Retrospective determination of primary feeding areas of Atlantic salmon (*Salmo salar*) using fingerprinting of chlorinated organic contaminants

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Atlantic salmon (*Salmo salar*) undertake extensive marine migrations. In the marine environment, the Atlantic salmon appears to suffer from heavy mortality, indicating the need for increased knowledge of its movements and habitat use at sea. Persistent organochlorine compounds (OCs) are found in measurable concentrations in all marine ecosystems. Geographically varying sources of OCs, transport, and transformation processes lead to different OC concentrations and compositions in the various ecosystems. As the principal source of organochlorine uptake in salmon is diet, populations utilizing different feeding areas may accumulate pollutant loads corresponding to their primary feeding areas. This hypothesis was tested by comparing the OC composition in Atlantic salmon from four locations: Lake Vättern (Sweden), Lake Vänern (Sweden), the Baltic Sea (off Denmark), and the River Imsa (Norway). Muscle and liver samples from each fish were analysed for 30 organochlorines (polychlorinated biphenyls, dichlorodiphenyltrichloroethanes, HCHs, hexachlorobenzene, and trans-nonachlor). Principal component analysis on normalized OC concentrations (OC pattern) showed separation of the salmon populations according to location; contaminant patterns were similar for liver and muscle tissue. It is therefore suggested that OC fingerprinting may be a valuable tool in identifying primary foraging areas of salmonids.

Keywords: Atlantic salmon, feeding areas, fingerprinting, migration, organochlorines.

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Introduction

There has been a serious and persistent decline of many fish stocks during the last few decades (ICES, 2006b), leading to some stocks dropping to a critical population size. One cause could be overexploitation of commercial species. A number of mitigating responses have been suggested, including establishment of marine protected areas (Roberts *et al.*, 2001; Halpern and Warner, 2002), no-take zones (Rowley, 1994), and quotas (Holland and Schnier, 2006). To apply these tools in an efficient way, knowledge of the spatial ecology of a fish stock is crucial, and this includes information on their feeding areas (ICES, 2006a).

Anadromous Atlantic salmon (*Salmo salar*) is one species that has experienced significant declines (ICES, 2006a). It is widely distributed in Europe, from Portugal to the north of the polar circle (Shearer, 1992). Spawning takes place in rivers, and the juveniles spend the following 1–6 years there before migrating to the sea (Hutchings and Jones, 1998). Then, having spent 1–5 years as a mainly pelagic forager at sea, they return to their native river to spawn. Currently, various methods of stock enhancement are applied to try to compensate for the considerable decline in natural spawning grounds and the general decline in water quality seen in the waterbodies of many countries (Ritter, 1997), including release of hatchery-reared fish to maintain populations in rivers. In the marine environment, the Atlantic salmon appears to suffer from heavy mortality as a consequence of both fishing and natural causes (Ritter, 1993). Therefore, as with many marine migrating teleosts, there is a need for more knowledge of its movements and habitat use at sea to secure its long-term protection.

Previous investigations of migration and distribution of the salmon at sea and in estuaries have applied various methods, including recapture of tagged fish in pelagic trawls (Holm *et al.*, 2000; Hansen and Jacobsen, 2003), telemetry using acoustic tags (Moore *et al.*, 1995; Thorstad *et al.*, 2004), and genetics (Shaklee *et al.*, 1999). These studies have indicated that salmon from Norway, Ireland, Scotland, Iceland, the US, Canada, Germany, France, and the west coasts of Denmark and Sweden migrate to feeding areas in the North Atlantic (Holm *et al.*, 2000; Hansen and Jacobsen, 2003), whereas salmon from the Baltic rivers stay in the Baltic Sea and feed mainly in the Baltic Main Basin (McKinnell and Lundqvist, 1998).

In addition to the impact of fishing, conservation of Atlantic salmon has been threatened by pollution of the sea by heavy metals and organic contaminants (Scott, 2001). Organochlorines (OCs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs) are found worldwide in the

marine environment, and accumulate in the lipid-rich tissue of aquatic organisms (AMAP, 1998; Borrell *et al.*, 2006). It has been suggested that the OC patterns accumulated in living tissue may be used to discriminate whale populations (de March *et al.*, 2004). Likewise, Innes *et al.* (2002) demonstrated that the eastern North American white whales (beluga) from various locations had noticeable differences in their pattern and level of OCs.

Aguilar and Jover (1993) discriminated between schools of long-finned pilot whales (*Globicephala malaena*) using ratios between PCBs and DDTs. In addition to these cetacean studies, some teleost studies have demonstrated differences in OC pattern between locations (Ashley *et al.*, 2003). Provided such differences exist for Atlantic salmon populations, it might be possible to address a number of questions related to feeding behaviour in the sea using OC patterns. To a large extent, previous methods designed to describe the marine migration of fish were dependent on capture or tracking at sea. Ideally, the method tested here might be used to identify the dominating marine foraging area in a salmon captured when returning to its natal spawning area.

The objective of our study was to test whether principal differences in OC patterns between different populations of Atlantic salmon exist, and to investigate if such differences, if found, could be used to identify foraging areas. Following the conclusions of Elskus *et al.* (1994) and Ashley *et al.* (2003), we hypothesized that Atlantic salmon foraging in different geographic areas would have different patterns of OCs that may reflect their respective foraging areas. Our study included the analysis of 30 OCs in Atlantic salmon captured in four distinct and separate geographic locations. Using principal component analysis (PCA), we tested whether the dominant marine foraging locations could be separated based on the OC pattern of individual fish.

Material and methods

Salmon were collected in Lake Vänern, Sweden, in the area between Spiken and Mellerud, in Lake Vättern close to Grannvik, in the Baltic Sea between Simrishamn (Sweden) and the island of Bornholm (Denmark), and in the River Imsa, a small river in southwestern Norway (Figure 1).

To determine foraging activity, the fish from Lake Vättern, Lake Vänern, and the Baltic Sea were caught using rod and line, and killed immediately after capture. Salmon from Lake Vättern (seven fish) and Lake Vänern (five fish) were caught in October or November 2004, and six salmon from the Baltic Sea (six fish) were caught in April 2004 and another two fish in February 2006. Ten returning salmon from River Imsa were caught in October 2005, in a fish trap controlling the ascent and descent of salmon situated 100 m upstream of the river mouth (Jonsson *et al.*, 1997). Of these ten salmon, six individuals were analysed for this study.

Following Larsson *et al.* (1996), samples from the dorso-anterior part of the fish were used for analysis. These samples and the livers were removed from each fish and stored in separate annealed glass containers at −20°C until analysis. The total weight (to the nearest 10 g) and total length (to the nearest millimetre) of each fish were determined.

All salmon from Lake Vänern and Lake Vättern were originally of hatchery origin, but had been released as either smolts or 0+ juveniles (Öberg *et al.*, 2003). The history of the fish from the Baltic Sea is unknown, but based on their size, they had spent at least 1 year in the marine environment. All salmon from the River Imsa were tagged and measured before release as smolt and had spent ∼1.5 years in the sea (data from NINA, the Norwegian Institute for Nature Research). These salmon experience a similar life cycle, consisting of 1–4 years of sea-foraging in the lake or the sea before their spawning migration. The food source of the four groups of salmon differs, however. Salmon from Lake Vänern primarily feed on European cisco (*Coregonus albula*), those in Lake Vättern on both European cisco and European stickleback (*Gasterosteus aculeatus*), salmon in the North Atlantic feed on herring (*Clupea harengus*) and a number of crustaceans, and salmon in the Baltic take herring and sprat (*Sprattus sprattus*) as their primary food (Shearer, 1992; Larsson *et al.*, 1996; Salminen *et al.*, 2001; Öberg *et al.*, 2003). All these prey fish are on a relatively similar trophic level.

Figure 1. Sampling locations for the study on organochlorine patterns in four geographically separate groups of Atlantic salmon (*Salmo salar*).

Chemical analysis

The lipid content of the muscle and liver samples was determined according to the method of Smedes (1999), and the dry matter content was calculated based on mass loss after drying at 105°C until constant weight.

OC analysis was performed as described previously for black guillemot (*Cepphus grylle*) samples (Vorkamp *et al.*, 2004a). Briefly, the salmon liver and muscle were homogenized, dried with diatomaceous earth, and spiked with the surrogate standards CB3, CB40, and CB198 (all purity 98.9%, Promochem, Wesel, Germany) for recovery calculations. Soxhlet extraction was performed using 350-ml glass-distilled acetone and *n*-hexane (1:4) (Rathburn, Walkerburn, Scotland). Following the extraction, the samples were concentrated to 1 ml using rotary evaporation and cleaned on a multilayer glass column (diameter 10 mm) consisting of 1 cm of anhydrous sodium sulphate, 5 g of silica impregnated with sulphuric acid (95–97% purity), 1 g activated silica (24 h at

160°C), and 5 g of aluminium oxide impregnated with 10% water. The column was eluted with 300 ml *n*-hexane, and the extracts concentrated by rotary evaporation under nitrogen. Following the addition of syringe standards (CB53 and CB155), the volume of the extracts was adjusted to 1 ml. Depending on least matrix interference, either CB53 or CB155 was chosen for the quantification of the OCs.

Using dual-column gas chromatography with electron-capture detector (GC-ECD), the samples were analysed for the following PCB congeners: CB28, CB31, CB52, CB101, CB105, CB110, CB118, CB128, CB138, CB149, CB153, CB156, CB170, CB180, CB187, CB194, and CB209; and the following organochlorine pesticides: α-HCH, β-HCH, γ-HCH, *o*, *p*′-DDT, *o*, *p*′-DDE, *p*, *p*′-DDT, *p*, *p*′-DDE, *p*, *p*′-DDD, hexachlorobenzene (HCB), and trans-nonachlor (TNC). More detailed information regarding the chemical analysis is available in Cleemann *et al.* (1999) and Vorkamp *et al.* (2004a).

The samples were processed in batches of 16 containing duplicate analyses of 1–2 samples, 2 blanks, and 2 sandeel (*Ammodytes tobianus*) oil samples used as internal reference material (Triple Nine Fish Protein in Esbjerg, Denmark). The concentrations in the internal reference material were monitored in control charts, and used to define "warning" (2 × s.d.) and "action" limits (3 × s.d.). Control charts serve the long-term monitoring of precision and allow the identification of instabilities in the analyses such as high or low values and increasing or decreasing trends. Compounds with concentrations outside the action limits were not included in the subsequent data treatment. Accuracy of the analyses was monitored by regular participation in the QUASIMEME laboratory proficiency tests for organochlorines in marine samples. Details on the QA/QC procedure and results are given by Asmund *et al.* (2004).

No recovery correction was performed because only samples with >80% recovery for CB40 and CB198 were accepted. The volatile congener CB3, added to the samples along with CB40 and CB198 for recovery calculations, indicated potential losses during the evaporation steps. The duplicate samples usually varied by <10% for compounds above the detection limit. The detection limits were ≤ 0.35 ng g^{-1} for all congeners and compounds. If the blanks showed concentrations >0.3 ng ml^{-1}, the original detection limit was doubled.

Data analysis

The dataset was examined before statistical analysis. The compound β-HCH was removed from the dataset because its concentrations were below the detection limit in >80% of the samples. In some samples, a few (<10%) of the congeners had concentrations below the detection limit, so to avoid introducing artefacts into the dataset through missing values, values below the detection limit were replaced by a random number between zero and the detection limit (Borga *et al.*, 2005).

To avoid effects caused by differences in OC concentration, OCs were normalized relative to CB153 (Muir *et al.*, 1988; Blanchard *et al.*, 1997). CB153 is a common PCB congener found in relatively high concentration in all samples, and has been used as a measure of total PCB concentration. As a congener, it is easily detectable on both columns in the GC analysis, and has proven to be stable in biota samples (Atuma *et al.*, 1996). Accordingly, concentrations were normalized to obtain an OC pattern for each sample:

$$y_{i,j} = \frac{x_{i,j}}{x_{i,\mathrm{CB153}}}, \tag{1}$$

where $y_{i,j}$ is the relative content of congener or compound j of the ith sample, $x_{i,j}$ the absolute concentration of congener or compound j of the ith sample, and $x_{i,\mathrm{CB153}}$ is the concentration of CB153 of the ith sample.

Initially, we wanted to determine whether the OC patterns in the liver and muscle samples differed. The dataset consisted of $p = 30$ variables (OCs) and $n = 24$ samples (fish). The number of samples was less than the number of variables ($n < p$), and the variables were tightly correlated. Owing to the multicollinearity problem (Timm, 2002), data were preprocessed using PCA, replacing the variables with fewer uncorrelated principal components (PC). Selection of an appropriate number of PCs constitutes a problem, but Langsrud (2002) suggested using the 50–50 Manova test. This test selects the lowest number of PC satisfying a limit of at least 50% of the explained variation (exVarPC), then chooses 50% of the remaining PC as buffer components (exVarBU). The exVarPC are then tested against an error term derived from the remaining PC (Langsrud, 2002). Before PCA, the variables in the OC-pattern data matrix were centred to zero mean. Hence, the 50–50 Manova test does not allow one to test hypotheses about the general mean, so the standardized sum test (Läuter, 1996; Läuter *et al.*, 1996) was applied in this case. An $n \times p$ matrix ($n = 24$, $p = 30$) of differences (normalized content) between liver and muscle were calculated, and the following hypothesis (no difference in OC pattern between liver and muscle samples) was tested:

$$H_{01} : \boldsymbol{\mu}_{\mathrm{diff}} = \mathbf{0} \quad \text{vs.} \quad H_{11} : \quad \boldsymbol{\mu}_{\mathrm{diff}} \neq \mathbf{0}, \tag{2}$$

where $\boldsymbol{\mu}_{\mathrm{diff}}$ is a $p \times 1$ vector of means of differences. This hypothesis was tested using the standardized sum test (Läuter, 1996; Läuter *et al.*, 1996). Calculations were performed in Microsoft Excel®2003.

Second, the effect of site was analysed by the 50–50 Manova procedure, including fish length, lipid content, and dry matter content as covariates. As no effect of covariates was detected, PCA using the software The Unscrambler for Windows v. 9.5 (Camo A/S, Trondheim, Norway) was applied to further examine differences among sites. The model was validated using the full cross-validation feature of the software.

Stability of the OC pattern over time was examined by comparing the OC patterns of salmon caught in the Baltic Sea in April 2004 and February 2006. The salmon from 2006 were introduced into the model by applying the original PCA model on the two new samples.

Results

All samples of liver and muscle contained measurable concentrations of OCs. Factors such as lipid content, weight, and length are correlated with the concentration of OCs in salmon, and these are therefore presented along with the concentrations of the different groups of OCs (Table 1). The concentrations of the individual compounds have been published elsewhere (Svendsen *et al.*, 2007b) but are summarized here. The concentrations (ng g^{-1} wet weight, ww) in the muscle samples of salmon from Lake Vänern, Lake Vättern, and the Baltic Sea were

Table 1. Weight, length, lipid content, and sum concentrations of compound groups of the samples of Atlantic salmon (*Salmo salar*) from four locations.

Parameter and unit	Lake Vänern (Sweden)	Lake Vättern (Sweden)	Baltic Sea (Denmark/Sweden) 2004	Baltic Sea (Denmark/Sweden) 2006	River Imsa (Norway)
n	7	5	6	2	6
Weight (kg)	5.10 (2.05)	4.43 (1.13)	4.59 (2.76)	7.9 (1.3)	1.33 (0.07)
Length (cm)	73.7 (10.6)	71.0 (4.5)	79.3 (11.9)	81.2 (7.8)	58.5 (1.5)
Lipid content muscle (%)	3.2 (2.5)	4.0 (0.17)	3.6 (0.84)	4.0 (1.9)	0.17 (0.11)
Lipid content liver (%)	6.1 (2.9)	5.2 (2.8)	3.6 (0.8)	N/A	2.6 (0.2)
$\sum$PCB muscle (ng g^{-1} ww)	53.1 (12.8)	56.8 (28.6)	58.1 (25.1)	82.7 (11.19)	6.9 (1.9)
$\sum$PCB liver (ng g^{-1} ww)	39.7 (25.3)	45.1 (27.8)	32.2 (8.5)	N/A	16.7 (8.5)
$\sum$DDT muscle (ng g^{-1} ww)	32.5 (8.1)	25.6 (13.5)	44.0 (28.1)	74.1 (13.41)	5.1 (1.7)
$\sum$DDT liver (ng g^{-1} ww)	22.4 (14.6)	19.7 (13.3)	22.5 (6.4)	N/A	11.2 (5.8)
HCB muscle (ng g^{-1} ww)	0.80 (0.55)	0.72 (0.13)	2.1 (1.2)	2.4 (0.21)	0.40 (0.13)
HCB liver (ng g^{-1} ww)	0.96 (0.63)	0.81 (0.57)	1.4 (0.45)	N/A	0.98 (0.19)
TNC muscle (ng g^{-1} ww)	0.79 (0.50)	1.0 (0.24)	1.5 (0.72)	2.5 (0.41)	0.91 (0.25)
TNC liver (ng g^{-1} ww)	0.65 (0.47)	0.74 (0.64)	0.66 (0.18)	N/A	2.1 (1.6)
$\sum$HCH muscle (ng g^{-1} ww)	0.64 (0.15)	0.59 (0.20)	1.6 (0.73)	0.8 (0.05)	0.15 (0.06)
$\sum$HCH liver (ng g^{-1} ww)	0.66 (0.25)	0.67 (0.36)	0.92 (0.31)	N/A	0.27 (0.06)

All values are means, and values in parenthesis are s.d. N/A = not analysed.

similar (Kruskal–Wallis test followed by Dunn's multiple comparison test) for all compound groups (PCB, HCH, DDT, and TNC), whereas the concentrations in the muscle samples from the River Imsa were significantly lower ($p < 0.05$). $\sum$PCB varied from 4.38 to 69.83 ng g^{-1} ww, and total OC concentration (30 compounds) from 8.73 ng g^{-1} ww in a salmon from the River Imsa to 219.7 ng g^{-1} ww in a salmon from the Baltic Sea. In the salmon from the Baltic Sea, Lake Vänern, and Lake Vättern, the concentration was higher in muscle than in the liver. River Imsa salmon showed the opposite, with lower concentrations in muscle tissue. Levels in the liver of the four groups of salmon were more comparable, probably related to the similar lipid content of the liver samples (Table 1). These findings indicate that the four groups of salmon cannot be separated using absolute concentrations of the different compound groups.

The salmon used in this study were of different size and lipid content (Table 1). Although these factors are known to influence the concentration and composition of OCs (Lundstedt-Enkel *et al.*, 2005), no effect of fish size (length), lipid content, or dry matter content was observed in either liver or muscle samples (Table 2), whereas the OC pattern differed significantly with site in both liver and muscle samples.

Based on raw concentrations (ng g^{-1} ww), the same congeners and compounds dominate the OC pattern for all four locations. For the PCBs, these were CB153, CB138, CB180, CB187, and CB101, and for the DDTs, it was *p*′*p*-DDE. These large concentration differences make it difficult to separate the locations based on the OC pattern of the raw concentrations. To remove this effect, the patterns were normalized to CB153 before PCA (Figure 2).

Four locations could be separated based on their congener and compound profile in both muscle and liver (Figure 2a). The first two PC accounted for 74% and 12%, respectively, of the total variation in muscle and liver samples. The two groups representing the salmon from the Swedish lakes seem to be located closer to each other in the scoreplot than to the groups representing salmon from the other two locations. There are indications of less variation within each group from the Swedish lakes than in salmon from the Baltic Sea and from Norway. Fish from Norway and the Baltic Sea are both influenced by *p*'*p*-DDE, whereas *p*'*p*-DDD seems to influence the samples from the Baltic Sea, and TNC affects the Norwegian samples. There was a significant difference ($p < 0.005$) in OC pattern between liver and muscle samples, but irrespective of this difference, samples grouped similarly (Figure 2a) on the first two PC, suggesting that either sample type is applicable for this type of study.

Table 2. 50–50 Manova results.

Site	Source	nPC	nBu	exVarPC	exVarBu	*p*-value
Muscle	Site	2	6	0.653	0.957	0.000
	Length	3	5	0.622	0.934	0.879
	Lipid	3	5	0.625	0.935	0.779
	Dry matter	3	5	0.632	0.933	0.216
Liver	Site	2	6	0.636	0.936	0.000
	Length	2	6	0.528	0.911	0.867
	Lipid	2	6	0.521	0.922	0.656
	Dry matter	2	6	0.529	0.922	0.865

nPC, number of PC extracted; nBu, number of buffer PCs excluded; exVarPC and exVarBu, explained variance by nPC and nPC+nBu, respectively.

Temporal stability of the pattern was investigated by analysing salmon from the Baltic Sea caught 2 years later (2006) and comparing the results with the salmon used for the original PCA model. The concentrations of OCs were similar to the older samples (2004) from the same area (Student's *t*-test, $p > 0.12$), and when these new samples were included in the PCA model, they grouped well with the other samples from the Baltic Sea (Figure 3).

Discussion

Using PCA to analyse OC patterns, this is the first study to indicate that Atlantic salmon utilizing different foraging areas may carry

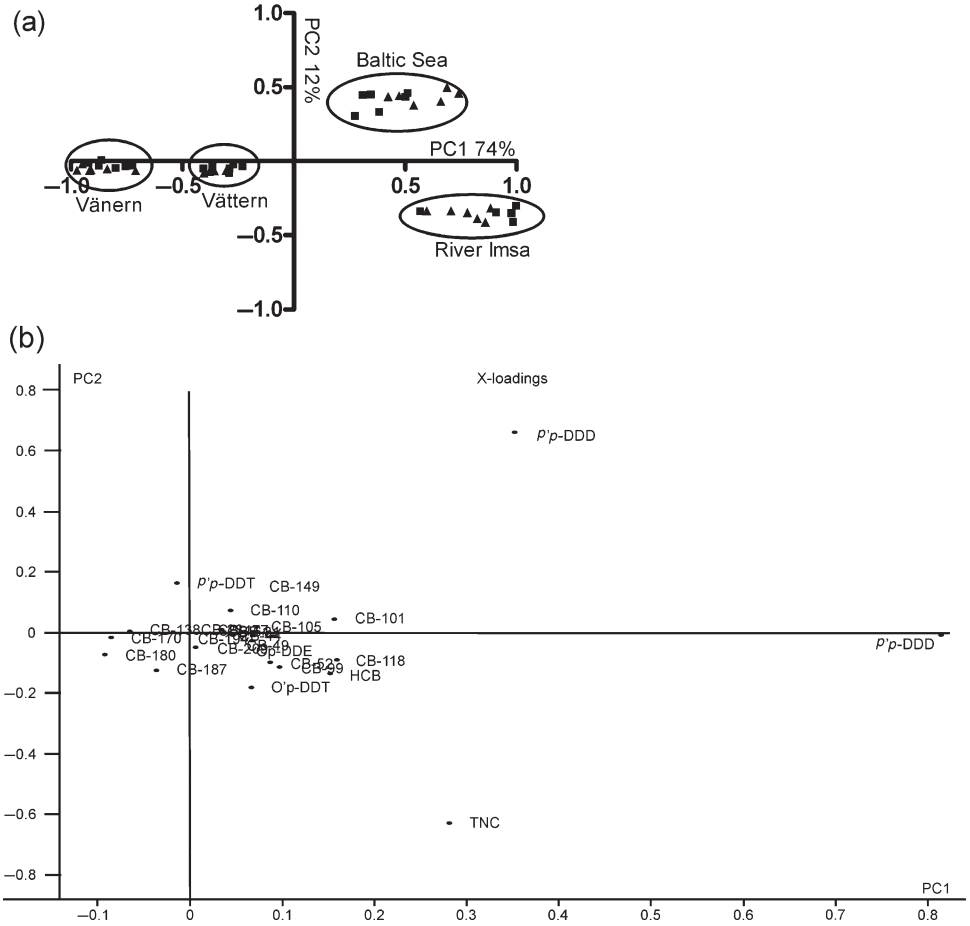

Figure 2. PCA analysis of organochlorine concentrations in muscle and liver samples of salmon (*Salmo salar*) from four sites normalized to PCB153. (a) Sample scores of the first two principle components. Squares represent the liver samples, triangles the muscle samples, and ovals the different sampling sites. (b) Variable loadings of the two first principle components. PC1 explains 74% of the variation, and PC2 explains 12%.

different OC patterns (Figure 2). Given the differences found, we suggest that the main foraging area of Atlantic salmon might be identified using OC fingerprinting retrospectively on post-foraging salmon.

The differences in OC pattern of the four groups of salmon we found are in accord with previous studies indicating differences in OC patterns among different populations of the same marine species (Elskus *et al.*, 1994; Hobbs *et al.*, 2001; Borrell *et al.*, 2006). Because fish foraging ecology and physiology may affect bioaccumulation of organochlorines through factors related to diet, feeding area, trophic position, growth rate, and lipid loss through metabolism (Burreau *et al.*, 2004, 2006), the effect of length, dry matter, and lipid content on the OC pattern in salmon was included in the analysis. The salmon from the River Imsa had smaller body mass and lower lipid content than three other groups (Table 1). The results indicated that the significant differences in patterns of the four groups (Table 2) could not be ascribed to differences in size, lipid, or dry matter content, because these factors were not significantly correlated with the OC pattern in the present study. The correlation between size, lipid, and dry matter content and the pattern may, however, also be influenced by the low sample number (5–7 from each location). When comparing levels of the individual compound groups between the four groups of salmon, three of the four groups could not be separated statistically. Additionally, concentrations were strongly correlated with factors such as the size of the salmon, also exemplifying the problems related to the use of absolute concentrations to separate fish samples. The measured concentrations of OCs were within the range reported previously (Vuorinen *et al.*, 1997; Öberg *et al.*, 2003; Burreau *et al.*, 2006). A manuscript on the concentrations of OCs and brominated flame retardants has been published already (Svendsen *et al.*, 2007b).

The OC patterns of salmon from the Baltic Sea captured 2 years apart indicated no differences (Figure 3). The 2006 samples grouped with the 2004 salmon from the same location, suggesting temporal stability of the OC composition in the salmon. Given the persistence of OCs, similar patterns over the time frame investigated may be anticipated in the environment, and results suggesting slow degradation times of months to years for OCs in salmon support the indication of stability in the patterns (Niimi, 1996; Morrison *et al.*, 1999). Potentially, the use of banned chemicals as tracers of foraging areas may be of limited use in future because these pollutants are likely to be removed from the environment over time. However, despite the present ban on the use of these chemicals, they are still found in the environment (Vorkamp *et al.*, 2004b). This, combined with observations of OC reaching stable levels in marine biota (Stow *et al.*, 1995;

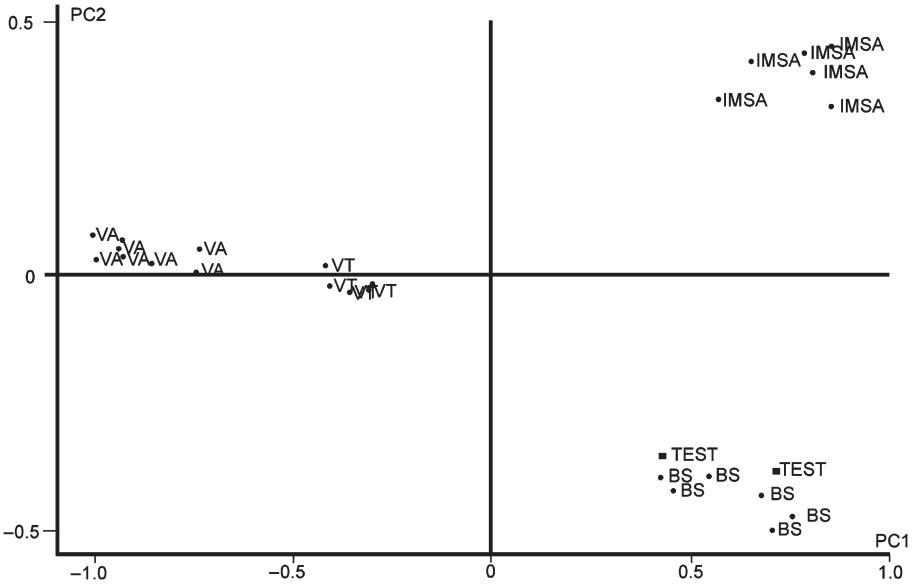

Figure 3. Two test salmon (TEST) from the Baltic Sea, caught 2 years after the original sample, grouped with the other salmon from the same location based on their organochlorine pattern (VA, Lake Vänern; VT, Lake Vättern; IMSA, River Imsa; BS, Baltic Sea). The results are based on muscle samples. PC1 explains 72% and PC2 15%.

Öberg *et al.*, 2003), suggests that substantial changes in the pattern even between years are not likely. Another factor possibly affecting the pattern found in Atlantic salmon might be a temporal change in the feeding areas of the fish as a consequence of spatial and temporal variation in food abundance (Kallio-Nyberg *et al.*, 1999). Such changes were, however, not mirrored in the OC pattern of the salmon compared here and caught in 2004 and 2006.

An effect of the OCs accumulated during their stay in fresh water before migration as smolts could hamper the pattern in such a way that it appeared to be the fresh-water pattern that was separating different groups. A recent investigation on OC patterns in different life stages of anadromous brown trout (*Salmo trutta*), which have a life cycle similar to that of Atlantic salmon, however, revealed that the pattern accumulated before smoltification does not have a significant effect on the pattern found in adult salmon (Svendsen *et al.*, 2007a). Additionally, if the weight of a smolt (20–40 g; Jokikokko *et al.*, 2006) is compared with the weight of the salmon we analysed here (1.2–10 kg), there is a difference factor of at least 30. Therefore, the OCs accumulated before smoltification would be diluted.

Although the OC patterns in liver and muscle differed, PCA indicated similar separation of groups of salmon using the muscle and liver of individual fish, suggesting that both types of tissue can be used to investigate in this way the primary feeding areas of Atlantic salmon. The observed difference between liver and muscle supports the findings of Blanchard *et al.* (1997) that some transformation of the OCs takes place in the liver of fish.

Our study sampled and identified differences in salmon foraging areas separated by hundreds of kilometres (Figure 1). Interestingly, Ashley *et al.* (2003) observed differences in OC pattern on a spatial scale ranging from 10 to 50 km, suggesting that OC fingerprinting may be used to separate fish populations that are foraging in areas closer to each other than those investigated here. It has been suggested that salmon from closely related rivers in the Baltic Sea watershed may share primary feeding areas in the marine environment (Kallio-Nyberg *et al.*, 1999), potentially leading to similar patterns of OCs within these fish. Provided feeding is continuous while migrating from the natal river to the main marine foraging area and back, even small differences in feeding locations may be mirrored in the OC pattern of the salmon. The pattern is probably an integration of the pattern from the different feeding grounds visited (Aguilar, 1987), so minor changes in feeding area may not be identified.

If the spatial resolution of the fingerprinting method is not sufficient to address a particular problem, methods that can reinforce the result could be applied. Some studies have managed these problems by applying additional methods. For instance, Herman *et al.* (2005) investigated the feeding ecology of North Pacific killer whales (*Orcinus orca*) using fatty acids, stable isotopes, and OCs. They were not able to differentiate populations using stable-isotope enrichment values, but when combining the results from the different techniques, separation was evident. Fisk *et al.* (2002) used organochlorines and stable isotopes to investigate the feeding ecology (trophic level) of Greenland sharks (*Somniosus microcephalus*), and de March *et al.* (2004) used a combination of organochlorine contaminant profiles and genetics for stock discrimination of white whales (*Delphinapterus leucas*). As these techniques have been applied primarily to address problems related to feeding behaviour and seldom to determine main feeding areas, a thorough review of the abilities of the methods has to be done before their use.

Salmon were used in this study because they offer an excellent opportunity for understanding the potential for OC-fingerprinting and are a likely test resource for future use of the method. All anadromous salmonids exhibit a population structure where genetically separated populations inhabit different rivers or tributaries (Shearer, 1992). This structure can be verified using different types of DNA method (e.g. Nielsen *et al.*, 1999; Hansen *et al.*, 2002). The different ecotypes normally found in most of the populations can be revealed by scale or otolith analysis (Kipling, 1962; Beall and Dovaine, 1988; Keeley *et al.*, 2005). The potential for OC-fingerprinting comes from the combination of all three

methods on the same sample of fish. This makes direct comparisons of feeding areas between different populations as well as between different ecotypes possible. In this context, the method described here supplements or replaces conventional tagging methods and offers the advantages of low price and quick results. Depending on the specific objective of an investigation, the two methods have both strengths and weaknesses, and have to be applied in full knowledge of those. Many of the weaknesses can, however, be overcome by a combination of both techniques.

To conclude, the results of this study have suggested that the feeding areas of Atlantic salmon populations may be separated using individual OC patterns. It is therefore suggested that OC-fingerprinting be used retrospectively to identify the primary foraging areas of Atlantic salmon. Separation of the fish using their OC patterns may have important implications for management of populations of all anadromous salmonids in the marine environment. During the past decade, several management tools have been applied to conserve populations of marine fish. For these methods to work as anticipated for salmon, knowledge of the spatial distribution of the fish populations at sea is crucial (ICES, 2004). Similarly, in terms of spatial siting of marine-protected areas, identification of primary feeding areas using OC-fingerprinting, which seems possible given the results presented here, may be a valuable tool in future fisheries management strategies. If a marine protected area is planned to protect a specific fish population, the effort of establishing it could be worthless if the primary feeding area of the fish is not within the marine protected area itself (Roberts, 2000). By the same token, there is a need for tools capable of relating individual fish to their population of origin to assist in policing regulations and identifying poachers. To address this problem, Nielsen *et al.* (2001) used microsatellite genetic markers to distinguish between different populations of cod (*Gadus morhua*), but the results shown here indicate that OC-fingerprinting could be used as a supplement to these techniques. Although much further refinement and validation of the OC-fingerprinting model is required, the method may be developed as a tool to identify the marine origin of individual fish in commercial landings or fish markets.

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