UNEXPLAINED GONAD ALTERATIONS IN WHITEFISH FROM LAKE THUN, SWITZERLAND: LEVELS OF PERSISTENT ORGANIC POLLUTANTS IN DIFFERENT MORPHS

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Introduction

Starting in the year 2000, a surprisingly high number of macroscopical gonad alterations has been observed in whitefish (*Coregonus* spp.) from Lake Thun, a prealpine lake located in the center of Switzerland. This unique phenomenon is still unexplained and has received much public attention. Six types of morphological states were described in these fish[1]. Gonadal adhesions/fusions to the peritoneal wall and to the lateral musculature (FUS), compartmentations (COM) and hermaphroditism (HER) were present at significantly higher prevalence in whitefish from Lake Thun than in the other lakes and are therefore considered as true deformation and typical for Lake Thun. Asymmetry (ASY), aplasia/atrophy of gonads (APL) and constrictions (CON) were also present in a comparable frequency in whitefish from other lakes, so that they represent rather a natural variation in gonad morphology of whitefish. The frequency of abnormal gonads varies between the different sympatric whitefish forms (so called morphs). Among the different whitefish morphs living in Lake Thun, the summer spawning morph Brienzlig showed more deformed gonads (41%) than the winter spawning morph Albock (26%). These two morphs differ additionally in the depth of their spawning areas (60-200 m for Brienzlig and 20-60 m for Albock), their growth rate (Brienzlig is slow growing and Albock is fast growing) and food patterns (Brienzlig feeds on coarser zooplankton and Albock on finer zooplankton).

As one possible cause for these effects, the presence of persistent, bioaccumulative and toxic compounds acting as endocrine disruptors in the lake has been discussed. In this study, concentrations of selected persistent organic pollutants (POP) were examined in whitefish from Lake Thun, and their link to the observed abnormalities was investigated. Two distinct morphs (Brienzlig, Albock) were considered, to study the influence of their distinct characteristics and behavior on their POP burden. Analyzed compounds included polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F), polychlorinated naphthalenes (PCN), polybrominated diphenyl ethers (PBDE) and hexabromocyclododecanes (HBCD).

Materials and Methods

Analyzed samples (n=20) included pooled whitefish muscle tissue from two different morphs (Brienzlig and Albock) with and without gonad alterations. Samples with gonad alterations were subdivided in pools affected either by FUS, COM and HER (alterations specific for Lake Thun) or by ASY, APL and CON (more commonly occurring alterations). The applied analytical method included liquid-liquid extraction[2], lipid content determination, extract cleanup and detection with gas chromatography coupled to high resolution mass spectrometry[3]. Quantification was based on the isotope dilution method.

Reported di-*ortho*-PCB include PCB 28, 52, 101, 138, 153 and 180. Dioxin-like PCB (dl-PCB) include the coplanar PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189. PCDD/F include all 17 2,3,7,8-substituted PCDD/F. PCN corresponds to the total of all tetra-CN to octa-CN. PBDE include PBDE 28, 47, 99, 100, 153 and 183 and HBCD include all stereoisomers. For dl-PCB and PCDD/F, the TEQ was determined based on the WHO-TEF from 1998[4]. Concentrations are reported on a lipid weight (lw) base.

Quality control included repeated analyses of blank samples (mean 1.8, 0.2, 0.6 and 0.4 ng/g lw for di-*ortho*-PCB, PCN, PBDE and HBCD; 1.9 and 0.6 pg TEQ/g lw for dl-PCB and PCDD/F), repeated analyses of identical fish samples (relative standard deviation 6% for the lipid content and 10%, 7%, 16%, 11%, 10% and 9% for di-*ortho*-PCB, dl-PCB, PCDD/F, PCN, PBDE and HBCD) and check for recovery of internal standards (mean 66%, 81%, 50%, 52%, 52% and 23% for di-*ortho*-PCB, dl-PCB, PCDD/F, PCN, PBDE and HBCD).

Results and Discussion

Figure 1 shows the range of concentrations (box plots) of the investigated POP in all whitefish samples from Lake Thun.

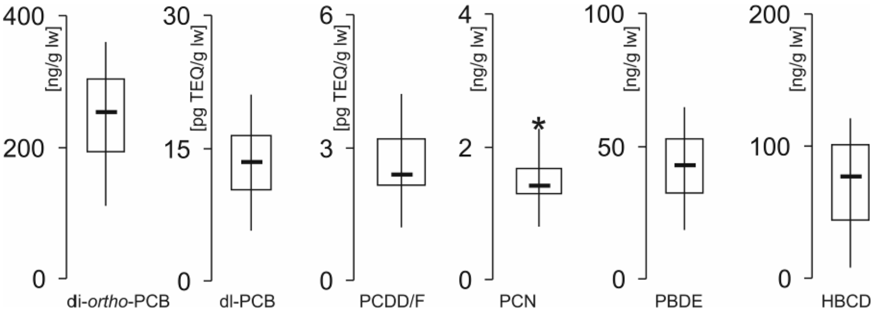

Figure 1: Boxplot with concentrations of POP in whitefish (n=20) from Lake Thun. The boxes stretch from the 25th-percentile to the 75th-percentile and contain the median in between. The whiskers end at the minimum and maximum observed value or at most 1.5 times the interquartile range. Outliers lower or higher than 1.5 times the interquartile range are represented separately by stars.

Concentrations ranged from 110-360 ng/g lw for di-*ortho*-PCB, 6-21 pg TEQ/g lw for dl-PCB, 1-4 pg TEQ/g lw for PCDD/F, 1-3 ng/g lw for PCN, 18-65 ng/g lw for PBDE and 8-121 ng/g lw for HBCD. Thus, due to the high concentrations PCB are still the most prominent POP accumulated in fish, although they are well regulated. First restrictions for the use of PCB were established in Switzerland in 1972. Complete ban of PCB followed in Switzerland in 1986 and internationally in 2004[5]. PCN represent another class of legacy organochlorines with similar former usage as PCB and banned or phased out in Europe in the 1970s. In Lake Thun fish, PCN concentrations are however considerably lower than PCB. Concentrations of PBDE are clearly lower than levels of the long time phased out PCB and also lower than HBCD. PBDE have been used in Europe as brominated flame retardants since 2004 and currently they are probably still present in high amounts in flame proofed materials. HBCD is one of the most widely used brominated flame retardant in Europe and the high levels in fish reveal the ongoing emissions and the strong bioaccumulative character of this compound.

Figure 2 shows the concentrations of dl-PCB + PCDD/F in whitefish morphs Brienzlig and Albock from the present study in comparison to our previous studies (same methodology used) of whitefish from other Swiss low altitude lakes[6] and fish from Swiss remote mountain lakes[3].

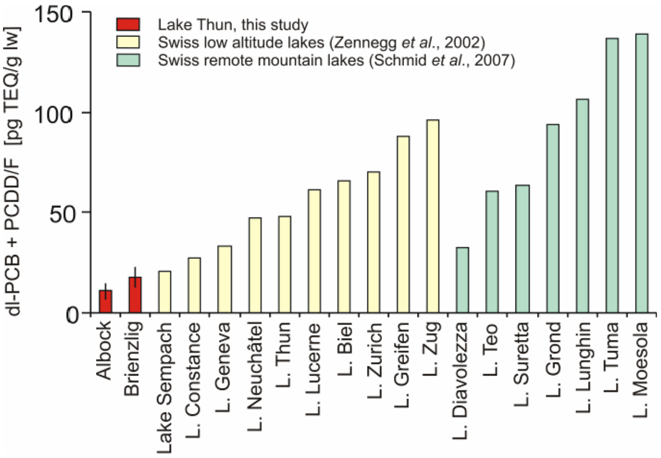

Figure 2: Concentrations of dl-PCB + PCDD/F in whitefish from Lake Thun (this study), in whitefish from Swiss low altitude lakes[6] and different fish species from Swiss remote mountain lakes[3].

Comparison with previous investigations shows that concentrations of the investigated POP in whitefish from Lake Thun are among the lowest concentrations in whitefish from comparable Swiss low altitude lakes. For dl-PCB + PCDD/F, the levels in Lake Thun whitefish are even lower than in other fish species from remote Swiss mountain lakes. Thus, Lake Thun can be considered as a background site with no evident important source for dioxins and dioxin-like compounds in its catchment area.

Regarding the two investigated whitefish morphs, Figure 3A shows the range of concentrations of dl-PCB + PCDD/F in Albock and Brienzlig. Among Brienzlig, concentrations in whitefish with no gonad alterations, in whitefish affected by ASY, APL and CON (common alterations) and in whitefish affected by FUS, COM and HER (specific alterations) are provided in Figure 3B.

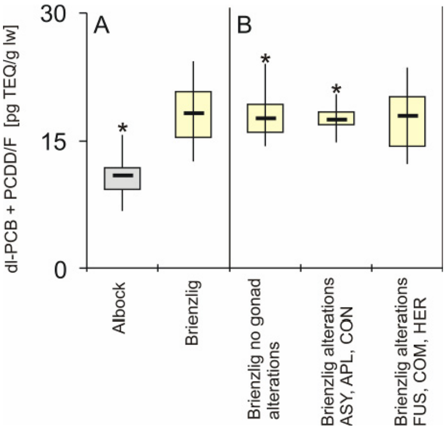

Figure 3: Boxplot with concentrations of dl-PCB + PCDD/F in morphs Albock and Brienzlig (A). Samples of the morph Brienzlig were subdivided in pools without alterations, with ASY, APL and CON as alteration types and FUS, COM and HER as alteration types (B). The boxes stretch from the 25th-percentile to the 75th-percentile and contain the median in between. The whiskers end at the minimum and maximum observed value or at most 1.5 times the interquartile range. Outliers lower or higher than 1.5 times the interquartile range are represented separately by stars.

Concentrations of dl-PCB + PCDD/F (as well as the other investigated compounds) differed significantly between the two whitefish morphs ($p < 0.0005$, Wilcoxon rank sum test). Concentrations were higher in Brienzlig than in Albock (Figure 3A). Possible explanations for the different concentrations in the two investigated whitefish morphs may include differences in exposure as consequence of diverse habitats, distinct food patterns, as well as differences in growth. The samples of the two morphs differ significantly in their age (mean age of Brienzlig 3.2 ± 0.8 years, Albock 2.6 ± 0.7 years) and thus in exposure time. The difference in mean age can be explained by the faster growth rate of the Albock, which therefore gets caught at a younger age. The faster growth rate of the Albock suggests an increased conversion ratio of food (consisting of fine zooplankton). Less uptake of food would also imply a lower uptake of lipophilic chemicals bound to ingested particles. Dilution by growth might simulate an increased elimination of the chemicals. For Brienzlig, the slower growth rate and the increased food uptake (coarse plankton) is a possible explanation for the higher POP burden.

Our investigations revealed no link between POP concentration in fish muscle tissue and gonad alterations (see Figure 3B for dl-PCB + PCDD/F in Brienzlig). Though, the separate analyses of the two whitefish morphs revealed that Brienzlig had significantly higher concentrations of the analyzed substances than Albock (Figure 3A). Gonad abnormalities occurred also more frequently in Brienzlig (41%) than in Albock (26%)[1]. However, a link between the investigated POP concentrations as a cause for the gonad alterations is still fairly unlikely for the following reasons: (i) within the Brienzlig morph, no differences in POP concentrations were observed in fish with and without gonad alterations; (ii) compared to other Swiss lakes, POP concentrations are clearly lower in Lake Thun whitefish (Figure 2). Differences in the lifestyle of the two morphs as discussed previously might cause both, gonad alterations and higher levels of contaminants.

Conclusions

Besides the investigation of the fish deformations, our data showed a significant dependency of the contaminant levels on the morph in a fish species from the same Lake. Inter-fish species variation (i.e. variation between different species) is known to be an important factor while studying fish contamination[7]. In the case of Lake Thun whitefish we also showed that variation within the fish species (i.e. variation between fish of different morphs within the same species) may be a relevant element to be considered while analyzing and comparing concentrations of pollutants in biota. The present study provides a relevant contribution to the numerous investigations about the unexplained occurrence of gonad malformations in whitefish from Lake Thun that are currently performed. No direct link between the investigated organohalogen compounds bioaccumulated in fish muscle tissue and the occurrence of gonad abnormalities in whitefish could be observed. An additional screening of selected samples of fish lipids revealed no unexpected lipophilic substances. Currently, whitefish from Lake Thun continue to be caught for commercial purpose as no contamination has been observed, which was confirmed in this study by the low levels of dioxins and dioxin-like compounds. However, additional investigations are needed to further investigate the hypothesis of exogenous substances as a reason for the gonad abnormalities.

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