The Otter (Lutra lutra) in Sweden

Contaminants and Health

ANNA ROOS
In the 1950s the otter started to decline in numbers and distribution in Sweden and other parts of Europe. In 1972 a game law came into force, listing otter as a species that if found dead should be reported and sent to the authorities. The numbers of dead otters reported from different areas indicate population status and distribution. Between 1970 and 2012, 832 otters were sent to the authorities, the majority (66%) during the last ten years. Most were killed in traffic accidents or drowned in fishing gear. However, the main cause of the decline is believed to be environmental contaminants. Experimental data show that a PCB residue level in muscle tissue of 12 mg/kg lw causes reproductive impairment in mink (Neovison vison), suggesting reproductive problems also in the highly PCB-exposed otters in Sweden. Since the bans of PCB and DDT in the mid-1970s, concentrations of these substances in otter and fish have decreased and the otter population is increasing.

Few pathological changes in otters have been found that can be related to high contaminant concentrations. However, we found a correlation between elevated PCB concentrations and alterations in bone mineral density. No relationship was seen between DDE and bone parameters.

The decline of the otter coincided with the decline of grey seals (Halichoerus grypus) and white-tailed sea eagles (Haliaeetus albicilla) in Sweden, all showing decreased reproductive outcome. Reproductive success started to increase for all of them around 1990 and during the same period concentrations of PCB and DDE have decreased in these species.

The body condition among female otters has increased over the study period, indicating an improved health status. However, we found a high prevalence (71%) of cysts on the spermatic duct in otters collected between 1999 and 2012, possibly caused by endocrine disrupting chemicals.

Although the organochlorine concentrations in otters have decreased, otters still face many problems. New threats to the otter population in Scandinavia are the perfluorinated chemicals, including PFOS and PFOA. Results in this thesis show an increasing trend for these compounds in otters up to 2011, including some extremely high concentrations of PFOS in otters from southern Sweden.

Keywords: Otter (Lutra lutra); PCB; DDT; PFAAs; reproduction; bone; time trends

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To my biggest supporter
-my Mother
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


V Roos, A., and Ågren, E. 2013. High prevalence of Müllerian duct cysts along the spermatic duct in wild Eurasian otters (*Lutra lutra*) from Sweden. *Submitted*

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Abbreviations

AMH  Anti Müllerian hormone
BMD  Bone mineral density
Cort-A  Cortical area (mm$^2$)
Cort-cnt  Cortical content (mg/mm)
Cort-den  Cortical density (mg/cm$^3$)
CB  Chlorinated biphenyl
DDD  Dichlorodiphenyl dichloroethane
DI  Desiccation index
$\Sigma$DDT  Sum of DDT (DDT+DDE+DDD)
DDT  Dichlorodiphenyl trichloroethane
DDE  Dichlorodiphenyl dichloroethylene
EDC  Endocrine disrupting chemical
ESB  Environmental Specimen Bank
lw  Lipid weight
MeHg  Methyl mercury
PCB  Polychlorinated biphenyls
PFAAs  Perfluoroalkyl acids
PFCA$s$  Perfluoroalkyl carboxylic acid
PFHxA  Perfluorohexanoic acid
PFHpA  Perfluoroheptanoic acid
PFOA  Perfluorooctanoic acid
PFNA  Perfluorononanoic acid
PFDA  Perfluorodecanoic acid
PFUnDA  Perfluoroundecanoic acid
PFDcDA  Perfluorododecanoic acid
PFTrDA  Perfluorotridecanoic acid
PFPeDA  Perfluoropentadecanoic acid
PFSA$s$s  Perfluoroalkane sulfonic acids
PFBS  Perfluorobutane sulfonic acid
PFHxS  Perfluorohexane sulfonic acid
PFOS  Perfluorooctane sulfonic acid
PFDS  Perfluorodecane sulfonic acid
FOSA  Perfluorooctane sulfonamide
SMNH  Swedish Museum of Natural History
TEQ  Toxic equivalent
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>Tot-cnt</td>
<td>Total bone mineral content (mg/mm)</td>
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<td>Tot-den</td>
<td>Total bone mineral density (mg/cm$^3$)</td>
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<td>Tot-A</td>
<td>Total bone cross sectional area (mm$^2$)</td>
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<tr>
<td>Trab-A</td>
<td>Trabecular area (mm$^2$)</td>
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<td>ww</td>
<td>Wet weight</td>
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Introduction

Otter

The otter is a medium-sized aquatic mustelid with short legs and a long muscular tail. In Sweden, adult males weigh 7–10 kg and are up to 120 cm long, and females weigh 4-7 kg and are approximately 100 cm long [1]. It mostly feeds on fish, but also on small mammals, birds and crayfish. The majority of the population in Sweden lives in limnic areas.

The male otter lives alone all year round, except during mating. The female gives birth to one or two cubs (up to five), about 63 days after mating. The cubs stay with their mother for approximately one year, but the period of lactation ends when the cubs are ~4 months old [2]. In Sweden, cubs are born all year round, although there might be a seasonal preference to summer and autumn, as in otters in some other areas, for example in Norway [3].

The otter grows to adult size within a year, and becomes sexually mature approximately at the age of two years [2,4]. Few otters reach the age of ten in the wild. In a study from Scotland, the overall mean age at death of otters was 4 years [5,6].

Population trends

During the first part of the 20th century the otter was common in Sweden (except for Gotland, the largest island in the Baltic Sea). However, during the second part of the century the population started to decline in numbers and area of distribution. Similar trends were also reported from other parts of western Europe where otters had been numerous prior to 1950. In some areas in central Europe it became endangered or very close to extinction, e.g., Denmark, Belgium, Netherlands, Germany, Italy, Luxembourg, and Switzerland whereas it was widespread in other areas, e.g., northern Finland and northwestern Norway, western France, Portugal, Greece, Albania, Hungary, Belarus and Ireland [7]. In 1968, the situation in Sweden was considered so serious that otter hunting was banned, except in connection with fish farms where hunting was allowed for another year. Thereafter the species was completely protected. The decrease continued after the ban, and
in 1977 the total Swedish population was estimated to 500-1500 otters, similar to the outcome of the annual hunt in the 1940–1950s (Figure 1).

![Hunting statistics - otter](image)

**Figure 1.** Annual number of otters (*Lutra lutra*) killed during otter hunt, indicating a drastic decline in the otter population in and after the 1950s [8].

Despite protection, otter numbers in Sweden continued to decrease [8,9,10,11]. The otter has been listed in Swedish game legislation since 1972 [12] which means that all dead otters found should be reported to the authorities, and the carcass sent to the Swedish Museum of Natural History (SMNH), or, in some cases, to other Swedish museums. Since the end of the 1960s, tissue samples from these animals are stored frozen in the Environmental Specimen Bank (ESB), whereas skins and skeletons are saved in the Zoology collections at the SMNH. From the end of the 1980s the otter population has increased, as indicated by the increase in number of dead otters sent to SMNH (Figure 2).

![Number of dead otters sent to the authorities 1974-2012](image)

**Figure 2.** Number of otters (*Lutra lutra*) found dead in Sweden and sent to museums of natural history from 1974–2012 (n=830). (Data from SMNH).
Many otter surveys have been carried out also during the last decade, showing that the otter population has increased (for summary see Figure 3a–d [13]. The surveys give an overview of the otter population status and distribution and most of them report an increasing – but still somewhat fragmented – otter population [13,14,15,16,17].

**Figure 3a–d.** Distribution of otter (*Lutra lutra*) in Sweden during different decades. The data includes several otter surveys as well as otters found dead. White areas = not surveyed, light grey = surveyed areas with no traces of otter, dark grey = surveyed areas with traces of otter. ▲ = locality of dead otters sent to the SMNH, 1970s: n=60; 1980s: n= 26, 1990s: n=126 and 2000–2010 n=454.

### Environmental contaminants

**PCB**

Polychlorinated biphenyls (PCBs) are a class of man-made organic compounds that consist of biphenyl molecules with one to ten chlorine atoms attached (Figure 4).

**Figure 4.** Chemical structure of PCB.
There are 209 congeners with varying numbers and positions of chlorines in the molecule. Many of the congeners are detected in the environment but their concentrations differ several magnitudes [18,19].

PCB was first manufactured in the US in 1929, and quickly came in use in, for example, electrical applications such as insulators for transformers and capacitors, as flame retardants, in heat transfer systems, hydraulic fluids, carbonless copying paper, compressors, plasticizers, pigments and as adhesives. It was also used in anti-fouling paint on boats. PCBs have a high thermal and chemical resistance, and some of the congeners are very stable. In general, the more chlorine atoms attached to the biphenyl the more stable the chlorinated biphenyl (CB) congener is. Also the position of the chlorine atoms on the molecule is of high importance [20]. Stable means that the molecule does not readily break down when exposed to heat or chemical treatment, or in various processes in the environment. The stable congeners remain in the environment for long periods of time, have low water solubility and accumulate in fatty tissues.

Also the toxicity of a CB-congener is dependent on the positions of the chlorines. There are three groups of CBs, that:

1) are chlorinated in two or more ortho positions (positions nr 2,2' and 6,6' in Figure 4);
2) are chlorinated in only one ortho position and are therefore called mono-ortho CBs;
3) lack chlorine atoms in ortho position, known as non-ortho or co-planar CBs. The co-planar CBs are considered the most toxic congeners, resembling the dioxins. They are found in very low concentrations in wildlife compared to the congeners with chlorine in ortho position [18,19].

PCB was banned in several steps in the mid-1970s in Sweden.

**Effects of PCB**

PCBs have many negative effects including an impact on the immune, reproductive, and endocrine systems [21,22,23,24,25,26,27,28].

Experimental studies on mink have shown that non- and mono-ortho CBs reduce the hepatic and pulmonary vitamin A concentrations [29]. Vitamin A deficiency may lead to a higher risk of infectious diseases, developmental disturbances and cancer [24,30]. In otters collected in the UK between 1988 and 1996 there was a negative relationship between concentrations of PCB and vitamin A in the liver [31]. Also, a negative correlation was found between hepatic vitamin A and concentrations of co-planar PCBs in otters from Denmark [24].

PCB has been shown to impair reproduction for the mink in laboratory experiments. Minks treated with a combination of CB congeners showed the most severe negative effects [21,22,23,27,28,32]. Pregnant minks either had spontaneous abortions or the newborn cubs died shortly after birth. Mink
with approximately 12.5 mg/kg PCB lw or higher in muscle showed severely impaired reproduction [21].

Due to the strong co-variation, it is often difficult to differentiate PCB and dichlorodiphenyl trichloroethane (DDT) and evaluate which of the chemicals that has caused an effect in nature, but a laboratory study on the mink concluded that it was PCB and not DDT that reduced reproductive success in mink [33].

It is also believed that it is mainly PCB that caused sterility among the seals in the Baltic [34,35].

DDT

DDT is an insecticide that came into use in 1942. The man who discovered its unique properties as an insecticide was Paul H. Müller, who received the Nobel Prize for this discovery in 1948. DDT is still used against insects spreading malaria and other diseases. It was used in Sweden up to 1970 when it was banned due to its negative effects on wildlife. However, DDT was allowed to be used in the Swedish forestry up to 1975 to fight insects harming fir tree plants, and it was used in lice killing shampoo until the 1980s.

DDT is a mixture of several similar isomers: \( p,p'-\text{DDT} \) is the major isomer (about 77% of the mixture, Figure 5), and approximately 15% of the DDT mixture is made up of \( o,p'-\text{DDT} \). In the environment DDT is degraded, fairly rapidly, to dichlorodiphenyl dichloroethylene (DDE) and dichlorodiphenyl dichloroethane (DDD). The half-life for DDT in soil is 2.5–5 years (depending on the type of soil) [36]. DDT, DDE, and DDD are, like PCBs and methyl mercury (MeHg), fat soluble, and are therefore found in adipose tissues in animals. In otters, it is most often only DDE that is detected, even during the 1970-1980s.

Figure 5. \( p,p'-\text{DDT} \) (left) and \( p,p'-\text{DDE} \), a metabolite of DDT (right).

Effects of DDT

DDT is an effective insecticide, with low acute toxicity to humans and wildlife. However, it has been associated with several negative effects in a longer perspective, such as eggshell thinning among many wild bird species.
A decreased eggshell thickness in correlation with elevated concentrations of DDTs was first demonstrated in eggs from peregrine falcons and Eurasian sparrowhawk (*Accipiter nisus*) in Great Britain [37]. Also peregrine falcons, ospreys (*Pandion haliaetus*) and white-tailed sea eagles (*Haliaeetus albicilla*) from Sweden showed decreased eggshell thickness with correlations to high concentrations of $\sum$DDT, (DDT+DDE+DDD), in the eggs. The reduced eggshell thickness had implications for reproductive success [38,39].

**Mercury**

In the 1920–1960s mercury (Hg) was used as seed-dressing agents in agriculture in Sweden. It was banned in 1966 due to the severe effect it had on many species of bird. Also the use of Hg within the paper mill industry was banned at approximately the same time.

Anthropogenic activities such as combustion of fossil fuels, mining and smelting, chlorine- and paper production as well as the use of amalgam in tooth fillings release large amounts of Hg into the ecosystems. The metal spreads locally from point sources such as industries, but also regionally and globally since Hg can be transported via the atmosphere and be deposited over land and oceans.

Metallic Hg can be transformed with the help of bacteria to organic forms, such as MeHg, which are more toxic than the inorganic, metallic Hg. In fish muscle almost all Hg is in the form of MeHg [40].

**Effects of mercury**

MeHg is highly toxic. It can pass the blood-brain barrier and affect the nervous system. MeHg biomagnifies through aquatic food chains [41], and can pose a threat to piscivorous species in certain areas.

In Sweden large numbers of birds were found dead or dying from Hg poisoning in the 1950s, which was related to the use of alkyl Hg compounds as seed-dressing agents [42]. A retrospective study on feathers from northern goshawk (*Accipiter gentilis*) showed a 10-fold increase of Hg in feathers collected 1940–1965 compared to Hg concentrations in feathers from 1815-1939 [43]. However, after the ban in 1966 the concentrations decreased quickly, as seen in for example feathers from yearlings of western marsh harrier (*Circus aeruginous*) sampled from 1965–1976 [44].

Experimental studies on the American mink have shown that mink receiving between 1-2 mg/kg of MeHg in their diet for a period of 100 days display signs of Hg toxicosis including anorexia, neural necrosis, and reproductive impairment [45].

River otters (*Lontra canadensis*) in a controlled laboratory experiment were fed a diet containing different concentrations of MeHg. When mean liver and muscle concentrations of MeHg reached 33 and 20 mg/kg ww
respectively, the otters developed signs of intoxication [46]. Two old otters from Shetland that died from Hg poisoning had concentrations higher than 30 mg/kg dry weight in liver, and showed an erratic behaviour before dying [5]. It was not reported whether it was MeHg or total-Hg that was measured in the Shetland study. Nevertheless, these otters showed high mercury concentrations. Certain individual otters from Sweden have shown very high concentrations of total Hg in liver. Probably only a fraction of total-Hg is MeHg in liver, and presumably the elevated concentrations seen in otters from Sweden (up to 119 mg/kg ww total-Hg in liver) are mostly inorganic Hg and not MeHg (Roos unpublished).

Perfluorinated alkyl acids

In the last decade a new group of chemicals has come in focus. Ever since Giesey and Kannan first described the world wide contamination of perfluorinated chemicals in wild animals [47], a lot of attention has been given to these compounds. Per- and polyfluoroalkyl substances (PFASs) are, in contrast to the chlorinated chemicals like PCB and DDT, both oil- and water repellent. They are highly fluorinated, man-made chemicals that have been in use for more than half a century in numerous industrial and consumer product applications [48] such as textile stain and soil repellents, grease-proofing for food-contact paper, processing aids in fluoropolymer manufacturing and in aqueous film-forming fire fighting foams. The PFASs can be divided into two groups: perfluoroalkyl acids (PFAAs) and their environmental or metabolic precursor compounds [49]. PFAAs are extremely persistent in the environment.

The two PFAA sub-groups of highest concern are the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkane sulfonic acids (PFSAs). Perfluorooctane sulfonic acid (PFOS) is the most widely investigated PFSA (Figure 6) and is usually found in by far the highest concentrations of the PFAAs in wildlife.

![Figure 6. Chemical structure of perfluorooctane sulfonic acid (PFOS).](image)

The largest historic producer of PFOS and PFOS-based compounds (i.e. PFOS precursors), the 3M company in US, phased out the production on a voluntary basis between 2000 and 2002 after evidence of elevated
concentrations of PFOS in blood from their workers and in wildlife [50]. Many time trends for concentrations of PFOS, including those in wildlife, have stopped increasing or started to level off since then. This is seen in for example Baltic grey seals [51] and guillemot eggs (Uria aalge) from the Baltic [52]. PFOS was banned in many applications within the EU in June 2008, but was partly replaced by other persistent PFAAs, for example the shorter chain homologue perfluorobutane sulfonic acid (PFBS). Another persistent PFAA receiving a lot of attention is perfluorooctanoic acid (PFOA), which has many manufacturing and industrial applications and is used primarily during the production of fluoropolymers.

**Effects of PFOS and PFOA**

The primary target organ of PFOS and PFOA is believed to be the liver. In mice PFOS and PFOA cause increased liver weight and hepatocytic hypertrophy [53,54]. Other species given PFOA (rats, monkeys, dogs and rabbits) showed the same effects [54]. Also, studies have shown abnormal behavior, weight loss and serious damage in liver and lung [55] as well as developmental neurotoxic effects [56] following exposure of PFOSA and PFOS. Early pregnancy loss, decreased postnatal survival, delays in general growth and development was also found for mice [57]. Excretion in urine appears to be a major route of elimination but the rate of clearance of PFOA differs between species [54].

**Bone**

The skeleton consists of two different types of bone tissue. The trabecular bone, also called the cancellous bone or “spongy bone”, typically occupies the interior region of bones (Figure 7). Approximately 25% of the trabecular bone is replaced yearly. It is highly vascularised and often contains red bone marrow where the production of blood cells occurs.

The other type of bone tissue is the cortical bone, synonymous with compact bone (Figure 7). The cortical bone supports the body, and forms the outer shell of most bones. It is very hard and dense and contributes about 80% of the weight of a human skeleton. Approximately 3% of the cortical bone is exchanged yearly.

Because bone is composed of dynamic tissues and bone remodelling (degradation and formation) continues constantly throughout life it is not surprising that pathological changes can be found also in the hard and compact cortical bone as well. Many factors have an influence on bone tissue growth and remodelling, such as hormones and vitamins but also stress, contaminants and endocrine disorders can affect the bone tissue homeostasis [58,59,60,61,62,63].
Organochlorines and bone

Environmental contaminants have been associated with pathological changes in bones from grey and harbour seals (*Phoca vitulina*) in Scandinavian waters [58,64,65]. A study of bone mineral density (BMD) in polar bears from Greenland revealed correlations between altered BMD and several different environmental contaminants such as PCBs, ∑DDT and chlordanes [62]. Another study on polar bears reported that penis bone weight and/or length decreased significantly with increasing levels of several organochlorines in adipose tissue in sub-adults and/or adults [66].

In the wild it is difficult to pinpoint a specific compound to a pathological lesion since there are so many confounding factors. However, several laboratory studies investigating effects of different PCB congeners on bone variables have been performed. For example, rats exposed to the coplanar congener CB-126 showed several effects, including thicker cortical bone [60,67]. A study on perinatal exposure to CB-153 and CB-126, respectively, in female goat (*Capra aegagrus hircus*) offspring concluded that CB-153 altered bone composition [61].
Aims

The otter used to be widespread in most parts of Sweden, but started to decline in the 1950s. Several reasons for the decline have been discussed, and one in particular is the role of contaminants. This thesis focuses on the role of contaminants, and their possible detrimental effects on otter health and reproduction. It also pinpoints new threats such as increasing concentrations of PFAAs and report high prevalence of cysts on the spermatic duct (vas deferens). The specific aims in the different papers were:

Paper I. To study PCB and $\sum$DDT concentrations in otters and otter feed (fish) from Sweden and to elucidate possible explanations to the decrease in otter population observed in Sweden after the 1950s.

Paper II. To elucidate whether otter bone alterations are correlated to PCB and/or DDE concentrations in the otters.

Paper III. To compare reproductive indices in three aquatic species in Sweden, otter, grey seal and white-tailed sea eagle, over time: otter, grey seal and white-tailed sea eagle in relation to organochlorine contaminants. The paper summarizes four decades of research on these species, and compares trends in reproductive success and time trends of PCB and DDE concentrations.

Paper IV. To study temporal trends in concentrations of perfluorinated contaminants in otters from southern Sweden (1972-2011), as well as to compare concentrations in otters from three areas: south-western Norway, southern and northern Sweden, respectively.

Paper V. To describe cysts found on the spermatic duct in otters, the prevalence and possible underlying cause.
Comments on methods for sampling and chemical analysis

Otters that are found dead are reported to the authorities, according to a game law. The police, or the public, send the carcasses to the SMNH or to the National Veterinary Institute in Uppsala. Otters that have died in traffic or drowned accidently in fishing gear are more likely to be reported and therefore the majority of the otters sent to SMNH have died from human activities.

Once at the museum the otter is registered, thawed and necropsied according to routine protocol. Also, as exact locality data as possible are noted along with other observations on circumstances around the finding. Several organs (liver, kidneys, heart, adrenals, ovaries, testes etc) are excised, sub sampled and stored at -30°C in the Environmental Specimen Bank (ESB). Otters that are sent to the National Veterinary Institute are necropsied there and then sent to the SMNH for sampling to the ESB.

For contaminant analysis, tissues are taken from ESB and sub sampled for various analyses (if not taken directly at necropsy). Organochlorines are analysed in muscle and PFAAs in liver. Heavy metals are analysed in muscle, liver and/or fur. Not all otters are analysed chemically, but until 2011 approximately 10/year were analysed.

The method for chemical analysis of PCB (and DDT) in otters was changed in 1992. Ten otters were analysed with both methods and the results were compared. The results from the latter method were then adjusted so that results from the two methods could be combined. How this is done is described in detail in Papers II and III. Previously the concentration of PCB was calculated based on seven of the major peaks in the chromatogram, which gave a sum of the major PCB congeners, “total-PCB”. This method was replaced by a method using capillary column gas chromatography, which separates the individual PCB congeners better. In this thesis, the PCB concentrations, determined after 1992 were based on analysis of CB-105, CB-118, CB-138+163, CB-153, CB-156, and CB-180. (The congeners 138 and 163 are not separated). These congeners are the most abundant, but not the most toxic CB congeners. However, the sum of PCBs gives a fair indication of the total-PCB burden. The results of the analysis of DDT and its metabolites did not differ between the two methods.
Also the method for PFAA analysis has changed, and 9 otters were analysed with both methods in order to compare the methods.
Results

Contaminant concentrations

PCB and DDT
A decrease in concentrations of $\Sigma$DDT and total-PCB in otters from northern Sweden was found for the period 1970–1994 (95% and 70%, respectively, Paper I). In southern Sweden the decreases in $\Sigma$DDT and total-PCB concentrations during this period were 75% and 50%, respectively. Also, the contaminant concentrations in fish from the 13 lakes included in the investigation decreased during the study period. The concentrations of $\Sigma$DDT decreased by approximately 80%, and total-PCB decreased by 60% in fish from most of the 13 lakes. The decrease of organochlorines has continued in otters as seen in Paper III, although the largest decrease took place early during the studied time period. In Paper III, the time trend of total-PCB and DDE is extended to year 2010. The concentrations of total-PCB decreased with 5.9% yearly during the last four decades. The estimated mean concentration of total-PCB has decreased in otters from approximately 70 mg/kg lw in 1968 to 8 mg/kg lw in 2010.

The annual decrease of mean $\Sigma$DDT concentrations in otters was estimated to be 8.8%. The estimated mean concentration of $\Sigma$DDT has decreased from approximately 3.4 mg/kg lw in 1968 to 0.2 mg/kg lw in 2010.

Fluorinated contaminants
Livers from 140 otters were analysed for PFAAs (Paper IV) to study time trends (1970–2011) and geographical trends (2005–2011). The time trends focused on otters from southern Sweden only, and the spatial study included otters from northern Sweden, southern Sweden and southwestern Norway.

PFOS was found in all samples in concentrations ranging from 19–16000 ng/g ww and was the predominant PFAA compound (approximately 80% of the analysed PFAAs were PFOS).

Perfluorononanoic acid (PFNA) was the second most abundant PFAA and the dominant PFCA with liver concentrations ranging between 0.51 and 637 ng/g ww. Among the PFCAs, the concentrations decreased gradually
with increasing chain length from PFNA to perfluoropentadecanoic acid (PFPeDA). Perfluoroctane sulfonamide (FOSA) was the only non-persistent precursor compound included in this study. It was found in all samples analysed (0.7-92 ng/g, n=41).

Significantly increasing concentrations between 1972 and 2011 were found for 9 of the 12 investigated compounds: PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), PFOS and PFDS (See Figure 8 for three examples: PFOA, PFNA and PFDA). The yearly increase for the different PFAAs was in the range 5.5–13%, resulting in doubling times between 5.5 and 13 years. When looking only at the last ten years of the time trend (2002–2011), the PFCAs still showed increasing trends and most PFCAs (with the exception of PFUnDA and PFTrDA) increased at an even faster rate during recent years compared to the whole study period. No significant trend for the concentrations of PFSAs was detected between 2002 and 2011.

No significant differences in PFAA concentrations between sub-adults and adults were found in otters apart from PFNA, where adults had slightly higher concentrations compared to the sub-adults (t-test, p=0.008). Also, no significant difference in concentrations between males and females was found for any of the compounds.

![PFAS (ng/g) in Otter liver](image)

*Figure 8. The concentrations of tree perfluorinated carboxylates: PFOA, PFNA and PFDA, all show increasing trends in otter (*Lutra lutra*, liver, ww) over time, even if only including the last ten years of the time series.*
A mother and her juvenile cub were analysed in Paper IV, and the liver concentration ratios cub/mother were below 1 for all PFAAs, indicating a limited transfer from mother to cub for several of the compounds. The highest ratios were found for the long-chain PFCAs (PFDoDA, PFTrDA and PFTeDA), with values between 0.80 and 0.99. Perfluorohexane sulfonic acid (PFHxS) showed the highest cub/mother ratio (0.72) among the PFSAs. The ratio of PFAA concentrations in lactiferous tissue/liver from a lactating female was ≤0.2 for most compounds. Only long-chain PFCAs (PFTrDA, PFTeDA and PFPeDA) and PFHxS displayed higher ratios, which can explain the elevated ratios of these PFAAs compared to the other analytes in the cub/mother comparison.

The general pattern (i.e. relative proportions) of PFAAs in otters in Paper IV was similar for all three sampling regions (northern Sweden, southern Sweden and south-western Norway). Among the PFCAs, PFNA dominated (33–40%) followed by PFDA and PFUnDA (20–30%). The other homologues did not contribute much to the sum of PFCAs.

The median concentration of PFOS in otters from southern Sweden was significantly higher than that in otters from northern Sweden and Norway. No significant difference between concentrations of PFOS in otters from northern Sweden and Norway was observed, although some very high (up to 3700 ng/g ww) concentrations were found in otters from northern Sweden, but not in Norway.

**Time trend for population status**

The otter population started to increase in northern Sweden when the median concentration of PCB in otter muscle was 10 mg/kg lw after 1990 (Paper I). The increases in southern Sweden came somewhat later. The increase in population is seen in otter surveys that are carried out over the country but it is also indicated in the number of otters sent to the authorities, from only few/year in 1990 to almost 100 in 2012.

Similar increases in the otter population, starting in the end of 1980s, are seen in the grey seal in the Baltic as well as the white-tailed sea eagle [13,68,69].
Health status

Few correlations between health status and contaminant concentrations in otters have been seen, but in Paper II it is shown that otters with elevated concentrations of PCB (but not DDE) have alterations in the cortical bone. There were positive relationships between three of the cortical bone variables analysed (area, mineral content (Figure 9) and thickness) and residue levels of PCB in muscle tissue, indicating that otters respond to elevated PCB concentrations. No correlations were found between PCB or DDE concentrations and trabecular bone parameters.

The alterations on the bone seen on the femurs were also seen on other bones in the skeleton.

Figure 9. Cortical mineral content (mg/mm) in correlation to log-PCB (mg/kg lw in muscle tissue, p<0.001, n=86) in otters (*Lutra lutra*) collected between 1974 and 2004 (Paper II).

The reproductive indices for otters have improved during the study period (1970s–2010, Paper III). The frequency of adult female otters with signs of reproduction increased from 0% in the beginning of the study period to 67% in 2010 (Figure 10). During the same period, body condition index showed an increasing trend among females (p<0.01) with about 0.65% yearly, indicating an improved health status (Figure 11).
Figure 10. Frequency of adult female otters (*Lutra lutra*) with signs of reproduction (lactating, pregnant and/or implantation scars) during the study period, from 1970 to 2010 (n=155). The line represents a logistic curve estimated by nonlinear regression.

Figure 11. Body condition index for female otters (*Lutra lutra*) showed an yearly increase of 0.65% during the study period (1970-2010, n=419, p<0.01).

In recent years I have noted that many males had cysts on the spermatic duct. This was seen in 71% of the males studied in Paper V. The number of cysts on each spermatic duct varied from one to over ten and was found in all age classes (juveniles, sub-adults and adults). Unilateral cysts were seen in 49% and bilateral cysts in 51% of otters with cysts. Among individual otters with one or more cysts present (n=168), 37, 28.6, and 34.4% had one, two, or three or more cysts, respectively (Figure 12).
Figure 12. Five cysts indicated on the spermatic duct (*vas deferens*) in an otter (*Lutra lutra*).
Discussion

Health parameters

Bone alterations

In paper II a positive correlation between altered cortical bone parameters and concentrations of PCB (but not DDE) in otter muscle was found. Alterations on seal bones from the Baltic have also been seen. For grey seals in the Baltic it is seen as bone loss [58]. In harbor seals on the Swedish west coast a deposition of bone tissue (exostosis of the alveolar bone) was seen in increasing prevalence over time [64], a bit similar to findings in otters in this study. The alterations seen on otter femurs in Paper II have been observed on other bones of the otter as well. The alteration seen on femurs might not have a negative effect on the individual, but similar alterations on the vertebral column might hinder the otter from moving freely.

Few other studies have been able to correlate a pathological alteration in otters with concentrations of environmental contaminants. In otters from Denmark, a negative correlation was detected between hepatic vitamin A concentration and co-planar PCB concentrations [24]. Otters with elevated concentrations of co-planar PCB, i.e. more than 2 ng TEQ/g lw in blood or liver, had strongly reduced hepatic vitamin A concentrations, and also showed a higher incidence of infectious diseases (although not statistically significant). Vitamin A deficiency can alter bone parameters similar to what was seen in otters, which has been shown in an experiment with dogs given a diet without vitamin A [70].

Reproduction

The prevalence of females with signs of recent reproduction has increased since approximately 1990. Also the reproductive health among grey seal females and white-tailed sea eagles has improved, during approximately the same time, while at the same time the concentrations of PCB and DDE have decreased substantially in the three animals.

During 1970-2011 the body condition index has increased in female otters, indicating a better health status.

However, a high prevalence of cysts (71%) on the vas deferens was seen in otters from most parts of Sweden in recent years (Paper V).
The presence of this type of cyst has not been described in otters previously, although it is possible that they have been overlooked earlier at necropsies. We propose that these cysts are Müllerian duct cysts, which are fetal remnants of the female genital ducts that normally regress under hormonal influence, once male differentiation is initiated. Anti-Müllerian hormone (AMH) induces the regression of the Müllerian ducts in male fetuses during sex differentiation and the timing of the AMH action on these ducts is very critical. In dogs (having similar gestation length as otter) the earliest evidence of Müllerian duct regression in male embryos was observed at 36 days of gestation, and the regression was completed by day 46 indicating the time window when the sex differentiation takes place [71].

In a laboratory study, pregnant mice were exposed to a synthetic estrogen – diethylstilbestrol (DES) and prominent Müllerian remnants were observed in 97% of the male offspring. These Müllerian remnants were often enlarged and cystic [72]. Possibly, the underlying causes for the cysts in otters are exposure to endocrine disrupting chemicals (EDCs) during the fetal stage.

Reason for the decline of the otter population

There are many factors that have been discussed in connection with the decline of the otter population including traffic, fishing, diseases and hunting [73].

Acidification of waters has been discussed as a problem for the otter, especially in the western and southern parts of Sweden which have been affected by acid rain with a subsequent decrease in pH in the waters. This has led to diminishing fish stocks in some lakes and thereby less food resources for the otter [73]. However, since the otter also disappeared from areas that were not affected by acidification, this could not be a general problem for the otter.

Although there are several possible reasons for a decline in the otter population, these above mentioned factors cannot explain why the otter disappeared from areas with little or no impact from human settlements. The large reduction in population sizes that has been seen for several animal species in Sweden, such as white-tailed sea eagle and seals in the Baltic Sea, coincided with a peak in levels of environmental contaminants, especially organochlorines and Hg. Therefore it was suggested that contaminants was the main factor causing the dramatic decline and posed the most serious threat also to the otter population [74,75,76,77]. The question is: which contaminants or groups of contaminants are to blame?
PCB and DDT

Otters with high residue levels of PCB have been found in decreasing and threatened populations, while otters with low residue levels of PCB have been found in thriving populations [11,75,76,77,78,79,80,81], indicating a sensitivity to PCB.

Severe reproductive failure in minks given PCB in laboratory studies has been found, as has been described earlier. Similar studies have not been performed with otter but it is reasonable to believe that the results are relevant for the otters as well, supporting the hypothesis that PCB could be one of the reasons for the decline of the otter.

However, Kruuk and Conroy [6] analysed PCB in livers from 116 otters (mostly traffic victims) collected between 1987 and 1992 from thriving otter populations in Scotland and their results were somewhat different from those of other studies. They reported low concentrations of dieldrin and DDE, and some otters with high concentrations of PCB. The highest PCB concentration was found in a lactating female. Therefore the authors cast doubt on the significance of published “critical levels” of PCBs to otter populations. The lactating female had a PCB concentration of 25 mg/kg ww (=1097 mg/kg lw) in liver. Data from Sweden show that lactating otters have very low residue levels of PCB in muscle [82], and that cubs have much higher residue levels of PCB in muscle compared to their mothers.

The concentrations of PCB in otters from Sweden have been very high but after the bans in the 1970s the concentrations have decreased substantially, and the otter population is now increasing.

Since residue levels of PCB and DDT were highly correlated it is difficult to discriminate between the two and elucidate which of them that caused reproductive failure, or indeed, if both contaminants contributed (Paper III).

Similar temporal trends of improved reproductive success and decreasing concentrations of \( \sum \)DDT and PCBs among otters, grey seals and white-tailed sea eagles further support the hypothesis that these compounds have had negative effects on the reproductive ability, and thereby also a negative effect on the population sizes. Not until the end of the 1980s or beginning of the 1990s could an improvement in reproductive success be seen, followed by an increase in the populations of the three species (Paper III).

Other contaminants

Mercury

Hg has been discussed as a possible reason for the decline of the otter population. Hg is analysed within the monitoring programme at SMNH, for
example in pike from Lake Bolmen in southern Sweden and Lake Storvindeln in northern Sweden and none of the localities show a trend over the last 40 years (Figure 13a+b). Also otters from Sweden have been analysed for Hg concentrations in a time trend from 1970-2002 and no trend was seen [83].

![Figure 13. Hg concentration in pike (Esox lucius) muscle (mg/kg ww) shows no trend over time a) pike from Lake Bolmen in southern Sweden and b) pike from Lake Storvindeln in northern Sweden. Redrawn from data from SMNH. [84].](image)

If Hg had a negative effect on the otter population one would expect decreasing concentrations of Hg in fish as well as in otters as the otter population increases but that was not the case.

**Dieldrin**

The decline of the otter population, and of many bird species in the UK, took place quickly and simultaneously over the whole country around 1957 and the most probable reason for the otter decline was pointed out to be the use of dieldrin [85]. One otter that died in 1972 had 14 mg/kg ww in liver (=237 mg/kg lw). This individual also had exceptionally high concentrations of DDE (20 mg/kg ww, or 332 mg/kg lw). All other otters in that study had much lower DDE concentrations [86] as had the otters from Sweden (Papers I–III). Jefferies and Hansson argued that dieldrin was the major threat to the otter population in the United Kingdom [86]. Clearly, at least parts of the UK environment were severely contaminated by dieldrin and DDT. Consequently, it is probable that dieldrin had a negative effect on otters and other wildlife in the UK.

Dieldrin was not commonly used in Sweden, and was banned in 1970. In Sweden, dieldrin has neither been used to a large extent in agriculture nor in sheep farming as in the UK. However, somewhat elevated concentrations of dieldrin were found in fish downstream textile industries in south-western Sweden at the end of the 1960s [87]. One thousand fish from Sweden, from both marine and freshwater areas, were analysed for dieldrin by the National
Institute of Public Health (later called National Food Agency) in the 1960s and in general low concentrations were found [87]. Furthermore, in the areas locally contaminated with dieldrin the concentrations decreased rapidly after the Swedish bans in 1970.

Dieldrin was analysed in a retrospective study recently performed, where a small sample of otters from Sweden (n=28), collected between 1968 and 2002, were included. It was shown that the concentrations were indeed very low even in the beginning of the study period when dieldrin was not yet banned. All but two individuals showed concentrations below 0.2 mg/kg ww (Figure 14, Roos unpublished data). In conclusion, there is no indication that dieldrin has caused any serious threat to the otter in Sweden.

![Figure 14](image.png)

*Figure 14. Concentrations (mg/kg ww) of dieldrin in liver of otters (*Lutra lutra*) from southern Sweden between 1968 and 2002 (n=28).*

**New threats to the otter population**

**Perfluorinated chemicals**

In accordance with the present study on otters, no difference in PFAA concentrations between adult male and female polar bears was found [88], which is different to what was seen for the chlorinated organic compounds [35,89]. Also, in both otters and polar bears, sub-adults had similar liver concentrations as adults for all compounds. This could indicate that lactation is not a major pathway of PFAA elimination among otters and polar bears or that a steady-state is reached quickly again after lactation. However, in harbour seals (*Phoca vitulina concolor*) in the northwest Atlantic, pups had higher concentrations of PFOS and PFDS compared to adults, and the authors suggested that maternal transfer is an important route for PFAAs to
pups [90]. It thus seems that otters are more like bears than seals in terms of lactational transfer of PFAAs.

Most studies on humans and wildlife are from marine areas and report decreasing trends for PFAAs such as PFOA and PFOS starting from the beginning of the 2000s. This applies also to the majority of investigated temporal trends from the Swedish environment, e.g. to grey seals and humans [51,91,92]. In the otters of the present study, however, all PFAAs show significantly increasing time trends (including PFOA and PFOS). The reason for this discrepancy is not known. It could possibly be due to an extraordinarily long elimination half-life of PFAAs in otters. This would explain both the potential to accumulate very high levels of PFOS as well as the continuously increasing trends of PFOA and PFOS despite potentially decreasing trends in the otter’s environment and diet.
Conclusions and outlook

The present thesis shows that the residue levels of PCB and DDT in Swedish otters have decreased since the 1980s. The numbers of dead otters sent to the authorities indicate an increase in population size after approximately 1990 and this is also seen in otter surveys carried out regularly. As in most countries, there are several tentative threats to the otter in Sweden. Dieldrin has had a negative impact on the otter population and other wildlife in the UK, where thousands animals were found dead in lethal doses of dieldrin or had elevated concentrations of dieldrin [85,86,93,94]. However, in Sweden organochlorines such as PCB and DDT appear to have been the main problems for the otter.

The otter is an aquatic top predator and as such heavily exposed to environmental contaminants. It is therefore important to continue monitoring contaminants in otters, as well as otter health. For example, we have shown that 71% of the males have cysts on their spermatic duct. The cysts do probably not impair the reproductive outcome but can be a sign of elevated EDC exposure to the fetus. At present, several studies are undertaken regarding otter health and contaminant concentrations in otters from Sweden (e.g. heavy metals, chlorinated and brominated compounds as well as some pharmaceuticals and parasites). The otter is recovering in many countries in Europe. However, in certain areas the population is decreasing. For example, the thriving population in Belarus decreased by 90% a few years ago, and the reason behind this is not known. This shows how rapidly some unknown, probably environmental, factor can affect an otter population. Consequently, the otter is a good indicator species showing the quality of the environment. Thus, it is important to continue monitoring otter populations in the future.
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I will never forget the first day I came to the Swedish Museum of Natural History, looking for a job. Mats Hjelmberg greeted me at the door and showed me the lab where Rickard Brook was blowing an osprey egg. I immediately liked both of them. It was in late 1987, and I had had many different types of jobs before, and saw this one in the lab as something fun and “in between”. I loved working with them in the lab, and I found most things we did very interesting. But I could not imagine staying there for so long! In those days, the Contaminant Research Group (CRG) was quite small, and tight. Mats Olsson became professor in 1989 and that was when I got a permanent position in the lab, as his assistant. Wawa Persson was the secretary and the two of us were the only females for several years, and we had a great time together. We went to the reindeer slaughter once a year to take samples, and while waiting for the slaughter to start we picked mushrooms and lingonberries in a stunningly beautiful environment. It was fantastic. Also, Anders Bignert worked at CRG at that time, and we became close friends. Tjelvar Odsjö had always time for a chat, and Alf Johnels, professor emeritus who actually started the CRG, came in every day and we had numerous of interesting talks and we shared many views such as an interest in animal welfare as well as the love of dogs. Alf died in 2010.

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The front photo is taken by Tomas Claesson in Skede: two otters on Emån (Småland), January 15, 2012. There were three cubs with their mother; the photo shows two of the cubs.

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❤

Denna doktorsavhandling fokuserar på uttern, diskuterar tänkbara orsaker till utterns minskning i landet och belyser dess hälsotillstånd. År 1972 infördes ”Kronans vilebråd” (senare ”Statens vilt”), ett begrepp som omfattar knappt 50 mer eller mindre ovanliga däggdjur och fåglar i Sverige. Om de hittas döda ska djuren enligt lagen rapporteras till myndigheterna. Polis och allmänhet hjälper till att skicka kadavren till Naturhistoriska riksmuseet. Där tas organprover som sparas i frysta i museets Miljöprovbank, och skeletten sparas i bensamlingarna. Mellan 1970 och 2012 rapporterades 832 uttrar, merparten av dem under de senaste tio åren (66%). De flesta uttrarna hade dött i trafiken (77%) eller fastnat i fiskeredskap och drunknat (8%).

Även om många uttrar dör i trafiken och/eller fastnar i fiskeredskap och drunknar så är den gemensamma orsaken till utterns minskning i stora delar av Europa förmodligen någon annan. De flesta forskare är övertygade om att orsaken är miljögifter i utterns föda. Forskare har emellertid olika uppfattningar om vilket miljögift det rör sig om. En del hänvisar till den stora användningen av insektsbekämpningsmedlet dieldrin, som sammanföll med massdöd av grävling, räv och olika fågelerarter i Storbritannien. Andra forskare har pekat ut kvicksilver som tänkbar bov i dramat. Många utterforskare är dock tämligen övertygade om att det är industri kemikalien PCB som medverkade till att uttern minskade så kraftigt i antal och utbredning.

PCB är en kemikalie som haft en mycket omfattande användning inom en rad olika applikationer såsom i transformatorer, kondensatorer, fogmassor,
flamskyddsmedel, självkopierande papper och båtbottenfärg. PCB förbjöds i Sverige i mitten av 1970-talet men finns fortfarande kvar i miljön på grund av dess stabilitet. Mink (*Neovison vison*) som i laboratorieförsök fått i sig PCB via födan uppvisar reproduktionsstörningar redan vid en halt i muskelfett på 12 mg/kg, och det är möjligt att ungefär samma PCB koncentration också påverkar fortplantningsförmågan hos uttern. I Sverige sågs ökningen av utterstammen först då medianhalterna av PCB föll under 10 mg/kg fet.


Kvicksilverhalter i fisk analyseras på årlig basis bland annat i två sjöar i Sverige sedan slutet av 1960-talet: Bolmen i södra Sverige och Storvindeln i norr. Under denna tidsperiod har inga förändringar i kvicksilverhalt observerats. Om kvicksilver varit orsak till populationsminskningen hos utter så skulle man förvänta sig att halterna minskar då uttern ökar men så är inte fallet.

Sammanfattningsvis så pekar det mesta på att det är just PCB och eventuellt DDT som har varit begränsande för utterstammen i Sverige, men det kan inte uteslutas att andra ämnen också kan ha påverkat uttern negativt i andra länder.

Exakt hur PCB har påverkat just uttern är svårt att avgöra, men troligen är det största problemet med PCB en negativ påverkan på reproduktionen. Störning av reproduktionen är också den mest uppenbara effekten av PCB i laboratorieförsök på mink. Få kopplingar mellan exponering av miljögifter och en patologisk förändring hos vilda uttrar har gjorts, men i ett av arbetena i denna avhandling beskrivs en koppling mellan höga halter av PCB – men inte DDE - i utter och pålagringar på utterns skelett. I vissa fall kan detta ha medfört en begränsad rörelseförmåga, vilket kan ha påverkat det allmänna hälsotillståndet.

En tydlig ökande trend av frekvensen honor som visar tecken på reproduktion har påvisats efter omkring 1990. Från att i stort sett inga av de inkomna vuxna honororna visade tecken på reproduktion 1970 medan andelen honor som visade tecken på reproduktion var närmare 80% år 2010. Även honornas kroppsindex har ökat över tid, vilket indikerar en bättre hälsostatus. Kroppsindex räknas ut i en formel som tar hänsyn till längd och

Utterpopulationen ökar nu i hela landet, vilket syns i utterinventeringar och antalet döda djur som inrapporterats till myndigheterna men också att uttern nu har återkommit till områden som tidigare varit tomma på utter under flera årtionden (t.ex. Skåne, Halland och Värmland).


Ett annat fynd som redovisas i denna avhandling är en hög frekvens cystor på sådesledarna hos uttrar. Hela 71% av alla undersökta hanar de senaste åren har en eller flera cystor på ena eller båda sådesledarna. Dessa cystor kan vara en rest från så kallade Müllerska gångarna vilka normalt tillbakabildas hos hanfoster under könsdifferentieringen. För närvarande finns ingen misstanke om att cystorna gör hanarna sterila, men deras förekomst visar på en möjlig påverkan av hormonestörande ämnen under fostertiden.


Uttern ökar nu i många områden i Europa, inte bara i Sverige. Det finns dock områden i Europa där utterpopulationen minskar. I Vitryssland, som tidigare hade en stark utterpopulation, har populationen minskat med 90% för några år sedan. Detta visar hur snabbt en förändring i populationstäthet kan ske utan att det finns en uppenbar förklaring. Uttern är en bra indikator på en god miljö, och det är viktigt att även i fortsättningen övervaka populationen, dess hälsostatus och djurens miljögiftsbelastning.
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