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Butyltin compounds in the food web: impacts on chironomids and Daubenton's bats

by

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Dedicated to my grandfather, Kalle Kantola.

TABLE OF CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts, referred to in the text by their Roman numerals:

I. Lilley T., Meierjohann A., Ruokolainen L., Peltonen J., Kronberg L. and Nikinmaa M. Reed beds facilitate transfer of tributyltin from aquatic to terrestrial ecosystems through insect vectors in the Archipelago Sea, SW Finland. Environmental Toxicology and Chemistry, 31, 1781–1787*.

II. Lilley T., Ruokolainen L., Pikkarainen A., Laine VN, Kilpimaa J, Rantala MJ. and Nikinmaa M. 2012. Impact of tributyltin on immune response and life history traits of *Chironomus riparius*: single and multi-generation effects and recovery from pollution. Environmental Science and Technology, 13, 7382–7389*

III. Lilley T., Ruokolainen L., Vesterinen E., Paasivirta L. and Norrdahl K. 2012. Sediment organic tin contamination promotes impoverishment of non-biting midge species communities in the Archipelago Sea, S-W Finland. Ecotoxicology, 5, 1333–1346*

IV. Vesterinen EJ, Lilley T, Thalmann O, Wahlberg N. Next Generation Sequencing reveals the diet of the Daubenton's bat (*Myotis daubentonii*). *Manuscript.*

V. Lilley T, Ruokolainen L, Meierjohann A, Kanerva M, Stauffer J, Laine VN, Nikinmaa M. Impacts of organic tin compounds on redox regulation and complement reaction in natural populations of Daubenton's bats (*Myotis daubentonii*). *Submitted manuscript.*

VI. Laine VN, Lilley T, Norrdahl K, Primmer CP. Population genetics of Daubenton's bat (*Myotis daubentonii*) in the Archipelago Sea, southwest Finland. *Submitted manuscript.*

1. INTRODUCTION

1.1. Study outline

When the discovery of high DDT and methyl mercury residues in birds and fish was made in the 1960's it was first realized that some pollutants, persistent organic pollutants, accumulate in terrestrial and aquatic food webs within ecosystems (Keith 1966; Cooke 1979). The observed mortalities and reproductive failures in fish and fish-eating birds and other associated effects were found to correlate with to DDT concentrations in these animals. In addition to this, top-level carnivores, especially predatory fish and birds, had higher residue concentrations than the food they consumed. Through this it was understood that persistent pollutants are accumulated within the food chain from one trophic level to the next and that contamination from multiple anthropogenic sources poses a significant hazard to terrestrial and aquatic ecosystems (Fent 1996).

The EU's new chemicals regulation, REACH, has 143 000 substances pre-registered for use inside the European Union (ECHA 2009). Some of these act as pollutants causing ecotoxicological effects, beginning with acute biochemical reactions in cells, which often determine the higher-level adverse effects, to chronic effects on the function and structure of ecosystems. Many of the more dangerous contaminants often share properties, that make them hazardous, such as high acute and/or chronic toxicity, high environmental persistence, high mobility often leading to contamination of groundwater, and high lipophilicity leading to bioaccumulation in food webs (El-Shahawi et al. 2010). Many of the effects may also be species-specific, thus increasing difficulty to pinpoint the causality and extrapolate to other organisms (Fent 1996). Also, so called "cocktail-effects" of multiple pollutants pose a significant threat to organisms and complicate the understanding of the behavior of contaminants in ecosystems (Celander 2011). However, ecotoxicological effects are dependent on the bioavailability, the fraction available for uptake by organisms.

Tributyltin (TBT) is a specific type of man-made organic tin compound (OTC), where three organic butyl groups form a molecule by covalently attaching to a tin atom. The commercial use of organotin compounds began in the 1940´s in the plastic industry, where organotin compounds are still used as additives to stabilise PVC against the effects of heat and light (Yngve 1940). The biocidal effects of organotin compounds were discovered in the late 1950´s and they were used as pesticides in wood preservatives, molluscicides, antihelminthics and as fungicides in textiles and various water systems (Cima et al. 2003). Furthermore, paints applied to the hulls of ships containing tributyltin (TBT) and triphenyltin (TPhT) were found to be extremely effective against barnacles, filamentous algae and other fouling organisms that hinder the efficient travel of large marine ships (Bosselman 1996; Terlizzi et al. 2001). A successful marketing program in the 1960s secured its use worldwide on smaller vessels in marine and inland waterways (Sayer et al. 2006). In 1985, the world production of OTC's was estimated at about 40,000 ton/year (Alzieu 1998), increasing to 50,000 ton/year by 1996 (OECD 2001). Unfortunately, OTC's are not only toxic to fouling organisms, but to all other organisms in the biota too. Tributyltin is one of the most toxic chemicals to have been intentionally released into the marine environment by man (Antizar-Ladislao 2008).

Antifouling paints are not the only source for OTC pollution. Organic tin compounds leaching from a variety of consumer products are of growing importance (Fent 1996). They are used for instance in wood preservation, antifungal action in textiles and industrial water systems, such as cooling tower and refrigeration water systems, wood pulp and paper mill systems, and breweries (Rudel 2003). The degradation products of TBT, monobutyltin (MBT) and dibutyltin (DBT), are mainly used as heat and light stabilizers for PVC materials. Dibutyltin is also increasingly used as binder in water-based varnishes (NCI 2000). Therefore, due to the various applications of TBT and its derivates, organic tin compounds have multiple pathways, direct and indirect, into the environment.

Tributyltin is characterized by high toxicity, environmental mobility, and a tendency to accumulate in living organisms (Fent 1996). Organic tin compounds, even at low concentrations, have numerous negative effects on the physiology organisms. These effects include hormone imbalance, ATP production failure and reproductive disorders (Alzieu et al. 1986; Gibbs & Bryan 1986; Stenalt et al. 1998). The adverse effects of organotin compounds have been shown in numerous studies, for insects (Vogt et al. 2007a; Vogt et al. 2007b; Nowak et al. 2009; Vogt et al. 2010), gastropods (Alzieu et al. 1986; Fioroni, Oehlmann, & Stroben 1991; Bauer et al. 1995; Schulte-Oehlmann et al. 2000; Gopalakrishnan et al. 2011), fish (Martin et al. 1989; Fent & Meier 1994; Nakayama et al. 2004; Greco et al. 2007) and marine mammals (Kim et al. 1996; Kannan et al. 1998; Kannan & Falandysz 1999; Nakata et al. 2002; Song et al. 2005). Despite the high concentrations of organic tin compounds found in, for instance aquatic invertebrates, less is known about the methods of accumulation and toxic effects of these substances in higher trophic vertebrate predators, which may be exposed to these pollutants via food ingestion (Fent 1996).

Once the harmful effects of organic tin compounds on wildlife were recognised, efforts to find a global solution to this problem were taken as well as an enforcement of legal requirements to protect the aquatic environment (Antizar-Ladislao 2008). The first step in the efforts was the prohibition of the use of TBT on boats under 25 meters in the mid 80's (European Community 1989). In 2001, The International Maritime Organization (IMO) called for a global treaty to ban the application, i.e. painting of organic tin compound-based paints on all vessels beginning in January 2003, and total prohibition by January 2008 (IMO 2001). The prohibition is currently in force in IMO member states. However, it is likely that organotin compounds will continue to be produced and used as effective biocides on vessels, especially in developing countries and countries that are not members of IMO. They also continue to be in use in plastic materials and wood preservatives (Antizar-Ladislao 2008).

Present and future restrictions will not immediately remove TBT and its degradation products from the marine environment, since they are very persistent in the sediments to which they are bound to. This has been recognized as an environmental problem in the Archipelago Sea, S-W Finland, parts of which contain higher than average concentrations of TBT in marine sediments (Ympäristöministeriö 2007). Relatively high concentrations of organic tin compounds have been measured in organisms living in the Archipelago Sea, such as the Baltic macoma (*Macoma balthica*), phytoplankton, the pike (*Esox lucius*), the perch (*Perca fluviatilis*), the pikeperch (*Sander lucioperca*), the Baltic herring (*Clupea harengus membras*) and the bream (*Abramis brama*, (Ympäristöministeriö 2007; Airaksinen et al. 2010).

Tributyltin biomagnifies in the food chain to some degree (Antizar-Ladislao 2008). Assessing the bioaccumulation of organic tin compounds in organisms is complex because they do not behave as metals but neither do they obey the organic-compound quantitative structure-activity relationship (QSAR) predictions for accumulation or toxicity. Many aquatic invertebrate species are known to have minimal metabolic capacity for OTC’s (Fent 1996). However, animals higher up in the food web, such as fish and other vertebrates, are known to extensively metabolize OTC’s, such as TBT, to lower tin moieties (Lee 1985). Consequently, due to their inability to metabolize OTC’s, invertebrates can act as vectors in transporting them higher up in the food web, if exposed to organic tin compounds in the environment (Looser et al. 2000).

The chironomids or non-biting midges (Diptera: Chironomidae) are an influential group of invertebrates, which form the base of aquatic and terrestrial food webs as food for fish, birds, bats and other invertebrates as well as being key components in the detritius cycle (Ferrington 2007). Chironomids are known to concentrate TBT during their larval period (Looser et al. 2000). Some effects of TBT on life history traits have been experimentally studied (Vogt et al. 2007b), but so far, for instance, the effects of sediment TBT concentrations on the abundance, species richness and species composition of this ecologically influential group has not been studied in detail. In addition, due to their aquatic and terrestrial life stages, this group could act as a vector for transporting organic tin compounds from aquatic to terrestrial ecosystems.

Many insectivorous bird and bat species rely at least to some degree on chironomids in their diets (Vaughan 1997). However, for the Daubenton’s bat (*Myotis daubentonii*), chironomids constitute the bulk of the diet (Flavin et al. 2001). The Daubenton’s bat is a common species in the Archipelago Sea, where it feeds on newly hatched adult chironomids trawled from the sea surface. As a consequence of this diet, the Daubenton’s bat may be exposed to organic tin compounds. Although OTC’s have previously been found in bats (Senthilkumar et al. 2001), their physiological, genetic or ecological effects on bats have not been documented.

Ecotoxicology is an interdisciplinary environmental science dealing with interactions between environmental chemicals and biota (Fent 1996). The food chain described above provides an interesting ecotoxicological framework for studying the fate and effects of OTC’s in aquatic and terrestrial ecosystems. It provides new information on the mechanisms, which facilitate the accumulation and transfer of OTC’s in an ecosystem.

1.2. Organic tin compounds

Organic tin compounds, except in rare cases, do not occur naturally. They are all industrially manufactured chemicals. They are a combination of an organic moiety and elemental tin (Sn). In OTC's, the tin atom is tetravalent and is bound to at least one carbon atom. The general formula for OTC's can be presented as $R_{4-n}SnX_n$, in which n = 0-3 (Rudel 2003). X is an inorganic substituent; usually a halogen (chloride- or fluoride ion), but depending on the prevailing environmental conditions may also be an oxide, hydroxide, sulphate or a carbonate. The organic moiety is expressed as R, and is an alkyl or aryl group. According to the number of moieties, the organic tin compound is named a mono-, di-, tri- or tetraorganotin (Pellerito et al. 2006). There can be several different kinds of organic groups, such as butyl-, phenyl-, octyl-, ethyl, propyl- or methyl groups (Meador 2011). The presence of organic moieties greatly enhances the compound's hydrophobicity, thus increasing its bioavailability and toxicity. The octanol-water partitioning coefficient (pK_{ow}) can be used to assess the bioaccumulation of a compound in an organism, as it measures the partitioning of a chemical between water and organic material, mainly lipids. A chemical is defined as slightly accumulating if the pK_{ow} is >3, moderately accumulating at >4 and highly accumulating at a pK_{ow} of >5. The pK_{ow} coefficient for TBT ranges between 2.9 and 4.4 and according to these values TBT is prone to bioaccumulation (Pellerito et al. 2006). Indeed, the octanol-water partition coefficient has been found to positively correlate with the OTC induced cytotoxicity (Bruschweiler et al 1995). Organic tin compounds also have a low Henry's law constant, which implies a very low tendency of organic tin compounds to leave the aqueous phase due to volatilization (Soderquist & Crosby 1980) and due to a high density (TBT 1.2kg/l), they are drawn to the bottom in aqueous environments (Rantala 2010).

The hydrocarbon moieties, attached to the tin atom cause the hydrophobicity of OTC's (Rudel 2003). The number of tin-carbon bonds and the length of the alkyl chain define the physical and chemical properties of the compounds. The water solubility of the compounds decreases as the number and length of the organic moieties (R) increase, but it also depends on the inorganic substitute, X (Hoch 2001). Environmental conditions such as pH, salinity and temperature also affect the water solubility of OTC's (Rudel 2003).

The most common OTC's found in aquatic environments are triorganotins, such as tributyltin (TBT), triphenyltin (TPT) or trimethyltin (TMT), or diorganotins (DBT, DPT or DMT respectively). In addition to these, a very large number of other potentially toxic OTC's exist. Many have been found in the environment and are considered significant contaminants, but still very little is yet known about the occurrence, bioaccumulation and toxicity of many of these chemicals. This is due to the fact that OTC-chemistry is very complex, owing to their polar, ionisable and hydrophobic structures (Meador 2011). The present work concentrates on di- and tri-moietic forms of butyltins, i.e. dibutyltin and tributyltin.

1.3. The degradation and transformation of organic tin compounds

The occurrence of TBT degradation products in water and sediments indicates that abiotic and biotic processes eventually lead to the removal of the compound from the environment (Fent 1996). Because of the toxicity of OTC's, organisms degrade them through metabolic processes. Organic tin compounds degrade through dealkylation or dearylation in which the number of organic groups in the compound decreases until only inorganic tin (Sn) is left. For instance in the case of tributyltin, inorganic tin is reached through dibutyltin and monobutyltin. The role of the inorganic substituent X in the degradation process is unknown.

R4Sn -> R3SnX -> R2SnX2 -> RSnX3 -> SnX4

The degradation can also be abiotic. Photolysis and hydrolysis are abiotic processes, but in the temperature- and pH-conditions of natural water environments only photolysis is significant as an abiotic degrader in breaking down organic tin compounds (Rudel 2003). The Sn-C bond is stable up to temperatures of 200°C (Kotrikla 2009). In photocatalytic degradation, UV-light and the resulting hydroxyl-radicals degrade TBT (Kotrikla 2009). However, water turbidity often effectively blocks photocatalytic degradation. For instance the water in northern Airisto, the setting of the present study, is turbid due to agricultural run-offs carried by rivers running into the sea (Jumppanen & Mattila 1994). In addition to photolysis, chemical degradation is an abiotic degradation process, which has some significance in natural conditions (Gadd 2000). Strong acids and electrophilic substances can cleave the bond between tin and carbon (Kotrikla 2009). Organic tin compounds can also transform due to methylation or dismutation (Rantala 2010).

Biotic processes are the most important mechanisms for tributyltin degradation in freshwater and estuarine water phases and sediments (Dowson et al. 1996). Some bacteria and fungi can degrade OTC's by removing organic chains one at a time, and thus making the substances less toxic. For instance, with the addition of an organic nutrient broth, the gram-negative bacteria *Pseudomonas diminuta* degrades up to 90% of TBT in three days in test conditions in an initial water concentration of 4-20 µg Sn/litre (Kawai et al. 1998). Sulphate-reducing bacteria have also been found to degrade OTC's in anaerobic sediments through methylation (Lascourreges et al. 2000). The marine alga, *Pavlova lutheri*, can also accommodate increasing concentrations of tributyltin, degrading it to less toxic mono- and dibutyltin (Saint-Louis et al. 1994). This suggests that the photolytic degradation of organotins may be indirectly due to the stimulation of photosynthetic microorganisms (Huang et al. 1993). However, in the case of many microorganisms, the end-product of degradation is a mono-organotin, such as monobutyltin, instead of inorganic tin. Also the ability of microorganisms to degrade organic tin compounds requires that the concentration of the toxicants is not high enough to prevent the function of microorganisms. Microorganisms may also remove these compounds from the environment by accumulating them (Gadd 2000), which may have consequences higher up in the food chain.

In summary, the half-lives of organic tin compounds depend on the prevailing conditions, the method of degradation and whether the compound is in water or bound to sediment.

Half-life estimates in sediments for TBT range from a year to ten years (Martinez-Llado et al. 2007; Kotrikla 2009). As mentioned above, TBT degradation occurs in aerobic and anaerobic conditions. However, degradation in anaerobic conditions, such as in sediments, in which the organic tin compounds often are in the Archipelago Sea, may take decades (Dowson et al. 1996). Therefore OTC's can be described as persistent pollutants in sediments due to their long half-life estimates (Roessink et al. 2008). Organic tin compounds degrade much faster in the water phase (Berto et al. 2007). The half-life estimates for TBT in water range from days to weeks (Alzieu 1998). When abiotic degradation, or degradation by microorganisms fails, OTC's may become available to higher organisms.

1.4. Organotins in the environment

Antifouling paints prevented fouling by continuous release of biocide and thus forming a thin layer of highly concentrated OTC-containing water around the hull of the ship. As a result OTC's leached off the hulls of ships as they travelled and also when ships moored at harbours, and because of the constant leaching, new layers of anti-fouling paint were added regularly. The leaching rate is controlled by paint characteristics, mainly by binding chemicals (Thouvenin et al. 2002). Organic tin compounds have a high tendency to adsorb onto suspended clay-rich sediments and organic matter (Poerschmann et al. 1997; Arnold et al. 1998; Hoch 2001). Approximately 95% of tributyltin in the water column is bound to suspended particles, including plankton and sediment particles, while the remainder is largely associated with dissolved organic matter and organic and inorganic ligands. Before the total ban of use on ships in 2008 (IMO 2001), TBT in the water column has been reported in several publications at concentrations commonly ranging from 1 to 200ng l^{-1} in harbors and marinas around the world (Seligman et al. 1988; Fent 1996; Harino et al. 1997; Antizar-Ladislao 2008). However, the concentration of TBT is approximately three orders of magnitude higher in sea floor sediments than in the water column (Valkirs et al. 1986). In addition, as the hulls of ships are refinished or subjected to other physical scraping (e.g. ice), paint particles containing OTC's are detached from hulls and rapidly settle on the sea floor.

Recent investigations suggest that TBT concentrations in water, sediment and biota have generally declined after the implemented bans (Fent & Hunn 1995; Champ 2000; Diez et al. 2006; Sayer et al. 2006), and the maximum concentrations in marine water rarely exceed 100 ng l^{-1} (Bhosle et al. 2004). For example, it has been reported that TBT concentrations in surface marine waters have declined in France (Alzieu 1998), in the UK (Dowson et al. 1996), in the US (Valkirs et al. 1991; Espourteille, Greaves, & Huggett 1993), in the Gulf of Mexico (Wade, Garcia-Romero, & Brooks 1991) and Australia (Batley et al. 1992). Even though water quality regarding OTC's has improved globally, given its strong affinity for suspended particulates and sediments, benthic sediments are regarded as the major sink for TBT in the environment (Clark et al. 1988; Hoch 2001). Because of the resistance to biodegradation of sediment-adsorbed organotin compounds (Sarradin et al. 1995; Page et al. 1996; Michel & Averty 1999), sediments represent a potential source for future exposure of OTC's still for years to come (Hoch 2001). In addition, concentrations in water and sediments are still high in countries, such as China, which have not fully regulated the use of organic tin compounds (Cao et al. 2009).

Sorption is considered to be one of the most important processes responsible for the reduction of TBT concentrations in water (Fent & Hunn 1995). However, although sediments are considered a sink for organic tin compounds, they are also remobilized from sediments back into the water phase, due to abiotic, biotic and human actions (Fent 2004). Compared to deeper waters, shallow waters have a relatively large interaction with the sediment compartment, which increases desorption due to differences in concentration, therefore putting coastal areas and freshwater lakes are at a higher risk of contamination due to remobilization (Hoch 2001). This is also reflected in accumulation into biota as coastal species generally exhibit higher butyltins accumulations than their relatives from off-shore areas (Hoch 2001). However, Borghi and Porte (Borghi & Porte 2002) showed that organotin pollution in the North-Western Mediterranean is not limited to coastal areas, but can also reach the deep-sea environment and inhabiting organisms (1000–1800 m depth).

In sediments, the rate of desorption through diffusion is affected by the pH and salinity of the water, where lower salinity decreases desorption. The organic moiety of the compound affects the intensity at which desorption occurs: di- and mono-moietic forms diffuse back in to water phase more readily than high moieties (Berto et al. 2007). Because of the long half-life of organotin compounds, desorption can occur along a large time span (Dowson et al. 1993). In addition to background desorption, organic tin compounds can be remobilized from sediments due to tidal actions, other water currents, a storm or due to human activities, such as dredging, dredge disposal or fishing (Eggleton & Thomas 2004).

1.5. Accumulation of organic tin compounds in biota

The availability of pollutants to organisms is a key determinant for the interactions of toxicants with biota, and thus uptake, bioaccumulation and toxicity (Fent 1996). Organic tin compounds can bioaccumulate via diet, water or sediments through passive diffusion or actively through membrane transport. The transfer of organic tin compounds from water or sediment through the surface of an organism is called bioconcentration, whereas transfer through the food chain is called biomagnification. Together they are called accumulation (Rantala 2010). Accumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is degraded. For instance, TBT, once released from an antifouling coating, is rapidly absorbed in the water by organic materials such as bacteria and algae or adsorbed onto suspended particles, such as clay and dissolved organic matter, in the water (Gadd 2000; Burton et al. 2004; Luan et al. 2006). Subsequently it is readily incorporated into the tissues of filter-feeding zooplankton, grazing invertebrates, and, eventually through diet, higher organisms such as fish, water birds, and mammals. A number of studies in industrialized coastal waters have reported biomagnification factors of over 1, indicating bioaccumulation through the food web does occur (Fent 1996, but see Lepper 2002). Accumulation is affected by many environmental factors, such as pH, humic acids and salinity (Looser et al. 2000). However, the comparison of results of various studies is often problematic due to the manner in which data are presented; e.g. analysis corrected with wet sample weights are not comparable to dry sample weight analyses.

Because organic tin compounds accumulate, or biomagnify, in the food web to some degree, they may eventually end up in humans, when food containing them, such as oysters and fish, are consumed (Hoch 2001). Three factors contribute to the extent of biomagnification in a food-chain, 1) the substance is persistent and can not be broken down by environmental processes, 2) the food chain energetics increase susceptibility, and 3) the organism has a low, or nonexistent, rate of internal degradation or excretion of the substance, which is generally caused by hydrophobicity of the substance. Contaminant uptake mechanisms and rates can vary among and within species and depend on development stage, season, behavior, sexual condition and the history of contamination (Luoma 1983; Tessier & Campbell 1987; Babukutty & Chacko 1995). The most common mode of TBT assimilation is generally thought to be via the diet at the sediment–water interface (Eggleton & Thomas 2004).

Organic tin compounds are generally adsorbed into fat containing tissues (Lespes et al. 2009). The lipophilic properties of butyltins promote their accumulation in organisms and their ionic properties improve their ability to bind on to macromolecules, such as proteins (Fent 1996). The intake and elimination of TBT define the concentration in tissues, but the lipid content regulates the toxic effect. However, some studies go as far as to claim that the affinity of TBT to proteins is higher than the affinity to lipids and will therefore be accumulated at higher concentrations in the liver (Stäb et al. 1996; Harino, Fukushima, & Kawai 2000).

The ability of an organism to degrade and excrete OTC's affects accumulation (Rudel 2003). If an organism cannot degrade or excrete the compound at the same speed as it is taken in, the compound accumulates in to the whole organism or certain tissues within it (Tremolada et al. 2009). Other species-specific factors, such as uptake efficiency, growth dilution and metabolism can affect the degree of accumulation (Fent 2004). Generally, once an organism exposed to TBT moves to a clean habitat and receives TBT-free food, TBT is removed from the organism effectively through a process called biotransformation. Insects are thought to be incapable of metabolizing TBT, but fish and mammals synthesize cytochrome P450 monoxygenase, which metabolizes TBT into DBT (Fish et al. 1976; but see Looser et al. 2000). However, many previous studies have provided evidence that TBT has significant and selective effects on the different components of the cytochrome P450 monoxygenase system. The presence of TBT stimulates the system but also decreases the quantity of cytochrome P450 and inhibits the catalytic activity of a cytochrome P450 isozyme, decreasing the ability of an organism to biotransform TBT into DBT (Morcillo & Porte 1998).

Little is still known about the long-term accumulation and ecotoxicological effects of OTC's on the structure and function of aquatic, or even less, terrestrial ecosystems with respect to biomagnification in food webs (Kannan et al. 1996; Kim et al. 1996; Stäb et al. 1996).

1.6. Factors affecting accumulation

Ecotoxicological effects are largely dependent on the bioavailability of the contaminants. Bioavailability means the proportion of organic tin compounds, which is ingested and reaches the target tissue. This is the proportion of organic tin compounds in water or sediment, which is available for uptake by an organism (Rudel 2003). The organic moiety of organic tin compounds affects their ability to accumulate. Several general factors, such as hydrophobicity and organism-specific properties determine the degree of bioconcentration (Fent 2004). For instance DBT and MBT accumulate to a lesser degree compared to TBT (Rudel 2003). Dissolved or weakly adsorbed organic tin compounds are the most bioavailable to organisms. More complex compounds, attached to, for instance minerals, may only be bioavailable through diet (Eggleton & Thomas 2004).

Organic tin compounds preferentially partition to organic matter in sediment and dissolved organic matter (DOM) in pore water (Eggleton & Thomas 2004). The composition of pore water is a critical component in organic contaminant partitioning because of the variability in colloidal material, dissolved organic and inorganic chemicals, redox potential, pH and temperature, which may differ significantly from that of the overlying water (Rand 1995). The bioconcentration of organic tin compounds decreases at high concentrations of DOM and humic acids (Looser et al. 2000). Because organic tin compounds are tightly bound to organic matter, its presence lengthens the time during which bioconcentration occurs and therefore organisms can reach a physiological equilibrium in which organic tin compounds are excreted as fast as they are accumulated. The slowing down of bioconcentration in the presence of DOM may due to TBT forming complexes with organic carboxyl-, thiol- and amino groups, and presumably are not available for uptake through the skin or gills. These complexes may be so large that they cannot move through the cell membrane. Movement through the cell membrane may also be hampered by the polarity of these molecules (Rudel 2003). The amount of organic matter in sediment also significantly affects the bioaccumulation of sediment-bound TBT. In addition, sediment grain size may also be of significance, as the amount of organic matter is reversely correlated to the mean grain size (Meador et al. 1997).

The redox-potential affects the partitioning of TBT between the sediment and the water phase. The high K_{ow}-value negatively affects the bioavailability of organic tin compounds from aqueous medium, because these compounds are hydrophobic. Therefore OTCs from sediments end up in organisms mostly through diet (Lamoureux & Brownawell 1999). Inside the organism, TBT moves through the cell membranes by an electrophoretic mechanism and disturbs the movement of electrons in oxidative phosphorylation in mitochondria. Data suggest that the TBT compound crosses the mitochondrial membrane as cation $Bu_3Sn(H_2O)_n^+$ through the lipid bilayer and not by making use of a physiological carrier (Bragadin et al. 2003).

1.7. Toxicity of organic tin compounds

Because of the high bioavailability of OTC's, they are harmful at low aqueous concentrations. The lowest toxic concentrations are in the range of 1-10ng/L in marine gastropods; effects in fish are at magnitudes higher (1-10 µg/L, Fent 2004). The varying toxicity levels of OTC's are mainly affected by the number of organic chains and their type. The toxicity reaches its maximum when three chains are attached to the tin atom. Triorganotins are significantly more toxic than di- or mono-organotins (Antizar-Ladislao 2008). This may be due to the increased surface area and lipophilicity of triorganotins. The most likely mode of action in organisms for triorganotins is therefore in interactions with lipid membranes. Tetraorganotins are of very low toxicity, which may be due to their non-polar composition. In general, organic tin compounds containing alkyl groups are more poisonous than those containing aryl groups (Song et al. 2006), and of these, the most toxic are the butyl- and propyltins (Pellerito et al. 2006). In addition to toxicity, the type of organic group also affects which organism the compound is toxic to. In general, mammals and insects are especially vulnerable to trimethyl compounds whereas fish, fungi and molluscs are more susceptible to triphenyl compounds (Kotrikla 2009). Also, the inorganic substituent may affect the toxicity. The toxicity is increased for instance when the inorganic substituent is biologically active or when it can assist in the transport of the molecule to the active site. However, the chelation of the inorganic substituent to the tin atom decreases toxicity, which may be due to the fact that a chelated tin atom cannot bind to an active site (Song et al. 2006).

In summary, the toxicity of organic tin compounds depends on their bioavailability and length of exposure, as well as the sensitivity of the target organism to the organic tin compound in question (Rudel 2003). Most importantly, the concentration of OTC's in the environment alone does not determine their toxicity, but rather the fraction of OTC's that is bioavailable (Shawky & Emons 1998). The bioavailability of OTC's decreases with increasing sediment organic matter (Antizar-Ladislao 2008). Also, pH and salinity affect the toxicity of OTC's albeit to a lesser degree (Shawky & Emons 1998; Gadd 2000). Temperature, however, has a greater effect on the toxicity of TBT than these. A rise in temperature speeds up metabolism in organisms and depletes energy reserves, which increases susceptibility to TBT (Kwok & Leung 2005).

The toxic effects of OTC's to various organisms have been thoroughly documented (Fent 1996). The significance of organic tin compounds to the environment was not realized until the early 1970s and already early research showed that the most commonly used OTC, TBT, is very toxic to a large number of aquatic organisms (Blaber 1970; Smith 1981). Tributyltin has several modes of action, which alone or in combination with others causes the consequent observed effects, such as imposex, where female gastropods develop male reproductive organs. The biochemical processes behind these are perturbation of calcium homeostasis, inhibition of mitochondrial oxidative phosphorylation, and ATP synthesis, inhibition of photophosphorylation in chloroplasts, genotoxicity and DNA damage, and inhibition of enzymes such as ATPases and cytochrome P450 monoxygenases (CYP, Fent 2004). All early toxicity studies were mainly conducted on gastropods and they concentrated on the occurrence of imposex (Alzieu et al. 1986; Gibbs & Bryan 1986). Later, TBT has also been demonstrated to cause impairments in growth, development, reproduction and survival of many marine species (Beaumont & Budd 1984; Bhosle et al. 2004; Hagger et al. 2005;

Martinez-Llado et al. 2007). The sensitivity of species to TBT varies among species, but already concentrations of 1 ng/l are known to cause imposex. The specific mode of action causing imposex is not understood. However, it may be explained by the known fact that TBT increases testosterone concentrations in female gastropods and inhibits the conversion of androgens to estrogen by the enzyme aromatase (Oehlmann et al. 2007).

Marine invertebrates are especially vulnerable to the effects of OTC's in their embryonic and larval stages (Antizar-Ladislao 2008). In addition to causing imposex, TBT causes the lysis of red blood cells, cytogenetic damage and apoptosis (Jia et al. 2009). TBT can also affect the expression of certain genes in invertebrates, such as the expression of glutathione-S-transferase (Feng et al. 2007), receptor activated C kinase in *Mya arenaria* (Siah et al. 2007), CuZn superoxide dismutase in *Haliotis diversicolor supertexta* (Zhang et al. 2009). Butyltins can also have toxic effects on haemocytes in the immune system of invertebrates. For example in the cultivated clam (*Tapes philippinarum*), the phagocytic activity was significantly reduced in an irreversible non-lethal manner depending on concentration and lipophilic affinity (Cima et al. 1998).

In spite of most of the studies having been conducted on invertebrates, many toxic effects on vertebrates have been documented. The structure and lipid solubility of triorganotins correlates with the toxic effects observed, and is in accordance with the theory that triorganotins exert toxic effects through interactions with membrane lipids (Gadd 2000). The scope of adverse toxic effects of organotins in vertebrates is quite broad and includes effects on macromolecular- and DNA synthesis and, primary immunosuppressive, endocrinopathic, neurotoxic metabolic, and enzymatic activity, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative, and possibly carcinogenic activity (Snoeij et al. 1986; Kannan & Falandysz 1997; Nakanishi 2008; Osman et al. 2009).

In particular, three main areas of study on the toxic effects of OTC's on vertebrates have emerged. The first concentrates on the effects of OTC's on energy metabolism. Butyltins especially, have been found to negatively affect the production of ATP in mitochondria. Of the butyltins, TBT presents the highest toxicity by disturbing the function of mitochondria with the F(1)F(0)-ATPase as main target (Nesci et al. 2011). DBT is less toxic and its toxicity action is generated by blocking the absorption of oxygen in the mitochondria, whereas MBT has no obvious toxic effect on mammals (Lei et al. 1998). The apoptosis of cells, involving caspase-3, modifications of cytoskeletal structure and the Bcl-2 family, may be due to butyltin-caused damage in the mitochondria (Zhu et al. 2007).

The second main area of interest are the effects of OTC's on cytochrome P450 enzymes of CYP1, 2 and 3 groups, which play a central role in the oxidative metabolism or biotransformation of a wide range of exogenous compounds, such as toxins, as well as a variety of endogenous compounds, including the metabolism of fatty acids and hormones (Nelson et al. 1996). Organic tin compounds, especially TBT, inhibit the activity of cytochrome

P450 enzymes. Tributyltin is able to penetrate the hydrophobic membrane environment, that envelopes the cytochrome P450 system. Here TBT binds to the haem moiety of cytochrome P450 (Rosenberg and Drummond, 1983; Fent and Bucheli, 1994). Additionally, it impedes the transfer of electrons from the hydrophobic binding site of cytochrome P450 reductase (Morcillo & Porte 1997). Furthermore, recent investigations of the effect of TBT at lower concentrations than those reported earlier from the subchronic test of fishes have suggested an involvement of cytochrome P450 enzymes in TBT metabolism and the androgenic effects of TBT in fish (Mortensen & Arukwe 2007).

The third major area of interest in the effects of OTC's in vertebrates is on the immune system. Butyltin compounds reduce cortical thymocyte numbers and as a consequence, the weight of the thymus. After extended exposure, butyltins suppress T-cell mediated immune responses (Seinen et al. 1977). Low doses inhibit immature thymocyte proliferation, whereas high doses cause a depletion of thymocytes by apoptosis (Raffray & Cohen 1991). Kinetic studies show that after the dealkylation of TBT by cytochrome P450, it is actually DBT, which causes these effects in thymocytes. Dibutyltin initiates an increase in intracellular Ca^{2+}, causing the generation of reactive oxygen species (ROS) and release of cytochrome c from the mitochondria. As a result caspases are activated, cleaving defined target proteins and leading to an irreversible apoptic damage of the cell. The aforementioned increase in Ca^{2+} may be caused by disruption of membrane or cytoskeletal functioning (Gennari et al. 2000).

1.8. Aims of the thesis

The brackish water Archipelago Sea, situated in the South-Western corner of Finland has previously been of interest due to its higher than average sediment organic tin compound concentrations (Ympäristöministeriö 2007). This is most likely due to the high shipping activity, with two major ports and large repair shipyard, a point source in the proximity of which the highest concentrations have been measured, and also due to of the large amount of sediments dumped by the rivers and lack of strong sea currents (Helminen et al. 1998), and large dredging and marine dumping actions that have to be carried out nearly every year to allow large ships to dock. In the Archipelago Sea, tributyltin has so far been found in a number of organisms including phytoplankton, the Baltic tellin (*Macoma balthica*), the pike (*Esox lucius*), the perch (*Perca fluviatilis*), the pikeperch (*Sander lucioperca*), the Baltic herring (*Clupea harengus membras*), and the bream (*Abramis brama,* Airaksinen et al. 2010). However, the transfer of organic tin compounds in the food web, and especially from an aquatic ecosystem to a terrestrial, and the effects on organisms during this process have not been studied in detail.

Ecotoxicology studies the interactions between environmental chemicals and the biota, including the fate of the chemicals and their effects on processes from genes to populations, which control the ecosystem. The relationship between cellular toxicological responses to toxicity at higher biological levels is a key question in ecotoxicology (Fent 1996). Ecotoxicological studies on say, tributyltin, require interdisciplinarity, considering the chemical, biological, toxicological, molecular and ecological processes included. Thus, ecotoxicology is essentially based on environmental chemistry, toxicology and ecology, which are combined with each other and integrated to form novel ecotoxicological concepts.

The aim here is to study the small-scale distribution of tributyltin and its effects on organisms by means of an ecotoxicological approach. More specifically, the thesis studies the distribution of tributyltin in the Archipelago Sea, and the physical and environmental features, which facilitate its accumulation into biota. Furthermore, the study aims to describe a possible pathway for the transfer of organic tin compounds from aquatic to terrestrial ecosystems through insect vectors, such as chironomids (Chironomidae). The thesis also studies the accumulation of tributyltin through the food web in to Daubenton's bats, and the possible physiological and genetic effects it afflicts upon these animals. This study broadens our understanding on the extent of the fate and consequences of organic tin compounds in the environment.

The following questions are addressed (Roman numerals refer to original papers presented as annexes):

1. What is the extent of tributyltin contamination in marine sediments in the northern part of the Archipelago Sea? (I)
2. What are the *in vitro* multigeneration effects of tributyltin on life history traits of a ecologically significant species group, the chironomids, in controlled laboratory conditions? (II)
3. Can the effects of tributyltin contamination be observed as differences in the abundance and species composition of chironomids? (III)
4. Can reed beds act as ecosystem boundaries, which facilitate the transfer of organic tin compounds from aquatic to terrestrial ecosystems through insect vectors such as chironomids? (I)
5. Do organic tin compounds accumulate in Daubenton's bats through their chironomid-rich diet and do the concentrations reflect local sediment organic tin compound concentrations? (IV, V)
6. What are the physiological consequences of exposure to organic tin compounds in Daubenton's bats? (V)
7. What can population ecology tell us about possible long-term exposure of Daubenton's bats to tributyltin? (VI)

2. SETTING OF THE STUDY

2.1. Study area

The study area of this thesis is situated in the Archipelago Sea on the Finnish coast of the northern Baltic Sea. It is situated in South-western Finland and covers a total area of 9436 km^2, of which islands constitute 2000 km^2 (Helminen et al. 1998). The area belongs to the hemi-boreal vegetation zone (Ahti et al. 1968) and exhibits strong seasonal patterns.

The Archipelago Sea is shallow with a mean depth of only 23 m. As with most of the Baltic Sea, there are no tides in the Archipelago Sea. Water levels may vary up to 1.7 m due to wind conditions and changes in wind and atmospheric conditions (Granö & Roto 1989). The weak near-bottom currents generally flow north to south and the area has a large river input of fresh water and making the salinity is very low (typically less than 5 psu). River input also carries a large amount of organic and clay-rich sediments, which accumulate on sea floors in the Archipelago Sea, especially in the northern part. Due to the nutrient runoff from rivers, the Archipelago Sea is slightly eutrophicated (Jumppanen & Mattila 1994). As a result of this and annual ice cover, light and oxygen levels are especially low at the bottom (Leppäkoski & Bornsdorff 1989).

There are two large ports with intense shipping activity in the area, in Turku and Naantali, with an oil terminal and cargo and passenger terminals (fig.1.). There are also several dry-docking facilities, a repair yard and a major shipyard. Because of the large amount of sediments dumped by the rivers and the lack of strong sea currents (Helminen et al. 1998), large dredging and marine dumping actions have to be carried out nearly every year in order to allow large ships to dock.

2.2. Description of the study species

2.2.1. Chironomidae

Chironomids are a widespread and species-rich family in aquatic ecosystems with at least a reported 6951 species worldwide (Pape et al. 2009). They are an ecologically important group of benthic macro-invertebrates playing a role in detritus processing and recycling organic matter and as prey species for fish, bats and aquatic birds (Coffman & Ferrington 1996; Vaughan 1997). The larvae occupy a wide variety of aquatic and semi-aquatic environments including fresh- and marine waters, and also brackish water such as the Baltic Sea. The within-family regional (Nilsson 1997) and local diversity (Ferrington 2007) is considerable, and in Finland alone, 784 species have been reported. Due to their aquatic and terrestrial life history traits and the fact that chironomid diversity and abundance is often higher than that of other common macroinvertebrate groups combined (Marziali

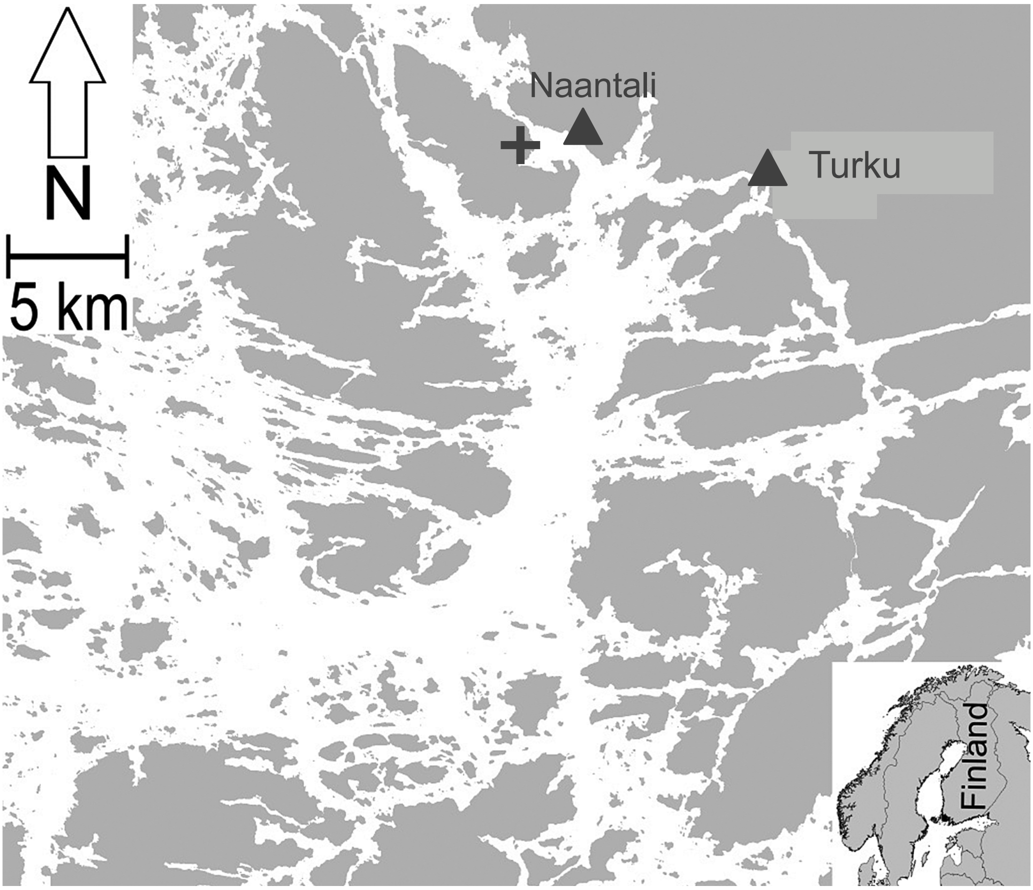

Figure 1. Map of the study area in the Archipelago Sea, S-W Finland. The cross indicates the location of the repair shipyard and the two triangles indicate the location of the major ports in the area.

et al., 2010), chironomid species are a very important group ecologically (Taenzler et al. 2007).

The life cycle of chironomids can be divided into four stages: egg, larval stage with complete metamorphosis (consisting of four instars), pupa and adult (Fig. 2). Often, the larvae live in the sediment in tubes constructed of algae, sediment particles and other available particles. Chironomids, for the most, are deposit feeders, projecting their head and the anterior part of their body outside of their tubes and ingesting silt, microdetritus, periphyton and other suitable particles on the sediment surface. The pupae rise to the surface before the adults hatch. The adults often form large mating swarms, after which the females attach their egg sacks to aquatic plants and other objects just below the water surface before dying. Although the larval stage of chironomids, depending on the climate, may take from weeks to months, the lives of adult chironomids are measured in days.

The non-biting midge, *Chironomus riparius,* is widely distributed in the northern hemisphere, most commonly at temperate latitudes. It is found in the Western Palearctic region, including Finland; and also in the Nearctic region. The study species is a collector-gatherer feeding on sediment-deposited detritus (Rasmussen 1984a, 1985). The larvae can tolerate a wide variation in pH, oxygen level, salinity and sediment grain size (Havas & Hutchinson 1982) and it often often occurs in polluted waters with observed population densities of over 50 000 juvenile individuals per square meter (Armitage et al. 1995). *C. riparius* winters in the third or fourth larval instar and the diapause is characterised by a halt in the development of the imaginal discs in the fourth subphase of the fourth larval instar. The synchronized emergence of the wintering cohort takes place in spring and the settlement of first instar new generation larvae occurs within a few weeks thereafter. The species exhibits multivoltine life cycles at temperate latitudes with repeated settling of larvae during April to November (Groenendijk et al. 1996), but larval development is strongly related to the water temperature and therefore the number of consecutive generations per year is strongly restricted by climatic conditions (Mackey 1977). The effect of temperature may be as dramatic as to lead to only one generation per year, such as in prairie ponds in the boreal zone of Canada, where the ice-free season lasts only six months and food supply is limited (Rasmussen 1984b). The wide tolerance range of the species to environmental conditions and the mating and egg-laying habits of the species have made the species a popular model organism in the laboratory. It has been used extensively in the past in sediment contamination tests (Cairns & Van der Schalie 1980; Sibley et al. 1997; Adams et al. 1999), and standard methods have been described for

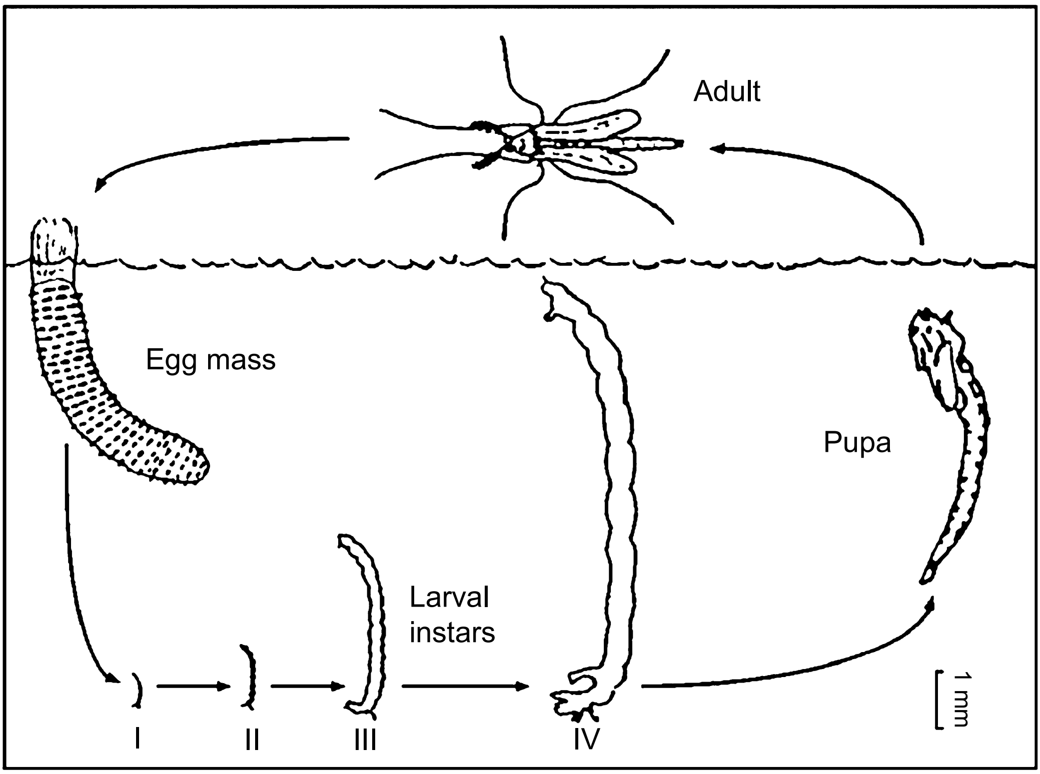

Figure 2. The life stages of chironomids. Adopted from Timmermans (1991).

conducting toxicity tests with the *C. riparius* and the related *Chironomus dilutus* (OECD 2004a; b; ASTM 2006).

2.2.2. The Daubenton's bat (*Myotis daubentonii*)

To date, thirteen species of bats have been recorded in Finland. All of the bats living in Finland, apart from the Northern bat (*Eptesicus nilssonii*), are at the northern-most limit of their range, and most of the species are restricted to southern- and central Finland. The Daubenton's bat (*Myotis daubentonii*; Vespertilionidae (Kuhl, 1817)) is one of the most common bats in Finland, with a latitudinal range extending to 70° in the north (Fig. 3). Outside Finland its range encompasses almost all of Europe and northern Asia, although the phylogeny of the daubentonii-group is unclear to the east of the Ural mountains (Kruskop 2004; Matveev et al. 2005).

Figure 3. Distribution of the Daubenton's bat in Finland, Europe and northern Asia. Adopted from IUCN Red List of Threatened Organisms (www.iucn.org)

Just like most temperate bats, the Daubenton's bat is a small (6-10 g) and insectivorous. It is a so-called "trawling bat" and has large, bristle covered hind feet, which make it well adapted to capturing insects from the surface of still water bodies. In Finland, individual animals may also forage in forests and woodlands in the vicinity of water bodies, especially in the spring before water temperature are high enough to produce an adequate amount of insects

(Nyholm 1965). The Daubenton's bat feeds opportunistically, i.e. insects of with a body size of approximately 7 mm are captured if available (Taake 1992). Chironomids (Chironomidae) constitute the bulk of their diet, but also other Diptera (crane-flies, mosquitoes), aphids, mayflies, lacewings, Hymenoptera, moths and caddis flies are seasonally captured (Swift & Racey 1983; Flavin et al. 2001). There are also reports of Daubenton's bat being able to feed on small fish (Siemers et al. 2001). The hunting flight is usually at 10-20 cm above the water surface and insects, usually emerging chironomids and even their pupae shells, are captured from the water surface. Daubenton's bats can only recognize the acoustic images of emerging insects if they are not masked by floating leaves or plant material. Because of these feeding habits, water bodies with extensive still and vegetation free water surfaces and trees on both banks represent key foraging patches for this species, as they provide high insect densities and a suitable foraging habitat during the seasonal activity period (Taake 1992; Warren et al. 2000). In Finland, due to the low salinity of the northern Baltic Sea, Daubenton's bats regularly forage over the sea surface.

In the study area Daubenton's bats typically hibernate until mid/late-April, but males may hibernate until mid-May (pers. observation). After hibernation Daubenton's bats typically migrate to their foraging areas and summer roost in their vicinity, which are often not situated very far from the hibernation site in Finland; typically under 10 km due to the high frequency of water bodies and suitable hibernation sites (Nyholm 1965). Outside Finland, in Central Europe, movements up to 304 km between summer roosts and hibernation sites have been recorded (Steffens et al. 2005), although mean movements are under 100-150 km (Tress et al. 2004). As with hibernation sites, Daubenton's bats show strong fidelity to their summer roosts and foraging grounds, returning to the same areas year after year (Nyholm 1965). Daubenton's bats prefer to roost in woodpecker cavities often found in Common Alder (*Alnus glutinosa*) or Birch (*Betula pendula*) in riparian forests often within a kilometer from the feeding grounds, although males often forage up to 6 km away (Senior et al. 2005). They also use small bird nest boxes very often; especially after the breeding birds vacate them in July. One roosting site in the study area is situated in an old mill, now in use as a museum.

Hibernation is an adaptation for overcoming food shortage in winter (Ransome 1990), and timing of reproduction is such that the periods of high energy demand in reproducing adults and in growing young coincide with those of high food availability (Racey 1982). Pregnancy and lactation in females and spermatogenesis in males are energy-demanding processes that require increased food intake compared with other times of the year (Anthony and Kunz 1977; Racey and Speakman 1987; Encarnacao and Dietz 2006; Encarnacao et al. 2006a; Dietz and Kalko 2007).

3. MATERIALS AND METHODS

3.1. Studying the extent of tributyltin contamination in marine sediments in the northern part of the Archipelago Sea

The data for manuscript (I) were collected during the summer of 2009 by dividing the northern Archipelago Sea into a 2 x 2 km grid and the southern, more channel-like section of Airisto was divided into 2 km segments representing sampling locations, from which triplicate sediment samples were collected from the shipping lane and *Phragmites australis* reed-beds close to the shore. Reed bed sediment samples from the top surface layer (0-5 cm) were collected with a small hand spade, being careful not to disturb the composition of the sediment. The shipping lane samples were taken by a core sampler from the surface layer (0-5 cm) and stored in -20 °C until HPLC/MS-analysis. The TBT concentrations of all three sediment samples from each collection point were analysed after solid phase extraction with HPLC/MS using a deuterated tributyltin (D27) internal standard. To statistically analyse the results, we calculated the distance between repair shipyard and the reed bed sampling sites, as well as the distance from these sites to the shipping lane. The effects of these distances, shipping lane depth, and shipping sediment TBT concentration on log-transformed reed bed sediment TBT concentrations were analysed in a linear regression.

3.2. Methods for studying multigeneration effects of TBT on *Chironomus riparius*

The aim of this experiment (II) was to experimentally test the multi-generation effects of sediment-bound tributyltin on life history and immunocompetence of *C. riparius*. The immediate- (single generation) and long-term (five generations) effects were tested as well as recovery from exposure after four generations. All *C. riparius* larvae used in the experiment were obtained from an in-house mass culture, which was established in 2009 with egg ropes from the University of Eastern Finland and University of Frankfurt, to avoid effects of inbreeding. Three exposure groups [low (30 µg/kg), intermediate (90 µg/kg) and high (180 µg/kg TBT in sediment dry weight)], a control and a solvent control with four replicates of each were used in 30 x 20 x 20 aquaria with 50 individuals in each. Fifty individuals of each exposure concentration were also raised in parallel to collect larvae for phenoloxidase assays and haemocyte counting. The selected TBT treatments were based on data collected from a random sampling of the Archipelago Sea, in Southwestern Finland (I). We took five individual freshly-hatched larvae from ten egg masses for each aquarium and from the 15th experimental day on, the number of emerged imogens were counted daily. The number of eggs per egg mass was counted, and dead adult chironomids were collected and weighed after drying on a precision scale. Actual sediment TBT concentrations were measured by HPLC/MS.

Larval chironomids in the final instar were sieved from the parallel aquarium sediments to collect haemolymph for PO-activity measurement and haemocyte counting. Phenoloxidase activity was measured using L-DOPA as a substrate and haemocytes were counted in a Bürker chamber without staining. Total protein concentration was measured using the BioRad protein assay kit based on the Bradford method and results were displayed as specific PO activity (U/mg protein).

To test the effects of five consecutive generations of exposure to the sediment TBT, egg ropes from the specific treatment and replicate were used for the next generation, again using 5 larvae per egg rope adding up to 50 individuals. In the fifth generation, due to space restrictions in the laboratory caused by the doubled number of containers needed for running the recovery-experiment in parallel, 30 larvae, as opposed to 50 in the previous four generations, were reared in smaller glass jars. The final recovery experiment tests whether the effect of TBT on immune defence and other life history traits are lost when a population exposed to TBT for multiple generations is reared in a clean environment (i.e. pollution of environment ends). Egg ropes were taken from TBT treatments in the fourth generation and reared in a TBT-free environment (i.e. Control, Solvent control, 30 Clean, 90 Clean and 180 Clean).

3.3. The effects of tributyltin on the species composition and abundance of natural populations of chironomids

The study examines the effects of sediment bound tributyltin on the species richness, species composition and abundance of chironomids in the northern Archipelago Sea (III). Sampling locations were based on the gradient obtained from the results of manuscript (I), ranging from very high contamination in the northern part of the sampling range to very low or clean in the southern. In order to minimize environmental variation, chironomid species living close to the shore in *Phragmites australis* –reedbeds were studied, and instead of larvae, only adult chironomids were sampled with emergence traps. This method provides information on chironomid species, that actually complete their aquatic larval stage in the study area (Raunio et al. 2011), which is important considering the effects of organic tin compounds on development.

Specifically designed floating emergence were used trap to catch emerging species, placed in triplicates approximately 5 meters from the shore. The collecting period started in late May and ended in late September 2009 with bottles being replaced with new ones weekly. The chironomids were identified to species level by Lauri Paasivirta based on nomenclature at Fauna Europaea (Saether & Spies 2011). Sediment TBT was analyzed from samples collected from under the traps using HPLC/MS, and environmental factors (loss on ignition, soluble phosphorus, sediment grain size and pH), which could affect chironomid distribution, were measured accordingly. The effect of increasing sediment TBT concentration on local chironomid species composition was tested using Mantel's test of matrix correspondence and partial Mantel's tests. Ecological dissimilarity in species composition was measured with the Sørensen index (Legendre & Legendre 1998). The information of local TBT concentration was converted into another distance matrix based on the Euclidean distance, and another Euclidean distance matrix was constructed from the geographical coordinates of each sample site. In addition to these matrices, a third explanatory matrix was constructed from the remaining environmental data.

3.4. Reed beds facilitating the transfer of organic tin compounds from aquatic to terrestrial ecosystems through chironomid vectors

Chironomids were sampled in select locations along the TBT gradient for HPLC/MS analysis of TBT concentration to gather information on whether chironomids could act as vectors in transferring organic tin compounds from aquatic to terrestrial ecosystems (I). Two custom emergence traps were placed per reed bed site to collect for chironomids during peak densities in early August. Aluminum foil covered vials filled with Etoh were used to collect and store samples. TBT was extracted from the chironomid samples by ultrasonification in methanol with internal standard of 1 µg of deuterated TBT (D_{27}). After evaporation, the residue was taken up in 500 µl of acetonitrile and 500 µl of purified water containing 0.1% trifluoroacetic acid (TFA, CF_3CO_2H). The sample was then run on a HPLC/MS and the internal standard and calibration curve were used to calculate the actual TBT concentration of the freeze dried and weighed samples

3.5. Accumulation of butyltins in Daubenton's bats through their diet

The following step in the thesis required proof of Daubenton's bats in the northern Archipelago Sea feeding on the emerging chironomids, which would expose them to tributyltin through their diet and actually measuring TBT, or its metabolite, DBT in the bats (IV). Here we collected bat fur for HPLC/MS-analysis and fecal pellets from bats sampled in the study area and used next generation sequencing to study diet DNA from the pellets. Sediment samples were also collected from all bat-sampling sites and analysed for TBT. In this preliminary study we sampled 15 bat individuals for fecal DNA, which was subsequently extracted in an insect-free DNA laboratory to prevent contamination. Individual libraries were prepared according to Meyer & Kircher (2010). Following this emulsion PCR and Ion Torrent Sequencing was carried out according to the most current protocol (Publication Part Number: 4471974 Rev. C). The resulting sequences were separated into different barcodes, that is, original individuals samples and the sequences were trimmed for original arthropod-specific primers. Sequences over 50 bp were clustered into haplotypes and identified to species using BLASTN 2.2.25+ algorithm (Zhang et al. 2000). The BLAST output was imported to program MEGAN (Encarnacao et al. 2005) to illustrate and compare differences between individual samples.

Butyltins were extracted from the fur samples with solid phase extraction after digestion with proteinase K and adding the internal standard of TBT $D_{27.}$ The final extract was taken up in 100 µl of ACN and 100 µl of 0.1% trifluoroacetic acid (TFA) in water before liquid chromatography mass spectrometry was run to measure for tributyltin and dibutyltin.

3.6. The physiological consequences of exposure to organic tin compounds in Daubenton's bats?

The organic tin compounds dibutyltin and tributyltin increase cellular reactive oxygen species (ROS) levels possibly by altering Ca^{2+} homeostasis (Corsini et al. 1996; Gennari et al. 2000) or by affecting biotransformation activity (Schmidt et al. 2005). Redox imbalances are also associated with immune function, which is hampered by organic tin compounds. This study examines whether the TBT-associated effects can be seen in general body condition and redox balance, redox enzyme activities, associated oxidative damage of red blood cells and complement function regardless of natural environmental variation in bat populations sampled in differently contaminated areas (V)? The blood samples were collected into 75 µl heparinized capillaries from the interfemoral vein. The capillaries were subsequently centrifuged (5 min, 1000 rpm/g) and the haematocrit was measured. Following this, the red blood cells were diluted into 0.9 % NaCl and the plasma in HBSS and stored in -80 °C.

The levels of glutathione reductase, glutathione peroxidase, glutathione-s-transferase, catalase and glucose-6-phosphate dehydrogenase activity and superoxide dismutase inhibition rate were measured. All levels and activities were measured in triplicate (intra-assay coefficient of variability [CV] < 10% in all cases) using 96-well and 384-well microplates. All values were correlated to protein content (Bradford 1976). Three control samples were used on every plate to correct for interassay variation (CV max 20 %). The ratio between reduced and oxidized glutathione species (GSH/GSSG) was measured with a glutathione detection reagent with reduced glutathione as the standard.

For measurement of the alternative complement pathway activity a recombinant strain of *Escherichia coli* K-12 was used by cloning with EGFPluxABCDEamp gene, which expresses the bacterioluciferase enzyme (Atosuo & Lilius 2011). The expression of the luciferase operon produces cells capable of emitting bioluminescence (BL) without the addition of substrate. Bioluminessence, i.e. the reduction of light as bacteria were destroyed, was used to measure the effectiveness of bat serum at killing bacteria (Kilpi et al. 2009). To determine a value for the activity, a slope from derivate values from points between 20 and 100 repeats, where the complement was visibly activated, was calculated.

The individual correlations between bat hair DBT concentrations and body condition index (weight/forearm length, BCI), haematocrit, protein carbonylation, complement and total ROS were calculated with a Spearman's correlation. To get an overall picture of the correspondence between sediment and fur organic tin content and the activity of the analysed set of antioxidant enzymes and redox status enzymes, we used Mantel tests of matrix correlation (Legendre & Legendre 1996).

3.7. Population structuring of Daubenton's bats in the Archipelago Sea

The study was initiated in order to understand the small-scale genetic population structure of the Daubenton's bat in a patchy environment of the Archipelago Sea with samples from sites in Finland and Europe (VI). The study reveals whether the Archipelago Sea, or another yet unidentified obstacle, poses a barrier to gene flow in Daubenton's bats, which are considered to have strong site fidelity and thought to fly only short distances during their seasonal movements, and to gain an insight on the possibility of chronic exposure to TBT through site fidelity. A 3 mm diameter tissue sample for genetical analyses was punched from both left and right wing membranes from selected sites in northern Archipelago Sea, eastern Finland, Spain, Switzerland and England. DNA was extracted from one biopsy punch by using the modified salt extraction protocol of Aljnabi & Martinez (1997). All samples were analysed at 9 microsatellite loci shown previously to be polymorphic in Daubenton's bats.

PCR products were electrophoresed on an ABI3130xl Genetic Analyzer with GeneScan -600 LIZ size standard and subsequently genotyped. Deviations from Hardy-Weinberg equilibrium (HWE) within all populations (within loci) and across all loci (within populations) were calculated and the genotypic linkage equilibrium was determined among every locus pair.

For every sample site the genetic diversity indices, observed number alleles (A), expected gene diversity (He), the observed proportion of heterozygotes (Ho) and the allelic richness (Rt) averaged across loci were calculated and the allelic richness (Rt) averaged across loci was calculated. Furthermore, population differentiation was estimated by calculating *P*-values for genic differentiation between each population pair at every locus and across all loci and pairwise FST values over all loci for each population pair were also implemented. Isolation by distance (IBD) is defined as a decrease in the genetic similarity between populations as the geographic distance between them increases. We used FST estimates to test for IBD using the Mantel test. IBD was determined for all individuals and also for males and females separately to detect sex-biased dispersal.

4. RESULTS AND DISCUSSION

4.1. The extent of tributyltin contamination in marine sediments in the northern part of the Archipelago Sea (I)

Tributyltin contamination in northern Archipelago Sea is very local, with the highest concentrations measured at the repair shipyard in Naantali and in its direct proximity. More importantly, tributyltin was also found in the ecologically important reed bed zone. The effect of distance on reed bed TBT differed according to shipping lane TBT concentrations. There was an interaction between shipping lane depth and distance from source.

Globally TBT and other OTCs are found at especially high concentrations around major ports, shipyards, and dry-docking facilities (Harino et al. 1997; Buggy & Tobin 2006), and the levels of TBT measured in the present study are in the same order of magnitude as measured in other recent studies on port areas (Chen et al. 2010; Eklund et al. 2010; de Oliveira et al. 2010; Liu et al. 2011). In accordance with this, the highest reed bed TBT concentrations were found close to the only active source in the area, the repair shipyard where ships are refinished, in the northern part of our study area. The large influx of clay-rich sediments from rivers feeding into the Archipelago Sea (Jumppanen & Mattila 1994) enforce the sedimentation of TBT by providing adsorption material in areas where TBT input from the dry-docking facility and shipping channels is already high. The concentrations in the main shipping channel were also highest in the northern part of the study area. The southern part of the study area is largely unaffected by TBT in both reed beds and the shipping lane. The discovery of TBT in reed beds suggests that TBT, and other OTCs, are transferred via insects from aquatic ecosystems to terrestrial ecosystems relatively close to an OTC source through ecosystem boundaries, such as Common reed beds, which are areas of high insect biomass production in the Archipelago Sea (Pitkänen 2006).

4.2. Multigeneration effects of tributyltin on life history traits of chironomids (II)

Because of its complex nature, TBT is a potent toxicant with multiple modes of action on insects and other organisms (Rantala 2010). Although many of the exact metabolic consequences are poorly understood, the effects on life history can be studied experimentally. The results of the single-, multigeneration- and recovery experiment of TBT on the life history of *C. riparius* indicates that TBT has both immediate and long-term effects on the parameters tested here (development time, weight, fecundity, survival, immune function), with additive effects over generations on survival and immune defence. However, the effects disappear in the first generation reared in clean conditions, apart from effects on development time, which is carried over generations.

In a single generation all sediment TBT concentrations (30, 90 and 180 µl kg $^{-1}$) lengthened the development time of individuals, decreased the weight of adults, the number of eggs laid and the number of haemocytes. To further test the possible effects of the different concentrations of TBT exposure on *C. riparius* over generations, the experiment was continued for another four generations. After five generations of TBT exposure larval development times continued to be elevated, compared to control, but no significant increases from the short-term exposure were evident. While TBT had a variable effect on the measured life-history variables (development time, fecundity, survival and weight) over generations, all variables differed significantly in comparison to the control treatment. Fecundity was higher in the treatments throughout the five generations and there was an increase in the fifth generation before extinction in the lowest and highest treatments. A similar trend was observed in the intermediate treatment, but it was not statistically significant. These effects are generally associated with toxicants, previously demonstrated with TBT on *C. riparius* (Vogt et al. 2007b). Survival was markedly lower in all TBT treatments, and decreased over the generations, until in the fifth generation under 20% of the highest treatment and under 40% of the lowest and intermediate treatments developed into adults. Also these results agree with the previous findings of (Vogt et al. 2007b).

Although the principles of the immune function in insects are analogous to vertebrates, there are fewer components, thus enabling more conclusive deductions of the effects of treatments. The number of haemocytes, the equivalent to vertebrate leukocytes, decreased with generation in all treatments. This multigeneration effect of TBT has not been described earlier, although the immediate effect of TBT on the immune system is documented in other arthropod groups (St-Jean et al. 2002; Harford et al. 2007; Misumi et al. 2009; Liu et al. 2009; Gopalakrishnan et al. 2011). Interestingly, as haemocyte numbers decreased, phenoloxidase activity increased, possibly as the animals attempted to compensate for the reduced cellular immunity with increased humoral immunity. Phenoloxidase activity was not significantly different to the control in the first generation in any TBT treatments, but in the second and subsequent generations, the phenoloxidase activity of all TBT treatments was higher than in the control. Again, this is the first experiment to demonstrate such an effect, although single generation studies have been conducted with similar results to our first generation (Tujula et al. 2001; Zhou et al. 2010). The decrease in haemocyte numbers could be caused by epigenetic effects, which build up over generations, or more direct maternal or paternal effects, which are delivered with the egg or sperm. However, since in the following recovery experiment we did not find any likelihood of previous generation TBT exposure to immune function when offspring were reared in a clean environment, this explanation is not likely. In fact all effects, apart from development time, returned to control levels in the recovery generation. Likewise, results of the recovery experiment exclude the effect of fast adaptation to the TBT as a possible explanation. This is somewhat surprising, as such effects passed over generations are the most feasible explanation for short-term changes in life-history in organisms with non-overlapping generations. However, the results on increased fecundity under exposure in particular suggest that TBT may act as a strong selective agent on chironomid life-history: only the most tolerant individuals manage to survive and reproduce under polluted conditions, while the stressful environment favours increased reproductive investment.

In general, the multigenerational effects observed can be due to physiological acclimation (phenotypic plasticity) varying between generations overall, genetic adaptation through micro-evolution (Medina et al. 2007) or inbreeding, probably caused by the death of TBT-intolerant phenotypes before reproduction. This would enhance the appearance of genetically determined traits for TBT tolerance, epigenetic effects, where TBT exposure affects the methylation/acetylation of histones/genes affecting gene transcription conferring resistance to TBT in consecutive generations. Also any combination of the foregoing are plausible. It seems TBT is such a potent toxicant—with a wide variety of effects on energy production, immune defence and hormone balance (Oehlmann et al. 2007) among others—that micro-evolution cannot produce a suitable genotype, at least in such small populations considered here, which may also be affected by inbreeding.

4.3. **The effect of sediment TBT contamination on chironomid community structure** (III)

TBT can act as a strong selective agent in populations. This study demonstrates that sediment TBT-contamination can also affect the species composition of communities, in this case, chironomid assemblages in the northern Archipelago Sea. More precisely, tributyltin causes a turnover in species composition and a reduction in the number of species and genera, but does not affect the overall abundance of chironomids, meaning chironomids are still available as food items for organisms higher up in the food chain. In terms of ecological importance of the results, it is probable that the abundance (i.e. total number of individuals) of chironomids is more important than the species composition of this functionally relatively coherent group as some species are able to tolerate higher concentrations and thus, for the lack of competition, are able to increase in abundance (Clements & Rohr 2009). As a result, the functional role of chironomids in the food web and detritus cycle may be preserved even when OTC- pollutants, such as TBT, have eliminated many species or even genera from the community. Our results suggest that the loss of biodiversity and loss of ecological function along the organic tin compound gradient are likely to occur at separate rates. This, however, means that chironomids can act as vectors transporting nutrients and environmental pollutants from aquatic ecosystems to terrestrial ecosystems and carry these far inland from their site of hatching (Mellbrand et al. 2011).

Tributyltin has also been found to have the same effect on marine periphyton in laboratory conditions (Dahl & Blanck 1996) and on species regime shifts in natural communities (Sayer et al. 2006; Smith et al. 2008). This suggests that our conclusion is not specific to chironomids, but may be valid for other species-rich groups with high functional redundancy. However, it should be noted that the simplification (i.e., reduced diversity) of an ecologically influential group might lower the resistance of the community to other environmental hazards (Clements & Rohr 2009). In addition, the study sites were selected based on sediment TBT concentrations, but we cannot fully exclude the possibility that associated pollutants (e.g. polyaromatic hydrocarbons (PAH), Martinez-Llado et al. 2007; Eklund et al. 2010) or other non-measured, environmental factors may have played a role (Vermeulen et al. 2000; Iwasaki et al. 2011) in the results obtained. Nevertheless, the results suggest a strong link between TBT and number of chironomid species and a strong effect on the chironomid community. Although associated pollutants, such as PAHs, may be present, they are often not associated with a species turnover as described here (Naes et al. 1999). Nevertheless, the “cocktail effect” of pollutants cannot be discounted.

Sediment TBT contamination influenced chironomid species composition separate to variation in measured environmental parameters; the effects did not overlap considerably. Thus, it is rather unlikely that the effect here associated with TBT is due to some other, unmeasured component in the environment, especially as there was minimal overlap between geographic location and TBT contamination (i.e., ruling out the possibility of spurious correlation due to spatial autocorrelation in TBT). Furthermore, the fairly constant number of chironomid individuals along the TBT gradient implies that all study sites were suitable habitat for the species studied.

4.4. Chironomids as vectors in transferring TBT from aquatic to terrestrial ecosystems (I)

Here we examined whether wild caught adult chironomids contained tributyltin, i.e. whether chironomids were capable of transferring the contaminant from an aquatic to a terrestrial ecosystem. Adult chironomids collected at sites closer to the main organic tin compound source in the study area, the repair shipyard in Naantali, were found to contain relatively high concentrations of TBT. In addition, the correlation between adult chironomid and sediment TBT concentration was very high. Concentrations in adult chironomids were higher than concentrations in the sediments they developed in, which suggest that TBT accumulates in chironomids.

The discovery of TBT in reed beds and chironomids inhabiting them, suggests that TBT, and other OTCs, are transferred via insects from aquatic ecosystems to terrestrial ecosystems relatively close to an OTC source through ecosystem boundaries, such as common reed beds, which are areas of high insect biomass production in the Archipelago Sea (Pitkänen 2006). Organisms, such as the non-biting midges (Chironomidae) analysed here, caddisfly (Trichoptera), and odonates (Anisoptera, Zygoptera), can act as vectors in transporting different compounds to some distance from their original site of development, the reed beds (Mellbrand et al. 2011). Insects cannot degrade TBT through metabolism; this compound is transferred to the next trophic level unchanged and in its most toxic form (Looser et al. 2000; Song et al. 2006). These insects are a primary food source of many bird and bat species (Vaughan 1997). The results suggest that accumulation in chironomids may only be local and occur close to a source though.

4.5. Organic tin compounds accumulate in Daubenton's bats through their chironomid-rich diet and the concentrations reflect local sediment organic tin compound concentrations (IV, V)

The study examined whether Daubenton's bats in the Archipelago Sea actually feed on the chironomids carrying tributyltin and whether local sediment TBT concentrations correlate with TBT or DBT concentrations in sampled bats. The most frequent Arthropoda group in the total diet is Chironomidae (30%) followed by Trichoptera (c. 10%) and the most frequent prey species was *Microtendipes pedellus*.

Traces of TBT were found in extracted bat hair samples, but the concentrations were below the confidence limit to allow quantification. Nevertheless, a significant difference was found between the mean bat fur DBT concentrations of sampling sites. Sediment TBT concentrations also correlated positively and significantly with bat fur DBT concentrations. This is not surpirising since TBT from marine sediments is accumulated in developing chironomid larvae (I) and adult chironomids act as vectors for transporting TBT from aquatic to terrestrial ecosystems. The local concentrations of sediment TBT do not affect the abundance of emerging chironomids, meaning food containing TBT is readily available for foraging bats (I). This suggests Daubenton's bats are exposed to TBT through their diet, but TBT is metabolised in the bat and excreted renally in the form of the more water-soluble and less toxic DBT. However, once TBT-containing adult chironomids are digested, it is quickly metabolized during absorption by epithelial intestinal cells into the more water soluble, and thus more easy to excrete, DBT by cytochrome P450 mono-oxygenase, which mammals, in comparison to arthropods, can synthesize (Fish et al. 1976). In this context, it is more plausible that the butyltin compound found in fur is in fact DBT and not TBT. Although bats molt annually (Tiunov & Makarikova 2007), storing in fur may be a secondary mode of action for avoiding the toxic effects of butyltins, which may also vary between individuals (Appel 2004). Considering this, liver samples would have provided a more informative picture on the exact concentrations of TBT and DBT the Daubenton's bats were dealing with.

4.6. The physiological consequences of exposure to organic tin compounds in Daubenton's bats? (V)

Because Daubenton's bats in northern Archipelago Sea feed on TBT contaminated chironomids, a study on whether physiological effects of TBT in bats could be found was warranted. No correlation was found between bat hair DBT concentrations or sampling site sediment TBT concentrations and BCI, haematocrit, total ROS or protein damage. Several possible explanations can explain the observed result. Although Daubenton's bats are known to forage locally, usually under 1 km from their roost (Encarnacao et al. 2005; Senior et al. 2005), it may still be that bats exhibit temporal and spatial variation in foraging behaviour, especially considering the very local contamination of TBT in the area, which decreases the intensity of accumulation and decreases oxidative stress accordingly. Also, bats in general, due to the high oxygen demand of flight, have a strong innate antioxidant defence system to handle autoxidation of red blood cells by reactive oxygen species produced as a consequence of high metabolic rates (Wilhelm Filho et al. 2007). Bats are also remarkably resistant to lipid peroxidation (Wilhelm Filho et al. 2007) and protein carbonylation (Salmon et al. 2009).

There was no correlation between sediment TBT concentration or fur DBT concentration with enzymatic antioxidant activity and redox status. Previously, the effect of TBT on the redox status of various organisms has been demonstrated in laboratory experiments (Corsini et al. 1996; Gennari et al. 2000), but the effect found in clinical studies is often masked in the wild by variation caused by changes in the natural environment and other, unmeasured environmental stressors. Also, considering the very low concentrations (ng/ml) disrupting vital metabolic functions of other wild organisms (Antizar-Ladislao 2008), it is surprising that no significant effect was found on any single parameter measured, although exposure via diet is considerable (I, IV) and the present study confirms butyltins end up in bat fur, strongly suggesting it is processed by the metabolism of the bats.

An association was found between complement activity and DBT concentrations in bat fur, where high concentrations of DBT resulted in low complement activity. The effect of butyltins has previously been intensively studied on other aspects of the vertebrate immune system (Raffray & Cohen 1991; Gennari et al. 2000; Frouin et al. 2008; Misumi et al. 2009). Yet the effect on the initiator and effective agent of many immune responses, the complement system, has not been studied before. Here, the cause of this effect cannot be fully directed at butyltins, as other unmeasured associated environmental variables may participate in the observed results (Schmidt et al. 2005). However, the very high concentrations at the TBT source suggest at least a partial role. Due to the lack of *in vitro* experiments, the mechanism of action through which butyltins cause a decrease in complement activity are not known. Study of the complement system suggests butyltin-caused disturbances in Ca^{2+} - homeostasis, which is involved in the initiation of the complement cascade, may be involved (Rivas et al. 1992; Suzuki et al. 2006, Gregory et al. 2003). Interestingly, one must recognise that here may be involved as Ca^{2+} - homeostasis (Rivas et al. 1992; Suzuki et al. 2006), which is involved in the initiation of the complement cascade (Gregory et al. 2003). Interestingly, one must recognise here that bat fur represents past exposure, and even after the growth of the butyltin containing fur, further degradation of the compound may have taken place with individual variance. This may affect the correlation with complement activity measured in the present study.

4.7. **Chronic exposure to TBT in Daubenton's bats** (VI)

Finally, considering the lower than expected butyltin concentrations and physiological effects caused by them, a study was conducted to investigate whether the reason for this could be in the mobility of Daubenton's bats, which are generally thought to show high site fidelity. Exploiting methods used in population genetics also allowed to an insight on the population structuring and possible conservation issues of Daubenton's bats in the northern

Archipelago Sea. The results indicate a generally low level of population structuring in Daubenton's bats, with high heterozygosity and low Fst values, even with all the European samples included, thus indicating that there are no evident barriers to gene flow in the Archipelago Sea, or the rest of Finland or Europe. However, we observed significant isolation by distance in both sexes, indicating some population differentiation on a larger, European scale. Isolation-by-distance was also observed within Finnish populations, indicating that there are higher levels of gene flow among local populations than among more distant ones. Sex-wise, significant IBD was observed only in females, indicating males disperse longer distances, as observed in Daubenton's bats and other species of *Myotis* elsewhere (Kerth et al. 2002; Ngamprasertwong et al. 2008).

Low pairwise Fst values and high gene diversity within sampling sites indicate low population structuring with very high levels of gene flow among local Daubenton's bat colonies, which could be due to the mating behavior or individual dispersal among local colonies. There have been reports of large numbers of Daubenton's bats, especially males, visiting the same underground sites in the late summer and autumn during a behavior called swarming (Parsons & Jones 2003). Only one swarming site has been found so far from the study area, which also showed high heterozygosity. Thus it is possible that the paucity of swarming sites mixes different colonies well. These results indicate that genetic isolation is not a conservation concern in the Daubenton's bat in the Archipelago Sea and as long as the mechanisms that maintain high heterozygosity are preserved. Also, the seasonal mobility of Daubenton's bats may partially explain the indistinct effects of tributyltin on their metabolism. Swarming and wintering sites are likely to play an important role as they ensure the genetic mixing of local populations. Our results highlight the need to preserve the swarming and wintering sites of bats to ensure the genetic diversity in local populations.

CONCLUSIONS

The present work demonstrates for the first time the effects and transfer of butyltin compounds in a food chain comprising of Daubenton's bats and their main food item, chironomids. The effects here, due to abiotic factors and other site dependent factors, are bound to the locality, and extrapolations to other arthropods and mammals should be dealt with scrutiny. The species-specific effects of chemicals, and hence species extrapolation, are among the major problems encountered in ecotoxicology that make effect, hazard, and risk assessment difficult. However, concerning study area and organisms studied here, some conclusions can be made in all probability. The study area is subject to strong point source contamination from the repair shipyard at the northern range of the study area. Other possible point sources, for instance the port in Turku, have reduced sediment TBT concentrations, most likely due to almost annual dredging actions, which have to be carried out in order to allow ships to dock. There may however be other smaller sources, e.g. pleasure boat marinas, which do not show up in the data. The contamination decreases in manner that seems almost logarithmic as distance from the repair shipyard increases. The sediments in the southern part of the study range are uncontaminated, and therefore it can be deduced that butyltin contamination in the Archipelago Sea is very local. An interesting, although self-evident, result is the occurrence of TBT in reed bed sediments, which facilitates the transfer of butyltins from aquatic to terrestrial ecosystems in the vicinity of the point source. In the present study, the role of an ecologically influential group of insects, the chironomids, as the vector for transfer was studied. Tributyltin was found in adult chironomids emerging from polluted sites.

Tributyltin caused a regime shift and decrease in species richness of chironomid communities, most likely caused by effects on life history traits observed in the multi-generation exposure experiment, but interestingly did not affect markedly the total abundance. Some species-specific effects of TBT play a part in the composition of chironomid communities and tolerant species are able to increase in abundance. These tolerant species are able to reach maturity carrying the accumulated butyltins they were exposed to in the sediment in their juvenile phase. Some sources suggest chironomids cannot degrade butyltins and others claim, at least *Chironomus riparius*, is able to do this (Looser et al. 2000). However, there may be species-specific differences in the ability of an organism to degrade TBT (Fent 2004).

Chironomids form a major part of the diets of many bats (Vaughan 1997), and especially the trawling bat, the Daubenton's bat (Flavin et al. 2001). However, to be certain of the possibility of transfer of butyltins from chironomids to bats, a genetic analysis of Daubenton's bats was carried out where it was in fact certified that 30% of the prey were chironomids, indicating a route for exposure. Following this butyltins were analysed from fur collected from Daubenton's bats, which was found to contain DBT, the degraded form of TBT. Only unmeasurable concentrations of TBT were found in some individuals. However, the fur DBT concentrations correlated with sampling site sediment TBT concentrations.

Perhaps due to an efficient xenobiotic metabolism and antioxidant system, no correlation of sediment TBT or fur DBT were found with the measured physiological parameters in bats. Total reactive oxygen species, oxidative damage to proteins, antioxidant enzyme activity and glutathione balance was not affected by sampling area or individual contamination. The reasons for this can only be speculated without further *in vitro* studies. However, previous studies have reported high resistance to oxidative damage and high antioxidant capacity in bats (Salmon et al. 2009). Also our further studies on population genetics of local Daubenton's bats suggest that the bats are mobile and may not be tied to contaminated sites. TBT is metabolized rapidly when toxic diet is not consumed (Fent 1996), which suggests that short-term exposure to TBT may not be a serious threat to the populations of Daubenton's bats.

Nevertheless, the study illustrates the difficulty of investigating the effects of toxicants in natural populations, where conditions are not controlled for. However, the innate immune system of Daubenton's bats appeared to be affected by individual bytultin exposure. The activity of this multioperational group of proteins forming the base of many immunological functions reduced with increasing exposure to butyltins. This is a result, which would also need further *in vitro* –studies to gain a better understanding of.

Ecosystems are complex, and large uncertainties exist when attempting to measure effects in the wild. However, because of the complexity of OTC's, extrapolations of laboratory results to natural conditions are also subject to criticism. The uncertainty should be reduced by investigations on the key processes that contribute and govern the effects of contaminants, and their mechanisms on different levels of biological organization, which is what was attempted here. The multipla of variables in natural conditions are unaccounted for and may mask many of the effects that are subject to investigations. And as to toxicants in the environment, although the point source for TBT in the study area is clearly acknowledged, one cannot disclose the possible effects and interactions of the thousands of other chemicals in daily consumption.

To conclude, the study demonstrates that 1) the contamination and effects of butyltins in the northern Archipelago Sea is very local and 2) given the suitable environment and vectors, butyltins can be transferred from aquatic to terrestrial ecosystems. The study also illustrates that studying the effects of exposure in natural populations is very difficult due to unaccounted environmental factors and other associated toxicants. Normally, in aquatic environments where TBT is present, other biocides e.g. Irgarol 1051 and toxicants, e.g. PAH may also be present, which however have different modes of action (Fernandez-Alba et al. 2002). Interactions with other toxicants, a "cocktail effect" is possible, but very difficult to study. Also, of especial concern, due to the agricultural run-offs carried into the sea by rivers, are the possible interactions with nutrients (Roessink et al. 2008).

The study demonstrates the potential of environmental toxins to travel from an ecosystem to another and that the possible effects of these toxicants in biota should be considered even at a distance from the source with more persistent and biomagnifying substances. Often, due to their lipophilicity, environmental toxicants are mobile and persistent as well as unpredictable. For instance in the case of OTC's, the length of the organic moiety determines whether organisms are able to metabolize it, which affects its potential to biomagnify in the food web. Factors such as these ultimately determine whether a substance is transferred from its original site of deposition. The study presents the need to acknowledge the mobility and transfer of toxicants in food web and facilitates further understanding of their fate in the environment.

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REFERENCES

Adams, S., Bevelhimer, M., Greeley, M., Levine, D. & Teh, S. (1999) Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. *Environmental Toxicology and Chemistry*, **18**, 628–640.

Ahti, T., Hämet-Ahti, L. & Jalas, J. (1968) Vegetation zones and their sections in northwestern Europe. *Annales Botanici Fennici*, **5**, 169–211.

Airaksinen, R., Rantakokko, P., Turunen, A.W., Vartiainen, T., Vuorinen, P.J., Lappalainen, A., Vihervuori, A., Mannio, J. & Hallikainen, A. (2010) Organotin intake through fish consumption in Finland. *Environmental Research*, **110**, 544–547.

Alzieu, C. (1998) Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean & Coastal Management*, **40**, 23–36.

Alzieu, C., Sanjuan, J., Deltreil, J. & Borel, M. (1986) Tin contamination in Arachon Bay - Effects on oyster shell anomalies. *Marine Pollution Bulletin*, **17**, 494–498.

Antizar-Ladislao, B. (2008) Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environment International*, **34**, 292–308.

Appel, K. (2004) Organotin compounds: Toxicokinetic aspects. *Drug Metabolism Reviews*, **36**, 763–786.

Armitage, P.D., Pinder, L.C. & Cranston, P.S. (Eds.). (1995) *Chironomidae: The Biology and Ecology of Non-Biting Midges*. Springer.

Arnold, C.G., Ciani, A., Müller, S.R., Amirbahman, A. & Schwarzenbach, R.P. (1998) Association of Triorganotin Compounds with Dissolved Humic Acids. *Environmental Science & Technology*, **32**, 2976–2983.

ASTM. (2006) Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. *In: Annual book of standards*. Philadelphia PA: ASTM.E. pp. 1706–1805.

Atosuo, J. & Lilius, E.-M. (2011) Escherichia coli K-12 (luxABCDEamp). A tool for analysis of bacterial killing by complement and myeloperoxidase activities in real-time basis. *European Journal of Clinical Investigation*, **41**, 37–37.

Babukutty, Y. & Chacko, J. (1995) Chemical partitioning and bioavailability of lead and nickel in an estuarine system. *Environmental Toxicology and Chemistry*, **14**, 427–434.

Batley, G.E., Scammell, M.S. & Brockbank, C.I. (1992) The impact of the banning of tributyltin-based antifouling paints on the Sydney rock oyster, *Saccostrea commercialis*. *Science of The Total Environment*, **122**, 301–314.

Bauer, B., Fioroni, P., Ide, I., Liebe, S., Oehlmann, J., Stroben, E. & Watermann, B. (1995) Tbt effects on the female genital system of *Littorina littorea* - a possible indicator of tributyltin pollution. *Hydrobiologia*, **309**, 15–27.

Beaumont, A. & Budd, M. (1984) High mortality of the larvae of the common mussel at low concentrations of tributyltin. *Marine Pollution Bulletin*, **15**, 402–405.

Berto, D., Giani, M., Boscolo, R., Covelli, S., Giovanardi, O., Massironi, M. & Grassia, L. (2007) Organotins (TBT and DBT) in water, sediments, and gastropods of the southern Venice lagoon (Italy). *Marine Pollution Bulletin*, **55**, 425–435.

Bhosle, N., Garg, A., Jadhav, S., Harjee, R., Sawant, S., Venkat, K. & Anil, A. (2004) Butyltins in water, biofilm, animals and sediments of the west coast of India. *Chemosphere*, **57**, 897–907.

Blaber, S.J.M. (1970) The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (l.). *Journal of Molluscan Studies*, **39**, 231–233.

Borghi, V. & Porte, C. (2002) Organotin pollution in deep-sea fish from the northwestern Mediterranean. *Environmental Science & Technology*, **36**, 4224–4228.

Bosselman, K. (1996) Environmental law and tributyltin in the environment (ed. De Mora S). *Tributyltin - Case study of an Environmental Contaminant*. Cambridge Environmental Chemistry Series. Cambridge University Press.

Bradford, M. (1976) A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Bragadin, M., Marton, D., Manente, S., Scutari, G. & Toninello, A. (2003) Tributyltin and mitochondria: new evidence in support of an uncoupling mechanism and a further characterisation of the transport mechanism. *Inorganica Chimica Acta*, **348**, 225–228.

Bruschweiler, B., Wurgler, F. & Fent, K. (1995) Cytotoxicity in-Vitro of Organotin Compounds to Fish Hepatoma-Cells Plhc-1 (*Poeciliopsis lucida*). *Aquatic Toxicology*, **32**, 143–160.

Buggy, C.J. & Tobin, J.M. (2006) Seasonal and spatial distributions of tributyltin in surface sediment of the Tolka Estuary, Dublin, Ireland. *Environmental Pollution*, **143**, 294–303.

Burton, E., Phillips, I. & Hawker, D. (2004) Sorption and desorption behavior of tributyltin with natural sediments. *Environmental Science & Technology*, **38**, 6694–6700.

Cairns, J.J. & Van der Schalie, W.H. (1980) Biological monitoring: Part 1. Early warning systems. *Water Res.*, 1179–1169.

Cao, D., Jiang, G., Zhou, Q. & Yang, R. (2009) Organotin pollution in China: An overview of the current state and potential health risk. *Journal of Environmental Management*, **90, Supplement 1**, S16–S24.

Celander, M.C. (2011) Cocktail effects on biomarker responses in fish. *Aquatic Toxicology*, **105**, 72–77.

Champ, M. (2000) A review of organotin regulatory strategies, pending actions, related costs and benefits. *Science of the Total Environment*, **258**, 21–71.

Chen, C.-F., Kao, C.-M., Dong, C.-D. & Chen, C.-W. (2010) Butyltin contamination in sediments and seawater from Kaohsiung Harbor, Taiwan. *Environmental Monitoring and Assessment*, **169**, 75–87.

Cima, F., Craig, P. & Harrington, C. (2003) Organotin compounds in the environment. *Organometallic Compounds in the Environment, Craig PJ (ed.)*. pp. 101–149. John Wiley and Sons, West Sussex.

Cima, F., Marin, M.G., Matozzo, V., Da Ros, L. & Ballarin, L. (1998) Immunotoxic effects of organotin compounds in *Tapes philippinarum*. *Chemosphere*, **37**, 3035–3045.

Clark, E.A., Sterritt, R.M. & Lester, J.N. (1988) The fate of tributyltin in the aquatic environment. *Environ. Sci. Technol.*, **22**, 600–604.

Clements, W. & Rohr, J. (2009) Community responses to contaminants: Using basic ecological principles to predict ecotoxicologial effects. *Environmental toxicology and chemistry*, **28**, 1789–1800.

Coffman, W.P. & Ferrington, L.C.J. (1996) Chironomidae. *in: R.W. Merritt and K.W. Cummins (Eds.) An introduction to the aquatic insects of North America*. pp. 635–754. Kendall/Hunt Publishing Company.

Cooke, A.S. (1979) Egg shell characteristics of gannets *Sula bassana*, shags *Phalacrocorax aristotelis* and great black-backed gulls *Larus marinus* exposed to DDE and other environmental pollutants. *Environmental Pollution (1970)*, **19**, 47–65.

Corsini, E., Schubert, C., Marinovich, M. & Galli, C. (1996) Role of mitochondria in tributyltin-induced interleukin-1 alpha production in murine keratinocytes RID B-5602-2011. *Journal of Investigative Dermatology*, **107**, 720–725.

Dahl, B. & Blanck, H. (1996) Pollution-induced community tolerance (PICT) in periphyton communities established under tri-n-butyltin (TBT) stress in marine microcosms. *Aquatc toxicology*, **34**, 305–325.

Diez, S., Jover, E., Albaiges, J. & Bayona, J.M. (2006) Occurrence and degradation of butyltins and wastewater marker compounds in sediments from Barcelona harbor, Spain. *Environment International*, **32**, 858–865.

Dowson, P.H., Bubb, J.M. & Lester, J.N. (1993) Temporal distribution of organotins in the aquatic environment: Five years after the 1987 UK retail ban on TBT based antifouling paints. *Marine Pollution Bulletin*, **26**, 487–494.

Dowson, P., Bubb, J. & Lester, J. (1996) Persistence and degradation pathways of tributyltin in freshwater and estuarine sediments. *Estuarine Coastal and Shelf Science*, **42**, 551–562.

ECHA. (2009) ECHA publishes an updated list of preregistered substances. ECHA/PR/09/03.

Eggleton, J. & Thomas, K. (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environment International*, **30**, 973–980.

Eklund, B., Elfstrom, M., Gallego, I., Bengtsson, B.-E. & Breitholtz, M. (2010) Biological and chemical characterization of harbour sediments from the Stockholm area. *Journal of Soils and Sediments*, **10**, 127–141.

El-Shahawi, M.S., Hamza, A., Bashammakh, A.S. & Al-Saggaf, W.T. (2010) An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta*, **80**, 1587–1597.

Encarnacao, J.A., Kierdorf, U., Holweg, D., Jasnoch, U. & Wolters, V. (2005) Sex-related differences in roost-site selection by Daubenton's bats (*Myotis daubentonii*) during the nursery period. *Mammal Review*, **35**, 285–294.

Espourteille, F.A., Greaves, J. & Huggett, R.J. (1993) Measurement of tributyltin contamination of sediments and *Crassostrea virginica* in the southern chesapeake bay. *Environmental Toxicology and Chemistry*, **12**, 305–314.

European Community. (1989) European Community. 145–155.

Feng, L., Miao, W. & Wu, Y. (2007) Differentially expressed genes of *Tetrahymena thermophila* in response to tributyltin (TBT) identified by suppression subtractive hybridization and real time quantitative PCR. *Aquatic Toxicology*, **81**, 99–105.

Fent, K. (1996) Ecotoxicology of organotin compounds. *Critical Reviews in Toxicology*, **26**, 3–117.

Fent, K. (2004) Ecotoxicological effects at contaminated sites. *Toxicology*, **205**, 223–240.

Fent, K. & Hunn, J. (1995) Organotins in Fresh-Water Harbors and Rivers - Temporal Distribution, Annual Trends and Fate. *Environmental Toxicology and Chemistry*, **14**, 1123–1132.

Fent, K. & Meier, W. (1994) Effects of triphenyltin on fish early life stages. *Archives of Environmental Contamination and Toxicology*, **27**, 224–231.

Fernandez-Alba, A.R., Hernando, M.D., Piedra, L. & Chisti, Y. (2002) Toxicity evaluation of single and mixed antifouling biocides measured with acute toxicity bioassays. *Analytica Chimica Acta*, **456**, 303–312.

Ferrington, L.C. (2007) Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia*, **595**, 447–455.

Fioroni, P., Oehlmann, J. & Stroben, E. (1991) The pseudohermaphroditism of Prosobranchs - Morphological aspects. *Zoologischer Anzeiger*, **226**, 1–26.

Fish, R.H., Kimmel, E.C. & Casida, J.E. (1976) Bioorganotin chemistry: Reactions of tributyltin derivatives with a cytochrome P-450 dependent monooxygenase enzyme system. *Journal of Organometallic Chemistry*, **118**, 41–54.

Flavin, D.A., Biggane, S.S., Shiel, C.B., Smiddy, P. & Fairley, J.S. (2001) Analysis of the diet of Daubenton's batMyotis daubentonii in Ireland. *Acta Theriologica*, **46**, 43–52.

Frouin, H., Lebeuf, M., Saint-Louis, R., Hammill, M., Pelletier, E. & Fournier, M. (2008) Toxic effects of tributyltin and its metabolites on harbour seal (*Phoca vitulina*) immune cells in vitro. *Aquatic Toxicology*, **90**, 243–251.

Gadd, G. (2000) Microbial interactions with tributyltin compounds: detoxification, accumulation, and environmental fate. *Science of the Total Environment*, **258**, 119–127.

Gennari, A., Viviani, B., Galli, C., Marinovich, M., Pieters, R. & Corsini, E. (2000) Organotins induce apoptosis by disturbance of [Ca2+](i) and mitochondrial activity, causing oxidative stress and activation of caspases in rat thymocytes RID B-5663-2011 RID B-5602-2011. *Toxicology and Applied Pharmacology*, **169**, 185–190.

Gibbs, P. & Bryan, G. (1986) Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, **66**, 767–777.

Gopalakrishnan, S., Huang, W.-B., Wang, Q.-W., Wu, M.-L., Liu, J. & Wang, K.-J. (2011) Effects of tributyltin and benzo[a]pyrene on the immune-associated activities of hemocytes and recovery responses in the gastropod abalone, *Haliotis diversicolor*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **154**, 120–128.

Granö, O. & Roto, M. (1989) The duration of shore exposure along the emerging Finnish coast. *Journal of Coastal Research*, **5**, 49–55.

Greco, L., Capri, E. & Rustad, T. (2007) Biochemical responses in *Salmo salar* muscle following exposure to ethynylestradiol and tributyltin. *Chemosphere*, **68**, 564–571.

Gregory, L., Thielens, N., Arlaud, G., Fontecilla-Camps, J. & Gaboriaud, C. (2003) X-ray structure of the Ca2+-binding interaction domain of C1s. *Journal of Biological Chemistry*, **278**, 32157–32164.

Groenendijk, D., Postma, J.F. & Admiraal, W. (1996) Influence of drift on population dynamics of metal exposed and reference populations of the midge *Chironomus riparius.* (MJ Sommeijer and PJ Francke, Eds.). *Nederlandse Entomologische Verniging* (N E V), ***7***: 203-208.

Hagger, J., Depledge, M. & Galloway, T. (2005) Toxicity of tributyltin in the marine mollusc Mytilus edulis

RID C-1662-2009. *Marine Pollution Bulletin*, **51**, 811–816.

Harford, A.J., O'Halloran, K. & Wright, P.F.A. (2007) Effect of in vitro and in vivo organotin exposures on the immune functions of murray cod (Maccullochella peelii peelii) RID D-4175-2011. *Environmental Toxicology and Chemistry*, **26**, 1649–1656.

Harino, H., Fukushima, M. & Kawai, S. (2000) Accumulation of butyltin and phenyltin compounds in various fish species. *Archives of Environmental Contamination and Toxicology*, **39**, 13–19.

Harino, H., Fukushima, M., Kurokawa, Y. & Kawai, S. (1997) Degradation of the tributyltin compounds by the microorganisms in water and sediment collected from the harbour area of Osaka City, Japan. *Environmental Pollution*, **98**, 163–167.

Havas, M. & Hutchinson, T. (1982) Aquatic Invertebrates from the Smoking Hills, Nwt - Effect of Ph and Metals on Mortality. *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 890–903.

Helminen, H., Juntura, E., Koponen, J., Laihonen, P. & Ylinen, H. (1998) Assessing of long-distance background nutrient loading to the Archipelago Sea, northern Baltic, with a hydrodynamic model. *Environmental Modelling and Software*, **13**, 511–518.

Hoch, M. (2001) Organotin compounds in the environment - an overview. *Applied Geochemistry*, **16**, 719–743.

Huang, G., Bai, Z., Dai, S. & Xie, Q. (1993) Accumulation and toxic effect of organometallic compounds on algae. *Applied Organometallic Chemistry*, **7**, 373–380.

IMO. (2001) International Convention on the Control of Harmful Antifouling Systems on Ships, IMO (the AFS Convention).

Iwasaki, Y., Kagaya, T., Miyamoto, K., Matsuda, H. & Sakakibara, M. (2011) Effect of zinc on diversity of riverine benthic macroinvertebrates: Estimation of safe concentrations from field data. *Environmental Toxicology and Chemistry*, **30**, 2237–2243.

Jia, X., Zhang, Z., Wang, G., Zou, Z., Wang, S., Huang, B. & Wang, Y. (2009) Expressed sequence tag analysis for identification and characterization of genes related to Tributyltin (TBT) exposure in the abalone Haliotis diversicolor supertexta. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, **4**, 255–262.

Jumppanen, K. & Mattila, J. (1994) The development of the state of the Archipelago Sea and environmental factors (in Finnish with English summary). *Lounais–Suomen vesiensuojeluyhdistyksen julkaisuja,* **82**, 1–206.

Kannan, K., Corsolini, S., Focardi, S., Tanabe, S. & Tatsukawa, R. (1996) Accumulation pattern of butyltin compounds in dolphin, tuna, and shark collected from Italian coastal waters. *Archives of Environmental Contamination and Toxicology*, **31**, 19–23.

Kannan, K. & Falandysz, J. (1997) Butyltin residues in sediment, fish, fish-eating birds, harbour porpoise and human tissues from the Polish coast of the Baltic Sea. *Marine pollution bulletin*, **34**, 203–207.

Kannan, K. & Falandysz, J. (1999) Response to the comment on: Butyltin residues in sediment, fish, fish-eating birds, harbour porpoise and human tissues from the Polish coast of the Baltic Sea. *Marine pollution bulletin*, **38**, 61–63.

Kannan, K., Guruge, K., Thomas, N., Tanabe, S. & Giesy, J. (1998) Butyltin residues in southern sea otters (*Enhydra lutris nereis*) found dead along California coastal waters. *Environmental science and technology*, **32**, 1169–1175.

Kawai, S., Kurokawa, Y., Harino, H. & Fukushima, M. (1998) Degradation of tributyltin by a bacterial strain isolated from polluted river water. *Environmental Pollution*, **102**, 259–263.

Keith, J.A. (1966) Reproduction in a population of herring gulls (*Larus argentatus*) contaminated by DDT. *Journal of Applied Ecology*, **3**, 57–70.

Kilpi, M.K., Atosuo, J.T. & Lilius, E.-M.E. (2009) Bacteriolytic activity of the alternative pathway of complement differs kinetically from the classical pathway. *Developmental and Comparative Immunology*, **33**, 1102–1110.

Kim, G.B., Lee, J.S., Tanabe, S., Iwata, H., Tatsukawa, R. & Shimazaki, K. (1996) Specific accumulation and distribution of butyltin compounds in various organs and tissues of the Steller sea lion (*Eumetopias jubatus*): Comparison with organochlorine accumulation pattern. *Marine Pollution Bulletin*, **32**, 558–563.

Kotrikla, A. (2009) Environmental management aspects for TBT antifouling wastes from the shipyards. *Journal of Environmental Management*, **90**, S77–S85.

Kruskop, S. (2004) Subspecific structure of *Myotis daubentonii* (Chiroptera, Vespertilionidae) and composition of the “daubentonii” species group. *Mammalia*, **68**, 299–306.

Kwok, K. & Leung, K. (2005) Toxicity of antifouling biocides to the intertidal harpacticoid copepod *Tigriopus japonicus* (Crustacea, Copepoda): Effects of temperature and salinity RID C-1055-2009. *Marine Pollution Bulletin*, **51**, 830–837.

Lamoureux, E. & Brownawell, B. (1999) Chemical and biological availability of sediment-sorbed hydrophobic organic contaminants. *Environmental Toxicology and Chemistry*, **18**, 1733–1741.

Lascourreges, J., Caumette, P. & Donard, O. (2000) Toxicity of butyltin, phenyltin and inorganic tin compounds to sulfate-reducing bacteria isolated from anoxic marine sediments. *Applied Organometallic Chemistry*, **14**, 98–107.

Lee, R. (1985) Metabolism of tributyltin oxide by crabs, oysters and fish. *Marine Environmental Research*, **17**, 145–148.

Legendre, P. & Legendre, L. (1998) *Numerical ecology. 2nd English Edition*. Elsevier.

Lei, H., Huang, G. & Dai, S. (1998) Toxicity and accumulation of tributyltin chloride on tilapia. *Applied Organometallic Chemistry*, **12**, 109–119.

Leppäkoski, E. & Bornsdorff, E. (1989) Ecosystem variability and gradients. Examples from the Baltic Sea as a background for hazard assessment. *Chemicals in the Aquatic Environment. Advanced Hazard Assessment*. pp. 6–58. Springer-Verlag, Berlin.

Lepper, P. (2002) Towards the derivation of quality standards for priority substances in the context of the Water Framework Directive. Preparatory work for the European Commission. 124 pp.

Lespes, G., Marcic, C., Heroult, J., Le Hecho, I. & Denaix, L. (2009) Tributyltin and triphenyltin uptake by lettuce. *Journal of Environmental Management*, **90**, S60–S68.

Liu, J., Pan, L.-Q., Zhang, L., Miao, J. & Wang, J. (2009) Immune responses, ROS generation and the haemocyte damage of scallop *Chlamys farreri* exposed to Aroclor 1254. *Fish & Shellfish Immunology*, **26**, 422–428.

Liu, L.-L., Wang, J.-T., Chung, K.-N., Leu, M.-Y. & Meng, P.-J. (2011) Distribution and accumulation of organotin species in seawater, sediments and organisms collected from a Taiwan mariculture area. *Marine Pollution Bulletin*, **63**, 535–540.

Looser, P., Fent, K., Berg, M., Goudsmit, G. & Schwarzenbach, R. (2000) Uptake and elimination of triorganotin compounds by larval midge *Chironomus riparius* in the absence and presence of Aldrich humic acid. *Environmental science and technology*, **34**, 5165–5171.

Luan, T., Jin, J., Chan, S., Wong, Y. & Tam, N. (2006) Biosorption and biodegradation of tributyltin (TBT) by alginate immobilized *Chlorella vulgaris* beads in several treatment cycles. *Process Biochemistry*, **41**, 1560–1565.

Luoma, S. (1983) Bioavailability of trace-metals to aquatic organisms - a review. *Science of the Total Environment*, **28**, 1–22.

Mackey, A. (1977) Growth and development of varval *Chironomidae*. *Oikos*, **28**, 270–275.

Martin, R.C., George Dixon, D., James Maguire, R., Hodson, P.V. & Tkacz, R.J. (1989) Acute toxicity, uptake, depuration and tissue distribution of tri-n-butyltin in rainbow trout, *Salmo gairdneri*. *Aquatic Toxicology*, **15**, 37–51.

Martinez-Llado, X., Gibert, O., Marti, V., Diez, S., Romo, J., Bayona, J.M. & de Pablo, J. (2007) Distribution of polycyclic aromatic hydrocarbons (PAHs) and tributyltin (TBT) in Barcelona harbour sediments and their impact on benthic communities RID A-4179-2010. *Environmental Pollution*, **149**, 104–113.

Matveev, V., Kruskop, S. & Kramerov, D. (2005) Revalidation of *Myotis petax* Hollister, 1912 and its new status in connection with *M. daubentonii* (Kuhl, 1817) (Vespertilionidae, Chiroptera). *Acta Chiropterologica*, **7**, 23–37.

Meador, J. (2011) Organotins in aquatic biota: Occurence in tissue and toxicological significance. *Environmental contaminants in biota*. pp. 255–286. CRC Press, Boca Raton, USA.

Meador, J., Krone, C., Dyer, D. & Varanasi, U. (1997) Toxicity of sediment-associated tributyltin to infaunal invertebrates: Species comparison and the role of organic carbon. *Marine Environmental Research*, **43**, 219–241.

Medina, M.H., Correa, J.A. & Barata, C. (2007) Microevolution due to pollution: Possible consequences for ecosystem responses to toxic stress. *Chemosphere*, **67**, 2105–2114.

Mellbrand, K., Lavery, P.S., Hyndes, G. & Hambäck, P.A. (2011) Linking Land and Sea: Different Pathways for Marine Subsidies. *Ecosystems*, **14**, 732–744.

Meyer, M. & Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*, **2010**, pdb.prot5448–pdb.prot5448.

Michel, P. & Averty, B. (1999) Distribution and Fate of Tributyltin in Surface and Deep Waters of the Northwestern Mediterranean. *Environmental Science & Technology*, **33**, 2524–2528.

Misumi, I., Yada, T., Leong, J.-A.C. & Schreck, C.B. (2009) The effect of in vitro exposure to tributyltin on the immune competence of Chinook salmon (*Oncorhynchus tshawytscha*) leukocytes. *Archives of Environmental Contamination and Toxicology*, **56**, 229–237.

Morcillo, Y. & Porte, C. (1997) Interaction of tributyl- and triphenyltin with the microsomal monooxygenase system of molluscs and fish from the western Mediterranean. *Aquatic Toxicology*, **38**, 35–46.

Morcillo, Y. & Porte, C. (1998) Monitoring of organotin compounds and their effects in marine molluscs. *Trac-Trends in Analytical Chemistry*, **17**, 109–116.

Mortensen, A.S. & Arukwe, A. (2007) Modulation of xenobiotic biotransformation system and hormonal responses in Atlantic salmon (*Salmo salar*) after exposure to tributyltin JBT). *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, **145**, 431–441.

Naes, K., Hylland, K., Oug, E., Forlin, L. & Ericson, G. (1999) Accumulation and effects of aluminum smelter-generated polycyclic aromatic hydrocarbons on soft-bottom invertebrates and fish. *Environmental Toxicology and Chemistry*, **18**, 2205–2216.

Nakanishi, T. (2008) Endocrine disruption induced by organotin compounds; organotins function as a powerful agonist for nuclear receptors rather than an aromatase inhibitor. *Journal of toxical sciences*, **33**, 269–276.

Nakata, H., Sakakibara, A., Kanoh, M., Kudo, S., Watanabe, H., Nagai, N., Miyazaki, N., Asano, Y. & Tanabe, S. (2002) Evaluation of mitogen-induced responses in marine mammal and human lymphocytes by in-vitro exposure of butyltins and non-ortho coplanar PCBs. *Environmental Pollution*, **120**, 245–253.

Nakayama, K., Oshima, Y., Yamaguchi, T., Tsuruda, Y., Kang, I.J., Kobayashi, M., Imada, N. & Honjo, T. (2004) Fertilization success and sexual behavior in male medaka, *Oryzias latipes*, exposed to tributyltin. *Chemosphere*, **55**, 1331–1337.

NCI. (2000) National Chemicals Inspectorate. Organotin stabilisers in PVC— assessment of risks and proposals for risk reduction measures. Summary of Report, vol. 6/00. Solna, Sweden: Swedish Chemicals Agency.

Nelson, D., Koymans, L., Kamataki, T., Stegeman, J., Feyereisen, R., Waxman, D., Waterman, M., Gotoh, O., Coon, M., Estabrook, R., Gunsalus, I. & Nebert, D. (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1–42.

Nesci, S., Ventrella, V., Trombetti, F., Pirini, M. & Pagliarani, A. (2011) Multi-site TBT binding skews the inhibition of oligomycin on the mitochondrial Mg–ATPase in Mytilus *galloprovincialis*. *Biochimie*, **93**, 1157–1164.

Nilsson, N. (1997) *Aquatic Insects of North Europe. A Taxonomic Handbook, vol. 2.* Apollo Books, Stenstrup, Denmark.

Nowak, C., Vogt, C., Pfenninger, M., Schwenk, K., Oehlmann, J., Streit, B. & Oetken, M. (2009) Rapid genetic erosion in pollutant-exposed experimental chironomid populations. *Environmental pollution*, **157**, 881–886.

Nyholm, E. (1965) Zur Ökologie von *Myotis mystacinys* (Leisl.) und *M.daubentoni* (Leisl.) (Chiroptera). *Annales Zoologici Fennici*, **2**, 77–123.

OECD. (2001) Organisation for Economic Co-operation and Development. Report on the test results of endocrine disrupting effects of tributyltin (TBT) on fish (Draft). http://www.oecd.org/dataoecd/7/1/2461193.pdf.

OECD. (2004a) OECD guidelines for the testing of chemicals, Sediment-water chironomid toxicity test using spiked sediment.

OECD. (2004b) OECD guidelines for the testing of chemicals, Sediment-water chironomid toxicity test using spiked water.

Oehlmann, J., Di Benedetto, P., Tillmann, M., Duft, M., Oetken, M. & Schulte-Oehlmann, U. (2007) Endocrine disruption in prosobranch molluscs: evidence and ecological relevance. *Ecotoxicology*, **16**, 29–43.

de Oliveira, C.R., dos Santos, D., dos Santos Madureira, L.A. & Rodrigues de Marchi, M.R. (2010) Speciation of butyltin derivatives in surface sediments of three southern Brazilian harbors. *Journal of Hazardous Materials*, **181**, 851–856.

Osman, A.M., van Kol, S., Peijnenburg, A., Blokland, M., Pennings, J.L.A., Kleinjans, J.C.S. & van Loveren, H. (2009) Proteomic analysis of mouse thymoma EL4 cells treated with bis(tri-n-butyltin) oxide (TBTO). *Journal of Immunotoxicology*, **6**, 174–183.

Page, D.S., Ozbal, C.C. & Lanphear, M.E. (1996) Concentration of butyltin species in sediments associated with shipyard activity. *Environmental Pollution*, **91**, 237–243.

Pape, T., Bickel, D. & Meier, R. (2009) *Diptera Diversity: Status, Challenges and Tools* (D Bickel, R Meier, and T Pape, Eds.). Brill.

Parsons, K. & Jones, G. (2003) Dispersion and habitat use by *Myotis daubentonii* and *Myotis nattereri* during the swarming season: implications for conservation. *Animal Conservation* **6**, 283–290.

Pellerito, C., Nagy, L., Pellerito, L. & Szorcsik, A. (2006) Biological activity studies on organotin(IV)(n+) complexes and parent compounds. *Journal of Organometallic Chemistry*, **691**, 1733–1747.

Pitkänen, T. (2006) Missä ruokoa kasvaa? *Turun ammattikorkeakoulun puheenvuoroja*, **29:** 1–82.

Poerschmann, J., Kopinke, F.-D. & Pawliszyn, J. (1997) Solid phase microextraction to study the sorption of organotin compounds onto particulate and dissolved humic organic matter. *Environmental Science and Technology*, **31**, 3629–3636.

Raffray, M. & Cohen, G.M. (1991) Bis(tri-n-butyltin) oxide induces programmed cell death (apoptosis) in immature rat thymocytes. *Archives of Toxicology*, **65**, 135–139.

Rand, G.M. (Ed.). (1995) *Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment*. CRC Press.

Rantala, M. (2010) Orgaaniset tinayhdisteet sedimenteissä ja kaloissa - Diploma work. , 1–95.

Rasmussen, J. (1984a) Comparison of gut contents and assimilation efficiency of 4th instar larvae of 2 coexisting chironomids, *Chironomus riparius* (Meigen) and *Glyptotendipes paripes* (Edwards). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **62**, 1022–1026.

Rasmussen, J. (1984b) The life-history, distribution, and production of *Chironomus riparius* and *Glyptotendipes paripes* in a prairie pond. *Hydrobiologia*, **119**, 65–72.

Rasmussen, J. (1985) Effects of Density and Microdetritus Enrichment on the Growth of Chironomid Larvae in a Small Pond. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1418–1422.

Raunio, J., Heino, J. & Paasivirta, L. (2011) Non-biting midges in biodiversity conservation and environmental assessment: Findings from boreal freshwater ecosystems. *Ecological Indicators*, **11**, 1057–1064.

Rivas, G., Ingham, K.C. & Minton, A.P. (1992) Calcium ion induced self-association of human complement C1s. *Biochemistry*, **31**, 11707–11712.

Roessink, I., Koelmans, A.A. & Brock, T.C.M. (2008) Interactions between nutrients and organic micro-pollutants in shallow freshwater model ecosystems. *Science of the Total Environment*, **406**, 436–442.

Rudel, H. (2003) Case study: bioavailability of tin and tin compounds. *Ecotoxicology and Environmental Safety*, **56**, 180–189.

Saether, O. & Spies, M. (2011) Fauna Europaea: Diptera, Chironomidae. *Fauna Europaea version 2.4, http://www.faunaeur.org*.

Saint-Louis, R., Pelletier, É., Marsot, P. & Fournier, R. (1994) Distribution and effects of tributyltin chloride and its degradation products on the growth of the marine alga *Pavlova lutheri* in continuous culture. *Water Research*, **28**, 2533–2544.

Salmon, A.B., Leonard, S., Masamsetti, V., Pierce, A., Podlutsky, A.J., Podlutskaya, N., Richardson, A., Austad, S.N. & Chaudhuri, A.R. (2009) The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. *The FASEB Journal*, **23**, 2317 –2326.

Sarradin, P.M., Lapaquellerie, Y., Astruc, A., Latouche, C. & Astruc, M. (1995) Long term behaviour and degradation kinetics of tributyltin in a marina sediment. *Science of the Total Environment*, **170**, 59–70.

Sayer, C.D., Hoare, D.J., Simpson, G.L., Henderson, A.C.G., Liptrot, E.R., Jackson, M.J., Appleby, P.G., Boyle, J.F., Jones, J.I. & Waldock, M.J. (2006) TBT causes regime shift in shallow lakes. *Environmental Science & Technology*, **40**, 5269–5275.

Schmidt, K., Staaks, G., Pflugmacher, S. & Steinberg, C. (2005) Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behavior, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). *Aquatic Toxicology*, **71**, 49–59.

Schulte-Oehlmann, U., Tillmann, M., Markert, B., Oehlmann, J., Watermann, B. & Scherf, S. (2000) Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. *Ecotoxicology*, **9**, 399–412.

Seinen, W., Vos, J.G., van Krieken, R., Penninks, A., Brands, R. & Hooykaas, H. (1977) Toxicity of organotin compounds. III. Suppression of thymus-dependent immunity in rats by di-n-butyltindichloride and di-n-octyltindichloride. *Toxicology and Applied Pharmacology*, **42**, 213–224.

Seligman, P., Grovhoug, J., Stang, P., Valkirs, A., Stallard, M. & Lee, R. (1988) Distribution and fate of tributyltin in marine-environment. *Abstracts of Papers of the American Chemical Society*, **195**, 70–GEOC.

Senior, P., Butlin, R.K. & Altringham, J.D. (2005) Sex and segregation in temperate bats. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2467–2473.

Senthilkumar, K., Kannan, K., Subramanian, A. & Tanabe, S. (2001) Accumulation of organochlorine pesticides and polychlorinated biphenyls in sediments, aquatic organisms, birds, bird eggs and bat collected from South India. *Environmental Science and Pollution Research*, **8**, 35–47.

Shawky, S. & Emons, H. (1998) Distribution pattern of organotin compounds at different trophic levels of aquatic ecosystems. *Chemosphere*, **36**, 523–535.

Siah, A., Masson, R., Loup, B., Bultelle, F., Pellerin, J., Leboulenger, F. & Danger, J.M. (2007) Receptor activated C kinase is down-regulated in the male gonad of the marine bivalve mollusc Mya arenaria exposed to tributyltin (TBT). *Aquatic Toxicology*, **83**, 295–305.

Sibley, P., Benoit, D. & Ankley, G. (1997) The significance of growth in *Chironomus tentans* sediment toxicity tests: Relationship to reproduction and demographic endpoints. *Environmental Toxicology and Chemistry*, **16**, 336–345.

Siemers, B., Dietz, C., Nill, D. & Schnitzler, H. (2001) *Myotis daubentonii* is able to catch small fish. *Acta Chiropterologica*, **3**, 71–75.

Smith, B.S. (1981) Tributyltin compounds induce male characteristics on female mud snails *Nassarius obsoletus = Ilyanassa obsoleta*. *Journal of Applied Toxicology: JAT*, **1**, 141–144.

Smith, R., Bolam, S.G., Rees, H.L. & Mason, C. (2008) Macrofaunal recovery following TBT ban. *Environmental Monitoring and Assessment*, **136**, 245–256.

Snoeij, N.J., Punt, P.M., Penninks, A. & Seinen, W. (1986) Effects of tri-n-butyltin chloride on energy metabolism, macromolecular synthesis, precursor uptake and cyclic AMP production in isolated rat thymocytes. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **852**, 234–243.

Soderquist, C.J. & Crosby, D.G. (1980) Degradation of triphenyltin hydroxide in water. *J. Agric. Food Chem.*, **28**, 111–117.

Song, L., Seeger, A. & Santos-Sacchi, J. (2005) On membrane motor activity and chloride flux in the outer hair cell: Lessons learned from the environmental toxin tributyltin. *Biophysical Journal*, **88**, 2350–2362.

Song, X., Zapata, A. & Eng, G. (2006) Organotins and quantitative-structure activity/property relationships. *Journal of Organometallic Chemistry*, **691**, 1756–1760.

St-Jean, S., Pelletier, E. & Courtenay, S. (2002) Very low levels of waterborne butyltins modulate hemocyte function in the blue mussel *Mytilus edulis*. *Marine Ecology-Progress Series*, **236**, 155–161.

Stäb, J., Traas, T., Stroomberg, G., vanKesteren, J., Leonards, P., vanHattum, B., Brinkman, U. & Cofino, W. (1996) Determination of organotin compounds in the foodweb of a shallow freshwater lake in the Netherlands. *Archives of Environmental Contamination and Toxicology*, **31**, 319–328.

Steffens, R., Zöphel, U. & Brockmann, D. (2005) 40 Jahre Fledermausmarkierungszentrale Dresden – methodische Hinweise und Ergebnisubersicht. *Sächsisches Landesamt fur Umwelt und Geologie*.

Stenalt, E., Johansen, B., Lillienskjold, S. v. & Hansen, B.W. (1998) Mesocosm study of *Mytilus edulis* larvae and postlarvae, including the settlement phase, exposed to a gradient of tributyltin. *Ecotoxicology and Environmental Safety*, **40**, 212–225.

Suzuki, N., Tabata, M., Kambegawa, A., Srivastav, A., Shirnada, A., Takeda, H., Kobayashi, M., Wada, S., Katsumata, T. & Hattori, A. (2006) Tributyltin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. *Life Sciences*, **78**, 2533–2541.

Swift, S. & Racey, P. (1983) Resource partitioning in 2 species of vespertilionid bats (Chiroptera) occupying the same roost. *Journal of Zoology*, **200**, 249–259.

Taake, K.-H. (1992) Strategien der Ressourcennutzung an Waldgewässern jagender Fledermäuse. *Myotis*, **30**, 7–74.

Taenzler, V., Bruns, E., Dorgerloh, M., Pfeifle, V. & Weltje, L. (2007) Chironomids: suitable test organisms for risk assessment investigations on the potential endocrine disrupting properties of pesticides. *Ecotoxicology*, **16**, 221–230.

Terlizzi, A., Fraschetti, S., Gianguzza, P., Faimali, M. & Boero, F. (2001) Environmental impact of antifouling technologies: state of the art and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **11**, 311–317.

Tessier, A. & Campbell, P. (1987) Partitioning of trace-metals in sediments - relationships with bioavailability. *Hydrobiologia*, **149**, 43–52.

Thouvenin, M., Peron, J., Charreteur, C., Guerin, P., Langlois, J. & Vallee-Rehel, K. (2002) A study of the biocide release from antifouling paints. *Progress in Organic Coatings*, **44**, 75–83.

Timmermans, K. (1991) *Trace metal ecotoxicokinetics of chironomids. PhD thesis,*. University of Amsterdam, Amsterdam, Holland.

Tiunov, M.P. & Makarikova, T.A. (2007) Seasonal molting in *Myotis petax* (Chiroptera) in the Russian Far East. *Acta Chiropterologica*, **9**, 538–541.

Tremolada, P., Sugni, M., Gilioli, G., Barbaglio, A., Bonasoro, F. & Carnevali, M.D.C. (2009) A dynamic model for predicting chemical concentrations in water and biota during the planning phase of aquatic ecotoxicological tests RID A-4365-2012. *Chemosphere*, **75**, 915–923.

Tress, J., Tress, C., Schorcht, W., Biedermann, M., Koch, R. & Iffert, D. (2004) Mitteilungen zum Wanderverhalten der Wasserfledermaus (*Myotis daubentonii*) und der Rauhhautfledermaus (*Pipistrellus nathusii*) aus Mecklenburg. *Nyctalus*, **9**, 236–248.

Tujula, N., Radford, J., Nair, S. & Raftos, D. (2001) Effects of tributyltin and other metals on the phenoloxidase activating system of the tunicate, *Styela plicata*. *Aquatic Toxicology*, **55**, 191–201.

Valkirs, A.O., Davidson, B., Kear, L.L., Fransham, R.L., Grovhoug, J.G. & Seligman, P.F. (1991) Long-term monitoring of tributyltin in San Diego Bay California. *Marine Environmental Research*, **32**, 151–167.

Valkirs, A., Seligman, P. & Lee, R. (1986) Butyltin Partitioning in Marine Waters and Sediments. *OCEANS '86*, 1165–1170.

Vaughan, N. (1997) The diets of British bats (Chiroptera). *Mammal Review*, **27**, 77–94.

Vermeulen, A.C., Liberloo, G., Dumont, P., Ollevier, F. & Goddeeris, B. (2000) Exposure of *Chironomus riparius* larvae (diptera) to lead, mercury and β-sitosterol: effects on mouthpart deformation and moulting. *Chemosphere*, **41**, 1581–1591.

Vogt, C., Belz, D., Galluba, S., Nowak, C., Oetken, M. & Oehlmann, J. (2007a) Effects of cadmium and tributyltin on development and reproduction of the non-biting midge Chironomus riparius (Diptera) - baseline experiments for future multi-generation studies. *Journal of Environmental Science and Health*, **42**, 1–9.

Vogt, C., Nowak, C., Diogo, J., Oetken, M., Schwenk, K. & Oehlinann, J. (2007b) Multi-generation studies with Chironomus riparius - Effects of low tributyltin concentrations on life history parameters and genetic diversity. *Chemosphere*, **67**, 2192–2200.

Vogt, C., Nowak, C., Diogo, J., Oetken, M., Schwenk, K. & Oehlinann, J. (2007) Multi-generation studies with *Chironomus riparius* - Effects of low tributyltin concentrations on life history parameters and genetic diversity. *Chemosphere*, **67**, 2192–2200.

Wade, T.L., Garcia-Romero, B. & Brooks, J.M. (1991) Oysters as biomonitors of butyltins in the Gulf of Nexico. *Marine Environmental Research*, **32**, 233–241.

Warren, R., Waters, D., Altringham, J. & Bullock, D. (2000) The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. *Biological Conservation*, **92**, 85–91.

Wilhelm Filho, D., Althoff, S.L., Dafré, A.L. & Boveris, A. (2007) Antioxidant defenses, longevity and ecophysiology of South American bats. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **146**, 214–220.

Ympäristöministeriö. (2007) Orgaaniset tinayhdisteet Suomen vesialueilla, Ympäristöministeriön työryhmän mietintö.

Yngve, V. (1940) US Patent 2219463.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000) A greedy algorithm for aligning DNA sequences. *Journal of computational biology: a journal of computational molecular cell biology*, **7**, 203–214.

Zhang, K., Wang, G., Zou, Z., Jia, X., Wang, S., Lin, P., Chen, Y., Zhang, Z. & Wang, Y. (2009) Cloning, characterization and TBT exposure response of CuZn superoxide dismutase from *Haliotis diversicolor supertexta*. *Molecular Biology Reports*, **36**, 583–594.

Zhou, J., Cai, Z., Zhu, X., Li, L. & Gao, Y. (2010) Innate immune parameters and haemolymph protein expression profile to evaluate the immunotoxicity of tributyltin on abalone *(Haliotis diversicolor supertexta*). *Developmental & Comparative Immunology*, **34**, 1059–1067.

Zhu, X., Xing, M., Lou, J., Wang, X., Fu, W. & Xu, L. (2007) Apoptotic related biochemical changes in human amnion cells induced by tributyltin. *Toxicology*, **230**, 45–52.