Improved Assessment in Environmental Monitoring of POPs

Using monitoring data from the aquatic ecosystem and human milk

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Abstract

The thesis deals with several aspects of monitoring of persistent organic contaminants (POPs) in biological matrices, for example choice of sample, sampling design, and statistical treatment of data both for temporal and spatial trends and for compliance towards a set target value. The efficiency has been evaluated through statistical power analyses. Contaminant data from more than 4 decades from the Swedish National Monitoring Programs for monitoring of contaminants in biota (marine, freshwater and human health), has been quantitatively evaluated both temporally and spatially and for compliance. The aim was also to evaluate the suitability of different matrices, i.e. herring (*Clupea harengus*), guillemot (*Uria aalge*) egg, cod (*Gadus morhua*), perch (*Perca fluviatilis*), eelpout (*Zoarces viviparous*), blue mussel (*Mytilus edulis*), pike (*Esox lucius*), Arctic char (*Salvelinus alpinus*) and human milk, for monitoring of POPs with the overall aim to improve the assessment within monitoring programs.

The results show that variation can be reduced by using pooled samples including more specimens but fewer chemical analyses, which in turn generate a higher statistical power to a lower cost, at least in cases where the cost of collection and sampling is considerably lower than the cost of chemical analysis. However, there are also a number of advantages using individual samples, such as information of sample variance and maximum value, which allows the choice of an appropriate central measure and direct adjustment of confounding factors.

Generally, the levels of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB) have decreased both in marine and freshwater biota but concentrations are still higher in the Baltic compared to e.g. the North Sea. The levels of dioxinlike-PCBs and polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDD/Fs) have decreased in human milk over time, but not to the same extent in fish and guillemot egg from the Baltic and the freshwater environment. This may be explained by the dietary advice developed by the Swedish Food Administration with the goal that girls, reproductive aged, and pregnant women should eat less food containing high levels of PCDD/Fs. Thus the levels in milk could continue to decrease at the same rate although the temporal trend in the environment has slowed down or leveled out.

The most essential regarding the choice of species and matrices for contaminant monitoring, is that the species and organ fit the purpose of the monitoring.

Keywords: *Environmental monitoring, temporal trends, power analysis, sampling strategy, PCBs, DDTs, HCHs, HCB, PCDD/Fs, fish, bird eggs, blue mussels, human milk.*

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Till mamma och pappa 

List of publications

This thesis is based on the following articles, which are referred to by roman numerals (I-V) in the text. Papers (I-V) are reprinted with kind permission of the publishers of Chemosphere, Ambio, Marine Environmental Research, and Environment International. My own contributions to each of the papers are presented in Appendix A.

I. Bignert A., Ericsson U., **Nyberg E.**, Miller A. and Danielsson S. 2014. Consequences of using pooled versus individual samples for designing environmental monitoring sampling strategies. Chemosphere 94: 177-182.

II. **Nyberg E.**, Danielsson S., Eriksson U., Faxneld S., Miller A. and Bignert A. 2014. Spatio-temporal trends of PCBs in the Swedish freshwater environment 1981-2012. Ambio 43: 45-57.

III. **Nyberg E.**, Faxneld S., Danielsson S., Eriksson U., Miller A. and Bignert A. 2015. Temporal and spatial trends of PCBs, DDTs, HCHs, and HCB in Swedish marine biota 1969-2012. Ambio 44: 484-497.

IV. Miller A., **Nyberg E.**, Danielsson S., Faxneld S., Haglund P. and Bignert A. 2014. Comparing temporal trends of organochlorines in guillemot eggs and Baltic herring: Advantages and disadvantages for selecting sentinel species for environmental monitoring. Marine Environmental Research 100: 38-47.

V. Fång J., **Nyberg E.**, Bignert A. and Bergman Å. 2013. Temporal trends of polychlorinated dibenzo-p-dioxins and dibenzofurans and dioxin-like polychlorinated biphenyls in mothers' milk from Sweden, 1972–2011. Environment International 60: 224-231.

Additional relevant publications by the author:

Fång J., **Nyberg E.**, Winnberg U., Bignert A. and Bergman Å. 2015. Spatial and temporal trends of the Stockholm Convention POPs in mothers' milk- a global review. Environmental Science and Pollution Research 22: 8989-9041.

Jörundsdóttir H.O., Jensen S., Hylland K., Holth T.F., Gunnlaugsdóttir H., Svavarsson J., Ólafsdóttir Á., El-Taliwy H., Rigét F., Strand J., **Nyberg E.**, Bignert A., Hoydal K.S. and Halldórsson. 2014. Pristine Arctic: Background mapping of PAHs, PAH metabolites and inorganic trace elements in the North-Atlantic Arctic and sub-Arctic coastal environment.
Science of The Total Environment 493: 719-728.

Miller A., Hedman J., **Nyberg E.**, Haglund P., Cousins I.T and Wiberg K. 2013. Temporal trends in dioxins (polychlorinated dibenzo-p-dioxin and dibenzofurans) and dioxin-like polychlorinated biphenyls in Baltic herring (*Clupea harengus*). Marine Pollution Bulletin 73: 220-230.

Dittman T., Becker P.H., Bakker J., Bignert A., **Nyberg E.**, Pereira M.G., Pijanowska U., et al. 2012. Large-scale spatial pollution patterns around the North Sea indicated by costal bird eggs within an EcoQO programme. Environmental Science and Pollution Research 19: 4060-4072.

Contents

Abbreviations

BMF	Biomagnification Factor
CL	Confidence Level
CV	Coefficient of Variation
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DL-PCBs	Dioxin-like polychlorinated biphenyls
EAC	Environmental Assessment Criteria
EcoQO	Ecological Quality Objectives
EPA	Environmental Protection Agency
EQS	Environmental Quality Standards
ESB	Environmental Specimen Bank
GES	Good Environmental Status
HCB	Hexachlorobenzene
HCHs	Hexachlorocyclohexanes
HELCOM	Helsinki Commission
LDT	Lowest detectable trend
LOD	Level or limit of detection
LOQ	Level or limit of quantification
MSFD	Marine Strategy Framework Directive
OSPARCOM	Oslo Paris Commission
PAH	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofurans
POPs	Persistent Organic Pollutants
SC	Stockholm Convention
SIA	Stable Isotope Analysis
SMNH	Swedish Museum of Natural History
SNMPCFB	Swedish National Freshwater Monitoring Program for Contaminants in Biota
SNMPCMB	Swedish National Marine Monitoring Program for Contaminants in Biota

SNMPHH	Swedish National Monitoring Program for Human Health
TDI	Tolerable Daily Intake
TGD	Technical Guidance Document
TL	Trophic Level
TMF	Trophic Magnification Factor
WFD	Water Framework Directive
YQR	Number of years required to detect and annual change of 10%

1 Introduction

American biologist Rachel Carson wrote the book *Silent Spring* in 1962. The book described the effects that the use of pesticides had on American wildlife, especially on birds (Carson 1962). The book put environmental issues on the table of the American public in particular, but was also globally noticed. Barely seven years later, Sören Jensen and co-workers found high levels of persistent organic pollutants (POPs) (e.g. PCBs and DDTs) in Swedish biota, and realised POPs were also a great threat to the Baltic Sea and Swedish freshwater ecosystems (Jensen et al. 1969). These findings were the starting point of continuous national monitoring programs for contaminants in biological matrices from the marine, freshwater and terrestrial environment in Sweden. At the same time, also as a result of the recognised pollution, Dr. Koidu Norén and co-workers started to collect and analyse organic contaminants in human milk from the Stockholm area to investigate human exposure.

The aim of the monitoring programs was primarily to monitor temporal trends to evaluate the rate of change in POPs when assessing measures taken to reduce the contaminant load. Since the start of the monitoring, overall significant decreases of about 70–90% have been observed for PCBs, DDTs, HCHs and HCB in fish and bird eggs from both the Baltic Sea and the Swedish freshwater environment, which implies that bans and restrictions implemented in the 1970s and 1980s have had the desired effect. Several of the classical POPs have also decreased considerably in human milk. However, concentrations of PCBs, DDTs and HCHs are still higher in the Baltic Sea compared to, for example, the North Sea.

When monitoring contaminants in biological matrices, there are several aspects that need to be considered, such as the choice of sample (species, organ etc.), sampling design, and how to evaluate the data statistically both for trend analysis and for compliance with a set target value. These aspects will be discussed in detail within this thesis.

1.1 Aim of thesis

The primary aim was to evaluate monitoring data collected for several decades within the national monitoring programs for contaminants in biological matrices, including the marine program, the freshwater program, and the human health program. The secondary aim was to assess the suitability of different matrices used for environmental monitoring of POPs for assessment of temporal and spatial trends, and to evaluate concentrations for compliance of set target values with the overall aim to improve the evaluation of results within the current monitoring programs. To evaluate and control efficiency and to guarantee quality in a quantitative way, statistical aspects have played a major role through e.g. power analysis.

Specific aims for each paper follow.

Paper I: To examine various scenarios using individual or pooled samples and the relationships between chemical analytical error and other sources of sample variance within the marine monitoring program for contaminants in biota; to outline advantages and disadvantages of pooled samples compared to individual samples. The article may be used as a technical guidance for countries setting up new monitoring programs.

Paper II: To examine temporal and spatial trends of PCB congeners in perch (*Perca fluviatilis*), pike (*Esox lucius*), and Arctic char (*Salvelinus alpinus*); to evaluate concentrations over time in relation to bans and restrictions, and in compliance with set environmental target levels; to investigate how monitoring design may affect the interpretation of these trends.

Paper III: To examine temporal, seasonal, and spatial relationships of PCBs, HCHs, HCB, and DDTs primarily in herring (*Clupea harengus*) and guillemot (*Uria aalge*) eggs, but also in cod (*Gadus morhua*), perch (*Perca fluviatilis*), eelpout (*Zoarces viviparous*), and blue mussel (*Mytilus edulis*); to evaluate concentrations over time in relation to imposed bans and restrictions; to investigate compound profiles that could indicate new releases or aging of residues, e.g. DDT/DDE, patterns of α, β, γ-HCH; and to examine concentrations in relation to environmental target values.

Paper IV: To evaluate the suitability of guillemot (*Uria aalge*) egg as a sentinel species for investigating Good Environmental Status (GES) within the Marine Strategy Framework Directive (MSFD) by comparing the tem-

poral trends of PCDD/Fs and DL-PCBs in guillemot egg with the temporal trends in herring (*Clupea harengus*).

Paper V: To assess temporal trends of PCDDs, PCDFs and DL-PCBs in human milk collected from nursing women in Stockholm since the 1960s; to compare the results with previous analysis of some of the older samples to evaluate if time series could be elongated if the sample population and analytical method remains the same.

2 Background

In the 1960s, the Baltic Sea was found to be severely polluted by PCBs and DDTs, two groups of persistent organic contaminants (Jensen et al. 1969, 1972). Jensen et al. (1969) analysed various marine species, including blue mussel *(Mytilus edulis)* at the bottom of the food chain to top predators such as grey, common, and ringed seals (*Halichoerus grypus*, *Phoca vitulina*, *Pusa hispida*). These contaminants were present in all species examined. At that time, little was known about the toxicological implications of the concentrations found in the environment (Jensen et al. 1969). Later studies revealed that the high concentrations of PCBs and DDT including its metabolites (DDE, DDD), caused severe reproduction problems among the Baltic seal species and white-tailed sea eagle (*Haliaeetus albicilla*) populations (Helle et al. 1976a, b; Helander et al. 2002; Bergman et al. 2003, Bredhult et al. 2008). These discoveries led to the start of an environmental contaminant research program at the Swedish Museum of Natural History (SMNH), which in turn initiated the launch of a comprehensive environmental monitoring program in the 1980s by the Swedish Environmental Protection Agency (SEPA). The Department of Environmental Research and Monitoring at the SMNH was given the responsibility of monitoring contaminants in marine and freshwater biota. The samples from these programs have been stored frozen in the Environmental Specimen Bank (ESB) at the SMNH.

The focus of these monitoring programs was primarily to follow temporal trends to estimate the rate of change in contaminant concentrations when following up bans and restrictions. The programs were also designed to indicate large-scale spatial differences, thereby detecting local contaminant incidents or widespread incidents.

Unlike the national strategy for monitoring levels of contaminants in the environment, a common national strategy was not set for monitoring human chemical exposure in Sweden. However, Dr. Koidu Norén, at Karolinska Institute, Sweden, initiated human health monitoring in Sweden when she began collecting and analysing organic contaminants in mothers' milk from

the Stockholm area in 1967 (Norén and Meironyté 2000). The milk was supplied by the Mothers´ Milk Centre in Stockholm (Meironyte et al. 1999). Dr Norén and her research group have analysed a wide range of persistent organic contaminants and their metabolites in human milk samples (Norén and Meironyté 2000, Meironyte et al. 1999, Norén et al. 1996, Norén and Lundén 1991). The samples, collected since 1972, were stored frozen for future re-analysis. In 1997, this milk collection was transferred to the ESB at SMNH.

In 1993, SEPA began a small scale program for human health monitoring after suggestions given in a report from the Institute for Environmental Medicine in 1992. SEPA's human health program examines heavy metals and organic contaminants in human material (blood, urine, milk) and also air pollutants, foodstuffs/drinking water, and road traffic noise. In 2010, the Department of Environmental Research and Monitoring at SMNH was assigned responsibility for the collection of human milk in the Stockholm area by the section for Health-related environmental monitoring (HÄMI) at the SEPA.

2.1 The Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPCMB)

2.1.1 The Baltic Sea/ Monitoring Stations

The majority of the SNMPCMB monitoring sites (18 of 23) are located in the Baltic Sea (Figure 2.1). The Baltic Sea is one of the largest brackish inland seas by surface area, being almost 400 000 km^2 with an average depth of 52 m (HELCOM, 2009). It has a water turn-over time of approximately 25 years (Stigebrandt 2001) due to the narrow connection to the North Sea via Kattegat and the Danish Straits (Winsor et al. 2001). The Swedish part of the Baltic Sea is generally divided into three basins – the Bothnian Bay and the Bothnian Sea together referred to as the Gulf of Bothnia, and the Baltic Proper (Figure 2.1). The basins differ by size (both volume and surface area), sea surface temperature, pH, depth and salinity amongst other abiotic factors. Surface salinity is around 3‰ in the most northern parts of the Gulf

of Bothnia, and almost 10‰ close to the Danish Straits in the Baltic Proper (Winsor et al. 2001). The water masses are also vertically stratified, which prevents the water layers from mixing. The stratification is caused by differences in salinity, and seasonally, also by temperature differences (HELCOM 2009). The catchment area of the Baltic Sea is larger than 1 700 000 km^2 and is inhabited by approximately 85 million people (HELCOM 2009). The population density is much higher in the southern and south western parts of the catchment area, with more than 100 people per km^2 compared to the northern and north-eastern parts, with fewer than 1 person per km^2 on average. Land-use follows the same pattern, with a high proportion of cultivated land in the south, and mainly forests, wetlands, and mountains in the north (HELCOM 2009).

The sampling sites within the SNMPCMB are all located in areas regarded as locally uncontaminated and, as far as possible, uninfluenced by major river outlets, ferry routes, or urban and industrial areas, and are thus considered reference sites for regional and local monitoring and recipient control.

Data from 23 sampling sites are discussed in this thesis, and in **Paper III and IV** (Figure 2.1). The year of initial analysis varies between the selected sites, but altogether they span over more than 40 years, 1969-2012. However, sampling and analysis has been carried out annually throughout the duration of the program, with few exceptions. The stations are generally located in coastal areas, but a few of them are situated in off-shore areas.

The contaminant monitoring is integrated with fish population and physiology monitoring, carried out by SLU AQUA and the University of Gothenburg, at three of the monitoring sites – Holmöarna and Kvädöfjärden in the Baltic Sea and Fjällbacka in the Skagerrak, with perch and eelpout being the common species monitored (sites number 4, 14 and 12, Figure 2.1).

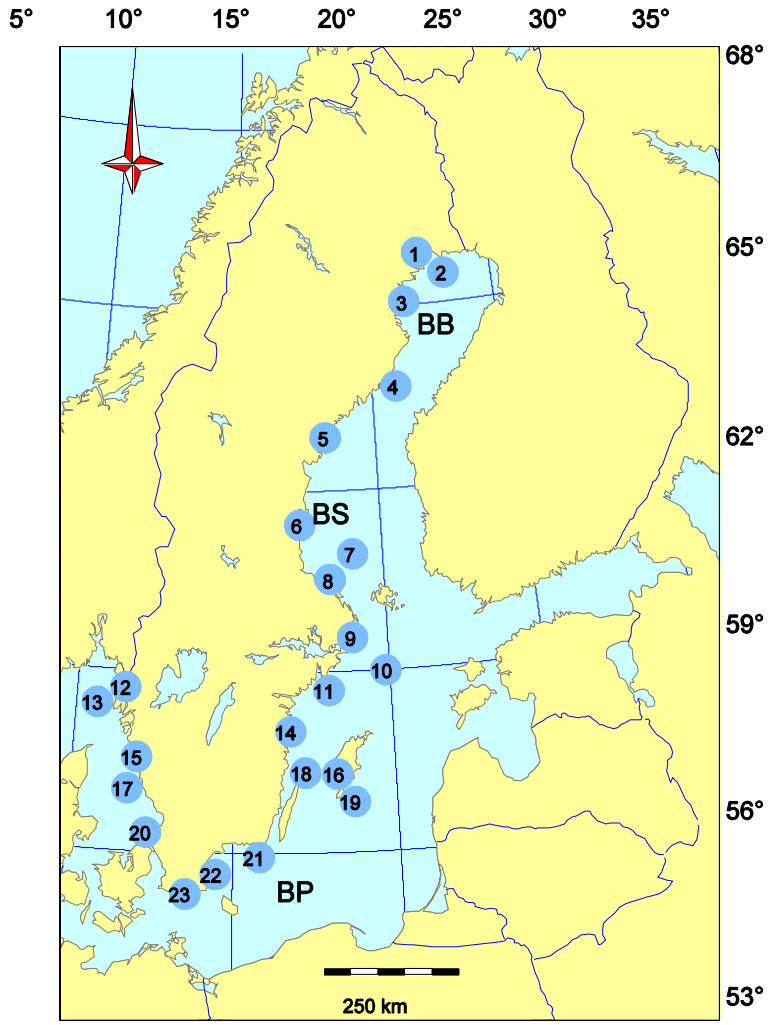

Figure 2.1. Sampling sites within the Swedish National Monitoring Program for Contaminants in Marine Biota. BB Bothnian Bay, BS Bothnian Sea, and BP Baltic Proper. ***Herring*****:** 1 Råneфjärden, 2 Harufjärden, 3 Kinnbäcksfjärden, 5 Gaviksfjärden, 6 Långvindsfjärden, 7 Bothnian Sea, offshore site, 8 Ängskärsklubb, 9 Lagnö, 10 Baltic Proper, offshore site, 11 Landsort, 13 Väderöarna, 17 Fladen, 18 Byxelkrok, 20 Kullen, 21 Utlängan, 22 Västra Hanöbukten, 23 Abbekås. ***Perch*****:** 4 Holmöarna, 14 Kvädöfjärden. ***Eelpout*****:** 4 Holmöarna, 14 Kvädöfjärden, 12 Fjällbacka. ***Cod:*** 19 SE Gotland, 17 Fladen. ***Blue mussel*****:** 12 Fjällbacka, 14 Kvädöfjärden, 15 Nidingen. ***Guillemot egg*****:** 16 Stora Karlsö

2.2 The Swedish National Monitoring Program for Contaminants in Freshwater Biota (SNMPCFB)

2.2.1 Swedish Lakes/Monitoring Stations

There are almost 100 000 lakes larger than 0.01 km^2 covering more than 9% of Sweden's total surface area. Most of the lakes are small (less than 0.1 km^2), however, 23 of the lakes have surface areas greater than 100 km^2, encompassing a third of the total lake surface area. Approximately 30% of all Swedish lakes are located in Norrbotten County bordering to the Bothnian Bay (www.smhi.se).

The 32 lakes monitored within the SNMPCFB (Figure 2.2) are distributed from the far north of Sweden (Lake Abiskojaure), located 200 km north of the Arctic Circle, to the southern-most parts (Lake Krageholmssjön), located 1800 km south of the Arctic Circle. The majority of the lakes are situated in the southern half of Sweden. The large distances between north and south (approximately 2000 km from the far northern border with Norway and Finland, to Malmö in the far south at the Danish border) leads to large temperature differences, thus some lakes are covered with ice for several months of the year, while a few in the far south remain ice-free. The length of time of ice coverage can have significant effects on oxygen conditions (Livingstone, 1993), which in turn influences phytoplankton diversity and production (Weyhenmeyer et al. 1999). The lakes also differ in size, nutrient status, general physical environment, and land use of surrounding areas. The smallest lake in the monitoring program is 0.06 km^2 (Lake Skärgölen), while the largest is 184 km^2 (Lake Bolmen) (Figure 2.2). These physical differences imply a great variability between the lakes concerning abiotic factors that might affect contaminant levels in fish.

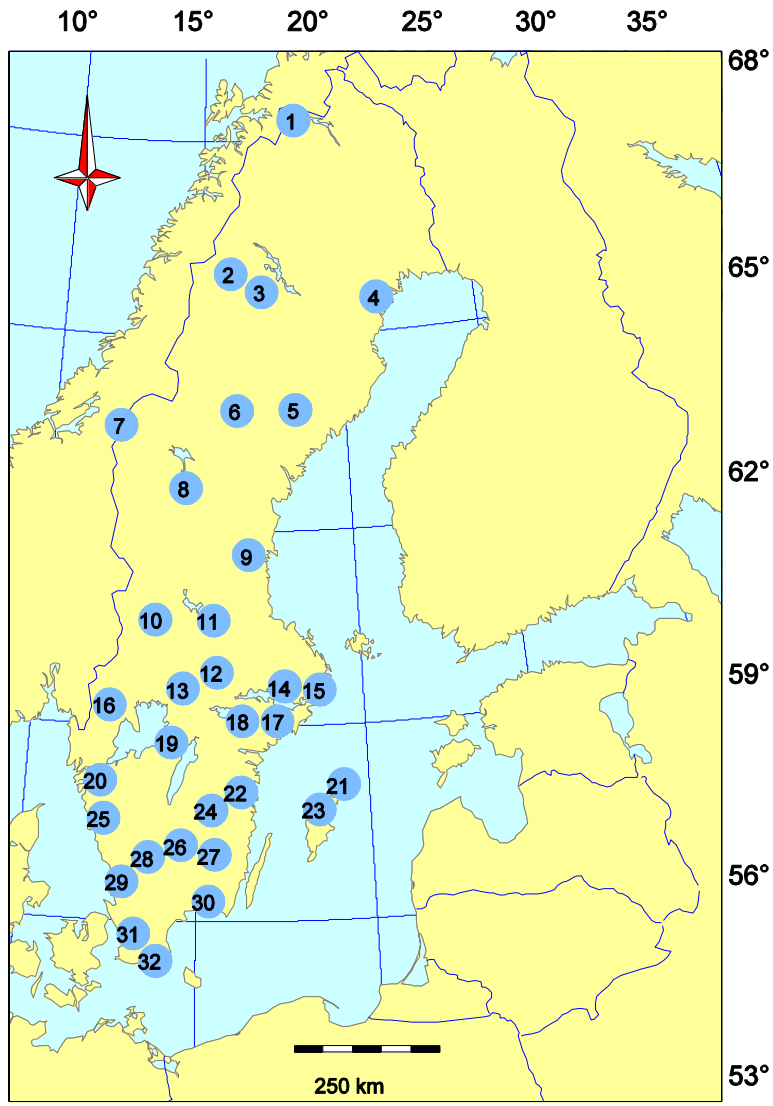

Figure 2.2. Sampling sites within the Swedish National Monitoring Program for Contaminants in Freshwater Biota. ***Arctic char:*** 1 Abiskojaure, 2 Tjulträsk, 7 Stor-Björsjön. ***Pike:*** 3 Storvindeln, 28 Bolmen. ***Perch:*** 4 Brännträsket, 5 Remmarsjön, 6 Degervattnet, 8 Stor-Backsjön, 9 Stensjön, 10 Gipsjön, 11 Spjutsjön, 12 Övre Skärsjön, 13 Limmingsjön, 14 Fysingen, 15 Tärnan, 16 Bysjön, 17 Stora Envättern, 18 Älgsjön, 19 Svartsjön, 20 Fräcksjön, 21 Bästeträsk, 22 Allgjuttern, 23 Horsan, 24 Skärgölen, 25 Lilla Öresjön, 26 Fiolen, 27 Hjärtsjön, 29 Stora Skärsjön, 30 Sännen, 31 Krankesjön and 32 Krageholmsjön.

The lakes within the monitoring program are selected using a number of criteria. The primary reason for the use of numerous criteria is that the lakes

should serve as reference sites, both nationally and regionally. The lakes must not be influenced by local contamination, thus land use in the surrounding areas is well investigated, and any areas with intensive agricultural activities are avoided. Preferably the lakes should be oligotrophic rather than eutrophic, because oligotrophic ecosystems display a faster response to changes in discharges of contaminants (Larsson et al. 2000). The lakes should also be placed as high up as possible in the drainage system to minimise the risk of local contamination. Liming activities in lakes (i.e. lime dumped into the lakes) have been common in Sweden since the late 1970s to counteract the negative effects of acidification (caused by atmospheric deposition of acidifying pollutants) on wildlife (Persson 2008). Areas where liming activities occur are avoided when selecting sampling sites. Preferably, the lakes should also have some protection against future exploitation. Another important factor is if other monitoring occurs (e.g. fish population, water chemistry), primarily for the evaluation of contaminant data, but sampling for the different programs could then also be coordinated for financial reasons.

2.3 The Swedish National Monitoring Program for Human Health (SNMPHH)

The SMNH is responsible for monitoring of human milk in the Stockholm and Gothenburg region; however, monitoring of human milk is also performed by the Swedish Food Administration in the Uppsala region. In 2000-2004 human milk was also collected from Lycksele in the north of Sweden, and Lund in the south of Sweden. A regional study of organohalogenated persistent compounds was performed, which included human milk from Uppsala and Gothenburg in the analysis. Statistically significant differences were found in most contaminant concentrations analysed between the four regions. However, the differences in concentrations between the regions were not large, hence indicating that there is a similar long-term exposure pattern of these compounds among nursing women from different regions in Sweden (Glynn et al. 2011). Due to similar exposure mechanisms, and also due to practical difficulties of collecting human milk samples, the current SNMPHH only consists of samples from Stockholm, Uppsala, and Gothenburg.

To reduce influence of confounding factors, the sample definition is narrow and restrictive. The selected mothers are healthy and non-smokers. They are

mainly primiparous, because studies have shown a correlation between contaminant level and the number of children a woman has given birth to (Dillon et al. 1981, Albers et al. 1996, Fitzgerald et al. 2001). Women of similar age are sampled because as age of the mother increases, levels of POPs in the fat generally also increase (Albers et al. 1996). However, the samples could be adjusted for age in the assessment. Samples are collected from 2 weeks up to three months after delivery to minimize variation in the milk composition. The mothers are born and have resided in Sweden for their entire lives to ensure that the contaminant level in the milk is representative of a Swedish contaminant load.

2.4 Monitoring matrices

2.4.1 Guillemot (*Uria aalge*) egg

The guillemot is a piscivorous bird species with a circumpolar distribution. It overwinters in the Baltic, mainly at Stora Karlsö, which is the largest breeding colony in the Baltic (Österblom et al. 2002) and preferably feed on sprat (*Sprattus sprattus)*, and herring (Österblom et al. 2001). Guillemot do not migrate far, thus their contamination concentrations are typically locally acquired. The breeding season begins in early May and normally only one egg is laid each year. However, if the first egg is lost, a second egg may be laid (Hedgren 1980). The egg has a high fat content, between 11-13%, which makes it suitable for analysis of substances that dissolve in the fat (Bignert et al. 1995). Guillemot eggs laid late in the season are presumably replacement eggs. These tend to contain significantly higher concentrations of organochlorines compared to eggs laid early (presumably first laid eggs) (Bignert et al. 1995).

2.4.2 Arctic char (*Salvelinus alpinus*)

Arctic char inhabit upland freshwaters of Swedish mountain areas. Their diet varies depending on prey availability, char size, and the presence of other competitive species. Small Arctic char generally feed on benthic invertebrates and plankton, while larger individuals feed on aquatic insects, and fish, including conspecifics (Hammar 2000). Large Arctic char are thus generally exposed to biomagnifying substances at the high end of the aquatic food chain. Arctic char muscle tissue is relatively fat, with 1-3% extractable

fat content (Nyberg et al. 2015). Spawning normally occurs during August-January (Kullander et al. 2012).

2.4.3 Cod (*Gadus morhua*)

Cod are found in both marine and brackish water but to be able to spawn successfully it demand a salinity and oxygen level above 11 PSU (Practical Salinity Unit) and 2 ml/l respectively (Nissling 1995). Baltic cod live below the halocline and feed mainly on clupeids (e.g. herring), but invertebrates and other fish species, as well as young cod, are also included in the diet (Pachur and Horbowy 2013). Spawning normally takes place between January (more common for Skagerrak cod) and July (more common for Baltic cod) (Nissling et al. 1998) on the Swedish west coast and in the Baltic Proper, which is the only area in the Baltic Sea where the salinity is sufficient for the eggs to remain afloat, which is necessary for successful spawning (Bagge and Thurow 1994). The cod liver is fat, which makes it appropriate for analysis of fat soluble contaminants, but the fat content is highly variable, between 12-52% extractable fat (95% confidence intervals) (Bignert et al. 2016a).

2.4.4 Eelpout (*Zoarces viviparous*)

The eelpout is also found in both brackish and marine waters and is regarded as a relatively stationary species. The eelpout is living quite close to the bottom (Jacobssen et al. 1993) and it feeds mainly on invertebrates and small fish (Ojaveer et al. 2004). The eelpout is viviparous (females give birth to fully developed fry) which enables measurements of number of eggs, fertilised eggs, larvae size, and embryonic development and in turn makes the species suitable for integrated studies of contaminants and reproduction (Jacobsson et al. 1993, Hedman et al. 2011). The mating takes place in August through September (Rasmussen et al. 2006). Eelpout muscle tissue is lean and contains approximately 0.5-0.7% of extractable fat (Bignert et al. 2016a).

2.4.5 Herring (*Clupea harengus*)

Herring is a pelagic fish species, living in both brackish and marine waters. Herring feed mainly on zooplankton, but fish and mysids are also included in the diet when herring size increases and availability of mysids is high (Casini et al. 2004). Herring is the most dominant commercial fish species in the

Baltic and important for a number of other fish eating species such as seals and guillemot (Lundin 2011). Herring is one of the core species for contaminant monitoring within the HELCOM convention area (HELCOM 2015). The herring muscle tissue has a fat content between 2 and 5%, which is considered as relatively fat, thus the species is suitable for analysis of fat soluble contaminants (Bignert et al. 2016a). The fat percentage is higher in herring on the Swedish west coast compared to herring in the Baltic Sea. Spawning typically occurs in spring or autumn, and in some cases it may even occur in summer and winter (Kullander et al. 2012).

2.4.6 Perch (*Perca fluviatilis*)

The European perch is found in both fresh and brackish waters (www.fishbase.org) and is sampled both within the SNMPCMB and the SNMPCFB. Perch is an opportunistic predatory fish that undergoes an ontogenetic shift in diet (Collette et al. 1977). Larger perch, at a higher level of the aquatic food chain, are thus exposed to higher concentrations of biomagnifying substances. The age at dietary shift is dependent on the growth rate of perch; growth rate can vary between lakes (Holmgren and Appelberg 2001) but also between sampling sites in the Baltic Sea (Bignert et al. 2016a). The perch is considered to be stationary (Thorpe 1977) and spawning takes place during April to June (Kottelat and Freyhof 2007). Perch muscle tissue is lean and contains approximately 0.6-0.8% of extractable fat (Bignert et al. 2016a).

2.4.7 Pike (*Esox lucius*)

Pike, sampled within the SNMPCFB, is a common fish in the Nordic countries and can be found in both fresh and brackish waters (Baltic Sea). Pike is a predatory, mainly piscivorous fish that will prey on conspecifics, but also feeds on frogs, small mammals and birds (www.fishbase.org). Spawning normally takes place during March to June (Kullander et al. 2012). Pike is a lean fish with an average muscle extractable fat content of 0.6% (Nyberg et al. 2015).

2.4.8 Blue mussel (*Mytilus edulis*)

Blue mussels are filter feeders, stationary, and function in a wide range of salinities and temperatures. These characteristics make them one of the most commonly used species for monitoring of contaminants. Size and biomass

decrease rapidly with lowered salinity, thus Baltic Sea mussels are much smaller than mussels from the Swedish west coast (Kautsky 1981). Blue mussel soft body contains approximately 1.0-1.5% of extractable fat (Bignert et al. 2016a).

2.4.9 Human milk

Women in Sweden or any other developed country have a highly variable diet. They consume products imported from all over the world and from different parts of the food chain. The composition of human milk, which apart from water mainly consists of carbohydrates, proteins, and fat, differs a lot depending on a number of factors. However, fat content is the most variable component (Mitoulas et al. 2002). The fat content has been shown to be affected by time post-partum (fat content increases with increasing time since birth), the portion of feeding (fat content increases during the course of a single feeding), number of children, and infections (La Kind 2002). A study by Jensen et al. (1996) reports an average fat content of 3-5% and similar numbers are also reported by Mitoulas et al. (2002). In the time trend study in **Paper V**, the fat content varied from 2.4-4.0 %, with a significantly higher fat content observed during more recent years.

2.5 Persistent Organic Pollutants (POPs)

The POPs discussed in this doctoral thesis (i.e. PCBs, DDTs, HCHs, HCBs and PCDD/PCDFs) have been of environmental and health concern for a number of decades. The POPs here in are presented in more detail under the Stockholm Convention (SC) (UNEP 2016).

Production of technical polychlorinated biphenyls (PCBs) started in 1929. Due to its chemical and physical properties (e.g. heat resistance) this group was mainly used in transformers, capacitors, hydraulic liquids and lubricants (UNEP 2016). The open use of PCBs have been nationally (SFS 1971:385) and internationally banned (OECD 1973) since the early 1970s, followed by a ban of all new uses in 1978, and finally, the total ban of PCBs in Sweden in 1995 (SFS, 1995: 1095).

Dichlorodiphenyltrichloroethane (DDT) is a potent insecticide that has mainly been used for mosquito control (UNEP 2016). DDT is transformed to dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloro-

ethane (DDD). The use of DDT has been banned since the 1970s in most countries. Currently it is only allowed to combat malaria (UNEP 2016), primarily in Africa and the Pacific Islands (Bogdal et al. 2013).

Hexachlorobenzene (HCB) has primarily been used as a fungicide and has been banned in the Baltic countries since mid-1970s (Gaul, 1992). HCB is also formed as a by-product in a number of industrial processes (e.g. electrolyte production, chlor-alkali processes, and waste incineration of materials containing chlorine), thus can still enter the environment (WHO 1997, Garı´ et al. 2014).

Hexachlorocyclohexane (HCH) has been produced since the late 1940s. Three of its isomers are of environmental interest, specifically α-, β- and γ-HCH. γ-HCH, known as lindane, is the most potent HCH isomer of this group; however, the technical mixtures of all three isomers have been widely used as commercial pesticides. The use of HCHs has been regulated or banned in a number of countries since the 1970-1990s (Willett et al. 1997). However, China and Romania did not cease lindane production until recently, while in India, lindane is still produced (Vijgen et al. 2011).

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) are unintentionally formed in a number of industrial processes and during most combustion processes, particularly if they are incomplete (e.g. waste incineration, forest fires, backyard burning, fossil fuel burning) (Baars et al. 2004). Pulp bleaching using chlorine gas has, in the past, been another source of PCDD/Fs to the Baltic Sea (Rappe et al. 1987, Wiberg et al. 1989) as were emissions related to the use of chlorinated pesticides (Baars et al. 2004). In the late 1970s, the use of dioxin-contaminated herbicides and chlorophenols (SFS 1977:246) were banned. During the 1990s, the efficiency of municipal waste incinerators in Sweden was greatly improved, and the pulp and paper industry gradually changed their bleaching technology towards a chlorine free process.

2.6 EU directives, conventions and the WHO's human milk program

2.6.1 The Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD)

The EU WFD was adopted in 2000, and primarily deals with freshwater, but the coastal-zone and estuary waters are also included in the directive, while the EU MSFD was adopted in 2008. The WFD aims to achieve good ecological and chemical status, with the common denomination of Good Environmental Status (GES) of all surface waters and ground water bodies in the EU. For the MSFD, the aim is GES in all European marine waters. GES, in accordance with the MSFD (MSFD, 2008/56/EC), is defined as "concentrations of contaminants at levels not giving rise to pollution effects".

The WFD requires that member states should report the chemical status of their water bodies to the EU Commission every six years (WFD, 2000/60/EC) concerning 45 priority substances. The evaluation of chemical status is done through monitoring of water, sediment, or biota, depending of the properties of the substance of concern. The EU has produced Technical Guidance Documents (TGDs) concerning different aspects of contaminant monitoring under the WFD umbrella, to facilitate the setup of new monitoring programs in countries with non-existent monitoring (European Commission 2010, 2011, 2013).

Within the MSFD, GES is evaluated through eleven qualitative descriptors that describe what the environment will look like when GES has been achieved. Two of the eleven descriptors deal with contaminants, namely "contaminants and pollution effects" and "contaminants in fish and other seafood" (MSFD, 2008/56/EC).

To evaluate if GES has been achieved, Environmental Quality Standards (EQSs) have been established for the priority substances. EQS values are set to protect the most sensitive organisms against harmful effects from hazardous substances. There are only 11 out of 45 priority substances that have EQS values set for biota. Biota standards are set for fish (except for polycyclic aromatic hydrocarbons (PAHs)), where the target species are crustaceans and molluscs, since PAHs are metabolised to a large extent in fish, in contrast to crustaceans and molluscs where the metabolic processes are less efficient (2013/39/EU, Elskus and Stegeman 1989). The EQS in fish is set

for the whole fish, if the most sensitive species consume the whole fish ($EQS_{biota)}$, and for muscle tissue if the most sensitive species is human ($EQS_{human\_health)}$, generally only consuming the fillet of most fish species. The EQS_{biota} in fish is set for fish of trophic level (TL) 3.5 in freshwater food webs, and TL 4.5 in marine food webs, and the EQS_{human_health} in fish is set for fish of TL 4, all with a default fat content of 5% and a default dry weight content of 26%. To put TL in a perspective, filter feeders (feeding on primary producers), such as mussels, are at trophic level 2 in the food web. Marine top predators are at a trophic level of 5.5 or higher. This implies that the analytical data needs to be recalculated to represent the correct TL and also be normalized to the default fat or dry weight content depending on analyte (European Commission 2013).

2.6.2 The Oslo Paris (OSPAR) Convention and the Helsinki Convention

The convention for the protection of the marine environment of the North-East Atlantic (the OSPAR convention) was adopted in 1992 and entered into force in 1998, and is governed by the OSPAR Commission (OSPARCOM). The OSPAR Convention has been signed by all European countries with a border or connection to the Atlantic. At the same time (1992), a convention for the protection of the marine environment in the Baltic Sea, the Helsinki Convention, was signed by all states bordering the Baltic Sea. The Helsinki convention entered into force in 2000 and is governed by the Helsinki Commission (HELCOM). Both conventions have the focus to protect the marine environment from all sources of pollution through intergovernmental cooperation. OSPARCOM has the specific aim to achieve levels of naturally occurring substances close to background level and levels of man-made substances close to zero, while HELCOM's specific aim strives towards a healthy Baltic Sea with a restored ecological status and concentrations of hazardous substances close to natural levels.

Monitoring of contaminants in marine biota in the North-east Atlantic Ocean and in the Baltic Sea is performed within the framework of these two conventions. The objectives are primarily to assess temporal trends and spatial differences within the convention area through harmonized sampling strategies and monitoring species. In the OSPAR Joint Assessment Monitoring Programme (JAMP) guidelines (OSPAR 2009) the quantitative objectives, sampling strategy, species selection, storage, practical sampling, and sample treatment is specified to get results as comparable as possible between the

different countries. HELCOM has specified similar issues for the Baltic Sea in the Manual for Marine Monitoring in the Cooperative Monitoring in the Baltic Marine Environment (COMBINE) Programme of HELCOM, often referred to as the COMBINE manual (HELCOM 2015). The TGD for monitoring of sediment and biota (European Commission 2010) is to a large extent based on the guidelines within OSPAR JAMP and HELCOM COMBINE.

Within the OSPAR convention, Environmental Assessment Criteria (EAC) have been developed for interpretation of chemical monitoring data in sediments and biota (OSPAR 2014) in the marine environment for the chemicals listed in the Coordinated Environmental Monitoring Programme (CEMP). OSPAR EACs intend to represent "the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species" and may be considered as related to EQSs. HELCOM uses EQSs for the core indicator substances (HELCOM 2016) where they are available, and when not, other target values are used, in some cases OSPAR EACs.

2.6.3 The Stockholm Convention

All substances discussed in this doctoral thesis are included in the initial 12 of the 26 Persistent Organic Pollutants (POPs) included in The Stockholm Convention (SC) on POPs. The SC is an international agreement that requires measures to reduce and prevent the release of organic compounds that are persistent, have a potential for long-range transport, accumulate in fatty tissues, are found in higher concentrations higher up the food chain, and are toxic both to wildlife and humans. The SC was adopted in 2001 and entered into force in 2004 (UNEP 2016).

2.6.4 WHO's Human Milk Monitoring

Monitoring of human milk is a non-invasive way to monitor the exposure of both infant and mother. WHO started to collect and evaluate data on levels of POPs in human milk in 1976. Since then, they have organized four international studies to evaluate concentrations and trends of PCDD/Fs, and DL-PCBs (Colles et al. 2008). This work, and the ratification of the SC in 2004, has resulted in an international protocol for monitoring human milk published in 2005, which aims to produce comparable and reliable results between participating countries and to measure the response in human milk of

measures taken under the SC to reduce the release of certain chemicals into the environment. The guideline deals with questions such as type of samples (pooled vs individual), number of samples, selection of donors, how to practically collect and transport the samples, ethical aspects etc. (WHO 2005).

To evaluate if the level of contaminants in the milk impose a risk for the infant, the tolerable daily intake (TDI) of the specific contaminant is used. It represents the tolerable amount of a contaminant in food and drinkingwater that a person can consume on a daily basis without it being a health risk. The average daily intake of a contaminant could be calculated using figures on average daily human milk consumption.

3 Designing an efficient monitoring program

Environmental monitoring is a prerequisite for international organizations, national authorities, environmental agencies, and others, to assess the effects of measures taken to protect the environment and to discover new threats and emerging substances. Environmental authorities and politicians trust the monitoring to be sensible enough to discover new threats or inefficient reductions of known problems e.g. as a basement for legislation. Large amounts of resources are spent on these activities, yet many ongoing monitoring programs fail to detect relevant changes in environmental exposure (Bignert et al. 2004, Rigét et al. 2010). This implies an unacceptable waste of resources.

Several measures can be taken that may lead to more effective monitoring programs in terms of statistical power. Objectives need to be clearly defined and formulated in a quantified way. It is imperative to consider the magnitude of change that is necessary to detect and the risks of making the wrong conclusion one is prepared to accept based on statistical tests (i.e. Type-I and Type-II errors). Furthermore, unnecessary noise (variance) should be reduced as much as possible by means of efficient sampling strategies, censoring, and adjustments using statistical techniques. Finally, the selection of appropriate statistical tests should be guided by the objectives and properties of the current data. These aspects will be discussed in detail below.

3.1 Objectives

The aim of a monitoring programme could, for example, focus on investigating changes over time, estimating geographical differences, or assessing compliance by comparing levels in a required matrix with the set environmental target value. In most cases, the objectives involve all three questions, and the program has to be designed so it can answer them at a high statistical power.

3.1.1 Temporal, spatial, or compliance to Quality Standards

Temporal trend monitoring aims to evaluate how levels of contaminants are changing over time and to estimate the rate of changes found. Knowledge about how levels are changing over time could give a good indication on the effectiveness of measures taken to reduce the discharge of various contaminants, and also if the contaminants that are monitored are approaching background levels (naturally occurring substances), or are close to zero (man-made substances) (OSPAR 2009). Spatial monitoring may aim to assess the contaminant status of an area or detect large scale geographical differences, or to identify and possibly explain spatial patterns and suggest potential sources or to identify areas of special concern (hot spots).

Compliance monitoring aims to evaluate if levels of contaminants are below or above certain quality standards (target values such as EQS or EAC) in order to assess whether the levels pose a threat to the most sensitive organism. Compliance monitoring also assists in the more general assessment on whether a specified region has reached or failed to reach the goals set for the region, e.g. GES within the WFD and the MSFD (OSPAR 2009, European Commission 2010). Temporal trend monitoring in combination with compliance monitoring provides a more reliable status assessment because the between year variability is then also considered.

3.2 Quantitative objectives

Before any monitoring is started, it is essential that explicit monitoring objectives are determined (Philips and Segar 1986). Quantitative objectives within a monitoring program generally states the size of a change that the program should be able to detect at a certain power and significance level. The quantitative objectives will thus vary with the purpose of the investigation. For example, if the purpose of the investigation is trend monitoring, the quantified objective includes information on the annual change that the program should be able to detect, and the time period that needs to be monitored to detect the specified trend at a specified power (OSPAR 2009). Quantitative objectives are necessary for guidance on frequency, continuance of monitoring, sample size etc. (European Commission 2010). The main quantitative objectives within the SNMPCMB and the SNMPCFB are presented in Nyberg et al. 2015:

- "To monitor long term time trends and to estimate the rate of changes found. Q*uantified objective:* to detect an annual change of 10% within a 10 year time period, with a power of 80% at a 5% significance level. "

- "To detect incidents of regional impact or widespread incidents and to act as watchdog monitoring to detect renewed use of banned contaminants. Q*uantified objective:* to detect an increase of 200% in a single year, with a power of 80% at a 5% significance level."

- "To indicate large scale spatial differences. *Quantified objective:* to detect differences of a factor of 2 between sites, with a power of 80% at a 5% significance level."

3.2.1 Power

Power analysis is the statistical tool used to investigate sensitivity of a monitoring programme after the objectives are quantified. Generally, a statistical test compares the data with a null hypothesis (H_0), which almost always is of the type "no difference/impact". The test provides a probability that H_0 is true. A critical level (α) is specified for the test. If the probability is less than α, H_0 is rejected. Two types of errors can appear when interpreting the statistical test – Type I and Type II. The Type I error within environmental monitoring is when a trend is indicated even though no true trend exists. The acceptable risk of conducting this error is determined by α. The related Type II error is when a true trend is not detected. The Type II error rate (β) is generally not fixed before the test is performed (Bignert 2002). However, in the quantified objectives regarding monitoring of contaminants in biota within OSPAR, the power ($1-\beta$) is set to 90% for some of the objectives (OSPAR 2009) and within AMAP (Arctic Monitoring and Assessment Programme) (AMAP 2014) and the Swedish national monitoring programs, power is set to 80%, while by convention, α is in all programmes set to 5%. The relevance of these fixed values of acceptable Type-I and Type-II-errors can be questioned. Mapstone (1995) suggests a different approach, where the critical changes that need to be detected are the primary focus, and the value of α is suggested to be determined by the balance of the cost of Type I/Type II errors.

Statistical power is the probability that a study will detect an effect, such as a temporal trend or a spatial difference, when there is a true trend or difference to be detected. High statistical power indicates that the probability of detecting a true trend, is high. Statistical power in temporal trend analysis is mainly affected by the magnitude of the trend, the length of the time-series, and the within- and between-year variation, which in turn is affected by sampling strategy, the number of samples, the type of statistical test applied, whether a two-or a one-tailed test is used, and the α-level. When interpreting results from statistical time series analyses, it is important to know with what power the temporal changes could be detected to be able to estimate the quality/sensitivity of the time series. However, even though a matrix is showing a high power, it is not always suitable for monitoring purposes if it does not respond to environmental changes in the contaminant being monitored fast enough (OSPAR 2009).

Reduction of noise (the unexplained variation between-samples and between-years) is an effective way to improve power. Noise reduction could be achieved, for example, by sample collection guidelines that aim at gathering as homogenous samples as possible regarding sex, age and other biological variables, guidelines for sample treatment, storage and preparation, and increased precision of the chemical analysis. Noise could also be reduced by using an appropriate base (i.e. fat, wet or dry weight) for expressing the contaminant concentrations, and by adjusting the data for relevant confounders. Reduction of noise will be discussed further in chapter 3.4, matrix selection.

3.3 Sampling strategy

The sampling strategy should be designed according to the quantitative objectives. As mentioned concerning the power, the recorded variability between samples should be reduced as much as possible. The accuracy and precision of the analytical performance should also be sufficient to meet the objectives, but normally the variation that originates from sample variation is much higher compared to variation due to uncertainty in the chemical analysis (**Paper I**).

Sample size is crucial for meeting the quantified objectives (**Paper I**). To estimate the required sample size when pooling of samples is considered, information on the precision of the chemical analyses (e.g. expressed as coefficient of variation (CVa)) and the total variance (e.g. expressed as coeffi-

cient of variation (CVt)) is needed. The specimen variance (expressed as coefficient of variation (CVs)) reflecting e.g. physiological difference, abiotic factors, and effects of long term storage and sample treatment, could be calculated from the analytical coefficient of variation and the total coefficient of variation using the relationship below (Råde and Westergren 1990):

$$CVt = (CVs^2+CVa^2)^{0.5}$$

The effect of various sample sizes on the random between-year variation (influenced by CVs and CVa) could then be estimated, and thus also a sample size that corresponds to the quantified objectives, represented here by the minimum trend that could be detected during a 10 year period with a power of 80% and α=0.05 (Figure 3.1). The examples in **Paper I** are based on fish samples from the regular monitoring programs (SNMPCMB, SNMPCFB), where all fish specimens at one sampling site were caught at the same time in the same place, and therefore show both temporal and spatial autocorrelation (i.e. they cannot be considered independent observations of the current levels in fish from the region and season). To avoid problems with autocorrelation, the regression analysis (Figure 3.1) is based on geometric means of the yearly sample sizes, not the individual measurements. In a situation where the total CV is about 50%, analysing 12 individual specimens per year (but using the geometric means) are sufficient to detect an annual change of about 5-6% during a ten year period.

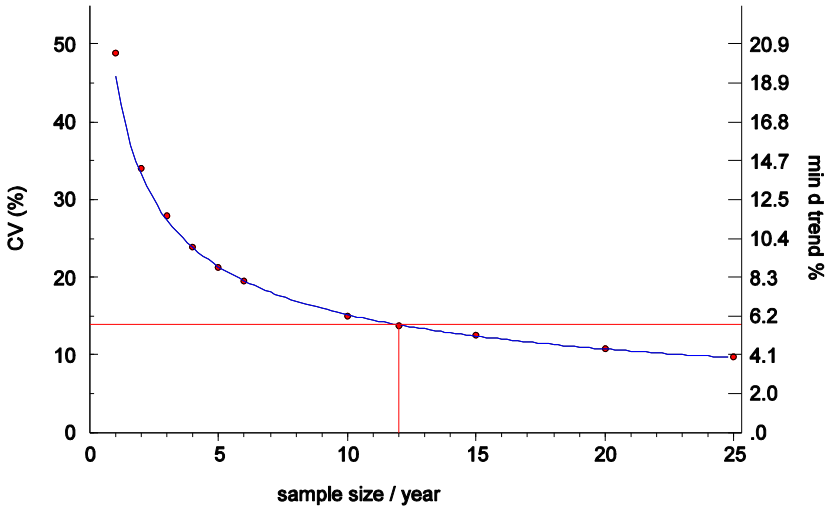

Figure 3.1. Individual samples from a log-normal distributed population, sampled over a 10 year period. In this scenario the total variance is set to 50% and analytical variance to 10%. The between-year variation is shown on the left y-axis, and minimum annual trend that could be detected at a statistical power of 80% is shown on the right y-axis. The red lines show the sample size needed to achieve a between-year variation of 14-15%, which is equivalent to a minimum detectable annual trend of 5-6%.

One important decision when selecting sampling strategy is the choice of individual or pooled samples (several individual samples homogenized into one pooled sample), both in regards to chemical analysis, and when storing samples for future analyses.

The effect of various numbers of pooled samples on the unexplained between-year variation was also investigated in **Paper I**. Analysing 2 pooled samples (i.e. increasing the number of collected specimens from 12 to 24 and reducing the number of chemical analyses from 12 to 2) per year, is sufficient to detect an annual change of about 5-6% during a ten year period at a maintained power of 80% (Figure 3.2).

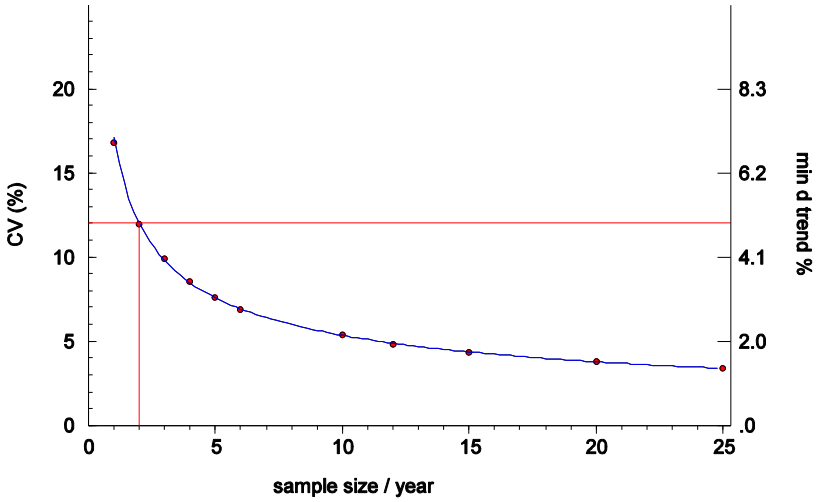

Figure 3.2. Pooled samples from a log-normal distributed population, using 12 individual specimens in each pool, over a 10 year period. The original total variance was set to 50%, and analytical precision to 10%. The between-year variation is shown on the left y-axis and minimum annual trend that could be detected with a statistical power of 80% is shown on the right y-axis. The red lines show the number of pools (consisting of 12 individual specimens each) needed to achieve a between-year variation of 14-15%, which is equivalent to a minimum detectable annual trend of 5-6%.

Advantages and disadvantages regarding the use of individual or pooled samples are discussed and evaluated in **Paper I** using monitoring data from the SNMPCMB. Situations when it is important to use individual samples are listed in **Paper I**, and are also as follows:

- When information about sample variance is important for its own sake e.g. identification of the sample distribution. Also because changes in variance are often the first sign of ongoing contamination (Bignert et al. 1993, Heffernan et al. 2014)

- When information on maximum value is crucial when the threshold level for a contaminant is set for the maximum concentration (**Paper I**).

- Individual samples allow the choice of an appropriate central measure, which is important because contaminants in biological matrices often show a right skewed distribution, in which case geometric or

median mean values are more appropriate. By contrast, pooled samples represent an arithmetic mean value (Caudill et al. 2007, Caudill 2012, Heffernan et al. 2014).

- Individual samples enable direct adjustments for confounding factors such as fat content and age, which could reduce variation within samples and in turn increase the sensitivity of the time-series (**Paper I**, Bjerkeng 2012).

- Individual samples facilitate the detection of extreme values (**Paper I,** Bjerkeng 2012).

- Individual samples allow an integrated comparison between contaminant concentrations and biological effects (Bignert et al. 1993).

The essential advantages of using pooled samples, as discussed in **Paper I**, are:

- Variation may be reduced and in turn the power increased within a smaller budget, if using pooled samples in cases where the cost of sampling and sample preparation are significantly lower than the cost of chemical analysis, and where the contribution of the biological variance is considerably larger than from the analytical error to the total variation (**Paper I**).

- Chemical analysis could be performed on very small samples, not providing enough material for individual analysis, if pooling them together (Gewurzt et al. 2011). A larger sample volume may also imply a reduced level or limit of detection (LOD) and thus a more precise estimation of the mean value than if individual samples, producing a number of LODs, were analysed (Schisterman et al. 2008).

One disadvantage of using pooled samples compared to individual samples is the so-called pooling error. The pooling error is discussed in Schisterman and Vexler (2008) and is a specific error connected to the physical process of pooling samples (e.g. effects of temperature, instrument, and technician variability).

Pooled samples are often preferable when the chemical analytical cost is considerably higher than the cost of sample preparation or if there is a wish

to reduce specimen variance. This is especially true when the individual measurements, at least to some degree, show autocorrelation, and it is questionable whether they can be considered independent observations. This is the case for most of the organic contaminants in the SNMPCMB and the SNMPCFB. Thus, during the last decade, a majority of the samples within the two programs are pooled. However, even though pooled analysis is performed, the samples are saved individually in the ESB to allow future individual analysis, if necessary.

3.4 Matrix selection

When choosing a suitable species for monitoring of contaminants there is a number of criteria that should be met for a species to qualify. These criteria have been highlighted in several publications (Moore 1966, Furness and Camphuysen 1997, Burger and Gochfeld 2004, Goodale et al. 2008, **Paper IV**) and monitoring guidelines (OSPAR 2009, European Commission 2010, HELCOM 2015).

The criteria include features such as the species should reflect the contaminant concentration in the surrounding environment and have the ability to accumulate the contaminant above the limit of detection, but without being toxicologically affected by the contaminant concentration (Moore 1966, OSPAR 2009). The species should be representative of and abundant throughout the whole study area and preferably also (fairly) stationary, thus representing the sampling location (Moore 1966, OSPAR 2009, European Commission 2010). It should be easy to identify, collect, transport, and handle, and large enough to yield sufficient amounts of material for individual chemical analysis (Moore 1966, Furness and Camphuysen 1997, OSPAR 2009, European Commission 2010, HELCOM 2015). The species should fit the demands of the marine conventions and the EU directives and therefore also be a potential food source for predators or humans, in order to be able to evaluate contaminant concentrations in the species against target values set for secondary poisoning or human consumption (European Commission 2010). In addition, it is also important that knowledge about the species biology and ecology is extensive to be able to decrease the variation within and between samples with the purpose of increasing statistical power (Moore 1966, Furness and Camphuysen 1997, Goodale 2008). It is also preferable if the species enable monitoring of biological effect parameters in addition to contaminant analysis. One must further consider if there are any ethical as-

pects involved in the sampling of the species, for example, if sampling affects the abundance of the species and thus future monitoring, or if permission is required to collect the samples.

For analysis of lipophilic organic contaminants, a high and stable fat content is an advantage. If the fish is too lean (fat content below 1%), the fat yield could be up to 25% too low, which in turn generates contaminant values presented on a fat weight basis that are correspondingly too high. The fat yield is very much dependent on the extraction method (Jensen et al 2003). The use of healthy looking specimens with undamaged skin is also preferable, because starved or unhealthy animals with very low fat content can show elevated concentrations of fat-soluble contaminants when expressed on a fat weight basis (Blomkvist et al. 1992, Bignert et al. 1993, HELCOM 2004).

When collecting biological specimens for the purpose of evaluating temporal changes in contaminant concentration, it is crucial to increase the comparability within and between years as much as possible by minimizing variability in the samples (OSPAR 2009) for increased power. A more recent contaminant load is generally represented by sub-adults, because many of these substances tend to bioaccumulate, and the contaminant levels often correlate with fish age (Dušek et al. 2005, Gewurtz et al. 2011). To increase the between-year comparability, younger specimens in the same age range are preferably collected. For herring, it has also been shown that a younger age class is less migratory compared to older age classes, thus are more representative of contamination at the sampling location (Parmanne 1990). Sex is another factor that can influence contaminant concentration in the sample, possibly explained by the elimination of lipophilic contaminants during roe release at spawning in female specimens (Scharma et al. 2009) or sex differences in energy allocation (Madenjian et al. 2011). Therefore, the same sex or the same proportion between sexes should be analysed each year. Contaminant concentrations could be affected by sampling season, especially close to spawning season (Bignert et al. 1993, Farkas et al. 2003), thus sampling close to or during spawning season should be avoided, and the sampling date should be kept consistent between years. Species within the SNMPCFB and SNMPCMB have been collected using the above criteria.

3.5 Statistical methods

One of the main aims of **Paper II-V** was to investigate the temporal trends of certain organic contaminants in biota and human milk. The trend analysis was carried out in three steps; first log-linear regression analysis was carried out, followed by the Mann-Kendall trend test, a non-parametric test, and finally non-linear trend components were identified.

Log-linear regression was performed for the entire investigated time period, and for the most recent 10 years, using the yearly geometric mean value in all papers, except **Paper V** where an arithmetic mean was applied due to having only one or two samples analysed per year in the study. Therefore, in this case, a geometric mean would have been equivalent to an arithmetic mean.

The non-parametric Mann-Kendall trend test was performed mainly to check that an indicated trend was not caused by points at the end of the line causing major leveraging. The test normally has a lower power than its parametric counterpart, but it is not affected by differences in magnitude of the concentrations, as it only measures the number of years where the concentration increases or decreases compared to the previous year (Gilbert 1987, Helsel and Hirsch 1995). Thus, if the outcome of the log-linear test is significant and the outcome of the Mann-Kendall test is not, it could either be because the Mann-Kendall test had a lower power or that extreme values at the end of the time series had an unjustifiably high influence on the slope.

In some cases, a non-linear trend is more suitable for description of contaminant development over time. In those cases, a running mean smoother was fitted to the annual geometric mean values. The significance of this line in comparison to the regression line was tested using ANOVA (Nicholson et al. 1998). In addition, non-linear trends could also be investigated using Change-Point detection. A method suggested by Sturludottir et al. (2015) has lately been applied to brominated and perfluorinated compounds that have been banned or phased out during the monitoring period. The method iteratively searches for a combination of two log-linear regression lines in different directions that explains significantly more of the total variance than that explained by a single regression line for the whole study period. In Figure 3.3, this method has been used for concentrations of the brominated flame retardant BDE-47 in guillemot eggs. A significant change point was detected in 1985. The advantage with the Change-point approach over the running

mean smoother, is that average slopes and halving/doubling times for two periods and a change-point year can be estimated quantitatively.

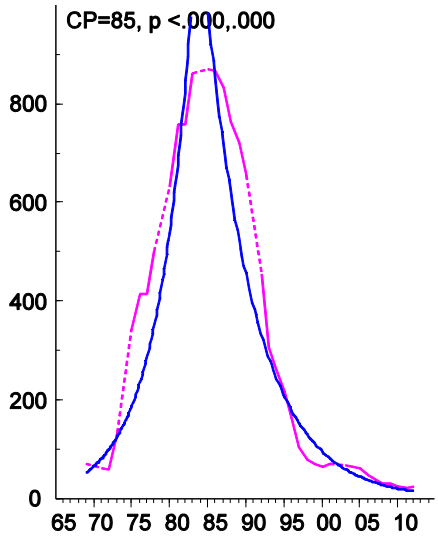

Figure 3.3. Temporal running mean smoother (5 years, purple line) and Change point (CP) detection (blue lines) of the brominated flame retardant, BDE-47, (ng/g fat weight) in guillemot egg 1969-2012.

In time series where contaminant concentrations are affected by confounders (confounding factors), adjustment for confounders is possible, at least if samples are analysed individually. Fat content can influence contaminant concentrations in cases where the fat content is very variable (Grimås et al 1985, Bignert et al 1993). Occasionally, this is of major importance for the evaluation of time series and sometimes it could even change the direction of a slope. The contaminant concentrations in cod liver and spring caught herring from Utlängan have hence been adjusted for varying fat content (**Paper II**). Age could also have a significant influence of contaminant concentration (e.g. Hg in herring muscle, Braune 1987). In these cases, an adjustment for age could improve the time series considerably. In Figure 3.4 the ∑PCDD/F (WHO-TEQ_{2005}) in human milk from Uppsala (individual analysis) is illustrated. The concentration is significantly affected not only by the change over time, but also by the age of the mothers, BMI (Body Mass Index), and the weight gained during pregnancy. In the time series adjusted for age, BMI, and weight gain, the CV has improved from 41% to 30%, and the lowest detectable trend (LDT) over a period of 10 years at a power of 80% im-

proved from 2.8% to 2.1%. The confounders could also be abiotic in character, such as temperature, pH, salinity, and productivity (Somero et al. 1977, Cusimano and Brakke 1986, Larsson et al. 1992).

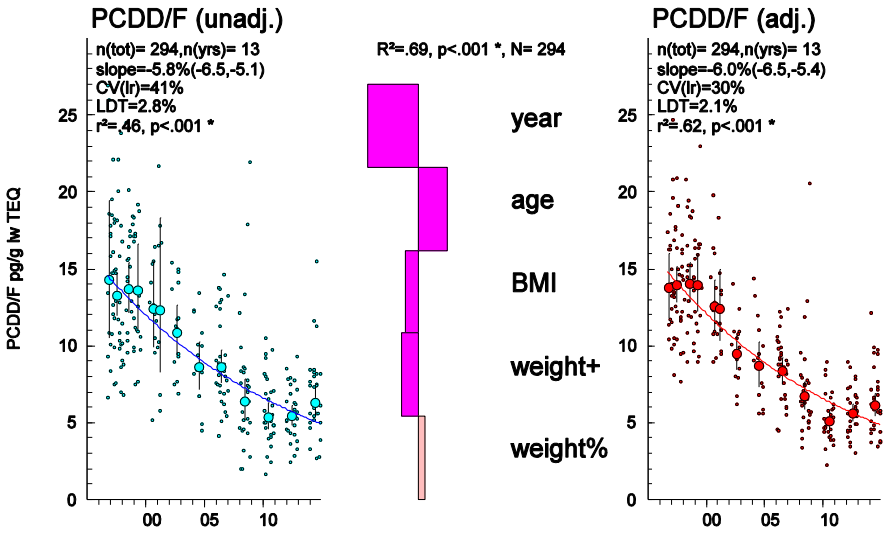

Figure 3.4. Temporal trend of ∑PCDD/F (WHO-TEQ$_{2005}$) (pg/g fat weight) in human milk from Uppsala. Unadjusted data on the left hand side, significant confounders in the middle and adjusted data on the right hand side. The colour of the blocks indicate p-values, purple:p<0.001 and pink: p<0.05 and confounders with blocks on the left of the central line affect the data negatively and on the right positively. **n(tot)** and **n(yrs)** are the total number of analysis and years, slope is the yearly change and its confidence intervals, **CV(lr)** is the coefficient of variation, **LDT** is the lowest detectable trend during 10 years at a power of 80%, and **r^2** is the coefficient of determination together with a p-value for a two sided test, (Figure slightly modified from Glynn et al 2016a).

Observations that are exceptionally far from the overall mean, the regression line, or the smoother, are of special concern. These observations are often referred to as outliers or extreme values. They could be caused by something unusual in the surrounding physical environment, a change in the pollution pattern, or errors in sampling procedure or chemical analysis. Outliers can have a major influence on the statistical power to detect temporal trends, either by changing the trend and/or masking its significance. A number of statistical methods have been proposed for the detection of outliers (Grubbs 1969, Hoaglin and Welsh 1978, Rosner 1983). However, it is very important that the removal of outliers is stated and motivated in order to avoid accusations of fraud (Wade 1976). In **Papers II-V**, the method described by

Hoaglin and Welsh (1978) was used; however, suspected outliers are indicated in the figures and still included in the statistical calculations.

Measurements below the quantification limit (LOQs) are calculated using LOD multiplied by 3. In the literature, there are several different ways describing how to treat LOQs, for example substituting them using the reported LOQ divided by 2, or divided by the square root of 2, but an even better technique is the regression based method described in Helsel (2005). However, the Helsel method requires a certain number of analysis above LOQ each year, and the method is thus not always suitable. In **Papers II-IV**, LOQs have been divided by the square root of 2; in **Paper V** LOQs have been divided by 2.

Assessing compliance towards a certain quality standard (i.e. comparing measured concentrations in biota with the standard) could play an important role in decision making, for example concerning permits, and when identifying risks from chemicals prior to implementing measures. Compliance checking could be performed in different ways, either by comparing the mean of a number of samples with the target value, or by using more statistical approaches that take into account the uncertainty in the measured values. The latter approach is required if the compliance assessment should be supported by some sort of estimate of the confidence of the outcome of the assessment (European Commission 2013).

Two scenarios assessing compliance using confidence intervals are illustrated (Figure 3.5, 3.6) and are referred to here as the "Brown" test (favours the polluter, Figure 3.5) and the "Green" test (favours the environment, Figure 3.6). The probable outcome, if a fail or pass decision is made on the basis of the lower confidence interval (Figure 3.5), is that a poor investigation with larger confidence intervals will give a pass even though the mean fails to reach levels below the quality standard. On the contrary, an investigation of high quality is awarded within the Green test, where smaller confidence intervals will lead to a pass where both the mean and the upper confidence level are below the quality standard. A poor investigation will provide a fail with the upper confidence level above the quality standard (Figure 3.6) (European Commission 2013).

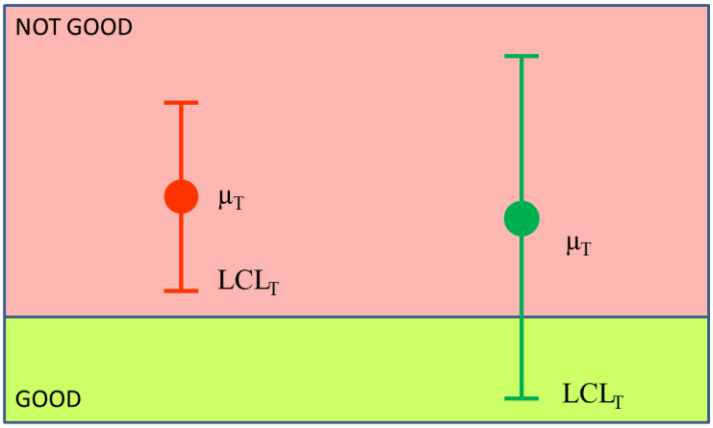

Figure 3.5. The "Brown" test within compliance assessment. To be significantly above the Quality Standard (indicated by the black line between good and not good status), the Lower Confidence Level (LCL_T) must not go below the Quality Standard.

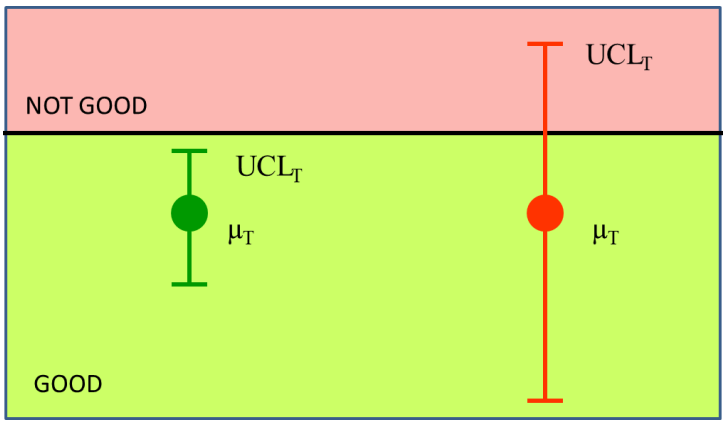

Figure 3.6. The "Green" test within compliance assessment. To be significantly below the Quality Standard (indicated by the line between good and not good status), the Upper Confidence Level (UCL_T) must not go above the Quality Standard.

When assessing compliance, the choice of value (e.g. mean, upper mean CL, or upper population CL) compared to the target level is central. Figure 3.7 is illustrating the difference between these three choices when assessing what year a contaminant (here ∑PCDD/F+DL-PCB) will reach compliance with a target level in human milk. The upper CL of the population will reach a body burden of ∑PCDD/F+DL-PCB regarded as safe, from a conservative fetal health point-of-view, in 2028.

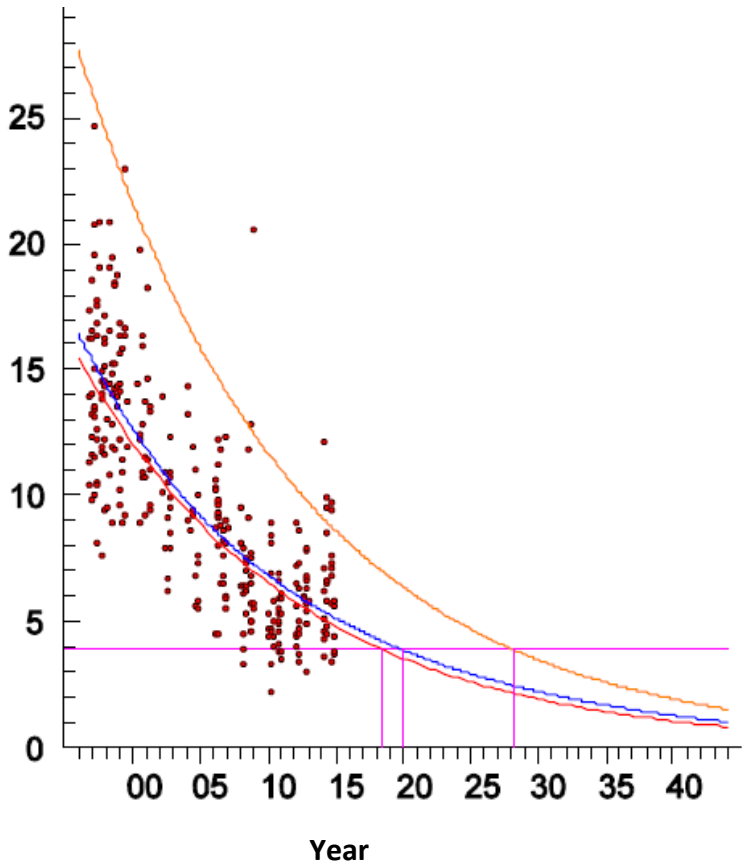

Figure 3.7. Extrapolated trends of temporal total ∑PCDD/F+DL-PCB (WHO-$_{TEQ2005}$ pg/g l.w.) concentrations in human milk, 1996-2014. The orange line represents the 95% confidence interval for the whole population, the blue line represents the 95% confidence interval for the mean, and the red line is representing the mean. The purple lines are showing when the trend reaches a body burden of 3.9 WHO-$_{TEQ2005}$ pg/g l.w.. The upper orange line is crossing a body burden of 3.9 pg WHO-$_{TEQ2005}$ pg/g l.w. in 2028. This maternal body burden, which is half of the US EPA non-cancer reference dose (Rfd) body burden of 7.9 pg WHO-$_{TEQ2005}$ pg/g l.w., could be regarded as safe from a fetal health point-of-view. The figure has been published in Glynn et al. 2016b.

4 Species/matrix comparison

One area where assessment could be improved is within the selection of monitoring species/matrices. In order to elucidate which species/matrices are more suitable than others, a number of issues related to key features for a monitoring species are discussed. Many of these features regarding guillemot egg and herring are discussed in more detail in **Paper III** and **IV**. The contaminants used for comparison of the species are PCBs (e.g. CB-153, the congener which is normally found in the highest concentrations in biological samples) and the sum of the toxic equivalents for PCDD/Fs, as data on these groups of substances are presented for most species/matrices in the publications in this thesis (**Paper II-V**).

Species within the SNMPCFB and SNMPCMB have all been chosen because of their suitability as monitoring species, hence many of the criteria presented in chapter 3.4, such as: their potential as a food source for top predators or humans; representativeness of recent contaminant load; abundance throughout the study area; size; ease to identify, transport and handle; good knowledge about species biology and ecology; suitability regarding the demands of the marine conventions and the EU directives; and their appropriateness regarding ethical considerations, are already in general fulfilled for all species. Therefore these features will not be discussed further within this thesis.

4.1 Representativeness of the sampling location

The species representativeness of the sampling location is central to temporal and spatial studies. Within the Baltic Sea, herring is a migratory fish species (Parmanne 1990) and the specific location where the contaminants are accumulated is therefore not known. However, a study by Bignert et al. (2007) on concentrations of PCDD/Fs in herring muscle sampled along the Baltic coast, showed spatial autocorrelation between samples in the range of about 100 km. Measured concentrations were relatively representative of the sampled region during the sampling season. Studies on Atlantic cod have

shown that the species could show both stationary and migratory behaviour depending on if it is a coastal population and the age of the cod (Godø 1995, Storr-Paulsen et al. 2004). Baltic cod also show variation in their migration pattern, with both stationary and long distance migration observed (Neuenfeldt et al. 2007). Perch and eelpout are considered fairly stationary fish species (Thorpe 1977, Jacobssen et al. 1993), hence they acquire their contaminant burden in the surrounding area. Guillemot remain in ice-free areas of the southern Baltic Proper year round (Stolt et al. 1991), thus is also relatively stationary. Contaminant concentration could be considered as locally acquired. Adult blue mussels are sessile, thus providing a time-integrated picture of local contamination (Cantillo 1998).

Human milk is not very representative of the sampling location. The major source of POPs in human milk is via food intake (Darnerud et al. 2006), with the exception of polybrominated flame retardants (PBDEs) where indoor dust may be considered as equally important for exposure (Fredriksen et al. 2009). Food consumption is becoming more and more globalized, at least in industrialized countries. In a study on regional differences of contaminants in human milk from four different cities in Sweden, concentrations of PCBs and PBDEs were quite similar even though there was spatial variability in the examined regions (Glynn et al. 2011). However, it should still be stressed that studies of human milk give representative and important measures of the range and average of the exposure to the fetus in the surveyed regions.

4.2 Fat content

All POPs discussed in this thesis dissolve in fat, hence the fat content of a monitoring matrix is therefore important. The advantages of high and stable fat content in a matrix/organ for monitoring of fat soluble contaminants are discussed in more detail in section 3.4.

Guillemot egg has a relatively high and stable fat content (extractable fat content 11-13%). Cod liver also has a high extractable fat content, however it is very variable (12-52%). Herring muscle tissue from the Bothnian Bay has an extractable fat content of around 2%. The fat content increases towards the Baltic Proper and the Swedish west coast where it can reach levels above 5%. The fat content in herring is not as stable as in guillemot egg, and stability varies between sampling sites, with the highest variability observed in spring caught herring from Utlängan (Baltic Proper) and autumn caught

herring from Väderöarna (Swedish west coast), 2-10% and 1-6% respectively (Bignert et al. 2016a). Arctic char muscle has extractable fat content in the same range as herring from the Bothnian Bay/Sea (1-3%). Blue mussel have slightly lower extractable fat content of 1-1.5%. Perch (0.6-0.8%), eelpout (0.5-0.7%), and pike (0.6%) muscle all have fat content below 1%.

4.3 Integrated monitoring

Integrated monitoring, including fish community and population variables, biological effect parameters and contaminant analysis, allows an assessment of any actual harm caused by contaminants from a subcellular to community level. Potential synergistic, antagonistic, and cocktail effects, and the influence of natural stressors such as salinity or temperature, are also included in the integrated assessment. Analysing and assessing the three classes of parameters at the same time has several advantages. If no correlations between monitored contaminant and biological effects are found, a search for new potential chemical candidates needs to be initiated. Changes in the biological effect parameters may also have an effect on growth, reproduction, and survival. Alterations at ecosystem level may be found, but possible explanations are often difficult to find from population studies alone (Sandström et al 2005).

The choice of effect parameters aims to reflect population characteristics and important physiological functions such as growth, energy storage and metabolism, reproduction, liver function, and immune defence. Effect parameters are in some cases related to specific groups of contaminants, for example certain organic chemicals and EROD (Ethoxyresorufin-O-deethylase) activity, and estrogenic substances, and vitellogenin in male plasma (Sandström et al 2005).

Species used for integrated monitoring should be fairly stationary, preferably during all life stages, since they are more suitable for monitoring effects of local pollution with one of the aims of linking observed effects in the field with the source/sources of the pollution. It is also important that the species provides the ability to separate contaminant-related effects from influences that could be caused by other factors such as natural variability, temperature, salinity, food availability, and so on. It should also be fairly sensitive to contaminants (ICES 2012). In Sweden, integrated fish monitoring is focused on perch and eelpout, two species which have a rather stationary behaviour

(Thorpe 1977, Jacobssen et al. 1993, Sandström et al 2005). The eelpout is viviparous, which enables a number of studies related to eggs (e.g. larval deformities and dead or malformed larvae) (Hedman et al 2011). Other parameters are monitored within the fish effect monitoring program such as EROD, GSI, vitellogenin, DNA-adducts, PAH-metabolites in bile, liver histopathology, intersex, and externally visible fish disease. Perch is not viviparous, thus only sampled for the latter analysis; however, perch is one of the most dominant species in the Baltic archipelagos and freshwater ecosystem.

Bird eggs are also suitable for integrated monitoring because the eggshell can be affected by chemicals (Bignert et al. 1995, Helander et al 2002, Bignert and Helander 2015) and measurements of physical parameters such as shell thickness, length and shell index, are sufficient for effect monitoring. Shell parameters have been measured in guillemot eggs since the start of monitoring in 1969. Furthermore, guillemot is relatively stationary since it remains in ice-free areas in the southern Baltic Proper all year.

Mussel is a species commonly used for measurements of biological effects of contaminants. Mussels are common, wide spread, sessile, and display a wide range of biological responses to contaminants (Salazar and Salazar, 1995, ICES 2012). However, blue mussel are not yet included in the Swedish integrated contaminant monitoring. Eelpout and blue mussel have been identified as key species for integrated monitoring within the OSPAR area (OSPAR 2012).

4.4 Temporal trends

CB-153 has been analysed temporally in the matrices discussed in this doctoral thesis. Results of the temporal analysis are shown (Table 4.1, Figure 4.1). Here, CB-153 was decreasing significantly over time, in the range of -2.2 to -8.8% per year, in all marine matrices except for cod liver (both sampling sites), eelpout muscle (Holmöarna and Fjällbacka) and herring muscle (Väderöarna). In the freshwater environment, CB-153 was decreasing significantly in Arctic char muscle (both sampling sites) and pike muscle from Storvindeln, between -3.6 to -7.2% annually. The concentrations of CB-153 in human milk samples from the Stockholm area were also decreasing significantly over time, at -4.8% per year. The most rapid decrease (-8.8%) was seen in eelpout muscle from Kvädöfjärden, most probably due to exceptionally high concentrations observed at the beginning of the monitoring period

(Table 4.1). Decreases in CB-153 concentration seen in a majority of matrices since the 1970s is a result of the ban of all new use of PCBs in 1978, and a number of measures taken to reduce concentrations in the environment.

In order to combine temporal trends for CB-153 at various contaminant levels in the same graph (Figure 4.1), data were transformed to percent of maximum concentration (within a specific time series and because the length of the time series varies, data were transformed to percent of the concentration recorded in 1995) for representative species (long time series or geographically close). Even though levels of CB-153 have decreased in most species and sites since the beginning of monitoring, the non-linear trends differ (Figure 4.1). Guillemot egg (St Karlsö), human milk (Stockholm), Arctic char (Abiskojaure), pike (Storvindeln) and eelpout (Kvädöfjärden) display quite similar trends, while herring (Utlängan), cod (south of Gotland), perch (Kvädöfjärden) and blue mussel (Kvädöfjärden) show quite large between-species variations.

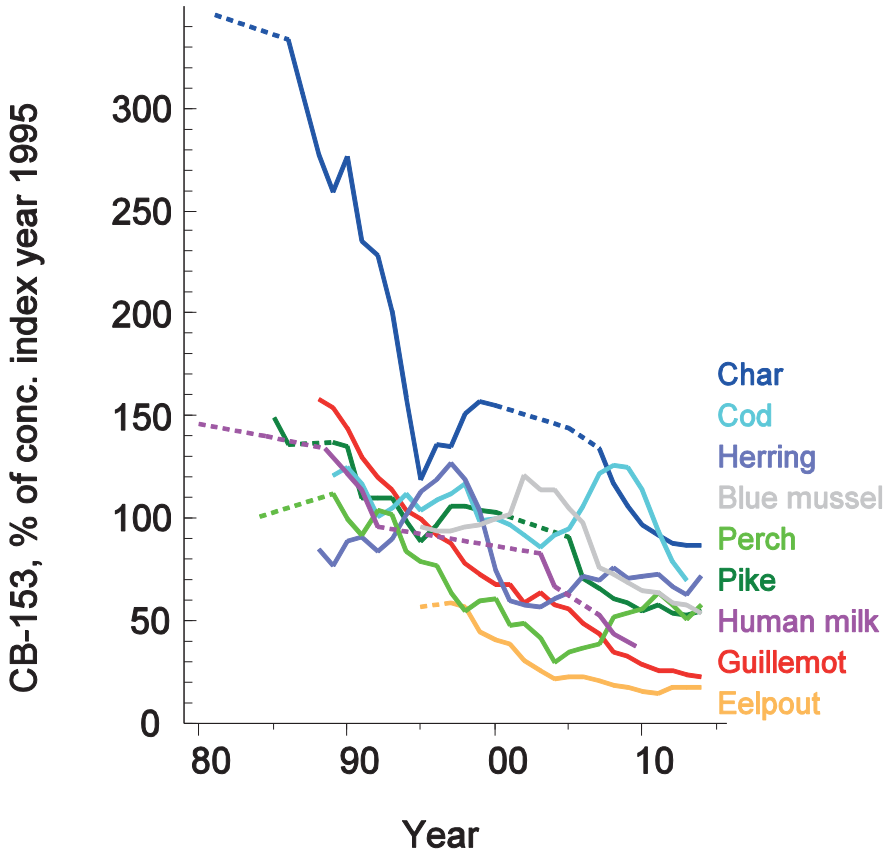

Figure 4.1. Temporal running mean smoother (5 years) of CB-153 (expressed as % of the value recorded in 1995) in autumn caught herring muscle (light blue line) from Utlängan (Baltic Proper); guillemot egg (red line) from St Karlsö (Baltic Proper); eelpout muscle (yellow line) from Kvädöfjärden (Baltic Proper); perch muscle (light green line) from Kvädöfjärden (Baltic Proper); blue mussel (grey line) from Kvädöfjärden (Baltic Proper); cod liver (turqoise line) from south of Gotland (Baltic Proper); pike muscle (dark green line) from Lake Storvindeln (northern Sweden); Arctic char muscle (dark blue line) from Lake Abiskojaure (northern Sweden) and human milk (purple line) from Stockholm. Dotted line segments indicate that one or more years are missing.

Temporal analysis of PCDD/Fs (represented by ∑PCDD/F (WHO-$_{TEQ1998}$)) has only been carried out for a few of the matrices and sampling sites discussed here (Table 4.2, Figure 4.2). Concentrations were decreasing significantly in guillemot egg, herring from Ängskärsklubb and Fladen, and in human milk from the Stockholm area (-2.8, -5.8, -0.77 and -5.9%, respec-

tively). By contrast, no trends were observed in the perch and pike time series from the freshwater environment. However, the levels were very high both in milk and herring from Ängskärsklubb in the beginning of the monitoring period, which was not the case for the freshwater fish.

Figure 4.2 illustrate the combined temporal trends of ∑PCDD/F (WHO-$_{TEQ1998)}$) for the species listed in Table 4.2 (because the length of the time series varies, data was transformed to percent of the concentration recorded in 1995). The figure shows that the temporal trend for guillemot egg and human milk are very similar until the late 1990s, after which it appears the ∑PCDD/F (WHO-$_{TEQ1998)}$) continue to decrease in milk while the trend in guillemot egg is levelling out. This may be explained by the fact that the advice concerning food for girls, reproductive aged, and pregnant women, developed by the Swedish Food Agency, has become more detailed and brought to the public's attention during the last decades. The dietary advice should, if reaching its goal, result in girls, reproductive aged, and pregnant women eating less food containing high levels of PCDD/Fs. Thus the levels in milk could continue to decrease at the same rate although the temporal trend in the environment has levelled out. In addition, food consumption patterns have changed considerably during recent years, representing a more global contaminant burden. The temporal trends in herring, perch and pike start much later and indicate a trend resembling the one seen in guillemot during the last decades.

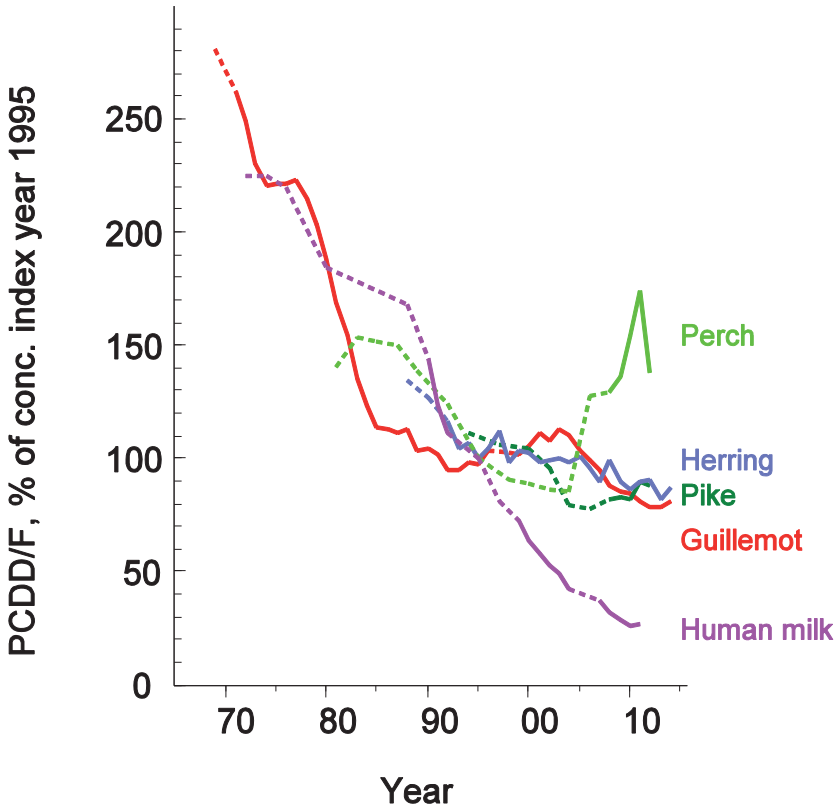

Figure 4.2. Temporal running mean smoother (5 years) of ∑PCDD/F (WHO-$_{TEQ1998}$) (expressed as % of the value recorded in 1995) in herring muscle (light blue line) from Utlängan (Baltic Proper); guillemot egg (red line) from St Karlsö (Baltic Proper); perch muscle (light green line) from Lake Skärgölen (southern Sweden); pike muscle (dark green line) from Lake Bolmen (northern Sweden); and human milk (purple line) from Stockholm. Dotted line segments indicate that one year or more is missing.

A small coefficient of variation (CV) is essential for detecting small changes in contaminant concentration (Gilbertson et al. 1987). The CV is, as mentioned before, affected by a number of things, e.g. physiological difference of the samples, abiotic factors, sampling strategy, as well as effects of long term storage, sample treatment, and analytical precision.

The time series presented in Table 4.1 and 4.2 are not always based on the yearly geometric means of individual analysis. In some cases, pooled samples are used and in others there is a variety of pooled and individual sam-

ples over the years. Time series with individual values are calculated using geometric mean values, which are less sensitive to extreme values compared to arithmetic mean values, which tend to decrease the CV slightly. Pooled samples are equal to an arithmetic mean value and in the case of a mixture of pooled and individual samples, geometric mean values are used for the years where individual analysis is performed and arithmetic for the years using pooled samples. The CV when using pooled samples is affected by the number of samples in the pool and also their sensitiveness to extreme values (**Paper I**).

Here, for CB-153, the median CV is 35%, ranging from 15 to 64%. A CV of 35% corresponds to a lowest detectable trend (LDT) in 10 years of 12-13% (ranging from 5.4-23%) and an estimated number of years required (YQR) to detect an annual change of 10% of 12 years (ranging from 8-16 years), both at a power of 80% (Table 4.1.).

The largest CV was seen in eelpout muscle from Holmöarna (64%) (Table 4.1). However, the monitoring of eelpout stopped in 2007 at Holmöarna, so that site may not be comparable to the other sites. The second largest CV was observed in perch from Lake Stensjön and Kvädöfjärden (55% and 54% respectively) followed by eelpout from Kvädöfjärden (49%) and herring from Harufjärden (44%). Kvädöfjärden has been subjected to intensified monitoring lately because some biological effect parameters have indicated that the area might be affected by contaminants. By contrast, blue mussel from Kvädöfjärden have in general a lower CV (26%) similar to the CV in blue mussel from the Swedish west coast (26-37%).

The lowest CV was seen in perch from Skärgölen (15%), but the temporal trend was based only on pooled samples (except one year) and it was a short temporal series (1999-2012, 7 years analysed), and no trends were indicated. In contrast to Skärgölen, Lake Stensjön had one of the highest CV, however here quite a low number of years were also analysed (1997-2012, 12 years analysed), but 50% of the samples analysed were individual and a trend was indicated during the last ten years. The second lowest CV was observed in guillemot egg from Stora Karlsö (17%) closely followed by pike from Storvindeln (19%). The CV seen in herring from the same area as the guillemot egg (Utlängan) was higher (36%).

The median CV for ∑PCDD/F (WHO-TEQ1998) is 30% (range 19 to 50%). A CV of 30% corresponds to a LDT in 10 years of 11% (range 8.1-18%),

and an estimated YQR to detect an annual change of 10% for 11 years (ranging from 9-14 years), both at a power of 80% (Table 4.2).

The highest CV seen for ∑PCDD/F (WHO-TEQ1998) was in herring from Ängskärsklubb (50%) (Table 4.2), most probably explained by the high and varying concentrations observed at the beginning of the monitoring period. The second largest CV was observed for pike from Storvindeln (42%) followed by perch from Skärgölen (39%). Human milk had the lowest CV (19%) closely followed by guillemot egg (22%). The temporal trend in milk was only based on pooled samples and the temporal trend in guillemot egg was mainly based on pooled samples.

4.5 Concentrations and compliance

In general, POPs bioaccumulate and biomagnify, which means that concentrations increase with increasing trophic level (HELCOM 2004, OSPAR 2007). All species within the SNMPCMB, SNMPCFB, as well as humans, bioaccumulate PCBs and PCDD/Fs to a certain extent. Regarding guillemots, the contaminant concentration seen in the adult females is mirrored in their eggs (Furness and Camphuysen 1997; Goodale et al. 2008)). This is also the case for human milk where the levels correlate well with levels measured in maternal serum (Darnerud et al. 2010, Darnerud et al. 2015). Hence, within the marine ecosystem the assumption could be made that the eggs are of similar trophic level as the adult birds. Guillemot from Stora Karlsö in the Baltic Sea mainly feed on sprat and herring (Österblom et al. 2001) and the levels (on a lipid weight l.w. basis) of ∑PCDD/F (WHO-TEQ_{1998}) (783 pg/g l.w.) and CB-153 (1.41 ug/g l.w) are about 30 and 18 times higher respectively in guillemot egg compared to herring muscle from sampling sites located in the south Baltic Proper (Utlängan and St Karlsö, Table 4.1, 4.2). This implies that PCDD/Fs and PCBs bioaccumulate in the egg to a high extent. Perch, eelpout (muscle), and blue mussel from Kvädöfjärden have concentrations of CB-153 in the same order of magnitude as in herring. The levels in cod liver from the south of Gotland are slightly higher than in herring (**Paper II and IV**; Table 4.1, 4.2). For the freshwater species it is difficult to make a general assumption because they are from different lakes where the contaminant input could vary a lot. However, the levels in pike (on a lipid weight basis) are slightly higher than in Arctic char from remote lakes in the north of Sweden and perch from the same area has levels comparable to the levels seen in Arctic char (**Paper III**,

Arctic char from remote lakes in the north of Sweden and perch from the same area has levels comparable to the levels seen in Arctic char (**Paper III**, Table 4.1). The levels seen in Swedish human milk after the year 2000 for PCDD/Fs (**Paper V**, Table 4.2) are only a tenth of the concentration seen in herring, whereas concentration of CB-153 (Fång et al 2015, Table 4.1) is only slightly lower than in herring.

The biota EQSs for evaluation of compliance are primarily set for fish within the WFD and MSFD, as mentioned in chapter 2.5.1 (European Commission 2013). OSPAR has target levels (EACs) for fish, mussel, and oyster for most CEMP substances (OSPAR 2014). Regarding the EQSs set for fish, it is not specified which species or TL that should be sampled. It is merely recommended in the guidelines (European Commission 2013) that the data should be transformed to a TL according to a protection goal of the established EQS, which in the freshwater environment is set to 3-4 and to 5 in the marine environment. To be able to adjust the data for a specific species to an accurate TL, knowledge about the species TL is needed and also information concerning the TMF, which are factors describing the average biomagnification (BMF) of a substance per trophic level. Stable isotope analysis (SIA) determining the ratio of the isotopes $^{15}N/^{14}N$ ($\delta^{15}N$) can be used to estimate the trophic level of a species (Peterson and Fry 1987). To establish the correct TL, a baseline also has to be determined using SIA (European Commission 2013).

The trophic position for the different fish species could be calculated using an equation from Post (2002);

Trophic position = $2 + (\delta^{15}N \text{ fish} - \delta^{15}N \text{ baseline organism})/3.4$

In the Baltic and at the Swedish west coast, blue mussel can be used as the baseline. However, both the $\delta^{15}N$ for the baseline (blue mussel) and the TL for herring, cod, and eelpout differed between the Baltic Sea and the Swedish west coast (Bignert et al. 2016a). This implies that the difference in TL between the same species from different lakes should not be underestimated.

TMFs are calculated using the relationship between the contaminant concentrations and the TL of organisms in the food web (Fisk et al 2001). Several studies on BMFs and TMFs for organochlorines in different food webs are available (e.g. Kiriluk et al. 1995, Kidd et al. 1995, Kidd et al. 1995b, Kidd et al.1998, Kucklick and Baker 1998, Kid et al. 2001). However, the TMF approach assumes that diet is the dominant route of exposure to contami-

nants and that TL is the major driver for accumulation of contaminants (Broman et al. 1992). This implies that other factors that are important for the contaminant concentration in a sample, such as age and size, can have a confounding effect on the regression line between TL and contaminant level (Borgå et al 2012). Borgå et al. (2012) discuss a number of factors that may affect TMFs, for example properties of the organism (affecting the metabolic rate), variables affecting the characterization of the food webs using stable isotopes, ecosystem characteristics (e.g. productivity, species composition, salinity) etc.

The EQSs in fish are set for either whole body, if the protection goal is secondary poisoning of a top predator, or the muscle fillet, if the protection goal is human. The most sensible matrix to monitor (e.g. regarding fat content or dry content) might not be whole body or muscle. Metals, with the exception of mercury and perfluorinated substances, are generally monitored in the liver because the concentrations are higher and generally above LOQ. Recalculations between organs are in these cases needed for compliance assessment and the recalculation factors might differ between species and also within species from different areas with varying contaminant concentrations (Boalt et al. 2014). The various adjustments and assumptions (TL, TMF, organ conversions) that facilitate comparisons between various media and matrices and are required to evaluate compliance with quality standards may add a lot of uncertainty. It is a prerequisite that robust and reliable methods are developed for this important issue to make these assessments meaningful.

EQSs for the PAHs represented by Benzo[a]pyrene and Fluoranthene are set for crustaceans and molluscs. No conversions between species or areas are suggested in the guidance document regarding the compliance against the set target levels (European Commission 2013). EQSs for bird eggs have not yet been established within the EU, and neither has OSPAR developed EACs for bird eggs. The guidance on biota monitoring states that solely pelagic food chain species, which excludes birds and mammals, could be adjusted by TMFs. However, OSPAR has done some work on Ecological Quality Objectives (EcoQOs) for eggs of Common and Arctic tern (*Sterna hirundo* and *Sterna paradisaea*) and oystercatchers (*Haematopus ostralegus*) in the North Sea to evaluate environmental health. The EcoQOs are target levels that estimate the expected status after a complete stop of any further input of pollutants.

Levels of contaminants in milk are straight forward when it comes to compliance. Concentrations of organic contaminants could be evaluated directly against the TDI set for infants if the daily milk consumption is known. It is often easier to evaluate human milk because infants generally feed exclusively on human milk, at least during the time period when the milk is collected.

Table 4.1. Trend for the entire period (in %) for **CB-153** assessed from the annual geometric mean if the number of analysis per year is > 2, otherwise it is based on the mean (μg g^{-1} lipid weight) in guillemot egg, Arctic char, cod, eelpout, herring, perch, pike, blue mussel, and human milk. P shows the p-value for a log-linear regression. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. CV% is the coefficient of variation around the regression line as a measure of between-year variation. Last year's CB-153 concentration values are estimated from the trend if $p<0.05$ and from the mean if no trend is present. The total number of years for the various time-series are shown in column three.

Matrix, Sampling site	Period	N years	Trend %	P	YRQ	LDT %	Power	CV %	Last year μg/g l.w.	Reference
Guillemot egg										
Stora Karlsö	88-12	25	-7.5	0.00	8	6.2	1.0	17	1.4	**Paper III**, Bignert et al. 2014
Arctic char muscle										
Abiskojaure	81-12	23	-4.4	0.00	11	11	1.0	31	0.009	**Paper II,** Nyberg et al. 2013
Tjulträsk	86-12	9	-7.2	0.00	12	13	0.45	34	0.014	**Paper II,** Nyberg et al. 2013
Cod liver										
SE Gotland	89-12	24	-1.2	0.11	10	9.1	1.0	25	0.16	**Paper III**, Bignert et al. 2014
Fladen	89-12	23	.39	0.72	12	13	1.0	37	0.47	**Paper III**, Bignert et al. 2014
Eelpout muscle										
Holmöarna	95-07	11	-3.0	0.54	16	23	0.32	64	0.12	**Paper III**, Bignert et al. 2014
Kvädöfjärden	95-12	17	-8.8	0.00	12	12	1.0	34	0.069	**Paper III**, Bignert et al. 2014
Fjällbacka	95-12	18	-.33	0.85	14	18	0.99	49	0.26	**Paper III**, Bignert et al. 2014
Herring muscle										
Harufjärden	87-12	24	-2.6	0.04	13	16	1.0	44	0.037	**Paper III**, Bignert et al. 2014
Ängskärsklubb	89-12	24	-5.2	0.00	13	14	1.0	39	0.064	**Paper III**, Bignert et al. 2014
Landsort	87-12	26	-5.9	0.00	13	15	1.0	43	0.042	**Paper III**, Bignert et al. 2014
Utlängan	88-12	25	-2.2	0.03	12	13	1.0	36	0.069	**Paper III**, Bignert et al. 2014
Fladen	88-12	25	-5.9	0.00	10	9.4	1.0	26	0.019	**Paper III**, Bignert et al. 2014
Väderöarna	95-12	17	-3.0	0.11	13	15	1.0	42	0.016	**Paper III**, Bignert et al. 2014

Perch muscle										
Holmöarna	95-12	18	-6.4	0.00	12	13	1.0	36	0.039	**Paper III**, Bignert et al. 2014
Kvädöfjärden	89-12	25	-3.3	0.03	15	19	1.0	54	0.033	**Paper III**, Bignert et al. 2014
Skärgölen	99-12	7	-1.6	0.30	8	5.4	0.77	15	0.017	**Paper II,** Nyberg et al. 2013
Stensjön	97-12	12	0.57	0.83	15	19	0.52	55	0.028	**Paper II,** Nyberg et al. 2013
Pike muscle										
Bolmen	88-12	21	-1.2	0.21	12	12	1.0	34	0.213	**Paper II,** Nyberg et al. 2013
Storvindeln	85-12	22	-3.6	0.00	9	6.9	1.0	19	0.061	**Paper II,** Nyberg et al. 2013
Blue mussel										
Nidingen	88-12	25	-8.1	0.00	10	9.4	1.0	26	0.015	**Paper III**, Bignert et al. 2014
Fjällbacka	88-12	24	-4.4	0.00	12	13	1.0	37	0.029	**Paper III**, Bignert et al. 2014
Kvädöfjärden	95-12	18	-2.9	0.00	10	9.5	1.0	26	0.043	**Paper III**, Bignert et al. 2014
Human milk										
Stockholm	72-09	13	-4.8	0.00	11	11	0.99	25	0.04	Fång et al 2015, unpub.data*

*The time series is presented in Fång et al. 2015, but not the data in the table.

Table 4.2. Trend for the entire period (in %) for **∑PCDD/F (WHO-$_{TEQ1998}$)** assessed from the annual geometric mean if the number of analysis per year is >2 otherwise it is based on the mean (pg g^{-1} lipid weight) in guillemot egg, herring, perch, pike and human milk. P shows the p-value for a log-linear regression. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. CV% is the coefficient of variation around the regression line as a measure of between-year variation. Last year's ∑PCDD/F (WHO-$_{TEQ1998}$) concentration values are estimated from the trend if $p<0.05$ and from the mean if no trend is present. The total number of years for the various time-series are shown in column three.

Matrix, Sampling site	Period	N years	Trend %	P	YRQ	LDT %	Power	CV %	Last year pg/g l.w.	Reference
Guillemot egg										
Stora Karlsö	70-11	41	-2.8	0.00	9	7.9	1.0	22	783	**Paper IV**, Bignert et al. 2013
Herring muscle										
Harufjärden	90-11	21	2.0	0.11	12	13	1.0	36	36.2	**Paper IV**, Bignert et al. 2013
Ängskärsklubb	79-11	29	-5.8	0.00	14	18	1.0	50	21.9	**Paper IV**, Bignert et al. 2013
Utlängan	88-11	22	-0.77	0.39	10	9.7	1.0	27	24.4	**Paper IV**, Bignert et al. 2013
Fladen	90-11	22	-1.9	0.03	10	8.7	1.0	24	6.52	**Paper IV**, Bignert et al. 2013
Perch muscle										
Skärgölen	81-11	16	-0.83	0.40	13	14	1.0	39	11.4	Nyberg et al. 2012
Pike muscle										
Bolmen	94-11	10	-1.7	0.34	11	11	0.75	30	62.2	Nyberg et al. 2012
Storvindeln	91-11	12	-3.6	0.09	13	15	0.59	42	13.7	Nyberg et al. 2012
Human milk										
Stockholm	72-11	22	-5.9	0.00	9	8.1	1.0	19	4.00	**Paper V**, unpub. data*

*The annual concentrations are presented in **Paper V**, but not the trend assessment.

5 Conclusion and future perspectives

A number of central aspects regarding the choice of samples and sampling design within environmental monitoring of contaminants in biological matrices are highlighted in this thesis. For example, it is essential to quantify the objectives before initiating a monitoring program; the quantitative objectives could differ depending of the aim of the monitoring e.g. if the monitoring program is focused on investigation of temporal or spatial differences or assessing compliance, or as in most cases all three types of questions; power analysis is an important tool for evaluation of the quality of the time series and the uncertainty of the trends that are observed; the estimation of sample size needed to meet the objectives and the choice of using pooled or individual samples are very much dependant on the precision of the analysis and the specimen variance within and between years; essential features regarding the choice of monitoring species; and that it is crucial to minimize the natural variability within and between samples by choosing samples of the same sex, age, sampling period etc. to increase the power of the time series.

Statistical methods for evaluation of temporal trends are discussed and the conclusion is that the choice of method for evaluating the trend is very dependent of the trend itself. Is it a linear trend, a non-linear trend, or is the trend changing direction over time? It is easy to miss something of importance by only using one method for trend evaluation. The importance of adjustment for possible confounding factors and the treatment of extreme values and levels below quantification within temporal trend assessment are also pointed out.

How to assess compliance against a target value is a hot potato for policy-makers. Should a test be used that favours the environment (a Green test) using high quality investigations, or a test that favours the polluter (a Brown test) where investigations of poor quality are rewarded? For me, as an environmental scientist, the choice is quite easy, but the chemical industry with its lobbyists seems to have a major influence on central decisions regarding environmental monitoring today.

The marine species most representative for their respective sampling site are the stationary eelpout and perch, the sessile blue mussel, and the relatively stationary guillemot. Herring and cod are, to a varying extent, integrating contaminant accumulation over a larger area. Eelpout, perch, and blue mussel are also key species within the integrated monitoring of contaminants, biological effects and fish populations. If fat soluble contaminants are analysed, a high and stable fat content is desirable, and therefore species such as guillemot (egg), herring (muscle) and Arctic char are preferred. Likewise, human milk has a relatively high fat content.

Variation within and between years (CV) for a time series is crucial for the power and the sensitivity of temporal trend analysis (e.g. the number of years it takes to detect a trend and the size of the trend that could be detected). The time series showing the lowest CV for PCBs were perch from Skärgölen (pooled samples, short and no trend), guillemot eggs from Stora Karlsö (individual analyses) and pike from Storvindeln (mainly individual analyses). In contrast, pike from Storvindeln had one of the highest CVs regarding PCDD/F analysis, but only pooled samples were analysed and the number of analyses performed were relatively few. Human milk and guillemot egg showed the lowest CVs for the PCDD/F (pooled analyses). Pooled samples will automatically generate a lower CV (**Paper I**) and Lake Skärgölen is in addition located high up in the drainage area and most likely only affected by diffuse contamination. Guillemot eggs have a high and stable fat content and have higher concentrations generating lower analytical errors, thus a lower CV. Changes in contaminant concentrations can be detected faster; alternatively, a smaller trend may be detected (Table 4.1, 4.2) when using a matrix with a lower CV (lower variability).

Target values established for assessments under the WFD/MSFD and OSPAR are primarily set for fish, but a few of the target values are also set for mussels. Regarding birds eggs, OSPAR has determined Ecological Quality Objectives for a few bird species. The TGD for biota monitoring under the WFD addresses several difficulties regarding compliance assessment with biota EQSs and gives advice on how to handle e.g. normalisation of data to the correct basis, recalculation of concentrations between different organs, and adjustment for trophic level. However, information on TMFs for different food webs and recalculation factors between organs for different fish species are lacking for many substances and species.

Regarding the choice of species and matrices within Swedish contaminant monitoring, it is essential that the species and organ fit the purpose of the monitoring. In summary, two of the clearest advantages for using guillemot eggs are the high lipid content and the relatively low CV in both time trends presented herein. The largest drawback of using guillemot egg lies in the limitations imposed on collection, that it is only present at a few sites in the Baltic Sea, and the lack of relevant target values. Regarding fish species, each has its own advantages and disadvantages. Perch is stationary, found all over Europe, and frequently used for biological effect monitoring, similar to eelpout, but both species have very low muscular fat content. Pike also has a low muscular fat content. Thus, they are not very suitable for analysis of fat soluble contaminants. Cod is a common species within the OSPAR area and the liver is high in fat but the fat content is very variable. However, adjustment for fat content is possible within the assessment. Herring is widely distributed, has a relatively high and stable fat content, and is commonly used for human consumption, but it is not very stationary. Blue mussel are stationary, but target levels are lacking for many substances.

The temporal trends presented for PCBs, representative for many classical organochlorines, for which concentrations have decreased since the 1970s, are good examples that measures taken to reduce concentrations have been effective. Regarding PCDD/Fs, the picture is not as clear. These contaminants have decreased in matrices in which the concentrations were very high in the beginning of the monitoring period, but show no significant changes in any direction in other matrices with lower levels in the beginning of the monitoring. This might imply that some of the measures taken to reduce the levels, such as the ban of dioxin contamination in herbicides, the cease of chlorine bleach within the pulp and paper industry and the improvement within waste incineration have had an effect, but there are still sources that are keeping these contaminants at a relatively stable level in marine and freshwater organisms compared to decreases seen in human milk.

In the future, for the assessment of compliance towards priority substances within OSPAR, HELCOM, and the WFD/MFSD, it would be of particular interest to develop relevant TMFs for the Baltic Sea food web. In addition, research regarding the distribution of contaminants in different organs is also needed to establish recalculation factors suitable for the most common monitoring species.

Sammanfattning (summary in Swedish)

Östersjön anses vara ett av världens smutsigaste havsområden. Det var under slutet av 1960-talet som det upptäcktes att Östersjön var svårt förorenat av långlivade organiska miljögifter såsom PCB och DDT. Det visade sig senare att dessa ämnen orsakat reproduktionsproblem hos både säl och havsörn, vilka är affischarter i den svenska marina miljön. Upptäckterna ledde till att ett omfattande miljöövervakningsprogram startades, finansierat av Naturvårdsverket. Enheten för Miljöforskning och Övervakning vid Naturhistoriska Riksmuseet fick ansvaret för övervakning av miljögifter i marin och limnisk biota (främst fisk, musslor, fågelägg). Idag ansvarar enheten också för den hälsorelaterade miljöövervakningen av miljögifter i bröstmjölk insamlade från Stockholmsområdet. Syftet med övervakningsprogrammen var i första hand att uppskatta förändringar över tid för att kunna utvärdera om åtgärder och förbud haft någon effekt.

De åtgärder som vidtogs har varit effektiva. Sedan övervakningen påbörjades noteras att halterna av PCB och DDT har minskat i både fisk, fågelägg och bröstmjölk, dock är koncentrationerna av majoriteterna av de klassiska miljögifterna (DDT, PCB, HCH) fortfarande avsevärt högre i Östersjön jämfört med andra havsområden.

Miljöövervakning är en förutsättning för att myndigheter och andra intressenter ska kunna bedöma hur effektiva åtgärdsprogrammen är och för att upptäcka nya hot i miljön. Stora summor läggs årligen på miljöövervakning, men dessvärre är många av programmen inte tillräckligt känsliga (har tillräckligt hög statistisk styrka) för att upptäcka förändringar i miljön i den storleksordning man skulle önska. För att förbättra bristfälliga program är det första steget att formulera syftet med miljöövervakningsprogrammet på ett kvantitativt sätt. Hur stor förändring måste kunna upptäckas och vilka risker kan accepteras när det gäller att dra fel slutsatser, att felaktigt påstå att det skett en förändring (Typ I fel) eller att felaktigt förmoda att ingen förändring sker fastän halterna ökar/minskar i miljön (Typ II fel). Dessutom bör onödigt brus (varians) minimeras genom en ändamålsenlig provtagningsstra-

tegi. I provtagningsstrategin ska provstorlek och val av individuella eller samlingsprov definieras. Matrisvalet, till exempel art, kön, storlek och organ, är också viktigt för att minska bruset och kan skilja sig åt för olika typer av miljögifter. Det är också mycket viktigt att den statistiska utvärderingen anpassas efter befintlig data.

Min avhandling syftar främst till att utvärdera övervakningsdata som samlats in under mer än 4 decennier inom de nationella övervakningsprogrammen för miljögifter i biologiska prov (marin, limnisk och human hälsa) i både tid och rum samt i förhållande till uppsatta miljögränsvärden som ska skydda den känsligaste arten mot toxiska effekter. Målet var även att utvärdera olika matrisers lämplighet för övervakning av långlivade organiska föroreningar i miljön med det övergripande målet att förbättra utvärderingen av resultaten i övervakningsprogrammen. Olika statistiska frågeställningar har en central roll i avhandlingen.

I **Paper I** undersöks hur valet av analys av individuella eller samlingsprov påverkar den statistiska styrkan. Den statistiska styrkan i temporala och geografiska studier bestäms av provvariationen, vilket i sin tur kan delas upp i analysfel och biologisk variation. Information om olika typer av variation är därmed avgörande för att kunna designa en effektiv provtagningsstrategi. Resultaten visar att variationen kan minskas med samlingsprov, vilket genererar en ökad statistisk styrka till en lägre kostnad i de fall där kostnaden för insamling och provtagning är betydligt lägre än kostnaden för kemisk analys. Emellertid finns det också ett flertal fördelar med individuella prov som bör beaktas.

Temporala (1981-2012) och geografiska trender av PCBer i abborre, gädda och röding från 32 svenska sjöar undersöks i **Paper II**. Generellt sett så minskar PCB koncentrationerna över tid, dock gäller detta inte alla PCB kongener och djurarter som studerats. Även kvoten mellan låg- och högklorerade kongener minskar över tid vilket indikerar att det inte sker någon större nyemission av PCB. Halterna av kongenerna CB-118 och CB-153 visar en geografisk gradient, med högre koncentrationer i stadsnära områden. Koncentrationen av CB-153 i abborre ligger under miljögränsvärdet, medan gränsvärdet för CB-118, som är dioxinlik och därmed mer toxisk, överskrids i vissa sjöar.

Paper III sammanfattar geografiska och temporala trender (1969-2012) av PCB, DDT, HCH och HCB i svensk marin biota (strömming, abborre, torsk,

tånglake, blåmussla och sillgrissleägg). Halterna av de undersökta ämnena har minskat över tid med 70-90% sedan övervakningen började. Miljögränsvärdet för CB-118 och DDE (metabolit till DDT) överskrids i vissa arter på några lokaler.

I **Paper IV** diskuteras fördelar och nackdelar med att använda strömming och sillgrissleägg för övervakning av klorerade fettlösliga föreningar genom att bland annat jämföra trender av dioxiner i de två arterna. Fetthalten i sillgrissleäggen var hög och variationen i proven låg, men äggen är relativt svåra att samla in. Strömming visade en högre variation i proven, men är lätt att samla in och halterna i strömming kan åldersjusteras.

Halten av dioxiner i bröstmjölk från Stockholmsområdet analyserades retrospektivt, 1972-2011, i **Paper V**. Halten av dioxiner minskar över tid och den årliga minskningen är större under de 10 sista åren. Resultaten från nyanalysen jämfördes också med gamla analysresultat av samma prov och överenstämmelsen var god, vilket indikerar att gamla tidsserier kan förlängas om samma analysmetod används.

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Appendix A

Contribution to **Paper I-V**

Paper I Planned the study together with the co-authors and participated in the data evaluation, discussions and the writing of the paper.

Paper II Planned the study together with the co-authors and was responsible for the data evaluation and the writing of the paper.

Paper III Planned the study together with the co-authors and was responsible for the data evaluation and the writing of the paper.

Paper IV Participated in the data evaluation, discussions and the writing of the paper.

Paper V Planned the study and sample selection together with first author Johan Fång. Participated in the data evaluation, discussions and writing of the paper.

All the papers rely on high quality chemical analyses for which environmental chemist have been responsible also for the choice of analytical methods. I have not been involved in these activities.

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Consequences of using pooled versus individual samples for designing environmental monitoring sampling strategies

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HIGHLIGHTS

- Use of individual or pooled samples is important when designing sampling strategies.
- Variation caused by e.g. chemical analysis or sample variation need to be considered.
- Various solutions are offered using different numbers of individual/pooled samples.
- Results allow the design of cost-efficient, statistically sound sampling strategies.

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ABSTRACT

Choosing an appropriate sampling strategy for chemical analysis within environmental monitoring includes the important decision of whether to sample and store individual or pooled samples. This choice impacts on future analyses from Environmental Specimen Bank samples. A number of advantages exist to support using either individual or pooled samples for temporal trend studies. However, it is important to know the total and analytical variance to be able to design the best sampling strategy. Statistical power in temporal or spatial studies is determined by the random/unexplained sample variation. The relationship between chemical analytical error and other sources of variation, as well as the cost for collection, preparation of samples and chemical analysis, will determine the number of individuals in each pool, and the number of pools that should be analysed to achieve high cost efficiency and good statistical power. Various scenarios of different numbers of individual samples, different numbers of pooled samples containing various numbers of individual specimens, the relationships between chemical analytical error and other sources of sample variance, have been compared by simulating random sampling from computer generated populations using realistic measures of variation from ongoing monitoring activities. These results offer guidance in the design of a cost-efficient, statistically sound sampling strategy.

1. Introduction

When choosing a sampling strategy for chemical analysis within environmental monitoring, or when storing samples in Environmental Specimen Banks (ESBs) for future analyses, the important decision of whether to use individual or pooled samples i.e., multiple individual samples homogenized into one sample for chemical analysis, should be emphasised. This decision can impact on many things, from the sensitivity to detect changes in contaminants in the environment, to the potential for future and/or retrospective analyses. Strategies outlines here are applicable to both environmental and human samples.

A number of advantages are gained from using individual samples (Bignert et al., 1993). Information about variance is important in itself to identify the sample distribution e.g., normal or log-normal distribution, and changes in variance are often the first sign of a change in contaminant burden. In this study, the variance estimates are based on biological samples from the Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPCMB). Information about the maximum value can be crucial when the threshold level for a substance is set at the maximum value. It may also be essential for risk analyses. Individual samples allow the freedom to choose an appropriate central measure (Caudill et al., 2007; Caudill, 2012). Environmental contaminant data often display a right skewed distribution, in which case geometric mean values are more appropriate. By contrast, pooled samples reflect approximate arithmetic means, although this can be compensated for to some extent (Caudill et al., 2007). Sampling of individual organisms facilitates direct adjustments for

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confounding factors, for example fat content, age, and size (length and weight), and the detection of extreme values. Further to this, storage of individual samples allows the estimation of within-specimen variation and distribution.

However, in cases where sampling and sample preparation are considerably less expensive than the chemical analytical costs, and where the contribution from inherent specimen variance due to e.g., physiological factors in biological samples, is considerably larger than the analytical error to the total random/unexplained variation, variation may be reduced, while keeping within the same budget, by using pooled samples. Hence, the statistical power to detect changes over time or differences between groups can be increased or the spatial distribution can be better defined for the same cost by using pooled samples. A reduction in the random/unexplained between-year variation implies that a smaller annual change can be detected, or that a specified lowest trend that needs to be detected will be identified in a shorter period of time (Bignert et al., 2004). Pooled samples may also allow for chemical analysis to be conducted when individual samples are too small to provide enough material e.g., small fish or zooplankton (Gewurzt et al., 2011), resulting in fewer non-detects. Further, a better defined spatial distribution enables e.g., comparison of quality status between lakes and other types of surface water within the Water Framework Directive (2000/60/EC) (WFD) and the Marine Strategy Framework Directive (2008/56/EC) (MSFD).

Within the SNMPCMB, temporal trend studies of polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexanes (α, β- and γ-HCH), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane (*p,p′*-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene (*p,p′*-DDE) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (*p,p′*-DDD) have been conducted. To study these contaminants, a number of individual samples have been collected from the same station within the same sampling season each year (Bignert et al., 2013). From the beginning, individual samples from 12 sampling sites were used, but during the last 10 years, due to the use of pooled samples, the SNMPCMB has been able to expand the number of sites sampled from 12 to 24. One of the objectives of the SNMPCMB is to estimate long-term time trends and the rate of the changes found. The quantified objective is to detect an annual change of approximately 5% within a time period of 10 years with a power of 80% at a significance level of 5%. This objective will be used as the target values in this study.

There are clearly a number of potential advantages and disadvantages in certain situations to using either pooled or individual samples. Here, we outline various sampling strategies for chemical analysis based on using different numbers of individual or pooled samples to provide several possible outcomes, which can then be used to guide those designing sampling strategies for environmental monitoring or working with the storage of specimens in ESBs. Although in this study the variance estimates are based on biological samples from the SNMPCMB, the results presented here should also be applicable for monitoring of human and abiotic samples if similar variance estimates are calculated for such matrices.

2. Materials and methods

This study was based on simulations using computer generated values. However, the estimated variances, the ratios between specimen variation and the chemical analytical measurement errors in the various scenarios were based on real values collected during the last 10 years of environmental contaminant monitoring within the SNMPCMB. This program is ongoing, and consists of locally unpolluted areas. Samples are collected annually. Herring (*Clupea harengus*) is the most frequently used species in the SNMPCMB, although cod (*Gadhus morhua*), eelpout (*Zoarces viviparous*), perch (*Perca fluviatilis*) and guillemot (*Uria aalge*) eggs are also monitored, as well as other species not included here. Since 1996, 10 individual cod, perch, eelpout, guillemot eggs and spring samples of herring, and 12 individual samples of autumn-caught herring from 12 sites along the Swedish coast were collected within the SNMPCMB and analysed and stored in an ESB. Since 2007, the SNMPCMB has expanded to include 24 sampling sites along the Swedish coast due to the resources saved by using pooled samples. However, all samples collected are individually stored in the ESB at the Department of Environmental Research and Monitoring, Swedish Museum of Natural History, Stockholm, Sweden. The chemical measurement errors handled in this study were estimated from the analyses carried out at the Department of Applied Environmental Science (ITM), Stockholm University, Sweden.

Beginning with 229 time series based on the above collected data, a number of filters were applied to eliminate series that were deemed unsuitable for this analysis. Total variances, expressed as coefficients of variation (CVt), were estimated from de-trended time series from the SNMPCMB (Bignert et al., 2013) for the last 10 years from 12 different sites. The removal of possible trends was achieved by subtracting the predicted annual mean value from a linear regression line from each individual observation in the same year. Eight time series with a CVt above 100% were removed because they were not representative of the majority of samples. A further 7 time series were excluded, mainly because of gaps (missing years) and non-monotonic trends. Another filter was applied to achieve robust data sets, which specified that a) the contaminant concentration should be quantified in at least 20 samples per site (23 time series excluded), and b) there should be at least 6 out of 10 years with quantifiable values (15 time series excluded). After filtering, 176 of the original 229 time series remained. The highest CVt observed for one time series was 97%, the lowest 12% and the median value 52% with an interquartile range of 35–62% (Table 1).

The precision of the chemical analysis, also expressed as a coefficient of variation (CVa) (within and between years), was based on approximately 6000 analyses from four different internal reference materials. These had a lipid content ranging from 0.5% to 11.3% analysed for 7–9 years over a period of 20 years i.e., not all reference materials were available for the entire period (two reference materials were used for 7 years; when these ran out, new ones were made and used for approximately 9 years, thus partly overlapping). A sample of 10 g of muscle tissue was extracted with a mixture of polar and non-polar solvents and analysed on a gas chromatograph equipped with a μ-electron capture detector (μECD) (Jensen et al., 1983; Eriksson et al., 1997) at ITM, Stockholm University. The standard deviation, expressed as CV%, was plotted against the arithmetic mean value for the log concentration on a lipid weight basis for each contaminant for each reference material. A regression line was fitted to the values for each contaminant group using a power function, so that the variation decreased when the concentration increased (Horwitz and Albert, 2006). The CV% of the geometric mean values for the contaminant concentrations for each species and sampling site within the SNMPCMB for the last 10 years was then calculated from the corresponding regression lines. The highest CVa was 16% and the lowest 4%. The median value was 10% with an interquartile range of 8–12% (Table 1).

Specimen variance (within and between years) reflecting e.g., physiological differences such as sex, age, condition and reproduction phase, or other factors possibly induced by abiotic factors e.g., temperature, and salinity, as well as long-term storage and sample treatment, expressed as coefficients of variation (CVs), were calculated from the CVt and the CVa using the relationship (Råde and Westergren, 1990):

$$CVt = (CVs^2 + CVa^2)^{0.5}.$$

Table 1
Examples of sampling sites, matrices, contaminants, variances, concentrations, number of measured values and years in time series from the Swedish National Monitoring Program for Contaminants in Marine Biota. These sites are chosen to represent some of the variation seen. CVt = total variance; CVa = chemical analysis variance; CVs = specimen variation; *n* = number of measured values; *n* years = total number of years.

Sampling site*	Matrix	Contaminant	CVt %	CVa %	CVs %	ng/g lw	*n*	*n* year
B.P., south	Cod	ß-HCH	12	10	7	15	100	10
B.B., south	Herring	y-HCH	13	12	4	8	87	9
B.B., north	Herring	α-HCH	15	13	7	5	68	7
B.P., north	Herring	y-HCH	16	12	11	9	89	9
St. Karlsö	Guillemot	HCB	21	5	21	829	99	10
Kattegatt	Cod	α-HCH	24	16	18	3	65	9
St. Karlsö	Guillemot	*p,p′*-DDE	28	4	27	16224	99	10
Kattegatt	Herring	CB52	33	12	31	6	78	8
B.B., south	Herring	CB28	34	12	32	3	67	9
B.B., south	Perch	*p,p′*-DDE	51	12	49	43	89	9
B.B., north	Herring	CB180	51	10	50	14	119	10
B.P., south	Herring	CB101	52	9	51	29	119	10
Skagerrak	Eelpout	*p,p′*-DDT	77	13	75	23	80	10
B.S.	Herring	CB138 + 163	77	6	77	147	100	10
B.P., south	Cod	*p,p′*-DDD	78	10	77	99	100	10
B.B., south	Eelpout	*p,p′*-DDT	96	12	96	42	86	9
Skagerrak	Eelpout	CB153	97	6	97	286	99	10

* B.B. = Bothnian Bay, B.S. = Bothnian Sea and B.P = Baltic Proper.

The highest CVs was 97% and the lowest 4%. The median value was 51% with an interquartile range of 33–61% (Table 1).

In order to estimate the effect of various sample sizes and the influence of CVs in relation to CVa on the reduction of the random between-year variation (CVby), and hence the statistical power of temporal trend analyses, a randomization experiment was designed (see e.g., Manly, 1997). Samples were taken randomly from computer generated populations of a fixed mean with added variance, simulating variances from both the sampled specimens and the chemical analytical error. Various scenarios were created for different combinations of magnitude from these sources, chosen from the SNMPCMB. The median values for CVt and CVa were 52% and 10% respectively. A CVt of 90% was chosen as it was the upper limit for a 95% confidence interval for all CVt values.

For variation to be reduced by using pooled samples, the contribution from CVs to total variance must be larger than the contribution from CVa. A CVt of 16% was the lowest value within the SNMPCMB where CVs was higher than CVa. A CVa of 5% and 15% were chosen as the lower and upper limits for the 95% confidence interval for all CVa values, respectively. All CVs values were calculated from the CVt and CVa (Table 1). Sampling was repeated 10 times to simulate 10 years. The number of individual samples in a pool and the number of pools per year were varied in the random sampling. The whole procedure was repeated 1000 times for each effort level i.e., the total number of pools analysed. Arithmetic mean values for the simulated between-year variance, expressed as the coefficient of variation (CVby), were calculated. A regression line was fitted to the mean values (Figs. 1 and 2).

To give an estimate of costs for the various scenarios, a cost of €51 per fish for sample preparation (age determination included, based on figures from the SNMPCB) and a cost of €456 per sample (extraction included, based on ITM figures) for organochlorine analysis are used in the figures.

3. Results

Probable between-year variation (CVby) achieved using different sampling sizes for individual samples are simulated (Fig. 1). Total variance (CVt) of 16%, 52% and 90% for a normal distribution was used, assuming an analytical error (CVa) of 10%. When a higher number of individual samples are analysed each year, a lower random between-year variation can be achieved (Fig. 1). According to Fig. 1, the expected CVby is approximately 14.5% if 12 individual

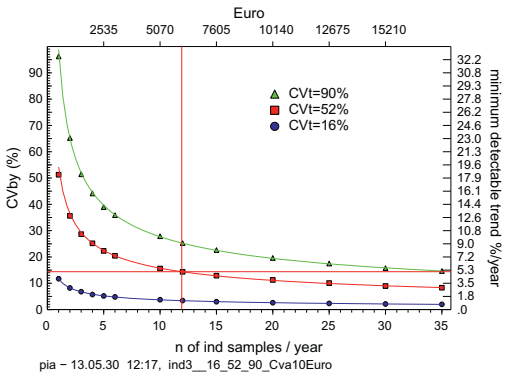

Fig. 1. Individual samples from a normally distributed population sampled for 10 years. Various variances for total variance (CVt), specimen variance (CVs) and an analytical variance (CVa) of 10% are used. The vertical lines shows the cost in Euro (€) and the number of samples needed to achieve a between-year variation (CVby) of 14.5%/annual change of approximately 5% (shown by the horizontal line) at a statistical power of 80%.

samples, as per the SNMPCMB, with a CVt of 52%, specimen variance (CVs) of 51% and an analytical error (CVa) of 10% are analysed. This is equivalent to being able to detect an annual change of approximately 5% over a period of 10 years with a power of 80%, one-sided test, $\alpha = 0.05$ (Bignert et al., 2013) i.e., our target value.

The CVby achieved using different numbers of pooled samples is shown (Fig. 2a–c). CVt varies from 16% to 90%, CVa from 5% to 15% and CVs from 6% to 90%. The number of individuals in each pool (Fig. 2a–c) was set to 12 to be comparable to the number used for pooled samples within the current SNMPCMB.

When a CVt of 16% was used (Fig. 2a), all combinations of CVa and CVs fulfill the criteria of being able to detect an annual trend of approximately 5% within a 10 year period by using only one pooled sample (material from 12 individual specimens). However, when CVt is as low as 16%, just one individual sample is needed to meet the set criteria (Fig. 1). Individual samples are therefore preferable in samples with a very low CVt (Figs. 1 and 2a). When the median values for CVs and CVa were used (51% and 10% respectively, Fig. 2b) only two pooled samples i.e., 24 individual

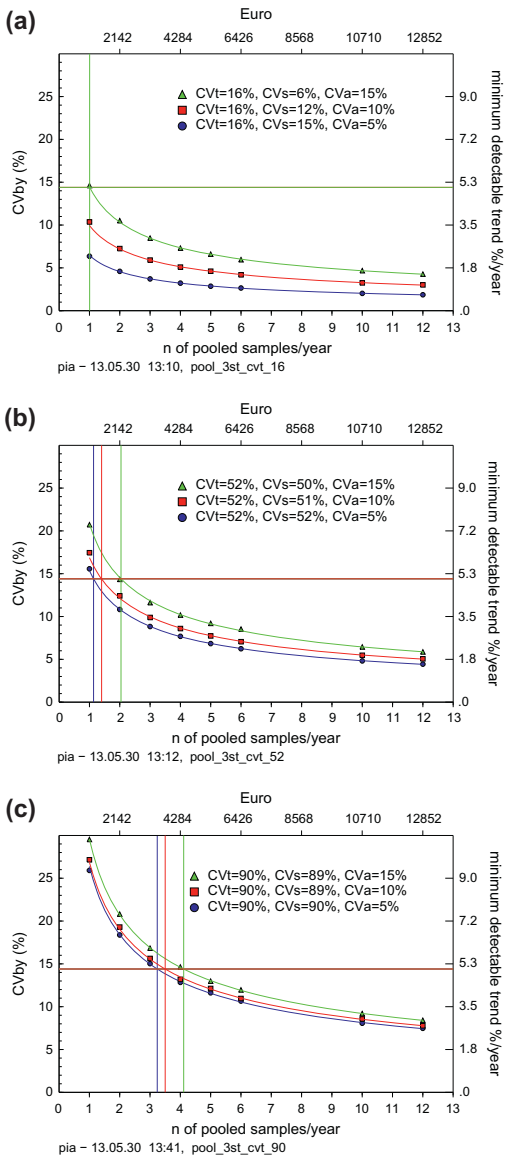

Fig. 2. (a–c) Pooled samples from a normally distributed population using 12 individual specimens in each pool sampled for 10 years. Various variances for total variance (CVt), specimen variance (CVs) and analytical variance (CVa) are used. The vertical lines shows the cost in Euro (€) and the number of samples required to achieve a between-year variation (CVby) of 14.5%/annual change of approximately 5% (shown by the horizontal line) at a statistical power of 80%.

specimens analysed but only two chemical analyses carried out annually, are needed to detect an annual change of 4%, slightly better than the target value of 5%. With the same CVa (10%) was used, but a CVt of 90% and a CVs of 89% was used (Fig. 2c), four pooled samples i.e., 48 individual specimens analysed but only four chemical analyses carried out annually, are required to achieve the target value of 5%. However, 34 individual samples are needed to achieve the same target value (Fig. 1) hence, pooled samples will still be more cost efficient (Figs. 1 and 2c). With rising CVa, the number of pooled samples required to maintain sensitivity to detect the same annual changes will increase slightly (Fig. 2a–c).

The cost of achieving the goal of detecting an annual change of 5% with a CVt of 52%, and different combinations of CVa and CVs is shown (Fig. 3a). With a CVa of 10% and a CVs of 51%, the cost varies from €1500 to €5600, depending on the combination of number of individuals per pool and the number of pooled samples. The highest cost, €5600, is for 11 individual samples. With three pools consisting of five individuals each, the cost will be €2100. The combination of one pool containing 21 individuals will have the lowest cost, at €1500; however, more fish must then be available. The cost when CVt is 90% is shown (Fig. 3b). With the same contribution of CVa as above (10%) and a CVs of 89%, a combination of 23 individuals per pool and two pools will have the lowest cost at €3300, but then 46 fish are needed.

The following examples show possible benefits of using pooled samples provided a CVt of 52%, a CVs of 51% and a CVa of 10% can be achieved.

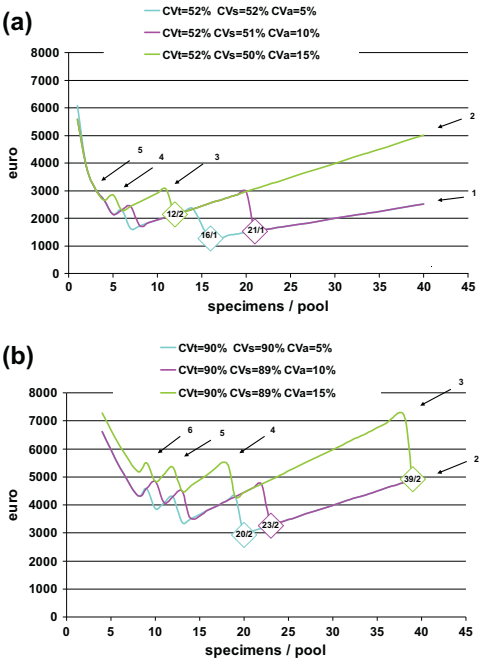

Fig. 3. (a and b) The cost for different combinations of number of specimens per pool and number of pooled samples needed to achieve a between-year variation (CVby) of 14.5%/annual change of approximately 5% at a statistical power of 80% from a normally distributed population sampled for 10 years. Various variances for total variance (CVt), specimen variance (CVs) and analytical variance (CVa) are used. The horizontal lines (marked with an arrow from one to six) represent different numbers of pooled samples. The number of specimens/pooled sample within the diamond represents the combinations with the lowest costs for CVt, CVs and CVa, respectively.

- To detect an annual change of 5% within 10 years, only two pools of 12 individuals each are needed, instead of 12 individual samples. Around €4000 is saved for one year at one site, implying that the spatial resolution could, by using pooled instead of individual samples, be improved from two to five sites.
- Five pooled samples, including the increased cost for sample preparation of 60 individual fish, could be analysed for the same cost as 12 individual samples. This means that even a less pronounced trend of 2% could be detected during a 10 year period, or that a trend of 5% could be detected faster.

4. Discussion

Here, various approaches that can be used as guidelines for designing sampling strategies for environmental monitoring have been outlined based on using different numbers of individual or pooled samples. Many different outcomes are shown to be suitable, depending on cost, and specimen, analytical, between-year and total variance. Pooled samples are deemed suitable in many situations, especially e.g., when chemical analytical costs are the largest proportion of the budget, or if variation due to specimen variance needs to be reduced. The cost of analysing multiple individual specimens compared to fewer pooled samples is higher within this study, and if cost is the major restraint, than pooled samples should be considered. However, thought should be given to storing specimens individually, as this allows for specimen variance to be analysed regularly. These sampling strategies can be used as a basis for making decisions on sample storage for retrospective studies made at a later time.

Differing combinations of numbers of individuals per pool, and number of pools to be analysed, based on different total variance (CVt), analytical variance (CVa) and specimen variance (CVs), can be used to meet various budgets while still achieving the target values (e.g., detecting a 5% annual change). In all cases more fish are needed compared to when individual samples are used, but the cost will be considerably lower. If the choice is between fewer individuals per pool or fewer pools, then the decision will be dependent on the number of available fish and the cost (analysis and sample preparation). Muñoz-Zanzi et al. (2006) examined the use of pooled samples as a low-cost alternative used for surveillance of infectious agents. The most cost-effective number of samples was eight samples per pool. Their result differs slightly from ours; however, their goal (detecting infectious diseases) was also different.

When an annual change of 5% needs to be detected within a period of 10 years with a CVt of 52%, two pools consisting of 12 individuals per pool, at a cost of €2142 can be applied, as per the strategy used in the SNMPCMB. Sundström et al. (2011) analysed human milk samples for temporal trends of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexansulfonate (PFHxS) from Swedish mothers. Twenty pooled samples i.e., one pooled sample per year, consisting of anywhere from 15 to 116 individual samples per pool, were used to conduct this research. The aim was to report the minimum trends in concentrations able to be detected within a 10 year period, with a statistical power of 80% and a Type I error of 5%. The annual change able to be detected was greater than 5% (6–12%) (Sundström et al., 2011). By analysing two pooled samples annually, instead of one, the goal of 5% could have been achieved, assuming the same CVt, according to results presented here.

After considering the best sampling design for cost-efficiency while maintaining good statistical power, one must not forget to consider storage of samples. Many ESBs store some samples already pooled and homogenized (J. Mueller, J. Koschorreck *pers. comm.*) due to the method of sample collection, but also out of ethical considerations, in particular in the use of human samples. An alternative, but more volume-costly strategy, would be to store the specimens individually to allow the estimation of within-specimen variance and distribution. This would allow optimisation of the pooling strategy in relation to future quantitative objectives and knowledge about chemical analytical errors in relation to specimen variance. The volume stored is a matter of detection limits for the specific contaminants to be analysed. In regards to retrospective studies, not knowing at the time of storage what chemicals will be analysed means that storing individual samples may be preferable. Also essential to consider is avoiding potential contamination of samples stored for future studies from chemicals that are currently not known, but are possibly present in equipment used for homogenization or in the ambient laboratory environment. The storage of blank samples should also be considered to allow for examination of contamination through the homogenization process or the laboratory environment. Storing individual specimens would also avoid any potential degradation of less persistent substances through the homogenization procedure.

Gewurzt et al. (2011) examined differences between monitoring for PCBs carried out using individual fish samples (number not specified) or pooled samples (consisting of 5 fish per pool) from data collected within two different monitoring programs. To keep results comparable, they selected data from each program generated from fish of the same size. Within-year variation was consistently higher in the individual samples compared to the pooled samples, most likely due to the higher specimen variance obtained when analysing individuals as compared to pooled samples. Furthermore, the use of pooled samples when generating fish consumption advice for human health was deemed inappropriate, given the need to evaluate variability and the risk posed by outliers in biasing pooled samples (Gewurzt et al., 2011). This is in agreement with results here, as pooled samples miss maximum values and specific information about variance.

The Co-ordinated Environmental Monitoring Programme (CEMP) Monitoring Manual (OSPAR, 2008) JAMP Guidelines for Monitoring Contaminants in Biota generally recommends sampling 12 individually analysed fish within the same age range and gender. This number is in accordance with this study, with a mean CVt of 50%, in order to reach the quantified objectives. However, within the SNMPCMB, if the CVt exceeds 52% we will be unable to fulfil the outlined goal within the given budget if only 12 fish are analysed, even with the suggested two pooled samples. If the specimen variance constitutes the larger part of the total variance and it is reasonably inexpensive to add extra specimens, we can improve the situation according to the presented scenarios, and possibly reach the quantitative objectives set in the programme, while remaining within budget. However, as shown (Figs. 1 and 2b), using two pools of 12 fish each would reduce the cost by more than half, therefore being more cost efficient.

Bjerkeng (2012) examined differences in trend detection ability for PCBs, DDE, Cd, Cu, Zn and Hg in cod liver, using individual or pooled samples. Data from the Norwegian Coordinated Environmental Program, which follows earlier JAMP (OSPAR, 2008) guidelines, was used to analyse how the precision of trend assessments would be affected by changes in the monitoring program. Previously, 25 individual fish were analysed annually for each site within this programme. Bjerkeng (2012) found that the ability to detect trends was lower if 20 fish were analysed individually compared to analysing 25 fish as 5 pools of 5 fish each. However, our results show that there is very little to be gained by sampling more than 15 fish, depending on initial specimen variance. Bjerkeng (2012) further stated that individual analysis might be better if exclusion of outliers or adjustment for biological characteristics were needed.

Turle and Collins (1992) examined the mean concentrations of a number of organochlorines from herring gull (*Larus arentatus*) eggs

collected yearly from multiple colonies, and pooled for analysis; however, every fifth year, all individual eggs were analysed. For most chemicals in most colonies, there was no significant difference between the pooled or individual samples; however nothing was mentioned of between-year, analytical or total variation between the individual and pooled samples. Nonetheless, the strategy of analysing individuals every few years to retain knowledge on specimen variance is recommended. This is not currently done within the SNMPCMB. However, as the SNMPCMB stores individual samples, this strategy could be included for the future.

It should be stressed that the way CVa is calculated here assumes that the same laboratory performs the chemical analysis each year. If analysis is carried out at different laboratories, the CVa can be higher. In proficiency tests, such as Quasimeme, the between-laboratory CV% for one contaminant for one exercise can differ from 22% to 132% (de Boer and Wells, 1997). Thus, it is important to ensure continuity by using the same laboratory each year for all analyses in a monitoring program to avoid increased analytical error and a corresponding decrease in statistical power in the monitoring program.

5. Conclusions

It is important to know the total and analytical variance to be able to design the best sampling strategy. Therefore, when analysing material from new sites or matrices, it is necessary to initially use individual samples to investigate the variance. There are a number of advantages to using individual samples, as outlined earlier. One possible strategy is to use a combination of individual samples for some time series and pooled samples for other time series, for example to be able to detect higher specimen variance, which can indicate a change in contaminant burden. This combination strategy could also be considered for sample storage.

The target of being able to detect an annual change of 5% within a 10 year period with a power of 80% at a significance level of 5% could be achieved by using, for example two pooled samples consisting of 12 individuals each, with a CVt of 52%, CVa of 15% and CVs of 50%. With a CVt of 90%, CVa of 15% and a CVs of 89%, five pooled samples containing 12 individuals each are needed. The same goal can also be achieved by many other combinations. For studies including upcoming years, it is relatively inexpensive to considerably increase the number of fish collected for most species, but for fish stored for many years in an ESB, the number of analysed pools and individual specimens in each pool will depend on the number of available fish.

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Spatio-temporal trends of PCBs in the Swedish freshwater environment 1981–2012

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Abstract Polychlorinated biphenyls (PCBs) have been monitored in perch (*Perca fluviatilis*), pike (*Esox lucius*), and Arctic char (*Salvelinus alpinus*) in reference lakes since the late 1960s. Temporal trends and spatial patterns are currently monitored in nine and 32 lakes, respectively. Overall, PCB concentrations are decreasing. However, this is not consistent for all congeners across all lakes and species. Perch has comparatively low PCB concentrations relative to suggested target levels, but individual congener concentrations in some lakes are concerningly high. No temporal trend is seen for CB-118 and CB-153 in perch, but significant decreasing trends exist for Arctic char and pike, for which monitoring started earlier than for perch. The lower/higher chlorinated congener ratio decreased over time in most lakes, indicating fewer new emissions. CB-118 and CB-153 concentrations in perch show spatial gradients across Sweden, with higher concentrations found near urban/industrial areas.

Keywords Fish · PCBs · Monitoring · Freshwater · Sweden · Trends

INTRODUCTION

Polychlorinated biphenyls (PCBs) are one of the 12 groups of persistent organic pollutants (POPs) originally included in the Stockholm Convention on POPs.[1] PCBs have been used in a wide variety of manufacturing processes, especially as plasticizers and insulators, and are widely distributed in the environment. In 1973, PCB use was banned in Sweden, except within sealed systems. In 1978, the ban was extended to prohibit all new use of PCBs.

PCBs can influence human health by affecting multiple organ systems (Carpenter 1998, 2006). Their toxicological effects on, for example, reproduction in mink are well documented (Aulerich and Ringer 1977; Bleavins et al. 1980). Animals in aquatic systems tend to biomagnify contaminants at a higher degree compared to terrestrial species due to the complexity and omnivory that characterizes aquatic food webs (Zanden and Rasmussen 1996). The concentrations of PCBs are generally positively correlated with the trophic position of a fish population within an aquatic food chain and are particularly high in predatory fish species (Brázová et al. 2012).

In the mid-1960s, research concerning environmental contaminant concentrations (e.g., chlorinated contaminants and heavy metals) and abundance, and their effects on wildlife began at the Swedish Museum of Natural History (SMNH) because of adverse health effects being observed in top predators such as Baltic grey seals (*Halichoerus grypus*) and white tailed sea eagles (*Haliaeetus albicilla*) (Helle et al. 1976a, b; Helander et al. 2002; Bredhult et al. 2008). During the 1970s, initial efforts were made to establish an environmental contaminant research program. In 1980, a comprehensive national monitoring program for environmental quality was formed by the Swedish Environmental Protection Agency (SEPA). SMNH was appointed the responsible institute for monitoring environmental contaminants in biological samples. The chemical analysis has been carried out at the same laboratory since the start of the monitoring program. The laboratory was initially a part of the SEPA and, since 1992, a part of Stockholm University (SU).

The primary objectives of the monitoring program were (1) to measure the concentrations of various contaminants and estimate normal variation in freshwater biota from

[1] http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx.

representative sites throughout the country that were uninfluenced by local sources; (2) to describe the general contaminant load and to supply reference values that could be used for comparison with data from regional and local monitoring programs; (3) to monitor long-term time trends and estimate the rate of changes found; (4) to estimate the response in biota to actions taken to reduce the discharge of various contaminants; (5) to detect incidents of regional, national or international influence; (6) to detect renewed usage of banned contaminants for compliance monitoring, and (7) to discover large scale spatial differences across the country.

Sweden is a country with many lakes that cover more than 9 % of the country's total surface area. The lakes within the Swedish National Monitoring Program for Contaminants in Freshwater Biota (SNMPCFB) are distributed from the northern parts of Sweden (Lake Abiskojaure) located 200 km north of the Arctic Circle, to the southern-most parts (Lake Krageholmssjön), with the majority located in the southern half of Sweden. The large distances between north and south leads to large temperature differences, thus some of the lakes are covered with ice for several months of the year, while others remain ice-free. The lakes also differ in size, nutrient status, general physical environment, and land use of surrounding areas. The smallest lake is 0.06 km^2 (Lake Skärgölen) and the largest is 184 km^2 (Lake Bolmen). These physical differences imply a great variability between the lakes concerning abiotic factors that might affect contaminant levels in fish.

Here we examine temporal and spatial relationships of PCB congeners (CB-28, CB-52, CB-101, CB-118, CB-138, CB-153 and CB-180) in pike (*E. lucius)*, Arctic char (*S. alpinus*) and perch (*P. fluviatilis*) to evaluate (1) concentrations over time in relation to imposed bans and restrictions; (2) spatial congener differences across Sweden; (3) concentrations against set environmental target levels; and (4) how monitoring design may affect the interpretation of these trends.

MATERIALS AND METHODS

Study species

The European perch is the most common freshwater fish in Sweden, and is also found in the brackish Baltic Sea (Kullander et al. 2012). Perch is an opportunistic predatory fish that undergoes an ontogenetic shift in diet (Collette et al. 1977; Kullander et al. 2012); small perch (5–30 mm) feed primarily on zooplankton, intermediate size (30–80 mm) on crustaceans, larvae and small fish, while large perch feed exclusively on fish and crayfish. Larger perch are thus exposed to biomagnifying substances at a high level of the aquatic food chain. The age at dietary shift is dependent on growth rate of perch, which can vary between lakes (Holmgren and Appelberg 2001). Perch muscle tissue is lean and contains approximately 0.3–0.6 % of extractable fat (Nyberg et al. 2013).

Pike is also a common fish in the Nordic countries and can be found in both fresh and brackish water (Baltic Sea). Pike is mainly piscivorous but occasionally also feeds on frogs, small mammals and sea birds (Kullander et al. 2012). As this species is located at a high trophic level, the concentrations of biomagnifying compounds such as POPs, are normally high. Pike is a lean fish with an average muscle fat content of 0.6 % (Nyberg et al. 2013).

Arctic char inhabits upland fresh waters of the Swedish mountain area. Diet varies depending on prey availability, fish size and the presence of other competitive species. Small individuals generally feed on benthic invertebrates and plankton, while larger individuals feed on fish, including conspecifics (Kullander et al. 2012). Because of the high trophic position of the large, piscivorous individuals, the concentrations of POPs might be high. Among the three investigated fish species, Arctic char has the highest lipid content: 1–3 % (Nyberg et al. 2013). All three species are fairly stationary, thus appropriate for studying local contaminant concentrations.

Sampling sites, number of samples, and sampling frequency

A total of 32 lakes are included in the Swedish National Monitoring Program for Contaminants in Freshwater Biota (SNMPCFB). Approximately 20 of these lakes are located in the southern half of Sweden (Fig. 1). In general, only one species per lake was sampled, with three exceptions. The year of initial PCB analysis varied among the selected lakes (Table 1). To facilitate regional comparisons, selected lakes were chosen to avoid possible confounding factors that could influence contaminant concentration in the sampled fish tissues e.g., (1) lakes should not be influenced by local contamination and must have some protection against future exploitation, (2) land use surrounding the lakes should be well investigated and intensively farmed rural areas avoided, (3) areas of liming activities should be avoided, and (4) lakes should preferably be placed high in the drainage system and be oligo- or mesotrophic.

The earliest time-series for PCBs (ΣPCBs) in the freshwater environment are from the late 1960s (pike from two lakes, including one that represents the Arctic region in Sweden). The ΣPCBs were estimated from 14 peaks on a packed column GC after calibration with Aroclor 1254 (Jensen et al. 1983). During 1988, analysis on a capillary column was introduced, allowing analysis of individual congeners (Eriksson et al. 1994). Pike has been analyzed for

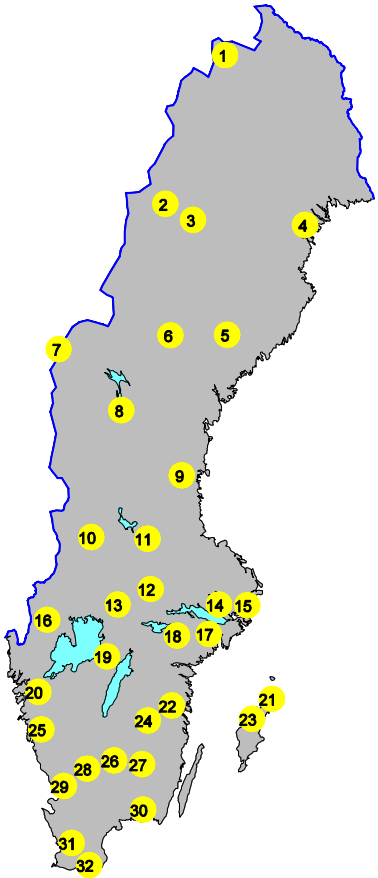

Fig. 1 Sampling sites within the Swedish National Monitoring Program for Contaminants in Freshwater Biota. See Table 1 for information about the different lakes

PCBs on an individual congener basis at two sites, Lake Bolmen, since 1988, and Lake Storvindeln since 1985 (Fig. 1; Table 1). Pike was collected in spring (April–May), during or soon after spawning. Arctic char was analyzed for PCBs on an individual congener basis at three sites—Lake Abiskojaure since 1981 (analyzed retrospectively), Lake Tjulträsk since 1986 and Lake Stor-Björsjön since 2007 (Fig. 1; Table 1). Char was sampled in the autumn (August-November), usually during spawning. In recent years, perch was the most frequently sampled species and is currently collected from 27 lakes within the program (Fig. 1; Table 1). Perch has been analyzed for PCBs on an individual congener basis since 1997 in Lake Stensjön and 1999 in Lake Skärgölen (Table 1). Sampling of perch was carried out in autumn (August–October), outside of the spawning season.

Sampling has been carried out annually throughout the duration of the program. Prior to 2011, ten individuals of each species were analyzed annually from each lake, either individually or as a pooled sample. However, since 2011, twelve individuals have been analyzed as a pool (Table 1) (Bignert et al. 2013). A lower sampling frequency and sampling size than twelve individuals would result in a considerable decrease of statistical and interpretational power (Bignert et al. 1993). During 2001–2005, several of the collected samples were not analyzed but instead stored frozen at −20 or −80°C in the Environmental Specimen Bank (ESB). Samples from nine of the 32 lakes were analyzed annually for PCBs since 2007 (Lake Abiskojaure, Lake Bolmen, Lake Horsan, Lake Krankesjön, Lake Skärgölen, Lake Stensjön, Lake Tjulträsk, Lake Storvindeln, and Lake Svartsjön), with the exception of Lake Svartsjön, which was analyzed annually since 2011. Of these nine lakes, six have been analyzed for more than 10 years (Table 1). The rest of the 32 lakes have been analyzed for PCBs only one to three times since sampling started.

Sample preparation and registered variables

For each fish, total body weight, body length, total length (body length plus the tail fin), sex, age, gonad weight, liver weight, and sample weight were recorded (Nyberg et al. 2013). To avoid surface contamination and to obtain a sample consisting of only muscle tissue, the epidermis and subcutaneous fatty tissue were carefully removed before the muscle tissue was excised. Muscle samples were taken from the middle dorsal muscle layer (TemaNord 1995). For the individual analyses, 10 g of muscle was taken from each fish; for the pooled samples, 1 g of muscle was taken from each fish (in total 10–12 g in each pool). The sampling and sample preparations were all performed according to the manual for collection, preparation, and storage of fish (SMNH 2012).

Chemical analysis

Samples were extracted using a mixture of polar and non-polar solvents. The lipid content of the organic phase was determined gravimetrically. After clean-up of the dissolved lipid extracts using concentrated sulfuric acid, the samples were analyzed on a gas chromatograph equipped with a μ-electron capture detector and two 60 m columns with different polarity used in parallel (Jensen et al. 1983; Eriksson et al. 1997). One internal laboratory reference material (LRM) of muscle from fish was used at every extraction event since 1994. Four different materials have been used during this period with lipid content from 0.54 to 5.9 %. Within-laboratory reproducibility was calculated from the LRMs for more than 8000 PCB values for all analyzed congeners, and

Table 1 Sampling sites, coordinates, species and number of years analyzed for PCBs (individual congeners) within the Swedish National Monitoring Program for Contaminants in Freshwater Biota. The first column refers to the sampling site numbers in Fig. 1

N in map	Lake	Latitude	Longitude	Species	Years analyzed for PCBs
1	Abiskojaure	68.31°	18.65°	Arctic char	81[a], 86–98[a], 00[a], 07[a], 08[b], 09–10[c], 11–12[d]
2	Tjulträsk	65.96°	16.07°	Arctic char	86–87[a], 95[a], 06[b], 08–10[c], 11–12[d]
3	Storvindeln	65.70°	17.13°	Pike	85–00[a], 05[a], 06[b], 07[a], 08[b], 09[c], 10[b], 11–12[d]
4	Bränträsket	65.53°	21.42°	Perch	07[b]
5	Remmarsjön	63.86°	18.27°	Perch	00[a], 05[a], 07[b]
6	Degervattnet	63.87°	16.23°	Perch	05[a], 07[b]
7	Stor-Björsjön	63.61°	12.23°	Arctic char	07[b]
8	Stor-Backsjön	62.68°	14.51°	Perch	07[b]
9	Stensjön	61.64°	16.58°	Perch	97–00[a], 05[a], 06[b], 07[a], 08[b], 09[c], 10[b], 11–12[d]
10	Gipsjön	60.65°	13.63°	Perch	07[b]
11	Spjutsjön	60.64°	15.45°	Perch	07[b]
12	Övre Skärsjön	59.84°	15.55°	Perch	05[a], 07[b]
13	Limmingsjön	59.59°	14.51°	Perch	07[b]
14	Fysingen	59.57°	17.92°	Perch	07[b], 10[b], 11–12[e]
15	Tärnan	59.56°	18.37°	Perch	05[a], 07[b]
16	Bysjön	59.30°	12.34°	Perch	05[a], 07[b]
17	Stora Envättern	59.11°	17.35°	Perch	00[a], 05[a], 07[b]
18	Älgsjön	59.09°	16.37°	Perch	07[b]
19	Svartsjön	58.76°	14.22°	Perch	07[b], 11–12[d]
20	Fräcksjön	58.15°	12.18°	Perch	07[b]
21	Bästeträsk	57.92°	18.94°	Perch	07[b]
22	Allgjuttern	57.95°	16.10°	Perch	07[b]
23	Horsan	57.87°	18.84°	Perch	07[a], 08[b], 09[c], 10[b], 11–12[d]
24	Skärgölen	57.78°	15.58°	Perch	99[a], 06[b], 08[b], 09–10[c], 11–12[d]
25	Lilla Öresjön	57.56°	12.34°	Perch	07[b]
26	Fiolen	57.09°	14.53°	Perch	07[b]
27	Hjärtsjön	57.05°	15.26°	Perch	00[a], 05[a], 07[b]
28	Bolmen	56.97°	13.77°	Pike	88–00[a], 05[a], 06[b], 07[a], 08[b], 09–10[c], 11–12[d]
29	Stora Skärsjön	56.67°	13.07°	Perch	97–98[a], 07[b]
30	Sännen	56.33°	15.36°	Perch	07[b]
31	Krankesjön	55.70°	13.47°	Perch	07[a], 08[b], 09[c], 10[b], 11–12[d]
32	Krageholmsjön	55.50°	13.75°	Perch	07[b]

[a] 10 individual specimens per year, minor deviations occur

[b] 1 pool per year of 10 individuals

[c] 2 pools per year of 10 individuals per pool

[d] 2 pools per year of 12 individuals per pool

[e] 1 pool per year of 12 individuals

resulted in a reproducibility of 14 % for all reported PCB congener values between 2 and 50 ng g^{-1} lipid weight (l.w.) and 8 % for values above 50 ng g^{-1} l.w. The laboratory has participated in the periodic QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency testing since 1993, with around 95 % of all reported values being within ±2 standard deviations of the assigned value. The quantification limit (defined as ten times the standard deviation of the measured concentration as the concentration approaches zero) is estimated to approximately 2 ng g^{-1} l.w. for all discussed PCB congeners.

Statistical analysis and maps

For the temporal trend analysis, log-linear regression was performed for the entire investigated period and for the most recent 10 years using the yearly geometric mean values. In cases where the regression line had a poor fit, a

Table 2 Arithmetic mean age, length, weight and extractable muscle fat content, ±95 % confidence intervals (CI) for samples within the Swedish National Monitoring Program in Freshwater Biota. For perch, the arithmetic mean value for 2007–2012 is presented. For Arctic char and pike, the arithmetic mean value for the whole monitoring period is presented. Presented in alphabetical order by lake

Lake	Species	Age (years) 95 % CI	Length (cm) 95 % CI	Weight (g) 95 % CI	Fat content (%) 95 % CI
Allgjuttern	Perch	4.6 (4.4–4.9)	18.0 (17.6–18.4)	56.6 (51.7–61.4)	0.54 (0.39–0.70)
Abiskojaure	Arctic char	5.1 (5.0–5.3)	26.9 (26.4–27.4)	216 (203–230)	1.7 (1.5–1.8)
Bolmen	Pike	5.2 (5.0–5.3)	54.1 (53.3–54.9)	1080 (1040–1120)	0.55 (0.54–0.57)
Brännträsket	Perch	7.6 (7.2–8.1)	18.7 (18.3–19.1)	68.1 (63.3–72.8)	0.62 (0.28–0.96)
Bysjön	Perch	5.4 (5.1–5.7)	17.3 (17.0–17.6)	52.9 (49.8–56.0)	0.68 (0.36–0.99)
Bästeträsk	Perch	3.9 (3.7–4.1)	17.5 (17.3–17.8)	53.4 (51.2–55.6)	0.70 (0.37–1.0)
Degervattnet	Perch	5.5 (5.2–5.8)	18.3 (18.0–18.6)	63.8 (60.6–67.0)	0.48 (0.31–0.65)
Fiolen	Perch	4.4 (4.2–4.7)	18.0 (17.6–18.3)	61.5 (57.5–65.5)	0.60 (0.43–0.76)
Fräcksjön	Perch	5.3 (5.0–5.5)	17.1 (16.8–17.4)	51.9 (48.3–55.5)	0.58 (0.43–0.76)
Fysingen	Perch	4.7 (4.4–4.9)	17.1 (16.8–17.4)	53.4 (50.0–56.8)	0.54 (0.46–0.62)
Gipsjön	Perch	6.4 (6.2–6.6)	17.3 (17.0–17.7)	56.1 (52.7–59.5)	0.52 (0.41–0.62)
Hjärtsjön	Perch	3.6 (3.4–3.7)	18.7 (18.3–19.0)	68.7 (64.9–72.6)	0.76 (0.44–1.1)
Horsan	Perch	5.3 (5.1–5.6)	19.0 (17.6–20.4)	58.4 (55.6–61.3)	0.68 (0.62–0.74)
Krageholmsjön	Perch	2.5 (2.3–2.7)	17.5 (16.9–18.1)	70.6 (61.0–80.2)	0.63 (0.49–0.77)
Krankesjön	Perch	3.1 (3.0–3.3)	19.5 (16.5–22.5)	59.6 (56.6–62.6)	0.50 (0.45–0.56)
Lilla Öresjön	Perch	5.6 (5.3–5.9)	18.3 (18.0–18.7)	63.5 (59.2–67.8)	0.70 (0.37–1.02)
Limmingsjön	Perch	4.9 (4.7–5.2)	17.5 (17.2–17.9)	55.8 (52.7–58.9)	0.65 (0.54–0.76)
Remmarsjön	Perch	6.8 (6.5–7.2)	18.9 (18.4–19.4)	72.7 (67.3–78.0)	0.66 (0.53–0.78)
Skärgölen	Perch	4.7 (4.5–5.0)	17.8 (16.9–18.7)	59.0 (54.8–63.1)	0.68 (0.64–0.72)
Spjutsjön	Perch	3.9 (3.7–4.1)	18.2 (17.9–18.4)	62.0 (59.1–64.9)	0.72 (0.52–0.92)
Stora Envättern	Perch	5.3 (5.1–5.6)	16.8 (16.5–17.2)	47.8 (44.2–51.4)	0.62 (0.30–0.93)
Stensjön	Perch	7.4 (7.0–7.7)	19.0 (18.3–19.7)	63.3 (61.1–65.6)	0.60 (0.54–0.65)
Stora Skärsjön	Perch	6.3 (6.1–6.6)	16.4 (16.2–16.6)	46.3 (44.4–48.2)	0.56 (0.45–0.68)
Stor–Backsjön	Perch	6.5 (6.2–6.7)	18.4 (18.1–18.6)	63.8 (61.1–66.5)	0.65 (0.47–0.82)
Stor–Björsjön	Arctic char	5.6 (4.5–6.7)	26.3 (25.6–27.1)	160 (146–175)	1.0 (0.46–1.6)
Storvindeln	Pike	5.8 (5.6–5.9)	61.3 (60.7–62.0)	1630 (1570–1680)	0.58 (0.57–0.60)
Svartsjön	Perch	7.3 (6.5–8.1)	15.6 (14.7–16.5)	39.1 (31.0–47.1)	0.70 (0.59–0.80)
Sännen	Perch	5.7 (5.5–6.0)	17.0 (16.7–17.3)	47.5 (44.1–51.0)	0.59 (0.39–0.79)
Tjulträsk	Arctic char	5.2 (5.1–5.3)	26.9 (26.3–27.6)	209 (193–225)	1.5 (1.3–1.6)
Tärnan	Perch	5.9 (5.6–6.2)	17.7 (17.3–18.1)	58.1 (53.1–63.0)	0.60 (0.50–0.69)
Älgsjön	Perch	5.8 (5.4–6.1)	16.7 (16.3–17.0)	46.1 (43.4–48.8)	0.69 (0.47–0.90)
Övre Skärsjön	Perch	6.4 (6.1–6.7)	18.5 (18.2–18.8)	64.6 (61.1–68.1)	0.63 (0.58–0.67)

3-point running mean smoother was checked for statistical significance in comparison to the regression using ANOVA (Nicholson et al. 1998). Potential outliers in the temporal trends were detected as described in Hoaglin and Welsch (1978). Suspected outliers are indicated in the figures but were included in the statistical calculations. Values below level of quantification (LOQ) were replaced by LOQ divided by the square root of 2 prior to all statistical analyses. Power was fixed to 80 %. The minimum possible trend that could be detected during a 10-year monitoring period at a significance level of 5 % was estimated and power analysis was also carried out. A significance level of 5 % was used for all tests.

Spatial differences in PCB concentrations were evaluated using bar maps. The height of the bars represents the arithmetic mean for 2007–2012, or shorter if results were not available. Principal component analysis (PCA) was performed on the proportions of the individual PCB-congener concentrations to the ΣPCBs to study differences in the species congener patterns and differences due to latitude. The percentage of each PCB-congener relative to the sum of congeners was calculated and log-transformed prior to PCA analysis. Before the PCA-scores were plotted they were centered and scaled to 100 %. Hotelling's T^2 test was used to check for possible significant differences in congener patterns. The statistical trend analysis and the bar maps were

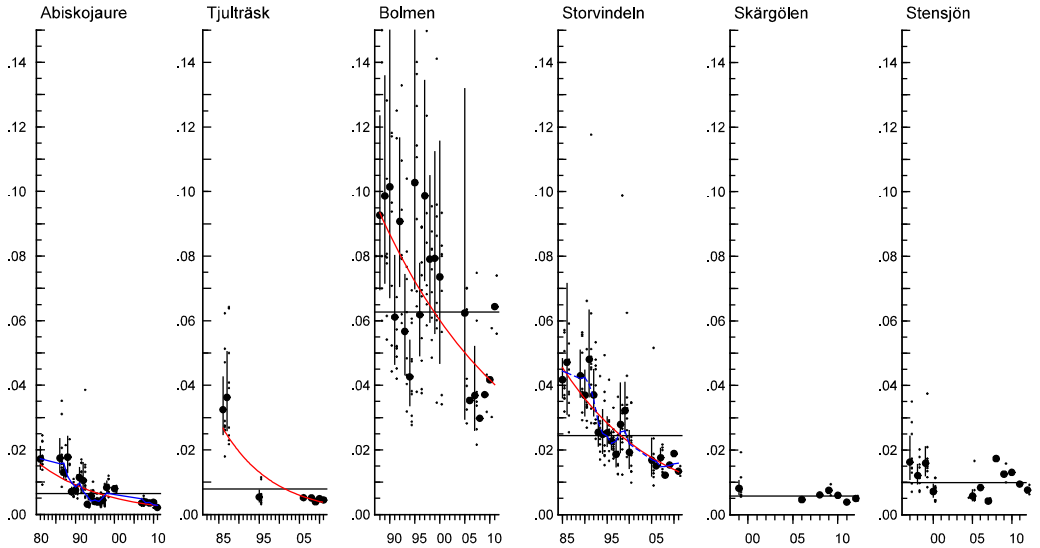

Fig. 2 Log-linear trends of CB-118 (ug g^{-1} lipid weight) in Arctic char muscle from Lake Abiskojaure and Lake Tjulträsk; in pike muscle from Lake Bolmen and Lake Storvindeln; and in perch muscle from Lake Skärgölen and Lake Stensjön (time series starting in 1981, 1986, 1988, 1985, 1997 and 1999, respectively). The *red lines* show a significant trend over the whole period and for the ten last years. The *dark blue lines* indicate non-linear trends ($0.05 < p < 0.1$). The *black horizontal line* shows the mean concentration over the whole period. Each figure displays the geometric mean concentration of each year (*circles*) together with the individual analyses (*small dots*) and the 95 % confidence intervals of the geometric means

performed for the dl-PCB, CB-118 (2,3′,4,4′,5-pentachlorobiphenyl), and for the non dl-PCB CB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), both of which are dominant congeners in fish, as well as the ratio between the more easily degradable CB-101 (2,2′4,5,5′-pentachlorobiphenyl) and the more stable CB-153. CB-28 (2,4,4′-trichlorobiphenyl) was included in the bar maps to show a divergent pattern. In the PCA analysis, CB-118 was chosen as the only dl-PCB, while CB-101, CB-153 and CB-180 (2,2′3,4,4′,5,5′-heptachlorobiphenyl) were chosen as representing PCBs with different degrees of chlorination. All statistics are based on lipid normalized values. Statistical software PIA (www.amap.no) was used for the trend analysis and the PCAs. All the results for perch (except within the PCA) are based on data from 2007 to 2012 because data are most complete during that period, while for Arctic char and pike, the results are based on data for the whole monitoring period.

Environmental assessment criteria

In accordance with the Marine Strategy Framework Directive 2008/56/EC (MSFD), Good Environmental Status (GES) is defined as "concentrations of contaminants at levels not giving rise to pollution effects." To determine GES, a number of target levels have been established representing a threshold that should not be exceeded. These target levels should protect the most sensitive organisms from the harmful effects of hazardous substances and have been developed within several groups or conventions e.g., Environmental Quality Standards (EQS) developed within the EC to evaluate GES (2008/105/EC), and the Environmental Assessment Criteria (EAC), developed within OSPAR (OSPAR 2009). For PCBs, EQSs are not established, so to evaluate concentrations of CB-118 and CB-153, OSPAR EACs are used in this study, and are 1.6 and 0.024 ug g^{-1} l.w., respectively (OSPAR 2009).

RESULTS

Biological variables

Arithmetic mean weight, age, length and muscle fat content are presented for samples from all lakes within the SNMPFB (Table 2). Perch size was similar among the lakes because they are chosen to be of similar size. By contrast, the age difference was large (2.5–7.6 years) between the lakes, with perch from the north being older. This is expected because fish grow more slowly in colder climates.

For Arctic char, both the age and total length were very similar between the three sampled lakes. For pike,

Table 3 Lake, species, contaminant, the annual percentage change ±95 % confidence interval (CI), r^2 reporting the coefficient of determination, p value (significant at <0.05), the lowest detectable change (% per year) for a ten year period with the current between year variation at a power of 80 %, coefficient of variation (CV) around the regression line as a measure of between-year variation, and the number of years required to detect a trend of 10 % at a power of 80 % for CB-118, CB-153 and the ratio between CB-101:CB-153 in Arctic char, pike and perch (muscle). Data for the whole monitoring period are presented in the first row and for the most recent 10 years (2003–2012) in the lower row for each lake. Presented in order of contaminant

Lake	Species	Contaminant	Annual % change (95 % CI)	r^2	p value	Lowest detectable change (%)	CV (%)	N years required to detect a trend of 10 %
Abiskojaure	Arctic char	CB-118						
1981–2012			−5.8 (−7.8, −3.9)	0.67	<0.001	14	40	13
2003–2012			−10 (−22, 2.2)	0.57	0.084	6.7	19	9
Tjulträsk	Arctic char	CB-118						
1986–2012			−7.8 (−11, −4.7)	0.83	<0.001	15	40	13
2003–2012			−5.0 (−12, 1.5)	0.54	0.098	4	11	7
Bolmen	Pike	CB-118						
1988–2012			−4.2 (−6.0, −2.4)	0.55	<0.001	12	32	11
2003–2012			−4.3 (−18, 9.5)	0.08	0.48	14	38	12
Storvindeln	Pike	CB-118						
1985–012			−4.9 (−6.0, −3.9)	0.83	<0.001	6.9	19	9
2003–2012			−4.4 (−12, 2.7)	0.28	0.18	6.9	19	9
Skärgölen	Perch	CB-118						
1999–2012			−3.6 (−9.1, 2.0)	0.35	0.16	8.4	24	10
2003–2012			−2.0 (−17, 13)	0.03	0.72	9.4	26	10
Stensjön	Perch	CB-118						
1997–2012			−2.1 (−7.8, 3.6)	0.06	0.44	17	48	14
2003–2012			6.7 (−11, 24)	0.13	0.39	18	49	14
Abiskojaure	Arctic char	CB-153						
1981–2012			−4.4 (−5.9, −2.9)	0.64	<0.001	11	31	11
2003–2012			−8.7 (−13, −4.8)	0.87	<0.01	3.2	9.0	6
Tjulträsk	Arctic char	CB-153						
1986–2012			−7.2 (−10, −4.5)	0.85	<0.001	13	36	12
2003–2012			−2.5 (−9.4, 4.6)	0.20	0.38	4.4	13	7
Bolmen	Pike	CB-153						
1988–2012			−1.2 (−3.2, 0.74)	0.08	0.33	12	34	12
2003–2012			−0.1 (−14, 14)	0.00	0.95	14	39	13
Storvindeln	Pike	CB-153						
1985–012			−3.6 (−4.6, −2.6)	0.73	<0.001	6.9	19	9
2003–2012			−4.7 (−9.5, 0.18)	0.48	0.055	4.6	13	7
Skärgölen	Perch	CB-153						
1999–2012			−1.6 (−5.2, 2.0)	0.21	0.30	5.4	15	8
2003–2012			−2.6 (−12, 6.9)	0.13	0.49	5.9	17	8
Stensjön	Perch	CB-153						
1997–2012			0.57 (−5.9, 7.0)	0.00	0.83	19	55	15
2003–2012			12 (−6.3, 31)	0.30	0.16	19	52	25
Abiskojaure	Arctic char	CB-101/CB-153						
1981–2012			−2.4 (−3.4, −1.5)	0.59	<0.001	6.7	19	9
2003–2012			2.6 (−4.6, 9.8)	0.15	0.40	6.0	17	8
Tjulträsk	Arctic char	CB-101/CB-153						
1986–2012			−0.98 (−2.0, 0.052)	0.42	0.058	4.6	13	7
2003–2012			0.37 (−5.9, 6.7)	0.00	0.85	3.9	11	7
Bolmen	Pike	CB-101/CB-153						
1988–2012			−2.5 (−3.2, −1.7)	0.71	<0.001	4.6	13	7

Table 3 continued

Lake	Species	Contaminant	Annual % change (95 % CI)	r^2	p value	Lowest detectable change (%)	CV (%)	N years required to detect a trend of 10 %
2003–2012			−2.7 (−8.4, −2.9)	0.19	0.28	5.4	15	8
Storvindeln	Pike	CB-101/CB-153						
1985–012			−2.0 (−2.7, 1.3)	0.63	<0.001	4.8	13	7
2003–2012			−0.41 (−2.2, 1.4)	0.04	0.61	1.7	4.9	5
Skärgölen	Perch	CB-101/CB-153						
1999–2012			−1.9 (−5.4, 1.5)	0.29	0.21	5.1	14	7
2003–2012			0.60 (−7.8, 9.0)	0.01	0.83	5.2	15	8
Stensjön	Perch	CB-101/CB-153						
1997–2012			−2.5 (−4.1, −0.97)	0.57	<0.01	4.4	12	7
2003–2012			−4.9 (−9.4, −0.34)	0.54	<0.05	4.3	12	7

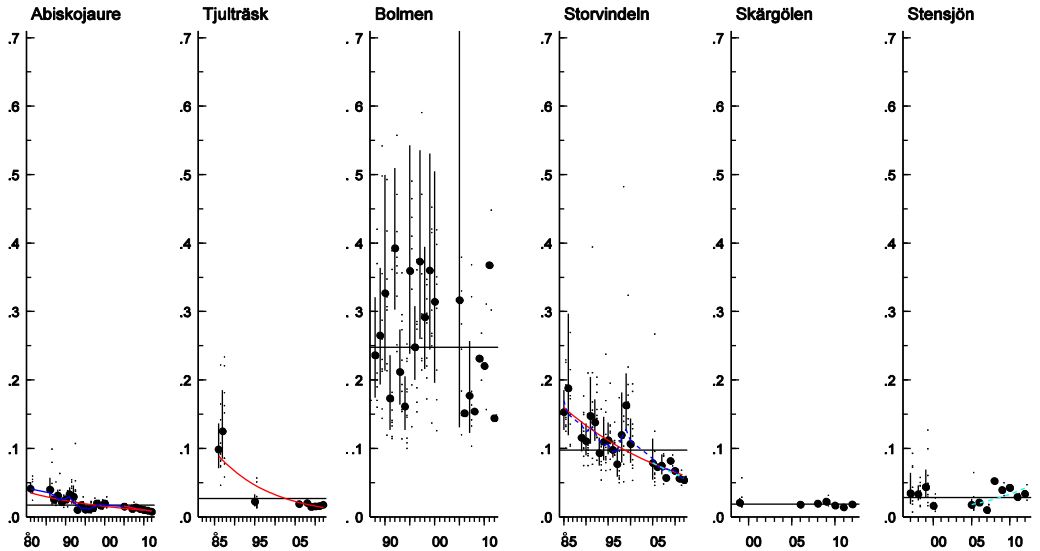

Fig. 3 Log-linear trends of CB-153 (ug g^{-1} lipid weight) in Arctic char muscle from Lake Abiskojaure and Lake Tjulträsk; in pike muscle from Lake Bolmen and Lake Storvindeln; and in perch muscle from Lake Skärgölen and Lake Stensjön (time series starting in 1981, 1986, 1988, 1985, 1997 and 1999, respectively). The *red lines* show significant linear trends over the whole period. The *light blue dotted line* indicates a trend for the last ten years ($0.05 < p < 0.2$). The *dark blue lines* indicate non-linear trends ($0.05 < p < 0.1$) and the *black horizontal line* the mean concentration over the whole period. Each figure displays the geometric mean concentration of each year (*circles*) together with the individual analyses (*small dots*) and the 95 % confidence intervals of the geometric means

specimens from Lake Storvindeln were somewhat larger and less lean than pike from Lake Bolmen.

Temporal trends

In perch muscle samples, no trends were seen for dl CB-118 in either sampled lake, but significant decreasing trends were seen for both Arctic char and pike muscle samples, of between 4.2 and 7.8 % per year (Fig. 2; Table 3). As with CB-118, no trends were seen for CB-153 in perch muscle from Lake Skärgölen or Lake Stensjön over the whole period (Fig. 3), but the statistical power to detect trends was relatively low. Concentrations of CB-153 in Arctic char decreased significantly in both lakes (between 4.4 and 7.2 % per year). CB-153 in pike showed no trend in Lake Bolmen, but decreased significantly in Lake Storvindeln (3.6 % per year) (Fig. 3; Table 3).

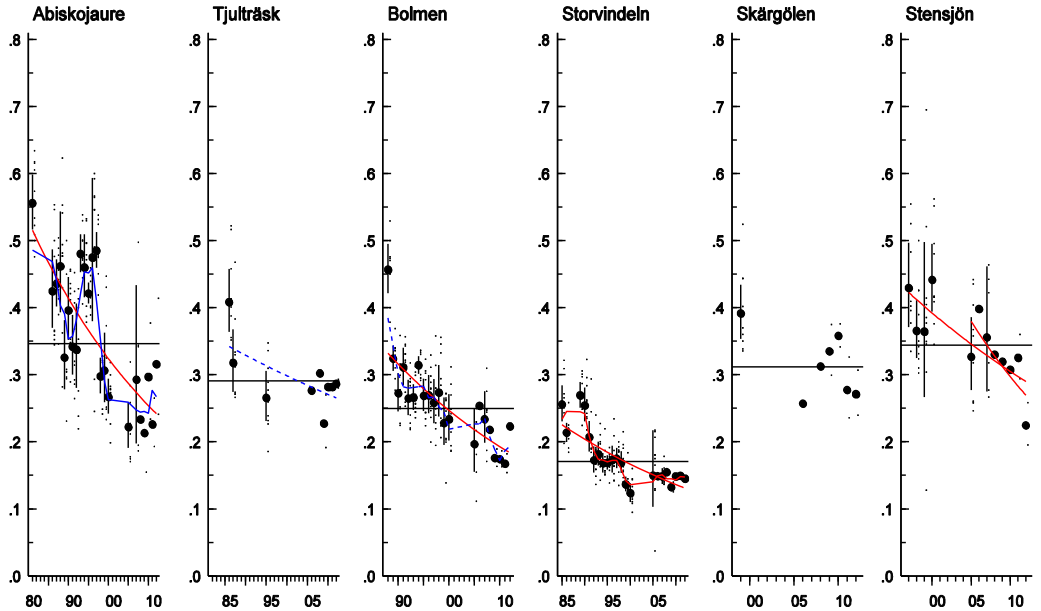

Fig. 4 Log-linear trends of the CB-101/CB-153-ratio in Arctic char muscle from Lake Abiskojaure and Lake Tjulträsk; in pike muscle from Lake Bolmen and Lake Storvindeln; and in perch muscle from Lake Skärgölen and Lake Stensjön (time series starting in 1981, 1986, 1988, 1985, 1997 and 1999, respectively). The *red linear and non-linear lines* show a significant trend over the whole period and for the ten last years. The *dark blue lines* indicate non-linear trends ($0.05 < p < 0.1$). The *black horizontal line* shows the mean concentration over the whole period. Each figure displays the geometric mean concentration of each year (*circles*) together with the individual analyses (*small dots*) and the 95 % confidence intervals of the geometric means

The number of years to detect a significant annual change of 10 % with 80 % statistical power for CB-153 and CB-118 varied: 11–13 years in Arctic char, 9–12 years in pike and 8–15 years in perch (Table 3). The statistical power to detect an annual change of 10 % was very close to 100 % in both the pike and Arctic char time series from Lake Abiskojaure for the entire period (not presented). Statistical power for the shorter perch time series and the Arctic char series from Lake Tjulträsk, which have fewer data points, varied between 37 and 77 %.

The ratio between the penta- and hexa-PCBs, as illustrated by CB-101/CB-153 (Fig. 4) has decreased over time in most lakes (between 2.0 and 2.5 % per year), with the exception of Lake Skärgölen and Lake Tjulträsk, where there are few data points.

Spatial patterns

For both CB-153 and CB-118 (Fig. 5a, b) concentrations in perch muscle were lowest in lakes in the north of Sweden, highest around urban centres of the three largest cities in Sweden, Stockholm, Gothenburg and Malmö, as well as in Karlskrona, an old naval city. Congener pattern differed between lakes. Lake Fysingen stands out with relatively high concentrations of the low chlorinated CB-28 (Fig. 5c). A similar pattern was observed when looking at the spatial distribution of CB-101/CB-153. Lower ratios were observed in the rural areas and higher ratios were observed in more densely populated regions (Fig. 5d).

Clear differences in congener pattern were seen between Arctic char and pike from the northern regions of Sweden (Fig. 6a). Pike generally has higher relative concentrations of CB-180 compared to the other two species, while Arctic char has relatively higher concentrations of CB-101. Perch from northern Sweden had a congener pattern between pike and Arctic char, and was significantly different from both of those species ($p < 0.05$, Hotelling's T^2 test).

Target levels

In all lakes and species, CB-153 concentration is below the OSPAR EAC of 1.6 ug g^{-1} l.w. (Figs. 3, 5a). The EAC for CB-118 of 0.024 ug g^{-1} lipid weight was exceeded in pike from Lake Bolmen and perch from Lake Krankesjön, Lake

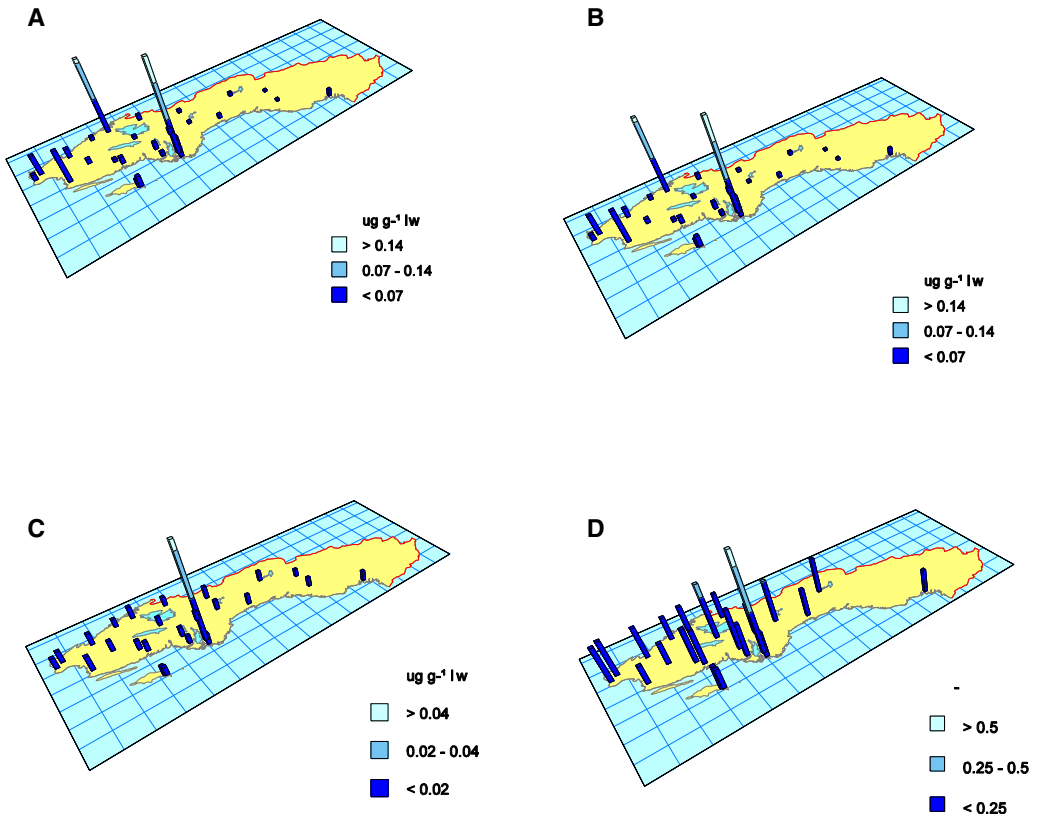

Fig. 5 Spatial variation in concentration (ug g^{-1} lipid weight) in freshwater perch muscle of **a** CB-153 **b** CB-118, **c** CB-28 and **d** the ratio between CB-101/CB-153; arithmetic mean values from 2007 to 2012

Sännen, Lake Fräcksjön, Lake Tärnan and Lake Fysingen (Figs. 2 and 5b) in 2007–2012.

DISCUSSION

The concentrations of PCBs, illustrated here by CB-118 and CB-153, were generally decreasing by about 3–8 % per year in both pike and Arctic char. No trend was observed for the perch time series, but this is most likely due to the short duration of these time series and because monitoring in perch started after the steep decrease, during the 1980s and 1990s, observed for pike and Arctic char. Decreasing trends for PCBs in biological samples of similar magnitudes have been reported in studies from the Baltic Sea (Bignert et al. 2013; Miller et al. 2013) and other countries (e.g., Braune et al. 2005; Ryan et al. 2005; Helgason et al. 2008; Rigét et al. 2010). In a review on temporal trends of PCBs in arctic biota, Rigét et al. (2010) found a mean annual decrease for CB-153 of 1.2 % based on all the time series analyzed in the review (40 in total), which was somewhat lower than the decrease found in the time series here. A number of the time series presented in Rigét et al. (2010) started in the 1990s, later than the time series of our study, the concentrations were lower and the decrease less steep, which might explain the different results and the mean power in our time series were also considerably higher, 71 % compared to 28 % in Rigét et al. (2010).

The decrease over time, seen for concentrations of PCBs in both freshwater and marine fish in Sweden, mirrors the measures taken (e.g., bans and restrictions) to reduce PCBs in the environment. Gewurtz et al. (2010) found similar results as in our study for $\sum$PCB, a steep decrease in the 1970s and 1980s, which levelled out in the mid-1990s, most probably as a result of bans and restrictions.

The ratio between the penta- (CB-101) and hexa-PCBs (CB-153) has decreased over time in most lakes. This

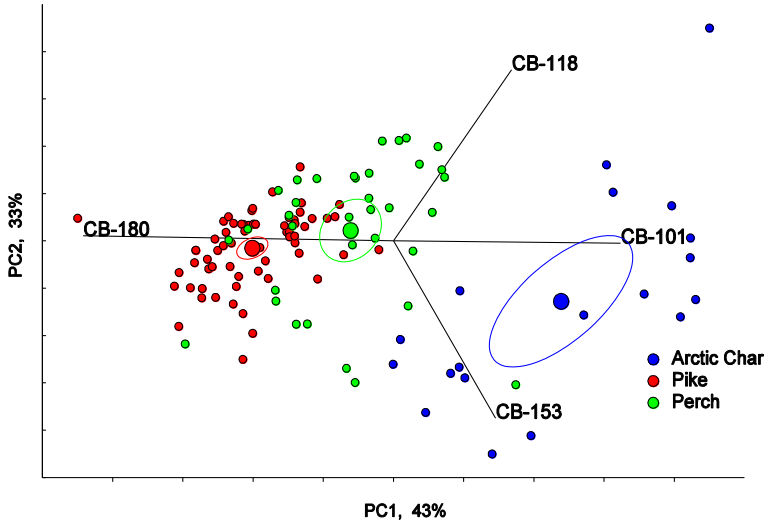

Fig. 6 PCA (principal component analysis), biplot and Hotellings 95 % confidence ellipses for center of gravity for each group. The figure shows PCB congeners (CB-101, CB-118, CB-153 and CB-180) in Arctic char, pike and perch (1997–2012) from the northern parts of Sweden, north between 61.00° and 69.00° latitude

decrease was expected due to a higher degree of volatilization and degradation of lower chlorinated PCBs. A decreasing ratio indicates that a temporal removal from the source has occurred. When locally contaminated lakes are identified, an increase in this ratio can be observed. During the last decade, the decrease appears to have levelled out at Lake Storvindeln and Lake Abiskojaure, which might indicate some change in PCB source in these areas. It is important that further studies are conducted to monitor the ratio of higher and lower chlorinated PCBs, as this may indicate releases of PCBs from new sources.

The concentrations of most PCBs (shown by CB-153 and CB-118) differ among lakes. It appears that lakes in the vicinity of urban and/or industrial areas (e.g., Lake Fysingen close to Arlanda airport and Lake Sännen close to the old naval city of Karlskrona), appear to be at greater risk of higher concentrations of PCBs compared to lakes in rural, less densely populated regions. Lake Fysingen stands out with not just high concentrations of CB-153 and CB-118, but also a high ratio of CB-101/CB-153 and relatively high concentrations of the lower chlorinated CB-28, indicating a new input of PCB. This needs to be investigated further to find out if this implies exposure from a new source or changes in land use around the lake. Turrio-Baldassarri et al. (1997) found that a relation between a persistent and a less persistent CB-congener could indicate new contamination using the ratio between CB-149/CB-153 in cow milk.

The principal component analysis on perch, pike, and Arctic char from the northern parts of Sweden showed clear differences in congener pattern between Arctic char and pike, which might be explained by differences in diet, uptake of contaminants and metabolism. Perch also differed from both pike and Arctic char, but were more similar to pike. Babut et al. (2012) found that fish ecological traits are important factors that could explain differences in the bioaccumulation of PCBs when examining 2848 samples of 36 freshwater fish species from approximately 300 sites in France. However, Babut et al. (2012) also found that congener pattern in different fish species was more related to their physiology and metabolism than to their ecological traits. Arctic char in our study showed a lot of within-species variance, which could be expected because this species varies to a great extent both between and within lakes, likely because these species are present in more lake habitats and feed at more variable trophic levels compared with the other two species (Kullander et al. 2012).

The EAC for CB-118 was exceeded in pike from Lake Bolmen and perch from Lake Krankesjön, Sännen, Fräcksjön, Tärnan and Fysingen, which shows that the levels were still too high in some parts of the freshwater environment to protect the most sensitive organisms. Fish from Latvian lakes (including perch) show similar levels to that observed here for CB-118, with a mean value of 0.026 ug g^{-1} l.w. (recalculated from fresh weight to lipid

weight basis) in fish muscle, which also exceeds the EAC (Zacs et al. 2013).

After >40 years of freshwater monitoring, some valuable lessons have been learned for developing the monitoring design of POPs. These include:

- Examination of individual congeners rather than only looking at the summed concentrations of a substance.
- The importance of annual monitoring.
- The choice of monitoring species, organ and selection of samples concerning e.g., age, sex, size, and sampling season.
- The importance of using the same laboratory for contaminant monitoring for temporal trend studies.

With a well-designed monitoring programme, small changes over time and space can be detected faster and this allows for focused remediation and policy efforts in specifically identified areas.

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Temporal and spatial trends of PCBs, DDTs, HCHs, and HCB in Swedish marine biota 1969–2012

Elisabeth Nyberg, Suzanne Faxneld, Sara Danielsson, Ulla Eriksson, Aroha Miller, Anders Bignert

Abstract In the 1960s, the Baltic Sea was severely polluted by organic contaminants such as PCBs, HCHs, HCB, and DDTs. Elevated concentrations caused severe adverse effects in Baltic biota. Since then, these substances have been monitored temporally and spatially in Baltic biota, primarily in herring (*Clupea harengus*) and in guillemot (*Uria aalge*) egg, but also in cod (*Gadus morhua*), perch (*Perca fluviatilis*), eelpout (*Zoarces viviparous*), and blue mussel (*Mytilus edulis*). These chemicals were banned in Sweden in the late 1970s/early 1980s. Since the start of monitoring, overall significant decreases of about 70–90 % have been observed. However, concentrations are still higher in the Baltic Sea than in, for example, the North Sea. CB-118 and DDE exceed the suggested target concentrations (24 μg kg^{-1} lipid weight and 5 μg kg^{-1} wet weight, respectively) at certain sites in some of the monitored species, showing that concentrations may still be too high to protect the most sensitive organisms.

Keywords Ecotoxicology · Baltic Sea biota · POPs · Monitoring

INTRODUCTION

In the 1960s, the Baltic Sea was found to be severely polluted by organic contaminants, e.g., polychlorinated biphenyls (PCBs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethane (DDT) including its metabolites (Jensen et al. 1969, 1972). High PCB and DDT concentrations most probably were major causes of severe reproduction problems in three Baltic seal species (*Halichoerus grypus*, *Pusa hispida*, and *Phoca vitulina*) as well as the white-tailed eagle (*Haliaeetus albicilla*) in the Baltic (Helle et al. 1976a, b; Helander et al. 2002; Bredhult et al. 2008). These discoveries led to the start of an environmental contaminant research program at the Swedish Museum of Natural History (SMNH). When a comprehensive national monitoring program for environmental quality was launched by the Swedish Environmental Protection Agency in 1980, SMNH was given the responsibility for monitoring of contaminants in marine biota (fish, blue mussel, and guillemot egg). The focus was not only to follow temporal trends of contaminants and to determine whether measures taken to reduce contaminant concentrations had an effect, but also to indicate large-scale spatial differences. Chemical analyses were carried out by the same laboratory during the whole study period. Results from this monitoring program for the period 1969–1995 were reported by Bignert et al. (1998). PCBs, HCHs, HCB, and DDTs are among the initial 12 persistent organic pollutants (POPs) included in The Stockholm Convention on POPs (UNEP 2008). They are all persistent, hydrophobic, bioaccumulative, and toxic, and can cause adverse effects in humans and wildlife (UNEP 2008). They are also widespread, being found in various matrices around the globe (Lohmann et al. 2007). The Stockholm Convention was adopted in 2001 and entered into force in 2004. In Sweden, these contaminants have been banned or their use restricted since the 1970s or the beginning of the 1980s.

Here we examine temporal, seasonal, and spatial relationships of PCBs (CB-153 for temporal, seasonal, and spatial trends; [the dioxin-like CB-118 congener for spatial trends]), DDTs [represented here by dichlorodiphenyldichloroethylene (DDE), by DDE we mean *p,p′*-DDE], HCB, and HCHs (α-, β-, and γ-HCH) in herring (*Clupea harengus*) muscle and guillemot (*Uria aalge*) eggs (temporal only). Concentrations of these chemicals in cod (*Gadus morhua*) liver, perch (*Perca*

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fluviatilis) muscle, eelpout (*Zoarces viviparous*) muscle, and blue mussel (*Mytilus edulis*) are included in the discussion to evaluate (1) concentrations over time in relation to imposed bans and restrictions; (2) concentration differences between sites; (3) compound profiles which could indicate new releases or aging of residues, e.g., DDT/DDE, patterns of α-, β-, γ-HCH; and (4) concentrations in relation to environmental target values set by EU and other international organizations.

MATERIALS AND METHODS

Studied species

Herring (*Clupea harengus*) is a pelagic species that feeds mainly on zooplankton (Casini et al. 2004). It is an important prey for several top predators, such as seals and guillemot (Lundin 2011), and by weight the dominant commercial fish species in the Baltic Sea. It is also one of the most used indicator species for contaminant monitoring in the Helsinki Commission (HELCOM) convention area.

European perch (*Perca fluviatilis*) is an opportunistic predator that undergoes an ontogenetic diet shift (Collette et al. 1977) and found in both fresh and brackish water (Baltic Sea) (Kullander et al. 2012).

Baltic cod (*Gadus morhua*) lives below the thermocline and feeds mainly on clupeids when adult (Pachur and Horbowy 2013).

Eelpout (*Zoarces viviparous*) is a relatively stationary, benthic species and feeds primarily on invertebrates and small fish (Ojaveer et al. 2004).

Blue mussel (*Mytilus edulis*) is a stationary filter feeder, found in a wide range of salinities and temperatures, and one of the most commonly used organisms for monitoring of contaminants in biota.

The guillemot (*Uria aalge*) is a piscivorous bird species with circumpolar distribution that in the Baltic feeds mainly on sprat (*Sprattus sprattus*) and herring (Österblom et al. 2001). It overwinters in the Baltic Sea, mainly in the southern Baltic Proper (Fransson et al. 2008), with Stora Karlsö being the largest breeding colony (Österblom et al. 2002). Since it is rather stationary, its contaminant load is locally acquired. Breeding starts in early May and normally only one lipid-rich (11–13 %) egg is laid. Late-laid replacement eggs tend to contain significantly higher concentrations of organochlorines (Bignert et al. 1995) and are consequently avoided in the present study.

The lipid content is an important co-variable for organic contaminants and varies among the sampled species: herring muscle 2–7 %, perch muscle 0.6–0.7 %, cod liver 16–71 %, eelpout muscle 0.4–0.8 %, blue mussel soft body 0.7–1.8 % (Bignert et al. 2014). Unlike, e.g., herring, the fat content in cod liver is highly variable.

Sampling sites and frequency

A total of 23 sampling sites were monitored for PCBs, DDTs, HCHs, and HCB within the Swedish National Monitoring Program for Contaminants in Marine Biota (Fig. 1). The year of first sampling differed among sites (Table S1 in Electronic Supplementary Material) but the last year of sampling is 2012 for all sites, except for eelpout from Holmöarna where sampling ended in 2007. Samples were taken annually, with a few exceptions. Sampling sites are located in areas where there are no known local sources of contamination and, as far as possible, uninfluenced by major rivers, ferry routes, or urban and industrial areas. The Swedish sampling stations are included in the network of HELCOM (Baltic Marine Environment Protection Commission–Helsinki Commission) stations in the Baltic and also in the Convention for the Protection of the Marine Environment of the North–East Atlantic (the OSPAR Convention) Joint Monitoring Programme (JMP) stations in the Kattegat/Skagerrak.

Sample preparation and registered variables

The specimens were collected and frozen as soon as practical, and then transported to the Environmental Specimen Bank (ESB) at the SMNH. All sampling and sample preparations were performed according to the manual for collection, preparation, and storage of fish (SMNH 2012).

Fish

For contaminants that bioaccumulate continuously, relatively young fish, 3–5 years, often give a more representative picture of the current contaminant load than adults. To minimize variation within and between years, relatively young fish of similar age and size (Table S1 in ESM) were collected annually and at the same time of year. Only healthy-looking specimens with undamaged skin were used. For each fish, total body weight, total length, sex, age, liver weight, and sample weight were recorded, as described in Bignert et al. (2014). Fish muscle samples were taken from the middle dorsal muscle layer (TemaNord 1995). For individual analyses, 10 g of muscle was taken. For pooled samples, 1 g of muscle was taken from each of 10–12 individuals fish (specified for each site in Table S1 in ESM). For cod liver, 5 g was taken from each fish.

Blue mussel

For each mussel, total shell length, shell weight, and soft body weight were registered. The whole soft bodies of 20 individuals were pooled, giving a final weight of c. 10 g.

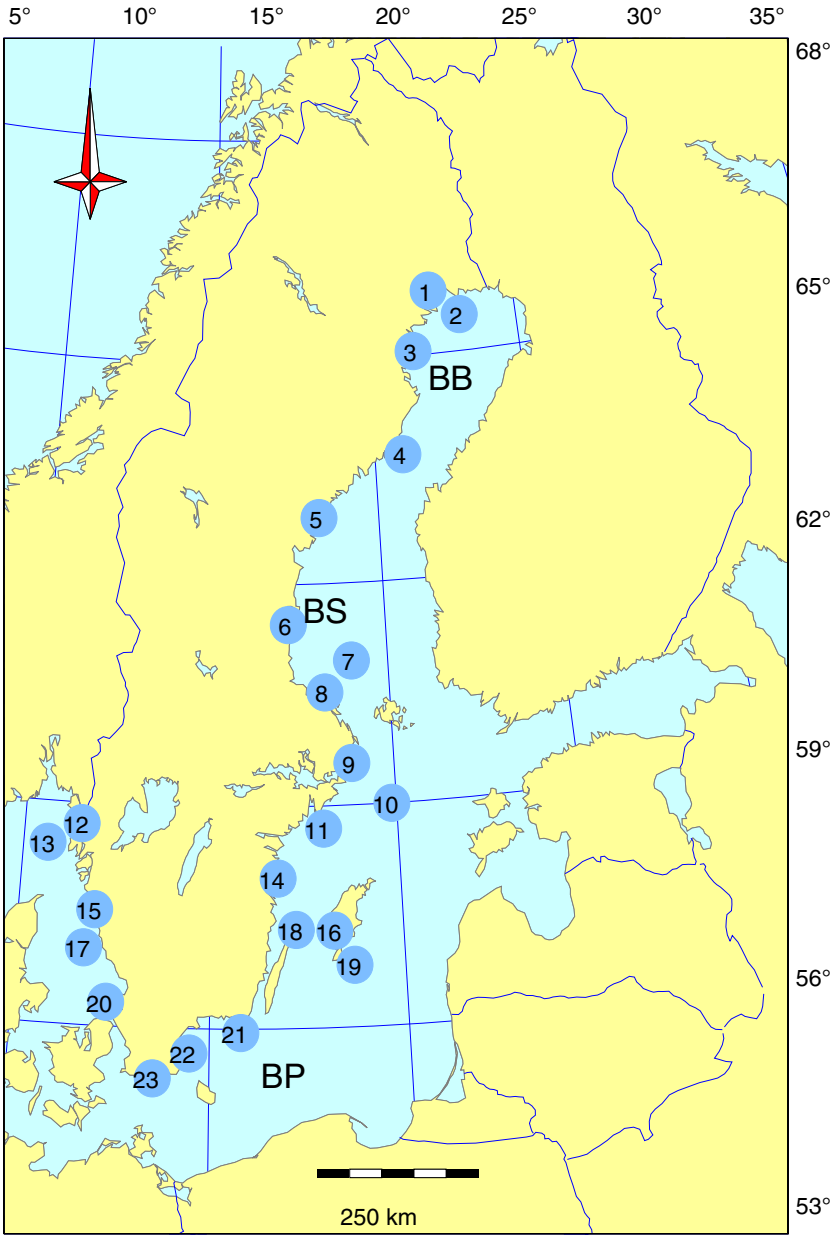

Fig. 1 Sampling sites within the Swedish National Monitoring Program for Contaminants in Marine Biota included in this study, *BB* Bothnian Bay, *BS* Bothnian Sea, and *BP* Baltic Proper. See Table S1 in ESM for information about the different sampling sites. 1 Råneфjärden, 2 Harufjärden, 3 Kinnbäcksfjärden, 4 Holmöarna, 5 Gaviksfjärden, 6 Långvindsfjärden, 7 Bothnian Sea, offshore site, 8 Ängskärsklubb, 9 Lagnö, 10 Baltic Proper, offshore site, 11 Landsort, 12 Fjällbacka, 13 Väderöarna, 14 Kvädöfjärden, 15 Nidingen, 16 Stora Karlsö, 17 Fladen, 18 Byxelkrok, 19 SE Gotland, 20 Kullen, 21 Utlängan, 22 Västra Hanöbukten, and 23 Abbekås

Guillemot egg

Guillemot egg contents were blown out through a hole drilled at the egg's equator. Egg length, width, total weight and weight of the empty, dried eggshell were recorded for each individual. Ten grams of homogenized egg content was prepared for analysis.

Chemical analyses

Samples were extracted by liquid–liquid extraction using a mixture of polar and non-polar solvents. The lipid content of the organic phase was determined gravimetrically. After clean-up of the dissolved lipid extracts using concentrated sulfuric acid, the samples were analyzed on a gas chromatograph equipped with a μ-electron capture detector (GC/ECD). Two 60-m columns with different polarities were used in parallel (Jensen et al. 1983; Eriksson et al. 1997). One internal laboratory reference material (LRM) of muscle from fish has been used at every extraction event since 1994. Five different materials have been used during this period with lipid contents from 0.54 to 11 %. Within-laboratory reproducibility was calculated from the LRMs for more than 8000 PCB and chlorinated pesticide values for all analyzed congeners and isomers. This resulted in a reproducibility of 14 % for HCB and all discussed PCB congener values ranging from 2 to 50 ng g^{-1} lipid weight and 8 % for values above 50 ng g^{-1} lipid weight, while corresponding values for HCH were 13 and 8 %, respectively and for *p*,*p*′-DDE, 19 and 11 %, respectively. Since 1993, the laboratory has participated in the periodic QUASIMEME proficiency testing with around 95 % of all reported values being within ±2 standard deviations of the assigned value. The quantification limit (LOQ) is estimated from the amount of contaminant injected on the GC/ECD and is defined as ten times the standard deviation of repeated measured concentration as the concentration approaches zero. Since the dissolved lipid varies from 0.05 to 0.3 g ml^{-1} solvent depending on the matrix and sampling site (based on amount of contaminant and lipid content), the LOQ varies from 0.001 to 0.01 μg g^{-1} lipid weight for all discussed PCB congeners and pesticides. CB-118, CB-153, and DDE have not been close to LOQ in any of the species. ß-HCH has not been close to LOQ in guillemot eggs but has approached or dropped below LOQ in blue mussel and fish and has been below LOQ in herring from the Bothnian Bay and the Swedish West coast during the last years. γ-HCH has been close to or below LOQ within (or during) the last 10–15 years in all species. HCB has approached LOQ during the last years and has in some cases been below LOQ in blue mussel, perch, and eelpout.

Statistical methods

All values below the limit of quantification (LOQ) were replaced by the LOQ divided by the square root of 2 prior to statistical analyses. For temporal trend analysis, log-linear regression was performed for the entire investigated period and for the most recent 10 years using the yearly geometric mean values on a lipid weight basis. In cases where the regression line had a poor fit, a three-point running mean smoother was checked for statistical significance in comparison to the regression using ANOVA (Nicholson et al. 1998). If the number of analysis per year exceeded 3, a 95 % CI for the geometric mean (asymmetric) was plotted. For matrices with variable fat content, i.e., spring-caught herring or cod liver, the concentrations expressed on a lipid weight basis were adjusted to concentrations estimated as if the fat content was fixed to the average fat content when appropriate (using the same technique as in an ANCOVA-analysis).

The lowest detectable trend within a 10-year monitoring period at a significance level of 5 % and a statistical power of 80 % was estimated. A significance level of 5 % was used for all tests. Temporal trend analyses were performed for CB-153, CB-118, DDE, γ-HCH, β-HCH, and HCB. These were chosen to represent their respective groups because CB-153 and DDE are normally found in the highest concentration in biological samples; β-HCH was chosen for guillemot egg because of the low measured concentration of γ-HCH when compared to that of β-HCH. Power analysis was also carried out for all the substances.

Principal component analysis (PCA) was performed on the proportions of single HCH isomer concentrations to the $\sum$HCH to study the change in isomer pattern. The percentage of each log-transformed HCH isomer relative to the sum of isomers was calculated and a centered log-ratio transformation (Aitchison 1994; Kucera and Malmgren 1998) was also applied prior to the PCA analysis (to avoid a possible bias due to the compositional nature of the percentages). Before the PCA scores were plotted, they were centered and scaled to 100 %. The eigenvector loadings were added to the PCA plot as vectors showing the magnitude of the relative concentrations for each isomer. The Hotelling's 95 % confidence ellipses for the center of gravity of each group were also calculated and plotted [i.e., the ellipse in which 95 % of the center of gravity for all equally sized samples from the same populations is expected to fall, see, e.g., Sokal and Rohlf (1995, pp. 586–593)]. A Hotelling's T^2 test (Zar 1999) including the individual HCHs was carried out to check for significant differences in the HCH composition over time.

The significant level for all tests was set to $\alpha = 0.05$. Since several pairwise comparisons were made with the Hotellings T^2-test, the significance level was Bonferroni-

adjusted to 0.0083 to maintain a true significance level of 0.05 (cf. Sokal and Rohlf 1995).

For comparing concentrations of different compounds in herring caught in spring and autumn at Ängskärsklubb and Utlängan, paired *t* tests were used, comparing the different years between spring and autumn.

Statistical software PIA (http://www.amap.no) was used for the trend analysis and the PCAs, and Statistica software for the paired *t* tests.

Target values

To protect the most sensitive organisms from harmful effects of hazardous substances, a number of target values that should not be exceeded have been developed within several groups or conventions, e.g., Environmental Quality Standards (EQS) developed within the European Commission (EC. Directive 2008; EU. Directive 2013), and Environmental Assessment Criteria (EAC) developed within OSPAR (OSPAR 2009). This study primarily uses internationally agreed target values such as EQSs and EACs. If reliable target values for Swedish environmental conditions have been produced, these have been used (i.e., HCHs).

Target values (EQS, EAC) are generally specified on a fresh weight basis. To allow temporal and spatial comparisons for lipophilic substances among samples from different species and tissues (e.g., cod liver, herring muscle) that vary in lipid content, the target values have been recalculated to lipid weight basis using the mean lipid content for each studied sample matrix. This has been done because the focus for this study has been the temporal and geographical variation in pollution load to the environment rather than assessment of risk to consumers of the studied species. Lipid weight concentrations make more sense when comparing concentrations of lipophilic substances—between seasons, over time, and between species—where the lipid content varies. The mean lipid content for the studied species is as follows: herring (autumn caught) 3.5 %, perch 0.64 %, eelpout 0.56 %, and cod liver 42 %.

Since there are no EQSs for PCBs, DDTs, and HCHs, OSPAR EACs were used to evaluate concentrations of CB-118, CB-153, and DDE in fish. The EACs for CB-118 and CB-153 are 24, and 1600 $\mu g\ kg^{-1}$ lipid weight, respectively (OSPAR 2009). The target value (OSPAR EAC) for DDE in fish is 5 $\mu g\ kg^{-1}$ wet weight (OSPAR 2009). The recalculated lipid weight target value for DDE is 143 $\mu g\ kg^{-1}$ for herring, 781 $\mu g\ kg^{-1}$ perch, 893 $\mu g\ kg^{-1}$ eelpout, and 11.9 $\mu g\ kg^{-1}$ cod liver. With regard to Swedish levels of organic carbon in the sediment and bioconcentration (BCF) and biomagnification factors (BMF), the Swedish Environmental Research Institute (IVL) has provided conversions between EQS for surface water to biota for HCHs (Lilja et al. 2010). The IVL target concentration used here is 2.6 $\mu g\ kg^{-1}$ wet weight for the sum of HCHs (α-, β- and γ-HCH) in the marine environment. The recalculated lipid weight values for HCH are as follows: herring 74.3 $\mu g\ kg^{-1}$, perch 406 $\mu g\ kg^{-1}$, eelpout 464 $\mu g\ kg^{-1}$, and cod liver 6.19 $\mu g\ kg^{-1}$.

The EQS used to evaluate HCB concentrations in this study is based on human health and set at 10 $\mu g\ kg^{-1}$ wet weight fishery product (2013/39/EU). The recalculated lipid weight value for HCB was 286 $\mu g\ kg^{-1}$ for herring, 1560 $\mu g\ kg^{-1}$ for perch, 1790 $\mu g\ kg^{-1}$ for eelpout, and 23.8 $\mu g\ kg^{-1}$ for cod liver.

RESULTS

PCBs

Temporal trends and seasonal differences

Concentrations of CB-153 decreased over the monitoring period for all species and sampling sites, with most trends significant. The significant decreases varied from 2 to 9 % per year, with the most rapid decrease in eelpout from Kvädöfjärden and the slowest in autumn-caught herring from Utlängan. In the last 10 years, concentrations decreased significantly in eelpout and blue mussel from Kvädöfjärden, in blue mussel from Nidingen, and in guillemot egg. By contrast, cod from Fladen showed a significant upward trend during the same period (Fig. 2, Fig. S1a–d in ESM; Table S2 in ESM). Trends were similar for CB-118, with concentrations decreasing over the monitoring period for most species and sampling sites (Table S3 in ESM). Also, as for CB-153, there was a significant upward trend in cod liver from Fladen during the latest 10 years and significant decreases in blue mussel from Kvädöfjärden and Nidingen and in guillemot egg. Concentrations of CB-153 and CB-118 in the Baltic decreased 55–85 % over the longest time series (herring and guillemot egg) that started in the late 1970s.

CB-153 concentration (geometric mean) showed a significant seasonal difference over the whole monitoring period, being higher in spring than in autumn, both at Ängskärsklubb ($p < 0.001$) and Utlängan ($p < 0.001$) (Fig. 2). In 2012, concentrations in herring from Ängskärsklubb were 0.12 and 0.064 $\mu g\ g^{-1}$ lipid weight in spring and autumn, respectively, and at Utlängan 0.095 $\mu g\ g^{-1}$ lipid in spring and 0.069 $\mu g\ g^{-1}$ lipid in autumn (Fig. 2; Table S2 in ESM). The fat content in autumn and spring was similar (Bignert et al. 2014).

Spatial trends

Herring muscle (arithmetic mean 2010–2012) had higher concentrations of both CB-118 and CB-153 at the Bothnian

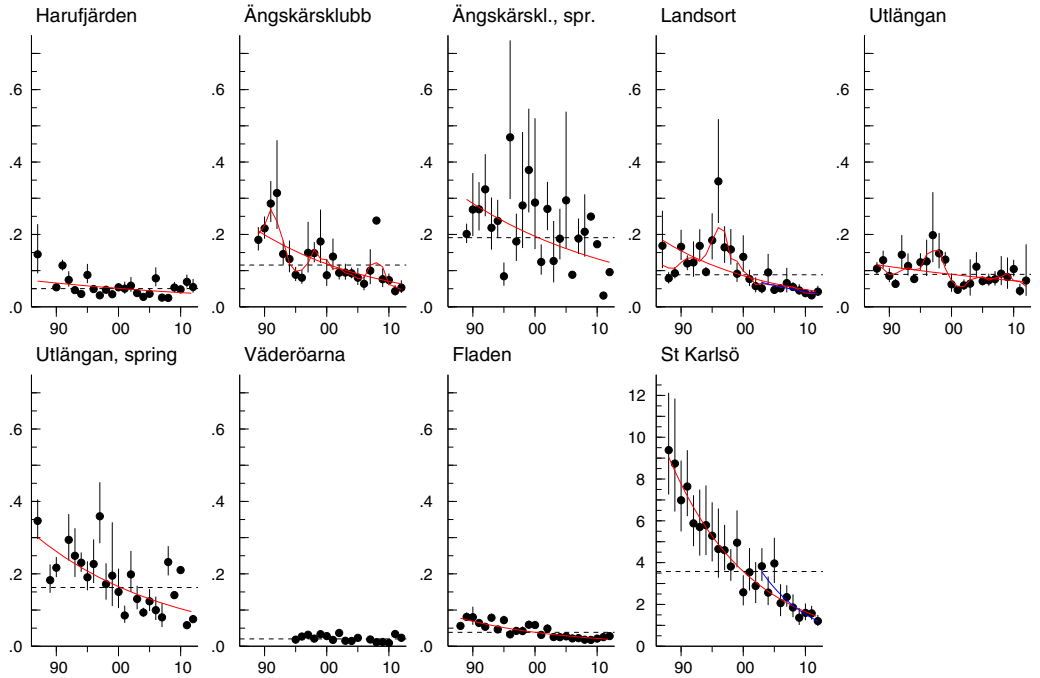

Fig. 2 CB-153 concentrations (μg g^{-1} lipid weight) in herring muscle from Harufjärden, Ängskärsklubb (autumn and spring), Landsort, Utlängan (autumn and spring), Fladen, and Väderöarna, and guillemot egg from Stora (St) Karlsö. The *linear red lines* show significant trends over the whole period and the *linear blue lines* significant trends for the last 10 years ($p < 0.05$). The *red smooth lines* show non-linear trends ($p < 0.05$). The *black dotted horizontal line* shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration for each year (*circles*) and 95 % CI for the geometric means

Sea offshore site and Lagnö in the northern Baltic Proper than in the Bothnian Bay and on the Swedish west coast (Fig. 3, Fig. S2 in ESM). Lagnö had the overall highest concentration of CB-118 (0.044 μg g^{-1} lipid weight) and CB-153 (0.14 μg g^{-1} lipid). Lowest concentrations of CB-118 (0.0068 μg g^{-1} lipid) and CB-153 (0.019 μg g^{-1} lipid) were found at Kullen on the southern west coast (Fig. 3, Fig. S2 in ESM).

Target values

Concentrations of CB-153 in 2012 were below the OSPAR EAC target value of 1.6 μg g^{-1} lipid weight in all fish species at all sites (Fig. S2; Table S2 in ESM). For CB-118, concentrations were above the OSPAR EAC of 0.024 μg g^{-1} lipid weight in cod from both sampling sites, in eelpout from Fjällbacka, in spring-caught herring and herring from Lagnö and the Bothnian Sea offshore site (Fig. 3; Table S3 in ESM), and just below or at the target value in eelpout, perch, and herring from all other Baltic Sea sites.

DDTs

Temporal trends and seasonal differences

DDE concentrations decreased over the monitoring period for all species and sites examined for temporal trends, with all trends significant, except for two eelpout stations. The significant decreases varied from 3.6 to 9.7 % per year. The most rapid decrease was in spring-caught herring from Utlängan, the second most rapid (9.4 % per year) in Guillemot egg, both from sites with comparatively high concentrations in the 1970s and 1980s. The slowest decrease was in the short time series of blue mussel from Kvädöfjärden. In the most recent decade, concentrations decreased significantly in herring at Landsort, guillemot egg, and blue mussel at Kvädöfjärden. By contrast, concentrations increased significantly in this period in Kvädöfjärden perch (Fig. S3a–g; Table S4 in ESM). The concentration of DDE in the Baltic has decreased 96–99 % in the longest time series (guillemot egg and herring) that started around 1970.

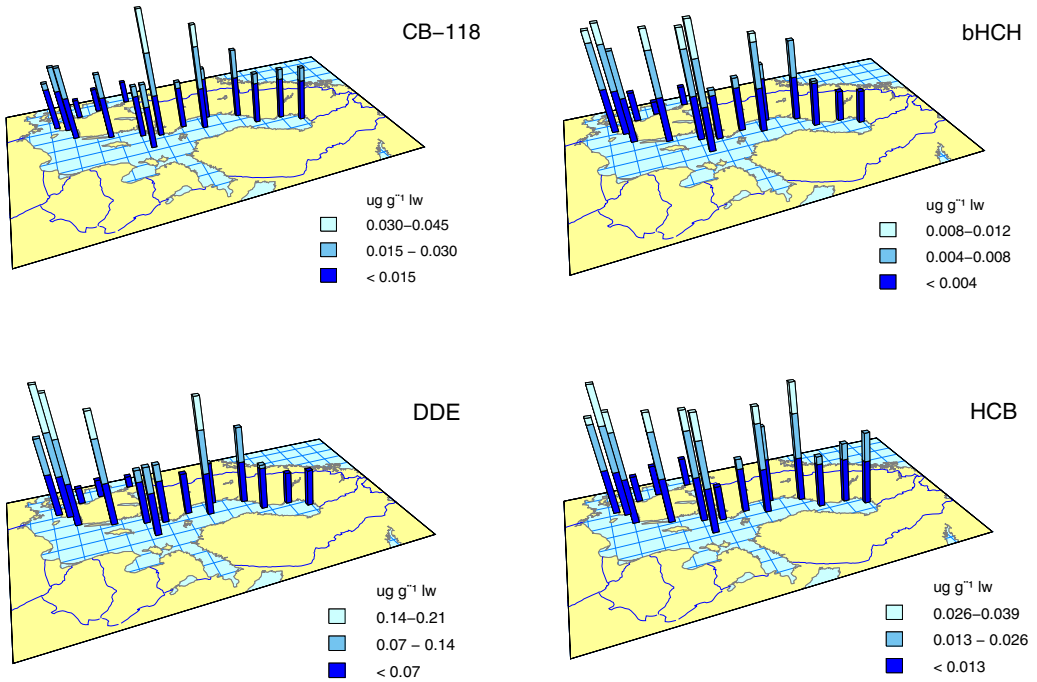

Fig. 3 Spatial variation in concentration (μg g^{-1} lipid weight) in herring muscle of CB-118, β-HCH, DDE, and HCB. Arithmetic mean values from 2010 to 2012

In herring, the DDE concentration showed a significant seasonal difference during the whole monitoring period and also during the latest 10 years at both Ängskärsklubb ($p < 0.001$ and $p < 0.05$ respectively) and Utlängan ($p < 0.001$ and $p < 0.01$, respectively), with higher values in spring than in autumn. In 2012, the DDE concentration in herring from Ängskärsklubb was 0.17 μg g^{-1} lipid weight in spring and less than a third, 0.054 μg g^{-1} lipid, in autumn (Fig. S3a, b; Table S4 in ESM).

Ratio DDT/DDE

The general trend for the ratio DDT/DDE over the whole study period is downward, except for some upward tendencies at the beginning of the 1980s and around the turn of the century, followed by a decrease in recent years (Fig. 4).

Spatial trends

DDE concentration in herring muscle was higher in the southern Baltic Proper than in the rest of the Baltic Sea, except for the Bothnian Sea offshore site, and lowest on the Swedish west coast. The highest concentrations in herring, 0.21 μg g^{-1} lipid weight, were found at Utlängan and Hanöbukten in the southern Baltic Proper, the lowest, 0.019 μg g^{-1} lipid, at Väderöarna in the Skagerrak (Fig. 3).

Target values

In 2010–2012, the DDE concentrations in herring from the Bothnian Sea offshore site, Byxelkrok, Utlängan, and Västra Hanöbukten were still above the target value of 0.143 μg g^{-1} lipid weight, based on the recalculated OSPAR EAC (Fig. 3). Values in spring-caught herring from Ängskärsklubb and Utlängan and in cod from Fladen and southeast Gotland (recalculated value 0.0119 μg g^{-1} lipid) were also above or near the target value. All perch and eelpout samples had concentrations below the recalculated target values of 0.781 and 0.893 μg g^{-1} lipid, respectively (Table S4 in ESM).

HCHs

Temporal trends and seasonal differences

Concentrations of γ-HCH in fish and blue mussel decreased significantly over the monitoring period at all sampling

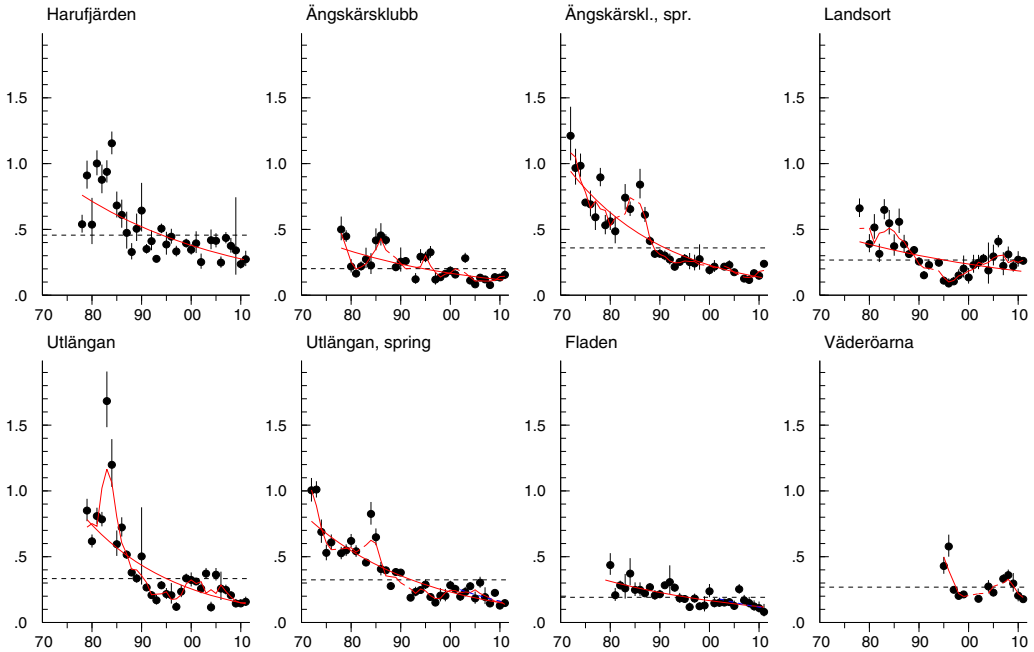

Fig. 4 DDT/DDE ratios in herring muscle from Harufjärden, Ängskärsklubb (autumn and spring), Landsort, Utlängan (autumn and spring), Fladen, and Väderöarna. The *red lines* show a significant ($p < 0.05$) trend over the whole period. The *red smooth lines* show non-linear trends ($p < 0.05$). The *black dotted horizontal line* shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration for each year (*circles*) and the 95 % CI of the geometric means

sites. Significant decreases varied 3.5–18 % per year, with the most rapid decrease in cod from Fladen and the slowest in perch from Holmöarna. During the last 10–15 years, γ-HCH concentrations were close to or below LOQ for most species and sites (Fig. S4a–f; Table S5 in ESM). Concentrations of β-HCH also decreased significantly in fish and blue mussel at most sites with between 1.6 and 7.7 % per year, slightly slower than for γ-HCH (Tables S5, S6 in ESM). In guillemot egg, β-HCH concentrations decreased significantly during the monitoring period by c. 8 % per year, and for the latest decade by c. 6 % per year (Fig. S4g; Table S6 in ESM). The concentration of γ-HCH in the Baltic has decreased 93–97 % in the longest time series (herring) that started in the late 1970s. The decrease of β-HCH was slightly smaller, c. 84–90 % in herring and guillemot egg.

For the whole monitoring period, the γ-HCH concentration in herring was significantly higher in spring than in autumn at Utlängan, but did not differ between seasons at Ängskärsklubb. In 2012, there was no seasonal difference at either sampling site (Fig. S4a, b; Table S5 in ESM), but values were now close to or below LOQ at both sites and seasons.

Spatial trends

Concentrations of β-HCH in herring muscle were higher in the Baltic Proper and the Bothnian Sea than in the Bothnian Bay and on the Swedish west coast (Fig. 3; Table S6 in ESM). The highest β-HCH concentration in herring (0.011 μg g^{-1} lipid weight) was found at the offshore site in the northern Baltic Proper and the lowest (0.0012 μg g^{-1} lipid) at Fladen in the Kattegat.

Target values

The highest β-HCH concentrations in this study were found in guillemot eggs. In 2010–2012, the highest concentration in fish, 0.013 μg g^{-1} lipid weight, was from herring sampled at Landsort in 2011. Most fish samples during this period were below LOQ for α- and γ -HCH (LOQ < 0.01 μg g^{-1} lipid weight). This implies that the total HCH concentration

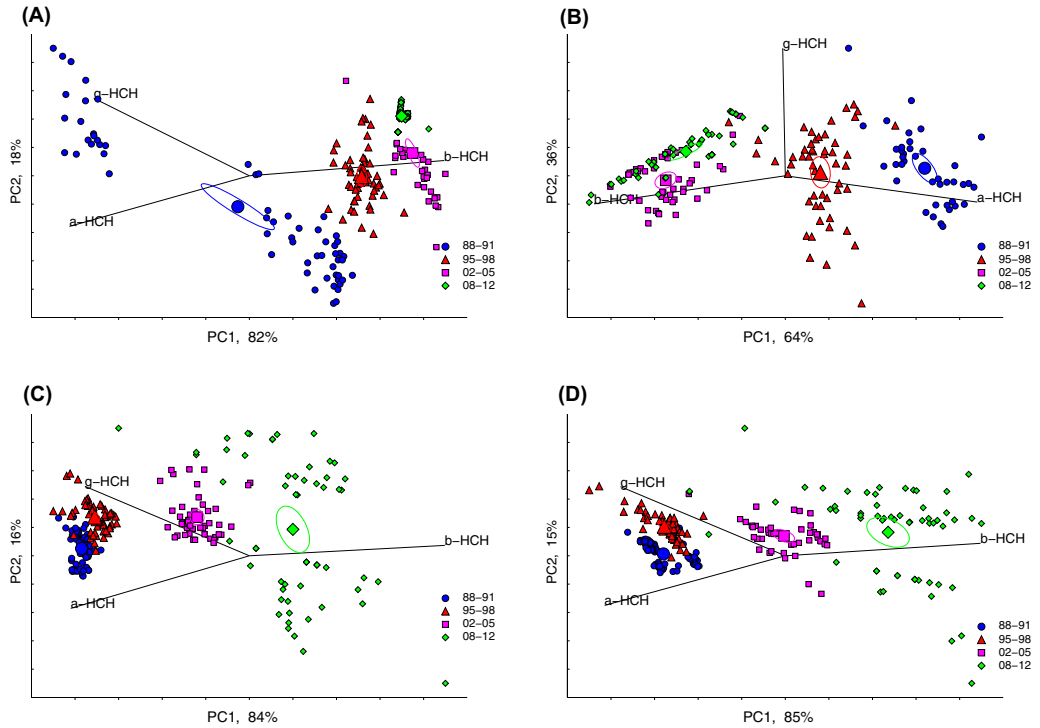

Fig. 5 Principal component analysis, biplot, and Hotelling's 95 % confidence ellipses for centers of gravity for each group. Changes over time in relative abundance of α-, β-, and γ-*HCH* to the sum in autumn-caught herring. **A** Harufjärden in the Bothnian Bay (*BB*), **B** Ängskärsklubb in the southern Bothnian Sea (*sBS*), **C** Landsort in the northern Baltic Proper (*nBP*), and **D** Utlängan in the southern Baltic Proper (*sBP*). A Hotelling's T^2 tests showed significant differences in patterns between all the time periods ($p < 0.0083$)

(α-, β-, and γ) was less than the target values in, e.g., herring (recalculated target value 0.074 μg g^{-1}), perch (0.41 μg g^{-1}), eelpout (0.46 μg g^{-1}), if the less than LOQ values for α- and γ -HCH were added to the highest β–HCH concentration. However, the target value was exceeded in cod liver from both Fladen and south east of Gotland (recalculated target value 0.0062 μg g^{-1} lipid weight) (Fig. 3, Fig. S4a–f in ESM; Tables S5, S6 in ESM).

Proportions of α-, β-, and γ-HCH

There was a clear shift with time in the HCH isomer pattern (α-, γ-, and β-HCH) at all four sites in the Baltic Sea (Fig. 5A–D). Before 2000, the HCH pattern was dominated by α- and γ-HCH, but with time the most persistent and bioaccumulative form, β-HCH, has increased. The centers of gravity for the four time periods were all significantly different (repeated Hotelling's T^2 test, $p <$ the Bonferroni-adjusted α value of 0.0083) (Fig. 5B). At Harufjärden, a shift in the isomer pattern seems to have occurred already during the first time period, 1988–1991 (Fig. 5A).

HCB

Temporal trends and seasonal differences

Concentrations of HCB decreased over the monitoring period in all fish species at all sampling sites and in guillemot egg, with most trends significant. Significant decreases ranged from 2.5 to 9.9 % per year. The most rapid decrease was in eelpout from Holmöarna, the slowest in autumn herring from Harufjärden. By contrast, a significant upward trend of 2.6 % per year was observed in blue mussel at Nidingen. During the last decade, significant upward trends were also seen in perch from Holmöarna and Kvädöfjärden (Fig. 6, Fig. S5a–d in ESM; Table S7 in ESM). The HCB concentration has decreased c. 90 % in the longest Baltic time series (guillemot egg) that started in the late 1970s.

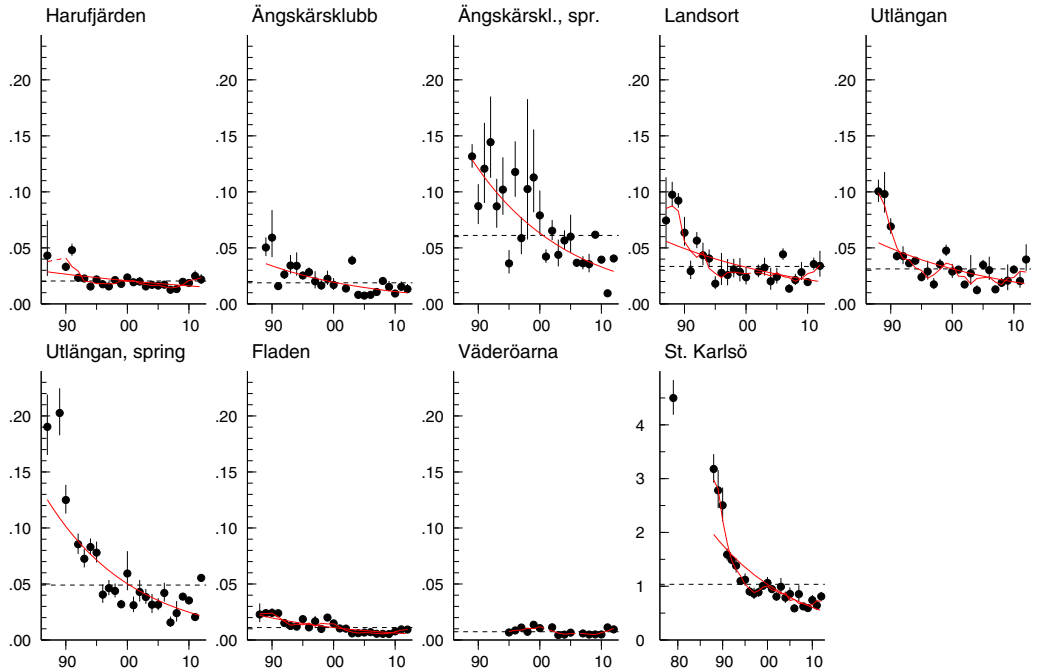

Fig. 6 *HCB* concentrations (μg g^{-1} lipid weight) in herring muscle from Harufjärden, Ängskärsklubb (autumn and spring), Landsort, Utlängan (autumn and spring), Fladen, and Väderöarna, and guillemot egg from Stora (St) Karlsö. The *linear red lines* show significant trends over the whole period and the *linear blue lines* significant trends for the last 10 years ($p < 0.05$). The *red smooth lines* show non-linear trends ($p < 0.05$). The *black dotted horizontal line* shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration of each year (*circles*) and the 95 % CI of the geometric means. The *bars* represent years where all values were below LOQ

The HCB concentration in herring differed seasonally for the whole monitoring period, being significantly higher in spring than in autumn at both Ängskärsklubb ($p < 0.001$) and Utlängan ($p < 0.001$) (Fig. 6). In 2012, the HCB concentration in herring from Ängskärsklubb was almost threefold higher in spring than in autumn (0.029 and 0.010 μg g^{-1} lipid weight, respectively), but concentrations at Utlängan were similar in autumn and spring (Fig. 6; Table S7 in ESM).

Spatial trends

Herring muscle from almost all sites in the Baltic Proper and the Bothnian Sea had higher HCB concentrations than in the Bothnian Bay and on the Swedish west coast. The highest concentration was found at Västra Hanöbukten in the southern Baltic Proper (0.037 μg g^{-1} lipid weight) and the lowest at Kullen in the Kattegat (0.0078 μg g^{-1} lipid weight) (Fig. 3).

Target values

Concentrations of HCB were below the target value (lowest recalculated target value is 0.0238 μg g^{-1} lipid weight, in cod liver) for all fish species at all stations (Fig. 3; Table S7 in ESM).

DISCUSSION

Temporal and spatial trends

Concentrations of PCBs, DDTs, HCHs, and HCB have decreased significantly in most time series at most sites during the last three to four decades, both in the Baltic Sea and on the Swedish west coast. CB-153 has generally decreased in the Baltic by 60–80 % since 1988, DDE by over 90 % since the late 1970s, and HCHs and HCB by 80–90 and 90 %, respectively, since 1979. This shows that measures taken, i.e.,

bans and restrictions implemented in the 1970s and 1980s, have had the desired effect. Downward trends of a similar magnitude for DDE, CB-153, and HCHs have been reported in fish from freshwater in Sweden (Nyberg et al. 2014a, b). By contrast, HCB has not decreased as much in freshwater as in the marine environment (Nyberg et al. 2014b). Downward trends in biota (mainly arctic) have also been reported from, e.g., Canada, Norway, Greenland, Iceland, Faroe Islands, and the United States (Alaska) for several of these substances (e.g., Braune et al. 2005; Ryan et al. 2005: Helgason et al. 2008; Rigét et al. 2010). Rigét et al. (2010) found a mean annual decrease of 1.2 and 1.9 % for CB-153 and DDE, respectively (both based on 40 time series); −2.9 % for β-HCH (24 time series); −7.3 % for γ-HCH (17 time series); and −2.5 % for HCB (40 time series). The annual decreases in arctic biota reported by Rigét et al. (2010) are all lower than or at the lower end of the annual decreases reported in this study for all of the substances. This difference might be due to the monitoring starting in more recent years for some of the time series in that study, meaning that concentrations were already lower and the decrease less steep. The lower Arctic temperature that slows degradation is also a possible explanation (Wania and Mackay 1993). Furthermore, some of the trends in the study by Rigét et al. (2010) were upward (three for CB-153, one for DDE and two for β-HCH), which weakens the overall results.

The efficiency of the program was evaluated and it was shown that due to its lower between-year variation over time, the guillemot time series could detect smaller changes compared to fish, over a 10-year period for CB-153, DDE, β-HCH, and HCB (range 6.2–11 %). The high and stable fat content of guillemot eggs make them a very suitable matrix for fat-soluble contaminants and explains the low variation. Rigét et al. (2010) summarized 316 temporal trends on legacy POPs in Arctic biota such as blue mussel, freshwater fish, terrestrial mammals, marine fish, seabirds, and marine mammals. The lowest detectable trend over a 10-year period varied from 11.9 to 20.5 % for CB-153, DDE, γ-HCH, β-HCH, and HCB. The statistical power to detect trends from these time series are in the same range as in this study. The statistical power to detect an annual change of 10 % was very close to 100 % for the entire monitoring period in all time series, except for the eelpout time series, which generally are shorter than the rest. A high statistical power—to detect changes, or show compliance with quality standards, or to increase the sensitivity to detect trends at a fixed sample size—is essential for improving the ability to discern changes in environmental data. Unless variance in environmental monitoring is minimized, there is a risk that resources will be wasted and important changes remain undetected.

Even though concentrations of all compounds in this study have decreased in recent decades, they are still higher in the Baltic Sea than in other marine areas. Jörundsdóttir et al. (2009) showed that concentrations in guillemot egg are still higher in the Baltic Sea than in, e.g., the North Atlantic for all studied substances except HCB. Concentrations of CB-153, DDE, and β-HCH in samples from the Baltic Proper were an order of magnitude higher than in North Atlantic samples. In this study, CB-153, CB-118, DDE, γ-HCH, and HCB all had higher concentrations in herring muscle in the Baltic Sea than on the Swedish west coast in 2010–2012. However, the spatial pattern in the Baltic Sea differs somewhat between compounds. Concentrations of CB-153 and CB-118 are rather homogenous in the Baltic Sea, with two sites showing elevated values (Lagnö and the Bothnian Sea offshore site).

In contrast, DDE has higher concentrations in the southern Baltic Proper than in the rest of the Baltic Sea, with a few exceptions. A possible explanation might be that the historical use of DDT in Sweden was focused in agricultural areas in the southern part of the country and the higher population density in the drainage area of the southern part of the Baltic Sea. Since DDT degrades to DDE and DDD and the use of DDT at present is banned in most countries and allowed only to combat malaria (UNEP 2008), primarily in Africa and the Pacific Islands (Bogdal et al. 2013), a continuous decrease in the DDT/DDE ratio is expected. A sudden increase would indicate the release of fresh DDT. The signal from the ratio is stronger (contains less noise) than from the concentration of DDT, since several confounding factors (e.g., fat content) cancel each other out. An increase in this ratio can possibly be discerned in the early 1980s, probably a consequence of DDT use in former East Germany (Kylin et al. 1996; Bignert et al. 1998). The possible upward trend seen during or shortly after the turn of the century (Fig. 4), most clearly at Landsort in the northern Baltic Proper, so far lacks a plausible explanation but it could be caused by a recent discharge. The most northern site, with lowest annual mean temperature, has the highest ratio of DDT/DDE. This may be explained by the somewhat lower volatility and degradation at lower temperature and stresses the importance of following time series of ratios from a climate change perspective.

Concentrations of β-HCH remain higher in the Baltic Proper than in the Bothnian Sea and Bay, but are approaching or below LOQ in the whole Baltic Sea. Higher levels in the Baltic Proper might, as for DDT, be explained by higher historical use in the more agricultural south of Sweden and the larger population of the Baltic Proper drainage area. The principal component analysis showed a clear shift in HCH pattern, from domination by α- and γ-HCH before 2000, to β-HCH after, even though the technical mixture containing β-HCH was banned before lindane (γ-HCH) (Li 1999). This may be because β-HCH is the most persistent of the HCH isomers, with a half-life of years compared to days for γ-HCH (Li 1999).

HCB concentrations are lower in the Bothnian Bay than in the rest of the Baltic Sea. Its use as a fungicide has been banned in the Baltic countries since the late 1980s, but HCB can still reach the environment as a by-product from the chlor-alkali industry (Garí et al. 2014) and from combustion of materials containing chlorine, which might explain the higher concentrations in the more densely industrialized regions in the Baltic Sea.

Seasonal differences

For the whole period, all substances, except γ-HCH, had significantly higher geometric concentration means in herring in spring than in autumn, both at Ängskärsklubb and Utlängan. In 2012, the mean concentrations of CB-153, DDE, and HCB in herring were higher in spring than in autumn at Ängskärsklubb and for CB-153 also at Utlängan, on a lipid weight basis. Similar seasonal differences in PCB concentrations recalculated to lipid weight have been reported for white croaker (*Genyonemus lineatus*) in San Francisco Bay (Greenfield et al. 2005) and herring from the Norwegian Sea (Frantzen et al. 2011). However, the percentage lipid varied a lot in these two studies and was much higher in the herring in autumn than in spring, making this a likely main cause of the seasonal difference in concentration. This was not the case in our study, where lipid content was relatively similar in spring and autumn (Bignert et al. 2014). Frantzen et al. (2011) suggested seasonal differences were due to differences in age between the spring- and the autumn-caught herring, but we selected fish of the same age ruling out this explanation. The differences may at least partly be due to seasonal variation in discharges to the Baltic, e.g., in precipitation, spring ice melt, and runoff, and organic contaminants could also be more easily dispersed due to higher volatility in summer. The seasonal concentration differences seen in this study could also be due to confounding factors, e.g., subpopulations differing in diets and migration patterns, or seasonal diet differences. Möllmann et al. (2004) found significant seasonal changes in the diet of Baltic herring, with more mysids in autumn and more copepods in spring. Bloater (*Coregonus hoyi*) in Lake Michigan ingested more *Diporeia hoyi* in summer, which correlated to an increased PCB burden (Stapleton et al. 2002). Further studies could provide more information on seasonal changes in herring diet, e.g., stomach content analysis and stable isotope analysis.

Target values

The recalculated OSPAR EAC of 0.024 μg g^{-1} lipid weight for CB-118 was exceeded at some sites in some species in the Baltic Sea while the others were at or just below the target concentration, indicating that levels may still be too high to protect the most sensitive organisms. The OSPAR EAC of 0.005 μg g^{-1} wet weight for DDE was exceeded at most cod and herring sites. Reindl et al. (2013) reported that the OSPAR EAC for DDE was exceeded also in herring from the Gulf of Gdansk. We found HCHs and HCB concentrations below the target values (IVL and EQS) of 0.026 μg g^{-1} and 0.010 μg g^{-1} wet weight, respectively, at all stations for all fish species, except for HCHs in cod liver. However, the high and very variable lipid content makes cod liver problematic for evaluation of the target concentration. In herring muscle from the Gulf of Riga, Reindl et al. (2013) also found concentrations of HCB and HCHs (0.0026 and 0.0013 μg g^{-1} wet weight, respectively, recalculated from lipid weight) far below the target concentrations.

CONCLUSIONS

Since monitoring started, PCBs, DDTs, HCHs, and HCB have all decreased by 60–90 % in the Baltic Sea, but concentrations are still higher than in the North Sea. CB-118 and DDE in the Baltic remain above the target concentrations, set to protect the most sensitive organisms.

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Electronic Supplementary Material

This supplementary material has not been peer reviewed.

Title: **Temporal and spatial trends of PCBs, DDTs, HCHs and HCB in Swedish marine biota 1969–2012**

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Table S1 Sampling sites, coordinates, species and start year/end year for analyses of PCBs, DDTs, HCHs and HCB (P=perch, H=herring, E=eelpout, C=cod, B=blue mussel) within the Swedish National Monitoring Program for Contaminants in Marine Biota. Month of sampling, age and size (total length for fish or total shell length for blue mussels) are shown the first time the species occur and cover all sites for that particular species except for blue mussels, which differ in age and size between the Baltic Sea site and the Swedish west coast sites. The first column refers to the sampling site numbers in Figure 1

No in map	Sampling site	Latitude	Longitude	Species	Start year for analyses of PCBs, DDTs, HCHs, HCB	Month of sampling	Age (years)	Total length or shell length (cm)
1	Rånefjärden	65° 45'N	22° 25'E	herring	07[b]	Aug–Oct	2–9 (most 3–5)	14–22
2	Harufjärden	65° 35'N	22° 53'E	herring	87a			
3	Kinnbäcksfjärden	65° 03'N	20° 53'E	herring	08[b]			
4	Holmöarna	63° 41'N	20° 53'E	perch herring eelpout	P:80[c], , H:09[b] E:95–07[c]	P:Aug–Oct, E:Nov	P:3–5, E:2–5	P:15–21, E:18–24
5	Gaviksfjärden	62° 52'N	18° 14'E	herring	07[b]			
6	Långvindsfjärden	61° 27'N	17° 10'E	herring	07[b]			
7	Bothnian Sea, offshore site	60° 57 N	18° 57'E	herring	08[b]			
8	Ängskärsklubb	60° 32'N	18° 09'E	herring	autumn: 78[a], spring: 72[b]			
9	Lagnö	59° 34'N	18° 50'E	herring	07[b]			
10	Baltic Proper, offshore site	58° 60'N	19° 52'E	herring	08[b]			
11	Landsort	58° 42'N	18° 04'E	herring	78[a]			
12	Fjällbacka	58° 37'N	11° 15'E	eelpout blue mussel	E: 95–07[c], 08[d], B: 84[e]	B:Sep–Dec	B:6–19	B:2–3
13	Väderöarna	58° 31'N	10° 54'E	herring	95[a]			

No in map	Sampling site	Latitude	Longitude	Species	Start year for analyses of PCBs, DDTs, HCHs, HCB	Month of sampling	Age (years)	Total length or shell length (cm)
14	Kvädöfjärden	58° 2'N	16° 46'E	perch eelpout blue mussel	P:80[c], E:95–07[c], 08[d], B:95 [e]		B:2–8	B:5–10
15	Nidingen	57° 18'N	11° 54'E	blue mussel	82 [e]			
16	Stora Karlsö	57° 16'N	17° 58'E	guillemot egg	69 [c]	May		
17	Fladen	57° 14 N	11° 50'E	herring cod	H: 80[a], C: 80[c]	C:Sep–Oct	C:2–4	C:33–66
18	Byxelkrok	57° 19'N	17° 30'E	herring	07[b]			
19	SE Gotland	56° 53'N	18° 38'E	cod	80[c]			
20	Kullen	56° 19'N	12° 23'E	herring	07[b]			
21	Utlängan	55° 57'N	15° 47'E	herring	autumn: 80[a], spring: 72[b]			
22	Västra Hanöbukten	55° 45'N	14° 17'E	herring	07[b]			
23	Abbekås	55° 18'N	13° 36'E	herring	07[b]			

[a] 12 individual specimens per year, [b] 2 pool per year of 12 individuals, [c] 10 individual specimens per year, [d] 2 pools per year of 10 individuals per pool, [e] 5 pools per year of 20 individuals. Deviations occurred during the monitoring period, especially at the beginning of the time series.

Table S2 Trend for the entire period and the last 10 years (in %) for **CB-153** assessed from the annual geometric mean (μg g^{-1} lipid weight) in herring, perch, cod, eelpout, blue mussel and guillemot egg. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ p<0.05, --/++ p<0.01, ---/+++ p<0.001. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's CB-153 concentration values are estimated from the trend if p<0.05 and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four.

Site number (from figure 1)	Matrix, Sampling site	N samples	N years	Period (Years)	Trend % (95% C.I.)	P	YRQ	LDT %	Last year μg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	333	24	87-12	-2.6(-5.1,-0.08)	0.0418 -	13	16	0.037 (0.027,0.052)
2	Harufjärden		10	03-12	6.1(-3.9,16)	0.1937	13	15	
8	Ängskärsklubb	332	24	89-12	-5.2(-7.5,-2.9)	0.0002 ---	13	14	0.064 (0.047,0.087)
8	Ängskärsklubb		10	03-12	-5.8(-17,5.6)	0.2746	14	17	
8	Ängskärsklubb, spring	277	24	89-12	-3.8(-7.1,-0.57)	0.0223 -	15	20	0.123 (0.079,0.19)
8	Ängskärsklubb, spring		10	03-12	-8.8(-25,7.3)	0.2435	17	25	
11	Landsort	354	26	87-12	-5.9(-8.1,-3.7)	0.0000 ---	13	15	0.042 (0.031,0.059)
11	Landsort		10	03-12	-6.7(-13,-0.67)	0.0327 -	10	7	
21	Utlängan	309	25	88-12	-2.2(-4.2,-0.16)	0.0337 -	12	13	0.069 (0.052,0.092)
21	Utlängan		10	03-12	-1.7(-8.6,5.2)	0.5861	10	9.9	
21	Utlängan, spring	272	24	87-12	-4.6(-6.8,-2.5)	0.0002 ---	12	14	0.095 (0.070,0.13)
21	Utlängan, spring		10	03-12	-2.1(-14,9.7)	0.6877	14	18	
17	Fladen	381	25	88-12	-5.9(-7.3,-4.4)	0.0000 ---	10	9.4	0.019 (0.015,0.023)
17	Fladen		10	03-12	-0.69(-5.0,3.7)	0.7203	8	6.1	
13	Väderöarna	291	17	95-12	-3.0(-6.9,0.90)	0.1181	13	15	0.016 (0.011,0.023) m
13	Väderöarna		9	03-12	2.1(-9.7,14)	0.6831	14	17	
	Cod liver								
19	SE Gotland	187	24	89-12	-1.2(-2.7,0.31)	0.1087	10	9.1	0.16 (0.13,0.20) m
19	SE Gotland		10	03-12	-0.54(-6.3,5.2)	0.8167	9	8.6	
17	Fladen	194	23	89-12	.39(-1.9,2.7)	0.7241	12	13	0.47 (0.35,0.64) m
17	Fladen		10	03-12	9.0(3.1,15)	0.0078 ++	10	8.5	
	Perch muscle								
4	Holmöarna	179	18	95-12	-6.4(-9.9,-3.0)	0.0013 --	12	13	0.039 (0.028,0.056)
4	Holmöarna		9	03-12	3.4(-4.2,11)	0.3228	11	11	
14	Kvädöfjärden	251	25	89-12	-3.3(-6.4,-0.24)	0 .034 -	15	19	0.033 (0.022,0.050)
14	Kvädöfjärden		10	03-12	9.3(-2.0,21)	0.0927	14	17	
	Eelpout muscle								
4	Holmöarna	96	11	95-07	-3.0(-14,7.7)	0.5416	16	23	0.12 (0.059,0.25) m
4	Holmöarna		9	98-07	-7.6(-23,8.1)	0.2893	17	23	

14	Kvädöfjärden	121	17	95-12	-8.8(-12,-5.3)	0.0001 ---	12	12	0.069 (0.050,0.095)
14	Kvädöfjärden		10	03-12	-6.4(-11,-1.8)	0.0123 -	8	6.5	
12	Fjällbacka	131	18	95-12	-.33(-4.8,4.2)	0.8524	14	18	0.26 (0.17,0.41) m
12	Fjällbacka		10	03-12	-3.4(-9.5,2.8)	0.2428	10	9.2	
	Blue mussel								
15	Nidingen	97	25	88-12	-8.1(-9.4,-6.7)	0.0000 ---	10	9.4	0.015 (0.013,0.019)
15	Nidingen		10	03-12	-12(-18,-5.5)	0.0034 --	11	10	
12	Fjällbacka	95	24	88-12	-4.4(-6.4,-2.4)	0.0002 ---	12	13	0.029 (0.022,0.039)
12	Fjällbacka		10	03-12	-1.6(-6.1,3.1)	0.451	8	6.6	
14	Kvädöfjärden	90	18	95-12	-2.9(-3.9,-1.9)	0.001---	10	9.5	0.043 (0.039,0.048)
14	Kvädöfjärden		10	03-12	-6.4(-9.1,-3.7)	0.001 ---	11	11	
	Guillemot egg								
16	Stora Karlsö	248	25	88-12	-7.5(-8.4,-6.5)	0.0000 ---	8	6.2	1.4 (1.2,1.6)
16	Stora Karlsö		10	03-12	-11(-16,-7.0)	0.0000 ---	9	6.8	

Table S3 Trend for the entire period and the last 10 years (in %) for **CB-118** assessed from the annual geometric mean (µg g^{-1} lipid weight) in herring, perch, cod, eelpout, blue mussel and guillemot egg. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ p<0.05, --/++ p<0.01, ---/+++ p<0.001. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's CB-118 concentration values are estimated from the trend if p<0.05 and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four

Site number (from figure 1)	Matrix, Sampling site	N samples	N years	Year	Trend % (95% C.I.)	P	YRQ	LDT %	Last year µg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	333	24	87-12	-4.5(-7.5,-1.5)	0.0055 --	15	19	0.011 (0.007,0.016)
2	Harufjärden		10	03-12	5.7(-5.3,17)	0.2643	14	16	
8	Ängskärsklubb	332	24	89-12	-6.6(-9.4,-3.7)	0.0001 ---	14	18	0.015 (0.010,0.022)
8	Ängskärsklubb		10	03-12	-6.0(-20,8.2)	0.3582	16	22	
8	Ängskärsklubb spring	267	23	89-12	-6.1(-9.7,-2.5)	0.0022 --	16	23	0.034 (0.021,0.055)
8	Ängskärsklubb spring		10	03-12	-11(-29,7.6)	0.2147	19	29	
11	Landsort	354	26	87-12	-7.8(-9.5,-6.1)	0.0001 ---	11	11	0.015 (0.012,0.020)
11	Landsort		10	03-12	-5.0(-8.9,-1.0)	0.0194	8	5.6	
21	Utlängan	309	25	88-12	-5.7(-7.7,-3.7)	0.0000 ---	12	13	0.018 (0.014,0.024)
21	Utlängan		10	03-12	-2.5(-9.7,4.6)	0.4387	11	10	
21	Utlängan spring	272	24	87-12	-7.8(-9.8,-5.8)	0.0000 ---	12	13	0.027 (0.020,0.035)
21	Utlängan spring		10	03-12	-5.1(-15,4.9)	0.2714	13	15	
17	Fladen	381	25	88-12	-8.7(-11,-6.9)	0.0000 ---	11	11.	0.006 (0.004,0.007)
17	Fladen		10	03-12	-2.0(-6.7,2.8)	0.3702	9	6.7	
13	Väderöarna	291	17	95-12	-4.2(-8.0,-0.41)	0.0309	13	15	0.006 (0.004,0.008)
13	Väderöarna		9	03-12	1.2(-9.5,12)	0.7909	13	15	
	Cod liver								
19	SE Gotland	187	24	89-12	-3.5(-5.4,-1.7)	0.0008 ---	11	11	0.054 (0.042,0.070)
19	SE Gotland		10	03-12	-1.1(-8.9,6.8)	0.7569	11	11	
17	Fladen	195	23	89-12	-1.5(-3.5,0.47)	0.1242	11	12	0.096 (0.074,0.13) m
17	Fladen		10	03-12	6.1(0.51,12)	0.0348 +	9	8.2	
	Perch muscle								
4	Holmöarna	179	18	95-12	-7.5(-11,-4.1)	0.0004 ---	12	13	0.010 (0.007,0.014)
4	Holmöarna		9	03-12	1.7(-6.1,9.5)	0.6222	11	11	
14	Kvädöfjärden	197	20	89-12	-1.8(-5.2,1.6)	0.2699	14	17	0.012 (0.008,0.018) m
14	Kvädöfjärden		10	03-12	9.2(-1.1,19)	0.0717	13	16	
	Eelpout muscle								
4	Holmöarna	96	11	95-07	-4.0(-14,6.0)	0.3913	16	21	0.025 (0.012,0.049) m
4	Holmöarna		9	98-07	-10(-23,3.4)	0.1195	15	20	
14	Kvädöfjärden	121	17	95-12	-7.7(-12,-3.6)	0.0013 --	13	15	0.019 (0.013,0.028)

14	Kvädöfjärden		10	03-12	-5.0(-10,0.41)	0.0637	9	7.7	
12	Fjällbacka	131	18	95-12	-1.5(-5.8,2.8)	0.4832	14	17	0.98 (0.064,0.15) m
12	Fjällbacka		10	03-12	-3.3(-11,4.3)	0.3483	11	12	
	Blue mussel								
15	Nidingen	97	25	88-12	-9.9(-11,-8.3)	0.0000 ---	11	11	0.004 (0.003,0.004)
15	Nidingen		10	03-12	-9.9(-15,-4.7)	0.0027 --	9	8.0	
12	Fjällbacka	95	24	88-12	-6.5(-9.1,-3.9)	0.0001 ---	14	18	0.011 (0.008,0.017)
12	Fjällbacka		10	03-12	-2.4(-7.7,3.3)	0.3600	9	8.1	
14	Kvädöfjärden	90	18	95-12	-4.2(-5.5,-2.9)	0.0021 --	12	12	0.011 (0.009,0.012)
14	Kvädöfjärden		10	03-12	-9.0(-12,-5.8)	0.001 ---	12	13	
	Guillemot egg								
16	Stora Karlsö	248	25	88-12	-7.9(-8.6,-7.2)	0.0000 ---	7	5.0	0.92 (0.83,1.0)
16	Stora Karlsö		10	03-12	-9.4(-14,-4.9)	0.0016 --	9	6.8	

Table S4 Trend for the entire period and the last 10 years (in %) for **DDE** assessed from the annual geometric mean (µg g^{-1} lipid weight) in herring, perch, cod, eelpout, blue mussel and guillemot egg. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ p<0.05, --/++ p<0.01, ---/+++ p<0.001. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's DDE concentration values are estimated from the trend if p<0.05 and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four

Site number (from figure 1)	Matrix Sampling site	N samples	N years	Year	Trend % (95% C.I.)	P	YRQ	LDT %	Last year µg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	513	33	78-12	-7.8(-9.6,-5.9)	0.0000 ---	15	20	0.024 (0.017,0.035)
2	Harufjärden		10	03-12	4.4(-6.2,15)	0.3686	13	16	
8	Ängskärsklubb	501	33	78-12	-7.4(-8.9,-5.9)	0.0000 ---	13	16	0.054 (0.041,0.072)
8	Ängskärsklubb		10	03-12	-6.9(-24,9.8)	0.3690	18	26	
8	Ängskärsklubb spring	650	39	72-12	-6.1(-7.5,-4.7)	0.0000 ---	15	19	0.17 (0.12,0.23)
8	Ängskärsklubb spring		10	03-12	-9.4(-30,11)	0.328	20	33	
11	Landsort	486	34	78-12	-5.6(-6.7,-4.4)	0.0000 ---	12	12	0.13 (0.11,0.17)
11	Landsort		10	03-12	-7.1(-13,-1.5)	0.0194-	9	8.1	
21	Utlängan	391	33	80-12	-4.1(-5.3,-2.9)	0.0000 ---	12	12	0.16 (0.12,0.20)
21	Utlängan		10	03-12	-1.3(-10,7.3)	0.7289	12	14	
21	Utlängan spring	633	38	72-12	-9.7(-11,-8.8)	0.0000 ---	11	12	0.14 (0.12,0.17
21	Utlängan spring		10	03-12	-3.1(-12,6.2)	0.468	12	14	
17	Fladen	556	33	80-12	-7.8(-9.0,-6.5)	0.0000 ---	12	13	0.018 (0.014,0.023)
17	Fladen		10	03-12	-0.06(-5.5,5.4)	0.9302	9	7.8	
13	Väderöarna	289	17	95-12	-4.8(-8.2,-1.5)	0.0074 --	12	13	0.014 (0.010,0.020)
13	Väderöarna		9	03-12	.00(-7.6,7.6)	0.9492	11	11	
	Cod liver								
19	SE Gotland	320	32	80-12	-4.7(-5.9,-3.4)	0.0000 ---	11	12	0.30 (0.24,0.37)
19	SE Gotland		10	03-12	-3.4(-14,7.0)	0.4783	13	15	
17	Fladen	371	32	80-12	-4.4(-5.6,-3.1)	0.0000 ---	12	12	0.18 (0.15,0.23)
17	Fladen		10	03-12	3.6(-1.3,8.5)	0.1281	9	7.0	
	Perch muscle								
4	Holmöarna	269	25	80-12	-9.2(-11,-7.8)	0.0000 ---	12	13	0.021 (0.016,0.028)
4	Holmöarna		9	03-12	2.0(-3.9,7.9)	0.4605	9	8.2	
14	Kvädöfjärden	281	30	80-12	-8.4(-11,-5.8)	0.0000 ---	18	27	0.024 (0.015,0.038)
14	Kvädöfjärden		10	03-12	11(0.31,23)	0.0439 +	14	16	
	Eelpout muscle								
4	Holmöarna	95	11	98-07	-2.0(-10,6.2)	0.595	14	17	0.10 (0.057,0.18) m
4	Holmöarna		10	03-12	-4.8(-17,7.9)	0.4020	15	18	
14	Kvädöfjärden	120	17	95-12	-8.2(-13,-3.5)	0.0022 --	14	17	0.070 (0.044,0.11)

14	Kvädöfjärden		10	03-12	-3.0(-7.9,1.8)	0.1833	9	6.9	
12	Fjällbacka	129	18	95-12	-1.1(-4.6,2.5)	0.5385	12	14	0.075 (0.053,0.11) m
12	Fjällbacka		10	03-12	-2.0(-5.2,1.3)	0.1937	7	4.6	
	Blue mussel								
15	Nidingen	96	28	82-12	-6.5(-8.2,-4.7)	0.0000 ---	14	16	0.018 (0.013,0.025)
15	Nidingen		10	03-12	1.5(-5.5,9.0)	0.6491	11	10	
12	Fjällbacka	99	28	84-12	-6.6(-8.1,-5.0)	0.0000 ---	12	13	0.012 (0.010,0.016)
12	Fjällbacka		10	03-12	1.5(-3.8,7.0)	0.5461	9	7.5	
14	Kvädöfjärden	90	18	95-12	-3.6(-4.7,-2.4)	0.0001 ---	11	11	0.046(0.041,0.051)
14	Kvädöfjärden		10	03-12	-7.4(-10,-4.4)	0.001 ---	12	12	
	Guillemot egg								
16	Stora Karlsö	440	42	69-12	-9.4(-10,-8.7)	0.0000 ---	11	11	6.5 (5.5,7.8)
16	Stora Karlsö		10	03-12	-9.0(-13,-4.7)	0.0020 --	8	6.5	

Table S5 Trend for the entire period and the last 10 years (in %) for **γ-HCH** in herring, perch, cod, eelpout, and blue mussel. assessed from the annual geometric mean (μg g^{-1} lipid weight) in herring, perch, cod, eelpout, and blue mussel. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ p<0.05, --/++ p<0.01, ---/+++ p<0.001. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's γ-HCH / β-HCH concentration values are estimated from the trend if p<0.05 and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four

Site number (from figure 1)	Matrix Sampling site	N samples	N years	Year	Trend % (95% C.I.)	P	YRQ	LDT %	Last year μg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	333	24	87-12	-9.7(-11,-8.3)	0.0000 ---	9	8.2	0.002 (0.002,0.002)
2	Harufjärden		10	03-12	-1.6(-3.9,0.71)	0.1451	6	3.2	
8	Ängskärsklubb	332	24	89-12	-12(-14,-10)	0.0000 ---	11	12	0.002 (0.001,0.002)
8	Ängskärsklubb		10	03-12	-2.4(-4.7,-0.03)	0 .0463 -	6	3.3	
8	Ängskärsklubb spring	276	24	89-12	-11(-13,-9.4)	0.0000 ---	11	11	0.002 (0.002,0.003)
8	Ängskärsklubb spring		10	03-12	0.55(-5.1,6.1)	0.8101	9	8.0	
11	Landsort	354	26	87-12	-13(-14,-12)	0.0000 ---	9	7.2	0.002 (0.002,0.003)
11	Landsort		10	03-12	-9.6(-14,-5.3)	0.0009 ---	8	6.1	
21	Utlängan	360	25	88-12	-14(-15,-13)	0.0000 ---	8	6.1	0.002 (0.002,0.003)
21	Utlängan		10	03-12	-8.8(-13,-4.4)	0.0019 --	8	6.3	
21	Utlängan spring	272	24	87-12	-13(-15,-12)	0.0000 ---	11	11	0.003 (0.002,0.003)
21	Utlängan spring		10	03-12	-2.0(-8.5,4.5)	0.5051	10	9.3	
17	Fladen	381	25	88-12	-15(-17,-13)	0.0000 ---	12	12	0.001 (0.001,0.002)
17	Fladen		10	03 - 12	-14(-21,-8.1)	0.0008 ---	10	9.0	
13	Väderöarna	290	17	95-12	-14(-18,-10)	0.0000 ---	13	15	0.001 (0.001,0.002)
13	Väderöarna		9	03-12	-8.5(-14,-2.9)	0.0090 --	9	7.7	
	Cod liver								
19	SE Gotland	187	24	89-12	-15(-16,-14)	0.0000 ---	8	6.0	0.002 (0.002,0.002)
19	SE Gotland		10	03-12	-11(-14,-6.8)	0.0002 ---	8	5.3	
17	Fladen	194	23	89-12	-18(-20,-16)	0.0000 ---	11	12	0.001 (0.001,0.001)
17	Fladen		10	03-12	-18(-26,-9.8)	0.0010 --	11	12	
	Perch muscle								
4	Holmöarna	179	18	89-12	-3.5(-5.7,-1.4)	0.0028 --	10	9.4	0.004 (0.003,0.005)
4	Holmöarna		9	03-12	-1.4(-3.7,1.0)	0.2181	6	3.2	
14	Kvädöfjärden	197	20	89-12	-4.9(-7.3,-2.5)	0.0004 ---	11	12	0.004 (0.003,0.005)
14	Kvädöfjärden		10	03-12	-1.6(-4.5,1.3)	0.2399	7	4.1	
	Eelpout muscle								
4	Holmöarna	95	11	95-07	-8.7(-12,-5.3)	0.0004 ---	9	7.1	0.005 (0.004,0.006)
4	Holmöarna		9	98-07	-7.7(-13,-2.5)	0 0104-	9	7.5	

14	Kvädöfjärden	120	17	95-12	-7.7(-10,-5.3)	0.0000 ---	10	9.1	0.004 (0.003,0.005)
14	Kvädöfjärden		10	03-12	-3.1(-6.5,0.31)	0.0670 -	7	4.9	
12	Fjällbacka	123	18	95-12	-5.5(-9.1,-1.8)	0.0067 --	13	15	0.004 (0.003,0.007)
12	Fjällbacka		10	03-12	4.5(-3.0,13)	0 2097	11	11	
	Blue mussel								
15	Nidingen	99	27	81-12	-12(-13,-9.8)	0.0000 ---	13	16	0.002 (0.002,0.003)
15	Nidingen		10	03-12	-1.6(-5.5,2.5)	0.3978	8	5.8	
12	Fjällbacka	96	25	83-12	-13(-14,-11)	0.0000 ---	13	15	0.002 (0.001,0.003)
12	Fjällbacka		10	03-12	-1.2(-5.2,2.9)	0.5128	8	5.8	
14	Kvädöfjärden	90	18	95-12	-11(-13,-9.1)	0.0000 ---	10	8.7	0.003 (0.002,0.003)
14	Kvädöfjärden		10	03-12	-7.3(-12,-2.2)	0 .0118 -	9	7.7	

Table S6 Trend for the entire period and the last 10 years (in %) for **β-HCH** in herring, perch, cod, eelpout, blue mussel and guillemot egg. Assessed from the annual geometric mean (μg g^{-1} lipid weight) in herring, perch, cod, eelpout, blue mussel and guillemot egg. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ $p<0.05$, --/++ $p<0.01$, ---/+++ $p<0.001$. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's γ-HCH / β-HCH concentration values are estimated from the trend if $p<0.05$ and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four.

Site number (from figure 1)	Matrix Sampling site	N samples	N years	Year	Trend % (95% C.I.)	P	YRQ	LDT %	Last year μg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	307	23	87-12	-6.3(-7.1,-5.5)	0.0000 ---	7	4.6	0.002 (0.002,0.003)
2	Harufjärden		10	03-12	-3.9(-6.0,-1.7)	0.0030 --	6	3.0	
8	Ängskärsklubb	302	22	89-12	-6.7(-8.4,-5.0)	0.0000 ---	10	9.3	0.005 (0.004,0.006)
8	Ängskärsklubb		10	03-12	-7.2(-13,-1.5)	0 .0200 -	9	8.3	
8	Ängskärsklubb spring	277	24	89-12	-6.5(-7.6,-5.4)	0.0000 ---	8	6.4	0.006 (0.005,0.007)
8	Ängskärsklubb spring		10	03-12	-4.2(-9.4,1.1)	0.1020	9	7.5	
11	Landsort	353	26	87-12	-6.0(-7.0,-5.0)	0.0000 ---	8	6.6	0.009 (0.008,0.010)
11	Landsort		10	03-12	-4.6(-8.8,-.44)	0.0330 -	8	5.9	
21	Utlängan	360	25	88-12	-7.6(-8.7,-6.4)	0.0000 ---	9	7.1	0.006 (0.006,0.008)
21	Utlängan		10	03-12	-5.4(-11,-.11)	0.0450 -	9	7.6	
21	Utlängan spring	272	24	87-12	-7.7(-9.2,-6.2)	0.0000 ---	10	9.6	0.006 (0.005,0.007)
21	Utlängan spring		10	03-12	-5.8(-13,1.2)	0.0900	11	10	
17	Fladen	381	25	88-12	-4.7(-7.2,-2.2)	0.0000 ---	14	16	0.003 (0.002,0.004)
17	Fladen		10	03 - 12	-15(-22,-8.3)	0.0000 ---	10	9.7	
13	Väderöarna	231	14	97-12	-5.4(-8.0,-2.8)	0.0000 ---	9	7.8	0.003 (0.002,0.002)
13	Väderöarna		9	03-12	-8.7(-14,-3.2)	0.0080 --	9	7.6	
	Cod liver								
19	SE Gotland	187	24	89-12	-7.7(-8.7,-6.7)	0.0000 ---	8	5.9	0.008 (0.007,0.009)
19	SE Gotland		10	03-12	-6.6(-11,-1.9)	0.0120 -	9	6.7	
17	Fladen	169	22	89-12	-6.5(-9.5,-3.6)	0.0000 ---	14	17	0.003 (0.002,0.004)
17	Fladen		10	03-12	-12(-21,-3.2)	0.0140 -	12	13	
	Perch muscle								
4	Holmöarna	179	18	89-12	-1.3(-1.4,4.0)	0.3110	12	12	0.004 (0.004,0.005) m
4	Holmöarna		9	03-12	-1.3(-1.4,4.0)	0.2180	6	3.2	
14	Kvädöfjärden	180	18	94-12	.38(-.76,1.5)	0.4990	7	4.3	0.005 (0.005,0.006) m
14	Kvädöfjärden		10	03-12	-1.6(-4.5,1.3)	0.2399	7	4.2	
	Eelpout muscle								
4	Holmöarna	87	11	95-07	-1.9(-8.6,4.9)	0.5500	12	14	0.006 (0.004,0.009) m

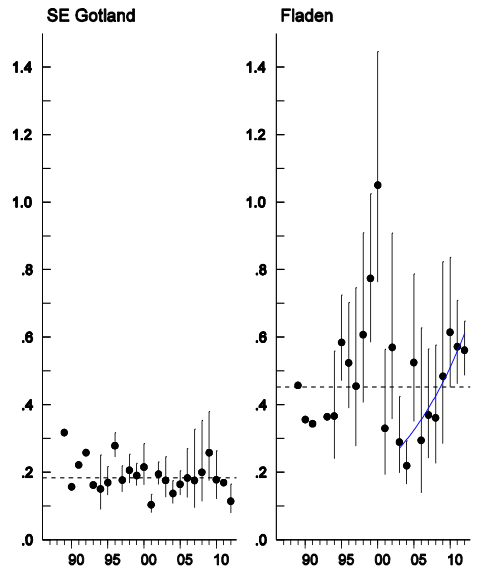
4	Holmöarna		9	98-07	-6.7(-14,.55)	0 0630	11	10	
14	Kvädöfjärden	120	17	95-12	-4.7(-6.1,-3.3)	0.0000 ---	7	4.8	0.005 (0.004,0.005)
14	Kvädöfjärden		10	03-12	-4.2(-7.7,.66)	0.0250	7	5.0	
12	Fjällbacka	105	16	97-12	.21(-3.8,4.2)	0.8770	12	13	0.005 (0.005,0.006) m
12	Fjällbacka		10	03-12	4.0(-4.3,12)	0 2950	12	12	
	Blue mussel								
15	Nidingen	87	23	88-12	-3.4(-6.0,-.91)	0.0100 -	14	16	0.003 (0.002,0.004)
15	Nidingen		10	03-12	-2.9(-8.4,2.5)	0.2460	8	6.4	
12	Fjällbacka	89	22	89-12	-1.6(-2.9,-.34)	0.0150 -	9	7.2	0.003 (0.002,0.003)
12	Fjällbacka		10	03-12	-2.1(-7.5,3.3)	0.3860	8	6.3	
14	Kvädöfjärden	80	16	96-12	-5.9(-8.5,-3.4)	0.0000 ---	10	8.8	0.006 (0.005,0.008)
14	Kvädöfjärden		10	03-12	-8.9(-15,-2.3)	0 .0150 -	9	7.8	
	Guillemot egg								
16	Stora Karlsö	248	25	88-12	-8.5(-9.5,-7.5)	0.0000 ---	8	6.3	0 .12 (0.11,0.14)
16	Stora Karlsö		10	03-12	-6.5(-8.5,-4.5)	0.0000 ---	6	2.6	

Table S7 Trend for the entire period and the last 10 years (in %) for **HCB** assessed from the annual geometric mean (µg g^{-1} lipid weight) in herring, perch, cod, eelpout, blue mussel and guillemot egg. P shows the p-value and – after the p-value means that the trend is negative and + means that the trend is positive; -/+ p<0.05, --/++ p<0.01, ---/+++ p<0.001. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. Last year's HCB concentration values are estimated from the trend if p<0.05 and from the mean (m) if no trend is present. Numbers in brackets are 95% confidence intervals (C.I.). The total number of samples and the number of years for the various time-series are shown in columns two to four

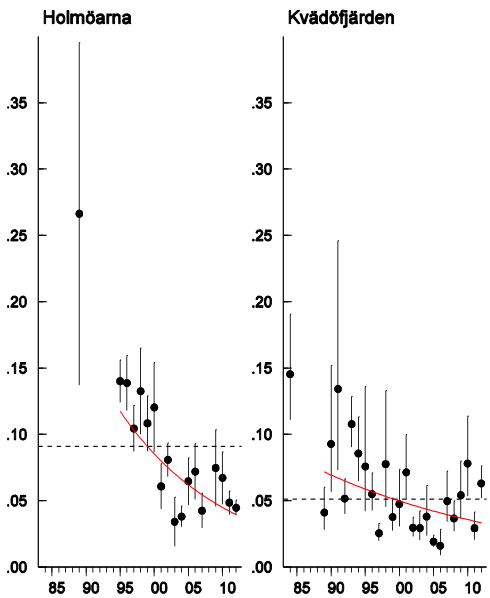
Site number (from figure 1)	Matrix Sampling site	N samples	N years	Year	Trend % (95% C.I.)	P	YRQ	LDT %	Last year µg/g l.w. (95% C.I.)
	Herring muscle								
2	Harufjärden	333	24	87-12	-2.5(-4.2,-0.86)	0.0046 --	11	10	0.015 (0.012,0.019)
2	Harufjärden		10	03-12	4.1(-0.42,8.7)	0.0679	8	6.4	
8	Ängskärsklubb	320	23	89-12	-5.6(-8.2,-3.0)	0.0002 ---	13	16	0.010 (0.007,0.014)
8	Ängskärsklubb		10	03-12	-0.95(-15,13)	0.8501	16	21	
8	Ängskärsklubb spring	277	24	89-12	-6.5(-9.0,-3.9)	0.0000 ---	13	16	0.029 (0.021,0.041)
8	Ängskärsklubb spring		10	03-12	-8.3(-21,4.3)	0.1648	15	19	
11	Landsort	342	25	87-12	-4.1(-6.3,-2.0)	0.0006 ---	13	15	0.020 (0.015,0.027)
11	Landsort		10	03-12	1.5(-7.9,11)	0.7225	12	14	
21	Utlängan	309	25	88-12	-4.6(-7.1,-2.2)	0.0007 ---	13	16	0.018 (0.013,0.025)
21	Utlängan		10	03-12	3.4(-7.0,14)	0.4805	13	15	
21	Utlängan spring	272	24	87-12	-7.0(-9.3,-4.8)	0.0000 ---	13	14	0.022 (0.016,0.029)
21	Utlängan spring		10	03-12	0.63(-9.4,11)	0.8592	13	15	
17	Fladen	380	25	88-12	-5.7(-7.4,-4.0)	0.0000 ---	11	11	0.006 (0.004,0.007)
17	Fladen		10	03-12	4.3(-0.18,8.7)	0.0563	8	6.3	
13	Väderöarna	270	16	95-12	-2.1(-5.8,1.6)	0.2402	13	14	0.006 (0.004,0.009) m
13	Väderöarna		9	03-12	6.7(-0.59,14)	0.0647	11	10	
	Cod Liver								
19	SE Gotland	187	24	89-12	-4.8(-7.1,-2.5)	0.0004 ---	13	14	0.020 (0.014,0.027)
19	SE Gotland		10	03-12	4.1(-3.5,12)	0.2474	11	11	
17	Fladen	194	23	89-12	-4.2(-6.3,-2.1)	0.0005 ---	12	12	0.009 (0.007,0.012)
17	Fladen		10	03-12	-1.4(-8.2,5.5)	0.6613	10	9.9	
	Perch muscle								
4	Holmöarna	179	18	89-12	-3.1(-6.2,-0.03)	0.0457-	13	14	0.007 (0.005,0.010)
4	Holmöarna		9	03-12	7.4(1.9,13)	0.0152 +	9	7.6	
10	Kvädöfjärden	241	25	84-12	-2.3(-5.1,0.46)	0.0936	15	19	0.006 (0.004,0.008)
10	Kvädöfjärden		10	03-12	12(2.7,21)	0.0170 +	12	13	
	Eelpout muscle								
4	Holmöarna	96	11	95-07	-9.9(-19,-1.2)	0.0295-	15	18	0.016 (0.009,0.028)
4	Holmöarna		9	98-07	-13(-27,0.026)	0.0491-	15	20	

14	Kvädöfjärden	121	17	95-12	1.0(-3.6,5.6)	0.6424	14	17	0.014 (0.009,0.022) m
14	Kvädöfjärden		10	03-12	9.4(-2.5,21)	0.1041	14	18	
12	Fjällbacka	131	18	95-12	-0.67(-2.8,1.5)	0.5263	9	8.2	0.008 (0.006,0.010) m
12	Fjällbacka		10	03-12	4.5(-0.51,9.6)	0.0701	9	7.2	
	Blue mussel								
15	Nidingen	97	25	88-12	2.6(0.78,4.5)	0.0068 ++	11	12	3.5 (2.7,4.4)
15	Nidingen		10	03-12	4.6(-0.58,10)	0.0738	9	7.2	
12	Fjällbacka	95	24	88-12	-0.82(-3.4,1.8)	0.5326	14	17	2.4 (1.7,3.4) m
12	Fjällbacka		10	03-12	.25(-4.4,5.2)	0.8734	9	6.8	
14	Kvädöfjärden	90	18	95-12	-1.6(-4.5,1.3)	0.2453	11	11	5.0 (3.7,6.7) m
14	Kvädöfjärden		10	03-12	-3.3(-11,5.0)	0.3813	12	12	
	Guillemot egg								
16	Stora Karlsö	258	26	79-12	-5.2(-6.5,-3.9)	0.0000 ---	10	8.7	0.55 (0.45,0.66)
16	Stora Karlsö		10	03-12	-2.5(-6.7,1.8)	0.2087	8	6.2	

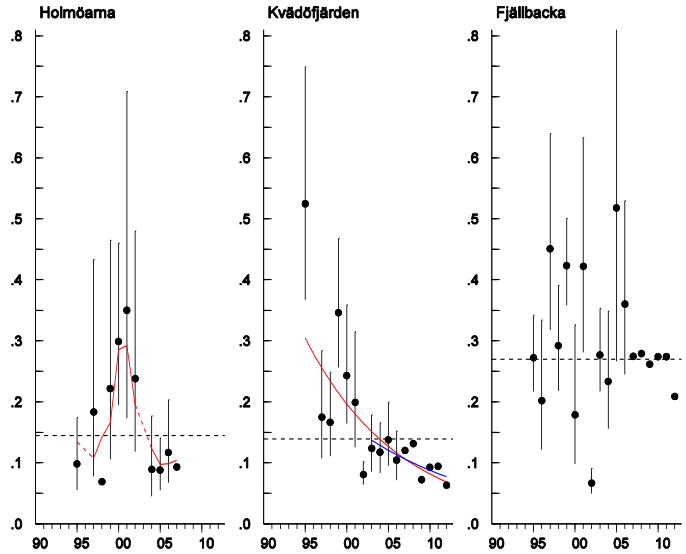
A)


SE Gotland
Fladen
1.4
1.2
1.0
.8
.6
.4
.2
.0
90
95
00
05
10

B)


Holmöarna
Kvädöfjärden
.35
.30
.25
.20
.15
.10
.05
.00
85
90
95
00
05
10

C)



D)

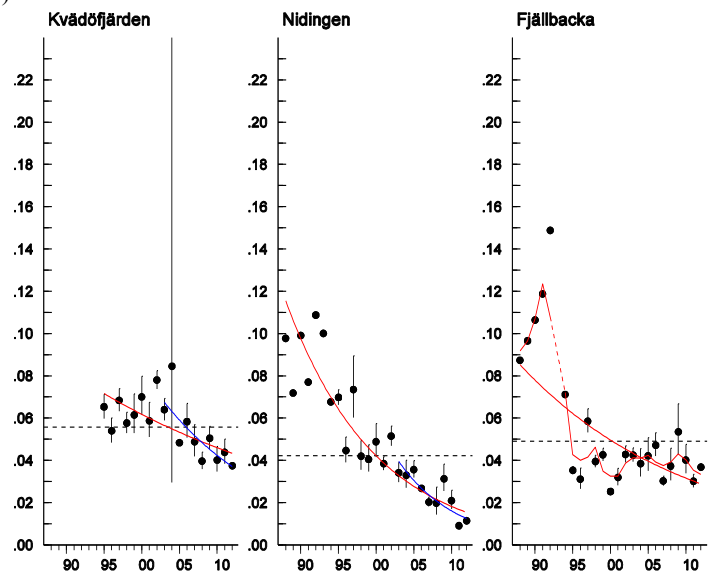


Fig. S1 CB-153 concentrations (µg g^{-1} lipid weight) in **A)** cod liver from SE Gotland and Fladen; **B)** perch muscle from Holmöarna and Kvädöfjärden; **C)** eelpout muscle from Holmöarna, Kvädöfjärden and Fjällbacka; **D)** blue mussels from Kvädöfjärden, Nidingen and Fjällbacka. The linear red lines show significant trends over the whole period and the linear blue lines significant trends for the ten last years ($p<0.05$). The red smoothed lines show non-linear trends ($p<0.05$). The black dotted horizontal line shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration for each year (circles) and 95% confidence intervals for the geometric means.

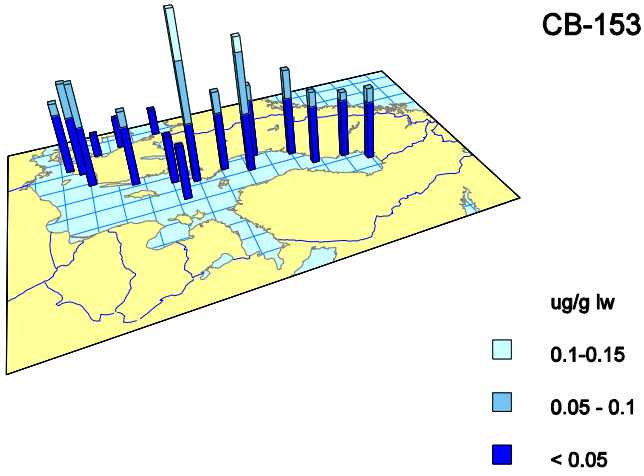
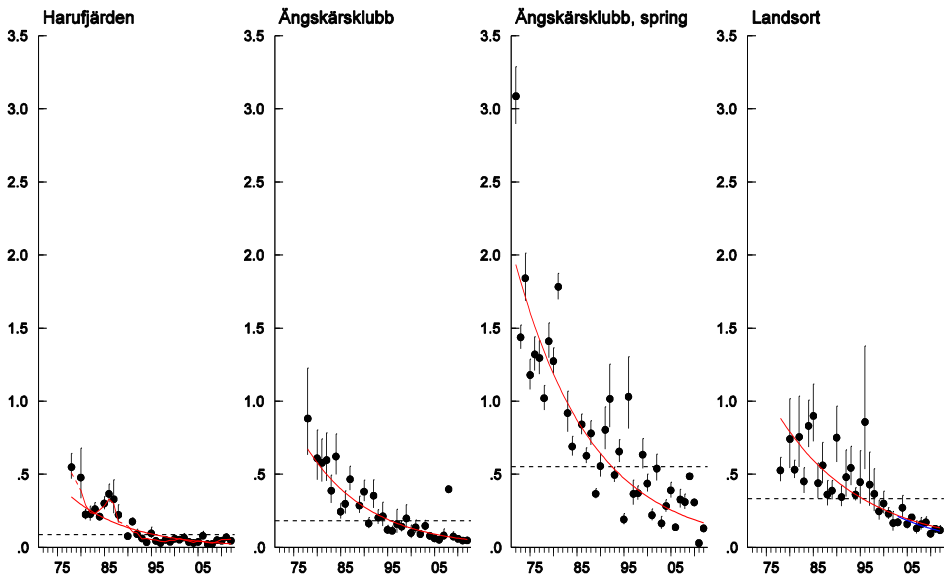


Fig. S2 Spatial variation in concentration (μg g^{-1} lipid weight) in herring muscle of CB-153. Arithmetic mean values from 2010-2012.

A)


Harufjärden
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Ängskärsklubb, spring
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1.0
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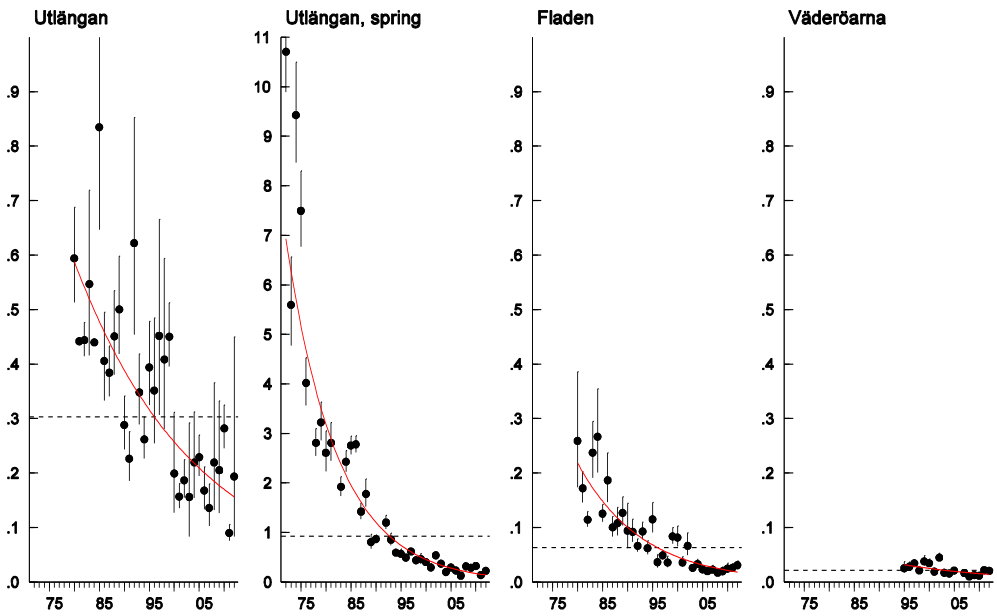
.0
75
85
95
05

B)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Utlängan, spring
Fladen
Väderöarna
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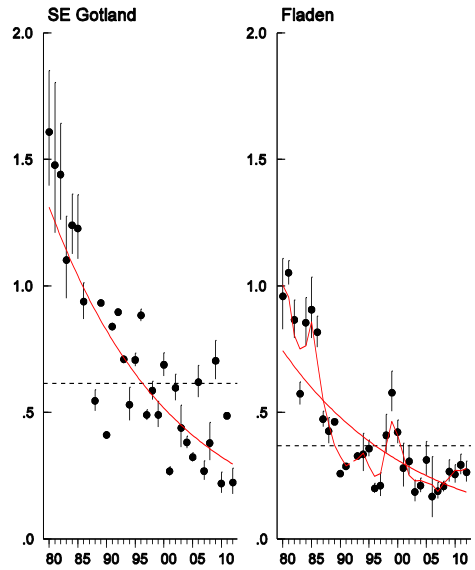
10
9
8
7
6
5
4
3
2
1
0
75
85
95
05

C)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Fladen
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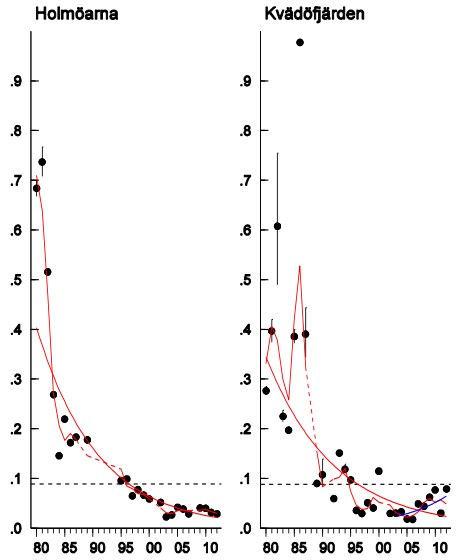
1.0
.5
.0
80 85 90 95 00 05 10

D)


Holmöarna
Kvädöfjärden
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.4
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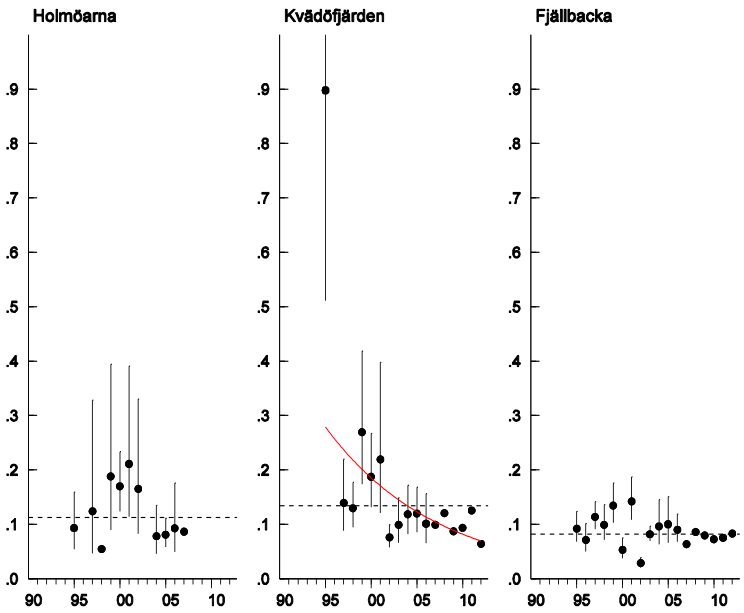
.2
.1
.0
80 85 90 95 00 05 10

E)


Holmöarna
Kvädöfjärden
Fjällbacka
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.6
.7
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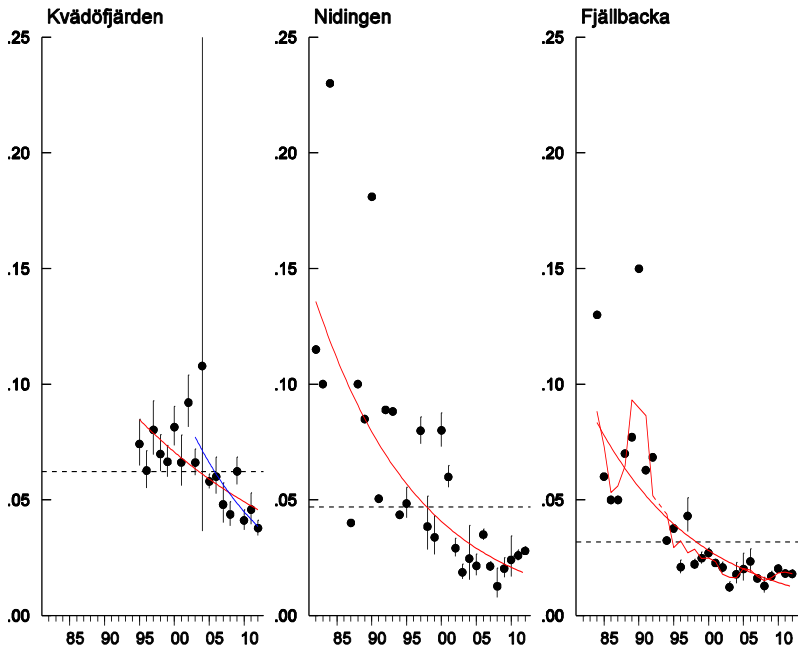
.9
90
95
00
05
10

F)


Kvädöfjärden
Nidingen
Fjällbacka
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.05
.10
.15
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.25
85
90
95
00
05
10

G)

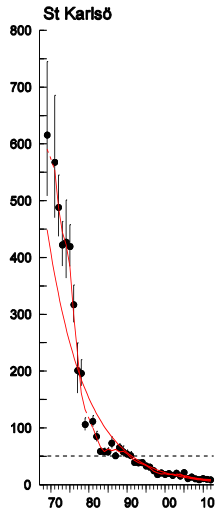
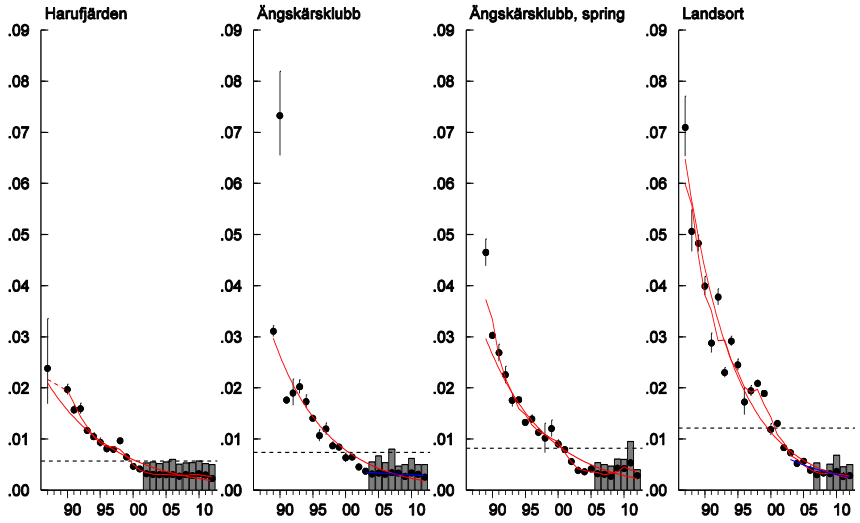


Fig. S3 DDE concentrations (µg g^{-1} lipid weight) in **A)** herring muscle from Harufjärden, Ängskärsklubb (autumn and spring, geometric means from spring are fat adjusted) and Landsort; **B)** herring muscle from Utlängan (autumn and spring, geometric means from spring are fat adjusted), Fladen, and Väderöarna (note the different scale for spring caught herring) **Note** the different scaling for Utlängan spring; **C)** cod liver from SE Gotland and Fladen, geometric means are fat adjusted; **D)** perch muscle from Holmöarna and Kvädöfjärden; **E)** eelpout muscle from Holmöarna, Kvädöfjärden and Fjällbacka; **F)** blue mussels from Kvädöfjärden, Nidingen and Fjällbacka; **G)** guillemot egg from Stora (St) Karlsö.. The linear red lines show significant trends over the whole period and the linear blue lines significant trends for the ten last years ($p<0.05$). The red smoothed lines show non-linear trends ($p<0.05$). The black dotted horizontal line shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration for each year (circles) and the 95% confidence intervals of the geometric means

A)


Harufjärden
Ängskärsklubb
Ängskärsklubb, spring
Landsort
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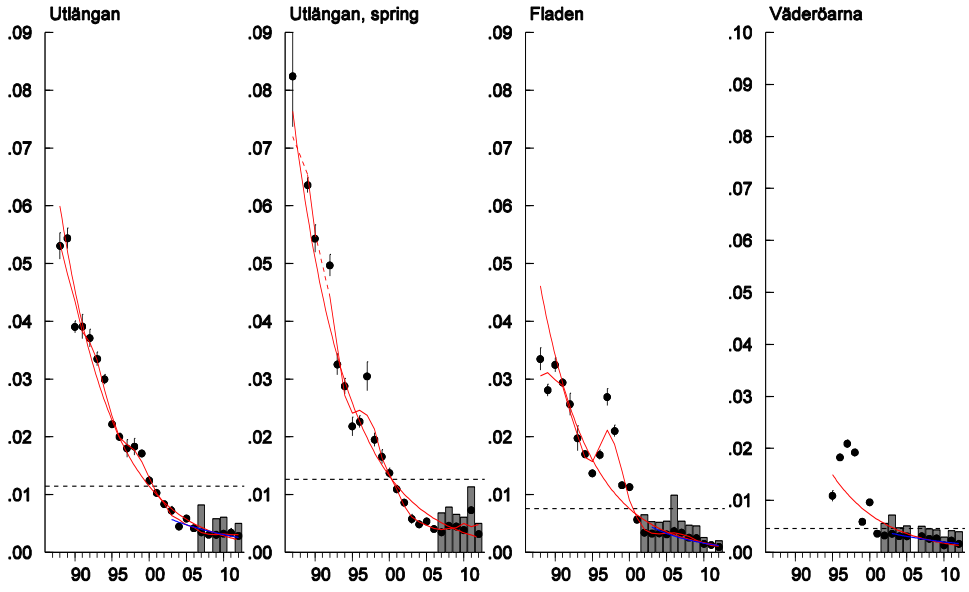
B)


Utlängan
Utlängan, spring
Fladen
Väderöarna
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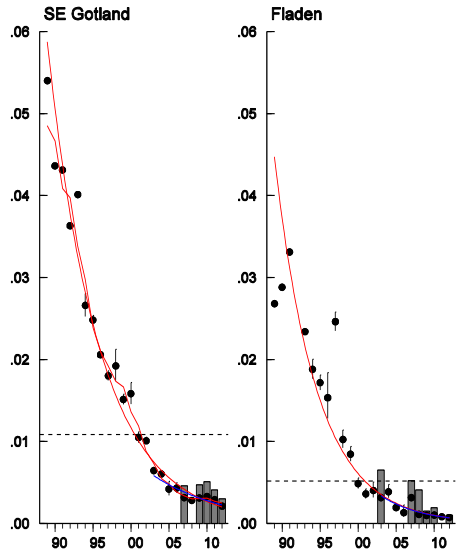
90 95 00 05 10

C)


SE Gotland
Fladen
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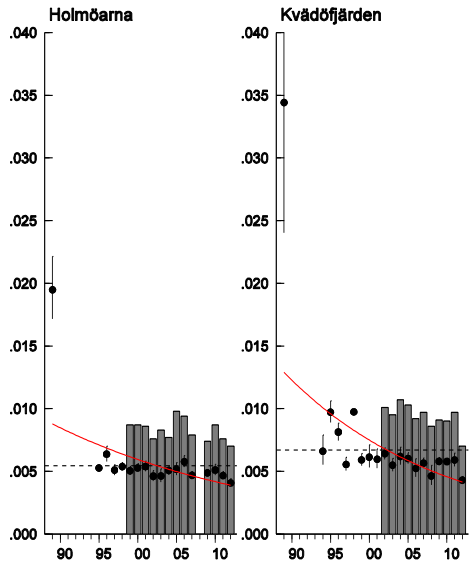
D)


Holmöarna
Kvädöfjärden
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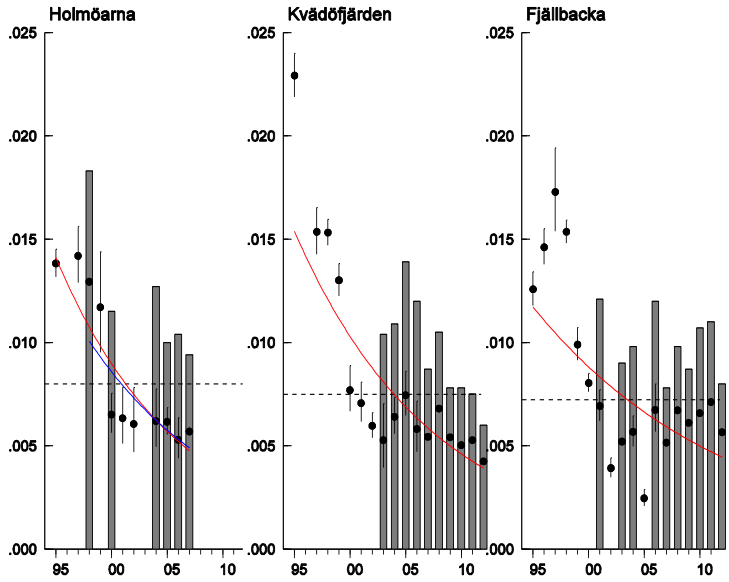
E)


Holmöarna
Kvädöfjärden
Fjällbacka
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.020
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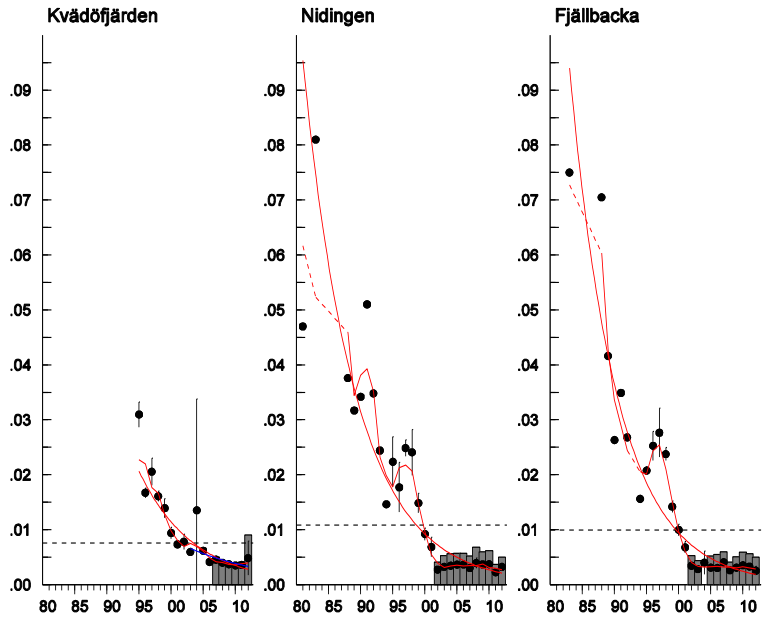
F)


Kvädöfjärden
Nidingen
Fjällbacka
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G)

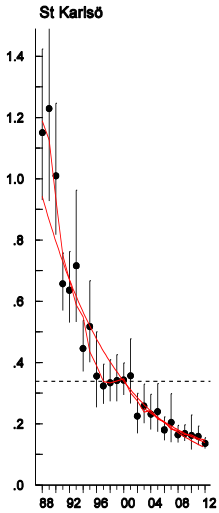
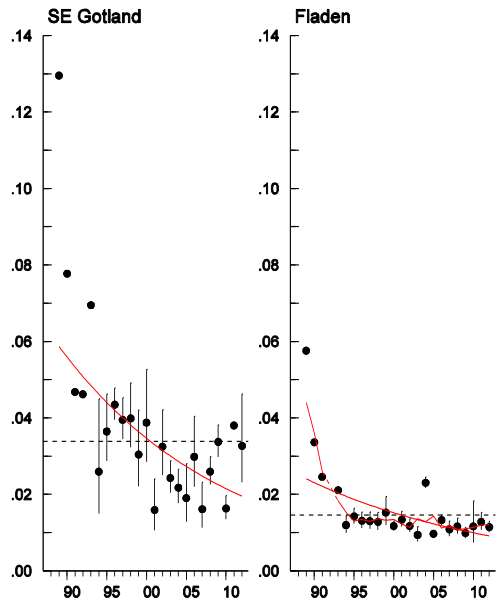


Fig. S4 γ-HCH concentrations (µg g^{-1} lipid weight) in **A)** herring muscle from Harufjärden, Ängskärsklubb (autumn and spring) and Landsort; **B)** herring muscle from Utlängan (autumn and spring), Fladen , and Väderöarna; **C)** cod liver from SE Gotland and Fladen, geometric means are fat adjusted; **D)** perch muscle from Holmöarna and Kvädöfjärden; **E)** eelpout muscle from Holmöarna, Kvädöfjärden and Fjällbacka; **F)** blue mussels from Kvädöfjärden, Nidingen and Fjällbacka. **β-HCH** concentrations in **G)** guillemot egg from Stora (St) Karlsö. The linear red lines show significant trends over the whole period and the linear blue lines significant trends for the ten last years ($p<0.05$). The red smoothed lines show non-linear trends ($p<0.05$). The black dotted horizontal line shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration for each year (circles) and the 95% confidence intervals of the geometric means. In time series where all values in one year were below LOQ, a grey bar shows the maximum LOQ and a dot represents the geometric mean value estimated from the individual LOQs divided by the square root of 2.

A)


SE Gotland
Fladen
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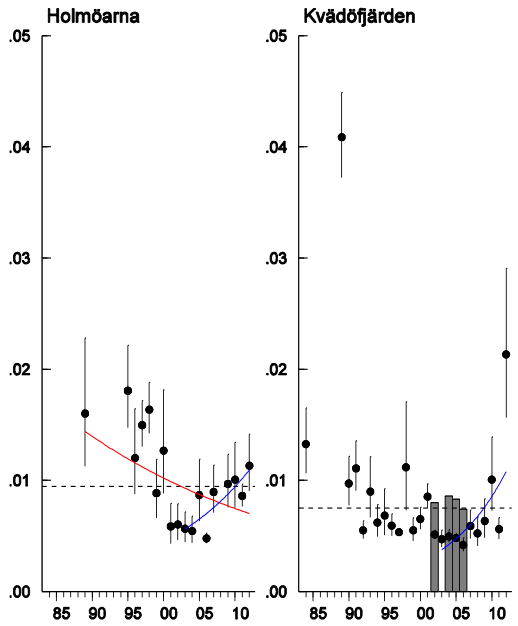
B)


Holmöarna
Kvädöfjärden
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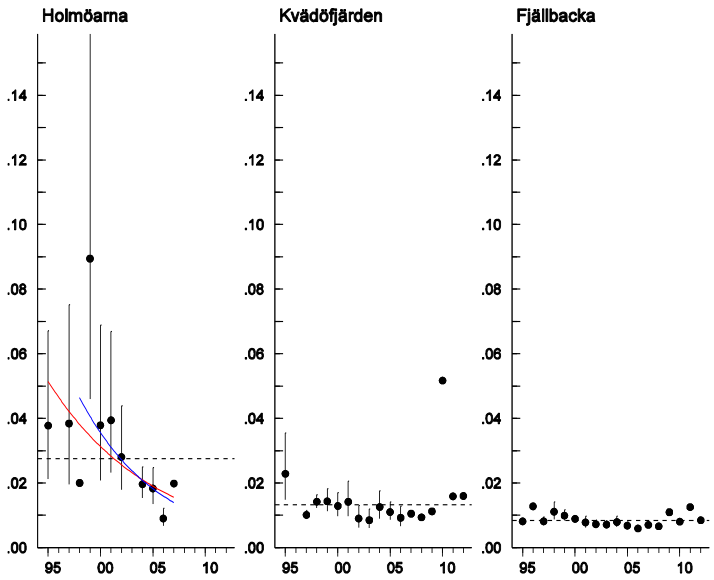
C)



D)

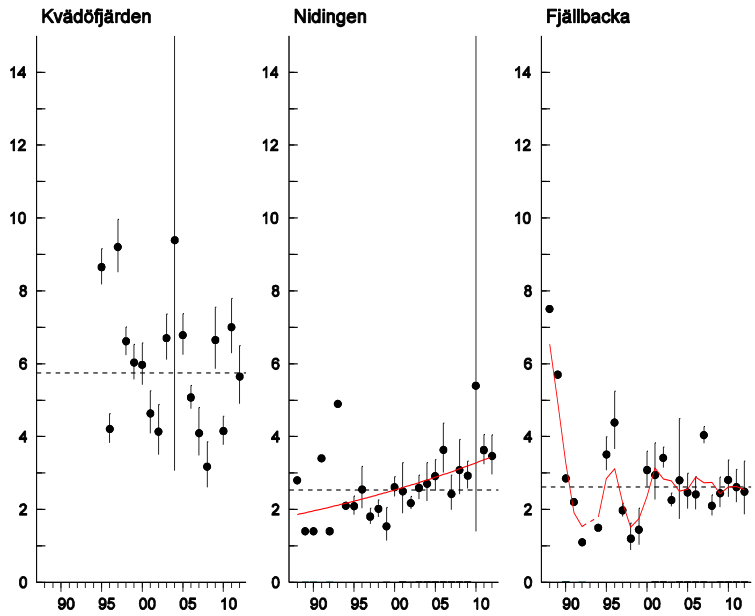


Fig. S5 HCB concentrations (µg g^{-1} lipid weight) in **A)** cod liver from SE Gotland and Fladen; **B)** perch muscle from Holmöarna and Kvädöfjärden; **C)** eelpout muscle from Holmöarna, Kvädöfjärden and Fjällbacka; **D)** blue mussels from Kvädöfjärden, Nidingen and Fjällbacka. The linear red lines show significant trends over the whole period and the linear blue lines significant trends for the ten last years ($p<0.05$). The red smoothed lines

show non-linear trends ($p<0.05$). The black dotted horizontal line shows the geometric mean concentration over the whole period. Each figure displays the geometric mean concentration of each year (circles) and the 95% confidence intervals of the geometric means. In time series where all values in one year were below LOQ, a grey bar shows the maximum LOQ and a dot represents the geometric mean value estimated from the individual LOQs divided by the square root of 2.

IV

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Comparing temporal trends of organochlorines in guillemot eggs and Baltic herring: Advantages and disadvantage for selecting sentinel species for environmental monitoring

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Uria aalge

ABSTRACT

Within Europe, the Marine Strategy Framework Directive (MSFD) is aimed at addressing the chemical status and quality of the marine environment. One of the main goals is to achieve Good Environmental Status (GES) in the marine environment. Environmental monitoring of biota e.g., Baltic herring and guillemot eggs, is conducted annually in Sweden to follow temporal changes in environmental contaminants. To determine the suitability of guillemot eggs as a sentinel species for investigating GES, we compared temporal trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in these two species from single sampling sites within Sweden.

Lipid content from guillemot eggs was consistently high and stable (yearly mean for >40 years, ~12%) compared to that of herring (yearly mean for >20 years, ~3%). A significant decreasing trend of ΣPCDD/F in TEQ WHO_{1998} was observed in guillemot eggs, but no trend was seen in herring. CB118 significantly decreased in both species, but in the last 10 years this decrease was not significant in herring. A number of advantages, such as high lipid content in the egg and a low coefficient of variation make guillemot suitable as a sentinel species. The advantages and disadvantages of using either guillemot eggs or Baltic herring are compared.

1. Introduction

Within Europe, the chemical status and quality of the marine environment are addressed through directives initiated by the European community, and include the Marine Strategy Framework Directive (MSFD; 2008/56/EC). One of the main goals within the MSFD is to achieve Good Environmental Status (GES) in the marine environment by 2020 in regards to eleven qualitative descriptors. Descriptor 8 refers to contaminants in the marine environment and is defined as "concentrations of contaminants at levels not giving rise to pollution effects" (MSFD; 2008/56/EC). This descriptor is determined from quality assessments based on Environmental Quality Standards (EQS), which represent a threshold that should not be exceeded (2008/105/EC). Within the Swedish National Marine Monitoring Programme (SNMMP), ongoing annual monitoring of numerous species from various sites within the Baltic Sea is conducted to follow temporal and spatial changes in environmental contaminants. Collected data contributes to quality assessments within HELCOM (Helsinki Commission) and OSPARCOM (Oslo and Paris Commission).

The Baltic Sea is a large, shallow, semi-enclosed body of water (Stigebrandt, 2001). It has been affected by high contaminant loads for many years. Included amongst these are polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs). These three groups of chemical compounds have caused concern for a number of years due to their high concentrations and potential to cause adverse effects in the environment and to human health (HELCOM, 2004). They are persistent, hydrophobic, bioaccumulate in organisms and sediment, biomagnify in organisms in the aquatic environment and are toxic to both humans and wildlife, and are thus one of the original 12 contaminants included in the Stockholm Convention for Persistent Organic Pollutants (POPs).

Because of the nature of PCDD/Fs and dl-PCBs, and because of the numerous other environmental pollutants in the Baltic Sea,

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0141-1136/

environmental monitoring of various biota is conducted by some EU countries. In Sweden, two species analysed annually are guillemot (*Uria aalge*) eggs and Baltic herring (*Clupea harengus membras*, *C. harengus* on the Swedish west coast), to follow temporal changes in environmental contaminants (OSPAR, 2007). Both of these species have been monitored within the SNMMP for long time periods (>30 years), allowing the comparison of PCDD/Fs and dl-PCBs in both species at sites in close proximity to each other, to investigate the use of guillemot as a sentinel species for GES.

Earlier publications highlight many criteria that should be met to qualify a species as suitable for use as a sentinel species in contaminant monitoring (e.g., Moore, 1966; Coulson, 1972; Peakall et al., 1980; Gilbertson et al., 1987; Oxynos et al., 1993; Furness and Camphuysen, 1997; Burger and Gochfeld, 2004; Goodale et al., 2008; Mondreti et al., 2013). They include features such as a high trophic feeding level in the selected species and thus the ability to accumulate the investigated contaminants to detectable concentrations (Moore, 1966; Coulson, 1972; Gilbertson et al., 1987); known migration and distribution of the species, suitable to the aims of the research (Coulson, 1972; Gilbertson, 1974a; Oxynos et al., 1993; Burger and Gochfeld, 2004); knowledge of and ability to decrease variation within and between samples to maintain good statistical power, even with a limited sample size (Gilbertson et al., 1987; Goodale et al., 2008); good abundance of the sampled population/species so long-term sampling and population conservation does not become an issue (Moore, 1966; Gilbertson et al., 1987; Burger and Gochfeld, 2004); ease of sampling, transport and handling (Moore, 1966; Coulson, 1972; Peakall et al., 1980; Gilbertson et al., 1987; Gilbertson, 1974a; Furness and Camphuysen, 1997; Mondreti et al., 2013); and good knowledge of the selected species biology and ecology (Moore, 1966; Furness and Camphuysen, 1997), useful when considering e.g., metabolism of contaminants, lipid content and possible effect monitoring. When thinking specifically of guillemot from the Baltic Sea as a sentinel species for GES, other suitable features can be identified e.g., high, stable lipid content, individually patterned eggs and ring marking of adult birds allowing age determination from the Stora Karlsö breeding colony.

It is preferable if as many criteria as possible can be met. By contrast, there are a number of less desirable features that should be avoided if feasible. Permission to collect samples of the preferred sentinel species, and limits on the number of samples allowed can hinder monitoring. As part of the knowledge of the biology and ecology of the species, age at first breeding may be important, if there is a known relationship between age and contaminant concentration, as has been shown in other species e.g., herring (Perttilä and Tervo, 1982; Kiviranta et al., 2003; Roots et al., 2003; Roots and Zitko, 2006; Pandelova et al., 2008; Miller et al., 2013). When considering the use of seabird eggs, it can be difficult to link specific adult age and concentration to that of the individual egg, which leaves a gap in knowledge. Besides this, contaminant concentrations are known to vary depending on egg-laying sequence in many bird species (e.g., Nisbet, 1982; Becker, 1992; Bignert et al., 1995; Pastor et al., 1995; Furness and Camphuysen, 1997), which is important to know when designing a sampling strategy, so as to avoid biasing samples.

1.1. Study species

The guillemot is a piscivorous bird species with a circumpolar distribution. Since 1968, guillemot eggs have been sampled from Stora Karlsö (Fig. 1) within the SNMMP. This species overwinters at a number of colonies in the Baltic region, with Stora Karlsö being the largest breeding colony in the Baltic (Österblom et al., 2002).

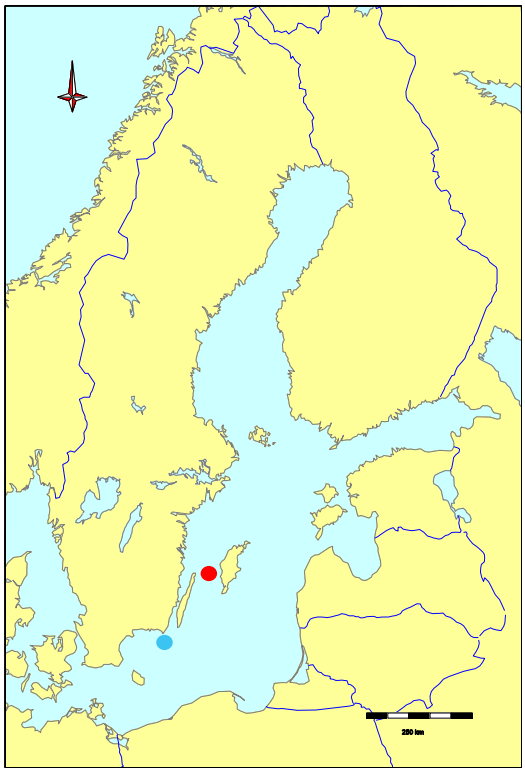

Fig. 1. Map of Scandinavia showing the Baltic Sea and surrounding countries. The red dot indicates the guillemot sampling site, Stora Karlsö. The blue dot indicates the herring sampling site, Utlängan. Both sites are located in the southern Baltic Proper.

Thus, these birds do not migrate far from Stora Karlsö, and their contamination concentrations are therefore locally acquired. Guillemots return to breed each year at the same colony, breeding for the first time in their fourth or fifth year (Birkhead and Hudson, 1977). Breeding starts in early May, and may continue until early June (Hedgren, 1980). Each brood laid typically consists of a single egg (Olsson et al., 2000), although if the first egg is lost, a second egg may be laid (Hedgren, 1980). The main prey species of guillemot from this colony is sprat, *Sprattus sprattus* (Hedgren and Linnman, 1979; Österblom et al., 2001) and herring.

Baltic herring are one of the most commonly used indicator species for contaminant monitoring programmes within the Baltic Monitoring Programme in the HELCOM convention area. Since 1988, Baltic herring have been sampled from Utlängan in the southern Baltic Proper (Fig. 1) for the SNMMP. Baltic herring are a migratory, pelagic species belonging to the family Clupeidae, and are a smaller sub-species of the Atlantic herring (*C. harengus*). When young, herring diet typically consists of zooplankton, with the proportion of nektobenthos e.g., mysids, and fish in the diet increasing as herring size increases (Casini et al., 2004; Parmanne et al., 2006), although season (Flinkman et al., 1992; Parmanne et al., 2006) and location (Arrhenius and Hansson, 1993; Strandberg et al., 1998) can influence diet. Given the bioaccumulation and biomagnification properties of PCDD/Fs and

dl-PCBs, diet is an important factor in these contaminant concentrations (HELCOM, 2004; OSPAR, 2007). Herring are the most dominant commercial fish species in the Baltic Sea. They are also important prey for several piscivorous marine species (Lundin, 2011), including guillemot.

Here we investigate the use of guillemot as a sentinel species for GES. We compare and contrast temporal trends of PCDD/F and dl-PCB concentrations in guillemot eggs from Stora Karlsö and Baltic herring from Utlängan, the nearest sampling site to Stora Karlsö within the SNMMP, and use these results as a basis for evaluating advantages and disadvantages of using guillemot eggs as a suitable matrix for investigating GES.

2. Materials and methods

2.1. Sampling sites and matrices

Since 1968, 20 guillemot eggs have been collected annually in mid-late May (spring) from Stora Karlsö and stored in the Environment Specimen Bank (ESB) at the Swedish Museum of Natural History (SMNH). Ten eggs have been analysed annually for PCDD/Fs and dl-PCBs since 1969. Stora Karlsö is an island approximately 6.5 km off the west coast of Gotland in the southern Baltic Proper (Table 1, Fig. 1). Permit for collection of the guillemot eggs was granted by the Swedish Environmental Protection Agency. Since 1988, Baltic herring have been collected in autumn (September–December) at Utlängan in the southern Baltic Proper to within a circumference of 3 nautical miles around the central coordinates (Table 1, Fig. 1). Herring collected during this period typically contain spring spawning specimens. There are no known local sources of contamination near either of these sites. Collections for both species have been conducted as part of a long-term national temporal trend monitoring programme, financed by the Swedish Environmental Protection Agency and conducted by the SMNH. Arithmetic mean and range (in parenthesis) for shell thickness was 0.61 (0.28–0.82) mm; herring age 4.2 (3–7) years; weight 41 (15–100) g; and total length i.e., length including the tail fin, was 19 (16–25) cm.

2.2. Sample treatment

Guillemot egg contents were removed by drilling a hole in approximately the same place on each egg's equator, and blowing the contents out. Eggs were collected soon after being laid. Embryos were therefore small and the total egg content was thus homogenized (Bignert et al., 2013). Egg length, width and total weight were recorded for each individual. Once the contents were removed, the egg shells were dried and dry shell weight was recorded. Egg shell thickness was measured using a modified micrometer at the site of the blow hole, and shell thickness index (weight (g)*1000/length (mm)*width (mm)) was calculated. Because replacement eggs have been shown to have higher organochlorine concentrations (Bignert et al., 1995), early laid eggs have been collected consistently since the late 1980s. Eggs from 1991, 1993–1996 and 1998–2006 were analysed as 7–10 homogenized individuals; eggs from 1969, 1970–1990, 1992 and 2007–2011 were analysed as pooled samples based on 7–10 individuals. Differences in the number of eggs analysed each year could affect the between-year variation and statistical power to detect a true trend. Approximately 30 g per egg/pool was sent for analysis.

Table 1
Sites, season and years when guillemot and herring have been sampled.

Site, location	Season	Years sampled
Stora Karlsö, southern Baltic Proper 57°17′N, 17°59′E	spring	1969, 1971–2011
Utlängan, southern Baltic Proper 55° 57′N, 15° 47′E	autumn	1988, 1990, 1992–2011

For Baltic herring, sampling and preparation procedures, and reasons for the choice of size and age classes used within the monitoring programme have been presented earlier (Bignert et al., 1998). To increase comparability between years, young specimens of 3–5 years of age were selected with few exceptions, because they tend to represent a more recent picture of contaminant load. To avoid variance due to gender, only female fish were used. For each specimen, body weight, total length, body length, sex, age (determined via scale reading), reproductive phase, state of nutrition, liver weight and sample weight were recorded. Methods for biometric measurements have been presented earlier (Miller et al., 2013). The number of herring analysed has varied over the years, with either 7–15 individuals being pooled, or 8–10 individual fish being analysed. As with the guillemot eggs, the differing numbers analysed in each year could affect the between-year variation and statistical power to detect a true trend (Bignert et al., 2014). To minimize the between-year and spatial variation in concentrations of lipid soluble contaminants due to differences in the amount of subcutaneous fat, pure muscle tissue without subcutaneous fat was analysed. Dorso-lateral herring muscle tissue of approximately 10 g per specimen/pool was removed under strict laboratory protocols, and sent for analysis. Due to the right-skewed distribution in PCDD/Fs and dl-PCB concentrations, geometric means have been calculated for each species to give a single concentration for each congener in each year.

2.3. PCDD/F and dl-PCB analytical methods

Analysis of PCDD/F and dl-PCBs for both species were carried out at the Department of Chemistry, Umeå University, Sweden. Congener specific results were produced using validated, accredited methods, using both traditional and simplified methods. Eggs collected between 1996 and 2000 were analysed according to a simplified method due to budgetary restrictions. In all other years, the traditional analysis method was used and included all 2,3,7,8 substituted congeners found in measurable concentrations. Both methods were included in analyses here as otherwise large gaps in temporal data existed, and by following the congeners measured using the simplified method, no large changes due to analytical methods were observed. For the traditional analysis, extraction method is described by Wiberg et al. (1998), the clean-up method by Danielsson et al. (2005), and the instrumental analysis (GC-HRMS) by Liljelind et al. (2003). Further details of analysis methods, including expanded measurement uncertainty and reproducibility can be found in Miller et al. (2013). Individual congeners analysed from herring and guillemot between 1996 and 2000 using the simplified method have not been included here i.e., only CB118, due to differences between traditional and simplified methods. However, TEQ values calculated based on these individual congeners between these years have been included so overall trends in contaminant concentration can be seen. Lipid content was determined for both sampling matrices during analysis.

For the simplified analysis method, samples were extracted, lipid contents determined, and lipids removed. Four marker congeners, specifically 2,3,4,7,8-PeCDF and PCBs 77, 126, and 157, were analysed using LC-LC-GC-ECD as described in Haglund et al. (2004). Two HPLC columns were used to obtain enough selectivity, a silica-column and a PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column, with *n*-pentane as the mobile phase. The appropriate eluent fraction from the silica column was heart-cut transferred to the PYE column, which was eluted in the forward direction until

15 s before the first marker (PCB 157) was expected to elute. The PYE column was then back-flushed via the silica column, and the resulting peak was transferred to the GC using a loop interface. The GC was equipped with an early vapour exit and was operated under concurrent solvent vaporisation conditions. Finally, the four markers were quantified and the remaining sixteen PCDD/Fs, seven mono-*ortho* PCBs, and PCB 169 were estimated using their ratios to 2,3,4,7,8-PeCDF, PCB 157, and PCB 126, respectively, which had been calculated using samples from 1995. These ratios were controlled with data from 2001 and found to be consistent (i.e., within the measurement uncertainty of the method). Thus, the simplified method should give a good estimate of individual congener concentrations as well as TEQ values. WHO-toxic equivalents (TEQs) were calculated using the individual congener concentrations and the 1998 toxic equivalency factors (WHO-TEFs) published by the World Health Organisation (van den Berg et al., 2006).

2.4. Statistical treatment of the data

For both species, yearly geometric means were calculated and plotted over the whole time period for 2,3,7,8-TCDD, 2,3,7,8-TCDF, ΣPCDD/F TEQ WHO_{1998} values and CB118. Where appropriate, log linear regression was used, and a 3-year running smoother applied. The annual decrease ±95% confidence intervals is shown, as well as the r^2 and p value, significant at $p < 0.05$. Statistical power was calculated for each temporal trend shown, as well as the number of years it takes to detect an annual change of 10% with a power of 80% and a significance level of $p < 0.05$. Because the time series for guillemot eggs is longer than the herring sampled at Utlängan, these same statistical tests were applied for the most recent 10 years of data (2002–2011) for both species to examine whether changes in these chemicals were comparable when investigating the same time period. Statistical software PIA (www.amap.no) was used for the trend analysis.

3. Results

3.1. Temporal trends and concentrations

Guillemot eggs have both a higher (mean 12.3%, range 9.6–16.1%) and more consistent lipid content compared to herring (Fig. 2A, B), with a significant decreasing trend over time seen in lipid content from herring of −3.7% per year (mean 2.8%, range 1.6–9.3%). The coefficient of variation (CV) for lipid content in guillemot is 10%, while in herring the lipid content is more variable with a CV of 27% (Table 2). Significant decreasing trends were seen for both 2,3,7,8-TCDD and 2,3,7,8-TCDF from guillemot eggs (figures not shown). 2,3,7,8-TCDD showed a relatively consistent decreasing trend over time, with an annual decrease of −5.1% (Table 2). In the most recent 10 years (2002–2011), 2,3,7,8-TCDD continued to show a significant decreasing trend (Table 3). By contrast 2,3,7,8-TCDF showed an increase in concentrations in the late 1970s to early 1980s (approx. 90–100 pg/g lipid weight (l.w.)), followed by a decrease to below 50 pg/g l.w. A further peak was observed in the early 2000s (approx. 50 pg/g l.w.). A significant decrease, with an annual change of −1.2% was seen for the whole monitoring period (Table 2). However, in the most recent 10 years, 2,3,7,8-TCDF showed no significant decreasing trend (Table 3). ΣPCDD/F TEQ WHO_{1998} shows a relatively consistent decrease over time (Fig. 3A) similar to 2,3,7,8-TCDD; however, the influence of 2,3,7,8-TCDF can be seen, in particular in the early 2000s, when 2,3,7,8-TCDF increased briefly, which is reflected in the ΣPCDD/F TEQ WHO_{1998} values. Nonetheless, a strong significant decrease was observed in the last 10 years (Table 3).

2,3,7,8-TCDD in Baltic herring from Utlängan showed an overall significant decreasing trend (Table 2). The highest concentration was observed in the first year of analysis, at just over 7 pg/g l.w. In the early 2000s, a slight increase was seen before concentrations continued decreasing (figure not shown). Concentrations of 2,3,7,8-TCDF started at approximately 56 pg/g l.w., with subsequent decreasing concentrations. A slight increase was seen again in the early 2000s, similar to that seen in 2,3,7,8-TCDD, with concentrations then continuing to decrease (figure not shown). In contrast both to the individual congener concentrations, and to ΣPCDD/F TEQ WHO_{1998} seen in guillemot eggs, ΣPCDD/F TEQ WHO_{1998} shows no trend over time, with mean values generally staying between 20 and 40 pg/g l.w. (Fig. 3B). An annual decrease of −0.8% was seen (Table 2). In the last 10 years, neither of the congeners or ΣPCDD/F TEQ WHO_{1998} showed significant decreases (Table 3).

CB118 was selected as representative of dl-PCBs because this is one of the most dominant congeners for this group of chemicals. A significant decreasing trend is seen for CB118 in guillemot eggs for

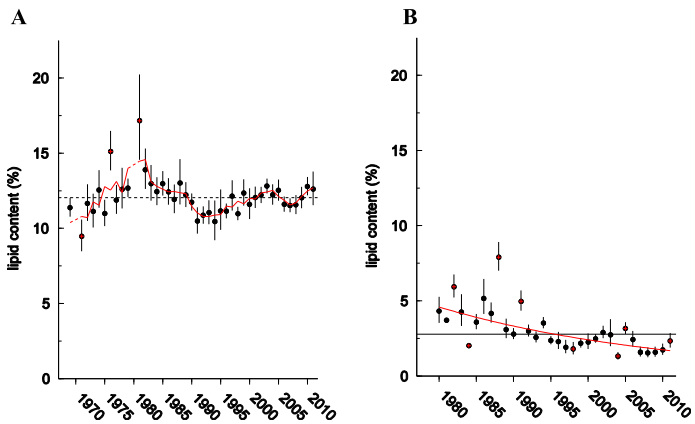

Fig. 2. A). Annual average lipid content ±95% confidence intervals, expressed as percentage fat, for guillemot eggs and B) herring muscle. The thin black line on both graphs represents the average lipid content over the entire time series. The red line in 2a is a 3 year running smoother, whilst in 2b only the regression line is shown.

Table 2
Total number of years that data has been analysed for each variable, the 2011 concentration estimated from the regression line and its ±95% confidence interval, annual percentage change ±95% confidence interval, r^2 reporting the coefficient of determination, *p*-value (significant at <0.05), the lowest detectable change (% per year) for a 10 year period with a between-year variation of 80%, coefficient of variation (CV) around the regression line as a measure of between-year variation for selected contaminants in guillemot eggs and Baltic herring for the whole time period. Statistical power for all presented time series is >99%.

Variable	Total *n* of years	Mean concentration 2011 (95% CI)	Annual change (%) (95% CI)	r^2	*p* Value	Lowest detectable change (%)	CV (%)
Guillemot egg							
Lipid content %		11.9 (11.2, 12.7)	−0.05 (−0.32, 0.23)	0.00	<0.733	3.7	10
2,3,7,8-TCDD, pg/g l.w.	37	80.3 (71.9, 89.5)	−5.1 (−5.6, −4.7)	0.94	<0.001	5.9	17
2,3,7,8-TCDF, pg/g l.w.	37	39.2 (31.6, 48.5)	−1.2 (−2.1, −0.3)	0.19	<0.007	12	33
ΣPCDD/F TEQ WHO_{1998}, ng/g l.w.	41	0.78 (0.68, 0.90)	−2.8 (−3.3, −2.2)	0.72	<0.001	7.9	22
CB118, μg/g l.w.	33	0.89 (0.76, 1.1)	−8.1 (−8.9, −7.4)	0.95	<0.001	9.1	25
Herring							
Lipid content %	32	1.6 (1.3, 1.9)	−3.7 (−4.7,−2.6)	0.63	<0.001	1.5	27
2,3,7,8-TCDD, pg/g l.w.	16	1.4 (1.1, 1.9)	−4.0 (−6.4, −1.7)	0.49	<0.003	12	33
2,3,7,8-TCDF, pg/g l.w.	17	21.9 (16.7, 28.8)	−1.8 (−4.0, −0.4)	0.17	<0.094	11	32
ΣPCDD/F TEQ WHO_{1998}, ng/g l.w.	22	0.024 (0.020, 0.031)	−0.8 (−2.6, 1.0)	0.03	<0.385	9.7	27
CB118, μg/g l.w.	15	0.018 (0.014, 0.023)	−5.3 (−7.8, −2.7)	0.6	<0.001	10	29

the whole time period and for the most recent 10 years (Fig. 4A). An annual decrease of −8.1% was seen (Table 2), with the decrease being relatively consistent over time. An outlier with a higher concentration (approximately 4 μg/g l.w.) was seen in 2001; however, the overall trend continued to decrease. In Baltic herring, CB118 showed a significant decreasing trend over time, with an annual decrease of −5.3% (Fig. 4B, Table 2). However, in the last 10 years, this decrease appeared to have levelled out.

In 2011, the geometric mean concentrations in guillemot eggs of 2,3,7,8-TCDD, 2,3,7,8-TCDF, ΣPCDD/F TEQ WHO_{1998}, and CB118, as estimated from the regression line (Table 2), were all approximately 30–60 times higher compared to herring, with the exception of 2,3,7,8-TCDF, which was only twice as high. The CV for 2,3,7,8-TCDD, 2,3,7,8-TCDF, ΣPCDD/F TEQ WHO_{1998} and CB118 in guillemot egg ranged from 17 to 33%, while the CV in herring ranged from 27 to 33% (Table 2). For the most recent 10 years the CV for guillemot eggs ranged from 9.9 to 20%, and for herring 21 to 34% (Table 3).

4. Discussion

For several reasons, guillemot eggs appear to be a very suitable sentinel species for investigating GES in regards to descriptor 8, here compared to Baltic herring, which has already been suggested as a sentinel species under descriptor 8. Herring also has many merits as a monitoring species - they are used for human consumption, support a large commercial fishery within the Baltic Sea, are a key prey species for marine mammals, fish and birds (Lundin, 2011) and are a commonly used species in HELCOM and OSPAR contaminant assessments. However, some biological and physical factors make guillemot eggs more suitable for investigating descriptor 8 in regards to GES. Both advantages and disadvantages of using guillemot eggs compared to Baltic herring for monitoring GES are outlined below. Temporal trends presented in the result section are discussed.

4.1. Temporal trends and concentrations of PCDD/Fs and dl-PCBs

4.1.1. Guillemot eggs

Concentrations of PCDD/Fs, illustrated here by 2,3,7,8-TCDD, 2,3,7,8-TCDF and ΣPCDD/F TEQ, were generally decreasing by 1–5% per year in guillemot eggs over the whole time period. 2,3,7,8-TCDD and ΣPCDD/F TEQ also showed decreasing trends of a similar magnitude for the most recent 10 years, whereas no significant trend was seen for 2,3,7,8-TCDF during that time period. The dl-PCBs, illustrated by CB118, decreased at a faster rate, approximately 8% per year during the whole time period and even faster during the most recent 10 years, around 14% per year. The rate of decrease for CB118 is of a similar magnitude as the decreases seen for non-dioxin like PCBs in guillemot eggs (Bignert et al., 2013). Decreases seen in these contaminants in guillemot since the 1970s is likely a result of a number of measures taken to reduce the concentrations of these substances. In 1977 and 1978, the use of two classes of dioxin contaminated herbicides, chlorinated phenoxyacetic acids (2,4,5-T containing products; SFS 1977: 246) and chlorophenols (Swedish EPA, 1977), were banned. During the

Table 3
For the most recent 10 years (2002–2011) of data, the annual percentage change ±95% confidence interval, r^2 reporting the coefficient of determination, *p*-value (significant at <0.05), the lowest detectable change (% per year) for the most recent 10 year period with a between-year variation of 80%, coefficient of variation (CV) around the regression line as a measure of between-year variation, and statistical power for the selected variables in guillemot eggs and Baltic herring for the whole time period.

Variable	Annual % change (95% CI)	r^2	*p* Value	Lowest detectable change (%)	CV (%)	Power
Guillemot egg						
Lipid content	−0.03 (−1.1, 1.1)	0.00	<0.911	1.5	4.4	1.0
2,3,7,8-TCDD	−5.7 (−8.2, −3.1)	0.77	<0.001	3.5	9.9	1.0
2,3,7,8-TCDF	−0.2 (−6.5, 6.1)	0.00	<0.91	9.0	25	0.87
ΣPCDD/F TEQ WHO_{1998}	−5.3 (−7.7. −2.8)	0.76	<0.001	3.4	9.6	1.0
CB118	−14 (−19, −8.8)	0.84	<0.001	7.0	20	0.98
Herring						
Lipid content	−5.1 (−12,1.9)	0.26	<0.13	10	28	0.78
2,3,7,8-TCDD	−6.0 (−14, −1.9)	0.28	<0.12	12	32	0.68
2,3,7,8-TCDF	2.8 (−5.6, 11)	0.06	<0.47	12	34	0.64
ΣPCDD/F TEQ WHO_{1998}	−0.10 (−5.5, 5.3)	0.00	<0.92	7.7	21	0.95
CB118	−2.0 (−8.4, 4.4)	0.6	<0.50	9.2	26	0.86

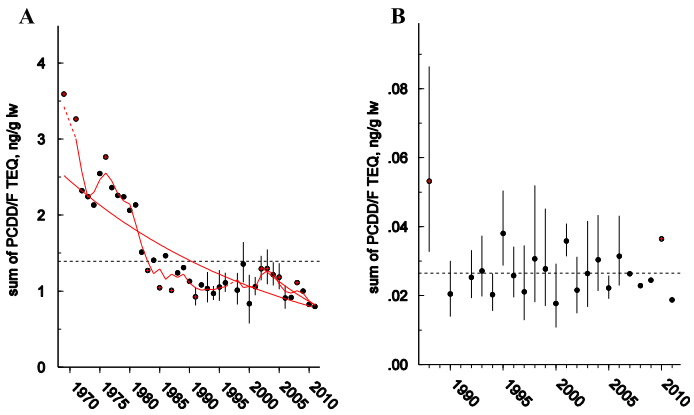

Fig. 3. Annual geometric mean ΣPCDD/F TEQ WHO_{1998} values (ng/g l.w.) ±95% confidence intervals for A) guillemot eggs from Stora Karlsö and B) Baltic herring from Utlängan. The thin black line in both graphs represents the geometric mean value over the entire time series. A 3 year running smoother and regression line is shown in 3a.

1990s, efficiency of incineration and flue gas treatment at municipal solid waste incinerators were greatly improved, and the pulp and paper industry gradually changed their bleaching technology from elemental chlorine (EC) bleaching to elemental chlorines free (ECF) and total chlorine free (TCF) bleaching (Strömberg et al., 1996). This appears to have had a positive effect towards decreasing concentrations. The steeper decrease observed for the dl-PCB, CB118, may be explained by the early national (SFS 1971: 385) and international action to ban open uses of PCB (OECD, 1973), the subsequent ban of all new uses in 1978 and, finally, the total ban of PCBs in Sweden brought into effect in 1995 (SFS, 1995: 1095).

There is no OSPAR Environmental Assessment Criteria (EAC) or Environmental Quality Standards (EQS) established for concentrations of PCDD/Fs or dl-PCBs in guillemot eggs. However, the concentrations of 2,3,7,8-TCDD (approximately 80 pg/g l.w.) and 2,3,7,8-TCDF (approximately 39 pg/g l.w.) recorded in 2011 and presented here were about eight to ten times higher compared to concentrations measured in guillemot eggs from the coast of California collected in 1993 (Jarman et al., 1997), when there were few actions taken worldwide to reduce concentrations of these substances. This implies that the Baltic is still severely polluted by PCDD/Fs. Concentrations of CB118 in common guillemot from two sampling sites in northern Norway reported mean concentrations between 392 and 450 ng/g l.w. in eggs sampled in 2003 (Helgason et al., 2008), approximately half of the mean concentration measured here in eggs from 2011 (890 ng/g l.w.).

4.1.2. Herring

The monitoring of PCDD/Fs and dl-PCBs in herring from Utlängan started almost two decades later than in guillemot eggs, which may be a contributing factor explaining why only 2,3,7,8-TCDD and CB118 show significantly decreasing trends over the whole monitoring period. The decrease seen for CB118, which ceased during the last 10 years, is similar to the time trend seen for the non-dioxin like PCB, CB153, in herring (Bignert et al., 2013). No trend was indicated during the last 10 years for any of the substances

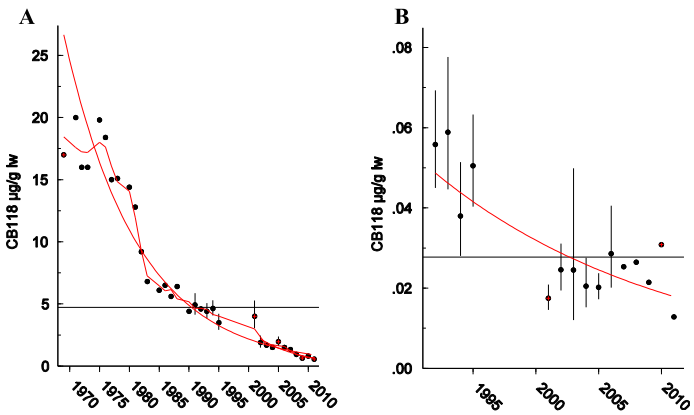

Fig. 4. Annual geometric mean CB118 (µg/g l.w.) ±95% confidence intervals in A) guillemot eggs from Stora Karlsö, and B) herring from Utlängan. The thin black line on both graphs represents the geometric mean value over the entire time series. The red line in 4a is a 3 year running smoother and the regression line, while in 4b only the regression line is shown.

measured in herring. Lipid content in herring from Utlängan showed a significant decreasing trend over time (this study, and Miller et al., 2013). This is surmised to play a role in observed contaminant concentrations (Miller et al., 2013) because leaner fish within the same species may have higher concentrations on a lipid weight basis compared to fat fish (HELCOM, 2004; Berntssen et al., 2005; Bignert et al., 2013), and is thus likely one contributing factor in the lack of temporal changes observed. Another possibility is herring age. PCDD/F and dl/PCB concentrations are known to be related to age (Isosaari et al., 2006; Karl and Ruoff, 2007; Pandelova et al., 2008), with older fish having higher concentrations compared to young fish due to longer exposure times. Average age of herring from Utlängan was 4.1 years.

The EQS currently used for PCDD/F concentrations in fish (3.5 pg WHO_{2005} TEQ/g w.w.) is based on human food limits set by the European Commission (COM 1259, 2011). The concentrations are normally overestimated compared to the threshold set for WHO_{2005} TEQs if estimated in WHO_{1998} TEQs. The concentrations measured in herring from Utlängan in 2011 are approximately six times lower than the EQS, although sample preparation here involves the removal of skin and subcutaneous fat, which indicates higher concentrations could be expected if these were included. By contrast, a study by Karl and Ruoff (2007) found highest concentrations of PCDD/F WHO_{1998} TEQ of 6.97 ng/kg w.w. from herring fillets off the coast of Latvia in 1999. Vuorinen et al. (2012) also found slightly higher concentrations compared to this study of PCDD/F WHO_{1998} TEQ in herring sampled in 2003 from the Baltic Proper (International Council for the Exploration of the Sea, ICES zone 29) and the Gulf of Finland (ICES zone 32), of 1.37 ng/kg w.w. and 1.68 ng/kg w.w. respectively. Herring in that study were younger, between 1 and 3 years, and skin and subcutaneous fat were included. For PCBs, EQSs are not established, so to evaluate concentrations of CB118, the OSPAR EAC of 24 ug/kg l.w. is used (OSPAR, 2009). The concentration of CB118 in herring from Utlängan in 2011 is slightly below the EAC, at 18 ug/kg l.w.

4.2. Advantages of using guillemot eggs

4.2.1. Trophic level

Concentrations of contaminants in the eggs of marine seabirds closely reflect concentrations seen in adult female birds (Furness and Camphuysen, 1997; Goodale et al., 2008) and therefore reflect the adult trophic level. Guillemot from Stora Karlsö in the Baltic Sea feed predominantly on sprat and herring (Hedgren and Linnman, 1979) thus making them a higher trophic level organism compared to herring; however, stable isotope data was not available for this study. Many pollutants such as PCDD/Fs and dl-PCBs bioaccumulate and biomagnify i.e., concentrations increase with increasing trophic level (HELCOM, 2004; OSPAR, 2007). Here, a significantly decreasing trend was seen for ΣPCDD/F TEQ WHO_{1998} in guillemot eggs over time, whereas no trend was detected in herring from Utlängan over the same period. Concentrations observed in guillemot eggs for these values were also an order of magnitude higher than in herring muscle tissue. For the individual congeners examined, concentrations were anywhere from 2 to 60 times higher in guillemot eggs compared to herring muscle tissue.

4.2.2. Migration and distribution

Knowledge of the range, distribution and migratory habits of the species under study is important when selecting a sentinel species (Gilbertson et al., 1987). Within the Baltic Sea, herring are a migratory (Parmanne, 1990) pelagic species, thus the specific location where contaminants are accumulated is not known. By contrast, guillemot remain in ice-free areas of the southern Baltic Proper year round (Stolt et al., 1991). Contaminant concentrations measured from guillemot eggs are therefore locally acquired, a criterion pointed out earlier (Coulson, 1972; Gilbertson, 1974a; Oxynos et al., 1993; Burger and Gochfeld, 2004). This is an advantage if the aim of the research is to investigate local contaminant concentrations. Furthermore, guillemot has a circumpolar distribution (Österblom, 2006), an advantage highlighted in Moore (1966). This allows comparisons from different geographic locations possible.

4.2.3. Low within- and between-year variation

Within- (Goodale et al., 2008) and between-year variation, specimen and chemical analytical variation (Bignert et al., 2014), as well as the necessity of a small coefficient of variation (CV) was stressed (Gilbertson et al., 1987) as criteria for being able to detect small changes in contaminant concentration even when only a small sample size is available. Here, the largest difference in CV between herring and guillemot eggs for the whole time period was seen for 2,3,7,8-TCDD, being higher in herring than in guillemot eggs (33% compared to 17%). Thus the estimated number of years required to detect an annual change of 10% with a power of 80%, one sided test with $\alpha = 0.05$, was also higher (12 years in herring as compared to 8 years in guillemot eggs). This overall trend of lower CV and therefore number of years to detect a trend being lower in guillemot eggs compared to herring held even when examining the most recent 10 years of data. Changes in contaminant concentrations can therefore be detected faster; alternatively, a smaller trend may be detected when using guillemot eggs as a matrix due to the lower variability, an advantage in any environmental monitoring programme.

4.2.4. Abundance

Guillemot populations in the Baltic Sea are healthy, comprising approximately 45,000 individual birds (Österblom et al., 2001). Ensuring the proposed sentinel species is abundant (Moore, 1966; Gilbertson et al., 1987; Burger and Gochfeld, 2004) regularly reproduces, and develops to reproductive age relatively quickly are key, in particular if long-term monitoring is the aim. A high rate of re-laying is essential for population conservation purposes when choosing to sample bird eggs (Gilbertson, 1974b). Guillemot are known to lay a second egg to replace early-laid eggs that are lost (Hedgren, 1980) e.g., removed for sampling. Re-laying helps to reduce the impact of egg collection on the population size although the late-laid egg may be more compromised due to e.g., a higher contaminant load. Using eggs also removes any need for killing breeding age birds, further decreasing negative impacts on population size (Oxynos et al., 1993) and avoiding ethical issues associated with killing birds (Furness and Camphuysen, 1997). Baltic herring are also numerous, being the dominant commercial fishery species in the Baltic Sea.

4.2.5. Individually patterned eggs, shell thickness and adult ring marking

The issue of whether contaminant concentrations in marine birds' eggs are a reflection of the life-time body burden of the laying female, rather than recent pollution uptake, has been raised by Furness and Camphuysen (1997). In the example of shags (*Phalacrocorax aristotelis*), the egg concentrations are known to be closely related to the concentration in the adult body tissue. An age-concentration relationship exists for PCDD/Fs and dl-PCBs in Baltic herring (e.g., Perttilä and Tervo, 1982; Kiviranta et al., 2003; Roots et al., 2003; Roots and Zitko, 2006; Pandelova et al., 2008; Miller et al., 2013) and for other contaminants and species, hence the effort to sample herring of a similar size and presumably age. An advantage of using guillemot eggs is that every egg is laid with a

distinct pattern of stripes and spots characteristic to the individual female, making it possible to identify which female the egg belongs to (Bignert et al., 1995). A large proportion of guillemot nesting at Stora Karlsö have been ring marked (Österblom et al., 2001), thus, with a little extra effort, the laying female of a collected egg can be identified, and information on e.g., age, recovered. Investigations of female age/egg contaminant concentrations are therefore possible. Further to this, a number of fledgling and adult guillemot are available either killed as bycatch in fishing equipment (Österblom et al., 2002), or killed when they jumped from the cliff at Stora Karlsö. This allows the possibility to investigate the tissue concentrations in adult birds recovered from equipment, as well as in fledglings, and compare with concentrations reported in the eggs.

Further to this, effect monitoring is less developed for herring compared to guillemot. By using guillemot eggs, simple physical parameters such as egg shell thickness, length and shell index can be measured. This allows for investigations between contaminant concentrations and physical changes over time, and has been used previously e.g., for investigating reproductive failure in white tailed sea eagles (*Haliaeetus albicilla*) due to contaminants (e.g., Helander et al., 2002).

4.2.6. Ease of sampling, handling, transport

Being abundant (Österblom et al., 2001), breeding on land in dense colonies (Olsson and Reutergårdh, 1986), being located comparatively close (80 km) to the Swedish east coast (Olsson and Reutergårdh, 1986) as well as the eggs being immobile, makes guillemot eggs from Stora Karlsö easy to collect, handle and transport, when compared to e.g., marine mammals, or even adult birds. These criteria have all been outlined previously, either for marine birds or their eggs (Moore, 1966; Coulson, 1972; Peakall et al., 1980; Gilbertson et al., 1987; Gilbertson, 1974a; Furness and Camphuysen, 1997; Mondreti et al., 2013).

4.2.7. Metabolism

Gilbertson (1974b) states that there is a small possibility of dieldrin and PCB metabolism occurring in the eggs of common terns (*Sterna hirundo*). Metabolism of PCDD/Fs and dl-PCBs in guillemot eggs cannot be ruled out here, however being able to quickly transport the sampled eggs and freeze them in the ESB is an advantage. Baltic grey seals (*Halichoerus grypus*), which feed on herring, have similar PCDD/F and dl-PCB concentrations as grey seals from the North Sea (unpublished data). By contrast, herring from the Baltic Sea have considerably higher concentrations than herring from the North Sea, indicating grey seals are able to metabolise these chemicals. Conversely, concentrations of these contaminants in adult guillemot from the Baltic region are considerably higher than in Baltic grey seals (Bignert et al., 1989), suggesting that guillemot do not metabolise PCDD/Fs or dl-PCBs (unpublished data), therefore metabolism of these contaminants in the eggs also seems unlikely. Here, concentrations of the analysed contaminants e.g., ΣPCDD/F TEQ WHO_{1998}, were an order of magnitude higher in guillemot eggs (ng/g l.w.) compared to in herring (pg/g l.w.). This result is likely due to trophic level, but a lack of metabolism of these contaminants in guillemot may also contribute.

4.2.8. Lipid content

Organochlorines dissolve in lipids, thus the lipid content of the organism being studied is important in detecting the true concentration of organochlorines. A very low lipid content, for example due to starving (Furness and Camphuysen, 1997), can result in elevated concentrations of organochlorines when expressed on a lipid weight basis. PCDD/Fs and dl-PCBs are lipophilic (fat soluble) persistent organic pollutants (HELCOM, 2004; Berntssen et al., 2005) and therefore accumulate to higher concentrations in biological material containing high lipid content. Guillemot eggs have a comparatively high (10–15%) lipid content when compared to herring from Utlängan (2–4%). Lipid content in the eggs was also reasonably stable for the last 40 years. By contrast, herring from Utlängan exhibited an average lipid content of <3% for the last 20 years of data collected, and overall, a significant decreasing temporal trend in lipid content was seen. Changes in lipid content can, as already mentioned, influence concentrations of PCDD/Fs and dl-PCBs on a lipid weight basis, as shown in Miller et al. (2013). Furthermore, as variable lipid content affects the ability to detect changes by increasing within- and between-year variation (Gilbertson et al., 1987), using a matrix with high, stable lipid content is advantageous for ensuring contaminant concentrations are accurately measured. Here, for example, an annual percentage decrease of −5.3% was detected for ΣPCDD/F TEQ WHO_{1998} in guillemot eggs in the most recent 10 years, but in herring, this annual percentage change was considerably lower, at only −0.1% decrease per year, as expected from both the implied trophic feeding level impact, and the lower, less stable lipid content in herring.

4.3. Disadvantages of using guillemot eggs

Alongside the many advantages of using guillemot eggs as a sentinel species for investigating GES when compared to herring, there are also some disadvantages that must be considered. These disadvantages are especially important if designing/starting a monitoring program.

4.3.1. Permission to collect

Within Sweden, permission must be granted by the Swedish Environmental Protection Agency to collect guillemot eggs, with a maximum of 20 eggs allowed to be collected yearly. Measurements of physical parameters from all 20 eggs are made, while chemical analysis is taken from 10 eggs annually. For the purposes of monitoring, 10 eggs per year are enough to achieve high statistical power. Here, statistical power to detect a trend of 10% in ten years was >99% for all of the time series presented, with annual changes of between −1.2 and −8.1% able to be detected in guillemot. However, statistical power decreased, in particular for herring, when only the most recent 10 years were examined, increasing the chance of committing a type I error. However, if other, non-monitoring research is planned then 20 eggs are rather restrictive. In terms of conservation of population numbers, it is advantageous to limit numbers collected each year. This ensures population numbers remain abundant, which in turn allows for ongoing monitoring. By contrast, Baltic herring are a commercial fishery species, and as such no limit is imposed on the number allowed. This makes herring preferable for other research purposes where greater numbers of individuals are necessary. However, permission to collect guillemot eggs is not required in every country. For example, no ethical permits are required for collection in Iceland (Jörundsdóttir et al., 2006; Jörundsdóttir, 2010), where eggs are collected for human consumption. Therefore this disadvantage is on a country-by-country basis.

4.3.2. Age at first breeding

The older age at first breeding of guillemots (4–5 years, Birkhead and Hudson, 1977) compared to herring (2–4 years) (Swedish Board of Fisheries, 2010) could be viewed as a disadvantage, because this implies a longer exposure time in guillemots prior to first egg laying. As already mentioned, contaminant concentrations in seabirds' eggs closely reflects that of the adult bird (Furness and Camphuysen, 1997; Goodale et al., 2008), although this may vary with species. However, concentrations in eggs may in some cases reflect recent exposure (Burger et al., 1999; Morrissey et al., 2010) rather than life-

time burden. While this has been addressed within the advantages of using guillemot eggs, a precise relationship between age of laying female and contaminant concentrations in eggs has not been established for guillemot. This should be kept in mind, in particular in regards to a point raised by Furness and Camphuysen (1997), of whether contaminant concentrations in marine birds' eggs are a reflection of the life-time body burden of the laying female, rather than recent pollution uptake. The relationship between guillemot age, contaminant concentration and concentrations in their eggs from the colony at Stora Karlsö warrants further attention. In contrast, age can be determined for herring using scales or otoliths, and thus a well-defined relationship between herring age and contaminant concentration has been established (e.g., Perttilä and Tervo, 1982; Kiviranta et al., 2003; Roots et al., 2003; Roots and Zitko, 2006; Pandelova et al., 2008; Miller et al., 2013). Thus age as a confounding factor can essentially be removed when examining e.g., PCDD/F and dl-PCB concentrations, which is not the case for guillemot eggs.

5. Conclusion

The decreasing trends, seen for PCDD/Fs and dl-PCBs in guillemot eggs since the beginning of the 1970s shows that measures taken to reduce concentrations have been successful. However, the concentrations in the Baltic are still elevated. For herring, only a few of the congeners have shown decreasing trends since the beginning of the 1990s and none during the most recent 10 years. The possibility to follow changes in pollutant load in the environment over time highlights the use of guillemot as a sentinel species. The advantages of using guillemot eggs clearly outweigh the disadvantages, with some disadvantages able to be avoided e.g., collection of early rather than late-laid eggs. Baltic herring have a number of merits to their use, and in fact are the most commonly used indicator species for contaminant monitoring in biota within the Baltic Monitoring Programme (BMP) in the HELCOM convention area (HELCOM, 2004). However, for the purposes of investigated GES, in particular Directive 8, the use of guillemot eggs has a number of advantages over herring e.g., effect monitoring, ease of collection and handling, low between and within-year variation, locally acquired contaminants etc. Two of the clearest advantages are the high lipid content and the lower CV generally for all of the analysed variables in eggs compared to herring. The largest drawback to using guillemot eggs lies in the limitations imposed on collection, and the need for information linking individual female age and contaminant concentration to eggs.

Worthwhile future research directions include matching sampled eggs to known, ringed female birds for the purpose of investigating the relationship between the laying female's age and contaminant concentration, and the egg concentration; examining detrimental effects of elevated organochlorine contaminants in late-laid eggs of guillemot; and exploring trophic level using stable isotope analysis. A number of other environmental pollutants e.g., perfluorinated substances and brominated flame retardants, have been analysed retrospectively in guillemot eggs during the last decade, resulting in both decreasing and increasing trends of high statistical power, which shows that guillemot eggs also are suitable for analyses of POPs other than PCDD/Fs and dl-PCBs. Guillemot eggs are clearly a very suitable matrix when examining essential attributes for a good sentinel species for investigating GES.

Author contributions

AM – wrote the manuscript.

EN – works with the data presented here, and reviewed earlier drafts of the manuscript.

SD – prepared graphs and statistics.

SF – works with the data presented here, and reviewed earlier drafts of the manuscript.

PH – in charge of the chemical analyses, reviewed earlier drafts of the manuscript.

AB – contributed ideas to the forming of the manuscript, and reviewed earlier drafts of the manuscript.

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Temporal trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and dioxin-like polychlorinated biphenyls in mothers' milk from Sweden, 1972–2011

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ABSTRACT

Temporal trends of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in mothers' milk are still quite rare. Data are particularly scarce when it comes to concentrations from the last decade, 2000 and onwards. The aims of the present study were to assess temporal trends of PCDD, PCDF and DL-PCB in mothers' milk from Stockholm, 1972–2011 and to compare the results with previous analysis of some of the older samples.

The samples were analyzed by high resolution GC/MS and results were statistically evaluated for the periods, 1972–2011 and 2002–2011. The rate of which ∑PCDDs, ∑DL-PCBs and the ∑TEQ are decreasing (on pg/g fat WHO-TEQ2005) is higher in the last decade compared to the 40 year period, 1972–2011. A similar trend is indicated, but not confirmed, for ∑TEQ of PCDFs, probably due to too many PCDF congeners below LOQ in the period 2002–2011. Concentrations of ∑PCDDs, ∑PCDFs, ∑DLPCBs and ∑TEQ, all expressed as pg/g fat on TEQ-WHO2005-basis, show a statistically significant decline over time, 5.8–6.8% per year, 1972–2011. The last ten years the annual declines for ∑PCDDs, ∑DL-PCBs and ∑TEQ are 9.2–11% and for ∑PCDF, 5.4%. Congener specific trend analysis, 2002–2001, of PCDDs and DL-PCBs showed the same pattern, while the PCDF congeners showed no such general trend. The results from the re-analysis showed good agreement with slightly lower ∑TEQ1998 pg/g fat concentrations in six out of seven samples and mean difference of 13% in ∑TEQ1998. The study shows that time series can be elongated from previous studies, as long as the sample population remains the same.

1. Introduction

WHO initiated biomonitoring of persistent organic pollutants (POPs) in mothers' milk in 1976 and so far five rounds of the global survey have been carried out during 1987–2010 (UNEP, 2012; WHO, 2009). The aim of the global survey is to assess the concentrations of POPs, so far with emphasis on polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like (DL) biphenyls (DL-PCBs), hereafter referred to as "dioxins" in this article. The mothers' milk program is now part of the Stockholm Convention (Stockholm Convention). Apart from on the Stockholm Convention website, dioxin concentrations in mothers' milk around the world have been summarized in several publications (LaKind, 2007; Srogi, 2008; Tanabe and Minh, 2010). In particular, LaKind (2007) has made a comprehensive compilation and analyzed global data on $\sum TEQ_{1998}$ concentrations, including temporal trends. To summarize the findings by LaKind, the concentrations are decreasing globally (1975–2005). However, the study did not report a decline in the last years, 2003–2005. National/regional time trend studies show similar decreasing trends of "dioxins", including studies from Australia (Harden et al., 2004), Russia (Mamontova et al., 2005), Norway (Becher et al., 2002), Sweden (Lignell et al., 2009), and Japan (Hori et al., 1999). In a review from 1996, temporal trends up to that point are summarized and in short, studies from Germany, The Netherlands, Norway and Sweden all show the same general declining trend of ∑TEQ values (Alcock and Jones, 1996). Croes and coworkers summarized the results from a WHO mothers' milk survey from the European countries which show decreasing trends of ∑TEQ concentrations (Croes et al., 2013). In contrast, a study from Japan reports *status quo* of the "dioxin" concentrations, 1998–2004 (Kunisue et al., 2006). Studies with samples from the last decade are more limited but show decreasing "dioxin" concentrations in Belgium (Croes et al., 2012), Ireland (Pratt et al., 2012) and Spain (Schuhmacher et al., 2009). In Sweden, Norén and coworkers started monitoring of mothers' milk already in 1970. A large number of articles/reports concerning levels and time trends of POPs in mothers' milk from this monitoring program are summarized (with some new original data included) in a review article from the year 2000 (Norén and

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Table 1
Data on sampling year, number of donating mothers in each pool, which samples that were previously analyzed for PCDD/Fs and DL-PCBs and double samples analyzed.

Year	Mothers	Mean age (years)	*Primiparae* (%)	Previously analyzed[a]	Two samples
1972	75	27–28	NA[b]	YES	
1974	90	27–28	NA[b]	YES	
1976	78	27–28	NA[b]	YES	
1978	87	27–28	NA[b]		
1980	116	27–28	NA[b]	YES	
1988/89	140	30	58		
1990	60	30	65	YES	
1991	60	28	56	YES	
1992	40	29	65	YES	
1995	20	30	65		
1997	40	31	65	YES	
1999	20	31	70		
2000	10; 10	30	75		YES
2001	20	30	80		
2002	10; 10	30	80		YES
2003	15	31	67		
2004	10; 10	30	80		YES
2007	10; 10	27	100		YES
2008	9; 9	28	100		YES
2009	10; 10	31	100		YES
2010	10; 9	30[c]	100		YES
2011	11; 11	30	100		YES

[a] Norén and Meironyte (2000).
[b] Not available.
[c] Only available for 5 out of 19 mothers.

Meironyte, 2000), which reports decreasing levels of "dioxins" over time. This is supported by Lignell et al. (2009), who report decreasing levels of POPs, including "dioxins", in mothers' milk from Sweden during 1996–2006.

Only a few time series with multiple year sampling of mothers' milk exists, especially with samples from the last decade. In this study we re-analyze a set of samples from the original (composite) time trend study (Norén and Meironyte, 2000), to test comparability, as well as new samples from 1999 to 2011. This will help us answer if the decreasing concentrations of "dioxins" are leveling off, i.e. what is the trend for the first decade of the 21st millennia, and if it is possible to compare the established concentrations from previous studies directly. Hence, the aims of the present study were to assess temporal trends of PCDD, PCDF and DL-PCB in mothers' milk from Stockholm, 1972–2011, and to compare the results with previous analyses of some of the older samples.

2. Materials and method

Three major concerns were considered when choosing mothers' milk samples; i) to ensure comparability between the new and the previous analyses of Swedish mothers' milk; ii) to add samples taken in the past to fill gaps in the previous time trend and iii) to expand the aforementioned time trend study ending in 1997 (Norén and Meironyte, 2000) to obtain data ranging from 1972 to 2011. In total, 30 samples were analyzed and eight of these were non-identical samples from a given year. The samples consisted of pooled mothers' milk from multiple donors, all healthy native Swedish, but were not exclusively from *primiparae*. Further information concerning sample composition is presented in Table 1.

The samples, 50 g each, were provided by the Swedish Environmental Specimen Bank, Department of Environmental Research and Monitoring, Swedish Museum of Natural History. The samples were prepared in house before they were shipped, on dry ice, to Eurofins GfA Lab Service GmbH, Hamburg, Germany, for analysis according to the method described by Reis et al. (2007). In brief the method could be described as follows: ^{13}C-labeled surrogate standards were added to the samples followed by liquid–liquid extraction and subsequent gravimetric lipid determination prior to multiple column clean-up, including carbon column purification. The purified extracts were analyzed by GC/HRMS.

2.1. Statistical methods

To test for significant log-linear trends for PCDDs, PCDFs and DL-PCBs, log-linear regression analysis was performed for the entire investigated time period and for the most recent 10 years using the yearly arithmetic mean values. In cases where the regression line had a poor fit, a 3-point running mean smother was checked for statistical significance in comparison with the regression through an ANOVA (Nicholson et al., 1998). Potential outliers in the temporal trends were detected using a method described by Hoaglin and Welsch (1978). The suspected outliers are merely indicated in the figures and were included in the statistical calculations. Values below level of quantification (LOQ) were replaced by LOQ/2 prior to the statistical analyses. Power analysis was also carried out. The power was fixed to 80% and the minimum possible trend to be detected during a monitoring period of 10 years at a significant level of 5% was estimated. A significance level of 5% was used for all tests.

3. Results

Individual PCDD and PCDF congener concentration data are presented in Table 2, for each of the pooled mothers' milk samples analyzed, and with concentrations given on a weight basis per gram fat. Table 2 also includes ∑PCDD/PCDF concentrations, but expressed on basis of WHO-TEQ$_{1998}$ and WHO-TEQ$_{2005}$, in pg/g fat (Van den Berg et al., 1998; Van den Berg et al., 2006). The corresponding data are reported in Table 3 for DL-PCBs, ∑DL-PCBs and ∑TEQ (WHO$_{1998}$ and WHO$_{2005}$).

Based on the results presented in Tables 2 and 3, it is possible to calculate and present temporal trends of the analytes as determined in Stockholm mothers' milk from 1972 to 2011. Time series analyses were performed for all analytes and selected temporal trend data are presented as graphs in Figs. 1–4.

Temporal trends, 1972–2011, for ∑PCDDs, ∑PCDFs, ∑DL-PCBs and ∑TEQ (i.e. the sum of ∑PCDDs, ∑PCDFs and ∑DL-PCBs), based on pg/g fat WHO-TEQ$_{2005}$ concentrations, are presented in Fig. 1a–d). The relative annual decrease over the 40 year period for PCDDs, PCDFs, DL-PCBs and ∑TEQs are 6.1%, 6.1%, 6.9% and 6.5% respectively, with $p < 0.001$ in each case. The relative annual decreases over the last ten years for PCDDs, PCDFs, DL-PCBs and ∑TEQs are 10% ($p < 0.001$), 7.3% ($p < 0.001$), 12% ($p < 0.012$) and 10% ($p < 0.002$), respectively. The number of years required to detect an annual change of 10% varied between 6 and 10 years for the groups in Fig. 1a–d). The power to detect a 10% annual change was 100% for all of the full time series. The smallest possible trend to detect varied between 3.7 and 9.4% change per year during a decade.

Temporal trends, 1972–2011, for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD and 1,2,3,6,7,8-HCDD, based on concentrations in pg/g fat, are presented in Fig. 2a–c). The relative annual decrease over the 40 year period for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD and 1,2,3,6,7,8-HCDD are 6.1%, 5.9% and: 6.0% with $p < 0.001$ in each case. The annual relative decrease over the last ten years for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD and 1,2,3,6,7,8-HCDD are 11%, 10% and: 10%, respectively, with $p < 0.001$ in each case. The number of years required to detect an annual change of 10% varied between 9–11 years for the three PCDD congeners and the power to detect a 10% annual change was 100% for the full time series. The smallest possible trend to detect varied between 6.9–19% change per year during a decade.

Temporal trends, 1972–2011, of 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, 1,2,3,7,8-PCDF and 2,3,4,6,7,8-HCDF, based on concentrations in pg/g fat, are presented in Fig. 3a–d). The relative annual decrease over the 40 year period for 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, 1,2,3,7,8-PCDF and 2,3,4,6,7,8-HCDF are 6.5%, 6.1%, 5.7% and 6.7%, respectively, with $p < 0.001$ in each case. The annual relative decrease over the last ten

Table 2
Concentrations (pg/g fat) of seven PCDD and ten PCDF congeners are presented. Also, WHO-TEQ_{1998} and WHO-TEQ_{2005} are presented for the sum of PCDDs and PCDFs (Van den Berg et al., 1998, 2006).

	1972	1974	1976	1978	1980	1988/89	1990	1991	1992	1995	1997	1999	2000:1	2000:2	2001
Fat content (%)	2.5	3.2	2.9	3.0	2.9	2.4	2.6	2.6	2.9	3.1	2.9	3.1	3.0	2.7	3.0
PCDD															
2,3,7,8-TetraCDD	<2.8	3.5	5.2	3.0	3.4	2.4	1.8	7.5	1.7	1.5	1.2	1.1	0.89	1.1	0.90
1,2,3,7,8-PentaCDD	9.4	7.2	7.3	7.8	7.1	5.2	4.8	4.1	3.8	3.6	3.3	2.7	2.0	2.2	2.3
1,2,3,4,7,8-HexaCDD	3.5	3.1	2.4	3.0	3.4	2.3	1.9	1.7	1.6	1.3	1.4	1.1	0.82	0.86	0.79
1,2,3,6,7,8-HexaCDD	28	23	24	27	25	20	20	15	16	14	12	9.8	7.3	8.1	8.3
1,2,3,7,8,9-HexaCDD	3.1	1.9	1.7	2.0	5.6	2.3	2.3	3.0	2.1	2.5	2.0	1.3	1.2	1.5	1.0
1,2,3,4,6,7,8-HeptaCDD	95	80	78	79	69	48	42	31	27	25	24	17	13	16	13
OctaCDD	430	340	320	380	330	230	240	170	140	110	110	96	74	80	77
PCDF															
2,3,7,8-TetraCDF	3.0	2.1	2.1	2.4	2.3	1.5	1.3	1.0	0.78	0.64	0.68	0.57	0.47	0.53	0.45
1,2,3,7,8-PentaCDF	1.3	0.84	0.76	1.0	0.66	0.46	0.43	0.39	0.30	0.26	0.28	0.24	0.23	<0.26	0.23
2,3,4,7,8-PentaCDF	31	23	25	26	22	14	13	10	10	10	8.7	7.2	5.5	6.1	6.3
1,2,3,4,7,8-HexaCDF	14	9.1	8.5	8.5	6.7	3.8	3.5	2.7	2.6	2.2	2.0	1.9	1.6	1.6	1.5
1,2,3,6,7,8-HexaCDF	7.7	5.5	5.4	5.6	4.7	3.1	2.8	2.4	2.3	1.9	1.9	1.7	1.4	1.5	1.4
1,2,3,7,8,9-HexaCDF	<0.28	<0.21	<0.27	<0.24	<0.22	<0.28	<0.28	<0.26	<0.24	<0.22	<0.24	<0.22	<0.25	<0.29	<0.22
2,3,4,6,7,8-HexaCDF	3.2	2.5	2.2	2.1	1.8	1.3	1.2	1.0	0.93	0.79	0.76	0.70	0.59	0.69	0.59
1,2,3,4,6,7,8-HeptaCDF	35	25.0	18	16	12	4.9	5.5	3.7	4.1	2.4	3.0	2.2	2.4	2.3	2.1
1,2,3,4,7,8,9-HeptaCDF	0.69	0.40	0.43	0.43	0.43	<0.27	<0.27	<0.26	<0.23	<0.21	<0.24	<0.21	<0.24	<0.28	<0.22
OctaCDF	1.9	1.2	1.6	0.81	1.0	2.2	<0.83	<0.79	<0.71	<0.66	<0.73	<0.65	<0.75	<0.88	<0.67
∑PCDD/F															
∑PCDD/F (WHO-TEQ_{1998})[a]	32	28	31	30	27	19	17	20	14	13	11	9.3	7.2	8.0	7.9
∑PCDD/F (WHO-TEQ_{2005})[a]	26	24	26	25	23	16	14	18	12	11	9.5	7.9	6.1	6.8	6.7

	2002:1	2002:2	2003	2004	2007:1	2007:2	2008:1	2008:2	2009:1	2009:2	2010:1	2010:2	2011:1	2011:2
Fat content (%)	3.4	3.6	2.8	3.7	3.9	3.9	4.1	3.5	4.0	3.0	4.0	3.8	3.2	3.7
PCDD														
2,3,7,8-TetraCDD	0.87	0.85	0.86	0.68	0.39	0.59	0.38	0.34	0.33	0.34	0.32	0.44	0.38	0.33
1,2,3,7,8-PentaCDD	2.3	1.9	2.1	1.7	1.2	1.5	1.1	0.78	1.1	0.87	0.92	1.1	1.0	0.85
1,2,3,4,7,8-HexaCDD	0.84	0.67	0.88	0.62	0.53	0.67	0.38	<0.34	0.39	0.40	0.30	0.51	0.42	0.36
1,2,3,6,7,8-HexaCDD	7.3	6.2	7.2	5.1	4.5	5.1	3.3	2.4	4.2	2.5	2.3	3.5	3.4	2.8
1,2,3,7,8,9-HexaCDD	1.1	1.0	0.94	0.80	0.96	1.3	0.67	0.74	0.71	0.68	0.55	0.70	0.73	0.69
1,2,3,4,6,7,8-HeptaCDD	14	9.2	12	7.2	6.1	8.6	5.8	4.0	4.8	3.8	4.2	4.2	5.8	6.2
OctaCDD	60	54	64	48	42	56	39	31	30	25	27	32	38	34
PCDF														
2,3,7,8-TetraCDF	0.41	0.60	0.62	0.38	<0.40	0.58	0.49	<0.47	<0.37	<0.50	<0.38	<0.40	<0.45	<0.40
1,2,3,7,8-PentaCDF	<0.19	0.26	0.27	<0.18	<0.28	<0.28	<0.28	<0.32	<0.26	<0.35	<0.26	<0.28	<0.31	<0.28
2,3,4,7,8-PentaCDF	6.1	5.4	5.7	4.6	3.2	4.7	3.4	2.5	2.8	3.0	3.0	3.9	3.1	2.9
1,2,3,4,7,8-HexaCDF	1.5	1.3	1.5	1.2	1.2	1.3	1.1	1.0	0.83	1.0	0.78	1.2	1.0	0.92
1,2,3,6,7,8-HexaCDF	1.4	1.3	1.4	1.1	1.0	1.1	1.0	0.85	0.81	0.89	0.76	1.1	1.0	0.84
1,2,3,7,8,9-HexaCDF	<0.21	<0.20	<0.26	<0.20	<0.31	<0.31	<0.31	<0.36	<0.29	<0.39	<0.29	<0.31	<0.35	<0.31
2,3,4,6,7,8-HexaCDF	0.70	0.54	0.54	0.52	0.45	0.63	0.61	0.53	0.48	0.49	0.37	0.51	0.52	0.51
1,2,3,4,6,7,8-HeptaCDF	9.5	1.6	1.6	1.6	2.7	3.4	1.8	1.9	1.0	1.1	1.1	1.1	2.2	1.2
1,2,3,4,7,8,9-HeptaCDF	<0.21	<0.19	<0.25	<0.19	<0.30	<0.30	<0.30	<0.35	<0.28	<0.38	<0.28	<0.30	<0.34	<0.30
OctaCDF	<0.63	<0.59	<0.78	<0.59	<0.93	<0.93	<0.92	<1.1	<0.86	<1.2	<0.87	<0.92	<1.0	<0.92
∑PCDD/F														
∑PCDD/F (WHO-TEQ_{1998})[a]	7.8	6.7	7.3	5.8	4.2	5.6	4.1	3.0	3.6	3.4	3.3	4.3	3.7	3.3
∑PCDD/F (WHO-TEQ_{2005})[a]	6.6	5.7	6.1	4.9	3.6	4.7	3.4	2.5	3.0	2.8	2.7	3.6	3.1	2.7

[a] Sums are calculated using LOQ/2 for analytes below LOQ.

years for 2,3,7,8-TCDF and 2,3,4,7,8-PCDF and 2,3,4,6,7,8-HCDF are 11% ($p < 0.002$) 7.9% ($p < 0.001$) and 5.3% ($p < 0.001$) respectively. No temporal concentration trend could be discerned for 1,2,3,7,8-PCDF, during the last ten years. The number of years required to detect an annual change of 10% varied between 7–9 years for the PCDF congeners and the power to detect a 10% annual change was 100% for all of the full time series. The smallest possible trend to detect varied between 3.4–7.9% change per year during a decade.

Temporal trends, 1972–2011, of the DL-PCB congeners, CB-118, CB-126 and CB-156 based on concentrations in pg/g fat are presented in Fig. 4a–c). The relative annual decrease over the 40 year period for CB-118, CB-126 and CB-156 are 7.6%, 7.0% and 5.8%, respectively, with $p < 0.001$ in each case. The annual relative decrease over the last ten years for CB-118, CB-126 and CB-156 are 9.7% ($p < 0.001$), 12% ($p < 0.015$) and 8.1% ($p < 0.003$), respectively. The number of years required to detect an annual change of 10% was 7–10 years, and the power to detect a 10% annual change was 100% for the full time series. The smallest possible trend to detect varied between 5.2%-9.0% change per year during a decade.

4. Discussion

The present study confirms decreasing temporal trends of the ∑TEQ of ∑PCDDs, ∑PCDFs and ∑DL-PCBs assessed herein (Table 2, Fig. 1). Likewise, it confirms significant concentration declines of the individual PCDD, PCDF and DL-PCB congeners analyzed. This was to be expected as the time series covers 40 years. The data are in accordance with previously obtained data for ∑PCDDs, ∑PCDFs and ∑DL-PCBs in mothers' milk from Stockholm, 1972–1997 (Norén and Meironyte, 2000). However, it is striking to see a steeper rate of decline over the most recent years,

Table 3
Concentrations (pg/g fat) of 12 DL-PCB congeners and the TEQ levels of each milk sample, using both the WHO TEQ_{1998} and TEQ_{2005} are shown (Van den Berg et al., 1998, 2006).

	1972	1974	1976	1978	1980	1988/89	1990	1991	1992	1995	1997	1999	2000:1	2000:2	2001
Fat content (%)	2.5	3.2	2.9	3.0.	2.9	2.4	2.6	2.6	2.9	3.1	2.9	3.1	3.0	2.7	3.0
PCB															
PCB 77	150[a]	33	21	28	28	21	36	120[a]	15	<9.8	<11	<9.7	<11	<13	<10
PCB 81	17	6.2	6.3	6.6	6.9	4.0	3.0	13	2.1	<1.3	1.5	1.7	<1.5	<1.8	1.5
PCB 105	14000	12000	13000	12000	9400	5200	5000	3900	3700	3400	2700	2200	1600	1900	1600
PCB 114	1900	1600	1700	2100	1600	1100	1000	860	790	780	670	490	350	490	480
PCB 118	64000	53000	56000	55000	43000	26000	25000	19000	18000	17000	14000	11000	8100	9400	8100
PCB 123	680	540	560	570	450	260	260	200	170	160	130	100	83	92	92
PCB 126	240	220	230	180	160	82	89	60	69	79	71	35	42	48	39
PCB 156	20000	15000	17000	19000	16000	12000	11000	9300	9300	8100	7700	6400	4900	6000	5400
PCB 157	3300	2800	3000	3400	2900	2200	2000	1700	1700	1500	1300	1200	880	1100	1000
PCB 167	7500	6000	6700	6800	5700	3900	3700	3000	2800	2600	2100	1900	1400	1500	1400
PCB 169	92	69	63	74	63	51	44	41	41	40	35	33	27	30	26
PCB 189	1600	1300	1400	1600	1300	940	1000	810	830	700	670	640	410	510	560
∑ DL-PCB (WHO-TEQ_{1998})[b]	46	39	42	38	32	20	20	15	16	16	14	9.3	8.6	10	8.7
∑ DL-PCB (WHO-TEQ_{2005})[b]	30	26	28	24	21	11	12	8.4	9.3	10	9.0	5.2	5.5	6.3	5.2
∑TEQ															
∑ TEQ (WHO-TEQ_{1998})[b]	78	67	73	68	60	38	37	35	29	28	25	19	16	18	17
∑ TEQ (WHO-TEQ_{2005})[b]	56	50	54	48	44	27	26	26	21	21	19	13	12	13	12

	2002:1	2002:2	2003	2004	2007:1	2007:2	2008:1	2008:2	2009:1	2009:2	2010:1	2010:2	2011:1	2011:2
Fat content (%)	3.4	3.6	2.8	3.7	3.9	3.9	4.1	3.5	4.0	3.0	4.0	3.8	3.2	3.7
PCB														
PCB 77	<9.5	<8.9	<12	<8.9	<14	<14	<14	<16	<13	<17	<13	<14	<16	<14
PCB 81	1.5	<1.2	1.8	1.3	<1.8	<1.9	<1.8	<2.2	<1.7	<2.3	<1.7	<1.8	<2.1	<1.8
PCB 105	1500	1700	1700	1400	930	1600	840	660	770	880	830	1100	680	810
PCB 114	430	360	450	300	190	300	200	150	150	200	220	280	180	150
PCB 118	8100	8100	8800	6400	4600	7000	4400	3400	3800	3700	3900	5100	3400	3900
PCB 123	78	89	94	66	45	79	47	38	51	45	43	62	35	43
PCB 126	34	54	38	32	28	43	13	10	20	12	19	20	17	17
PCB 156	4500	4100	4600	3200	2600	3700	2600	1900	2300	1800	2500	2900	2500	1800
PCB 157	880	680	880	640	460	650	460	330	360	330	450	540	430	320
PCB 167	1300	1200	1400	970	710	910	610	460	570	460	540	630	520	480
PCB 169	24	23	21	17	16	18	14	<10.8	13	<11.6	13	13	16	<9.2
PCB 189	390	360	410	300	220	340	190	150	220	130	190	230	200	140
∑DL-PCB (WHO-TEQ1998)[b]	7.6	9.2	8.1	6.3	5.2	7.6	3.6	2.6	4.0	2.8	4.1	4.6	3.8	3.3
∑DL-PCB (WHO-TEQ2005)[b]	4.7	6.6	5.0	4.1	3.6	5.2	2.0	1.2	2.6	1.4	2.5	2.7	2.4	1.9
∑TEQ														
TOTAL-TEQ (WHO-TEQ_{1998})[b]	15	16	15	12	9.4	13	7.7	5.6	7.6	6.1	7.4	8.9	7.5	6.6
TOTAL-TEQ (WHO-TEQ_{2005})[b]	11	12	11	8.9	7.2	9.9	5.4	3.7	5.7	4.2	5.2	6.2	5.5	4.6

[a] Values contain a degree uncertainty due to poor recovery of the corresponding ^{13}C-labeled surrogate standard.
[b] Sums are calculated using LOQ/2 for analytes below LOQ.

2002–2011 for the ∑PCDD and ∑DL-PCB TEQs, than for the full period. In contrast, the steepness of the ∑PCDF TEQ decreasing time trend has not changed much over time. Several of the PCDFs are below LOQ during the latter 10 years, allowing no trend analysis, while 2,3,7,8-TCDF and 2,3,4,7,8-PCDF (WHO-TEF_{2005} = 0.1 and 0.3) both show stronger and significant declines over the recent 10 years. However, 1,2,3,4,7,8-HCDF and 1,2,3,6,7,8-HCDF (WHO-TEF_{2005} = 0.1 and 0.1) show a similar significant decrease as over the 40 year period. 2,3,4,6,7-HCDF and 2,3,4,6,7,8-HCDF show no statistical significant trend ($p > 0.05$) for the last decade. Still, a majority of the PCDD, PCDF and DL-PCB congeners decrease faster during the last decade than during the whole 40 year period and the congeners that do not decrease are difficult to assess since they all have several concentration points below LOQ. Altogether we therefore conclude that the "dioxins" decline faster today than previous decades. This is to be regarded as a positive outcome of the management of dioxin sources and the official advice given to pregnant and nursing women in Sweden (NFA, 2013). The results reported herein are particularly good news for nursing mothers and their children, both in Sweden and beyond. The latter, because the steeper decline of dioxins in mothers' milk over the last decade confirms that it is possible to make a change through strict management of dioxin sources.

It is previously reported that certain, in particular PCDF, congeners do not show decreasing time trends (Lignell et al., 2009; Pratt et al., 2012; Solomon and Weiss, 2002). In these reports no statistically significant decreases of temporal trends were observed for 2,3,7,8-TCDF, 1,2,3,7,8-PCDF and 2,3,4,6,7,8-HCDF, between 1996 and 2006. The increase in concentration of 2,3,4,7,8-PCDF between sampling times reported in Irish mothers' milk (Pratt et al., 2012) could not be observed in the present study. In fact, a tenfold decrease for the whole series and a halving of the concentrations between 2002 and 2010, see Table 2, duplicate samples for 2002: 5.4; 6.1 and 2010: 3.0; 3.9 pg/g fat, for the same sampling years as the Irish study. The authors of the Irish study explain the increase of 2,3,4,7,8-PCDF as a result of the selected sampling groups, changing from a rural to a more urban population.

When looking into the actual concentrations of "dioxins" in mothers' milk reported in recent years (milk that includes samples from 2008 and later) the $\sum TEQ_{2005}$ place the Swedish concentrations (this study) in the lower end of dioxin exposures (Table 4). This is supported by the $\sum TEQ_{1998}$ concentrations, all of which are higher than the Swedish concentrations. The ∑PCDD/Fs (both TEQ_{1998} and TEQ_{2005}) concentrations reported in the current study are comparatively low, but still

Table 4
Concentrations of "dioxins" reported in selected, recent studies with concentrations expressed in pg/g fat (Van den Berg et al., 1998, 2006).

Country	Year	∑PCDD/F (mean) TEQ_{1998}	∑PCDD/F (mean) TEQ_{2005}	∑PCDD/F (median) TEQ_{2005}	∑DL-PCB (mean) TEQ_{1998}	∑DL-PCB (mean) TEQ_{2005}	∑TEQ (mean) TEQ_{1998}	∑TEQ (mean) TEQ_{2005}	Reference
Africa	'05–'11			3.6					UNEP (2011)
Antigua & Barbuda	'07–'09							4.3	UNEP (2009)
Asia & the Pacific	'05–'11			4.5					UNEP (2011)
Belgium	'09–'10	8.4[a]	6.9[a]		5.8[a]	3.7[a]	14[a]	11[a]	Croes et al. (2013)
Central & Eastern Europe	'05–'11			5.9					UNEP (2011)
Chile	'07–'09							9.7	UNEP (2009)
Ghana	'07–'09							3.2	UNEP (2009)
GRULAC[b]	'05–'11			5.6					UNEP (2011)
Ireland	2010	6.32			3.34		9.66		Pratt et al. (2012)
Italy	'08–'09	4.6–6.1	3.8–4.9		6.2–6.9	4.8–5.7	11–13	8.6–11	Ulaszewska et al. (2011)
Italy	'08–'09							4.3–15[c]	Bianco et al. (2013)
Korea	'07–'09							4.0	UNEP (2009)
Nigeria	'07–'09							3.1	UNEP (2009)
Senegal	'07–'09							7.2	UNEP (2009)
Sweden	2011	3.3; 3.7	2.7; 3.1		3.3; 3.8	1.9; 2.4	6.6; 7.5	4.6; 5.5	Current study
Uruguay	'07–'09							6.9	UNEP (2009)
Western Europe & other States	'05–'11			6					UNEP (2011)
Vietnam	2008			2.7; 6.6					Nhu et al. (2011)
Vietnam	2008			5.2					Manh et al. (2013)

[a] Geometric mean concentrations.
[b] Group of Latin America & Caribbean Countries.
[c] TEQ not specified.

it is a limited data set to compare with. Only a few studies report concentrations of ∑DL-PCBs, but the concentrations obtained in this study are low to medium. The study from Croes et al. (2013) also includes concentrations obtained from CALUX-assays. These are not included in Table 4 since more comparable, GC–MS analysis derived, results from the same samples are available.

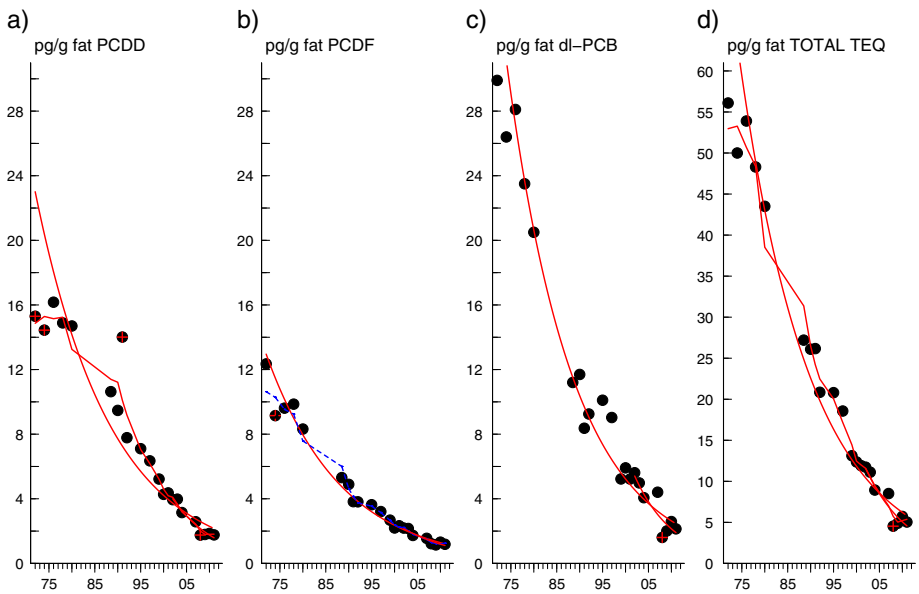

Fig. 1. Temporal trends of $TEQ_{(WHO-2005)}$ for PCDDs, PCDFs, DL-PCBs and ∑TEQ (∑TEQ PCDDs + ∑TEQ PCDFs + ∑TEQ DL-PCBs) (pg/g fat) in mothers' milk from Stockholm, 1972–2011. The linear red lines ($p < 0.05$) are based on log-linear regression analyses. The red, as well as the blue dotted, non-linear lines ($p < 0.05$ and $0.05 < p < 0.1$, respectively) are smoothers fitted to the annual mean values. A red cross represents a suspected outlier.

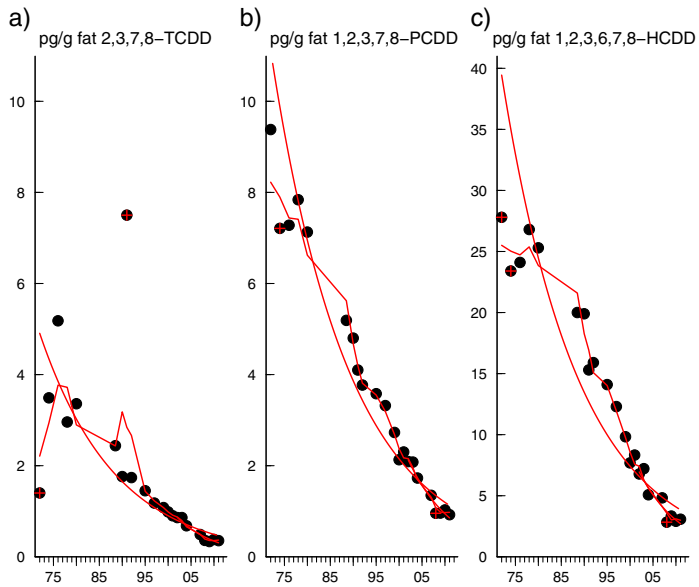

Fig. 2. Temporal trends of 2,3,7,8-TCDD, 1,2,3,7,8-PCDD and 1,2,3,6,7,8-HCDD (pg/g fat) in mothers' milk from Stockholm, 1972–2011. The linear red lines ($p < 0.05$) are based on log-linear regression analyses. The red non-linear lines ($p < 0.05$) are smoothers fitted to the annual mean values. A red cross represents a suspected outlier.

In order to certify the analytical results between previously reported data (Norén and Meironyte, 2000) and the results presented herein, several samples were selected for reanalysis. The results from the two occasions for analyses, 2000 and 2013, respectively, are visualized in Fig. 5. The concentration differences are rather small, with the highest discrepancy observed for the sample taken in 1972 (Fig. 5). Also, the

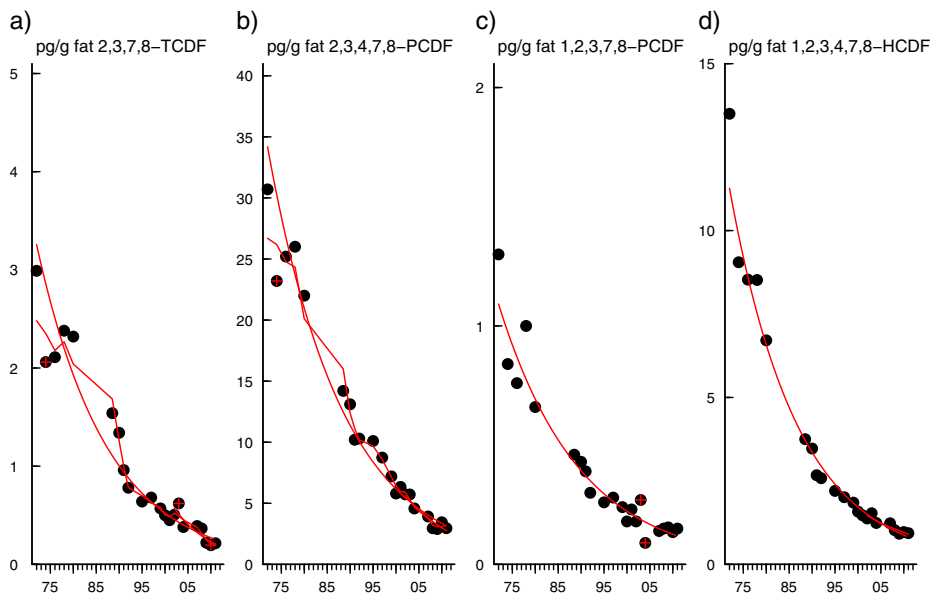

Fig. 3. Temporal trends of 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, 1,2,3,7,8-PCDF and 1,2,3,4,7,8-HCDF (pg/g fat) in mothers' milk from Stockholm, 1972–2011. The linear red lines ($p < 0.05$) are based on log-linear regression analyses. The red non-linear lines ($p < 0.05$) are smoothers fitted to the annual mean values. A red cross represents a suspected outlier.

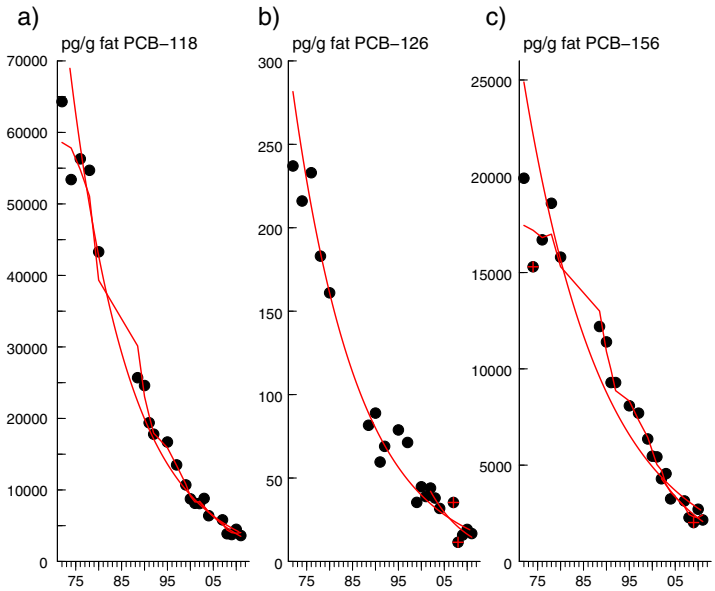

Fig. 4. Temporal trends of CB-118, CB-126 and CB-156 (pg/g fat) in mothers' milk from Stockholm, 1972–2011. The linear red lines ($p < 0.05$) are based on log-linear regression analyses. The red non-linear lines ($p < 0.05$) are smoothers fitted to the annual mean values. A red cross represents a suspected outlier.

relative proportions of ∑PCDDs, ∑PCDFs and ∑DL-PCBs, are in rather good agreement for all years. In some further detail, the previous analysis seems to have yielded a somewhat higher result on pg/g fat $\sum TEQ_{1998}$ basis compared to the current study with the exception of one of the samples, the one from 1980. Further, the mean and median differences between the studies were 13 and 15%, respectively, on $\sum TEQ_{1998}$ basis. The largest difference was found for ∑PCDDs, mean and median difference of 20% and 25% respectively. The differences for ∑DL-PCBs and ∑PCDFs were lower, approximately 10% mean and median difference for both groups of compounds. The result of this part of the present study shows that direct comparison between historical data and new data is possible for monitoring of PCDDs, PCDFs and DL-PBCs by applying the methodology described herein. Accordingly, it is possible to elongate existing time trends with new samples.

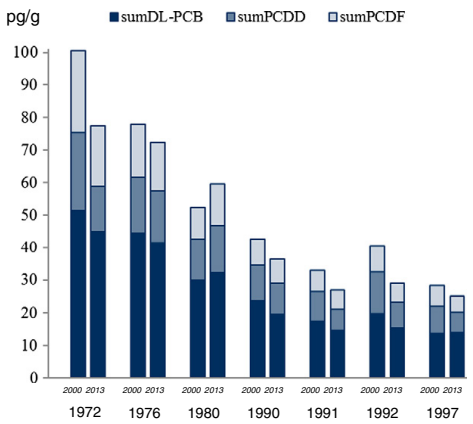

Fig. 5. The concentrations of PCDD, PCDFs and DL-PCBs previously analyzed and reported (Norén and Meironyte, 2000) compared to those presented in this study in the same samples, denoted 2000 and 2013 respectively. The TOTAL-TEQ-value (WHO_{1998}) of each sample is divided in three segments: ∑PCDDs, ∑PCDFs and ∑DL-PCBs.

Fig. 6 shows the quotas of the PCDDs, PCDFs and DL-PCBs of the $TOTAL\text{-}TEQ_{2005}$ for each sample of the time trend, 1972–2011, presented herein. It can be generalized that half of the ∑TEQ is made up of DL-PCBs, and the other half comprise of somewhat more PCDDs than PCDFs. Time trend analyses of the three fractions show a relative annual decrease over the 40 year period for the DL-PCBs, 0.44% per year ($p < 0.49$), but show no statistical significant trend for the last decade. The PCDDs and PCDFs show no statistical significant trend for either time period.

Comparability between studies from the literature, even when it comes to the same matrix — mothers' milk, is strongly hampered by several facts. First, the present lack of original congener specific data, presented either on a weight basis or on a molar basis, that is necessary to allow calculations of TEQs when new TEFs are applied, is not reported. Further, congener specific data are the most reliable data as a base for assessing temporal trends. Sum of analyte data may hide interesting and relevant temporal trends, as discussed for the PCDFs above. Second, the lack of unified sampling strategies influences the results. To promote the best possible sampling strategy it is relevant to apply the instructions from the WHO milk program (UNEP, 2012) or something as close to this as possible. Third, the lack of long term temporal trend analysis strongly hampers spatial comparisons of such trends.

5. Conclusions

The rate of which ∑PCDDs, ∑PCDFs ∑DL-PCBs and the ∑TEQ are decreasing (on pg/g fat $WHO\text{-}TEQ_{2005}$) is steeper in the last decade compared to the 40 year period, 1972–2011. The declines for PCDDs, PCDFs, DL-PCBs and ∑TEQs are 10%, 7.3%, 12% and 10% per year, last decade, compared to 6.1%, 6.1%, 6.9%% and 6.5% per year, 1972–2011. The

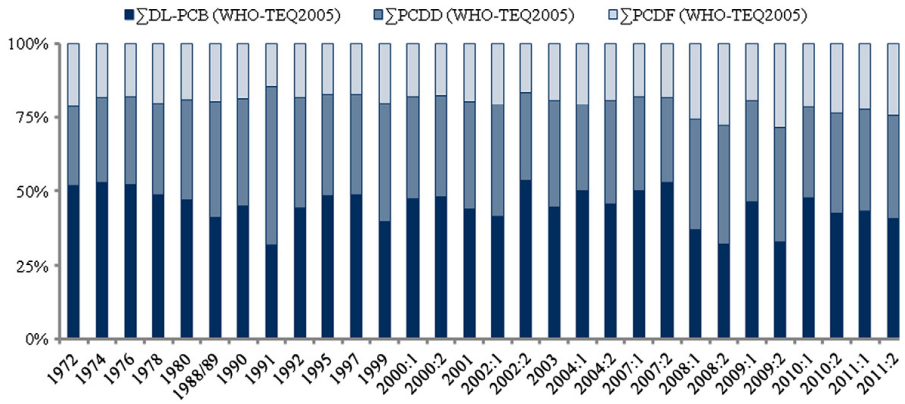

Fig. 6. Composition of the time series from 1972 to 2011. The TOTAL-TEQ-value (WHO_{2005}) of each sample is divided into three segments: ∑PCDDs, ∑PCDFs and ∑DL-PCBs.

difference in steepness, between the whole time period and the last ten years, is much smaller for ∑TEQ of PCDFs than for the other groups, likely due to too many PCDF congeners below LOQ, 2002–2011. The faster rate of decline over this period of time is confirmed by the temporal trends of the individual "dioxins", as determined on a weight basis. The faster drop in "dioxin" concentrations in mothers' milk in Sweden is a mirror of successful measures for lowering dioxin exposures. The good agreement between historical data and new data supports the elongation of existing monitoring series or data points, within the monitoring program. In contrast, it is still very difficult to compare results between monitoring programs due to differences in sampling strategies, reporting and lack of long term temporal studies of dioxins in mothers' milk.

Acknowledgments

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