DIFFERENT PCDD/PCDF CONGENER COMPOSITION IN SALMON AND BROWN TROUT FROM SWEDISH WATERS

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Introduction

The Swedish National Food Administration (SNFA) is undertaking a survey to analyse the concentration of polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (DFs) and dioxin-like polychlorinated biphenyls (PCBs) in fish from Swedish waters[1]. Here we present a part of the results from this survey for two species, salmon (*Salmo salar*) and brown trout (*Salmo trutta*) caught south east of Gotland in the Baltic proper and in Swedish rivers and lakes.

In most scientific work comparing the variables one by one separately performs the comparison of groups. This is a time-consuming and difficult approach that might lead to spurious and confusing results, especially where there are a higher number of variables measured (>3). In this investigation the resulting data set is multivariate (21 measured PCDD/DFs and PCBs) and this, together with a large natural variation within variables, leads to challenges in the data analysis. By applying multivariate methods such as principal component analysis (PCA) and partial least squares regression to latent structures (PLS) an overview of the data is rapidly obtained as well as relationships between different biological factors that can lead to a deeper understanding of underlying mechanisms[2, 3]. The multivariate statistical method, PLS discriminant analysis[3] (PLS DA), is a valuable tool to identify the differences in content of measured substances between different groups of objects. In this work, the chemical composition of contaminants in salmon and brown trout caught in the same area, fish of the same species but from different locations, female fish *vs* male fish and finally wild fish *vs* farmed were evaluated.

Methods and Materials

The fish were caught during fall 2000 and 2001 in the Baltic proper south east of Gotland (feeding-salmon), in the Swedish rivers Luleälven, Ångermanälven, Dalälven, and Mörrumsån, (spawning migrating fish) as well as the Swedish lakes, Vänern and Vättern. For the chemical analyses, dorsal muscle tissue was used. The analysis of salmon and brown trout from SE of Gotland were performed on individual samples (n=10 and n=6 respectively) while the analysis of the fish from other sampling sites was carried out using pooled, sex separated samples (n=5-9 / pool) to a total of 19 pooled samples. Two individual male brown trout were statistically outlayers (due to very low age) and therefore excluded from the statistical analysis.

The analysed substances are 17 PCDDs, PCDFs and four *non-ortho* substituted PCBs (see table 1). Extraction, clean-up and analysis was done according to validated methods at Environmental Chemistry, Umeå University, Sweden. The PCDD/DFs and dioxin like PCBs levels are expressed in pg/g fresh weight. In the calculations for non-detectable values, the upper-bound level has been used (1 x level of detection).

Table 1. Substances analysed, included in all modelling in this work, and their abbreviations

Analysed Substance	Abbreviation
2,3,7,8-TCDF	TCDF
2,3,7,8-TCDD	TCDD
1,2,3,7,8-PeCDF	PeCDF1
2,3,4,7,8-PeCDF	PeCDF2
1,2,3,7,8-PeCDD	PeCDD
1,2,3,4,7,8-HxCDF	HxCDF1
1,2,3,6,7,8-HxCDF	HxCDF2
2,3,4,6,7,8-HxCDF	HxCDF3
1,2,3,7,8,9-HxCDF	HxCDF4
1,2,3,4,7,8-HxCDD	HxCDD1
1,2,3,6,7,8-HxCDD	HxCDD2
1,2,3,7,8,9-HxCDD	HxCDD3
1,2,3,4,6,7,8-HpCDF	HpCDF1
1,2,3,4,7,8,9-HpCDF	HpCDF2
1,2,3,4,6,7,8-HpCDD	HpCDD
3,3',4,4' PCB	PCB 77
3,4,4',5 PCB	PCB 81
3,3',4,4',5 PCB	PCB 126
3,3',4,4',5,5' PCB	PCB 169

Statistics PLS DA is a method for allocating observations (here fish) to one class a priori the analysis (eg. salmon=Class 1, brown trout Class=2) to identify what variables contributes to eventual class separation. The PLS DA identify the variables which best separates the classes, this is illustrated in a coefficient plot (95% CI denoted) and the validity of the model is estimated by R^2 and Q^2 (cross validated R^2) values. For all multivariate modelling the software SIMCA-P 9.0 from www.Umetrics.com was used.

Results and discussion

The presented data evaluations are based on the measured PCDD/DFs and PCBs in fish of different species and/or populations. The concentration of dioxin-like compounds, in this case expressed as sum WHO TEQ, does not differ between salmon and brown trout from SE of Gotland (6.8 ± 1.8 and 4.8 ± 2.7, respectively, mean pg/g ww ± SD) indicating that the exposure of salmon and brown trout caught in this region is similar. Further, in the fish material from the rivers and from SE of Gotland, no statistically significant north-south gradient could be found, nor differences due to gender.

In contrast, when performing a PLS DA analysis based on the individual salmon and brown trout analyses from SE of Gotland, including all 21 individual substances in the model (Table 1), the two species are completely separated (3 components, $R^2X=0.83$, $R^2Y=0.94$, $Q^2=0.71$). This describes a similar interspecies congener composition but a different distribution pattern between species for this site. In the resulting coefficient plot (Fig 1) it is shown that salmon have significantly higher concentration than brown trout of PeCDF1, HxCDF2, HxCDF3 and HxCDD2 and that brown trout have higher concentration than salmon of HxCDF4, HpCDD, OCDF, PCB81 and 126. The distribution pattern, with higher concentrations of the “lower chlorinated” PCDD/DFs in salmon and the “higher chlorinated” PCDD/DFs in brown trout is interesting. The mechanism(s) of this difference will have to be investigated more thoroughly, but species specific differences in uptake rates, metabolism, choice of feed and feeding areas could be suggested.

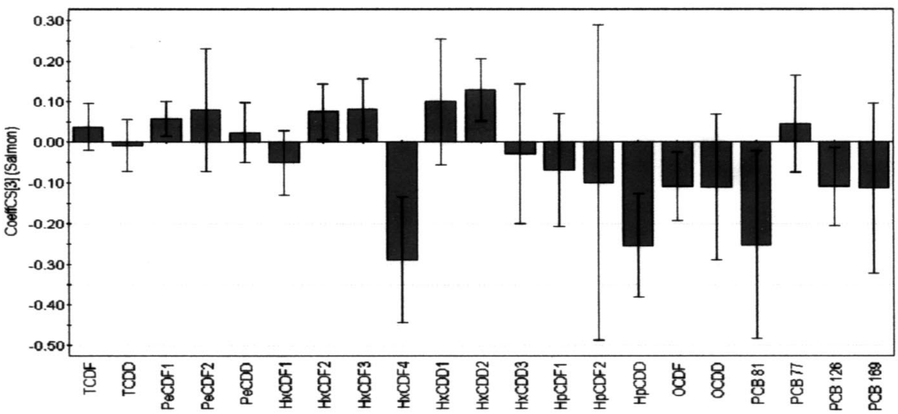

Figure 1. PLS DA, Salmon *vs* Brown trout, all from SE of Gotland. Coefficient plot (±95% CI). For abbreviations of substances see Table 1.

Further, a PLS DA modelling salmon from SE of Gotland *vs* salmon from Lake Vättern (substances in table 1) completely separate the two populations (3 components, $R^2X=0.78$, $R^2Y=0.99$, $Q^2=0.92$) (Fig 2 top). In accordance, a corresponding modelling of brown trout from SE of Gotland *vs* brown trout from Lake Vättern also results in a complete class separation (3 components, $R^2X=0.88$, $R^2Y=0.98$, $Q^2=0.84$) (Fig 2 bottom). This indicate that the distribution pattern of the 21 congeners are similar on basis of the catching area and that the exposure pattern has a greater influence on the differences in congener profile than the difference between species. The salmon and brown trout from SE of Gotland showed significantly higher concentrations of. PeCDF2, HxCDF2 and PCB77 and significantly lower concentrations of. HxCDF4 and PCB81, PCB126, and PCB169 compared to the salmon and brown trout from Lake Vättern.

Most interestingly, two out of 5 female salmon from SE of Gotland were born in the wild, while the others were compensatory reared (*i.e.* released at smolt stage). When comparing these two different groups, compensatory reared *vs* wild (substances in table 1) by a PLS DA (2 components, R2X = 0.73, R2Y=0.99, Q2=0.71) a pattern emerge where the compensatory reared fish had higher concentrations in almost all measured substances (none of the substances was significantly higher in the wild fish) especially for the lower chlorinated PCDDs, PCDFs and PCBs. This indicates that the exposure patterns differ between wild and compensatory reared fish.

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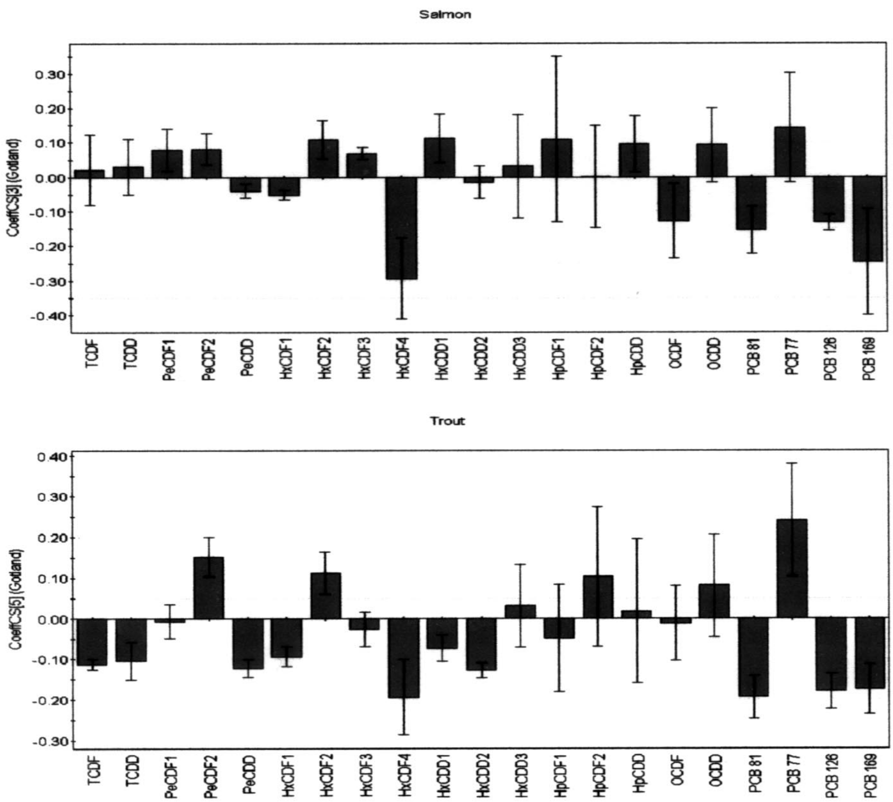

Figure 2. Salmon and Brown trout respectively. PLS DA fish from SE of Gotland *vs* fish from lake Vättern. Coefficient plots (±95% CI). For abbreviations of substances see table 1.