturbation, which adds information to the understanding of how the invasion of *Marenzelleria* can affect important benthic processes in the Baltic Sea.
**DISCUSSION**

**Bioturbation and the fate of contaminants in the Baltic Sea**

The three infaunal species studied in paper I-IV displayed distinctly different modes of bioturbation and had significantly different effects on the burial and remobilization of contaminants. The active particle mixing of the upper 2-3 cm of the sediment by the amphipod *Monoporeia affinis* resulted in both increased burial and retention of surface deposited contaminants in the sediment (I), as well as an enhanced release of sediment-bound PCB from sediment to water (III). They caused substantial water column turbidity by bioresuspension of sediment particles (I, III) and thus also particle-associated contaminants (III). The clam *Macoma balthica* is less motile than *M. affinis* but can also be categorized as a biodiffuser (Bradshaw et al. 2006, I). Their main effect on the sediment contaminant distribution is probably by passive transport of particle-associated contaminants as a result of particles falling into the space created around their shells when they move around (I). The clams caused little particle resuspension, in fact suspension-feeding may instead filter out both suspended particles and contaminants present in the water and increase the deposition of contaminants on the sediment surface through biodeposition (Gilek et al. 1997, I). Bivalves have been found to have limited effects on contaminant fate (Riedel et al. 1997, I) compared to other groups of infauna (e.g. polychaetes), probably due to generally lower motility and lack of bio-irrigation. The polychaete *Marenzelleria* sp. also appeared to have a low motility, largely remaining in their burrows except when venturing out searching for food around their burrow entrance. They did not cause any significant particle mixing below the top surface layer (<0.5 cm), although a localized transport within their burrow structures was indicated (I, V). On the contrary, the worms were effective bio-irrigators (V). The most important effect on contaminant transport by *Marenzelleria* sp. is thus via bio-irrigation, thereby primarily influencing the fate of dissolved and/or DOM-associated contaminants (I-IV).

Infauna are often not considered important when studying the large-scale fate of contaminants and the main focus is usually on physical processes (Jonsson 2000). However, on a local scale, the activities of benthic invertebrates can both enhance (Ciutat et al. 2006) and even exceed (Riedel et al. 1987, III) the effects of physical
processes for contaminant transport. The bottom current and wave induced physical disturbance of the sediment in the deeper accumulation areas of the Baltic Sea is generally weak and sporadic (Stips et al. 1998, Danielsson et al. 2007). Compared to the continuous process of biologically mediated sediment resuspension, periodic and short-lived physical resuspension swiftly entrain a lot of particles that rapidly re-settle back on to the sediment surface, suggesting that over time bioresuspension can dominate the particle flux in these environments. Results in paper III support this assumption, showing that bioturbation by *Monoporeia affinis* caused a higher flux of particles and particle-associated PCB from sediment to water than simulated physical resuspension. The release of dissolved PCB after physical resuspension is probably mainly due to desorption from resuspended particles and a rapid release of surface pore water (Schneider et al. 2007). Despite a lack of particle resuspension by *Marenzelleria* sp., bioturbation by the worm and physical resuspension caused a similar flux of dissolved PCB to the water, showing the importance of a slower but continuous advective transport via bio-irrigation (III). The relative effects of physical vs. biological disturbance for the fate of contaminants is clearly dependent on the specific hydrographic, geochemical, and biological properties of the benthic system, as well as the physiochemical properties of the contaminant. Results presented here suggest that bioturbation by common Baltic invertebrates affects contaminant fate in the benthic environment in a species-specific way, both increasing the retention and enhancing remobilization, and that it can be just as, or even more, important than physical resuspension for the remobilization of sediment-bound contaminants.

**The multiple roles of organic matter for contaminant fate**

Settling organic matter (OM) is the main food source for deposit feeding infauna below the photic zone and the supply typically varies in time, quantity and quality. Moreover, OM is one of the most important sorbents for HOCs and metals and contaminants thus often reach the sediment associated to OM of various origins. Ingestion of contaminated food-particles is an important route of exposure for deposit-feeding infauna and it has been shown that bioaccumulation of HOCs (Gunnarsson et al. 1999) and metals (Maloney 1996) can be correlated to the nutritious value of the sediment OM. The bioaccumulation of BDE-99 and cadmium by Baltic infauna was significantly higher when associated to a labile
microalgae, simulating the deposition of contaminants to the benthic system with a settling phytoplankton bloom, compared to when associated with lignin (cf. terrestrial run-off) or sediment (cf. sediment resuspension) (II). This result was probably largely due to selective feeding by the organisms and a higher feeding rate in the algae treatment, although differences in sorption capacity of the various OM types also needs to be considered. For example, contaminants were likely more readily desorbed from the labile algae than the lignin or sediment, which increased the animals’ exposure from dissolved compounds in the pore water. This was supported by the concurrent higher bioaccumulation and transport of soluble cadmium in the algae treatment by *Marenzelleria* sp. compared to the other species (I, II). Therefore, there are likely seasonal differences in the bioaccumulation and transport of contaminants in the Baltic benthic ecosystem as well as differences depending on community composition (Gunnarsson et al. 2000, I, II).

Eutrophication has increased the load of sinking OM in the Baltic Sea and the bottom areas suffering from hypoxic/anoxic bottom water conditions and depletion of benthic macrofauna has increased as a result (Karlson et al. 2002). Several actions are currently being taken by countries around the Baltic Sea to reduce the input of nutrients and decrease eutrophication (Backer & Leppänen 2008). The recolonisation of these sediments following improved oxygen conditions could lead to a significant increase in the release of contaminants (IV). A colonization dominated by the opportunistic and low-oxygen tolerant *Marenzelleria* spp. (Hahlbeck et al. 2000) would not result in a general mixing of the sediment but rather an enhanced diffusive flux of dissolved contaminants from the sediment to the water (I, III-V). The establishment of *Marenzelleria* spp. can enhance the overall sediment redox condition and allow subsequent colonization by other less tolerant species, like *Monoporeia affinis*. Results from paper I-III highlighted the importance of understanding the complex interactions between ecological (e.g. infaunal feeding and bioturbation, OM cycling) and physicochemical processes (e.g. contaminant partitioning) when assessing the fate of contaminants in a sediment ecosystem.
Potential effects of the invasion of *Marenzelleria*

Since their invasion in the 1980s, the polychaete genus *Marenzelleria* has completely changed the benthic community structure and they are now the dominant species in many areas (Perus & Bonsdorff 2004). The previously abundant *Monoporeia affinis* has concomitantly decreased (Perus & Bonsdorff 2004), and although there are indications of inter-specific competition between the two species (Kotta & Olafsson 2003) the exact reasons behind the decline of the amphipod populations are not fully understood (Jacobson 2008). Based on results presented in this thesis, a shift from a community dominated by *M. affinis/Macoma balthica* to one dominated by *Marenzelleria* spp. could affect the contaminant fate in different ways depending on the type of contaminant and the specific situation. Bioturbation by *Monoporeia/Macoma* contribute to the incorporation of ‘new’ contaminants, deposited on the sediment surface or from the water column, into the sediment, which will enhance the retention and thus decrease the risk of remobilization. On the other hand, particle-mixing and altering effects on the sediment biogeochemistry, in particular by *Monoporeia*, also result in the release of sediment-bound contaminants back to the water column. In areas with on-going pollution and high contaminant concentrations in the sediment surface layer, this could probably cause a rapid recycling of contaminants between the sediment and the overlying water. However, reduced inputs of many POPs and metals during the last decades has resulted in a gradual decrease of contaminant concentrations in the upper sediment layers in most areas, and the vertical distribution of many POPs and metals typically shows a sub-surface peak below 5 cm, i.e. below the maximum burial depth of most native species in deep soft-bottoms. *Marenzelleria*, however, can reach these deposits through their creation of deep burrows (>35 cm) and thus significantly increase the remobilization of historical contaminants. In addition, *Marenzelleria* does not contribute to the burial and retention of new surface deposited contaminants, although they might increase the incorporation of soluble contaminants via irrigation. Ecological studies on the effects of the invasion by *Marenzelleria* spp. are inconclusive (Orlova et al. 2006); it is clear that the benthic community structure has changed but whether this is positive (increased biodiversity, functional complexity) or negative (inter-specific competition, enhanced recycling of contaminants and nutrients) for the Baltic Sea ecosystem remains unclear.
CONCLUSIONS

Long-term environmental monitoring studies show a significant decline of contaminants in Baltic biota (Bignert et al. 1998). Apart from successful source control and reduced environmental input, this might suggest little or no remobilization of historic contaminants from the large sediment deposits. No bioturbation in the widespread anoxic/hypoxic bottom areas and weak physical forcing in the deep accumulation basins potentially make these sediments efficient traps of contaminants. However, improved benthic redox conditions and recolonisation by sediment-living organisms could instead turn the sediments from a sink into a source. This issue is of particular concern when combined with the invasion of *Marenzelleria* spp., since these worms can burrow deep into the sediment to remobilize historic deposits. In addition, declining loads of contaminants to the Baltic Sea increase the relative importance of sediments as secondary sources to the ecosystem. In summary, bioturbation is an important process influencing the fate of contaminants in the Baltic Sea benthic system and its importance may increase with the invasion of new deeper-digging species. Such new additions to the Baltic fauna can lead to changes in functional diversity (bioturbation mode and depth) as well as the food-web structure (additional prey items) that could alter the pathways and distribution of contaminants in the Baltic Sea ecosystem.
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